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# Microbial diversity in restored wetlands of San Francisco Bay

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## ABSTRACT

Wetland ecosystems may serve as either a source or a sink for atmospheric carbon and greenhouse gases. This delicate carbon balance is influenced by the activity of below-ground microbial communities that return carbon dioxide and methane to the atmosphere. Wetland restoration efforts in the San Francisco Bay-Delta region may help to reverse land subsidence and possibly increase carbon storage in soils. However, the effects of wetland restoration on microbial communities, which mediate soil metabolic activity and carbon cycling, are poorly studied. In an effort to better understand the underlying factors which shape the balance of carbon flux in wetland soils, we targeted the microbial communities in a suite of restored and historic wetlands in the San Francisco Bay-Delta region. Using DNA and RNA sequencing, coupled with greenhouse gas monitoring, we profiled the diversity and metabolic potential of the wetland soil microbial communities along biogeochemical and wetland age gradients. Our results show relationships among geochemical gradients, availability of electron acceptors, and microbial community composition. Our study provides the first genomic glimpse into microbial populations in natural and restored wetlands of the San Francisco Bay-Delta region and provides a valuable benchmark for future studies.

## OBJECTIVES

The aims of our study are to:

- Sample microbial diversity across wetland age and salinity gradients
- Detect microbial response to geochemical fluctuation and the influence of above-ground plant species
- Link microbial diversity to carbon flux measurements from wetland soils
- Evaluate wetland carbon source/sink potential and associated indicator microbial species

## SITE DESCRIPTIONS

Twitchell Island wetland (121.65°W, 38.11°N) is a pilot-scale wetland restoration project established in 1997 to assess carbon storage land subsidence-reversal potential of converted agricultural land in the Sacramento-San Joaquin River Delta (Figure 1a). The wetland consists of two adjacent ponds, each about 3 ha (Figure 1b) with artificially maintained water depths of 25cm and 55cm. We sampled the West Pond along a transect from the water source inlet (A) to points furthest from the inflow (C/L, Figure 1b) in February and August 2011.

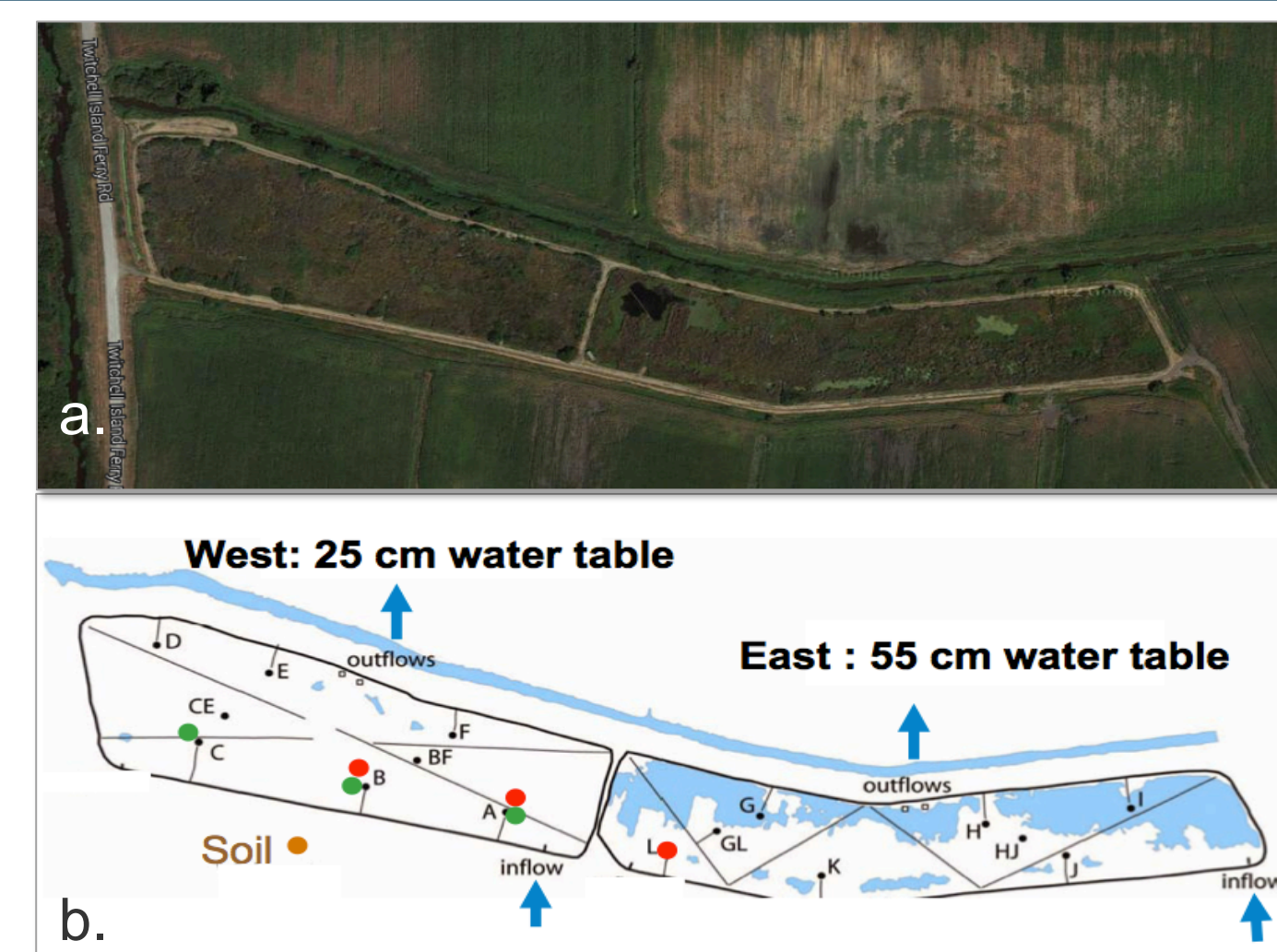


Figure 1. Twitchell wetland (a) and sampling locations (b).

In Phase II of the project, we sampled a suite of wetlands in San Pablo Bay, Suisun Bay and in the Delta region of San Francisco Bay (Figure 2). These wetlands encompassed a salinity gradient ranging from 0ppt (Sandmound Slough) to 40ppt (China Camp). Five wetlands sampled were historical wetlands (blue markers, Figure 2) and six wetlands were restored wetlands (red markers, Figure 2).

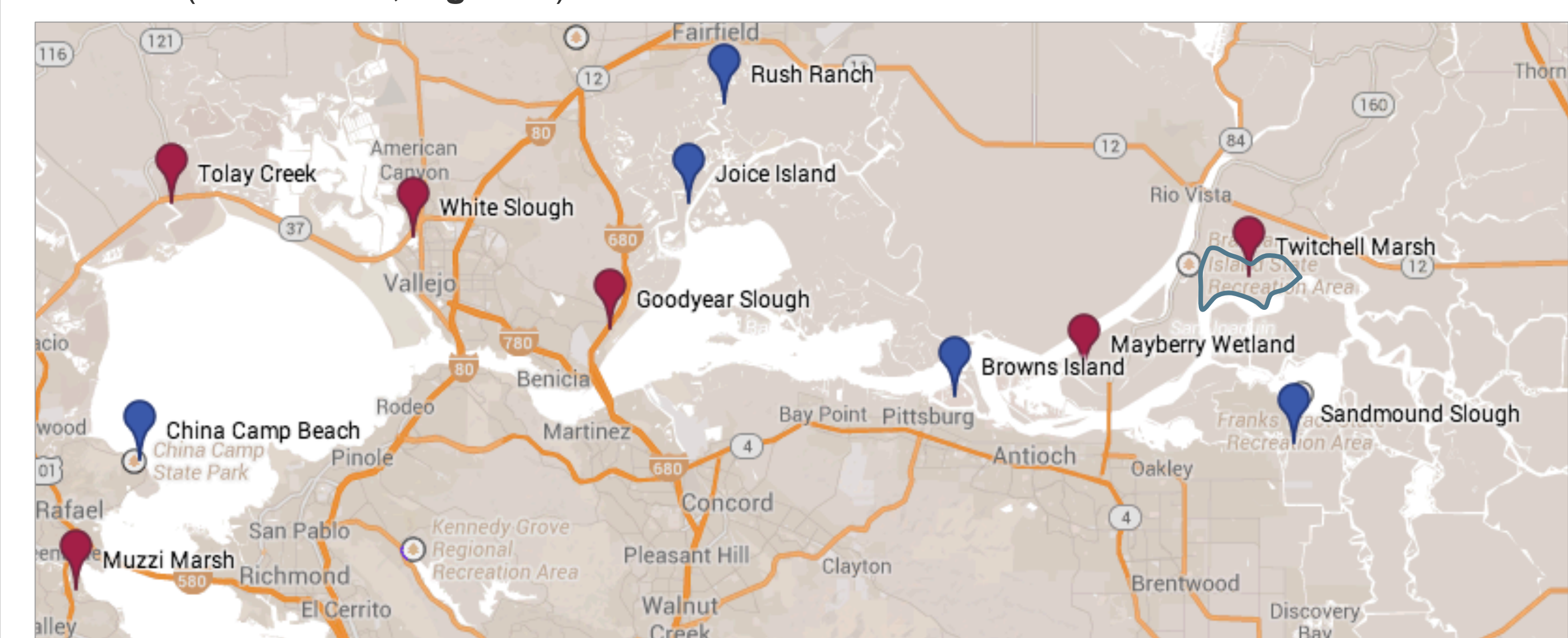


Figure 2. Wetland sampling locations in San Pablo Bay, Suisun Bay and the SF Delta. Blue markers are historical wetlands, red markers are restored wetlands.

## Phase I Results: Twitchell Wetland

Increasing methane production rates were observed at Twitchell wetland along the transect from site A→C/L (Figure 1). Methane emissions were highest at the Twitchell wetland sites C/L which were furthest from the water inlet (Figure 3). These higher CH<sub>4</sub> emissions corresponded to sites with lowest oxygen and nitrate availability (He et al., in review).

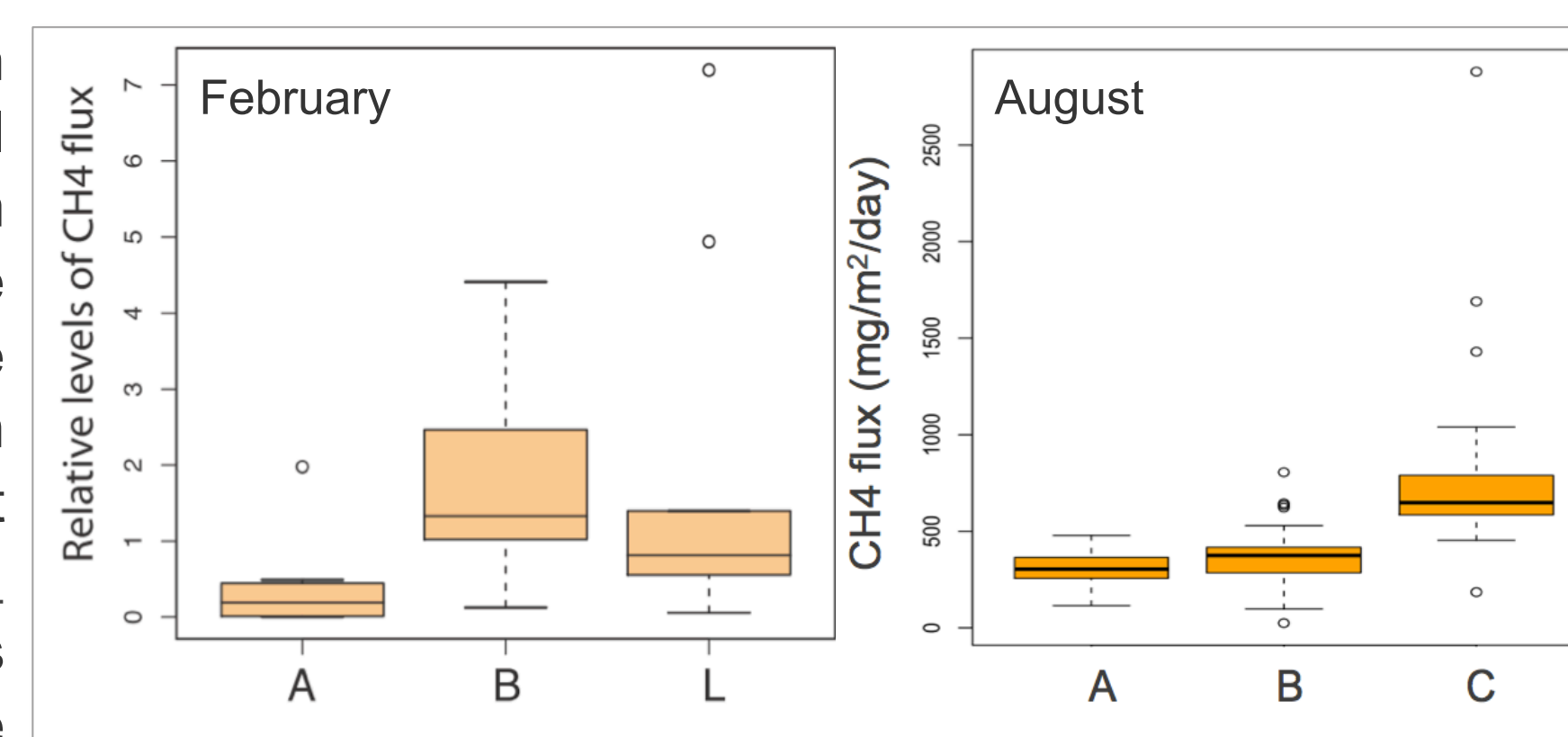


Figure 3. Methane production at Twitchell sites in February and August.

Microbial communities were strongly associated with sampling site (A, B, C/L) and plant type (Cattail, Tule, Bulk soil, Figure 4). High methane-production sites were enriched for phyla typically observed in anoxic environments (Riviere et al., 2009), consistent with the expectation that after inundation, soil environments become more anaerobic and methanogenic.

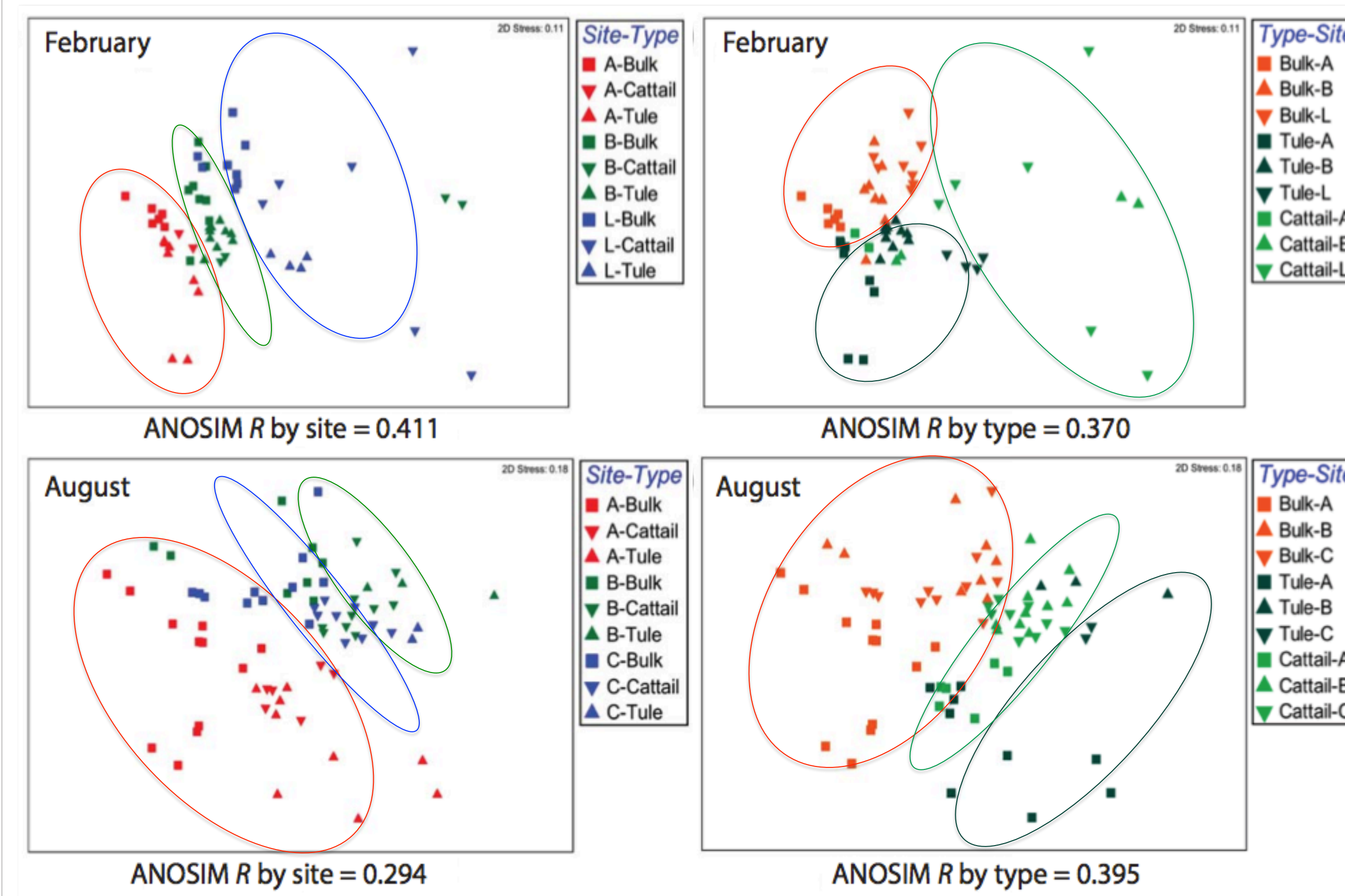


Figure 4. Nonmetric multi-dimensional scaling (NMDS) of relative Operational Taxonomic Unit (OTU) abundance.

## MATERIALS AND METHODS

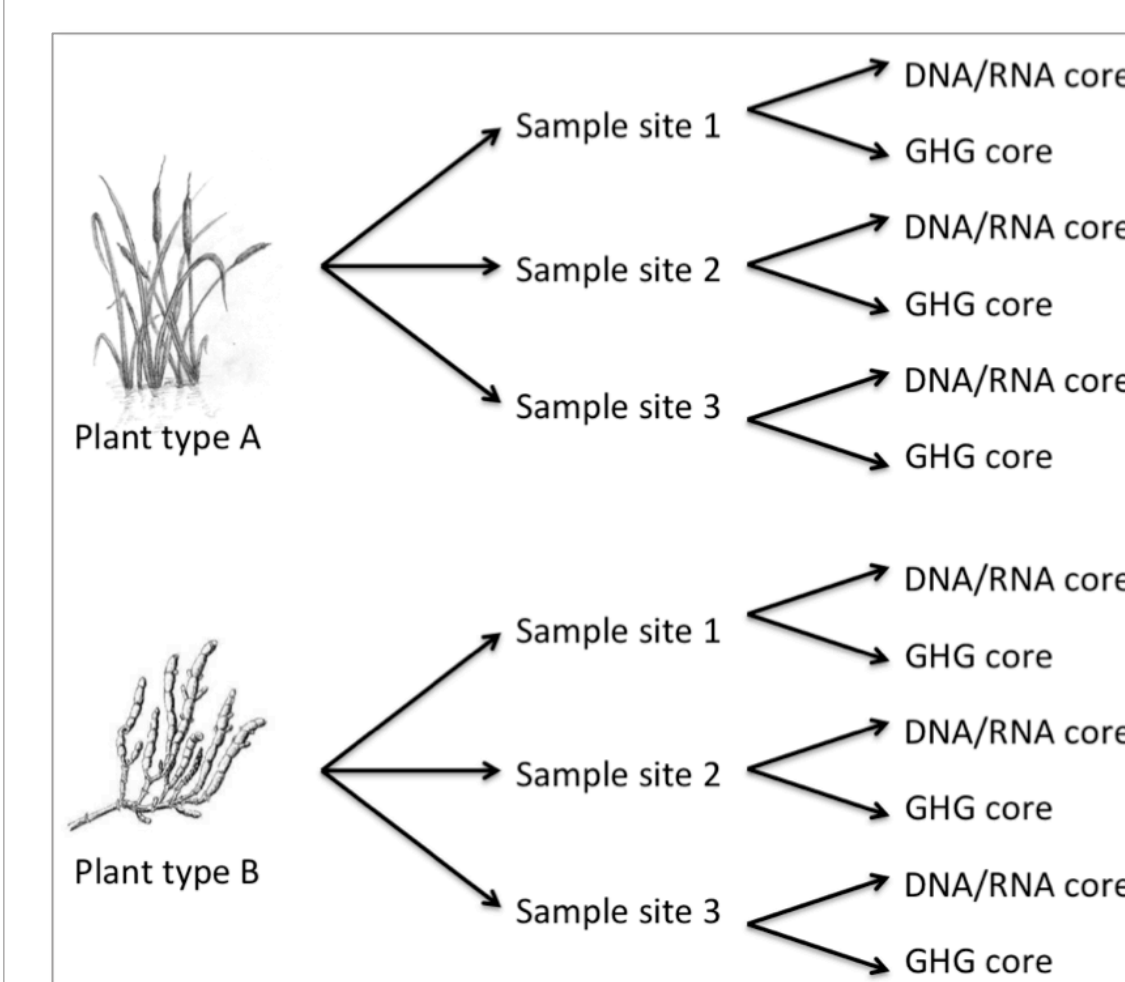


Figure 5. Sampling schematic for San Pablo Bay, Suisun Bay, and Delta sites.

**Twitchell wetland:** Soils were dissected into bulk, tule rhizome and cattail rhizome samples. A subsample was taken from February 2011 samples for 12-day lab incubation studies to quantify greenhouse gas (GHG) flux. For August 2011 samples, stationary whole-plant chambers were used to measure GHG flux. Soil samples were extracted for DNA and microbial community profiles were generated by pyrotag sequencing targeting the 16S rRNA gene.

**San Pablo/Suisun/Delta wetlands:** Soil cores were collected using an AMS split core sampler. Adjacent DNA/RNA and GHG cores were collected in triplicate for two plant types at each site (Figure 5). Intact soil cores were analyzed for CO<sub>2</sub>/CH<sub>4</sub> flux on a Los Gatos Research GHG Analyzer.



Bulk Decomposed



Cattail Rhizome



Tule Rhizome

## Phase II Results: San Pablo Bay, Suisun Bay, Delta Wetlands

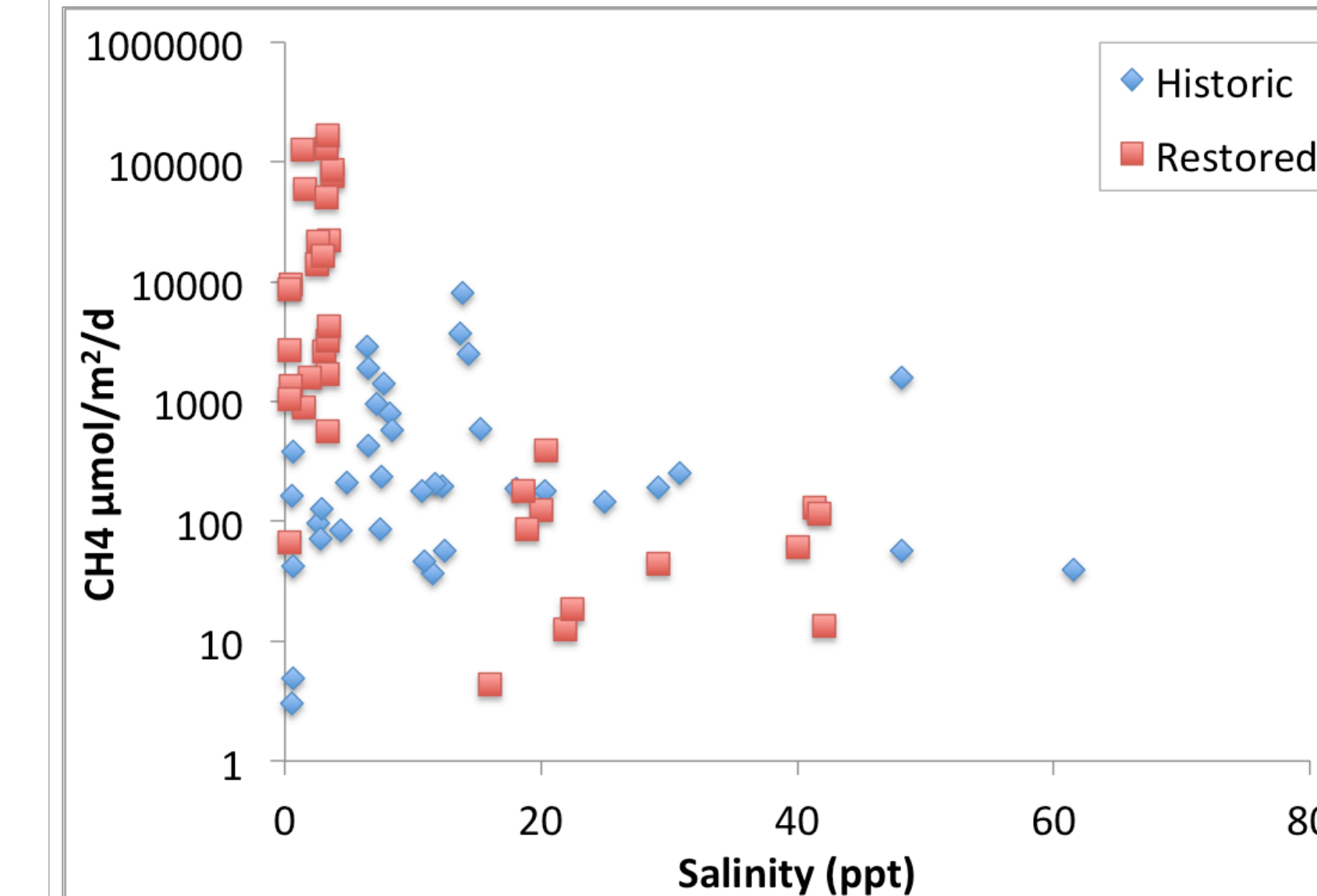


Figure 6. Methane production versus salinity for historic and restored wetlands.

Historic wetland soils yielded lower average methane production rates than restored wetlands (Figure 6). Historic wetlands averaged 663 µmol/m<sup>2</sup>/d and restored wetlands averaged 19274 µmol/m<sup>2</sup>/d of methane, potentially the result of larger labile carbon pools and more saturated soils in restored wetlands. Soils below 18ppt accounted for 99.5% of the total regional methane production, in agreement with previous observations in wetland soils (Poffenberger et al., 2011).

Methane production rates clustered along salinity and plant type gradients (Figure 7a). Tule (*Schoenoplectus acutus*) and cattail (*Typha* spp.) were associated with the highest rates of methane production. Net methane consumption was measured at the oligohaline, historic wetland Sandmound Slough in the tule and three-square bulrush soils.

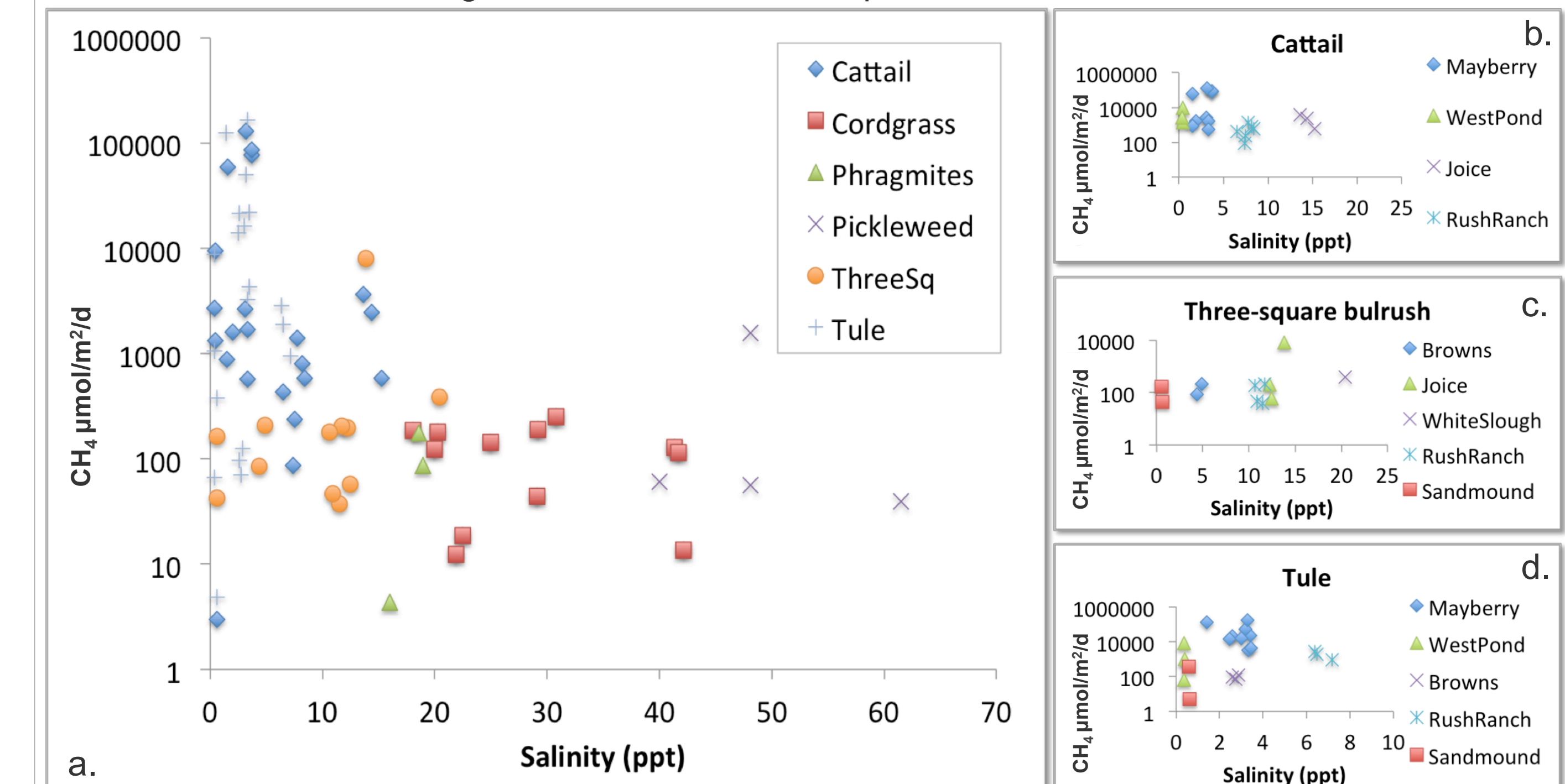


Figure 7. Methane production versus salinity for historic and restored wetlands separated by plant type (a) and by sampling location for individual plant types (b-d).

The influence of sampling location (7b-d), salinity, and plant type on methane production were all statistically significant (ANOVA,  $p > 0.0001$ ), although these factors co-vary. Site was a stronger predictor of methane flux ( $R^2=0.62$ ) than salinity ( $R^2=0.144$ ) or plant type ( $R^2=0.40$ ), although only methane flux from freshwater restored wetlands was significantly greater than the mean.

## CONCLUSIONS

- At Twitchell wetland, microbial community composition is primarily governed by sampling location and sample types (bulk soil, tule rhizome, cattail rhizome).
- Low methane production samples at Twitchell wetland were associated with the site closest to the inflow, correlated to higher availabilities of sulfate, nitrate and oxygen. Lower numbers of methanogen OTUs were detected at these sites.
- Soil methane production clustered according to sampling location, plant type and salinity along the San Pablo/Suisun/Delta wetland sampling survey. Sampling location had the strongest influence on methane production.
- Restored wetlands had higher average methane production rates than historic wetlands possibly due to inundation regime, larger labile carbon pools or decreased availability of terminal electron acceptors.

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