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

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Key points

Widespread H_2 supersaturations reported in Atlantic Ocean between 30N and 40S

Supersaturations are similar N and S, suggesting N_2 fixation S is similar to N of equator

Measured N_2 fixation is low S of equator, suggesting gaps in sampling or a novel source

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 modelled 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*; under-sampling of nitrogen fixation south of the equator related to excessive focus on *Trichodesmium*; and methodological shortcomings of nitrogen fixation techniques that cause a bias towards

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.

Index Terms
Oceanography: Biological and Chemical
Gases
Biogeochemical cycles and modelling
Nutrients and nutrient cycling

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 timescales 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 nanomolar ammonia would result in a supersaturation of hydrogen by 147% at 25°C (i.e. $[H_2]$ about 2.5 times atmospheric equilibrium).

$$N_2 + 8H^+ + 8e^- + 16ATP -> 2NH_3 + H_2 + 16ADP + 16\ Pi \qquad \text{(Eq. 1)}$$

-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 sub-nanomolar 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 indicate a range of release rates varying widely from a high of about 0.3 mol of net hydrogen production per mol of N_2-fixed for the relatively well-studied diazotroph, *Trichodesmium*, to yields one and two 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*., 2009) 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.* (2010) 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 (Moore *et al*., 2009) 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 semi-continuous 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.

Methods

Dissolved hydrogen was measured between Southampton, UK, and Punta Arenas, Chile during the AMT-20 cruise, October 12 – November 25, 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 (Wiesenberg 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 two 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 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 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 (Moore *et al*., 2009) that H_2 could be lost from the ship's water supply (an effect presumed to result from the presence of organisms colonising 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 3 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 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 non-diazotrophic autotrophs, two diazotrophs (analogs of *Trichodesmium* and unicellular) and two grazers. The diazotrophs are parameterized as growing more slowly and needing more iron than non-diazotrophs, but are not restricted in distribution by temperature (Monteiro *et al*., 2011). Two simulations come from two different physical model resolutions (1°x1°, 23 levels and 2°x2.5°, 22 levels) (Dutkiewicz *et al*., 2012; Dutkiewicz *et al*., 2014). Within these two physical resolutions are also included simulations from 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.

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 10s 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 micromoles/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 10 and 100 nmol/L consistent with contamination from sacrificial zinc anodes on the ship's hull (but, as noted in Methods 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, were 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 coloured 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 (Moore *et al*., 2009) from a transect between Fiji (18°S) and Hawaii (19°N) (Figure 4) were 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 1mol H_2 produced:1mol of N_2 fixed (Equation 1), much of the reducing power in H_2 is reutilized intracellulary through the action of uptake hydrogenases (Wilson *et al*., 2010b, Wilson *et al*., 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 - 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; Wilson *et al*., 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 Wanninkof (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} y^{-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 $m^{-2}y^{-1}$ (range 40 – 120 mmol N $m^{-2}y^{-1}$), 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} y^{-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*., 2009) 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. Figures 2b and 2c in C.M. Moore *et al*. (2009) show elevated N* and decreased P*, both indicators of nitrogen fixation, in the western South Atlantic from ~25°S-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 $m^{-2}y^{-1}$ 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*), that 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*., 2010; 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 3 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 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 modelling 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 J.Moore and Doney (2007) and Krishnamurphy *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 20-35°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 and 15°N around 15-25°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 (Moore *et al*. 2009), 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 under-represent it in others? Historically there has been a bias towards 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, Breibarth (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 non-filamentous 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 modelling study of Monteiro *et al*. (2010) also suggested a wider range and almost equal importance of unicellular diazotrophs to *Trichodesmium*.

If it is the case that unicellular diazotrophs have been under-represented 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.

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 towards 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 modelled 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 are hampered by 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.

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Figures

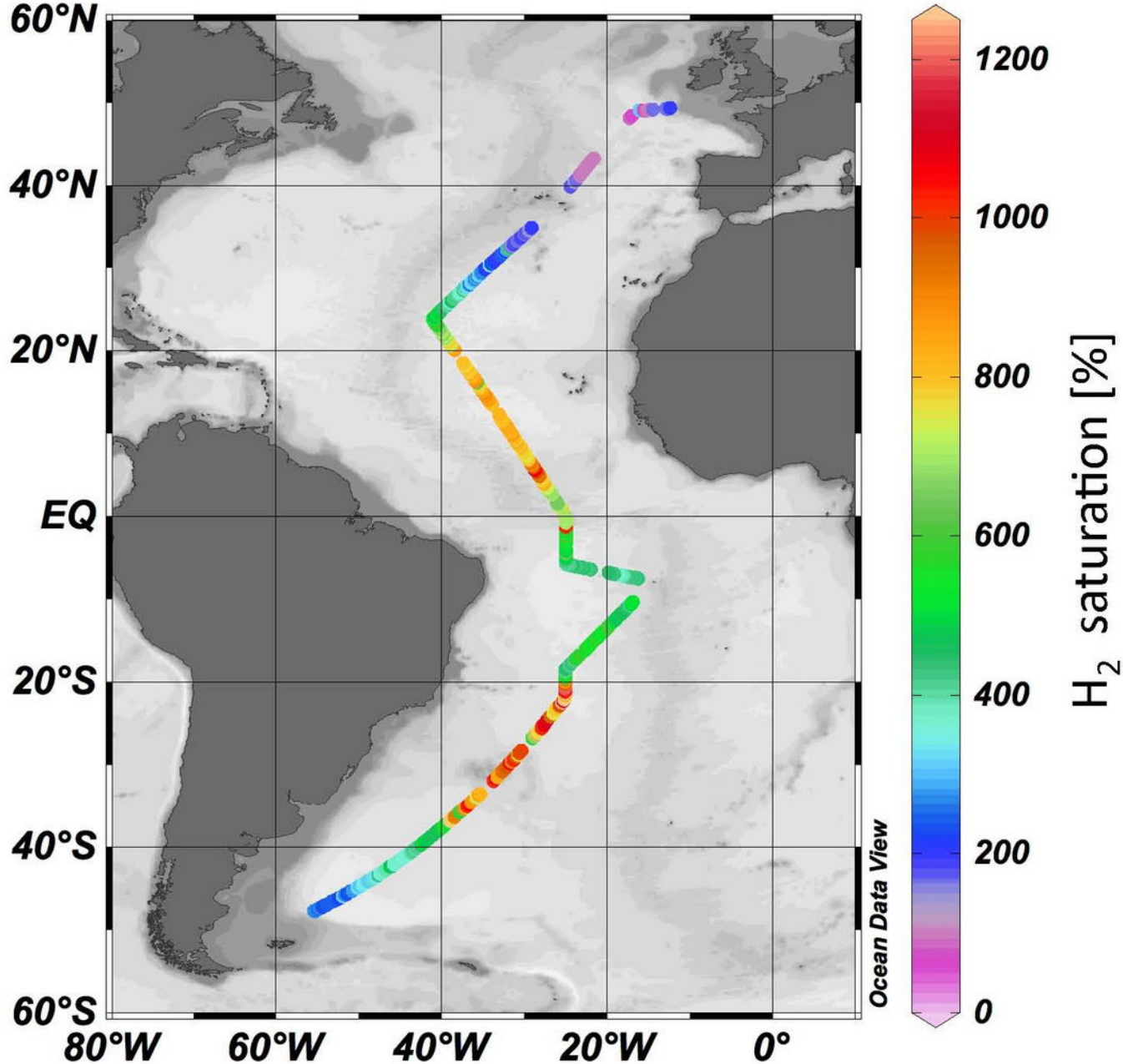
Figure 1. AMT20 cruise track of RRS James Cook from Southampton to Punta Arenas, October 12 – November 25, 2010. Breaks occur where sampling stopped as the ship traversed the territorial waters of the Azores and Ascension Island. Coloured circles show saturation of dissolved hydrogen (%) along track, obtained by resampling the full data set, shown in Fig. 2 below, approximately every 15 nautical miles.

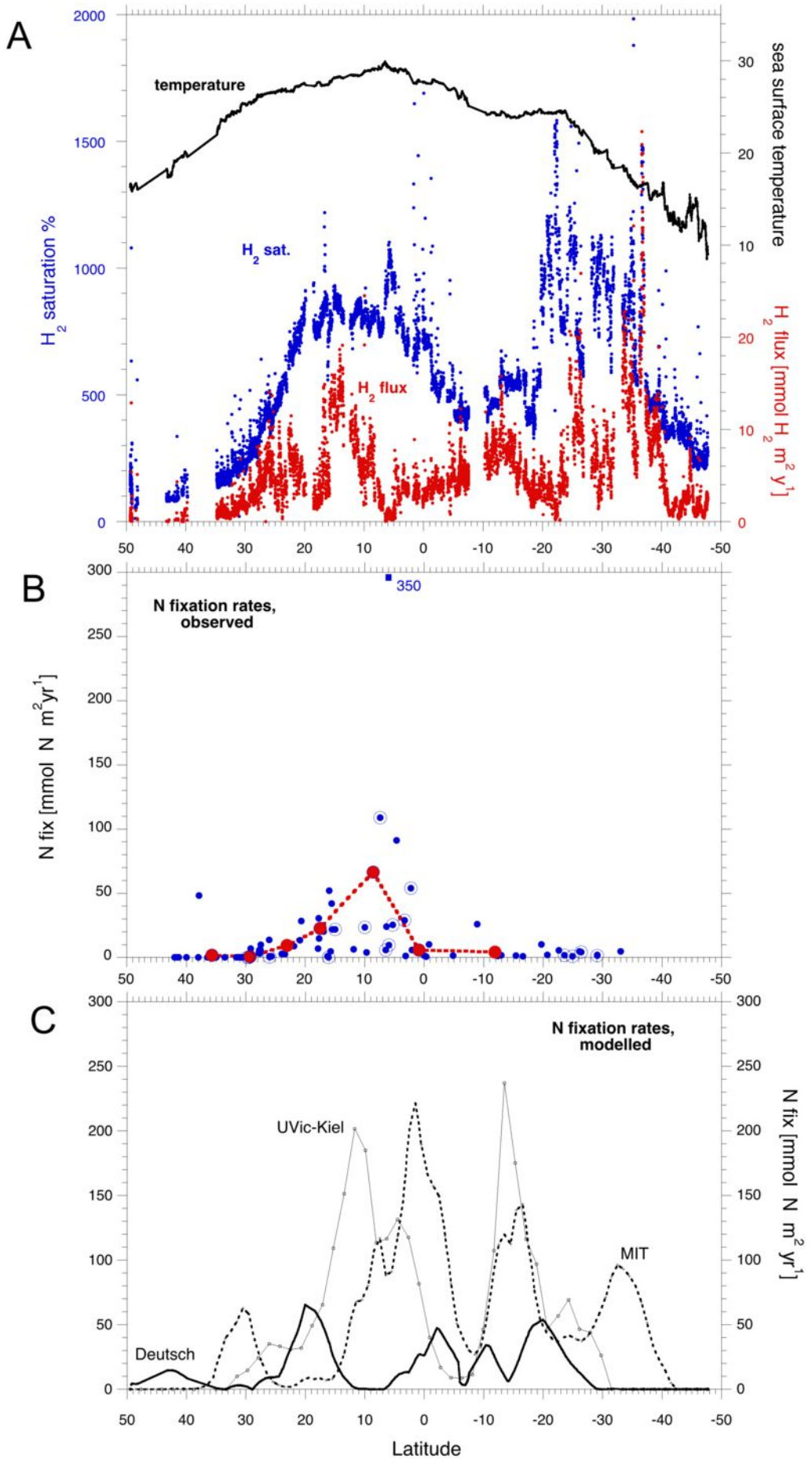
Figure 2. A. Saturation (%) of dissolved hydrogen in surface waters, SST, and H_2 flux to the atmosphere mmol m^{-2} yr^{-1}, which can be used to estimate a minimum N_2 fixation rate assuming a 1:4 ratio of H_2 released to N_2 fixed; B. measured nitrogen fixation rates (mmol N m^{-2} yr^{-1}) on the same cruise track of AMT17 to 20°S (C.M. Moore et al., 2009) joined red circles; fixation rates from the PANGAEA database binned to 3° grid squares, measurements within 3° of the cruise track shown a 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*.

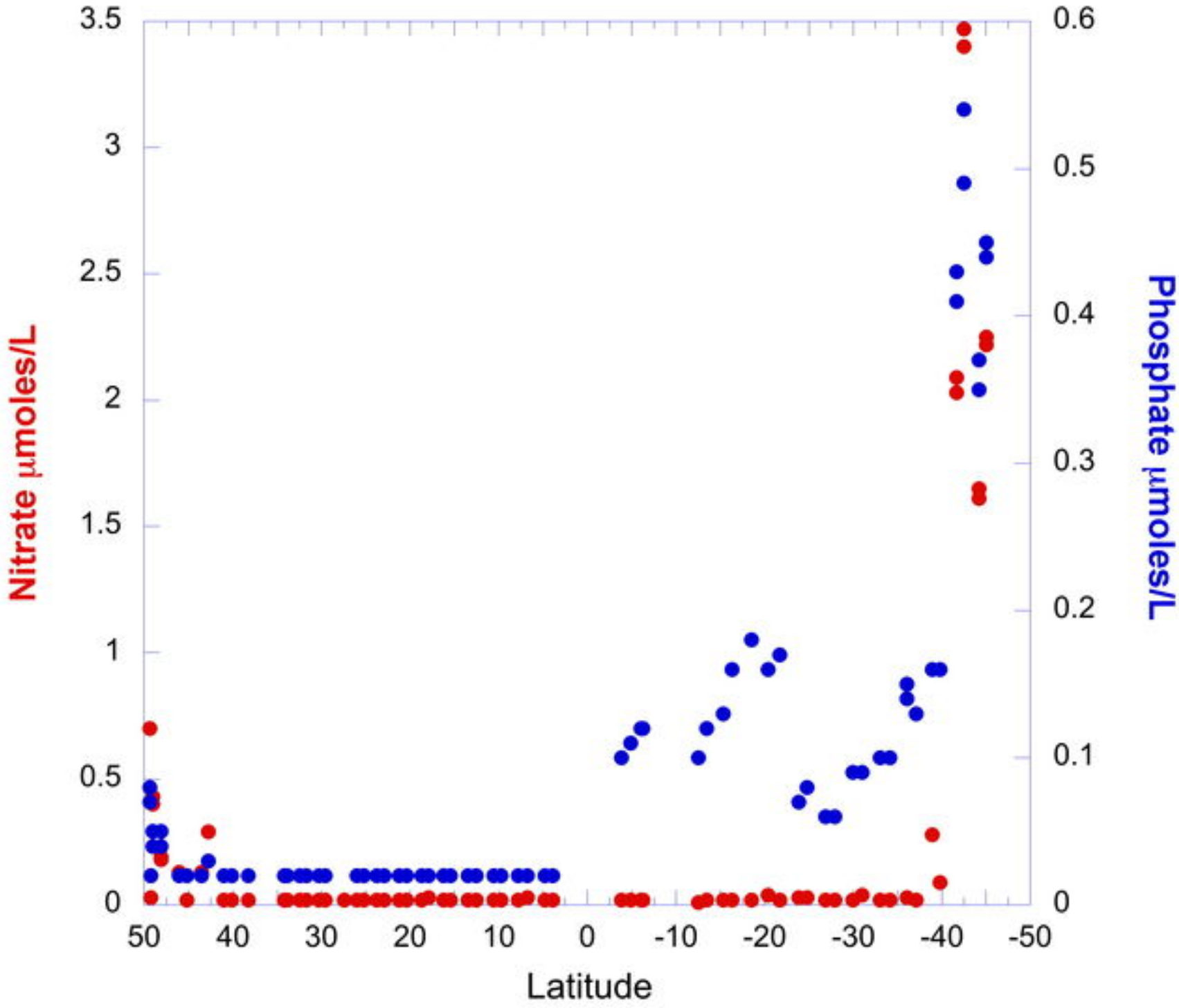
Figure 3. Plot of surface NO_3^- and PO_4^{3-} from AMT 20 (μmol/L) against latitude

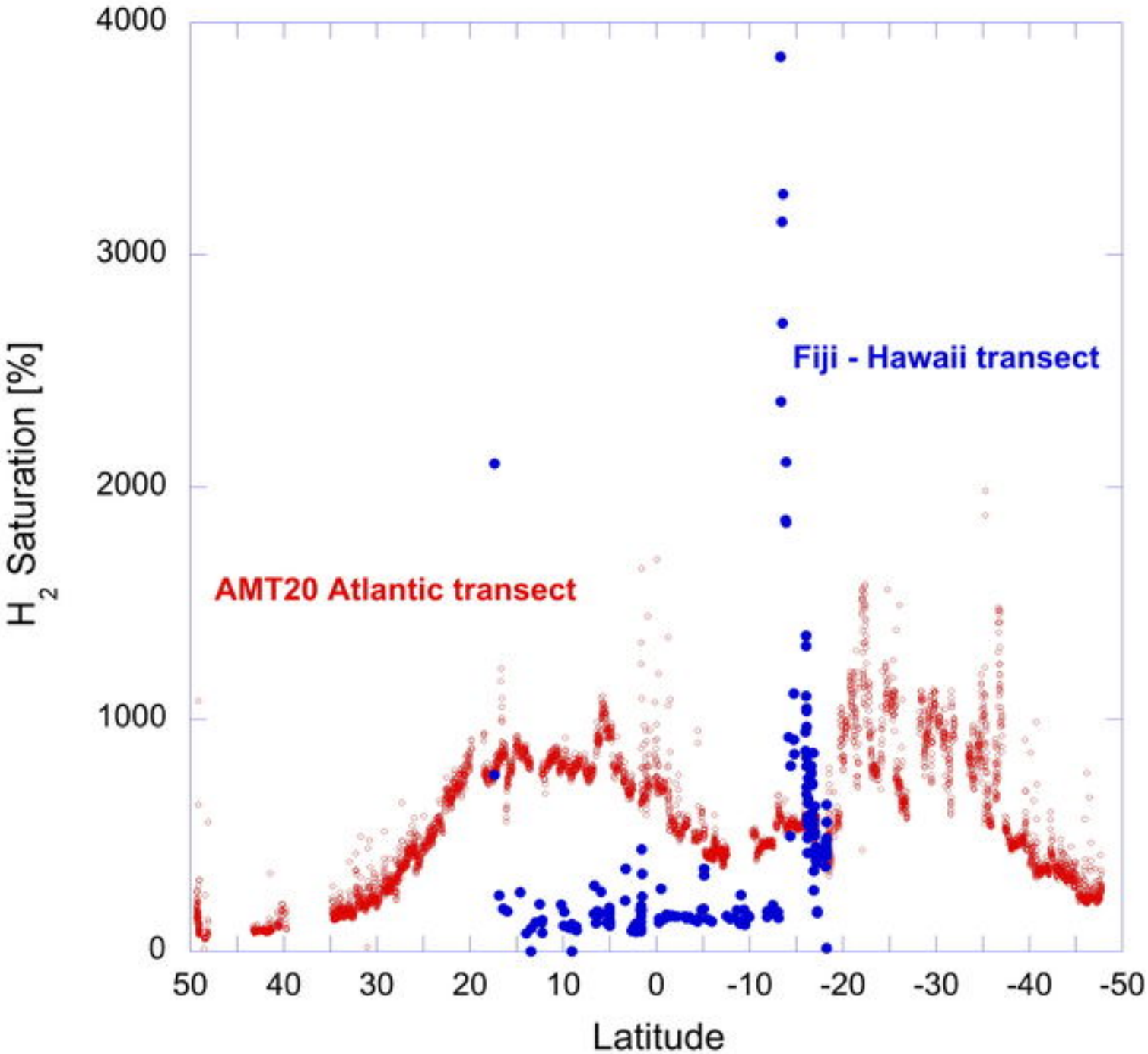
Figure 4 H_2 supersaturations along the AMT 20 transect and a transect between Fiji and Hawaii (Moore *et al*., 2009)

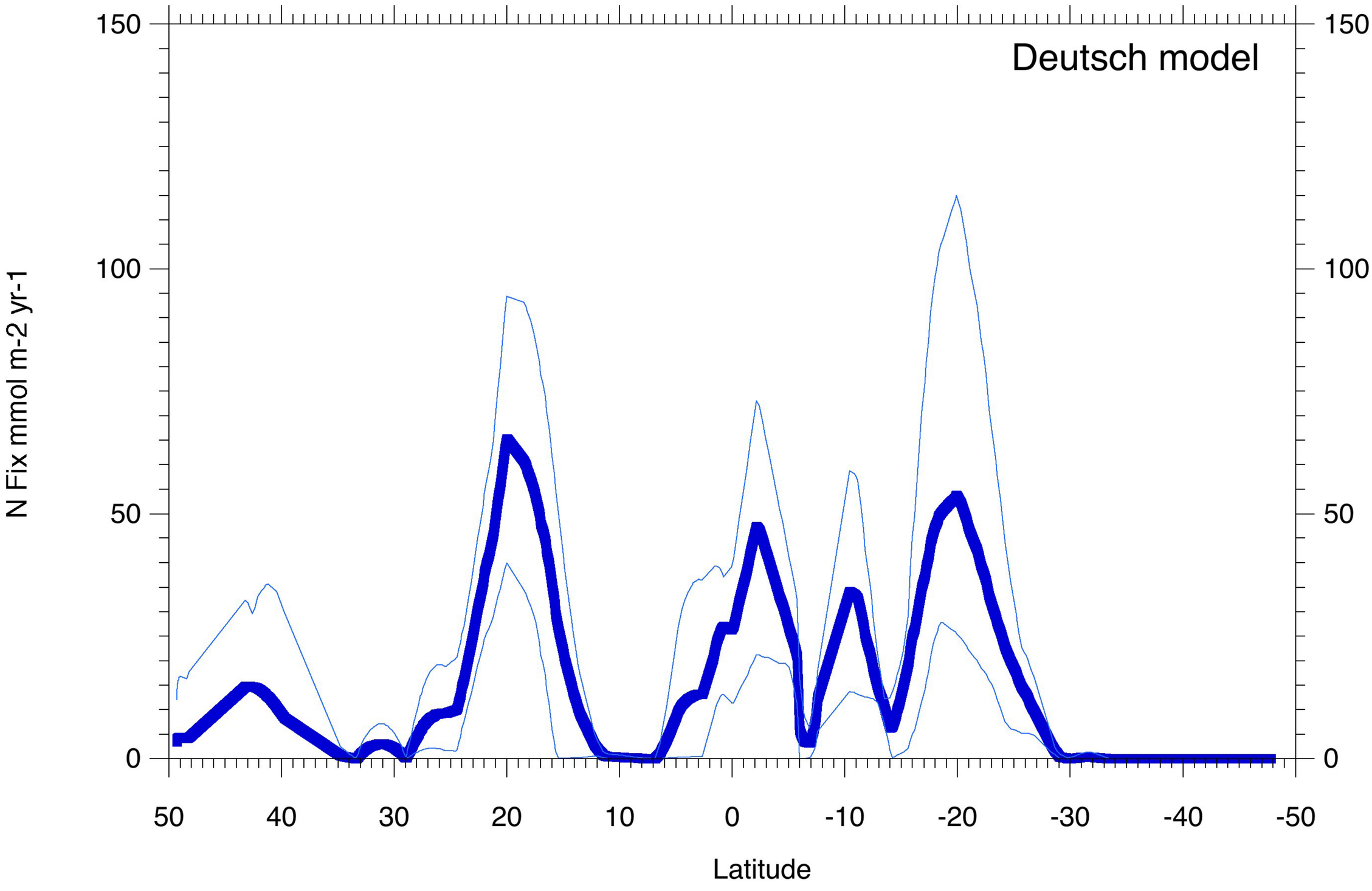
Figure 5. Upper panel – the modelled mean nitrogen fixation rate (mmol N m^{-2} yr^{-1}) along the AMT20 cruise track and range of uncertainties resulting from seasonal variations (Deutsch model); lower panel, the modelled mean and range along the same track from an ensemble of MIT model runs.

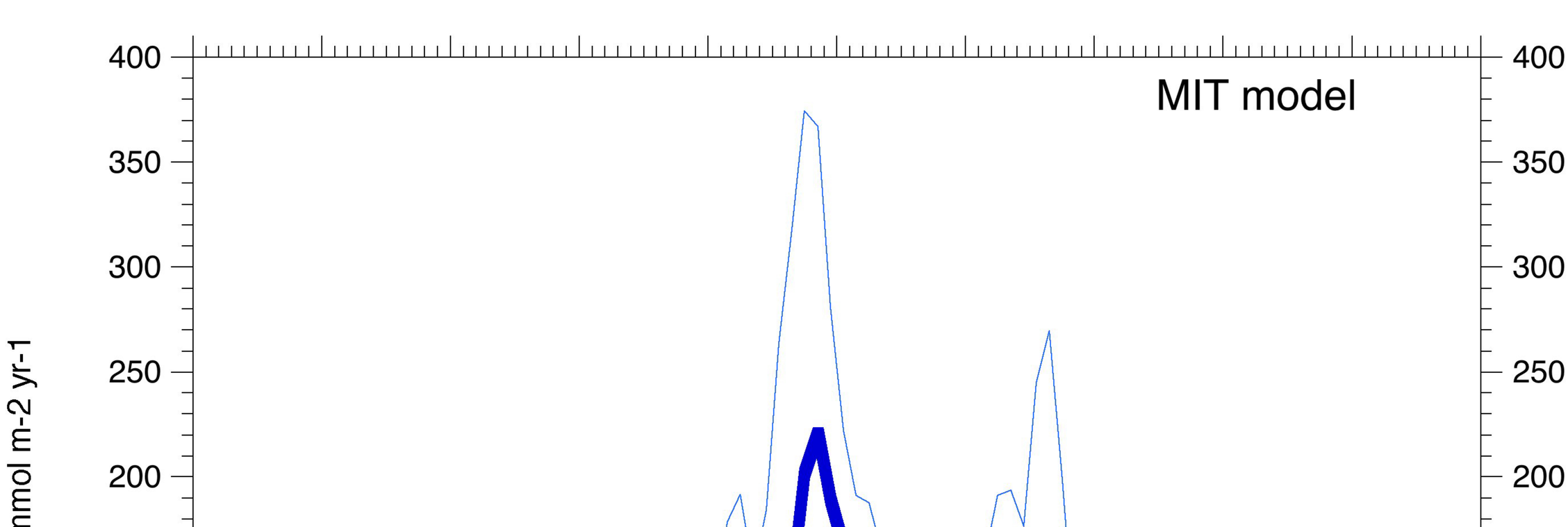

60°N
40°N
20°N
EQ
20°S
40°S
60°S
80°W
60°W
40°W
20°W
0°
Ocean Data View
1200
1000
800
600
400
200
0
H2 saturation [%]


A
temperature
H2 sat.
H2 flux
H2 saturation %
sea surface temperature
H2 flux [mmol H2 m2 y1]
B
N fixation rates, observed
350
N fix [mmol N m2yr1]
C
N fixation rates, modelled
UVic-Kiel
MIT
Deutsch
N fix [mmol N m2 yr1]
Latitude


Nitrate µmoles/L
Phosphate µmoles/L
Latitude
3.5
3
2.5
2
1.5
1
0.5
0
0.6
0.5
0.4
0.3
0.2
0.1
0
50
40
30
20
10
0
-10
-20
-30
-40
-50


4000
3000
2000
1000
0
H2 Saturation [%]
Fiji - Hawaii transect
AMT20 Atlantic transect
50
40
30
20
10
0
-10
-20
-30
-40
-50
Latitude


Deutsch model
N Fix mmol m-2 yr-1
150
100
50
0
50
40
30
20
10
0
-10
-20
-30
-40
-50
Latitude


MIT model
N Fix mmol m-2 yr-1
400
350
300
250
200
150
100
50
0
50
40
30
20
10
0
-10
-20
-30
-40
-50
Latitude