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Article

Copper and Gold Nanoparticles Increase Nutrient Excretion Rates of Primary Consumers

Brittany G. Perrotta,* Marie Simonin, Jeffrey A. Back, Steven M. Anderson, Astrid Avellan, Christina M. Bergemann, Benjamin T. Castellon, Benjamin P. Colman, Gregory V. Lowry, Cole W. Matson, Emily S. Bernhardt, and Ryan S. King*

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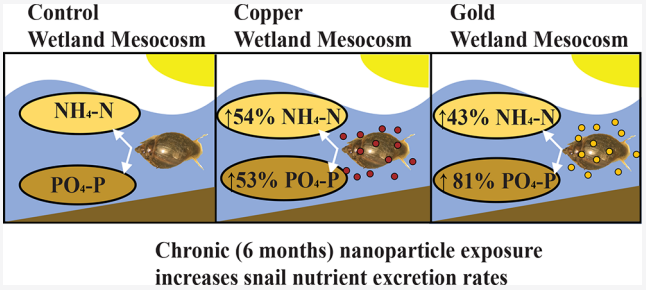
ABSTRACT: Freshwater ecosystems are exposed to engineered nanoparticles through municipal and industrial wastewater-effluent discharges and agricultural nonpoint source runoff. Because previous work has shown that engineered nanoparticles from these sources can accumulate in freshwater algal assemblages, we hypothesized that nanoparticles may affect the biology of primary consumers by altering the processing of two critical nutrients associated with growth and survivorship, nitrogen and phosphorus. We tested this hypothesis by measuring the excretion rates of nitrogen and phosphorus of *Physella acuta*, a ubiquitous pulmonate snail that grazes heavily on periphyton, exposed to either copper or gold engineered nanoparticles for 6 months in an outdoor wetland mesocosm experiment. Chronic nanoparticle exposure doubled nutrient excretion when compared to the control. Gold nanoparticles increased nitrogen and phosphorus excretion rates more than copper nanoparticles, but overall, both nanoparticles led to higher consumer excretion, despite contrasting particle stability and physiochemical properties. Snails in mesocosms enriched with nitrogen and phosphorus had overall higher excretion rates than ones in ambient (no nutrients added) mesocosms. Stimulation patterns were different between nitrogen and phosphorus excretion, which could have implications for the resulting nutrient ratio in the water column. These results suggest that low concentrations of engineered nanoparticles could alter the metabolism of consumers and increase consumer-mediated nutrient recycling rates, potentially intensifying eutrophication in aquatic systems, for example, the increased persistence of algal blooms as observed in our mesocosm experiment.



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■ INTRODUCTION

Engineered nanoparticles (NPs) are ubiquitous in many commercial products and enter the aquatic environment where they have been shown to have a wide variety of effects. Nanomaterials represent a sizeable fraction of materials used in the global economy and consequently have multiple pathways into the environment. Flow into aquatic ecosystems by runoff and wastewater is estimated to contribute up to 21,000 metric tons of NPs annually.[1] Once NPs are released into the environment, they interact with a complex aquatic ecosystem. At present, more extensive research has been conducted on NPs in laboratory settings.[2,3] Less research has been conducted on the ecosystem-level effects of NPs under realistic environmental conditions, such as chronic long-term exposure and NP interactions with food webs, particularly primary producers and consumers.[4,5]

Primary producers (plants and algae) and consumers (grazers) interact extensively with NPs discharged into aquatic ecosystems. This interaction allows for the transfer of NPs from the water column or sediments into aquatic food webs, resulting in possible negative effects on ecosystems.[6,7] Although most studies have shown relatively benign effects of NPs, some NPs have toxic effects on organisms including algae, cladocerans, snails, and fish.[8−10] NPs have been shown to alter organism metabolism and bind to ions such as phosphate, limiting essential nutrients from interacting with cells.[11−13] Changes to organism metabolism—how organisms take up, transform, and expend energy and materials—can lead to indirect effects on the ecosystem.[14,15]

Consumers are an integral part of nutrient dynamics in ecosystems and may affect primary productivity by altering the supply and quality of resources such as nutrients, primarily nitrogen and phosphorus.[16] Consumer-mediated nutrient recycling is an ecological process, which includes the excretion of dissolved nitrogen and phosphorus by an organism into its

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surrounding environment over time. It is commonly used for testing the predictions of important ecological frameworks, such as the metabolic theory of ecology or ecological stoichiometry.[17] Both of these frameworks use principles of physics, chemistry, and biology to link the biology or elemental composition of individual organisms to the ecology of populations, communities, and ecosystems.[15,18,19]

Previous studies measuring consumer-mediated nutrient recycling have contributed considerably to our understanding of interactions among consumers, algae, and nutrients in aquatic ecosystems.[20,21] Individual consumer species can directly alter ecosystem function through grazing stored forms of organic nutrients which they then excrete in simplified forms at rates comparable to major nutrient sources. These nutrients excreted by consumers are then immediately available for plant, algal, and microbial uptake and drive consumer-mediated nutrient recycling.[16,22−24] Further, the processing of food under varying environmental conditions, particularly contrasting background nutrient enrichment regimes, can lead to differential consumer excretion of nutrients into aquatic ecosystems.[25]

Ecological processes have nutrient requirements, and the excess nutrients, specifically nitrogen and phosphorus, may result in altered ecosystem function. Surface waters have become nutrient-enriched, largely because of urban and agricultural practices, leading to widespread eutrophication of freshwater ecosystems. These eutrophic ecosystems have an increased capacity for algal biomass and primary production.[26−28] The increase in primary productivity exacerbates diel dissolved oxygen fluctuations and contributes to an overall decline in species richness while encouraging proliferation of more pollution-tolerant species.[29−31]

Proliferation of pollution-tolerant species that recycle nitrogen quickly may affect overall biogeochemical cycling through consumer-mediated nutrient recycling pathways.[32] Pollution-tolerant species, such as the aquatic snail, *Physella acuta*, have been shown to persist where NPs co-occur with high nutrient loads in wastewater streams and agricultural runoff.[33] *P. acuta* is a freshwater pulmonate snail detritivore and herbivore that reproduces quickly, can occur at high densities, and thrives under a wide range of food and cultural conditions.[34−37] Previous work showed that they accumulate substantial amounts of NPs relative to other primary consumers and are a good model to study uptake of NPs.[9,38−46]

We know very little of the effects of NPs and nutrients on the relationships among consumers and nutrient remineralization through consumer-mediated nutrient recycling, despite growing evidence that metal-based NPs accumulate in snails and that metals have been found to influence the physiology of snails at high concentrations.[47,48] Investigations of contaminants within the metabolic theory of ecology, ecological stoichiometry, and consumer-mediated nutrient recycling framework provide an understanding of contaminant effects on aquatic food webs while also assessing the potential utility of these established ecological responses to complex mixtures of NPs and nutrient contaminants in freshwater ecosystems.

Here, we studied the interactive effects of NPs and nutrient enrichment on *P. acuta* with a chronic long-term, low-concentration dosing scheme to provide a better understanding of NP effects on aquatic food webs and of how NPs may indirectly impact aquatic ecosystems by altering consumer metabolism. We investigated how chronic exposures to copper hydroxide-based (CuNP) or gold (AuNPs) (weekly additions) crossed with two levels of nutrient enrichment influenced the nutrient excretion of consumers in outdoor wetland mesocosms over 6 months. We used CuNPs found in a commercially available fungicide at a realistic dose to investigate particle and biotic interactions likely to be occurring in aquatic ecosystems. We chose AuNPs as a model NP that is easy to detect in complex environmental matrices, in part because of its low natural background, enabling us to study the fate of these particles.[49] We hypothesized that consumer nitrogen (as NH_4^+−N) and phosphorus (as PO_4^{3-}−P) excretion would be elevated in response to CuNP exposure relative to AuNPs and control treatments. We hypothesized that copper would increase excretion rates of consumers relative to gold because CuNPs have antimicrobial/antifungal properties and previous research demonstrated that the antimicrobial/antifungal agent triclosan increased snail nutrient excretion.[32] We hypothesized that consumers in the AuNP mesocosms would excrete similarly to the control mesocosms, as previous studies have used AuNPs as inert NP tracers because of their low dissolution rates in abiotic water and interaction with organisms as a particle.[50−52] Furthermore, we hypothesized that snails at higher background nutrient treatments would excrete at higher rates than ambient nutrient treatments because consumer food sources in these treatments are nutrient-enriched and more available.[53] In our study, we saw an increased internal eutrophication in the mesocosms exposed to the combination of NPs and nutrient enrichment.[5] We wanted to investigate ecosystem level changes, which could drive increased eutrophication, other than primary producers. Consumer responses including consumer-mediated nutrient recycling might be one of the key parameters explaining this phenomenon. Consumer metabolic responses to NPs are largely unknown, but consumer metabolic responses to environmental variables have been known to alter ways in which nutrients are processed in consumers, leading to a differential excretion of nutrients to the water column.[54,55]

■ MATERIALS AND METHODS

Wetland Mesocosm Experimental Design. This study took place in the Center for the Environmental Implications of NanoTechnology (CEINT) experimental wetland mesocosm facility located in the Duke Forest in Durham County, North Carolina, USA. We employed the same slant-board mesocosm design that has been described previously.[56,57] Mesocosms were built to dimensions (3.66 m × 1.22 m × 0.81 m), were filled with sandy-loam soil, and then with ∼250 L of ground water. This design yields an upland zone with minimal inundation, a transitional zone that is periodically inundated with water, and an aquatic zone that is permanently inundated. The detailed mesocosm setup is found in the Supporting Information, section "Mesocosm Setup", and has been previously reported.[5,38,40] In June 2015, we added the following organisms to the aquatic zone: rooted macrophyte (*Egeria densa*), aquatic snails (*P. acuta* and *Lymnaea* sp.), and eastern mosquito fish (*Gambusia holbrooki*).

On September 28, 2015, two levels of nutrients (ambient and enriched) were established through weekly additions. The enriched level received 1 L of mesocosm water supplemented with 88 mg of N (as KNO_3) and 35 mg of P (as KH_2PO_4) added throughout the entire aquatic zone.

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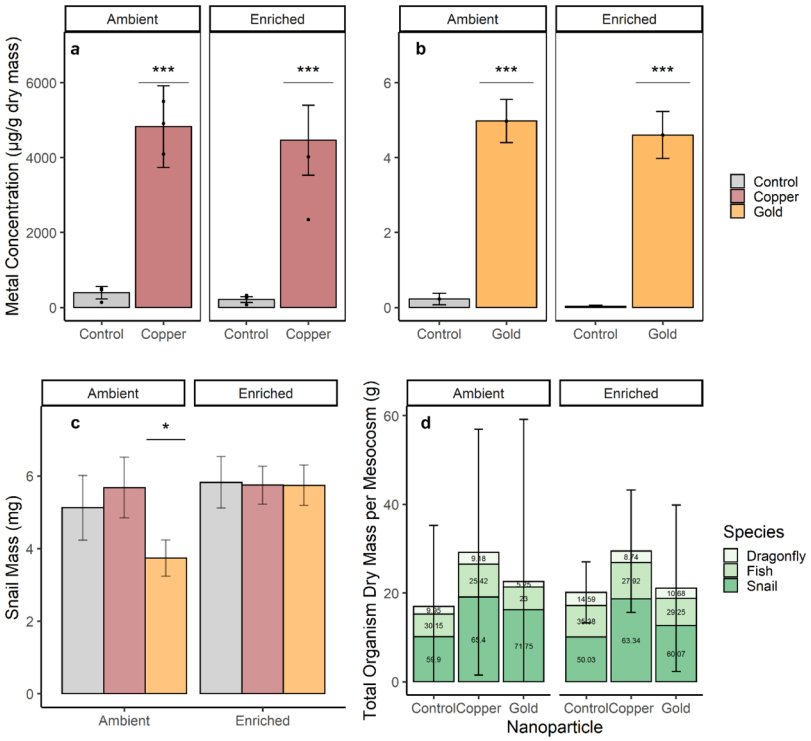

Figure 1. Snail metal concentration ± 95% CI for Cu (a) and Au (b) in snail tissues from NP treatments. Axes and the scale are different for each plot. Black points represent replicate values. NP treatments are distinguished by bar colors. (c) Average DM (mg ± 95% CI) of snails included in excretion experiment per treatment. (d) Average total organismal DM in each mesocosm per treatment (g ± 95% CI) at the end of the mesocosm experiment.

On January 18, 2016, mesocosms were randomly assigned to NP treatments across the two nutrient treatments to the NP treatments (control, copper, gold). The fully factorial experiment resulted in three treatments with no nutrient additions (ambient): control, CuNP, and AuNP and three treatments with nutrient additions (enriched): control, CuNP, and AuNP. Each treatment was replicated in three independent mesocosms, resulting in a total of 18 mesocosms that were exposed for 192 days (about 6 months). NPs were dosed weekly into mesocosms by mixing freshly sonicated NPs with 1 L of mesocosm water and distributed throughout the entire aquatic zone.[5,40]

The CuNP mesocosms received a weekly dose of 35 mg of Kocide for 6 months, except for the first week of treatment when they received an initial pulse of 374 mg that resulted in a total dose of 0.9816 g of Kocide per mesocosm over the duration of the study. Kocide contains 27% Cu by dry weight, so 358 mg of Cu from $CuOH_2$ was added to the mesocosms. The concentration of Cu was intended to be environmentally realistic by simulating field application rates and losses.[5] This resulted in an average water column Cu ± SE concentration in CuNP mesocosms of 11 ± 1.1 and 13 ± 2.0 μg/L in the ambient and enriched mesocosms, respectively, over the course of the experiment (Table S1). The mesocosms exposed to AuNPs received a weekly dose of 19 mg Au, resulting in a total dose of 532 mg Au added over the 6 months of the experiment.[5] This resulted in an average Au ± SE concentration in the AuNP mesocosms of 6 ± 1.0 and 9 ± 1.8 μg/L in the ambient and enriched mesocosms, respectively, over the course of the experiment (Table S1).

Consumer Nutrient Excretion. The nutrient excretion study was conducted during the peak of growing season in July and after snails had been exposed to NPs for 6 months and 3 months prior to the end of the mesocosm experiment. We chose to focus on snails because nutrient excretion is well-studied in snails,[22,32,58−61] and snails represented 68% of the nonplant biomass in our mesocosms. Ten *P. acuta* snails were collected from each control, Cu and Au mesocosm and placed into mesocosm-specific plastic bins with mesocosm water. We then placed single snails into 15 mL conical tubes with 7 mL of filtered ambient nutrient, control mesocosm water (0.2 μm Nalgene Filter, Thermo Scientific, USA). All snails were incubated at a water temperature of 30 °C for 95 ± 0.5 min (mean ± SE). After incubation, snails and fecal matter were removed from excretion water. Snails and excretion water were immediately frozen and shipped to Baylor University. Excretion water samples were thawed and filtered (0.45 μm Whatman Filter, Cytiva, USA) to remove any remaining particulates (*e.g.* feces) prior to analysis. Frozen snails were analyzed for NP uptake by quantifying body burdens of Cu and Au using inductively coupled plasma mass spectrometry (ICP−MS). The ICP−MS methods are detailed in the Supporting Information, section "ICP−MS Methods", and were previously described from this mesocosm experiment.[38,40]

Cu speciation was measured on the dried powder of soft tissues that was kept under a N_2 atmosphere. Prior to analysis, the powder was homogenized, pressed into 6 mm pellets, and sealed with Kapton tape. Pellets were analyzed at the Cu K-edge (8.98 keV) for X-ray absorption near-edge structure (XANES) spectroscopy under 77 K cryostat conditions to avoid beam damage. Additional beamline, sample information, and LCF modeling are detailed in the Supporting Information, section "Cu Speciation", and were previously described from this mesocosm experiment.[38,40]

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Background water nutrient concentrations (NH_3−N and PO_4−P) were measured in 25 replicates of filtered, control mesocosm water that was not exposed to snails. Ammonia was determined using the phenolate method and phosphorus was determined using the molybdate colorimetric method and on a Lachat QuikChem 8500 flow-injection autoanalyzer.[65,66] The snail excretion water was analyzed for NH_3−N and PO_4−P in the same manner. The mean background NH_3−N and PO_4−P and concentrations were subtracted from the excretion concentrations to determine the snail-excreted NH_3−N and PO_4−P. NH_3−N was measured, but NH_4−N was estimated using differences in the molecular weight. Hence, we measured ammonia, the predominant form of nitrogen excreted by aquatic mollusks, and reported ammonium, which is the standard practice for measuring nitrogen excretion in aquatic animals.[22,32,58,59,62−64]

Snail and food source (floc, *E. densa*, periphyton from *E. densa*, and the sediment) were oven-dried at 60 °C, homogenized, and weighed into silver capsules. Capsules were then exposed to HCl to remove inorganic carbon, oven-dried at 60 °C for 48 h, and wrapped in tin capsules for stable carbon and nitrogen analysis.[67] Analysis was conducted on a Thermo-Electron Delta V Advantage isotope ratio mass spectrometer (IRMS) with a dual inlet system.

Statistical Analysis. Snail excretion rate (NH_4−N and PO_4−P μg/h) and metal accumulation in snail tissues (Cu and Au μg/g DM) were modeled using linear mixed models (LMMs) to determine the effects of the NP exposure, nutrient status, snail biomass, and their interaction on both nutrient excretion and bioaccumulation.[68] The main effects were NP treatment (control, Au, Cu), and nutrient treatment (ambient and enriched), whereas dry mass (DM, mg) was modeled as a covariate because excretion rate was expected to be dependent on snail body mass.[63,69] Mesocosm number (n = 18) was a random effect, which nested individual snail measurements within mesocosms and subsequently modeled the random effect of each mesocosm on these values.[68] The models were fit using the lmer function of the lme4 package in R.[70,71] Model fits were evaluated using residual plots, which demonstrated that the model fit the data and met the assumptions of a LMM. Conditional R^2 values were calculated for the selected model.[72] Significance of main effects and interactions were estimated using the analysis of variance (ANOVA) function in the car package (Table S2).[68,73] The function predict.se.MERmod in the lme4 package was used to predict NH_4−N or PO_4^-−P excretion rates using the model and different combinations of levels of significant main effects or interactions ($p < 0.05$). The fitted models were used to graphically illustrate the predicted mean and 95% confidence interval (CI) excretion rates by snail DM. Results were plotted in ggplot2 for both NH_4−N (Figure 3a,b) and PO_4−P excretion (Figure 4a,b).[74]

Average snail nutrient recycling rates were determined by averaging snail NH_4−N and PO_4−P excretion values by treatment (Figures 3c,d and 4c,d). Snail biomass was determined by averaging snail DM from the excretion experiment (Figure 1c). The lsmeans package in R was used on the full excretion models described in Table S2 to look at pairwise differences between nutrient and NP treatments.[75]

Stable isotope analysis, including snail diet composition, was determined using the MixSIAR package in R.[76] The δ^{13}C and δ^{15}N values for the snail tissue and food sources were considered tracers, and the NP was treated as a fixed effect (Figure S2).

RESULTS

Metal Accumulation in Snails. Cu and Au concentrations in NP-exposed snail tissues were more than 10-fold higher than control snails (Figure 1a,b, $p < 0.001$). Metal concentrations for CuNP-exposed snails averaged 4622 μg Cu/g snail and 4.78 μg Au/g snail for AuNP-exposed snails, with Cu accumulating at 1000-fold higher concentrations compared to Au. Nutrient status had no effect on metal accumulation in snails (Figure 1a, $p < 0.68$ and Figure 1b, $p < 0.11$).

Average snail body DM ranged from 3.73 to 5.82 mg in the ambient AuNP mesocosms and the enriched control mesocosms, respectively. While both types of NPs accumulated in the snail tissue, CuNPs did not influence the body size of snails either in the ambient or enriched mesocosms (Figure 1c, $p < 0.34$ and $p < 0.99$). Chronic AuNP exposure in the ambient mesocosms led to a reduction of snail body DM (Figure 1c, $p < 0.05$), but there was no difference in DM between the enriched AuNP snails and the enriched control snails (Figure 1c, $p < 0.99$). Without chronic NP dosing, there was no difference in snail body size between nutrient treatments (Figure 1c, $p < 0.08$). Snails represented 68% of the nonplant, living organism biomass, and *P. acuta* represented 83% of the snail biomass (Figure 1d).

Measurements of Cu speciation in the snail tissue (Figure 2) indicate that the Cu uptake was predominately of an ionic form

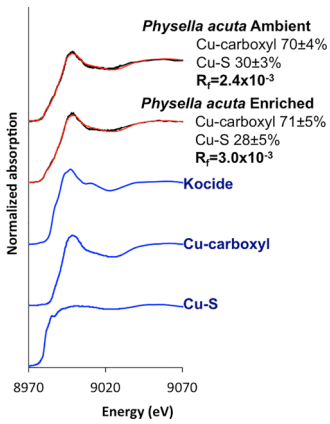

Figure 2. XANES at Cu K-edge for *P. acuta* sampled in either ambient or enriched mesocosms in comparison with Kocide, Cu−carboxyl, and Cu−S references, as described in (Avellan et al., 2020).[40] Results of the linear combination analysis (LCA) of the *P. acuta* spectra are also presented along with their quality factor (R_f) as detailed in the Methods section. More details of Cu speciation can be found in the Supporting Information, and the references used as potential additional compounds for the LCA can be found in Figure S1.

bound to carboxyl groups (~70%), with the remainder represented by sulfidized Cu (~30%). Kocide CuNPs are primarily $Cu(OH)_2$, yet there was no contribution of $Cu(OH)_2$ in the XANES spectra, suggesting that snails are not taking up the CuNPs directly through grazing. The addition of nutrients in the system did not change the speciation of copper associated with the snails. Tissue concentrations were too low to measure Au speciation in snails.

Nitrogen Excretion. Nitrogen excretion rates increased linearly as snail DM (covariate) increased across all NP and nutrient mesocosms (Figure 3a,b). NPs had the largest effect on N excretion, but nutrient and the interaction between DM

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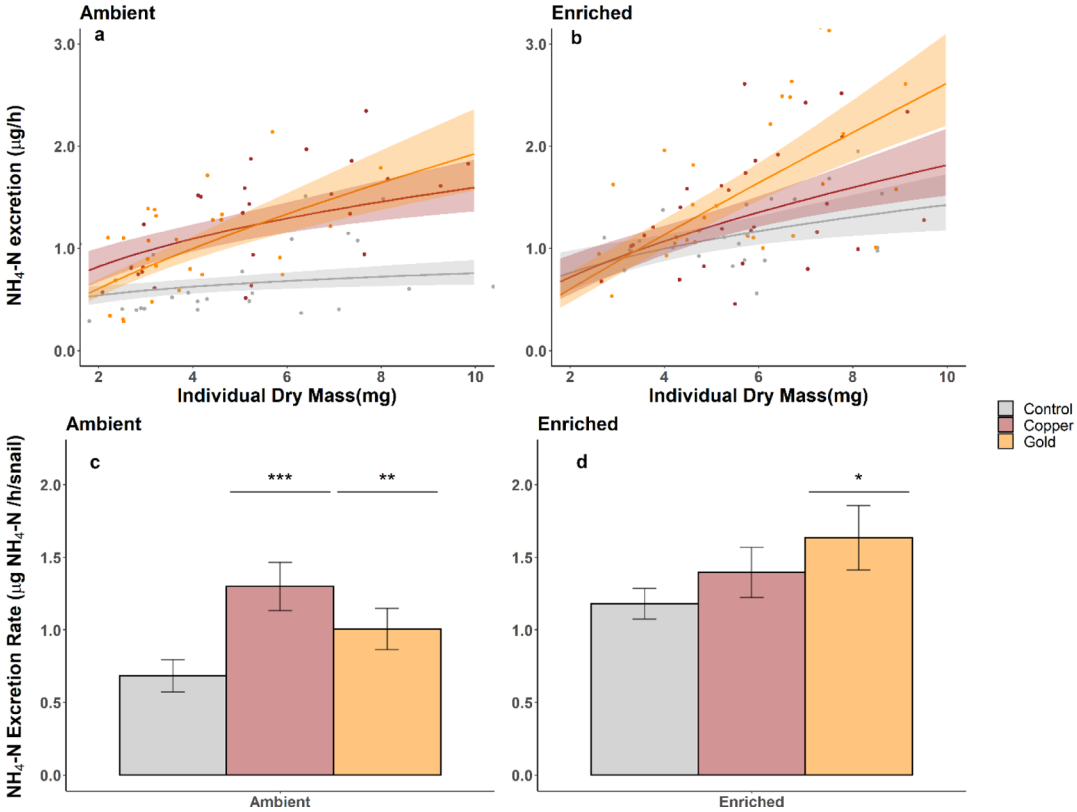

Figure 3. Nitrogen excretion rates expressed by individual snail body DM (mg) in the (a) ambient and (b) enriched mesocosms. NPs are distinguished by shading color. Lines and shaded areas represent predicted means and 95% CIs from LMM. Mean nitrogen excretion rates by *P. acuta* after NP exposure in the (c) ambient and (d) enriched mesocosms. Means and 95% CIs are presented, and significant differences (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$) are indicated by asterisks.

and NPs also had significant effects (Table S2, $p < 0.001$, $p < 0.05$). The conditional R^2 value for the nitrogen excretion LMM is 0.57, which describes the proportion of variance explained by the fixed and random factors (Table S2). Although N excretion rates were similar for snails with low DM in all treatments, as DM increased, N excretion rates in AuNP-exposed snails had larger slopes than those observed for CuNP-exposed snails. Both AuNP and CuNP-exposed snails had larger slopes than those observed for control snails.

In ambient mesocosms, the amount of N excreted per mean individual snail was 90 and 47% higher in the CuNPs and AuNPs mesocosms, respectively, compared to the ambient control mesocosms (Figure 3c, $p < 0.001$, $p < 0.01$). However, in enriched mesocosms, the amount of N excreted per average individual snail was 38% higher in the AuNP mesocosms (Figure 3d, $p < 0.05$). Snails from the enriched-CuNP mesocosms experienced an 18% increase in N excretion, but this increase was not statistically different from the enriched control mesocosms. After accounting for the effect of NPs, snails from the enriched mesocosms excreted 72% more N than snails from the ambient mesocosms (Figure 3c,d, $p < 0.001$).

Phosphorus Excretion. There were significant NP and nutrient effects on P excretion rate, with NPs having a larger influence on snail P excretion than nutrients (Table S2, $p < 0.001$, $p < 0.05$). There was a significant DM and NP interaction, indicating that control, Au, and Cu NP mesocosms had different effects on snail P excretion as a function of snail DM (Table S2, $p < 0.05$). The conditional R^2 value for the phosphorus excretion LMM is 0.45 (Table S2). Phosphorus excretion increased significantly with increasing DM in the ambient CuNP mesocosms (Figure 4a). Conversely, ambient and enriched AuNP and enriched CuNP mesocosms showed little change in P excretion with increasing DM (Figure 4a,b). Ambient and enriched control mesocosms had a slightly negative relationship in P excretion with increasing DM.

P excretion per mean individual snail was 94 and 110% higher in the ambient CuNP and AuNP mesocosms, respectively, compared to the ambient control mesocosms (Figure 4c, $p < 0.001$). The mass of P excreted per mean individual snail was 52% higher in the enriched AuNP mesocosms, respectively, compared to the enriched control mesocosms (Figure 4d, $p < 0.01$). Snails from the enriched CuNP mesocosms had a slight increase in P excretion (11%), but it was not statistically different from the enriched control mesocosms (Figure 4d, $p < 0.52$). After accounting for the effect of NPs, snails excreted 71% more P per mean individual in the enriched than in the ambient mesocosms (Figure 4c,d, $p < 0.001$).

■ DISCUSSION

Consumer-mediated nutrient recycling balances the requirements of organisms for energy and nutrients and the resulting relationship between diet, growth rates, and excretion.[17,18,32] Through excretion, consumers can be important drivers of nutrient cycling and can supply nutrients at rates comparable to other major nutrient sources in aquatic ecosystems.[22,59,69] Previous analyses have found the prediction of nutrient excretion for any animal taxon or aquatic ecosystem to be

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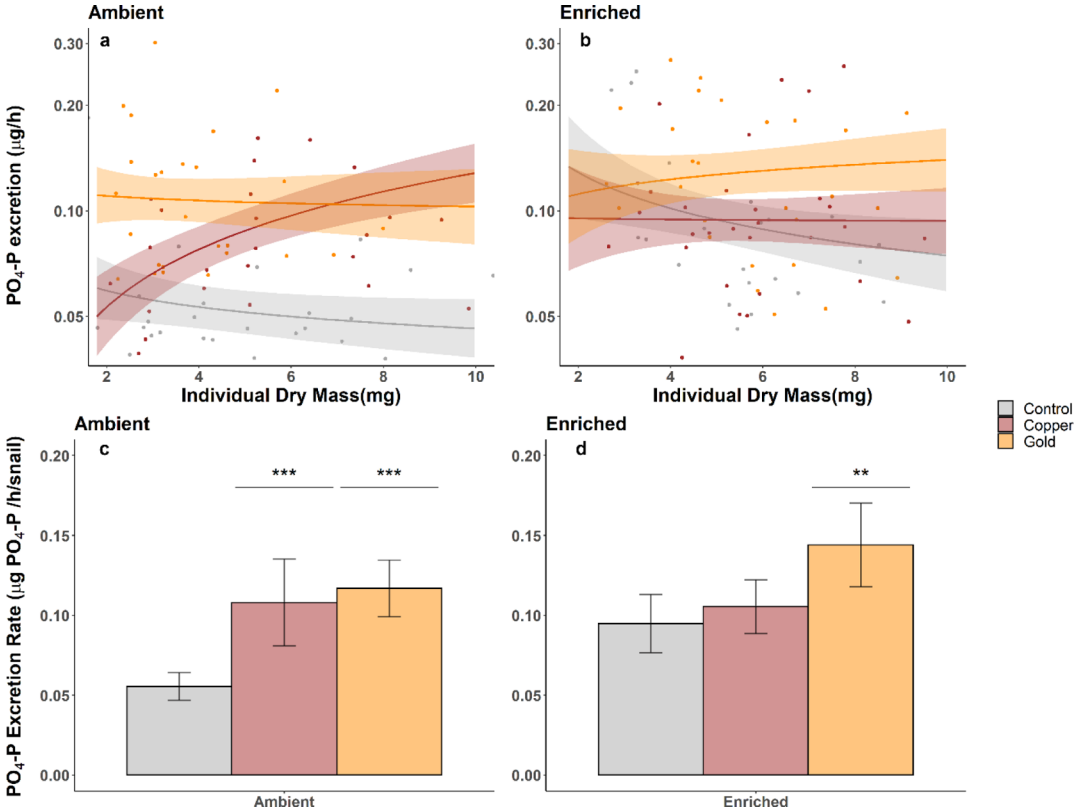

Figure 4. Phosphorus excretion rates expressed by individual snail body DM (mg) in the (a) ambient and (b) enriched mesocosms. NPs are distinguished by shading color. Lines and shaded areas represent predicted means and 95% CIs from LMMs. Mean phosphorus excretion rate of *P. acuta* after NP exposure in the (c) ambient and (d) enriched mesocosms. Means and 95% confidence intervals are presented and significant differences (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$) are indicated by asterisks.

best described using body size, water temperature, trophic guild, and vertebrate classification.[17]

Here, we see that N and P excretion rates of consumers increased as much as two-fold in response to chronic NP exposure in both ambient and enriched mesocosms between snails of the same species, sizes, and at the same water temperature. The best predictors of aquatic organism excretion were all equivalent in our study, yet we see a doubling of N and P excretion, strongly indicating that NP exposure is the driving variable behind this increased excretion. This two-fold change in cycling of nutrients by consumers means that algal communities have nutrients available to them at twice the rate when consumers are exposed to chronic NPs. Thus, NP exposure to consumers may result in increased primary production rates, and in our system, it resulted in NP mesocosms spending a larger proportion of time above the nutrient threshold where blooms occur.[5,32,77,78] While body size is typically the primary driver of nutrient excretion, we saw no difference in snail body sizes between treatments, with the exception of smaller snails in the ambient AuNP mesocosms, although we saw a difference in nutrient excretion. Stable isotope analysis of snail food sources and snail body tissue across treatments showed an indistinguishable preference for periphyton, Egeria, and "floc" compared to other food sources (Figure S2). Floc is a matrix of amorphous detritus, periphyton, and animal feces (particularly snails), which is why snail isotopes aligned so closely to the floc—it was likely the signature of their own waste product rather than their dominant food, which was periphyton. This indicates that there is likely a disparity in how snails are compensating for excreted phosphorus under NP treatments, whether it be compensatory feeding, increased energy demands to maintain homeostasis, increased P associated with fecal matter, or another mechanism to be determined in future work.[60,79]

Particle dissolution rate is important because it determines if the organism interacts with a dissolved or a particulate metal in aquatic ecosystems. Although both NPs experienced some dissolution in different compartments of the mesocosms, CuNPs dissolve more rapidly in the water column than AuNPs.[40] Measurements during this experiment indicate that CuNPs rapidly dissolve ($t_{1/2}$ = 33 h in our mesocosms), releasing Cu ions that form a complex with organic matter and sediment onto *E. densa* and the floc.[40,80] The surface of *E. densa* and the floc is where the snails have high grazing activity and encounter the complexed ionic Cu and some CuS species (Figures 2 and S2). While AuNPs have low dissolution rates in abiotic water and generally interact with organisms as particles, this was not true in our mesocosms containing *E. densa*.[50−52] The dosed AuNPs in the mesocosms associated with *E. densa* were transformed into oxidized species by periphyton.[81] At the time of the snail excretion study, most of the added AuNPs were associated with the *E. densa* periphyton and the floc, the primary grazing areas for the snails. All of the Au associated with *E. densa* and ∼10% of the Au in the floc had dissolved and become associated with new ligands.[40] Therefore, snails were likely to be feeding on a combination of ionic Au species and AuNPs. While accumulation into the consumer tissue type varied by the NP type and differences in their rates of dissolution in the water column, consumer excretion rates in the mesocosms exposed to NPs are similar. This may be

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because snails primarily uptake dissolved and complexed forms of the metals and not pristine NPs.[82,83]

Snails accumulated high amounts of metals in their soft tissues after 6 months of exposure, especially for CuNPs (4622 μg/g). Cu speciation in the snail tissue was mainly associated with carboxyl groups (Figure 2). Copper is a biologically relevant element to consumers; it is actively and intentionally taken up and tightly regulated by organisms. It has direct interactions with amino acid and carboxyl side chains within peptide chains and is critical to organismal biochemical reactions.[84] Conversely, gold is not a biologically relevant metal in consumers. Organisms in aquatic ecosystems are unlikely to encounter gold NPs or complexed Au ions, and therefore, it is a novel contaminant.[85] This could explain the lower amount of Au in snail tissues compared to Cu. However, these differences may also be the result of the faster dissolution rate of CuNPs in the water column and the resulting differences in their distribution in the mesocosms.[40] While NP body burdens were studied, they did not correlate well with consumer excretion. In future work, we intend to investigate possible mechanisms for the increase in nutrient excretion.

While we understand that nutrient recycling increases with NP exposure, we do not know the mechanism(s) behind these alterations. Previous studies have reported an increase in consumer nutrient excretion after exposure to other contaminants, including triclosan.[32] Triclosan is a well-known antimicrobial and has a probabilistic mechanism to alter the gut microbiome within the consumer. In our study, CuNPs have known antimicrobial properties and, while not typically thought of as an antimicrobial, AuNPs have some effect on microbial communities.[81] While we could not confirm the direct uptake of AuNPs, some of the Au in snails is likely AuNPs because the floc contained primarily AuNPs.[40] In our study, both NPs likely exhibited some antimicrobial properties.[81,86−89] These properties could alter the gut microbiome in snails and alter food assimilation efficiency.[90−92] However, most Cu (and perhaps Au) accumulated by snails was as dissolved species. Both ionic Cu and Au have demonstrated toxicity in aquatic organisms and may exhibit differential toxicity to snails.[48,88,93] Another mechanistic explanation is that metal exposure may result in increased energy demands because of metal transport and sequestration, limiting the amount of energy available for nutrient uptake processes. This could be analyzed via metabolite biomarkers and gene expression.[2,94,95] While NPs are novel additions to consumer environments, nutrient enrichment has been well-studied and identified as a water quality issue worldwide.

Previous studies have found that nutrient enrichment increases consumer excretion and these studies concur with our results. Nutrient enrichment increases consumer excretion because consumers are not sequestering nutrients, as there is a surplus in their food.[96] Independent of NP exposure, N and P excretion rates increased by 70% in the enriched mesocosms compared to ambient mesocosms. This indicates that there is an enriched versus ambient modulated effect. Previous literature has found that organismal excretion increased across agricultural gradients in mayfly species and fish assemblages.[97,98] The excretion rates that we saw in the control snails in this study were similar to those reported in *Physella* sp. collected from wetland ecosystems, and our excretion pattern of increasing N excretion as size increases is consistent with those reported in previous studies.[24,59] Nutrient enrichment from wastewater streams are frequently mixed with other emerging contaminants, including NPs.

NPs are likely to enter aquatic ecosystems as part of waste streams that include high nutrients (*e.g.* agricultural fertilizers and wastewaters). This nonlethal impact of NPs on consumers has the potential to exacerbate nutrient pollution and hypoxia in aquatic ecosystems. The doubling of nutrient excretion by consumers could be an overlooked factor in the intensification of eutrophication driven by consumer exposure to contaminants such as NPs. Although our experiment focused on snails, other aquatic consumers interact with NPs and the metal species they transform into through the same exposure pathways. It is likely that this increase in nutrient excretion is applicable to other aquatic consumers who live in similar waste streams with high nutrient loading and contaminant mixtures. In our study, this resulted in an increased frequency of algal blooms in NP mesocosms.[5] If our experimental mesocosm findings are indicative of global processes, NP and consumer interactions could contribute to widespread increases in aquatic productivity, resulting in more frequent algal blooms and more rapid degradation of aquatic ecosystems.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.0c02197.

Additional information concerning the mesocosm setup, ICP−MS methods, water column metal concentrations, ANOVA results from LMMs, Cu speciation, normalized absorption of XANES spectra of reference compounds, and stable isotope figure (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Brittany G. Perrotta − *Center for the Environmental Implications of NanoTechnology, Duke University, Durham, North Carolina 27708, United States; Center for Reservoir and Aquatic Systems Research (CRASR) and Department of Biology, Baylor University, Waco, Texas 76798, United States;* orcid.org/0000-0003-2669-3047; Email: Brittany_Perrotta@baylor.edu

Ryan S. King − *Center for the Environmental Implications of NanoTechnology, Duke University, Durham, North Carolina 27708, United States; Center for Reservoir and Aquatic Systems Research (CRASR) and Department of Biology, Baylor University, Waco, Texas 76798, United States;* Email: Ryan_S_King@baylor.edu

Authors

Marie Simonin − *Center for the Environmental Implications of NanoTechnology and Biology Department, Duke University, Durham, North Carolina 27708, United States;* orcid.org/0000-0003-1493-881X

Jeffrey A. Back − *Center for Reservoir and Aquatic Systems Research (CRASR), Baylor University, Waco, Texas 76798, United States*

Steven M. Anderson − *Center for the Environmental Implications of NanoTechnology and Biology Department, Duke University, Durham, North Carolina 27708, United States; Department of Forestry & Environmental Resources,*

https://dx.doi.org/10.1021/acs.est.0c02197
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North Carolina State University, Raleigh, North Carolina 27695, United States

Astrid Avellan − *Center for the Environmental Implications of NanoTechnology, Duke University, Durham, North Carolina 27708, United States; Civil & Environmental Engineering, Carnegie Mellon University, Pittsburgh, Pennsylvania 15213, United States;* orcid.org/0000-0001-6081-4389

Christina M. Bergemann − *Center for the Environmental Implications of NanoTechnology and Biology Department, Duke University, Durham, North Carolina 27708, United States*

Benjamin T. Castellon − *Center for the Environmental Implications of NanoTechnology, Duke University, Durham, North Carolina 27708, United States; Center for Reservoir and Aquatic Systems Research (CRASR) and Department of Environmental Science, Baylor University, Waco, Texas 76798, United States;* orcid.org/0000-0001-8883-076X

Benjamin P. Colman − *Center for the Environmental Implications of NanoTechnology, Duke University, Durham, North Carolina 27708, United States; Department of Ecosystem and Conservation Sciences, University of Montana, Missoula, Montana 59812, United States*

Gregory V. Lowry − *Center for the Environmental Implications of NanoTechnology, Duke University, Durham, North Carolina 27708, United States; Civil & Environmental Engineering, Carnegie Mellon University, Pittsburgh, Pennsylvania 15213, United States;* orcid.org/0000-0001-8599-008X

Cole W. Matson − *Center for the Environmental Implications of NanoTechnology, Duke University, Durham, North Carolina 27708, United States; Center for Reservoir and Aquatic Systems Research (CRASR) and Department of Environmental Science, Baylor University, Waco, Texas 76798, United States;* orcid.org/0000-0002-6472-9357

Emily S. Bernhardt − *Center for the Environmental Implications of NanoTechnology and Biology Department, Duke University, Durham, North Carolina 27708, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.est.0c02197

Notes

The authors declare no competing financial interest.

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