

# Identification of Microbial Carbon Cyclers Using Stable Isotope Approaches

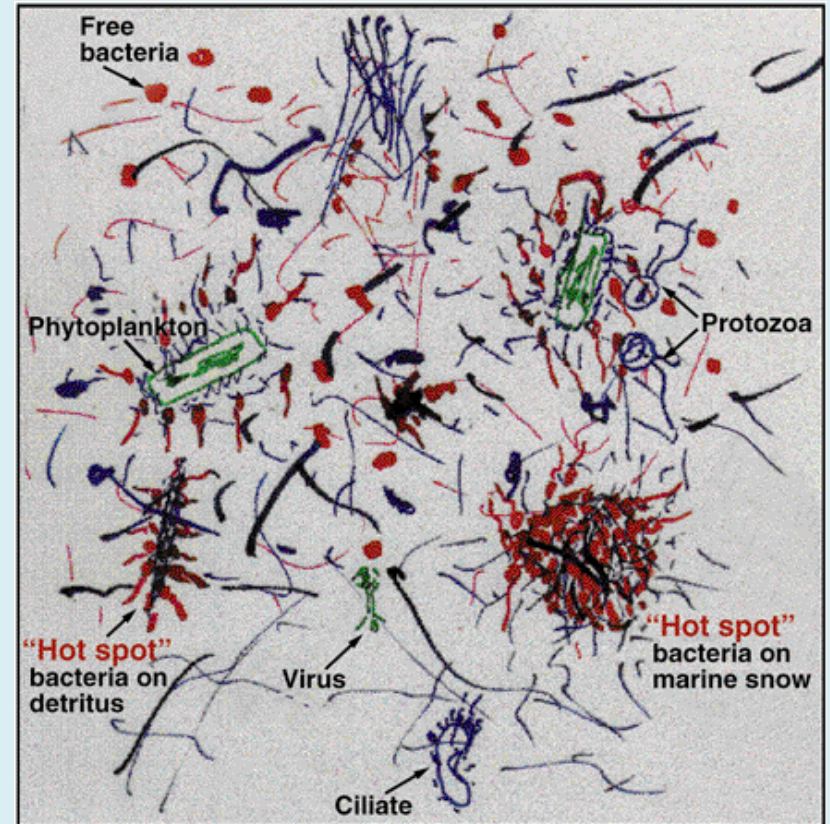
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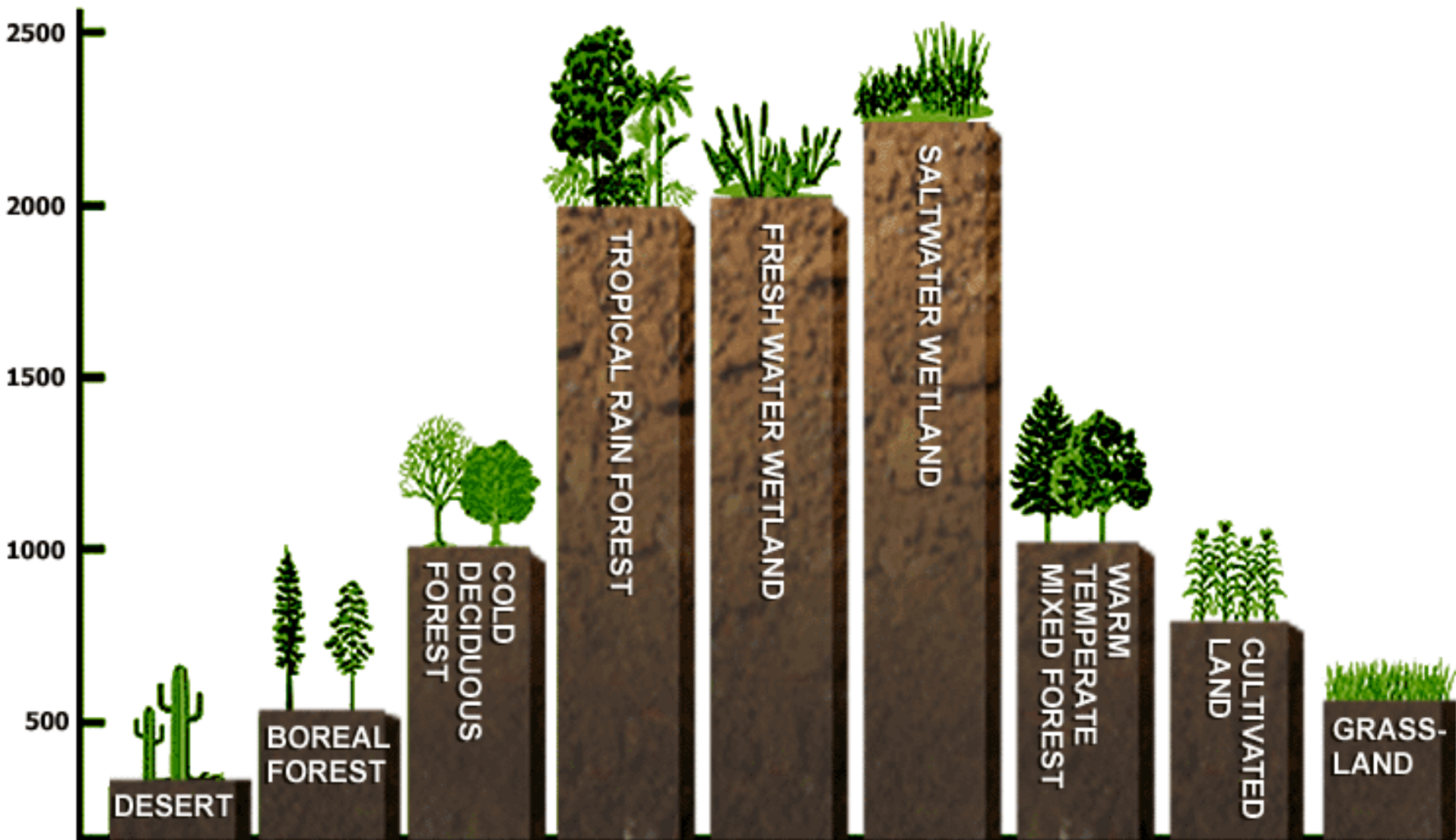
# Microbial Ecosystem Functions

- Biogeochemical cycling
  - Nitrogen
  - Sulfur
  - **Carbon**
- Primary production
- Trophic support



Azam et al. 1998

# NET PRIMARY PRODUCTIVITY OF SELECTED ECOSYSTEMS (g/m<sup>2</sup>/year - amount of photosynthesis)



# Sources of Primary Production



*Spartina foliosa*



*Sarcocornia pacifica*



Algal mats



*Spartina* plant detritus (lignocellulose)

**Lignin**

**Cellulose**

**Hemicellulose**

(Benner et al. 1987)

Algal detritus (labile photosynthates)

**Carbohydrates**

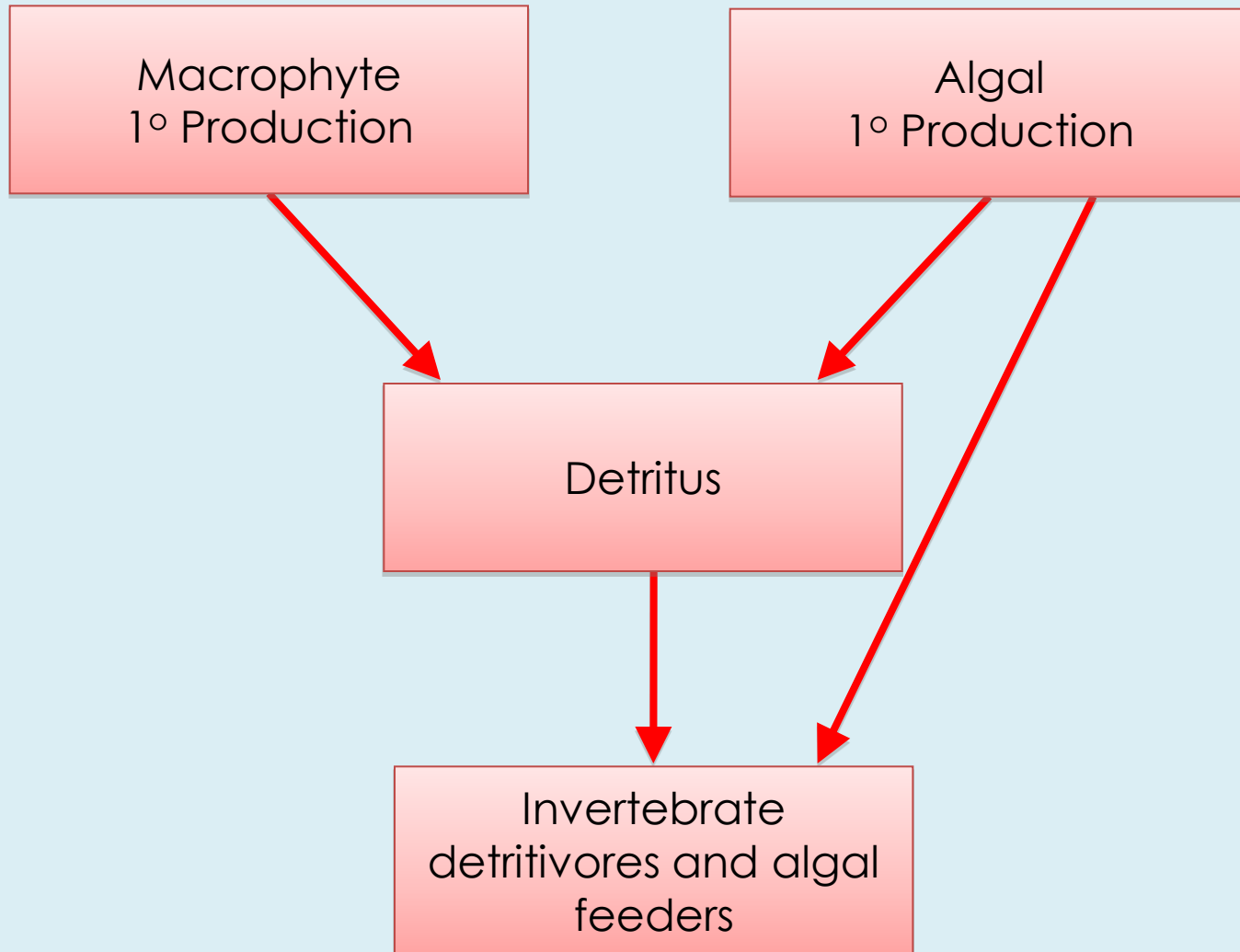
**Polysaccharides**

**Sugars**

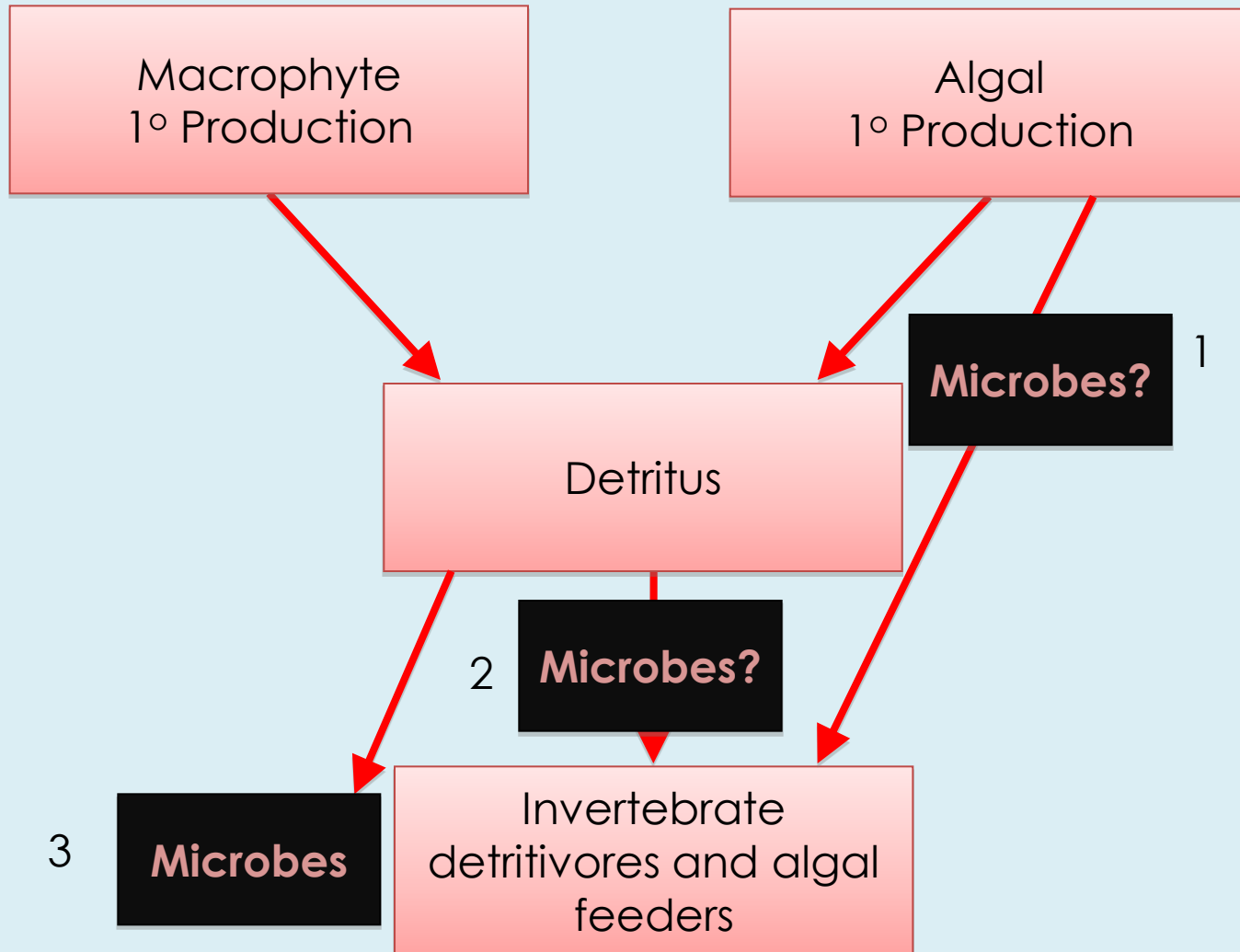
(Jones and Cannon 1986)

Greater algal productivity on west coast (Zedler et al.1980; Bouillon and Boschker, 2006 )

# Fate of Organic Matter



# Fate of Organic Matter



# Objectives

- Identify **bacteria** responsible for the utilization of marsh macrophyte derived carbon
- Identify **invertebrates** responsible for *Spartina* and algal utilization
- Place microbial carbon cyclers within benthic trophic structure

# Study Site





# Stable Isotope Analysis (SIA)

$$\delta^{13}\text{C} = \left( \frac{^{13}\text{C}/^{12}\text{C}_{\text{sample}}}{^{13}\text{C}/^{12}\text{C}_{\text{standard}}} \right) - 1 \times 1000$$

1 per mil shift in C per trophic level

## Phototrophs

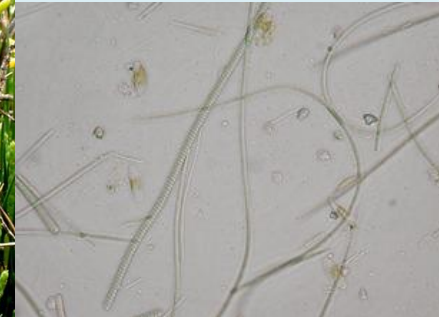
$\delta^{13}\text{C}$  *Salicornia*      Diatoms      *Spartina*      Cyanobacteria

-26

-20

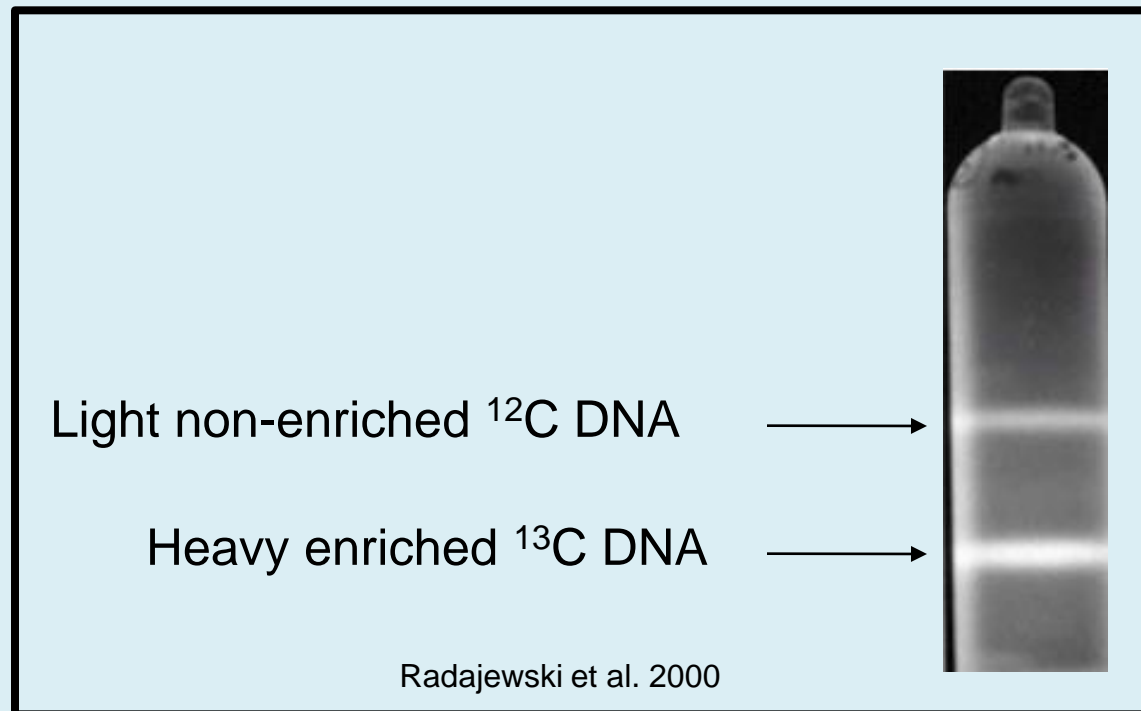
-13

-10



# I. DNA Stable Isotope Probing (SIP)

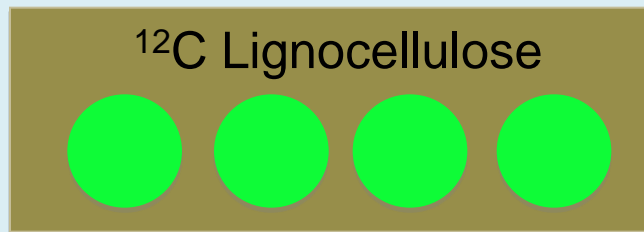
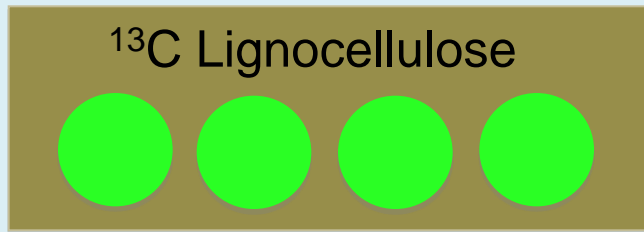
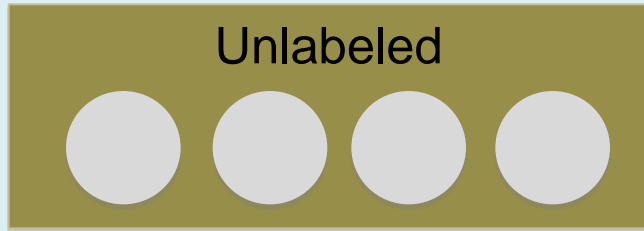
- Directly links **microbial identity with function** in a natural community (Radajewski et al. 2000; Neufeld, 2007)



# Applications of DNA SIP

Metabolizer type	Substrate	Habitat	References
Methanol utilizers	$^{13}\text{C}$ methanol	Forest soil sample	Radajewski et al. 2000
Phytodetritus degraders	$^{13}\text{C}$ cyanobacteria	Marine sediment	Gihring et al. 2009
Pollutant degraders	$^{13}\text{C}$ naphthalene	Bioreactor	Singleton et al. 2005
Root leachate utilizers	$^{13}\text{CO}_2$	Grassland soil	Rangel-Castro, 2005
Fungal methanol degraders	$^{13}\text{C}$ methanol	Rice field soil	Lueders et al. 2004
Methanotrophs	$^{13}\text{C}$ methane	Peat Bog	Morris et al. 2002
Cellulose degraders	$^{13}\text{C}$ cellulose	Pine soils	Eichorst and Ruske, 2012
Benzene degraders	$^{13}\text{C}$ benzene	Enrichment cultures	Herrmann et al. 2010

# Sampling For SIP



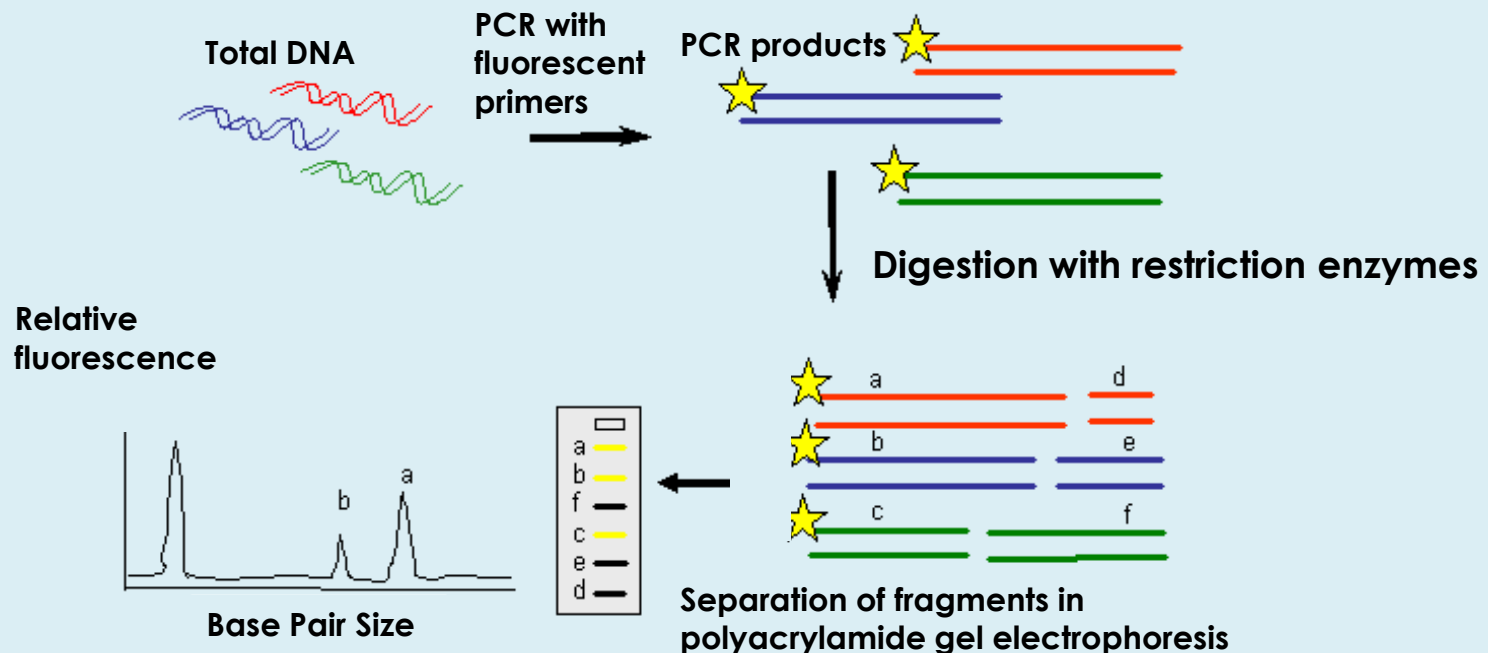
- Used surface sediment cores
- Amended microcosms in lab
- Sampled at 0 and 30 days
- DNA extraction
- Density gradient centrifugation
- Fractionation and collection of DNA
- Molecular analyses

Total=12 x 1 cm cores divided into 3 treatments



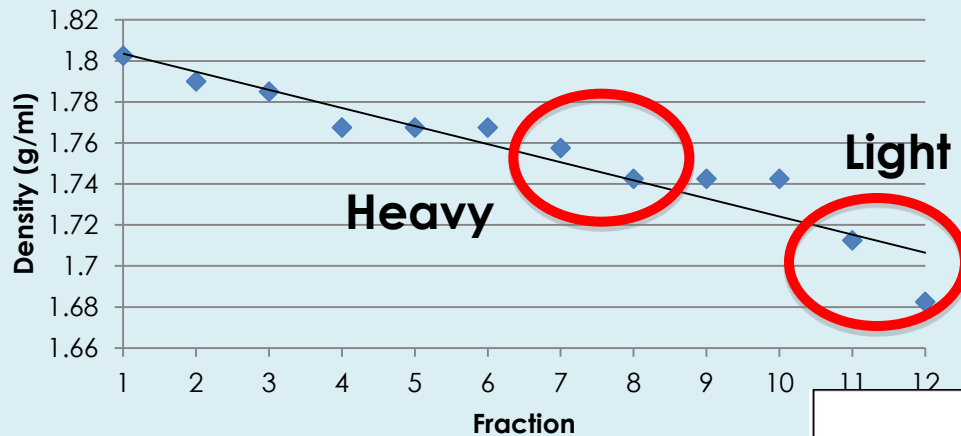
# DNA SIP Molecular Analysis

- PCR amplification of 16S rRNA genes for bacteria
- Terminal Restriction Fragment Length Polymorphism (**T-RFLP**) to identify general community differences
- Clones libraries created for the heavy (utilizers) and light (non-utilizers) fractions

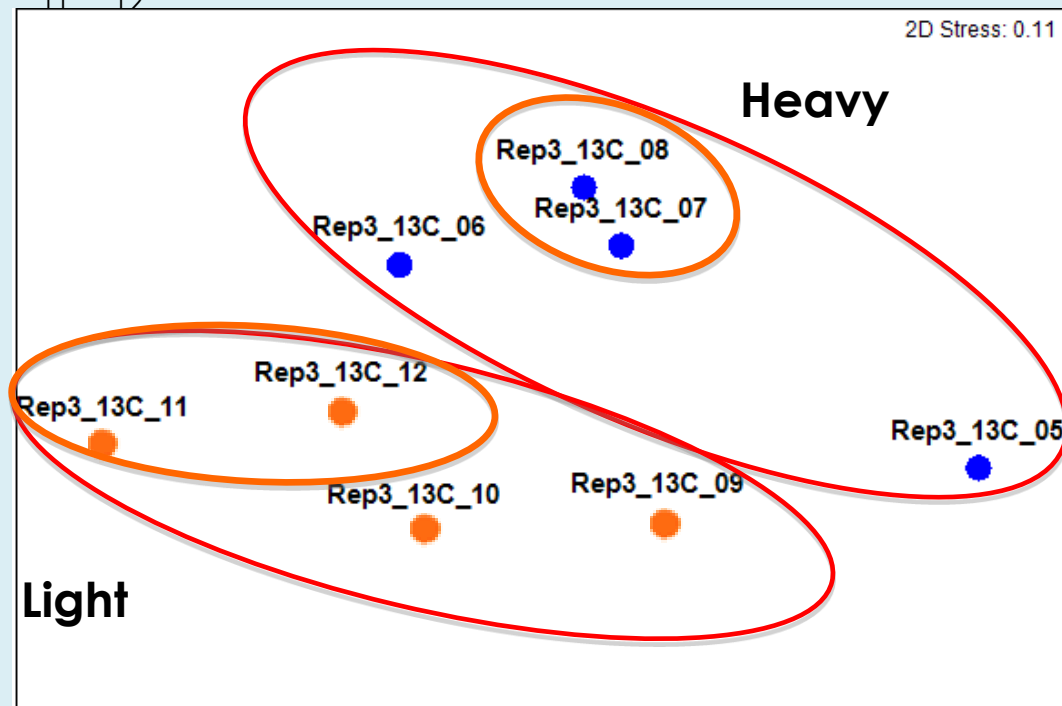


# Identifying Enriched Fractions

$^{13}\text{C}$  Tube Fraction Density

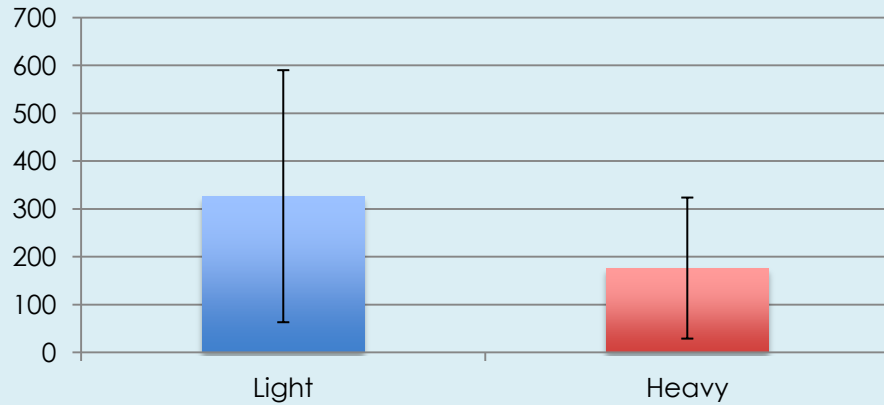


• Verified  $^{12}\text{C}$  Lignocellulose sediment value was not enriched

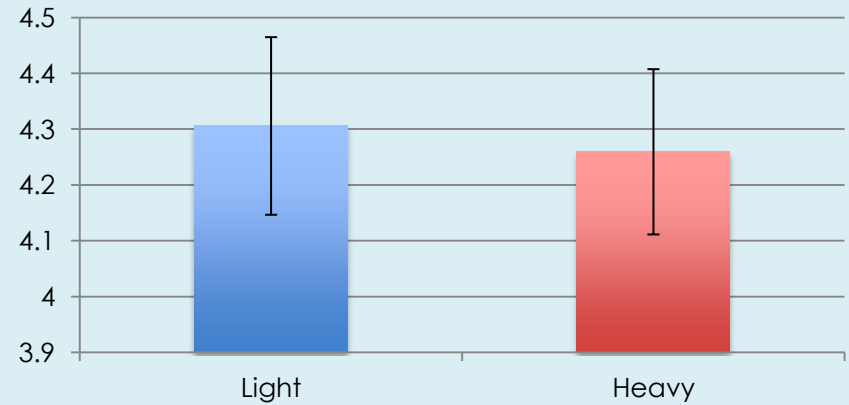


# Similar Richness and Diversity

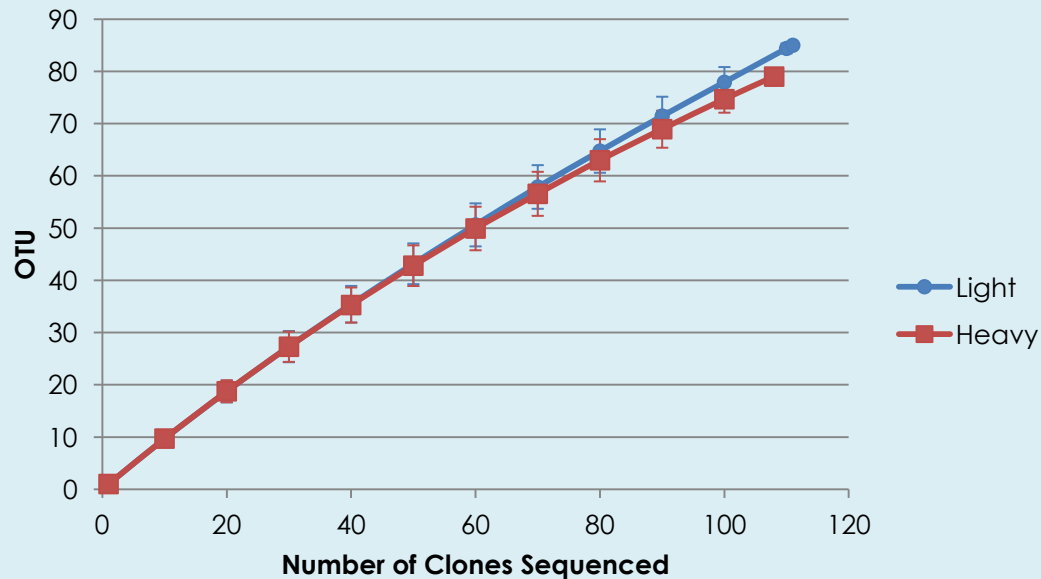
## Chao



## Shannon

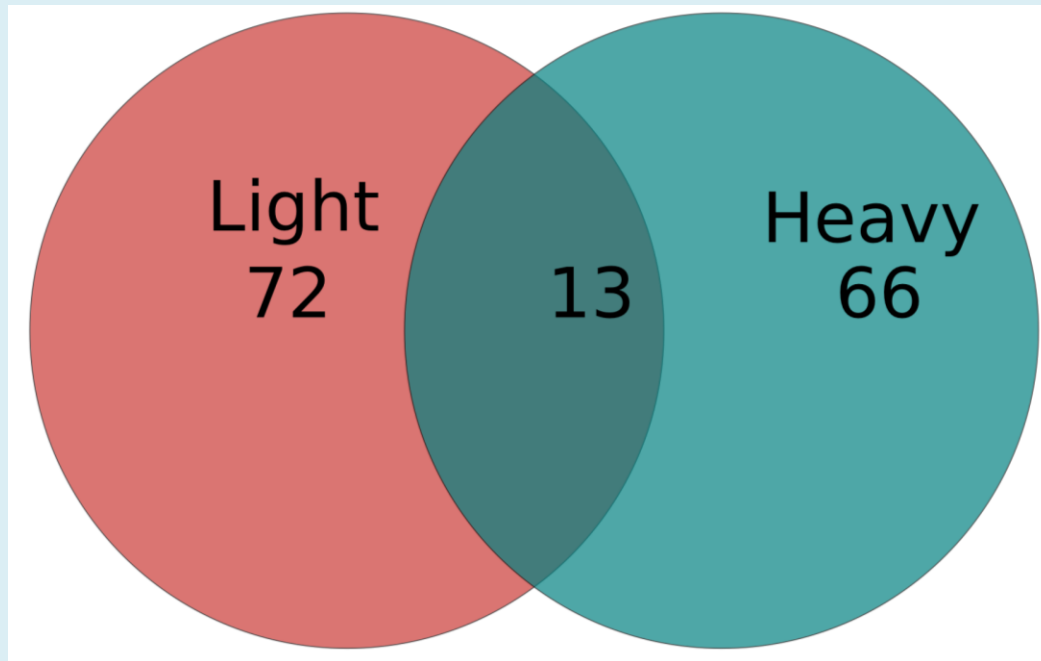


## Rarefaction Curve



# Distinct Community Structure

Species Level



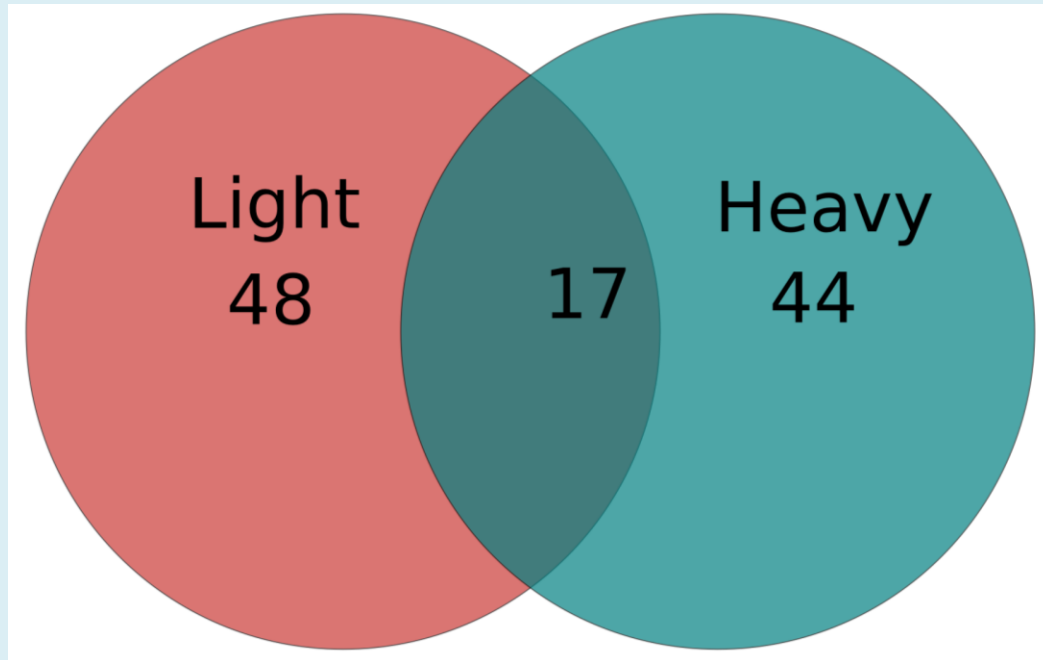
**Libshuff:** determines if clone libraries are significantly different based on operational taxonomic units (OTU)

**Libshuff  $p < 0.001$**



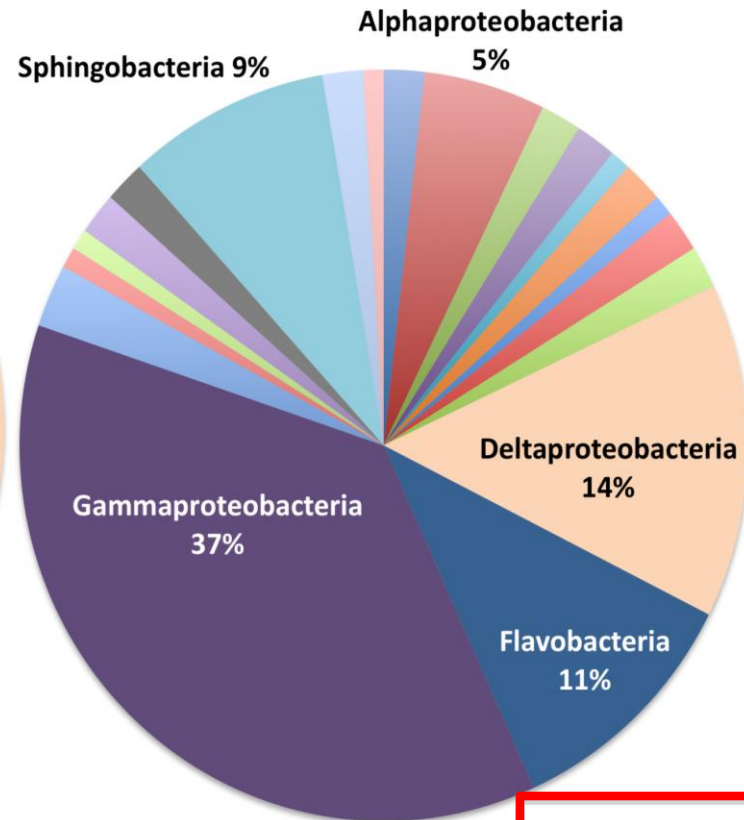
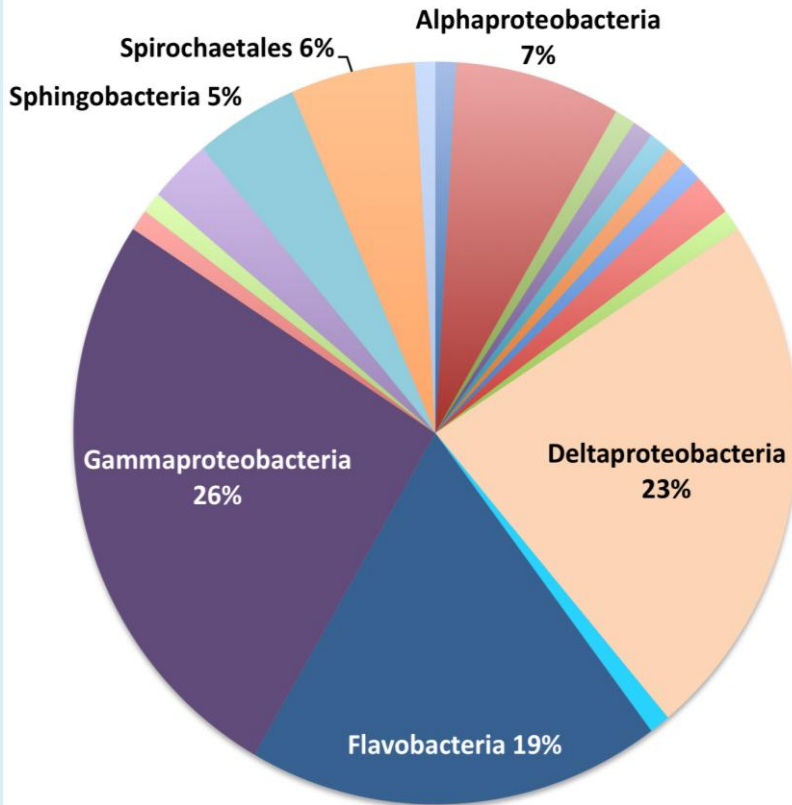
# Distinct Community Structure

Genus Level



# Heavy

# Light



- |                       |                       |                         |                 |                  |
|-----------------------|-----------------------|-------------------------|-----------------|------------------|
| ■ Acidobacteriaceae   | ■ Alphaproteobacteria | ■ Betaproteobacteria    | ■ Cyanobacteria | ■ Clostridia     |
| ■ Deltaproteobacteria | ■ Flavobacteria       | ■ Gammaproteobacteria   | ■ Holophagae    | ■ Verucomicrobia |
| ■ Planctomycetacia    | ■ Sphingobacteria     | ■ Epsilonproteobacteria | ■ Holophagae    | ■ Spirochaetales |

## II. *In Situ* Enrichment Experiment

- Tracked enriched  $^{13}\text{C}$  algal mats and  $^{15}\text{N}$  *Spartina* into the organisms that utilized these different sources of carbon



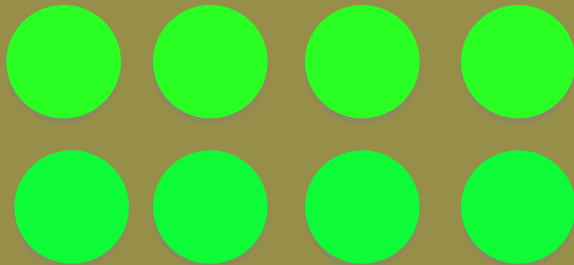
Enriched algal mat - 6X background  
Enriched *Spartina* -100X background

# Sampling For SIA

$^{13}\text{C}$  Bicarbonate Algal Mats



Litter bags of  $^{15}\text{N}$  *Spartina*



100  $\mu\text{m}$   
mesh

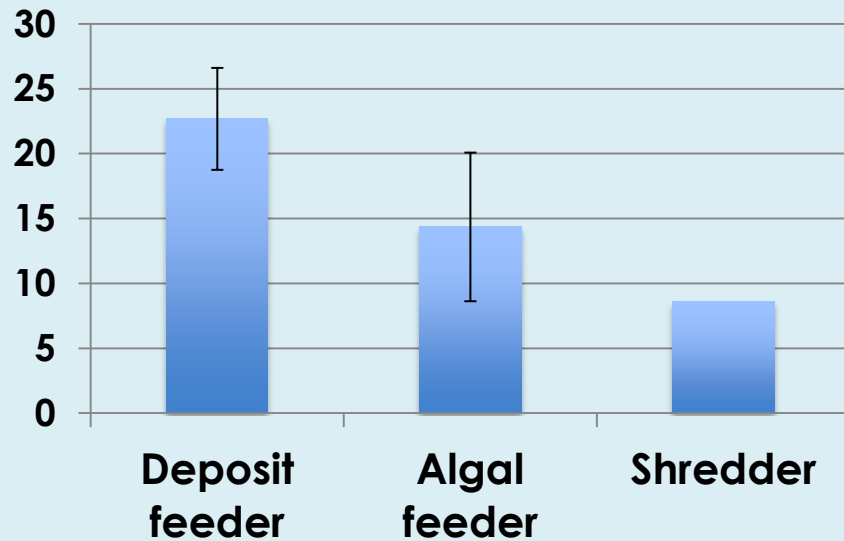
~2 mm  
mesh

Sampled sediment from below litter bags at 0 and 30 days for:

Inverts  
Microbes  
Sediment  
Algae  
*Spartina*

# Enrichment May Vary by Feeding Type

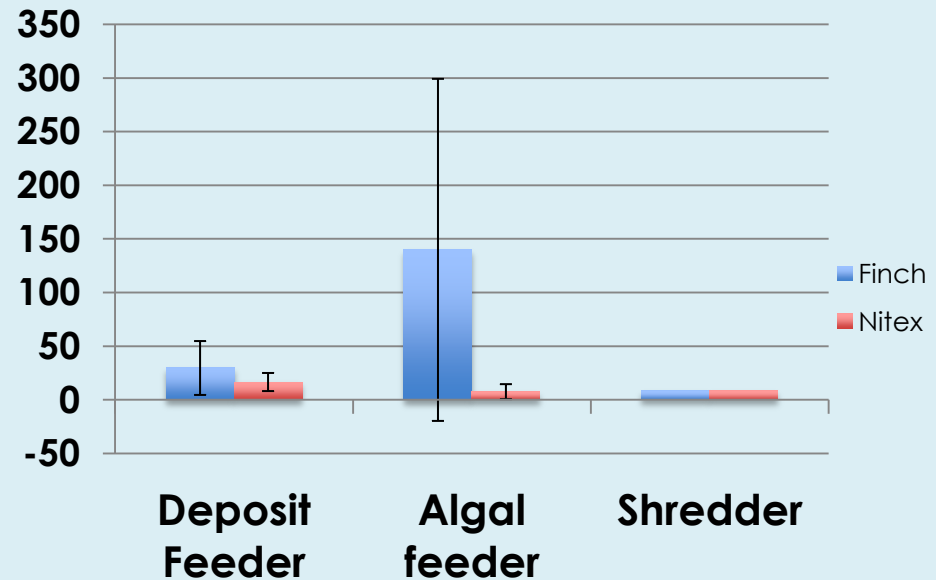
$\Delta$  in  $\delta^{13}\text{C}$  Enriched Algal Treatment



$F_{2,10}=3.63$

$P=0.065$

$\Delta$  in  $\delta^{15}\text{N}$  Enriched Spartina Treatment



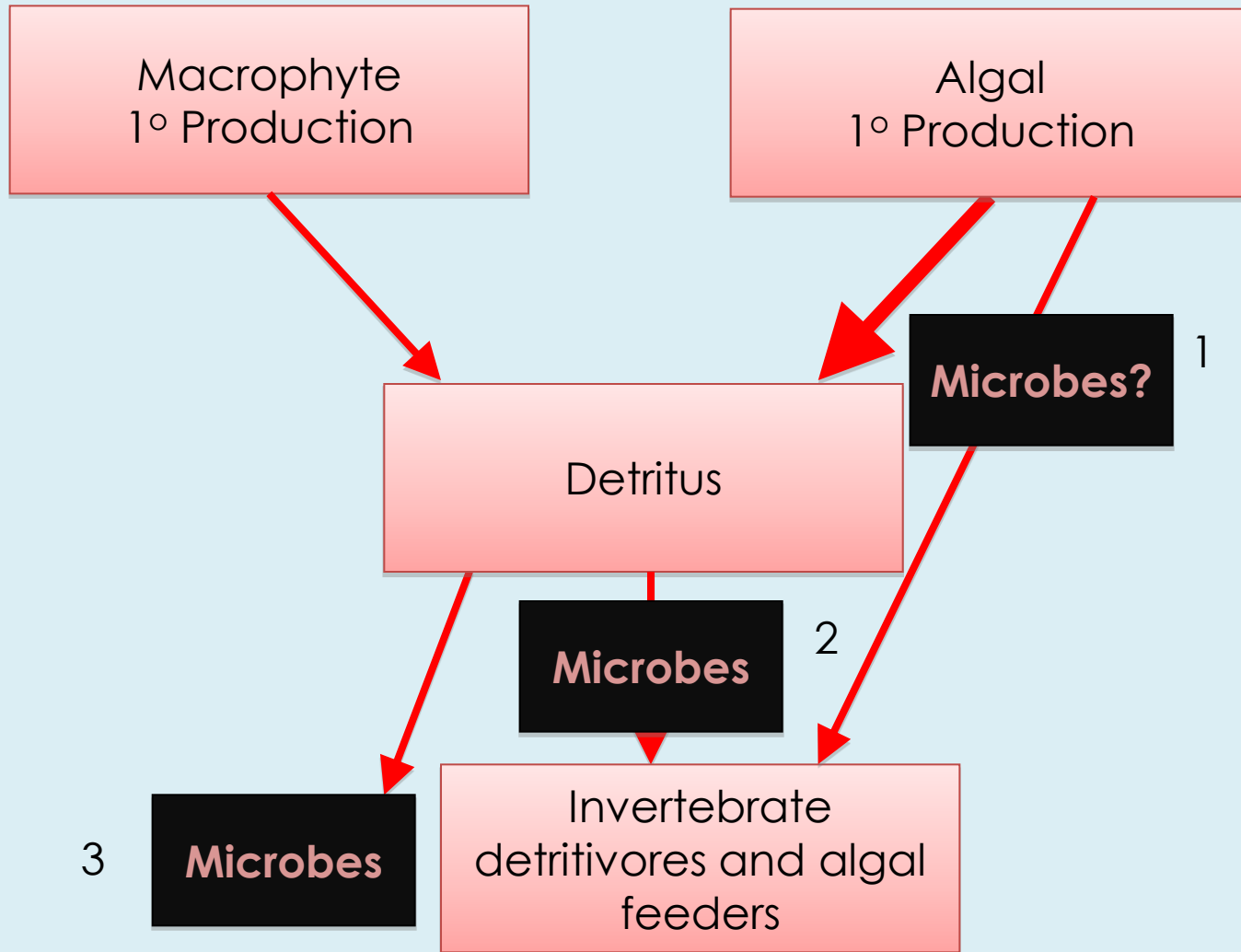
**Finch**  $H_2=0.74$   $P=0.69$

**Nitex**  $F_{2,9}=1.87$   $P=0.209$

# Conclusions

- This study is the first to use SIP with salt marsh sediment
- Lignocellulose SIP successfully identified bacterial carbon utilizers
  - community structure differences
- DNA SIP paired with enrichment studies has the potential to elucidate microbial mediated pathways

# Fate of Organic Matter



# Further Investigation

- Direct counts for microbial biomass and abundance
- Fungal characterization via DNA SIP
- Mixing models of stable isotope data





# Acknowledgements

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