Metagenomics Characterization of Periphyton Nutrient Cycling in the STAs

The Greater Everglades Ecosystem Restoration Conference: Restoration in a Changing Climate

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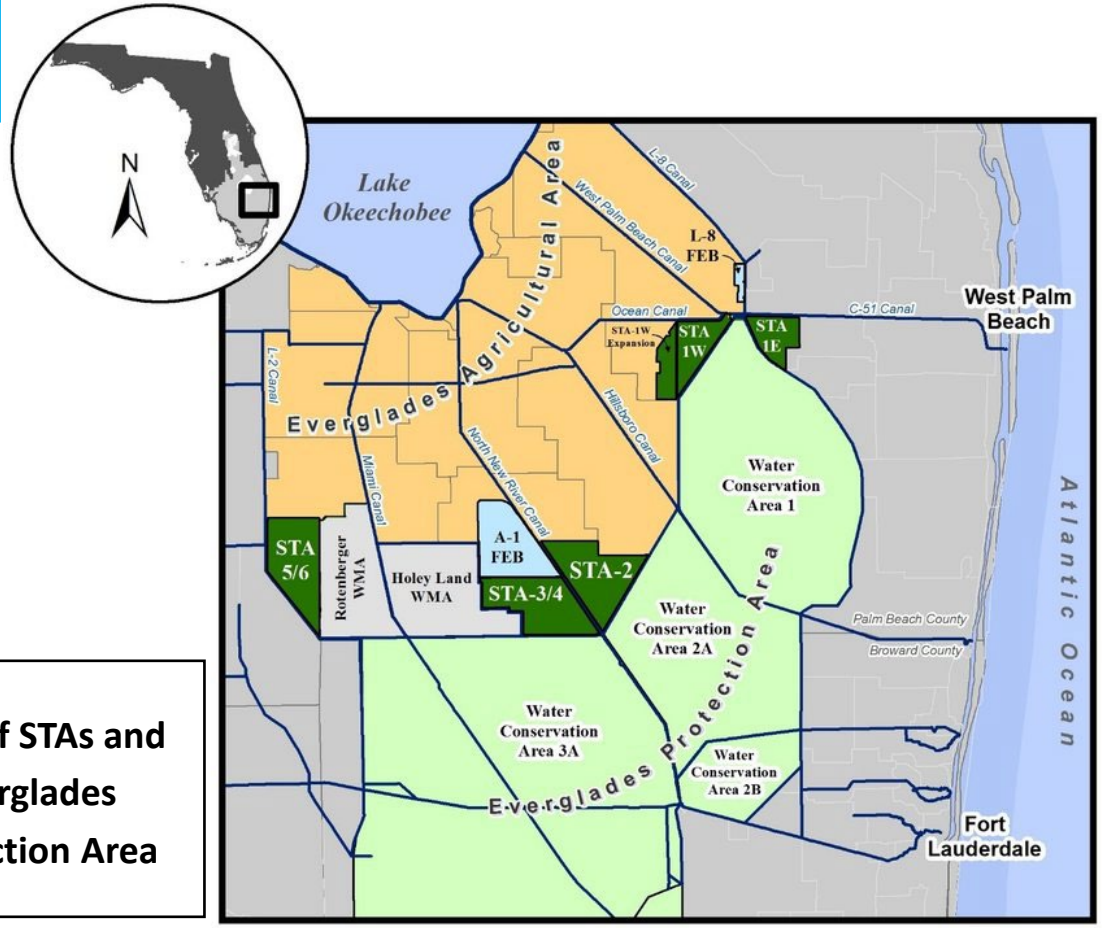
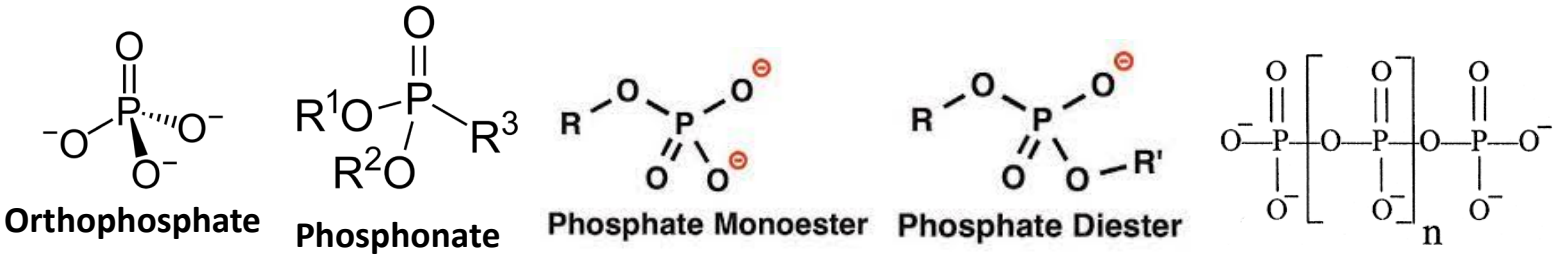
Organic P in the Everglades STAs

- **STA outflows meet strict TP limits (WQBEL)**
- **Outflow TP is low, <u>primarily organic P</u>, inorganic P below detection limits[1]**
- **All organisms need inorganic P, scavenge with <u>phosphatase enzymes</u>[2]**



Map of STAs and Everglades Protection Area

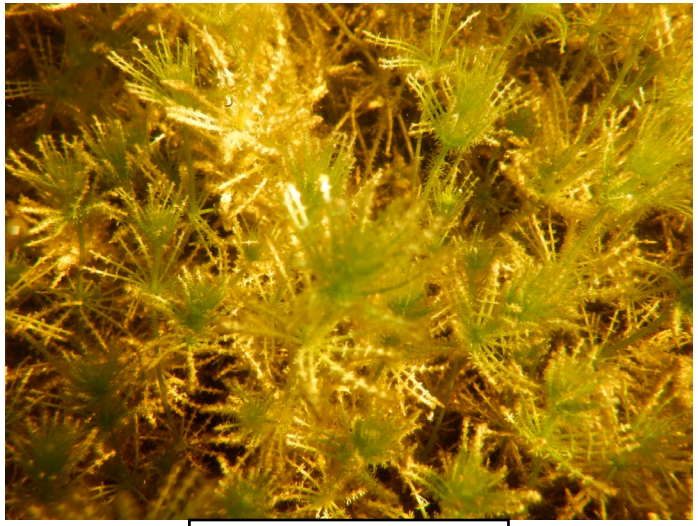


Inorganic P and organic P compounds

P Cycling in the Everglades STAs

- **<u>Rapid microbial processes</u> (periphyton, phytoplankton) dominate short-term P cycling where P concentrations are low[1]**
- **P cycling important but poorly understood**
- **SAV – submerged aquatic vegetation**
  EAV – emergent aquatic vegetation

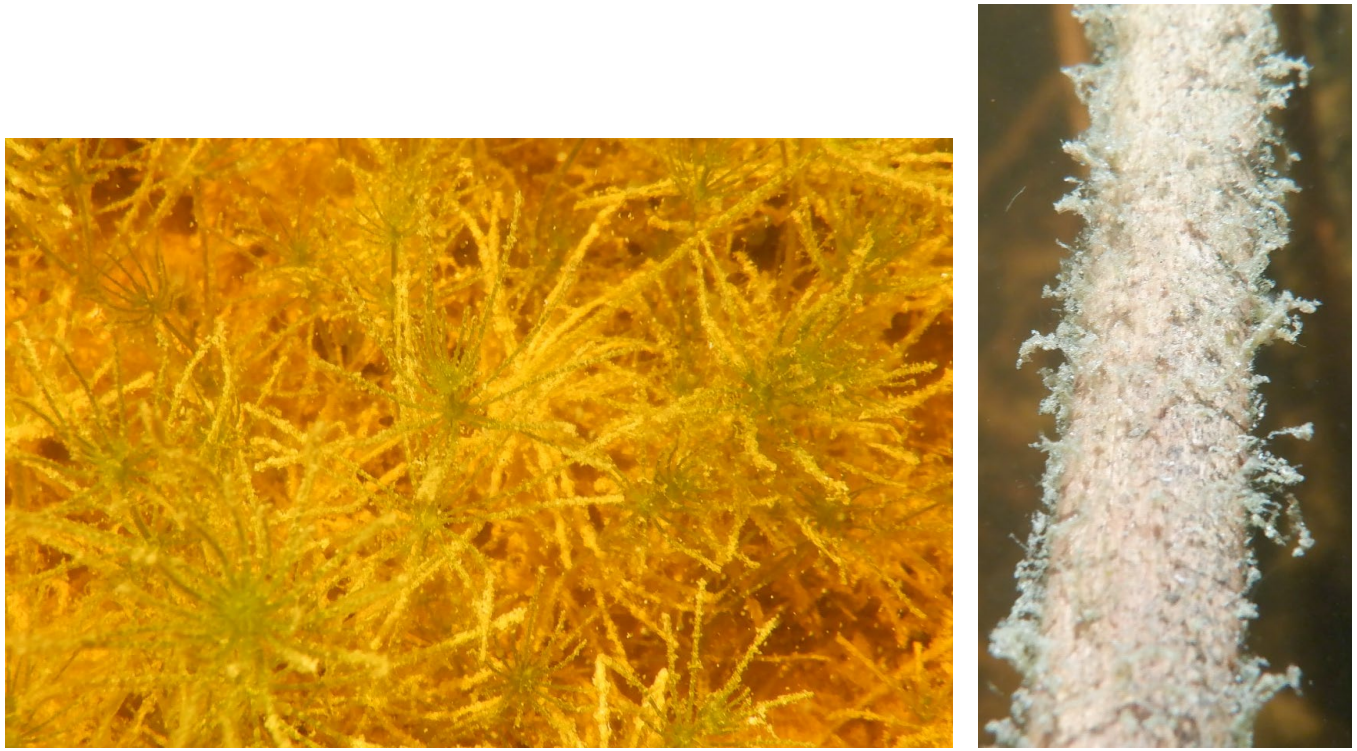
EAV (*Typha* spp.)



SAV (*Chara* spp.)

Why Further Investigation is Needed

- **Phosphatase activity differs from inflow to outflow, between SAV and EAV areas[1]**
- **STA-3/4 pilot study: some differences in P metabolism genes between SAV and EAV periphyton[2]**
- **Further understanding is needed**
  - **Differences in potential for SAV and EAV periphyton to use different organic P forms?**



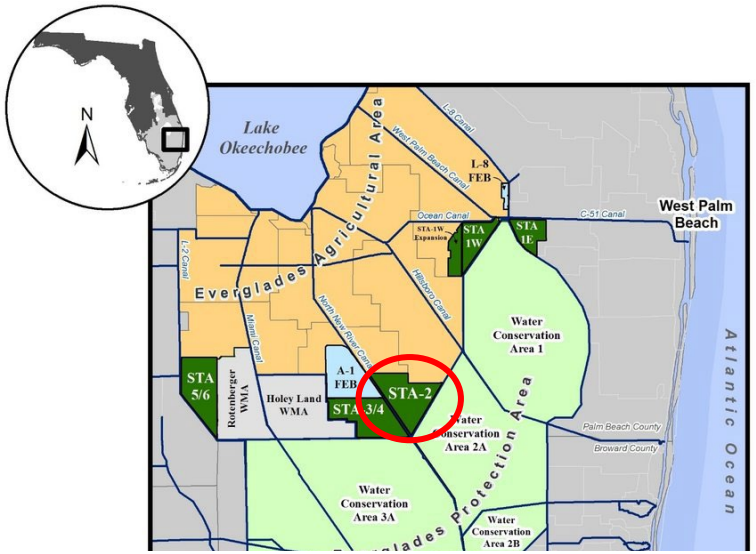
Periphyton on SAV (*Chara* spp., right) and on EAV (*Typha* spp., left)

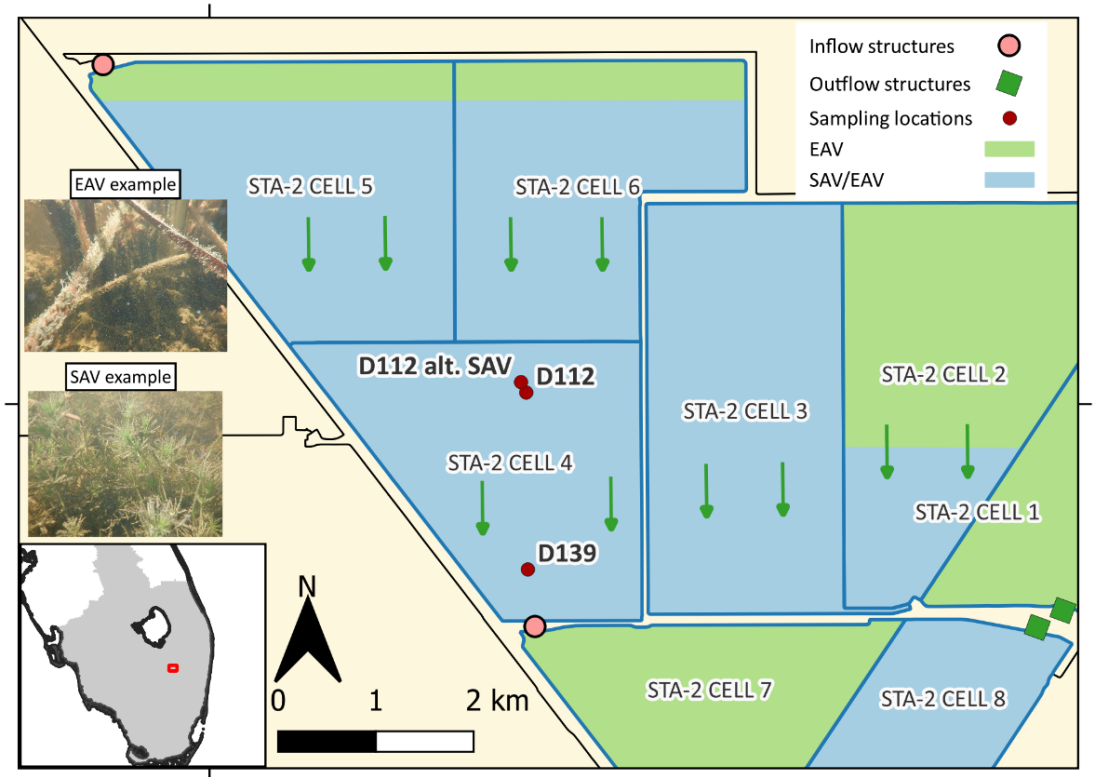
Study Objectives

- **Compare SAV and EAV periphyton community functions**
- **Understand periphyton's effect on P cycling**
- **Evaluate spatial (2 sites) and temporal (wet and dry season) dynamics of community diversity and functional potential**

Field Work: Bimonthly Sampling

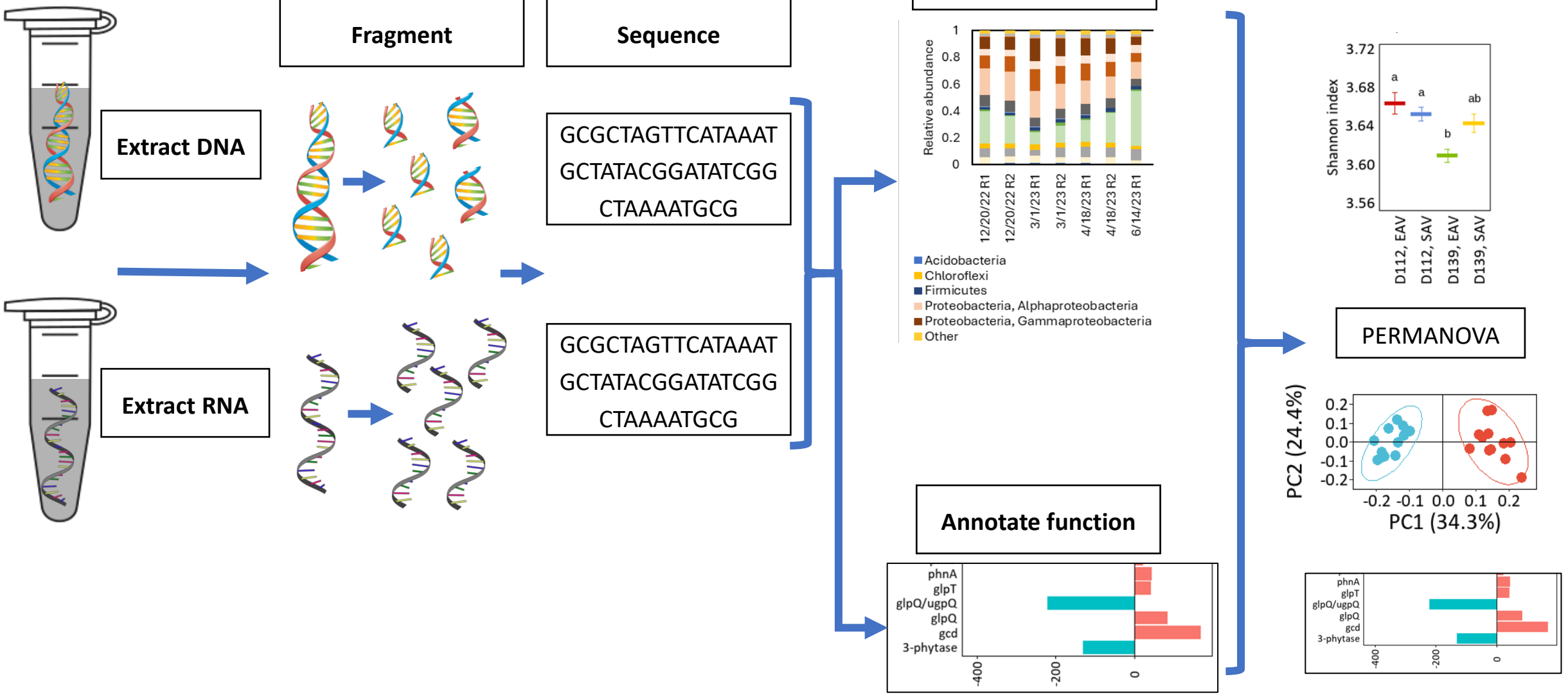
- **Dec. 22'-Nov. 23' from two sites (mid-flow site D112 and outflow site D139)**
- **Surface water nutrients samples[1]**
  - Temp, pH, depth, TP, TDP, SRP, dCa, NH3-N, NOx-N, TN
  - Phosphatase enzyme activity (APA - alkaline phosphatase; PDE - bis-phosphodiesterase)
- **<u>DNA (and RNA) samples</u>[1]**
  - **<u>Metagenomics:</u> total DNA of microbial community**
  - **<u>Metatranscriptomics</u>: total RNA of microbial community**
- **Microscopic algal ID periphyton samples[1]**
  - **Cells/surface area on SAV and EAV**





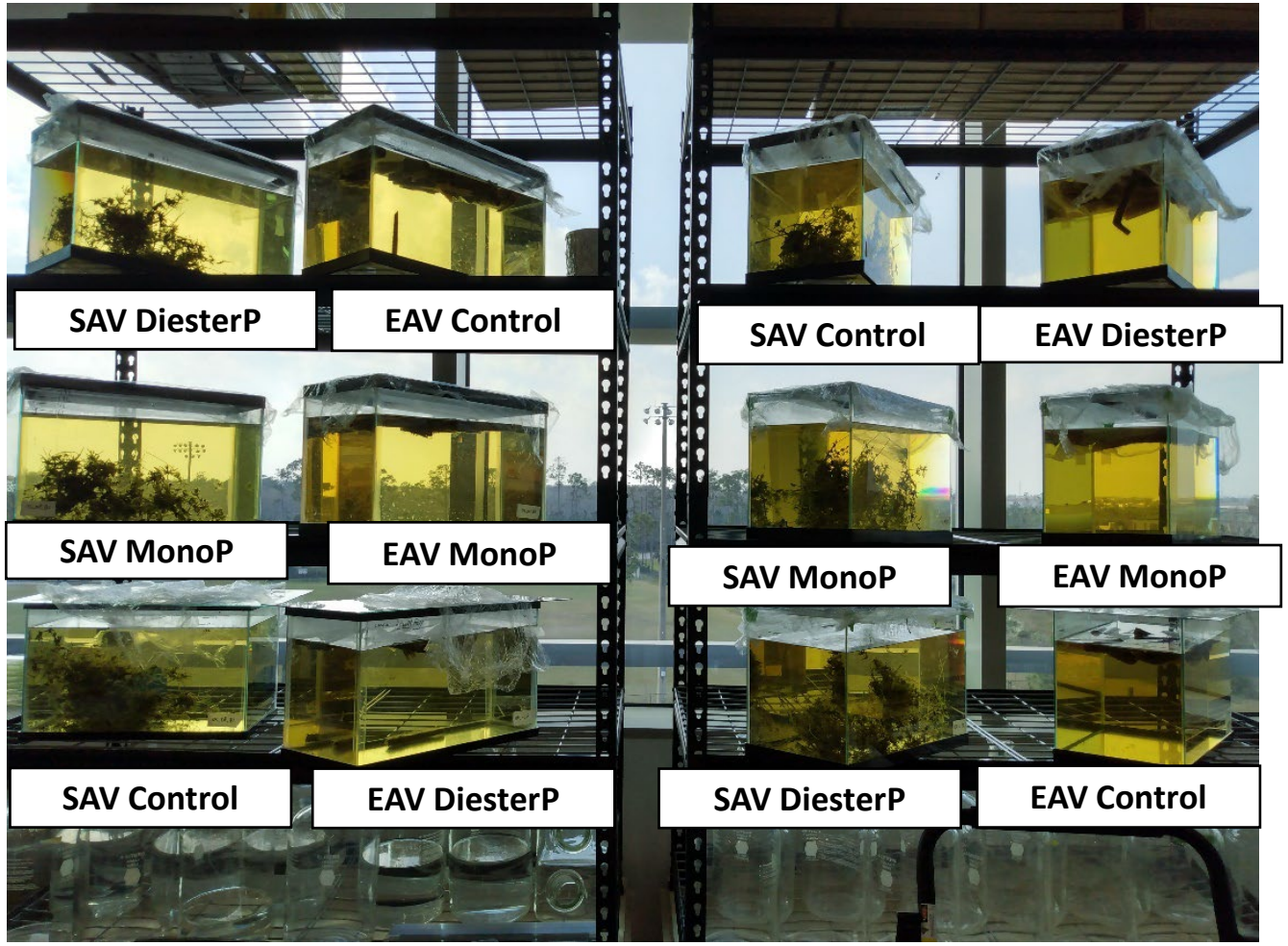
Site map of STA-2 sampling sites. Cell 4 receives inflow from Cells 5 and 6, and water discharges from Cell 4. Arrows show flow direction.

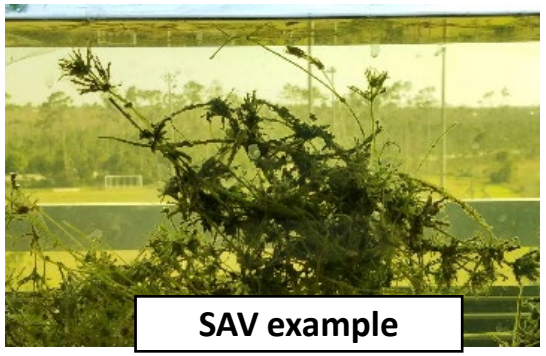
Metagenomic/metatranscriptomic sequencing



Microcosm Experiments

- **Dry season (March), wet season (August) using live material from D139**
- **Dosed daily over 96 hrs. at 5x average DOP values from D139[1]**
  - **2 control aquaria**
  - **2 monoP aquaria: Disodium-α-glycerophosphate**
  - **2 diesterP aquaria: refined lecithin**
  - March: 0.0375 mg P/L
  - August: 0.0275 mg P/L

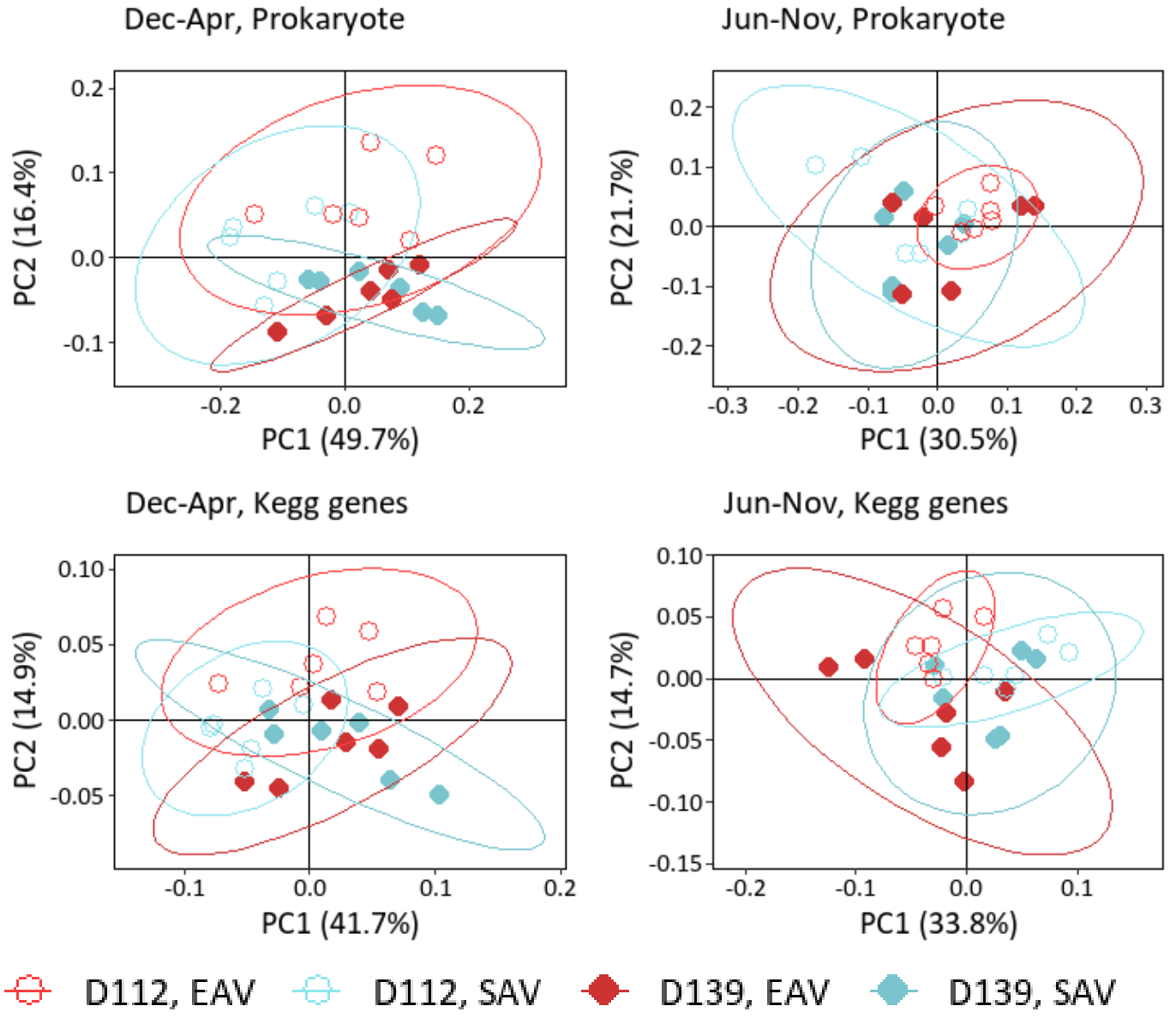




(Top) Mesocosm setup showing treatment and plant type . (Bottom) example of SAV and EAV aquaria

Field Prokaryotic Community Differences: Field SAV vs. EAV

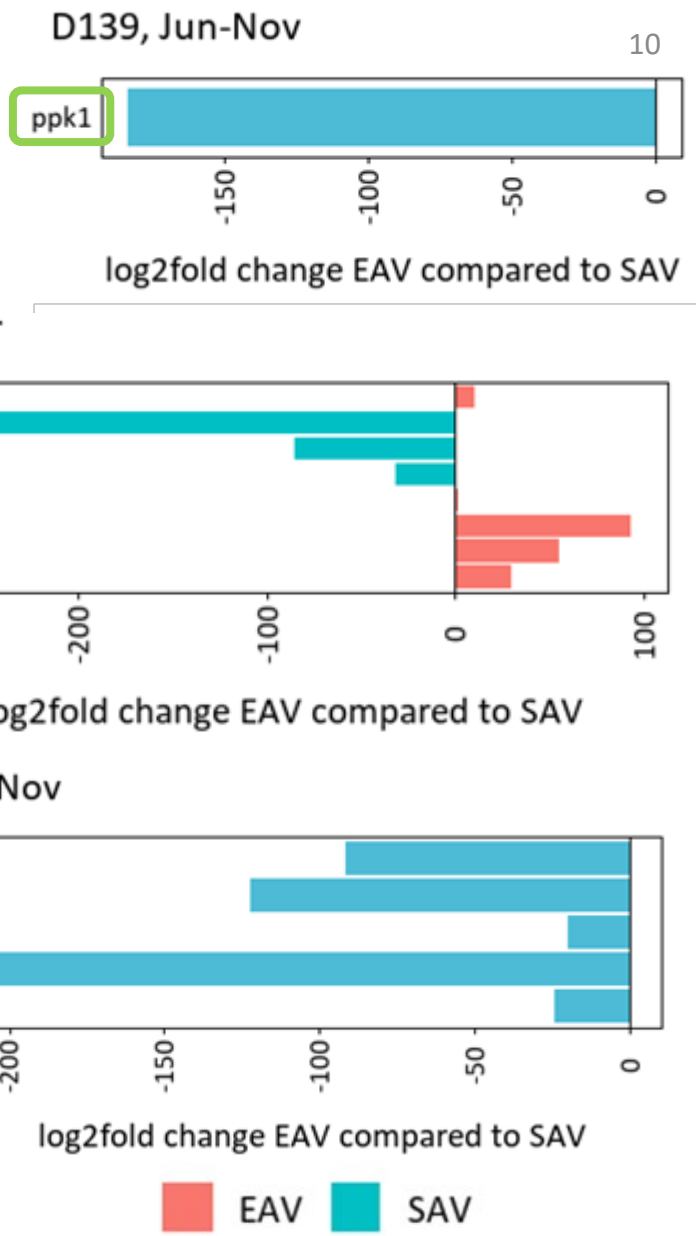
- **Communities did not cluster as established wet/dry season, rather: dry = Dec-Apr, wet = Jun-Nov**
  - **Strongest differences with communities was with season**
- **SAV and EAV communities differed at D112 and not D139 (PERMANOVA, p<0.05)**



PCoA diagrams using Bray Curtis dissimilarity for prokaryote community and total functional annotations.

Field Differences in Composition of P genes: SAV vs. EAV

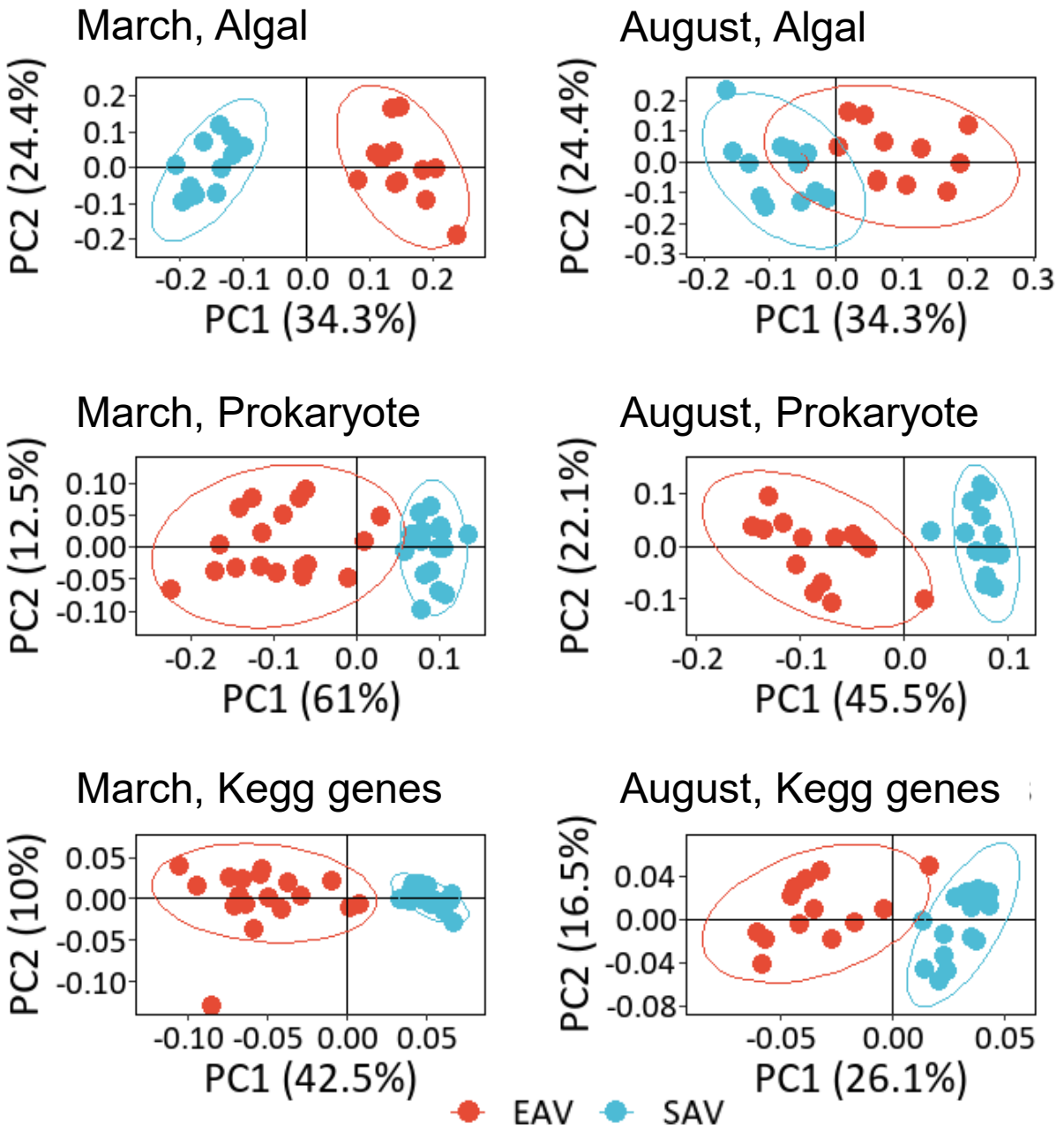
- **Differences in P genes between SAV and EAV at D112 (PERMANOVA, p<0.05)**
  - **Dry season:**
    - **SAV = P storage (green)**
    - **EAV = phosphonate metabolism (red)**
  - **Wet season:**
    - **SAV = phosphonate metabolism (red) and diesterP utilization (purple)**



Log2fold change of estimates of coefficients from MaAsLin2 output, where only significant differences were plotted.

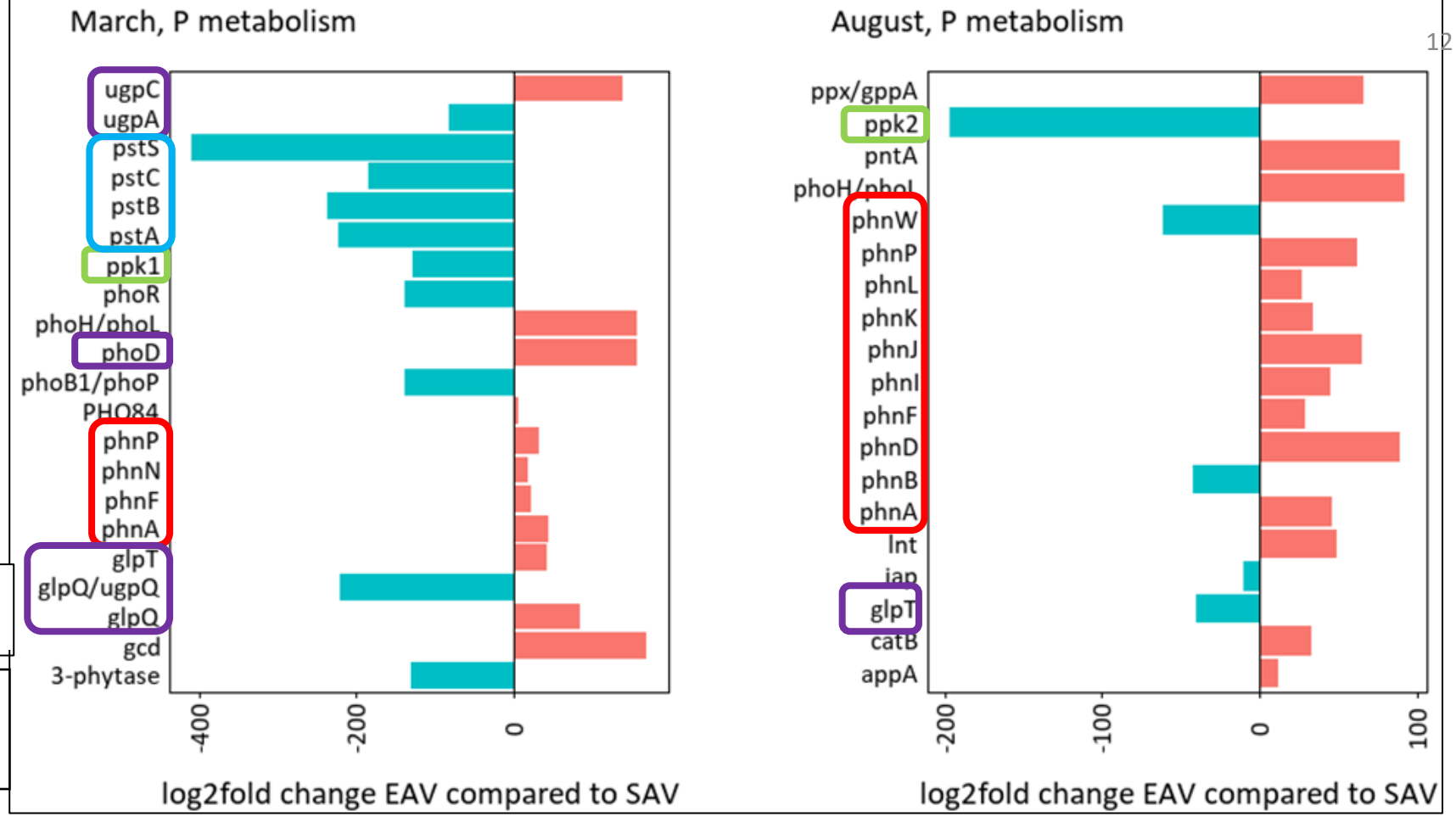
Microcosm SAV vs. EAV

- **SAV and EAV communities differed without regard to treatment (PERMANOVA, p<0.05)**
- Live material from D139
- Little differences in expression or algal community with treatment



PCoA diagrams using Bray Curtis dissimilarity for algal community, prokaryote community, and total functional annotations.

Differences in P gene Activity: SAV vs. EAV

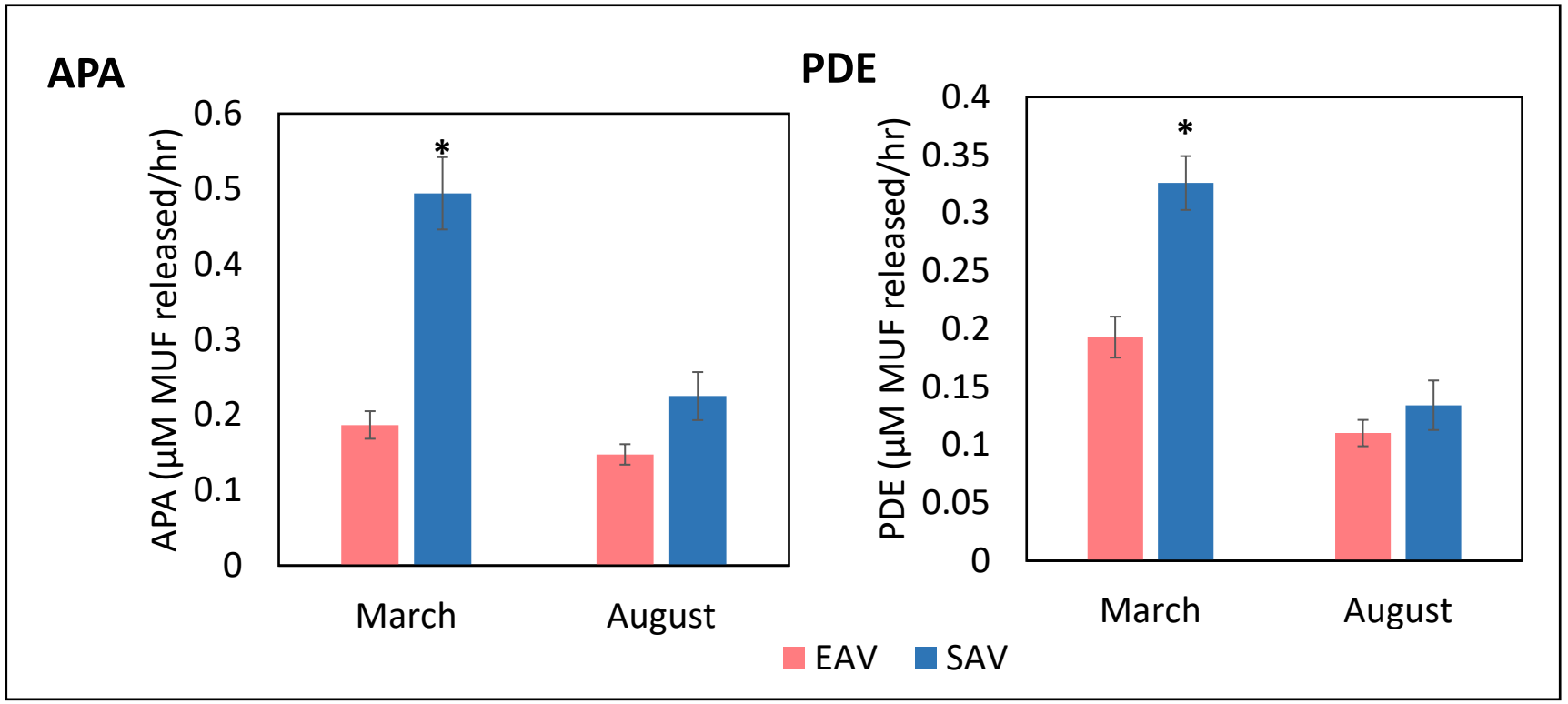


EAV SAV

Log2fold change of estimates of coefficients from MaAsLin2 output, where only significant differences were plotted.

- **Differences in P gene expression between SAV and EAV (PERMANOVA, p<0.05)**
  - **EAV = phosphonate metabolism (red)**
  - **SAV = P storage (green), inorganic P transport (blue)**
  - **Genes related to diesterP utilization (purple) differentially expressed in SAV or EAV**

Phosphatase Activity Higher in March SAV Microcosms

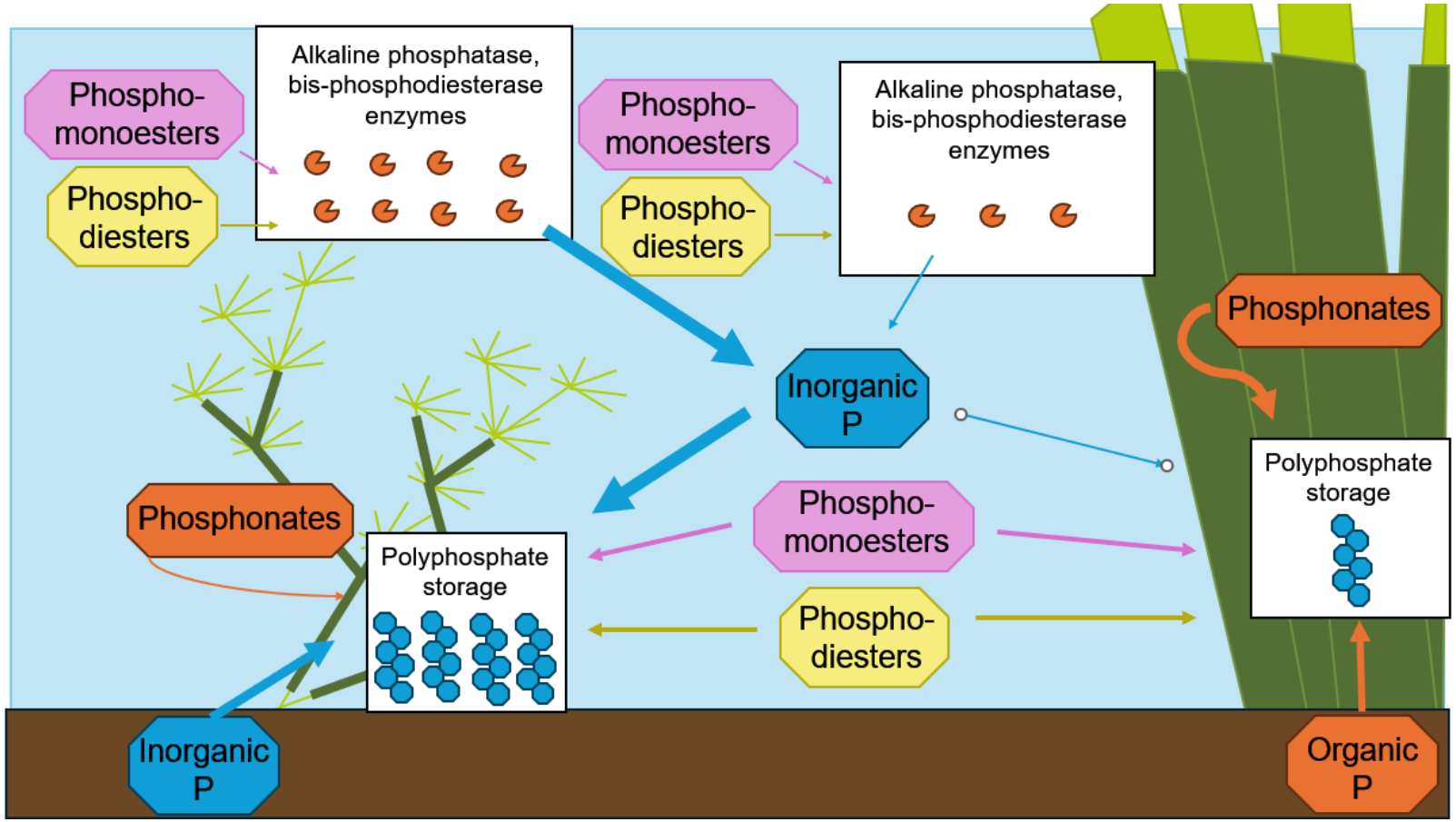


Microcosm APA and PDE enzyme activity, averaged with ± standard error.

- **Enzyme activity (APA, PDE) greater in SAV aquaria in March**

SAV/EAV Can Use Different P Forms

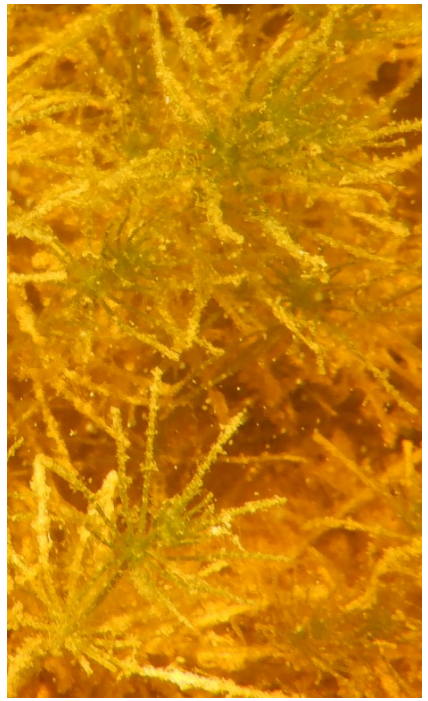
- **Inorganic P uptake, P storage more expressed in SAV periphyton[1]**
- **Inorganic P freed from organic P via enzyme activity**
- **Phosphonate metabolism expressed more in EAV periphyton**
- **Phosphonates not detected in STA surface waters, EAV periphyton may scavenge P from surrounding biomass[2]**



Graphical abstract showing inorganic P uptake and storage by SAV, and phosphonate uptake by EAV.

Differences in Gene Composition of Periphyton from SAV and EAV Supports Mixed Marsh

- **SAV and EAV field samples differ at D112 and not D139**
  - **Higher P at D112, seasonal differences in nutrients at D112[1]**
- **SAV and EAV differ in microcosms (D139 material)**
- **Both macrophytes needed to process diversity of organic P molecules**
- **Mixed marsh conditions could be most effective for P removal at both sites**



Structural differences between SAV and EAV leaves influences community composition.

Conclusions

Mixed marsh conditions could be best for P removal in the STAs because of differences in P metabolism genes and activity between SAV and EAV periphyton

Questions?

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