Enzymatic hydrolysis of dissolved organic phosphorus in the Everglades Stormwater Treatment Area source waters

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BACKGROUND

- Stormwater treatment areas (STAs) retain much of the total P loads before discharge to the Everglades
- While the soluble reactive P fraction is removed quickly, particulate and dissolved organic P (DOP) constitute the majority of P in outflow waters.
- Characterizing dissolved organic P forms and biotic conversion, thus, remains critical to addressing P retention performance of the Everglades STAs.

RESEARCH QUESTIONS

- Do STA source waters differ in enzymatically hydrolysable dissolved organic P fractions?
- Does microbial turnover of dissolved organic matter differ among STA source waters?

STUDY DESIGN AND METHODS

Study sites:

- STA 3/4 Inflow (G-370)
- L8 FEB (G-538)
- Lake Okeechobee (S-354)
- STA 2 Inflow (S-6)

Methods:

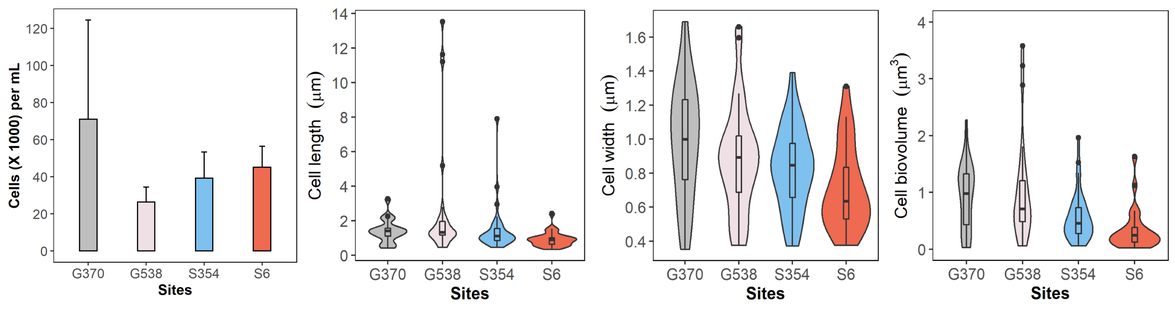
- Water samples immediately filtered using 0.2 µm filter, stored at 4C.
- Dissolved organic carbon (DOC) quality assessed with UV-Visible spectroscopy using **spectral slope ratios** (Helms et al., 2008)
- **Enzyme Hydrolyzable DOP** determined by following Monbet et al. (2007) by hydrolyzing samples with P enzymes over night at 37ºC and measurement of SRP produced after hydrolysis.

- **Microbial bioassays**: Samples incubated in 120 mL glass serum bottles, with 1 mL of unfiltered sample as inoculum, for 4 weeks at 35ºC.
- **Respiration rate** determined by periodic measurement of headspace CO_2.
- At the end of the bioassay microbial **cells were counted** under 1000X magnification.
- Cell length and width were estimated for representative cell samples (n=110).
- **Cell biovolume estimated** assuming cell shape of cylinder after Fry and Davies (1985).
- **Data analyzed** with one-way analysis of variance.

RESULTS

1. Microbial characteristics after 4-week incubation of STA source waters

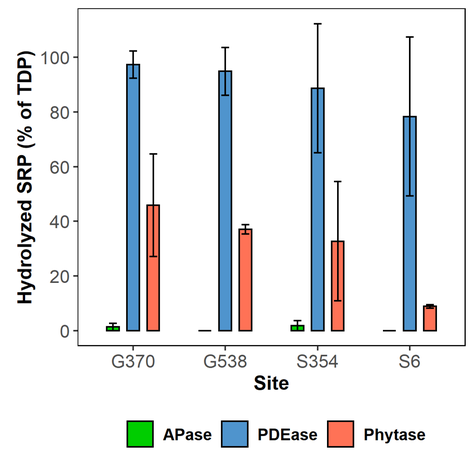
- Microbial communities were different for the inflow sources based on cell size/shape and production.
- Microbial cell biovolume in L8 FEB (G-538) was 2.7- fold larger than in STA 2 (S-6).
- The lower microbial biomass C, cell counts, and smaller cells in STA 2 (S-6) relative to STA 3/4 (G-370) suggests microbial mineralization, rather than growth, may have been occurring at STA 2 (S-6) site.



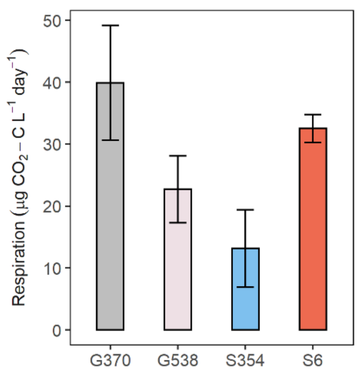
2. Recovery of total dissolved phosphorus as soluble reactive phosphorus across sites following P enzyme additions.

Sites	APase-hydrolysed SRP (µg L^{-1})	PDEase-hydrolysed SRP (µg L^{-1})	Phytase-hydrolysed SRP (µg L^{-1})
STA 3/4 Inflow (G-370)	0.2 ± 0.2	8.4 ± 3.5 b	3.3 ± 0.2
L8 FEB (G-538)	0.0 ± 0.0	7.9 ± 0.5 b	3.2 ± 0.6
Lake Okeechobee (S-354)	0.5 ± 0.5	12.1 ± 4.9 ab	3.2 ± 0.3
STA 2 Inflow (S-6)	0.0 ± 0.0	24.1 ± 3.6 a	3.0 ± 0.8

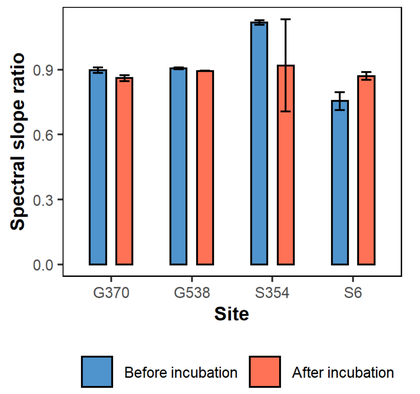
- Very little hydrolysis by phosphomonoesterase indicates a scarcity of highly available P forms.
- Most DOP was hydrolyzed by Phosphodiesterase or Phytase, indicating mostly diester-P in the inflows.



3. Microbial respiration responses and changes in DOM spectral characteristics

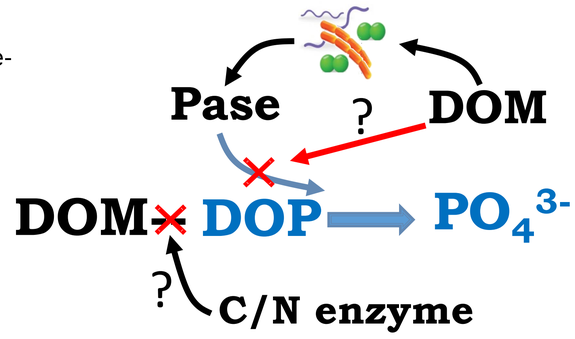


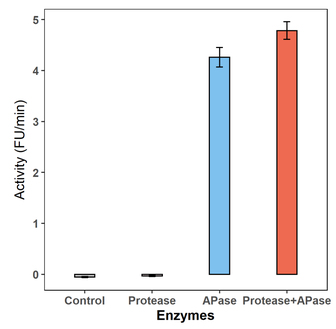
- Microbial respiration was not significantly different during incubation at 35°C (p = 0.133).
- STA 3/4 (Site G-370) had a respiration rate 3 times that of Lake Okeechobee outflow (S354 site).
- DOM slope ratio decreased with consumption of bioavailable C.
- STA 2 (S-6) likely had higher terrigenous (less bioavailable) DOM at the beginning of the incubation.



CONCLUSIONS AND FUTURE RESEARCH

- Microbial growth on DOM sources differed between sites and did not correlate with enzyme-hydrolyzed P.
- Low monoester P and high diester P in 0.2 µm filtered waters indicates recycling of P through biota.
- Unlike DOP in 0.2 µm filtered samples, preliminary analysis of 0.45 µm filtered samples yielded only 18-40% of DOP as hydrolysable P.
- Most DOP (<0.45 µm) remains unavailable and poorly understood.





- Future experiments will explore factors affecting enzymatic attack of larger molecular weight DOP (>0.45 µm), including enzyme complexation by DOM and synergism of multi-enzyme systems on the activity of P hydrolyzing enzymes.
- We are currently evaluating if other enzymes facilitate or hinder the activity of monoesters and diesters.

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