Does Agricultural Runoff Influence Anaerobic Methanotrophy in a Southern Californian Coastal Wetland?

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Introduction

Coastal wetlands are productive ecosystems hosting diverse wildlife, including many endangered birds, plants, and aquatic organisms. Anthropogenic activity, including agricultural runoff, may feed high concentrations of nitrate (NO_3^-) into these wetlands and spark harmful eutrophication events. As anthropogenic activity persists and intensifies, investigating nitrate remediation is essential to controlling the frequency of eutrophication events and maintaining coastal wetland habitats. The anaerobic oxidation of methane (AOM) by wetland soil microbes may be a significant sink to the wetland nitrate burden as it may utilize nitrate as an electron acceptor[1]. AOM is also a significant sink to the emission of methane – a potent greenhouse gas – from coastal wetlands[2]. This project will assess the role of AOM in alleviating anthropogenic nitrate input to a Southern Californian coastal wetland, and the effect of anthropogenic nitrate input on wetland AOM in turn.

Figure 1. Images from the Carpinteria Salt Marsh Reserve. From left to right: a brackish stream with signs of eutrophication (light-green algal growth), surface 3 cm sediment collected from Site A (refer to **Fig. 5**). for sediment slurry incubations, a drainage pipe near Site A.

Background

The Carpinteria Salt Marsh Reserve (CSMR) is a southern Californian coastal wetland influenced both by the Pacific Ocean and human activity via runoff. Nitrate concentrations in a brackish region of the wetland, Site A (refer to **Fig. 5**), reach as high as ~803 µM (G. Vetushko, unpubl. data). Previous literature suggests nitrate concentrations may reach over 4000 µM in a freshwater region[3].

On 08/12/2024, we collected surface 3 cm sediment from Site A for anoxic sediment slurry incubations. We utilized $^{15}N\text{-}NO_3^-$ and $^{14}C\text{-}CH_4$ to evaluate the significance of AOM to removing nitrate from the sediment and $^{35}S\text{-}SO_4^{2-}$ to evaluate the effect of high nitrate input on sulfate reduction, a metabolism that may be coupled to AOM[4]. We flushed our incubations with and without methane to distinguish AOM activity.

$$5CH_4 + 8NO_3^- + 8H^+ \rightarrow 5CO_2 + 4N_2 + 14H_2O \quad (1)$$
$$\Delta G° = -765 \text{ kJ mol}^{-1} CH_4$$

$$CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O \quad (2)$$
$$\Delta G° = -14 \text{ kJ mol}^{-1} CH_4$$

Figure 2. Gibb's free energy for the following reactions: AOM coupled to sulfate reduction and nitrate reduction respectively[1,4].

Previous Findings (Site A)

On 08/12/2024 , we measured ~187 µM NO_3^- in the water column at Site A (refer to **Fig. 5**). We utilized this nitrate concentration in our $^{15}N\text{-}NO_3^-$ incubations to simulate runoff-associated nitrate input.

- Starved Site A sediment of nitrate ($[NO_3^-] < 0.5$ µM)
- Created sediment slurry with anoxic, artificial sterile brackish media (3:1 bulk sediment:media)
- Targeted 187 µM $^{15}N\text{-}NO_3^-$ in 165 mL serum vial incubations

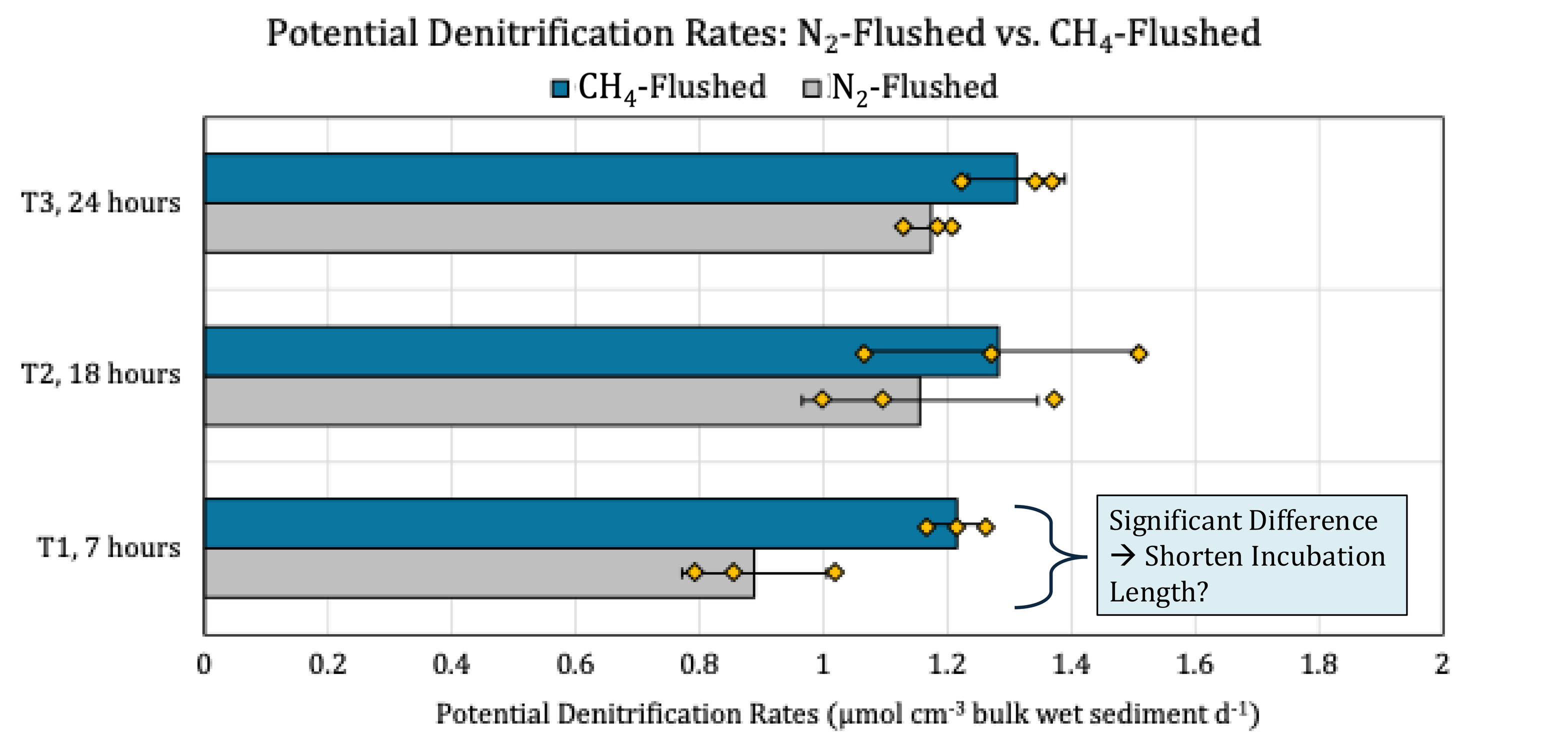

Figure 3. A comparison of potential denitrification rates in sediment slurry incubations. Blue columns represent averaged values from CH_4-flushed incubations and grey columns represent averaged values from N_2-flushed incubations . Yellow diamonds represent individual replicate values. All values are relative to killed controls. Standard deviation bars represent +/- 1σ.

We targeted the lowest and highest concentrations of nitrate (7.6 and 803 µM respectively) measured at Site A to evaluate the effect of agricultural runoff on sulfate reduction and potentially sulfate-dependent AOM.

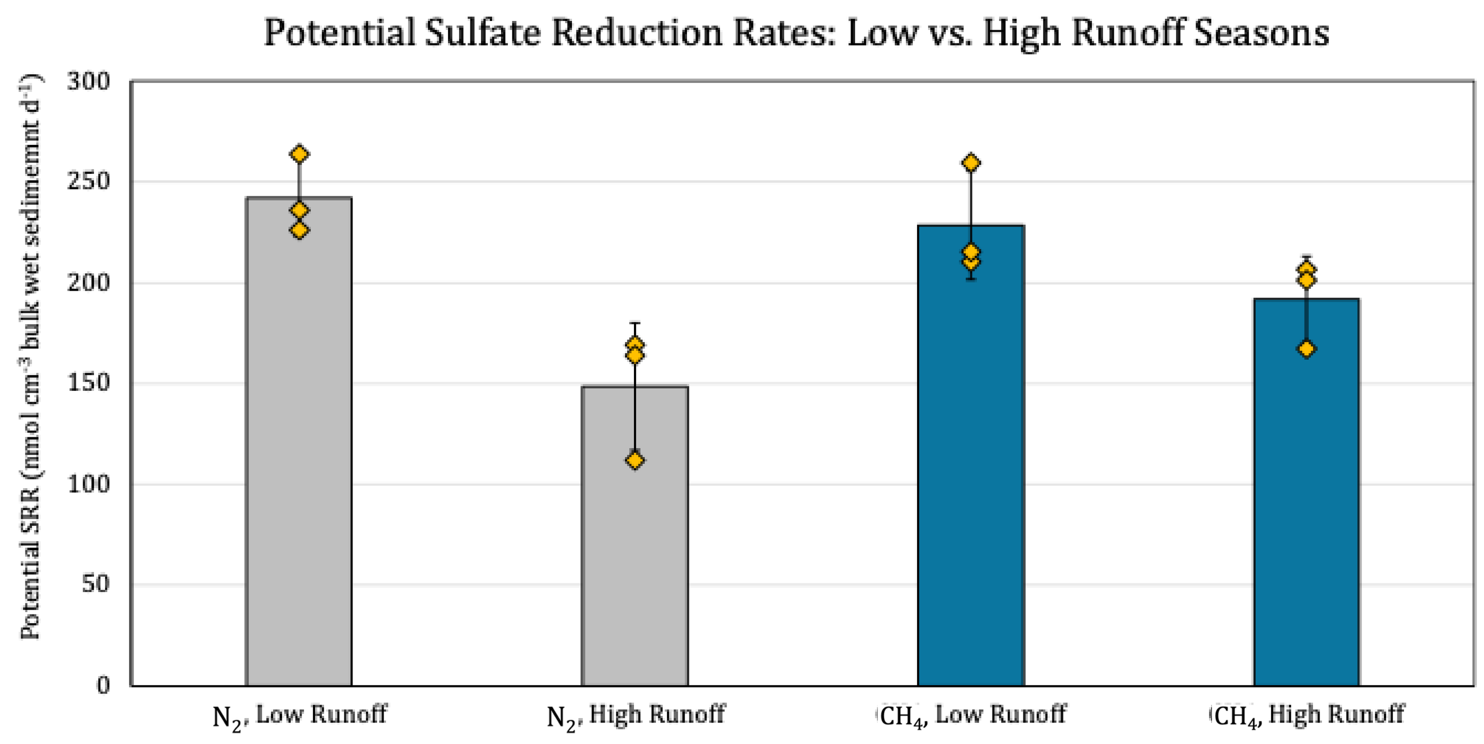

Figure 4. A comparison of potential sulfate reducion rates (SRR) in sediment slurry incubations. Blue columns represent averaged values from CH_4-flushed incubations and grey columns represent averaged values from N_2-flushed incubations. The yellow diamonds represent individual replicates. Low runoff incubations were injected to target 7.6 µM nitrate; high runoff incubations were injected to target 803 µM nitrate. All values are relative to killed controls. Standard deviation bars represent +/- 1σ.

Present and Future Directions

Figure 5. A map of the Carpinteria Salt Marsh Reserve and its surrounding vicinity. Algal growth in the wetland is evident by light-green 'blooms'. Coordinates and samples were taken at sites marked with a blue checkpoint, ranging from A to E. This map was made by Yuzuna Kudo using ArcGIS.

Visit I: Identify Regions with High Runoff Exposure (05/19/2025)
Identify 5 to 6 brackish wetland sites potentially exposed to runoff. Collect water column samples and sediment cores to measure nitrate concentration, nitrate penetration depth into sediment, and sediment methane concentrations.

Visit II: Collect Sediment for Anaerobic Oxidation of Methane Rates
Return to 3 to 4 brackish wetland sites with highest nitrate and collect surface, runoff-exposed sediment. Incubate in-lab to measure how nitrate input influences AOM rates.

Visit III: Collect Sediment for Nitrate Reduction Rates
Return to and collect sediment from 1 brackish wetland region where nitrate addition significantly increased AOM rates. Incubate in-lab to compare nitrate reduction rates (denitrification and DNRA) with and without methane.

References and Acknowledgements

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