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Spatial variation in N_2-fixation rate and diazotroph activity in the Tropical Atlantic

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Abstract

A variety of N_2-fixers occur in the tropical Atlantic and these diazotrophs make a substantial contribution to the nitrogen budget of the upper water column. A synthesis of previously published and novel rate measurements for the Tropical North Atlantic provides insight into the role of two different diazotroph groups in supporting N_2 fixation in the tropical Atlantic. The overall rate of N_2-fixation by the two groups of diazotrophs was similar in the eastern and western parts of the basin, but N_2-fixation by *Trichodesmium* was strongly dominant in the western part of the basin while small diazotrophs played a much larger role to the east of 40° W. The reasons for this shift in dominance are unclear, as is the identity of the small organisms fixing N_2 in the water column.

1 Introduction

A variety of lines of evidence, including biological surveys and rate measurements as well as geochemical estimates of major N cycle fluxes, have demonstrated that N_2-fixation plays a critical role in supplying new nitrogen to support biological production in nutrient-poor open ocean ecosystems. Our knowledge of the diversity of N_2 fixing organisms (diazotrophs) has grown rapidly in recent years through the increasing application of molecular tools to characterize the marine microbial community (e.g., Zehr et al., 1998, 2001), but significant gaps remain in our understanding of the distribution and activity of different diazotrophic organisms.

The best-known marine diazotrophs are colonial cyanobacteria of the genus *Trichodesmium*, which occurs broadly in subtropical and tropical waters throughout the world (Capone et al., 1997). *Trichodesmium* often forms dense surface blooms of large spatial extent and can clearly make a significant contribution to the local and global budget of oceanic nitrogen (Karl et al., 2002; Capone et al., 2005). Because of its size and prominence at sea, *Trichodesmium* has received a great deal of attention in both field (Carpenter, 1992; Capone et al., 1997, 2005) and laboratory studies (Ohki

et al., 1986; Chen et al., 1996; Mulholland and Capone, 1999; Mulholland et al., 2004). As a result, we now have a much better understanding of the environmental factors and physiological underpinnings of N_2-fixation by *Trichodesmium* than we do for any other marine diazotroph. Compilation of extensive data from multiple cruises also provides substantial information on the spatial distribution of N_2-fixation by *Trichodesmium* within the North Atlantic: *Trichodesmium* biomass and N_2-fixation rates both tend to be highest in the western part of the basin (Capone et al., 2005), as is the overall contribution of N_2-fixation to the water column N budget (Montoya et al., 2002; McClelland et al., 2003; Capone et al., 2005)

Despite its clear importance, N_2 fixation by *Trichodesmium* cannot account for the total rate of N_2 fixation implied by geochemical budgets for the oligotrophic ocean in general or the North Atlantic in particular (Gruber and Sarmiento, 1997; Deutsch et al., 2001; Capone et al., 2005). Other diazotrophs must be making a significant contribution to N_2-fixation on both a basin and a global scale. Over the last decade, the application of molecular approaches to characterizing microbial communities has led to a dramatic increase in the known diversity of marine N_2-fixers (e.g., Zehr et al., 1998, 2001). The importance of N_2 fixation by unicellular cyanobacteria was first demonstrated at the Hawaii Ocean Time-series (HOT) Station ALOHA in the North Pacific Subtropical Gyre (Zehr et al., 2001; Dore et al., 2002), and more recent studies have demonstrated that these organisms are broadly distributed and can make a substantial contribution to the nitrogen budget of the upper water column of the oligotrophic Pacific (Montoya et al., 2004).

The diazotrophic community of the tropical Atlantic has been described by Langlois et al. (2005), who found that filamentous forms (e.g., *Trichodesmium*) are common in surface samples across the basin, while unicellular cyanobacteria and γ-proteobacteria appear to dominate the community of N_2 fixers below the surface. Several recent papers report measurements of N_2 fixation by small diazotrophs in the Atlantic and provide some information on the spatial distribution of N_2-fixation. For example, Falcon et al. (2004) used the acetylene reduction assay on concentrated water samples to

measure rates as high as 0.15 nmol N L^{-1} h^{-1} in the Western Tropical North Atlantic, with the highest rates apparently occurring at night. Voss et al. have used the $^{15}N_2$ tracer technique to measure N_2-fixation rates as high as 2–3 nmol N L^{-1} h^{-1} in unconcentrated water samples from the Eastern Tropical North Atlantic (Voss et al., 2004). Although Voss et al. did not screen their samples to remove *Trichodesmium* and other large diazotrophs, they reported elevated rates of N_2-fixation at 50 m depth in waters with few *Trichodesmium* trichomes (Voss et al., 2004). On the same cruise, Mills et al. (2004) carried out an extensive set of manipulations to test the controls on N_2-fixation and concluded that diazotrophic activity was limited by both Fe and P availability.

Here we examine the patterns of activity by different groups of diazotrophs in the tropical and subtropical North Atlantic, integrating our own rate measurements with data drawn from the literature. The majority of published rate measurements focus on *Trichodesmium*, but recent field efforts have begun to illuminate the role of other, smaller diazotrophs in N_2-fixation, making a comparison of the rates and patterns of N_2 fixation by these different groups both timely and feasible.

2 Materials and methods

Samples for N_2-fixation rate measurements were collected on cruises to the tropical North Atlantic (Table 1, Fig. 1) using methods described in detail elsewhere (Voss et al., 2004; Capone et al., 2005). In brief, rate measurements were carried out under simulated in situ conditions in shipboard incubators using either isolated colonies of *Trichodesmium* or unconcentrated water samples collected with a CTD-rosette. We used both the acetylene reduction assay (ARA, Capone and Montoya, 2001) and $^{15}N_2$ tracer methods (Montoya et al., 1996) to quantify the rate of N_2 fixation in discrete samples. These assays are complementary in the sense that ARA provides a measure of total nitrogenase activity while the $^{15}N_2$ tracer assay provides a measure of the net incorporation of N_2 into biomass (Mulholland et al., 2004) and has a lower limit of

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detection in oligotrophic waters (Montoya et al., 1996). The tracer method is especially well suited to measuring the rate of N_2 fixation in dilute cell suspensions, including unconcentrated seawater samples, while ARA is frequently the method of choice in studies of larger and/or more abundant diazotrophs (e.g., *Trichodesmium* colonies). We took care to minimize the handling and perturbation of our samples and incubation bottles as much as possible at all stages of the sampling and experimental process.

For geochemical context, we calculated distributions of N* based on the World Ocean Atlas 2001 (Conkright et al., 2002) climatology for the North Atlantic. In brief, we used Ocean Data View (Schlitzer, 2005) to calculate N* based on objectively mapped nutrient fields, then integrated the resulting N* fields vertically through the upper water column. We recognize the limitations of this approach, including the potential artifacts associated with calculating N* distributions from nutrient fields that have undergone objective analysis independently, and therefore use the N* distribution as a strictly qualitative indicator of the distribution of N_2-fixation activity on a basin scale.

3 Results

3.1 Trichodesmium N_2-fixation rates

The rates of N_2 fixation by *Trichodesmium* spanned a broad range with the bulk of the values falling in a range up to about 200 μmole N m^{-2} d^{-1} (Fig. 2), The distribution of rates for cruises SJ9603, SJ9612, and MP01were skewed, with a few stations showing areal rates much higher than the mean (Fig. 2). To test for differences among cruises, we performed a one-way ANOVA for rates measured within the region of overlap of the four cruises (0°<latitude<15° N and 40°<longitude<60° W). Our ANOVA showed a significant ($p<0.05$) overall difference among the four cruises (ANOVA: factor df=3, total df=112, $p=0.025$). A post-hoc Tukey's HSD multiple-comparison test revealed only one significant difference, between cruises SJ9612 and MP03.

3.2 Bulk water N_2-fixation rates

In the Western portion of the basin, rates of N_2-fixation measured in bulk (i.e., unconcentrated) water samples were substantially lower than the rates measured for *Trichodesmium* in the same region (Fig. 3). Rates measured in unconcentrated water samples on different cruises to the Western Tropical North Atlantic were very similar (Fig. 3) despite differences in year, season, and some experimental details (see Discussion). N_2 fixation rates in bulk water generally increased to the east.

3.3 N* fields

N* values integrated through the upper 300 m of the water column showed relatively little variation north of 10° N (Fig. 4a). In contrast, N* values integrated through the upper 750 m of the water column showed clear zonal structure with a band of high values (>2 μmole m^{-2}) in the subtropics (Fig. 4b).

4 Discussion

N_2-fixation plays a critical role in supporting biological production in the open ocean. We have integrated previously published and new sets of measurements carried out in the Tropical North Atlantic to provide a broad overview of the relative importance of N_2 fixation by different diazotrophs across the basin. We recognize the challenge inherent in comparing data from cruises carried out in different years and seasons but believe that our approach is justified because: a) two of the five cruises occurred in the autumn while two others fell within two months of the autumn cruises; b) data from different cruises to the western portion of the basin show similar integrated rates of N_2-fixation despite being from different years and seasons; c) our goal is to evaluate basin scale distributions of N_2-fixation by different groups of diazotrophs; and d) the spatial differences we are interested in appear to be larger than the seasonal and annual

variations in our data set. We will examine several issues in detail before moving on to our comparison of N_2 fixation by different diazotroph groups.

4.1 Comparisons among cruises and between methods

The five cruises we have focused on for this study occurred over a span of six years and in three different seasons. We carried out a comparison of the *Trichodesmium* rates measured on four of the five cruises to test whether they could be treated as a single combined set for comparison with rates measured in bulk (unconcentrated) water samples on three of the five cruises. Our ANOVA for the region of overlap of the four cruises revealed only one significant pairwise difference, between cruises SJ9612 and MP03. Inspection of Fig. 2 shows that this difference reflects the absence of a tail extending to high rates in the data from cruise MP03, leading to an overall lower mean areal rate of N_2 fixation for that cruise. The other three cruises (SJ9603, SJ9612, and MP01) were statistically indistinguishable and can be combined without further consideration. Including cruise MP03 in the aggregate data set will tend to reduce the contrast with the rates measured in bulk water samples, which are generally lower than the rates measured for *Trichodesmium*. Since we wished to test the hypothesis that areal rates of N_2 fixation by *Trichodesmium* are significantly higher than rates associated with other diazotrophs in the western part of the basin (see below), combining the four *Trichodesmium* data sets for this comparison will lead to a conservative test.

An additional concern is methodological in nature: our data set includes direct measurements of N_2 fixation by isolated colonies of *Trichodesmium* as well as assays carried out using bulk (unconcentrated) water samples from the water column. On the earlier cruises, our focus was entirely on *Trichodesmium* and all assays were done using ARA. The discovery of abundant small diazotrophic cyanobacteria in the Pacific (Zehr et al., 2001) provided a strong incentive to investigate the activity of such small organisms in the Atlantic. This discovery also coincided with the development of the $^{15}N_2$ tracer method, which provided the sensitivity necessary for quantifying N_2 fixation activity by populations of small diazotrophs dispersed in the water column. We

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have carried out extensive intercomparisons of the ARA and $^{15}N_2$ tracer methods for measuring N_2-fixation in *Trichodesmium* (Capone et al., 2005) but the low rates typically associated with unconcentrated water samples makes a direct intercomparison between the two assays difficult for bulk water samples. A direct comparison of different methods for measuring N_2 fixation rates will require isolation and cultivation of the common small diazotrophs for focused lab experiments, but the most common small diazotrophs (Group A) are not yet available in lab culture. At this time, we have no reason to suspect that rates measured in $^{15}N_2$ tracer and ARA incubations are biased in one way or another or are unsuitable for direct comparison.

Finally, the details of the $^{15}N_2$ tracer incubations carried out on different cruises require consideration. On Cruises MP01 and MP03, samples were passed through a 10 μm filter to remove any larger diazotrophs (e.g., free trichomes of *Trichodesmium*) from the sample prepared for mass spectrometry. As a result, the rates reported for bulk water incubations on these cruises are clearly related to the activity of small diazotrophs. In contrast, the incubation protocol on Cruise ME55 did not involve any size-fractionation of the sample, which means that the rates reported may include a contribution from *Trichodesmium* or any other large diazotrophs present in the water column (e.g., diatom-diazotroph associations). Langlois et al. (2005) reported that filamentous forms were present in surface samples at most stations sampled on this cruise, while unicellular cyanobacteria and bacteria were dominant deeper in the water column. Along the same lines, many of the profiles from this cruise showed significant rates of N_2 fixation throughout the mixed layer and without the strong bias toward fixation at the surface which is typical of *Trichodesmium*.

Despite the potential contamination by large diazotrophs, the rates measured in bulk water incubations in the western portion of the cruise ME55 track were much lower than the rates measured for *Trichodesmium* in the same region and were very similar to bulk water rates measured on cruises MP01 and MP02. An analysis of variance showed no significant differences among these three cruises ($p>0.05$), supporting the idea that data from all three cruises measured the same underlying phenomenon and could

therefore be combined into a single data set for comparison with the *Trichodesmium* data.

In summary, consideration of the available data revealed that: a) we can pool all of our *Trichodesmium* rate measurements from different cruises without biasing the combined data set toward high values and b) we can pool the whole water rate measurements despite differences in experimental detail among the cruises. These pooled data sets then allow us to examine the basin scale distribution of N_2-fixation activity by two major groups of N_2-fixers: *Trichodesmium* and smaller diazotrophs.

4.2 Distribution of N_2-fixation activity

Our combined data set shows the highest rates of N_2-fixation in the western part of the Tropical North Atlantic where dense populations of *Trichodesmium* are well-documented (Carpenter et al., 2004; Capone et al., 2005). The areal rate of N_2-fixation by *Trichodesmium* showed a great deal of spatial variation but the maximal values decreased markedly to the east (Fig. 3). In contrast, most of the rates measured in bulk water samples were substantially lower than the comparable *Trichodesmium* rates (Fig. 3). This is particularly noticeable in the region west of 40° W where the mean rate of N_2-fixation by *Trichodesmium* was roughly 8-fold higher than the mean rate measured in whole water incubations from all three cruises (Table 3). The rates of N_2 fixation by *Trichodesmium* and small diazotrophs were more similar in the eastern part of our study region (Table 3).

We can compare the two diazotroph types by collapsing the distributions shown in Fig. 3 into zonal transects of N_2 fixation activity in *Trichodesmium* (Fig. 5a) and small diazotrophs (Fig. 5b). This zonal comparison highlights the difference in areal rate of N_2 fixation by the two different types of diazotroph. *Trichodesmium* is capable of much higher rates of N_2 fixation in regions where it is abundant, but its contribution to the water column N budget clearly decreases markedly to the east, where its biomass is generally lower (Carpenter et al., 2004). We tested for longitudinal differences in N_2-fixation rate by comparing mean rates for waters to the east and west of 40° W,

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which divides our study region roughly in half. Although the mean rate of N_2-fixation by *Trichodesmium* is substantially lower in the eastern part of the basin, the difference between regions is not statistically significant (p=0.4433, Table 3)

The small diazotrophs in our whole water incubations account for generally low areal rates of N_2 fixation (<50μmol N m^{-2} d^{-1}) in the western part of the basin, with an increase to the eastward along 10° N (Fig. 5, Table 3). The overall difference in rate of N_2-fixation by small diazotrophs in the eastern and western portions of the Tropical Atlantic is highly significant (p=0.0093, Table 3). Note that most of the low rates in the eastern part of the ME55 transect (10° to 30° W) are from experiments conducted between the equator and 10° N (Fig. 3). This decrease toward the equator may reflect the increased nutrient availability associated with the equatorial upwelling and circulation system (Voss et al., 2004), but it's notable that Voss et al. (2004) found low but measurable rates of N_2 fixation even at the equator.

The basin-scale pattern that emerges from our data thus reveals a very interesting contrast between *Trichodesmium* and small diazotrophs, which show opposite trends in N_2 fixation rate with longitude. Because *Trichodesmium* N_2-fixation rates show a general decline to the east, the net effect is a shift from strong dominance of N_2-fixation by *Trichodesmium* in the western part of the tropical North Atlantic to a more even partitioning of N_2-fixation between *Trichodesmium* and small diazotrophs in the eastern part of the basin. Note that we have focused on just two diazotrophic groups in making our regional comparison and have ignored the diatom-diazotroph associations (DDAs) that often form very dense and highly productive blooms in the region affected by the plume of the Amazon River (Carpenter et al., 1999). Including DDAs in our analysis would only increase the contrast between the eastern and western parts of the Tropical North Atlantic.

5 Implications and conclusions

Our rate measurements were carried out during multiple cruises in different years and seasons, but a careful consideration of the data showed that we could combine our data to allow a comparison of distribution of N_2-fixation by two major groups of diazotroph. We do not know the factors producing this zonal shift in dominance of N_2-fixation, but it likely reflects a basin scale pattern of variation in environmental parameters affecting N_2-fixation.

The best current information on the environmental factors controlling N_2-fixation in the Atlantic comes from an elaborate set of bioassay experiments carried out by Mills et al. (2004) on Cruise ME55. These experiments were designed to explore the role of iron and phosphorus in limiting N_2 fixation in surface waters of the Eastern Tropical Atlantic. These bioassays provided strong evidence that N_2 fixation is co-limited by both Fe and P availability, both of which are injected into surface waters by African dust (Mills et al., 2004). Unfortunately, experimental differences make it difficult to relate these bioassays directly to the rate measurements carried out on the same cruise by Voss et al. (2004). Firstly, the bioassays were set up with water collected from the surface with a pump system, an approach that is much more likely to sample large diazotrophs like *Trichodesmium* than the CTD-rosette system used by Voss et al. (2004) to collect their experimental water. This difference in sampling approach may in turn be responsible for a strong contrast between the diazotrophs active in the two sets of experiments; our consideration of data from different cruises strongly suggests that the measurements carried out by Voss et al. (2004) reflected the activity of small diazotrophs rather than the *Trichodesmium* or *Katygnemene* that were abundant in the bioassays (Mills et al., 2004). Finally, the bioassay incubations lasted 48 h, providing opportunity for community changes and recycling processes that are unlikely in the much shorter experiments (6–7 h) of Voss et al. (2004). Although the two sets of experiments carried out on Cruise ME55 are not directly comparable, the eastward increase in N_2-fixation rates observed on cruise ME55 (Voss et al., 2004) is consistent with the

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impact of inputs of N and P entering the surface ocean in the form of African dust, a flux that should increase to the east.

Comparing the mean rates of N_2-fixation by *Trichodesmium* and small diazotrophs reveals similar total rates of N_2-fixation across the basin (Table 3). This may well be coincidental since we excluded other important groups of diazotrophs (e.g., DDAs), but it is tempting to speculate that the rate of N_2-fixation is constant across the basin with an east-west change in dominance reflecting different selective pressures acting on the diazotrophs. This scenario is qualitatively consistent with the distribution of N* values in the Tropical Atlantic, which shows relatively little zonal variation in comparison to very strong meridional gradients (Fig. 4). The complex structure in the N* distribution in the Eastern Tropical Atlantic may also reflect losses of N through sedimentary denitrification, which will tend to decrease N* locally. We used integrated N* values to provide a measure of the overall impact of N_2-fixation on the water column N budget via remineralization of sinking organic matter, recognizing that the time scales implicit in the nutrient climatology are very different than the time scale of the rate measurements we discuss here (see Methods for other caveats).

At this time, the identity of the small diazotrophs fixing N_2 in the whole water incubations remains poorly known, but is the focus of ongoing field programs in the Atlantic. Efforts are also continuing to isolate and characterize the N_2-fixation physiology of these organisms, in particular the Group A cyanobacteria that play a major role in N_2-fixation in the Pacific, and perhaps the Atlantic (Zehr et al., 2001; Montoya et al., 2004; Langlois et al., 2005).

Our results suggest a large scale pattern of niche partitioning between diazotroph groups, but the environmental factors leading to these spatial differences remain unclear. Continuing field programs to measure N_2-fixation across a broad range of longitude and lab efforts aimed at isolation and characterization of small diazotrophs are both critical to resolving the interactions among environmental factors and the different groups of diazotrophs that we now know are active in the North Atlantic. This is among the most intriguing and pressing issues in the supply side of the marine nitrogen

budget.

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Table 1. Cruises included in this study.

Dates	Ship	Cruise	Region	Rates Measured
18 March–25 April 1996	R/V Seward Johnson	SJ9603	Tropical North Atlantic	*Trichodesmium*
10 Oct–8 Nov 1996	R/V Seward Johnson	SJ9612	SW Tropical N. Atlantic	*Trichodesmium*
9 Jan–20 Feb 2001	R/V Seward Johnson	MP01	SW Tropical N. Atlantic	*Trichodesmium*, Bulk water
27 June–15 Aug 2001	R/V Knorr	MP03	SW Tropical N. Atlantic	*Trichodesmium*, Bulk water
12 Oct–17 Nov 2002	F/S Meteor	ME55	Tropical North Atlantic	Bulk water

Table 2. ANOVA comparing areal rates of N_2 fixation by *Trichodesmium* measured on different cruises (SJ9603, SJ9612, MP01, and MP03) within the region defined by 0°<latitude<15° N and 40° <longitude<60° W. A Tukey's HSD multiple comparisons test revealed only one significant difference, between SJ9612 and MP03.

Source	DF	SS	MS	F	p
Cruise	3	561 993.6	187 331	3.2797	0.0249
Error	83	4 740 872.4	57 119		
Total	86	5 302 866.0			

Table 3. Summary of mean areal rates (mean±SE) of N_2 fixation by *Trichodesmium* and small diazotrophs in the western (longitude>40° W) and eastern (longitude<40° W) portions of our study area. Number of measurements shown in parentheses. The regional difference was not significant for *Trichodesmium* (p=0.4433, df=111) and highly significant for the small diazotrophs (p=0.0093, df=32).

Diazotroph Type	West	East
Trichodesmium	172±28 (100)	108±78 (13)
Small diazotrophs	23±4.7 (23)	72±23 (11)

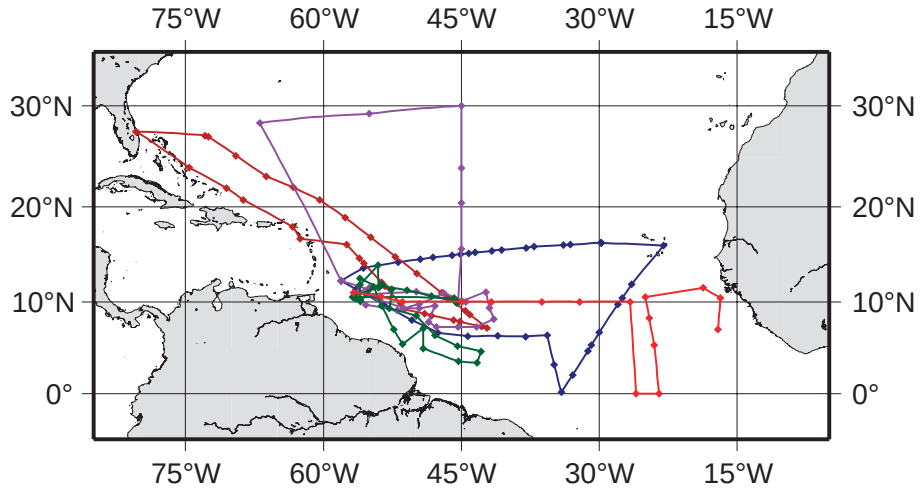

Fig. 1. Tracks of the five cruises included in this study: SJ9603 (dark blue), SJ9612 (brown), MP01 (purple), MP03 (green), and ME55 (red). Chart prepared with GMT (Wessel and Smith, 1998).

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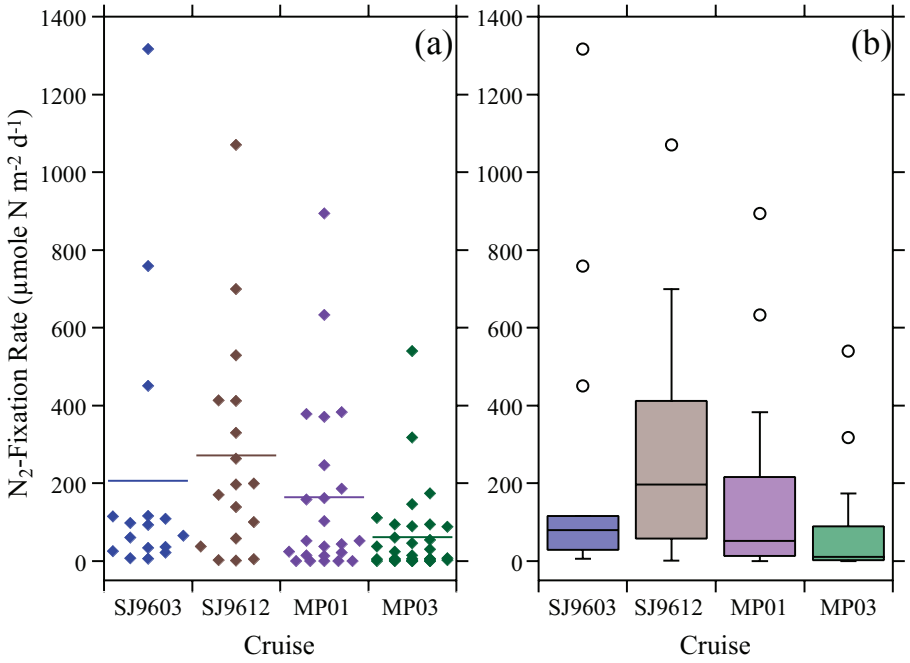

Fig. 2. Areal N_2 fixation rate measurements for *Trichodesmium* on cruises SJ9603, SJ9612, MP01, and MP02 within the region sampled by all four cruises (0°<latitude<15° N and 40°<longitude<60° W). **(a)**: Summary showing distribution of rates and overall mean rate (horizontal line) for each cruise. Individual points are offset laterally for clarity. **(b)**: Box plot summaries for the rate data from the four cruises. Each box spans the 25th and 75th quantiles of the data with the median shown by the line crossing the box. The whiskers extend to the 10th and 90th quantiles of the data.

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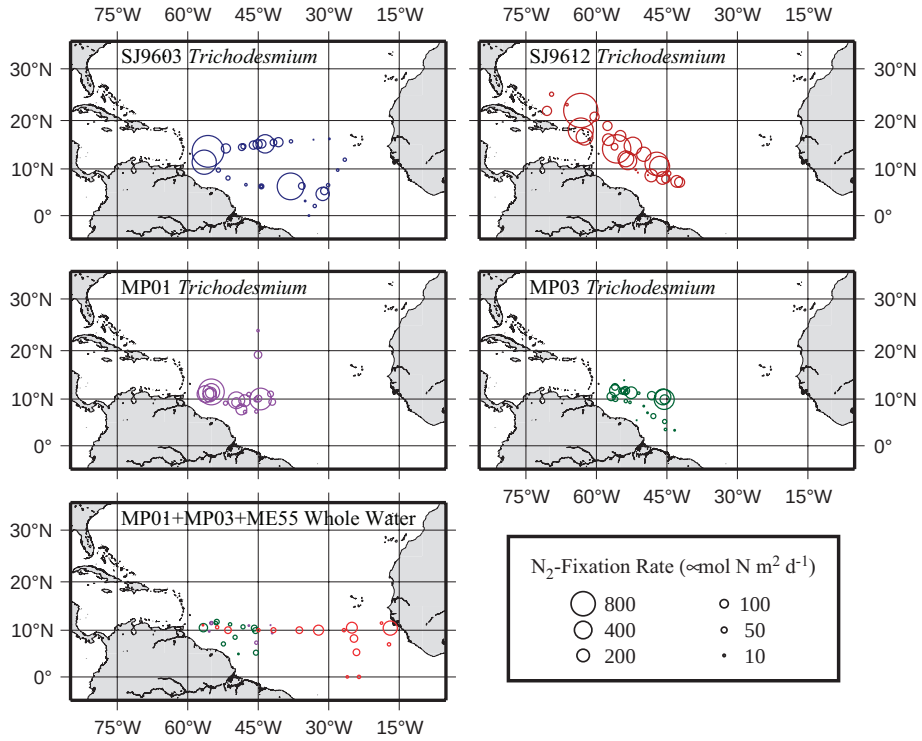

Fig. 3. Areal N_2 fixation rate measurements for *Trichodesmium* (cruises SJ9603, SJ9612, MP01, and MP02), and whole water incubations (MP01, MP03, and ME55). Area of each circle is proportional to the integrated rate of N_2 fixation at that station. Colors as in Fig. 1. Charts prepared with GMT (Wessel and Smith, 1998).

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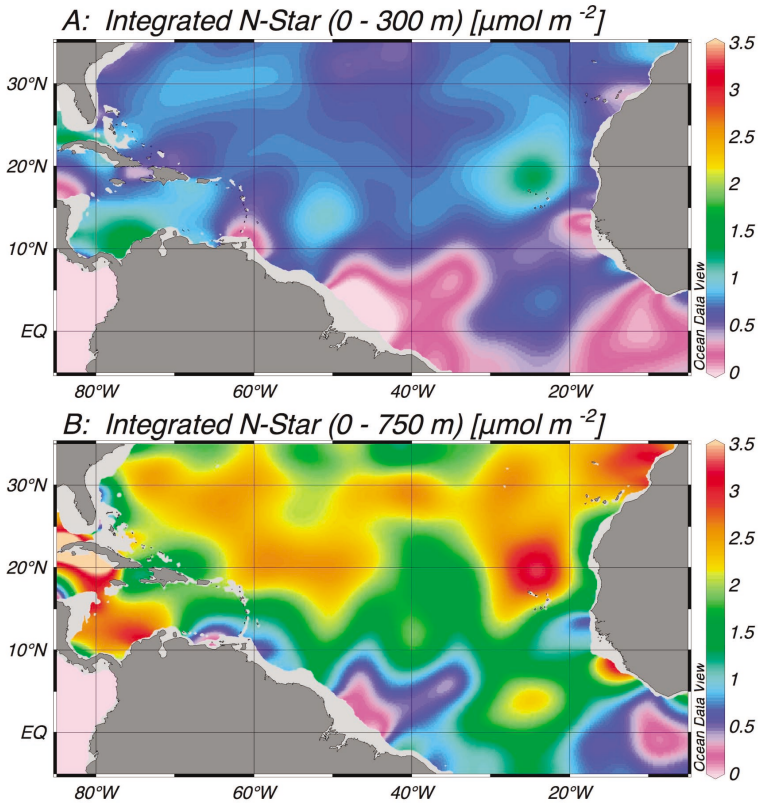

Fig. 4. Integrated N* values for the region covered in this study. N* values were calculated based on objectively analyzed 1° nutrient fields from the World Ocean Atlas 2001 (Conkright et al., 2002), then integrated vertically through the upper 300 m **(a)** or 700 m **(b)** of the water column using Ocean Data View (Schlitzer, 2005).

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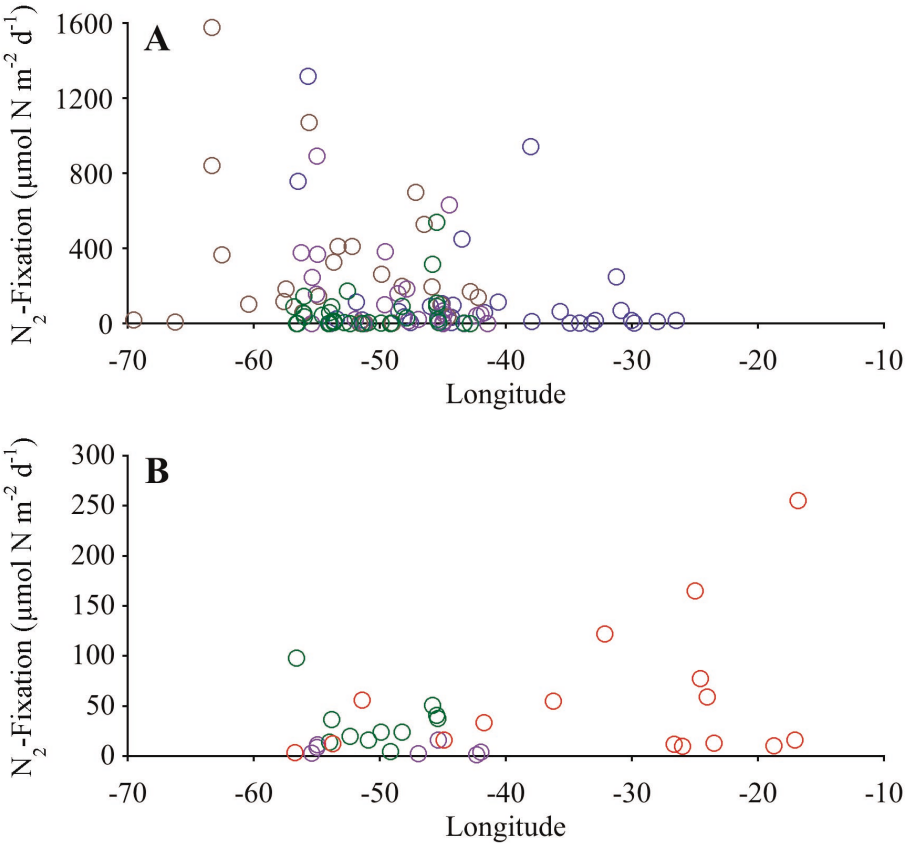

Fig. 5. Areal N_2 fixation rate as a function of longitude for *Trichodesmium* **(a)** and whole water incubations **(b)**. Colors as in Fig. 1.