#### Microbial diversity and carbon cycling in San Francisco Bay wetlands

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#### **ABSTRACT**

Wetland restoration efforts in San Francisco Bay aim to rebuild habitat for endangered species and provide an effective carbon storage solution, reversing land subsidence caused by a century of industrial and agricultural development. However, the benefits of carbon sequestration may be negated by increased methane production in newly constructed wetlands, making these wetlands net greenhouse gas (GHG) sources to the atmosphere. We investigated the effects of wetland restoration on belowground microbial communities responsible for GHG cycling in a suite of historic and restored wetlands in SF Bay. Using DNA and RNA sequencing, coupled with real-time GHG monitoring, we profiled the diversity and metabolic potential of wetland soil microbial communities. The wetland soils harbor diverse communities of bacteria and archaea whose membership varies with sampling location, proximity to plant roots and sampling depth. Our results also highlight the dramatic differences in GHG production between historic and restored wetlands and allow us to link microbial community composition and GHG cycling with key environmental variables including salinity, soil carbon and plant species.

## **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

## 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

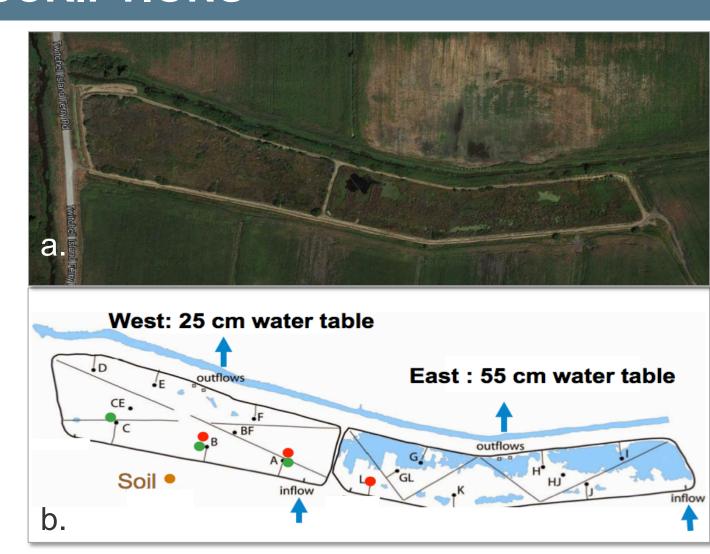


Figure 1. Twitchell wetland (a) and sampling locations (b).

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).

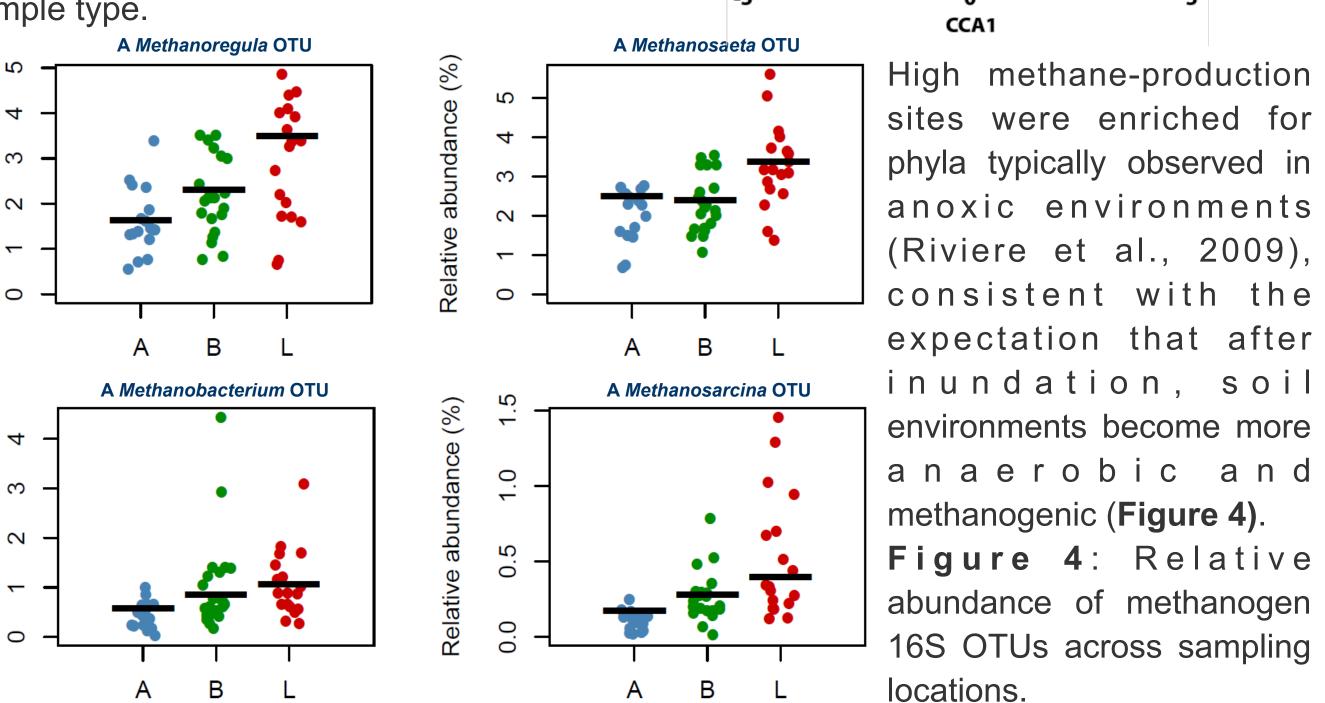


Figure 2. Wetland sampling locations in San Pablo Bay, Suisun Bay and the SF Delta. Blue markers are historical wetlands, red markers are restored wetlands.

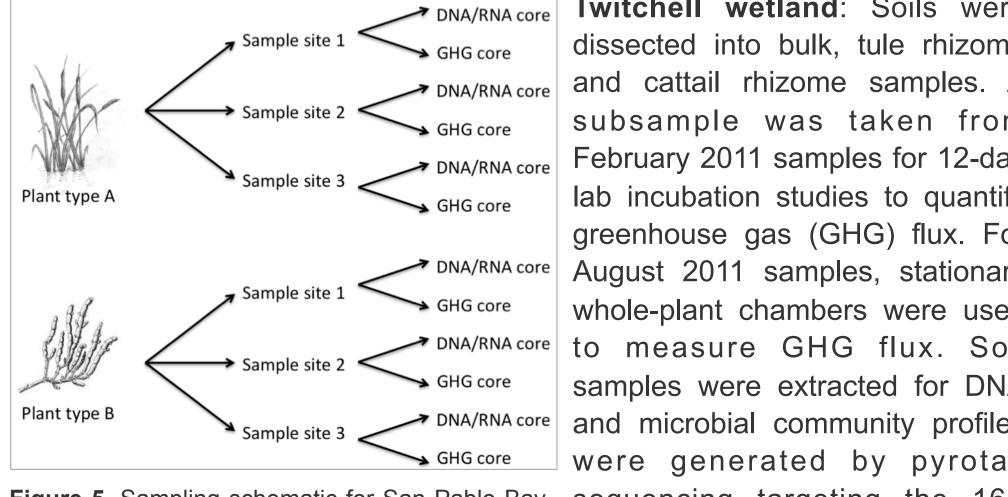
# Phase I Results: Twitchell Wetland

Increasing methane production rates were observed at Twitchell wetland along the transect from site A→C/L (Figure 1). o-Methane emissions were highest at the Twitchell wetland site C which was furthest 2 -2from the water inlet (Figure 3a). These higher CH<sub>4</sub> emissions corresponded lower oxygen, nitrate, and sulfate availability (He et al., in review). Microbial communities clustered according to sampling site (Figure 3a) and sample type (Figure 3b), although sampling site was a stronger predictor of community composition than sample type (ANOSIM 0.38 vs. ANOSIM 0.22, respectively).

Figure 3. Canonical correspondence analyses (CCA) of wetland 16S Operational Taxonomic Units (OTUs) and geochemical parameters; a) samples colored by -6sampling location, b) samples colored by sample type.



## MATERIALS AND METHODS



Suisun Bay, and Delta sites.

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 Figure 5. Sampling schematic for San Pablo Bay, sequencing targeting the 16S rRNA gene.

Twitchell wetland: Soils were

dissected into bulk, tule rhizome

subsample was taken from

February 2011 samples for 12-day



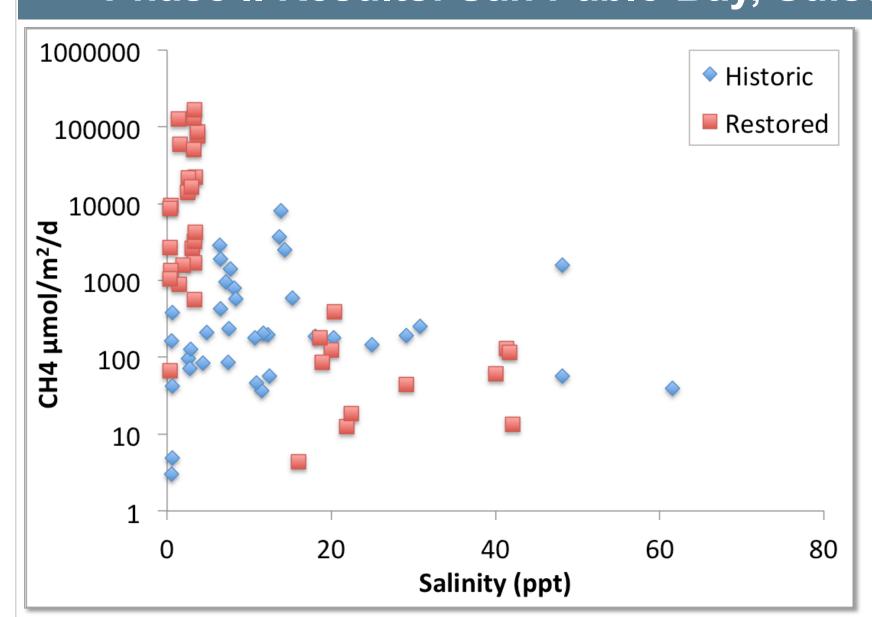
San Pablo/Suisun/Delta wetlands: Soil cores were collected using an AMS split core sampler. Adjacent DNA/RNA and GHG cores were collected in triplicate for two plant types at each site (Figure 5). Intact soil cores were analyzed for CO<sub>2</sub>/CH<sub>4</sub> flux on a Los Gatos Research GHG Analyzer.







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



Historic wetland soils yielded lower average methane production rates than restored wetlands (Figure 6). Historic wetlands averaged 663 umol/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

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

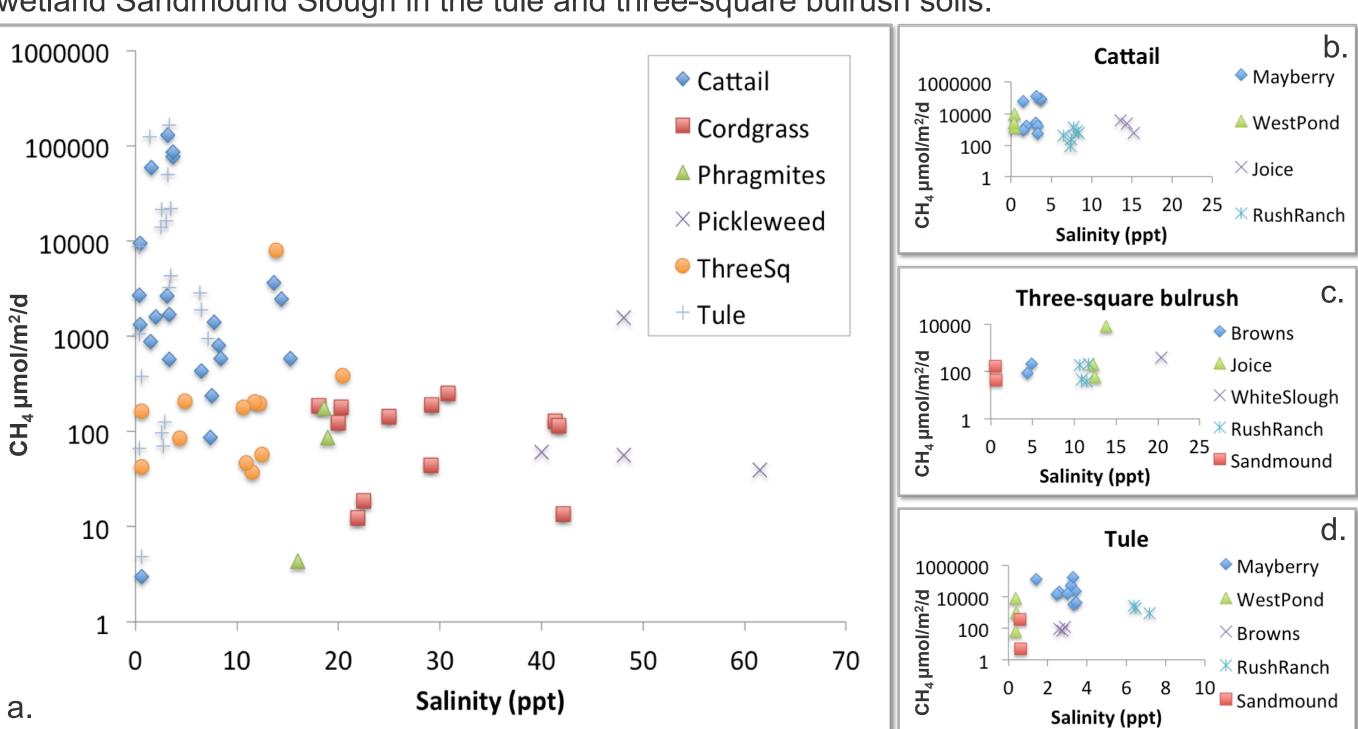


Figure 7. Methane production versus salinity for historic and restored wetlands separated by plant type (a) and by sampling location for individual plant types (b-d).

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<sup>2</sup>=0.62) than salinity (R<sup>2</sup>=0.144) or plant type (R<sup>2</sup>=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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