Extensive hydrogen supersaturations in the western South Atlantic Ocean suggest substantial underestimation of nitrogen fixation

Robert M. Moore[1], Markus Kienast[1], Michael Fraser[1], John J. Cullen[1], Curtis Deutsch[2], Stephanie Dutkiewicz[3], Michael J. Follows[3], and Christopher J. Somes[4]

[1]Department of Oceanography, Dalhousie University, Halifax, Nova Scotia, Canada, [2]School of Oceanography, University of Washington, Seattle, Washington, USA, [3]Department of Earth, Atmospheric and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA, [4]GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel, Germany

Abstract The nitrogen cycle is fundamental to Earth's biogeochemistry. Yet major uncertainties of quantification remain, particularly regarding the global oceanic nitrogen fixation rate. Hydrogen is produced during nitrogen fixation and will become supersaturated in surface waters if there is net release from diazotrophs. Ocean surveys of hydrogen supersaturation thus have the potential to illustrate the spatial and temporal distribution of nitrogen fixation and to guide the far more onerous but quantitative methods for measuring it. Here we present the first transect of high resolution measurements of hydrogen supersaturations in surface waters along a meridional 10,000 km cruise track through the Atlantic. We compare measured saturations with published measurements of nitrogen fixation rates and also with model-derived values. If the primary source of excess hydrogen is nitrogen fixation and has a hydrogen release ratio similar to *Trichodesmium*, our hydrogen measurements would point to similar rates of fixation in the North and South Atlantic, roughly consistent with modeled fixation rates but not with measured rates, which are lower in the south. Possible explanations would include any substantial nitrogen fixation by newly discovered diazotrophs, particularly any having a hydrogen release ratio similar to or exceeding that of *Trichodesmium*; undersampling of nitrogen fixation south of the equator related to excessive focus on *Trichodesmium*; and methodological shortcomings of nitrogen fixation techniques that cause a bias toward colonial diazotrophs relative to unicellular forms. Alternatively, our data are affected by an unknown hydrogen source that is greater in the southern half of the cruise track than the northern.

1. Introduction

Hydrogen is rather uniformly distributed in the atmosphere at mixing ratios around 0.5 ppm in the northern hemisphere and 0.52 in the southern [*Simmonds et al.*, 2000]. Its sources include biomass burning and atmospheric photolysis of formaldehyde, as part of the oxidation sequence of methane, and its primary sink is uptake by soils (indicative of bacterial consumption) [*Rhee et al.*, 2006]. Were there no substantial marine sources or sinks, hydrogen concentrations in the surface waters would be around 0.3 nmol/L. But nitrogen fixation, a key process in the ocean that over various time scales brings into balance losses of combined nitrogen from denitrification [*Karl et al.*, 2002] and anammox, has long been known to produce hydrogen [e.g., *Hadfield and Bulen*, 1969]. If hydrogen were released stoichiometrically with fixation (equation (1)), the instantaneous production of 1 nM ammonia would result in a 147% supersaturation of hydrogen at 25°C (i.e., [H$_2$] about 2.5 times atmospheric equilibrium).

$$N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16 \ Pi \tag{1}$$

where Pi is inorganic phosphate [*Wilson et al.*, 2010a]. It follows that surface waters in oceans regions where nitrogen fixation is occurring would be expected to be supersaturated in hydrogen unless there are consumption processes in the ocean that exactly and instantaneously balance the hydrogen produced from nitrogen fixation. Currently there is no means of rapidly detecting nitrogen fixation in the oceans but

hydrogen is easily analyzed at subnanomolar concentrations, so it would seem that surveys of saturation levels might offer a way of at least qualitatively revealing the pattern of nitrogen fixation in the ocean.

Few studies have yet been made of the net release of hydrogen by marine diazotrophs and those that have indicated a range of release rates varying widely from a high of about 0.3 mol of net H_2 production per mol of N_2 fixed for the relatively well-studied diazotroph, *Trichodesmium*, to yields 1 and 2 orders of magnitude less for the unicellular *Cyanothece* sp. and *Crocosphaera watsonii*, possibly due to efficient recycling of hydrogen as a source of energy [*Wilson et al.*, 2010a]. While there are no reports of hydrogen release by endosymbiotic diazotrophs such as *Richelia intracellularis*, such diazotrophs are less likely to contribute to net hydrogen release to the host's environment as there are two organisms that might utilize it as an energy source. No fully quantitative relationship can be expected between nitrogen fixation rates and supersaturation on account of such variations in release rates driven by hydrogen recycling by diazotrophs, as well as utilization by other bacteria as an energy source, and the dependence of hydrogen concentrations on the ventilation rate of the mixed layer, a process itself dependent on mixed layer depth and wind speed. Nonetheless, it seems reasonable to suggest that nitrogen fixation will lead to supersaturation of hydrogen and that observed supersaturations of hydrogen might be used to calculate lower limits on underlying rates of nitrogen fixation.

Other marine sources of hydrogen that must be considered in accounting for any observed supersaturations are fermentation, ocean vents, photochemical production, microbial oxidation of phosphite, and contamination. Of this list, fermentation can be dismissed in oxygenated surface waters of the deep ocean. The influence of deep sea vents does not extend high into an oxygenated water column on account of microbial oxidation of hydrogen as demonstrated by many deep water profiles [e.g., *Schropp et al.*, 1987; *Moore et al.*, 2009b] that show extremely low concentration beneath a depth of a few hundred meters. *Karl* [2014] refers to the possible release of hydrogen during microbial oxidation of phosphite [*Yang and Metcalf*, 2004], but points out that there are no estimates of phosphite concentrations or fluxes in the ocean, so at present this potential source is very speculative. While hydrogen can be produced by cyanobacterial bidirectional hydrogenase, *Bothe et al.* [2010a] report that the enzyme appears not to be present in open ocean cyanobacteria. We will discuss the other possible hydrogen sources: nitrogen fixation, photochemistry, and contamination.

A series of measurements of dissolved hydrogen in the equatorial Pacific [*R. M. Moore et al.*, 2009b] supported a relationship between observed supersaturations and nitrogen fixation activity measured by $^{15}N_2$-uptake incubations. That study has motivated both a streamlining of the hydrogen analysis to permit semicontinuous measurements and a longer, more detailed survey across 10,000 km in the Atlantic Ocean as part of the Atlantic Meridional Transect programme, the results of which are presented here.

2. Methods

Dissolved hydrogen was measured between Southampton, UK, and Punta Arenas, Chile during the AMT20 cruise, 12 October to 25 November 2010 (Figure 1). Seawater from the ship's continuously pumped supply was passed at a controlled rate as a bubble-segmented stream through a glass coil equilibrator [*Xie et al.*, 2001] held in a water jacket, the temperature of which was recorded. The gas phase in the equilibrator (supplied as ultrahigh purity air) was separated from the water stream and fed to the sample loop (1 mL) of an analyzer comprising a molecular sieve column, a heated mercuric oxide bed, and a UV detector (Peak Laboratories, USA). Using the hydrogen solubility at the appropriate temperature, pressure, and salinity [*Wiesenburg and Guinasso*, 1979] and the measured concentration of hydrogen in the gas stream leaving the equilibrator, the equilibrium concentration of hydrogen in the aqueous phase was calculated. The calculated concentration of hydrogen in the seawater entering the equilibrator is the sum of the concentration in the aqueous phase leaving the equilibrator and the amount transferred into the gas stream per unit water volume. A correction factor (100/E) was then applied to account for the efficiency (E) of the equilibrator being less than 100%. The efficiency was measured daily by analyzing a water sample having a known hydrogen concentration, prepared by purging seawater with a 5 ppm hydrogen standard. The average efficiency was 78% (standard deviation 12%, n = 111). For samples collected over 2 days south of 43°S, efficiencies were anomalously low (average 56%)—a result that could be attributable to incomplete equilibration during the preparation of the seawater standard. This could result in saturations being overestimated by a
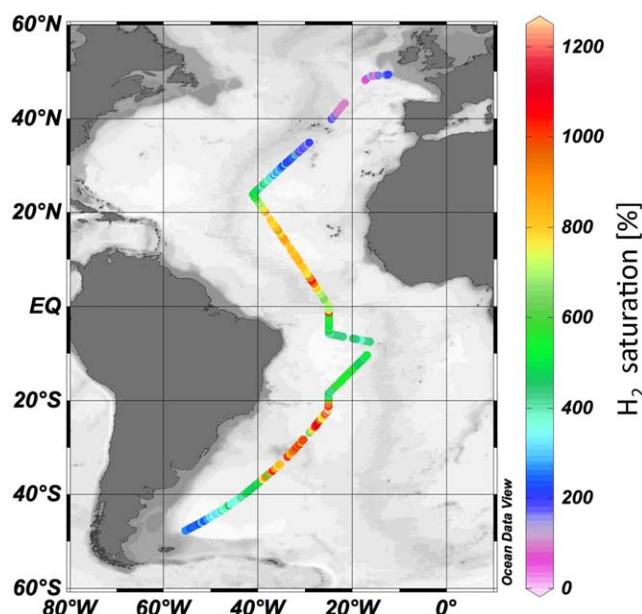
Figure 1. AMT20 cruise track of RRS James Cook from Southampton to Punta Arenas, 12 October to 25 November 2010. Breaks occur where sampling stopped as the ship traversed the territorial waters of the Azores and Ascension Island. Colored circles show saturation of dissolved hydrogen (%) along track, obtained by resampling the full data set, shown in Figure 2, approximately every 15 nautical miles.

factor of 1.4 for the southernmost samples; applying such a correction would reduce saturations at this end of the transect from around 250% to 180%. The supersaturation in the surface ocean is the measured hydrogen concentration in seawater minus that calculated to be in equilibrium with the atmosphere (C_{eq}) at ambient temperature and atmospheric pressure expressed as a percentage of C_{eq}. Measurements of equilibrated air were interspersed with scrubbed air and gas standard that had been made up by gravimetric dilution of a measured volume of hydrogen in zero air. Blanks (0.1 nmol/L, standard deviation 0.02, n = 30) were determined by running seawater purged with zero air that had been scrubbed of hydrogen.

A major potential source of hydrogen contamination in waters pumped into the vessel's supply is corrosion of zinc anodes present on the hull. Prior to the AMT20 cruise, anodes located in front of the ship's water intake were removed. Nevertheless, as hydrogen released from other anodes can result in a spreading cloud of contamination around the hull while the ship is stationary, no samples are included in the data set that were collected while the ship was on station or within 30 minutes of approaching and leaving.

Because it had earlier been found [*R. M. Moore et al.*, 2009b] that H_2 could be lost from the ship's water supply (an effect presumed to result from the presence of organisms colonizing the ship's plumbing), the plumbing was treated with bactericide (Decon) initially and twice during the cruise. Occasional extremely high measurements (23 of 5987) that could not be accounted for were identified and removed by determining a running median (in blocks of 11 measurements) for all underway measurements and identifying as anomalous all measurements that were 3.6 standard scores above or below the median; these were excluded from the analysis. As the analytical system does not respond instantaneously to a change in hydrogen concentrations, a number of peaks immediately following these 23 identified outliers were also excluded, giving a total of 42 points excluded in 5987 surface measurements.

Estimates of nitrogen fixation rates along the cruise track were obtained from three models; two are ecosystem models ("MIT" and "UVic-Kiel") [*Dutkiewicz et al.*, 2012; *Somes et al.*, 2013] that simulate the distribution and activity of diazotrophs based on resource availability, and one [*Deutsch et al.*, 2007] from a model that uses nutrient data to infer N_2 fixation rates needed to account for observed excess P consumption.

The UVic-Kiel results are from a coupled ocean-atmosphere-sea ice-biogeochemical Earth System Climate model of intermediate complexity [*Weaver et al.*, 2001]. The ocean biogeochemical-ecosystem component is based on *Somes et al.* [2013] and incorporates the ecosystem improvements outlined in *Keller et al.* [2012]. The state variables included are two nutrients (nitrate and phosphate), two phytoplankton classes (N_2-fixing diazotrophs and other phytoplankton), zooplankton, sinking detritus, dissolved organic matter as well as carbon and oxygen. Iron limitation is calculated using monthly surface dissolved iron concentrations from the BLING model [*Galbraith et al.*, 2010]. Diazotrophs grow more slowly relative to other phytoplankton, but they are not nitrogen limited. Denitrification, the consumption of nitrate in suboxic zones ($O_2 < 5$ μM) in the water column and seafloor sediments, decreases the nitrate:phosphate ratio in deep source waters and thus increases nitrogen limitation in the euphotic zone, thereby creating an ecological niche for
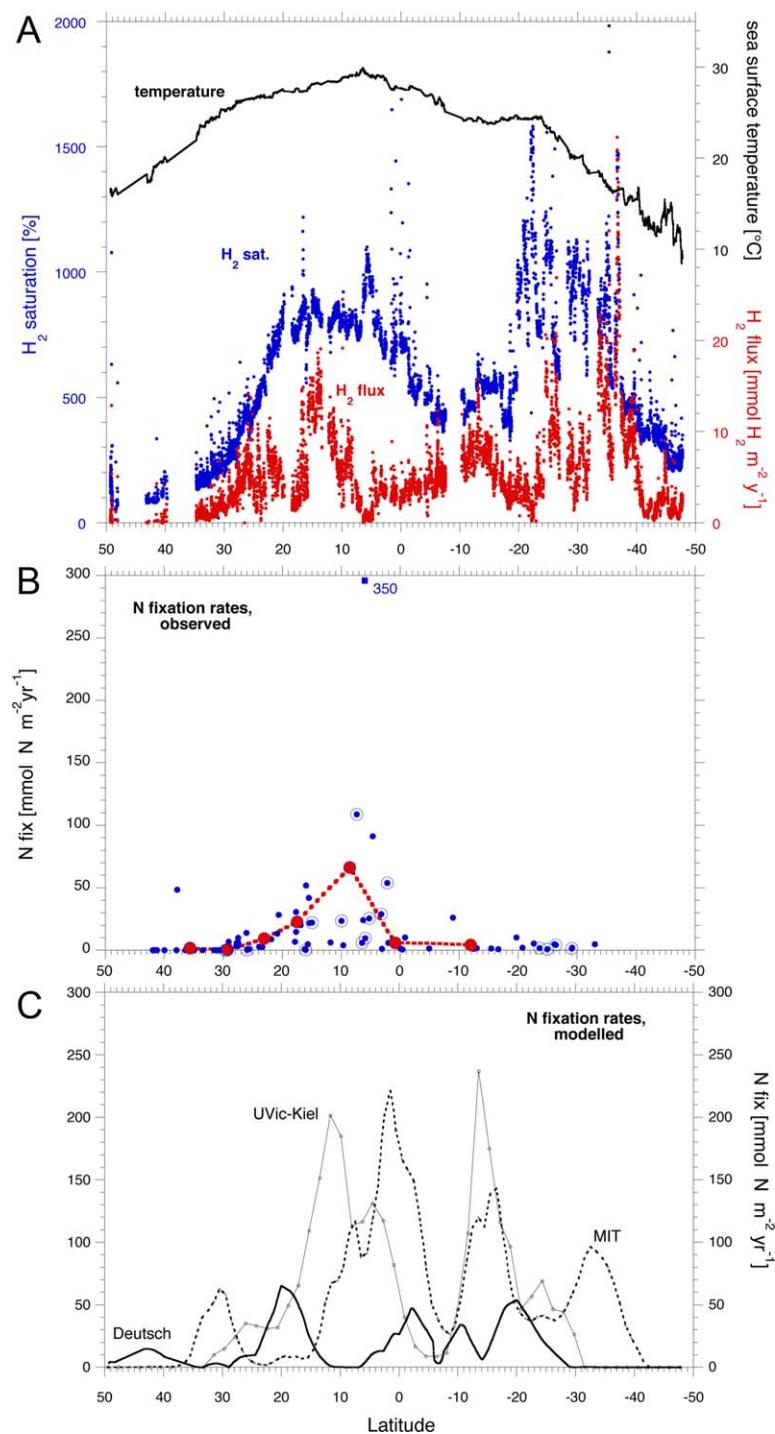
Figure 2. (a) Saturation (%) of dissolved hydrogen in surface waters, SST, and H_2 flux to the atmosphere mmol m^{-2} yr^{-1}, which can be used to estimate a minimum N_2 fixation rate assuming a 1:4 ratio of H_2 released to N_2 fixed; (b) measured nitrogen fixation rates (mmol N m^{-2} yr^{-1}) on the same cruise track of AMT17 to 20°S [*C. M. Moore et al.*, 2009a], shown as joined red circles; fixation rates from the PANGAEA database binned to 3° grid squares, measurements within 3° of the cruise track shown as circled blue points, and all other N. Atlantic measurements shown as blue dots. (c) Annual mean nitrogen fixation rates integrated to 120 m predicted by three models *UVic-Kiel/Deutsch/MIT*.

diazotrophs in the model. Diazotrophs are also capable of consuming dissolved organic phosphorus after phosphate is depleted, which can stimulate additional N_2 fixation in the oligotrophic ocean where iron is sufficiently available, most notably in the tropical/subtropical North Atlantic. Nitrogen fixation has a strong seasonal cycle with highest N_2 fixation rates occurring in summer/early fall when stratification is strong and temperatures are high. The global rates for nitrogen fixation, water column denitrification, and benthic denitrification are 175, 61.7, and 113 Tg N yr^{-1}, respectively, in the model of *Somes et al.* [2013].

The MIT results are from simulations of the MIT general circulation model (MITgcm) coupled to a biogeochemical-ecosystem model. The model [*Dutkiewicz et al.*, 2012] includes the cycling of carbon, phosphorus, nitrogen, silica, iron, and oxygen through inorganic, living, dissolved, and particulate organic phases. It resolves several nondiazotrophic autotrophs, two diazotrophs (analogs of *Trichodesmium* and unicellular), and two grazers. The diazotrophs are parameterized as growing more slowly and needing more iron than nondiazotrophs, but are not restricted in distribution by temperature [*Monteiro et al.*, 2011]. Two simulations come from two different physical model resolutions (1° × 1°, 23 levels and 2° × 2.5°, 22 levels) [*Dutkiewicz et al.*, 2012, 2014]. Within these two physical resolutions are also included simulations from
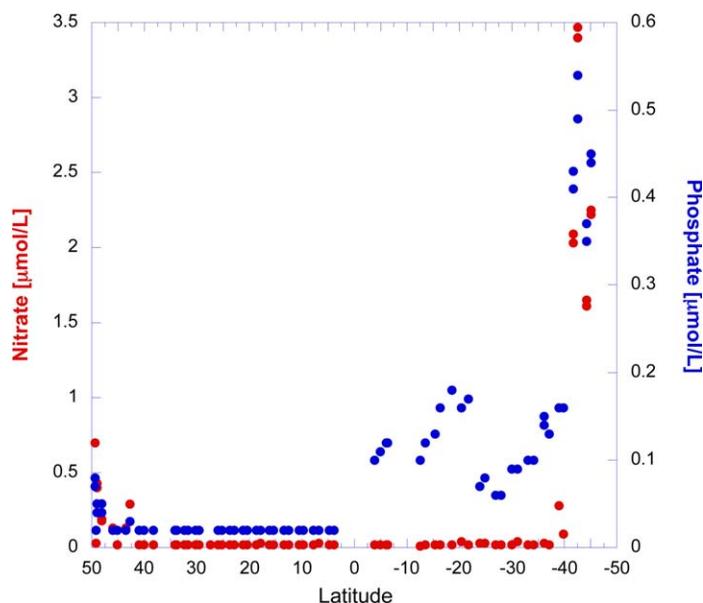
Figure 3. Plot of surface NO_3^- and PO_4^{3-} from AMT20 (μmol/L) against latitude.

sensitivity studies involving initial conditions and key parameters that are difficult to constrain with certainty (such as remineralization rates, and zooplankton grazing rates).

The approach of the *Deutsch et al.* [2007] model is entirely different in that it has no dependence on the growth dynamics of diazotrophs, but rather it identifies those regions of the ocean in which mass balance for dissolved phosphorus can only be achieved by a process that removes it independently of nitrate. In these regions the loss of excess phosphorus is attributed to diazotrophy, and the nitrogen fixation is calculated using a ratio of N/P consistent with the composition of diazotrophs. The main sources of uncertainty in the model are deficiencies in the underlying ocean circulation model and uncertainties in the seasonal distributions of nitrate and phosphate in both inorganic and organic forms.
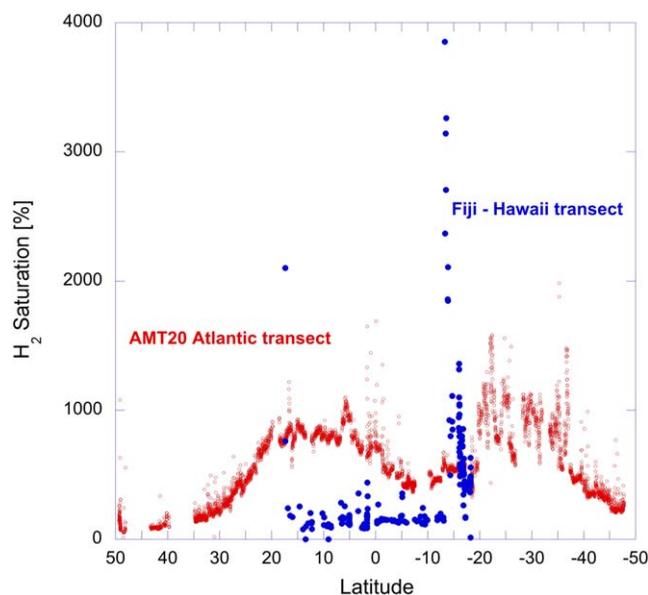
3. Results and Discussion

Hydrogen saturations are shown in Figure 2a as a function of latitude along the ship's track. Breaks occur where sampling stopped as the ship traversed the territorial waters of the Azores and Ascension Island. Prominent features are high saturations (up to ca. 800%) in two bands lying between 25°N and 35°S, separated by a section with saturations around 500% between 3°S and 20°S. Southern hemisphere saturations are markedly more variable on a scale of tens of km. Nitrate concentrations are uniformly low between 40°N and 37°S (Figure 3), but phosphate concentrations are significantly higher (0.05–0.2 μmol/L) between the equator and 40°S. Saturations of 500% and higher occur over a temperature range of around 26°C north of the equator to around 17°C to the south.

It is difficult to fully assess the possibility of contamination effects and their magnitude on our underway samples. Samples collected by Niskin bottles when the vessel was stationary showed that levels of hydrogen in the surface layer were highly variable and frequently in the range between 10 and 100 nmol/L consistent with contamination from sacrificial zinc anodes on the ship's hull (but, as noted in section 2 none was forward of the seawater intake). Inspection of Figure 2a shows that some scatter occurs above a narrow, dense band of values, but minimal scatter beneath. Contamination could contribute to positive scatter, but is not consistent with the tightly packed values forming the broad bimodal pattern with minimum values still showing high supersaturations of hydrogen.

Photochemistry has been shown to yield hydrogen from freshwater high in humic materials, and to a lesser extent from coastal seawater [*Punshon and Moore*, 2008]. This process, together with possible biological effects, was offered by *Herr et al.* [1984] as possible explanations of a relationship between hydrogen concentrations and solar radiation in waters of the tropical S. Atlantic. But extrapolation of measured production rates in colored water to the published absorption coefficients at 350 nm of oligotrophic waters led *Punshon and Moore* [2008] to conclude that only a fraction of the increase in hydrogen concentration reported by *Herr et al.* [1984] could be attributable to photochemical production. We are not aware of any thorough study involving direct measurements of photochemical production of H_2 in open ocean waters, but H_2 data [*R. M. Moore et al.*, 2009b] from a transect between Fiji (18°S) and Hawaii (19°N) (Figure 4) were

Figure 4. H_2 supersaturations along the AMT20 transect and a transect between Fiji and Hawaii [*R. M. Moore et al.*, 2009b].

tightly correlated with measures of nitrogen fixation rates, so there was no need to invoke any significant photochemical production.

The production of H_2 from N_2 fixation has been known for decades [e.g., *Hadfield and Bulen*, 1969; *Scranton et al.*, 1987] even though details of the production mechanism have been slow to emerge. *Ogo et al.* [2004] proposed that H_2 is evolved by reductive elimination from a dihydride species in the reactive centre of the nitrogenase that leaves the centre in an activated state, primed for binding of N_2 and its subsequent reduction. This mechanism is supported by *Hoffman et al.* [2014]. While the primary stoichiometry of the mechanism is 1 mol H_2 produced:1 mol of N_2 fixed (equation (1)), much of the reducing power in H_2 is reutilized intracellularly through the action of uptake hydrogenases [*Wilson et al.*, 2010b, 2012]. It can be speculated that if the diazotroph itself does not consume some of the waste H_2, it is likely to be consumed by other bacteria in oxygenated water. It is the net release rates from diazotrophs to solution that is relevant here. Laboratory measured ratios of net H_2 production to N_2 fixation range from 0.2 to 0.3 for *Trichodesmium*, which fixes N_2 during the day [*Wilson et al.*, 2012] to 0.05 for the unicellular cyanobacterium *Cyanothece*, and 0.03 for the unicellular *Crocosphaera* [*Wilson et al.*, 2010a]. Both of these unicellular diazotrophs fix N_2 at night, thereby separating photosynthetic production of oxygen from oxygen-sensitive nitrogen fixation. In turn, lower nocturnal availability of reductant would be expected to limit rates of hydrogen release [*Wilson et al.*, 2010b, 2012]. It should be noted that *Cyanothece* has a coastal and benthic distribution rather than oceanic.

What is the minimum rate of nitrogen fixation that would be consistent with our observed H_2 concentrations? Estimating that rate is done in two steps, both of which have major uncertainties. The first is converting the observed supersaturations to fluxes, and the second is choosing a conversion factor. The net production of H_2 in the water column is set equal to its flux to the atmosphere (Figure 2a), that being the product of the excess H_2 and an exchange velocity calculated from measured wind speed using the relationship of *Wanninkhof* [1992]. Our calculated rate of N_2 fixation will be an underestimate on account of N_2 fixation beneath the mixed layer that will not contribute significantly to the H_2 flux to the atmosphere, and some unknown fraction of the H_2 produced throughout the water column would be consumed by microbes. Most flux values lie in the range 10 ± 5 mmol H_2 m^{-2} yr^{-1}. As we are estimating the minimum nitrogen fixation rate capable of accounting for the estimated hydrogen production, we select the release ratio of H_2 to N_2 fixed reported for *Trichodesmium*, 1:4 [*Wilson et al.*, 2012]. This leads to an estimated 80 mmol N m^{-2} yr^{-1} (range 40–120 mmol N m^{-2} yr^{-1}), close to a weighted grand average of N fixation by *Trichodesmium* for 154 stations in the tropical North Atlantic, 87.2 ± 13.9 (s.e.) mmol N m^{-2} yr^{-1} [*Capone et al.*, 2005], and thus consistent with expectations if *Trichodesmium* dominates N-fixation in the tropical North Atlantic, as has been suggested by *Goebel et al.* [2010], based on quantification of *nifH* genes from different taxa. The calculated H_2 fluxes in the southern hemisphere show higher mesoscale variability (Figure 2) but require N_2 fixation of a similar magnitude, or more if H_2:N_2 release rates are lower than assumed.

Measured nitrogen fixation rates for the Atlantic Ocean binned to 3° grid squares extracted from the PANGAEA database assembled by *Luo et al.* [2012] are shown in Figure 2b. Measurements within 3° of the cruise track are identified and also some along the same cruise track (as far as 20°S) from AMT17 [*C. M. Moore et al.*, 2009a] when nitrogen fixation was strongly biased to the northern hemisphere and was reported to

closely match the distribution of *Trichodesmium*, the abundance of which was at, or close to, zero south of the equator, a fact attributed to deeper mixed layers (from ∼3°N to ∼10°S) and, in particular, to iron limitation. It should be noted that the track of AMT17 diverged from that of AMT20 at 20°S and proceeded in a southeasterly direction. *C. M. Moore et al.* [2009a, Figures 2b and 2c] show elevated N* and decreased P*, both indicators of nitrogen fixation, in the western South Atlantic from ∼25°S to 35°S in an area traversed by our cruise track.

It is apparent that an estimate of nitrogen fixation in the range 40–120 mmol N m^{-2} yr^{-1} is consistent with the data between 30°N and the equator, but in the southern hemisphere the very sparse measurements available are much below this range. The implications of the comparison between hydrogen production estimates and measured nitrogen fixation measurements are (i) that the observed hydrogen supersaturations in the northern hemisphere Atlantic could be accounted for by nitrogen fixation if the molar ratio of H_2 released to N_2 fixed is near 0.25—a value reported for *Trichodesmium* in culture, and (ii) that N_2 fixation is markedly higher in the southern hemisphere western Atlantic than indicated by the PANGAEA database, and/or the source of H_2 in the southern hemisphere is different from that in the northern.

A deficiency of published nitrogen fixation rates is that some unknown fraction of them are likely to be underestimates on account of methodological shortcomings, namely, the incomplete equilibration of $^{15}N_2$ gas added to the incubated seawater [*Großkopf et al.*, 2012]. These authors report evidence that the underestimation can be up to 6 times greater for unicellular diazotrophs than for *Trichodesmium*. Any general underestimation of N_2 fixation rates would strengthen the case for attributing hydrogen supersaturations to diazotrophy, certainly in the case of a *Trichodesmium* source, and for unicellular diazotrophs that have a greater release ratio than the two species that have so far been studied (*Cyanothece* and *Crocosphaera*), which fix N_2 at night and hence derive greater benefit from recycling H_2. It is interesting that a recently discovered and so far uncultivated unicellular diazotroph UCYN-A seems to have adapted to daytime N_2 fixation, when proximity to photosynthetic oxygen production is a problem, by lacking PSII and being symbiotically associated with a Prymnesiophyte [*Bothe et al.*, 2010b; *Thompson et al.*, 2012]. These traits could have consequences for net H_2 production. Besides reducing exposure to high concentrations of photosynthetically produced oxygen, a loose, surface association of UCYN-A with the alga could permit ready loss of H_2 to the surrounding water. Fixation of nitrogen during the day diminishes the need for energy production from hydrogen recycling, suggesting that the ratio of net H_2 production to N_2 fixation might be higher than for other coccoid cyanobacteria that fix N_2 at night [*Wilson et al.*, 2010a]. Evaluation of these suggestions, and related predictions for diatom-diazotroph symbioses, might have to await the successful culturing of UCYN-A and further studies of the associations of cyanobacteria with symbiotic algae [cf. *Goebel et al.*, 2010].

In view of the sparse measurements of nitrogen fixation in the South Atlantic and questions regarding their reliability, we have considered three model-based estimates of N_2 fixation rates along the cruise track (Figure 2c). In the case of the MIT model a measure of uncertainty is provided from a range of simulations that yield variations in the ratio of supply rates of iron and phosphate relative to dissolved inorganic nitrogen. These ratios are crucial in dictating where diazotrophs can flourish [*Ward et al.*, 2013], so their variation leads to the spread of results seen in Figure 5. The envelope of uncertainty from the Deutsch model is provided by the maximum and minimum for each month. Despite the differences in assumptions and approach, in all these models the mean values of N_2 fixation along the cruise track (integration depth 120 m) support the occurrence of widespread N_2 fixation between latitudes 30°N and 30°S (Figure 2c). On an annual basis, none of the models predicts significantly higher N_2 fixation in the northern hemisphere than southern along our cruise track (Figure 1). Unless there is a large-scale difference in the hydrogen release ratios for diazotrophs between the two regions, the diazotrophic contribution to hydrogen supersaturations would be expected to be similar. This would be broadly consistent with our observed pattern of hydrogen supersaturations and suggests that nitrogen fixation has been underestimated in the southern hemisphere Atlantic, resulting perhaps from too restricted sampling, or use of methods that tend to underestimate nitrogen fixation from important classes of diazotrophs, such as unicellular forms. Any such underestimation would need to be associated with diazotrophs that have a hydrogen release ratio similar to (or higher than) that of *Trichodesmium*. Alternatively, it would seem necessary to argue that the three models all have shortcomings that cause them to overestimate nitrogen fixation in the southern hemisphere Atlantic. Certainly, the models are not consistent with each other in their more detailed latitudinal simulations of
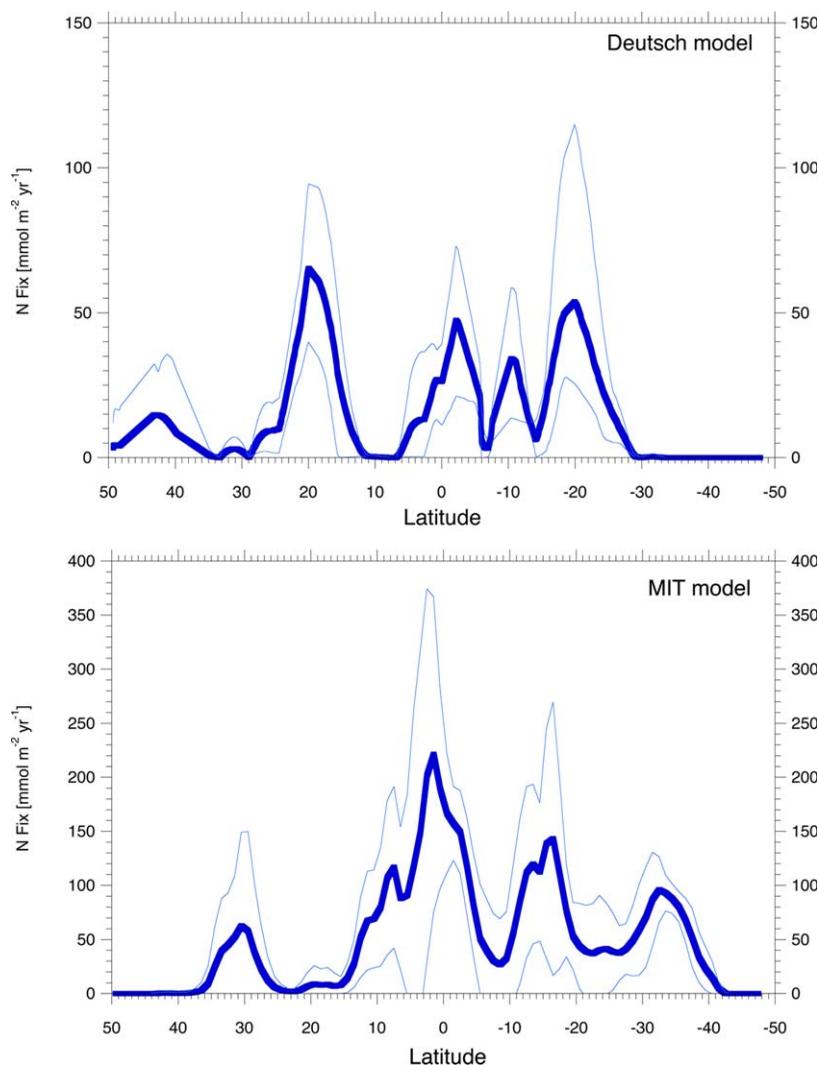
Figure 5. (top) The modeled mean nitrogen fixation rate (mmol N m^{-2} yr^{-1}) along the AMT20 cruise track and range of uncertainties resulting from seasonal variations (Deutsch model); (bottom) the modeled mean and range along the same track from an ensemble of MIT model runs.

nitrogen fixation, but they do combine our current knowledge of the controls on diazotrophs and their ecology, and our knowledge of the distribution of excess phosphate (P*) in the Atlantic [*Deutsch et al.*, 2007].

Other modeling studies also suggest nitrogen fixation south of the equator: the models of *Hood et al.* [2004] and *Stukel et al.* [2013] indicated *Trichodesmium* in the S. Atlantic, mainly in the eastern basin, but also around 10°S in the longitude range of our models and cruise track, and the models of *Moore and Doney* [2007] and *Krishnamurthy et al.* [2009] indicate very similar nitrogen fixation as our models and along much of the cruise track. The compilation of data on *Trichodesmium* distribution in *LaRoche and Breitbarth* [2005] also shows it to have been observed in the latitude range 20°S–35°S close to the AMT20 cruise track. However, while *Tyrrell et al.* [2003] report a moderate abundance of *Trichodesmium* at ca. 35°S, also close to the AMT20 track, they found generally low abundances south of the equator in contrast to high abundances between 0°N and 15°N around 15°W–25°W.

Can it be the case that nitrogen fixation plays no significant role at all in the large-scale distribution of hydrogen supersaturations? This proposal is inconsistent with field results reported from a lower resolution study of hydrogen supersaturations and nitrogen fixation in the equatorial Pacific [*C. M. Moore et al.*, 2009a], and also with laboratory studies that show *Trichodesmium* to release H$_2$ at a ratio to N$_2$ fixed of around 0.25.

Could the database of nitrogen fixation measurements in the Atlantic be biased such as to emphasize diazotrophy in some areas and underrepresent it in others? Historically there has been a bias toward making nitrogen fixation measurements in warm waters and a focus on *Trichodesmium* spp., the colonies of which are conspicuous and undoubtedly a major contributor to marine nitrogen fixation. *Trichodesmium* spp. distribution are reported [*Carpenter and Capone*, 2008] to be roughly limited by the 20°C isotherm, and while the organisms may be found in cooler water their activity is low and their growth slow. While laboratory studies [*Staal et al.*, 2003] have shown acetylene reduction in a *Trichodesmium* culture (strain IMS 101) increasing right up to 35°C,

MOORE ET AL.   4347

Breitbarth [2005] reports maximum growth rates between 24 and 30°C. *Karl et al.* [2002] comment that most reported *Trichodesmium* blooms occur in waters at or warmer than 25°C. But there is now evidence for the presence of unicellular diazotrophs over a wider temperature range. For example, while *Langlois et al.* [2005] found filamentous cyanobacterial nifH sequences, with one exception, in waters having temperatures between 26.5 and 30°C and undetectable nitrate, they found nifH sequences of nonfilamentous diazotrophs in waters from 15 to 30°C and sometimes containing detectable nitrate. Unicellular diazotrophs have also been reported to be active in the temperature range 19–25°C, though typically showing lower N_2 fixation rates than *Trichodesmium* [*Needoba et al.*, 2007]. The modeling study of *Monteiro et al.* [2011] also suggested a wider range and almost equal importance of unicellular diazotrophs to *Trichodesmium*.

If it is the case that unicellular diazotrophs have been underrepresented in studies of nitrogen fixation in the Atlantic, or their activity underestimated, it can begin to account for our reported distribution of hydrogen only if the ratio of H_2 released to N_2 fixed is higher than that of the only two species so far examined. This in turn strongly suggests that for any missing unicellular diazotroph to be significant in this respect, it would likely be a daytime fixer of nitrogen, so having a reduced need to recycle the hydrogen that it produces.

4. Conclusions

Supersaturations of hydrogen measured in the Atlantic Ocean north of the equator can be accounted for by typical published nitrogen fixation rates when a hydrogen:nitrogen release ratio matching that for *Trichodesmium* is used.

Our data show similar hydrogen supersaturations in the western Atlantic south of the equator. These are not consistent with published nitrogen fixation rates, even using release ratios around 0.25, typical of *Trichodesmium*. Possible explanations could include (i) an unknown source of hydrogen that does not play a major role in the North Atlantic, (ii) underestimation of nitrogen fixation south of the equator associated with a diazotroph that has a low efficiency of recycling hydrogen—this suggests a daytime fixer of N_2. Possible reasons for nitrogen fixation having been underestimated include a bias toward sampling in waters warm enough to support *Trichodesmium* growth, and the use of methods for measuring fixation rates that discriminate against unicellular diazotrophs.

Our hydrogen distribution in the Atlantic is consistent with modeled nitrogen fixation in that the distribution of each is approximately symmetrical about the equator—but with a requirement that the hydrogen:nitrogen release ratio cannot substantially differ between the north and south.

Interpretation of our data is hampered by the absence of information of hydrogen release rates of any unicellular diazotrophs that fix nitrogen during the day. Of particular interest is a UCYN-A that lacks photosystem II, thereby reducing the constraint imposed by photosynthetic oxygen production on the activity of oxygen-sensitive nitrogenase. Measurement of hydrogen release by this bacterium would be a great interest, but the organism has yet to be successfully cultured.

References

Bothe, H., H. J. Tripp, and J. P. Zehr (2010a), Unicellular cyanobacteria with a new mode of life: The lack of photosynthetic oxygen evolution allows nitrogen Fixation to proceed, *Arch. Microbiol.*, *192*, 783–790, doi:10.1007/s00203-010-0621-5.

Bothe, H., O. Schmitz, M. G. Yates, and W. E. Newton (2010b), Nitrogen fixation and hydrogen metabolism in cyanobacteria, *Microbiol. Mol. Biol. Rev.*, *74*, 529–551, doi:10.1128/MMBR.00033-10.

Breitbarth, E. (2005), Ecophysiology of the cyanobacterium *Trichodesmium*, PhD thesis, Christian Albrechts Univ. of Kiel, Kiel, Germany.

Capone, D. G., J. A. Burns, J. P. Montoya, A. Subramaniam, C. Mahaffey, T. Gunderson, A. F. Michaels, and E. J. Carpenter (2005), Nitrogen fixation by *Trichodesmium* spp.: An important source of new nitrogen to the tropical and subtropical North Atlantic Ocean, *Global Biogeochem. Cycles*, *19*, GB2024, doi:10.1029/2004GB002331.

Carpenter, E. J., and D. G. Capone (2008), *Nitrogen in the Marine Environment*, 2nd ed., Academic, San Diego, Calif., doi:10.1016/B978-0-12-372522-6.00004-9.

Deutsch, C., J. L. Sarmiento, D. M. Sigman, N. Gruber, and J. P. Dunne (2007), Spatial coupling of nitrogen inputs and losses in the ocean, *Nature*, *445*, 163–167, doi:10.1038/nature05392.

Dutkiewicz, S., B. A. Ward, F. Monteiro, and M. F. Follows (2012), Interconnection between nitrogen fixers and iron in the Pacific Ocean: Theory and numerical model, *Global Biogeochem. Cycles*, *26*, GB1012, doi:10.1029/2011GB004039.

Dutkiewicz, S., B. A. Ward, J. Scott, and M. Follows (2014), Understanding predicted shifts in diazotroph biogeography using resource competition theory, *Biogeosci. Discuss.*, *11*, 7113–7149, doi:10.5194/bgd-11-7113-2014.

Galbraith, E. D., A. Gnanadesikan, J. P. Dunne, and M. R. Hiscock (2010), Regional impacts of iron-light colimitation in a global biogeochemical model, *Biogeosciences*, *7*, 1043–1064, doi:10.5194/bg-7-1043-2010.

Goebel, N. L., K. A. Turk, K. M. Achilles, R. Paerl, I. Hewson, A. E. Morrison, J. P. Montoya, C. A. Edwards, and J. P. Zehr (2010), Abundance and distribution of major groups of diazotrophic cyanobacteria and their potential contribution to N_2 fixation in the tropical Atlantic Ocean, *Environ. Microbiol.*, *12*, 3272–3289.

Großkopf, T., W. Mohr, T. Baustian, H. Schunck, D. Gill, M. M. M. Kuypers, G. Lavik, R. A. Schmitz, D. W. R. Wallace, and J. LaRoche (2012), Doubling of marine dinitrogen-fixation rates based on direct measurements, *Nature*, *488*, 361–364, doi:10.1038/nature11338.

Hadfield, K. L., and W. A. Bulen (1969), Adenosine triphosphate requirement of nitrogenase from *Azotobacter vinelandii*, *Biochemistry*, *12*, 5103–5108.

Herr, F. L., E. C. Frank, G. M. Leone, and M. C. Kennicutt (1984), Diurnal variability of dissolved molecular hydrogen in the tropical South Atlantic Ocean, *Deep Sea Res., Part A*, *31*, 13–20.

Hoffman, B. M., D. Lukoyanov, Z.-Y. Yang, D. R. Dean, and L. C. Seefeldt (2014), Mechanism of nitrogen fixation by nitrogenase: The next stage, *Chem. Rev.*, *114*, 4041–4062, doi:10.1021/cr400641x.

Hood, H. R., V. J. Coles, and D. G. Capone (2004), Modeling the distribution of *Trichodesmium* and nitrogen fixation in the Atlantic Ocean, *J. Geophys. Res.*, *109*, C06006, doi:10.1029/2002JC001753.

Karl, D. M. (2014), Microbially mediated transformations of phosphorus in the Sea: New views of an old cycle, *Annu. Rev. Mar. Sci.*, *6*, 279–337, doi:10.1146/annurev-marine-010213-135046.

Karl, D. M., A. Michaels, B. Bergman, D. Capone, E. Carpenter, R. Letelier, F. Lipschultz, H. Paerl, D. Sigman, and L. Stal (2002), Dinitrogen fixation in the world's oceans, *Biogeochemistry*, *57/58*, 47–98.

Keller, D. P., A. Oschlies, and M. Eby (2012), A new marine ecosystem model for the University of Victoria Earth System Climate Model, *Geosci. Model Dev.*, *5*, 1195–1220, doi:10.5194/gmd-5-1195-2012.

Krishnamurthy, A., J. K. Moore, N. Mahowald, C. Luo, S. C. Doney, K. Lindsay, and C. S. Zender (2009), Impacts of increasing anthropogenic soluble iron and nitrogen deposition on ocean biogeochemistry, *Global Biogeochem. Cycles*, *23*, GB3016, doi:10.1029/2008GB003440.

Langlois, R. J., J. LaRoche, and P. A. Raab (2005), Diazotrophic diversity and distribution in the tropical and subtropical Atlantic Ocean, *Appl. Environ. Microbiol.*, *71*, 7910–7919, doi:10.1128/AEM.71.12.7910-7919.

LaRoche, J., and E. Breitbarth (2005), Importance of the diazotrophs as a source of new nitrogen in the ocean, *J. Sea Res.*, *53*, 63–91.

Luo, Y., et al. (2012), Database of diazotrophs in global ocean: Abundance, biomass and nitrogen fixation rates, *Earth Syst. Sci. Data*, *4*, 47–73, doi:10.5194/essd-4-47-2012.

Monteiro, F., S. Dutkiewicz, and M. F. Follows (2011), Biogeographical controls on the marine nitrogen fixers, *Global Biogeochem. Cycles*, *25*, GB2003, doi:10.1029/2010GB003902.

Moore, C. M., et al. (2009a), Large-scale distribution of Atlantic nitrogen fixation controlled by iron availability, *Nat. Geosci.*, *2*, 867–871, doi:10.1038/NGE0667.

Moore, J. K., and S. C. Doney (2007), Iron availability limits the ocean nitrogen inventory stabilizing feedbacks between marine denitrification and nitrogen fixation, *Global Biogeochem. Cycles*, *21*, GB2001, doi:10.1029/2006GB002762.

Moore, R. M., S. Punshon, C. Mahaffey, and D. M. Karl (2009b), The relationship between dissolved hydrogen and nitrogen fixation in ocean waters, *Deep Sea Res., Part I*, *56*, 1449–1458.

Needoba, J. A., R. A. Foster, C. Sakamoto, J. P. Zehr, and K. S. Johnson (2007), Nitrogen fixation by unicellular diazotrophic cyanobacteria in the temperate oligotrophic North Pacific Ocean, *Limnol. Oceanogr. Methods*, *52*, 1317–1327.

Ogo, S., B. Kure, H. Nakai, Y. Watanabe, and S. Fukuzumi (2004), Why do nitrogenases waste electrons by evolving dihydrogen? *Appl. Organometal. Chem.*, *18*, 589–594, doi:10.1002/aoc.744.

Punshon, S., and R. M. Moore (2008), Photochemical production of molecular hydrogen in lake water and coastal seawater, *Mar. Chem.*, *108*, 215–220.

Rhee, T. S., C. A. M. Brenninkmeijer, and T. Rockmann (2006), The overwhelming role of soils in the global atmospheric hydrogen cycle, *Atmos. Chem. Phys.*, *6*, 1611–1625.

Schropp, S. J., M. I. Scranton, and J. R. Schwarz (1987), Dissolved hydrogen, facultatively anaerobic, hydrogen-producing bacteria, and potential hydrogen production rates in the western North Atlantic Ocean and Gulf of Mexico, *Limnol. Oceanogr.*, *32*, 396–402.

Scranton, M. I., P. C. Novelli, A. Michaels, S. G. Horrigan, and E. J. Carpenter (1987), Hydrogen production and nitrogen fixation by *Oscillatoria thiebautii* during in situ incubations, *Limnol. Oceanogr.*, *32*, 998–1006.

Simmonds, P. G., R. G. Derwent, S. O'Doherty, D. B. Ryall, L. P. Steele, R. L. Langenfelds, P. Salameh, H. J. Wang, C. H. Dimmer, and L. E. Hudson (2000), Continuous high-frequency observations of hydrogen at the Mace Head baseline atmospheric monitoring station over the 1994–1998 period, *J. Geophys. Res.*, *105*, 12,105–12,121.

Somes, C. J., A. Oschlies, and A. Schmittner (2013), Isotopic constraints on the pre-industrial oceanic nitrogen budget, *Biogeosciences*, *10*, 5889–5910, doi:10.5194/bg-10-5889-2013.

Staal, M., F. J. R. Meysman, and L. J. Stal (2003), Temperature excludes N_2-fixing heterocystous cyanobacteria in the tropical oceans, *Nature*, *425*, 504–507.

Stukel, M. R., V. J. Coles, M. T. Brooks, and R. R. Hood (2013), Top-down, bottom-up and physical controls of diatom-diazotroph assemblages in the Amazon River Plume, *Biogeosci. Discuss.*, *10*, 13,931–13,976, doi:10.5194/bgd-10-13931-2013.

Thompson, A. W., R. A. Foster, A. Krupke, B. J. Carter, N. Musat, D. Vaulot, M. M. M. Kuypers, and J. P. Zehr (2012), Unicellular cyanobacterium symbiotic with a single-celled eukaryotic alga, *Science*, *337*, 1546, doi:10.1126/science.1222700.

Tyrrell, T., E. Marañón, A. J. Poulton, A. R. Bowie, D. S. Harbour, and E. M. S. Woodward (2003), Large-scale latitudinal distribution of *Trichodesmium* spp. in the Atlantic Ocean, *J. Plankton Res.*, *25*, 405–416.

Wanninkhof, R. (1992), Relationship between wind speed and gas exchange over the ocean, *J. Geophys. Res.*, *97*, 7373–7382.

Ward, B. A., S. Dutkiewicz, C. M. Moore, and M. J. Follows (2013), Iron, phosphorus and nitrogen supply ratios define the biogeography of nitrogen fixation, *Limnol. Oceanogr. Methods*, *58*, 2059–2075.

Weaver, A. J., et al. (2001), The UVic earth system climate model: Model description, climatology, and applications to past, present and future climates, *Atmos. Ocean*, *39*, 361–428.

Wiesenburg, D. A., and N. L. Guinasso (1979), Equilibrium solubilities of methane, carbon monoxide and hydrogen in water and seawater, *J. Chem. Eng. Data*, *24*, 356–360.

Wilson, S. T., R. A. Foster, J. P. Zehr, and D. M. Karl (2010a), Hydrogen production by *Trichodesmium erythraeum Cyanothece* sp. and *Crocosphaera watsonii*, *Aquat. Microb. Ecol.*, *59*, 197–206, doi:10.3354/ame01407.

Wilson, S. T., S. Tozzi, R. A. Foster, I. Ilikchyan, Z. S. Kolber, J. P. Zehr, and D. M. Karl (2010b), Hydrogen cycling by the unicellular marine diazotroph *Crocosphaera watsonii* strain WH8501, *Appl. Environ. Microbiol.*, *76*, 6797–6803.

Wilson, S. T., Z. S. Kolber, S. Tozzi, J. P. Zehr, and D. M. Karl (2012), Nitrogen fixation, hydrogen cycling, and electron transport kinetics in *Trichodesmium erythraeum* (Cyanobacteria) strain IMS 1011, *J. Phycol.*, *48*, 595–606.

Xie, H., O. C. Zafiriou, W. Wang, and C. D. Taylor (2001), A simple automated continuous-flow-equilibration method for measuring carbon monoxide in seawater, *Environ. Sci. Technol.*, *35*(7), 1475–1480, doi:10.1021/es001656v.

Yang, K., and W. W. Metcalf (2004), A new activity for an old enzyme: *Escherichia coli* bacterial alkaline phosphatase is a phosphite-dependent hydrogenase, *Proc. Natl. Acad. Sci. U. S. A.*, *101*, 7919–7924.