Differential Sensitivity of Wetland-derived Nitrogen Cycling Microbes to Copper Nanoparticles

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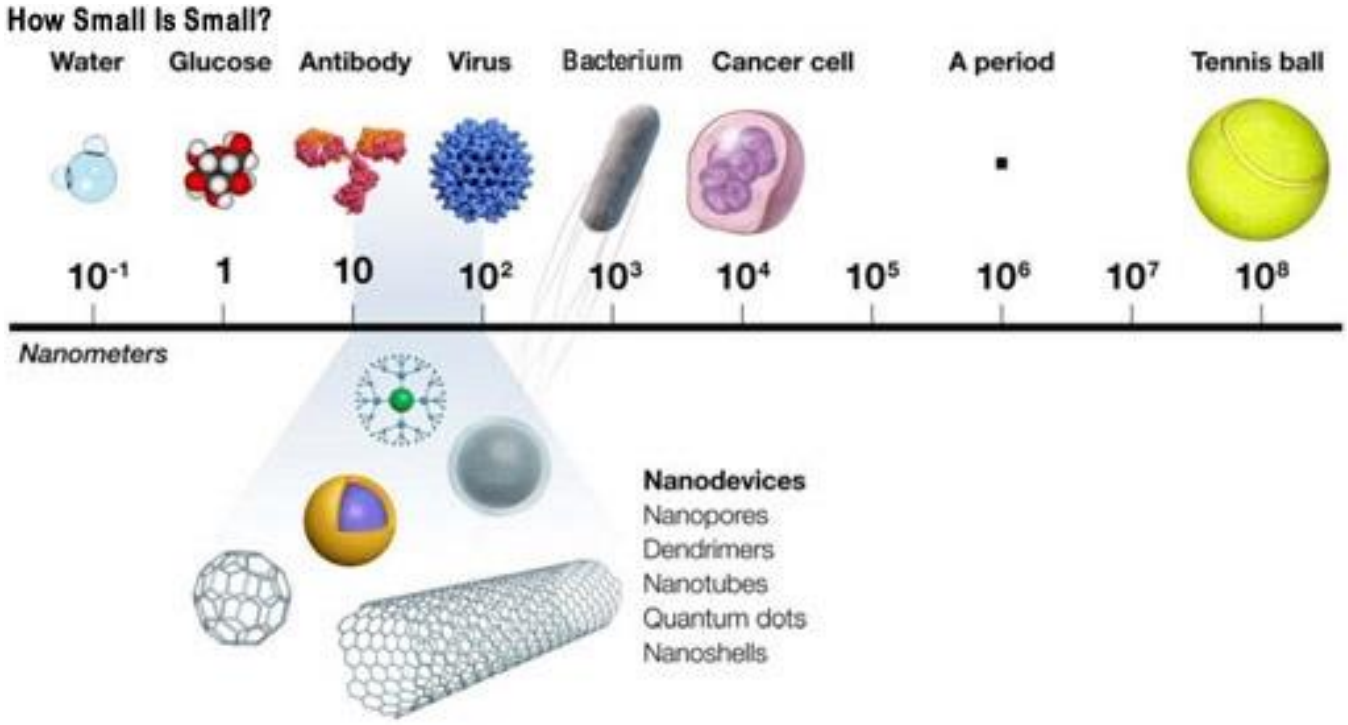
2017 SNO CONFERENCE

Nanoscale: Size Matters

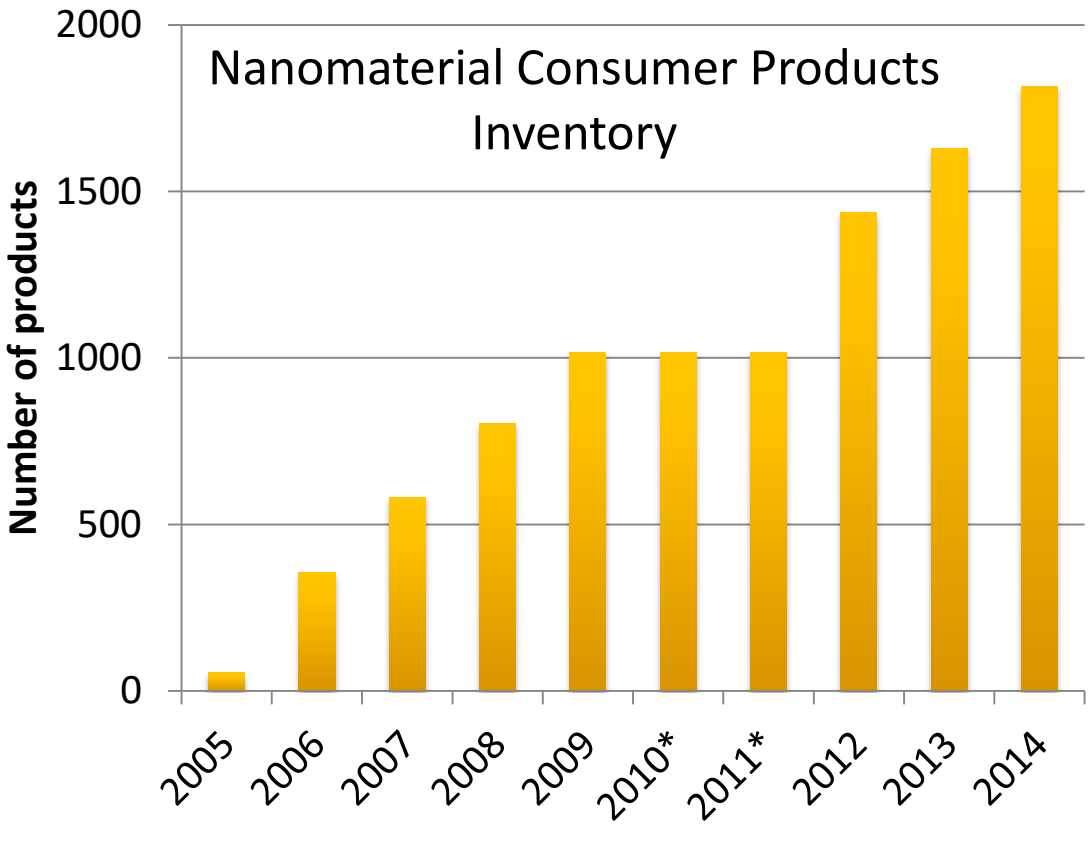
- Nanoscale = 1-100 nm in at least 1 dimension
- Increased surface area = increase reactivity
- Unique nanoscale properties



The ratio of a nanomaterial to a tennis ball is the same as the ratio of tennis ball to the moon

Klaine et al. 2011

>1800 Products Use Nanomaterials



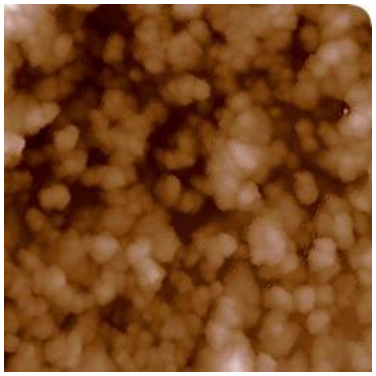
Source: Woodrow Wilson International Center for Scholars' Project on Emerging Nanotechnologies

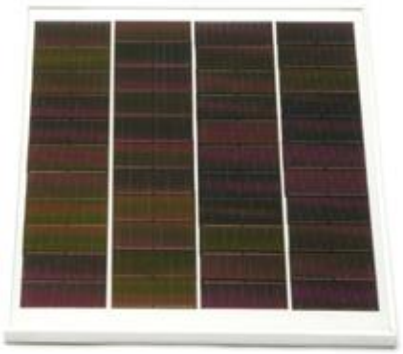
(Vance *et al., Beilstein J. Nanotechnol.* 2015, 6, 1769–1780.)

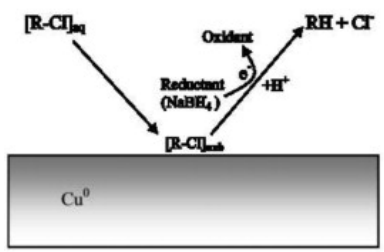
*No data collected.

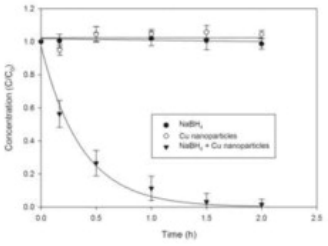
Copper-containing Nanoparticles

- Zerovalent copper (Cu-NPs) & copper (II) oxide (CuO-NPs)
- Applications: filtration devices, cosmetics, electrodes, alloys, steel manufacturing, coatings and sealants, solar panels, and remediation of water contaminants, e.g. dichloromethane
- Cheaper alternative to Ag (most used NP)

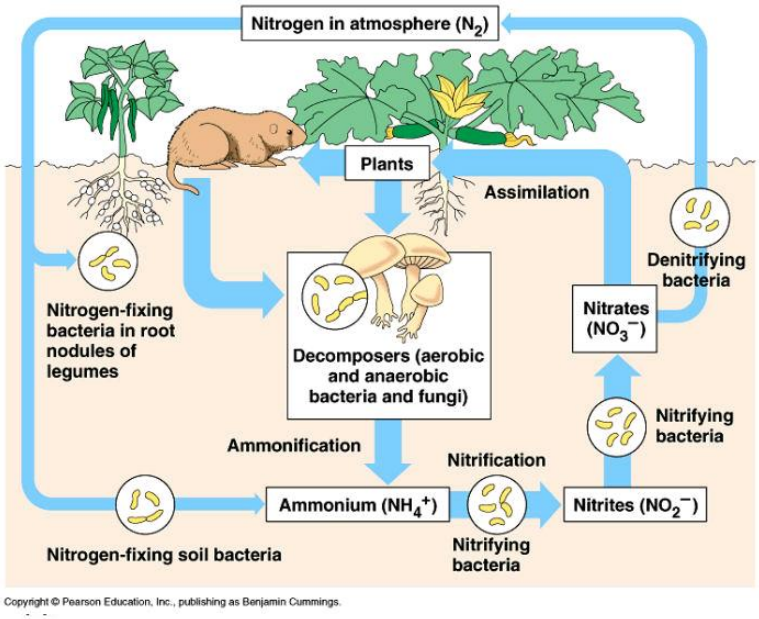








Microbial Interactions with NPs



(b)

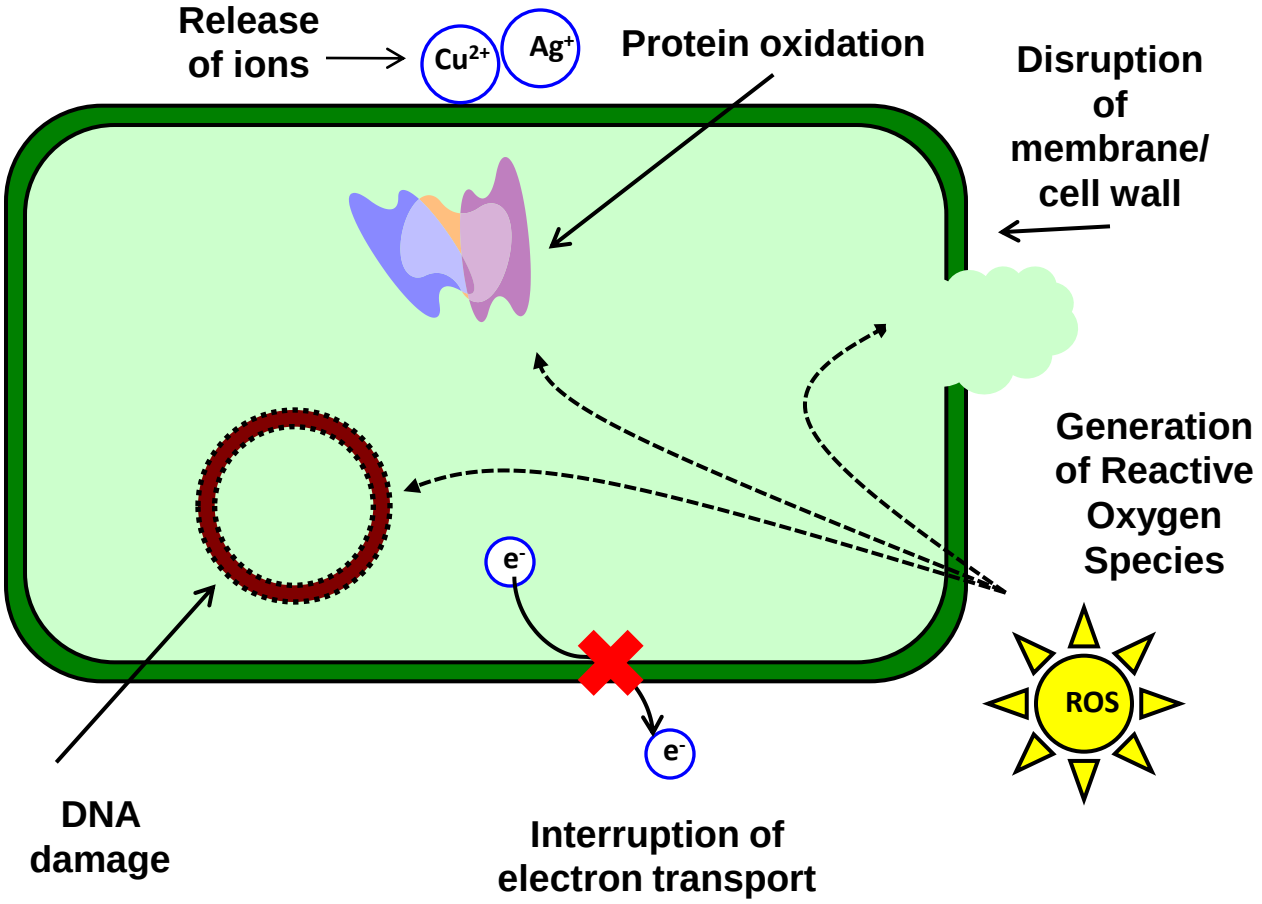
Environmental Impacts

- Disposal/accidental discharge can affect microbial ecology and disrupt biogeochemical cycles.
- Antimicrobial activity indicative of toxicity to higher level organisms.

Microbial Control

- NPs control fouling and pathogens in various products
- Novel antimicrobials
- Nano-bio remediation

Toxicity Mechanisms



(Mahendra *et al*., Nanotech. Apps. For Clean Water, 2008)

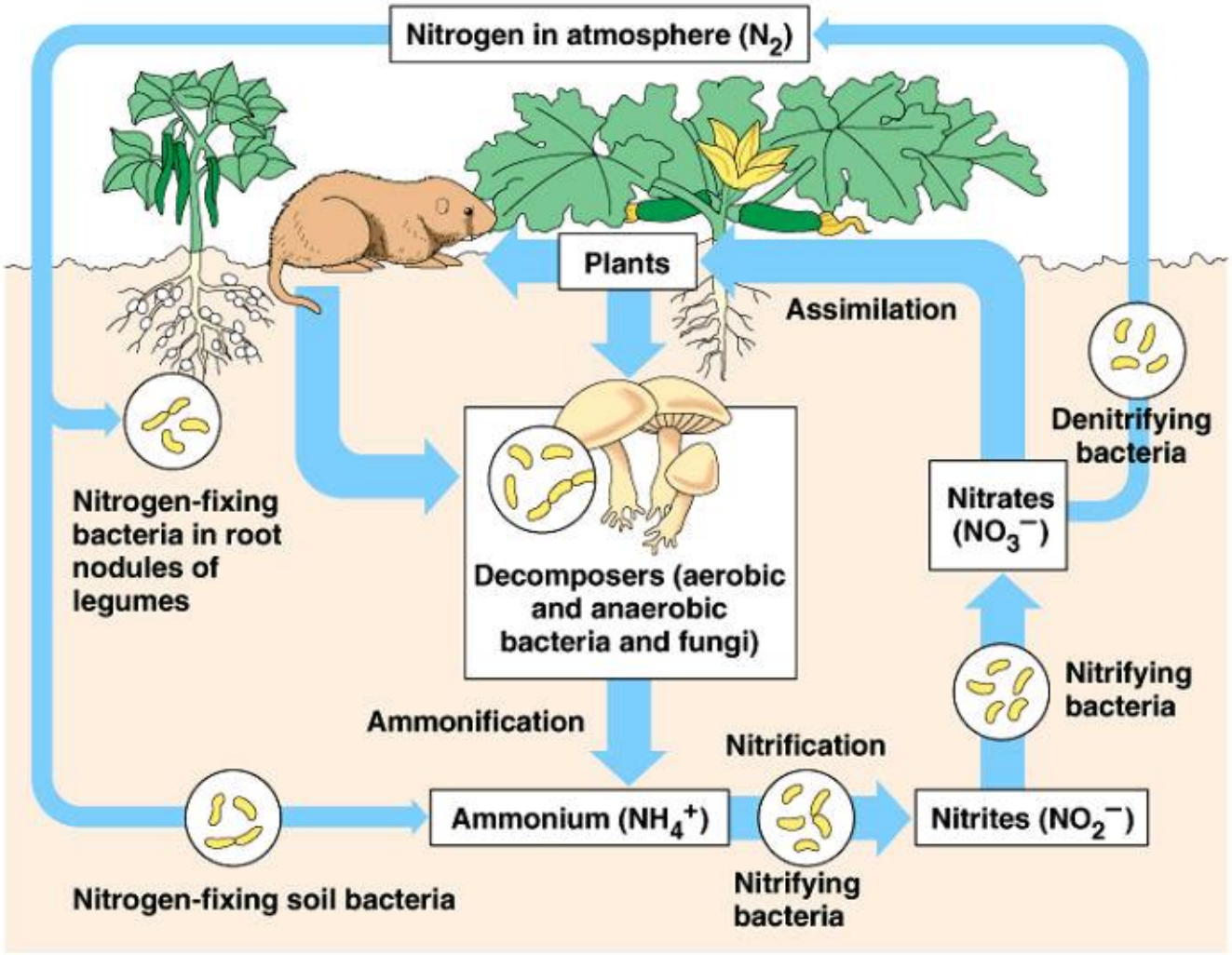
Risk = Hazard X Exposure

Hazard, but no exposure

Exposure but no hazard

Hazard as well as exposure

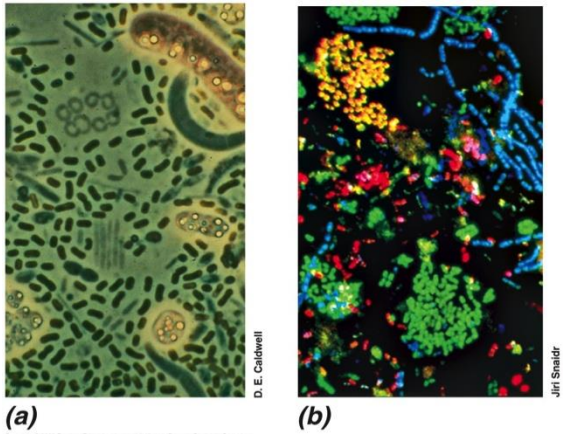
Potential NP Impacts to the Nitrogen Cycle



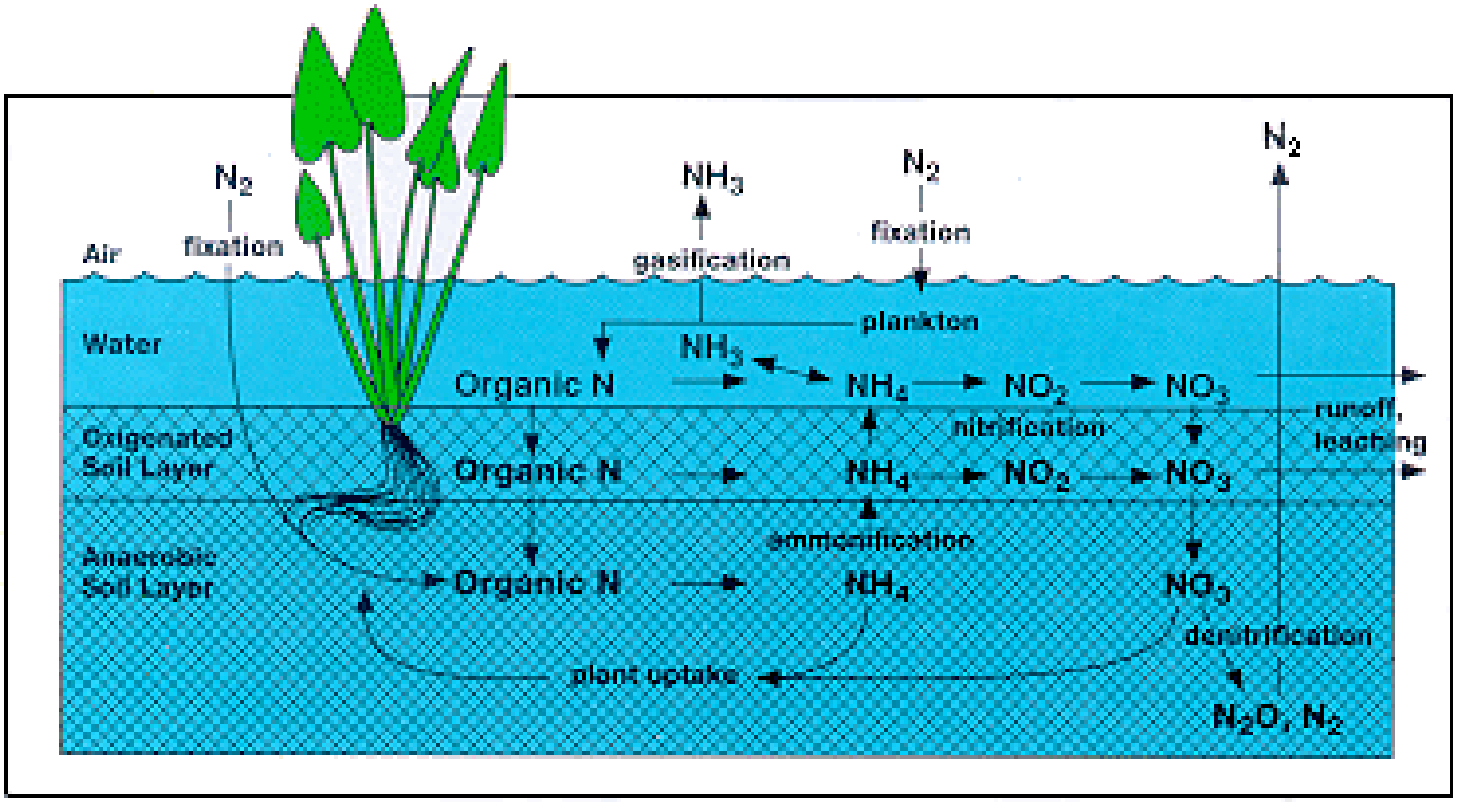
Potential NP Impacts to the Nitrogen Cycle

Overall Hypothesis

Microbial growth characteristics will affect their interactions with metal containing NPs. These sensitivities will affect population & diversity with time.



Wetlands Contain Diverse Nitrogen Cycling Microorganisms



Nitrogen cycling in wetlands progresses more rapidly where there is a thin oxygenated soil layer present.

After Mitsch & Gosselink 1993

Samples Collected from Malibu Lagoon

Impacts of Cu-NPs on Wetland Derived Microcosms

Metal sources: Cu-NP and $CuCl_2$

Model Systems: Wetland Derived Microcosms

Objective: Determine which environmental nitrogen cycling microorganisms are most sensitive to Cu-NPs in mixed communities

Experimental Design and Data Analysis

Sample Collection
Malibu Lagoon, CA, USA

Homogenize

Microcosms

Control (0 Copper) | 100 mg/L Cu-NP | 100 mg/L $CuCl_2$

Every 10 days:
- Flush with N_2
- Sucrose (100 mg/L)
- Nitrate (350 mg/L)

Days
0, 10, 100

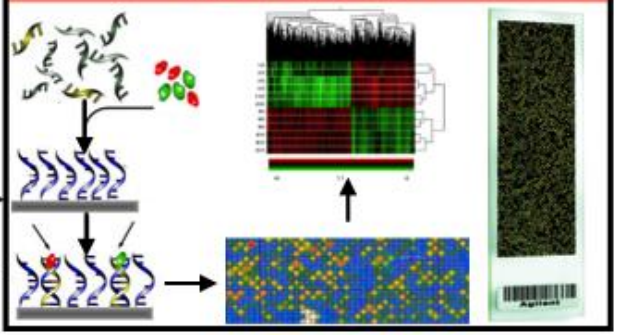
DNA Extraction

RNA Extraction

Water Quality Characteristics

- Nitrate
- Nitrite
- Ammonia
- pH
- Conductivity
- COD
- Total Dissolved Cu
- Total Dissolved Fe

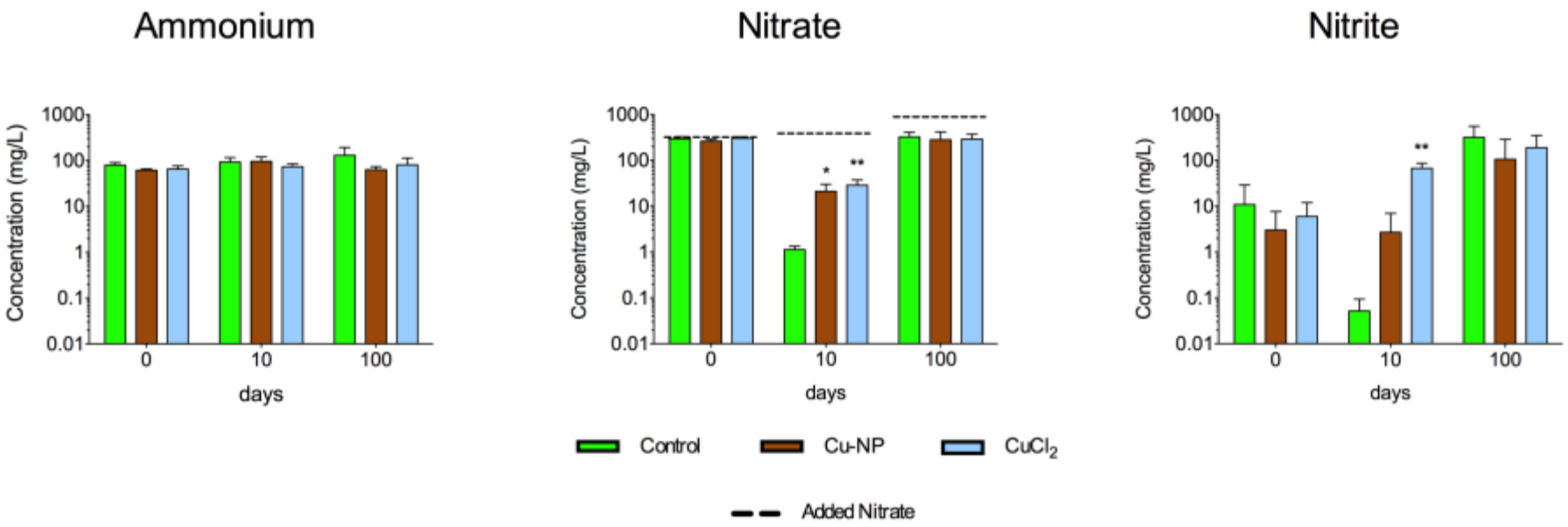
DNA Microarray and Analysis (GeoChip 5.0)



Metatranscriptomic Sequencing and Analysis (MG-RAST)

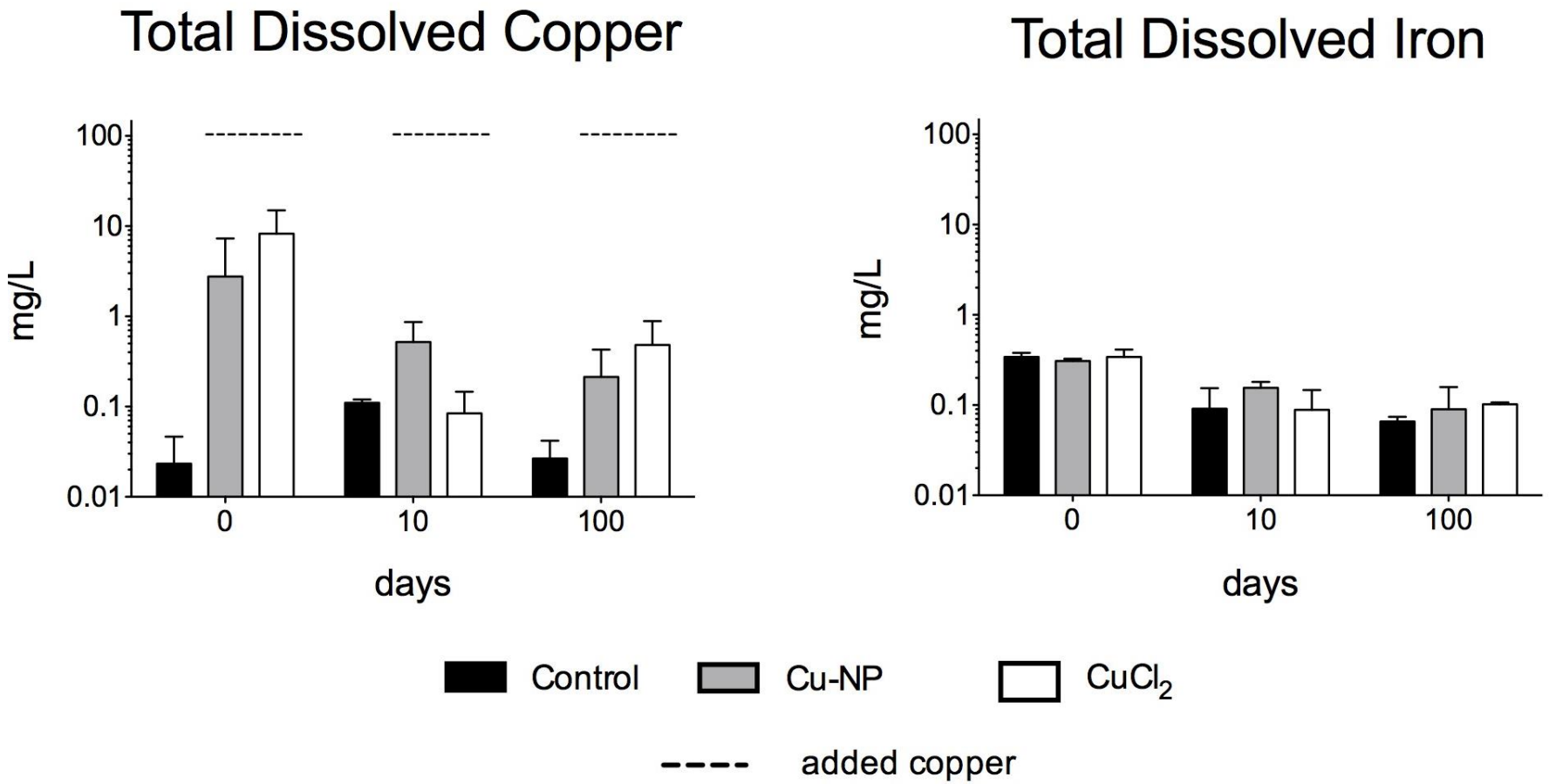
	Control	Cu-NP
Dissimilatory N Reduction	0.37	0.23
Denitrification	0.43	0.34
Ammonification	0.42	0.54
Nitrogen Fixation	0.33	0.32
Assimilation	0.50	0.49

Nitrite and Nitrate Accumulated in Copper Exposed Microcosms



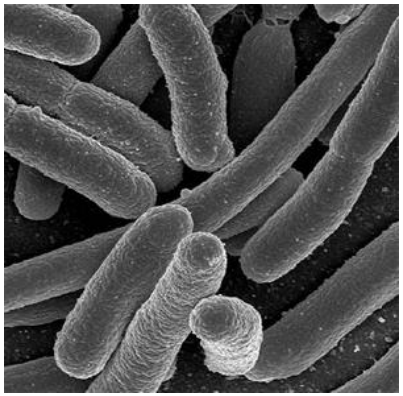
(Reyes *et al*., in review, 2017)

Soluble Species Released from Cu-NPs



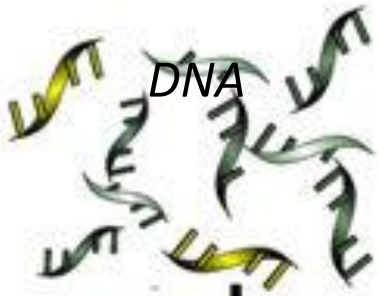
(Reyes *et al*., in review, 2017)

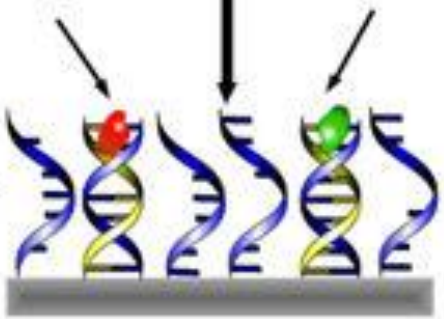
How Microarrays Work



Cell lysis

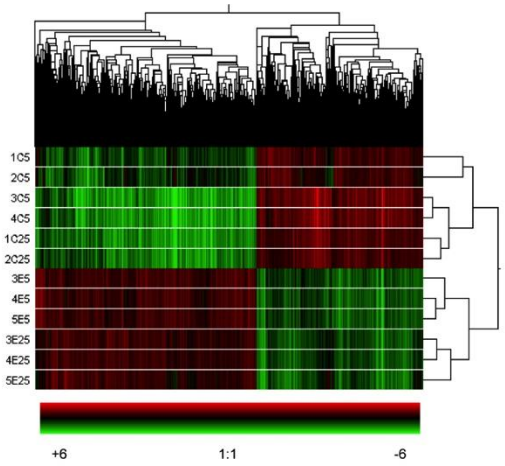
DNA extraction





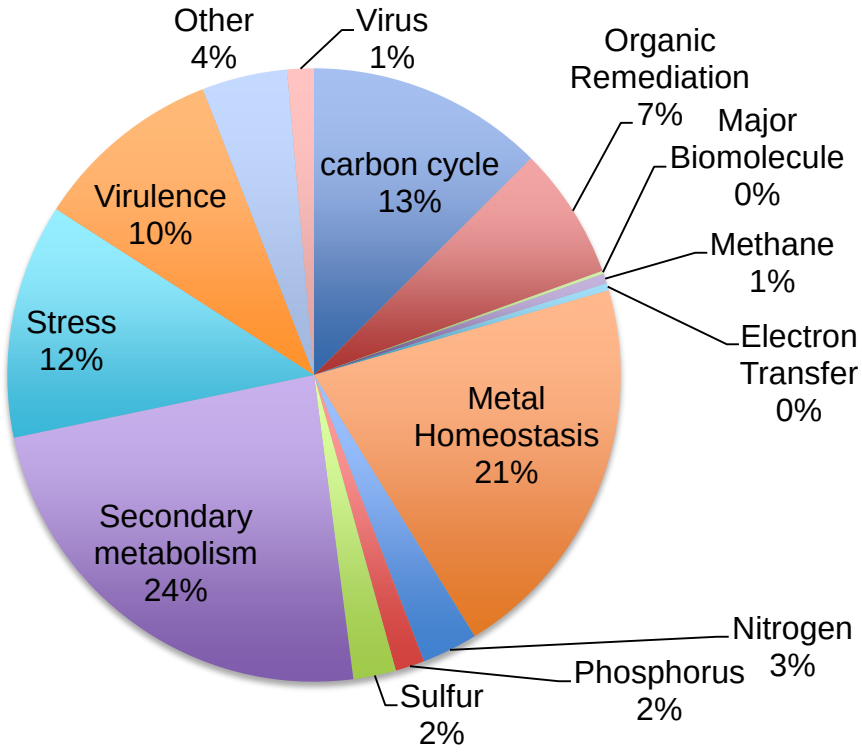
Fluorescence

Data analysis

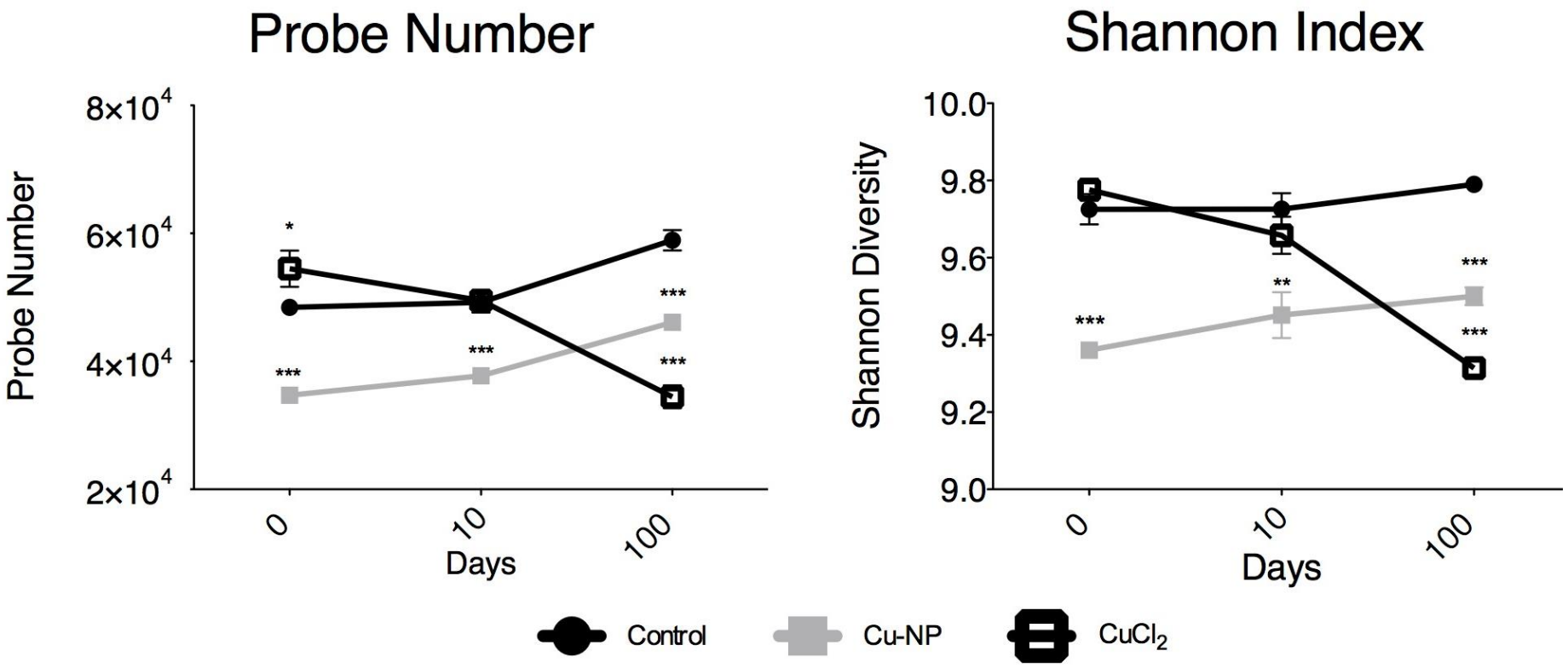


GeoChip 5.0 Probe Distribution

- FGA = Functional Gene Array
- 212,649 oligonucleotide (50 mer) Probes
  - Gene specific and group specific
  - Multiple probes for each sequence
- >10,000 genes
- 150 functional groups
  - Nutrient cycling: Nitrogen, Carbon, Sulfur, and Phosphorus
  - Metal reduction and resistance
  - Organic contaminant degradation

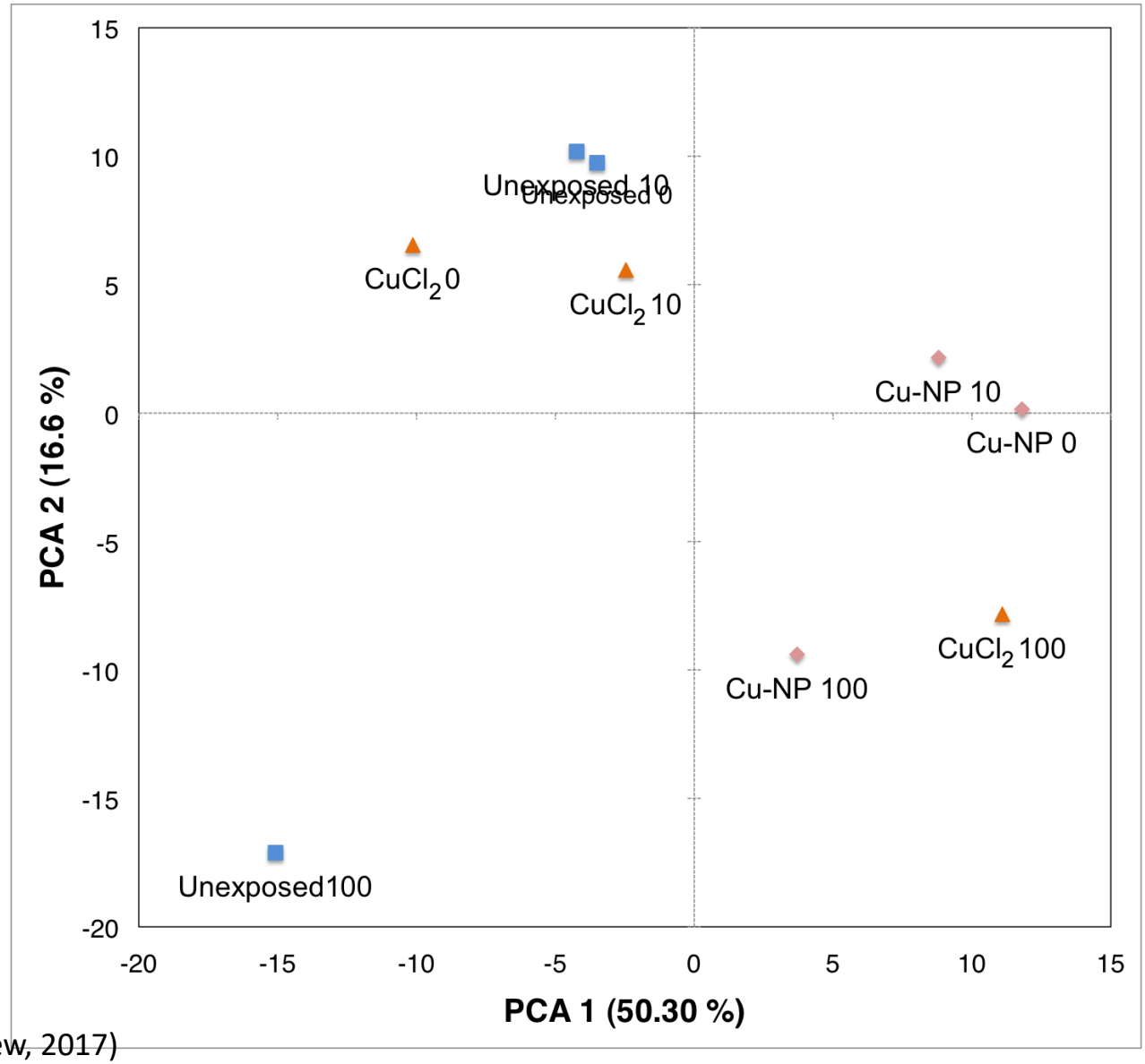


Reduced Probe Number & Diversity for Copper Exposed Microcosms



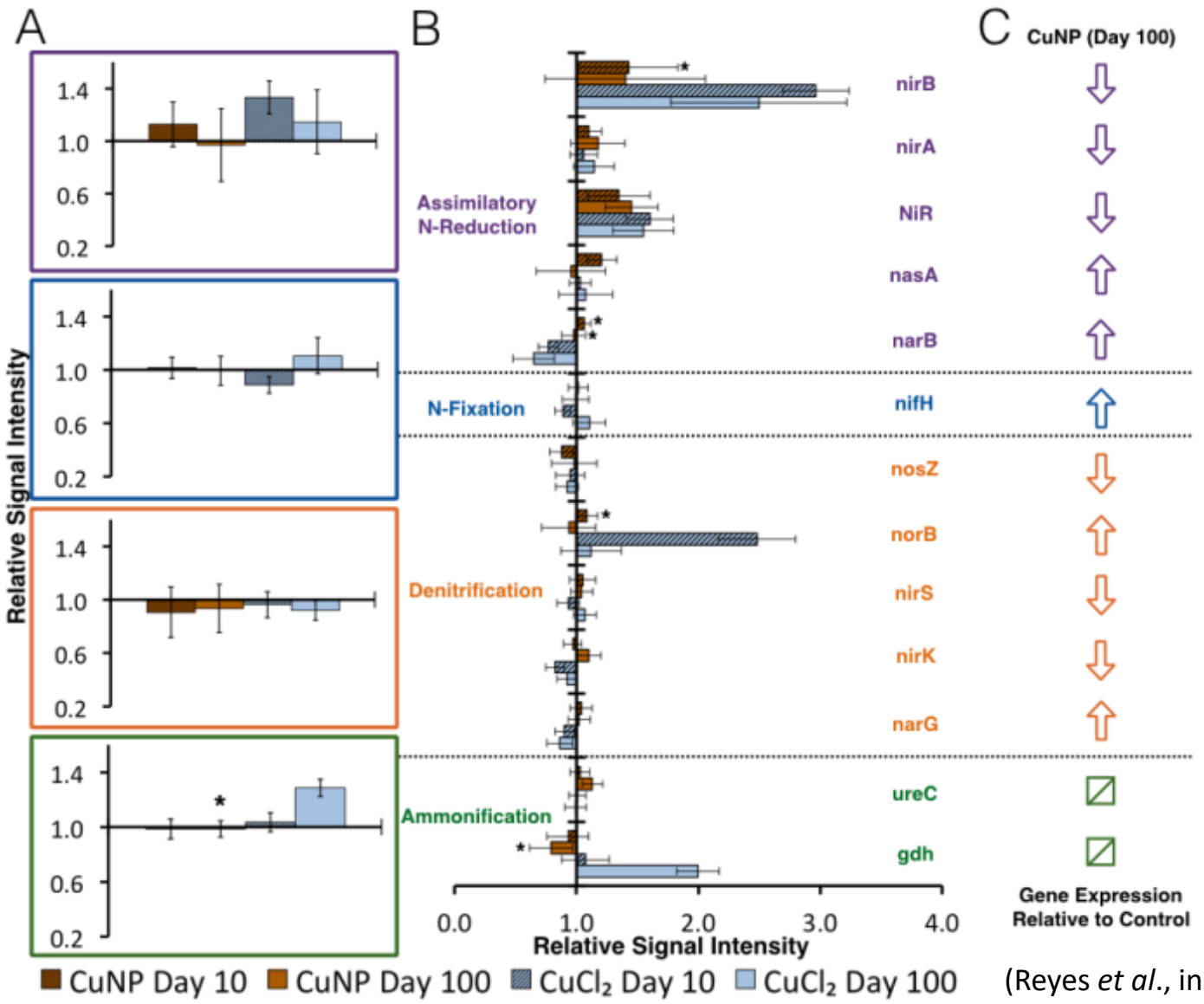
(Reyes *et al*., in review, 2017)

PCA Showed Divergence in 100 Day Samples



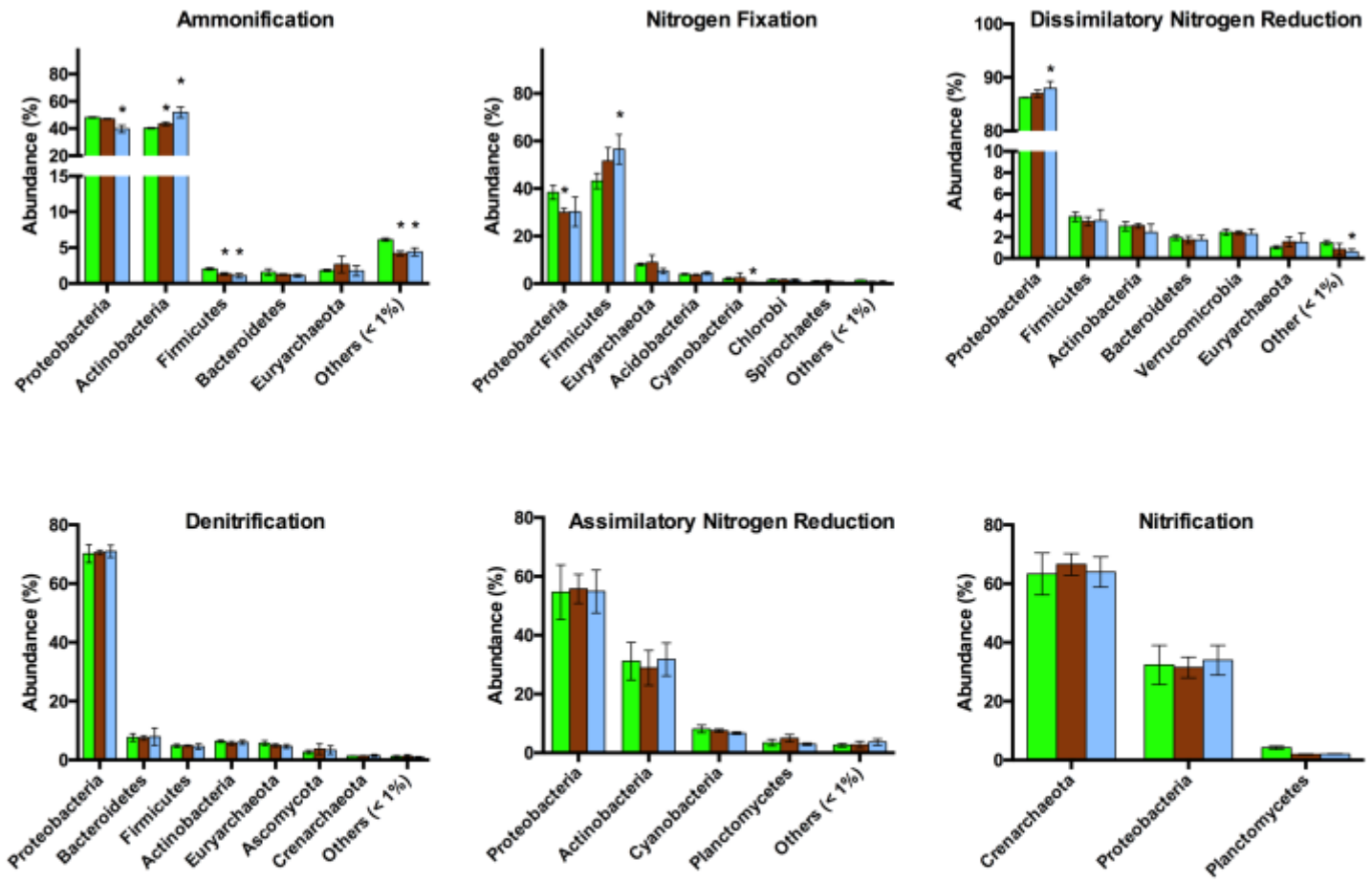
(Reyes *et al.*, in review, 2017)

Reduced Signal from Nitrogen Cycling and Electron Transfer genes



(Reyes *et al.*, in review, 2017)

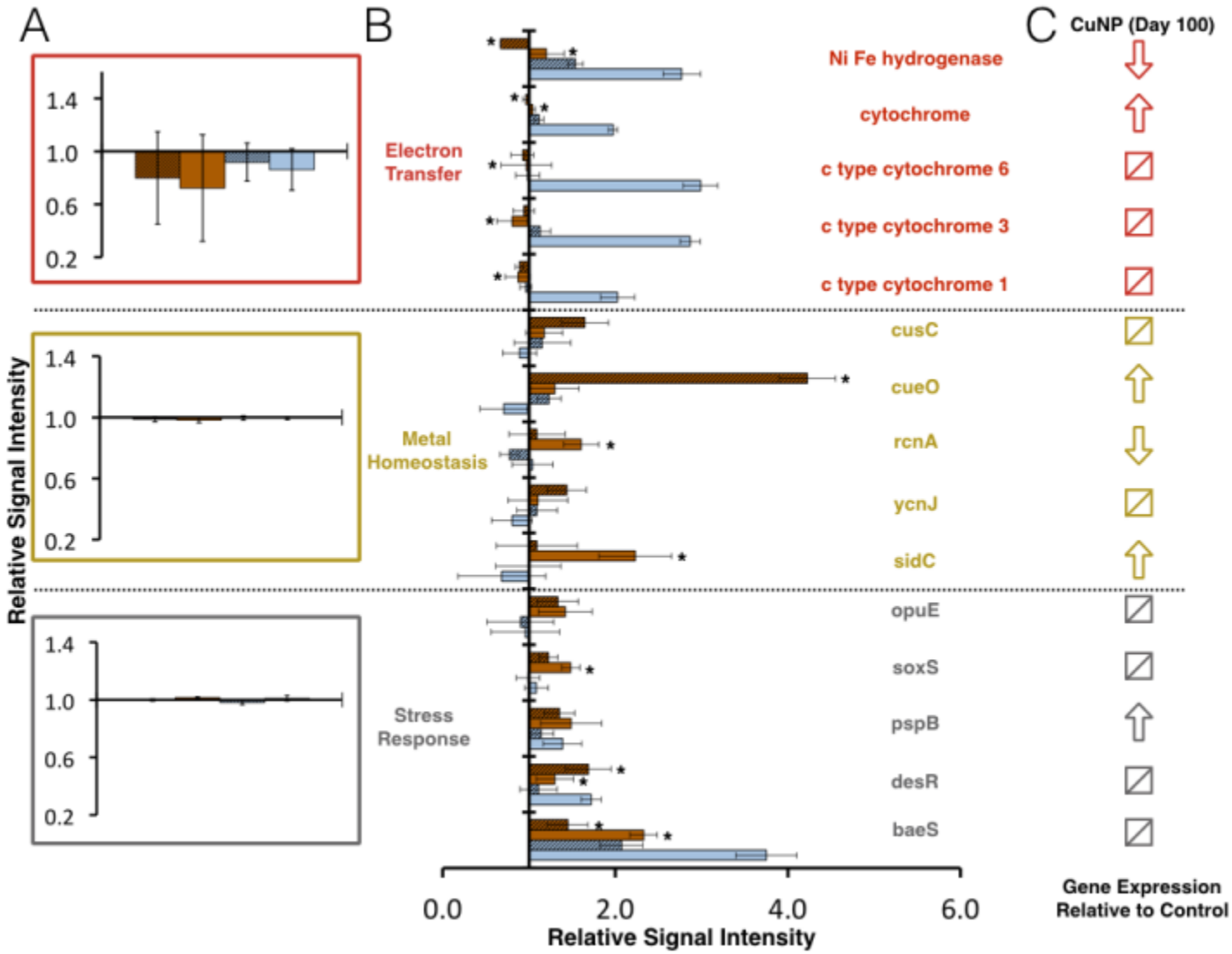
DNRA < Nitrogen Fixation < Ammonification



*Indicates significantly different from control ($p < 0.05$)

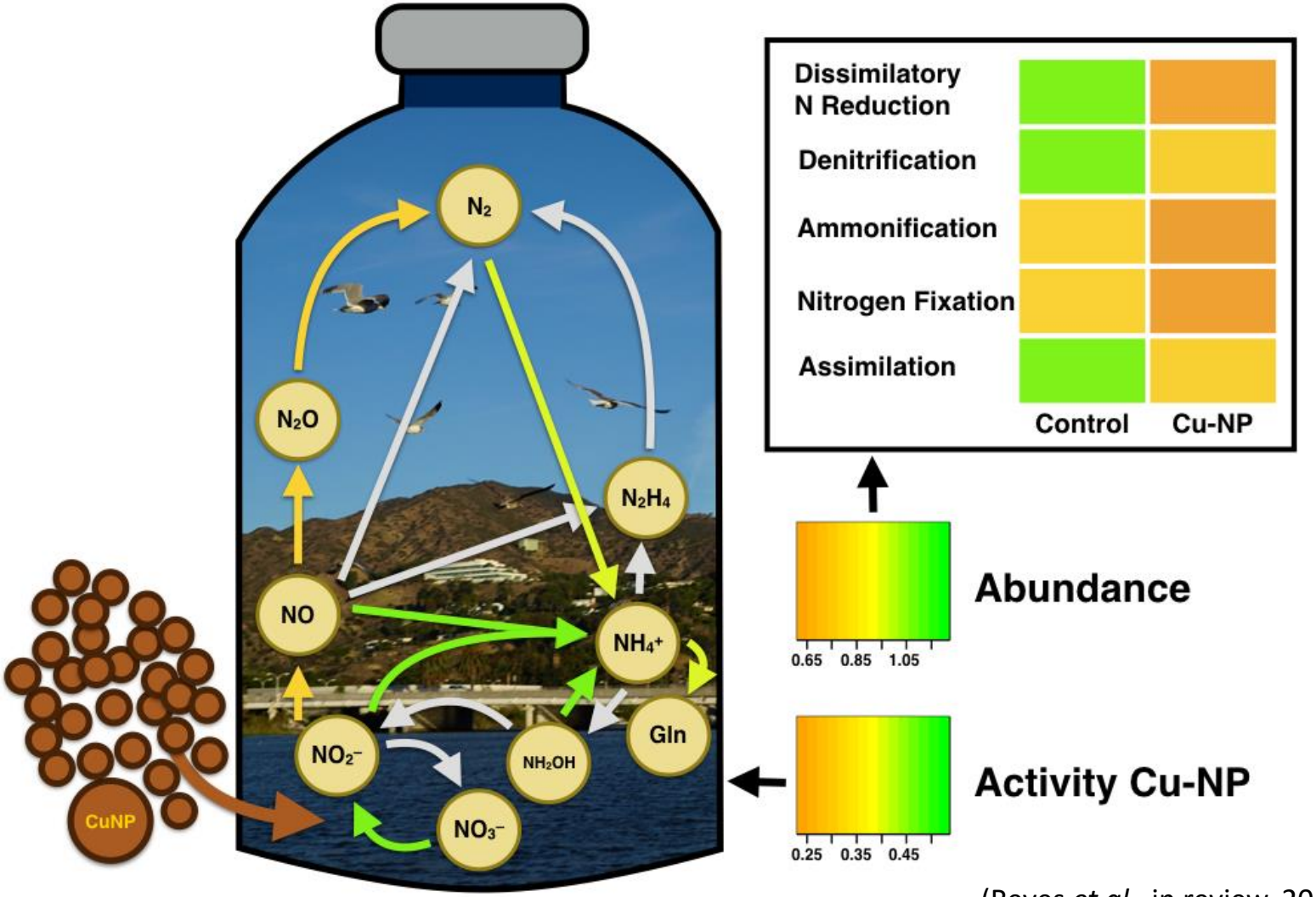
(Reyes *et al.*, in review, 2017)

Impacts to Electron Function, Metal Homeostasis, and Stress Response



(Reyes *et al.*, in review, 2017)

The Microbial Community was Resilient after Long-term Exposure to Cu-NPs



(Reyes *et al*., in review, 2017)

Summary and Significance

- Acute exposure to Cu-NPs negatively impact wetland microbial communities and N-cycling processes.
  - Accumulation of NO_3^- + NO_2^-
- Microbial communities demonstrated resilience over 100 days.
- Cu-NPs may shape long-term nitrogen transformation by selecting for resilient and metal-tolerant N-cycling microorganisms.
  - Increases in denitrification promote the release of N_2O (~ 300 fold greater global warming potential than CO_2).
  - Decreases in N-fixation combined with increases in denitrification may limit wetland productivity.

Acknowledgements

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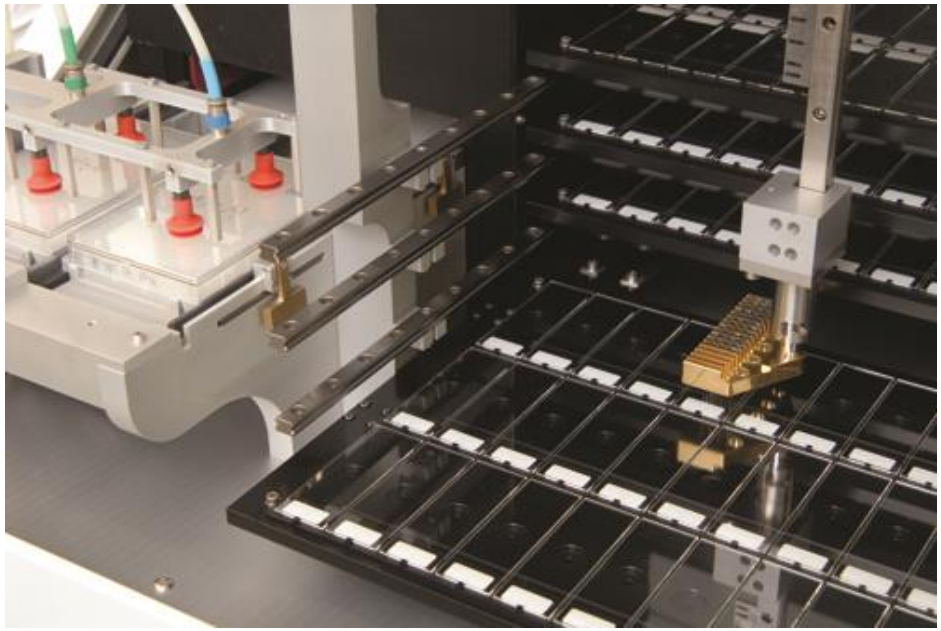
Geochip Data Processing

- Signals analyzed on Online Geochip Data Analysis Pipeline
- Poor quality spots were flagged by Imagene or signal:noise < 2
- Normalized signal intensity normalized by the total intensity of microarray followed by dividing by a constant
- Probe must be detected in at least 2 replicates



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