

# Wetland microbial community response to restoration

Susanna Theroux (stheroux@lbl.gov)<sup>1\*</sup>, Wyatt Hartman<sup>1</sup>, Susannah Green Tringe<sup>1</sup>

1. DOE Joint Genome Institute, Walnut Creek, CA



## INTRODUCTION

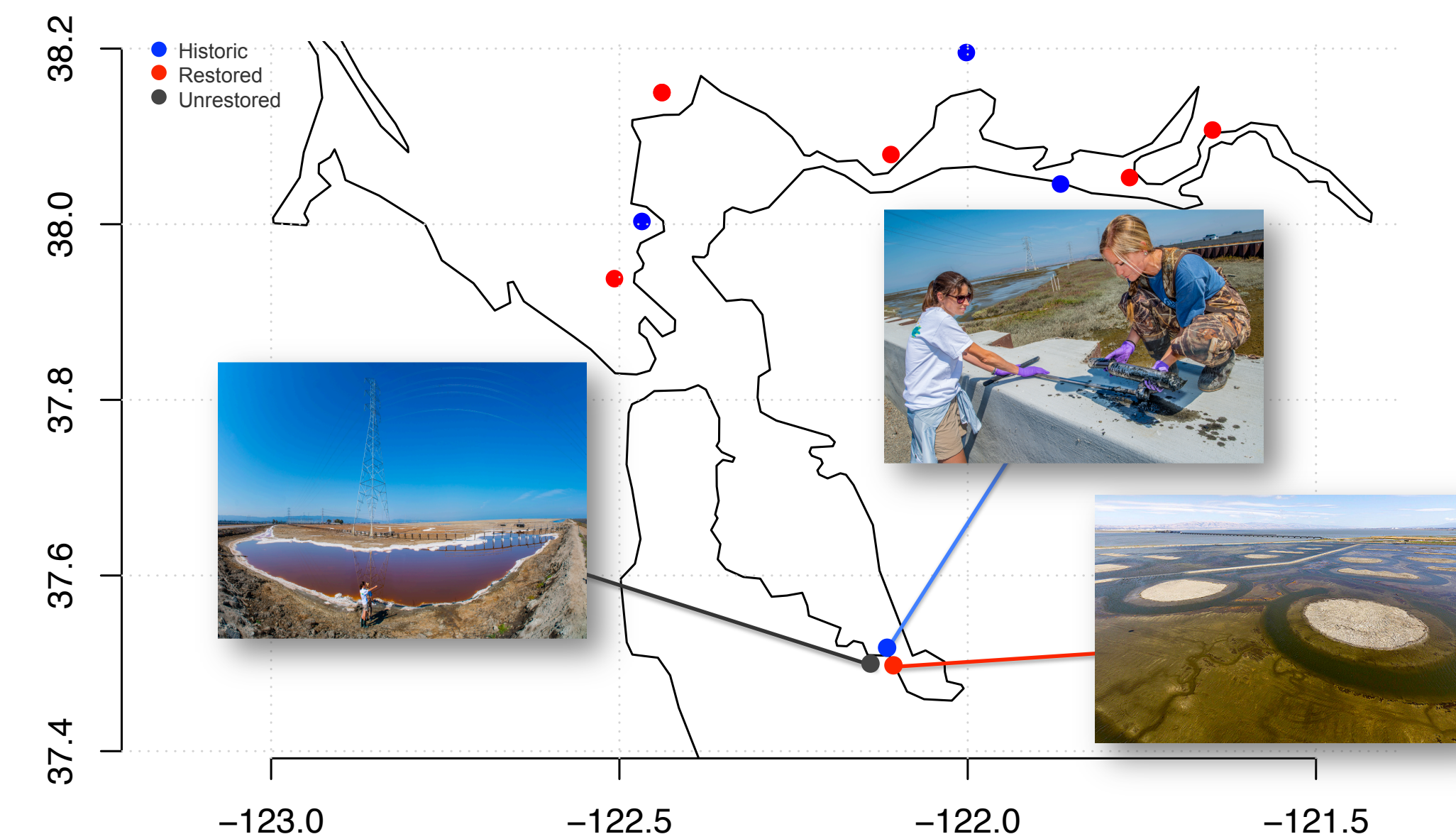
Wetland restoration has been proposed as a potential long-term carbon storage solution, with a goal of engineering geochemical dynamics to accelerate peat accretion and encourage greenhouse gas (GHG) sequestration. However, wetland microbial community composition and metabolic rates are poorly understood and their predicted response to wetland restoration is unknown. In an effort to better understand the underlying factors that shape the balance of carbon flux in wetland soils, we targeted the microbial communities along a salinity gradient ranging from freshwater tidal marshes to hypersaline ponds in the San Francisco Bay-Delta region. Using 16S rRNA gene sequencing and shotgun metagenomics, coupled with greenhouse gas measurements, we sampled sixteen sites capturing a range in salinity and restoration status. Our study links belowground microbial communities and their aboveground greenhouse gas production and highlights the inherent complexity in predicting wetland microbial response in the face of both natural and unnatural disturbances.

## OBJECTIVES

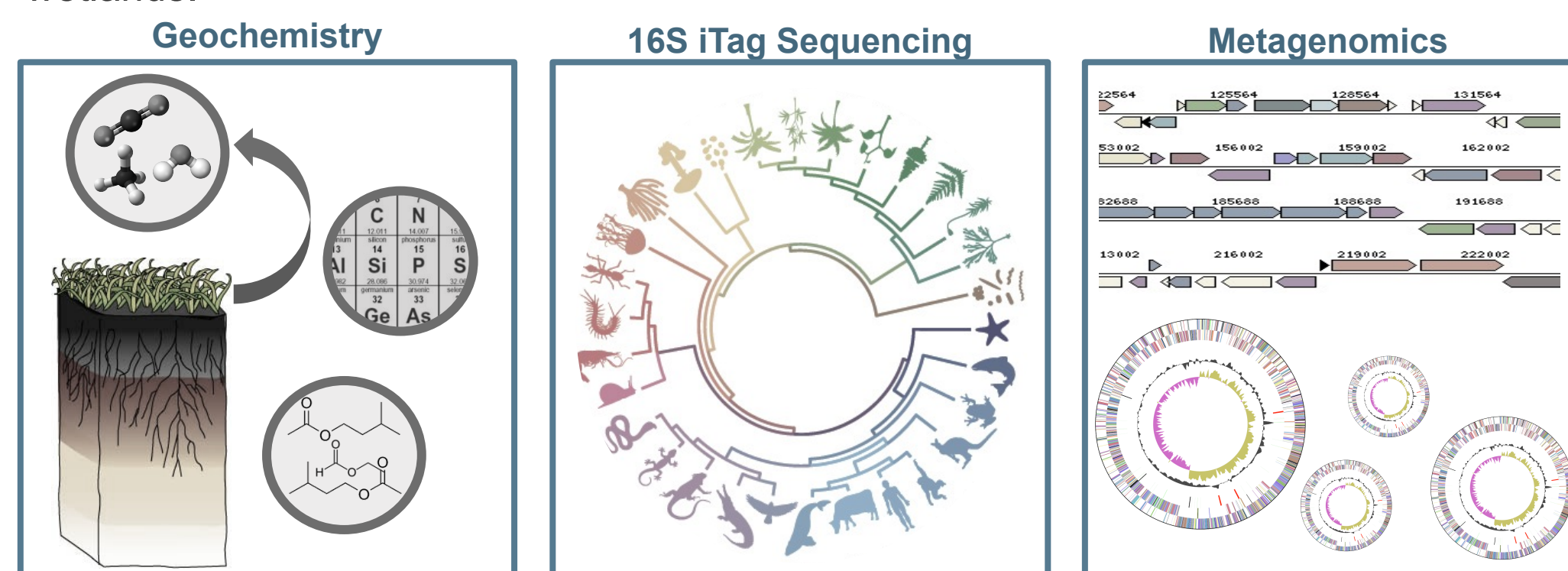
The aims of our study are to:

- Sample microbial diversity across wetland age and salinity gradients
- Link microbial diversity to carbon flux measurements from wetland soils
- Evaluate the role of restoration in shifting the net greenhouse gas source-or-sink potential of wetlands

## METHODS

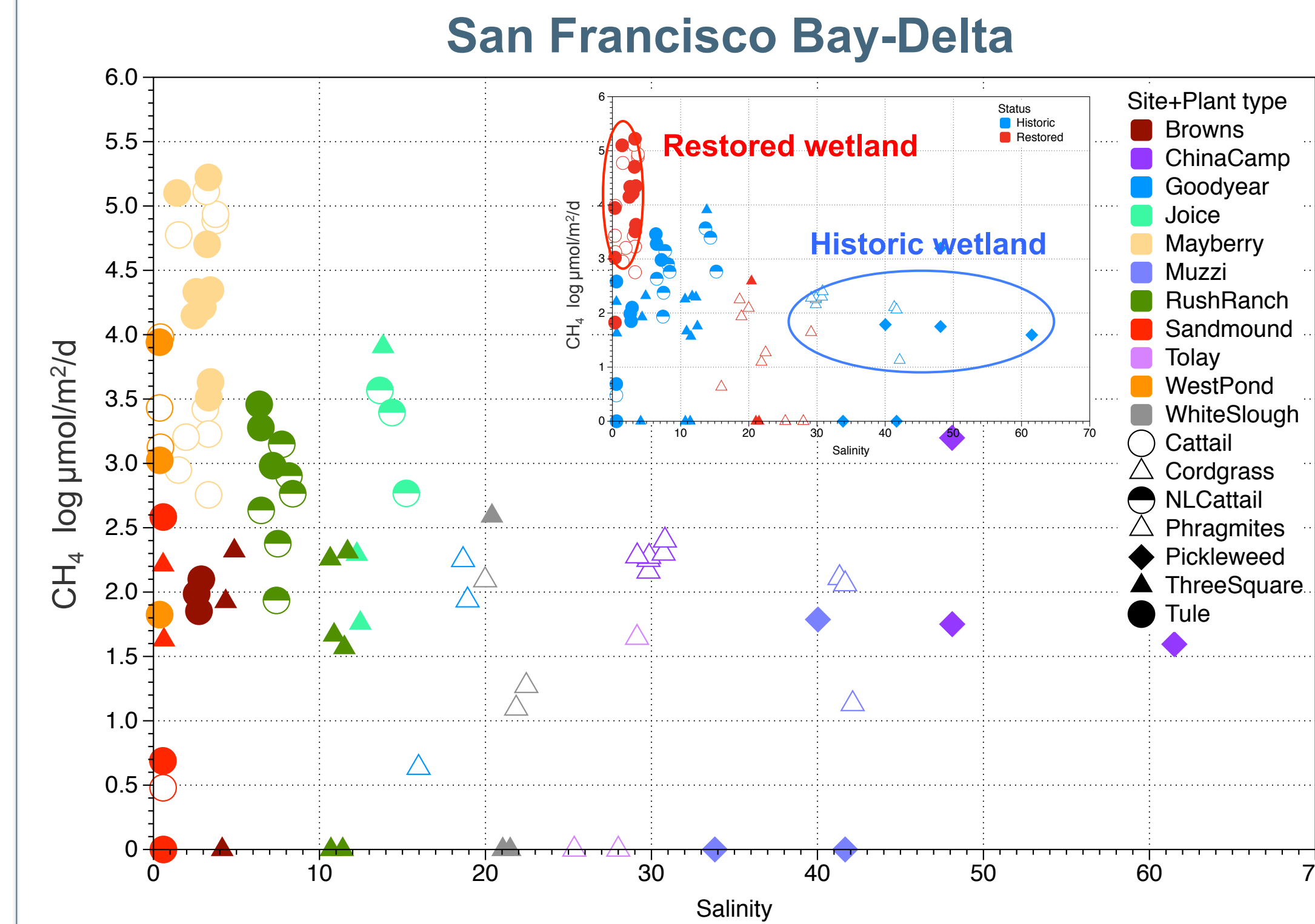


**Figure 1.** Wetland sampling locations in San Francisco Bay-Delta. Blue markers are historical wetlands, red markers are restored wetlands, grey markers are unrestored wetlands.

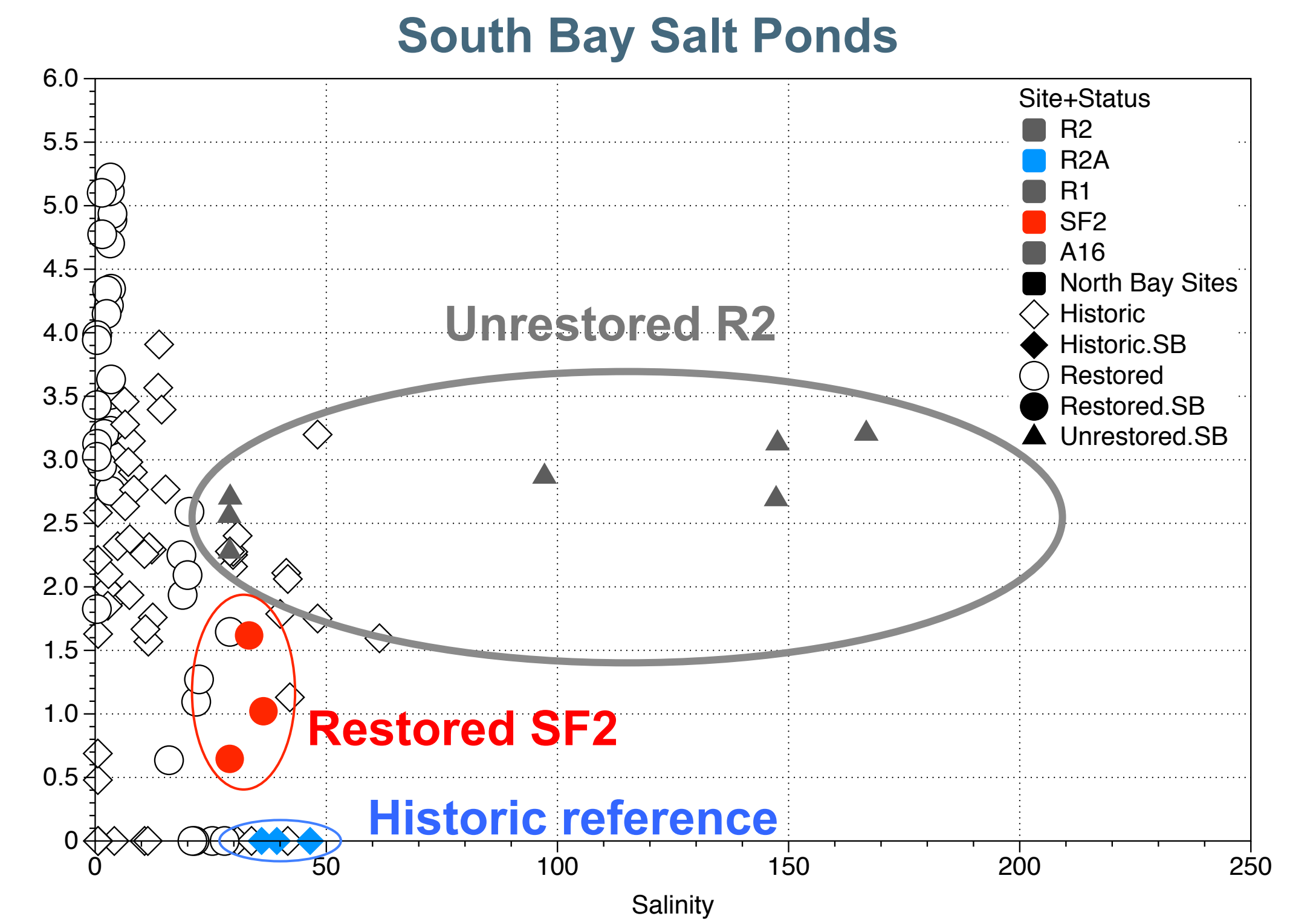


**Figure 2.** Sampling schematic for soil collection, GHG (CO<sub>2</sub>, CH<sub>4</sub>, H<sub>2</sub>O) and soil geochemistry measurements, and 16S SSU rRNA gene sequencing and shot-gun metagenomics.

## RESULTS: Methane production across wetlands

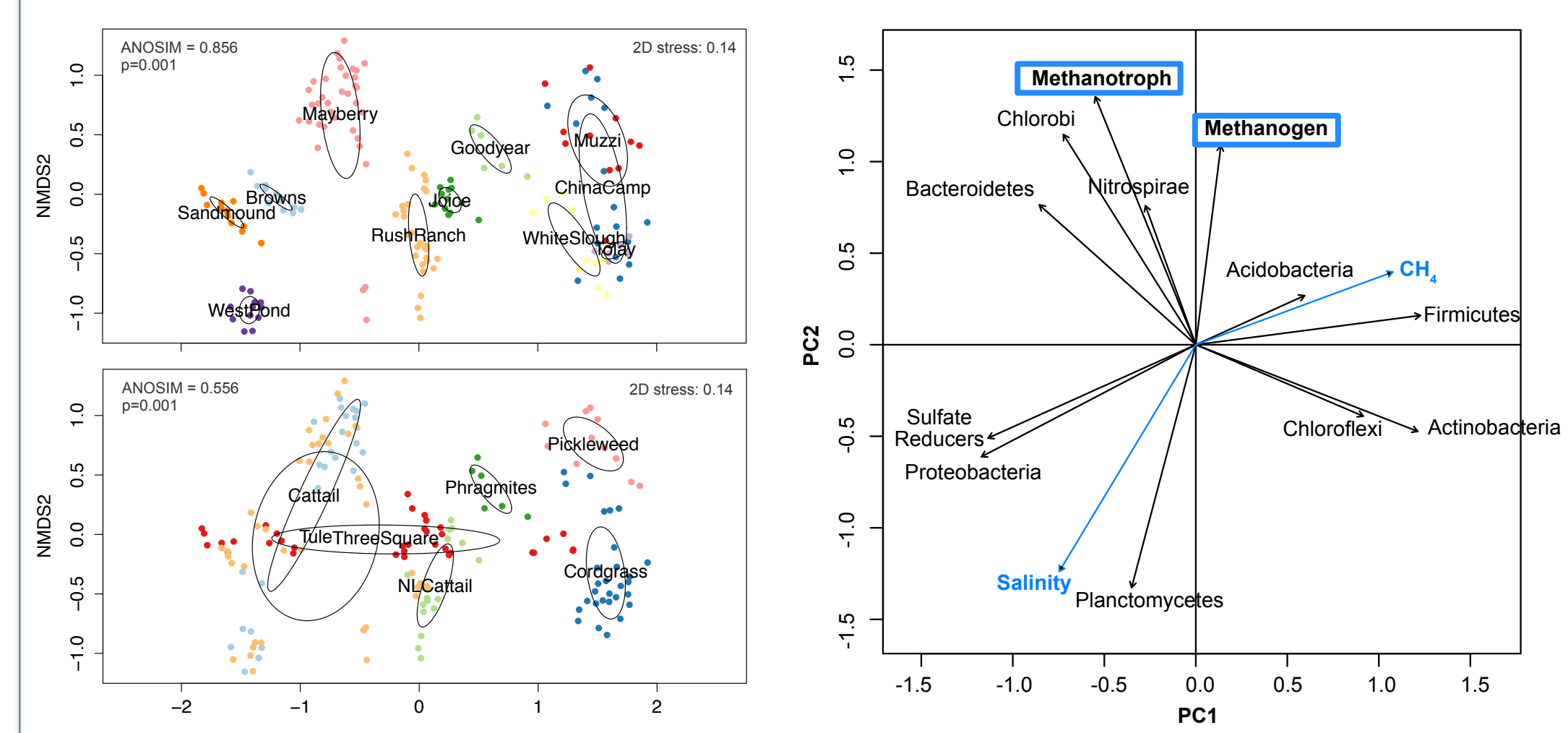


**Figure 3.** Methane production versus salinity in SF Bay-Delta wetlands by sampling location and restoration status (inset). Highest rates of methane production were observed in restored freshwater wetlands.



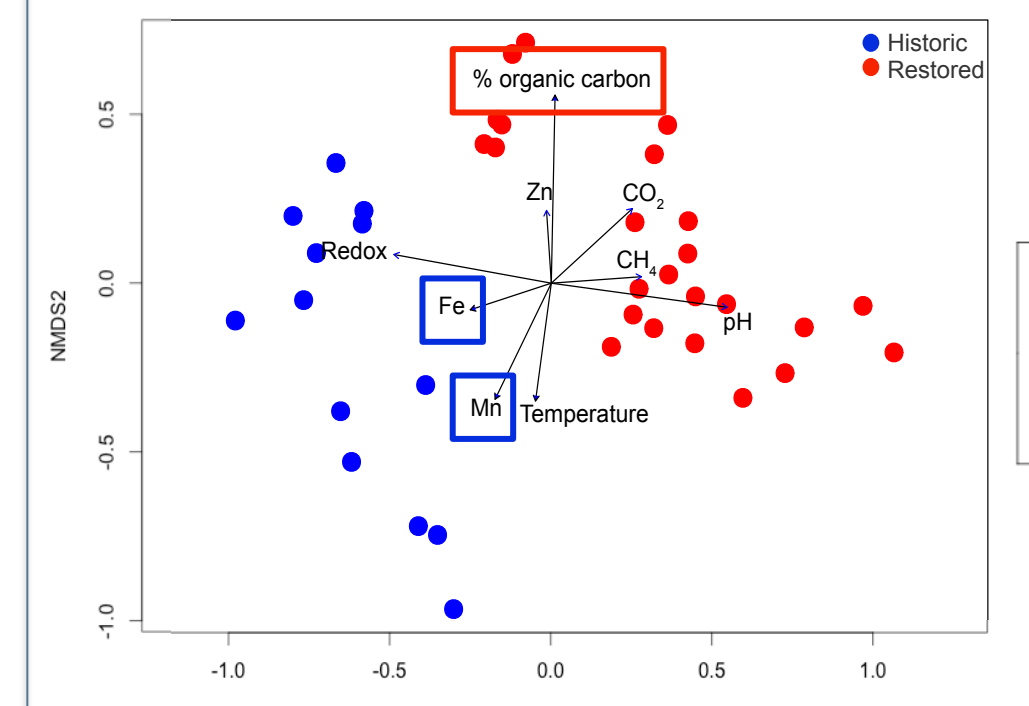
**Figure 6.** Methane production versus salinity in South Bay Salt Ponds. Highest rates of methane production were observed in unrestored, hypersaline ponds.

## SF Bay-Delta microbial communities

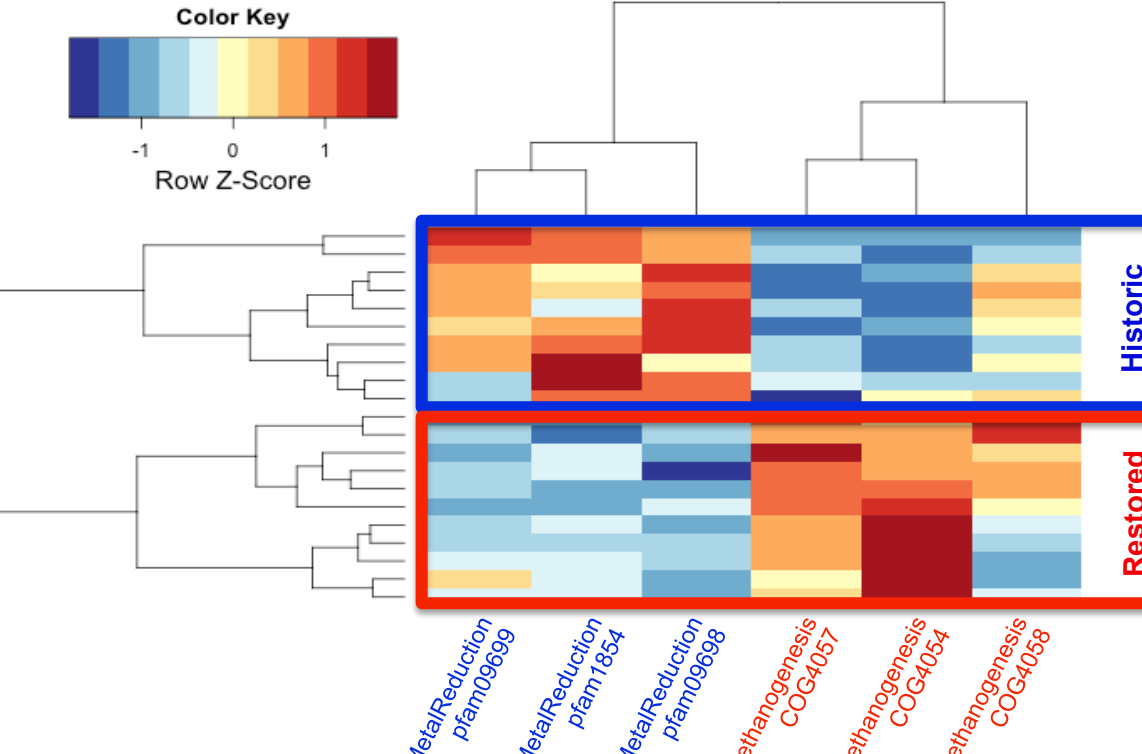


**Figure 4A.** NMDS of Bray-Curtis dissimilarities for wetland bacterial and archaeal populations by site (top) and plant type (bottom).

**Figure 4B.** Correlation biplot of relative abundances of key microbial groups along salinity/CH<sub>4</sub> gradients.

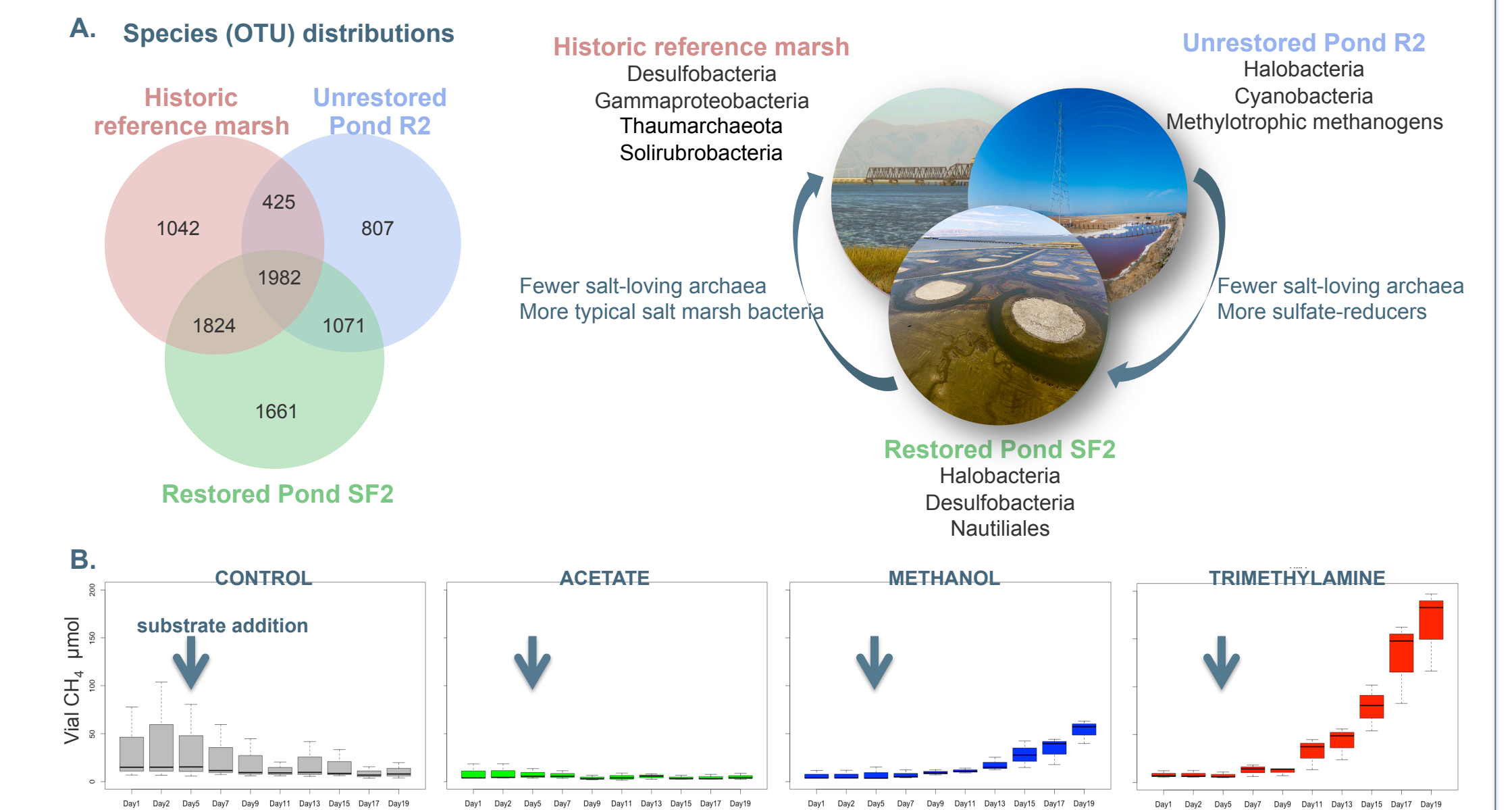


**Figure 5A.** NMDS of freshwater methanogen OTUs with environmental variables (bioenv) that contribute significantly to differences between historic and restored communities.



**Figure 5B.** Heatmap of select gene pathways in freshwater wetlands. Restored sites were enriched in genes for methanogenesis, historic sites were enriched for metal reduction.

## South Bay Salt Pond microbial communities



**Figure 7A.** Species overlap comparisons across three sampling sites (left) and dominant phyla across sites (right). **7B.** Results of methanogen enrichment culture substrate-addition experiment confirming the presence of methylophilic methanogens, *Methanobus* and *Methanococcoides*, in unrestored Pond R2.

## CONCLUSIONS

- Microbial community composition and metabolic potential clustered strongly according to sampling site, plant type, and salinity.
- The availability of trace metal terminal electron acceptors and total organic carbon may help explain large variations in methane production between restored and historic freshwater wetlands.
- Methylophilic methanogens using "alternative substrates" are responsible for elevated methane production in the hypersaline Pond R2.
- Restoration drastically decreased methane production in the hypersaline ponds. Microbial communities in a recently restored pond were similar to those in a remnant historic marsh.