Iron and phosphorus co-limit nitrogen fixation in the Eastern Tropical North Atlantic

Matthew M. Mills, Céline Ridame, Margaret Davey, Julie La Roche, Richard J. Geider

Iron and phosphorus co-limit nitrogenfixationinthe eastern tropical North Atlantic

Matthew M. Mills[1], Celine Ridame[1], Margaret Davey[2,3], Julie La Roche[1] & Richard J. Geider[2]

[1]*Marine Biogeochemistry, IFM-GEOMAR Leibniz-Institut für Meereswissenschaften, Düsternbrooker Weg 20, D-24105 Kiel, Germany*
[2]*Department of Biological Sciences, University of Essex, Colchester CO4 3SQ, UK*
[3]*Marine Biological Association of the United Kingdom, Plymouth PL1 2PB, UK*

The role of iron in enhancing phytoplankton productivity in high nutrient, low chlorophyll oceanic regions was demonstrated first through iron-addition bioassay experiments[1] and subsequently confirmed by large-scale iron fertilization experiments[2]. Iron supply has been hypothesized to limit nitrogen fixation and hence oceanic primary productivity on geological timescales[3], providing an alternative to phosphorus as the ultimate limiting nutrient[4]. Oceanographic observations have been interpreted both to confirm and refute this hypothesis[5,6], but direct experimental evidence is lacking[7]. We conducted experiments to test this hypothesis during the *Meteor 55* cruise to the tropical North Atlantic. This region is rich in diazotrophs[8] and strongly impacted by Saharan dust input[9]. Here we show that community primary productivity was nitrogen-limited, and that nitrogen fixation was co-limited by iron and phosphorus. Saharan dust addition stimulated nitrogen fixation, presumably by supplying both iron and phosphorus[10,11]. Our results support the hypothesis that aeolian mineral dust deposition promotes nitrogen fixation in the eastern tropical North Atlantic.

There is long-standing uncertainty as to whether N or P is the nutrient that limits phytoplankton productivity in the sea[12]. On timescales of one or two days, nutrient-enrichment experiments indicate that primary productivity is N-limited in the Sargasso Sea[13]. On geological timescales, however, N_2 fixation can increase the nitrate inventory of the ocean, thus increasing primary production. In turn, N_2 fixation may be limited by either P (ref. 4) or Fe (ref. 3). These two essential nutrients are in sparse supply in oligotrophic oceans, and both P and Fe have been implicated individually as the nutrient that ultimately limits oceanic primary production and carbon dioxide sequestration[3,4]. However, direct experimental assessment of the relative importance of P and Fe in controlling N_2 fixation in natural plankton populations is lacking[7].

In the oligotrophic North Atlantic[5,6,14] N_2 fixation may fuel 50% of the export production[15]. Atmospheric transport of Saharan dust is a major source of dissolved Fe to the tropical North Atlantic[9] and *Trichodesmium* spp. are most abundant in areas of high Saharan dust deposition[8,16]. In this region, Fe may be supplied in excess of the *Trichodesmium* spp. growth requirements[5,14] and this has been interpreted to support the P-limitation hypothesis[5]. Furthermore, in the subtropical and tropical Atlantic, the rate of nitrogen fixation has been correlated with the phosphorus content (but not the iron content) of filamentous non-heterocystous *Trichodesmium* spp[5]. These oceanographic observations have been interpreted to support P-limitation of diazotrophy. In fact, the low dissolved inorganic phosphate concentrations in surface waters of the Sargasso Sea have been used to argue that the phytoplankton community, as a whole, is P-limited in this region[6].

Nutrient-addition bioassays, designed to investigate which nutrient (N, P or Fe) limits primary production and nitrogen fixation, were conducted at three stations in the tropical Eastern Atlantic (see Supplementary Information) during October–November 2002. A fully replicated, factorial design of the nutrient additions using trace-metal clean techniques was implemented to assess the individual and combined effects of N, P and Fe on the community as a whole and on the diazotrophs. Nitrogen was supplied as NH_4^+ and NO_3^- to allow for species-specific preferences, common among the picophytoplankton[17]. Labelling with $^{15}N_2$ and $^{14}CO_2$ permitted the assessment of nitrogen fixation[18] by diazotrophs and carbon fixation[19] by whole plankton community. Biomass was assessed from chlorophyll *a* concentration[20]. Additions of Saharan soils were made to assess the potential impact of dust deposition on the primary and diazotrophic production.

At all locations, chlorophyll and nutrient concentrations reflected oligotrophic conditions (see Supplementary Information). Iron concentrations ranged between 1 and 3 nM (P. Croot, personal communication), and are typical of surface waters around these areas[16]. Non-heterocystous filamentous cyanobacteria of the genera *Trichodesmium* and *Katagnymene* were the dominant diazotrophs, but based on *nifH* gene sequencing[21], unicellular cyanobacterial and heterotrophic diazotrophs were also present.

In all experiments, phytoplankton production and biomass were limited by the availability of fixed nitrogen as demonstrated by the 2–3-fold stimulation of CO_2 fixation and the 1.5–2.5-fold increase of chlorophyll concentration in the treatments amended with N. In contrast, additions of P, Fe, or P + Fe did not increase carbon fixation or chlorophyll concentration (Fig. 1). However, once N-limitation was artificially removed by adding nitrate and ammonium, additions of P and Fe further increased productivity and chlorophyll. The largest effect was found when N, P and Fe were added simultaneously, which led to 5–10-fold increases in CO_2 fixation rates (Fig. 1a–c) and corresponding increases in chlorophyll (Fig. 1d–f).

Our experiments demonstrate that N was the proximate limiting nutrient in this region, contrary to recent suggestions of P-limitation throughout the North Atlantic[6,22]. Nitrogen limitation of primary production accentuates the potential importance of diazotrophy throughout this region. Nitrogen fixation was detected at all stations, but was lowest at the westernmost site (Fig. 1g). Addition of P and Fe together resulted in a 2–3-fold enhancement of the N_2 fixation rate relative to the control at all three stations (Fig. 1g–i). Addition of either P alone or Fe alone did not stimulate N_2 fixation at the two easternmost sites. At these locations, N_2 fixation was co-limited by both P and Fe.

Saharan dust additions stimulated N_2 fixation as much as twofold (Fig. 1g–i), implying that these treatments relieved the co-limitation of N_2 fixation by P and Fe. We calculated that the supply of dissolved P and Fe from the dust additions was sufficient to meet the demands of the enhanced N_2 fixation rates (see Methods and Supplementary Information). We cannot rule out, however, that the alleviation of P-limitation of diazotrophy by the dust addition was due to the addition of another micronutrient, such as Zn, which is a cofactor in many alkaline phosphatases[7].

The additions of ammonium and nitrate inhibited N_2 fixation (Fig. 1h, i). Diazotrophy may have been suppressed through physiological feedback inhibition of the nitrogenase by dissolved inorganic nitrogen[8] in these treatments. Alternatively, other microorganisms may have outcompeted the diazotrophs for the limited P and Fe on alleviation of N-limitation. We cannot differentiate between these alternate explanations, but the repression of N_2 fixation by dissolved fixed nitrogen suggests that N-limitation of community primary productivity is a pre-requisite for high rates of diazotrophy in surface waters.

The tropical Atlantic is subjected to some of the highest mineral dust deposition rates in the world[9], and has high dissolved iron concentrations in surface waters relative to other oceanic basins. As such, this is a region where the phytoplankton community as a whole[23], and the diazotrophs in particular[14], are least likely to be Fe-limited. Given the reported importance of P-limitation in the subtropical and tropical Atlantic[5,6] and the high Fe concentrations

measured in our study (see Supplementary Information), we were surprised to find that Fe addition was required to stimulate diazotrophy. However, our results are consistent with the recent suggestion that iron may control the abundance of diazotrophs in the South China Sea, another region subject to high dust deposition[24]. As it is generally argued that Fe concentrations in our study area are in excess of diazotroph iron requirements[14,25], our findings suggest that total dissolved Fe concentration is a poor index of bioavailability[26], perhaps due to temporal variation in the chemical speciation of dissolved Fe (ref. 7). It is also possible that the level of iron required to saturate diazotroph growth has been underestimated. The important role of Fe in our study region implies that the control of N_2 fixation by Fe should be even greater in other oceanic regions[6,14] that receive less dust deposition.

Whether P and Fe co-limitation of N_2 fixation is peculiar to the eastern tropical North Atlantic during the time that we sampled, or is common throughout the oligotrophic ocean, can only be ascertained by further research. It is usually assumed that P is supplied primarily from deep waters by mixing or diffusion, whereas Fe is supplied both from deep waters and by mineral dust deposition from the atmosphere. Temporal variability in these sources of nutrients may cause transients in the ratio of P supply to Fe supply, and thus affect the relative importance of these two elements in controlling N_2 fixation. Previous suggestions of P-limitation of diazotrophy in the North Atlantic[5,6] were based on observations made during spring when dust deposition is at its highest. Although dust deposition in the tropical Atlantic is high relative to other locations, the seasonal low deposition during our study in autumn may have shifted the balance towards P and Fe co-limitation. (see Supplementary Information).

A common source of P and Fe, through dust deposition, could also lead to co-limitation of nitrogen fixation. Our observation of stimulation of nitrogen fixation by the addition of Saharan dust is consistent with this suggestion. To be valid, this hypothesis requires that aeolian supply of the bioavailable forms of P and Fe must roughly match the diazotrophic demand for P and Fe. It is commonly assumed that the dissolution of dust can supply the Fe required to support nitrogen fixation, but would not be able to supply the necessary P. This is because crustal material contains about 30 times as much Fe as P (3.5% versus 0.11%), whereas diazotrophs require 30–300 times less Fe than P. However, there are uncertainties on both the supply and demand sides of this comparison. The availability of these nutrients on contact with sea water depends on the dust source, the dust load to the surface of the ocean, prevailing pH conditions during atmospheric processing as well as the mode of deposition (wet or dry)[11]. Due to solubility differences, more phosphate then Fe can be released from dust (see Supplementary Information). Furthermore, we expect the dissolved phosphate to be immediately bioavailable. The bioavailability of the Fe released from dust is less certain[26]. On the demand side, estimates of P and Fe requirements of *Trichodesmium*[14,27] vary, whereas those of the recently discovered marine diazotrophs[21] are not yet characterized. The possibility that simultaneous release of P and Fe from dust may stimulate diazotroph production in regions of high dust deposition remains an open question (see Supplementary Information).

The results presented here have important implications for understanding controls on marine N_2 fixation and how it relates to CO_2 fixation in the North Atlantic. First, contrary to recent suggestions[6,22], our experiments demonstrate that the total primary productivity of the natural plankton community in the tropical Atlantic is N-limited. Second, they demonstrate that N_2 fixation is co-limited by Fe and P in a region where mineral dust deposition is high and iron should be in excess. This has not been reported before. Further studies are required to determine whether this co-limitation is widespread. Finally, our results suggest that dust, when supplied at high levels locally, can relieve Fe and P co-limitation of diazotrophy. The tropical North Atlantic is a region of high dust deposition. It is also considered one of the most important areas

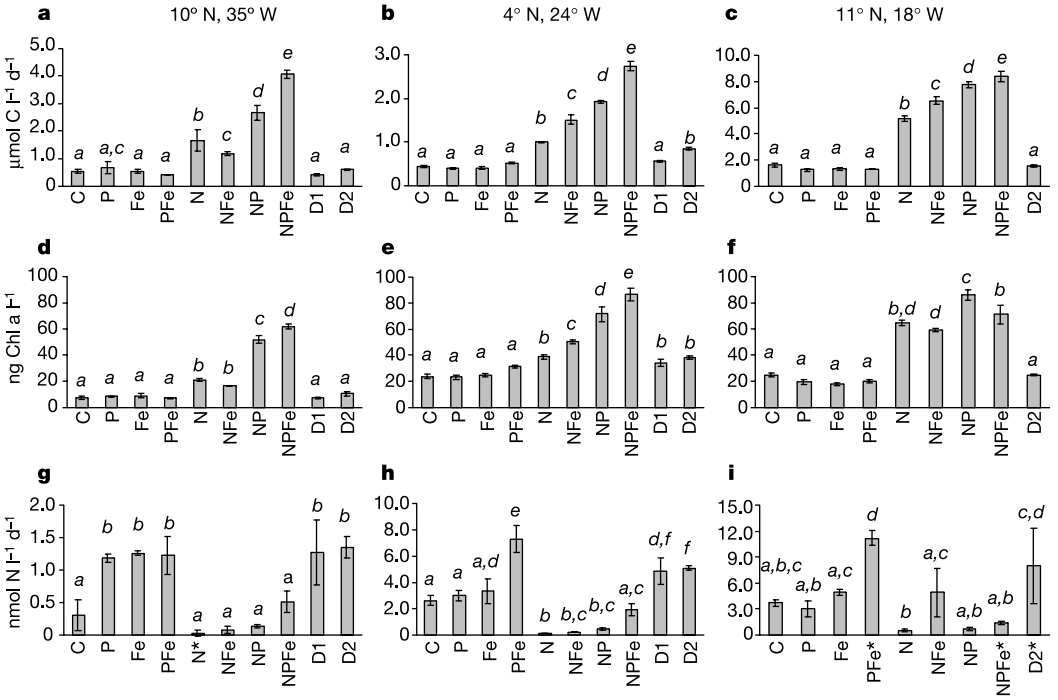

Figure 1 Effect of nutrient additions during bioassay experiments. Measurements were taken at three sites in the tropical Atlantic during October–November 2002. **a**–**c**, Net CO_2 fixation rates; **d**–**f**, chlorophyll *a* concentration; **g**–**i**, N_2 fixation rates. Carbon fixation, nitrogen fixation and chlorophyll were measured from separate triplicate bottles, such that nine bottles were incubated for each nutrient treatment. Shown are means ± standard errors, $n = 3$ for all variables except where $n = 2$ (as indicated by an asterisk). Treatment means were compared using a one-way ANOVA and a Fisher PLSD means comparison test. Means that are not significantly different are labelled with the same letter ($\alpha = 0.05$).

globally for N_2 fixation[15]. Dust deposition at this location is highly episodic, and has varied widely on geological timescales[28]. If dust deposition can to some extent relieve both P and Fe limitation of diazotrophy, the postulated link between climate-driven changes in dust deposition and N_2 fixation may be even stronger then initially suggested[3,29]. □

Methods

Trace-metal clean techniques were strictly used throughout the preparation and execution of the experiments as this is crucial to the good survival of diazotrophs and for *Trichodesmium* spp. in particular[27]. Surface sea water was collected (1–3 m) after dark using a trace-metal clean diaphragm pump. Sea water was pumped into 60-l carboys from which it was siphoned into 1.18 l acid-washed polycarbonate bottles. Under a laminar flow hood, nutrients were added alone and in combination to final concentrations of 1.0 μM NH_4^+ + 1.0 μM NO_3^-, 0.2 μM NaH_2PO_4, and 2.0 nM $FeCl_3$. Saharan dust treatments were also conducted with final concentrations of 0.5 mg l^{-1} (D1) and 2 mg l^{-1} (D2). These concentrations were chosen to simulate concentrations in the upper 1 m of the water column after a strong Saharan aerosol deposition event[11]. The dust consisted of the fine fractions of surface soils collected in the Hoggar region (Southern Algeria) with a grain-size distribution and chemical composition typical for Saharan aerosols collected far from the source[10,11]. The measured P and Fe content of the dust was 0.14 ± 0.01% and 4.97 ± 0.49% (± one standard error). The phosphate liberated from the dust treatments was approximately 2.7 and 10.8 nmol l^{-1}, and Fe released was 0.9 and 3.6 nmol l^{-1}, for the 0.5 and 2.0 mg l^{-1} dust treatments respectively (see Supplementary Information). The bottles were then sealed gas tight and placed in an on-deck incubator with circulating surface sea water. Light was attenuated to 20% of incident surface values with blue filters (Lagoon Blue, Lee Filters #172). For each treatment, parallel incubations for each variable (carbon fixation, nitrogen fixation and biomass) were run in triplicate over 48 h with rate measurements made during the final 24 h and chlorophyll concentration determined at 48 h. At each of the three study sites, over 100 bottles were incubated. For measuring net nitrogen fixation rates, 1.0 ml of 99% $^{15}N_2$ was introduced to each bottle through a butyl septum using a gas-tight syringe. $^{15}N_2$ uptake measurements may underestimate nitrogen fixation if significant release of dissolved nitrogen occurs[30]. However, this effect should be minimal in our experiments because, in a N-limited oligotrophic system, our 24-h rate measurements should allow released labile dissolved N to be reincorporated into particulate matter. For measuring primary productivity, 0.1 mCi ^{14}C-bicarbonate was added to each bottle. All incubations were conducted from dawn-to-dawn and stopped by gentle filtration.

1. Martin, J. H. & Fitzwater, S. E. Iron deficiency limits phytoplankton growth in the north-east Pacific subarctic. *Nature* **331,** 341–343 (1988).
2. Boyd, P. *et al.* A mesoscale phytoplankton bloom in the polar Southern Ocean stimulated by iron fertilization. *Nature* **407,** 695–702 (2000).
3. Falkowski, P. G. Evolution of the nitrogen cycle and its influence on the biological sequestration of CO_2 in the ocean. *Nature* **387,** 272–275 (1997).
4. Tyrrell, T. The relative influences of nitrogen and phosphorus on oceanic primary production. *Nature* **400,** 525–531 (1999).
5. Sanudo-Wilhelmy, S. A. *et al.* Phosphorus limitation of nitrogen fixation by *Trichodesmium* in the central Atlantic Ocean. *Nature* **411,** 66–69 (2001).
6. Wu, J., Sunda, W., Boyle, E. A. & Karl, D. M. Phosphate depletion in the Western North Atlantic Ocean. *Science* **289,** 759–762 (2000).
7. Morel, F. M. M. & Price, N. M. The biogeochemical cycles of trace metals in the oceans. *Science* **300,** 944–947 (2003).
8. Capone, D. G., Zehr, J. P., Paerl, H. W., Bergman, B. & Carpenter, E. J. *Trichodesmium*, a globally significant cyanobacterium. *Science* **276,** 1221–1229 (1997).
9. Gao, Y., Kaufman, Y. J., Tanre, D., Kolber, D. & Falkowski, P. G. Seasonal distributions of aeolian iron fluxes to the global ocean. *Geophys. Res. Lett.* **28,** 29–32 (2001).
10. Guieu, C., Loye-Pilot, M. D., Ridame, C. & Thomas, C. Chemical characterization of the Saharan dust end-member: Some biogeochemical implications for the western Mediterranean Sea. *J. Geophys. Res. Atmos.* **107,** 4258 (2002).
11. Ridame, C. & Guieu, C. Saharan input of phosphate to the oligotrophic water of the open western Mediterranean Sea. *Limnol. Oceanogr.* **47,** 856–869 (2002).
12. Redfield, A. C. The biological control of chemical factors in the environment. *Am. Sci.* **46,** 205–221 (1958).
13. Graziano, L. M., Geider, R. J., Li, W. K. W. & Olaizola, M. Nitrogen limitation of North Atlantic phytoplankton: Analysis of physiological condition in nutrient enrichment experiments. *Aquat. Microb. Ecol.* **11,** 53–64 (1996).
14. Berman-Frank, I., Cullen, J. T., Shaked, Y., Sherrell, R. M. & Falkowski, P. G. Iron availability, cellular iron quotas, and nitrogen fixation in *Trichodesmium*. *Limnol. Oceanogr.* **46,** 1249–1260 (2001).
15. Gruber, N. & Sarmiento, J. L. Global patterns of marine nitrogen fixation and denitrification. *Glob. Biogeochem. Cycles* **11,** 235–266 (1997).
16. Tyrrell, T. *et al.* Large-scale latitudinal distribution of *Trichodesmium spp.* in the Atlantic Ocean. *J. Plankton Res.* **25,** 405–416 (2003).
17. Palinska, K. A. *et al.* The signal transducer P-II and bicarbonate acquisition in *Prochlorococcus marinus* PCC 9511, a marine cyanobacterium naturally deficient in nitrate and nitrite assimilation. *Microbiology* **148,** 2405–2412 (2002).
18. Montoya, J. P., Voss, M., Kähler, P. & Capone, D. G. A simple, high-precision, high-sensitivity tracer assay for N_2 fixation. *Appl. Environ. Microbiol.* **62,** 986–993 (1996).
19. Lohrenz, S. *et al.* A comparison of *in situ* and simulated *in situ* methods for estimating primary production. *J. Plankton Res.* **14,** 201–221 (1992).
20. Welschmeyer, N. Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments. *Limnol. Oceanogr.* **38,** 1985–1992 (1994).
21. Zehr, J. P. *et al.* Unicellular cyanobacteria fix N_2 in the subtropical North Pacific Ocean. *Nature* **412,** 635–638 (2001).
22. Ammerman, J. W., Hood, R. R., Case, D. A. & Cotner, J. B. Phosphorus deficiency in the Atlantic: An emerging paradigm in oceanography. *EOS Trans. Am. Geophys. Union* **84,** 165–170 (2003).
23. Coale, K. H., Fitzwater, S. E., Gordon, R. M., Johnson, K. S. & Barber, R. Control of community growth and export production by upwelled iron in the equatorial Pacific Ocean. *Nature* **379,** 621–624 (1996).
24. Wu, J. F. *et al.* Dissolved inorganic phosphorus, dissolved iron, and *Trichodesmium* in the oligotrophic South China Sea. *Global Biogeochem. Cycles* **17,** 1008 (2003).
25. Kustka, A., Sañudo-Wilhelmy, S., Carpenter, E. J., Capone, D. G. & Raven, J. A. A revised estimate of the iron use efficiency of nitrogen fixation, with special reference to the marine cyanobacterium *Trichodesmium* spp. (Cyanophyta). *J. Phycol.* **39,** 12–25 (2003).
26. Rue, E. & Bruland, K. Complexation of Fe(III) by natural ligands in the central north Pacific as determined by a new competitive ligand equilibrium/absorptive cathodic voltammetry method. *Mar. Chem.* **50,** 117–138 (1995).
27. Kustka, A. *et al.* Iron requirements for dinitrogen- and ammonium-supported growth in cultures of *Trichodesmium* (IMS 101): Comparison with nitrogen fixation rates and iron: carbon ratios of field populations. *Limnol. Oceanogr.* **48,** 1869–1884 (2003).
28. Mahowald, N. *et al.* Dust sources and deposition during the last glacial maximum and current climate: A comparison of model results with paleodata from ice cores and marine sediments. *J. Geophys. Res. Atmos.* **104,** 15895–15916 (1999).
29. Karl, D. *et al.* Dinitrogen fixation in the world's oceans. *Biogeochemistry* **57,** 47–98 (2002).
30. Glibert, P. M. & Bronk, D. A. Release of dissolved organic nitrogen by marine diazotrophic cyanobacteria, *Trichodesmium* spp. *Appl. Environ. Microbiol.* **60,** 3996–4000 (1994).

Acknowledgements We would like to acknowledge the captain and crew of the F/S *Meteor* and the scientists aboard the *Meteor 55* SOLAS cruise, especially D. Wallace, K. Lochte, H. Bange, P. Croot, M. Voss, F. Malien, R. Langlois and P. Fritsche. We thank D. Wallace for insightful comments on this manuscript. Additionally, we acknowledge W. Balzer for loaning the clean container used during M55. This work was supported by the Deutsche Forschungsgemeinschaft's Meteor Schwerpunktprogramm, a Natural Environment Research Council grant to R.J.G., and a Marie Curie Post Doctoral fellowship to C.R.

Authors' contributions The first three authors made equal contributions to the success of the experiments. This manuscript is the product of an equal collaboration between the groups of J.L. at IfM-Geomar and R.J.G. at University of Essex.

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

Correspondence and requests for materials should be addressed to R.J.G. (geider@essex.ac.uk).

Supplementary Information

Iron and phosphorus co-limit nitrogen fixation in the Eastern Tropical North Atlantic

Matthew M. Mills, Celine Ridame, Margaret Davey, Julie La Roche, Richard J. Geider

Supplementary Results and Discussion

Aerosol loads over the tropical North Atlantic and at other locations

Aerosol optical thickness for the tropical Atlantic during Meteor-55 (Fig S1) was obtained from the MODIS Online Visualization and Analysis System (MOVAS). Higher concentrations of aerosols are typically observed over the tropical and subtropical North Atlantic in Spring than in Autumn (Figure S2a & b). The minimum monthly-integrated aerosol optical thickness along the Meteor 55 cruise (Figure S2c) is approximately equal to the maximum values observed at the Bermuda Atlantic Time Series (Figure S3a) and Hawaii Ocean Time-Series sites (Figure S3b). The seasonal and spatial variability in dust load may lead to seasonal and spatial variability in the degree to which Fe and P limit N_2 fixation.

Initial conditions for bioassay experiments

The initial dissolved and particulate nutrient concentrations for bioassay experiments are given in Table S1 and sample locations are shown in Figure S1.

Comparison of dissolved P and Fe released by the dust in our incubations with particulate P:Fe of *Trichodesmium*

We added two concentrations of Saharan dust (0.5 and 2.0 mg L^{-1}) to our incubations. The measured P and Fe contents of the dust were 0.14 ± 0.01% and 4.97 ± 0.49% respectively. Ridame and Guieu[S1] found that 10% of the P in the dust dissolved within 6 hours and 14% within one week when dust was added to seawater at a concentration of 5 mg L^{-1}. Thus, for our two day incubations, we estimate that approximately 12% of the P would have dissolved, increasing phosphate concentrations by approximately 2.7 and 10.8 nmol P L^{-1} in the low and high dust treatments respectively. Estimates of Fe released from the equation given by Bonnet and Guieu[S2] are 0.8 and 1.4 nmol Fe L^{-1} for the respective low and high dust treatments. Additional dissolution experiments were carried out in filtered seawater during the Meteor-55 cruise using dust concentrations of 5 and 10 mg L^{-1} (Ridame unpublished) and yielded results very similar to the published values[S1,S2]. Thus, for our experimental conditions, we estimate that the ratio of dissolved P to dissolved Fe that arose from dust dissolution was about 3 mol P:mol Fe in the low dust addition and 8 mol P:mol Fe in the high dust addition.

The ratio of P:Fe that is released via dust dissolution can be compared with measurements of the P:Fe of natural and laboratory cultures of *Trichodesmium*. In nature, C:Fe of *Trichodesmium* colonies ranges from about 1,000 to 100,000 mol C:mol Fe[S3]. Assuming a Redfield C:P of 106, this translates to about 10 to 1,000 mol P:mol Fe. In laboratory conditions, the P:Fe of *Trichodesmium* growing at 70-100% of its maximum growth rate ranges from about 30 to 300 mol P:mol Fe, although higher values have been observed under extreme Fe-limitation in P-replete cultures[S4,S5]. Thus, the range of P:Fe observed in *Trichodesmium* in nature compares

well with the range observed in the laboratory. However, Fu and Bell[S6] report that in culture, *Trichodesmium* GBR-TRLI101 grows best in medium with low initial dissolved P concentration of around 1 μM, yielding a P:Fe supply ratio of 5.

Although the Fe content of dust is approximately 37 times higher then that of P, the differential solubilities for P and Fe leads us to expect that more P then Fe should be released from the dust, as shown from the values presented above. Moreover, P is released as phosphate (this was the only form of dissolved P that was measured in the dissolution experiments cited above) and should be available for immediate uptake by phytoplankton while only a fraction of the total dissolved Fe is likely to be immediately bioavailable. Typically, >99% of the dissolved Fe in seawater is complexed to ligands[S7]. Alternatively, the Fe coming from dust may be in the form of colloids [S8]. Complexed and colloid Fe may not be immediately available to the phytoplankton. If 99% of the Fe that dissolves from dust is not immediately available, then the bioavailable P:Fe may be as high as 300 mol P:mol Fe. The likely range of bioavailable P:Fe in dust of 3-300 mol P:mol Fe overlaps with the range of particulate P:Fe that is required to support maximal growth of *Trichodesmium*. We conclude that the possibility that dust supplies the necessary P and Fe to support the high biomass-specific rates of N_2 fixation that are only observed when *Trichodesmium* is growing near its maximum growth rate is an open question.

Potential nitrogen release from Saharan Dust

We also evaluated the potential supply of nitrogen from Saharan dust. The Saharan dust that we used in our experiments contained 0.12%± 0.003% (by weight) of total nitrogen. For dust additions of 0.5 and 2 mg L^{-1}, the maximum N enrichment, assuming that the N dissolves completely and all N is bioavailable would have been

0.04 and 0.17 µmol L^{-1}, respectively. This compares with 1 µmol L^{-1} nitrate plus 1 µmol L^{-1} ammonium that we used in our +N treatments. In dissolution experiments (Ridame, unpublished) using 5 and 10 mg L^{-1} Saharan dust, the ammonium concentration liberated by dust after 48h contact time was undetectable (<0.05 µM), as expected given the low nitrogen content of the dust. Saharan dust addition led to an increase in chlorophyll concentration and primary production relative to the control in the experiment conducted at 4 ^{o}N 24 ^{o}W, but not in the other two experiments. This suggests that the Saharan dust that we used may have provided nitrogen to the phytoplankton in this experiment. None-the-less, enhancement of nitrogen fixation by dust addition was also observed at this location.

Comparison of our dust additions with dust concentrations in the North Atlantic

We used Saharan dust enrichments of 0.5 to 2 mg L^{-1} in our incubations. The lowest addition was dictated by the need to add a measurable and representative quantity of Saharan soil fraction to the bottles. These concentrations of added dust can be compared with estimates of *in situ* dust concentrations derived from the measured dissolved aluminium concentration in surface ocean waters from the tropical North Atlantic[S9]. Measures and Vink[S9] converted Al concentration in the surface layer to dust concentration by assuming that dust was 8.1% aluminium by weight and that 1.5 to 5% of the aluminium was soluble. For the highest concentration of Al measured (70 nM in Gulf of Guinea)[S9], we estimate that the dust concentration would be 0.04 to 0.13 mg L^{-1}. For the Sargasso Sea, with 30.6 nM Al[S9], the dust concentration would be 0.02 to 0.06 mg L^{-1}.

The estimates of dust concentration based on surface aluminium may not represent the peak values observed during and immediately after a dust deposition

event. Zender and co-workers[S10] give annual average dust deposition rates of about 0.1 to 1 μg m^{-2} s^{-1} (e.g., 8.6 to 86 mg m^{-2} d^{-1}) in the tropical North Atlantic, with the highest rates in the east. If we assume that a dust deposition event would have 10 fold higher dust concentration than the average, the event would deliver up to 860 mg m^{-2} d^{-1}. If confined to the upper 1 m of the water column, the dust concentration would be about 0.86 mg dust L^{-1}. Distributed over the upper 20 m of the water column (i.e., a typical mixed layer depth), the concentration would be reduced to about 0.04 mg dust L^{-1}. This is of the same order as the dust concentrations estimated for the Sargasso Sea and Gulf of Guinea[S9].

We feel that the calculations made above provide conservative estimates of dust deposition as dissolved aluminium concentration reflects the average dust deposition over the lifetime of aluminium in the surface ocean, and dust deposition may be much more episodic than we have assumed in our calculation of dust load. Other estimates of dust deposition are higher. For example, Bonnet & Guieu[S2] give concentrations of 0.01, 0.1 and 0.5 mg L^{-1} as dust concentrations that would accompany low, median and high Saharan dust deposition events to the upper 10 m of the water column.

Previous estimates of contribution of Saharan dust released P to primary productivity

Other investigators have reported that input of P via dust deposition stimulates primary production at the event scale. Ridame and Guieu[S1] calculate that on the event scale, dust associated P can account for 15% of the integrated new production and 14% of the primary production in the mixed surface layer of the western Mediterranean. Likewise, Markaki and co-workers[S11] show that atmospheric

deposition of DIP could account for up to 38% of new production in the Levantine Basin (Western Mediterranean) during summer and autumn. Finally, Herut and co-workers suggest atmospheric inputs of P may account for 4-11% of new production in the SE Mediterranean[S12], and estimate that leachable fluxes of inorganic P might account for ~15% of new production in the SE Mediterranean[S13].

Potential contributions of dust deposition of P and Fe to N_2 fixation in the North Atlantic

We used estimates of dust deposition, dust solubility and the C:N:P:Fe ratio for diazotrophic growth to calculate the potential for dust to support N_2 fixation in the North Atlantic. We calculate that the P in dust can support up to 30% of the most recent estimate of N_2 fixation in the North Atlantic[S14], whereas Fe in dust can support >100% of this rate.

Zender and co-workers[S10] estimate atmospheric deposition of 0.19 Tg P yr^{-1} (= 6.1 10^9 mol P yr^{-1}) to the North Atlantic. At a Redfield N:P of 16:1, and assuming that 10% to 100% of the P is used to support N_2 fixation, atmospheric deposition could support a N_2 fixation rate of 0.98 to 9.8 10^{10} mol N yr^{-1}. This can be compared with a recent estimate[S14] of N_2 fixation in the North Atlantic of 31 10^{10} mol N yr^{-1}, or the earlier[S15], higher estimate of 200 10^{10} mol N yr^{-1}. Thus, for the North Atlantic as a whole, P deposited in dust can support at most 31% (e.g., 9.8 10^{10}/31 10^{10}) of the annual N_2 fixation. The amount of N_2 fixation that could be supported by atmospheric dust deposition would be three fold higher if the measured N:P for *Trichodesmium* of 40-50 mol N:mol P[S16] is used in these calculations instead of the Redfield N:P. The amount of N_2 fixation that could be supported by dust deposition would also be higher if the dust load to the ocean is higher than estimated by Zender

and co-workers[S10]. Earlier estimates of dust deposition to the ocean are 1.5 to 3 fold higher[S17, S18] than this most recent estimate[S10].

Zender and co-workers[S10] estimate aeolian transport of 6.2 Tg Fe yr^{-1} (= 1.1 10^{11} mol Fe yr^{-1}) to the North Atlantic. Assuming a N:Fe ratio for diazotrophic growth of 4000 for *Trichodesmium* at a moderate rate of 0.1 d^{-1} (based on 38 μmol Fe:mol C)[S5] and assuming C:N = 106/16), and that 0.2% to 2.0% of the Fe in dust can be used to support diazotrophy, atmospheric deposition of Fe could support a N_2 fixation rate of 9 - 99 10^{11} mol N yr^{-1}.

These estimates are subject to considerable uncertainty. They depend on the accuracy of current estimates of dust deposition[S10,S17,S18], the type of dust[S19], the concentration, solubility[S1,S2] and bioavailability[S19] of P and Fe in dust, and the C:N:P:Fe stoichiometry of diazotroph growth[S3,S4,S5]. They also depend on the match or mismatch between the geographical and temporal distribution of dust deposition and diazotrophy. The maximum impact will be achieved if the geographical distribution and timing of the dust input matches the distribution of the diazotrophs. The contribution to nitrogen fixation of P from dust could be higher if one considers only the eastern tropical Atlantic rather then the North Atlantic as a whole. The impact of dust must also be considered relative to vertical and horizontal inputs of available P, Fe and N.

Table S1. Initial conditions for bioassay experiments (mean ± SE).

	Experiment 1	Experiment 2	Experiment 3
Latitude	10° 00' N	4° 24' N	11° 01' N
Longitude	34° 42' W	23° 51' W	18° 14' W
Chl a (ng L^{-1})	18.1 ± 2.5	37.9 ± 0.8	21.5 ± 0.7
NO_3 (µmol L^{-1})[1]	<0.3	<0.3	<0.3
TDN (µmol L^{-1})	1.1 ± 0.23	2.5 ± 0.07	4.0 ± 0.01
PO_4 (µmol L^{-1})	<0.03	<0.03	<0.03
TDP (µmol L^{-1})	0.2 ± 0.01	0.2 ± 0.01	0.2 ± 0.01
SiO_2 (µmol L^{-1})	1.6	1.0 ± 0.02	0.5 ± 0.05
Fe (nmol L^{-1})[2]	2.6	1.3	1.1
PC (µmol L^{-1})	9.9 ± 3.4	8.0 ± 0.08	14.3 ± 7.7
PN (µmol L^{-1})	0.6 ± 0.02	0.6 ± 0.02	1.6 ± 1.0
Diazotrophs detected[3]	Not determined	*Trichodesmium sp.*	*Trichodesmium sp.*
		Katagnemene sp.	*Katagnemene sp.*
			Vibrio diazotrophicus

[1] Nitrate , phosphate and silicate concentrations were measured by F. Malien
[2] Fe measurements were made in collaboration with P. Croot
[3] *nifH* sequence information was provided by R. Langlois

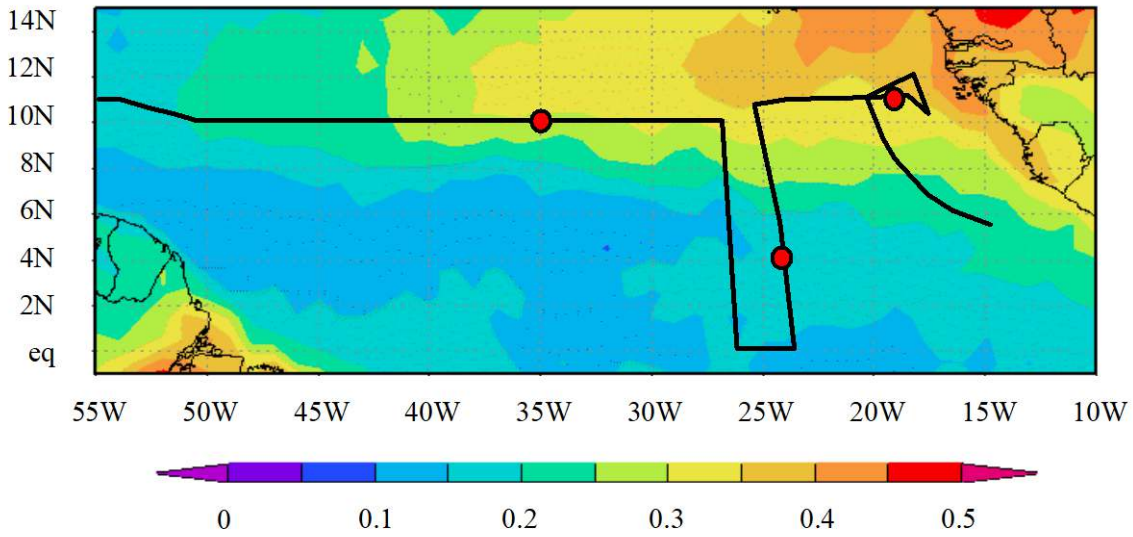

Figure S1. Location of bioassay experiments and integrated map of aerosol optical thickness along the Meteor 55 cruise track. Given residence time within the surface mixed layer of approximately one month for Fe, this map represents, at least qualitatively, the relative dust loading during our experiments. Aerosol optical thickness obtained from the MODIS Online Visualization and Analysis System (MOVAS) (http://lake.nascom.nasa.gov/www/online_analysis/movas/monthly/index.shtml), developed by the Goddard Earth Sciences Distributed Active Archive Center (GES DAAC) and the MODIS Atmosphere Science Team. The original MODIS data were acquired as part of the NASA's Earth Science Enterprise (algorithms by the MODIS Science Teams; data processing by the MODIS Adaptive Processing System (MODAPS) and GES DAAC; and archive and distribution by the GES DAAC).

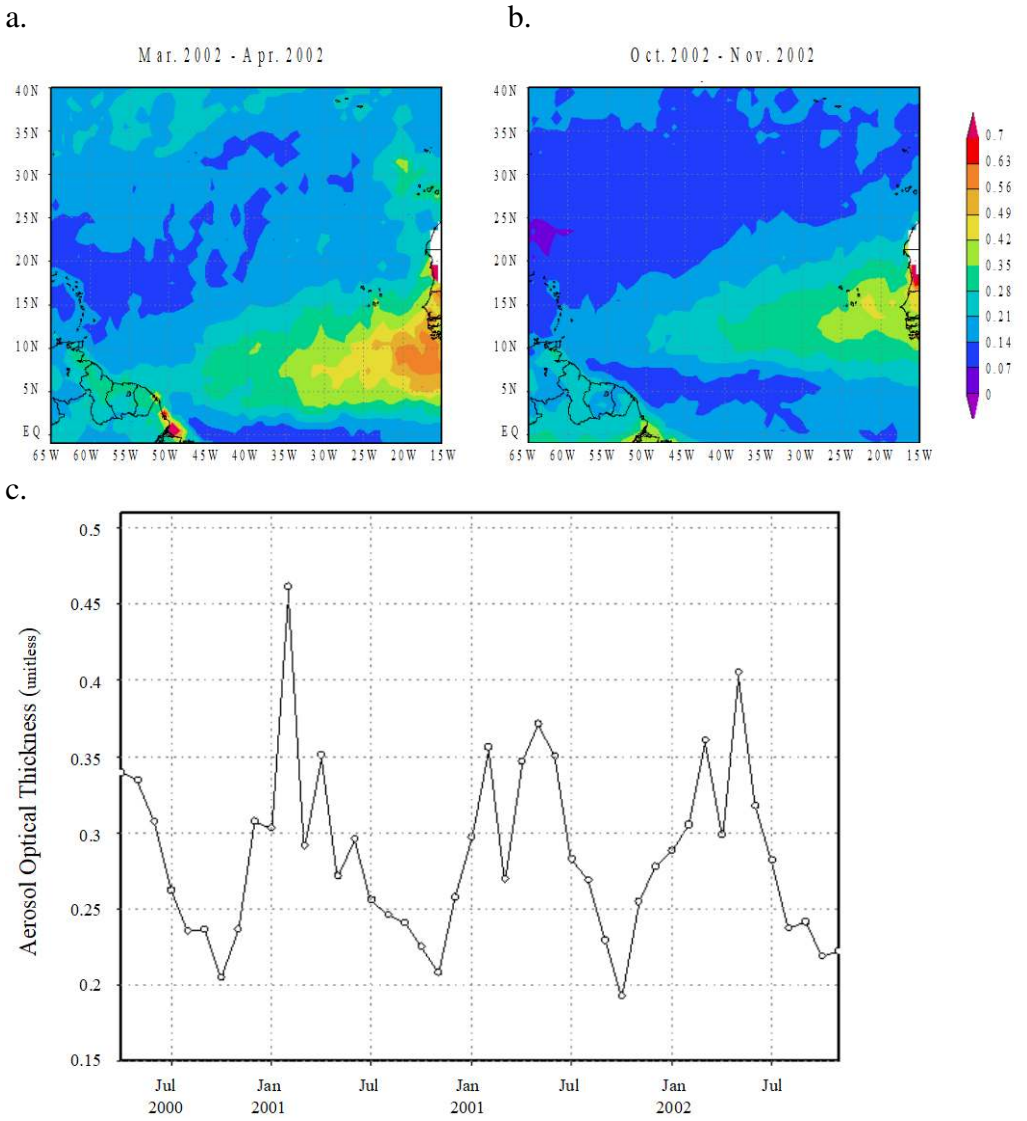

Figure S2. Monthly-integrated aerosol optical thickness for the North Atlantic in (a) March to April 2002, (b) October to November 2002 and (c) a time plot image from April 2000 to November 2003 along the Meteor 55 cruise track. (http://lake.nascom.nasa.gov/www/online_analysis/movas/monthly/index.shtml), developed by the Goddard Earth Sciences Distributed Active Archive Center (GES DAAC) and the MODIS Atmosphere Science Team. The original MODIS data were acquired as part of the NASA's Earth Science Enterprise (algorithms by the MODIS Science Teams; data processing by the MODIS Adaptive Processing System (MODAPS)and GES DAAC; and archive and distribution by the GES DAAC).

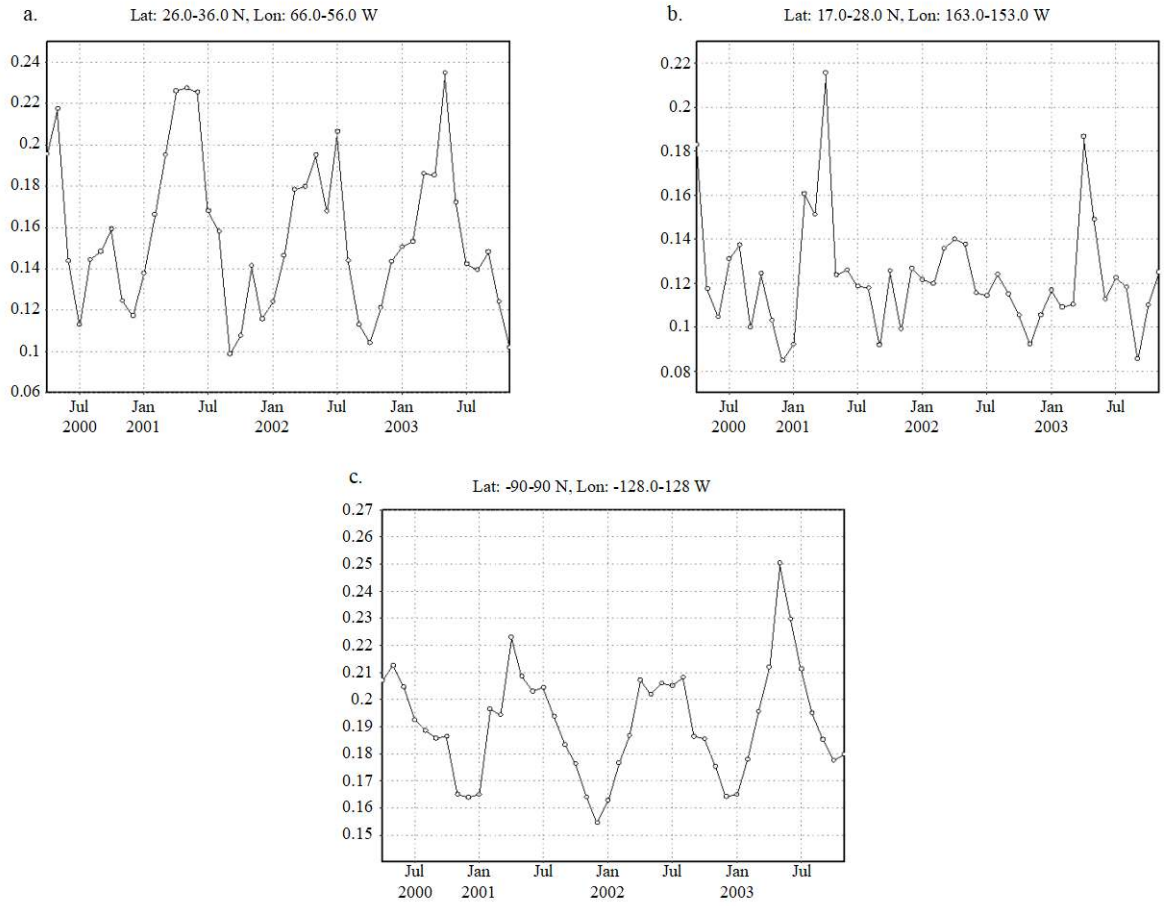

Figure S3. Monthly integrated aerosol optical thickness between April 2000 and November 2003 at oligotrophic waters surrounding (a) the Bermuda Atlantic Time Series site, (b) Hawaii Ocean Time-Series site and (c) a global average. (http://lake.nascom.nasa.gov/www/online_analysis/movas/monthly/index.shtml), developed by the Goddard Earth Sciences Distributed Active Archive Center (GES DAAC) and the MODIS Atmosphere Science Team. The original MODIS data were acquired as part of the NASA's Earth Science Enterprise (algorithms by the MODIS Science Teams; data processing by the MODIS Adaptive Processing System (MODAPS) and GES DAAC; and archive and distribution by the GES DAAC).

Supplementary References

S1. Ridame, C. & Guieu, C. Saharan input of phosphate to the oligotrophic water of the open western Mediterranean Sea. *Limnol. Oceanogr.* **47**, 856-869 (2002).

S2. Bonnet, S. & Guieu, C. Dissolution of atmospheric iron in seawater. *Geophy. Res. Lett.* **31** doi:10.1029/2003GL018423. (2004).

S3. Kustka A., Carpenter E. J., Sanudo-Wilhelmy S. A. Iron and marine nitrogen fixation: progress and future directions. *Res. Microbiol.* **153**: 255-262 (2002).

S4. Berman-Frank I., Cullen J. T., Shaked Y., Sherrell R.M. & Falkowski P.G. Iron availability, cellular iron quotas, and nitrogen fixation in *Trichodesmium*. *Limnol. Oceanogr.* **46**: 1249-1260 (2001).

S5. Kustka, A. et al. Iron requirements for dinitrogen- and ammonium-supported growth in cultures of *Trichodesmium* (IMS 101): Comparison with nitrogen fixation rates and iron: carbon ratios of field populations. *Limnol. Oceanogr.* **48**, 1869-1884 (2003).

S6. Fu, F. X. & Bell P. R. F. Factors affecting N-2 fixation by the cyanobacterium *Trichodesmium* sp GBR-TRLI101. *Fems Microbiol. Ecol.* **45**(2): 203-209 (2003).

S7. Macrellis H. M., Trick C. G., Rue E. L., Smith G. & Bruland K. W. Collection and detection of natural iron-binding ligands from seawater. *Mar. Chem.* **76**: 175-187 (2001).

S8. Wu, J., Boyle, E., Sunda, W. & Wen, L.-S. Soluble and colloidal iron in the oligotrophic North Atlantic and North Pacific. *Science* **293**, 847–849 (2001).

S9. Measures C. I. & Vink S. On the use of dissolved aluminium in surface waters to estimate dust deposition to the ocean. *Glob. Biogeochem. Cyc.* **14**: 317-327 (2000).

S10. Zender, C. S., Bian, H. S. & Newman, D. Mineral dust entrainment and deposition (DEAD) model: Description and 1990s dust climatology. *J. Geophys. Res.-Atmos*. 108, art. no.-4416 (2003).

S11. Markaki, Z. et al. Atmospheric deposition of inorganic phosphorus in the Levantine Basin, eastern Mediterranean: Spatial and temporal variability and its role in seawater productivity. *Limnol. Oceanogr.* **48**, 1557-1568 (2003)

S12. Herut, B., Krom, M. D, Pan, G. & Mortimer, R. Atmospheric input of nitrogen and phosphorus to the Southeast Mediterranean: Sources, fluxes, and possible impact. *Limnol. Oceanogr.* **44**: 1683-1692 (1999).

S13. Herut, B., Collier, R., Krom, M. D. The role of dust in supplying nitrogen and phosphorus to the southeast Mediterranean. *Limnol. Oceanogr.* **47**, 870-878 (2002).

S14. Hansell, D. A., Bates, N. R. & Olson, D. B. Excess nitrate and nitrogen fixation in the North Atlantic Ocean. *Mar. Chem.* **84**, 243-265 (2003).

S15. Gruber, N. & Sarmiento, J. L. Global patterns of marine nitrogen fixation and denitrification. *Glob. Biogeochem. Cyc.* **11**, 235-266 (1997).

S16. Letelier, R. & Karl, D. M. Role of *Trichodesmium* spp. in the productivity of the subtropical North Pacific Ocean. *Mar. Ecol. Prog. Ser.* **133**: 263-273 (1996).

S17. Duce, R. A. et al. The atmospheric input of trace species to the world ocean. *Glob. Biogeochem. Cyc.* **5**: 193-259 (1991).

S18. Ginoux P., Chin M., Tegen I., Prospero J. M., Holben B., Dubovik O. & Lin S. J. Sources and distributions of dust aerosols simulated with the GOCART model. *J. Geophys. Res.* **106**: 20255-20273 (2001).

S19. Visser F., Gerringa L. J. A., Van der Gaast S. J., de Baar H. J .W. & Timmermans K. R. The role of the reactivity and content of iron of aerosol dust on growth rates of two Antarctic diatom species. *J. Phycol.* **39**: 1085-1094 (2003).