The Effect of Saltwater Intrusion on Microbial Community Structure and Function in a Tidal Freshwater Marsh

DOE National Institute for Climatic Change Research

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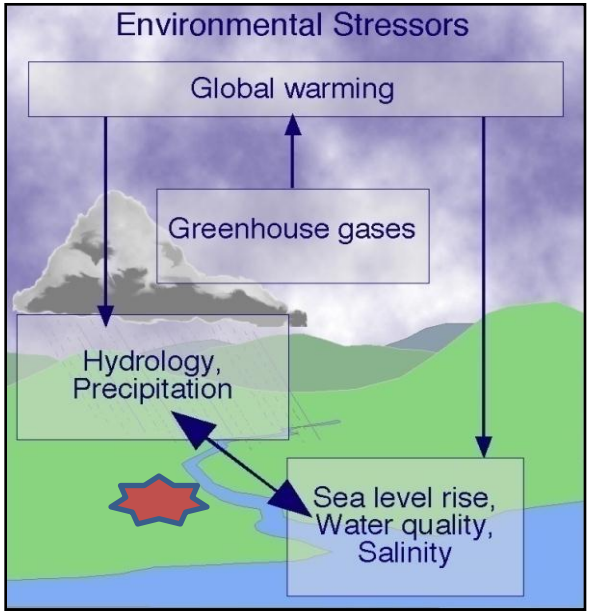
Salinity Gradients & Climate Change



Neubauer & Craft. 2009. Global change and TFW.

- Freshwater wetlands
  - Saltwater intrusion
    - Low freshwater flow (e.g., drought)
    - Sea level rise from global warming
- Salt marsh
  - Salinity decrease from increased freshwater discharge

Ecosystem Responses

- **Physico-chemical environment**
  - Altered hydrologic regime
  - Change in soil moisture & redox
  - Ionic strength effects sorption & cation exchange
- **Vegetation**
  - Salt tolerance & preferred flooding regimes
  - Shift in community composition
  - Changes in productivity & respiration
- **Biogeochemistry**
  - Major impact on C, N, and S cycles
  - Altered rates of C sequestration, quality of the stored C, and fluxes of CH_4 and CO_2 to the atmosphere

Microbial Community Response

Changes in hydrology & salinity could alter…

Microbial Community Response

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- **Organic C quality and quantity (direct & indirect effects)**

Microbial Community Response

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- **Available terminal e^- acceptors for anaerobic decomposition**

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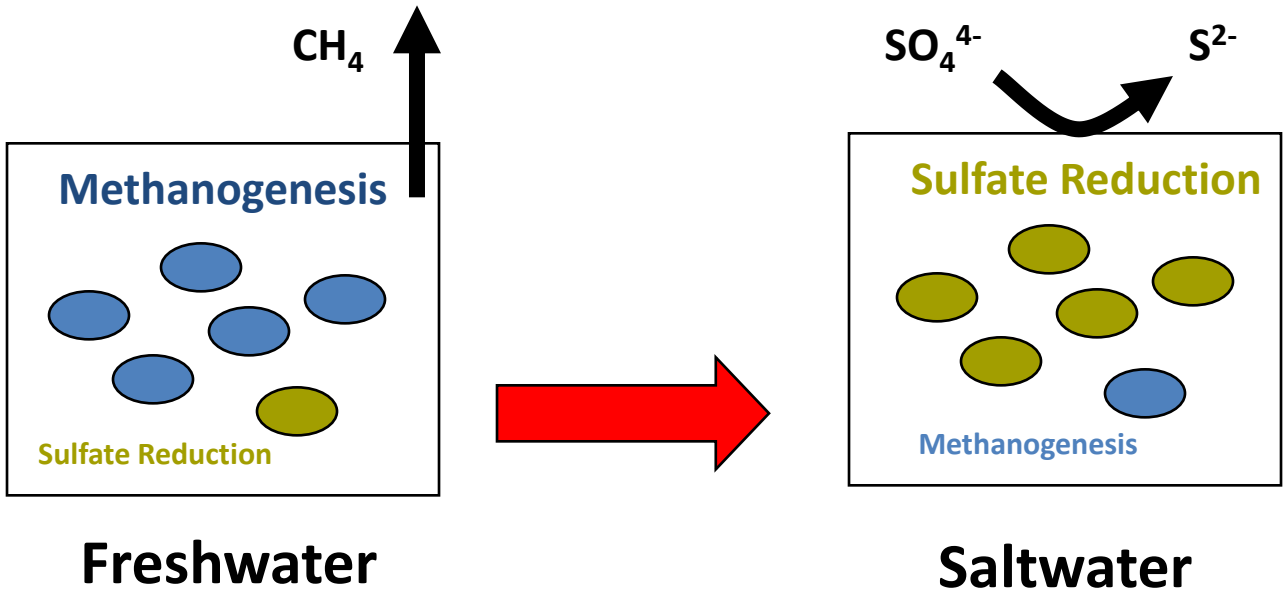
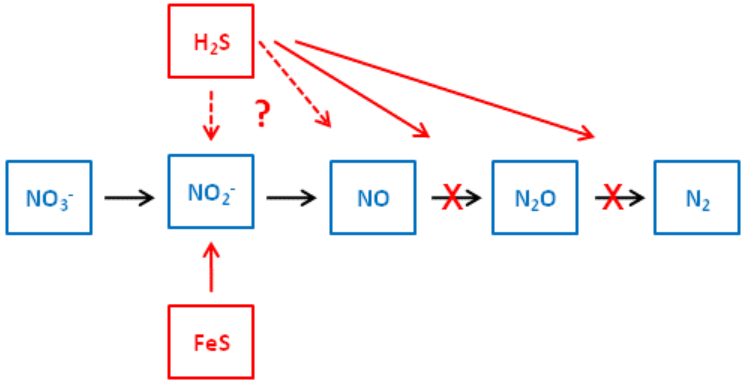


Microbial Community Response

Changes in hydrology & salinity could alter…

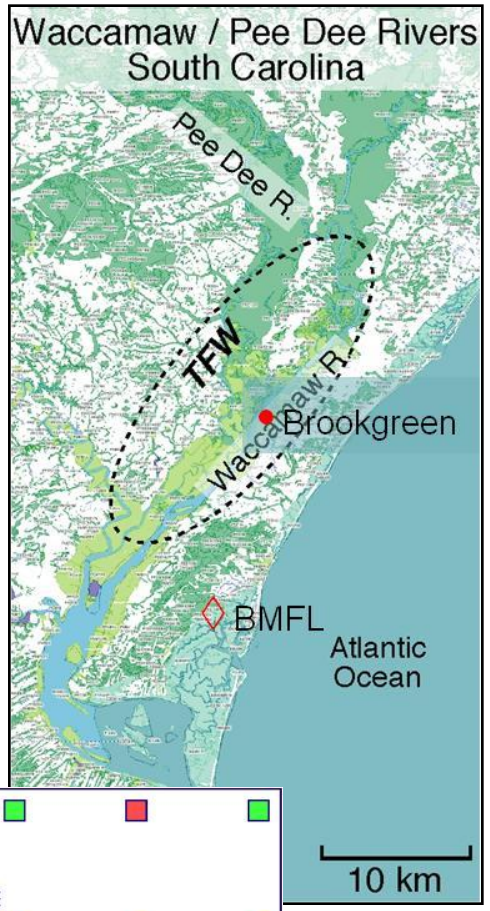
- Organic C quality and quantity (direct & indirect effects)
- Available terminal e^- acceptors for anaerobic decomposition
- **H_2S toxicity, produced by SO_4^- reduction**
  - Inhibits nitrification, less NO_3^- for denitrification
  - Direct toxicity to enzymes in the denitrification sequence

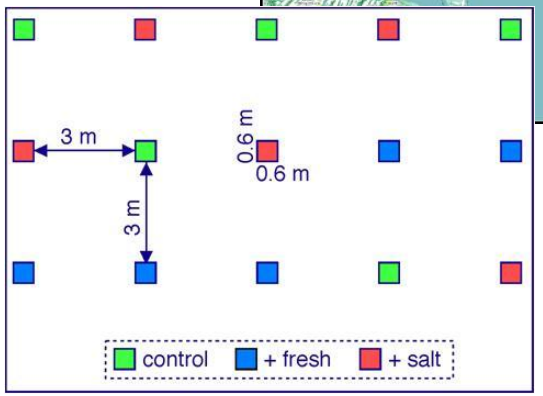


Experimental Approach

- Brookgreen Marsh
  - Tidal freshwater
  - Semi-diurnal tides
  - 30+ herbaceous plant species
  - Organic-rich soils (~60% OM, ~30% C)

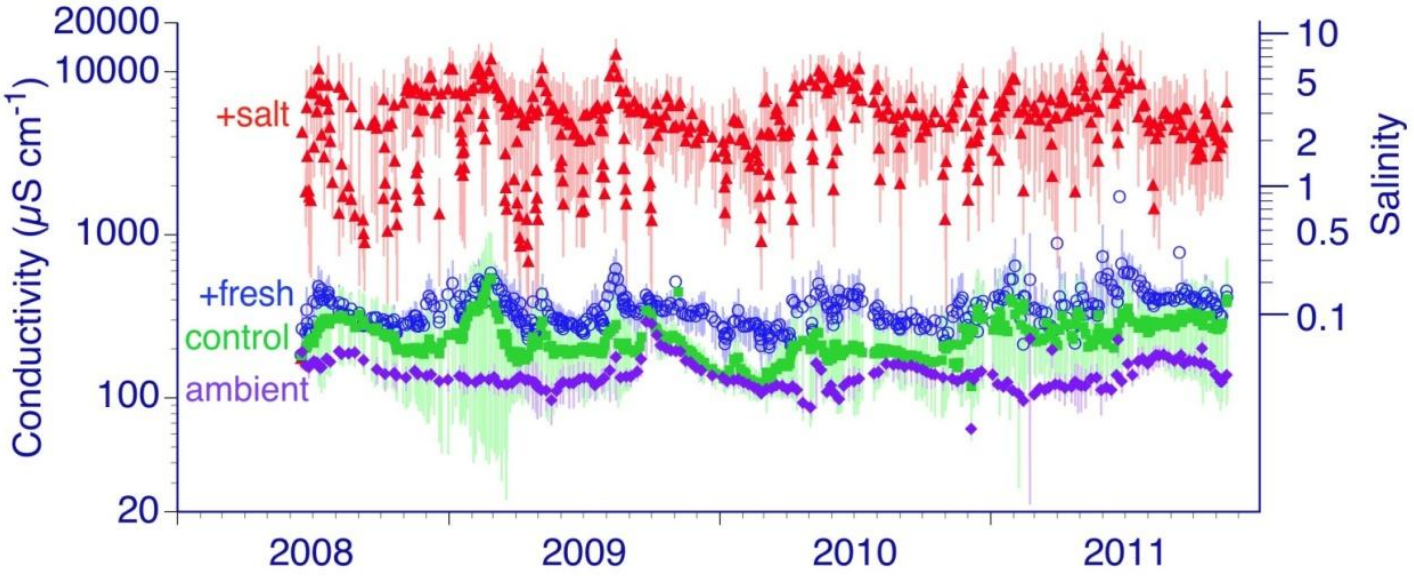
- Field manipulation
  - 40 L fresh or brackish water, 2X per week
  - Control, +Fresh, +Salt (N=5 of each)
  - June 2008 through Nov 2011





Treatment Effective

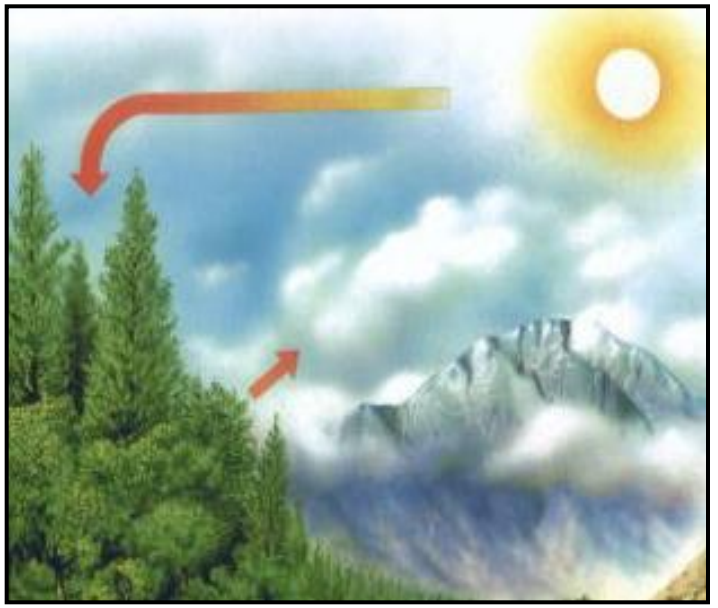
Porewater showed consistent increase in salinity



Control, +fresh, and +salt data: n = 5 plots x 2 depths (10 & 25 cm) per plot;
Ambient data: n = 2 locations x 2 depths per location

Analyses Performed

- Vegetation
  - Community composition & biomass
  - Photosynthesis and leaf fluorescence
- Biogeochemistry
  - CO_2 and CH_4 fluxes
  - Sediment accretion (^{137}Cs dating)
  - Soil CN analysis, % organic, bulk density
  - Porewater chemistry
- Microbial Community
  - Extracellular enzyme activity
  - Soil O_2 Demand (SOD)*
  - Denitrification rates (N_2 via MIMS)*
  - Denitrifies, Methanogens, and Sulfate Reducers



Saltwater Intrusion into Tidal Freshwater Marshes Drives Shifts at all Levels of Ecosystem Organization

Scott Neubauer
Thursday at 1:40
In Bonaire 5&6

** Data from Mike Piehler, UNC*

Analyses Performed

- Vegetation
  - Community composition & biomass
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- Biogeochemistry
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  - Soil CN analysis, % organic, bulk density
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- **Microbial Community**
  - **Extracellular enzyme activity**
  - **Soil O_2 Demand (SOD)***
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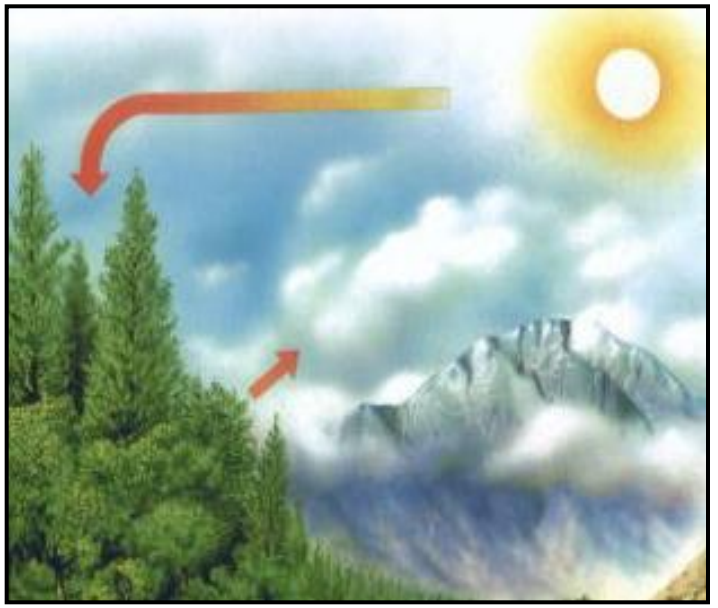

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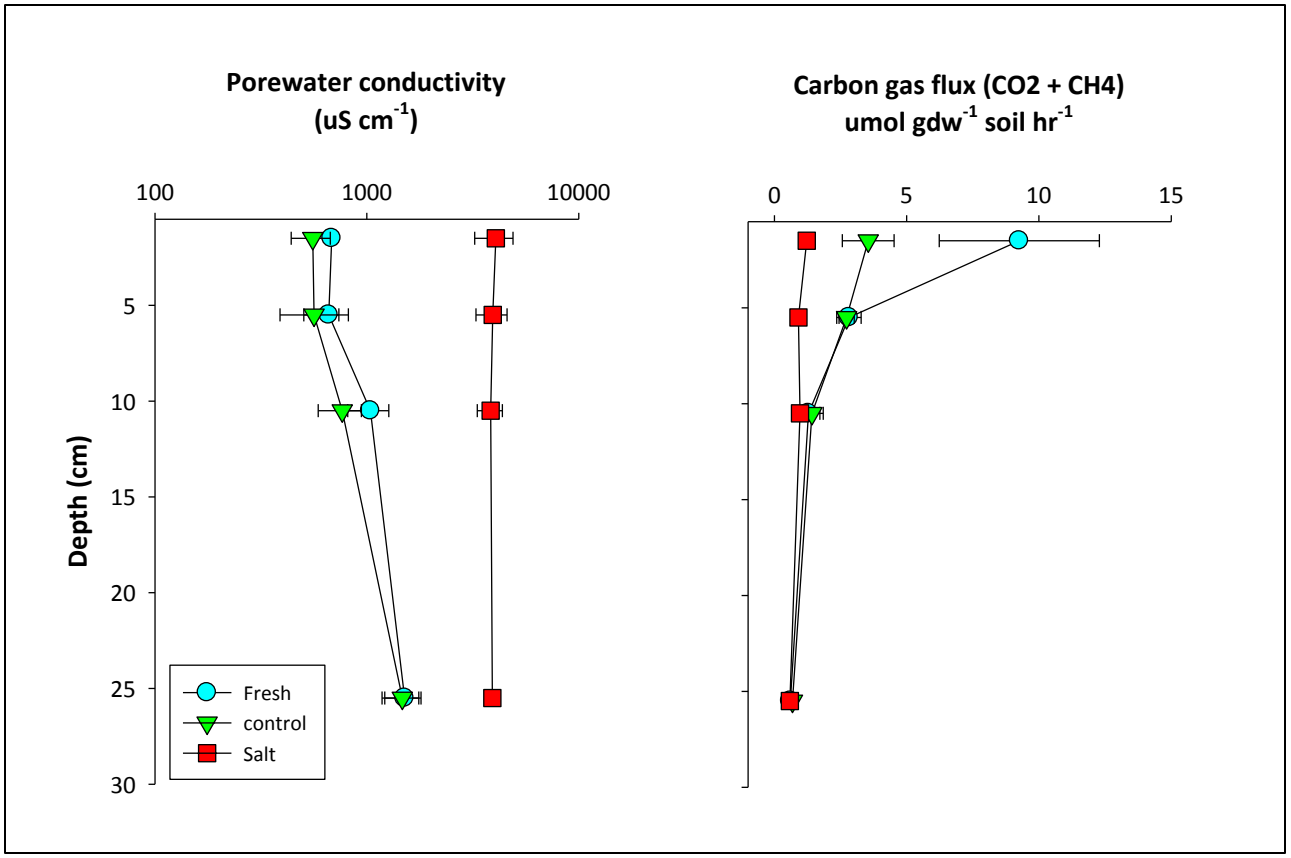
Sampling Approach

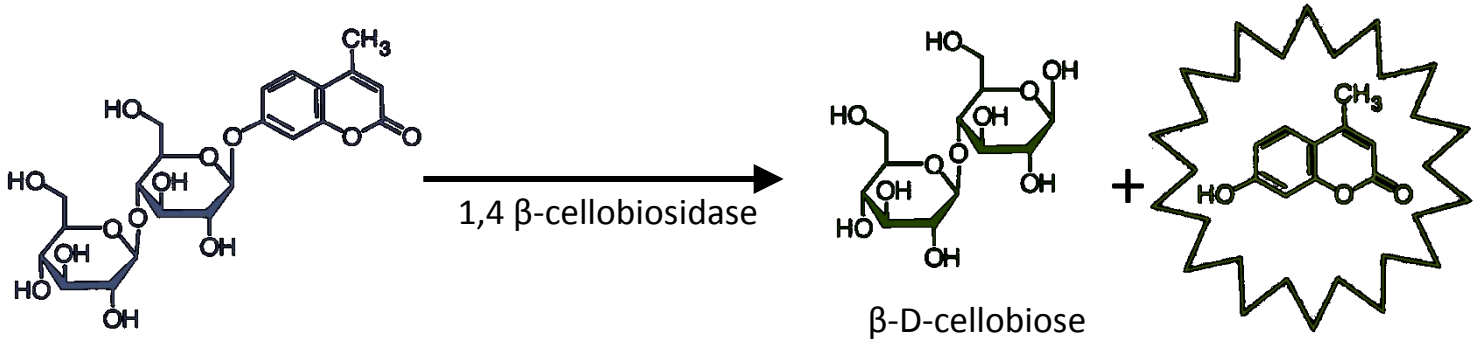
- 0-3, 3-8, 8-13 and 23-28 cm below surface
- Small plots precluded full suite of analyses at all sampling events
- Today: 0-3 cm (greatest activity) and Nov 2011 (final conditions)



Extracellular Enzyme Assays

Produced by microbes to target local substrate conditions, community function assay & proxy for OM quality

Targets labile carbon	**Targets recalcitrant carbon**
• β-1,4-glucosidase E.C.# 3.2.1.21	• β-D-xylosidase E.C.# 3.2.1.37
• 1,4-β-cellobiosidase E.C.# 3.2.1.37	• Phenol oxidase E.C.# 1.10.3.2

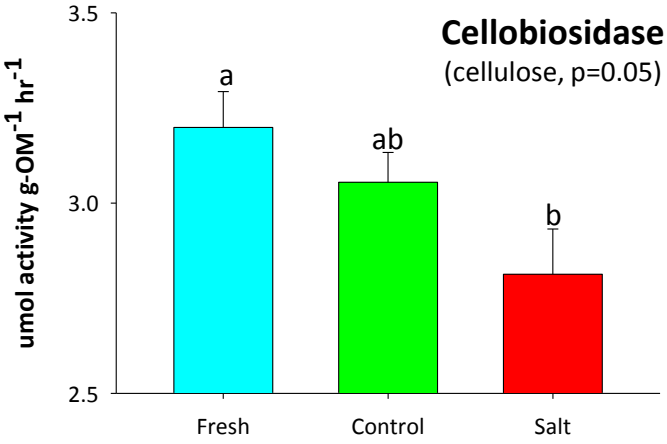


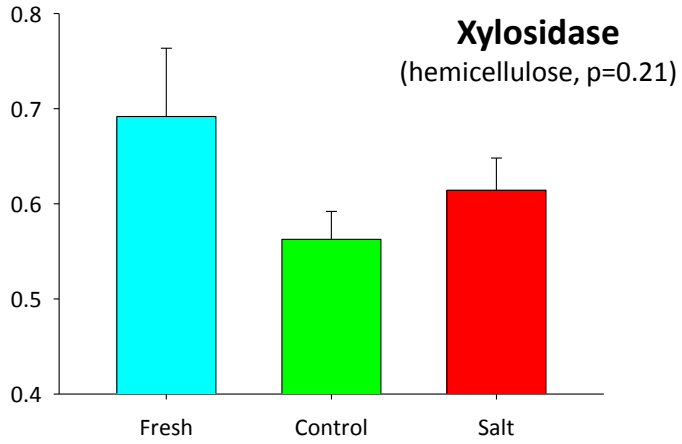
4-Methylumbelliferyl β-D-cellobioside
(proxy for cellulose)

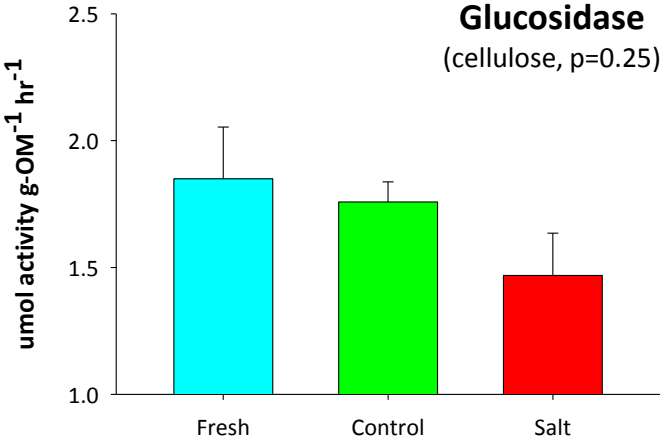
β-D-cellobiose

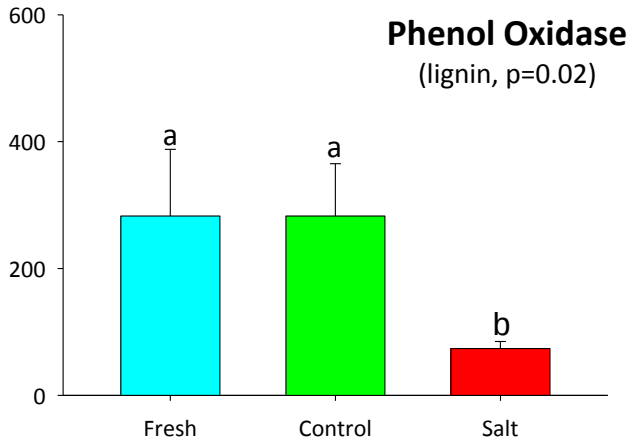
Methylumbelliferone
+ fluorescence

Treatment Effects - Soil Enzyme Activity

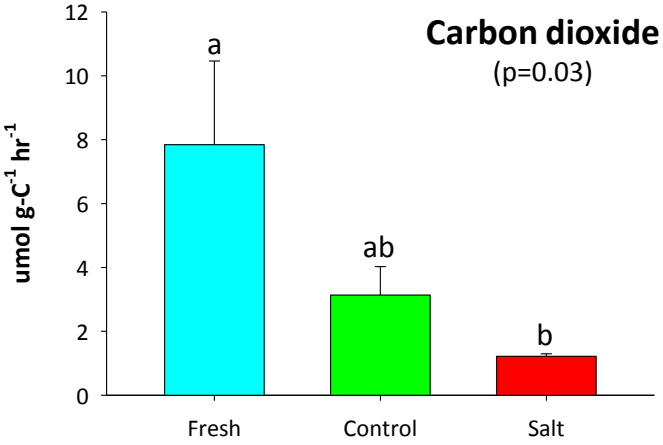


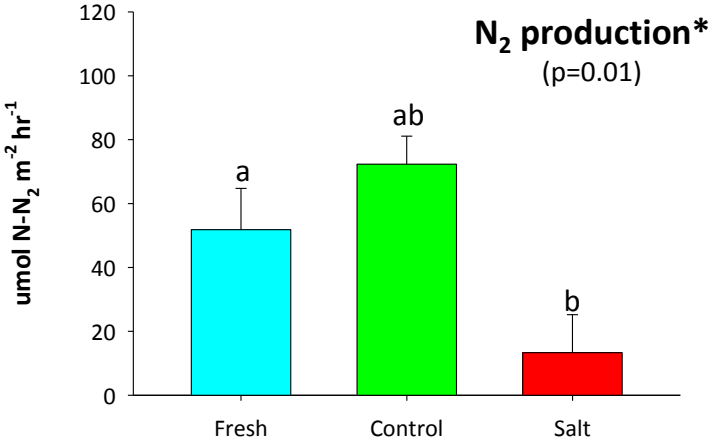


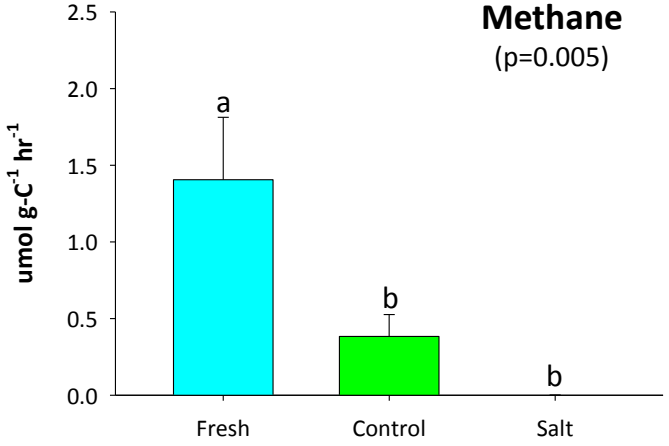


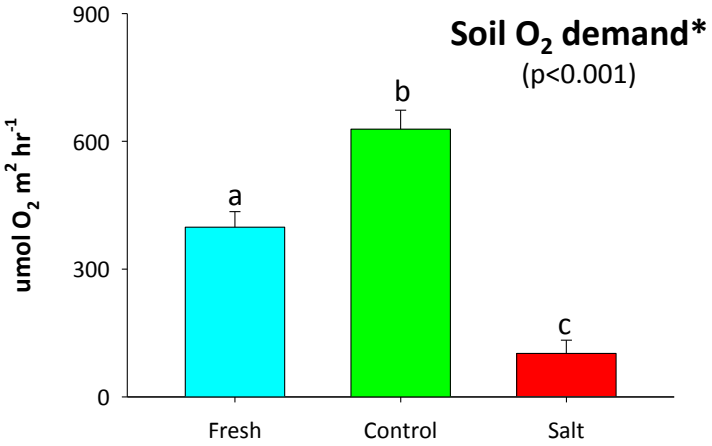


Treatment Effects – Gas Fluxes & SOD









** Data from Mike Piehler, UNC*

Summary - Function

- Enzyme assays
  - Salt = activity decreased in 3/4 substrates
  - Fresh = same or slightly greater than controls
  - Similar for labile & recalcitrant material?

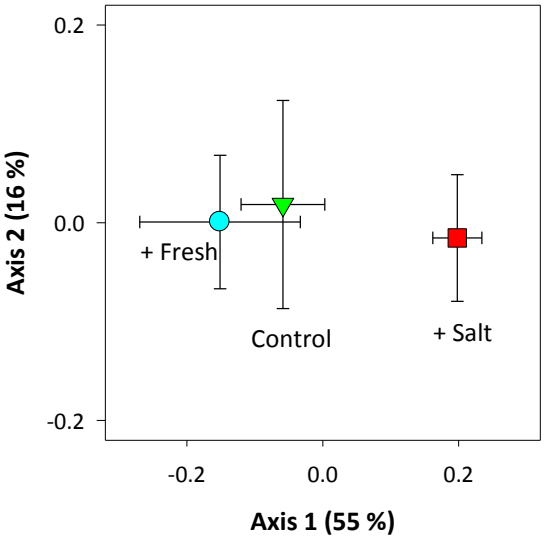
- Gas fluxes and SOD
  - Salt = always lower
  - Fresh = CO_2 & CH_4 flux increased over control

- Do we also see changes in microbial community structure? Can we link composition shifts to function?

Treatment Effects – Microbial Community Structure

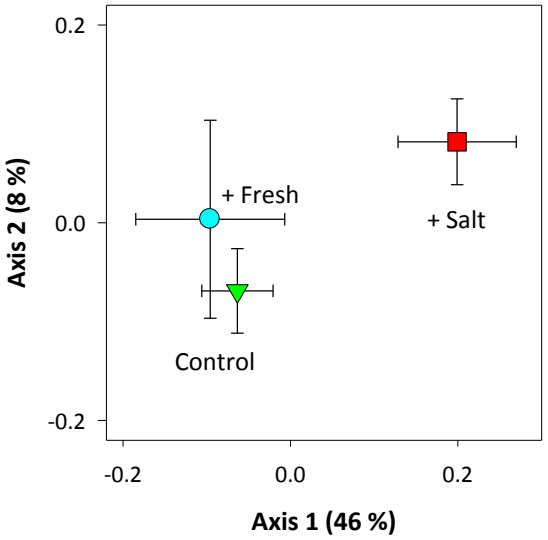
T-RFLP targeting conserved functional genes for each redox group

Sulfate Reducers



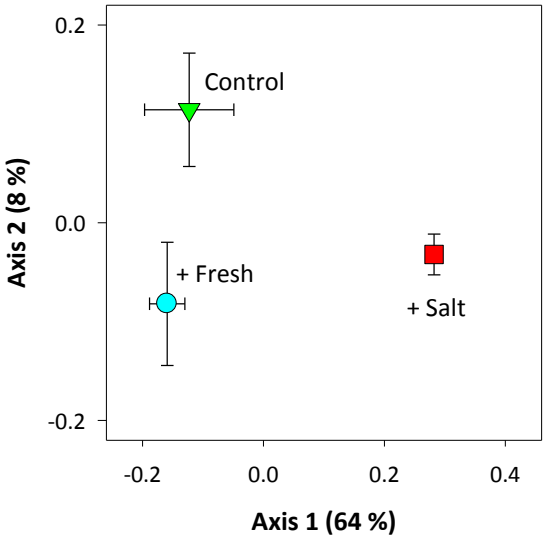
NDMS 2D Stress = 0.17
Saltwater addition vs others ($p<0.05$)
dsrA (Santillano et al 2010)

Methanogens

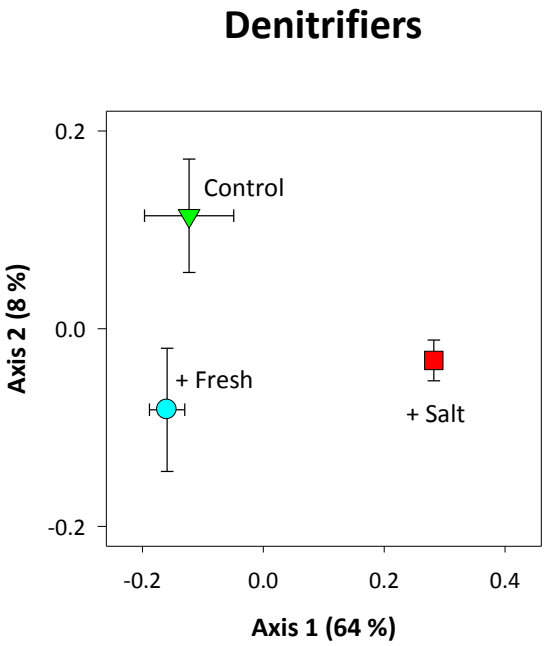


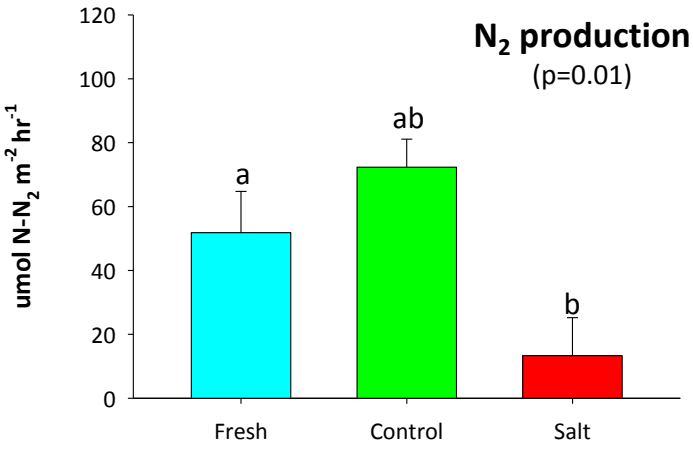
NDMS 2D Stress = 0.19
Saltwater addition vs others ($p<0.05$)
mcrA (Marchesi et al. 2001)

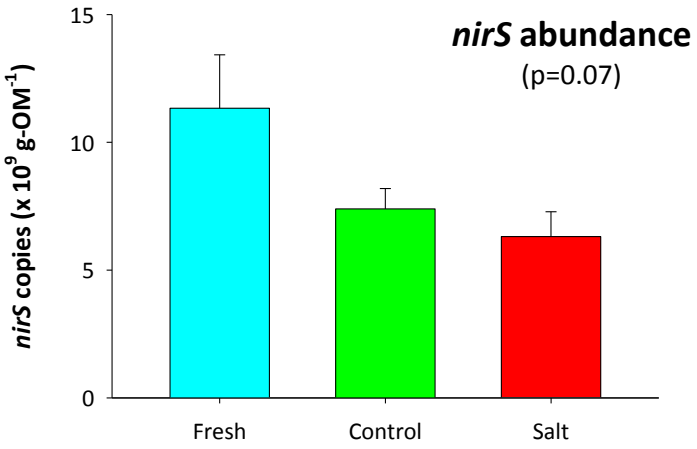
Denitrifiers



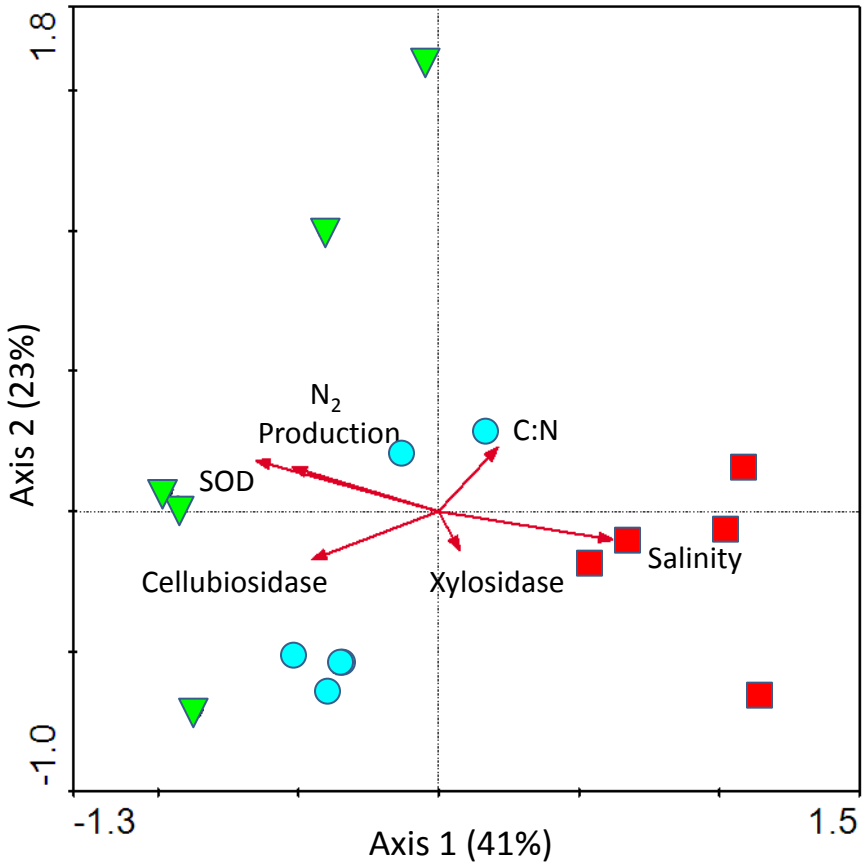
NDMS 2D Stress = 0.16
All treatments differ ($p<0.02$)
NirS (Wolfsing & Prieme 2004)


Denitrifiers
0.2
0.0
-0.2
Axis 2 (8 %)
Control
+ Fresh
+ Salt
-0.2
0.0
0.2
0.4
Axis 1 (64 %)


N2 production
(p=0.01)
umol N-N2 m-2 hr-1
120
100
80
60
40
20
0
a
ab
b
Fresh
Control
Salt


nirS abundance
(p=0.07)
nirS copies (x 10^9 g-OM^-1)
15
10
5
0
Fresh
Control
Salt

Structure-Function Relationships



- Redundancy analysis of denitrifier community composition
- 40% of community variation explained by these variables
- C:N and SOD included – any variation explained by carbon quality differences as compared to function?
- Axis 1 (N_2 Production, SOD, cellobiosidase, and salinity) explains the greatest amount of variation

Conclusions

- Microbial community structure-function linked
  - *Composition of functional groups significantly affected by saltwater intrusion*
  - *Composition correlated with changes in OM quality and Nitrogen gas production.*

- Added freshwater inputs
  - *Few changes for functional assays (denitrification & EEA) or community composition, but increases in CO_2 & CH_4.*

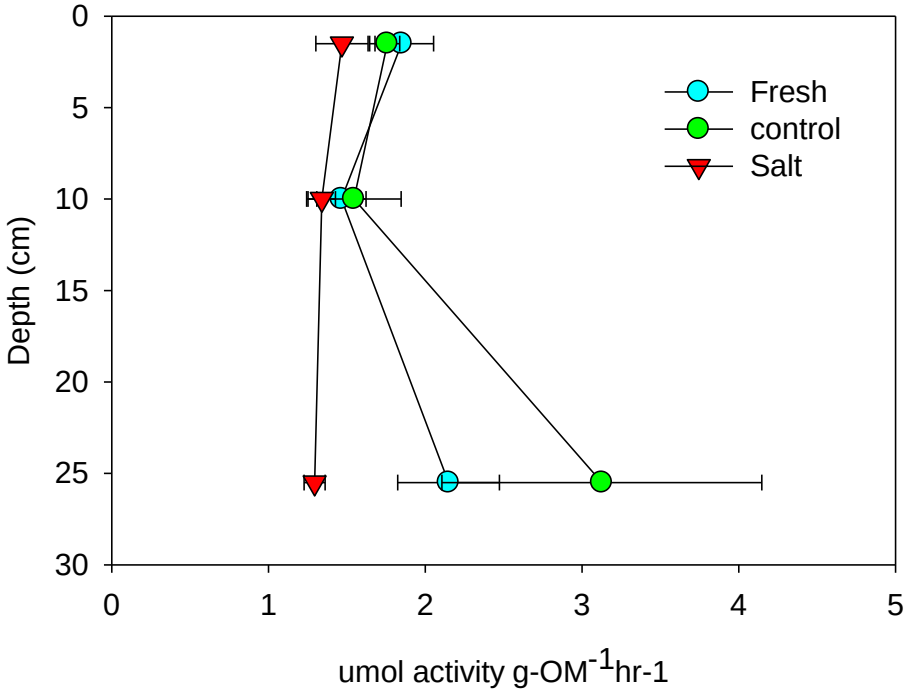
- Saltwater intrusion
  - *Breakdown of both labile and recalcitrant OM may be reduced. Potential changes in C storage and greenhouse gas emissions?*
  - *Lower N_2 production suggests denitrification rates maybe reduced in cases of salt water intrusion – impact on coastal eutrophication?*

Thanks:

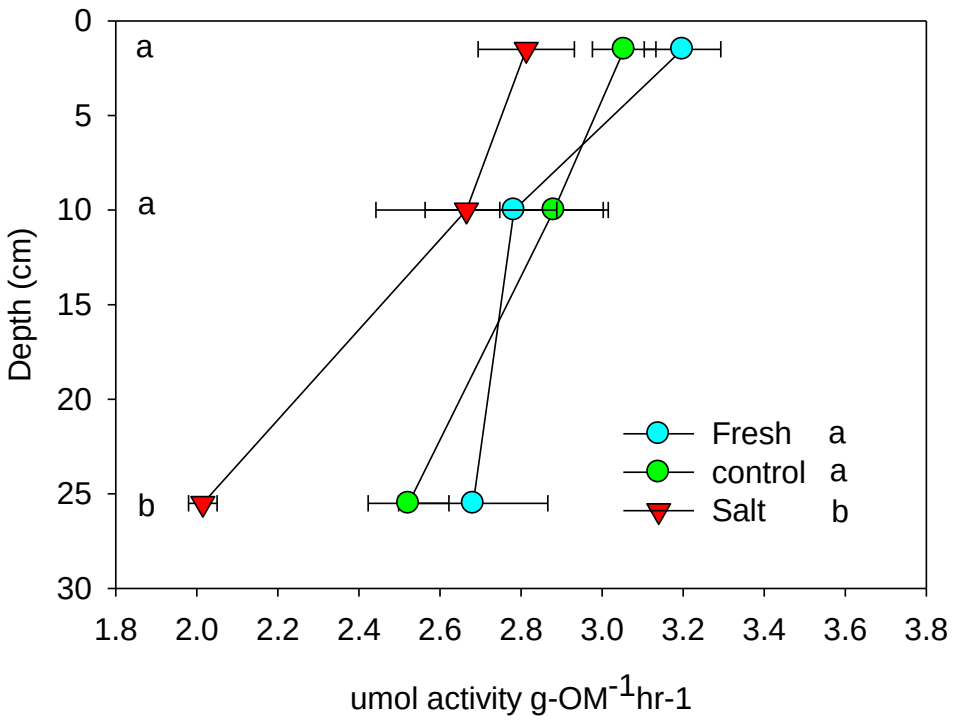
- Scott C. Neubauer for the opportunity to collaborate
- Ember Morrissey for patience and ideas
- Christine Prasse for enzyme wizardry
- And to Rima and the Franklin lab for putting up with my antics

Labile Enzyme Activity

Glucosidase



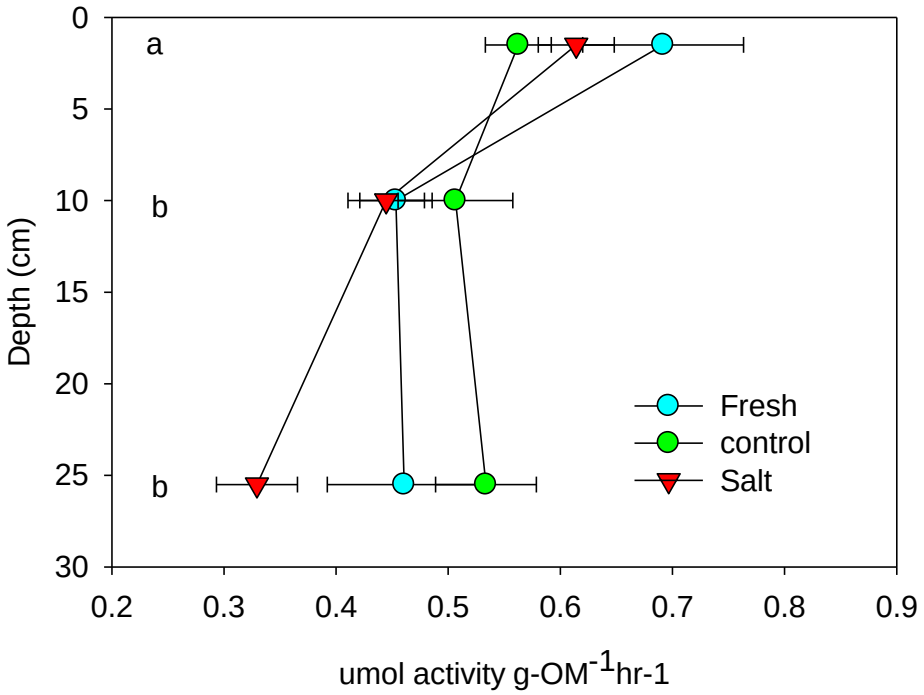
Cellobiosidase



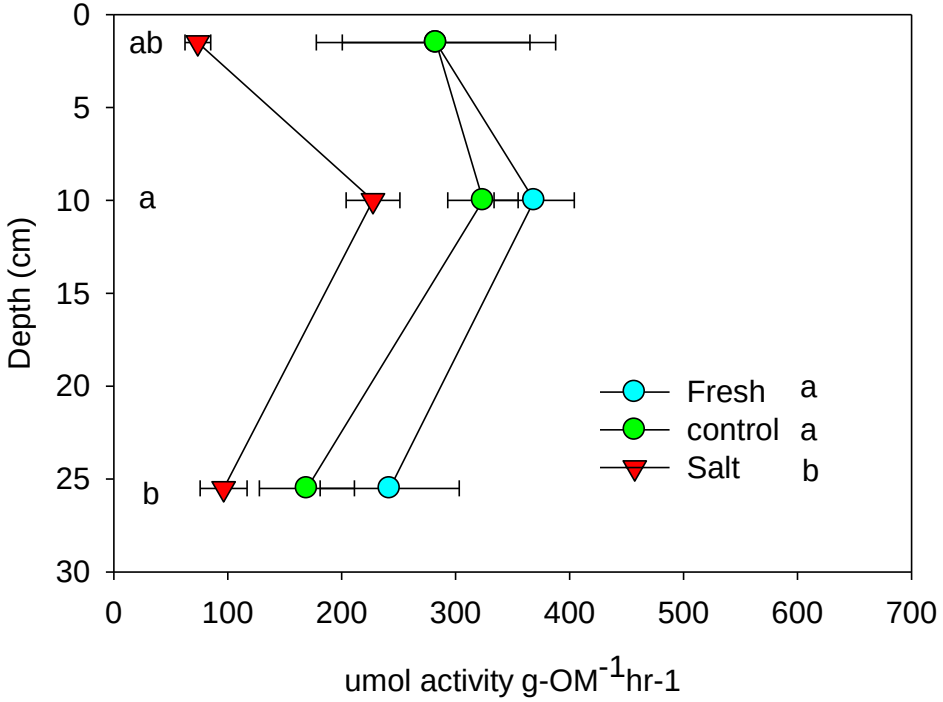
Significant decrease in Cellobiosidase activity with depth
Significant decrease in Cellobiosidase activity with Salt treatment

Recalcitrant Enzyme Activity

Xylosidase



Phenol Oxidase



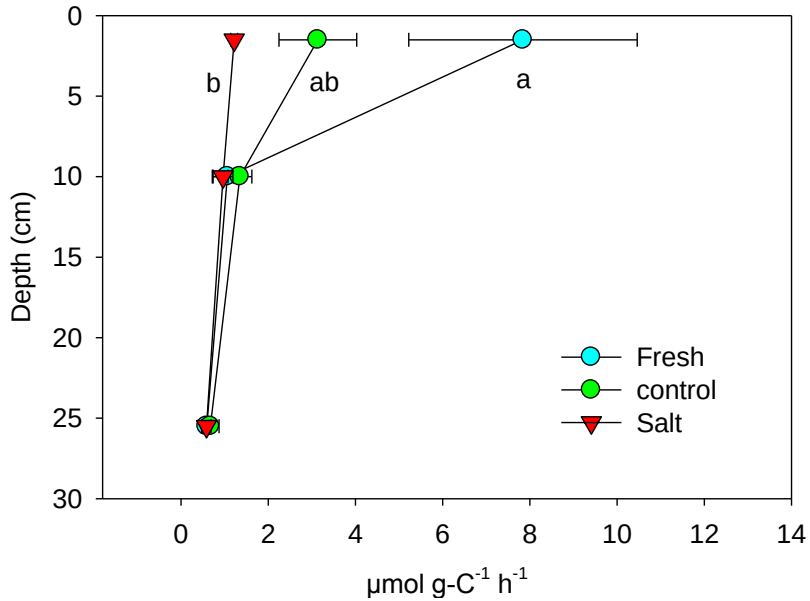
Significant decrease in activity with depth

Significant decrease in Phenol Oxidase activity with Salt treatment

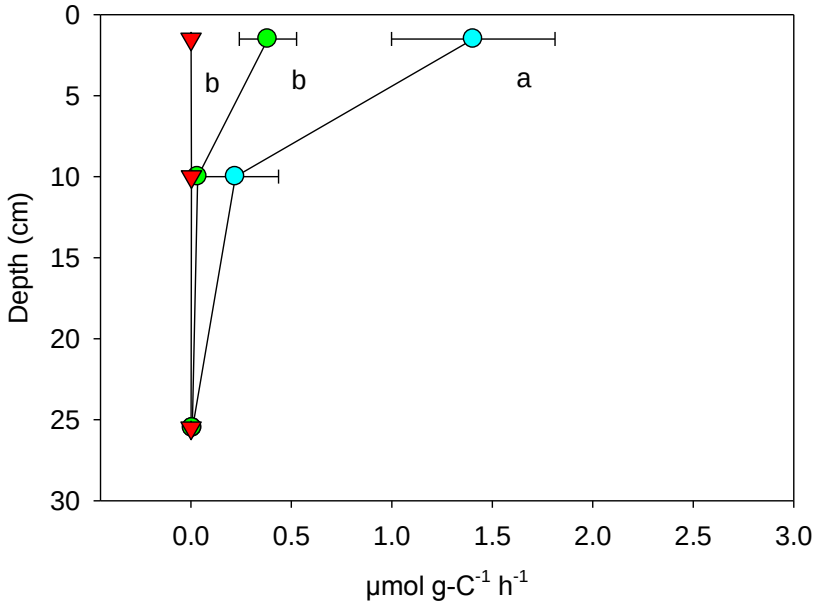
Salinity Treatments effects on carbon and nitrogen cycling end products

Salinity treatment decreases end products

Carbon Dioxide Flux



Methane Flux



Dinitrogen Gas Prodction