

# Utility of biofilm fatty acid signatures as a tool to characterize watershed environmental stress

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# *An immense challenge*



Population predicted to shift from 17.7 to 20 million people by 2030  
Associated demands for food, energy, clean water.

Chesapeake TMDL program (a pollution diet)  
Watershed specific (EPA mandates to the state level)

*“generate less pollution yet plan for the same levels given a 3-5 million population increase”*

## *Can we complement nutrient monitoring?*

(1) How do we characterize disturbed ecosystems?

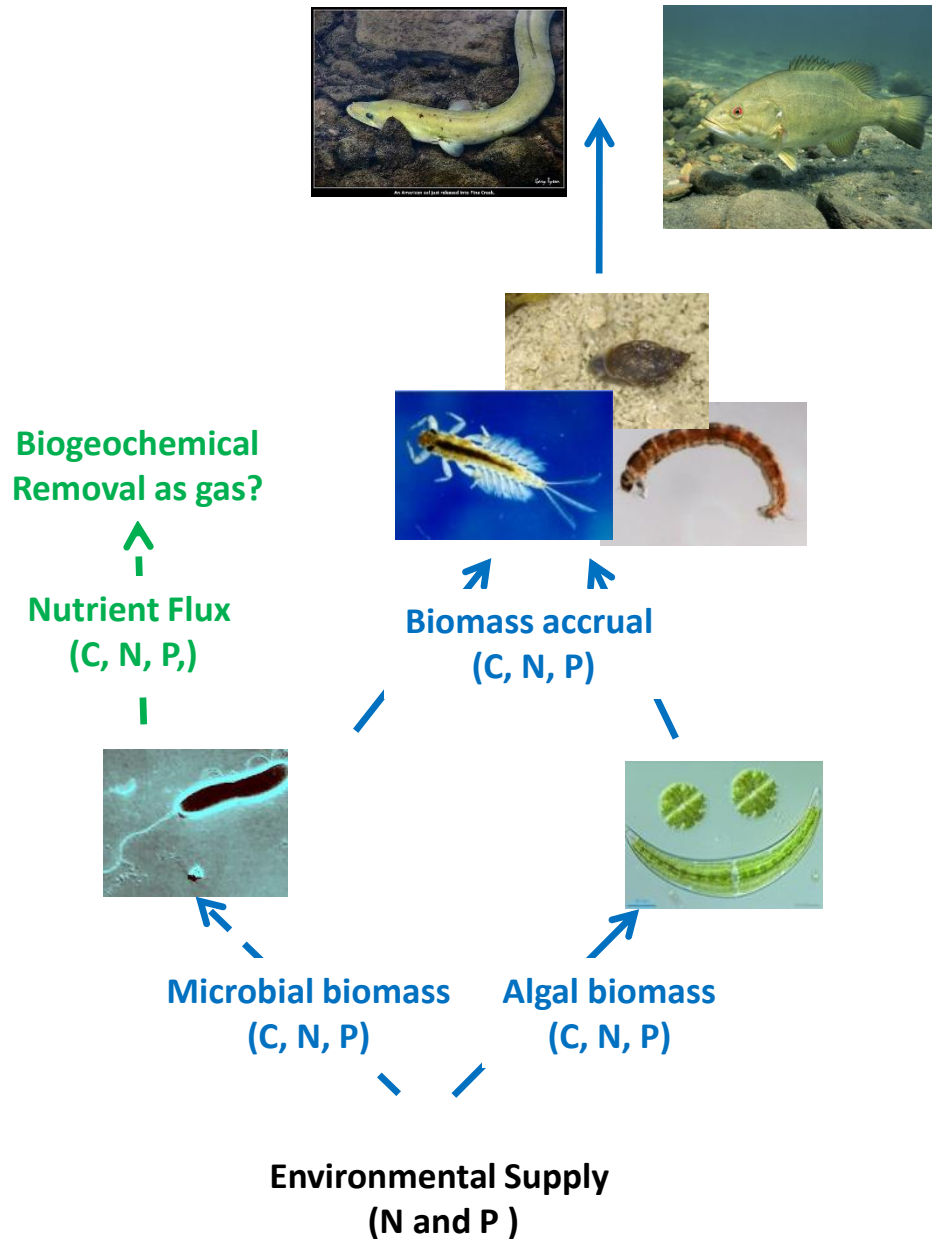
(2) What benchmarks:

Do we use to characterize the extent of disturbance?

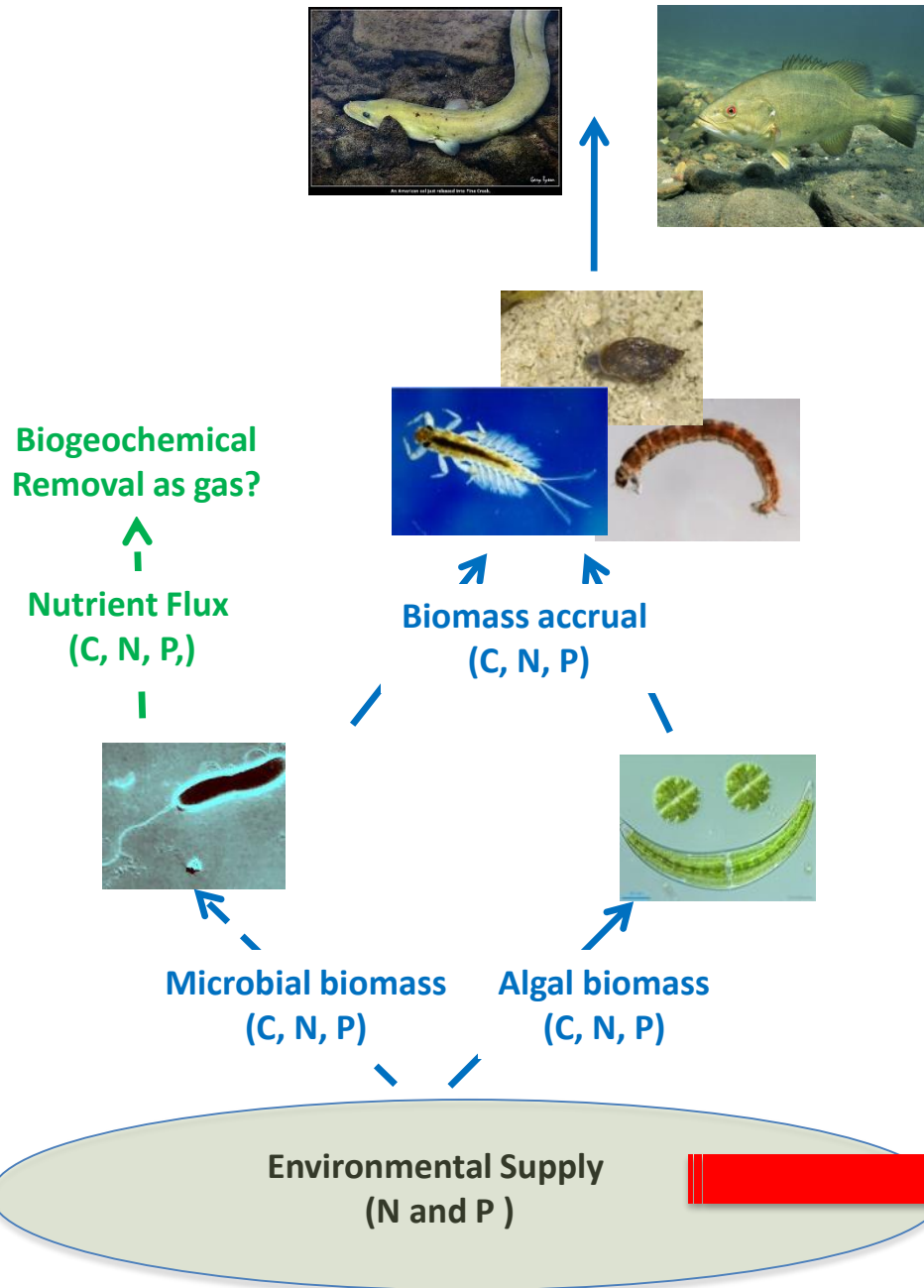
Do we use to evaluate the success of restoration?



# Approaches to ecological assessment



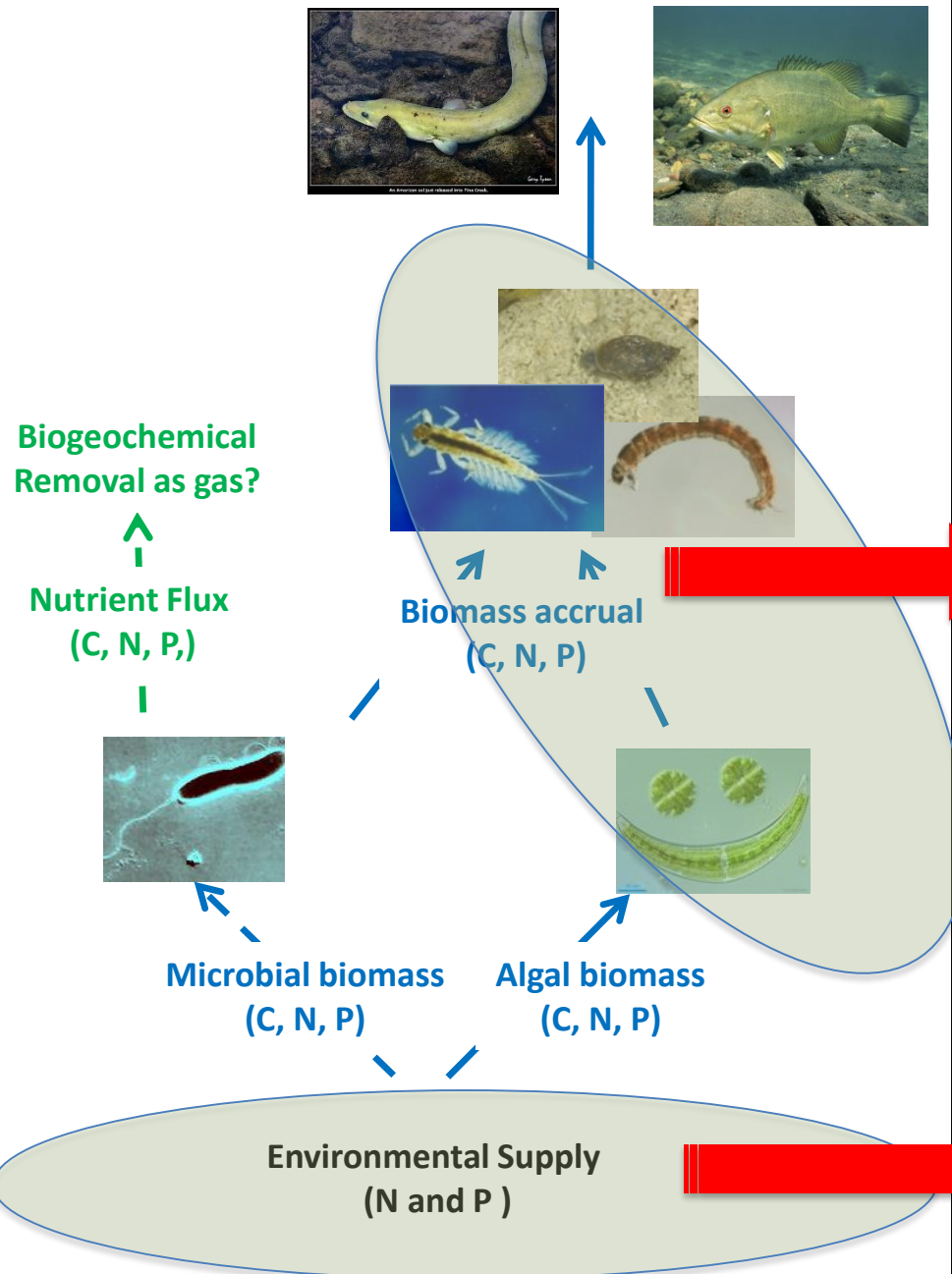
# Approaches to ecological assessment



Supply side-monitor point sources and total maximum delivered loads



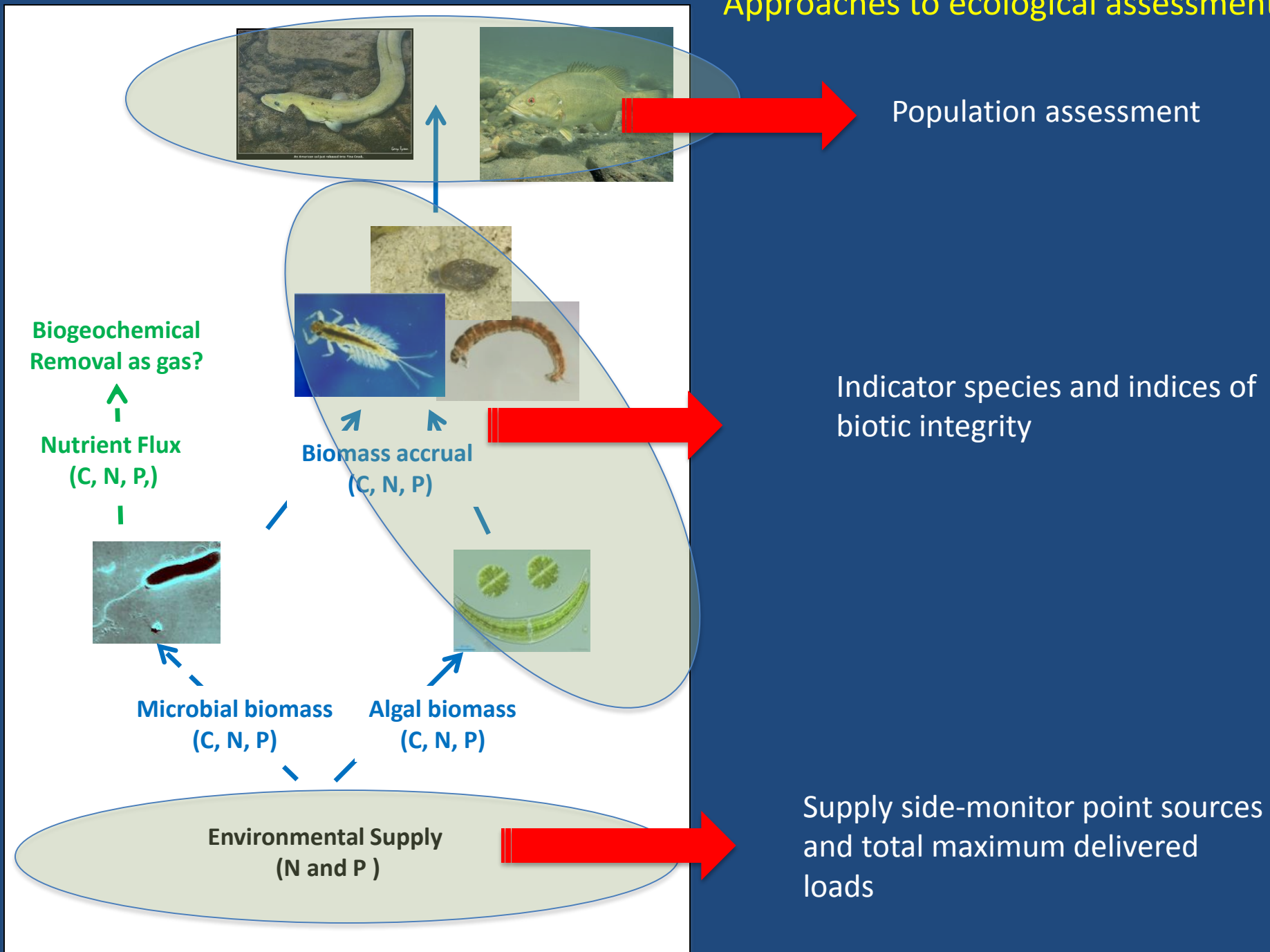
# Approaches to ecological assessment

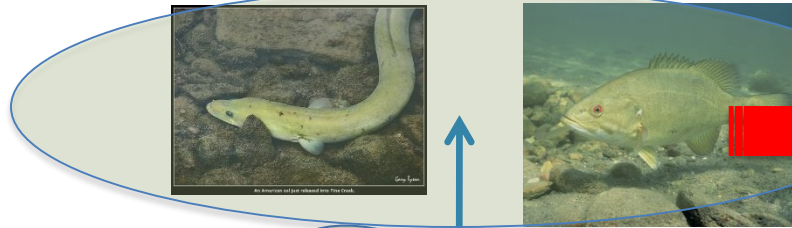


Indicator species and indices of biotic integrity

Supply side-monitor point sources and total maximum delivered loads

# Approaches to ecological assessment



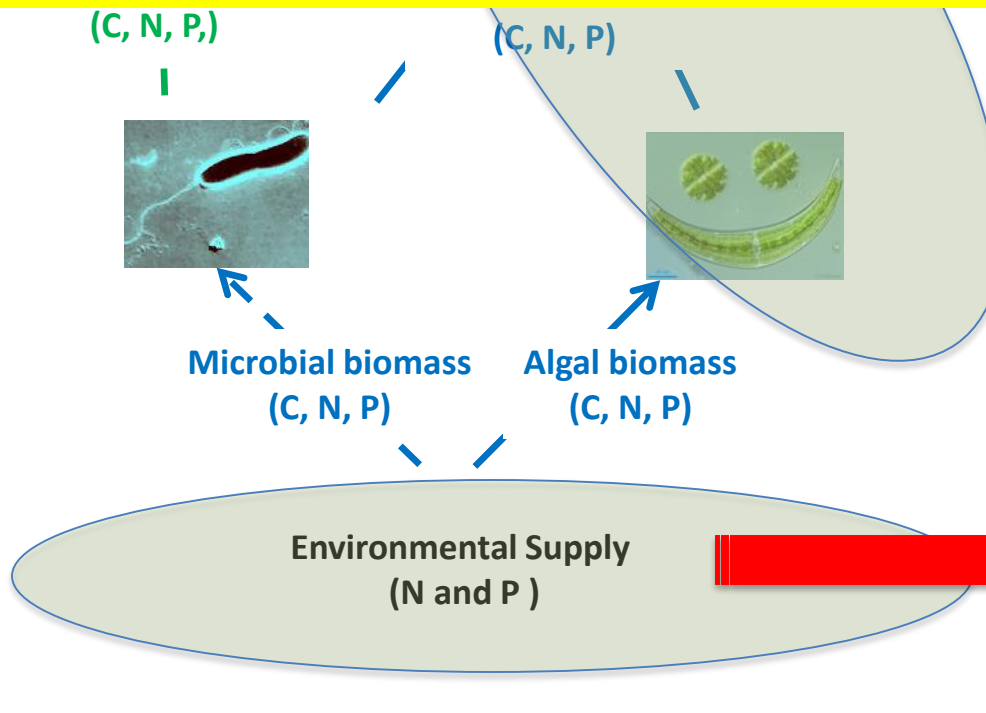


Population assessment

Biogeochemical  
Removal as gas?



What about linkages among compartments?



Supply side-monitor point sources  
and total maximum delivered  
loads



**ECOLOGICAL NUTRITION:** Study of macromolecules (lipids, carbohydrates, proteins); nutrients (carbon, nitrogen, phosphorus); and vitamins within a foodweb or ecosystem.

- **Fatty acids** are a group of lipids with a distinct chemical structure. Often used to assess the quality of carbon.
- Typically are bookended by a methyl and carbonyl group.

**SAFA:** Saturated fatty acid

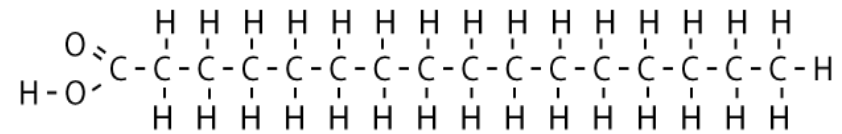
**MUFA:** Mono-unsaturated fatty acids

**PUFA:** Poly-unsaturated fatty acids

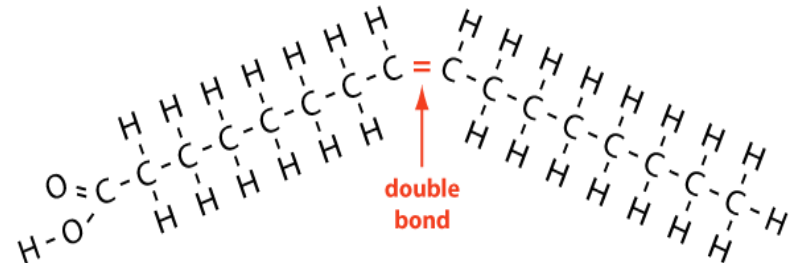
**HUFA:** Highly unsaturated FA

**w3** and **w6:** placement of the first double bond

**saturated fatty acid**



**unsaturated fatty acid**



## Fatty acids serve many physiological and ecological functions:

- Fundamental building blocks  
(macromolecule and growth)
- Promote physiological processes  
(visual acuity)
- Govern physiological processes  
(membrane fluidity)

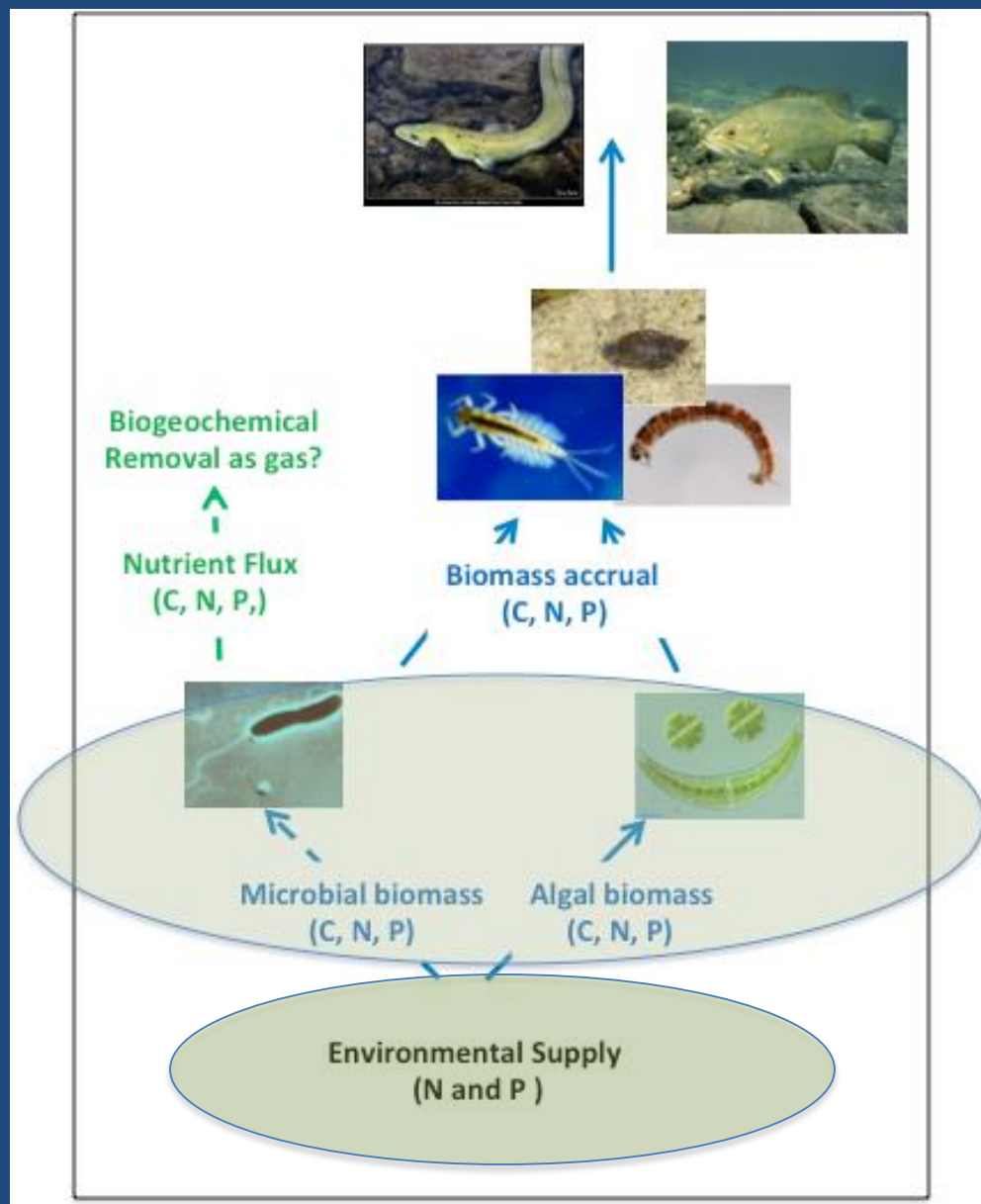
## Ecologically important FA's

HUFA, PUFA and long chain fatty acids are generated via elongation and desaturation.

Only certain taxa at the base of the food web can do it *de novo*.

Ecologically important FA's are an important commodity within foodwebs.

How do watershed stressors influence fatty acid composition?



# Can we use biofilms to characterize nitrogen, phosphorus, and sediment stressors?

## Approach:

- Flow through 110 L mesocosms connected to solenoids.
- Manipulated sediment, nitrogen, phosphorus
- Colonized each stream with the same slurry collected from nearby Straight Run.
- Incubated the streams for 30 days



# Experimental design

*Analogous to a 3 way ANOVA*

		No Sediment		Sediment	
		Low N 0.3 mg N	High N 6.0 mg N	Low N 0.3 mg N	High N 6.0 mg N
Low P	nps	Nps	Low P	npS	NpS
High P	nPs	NPs	High P	nPS	NPS

## Individual treatments

nps, Nps, nPs, NPs, npS, NpS, nPS, NPS

***n = 4***

## Specific treatments

n, N

p, P

s, S

***n =16***





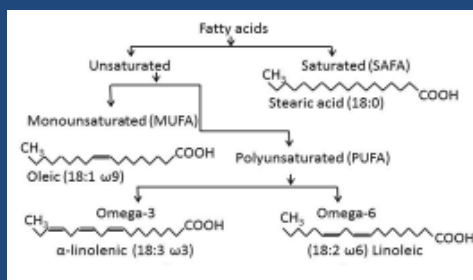
## Response measures (conventional)

- Biomass accrual
- Algal community structure
- Total and dissolved nutrients

## Functional responses



### Fatty acids



### Enzyme activity



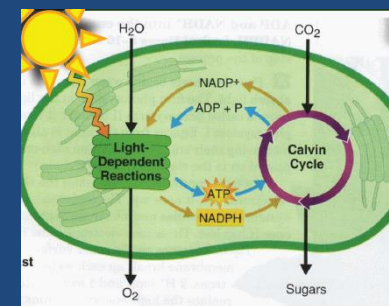
P-ase  
N-ase  
C-ase

### Stable isotopes

$^{14}\text{N}$ 14.00307 99.63%	$^{15}\text{N}$ 15.0001 0.37%
$^{12}\text{C}$ 12.00000 98.89%	$^{13}\text{C}$ 13.00335 1.11%

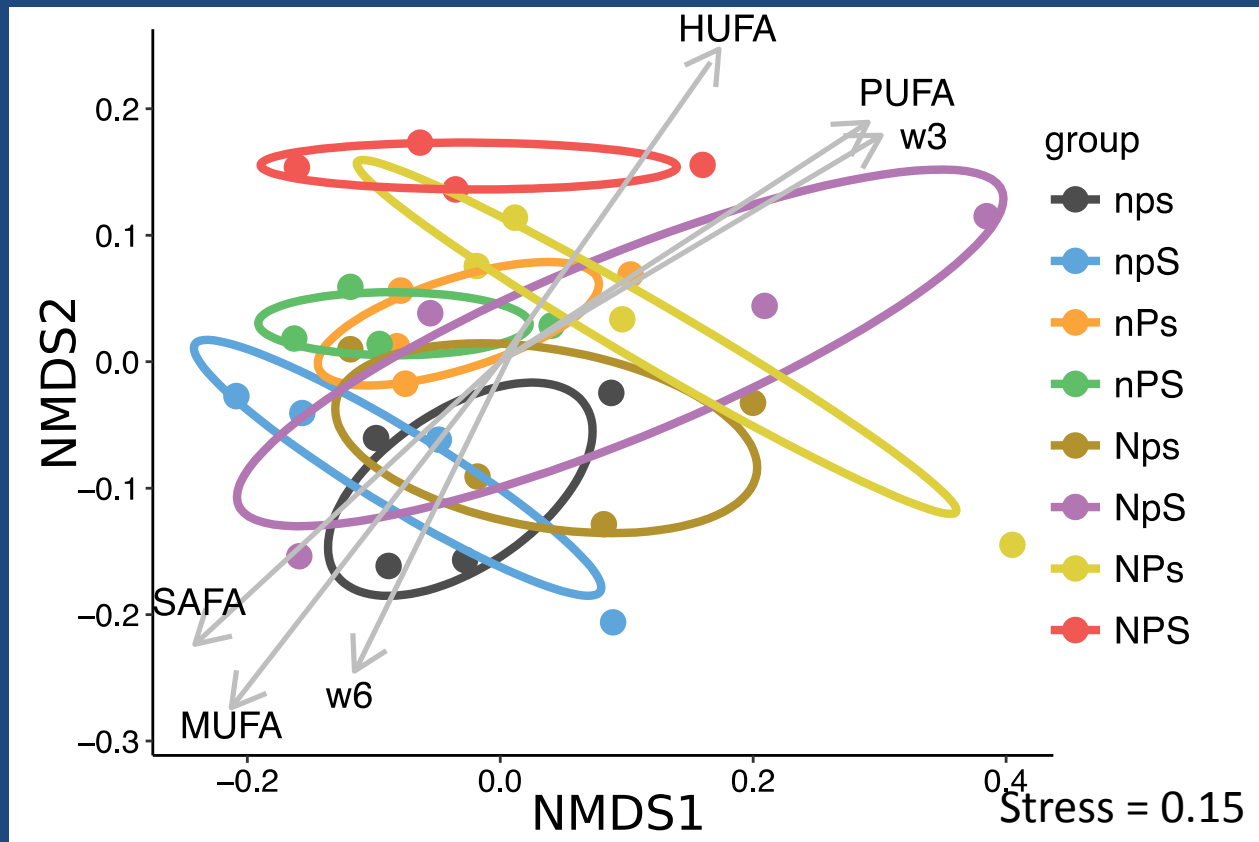
ambient  
labeling study  
(N uptake)

### Metabolism

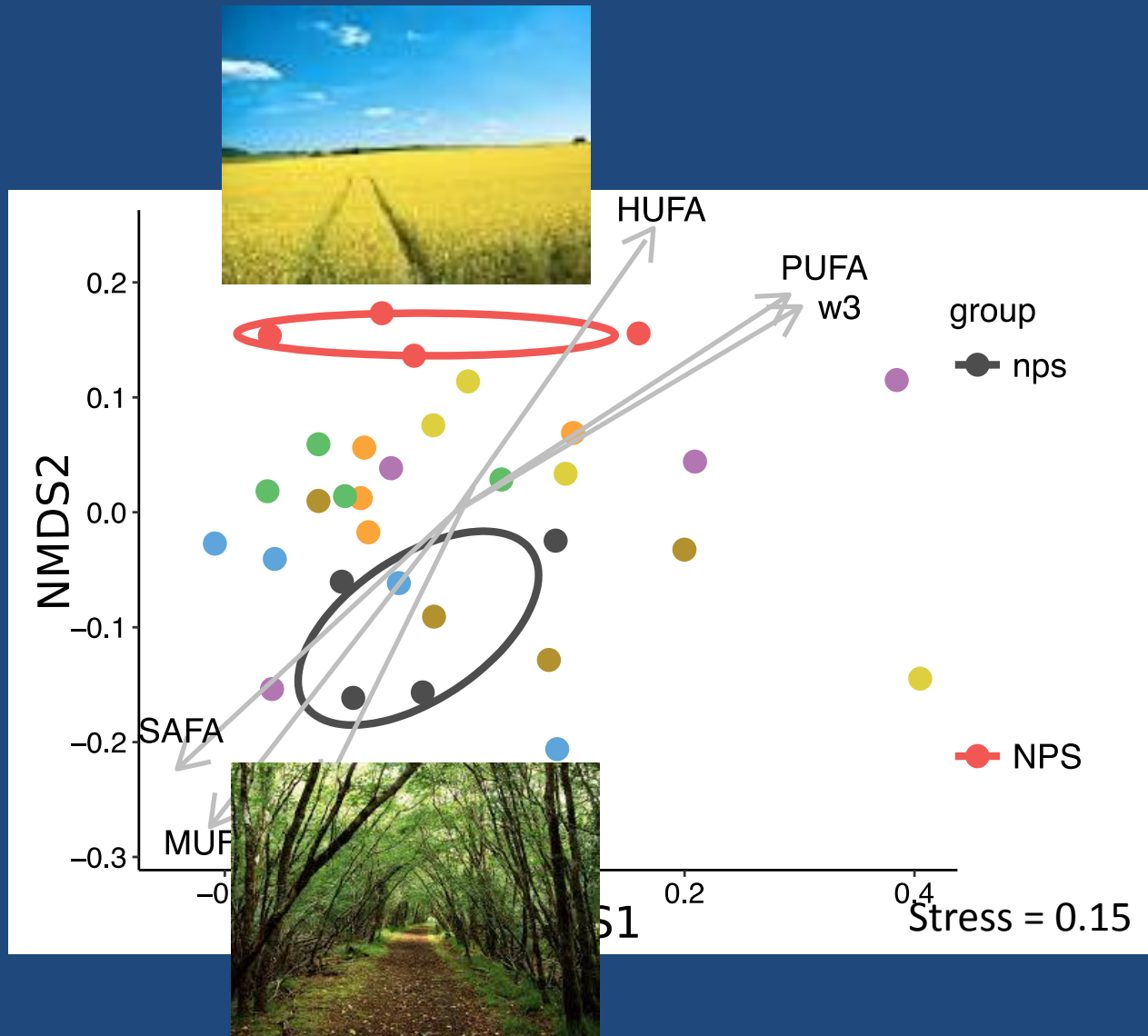


GPP  
Respiration

## Fatty acids



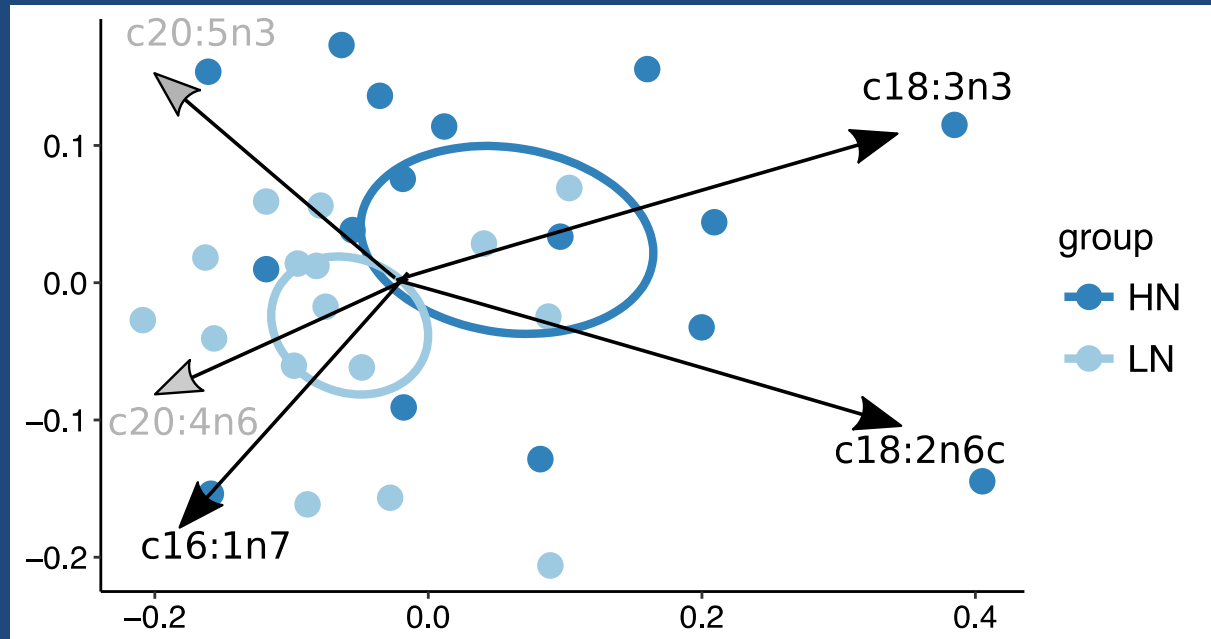
# Fatty acids



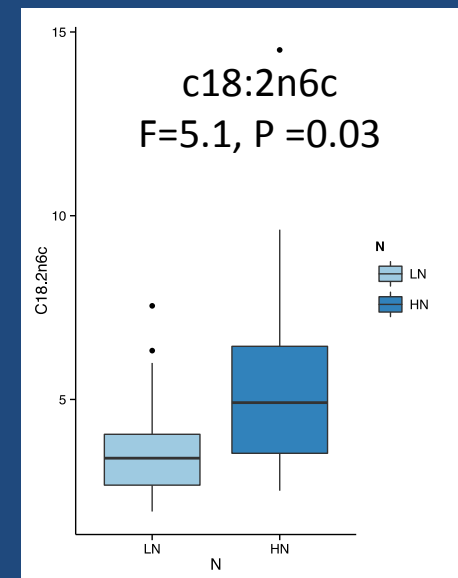
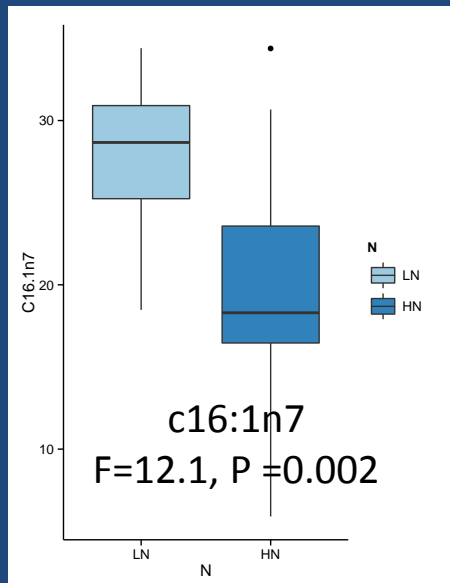
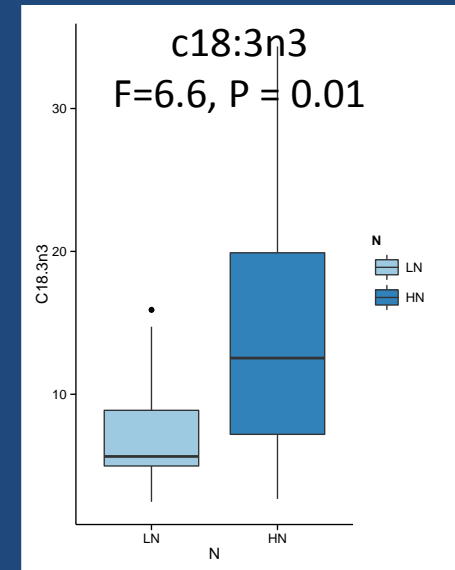
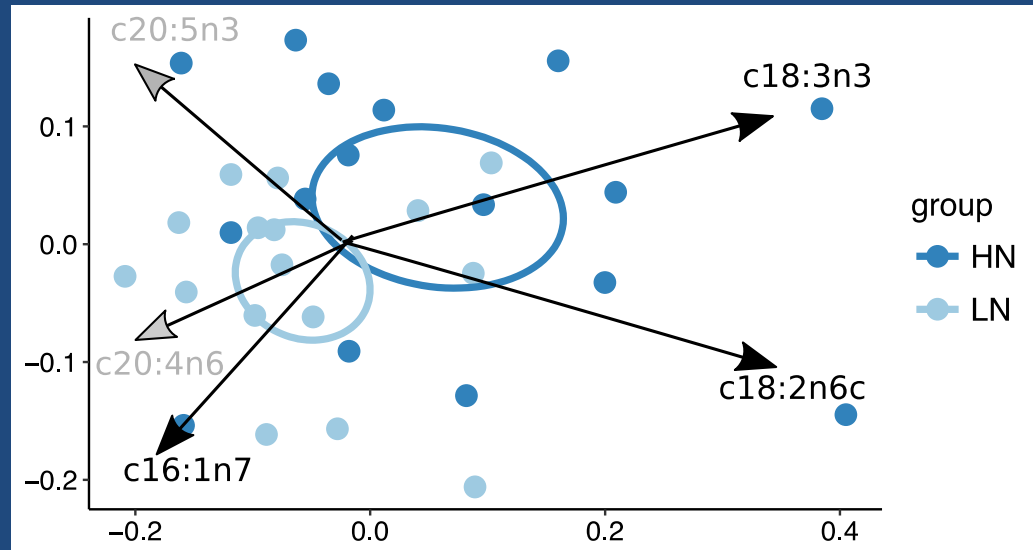
## SIMPER Analysis (Similarity of Percentages)

Characterize which fatty acids explain differences in treatment assignment.

Low versus high nitrogen

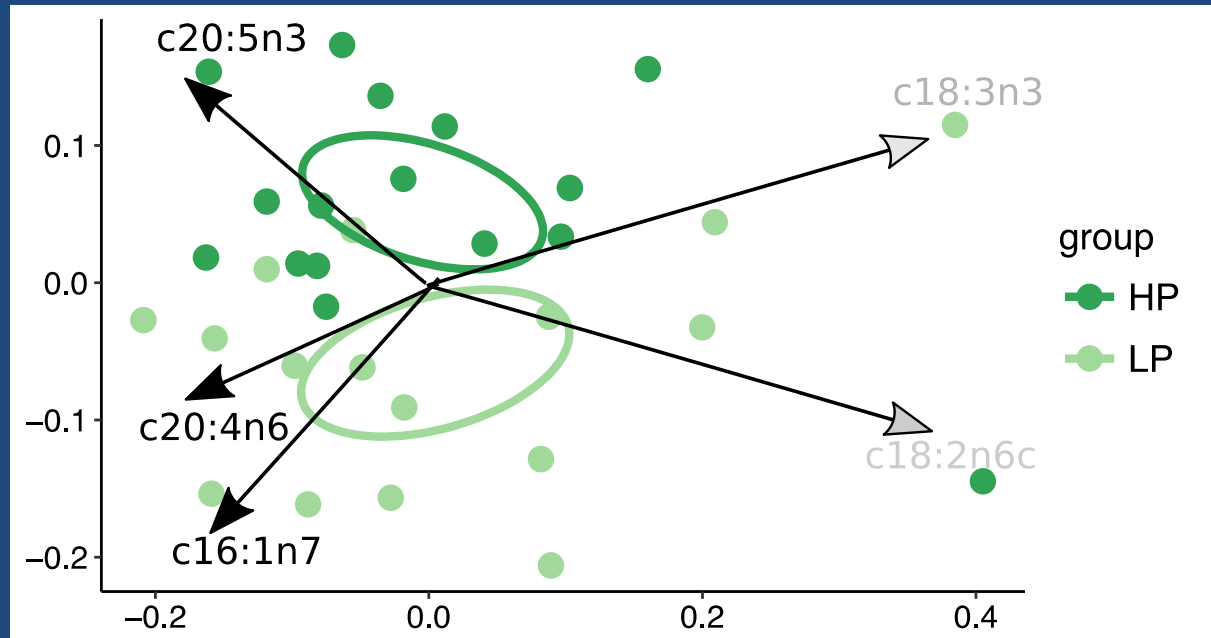


# Treatments organized according to low versus high nitrogen

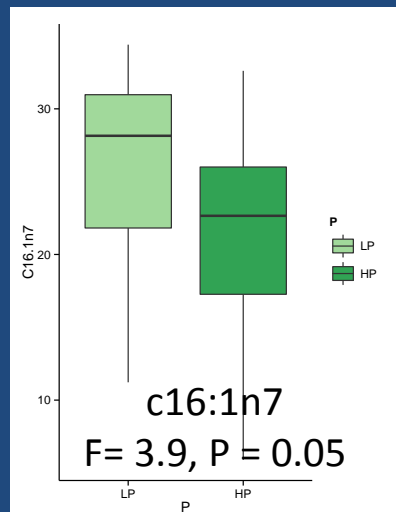
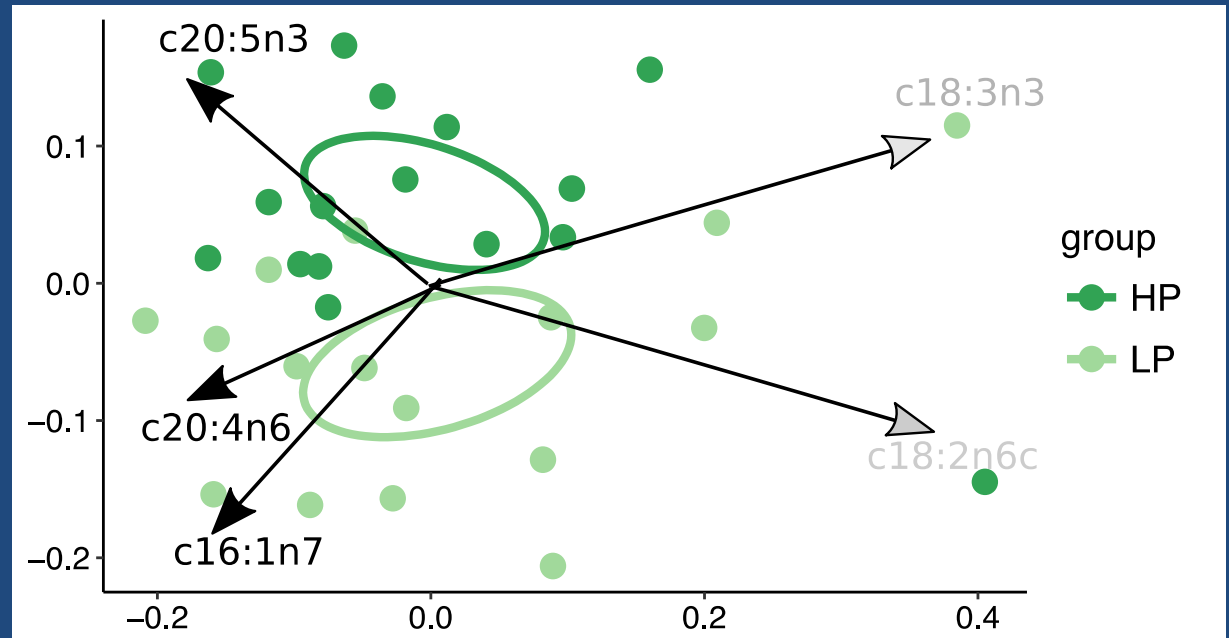
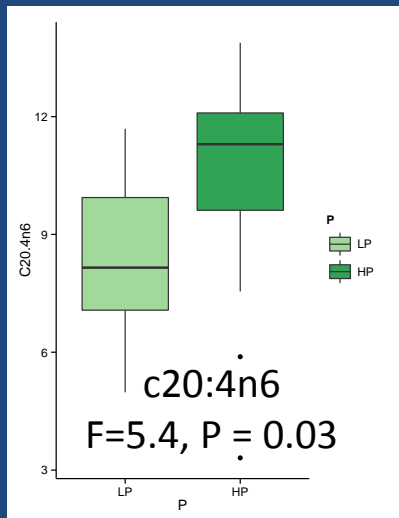
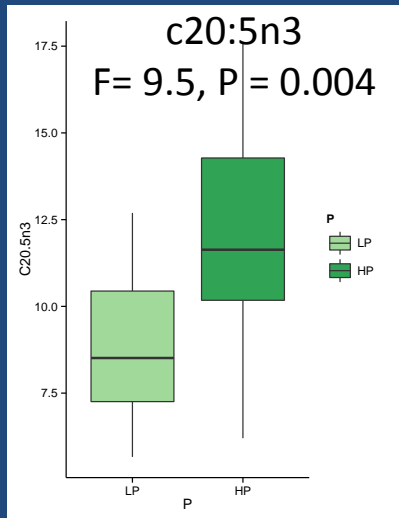




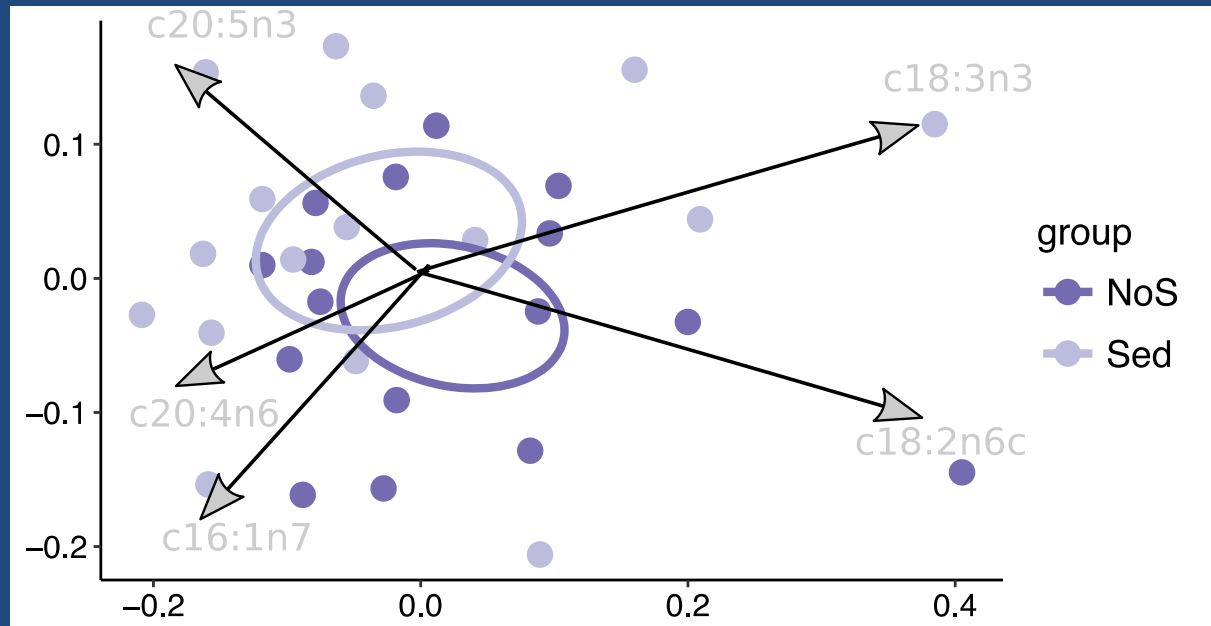
## Treatments organized according to low versus high phosphorus



Treatments organized according to low versus high phosphorus



## Treatments organized according to low versus high sediment



# Can we detect a threshold ?

ANCOVA approach

Mussels

Eels

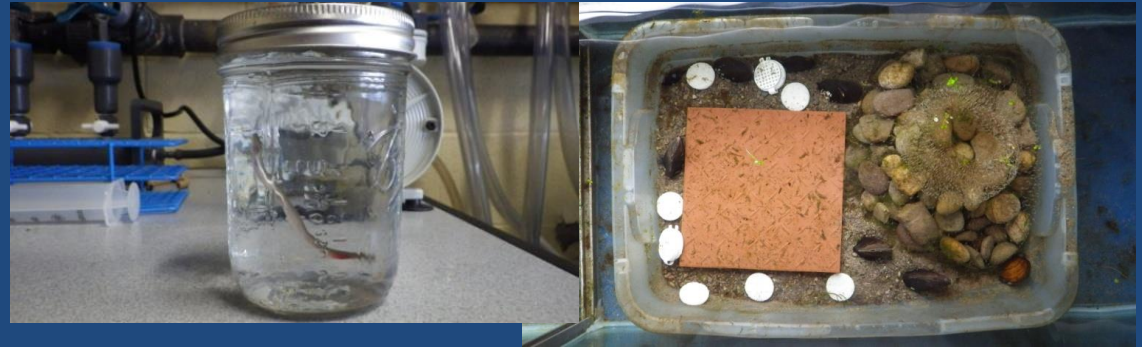
Control (no mussels or eels)

Mussels +eels

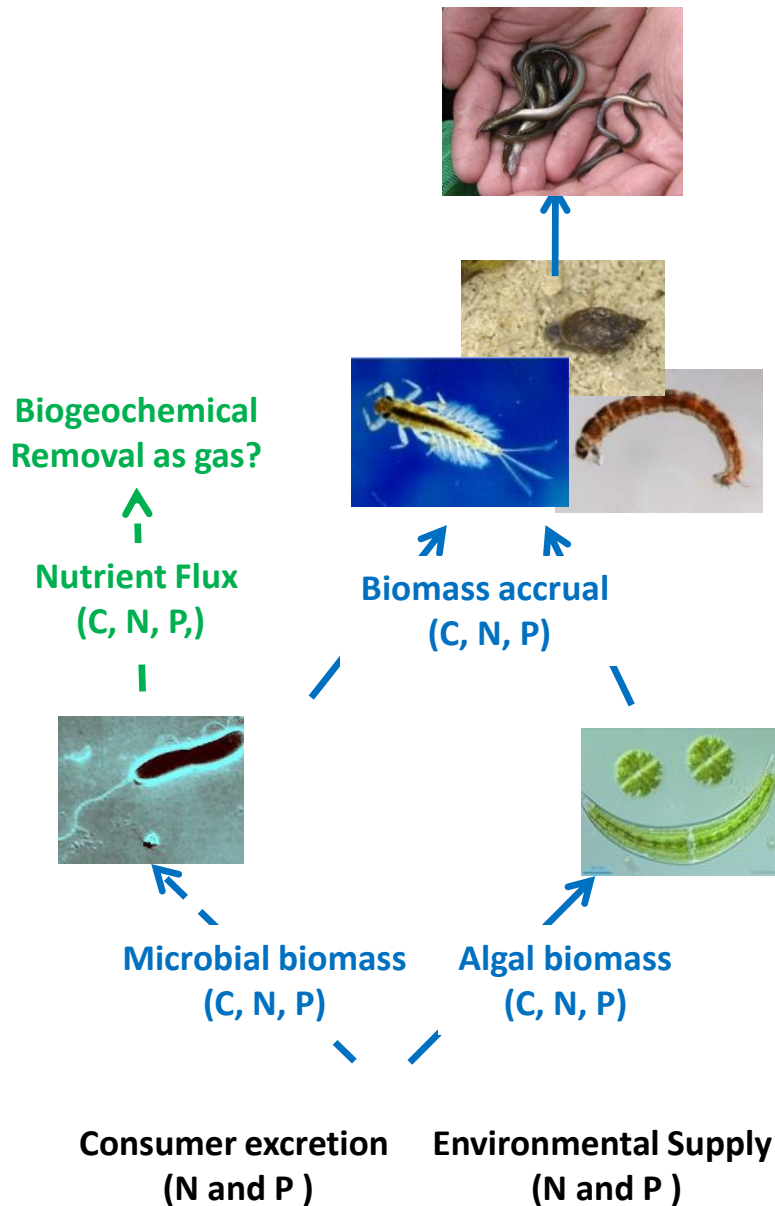
24 different nitrogen loading regimes

0.005-6 mg L  $\text{NO}_3^{-1}$

Blocks of eight loadings per treatment per round



# Can we detect a threshold of ecological function?

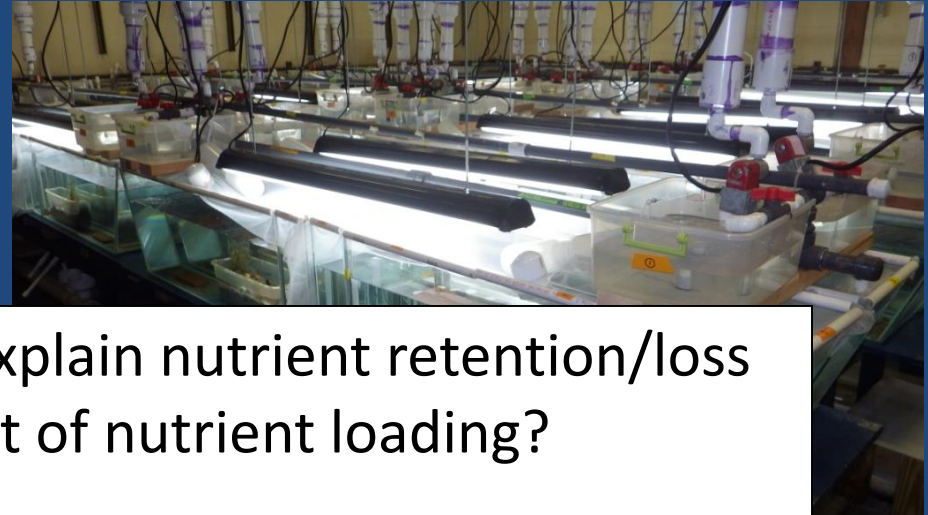


Measured:

- 1) Nutrient uptake (isotope labeling)  
Gas emission ( $N_2O$ ,  $N_2$ ,  $CH_4$ ,  $CO_2$ )
- 2) Macroinvertebrate community structure
- 3) Microbial and algal community  
(phenotype and fatty acid composition)
- 4) Mussel/Eel physiology and excretion
- 5) Dissolved nutrient flux (inflow vs. outflow)



# Can we detect a threshold of ecological function?

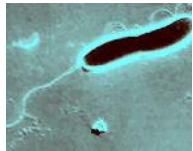


(1) What factors best explain nutrient retention/loss along a gradient of nutrient loading?

(2) Do consumers have the ability to alter this threshold?

Biogeochemistry  
Removal as gas

Nutrient Flux  
(C, N, P)



Microbial biomass  
(C, N, P)



Algal biomass  
(C, N, P)

Consumer excretion  
(N and P)

Environmental Supply  
(N and P)

Gas emission ( $\text{N}_2\text{O}$ ,  $\text{N}_2$ ,  $\text{CH}_4$ ,  $\text{CO}_2$ )

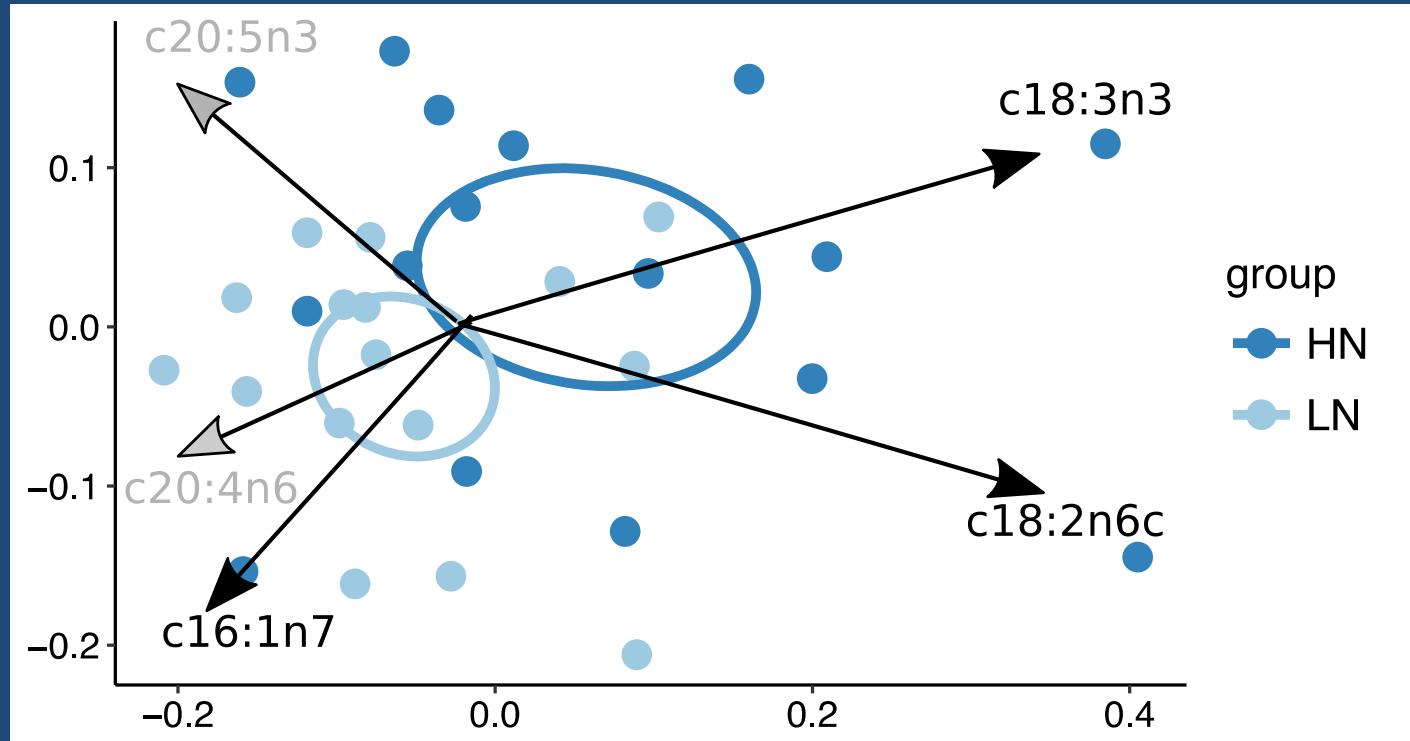
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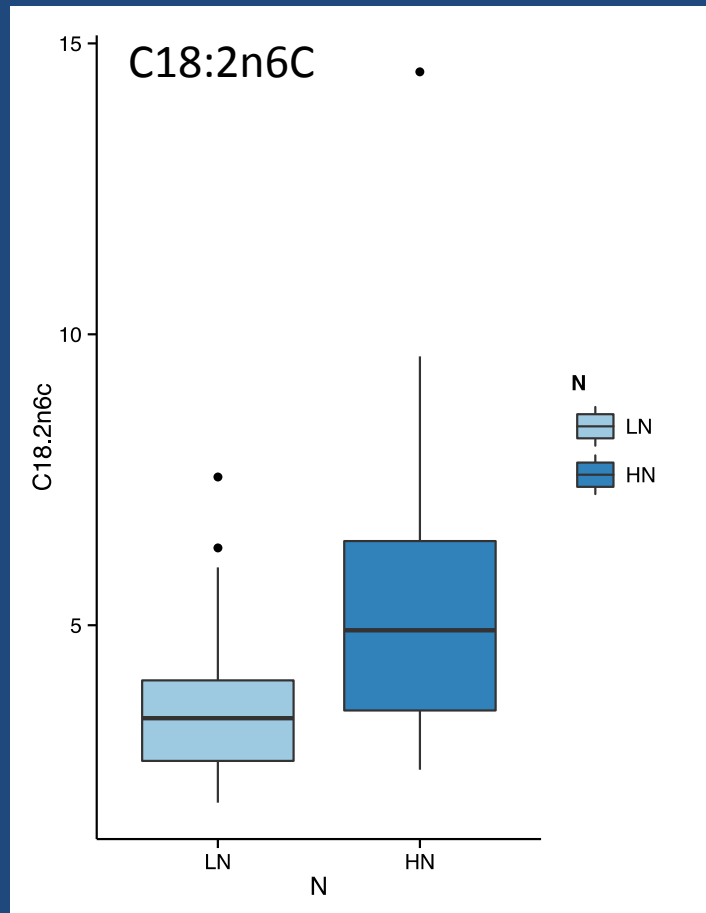
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## Key fatty acids that may be indicative of nitrogen stressors

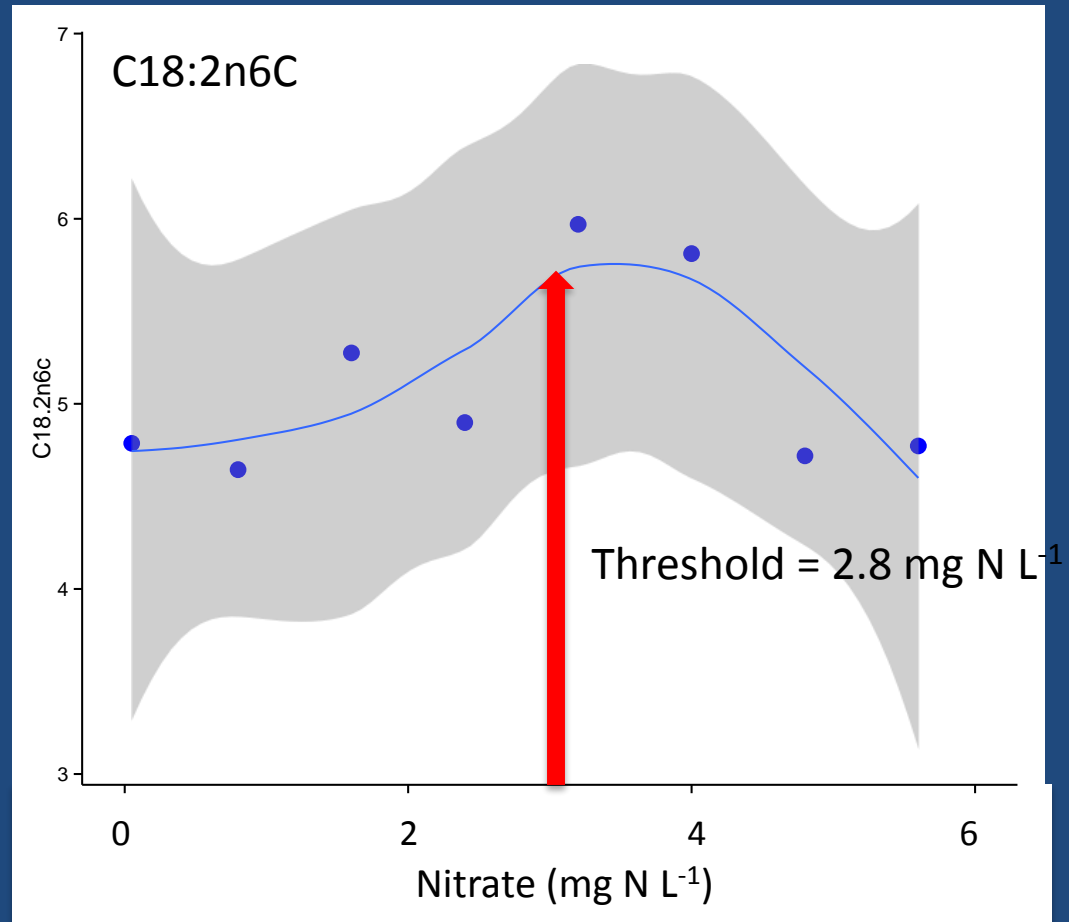


# Key fatty acids that may be indicative of nitrogen stressors

## ANOVA (N,S,P) study

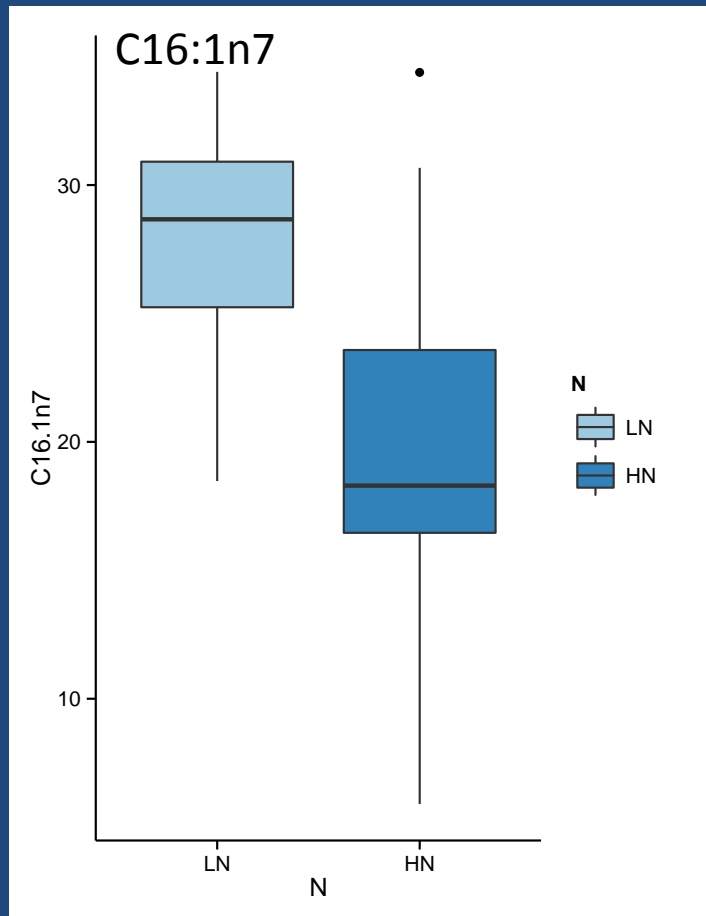


## Threshold study

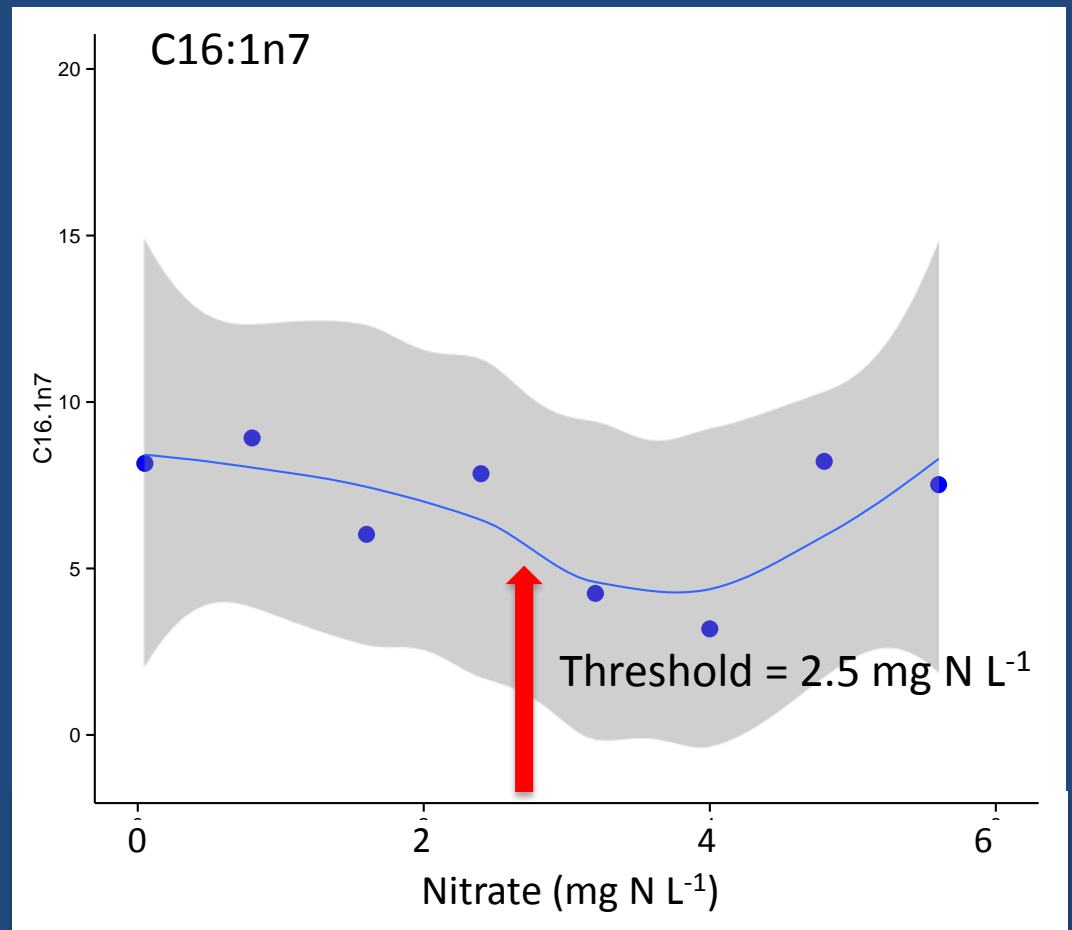


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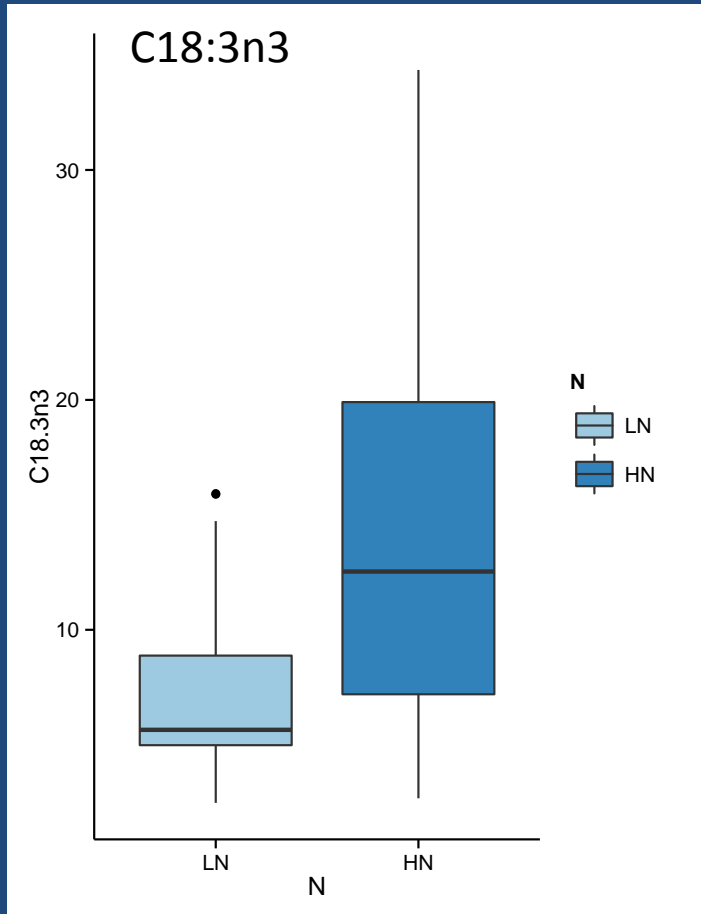


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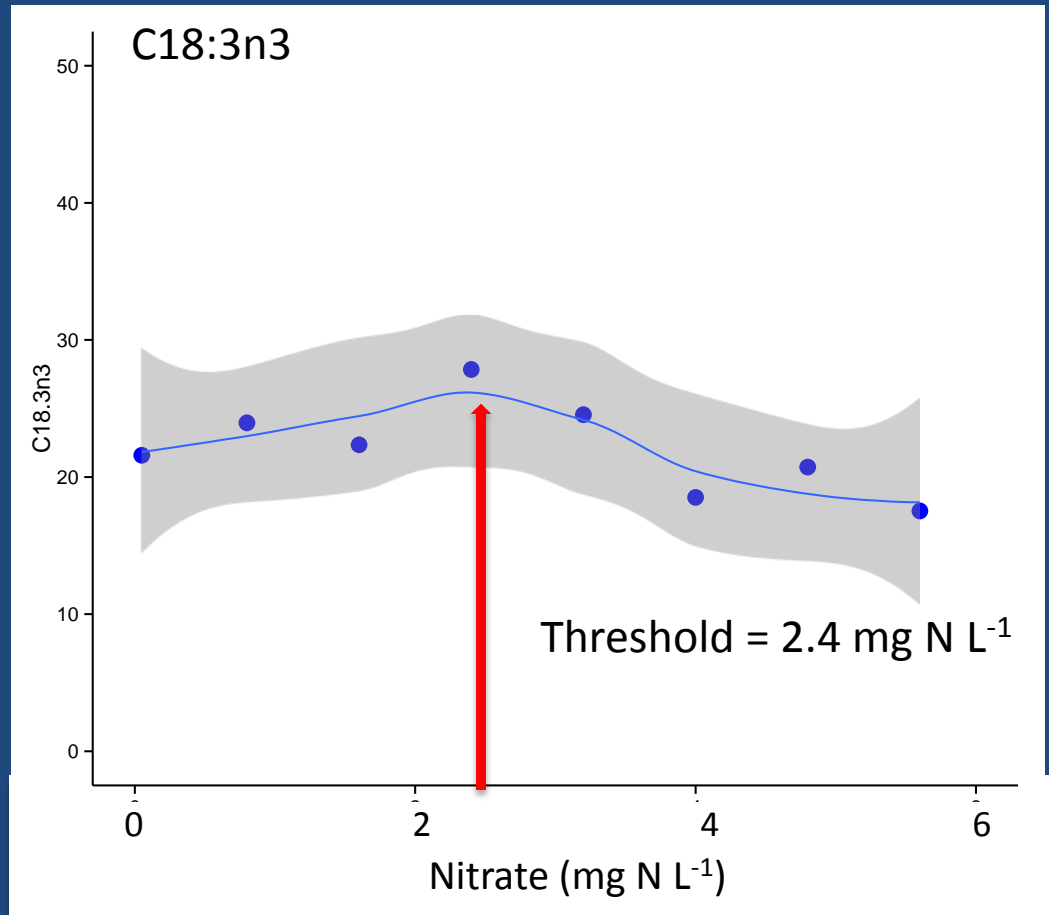


# Key fatty acids that may be indicative of nitrogen stressors

## ANOVA (N,S,P) study



## Threshold study



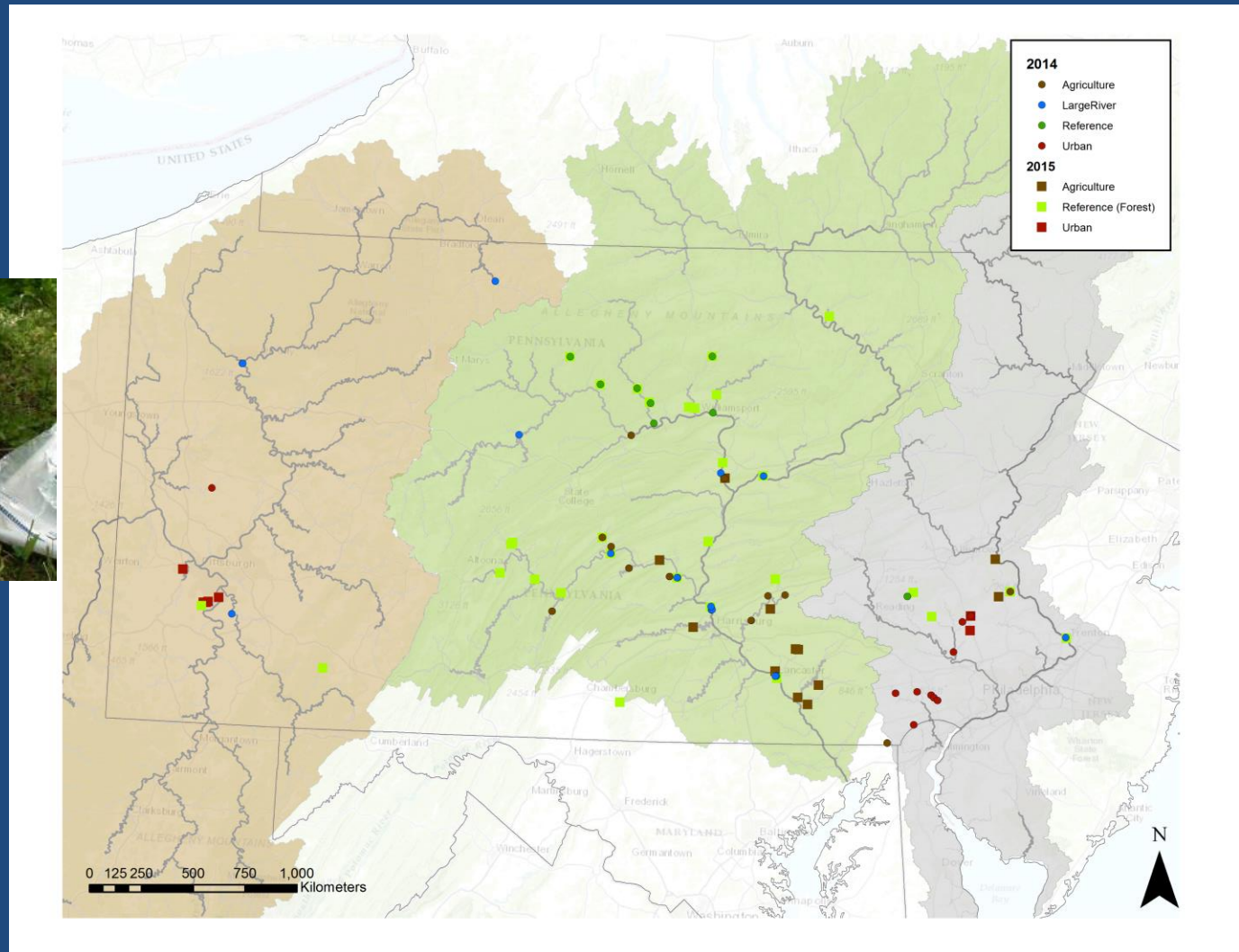


# Scaling up to the real world!

Collaboration with DEP 2014 and 2015

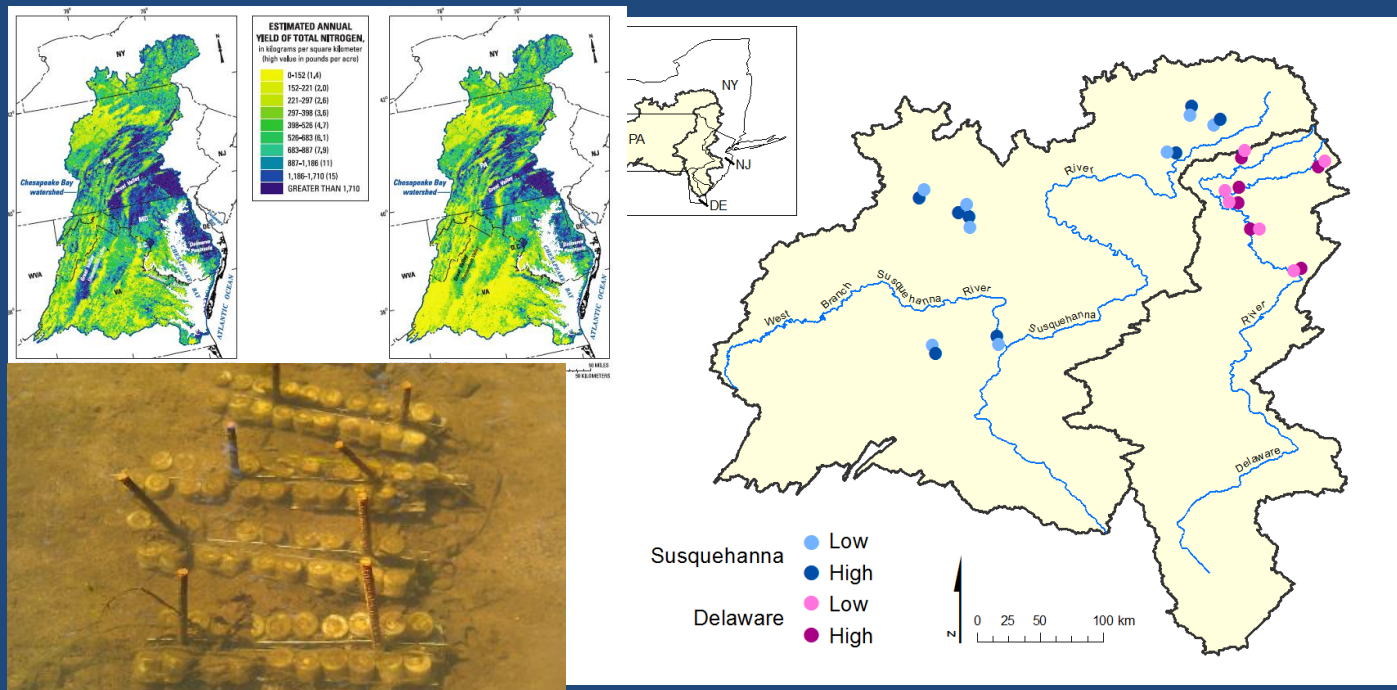
Biofilm Fatty acids

Gradient of watershed land-use and nutrient loading



# Integrating nutritional indices into other USGS capabilities

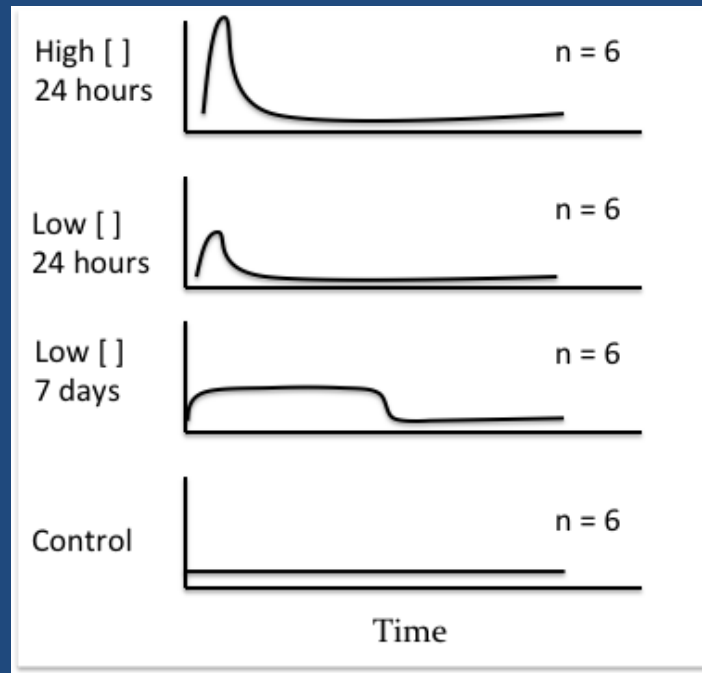
## Linking nutrition to ecological function and SPARROW



# Nutritional studies in other systems with different stressors

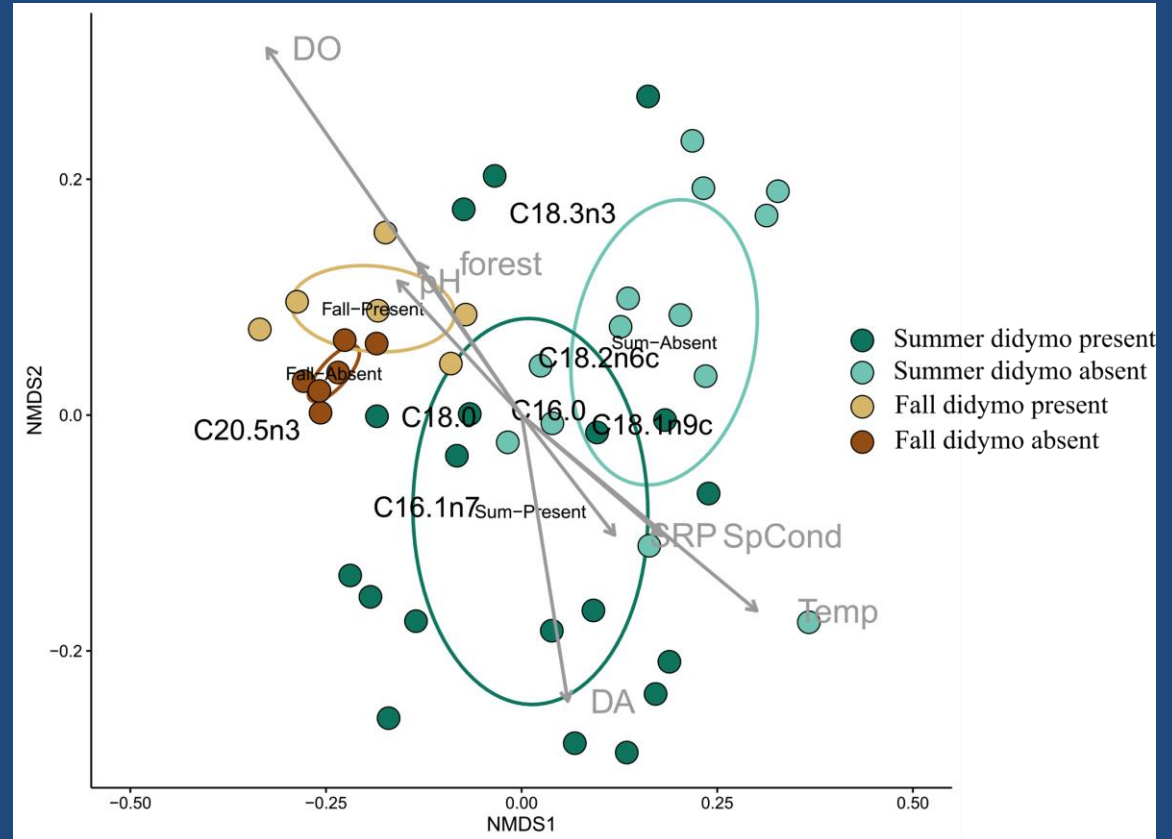
## Simulated brine spill

Ecosystem approach to understanding unconventional gas exploration



## Collaboration with Susquehanna River Basin Commission (SRBC)

### Fatty acid profiles of biofilms with and without Didymo





## Still a lot of science needs to be done!

### Methodological caveats

- How to sample and where to sample?
- Contamination?
- Lab-specific variability in chemistry and interpretation?



**Thank you!**  
**Field and lab assistance**

Cara Campbell  
David Dropkin  
Heather Galbraith  
Gary Walters  
Carrie Blakeslee  
Hillary Abraham  
Andrew Hughes  
Kelly Maloney  
Matt Shank

