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

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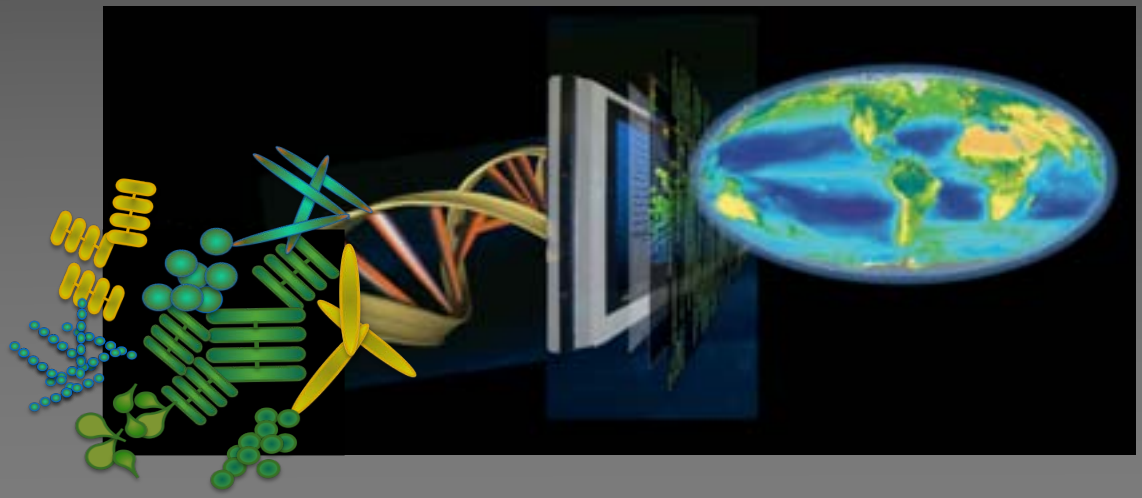

COLUMBIA UNIVERSITY
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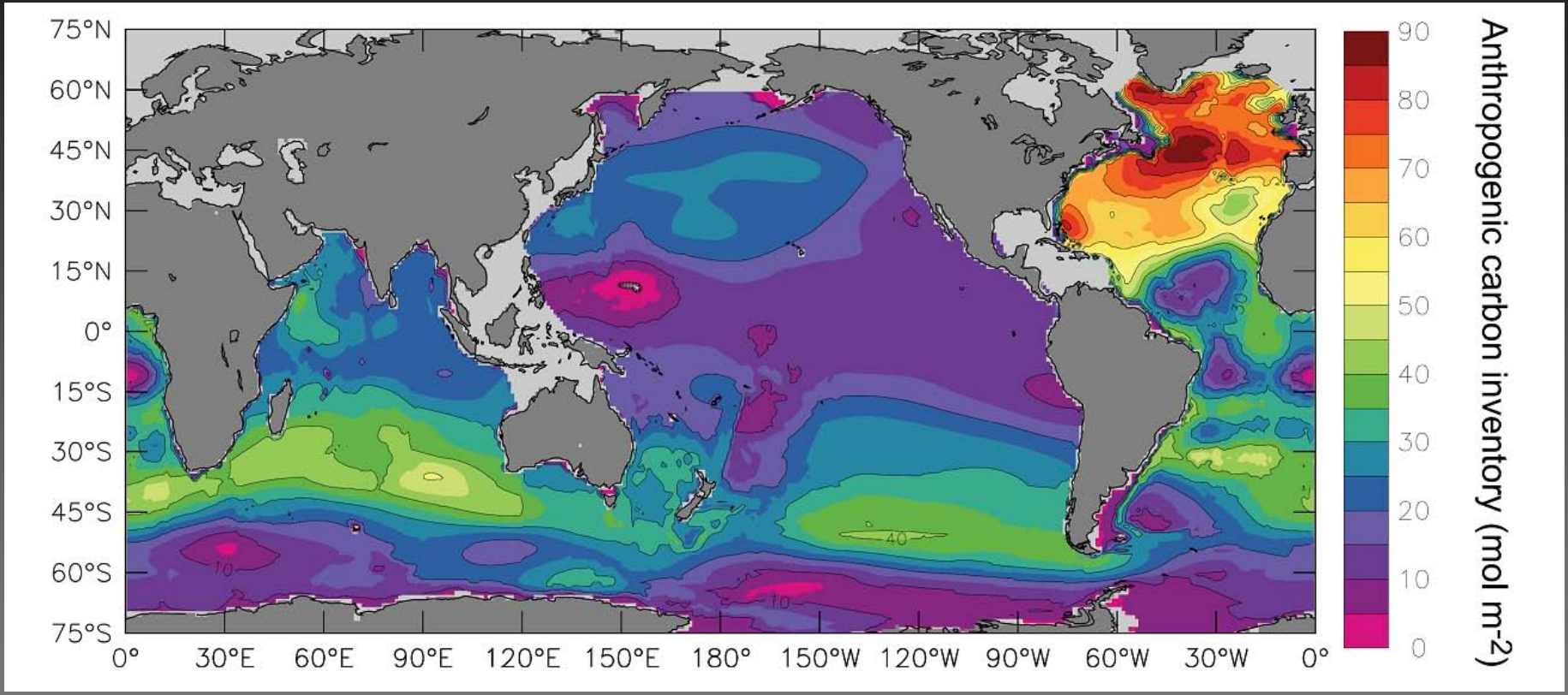
EARTH OBSERVATORY

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

Microbial biogeochemistry - 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*

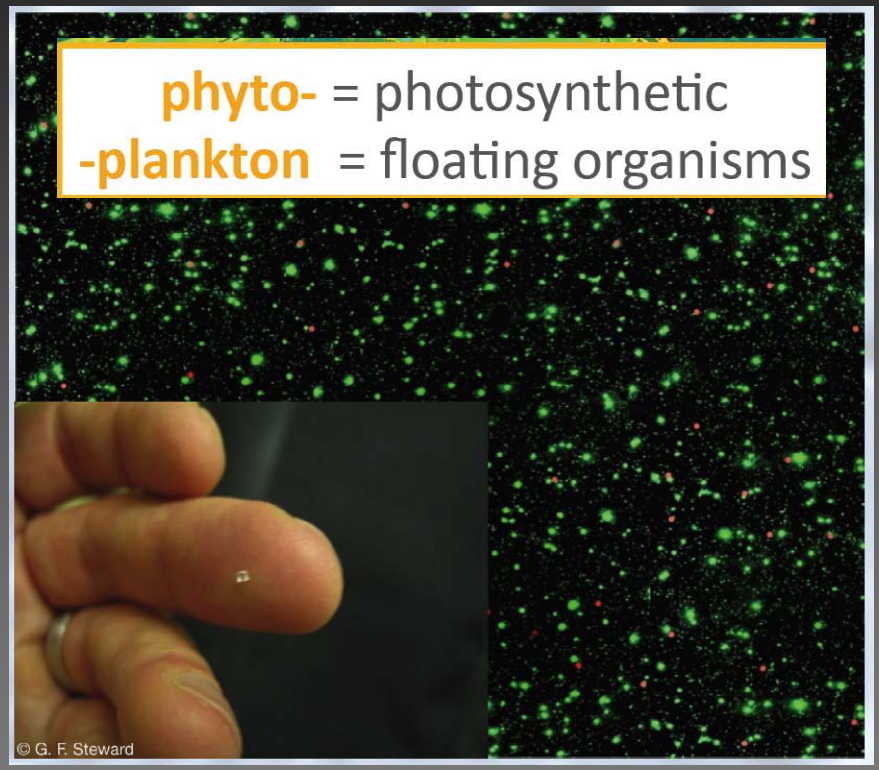
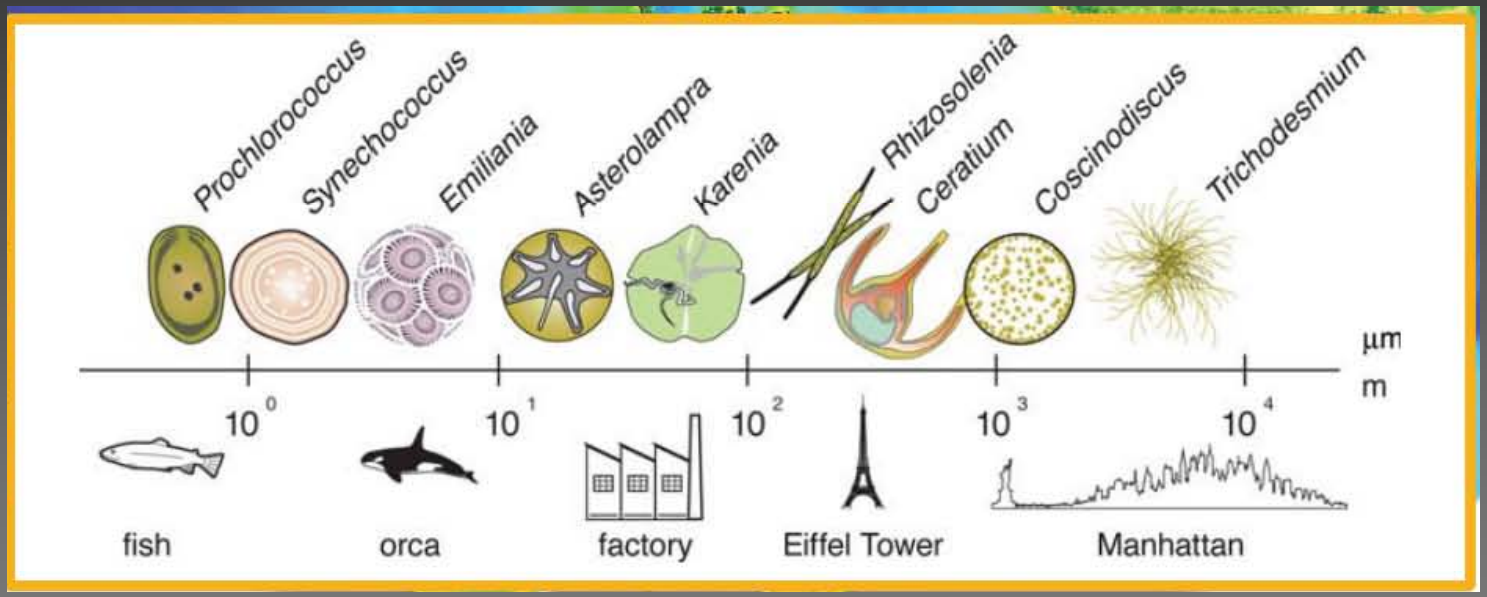
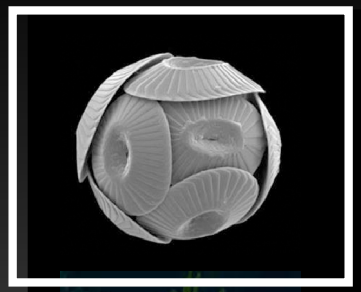
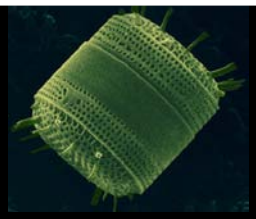


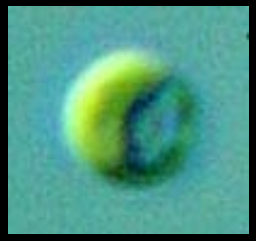
Image courtesy C-MORE

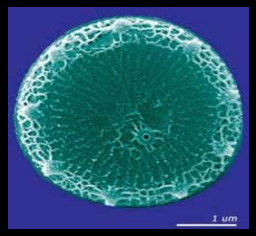
Marine phytoplankton are highly diverse







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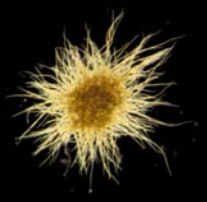
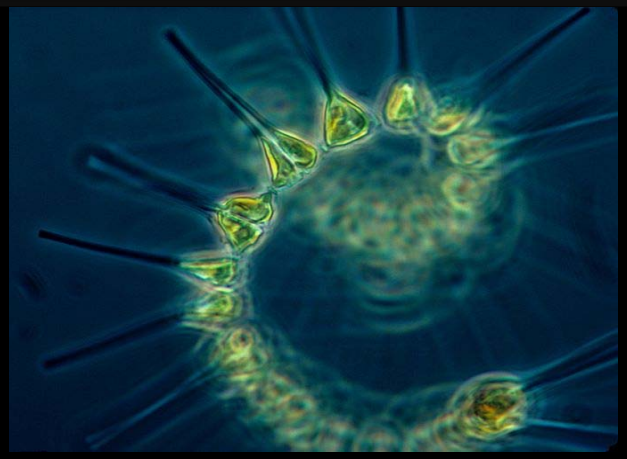
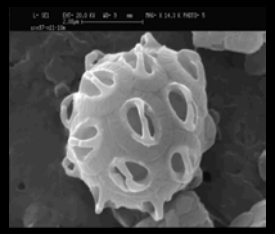
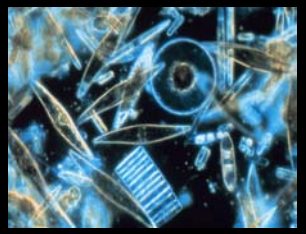
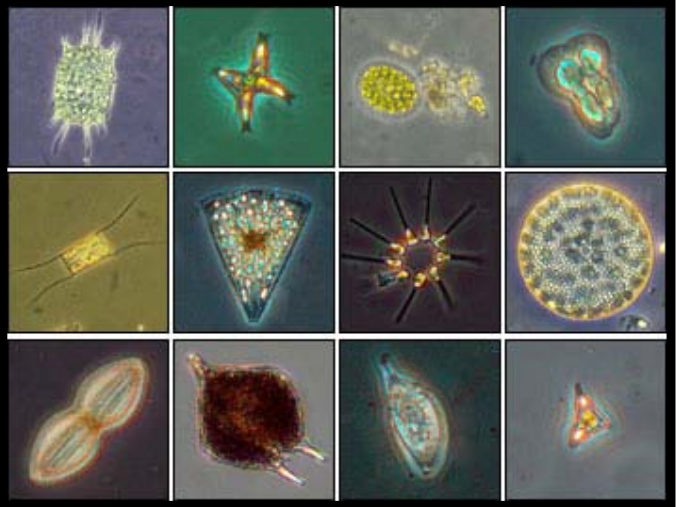
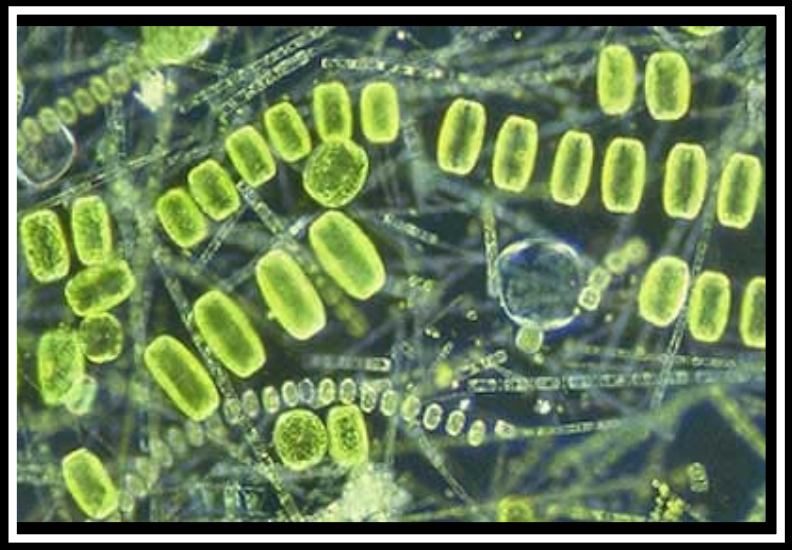






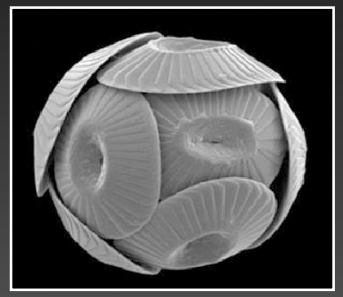






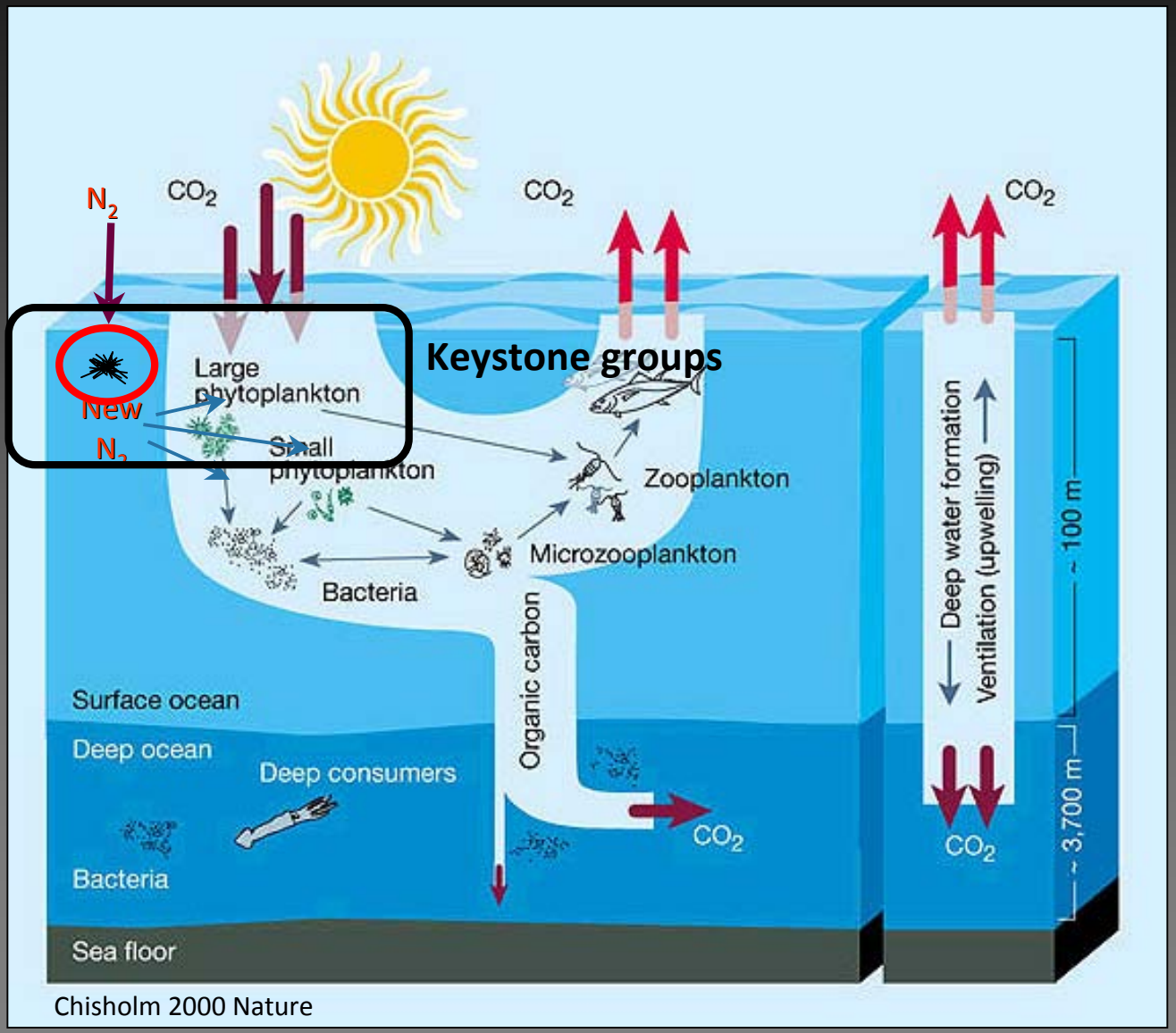
Focus on keystone groups

Haptophytes



Diatoms

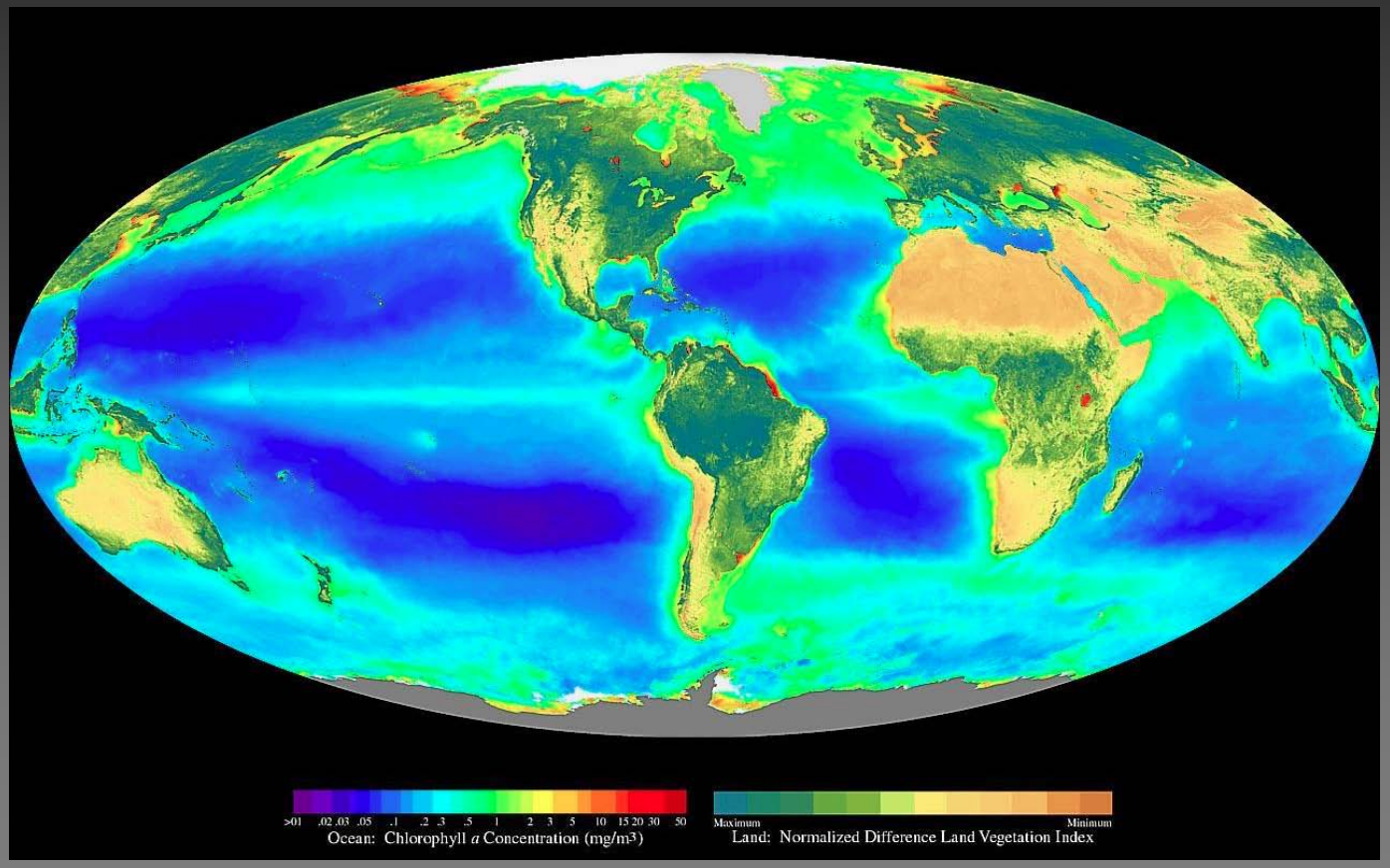
N_2 Fixers



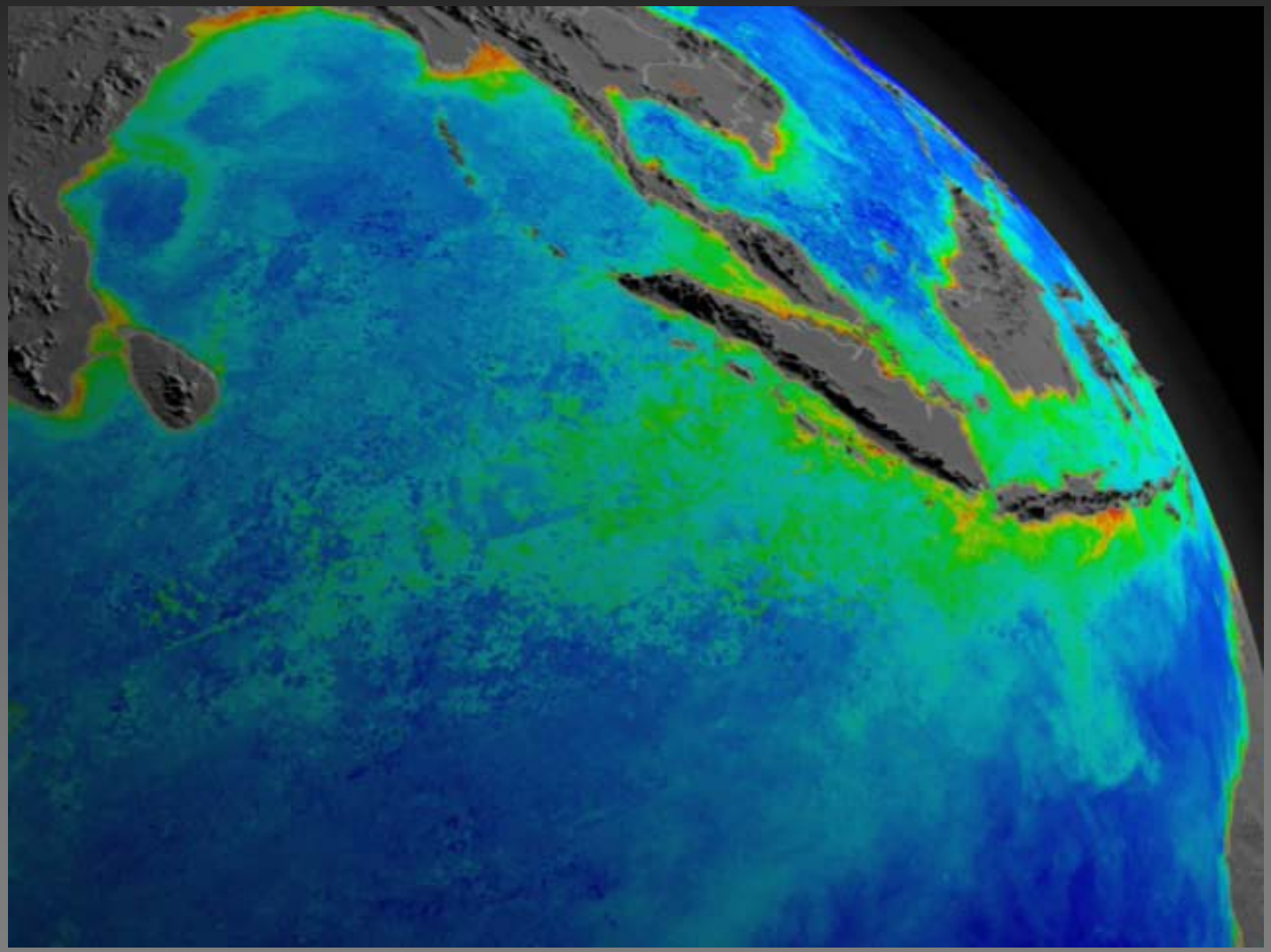
Chisholm 2000 Nature

Phytoplankton play a profound role in the earth system

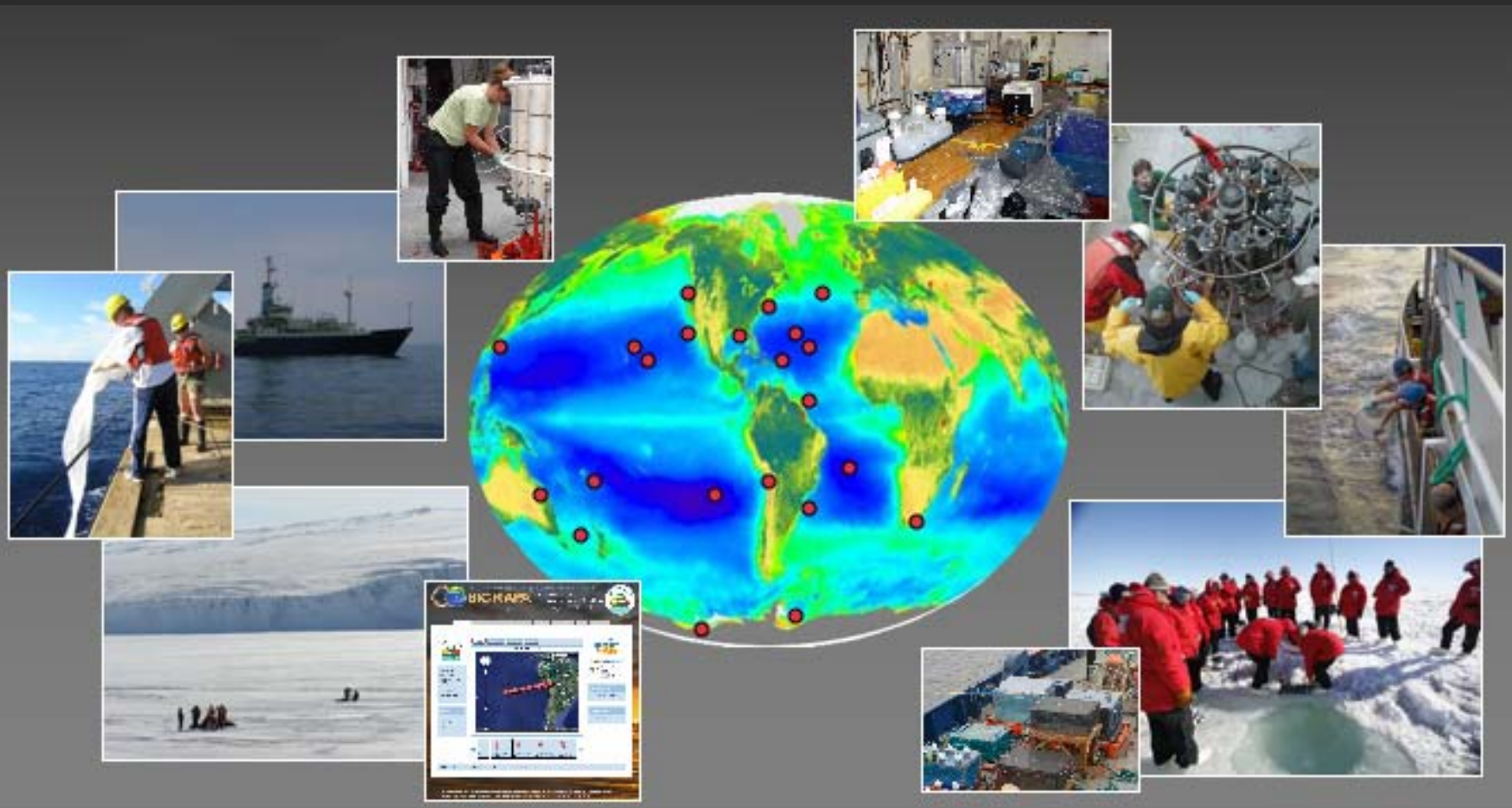
Half of global primary production



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



Sampling microbes across marine ecosystems



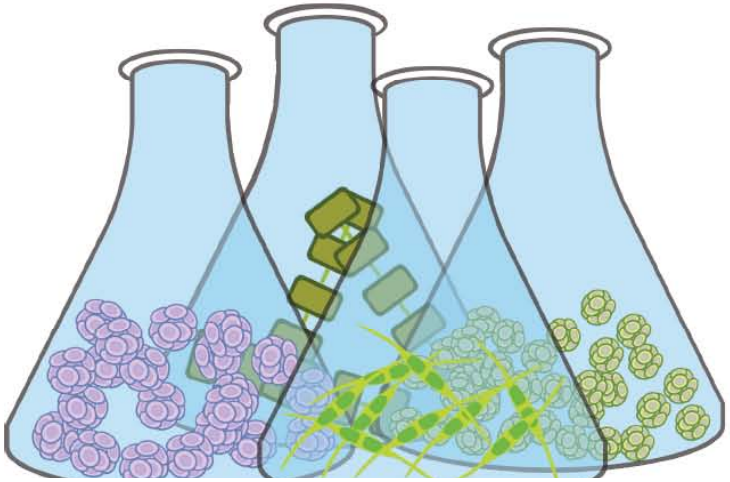
Culture and field based approaches to physiological ecology

Culture-based experiments

Species-specific responses to well-controlled environment

Limitations:

- Species must be in culture
- Time consuming
- Extrapolations to the field

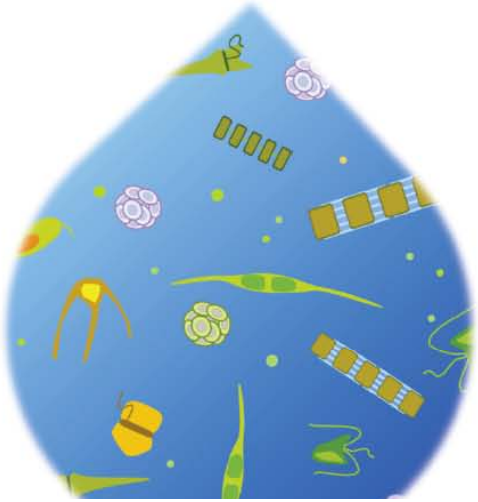


Field-based studies

Assess whole community dynamics in a natural environment

Limitations:

- Not species-specific



Genome and transcriptome-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

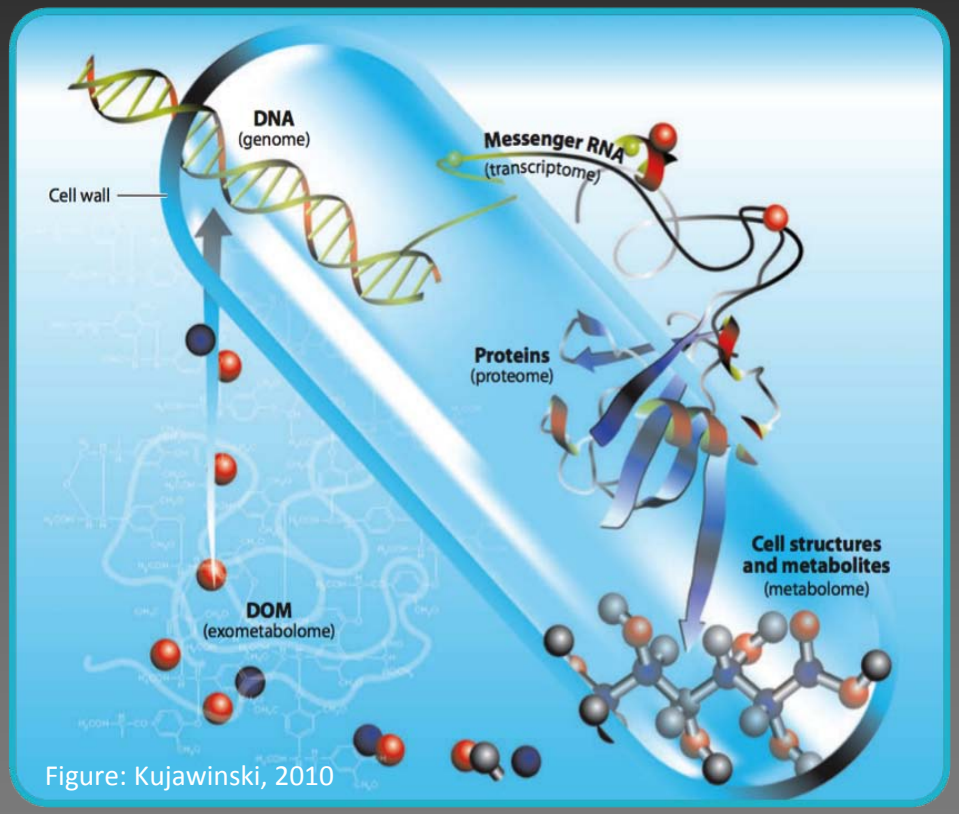


Figure: Kujawinski, 2010

Increasingly able to use 'omic and 'metaomic approaches!

Leveraging 'omic data to study marine microbes

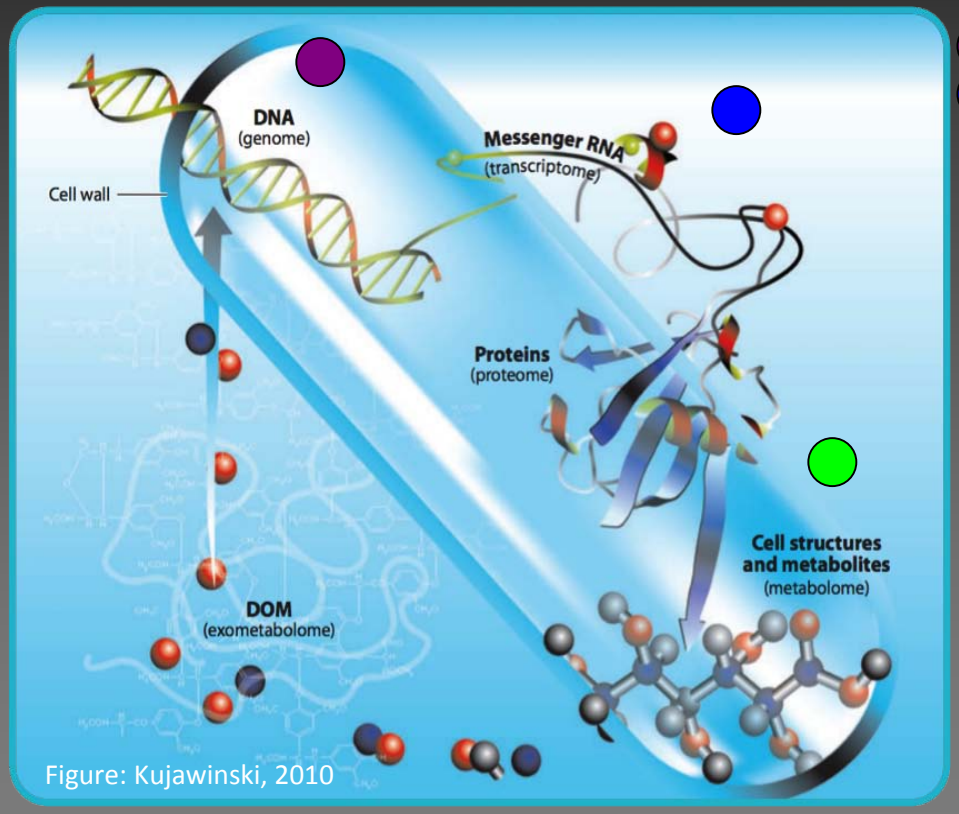


Figure: Kujawinski, 2010

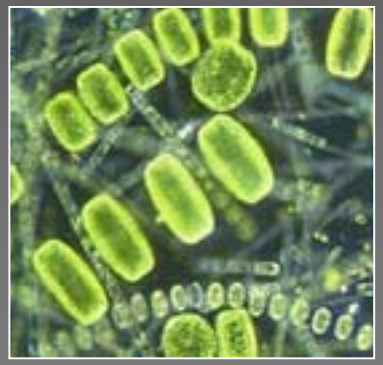
- **Taxonomic Diversity:** Who is there?
- **Metabolic capacity:** What are the molecular underpinnings of resource metabolism?
- **Metabolic plasticity:** How are those pathways regulated?
- **Functional diversity:** How are the pathways expressed *in situ*?
- **Niche space**: How are resources partitioned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

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

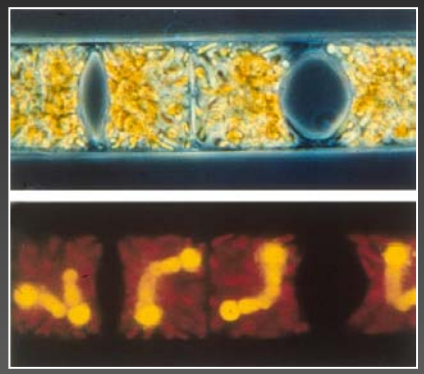


Transcriptome - enabled

Nitrogen-fixing marine cyanobacteria

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

Richelia



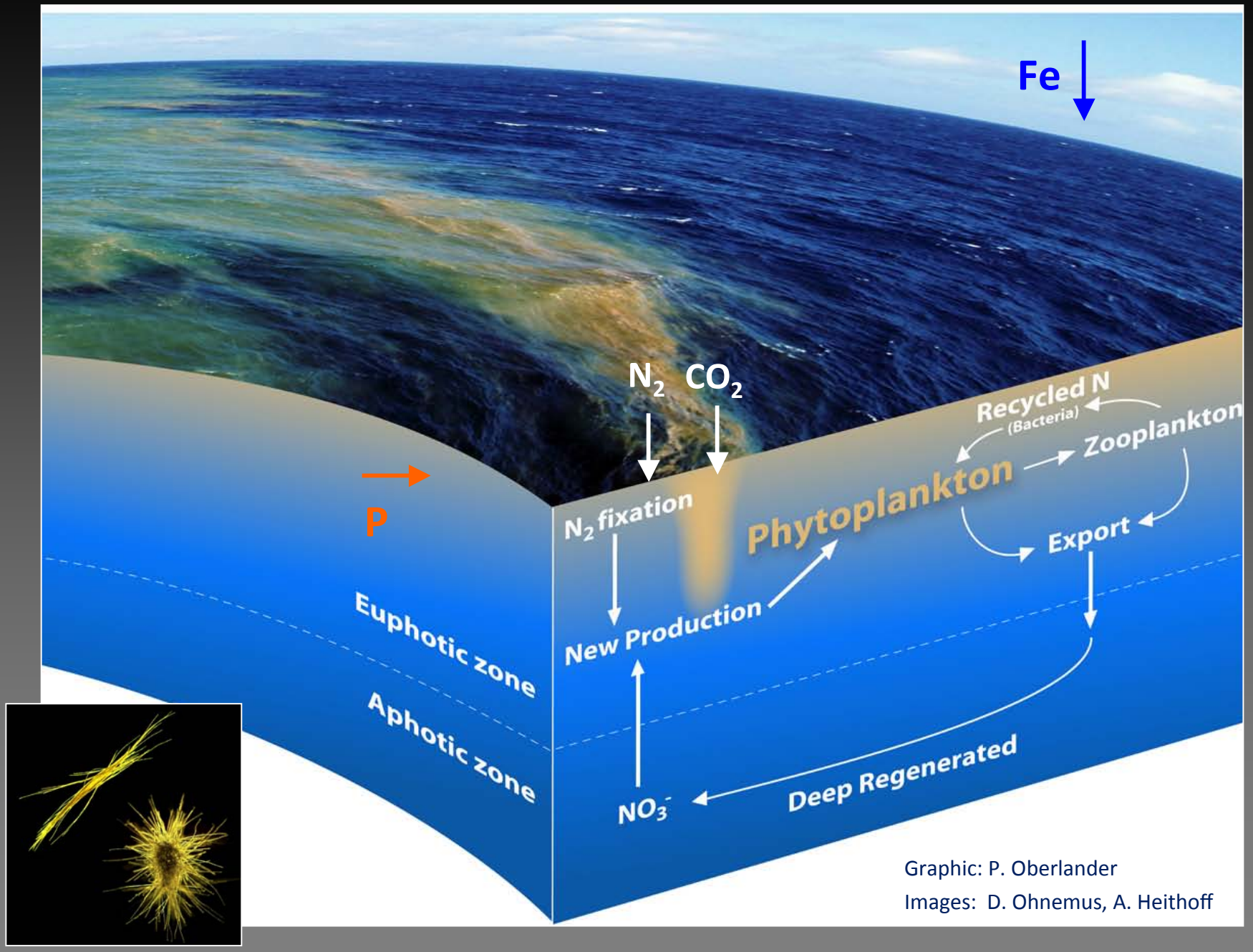
Crocosphaera

Trichodesmium

-Trichodesmium contortum
-Trichodesmium erythraeum
} *T. erythraeum* - like Clade

-Trichodesmium tenue
-Trichodesmium thiebautii
} *T. theibautii* - like Clade

-Trichodesmium spiralis
-Trichodesmium hildebrandtii



Graphic: P. Oberlander

Images: D. Ohnemus, A. Heithoff

Key themes

Taxonomic diversity

- How are the major clades of *Trichodesmium* distributed?

Metabolic traits and trade-offs

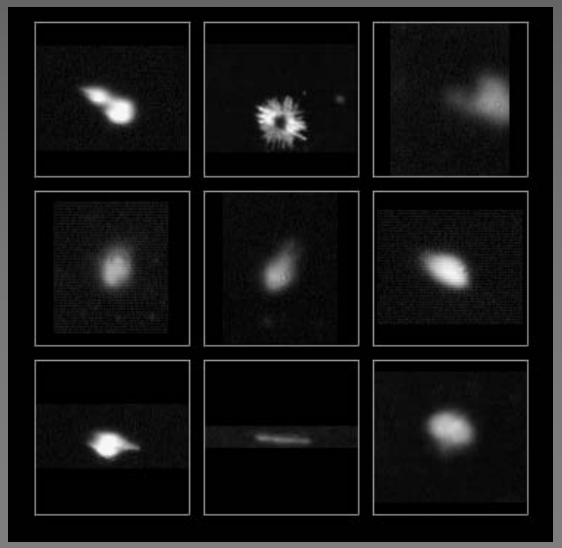
- What phosphorus is bioavailable?
- Is phosphorus supply sufficient to support maximal N_2 fixation in the North Atlantic?

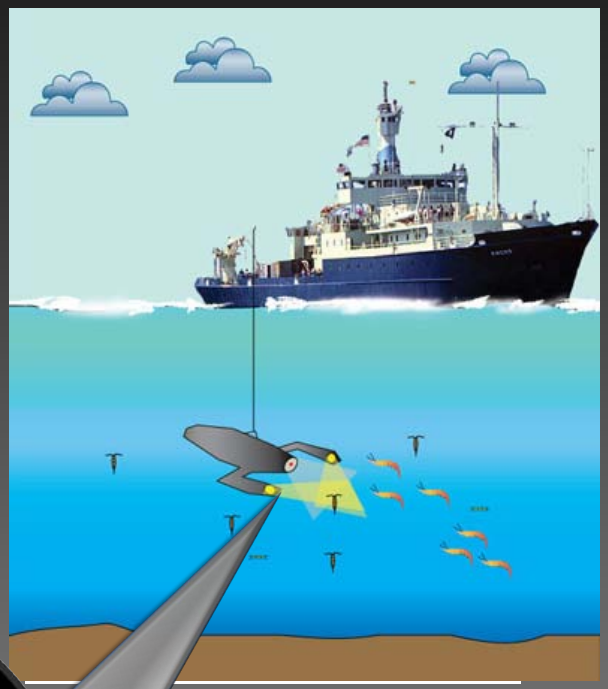
Niche space

- What is the composition of the colony and are activities influenced by communication among epibionts and *Trichodesmium*?

Enumerating *Trichodesmium* for N_2 fixation models

- Video Plankton Recorder
  - Underway
  - High throughput
  - Only detects colonies
  - Image classification a challenge
  - No clade specificity

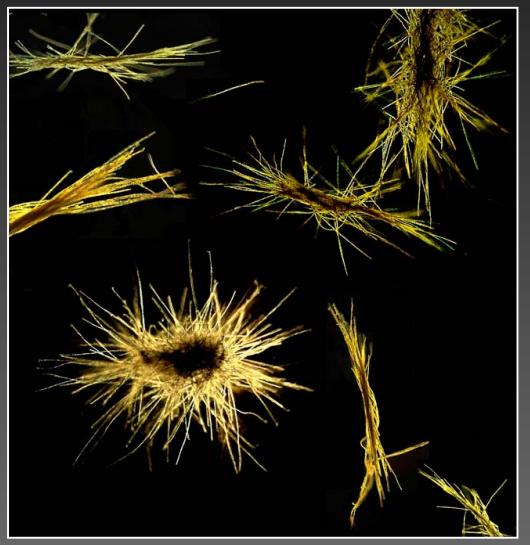




Elise Olson

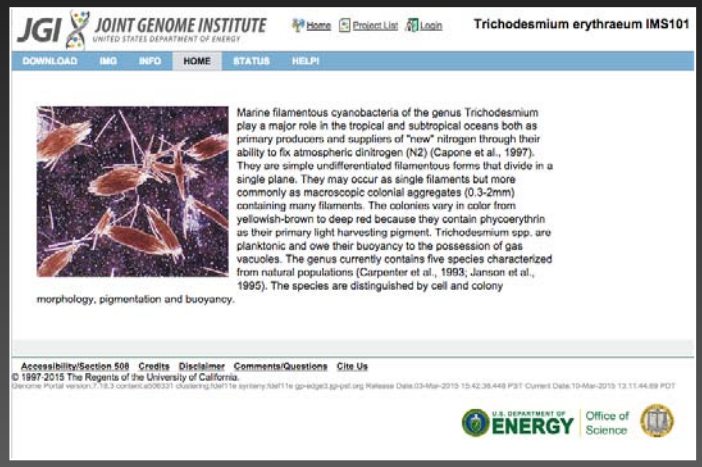
Enumerating *Trichodesmium* beyond the colony

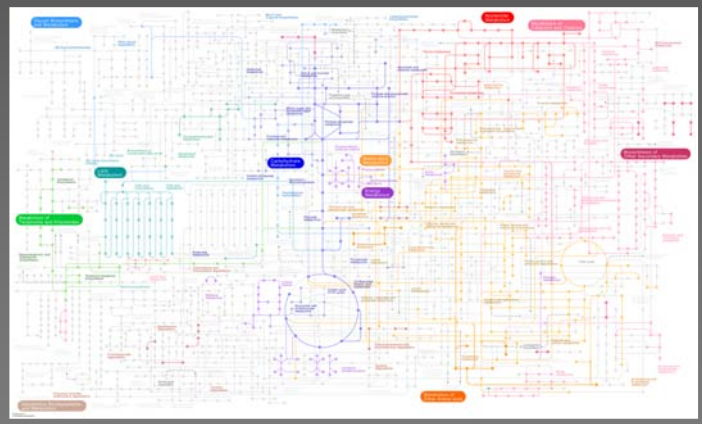
- VPR
  - High throughput
  - Misses filaments
  - No clade specificty
- Microscopy on discrete samples
  - Low throughput
  - Detects colonies and filaments
  - No clade specificity
- Molecular diagnostic (qPCR)
  - High throughput
  - Detects colonies and filaments
  - Sensitive
  - Clade-specific



Mónica Rouco

Leveraging the *Trichodesmium erythraeum* genome





2006 Genome sequence provided new and continued insight and a blue-print for developing molecular tools

Trichodesmium clades have different activities

- Clades may have different capacity and unique distributions like other marine cyanobacteria
- Different clades:
  - Make different phosphorus compounds (Dyhrman et al. 2009 *Nature Geosci*.)
  - Reduce phosphate differently (Van Mooy et al. 2015 *Science*)
  - Respond differently to increased CO_2 (Hutchins et al. 2015 *PNAS*)

Culture cells

↓

Harvest and preserve samples

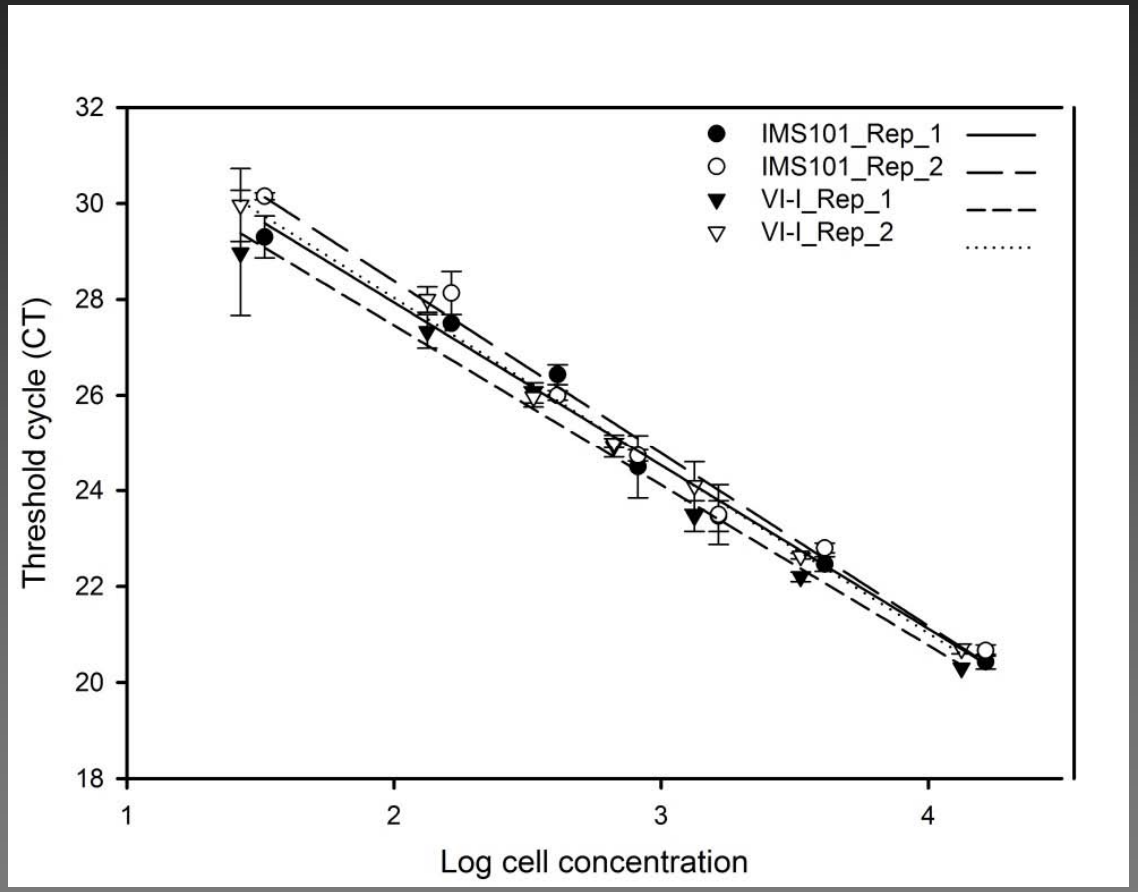
↙ ↘

Microscopy qPCR

↓

Standard curve

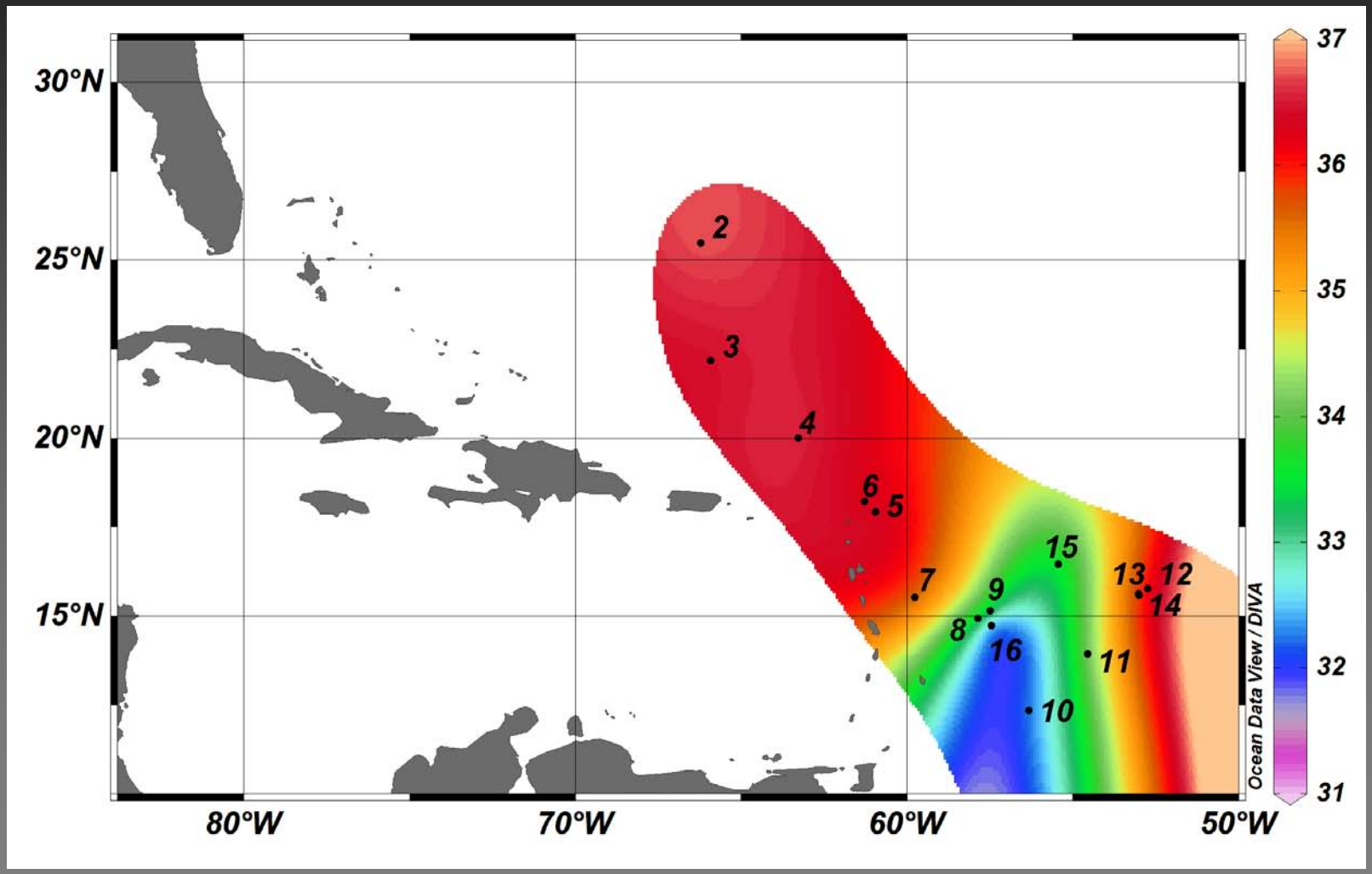
Standard curve from cultured isolates



Rouco et al. 2014 Limnol. Oceanogr.

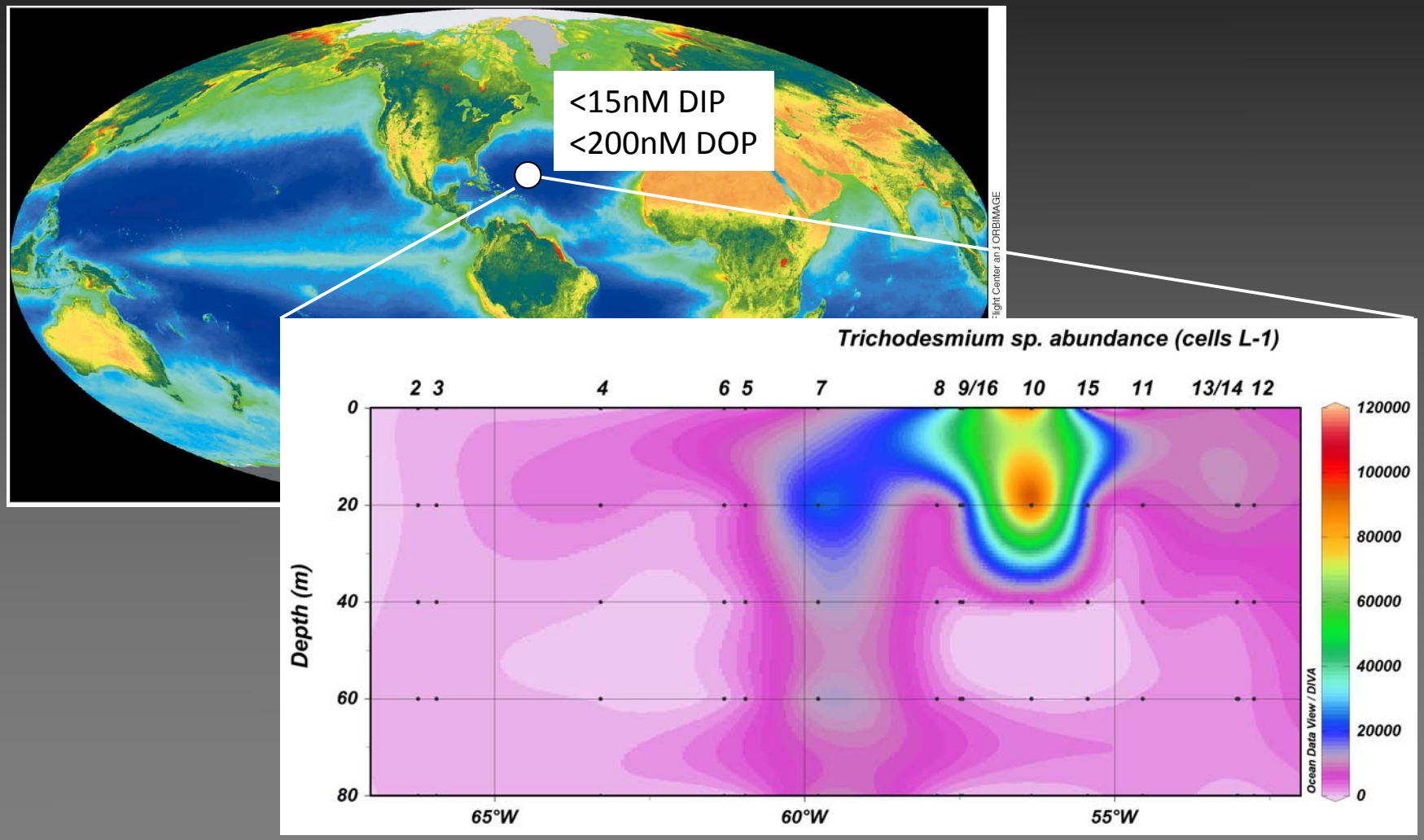
The *rnpB* gene amplifies specifically from each clade with ~100% efficiency

How are the major clades distributed?



Rouco et al. 2014 Limnol. Oceanogr.

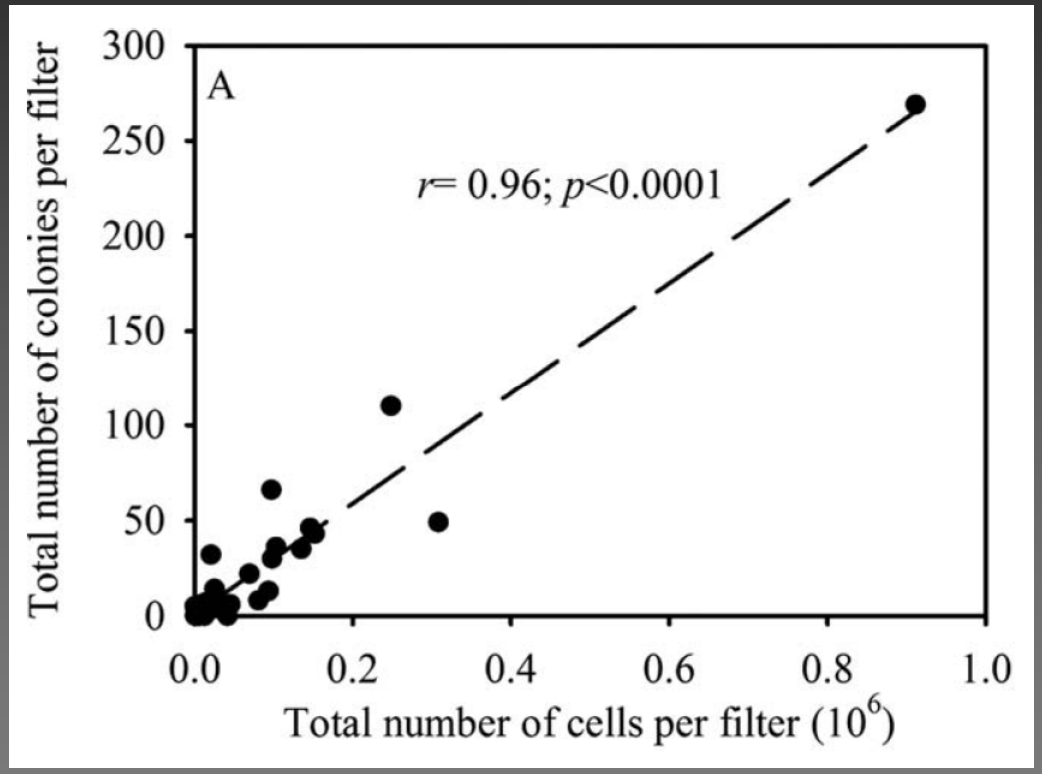
Trichodesmium is found in the low phosphorus - oligotrophic western North Atlantic



Rouco et al. 2014 Limnol. Oceanogr.

Trichodesmium clade abundance correlates with colony counts

- Microscopy and VPR-based colony abundance correlate with qPCR cell count
- Counts suggest the presence of free filaments (so VPR is missing biomass)
- Counts suggest that the majority of the population falls into one of the two clades



Rouco et al. 2014 Limnol. Oceanogr.

Clade distribution and abundance trends

Drain 10L

↓

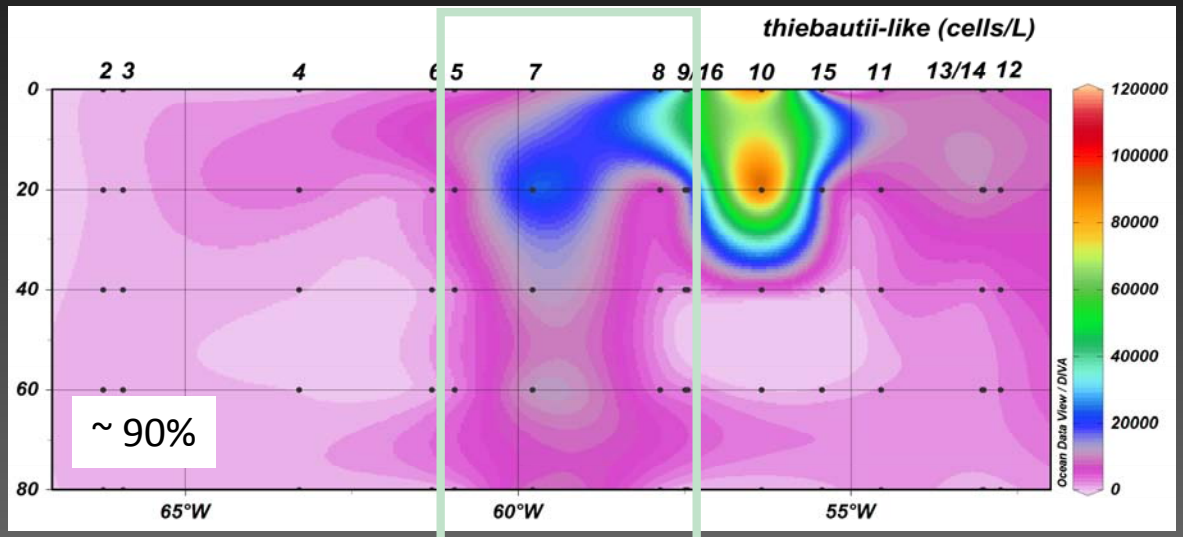
Preserve filter

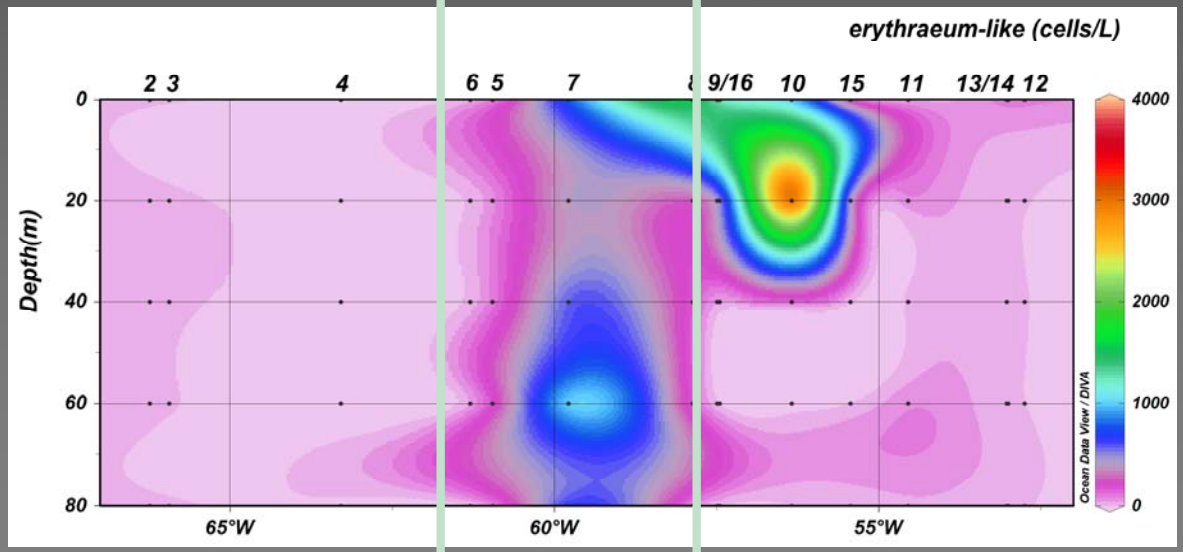
↓

Extract DNA

↓

qPCR/QC

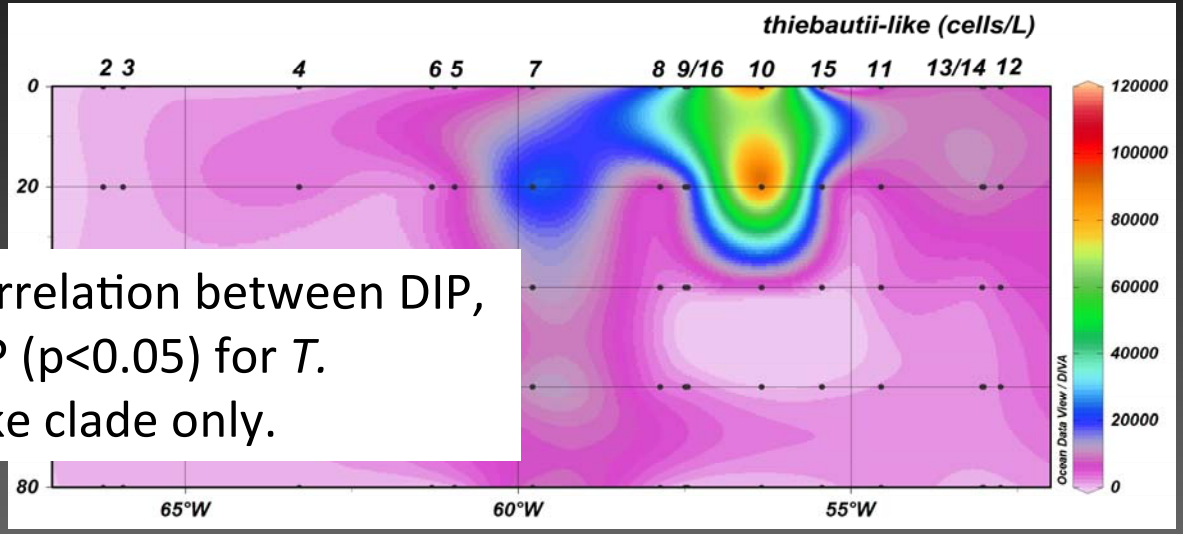


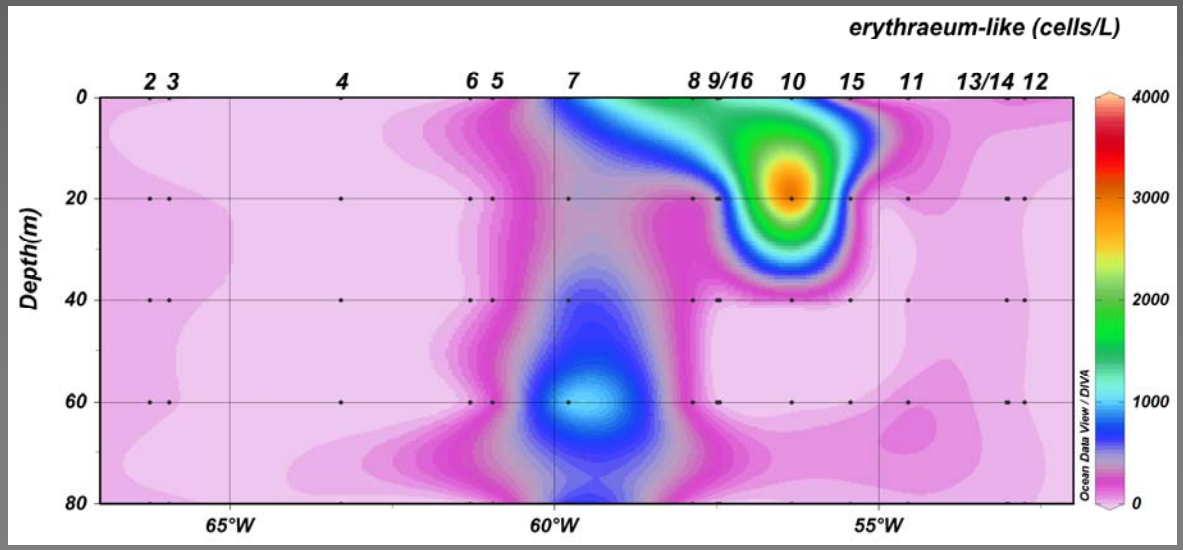


Rouco et al. 2014 *LO*

Clade distribution

Significant correlation between DIP, DOP, and TDP ($p<0.05$) for *T. thiebautii* - like clade only.





Summary - Taxonomic diversity

How are the major clades distributed?

- *T. theibautii* - like species are dominant (~90%) in the western North Atlantic (~50% in the North Pacific) - NOT THE CLADE OF THE GENOME STRAIN

- Distribution and correlations with temperature and phosphorus pools hint at the importance of P bioavailability in driving the dynamics

-Trichodesmium contortum
-Trichodesmium erythraeum
} *T. erythraeum* - like Clade

-Trichodesmium tenue
-Trichodesmium thiebautii
} DOMINANT

-Trichodesmium spiralis
-Trichodesmium hildebrandtii

Key themes

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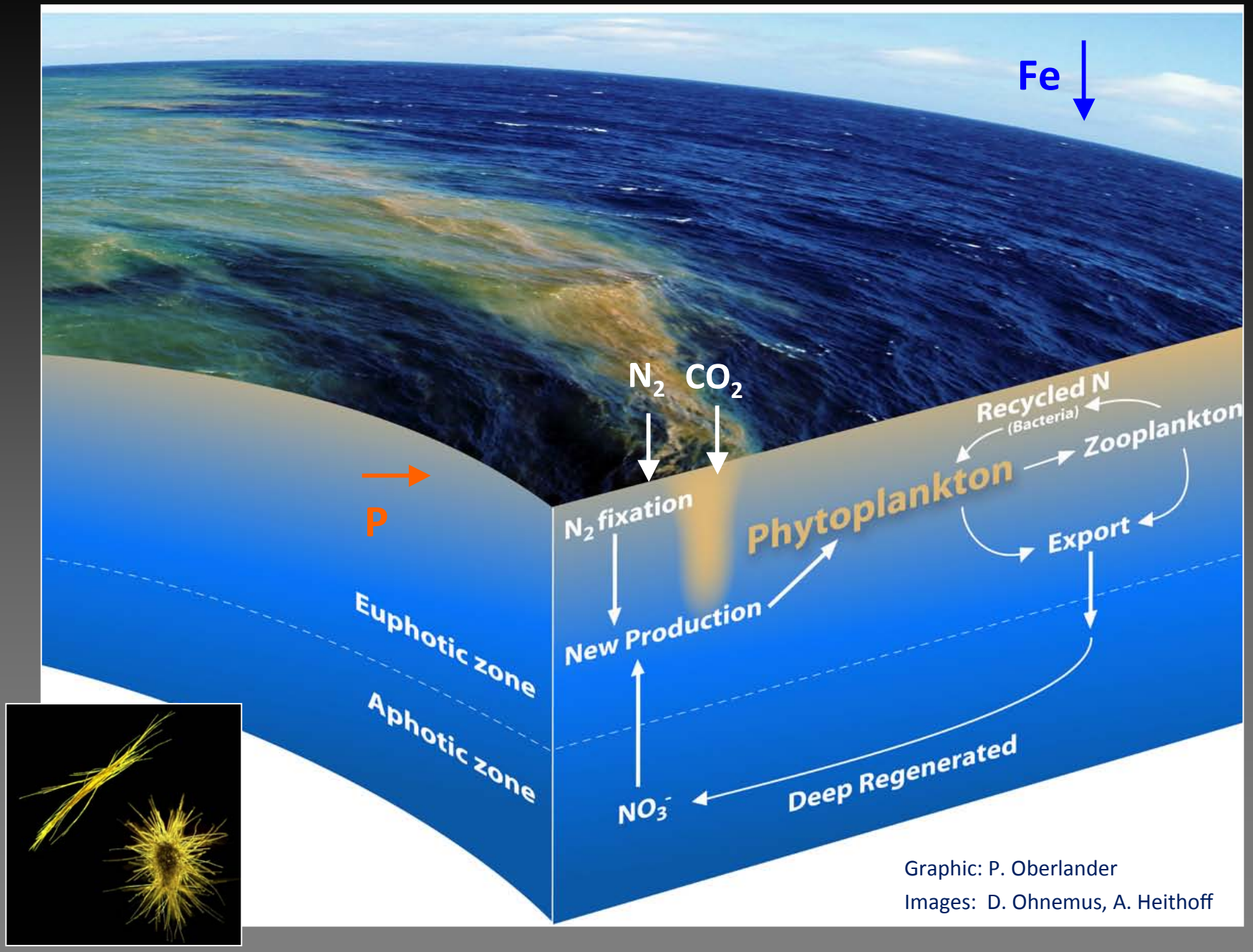


Graphic: P. Oberlander

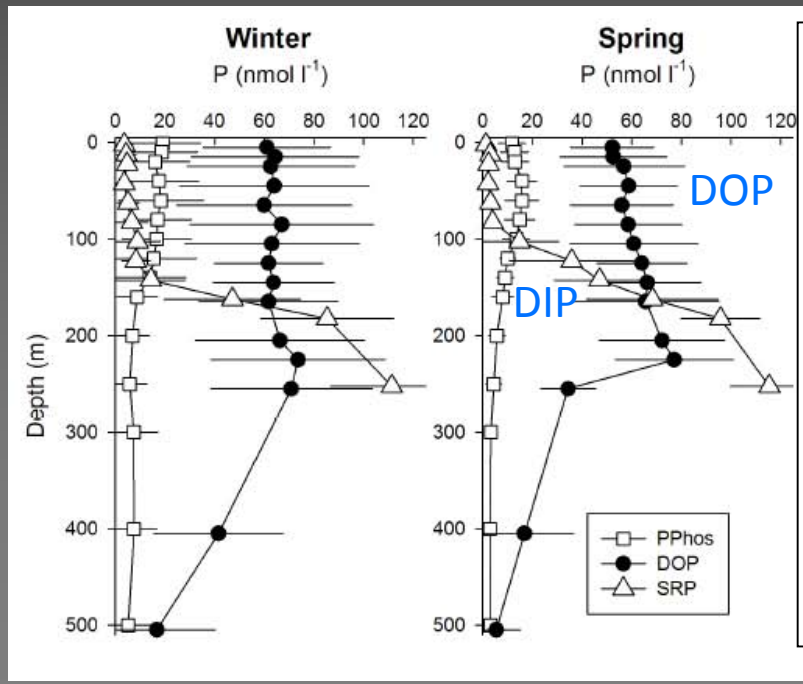
Images: D. Ohnemus, A. Heithoff

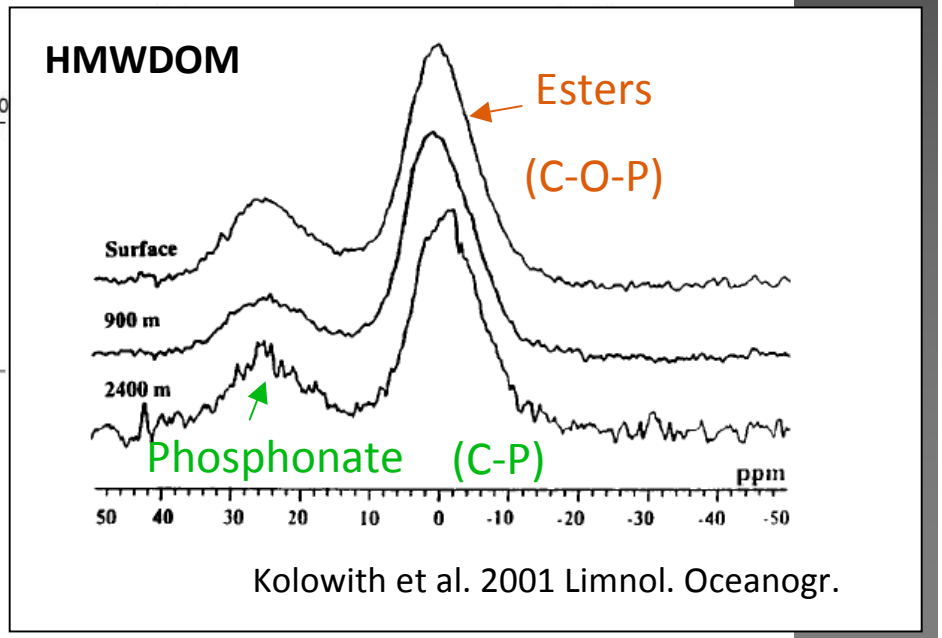
Phosphorus pools in the western North Atlantic

Particulate phosphorus

PPhos

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

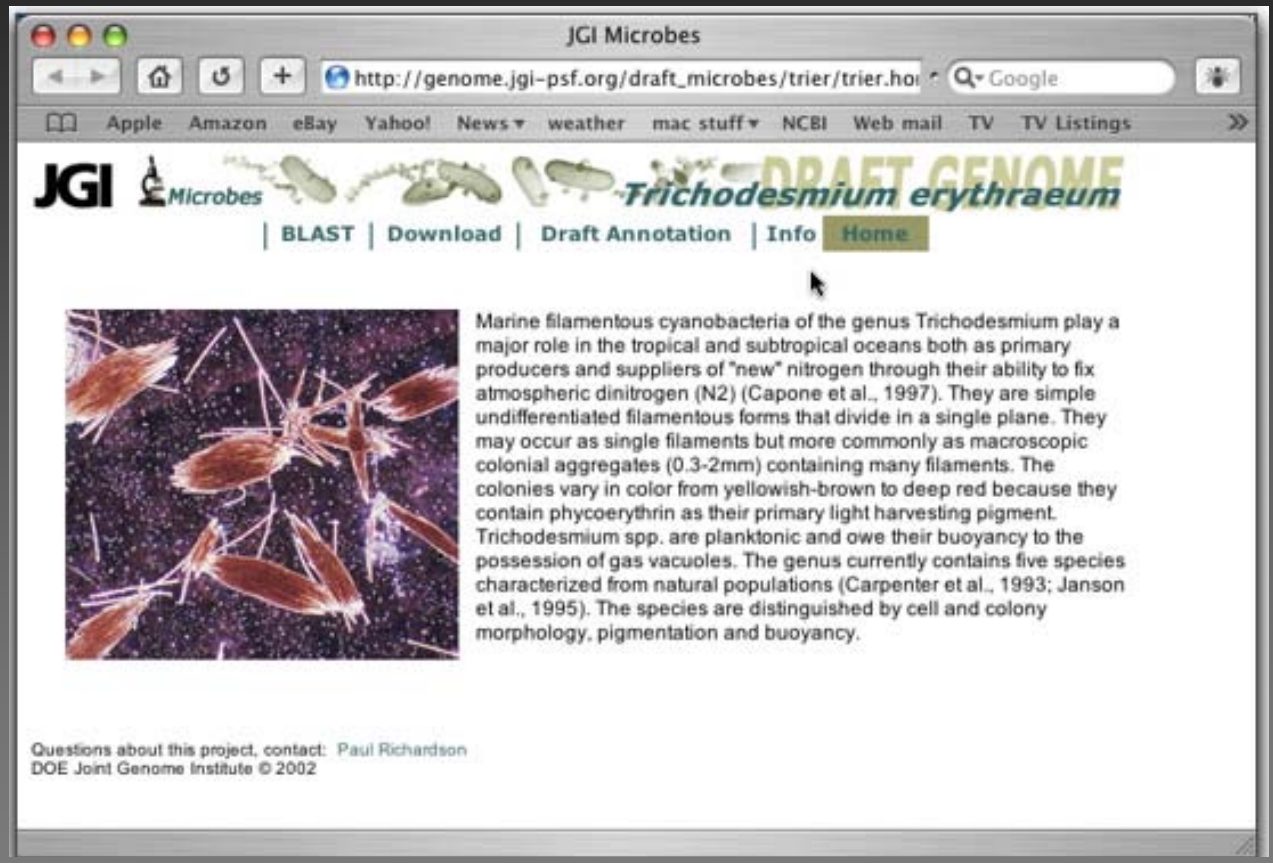




Kolowith et al. 2001 Limnol. Oceanogr.

Lomas et al. (2010) *Biogeosciences*

Trichodesmium erythraeum IMS101 genome page



www.jgi.doe.gov

Phosphorus metabolic traits and trade-offs

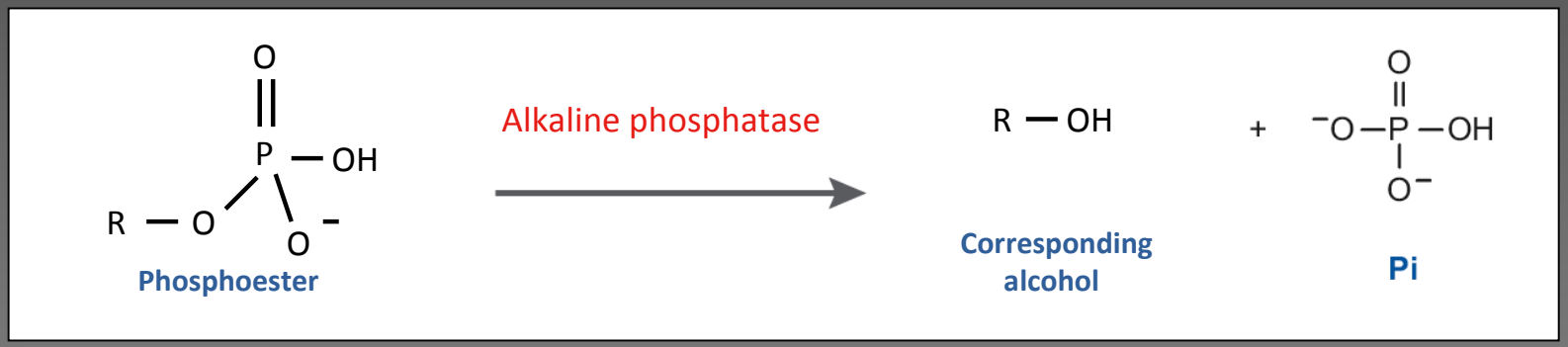
- Phosphonate
  - C-P Lyase (Fe co-factor)

C-P

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

COP

COP

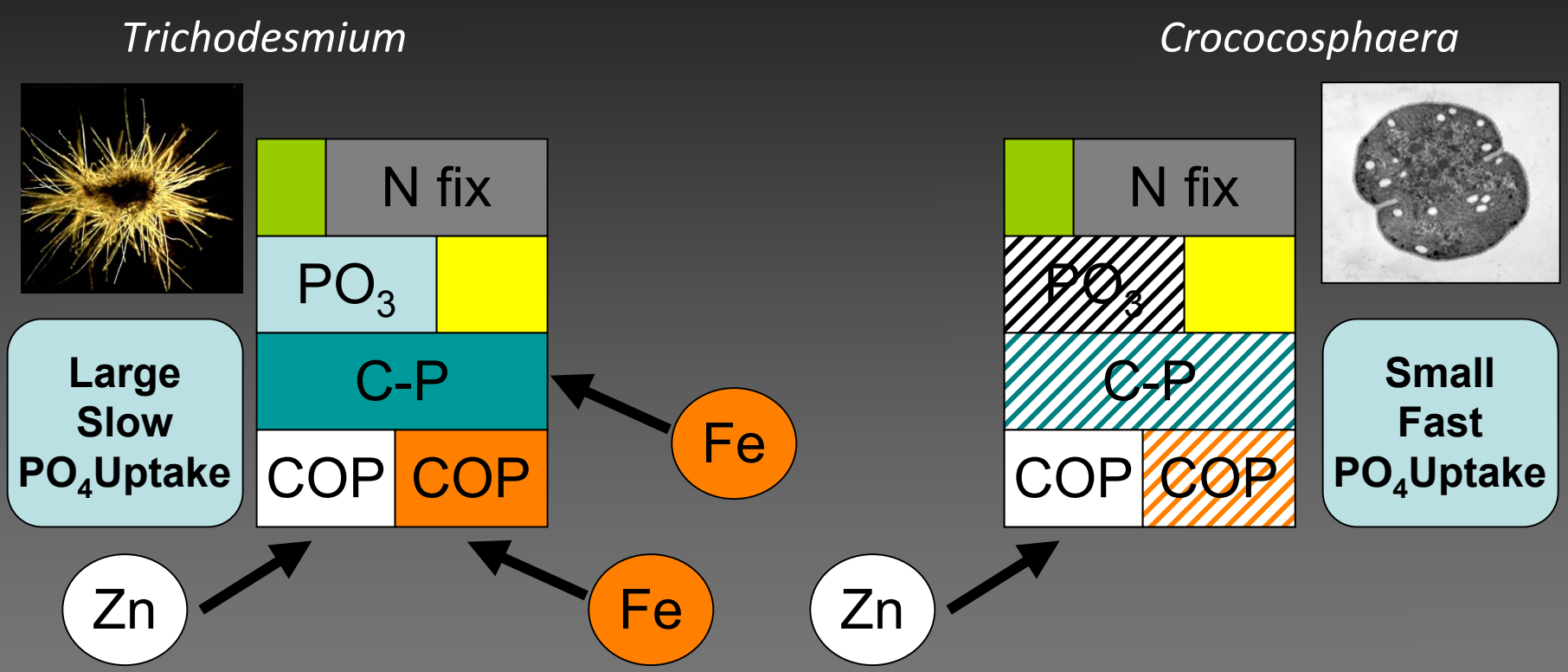


- Phosphite
  - *ptxD* gene cluster

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

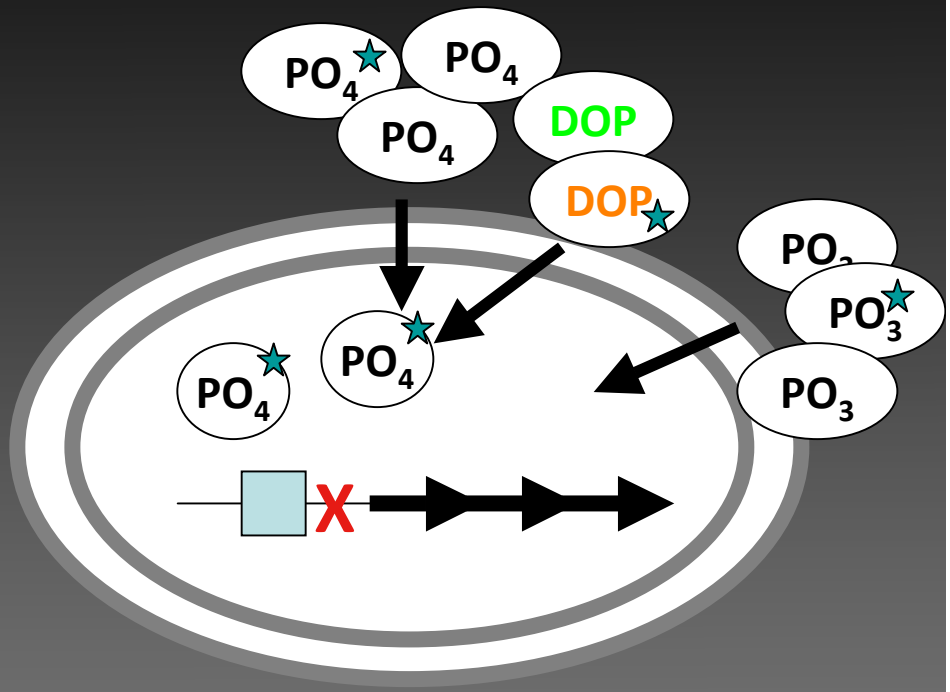


Other N_2 fixing cyanobacteria genomes do not encode the same pathways for phosphorus metabolism - less available substrates, but less metal requirement

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

Assaying P supply with isotope tracers

- Rates are difficult to constrain with tracer studies
  - No tracers for DOP
  - Dynamic P requirement



Trichodesmium:

~ 0.5 nmol $PO_4 h^{-1} ugChla^{-1}$

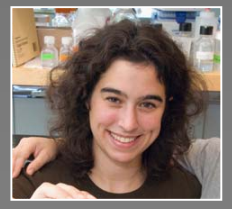
~ 29 - 80 % ester DOP

~ ?% phosphonate DOP

~ ?% phosphite (PO_3)

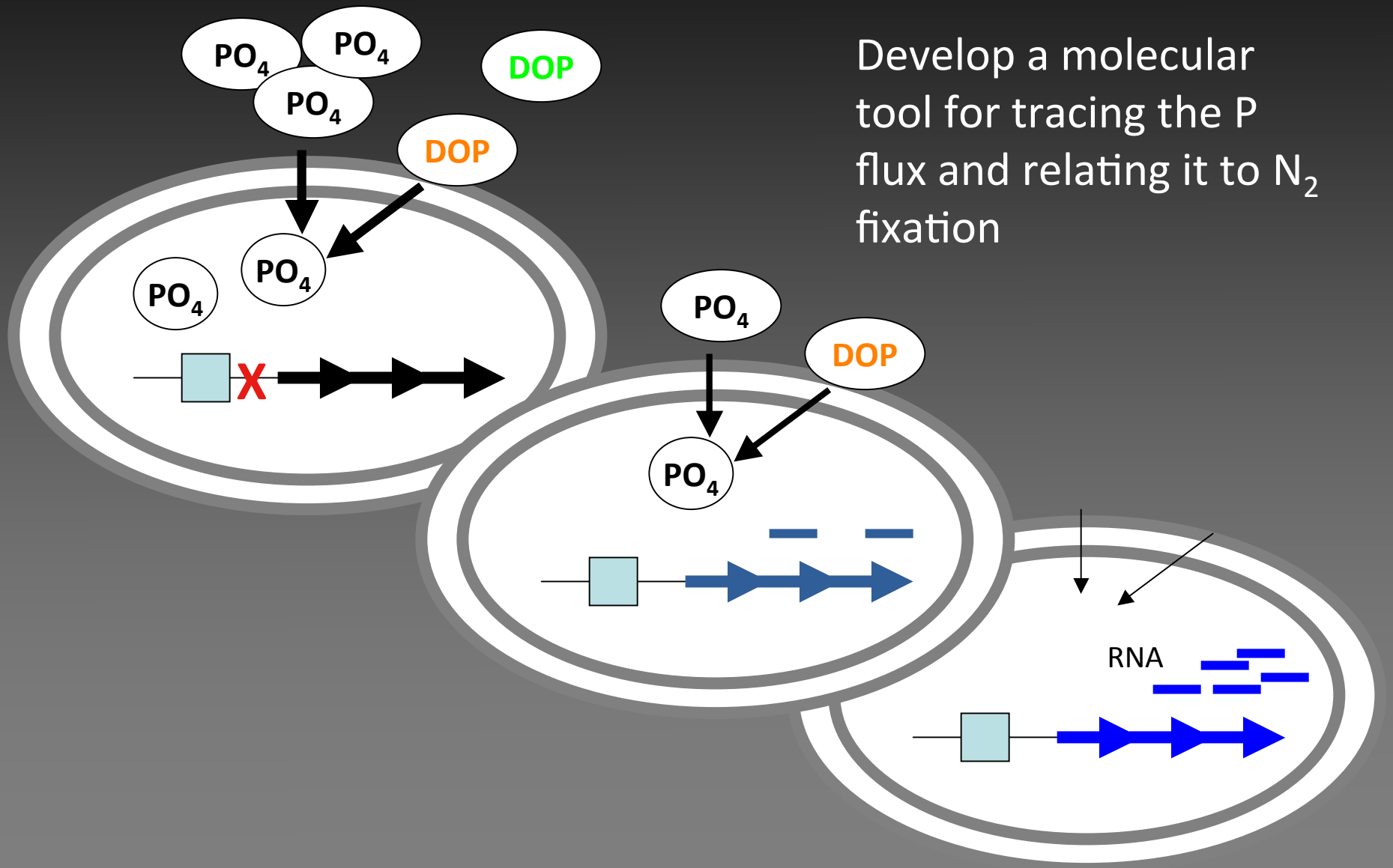
Orchard et al. 2010 *Limnol and Oceanogr*

Van Mooy et al. 2015 *Science*



Liz Orchard

Assaying P supply with a genome-enabled tool



Develop a molecular tool for tracing the P flux and relating it to N_2 fixation

Tracking genomic potential with expression studies

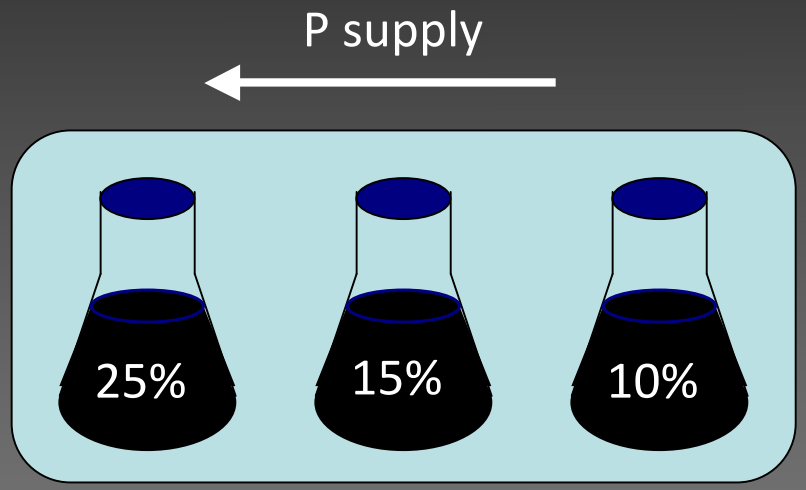
Culture cells

↓

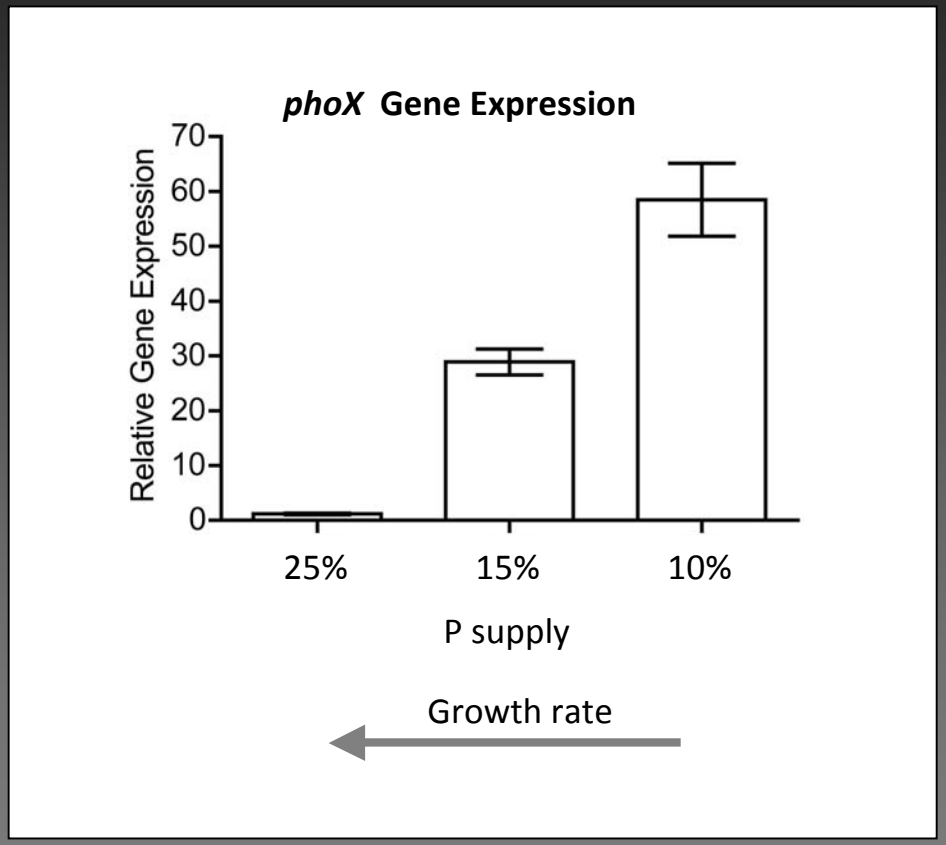
Harvest and preserve samples

↓

qRT-PCR of *phoX* Activity



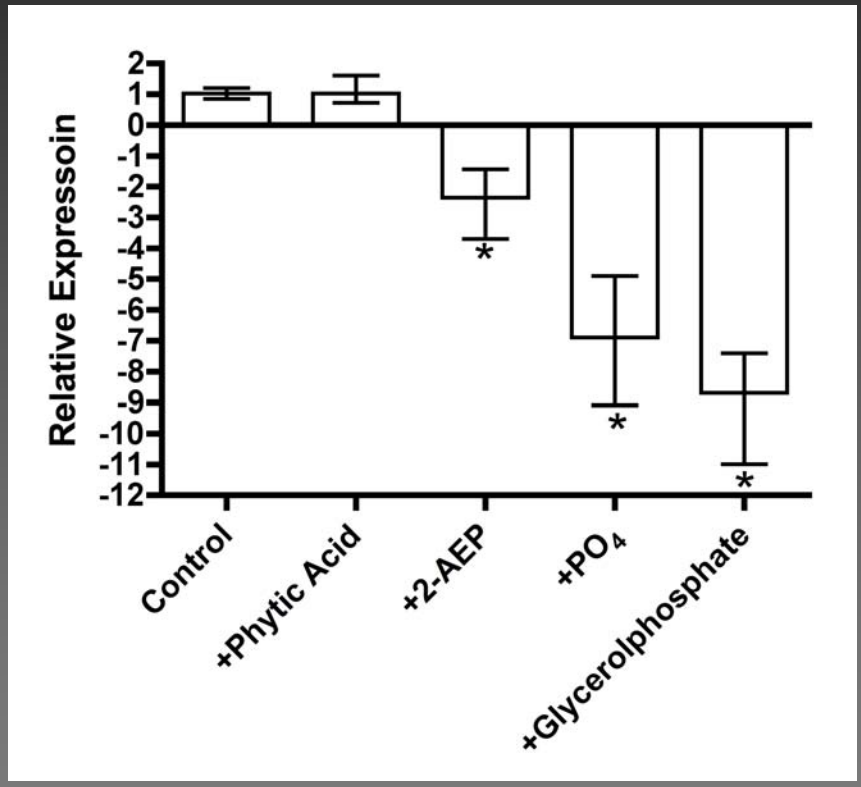
Calibrating gene expression to growth and N_2 fixation



Orchard and Dyhrman unpublished

Calibrating gene expression to P supply

- *phoX* expression is responsive to cellular P regardless of exogenous source.
- Transcripts are rapidly turned over.
- Response is similar in culture experiments with both clades



Orchard and Dyhrman unpublished

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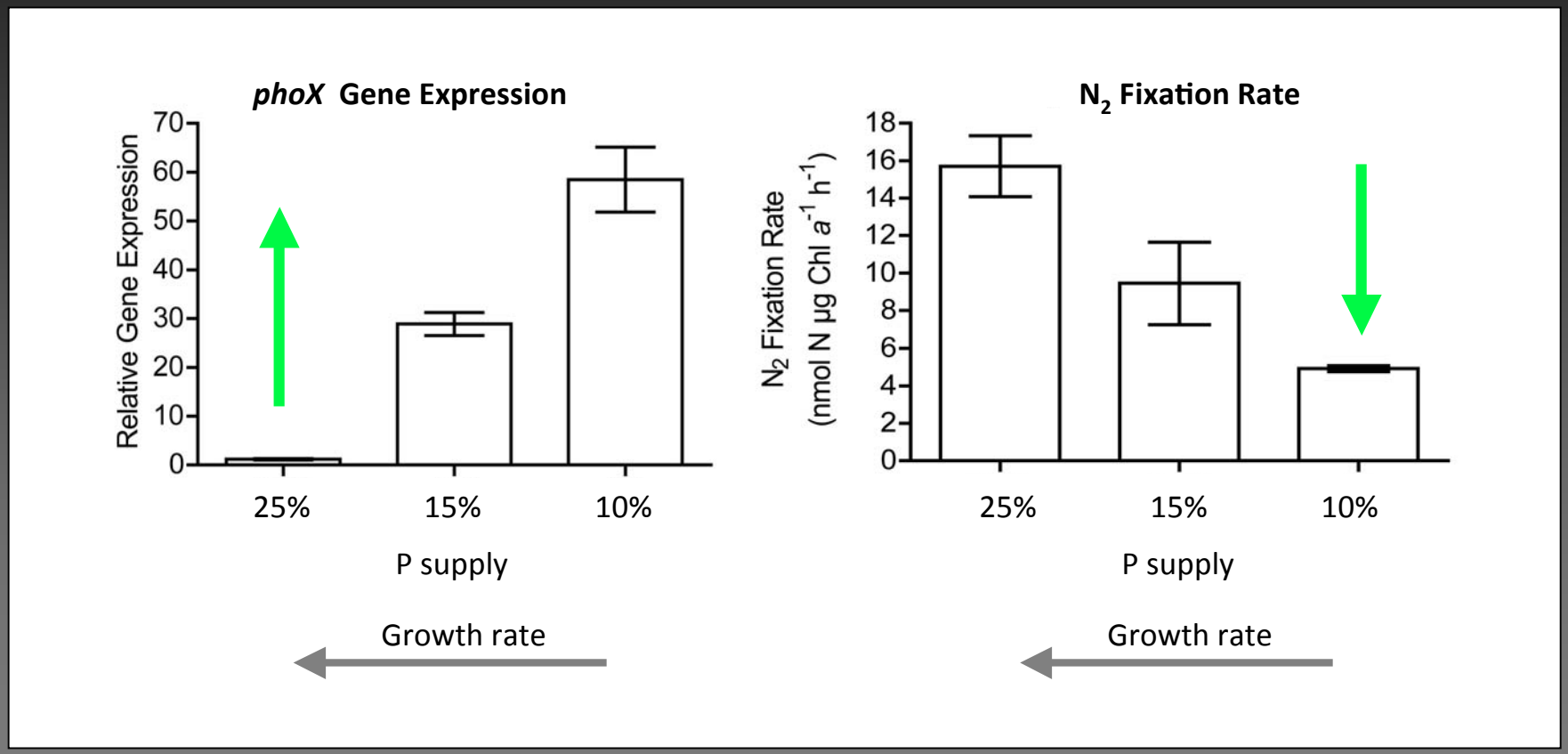

Orchard and Dyhrman unpublished

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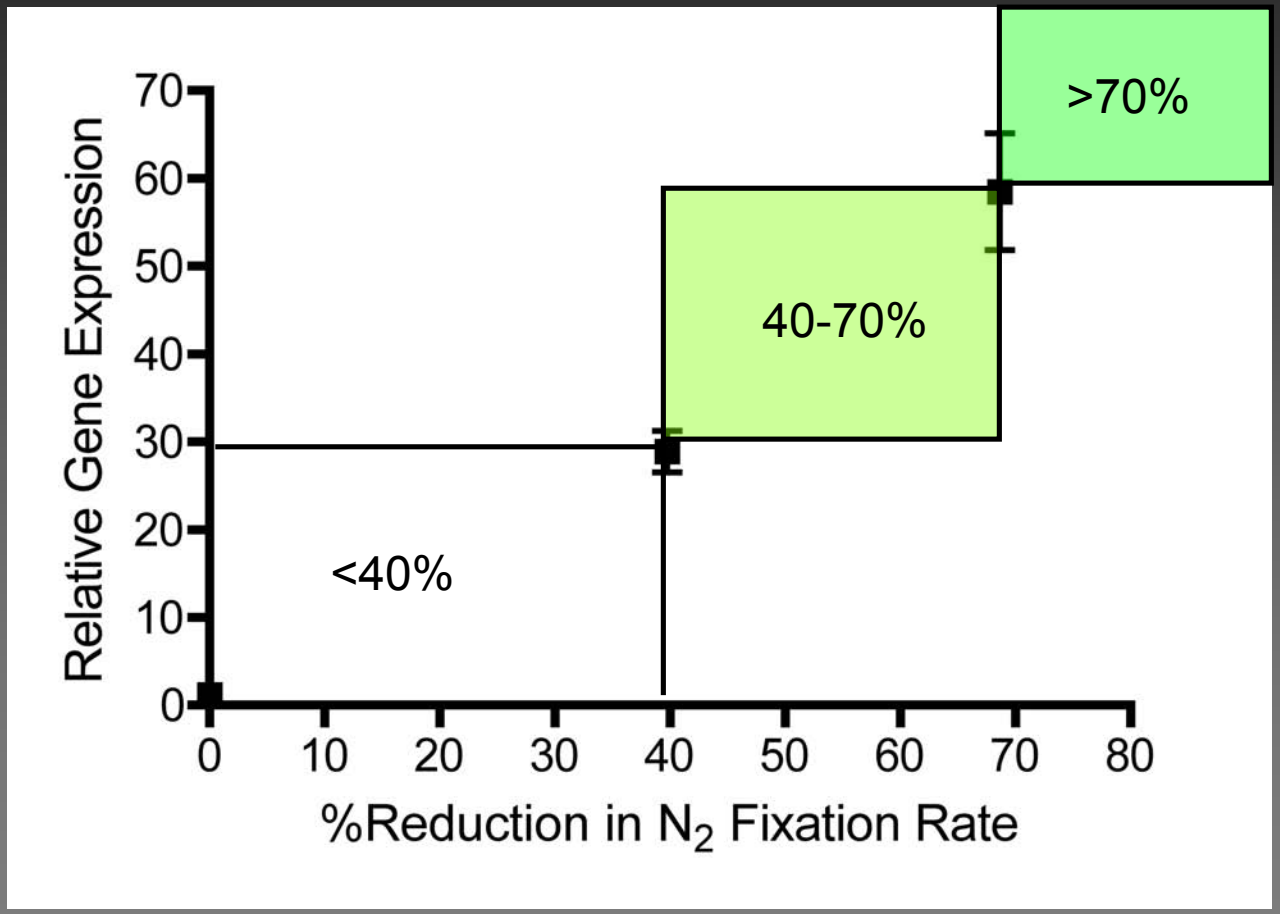
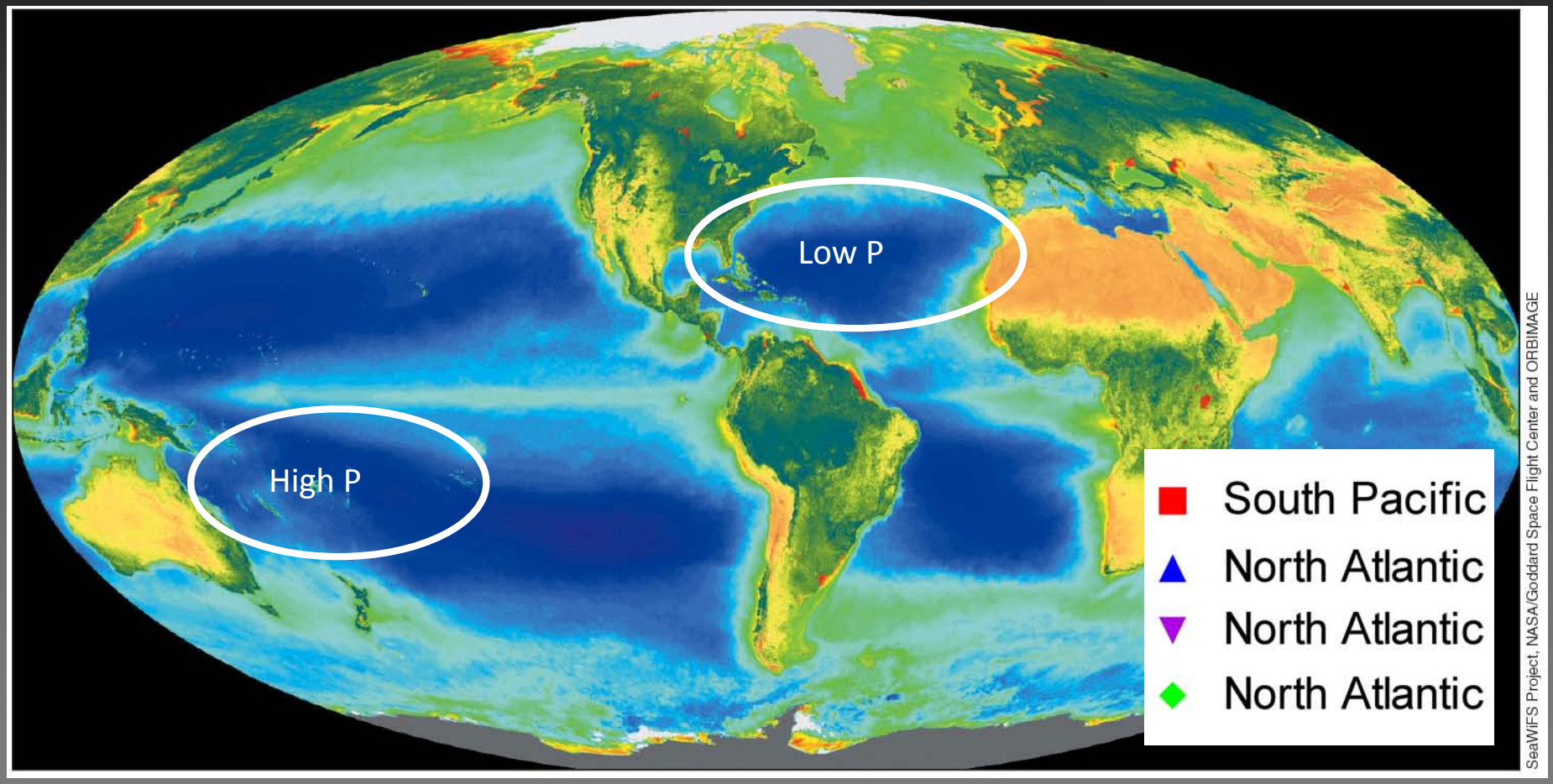


Orchard et al. in prep

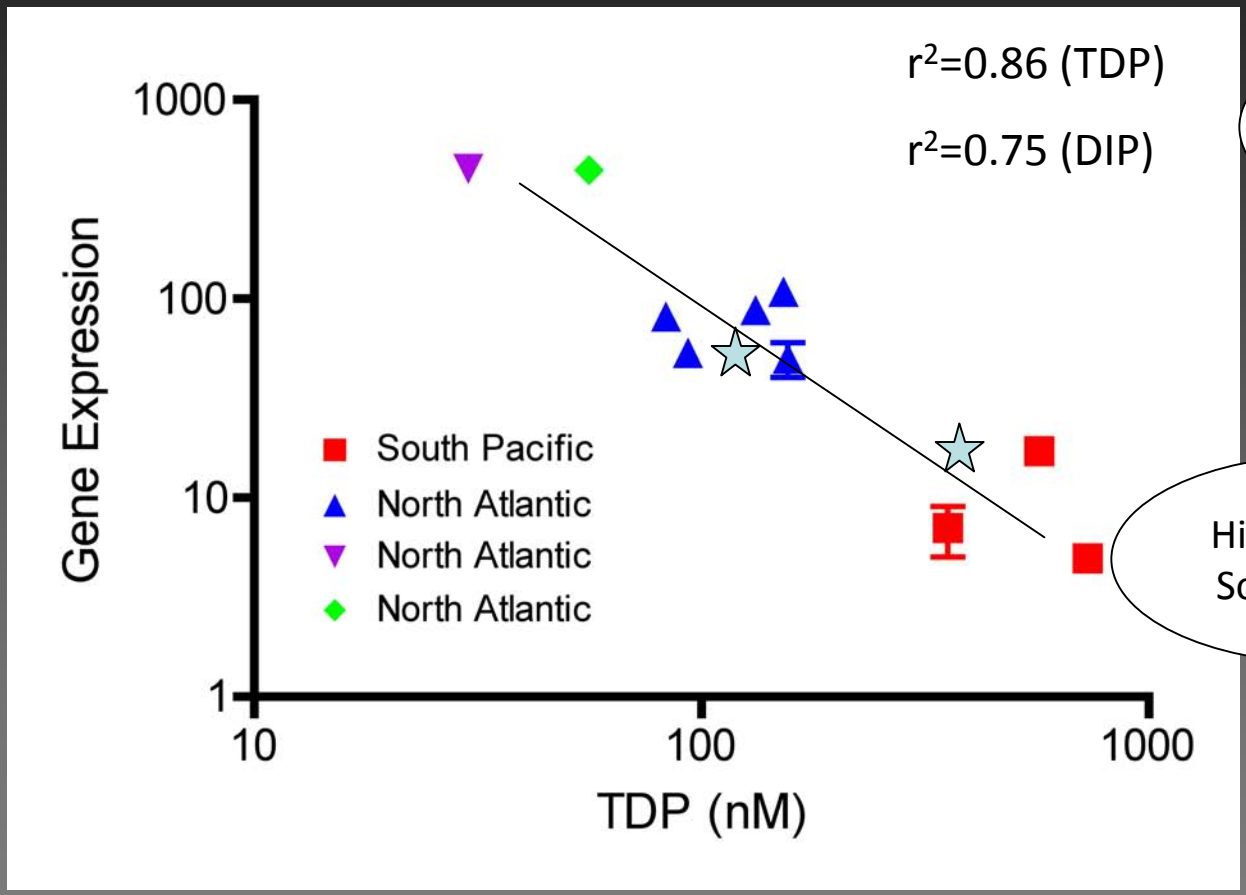
Sampling different P regimes



DIP, TDP Measurements

Measurements of quantitative gene expression for *Trichodesmium sp.*

Gene expression increases at low phosphorus

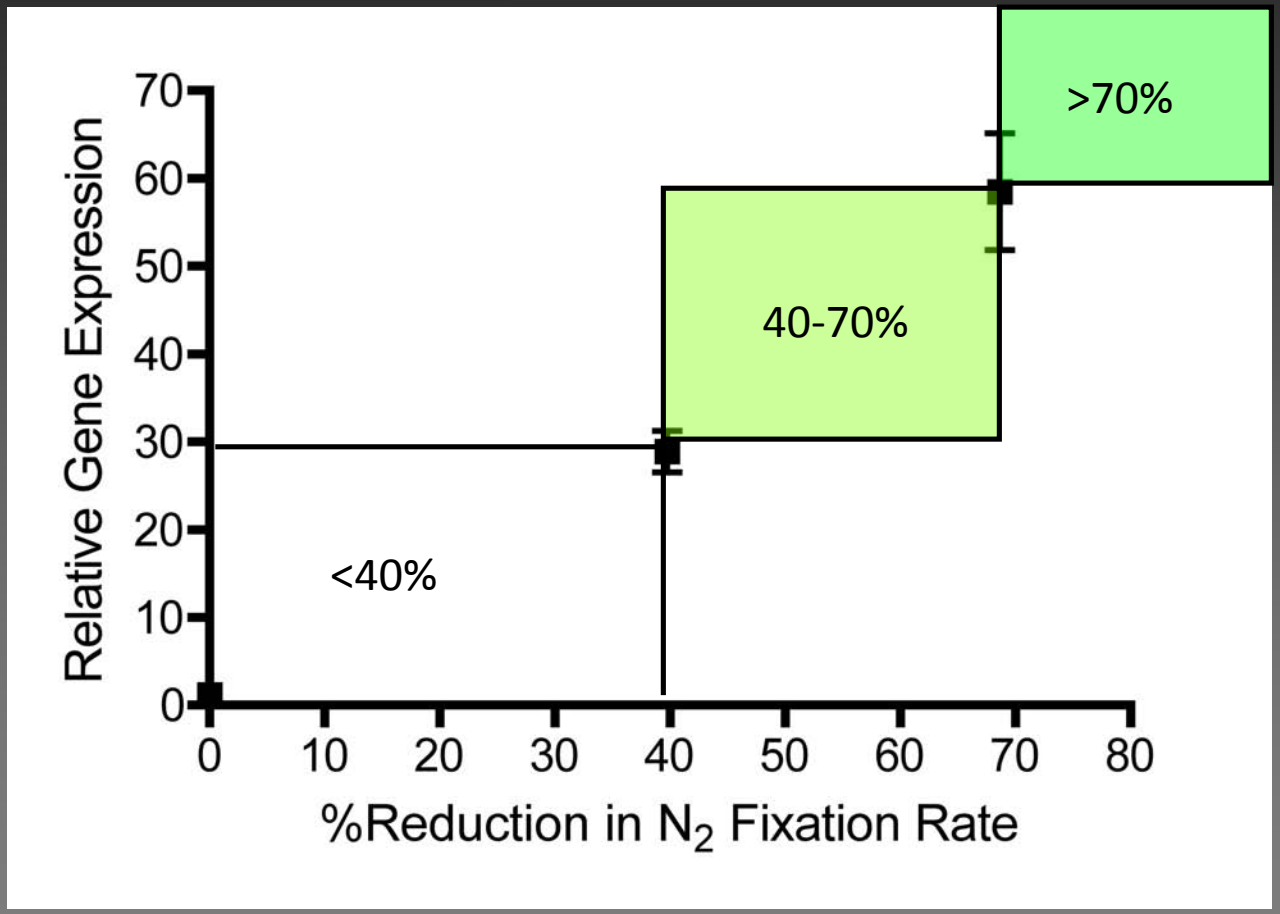


Low P supply
North Atlantic

High P supply
South Pacific

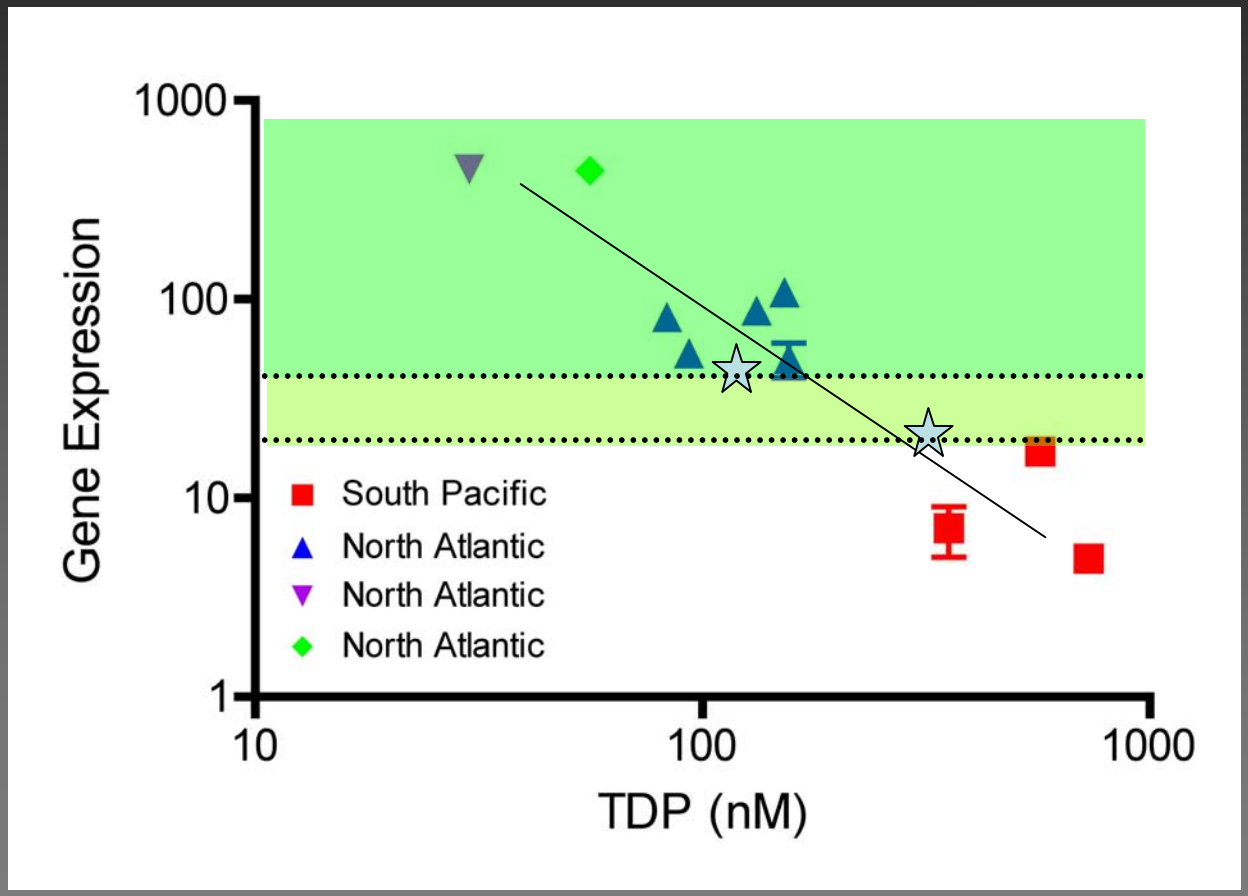
Orchard and Dyhrman unpublished

Calibrating gene expression to N_2 fixation



Orchard and Dyhrman unpublished

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



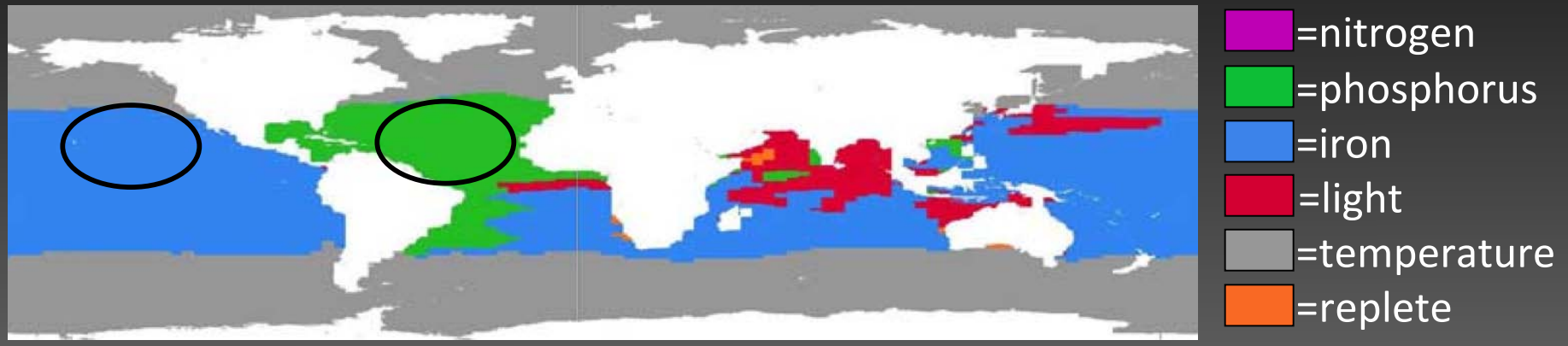
>70%

40-70%

<40%

Orchard and Dyhrman unpublished

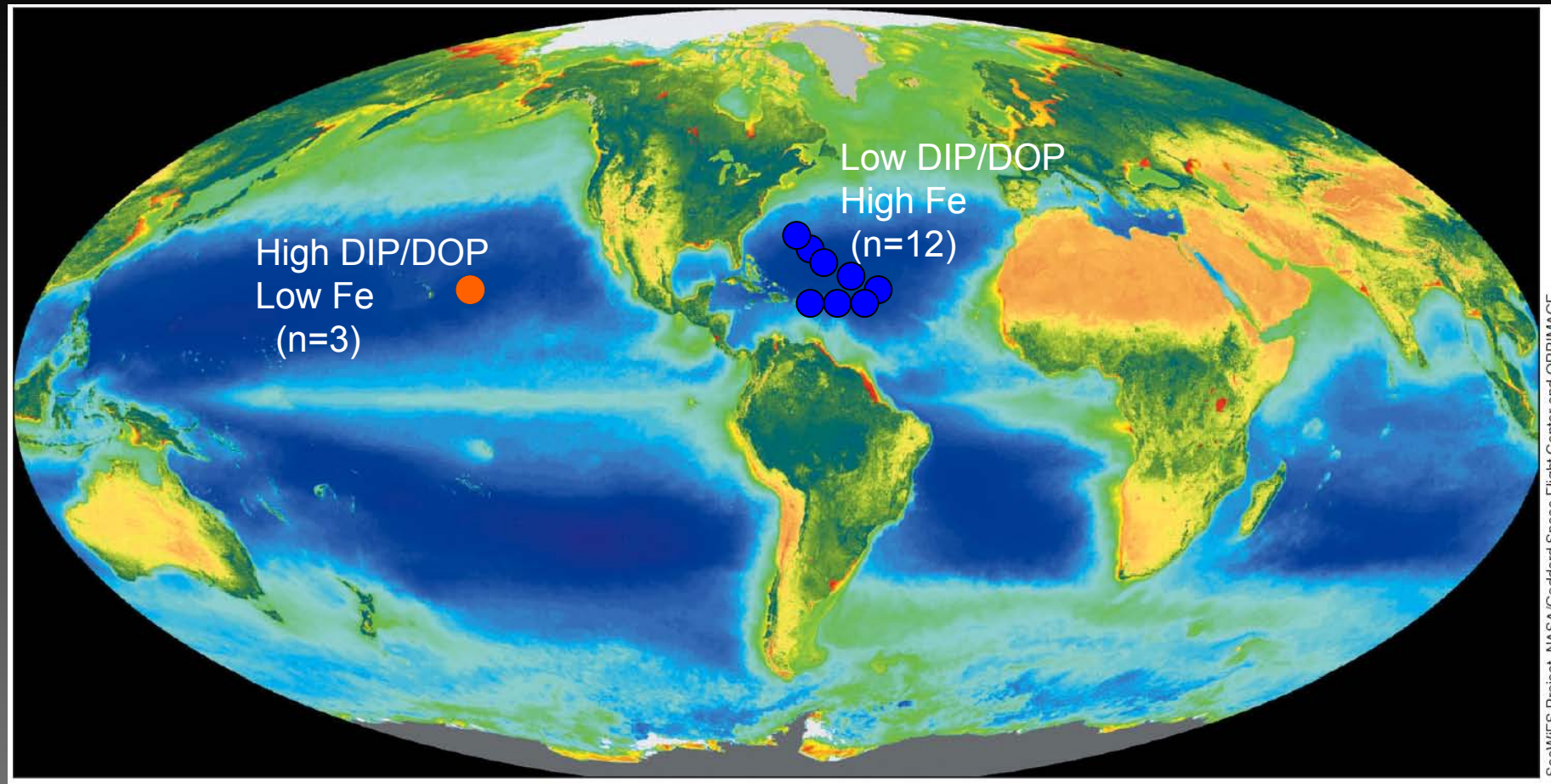
Constraints on *Trichodesmium* N_2 fixation



(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)
SeaWiFS Project, NASA/Goddard Space Flight Center and ORBIMAGE

Sampling and pipeline

Collect samples

Remove rRNA

Submit samples to sequencing center

Sequence using RNAseq and map reads to genomes

Target 30 - 60 million 100 bp paired-end reads

Stringent mapping

Removed reads with ambiguous mapping

IMS101 Genome - Need to move to a curated database which better captures sequence diversity

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
iihiiihiihiihiiiihiiiidggggeeeecddddccbcccccccc_
@DHT4KXP1:1:1101:5781:2386#0/1
CTTTCAGATACAGTAGGATTTATTCAGGATCTTCCGACGACATTGATTGCTG
CATTCCGCTCAACGCTTGAGGAAGTAAAAGAAGCGGATTTAATTCTGCA

Summary - Metabolic traits and trade-offs

What phosphorus forms are bioavailable?
Is P supply sufficient to support maximal N_2 fixation in the North Atlantic?

- *Trichodesmium* genome suggests bioavailabiliy of phosphonate, ester, phosphite etc.
- *Trichodesmium phoX* expression levels suggests that supply of bioavailable P is low in the western N. Atlantic, which could constraint N_2 fixation
- Predicted biogeochemical drivers of N_2 fixation are reflected in *Trichodesmium* physiology

Key themes

Taxonomic diversity

- How are the major clades of *Trichodesmium* distributed?

Metabolic traits and trade-offs

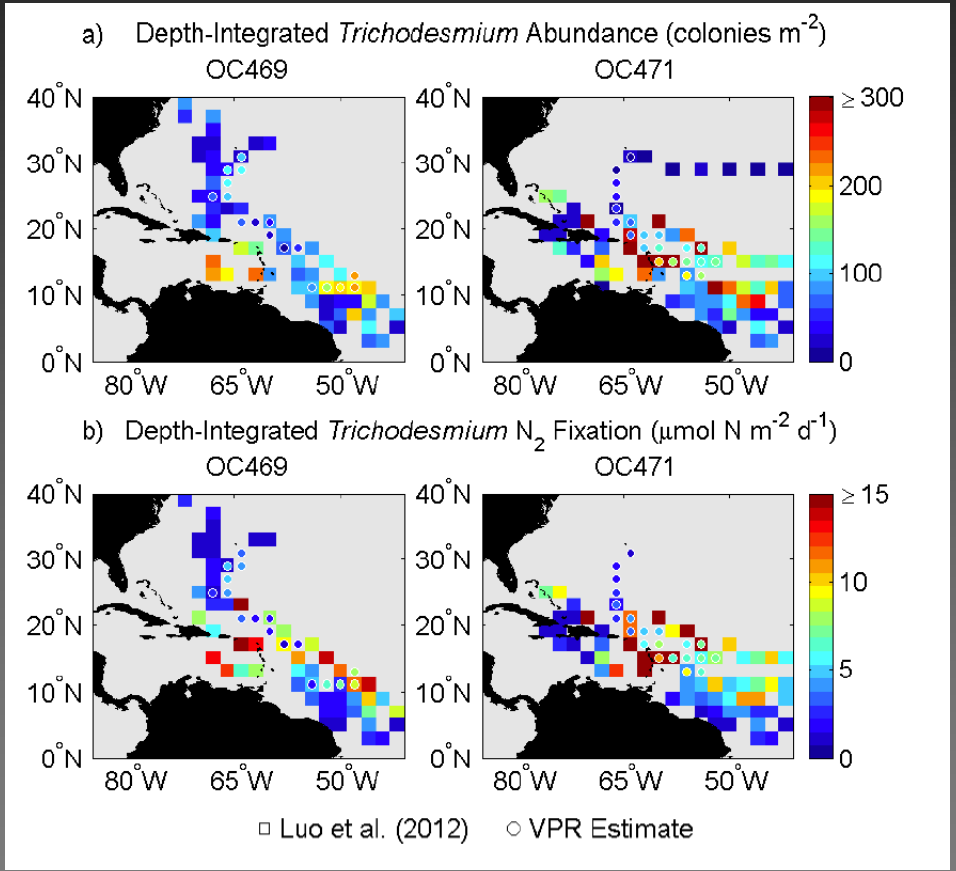
- What phosphorus is bioavailable?
- Is phosphorus supply sufficient to support maximal N_2 fixation in the North Atlantic?

Niche space

- What is the composition of the colony and are activities influenced by communication among epibionts and *Trichodesmium*?

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

Could there be other factors? - Epibiont interactions

- *Trichodesmium* colonies harbor epibionts in cultures and field populations (Hmelo et al. 2012 *AME*)
- Quorum sensing communication molecules (acylated homoserine lactones - AHL) detected in colonies (Van Mooy et al. 2012 *ISME J)*
- What is epibiont diversity and the role of the *Trichodesmium* microbiome in its physiological ecology

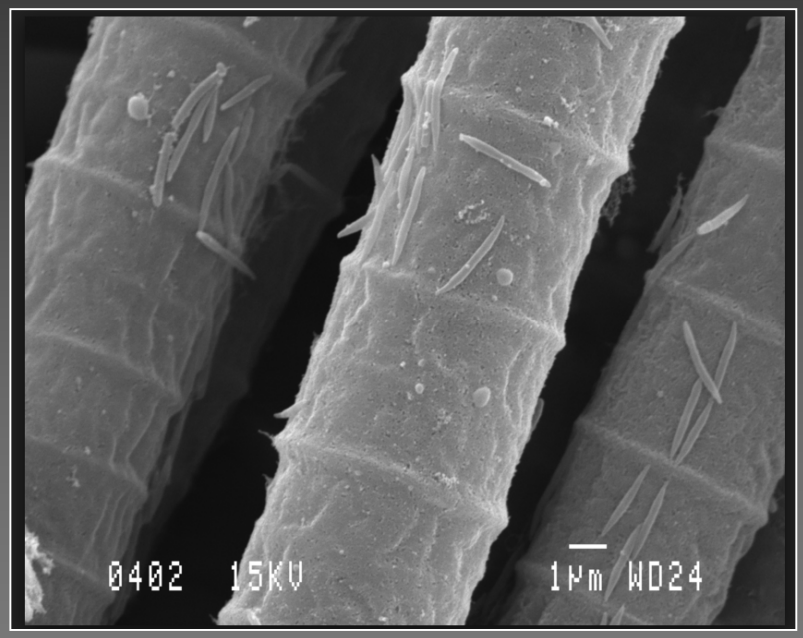


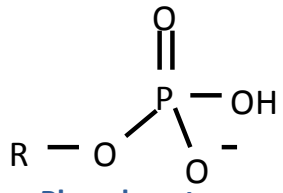
Image courtesy Tracy Mincer

Epibiont interactions - alkaline phosphatase activity

Collect colonies

Incubate with and without AHL

Alkaline phosphatase activity

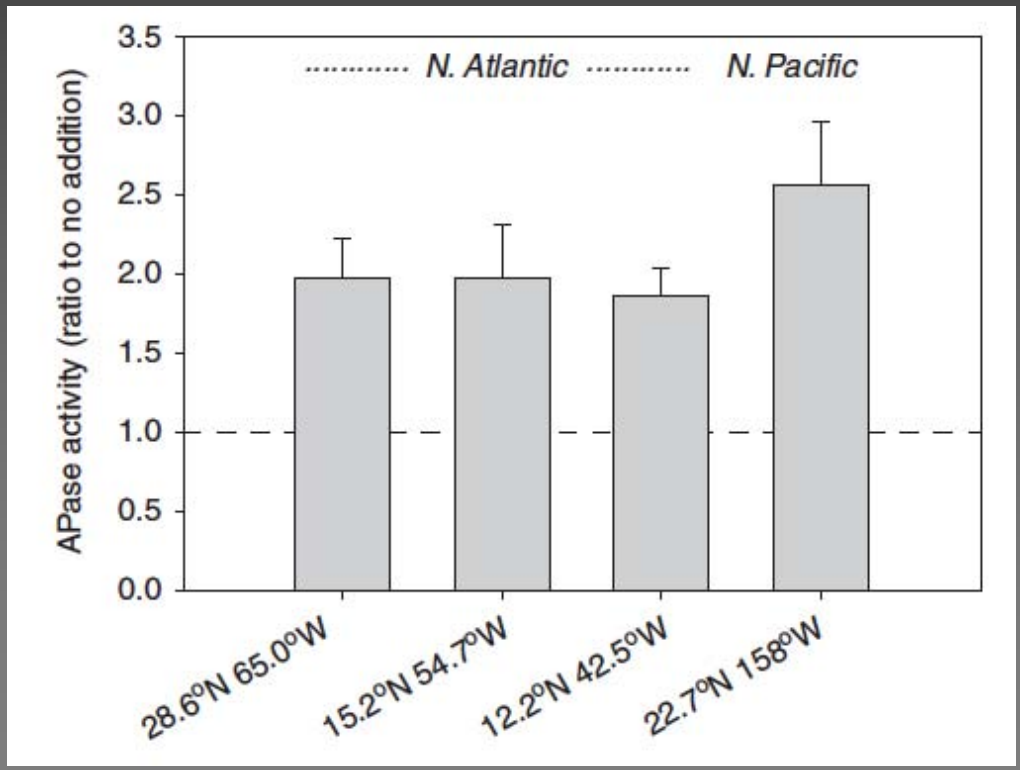


Phosphoester

Alkaline phosphatase

R — OH

Corresponding alcohol



Van Mooy et al. (2012) *ISME J*

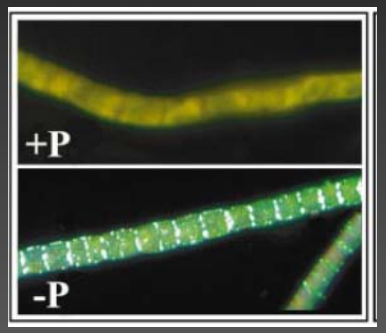
Epibiont interactions - alkaline phosphatase activity

Collect colonies

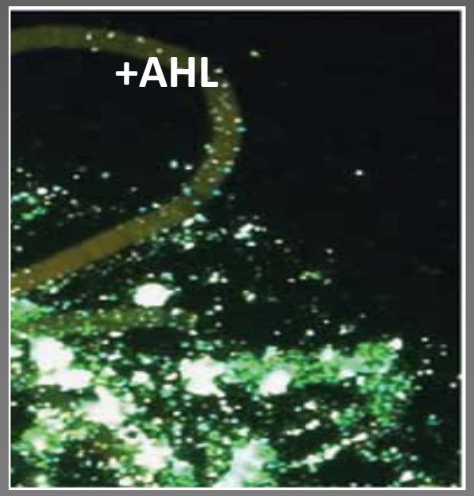
Incubate with and without AHL

Alkaline phosphatase activity

2.5 nmol $PO_4h^{-1}ugChla^{-1}$



17.5 nmol $PO_4h^{-1}ugChla^{-1}$



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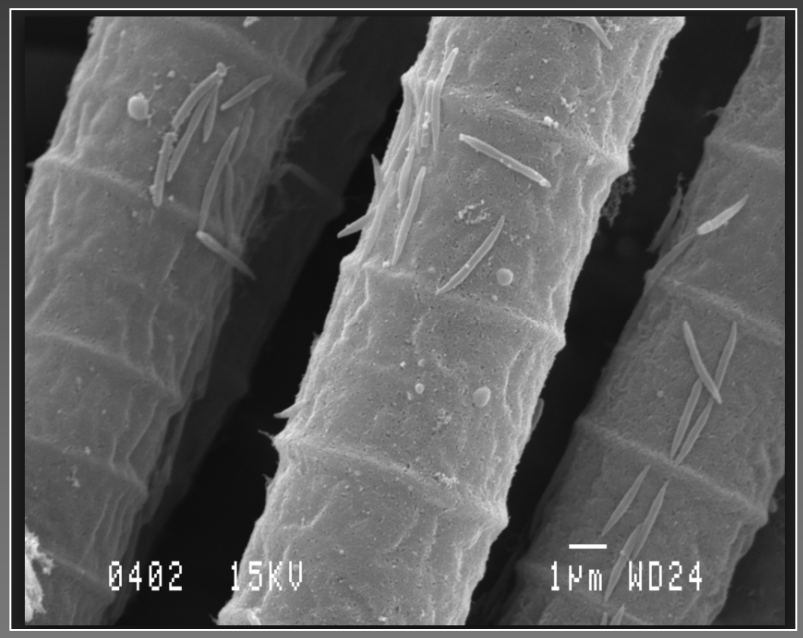
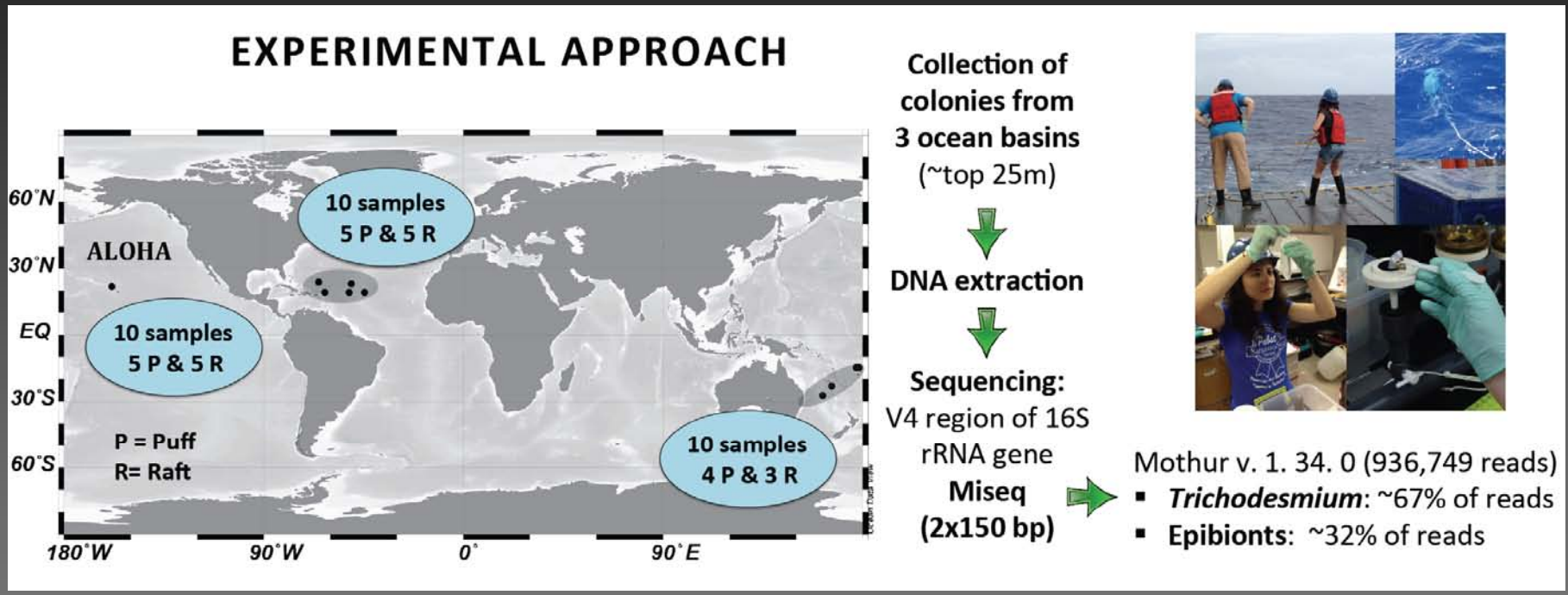


Image courtesy Tracy Mincer

Epibiont diversity

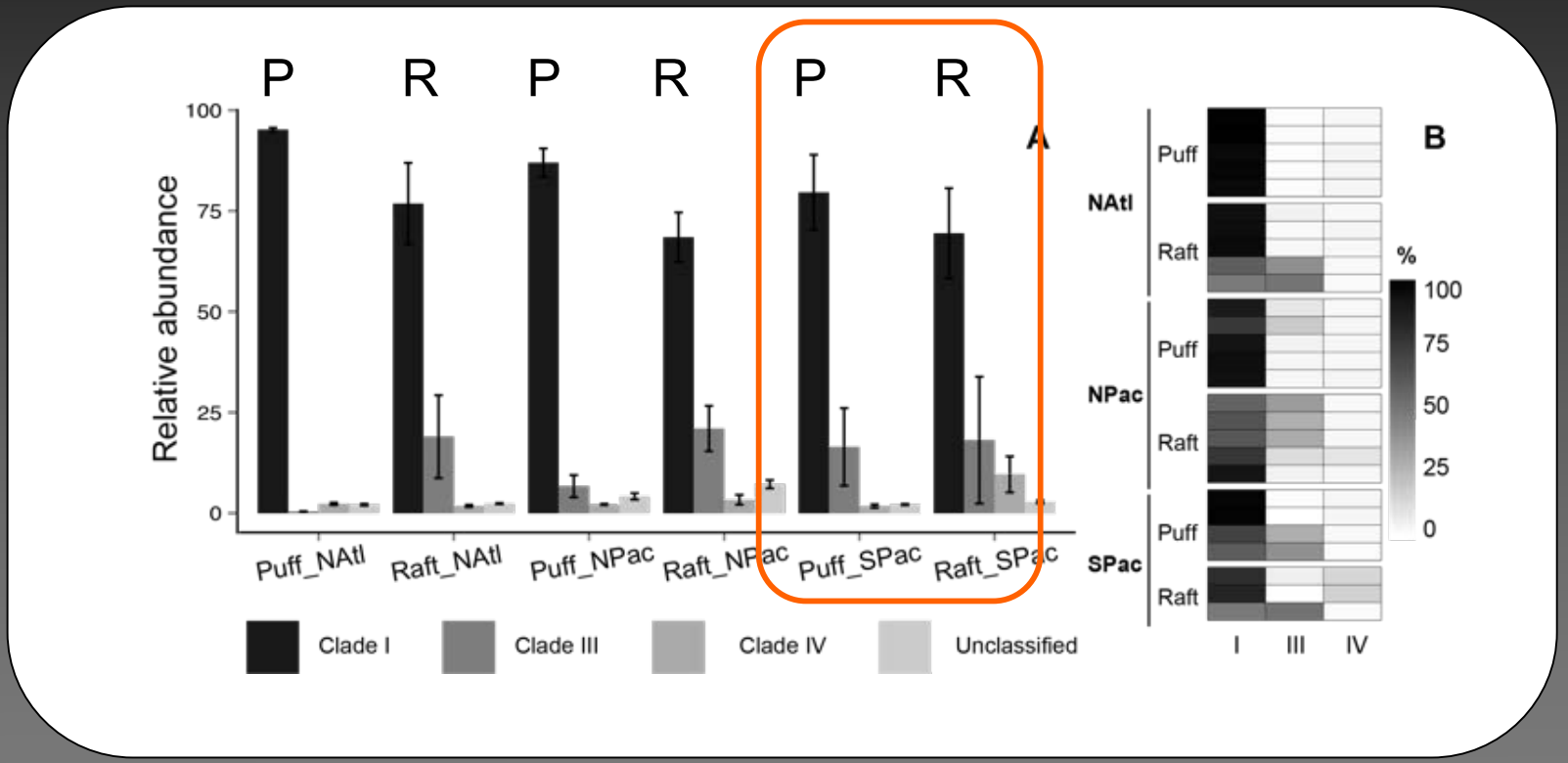


Are epibiont communities distinct as a function of colony morphology or environment?

Mónica Rouco

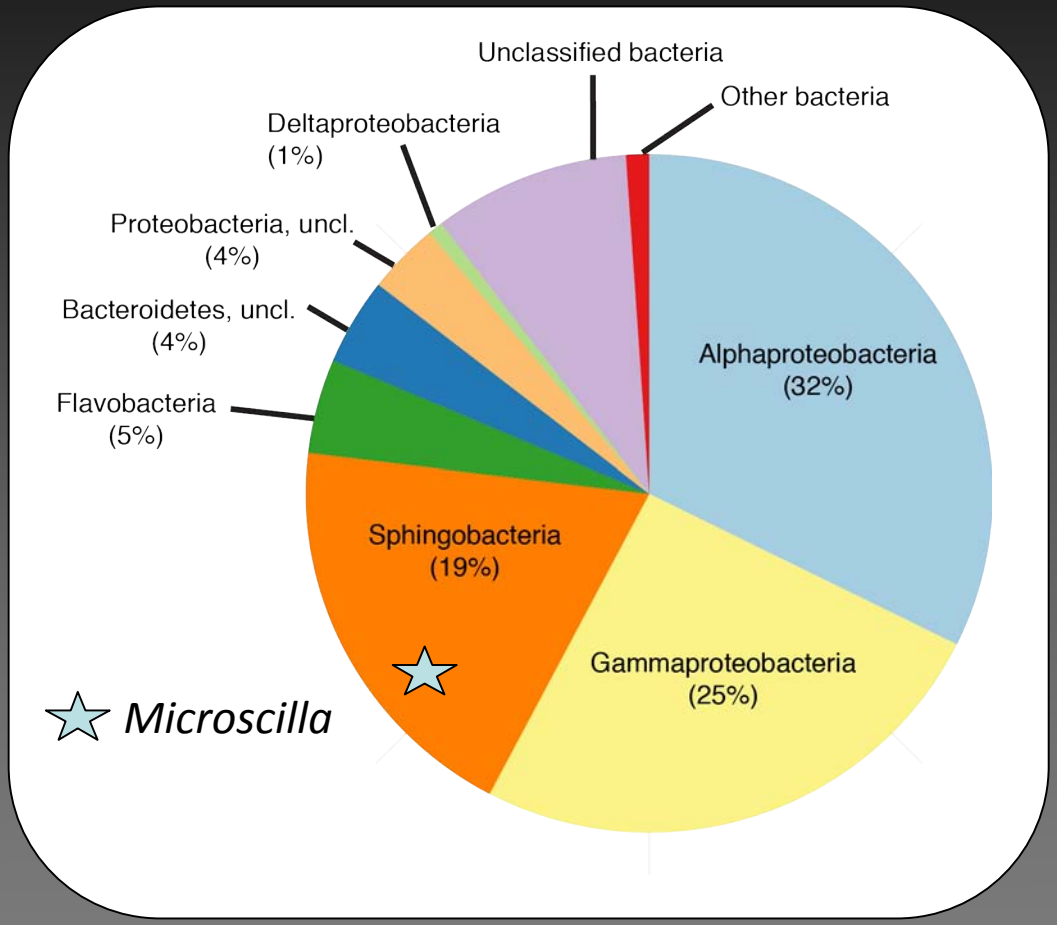
Colony composition by region

N. Atlantic N. Pacific S. Pacific



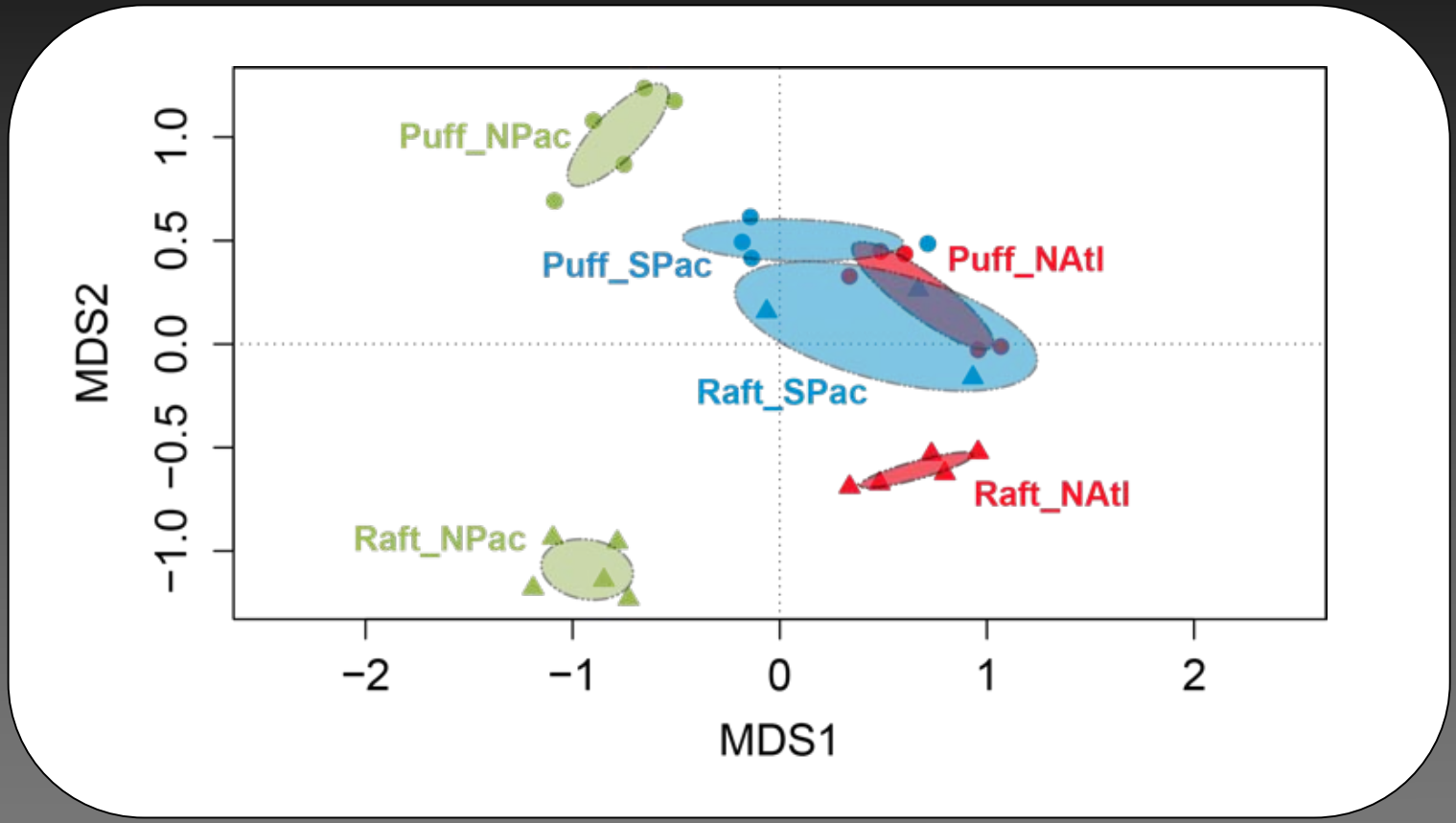
Colonies are not likely species specific, the raft morphology is more diverse except in the S. Pacific

Average epibiont community



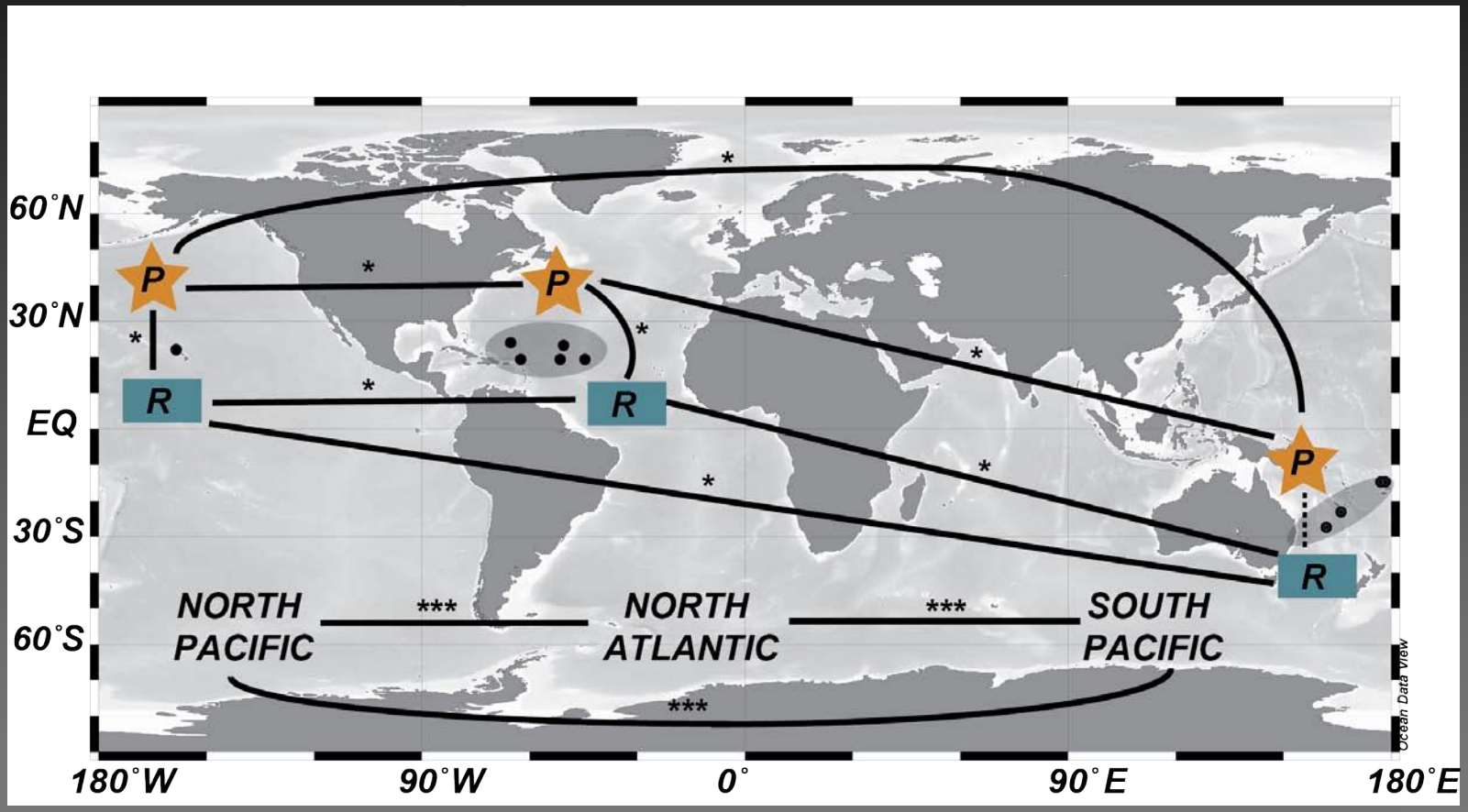
Trichodesmium colonies harbor diverse epibionts distinct from common water column bacteria.

Epibiont community diversity



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

Epibiont community diversity



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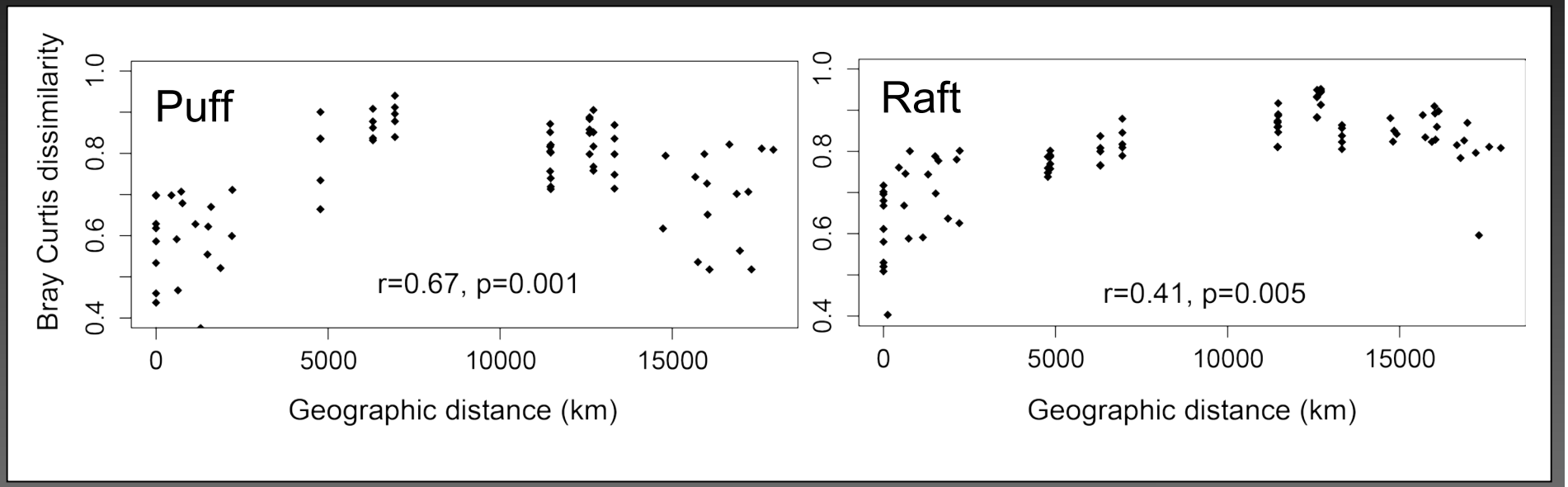
Epibiont community diversity

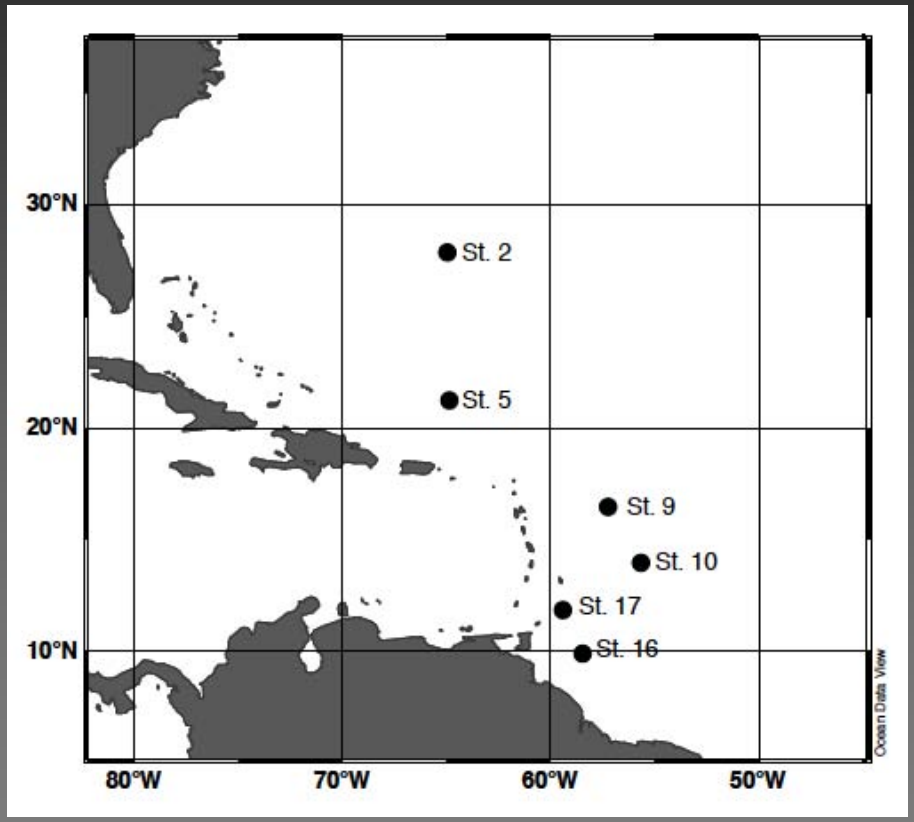


Epibiont communities are isolated by distance across ocean basins within a morphology so both neutral and selective processes may drive the holobiont structure

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.

Metagenome of the *Trichdoesmium* holobiont



1 Lane Illumina PE 100 bp
~26M PE reads / sample

↓

Assemble by station/full dataset with IDBA-UD

↓

Bin full dataset assembly by TNF and coverage using MaxBin

↓

Predict proteins from individual station assemblies using Prodigal

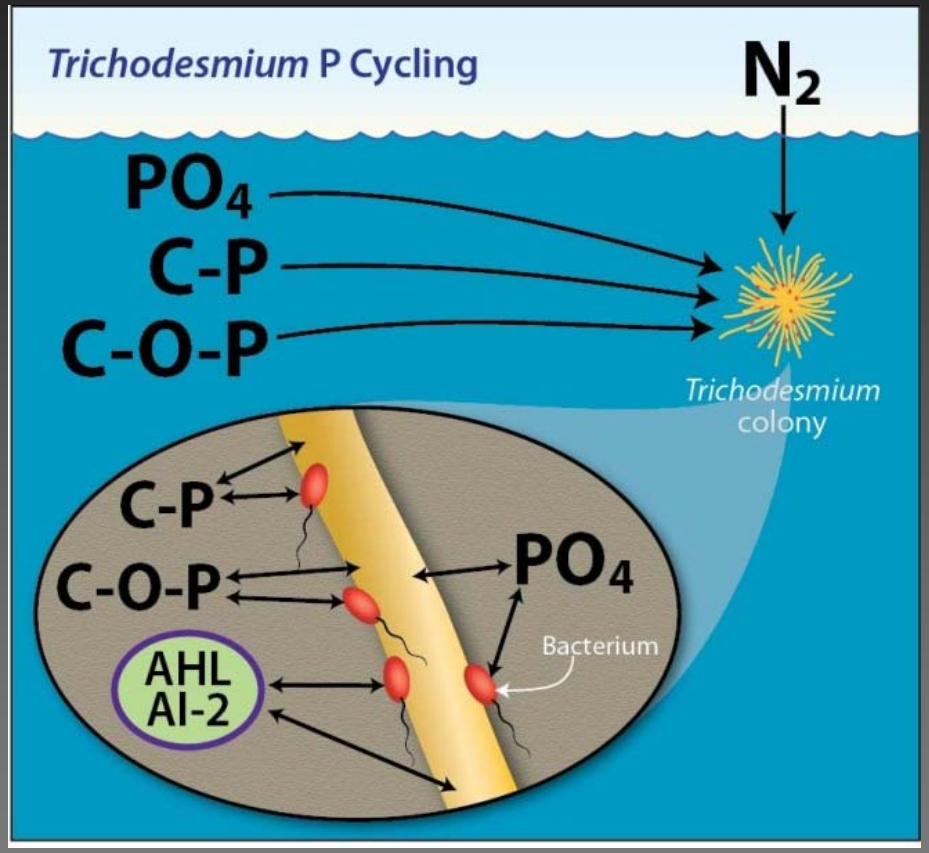
↓

Cluster predicted proteins using MCL (inflation value of 1.4)

↓

Annotate bins and predicted proteins using DIAMOND against UniRef90 etc.

Metabolic partitioning in the holobiont



Metagenomics will emphasize how metabolic potential is segregated between *Trichodesmium* clades and epibionts, and how these communities are organized

Summary - Niche spece

What is the composition of the colony and are activities influenced by communication among epibionts and *Trichodesmium*?

- Colonies harbor diverse epibionts distinct from water column, which vary by ocean region and colony morphology
- AHLs can modify epibiont alkaline phosphatase activity independent of phosphorus chemistry
- Many orthologous groups are unique to the epibionts, suggestive of their role in creating metabolic diversity
- Next steps - involve investigating these interactions in more detail - including N_2 fixation and coordinated metabolism with metagenomics and metatranscriptomics

Conclusions

The physiological ecology and metabolic traits and trade-offs of different *Trichodesmium* species or clades may be more complex than previously appreciated, and genome-enabled approaches are providing new tools to trace these relationships in the field.

Taxonomic diversity

Metabolic traits and trade-offs

Niche space