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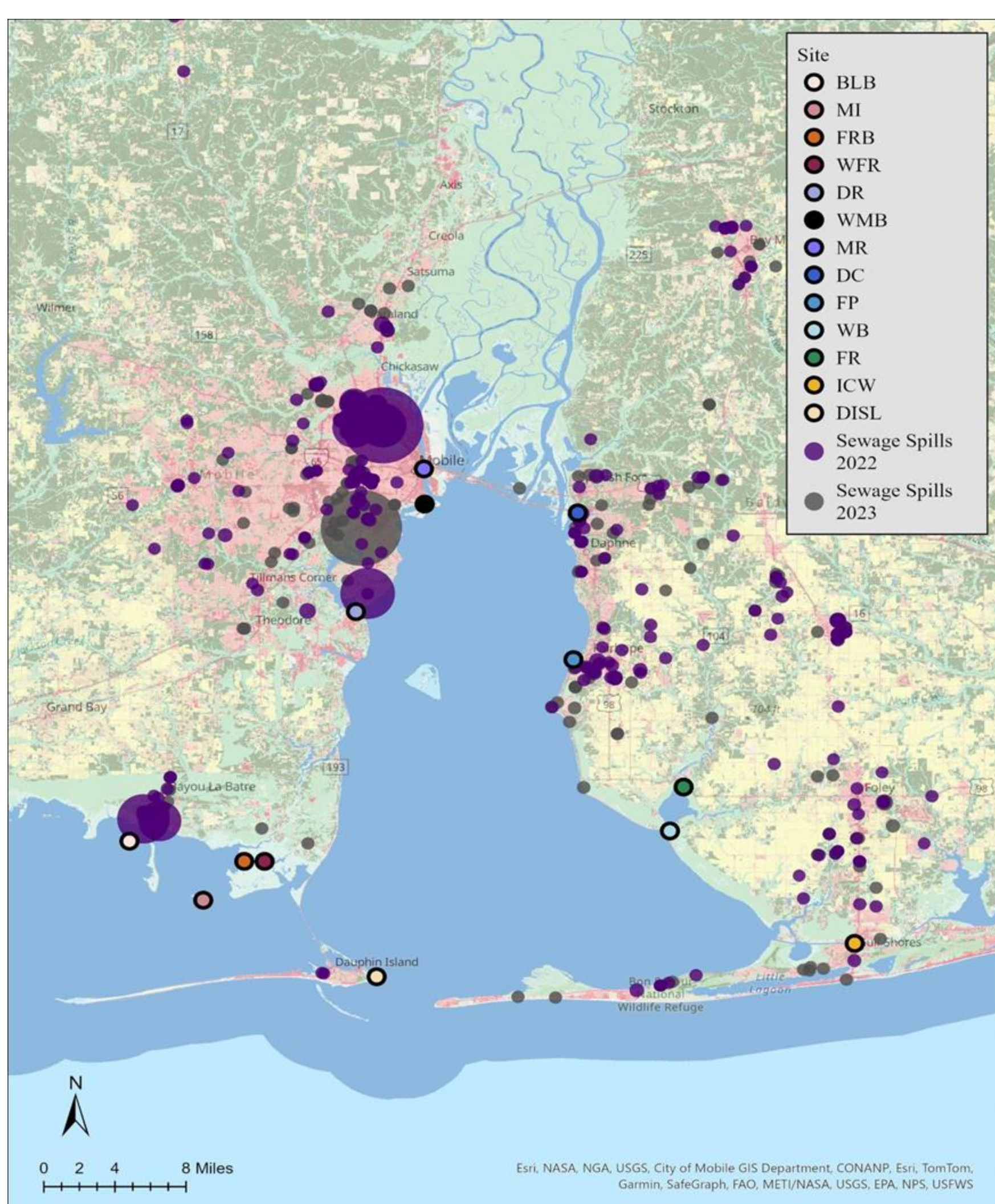
## Introduction

- Urbanization of coastlines can introduce potential human pathogens from various terrestrial sources
- Management of potential pathogens is difficult when fecal sources are unknown
- Molecular microbial source tracking (MST) methods can identify DNA-based communities and quantify fecal-associated bacteria, such as *Bacteroides*
  - *Bacteroides* is a group of non-pathogenic gut bacteria with high host specificity
- Currently, microbial sources to the Mobile Bay watershed are largely undefined

**Objective:** To identify major fecal sources to the Alabama coast using molecular source tracking.

## Methods

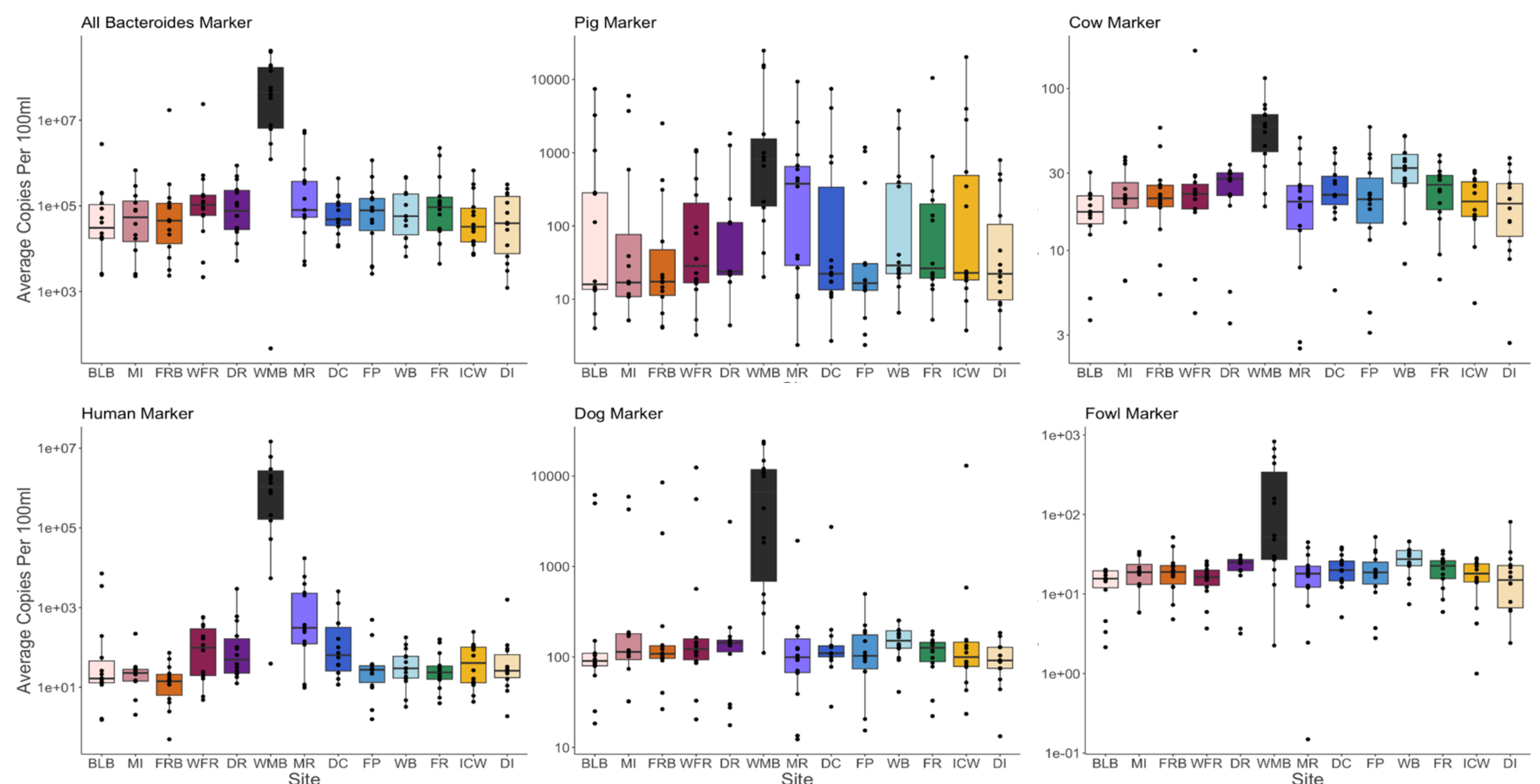
- Water samples were collected monthly for one year from 13 sites that represent potential fecal inputs to Alabama's coast (Fig. 1)
  - WMB samples were collected from the treated effluent pipe of the Mobile area wastewater treatment plant
- Samples were processed via two molecular MST techniques:
  - 6 *Bacteroides* qPCR assays (total, human, dog, pig, cow, fowl)
  - eDNA metagenomics
- Environmental parameters and nutrient measurements (TDN, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>) were also collected



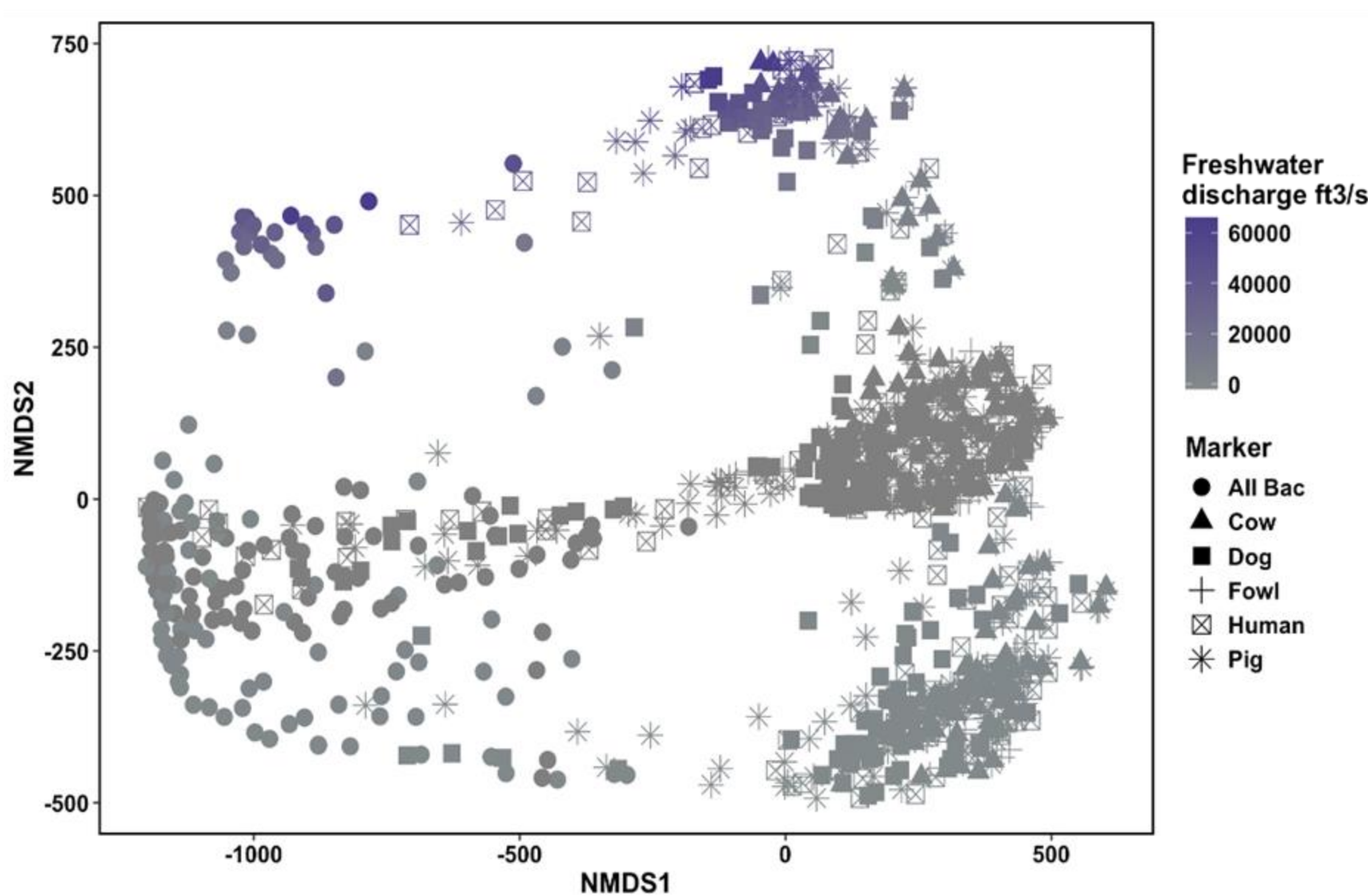
**Figure 1.** Sampling sites and locations of known sewage spills in 2022-2023 (Basemap: USGS National Land Cover Database).

## Bacteroides in Mobile Bay

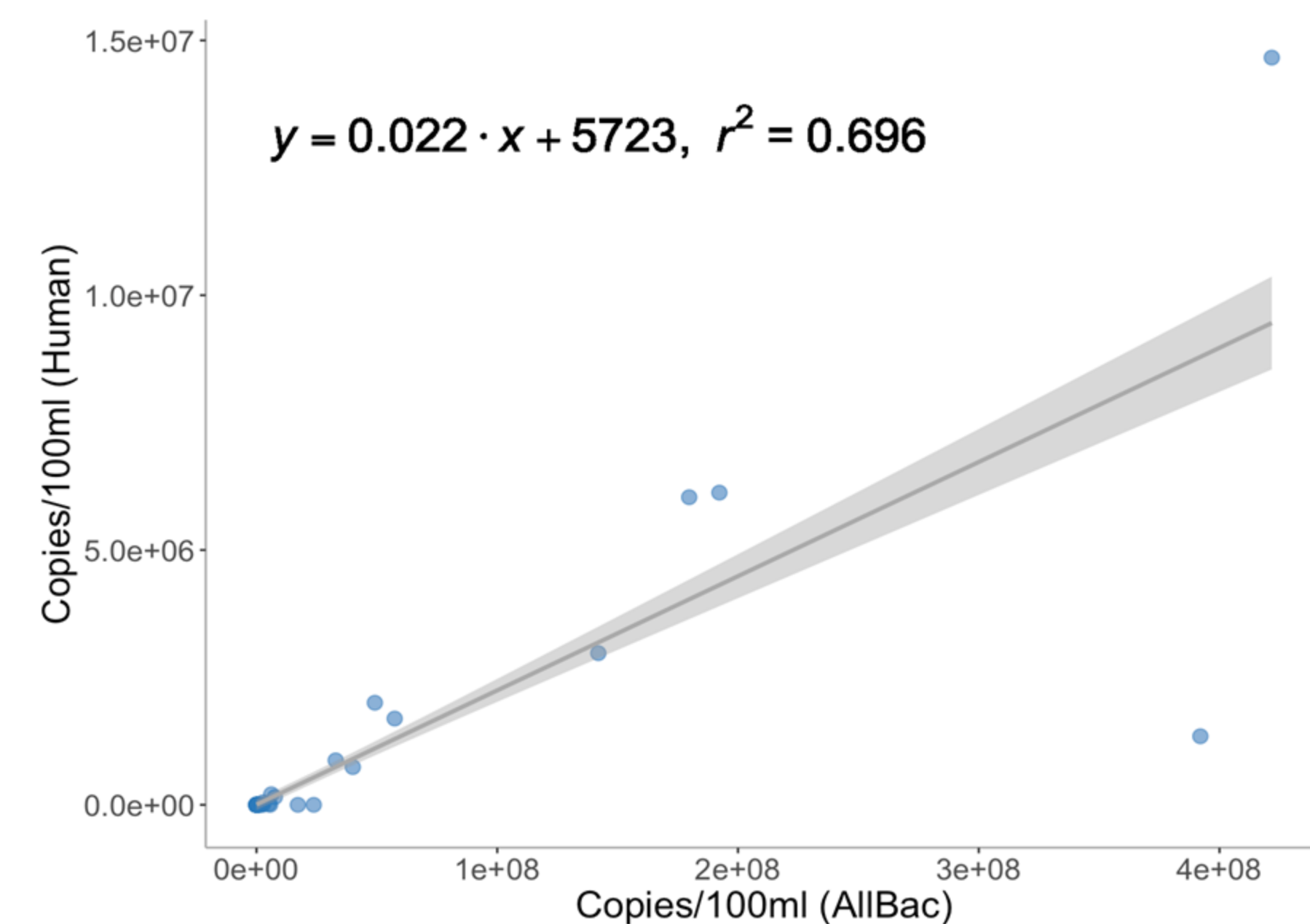
- Bacteria associated with feces were prevalent in Mobile Bay year-round (Fig. 2)
- The *Bacteroides* community was reflective of inter-site differences and freshwater discharge (Fig. 3)
- Human-associated *Bacteroides* were the dominant fecal source (Fig. 4)



**Figure 2.** Inter-site differences in *Bacteroides* concentrations from each biological fecal source during all timepoints. Note that axis scales are not consistent.



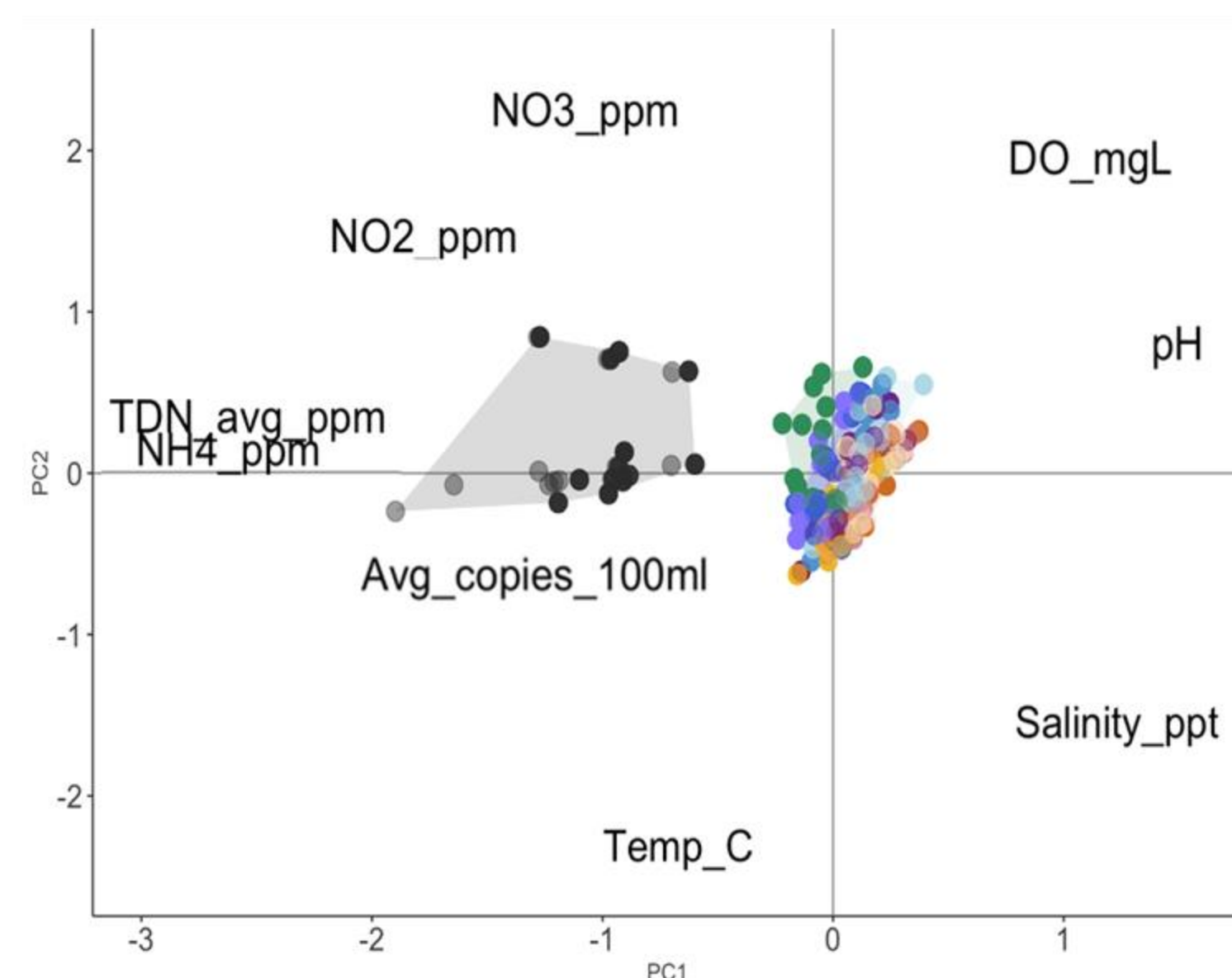
**Figure 3.** NMDS of *Bacteroides* copy numbers and environmental parameters colored by freshwater discharge (ft<sup>3</sup>/s).



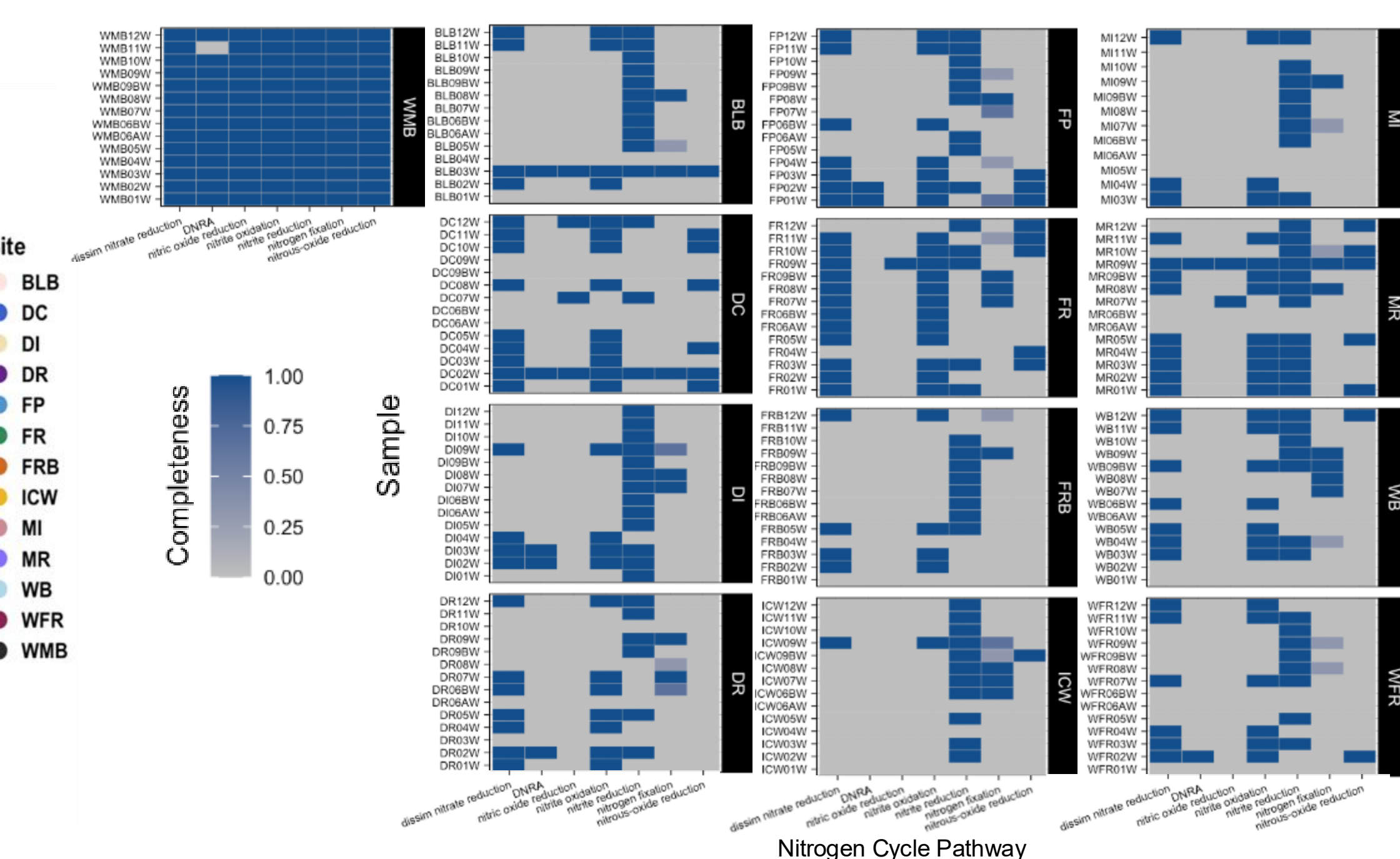
**Figure 4.** Significant positive correlation between human-specific and general *Bacteroides* gene markers ( $r^2=0.696$ ,  $p<0.00001$ ).

## Wastewater Effluent as a Guide

- Multivariate analyses showed a distinct treated wastewater effluent community, driven by nitrogen constituents (Fig. 5) and further supported by complete or near complete N cycle pathways (Fig. 6)
- Microbial taxa and metabolic pathways present in the effluent can serve as an indicator of human sewage signatures at other sites
- The depth of information generated from this integrated MST approach may allow for the implementation of better management practices and protection for coastal communities



**Figure 5.** PCA of water chemistry and nutrient data with *Bacteroides* copy numbers from all sources. Wastewater effluent (black) was driven by nitrogen.



**Figure 6.** Completeness of nitrogen cycle pathways in eDNA sequences. All nitrogen cycling pathways were complete or near complete in treated effluent (WMB) samples.

## Acknowledgments

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