

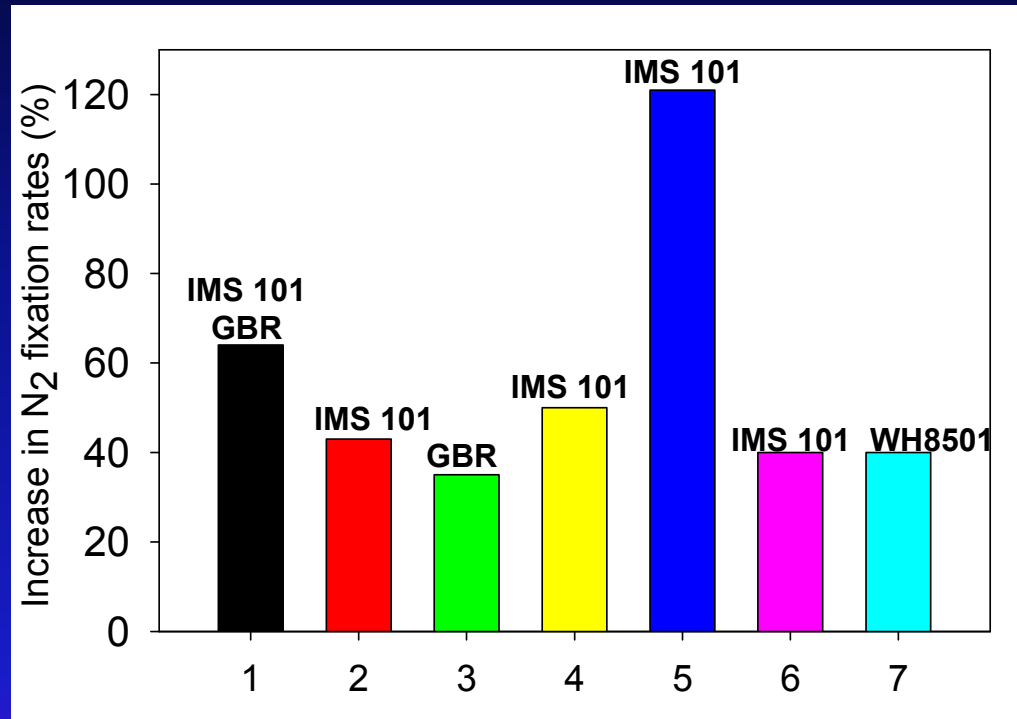
**Stuck in the fast lane:
exploring the evolutionary consequences
of long-term selection by elevated CO₂ in
*Trichodesmium***

**Dave Hutchins
Nate Walworth, Eric Webb,
Mak Saito, and Feixue Fu**

The colonial cyanobacterium *Trichodesmium* accounts for perhaps $\frac{1}{4}$ to $\frac{1}{2}$ of N_2 fixation in the oligotrophic oceans, and can be limited by Fe, P or light.



N₂ fixation rates of marine cyanobacteria can increase at high pCO₂ -but all of these studies have been brief (weeks) and used only one or two isolates.



From Hutchins et al. (2009)

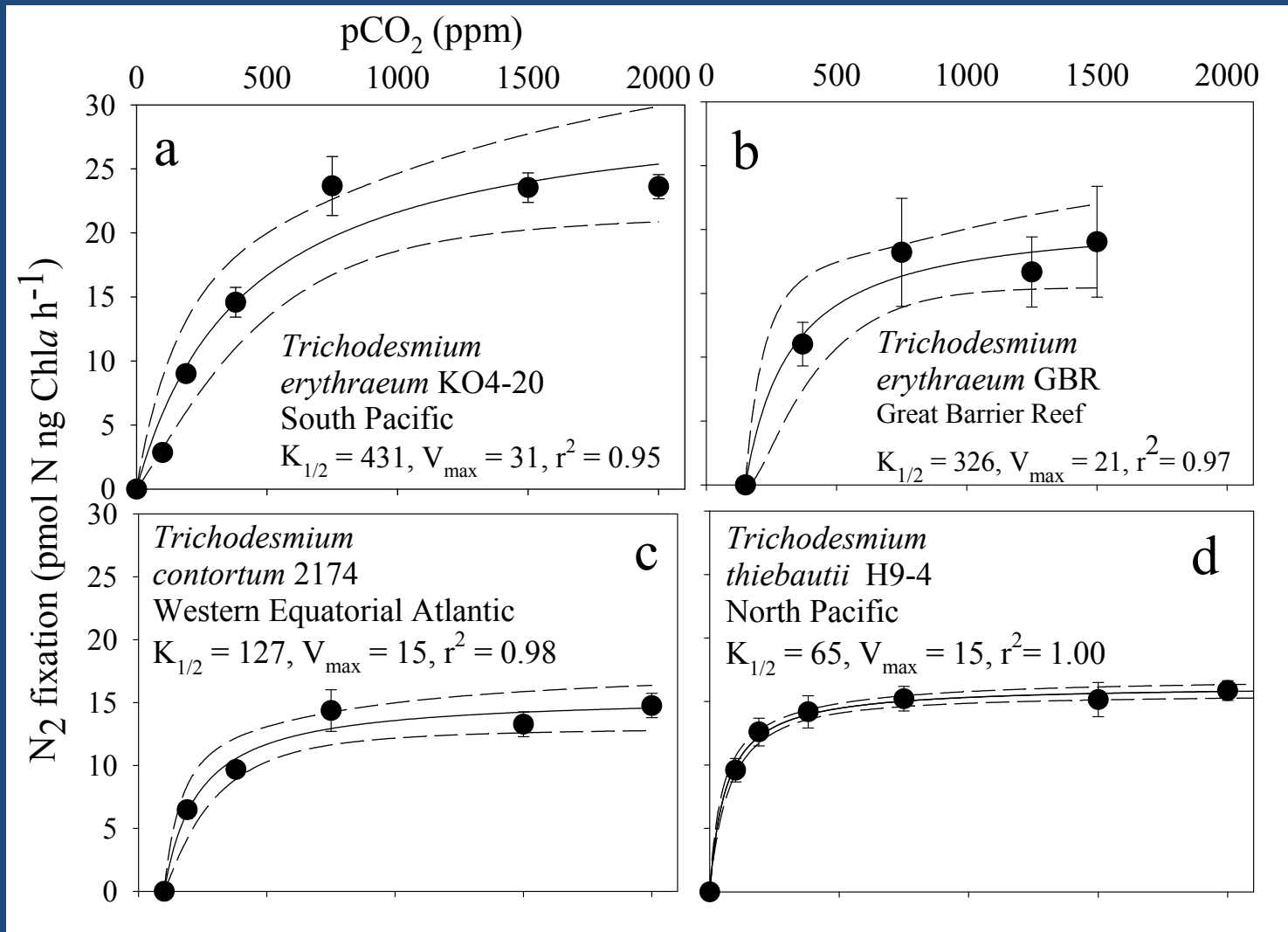
***Trichodesmium*: 1, 2, 3, Hutchins et al. 2007; 4, Barcelos e Ramos et al. 2007; 5, Levitan et al. 2007; 6, Kranz et al. 2009. *Crocospaera*: 7, Fu et al. 2008.**

Our goals

- Examine the existing diversity within the genus *Trichodesmium* for evidence that CO₂ availability has been a selective factor in its evolutionary history

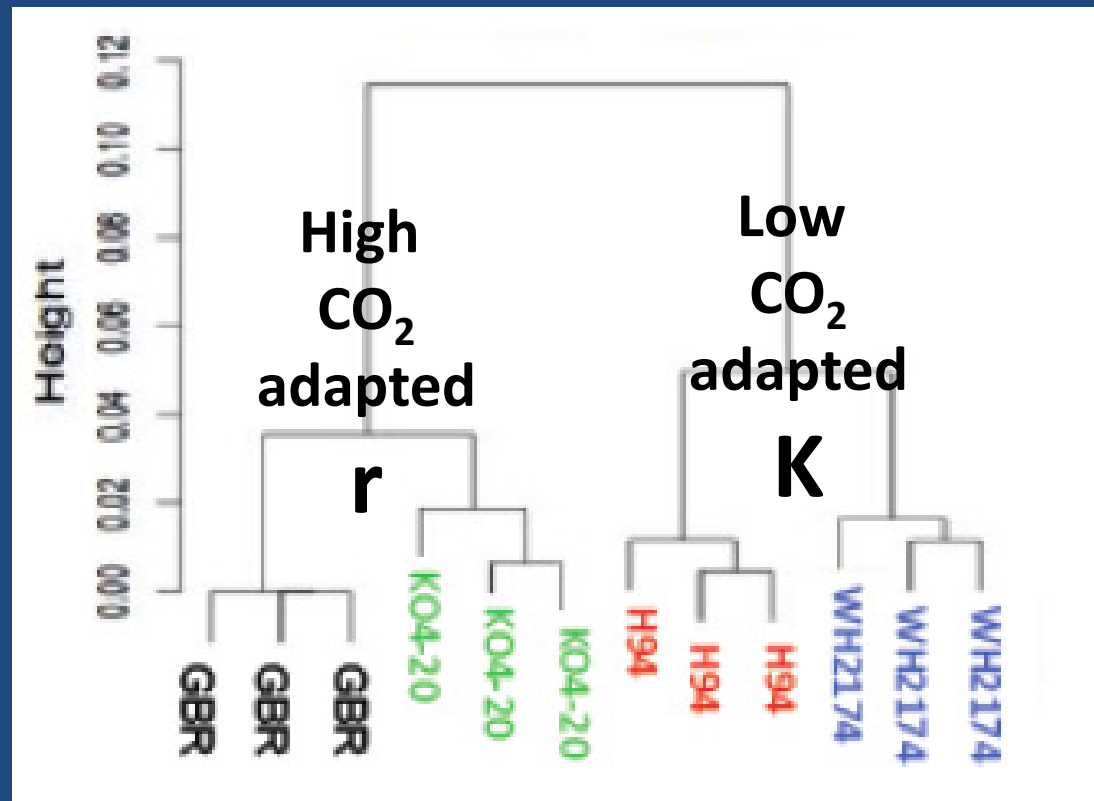
- Long-term experimental evolution studies using *Trichodesmium* cultures selected under multiple CO₂ levels

The genus *Trichodesmium* exhibits a wide diversity of responses to CO₂



Hutchins, Fu, Webb, Walworth and Tagliabue (2013)
Nature Geoscience doi: 10.1038/ngeo1858

Heirarchical clustering analysis groups *Trichodesmium* strains into “r-selected” and “K-selected” clusters



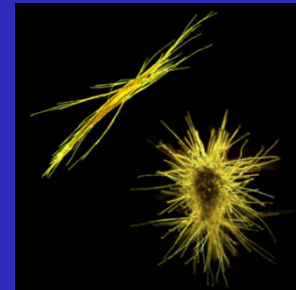
Hutchins, Fu, Webb, Walworth and Tagliabue (2013)

Nature Geoscience

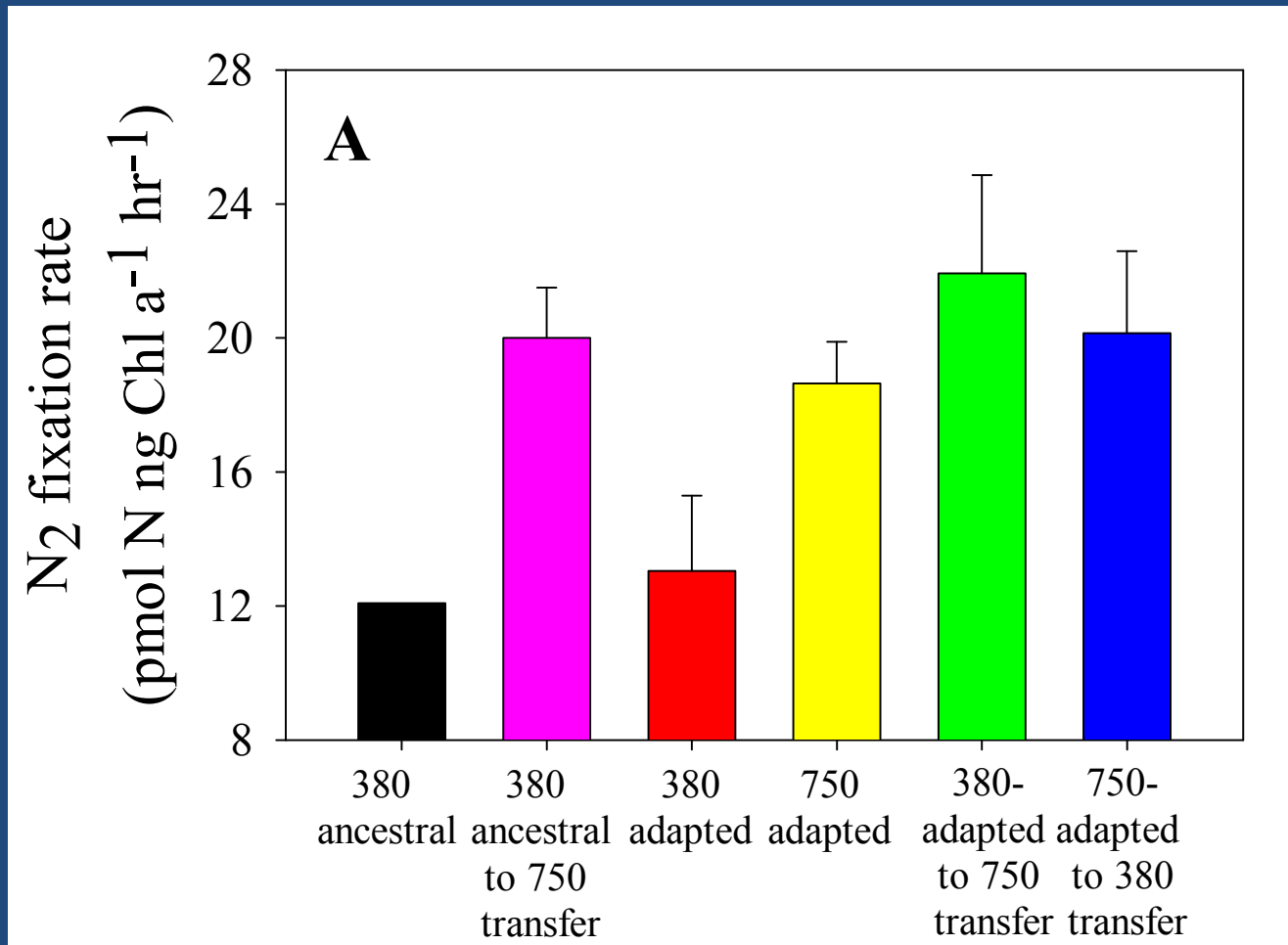
Trichodesmium CO₂ experimental evolution study

- *T. erythraeum* IMS 101 grown in steady-state cultures for 4 1/2 years (600-850 generations) at either 380 or 750 ppm CO₂ (six replicates each)
- After the adaptation period, we measured growth and N₂ fixation rates, and sampled for transcriptomics, genomics, and proteomics in four different treatments:

1. 380-adapted cell lines
2. 750-adapted cell lines
3. 380-adapted cell lines switched to 750 for two weeks.
4. 750-adapted cell lines switched to 380 for two weeks.

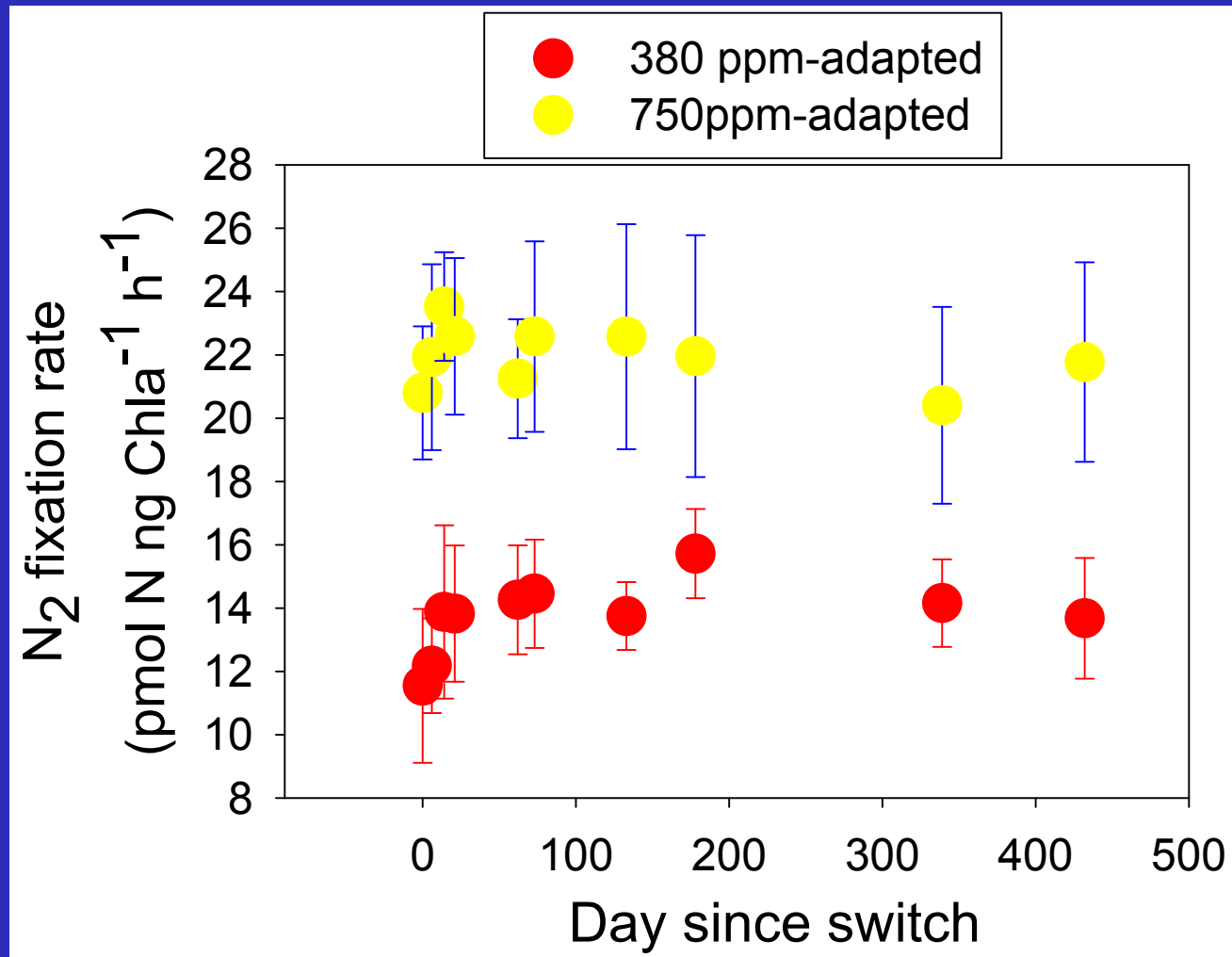


N_2 fixation rates of *Trichodesmium* ancestral, adapted, and “switch” CO_2 experimental evolution cell lines



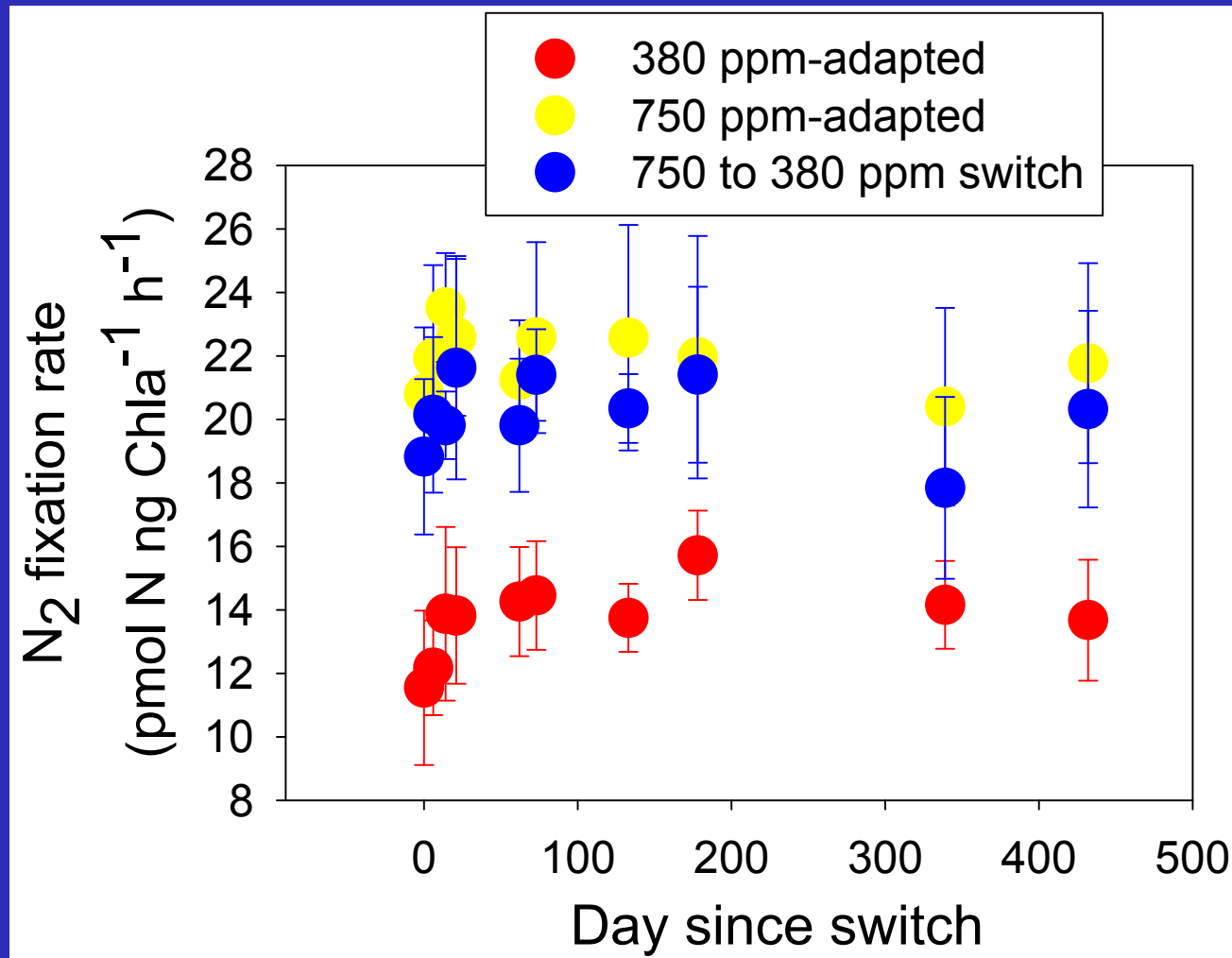
In the 750 to 380 switch cell lines, N_2 fixation rates persisted at very high levels, indistinguishable from cell lines maintained at 750

N_2 fixation rates of *Trichodesmium* 380- and 750- adapted cell lines over the subsequent 15 months



Hutchins,
Walworth,
Webb
and Fu
in prep.

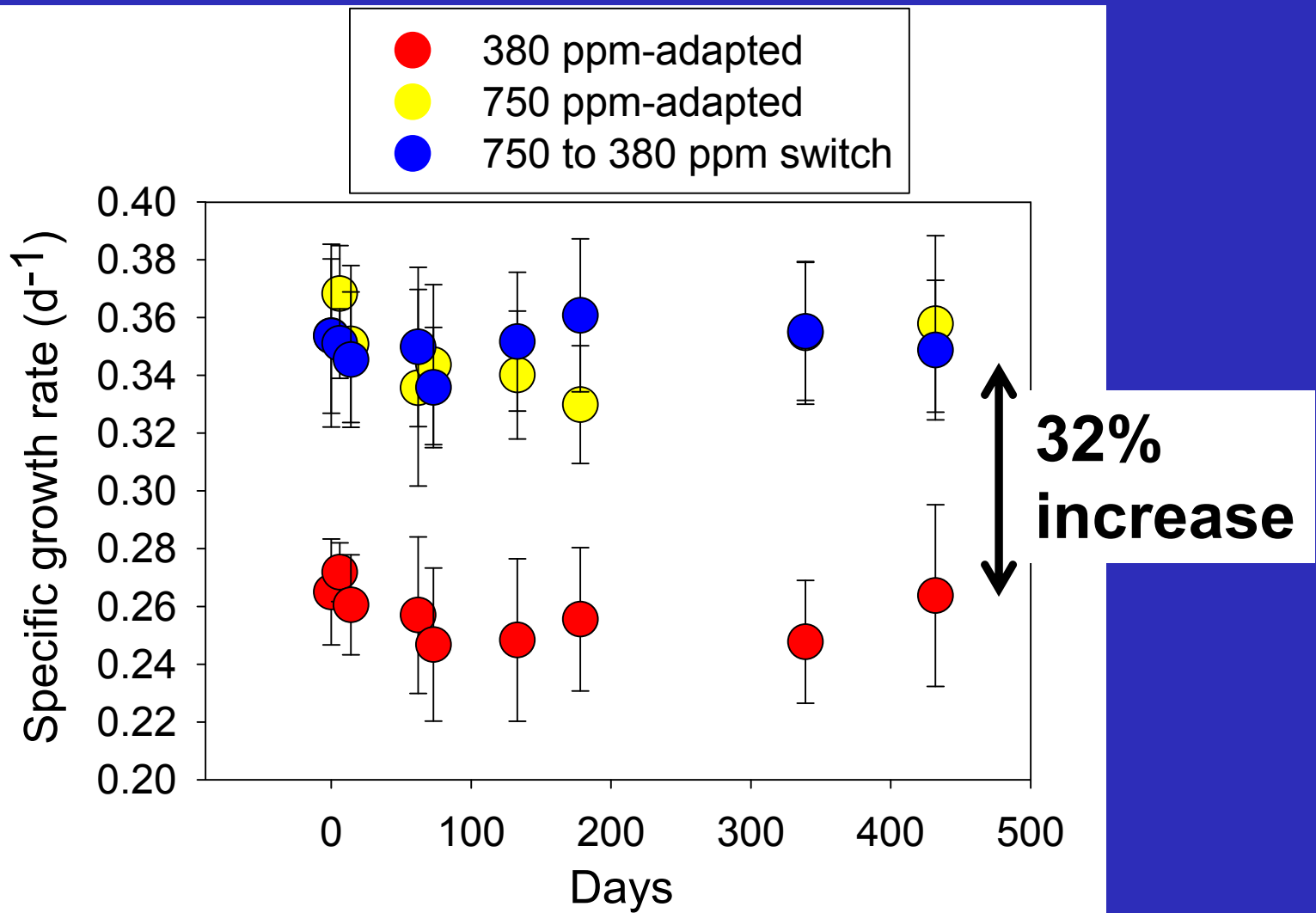
N_2 fixation rates of *Trichodesmium* 380- and 750-adapted and 750 to 380 switch cell lines over the subsequent 15 months



Hutchins,
Walworth,
Webb and
Fu
in prep.

Constitutive up-regulation of N_2 fixation rates
in all six 750 to 380 switch cell lines...

Growth rates over the subsequent 15 months



Hutchins,
Walworth,
Webb and
Fu
in prep.

In evolutionary terms, a 32% increase in fitness!

Phenotypic responses of *Trichodesmium* to long-term selection by high CO₂

- ◆ *Trichodesmium* growth and N₂ fixation rates are still strongly stimulated by elevated CO₂, even after ~600-850 generations of adaptation
- ◆ Unexpectedly, when 750 ppm-adapted cell lines are switched back to 380 ppm CO₂, high rates of N₂ fixation are maintained apparently indefinitely.
- ◆ This “high CO₂ phenotype” consisting of elevated N₂ fixation and growth rates is shared by three different treatments:
 - ◆ 750-adapted
 - ◆ 380-adapted switched to 750
 - ◆ 750-adapted switched back to 380



How can we mine the transcriptome data set for the mechanisms behind this constitutive up-regulation response?

Focus on finding genes that are expressed in the same treatment-related patterns as the phenotype (N₂ fixation and growth rate)

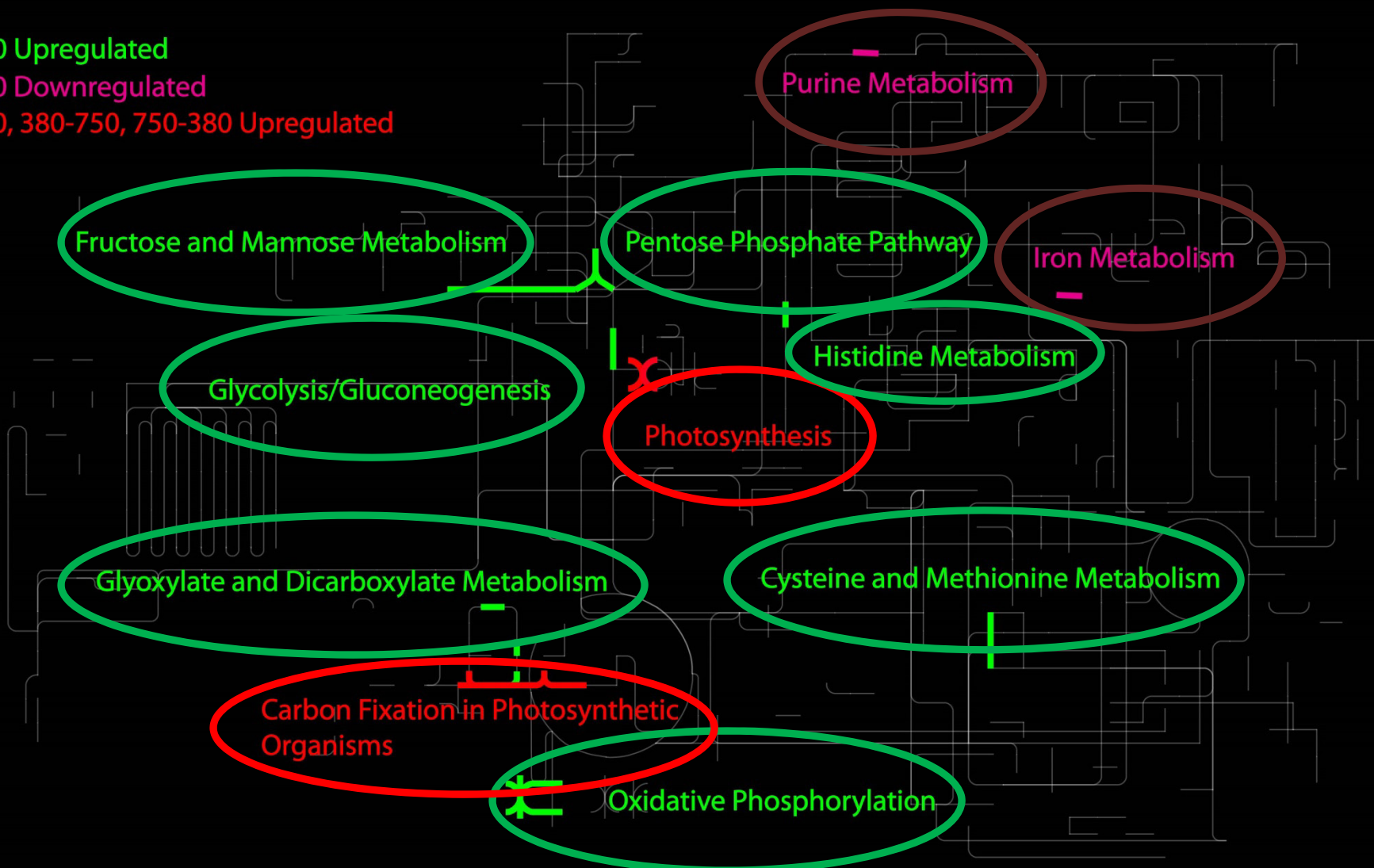
That is, they are up-regulated in the 750-adapted and 380-to-750 switch,

But aren't down-regulated again in the 750-to-380 switch...

In other words, they are shared by all of the "high CO₂ phenotype" cell lines

Metabolic map of up- and down-regulated pathways in *Trichodesmium* CO₂ evolution experiment

- 750 Upregulated
- 750 Downregulated
- 750, 380-750, 750-380 Upregulated



Genes up-regulated in the 750-adapted, 380-750 switch, and 750-380 switch:

- Various photosystem and carbon fixation genes are up-regulated in these 3 treatments with the same phenotype, including 2 that they all share:

Photosynthesis: Photosystem II protein PsbK

Carbon fixation: Ribulose-bisphosphate-carboxylase

- Not one photosynthetic or carbon fixation gene is up-regulated in the 380-adapted cell lines...

Genes up-regulated in cell lines exhibiting the increased N₂ fixation phenotype

Conspicuously lacking under high CO₂ :

Consistent up-regulation of genes coding for nitrogenase (*nif* operon), or other nitrogen metabolism pathways

The stimulation of N₂ fixation by high CO₂ appears to be based entirely on up-regulation of cellular energy-related pathways:

photosynthesis, carbon fixation,
oxidative phosphorylation, glycolysis.....

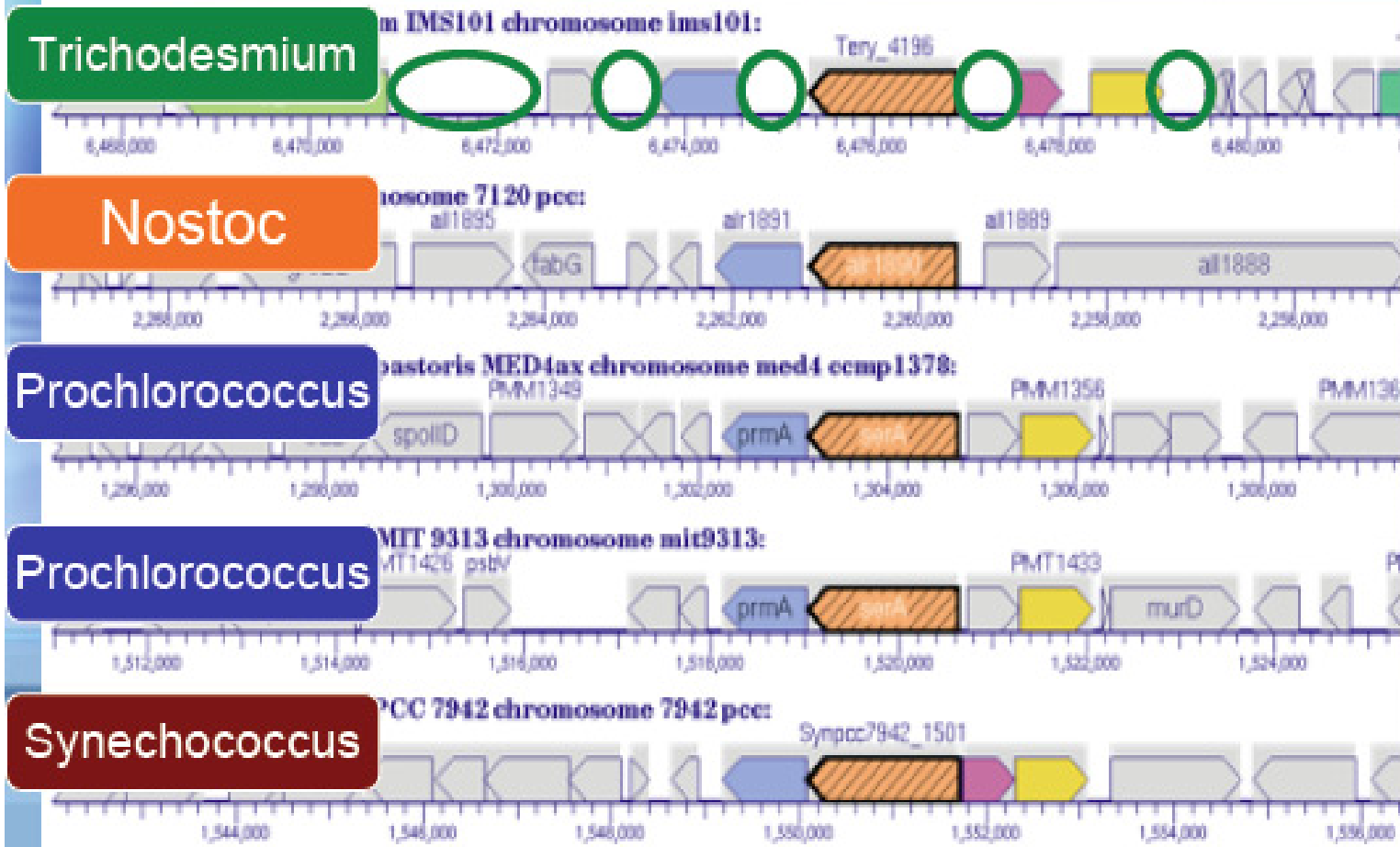
Another commonality between every
single replicate cell line of all 3
“high CO₂ phenotype” treatments:

- A putative methyltransferase is highly expressed
- Transfers a methyl group to nitrogen, carbon, or oxygen atoms in DNA or protein.
- Consistent with up-regulation of methionine metabolism in high CO₂-adapted cell lines (methyl donor)
- Indication of enhanced DNA or protein methylation in treatments showing up-regulated N₂ fixation?

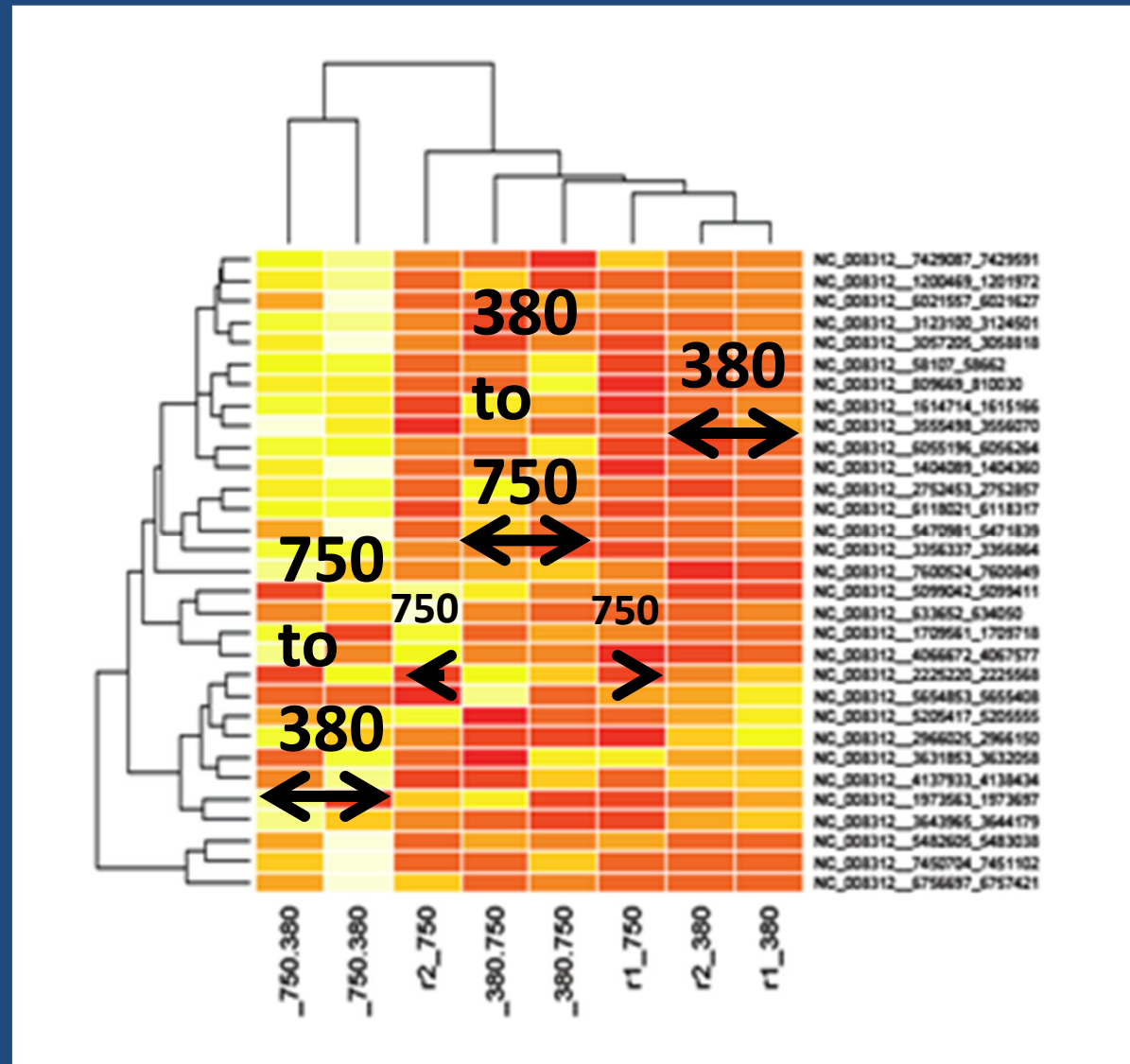
**A potential gene regulatory or
post-translational modification mechanism
that becomes “stuck in the on position”
in the 750-adapted cell lines?**

Trichodesmium has an unusual amount of intergenic DNA

A Unique Bacterial Genome



Heat map showing relative expression levels of intergenic non-coding regions in *Trichodesmium* adapted and “switch” cell lines



Walworth
et al.
in prep.

Intergenic expression is strongly upregulated in the 750 to 380 switch cultures (brighter yellow colors in the two left-hand columns)

Highly expressed intergenic regions

Some are known gene regulatory regions such as promoters

Many others are different- they are expressed as small non-coding RNAs (ncRNAs- confirmed by northern blot analyses)

Small ncRNAs have been shown to perform gene expression regulatory functions during stress responses in other bacteria

These expressed intergenic regions are not just “junk DNA”!

They may play a crucial role in controlling the responses of *Trichodesmium* to environmental change...

Preliminary genomics results

Illumina sequencing allows us to compare the IMS 101 genome:

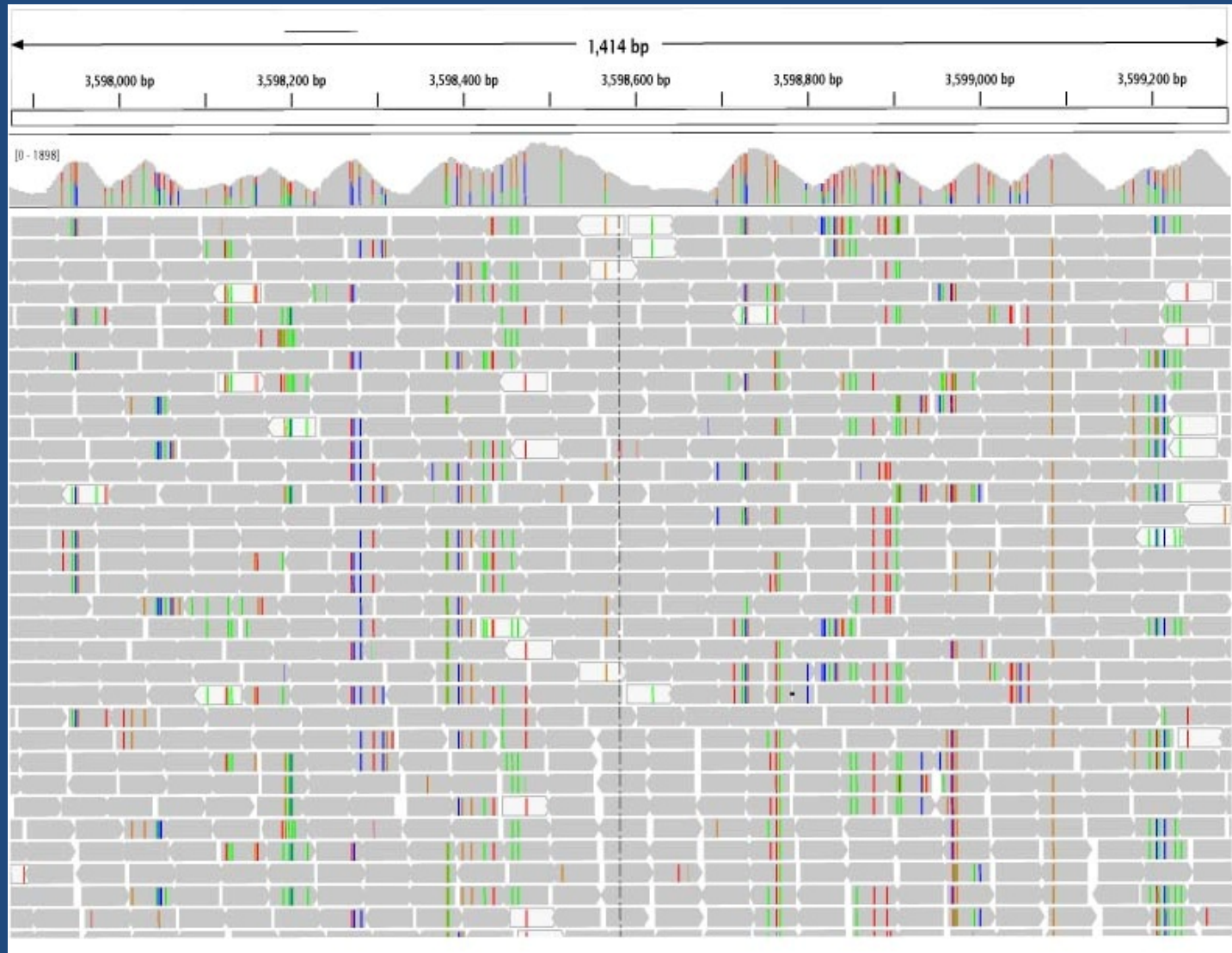
- 1) When the original reference genome was sequenced (~15 years ago)
- 2) At the beginning of our evolution experiment (T_0 , ~5 years ago)
- 3) Now, in the 380-adapted and 750-adapted cell lines after ~5 years of selection

Possible types of mutations include single nucleotide polymorphisms (SNPs), insertions, and deletions

Considerable evolution has occurred between the original IMS 101 reference genome and the beginning of our experiment 10 years later...

The original clonal cell line has diverged into many sub-populations with differing single nucleotide polymorphism (SNP) patterns

Illumina read pileup figure of SNPs in the gas vesicle region of the *Trichodesmium* genome, in a single replicate cell line



A surprising amount of genetic diversity for a “clonal” culture!

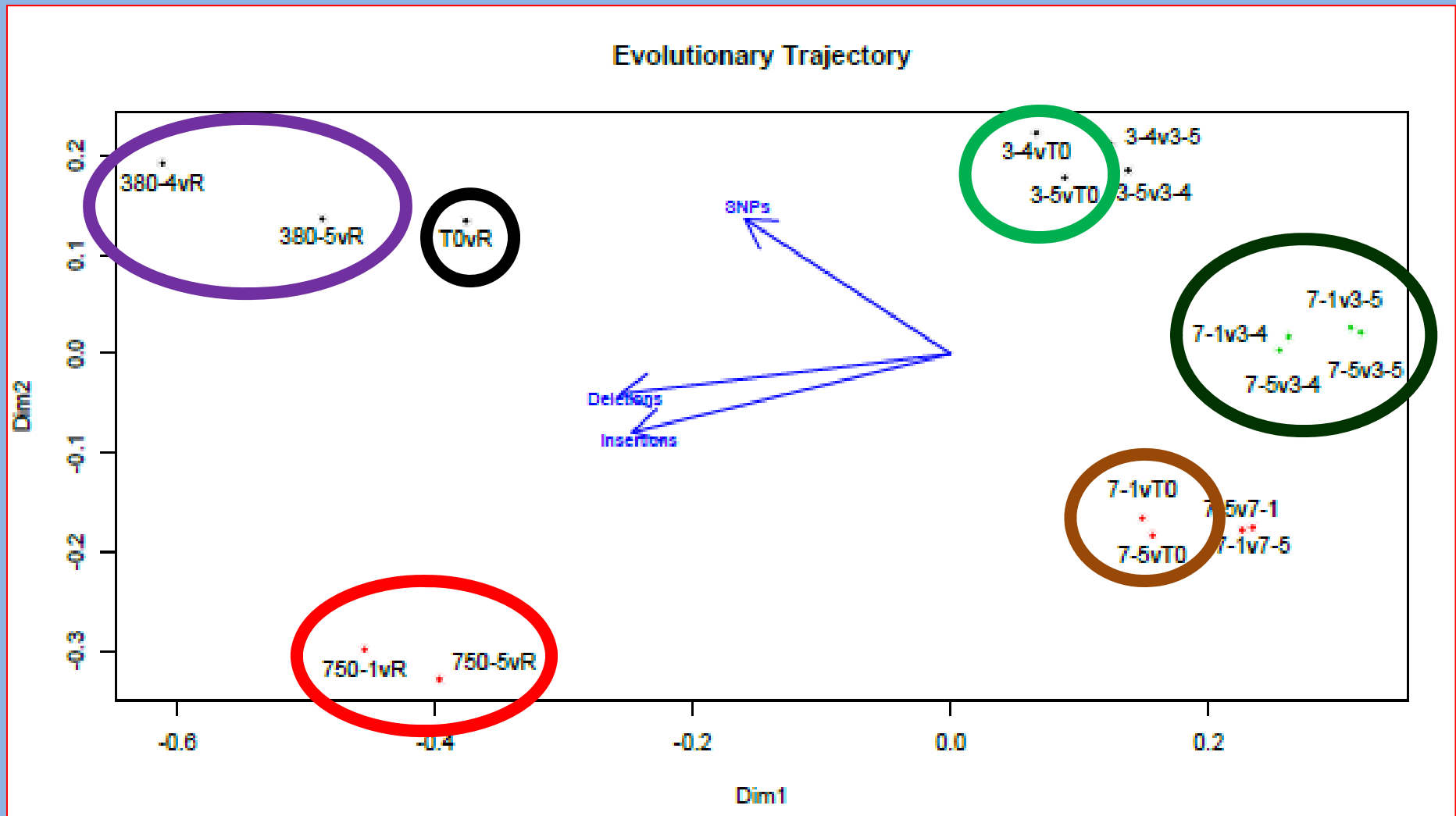
Despite this unexpected diversity, some new SNPs (relative to the reference genome) are ubiquitous across all the sub-populations-

“Selective sweeps” have occurred...

One is in the ferric uptake regulator gene (fur)

An adaptation to being grown
in artificial trace metal-buffered medium?

The replicate cell lines in each treatment appear to have unique evolutionary trajectories relative to every other treatment, in terms of numbers and types of mutations (MDA plot)



Our T0 versus the reference genome
 380-adapted versus the reference genome
 750-adapted versus the reference genome

380-adapted versus the T0 genome
 750-adapted versus the T0 genome
 750-adapted versus 380 adapted

Biogeochemical questions and their implications

- If *Trichodesmium* adapts “irreversibly” to future higher CO₂ levels, when and how will it be able to re-adapt to lower levels?
- Can this help explain the apparent existence of high- and low-CO₂ adapted strains of *Trichodesmium*?
- What are the implications of constitutive up-regulation of N₂ fixation for Fe and P limitation of diazotrophs?
- What other kinds of evolutionary surprises are in store from “well-understood” biogeochemically-critical organisms like *Trichodesmium*?



Current and future work

- 1) Genome and proteome comparisons with transcriptomes, targeted rt-QPCR for specific genes of interest
- 2) Isolation and comparison of new clonal cell lines isolated from our “mixed population” CO₂-adapted *Trichodesmium* cultures (N₂ fixation physiology, genome sequencing)
- 3) Responses of our 350- and 750-adapted cell lines to Fe and P limitation
- 4) Characterization of associated bacterial meta-communities in the adapted cell lines.
- 5) Experimental evolution of other *Trichodesmium* species/strains (high- versus low-CO₂ adapted strains, fresh isolates)
- 6) Experimental evolution of unicellular *Crocospaera* (faster growing, amenable to cryopreservation)

Acknowledgements



Support from NSF OCE Biological Oceanography

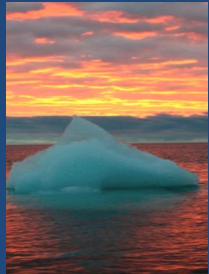
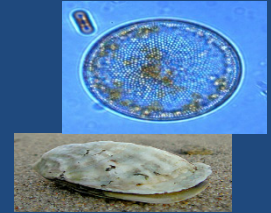
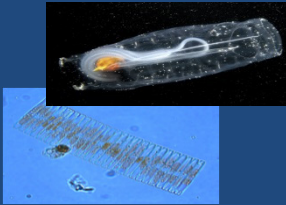
New Gordon Research Conference: Ocean Global Change Biology

Dave Hutchins (chair), Phil Boyd (vice-chair)
Adina Paytan and Shannon Meseck (co-organizers)

July 6-11, 2014 at the Waterville Valley Resort, NH.

Session overview:

1. Differential vulnerability of ecosystems to global change
2. Paleo proxies for multiple environmental stressors
3. Adaptation and evolution under selection by global change
4. Ocean acidification effects on nutrients and trace metal supply
5. The interplay between warming, ocean acidification and ocean hypoxia
6. Interactive effects- evidence of biological synergism and antagonisms
7. Remote sensing and ecosystem modeling of multiple environmental stressors
8. Physiological basis for interactions between anthropogenic stressors
9. The socio-economic impacts of ocean acidification



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or visit the GRC website: <http://www.grc.org/programs.aspx?year=2014&program=oceanglob>

