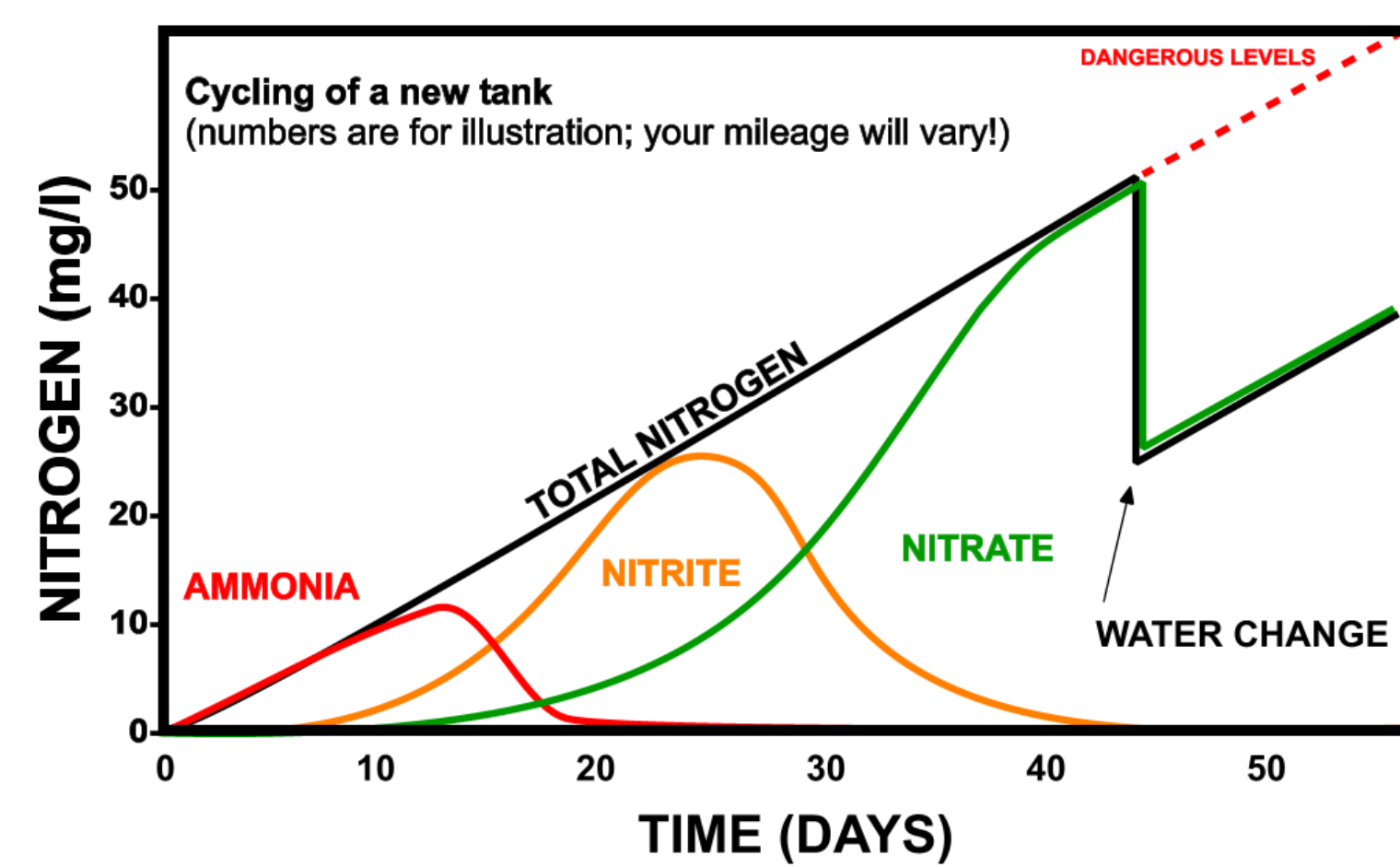
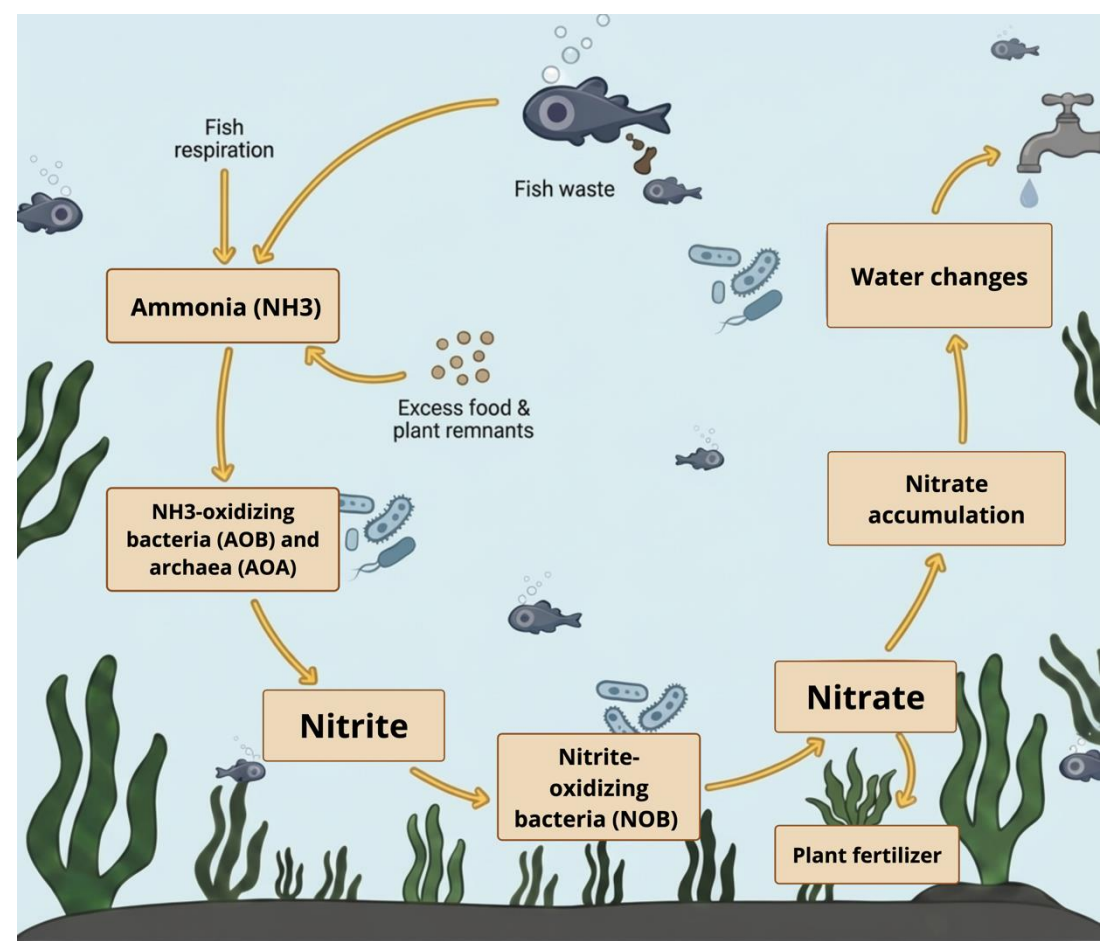


INTRODUCTION

- **Copper sulfate (CuSO₄)**
- Quarantine treatment for saltwater fishes
- Antibacterial, antifungal, and antiparasitic
- Ectoparasitic infections
- *Cryptocaryon sp.* (marine white spot disease)
- Therapeutic levels of Cu²⁺: 0.15 – 0.22 mg/L
- Maintained **21 days**
- Risks, adverse effects
- Elasmobranchs, invertebrates, teleost fishes
- Microorganisms responsible for **nitrification and biofiltration** of aquaria
- Nitrogen cycling in aquarium systems:



RESULTS

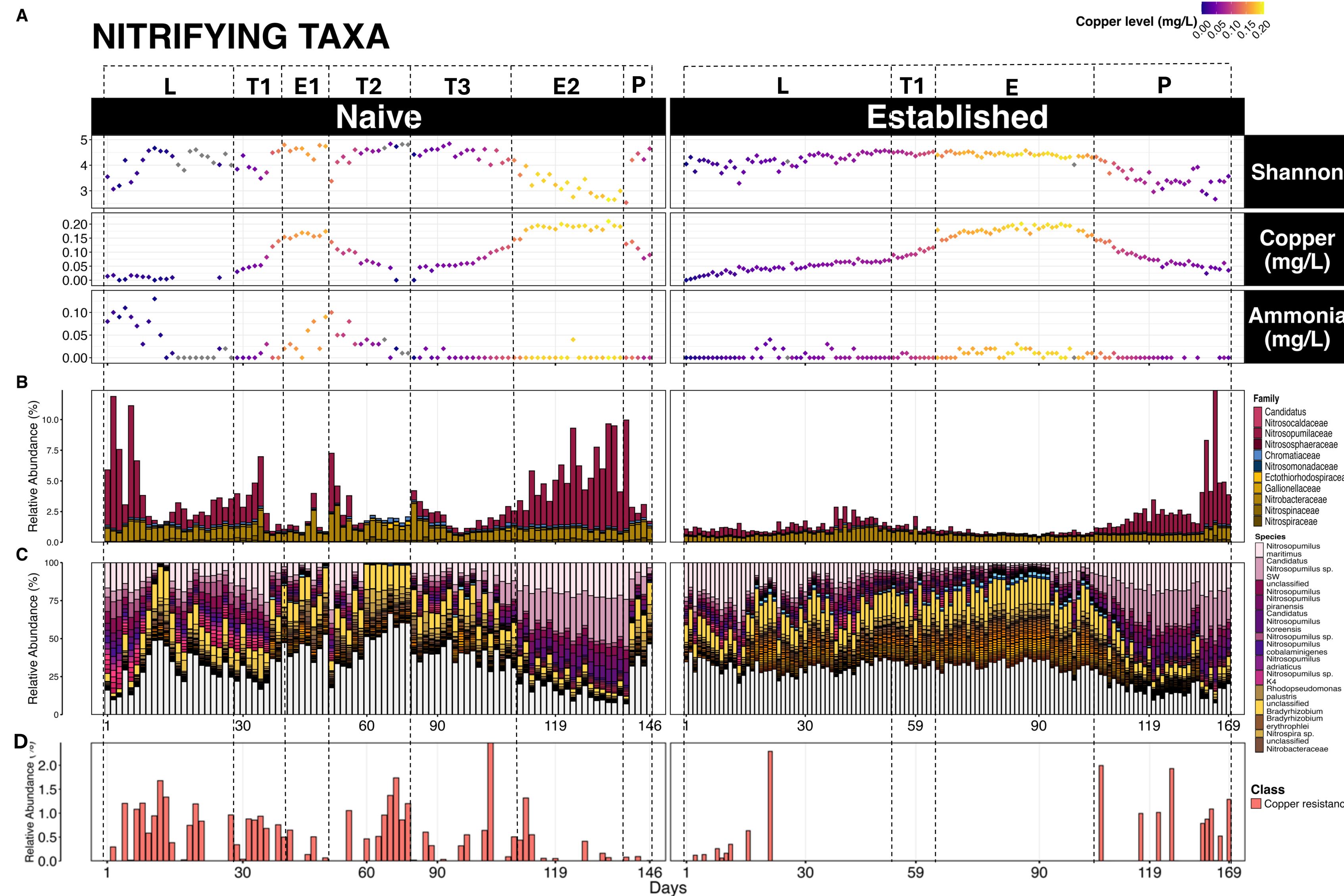


Figure 1. (A) Shannon's diversity index of nitrifying communities. Cu²⁺ and ammonia levels in water over the study period. (B) Relative abundance of nitrifying taxa within the overall microbial community. Ammonia-oxidizing archaea (AOA) are shown in shades of pink, ammonia-oxidizing bacteria (AOB) in shades of blue, and nitrite-oxidizing bacteria (NOB) in shades of yellow. (C) Species-level compositions within the nitrifying communities. (D) Relative abundance of copper resistance gene groups.

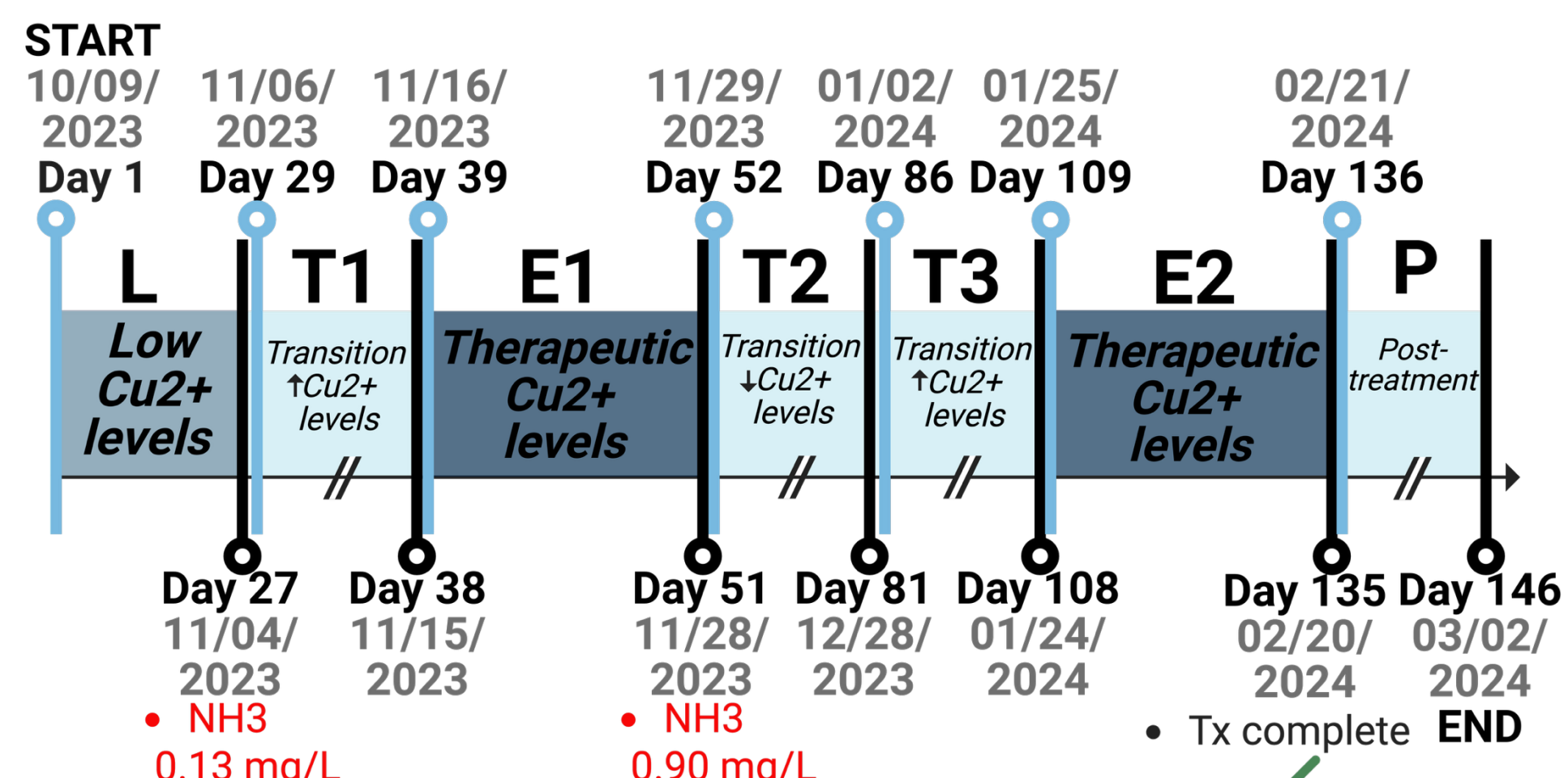
OBJECTIVE

Characterize microbial communities, with a focus on nitrifiers, in naïve and established saltwater aquarium systems during copper sulfate (CuSO₄) treatment

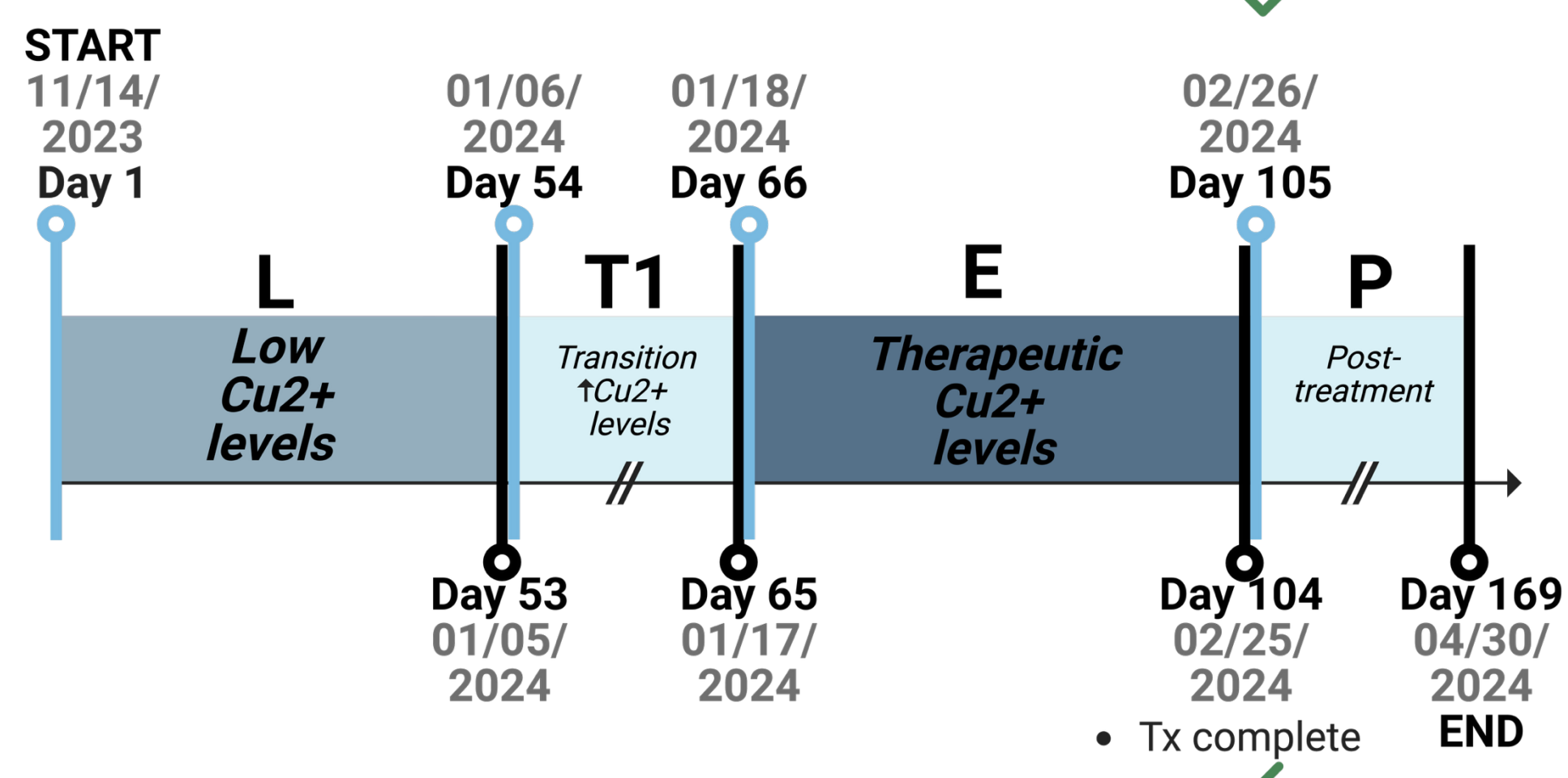
METHODS

Sample Collection

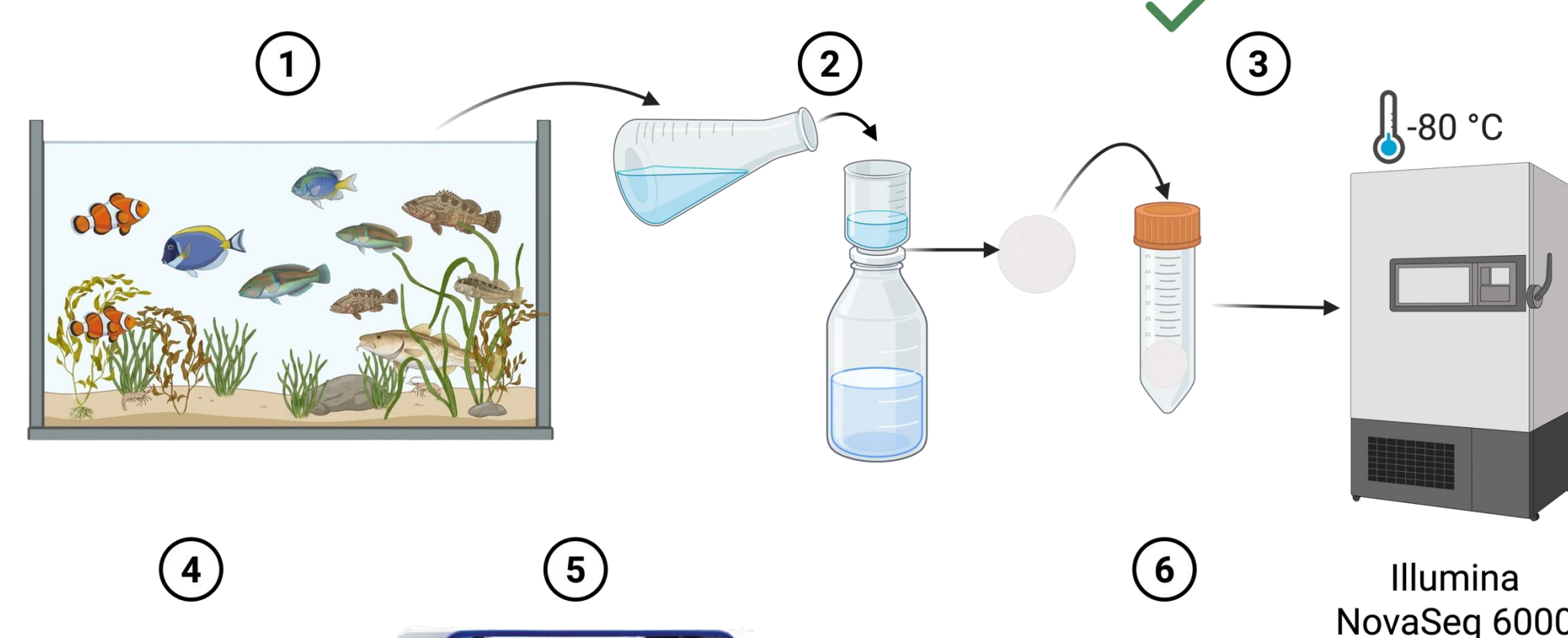
- Sampled newly commissioned (naïve) and established systems (>10 y/o)
- During and after CuSO₄ treatment
- **Naïve system**



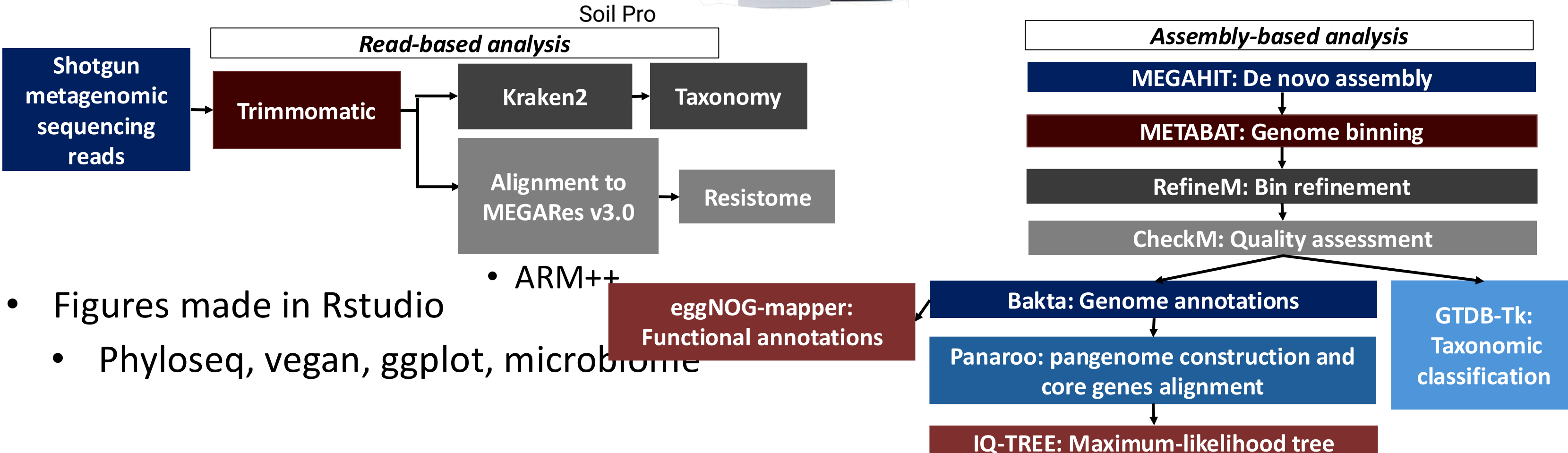
- **Established system**



Sample Processing



Bioinformatics



- Figures made in Rstudio
- Phyloseq, vegan, ggplot, microbiome

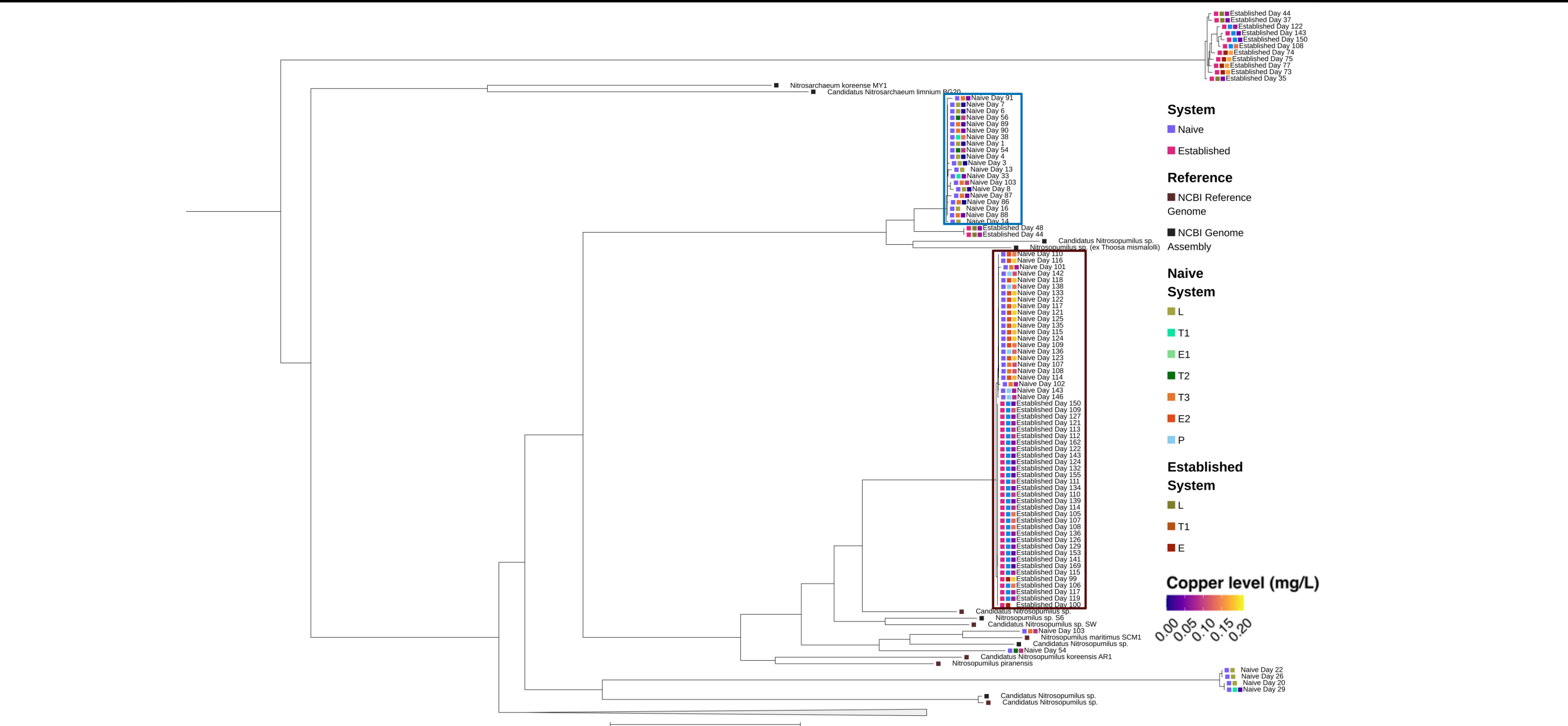


Figure 2. Phylogenetic analysis of AOA MAGs. High quality MAGs (>90% completeness, <5% contamination) recovered before the second therapeutic Cu²⁺ exposure (early-phase; blue box) in the naïve system formed a distinct monophyletic clade, separate from those recovered during or after the second exposure (late-phase). MAGs recovered after Cu²⁺ exposure in the established system clustered closely with late-phase MAGs from the naïve system (maroon box).

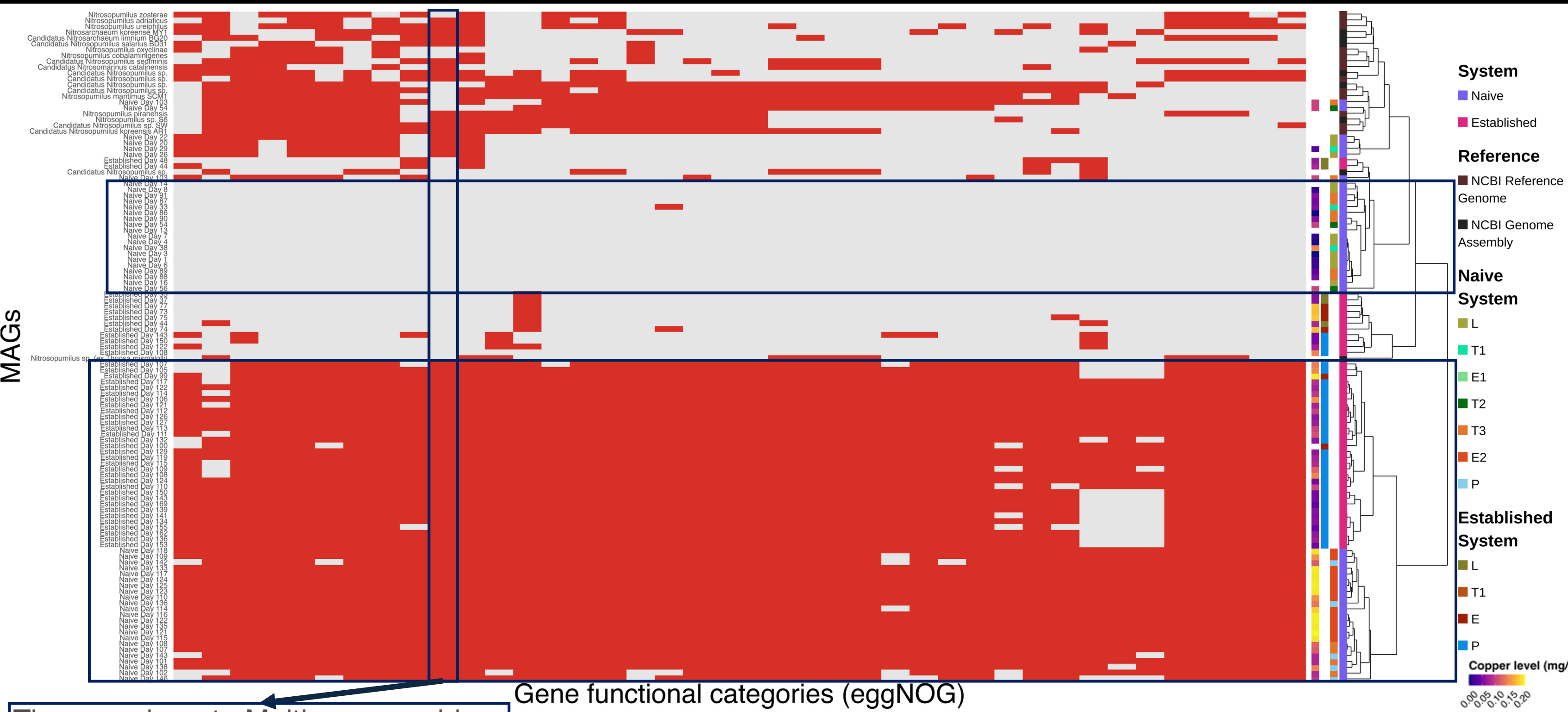


Figure 3. Heatmap of gene annotations for high-quality AOA MAGs, grouped into functional categories. Red indicates presence and gray indicates absence. The heatmap is zoomed to emphasize a region of notable clustering. Interestingly, all early-phase AOA MAGs from the naïve system lack genes within the multicopper oxidase functional group, whereas they are consistently present in all late-phase MAGs from the naïve system, as well as post-exposure MAGs from the established system.

TAKE-AWAYS

- Effects on the nitrifying communities differed between 1st and 2nd exposures to therapeutic Cu²⁺ concentrations in the naïve system.
- Effect in the established system was seen during and after exposure.
- *Nitrosopumilaceae* (AOA) were the dominant ammonia-oxidizers in both systems.
- Although the naïve system had an overall higher prevalence of copper resistance genes, Cu²⁺ concentration was not correlated.
- AOA MAGs were recovered from both systems. Clustering:
 - Naïve system: driven by phase-related Cu²⁺ exposure (E1 vs E2)
 - Established system: post-exposure (P)

ACKNOWLEDGEMENTS

The authors wish to the Shedd Aquarists and Water Quality Team, as well as employees from the VERO Research laboratory. Computing resources for this work were provided by Texas A&M High Performance Research Computing.