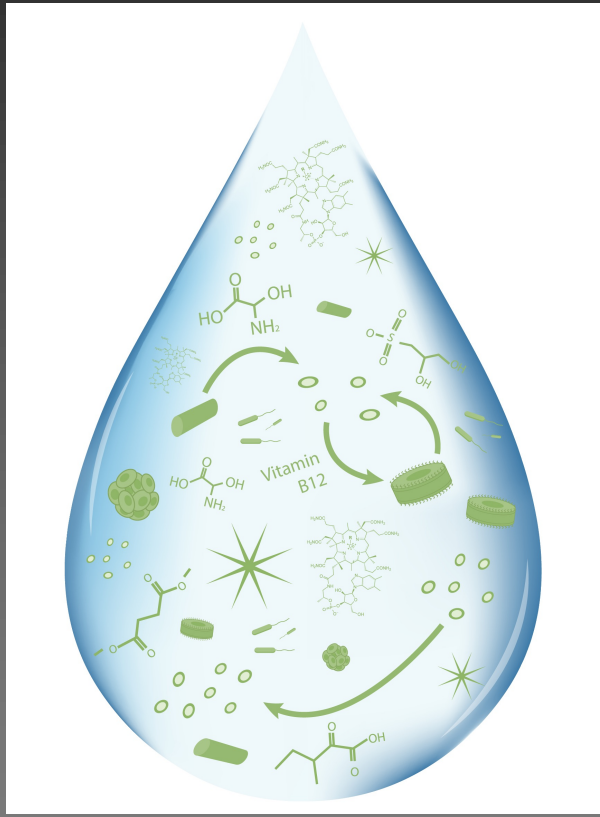
What are the rules that govern the chemical – microbe network? A focus on interactions…



Who is there?
What are they doing/capable of doing?
How are they interacting?

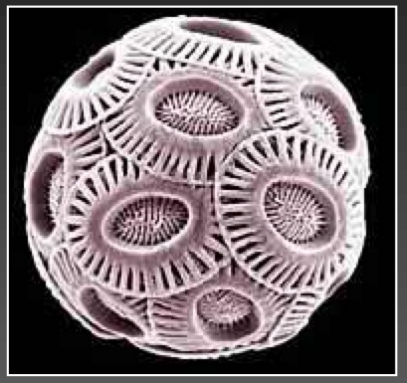
If we learn the rules for how the system operates – then we have a better chance of predicting future ocean responses

'Omic-enabled advances allowing to query cells in their environment in a species-specific way

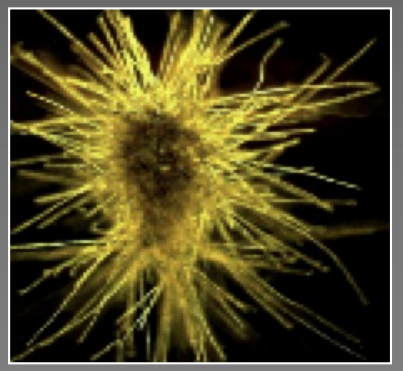
Graphic: WHOI

Vignettes

- Co-existing in a sea of competition: tracing drivers of phytoplankton physiological ecology in the open ocean



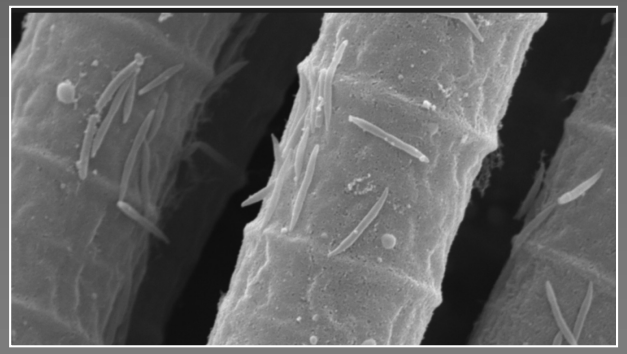
- Microbiome interactions: Tracking the microbiome of a keystone N_2 fixer



Core questions

Host-microbiome interactions

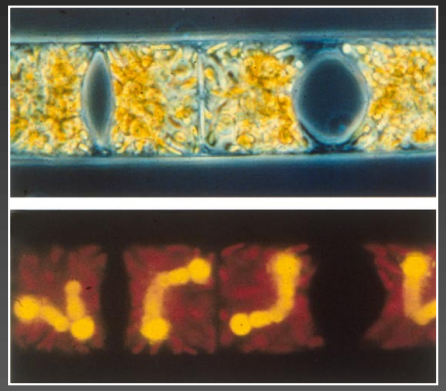
- What is the role of the microbiome in *Trichodesmium* physiological ecology
  - Who is there?
  - What are they doing?
  - Are they interacting?



Nitrogen-fixing marine cyanobacteria

- Symbionts
  - UNCYN-A
  - *Richelia*
  - UCYN-C
- Free-living
  - *Crococosphaera*
  - *Trichodesmium*

Richelia



Crocosphaera

-*Trichodesmium erythraeum* — Clade III

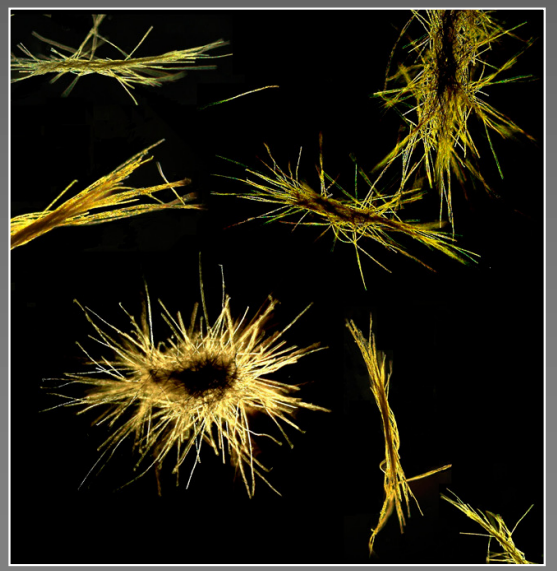
-*Trichodesmium tenue*
-*Trichodesmium contortum*
-*Trichodesmium spp. (miru, nobis)** — Clade II

* Delmont et al. (2021) PNAS

-*Trichodesmium thiebautii* — Clade I

-*Trichodesmium spiralis*
-*Trichodesmium hildebrandtii* — Clade IV

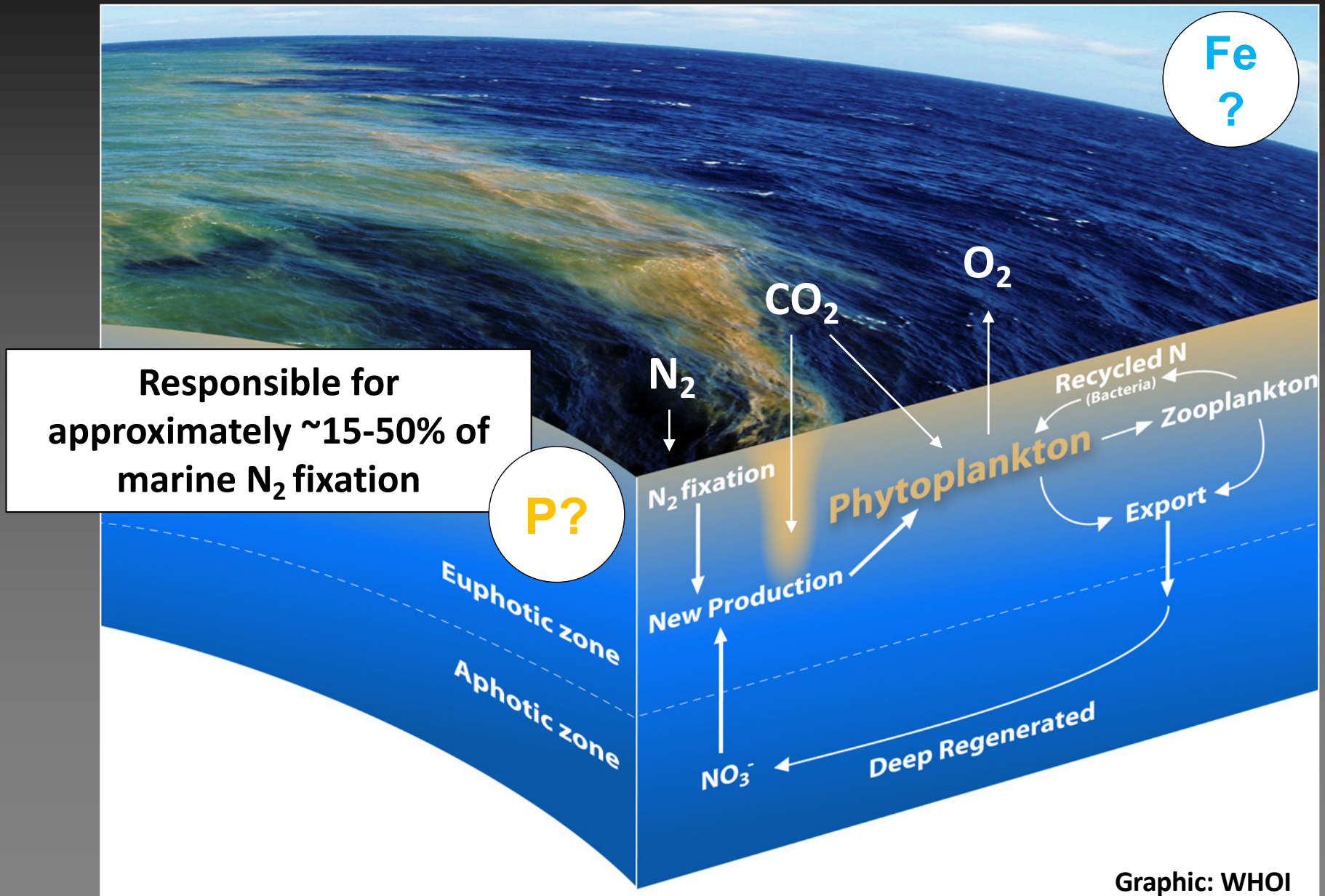
Trichodesmium



Trichodesmium: critical to ecosystem function

Photo: Chris Wade
Tricho. micrograph: WHOI

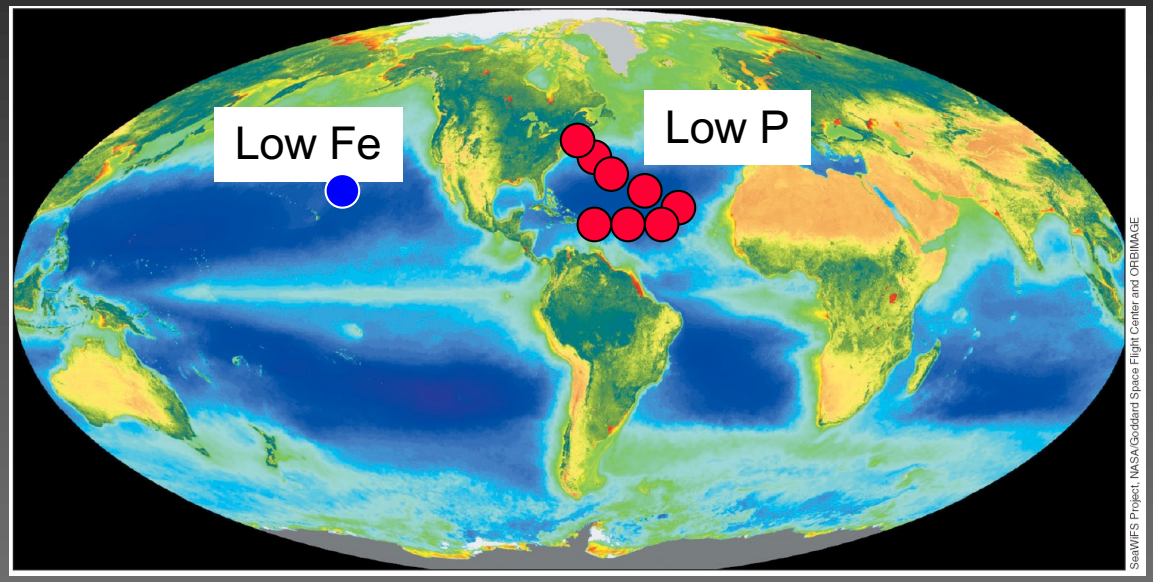
Trichodesmium: critical to ecosystem function



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

Graphic: WHOI

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



North Atlantic

P-limited?

North Pacific

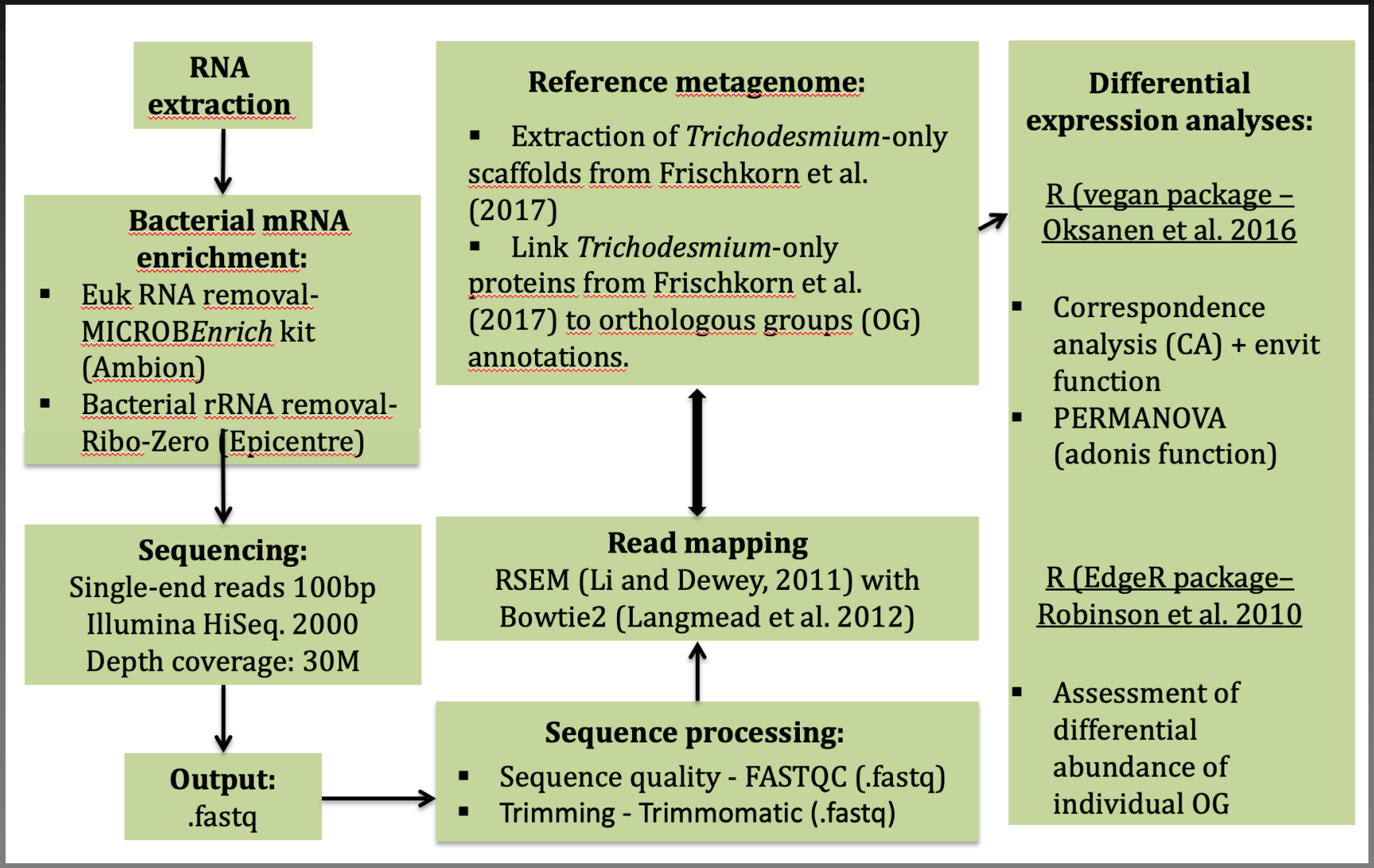
Fe-limited?

2000s

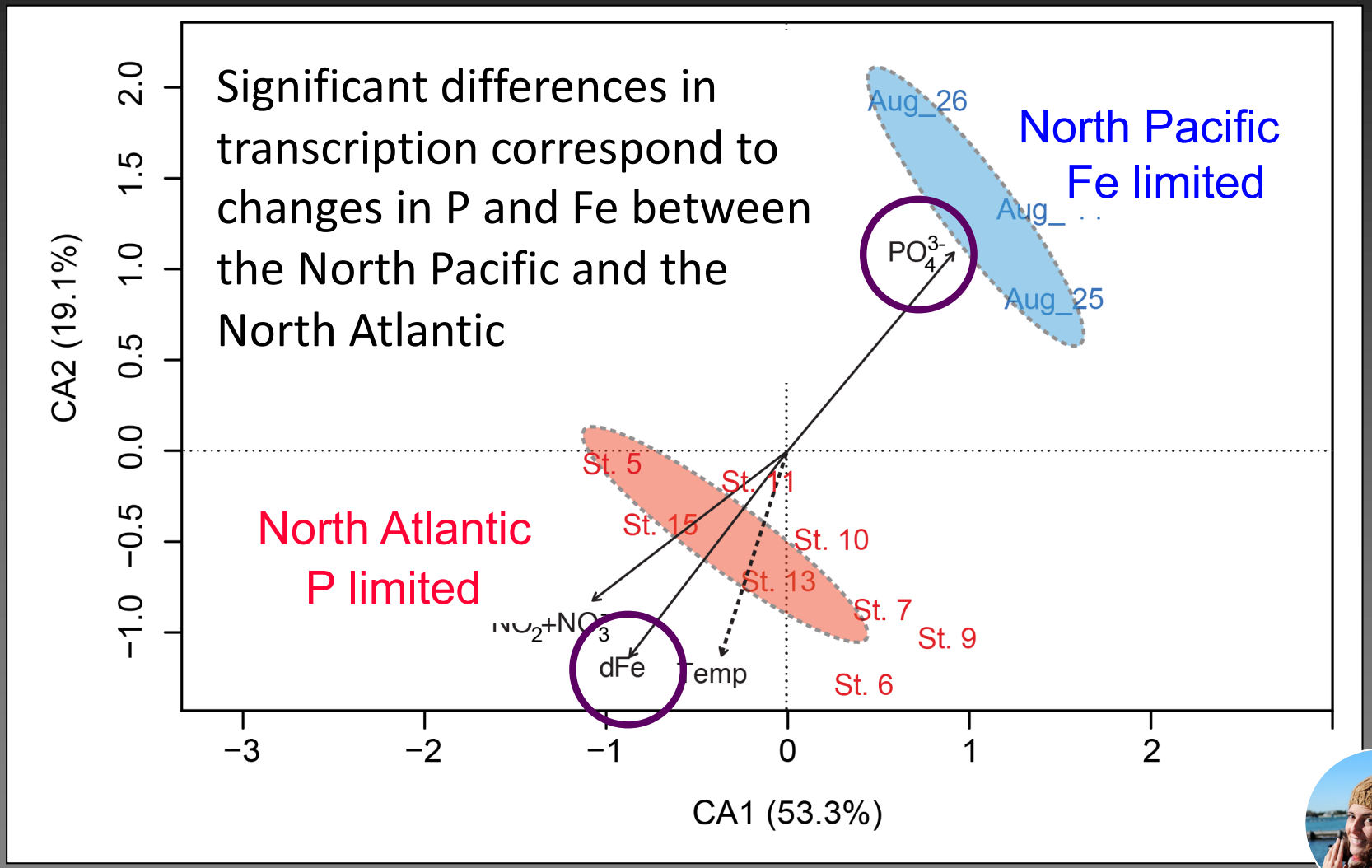
- Protein biomarkers: Dyhrman et al. (2002) *L&O*
- qRT-PCR of transcript biomarkers: Dyhrman et al. (2006) *Nature*

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

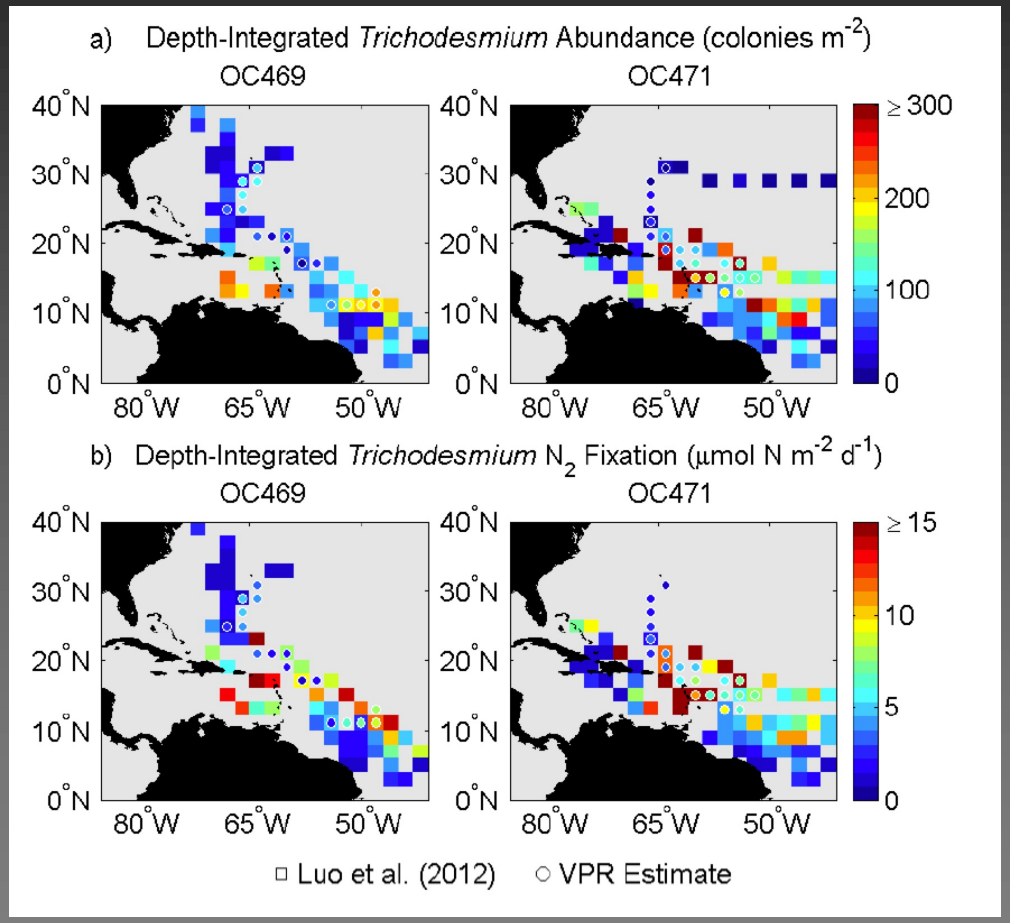
Trichodesmium transcriptional patterns between the North Pacific and the North Atlantic



Ruoco et al. (2018) *ISME J*

Modeling N_2 fixation is still a challenge

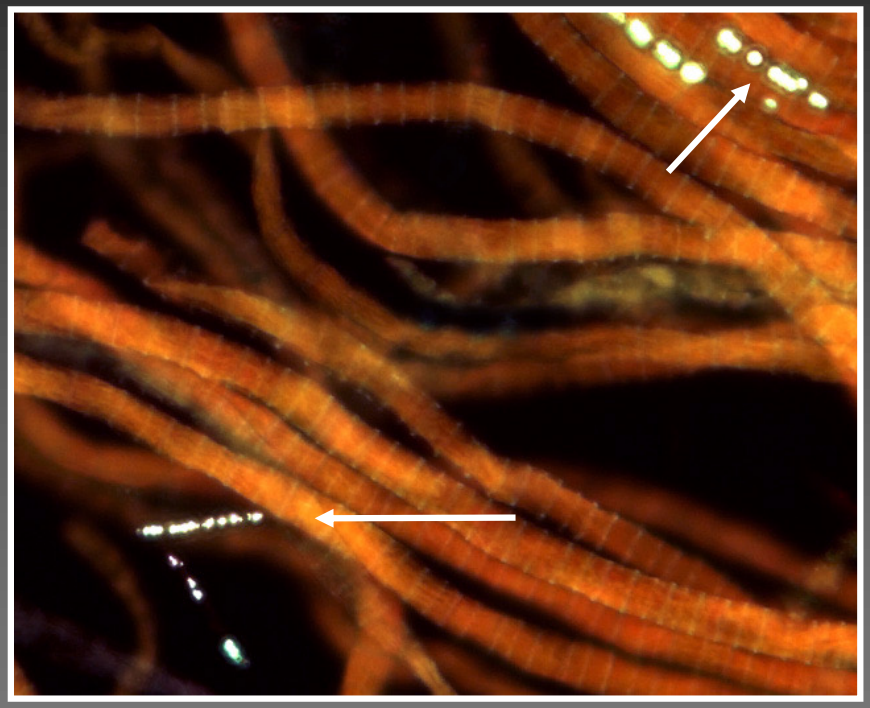
- Models do not balance the N cycle in the ocean or recapitulate patterns well
- Assays of nitrogen fixation are technically difficult = variability
- Information on distribution over time and with depth is still patchy
- **Geochemistry is not often a good predictor of distribution or N_2 fixation**



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**

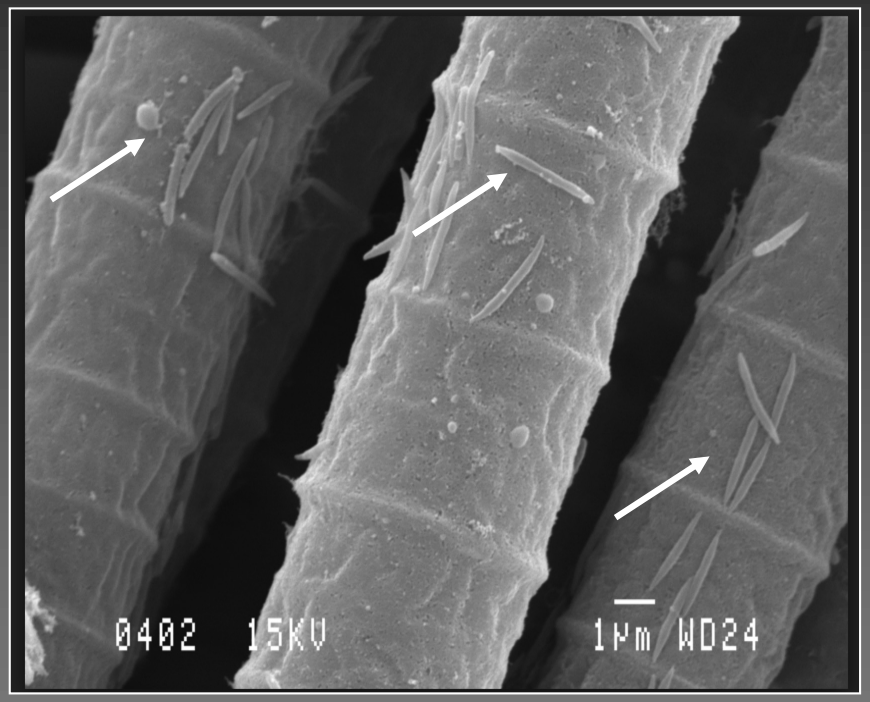


Image courtesy Tracy Mincer

Trichodesmium colonies harbor other microbes with distinct physiology.

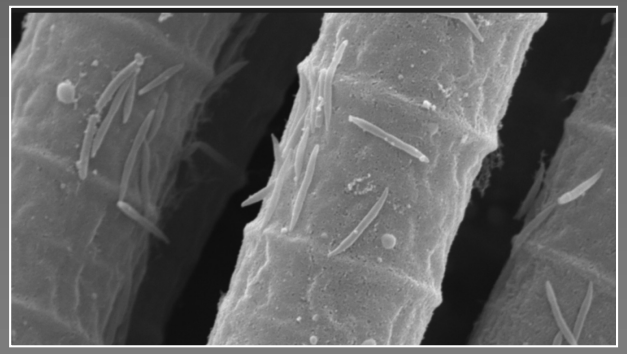
Core questions about the *Trichodesmium* microbiome

Who is there?

- Microbiome diversity
- *16S amplicon sequencing*

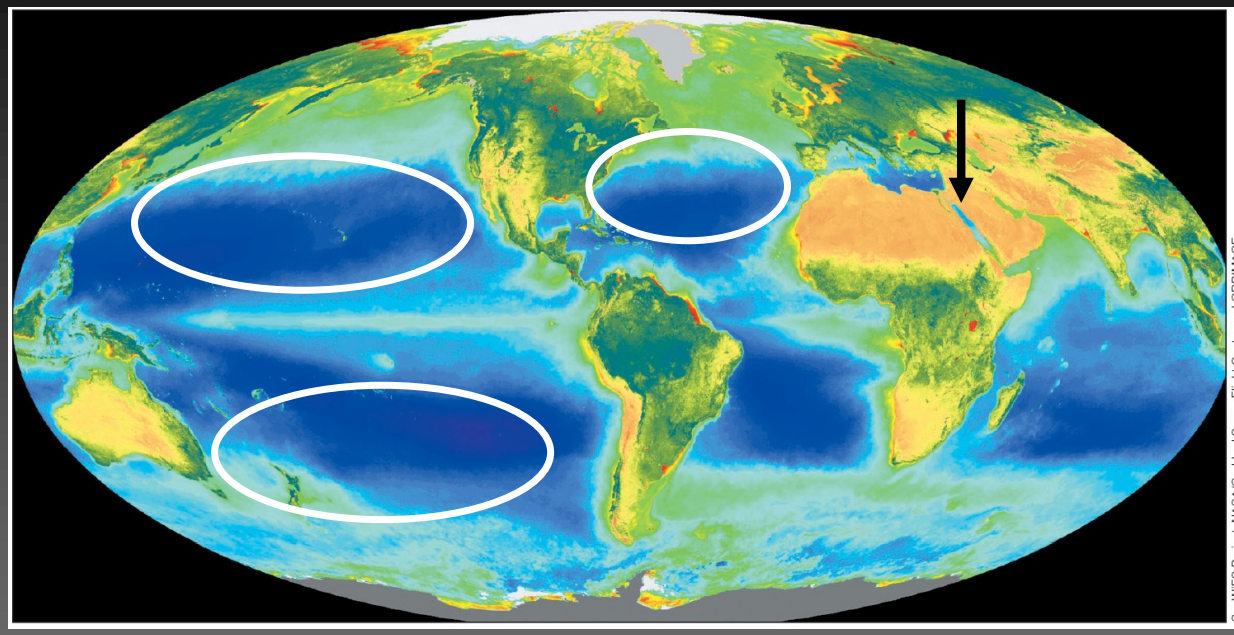
What are they doing?

- Microbiome functional diversity
- *Metagenome assembled genomes*



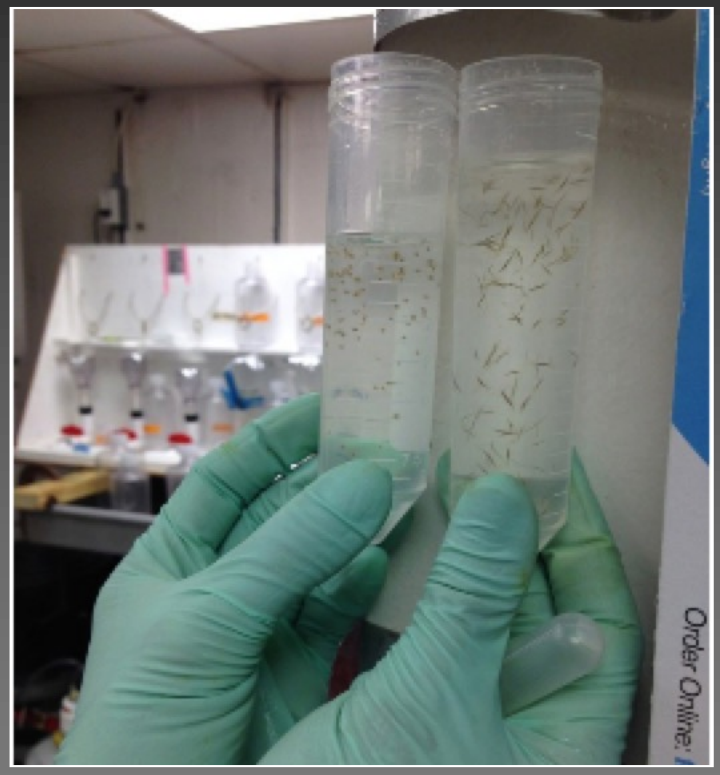
Are they interacting?

- Coordinated diel signaling
- *Metatranscriptomics*



SeaWIFS Project, NASA/Goddard Space Flight Center and ORBIMAGE

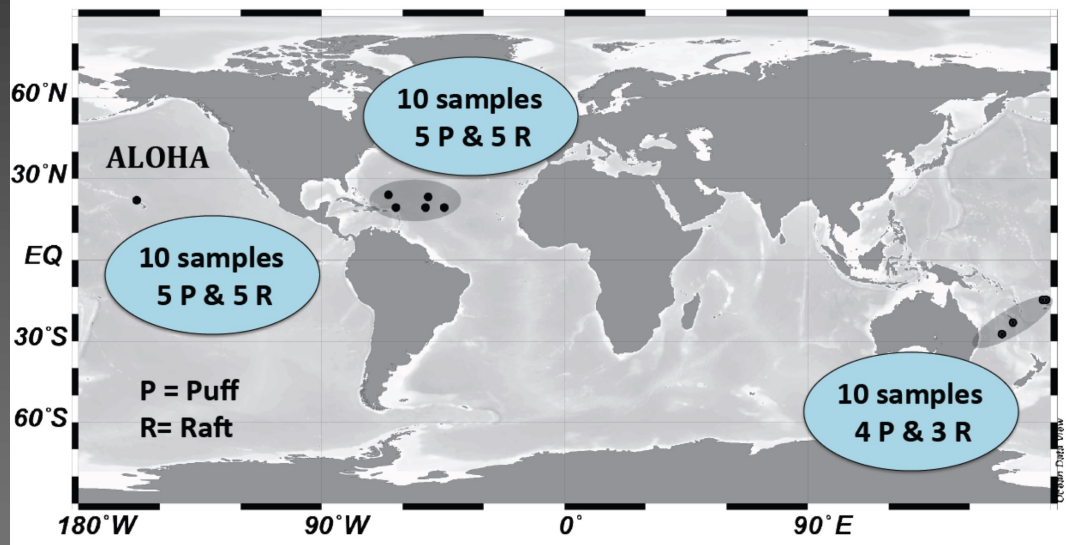
Trichodesmium microbiome diversity



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)** →

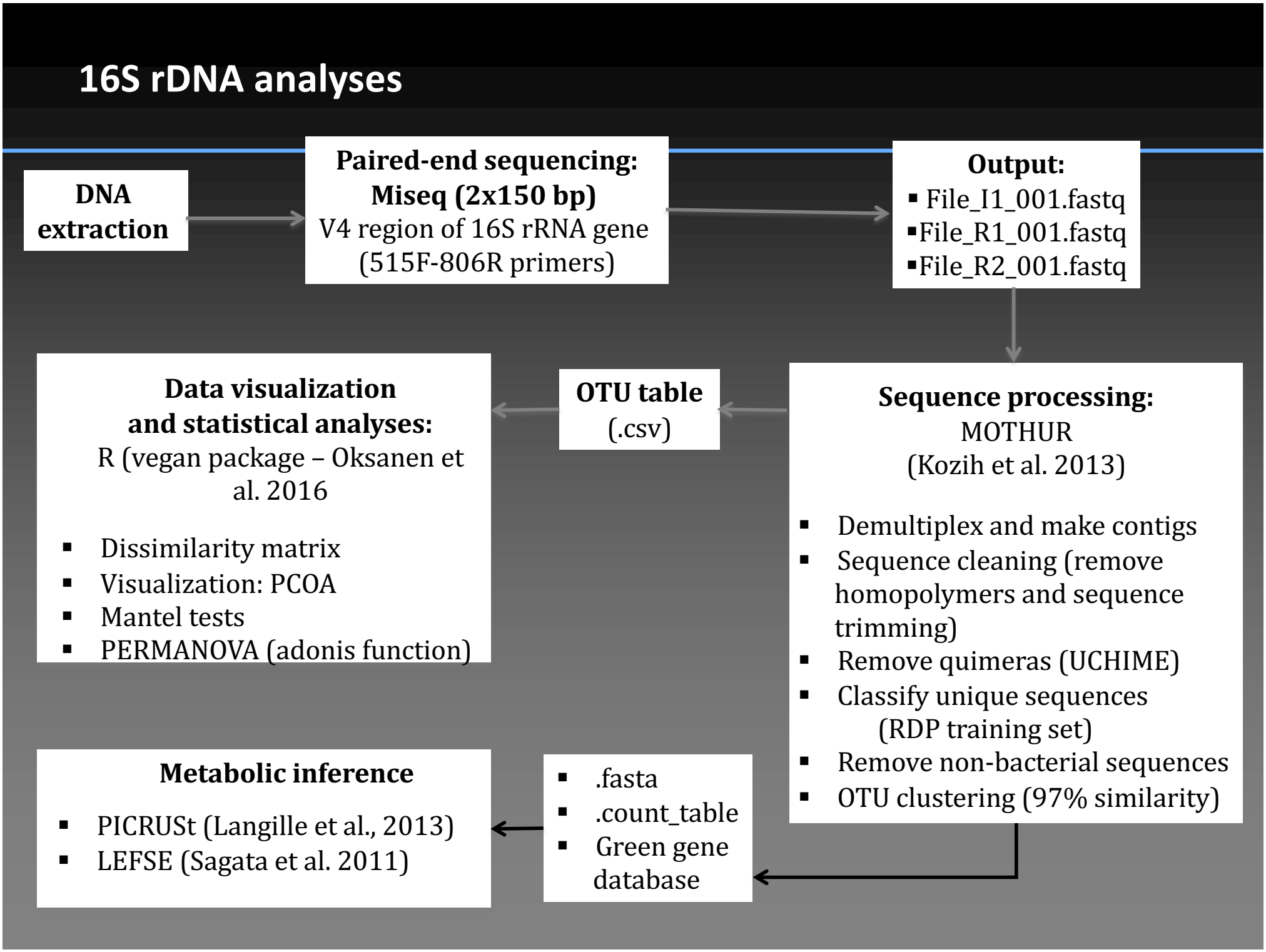
Mothur v. 1. 34. 0 (936,749 reads)

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

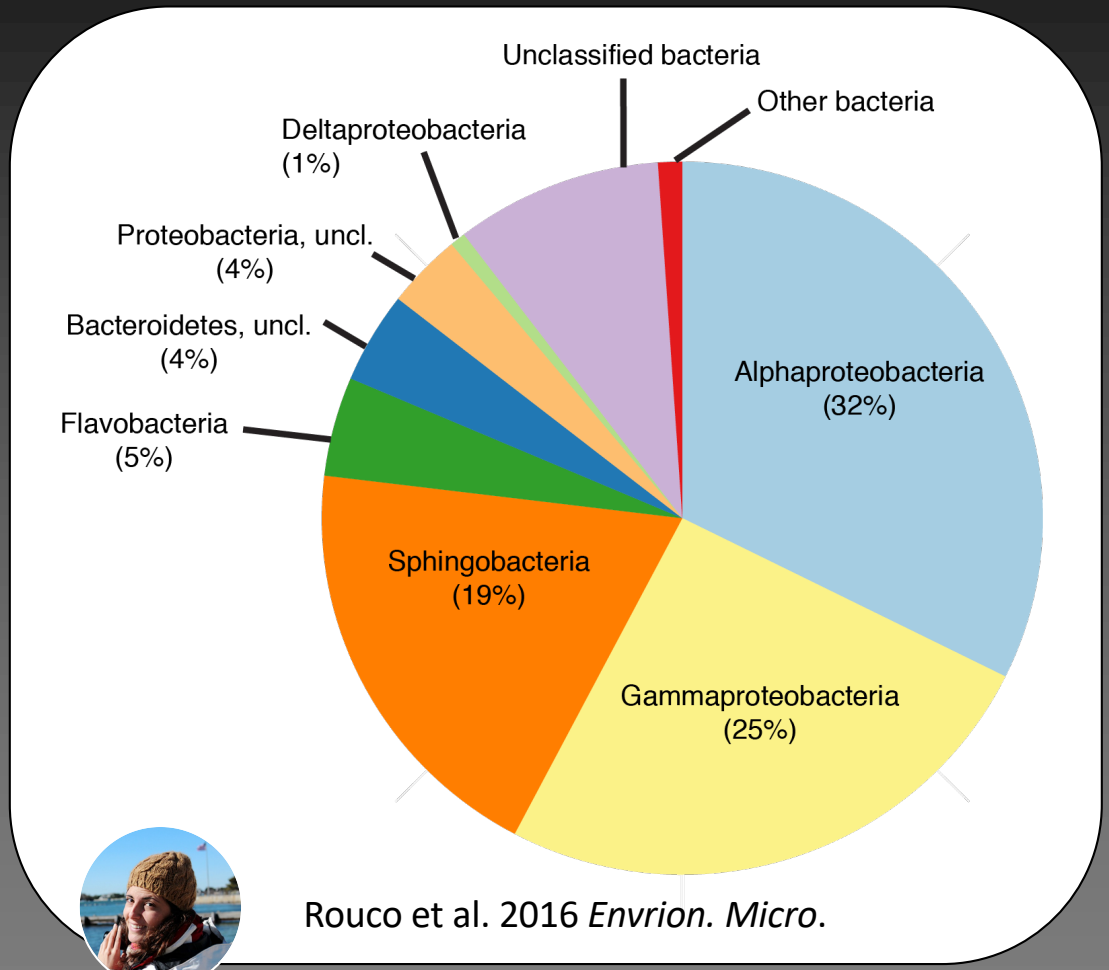
What groups co-occur with *Trichodesmium*?

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

16S rDNA analyses



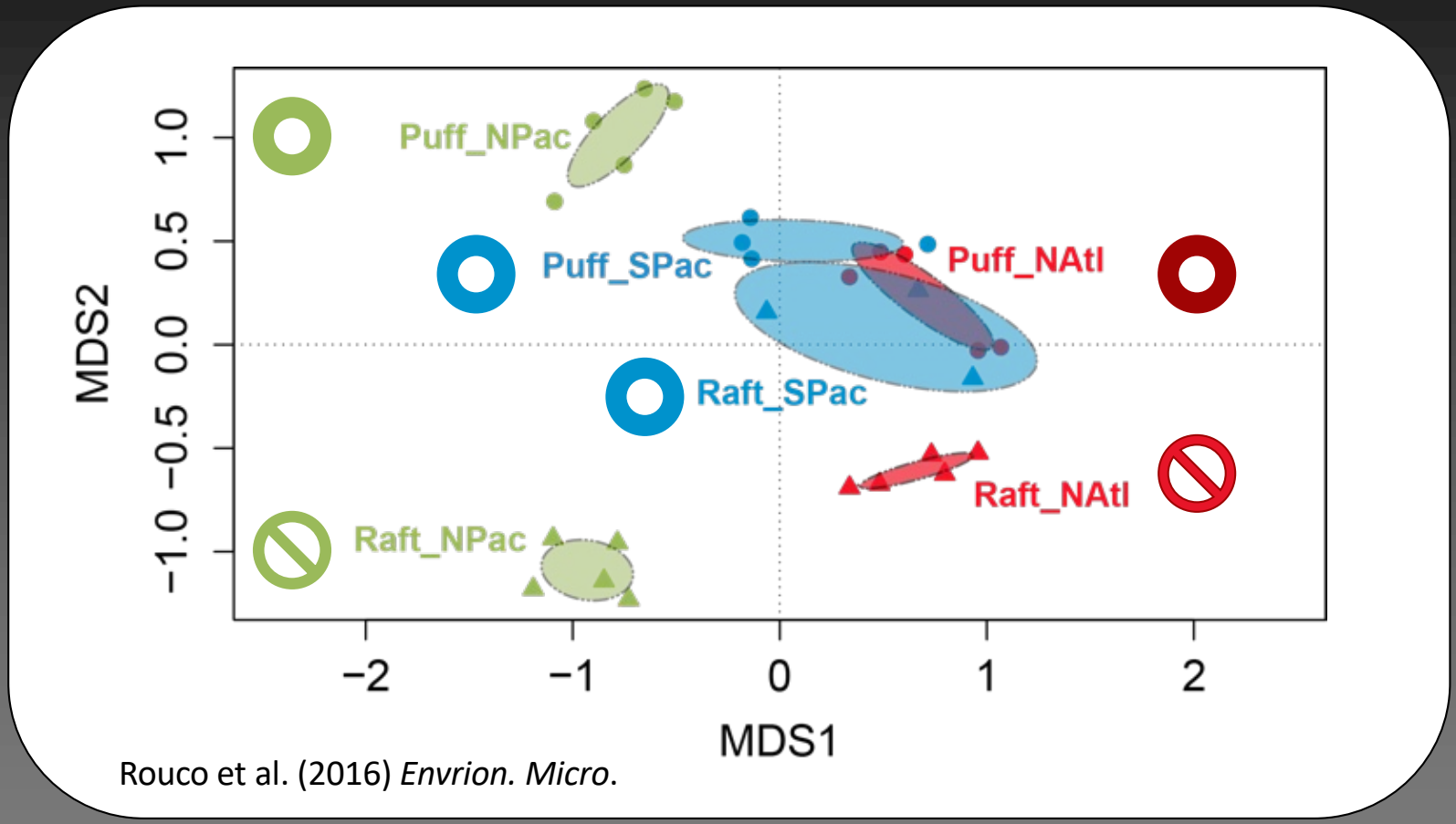
Average epibiont community



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?*

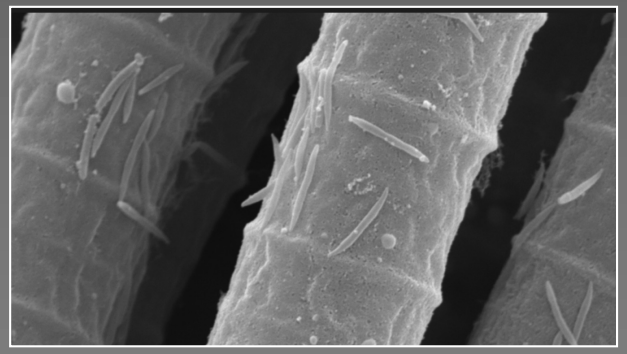
Core questions about the *Trichodesmium* microbiome

Who is there?

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

What are they doing?

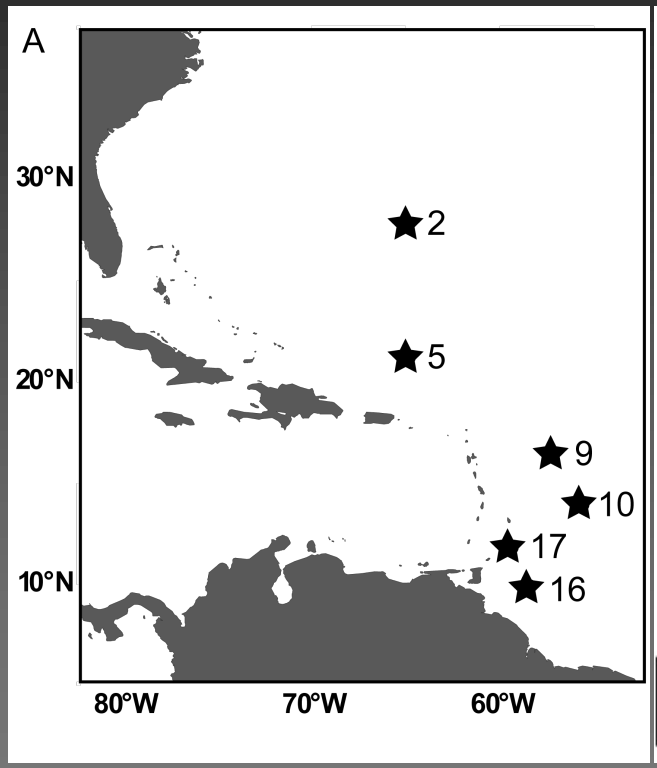
- Microbiome functional diversity

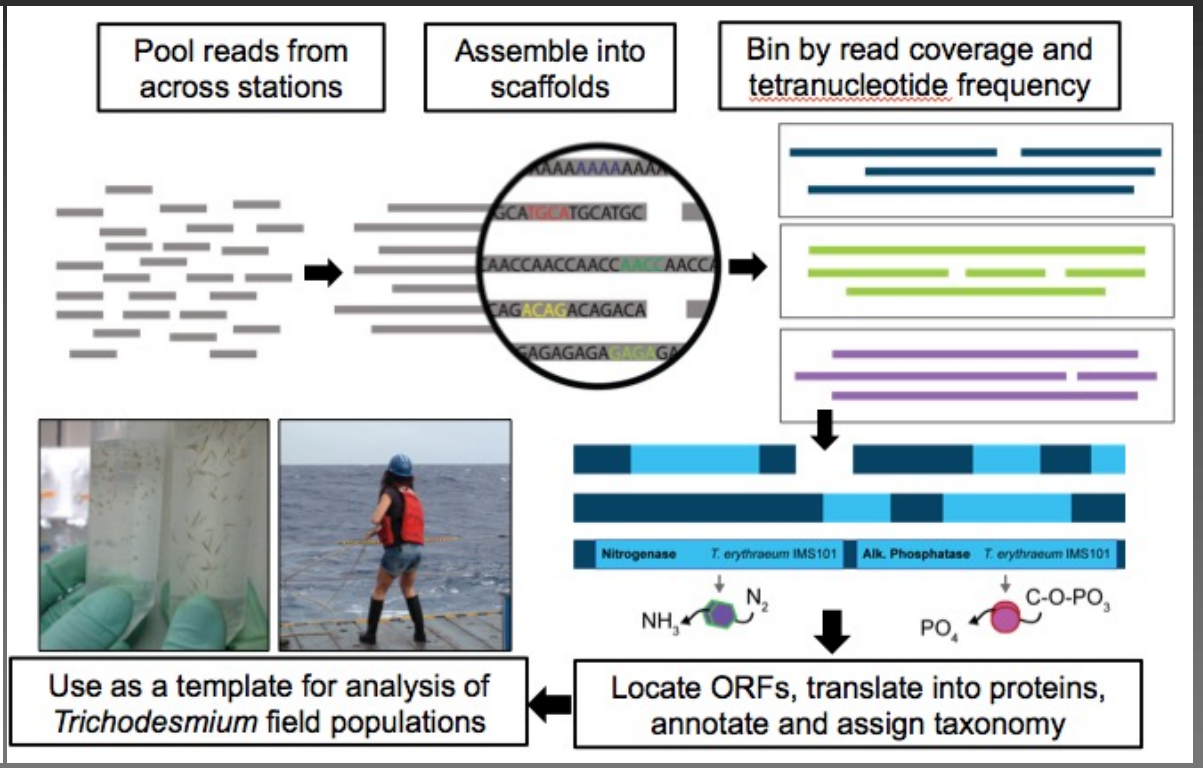


Are they interacting?

- Diel patterns in the consortia

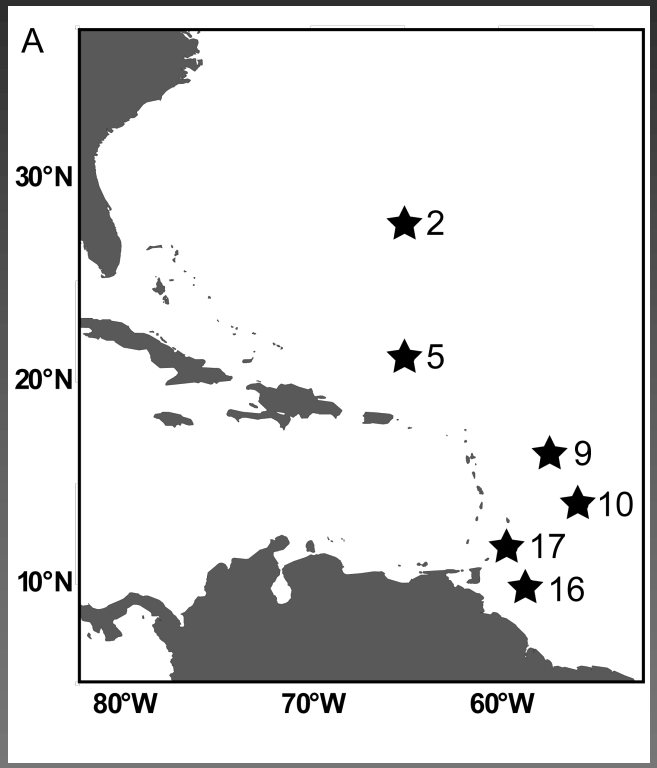
Metabolic potential in the *Trichodesmium* holobiont

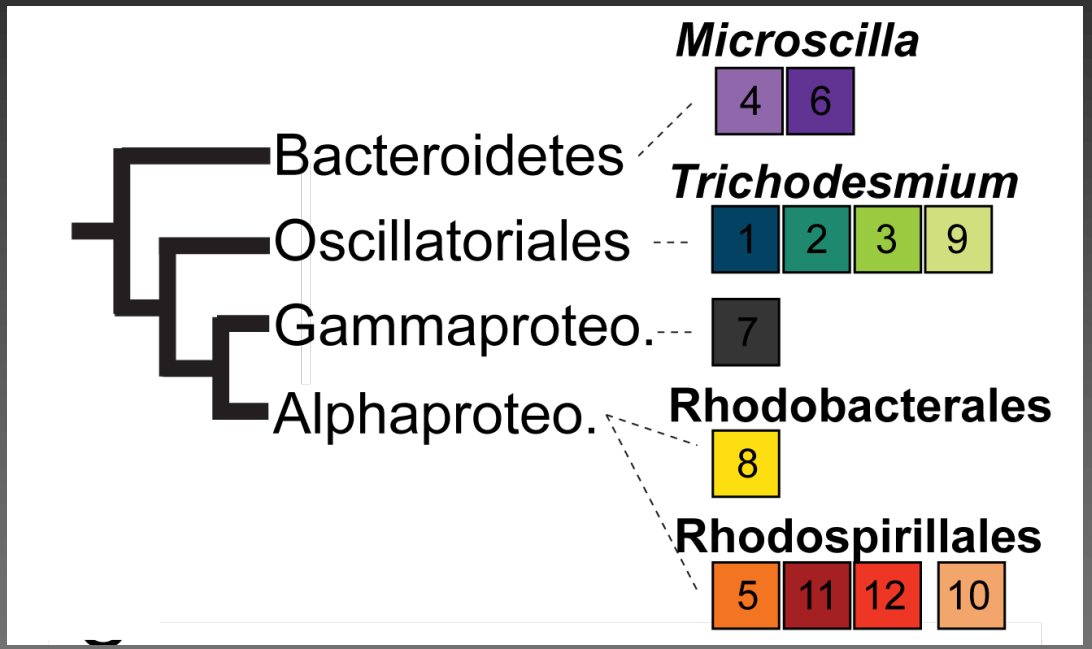




Trichodesmium colonies were isolated for metagenome sequencing along a phosphorus gradient in the western north Atlantic.

Composition of the holobiont

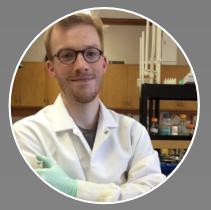




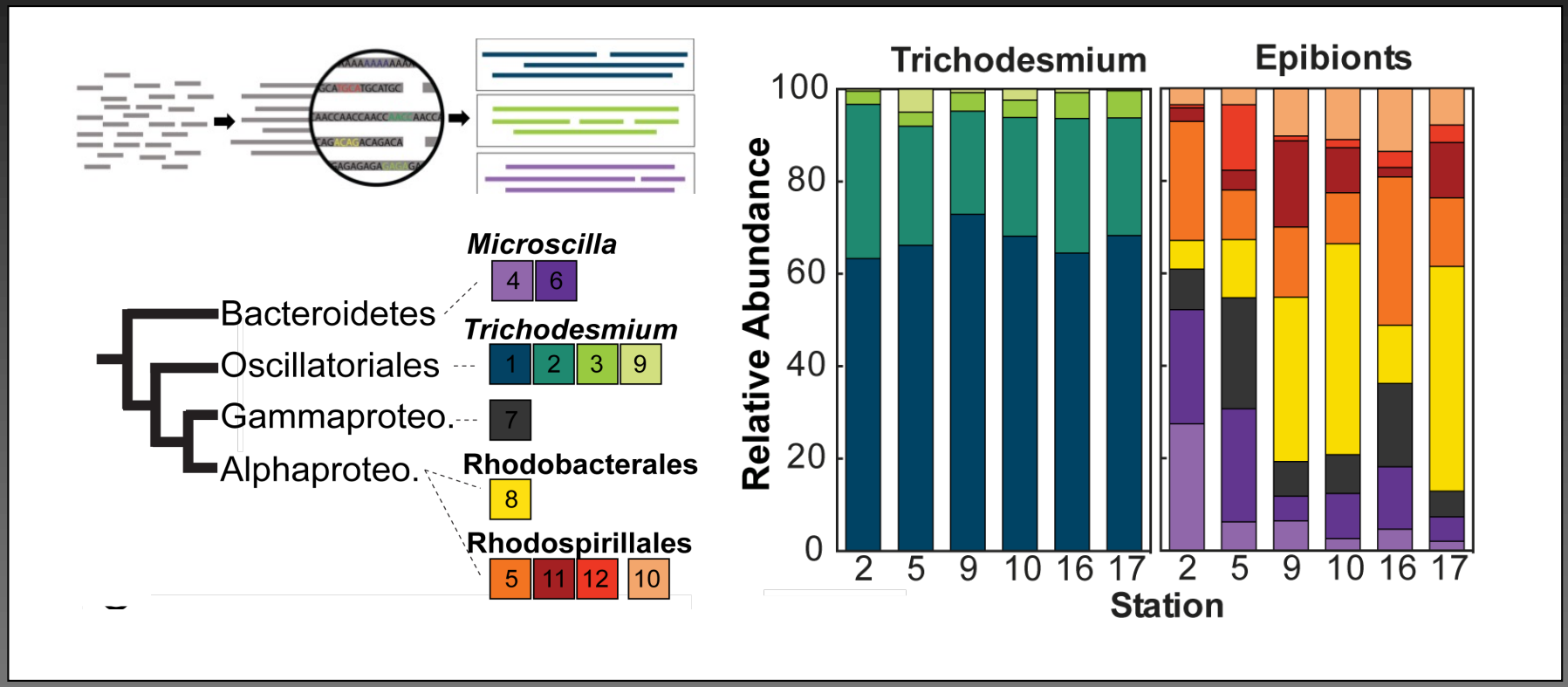
Nearly complete (~90%) genome bins were reconstructed from a merged assembly and results are consistent with 16S data

= Metagenome assembled genome or MAG

Frischkorn et al. (2017) *ISMEJ*



Distribution of MAGs with read mapping

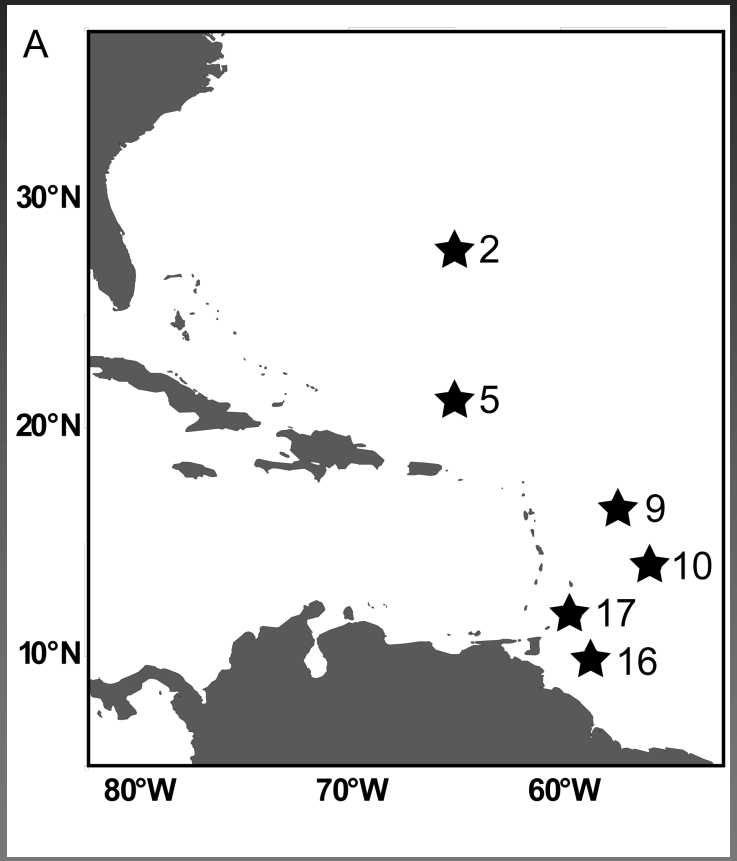


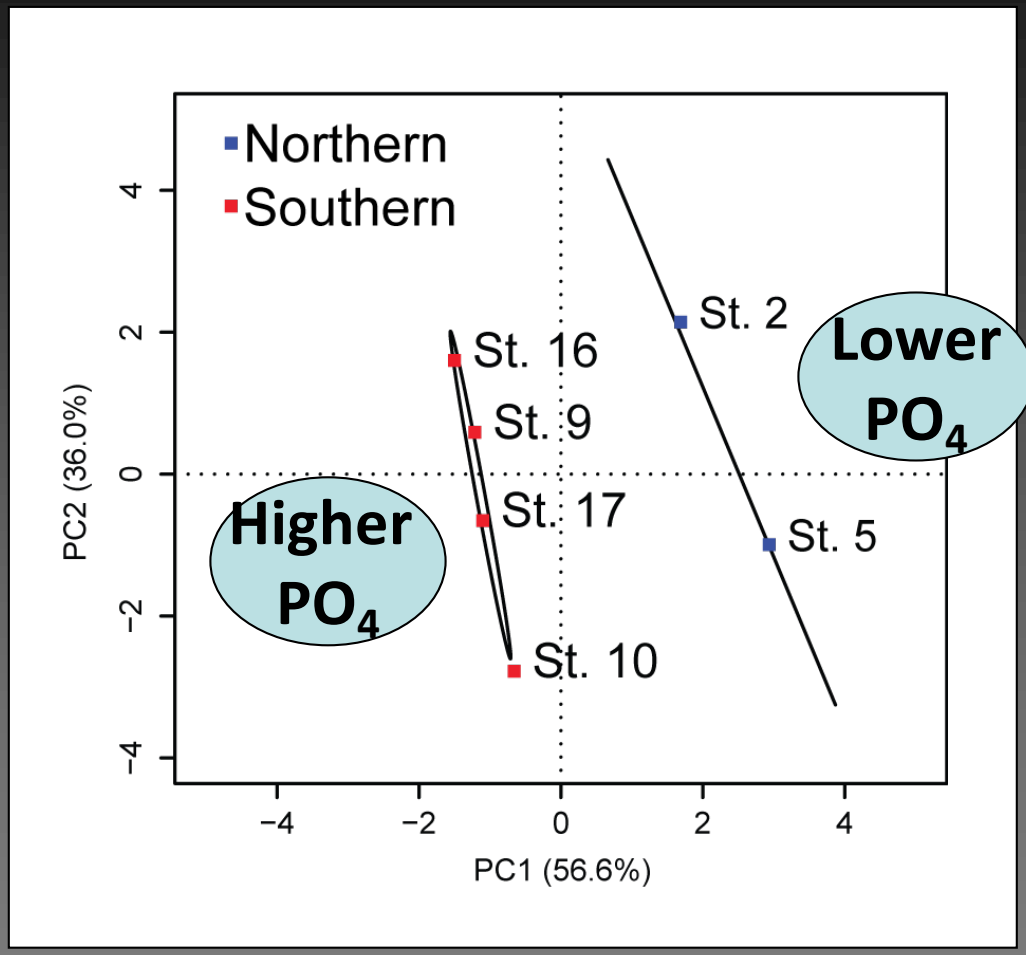
Frischkorn et al. (2017) *ISMEJ*

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



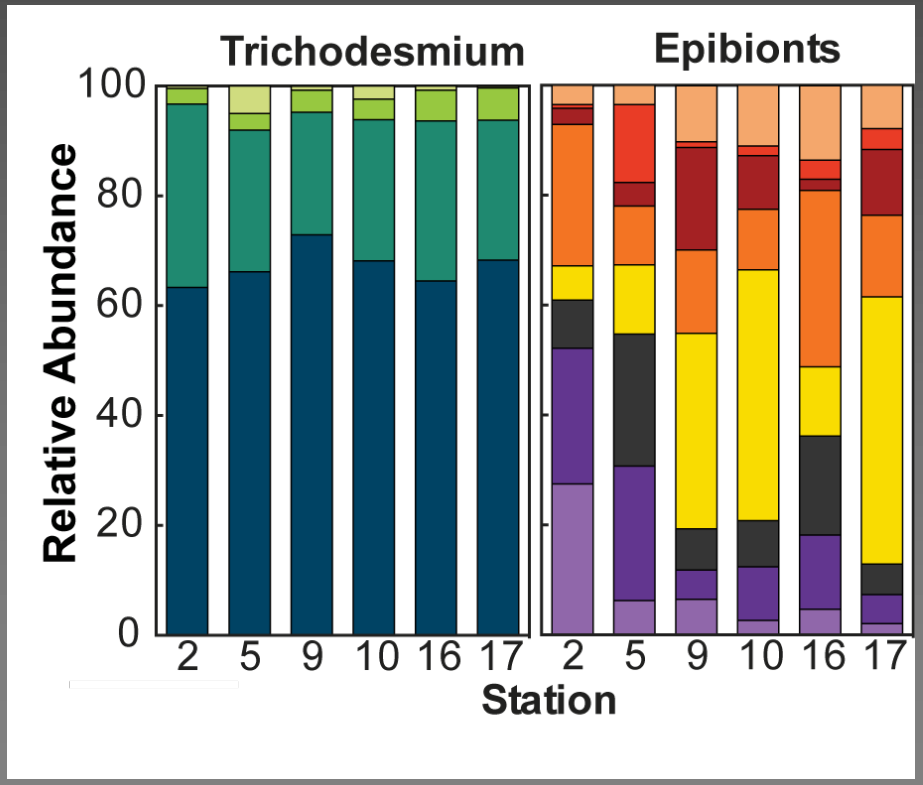


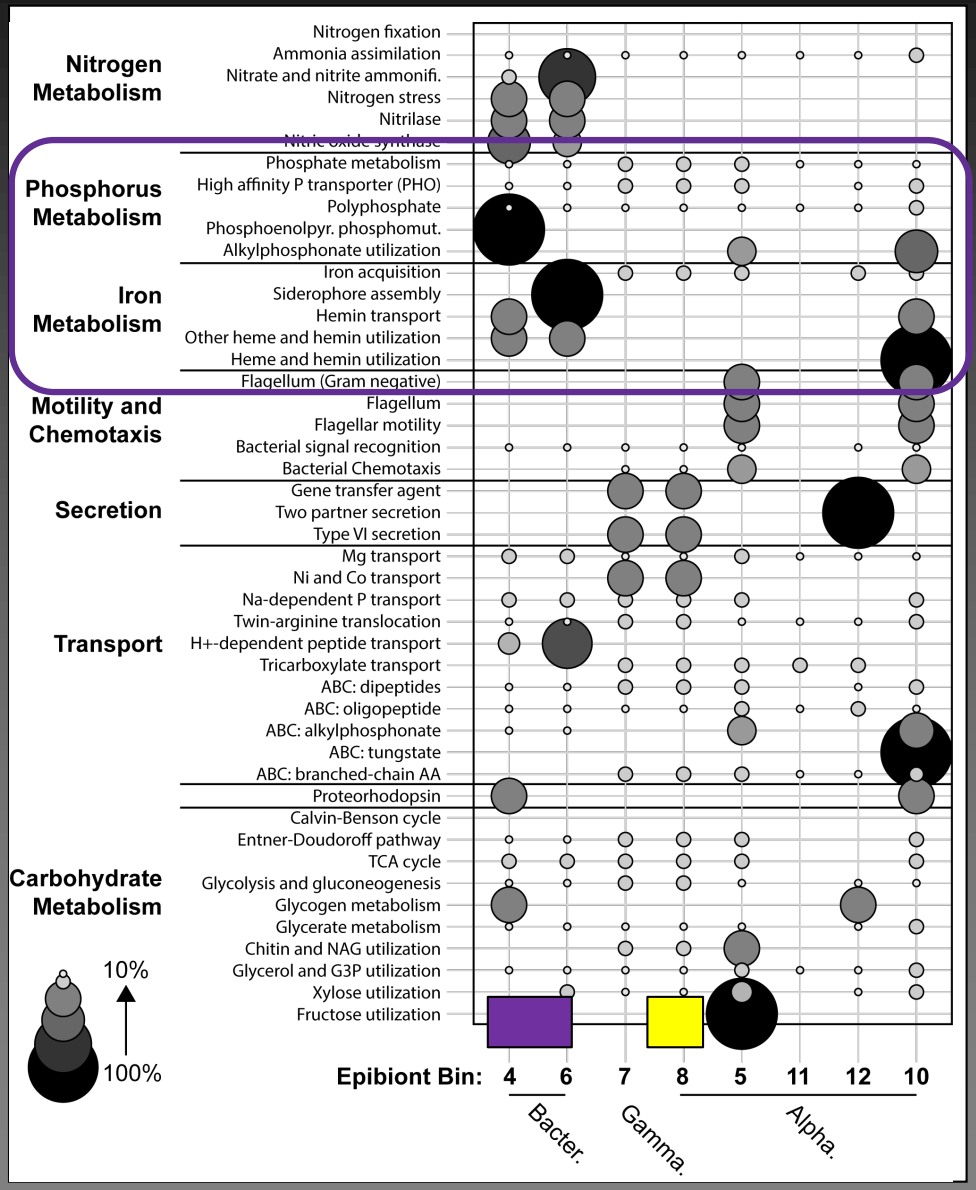
Frischkorn et al. (2017) *ISME J*

Significant difference in epibiont relative abundance with total dissolved phosphorus

Variable distribution of functional pathways among epibionts

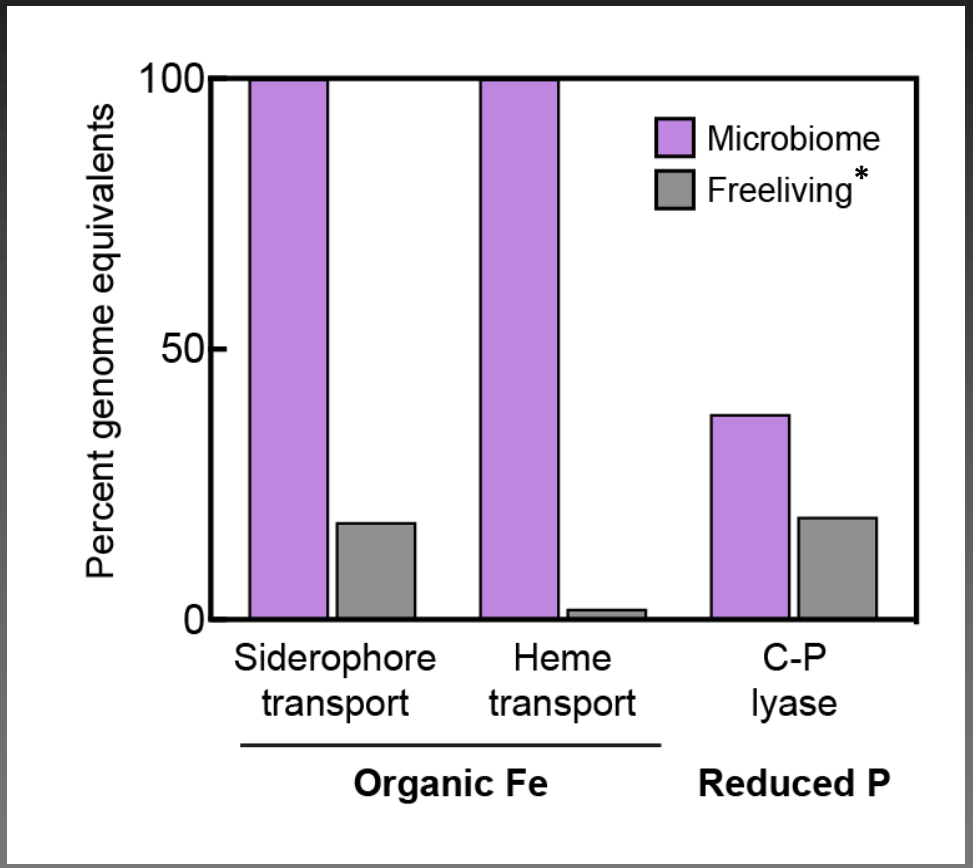
- Differential pathway enrichment consistent with a microbiome that is modulated as a function of environment





Frischkorn et al. (2017) *ISMEJ*

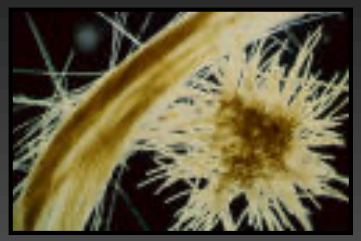
Epibionts enrich holobiont for key functions



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*

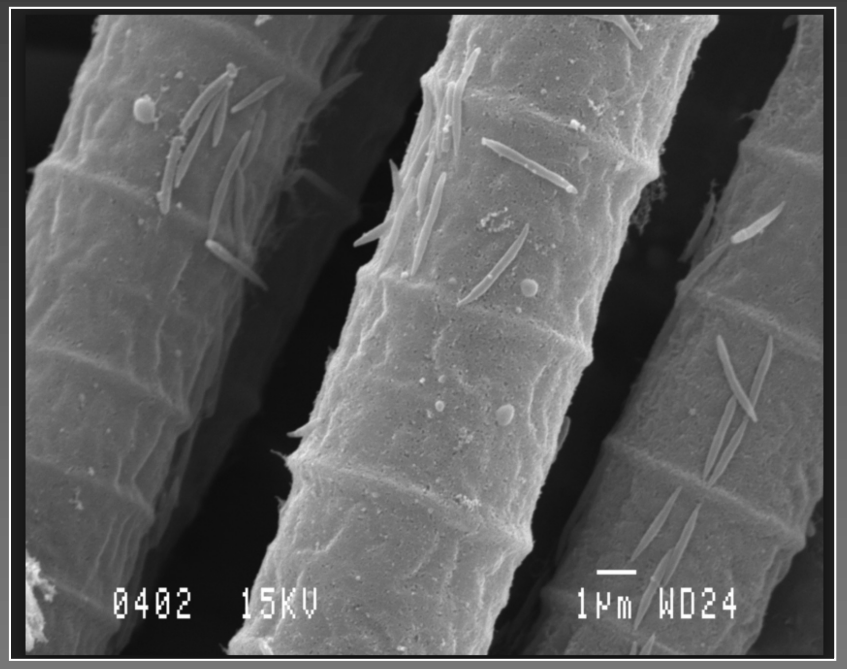
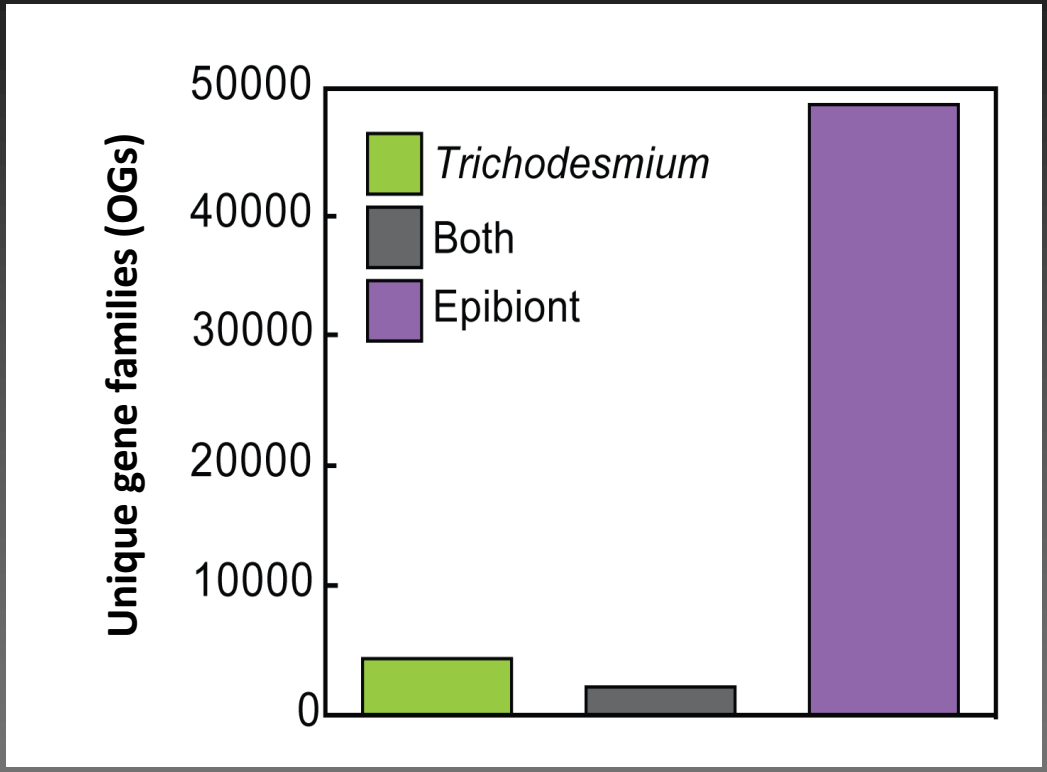


Image courtesy Tracy Mincer

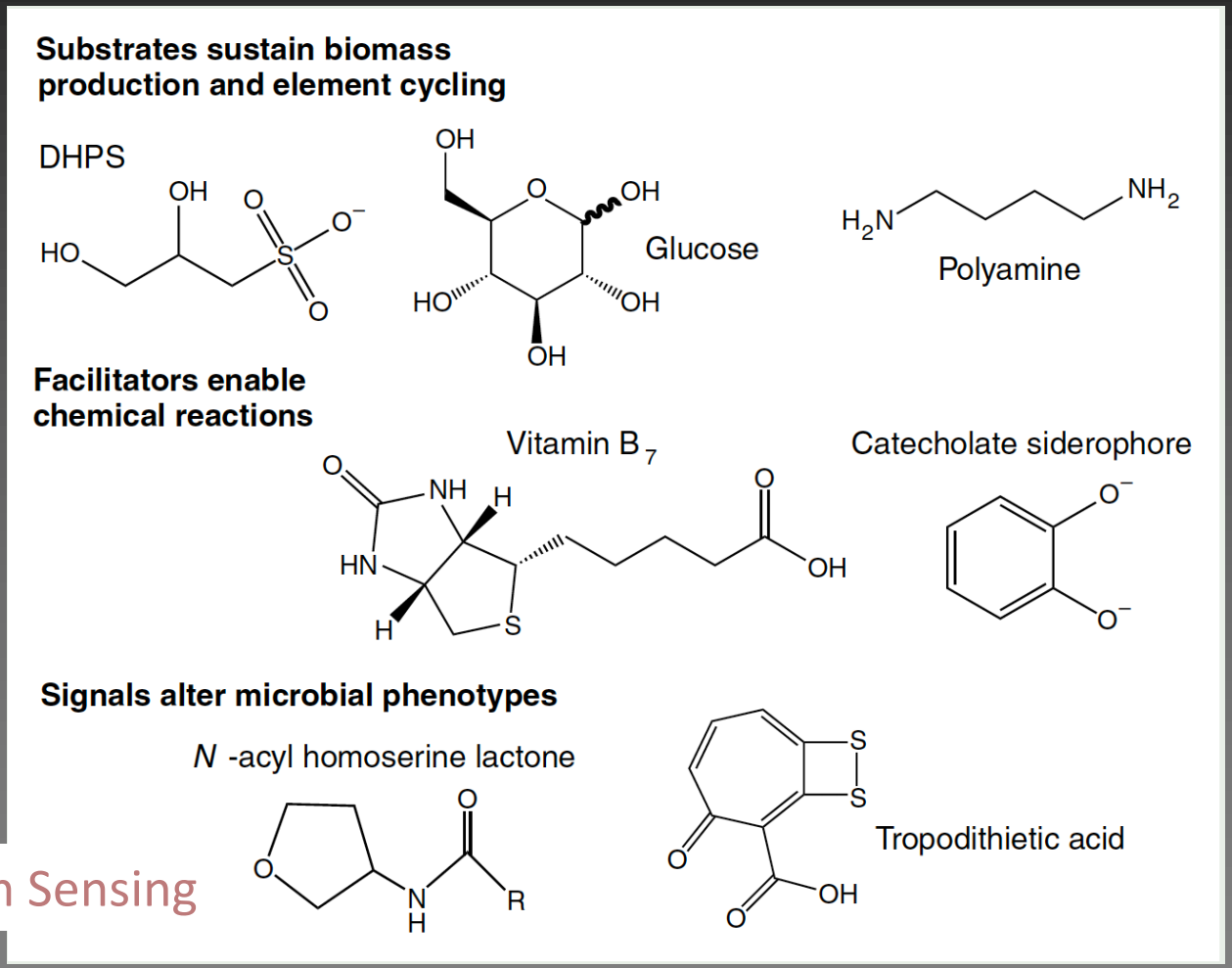
Epibionts significant metabolic potential



Frischkorn et al. (2017) *ISME J*

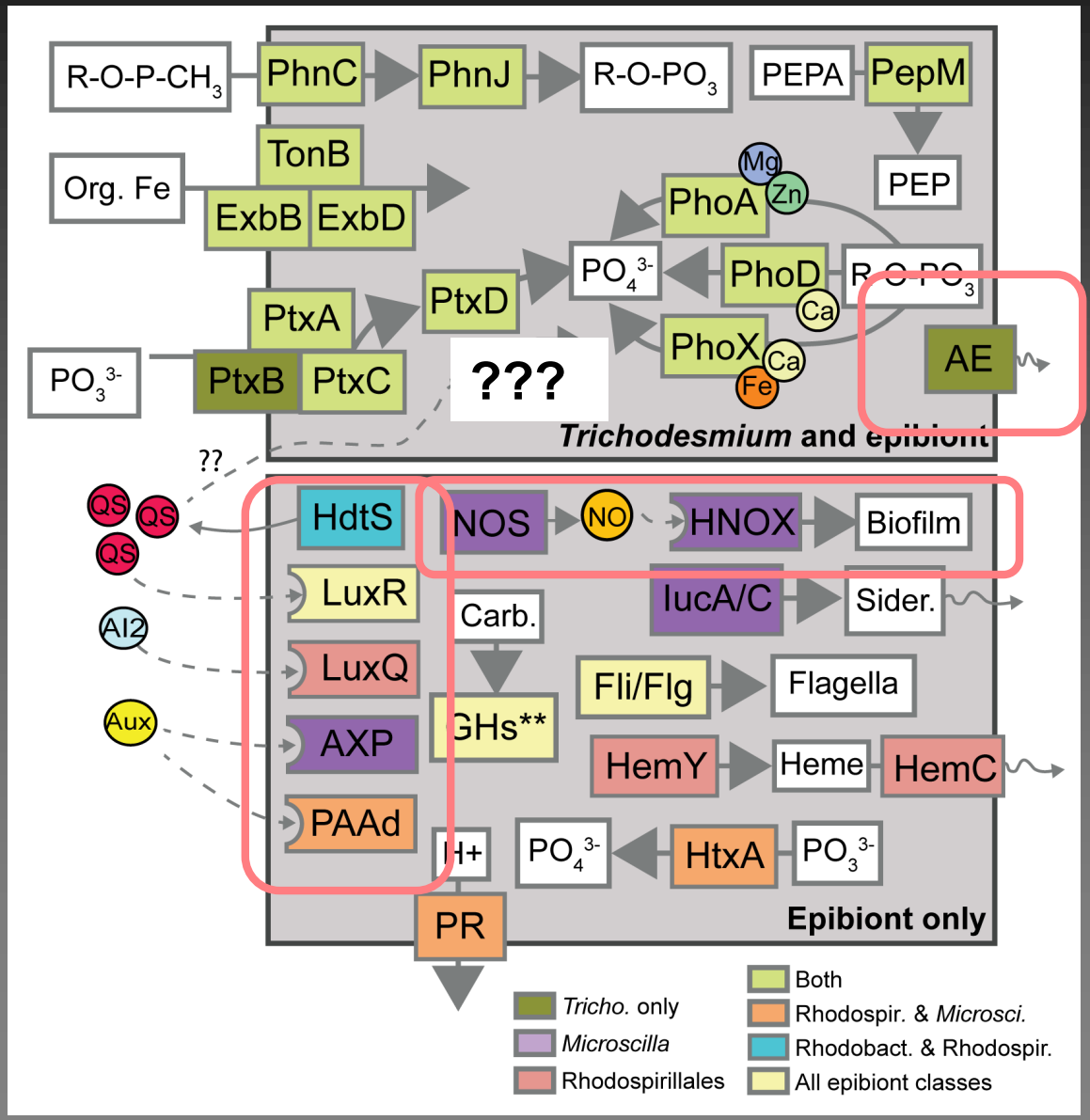
Orthologous (OG) group analysis suggests that epibionts confer the vast majority (>90% of OGs) of metabolic *functions* to the holobiont.

Chemical-microbe network within the *Trichodesmium* holobiont



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

Microbial cross talk within the *Trichodesmium* holobiont



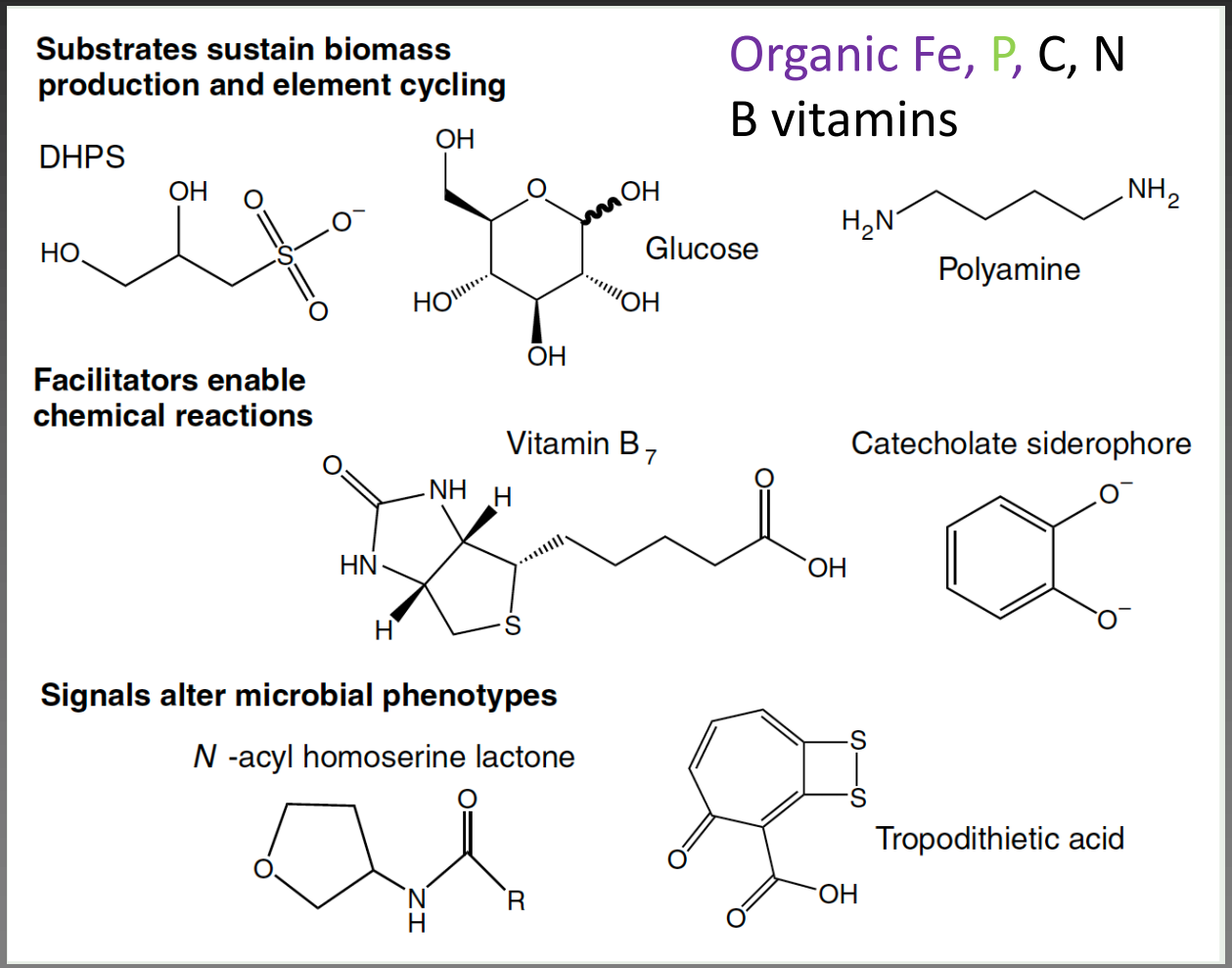
Auxin sensing and response

NO production

AHL QS Pathways are isolated to microbiome

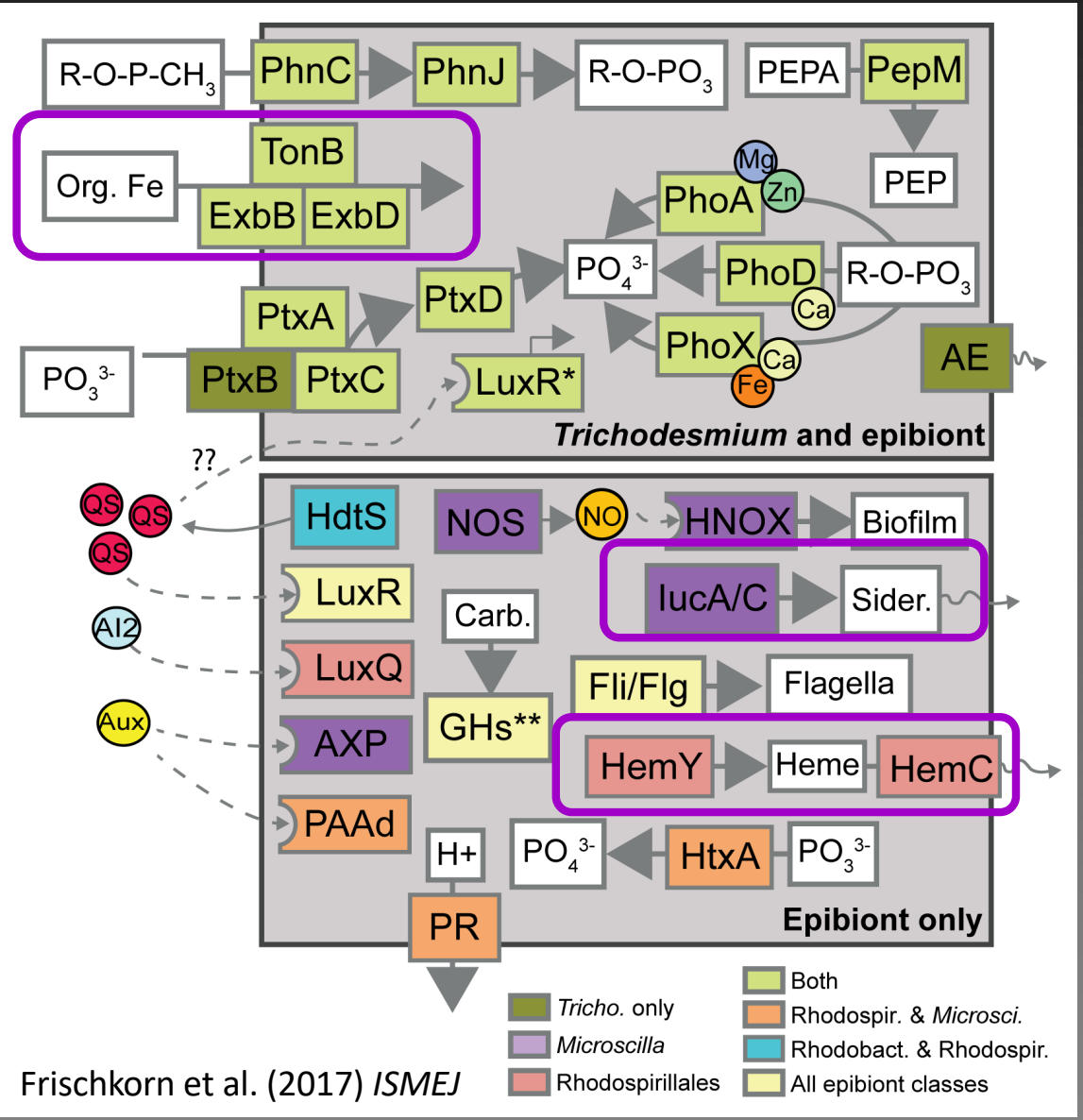
QS and cell signaling

Chemical-microbe network within the *Trichodesmium* holobiont



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

Metabolic partitioning within the *Trichodesmium* holobiont

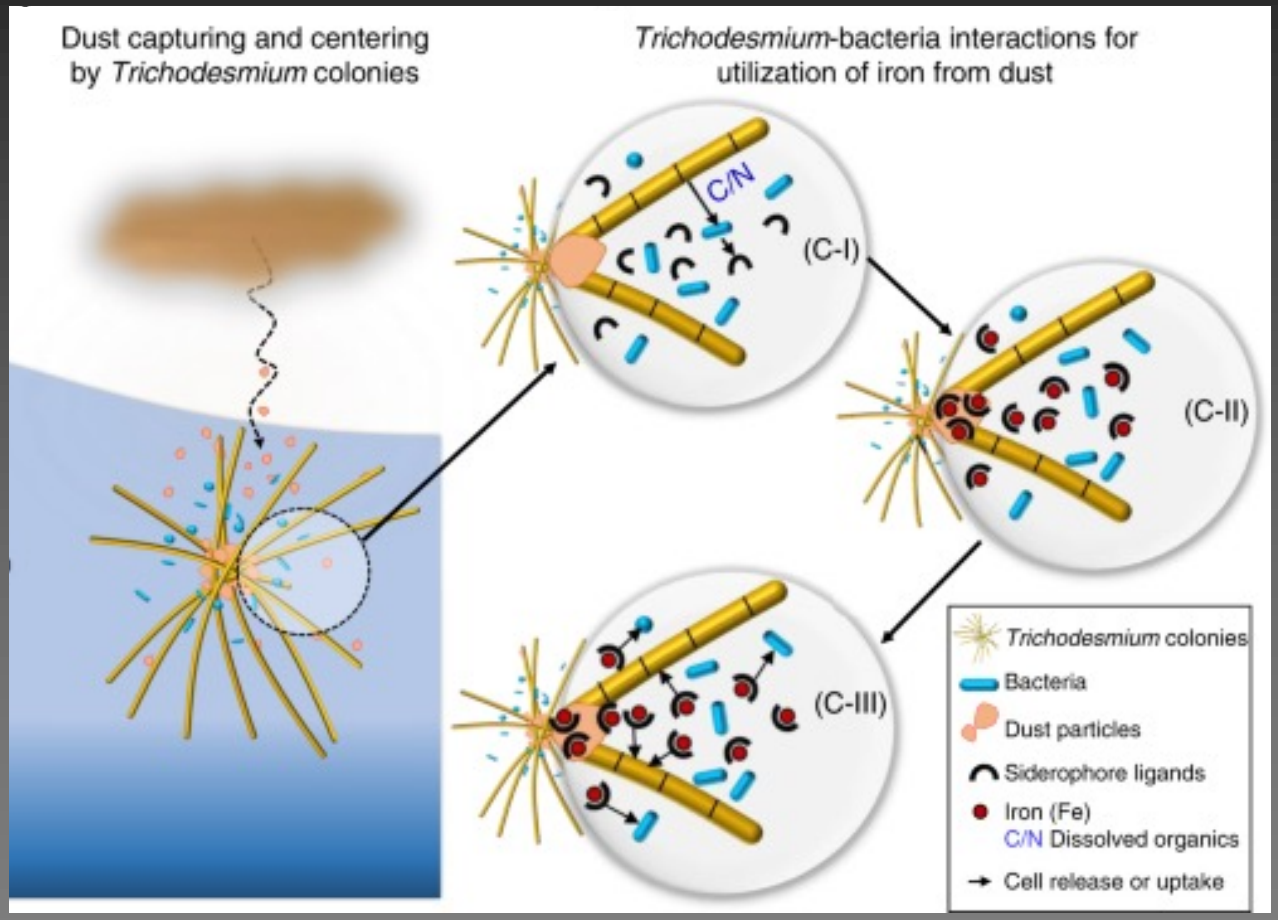


Frischkorn et al. (2017) *ISMEJ*

Organic Iron

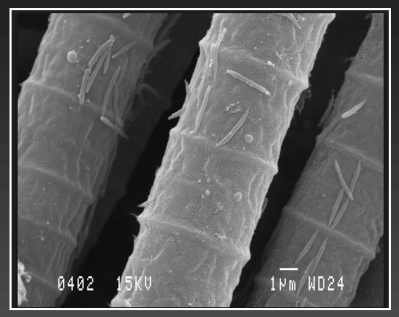
Epibionts can produce organic iron complexes that likely modulate iron in the holobiont microenvironment

Siderophore mining of iron from dust

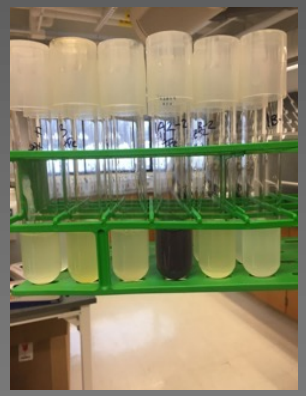


Basu et al. (2019) *Communication Biol.*

Cultured epibionts produce siderophores in low iron media

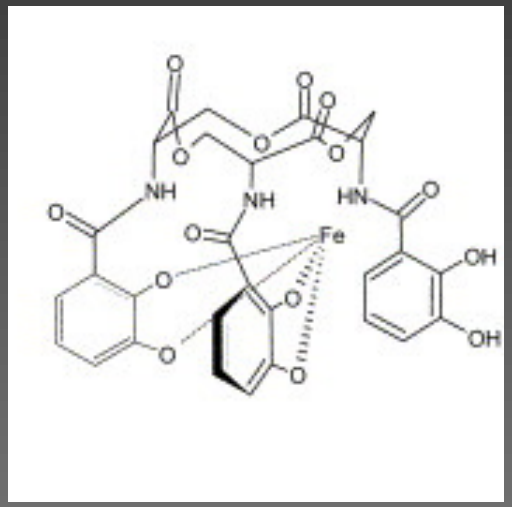


Isolate epibionts

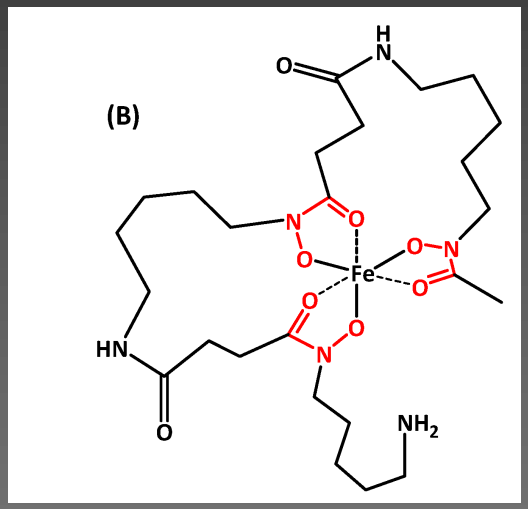


LC-MS

Aerobactin



Desferrioxamine B



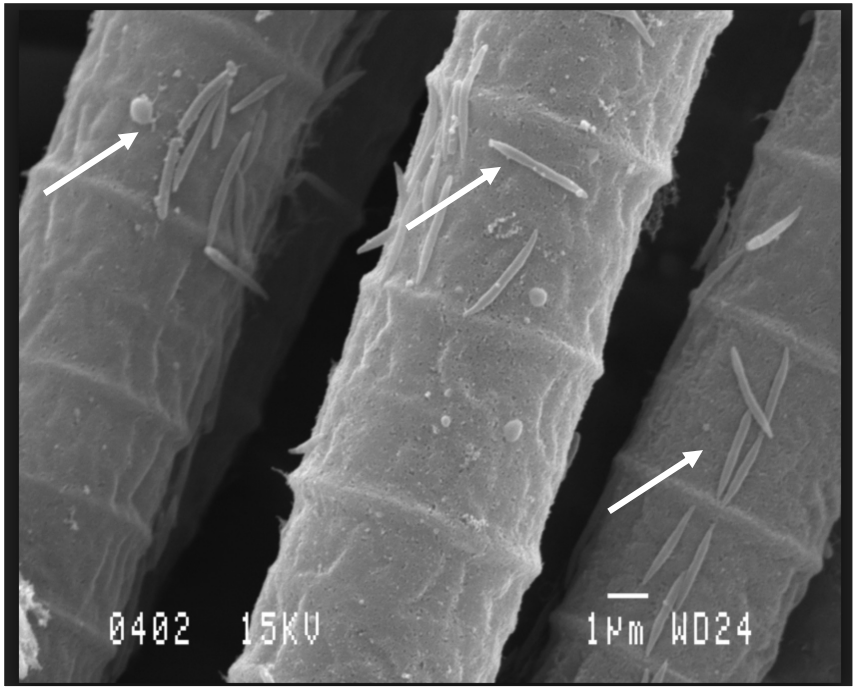
All six epibionts produce siderophores including those related to aerobactin and desferrioxamine consistent with MAG predictions and iron experiments.

O. Baars et al. unpublished

Lessons learned

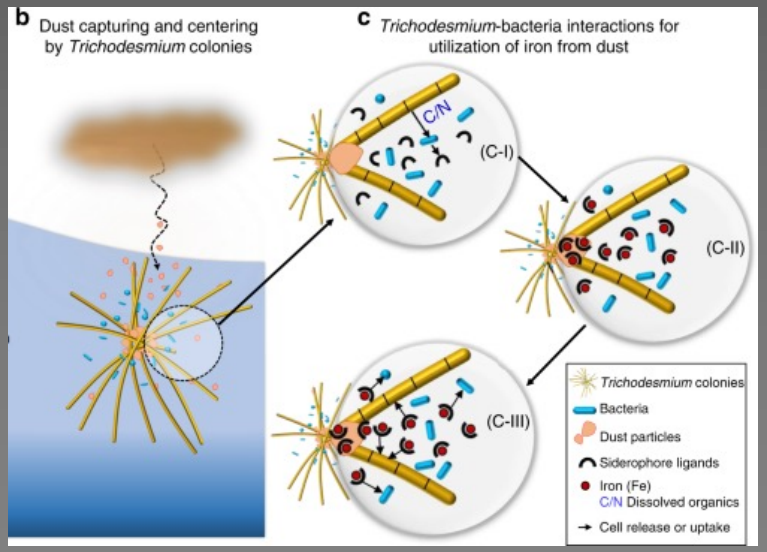
- *Trichodesmium* epibionts are functionally diverse, and these functions suggest complex interactions within holobiont (substrates, signals)

- There is an amazing microbiome on *Trichodesmium*! But we need to sequence deeper.



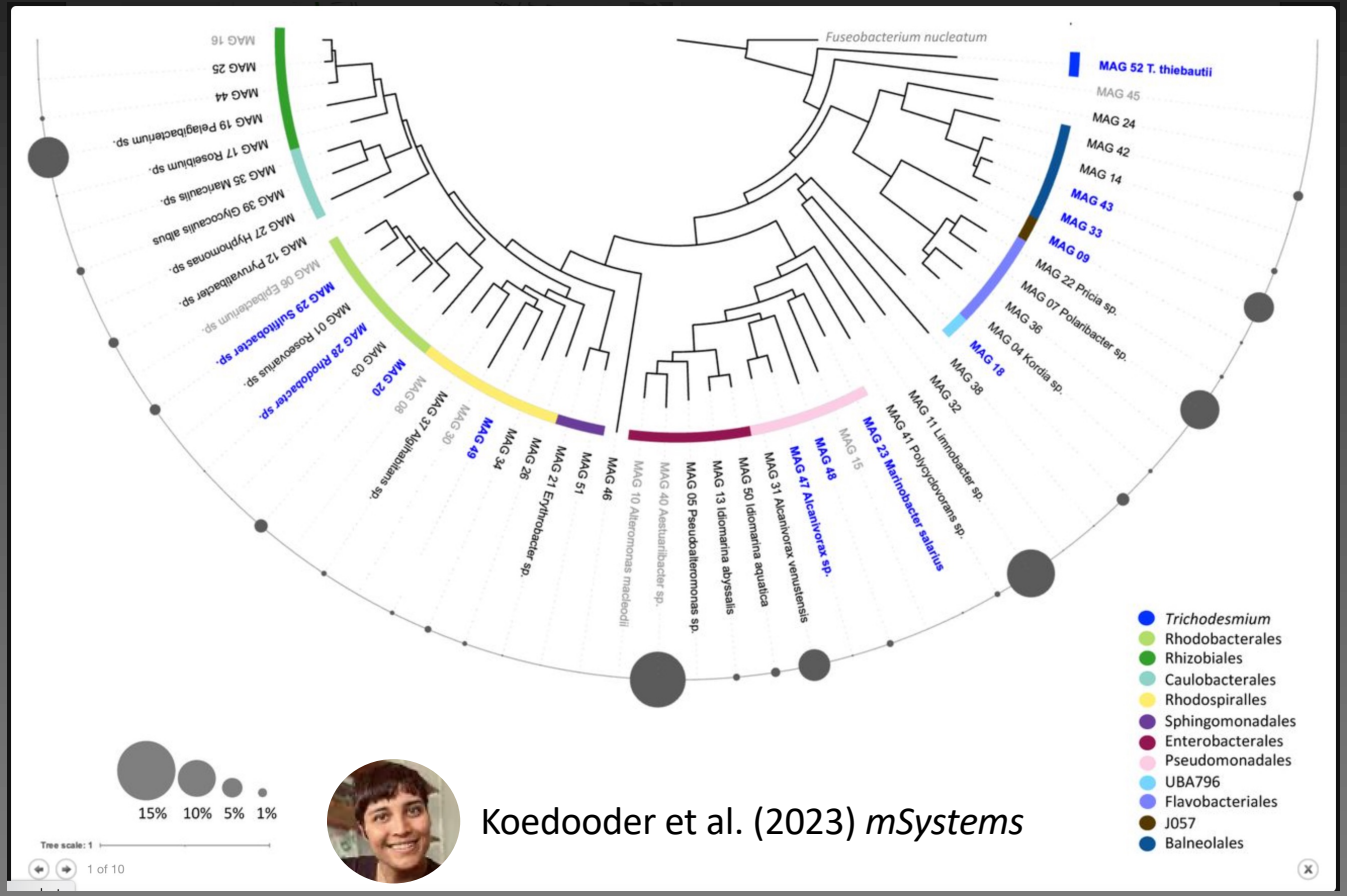
More microbiome sequencing…..

- Expanded biogeography in collaboration with Yeala Shaked of Hebrew University
- Seasonal *Trichodesmium* microbiome sequencing in Gulf of Aqaba



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

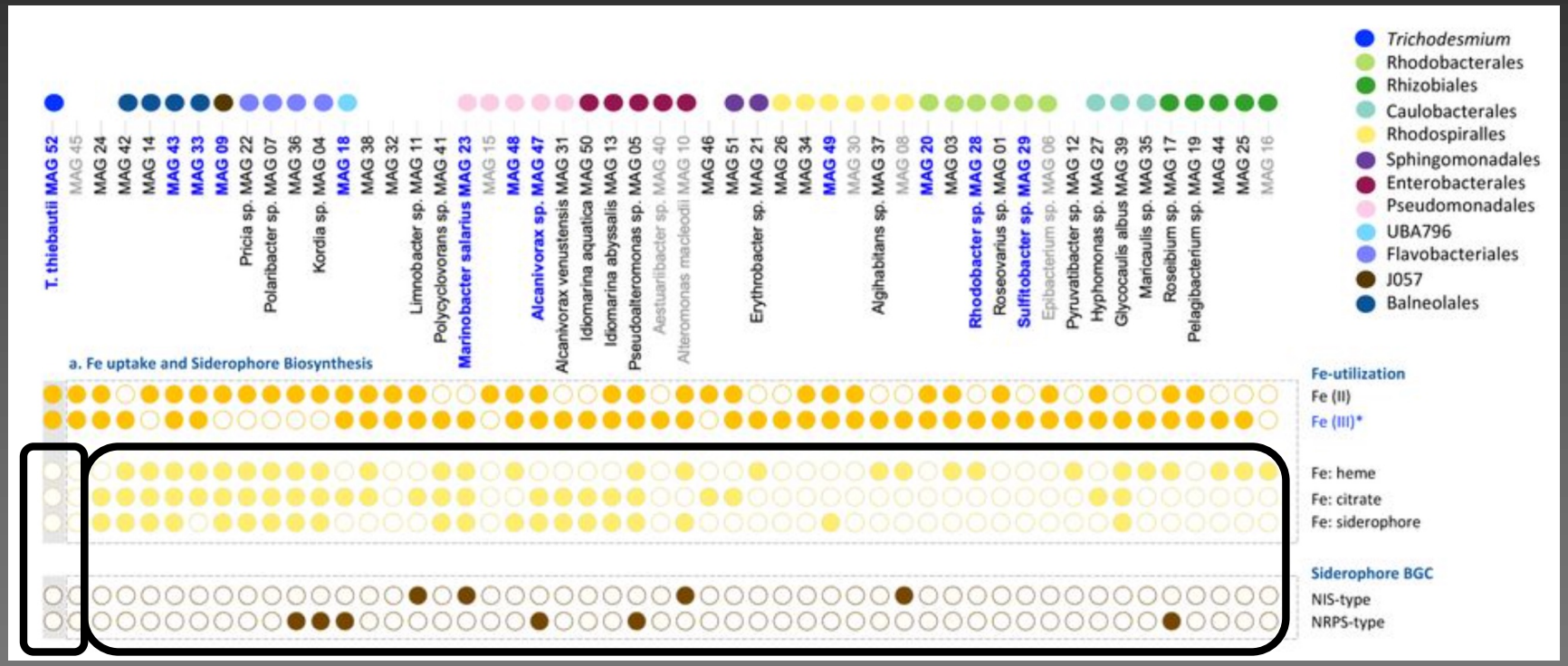
Epibiont distribution in Red Sea samples



Koedooder et al. (2023) *mSystems*

43 >98% complete MAG
11 MAG the same across time (blue)
Broad similarities with North Atlantic samples

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



Koedooder et al. (2023) *mSystems*

Red Sea epibionts are enriched in siderophore production (common goods) and organic Fe functions relative to *Trichodesmium*

User need for genome/pan genome data integration

- **Common frameworks and accessible data types facilitate team-science.**

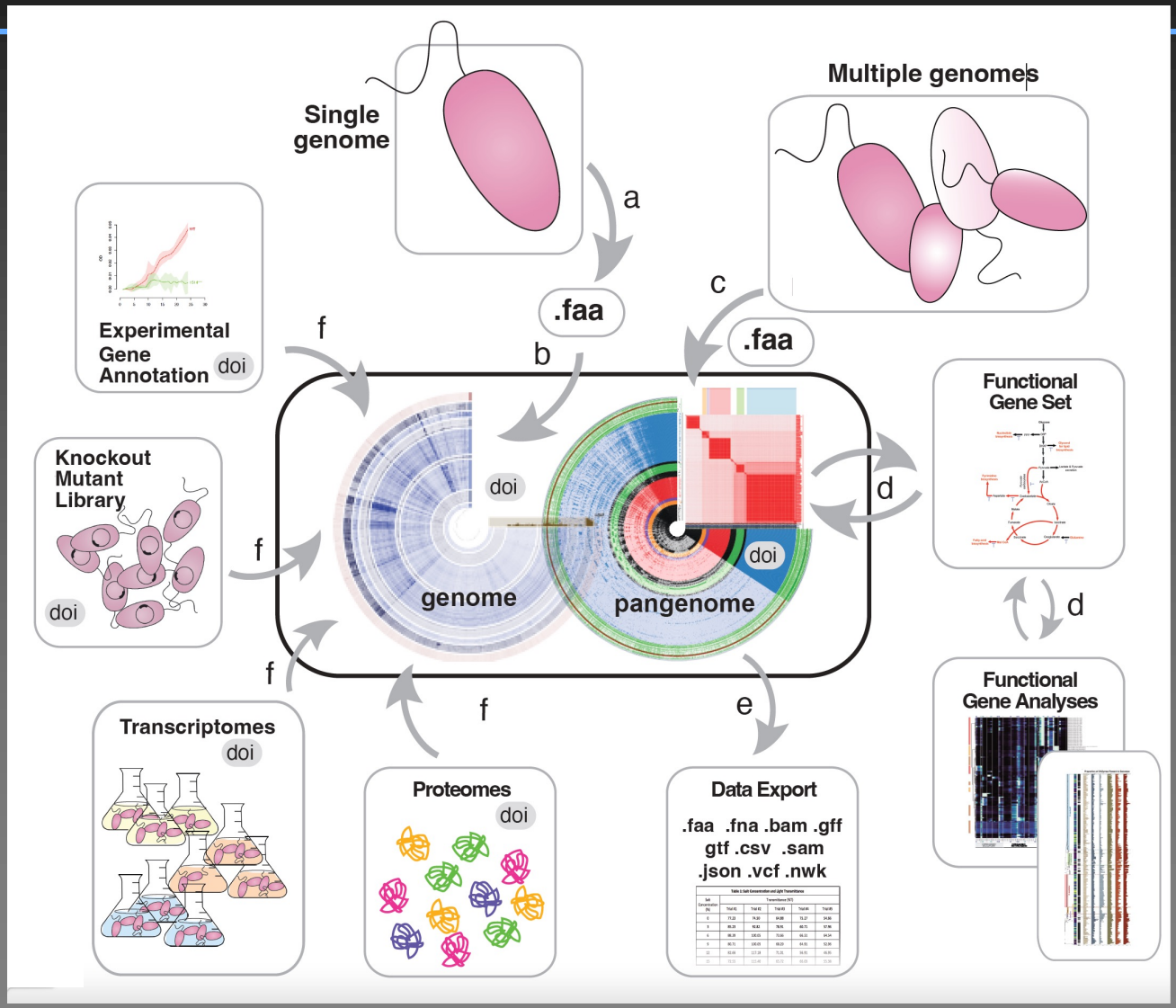
Digital Microbe: A Genome-Informed Data Integration Framework for Collaborative Research on Emerging Model Organisms

Iva Veseli[1,‡], Zachary S. Cooper[2,‡], Michelle A. DeMers[3,‡], Matthew S. Schechter[4], Samuel Miller[5], Laura Weber[6], Christa B. Smith[2], Lidimarie T. Rodriguez[7], William F. Schroer[2], Matthew R. McIlvin[6], Paloma Z. Lopez[6], Makoto Saito[6], Sonya Dyhrman[8], A. Murat Eren[5,9,10,11,†], Mary Ann Moran[2,†], Rogier Braakman[3,†]

C-CoMP

2021 2030 United Nations Decade of Ocean Science for Sustainable Development

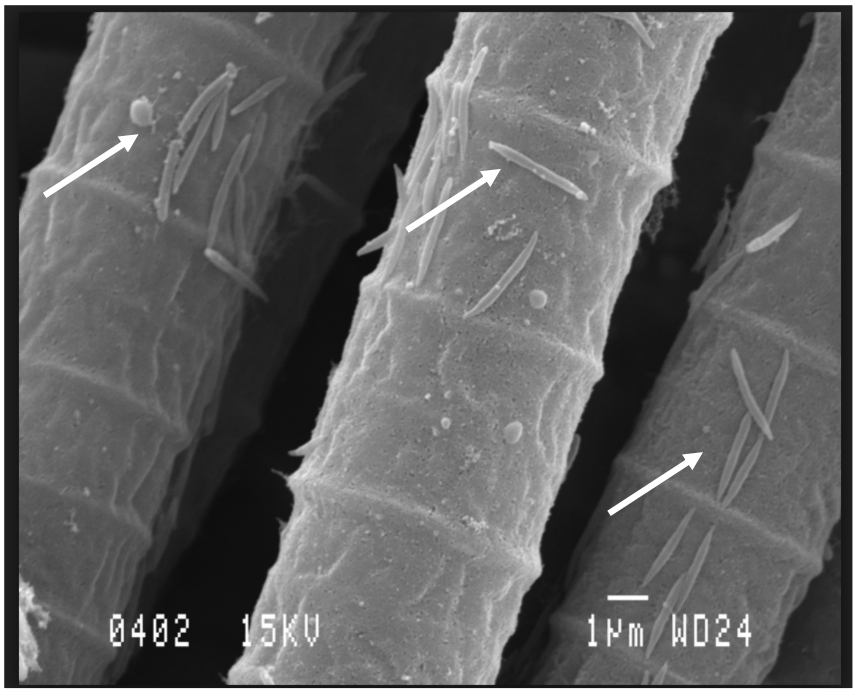
Digital microbes – a data package with data layers referenced to genes



Veseli et al. (pending submission) *Scientific Data*

Lessons learned….

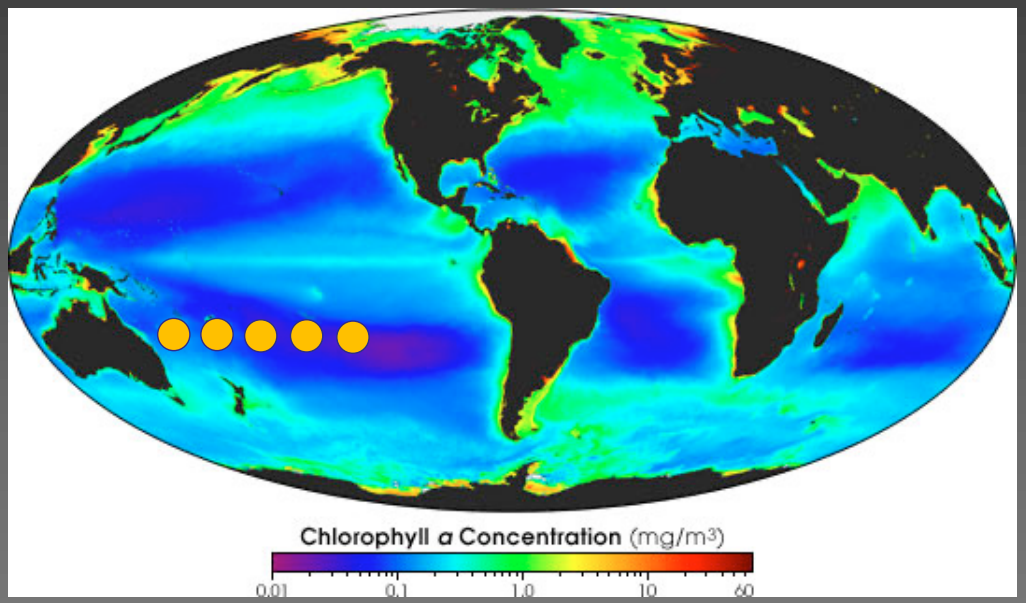
- Traits are consistent with regional geochemistry and host physiology
- Common workflows and accessible data types facilitate team-science.
- Now we are looking at how those metagenomic functions are expressed.



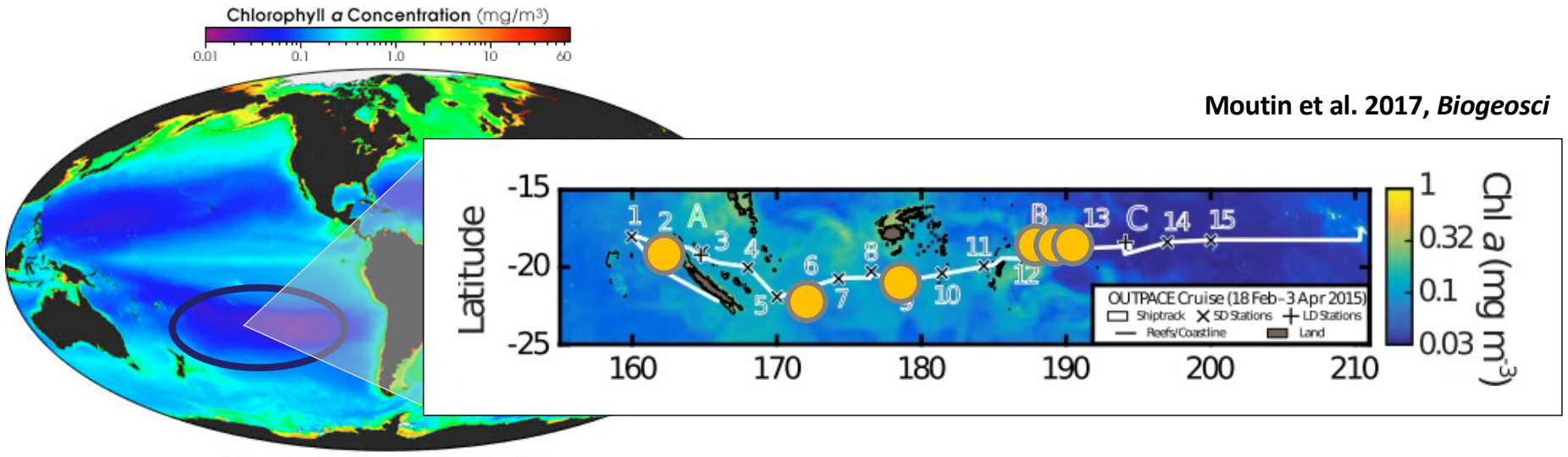
Coordinated analysis of phosphorus cycling

- 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

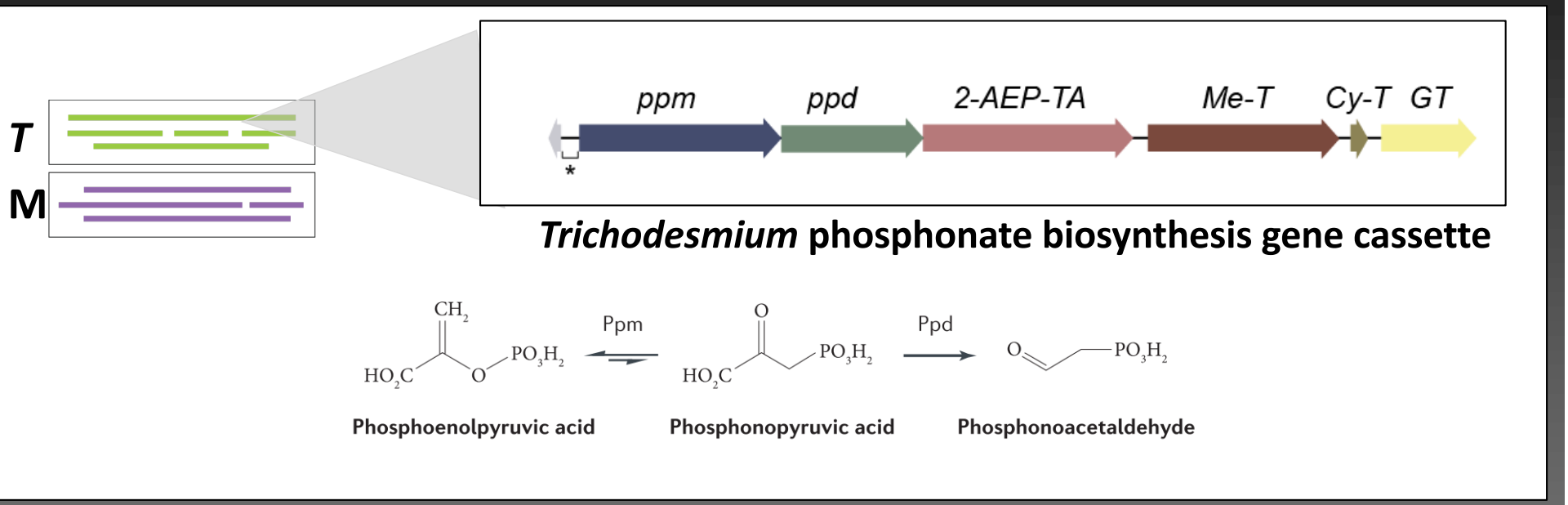


Moutin et al. 2017, *Biogeosci*

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

Metatranscriptomes

Metagenomic evidence of P reduction



Frischkorn et al. (2018) *Biogeoscience*

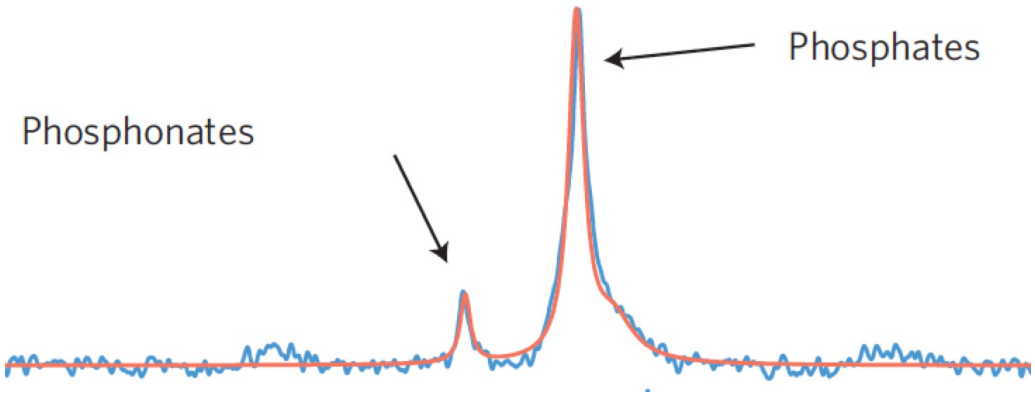
Complete gene set for phosphonate biosynthesis assembled in *Trichodesmium* genome bin (MAG).

Not present in microbiome MAGs

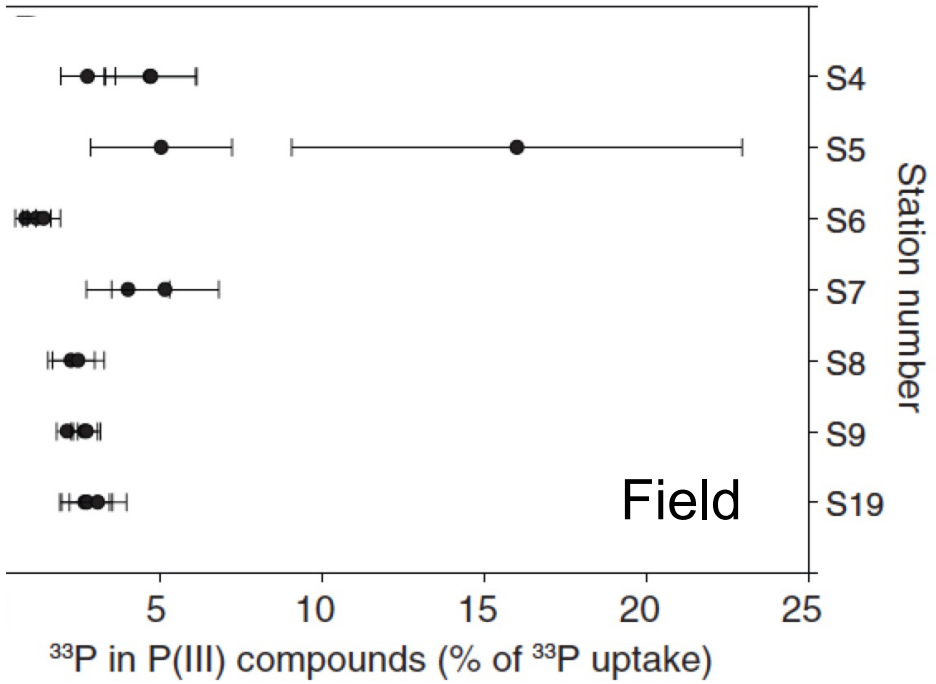
Answers to enduring mysteries… who makes C-P compounds?

Phosphonate (C-P) biosynthesis

Enrichment Culture



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



Van Mooy et al. (2015) *Science*

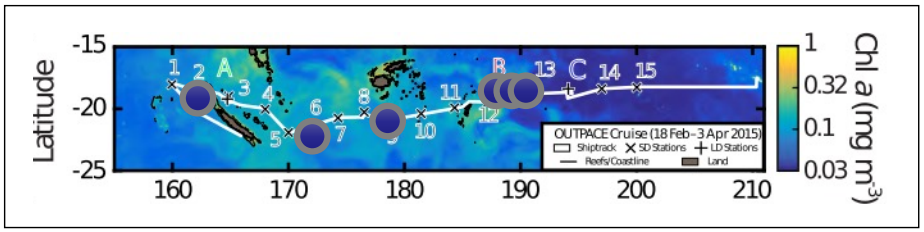
Phosphonates are produced at high rates in the holobiont - hot spot for reduced phosphorus cycling. Is it *Trichodesmium* or the epibionts?

Genes are expressed with P reduction

Measure gene expression

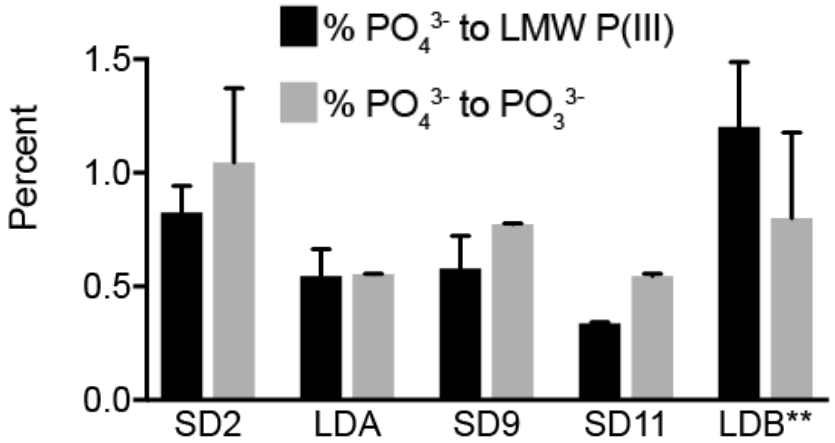
Metatranscriptome reads mapped to the P reduction gene cassette

Frischkorn et al. (2018) *Biogeoscience*



Measure phosphate reduction

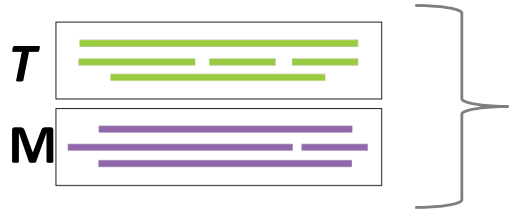
Percentage of radiolabeled phosphate taken up and reduced by *Trichodesmium* colonies



***methylphosphonate, phosphonoacetylaldehyde, or 2-aminoethylphosphonate**

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

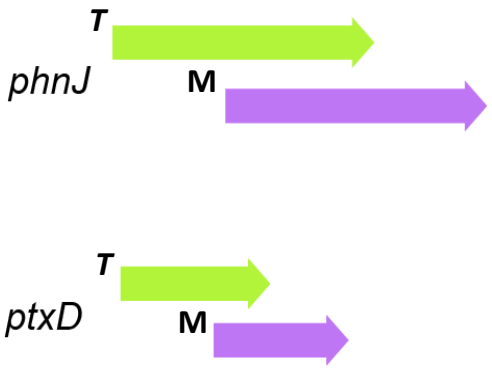
Evidence for metabolism of reduced phosphorus compounds in *Trichodesmium* and the microbiome



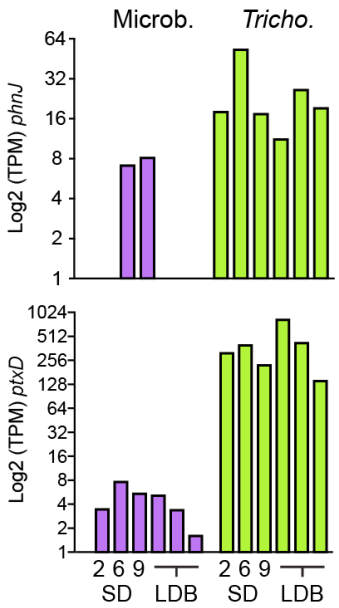
Query for genes:

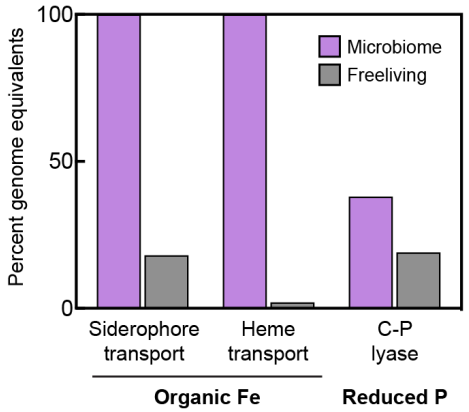
- **Phosphonate C-P lyase (*phnJ*)**
- **Phosphite dehydrogenase (*ptxD*)**

✓ **Genes detected**



✓ **Genes expressed**

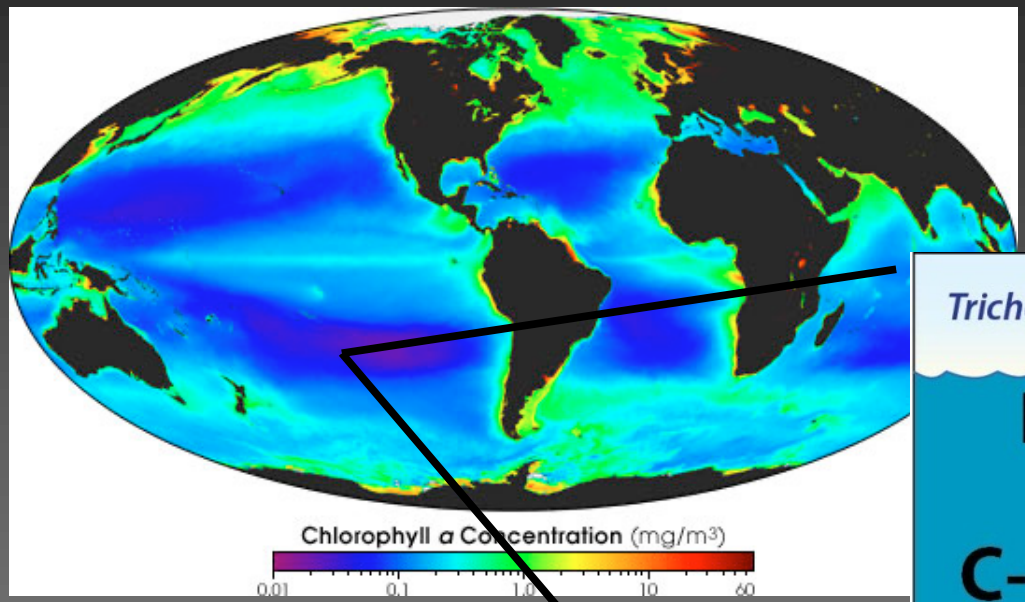


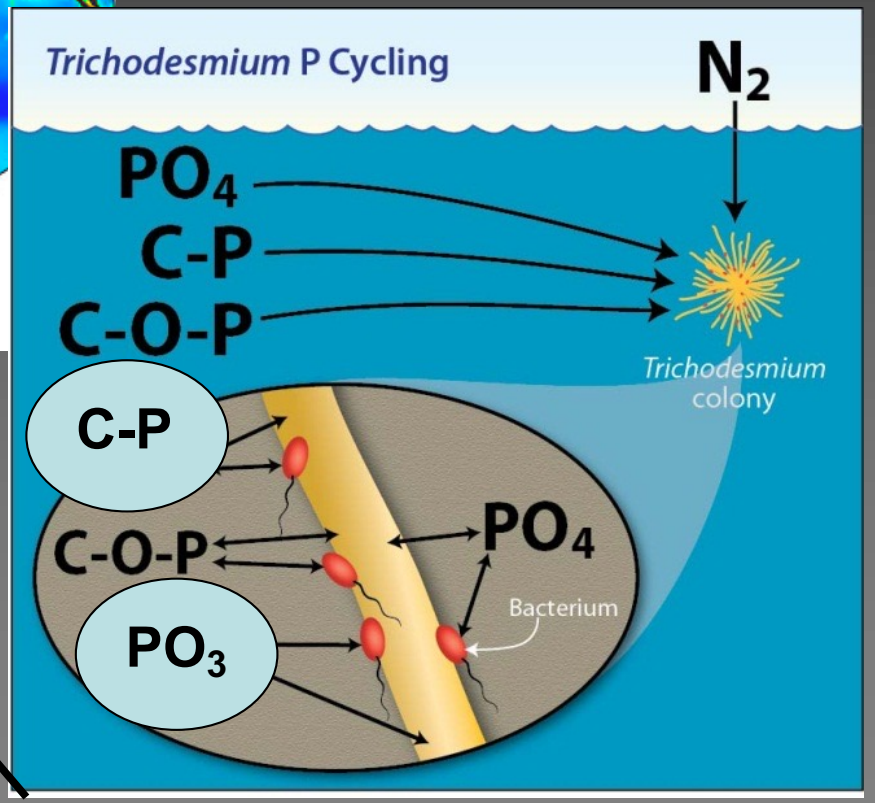


Ability to access reduced P is enriched in *Trichodesmium* consortia

Frischkorn et al. (2018) *Biogeoscience*

Novel P currencies used in holobiont….





Mitigate competition for scarce resource?

Graphic: WHOI

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

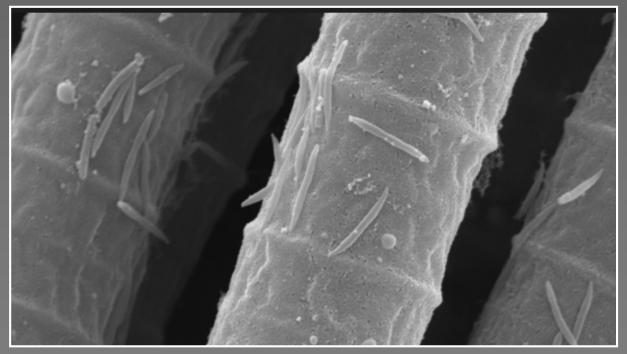
Core questions about the *Trichodesmium* microbiome

Who is there?

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

What are they doing?

- **Microbiome contributes functional diversity to community**

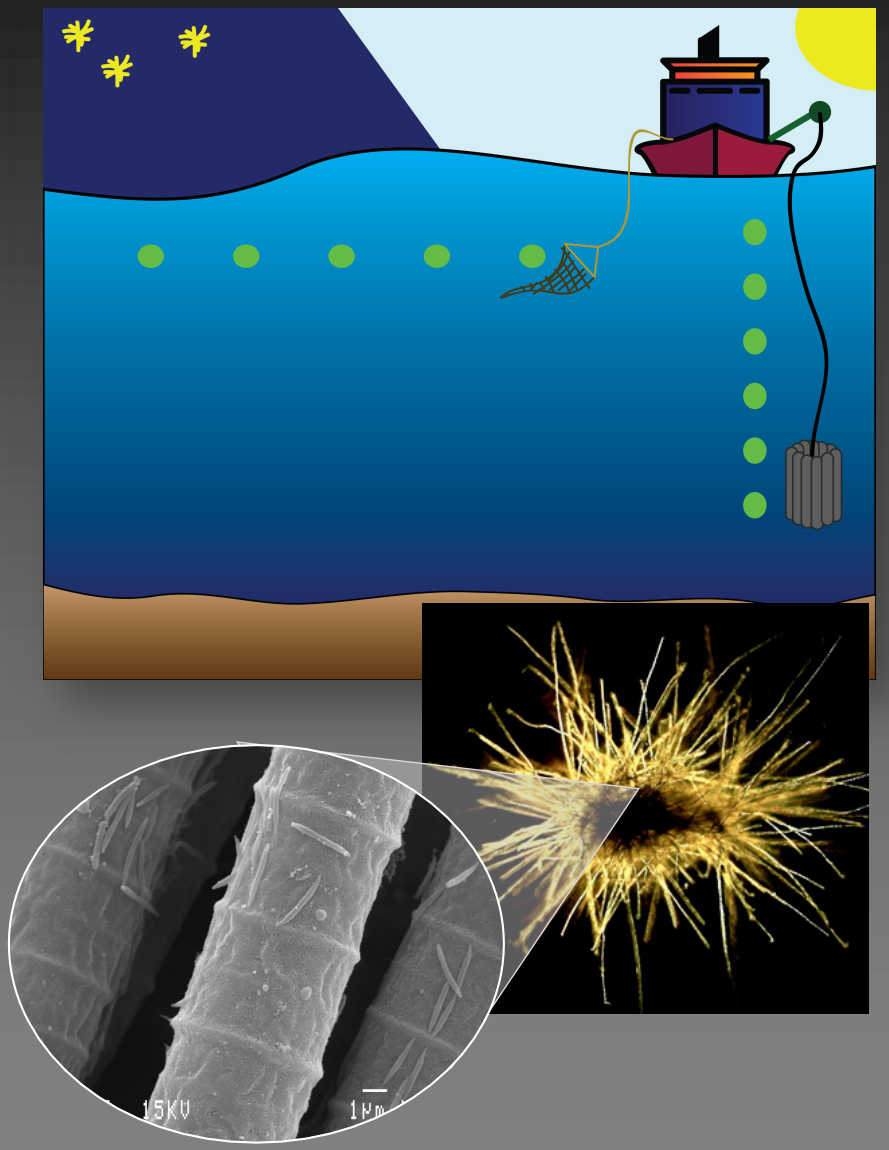


Are they interacting?

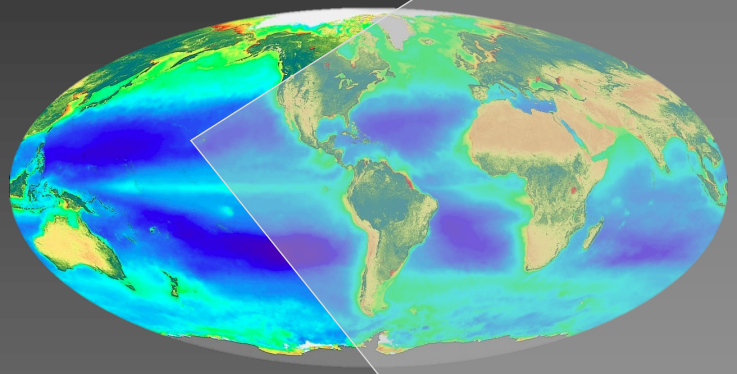
- Diel patterns in the consortia

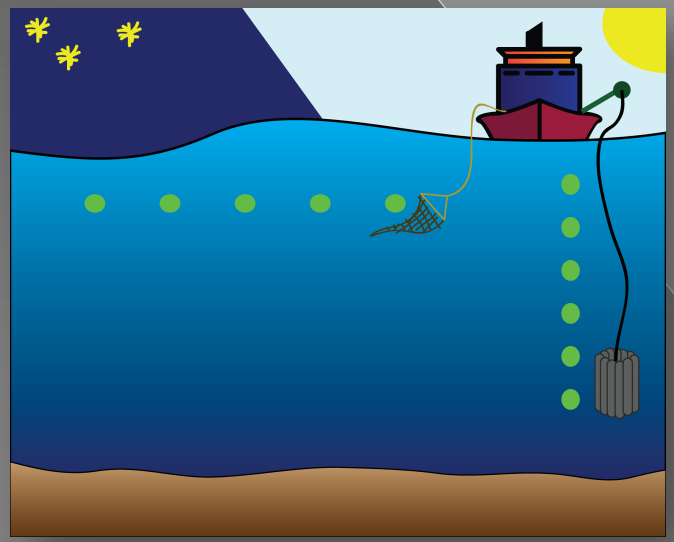
Using light to study interactions

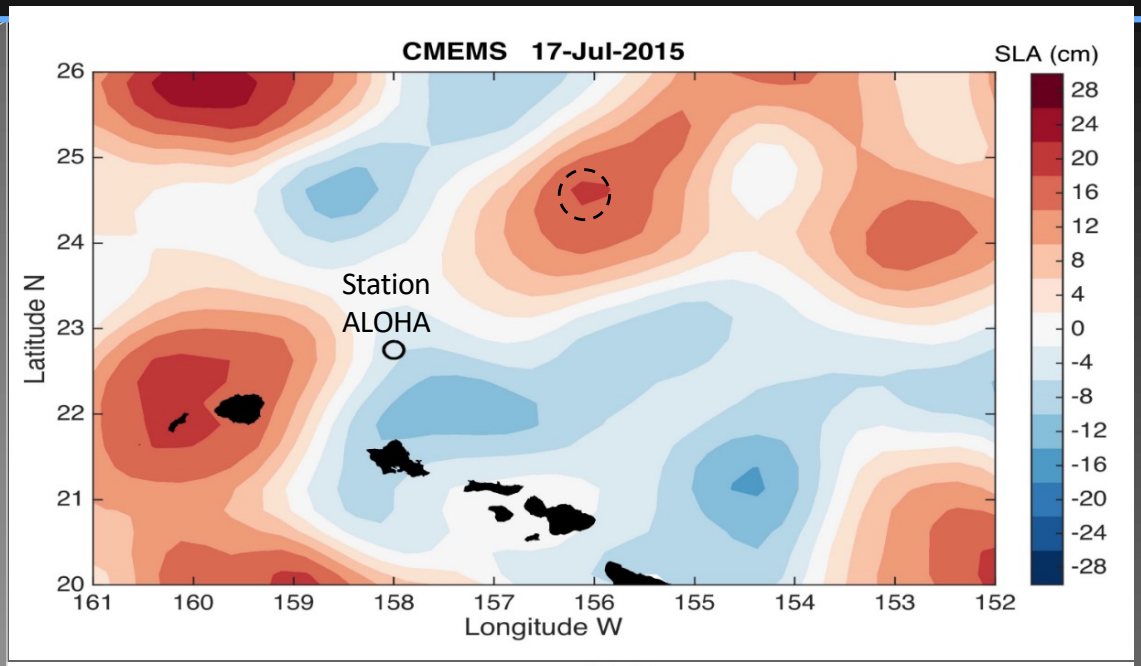
- Light drives diel changes in *Trichodesmium* CO_2 and N_2 fixation
- Do epibionts have diel responses, and what might they tell us about interactions between the host and microbiome?

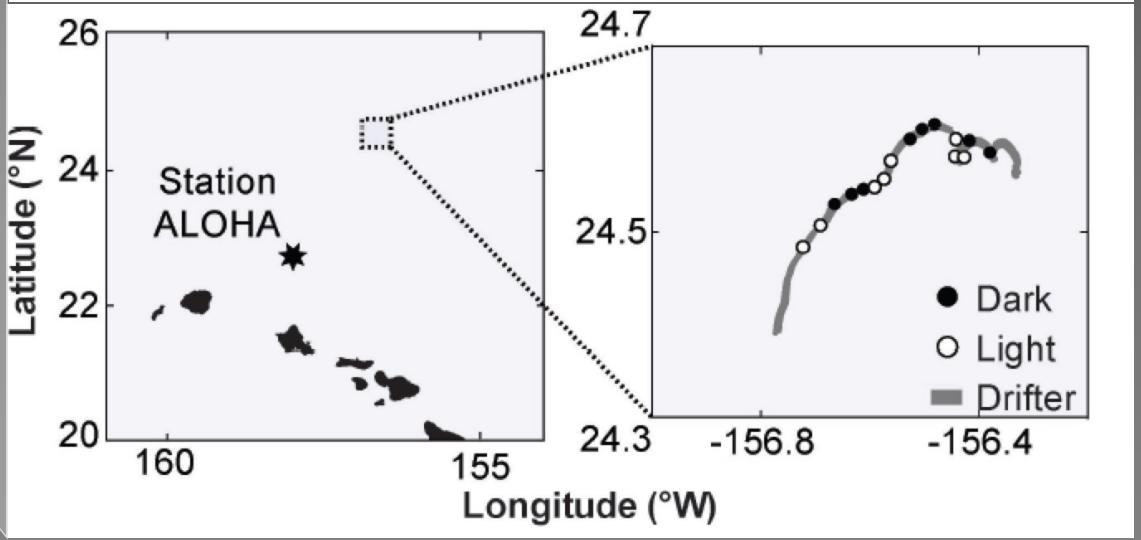


Sampling light driven responses in the NPSG

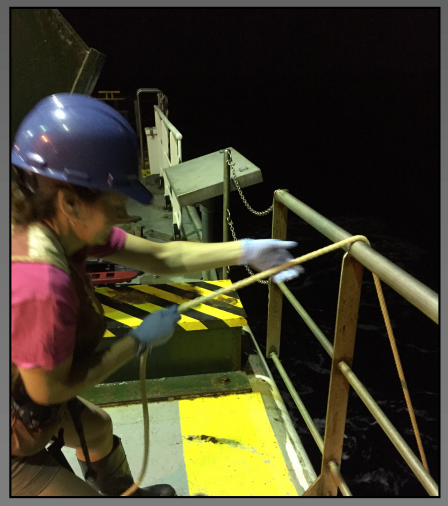


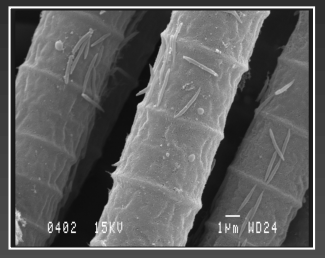






Coordinated expression dynamics in host and microbiome





Metatranscriptomes

↓

Orthologous group analysis

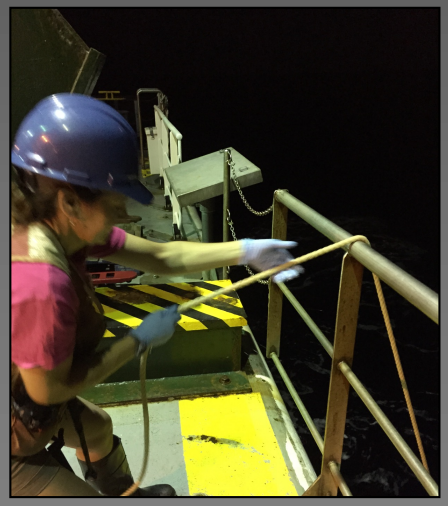
↓

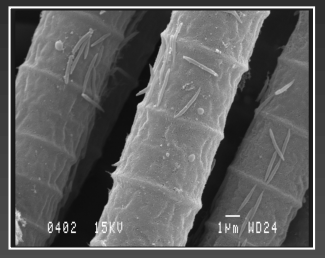
Periodicity: RAIN
Co-expression: WGCNA

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

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

Coordinated expression dynamics in host and microbiome





Metatranscriptomes

↓

Orthologous group analysis

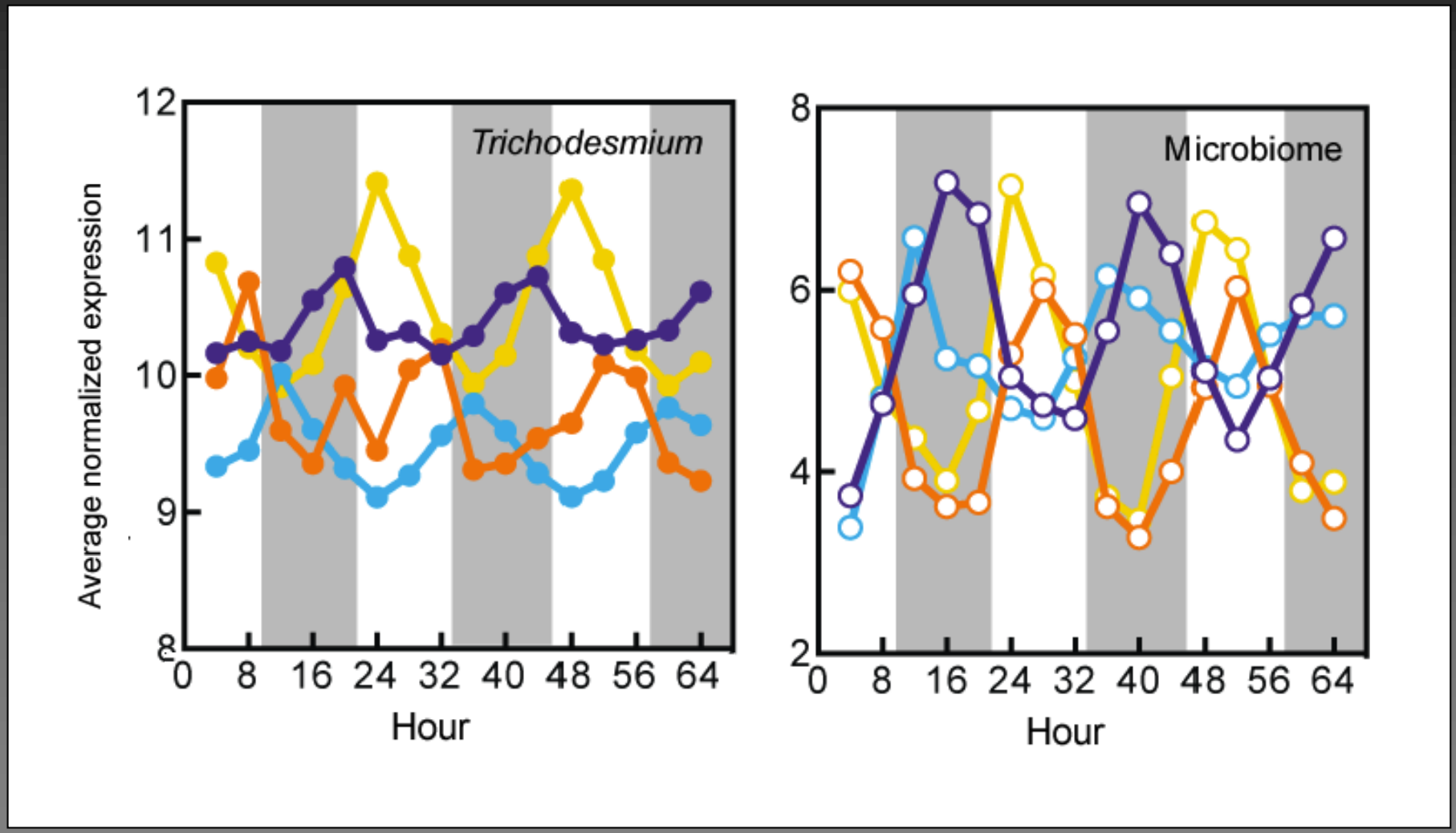
↓

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

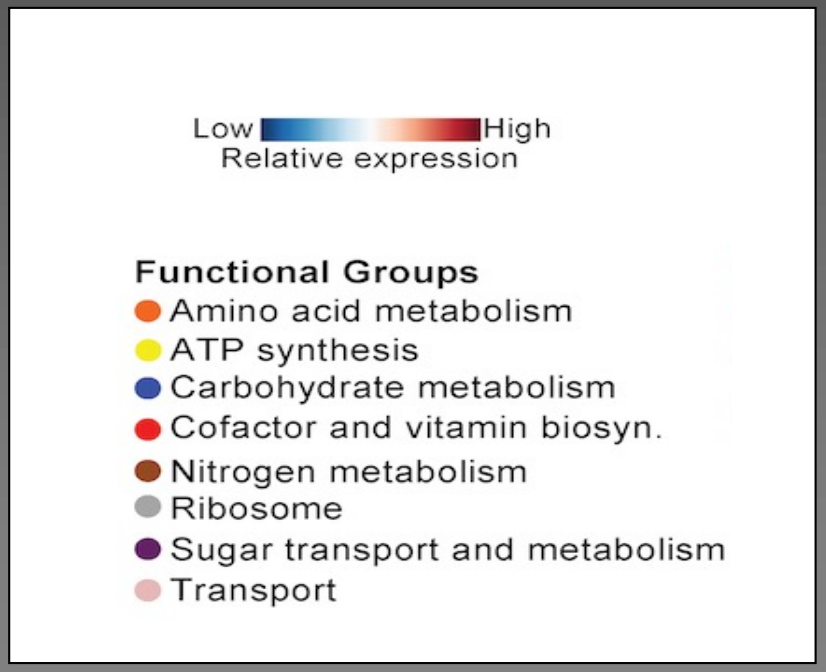
Diel modulation of transcripts in *Trichodesmium*

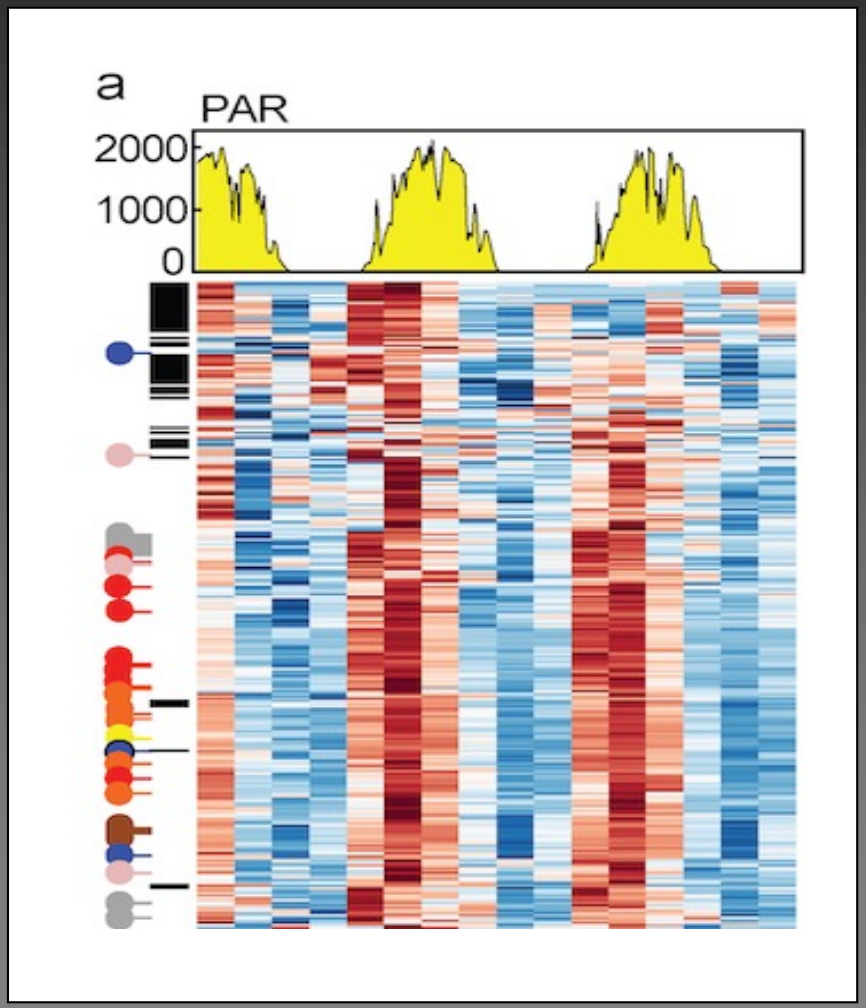


Frischkorn et al. (2018) *ISMEJ*

WGCNA co-expression network

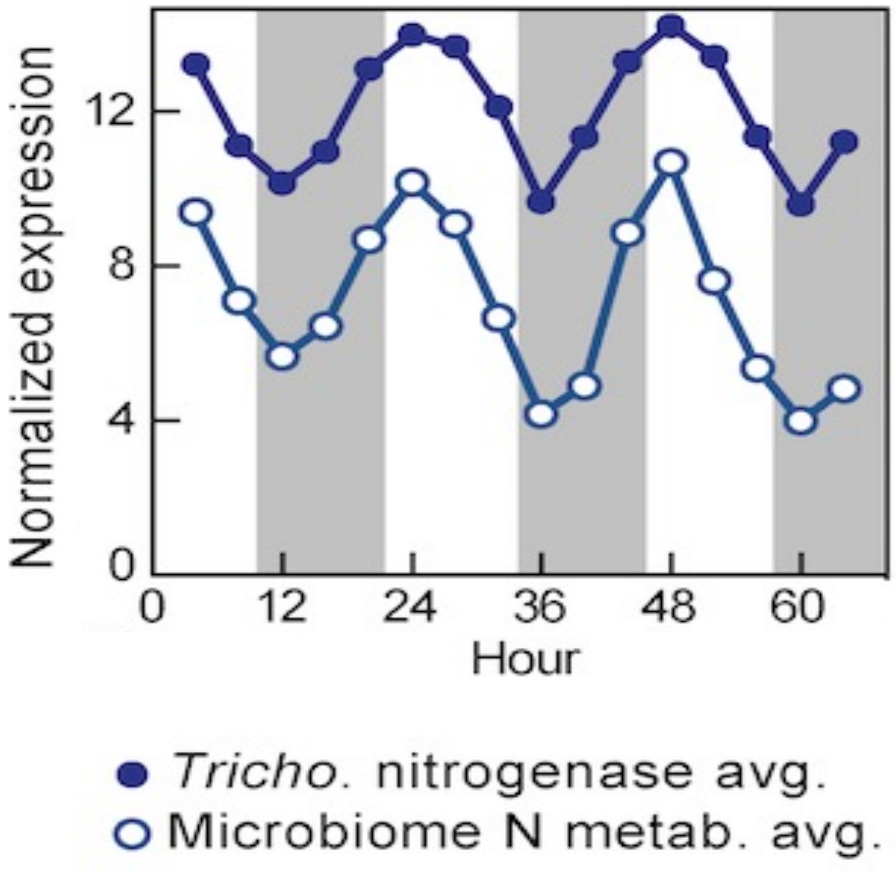
Module example: Significantly coordinated expression patterns between *Trichodesmium* and microbiome.

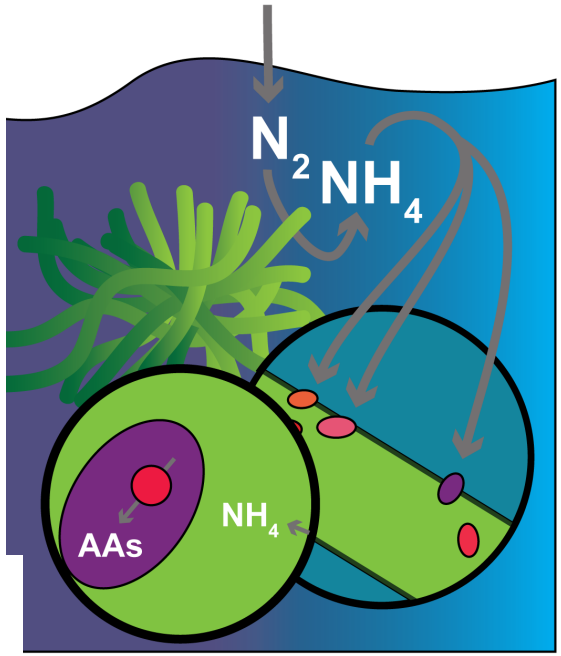




Frischkorn et al. (2018) *ISMEJ*

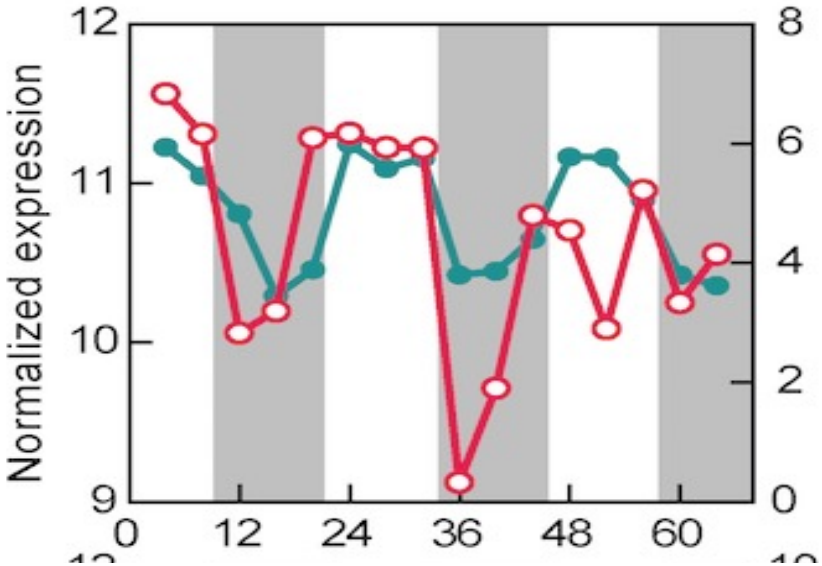
Diel modulation of transcripts suggest coordination: Nitrogen





Frischkorn et al. (2018) *ISMEJ*

Diel modulation of transcripts suggests coordination: Carbon



$CO_2 \rightarrow$ DOC $\rightarrow$ CO_2

Trichodesmium Microbiome

- *Tricho.* photosystem & C fixation avg.
- Microbiome respiration avg.

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, substrate exchanges and other activities in the holobiont.

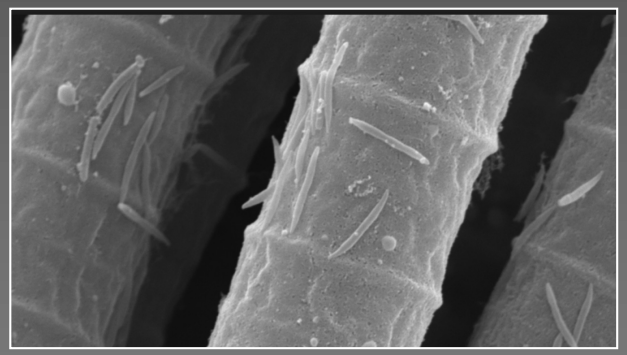
Core questions about the *Trichodesmium* microbiome

Who is there?

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

What are they doing?

- **Microbiome contributes functional diversity to community**



Are they interacting?

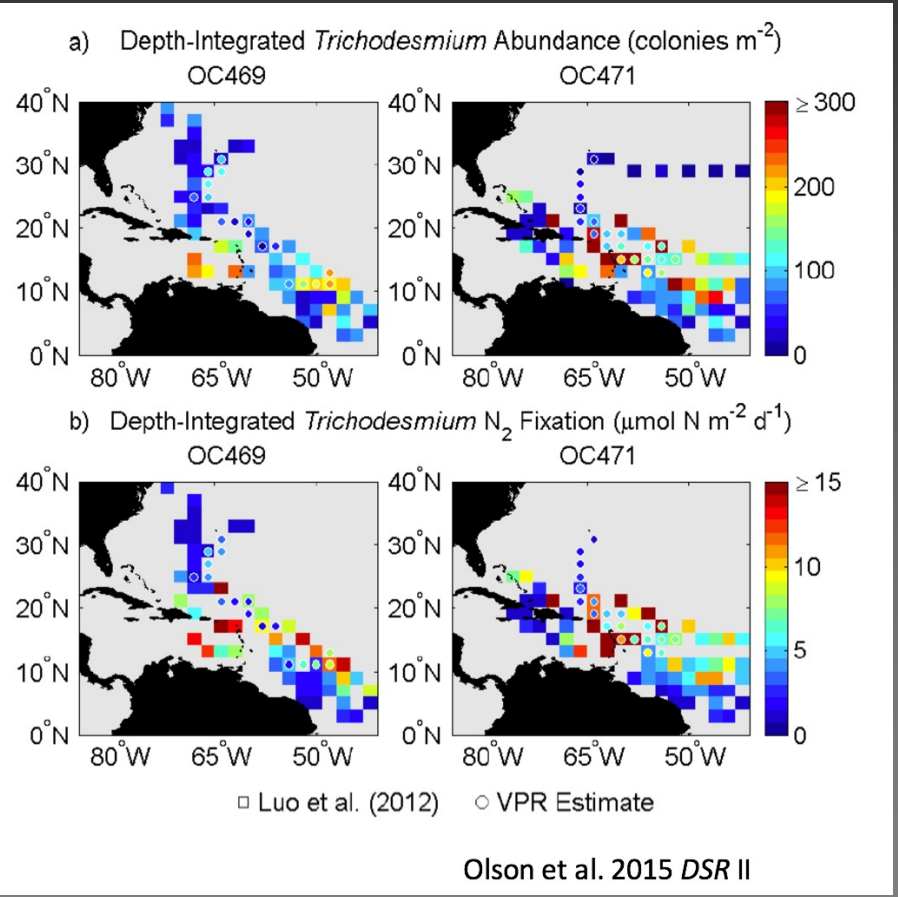
- **Yes! Significant co-expression of host-microbiome genes**

Host → Microbiome

Microbiome → Host

Can the microbiome influence host N_2 fixation?

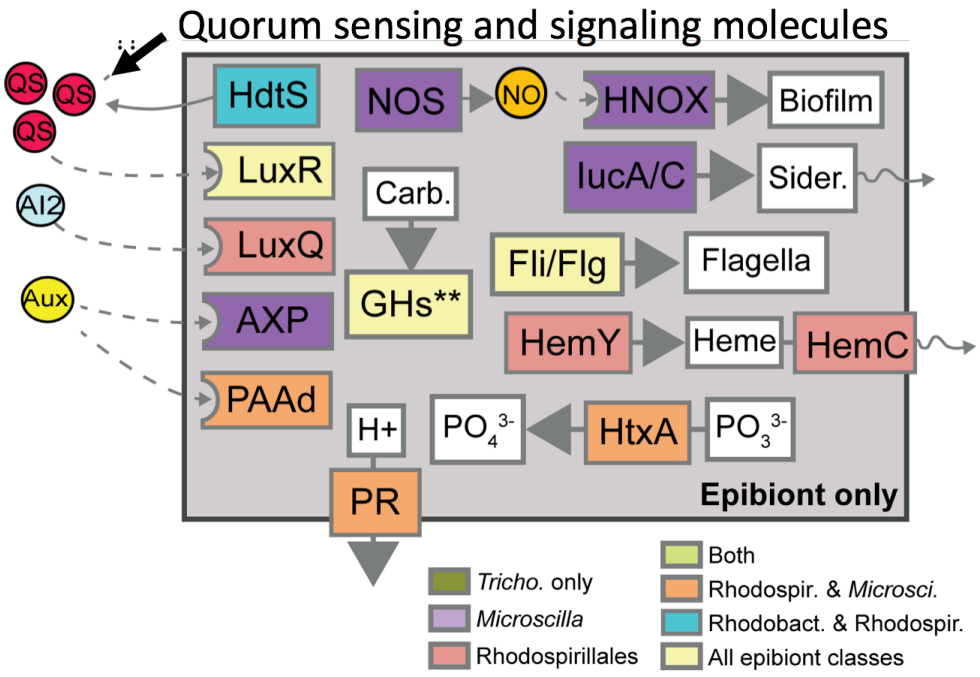
Maybe the models are missing the role of the microbiome?



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*

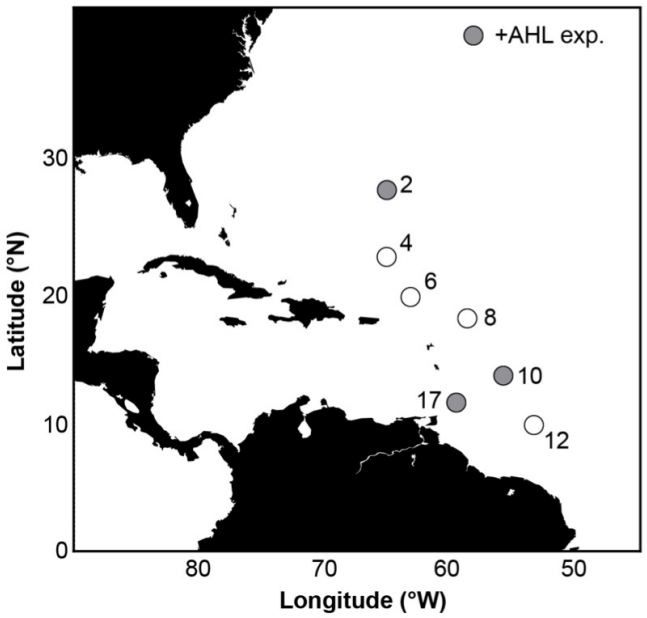


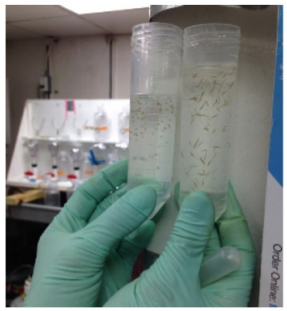
Frischkorn et al. (2017) *ISMEJ*

Selective manipulation of the microbiome…

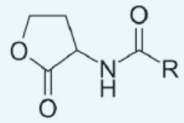
Do microbiome infochemicals influence Trichodesmium phenotype?

Selective manipulation of the epibiont activities with AHLs

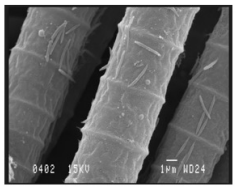




N-acyl homoserine lactone



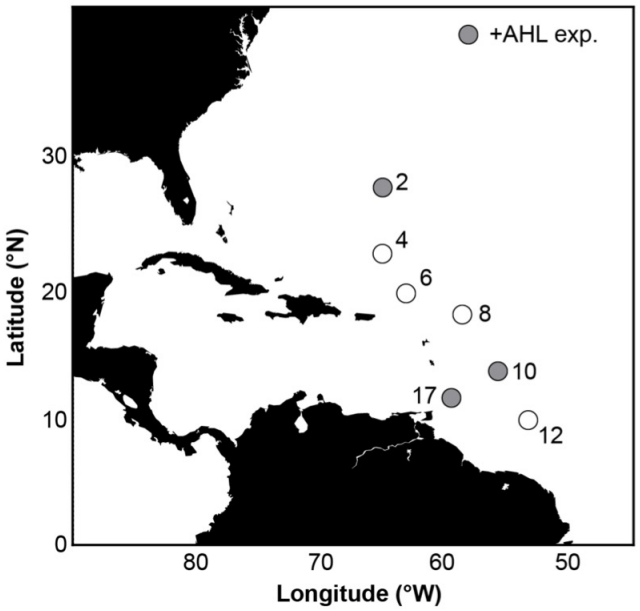
Microbiome

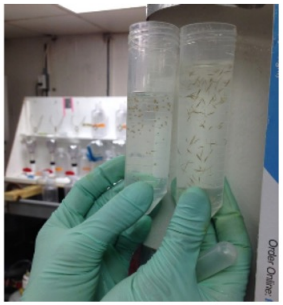


Trichodesmium N_2 Fixation

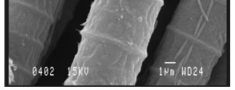
AHL incubations modulate *Trichodesmium* N_2 fixation

Epibiont infochemicals influence host phenotype



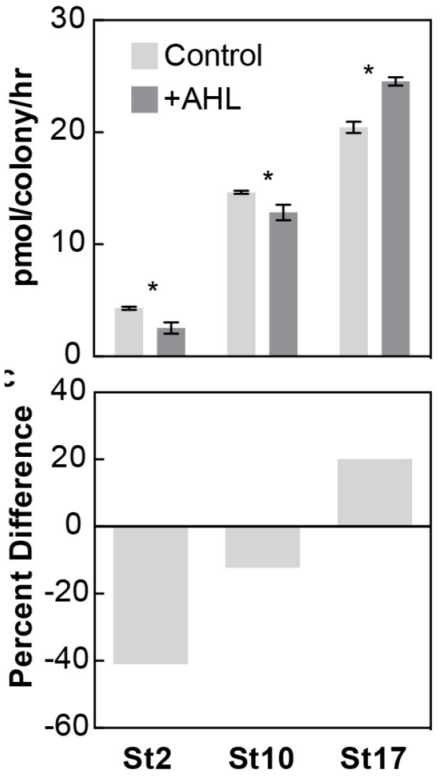


N-acyl homoserine lactone



Microbiome

Trichodesmium N_2 Fixation



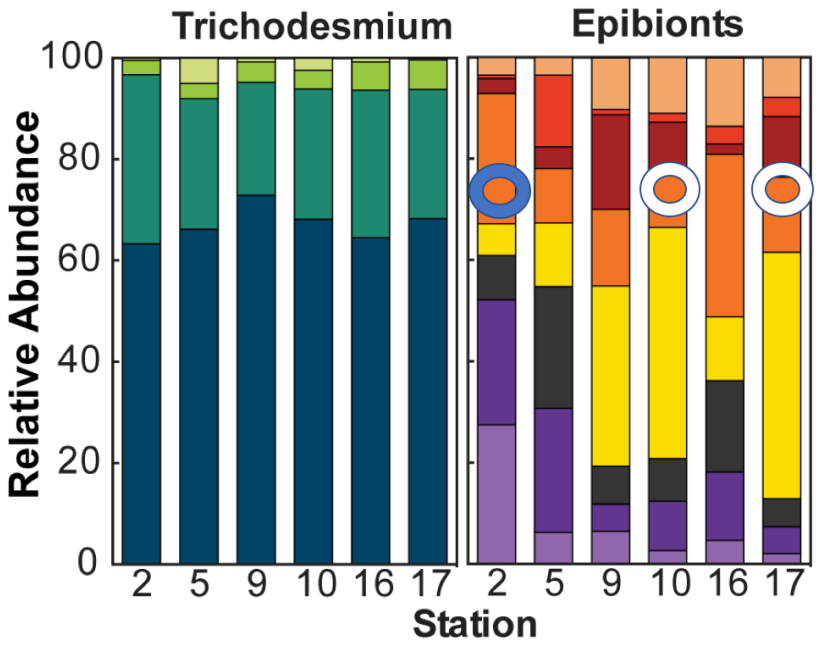
Frischkorn et al. (2018) *L&O Letters*

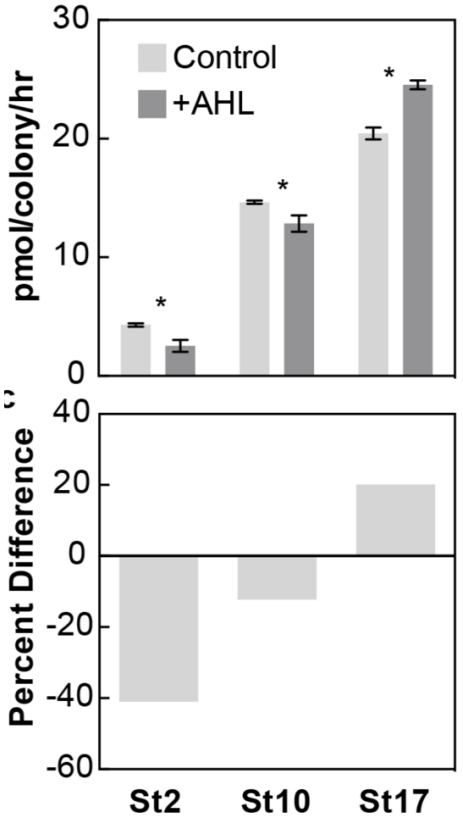
Microbiome can modulate host N_2 fixation rates up to 40%, in orthogonal directions, and independently of known drivers (P, Fe, light, temperature)

Mechanisms still uncertain…

What is the mechanism driving the N_2 fixation response?

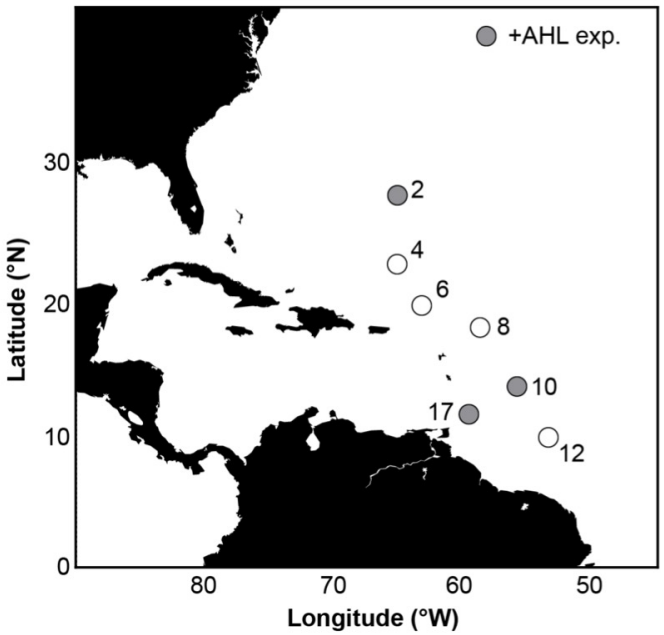
Changes in epibiont community structure, and *Trichodesmium* physiology could play a role.

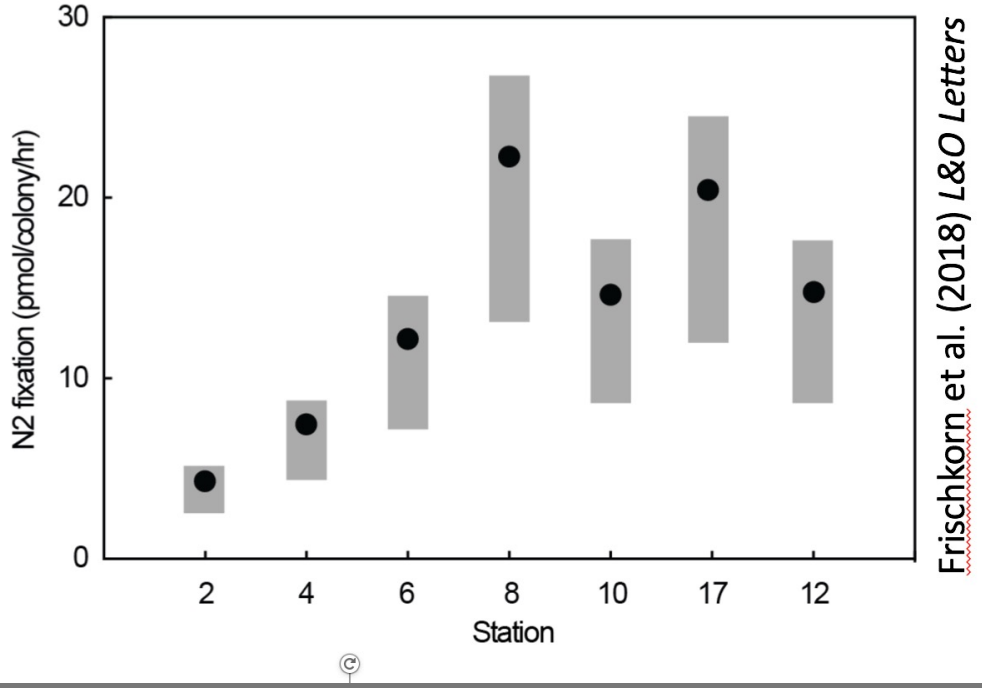




Microbiome activities modulate N_2 fixation in host

Epibiont infochemicals influence ecosystem drivers



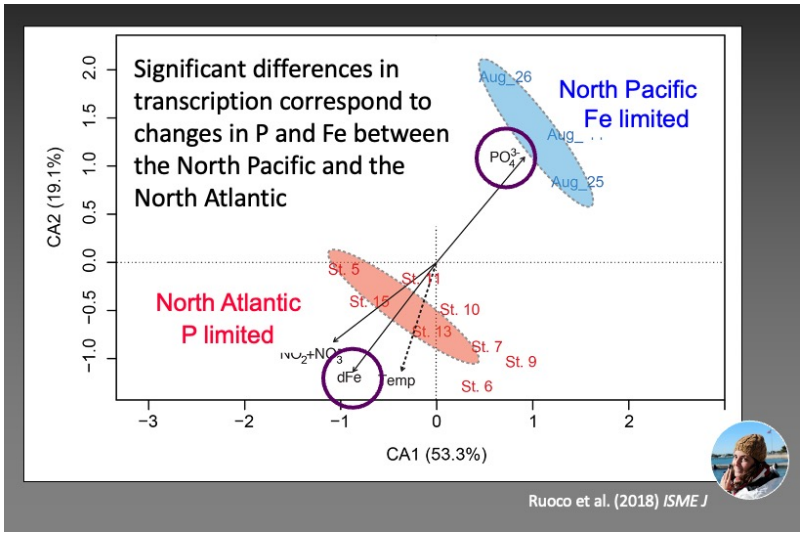


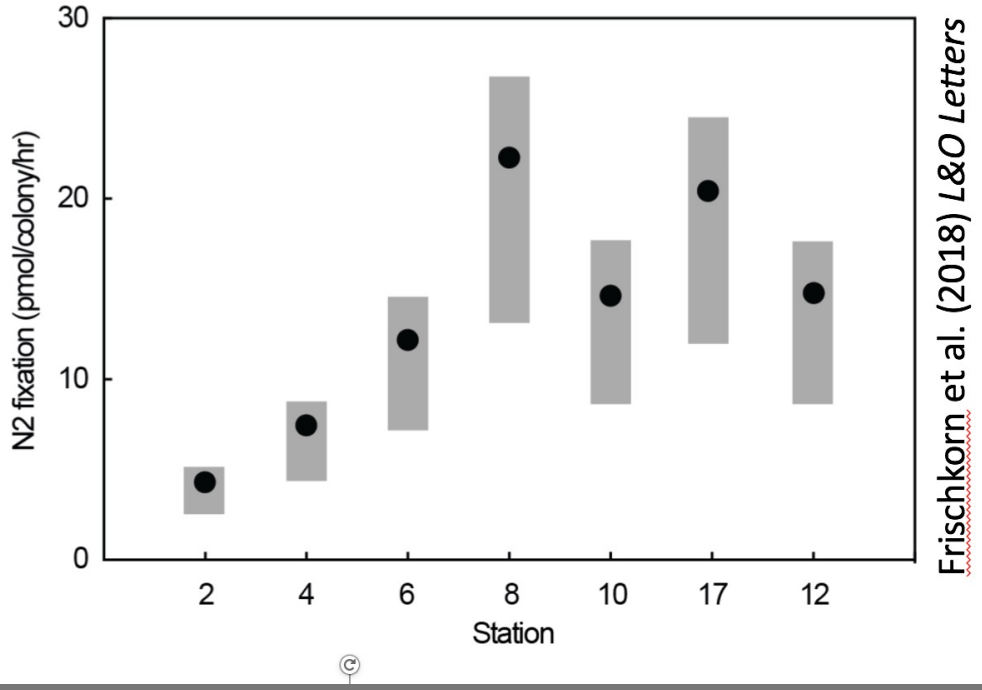
Biological interactions are a previously unrecognized driver of N_2 fixation

Need to revise modeling efforts focused on drivers of N_2 fixation

Microbiome activities modulate N_2 fixation in host

Epibiont infochemicals influence ecosystem drivers





Biological interactions are a previously unrecognized driver of N_2 fixation

Need to revise modeling efforts focused on drivers of N_2 fixation to include the geochemistry (e.g. Fe and P) and the biology (interactions)!

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

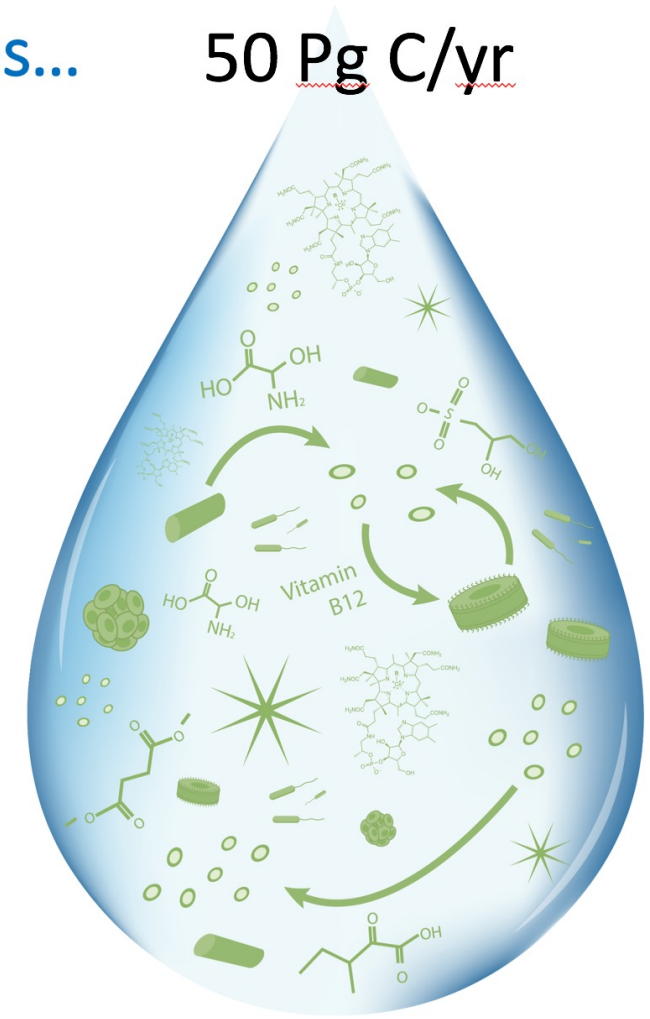
- **16S community amplicon sequencing**: Colonies harbor diverse epibionts distinct from water column, that are dynamically curated across gradients in the environment
- **Metagenomics**: Epibionts confer substantial metabolic potential which likely underpins *Trichodesmium* fitness
- **Metatranscriptomics**: 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…

- 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*



Graphic: WHOI

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!