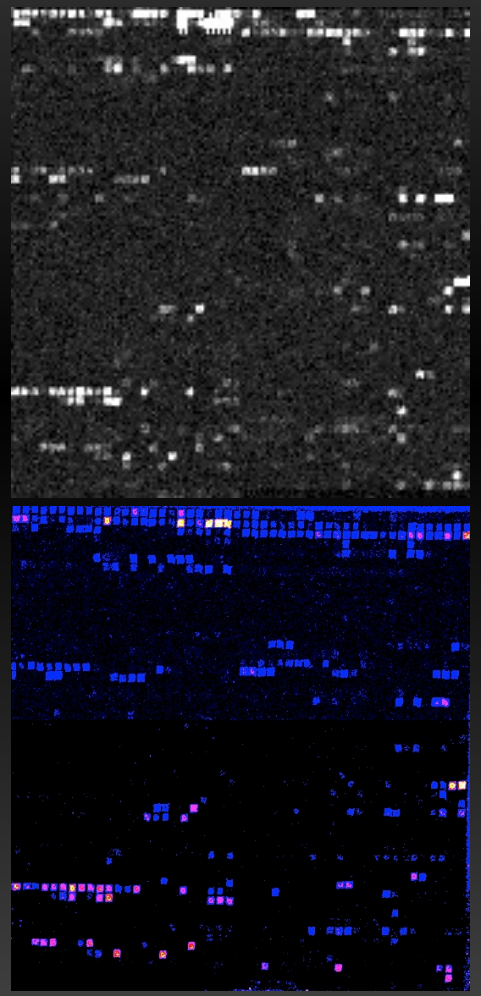
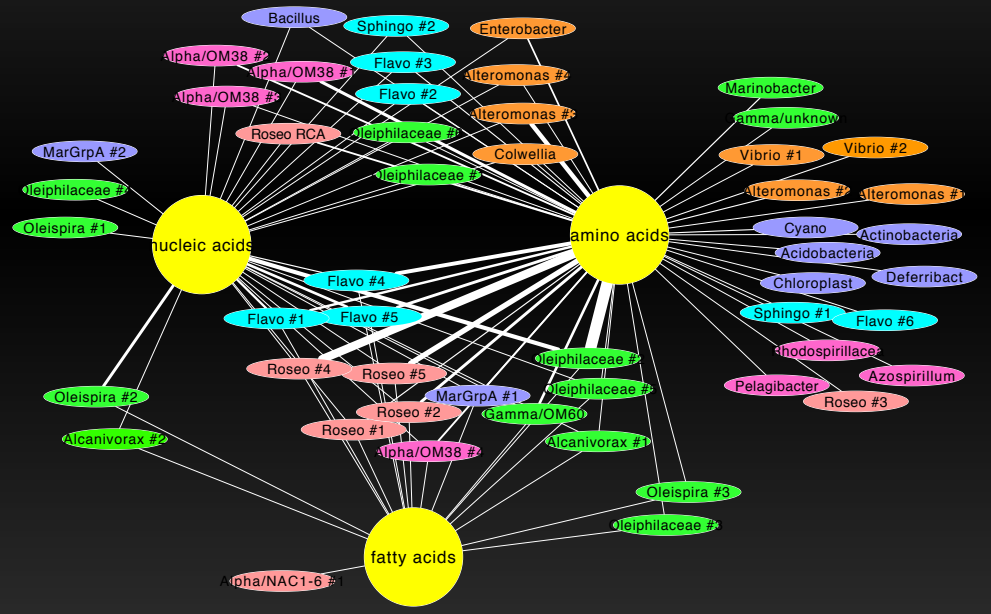
NanoSIMS and stable isotope probing for quantitative microbial biogeochemistry



Xavier Mayali
Lawrence Livermore National Laboratory



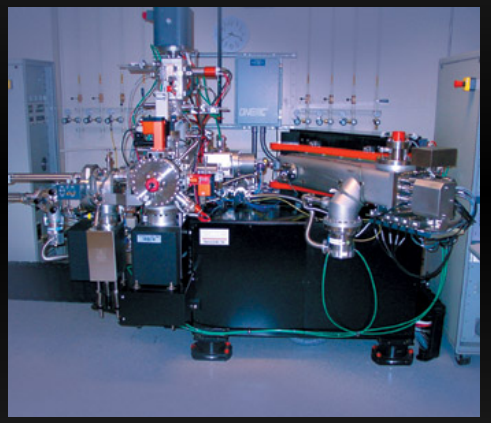
Woods Hole Oceanographic Institution,
February 6 2018

This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344

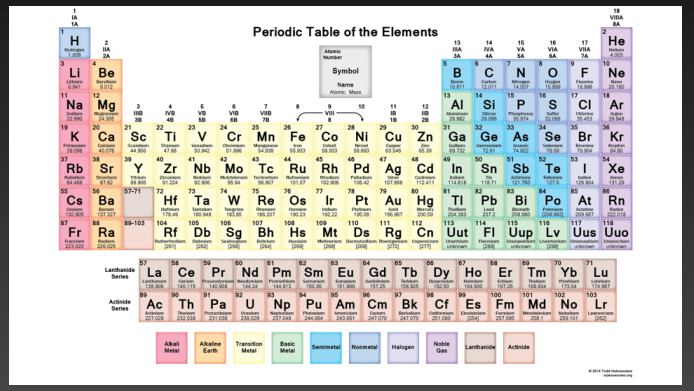
Talk Outline

1. Science at Department of Energy National Laboratories

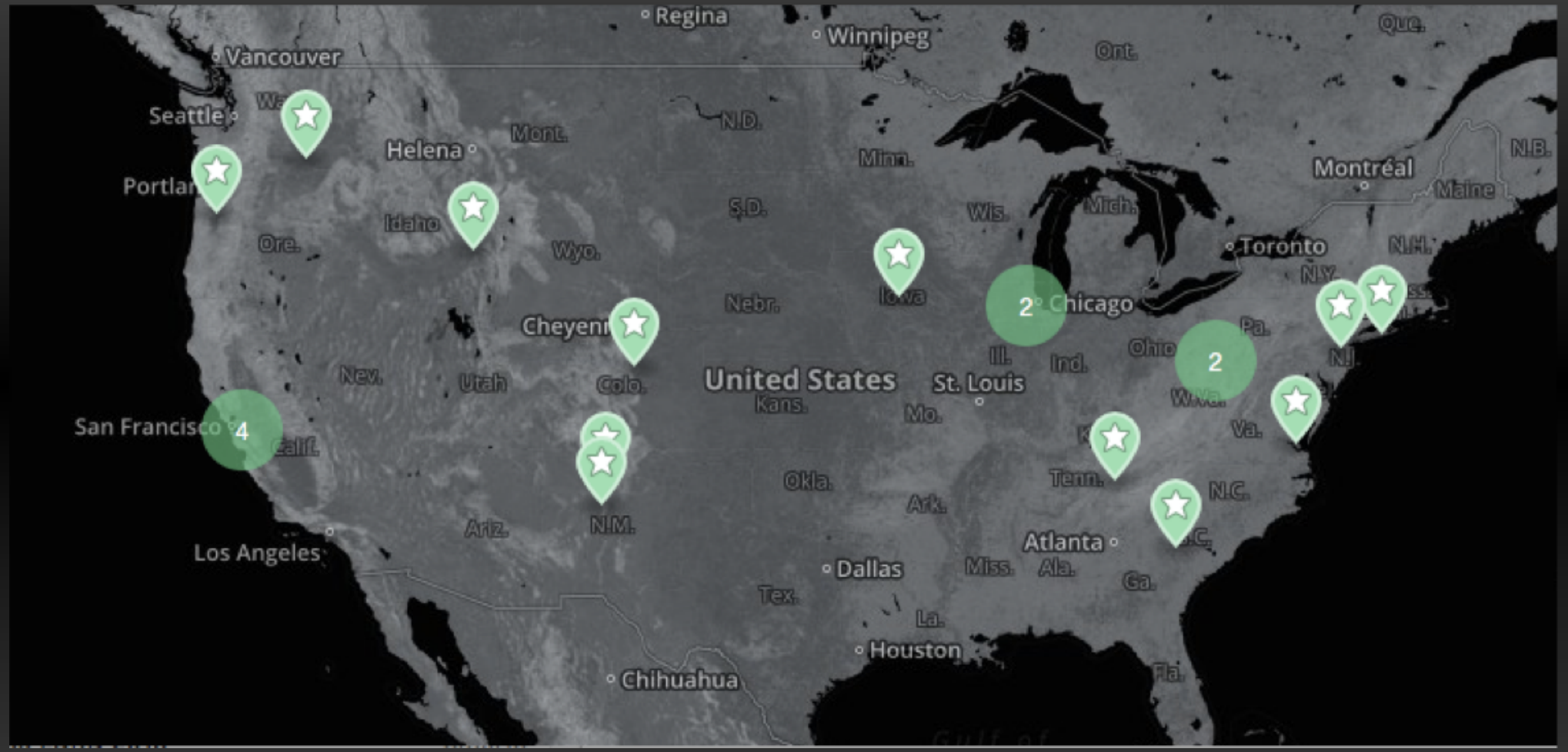
2. Approach to linking microbial identity and biogeochemical function (stable isotopes + NanoSIMS)



3. A few examples of applying this approach
    1. Bacterial organic matter cycling
    2. Interactions between bacteria and microalgae



DOE National Laboratories



Total = 60,000 employees

https://www.energy.gov/maps/doe-national-laboratories

Lawrence Livermore National Laboratory

5,800 employees
Armed guards at the gate
Free bikes!

Funding structure

All scientists are 100% soft money positions

-Programmatic (LLNL-mission driven): non-competitive

-Competitive (external, internal)

DOE's Genomic Sciences program (in Biological and Environmental Research)

DOE's Bioenergy Technologies Office (in Energy Efficiency and Renewable Energy)

LLNL Laboratory Directed Research and Development program

Gordon and Betty Moore Foundation

NSF/NASA

Lawrence Livermore National Laboratory

DOE GENOMIC SCIENCE
SYSTEMS BIOLOGY FOR ENERGY AND ENVIRONMENT
OFFICE OF SCIENCE
U.S. DEPARTMENT OF ENERGY

GORDON AND BETTY MOORE FOUNDATION

Work structure

Most projects involve combinations of staff scientists, postdocs, technical staff, and outside collaborators (academia, government labs, private companies)

Staff scientists


Peter Weber


Erin Nuccio


Jennifer Pett-Ridge


Ty Samo


Rhona Stuart

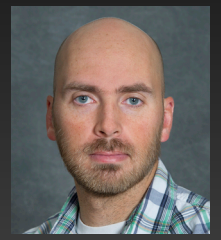

Steve Blazewicz


Xavier Mayali


Ben Stewart

Postdocs

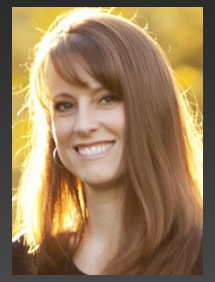

Chris Ward


Jeff Kimbrel

Technical staff

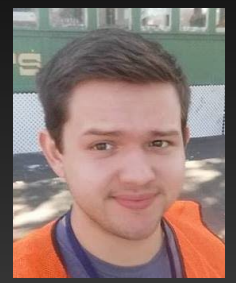

Shalini Mabery


Christina Ramon


Jessica Wollard

Students

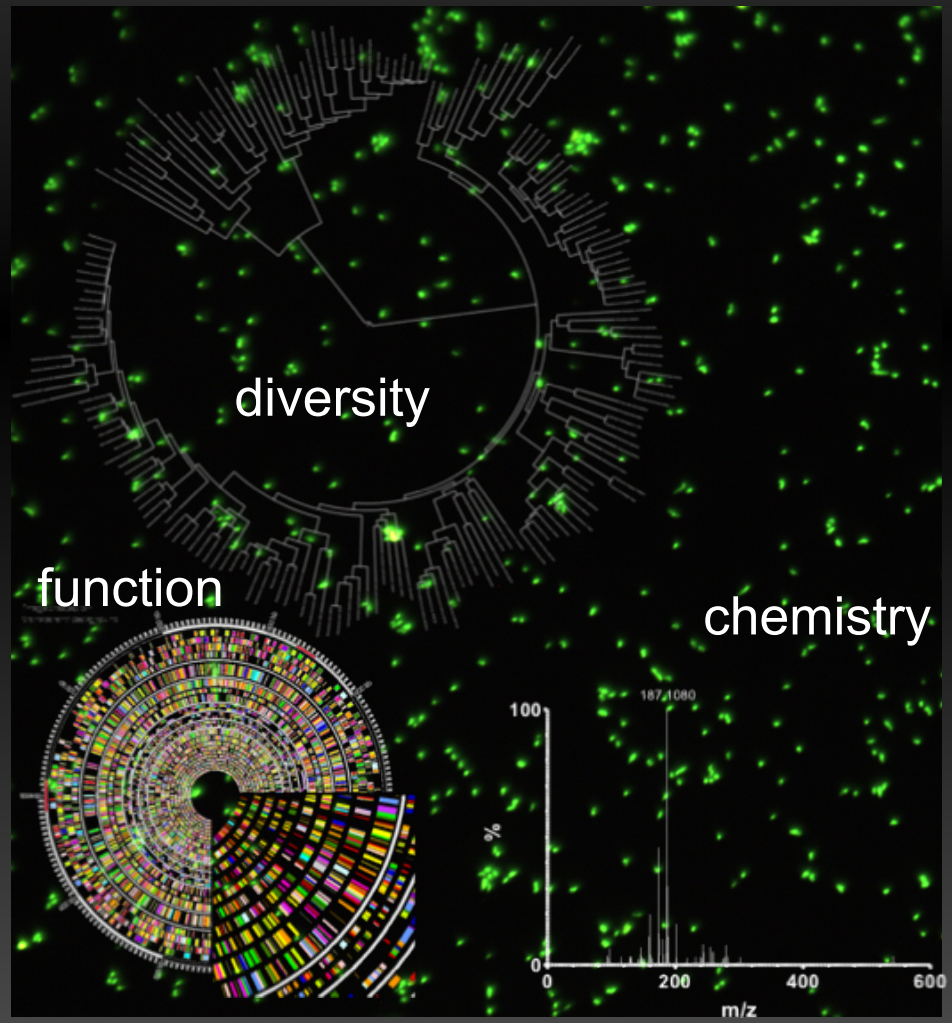

Nestor Arandia, U. Gijon, Spain


Jorge Ligeti, Las Positas College

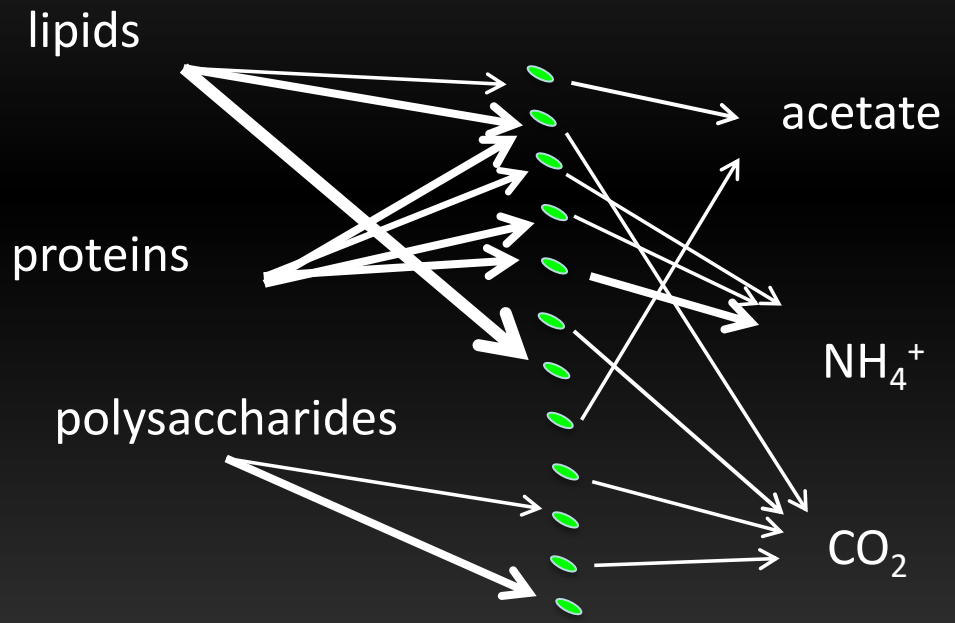
Student fellowships are available: https://lgsp.llnl.gov/ http://students.llnl.gov/

Research Goals: quantitative understanding of microbial control of biogeochemistry

Current capabilities

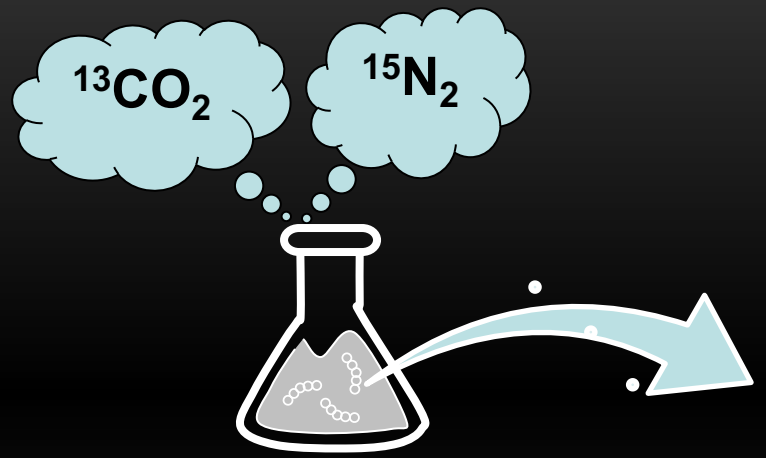


Ultimate goal

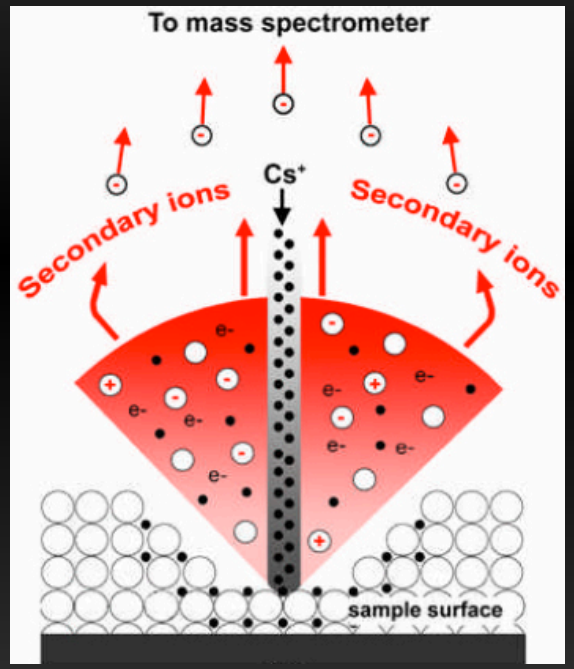


Organism-specific quantitative measures of biogeochemical activity

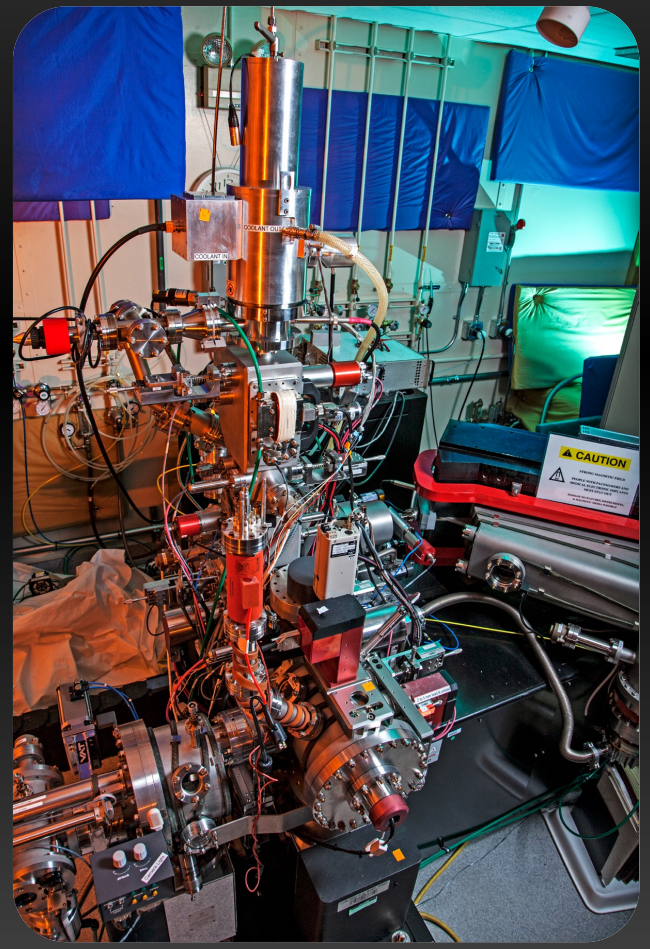
General Approach: Stable isotope additions to trace metabolism in complex communities



1. Incubate sample in stable isotope labeled substrates
2. Organisms that use the substrates get labeled
3. Harvest, remove excess substrate
4. Use NanoSIMS to quantify substrate use by microbes



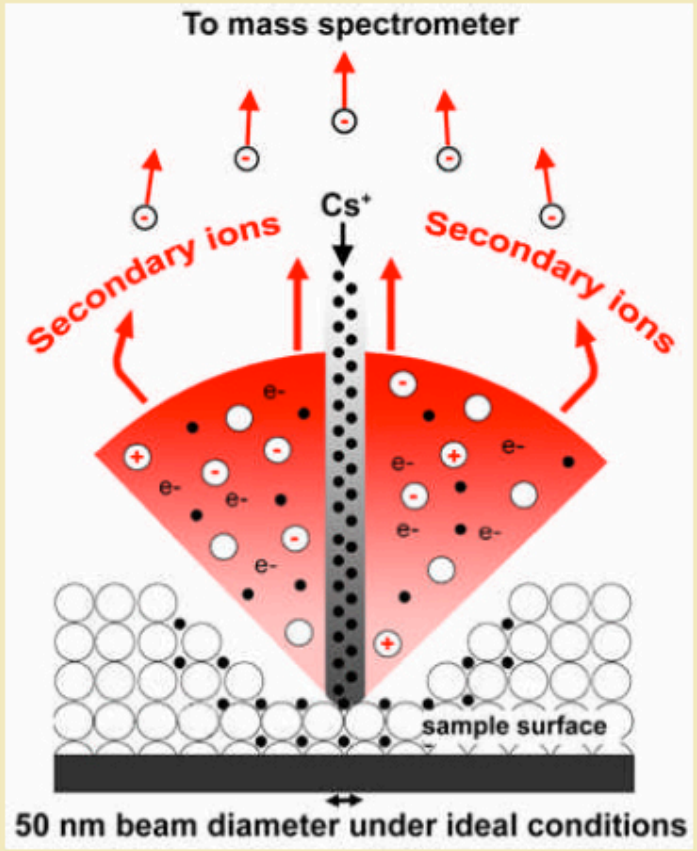
NanoSIMS analysis quantifies isotope incorporation

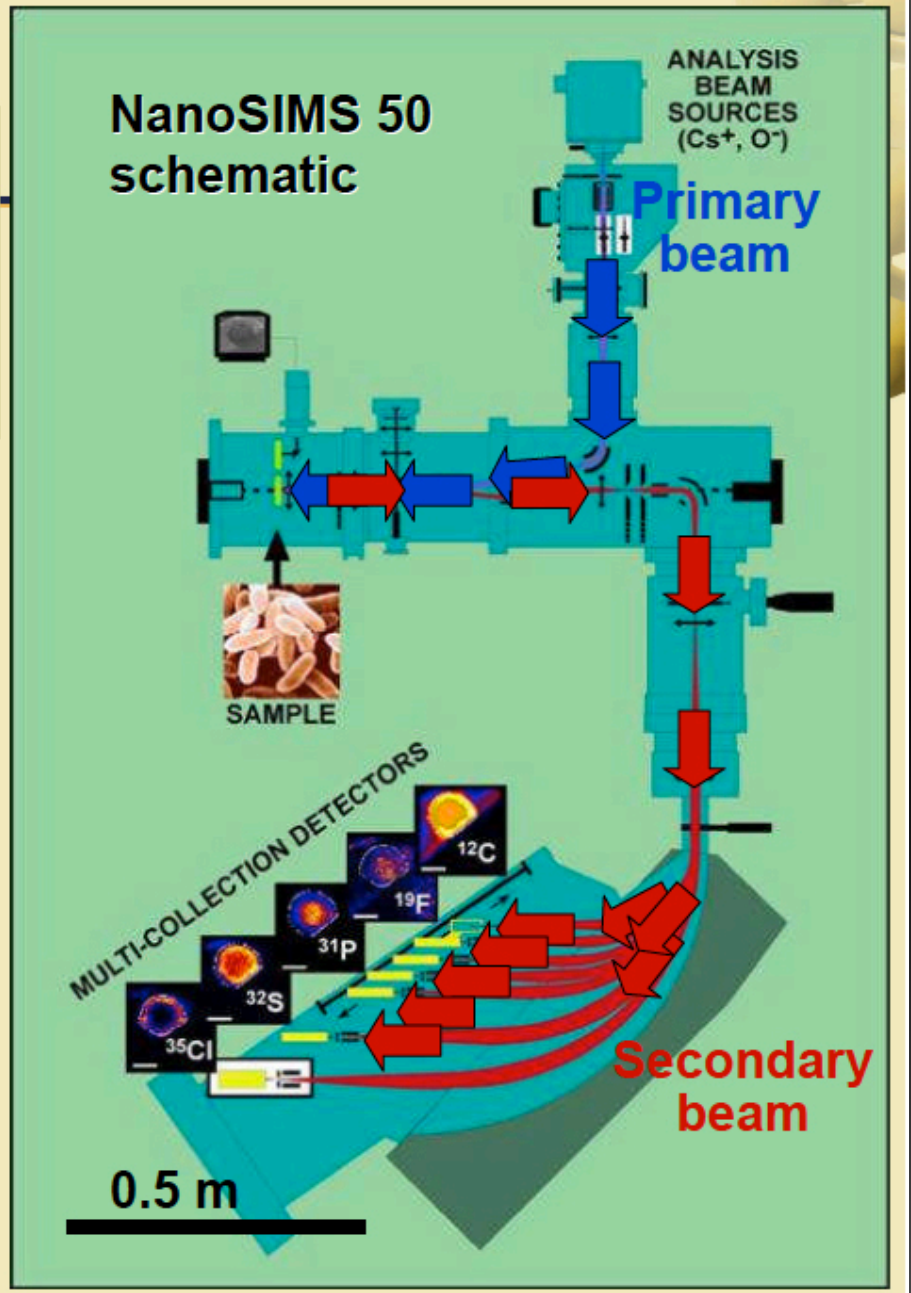


The LLNL NanoSIMS

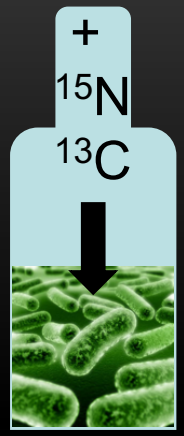
A surface sputtering technique

- Primary beam scans sample surface to produce secondary ions
- Secondary ions detected to produce quantitative digital images
- Simultaneous detection of 5 species
- High sensitivity: → 5% useful yield





Method #1: Chip-SIP (Stable Isotope Probing of Microarrays)

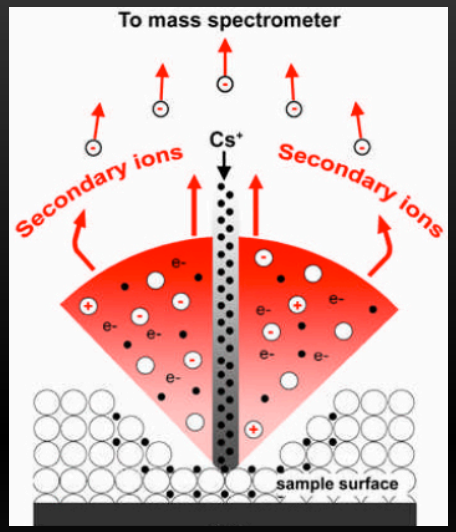


Incubation over time

Isotopically enriched

RNA

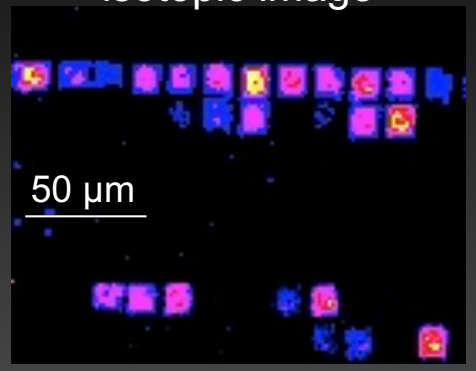
RNA binds to complementary DNA on microarray according to sequence

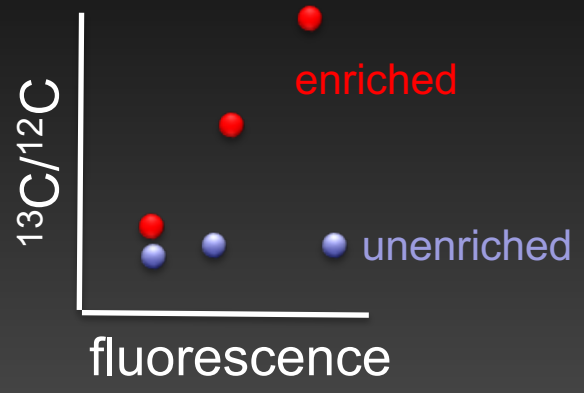


Detection of isotope signal by NanoSIMS

Fluorescence signal (how much RNA?)

NanoSIMS $^{13}C/^{12}C$ isotopic image

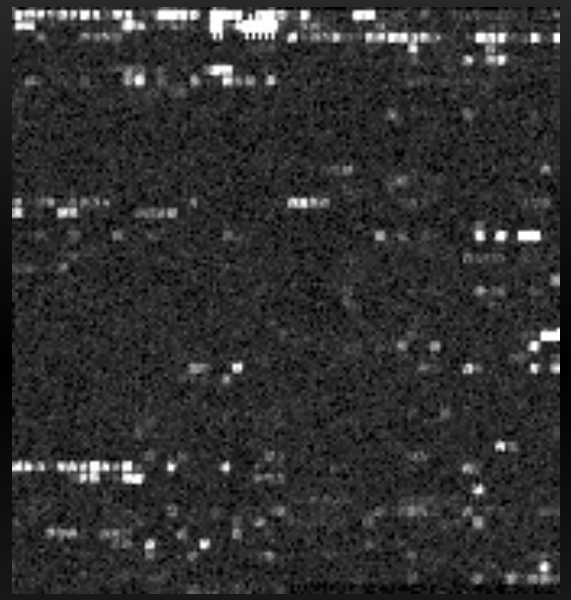




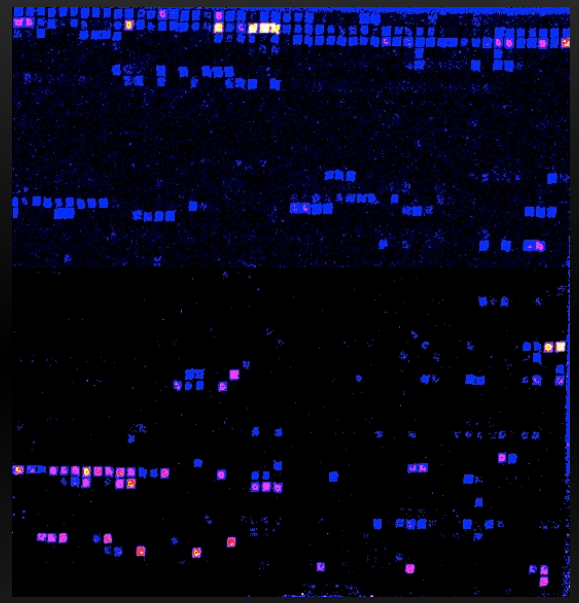
Mayali et al. 2012 ISME J

We can quantify the relative isotope incorporation by multiple organisms living together

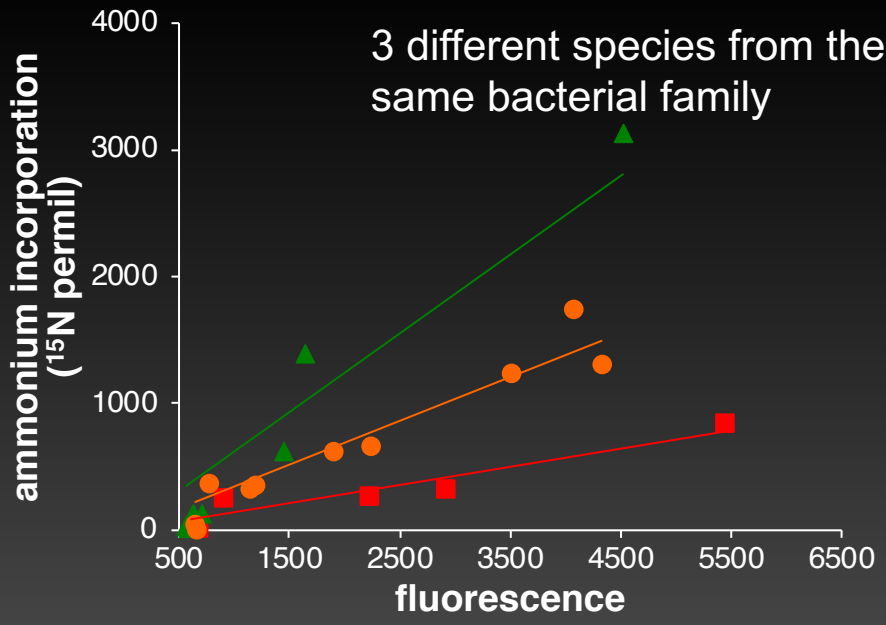
SF Bay water + $^{15}NH_4^+$



Fluorescence (how much RNA is hybridized)



$^{15}N/^{14}N$ (how much isotope enrichment)



Mayali et al. 2012 ISME J

Quantifying the relative isotope incorporation of multiple substrates incubated side by side

^{13}C fatty acids

^{13}C nucleic acids

^{13}C amino acids

Colors =
Oceanospirillales
Other Gammaproteobacteria
Rhodobacteriacea
Other Alphaproteobacteria
Bacteroidetes

Bacillus
Sphingo #2
Enterobacter
Alpha/OM38 #2
Alpha/OM38 #1
Flavo #3
Alteromonas #4
Alpha/OM38 #3
Flavo #2
Alteromonas #3
Roseo RCA
Oleiphilaceae #
Colwellia
Oleiphilaceae #
MarGrpA #2
Oleiphilaceae #
Oleispira #1
nucleic acids
generalists
amino acids
Marinobacter
Gamma/unknown
Vibrio #1
Vibrio #2
Alteromonas #2
Alteromonas #1
Cyano
Actinobacteria
Acidobacteria
Chloroplast
Deferribact
Sphingo #1
Flavo #6
Rhodospirillacea
Azospirillum
Pelagibacter
Roseo #3
Flavo #4
Flavo #1
Flavo #5
Roseo #4
Roseo #5
Oleiphilaceae #2
Oleiphilaceae #5
MarGrpA #1
Roseo #2
Gamma/OM60
Roseo #1
Alcanivorax #1
Alpha/OM38 #4
Oleispira #2
Alcanivorax #2
Oleispira #3
Oleiphilaceae #3
fatty acids
Alpha/NAC1-6 #1

52 positives /107 targets

Mayali et al. 2012 ISME J

Quantifying the relative incorporation of 14 substrates?

^{13}C glucose

^{13}C maltose

^{13}C starch

^{13}C lactose

^{13}C lipids

^{13}C fatty acids

^{13}C nucleic acids

$^{13}CO_3^-$ +light

^{13}C cellulose

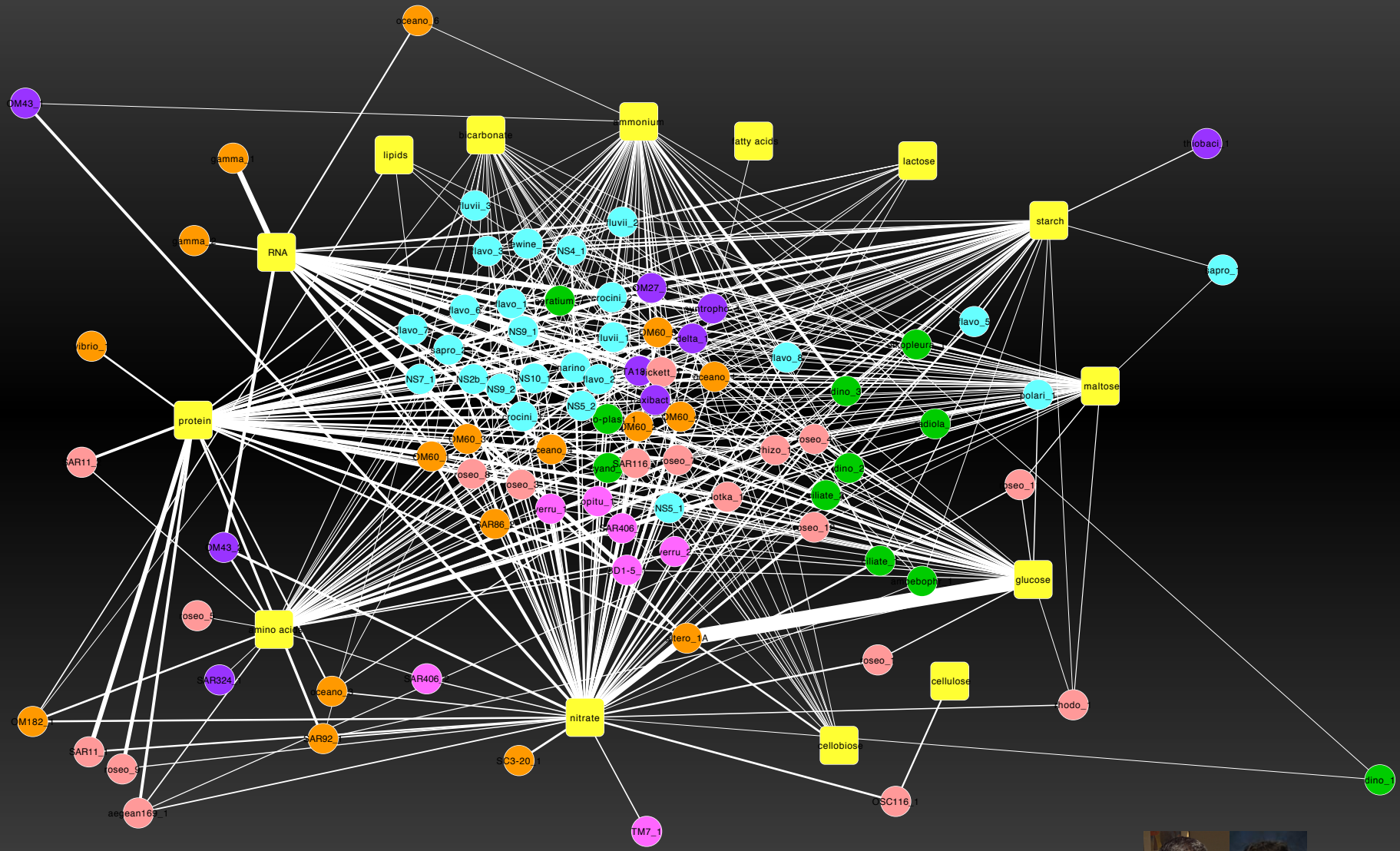
^{13}C cellobiose

^{13}C protein

^{13}C amino acids

$^{15}NO_3^-$

$^{15}NH_4^+$



Colors =

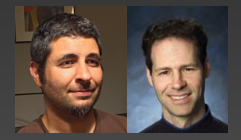
Protists/cyanobacteria

Gammaproteobacteria

Alphaproteobacteria

Bacteroidetes

Other



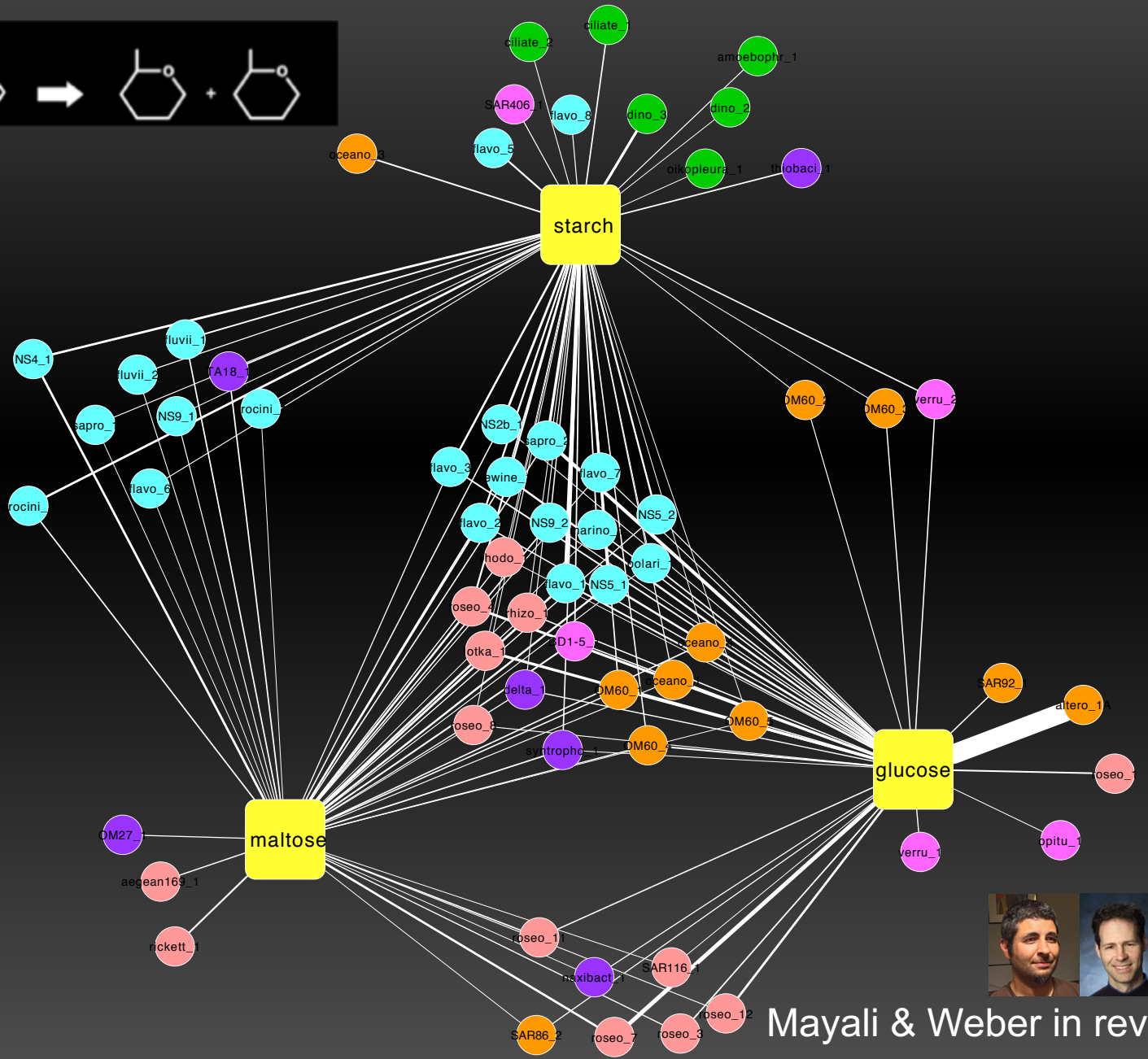
Mayali & Weber in review

We can zoom in on a specific process (e.g. starch degradation)

^{13}C starch

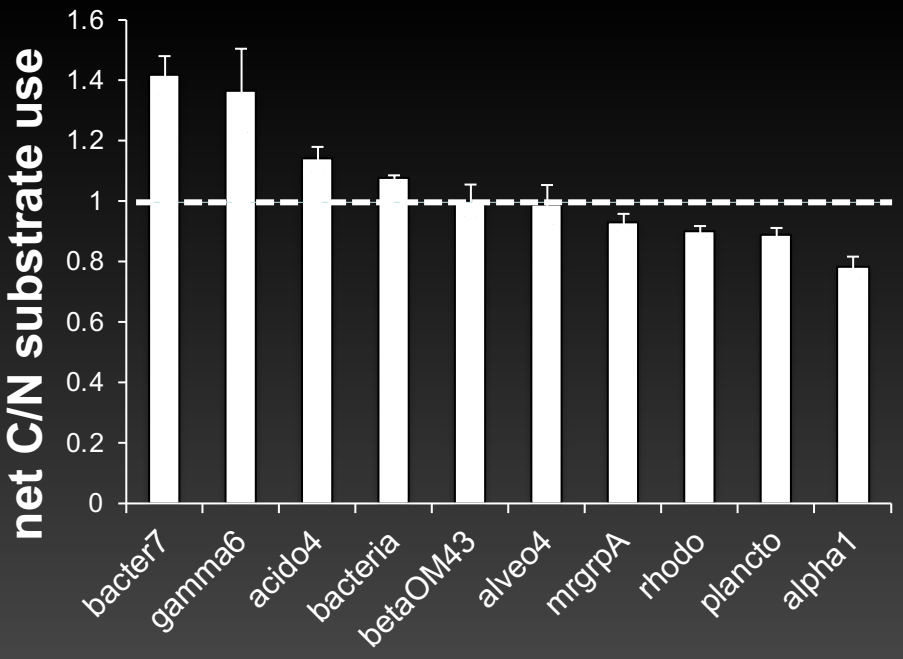
^{13}C maltose

^{13}C glucose

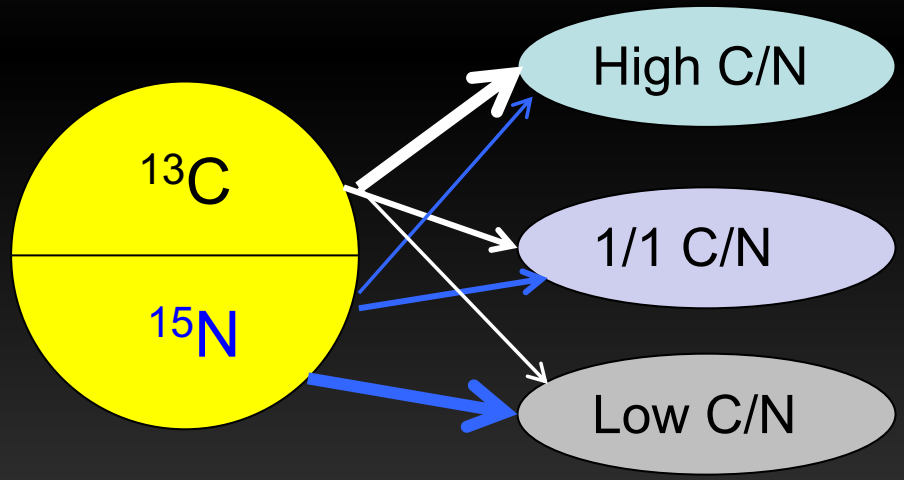


Mayali & Weber in review

Simultaneous ^{15}N and ^{13}C analysis to quantify C/N resource use

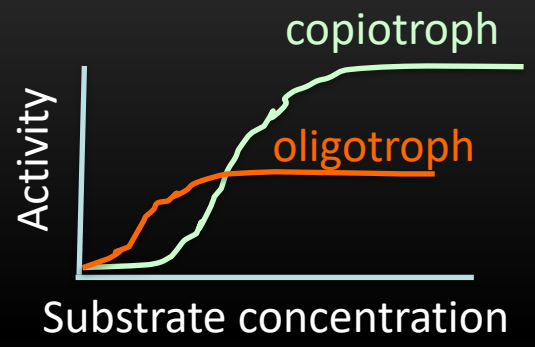


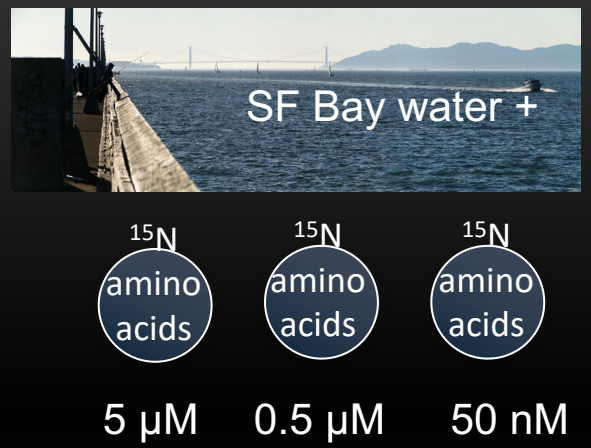
1 to 1 line

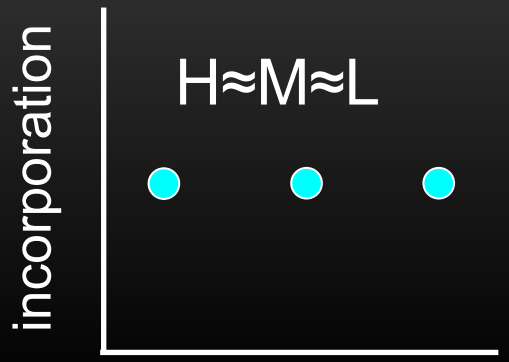


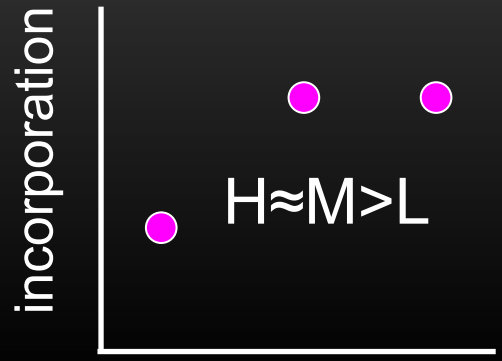
Mayali et al. 2013 FEMS Microb Ecol

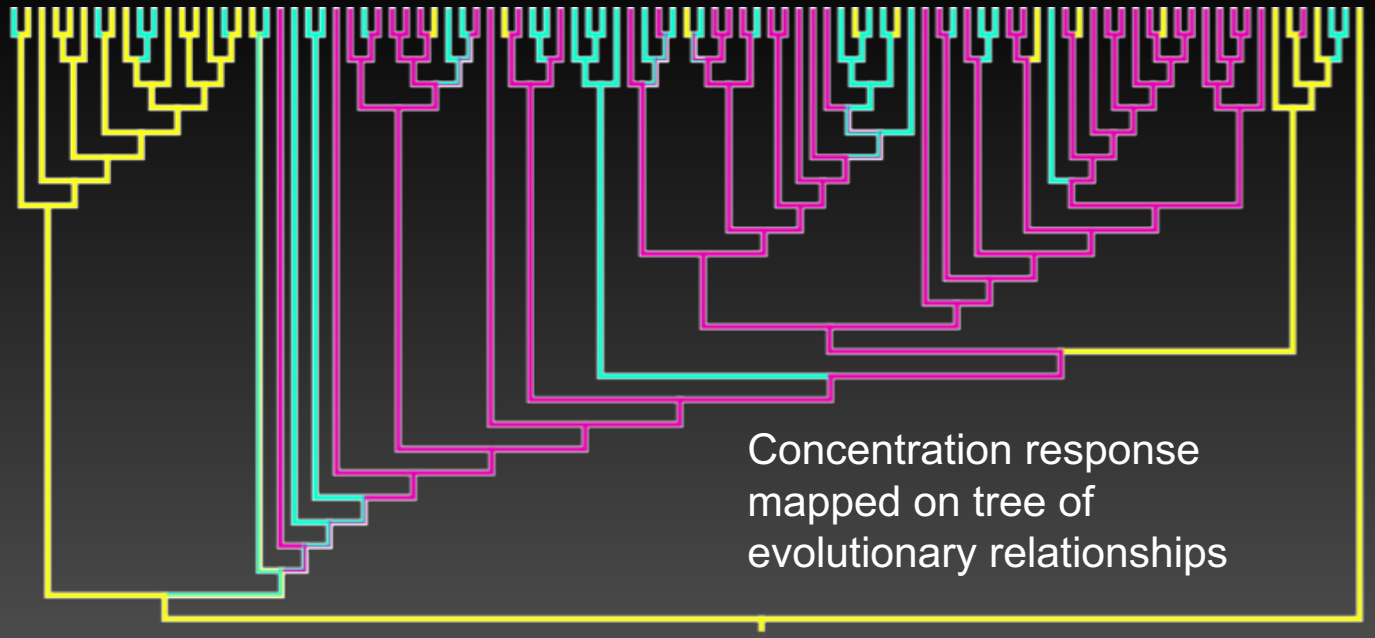
Different microbial groups more active at different substrate concentrations







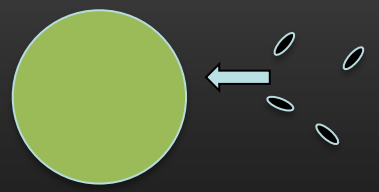


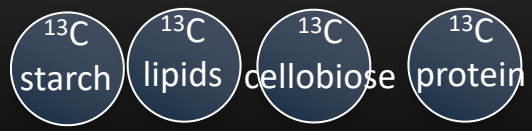


Mayali et al. 2014 PLoS ONE

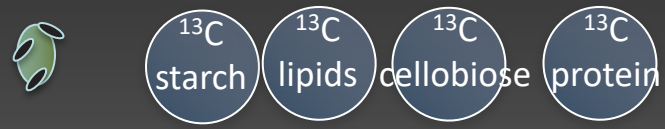
Microbial activity changes over time

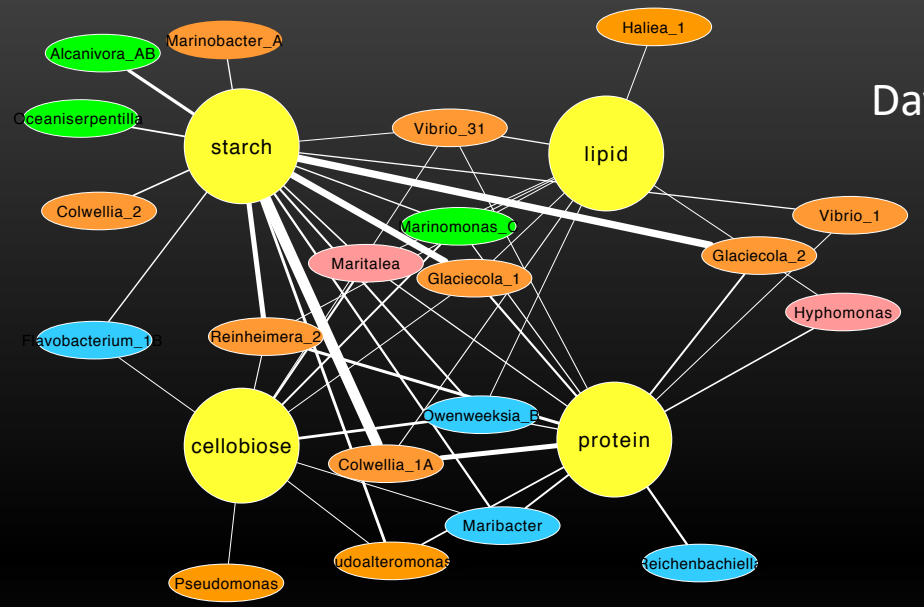
uncolonized diatom detritus

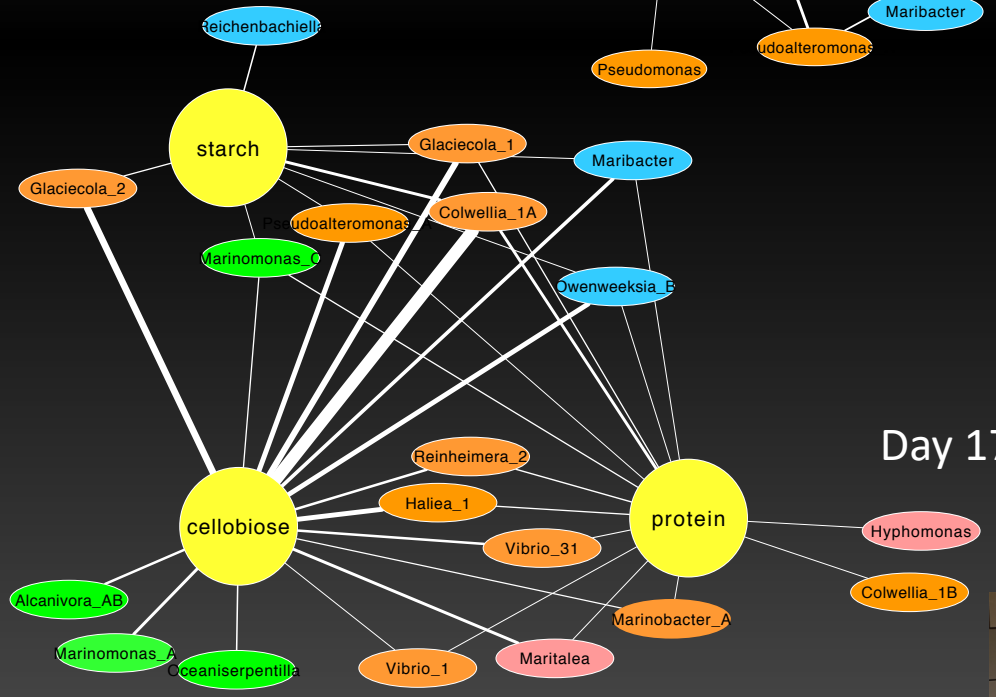


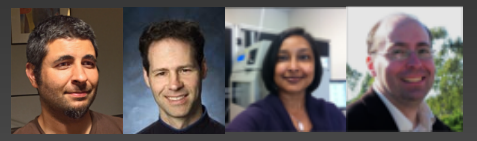


Pacific coast (1 micron filtered), incubated for ~2 weeks with diatom particles in the laboratory



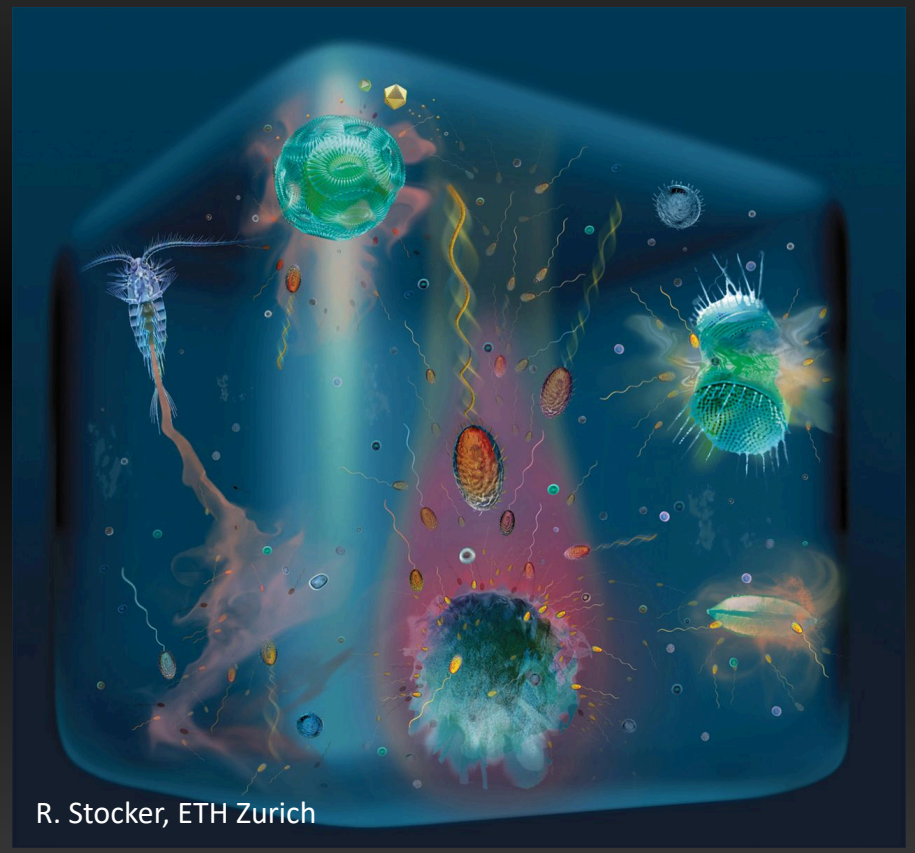


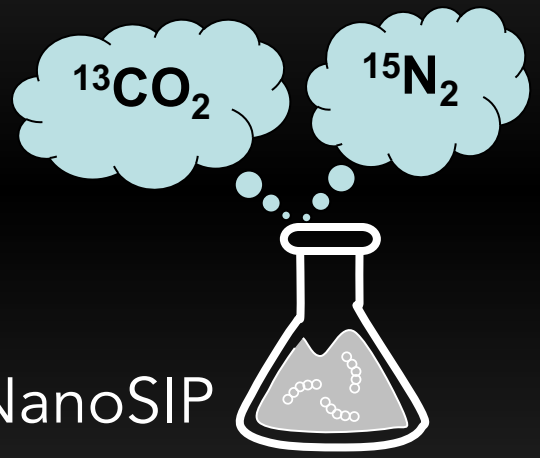




Mayali et al. 2016 Environ Microbiol

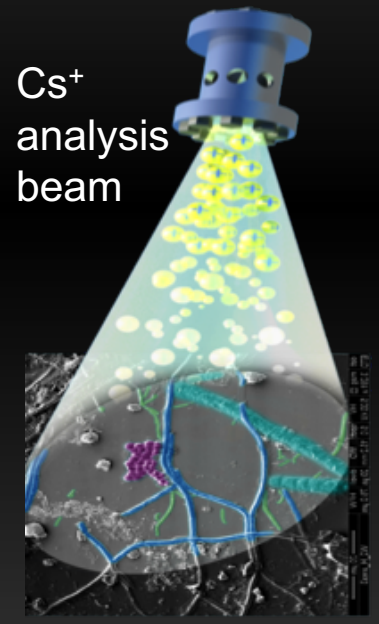
Method 2: NanoSIP (analysis of whole cells)





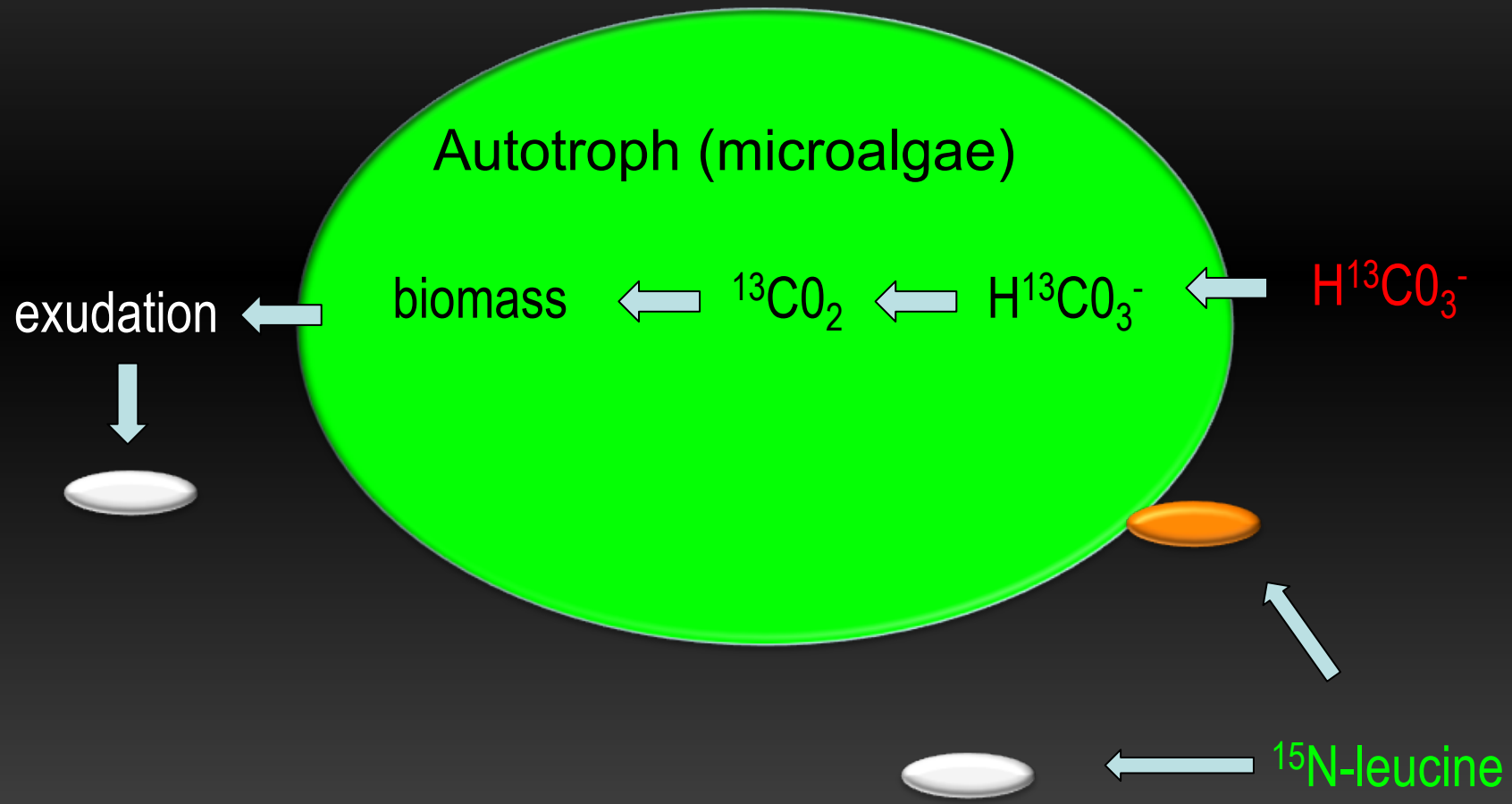
NanoSIP

1. Incubate sample in stable isotope labeled substrates
2. Harvest, remove excess substrate and disperse
3. Use NanoSIMS to quantify substrate use by single cells



NanoSIP analysis locates cells that use the substrate

Using NanoSIP to study interactions between heterotrophic bacteria and autotrophic algae (and their organic matter)



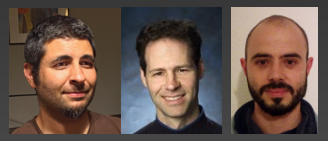
Impact of temperature on marine microbial activity

Hypothesis: increased temperature influences the coupling between autotrophy and heterotrophy

control

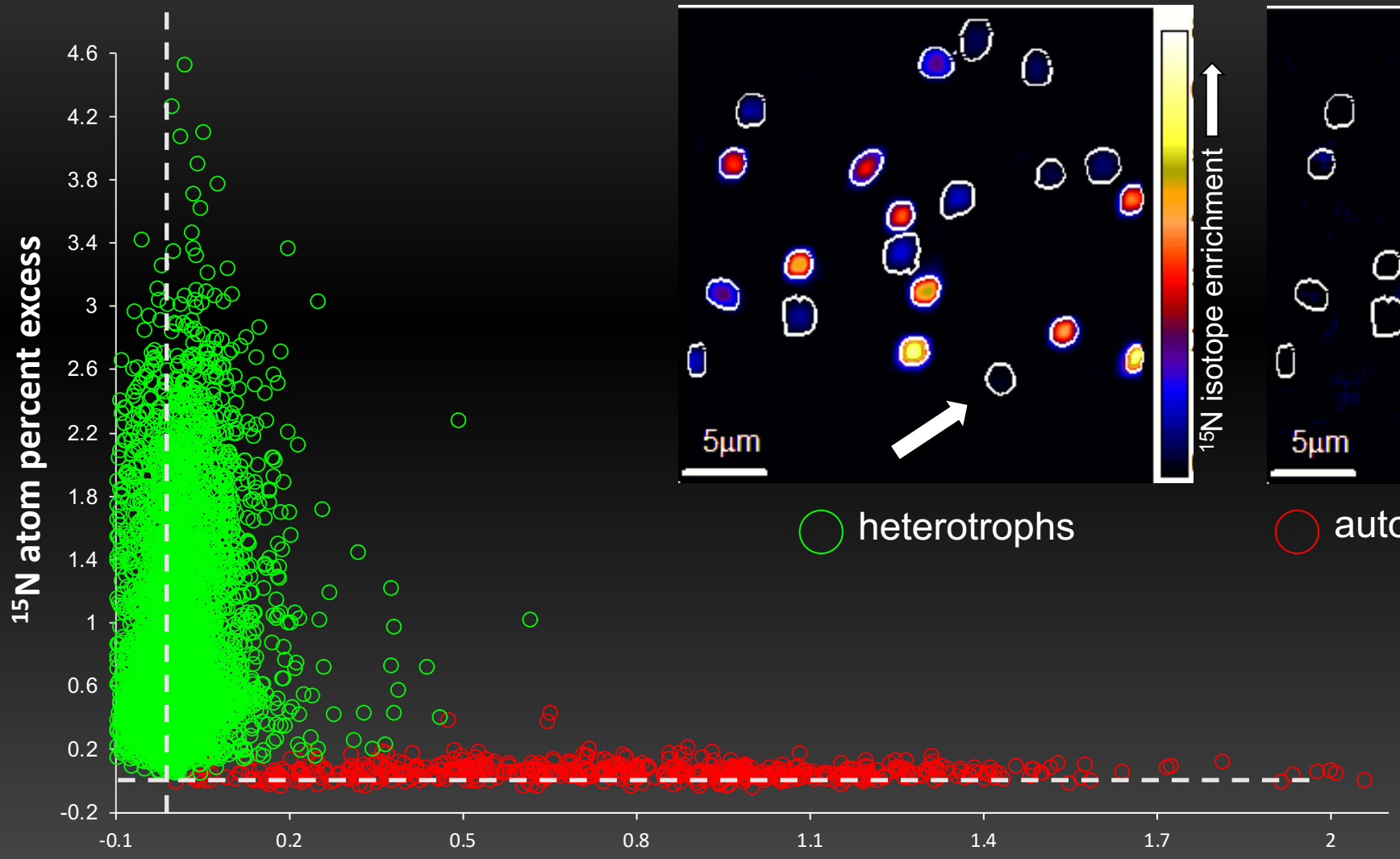
+ 4°C

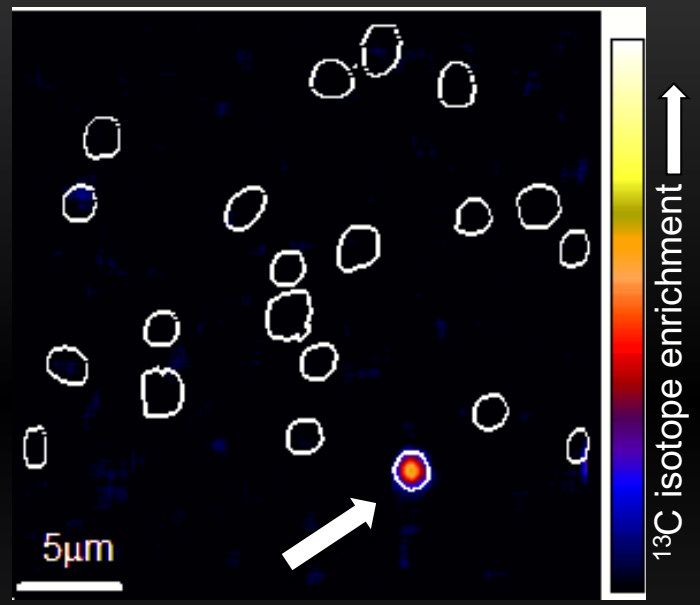
+ ^{15}N-leucine (50 nM), + $H^{13}CO_3^-$ (100 µM), 12 hour incubations



Arandia et al. 2017 ISME J

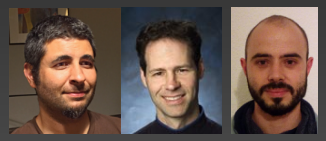
NanoSIMS quantifies cell-specific C fixation and bacterial production





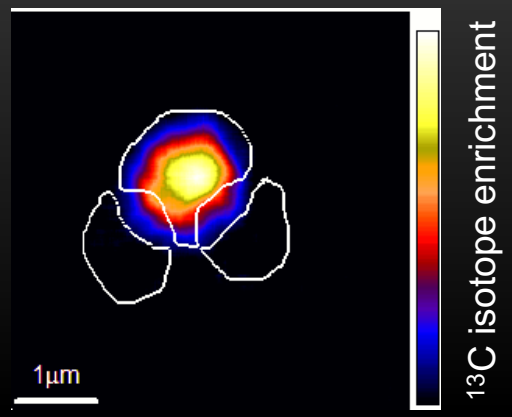
heterotrophs

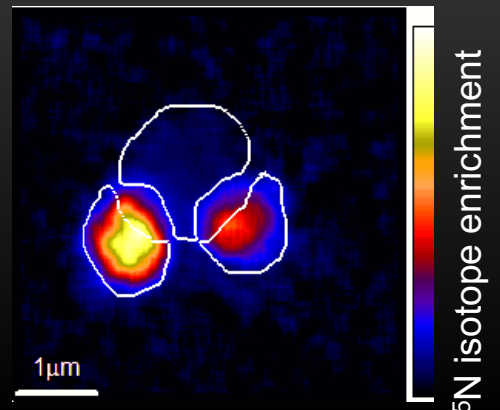
autotrophs

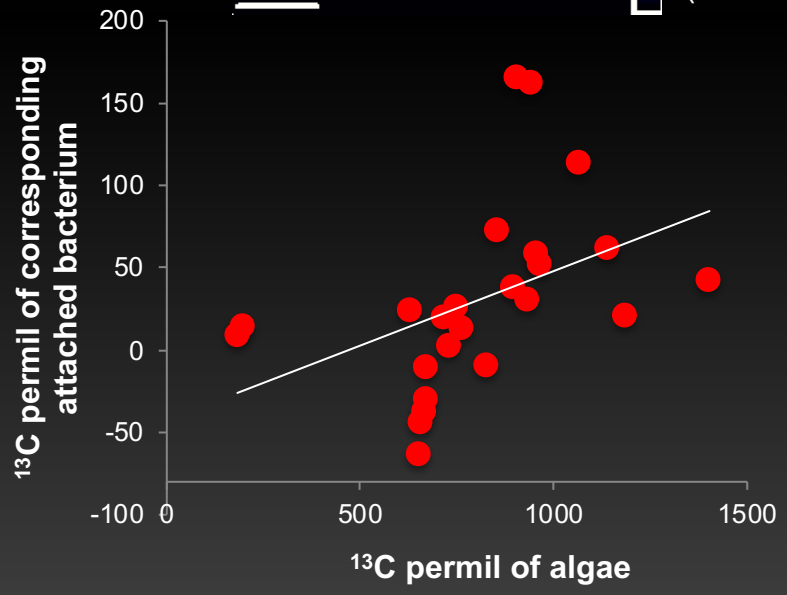


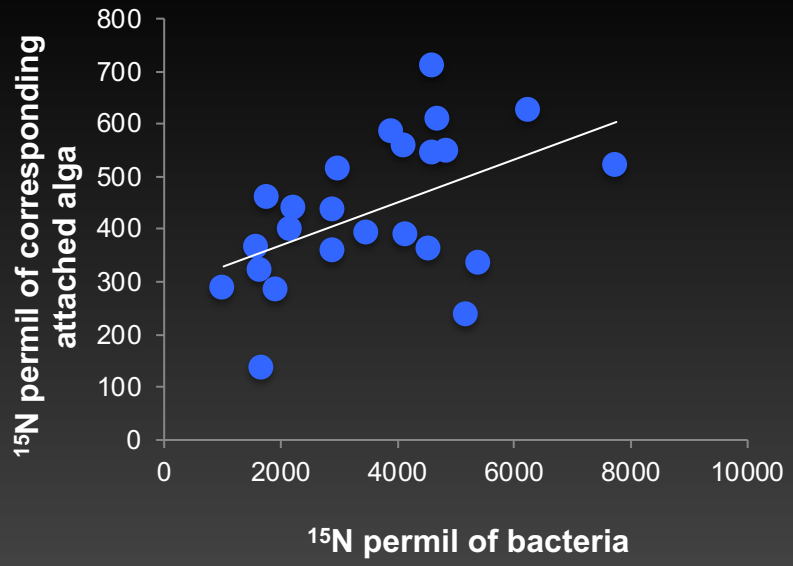
Arandia et al. 2017 ISME J

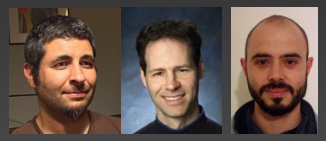
Isotope imaging identifies heterotrophs attached to autotrophs and quantifies their C/N exchange





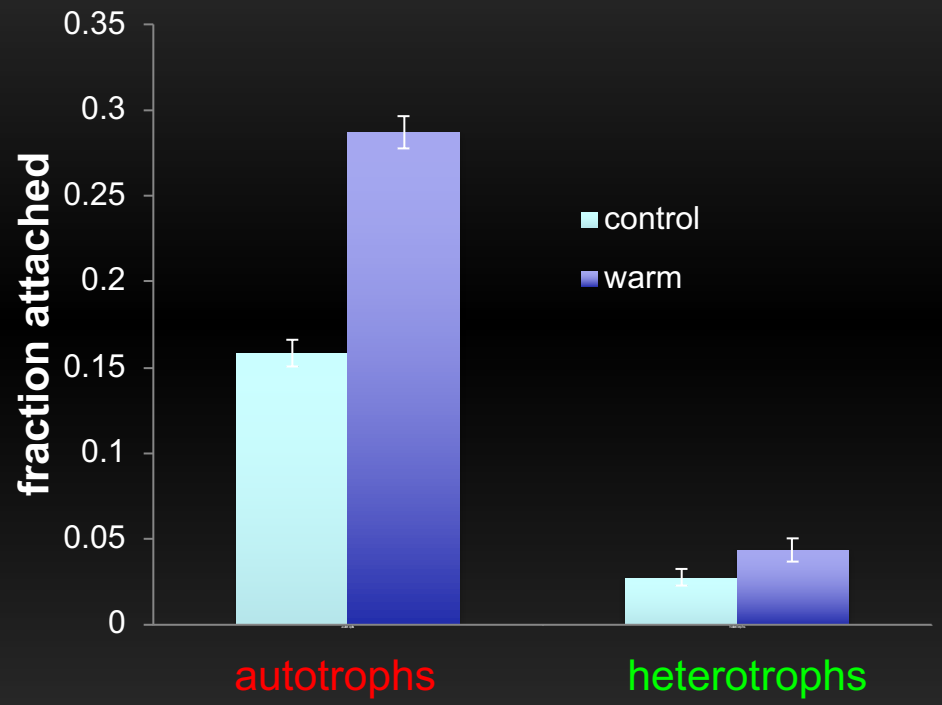




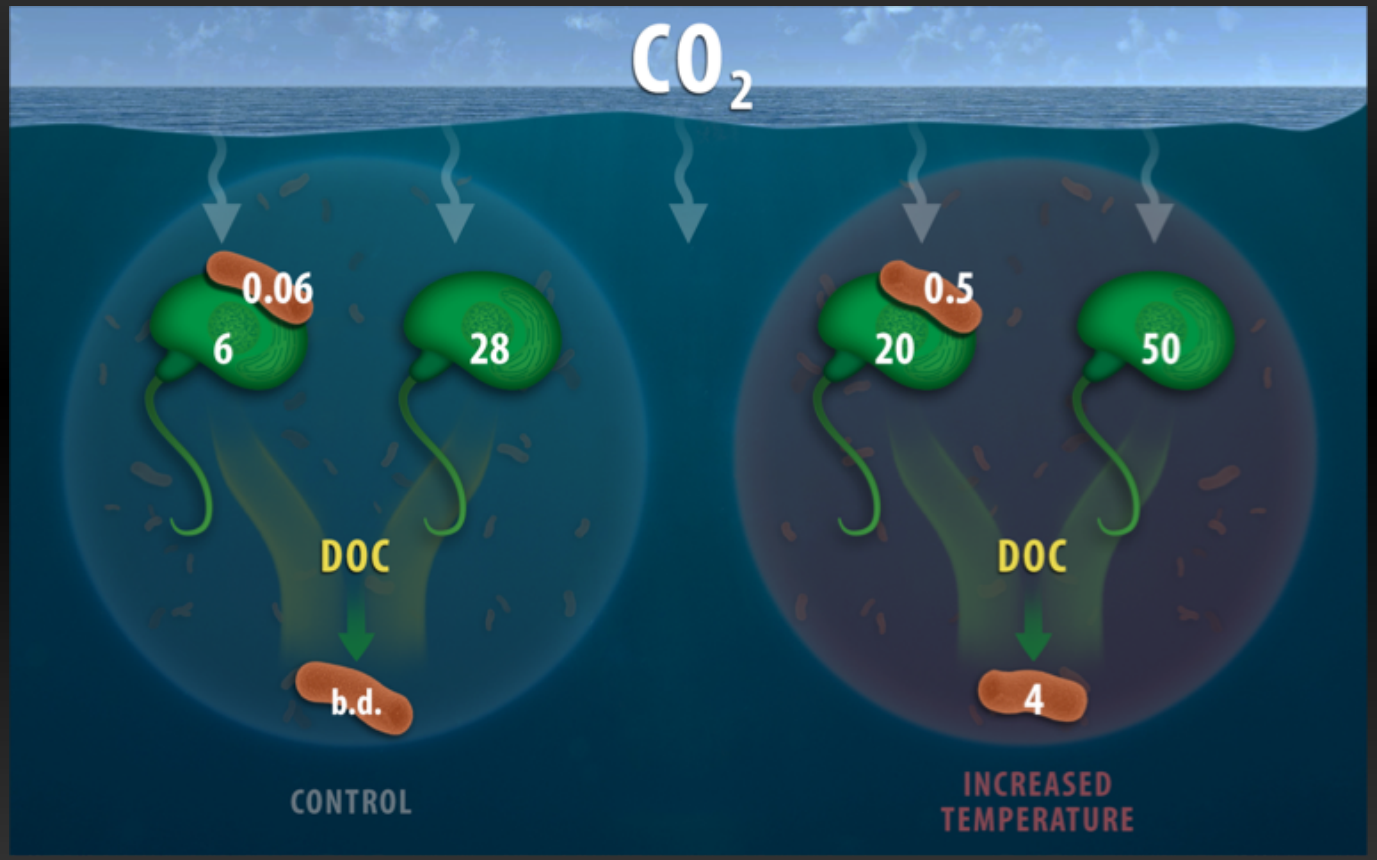


Arandia et al. 2017 ISME J

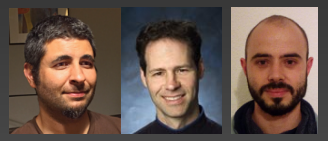
Temperature affects autotroph-heterotroph attachment and cell-specific activities



+ 4°C = 2X attachment



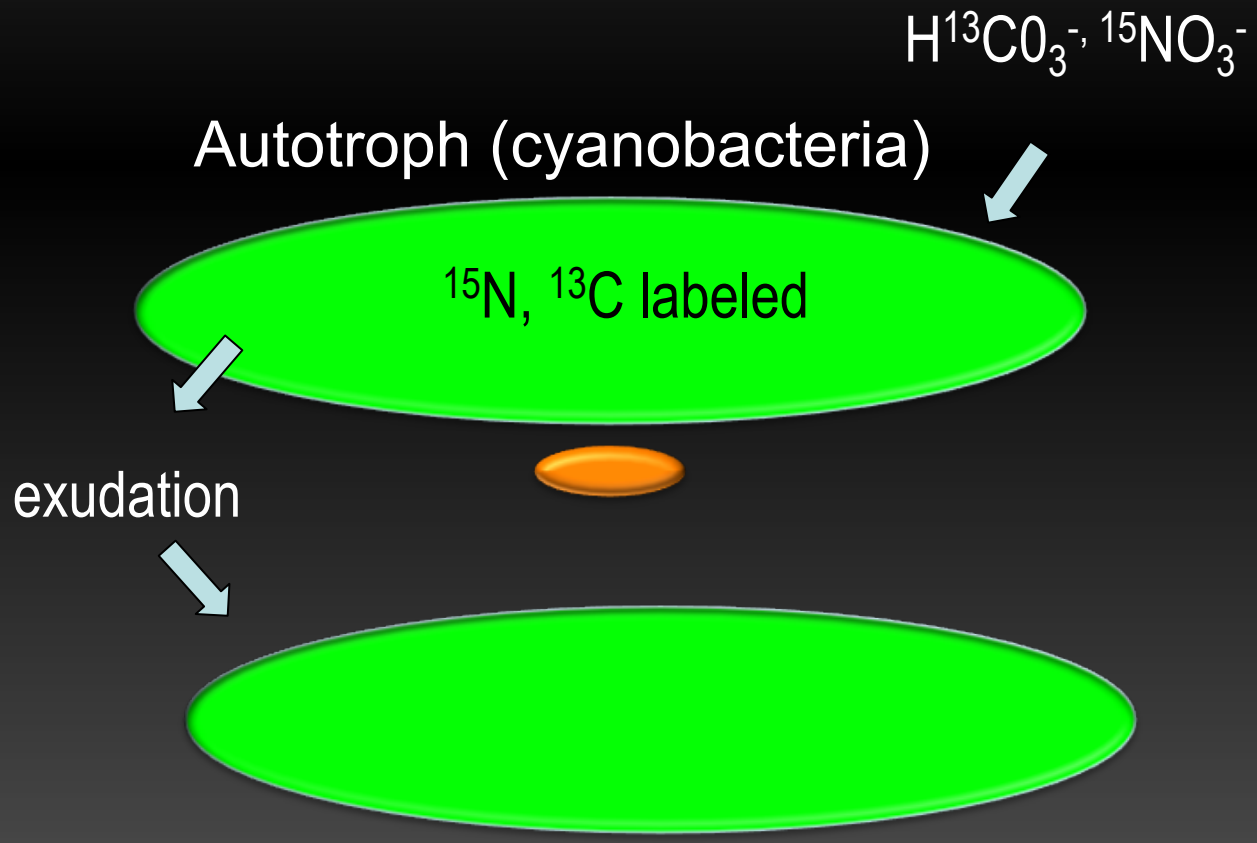
+ 4°C = increased bacterial C and N incorporation
Attachment increased C incorporation

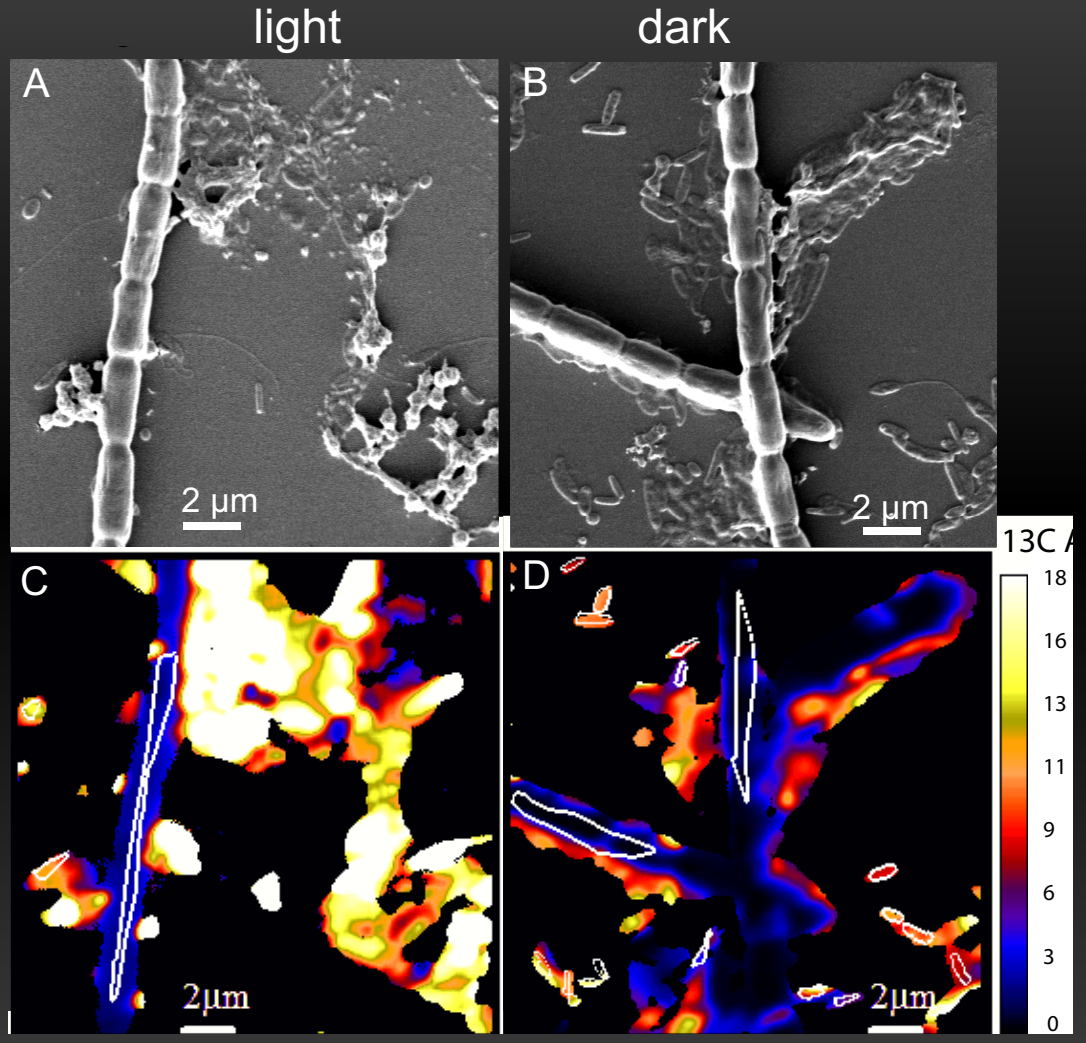


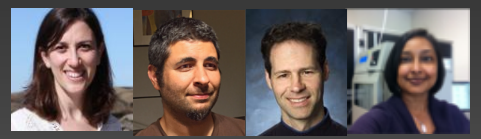
Arandia et al. 2017 ISME J

Cyanobacteria incorporate extracellular C and N

Hypothesis: EPS biofilm matrix is used as a C and N storage mechanism for cyanobacteria







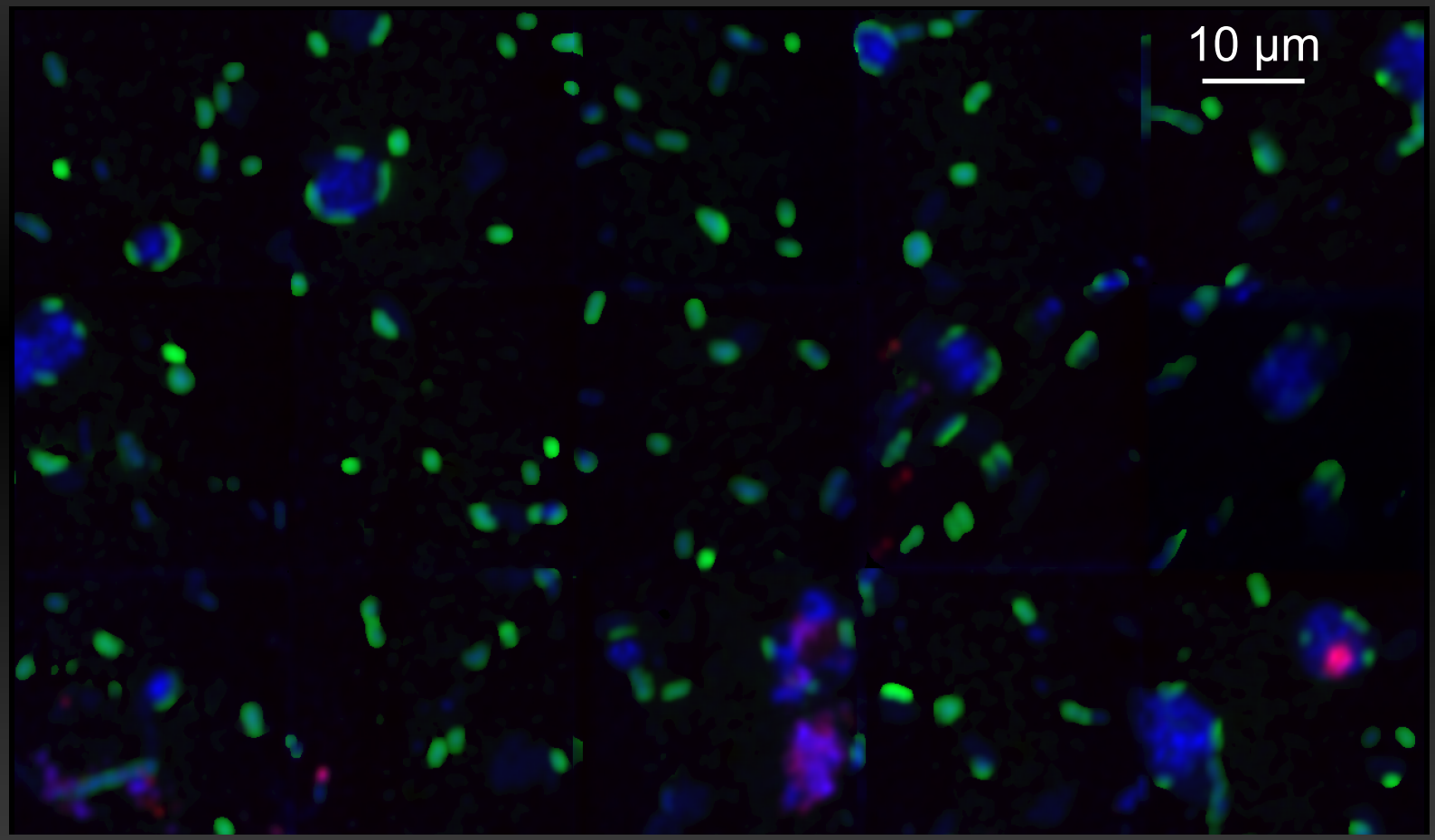
Stuart et al. 2015 ISME J, Stuart et al. 2016 mBio

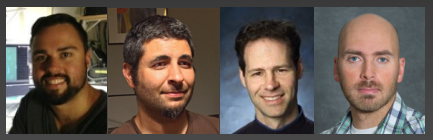
Bacterial-algal interactions in biofuel producing algal ponds

collect bacterial size fraction
from algal ponds

+^{15}N-leucine (50 nM)
+ $H^{13}CO_3^-$ (1 mM)
18 hour incubations

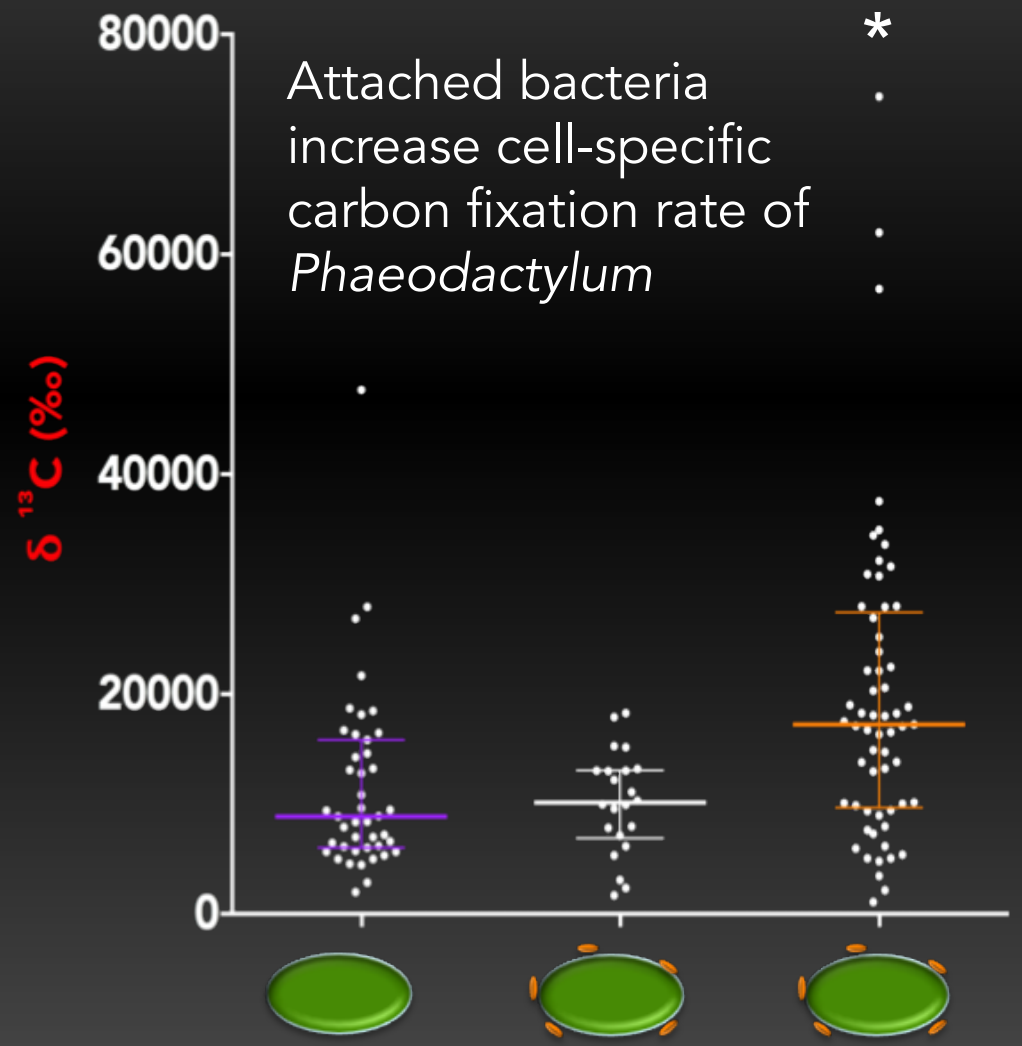
Add to bacteria-free
algal cultures



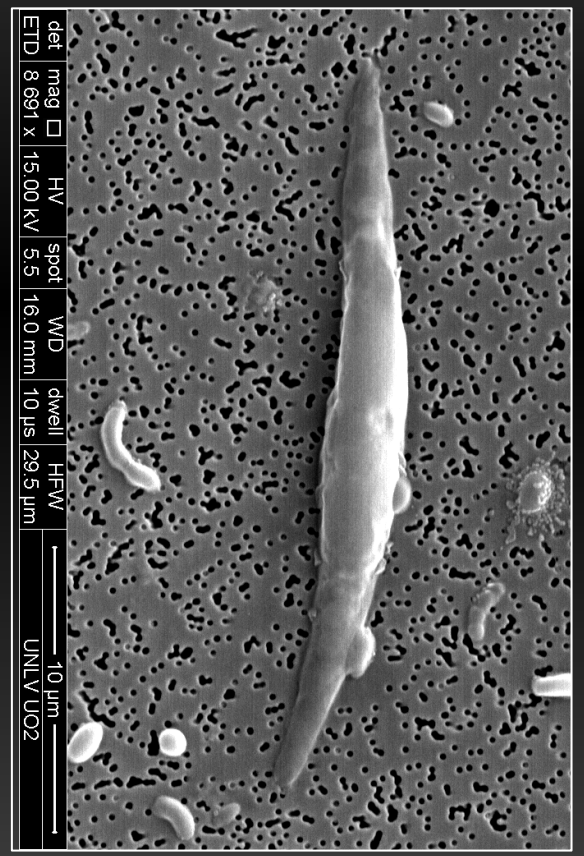


Samo et al. in revision

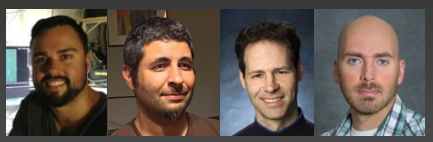
Bacterial attachment has a variable effect on algal primary productivity



P. tricornutum



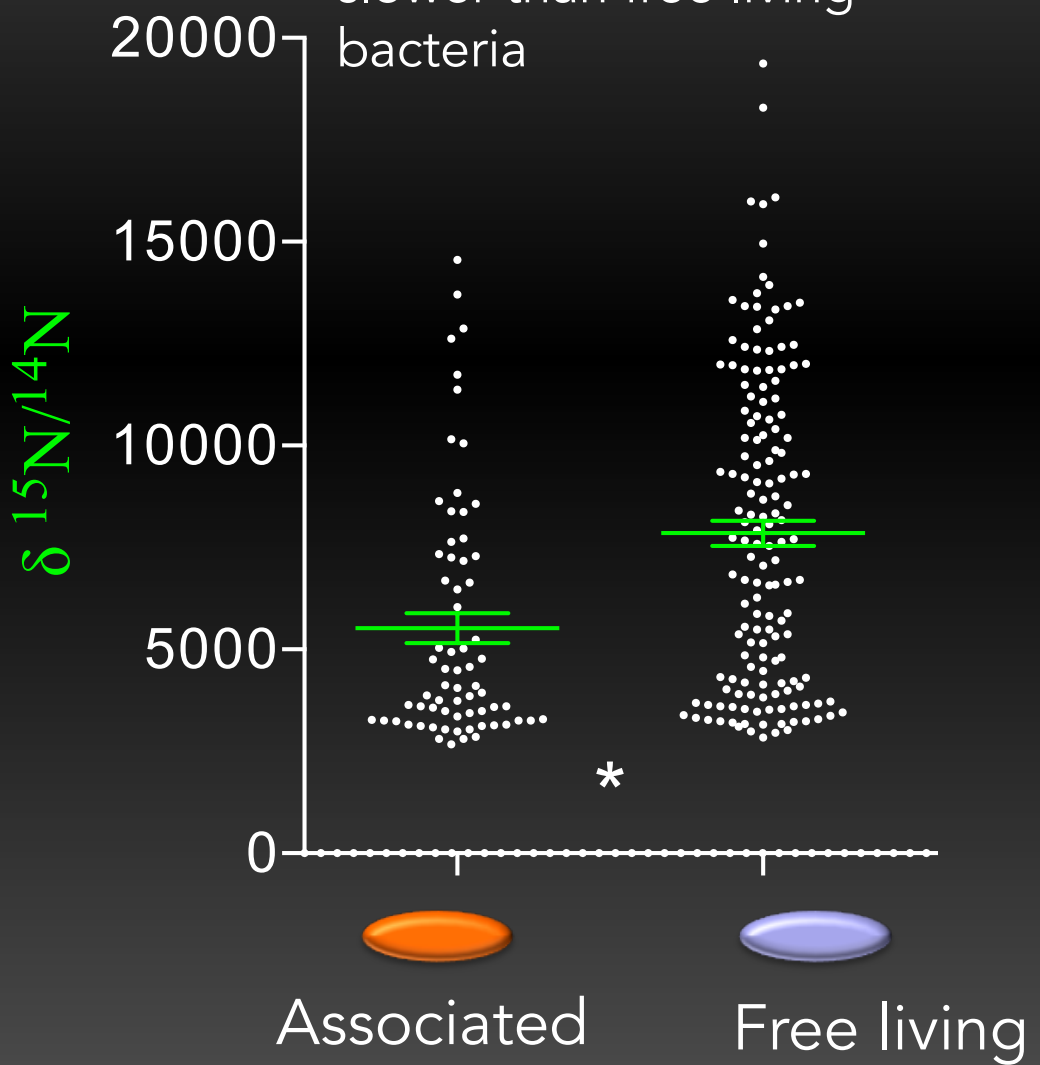
* = statistical significance (non-parametric Mann-Whitney U test)



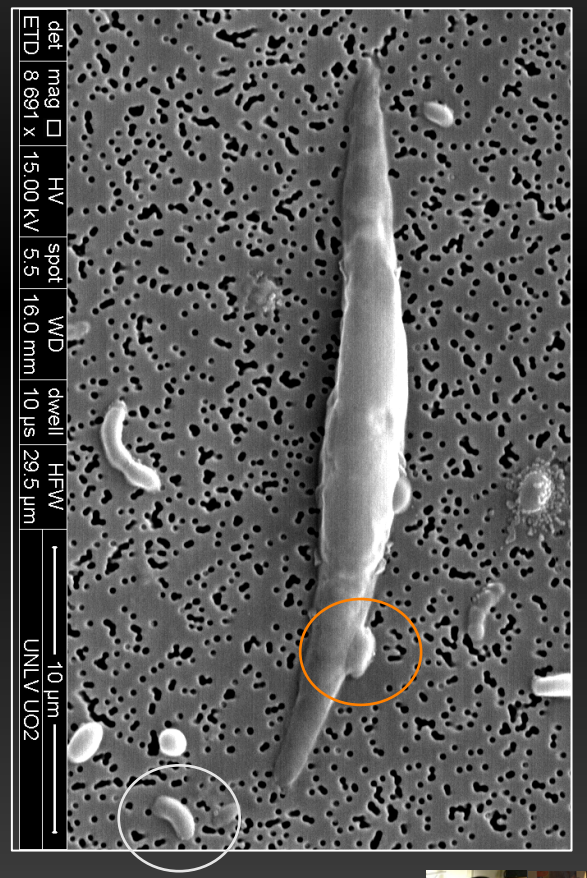
Samo et al. in revision

Effect of attachment on bacterial growth

Algal attached bacteria grow slower than free living bacteria



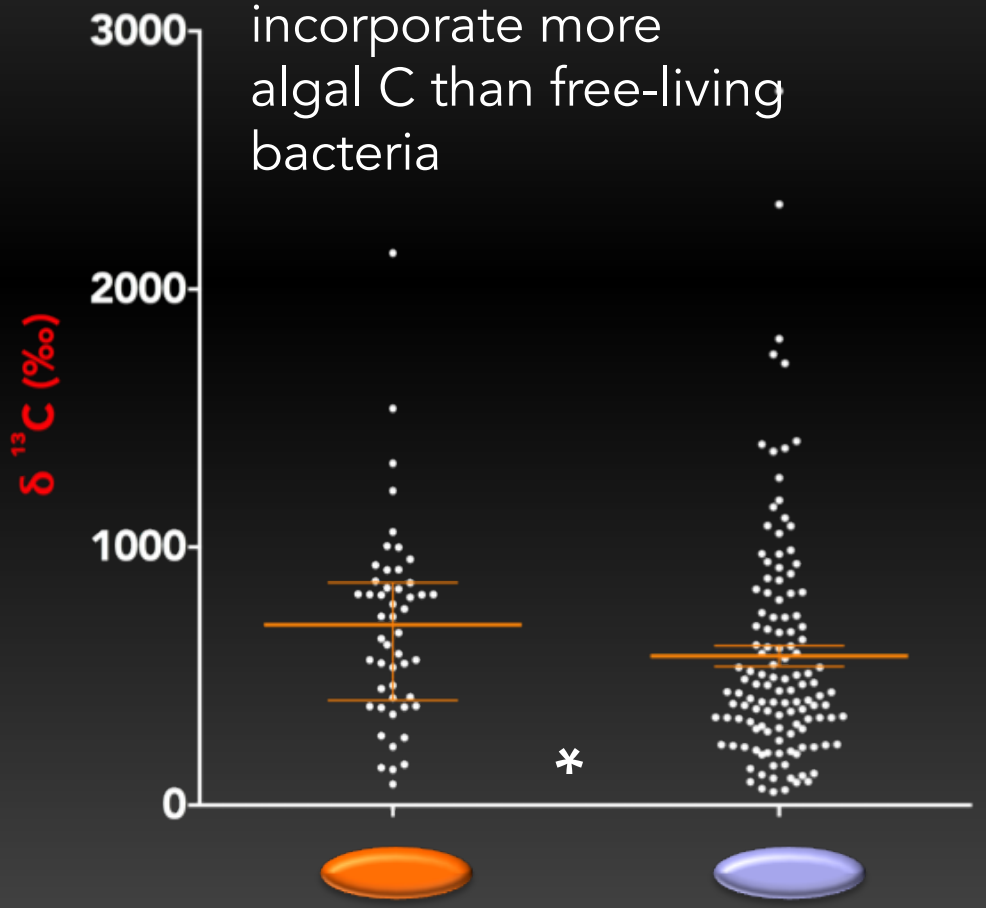
P. tricornutum



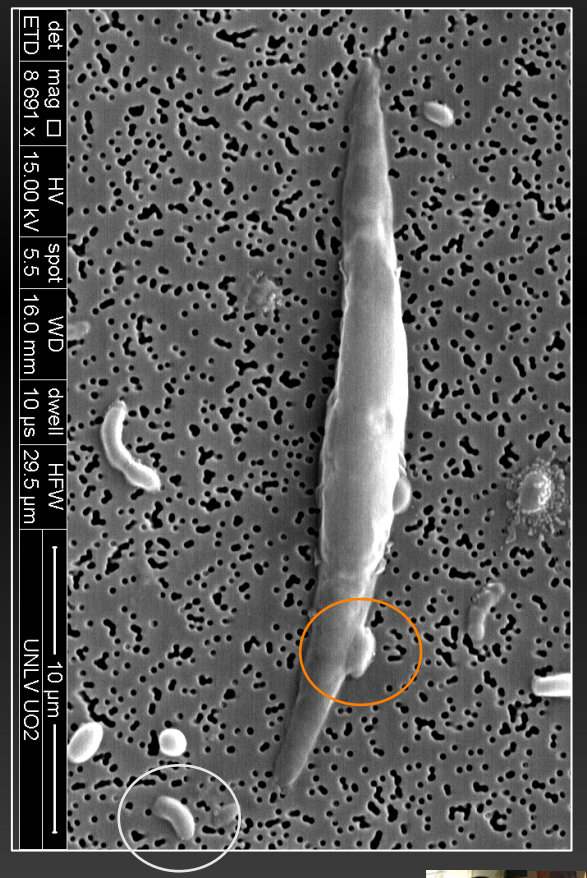
Samo et al. in revision

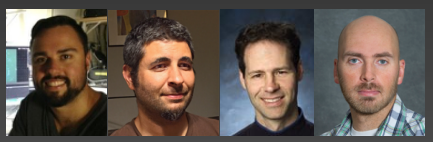
Effect of attachment on transfer from autotrophs to heterotrophs

Attached bacteria incorporate more algal C than free-living bacteria



P. tricornutum





Samo et al. in revision

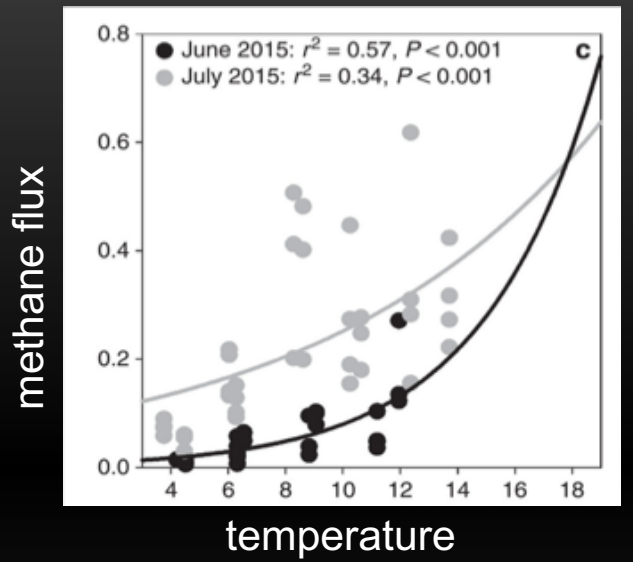
What does NanoSIMS quantitative imaging add to the environmental microbiologist's toolbox?

- Enables species-specific (or cell specific) quantitative analysis of isotopic incorporation after incubation with labeled substrates

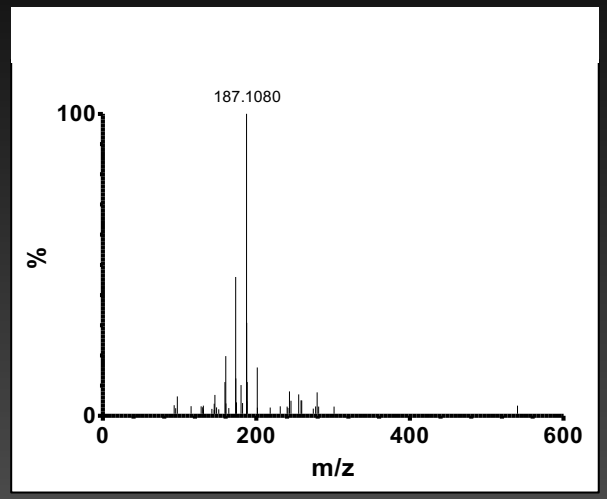
- Comparison of relative incorporation of different substrates by the same community incubated side-by-side
- Identify generalists vs. specialists
- Species-specific differences in C:N substrate use efficiency
- Response to increasing substrate concentrations
- Examine how incorporation varies over time or in response to perturbations
- Quantify exchange between heterotrophs and autotrophs

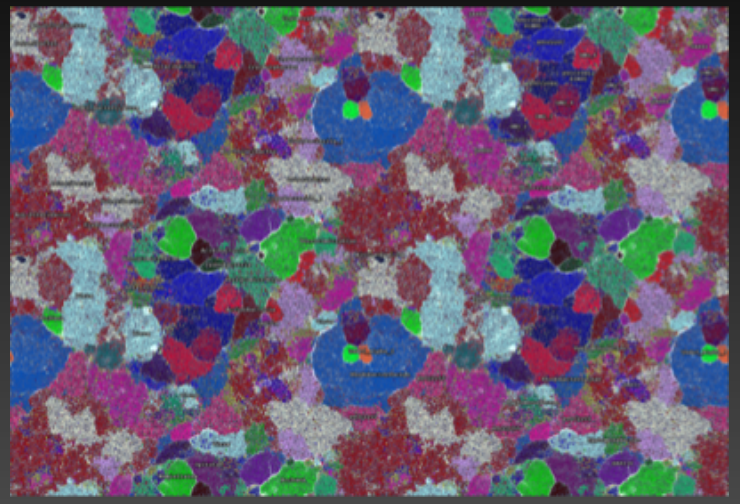
Complementary tools are needed for context or for hypothesis generation

Physiological or biogeochemical measurements



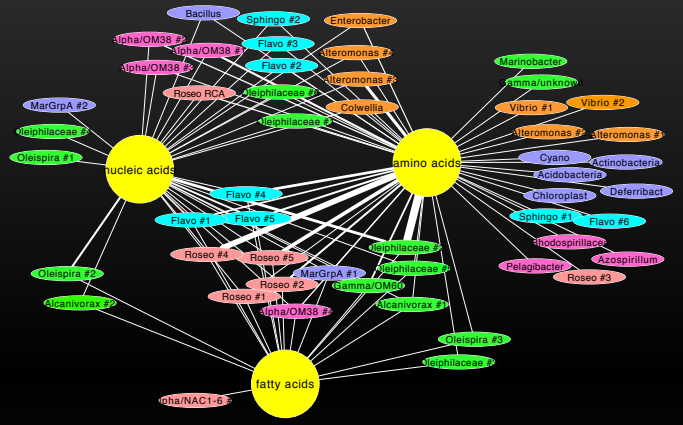
Functional gene/protein expression



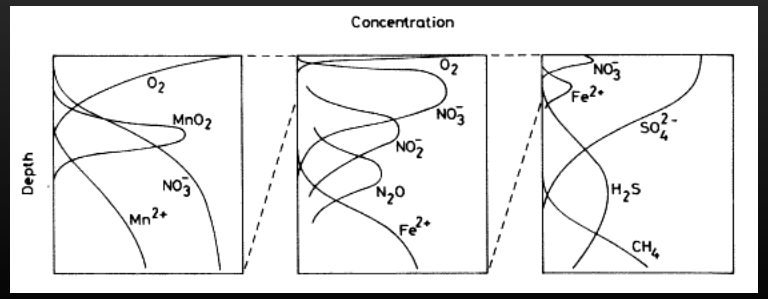


Metabolite characterization

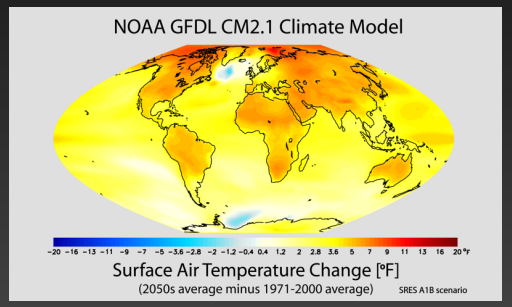
How do we make quantitative microbial data useful?



Microbial ecology



biogeochemistry



Climate modeling