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EFFECT OF GOLD NANOPARTICLES AND CIPROFLOXACIN ON MICROBIAL CATABOLISM: A COMMUNITY-BASED APPROACH

KELA P. WEBER,*† ELIJAH J. PETERSEN,‡ SONJA BISSEGGER,† IRIS KOCH,† JUN ZHANG,† KENNETH J. REIMER,† LARS REHMANN,§ ROBIN M. SLAWSON,|| RAYMOND L. LEGGE, # and DENIS M. O'CARROLL††‡‡

† Department of Chemistry and Chemical Engineering, Royal Military College of Canada, Kingston, Ontario, Canada
‡ National Institute of Standards and Technology, Material Measurement Laboratory, Gaithersburg, Maryland, USA
§ Department of Chemical and Biochemical Engineering, University of Western Ontario, London, Ontario, Canada
|| Department of Biology, Wilfrid Laurier University, Waterloo, Ontario, Canada
Department of Chemical Engineering, University of Waterloo, Waterloo, Ontario, Canada
†† Department of Civil and Environmental Engineering, University of Western Ontario, London, Ontario, Canada
‡‡ Water Research Laboratory, School of Civil and Environmental Engineering, University of New South Wales, Manly Vale, New South Wales, Australia

Running title: Effect of Emerging Contaminants on Microbial Catabolism

*Address correspondence to Kela.Weber@rmc.ca

Additional Supporting Information may be found in the online version of this article.

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Abstract: The effect of gold nanoparticles (AuNPs) and ciprofloxacin on the catabolism of microbial communities was assessed. This was accomplished through an *ex-situ* methodology designed to give *a priori* knowledge on the potential for NPs, or other emerging contaminants, to affect the catabolic capabilities of microbial communities in the environment. Microbial communities from a variety of sources were incubated with 31 pre-specified carbon sources and either National Institute of Standards and Technology (NIST) reference material 10 nm AuNPs, or ciprofloxacin on 96 well microtiter plates. From the ciprofloxacin study, dose response curves were generated and exemplified how this method can be used to assess the effect of a toxicant on overall catabolic capabilities of microbial communities. When adding 10 nm AuNPs at concentrations ranging from 0.01 to 0.5 µg/mL, rhizosphere communities from *Typha* roots were only slightly catabolically inhibited at a single concentration (0.05 µg/mL), no effects were seen on wetland water communities, and a minor positive (i.e. enhanced catabolic capabilities) effect was observed for loamy soil communities. This positive effect may have been due to a thin layer of citrate found on these AuNPs which initiated co-metabolism with some of the carbon sources studied. Based upon the conditions considered the possible adverse effects of AuNPs on the catabolic capabilities of microbial communities appears to be minimal.

Keywords: Ecotoxicology, Emerging contaminants, Microbial community, Nano-gold, Ciprofloxacin

INTRODUCTION

Nanoparticles (NPs) such as gold nanoparticles (AuNPs) have a wide range of potential applications with one of the more popular being biomedical devices [1,2]. The size range of NPs gives them enhanced catalytic, physical strength, thermal, and optical properties when compared to their micro-scale counterparts with the same elemental composition. However, the expected release of NPs into the environment may pose a risk. As such, substantial research has been conducted in recent years to assess the ecotoxicity of carbonaceous, metal, and metal oxide nanoparticles [3,4,5]. Of recent interest are the ecotoxicological effects of NPs on environmental microbial communities, which play key roles in ecosystem function including primary production, nutrient cycling and waste decomposition.

It is the community as a whole and the complexities surrounding their interactions which provide ecosystem services. Some of the earlier work regarding toxicological effects of nanoparticles on microbial life were completed using single bacterial species [6,7,8,9,10,11]. Other research has investigated the effects of different NPs on microbial communities using a mixture of respiration, enzymatic activity, enumeration or community structure endpoints [12,13,14,15,16,17,18]. However, no studies have yet been conducted on the catabolic characteristics and overall catabolic functions of microbial communities, which are one of their most important functions. Commercially available plates, such as the BIOLOG^{TM} ECO plate [19], are available for assessing a community's ability to utilize a variety of different carbon sources [20]. Some recent studies have used these plates to investigate pollution induced tolerance of environmental microbial communities [21,22] or the effect of a perturbation event on microbial communities [23,24,25,26]. This type of microtiter plate system, however, has not been used as an *ex-situ* vessel for assessing *a priori* the effects of chemicals or materials on the catabolic capabilities of microbial communities of any type. In order for any one carbon source in a well on the BIOLOG^{TM} ECO plate to be utilised, the community as a whole needs to be able to utilize that carbon source. This can come from a single species or group directly metabolising the carbon source or from a cooperative or synergistic catabolic utilisation where exoenzymes are expressed and/or metabolites created and utilised. This sequence of catabolic and metabolic events is one way in which microbial community function can be described. Thus, one of the primary advantages to this type of method is that a whole community response is better

represented and understood, and is more typical of community and biofilm behaviour, predominant in natural settings.

The objective of this study was to develop, test, and utilize an *ex-situ* method to assess the effects NPs may have on the overall catabolic capabilities and function of naturally occurring microbial communities. The proposed microtiter approach was used to produce a number of dose-response curves. First, the broad spectrum antibiotic ciprofloxacin was used to evaluate the catabolic inhibitory effects of the antibiotic on both antibiotic-resistant and non-resistant microbial communities from laboratory mesocosm wetlands. This is an important topic itself given that antibiotic residues have been detected in the final effluents of wastewater treatment plants (WWTPs) worldwide and are themselves emerging contaminants that may have significant environmental impacts [27,28]. This type of *ex-situ a-priori* study has never before been done assessing the effects of ciprofloxacin on the overall catabolic function of microbial communities. Second, the catabolic inhibitory effects of National Institute of Standards and Technology (NIST) reference material 10 nm nominal AuNPs on microbial communities from both wetland and soil environments were assessed utilizing this new approach. This investigation provides the first data of this type on the impacts of NPs on the catabolic capabilities of microbial communities.

MATERIALS AND METHODS

Experimental design

Two experimental regimes are described. First, an experiment assessing the effects of ciprofloxacin on both antibiotic resistant and non-resistant microbial communities is presented to help introduce the conceptual usage of the methodology. Second, the effect of AuNPs on several different naturally occurring communities is described.

Ciprofloxacin study. Eight different mesocosm wetlands (MW1 through MW8) were sampled to gather representative interstitial water samples containing mixed microbial communities. The types of mesocosms used have been previously described in Weber *et al.* [26]. The 4 mesocosm wetlands MW1, MW2, MW3, MW4 all contained microbial communities that had demonstrated antibiotic tolerance, while the 4 mesocosm wetlands MW5, MW6, MW7, and MW8 did not contain induced antibiotic resistant microbial communities [29]. These 8 different MW microbial communities were exposed to 0, 0.5 µg/mL, 1.0 µg/mL, or 2.0 µg/mL ciprofloxacin in triplicate in BIOLOG^{TM} ECO plates and their catabolic profile and function quantified. Concentrations were chosen based on previous studies where non-antibiotic resistant environmental communities exhibited an EC50 of

0.56 µg/mL with reductions in activity or growth recorded at concentrations as high as 2 µg/mL [26,30,31]. The carbon sources contained in the wells of BIOLOG™ ECO plates are listed in Table 1, and general groupings of the carbon sources are also provided to reveal the general diversity of carbon sources on any one plate.

Gold nanoparticle study. Experiments were also designed to elucidate the effects of AuNPs on the catabolic capabilities of naturally occurring microbial communities. AuNPs were National Institute of Standards and Technology (NIST) 10 nm reference material (RM) nanoparticles [32]. Three different microbial community types were used for the assessment: communities detached from wetland residing *Typha* roots, communities detached from a loamy soil, and communities residing in wetland water from an area predominately composed of *Typha*. All 3 community types were gathered from the Kingston area near the Royal Military College of Canada (RMCC) (latitude, longitude: 44.234,-76.465) in August, 2011. A local wetland dominated by *Typha* was used to gather root samples and free water samples. Loamy soil was taken from 10 cm below a local RMCC garden bed. These three different microbial community types were chosen to demonstrate the suitability of this method for different microbial community types. Each sample was taken in triplicate and processed in triplicate throughout the study. All three different microbial composite communities were exposed to 0, 0.01, 0.05, 0.1, or 0.5 µg/mL AuNPs, and their catabolic capabilities assessed. These concentrations were selected based upon the highest AuNP that could be readily utilized based on the concentration in the RM (51.56 µg/mL).

Aggregation of the AuNPs in phosphate buffer was tested under conditions similar to the microbial community experiments using dynamic light scattering (DLS, NICOMP 380 ZLS; Particle Sizing Systems, Santa Barbara, CA). Each sample was tested three times with each run lasting 2 min. Four samples were tested at 0 and 24 h. The uncertainty of size distribution by DLS represented as standard deviations and propagation of errors was used to combine uncertainties from each individual measurement and the uncertainty from combining the individual measurements. When a single sample was tested twice with two sets of three runs, the initial size was (14.5 ± 5.7) nm and (15.1 ± 5.5) nm, thus indicating good instrument reproducibility despite the high standard deviations which were mainly from uncertainty from the individual measurements. Additional methodology details can be found in the supplemental data.

AuNP solutions were analyzed for Au concentrations by inductively coupled plasma-mass spectrometry (ICP-MS) on a quadrupole ICP-MS instrument (Elan DRC II, PerkinElmer, Canada), equipped with a concentric

nebulizer and a cyclonic spray chamber. AuNP concentrations were quantified at times 0h and 24h in the same phosphate buffer solution used in the toxicity experiments (exposures run in additional plates for the sole purpose of sampling for AuNP concentration). Additional methodology details are provided in the supplemental data.

Assessment of the catabolic capabilities of microbial communities

Assessment of the catabolic capabilities was performed using BIOLOG™ plates. In general BIOLOG™ plates are 96-well microtiter plates where each well contains a different carbon source and a redox dye indicator, tetrazolium violet. When a mixed microbial community sample is inoculated into each of the wells, the production of nicotinamide adenine dinucleotide (NADH) via cell respiration reduces the tetrazolium dye to formazan, resulting in a colour change within each individual well, which can be detected photometrically. This study specifically used BIOLOG™ ECO plates (Biolog Inc., Hayward CA., USA) which contain 31 different carbon sources, and a blank in triplicate. The plate wells were inoculated with 140 µL of a suspended microbial community sample and then incubated at room temperature and read at an absorbance of 590 nm every 6 hours for 96 hours. Where microbial community detachment was required (root samples and soil samples), the methods outlined in Weber and Legge [31] were followed using a slow shaking method in phosphate buffer prepared to 10mM with a pH of 7 and 8.5 g/L NaCl.

Analysis of the catabolic data was performed in a similar manner as described by Weber and Legge [20] and Weber *et al.* [33]. For the ciprofloxacin study (MW1-8 samples) absorbance readings at 84 h were identified as the metric for further data analysis while absorbance after 24 h was used for the AuNP study (*Typha* roots, soil, wetland water) (see Weber and Legge [20] for a detailed explanation of metric choices). In short, when choosing a specific time point for further analysis a balance is needed between the amount of information contained in a specific data set from any one time point (which can be measured by calculating the amount of variation between all absorbance values) and the number of absorbance values within the linear range of the instrument. As incubation time progresses the amount of information in the data set from any one time point increases. This concept can be demonstrated by considering the incubation time at zero minutes where the data set contains no useful information as all absorbance values read the same value. However, after the incubation time period progresses past a certain point some absorbance values for specific carbon sources (wells) will also fall outside of the linear absorbance range (absorbance values above 2 are considered to fall outside the linear range). Therefore

when choosing a time point for further analysis, careful examination of all data collected is required before deciding on which exact time point is to be used for further data analysis. While data was also collected for other time points in this study, the values for some of these data sets included absorbance values which extended beyond the linear range. Thus, only the 24 h data for the AuNPs was utilized because data after longer time periods was outside of the linear range. For the ciprofloxacin experiment, the communities were still in the lag phase after 24 h, and therefore data from 84 h was used. This data set represents a carbon source utilisation pattern which is unique to the microbial community being assessed on a plate.

Two different metrics were extracted from the carbon source utilisation patterns (CSUPs) gathered and used for further analysis: 1) The average well colour development (AWCD), and 2) the number of carbon sources utilised (substrate richness). The AWCD represents the average catabolic activity over all wells of the microbial community being assessed, and is calculated as:

$$AWCD = \frac{1}{31}\sum_{i=1}^{31}\left(A_i - A_0\right) \quad (1)$$

Where A_i represents the absorbance reading of well i and A_0 is the absorbance reading of the blank well (inoculated, but without a carbon source).

The number of carbon sources which a microbial community is able to utilize on each plate provides a representation of the catabolic potential of a particular community. This can also be referred to as substrate richness which is calculated as the number of wells with a corrected absorbance ($A_i - A_o$) greater than 0.25 [20]. Where the AWCD represents overall catabolic activity, the substrate richness metric identifies the catabolic capability range of the microbial community assessed.

RESULTS AND DISCUSSION

Assessing the catabolic capabilities of microbial communities during exposure to Ciprofloxacin

The ciprofloxacin study was performed to evaluate the effect of ciprofloxacin on the catabolic capabilities of microbial communities, but also serves as a good demonstration for the type of data which can be gathered using this methodology (see Figure 1). Figure 1A and Figure 1B present data which represent standard dose-response

curves. Both the overall catabolic activity (AWCD) and the number of carbon sources utilised (richness) for the antibiotic resistant microbial populations decreased with increasing doses of ciprofloxacin. At a concentration of 0.5 μg/mL a community level catabolic inhibitory effect is seen, with 1 μg/mL and 2 μg/mL exhibiting even greater (yet similar to each other) inhibitory effects. For the antibiotic resistant microbial communities a minimum AWCD between 0.2 and 0.4, and a minimum richness between 7 and 14, out of 31, at 1 μg/mL and 2 μg/mL is observed. Figure 1C and 1D present the same type of data for non-antibiotic resistant microbial populations, here it can be seen that a sharp drop-off in AWCD and richness occurs at 0.5 μg/mL. At concentrations of 0.5 μg/mL, 1 μg/mL and 2 μg/mL, AWCDs are between 0 and 0.2, and richness values are between 0 and 8. This data indicates the antibiotic resistant community's ability to stay catabolically active in the presence of higher concentrations of the antibiotic ciprofloxacin, when compared to the non-antibiotic resistant community.

In addition to the AWCD and richness metrics presented as dose-response curves, carbon source specific dose-response curves can also be visualised. Figure 2 presents the carbon source specific data for MW2 (antibiotic resistant). Figures 1A and 1B presented general metrics to describe the effect of ciprofloxacin on the catabolic capabilities of the MW2 microbial community as a whole. Figure 2 gives explicit detail regarding the community's ability to utilise each individual carbon source. It can be seen from Figure 2 that the dose-response curves for the each individual carbon source is unique.

Figure S1 displays the data where carbon sources are grouped into guilds as described in Table 1. The average guild response as displayed here is calculated in the same way as AWCD, but using only the specific carbon sources from the different guilds. This type of analysis allows for rapid investigations to understand if one general type of catabolic function is either being hindered or removed, while others are perhaps not affected. In this case the responses for the carbon source guilds were all similar as shown in Figure S1.

Assessing the catabolic capabilities of microbial communities during exposure to gold nanoparticles

Naturally occurring microbial communities were gathered from wetland *Typha* roots, soil, and wetland water and their catabolic capabilities assessed at varying AuNP doses (see Figure 3). Under the conditions tested, the effect of AuNPs on the catabolic capabilities of environmental communities was minimal. The first observation to be noted is the general lack of a visually noticeable effect of AuNP dose on the catabolic responses of any of the composite communities. Figure 3A shows the AWCD of the *Typha* root community and the wetland

water community to be largely unaffected by the AuNPs, whereas the soil community is in fact positively influenced by an increasing AuNPs dosage. Similar results are visually seen in Figure 3B when observing the substrate richness results, although in this case the *Typha* root community may be affected negatively to a small degree.

One important factor which needs to be considered in performing NP studies is the stability of the NP in solution and the relative size of the suspended NPs during the course of the experiments. Using DLS there did appear to be agglomeration of the AuNPs in the phosphate buffer solution during a 24 h period, the length of the toxicity experiment with the microbial community. However, it was not straightforward to unambiguously quantify the extent of agglomeration using the DLS software. When fitting the data with a Gaussian distribution as is most common with DLS software and using the intensity-weighted size distribution, the size increased from (14.7 ± 5.5) nm to (19.7 ± 12.5) nm (n=12 runs) after 24 h (see the Supplementary Material for additional discussion). ICP-MS was also used to measure the concentration of Au in solution during the 24h exposure period. Results showed a decrease of less than 1% of the Au in solution over the 24h exposure period (pooled results from 3 separate exposure experiments). These results indicate that aggregation was relatively minor and is not expected to have substantially impacted the results obtained. Thus, it can be said that the microbial communities were in contact with AuNPs for the duration of the study.

Table 2 summarizes minimum community level catabolic effect concentration (MCLCEC) results. The MCLCEC is a metric presented here to assist in extracting simple data from such a complicated data set. The MCLCEC is defined here as the minimum tested concentration of a material which creates a statistically significant reduction in the catabolic capabilities of a microbial community, and are reported here to semi-quantify the effects of AuNPs on the catabolic capabilities of the communities studied herein. As stated by Jager [34] use of ECx (the concentration showing x% effect) values are perhaps more useful for extrapolating ecotoxicological results to policy. In this case the effect of AuNPs on the catabolic capabilities of the selected microbial communities was quite small, and therefore EC50 values were not calculated as they would either 1) be outside of the environmentally relevant range of concentrations tested here, or 2) be non-existent given the data trend reported here (plateau).

The general lack of a negative response to AuNPs was similar to results previously obtained using these same AuNPs when testing their cytotoxic and genotoxic effects on HepG2 cells and calf-thymus DNA [35]. It is noteworthy that substantial differences in toxicological responses to NPs have been observed for different cell lines and bacteria. In particular, a recent review showed that median minimal inhibitory concentrations varied by over an order of magnitude between cells and bacteria, and thus a lack of an effect on an individual cell line does not conclusively indicate that they won't be toxic to a bacterial community [36]. In this study, a decrease in the richness was observed for the *Typha* roots sample at a dose of 0.05 μg/mL. However, a dose-response behavior was not observed because the two higher concentrations were not significantly different from the control. Therefore, this very small inhibitive effect is only reported here as a preliminary result. To report this result with confidence, additional investigation is required using a broader range of conditions (e.g. different water chemistries).

The positive response seen by the soil community can perhaps be attributed to both the microbial community type and the very small amount of citrate coating found on the outside of NIST reference AuNPs. Richness for soil communities was shown to increase from 16 to 19 given the increasing dosage of AuNPs. The specific carbon sources which were additionally utilised given the AuNP dose were D-glucosaminic acid, 2-Hydroxy benzoic acid (salicyclic acid), and Itaconic acid; these compounds are all classified as carboxylic & acetic acids (see Table 1). Citrate is a key compound in the Kreb's cycle and a readily metabolised carbon source for many microorganisms. It is suggested that perhaps the very small amount of added citrate in these 3 wells allowed for a co-metabolism to occur by a specific species or group of microorganisms and possible subsequent synergistic metabolite utilisation by additional microorganisms. It is also suggested that in comparison to the wetland water or *Typha* root communities, the soil microbial communities were not hindered to any great degree by the 10 nm AuNPs perhaps due to the adaptation to their original ecosystem environment which contains a wide range of particle sizes with some (although by no means the majority) being in the nano-size range.

In contrast to many other methods which aim to understand effects of a chemical on growth rate or viability, this method investigates effects on the catabolic function and capabilities of a community as a whole. Single organism testing can be useful as a basis, but community testing, especially in the case of microbial communities, is preferred for an assessment of environmental response due to the synergistic and cooperative

relationships between community components. This method has been developed and demonstrated here as a means of understanding the effects of contaminants on the function of microorganisms at the community level. Given care in sample preparation and characterizing the contaminant exposure over time in the plates, the method can provide relevant information regarding the potential effects of contaminants on the functional abilities, in this case the catabolic capabilities, of microbial communities. It is proposed that this method could be used as a means of screening large numbers of contaminants for their effects on the functional capabilities of many different microbial community types. Contaminants then identified as having a greater capacity for inducing a negative effect on a microbial community could be studied in greater depth via more time, energy and material intensive meso-scale or full-scale ecosystem exposure experiments.

As with all ecotoxicological testing, there are some limitations to this method. When completed *ex-situ* not all factors present in the environment will be quantified or tested. Factors such as water pH, ionic strength, background nutrient levels (when detaching communities into PBS or another buffer), or additional toxic environmental compounds were not measured here. These types of factors could be varied within the carbon source plates and factorial design type studies accomplished for more extensive testing. One of the more cited limitations of this type of methodology is the reliance on media culturing, where media culturing has long been known to not capture all microorganisms in a community. It should be added though that this limitation is cited with respect to enumeration, and enumeration is not the goal of this methodology. It is true that some microorganisms in the population may not contribute to the catabolic potential as measured; however, the focus here is on the large diversity of carbon source substrates and the community's ability to catabolically utilise the selected carbon sources. The BIOLOG^{TM} ECO plate was developed for the purpose of classifying and differentiating the differences in carbon source utilisation patterns (CSUPs) of different soil microbial communities [19]. Therefore, although the usefulness of this method may be observed and described here, the carbon sources themselves could be re-chosen to represent more ecotoxicologically relevant catabolic transformations important to nutrient cycling, carbon cycling, and waste decomposition in the environment. It should also be noted that this method does not require, and is not suggested to, precondition microbial community cultures in any way before microtiter plate inoculation. This method is being explored to assess the catabolic capabilities of the intrinsic environmental community as accurately as possible. Additional work via analogous studies tracking structural shifts in the

microbial communities would also help in understanding the potential effects of emerging contaminants on environmental microbial communities.

CONCLUSIONS

The concept behind this method, determining *a priori* the potential effects of NPs or chemicals on naturally occurring microbial communities, differs from other methodologies where microbial communities from environmental waters or soils are gathered and assessed as either control communities or stressed communities. The method proved useful in generating dose-response type curves when evaluating the effect of an antibiotic on microbial communities. The method can be used to report overall community-type catabolic capability metrics such as overall average activity (AWCD) and substrate richness. Additionally, more detailed analysis can be completed for each individual carbon source evaluated or for sets of carbon sources when discussed as guild groupings. This method was used to successfully show the impact of emerging contaminants on the catabolic activities of microbial communities. Ciprofloxacin showed distinctly different effects on microbial communities that were and were not resistant to antibiotics. When used to evaluate the effect of AuNPs on environmental microbial communities, only small inhibitory effects at the concentration of 0.05 μg/mL were seen for rhizospheric communities from *Typha* roots, no effect was seen on wetland water communities, and a slight positive effect was seen with soil communities. Under the conditions studied, it is suggested that the possible negative effect of AuNPs on the catabolic capabilities of microbial communities is minimal. Thus, the NIST RM AuNPs may serve as a potential NP negative control for future microbial toxicity studies. This was the first *ex-situ a-priori* study on the impacts of NPs on the catabolic capabilities of microbial communities, and the methodology described here can likely serve as a basis for rapid screening of the potential effects of NPs on this endpoint.

SUPPLEMENTAL DATA

Sections S1–S2.

Table S1.

Figure S1. (50 KB DOC).

Acknowledgment—Certain commercial equipment or materials are identified in this paper in order to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose. The authors would like to express their appreciation for funding of this work to NSERC in the form of Discovery Grants to KPW, KJR, LR, RLL and DMO. Additional support from ORF in the way of funding to the Centre for Control of Emerging Contaminants (CCEC) to RMS and RLL is gratefully acknowledged. Dr. Vytas Reipa is thanked for assistance with dynamic light scattering measurements.

REFERENCES

1. Huang X, Jain PJ, El-Sayed IH, El-Sayed MA. 2007. Gold nanoparticles: intersting optical properties and recent applications in cancer diagnostics and therapy. *Nanomedicine.* 2:681-693.
2. DeLong RK, Reynolds CM, Malcolm Y, Schaeffer A, Severs T, Wanekaya A. 2010. Functionalized gold nanoparticles for the binding, stabilization, and delivery of therapeutic DNA, RNA, and other biological macromolecules. *Nanotechnology, Science and Applications*. 3:53–63.
3. Petersen EJ, Zhang L, Mattison NT, O'Carroll DM, Whelton AJ, Uddin N, Nguyen T, Huang Q, Henry TB, Holbrook RD, Chen KL. 2011. Potential Release Pathways, Environmental Fate, And Ecological Risks of Carbon Nanotubes. *Environ Sci Technol*. 45:9837-9856.
4. Thomas CR, George S, Horst AM, Ji Z, Miller RJ, Peralta-Videa JR, Xia T, Mädler L, Gardea-Torresdey JL, Holden PA, Keller A, Lenihan HS, Nel AE, Zink JI. 2011. Nanomaterials in the environment: From materials to high throughput screening to organisms. *ACS Nano*. 5:13-20.
5. Petersen E.J, Henry TB. 2012. Methodological considerations for testing the ecotoxicity of carbon nanotubes and fullerenes: Review. *Env Tox and Chem*. 31:60-72.
6. Adams LK, Lyon DY, Alvarez, PJJ. 2006. Comparative eco-toxicity of nanoscale TiO2, SiO2, and ZnO water suspensions. *Water Res.* 40:3527-3532.
7. Choi O, Deng KK, Kim N-J, Ross L, Surampalli RY, Hu Z. 2008. The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth. *Water Res*. 42:3066-3074.
8. Jones N, Ray B, Ranjit K, Manna AC. 2008. Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. *FEMS Microbiol Letters.* 279:71-76.
9. 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*. 43:7285-7290.
10. Diao M, Yao M. 2009. Use of zero-valent iron nanoparticles in inactivating microbes. *Water Res.* 43:5243-5251.
11. Li Z, Greden K, Alvarez P, Gregory K, Lowry G. 2010. Adsorbed polymer and NOM limits adhesion and toxicity of nano scale zero-valent iron (nZVI) to E. coli. *Environ Sci Technol.* 44:3462-3467.

12. Tong Z, Bischoff M, Nies L, Applegate B, Turco R. 2007. Impact of fullerene (C60) on a soil microbial community. *Environ Sci Technol.* 42:2985-2991.

13. Johansen A, Pedersen A, Jensen KA, Karlson U, Hansen BM, Scott-Fordsmand JJ, Winding A. 2008. Effects of C60 fullerene nanoparticles on soil bacteria and protozoans. *Env Tox And Chem.* 27:1895-1903.

14. Ge Y G, Schimel J P, Holden P A. 2011. Evidence for Negative Effects of TiO2 and ZnO Nanoparticles on Soil Bacterial Communities. *Environ Sci Technol.* 45:1659-1664.

15. Bradford A, Handy RD, Readman JW, Atfield A, 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.

16. Sheng Z, Liu Y. 2011. Effects of silver nanoparticles on wastewater biofilms. *Water Res*. 45:6039-6050.

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

18. Hänsch M, Emmerling C. 2010. Effects of silver nanoparticles on the microbiota and enzyme activity in soil. *J Plant Nutrit Soil Sci*. 173:554-558.

19. Insam H. 1997. A new set of substrates proposed for community characterization in environmental samples. In: Insam H, Rangger A, editors. *Microbial Communities: Functional Versus Structural Approaches*. Berlin: Springer-Verlag.

20. Weber KP, Legge RL. 2010b. Community level physiological profiling. In: Cummings SP editor. *Methods in Molecular Biology: Bioremediation*. New Jersey : The Humana Press Inc; pp. 263-281.

21. Rutgers M, Verlaat IMV, Wind B, Posthuma L, Breure AM. 1998. Rapid method for assessing pollution-induced community tolerance in contaminated soil. *Env Tox and Chem*. 17:2210-2213.

22. Demoling LA, Baath E, Greve G, Wouterse M, Schmitt H. 2009. Effects of sulfamethoxazole on soil microbial communities after adding substrate. *Soil Biol. and Biochem*. 41:840-848.

23. Weber KP, Gehder M, Legge RL. 2008. Assessment of the changes in the microbial community in response to acid mine drainage exposure. *Water Res*. 42(1-2):180-188.

24. Shah V, Belozerova I. 2009. Influence of Metal Nanoparticles on the Soil Microbial Community and Germination of Lettuce Seeds. *Wat Air Soil Pollut.* 197:43-148.

25. Kumar N, Shah V, Walker V. 2011. Perturbation of arctic soil communities by metal nanoparticles. *J Haz Mat.* 190:816-822.

26. Weber KP, Mitzel MR, Slawson RM, Legge RL. 2011. Effect of ciprofloxacin on microbiological development in wetland mesocosms. *Water Res.* 45:3185-3196.

27. Batt AL, Kim S, Aga DS. 2007. Comparison of the occurrence of antibiotics in four full-scale wastewater treatment plants with varying designs and operations. *Chemosphere.* 68:428-435.

28. Carballa M, Omil F, Lema JM, Llompart M, García-Jares C, Rodríguez I, Gómez M, Ternes T. 2004. Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant. *Water Res.* 38:2918-2926.

29. Helt CD, Weber KP, Legge RL, Slawson RM. 2012. Antibiotic resistance profiles of wetland bacteria and fecal indicators following ciprofloxacin exposure in lab-scale constructed mesocosms. *Ecol Eng.* 39:113-122.

30. Näslund J, Hedman JE, Agestrand C. 2008. Effects of the antibiotic ciprofloxacin on the bacterial community structure and degradation of pyrene in marine sediment. *Aquatic Toxicol.* 90:223-227.

31. Weber KP, Legge RL. 2010a. Method for the detachment of culturable bacteria from wetland gravel. *J Microbiol Methods.* 80:242-250.

32. National Institute of Standards and Technology. 2012. Report of Investigation: Reference Material® 8011, Gold Nanoparticles, Nominal 10nm Diameter. U.S. Department of Commerce, Gaithersburg, Maryland.

33. Weber KP, Grove JA, Gehder M, Anderson WA, Legge RL. 2007. Data transformations in the analysis of community-level substrate utilisation data from microplates. *J Microbiol Methods*. 69:461-469.

34. Jager T. 2012. Bad habits die hard: The NOEC's persistence reflects poorly on ecotoxicology. *Env Tox and Chem.* 31:228-229.

35. Nelson BC, Petersen EJ, Marquis BJ, Atha DH, Elliott JT, Cleveland D, Watson SS, Tseng I-H, Dillon A, Theodore M, Jackman J. 2013. NIST gold nanoparticle reference materials do not induce oxidative DNA damage. *Nanotoxicol.* 7(1):21-29.

36. Bondarenko O, Juganson K, Ivask A, Kasemets, K, Mortimer, M, Kahru, A. 2013. Toxicity of Ag, CuO and ZnO nanoparticles to selected environmentally relevant test organisms and mammalian cells in vitro: a critical review. *Arch Toxicol.* 87(7): 1181-1200.

Figure 1: Dose-response curves for microbial communities from 8 different mesocosm wetlands (MWs). (A) Average well colour development (AWCD) and (B) the number of carbon sources utilized (richness) for antibiotic resistant MW microbial communities over increasing ciprofloxacin dose. (C) AWCD and (D) the number of carbon sources utilized (richness) for non-antibiotic resistant mesocosm wetland microbial communities over increasing ciprofloxacin dose. Data points are the average of triplicate measurements and uncertainties indicate one standard deviation.

Figure 2: Well colour development (corrected absorbance at 590nm) data (84h), given varying ciprofloxacin dose, for all 31 carbon sources collected for an antibiotic resistant microbial community (mesocosm wetland 2– MW2). Data points are the average of triplicate measurements and uncertainties indicate one standard deviation.

Figure 3: Dose-response curves for microbial communities from *Typha* roots, soil, and wetland water. Responses represented by (A) average well colour development (AWCD) and (B) the number of carbon sources utilized (richness) over increasing AuNP dose. Data points are the average of triplicate measurements and uncertainties indicate one standard deviation.

Table 1: BIOLOG™ EcoPlate carbon sources and guild groupings

Well No.	ID	C-Source	Guild
Well 1	C0	Water (Blank)	
Well2	C1	Pyruvic Acid Methyl Ester	Carbohydrate
Well3	C2	Tween 40	Polymers
Well4	C3	Tween 80	Polymers
Well5	C4	Alpha-Cyclodextrin	Polymers
Well6	C5	Glycogen	Polymers
Well7	C6	D-Cellobiose	Carbohydrates
Well8	C7	Alpha-D-Lactose	Carbohydrates
Well9	C8	Beta-Methyl-D-Glucoside	Carbohydrates
Well10	C9	D-Xylose	Carbohydrates
Well11	C10	i-Erythritol	Carbohydrates
Well12	C11	D-Mannitol	Carbohydrates
Well13	C12	N-Acetyl-D-Glucosamine	Carbohydrates
Well14	C13	D-Glucosaminic Acid	Carboxylic & Acetic Acids
Well15	C14	Glucose-1-Phosphate	Carbohydrate
Well16	C15	D,L-alpha-Glycerol Phosphate	Carbohydrate
Well17	C16	D-Galactonic Acid-Gamma-Lactone	Carboxylic & Acetic Acids
Well18	C17	D-Galacturonic Acid	Carboxylic & Acetic Acids
Well19	C18	2-Hydroxy Benzoic Acid	Carboxylic & Acetic Acids
Well20	C19	4-Hydroxy Benzoic Acid	Carboxylic & Acetic

			Acids
Well21	C20	Gamma-Hydroxybutyric Acid	Carboxylic & Acetic Acids
Well22	C21	Itaconic Acid	Carboxylic & Acetic Acids
Well23	C22	Alpha-Ketobutyric Acid	Carboxylic & Acetic Acids
Well24	C23	D-Malic Acid	Carboxylic & Acetic Acids
Well25	C24	L-Arginine	Amino acids
Well26	C25	L-Asparagine	Amino acids
Well27	C26	L-Phenylalanine	Amino acids
Well28	C27	L-Serine	Amino acids
Well29	C28	L-Threonine	Amino acids
Well30	C29	Glycyl-L-Glutamic Acid	Amino acids
Well31	C30	Phenylethylamine	Amines/Amides
Well32	C31	Putrescine	Amines/Amides

Table 2: Summary of AuNP does-response data and associated p-values from a one way ANOVA for each dose-response data set, and p-values from a subsequent 2-sided Dunnett's test comparing dose concentration response (0.01-0.5 µg/mL) with the control response (0 µg/mL). [a] Indicates the MCLCEC is reported for a positive response. Results where $p<0.05$ are marked with a *. Results compiled using Statistica 8.0.

		Dose (µg/mL)					
		0	0.01	0.05	0.1	0.5	MCLCEC
Typha roots							
	AWCD	1.36	1.38	1.34	1.37	1.41	
	ANOVA p-value	0.229					
	Dunnett p-value	-	0.942	0.872	0.993	0.285	N/A
	Richness	30.67	29.67	28	29	29	
	ANOVA p-value	0.035*					
	Dunnett p-value	-	0.453	0.011*	0.113	0.113	0.05 µg/mL
Soil							
	AWCD	0.55	0.62	0.70	0.68	0.73	
	ANOVA p-value	0.003*					
	Dunnett p-value	-	0.280	0.007*	0.014*	0.001*	0.05 µg/mL[a]
	Richness	16	18.33	19.67	18.67	19	
	ANOVA p-value	0.044*					
	Dunnett p-value	-	0.138	0.017*	0.083	0.049*	0.05 µg/mL[a]
Wetland water							
	AWCD	0.47	0.47	0.43	0.43	0.43	
	ANOVA p-value	0.162					
	Dunnett p-value	-	0.999	0.829	0.335	0.827	N/A
	Richness	16	16	15.33	15	15	
	ANOVA p-value	0.063					
	Dunnett p-value	-	1.000	0.980	0.096	0.922	N/A

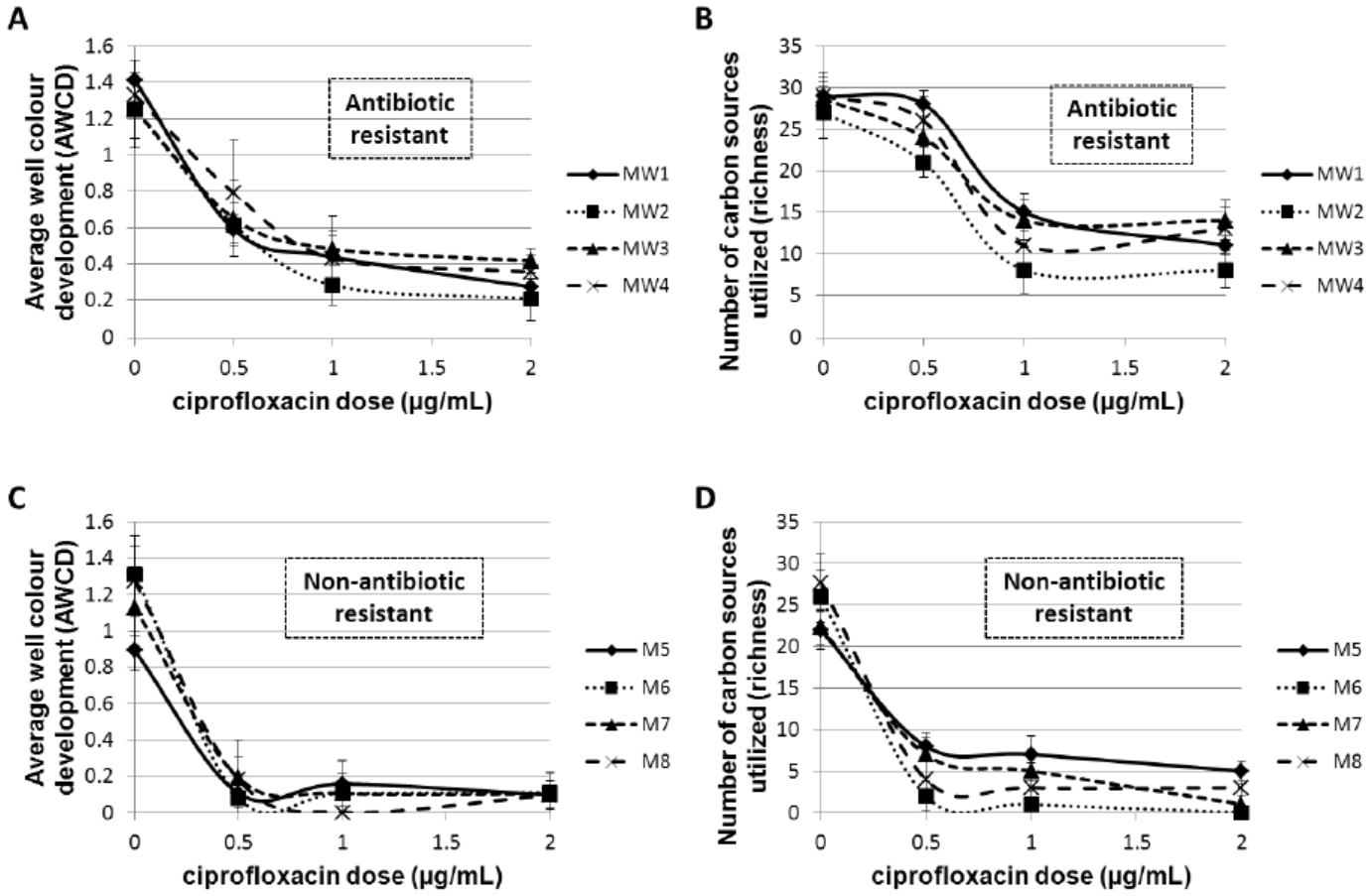

Figure 1

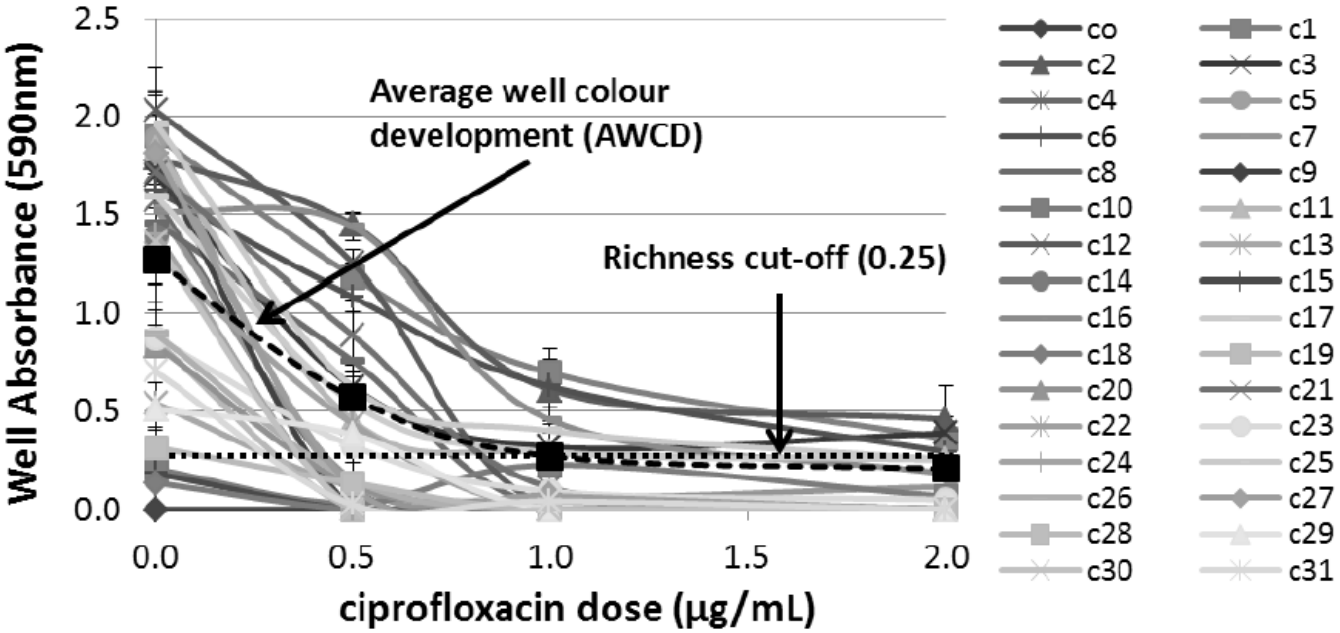

Figure 2

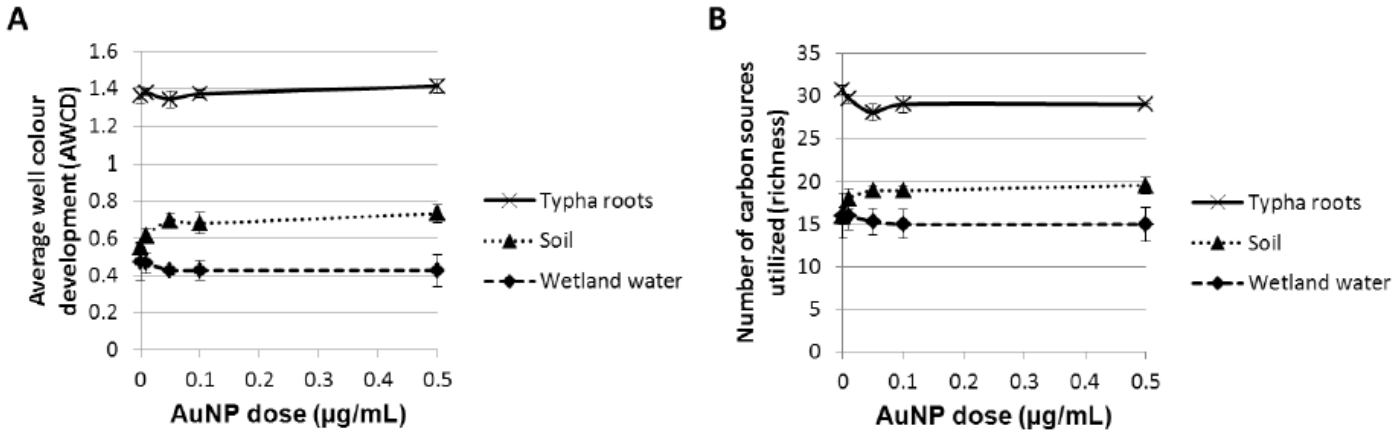

Figure 3