Copper and Zinc Nanoparticle Interactions with Nitrogen Cycling Bacteria

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Abstract

Interactions of engineered nanoparticles (NPs) with microorganisms driving the nitrogen cycle might have implications for food production, eutrophication of natural waters, and atmospheric nitrous oxide generation processes. Previous studies have shown that copper and zinc NPs can affect microbial communities differentially. In this study, copper containing nanomaterials, specifically nCu & nCuO, were tested because metal containing nanomaterials comprise the highest percentage of consumer NPs produced. Pure cultures of the nitrifying bacterium, *Nitrosomonas europaea,* and the nitrogen-fixing bacterium, *Azotobacter vinelandii* were selected as model microorganisms mediating two important processes in the nitrogen cycle. High throughput fluorescence assays based on ATP content determined that nCu showed similar effects across organisms. IC_{50} values decreased between time points; from 75.5 ± 8.2 to 54.2 ±7.1 mg/L for *N. Europaea* and 49.8 ± 9.8 to 21 ± 14.2 mg/L in *A. vinelandii*. In the range tested, nCuO was not toxic to these bacteria. Particle size and zeta potential measurements imply that the stability of nCu and nCuO affects their differential toxicity. Copy numbers of nitrogen fixation (nif) genes in exposed cells were lower at 48 hr, suggesting greater stress at late exponential/stationary phase. This confirmed time-dependent behavior observed in the ATP assays. These results are valuable for designing our ongoing and future studies on environmental microbial communities involved in nitrogen cycling.

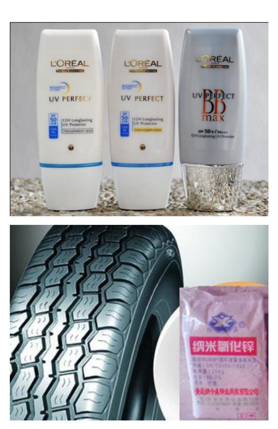
Background

Novel NP properties encourage use and product development

Copper Nanoparticles

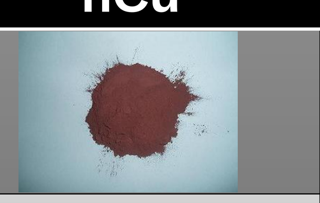
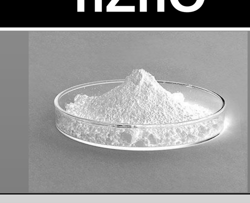
- Applications: filtration devices, cosmetics, electrodes, alloys, steel manufacturing, coatings and sealants.
- 12k publications within the past decade, > 2k in 1 year

Zinc Nanoparticles

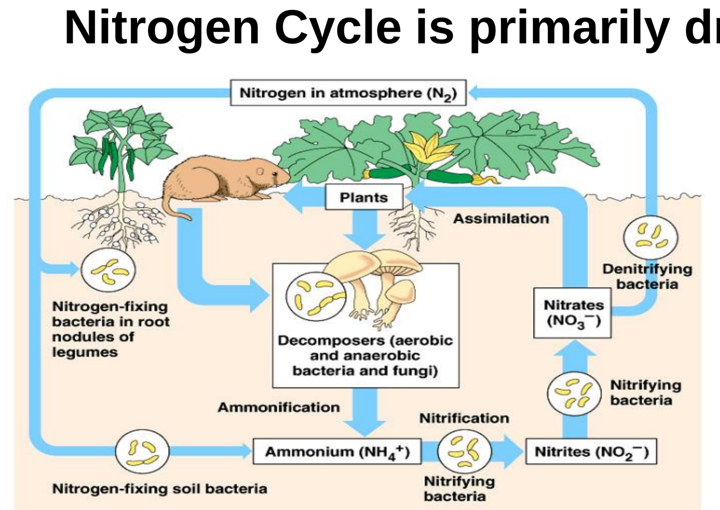


- Applications: cosmetics, tires, paints, plastics additives, ceramics, and semiconductors
- 2010: 5th most used Consumer Nanoparticle (nanotechproject.org)

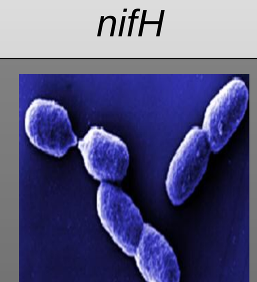
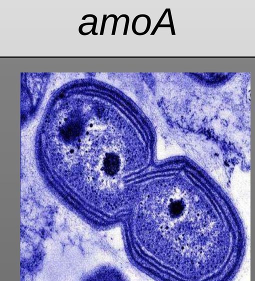
Nanoparticles tested:

	nCu	nCuO	nZnO
			
Size (nm)	50	40	10
Control Salt	$CuCl_2$	$CuCl_2$	$ZnCl_2$

Nitrogen Cycle is primarily driven by microorganisms



- Management of Nitrogen Cycle is a Grand Challenge for 21st Century (NAE).
- Nitrogen imbalances have implications for food production, eutrophication, & atmospheric nitrous oxide generation.
- NPs may differentially impact nitrogen cycling bacteria resulting in sinks

	Nitrogen Fixation	Nitrification	Denitrifcation
Organism	*A. vinelandii*	*N. europaea*	*P. stutzeri*
Gene of Interest	*nifH*	*amoA*	*nirK*
			

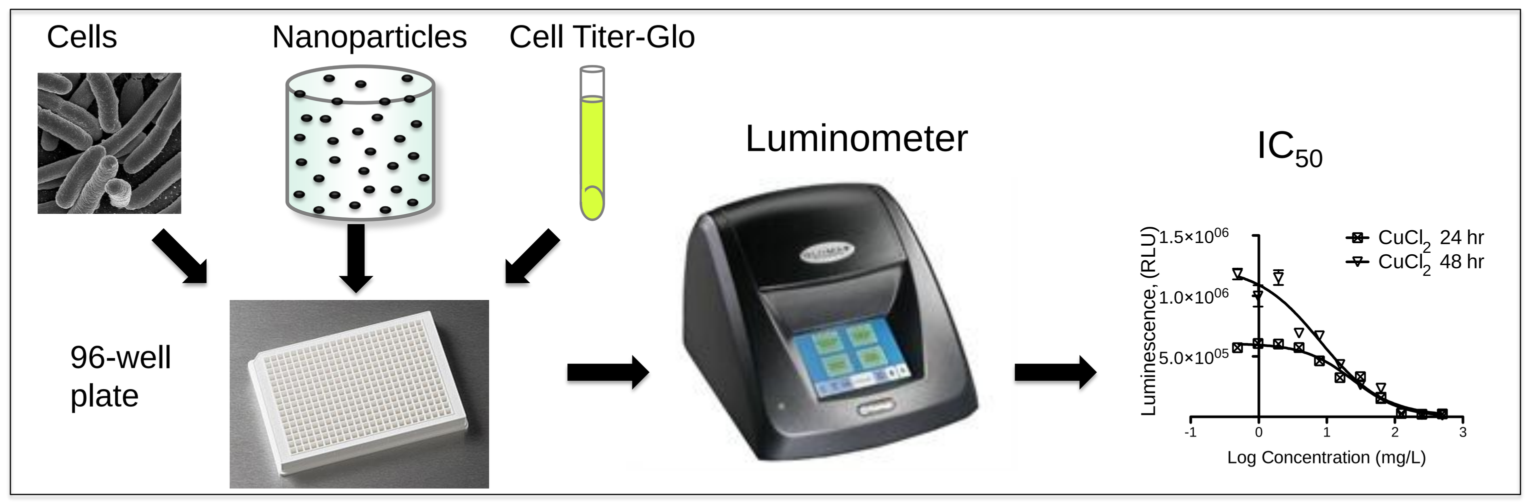
Experimental Design

Objectives:

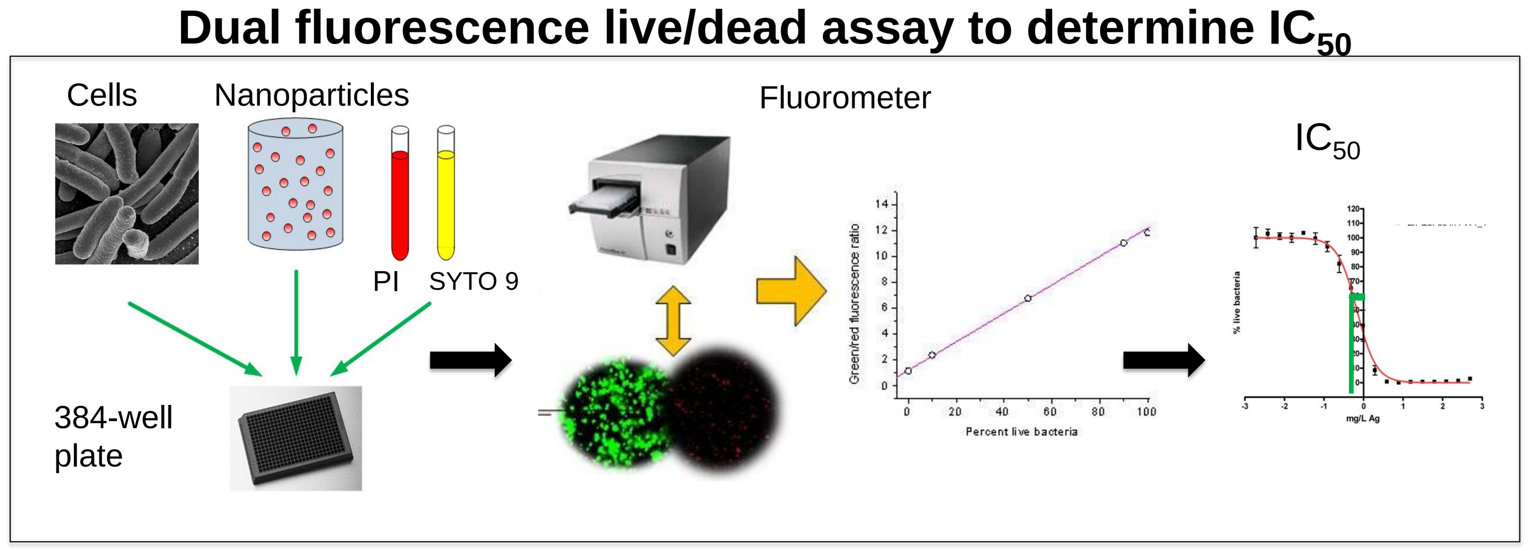
- Determine differential sensitivities of nitrogen cycling bacteria to copper & zinc nanoparticles
- Determine effect of nanoparticles on nitrogen transformation genes in model organisms.

Assay Development

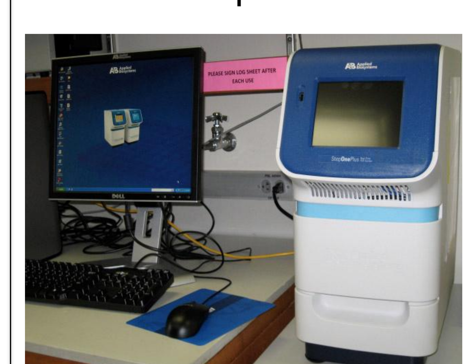
Metabolic Luminescence based assay to determine IC_{50}



Dual fluorescence live/dead assay to determine IC_{50}

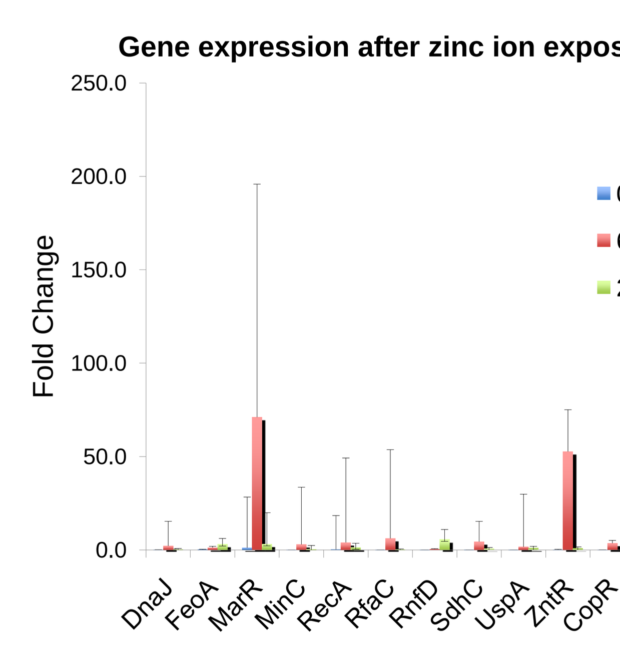


At IC_{50}, determine genetic and physico-chemical impacts

Expression of genes of interest: RT-qPCR	Metal ion concentration in media ICP-OES	Zeta potential & particle size in media ZetaPals
		

Stress Pathways

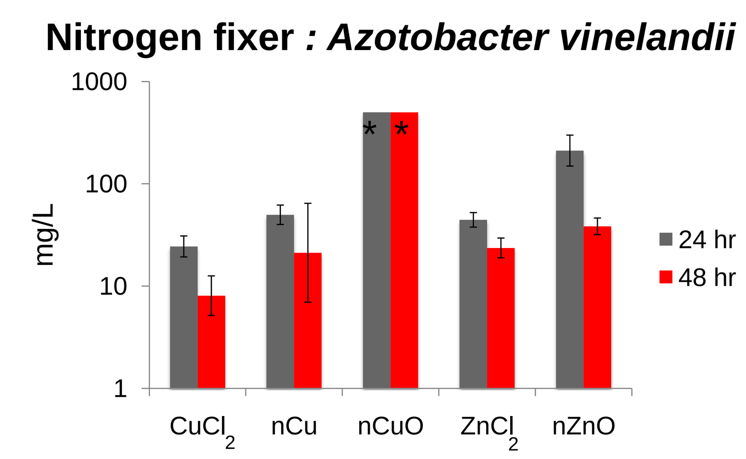
Gene	Name	Pathway
FeoA	Ferrous iron transport protein A.	Metal Homeostasis
CopR	CueR transcriptional dual regulator	Metal Homeostasis
ZntR	Zn(II)-responsive regulator of zntA.	Metal Homeostasis
RfaC	Lipopolysaccharide heptosyltransferase-1	Membrane Damage
RnfD	Electron transport complex protein rnfD.	Membrane Damage
SdhC	Succinate dehydrogenase membrane protein	Energy Stress
MinC	Septum site-determining protein minC.	Cell division
MarR	Multiple antibiotic resistance protein marR.	Drug Resistance
RecA	RecA protein	DNA Damage
DnaJ	Chaperone protein dnaJ (Heat shock protein J) (HSP40).	Protein Damage
UspA	Universal Stress Protein A	Universal Stress
DnaE	Catalytic α subunit of DNA polymerase II	Housekeeping/reference
GapA	D-glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	Housekeeping/reference

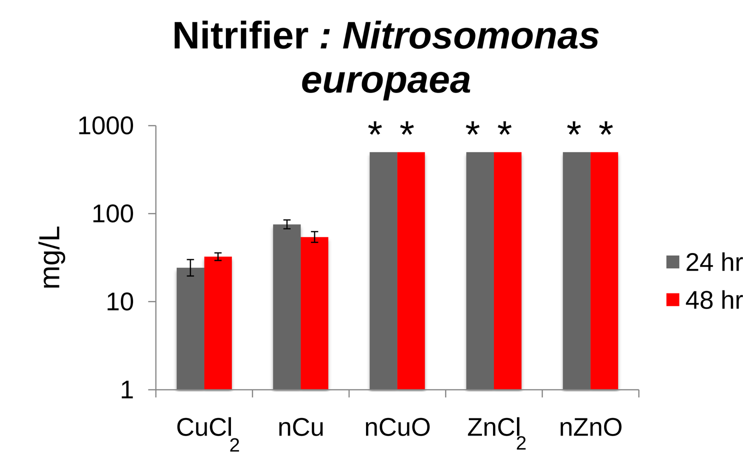


Results

IC_{50} Concentration:

Sensitivities change across species and time

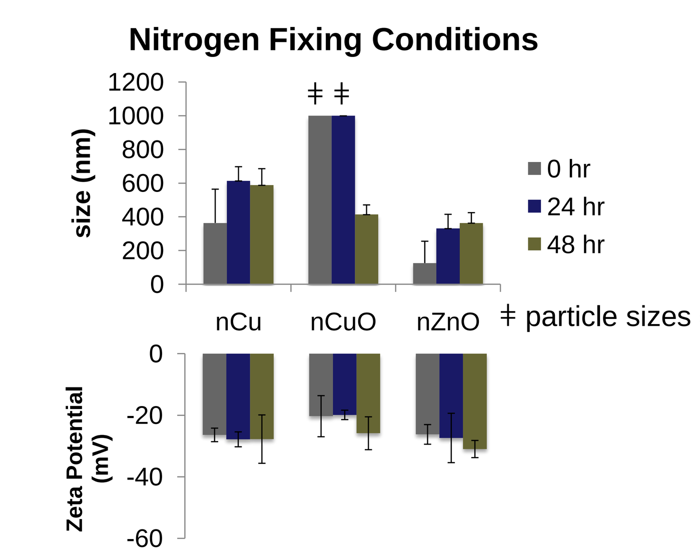


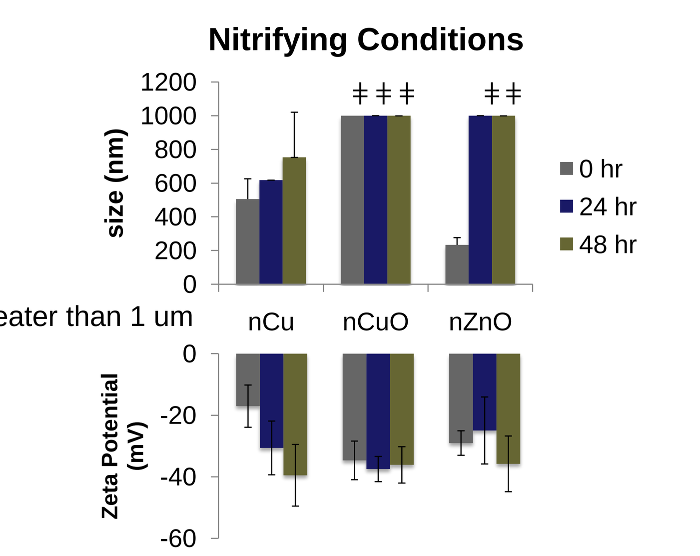


* Toxicity not observed in the range tested

Particle Size & Zeta Potential:

Larger particle aggregates are less toxic

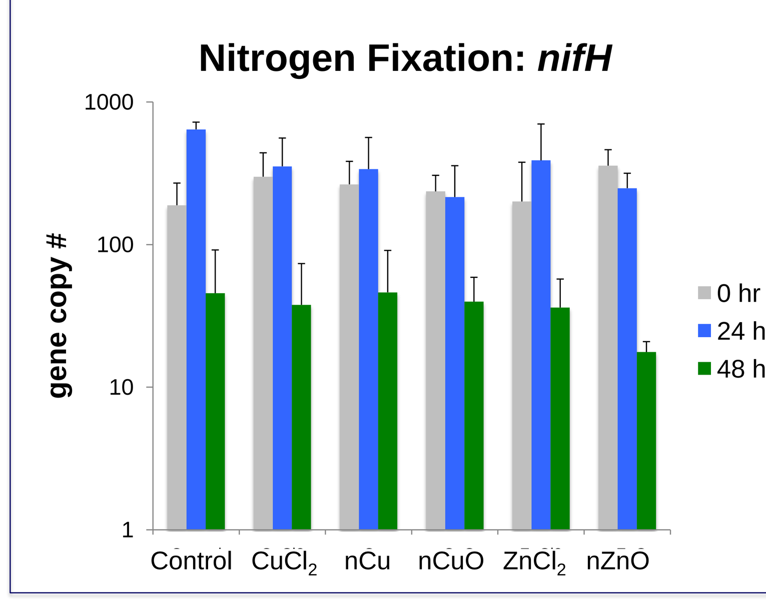


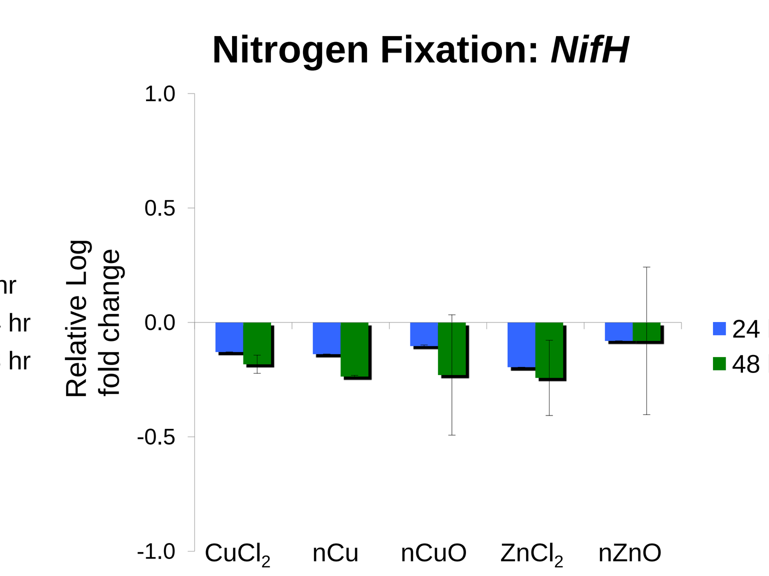


‡ particle sizes greater than 1 um

Gene Abundance:

Sub-lethal concentrations also cause inhibition of bacterial function





Summary and Future Directions

- Under conditions tested, nanoparticle aggregates were less toxic than ions.
- Both *A. vinelandii* and *N. europaea* were sensitive to nCu. In contrast, only *A. vinelandii* showed sensitivity to nZnO.
- nCuO appeared to not affect ATP content; however, *nif* gene data suggest that nCu had inhibitory effect on *A. vinelandii*.
- IC_{50}s and gene abundance were affected by the growth phase of organism during exposure.
- Data from pure culture work will be used to design experiments for microcosms of nitrogen cycling bacteria derived from wastewater treatment plants and wetlands. Microbial functional diversity will be assessed with GeoChip platform.

Acknowledgements

- This work was funded by NSF award #1134355.
- We thank Dr. Eric M. Hoek, Dr. Jenny A. Jay and Dr. Michael K. Stenstrom for their input and assistance with particle characterization and ATP luminescence assays.