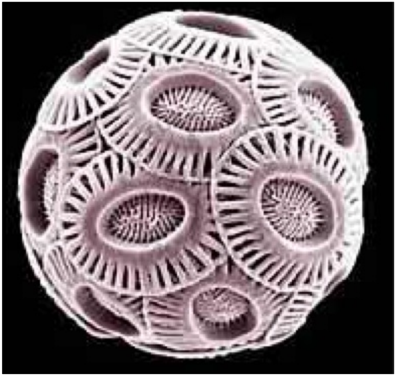
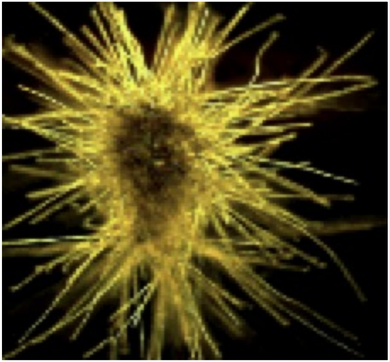
# Vignettes

- Light and nutrients: defining the drivers of physiological ecology and competition in microeukaryotes



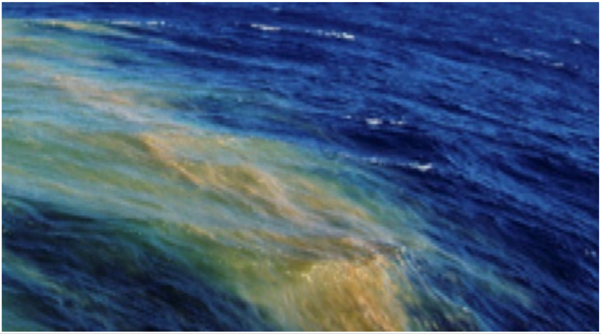
- Host-microbiome interactions: Tracking the microbiome of a keystone $N_2$ fixer

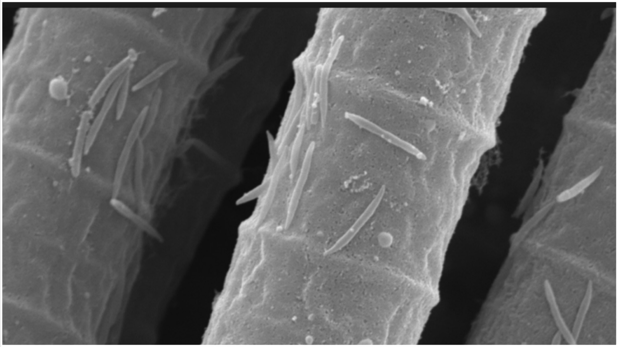


# Core questions

Host-microbiome interactions

- What is the role of the microbiome in *Trichodesmium* physiological ecology
  - Who is there?
  - What are they doing?
  - Are they interacting?

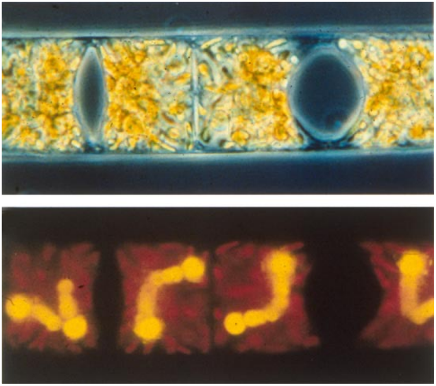




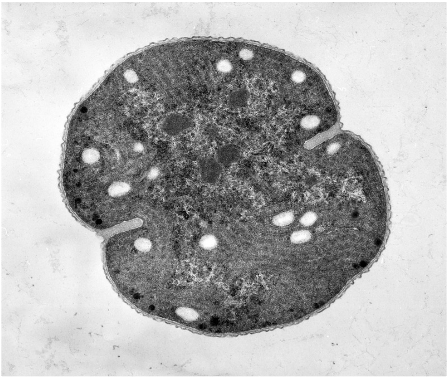
# Nitrogen-fixing marine cyanobacteria

- Symbionts
  - UNCYN-A
  - *Richelia*
- Free-living
  - *Crocococsphaera*
  - *Trichodesmium*

*Richelia*



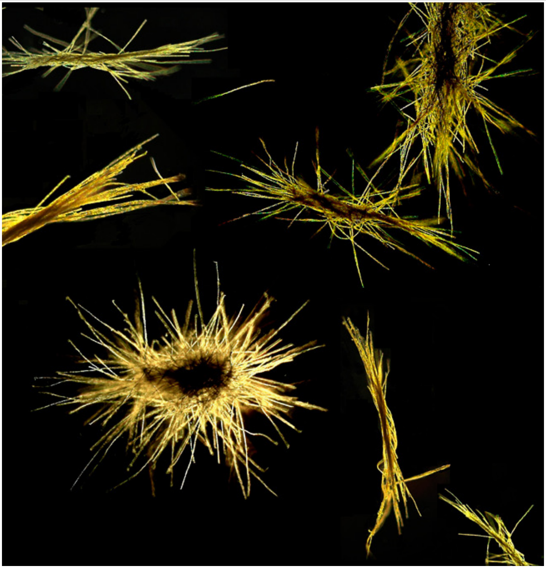
*Crocosphaera*

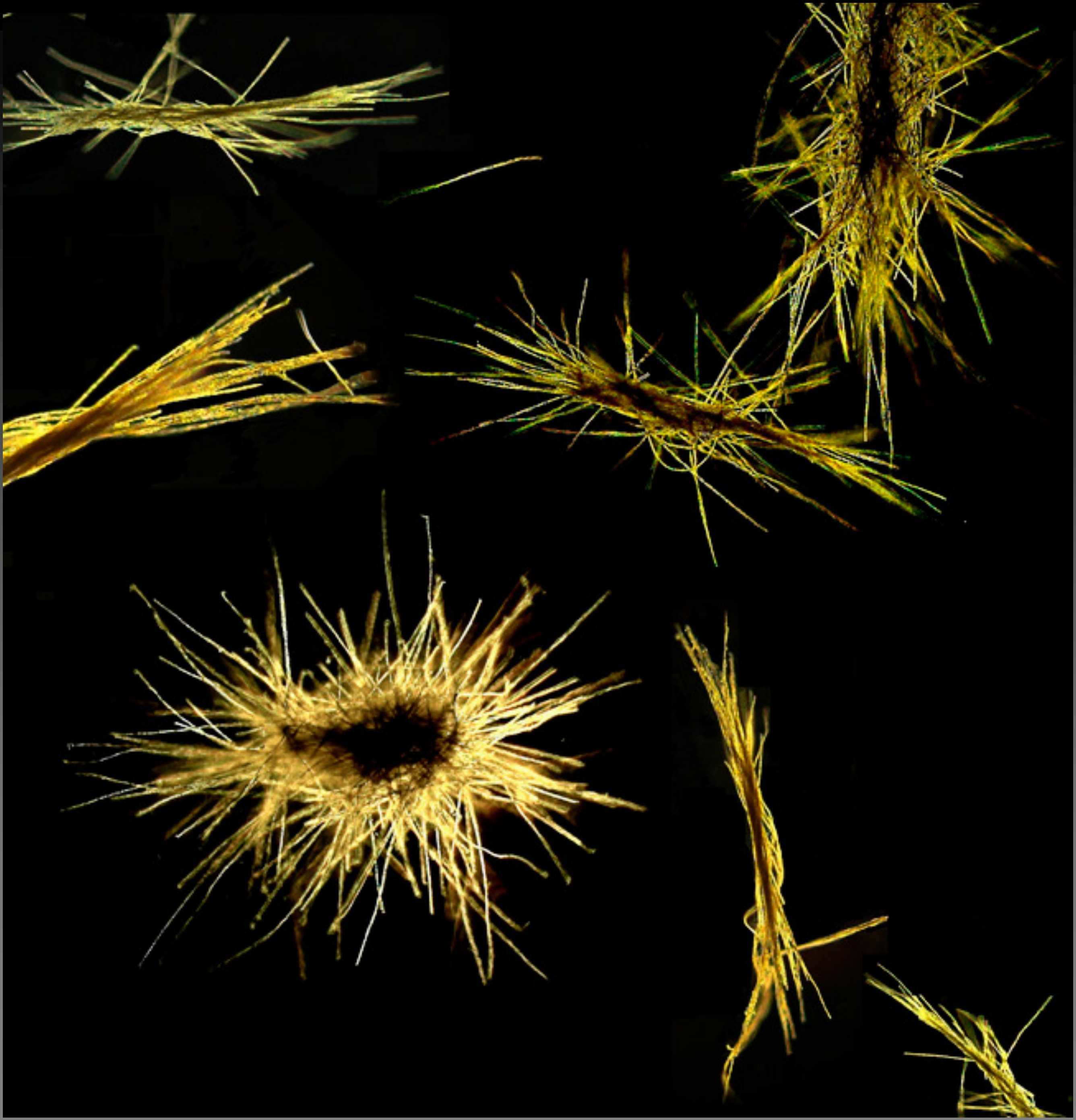


| | |
|---|---|
| -*Trichodesmium erythraeum* | Clade III |
| -*Trichodesmium tenue*<br>-*Trichodesmium contortum*<br>-*Trichodesmium spp. (miru, nobis)** | Clade II |
| -*Trichodesmium thiebautii* | Clade I |
| -*Trichodesmium spiralis*<br>-*Trichodesmium hildebrandtii* | Clade IV |

* Delmont et al. (2021) PNAS

*Trichodesmium*



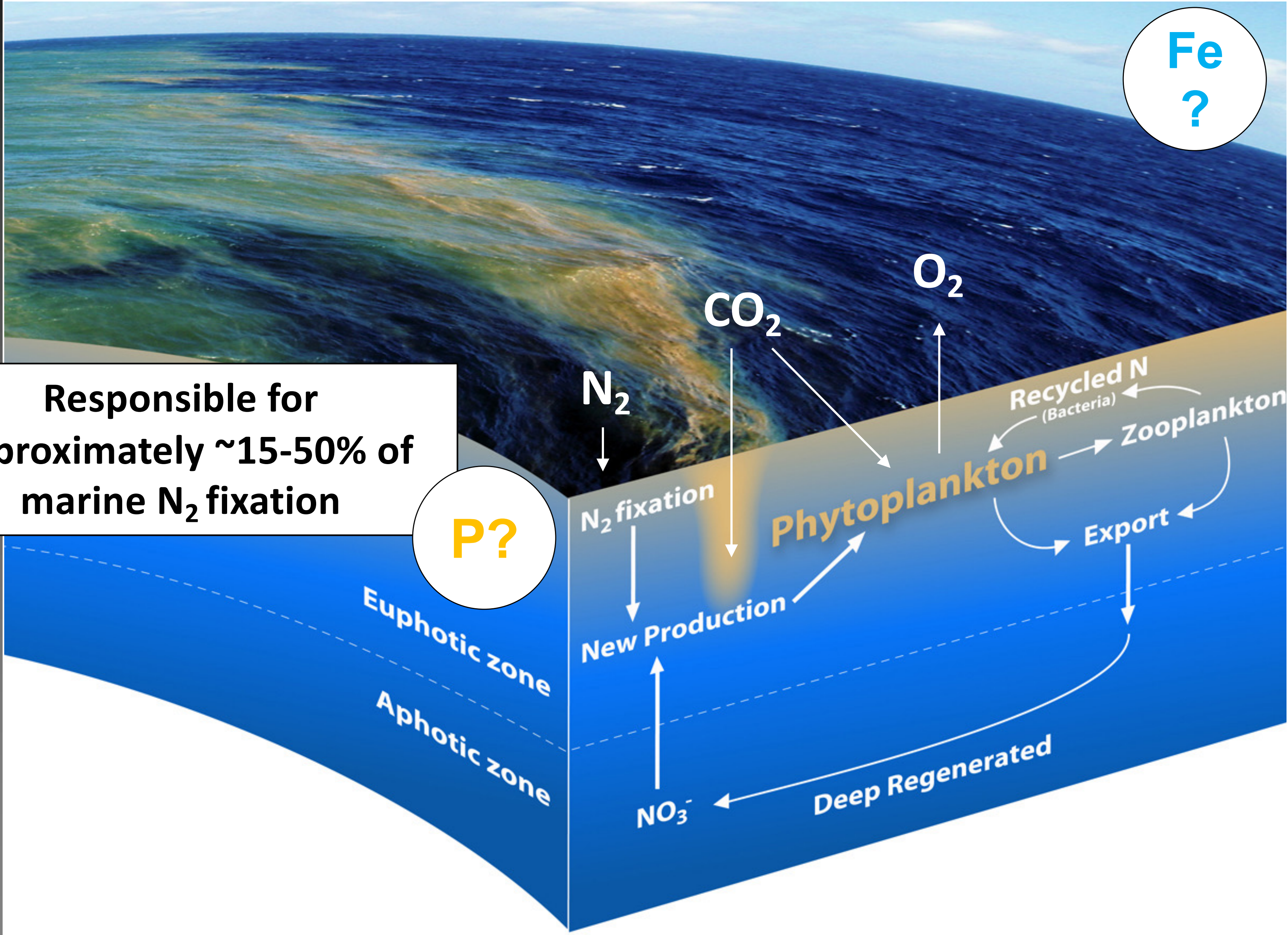


# *Trichodesmium:* critical to ecosystem function

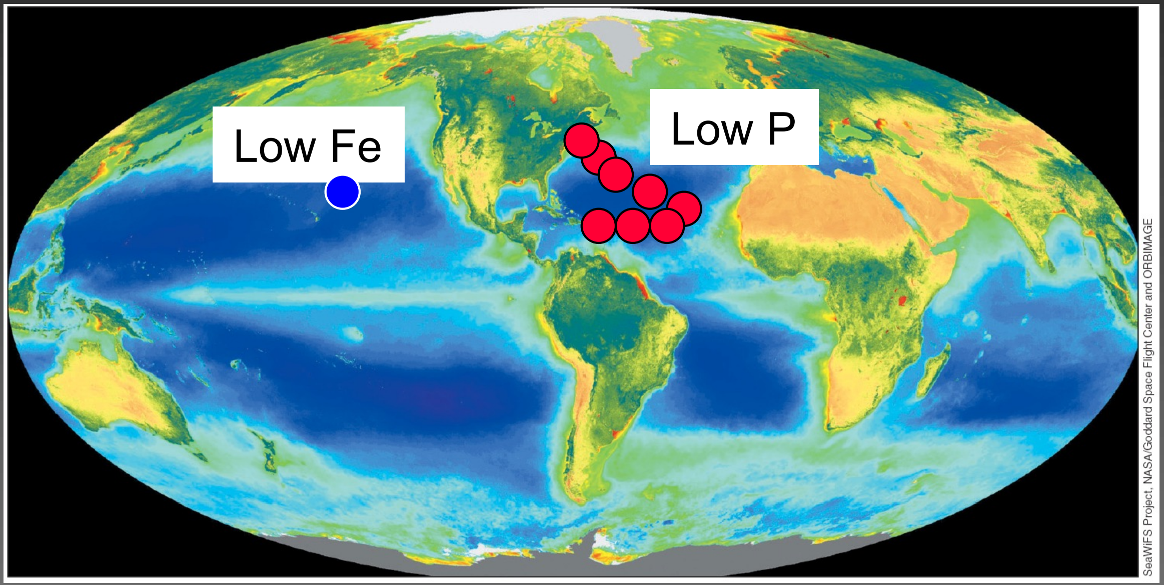
Photo: Chris Wade
*Tricho.* micrograph: WHOI

# *Trichodesmium:* critical to ecosystem function

Responsible for approximately ~15-50% of marine $N_2$ fixation



# Predicting the role of Fe and P in *Trichodesmium* distribution and $N_2$ fixation



SeaWiFS Project, NASA/Goddard Space Flight Center and ORBIMAGE

North Atlantic

P-limited?

North Pacific

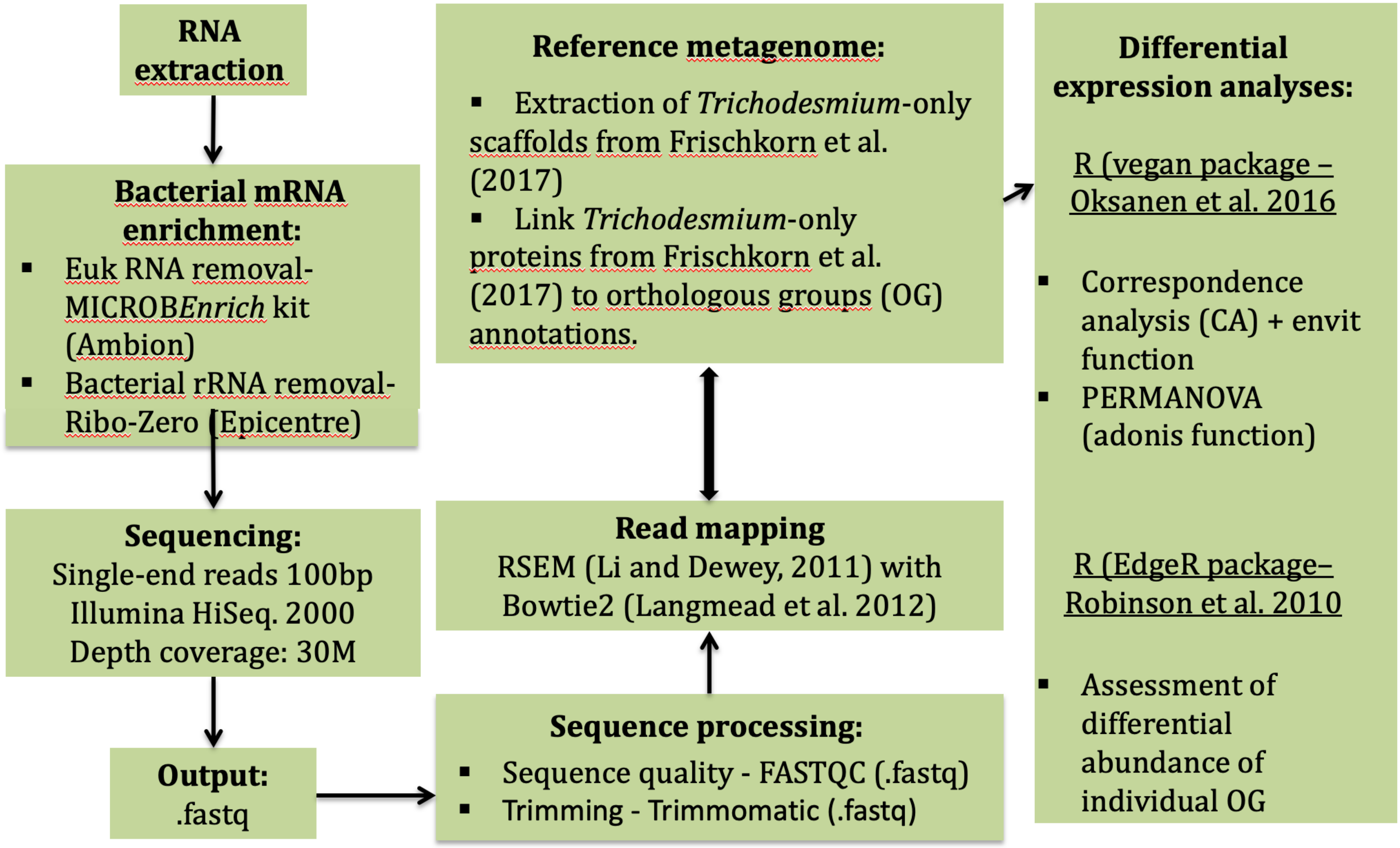
Fe-limited?

2000s

- Protein biomarkers: Dyhrman et al. (2002) *L&O*
- qRT-PCR of transcript biomarkers: Dyhrman et al. (2006) *Nature*

2010s

- Metagenomics and Metatranscriptomics: Rouco et al. (2018) *ISME J*.



**RNA extraction**

**Bacterial mRNA enrichment:**

- Euk RNA removal- MICROB*Enrich* kit (Ambion)
- Bacterial rRNA removal- Ribo-Zero (Epicentre)

**Sequencing:**
Single-end reads 100bp
Illumina HiSeq. 2000
Depth coverage: 30M

**Output:**
.fastq

**Sequence processing:**

- Sequence quality - FASTQC (.fastq)
- Trimming - Trimmomatic (.fastq)

**Read mapping**
RSEM (Li and Dewey, 2011) with
Bowtie2 (Langmead et al. 2012)

**Reference metagenome:**

- Extraction of *Trichodesmium*-only scaffolds from Frischkorn et al. (2017)
- Link *Trichodesmium*-only proteins from Frischkorn et al. (2017) to orthologous groups (OG) annotations.

**Differential expression analyses:**

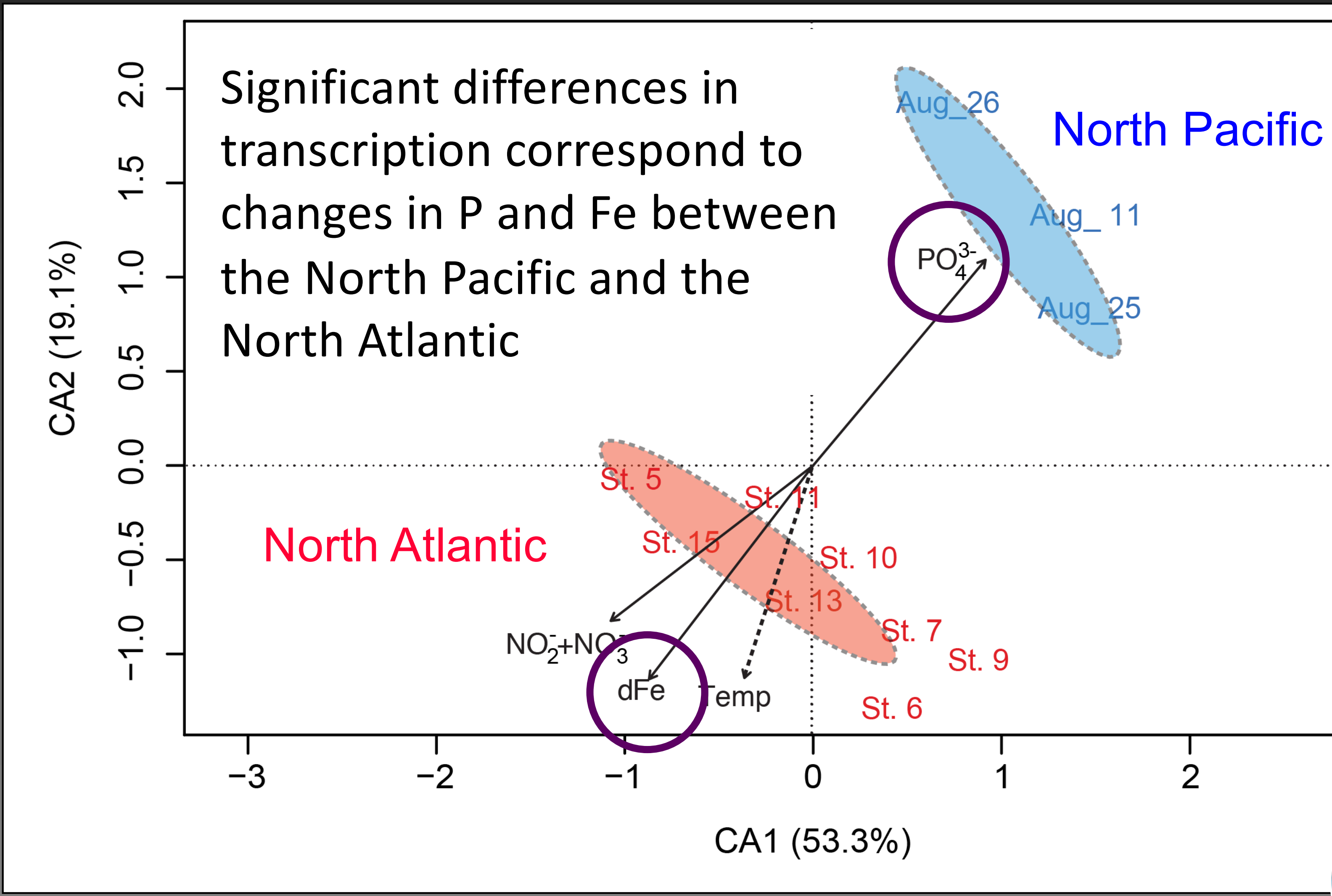
R (vegan package – Oksanen et al. 2016

- Correspondence analysis (CA) + envit function
- PERMANOVA (adonis function)

R (EdgeR package– Robinson et al. 2010

- Assessment of differential abundance of individual OG

# *Trichodesmium* transcriptional patterns between the North Pacific and the North Atlantic

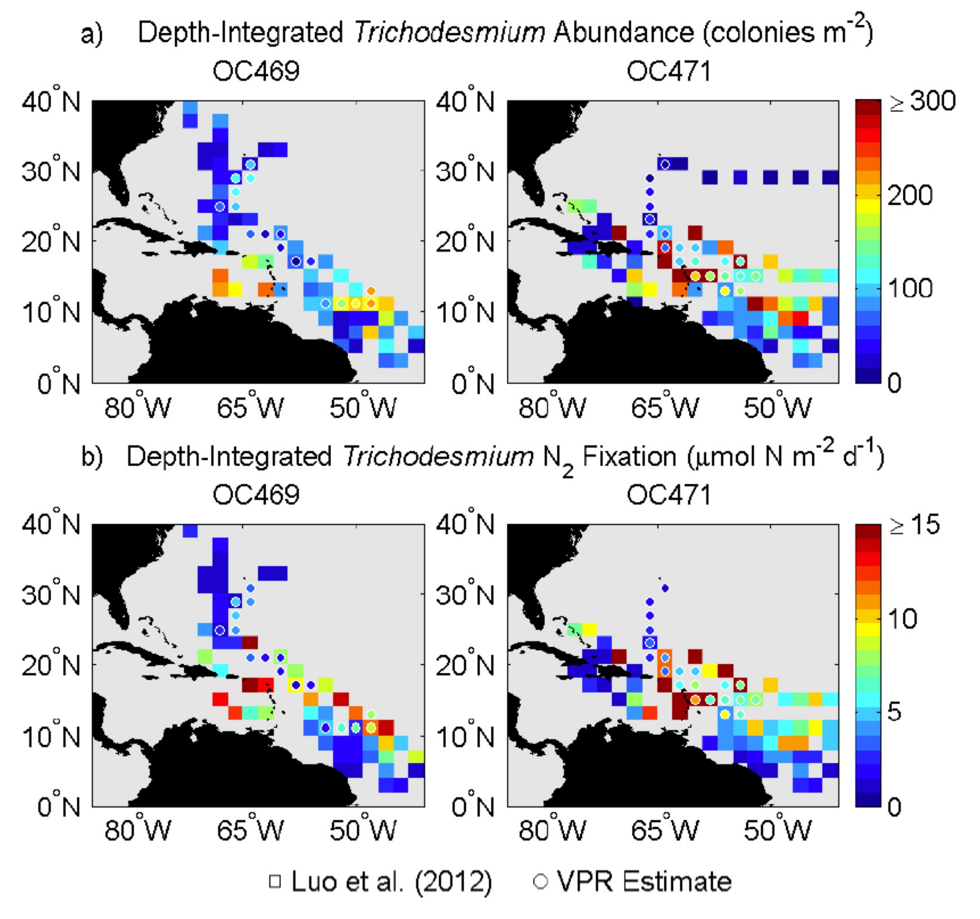


Significant differences in transcription correspond to changes in P and Fe between the North Pacific and the North Atlantic

Ruoco et al. (2018) *ISME J*

# Modeling $N_2$ fixation is still a challenge

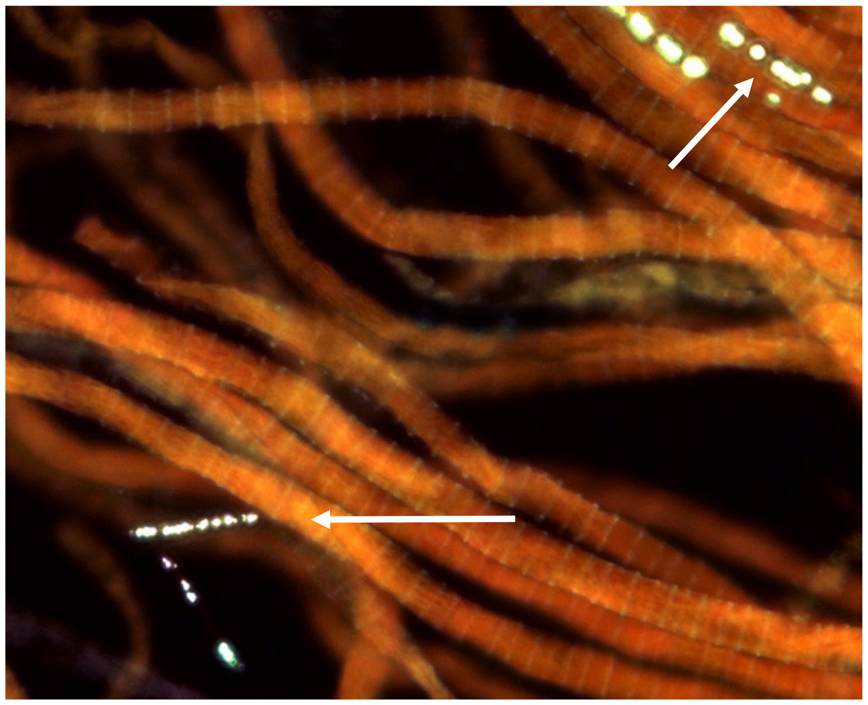
- Models do not balance the N cycle in the ocean or recapitulate patterns well
- Assays of nitrogen fixation are technically difficult = variability
- Information on distribution over time and with depth is still patchy
- **Geochemistry is not often a good predictor of distribution or $N_2$ fixation**



Olson et al. 2015 *DSR* II

# *Trichodesmium* hosts a community of epibiotic microbes

### *Trichodesmium* and *Plectonema*



Dyhrman et. al. (2002) *L&O*

### *Trichodesmium* and Epibionts

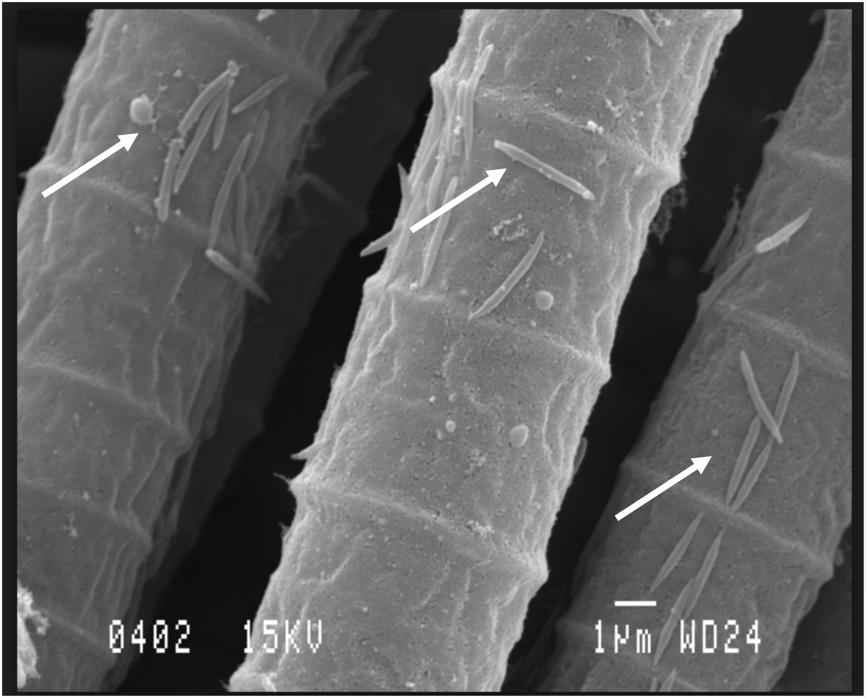


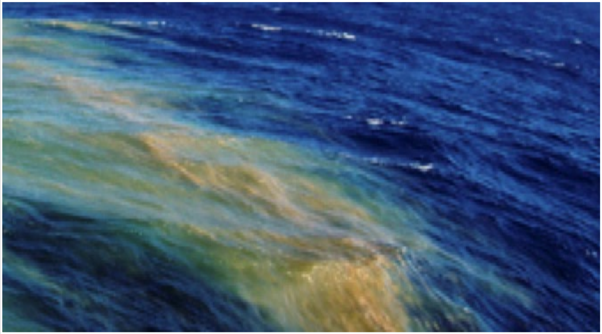
Image courtesy Tracy Mincer

*Trichodesmium* colonies harbor other microbes with distinct physiology.

# Core questions about the *Trichodesmium* microbiome

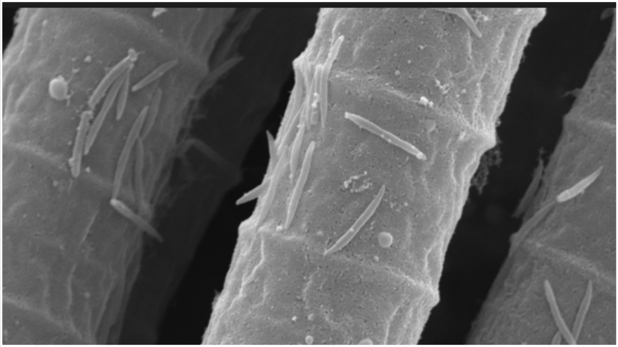
Who is there?

- Microbiome diversity
- *16S amplicon sequencing*



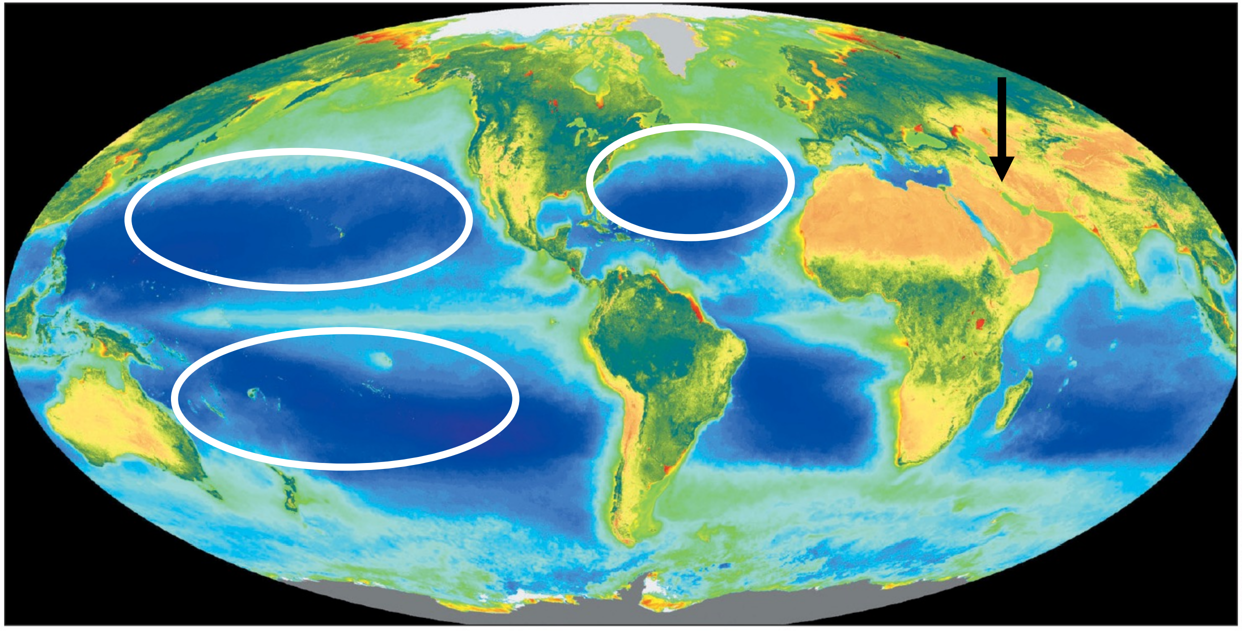
What are they doing?

- Microbiome functional diversity
- *Metagenome assembled genomes*

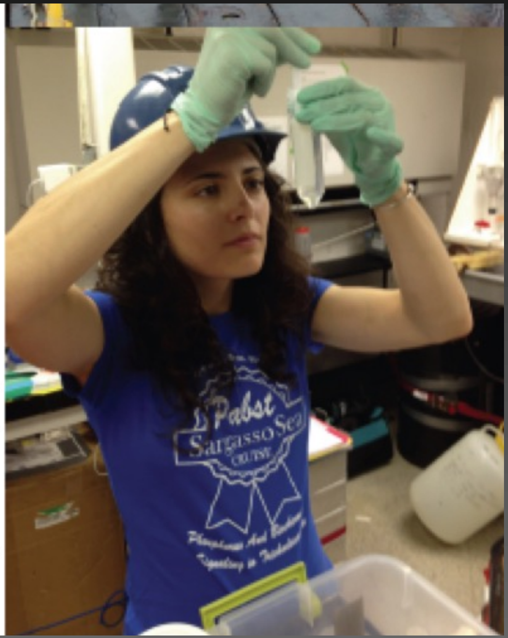


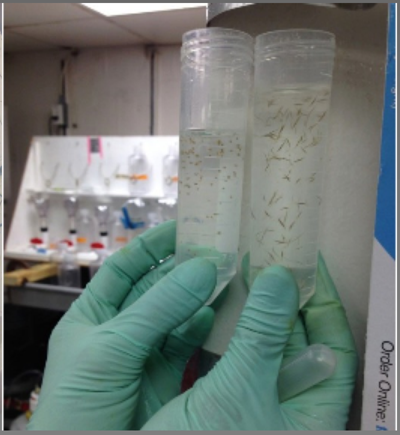
Are they interacting?

- Diel patterns in the consortia
- *Metatranscriptomics*

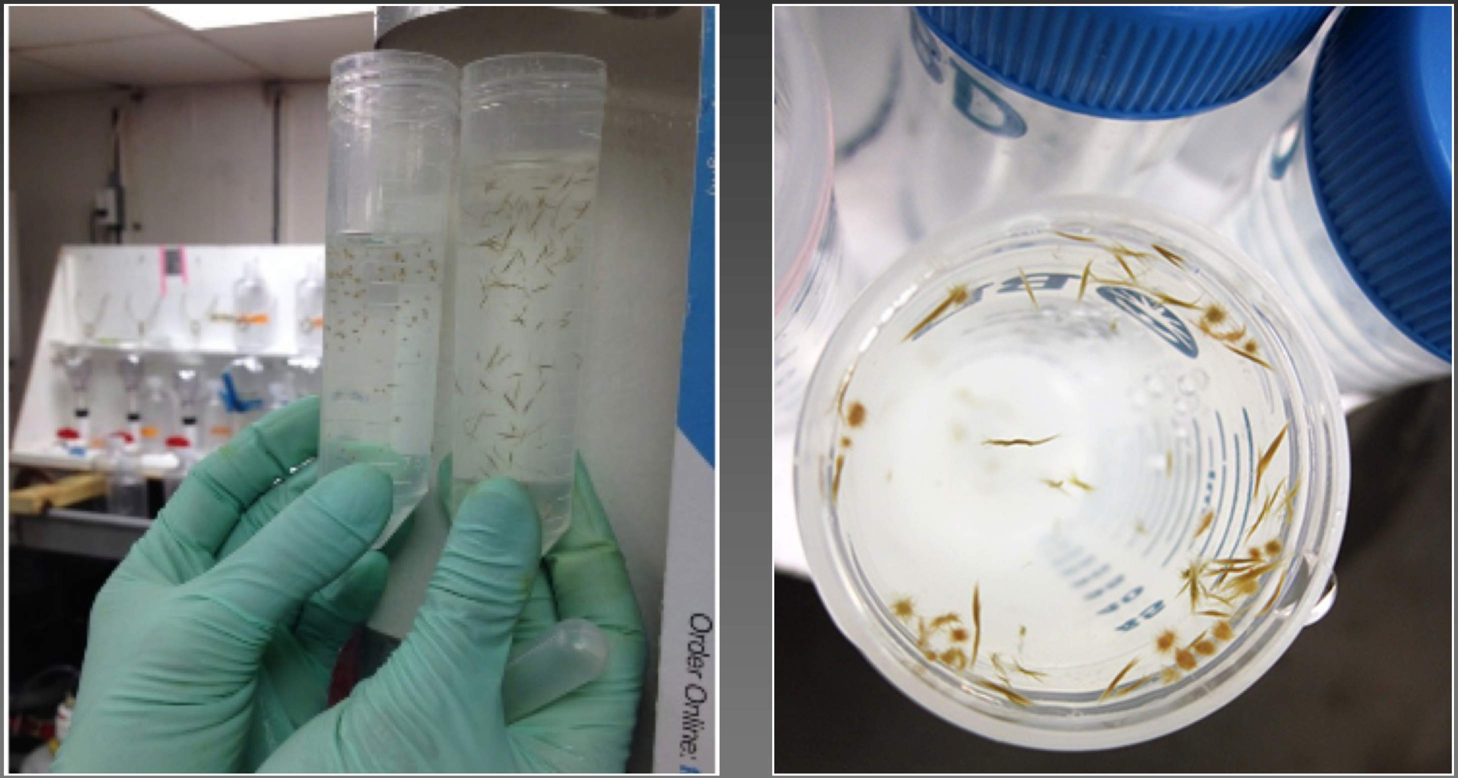


SeaWiFS Project, NASA/Goddard Space Flight Center and ORBIMAGE





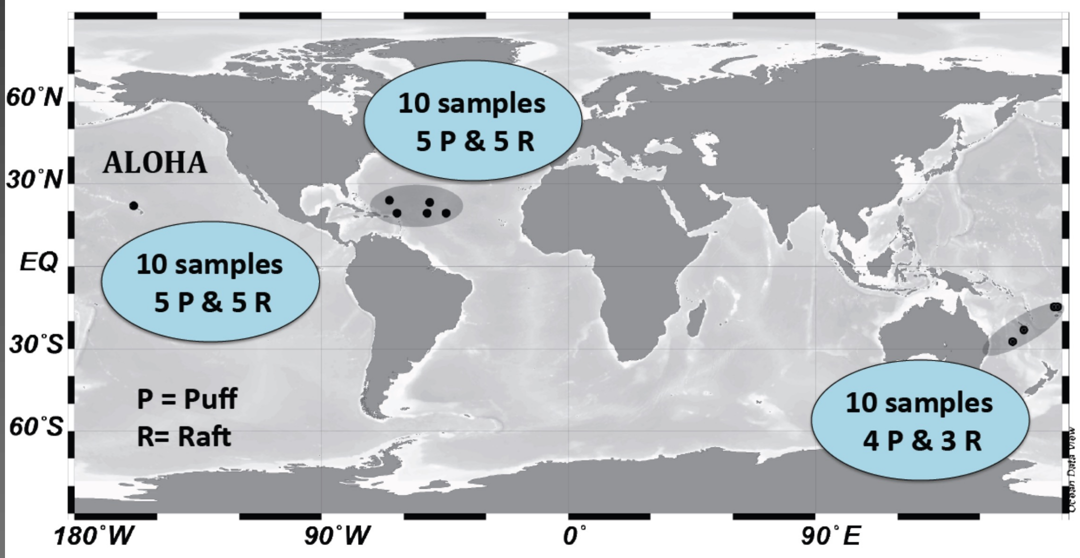
# *Trichodesmium* microbiome diversity



*Trichodesmium* colonies are picked and rinsed from net tows for 16S sequencing to remove all but the tightly associated epibionts.

# Epibiont diversity

## EXPERIMENTAL APPROACH



**Collection of colonies from 3 ocean basins**
(~top 25m)

**DNA extraction**

**Sequencing:**
V4 region of 16S rRNA gene
**Miseq**
**(2x150 bp)**

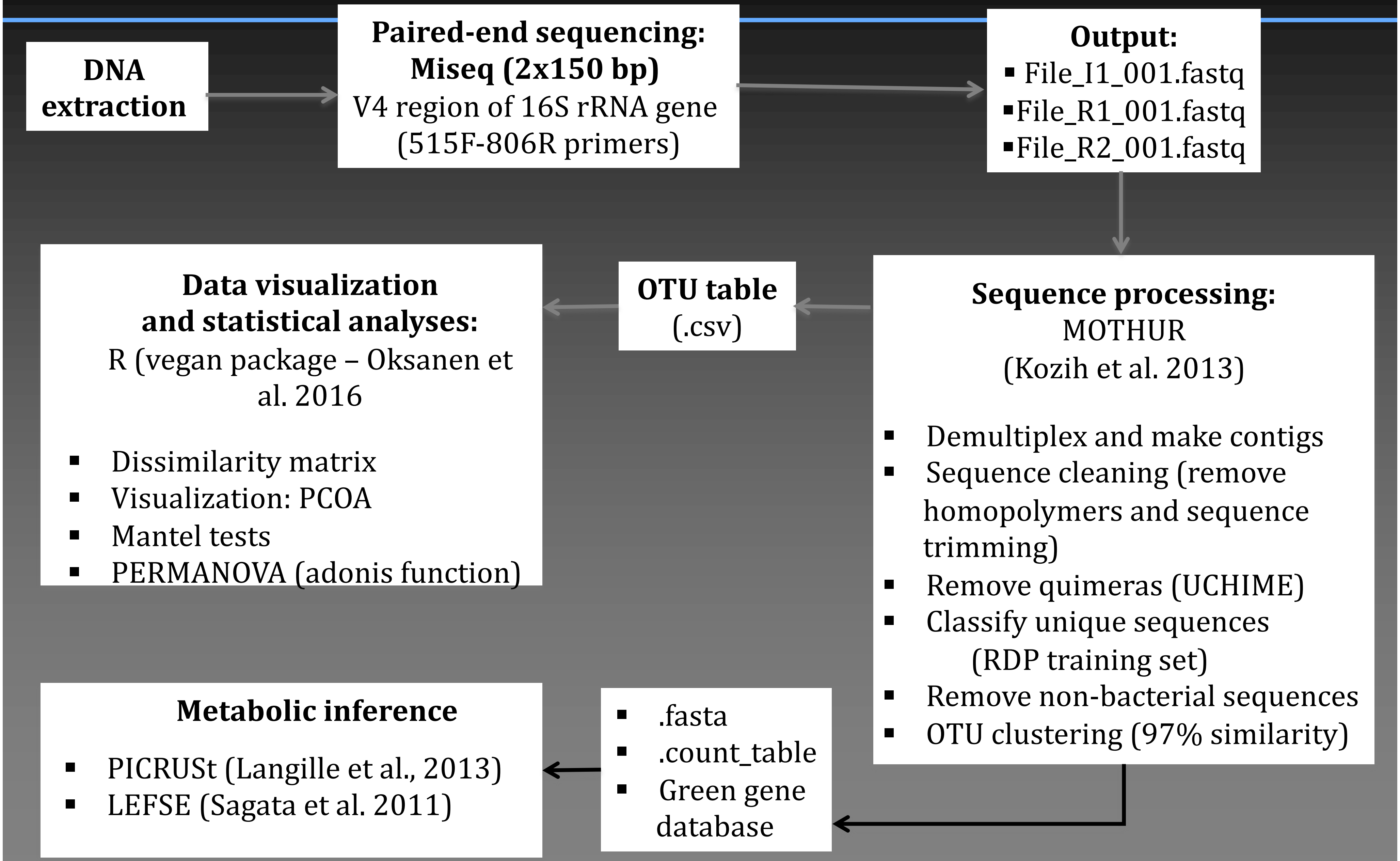
Mothur v. 1. 34. 0 (936,749 reads)

- ***Trichodesmium***: ~67% of reads
- **Epibionts**: ~32% of reads

What groups co-occur with *Trichodesmium*?

Are epibiont communities distinct as a function of colony morphology or environment?

# 16S rDNA analyses



**DNA extraction**

**Paired-end sequencing:**
**Miseq (2x150 bp)**
V4 region of 16S rRNA gene
(515F-806R primers)

**Output:**
- File_I1_001.fastq
- File_R1_001.fastq
- File_R2_001.fastq

**Sequence processing:**
MOTHUR
(Kozih et al. 2013)

- Demultiplex and make contigs
- Sequence cleaning (remove homopolymers and sequence trimming)
- Remove quimeras (UCHIME)
- Classify unique sequences (RDP training set)
- Remove non-bacterial sequences
- OTU clustering (97% similarity)

**OTU table**
(.csv)

**Data visualization and statistical analyses:**
R (vegan package – Oksanen et al. 2016

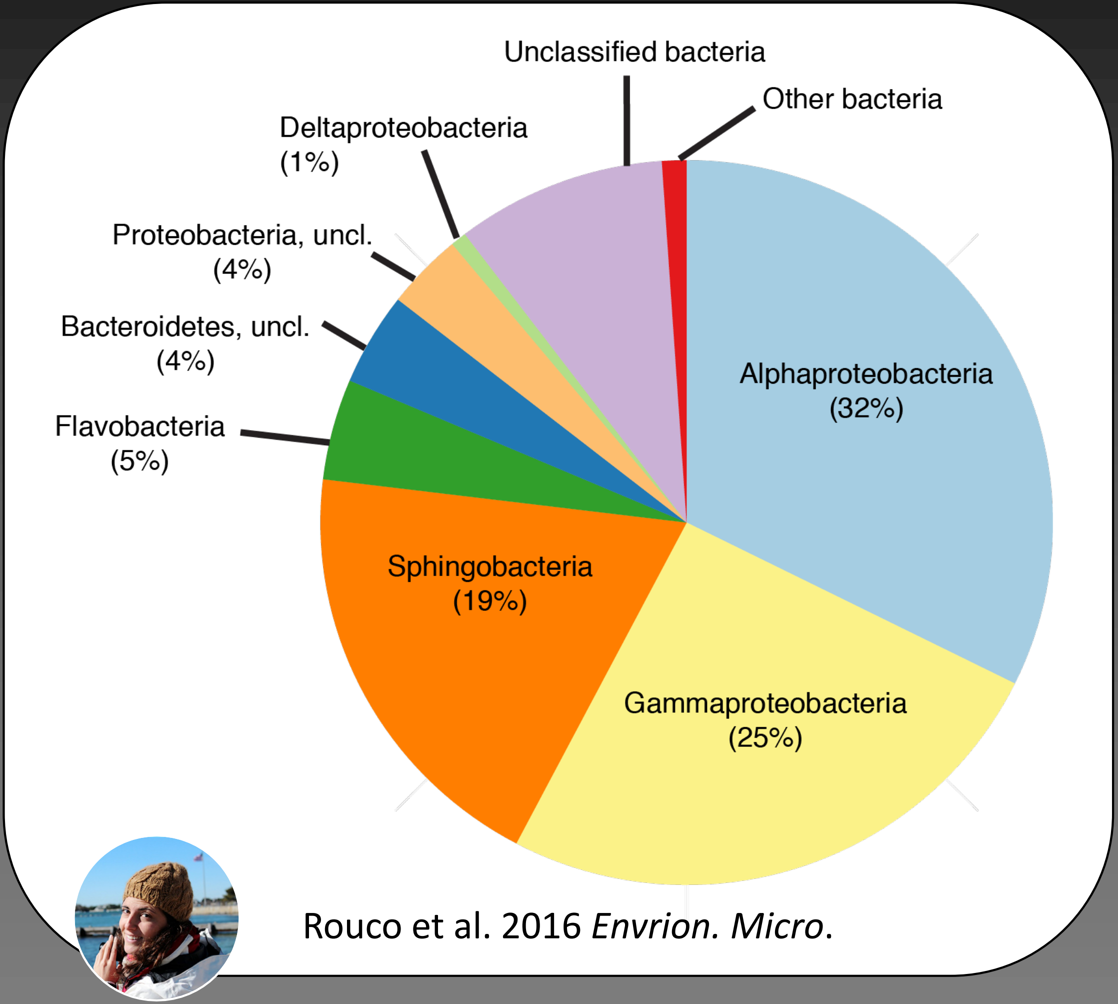
- Dissimilarity matrix
- Visualization: PCOA
- Mantel tests
- PERMANOVA (adonis function)

- .fasta
- .count_table
- Green gene database

**Metabolic inference**

- PICRUSt (Langille et al., 2013)
- LEFSE (Sagata et al. 2011)

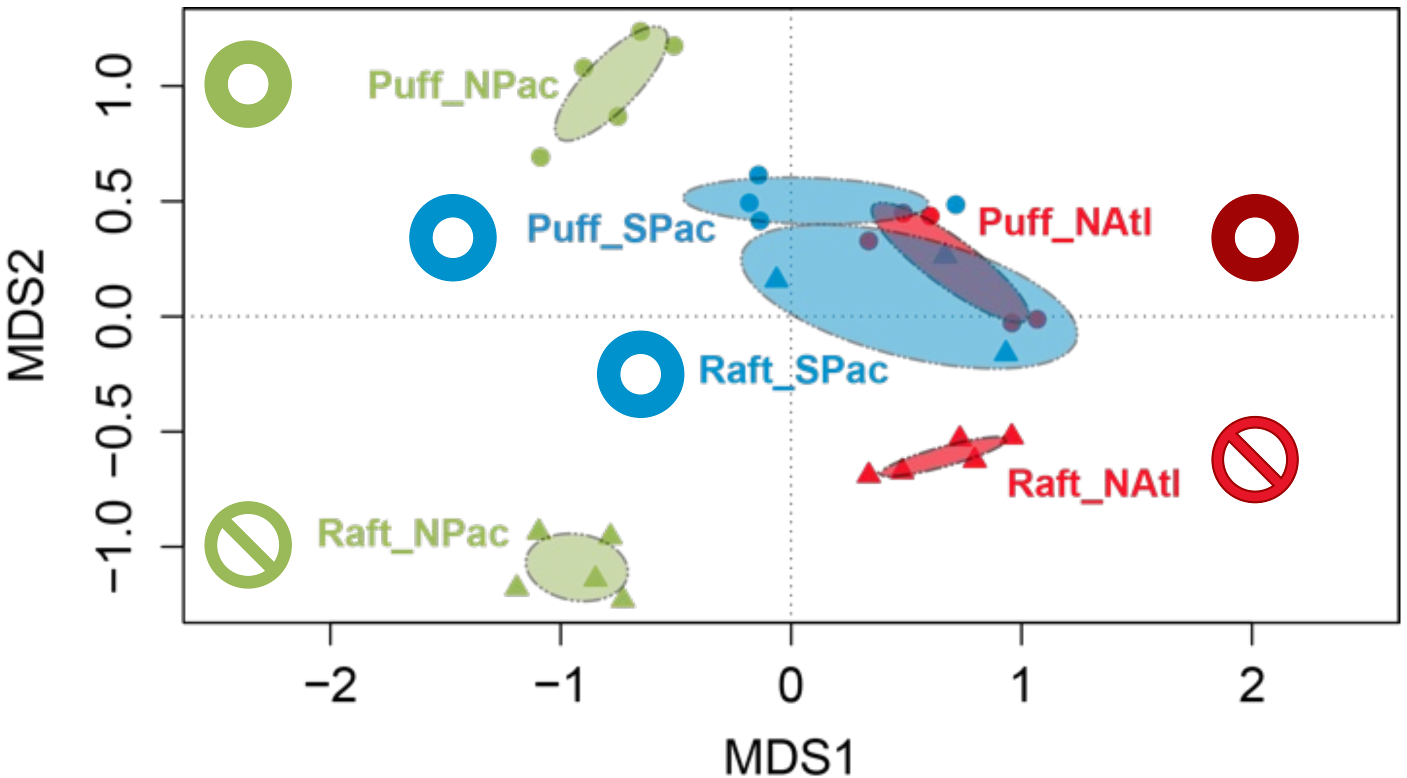
# Average epibiont community



Rouco et al. 2016 *Envrion. Micro*.

16S amplicon sequencing indicates that *Trichodesmium* colonies harbor diverse epibionts distinct from common water column bacteria, and those found on sinking particles.

# Microbiome community diversity (16S)



Rouco et al. (2016) *Envrion. Micro*.

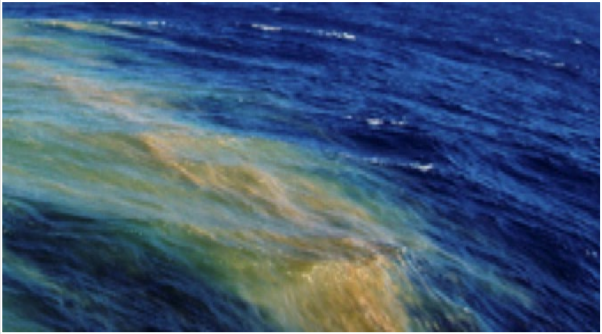
Microbiome communities significantly differ by ocean basin, and with colony morphology, except for the S. Pacific where the *Trichodesmium* composition of rafts and puffs were not significantly different.

# Summary

- **Need to think about *Trichodesmium* as a holobiont**

- *What drives community assembly?*
  - *Unique niche?*
    - Type of *Trichodesmium*
    - Physiological ecology in the colony
    - Environment
  - *Lottery?*
    - Random selection of potential copiotrophs with certain gene functions?
- *Stability over time?*

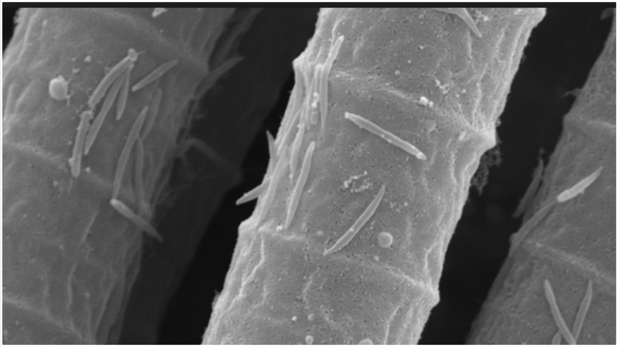
# Core questions about the *Trichodesmium* microbiome



Who is there?

- **Diverse community, distinct from water column**

What are they doing?



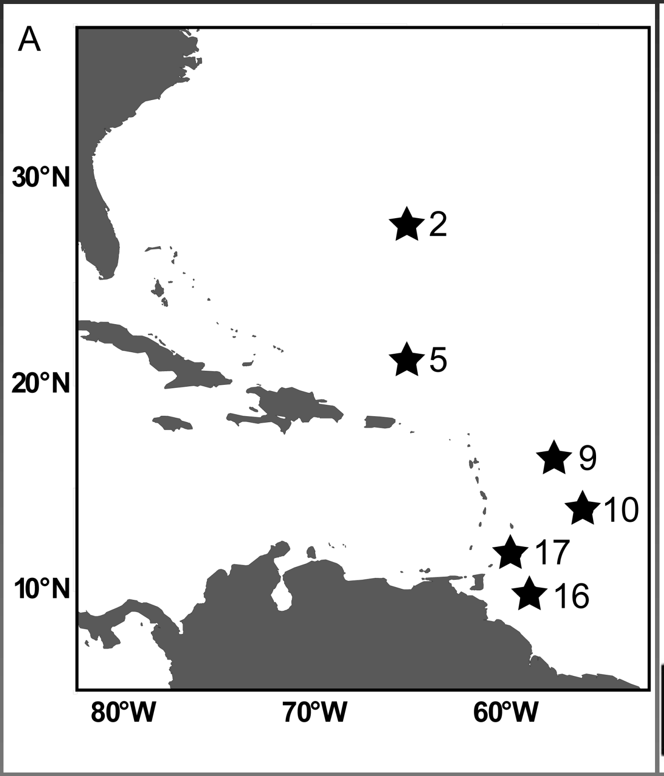
- Microbiome functional diversity

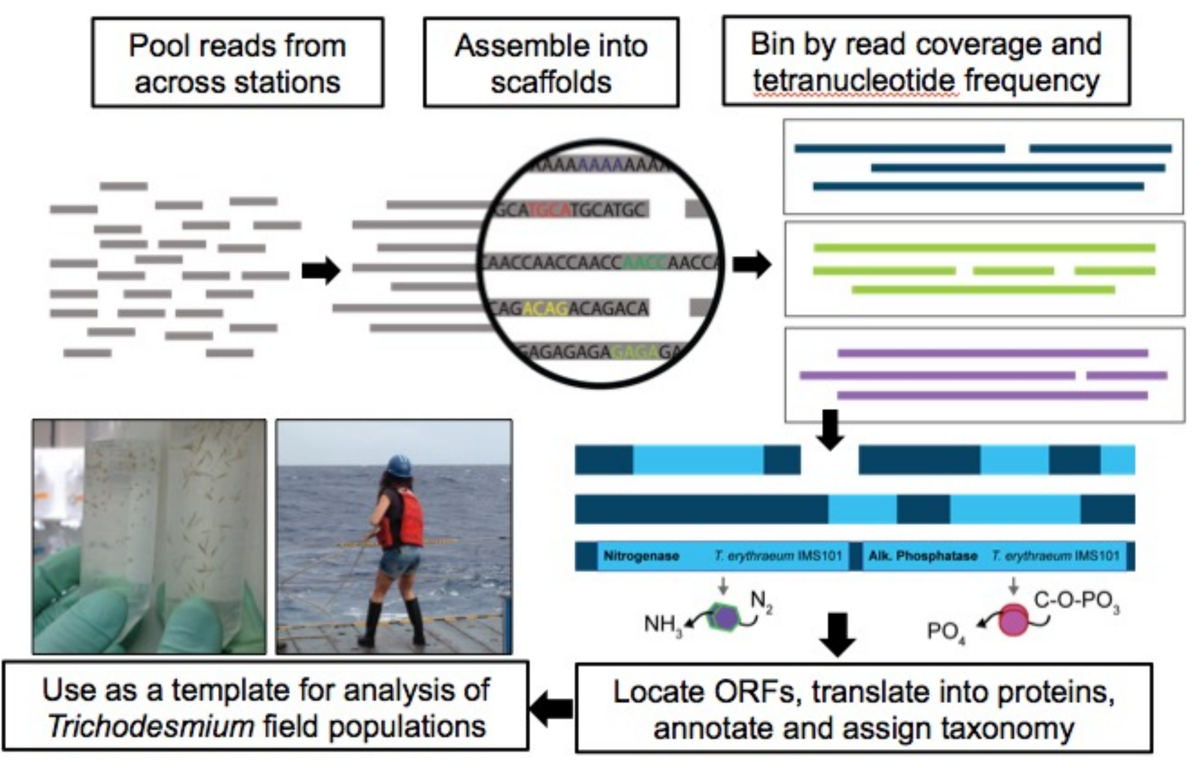
Are they interacting?

- Diel patterns in the consortia

# Metabolic potential in the *Trichodesmium* holobiont

**IDBA-UD** **MaxBin2**




Pool reads from across stations

Assemble into scaffolds

Bin by read coverage and tetranucleotide frequency

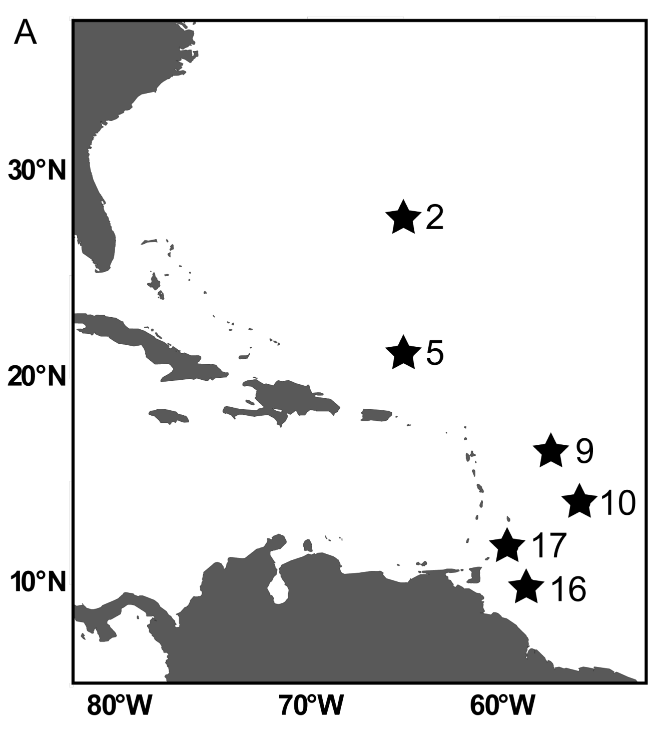
Locate ORFs, translate into proteins, annotate and assign taxonomy

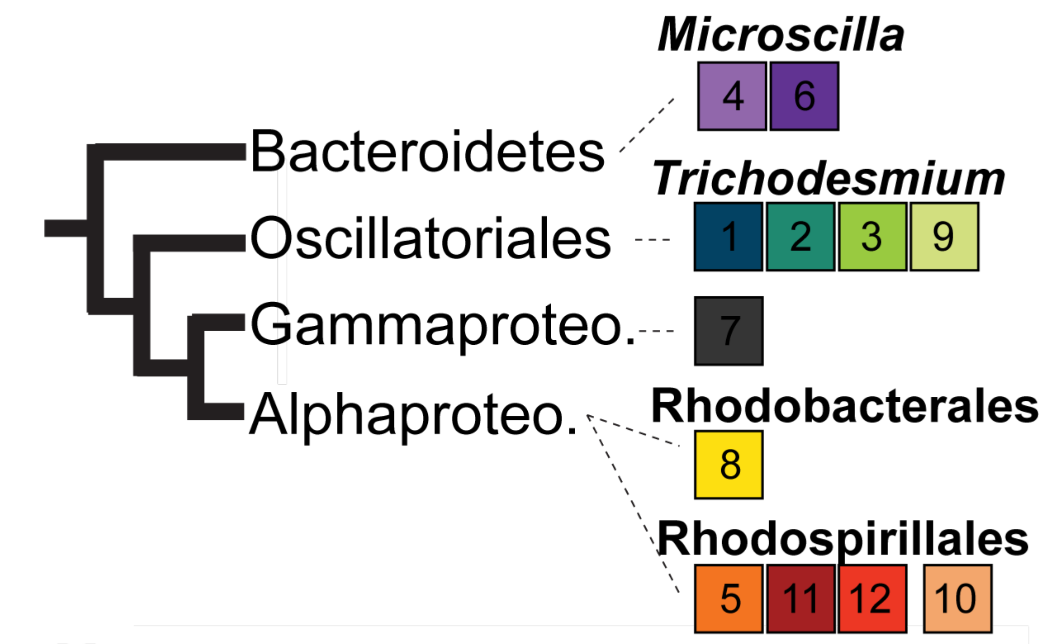
Use as a template for analysis of *Trichodesmium* field populations

**Prodigal** **DIAMOND-BLAST**
**MEGAN KEGG**

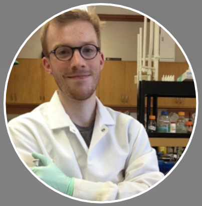
*Trichodesmium* colonies were isolated for metagenome sequencing along a phosphorus gradient in the western north Atlantic.

# Composition of the holobiont



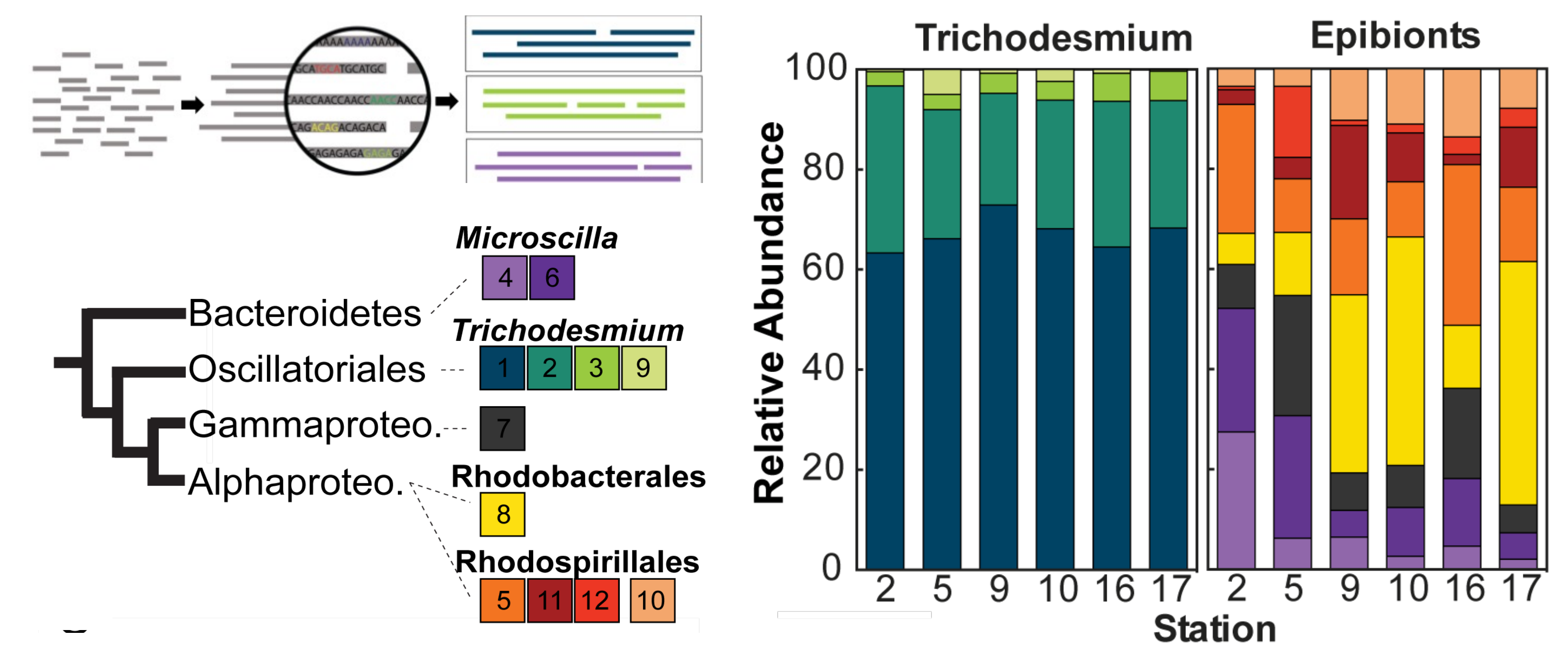


Nearly complete (~90%) genome bins were reconstructed from a merged assembly and results are consistent with 16S data



Frischkorn et al. (2017) *ISMEJ*

# Distribution of MAGs with read mapping

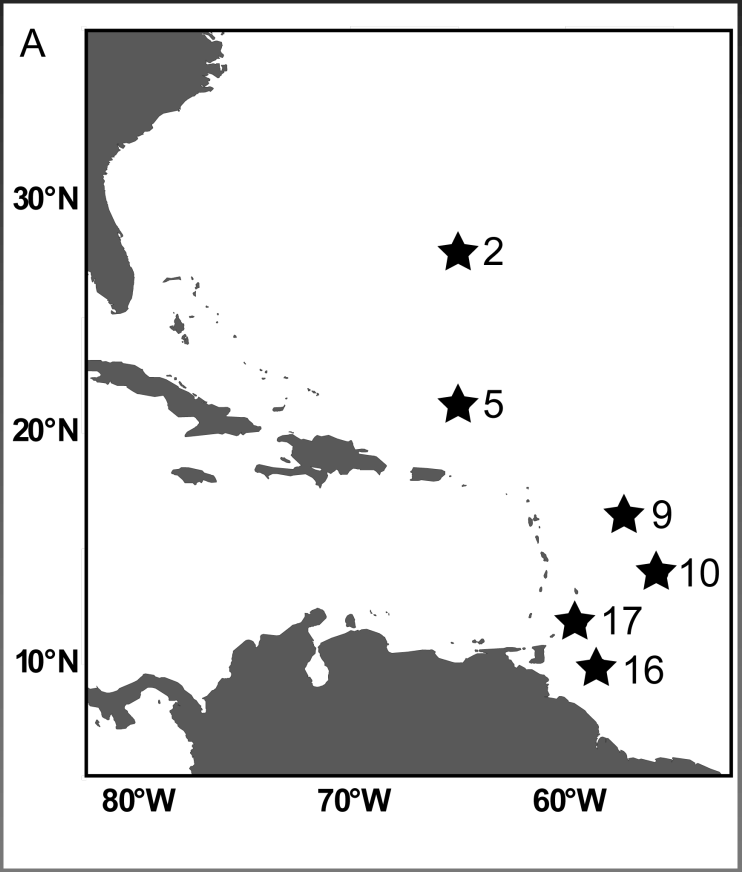


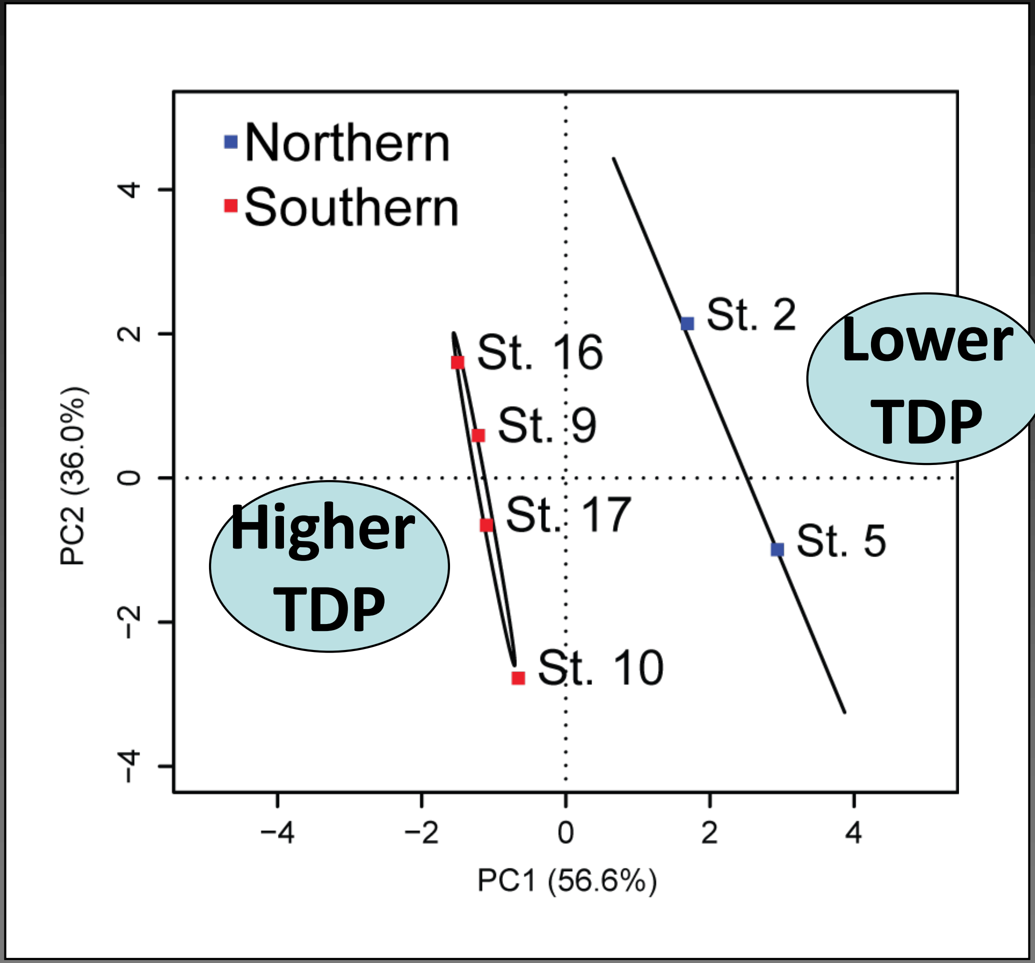
Frischkorn et al. (2017) *ISMEJ*

Relative proportion of *Trichodesmium* bins consistent across stations

Epibiont genome bins are detected at all stations, but the relative abundance varies

# Microbiome MAGs differ significantly between regions



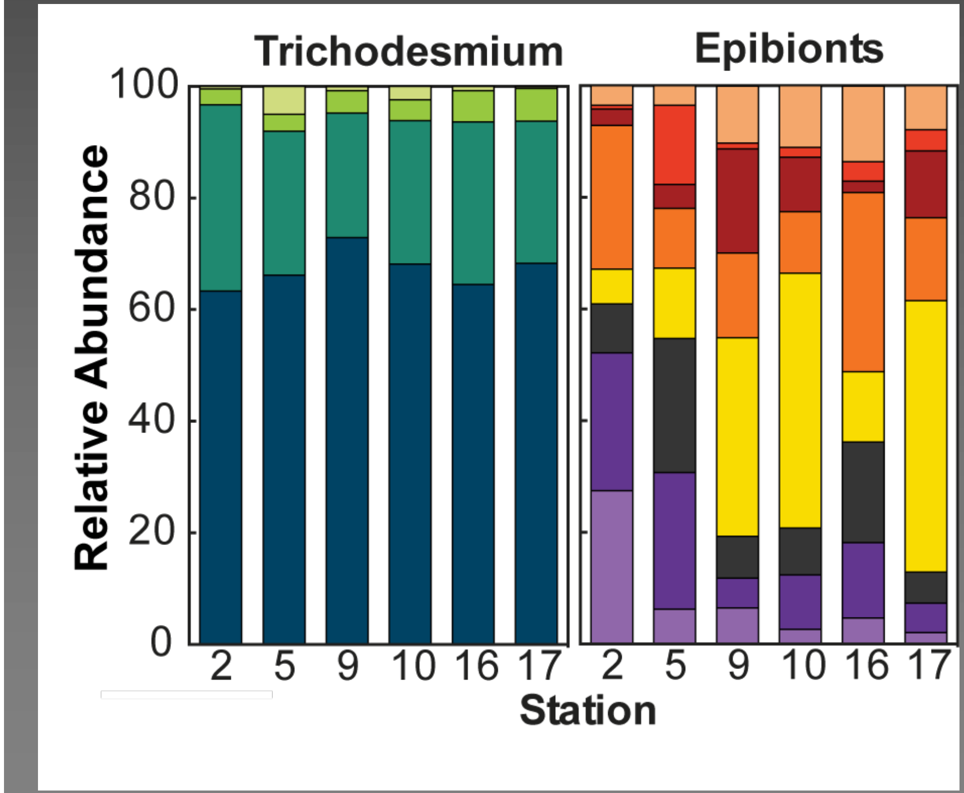


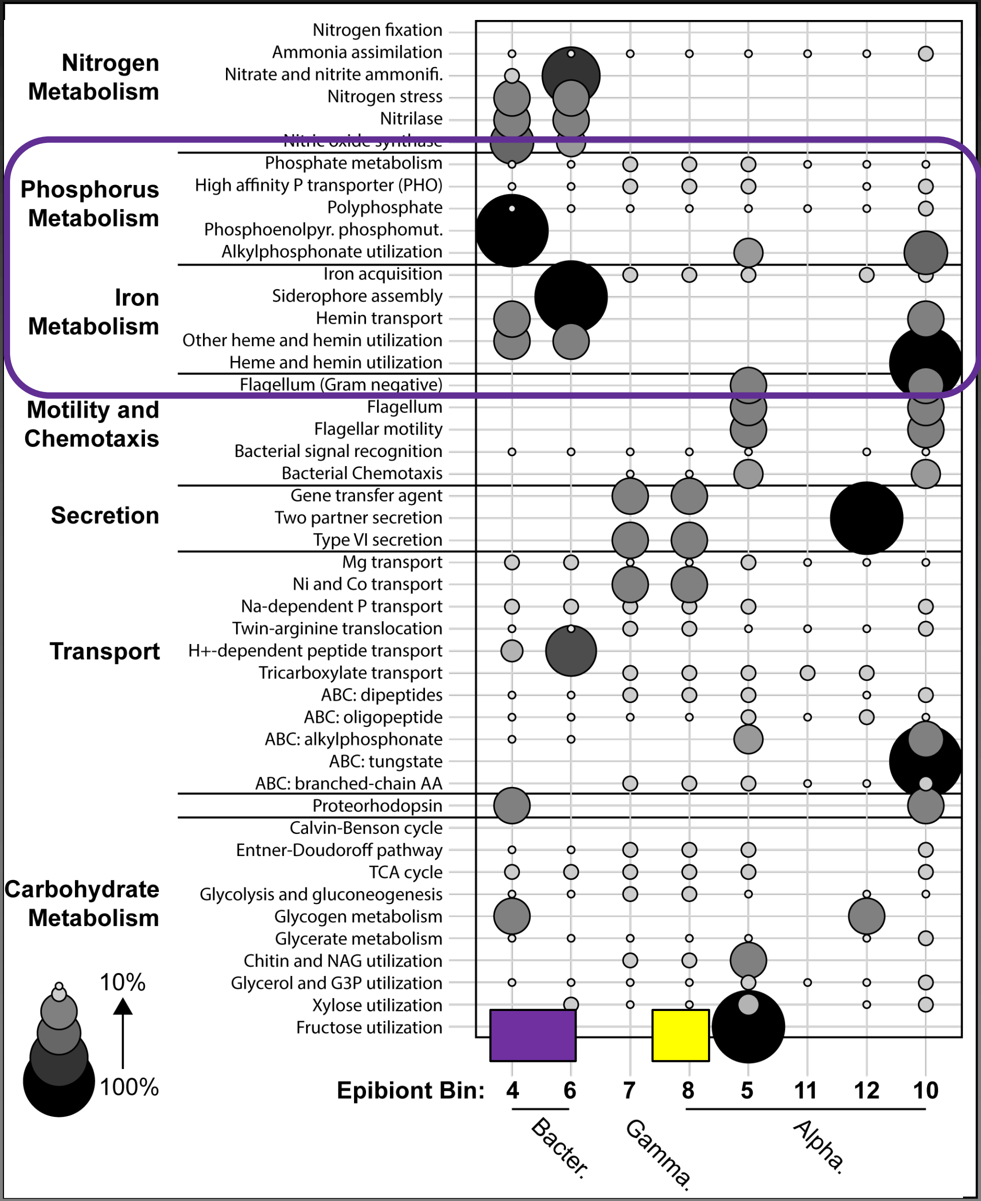
Frischkorn et al. (2017) *ISME J*

Significant difference in epibiont relative abundance with total dissolved phosphorus (TDP)

# Variable distribution of functional pathways among epibionts

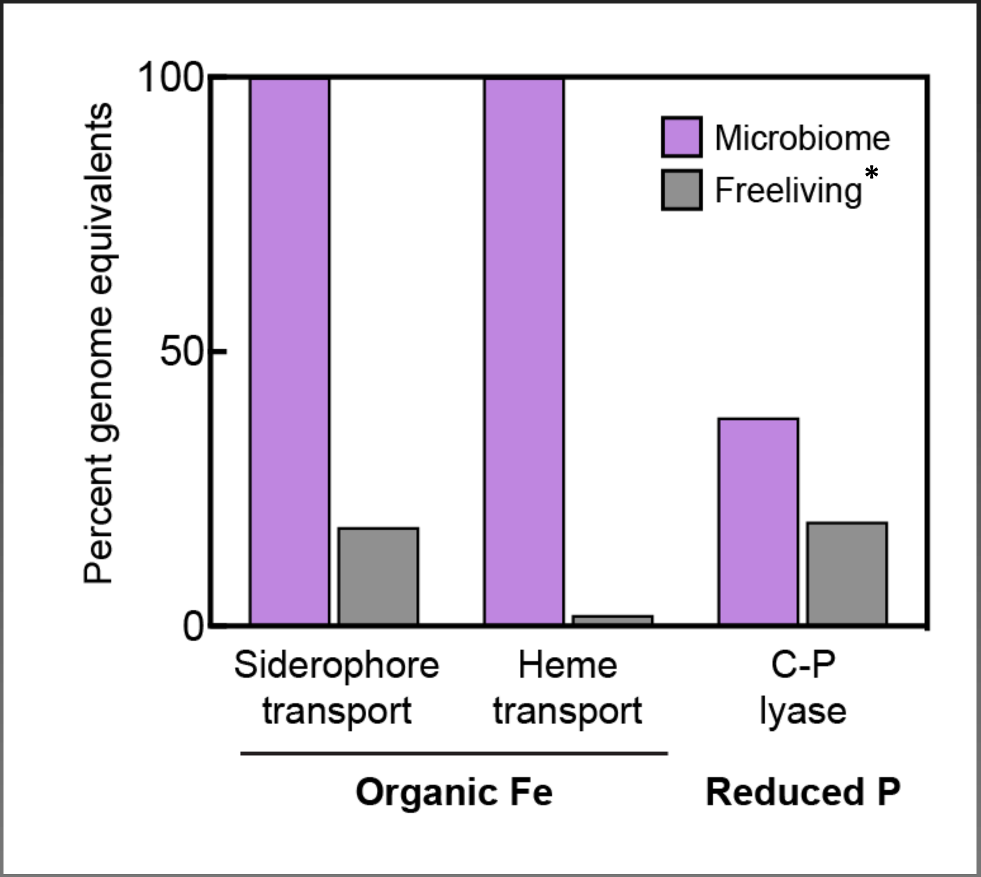
- Differential pathway enrichment consistent with a microbiome that is modulated as a function of environment





Frischkorn et al. (2017) *ISMEJ*

# Epibionts enrich holobiont for key functions



Frischkorn et al. (2017) *ISME J*

Phosphonate, heme and siderophore functions are enriched in the epibionts relative to water column microbes in the Sargasso Sea.

# Comparing metabolic potential in the holobiont

Metagenomes

Orthologous group analysis

Epibionts v. *Trichodesmium*

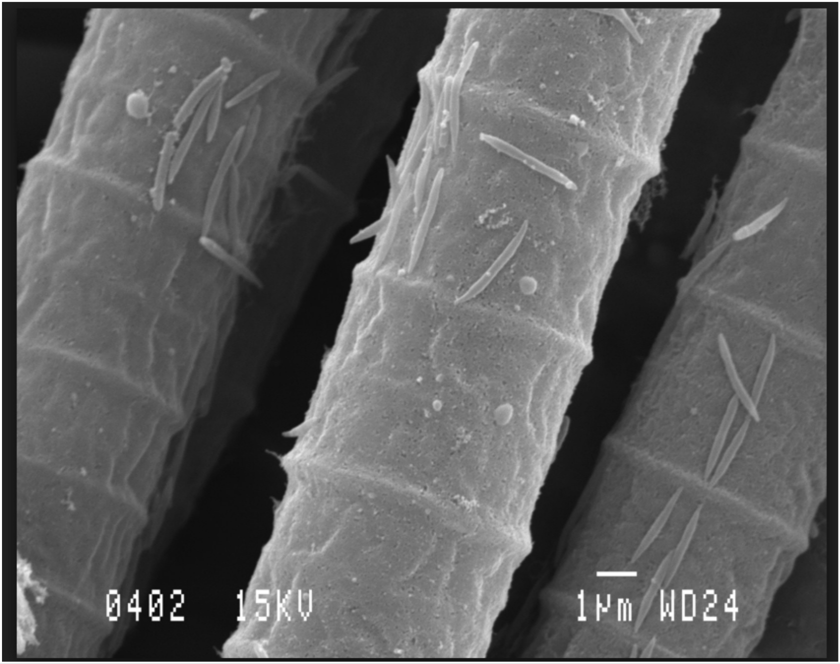
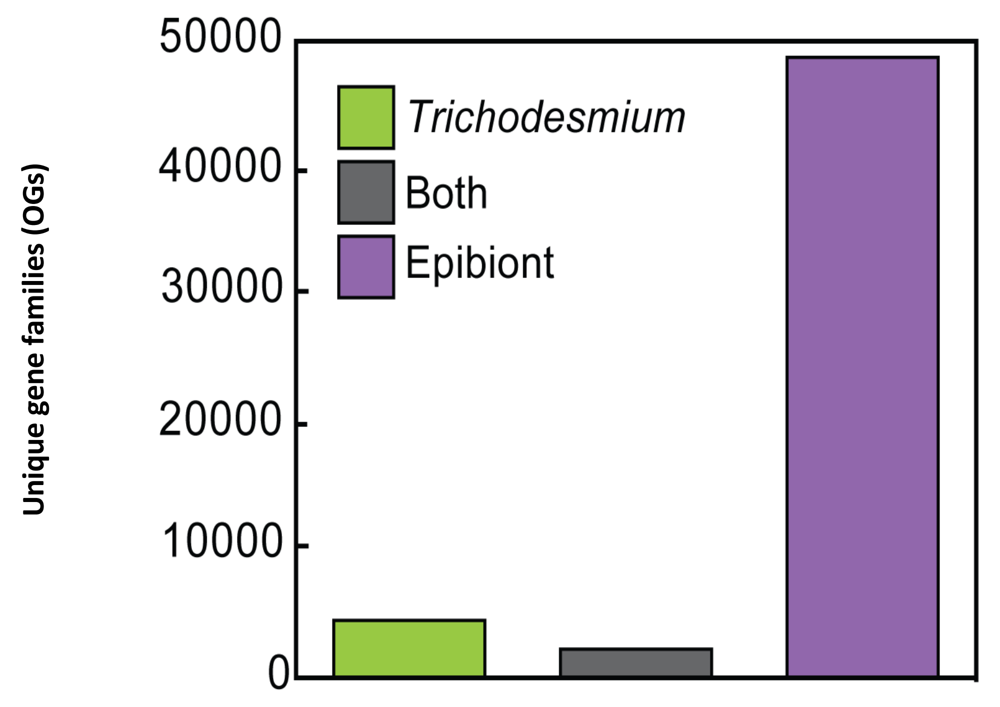


Image courtesy Tracy Mincer

# Epibionts significant metabolic potential

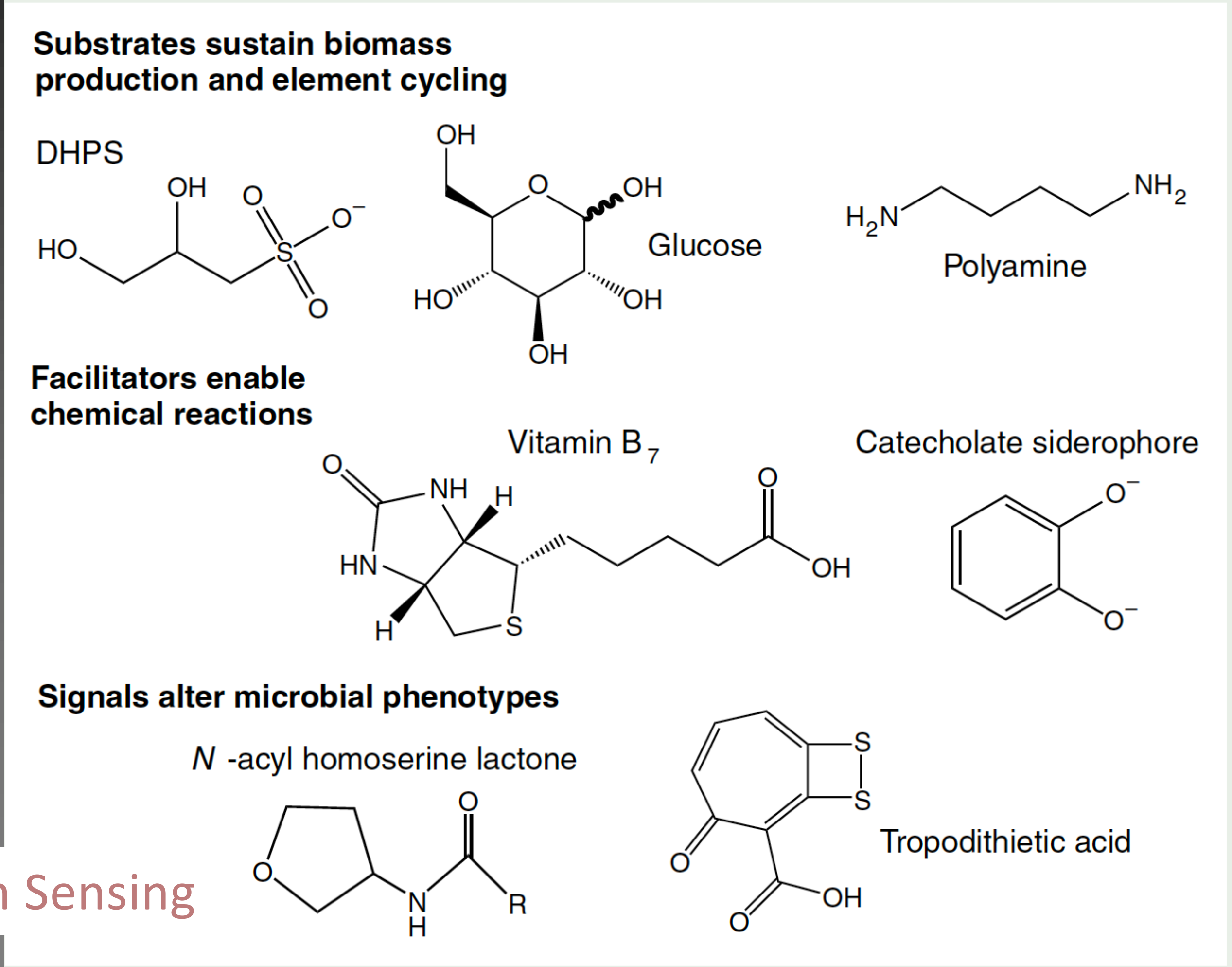


Frischkorn et al. (2017) *ISME J*

Orthologous (OG) group analysis suggests that epibionts confer the vast majority (>90% of OGs) of metabolic *functions* to the holobiont.

Caveat… "Orthology not equal to function." Rob Waterhouse

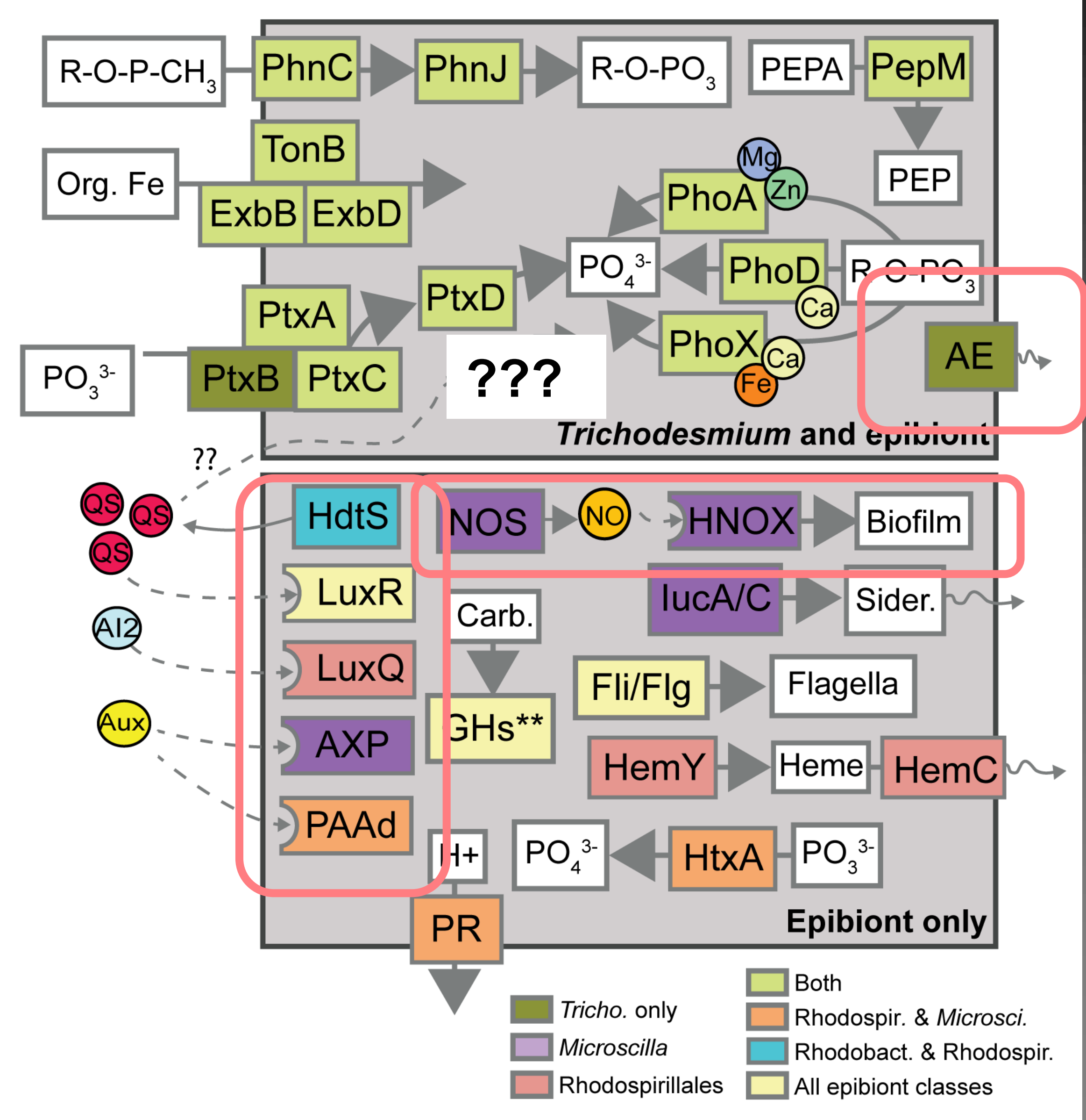
# Chemical-microbe network within the *Trichodesmium* holobiont



Quorum Sensing

Moran et al. (2022) *Nature Micro.*

# Microbial cross talk within the *Trichodesmium* holobiont



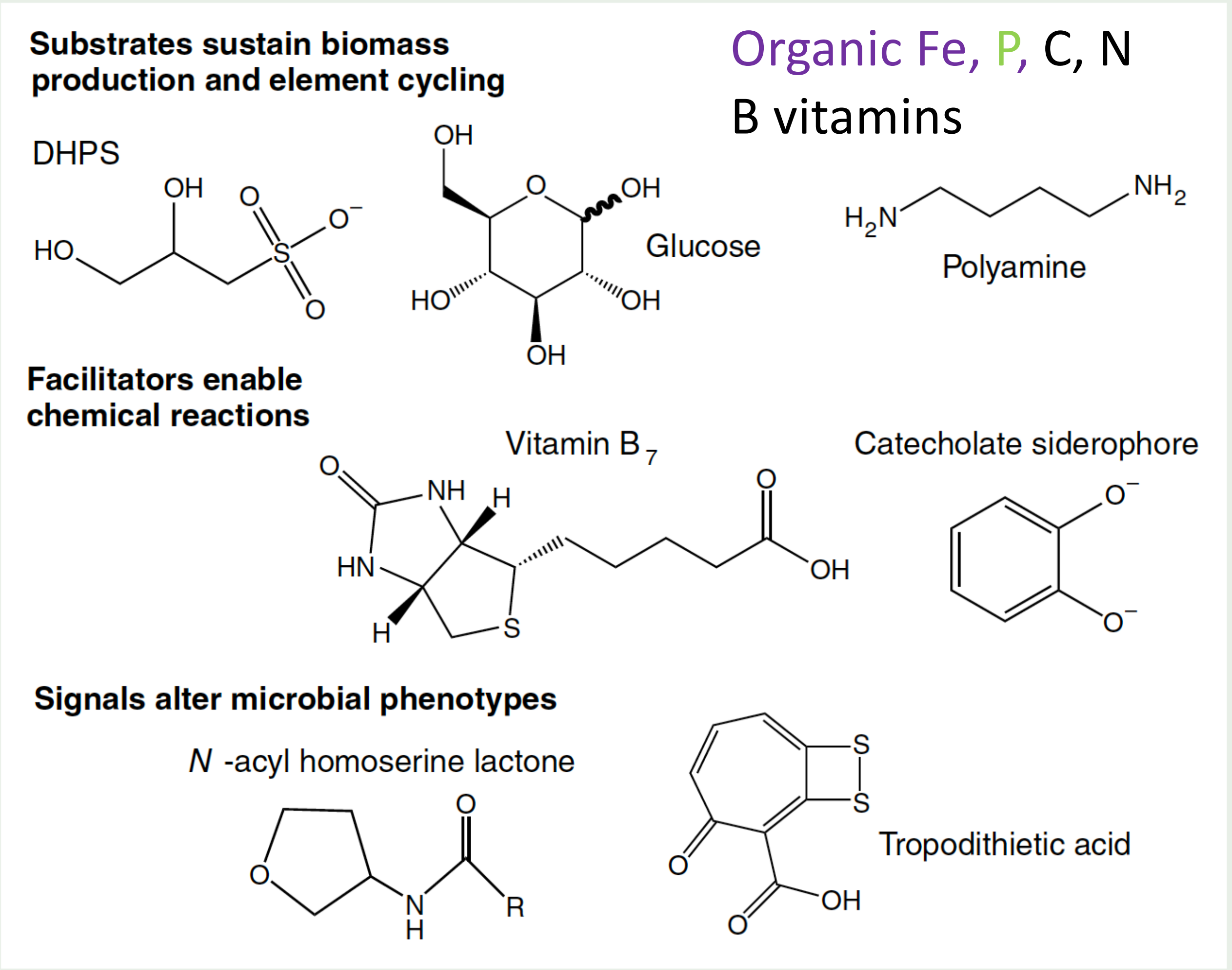
Auxin sensing and response

NO production

AHL QS Pathways are isolated to microbiome

QS and cell signaling

# Chemical-microbe network within the *Trichodesmium* holobiont

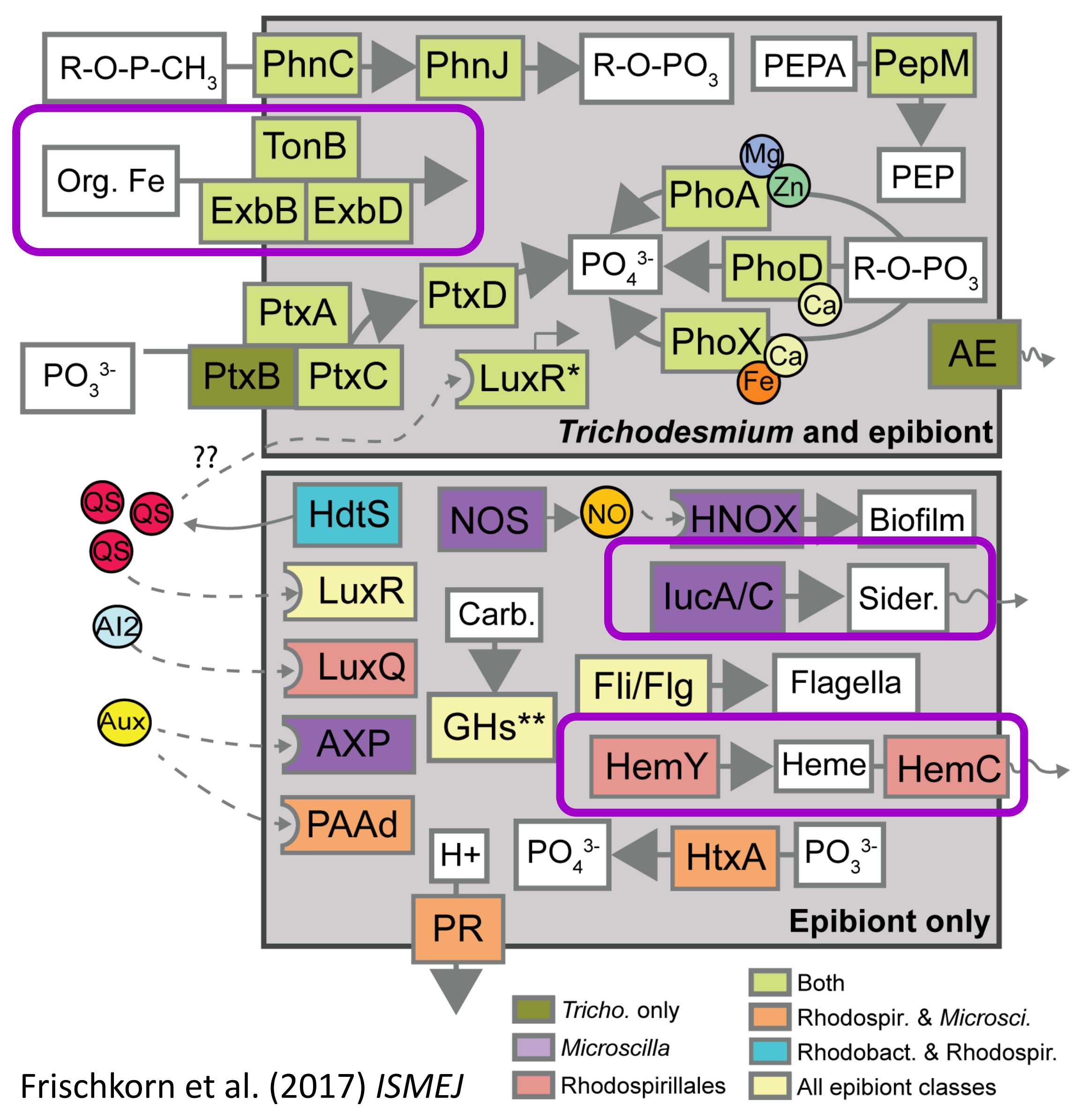


Organic Fe, P, C, N

B vitamins

Moran et al. (2022) *Nature Micro.*

# Metabolic partitioning within the *Trichodesmium* holobiont

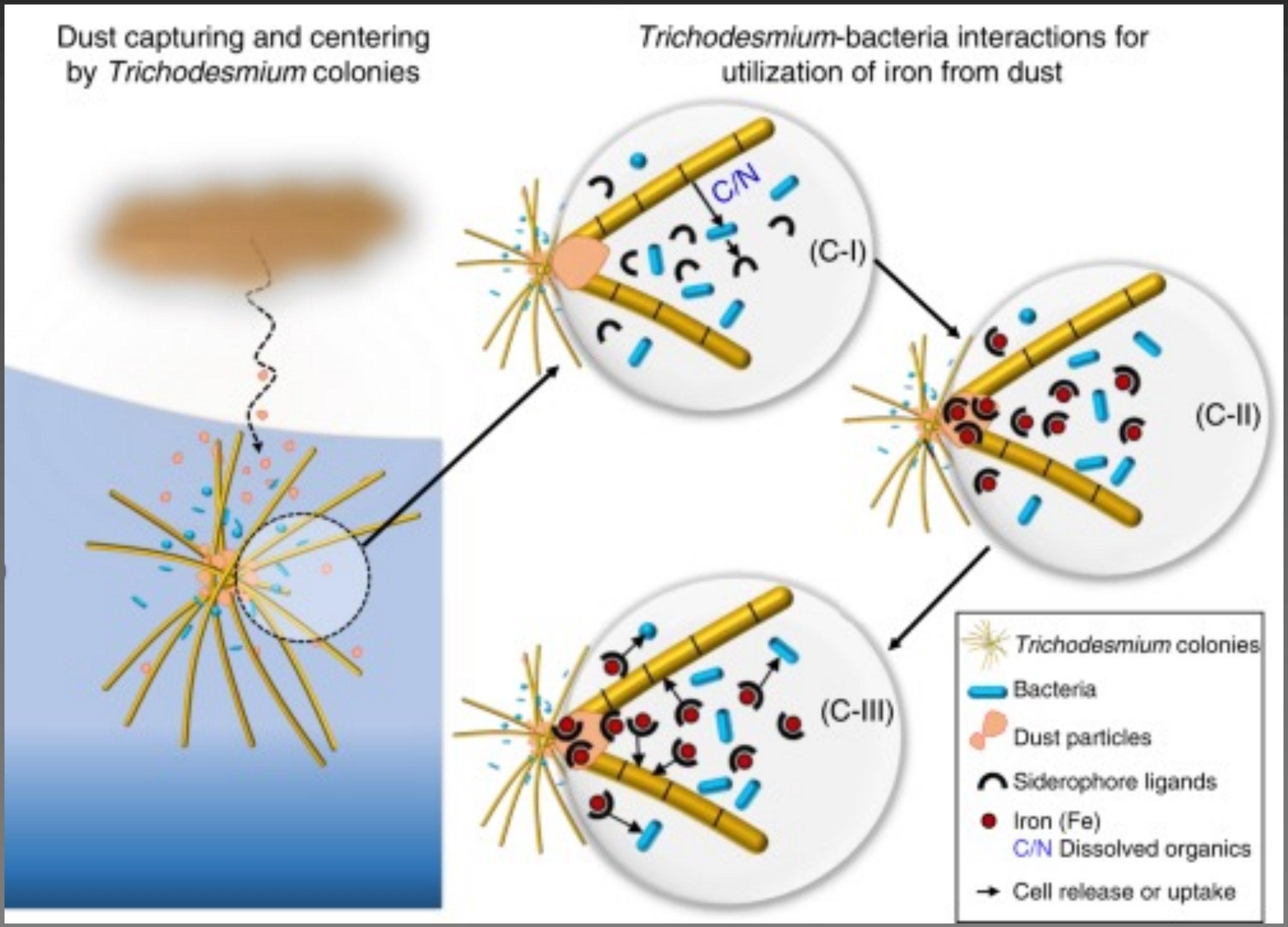


Frischkorn et al. (2017) *ISMEJ*

## Organic Iron

Epibionts can produce organic iron complexes that likely modulate iron in the holobiont microenvironment

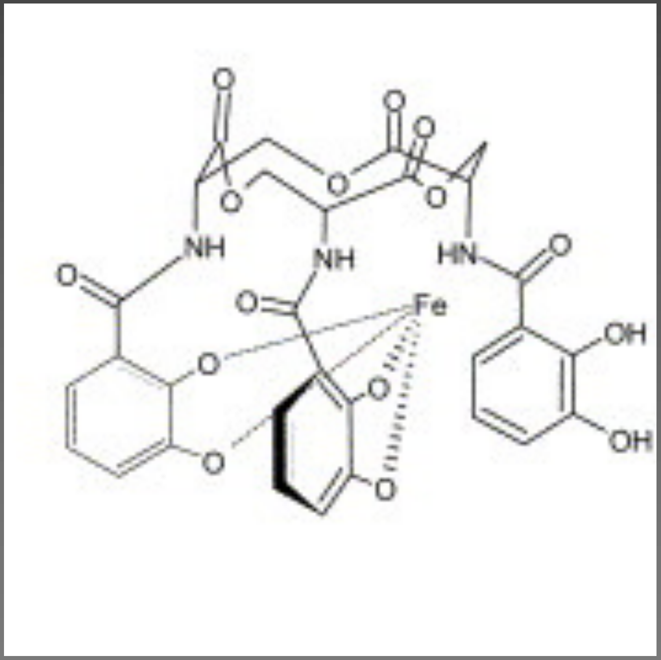
# Siderophore mining of iron from dust



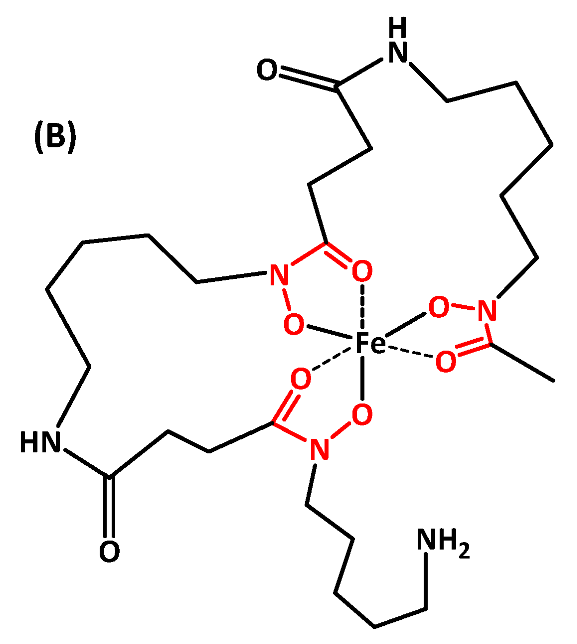
Basu et al. (2019) *Communication Biol.*

# Siderophores are C and N rich compounds

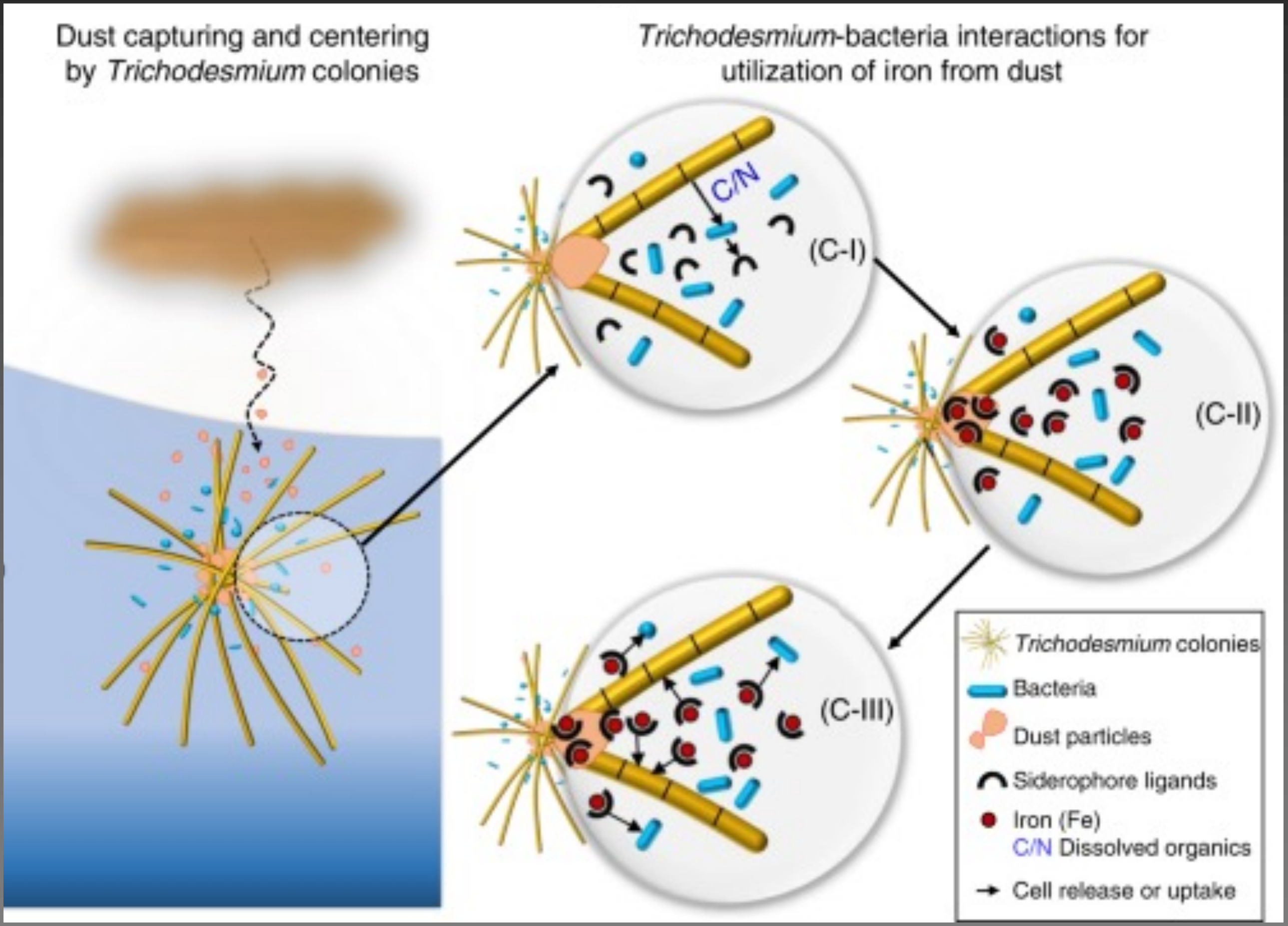
Aerobactin



Desferrioxamine B

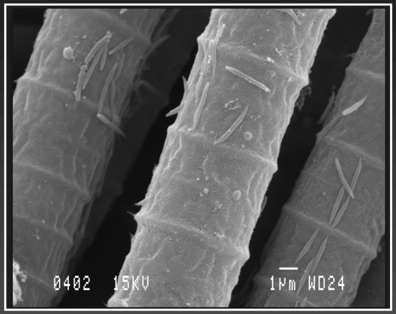


# Siderophore mining of iron from dust



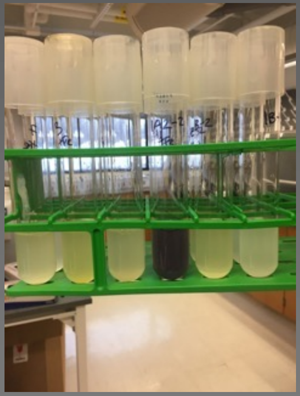
Basu et al. (2019) *Communication Biol.*

# Cultured epibionts produce siderophores in low iron media



Isolate epibionts

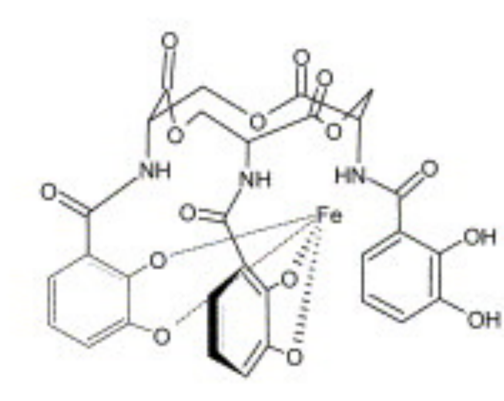
↓



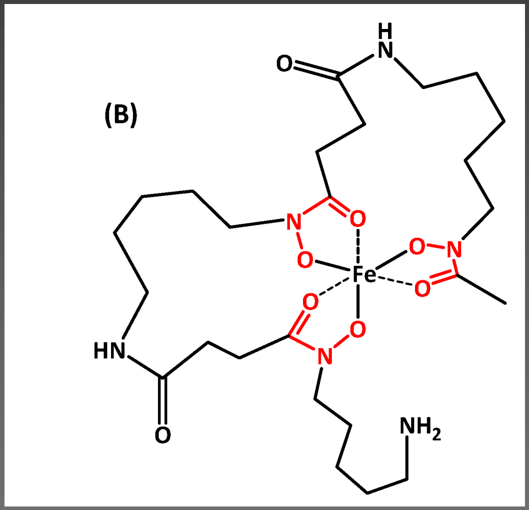
↓

LC-MS

Aerobactin



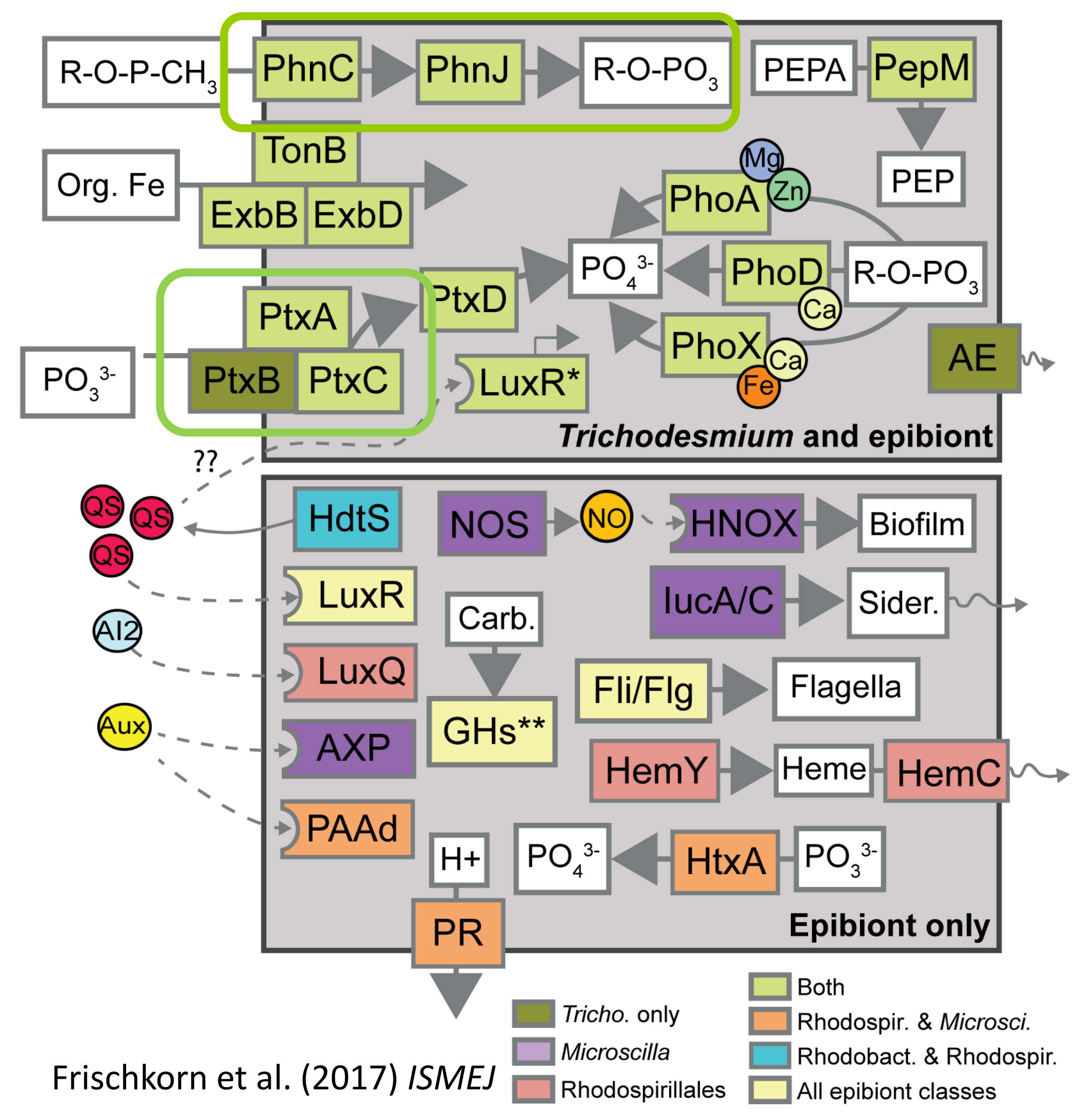
Desferrioxamine B



All six epibionts produce siderophores including those related to aerobactin and desferrioxamine consistent with MAG predictions and iron experiments.

O. Baars et al. unpublished

# Uptake and metabolism of organic and reduced phosphorus forms



Frischkorn et al. (2017) *ISMEJ*

C-P Lyase

PhoX

PhoA

**C-P**

**C-O-P**

Phosphite uptake

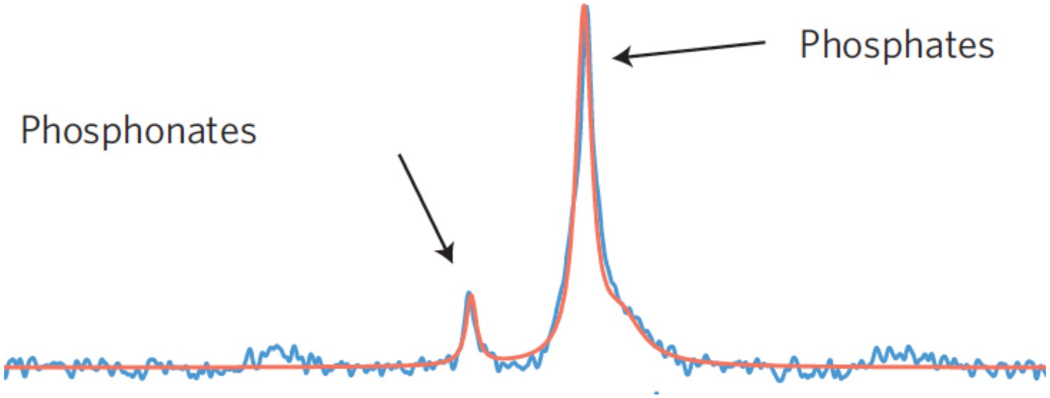
**PO$_3$**

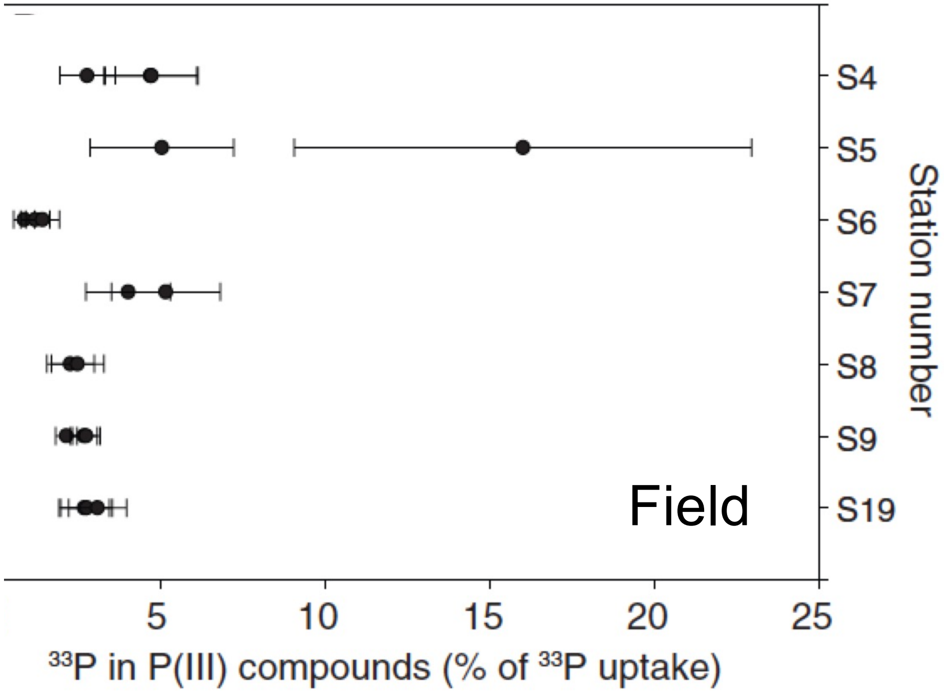
Phosphorus metabolisms are present in both *Trichodesmium* and the microbiome.

# Answers to enduring mysteries… who makes C-P compounds?

## Phosphonate (C-P) biosynthesis

Enrichment Culture



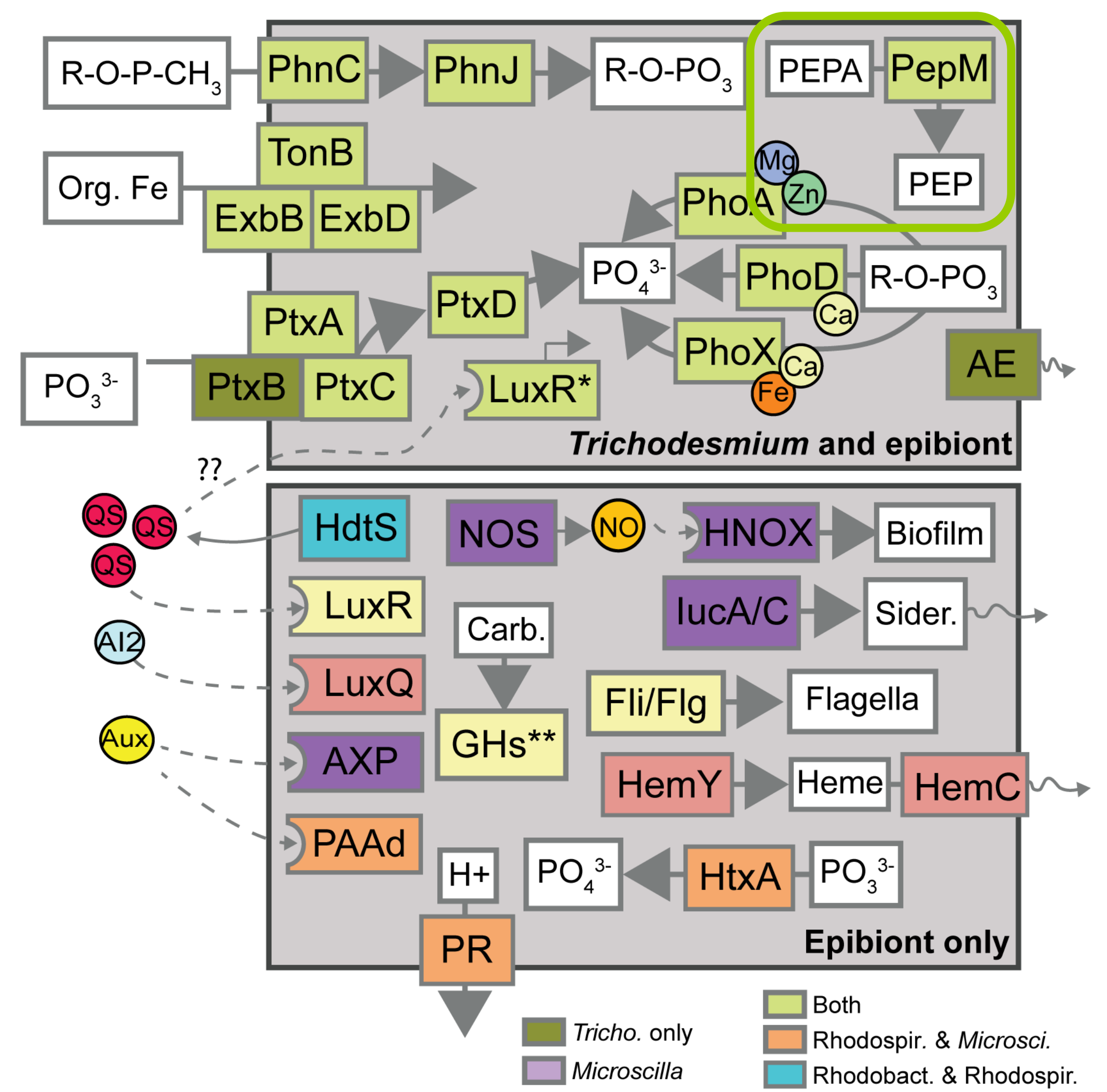


Dyhrman et al. (2009) *Nature Geo.*

Van Mooy et al. (2015) *Science*

Phosphonates are produced at high rates in the holobiont - hot spot for reduced phosphorus cycling. Is it *Trichodesmium* or the epibionts?

# Phosphonate production is a shared metabolism



Frischkorn et al. (2017) *ISMEJ*

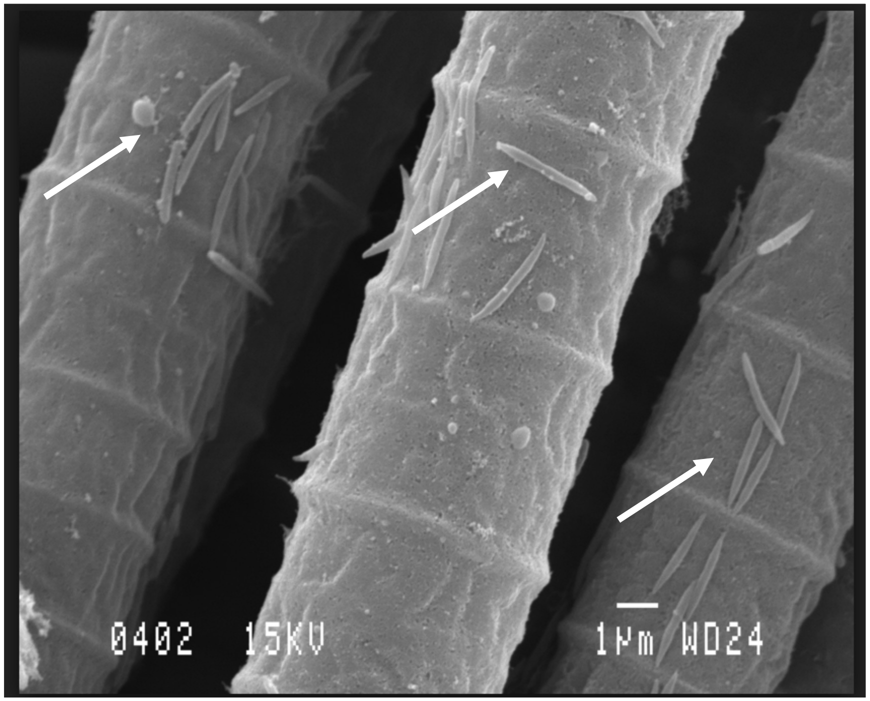
## Phosphonate biosynthesis

**C-P**

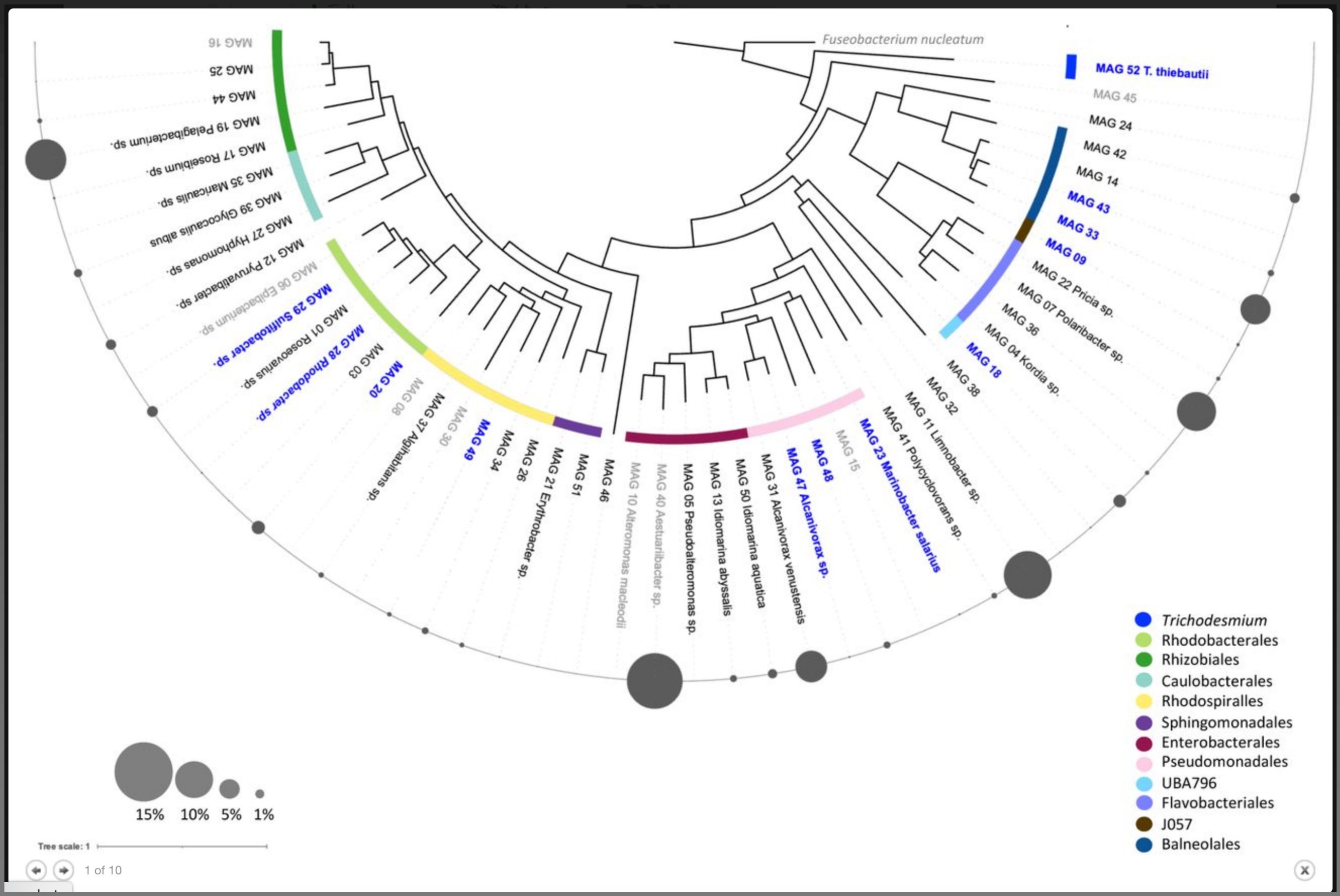
Phosphonate can be produced by both *Trichodesmium* and the epibionts – at least in this environment

# Lessons learned…

- **There is an amazing microbiome on *Trichodesmium*! But we need to sequence deeper.**
- *Trichodesmium* epibionts are functionally diverse, and these functions suggest complex interactions within holobiont (substrates, signals)



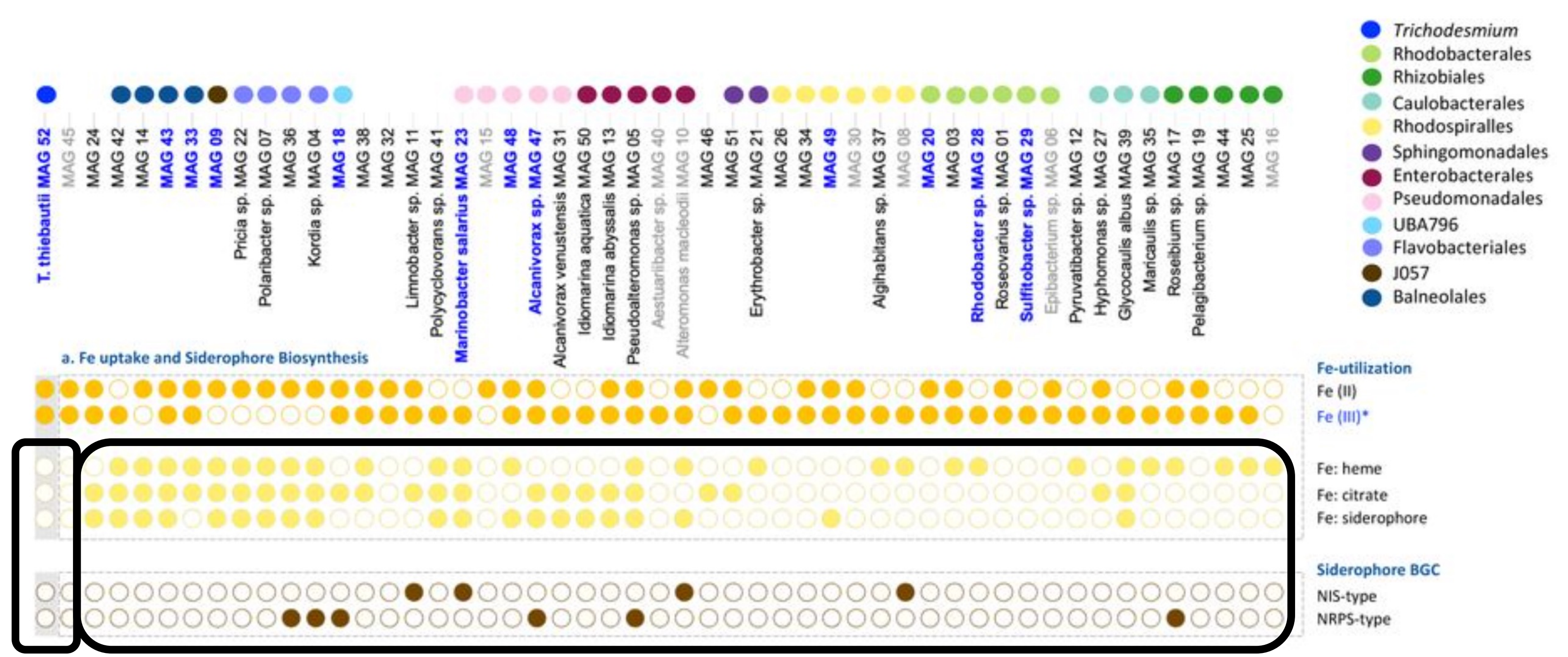
# Epibiont distribution in Red Sea samples



Koedooder et al.

https://www.biorxiv.org/content/10.1101/2023.03.15.532517v4.full

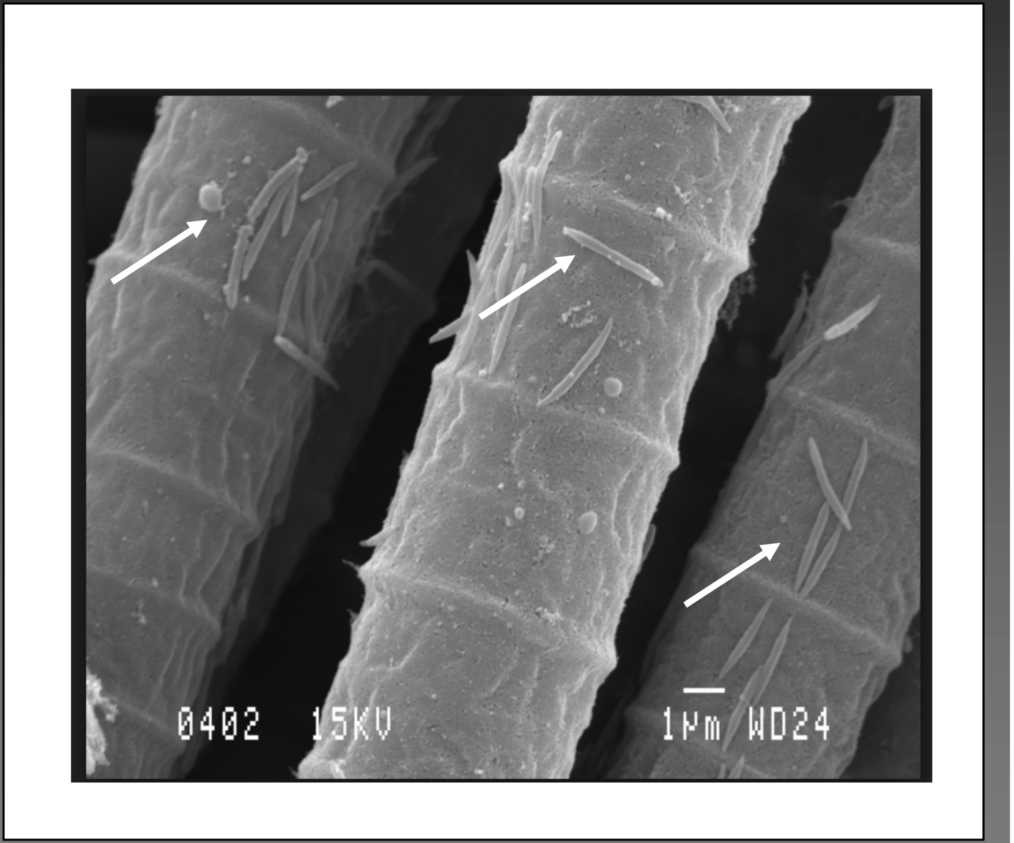
# Red Sea epibionts carry enriched Fe traits relative to *Trichodesmium*



https://www.biorxiv.org/content/10.1101/2023.03.15.532517v4.full

# Lessons learned…

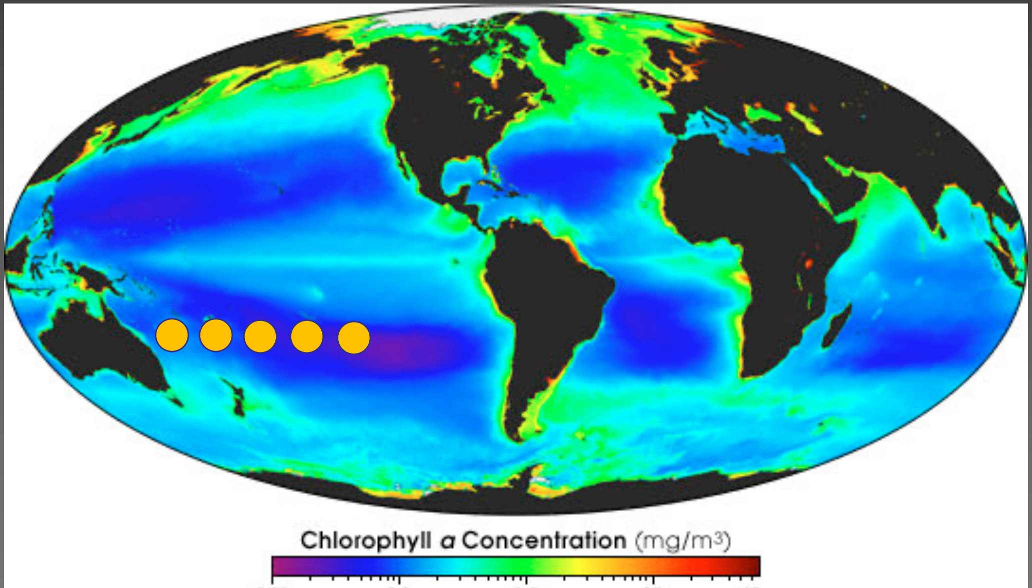
- We can do it!  But we need to sequence deeper.
- ***Trichodesmium* epibionts are functionally diverse, and these functions suggest complex interactions within holobiont (substrates, signals)**
- **Now we are looking at how those metagenomic functions are expressed.**



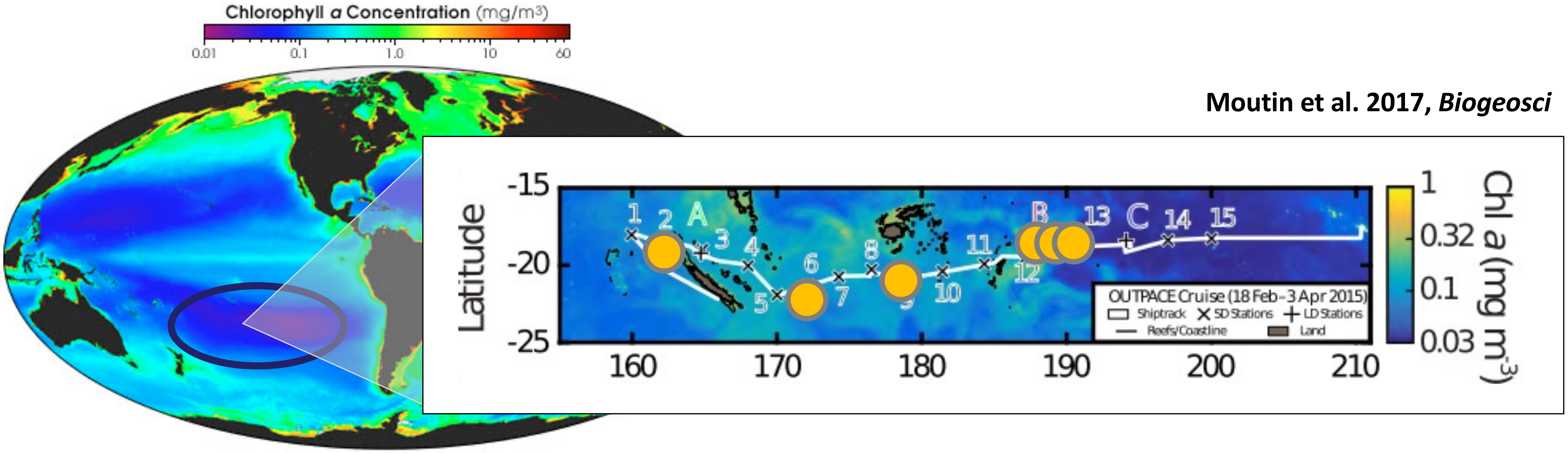
# Coordinated analysis of phosphorus cycling

- South Pacific is undersampled and the dynamics of the *Trichodesmium* holobiont are not well understood
- Unique opportunity to sample metagenome, metatranscriptome, and key activities.
- Is there evidence of holobiont phosphorus reduction and cycling in this environment?

**OUTPACE**



# Physiological ecology of *Trichodesmium* and its microbiome in the western tropical South Pacific

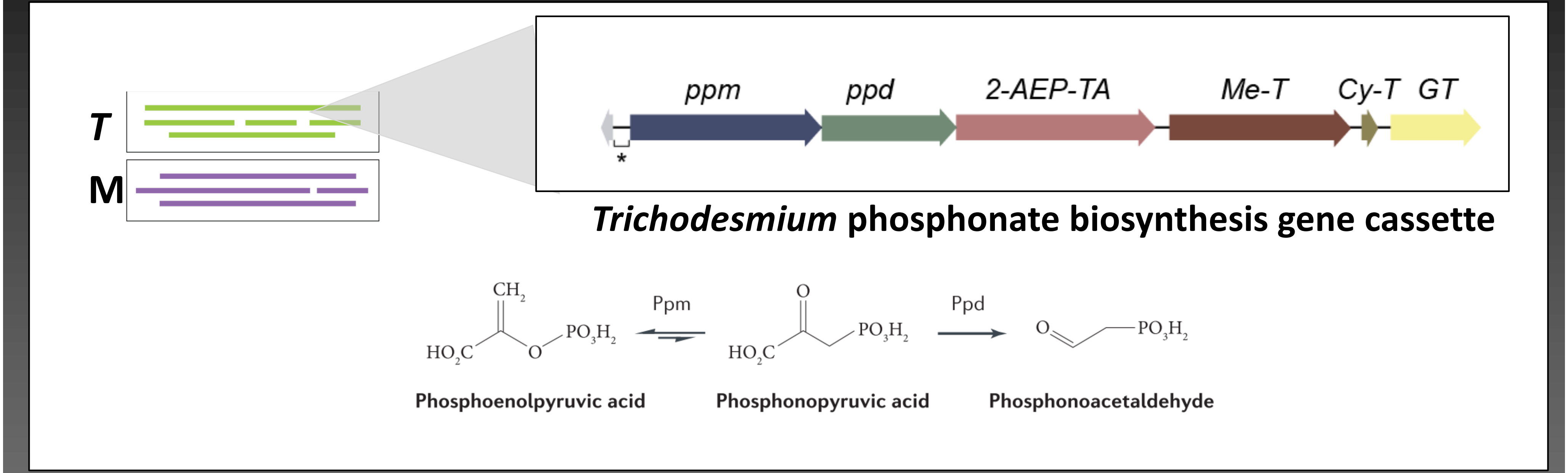


Moutin et al. 2017, *Biogeosci*

● ***Trichodesmium***
- ***Metagenome***
- ***Metatranscriptome***
- ***P reduction***

**Metatranscriptomes**

# Metagenomic evidence of P reduction



***Trichodesmium* phosphonate biosynthesis gene cassette**

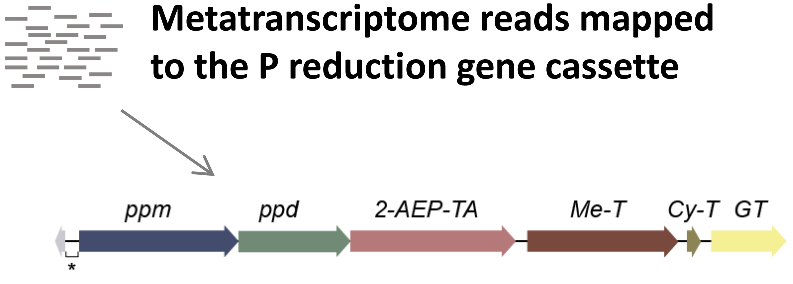
Frischkorn et al. (2018) *Biogeoscience*

Complete gene set for phosphonate biosynthesis assembled in *Trichodesmium* genome bin (MAG).

Not present in microbiome MAGs

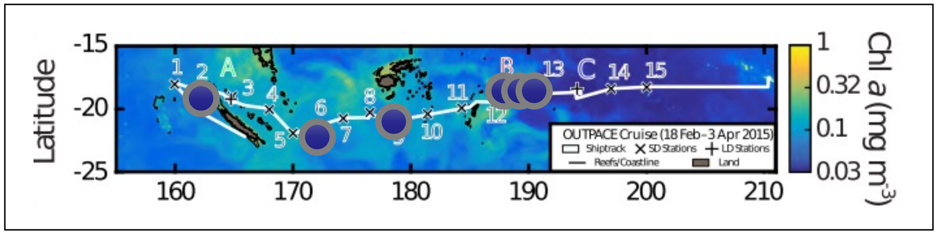
# Genes are expressed with P reduction

## Measure gene expression



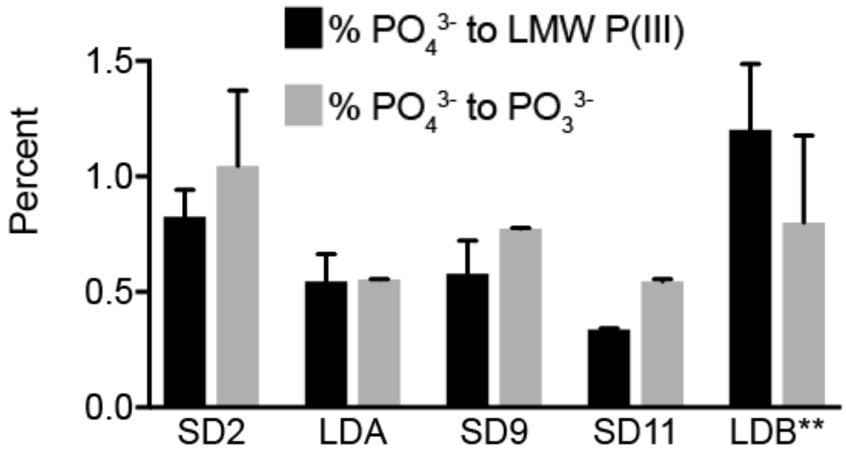
Metatranscriptome reads mapped to the P reduction gene cassette

Frischkorn et al. (2018) *Biogeoscience*



## Measure phosphate reduction

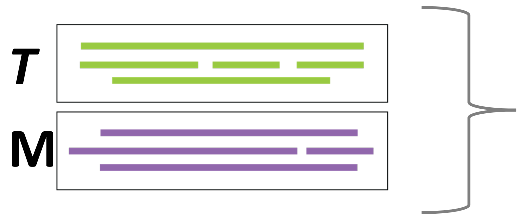
Percentage of radiolabeled phosphate taken up and reduced by *Trichodesmium* colonies



*methylphosphonate, phosphonoacetylaldehyde, or 2-aminoethylphosphonate

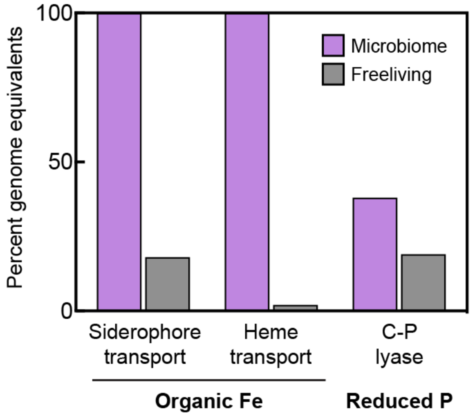
- ✓ Genes detected
- ✓ Genes expressed
- ✓ Activity measured
- ? P cycling

# Evidence for metabolism of reduced phosphorus compounds in *Trichodesmium* and the microbiome



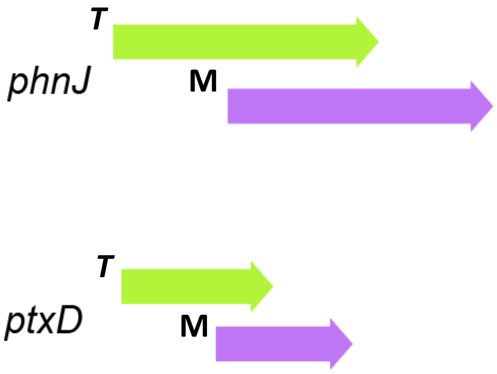
**Query for genes:**

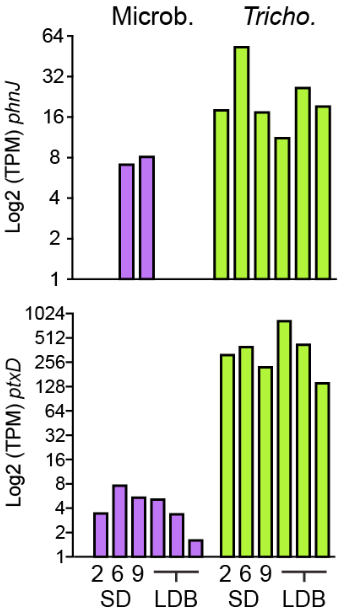
- **Phosphonate C-P lyase (*phnJ*)**
- **Phosphite dehydrogenase (*ptxD*)**



✓ **Genes detected**

✓ **Genes expressed**

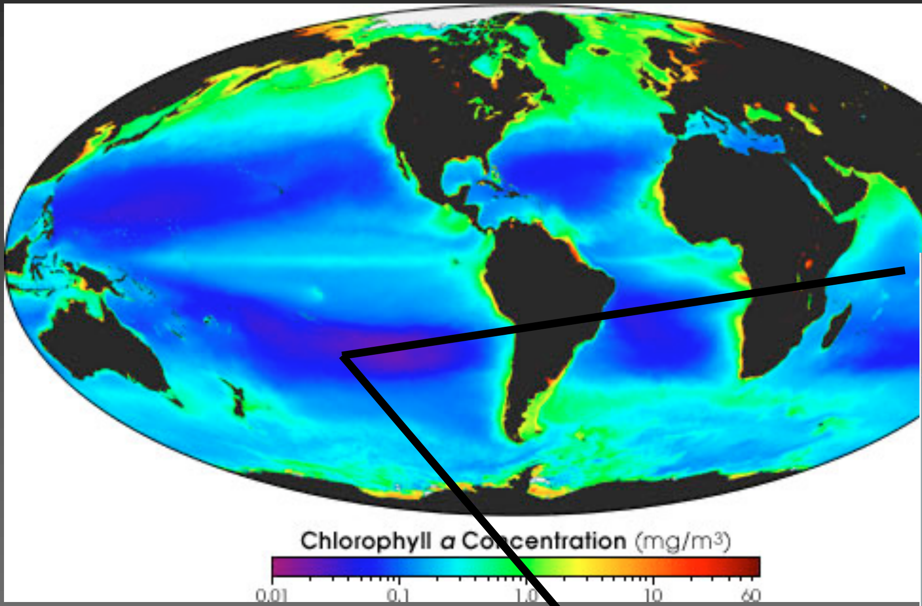


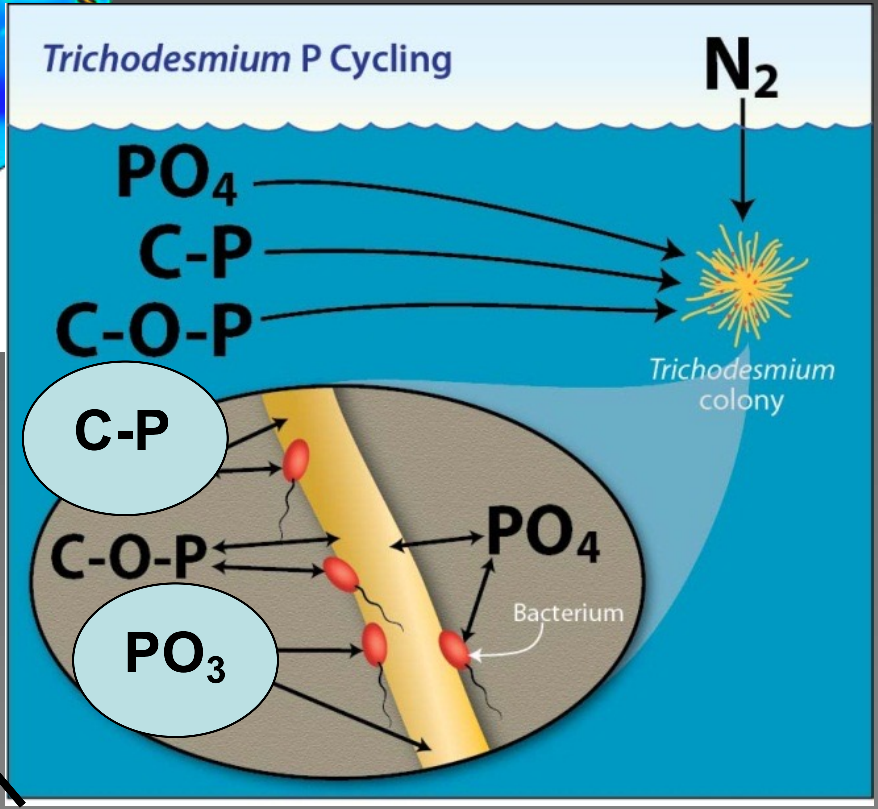


**Ability to access reduced P is enriched in *Trichodesmium* consortia**

Frischkorn et al. (2018) *Biogeoscience*

# Novel P currencies used in holobiont….





Mitigate competition
for scarce resource?

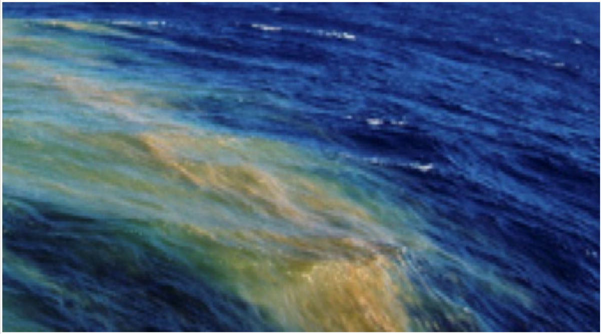
## Summary

- Need to think about *Trichodesmium* as a holobiont with a broader metabolic potential than just *Trichodesmium* alone.
- Microbiome is distinct and varies as a function of environment
- Holobiont is a potential hot-spot of reduced phosphorus cycling.
- *Consistency of epibiont diversity and functional capacity across environments?*
    - Metagenomes and metatranscriptomes from different environments

# Core questions about the *Trichodesmium* microbiome

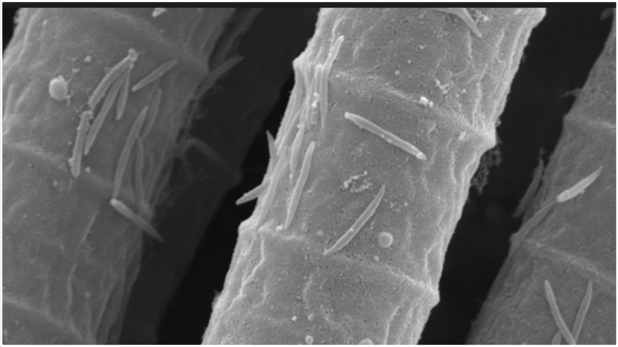
Who is there?

- **Diverse community, distinct from water column**



What are they doing?

- **Microbiome contributes functional diversity to community**



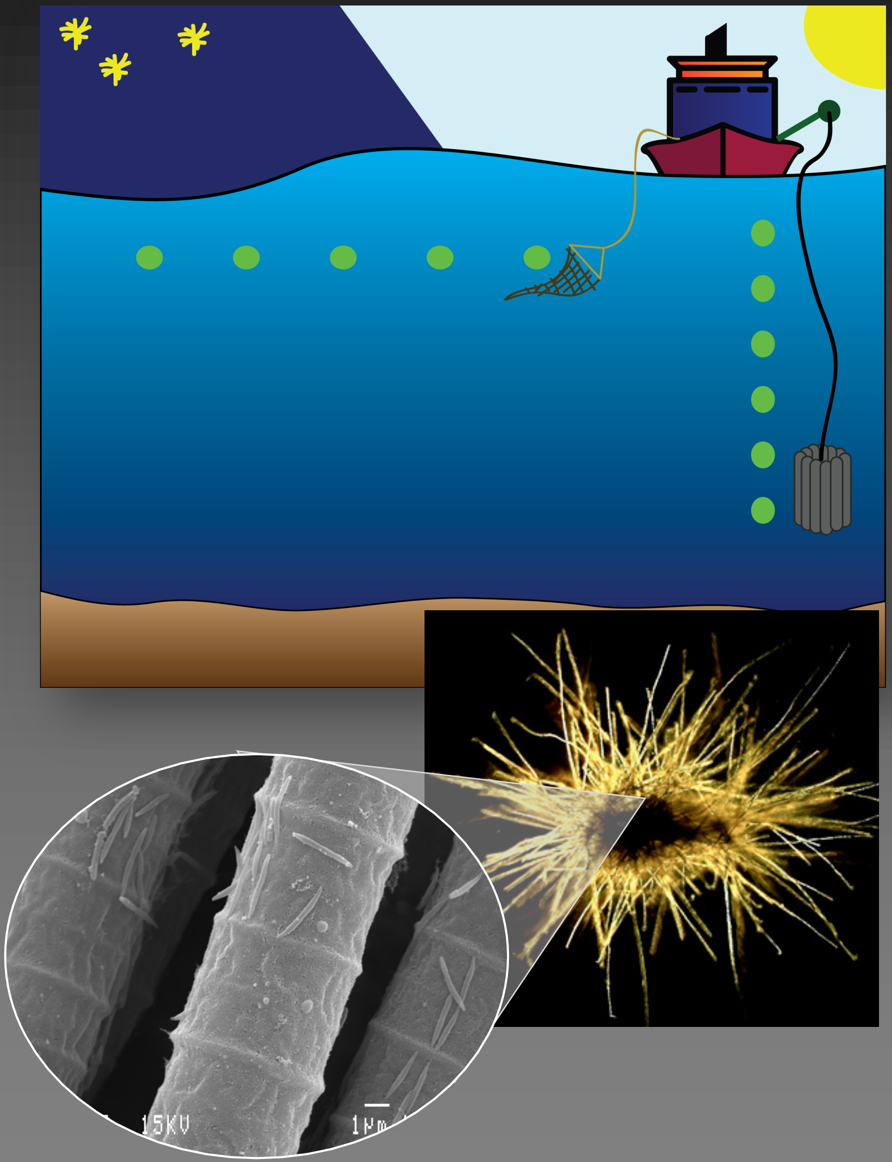
Are they interacting?

- Diel patterns in the consortia

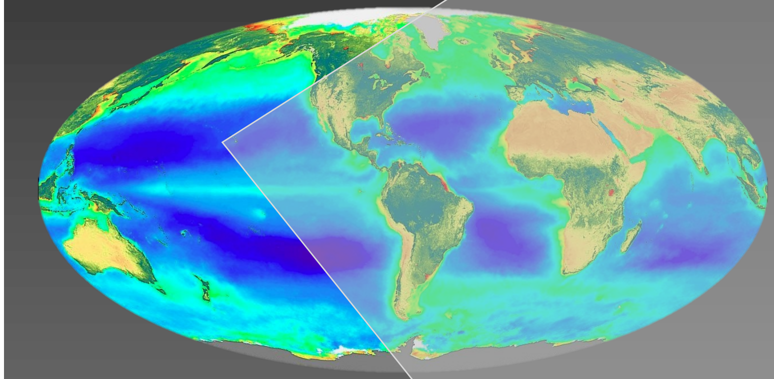
# Using light to study interactions

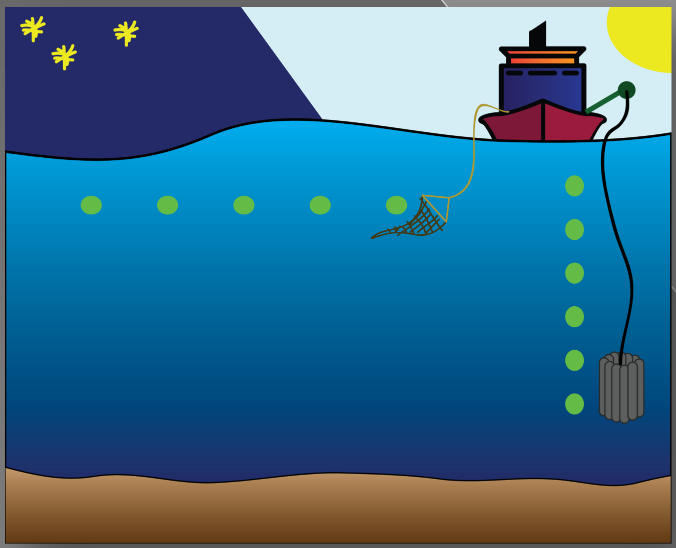
- Light drives diel changes in *Trichodesmium* $CO_2$ and $N_2$ fixation

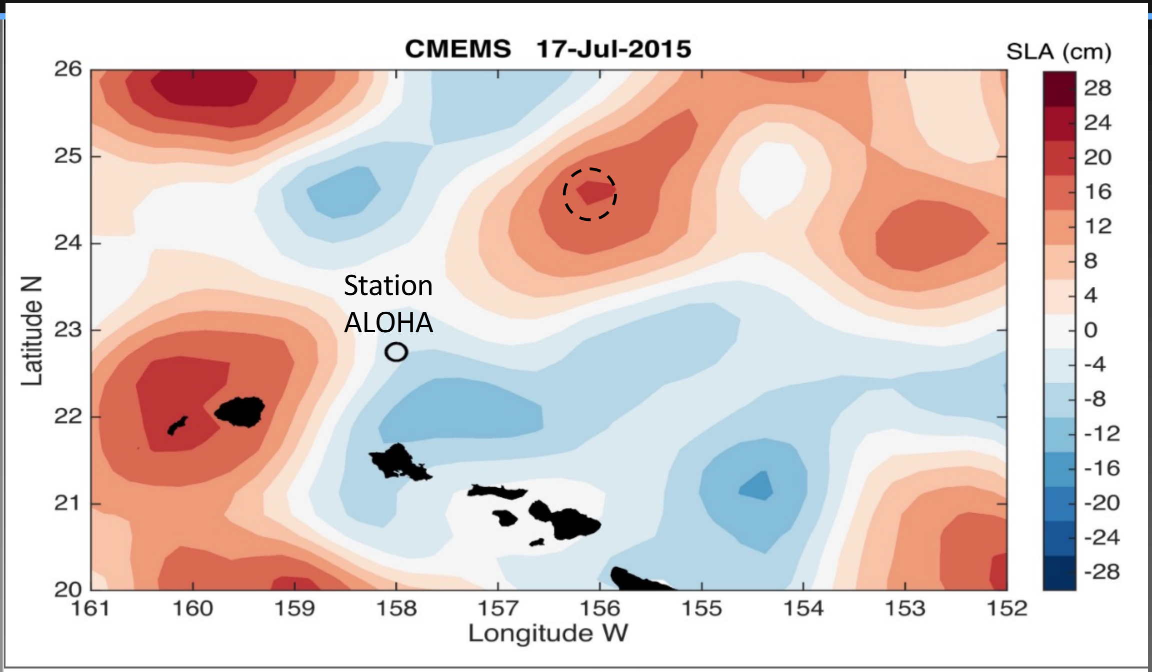
- Do epibionts have diel responses, and what might they tell us about interactions between the host and microbiome?

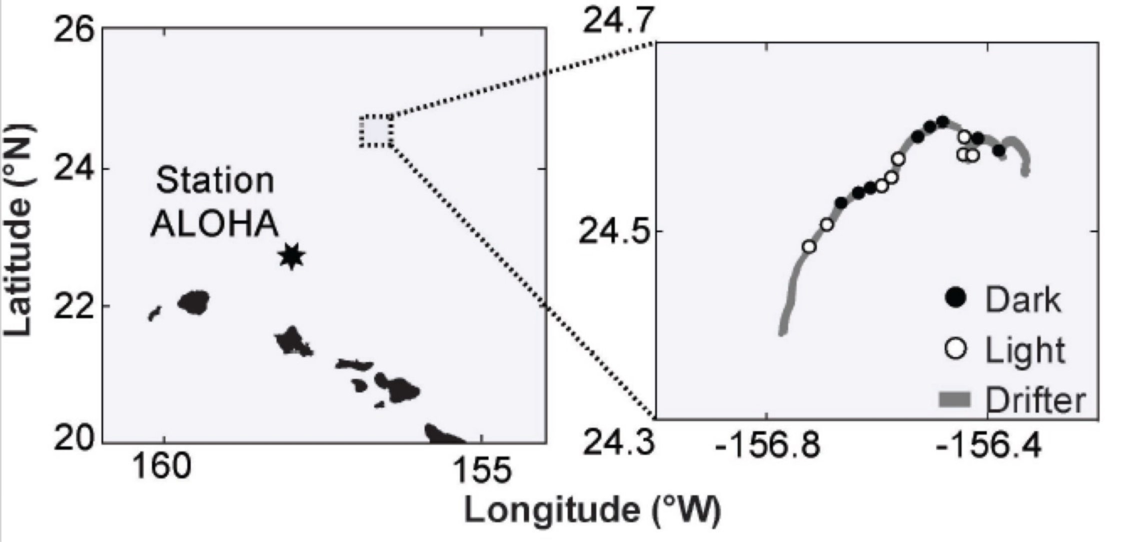


# Sampling light driven responses in the NPSG

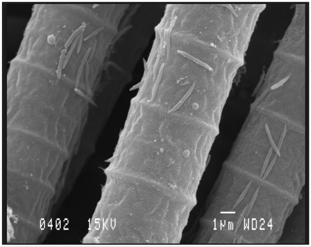








# Coordinated expression dynamics in host and microbiome



Metatranscriptomes

↓

Orthologous group analysis

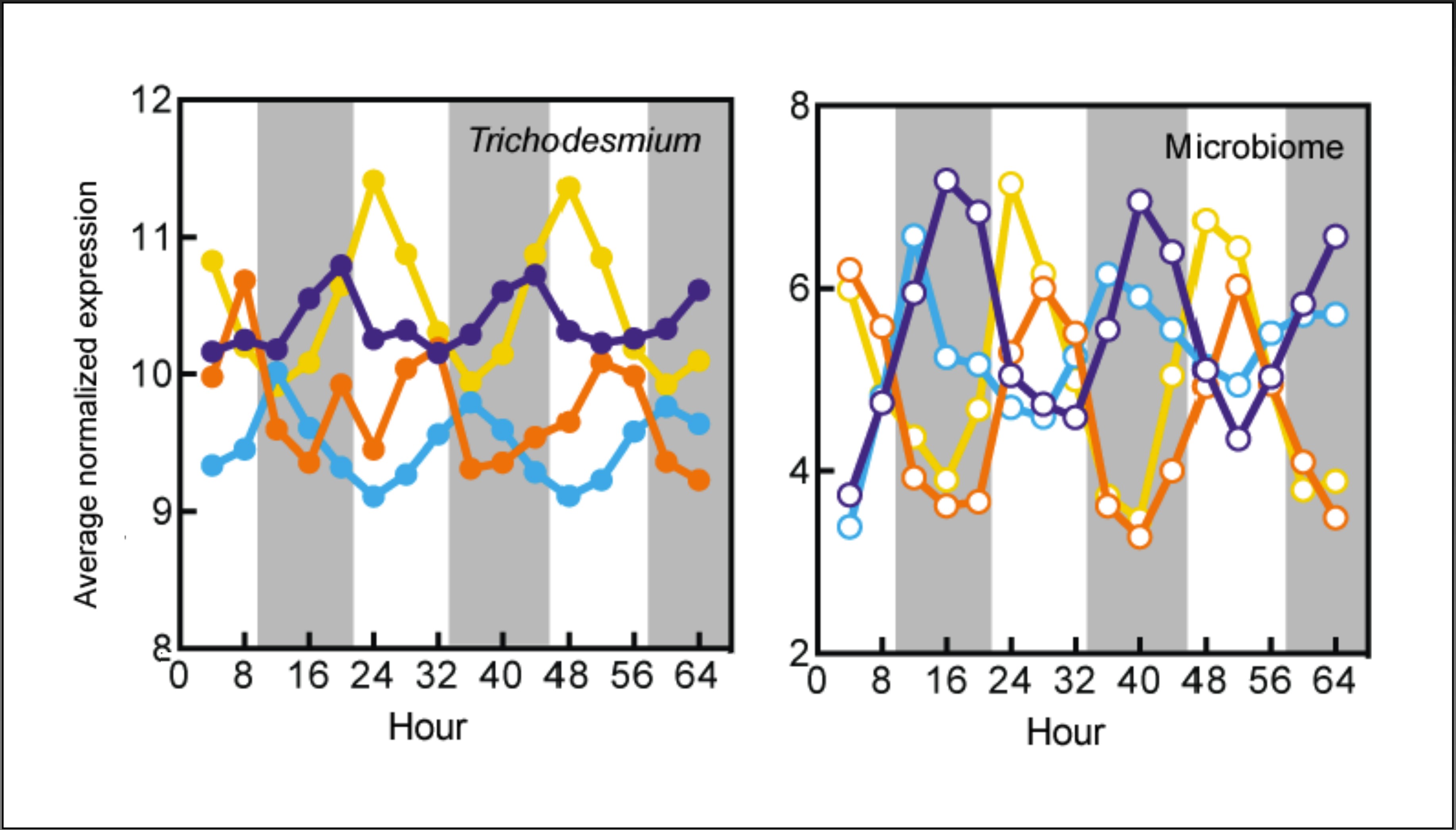
↓

Periodicity: RAIN
Co-expression: WGCNA

https://omictools.com/rhythmicity-analysis-incorporating-nonparametric-methods-tool

https://bmcbioinformatics.biomedcentral.com/articles/10.1186/1471-2105-9-559

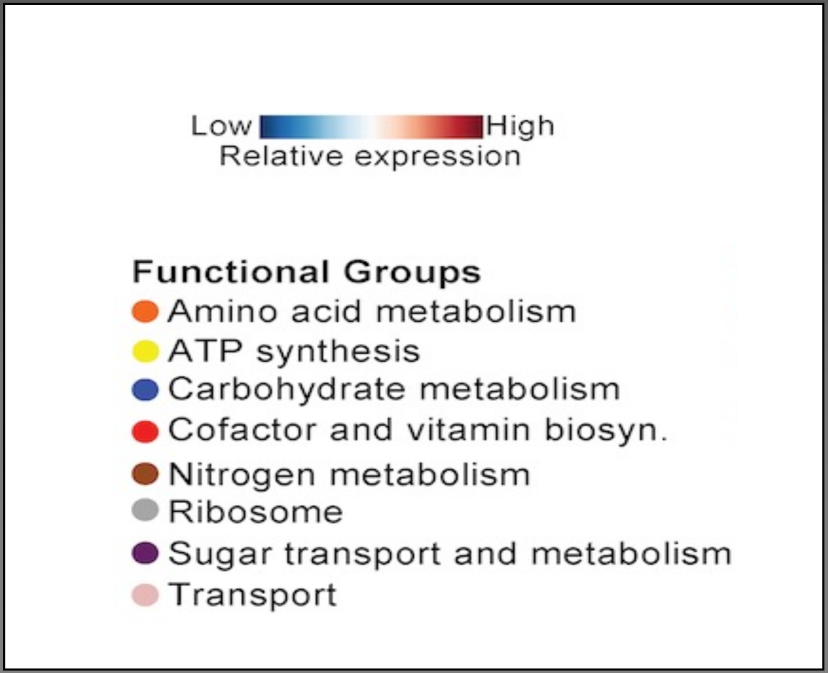
# Diel modulation of transcripts in *Trichodesmium*

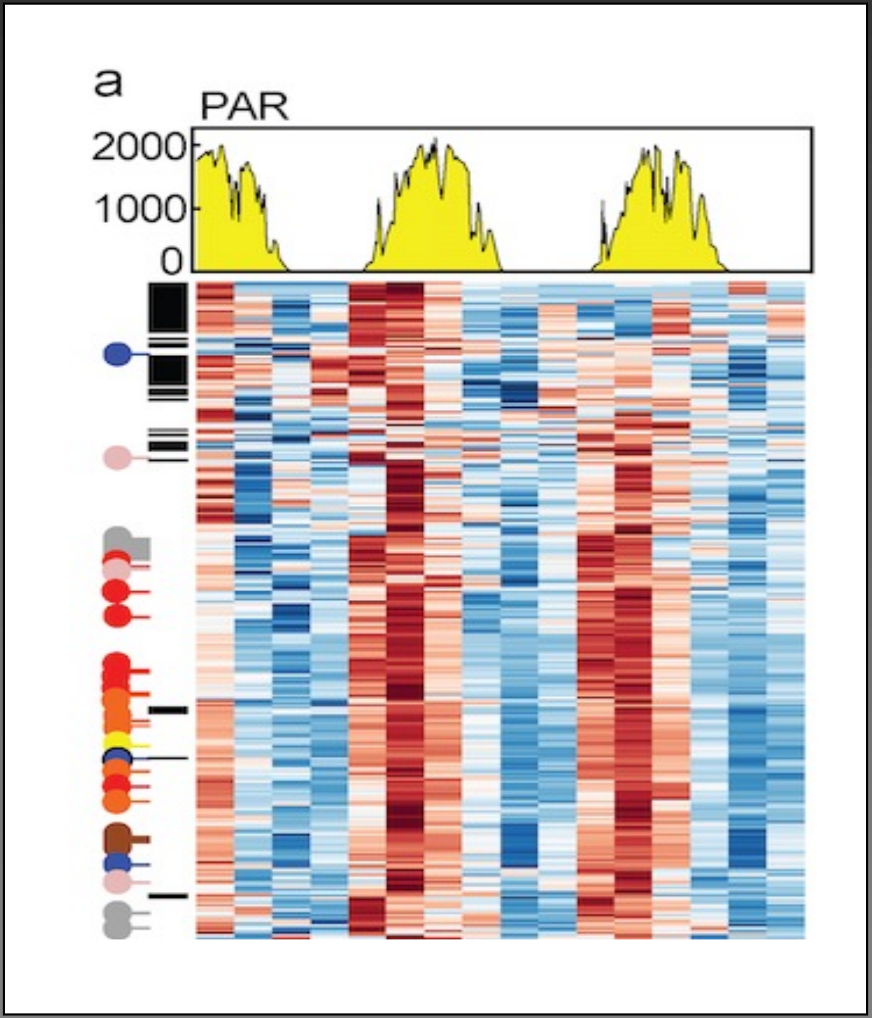


Frischkorn et al. (2018) *ISMEJ*

# WGCNA co-expression network

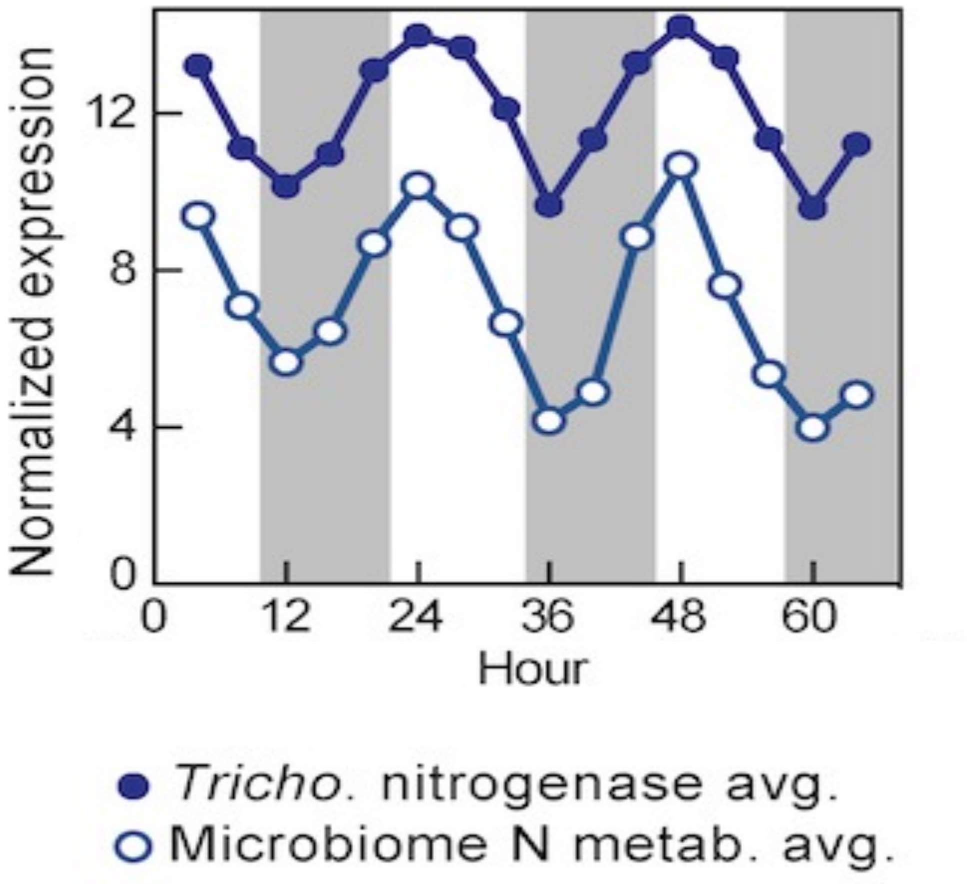
Module example: Significantly coordinated expression patterns between *Trichodesmium* and microbiome.

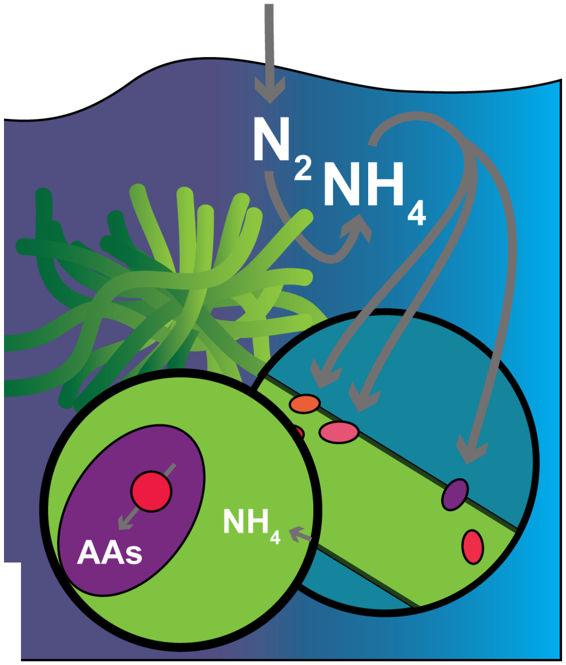




Frischkorn et al. (2018) *ISMEJ*

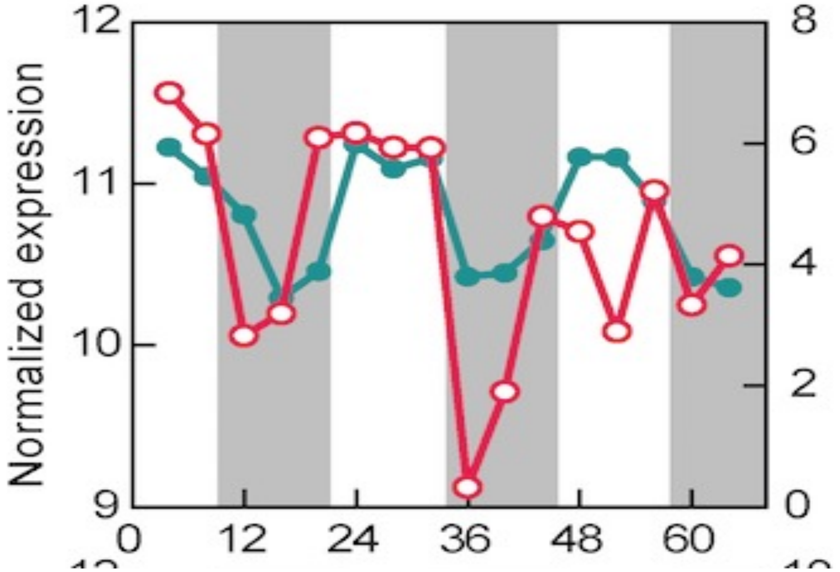
# Diel modulation of transcripts suggest coordination: Nitrogen





Frischkorn et al. (2018) *ISMEJ*

# Diel modulation of transcripts suggests coordination: Carbon



$CO_2 \rightarrow$ DOC $\rightarrow$ $CO_2$

*Trichodesmium* Microbiome

Frischkorn et al. (2018) *ISME J*

# Summary

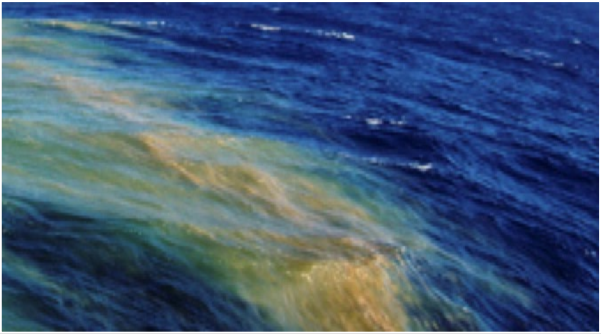
- The microbiome and *Trichodesmium* are interacting!
  - Nitrogen
  - Carbon
  - Vitamin B12, P, Fe etc.

- Heterotrophic epibionts have striking diel signals that likely underpin signaling, exchanges and other activities in the holobiont.

# Core questions about the *Trichodesmium* microbiome

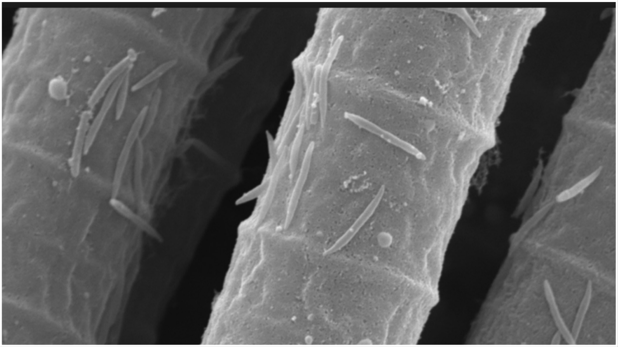
Who is there?

- **Diverse community, distinct from water column**



What are they doing?

- **Microbiome contributes functional diversity to community**



**Host →Microbiome**

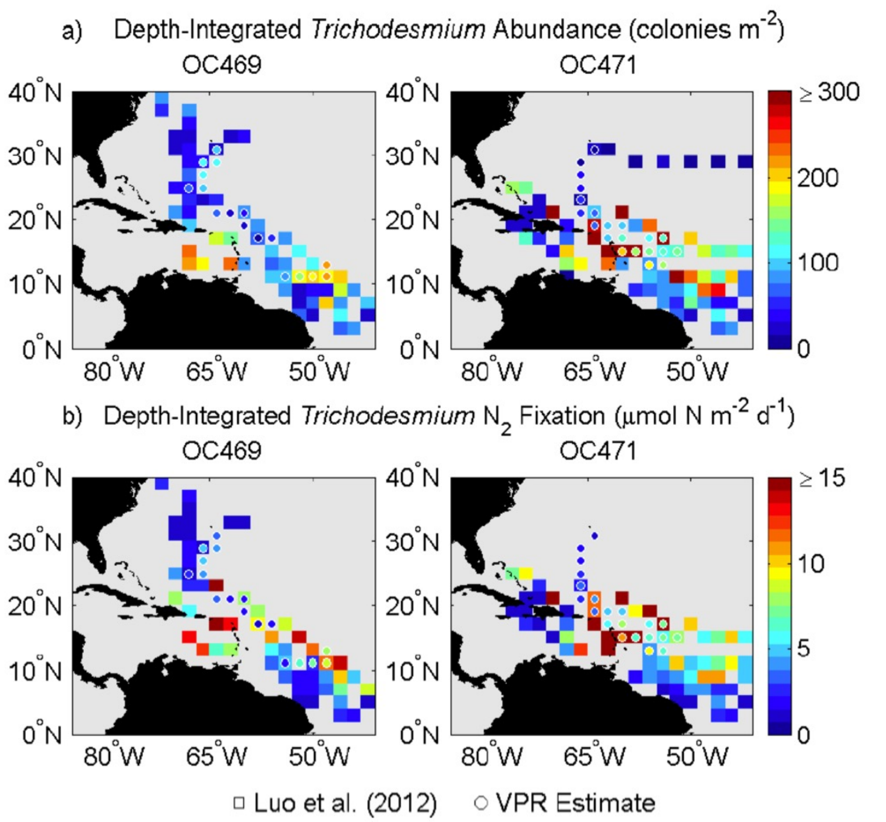
**Microbiome → Host**

Are they interacting?

- **Yes! Significant co-expression of host-microbiome genes**

# Can the microbiome influence host $N_2$ fixation?

Maybe the models are missing the role of the microbiome?



a) Depth-Integrated *Trichodesmium* Abundance (colonies $m^{-2}$)

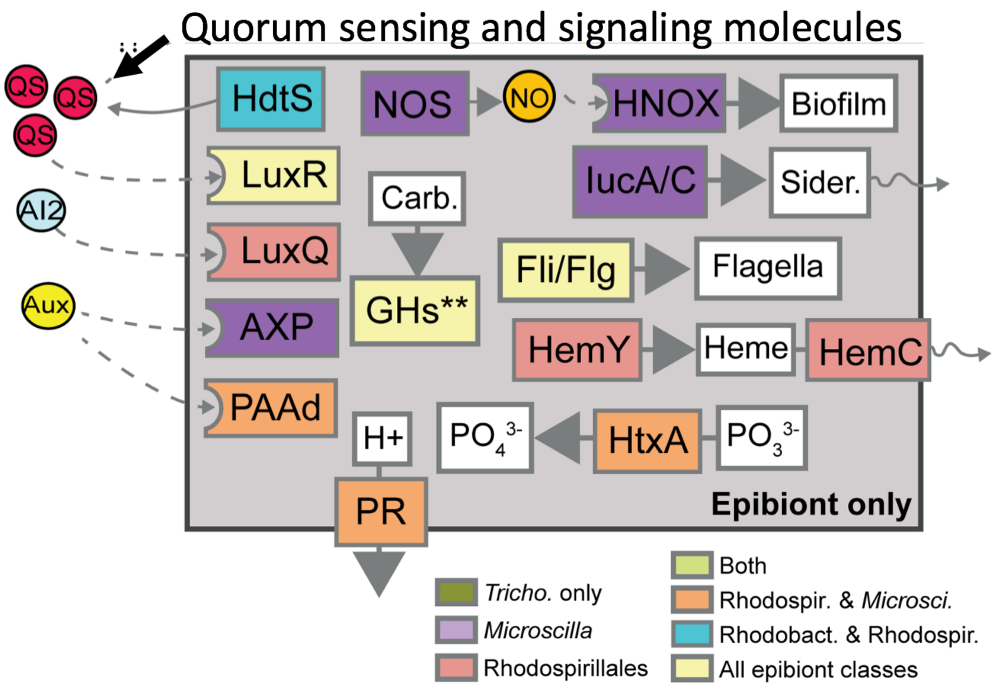
b) Depth-Integrated *Trichodesmium* $N_2$ Fixation (µmol N $m^{-2}$ $d^{-1}$)

Olson et al. 2015 *DSR* II

# Selective manipulation of the microbiome…

## The epibiont community is talking to itself…

- Quorum sensing communication molecules (acylated homoserine lactones - AHL) detected in colonies (Van Mooy et al. 2012 *ISME J*)
- Addition of AHLs to field colonies changes activity independent of geochemistry (Van Mooy et al. 2012 *ISME J*)
- Genes for AHL biosynthesis and AHL receptors detected in epibionts – not *Trichodesmium*

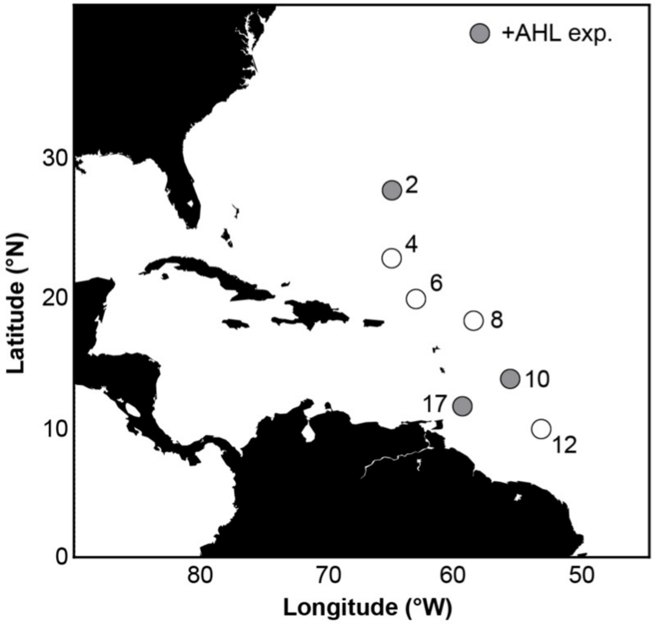


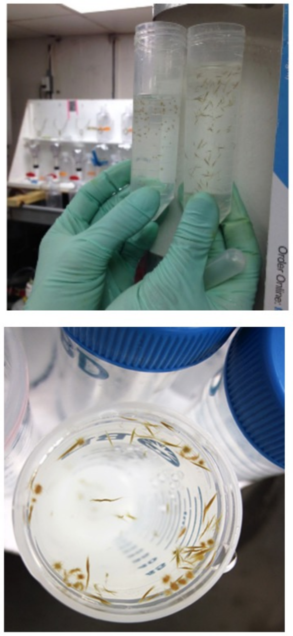
Frischkorn et al. (2017) *ISMEJ*

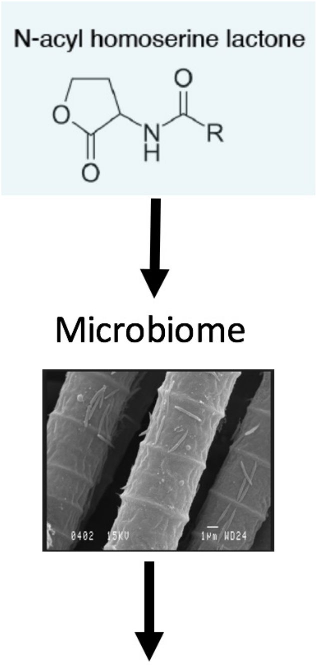
# Selective manipulation of the microbiome…

## *Do microbiome infochemicals influence Trichodesmium phenotype?*

Selective manipulation of the epibiont activities with AHLs





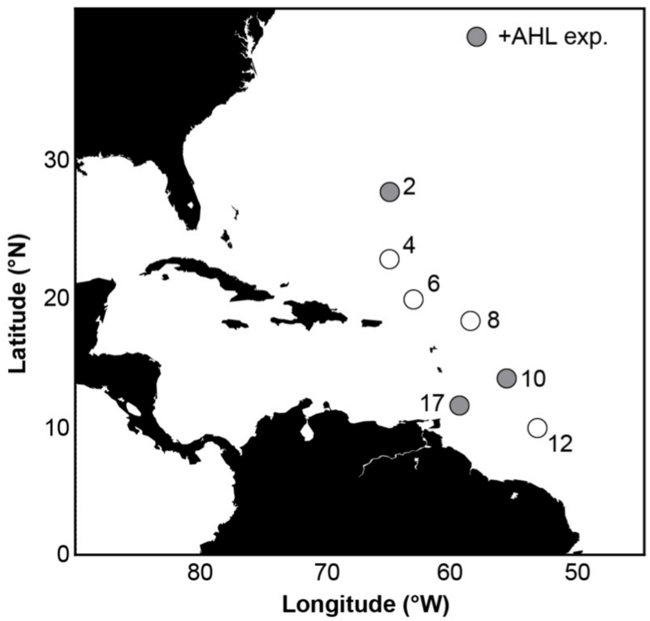


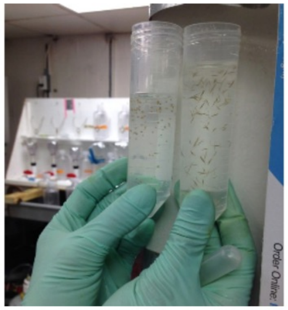
Microbiome

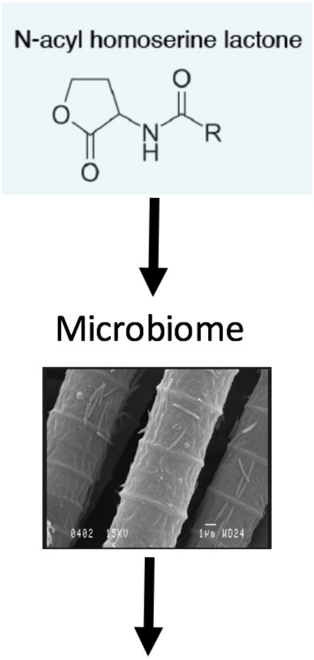
*Trichodesmium* $N_2$ Fixation

# AHL incubations modulate *Trichodesmium* $N_2$ fixation

## Epibiont infochemicals influence host phenotype



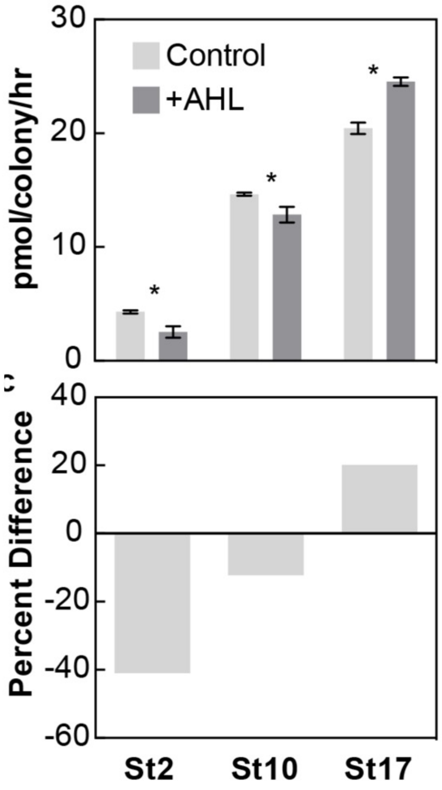




N-acyl homoserine lactone

Microbiome

*Trichodesmium* $N_2$ Fixation



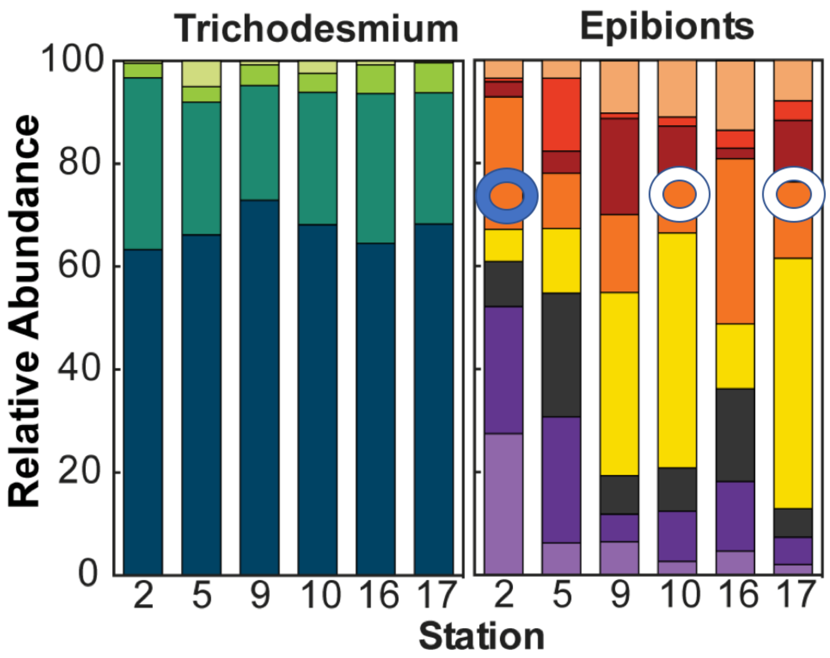
Frischkorn et al. (2018) *L&O Letters*

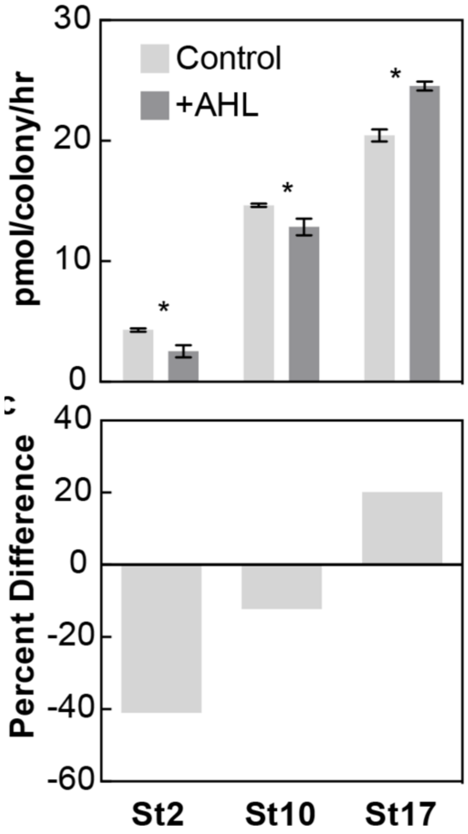
Microbiome can modulate host $N_2$ fixation rates up to 40%, in orthogonal directions, and independently of known drivers (P, Fe, light, temperature)

# Mechanisms still uncertain…

## *What is the mechanism driving the $N_2$ fixation response?*

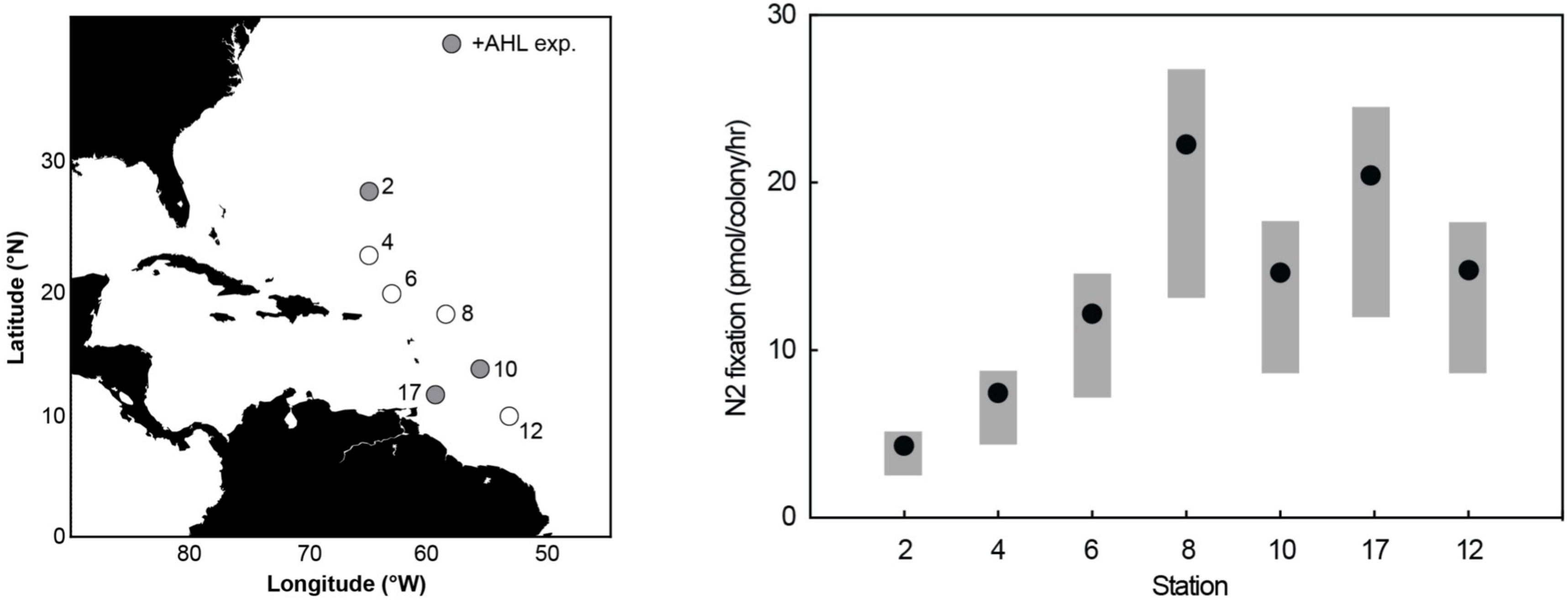
Changes in epibiont community structure, and *Trichodesmium* physiology could play a role.





# Microbiome activities modulate $N_2$ fixation in host

## Epibiont infochemicals influence ecosystem drivers



Frischkorn et al. (2018) *L&O Letters*

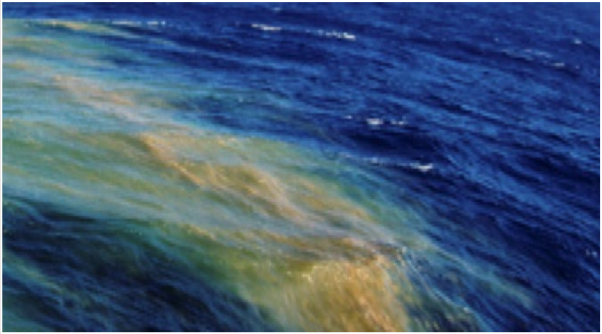
Biological interactions are a previously unrecognized driver of $N_2$ fixation

**Need to revise modeling efforts focused on drivers of $N_2$ fixation**

# Summary

- The microbiome and *Trichodesmium* are interacting!
- Epibionts can influence $N_2$ fixation, but the full mechanisms that underpin this still need to be identified.

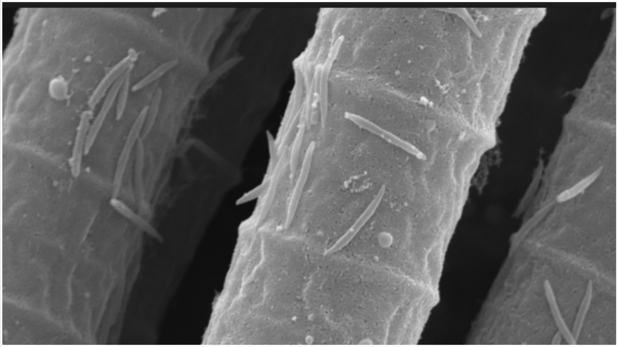
# Core questions about the *Trichodesmium* microbiome



Who is there?

- **Diverse community, distinct from water column**

What are they doing?



- **Microbiome contributes functional diversity to community**

Are they interacting?

- **Yes! Significant co-expression of host-microbiome genes**

**Microbiome can influence host $N_2$ fixation!**

# Summary

- **16S community amplicon sequencing**: Colonies harbor diverse epibionts distinct from water column, that are dynamically curated across gradients in the environment
- **Metagenomics**: Epibionts confer substantial metabolic potential which likely underpins *Trichodesmium* fitness
- **Metatranscriptomics**: Novel P cycling currencies may avoid competition
- Apparent interactions between *Trichodesmium* and its microbiome

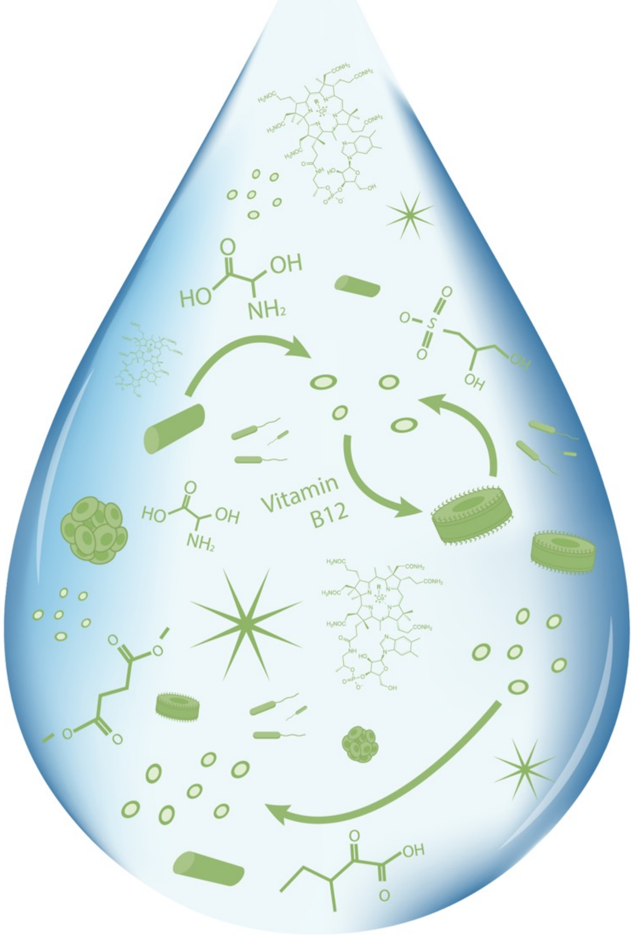
**Microbiome plays a critical role in *Trichodesmium* physiological ecology and biogeochemical cycling**

# What comes next?

## We still need to understand the rules…

50 Pg C/yr

- The network of interacting microbes, chemicals and their ecological and biogeochemical relationships
- How taxonomic and functional diversity underpin processes at different scales
- Sensitivity to disturbance and longer term changes – *resilience*
- *The time is now*



# Acknowledgements

- Dyhrman Lab and many extraordinary collaborators

- NSF
- DOE
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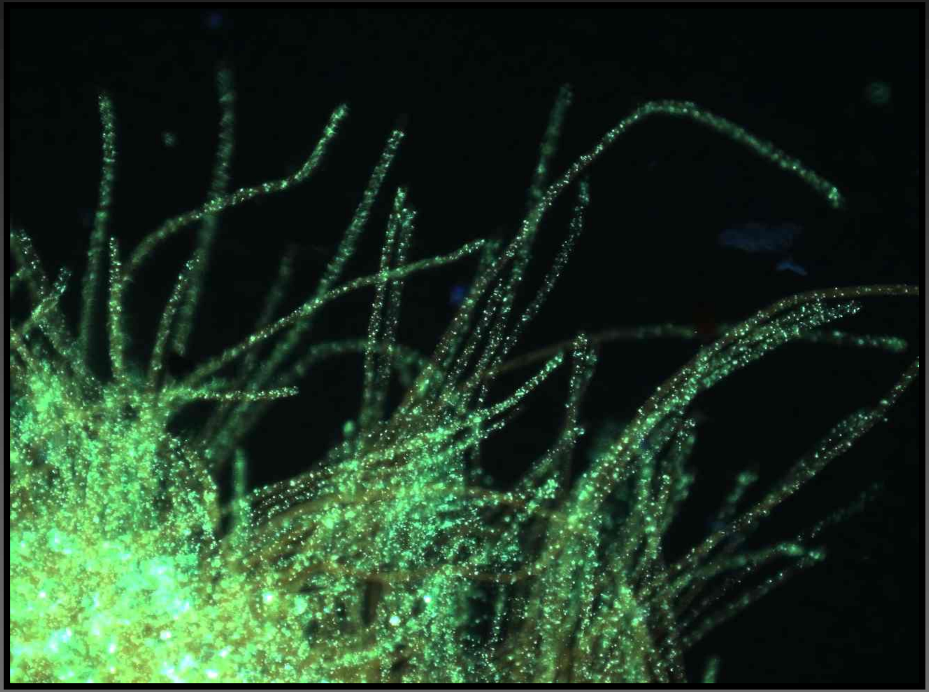

# Conclusions

'Omics approaches are providing new insights into the chemical-microbe network that drive ocean ecosystem structure and function

Lessons learned: Where there is a will there is a way!

Enjoy the rest of the workshop!