Assessing how disruption of methanogenic communities and their syntrophic relationships in tidal freshwater marshes via saltwater intrusion may affect CH4 emissions

David J. Berrier  
*Virginia Commonwealth University*, berrierdj@vcu.edu

Scott C. Neubauer  
*Virginia Commonwealth University*, sneubauer@vcu.edu

Rima B. Franklin  
*Virginia Commonwealth University*, rbfranklin@vcu.edu

Follow this and additional works at: [http://scholarscompass.vcu.edu/rice_symp](http://scholarscompass.vcu.edu/rice_symp)  
Part of the [Botany Commons](http://scholarscompass.vcu.edu/rice_symp), [Forest Biology Commons](http://scholarscompass.vcu.edu/rice_symp), [Plant Biology Commons](http://scholarscompass.vcu.edu/rice_symp), and the [Terrestrial and Aquatic Ecology Commons](http://scholarscompass.vcu.edu/rice_symp)

© The Author

Downloaded from
[http://scholarscompass.vcu.edu/rice_symp/16](http://scholarscompass.vcu.edu/rice_symp/16)

This Poster is brought to you for free and open access by the Rice Rivers Center at VCU Scholars Compass. It has been accepted for inclusion in Rice Rivers Center Research Symposium by an authorized administrator of VCU Scholars Compass. For more information, please contact *libcompass@vcu.edu*. 
Figure 2. The degradation of organic matter in wetlands, both the presence of SRB (a) and in freshwater (b). (Diagram from Muzyer and Stams (2008).)

**Approach**
- Freshwater 30% (w/v) anaerobic microcosms were constructed with soil and pore water from Cumberland Marsh, a TFW located on the Rappahannock River, Virginia.
- Treated using various combinations of the following amendments:
  - 4 mM Na2SO4 to increase [SO4²⁻] as would occur with saltwater intrusion
  - 12 mM NaCl to control for the effect of increased ionic strength without increasing SO4²⁻ availability
  - 2.5 mM NaMoO4 (Na2MoO4), a SRB inhibitor
  - Additions of 2.5 mM butyrate (n-butyric acid) in combination with inhibitors was used to determine the role of SRB and MG.
    - 5 mM BEISA (2-Bromoethanesulfonic acid) a SRB inhibitor
    - 5 mM MoO4²⁻ (Na2MoO4)
    - H+ > 100 Pa
- We followed the response of the microbial community by monitoring:
  - CH4 and CO2 production - gas chromatography
  - Butyrate, acetate, and formate concentrations - on chromatograph

**Objectives**
- 1. Determine the effect of chloroaliphatic SO2⁻⁴ on concentrations of MG community functions (i.e., CH4 production and syntrophic butyrate degradation).
- 2. Assess whether these functions recover after competition with SRB has been removed.

**Introduction**
- Tidal freshwater wetlands (TFW), which lie at the interface of saltwater and freshwater ecosystems, are predicted to experience moderate salinity increases due to sea level rise.
- Increases in salinity generally suppress CH4 production, but it is uncertain to what extent elevated salinity will affect CH4 cycling in TFW. It is also unknown whether CH4 production will resume when freshwater conditions return.
- The ability to produce CH4 is limited to a monophyletic group of the Euryarchaeota phylum called methanogens (MG), who are limited to a small number of substrates (e.g., acetate, H2 and formate) produced from the breakdown of fermentation products.
- In freshwater anaerobic soils, the degradation of certain fermentation products (e.g., butyrate, propionate) is only energetically favorable when their catabolic byproduct, H2 or formate, is consumed to low concentrations by MGs. This is considered a form of obligate syntrophy (Table 1).
- Sulfate reducing bacteria (SRB) are capable of utilizing a larger variety of substrates than MG, including substrates degraded by methanogenic syntrophy (e.g., butyrate, propionate).
- The introduction of sulfate (SO4²⁻) into TFW via saltwater intrusion events may allow SRB competition to enter the community and have a significant impact on the rate at which CH4 is produced. The critical role of SRB in the breakdown of butyrate and its contribution to the microbial communities in the Cumberland tidal freshwater marshes (Figure 1). This may select for MG taxa that differ in their CH4 production.

**Experimental design**
- SRB competition was introduced in the SO4²⁻ / NaCl treatment (Fig. 3).
- The SRB competition was then removed using the SRB inhibitor MoO4²⁻ to allow MG and syntrophic bacteria to recover (Fig. 3).
- At each sampling event, the gas production rates from the microcosms (n=3) from the “S” labeled treatment groups were taken (Fig. 3).
- The ability of the microcosms (n=3) from the “S” labeled treatment groups to breakdown 2.5 mM butyrate and the contribution of the different microbial groups was determined at each sampling event (Fig. 3).
- The percentage of measurable carbon species relative to the initial total carbon measured for the proportion of measurable carbon species relative to the initial total carbon measured for the microcosms were incubated in 2.5 mM butyrate and 50 mM BEISA to inhibit MG activity (Fig. 4b). H2 > 100 Pa to inhibit syntrophic bacteria (Fig. 4c), or in no inhibitor as a control (Fig. 4a).
- Butyrate breakdown occurred more slowly in the SRB treatment (Fig. 6c) compared to the fresh control (Fig. 6d). The SRB inhibition of MG via BESA (Fig. 6d & 6e) in the SRB treatment resulted in slower butyrate breakdown and significantly less CH4 production rate was decreased by the inhibition of MG (Fig. 6c).
- The CH4 production rates did not recover to similar levels of the fresh control after SRB competition had been removed. However, CH4 production rates were also lower in the SRB treatment indicating that the inhibition of CH4 production rates to recover may be a result of salinity stress rather than the last effect of SRB competition (Fig. 5).

**Table 1.** The Gibbs free energy of syntrophic butyrate degradation. * Table modified from Stams and Plugge (2005) and Muzyer and Stams (2008). 

<table>
<thead>
<tr>
<th>Reaction</th>
<th>ΔG°</th>
<th>298K</th>
<th>308K</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2 + CO2 → CH4 + H2O</td>
<td>-214.5</td>
<td>-216.2</td>
<td></td>
</tr>
<tr>
<td>H2 + CH3COO⁻ → CH4 + H+ + CO3²⁻</td>
<td>-204.3</td>
<td>-206.0</td>
<td></td>
</tr>
<tr>
<td>H2 + CH3COO⁻ → CH4 + H+ + CO3²⁻</td>
<td>-204.3</td>
<td>-206.0</td>
<td></td>
</tr>
<tr>
<td>H2 + CH3COO⁻ → CH4 + H+ + CO3²⁻</td>
<td>-204.3</td>
<td>-206.0</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 3.** Green bottles indicate 1.) fresh controls receiving no treatment, blue bottles received 2.5 mM NaCl treatment. Brown bottles received 4.) NaCl treatment. Purple bottles received 3.) MoO4²⁻ treatment. Treatments marked marked “S” were sampled during the respective sampling event.

**Figure 4.** The percentage of measureable carbon species relative to the initial total carbon measured for microcosms assessed during the initial sampling event. Fresh control microcosms were incubated in 2.5 mM butyrate with no inhibitors. The SO4²⁻ treatment group was incubated in 2.5 mM butyrate and 2.5 mM MoO4²⁻ to determine the role of MG (b), 50 mM BEISA to determine the role of MG (a), or no inhibitor control (c). (A) graph depicts formate as a percentage of initial carbon for the butyrate assay in (a-d).

- While the uninhibited SO4²⁻ treatment broke down butyrate down the fastest (Fig. 6c), the breakdown appeared to be mediated through both SRB and syntrophy. This is evident by the appreciable accumulation of CH4 and formate (Fig. 6c & 6e) in the SRB²⁻ treatment. The inhibition of MG via BEISA (Fig. 6d & 6e) in the SO4²⁻ treatment resulted slower butyrate breakdown and significantly less formate production than when both MG and SRB were uninhibited in the SO4²⁻ treatment (Fig. 6c).
- Although SRB are capable of utilizing acetate. MG seem to be the primary agent as significantly larger quantities of acetate accumulated when MG was inhibited (Fig. 6d).

**Figure 5.** The CH4 production rates for each of the treatment groups at each sampling event. Colours correspond to the treatment groups in figure 2.

**Figure 6.** The percentage of measureable carbon species relative to the initial total carbon measured for microcosms assessed during the treatment sampling event. Fresh control microcosms were incubated in 2.5 mM butyrate with no inhibitors. The SO4²⁻ treatment group was incubated in 2.5 mM butyrate and 2.5 mM MoO4²⁻ to determine the role of MG (b), 50 mM BEISA to determine the role of MG (d), or no inhibitor control (a). (b) graph depicts acetate as a percentage of initial carbon for the butyrate assay in (a-d).

- Although soil slurries recovering from SRB competition produced slightly less CH4, and broke down butyrate at a slightly slower rate, these differences were not enough to conclude that the syntrophic bacteria and MG had not recovered similar function to the fresh control (Fig. 7).
- Conclusions: The syntrophic bacteria, MG, and SRB all seem to active in breaking down butyrate when 4 mM SO4²⁻ is present. The ability of the MG and syntrophic bacteria to functionally recover from SRB competition is limited, possibly as a result of their ability to maintain a metabolic functions during this competitive stress. There is a decrease in CH4 production rates but it is difficult to determine whether this is a result of changes in the MG community as a result of SRB competition or salinity affecting stimulation.

**Acknowledgements:** Special thanks to the VCU Rice Center for their generous funding.

**Work Cited**

**Figure 7.** The percentage of measureable carbon species relative to the initial total carbon measured for microcosms assessed during the recovery sampling event. Fresh control (a) and recovery treatment (b) microcosms were incubated in 2.5 mM butyrate. *indicates no gas measurement were taken.