

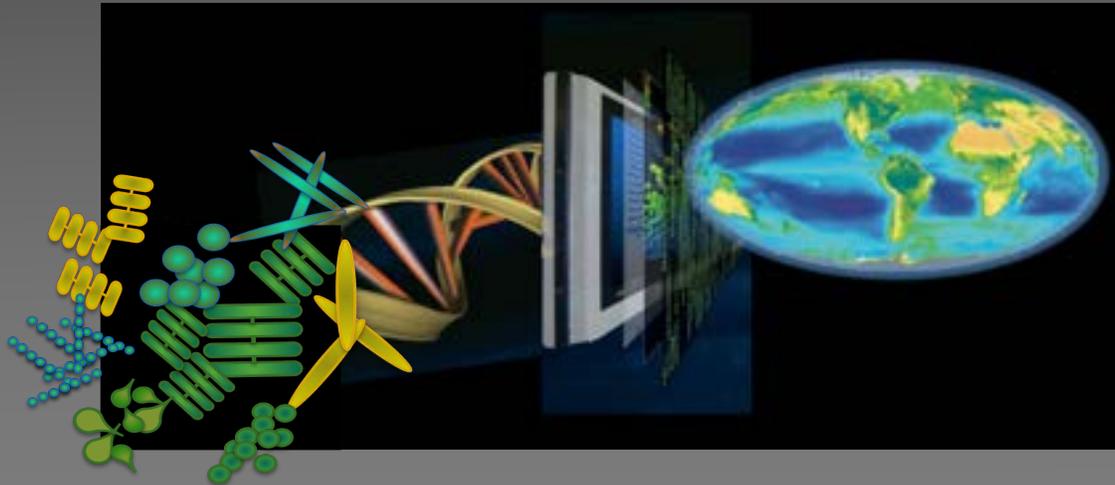
From flask to field: tracking the drivers of phytoplankton physiological ecology across marine ecosystems

Sonya Dyhrman

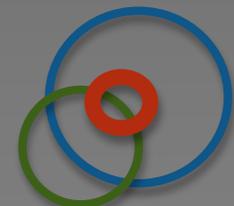
Department of Earth and Environmental Sciences

Lamont Doherty Earth Observatory

Columbia University



COLUMBIA UNIVERSITY
IN THE CITY OF NEW YORK

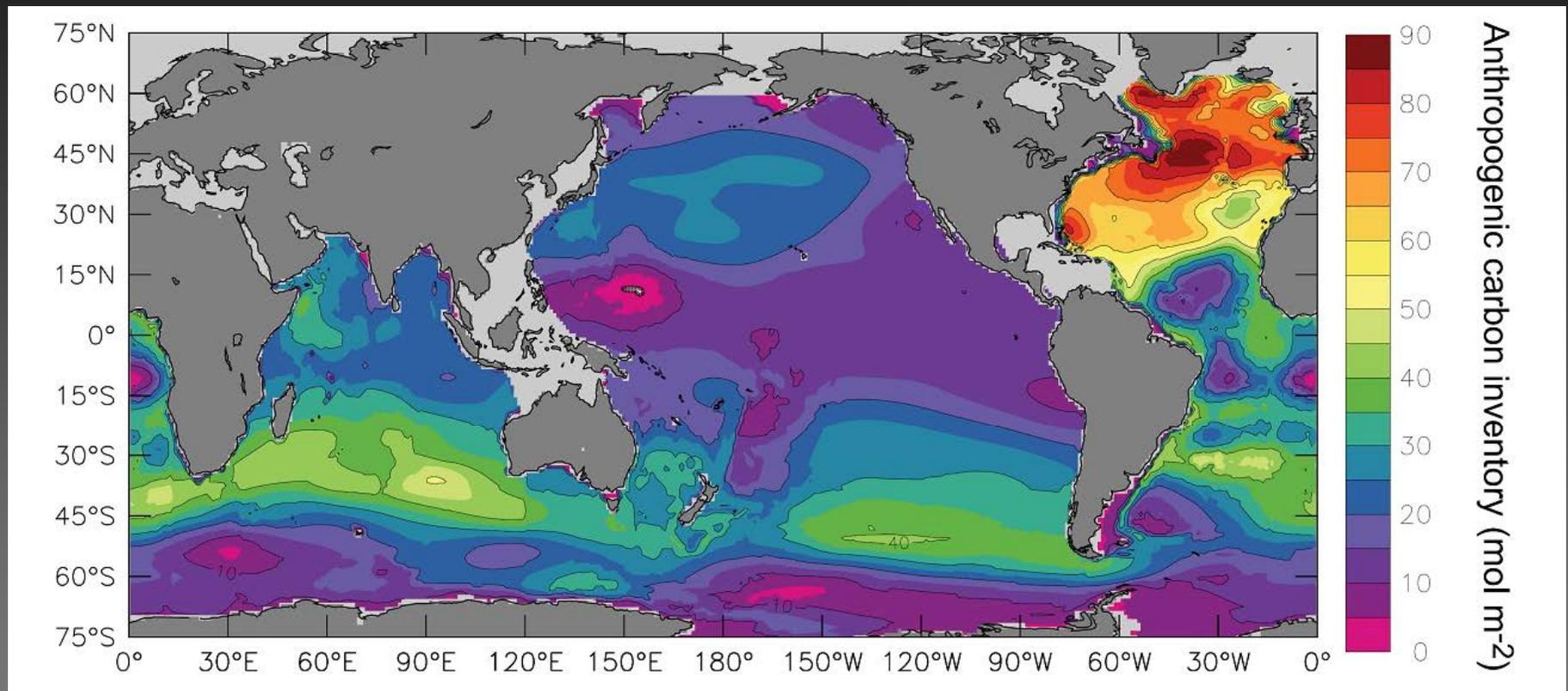


LAMONT-DOHERTY
EARTH OBSERVATORY

The ocean makes our planet livable



The ocean acts as a buffer for CO₂ in the atmosphere



Sabine et al. (2004) *Science*

Between 1800 - 1994, ocean has absorbed ~120 petagrams of CO₂
Oceanic sink accounts for ~48% of fossil-fuel emissions

The vast unseen microbial populations play a critical role in ocean function



Marine Microbes - fundamental to ocean ecosystem function

- Marine microbes...
 - Produce and consume green house gases
 - Supply the marine food web
 - Recycle organic matter
 - Account for roughly half of global primary production

- *make the planet habitable*

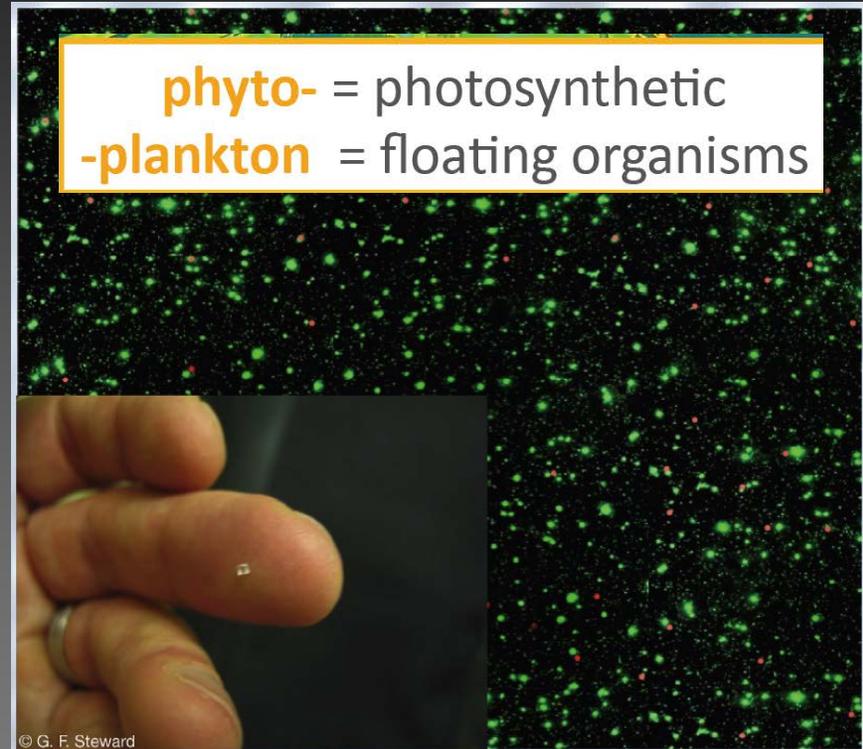
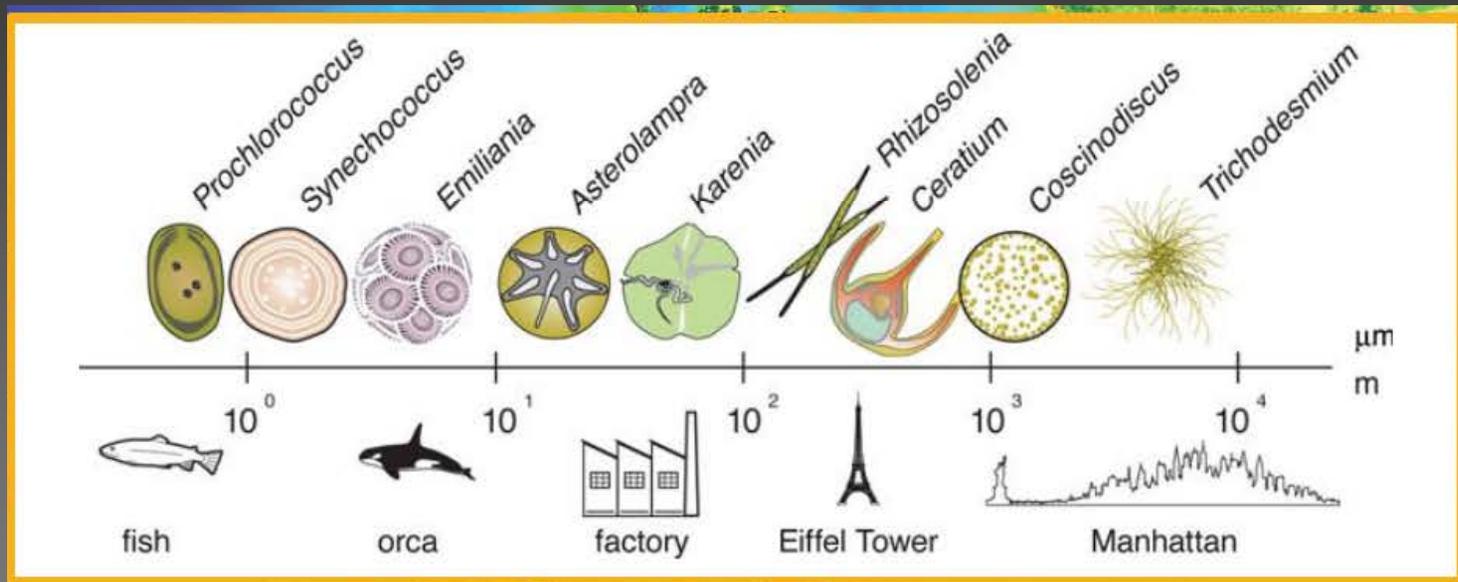
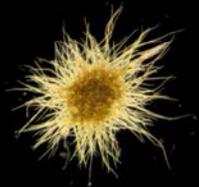
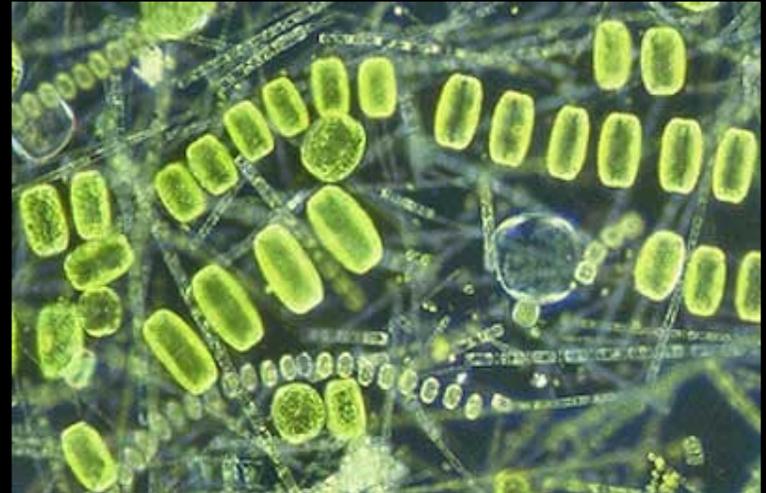
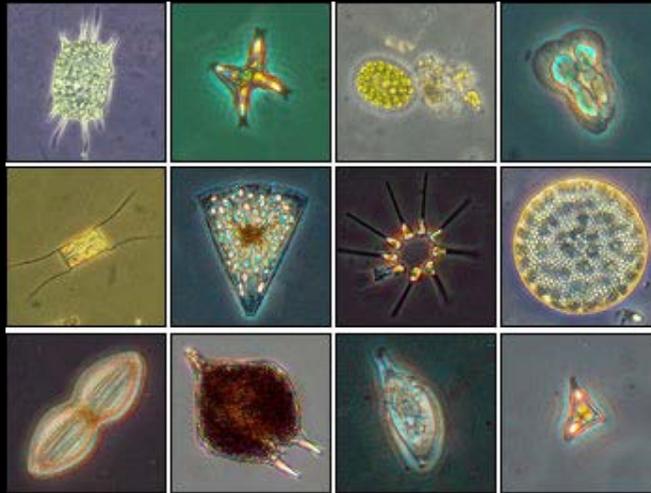
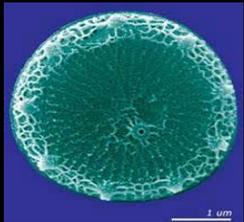
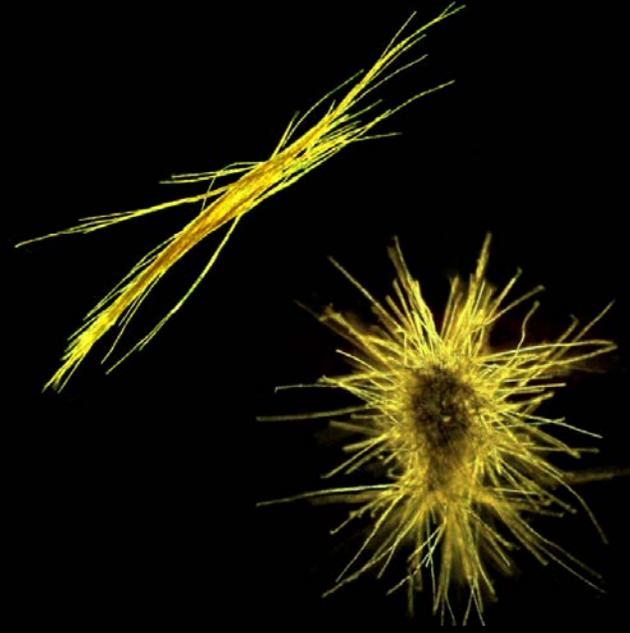
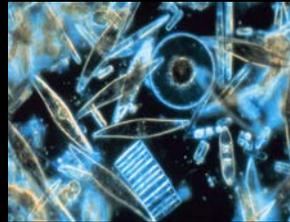
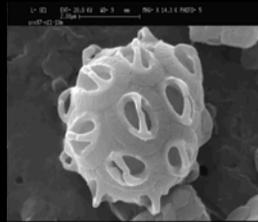
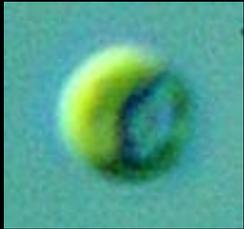
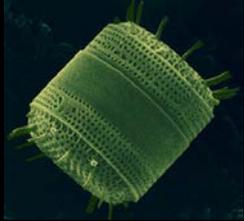


Image courtesy C-MORE

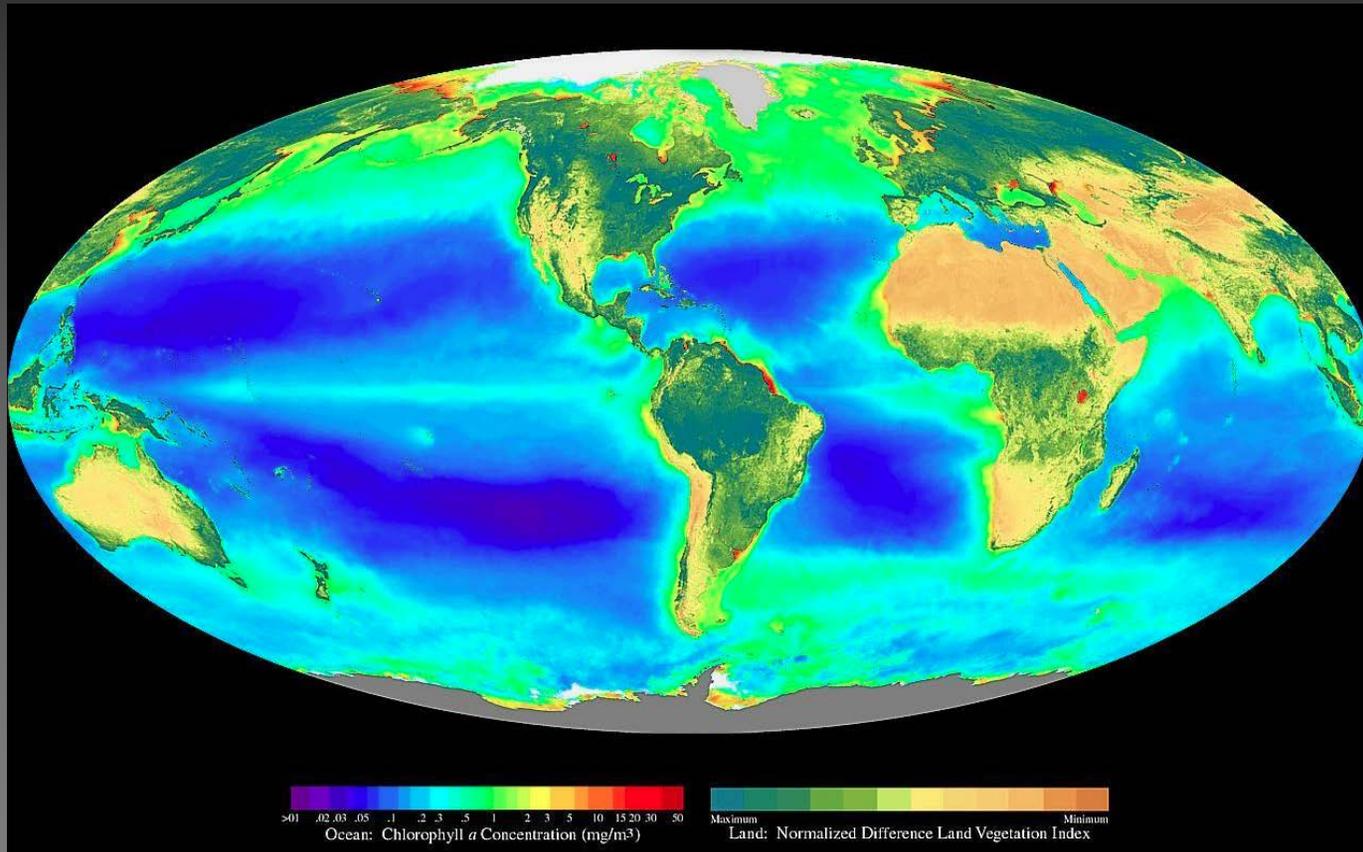
Marine phytoplankton are highly diverse



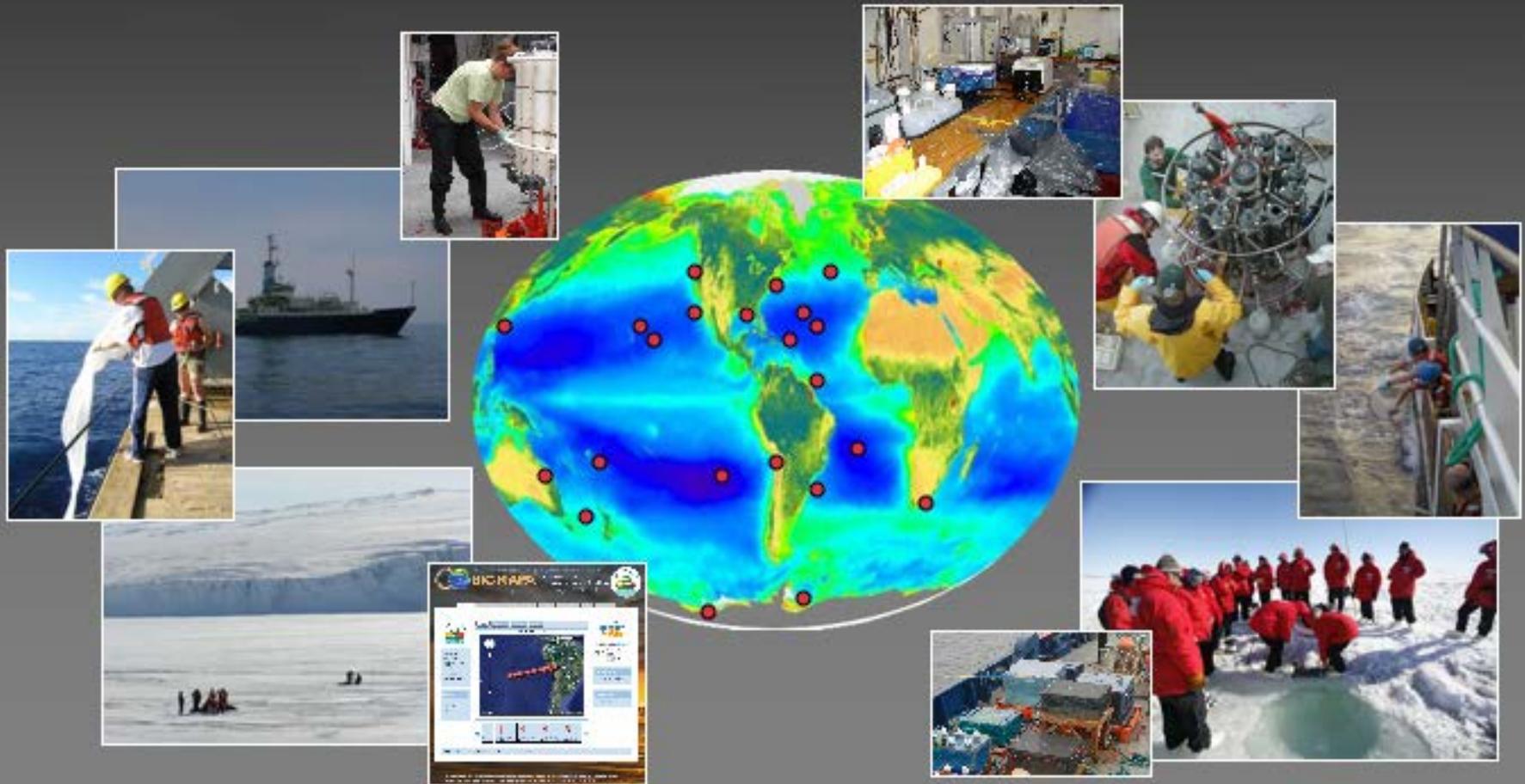


Phytoplankton play a profound role in the earth system

Half of global primary production



Sampling microbes across marine ecosystems



Tracking physiological ecology: from the flask to the field

Culture-based experiments

Species-specific responses to well-controlled environment

Limitations:

Species must be in culture

Time consuming

Extrapolations to the field

Field-based studies

Assess whole community dynamics in a natural environment

Limitations:

Not species-specific

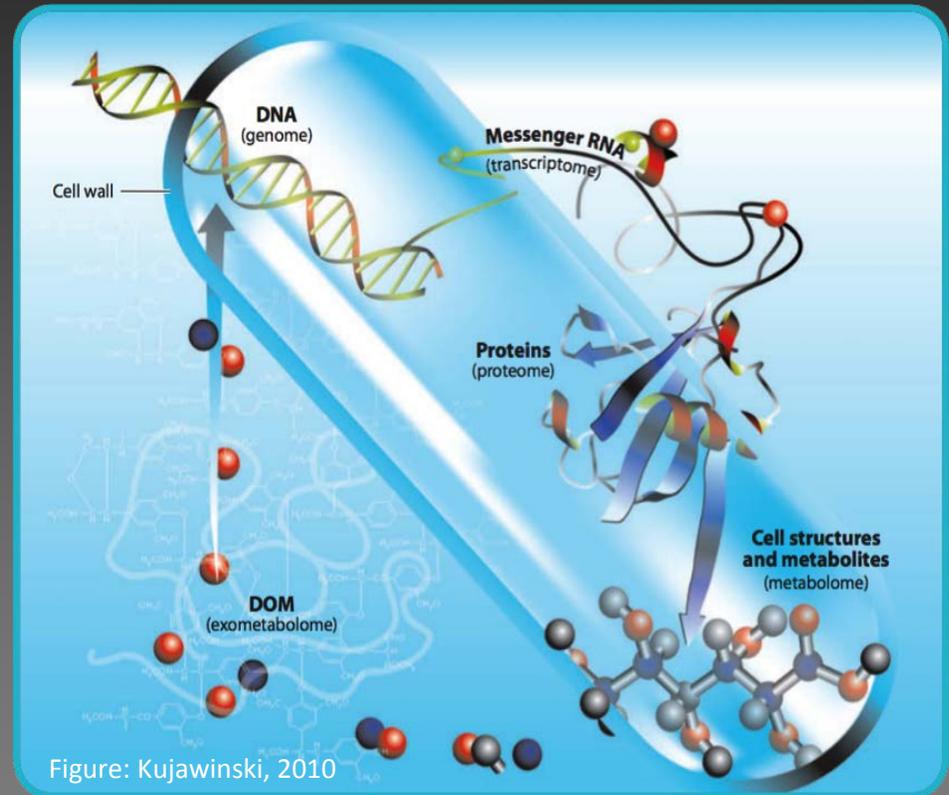
Micro/Mesocosm



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

Challenges and opportunities in microbial oceanography

- Long standing challenges:
 - Populations are dilute
 - Few species-specific assays
 - Few genome or transcriptome sequences
- New opportunities
 - Novel concentration and detection strategies
 - Increases in whole genome sequences
 - Increases in transcriptomes for eukaryotic taxa



Increasingly able to use 'omic and 'metaomic approaches!

Leveraging 'omic data to study marine microbes

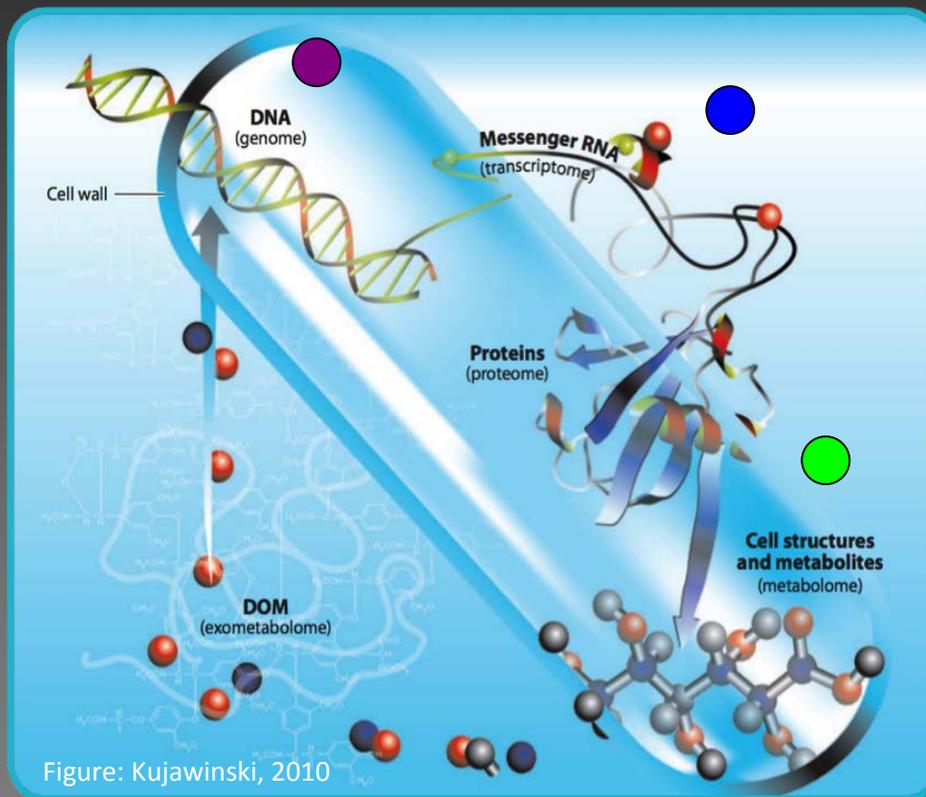


Figure: Kujawinski, 2010

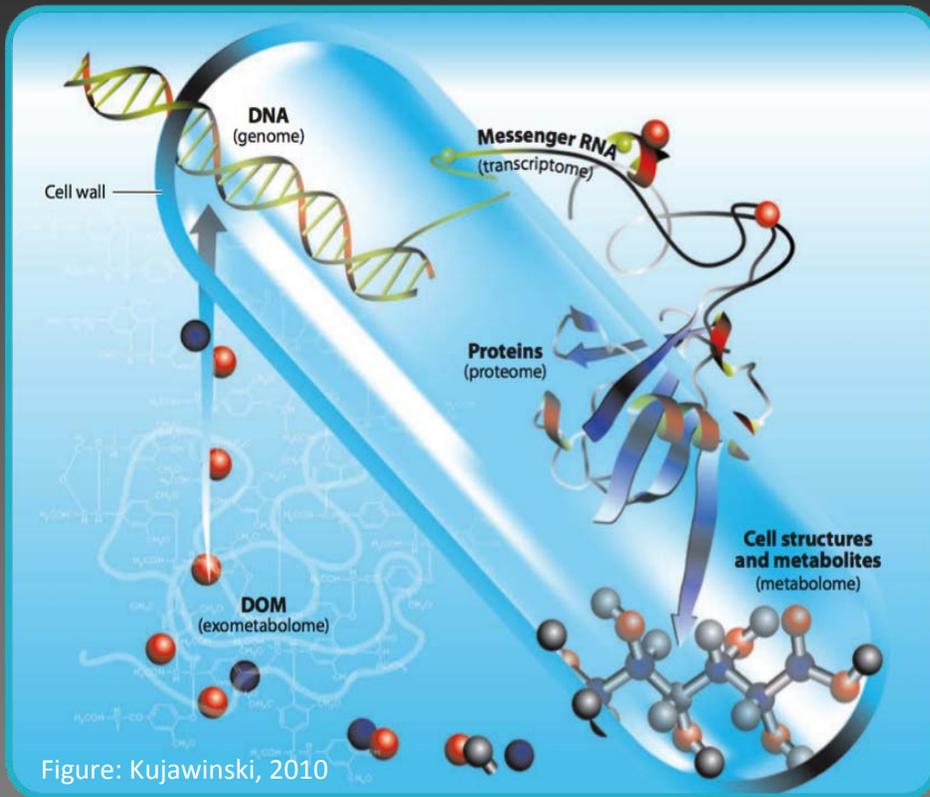
- **Taxonomic Diversity:** Who is there?
- **Metabolic capacity:** What are the molecular underpinnings of resource metabolism?
- **Metabolic plasticity:** How are those pathways regulated?
- **Functional diversity:** How are the pathways expressed *in situ*?
- **Niche space:** How are resources partitioned over time and between species?

How did we get here?

- 1990s
 - First marine bacterial WGS
 - Fosmid cloning of community DNA
- 2000s
 - BAC libraries of community DNA
 - Sequencing and WGS assembly of whole community DNA
 - First marine microbial eukaryote WGS
- 2010s
 - Bacterial community RNA sequencing ★
 - Bacterial community proteomics and metabolomics
 - SAGs
 - MMETSP - marine microbial eukaryote transcriptomes
 - Eukaryotic community RNA sequencing



Ongoing challenges



We need to grow the inventory of species and genes

We have too few samples, and too few contiguous datasets

Processing loses ecological context

Lots of “dark matter”

Still too expensive to generate and store data

Vignettes

- From genome to biome: Tracking the metabolism and microbiome of a keystone N_2 fixer

Genome - enabled



- Co-existing in a sea of competition: Leveraging transcriptome data to identify the physiological ecology of phytoplankton from key groups

Transcriptome - enabled



Thank you



Gwenn Hennon



Mónica Rouco



Sheean Haley



Kyle Frischkorn



Matt Harke



Maria Hernandez



Harriet Alexander



Two themes

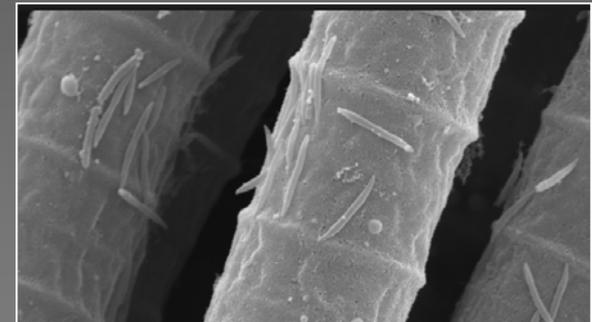
Metabolic traits and trade-offs

- What phosphorus is bioavailable?
- What are the biogeochemical constraints on N_2 fixation?



Host microbiome interactions

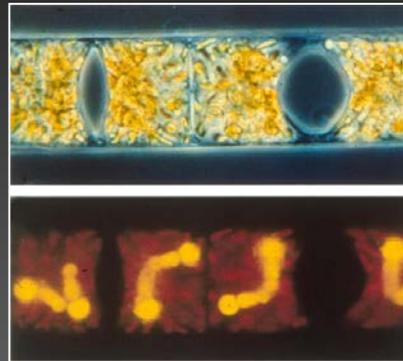
- Who is there? Microbiome diversity
- What are they doing? Microbiome functional diversity and interactions



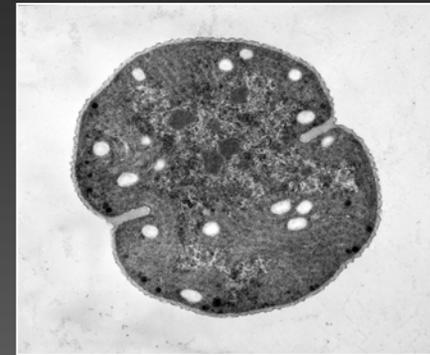
Nitrogen-fixing marine cyanobacteria

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

Richelia



Crocospaera



Trichodesmium



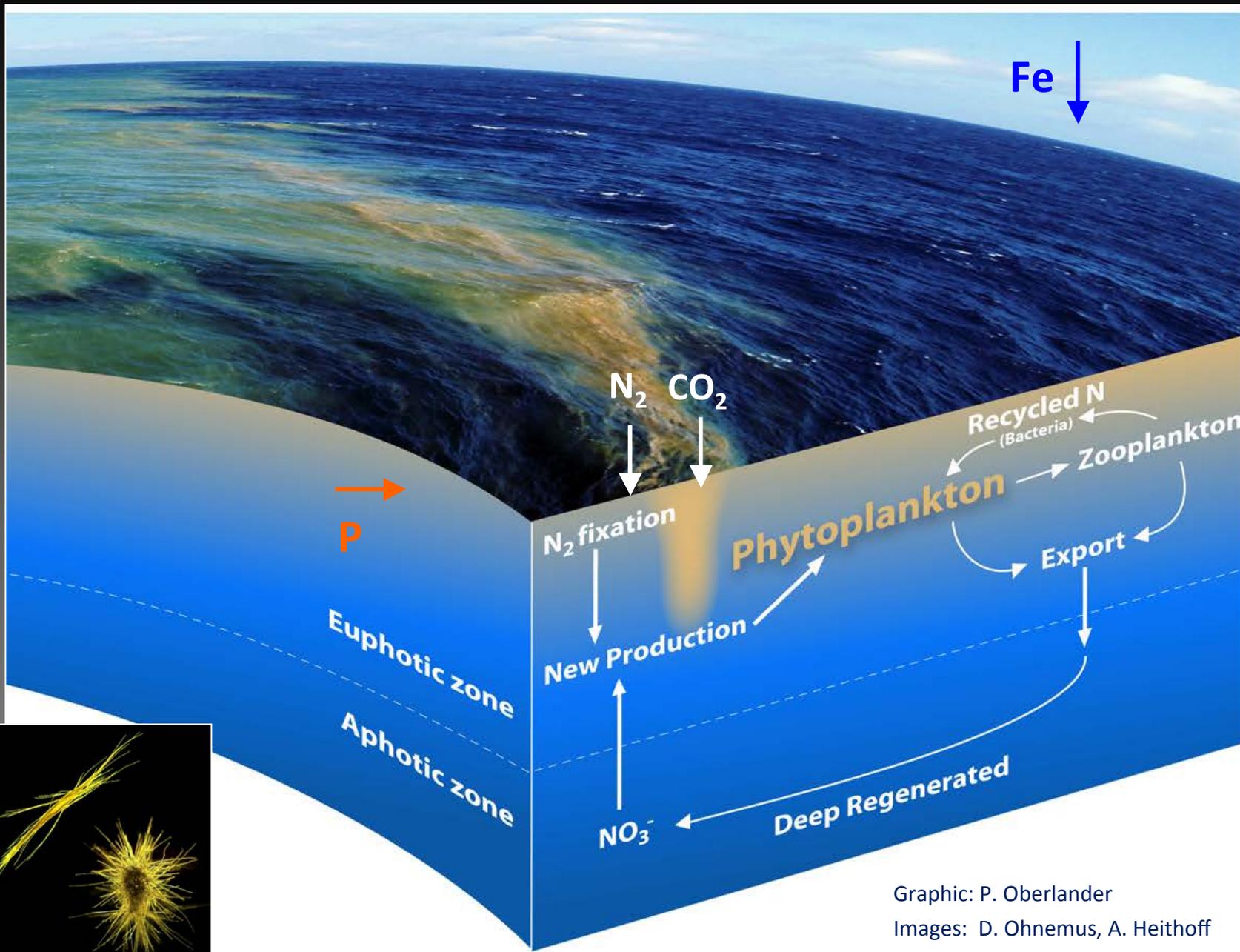
-*Trichodesmium contortum*
-*Trichodesmium erythraeum*

} *T. erythraeum* -
like Clade

-*Trichodesmium tenue*
-*Trichodesmium thiebautii*

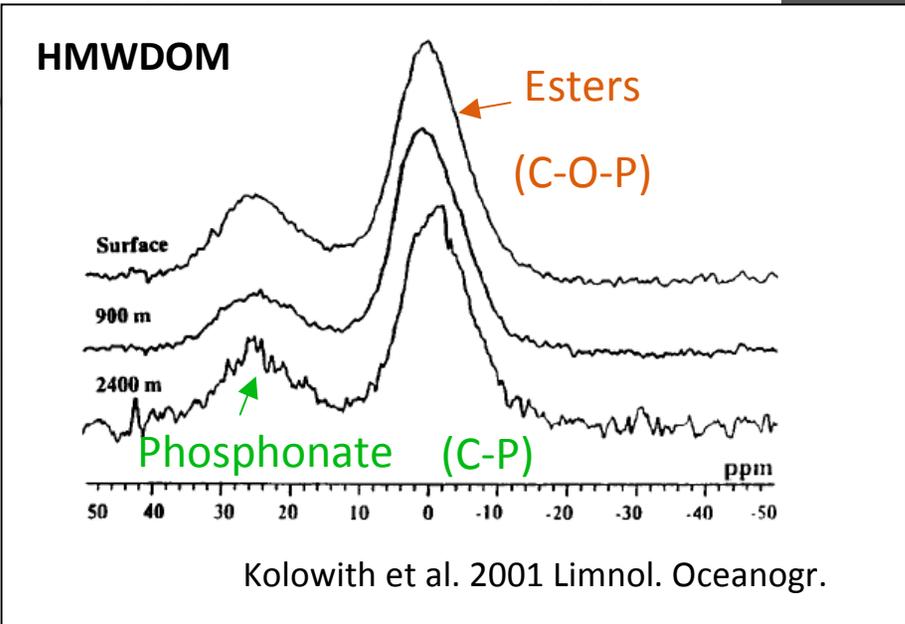
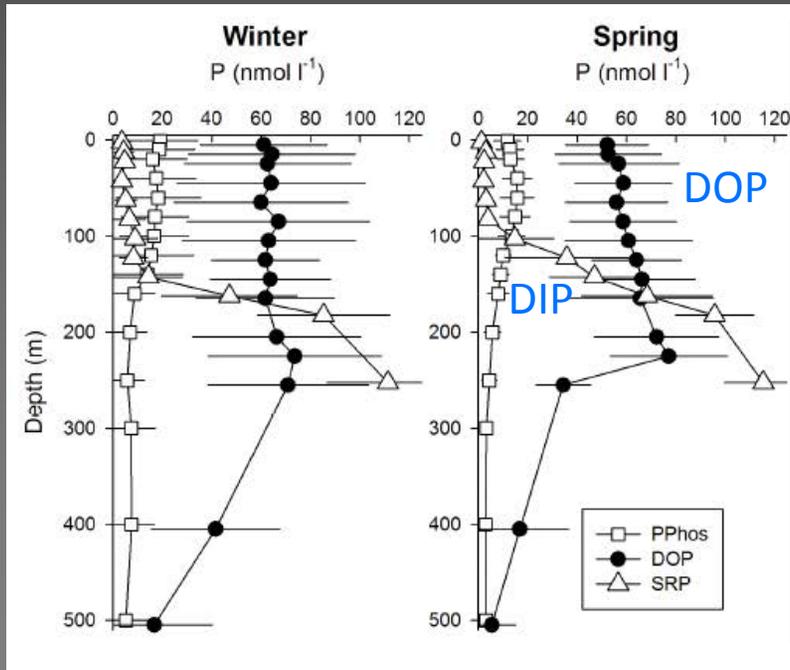
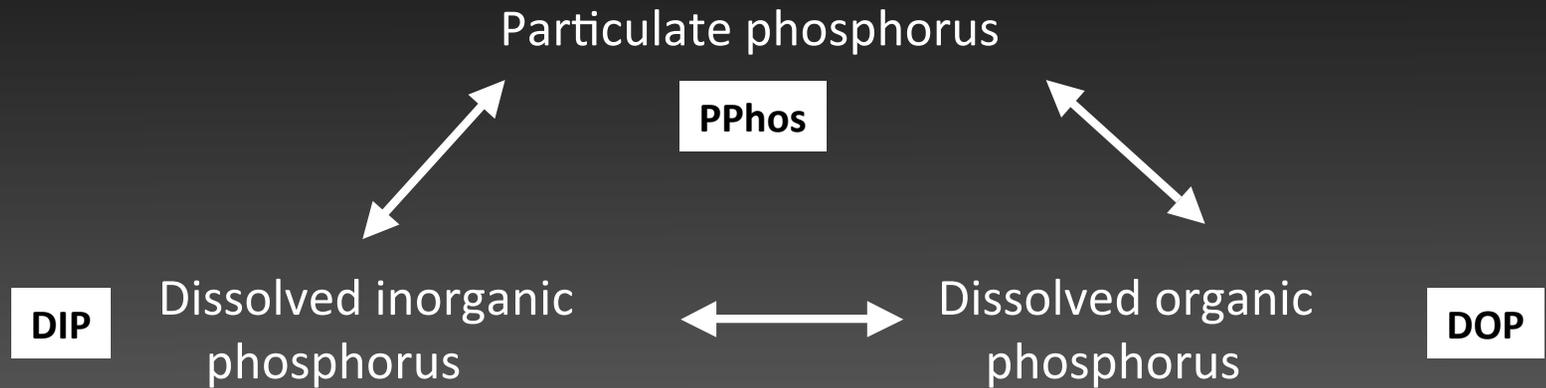
} *T. thiebautii* -
like Clade

-*Trichodesmium spiralis*
-*Trichodesmium hildebrandtii*

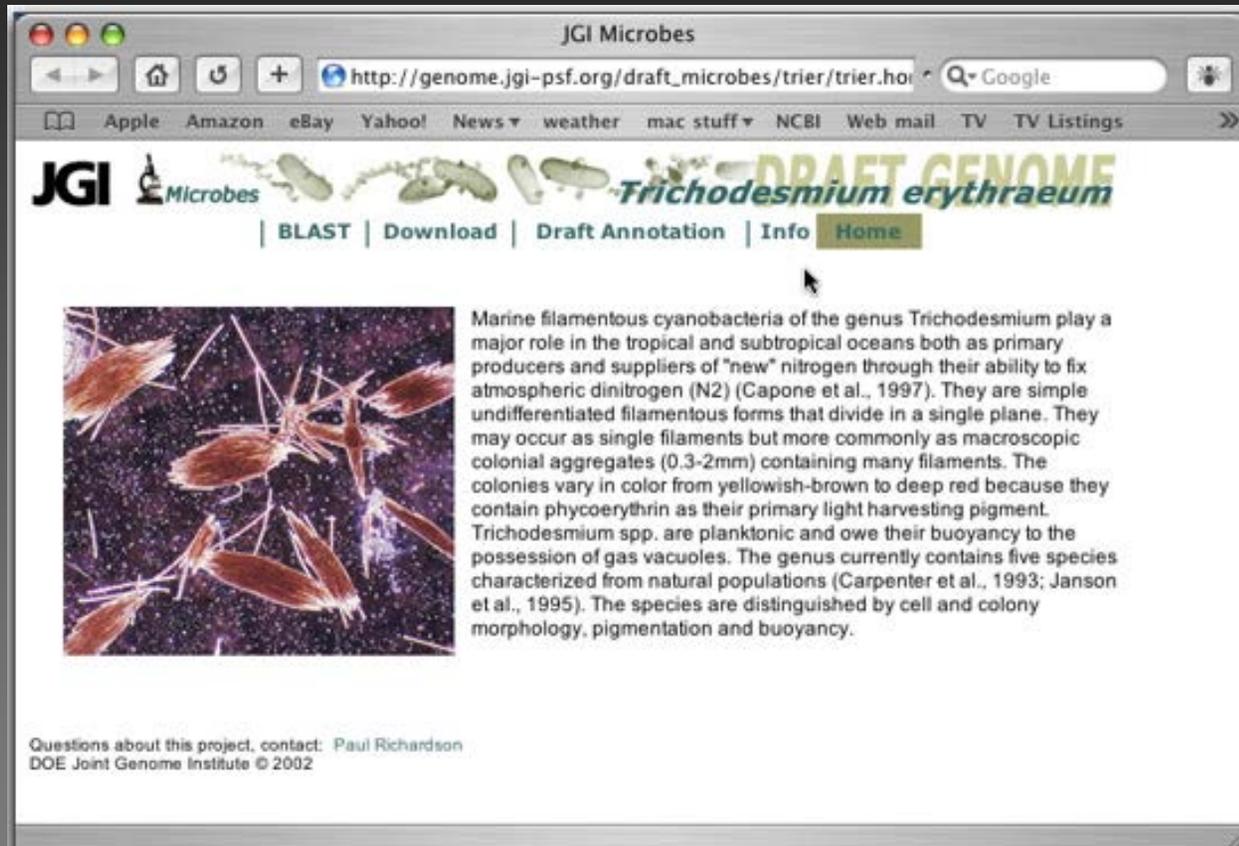


Graphic: P. Oberlander
 Images: D. Ohnemus, A. Heithoff

Phosphorus pools in the western North Atlantic



Trichodesmium erythraeum IMS101 genome page



The screenshot shows a web browser window titled "JGI Microbes". The address bar contains the URL "http://genome.jgi-psf.org/draft_microbes/trier/trier.hor". The browser's search bar has "Google" entered. Below the browser window, the website header features the "JGI Microbes" logo on the left and a large banner for "DRAFT GENOME Trichodesmium erythraeum" on the right. A navigation menu below the banner includes links for "BLAST", "Download", "Draft Annotation", "Info", and "Home".



Marine filamentous cyanobacteria of the genus *Trichodesmium* play a major role in the tropical and subtropical oceans both as primary producers and suppliers of "new" nitrogen through their ability to fix atmospheric dinitrogen (N₂) (Capone et al., 1997). They are simple undifferentiated filamentous forms that divide in a single plane. They may occur as single filaments but more commonly as macroscopic colonial aggregates (0.3-2mm) containing many filaments. The colonies vary in color from yellowish-brown to deep red because they contain phycoerythrin as their primary light harvesting pigment. *Trichodesmium* spp. are planktonic and owe their buoyancy to the possession of gas vacuoles. The genus currently contains five species characterized from natural populations (Carpenter et al., 1993; Janson et al., 1995). The species are distinguished by cell and colony morphology, pigmentation and buoyancy.

Questions about this project, contact: Paul Richardson
DOE Joint Genome Institute © 2002

Phosphorus metabolic traits and trade-offs

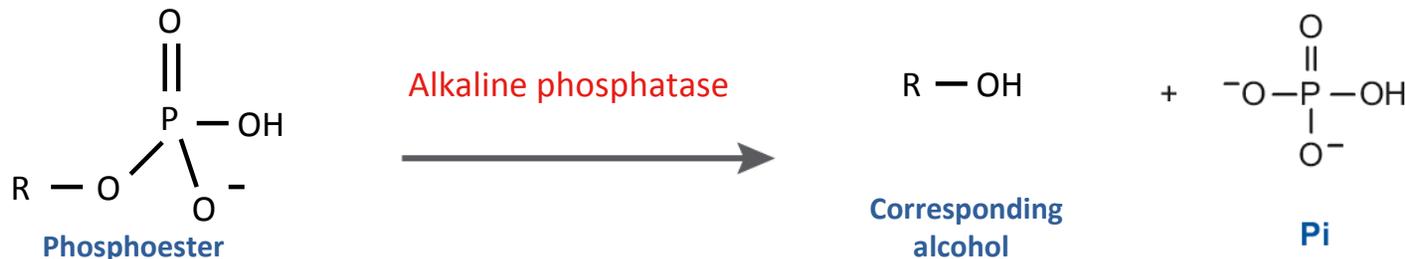
- Phosphonate
 - C-P Lyase (Fe co-factor)

C-P

- Ester
 - *phoX* type alkaline phosphatase (Ca Fe co-factor)
 - *phoA* type alkaline phosphatase (Zn co-factor)

COP

COP

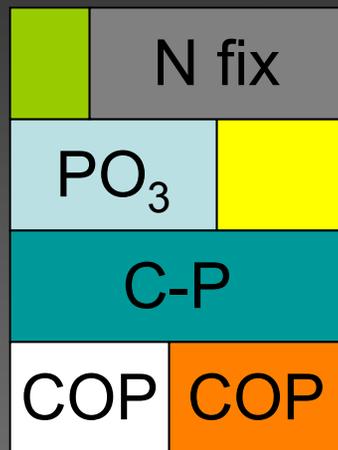


- Phosphite
 - *ptxD* gene cluster

PO₃

Comparative genomics: phosphorus traits and trade offs

Trichodesmium



Fe

Zn

Fe

Crococosphaera



Zn

Other N₂ fixing cyanobacteria genomes do not encode the same pathways for phosphorus metabolism - less available substrates, but less metal requirement

Tracking genomic potential with expression studies



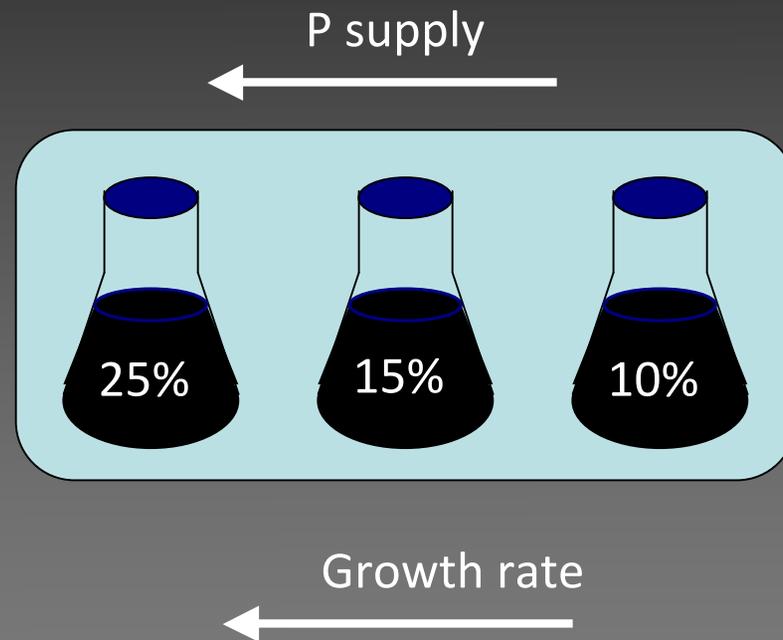
Culture cells



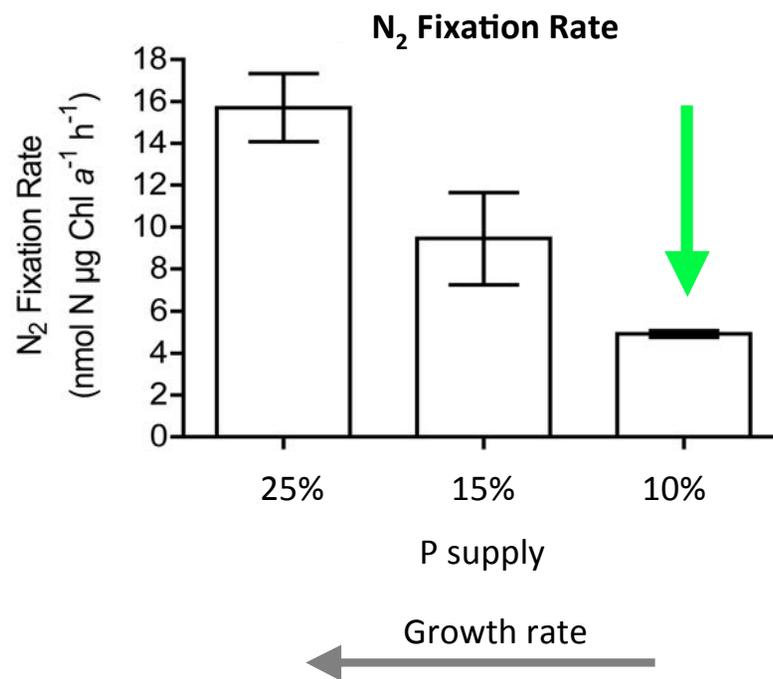
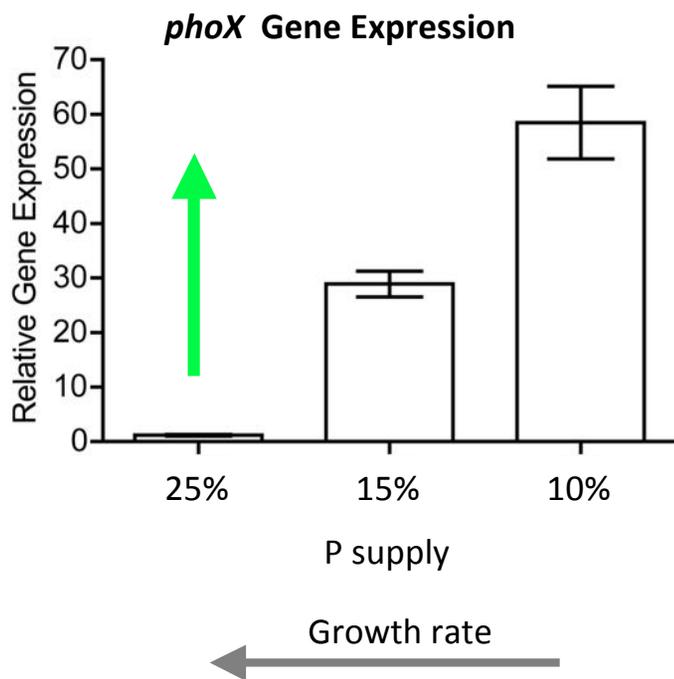
Harvest and preserve samples



qRT-PCR of *phoX*
Activity

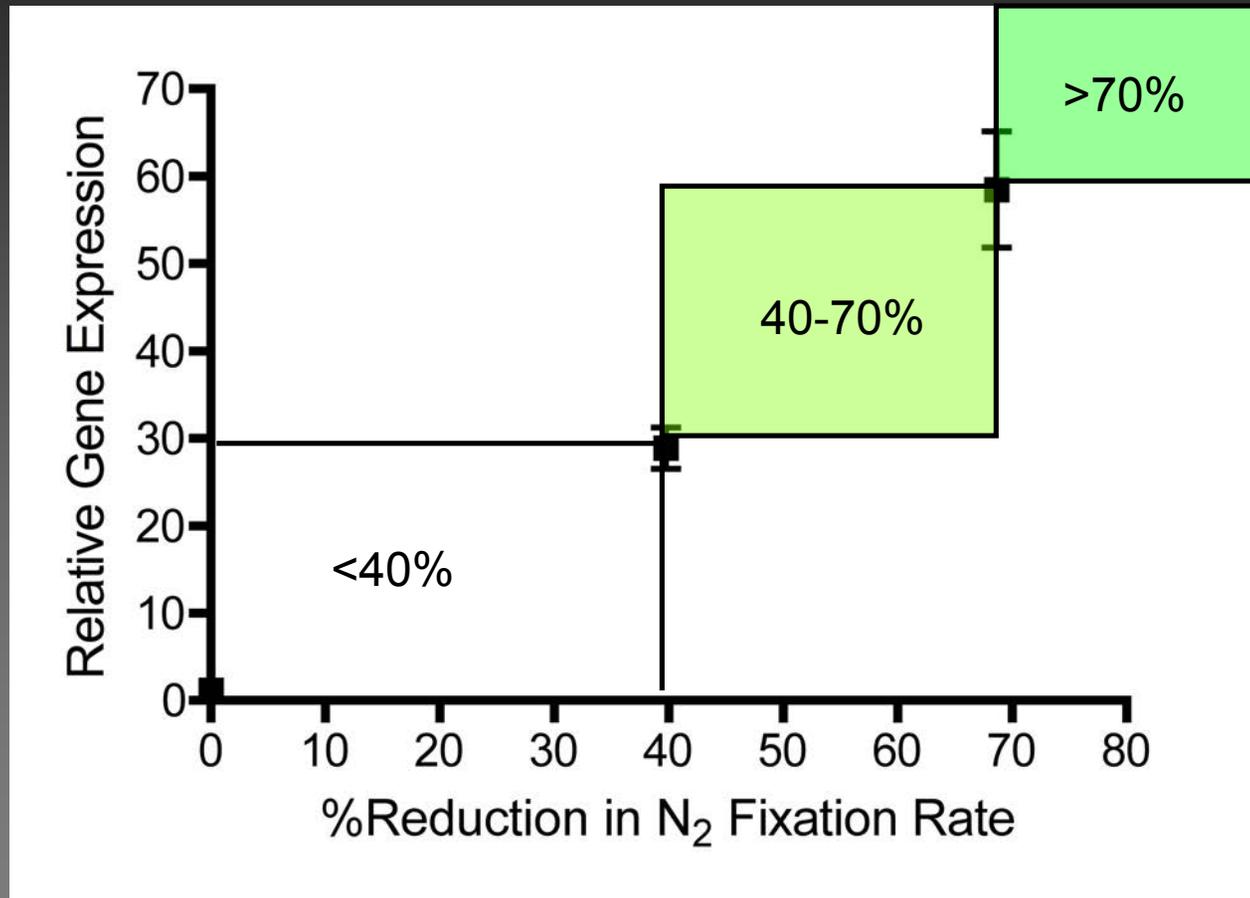


Calibrating gene expression to growth and N₂ fixation



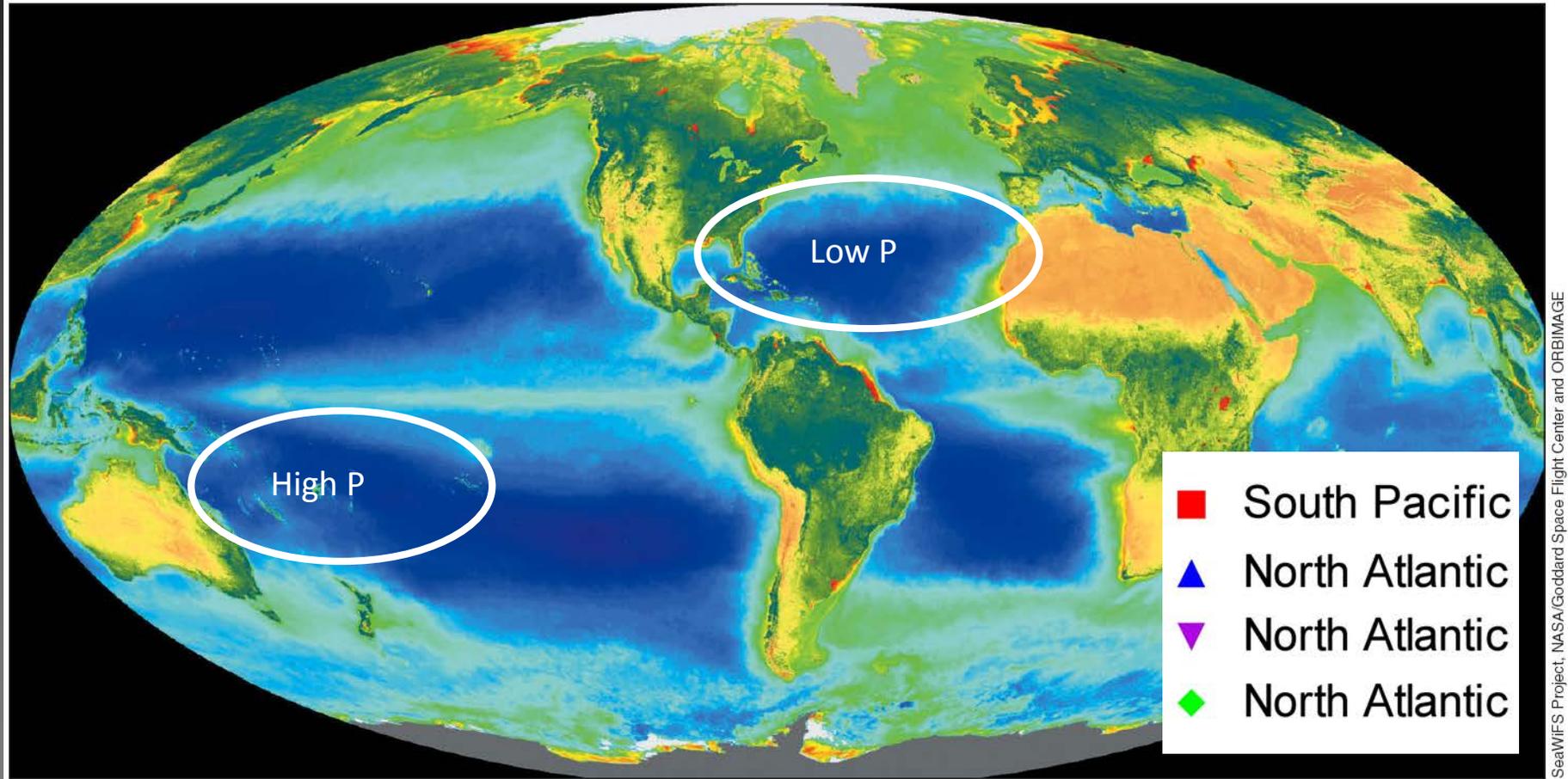
Orchard and Dyhrman unpublished

Calibrating gene expression to N₂ fixation



Orchard et al. in prep

Sampling different P regimes





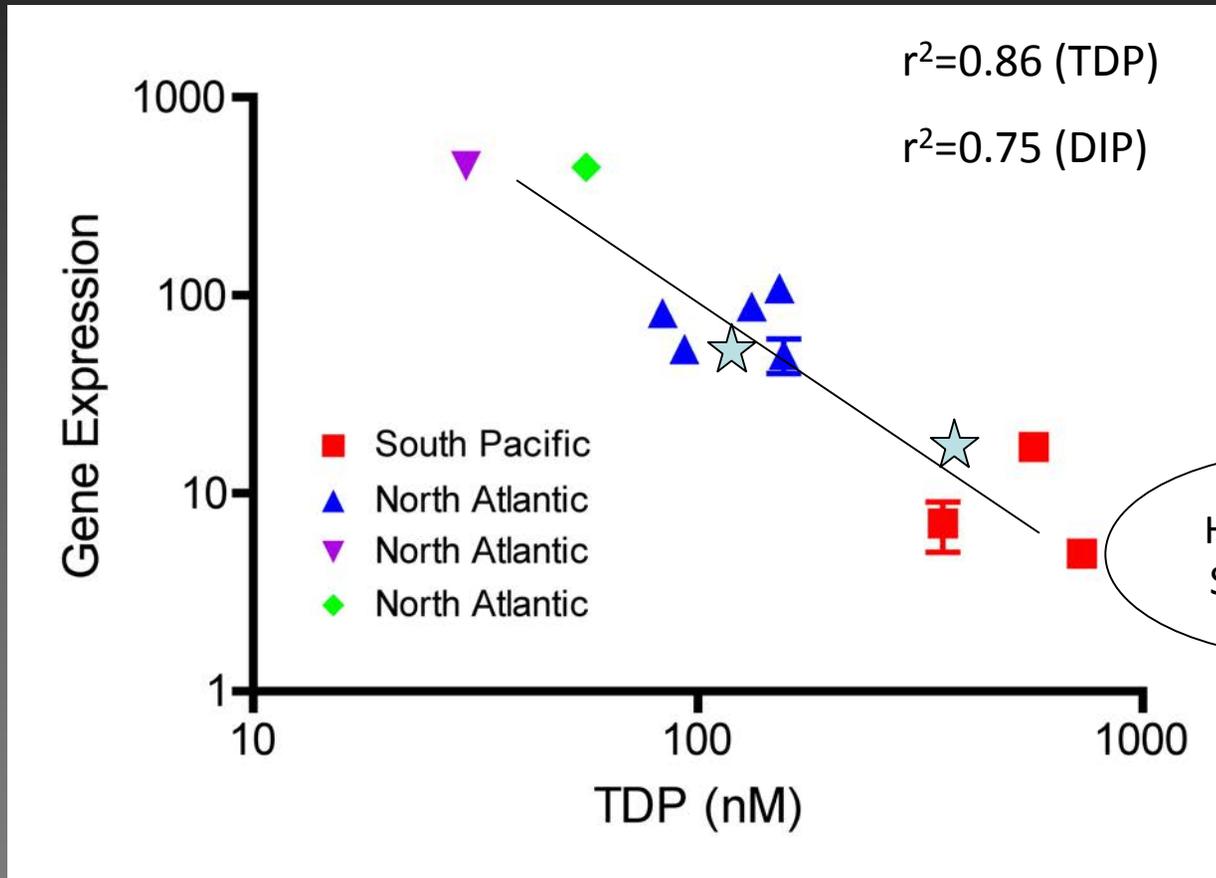


DIP, TDP
Measurements



Measurements of
quantitative gene
expression for
Trichodesmium sp.

Gene expression increases at low phosphorus

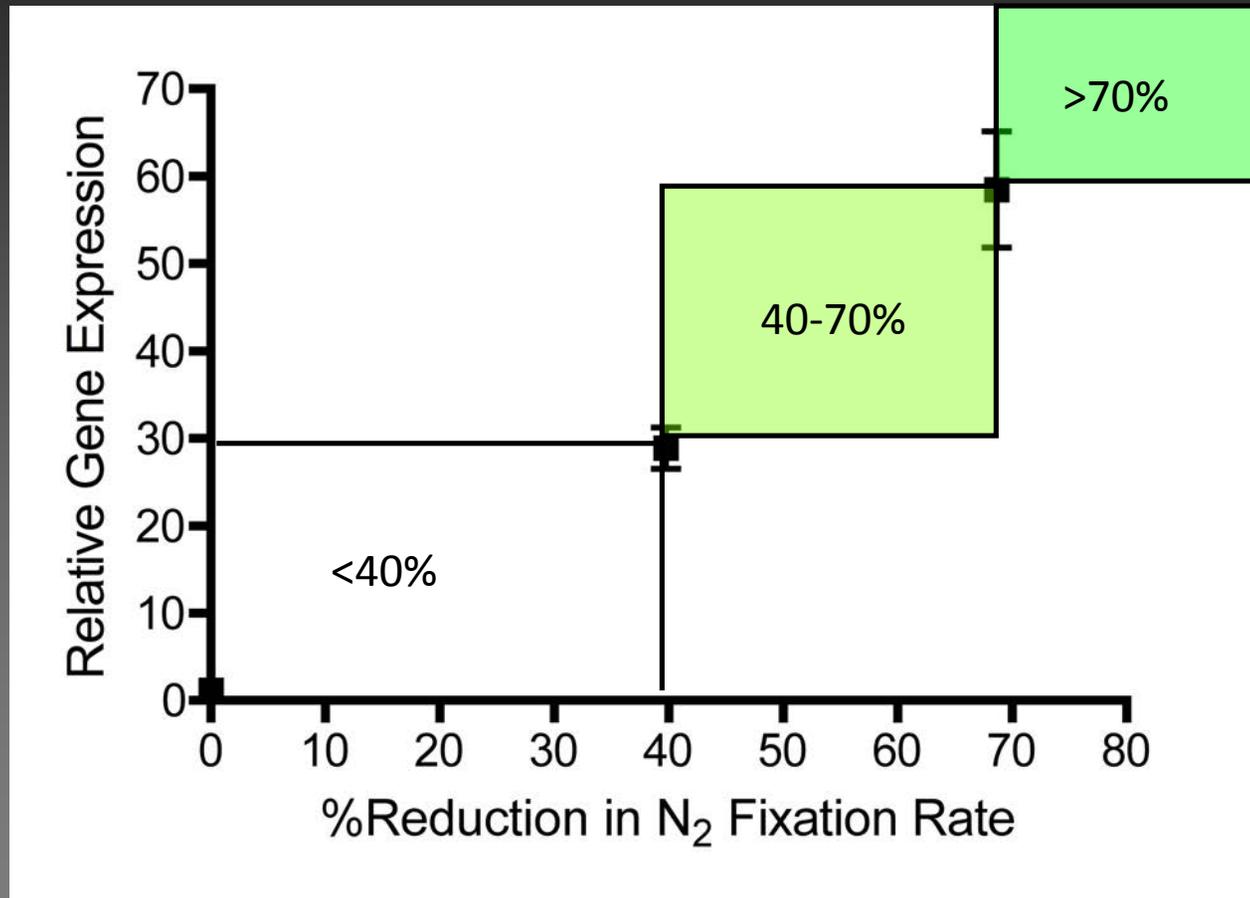


Low P supply
North Atlantic

High P supply
South Pacific

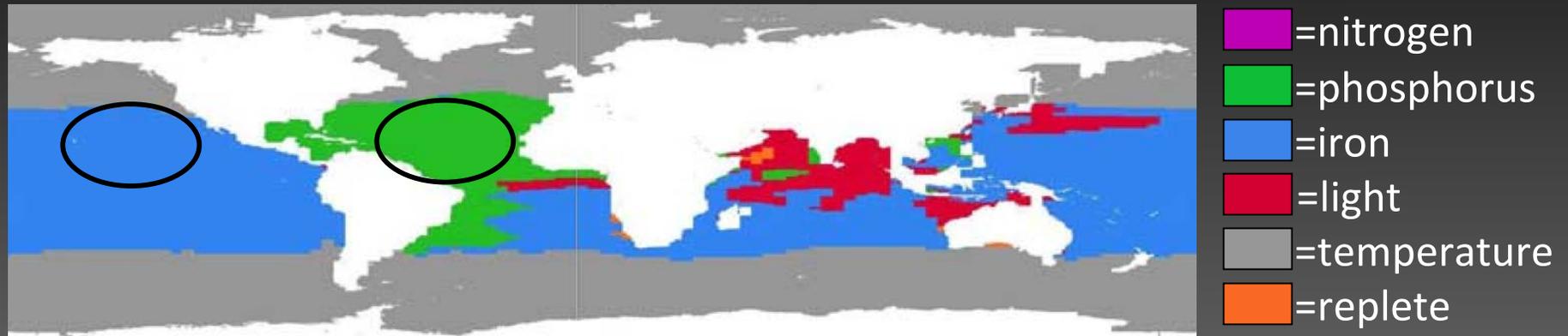
Orchard and Dyhrman unpublished

Calibrating gene expression to N₂ fixation



Orchard and Dyhrman unpublished

Constraints on *Trichodesmium* N₂ fixation



(Moore et al. 2004)

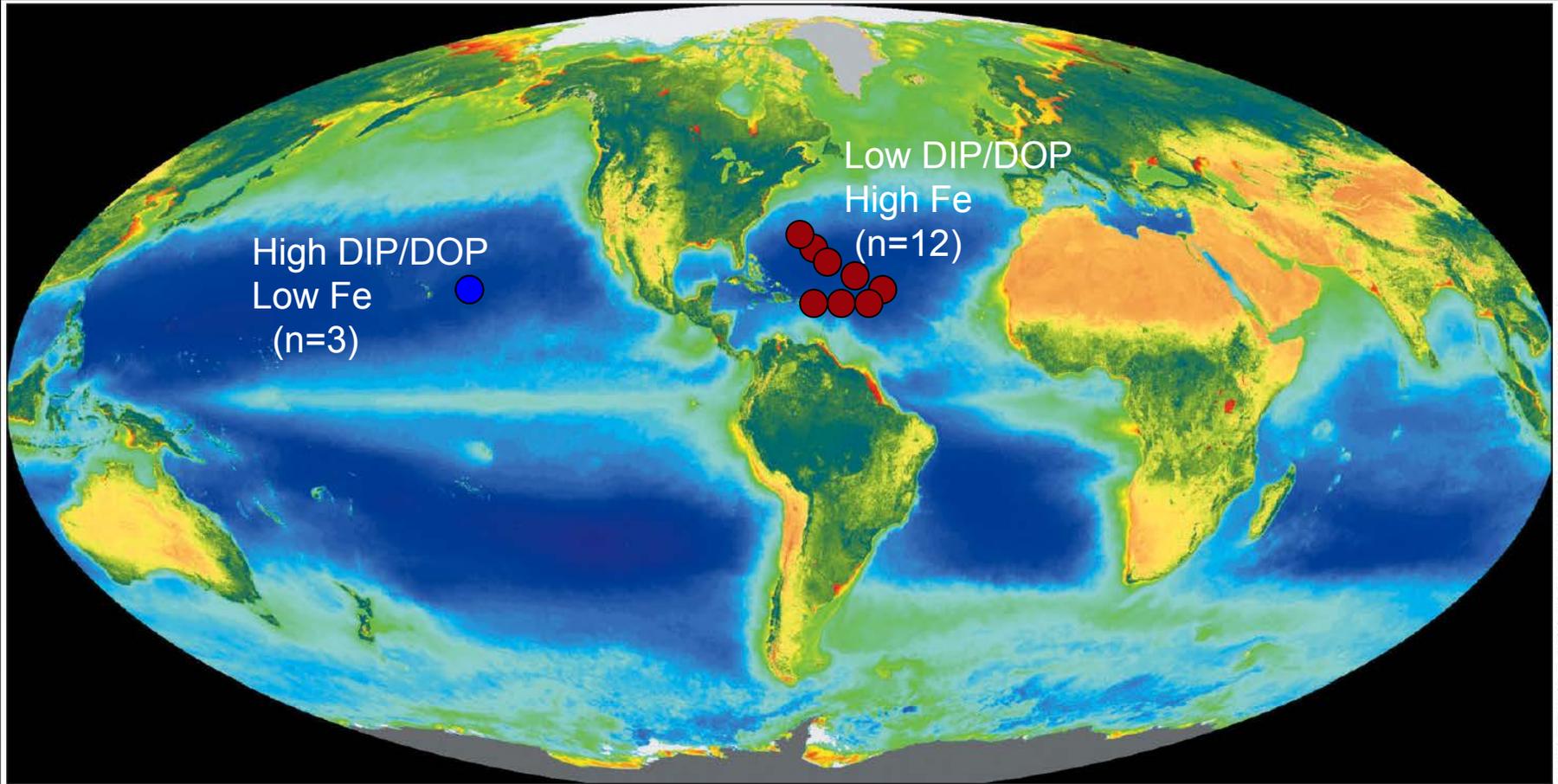
Molecular patterns corroborate predictive models in the western north Atlantic

phoX - P regulated ester metabolism (Orchard et al. 2009 *Environ. Micro.*)

idiA - Fe regulated iron metabolism (Chappell et al. 2013 *ISME J.*)

rnpB - reference gene

nifH - N₂ - fixation

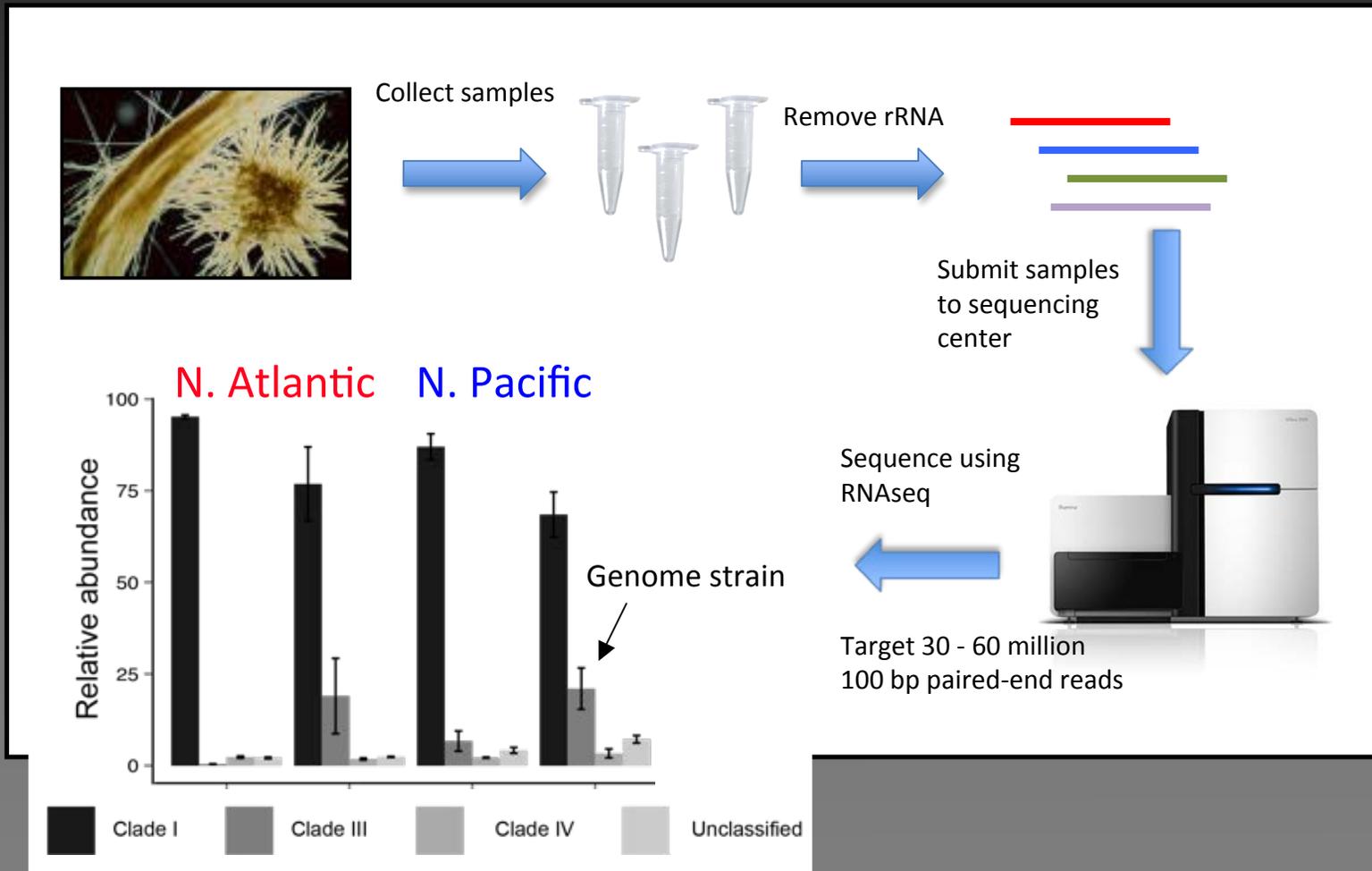


SeaWiFS Project, NASA/Goddard Space Flight Center and ORBIMAGE



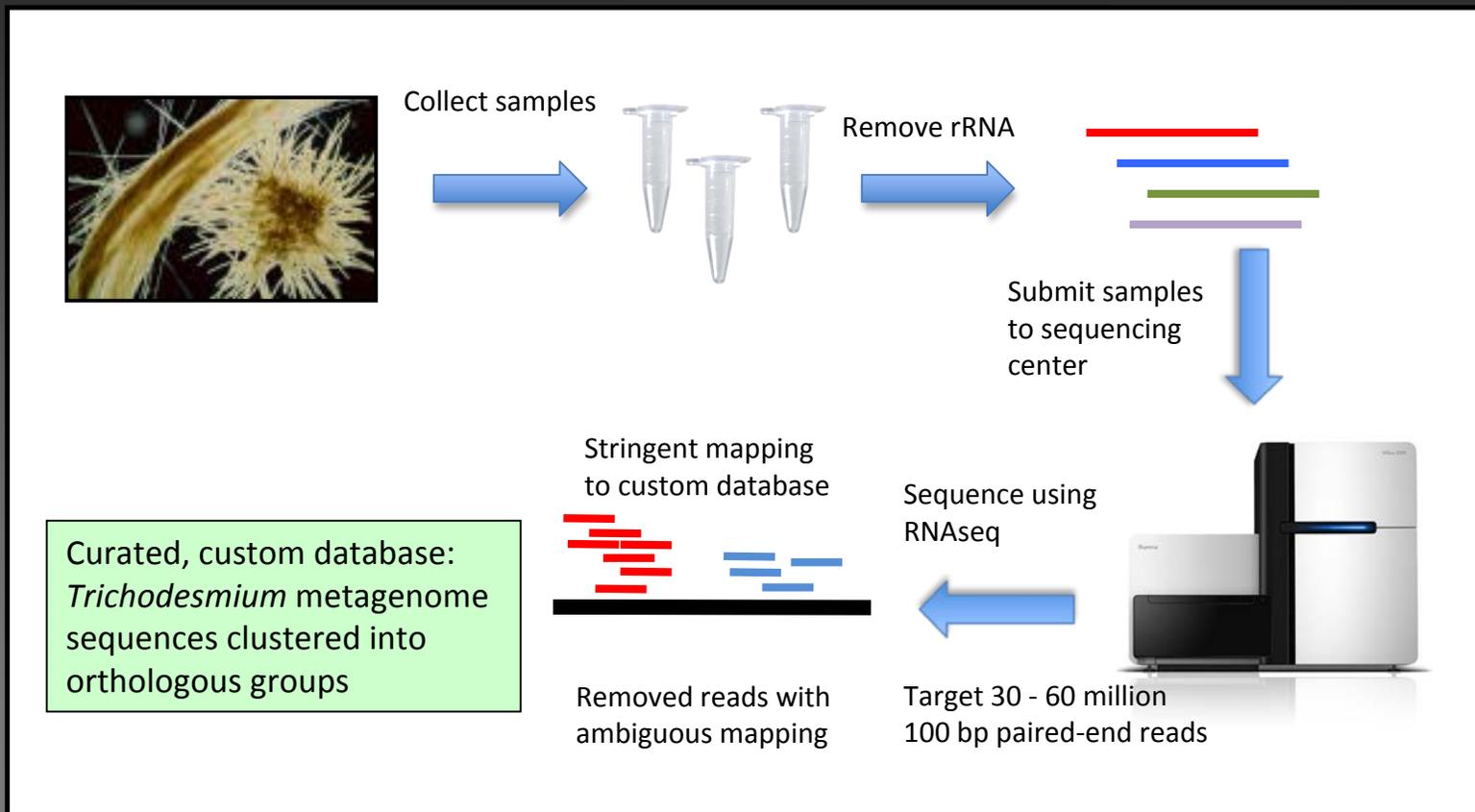
Order Online

Metatranscriptome analysis pipeline



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

Metatranscriptome analysis pipeline



The reality.....

```
CAACAACATCATACCCTTCCAAGGCACGGGCTTCAACGCCCGCCAAATCTCC
TGCGGCAGCAGGGATGGGACCCTGGGACTGTGTGGCGCTGTGGAGGCCG
+DHT4KXP1:1:1101:5930:2353#0/1
CAACAACATCATACCCTTCCAAGGCACGGGCTTCAACGCCCGCCAAATCTCC
TGCGGCAGCAGGGATGGGACCCTGGGACTGTGTGGCGCTGTGGAGGCCG
+DHT4KXP1:1:1101:5930:2353#0/1
CAACAACATCATACCCTTCCAAGGCACGGGCTTCAACGCCCGCCAAATCTCC
TGCGGCAGCAGGGATGGGACCCTGGGACTGTGTGGCGCTGTGGAGGCCG
+DHT4KXP1:1:1101:5930:2353#0/1
CAACAACATCATACCCTTCCAAGGCACGGGCTTCAACGCCCGCCAAATCTCC
TGCGGCAGCAGGGATGGGACCCTGGGACTGTGTGGCGCTGTGGAGGCCG
+DHT4KXP1:1:1101:5930:2353#0/1
CAACAACATCATACCCTTCCAAGGCACGGGCTTCAACGCCCGCCAAATCTCC
TGCGGCAGCAGGGATGGGACCCTGGGACTGTGTGGCGCTGTGGAGGCCG
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TACCAATTTTCATTTCCATGTCTCTCCCATCTTGACACATTATTTTCT
ACCTCCATCACATCATGATCACGTTATACGATCTCTACAGTAGCCCCCA
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obbeeeegggegiiiiighhhhiiifhiiifgiifhiiiiihhiii
lihihiihiihiihiiidggggeeeecdddddccbccccc_
+DHT4KXP1:1:1101:5781:2386#0/1
```

Metatranscriptome analysis

RNA extraction

Bacterial mRNA enrichment:

- Euk RNA removal- MICROBEnrich kit (Ambion)
- Bacterial rRNA removal- Ribo-Zero (Epicentre)

Sequencing:

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

Output:
.fastq

Preparation of reference metagenome:

- Extraction of *Trichodesmium*-only scaffolds from metagenome data

Read mapping

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

Sequence processing:

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

Differential expression analyses:

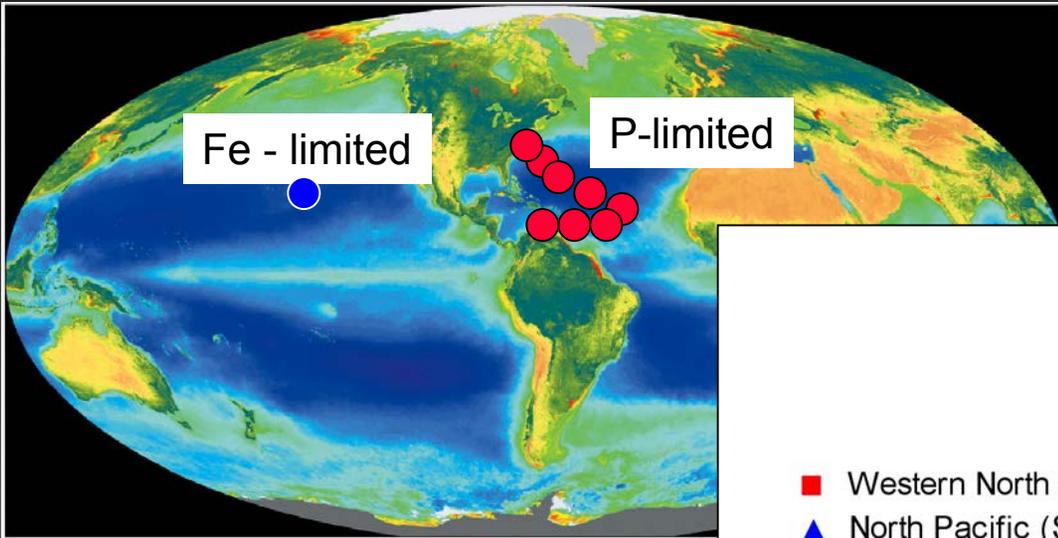
R (vegan package – Oksanen et al. 2016)

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

R (EdgeR package– Robinson et al. 2010)

- Assessment of differential abundance of individual OG

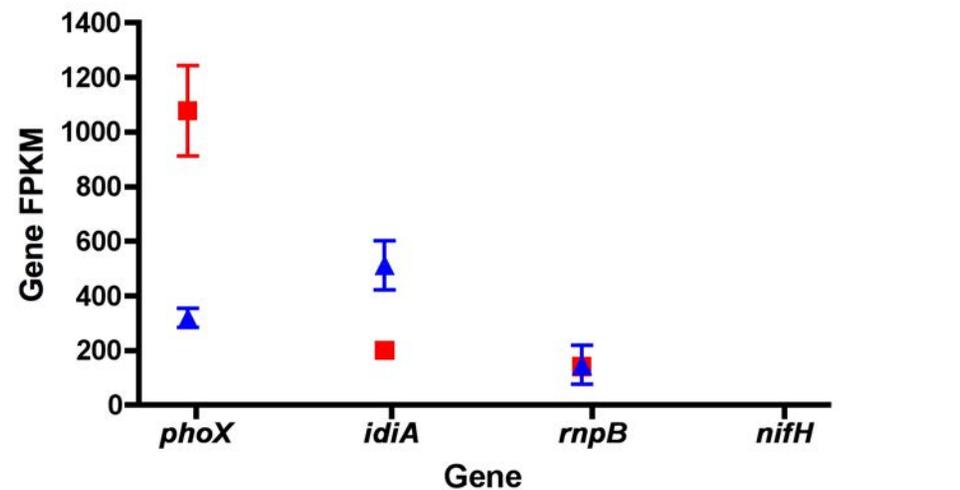
Biomarkers consistent with model prediction



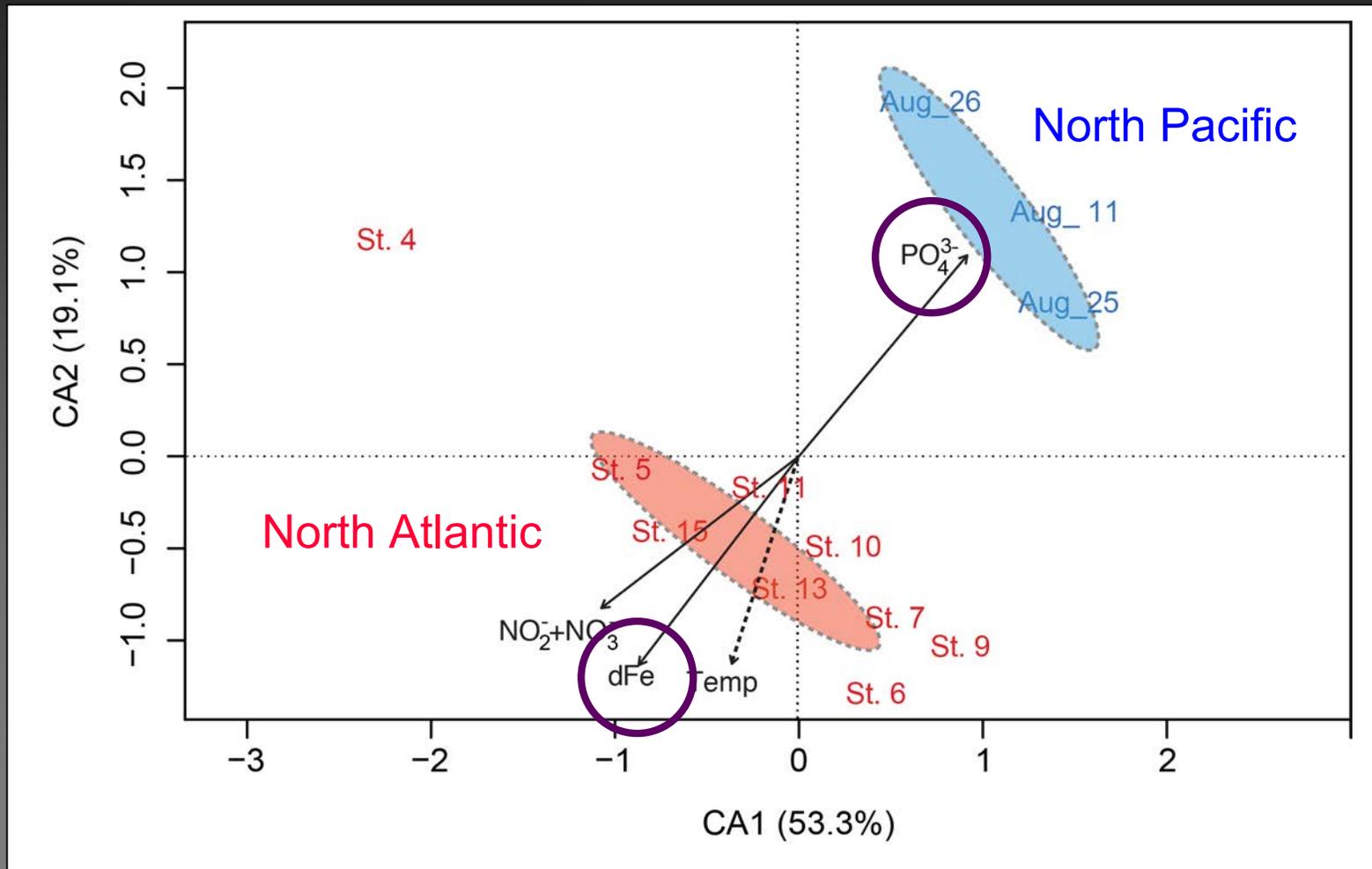
Biomarkers confirm model predictions.

Trichodesmium sp. transcripts

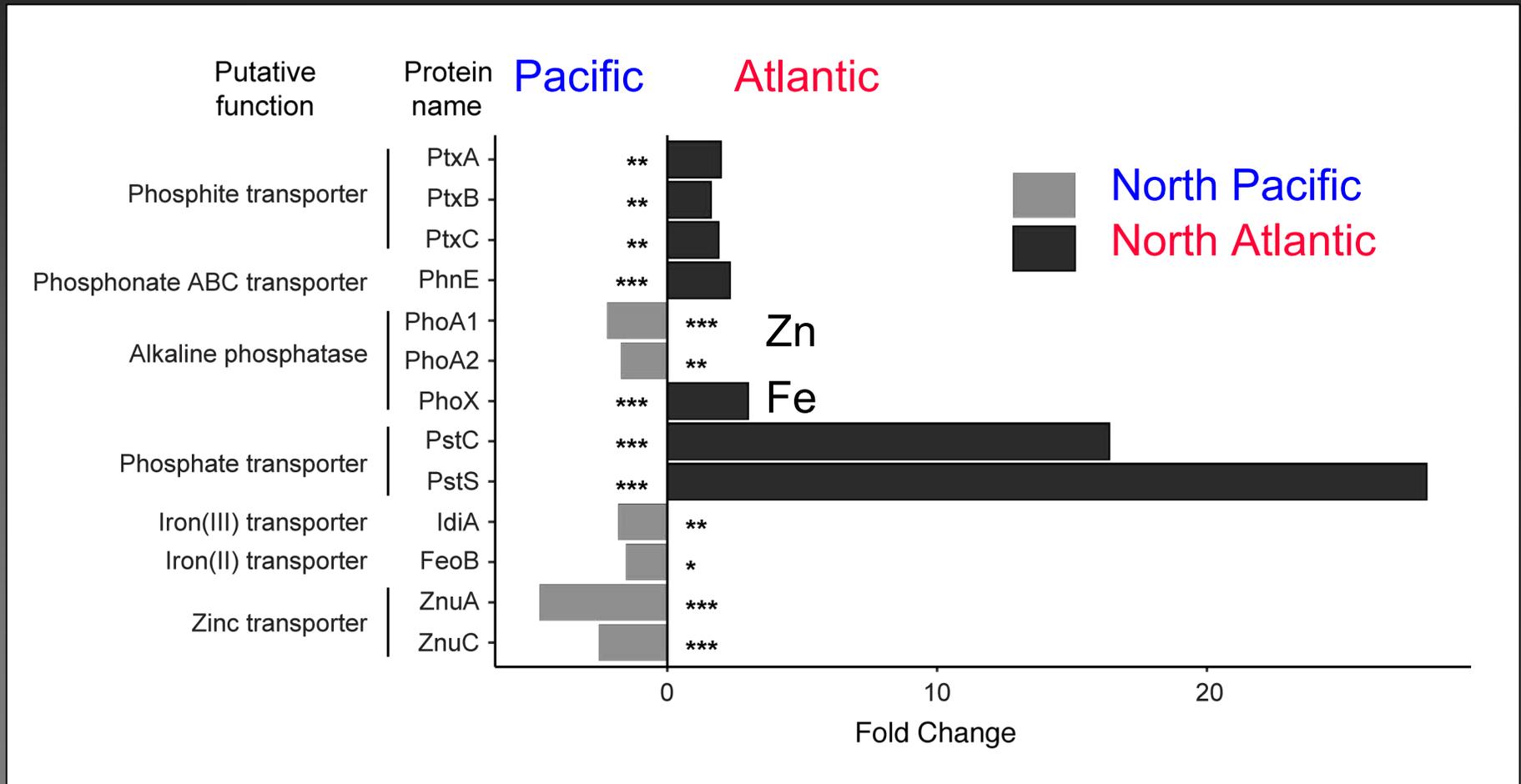
- Western North Atlantic
- ▲ North Pacific (St. ALOHA)



Significant differences in global transcription between the North Pacific and the North Atlantic



Transcription patterns indicate metalloenzyme trade-offs and geochemical controls



Summary - Metabolic traits and trade-offs

What phosphorus forms are bioavailable?

What are the biogeochemical constraints on N₂ fixation?

- **Genome:** *Trichodesmium* genome suggests bioavailability of phosphonate, ester, and phosphite
- **Marker transcripts:** *Trichodesmium phoX* expression levels suggests that supply of bioavailable P is low in the western N. Atlantic, which could constraint N₂ fixation
- **Metatranscriptome:** Predicted biogeochemical drivers of N₂ fixation are reflected in *Trichodesmium* transcriptional signals including likely metalloenzyme switching

Two themes

Metabolic traits and trade-offs

- What phosphorus is bioavailable?
- What are the biogeochemical constraints on N₂ fixation?



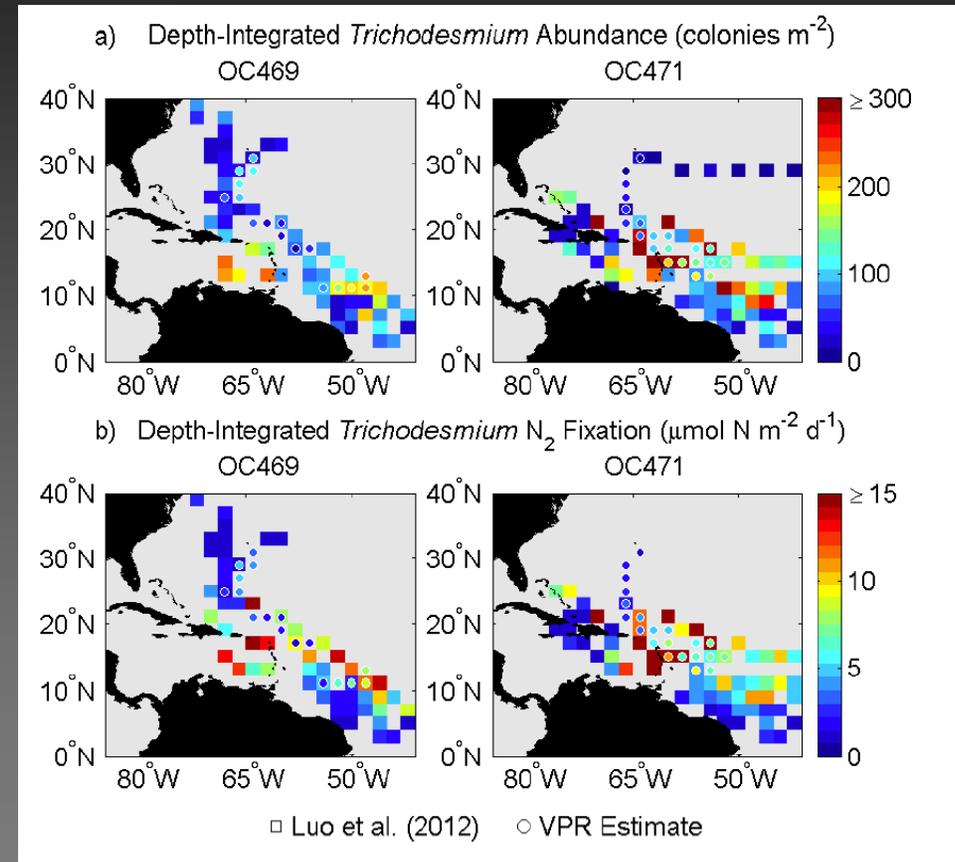
Host microbiome interactions

- Who is there? Microbiome diversity
- What are they doing? Microbiome functional diversity and interactions



Modeling N₂ 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 necessarily a good predictor of distribution or N₂ fixation



Olson et al. 2015 *DSR II*

Host microbiome interactions

- *Trichodesmium* colonies harbor epibionts in cultures and field populations (Ruoco et al. 2016 *EM*)
- 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*)

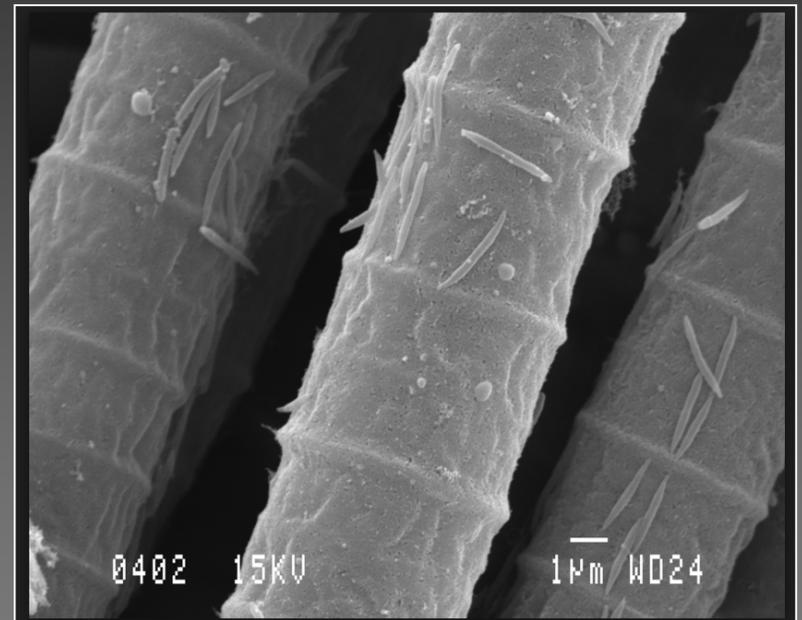
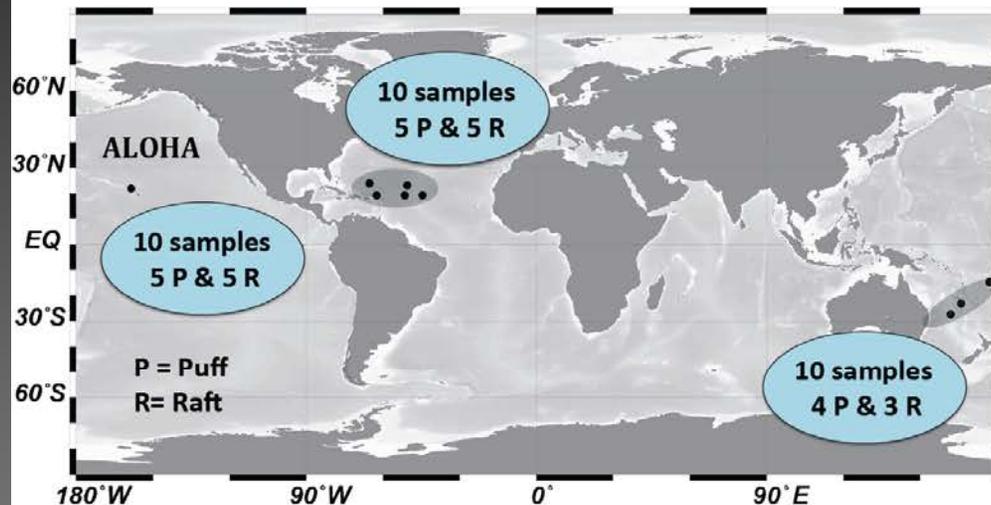


Image courtesy Tracy Mincer

Epibiont diversity

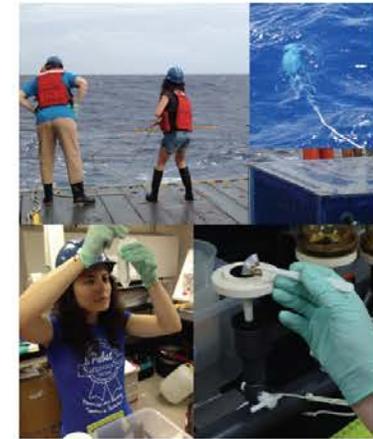
EXPERIMENTAL APPROACH



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

DNA extraction

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

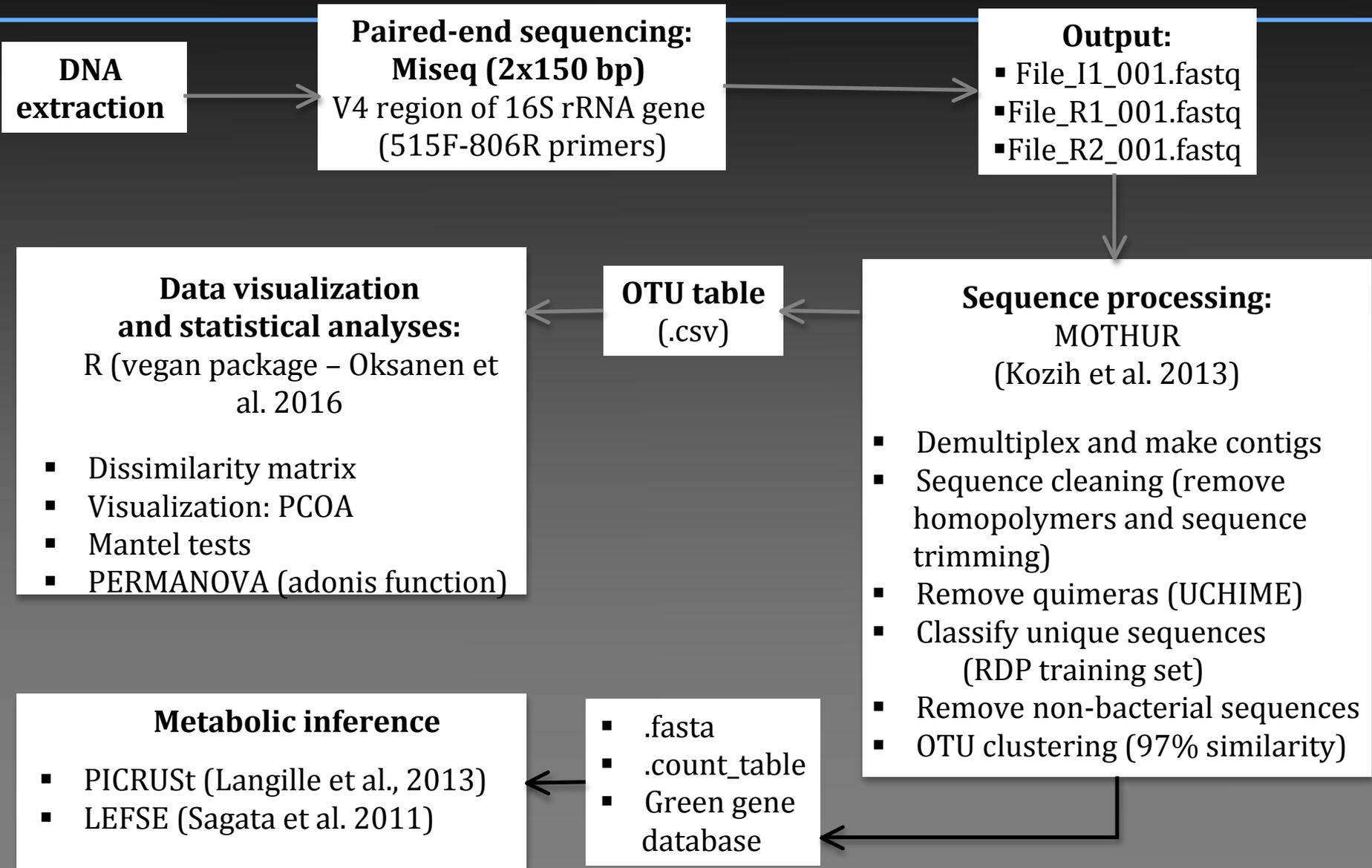


Mothur v. 1. 34. 0 (936,749 reads)

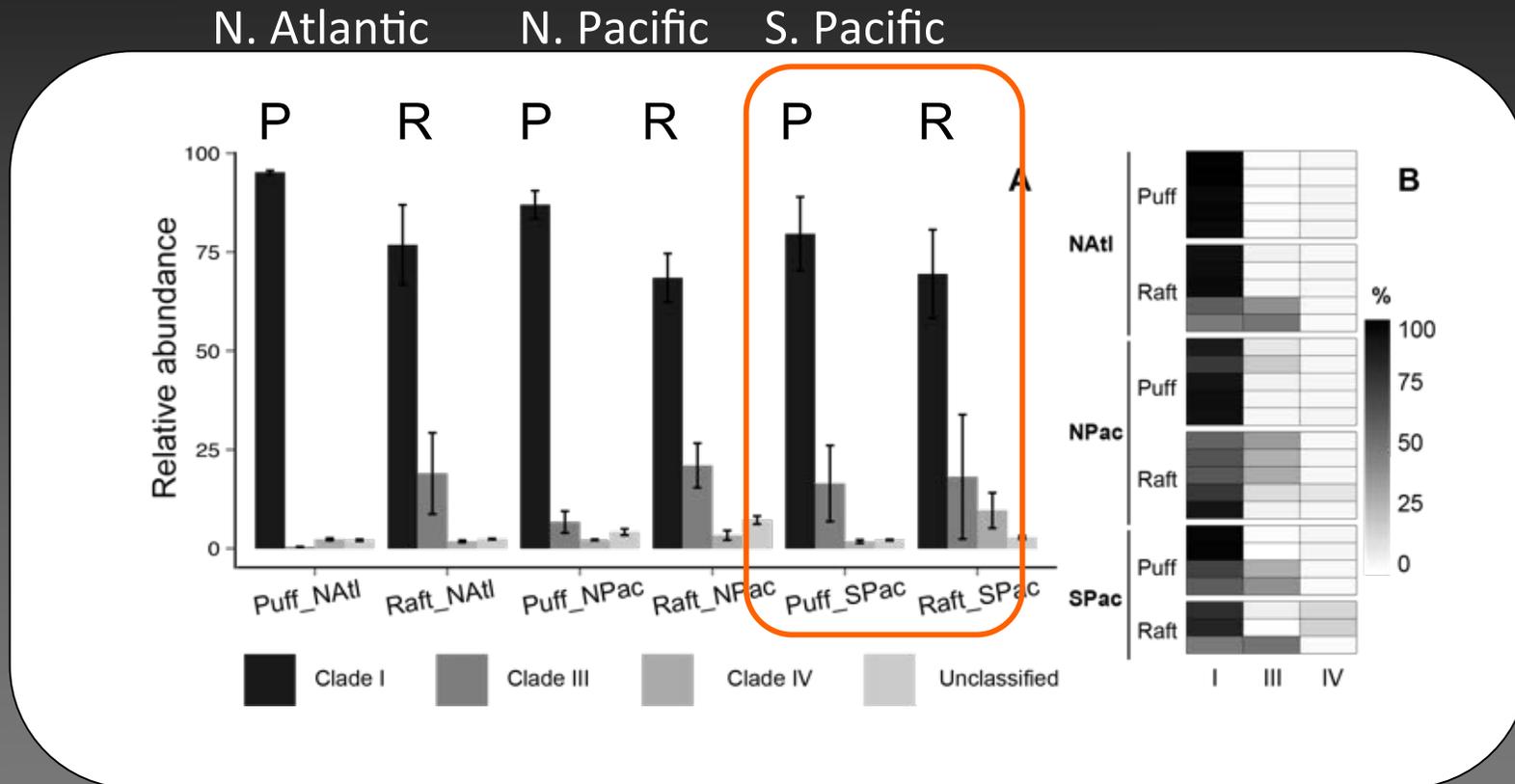
- *Trichodesmium*: ~67% of reads
- Epibionts: ~32% of reads

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

16S rDNA analyses

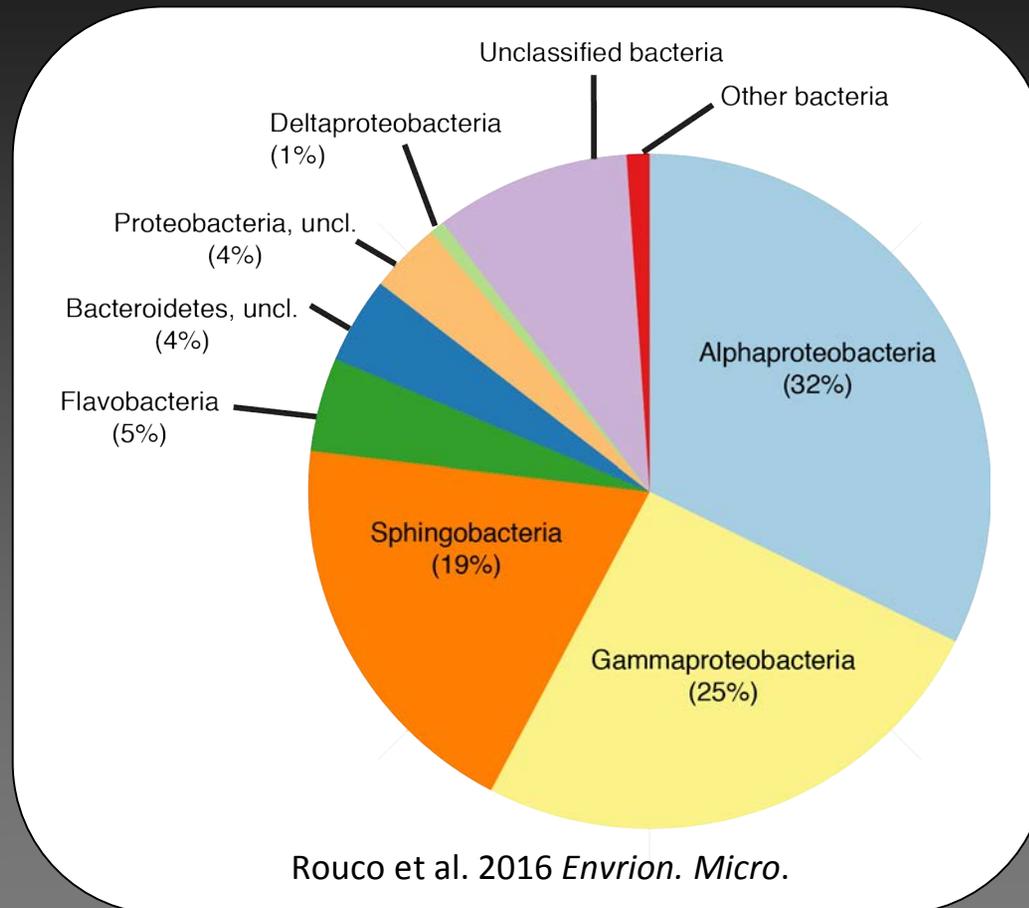


Colony composition by region



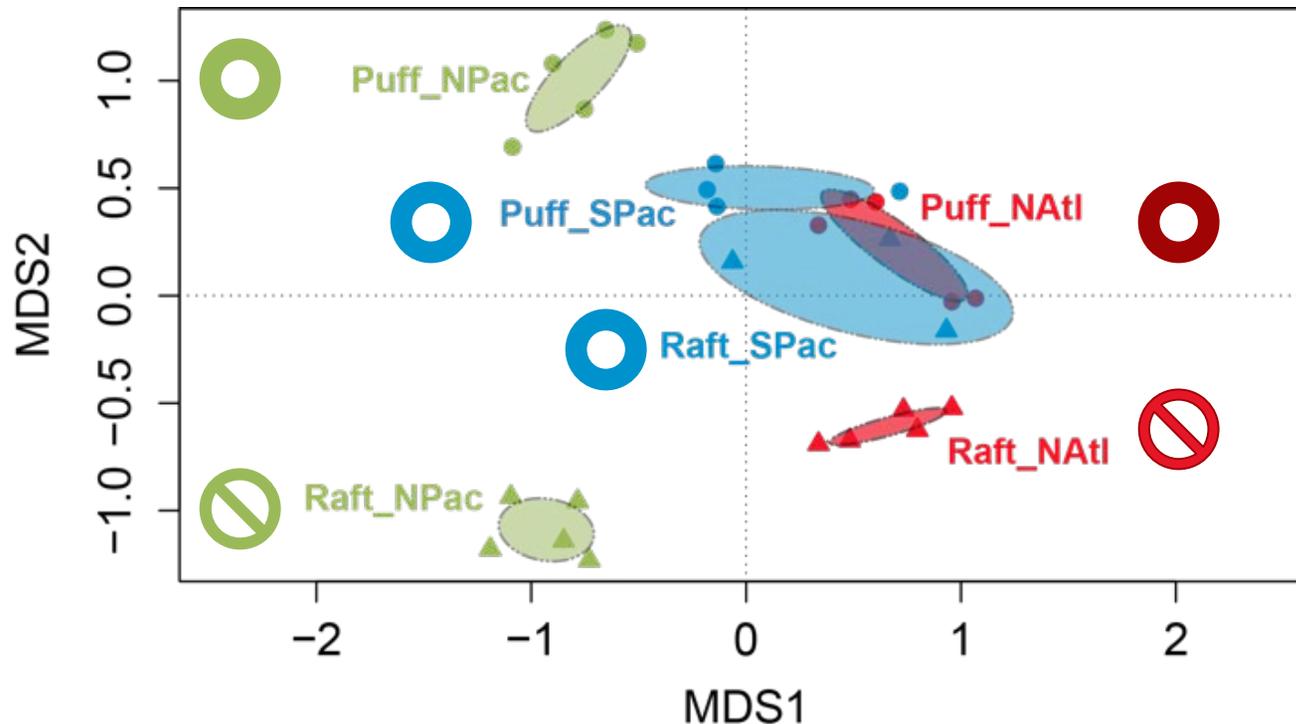
Colonies are not likely species specific, the raft morphology is more diverse except in the S. Pacific

Average epibiont community



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

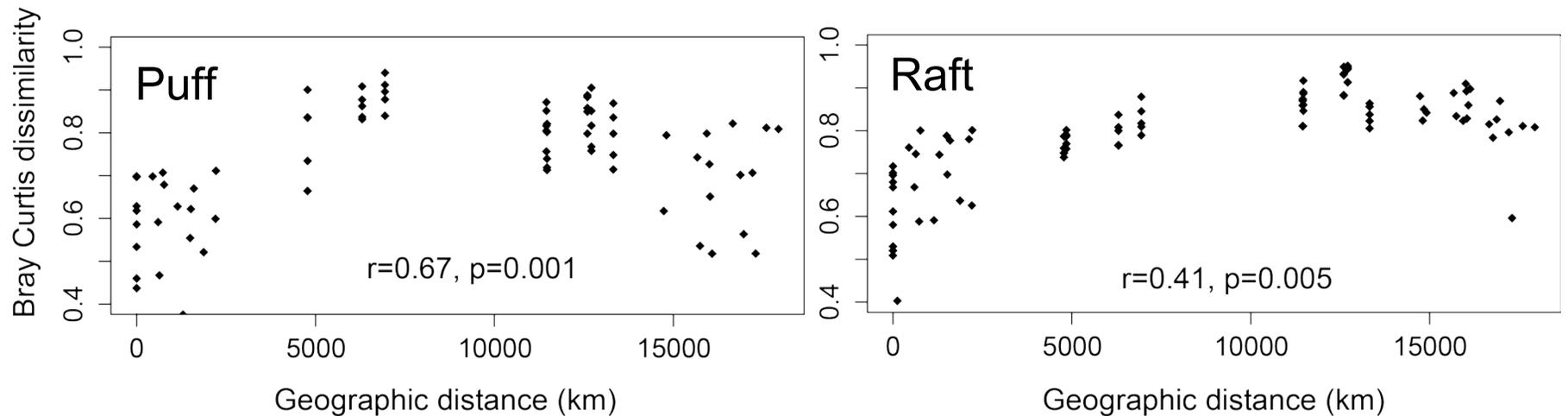
Microbiome community diversity (16S)



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

Microbiome communities significantly differ by ocean basin, and with colony morphology, except for the S. Pacific. Communities are distinct from the water column, and sinking particles.

Epibiont community diversity

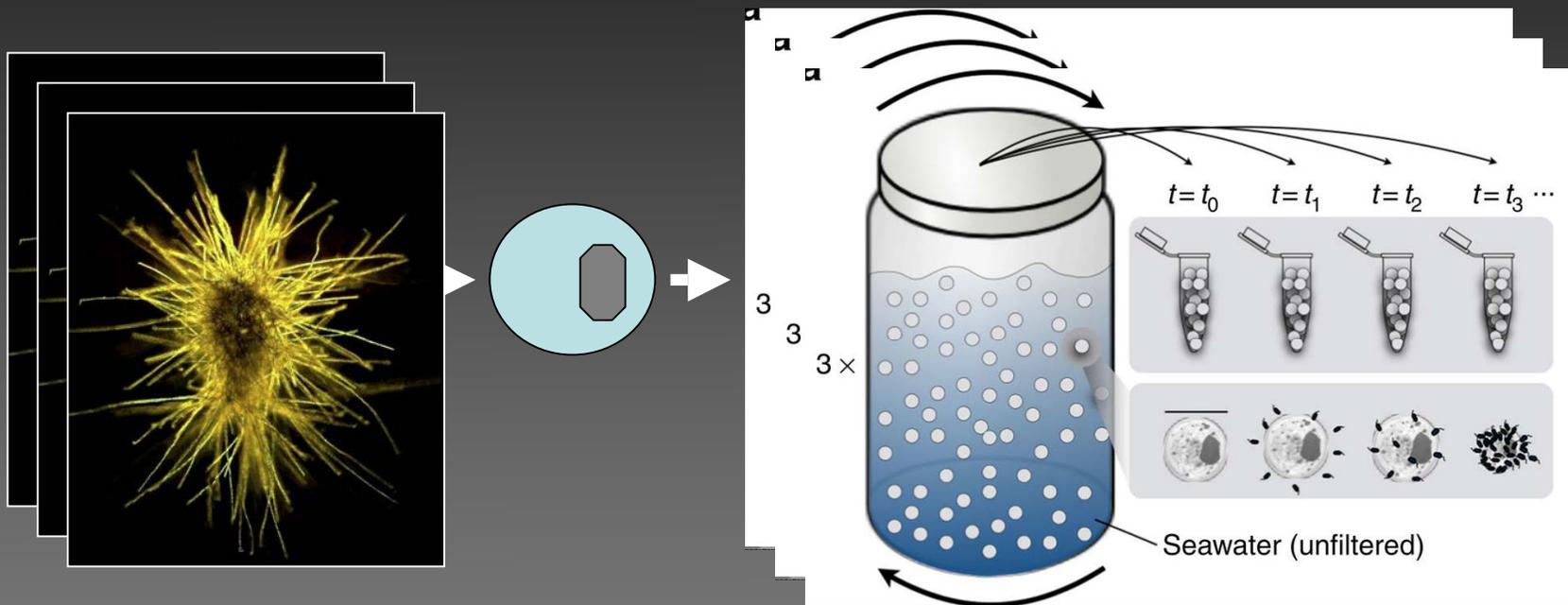


Epibiont communities are isolated by distance across ocean basins within a morphology so both neutral and selective processes may drive the holobiont structure

What drives community assembly?

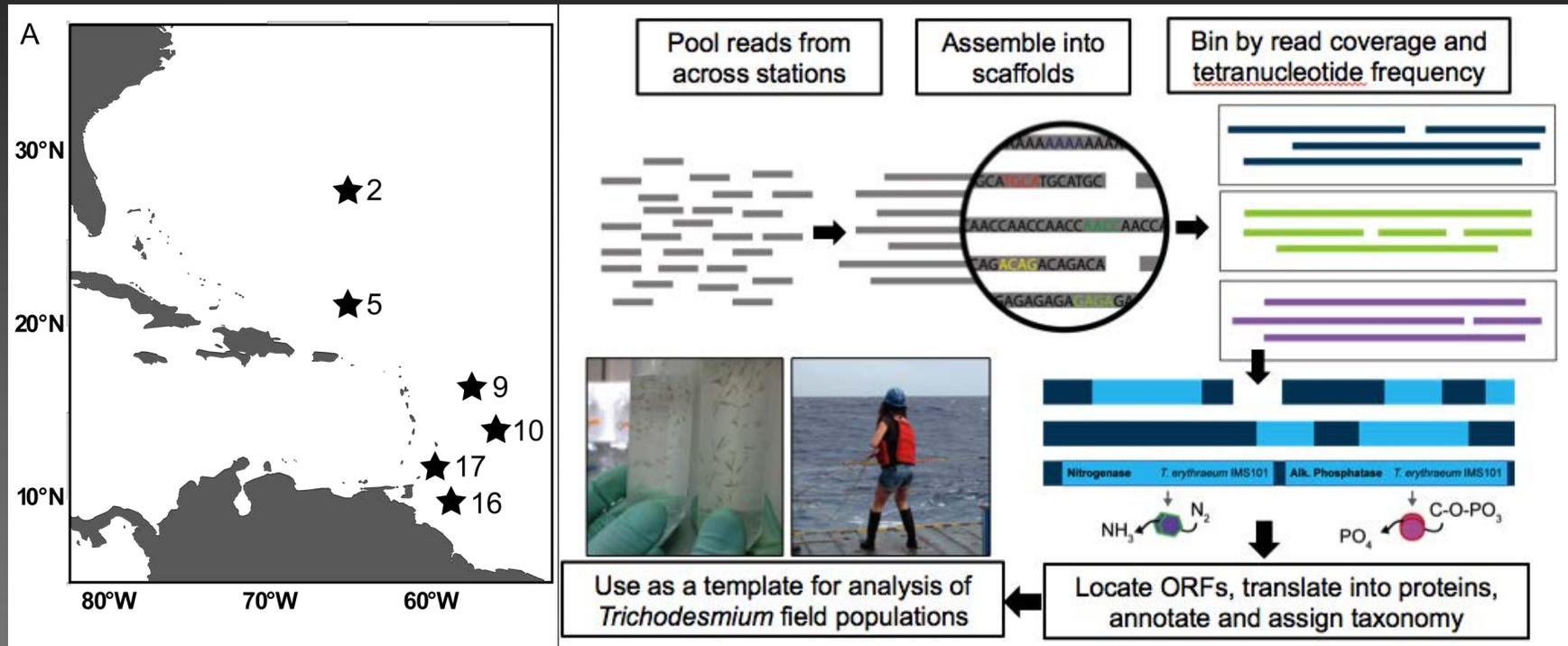
- *Niche?* What type of *Trichodesmium*, physiological ecology in the colony, environment..
- *Lottery?* Random selection of potential copiotrophs, role of taxonomic v. functional group uncertain...
- Working to examine the *Trichodesmium* holobiont with metagenomics/metatranscriptomics and “germ-free” *Trichodesmium*

Drivers of community assembly



Initial trial of “germ-free” *Trichodesmium* ran into problems in the NPSG - phase two schedule for spring 2018.

Metabolic potential in the *Trichodesmium* holobiont



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

Metagenome Pipeline

Pool reads and assemble

IDBA-UD

Bin by read coverage and tetranucleotide frequency, assess bin completeness & length

MaxBin

Locate ORFs, translate into proteins

Prodigal

Annotate proteins and assign taxonomy

DIAMOND-BLAST
MEGAN?
KEGG

Cluster binned scaffolds into orthologous groups

MCL

Metatranscriptome Pipeline

Pool reads and assemble

Eel Pond mRNAseq Protocol (Titus Brown)

Map reads from each sample to de novo assembly

RSEM

Assign taxonomy and annotate (and maybe sum read counts by species/group)

DIAMOND-BLAST (vs NR)
MEGAN
KEGG

Find genes/OGs with differential expression between samples

ASC (no reps)
EdgeR (reps)

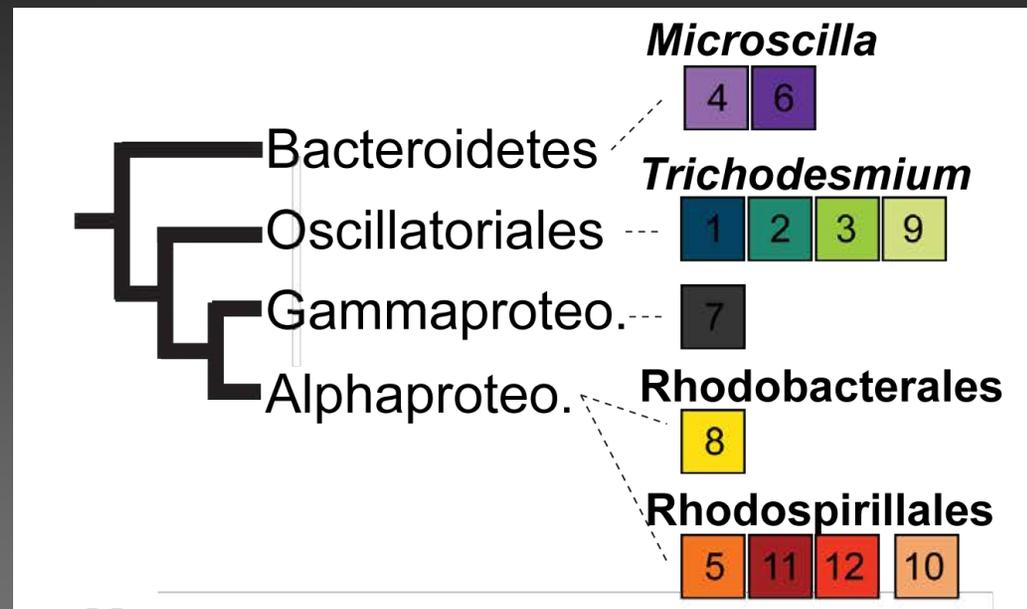
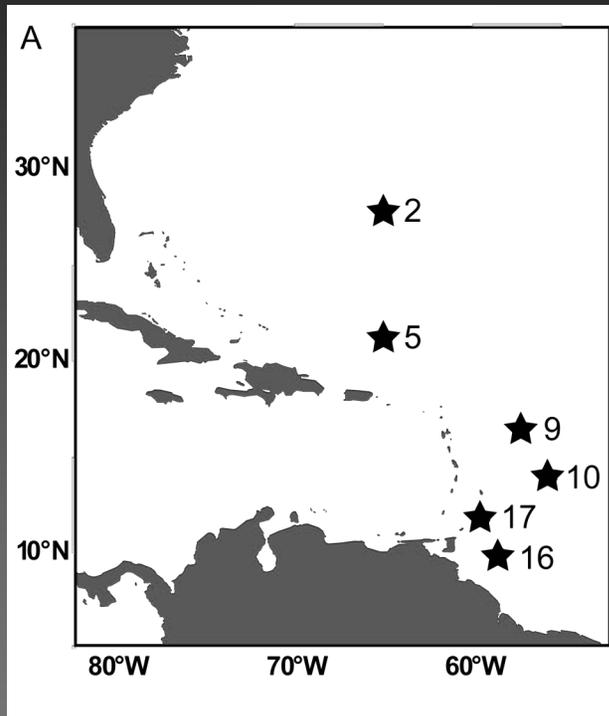
Normalize expression between different samples

EdgeR (variance stabilize)
TMM (NOISeq in R)
TPM

Find genes/OGs with key expr. patterns

RAIN (R)
Thaben and Westermark, 2014

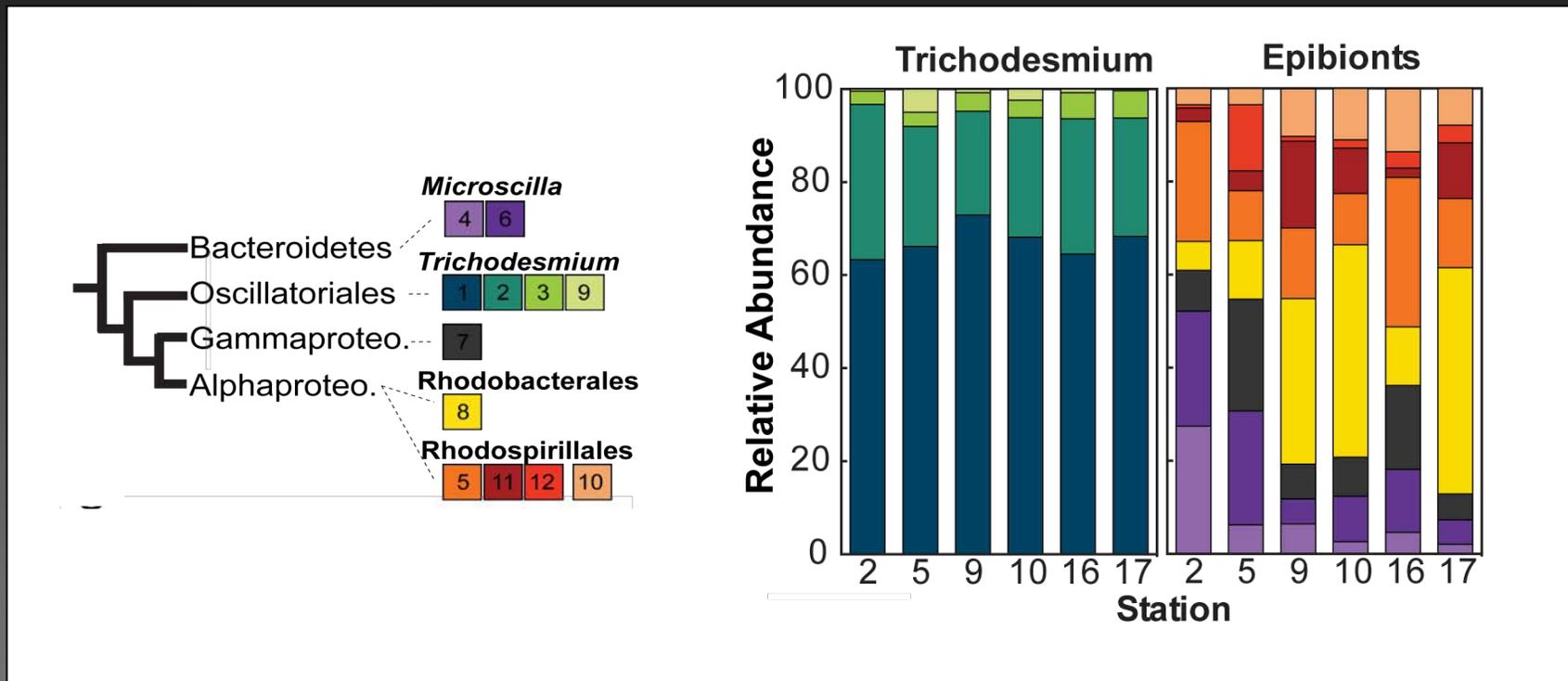
Composition of the holobiont



Frischkorn et al. (2017) ISMEJ

Nearly complete (65-90%) genome bins were reconstructed from a merged assembly and results are consistent with 16S data

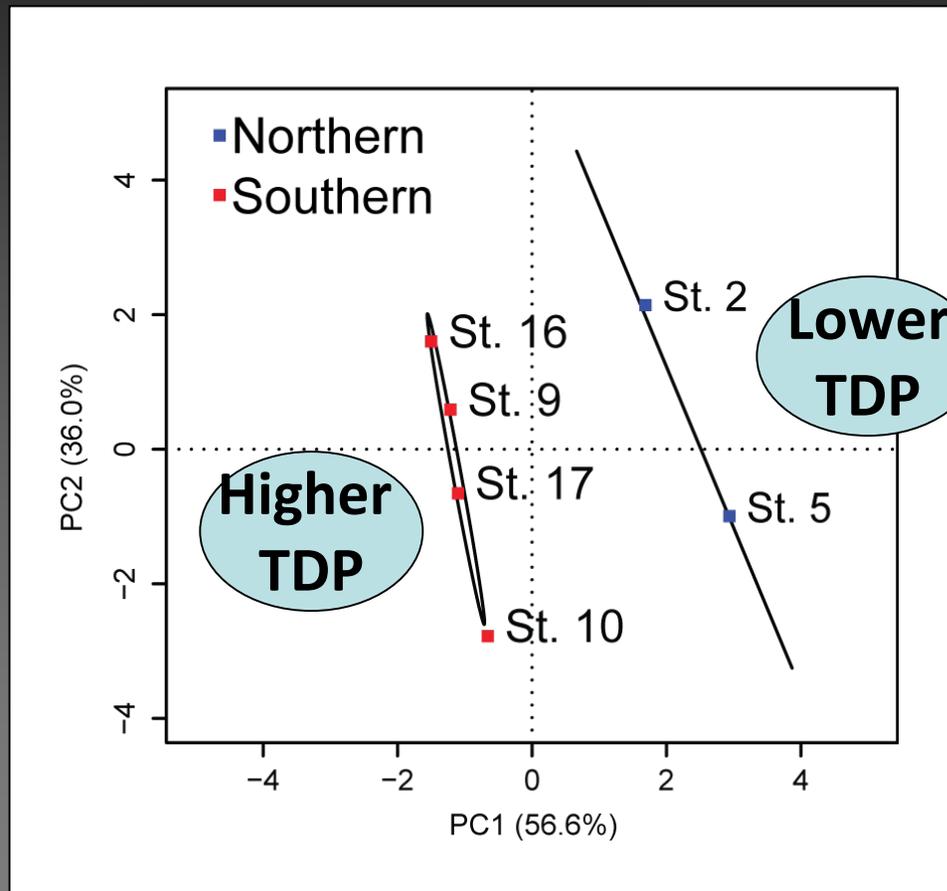
Distribution of holobiont



Frischkorn et al. (2017) ISMEJ

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

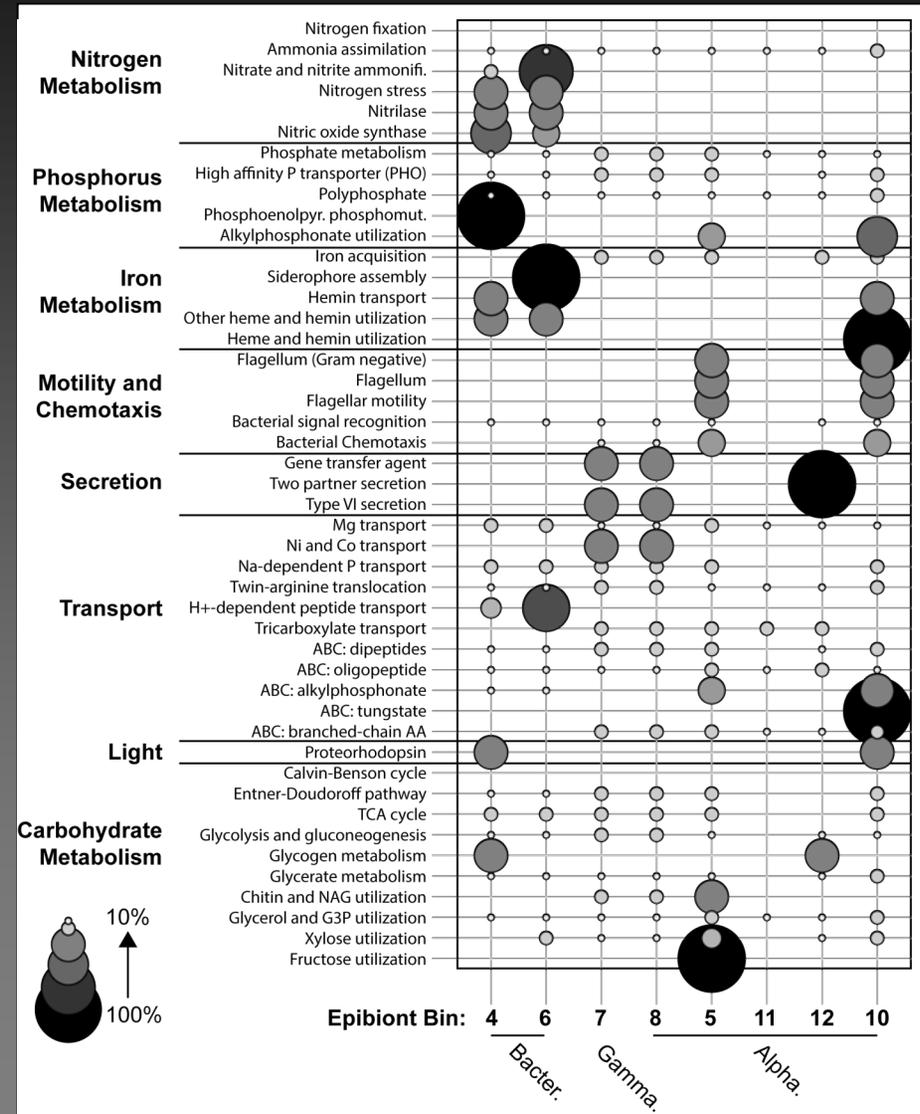
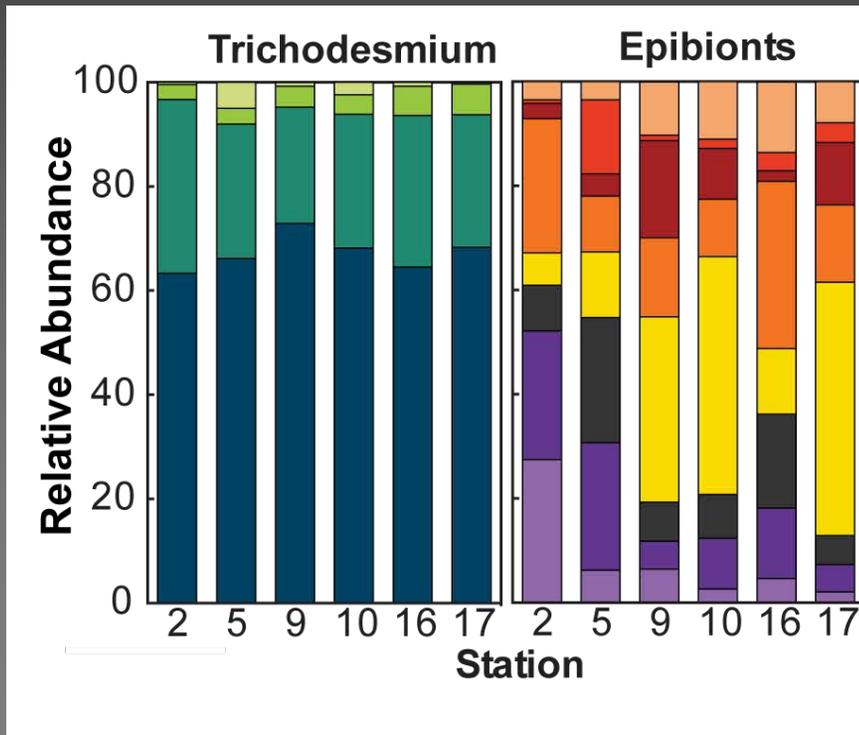
Microbiome diversity differs significantly between regions



Frischkorn et al. (2017) *ISMEJ*

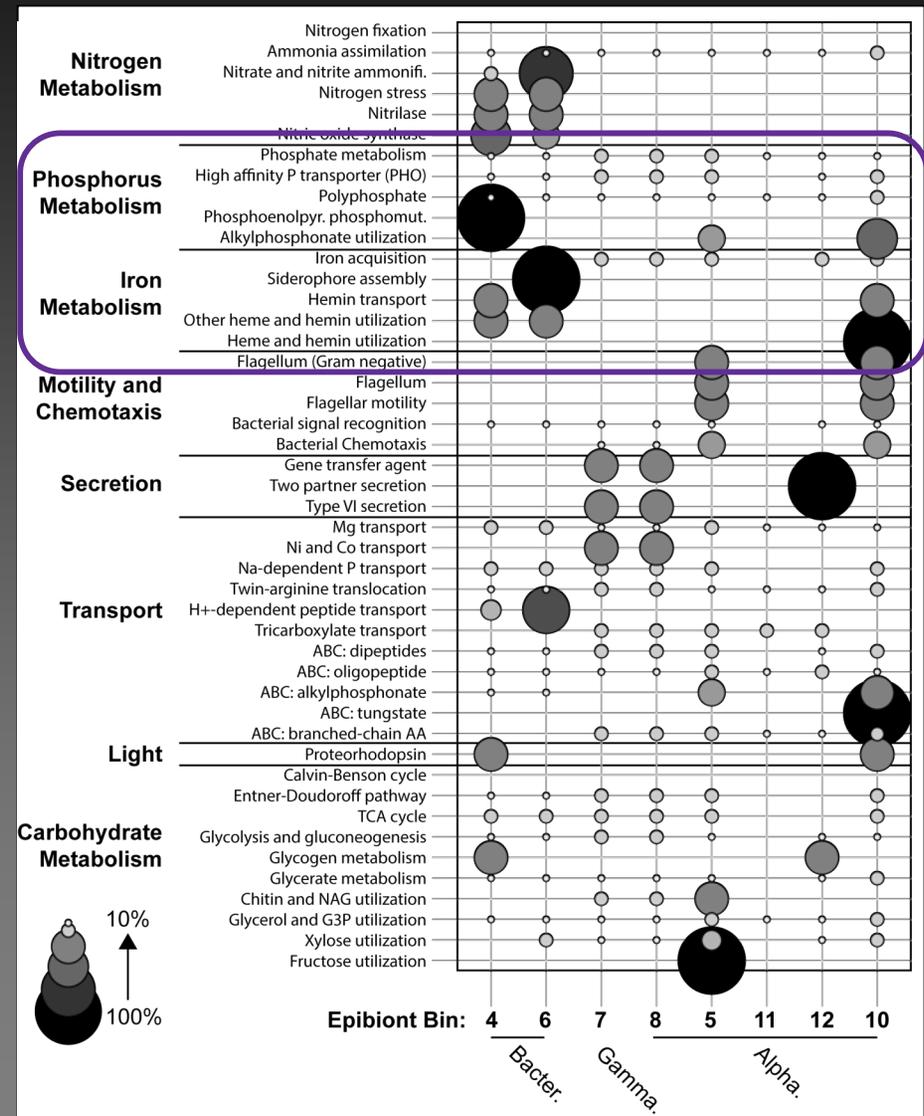
Variable distribution of functional pathways among epibionts

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



Variable distribution of functional pathways among epibionts

- Differential pathway enrichment consistent with a microbiome that is modulated as a function of environment
- Phosphonate, heme and siderophore functions are enriched relative to water column microbes in the Sargasso Sea.



Comparing metabolic potential in the holobiont



Metagenomes



Orthologous group
analysis



Epibionts v. *Trichodesmium*

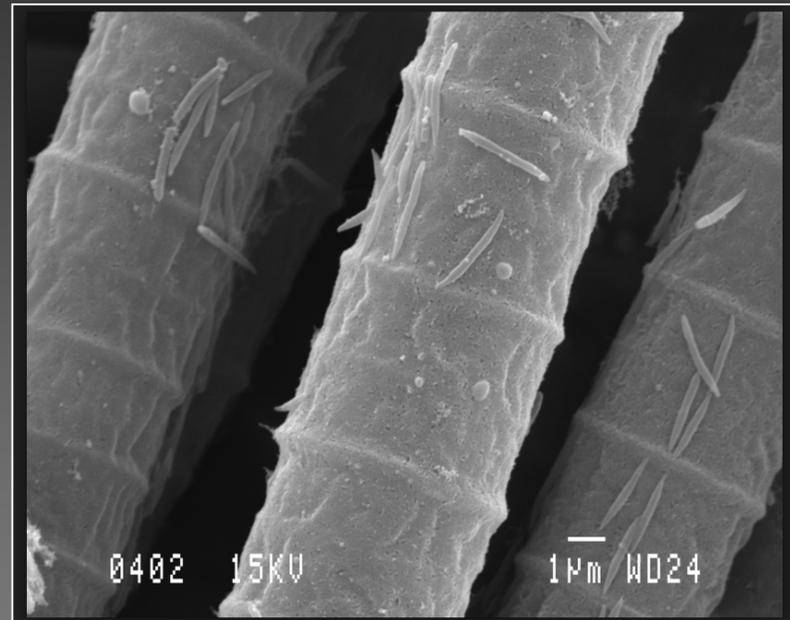
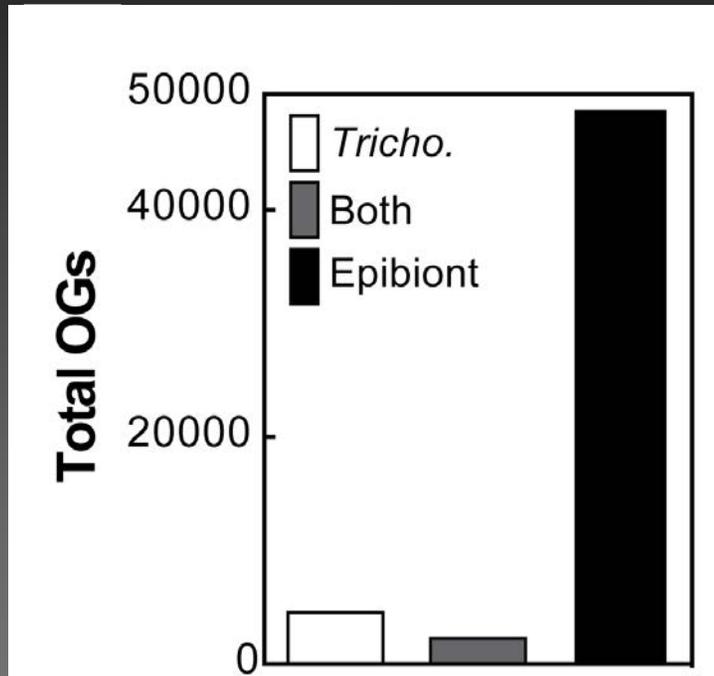


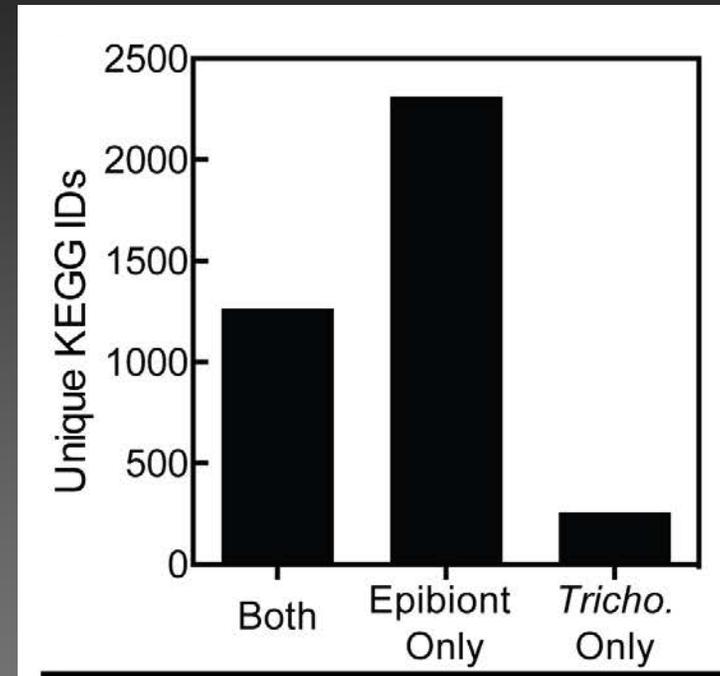
Image courtesy Tracy Mincer

Epibionts confer the majority of the metabolic potential

>90% OGs



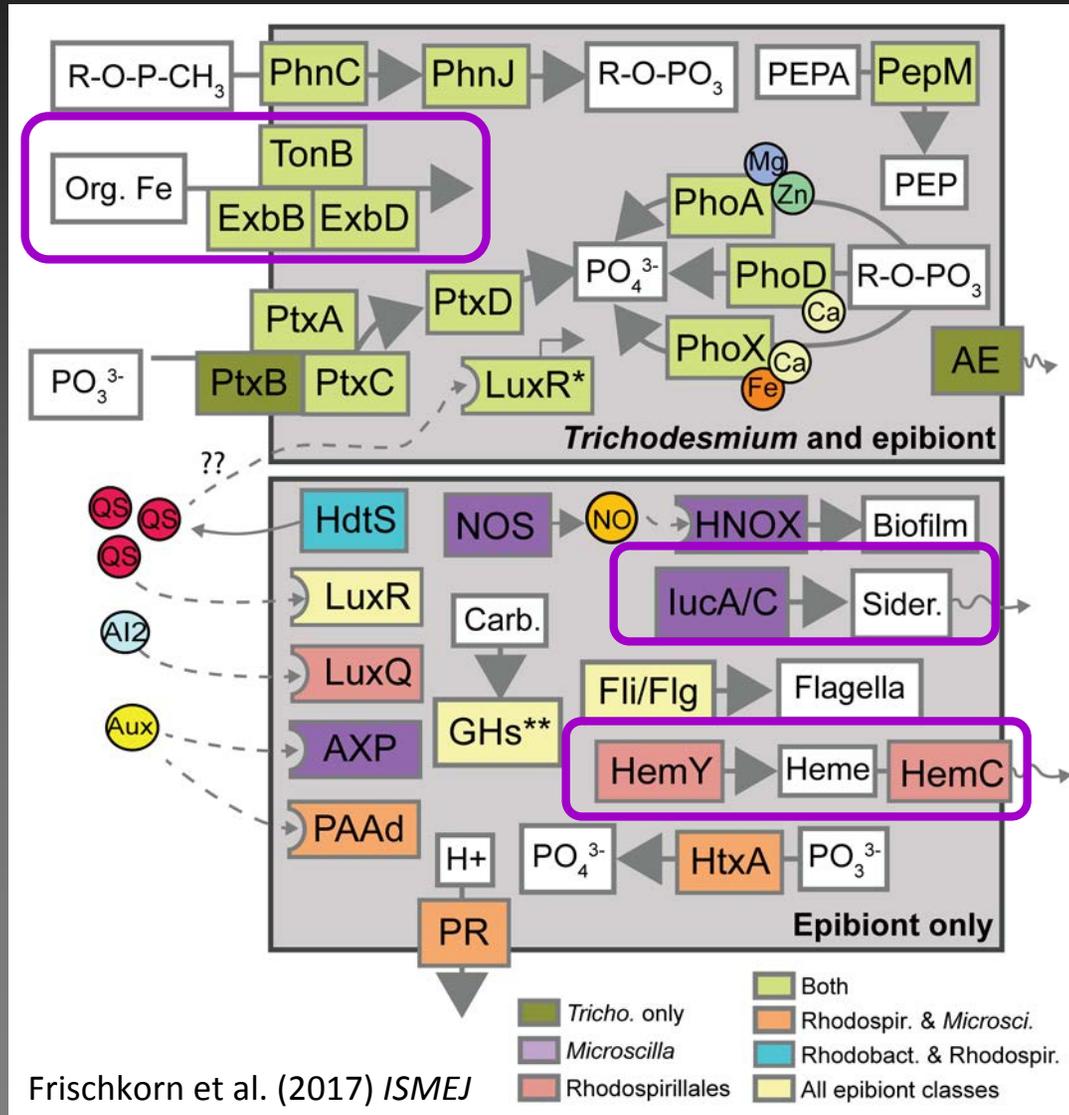
>10x Functions



Frischkorn et al. (2017) ISMEJ

Orthologous (OG) group analysis suggests that epibionts confer the vast majority of metabolic functions to the holobiont.

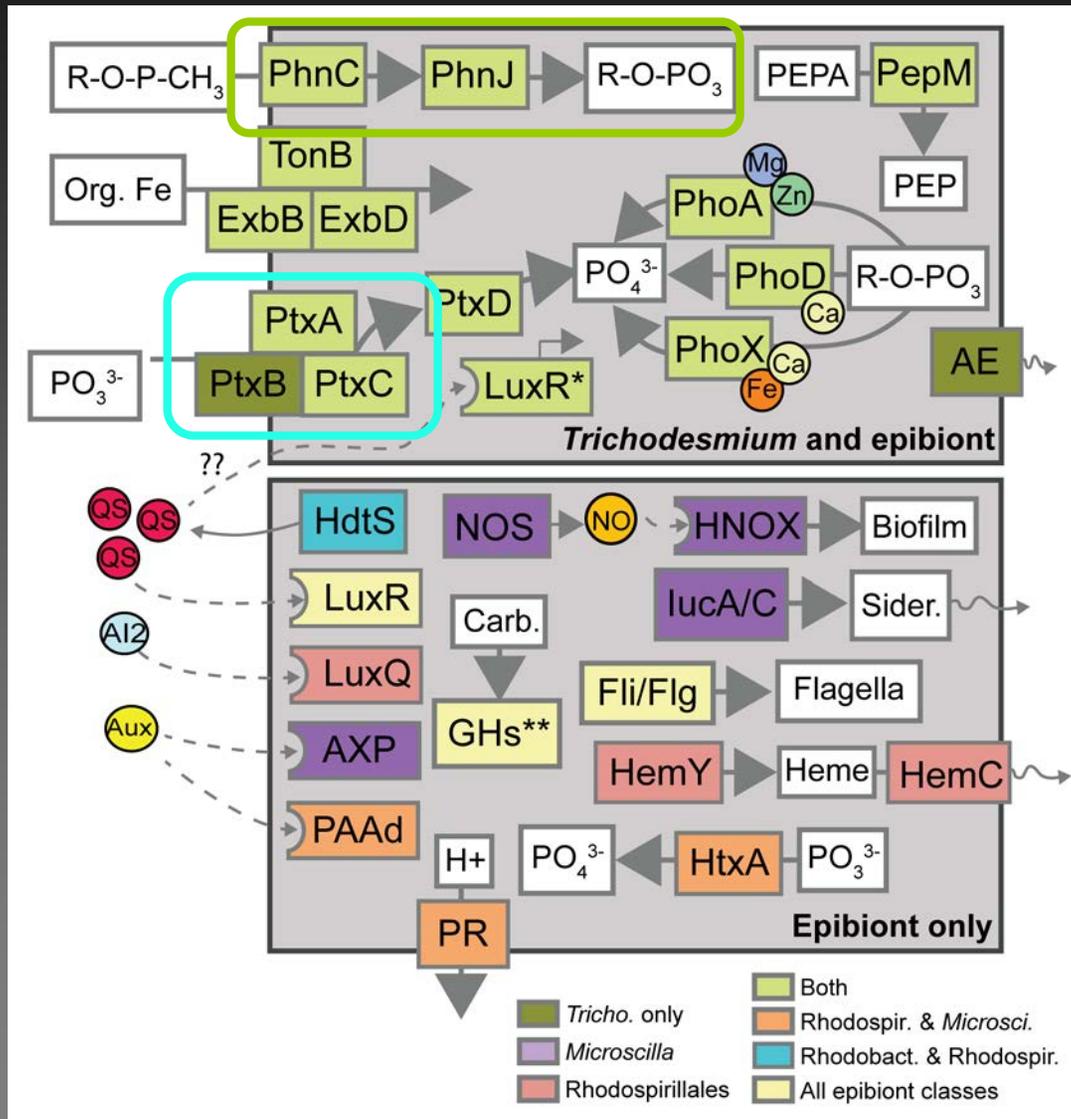
Metabolic partitioning within the *Trichodesmium* holobiont



Organic Iron

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

Uptake and metabolism of reduced phosphorus forms



C-P Lyase
PhoX
PhoA

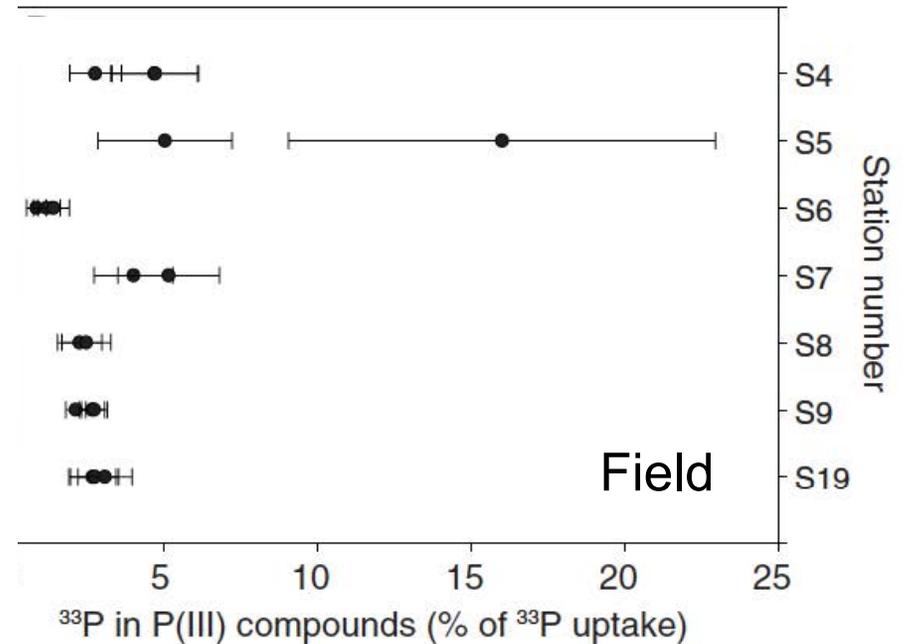
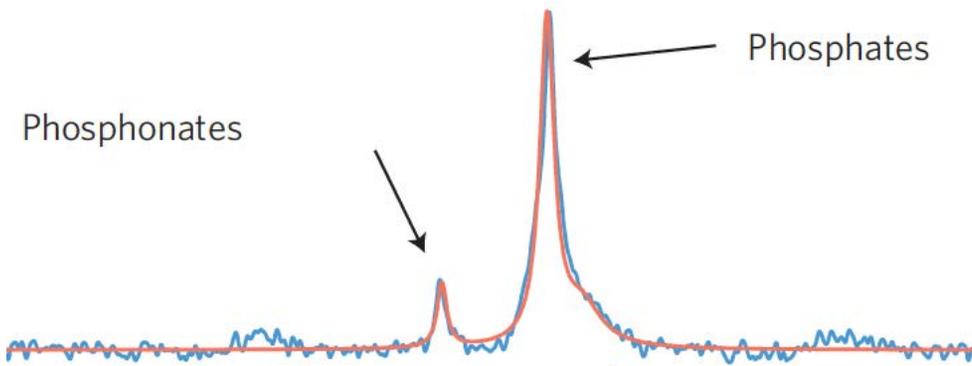
Phosphite
uptake

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

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

Phosphonate (C-P) biosynthesis

Culture

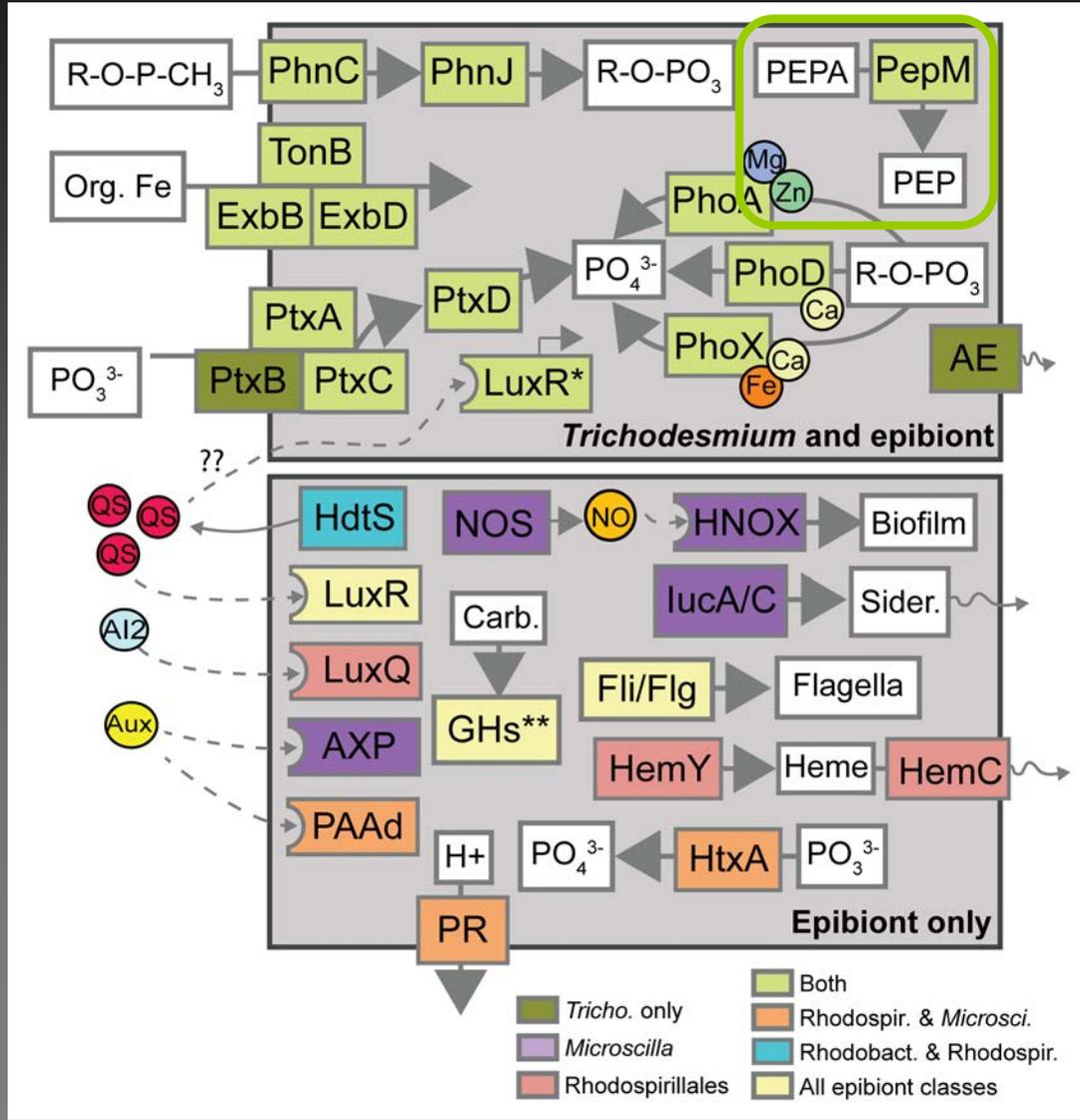


Dyhrman et al. (2009) *Nature Geo.*

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?

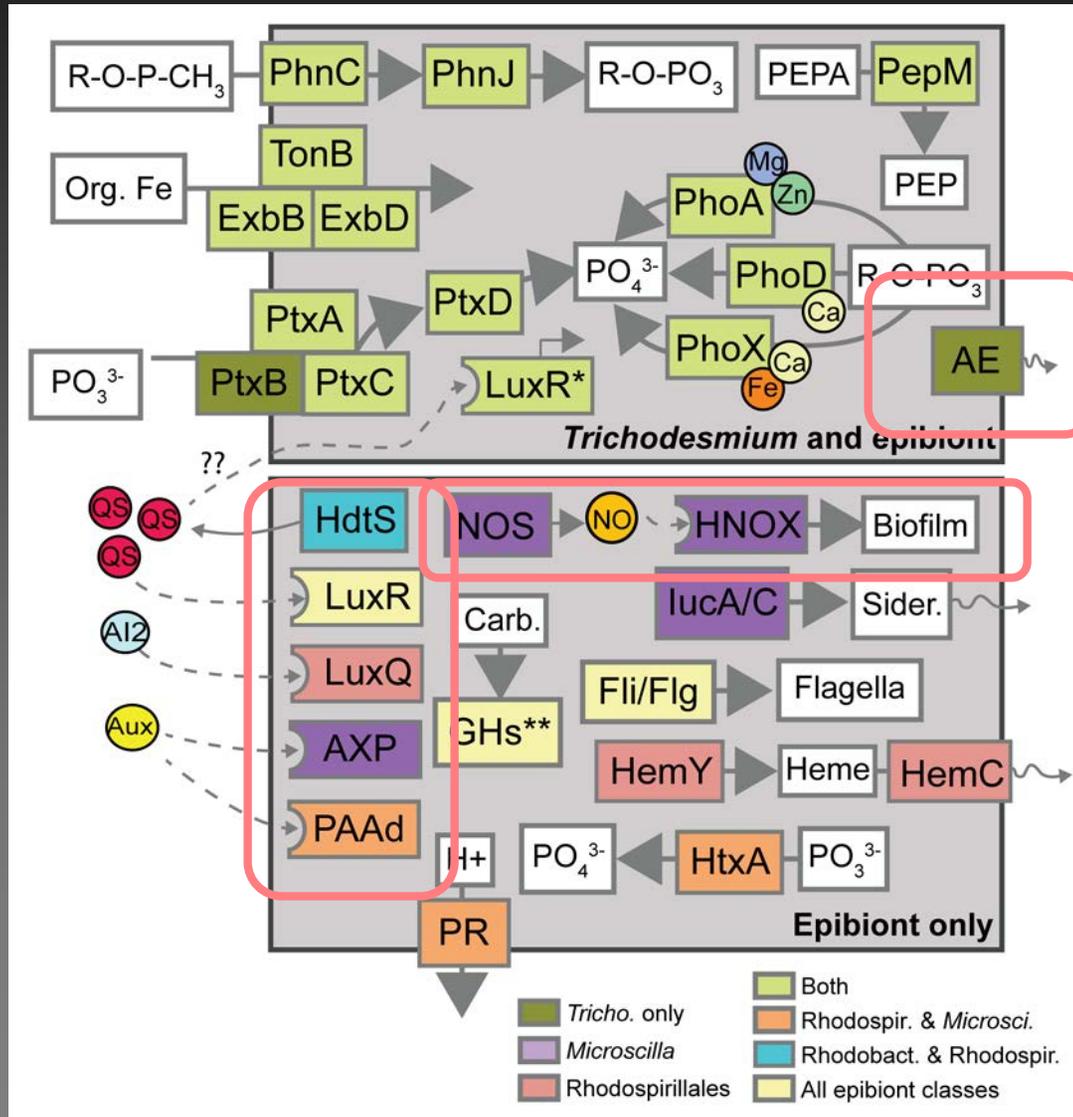
Phosphonate production is a shared metabolism



Phosphonate biosynthesis

Phosphonate produced by both *Trichodesmium* and the epibionts

Microbial cross talk within the *Trichodesmium* holobiont



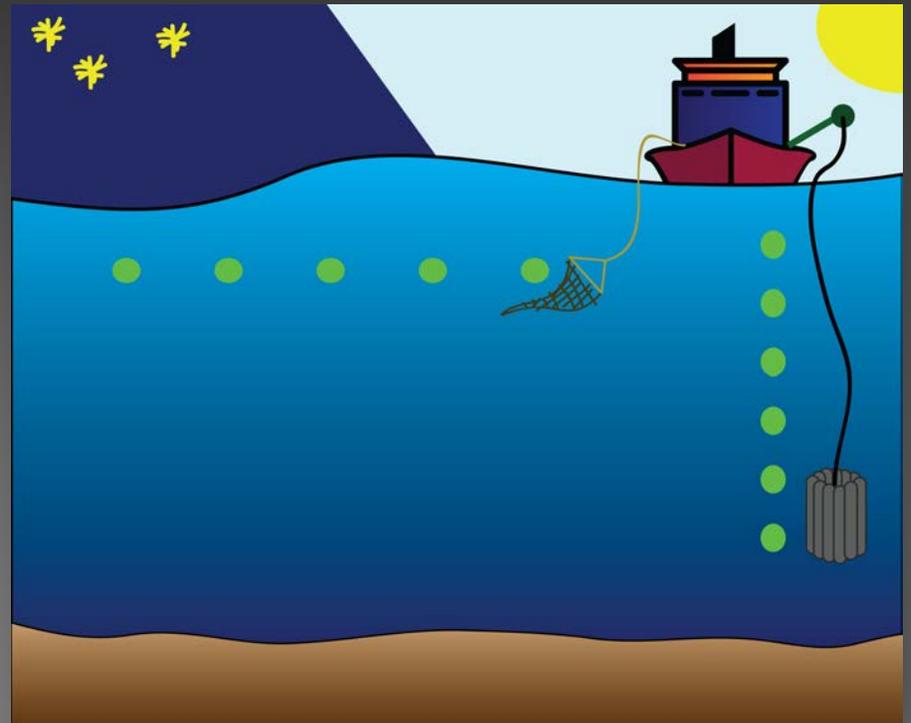
Tracking the interactome... with metatranscriptome profiling after addition of NO and QS molecules

QS and cell signaling

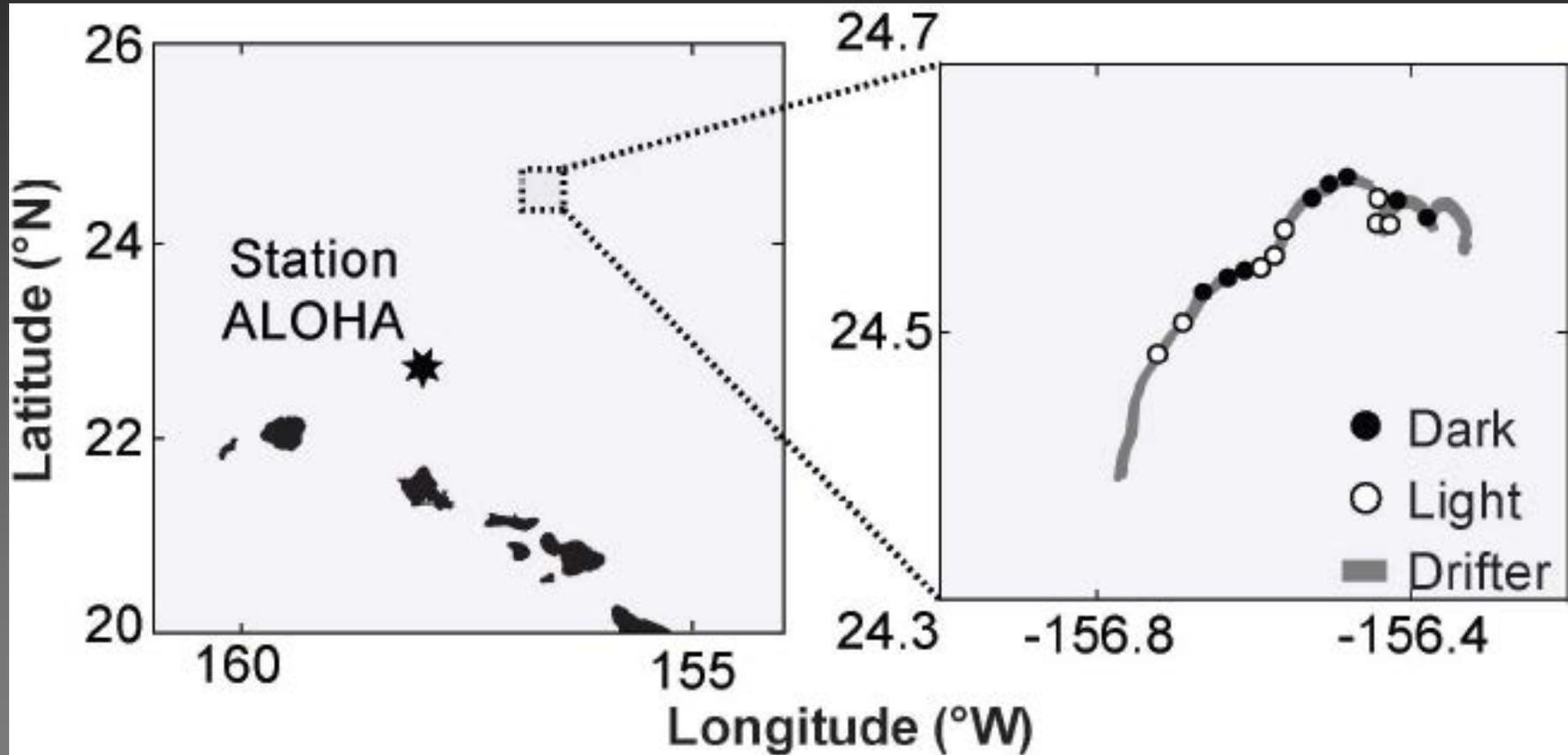
Trichodesmium holobiont interactome...

Using diel metatranscriptome sampling to tease apart possible interactions between *Trichodesmium* and the epibionts.

Are there diel signatures to epibiont genes?

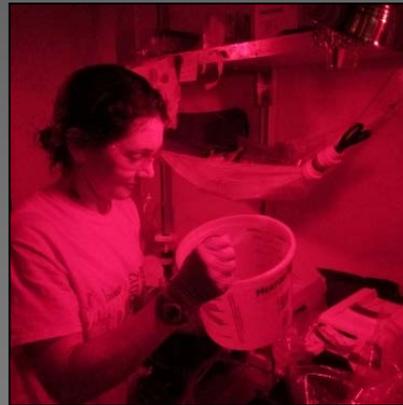
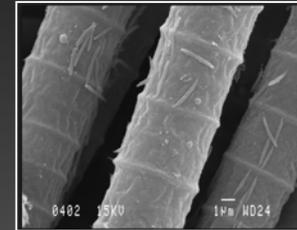


Sampling the interactome... using a time series of daily rhythms



Colony samples were taken every 4 hours for 68 hours, twice!

Coordinated expression dynamics in host and microbiome



Metatranscriptomes



Orthologous group
analysis

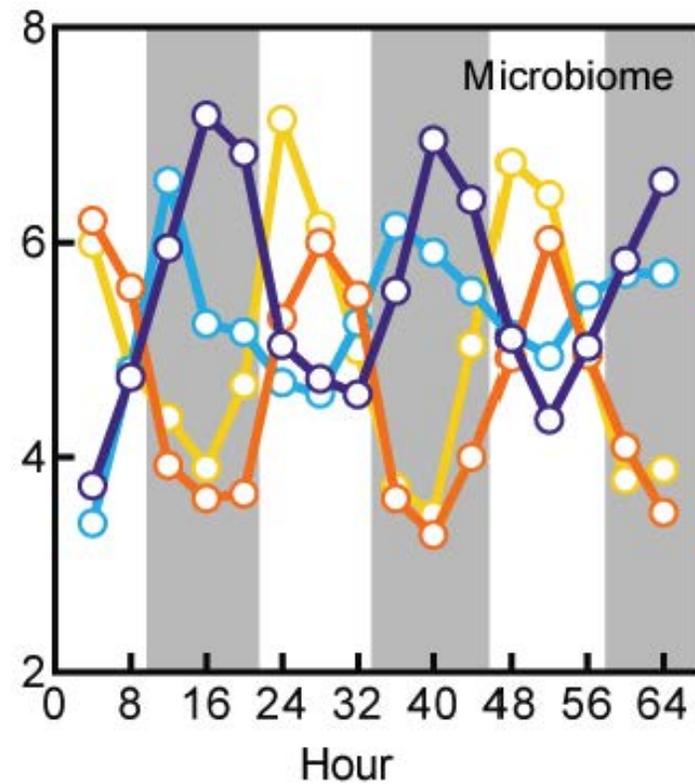
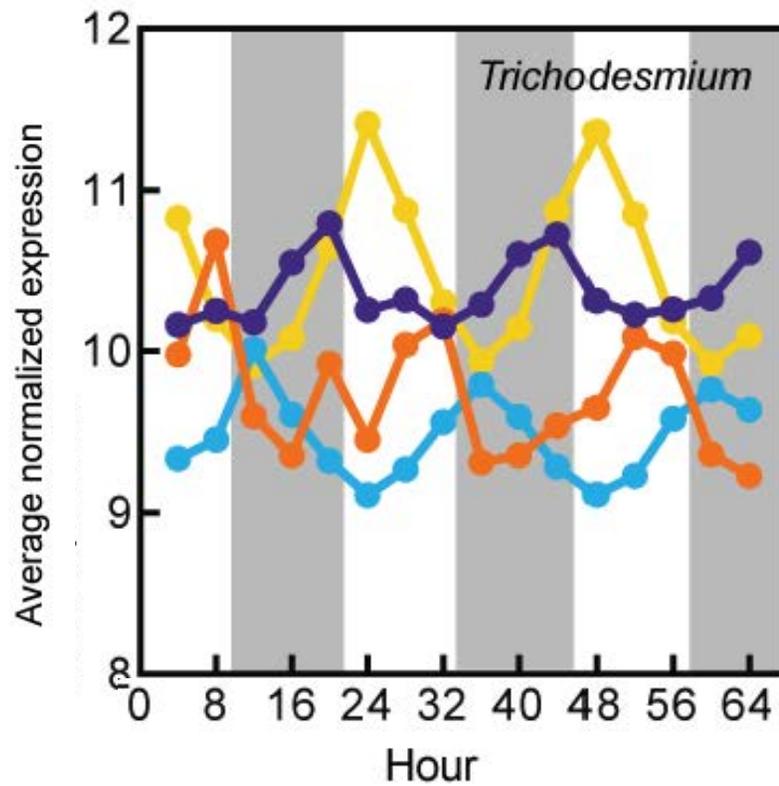


Periodicity: RAIN
Co-expression: WGCNA

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

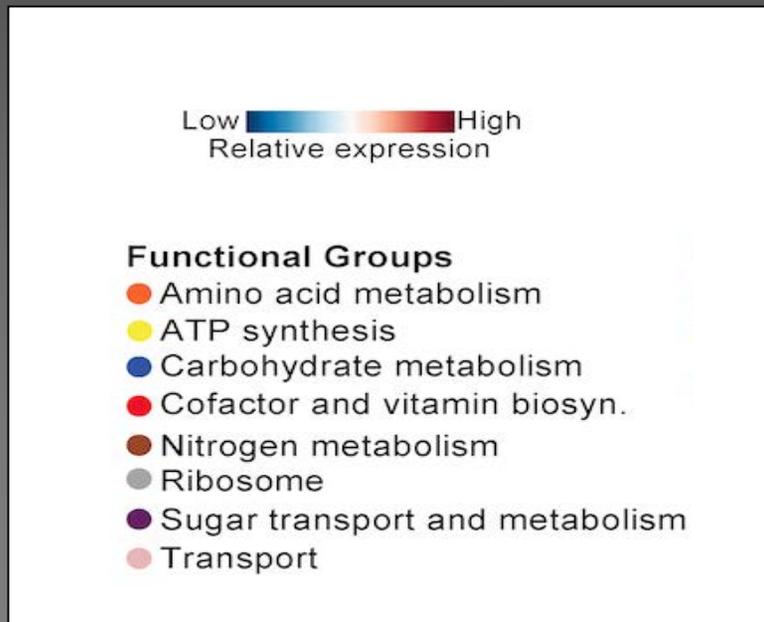
<https://bmcbioinformatics.biomedcentral.com/articles/10.1186/1471-2105-9-559>

Diel modulation of transcripts in *Trichodesmium*

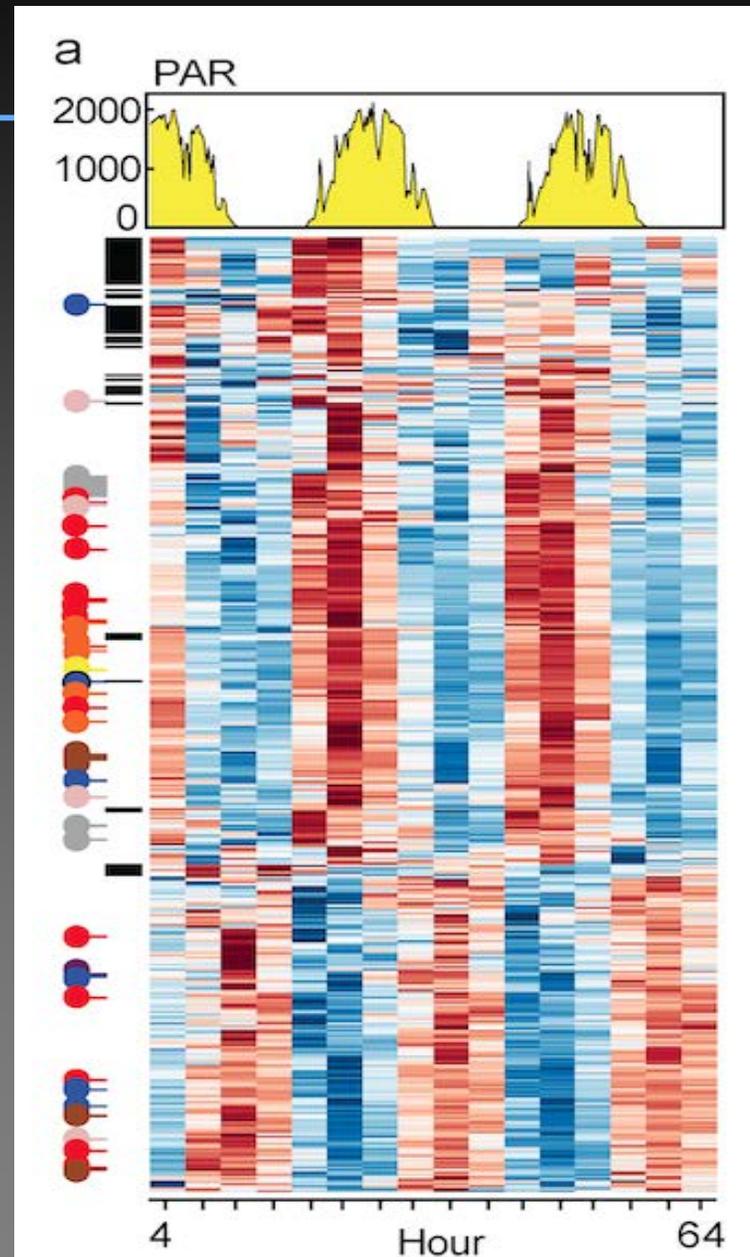


WGCNA co-expression network

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

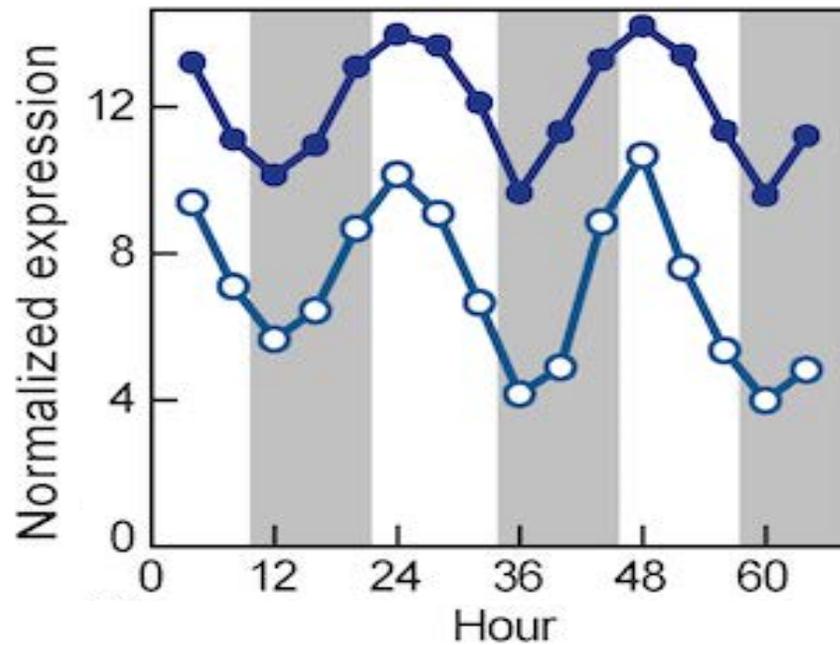


Frischkorn et al. (2018) *ISMEJ*

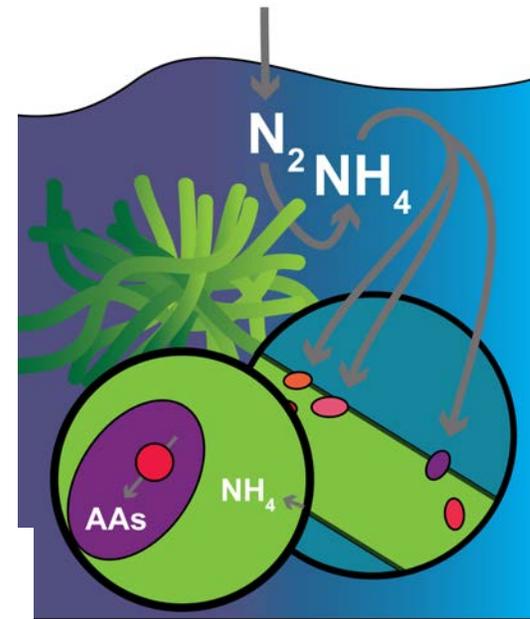


Analyze key functions for coordination within the consortium

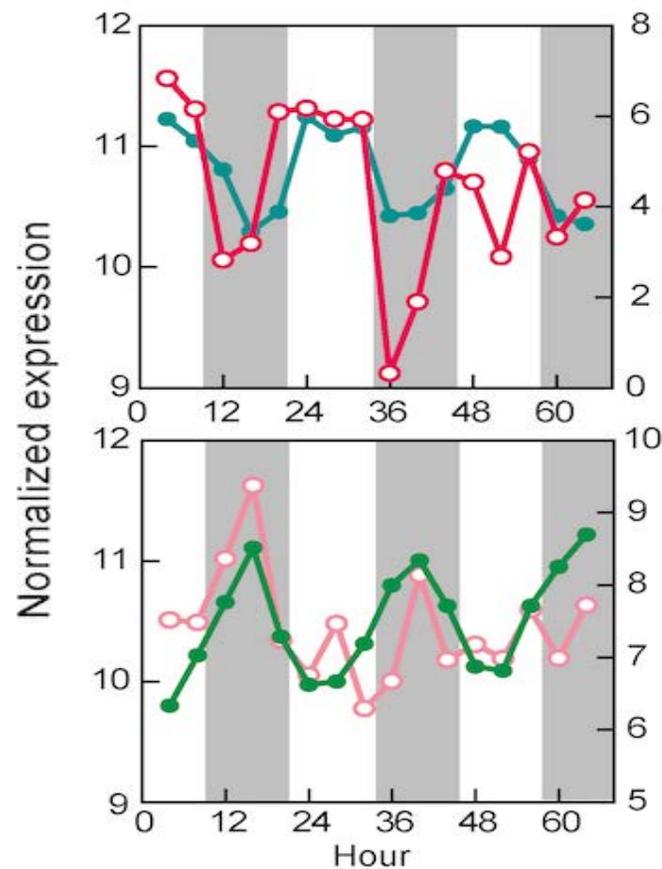
Diel modulation of transcripts suggest coordination: Nitrogen



- *Tricho. nitrogenase avg.*
- *Microbiome N metab. avg.*



Diel modulation of transcripts suggest coordination: Carbon



Trichodesmium Microbiome

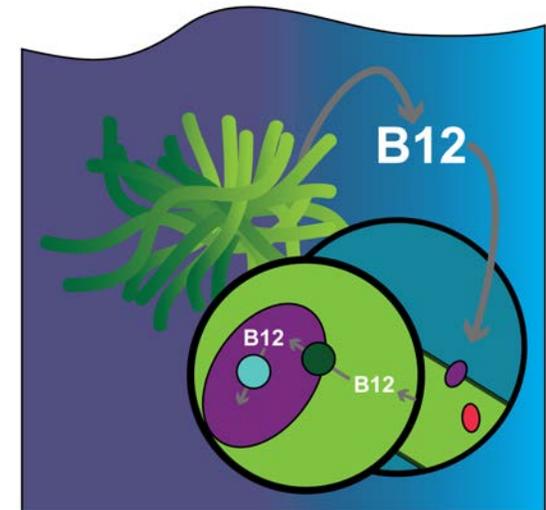
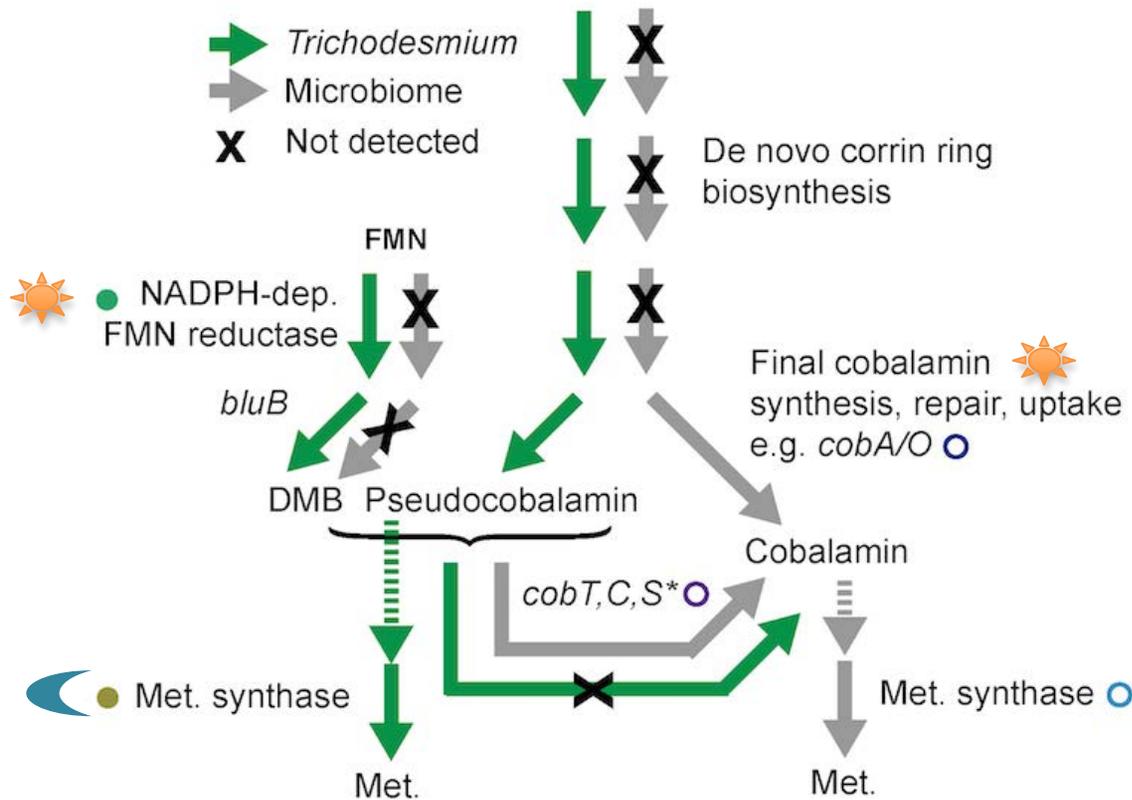
Light Peak

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

Dark Peak

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

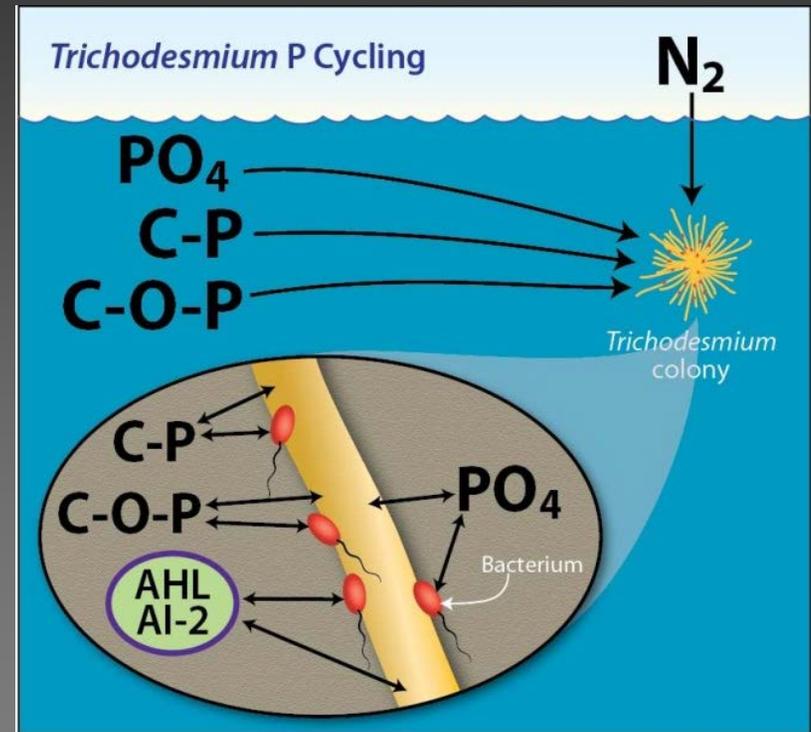
Diel modulation of transcripts suggest coordination: Vitamins



Summary - Host microbiome interactions

What is the role of the microbiome in *Trichodesmium* physiological ecology?

- **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:** Apparent interactions between *Trichodesmium* and its microbiome



Conclusions

Genome-enabled approaches are providing new tools to trace the activities and physiological ecology of *Trichodesmium* and its microbiome.

Lessons learned: There is value in comparisons/time-series and having coincident datasets (Mult-omics)

