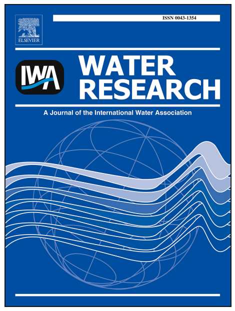

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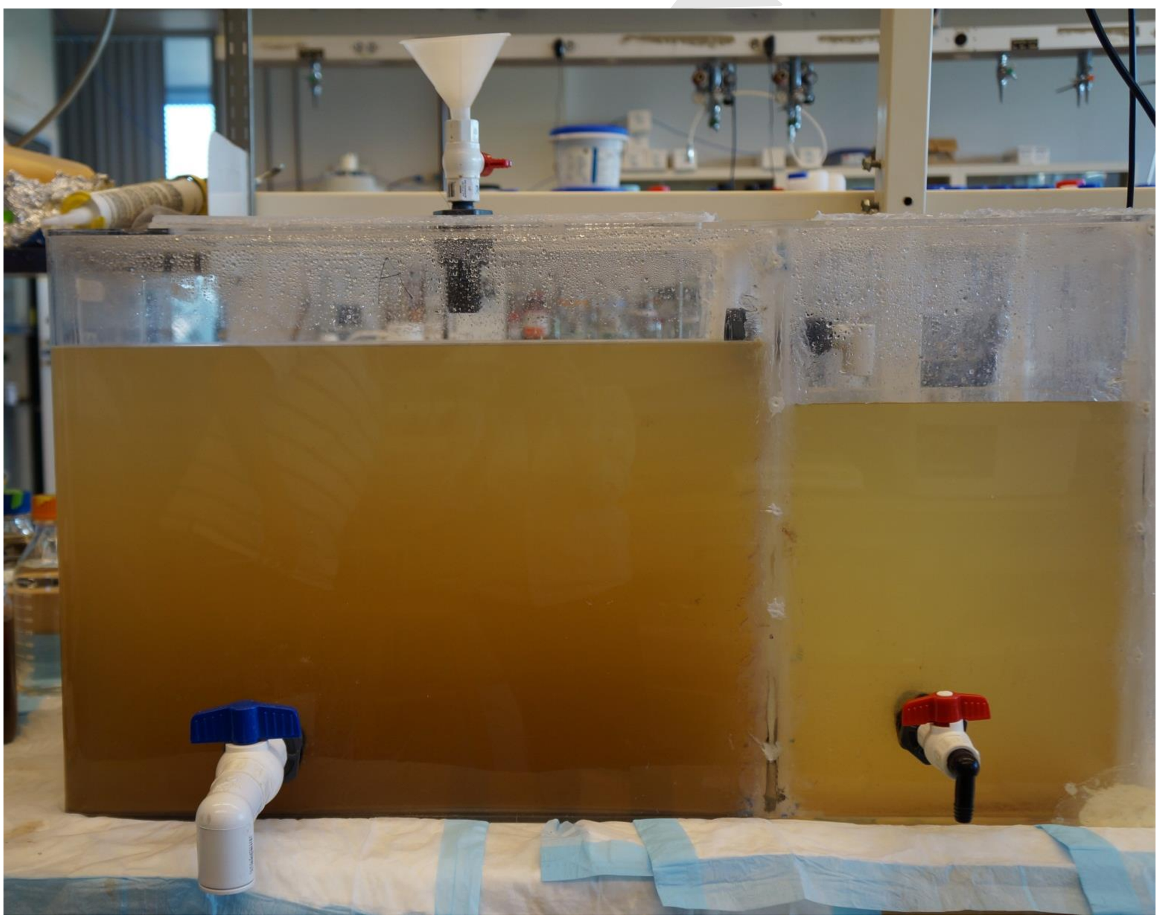
Graphical Abstract



Effects of copper particles on a model septic system's function and microbial community

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Abstract

There is concern surrounding the addition of nanoparticles into consumer products due to toxicity potential and the increased risk of human and environmental exposures to these particles. Copper nanoparticles are found in many common consumer goods; therefore, the disposal and subsequent interactions between potentially toxic Cu-based nanoparticles and microbial communities may have detrimental impacts on wastewater treatment processes. This study investigates the effects of three copper particles (micron- and nano-scale Cu particles, and a nano-scale $Cu(OH)_2$-based fungicide) on the function and operation of a model septic tank. Septic system analyses included water quality evaluations and microbial community characterizations to detect changes in and relationships between the septic tank function and microbial community phenotype/genotype. As would be expected for optimal wastewater treatment, biological oxygen demand (BOD_5) was reduced by at least 63% during nano-scale Cu exposure, indicating normal function. pH was reduced to below the optimum anaerobic

fermentation range during the micro Cu exposure, suggesting incomplete degradation of organic waste may have occurred. The copper fungicide, $Cu(OH)_2$, caused a 57% increase in total organic carbon (TOC), which is well above the typical range for septic systems and also corresponded to increased BOD_5 during the majority of the $Cu(OH)_2$ exposure. The changes in TOC and BOD_5 demonstrate that the system was improperly treating waste. Overall, results imply individual exposures to the three Cu particles caused distinct disruptions in septic tank function. However, it was observed that the system was able to recover to typical operating conditions after three weeks post-exposure. These results imply that during periods of Cu introduction, there are likely pulses of improper removal of total organic carbon and significant changes in pH not in the optimal range for the system.

1. Introduction

The release of engineered nanomaterials is an emerging ecological concern since initial studies indicate significant toxicity to multiple species including bacteria, mice, fish, and humans (Kang et al., 2007, Nel et al., 2006, Wiesner et al., 2006 and Xia et al., 2008). These nanomaterials may be released from consumer products into the environment at any point of a product's life, from manufacture to disposal. Nanoparticles are becoming more prevalent in common consumer goods such as foods and cosmetics. These particles are likely to enter household drains from the disposal and use of consumer products, and ultimately, may be released from wastewater treatment plants (WWTPs) into the environment (Keller et al., 2013, Keller and Lazareva, 2013 and Keller et al., 2014). Therefore, understanding the interactions between nanomaterials and bacteria in engineered systems (e.g. laboratory-scale septic tank) can indicate the consequences of nanomaterials on wastewater treatment processes and the potential for environmental release. Here, the effect of nanoparticles on the function and operation of a septic tank will be investigated.

A septic tank, which is an onsite, decentralized wastewater system (EPA, 2011), was chosen for the nanoparticle exposure experiments because it is estimated that 20-30% of American households have this type of onsite treatment method, and a projected 25% of predicted planned developments will also use septic tanks for waste disposal (EPA, 1999 and EPA, 2011). Additionally, 26% of households in Europe and 20% of Australian households also use septic systems (Beal et al., 2005 and Williams et al., 2013). It is critical that the function of these systems is maintained for sanitation and health. Improperly functioning septic systems can result in groundwater contamination and disease outbreaks (Beal et al., 2005 and Yates, 1985). Essentially, the septic tank provides a waste separation process between the sludge, the floating material, and the wastewater in an anaerobic environment in which digestion of the sludge and floating material occurs (Canter and Knox, 1985). To date, no such studies have been performed to assess the effect of nanoparticles on septic system function.

Nanoparticles have been detected in municipal wastewater treatment systems (Kiser et al., 2009 and Westerhoff et al., 2011), and therefore, nanoparticles are also likely being introduced into onsite septic systems. At one sampling site, the raw sewage from a wastewater treatment plant (WWTP) contained 100-3000 µg/L of nano and larger-sized titanium which absorbed into sludge biomass (Kiser et al., 2009). Additionally, many studies have looked at nanoparticle behavior in WWTPs (Kaegi et al., 2011) such as aggregation (Kiser et al., 2010), removal efficiency (Limbach et al., 2008), and fate (Benn and Westerhoff, 2008 and Jarvie et al., 2009), as well as the effects of NPs on wastewater biofilms (Choi et al., 2008, García et al., 2012, Mu et al., 2011 and Sheng and Liu, 2011), which in turn may affect the removal of nitrogen and phosphorus (Zheng et al., 2011). Recently, published work has highlighted the predicted fate of nanomaterials in the environment. Of the 22-200 metric tons/year of Cu and CuO_x produced, approximately 37 metric tons/year are predicted to enter WWTPs (Keller et al., 2013 and Lazareva and Keller, 2014). Concentrations of 0.03 μg Cu/L may be present in WWTP effluent and up to 0.24 mg Cu/kg in biosolids (Keller et al., 2013 and Lazareva and Keller, 2014). In the San Francisco Bay region, it is predicted that 0.05 μg Cu/L is found in WWTP effluent and between 0.01 – 0.5 mg Cu/kg is in biosolids (Keller and Lazareva, 2013).

Copper nanoparticles (Cu NPs) were chosen for introduction into the model septic tank in this work because Cu NPs are one of the most commonly used nanoparticles. Cu NPs are used in a wide range of applications including electronics, ceramics, inks, polymers, films, coatings, fungicides, cosmetics, personal care products, and other metal containing products (Maynard and Michelson, 2006, Nasibulin et al., 2001 and Yang et al., 2006). Cu NPs are also used as a bacteriocide (Grosell et al., 2006), which can be incorporated into coatings, plastics, paints, and textiles. Recent work has shown Cu to be found in personal care products, and the predicted fate of these particles at the end of the product lifecycle is disposal into WWTPs (Keller et al., 2014 and Kressler, 2011). Another source of copper into septic tanks, besides from personal care products, may be through the leaching of copper from household pipes (Hong et al., 1998 and Subramanian et al., 1991). The potential impact of these commonly sourced nanoparticles on septic system operation is a concern since treated effluent from a decentralized treatment process, or septic system, is emitted directly into a soil leachfield and groundwater (EPA, 1977). As only 40% of all septic tanks are estimated to be properly functioning (Canter and Knox, 1985) it can be inferred that management and regular monitoring of septic tanks are not practically enforced.

Due to the lack of consistent monitoring of septic tanks, it is hypothesized that nanoparticles entering septic systems from the disposal of common consumer products via household drains may cause unknown, deleterious effects on the function and operation of the septic tank, resulting in either the release of untreated waste or nanoparticles into the groundwater. Traditionally, laboratory nanoparticle toxicity and transport studies are conducted in idealized lab settings (Chowdhury et al., 2011, Maurer-Jones et al., 2010 and Samberg et al., 2010), which does not account for the complex matrix found within a septic system. This work was developed to evaluate the impact of nanoparticle exposure on a septic tank, specifically, to determine if Cu exposure leads to deviation from baseline conditions and causes the septic system to insufficiently and unreliably treat wastewater. Tracking changes in septic tank influent and effluent using traditional wastewater quality tests, microbial community characterization, and microbial community sequencing allowed for the assessment of septic tank performance for each Cu exposure scenario. First, the baseline conditions of the septic system were defined, and subsequent impacts caused by the various Cu forms added in the system were measured. This work will have meaningful implications for improving wastewater treatment for decentralized septic systems.

2. Materials and Methods

2.1 Model Septic System

The septic system used for these experiments was developed and reported on previously (Marcus et al., 2013). The model septic tank was designed to have a typical residence time of three weeks in the primary compartment (Marcus et al., 2013 and EPA, 2002). The system used here mimics a full-scale septic system because it represents a scaled down two-chambered system with the correct 2:1 ratio for the chamber volumes, uses synthetic greywater representing household waste , and contains “toilet waste” which is simulated by adding fecal bacteria from a model human colon reactor. This system differs from previous laboratory septic systems in that it is not seeded with sludge from wastewater (Zaveri and Flora, 2002), but uses a fresh representative microbial community. The system was confirmed to be anoxic by using a dissolved oxygen (DO) probe (Thermo Electron corporation Model 033005D). Liquid in both the primary and secondary chambers and in the effluent had <1.0 mg/L of dissolved oxygen, well within the typical range of anaerobic systems (Bertanza, 1997). More details and a digital image of the septic system are found in the supporting information (SI, Fig. S1). Briefly, the influent added to the septic tank is composed of three components: deionized water (DI H_2O), synthetic greywater, and colon waste (Marcus et al., 2013). The composition of the colon waste and synthetic greywater is listed in the SI.

2.2 Nanoparticle Selection

Cu particles were chosen for this study as a potential perturbance in a septic tank system. The purpose of this work was to characterize the responses within the septic system, rather than to characterize the Cu materials. A previous study conducted physicochemical characterizations of the three Cu particles used in this work (Lin et al., 2015). The characterization work has shown the following size in deionized water (DI H_2O): nano Cu as 1164 ± 202 nm and $Cu(OH)_2$ as 889 ± 156 nm using HT-DLS (high throughput dynamic light scattering instrument, Dynapro Plate

Reader, Wyatt Technology) (Lin et al., 2015). Micro Cu size was not collected due to fast particle sedimentation (Keller and Lazareva, 2013). The primary size of the particles are as follows: 10 nm $Cu(OH)_2$, >1000 nm micro Cu, and 200-1000 nm nano Cu (Lin et al., 2015). Additional characterization details from this study are also listed in the SI (Lin et al., 2015). All Cu particles (nano Cu, micro Cu, and $Cu(OH)_2$) have been obtained through collaboration with the University of California Center for Environmental Implications of Nanotechnology (UC-CEIN). The three Cu particles were manufactured by US Research Nanomaterials, Inc. (nano Cu), Sigma Aldrich (micro Cu), and TreeGeek ($Cu(OH)_2$). These three model particles were chosen to elicit effects between the nano-scale size (nano Cu), bulk-size (micro Cu), and a nano-scale fungicide commercial product ($Cu(OH)_2$). Previous work conducted with these particles has measured the degree of dissolution, with nano Cu and $Cu(OH)_2$ being more soluble (>8 wt% dissolution) than micro Cu (<2 wt% dissolution) (Lin et al., 2015).

For the three individual septic tank experiments, 100 mg of the chosen Cu particle was added once per day during five consecutive weekdays, for three weeks, for a total of 1500 mg of the Cu particle per experiment. This dosing design was chosen to represent low, uniform, daily doses of Cu found in WWTP effluents (Keller et al., 2013, Keller and Lazareva, 2013 and Lazareva and Keller, 2014). This equated to a final concentration of 10 ppm Cu within the septic tank over the course of three weeks with the assumption of equal distribution in the primary and secondary chambers. 10 ppm was chosen based upon predicted concentrations of Cu found in WWTP effluent and biosolids (Keller et al., 2013, Keller and Lazareva, 2013 and Lazareva and Keller, 2014). Three individual experiments (one experiment per Cu particle) were conducted in the system and consisted of: four weeks of baseline (no Cu) and three weeks of Cu addition (total of 1500 mg added and denoted as Cu weeks 1-3) which were followed by three weeks where Cu was no longer added to the system (denoted as post-Cu weeks 1-3). The model septic tank was designed to have a typical residence time of three weeks in the primary compartment (Marcus et al., 2013 and EPA, 2002). The purpose of the three weeks of analysis after the Cu addition (post-Cu weeks 1-3) was to determine if the effluent quality would return to values recorded during the baseline period. If the system returns to baseline values during the post-Cu exposure, this indicates that the system was able to re-establish its function. Because Cu and nano-scale Cu are known to have antibacterial effects, immediate changes, such as a loss of septic system function, are anticipated during the Cu exposure weeks (Cu weeks 1-3) (Grass et al., 2011 and Ruparelia et al., 2008). While Cu particles were no longer added during post-Cu exposure, the assumption was made that Cu added during the prior three weeks (Cu weeks 1-3) was likely still present in the system or effluent. The system was dismantled and cleaned between each experiment. More details on the Cu particle dosing procedure of the septic system are listed in the SI.

2.3 Water Quality Tests

For brevity, the water quality methodology and the water quality sampling schedule (Table S1) are presented in detail in the SI. Water quality tests include pH, total organic carbon (TOC), turbidity, total suspended solids (TSS), and the five-day biological oxygen demand test (BOD_5). These specific water quality tests were selected because they are traditionally associated with monitoring both WWTPs and septic tank systems (Canter and Knowx, 1985, Crites and Tchnobanoglous, 1998 and Brandes, 1978). Also, these tests are used for the regulation and monitoring purposes of these systems (EPA, 1999 and Brandes, 1978). Therefore, using literature values as a comparison, these water quality tests were used to relate observable changes to the accepted reported literature values. This also ensured the measured range in the system was within reason of the reported values.

To determine the system's influent values, measurements were taken during baseline week 4, Cu week 3, and post Cu week 3 and were averaged. The baseline effluent average was determined by collecting measurements during the four weeks of pre-Cu measurements and averaging the data.

Additional water quality tests (alkalinity, conductivity, and hardness) were also measured in the system but are reported in another study that determined the transformation of Cu particles and subsequent alterations on Cu toxicity (Lin et al., 2015).

2.4 Bacteria Characterization

Microbial community phenotype can be affected by perturbances in aquatic systems (Marcus et al., 2013). Therefore, because septic system function is linked directly to metabolic activity of the microbial community, changes occurring to the community phenotype were also measured. Microbial characterization techniques were selected based upon previous work with environmental microbial isolates and communities (Marcus et al., 2013 and Taylor et al., 2015). All microbial community testing for this study was conducted as reported in a prior study with the model septic system (Marcus et al., 2013). Electrophoretic mobility (EPM, a surrogate for relative cell surface charge), hydrophobicity, cell concentration, and cell size were measured from cells emitted in the effluent. The purpose of the bacteria characterization was to monitor changes in the microbial community as a function of the copper particle exposure. For consistency, all microbial characterization experiments were conducted once a week on the same day in triplicate. More details on the bacteria characterization tests, further methods, and the sampling schedule (Table S1) are located in the SI.

An additional microbial community analysis conducted was pyrosequencing; this analysis evaluated the microbial community structure using the 16S rRNA gene. The purpose of this analysis was to determine the changes in the structure experienced by the community as a function of Cu particle exposure when compared to the baseline community structure. Extraction, preparation, and pyrosequencing of all microbial samples collected from the septic system were followed exactly as in previous research with this model septic system (Marcus et al., 2013). Additional details on the methods used for sequencing are located in the SI.

2.5 Copper Analysis

The amount of free Cu^{2+} ions emitted in the effluent from the septic system was determined using an Orion™ cupric solid state half-cell ion specific electrode (Cu ISE, Thermo Scientific), an Orion™ double junction Sure-Flow™ reference electrode (Thermo Scientific), and an Orion Star™ A214 pH/ISE meter (Thermo Scientific). This measurement did not include any Cu^{2+} ions bound to organic matter and inorganic species (Sanders, 1982 and Temminghoff et al., 1997). Measurements were made in 10 mL samples from the effluent twice a week in triplicate. Further information is provided in the SI on the calibrations, concentrations, and sample preparation.

A mass balance of Cu within the septic system was reported previously in an additional study (Lin et al., 2015).

2.6 Statistical Analyses

Statistical analyses were conducted individually for each test parameter mentioned in the material and methods (e.g., all water quality tests, bacteria characterizations, and community sequencing) to identify significant trends as a function of Cu exposure and type. A Student t test was used and if p values were <0.05, the difference between the average of grouped data was considered significant. Data were analyzed on a per week basis. All experimental conditions (Cu weeks 1-3, post-Cu weeks 1-3) were individually compared to the baseline conditions. Data were assessed for a normal distribution using a Shapiro-Wilk test and equal variance using a F-test. A student t test was also run on grouped data (e.g., all baseline weeks vs Cu weeks 1-3, and baseline weeks vs post-Cu weeks 1-3). The average and standard deviation of all data values collected and used in statistical analyses are presented in Table S2. Statistical analyses were conducted with Excel 2011 (v.14.3.9, Microsoft, Redmond, WA) and with StatPlus®:mac LE.2009 (v.5.8.2.0, AnalystSoft Inc.).

The septic system was considered to undergo a loss of function if the grouped data for the Cu exposure (Cu weeks 1-3) or post-copper exposure conditions (post-Cu weeks 1-3) were statistically different when compared to the baseline, and by comparisons to known septic system performance (Beal et al., 2005, Brandes, 1978, Crites and Tchnobanoglous, 1998, and 1966, Zaveri and Flora, 2002). But, because of the three-week residence time in septic systems (Marcus et al., 2013), only the final copper exposure week, (post-Cu week 3) was presented in the results (post-Cu weeks 1 and 2 values can be found in Table S2). This is because recovery of the microbial community was anticipated to be associated with the residence time of the system.

3. Results

3.1 Water Quality Test Results

Five water quality tests (pH, TOC, turbidity, TSS, and BOD_5) commonly used to evaluate effluent quality from WWTPs and septic tanks were used in this study (Beal et al., 2005 and Zaveri and Flora, 2002). The baseline data determined if there were any statistically significant deviations in typical operating parameters when compared to a perturbance, here the Cu and post-Cu exposures. For all results (water quality tests and bacteria characterization tests), variation within data for the baseline weeks (no Cu present in the system) was not significantly different ($p > 0.05$). Therefore, all baseline data were averaged for the sake of comparison to other experimental conditions with the Cu particles. Water quality measurements, pH, TOC, turbidity, and TSS are found in Fig. 1A-1D. BOD_5 data are found in Fig. 2B. The typical range of water quality values for septic tank effluent from previous literature studies can be found in Table 1. Throughout the results, data are report as average ± standard deviation.

pH. For pH, the baseline effluent average was 7.0 ± 0.1 and the influent pH average was 7.6 ± 0.2. Both of these values fall within the typical functioning range of a septic tank (Table 1). Due to consistency of the influent material, the influent pH average remained constant at pH 7.6 ± 0.2 during the course of the experiments. The septic tank pH averages during and following the nano Cu addition were between pH 6.8-7.2 and did not significantly change when compared to the data for the baseline (pH = 7.0 ± 0.1, Fig. 1A). Experimental values during and after the nano Cu exposure are also within the accepted range of septic tank pH values (Table 1). The final experimental week (post-Cu week 3) following exposure had an average pH of 6.7 ± 0.0.

Over the three weeks of micro Cu addition, pH data was significantly lower when compared to the baseline data (Fig. 1A). For example, after the initial week of the micro Cu introduction, data for the pH average (6.3 ± 0.1) significantly decreased when compared to the data comprising the average baseline value ($p<0.001$). In the second and third weeks of the micro Cu exposure, the pH was also below typical pH range for septic tanks, albeit these values were not significantly different from the baseline ($p>0.05$). However, when evaluated together and analyzed, the pH data for the three weeks of micron-scale Cu injection (pH 6.5 ± 0.1) was also significantly lower compared to the baseline data ($p<0.05$). After three weeks without further micro Cu addition, the effluent pH had a value of 6.8 ± 0.2 for the final post micro Cu week 3.

The final week in the $Cu(OH)_2$ exposure had the highest pH average (7.5 ± 0.1) for all experimental conditions (Fig. 1A) and while this value is significantly higher when compared to the baseline data ($p<0.001$), it is still within the typical pH range for a septic system. All other experimental pH values were within the anticipated pH range for septic systems. The post-$Cu(OH)_2$ week 3 average is pH 7.1 ± 0.1.

TOC. For TOC, the baseline effluent average value and standard deviation was 54.8 ± 17.7 mg/L. The average influent TOC value was 89.1 ± 28.3 mg/L and was consistent for all experiments. Accepted effluent TOC values for a properly functioning septic tank are listed in Table 1. The only significant difference during the nano Cu experiment (Fig. 1B) was the comparison between nano Cu week 2 (16.7 ± 0.2 mg/L) and the post-nano Cu week 3 condition (91.1 ± 4.0 mg/L) ($p<0.0001$). TOC was considered to maintain the baseline condition for all other nano Cu experimental weeks. While the final week in the post-nano Cu exposure condition does have a significantly higher value when compared to the baseline data (91.1 mg/L vs. 54.8

mg/L), all TOC values for the nano Cu exposure were within anticipated levels for septic system function.

TOC averages for the micro Cu exposure (weeks 1-3) were between 47.0-49.8 mg/L (Fig. 1B) and the final post-micro Cu exposure value was 47.0 ± 20.8 mg/L. The data for all of these weeks were considered statistically insignificant when compared to the baseline data (p=0.3). The typical range of TOC in septic tank effluent during the micro Cu experiment was well within the anticipated values based upon real world septic systems (Table 1).

The TOC data for the $Cu(OH)_2$ exposure ranged from 82.5-208.2 mg/L, and were significantly higher when compared to the baseline data (54.8 ± 17.7 mg/L) and to the post-$Cu(OH)_2$ data (75.8 ± 2.8 mg/L) (p=0.0009 and p=0.02, respectively). However, the post-$Cu(OH)_2$ exposure data were not significantly different when compared to the baseline. The $Cu(OH)_2$ exposure resulted in the two highest TOC averages, 208.2 ± 136.4 and 94.2 ± 5.9 mg/L, for all experimental conditions with all Cu particles. Yet the TOC values measured under all $Cu(OH)_2$ conditions were within the expected range for TOC in septic tank effluent (Table 1), which indicates the system is still operating within typical conditions.

Turbidity. The baseline effluent turbidity average and standard deviation was 11.3 ± 1.1 NTU. The turbidity influent average value for all experiments was 78.5 ± 3.7 NTU (Fig. 1C), and is in the range for expected influent values for household greywater, which is 22-72 NTU (Casanova et al., 2001). The effluent turbidity averages (Fig. 1C) for the nano Cu experiment show fluctuations during the nano Cu weeks 1-3 (2.5-4.6 ± 0.4 NTU). Turbidity data during the nano Cu exposure were significantly lower when compared to the baseline and post-nano Cu exposure data (p<0.0005). The final post-nano Cu exposure average value was 10.0 ± 0.1 NTU.

Effluent turbidity decreased during the micro Cu experiment when compared to the baseline average (Fig. 1C). When grouped and analyzed, the overall micro Cu exposure average (2.5 ± 0.3 NTU) was significantly lower when compared to the baseline turbidity data (11.3 ± 1.1 NTU). The final post-micro Cu exposure week had an average of 1.5 ± 0.6 NTU. While not displayed in Fig. 1C, the post-micro Cu week 1 exposure has the highest turbidity value for all experiments with an average of 24.0 ± 0.2 NTU (SI, Table S2, $p<0.001$).

Turbidity increased during the $Cu(OH)_2$ experiment when compared to the baseline average. The second highest turbidity average for all experiments was during the third week of the $Cu(OH)_2$ exposure (22.2 ± 1.4 NTU, Fig. 1C). Overall, when grouped and analyzed, the $Cu(OH)_2$ exposure data (10.1-22.2 ± 0.7 NTU) was significantly higher when compared to the baseline data (11.3 ± 1.1 NTU, $p<0.006$). $Cu(OH)_2$ experiments had higher turbidity averages overall when compared to the micro Cu and nano Cu experimental averages. The final week of the post-$Cu(OH)_2$ condition average was 6.4 ± 0.2 NTU. All turbidity values for the $Cu(OH)_2$ exposure were above the recorded baseline average. However, these effluent values were well below the range for expected influent values for household greywater (Casanova et al., 2001).

TSS. The TSS effluent baseline average value and standard deviation was 52.7 ± 7.6 mg/L (Fig. 1D). The TSS influent average was 57.0 ± 2.9 mg/L. TSS typically ranges from 40-140 mg/L in effluent, with a removal efficiency of 60-80% expected (Table 1). During baseline conditions, only an 8% reduction occurred from the influent to the effluent for TSS. There were no significant trends in the TSS data for any of the Cu particles, but some changes in the data are worth noting.

The nano Cu exposure TSS values decreased over time during the nano Cu exposure (38.1-41.6 ±10.4 mg/L) to the final post-nano Cu exposure (29.0 ± 10.6 mg/L). TSS averages were well below the anticipated range for septic tank effluent and a 28-49% reduction occurred during this experiment. The post-nano Cu grouped data was overall significantly lower (31.6 mg/L) when compared to the baseline weeks ($p<0.01$). The final post-nano Cu week 3 exposure weeks had the lowest recorded TSS value of 29 mg/L.

The micro Cu particles had a decrease in TSS over time. There was a 78% reduction of TSS from the influent to the effluent during the micro Cu exposure weeks. The final post-micro Cu exposure week had an 87% reduction in TSS. These are in the expected range of TSS reduction in a functioning septic system (Table 1).

$Cu(OH)_2$ TSS averages increased during weeks 2-3 of the $Cu(OH)_2$ exposure when compared to the baseline data. For $Cu(OH)_2$ exposure week 1 and for the post-$Cu(OH)_2$ final exposure week, a 19% and 26% reduction occurred in TSS from the influent to the effluent. The increase in TSS during $Cu(OH)_2$ exposure weeks 2 and 3 correlates with an increase seen in TOC. $Cu(OH)_2$ did not cause significant changes in TSS during the $Cu(OH)_2$ exposure. The highest TSS value (60 mg/L, Figure 1D) was recorded for this form of Cu.

BOD_5. The five-day biological oxygen demand test (BOD_5, Fig. 2B) baseline effluent average and standard deviation was 82.0 ± 5.6 mg/L and the influent BOD_5 average value was 127.0 ± 0.10 mg/L. BOD_5 was reduced by ~35% in baseline conditions which is within the estimated range for septic tanks (Table 1).

The lowest BOD_5 average recorded for the nano Cu experiment was during week 1 of the nano Cu exposures (34.0 ± 6.4 mg/L, Fig. 2B). For all conditions (baseline, nano Cu exposure, and post-nano Cu exposure), the BOD_5 experienced a reduction ranging from 35-73% when compared to the influent. During the micro Cu exposure, there were two significant increases in BOD_5 effluent averages for the first (241.7 ± 7.6 mg/L) and third exposure weeks (223.3 ± 1.1

mg/L) when compared to the baseline data. The final week in the post-micro Cu exposure had a BOD_5 average of 26.8 ± 8.3 mg/L, which was the lowest BOD_5 value recorded for all of the experiments. During the micro Cu experiment, a 30-78% reduction in BOD_5 from the influent to the effluent was experienced only for the baseline, micro Cu exposure week 2, and the final post-micro Cu exposure week. All other weeks did not experience the anticipated percent reduction associated with a functioning septic system.

Half of the $Cu(OH)_2$ exposure had significantly higher BOD_5 averages (267.0 ± 4.8 and 208.5 ± 12.0 mg/L, $p<0.05$) when compared to the baseline data. The 267.0 mg/L average was the highest BOD_5 value recorded for all Cu experiments conducted. These two weeks were above the expected BOD_5 values for septic tank systems (Table 1) and did not have the anticipated precent reduction in BOD. Two weeks ($Cu(OH)_2$ week 1 and post-$Cu(OH)_2$ week 3) were significantly lower than the baseline effluent BOD_5 average. BOD_5 experienced a 76% reduction for these two weeks during the $Cu(OH)_2$ exposure.

3.2 Bacteria Characterization Results

Results for the bacteria characterization tests (hydrophobicity, electrophoretic mobility, cell size, and cell concentration) are found entirely in the SI (Fig. S2). While the bacteria characterization tests did not overall have meaningful trends and did not correlate with changes in the water quality tests, significant changes did occur. For example, the cell hydrophobicity grouped data increased during the nano Cu exposure (64.4 ± 0.3%) when compared to the baseline grouped data (24.3-45.8 ± 0.6-5.0%) and had the highest hydrophobicity values for any Cu exposure. In the micro Cu experiment, cells experienced a change in electrophoretic mobility (EPM, a surrogate for surface charge) and data became more negative during the micro Cu exposure (-1.5 ± 0.3 to -1.6 ± 0.3 [(µm/s)/(V/cm)]) when compared to the baseline data (-1.0 ± 0.2 to -1.4 ± 0.3 [(µm/s)/(V/cm)]). During the $Cu(OH)_2$ exposure, the most negative EPM average and standard deviation was recorded for the bacterial cells (-2.4 ± 0.4 [(µm/s)/(V/cm)]).

Pyrosequencing. The microbial community structure was determined using pyrosequencing. The baseline averages for the microbial community structure at the phyla level for all Cu experiments was as follows: Proteobacteria 83.9%, Firmicutes 4.2%, and Bacteriodetes 12.5% (Fig. 2A). For the sequencing data in this study, the following phyla: Acidobacteria, Actinobacteria, Chloroflexi, Synergistetes, and Cyanobacteria were not statistically analyzed in the community structure because when combined, these three phyla made up less than 1% of the community structure for all experimental conditions and weeks. The phyla that composed 99% of the community were Proteobacteria, Firmicutes, and Bacteriodetes. Proteobacteria remained the dominant phyla during all Cu experiments. Only one phylum, Firmicutes, had a significant change when compared to the baseline data during the course of the nano Cu experiment (Fig. 2A). The amount of Firmicutes (4.2%) present in the baseline significantly increased during the nano Cu weeks (11.1-19.8%, $p=0.003$) and the Proteobacteria data decreased from 83% in the baseline to between 67.3-76.7% in during the majority of the nano Cu exposures and during the final week of the post exposure condition (nano Cu weeks 2 and 3, and post-nano Cu week 3). However, all other changes in the community during the nano Cu exposure and post-nano Cu weeks were not significant.

The community structure maintained stable conditions during the micro Cu exposure (Fig. 2A). The micro Cu exposure showed Firmicutes average values maintained a constant level in the community between 1.7-3.4%. Proteobacteria was the dominant phyla in the community for the duration of micro Cu addition (64.4-74.6% of population) and Bacteriodetes averages ranged between 23.6-32.2%. All post-micro Cu microbial community structure data were within the same range as the baseline and micro Cu exposure.

The $Cu(OH)_2$ particle affected both Proteobacteria and Firmicutes. Phyla results (Fig. 2A) show that Proteobacteria data significantly decreased during the $Cu(OH)_2$ addition (60.0-68.6%) and for post-$Cu(OH)_2$ exposure (60.8%) when compared to the baseline Proteobacteria data (83.9%). Firmicutes was significantly higher for the $Cu(OH)_2$ exposure (8.5-10.7%) when compared to the Firmicutes baseline (4.2%). The post-$Cu(OH)_2$ Firmicutes percentage was also significantly higher (25.3%) compared to the baseline.

3.3 Copper Analysis

The purpose of the copper analysis using the copper ion specific electrode (Cu ISE) was to determine the amount of free Cu^{2+} ions emitted in the effluent (Fig. 1E). Cu^{2+} ions were not present in the effluent under baseline conditions. The highest recorded Cu^{2+} ion concentration average and standard deviation in the effluent was during $Cu(OH)_2$ week 3 at 4.9 ± 0.8 ppm. Micro Cu effluent was measured as having 1.0 ± 0.3 ppm during micro Cu week 3 and 1.8 ± 2.5 ppm during post-micro Cu week 3. Post-nano Cu week 3 had an average value of 1.5 ± 1.1 ppm. All other averages were below 0.2 ppm. Both the micro Cu and nano Cu showed an increase in Cu^{2+} ion concentration over the course of the experiments. Data are not available for micro Cu week 1. These values are in agreement from previous published work determining the total amount of Cu emitted from a septic system (Lin et al., 2015). Using ICP-MS to perform a mass balance of Cu in the system, the previous work also demonstrated that less than 1% of all the forms of Cu added to the septic tank per experiment was emitted in the effluent, with 99% of the Cu being found in the sludge in the primary chamber of the septic system (Lin et al., 2015).

4. Discussion

4.1 Water Quality Tests

Of the five water quality tests performed (pH, TOC, turbidity, TSS, and BOD_5), it should be noted that each of these parameters had a range of responses that also varied per Cu particle exposure. The septic tank was affected minimally for the pH parameter except for the micro Cu exposure weeks. Since pH is used as an indicator for septic system operation as a function of microbial activity, the data indicate that septic system performance was minimally impacted with the exception of the micro Cu exposure. Typical values associated with real world septic tanks shows the average effluent pH to be between 6.7-7.6 for optimal treatment of waste; this is in agreement with the current study and additional septic tank effluent characterization (Bitton, 2005, Brandes, 1978 and Marcus et al., 2013). The addition of micro Cu resulted in the greatest pH disturbance (decrease) from baseline conditions (6.4-6.6 vs. 6.7-7.6). The micro Cu pH was not in the ideal range for methanogenesis (Bitton, 2005) for two weeks of the micro Cu exposure. Here, the data demonstrate that consumer products with different types of Cu materials (micro Cu vs. nano Cu vs. $Cu(OH)_2$) can have a range of effects on an anaerobic treatment system.

However, recent work has shown that regardless of the type of Cu material, organic waste and pH within the septic system mitigate toxicity effects due to transformation, speciation, and sedimentation of the Cu particles (Lin et al., 2015). In anaerobic treatment, decreases in pH can be an indirect measurement of an accumulation of volatile fatty acids (VFAs) within the system, which are produced by bacteria through anaerobic degradation of waste (American Public Health Association, 1989). VFAs can lower the pH in the system. Methanogenesis, the last process in septic system fermentation and an important step in the degradation of waste (Zaveri and Flora, 2002), occurs ideally between pH 6.7-7.4. Hence, methanogenesis is often the most sensitive phase and the rate-limiting step in the fermentation process (Bitton, 2005 and Murto et al., 2004), and will be affected by bacterial acid production (Grass et al., 2011). If the Cu particles affect the bacteria necessary for specific steps in fermentation (Chen et al., 2008 and Murto et al., 2004), the system may experience changes in acid production, causing a decrease in pH. Disrupting methanogenesis can lead to untreated effluent emitted into the leachfield. Because methanogenic and acidogenic microorganisms have an optimal pH range within septic systems (Bitton, 2005), failure to maintain this optimal range can lead to septic system failure, e.g., improper treatment of the wastewater. Therefore, pH is an important indicator of distress in the system, and the release of incompletely treated waste into groundwater has consequences regarding human and environmental health. While in this study methane production and methanogen population of the microbial community structure were not directly measured, previous research using activated sludge indicated that free Cu has a IC_{50} at a concentration of 0.02 mg of free Cu per L activated sludge to specific bacteria populations necessary for the breakdown of waste (Braam and Klapwijk, 1981). This toxic concentration of Cu is one order of magnitude lower than the free Cu concentrations found in the effluent of the septic tank. This indicates that microbial toxicity is likely within the septic system based upon the amount of free Cu found in the effluent.

In engineered wastewater settings, TOC values are typically related to the amount of natural organic matter (NOM), such as humic and fulvic acids; the presence of acids can influence the pH of the system. These acids are present in wastewater and impact turbidity readings. However, in this study effluent TOC is based upon the acid and sugar content of the degraded organic constituents from the model colon which may be less complex in structure. The TOC effluent value was anticipated to be between 50-350 mg/L (Brandes, 1978 and Crites and Tchnobanoglous, 1998). All conditions (baseline and all Cu experiments) maintained a TOC value below 350 mg/L, indicating that the system in this study stayed within the typical range for real world septic systems. One trend noted during the $Cu(OH)_2$ exposure shows an increase in TOC that corresponds to an increase in BOD_5. Overall, the TOC measurements indicate the lack of any system failure. Here, failure was defined as the system's inability to treat waste; one attribute indicating failure is increased loads of organic material (TOC) present in the effluent.

High TOC values are also associated with increased concentrations of acids from the degradation of organic waste. These acids can lower the pH within the septic system. Therefore, the presence of increased amounts of TOC can result in lower pH. This phenomena is noted for week 1 of the $Cu(OH)_2$ exposure. During week 2 of the nano Cu exposure, a decrease in TOC may be related to the increase in pH, yet these values are still within the documented typical ranges for TOC and pH for septic tanks. There is no clear relationship between TOC and pH trends with the micro Cu exposure. Since each Cu particle causes distinct differences in the TOC data, this may indicate that changes in wastewater quality are likely caused by the particle properties such as size or chemical composition.

Domestic wastewater influent for a WWTP can have a turbidity ranging from 186-328 NTU (Chu and Li, 2006) and a reduction of turbidity from the influent to the secondary effluent is typically between 80-98% (Delgado et al., 2003). In the septic tank with the absence of Cu particles, the system reduced influent turbidity by ~90%. In fact, for the nano Cu experiments, the system was capable of achieving a >90% reduction of turbidity, while the micro and $Cu(OH)_2$ showed turbidity reduced between 70-90%. Here, turbidity was reduced to within the accepted ranges; this measurement indicated that the system was well conditioned to handle the perturbances experienced.

Typical values from the literature demonstrate that the secondary effluent BOD_5 from a septic tank should be between 35-200 mg/L (Brandes, 1978). In agreement with what is expected for functioning septic tanks, BOD_5 in this study showed a 30-50% reduction between the influent and the baseline conditions (Bounds, 1997, Brandes, 1978, EPA, 2002, Rock and Boyer, 1997 and Siegrist et al., 1984).

Micro Cu and $Cu(OH)_2$ caused the largest increase in average effluent BOD_5 and did not result in a subsequent reduction in BOD_5 for four weeks. These high BOD_5 values indicate that there is an increased oxygen demand for the oxidation and degradation of organic waste in the effluent and verifies the presence of organic waste in the effluent. However, in this study the TOC remained relatively constant, indicating that BOD_5 was affected by an increase in microbial enzyme activity related to Cu availability as a micronutrient rather than the presence of increased TOC. In contrast, low BOD_5 values mean the presence of less organic material. At the end of the post-Cu week exposures, all BOD_5 values were relatively low (between 26-46 mg/L) and showed a 64-80% reduction in BOD_5. This indicates that the septic system was able to return to baseline conditions in terms of the amount of organic waste present in the secondary effluent. One limitation of the BOD_5 test is the inability to differentiate between amount of organic material and microbial activity, since the test is effectively measuring oxygen consumed by bacteria over a five-day period. The amount of oxygen consumed is often a proxy for the amount of organic material present in the system.

4.2 Bacteria Characterization

The microbial community was monitored throughout the course of the experiments to determine phenotypic changes associated with the Cu particle perturbances. These measurements were selected based upon previous work that used these methods to characterize bacteria in aqueous environments (Marcus et al., 2013 and Taylor et al., 2015), specifically assessments for environmental microbial community analyses such as hydrophobicity, surface charge (electrophoretic mobility, EPM, a surrogate of surface charge), and cell size were chosen (Bolster et al., 2010 and Tazehkand et al., 2008). While significant changes did occur in the characterization tests, there were no meaningful trends that correlated with the water quality data. Traditionally, these tests can give insight into changes occurring to the microbial community that are often associated with stress conditions, and these fluctuations could be used as additional indicators that the septic system is not operating optimally. A few of the bacteria characterization results are worth mentioning in more detail.

For example, an increase in cell hydrophobicity is associated with biofilm formation (Marcus et al., 2013 and Schäfer et al., 1998). Previous work with the septic system showed a baseline hydrophobicity cell value of 40.1 ± 2.8% and an increase in hydrophobicity was noted with a pathogen perturbance (51.8 ± 8.4%) (Marcus et al., 2013). Here, the average baseline hydrophobicity fluctuated between 24-45%. Experimental Cu particle conditions hydrophobicity averages were between 20-64%. Here, no distinct pattern was observed when comparing the hydrophobicity responses to the three Cu particles.

Another characterization test, surface charge (EPM), is an indicator of the stability and attachment potential for the microbial community, with higher absolute values indicating greater stability (Elimelech et al., 1998). EPM values for bacteria in aqueous environments are typically negative (Wilson et al., 2001). A positive EPM value recorded during the nano Cu exposure suggests aggregate formation of the bacteria with the Cu particles, with Cu likely coating the bacteria, thus changing its surface charge (Jiang et al., 2009). Overall the septic system had greater fluctuations in EPM ranging from -2.38 to 0.90 (µm/s)/(V/cm)) in the presence of Cu particles when compared to previous work in the system (Marcus et al., 2013). $Cu(OH)_2$ had the greatest change in surface charge since positive values were measured. Nano Cu had the greatest variation in bacterial surface charge. The fluctuations in the EPM data suggest greater potential for Cu and bacteria aggregation. Therefore, EPM may be a useful tool to determine changes within septic systems and to predict the fate or aggregation of nanomaterials and bacteria in the effluent (Bolster et al., 2010 and Haznedaroglu et al., 2009).

Finally, changes in cell size, particularly a decrease in cell size, can be attributed to stress on the cell or changes in cell viability (Bakken and Olsen, 1987, Kjelleberg et al., 1987, Palumbo et al., 1984, Tate, 1986 and Torrella and Morita, 1981). The greatest decrease in cell size compared to the baseline was during post-$Cu(OH)_2$ exposure. Of the three Cu experiments, the micro Cu resulted in the least amount of fluctuation in bacterial size over the course of the experiments.

The microbial community was analyzed using pyrosequencing to determine the changes that the community experienced as a function of Cu particles when compared to the baseline community characteristics. This study is not the first to observe changes in the microbial community structure at the phyla level. In fact, many studies have reported changes at the phyla level and have suggested analyses at this taxonomic group as a monitoring tool for various types of wastewater treatment (Hu et al., 2012, Nielsen et al., 2012, Wagner et al., 2002 and Yang et al., 2014). In this work, community structure changes were noted during the same experimental weeks that high pulses in BOD_5 occurred ($Cu(OH)_2$ weeks 1-3, Proteobacteria decreased and Firmicutes increased). At the phyla level, Proteobacteria made up the majority of the community in all conditions, even though $Cu(OH)_2$ did cause a significant decrease in this phylum (15-23% decrease from baseline condition). Other studies have confirmed the dominance of Proteobacteria in wastewater conditions (Marcus et al., 2013, Hu et al., 2012 and Tomaras et al., 2009). Firmicutes are also regularly found in wastewater and have been reported to have low resistance to the shear forces present in WWTPs, therefore occupying a select niche in wastewater microbial communities (Larsen et al., 2008 and Wilén et al., 2008). The phyla Firmicutes includes fermenting bacteria (Diaz et al., 2006), which is important for septic tank systems. Both nano Cu and $Cu(OH)_2$ caused significant increases in the Firmicutes phylum.

In the current work, the microbial community at the phyla level was significantly altered for the nano Cu exposure (Firmicutes increased) and during the $Cu(OH)_2$ exposure (Proteobacteria decreased and Firmicutes increased). These fluctuations indicate that the community structure is not stable. In multiple studies it has been shown that community stability is not often associated with the functional stability of the system, possibly due to functional redundancy, and that microbial community structure in multiple types of WWTPs is highly and continuously variable despite stable function (Cytryn et al., 2005, Fernández et al., 1999, Gentile et al., 2006, Miura et al., 2007, Wittebolle et al., 2009 and Zumstein et al., 2000). In fact, one study suggests that the less stable a community's structure, the more stable the waste degradation performance (Fernandez, 2000). This work indicates a strong functional redundancy in the system. Additionally, since Cu is well documented to settle into sediment and sludge layers (Lin et al., 2015), it was anticipated that Cu was not bioavailable or causing disruption to the microbes within the liquid layer in the primary chamber.

4.3 Copper Analysis

The Cu^{2+} ion concentration emitted in the effluent was measured during all experimental conditions. These Cu^{2+} ion concentrations released in the effluent are in agreement from previous published work determining the overall mass balance and concentration of Cu emitted from a septic system, and are in the 1-5 ppm range (Lin et al., 2015). Cu^{2+} ions have demonstrated toxicity to organisms such as bacteria (Flemming and Trevors, 1989), and therefore, effects seen in the system that are related to microbial activity may be due to free ions released from the Cu particles rather than the Cu particles. Research has also shown that a decreasing pH will increase the solubility of copper and lead to greater dissolution and an increase in the presence of Cu^{2+} ions (Adeleye et al., 2014). Here, the Cu^{2+} ion concentration during the micro Cu experiment may have increased due to the drop in pH during this experimental condition. Another reason for the increase in Cu^{2+} ion concentration is due to the gradual increase in Cu particle concentration throughout the experiment. It should be noted that results might have some inaccuracy due to interfering constituents such as Fe^{2+} (Fe^{2+} ions are a minimal component of the medium), divalent ions, and the complexity of the sewage matrix, which includes high concentrations of organics (Sterritt and Lester, 1984).

5. Conclusion and Environmental Implications

Septic system failure is defined as the release of nutrients and pathogens in effluent discharge (Ahmed et al., 2005). Therefore, it is important to understand the effects of any contaminants that may enter and alter septic system function, such as various Cu particles present in common consumer items. Here, multiple testing strategies were used to thoroughly characterize a septic system with and without copper particles. The septic system experienced various transformations during the three Cu exposures such as fluctuations in the water quality (pH, BOD_5, and turbidity), microbial community phenotypic changes (hydrophobicity, surface charge, and size), and variation in the microbial community composition.

Overall, the septic system function was robust and managed the various Cu perturbances. Even with weekly fluctuations in the experiments, the data suggest that 100% of the time, the water quality parameters and microbial composition were recovering towards baseline conditions by the final week in the experiment (post-Cu week three) and most likely would return to, or maintain the baseline conditions after such a perturbance, regardless of the particle type. The release of untreated wastewater or Cu particles into the leachfield may occur on a week-by-week basis and may vary depending on specific conditions within the system (i.e., microbial community composition, pH, BOD_5, TOC), and it likely to differ between septic systems.

Based upon this work, the subsequent entry of the effluent into the leachfield and groundwater may have low concentrations of copper (~0.2 ppm) with occasional pulses of higher Cu concentrations, which may or may not meet current regulation discharge limits for Cu. The release of engineered nanomaterials into aqueous environments does have known (Hagedorn et al., 1981 and Yates, 1985) impacts and may have additional unknown effects on the environment and on human health. Additionally, in anaerobic aqueous environments the sulfidation of metals has been reported to eliminate metal toxicity (Kim et al., 2010 and Sterritt, and Lester, 1984) and sulfidation readily occurs in anaerobic environments with organic matter present, such as a septic system. Finally, the approach of using a realistic engineered system in the laboratory that provides environmentally representative conditions should be considered in future experiments.

Supporting Information

Further information on methodologies and results are discussed within the SI.

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Notes

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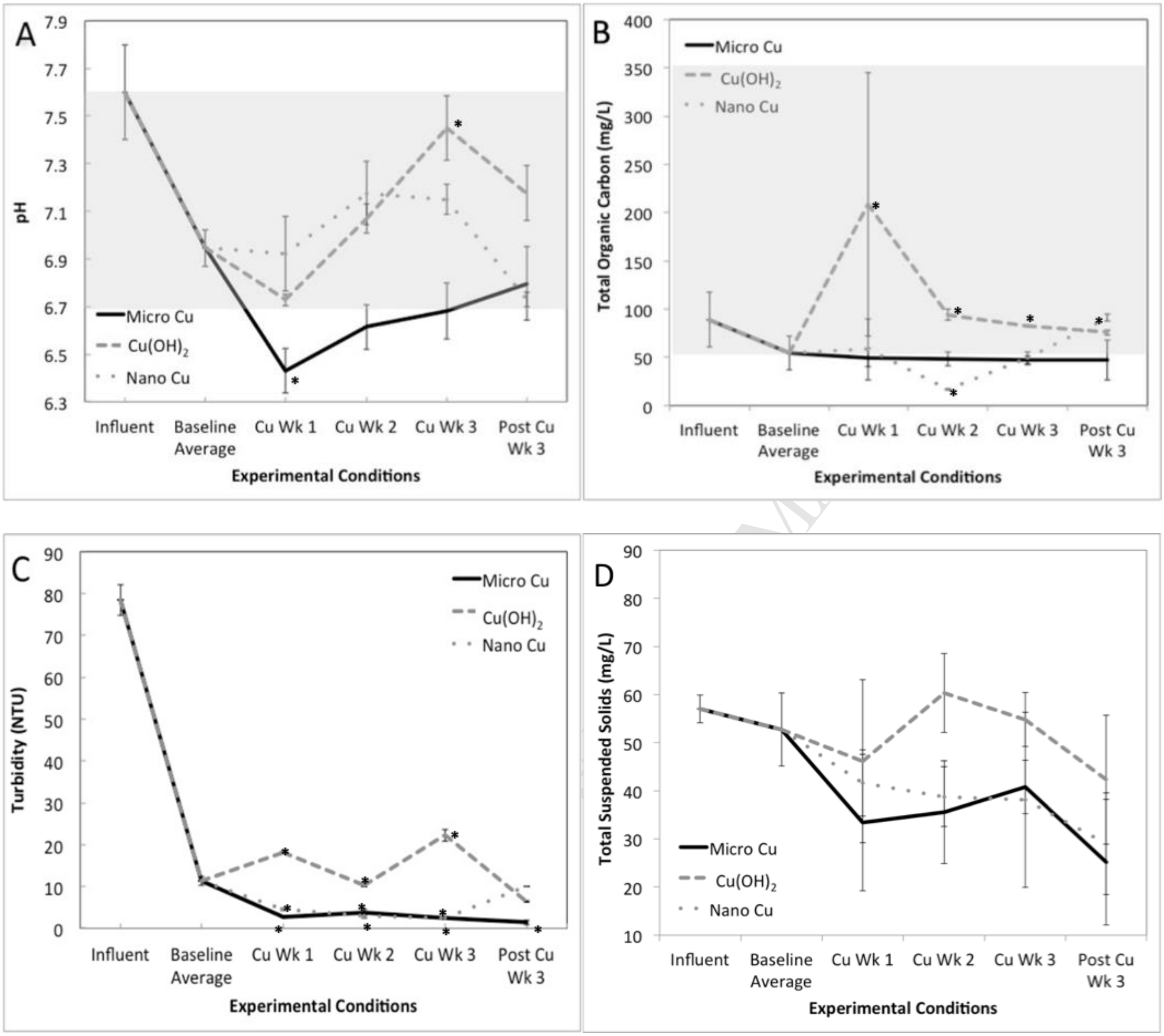
Table 1. Range of typical water quality values for a functioning septic system.

	Septic Tank Secondary Effluent Values			
Water Quality Tests	Literature Values	% Reduction Expected	Source	Baseline Values from this Study
pH	6.7-7.6	NA	Brandes, 1978 Crites and Tchnobanoglous, 1998	7.0 ± 0.1
TOC	50-350 mg/L	N/A	Brandes, 1978 Crites and Tchnobanoglous, 1998	54.8 ± 17.7 mg/L
Turbidity	N.A	N/A		11.3 ± 1.1 NTU
TSS	40-140 mg/L	60-80%	Crites and Tchnobanoglous, 1998 Bounds, 1997 Rock and Boyer, 1995	52.7 ± 7.6 mg/L
BOD_5	35-200 mg/L	30-50%	Brandes, 1978 Crites and Tchnobanoglous, 1998	82.0 ± 5.6 mg/L

N/A indicates data not available for septic systems. Baseline values listed for this study are an average and standard deviation of the data collected over four weeks.


A
7.9
7.7
7.5
7.3
7.1
6.9
6.7
6.5
6.3
pH
Micro Cu
$Cu(OH)_2$
Nano Cu
Influent
Baseline Average
Cu Wk 1
Cu Wk 2
Cu Wk 3
Post Cu Wk 3
Experimental Conditions
B
400
350
300
250
200
150
100
50
0
Total Organic Carbon (mg/L)
Micro Cu
$Cu(OH)_2$
Nano Cu
Influent
Baseline Average
Cu Wk 1
Cu Wk 2
Cu Wk 3
Post Cu Wk 3
Experimental Conditions
C
90
80
70
60
50
40
30
20
10
0
Turbidity (NTU)
Micro Cu
$Cu(OH)_2$
Nano Cu
Influent
Baseline Average
Cu Wk 1
Cu Wk 2
Cu Wk 3
Post Cu Wk 3
Experimental Conditions
D
90
80
70
60
50
40
30
20
10
Total Suspended Solids (mg/L)
Micro Cu
$Cu(OH)_2$
Nano Cu
Influent
Baseline Average
Cu Wk 1
Cu Wk 2
Cu Wk 3
Post Cu Wk 3
Experimental Conditions

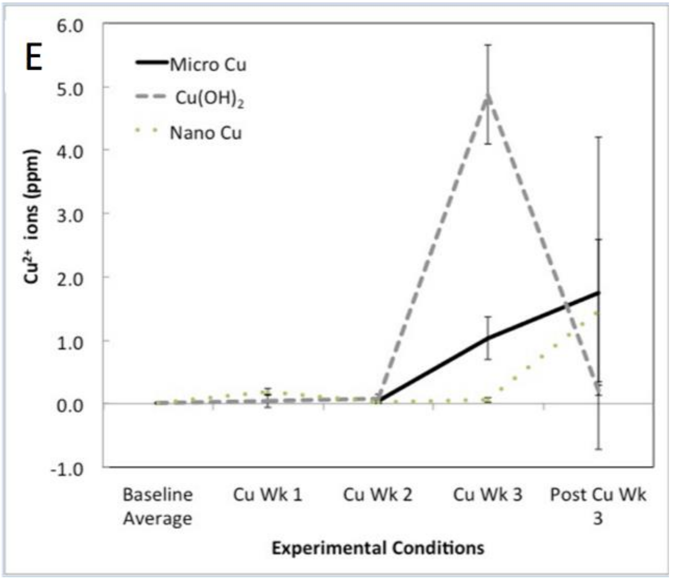


Fig. 1. Changes in water quality parameters pH (A), total organic carbon, or TOC (B), and turbidity (C), Cu^{2+} free ion concentration (D), and TSS (E) were measured over the course of three independent ten-week experiments for micro Cu (solid line), $Cu(OH)_2$ (dashed line), and nano Cu (dotted line). A shaded box indicates the typical septic system range for each test to give better clarification on when the septic system is out of range. Influent pH was maintained at 7.6 during all experimental conditions due to consistency of influent material (colon waste, DI H_2O, and greywater). The points plotted are the recorded average and bars are the standard deviation. The * symbol denotes statistically different data when compared to the baseline. Statistical analyses were not conducted for the Cu ion data. The average and standard deviation values are listed in full within the SI.

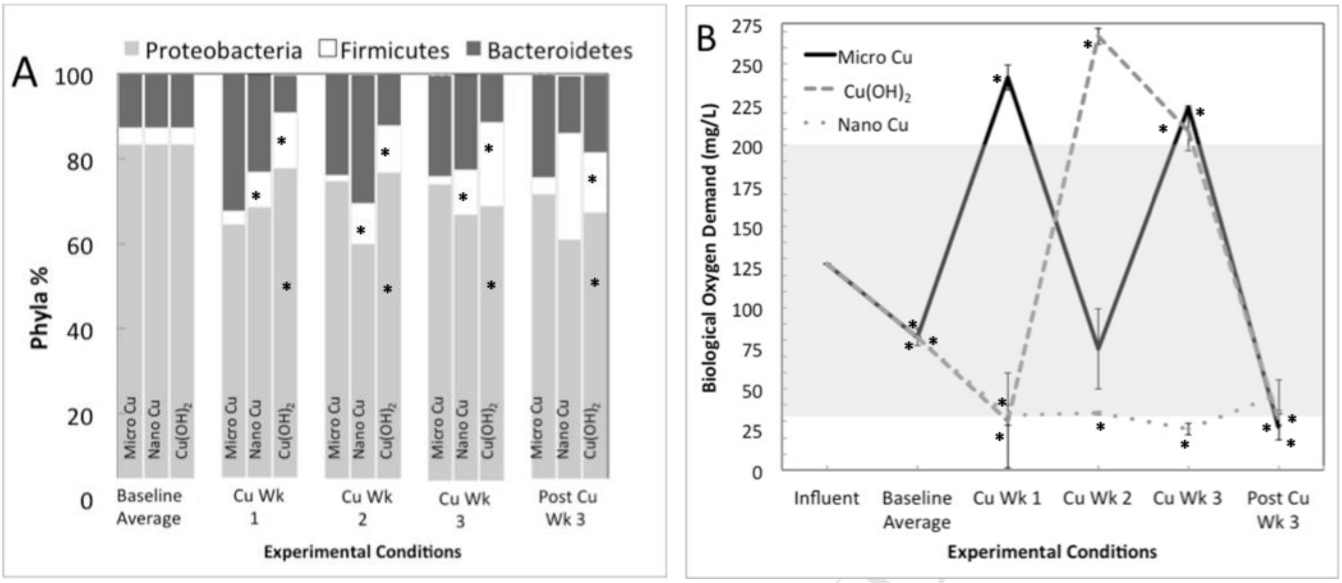


Fig. 2. Changes in microbial community phyla (A) and biological oxygen demand, or BOD_5 (B) over the course of three independent ten-week experiments for micro Cu (solid line), $Cu(OH)_2$ (dashed line), and nano Cu (dotted line). A shaded box indicates the typical septic system range for each test to give better clarification on when the septic system is out of range. The * symbol denotes statistically different data when compared to the baseline data, or the influent for the BOD data. The average and standard deviation values are listed in full within the SI.

Effects of copper particles on a model septic system's function and microbial community

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Highlights

- 20-30% of US households have decentralized wastewater treatment systems.
- A laboratory model septic system was used to study three Cu-based particles.
- Cu particles affected the pH, BOD, and TOC measurements.
- Overall, septic systems can manage new emerging perturbances.
- Pulses of untreated waste effluent may be released into the leachfield.