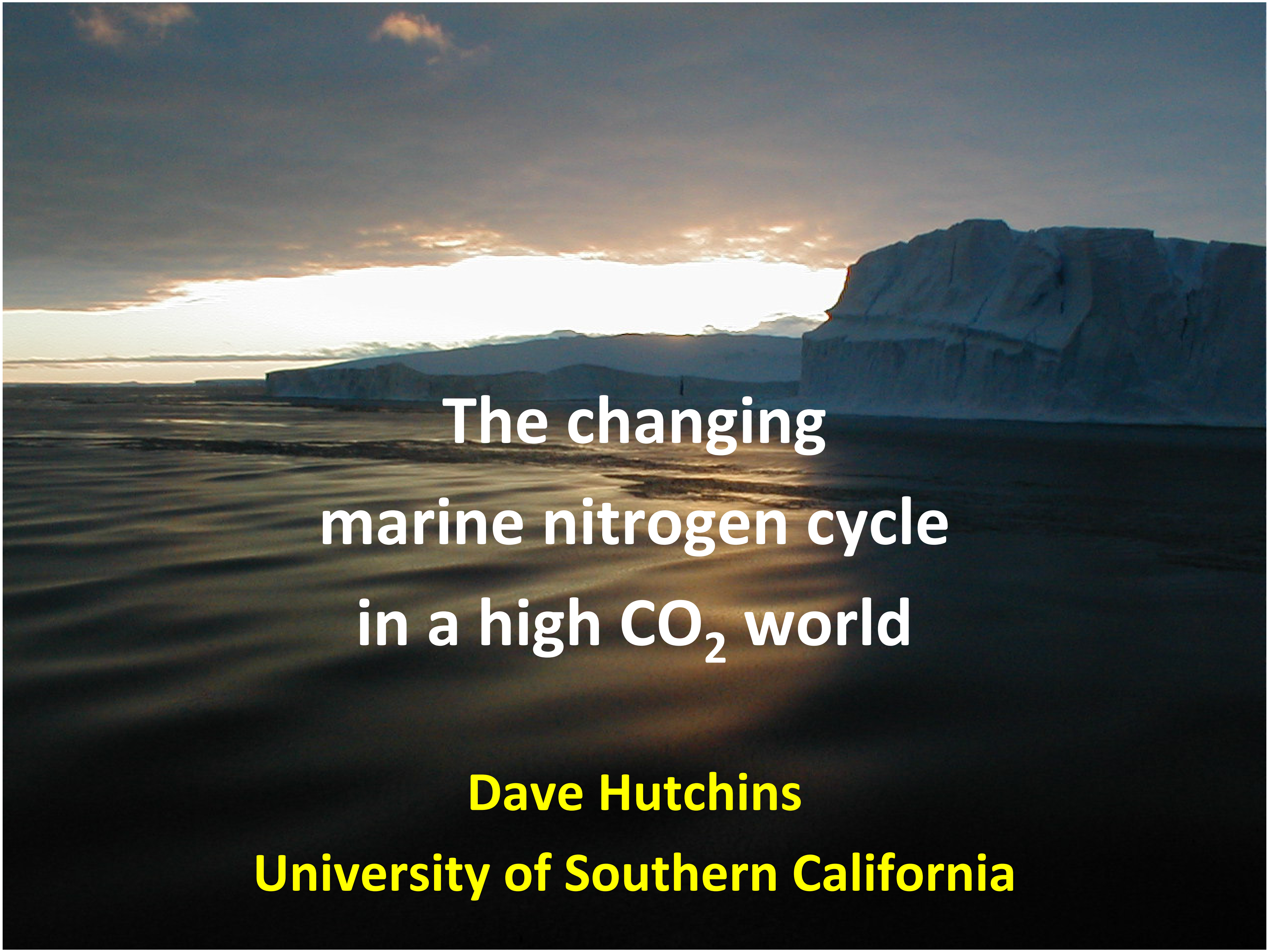
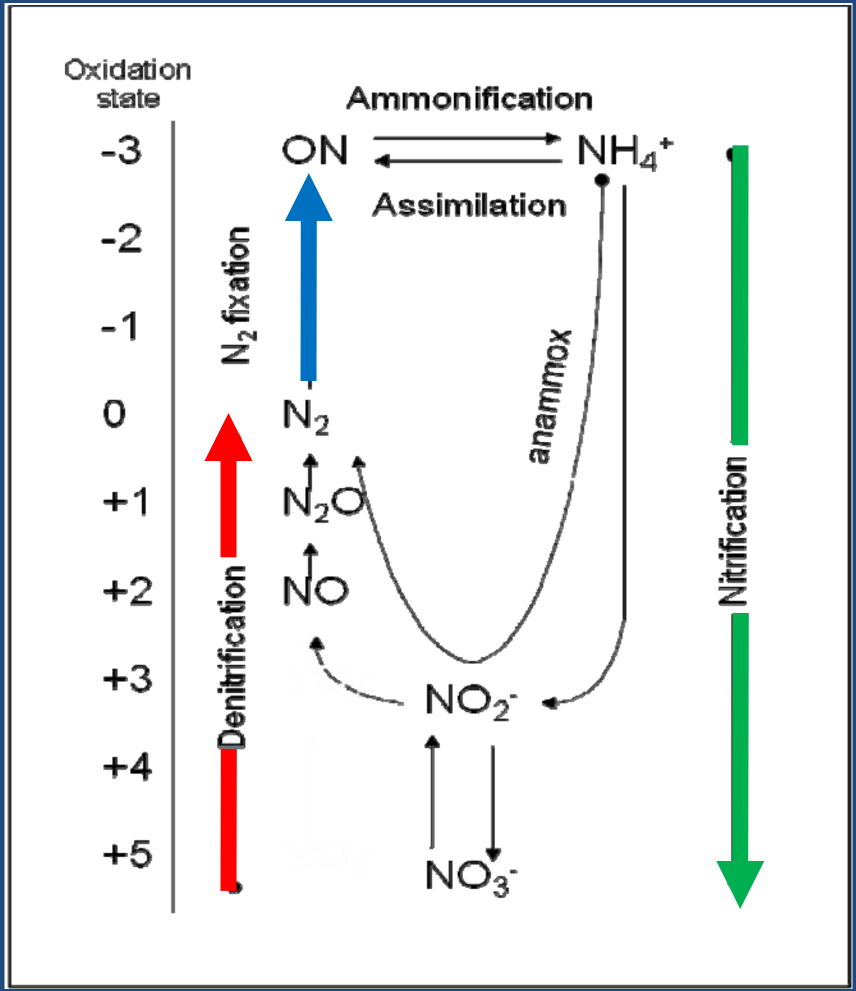
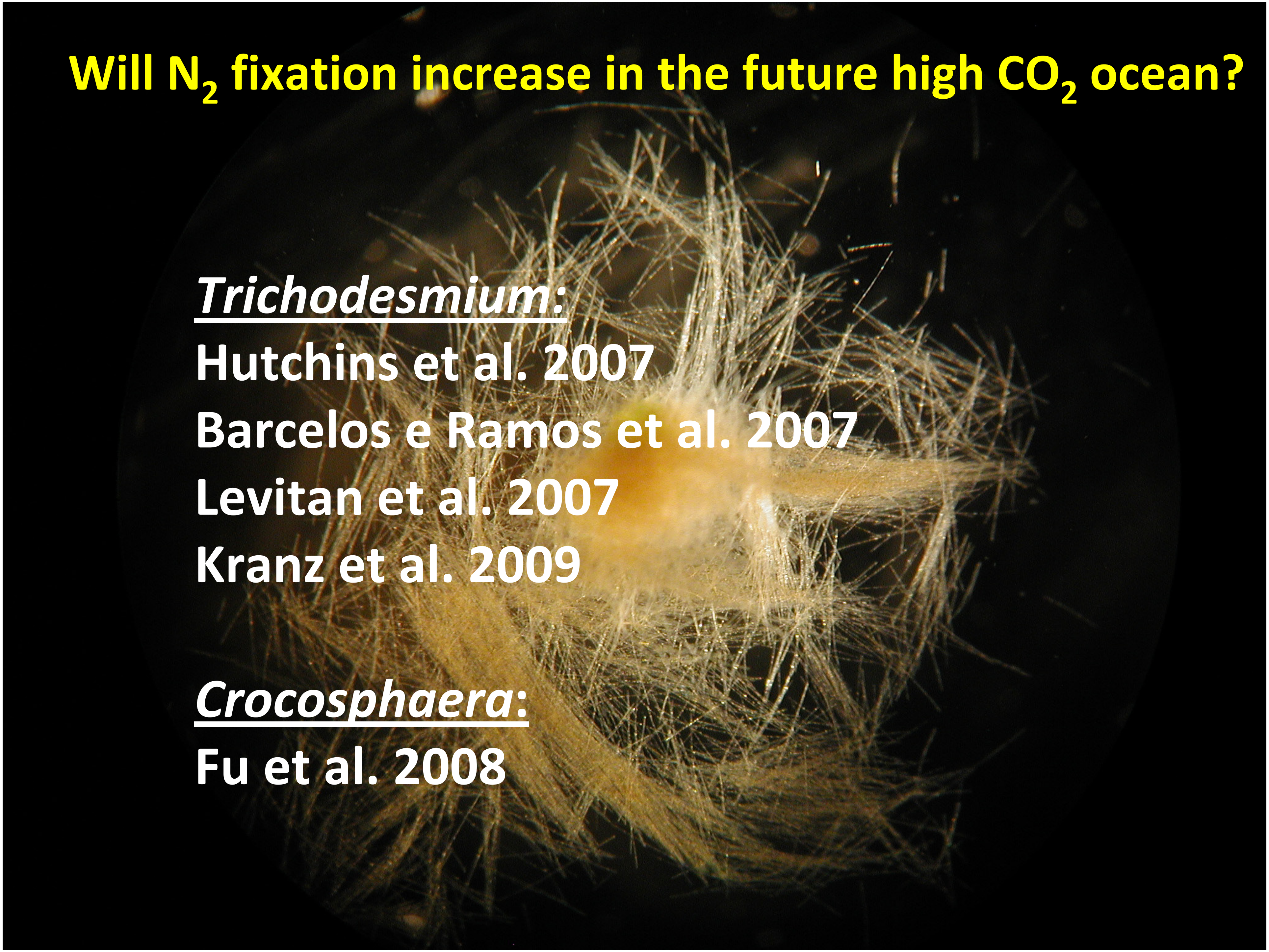

The changing
marine nitrogen cycle
in a high CO_2 world
Dave Hutchins
University of Southern California

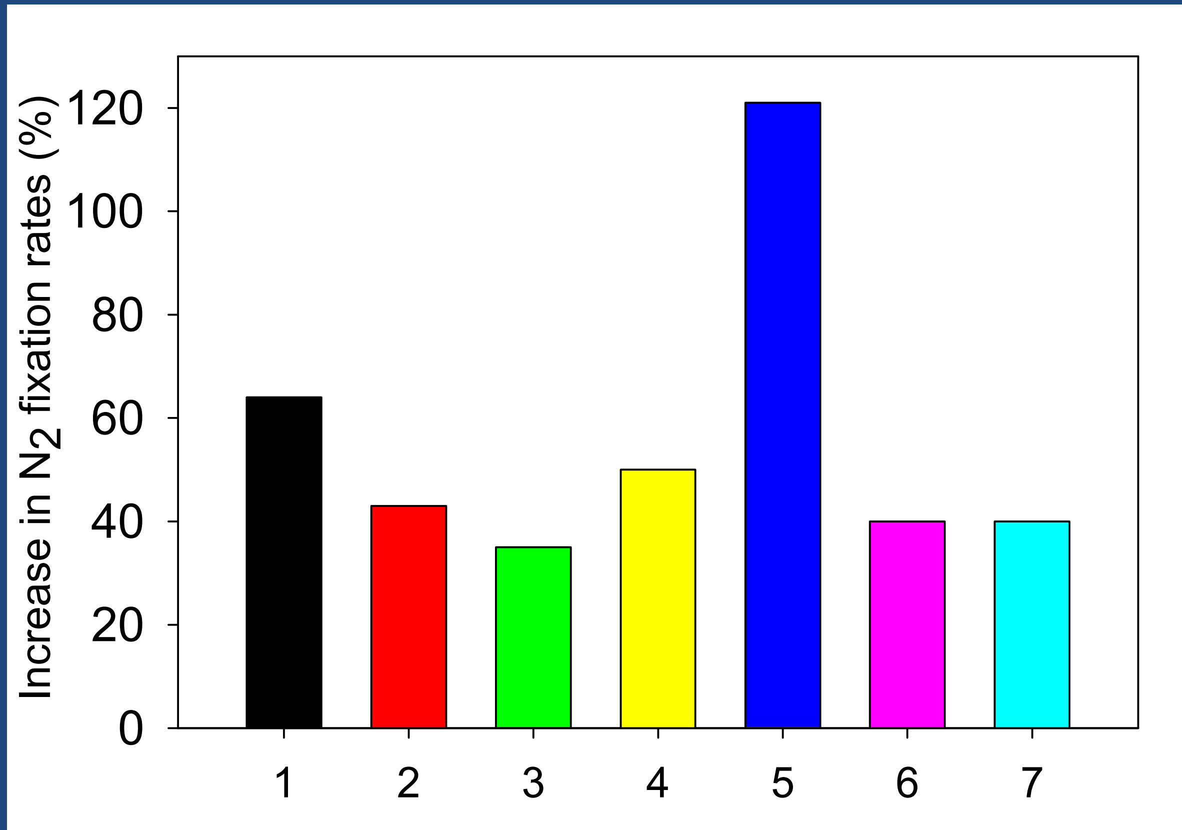
The marine N cycle



C : N : P


Will N_2 fixation increase in the future high CO_2 ocean?
Trichodesmium:
Hutchins et al. 2007
Barcelos e Ramos et al. 2007
Levitan et al. 2007
Kranz et al. 2009
Crocosphaera:
Fu et al. 2008

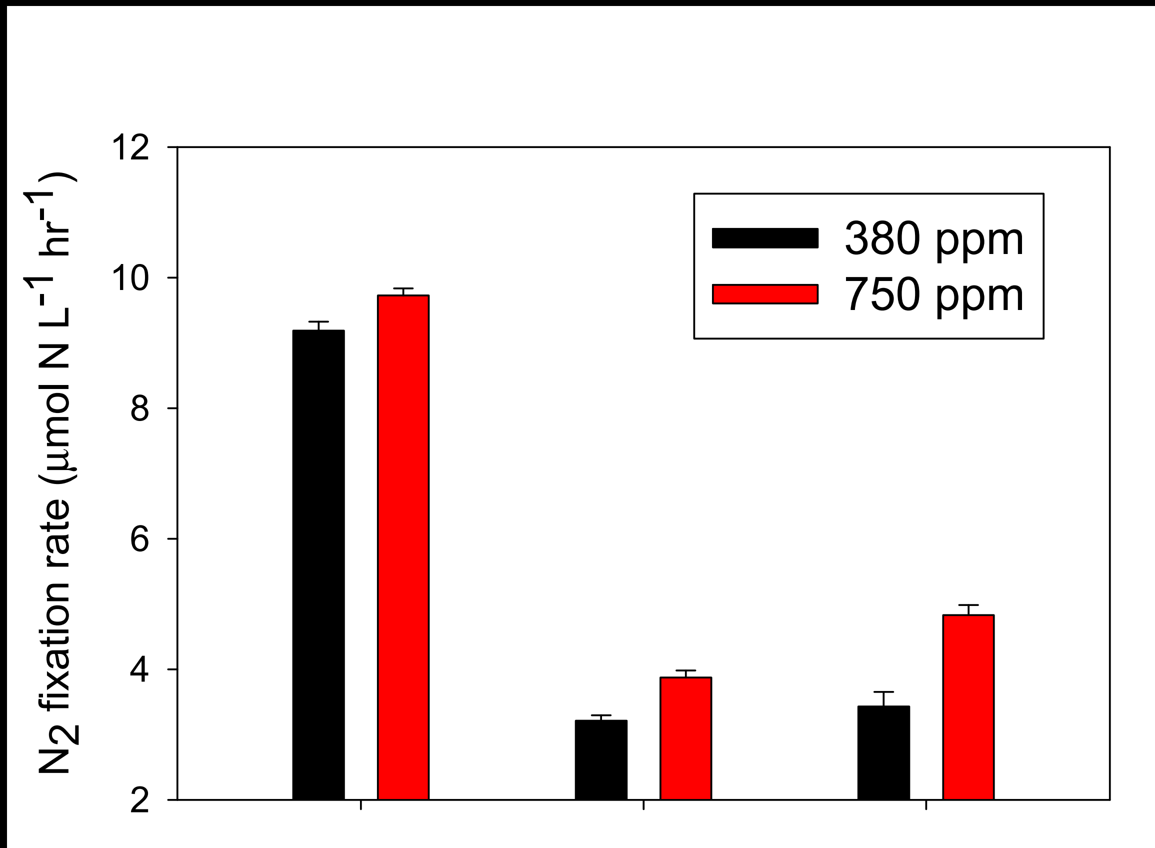
cyanobacteria



1) *Trichodesmium erythraeum* strain GBR at 29°C, 380-750 ppm CO_2 (Hutchins et al. 2007)
2) *T. erythraeum* strain GBR at 25°C, 380-750 ppm CO_2 (Hutchins et al. 2007)
3) *T. erythraeum* strain IMS 101 at both 25°C and 29°C, 380-750 ppm CO_2 (Hutchins et al. 2007)
4) *T. erythraeum* strain IMS 101 at 25°C, 380-750 ppm CO_2 (Barcelos e Ramos et al. 2007)
5) *T. erythraeum* strain IMS 101 at 25°C, 400-900 ppm CO_2 (Levitan et al. 2007)
6) *T. erythraeum* strain IMS 101 at 25°C, 370-1000 ppm CO_2(Kranz et al. 2009)
7) *Crocosphaera watsonii* strain WH8501 at 28°C, 380-750 ppm CO_2 (Fu et al. 2008)

Hutchins et al. submitted, Oceanography

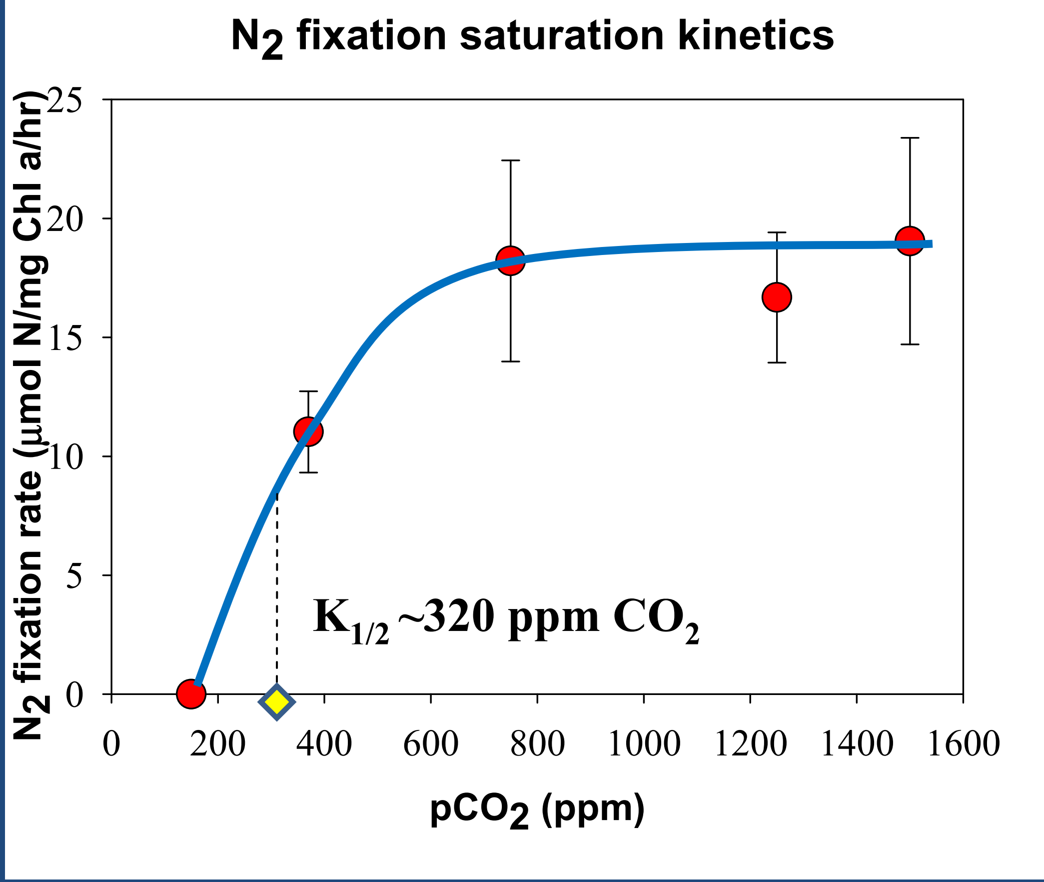
Short-term CO_2 enrichments using natural *Trichodesmium* colonies from the Gulf of Mexico

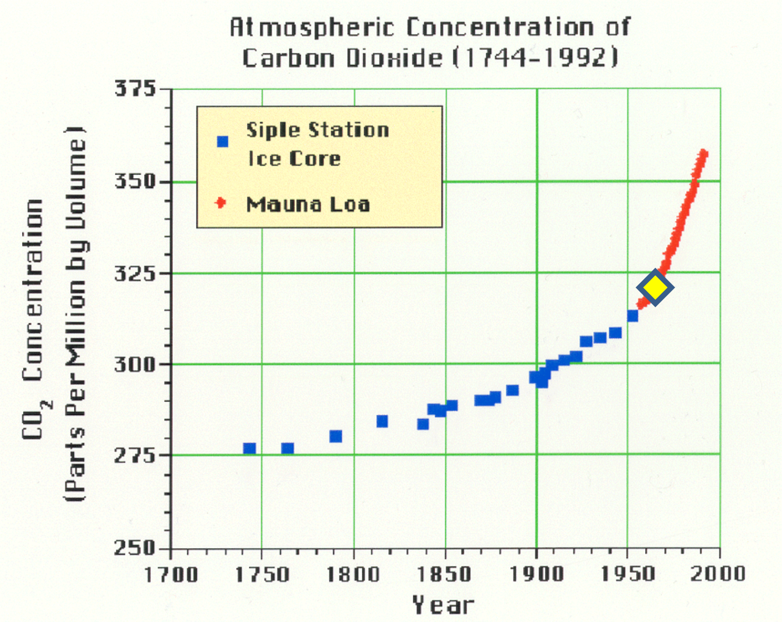


N_2 fixation rates increased 6- 41% within a few hours of elevating pCO_2 to 750 ppm

Hutchins et al. submitted, Oceanography

CO_2 as a "limiting nutrient" for N_2 fixation: Michaelis-Menten kinetics

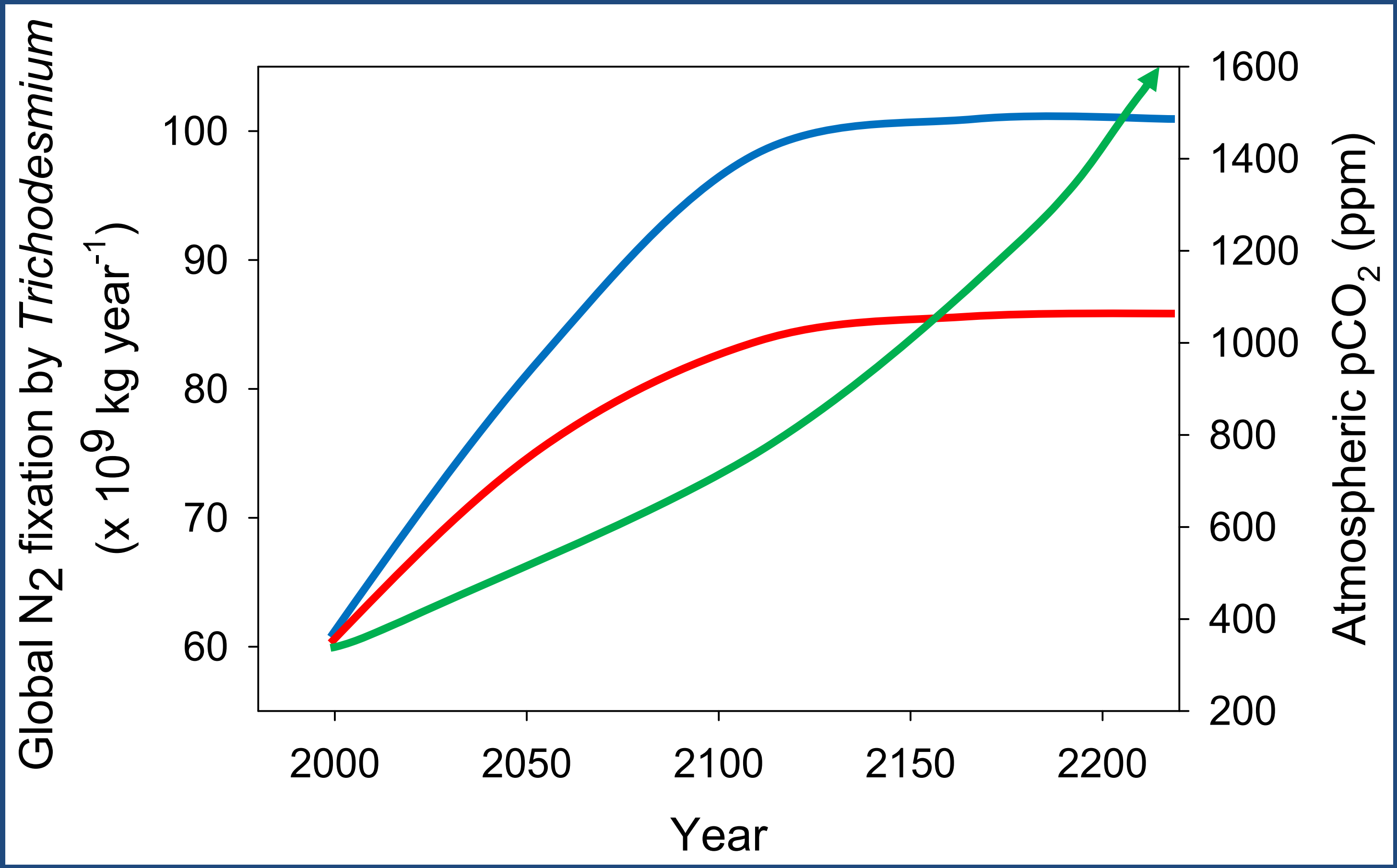




Data for *Trichodesmium* strain GBR

Hutchins et al. 2007, Limnology and Oceanography 52

Future trends in global N_2 fixation by *Trichodesmium*?

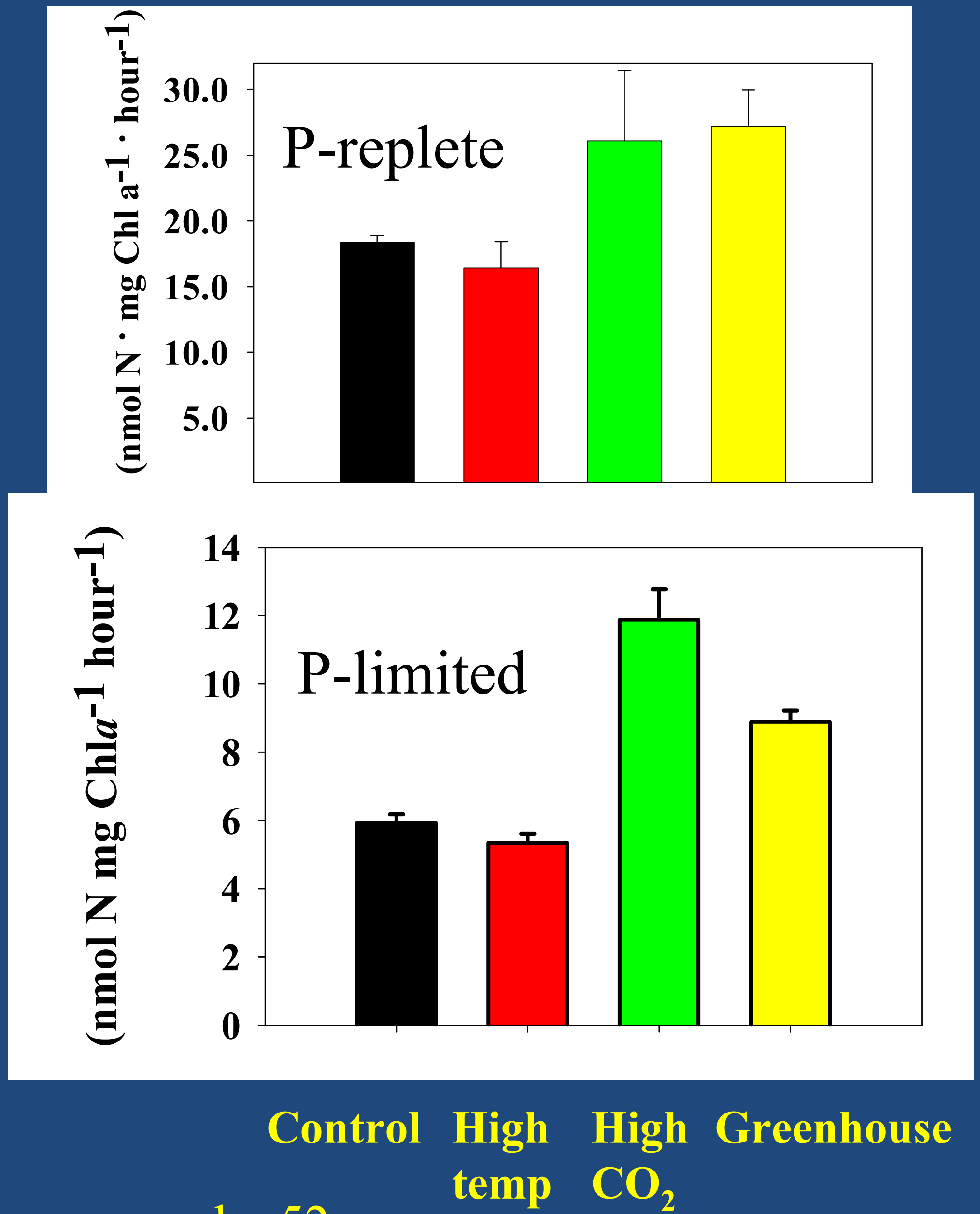


Maximum (blue) and minimum (red) projected annual global N_2 fixation increases versus pCO_2 (green)

Hutchins et al. submitted, Oceanography

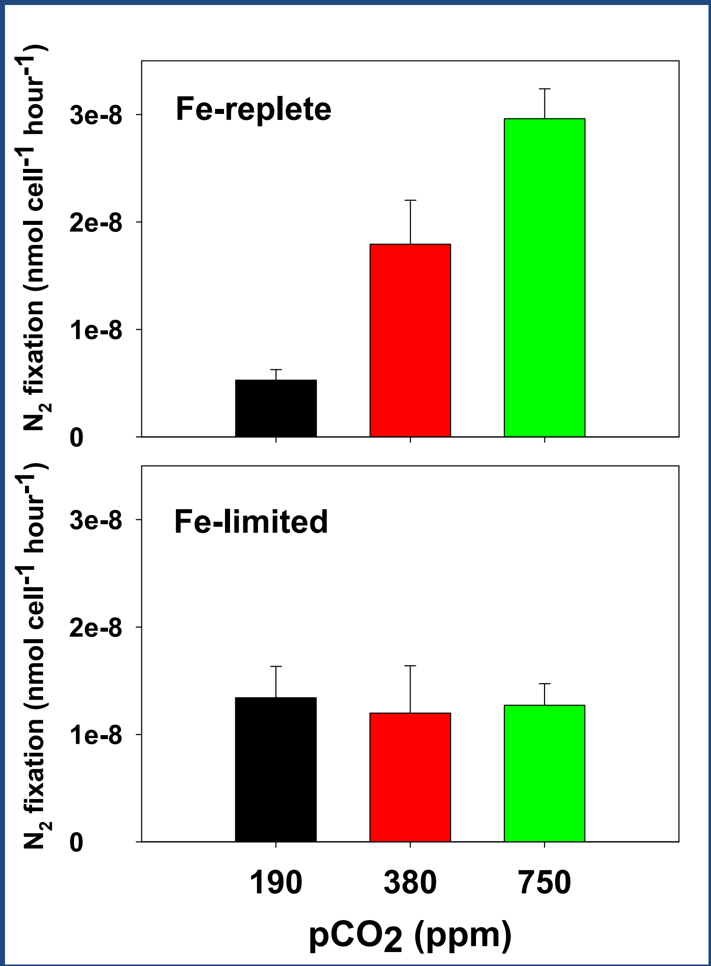
pCO_2 and P co-limitation of *Trichodesmium* N_2 fixation

Adding <u>either</u> P or CO_2 will increase N_2 fixation and growth rates of P-limited cultures at present day pCO_2



Hutchins et al. 2007, Limnology & Oceanography 52

Crocosphaera: N_2 fixation rates as a function of Fe and CO_2



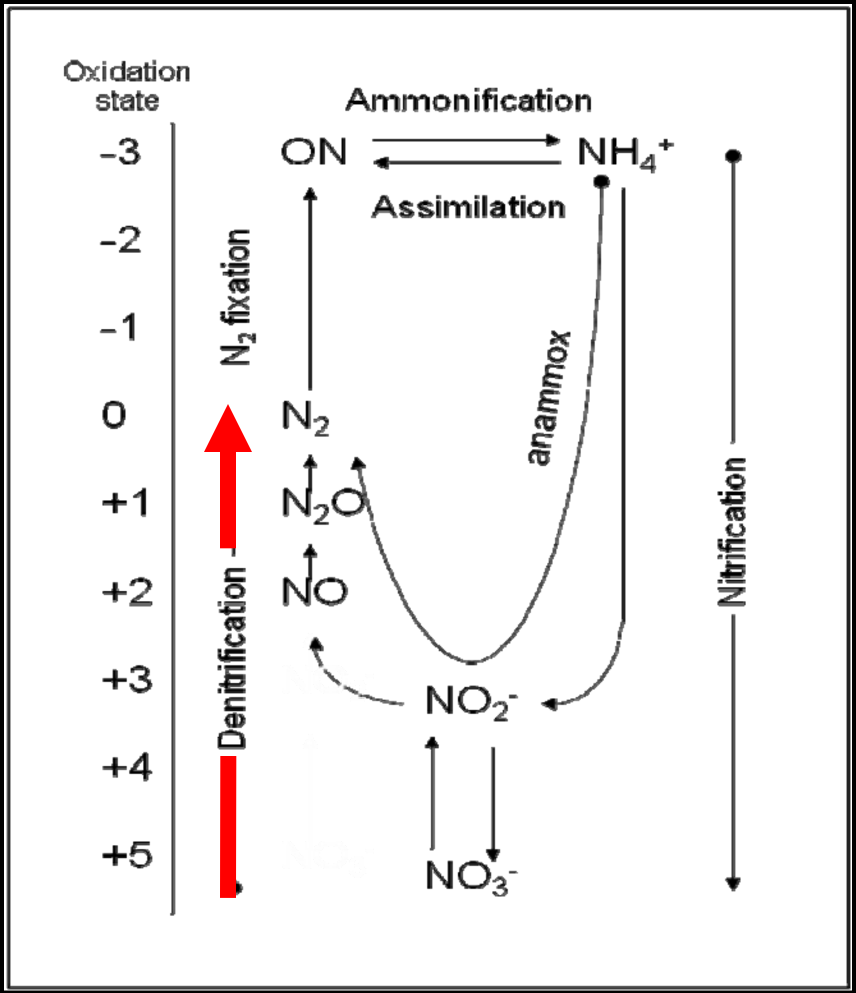
A traditional single limiting nutrient, Liebig-type relationship, in which increasing CO_2 stimulates N_2 fixation only after Fe limitation is first relieved

Fu et al. 2008, L&O 53

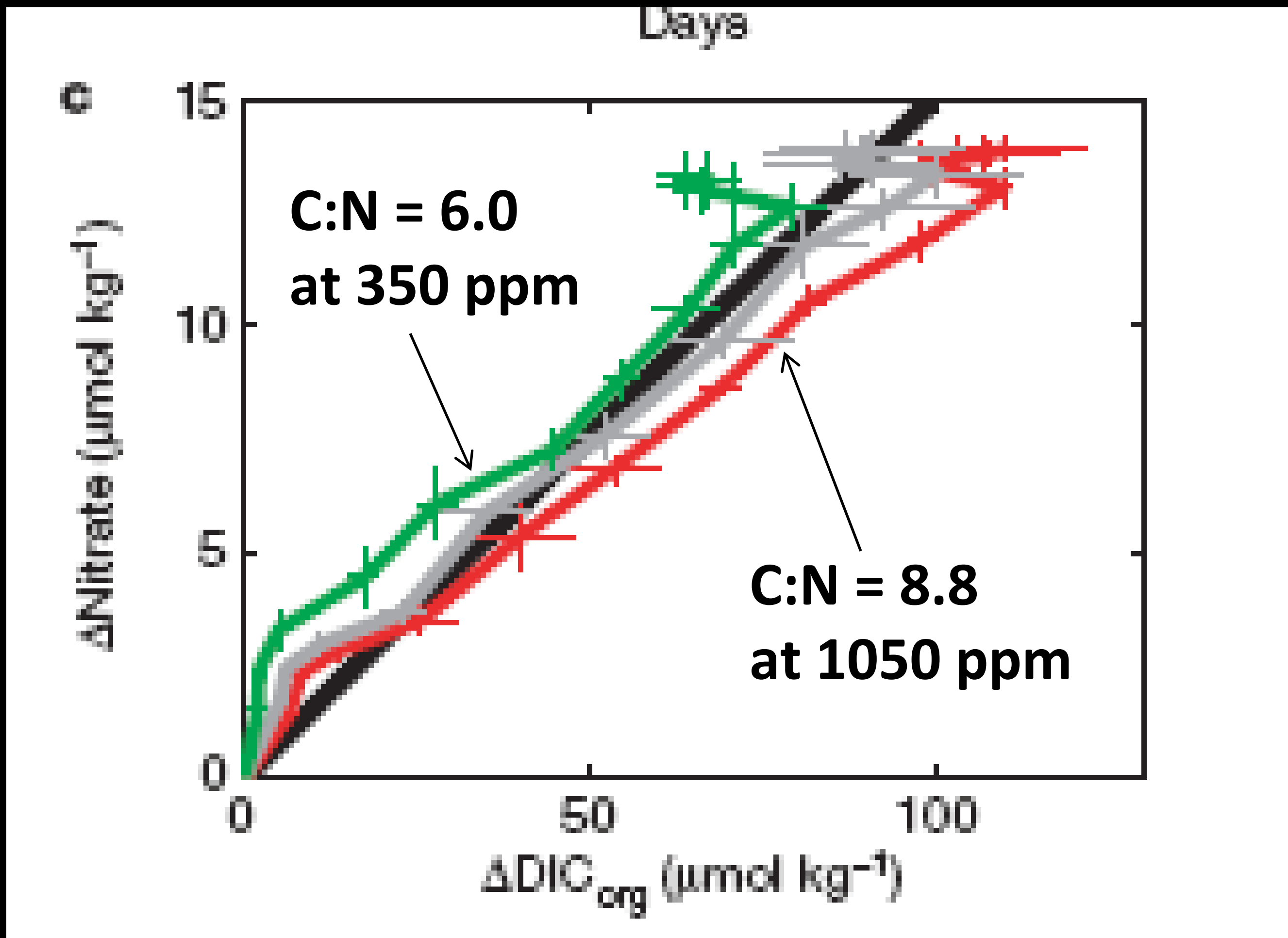
- Global marine N_2 fixation could increase dramatically (~50%?) with doubled pCO_2 over the next 100 years
- The relationship between N_2 fixation and pCO_2 appears to fit a classical Michaelis-Menten saturation curve, with a $K_{1/2}$ of ~320 ppm CO_2
- CO_2-mediated N_2 fixation increases may occur despite widespread growth limitation by P-

  but perhaps Fe availability will be the ultimate limiting factor?

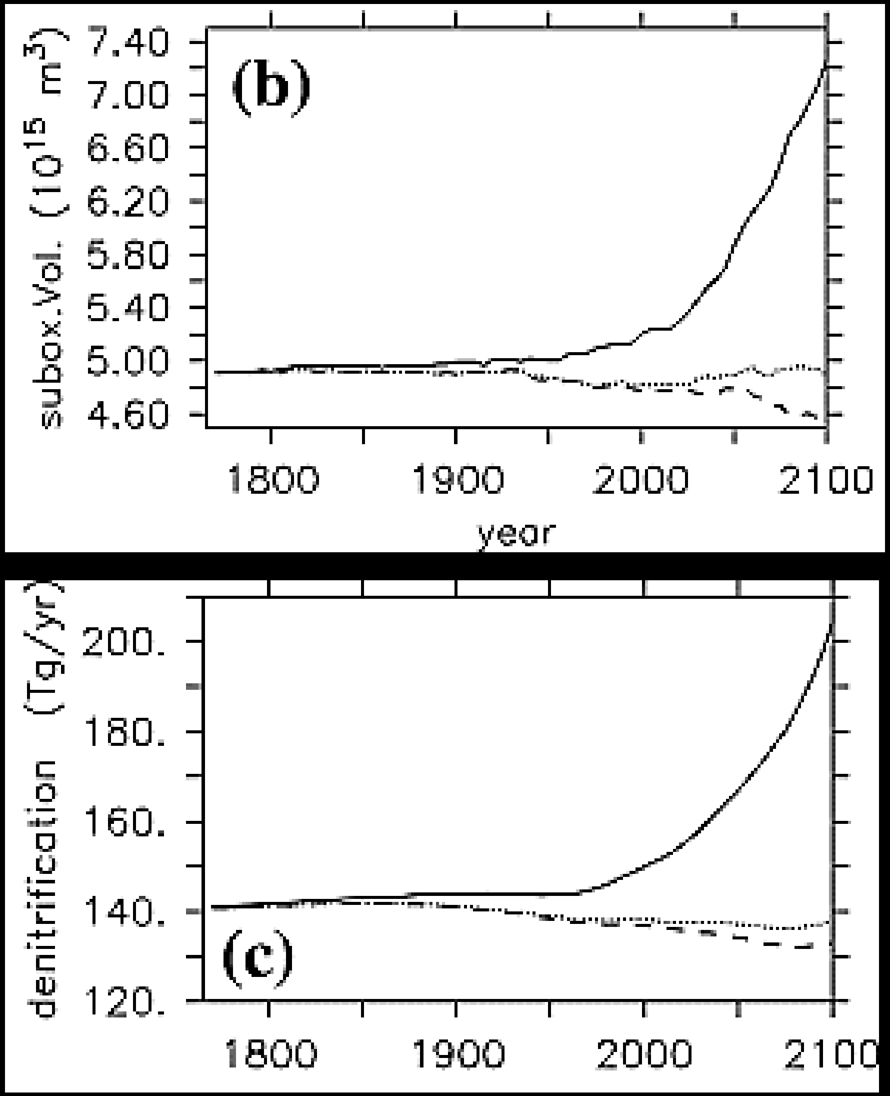
How will denitrification change in a future acidified ocean?



Carbon "overconsumption" at high pCO_2?



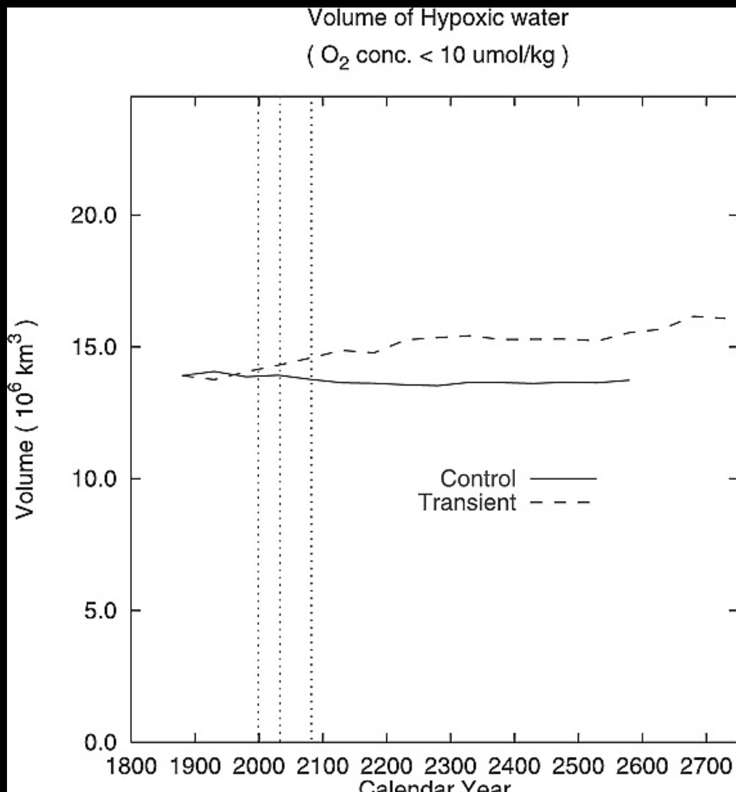
Riebesell et al. 2007, Nature 450: 545-548



Oschlies et al. 2008, Global Biogeochemical Cycles 22

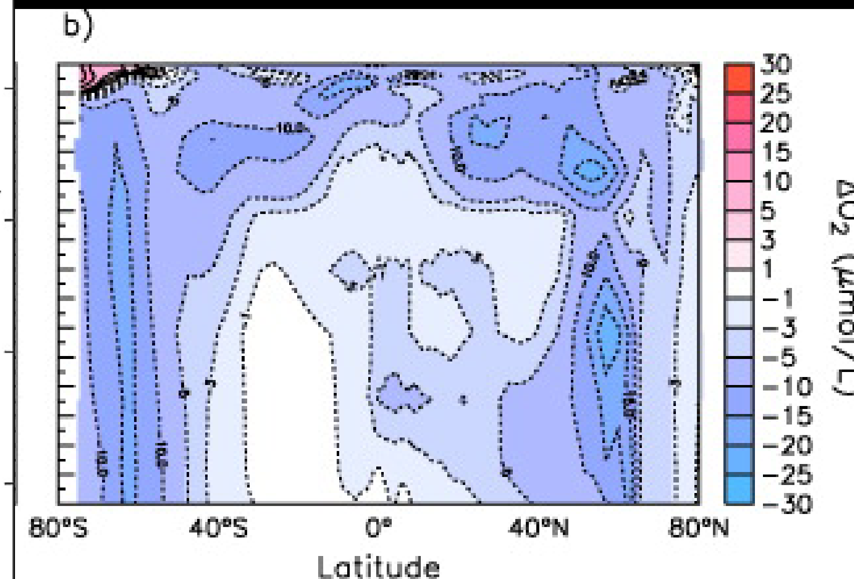
“Simulated 21st century's increase in oceanic suboxia by CO_2-enhanced biotic carbon export”

Simulated effects of warming and stratification on global suboxic water volume



Future increases in the global volume of hypoxic water

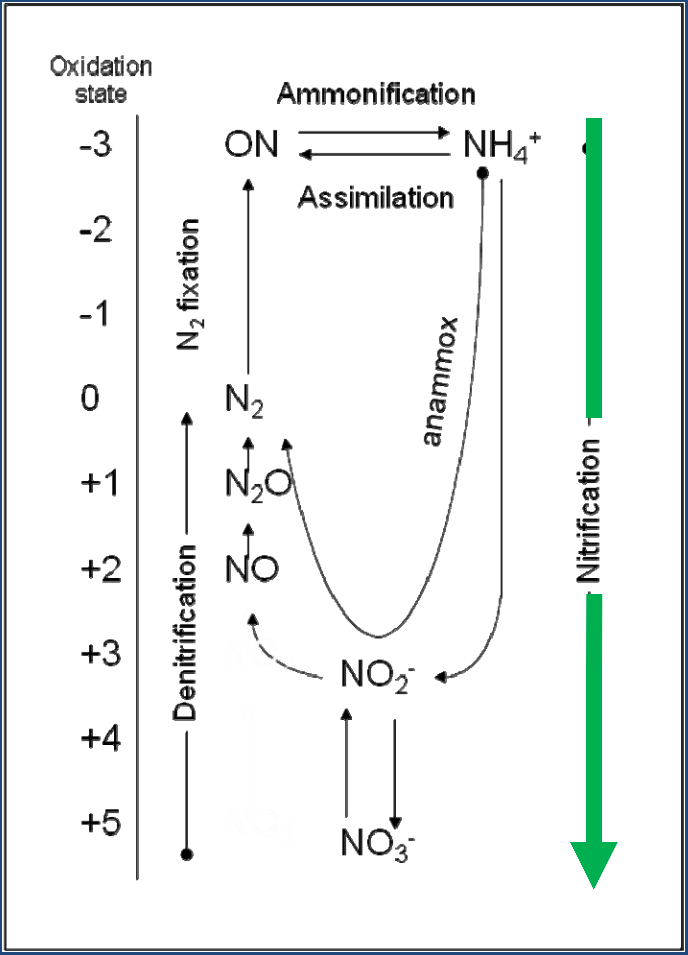
Matear and Hirst 2003,
Global Biogeochemical Cycles 17



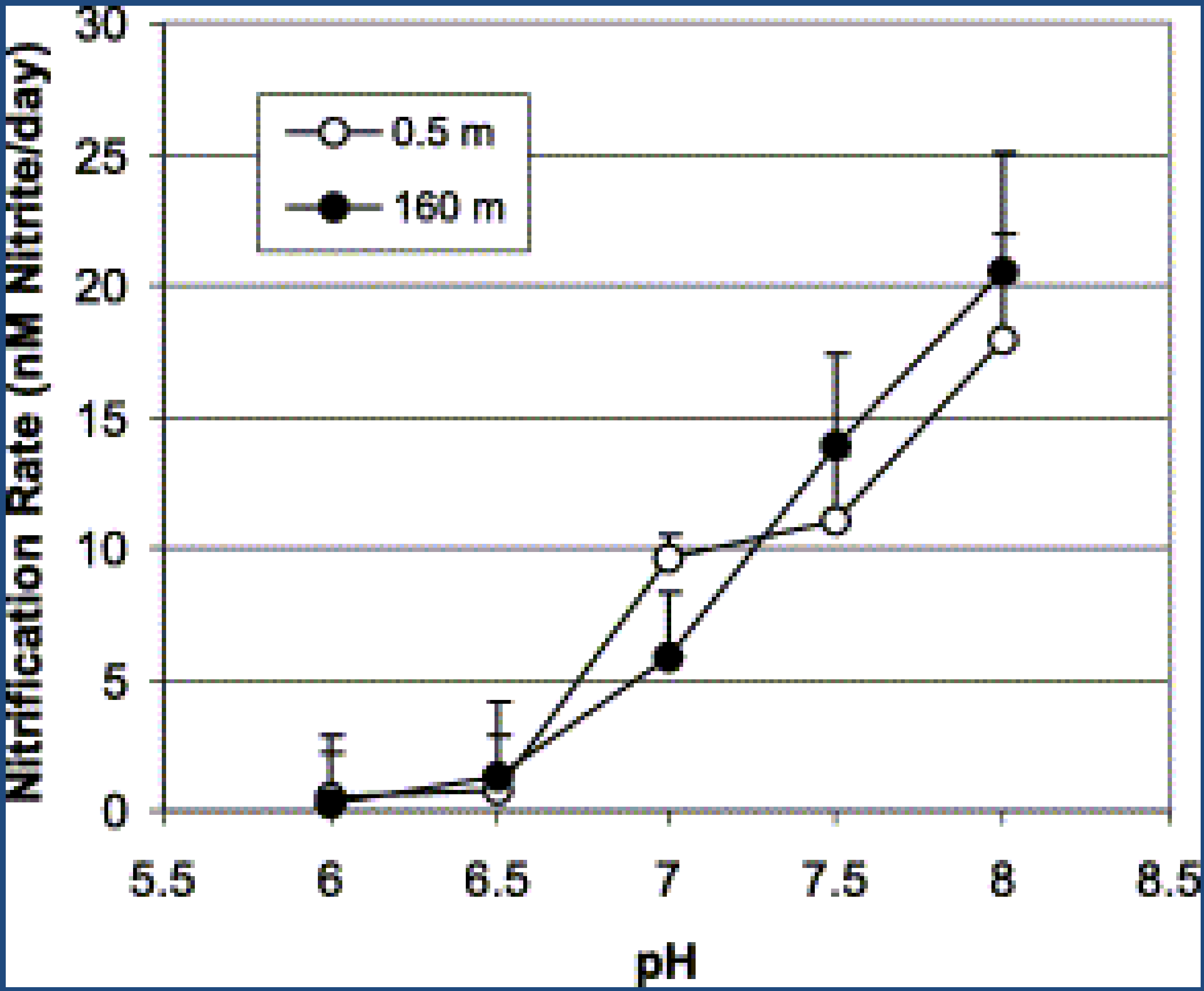
Differences in zonal mean of ocean dissolved O_2 between 2080–2100 and 1980–2000

Bopp et al. 2002,
Global Biogeochemical Cycles 16

Will nitrification respond to CO_2-enriched conditions?



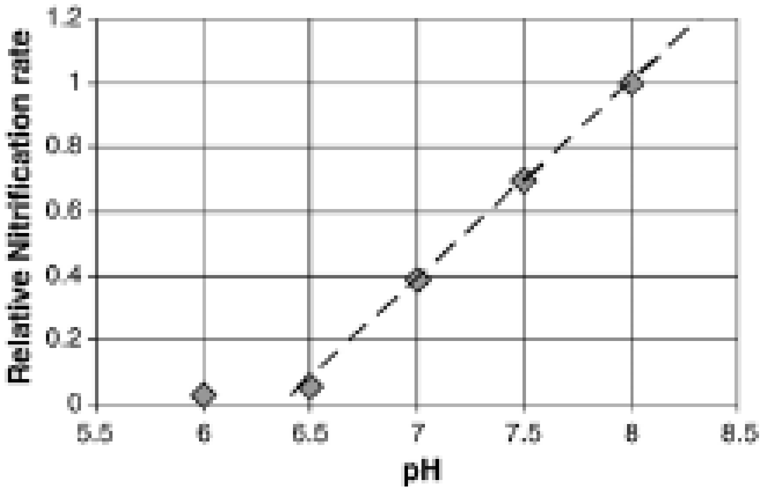
Nitrification rates decrease with acidification

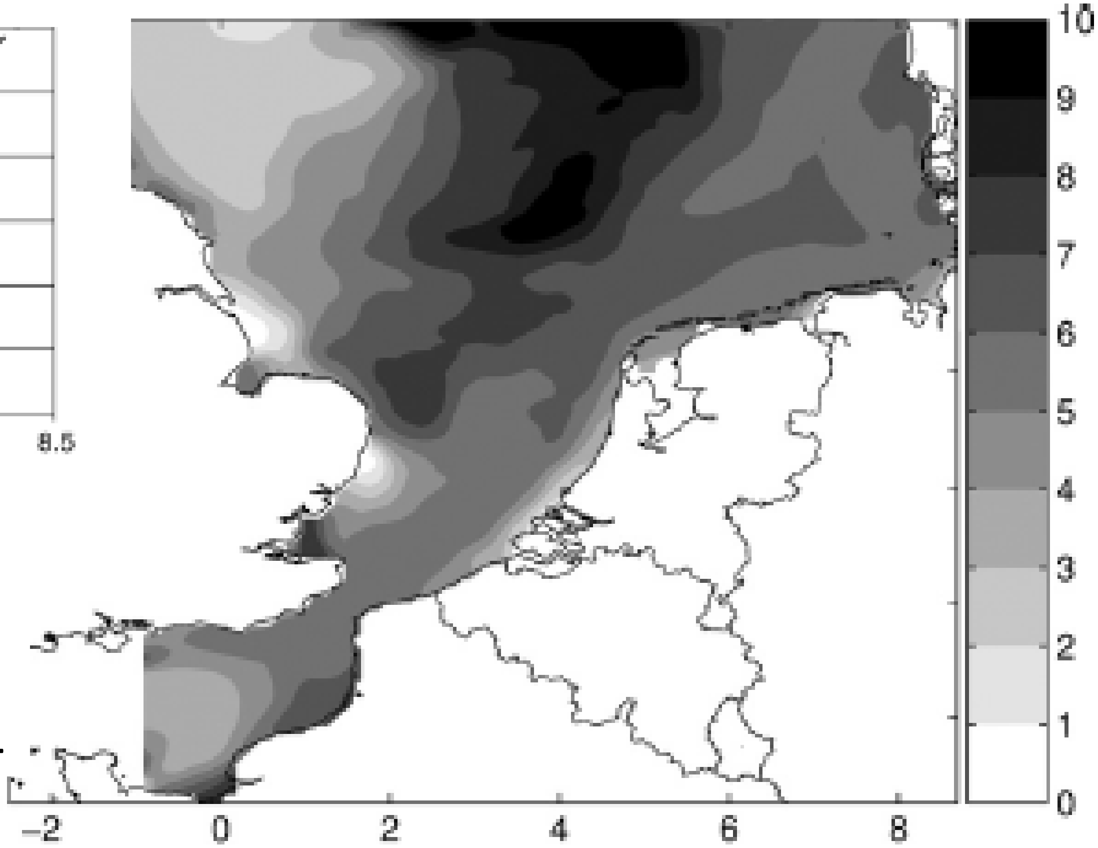


Huesemann et al. 2002, Marine Pollution Bulletin 44: 142-148

Modeled effects of a 20% reduction in North Sea nitrification rates at 1000 ppm CO_2

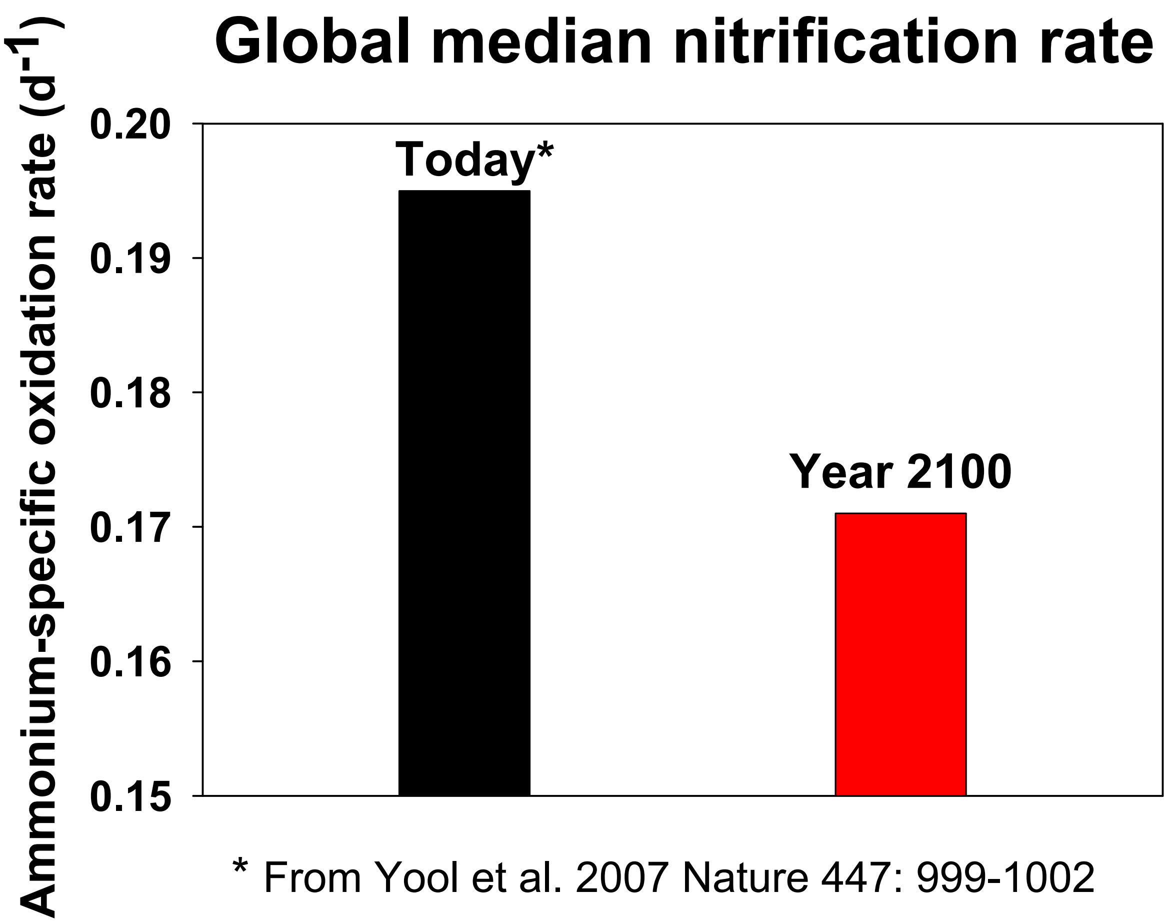
Change in the ratio of nitrate: total DIN (%)





Blackford and Gilbert 2007

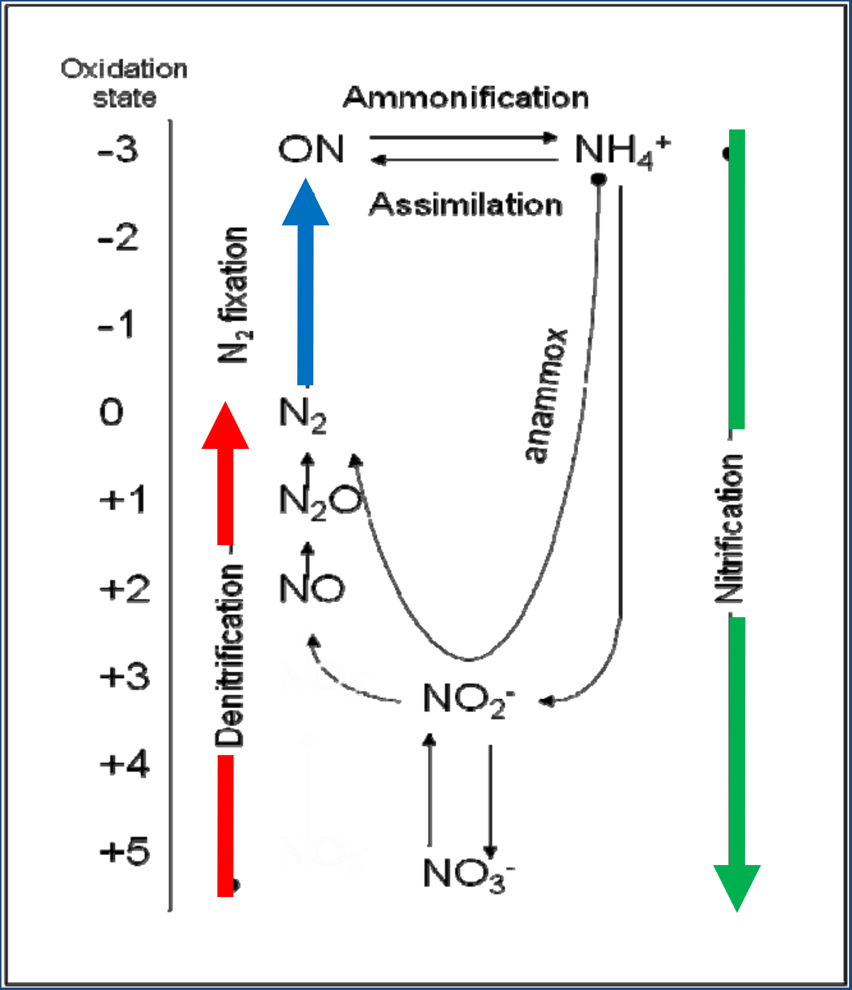
Journal of Marine Systems 64: 229-241



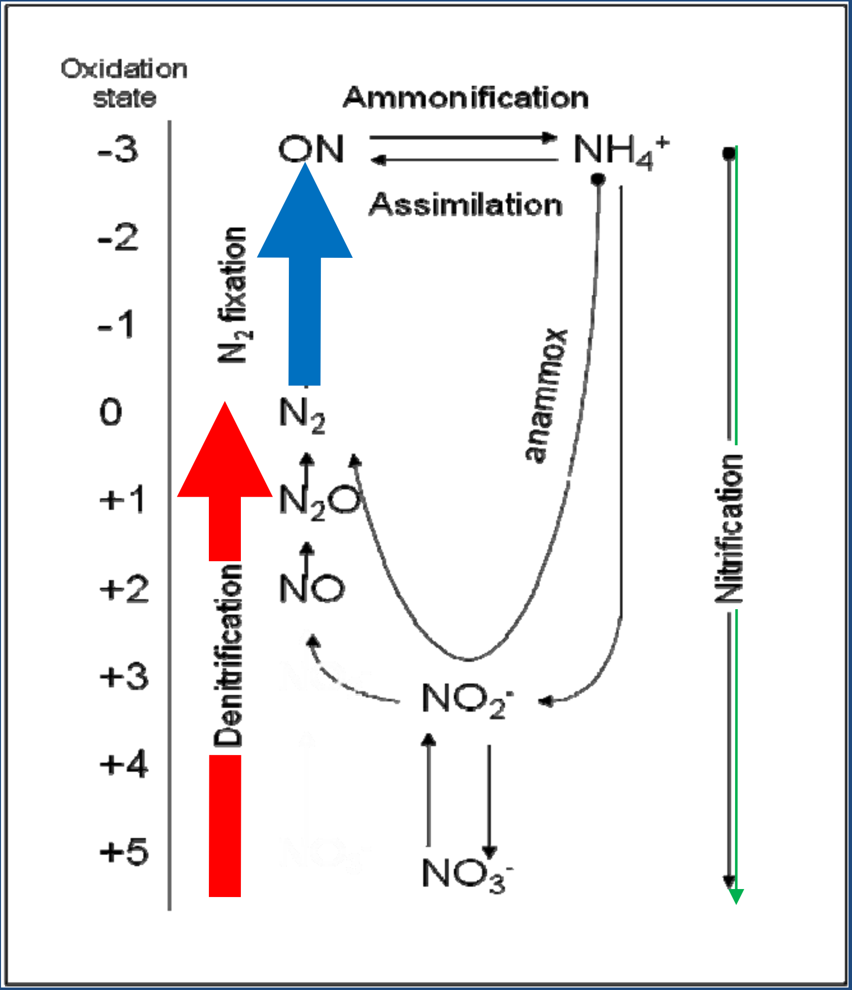
* From Yool et al. 2007 Nature 447: 999-1002

How will today's N cycle change in a future acidified ocean?

Today



Year 2100

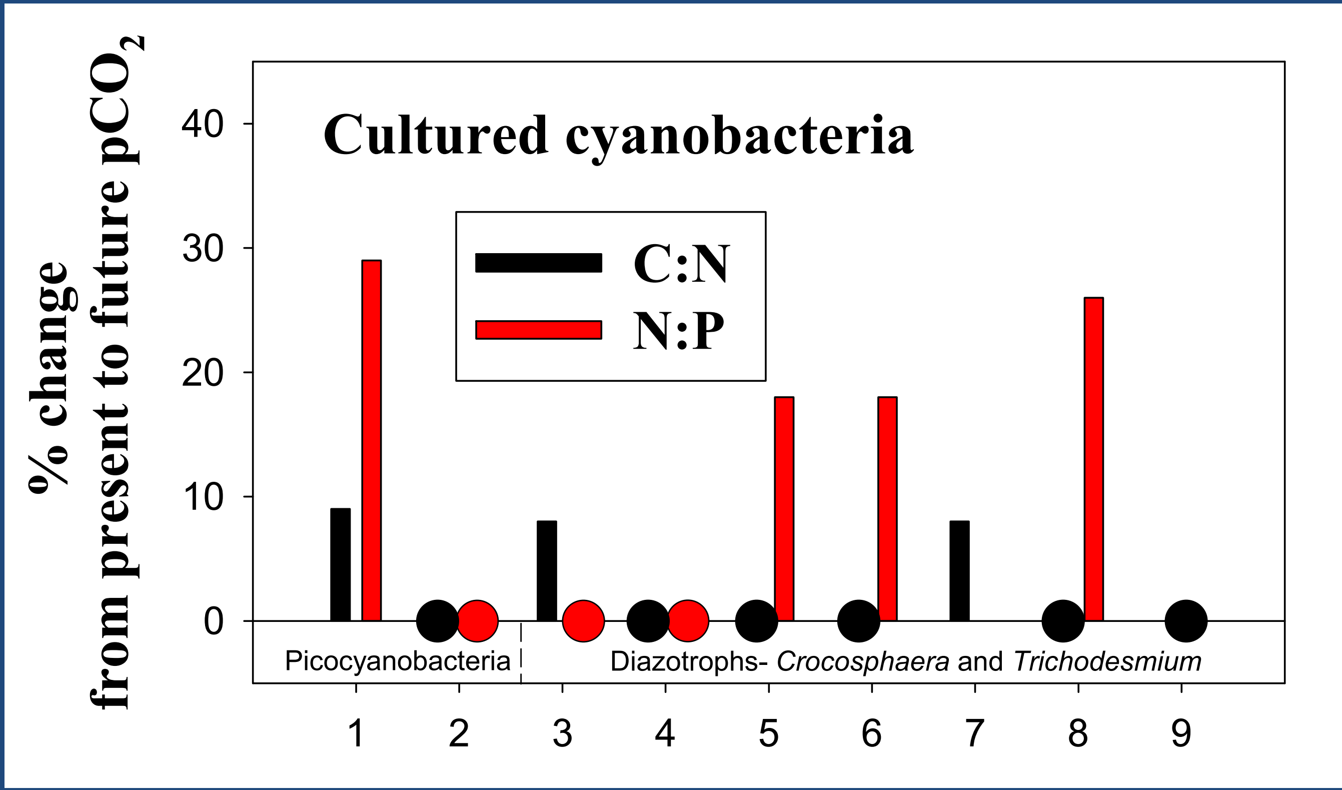


Phytoplankton elemental stochiometry in the high CO_2 ocean

C:N and N:P

1.Evidence from culture studies of cyanobacteria and eukaryotic algae

2. Evidence from natural phytoplankton community manipulation experiments



1 &2, *Synechococcus* and *Prochlorococcus* at 380 and 750 ppm CO_2 (Fu et al. 2007)

3 &4, Fe-replete and Fe-limited *Crocosphaera* at 380 grown aand 750 ppm CO_2 (Fu et al. 2008)

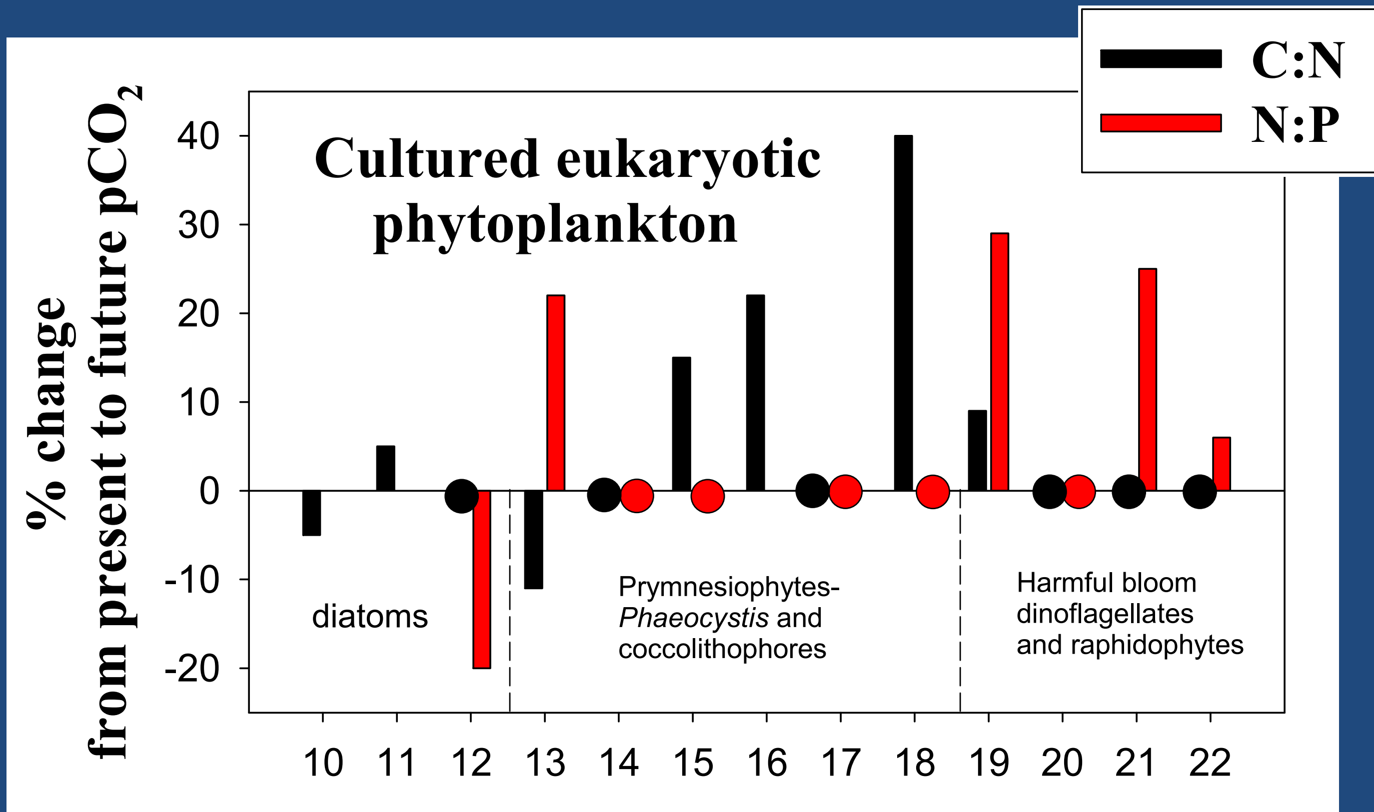
5&6 P-replete and P-limited *Trichodesmium* at 380 and 750 ppm CO_2 (Hutchins et al. 2007)

7 *Trichodesmium* at 400 and 900 ppm CO_2 (Levitan et al. 2007)

8 *Trichodesmium* at 380 and 750 ppm CO_2 (Barcelos e Ramos et al. 2007)

9 *Trichodesmium* at 370 and 1000 ppm CO_2 (Kranz et al. 2009)

Hutchins et al. submitted, Oceanography



10 &11 Diatoms *Asterionella* at 430 and 820 ppm CO_2 and *Skeletonema* at 400 and 720 pCO_2 (Burkhardt et al. 1999)

12 &13 Antarctic diatom *Chaetoceros* and prymnesiophyte *Phaeocystis* at 430 and 820 ppm CO_2 (Fu et al. unpubl results)

14&15 Coccolithophorid *Emiliania huxleyi* under low and high light at 375 and 750 ppm pCO_2 (Feng et al. 2008)

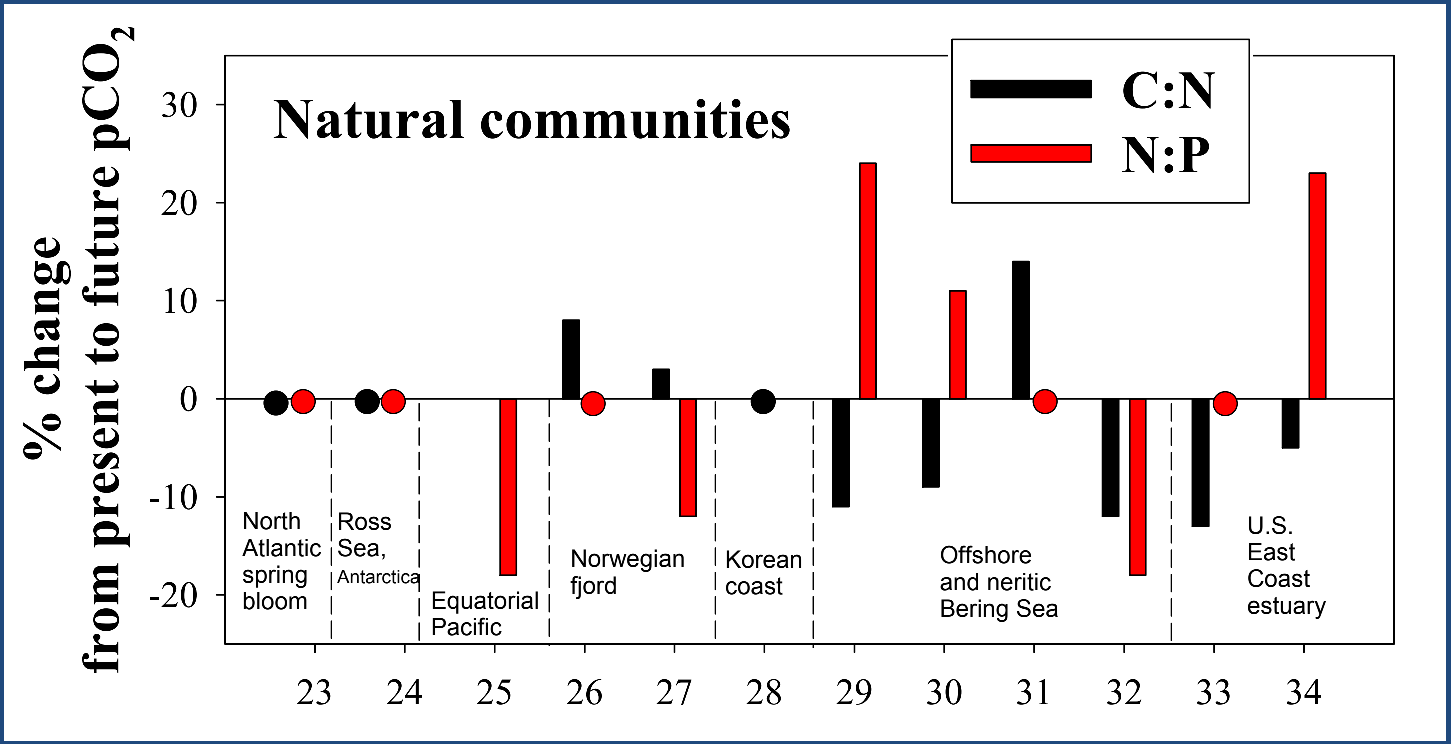
16 Coccolithophorid *Emiliania huxleyi* at 490 and 750 ppm pCO_2 (Iglesias-Rodriguez et al. 2008)

17&18 Non-calcifying *Emiliania huxleyi* gunder low and high light at 360 and 2000 ppm CO_2 (Leonardos and Geider 2005)

19 &20 Toxic raphidophyte *Heterosigma* and the dinoflagellate *Prorocentrum* at 375 and 750 ppm pCO_2 (Fu et al. 2008)

21 &22 P-replete and P-limited dinoflagellate *Karlodinium* at 430 and 745 ppm CO_2 (Fu et al. in review)

Hutchins et al. submitted, Oceanography

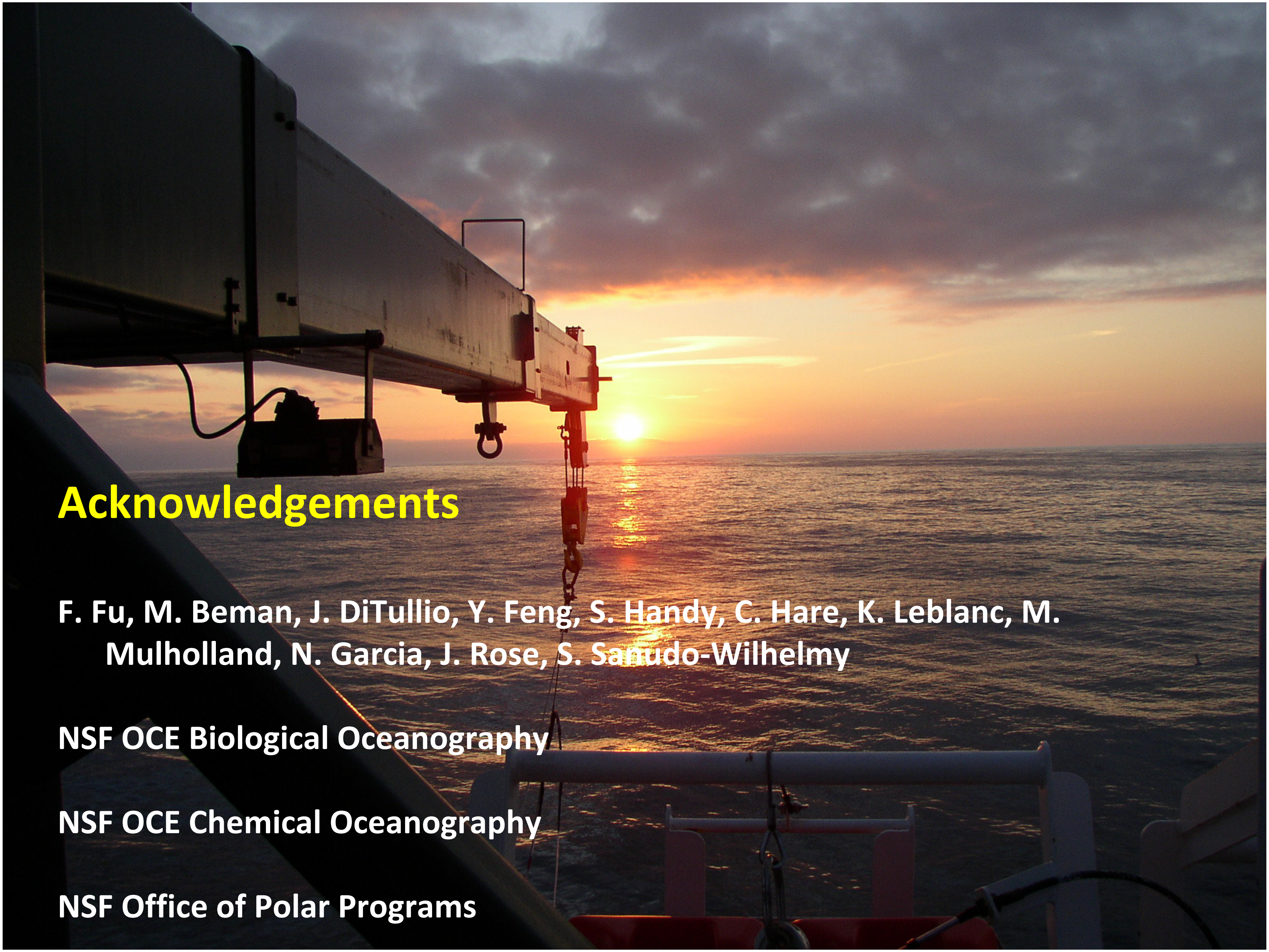


23. North Atlantic spring bloom, 390 and 690 ppm CO_2 (Feng et al. 2009)
24. Ross Sea, Antarctica, 380 and 750 ppm CO_2 (Feng et al. in review)
25. Equatorial Pacific, 150 and 750 ppm CO_2 (Tortell et al. 2002)
26. Norwegian fjord, 350 and 700 ppm CO_2 (Riebesell et al. 2007)
27. Norwegian fjord, 410 and 710 ppm pCO_2 (Engel et al. 2005)
28. Korean coastal waters, 400 and 750 ppm CO_2 (Kim et al. 2006)
29 &30. Bering Sea shelf at 10°C and 15°C, 370 and 750 ppm pCO_2 (Hare et al. 2007)
31 &32. Bering Sea offshore at 10°C and 15°C, 370 and 750 ppm pCO_2 (Hare et al. 2007)
33 &34. U.S. East Coast estuary, 380 and 750 ppm pCO_2 (Fu et al. unpubl.results)

Hutchins et al. submitted, Oceanography

Conclusions

- Ocean acidification seems likely to drive major changes in the marine nitrogen cycle
  - Increased N_2 fixation?
  - Increased denitrification?
  - Decreased nitrification?
  - Unknowns: N assimilation, ammonification, anammox
- C:N and N:P ratios of individual phytoplankton species often increase at high pCO_2, but the trends in whole community stoichiometry are much more variable
- Be cautious when extrapolating from any particular experiment or regime to the whole future ocean!



Acknowledgements

F. Fu, M. Beman, J. DiTullio, Y. Feng, S. Handy, C. Hare, K. Leblanc, M. Mulholland, N. Garcia, J. Rose, S. Sanudo-Wilhelmy

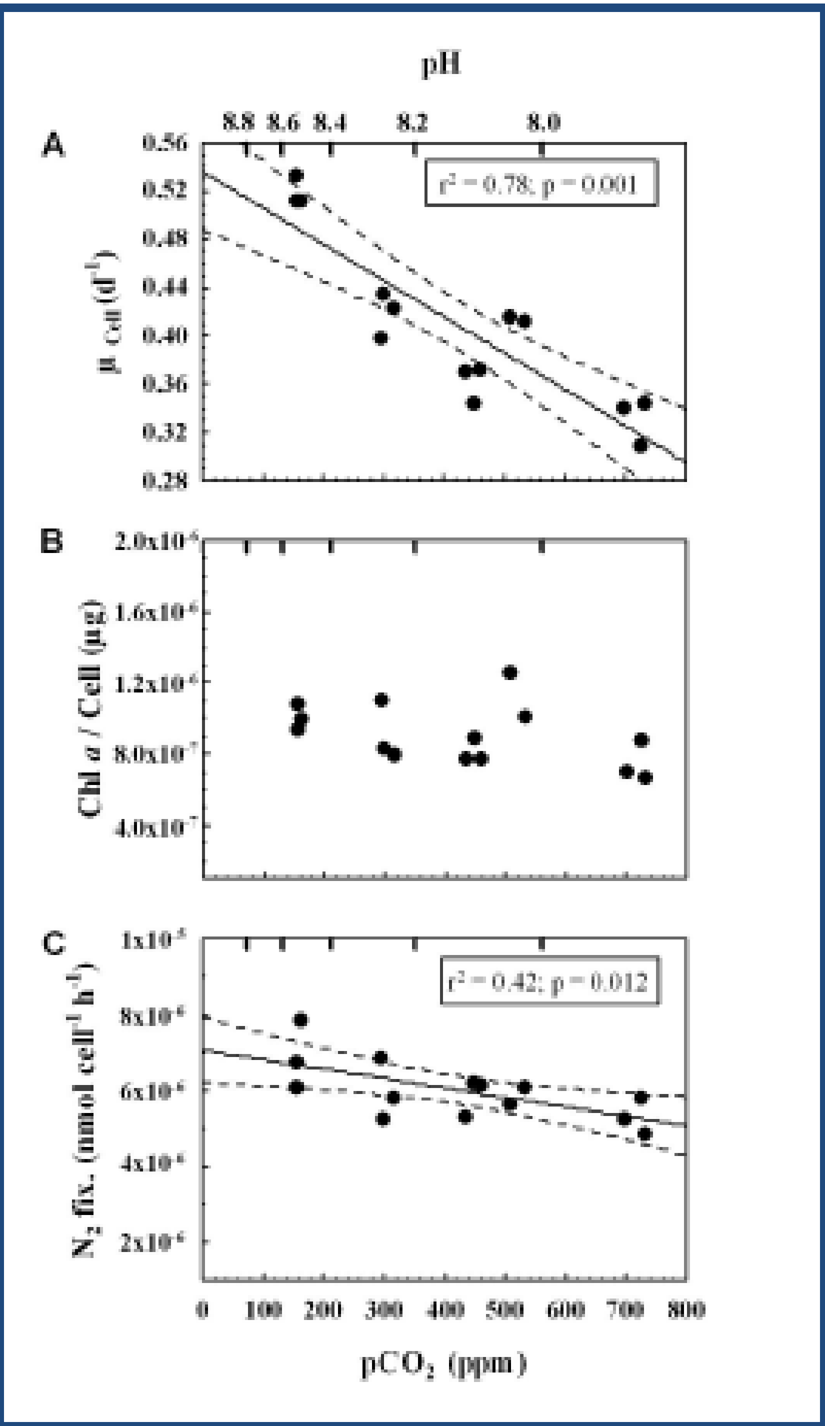
NSF OCE Biological Oceanography

NSF OCE Chemical Oceanography

NSF Office of Polar Programs

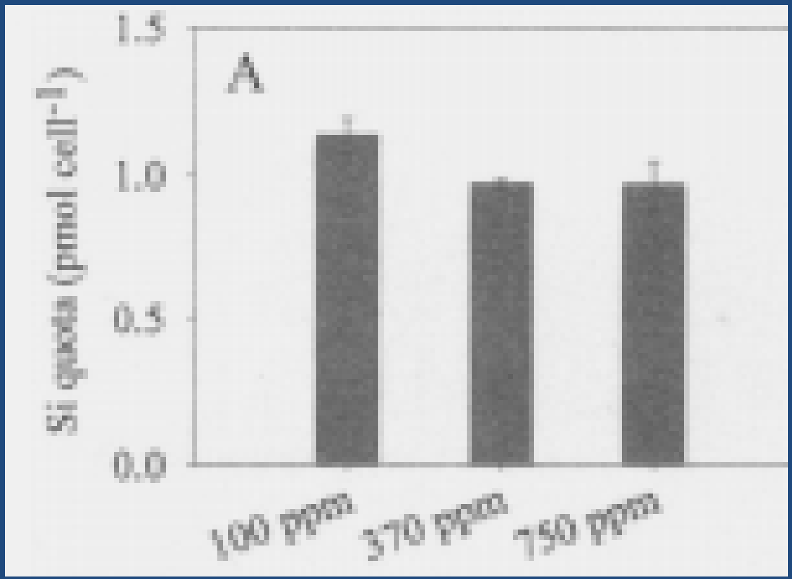
Not all N_2-fixing cyanobacteria will necessarily benefit from high pCO_2:

The harmful bloom species *Nodularia spumigena* from the brackish Baltic Sea



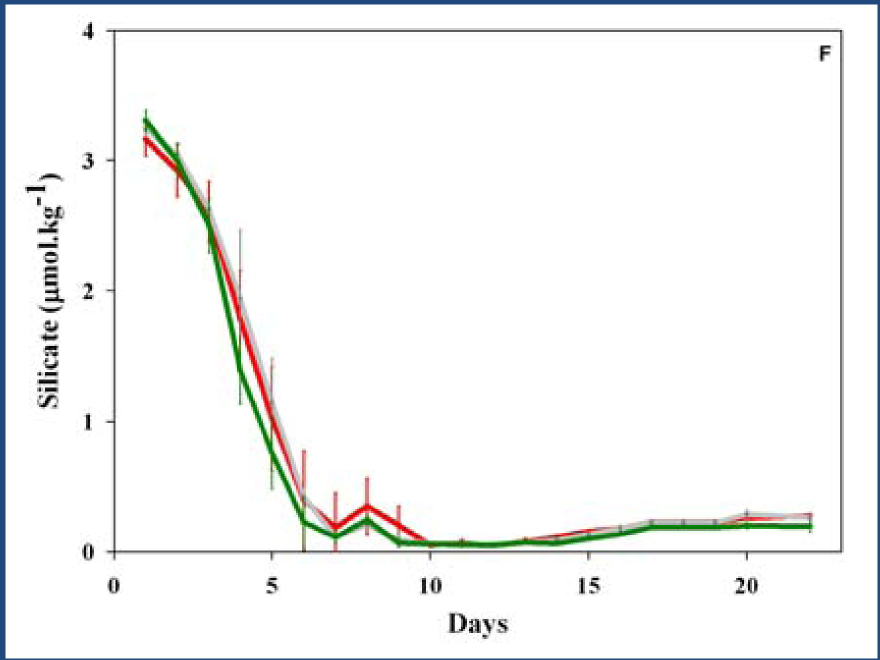
Czerny et al. 2009,
Biogeosciences Discussions 6: 4279-4304

Little or no direct impact of changing pCO_2 on diatom Si utilization



Cellular Si quotas of a cultured diatom are unchanged between 370 and 750 ppm CO_2

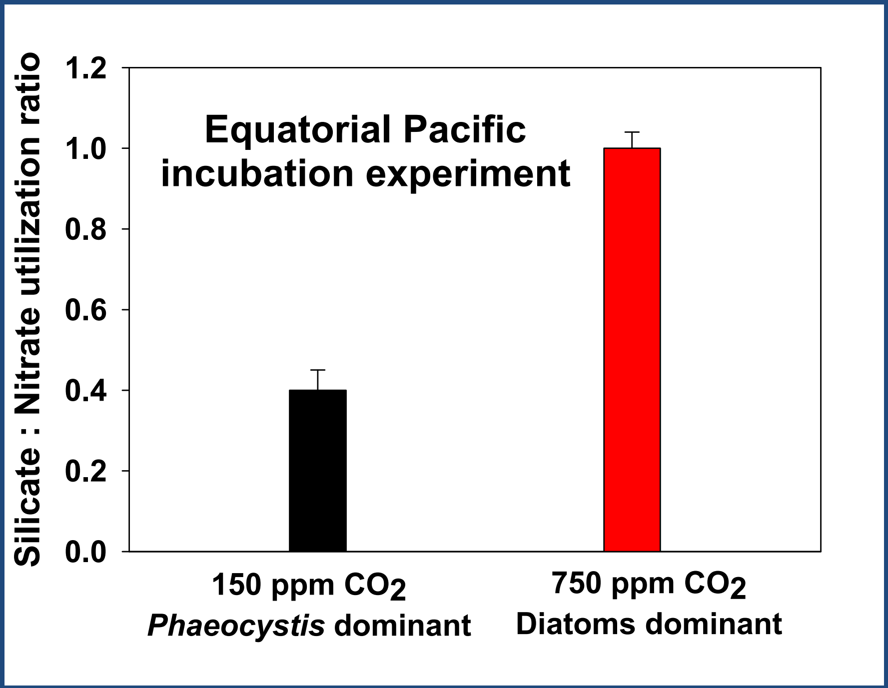
Milligan et al. 2004,
Limnology and Oceanography 42: 322-329



Silicate drawdown is identical at 350, 700 and 1050 ppm CO_2 in a Bergen mesocosm experiment

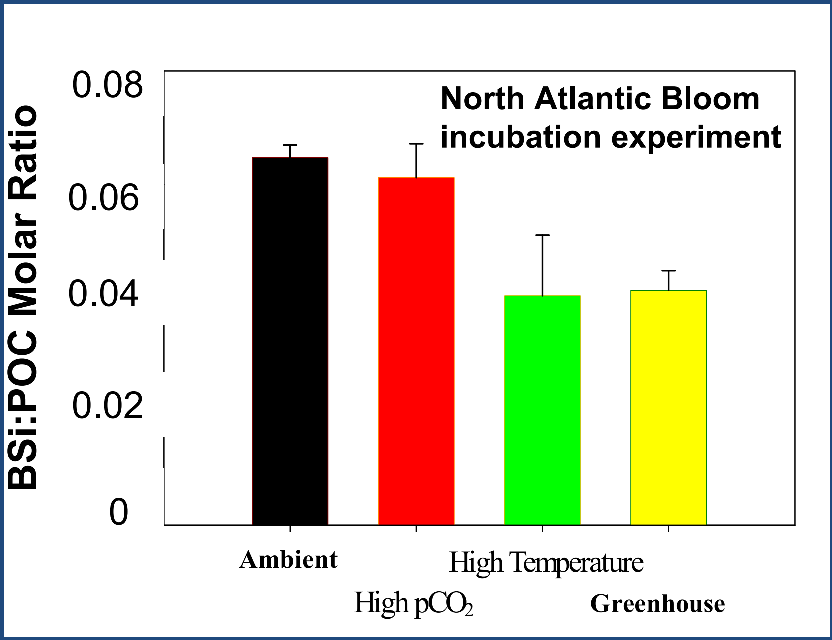
Bellerby et al. 2008,
Biogeosciences Discussions 4

pCO_2 and temperature indirectly change Si cycling due to phytoplankton community shifts



Changing Si:N utilization ratios due to a CO_2-driven community shift between diatoms and *Phaeocystis*

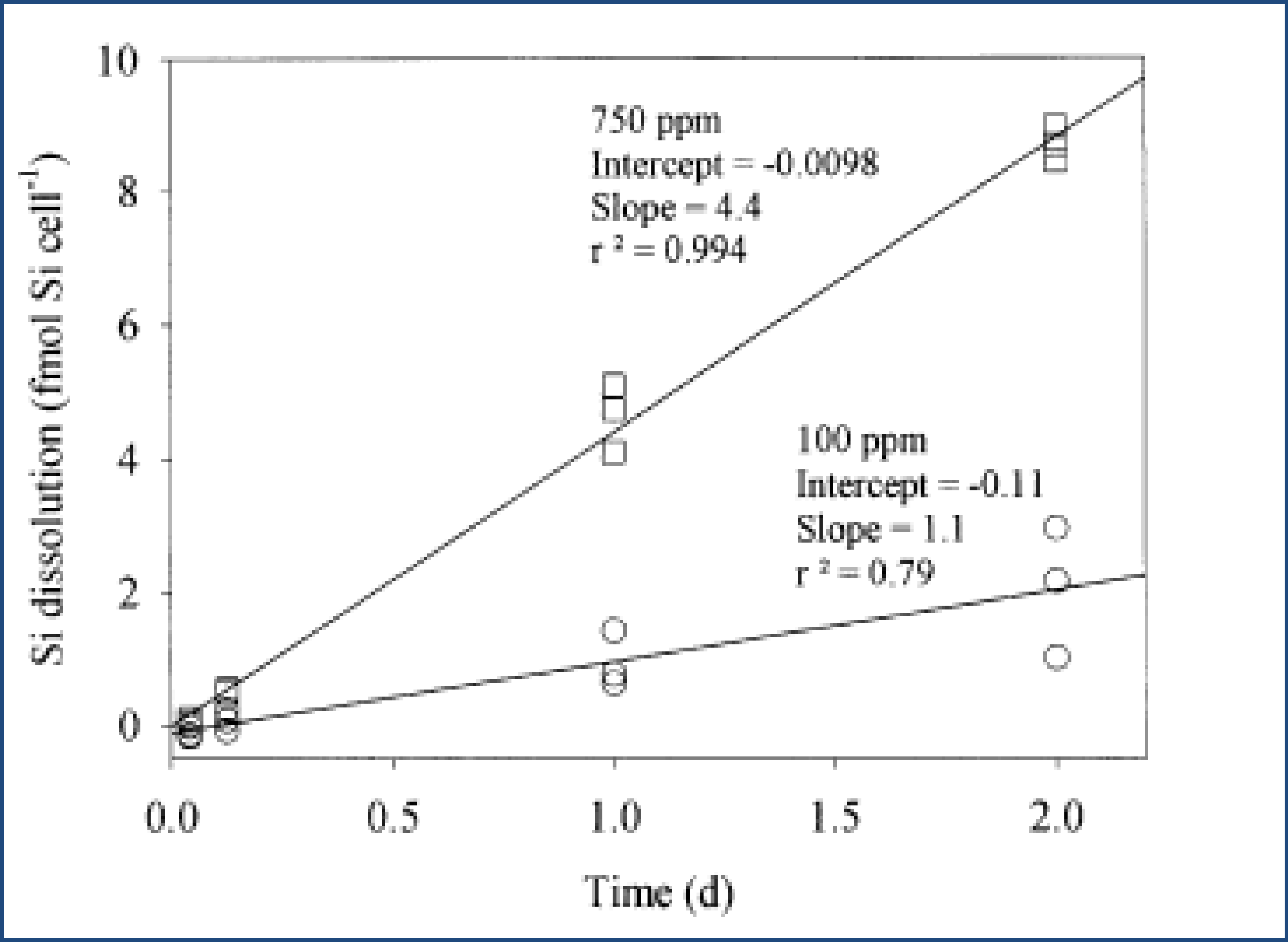
Adapted from Tortell et al. 2002, MEPS 236



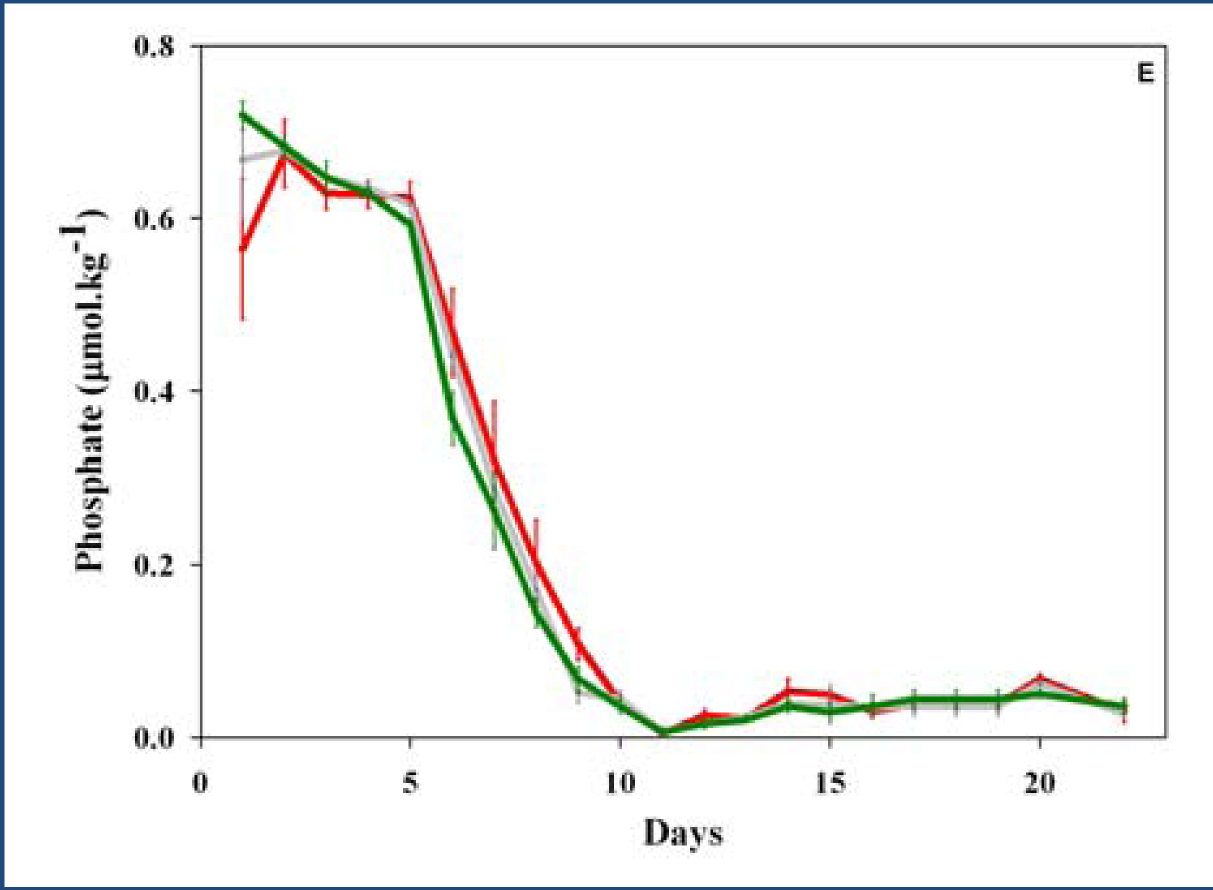
Changing particulate Si: C ratios due to a temperature-driven community shift between diatoms and coccolithophores

Feng et al. 2009, MEPS in press.

Ocean acidification enhances the silica dissolution rates of empty diatom frustules



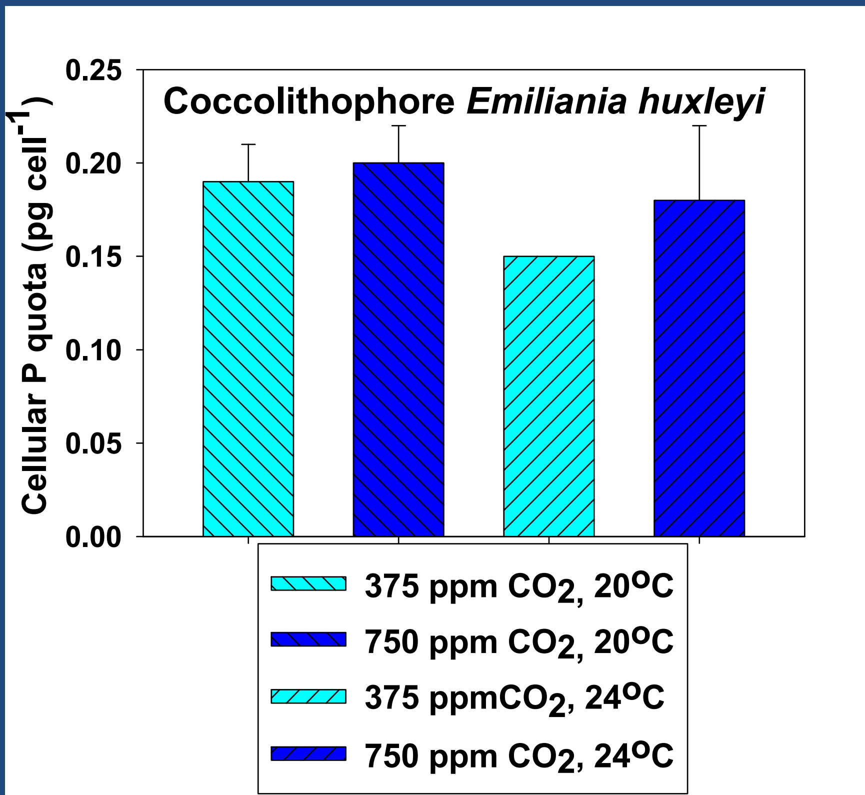
Milligan et al. 2004, Limnology and Oceanography 42: 322-329



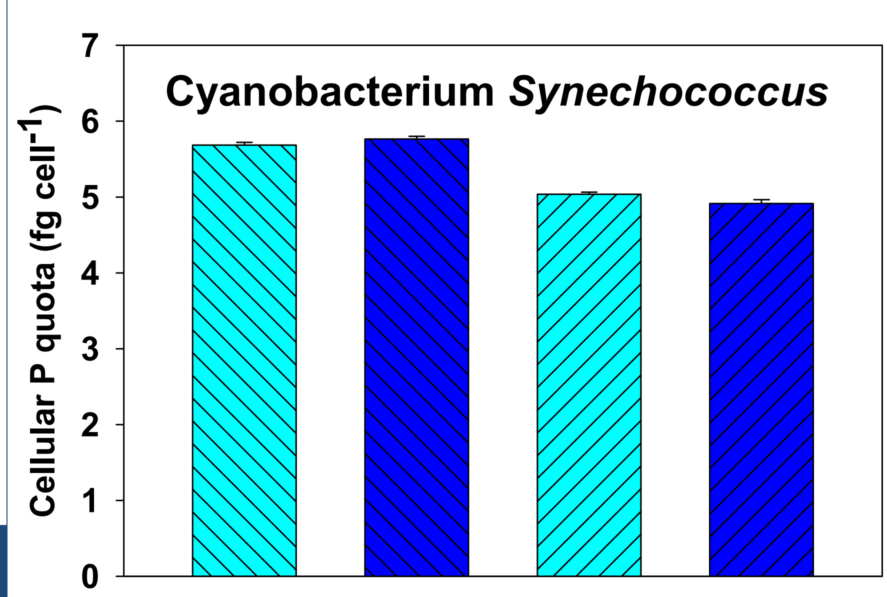
Phosphate drawdown is unchanged at 350, 700 and 1050 ppm CO_2 in a Bergen mesocosm experiment

Bellerby et al. 2008,
Biogeosciences Discussions 4

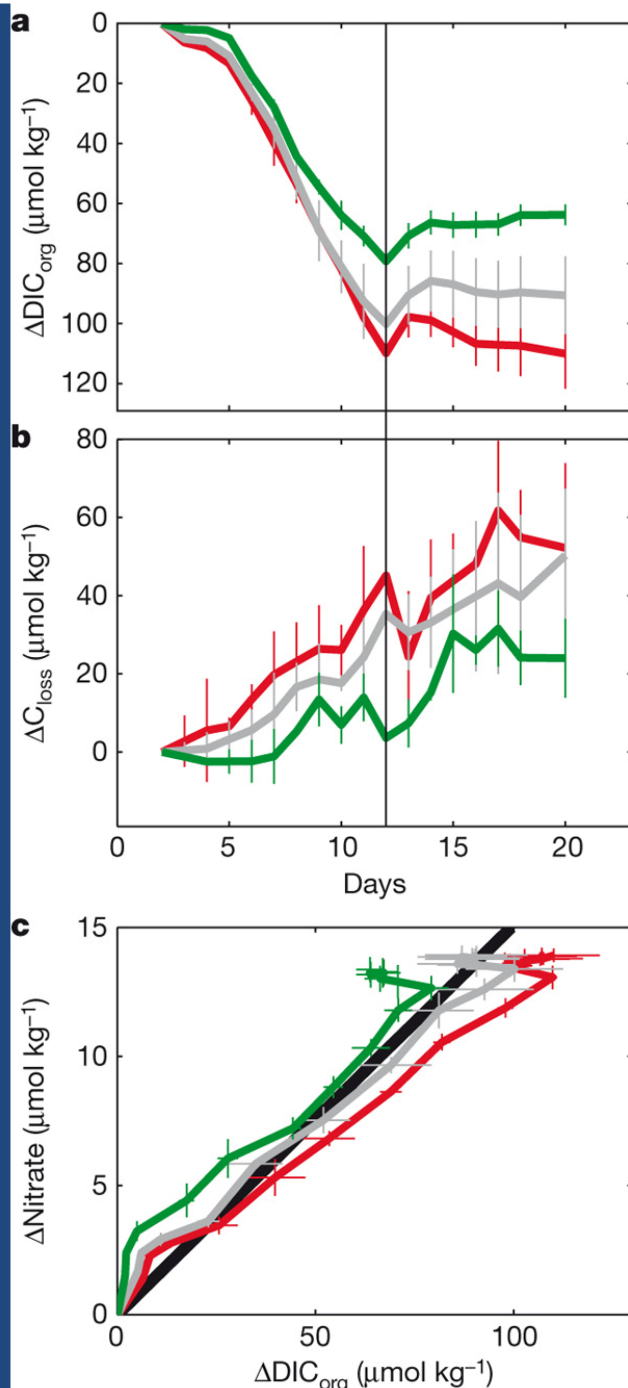
Phytoplankton P requirements: Little or no response to pCO_2 increases



Feng et al. 2008
European Journal of Phycology 43: 87-98



Fu et al. 2007
Journal of Phycology 43: 485-496



a
ΔDIC$_{org}$ (μmol kg^{-1})
0
20
40
60
80
100
120
b
ΔC$_{loss}$ (μmol kg^{-1})
80
60
40
20
0
0
5
10
15
20
Days
c
ΔNitrate (μmol kg^{-1})
15
10
5
0
0
50
100
ΔDIC$_{org}$ (μmol kg^{-1})

How predictive are short term manipulation experiments of future long-term changes?

How do we account for adaptation and evolution of populations and communities?

Algal evolution in response to changing pCO_2

21. Jorde, L. B. in *Current Developments in Anthropological Genetics* (eds Mielke, J. H. & Crawford, M. H.) 135–208 (Plenum, New York, 1980).
22. Notohara, M. The coalescent and the genealogical process in geographically structured populations. *J. Math. Biol.* 29, 59–75 (1990).
23. Wilkinson-Herbots, H. M. Genealogy and subpopulation differentiation under various models of population structure. *J. Math. Biol.* 37, 535–585 (1998).
24. Hey, J. & Machado, C. A. The study of structured populations—new hope for a difficult and divided science. *Nature Rev. Genet.* 4, 535–543 (2003).

Supplementary Information accompanies the paper on **www.nature.com/nature**.

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Competing interests statement The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to D.L.T.R. (dr@tedlab.mit.edu).

Phenotypic consequences of 1,000 generations of selection at elevated CO_2 in a green alga

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Biology Department, McGill University, Montreal, Quebec H3A 1B1, Canada

Estimates of the effect of increasing atmospheric CO_2 concentrations on future global plant production rely on the physiological response of individual plants or plant communities when exposed to high CO_2 (refs 1–6). Plant populations may adapt to the changing atmosphere, however, such that the evolved plant communities of the next century are likely to be genetically different from contemporary communities[7–12]. The properties of these future communities are unknown, introducing a bias of unknown sign and magnitude into projections of global carbon pool dynamics. Here we report a long-term selection experiment to investigate the phenotypic consequences of selection for growth at elevated CO_2 concentrations. After about 1,000 generations, selection lines of the unicellular green alga *Chlamydomonas* failed to evolve specific adaptation to a CO_2 concentration of 1,050 parts per million. Some lines, however, evolved a syndrome involving high rates of photosynthesis and respiration, combined with higher chlorophyll content and reduced cell size. These lines also grew poorly at ambient concentrations of CO_2. We tentatively attribute this outcome to the accumulation of conditionally neutral mutations in genes affecting the carbon concentration mechanism.

responses[9–12], but have been limited to fewer than ten generations. The long-term response to selection and the properties of populations adapted to elevated CO_2 remain unknown, and constitute an important limit on our ability to predict future plant productivity.

We used a microbial model system in which large population size and short generation time make it possible to evaluate evolutionary change caused by the spread of novel mutations over hundreds of generations. *Chlamydomonas reinhardtii* is a unicellular green alga that has been extensively used to study the physiology and genetics of photosynthesis[13]. It possesses a carbon-concentrating mechanism (CCM), which increases the concentration of CO_2 near the active site of ribulose 1,5-bisphosphate carboxylase–oxygenase (Rubisco), in common with most other eukaryotic microalgae that have been studied[14]. We set up ten isogenic selection lines from each of two ancestral genotypes, half being grown at ambient CO_2 (ambient lines) and half at a concentration that increased from ambient to 1,050 p.p.m. over about 600 generations and was then maintained at this level for a further 400 generations (high lines). At least 10^5 cells per line were transferred for 125 transfers in a buffered, nutrient-rich medium. The history of these lines thus emulates the conditions that photosynthetic organisms are likely to experience during the next century or so, with respect to CO_2 levels alone.

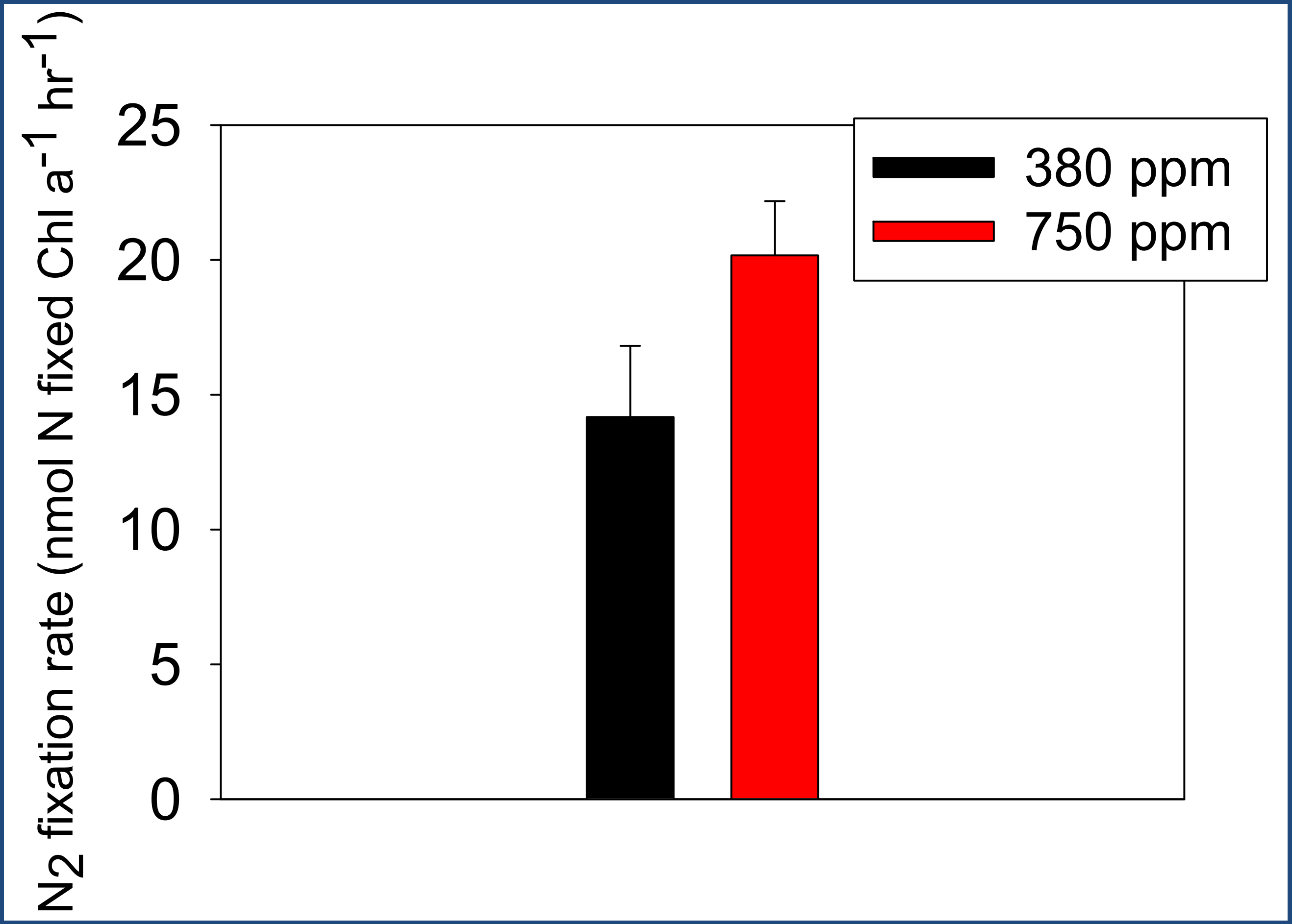
The physiological effect of elevated CO_2 concentration is expected to be an increase in photosynthesis, causing an increase in growth. Net photosynthesis in the ambient lines increased by about 30% when they were grown at high CO_2 (Fig. 1a). The ambient lines diverged through time so that by the end of the experiment they varied significantly in the rate of photosynthesis (one-way analysis of variance (ANOVA): $F_{9,18} = 9.0$, $P < 0.001$) when grown at ambient CO_2 concentrations. The high lines had normal rates of photosynthesis at ambient CO_2, which increased by more than 50% as an average over all lines at high CO_2. However, this effect was very inconsistent: one group of high lines had low rates whereas a second group had very high rates of photosynthesis at high CO_2 concentration (Fig. 1a). This distinction was not related to the identity of the ancestor, and represented significantly more divergence in photosynthetic rates than was seen in the ambient lines ($F_{1,16} = 10.5$, $P = 0.005$).

The growth rate of cultures grown at elevated CO_2 was correlated with their photosynthetic rate among the ambient lines, but not among the high lines (Fig. 1b). The physiological effect of CO_2 on photosynthesis was reflected by growth in pure culture, where the maximal rate of increase (Fig. 1c) and the limiting density (Fig. 1d) of both the ambient and the high lines are enhanced substantially by high CO_2. However, there was no indication of a parallel evolutionary response: by the end of the selection experiment, the high lines had not become specifically adapted to growth at high CO_2; their growth at high CO_2 being no greater than, and perhaps even less than, the growth of the ambient lines. There was nevertheless an indirect response: the growth of some high lines was markedly impaired at ambient CO_2 concentrations where two of the lines

Laboratory adaptation and evolution experiment

- *Trichodesmium* (cyanobacterium), *Emiliania huxleyi* (coccolithophorid), and *Thalassiosira weissflogii* (diatom)
- Long-term growth (100-1000s of generations) at a range of pCO_2
- Physiological, biochemical and molecular assessments of adaptive changes in response to CO_2 selection

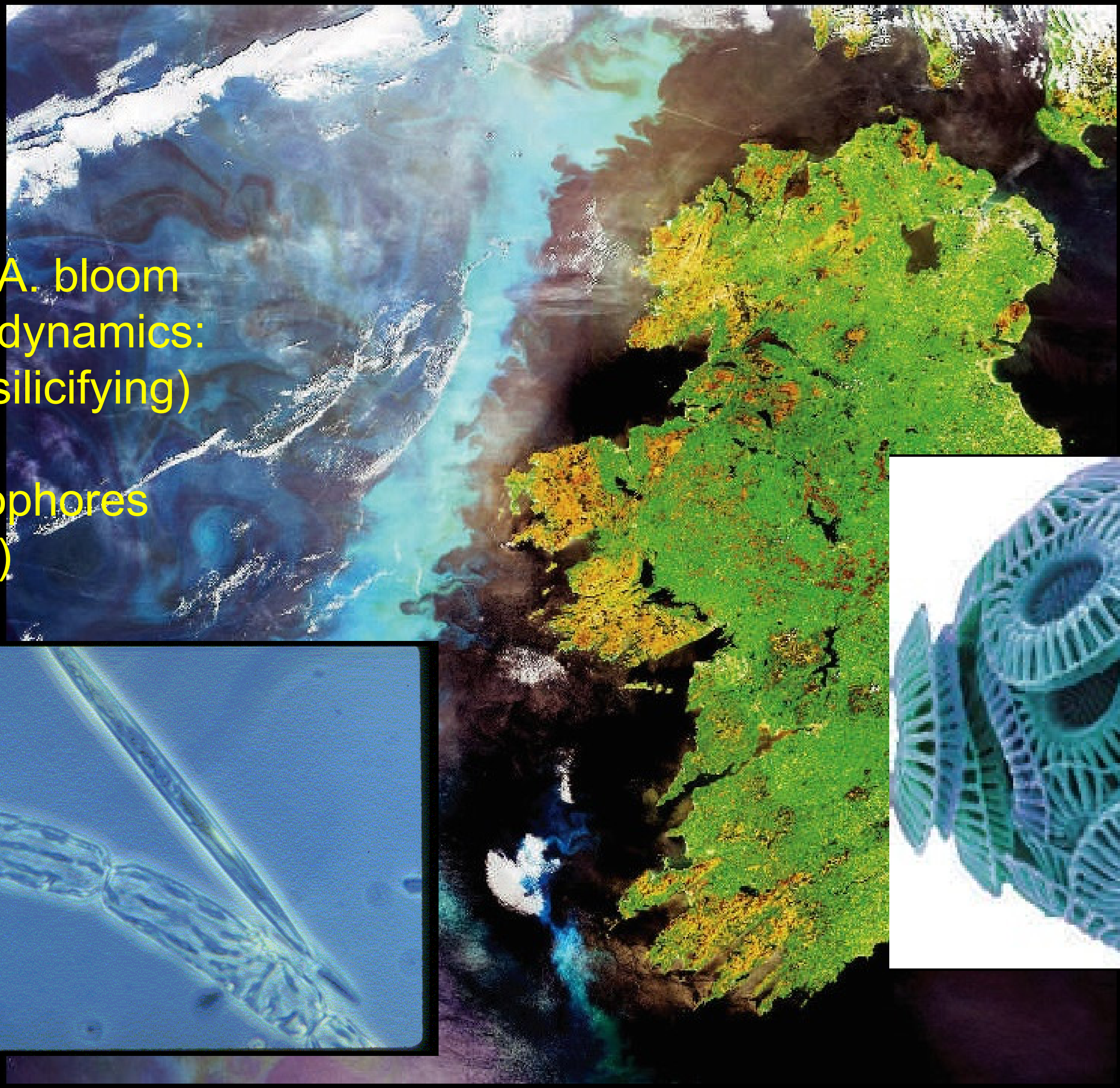
Trichodesmium adaptation experiment: N_2 fixation rates after 100 generations of high CO_2 growth

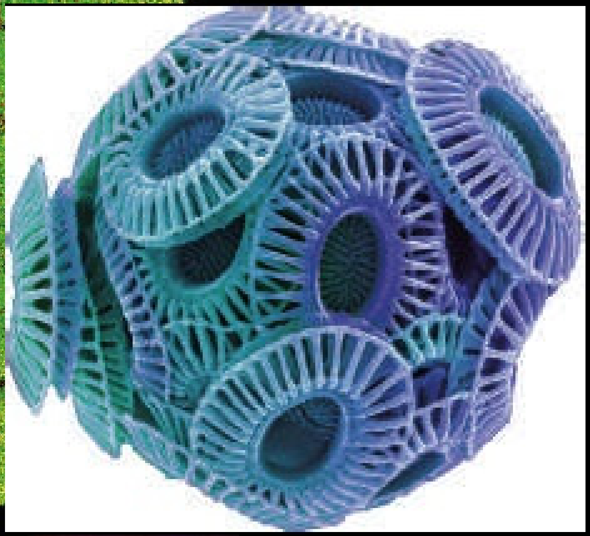


2006 North Atlantic coccolithophore bloom

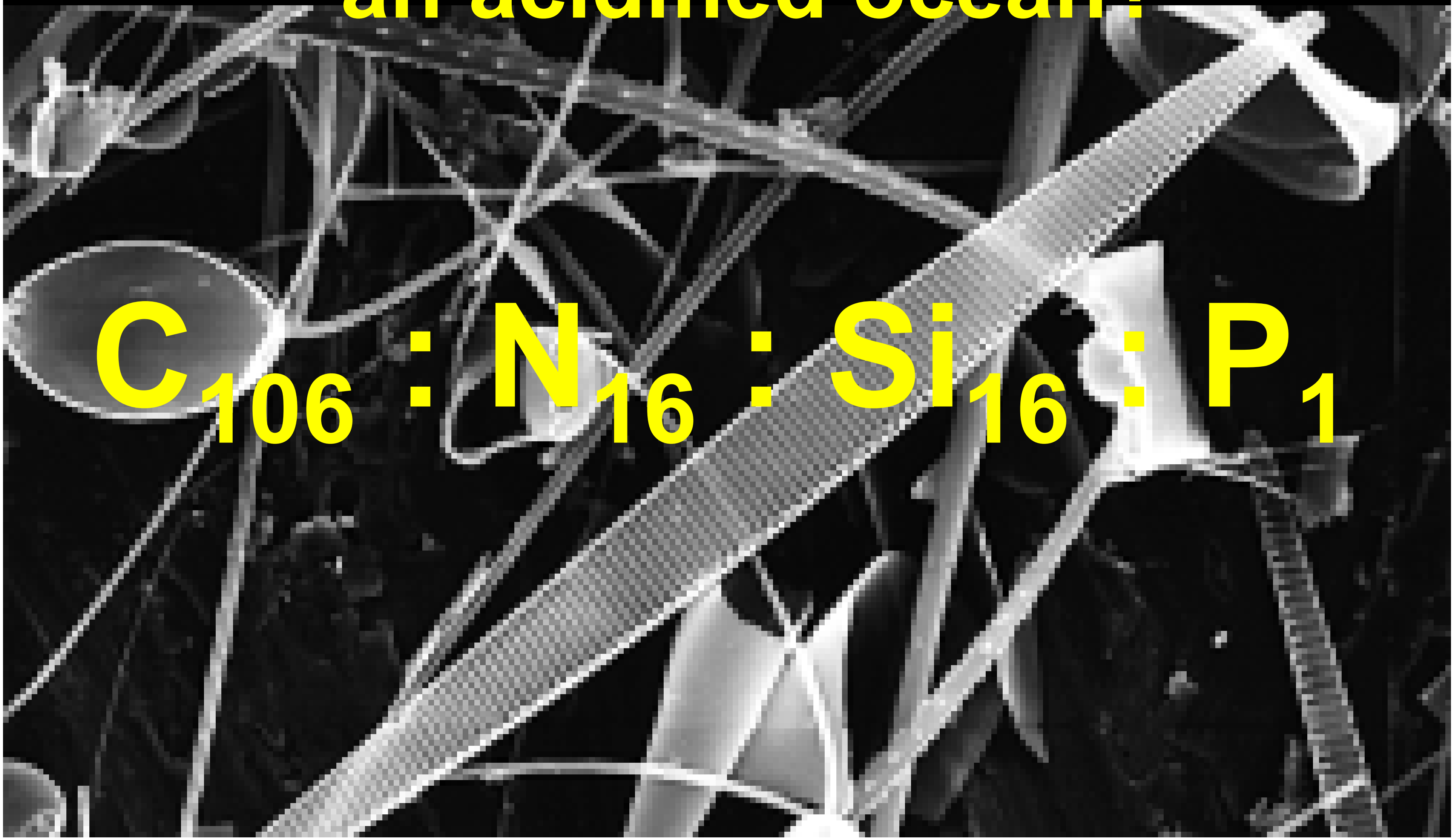
(Envisat image, ESA)

Typical N.A. bloom biological dynamics:
Diatoms (silicifying)
versus
Coccolithophores
(calcifying)





How will nutrient biogeochemistry change in an acidified ocean?



$$C_{106} : N_{16} : Si_{16} : P_{1}$$