Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic

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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

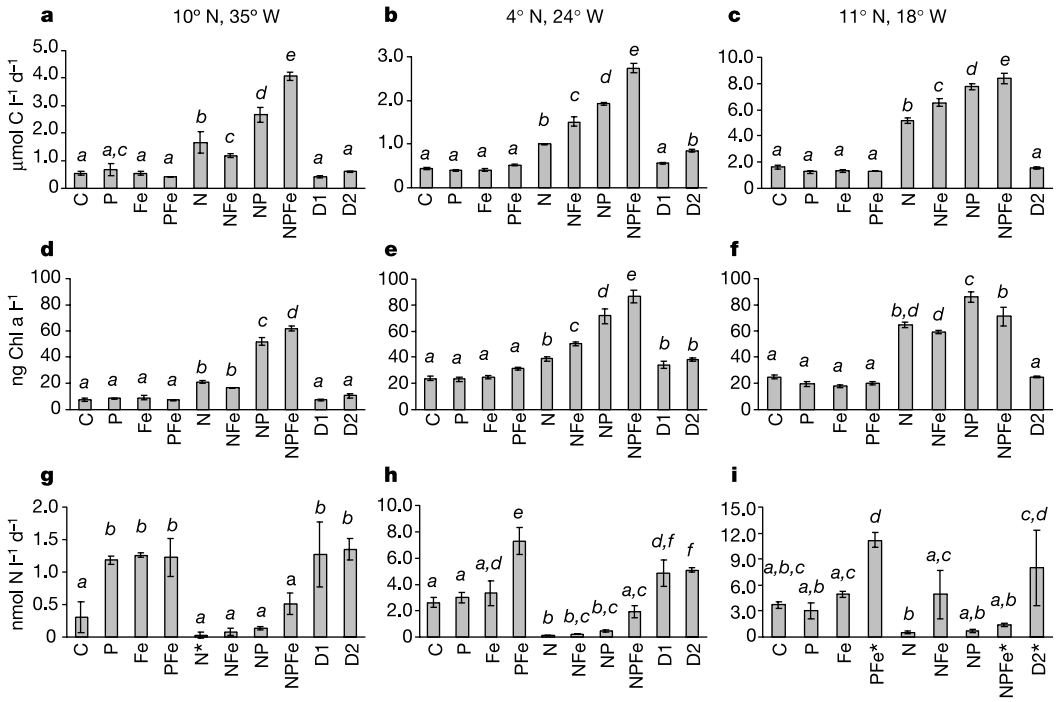

Figure 1 Effect of nutrient additions during bioassay experiments. Measurements were taken at three sites in the tropical Atlantic during October–November 2002. **a**–**c**, Net CO_2 fixation rates; **d**–**f**, chlorophyll *a* concentration; **g**–**i**, N_2 fixation rates. Carbon fixation, nitrogen 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.

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Evidence for ecology's role in speciation

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A principal challenge in testing the role of natural selection in speciation is to connect the build-up of reproductive isolation between populations to divergence of ecologically important traits[1,2]. Demonstrations of 'parallel speciation', or assortative mating by selective environment, link ecology and isolation[3–5], but the phenotypic traits mediating isolation have not been confirmed. Here we show that the parallel build-up of mating

cells fully induced with 0.5% galactose) of Gal3p and Gal80p, respectively (Supplementary Figs S2 and S4).

Determination of galactose consumption rate

To determine the galactose consumption rate, aliquots from cultures were filtered and the galactose concentration of the cell-free medium was analysed as follows. β-Galactose dehydrogenase was used to oxidize galactose in the presence of 2.5 mM NAD^+ dissolved in a buffer containing 50 mM imidazole and 5 mM $MgCl_2$ pH 7.0 (ref. 30). Conversion of NAD^+ into NADH was followed spectrophotometrically at 340 nm.

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corrigendum

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In this Letter to *Nature*, the chlorophyll *a* data presented in Fig. 1d–f and in Table S1 of the Supplementary Information are an order of magnitude too low owing to a calculation mistake. This error does not alter the conclusions of our paper. □