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Assessing how disruption of methanogenic communities and their syntrophic relationships in tidal freshwater marshes via saltwater intrusion may affect CH_4 emissions

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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 CH_4 production, but it is uncertain to what extent elevated salinity will affect CH_4 cycling in TFW. It is also unknown whether CH_4 production will resume when freshwater conditions return.
- The ability to produce CH_4 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, H_2, 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, H_2 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 (SO_4^{-2}) into TFW via saltwater intrusion events may allow SRB to disrupt syntrophic relationships between hydrogenotrophic MG and syntrophic fermenters (Figure 1). This may select for MG taxa that differ in their rate of CH_4 production.

Objectives

1. Determine the effect of oligohaline SO_4^{-2} concentrations on MG community functions (i.e., CH_4 production and syntrophic butyrate degradation).
2. Assess whether these functions recover after competition with SRB has been removed.

Approach

- Freshwater 30% (wt/vol) anaerobic microcosms were constructed with soil and pore water from Cumberland Marsh, a TFW located on the Pamunkey River, Virginia.
- Treated using various combinations of the following amendments:
  - 4 mM Na2SO4 to increase [SO4^{-2}] as would occur with saltwater intrusion
  - 12 mM NaCl to control for the effect of increased ionic strength without increasing SO4^{-2} availability
  - 2.5 mM MoO4^{-2} (Na2MoO4), a SRB inhibitor
- Additions of 2.5 mM butyrate (n-butyric acid) in combination with inhibitors were used to determine the role of SRB and MG in butyrate breakdown.
  - 5 mM BESA (2-Bromoethanesulfonic acid) a MG inhibitor
  - 5 mM MoO_4^{-2} (Na_2MoO_4)
  - H_2 > 100 Pa

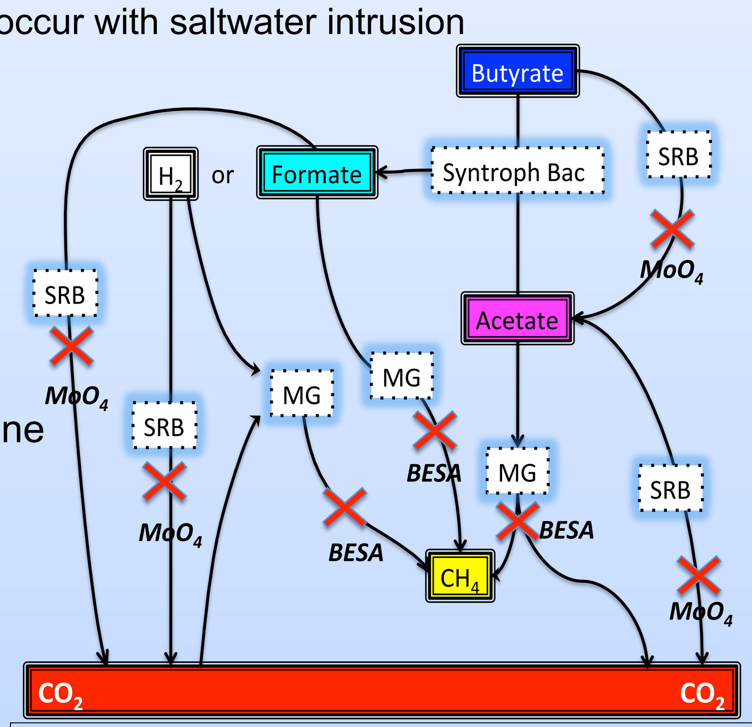


Figure 1. Pathways of butyrate breakdown in anaerobic environments, the microbial groups responsible, and the pathways affected by inhibitors

- We followed the response of the microbial community by monitoring:
  - CH_4 and CO_2 production - gas chromatography
  - Butyrate, acetate, and formate concentrations - ion chromatograph

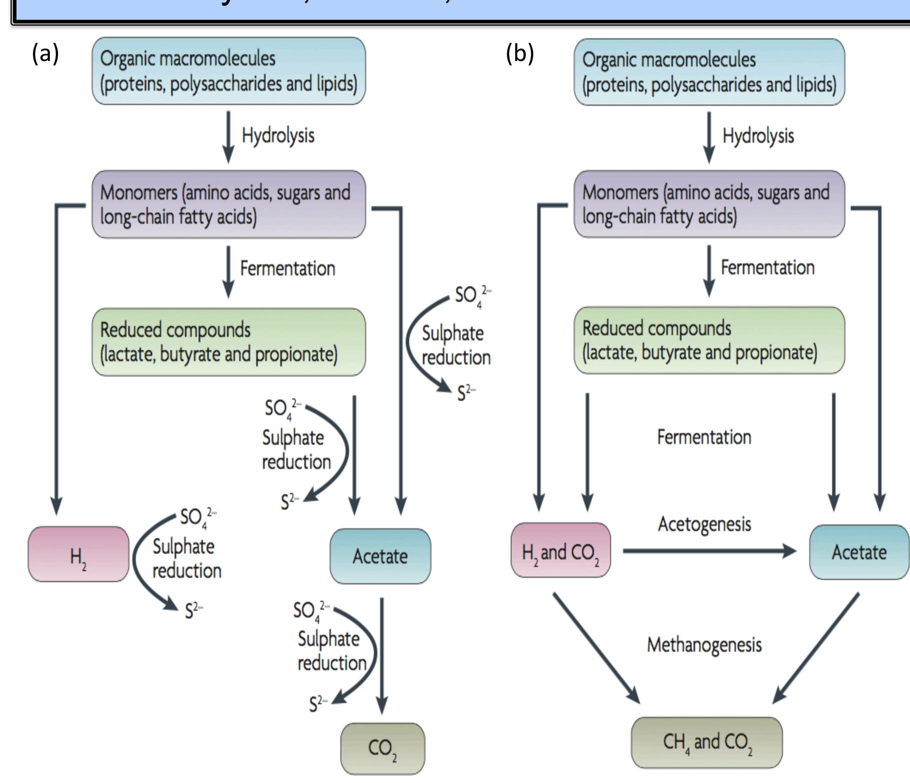


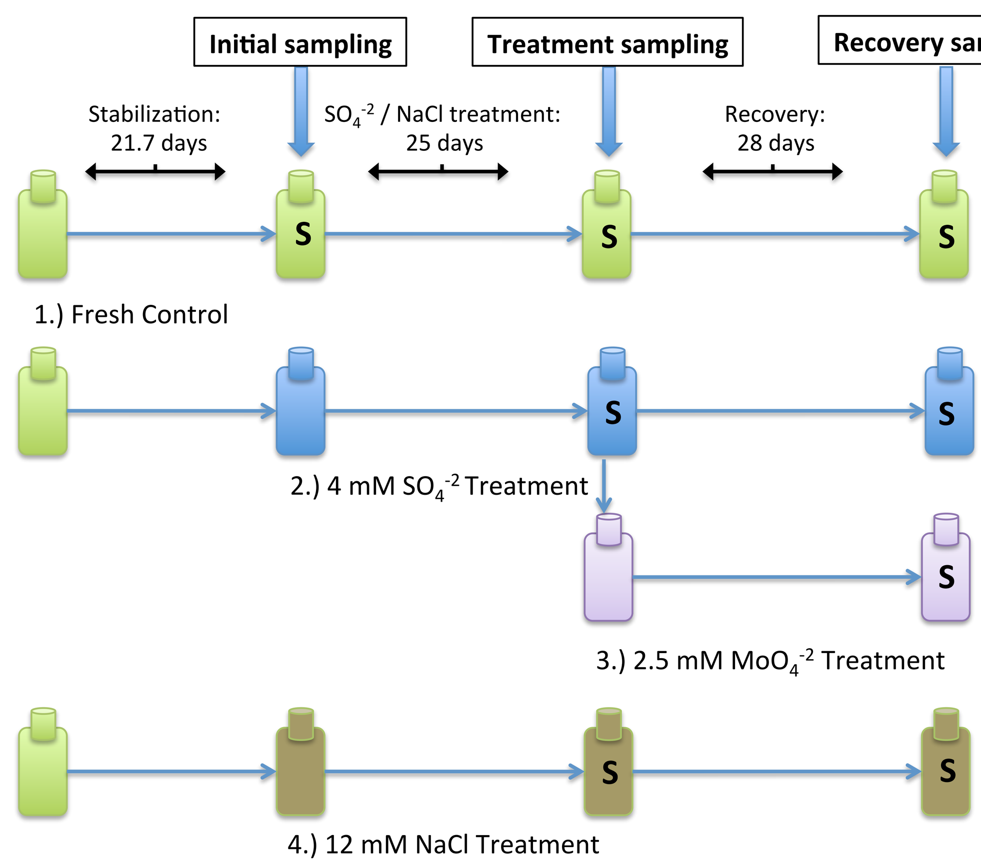
Figure 2. The degradation of organic matter in wetlands, both in the presence of sulfate (a) and in freshwater (b). Diagram from Muyzer and Stams (2008).

Table 1. The Gibb's free energy of syntrophic butyrate degradation.* Table modified from Stams and Plugge (2009) and Muyzer and Stams (2008).

Reaction	ΔG°'	ΔG at 1 Pa H_2
Proton-reducing bacteria		
Propionate⁻ + $2H_2O$ → acetate⁻ + CO_2 + $3H_2$	+72 kJ	–21 kJ
Butyrate⁻ + $2H_2O$ → 2 acetate⁻ + H^+ + $2H_2$	+48 kJ	–22 kJ
Methanogens		
$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	–131 kJ	–15 kJ
Acetate⁻ + H^+ → $CO_2 + CH_4$	–36 kJ	–36 kJ
Sulfate reducers		
Propionate⁻ + 0.75 SO_4^{-2} → Acetate⁻ + HCO_3^- + 0.75 HS^- + 0.25 H^+	–37.7 kJ	–37.7 kJ
Butyrate⁻ + 0.5 SO_4^{-2} → 2Acetate⁻ + 0.5 HS^- + 0.5 H^+	–27.8 kJ	–27.8 kJ

*ΔG°' (Standard Gibbs free energy change) is expressed in kJ mol^{-1} and calculated for H_2 in the gaseous state at 1 Pa, and CH_4 and CO_2 in the gaseous state at 10^4 Pa. All other compounds are calculated at 10 mM.

Experimental design



- SRB competition was introduced in the SO_4^{-2} / NaCl treatment (Fig. 3). The SRB competition was then removed using the SRB inhibitor MoO_4^{-2} to allow MG and syntrophic bacteria to recover (Fig. 3).
- At each sampling event, the gas production rates from the microcosms (n=5) 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)

Figure 3. Green bottles indicate 1.) fresh controls receiving no treatment. Blue bottles received 2.) SO_4^{-2} treatment. Brown bottles received 4.) NaCl treatment. Purple bottles received 3.) MoO_4^{-2} treatment. Treatments marked marked "S" were sampled during the respective sampling event

Demonstrating Syntrophy in a Tidal Freshwater Marsh

- Fresh control microcosms from the the initial sampling event were incubated in 2.5 mM butyrate and 50 mM BESA to inhibit MG activity (Fig. 4b), H_2 > 100 Pa to inhibit syntrophic bacteria (Fig. 4c), or in no inhibitor as a control (Fig. 4a).
- Butyrate remained above 80% of total initial measured carbon for more than 10 days in MG inhibited soil slurries (Fig. 4b). In contrast, fresh control slurries brought butyrate concentrations below 8% over the same time period indicating that methanogens are critical to butyrate breakdown in freshwater environments (Fig 4a).
- Additions of H_2 >100 Pa did not inhibit butyrate breakdown (Fig 4c). This likely reflects the use of formate as an alternative molecule for interspecies electron transport. Formate production was measurable byproduct of butyrate breakdown (Fig 4d).
- **Conclusion: The important role of methanogens in butyrate breakdown and the accumulation of formate indicates the butyrate is degraded by syntrophic fermenters in the Cumberland tidal freshwater marsh.**

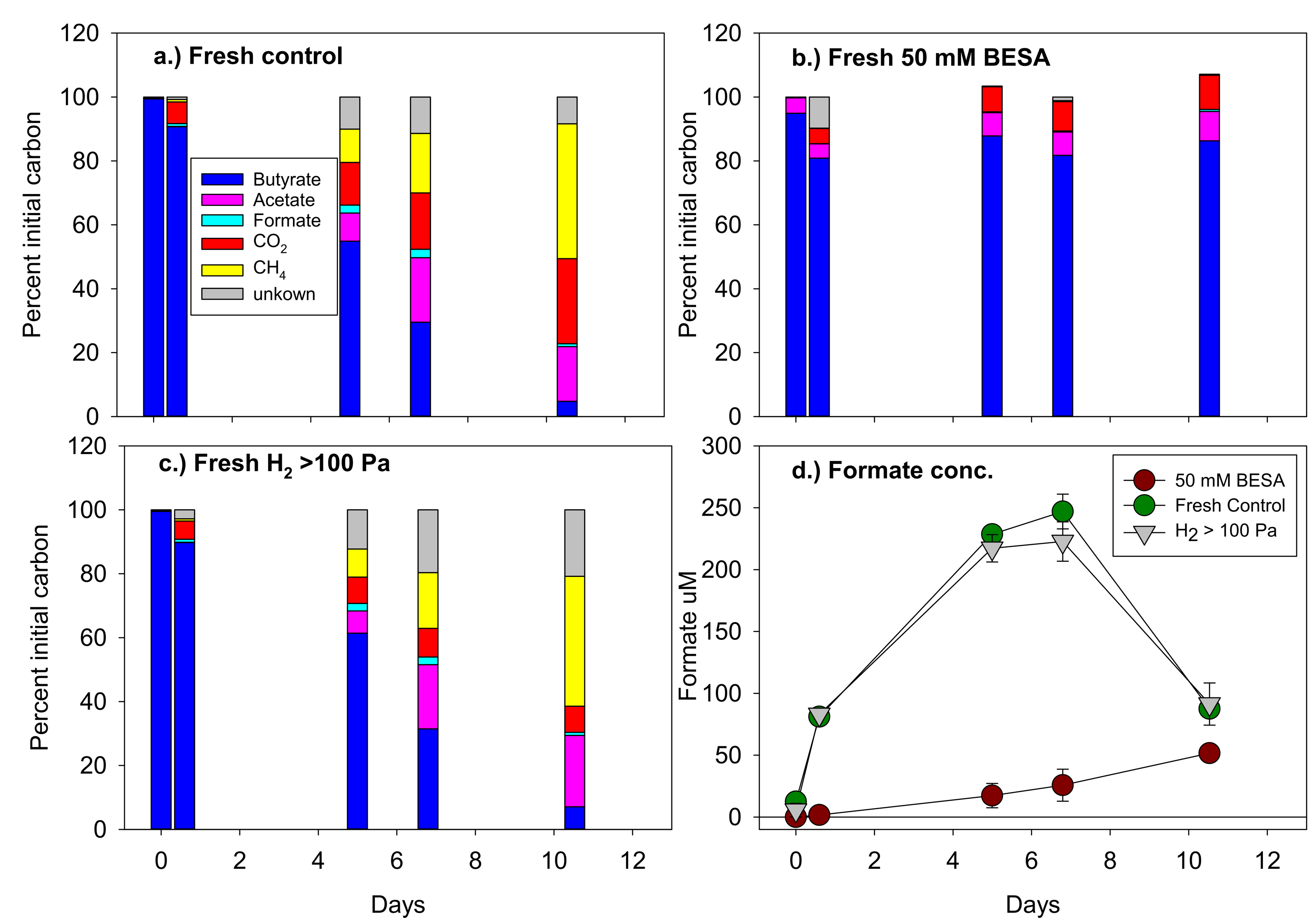


Figure 4. The percentage of measurable carbon species relative to the initial total carbon measured for microcosms assayed during the initial sampling event. Fresh control microcosms were incubated in 2.5 mM butyrate and 50 mM BESA to inhibit MG activity (b), H_2 > 100 Pa to inhibit syntrophic bacteria (c), or in no inhibitor as a control (a). Graph (d) depicts the formate concentrations over these butyrate assays.

Work Cited

Muyzer, G., & Stams, A. J. (2008). The ecology and biotechnology of sulphate-reducing bacteria. Nature Reviews Microbiology, 6(6), 441-454.

Stams, A. J., & Plugge, C. M. (2009). Electron transfer in syntrophic communities of anaerobic bacteria and archaea. Nature Reviews Microbiology, 7(8), 568-577.

The functional response and recovery of microbial communities to SO_4^{-2} availability

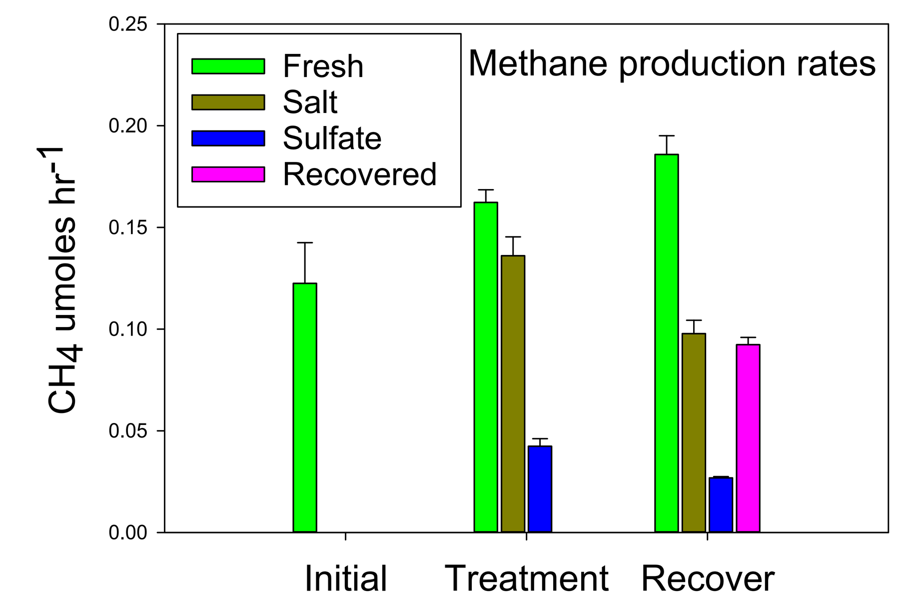


Figure 5. The CH_4 production rates for each of the treatment groups at each sampling event. Colors correspond to the treatment groups in figure 2.

- The CH_4 production rate was decreased by greater than 75% in the 4 mM SO_4^{-2} treatment group relative to the fresh control for both the treatment sampling event and the recovery sampling event (Fig. 5).
- The CH_4 production rates did not recover to similar levels of the fresh control after SRB competition had been removed. However, CH_4 production rates were also lower in the salt control indicating that the inability of CH_4 production rates to recover may be a result of salinity stress rather than the lasting effect of SRB competition (Fig. 5).

Treatment sampling butyrate assay:

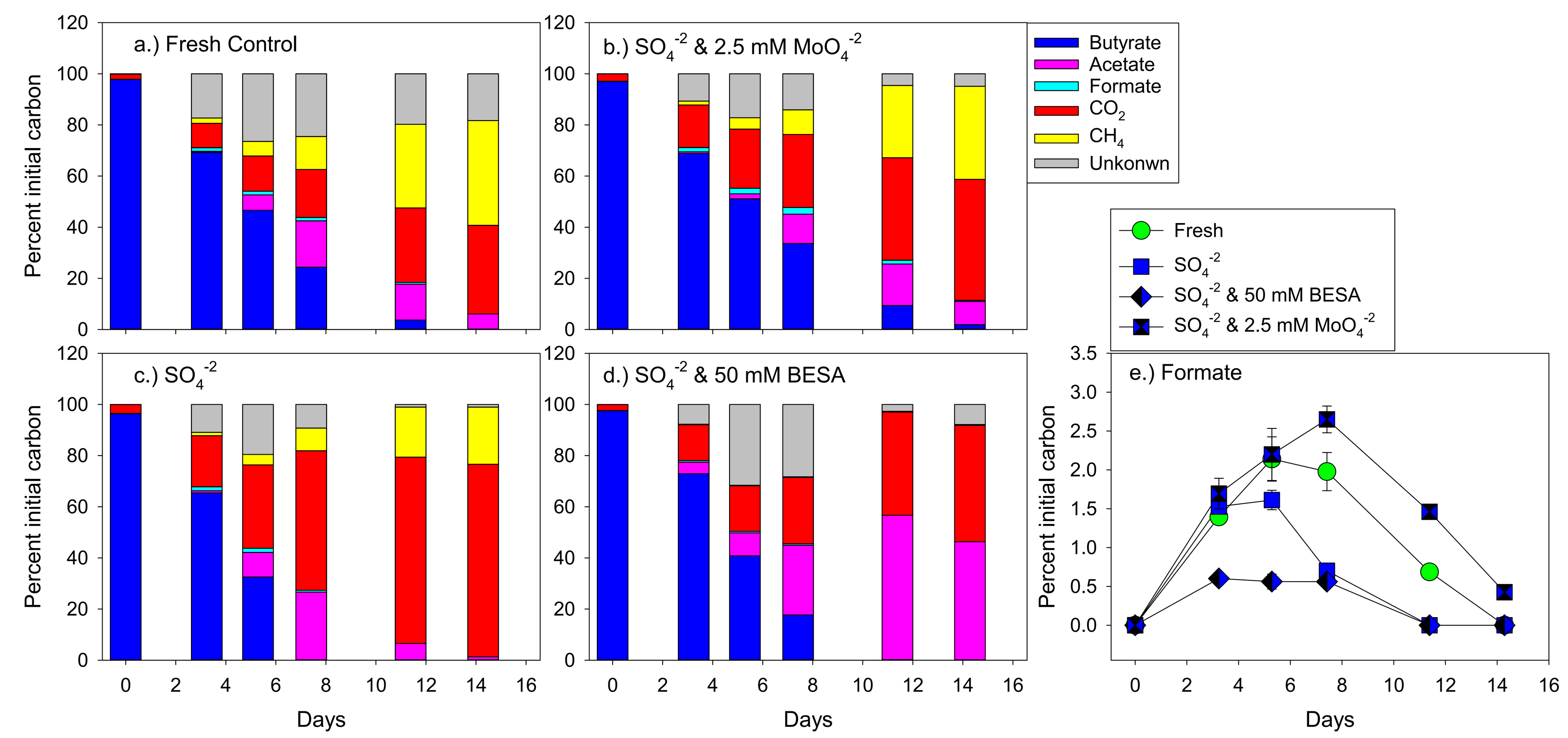


Figure 6. The percentage of measurable carbon species relative to the initial total carbon measured for microcosms assayed during the treatment sampling event. Fresh control microcosms were incubated in 2.5 mM butyrate with no inhibitor(a). The SO_4 treatment group was incubated in 2.5 mM butyrate and 2.5 mM MoO_4^{-2} to determine the role of SRB (b), 50 mM BESA to determine the role of MG (d), or no inhibitor control (c). The (e) graph depicts formate as a percentage of initial carbon for the butyrate assays in (a-d).

- While the uninhibited SO_4^{-2} treatment broke 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 CH_4 and formate (fig. 6c & 6e) in the SO_4^{-2} treatment. The inhibition of MG via BESA (Fig. 6d & 6e) in the SO_4^{-2} treatment resulted slower butyrate breakdown and significantly less formate production than when both MG and SRB were uninhibited in the SO_4^{-2} 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. d)

Recovery sampling butyrate assay:

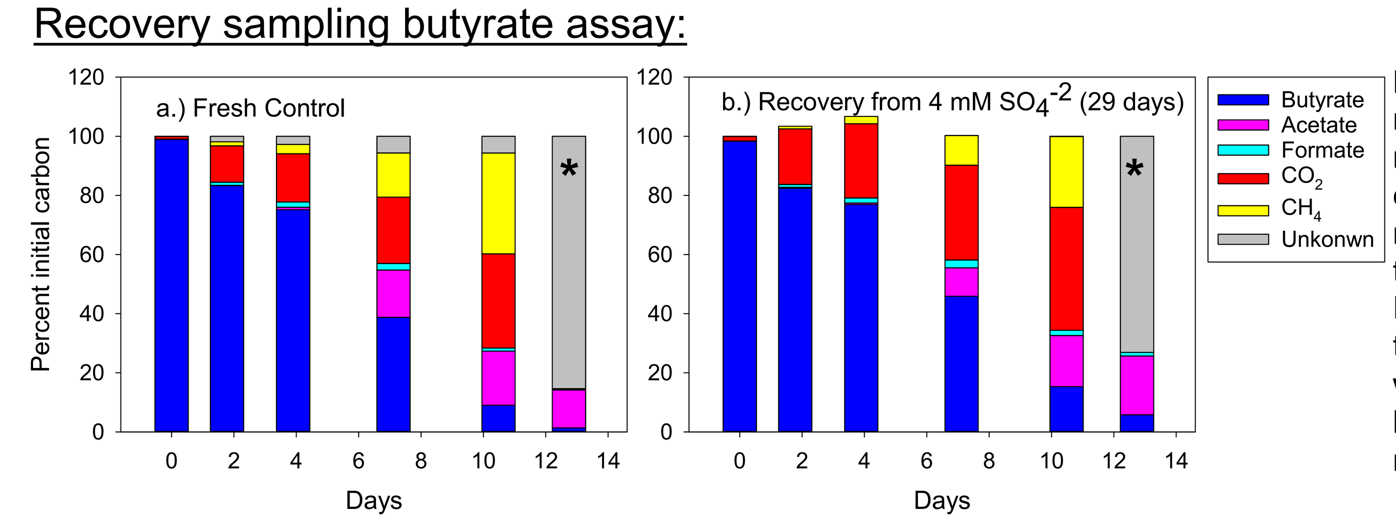


Figure 7. The percentage of measurable carbon species relative to the initial total carbon measured for microcosms assayed during the treatment sampling event. Fresh control (a) and recovery treatment (b) microcosms were incubated in 2.5 mM butyrate. " * " indicates no gas measurement were taken.

- Although soil slurries recovering from SRB competition produced slightly less CH_4, and broke down butyrate at slightly slower rate, these differences were not great 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 be active in breaking down butyrate when 4 mM SO_4 is present. The ability of the MG and syntrophic bacteria to functionally recover from SRB competitive stress is likely a result of their ability to maintain a metabolic functions during this competitive stress. There is a decrease in CH_4 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 metabolic activity.**

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