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

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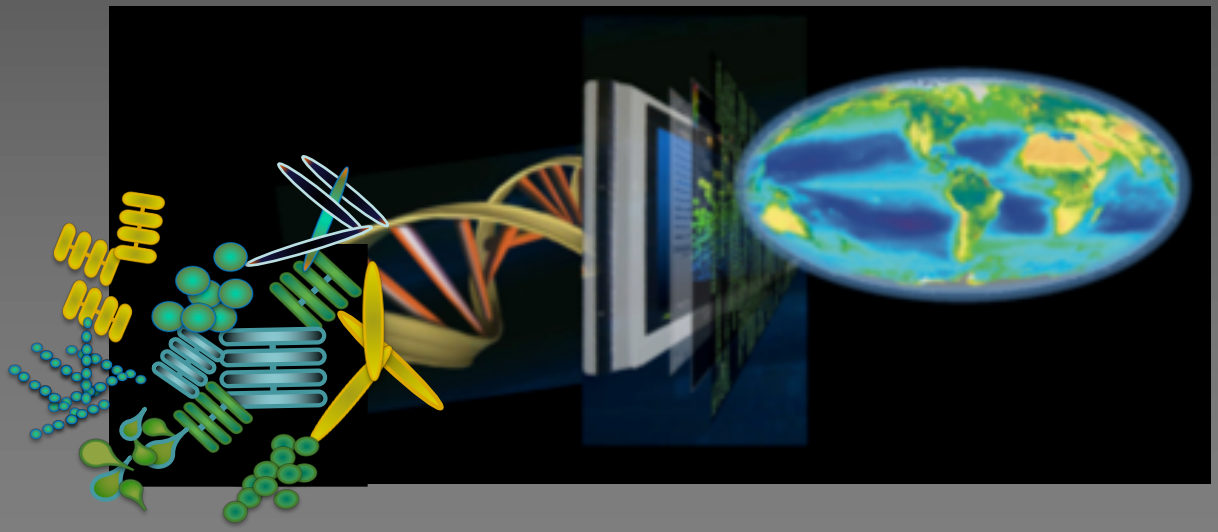
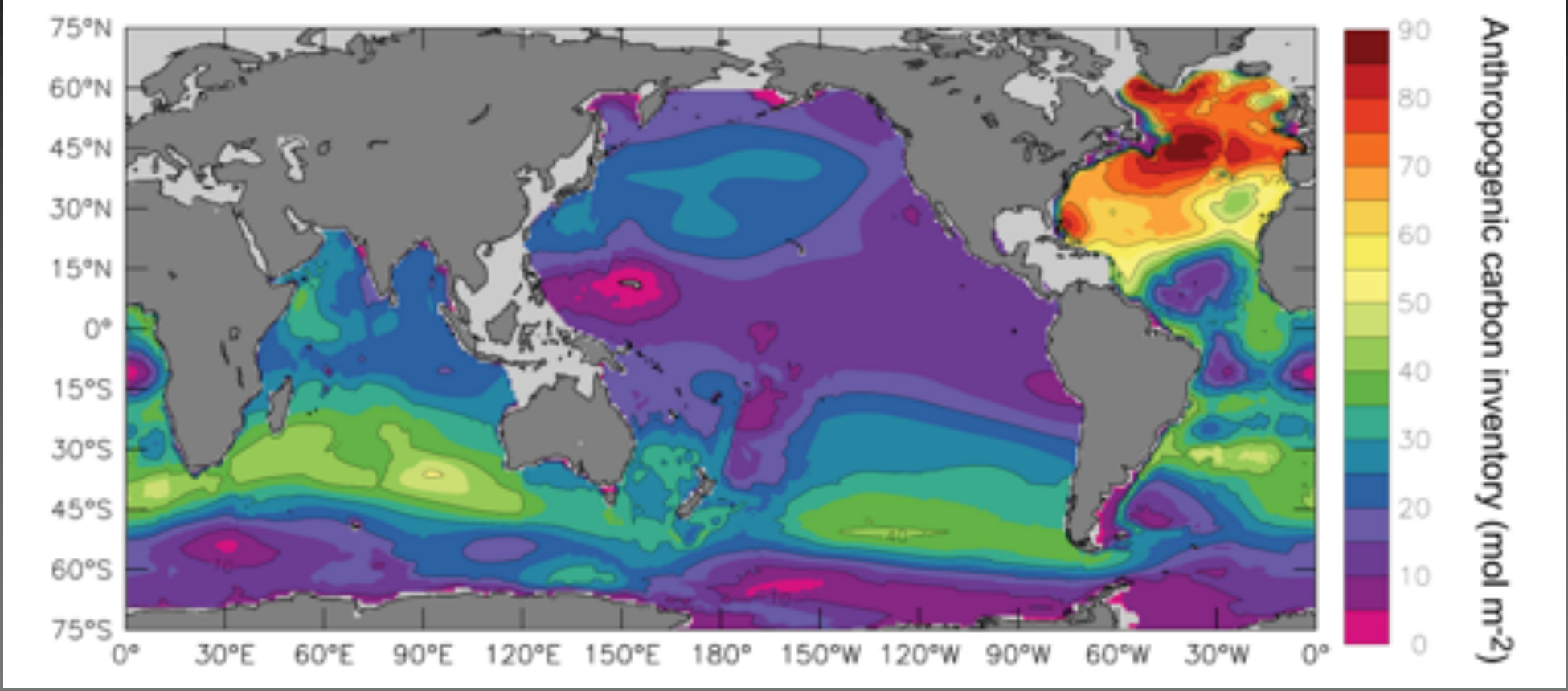


COLUMBIA UNIVERSITY
IN THE CITY OF NEW YORK

LAMONT-DOHERTY
EARTH OBSERVATORY

The ocean makes our planet livable

The ocean acts as a buffer for CO_2 in the atmosphere



Sabine et al. (2004) *Science*

Between 1800 - 1994, ocean has absorbed ~120 petagrams of CO_2
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*

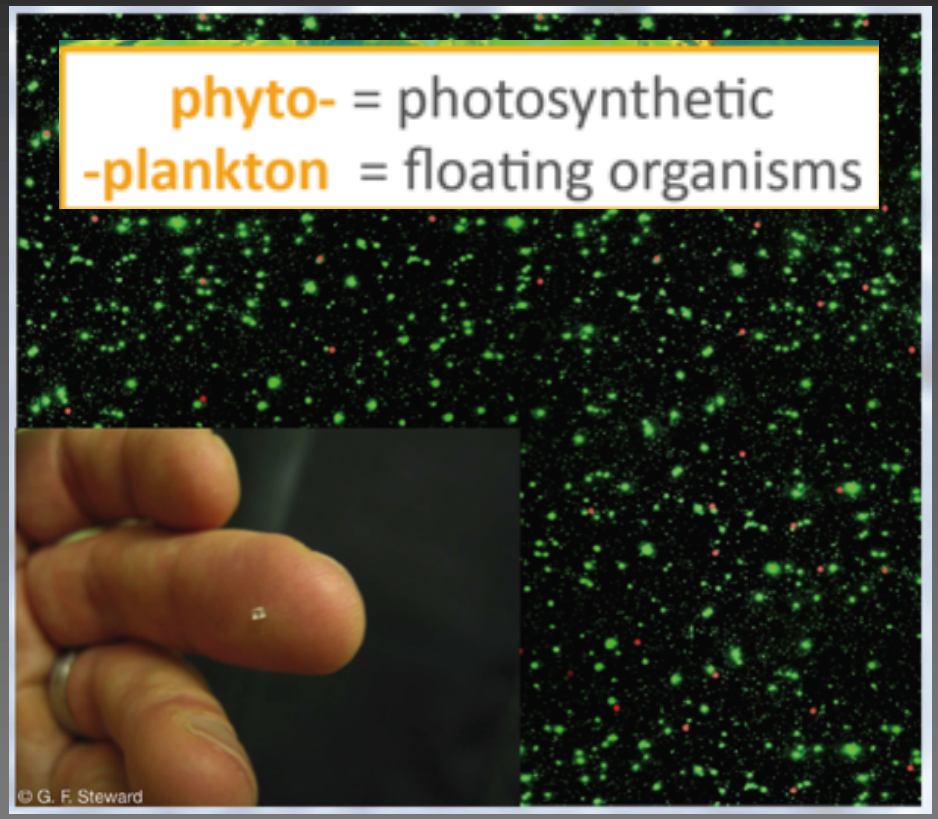
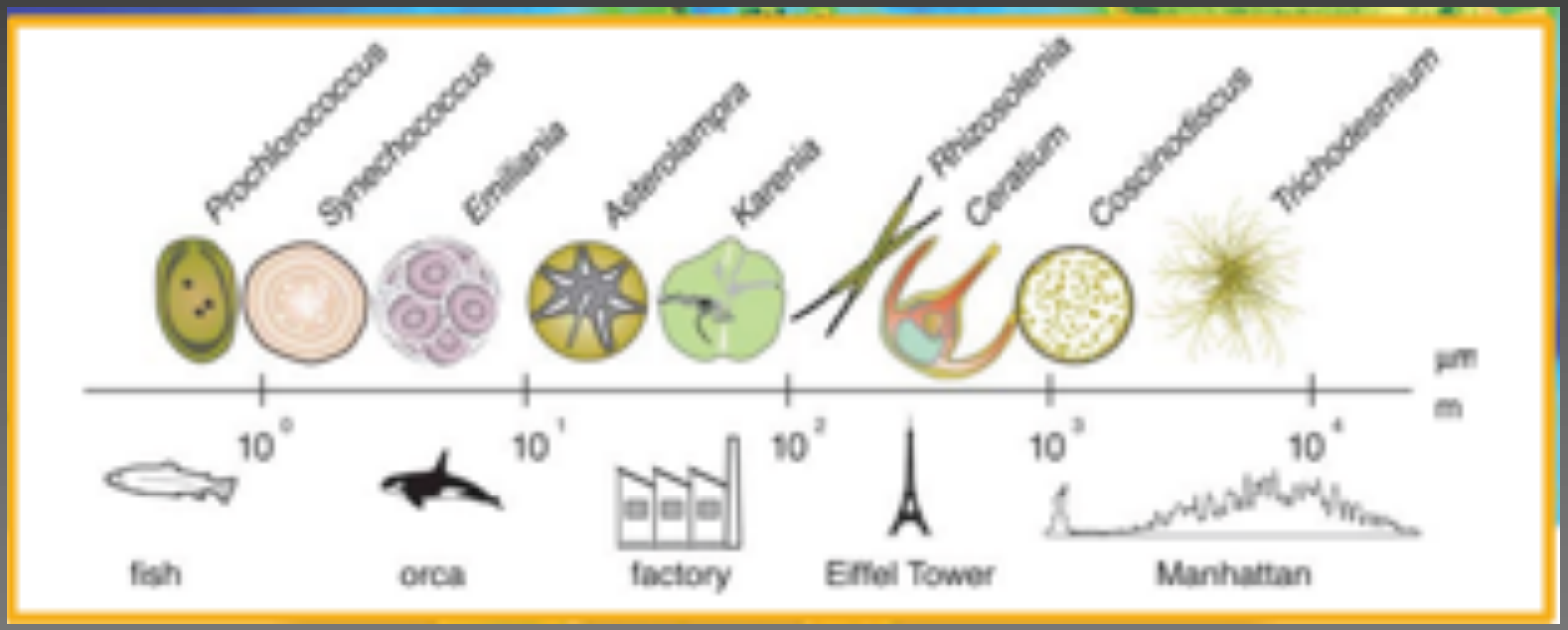
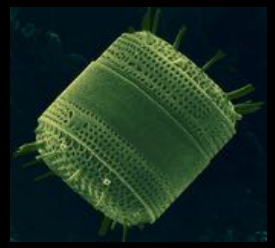
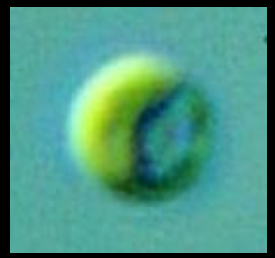


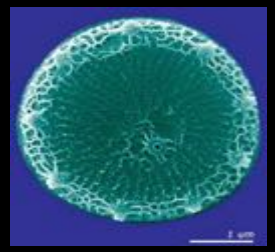
Image courtesy C-MORE

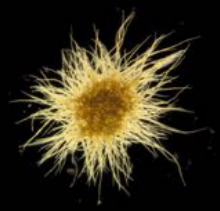
Marine phytoplankton are highly diverse

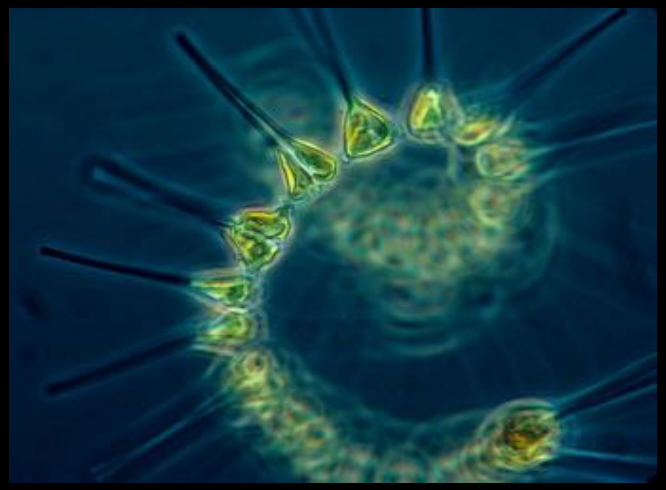


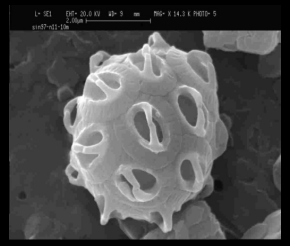


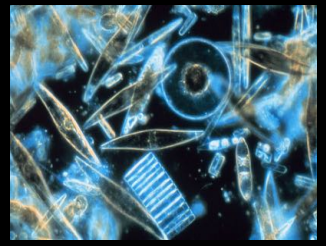


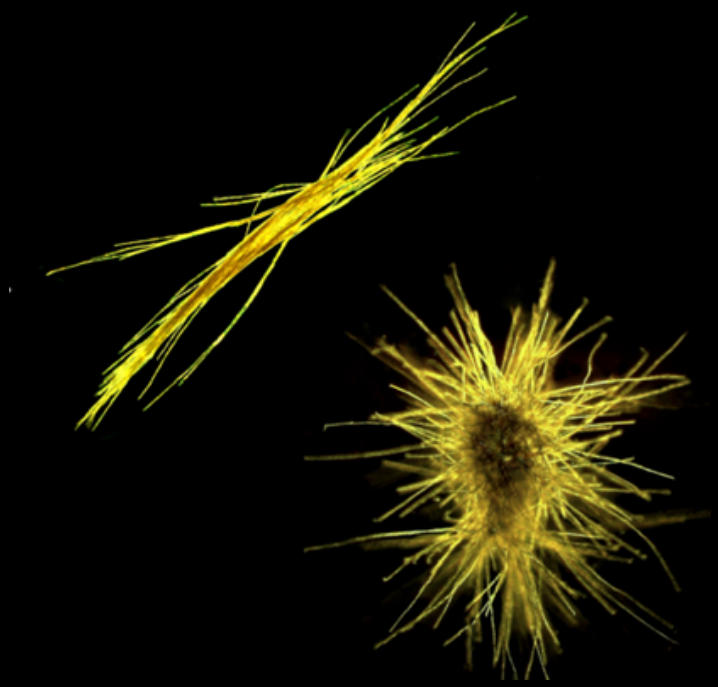










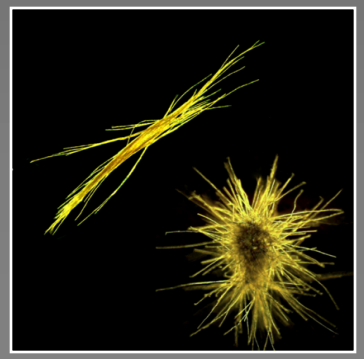


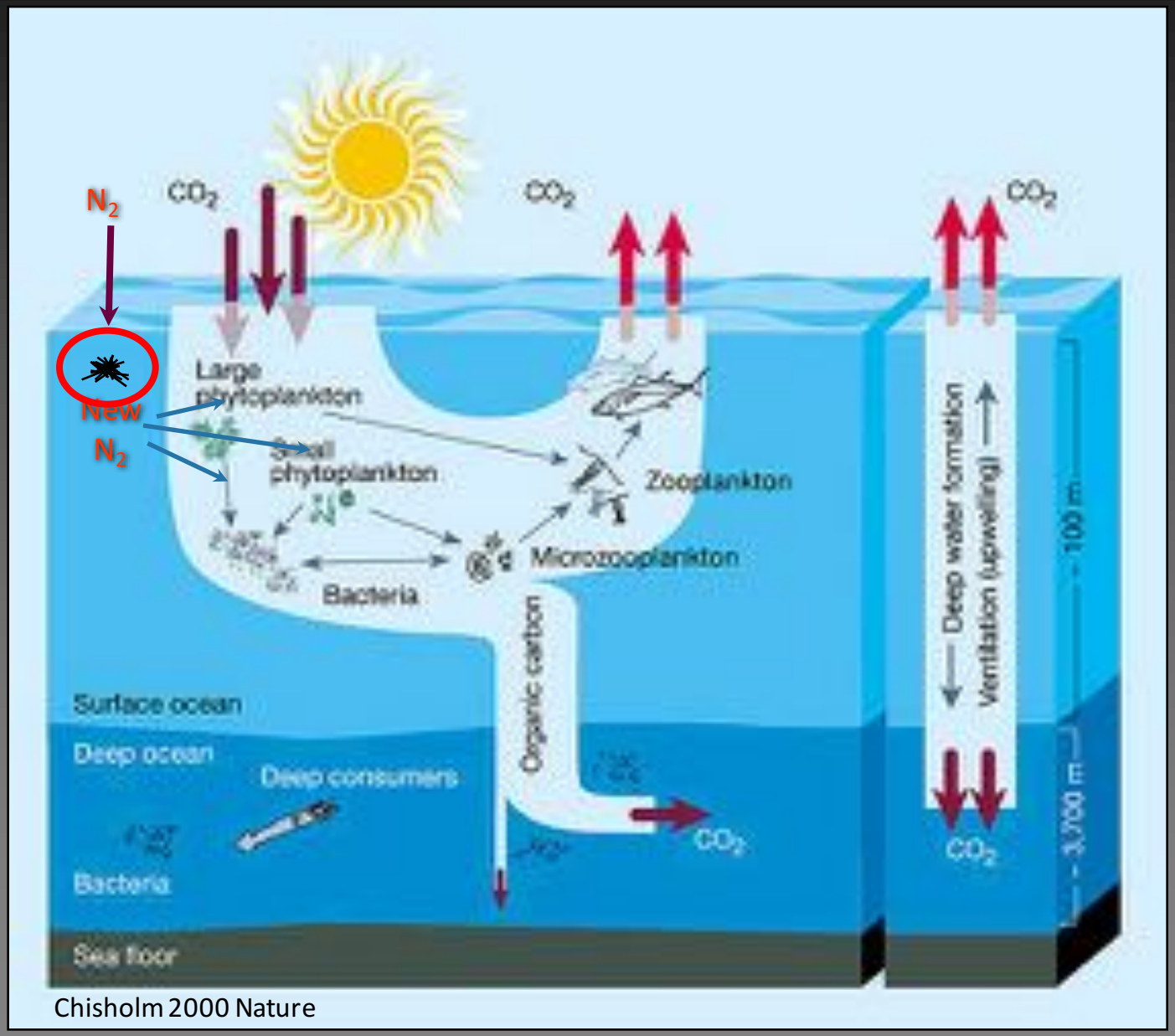
Phytoplankton underpin ocean ecosystem function

Haptophytes

Diatoms

N_2 Fixers

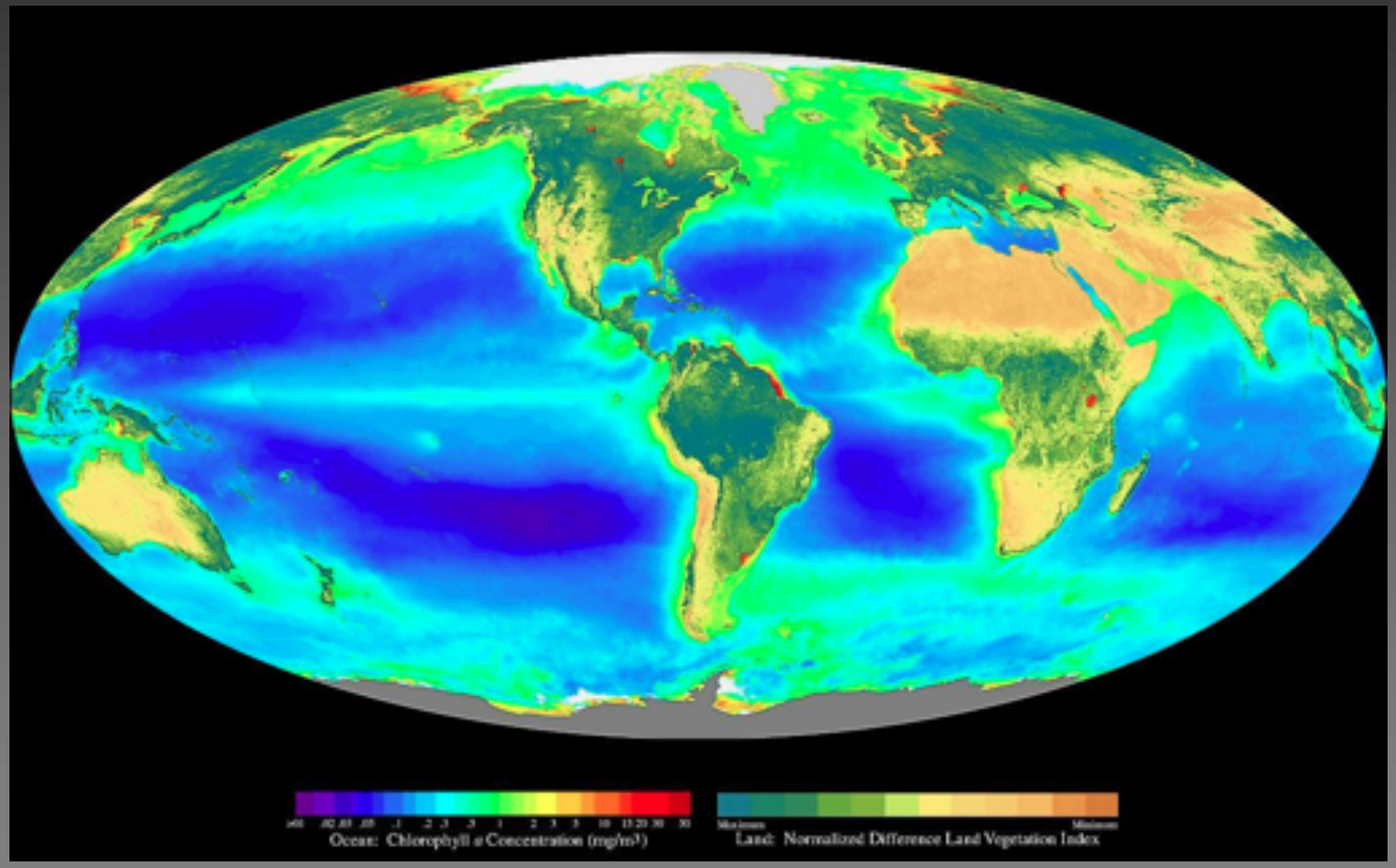




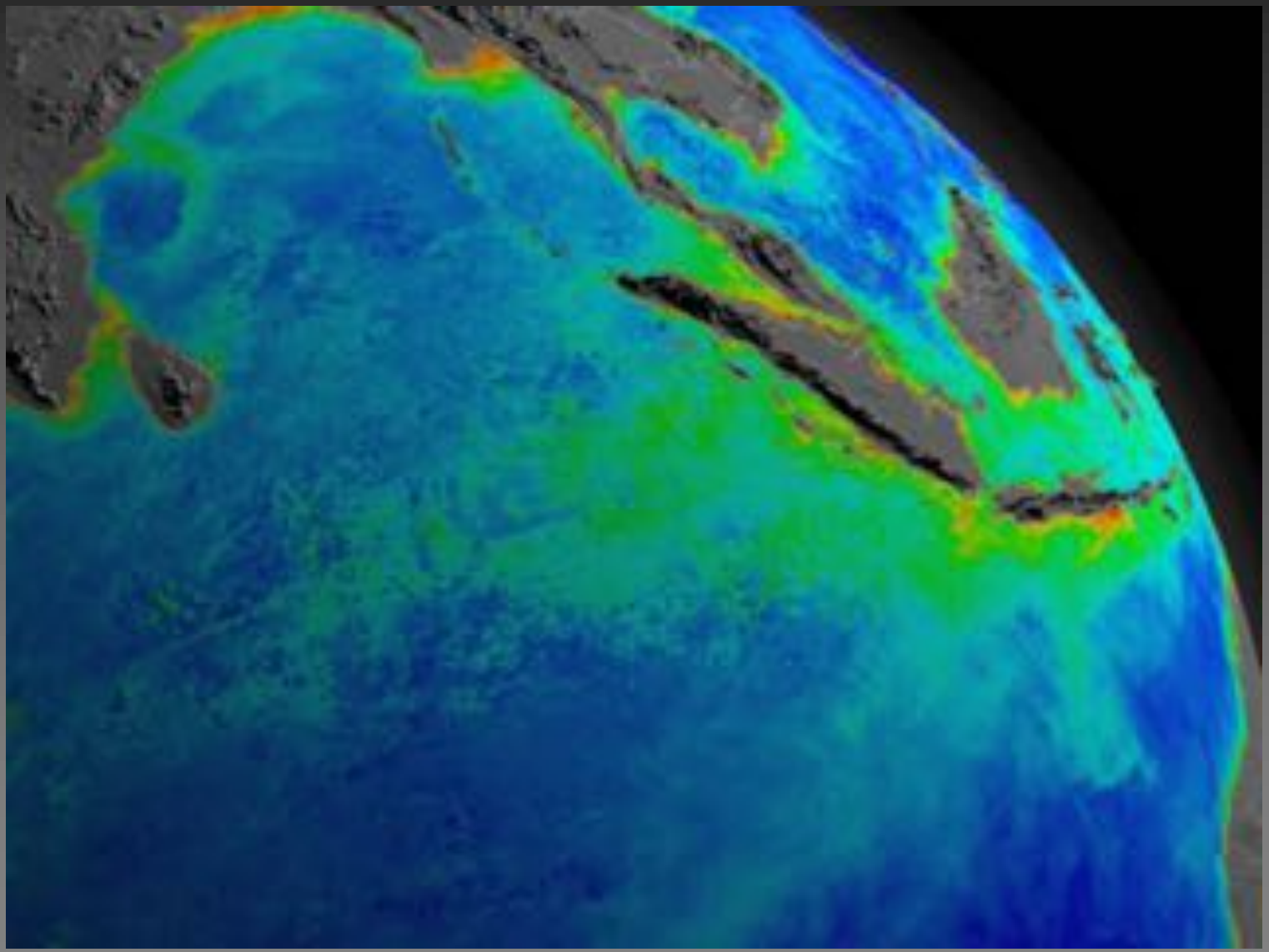
Chisholm 2000 Nature

Phytoplankton play a profound role in the earth system

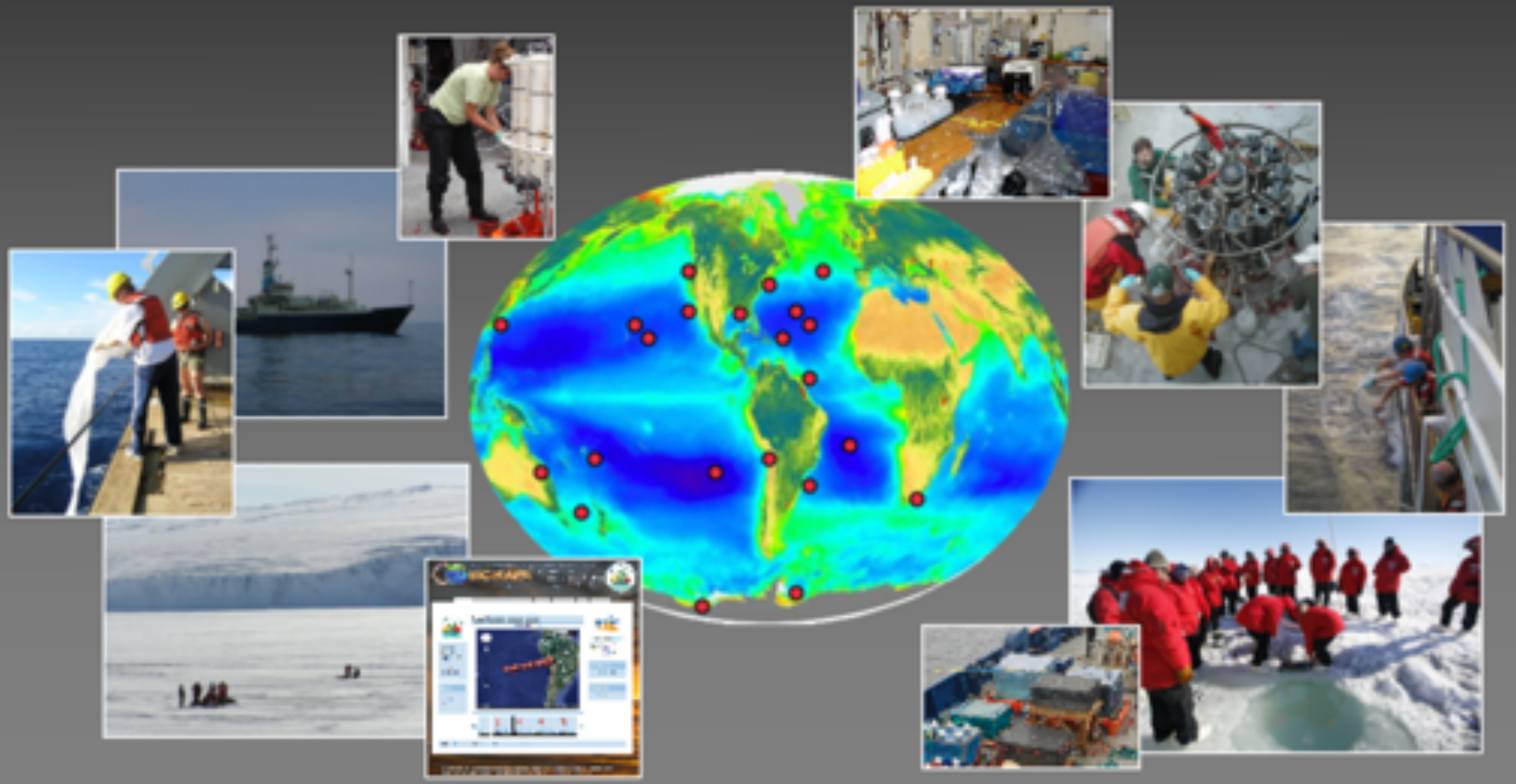
Half of global primary production



Seasonal chlorophyll distributions in the sea - highlights the global significance of phytoplankton



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

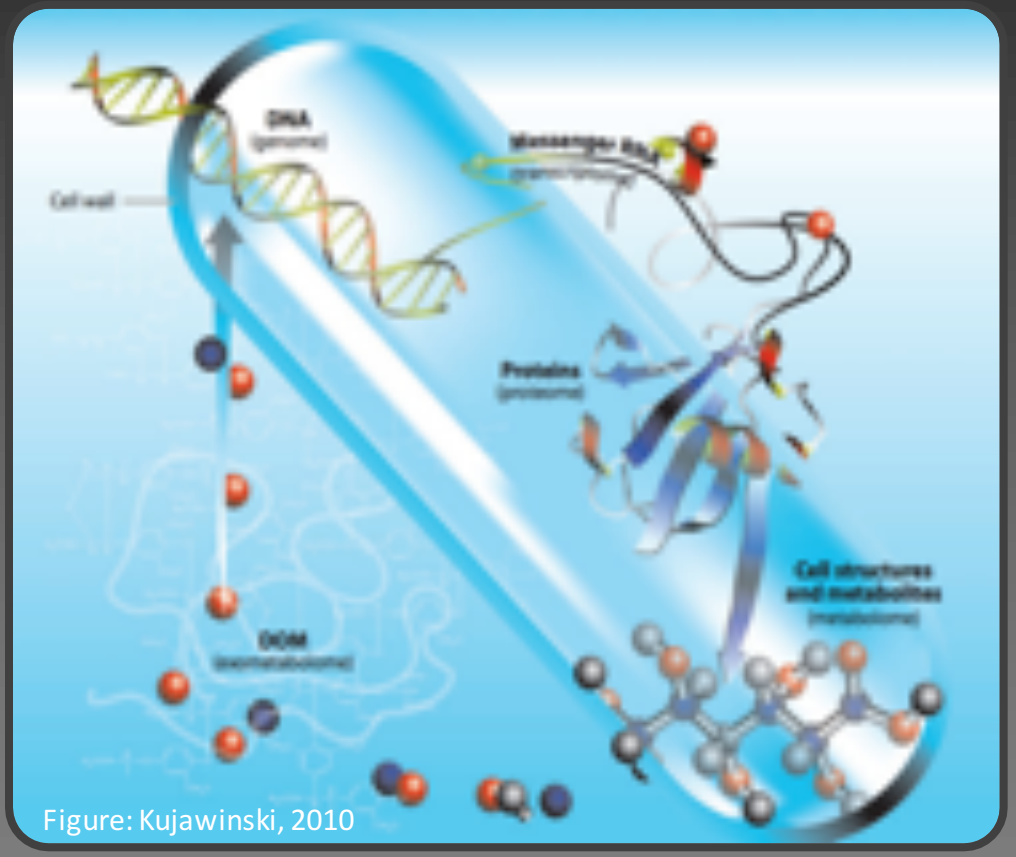


Figure: Kujawinski, 2010

Increasingly able to use 'omic and 'metaomic approaches!

Leveraging ‘omic data to study marine microbes

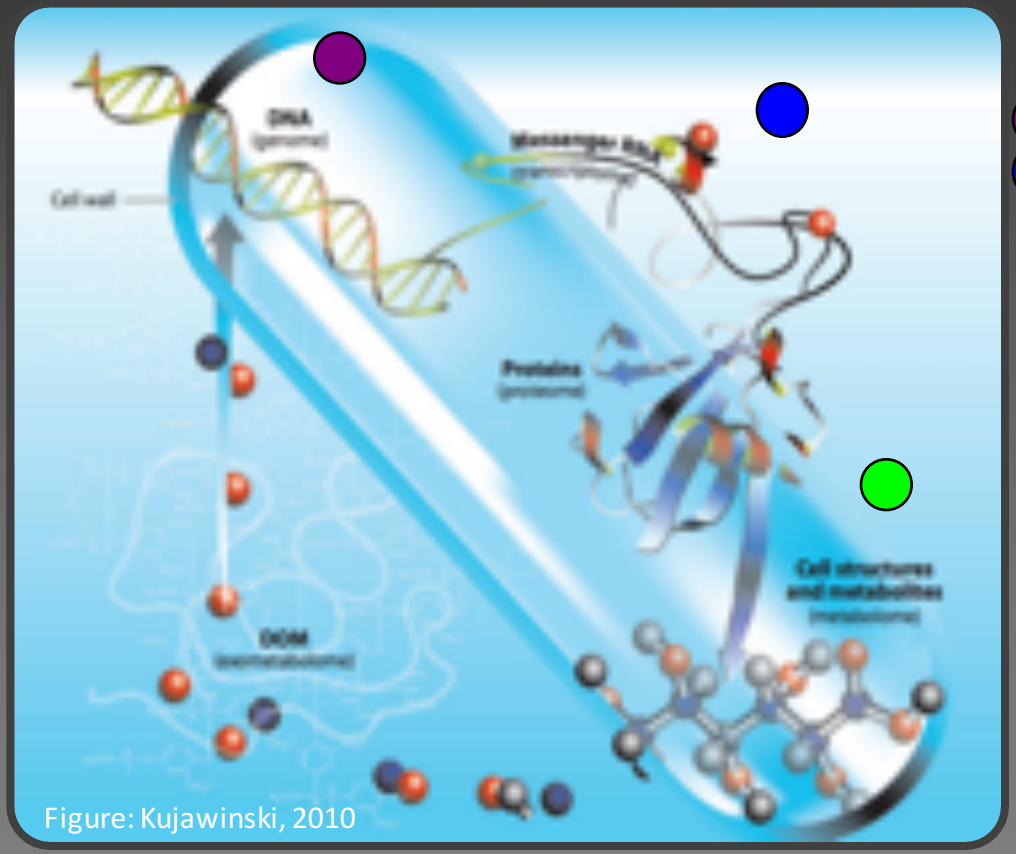


Figure: Kujawinski, 2010

Taxonomic Diversity: Who is there?

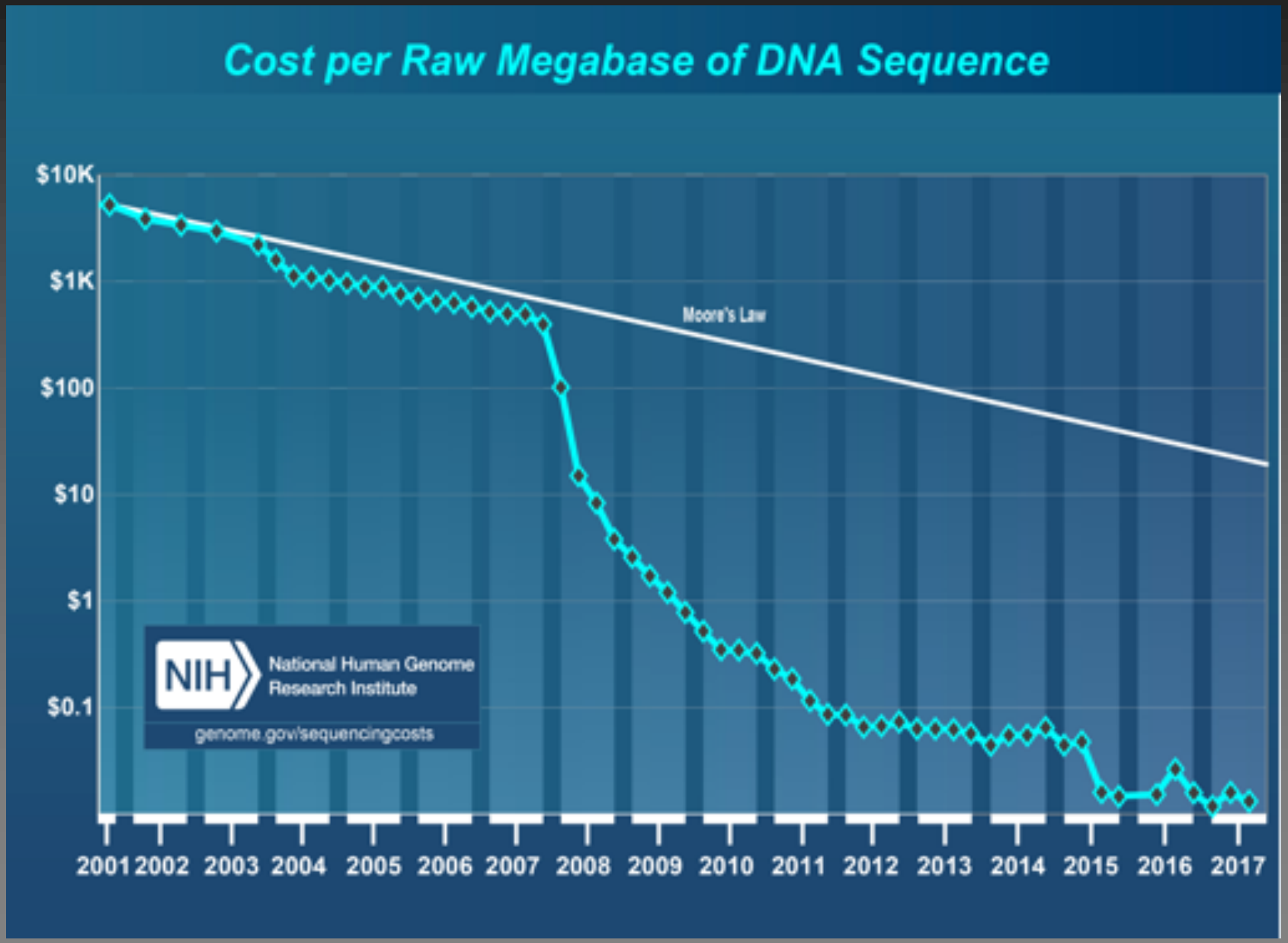
Metabolic capacity: What are the molecular underpinnings of resource metabolism?

Metabolic plasticity: How are those pathways regulated and expressed *in situ*?

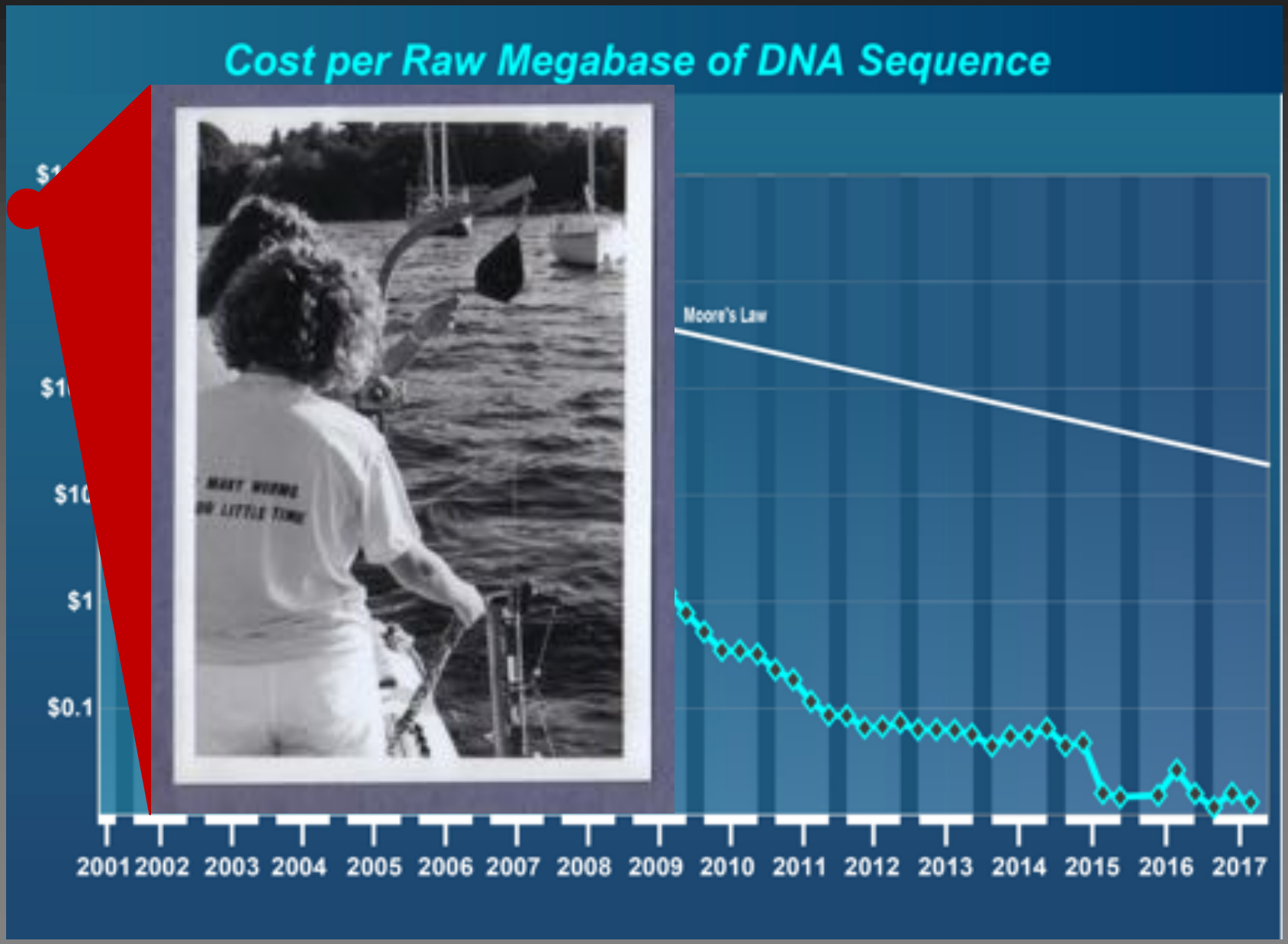
How did we get here?

- 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

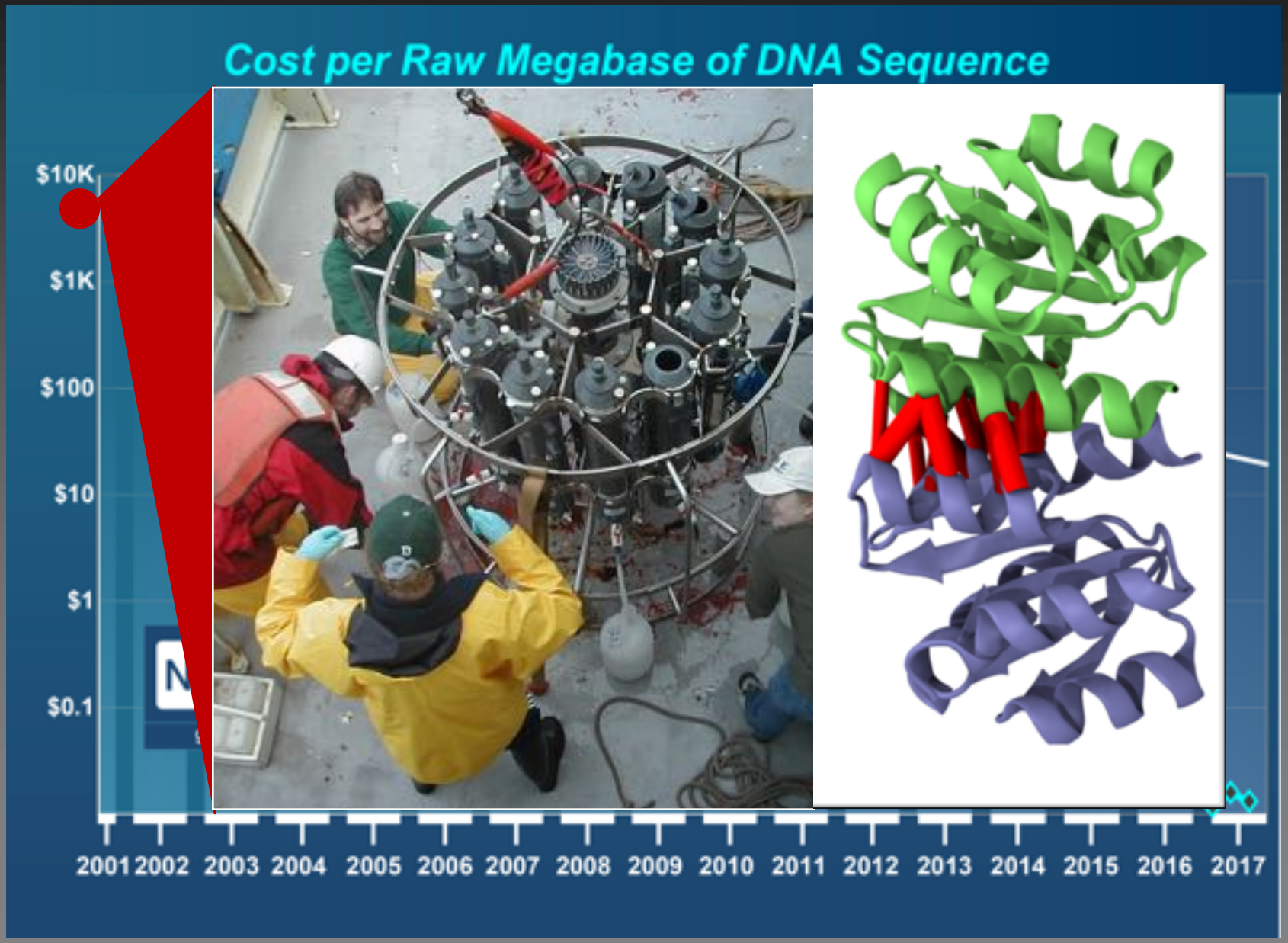
Sequencing advances opens new ’omic approaches



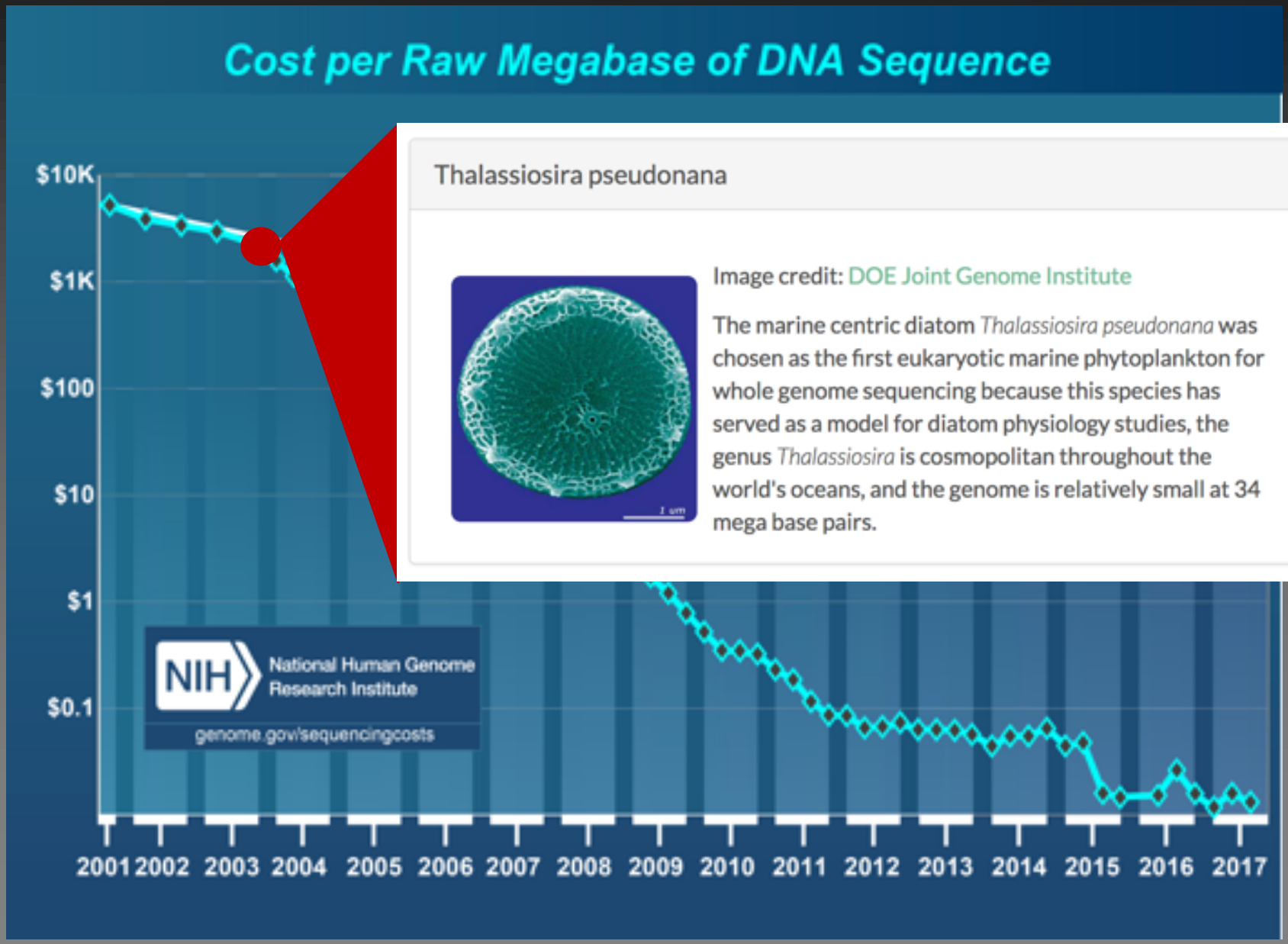
Sequencing advances opens new ’omic approaches



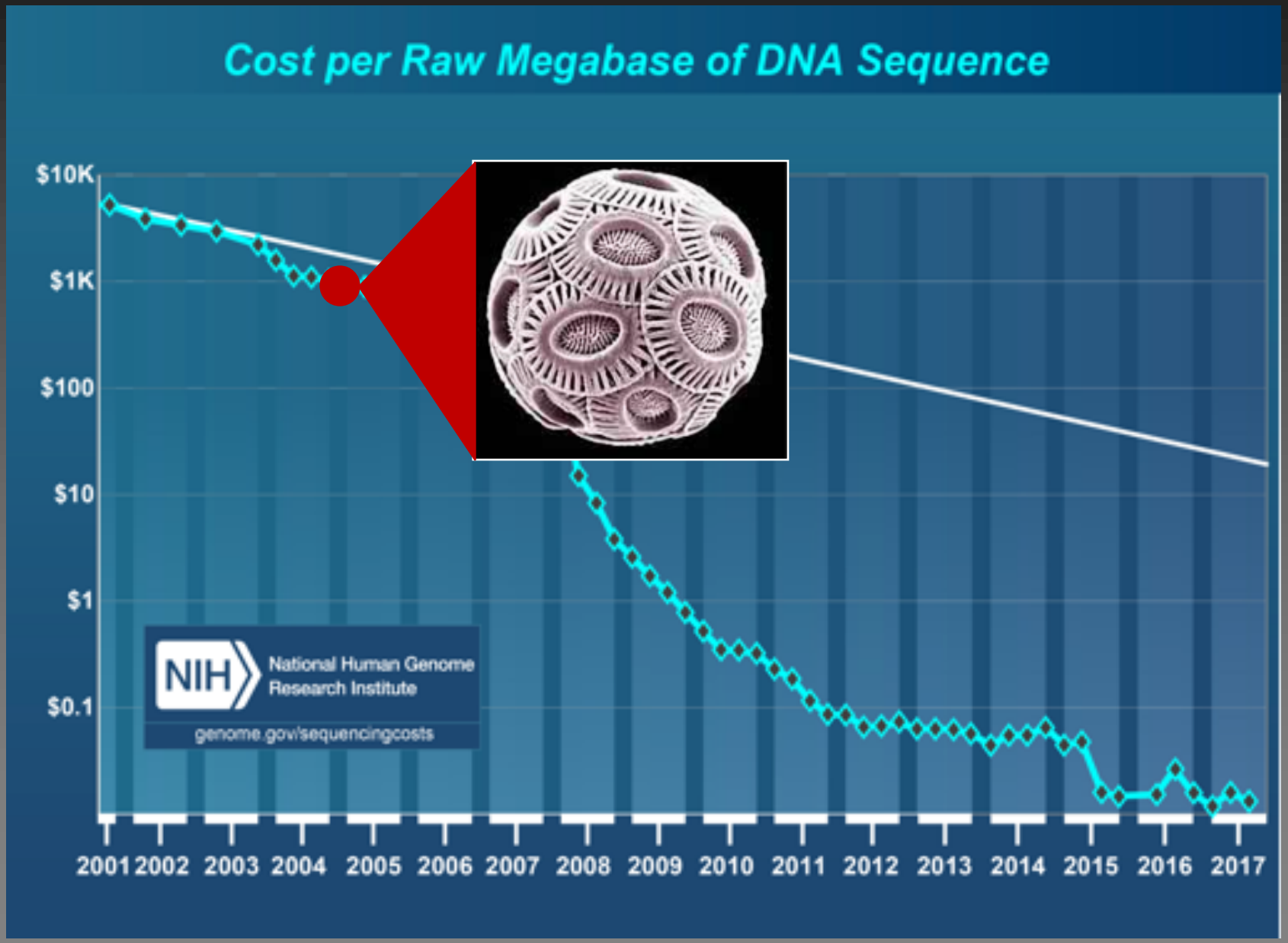
Sequencing advances opens new ’omic approaches



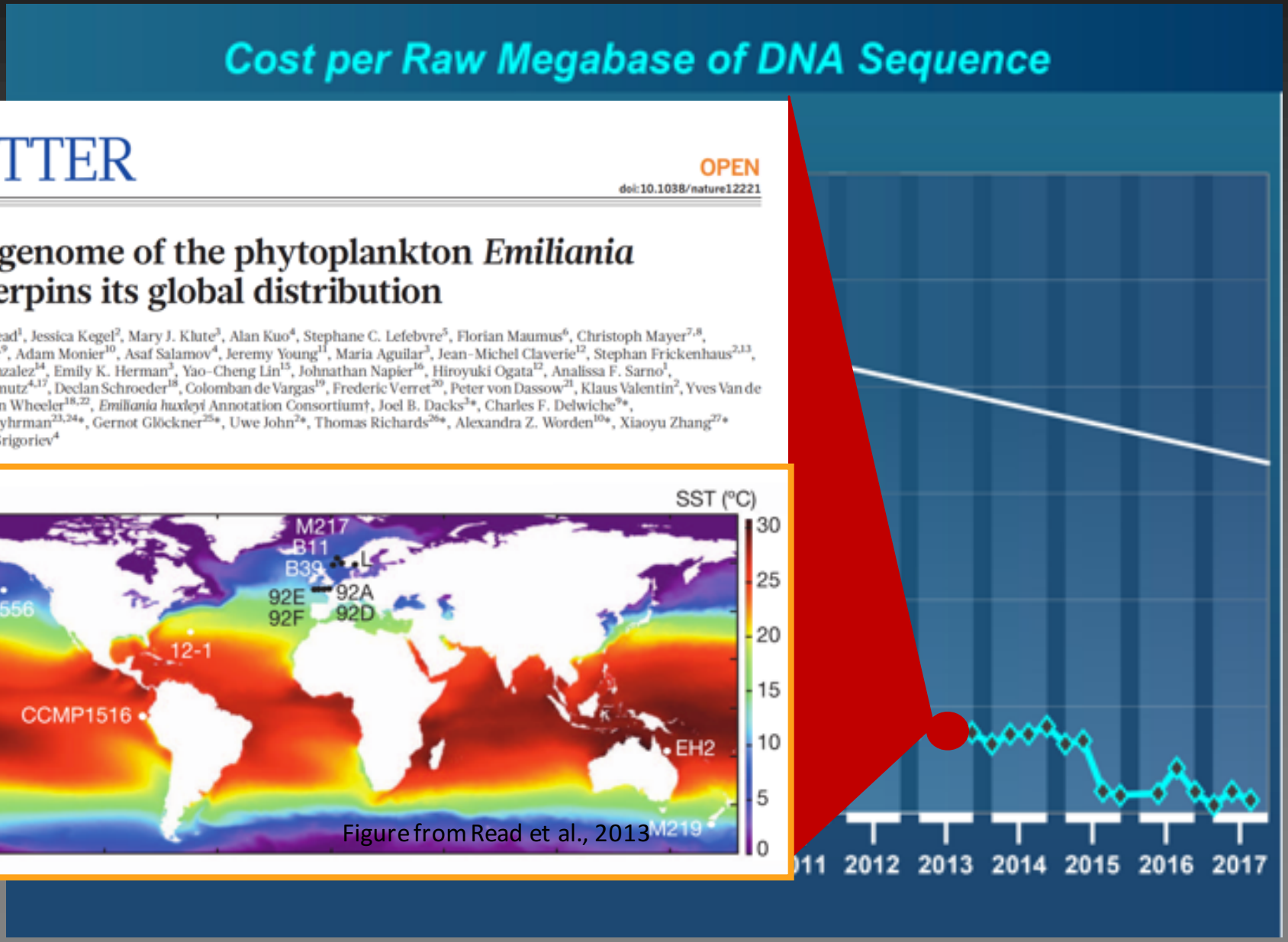
Sequencing advances opens new ’omic approaches



Sequencing advances opens new ’omic approaches



Sequencing advances opens new ’omic approaches



LETTER

OPEN

doi:10.1038/nature12221

Pan genome of the phytoplankton *Emiliania* underpins its global distribution

Betsy A. Read[1], Jessica Kegel[2], Mary J. Klute[3], Alan Kuo[4], Stephane C. Lefebvre[5], Florian Maumus[6], Christoph Mayer[7,8], John Miller[9], Adam Monier[10], Asaf Salamov[4], Jeremy Young[11], Maria Aguilar[3], Jean-Michel Claverie[12], Stephan Frickenhaus[2,13], Karina Gonzalez[14], Emily K. Herman[3], Yao-Cheng Lin[15], Johnathan Napier[16], Hiroyuki Ogata[12], Analissa F. Sarno[1], Jeremy Shmutz[4,17], Declan Schroeder[18], Colomban de Vargas[19], Frederic Verret[20], Peter von Dassow[21], Klaus Valentin[2], Yves Van de Peer[15], Glen Wheeler[18,22], *Emiliania huxleyi* Annotation Consortium†, Joel B. Dacks[3]*, Charles F. Delwiche[9]*, Sonya T. Dyhrman[23,24]*, Gernot Glöckner[25]*, Uwe John[2]*, Thomas Richards[26]*, Alexandra Z. Worden[10]*, Xiaoyu Zhang[27]* & Igor V. Grigoriev[4]

Figure from Read et al., 2013

Ongoing challenges

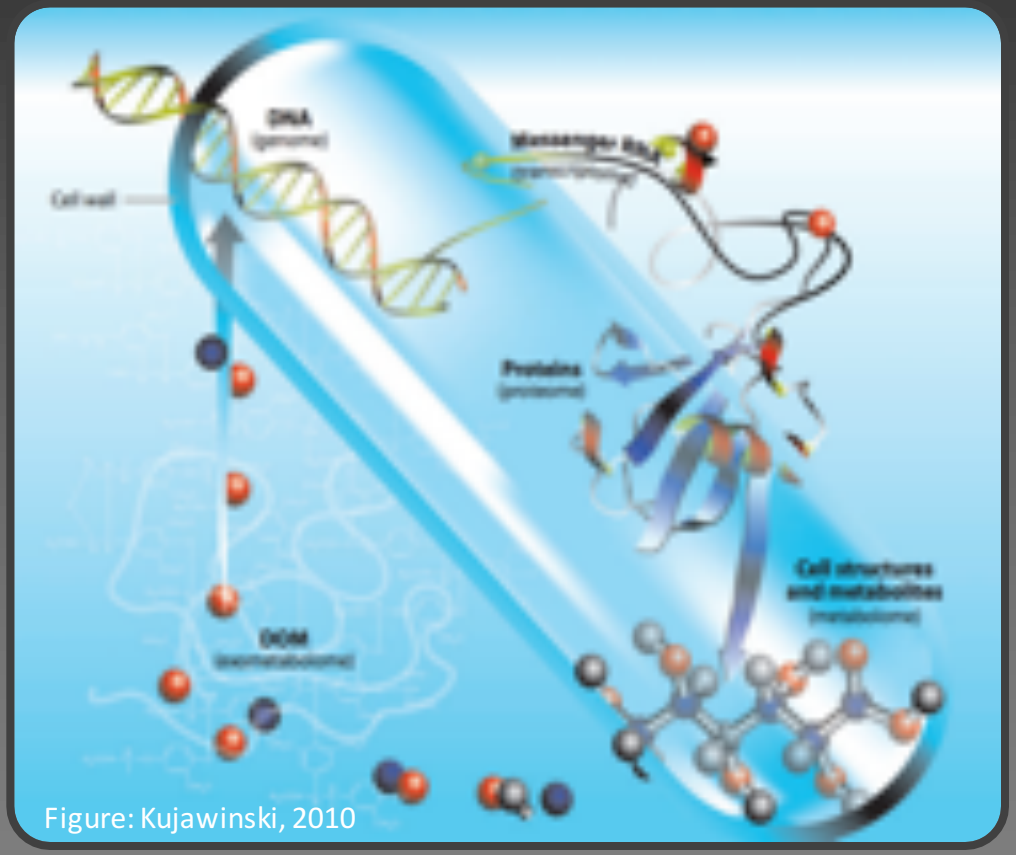


Figure: Kujawinski, 2010

We need to grow the inventory of species and genes

We have too few samples, and too few contiguous datasets

Processing loses ecological context

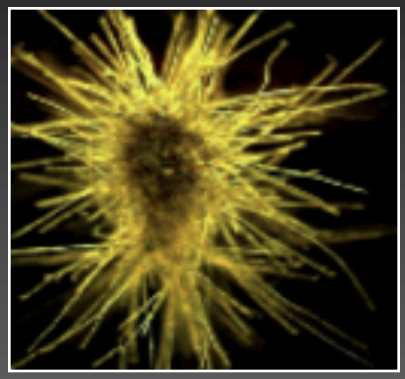
Lots of “dark matter”

Still too expensive to generate and store data

Vignettes

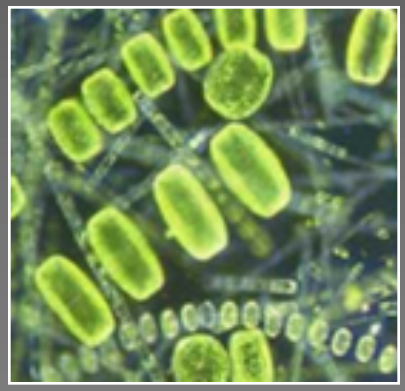
- From genome to biome: Tracking the metabolism and microbiome of a keystone N_2 fixer

Genome - enabled



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

Transcriptome - enabled

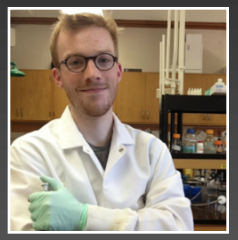


Thank you

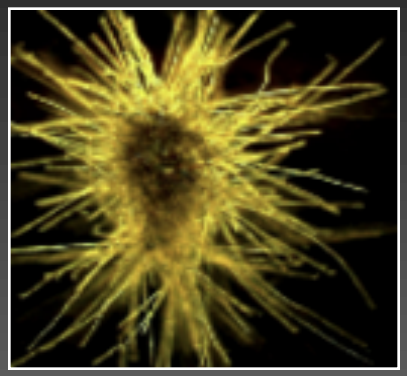
Gwenn Hennon

Mónica Rouco

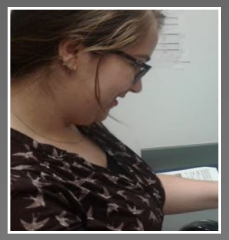
Sheean Haley



Kyle Frischkorn

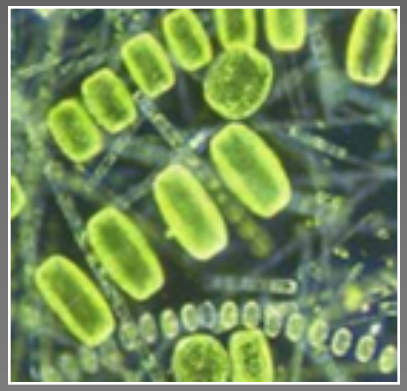


Matt Harke



Maria Hernandez

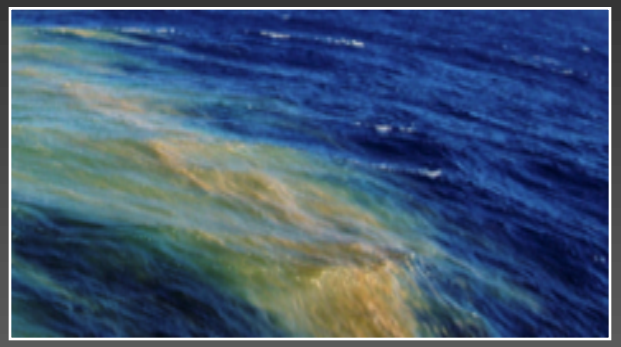
Harriet Alexander



Core questions

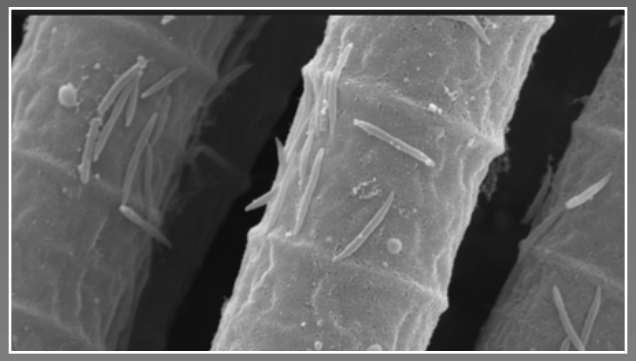
Metabolic traits and trade-offs

- 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, holobiont physiology and controls on N_2 fixation



Nitrogen-fixing marine cyanobacteria

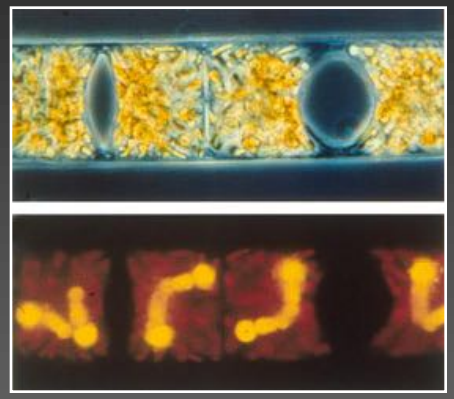
- Symbionts
  - UNCYN-A
  - *Richelia*
- Free-living
  - *Crococosphaera*
  - *Trichodesmium*

-Trichodesmium contortum
-Trichodesmium erythraeum

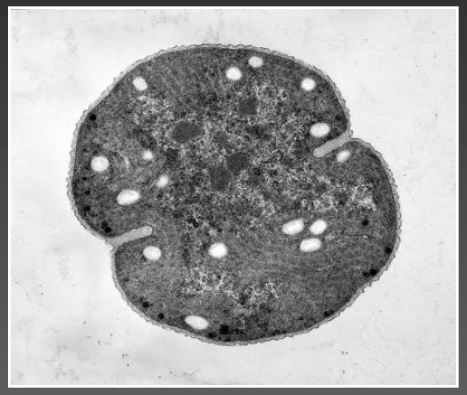
-Trichodesmium tenue
-Trichodesmium thiebautii

-Trichodesmium spiralis
-Trichodesmium hildebrandtii

Richelia



Crocosphaera

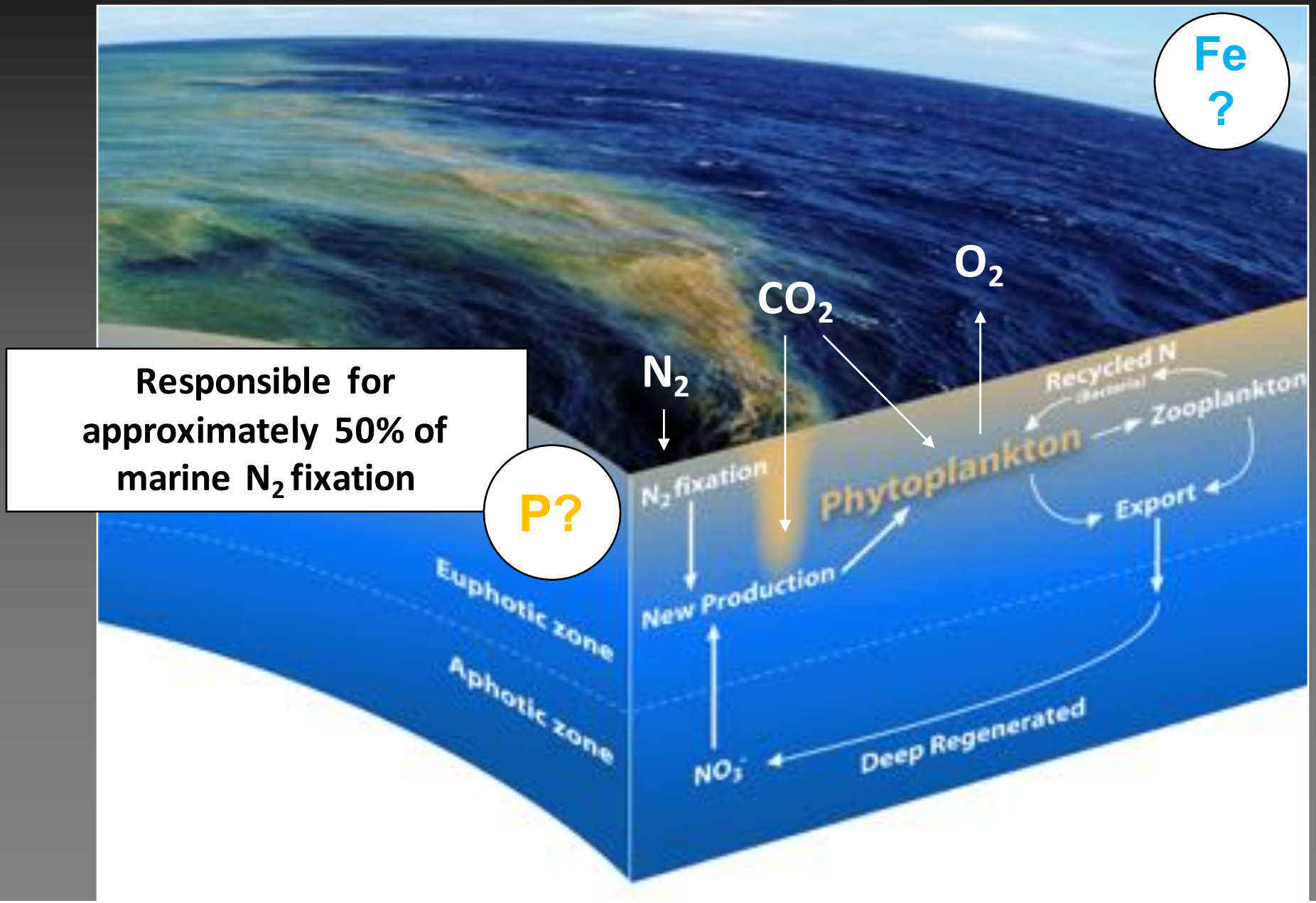


Trichodesmium

Trichodesmium: critical to ecosystem function

Photo: Chris Wade
Tricho. micrograph: WHOI

Trichodesmium: critical to ecosystem function



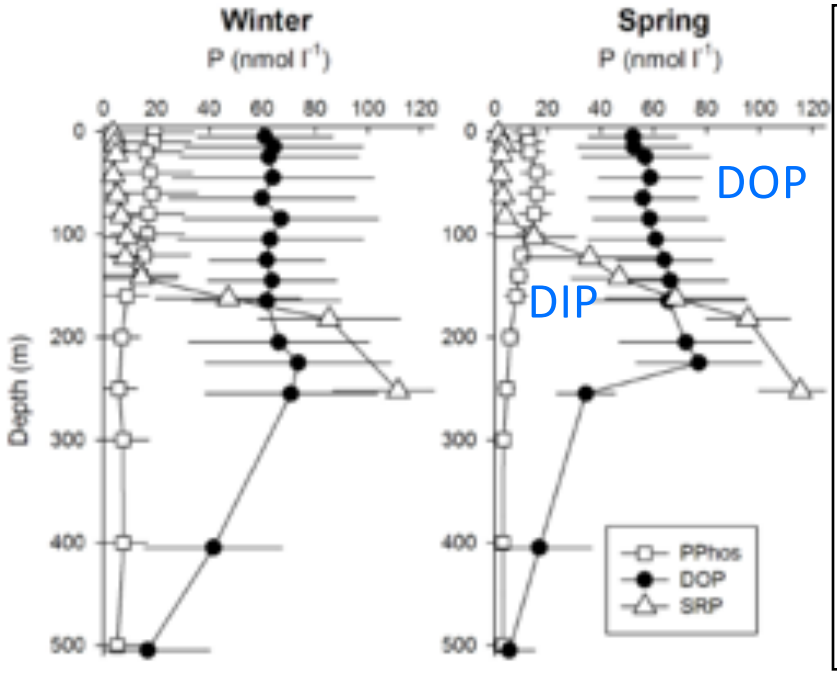
Responsible for approximately 50% of marine N_2 fixation

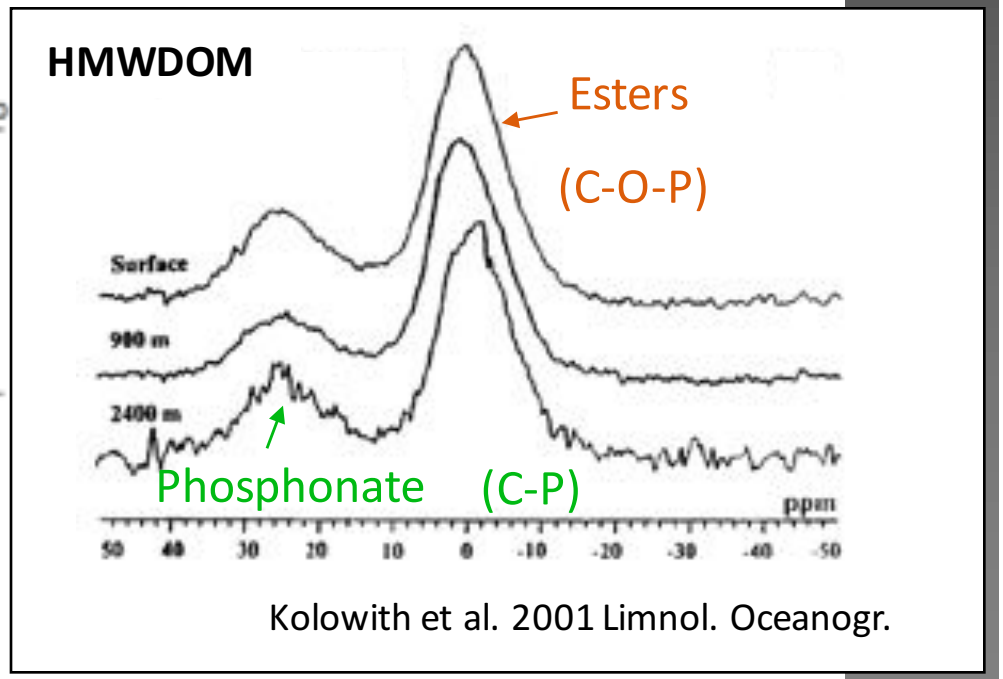
Phosphorus pools in the open ocean

Particulate phosphorus

PPhos

DIP Dissolved inorganic phosphorus ⟷ Dissolved organic phosphorus **DOP**

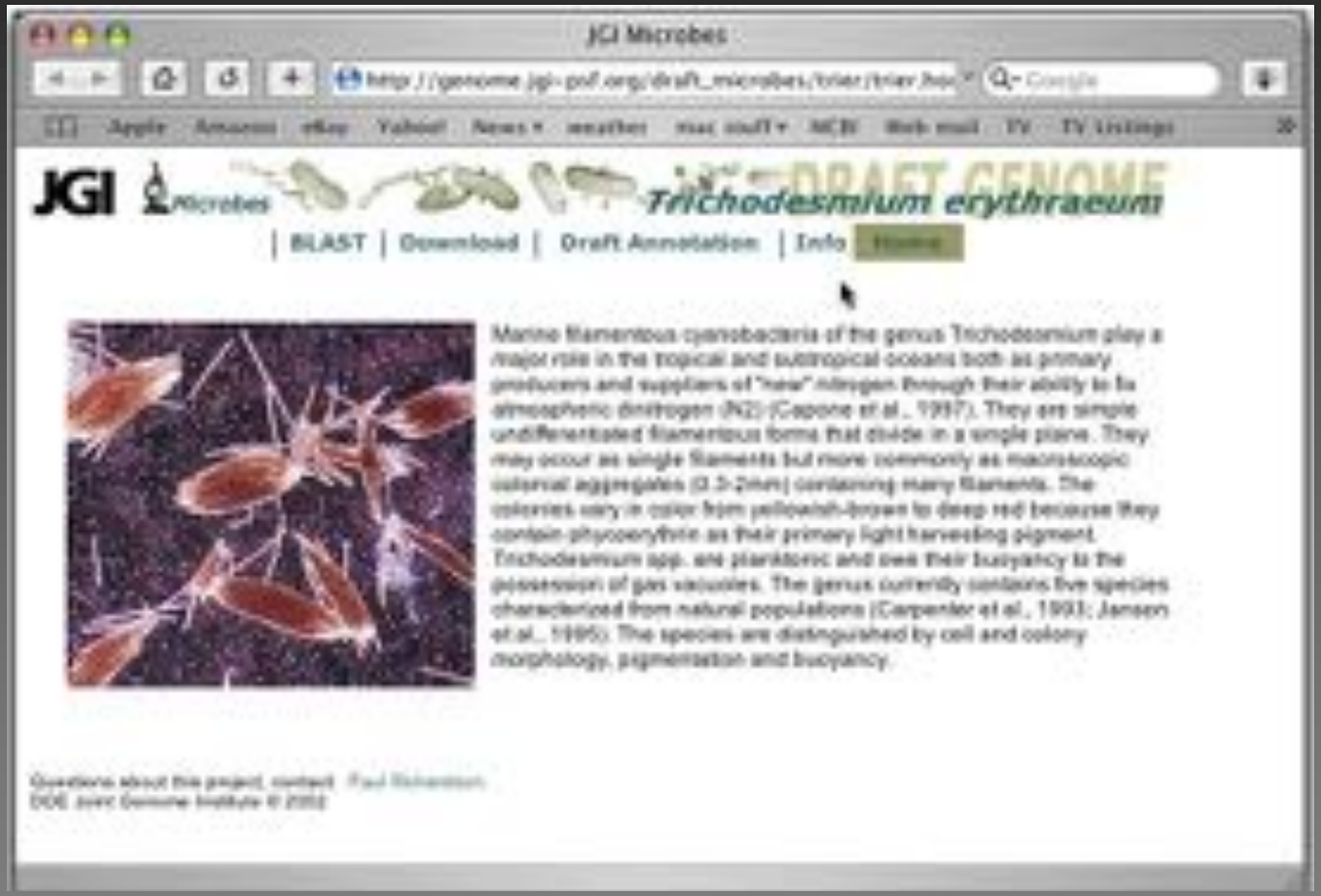




Kolowith et al. 2001 Limnol. Oceanogr.

Lomas et al. (2010) *Biogeosciences*

Trichodesmium erythraeum IMS101 genome page



www.jgi.doe.gov

Phosphorus metabolic traits and trade-offs

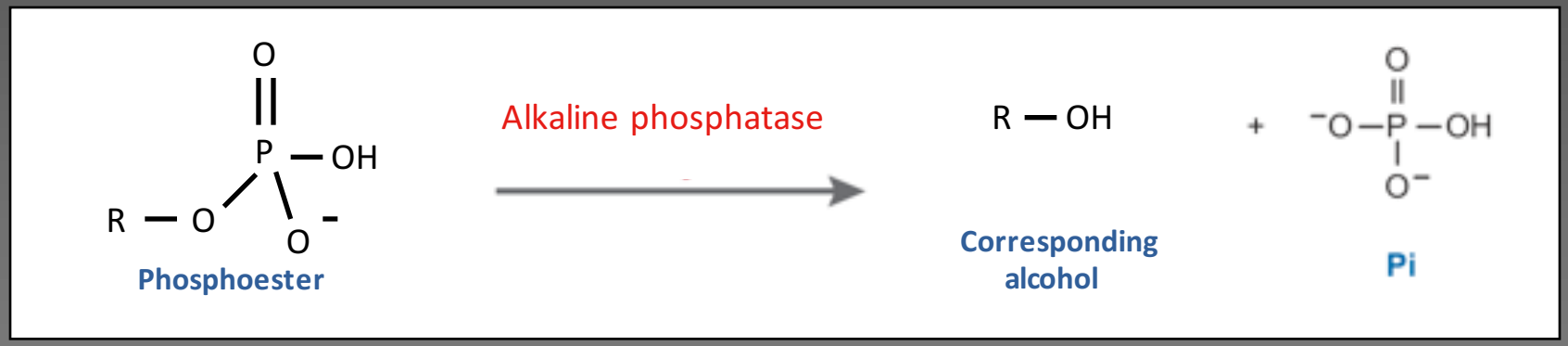
- Phosphonate
  - C-P Lyase (Fe co-factor)

C-P

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

COP

COP



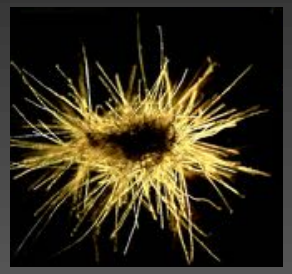
- Phosphite
  - *ptxD* gene cluster

PO_3

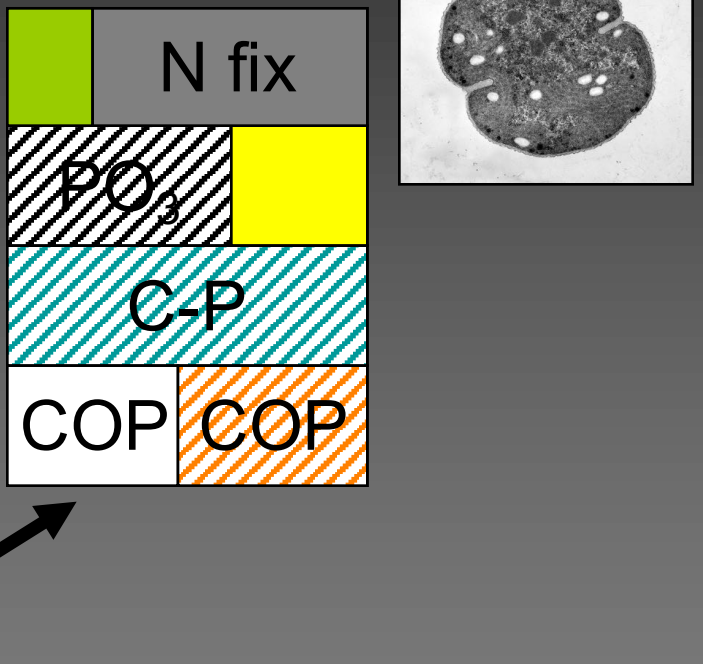
Dyhrman et al. 2006 *Nature*, Dyhrman and Haley 2010 *AEM*, Orchard et al. 2010 *L&O*, Van Mooy et al. 2015 *Science*

Comparative genomics: phosphorus traits and trade offs

Trichodesmium



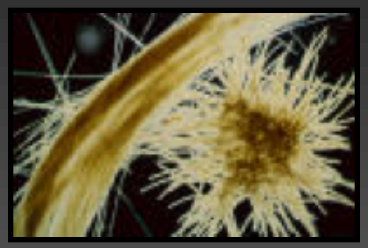
Crococosphaera



Other N_2 fixing cyanobacteria genomes do not encode the same pathways for phosphorus metabolism - less available substrates, but less metal requirement

Dyhrman et al. 2006 *Nature*, Dyhrman and Haley 2010 *AEM*, Orchard et al. 2010 *L&O*, Van Mooy et al. 2015 *Science*

Tracking genomic potential with expression studies



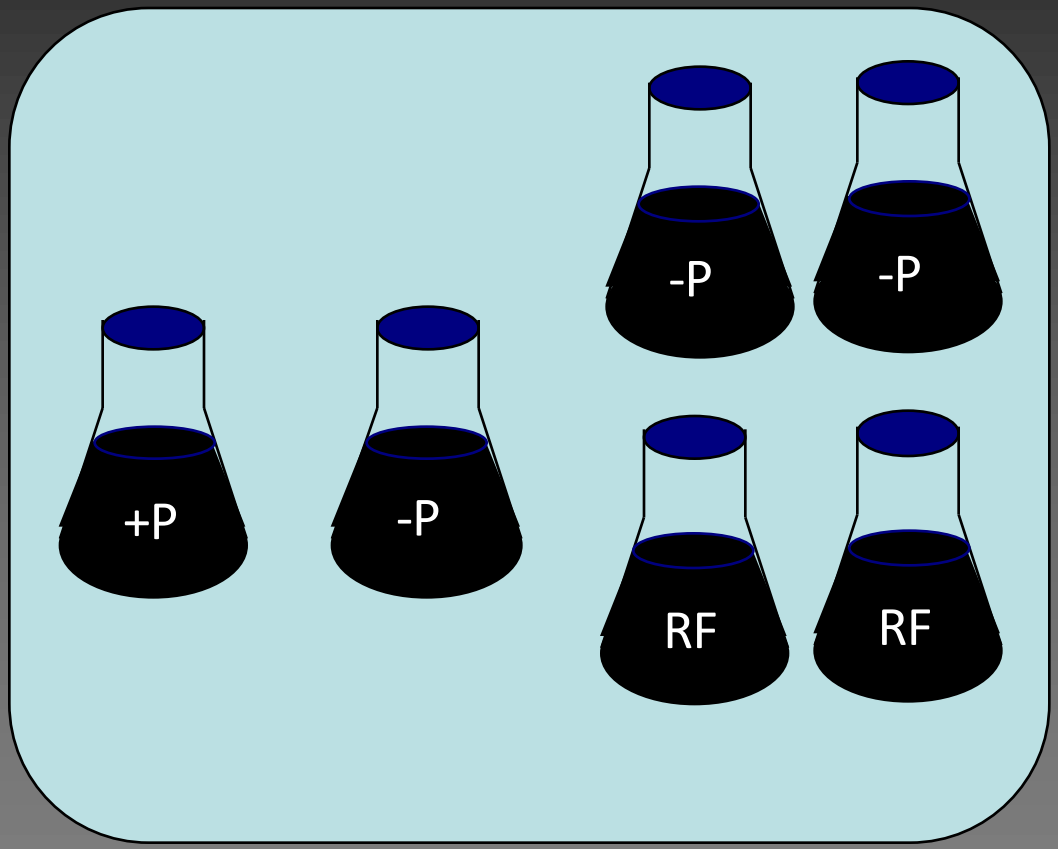
Culture cells

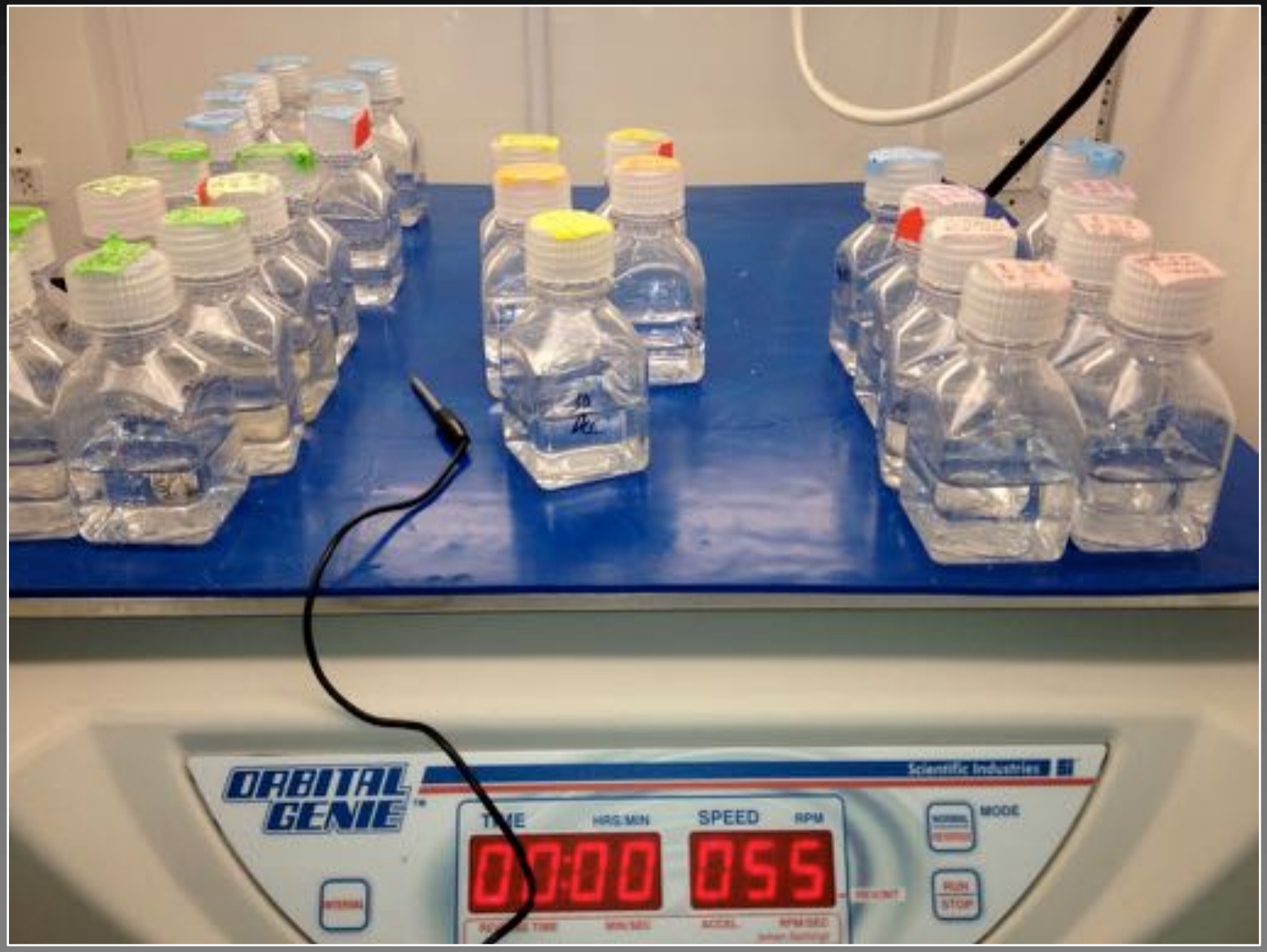
↓

Harvest and preserve samples

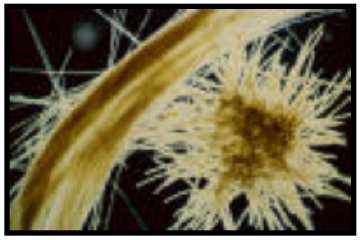
↓

Transcriptome
Proteome




ORBITAL GENIE
Scientific Industries
TIME
HRS:MIN
SPEED
RPM
MODE
RUN
STOP

Tatranscriptome analysis pipeline



Collect samples

Remove rRNA

Submit samples to sequencing center

Sequence using RNAseq

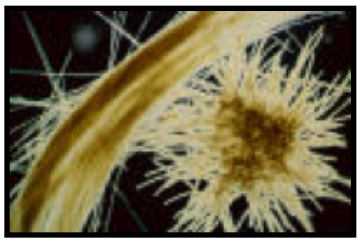
Target 30 million 100 bp paired-end reads

Stringent mapping to IMS101 genome

Removed reads with ambiguous mapping

Differential expression evaluated with EdgeR

Proteome analysis pipeline



Collect samples

Extract protein
Generate peptide

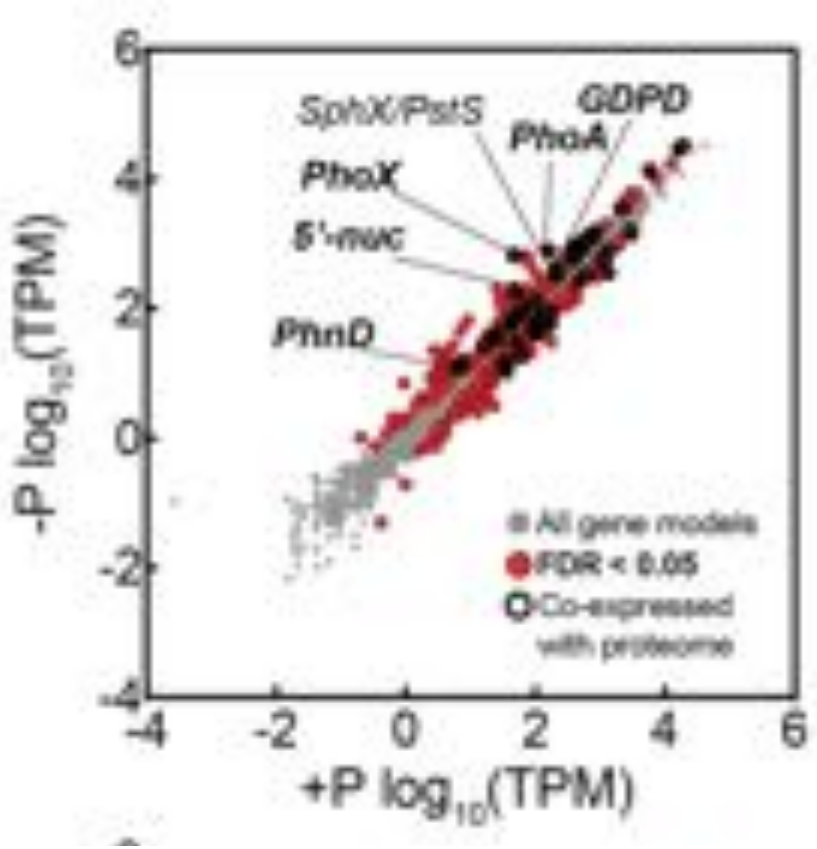
LC-MS/MS

ID MS against gene models

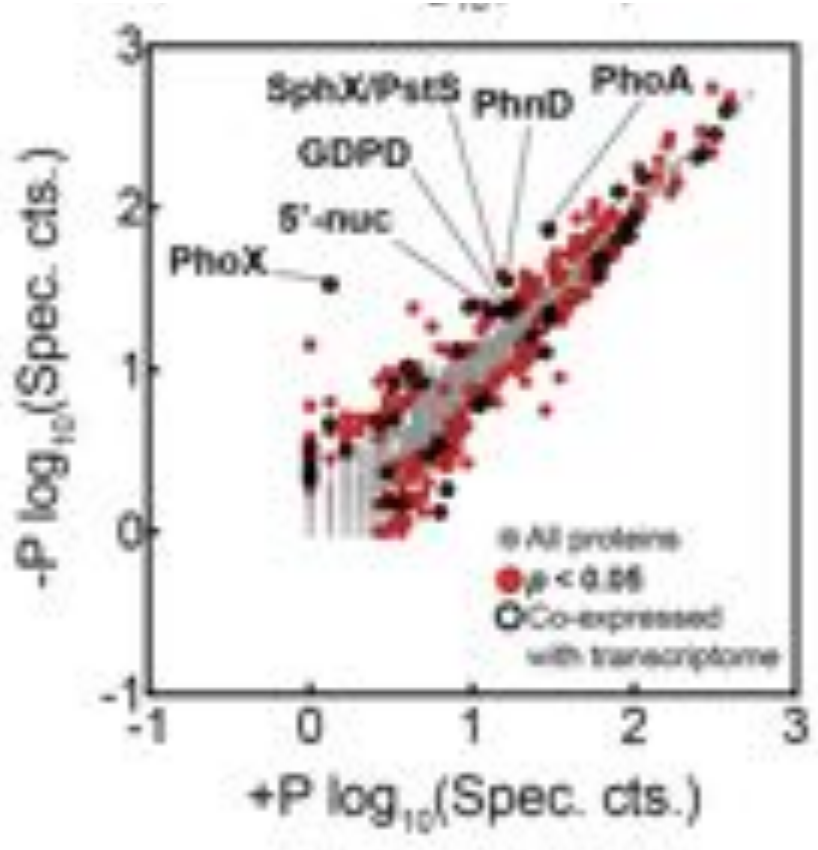
Relative differences of spectral counts with Scaffold

How does *Trichdoesmium* respond to low phosphorus?

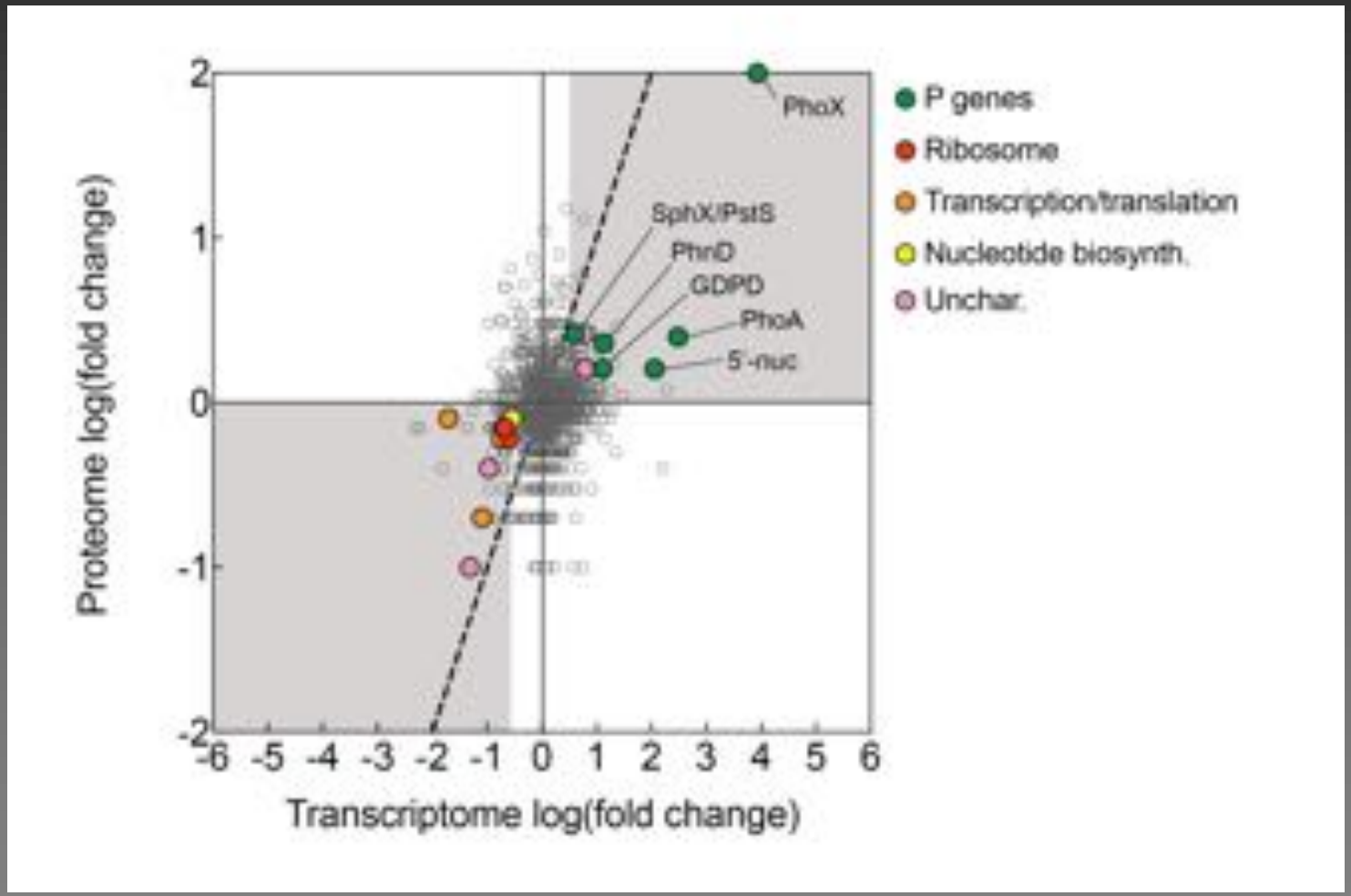
Transcriptome



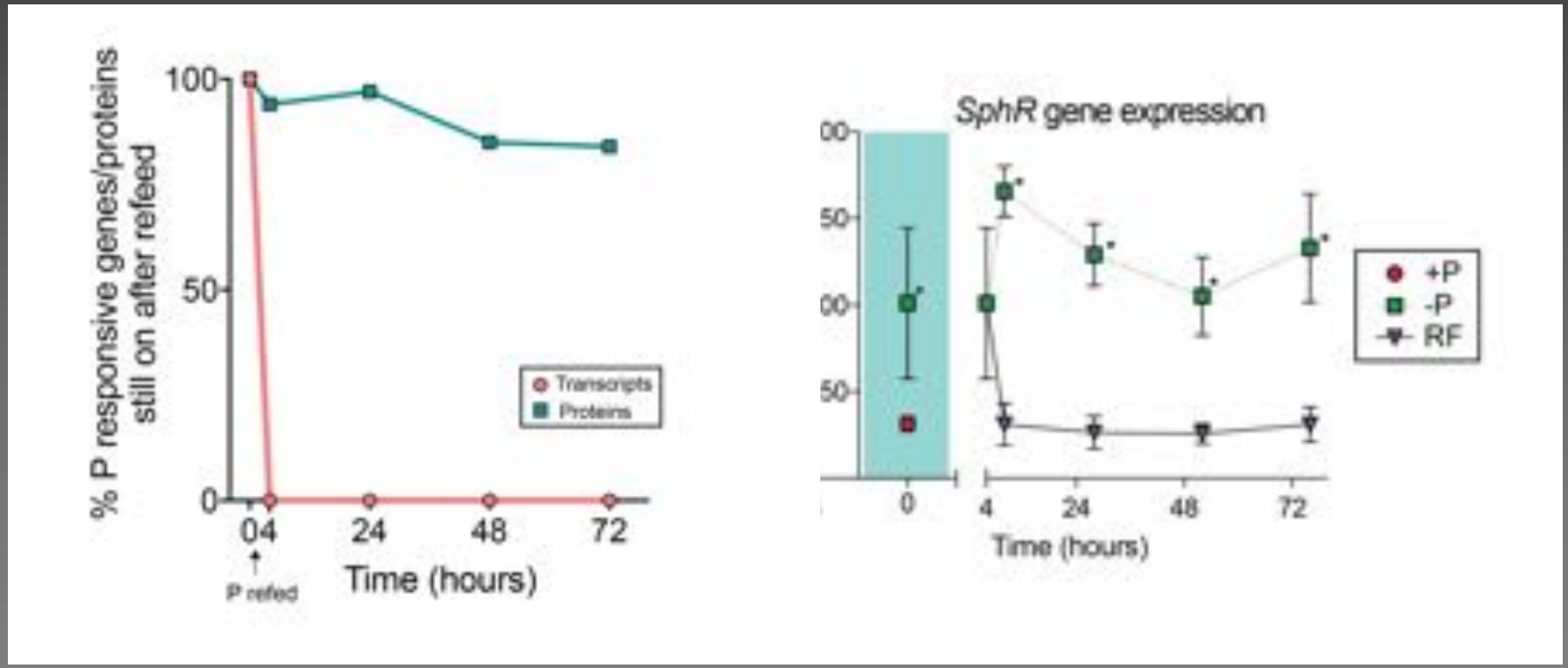
Proteome



Transcriptome - proteome coordination

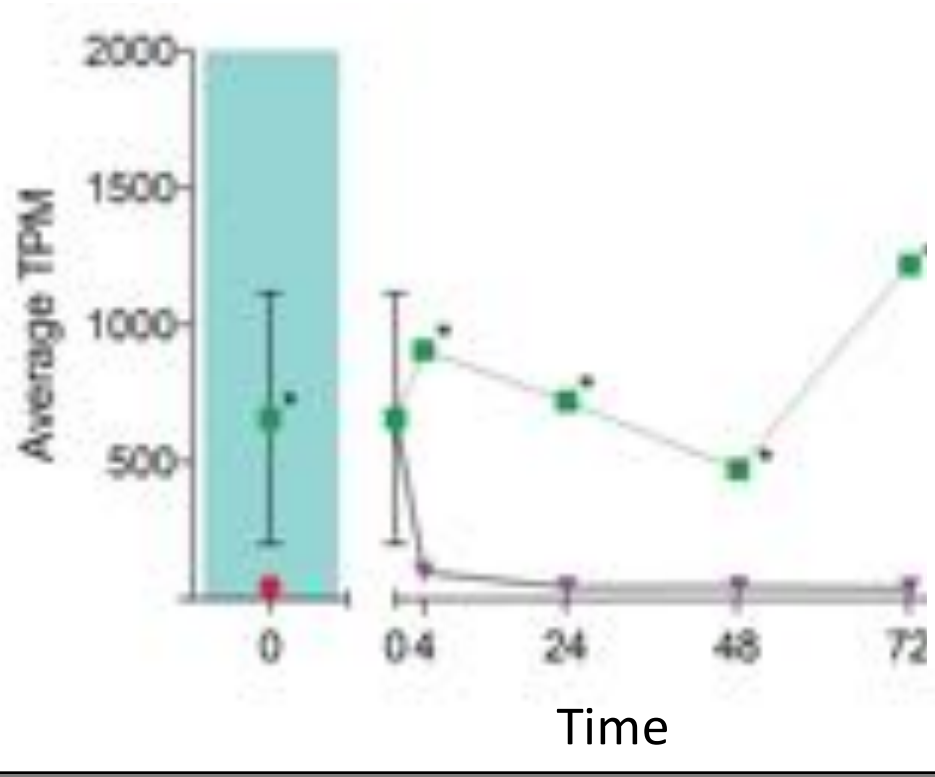


Transcripts turnover more quickly than proteins

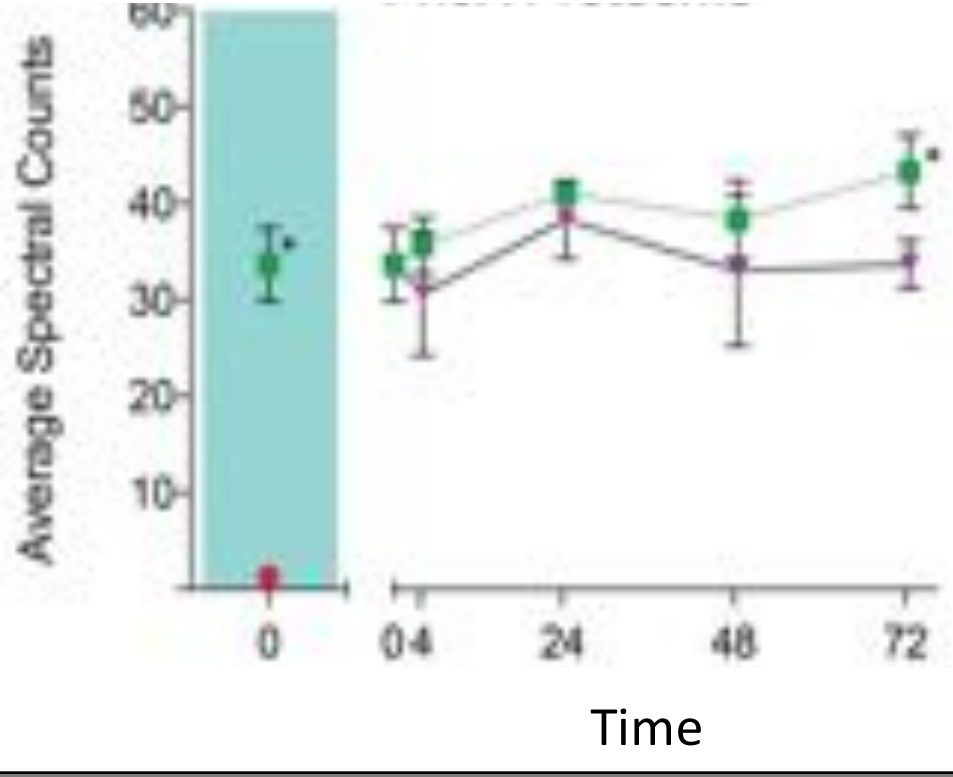


How does *Trichdoesmium* respond to low phosphorus?

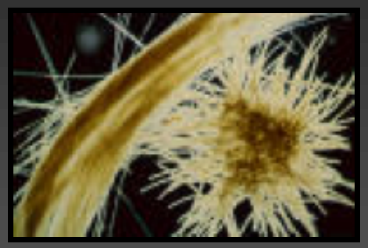
***phoX* transcripts**



PhoX proteins



Tracking genomic potential with expression studies



Culture cells

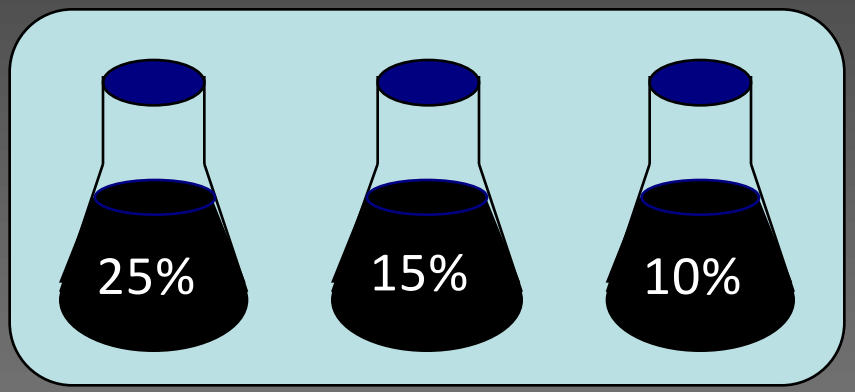
↓

Harvest and preserve samples

↓

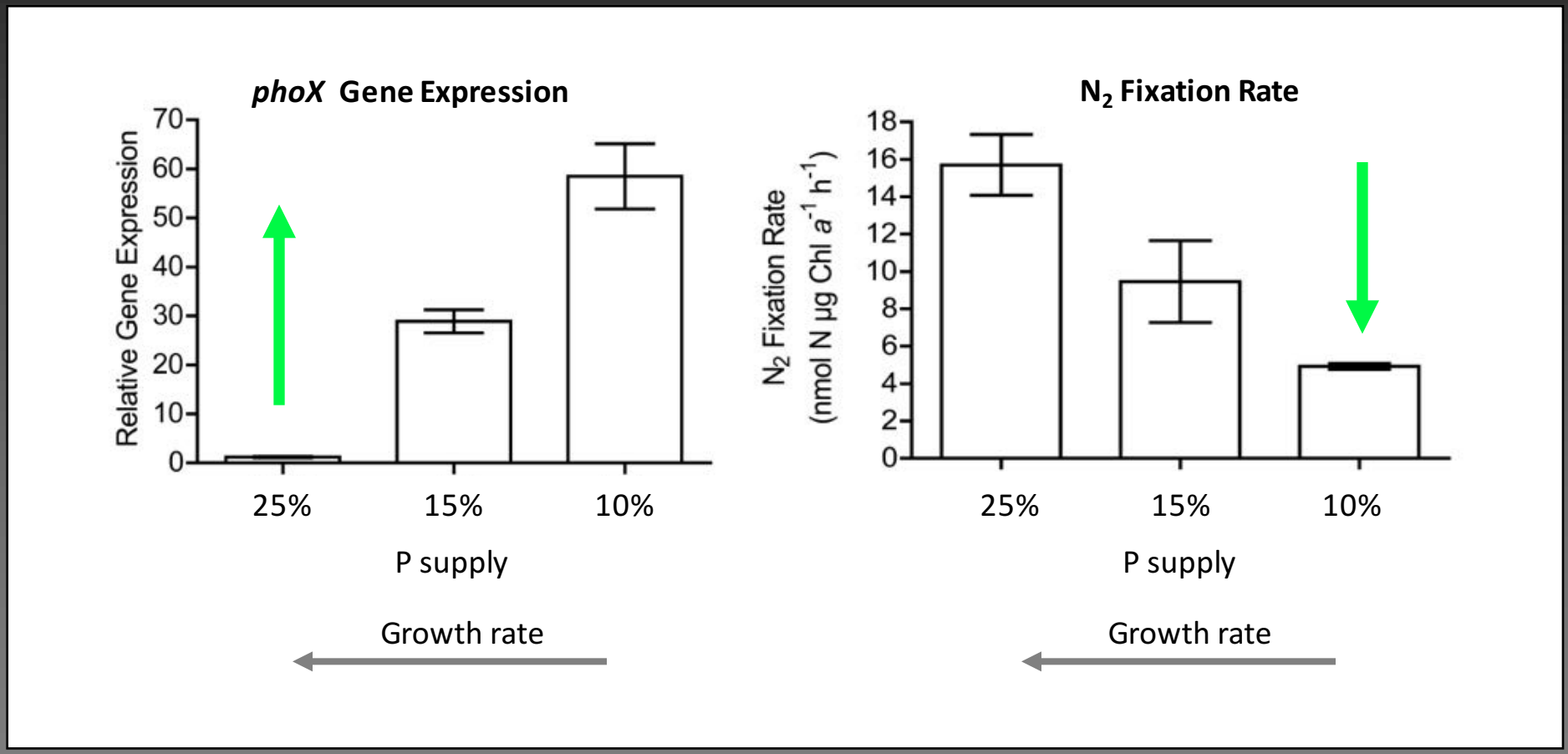
qRT-PCR of *phoX* Activity

P supply



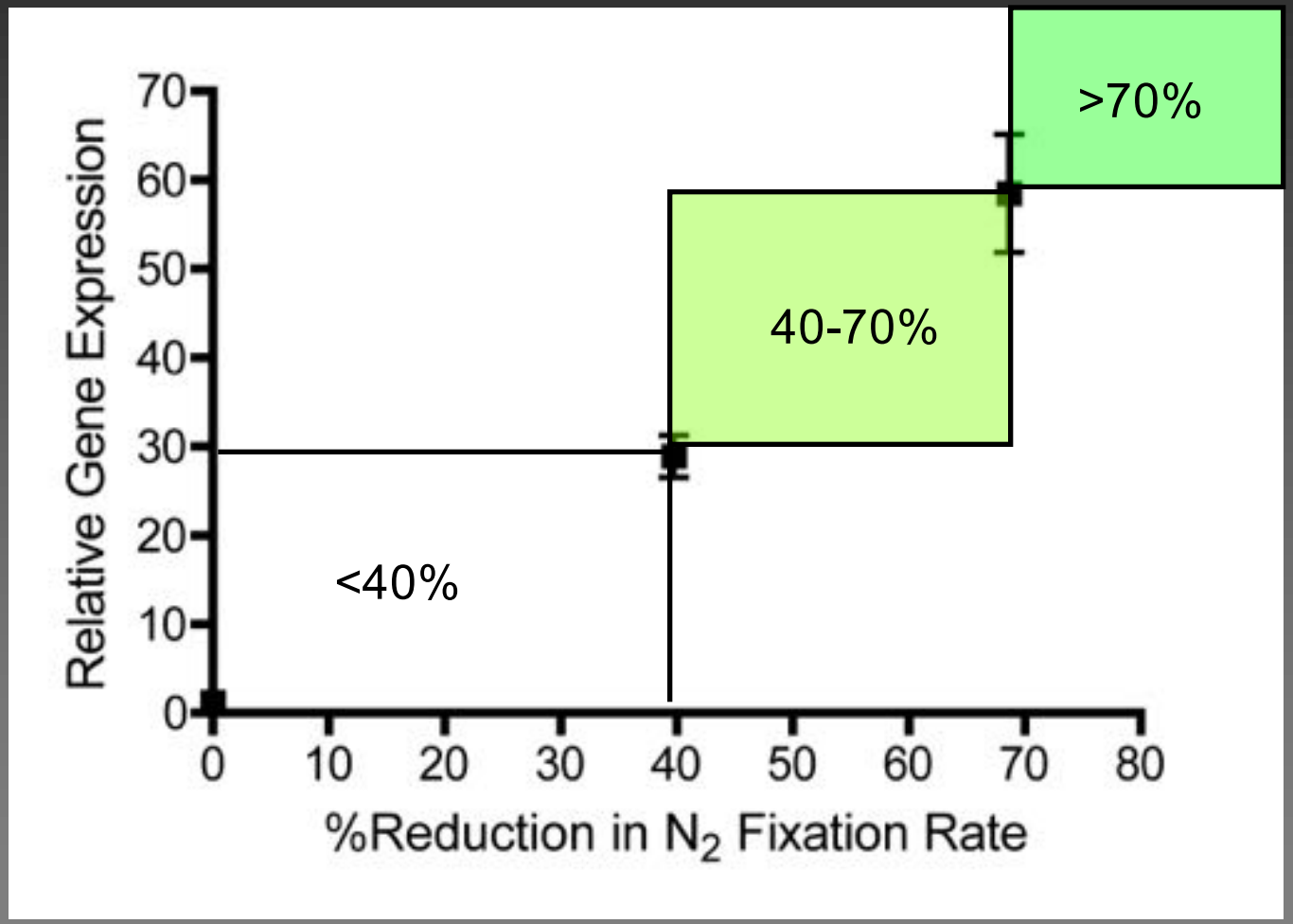
Growth rate

Calibrating gene expression to growth and N_2 fixation



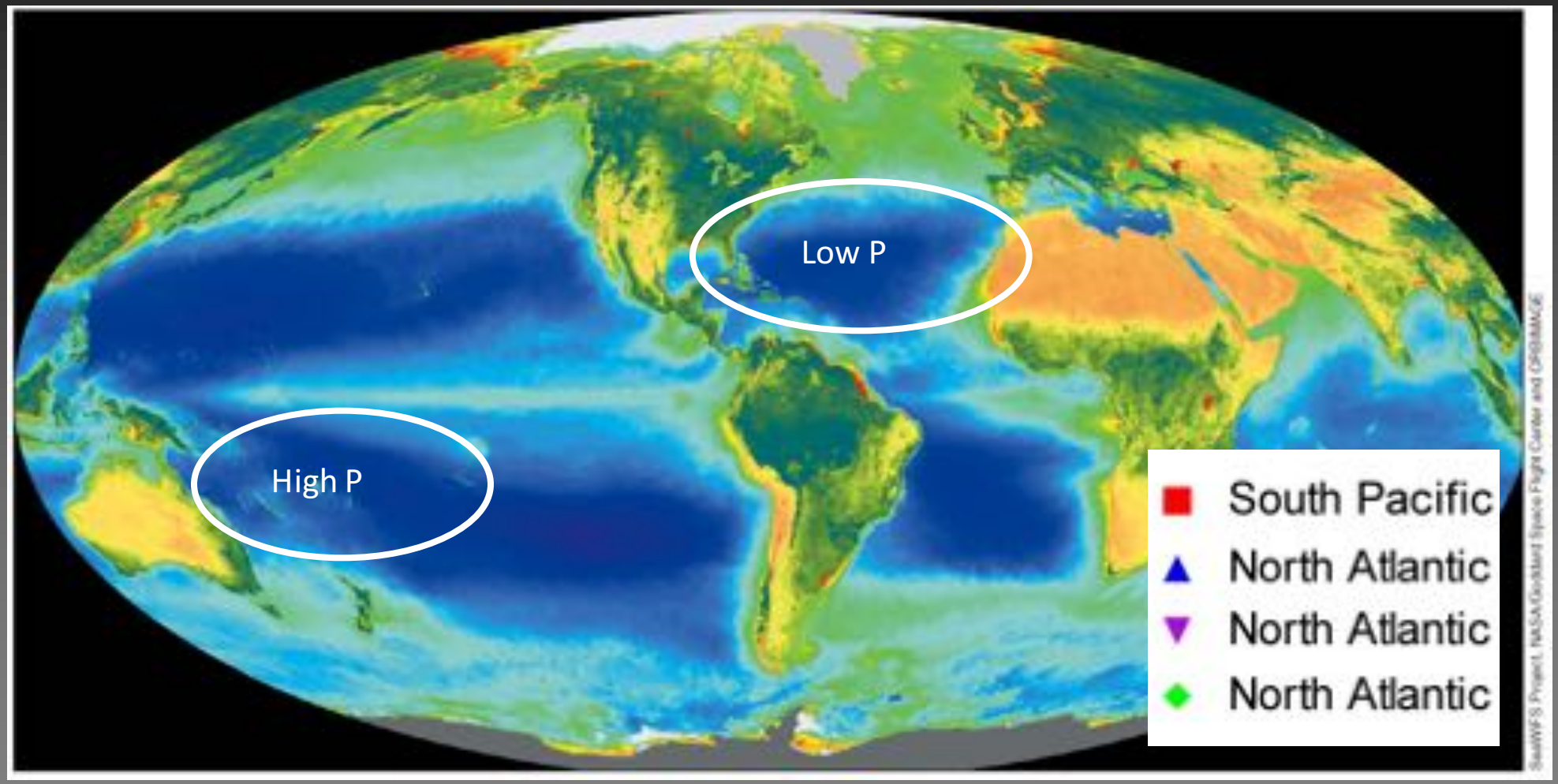
Orchard and Dyhrman unpublished

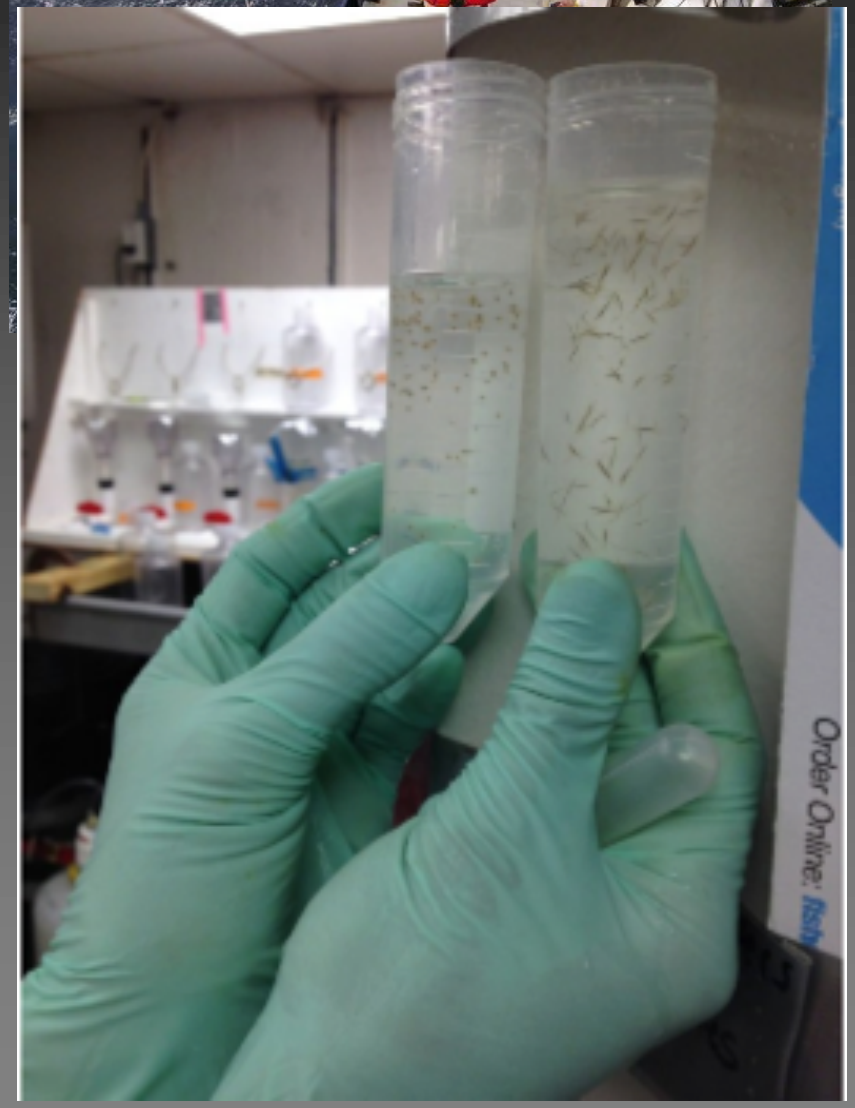
Calibrating gene expression to N_2 fixation



Orchard et al. in prep

Sampling different P regimes

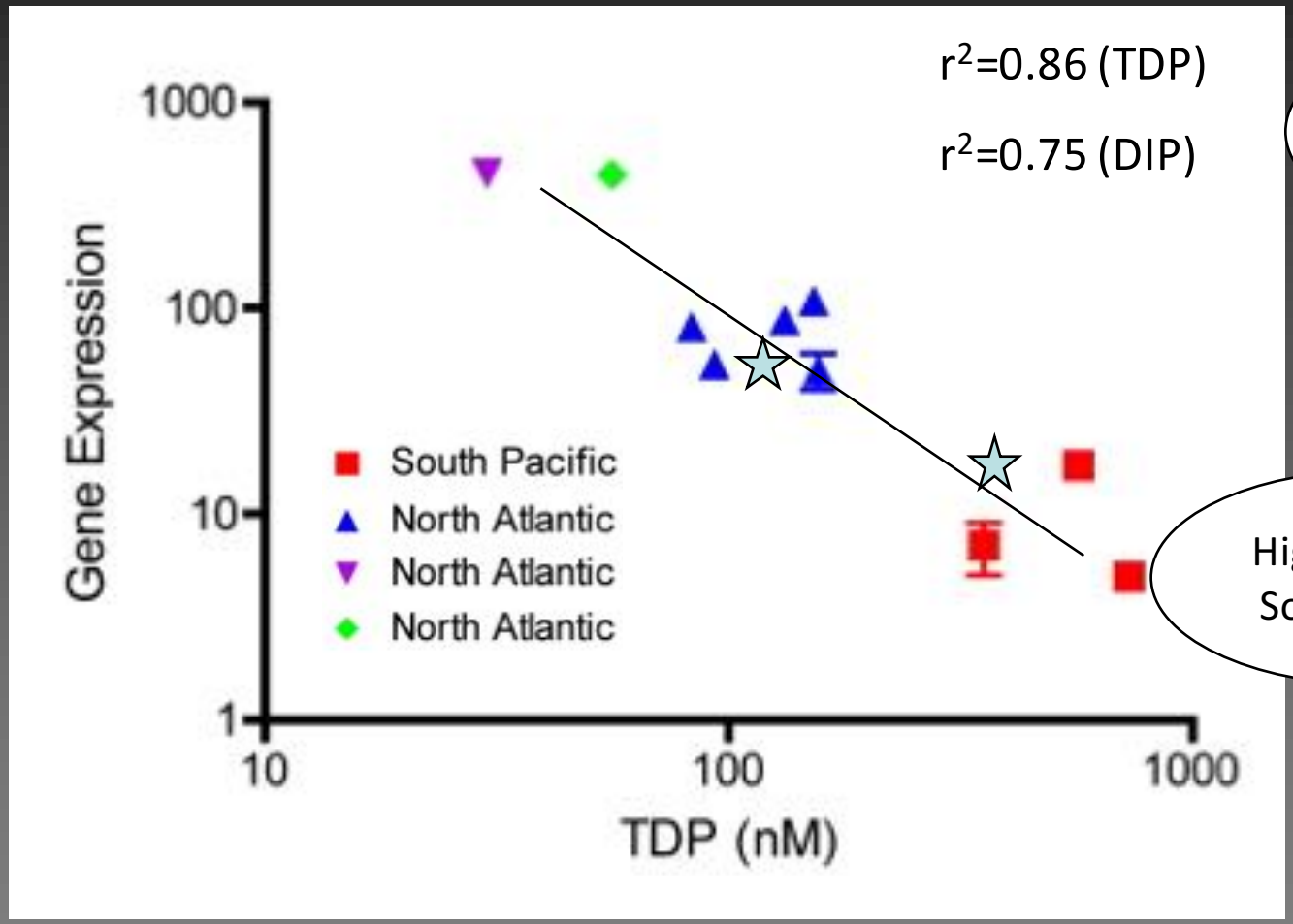




DIP, TDP Measurements

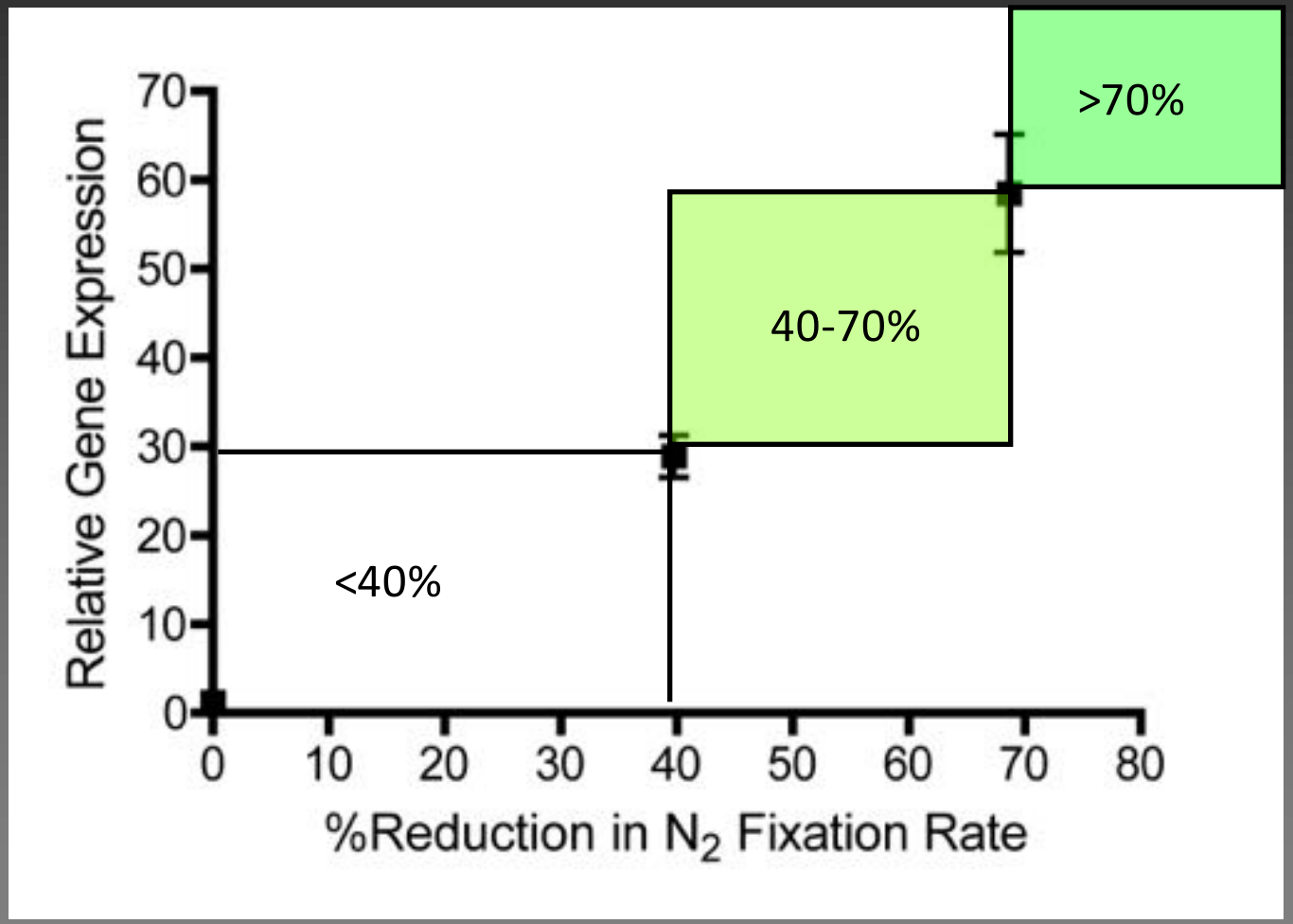
Measurements of quantitative gene expression for *Trichodesmium sp.*

Gene expression increases at low phosphorus



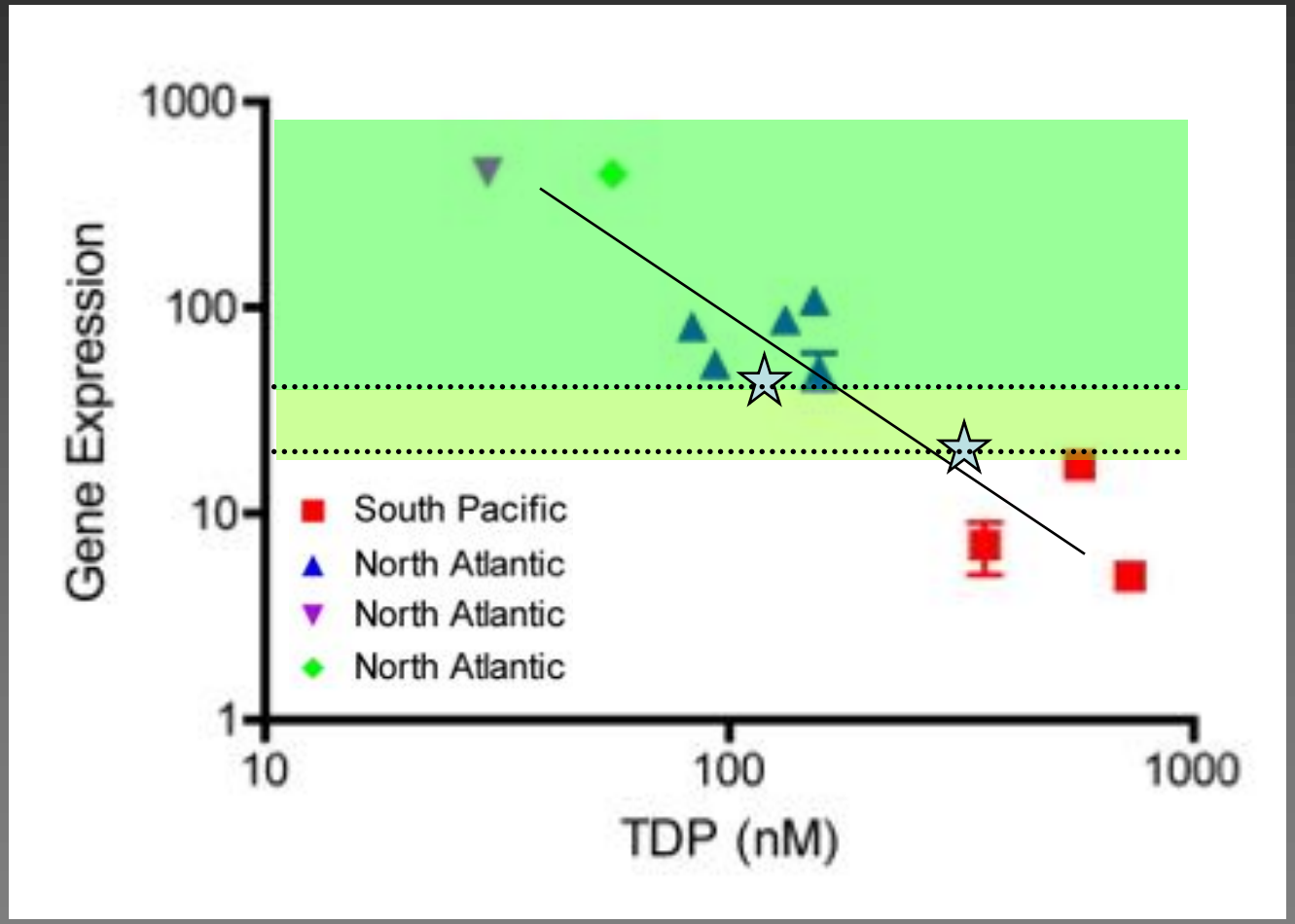
Orchard and Dyhrman unpublished

Calibrating gene expression to N_2 fixation



Orchard and Dyhrman unpublished

Data predicts that P supply limits N_2 fixation in the western N. Atlantic



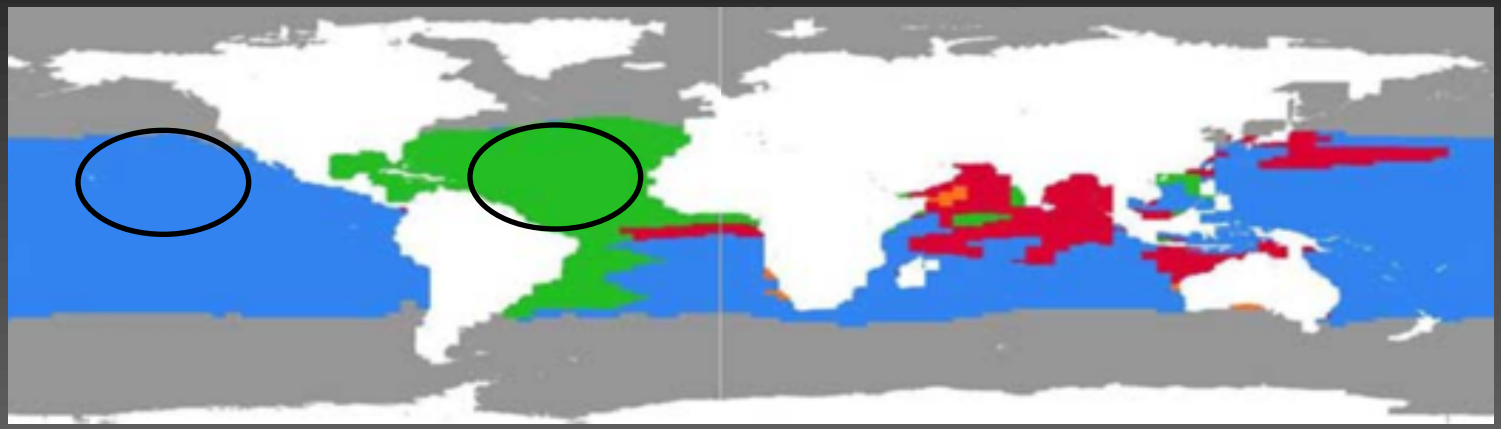
>70%

40-70%

<40%

Orchard and Dyhrman unpublished

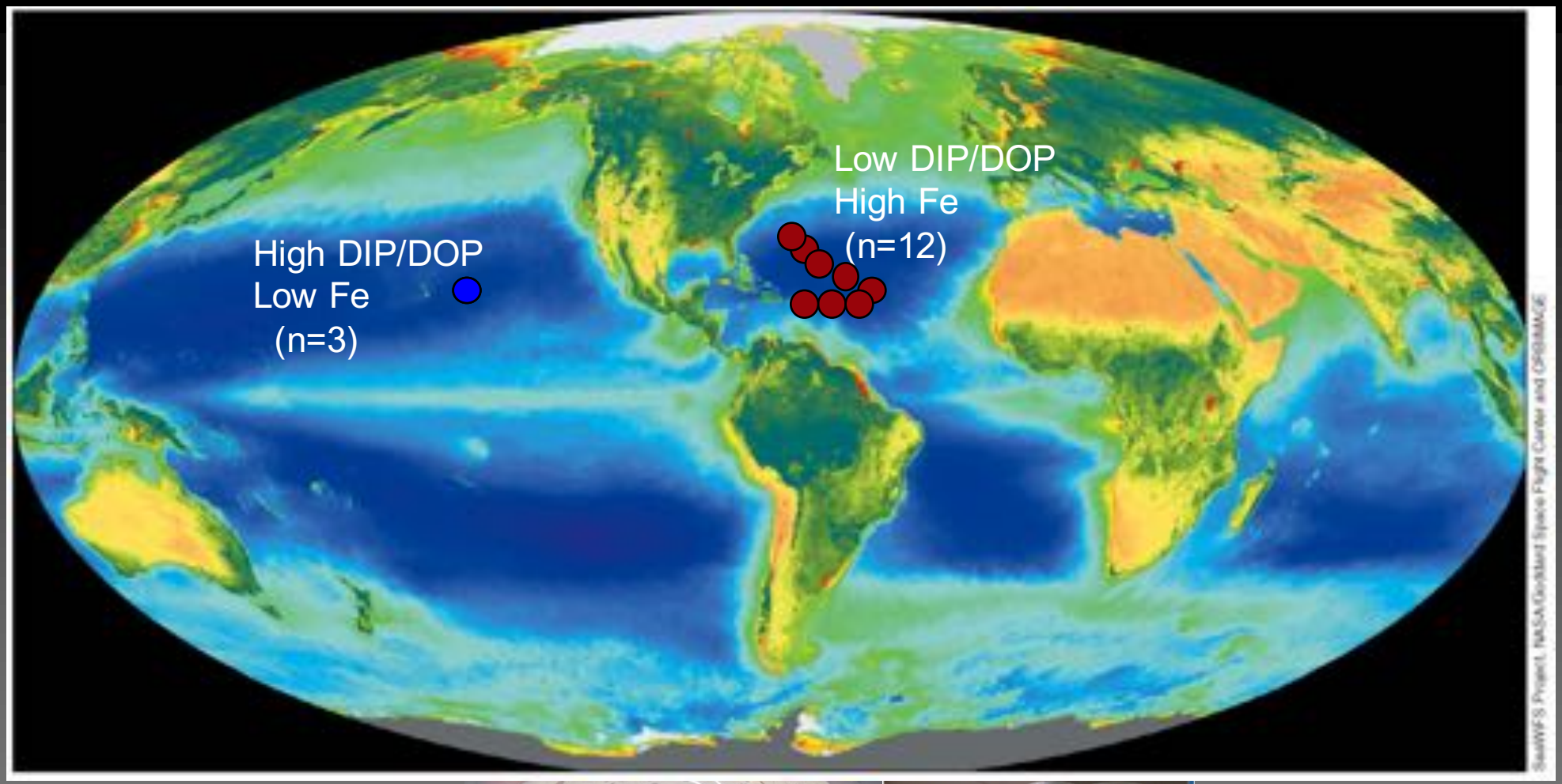
Constraints on *Trichodesmium* N_2 fixation

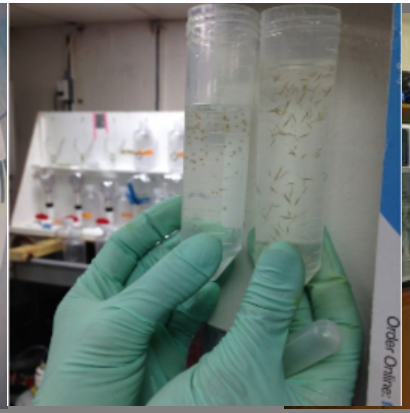


(Moore et al. 2004)

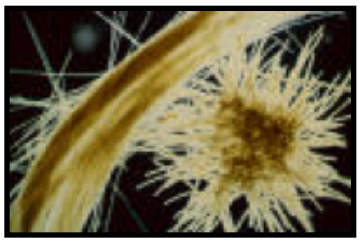
Molecular patterns corroborate predictive models in the western north Atlantic

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 - N_2 - fixation


High DIP/DOP
Low Fe
(n=3)
Low DIP/DOP
High Fe
(n=12)



Metatranscriptome analysis pipeline



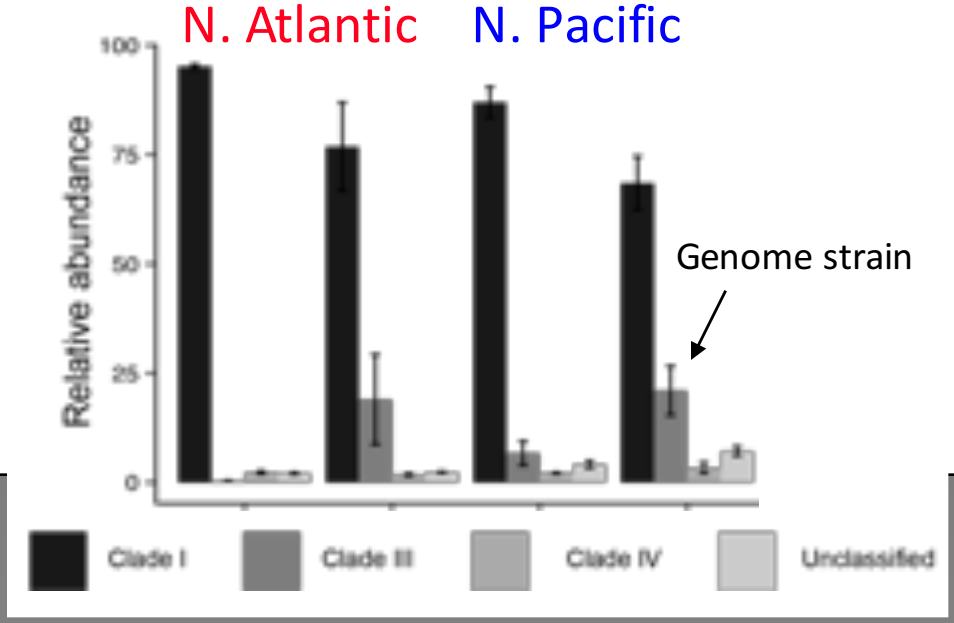
Collect samples

Remove rRNA

Submit samples to sequencing center

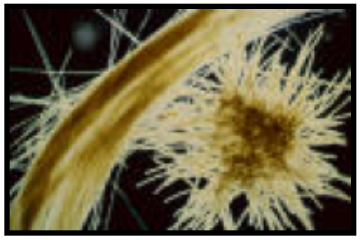
Sequence using RNAseq

Target 30 - 60 million
100 bp paired-end reads



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

Metatranscriptome analysis pipeline



Collect samples

Remove rRNA

Submit samples to sequencing center

Sequence using RNAseq

Target 30 - 60 million 100 bp paired-end reads

Stringent mapping to custom database

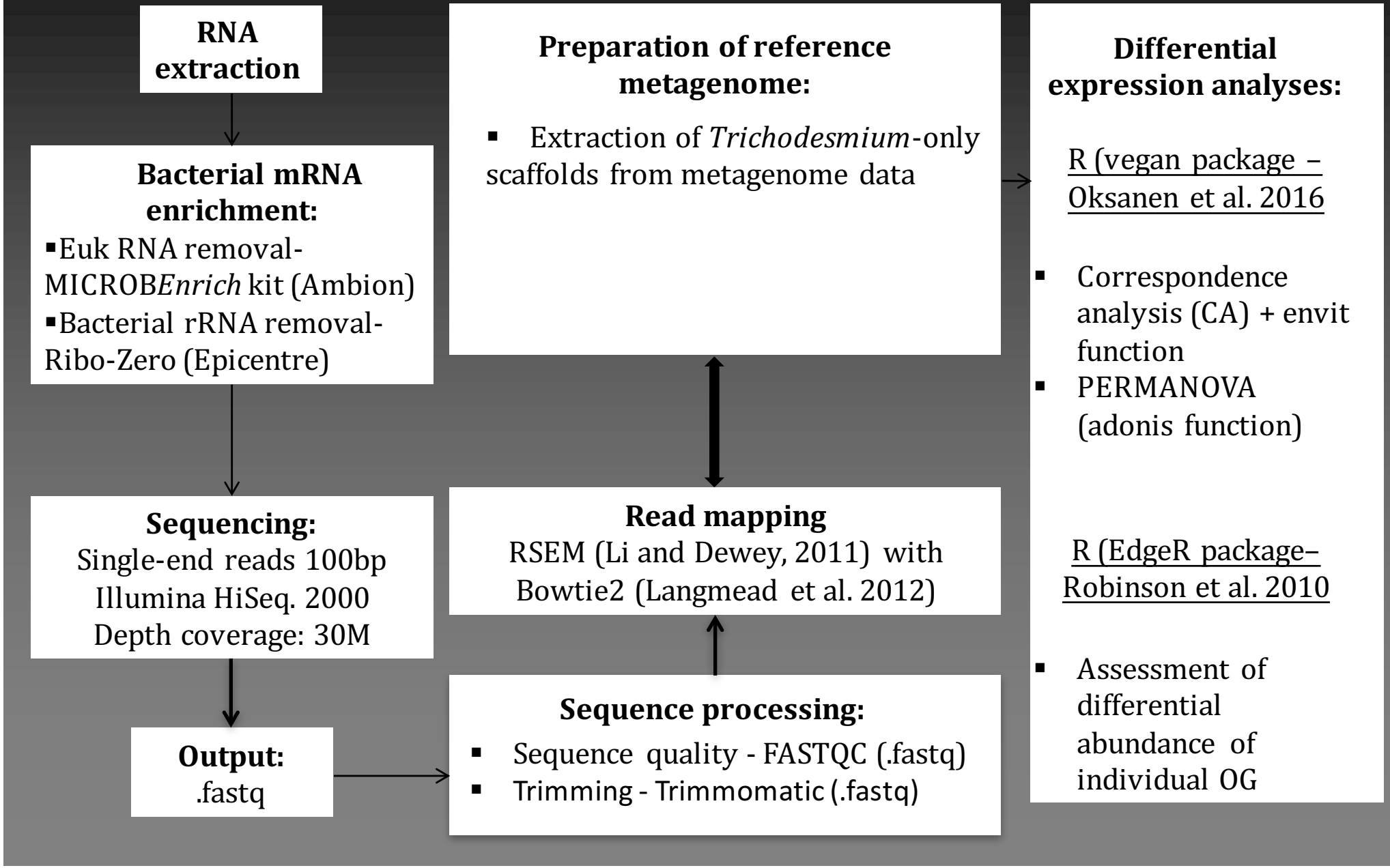
Removed reads with ambiguous mapping

Curated, custom database: *Trichodesmium* metagenome sequences clustered into orthologous groups

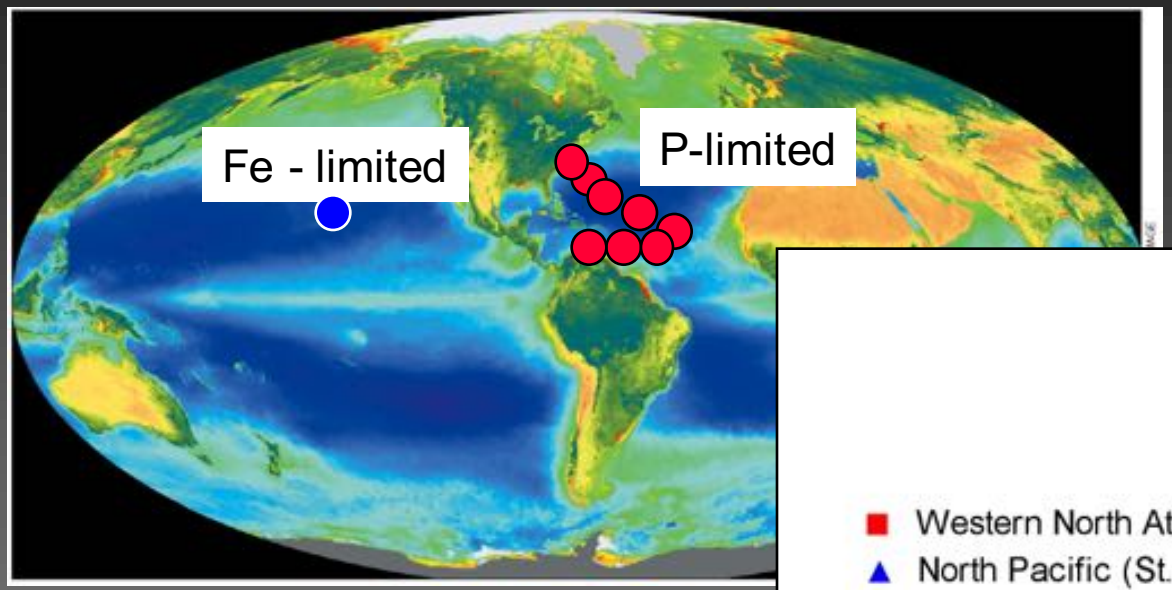
The reality…..


CAACAACATCATACCCTTCCAAGGCACGGGCTTCAACGCCCGCCAAATCTCC
TGCGGCAGCAGGGATGGGACCCTGGGACTGTGTGGCGCTGTGGAGGCCG
+DHT4KXP1:1:1101:5930:2353#0/1
\W\^^aac^[`[cfhh_Z`^eg[RJ`W^^OOO^aeaee0_eehhULVMV\H\
\`ZSNWW^aaQTRGX^BBBBBBBBBBBBBBBBBBBBBBBBBBBBBB
@DHT4KXP1:1:1101:5904:2385#0/1
CTACCATTTTCATTTTCCATGTCTCTCCCCATCTTGACACATTATTTTTTCT
ACCTCCATCACATCATGATCACGTTATACGATCTCTACAGTAGCCCCCA
+DHT4KXP1:1:1101:5904:2385#0/1
bbbeeeeegggegiiiiiiiighhhhiiiifhihiifgiifhiiiiihhiii
iihiiihiihiihiiiihiiiidggggeeeecddddccbccccccc_
@DHT4KXP1:1:1101:5781:2386#0/1
CTTTCAGATACAGTAGGATTTATTCAGGATCTTCCGACGACATTGATTGCTG

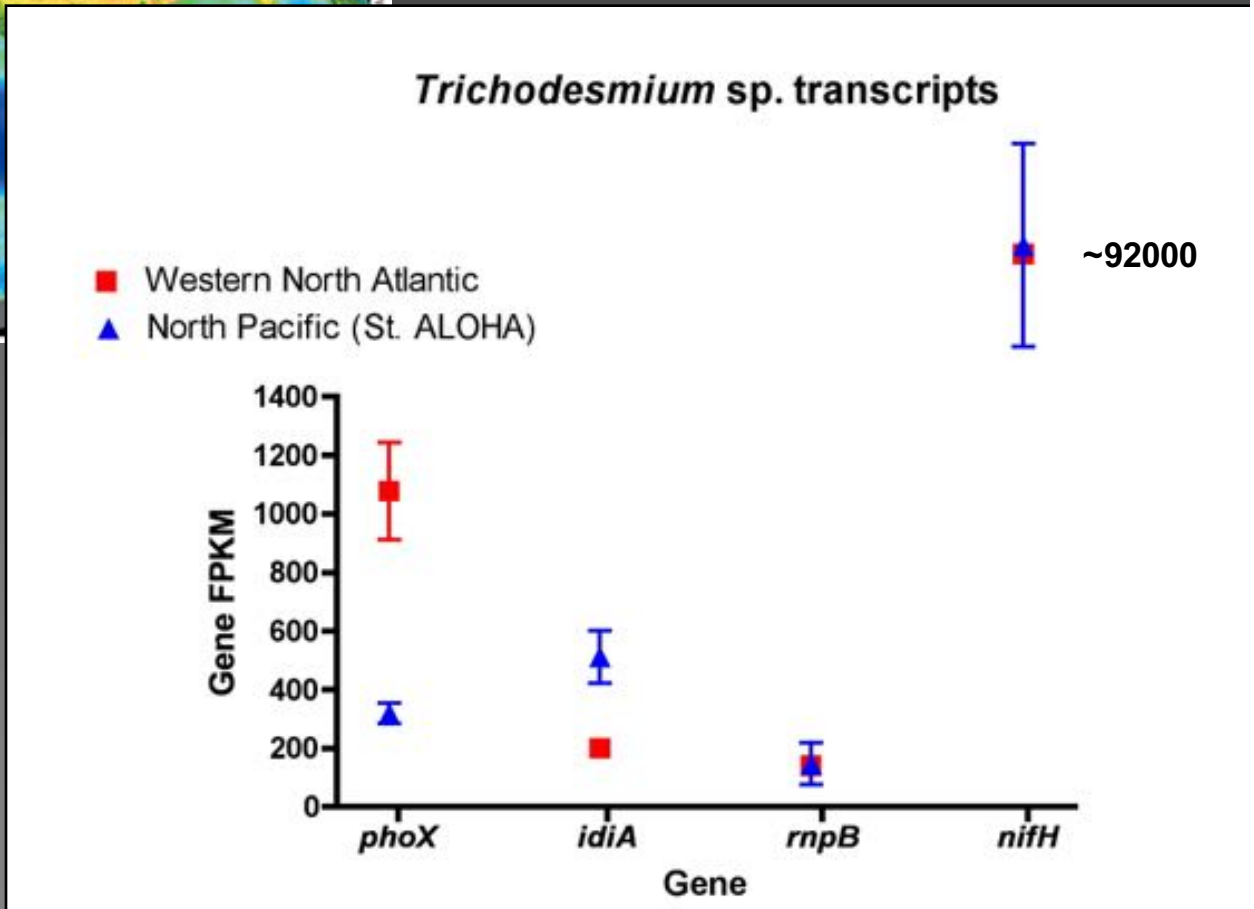
Metatranscriptome analysis



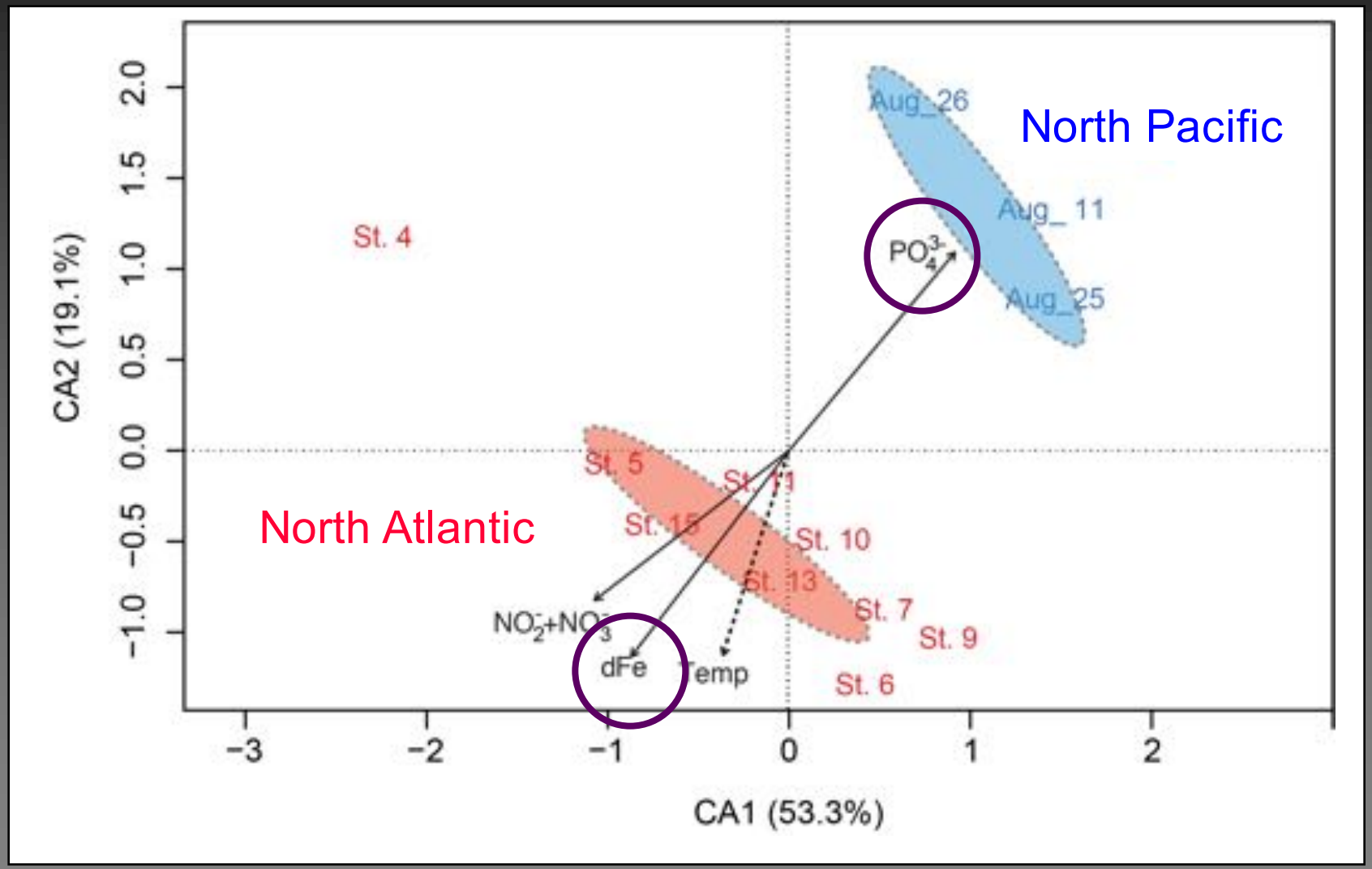
Biomarkers consistent with model prediction



Biomarkers confirm model predictions.

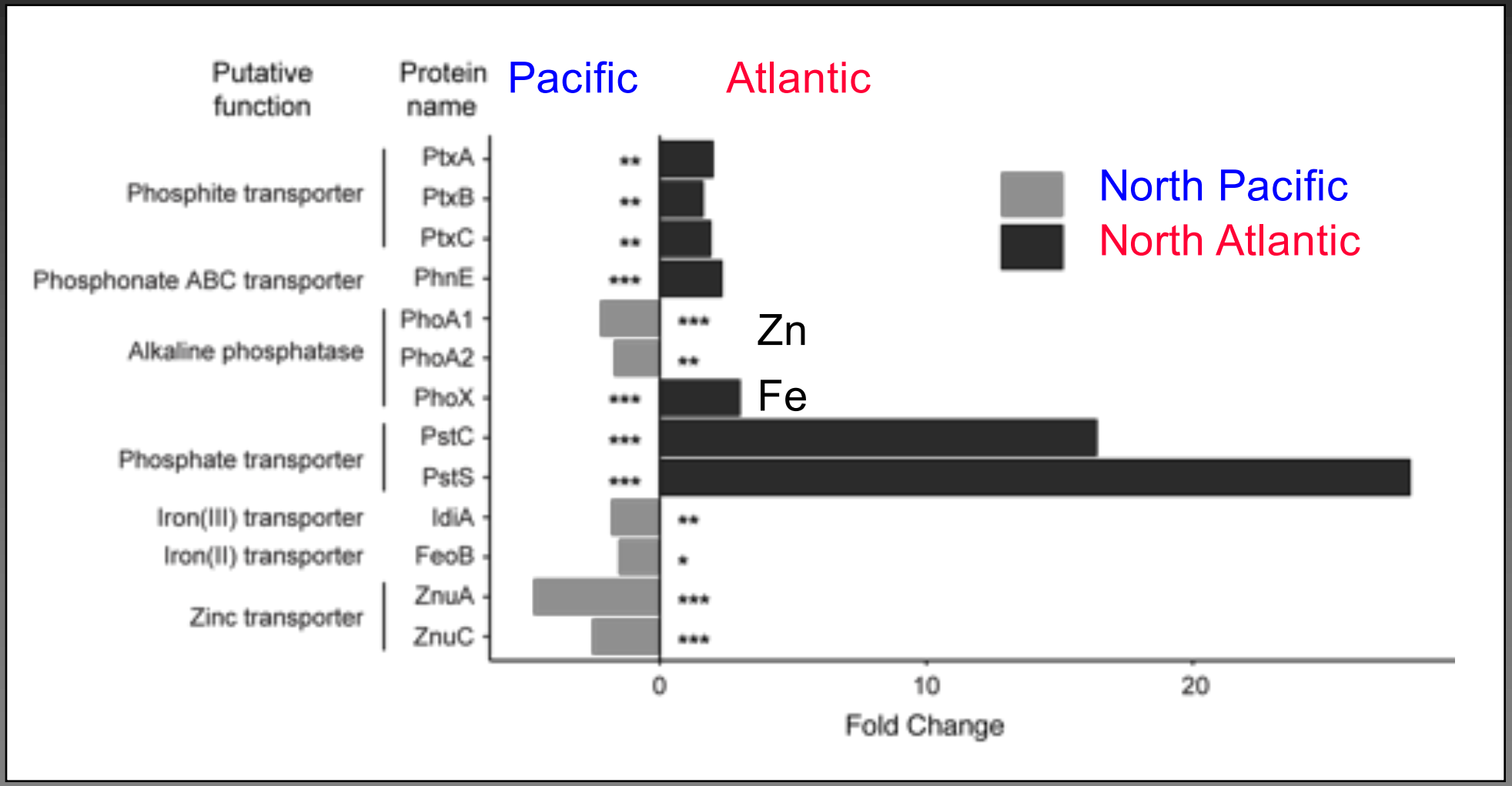


Significant differences in global transcription between the North Pacific and the North Atlantic



Ruoco et al. (2018) *ISMEJ*

Transcription patterns indicate metalloenzyme trade-offs and geochemical controls



Ruoco et al. (2018) *ISMEJ*

Summary - Metabolic traits and trade-offs

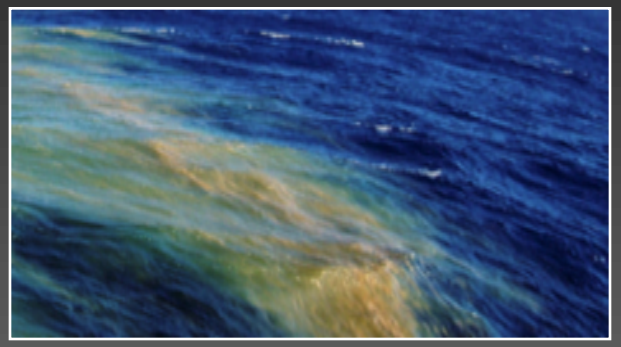
What phosphorus forms are bioavailable?
What are the biogeochemical constraints on N_2 fixation?

- **Genome***: Trichodesmium* genome suggests bioavailabiliy 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 N_2 fixation
- **Metatranscriptome**: Predicted biogeochemical drivers of N_2 fixation are reflected in *Trichodesmium* transcriptional signals including likely metalloenzyme switching

Core questions

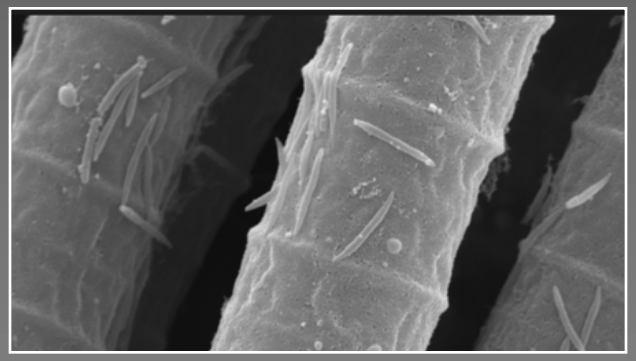
Metabolic traits and trade-offs

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



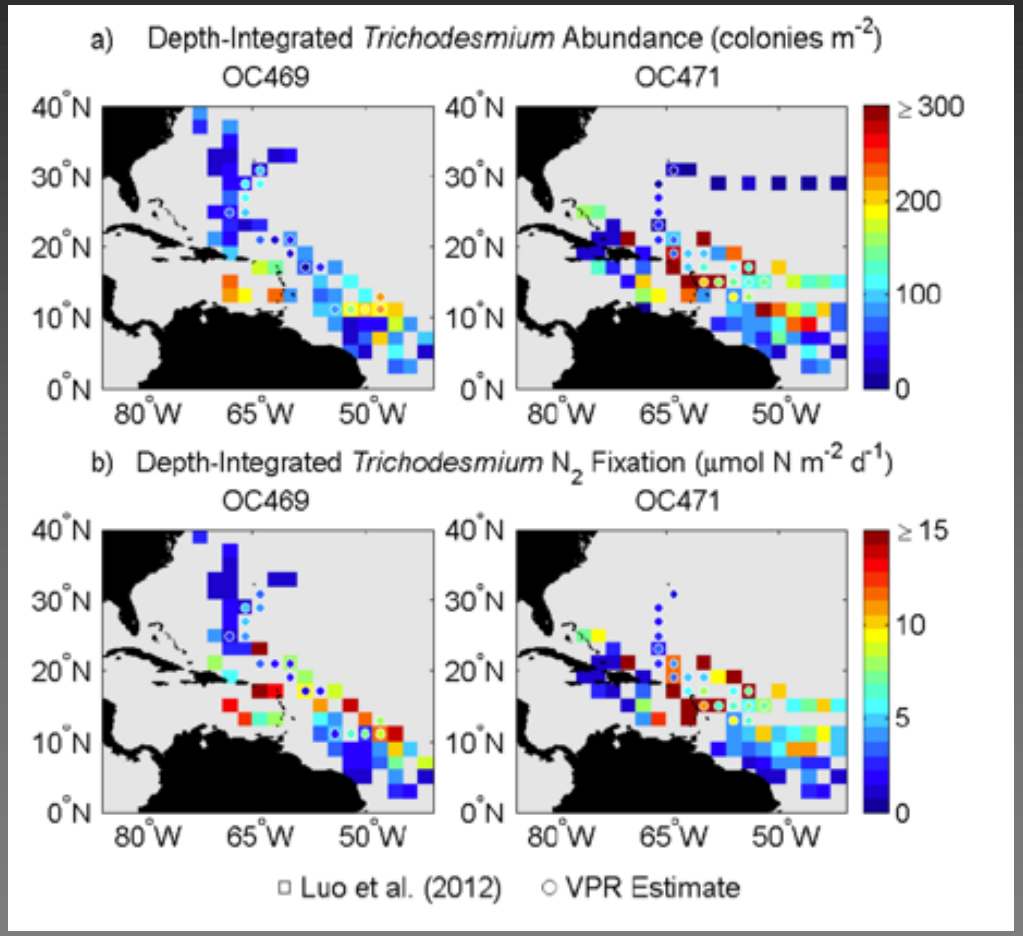
Host microbiome interactions

- Who is there? Microbiome diversity
- What are they doing? Microbiome functional diversity, holobiont physiology and controls on N_2 fixation



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 necessarily a good predictor of distribution or N_2 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*)

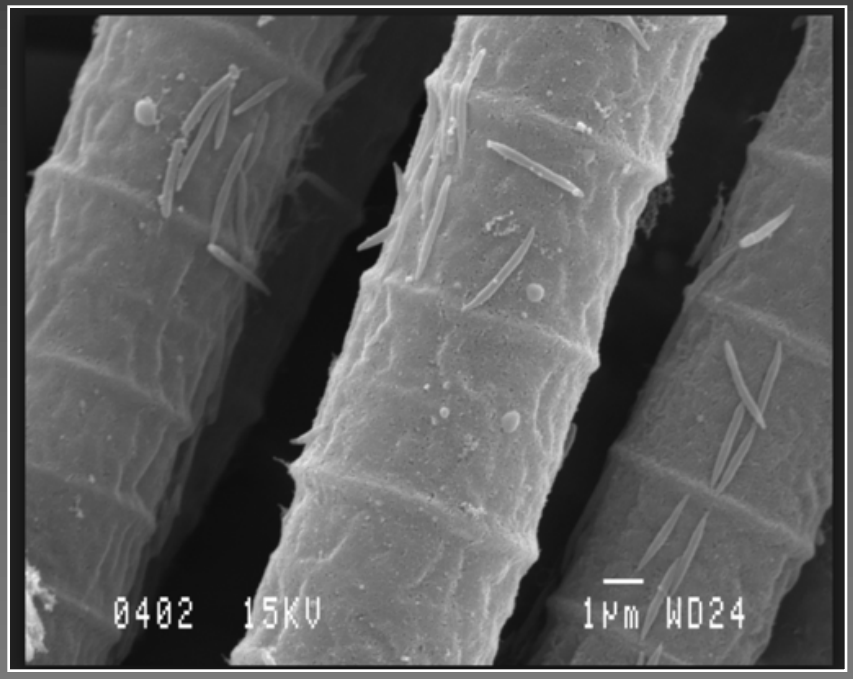
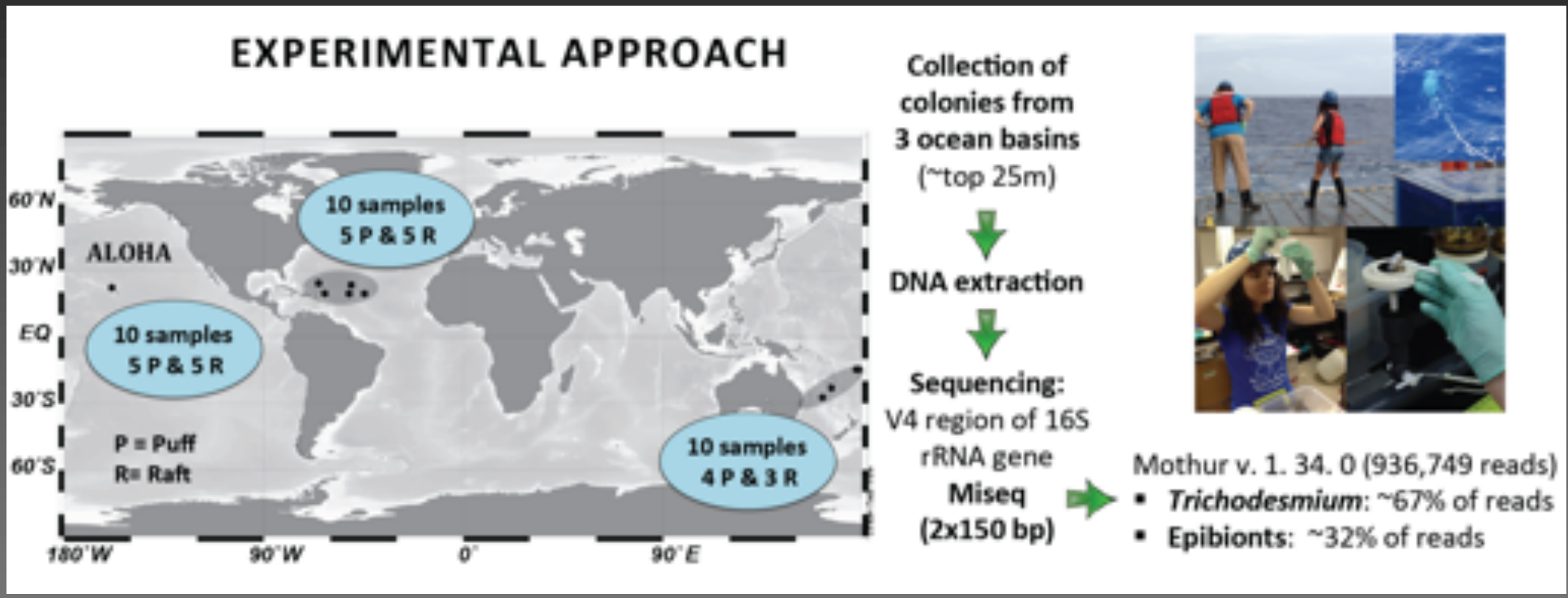


Image courtesy Tracy Mincer

Epibiont diversity



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)

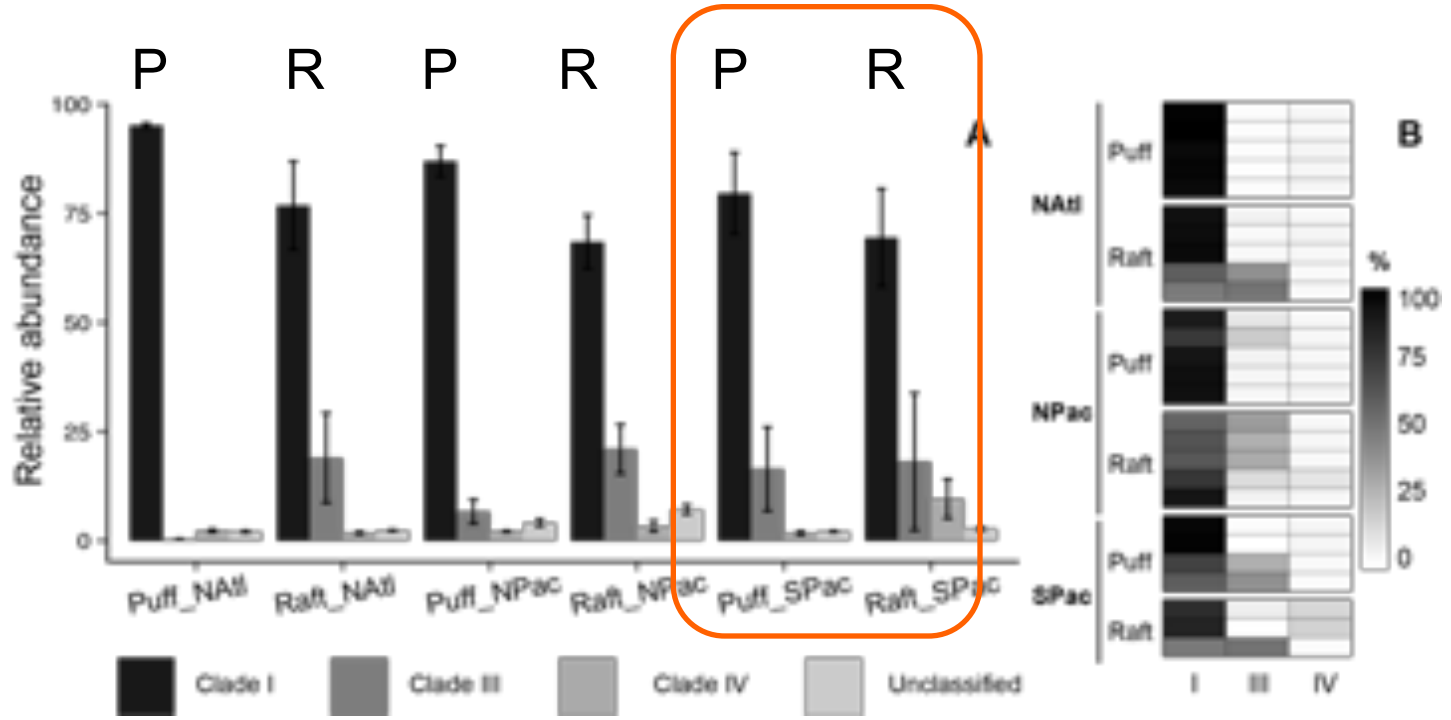
- .fasta
- .count_table
- Green gene database

Metabolic inference

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

Colony composition by region

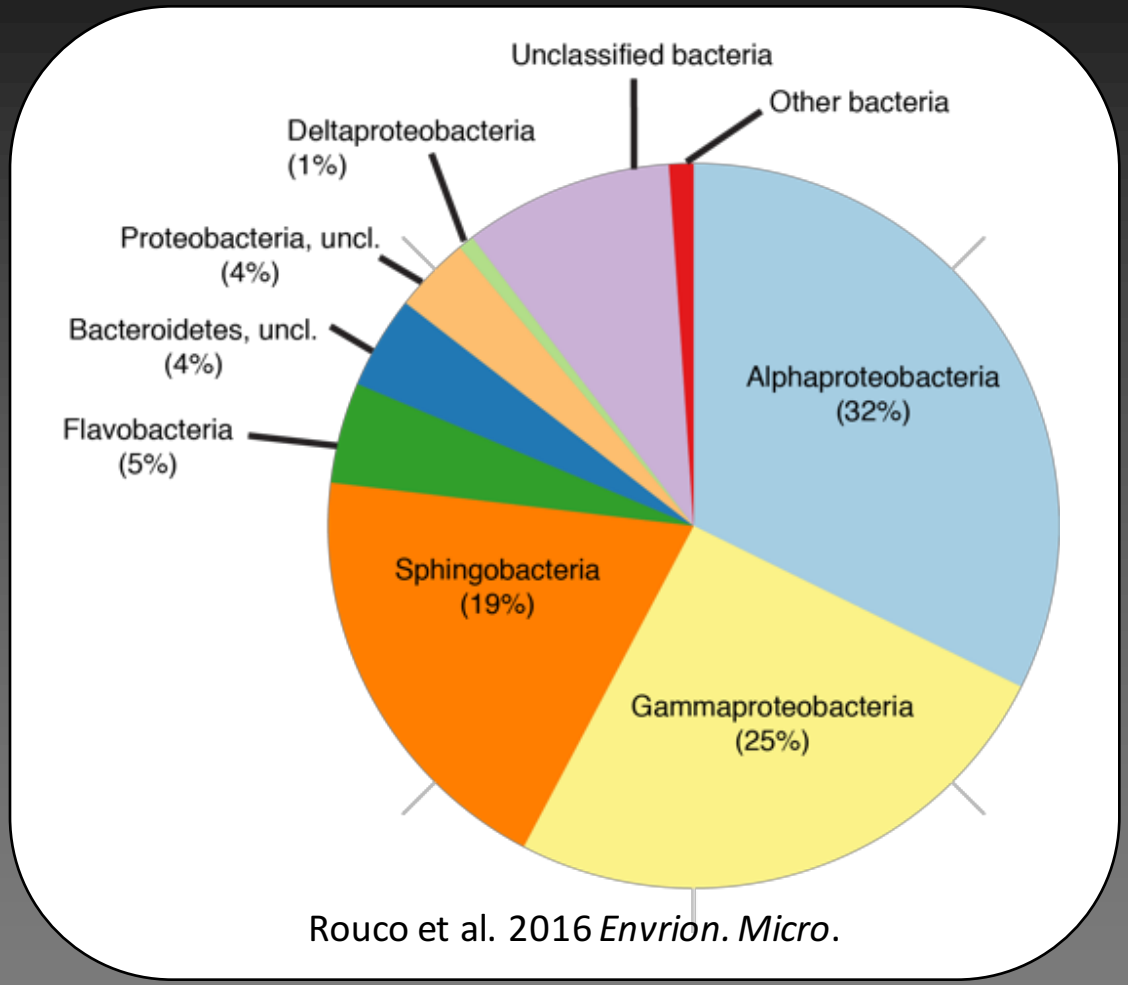
N. Atlantic N. Pacific S. Pacific



Rouco et al. 2016 *Envrion. Micro*.

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

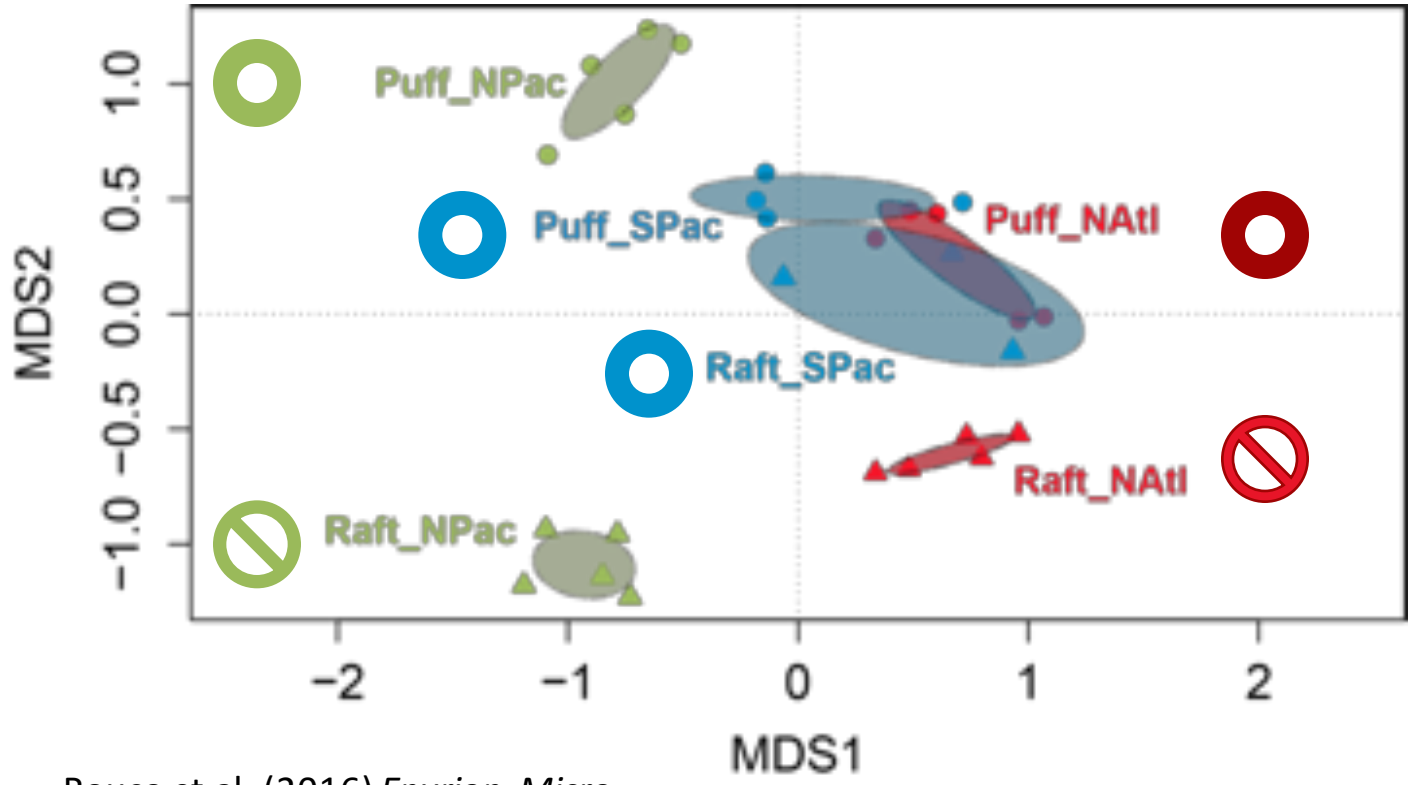
Average epibiont community



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)



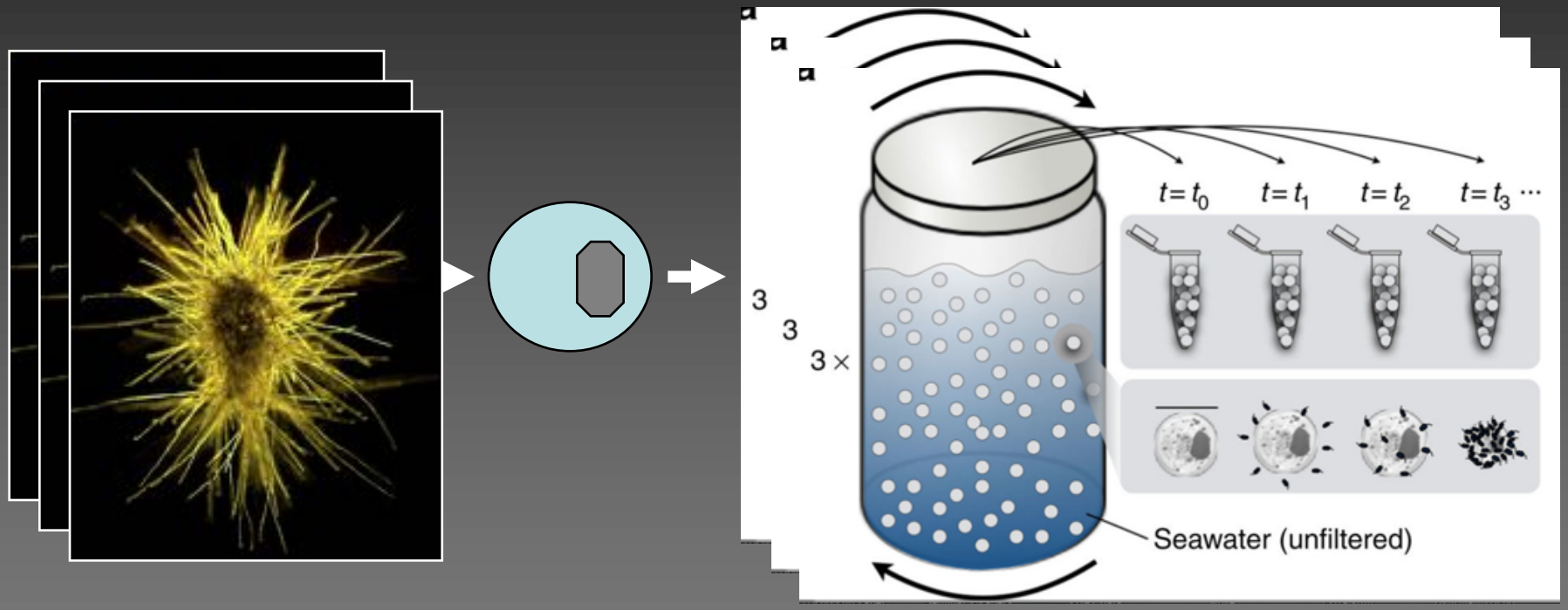
Rouco et al. (2016) *Envrion. Micro*.

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

What drives community assembly?

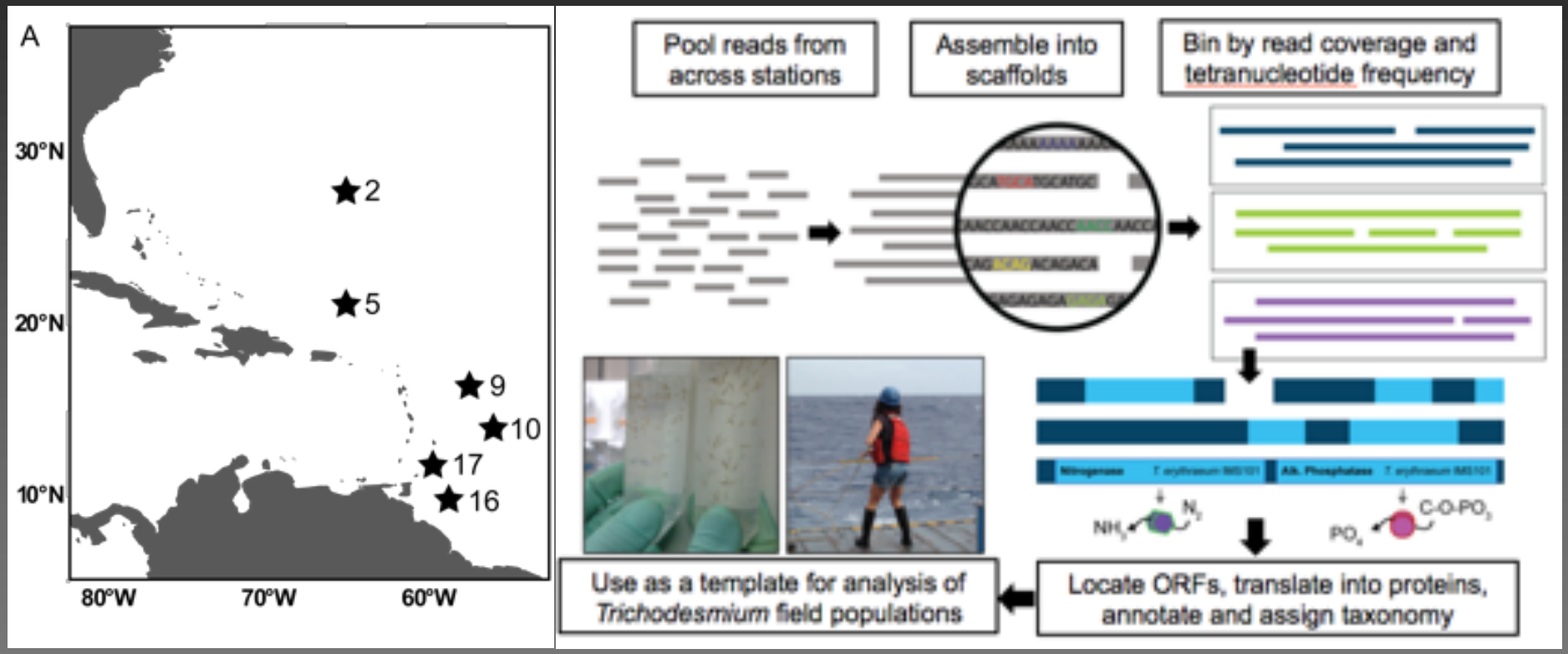
- *Niche?* What type of *Trichodesmium*, physiological ecology in the colony, environment..
- *Lottery?* Random selection of potential copiotrophs, role of taxonomic v. functional group uncertain…
- Working to examine the *Trichodesmium* holobiont with metagenomics/metatranscriptomics and “germ-free”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

Drivers of community assembly



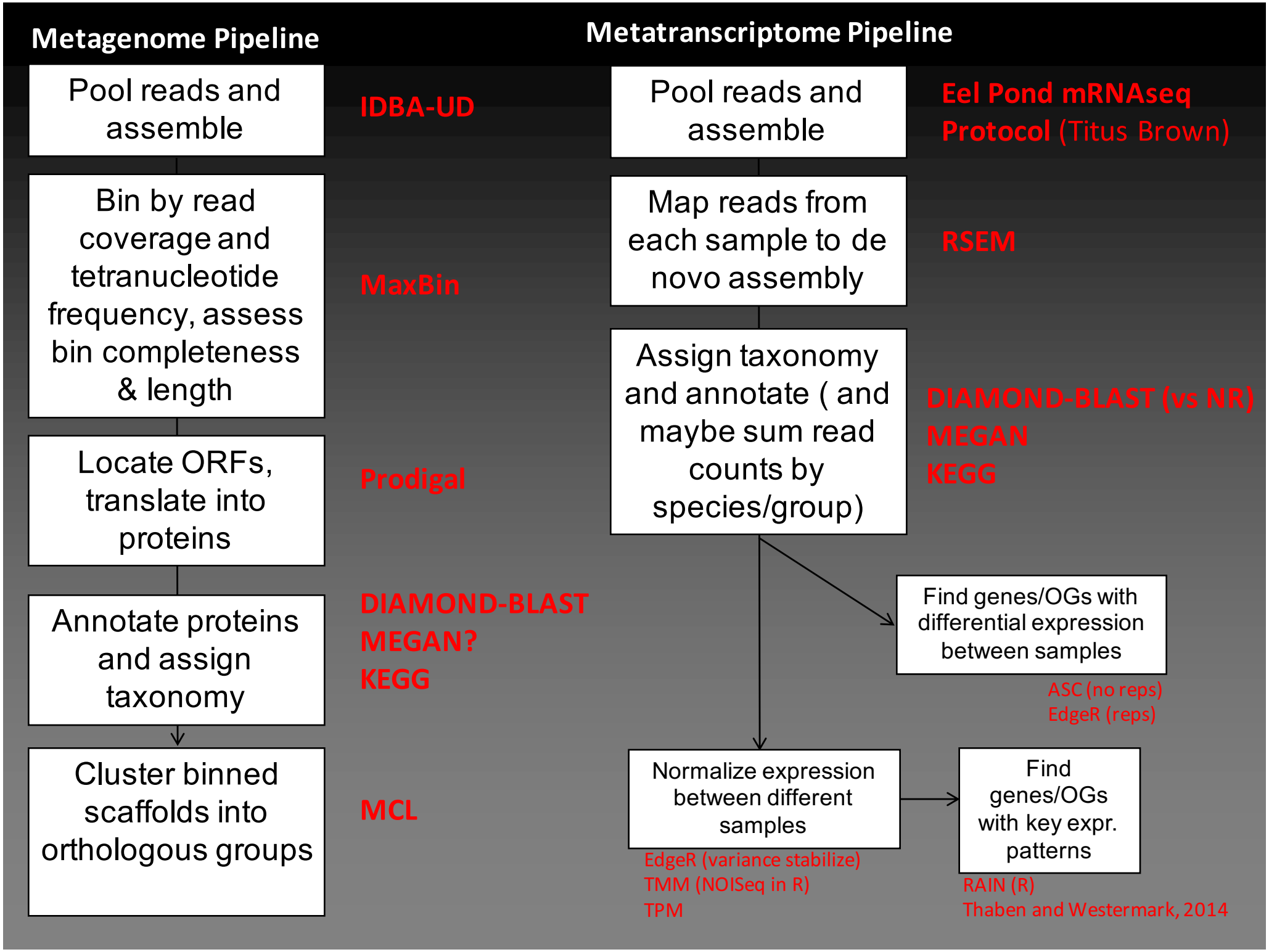
Initial trial of “germ-free” *Trichodesmium* ran into problems - phase two scheduled for spring 2019.

Metabolic potential in the *Trichodesmium* holobiont

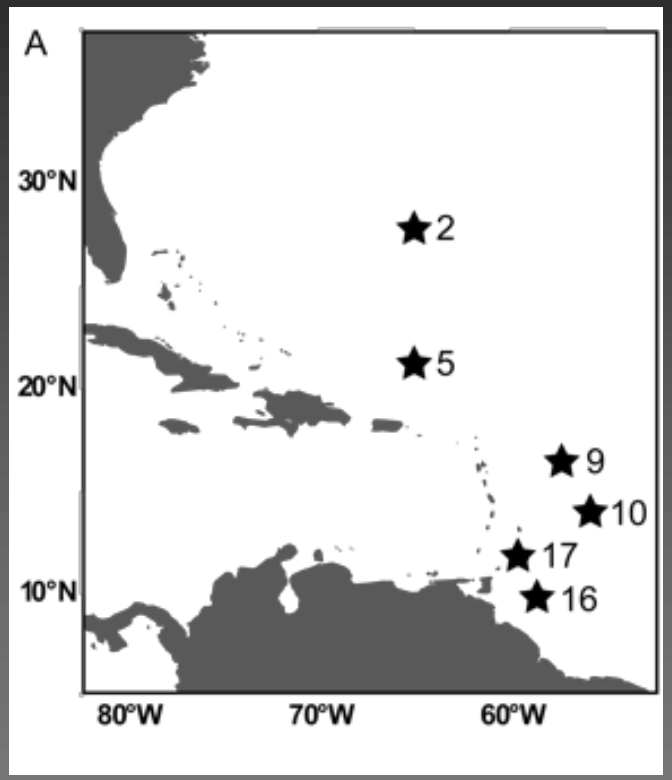


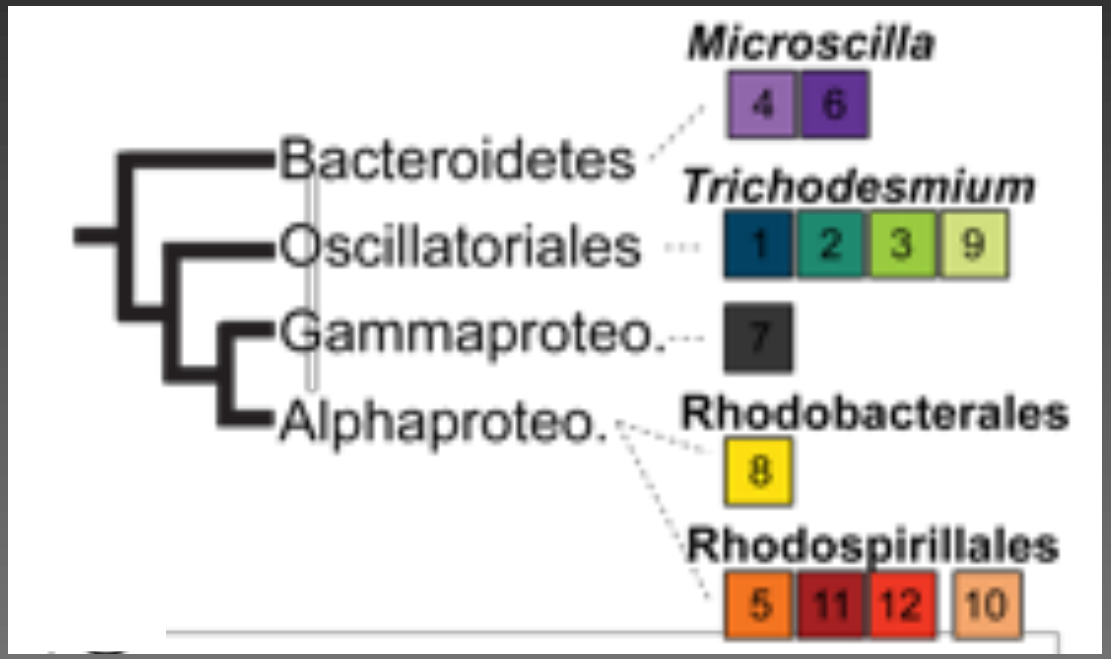
Each sample:

- **20 gigabytes**
- **~120 million reads**
- **>200,000 protein coding genes**


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

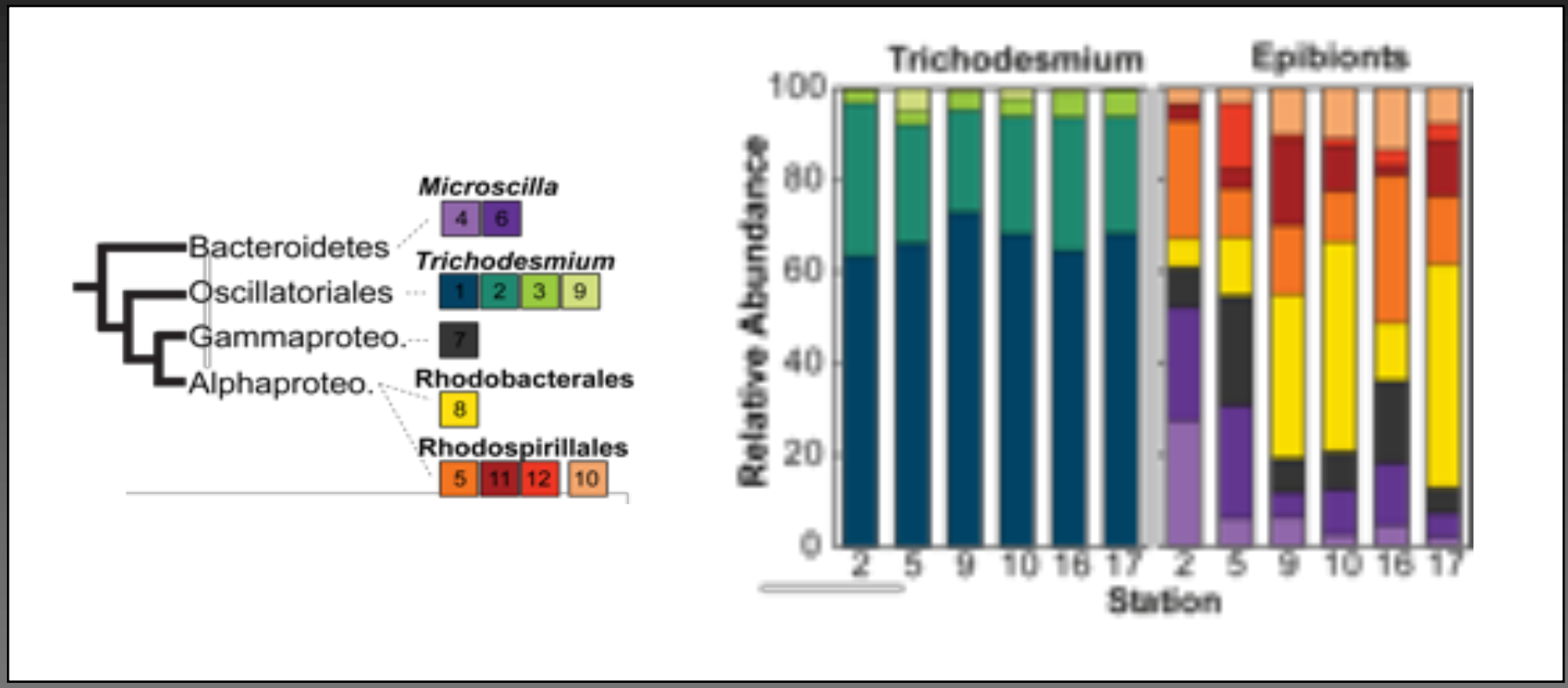




Frischkorn et al. (2017) *ISMEJ*

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

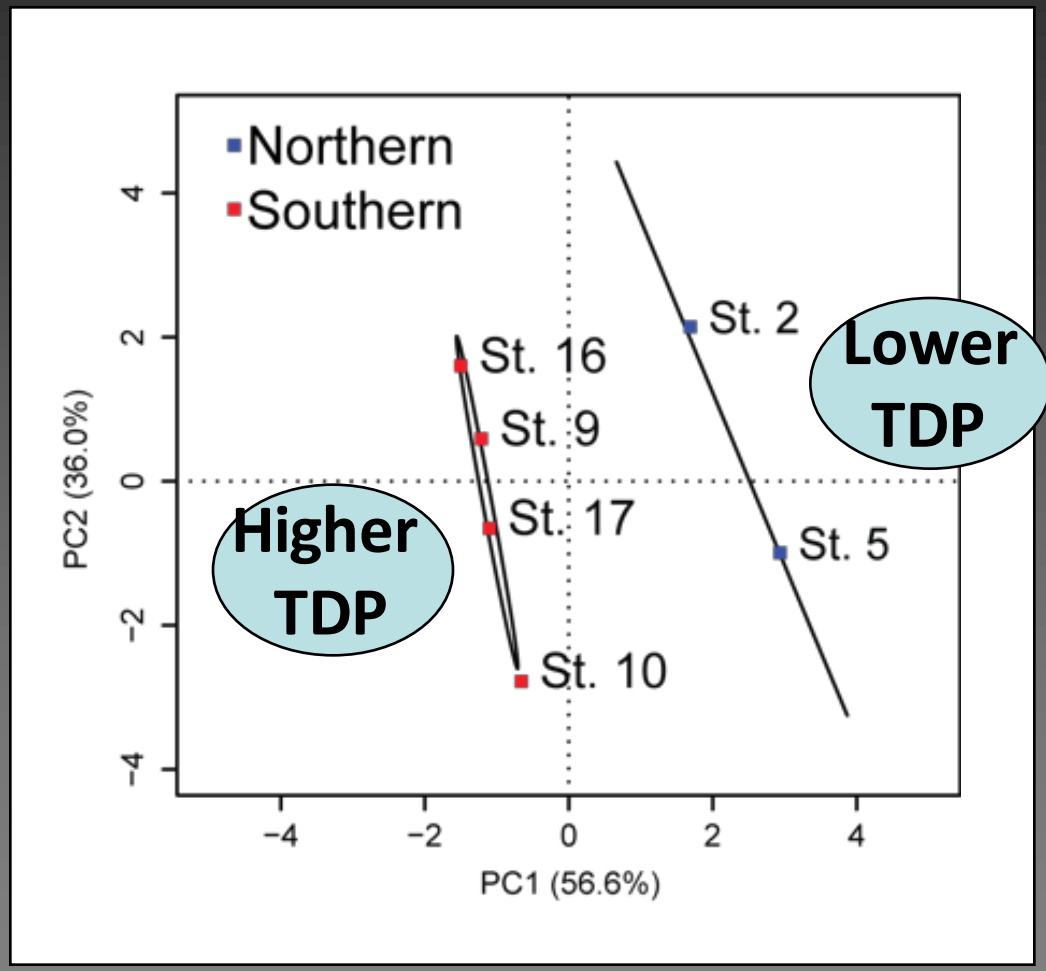
Distribution of holobiont



Frischkorn et al. (2017) *ISMEJ*

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

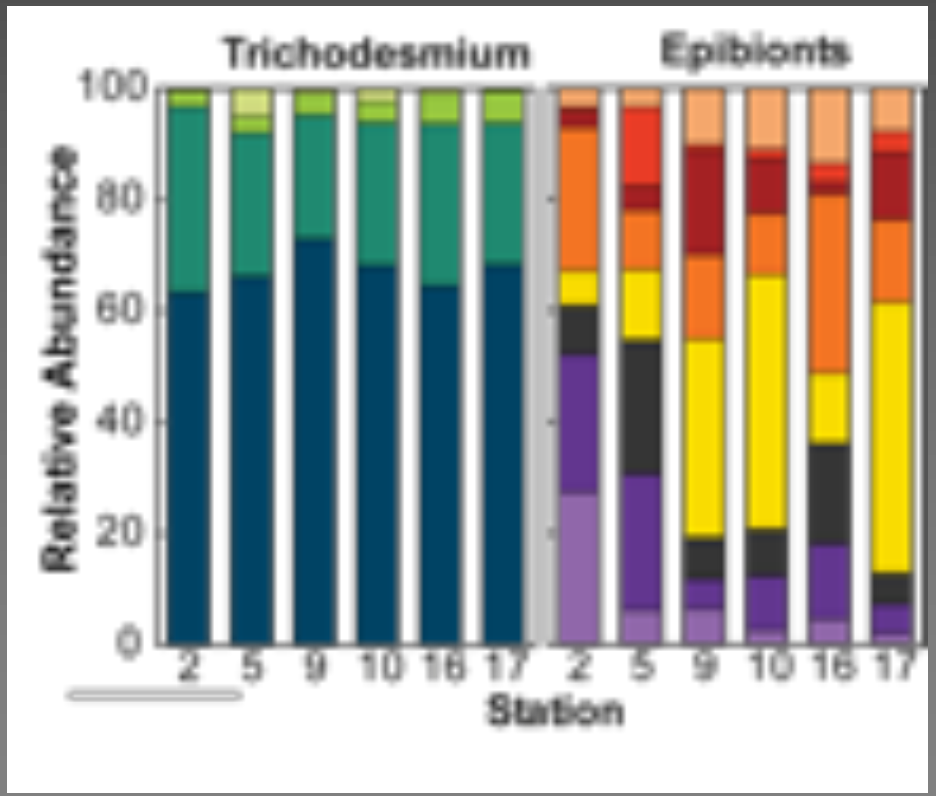
Microbiome diversity differs significantly between regions

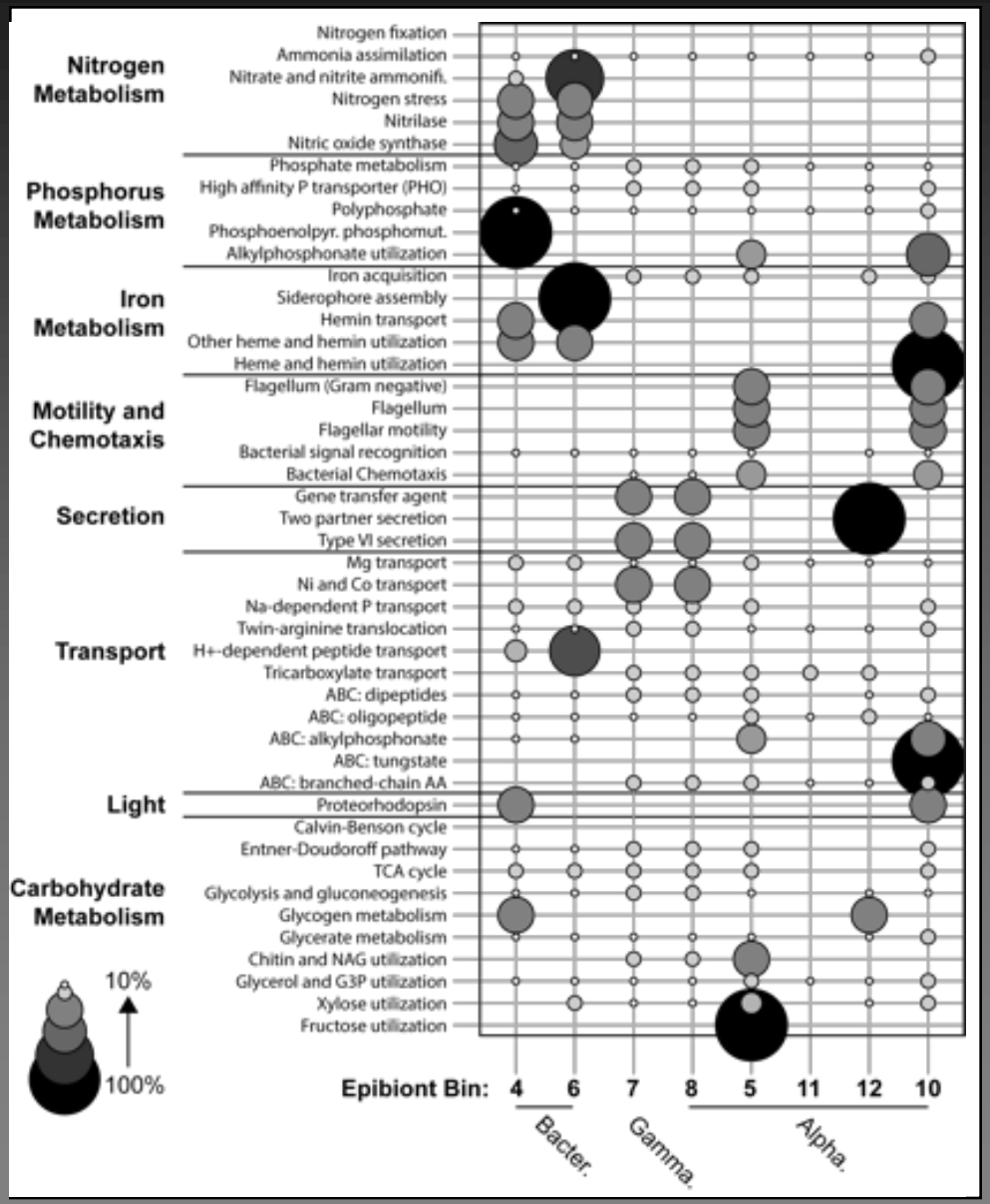


Frischkorn et al. (2017) *ISMEJ*

Variable distribution of functional pathways among epibionts

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



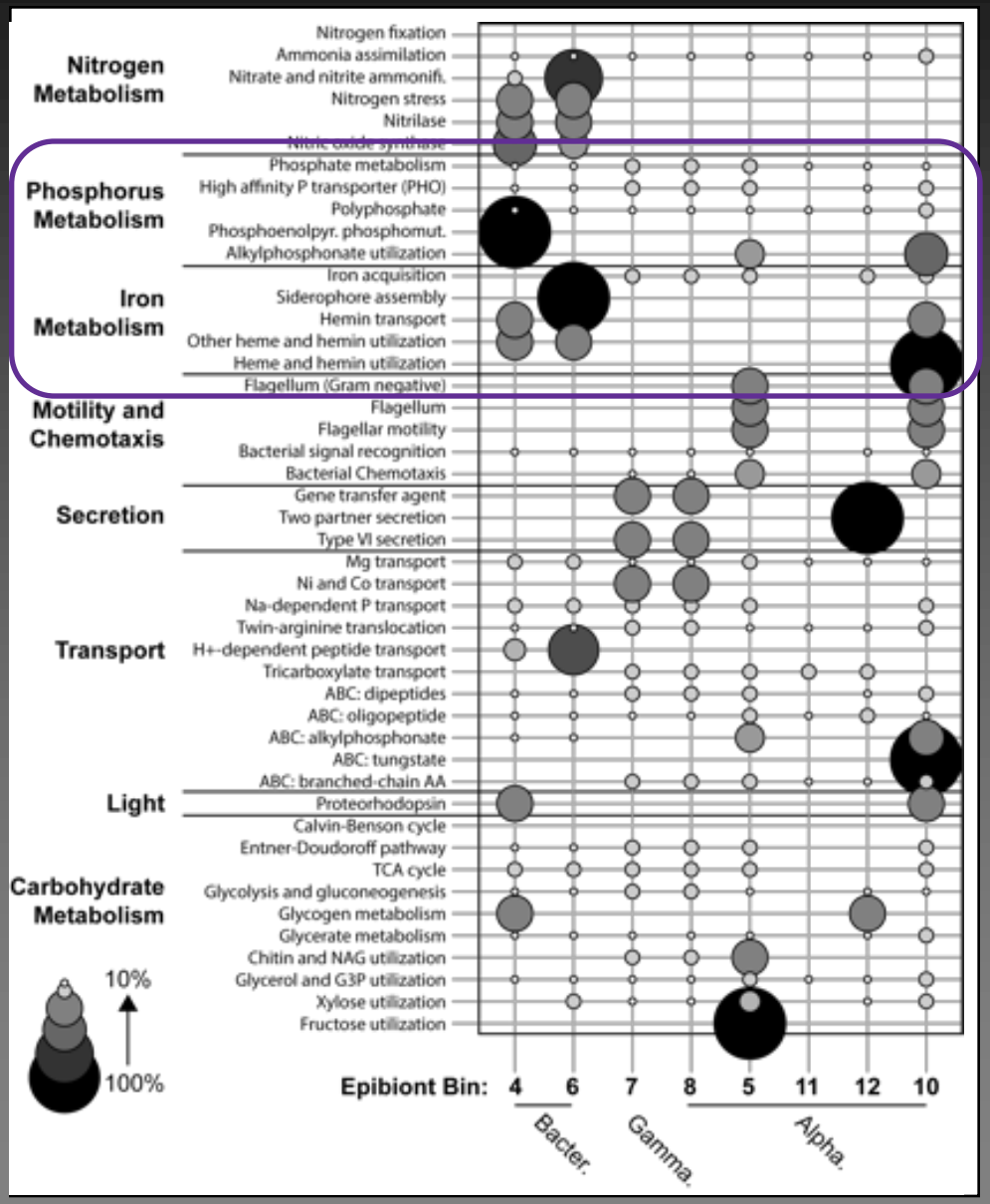


Frischkorn et al. (2017) *ISMEJ*

Variable distribution of functional pathways among epibionts

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

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



Frischkorn et al. (2017) *ISMEJ*

Comparing metabolic potential in the holobiont

Metagenomes

↓

Orthologous group analysis

↙ ↘

Epibionts v. *Trichodesmium*

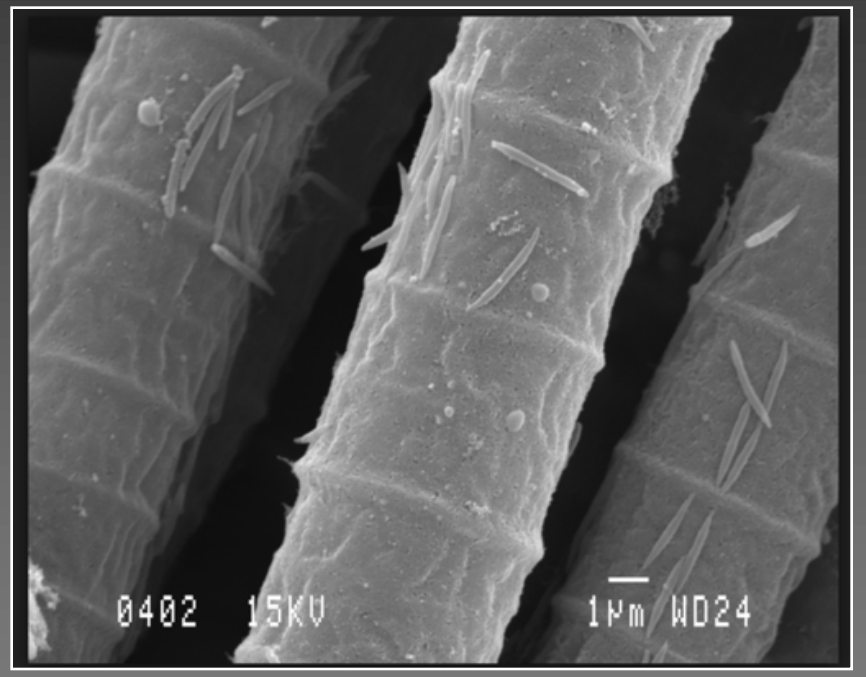
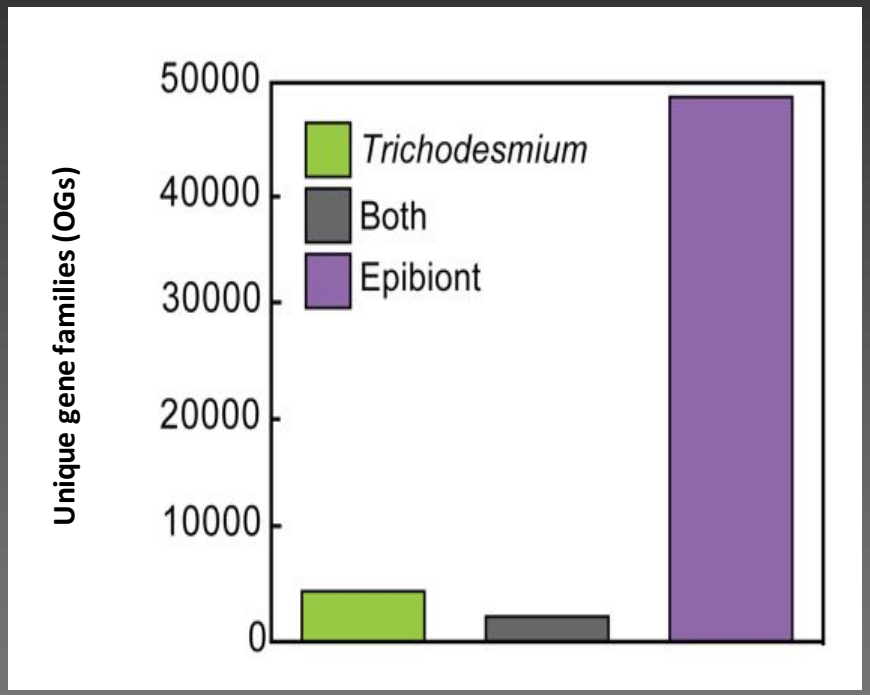
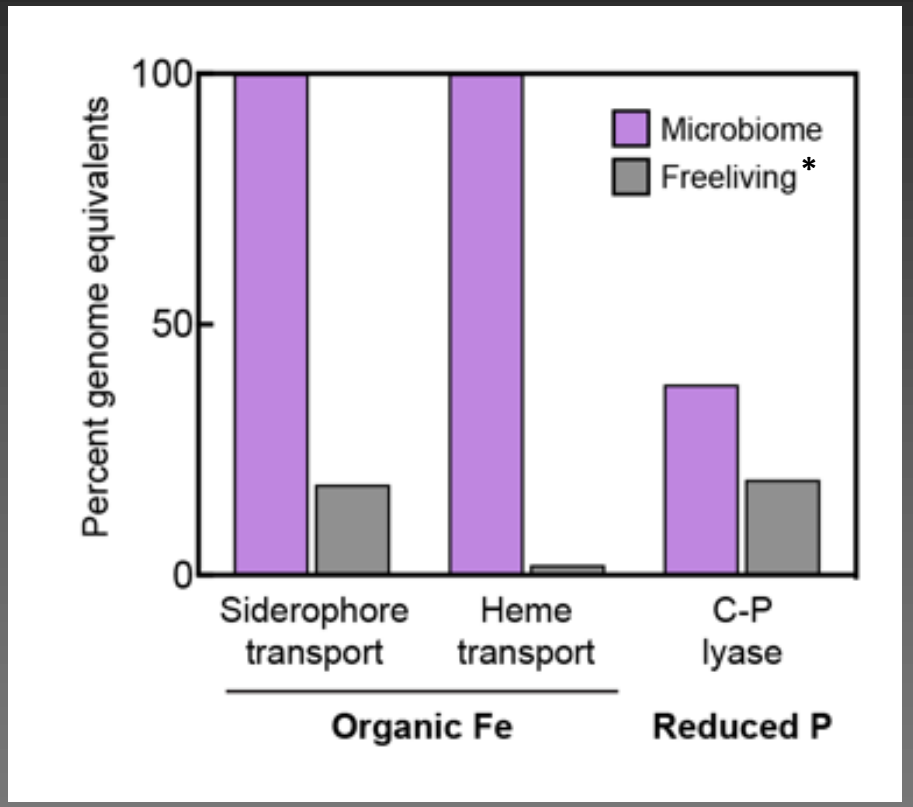


Image courtesy Tracy Mincer

Epibionts confer the majority of the metabolic potential

>90% OGs

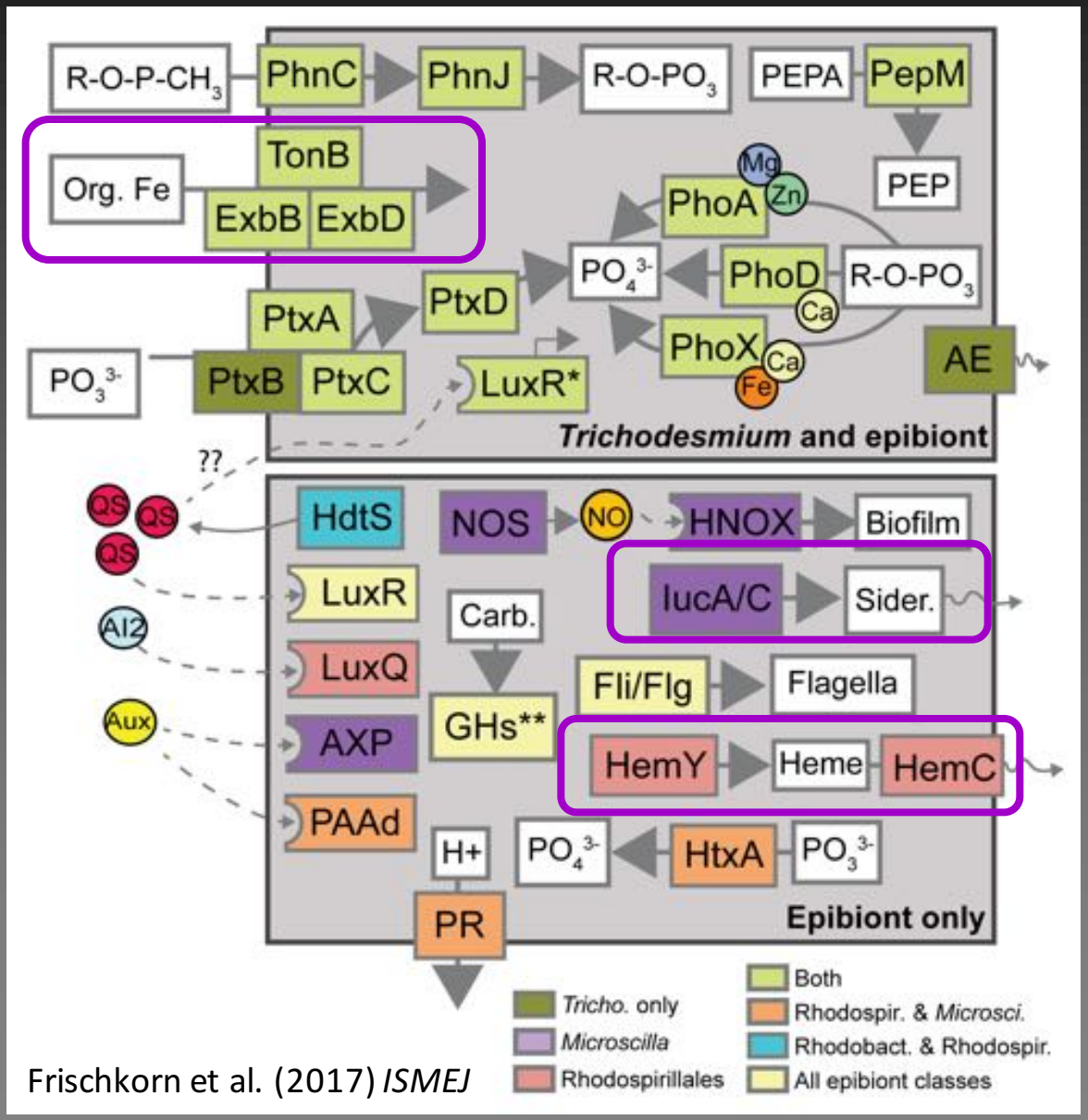




Orthologous (OG) group analysis suggests that epibionts confer the vast majority of metabolic functions to the holobiont.

Epibiont metabolism expands Fe and P functions for the holobiont.

Metabolic partitioning within the *Trichodesmium* holobiont

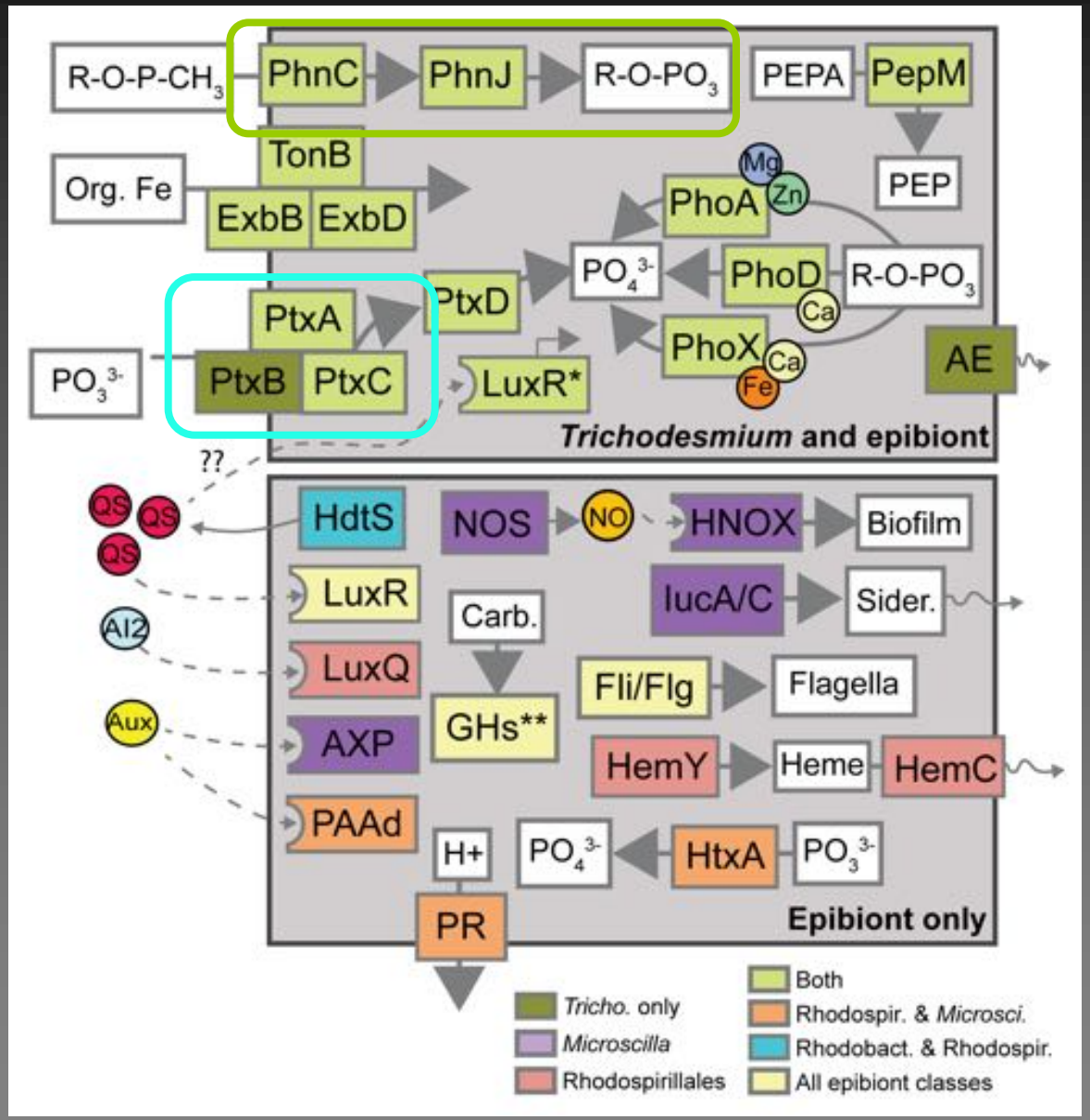


Frischkorn et al. (2017) *ISMEJ*

Organic Iron

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

Uptake and metabolism of reduced phosphorus forms



C-P Lyase
PhoX
PhoA

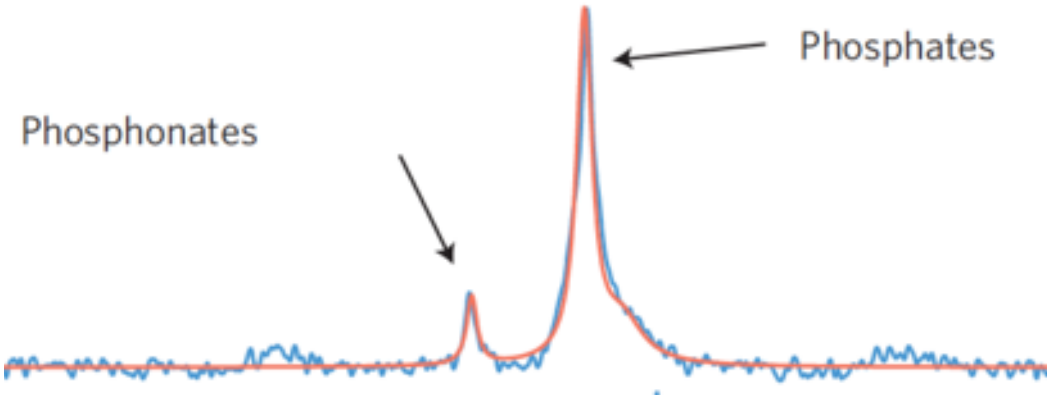
Phosphite uptake

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

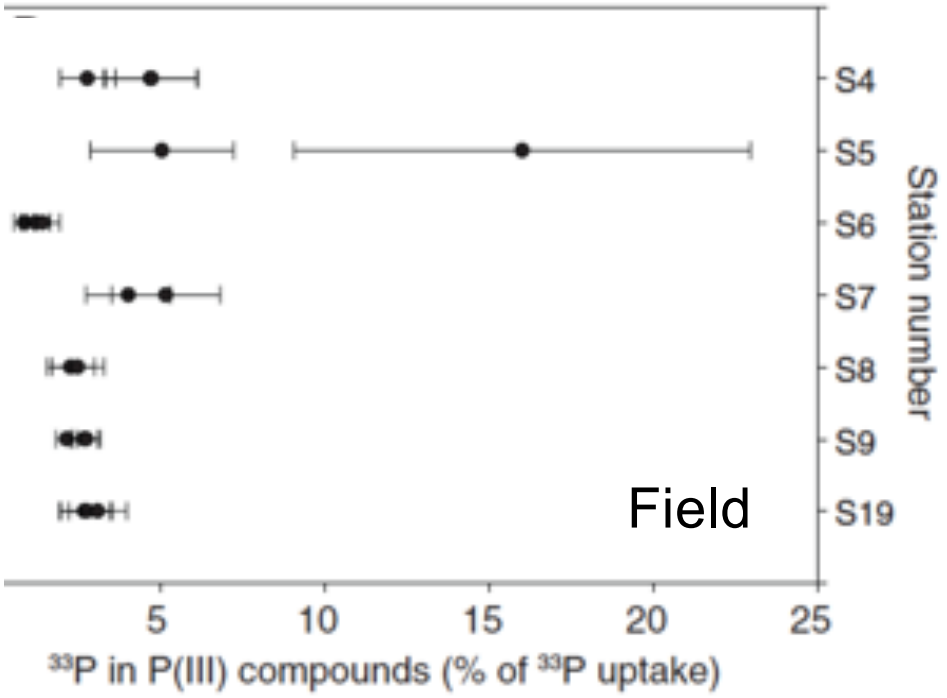
Answers to enduring mysteries… who makes C-P compounds?

Phosphonate (C-P) biosynthesis

Culture



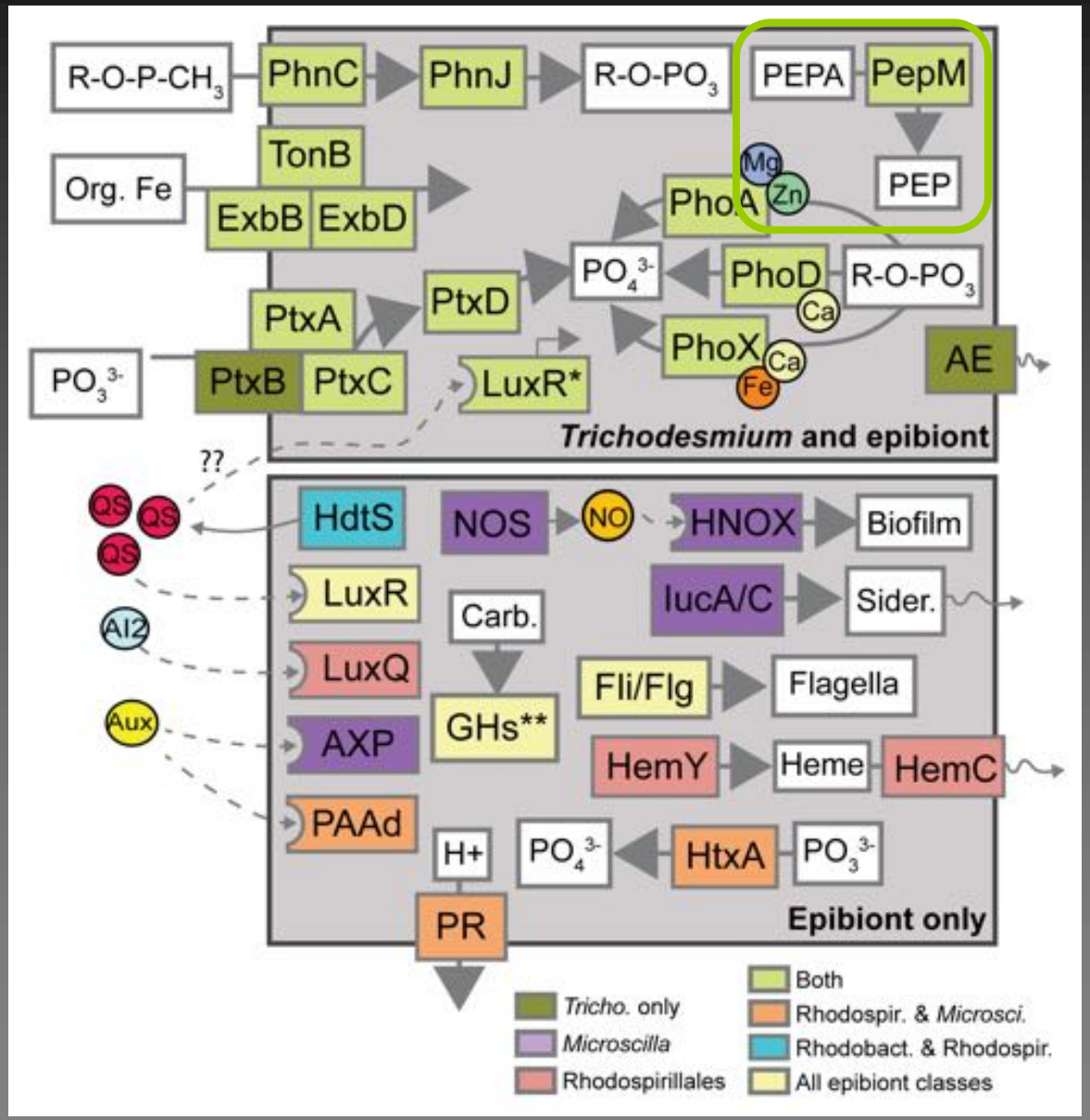
Dyhrman et al. (2009) *Nature Geo.*



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?

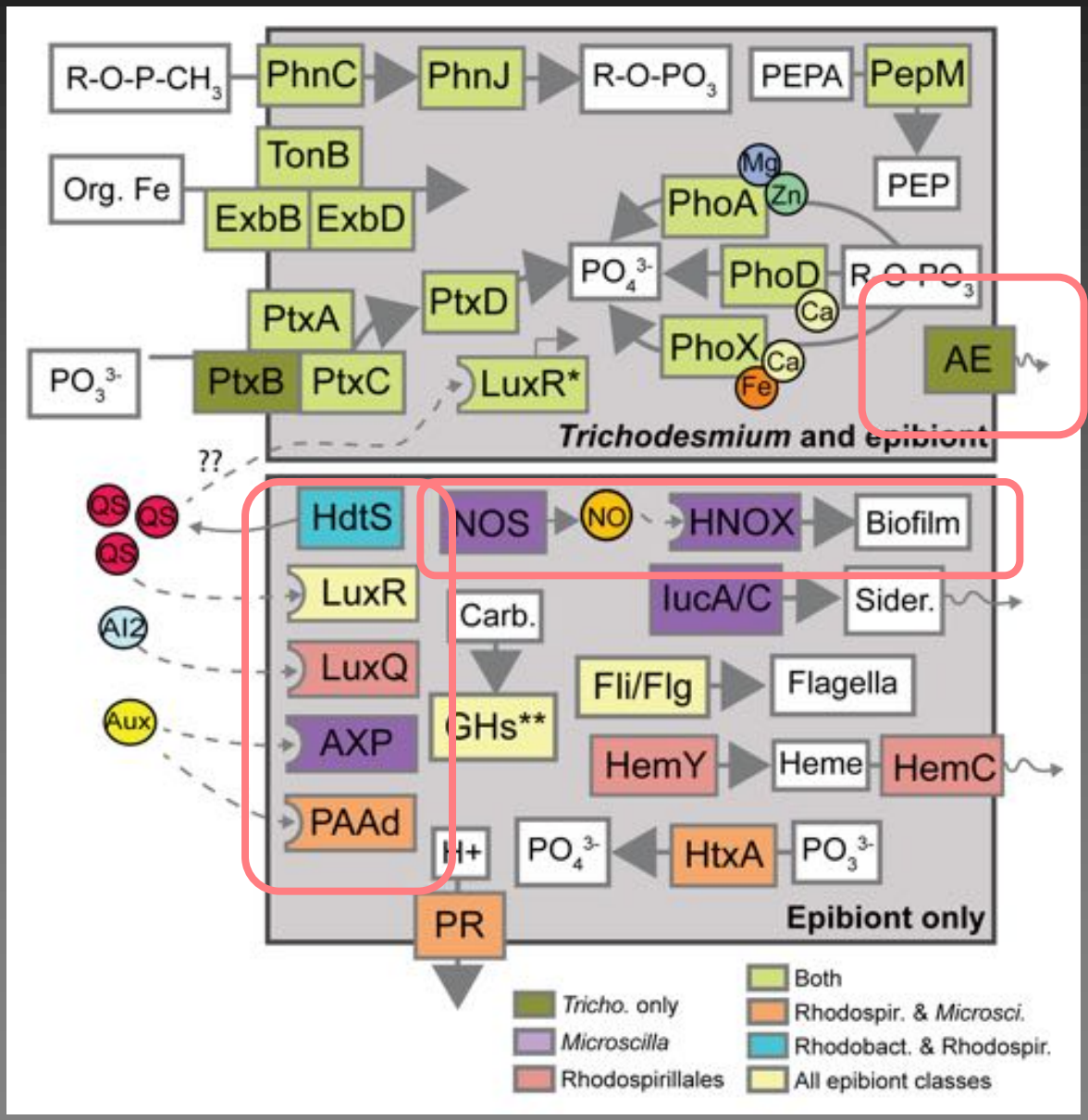
Phosphonate production is a shared metabolism



Phosphonate biosynthesis

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

Microbial cross talk within the *Trichodesmium* holobiont



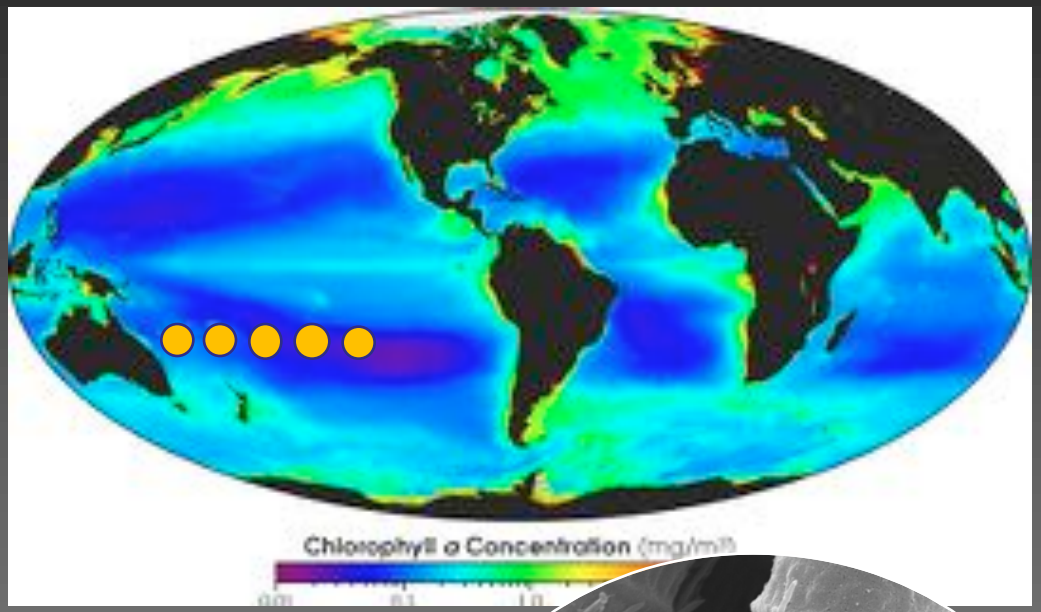
Tracking the interactome… with metatranscriptome profiling after addition of NO and QS molecules

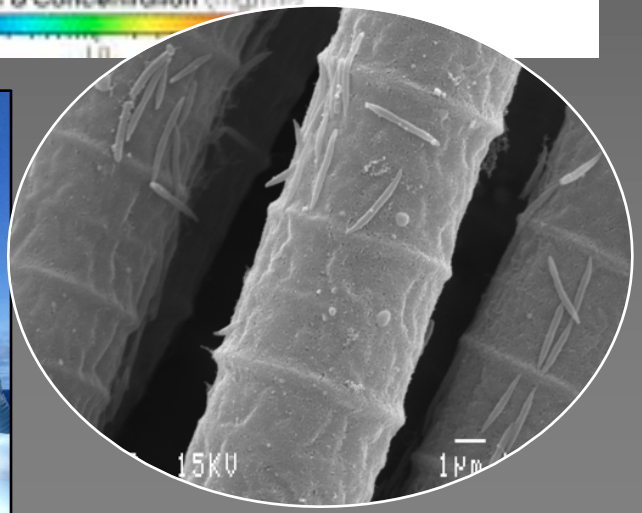
QS and cell signaling

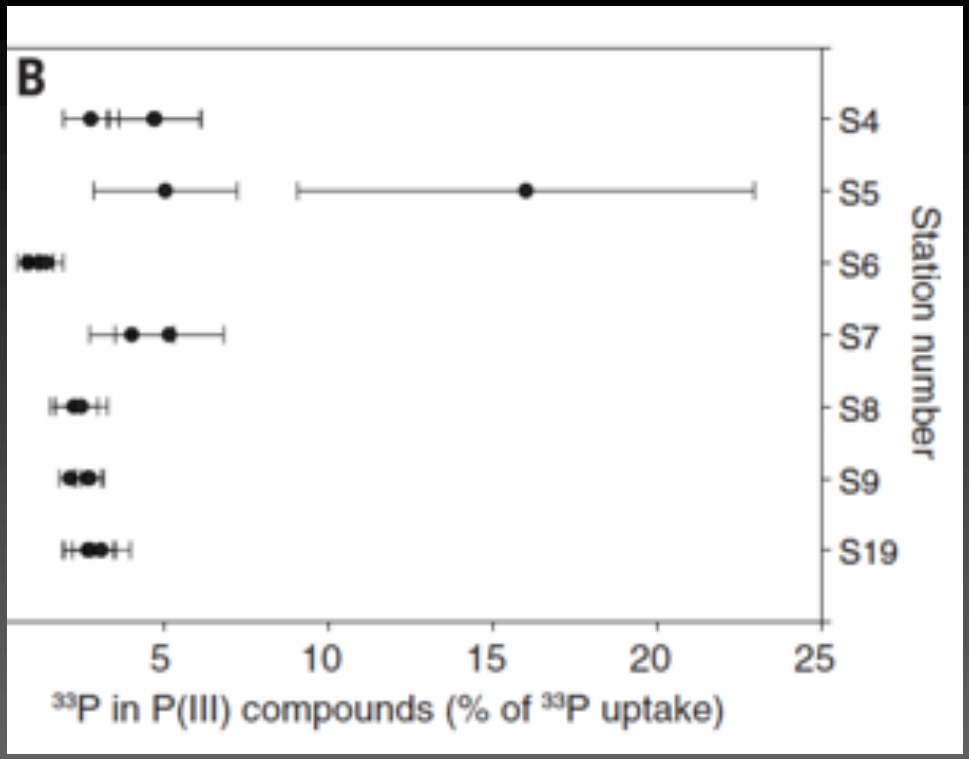
Joint metagenome and metatranscriptome analysis

- 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 phosphorus reduction and cycling in this environment?

OUTPACE



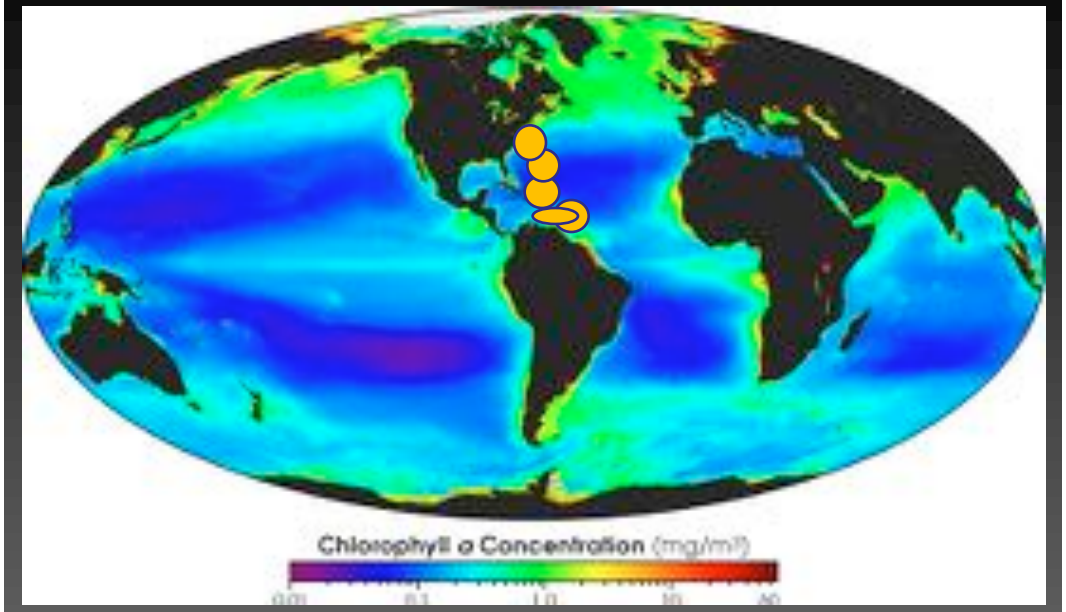




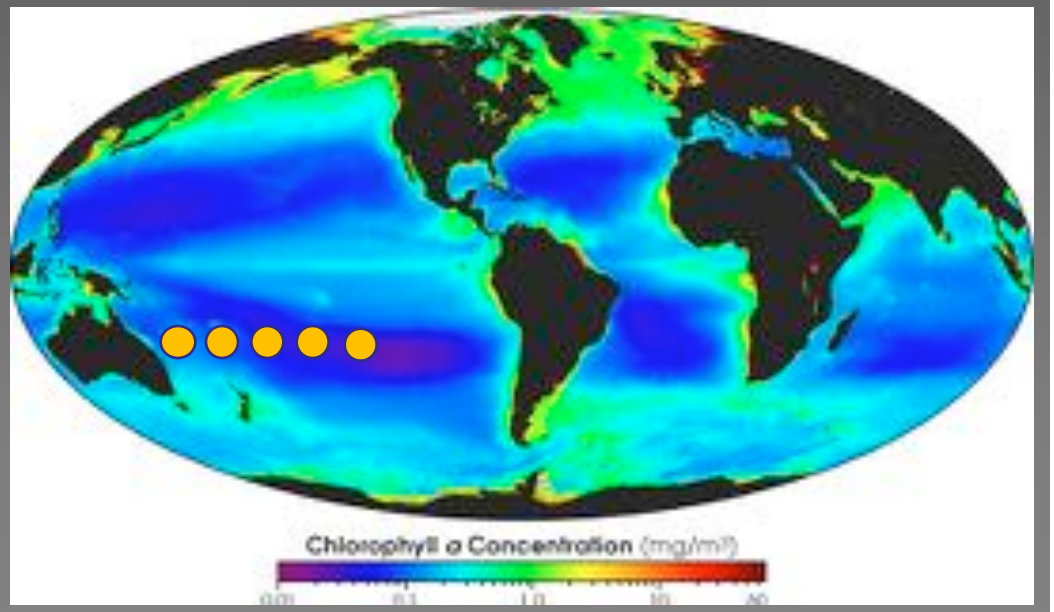
Van Mooy et al. (2015) *Science*

?

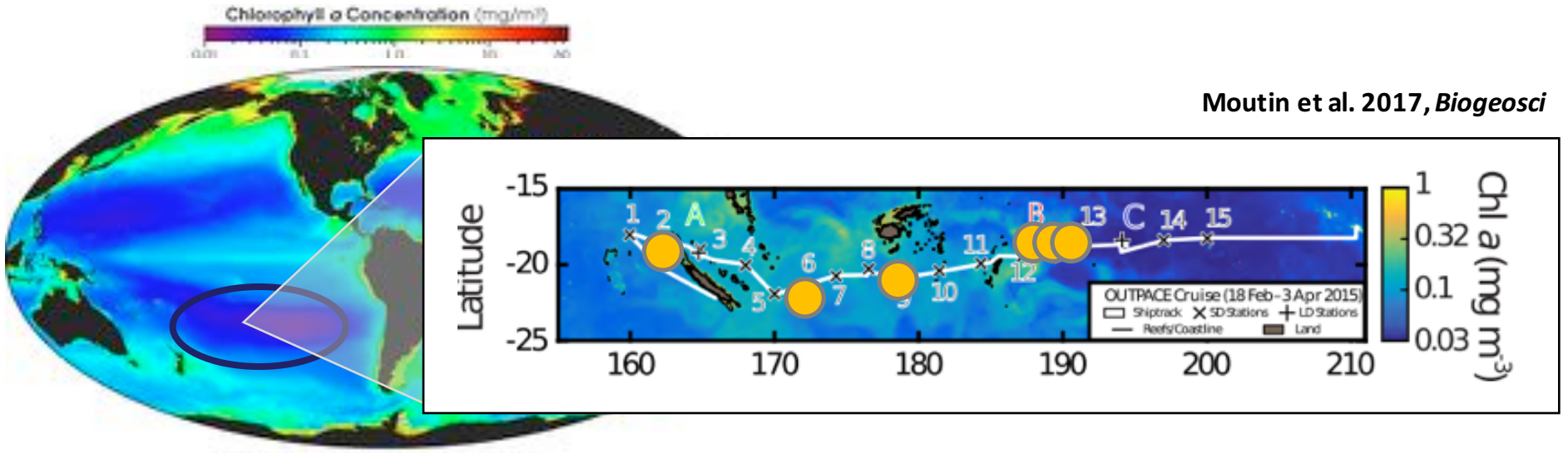
PABST



OUTPACE



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

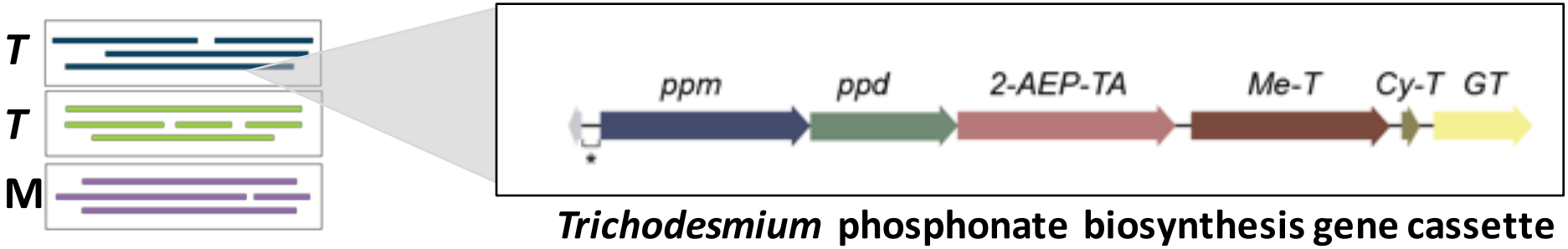


Moutin et al. 2017, *Biogeosci*

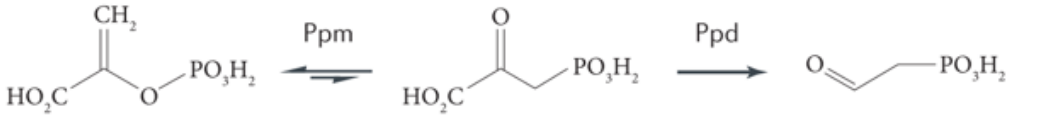
Trichodesmium **samples taken**

Metatranscriptomes

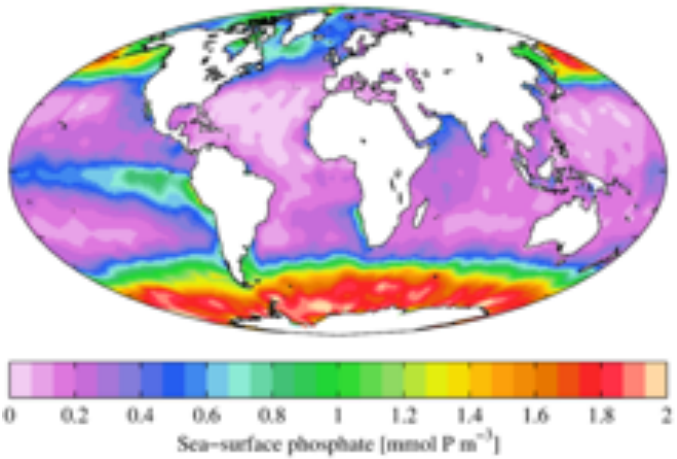
Metagenomic evidence of P reduction



Trichodesmium **phosphonate biosynthesis gene cassette**



Phosphoenolpyruvic acid Phosphonopyruvic acid Phosphonoacetaldehyde



Phosphonates in the oligotrophic ocean

- **25% DOP is in the C-P bond class**
- **Recalcitrant**
- **Mysterious origins**
- ***Trichodesmium*** **colonies are hotspots**

McGrath et al. 2013

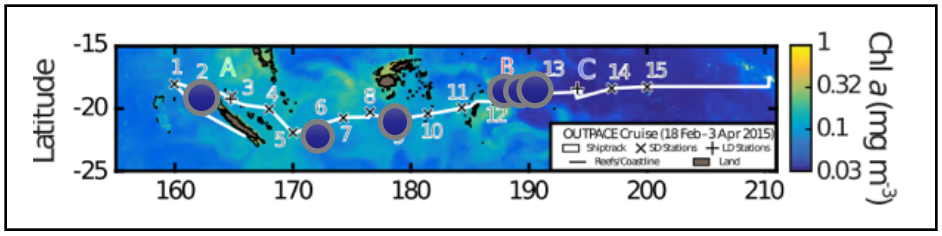
Dyhrman et al., 2006

Van Mooy et al., 2015

Genes are expressed with P reduction

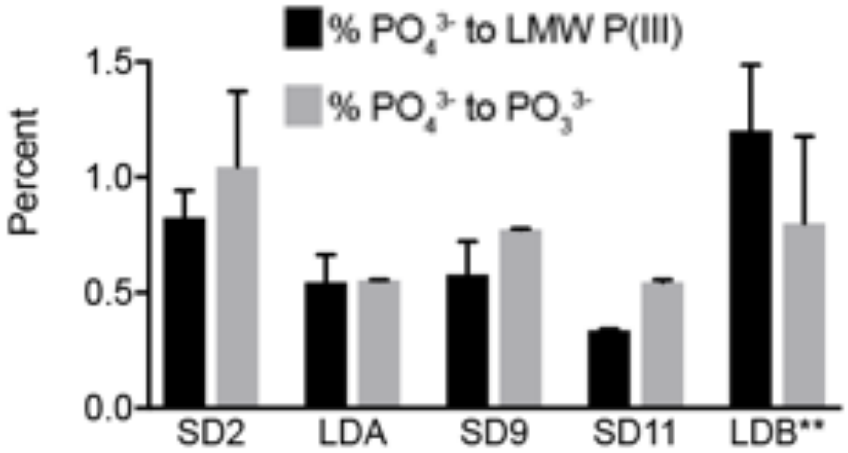
Measure gene expression

Metatranscriptome reads mapped to the P reduction gene cassette



Measure phosphate reduction

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



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

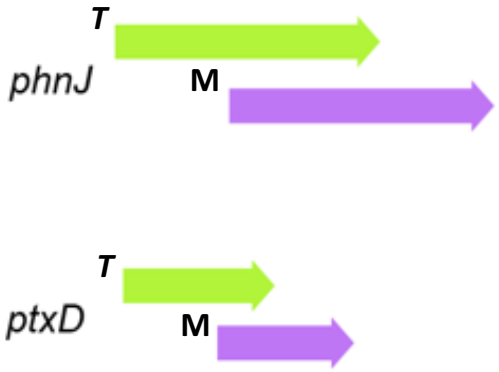
- ✓ **Genes detected**
- ✓ **Genes expressed**
- ✓ **Activity measured**
- ? **Interactions & ecology?**

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

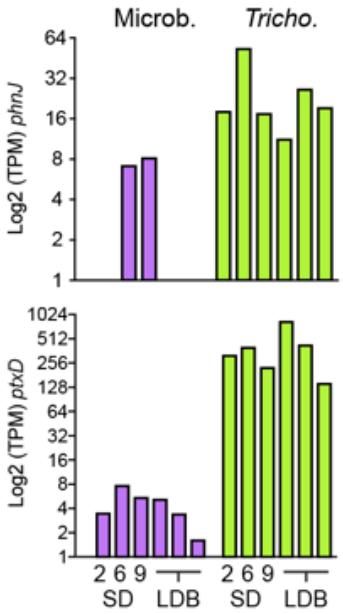
Query for genes:

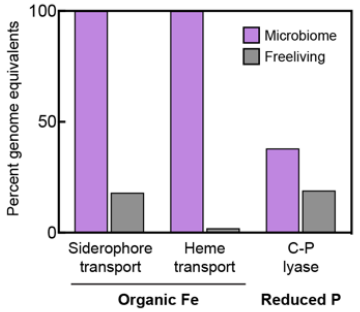
- **Phosphonate C-P lyase (*phnJ*)**
- **Phosphite dehydrogenase (*ptxD*)**

✓ **Genes detected**



✓ **Genes expressed**





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

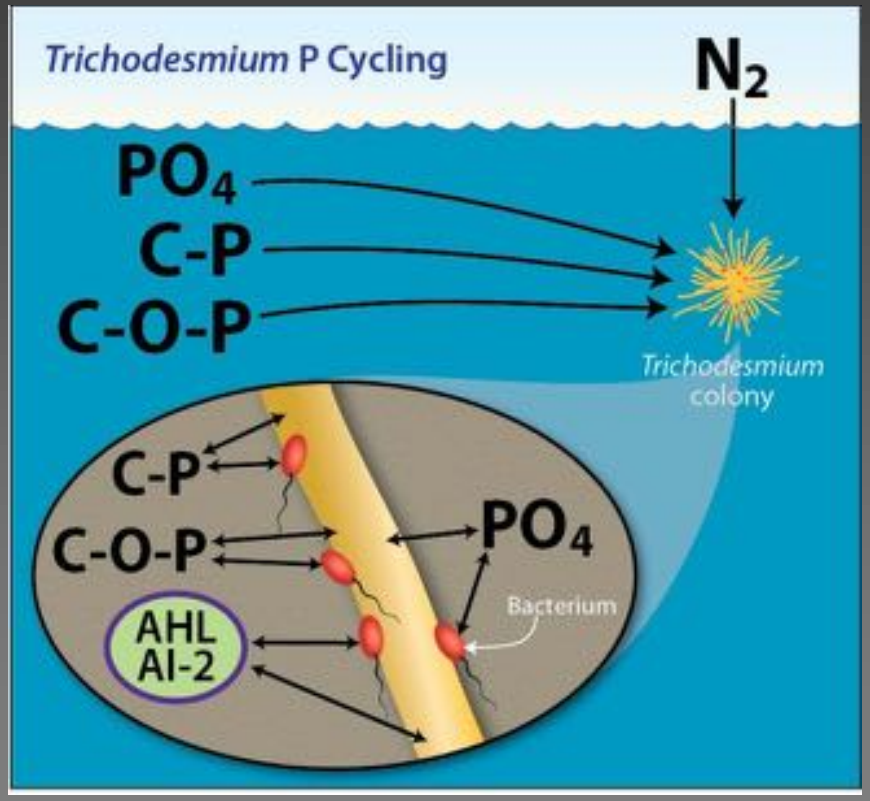
A cryptic P currency?

Frischkorn et al. (2018) *Biogeoscience*

Summary - Host microbiome interactions

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

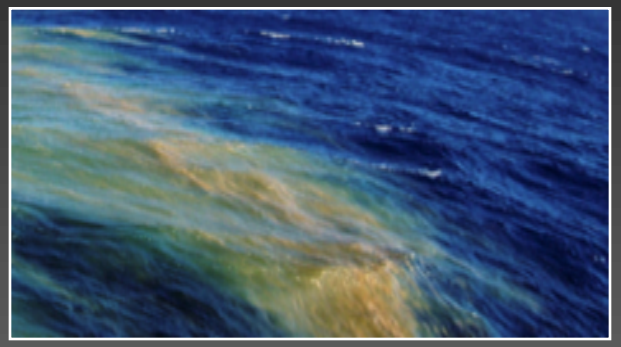
- **16S community amplicon sequencing**: Colonies harbor diverse epibionts distinct from water column, that are dynamically curated across gradients in the environment
- **Metagenomics**: Epibionts confer substantial metabolic potential which likely underpins *Trichodesmium* fitness
- **Metatranscriptomics**: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



Core questions

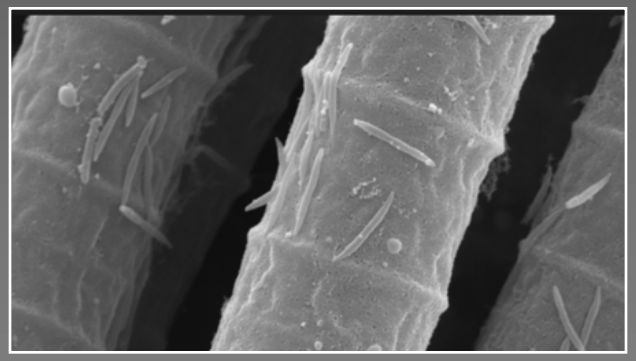
Metabolic traits and trade-offs

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

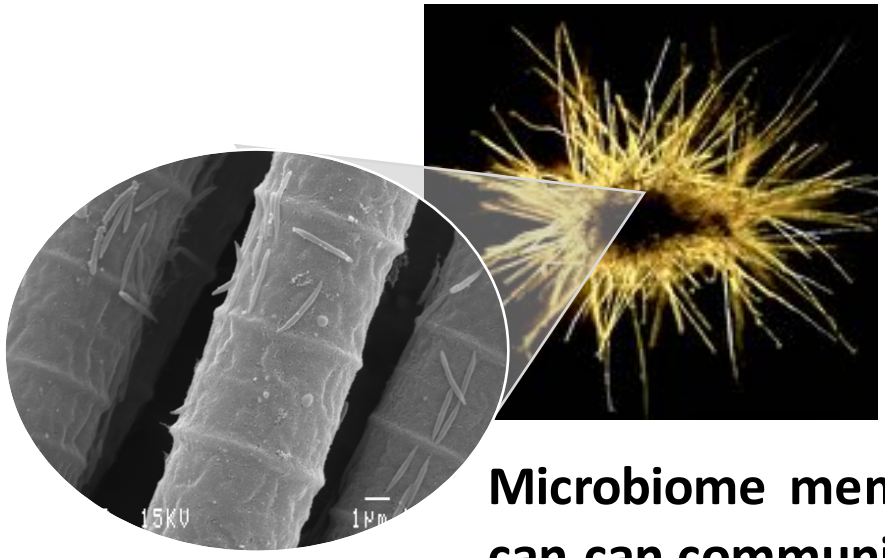


Host microbiome interactions

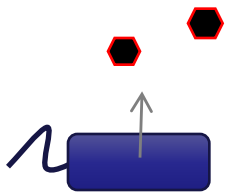
- Who is there? Microbiome diversity
- What are they doing? Microbiome functional diversity, holobiont physiology and **controls on N_2 fixation**

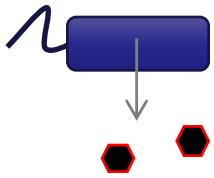


A strategy for assessing the physiological impact of the microbiome on *Trichodesmium* N_2 fixation



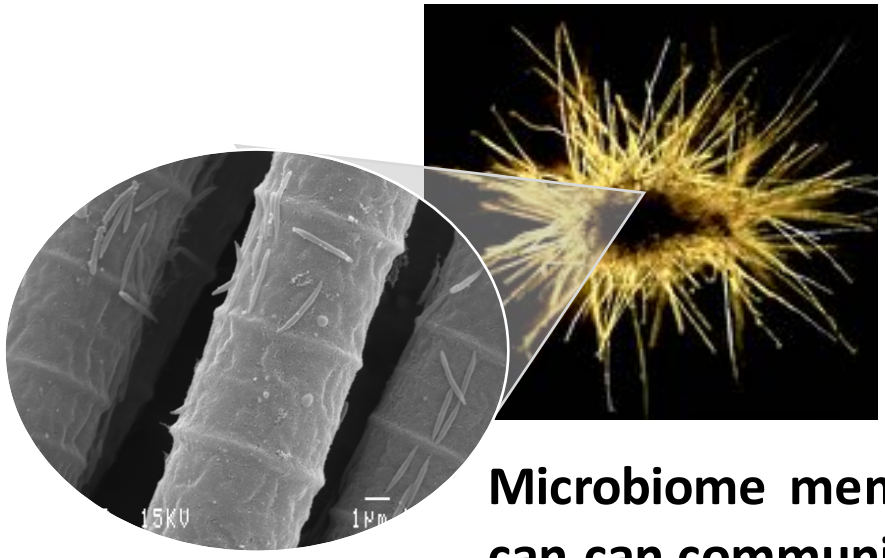
- AHLs





Microbiome members can can communicate using a mechanism called quorum sensing

A strategy for assessing the physiological impact of the microbiome on *Trichodesmium* N_2 fixation



- AHLs

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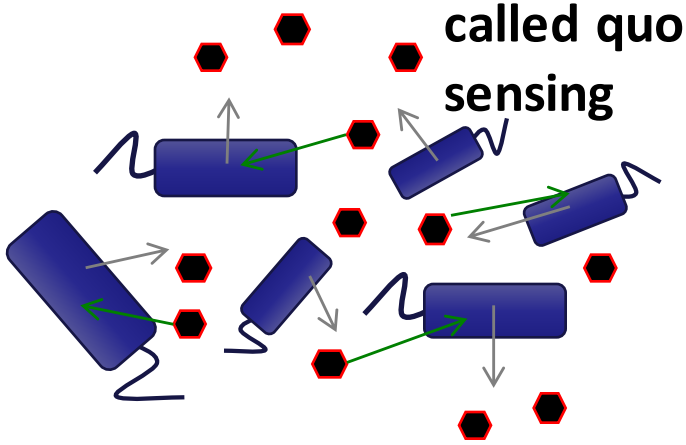
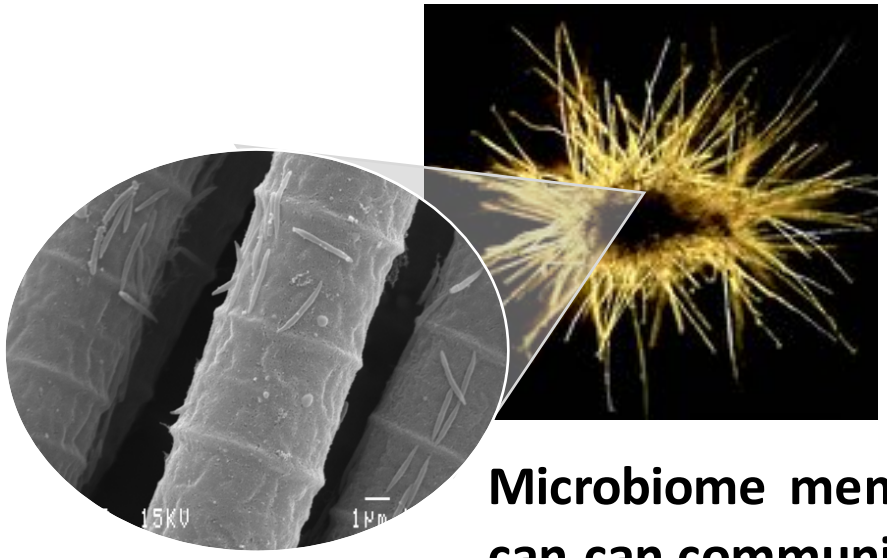
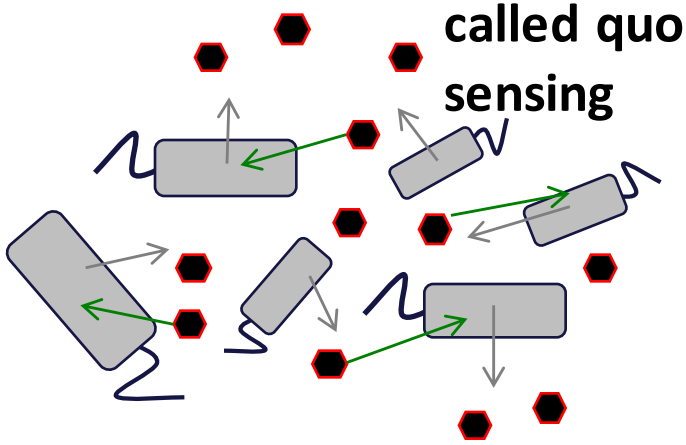


A strategy for assessing the physiological impact of the microbiome on *Trichodesmium* N_2 fixation

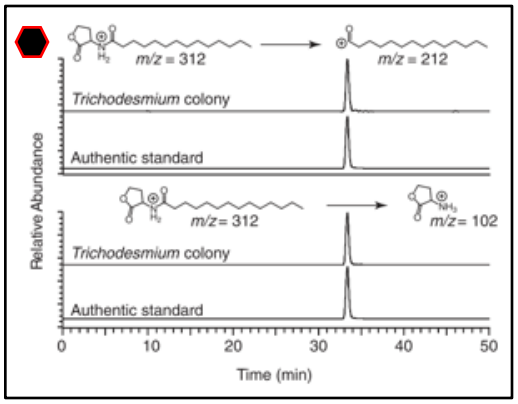


● AHLs

Microbiome members can can communicate using a mechanism called quorum sensing



AHLs found in colonies



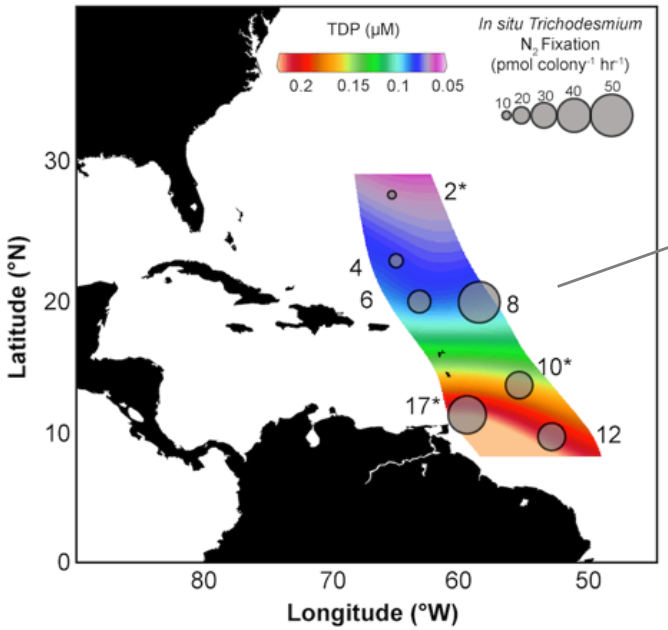
Van Mooy et al., 2012

******Trichodesmium* can't "hear" QS molecules*****

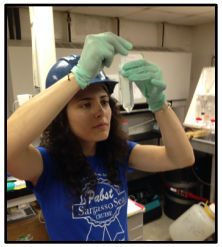
↓

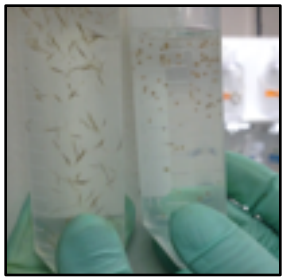
AHLs can be used to selectively modify microbiome behavior

Selectively modifying the *Trichodesmium* microbiome with AHLs in the North Atlantic

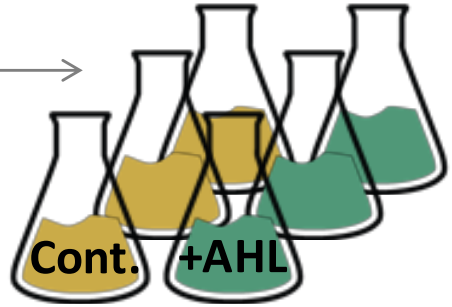


Isolate *Trichodesmium*





Incubate with a cocktail of AHL molecules

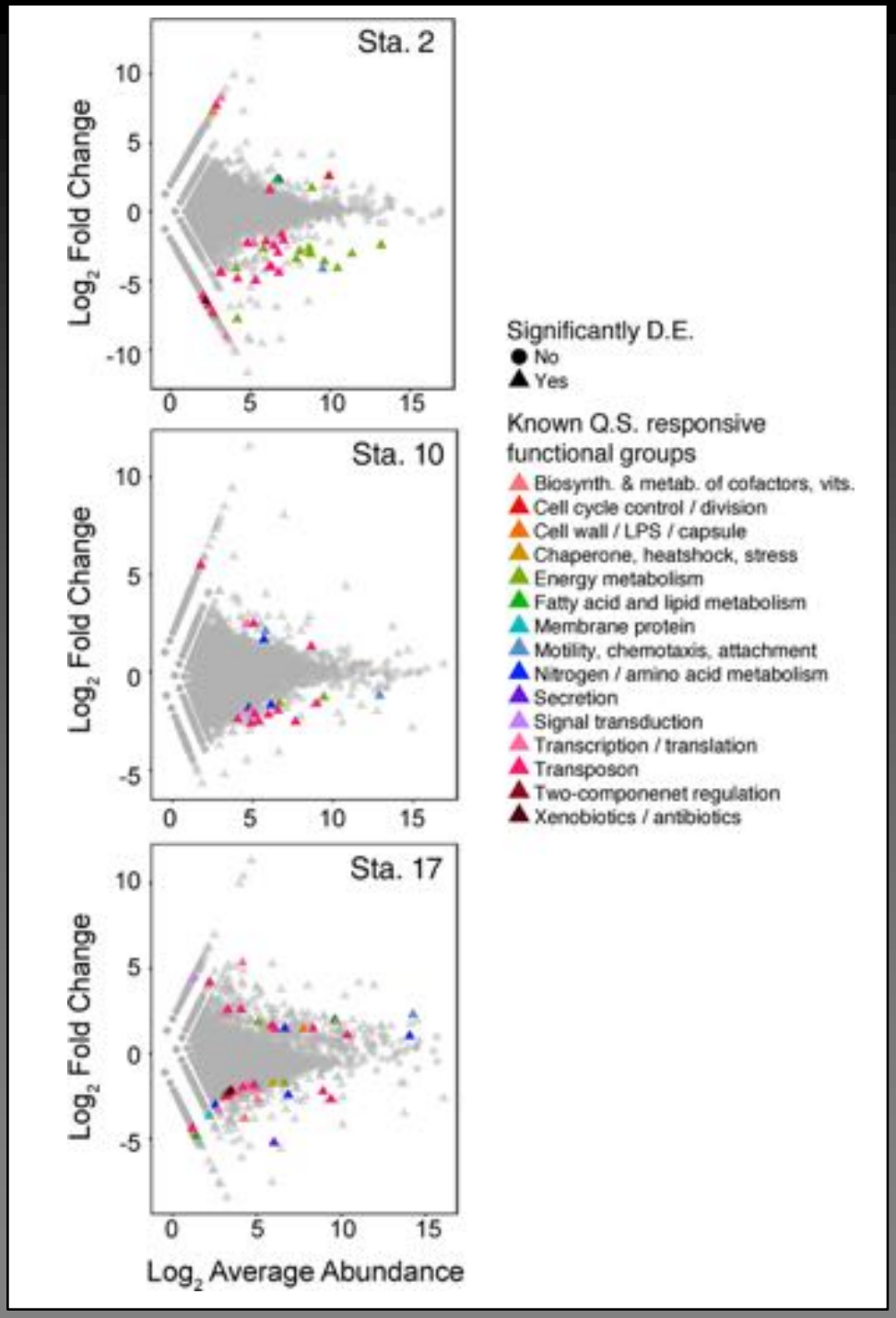


Does AHL addition influence microbiome gene expression?


AHLs t=0
LIPIDS t=0
AHLs t=0
Lipids t=0
KP HYAA
2543
BOX 1 of 18

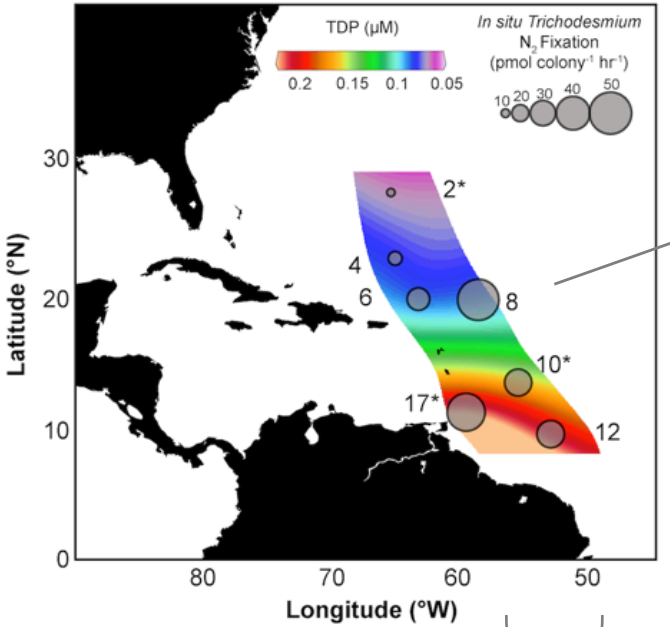

STAR WARS STAR WARS STAR


I
PO
4



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

Microbiome modulates N_2 fixation



Isolate *Trichodesmium*

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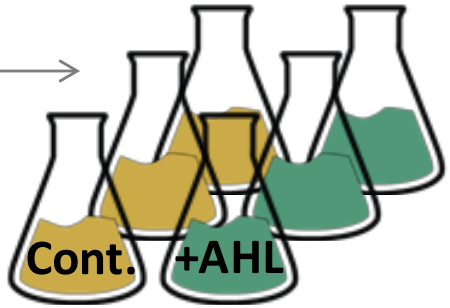
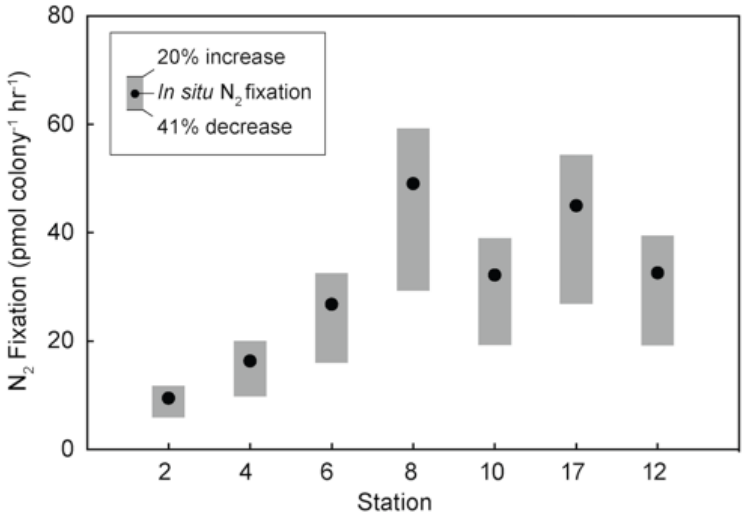
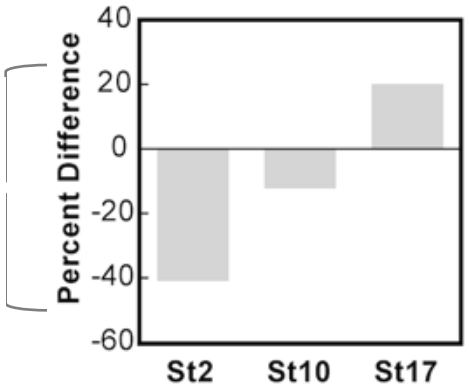
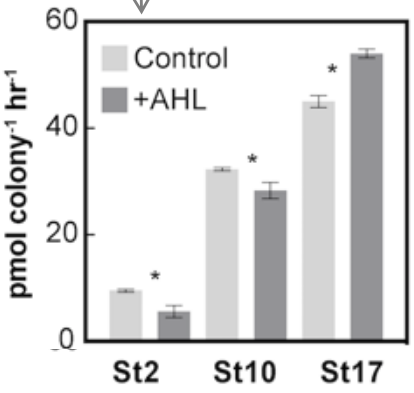






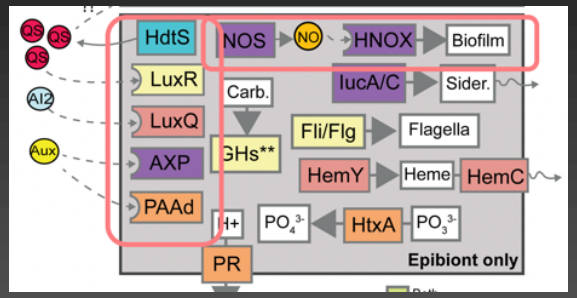


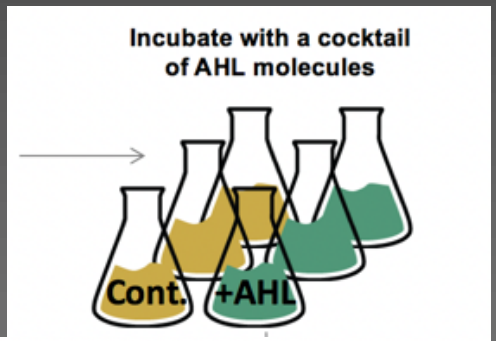
Frischkorn et al. (2018) *L&O Letters*

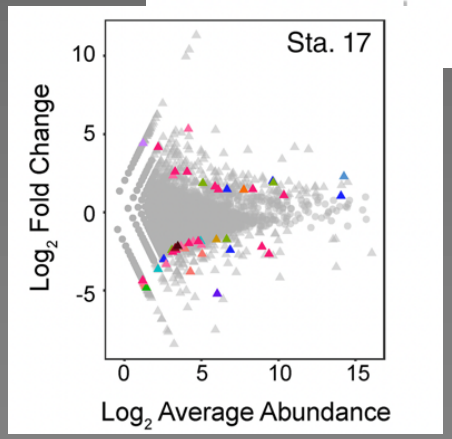
Summary – host microbiome interactions focus on N_2 fixation

Can the microbiome modulate host N_2 fixation?

- **Metagenome**: *Trichodesmium* can't "hear" AHLS, while the microbiome can
- **Metatranscriptome**: AHL addition induces known quorum sensing responses and shifts host N_2 fixation
- Understanding the mechanisms that underlying interactions will help models and predictions of *Trichodesmium* distribution and N_2 fixation



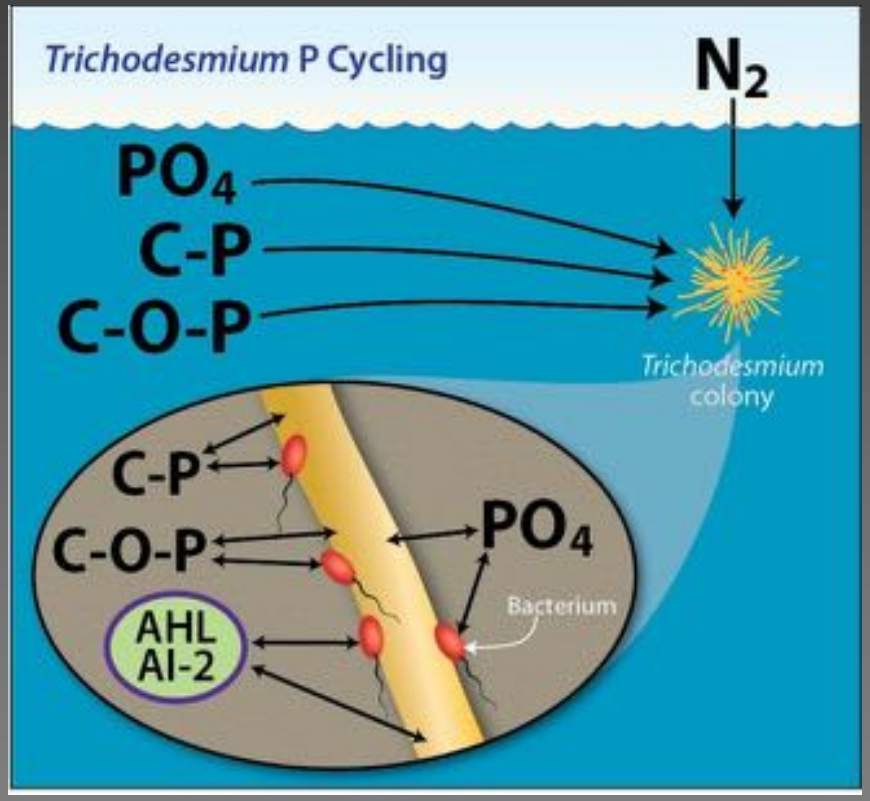




Summary - Host microbiome interactions

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**: Distinct physiology and 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 (Mulit-omics)