plateau and the marine records from the Indian Ocean, interpreted with the aid of climate-model simulations that take into account both uplift and lateral extension of the Tibetan plateau, support and extend earlier conclusions\(^1,17\) concerning the nature and probable causes of the multi-stage evolution of Asian climates.

Received 8 January; accepted 9 March 2001.


Marine fixation of atmospheric nitrogen is believed to be an important source of biologically useful nitrogen to ocean surface waters\(^1\), stimulating productivity of phytoplankton and so influencing the global carbon cycle\(^2\). The majority of nitrogen fixation in tropical waters is carried out by the marine cyanobacterium Trichodesmium\(^3\), which supplies more than half of the new nitrogen used for primary production\(^4\). Although the factors controlling marine nitrogen fixation remain poorly understood, it has been thought that nitrogen fixation is limited by iron availability in the ocean\(^5,6\). This was inferred from the high iron requirement estimated for growth of Trichodesmium where aeolian iron inputs are plentiful\(^7\). Here we report that nitrogen fixation rates in the central Atlantic appear to be independent of both dissolved iron levels in sea water and iron content in Trichodesmium colonies. Nitrogen fixation was, instead, highly correlated to the phosphorus content of Trichodesmium and was enhanced at higher irradiance. Furthermore, our calculations suggest that the structural iron requirement for the growth of nitrogen-fixing iron organisms is much lower than previously calculated\(^8\). Although iron deficiency could still potentially limit growth of nitrogen-fixing iron organisms in regions of low iron availability—for example, in the subtropical North Pacific Ocean—our observations suggest that marine nitrogen fixation is not solely regulated by iron supply.

We collected surface water samples and colonies of Trichodesmium spp. using trace-metal clean methods along two transects in the tropical (0–6°N latitude; 50–28°W longitude) and subtropical (10–16°N; 30–55°W) Atlantic Ocean in April 1996, and analysed them for C, N, P and Fe content. We also measured N2 fixation rates of colonies (Methods).

Strong spatial gradients in the N2-fixing diazotrophic activity were observed along the tropical and subtropical transects. Cell C specific N2 fixation in the subtropical northern transect (median was 1.44 per mg chl a) was four times higher than in the tropical transect (median was 38 μmol N per mol C per h) (Fig. 1a). Trichodesmium biomass (Fig. 1b) was also seven times higher in the northern subtropical transect (subtropical median was 1.44 per mg chl a per m²; tropical median was 0.20 mg chl a per m²).

In contrast to N2 fixation, dissolved Fe concentrations in surface waters of the sub-tropical (median was 0.77 nM) and tropical (median was 0.95 nM) Atlantic were relatively constant (Fig. 1c). Similarly, levels of Fe in field-collected Trichodesmium colonies

Phosphorus limitation of nitrogen fixation by Trichodesmium in the central Atlantic Ocean

Sergio A. Sañudo-Wilhelmy*, Adam B. Kustka†, Christopher J. Gobler‡, David A. Hutchins‡, Min Yang*, Kamazima Lwiza*, James Burns§, Douglas G. Capone*, John A. Raven† & Edward J. Carpenter‡

* Marine Sciences Research Center, State University of New York, Stony Brook, New York 11794-5000, USA
† Southampton College, Natural Science Division, Long Island University, Southampton, New York 11968, USA
‡ College of Marine Studies, University of Delaware, Lewes, Delaware 19958, USA
§ Wrigley Institute for Environmental Studies and Department of Biological Sciences, University of Southern California, Los Angeles, California 90089, USA
© Division of Environmental and Applied Biology, School of Life Sciences, University of Dundee, Dundee DD1 4HN, UK
‡ Romberg Tiburon Center, San Francisco State University, Tiburon, California 94920, USA
ranged from 3 to 13 pmol per colony (median was 6 pmol per colony), and were not significantly different in the two transects (Fig. 1d). These Fe levels in the colonies were an order of magnitude lower than those previously reported, owing to our implementation of trace-metal-clean techniques for collection and analysis. Moreover, _Trichodesmium_ N₂ fixation and biomass were independent of the dissolved Fe concentrations and the Fe content of the colonies (Fig. 2a, b).

High N₂ fixation rates in the Atlantic were measured at relatively low Fe concentrations in the colonies (median was 36 µmol Fe per mol C; range 22–72 µmol Fe per mol C; Fig. 1d). Although the Fe requirements of _Trichodesmium_ and other oceanic algae are not yet well known, the Fe:C ratios of the _Trichodesmium_ colonies we measured in the central Atlantic Ocean were similar to the published Fe:C values for prokaryotic cyanobacteria (~20 µmol Fe per mol C; ref. 8), growing on N (from nitrate) in cultures. Furthermore, the _Trichodesmium_ Fe:C ratios we measured were nearly identical to the Fe:C uptake ratios of coastal diatoms (34–88 µmol Fe per mol C; ref. 9), despite the fact that coastal species might be expected to have higher Fe requirements than open-ocean organisms, owing to their adaptation to higher ambient Fe levels in coastal waters. These observations suggest that the Fe requirements for diazotrophic marine cyanobacteria such as _Trichodesmium_ may not be 100-fold higher than for NH₃-fixing phytoplankton, as previously calculated.

We recalculated the theoretical iron use efficiency (IUE) for growth supported by N₂ fixation (Table 1) previously reported by Raven. We found that the mol Fe required (specific to the nitrogenase complex only and assuming comparable structural and bioenergetic requirements between autotrophic and heterotrophic diazotrophs) to fix 1 mol C per second via N₂ fixation must be between 5.2 and 5.5 times greater than NH₃-assimilating phytoplankton. This range of Fe requirements is still rather higher than values calculated for phytoplankton growing on N from nitrate. However, we cannot discount any additional Fe requirement due to physiological phenomena not directly related to the structural and bioenergetic Fe demand of nitrogenase, such as variations in Fe catalyst stoichiometry.

Our results suggest that N₂ fixation in the subtropical Atlantic Ocean may not be an iron-limited process at this time of the year, when Fe inputs are relatively low. The apparent absence of a deep layer control over these biological processes is consistent with the relatively high background levels of dissolved Fe found in surface waters of the central Atlantic Ocean (~1 nM; ref. 1c). Those levels are between 2 and 5 times higher than the levels reported in the subtropical north Pacific (0.2–0.5 nM; ref. 13), probably owing to the high Saharan aeolian fluxes and tropical river inputs to the Atlantic.

In contrast to the relatively invariant levels of dissolved Fe and Fe content in the colonies measured along the two Atlantic transects.
letters to nature

(Fig. 1c and d), phosphorus levels in the *Trichodesmium* colonies in the subtropical transect (median was 1.10 nmol P per colony) were two times higher than in the tropical transect (median was 0.51 nmol P per colony; Fig. 1e). Furthermore, although our analyses suggested no relationship between Fe levels and N₂ fixation in the subtropical and tropical Atlantic, rates of N₂ fixation were significantly correlated with the P levels in the colonies (Fig. 2b). Although the importance of P for N₂ fixation in the open ocean has been hypothesized²⁹, our data provide the first direct evidence of such a link. The N:P molar ratios measured in the colonies (median was 51; range was 35–61) and in the subtropical (median was 21; range was 14–30, Fig. 1f) were higher than the Redfield ratio of 16:1, and these elemental ratios were within the range reported for dissolved NO₃⁻:PO₄ (that is, 20–40) of the Sargasso Sea²⁷. Therefore, N₂-fixing diazotrophs may contribute to the high dissolved N:P ratios observed in some areas of the Atlantic³¹.

Analysis of the physical regime in the central Atlantic Ocean suggested that N₂ fixation in this region could also be influenced by upper-ocean mixed-layer depth, as previously reported³₂,³³ (Fig. 2c). The observation that N₂ fixation rates were higher at shallower mixed-layer depth is consistent with the relatively high light requirement of *Trichodesmium*³⁴, suggesting that inadequate mean irradiance for photosynthesis may directly affect the energetically expensive N₂ fixation process³⁵. Our results showed that when irradiance was increased, N₂ fixation was also enhanced (Fig. 2c); therefore, light may also have limited N₂ fixation during our cruise. Surface water temperatures in the central Atlantic were relatively constant during our cruise (26.23 ± 1.27°C) and no relationship with N₂ fixation was observed.

Although the importance of Fe in controlling primary productivity in high-nitrate, low-chlorophyll regions of the world ocean is well established³⁶, our results suggest that other factors (such as P and light) may control N₂ fixation under the high-Fe conditions of the central Atlantic Ocean. Although no other measurements of Fe and P in *Trichodesmium* are available in the literature, the C:N:P:Fe stoichiometry (99.6:18.3:1:3.7 × 10⁻⁴) in our field-collected *Trichodesmium* colonies (Fig. 3) suggests that P should limit N₂ fixation only after the Fe quota is met. For example, an average ambient dissolved P concentration of 75 ± 42 nM in the Atlantic³⁷ and our average dissolved Fe of 0.89 ± 0.41 nM yield a dissolved P:Fe ratio of between 1:1.1 × 10⁻² and 1:1.5 × 10⁻². Those dissolved ratios are 3–4 times lower than the ratio measured in *Trichodesmium*, suggesting that the colonies in the Atlantic were not Fe-limited during our study. However, Fe deficiency could limit the growth of this organism in other ocean basins when Fe inputs are much lower. The calculated dissolved P:Fe ratio for the subtropical north Pacific ranges from a Fe-limitation ratio of 1:9.6 × 10⁻⁴ to a near-Fe-limitation ratio of 1:2.1 × 10⁻³ (based on dissolved P and Fe concentrations of 222 ± 14 nM (ref. 16) and 0.2–0.5 nM (ref. 13) respectively).

Our results suggest that N₂ fixation by *Trichodesmium* in the central Atlantic Ocean appears to be influenced by a series of factors (such as P or light). However, in situ Fe and P addition experiments at various irradiance levels are needed to further substantiate these conclusions. Rising atmospheric CO₂ may result in lower near-surface wind speeds³³ and shallower mixed layers³⁴ in the central Atlantic, consequently allowing more N₂ fixation to occur. Increased N₂ fixation could therefore potentially provide a negative feedback mechanism to climatic warming by sequestering anthropogenic CO₂ from the atmosphere.

### Methods

Surface water samples and *Trichodesmium* colonies were collected from a Zodiac deployed with the RV *Seward Johnson*. Water samples were filtered on-board in a portable class-100 clean unit using 0.45 μm acid-washed polycarbonate filters into acid-washed bottles. Colonies were collected at a depth of about 5 m by towing an acid-washed 102-μm plankton net at about 1 knot. In a class-100 portable bench, an average of about 100 colonies per station were hand-picked from the acid-washed cod-end using a plastic inoculating loop, deposited into 3-mL acid-washed Teflon vials, and digested in acid using a combination of Q-HCl, Q-HNO₃ and Q-HF. Fe content in the *Trichodesmium* colonies was then measured by graphite furnace atomic absorption spectrometry (GFAAS). The Fe blank was 39 ± 11 pmol per vial. Dissolved Fe was also measured using GFAAS after an organic extraction³¹. Dissolved Fe blanks were 158 ± 88 ppm (mean ± 1 s.d.). Phosphorus content in digested colonies was determined by spectrophotometric techniques developed for small volumes of intersitial waters³⁸. Nitrogen fixation was determined on isolated colonies collected between 10 and 20 m by slow (1 knot) plankton tow using a 1-m diameter, 202-μm mesh net using the C₂H₂ reduction technique³⁹. Results were converted to mol N fixed using a 3:1 ratio of C₂H₂ reduced to N₂ fixed. Particulate organic carbon

### Table 1 Iron requirements for diazotrophic growth

<table>
<thead>
<tr>
<th>Diazotroph</th>
<th>Fe in MoFe protein[1] (mol mol⁻¹)</th>
<th>Fe in nitrogenase complex (mol mol⁻¹)</th>
<th>Relative molecular mass of MoFe</th>
<th>Specific activity (nmol C₂H₂ min⁻¹ per mg MoFe)</th>
<th>N fixed (per mol enzyme s⁻¹)</th>
<th>N fixed (per mol enzyme s⁻¹)</th>
<th>Fe required (per mol fixed C s⁻¹)?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azotobacter vinelandii</td>
<td>34–38</td>
<td>54–58</td>
<td>216–270K</td>
<td>1,400</td>
<td>3.78</td>
<td>0.065</td>
<td>2.12</td>
</tr>
<tr>
<td>Azotobacter</td>
<td>&gt;22</td>
<td>42</td>
<td>222K</td>
<td>2,000</td>
<td>4.93</td>
<td>0.117</td>
<td>1.22</td>
</tr>
<tr>
<td>chroococcum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kleiella pneumoniae</td>
<td>32</td>
<td>52</td>
<td>218K</td>
<td>2,150</td>
<td>5.21</td>
<td>0.100</td>
<td>1.43</td>
</tr>
<tr>
<td>Clostridium pasteurianum</td>
<td>24</td>
<td>44</td>
<td>220K</td>
<td>2,500</td>
<td>6.11</td>
<td>0.139</td>
<td>1.03</td>
</tr>
<tr>
<td>Rhizobium japonicum</td>
<td>29</td>
<td>49</td>
<td>200K</td>
<td>1,000</td>
<td>2.22</td>
<td>0.045</td>
<td>3.15</td>
</tr>
</tbody>
</table>

[1] Based on the optimal ratio of 5 mol nitorgenase reductase (Fe protein) to 1 mol nitrogenase (MoFe protein),[2] and 4 mol of Fe per Fe protein.[3]

[2] Calculated using the molecular mass, the specific activities and an ethylene production: N₂ fixed stoichiometry of 3:1.


[4] In order to express the nitrogenase Fe requirements in terms of C fixation, a C:N fixation ratio of 7:1 is assumed as in ref. 6. Therefore, the photoautotrophic Fe requirement for C and N fixation (that is, growth) is the sum of the Fe bound in the nitrogenase complex, the bioenergetic Fe needed for C fixation and respiration to fuel nitrogenase, as well as the Fe needed for net C fixation. For the latter, we used 0.9 mol Fe (per mol C s⁻¹) to compute the estimated Fe requirement for diazotrophic growth for photoautotrophs as 1.9–4.1 mol Fe (per mol C s⁻¹) (see ref. 6). These enzymatic iron use efficiencies are derived from a variety of terrestrial heterotrophic diazotrophs because these data are lacking for marine photoautotrophic diazotrophs. However, the functional regions of the modelled tertiary structure of Trichodesmium Fe protein appear to be highly conserved with respect to a heterotrophic diazotroph such as Azotobacter vinelandii (ref. 30).
levels in the field-collected colonies were determined using a Carl Zebr HeLa NAI.500 NCS system. For Trichodesmium chlrophyl biomass, the contents of whole 10.1 Niskin bottles from stratified depths were gravity filtered onto 5- to 10-μm polycarbonate filters and chlrophyl density determined by direct microscopic enumeration using phycocerythrin epifluorescence. Trichodesmium chlrophyl density was converted to chlrophyl terms by a factor derived from direct extraction and determination of chlrophyl per trichome and per colony at each station. Trichodesmium biomass was then integrated to the upper 50 m. Standard hydrographic parameters (temperature, salinity and density) were measured by CTD (conductivity–temperature–depth) at each sampling location.

Received 4 January; accepted 14 March 2001.


Supplementary information is available from Nature’s World-Wide Web site (http://www.nature.com) or as paper copy from the London editorial office of Nature.

Acknowledgements
This work was supported by NSF Chemical and Biological Oceanography.

Correspondence and requests for materials should be addressed to S.A.S.W.
(e-mail: sanudo@notes.cc.scuny.edu).

Preservation of ancient and fertile lithospheric mantle beneath the southwestern United States

Cin-Ty Lee†, Qingzhou Yin†, Roberta L. Rudnick† & Stein B. Jacobsen*†

* Department of Earth and Planetary Sciences, Harvard University, 20 Oxford Street, Cambridge, Massachusetts 02138, USA

Stable continental regions, free from tectonic activity, are generally found only within ancient cratons—the centres of continents which formed in the Archaean era, 4.0–2.5 Gyr ago. But in the Cordilleran mountain belt of western North America some younger (middle Proterozoic) regions have remained stable1, whereas some older (late Archaean) regions have been tectonically disturbed3, suggesting that age alone does not determine lithospheric strength and crustal stability. Here we report rhenium–osmium isotope and mineral compositions of peridote xenoliths from two regions of the Cordilleran mountain belt. We found that the younger, undeformed Colorado plateau is underlain by lithospheric mantle that is ‘depleted’ (deficient in minerals extracted by partial melting of the rock), whereas the older (Archaean), yet deformed, southern Basin and Range province is underlain by ‘fertile’ lithospheric mantle (not depleted by melt extraction). We suggest that the apparent relationship between composition and lithospheric stability, inferred from different degrees of crustal deformation, occurs because depleted mantle is intrinsically less dense than fertile mantle (due to iron having been lost when melt was extracted from the rock). This allows the depleted mantle to form a thicker thermal boundary layer4 between the deep convecting mantle and the crust, thus reducing tectonic activity at the surface. The inference that not all Archaean crust has developed a strong and thick thermal boundary layer leads to the possibility that such ancient crust may have been overlooked because of its intensive reworking or lost from the geological record owing to preferential recycling.

The North American Cordillera is a broad continental region marked by a long period of tectonic activity, which began in the Palaeozoic era with a series of mountain-forming events and culminated in the Cenozoic era with extension5. Deformation appears to be heterogeneously distributed (Fig. 1). The Basin and Range province, which includes much of Nevada and southeastern California, has experienced robust crustal thickening and subsequent large-scale extension (possibly up to 200%)6. In contrast, the Colorado plateau, an elevated circular region surrounded on all sides by deformed crust, has remained an island of tectonic quiescence, as evidenced by flat-lying, unfolded and largely unaffected Palaeozoic sedimentary strata7.

Given the relative differences in the degree of deformation seen in the overlying crust7 and the correlation between age and stability observed elsewhere in the continents, the more-tectonized Basin and Range lithospheric might be expected to be younger than that beneath the less-tectonized Colorado plateau. However, Sm–Nd model ages indicate that the crust in the southern Basin and Range (referred to here as Mojavia) is older, formed in Palaeoproterozoic to Archaean times (~2.0–2.6 Gyr ago)1, whereas the Colorado plateau crust formed subsequently in the middle Proterozoic (1.6–2.0 Gyr ago)3,9. There are two possible explanations for this unexpected relationship. First, the lithospheric mantle beneath Mojavia may not be as old as the crustal model ages indicate. This might