Relative influence of nitrogen and phosphorus availability on phytoplankton physiology and productivity in the oligotrophic sub-tropical North Atlantic Ocean

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Abstract

Nutrient addition bioassay experiments were performed in the low-nutrient, low-chlorophyll oligotrophic subtropical North Atlantic Ocean to investigate the influence of nitrogen (N), phosphorus (P), and/or iron (Fe) on phytoplankton physiology and the limitation of primary productivity or picophytoplankton biomass. Additions of N alone resulted in 1.5–2 fold increases in primary productivity and chlorophyll after 48 h, with larger (~threefold) increases observed for the addition of P in combination with N (NP). Measurements of cellular chlorophyll contents permitted evaluation of the physiological response of the photosynthetic apparatus to N and P additions in three picophytoplankton groups. In both *Prochlorococcus* and the picoeukaryotes, cellular chlorophyll increased by similar amounts in N and NP treatments relative to all other treatments, suggesting that pigment synthesis was N limited. In contrast, the increase of cellular chlorophyll was greater in NP than in N treatments in *Synechococcus*, suggestive of NP co-limitation. Relative increases in cellular nucleic acid were also only observed in *Synechococcus* for NP treatments, indicating co-limitation of net nucleic acid synthesis. A lack of response to relief of nutrient stress for the efficiency of photosystem II photochemistry, $F_v : F_m$, suggests that the low nutrient supply to this region resulted in a condition of balanced nutrient limited growth, rather than starvation. N thus appears to be the proximal (i.e. direct physiological) limiting nutrient in the oligotrophic sub-tropical North Atlantic. In addition, some major picophytoplankton groups, as well as overall autotrophic community biomass, appears to be co-limited by N and P.

Nutrient limitation can affect phytoplankton community structure and constrain the extent to which oceanic primary production influences global nutrient and carbon cycles. Establishing the limiting nutrient(s) under different oceanographic conditions, and the causes and nature of such limitation, is thus an important goal for understanding feedbacks between oceanic biota and the environment (Arrigo 2005).

The nature of phytoplankton nutrient stress has important consequences, both for understanding ecosystem functioning and for the interpretation of experimental and in situ data (Cullen et al. 1992; Arrigo 2005). In particular, a low nutrient supply can be hypothesized to

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limit the yield of phytoplankton biomass (cf. Liebig's Law of the Minimum) and/or phytoplankton growth rate (Blackman's type limitation; *see* Cullen 1991). Interpretation of bioassay experiments must take account of these and other possible confounding factors, including containment effects (Graziano et al. 1996). For example, a lack of biomass accumulation may not represent the absence of growth rate limitation in a system where source and sink terms are tightly coupled by efficient grazing and nutrient recycling (Goldman 1980; Graziano et al. 1996). Conversely, biomass increases in controls may reflect a reduction in grazing pressure (Cullen et al. 1992).

Geochemical arguments suggest that phosphorus (P) should limit oceanic production because nitrogen (N) can be supplied to the biota via N_2 fixation (Redfield 1958). In contrast, N typically limits phytoplankton growth in the oligotrophic sub-tropical and tropical oceans (Ryther and Dunstan 1971; Graziano et al. 1996; Mills et al. 2004). Tyrrell (1999) attempted to reconcile these two points of view by suggesting that P-limitation of N_2 fixation leads to the N-limitation of phytoplankton growth. The modeling study of Tyrrell (1999) concluded that P was the ultimate limiting nutrient on geochemical time scales (100–1,000+ years), whereas N was the proximal (i.e., directly) limiting nutrient on physiological (day) and ecological (month to year) time scales. Alternatively, Falkowski (1997) hypothesized that oceanic primary production was N-limited because low iron (Fe) availability limits N_2 fixation.

In contrast to N, recent work has emphasized the potential role of P as the proximal limiting nutrient on physiological time scales in the sub-tropical North Atlantic (Wu et al. 2000; Ammerman et al. 2003; Lomas et al. 2004). Biological evidence for P limitation in the western sub-tropical North Atlantic comes from observations of high activities of the alkaline phosphatase (AP). AP is an enzyme that increases P availability through hydrolysis of components in the dissolved organic P pool (Ammerman et al. 2003; Lomas et al. 2004). However, interpretation of AP activity is not straightforward, and high activities may not be indicative of overall community P limitation (Moore et al. 2005*b*). Similarly, the observed expression of the inorganic P binding protein (Scanlan and Wilson 1999) and the prevalence of genes implicated in P acquisition within natural prokaryotic populations (Martiny et al. 2006) both indicate a degree of P stress resulting from low ambient concentrations in this region, but may not necessarily represent proximal sole P limitation. Indeed, up-regulation of AP activity and inorganic P acquisition mechanisms presumably serves to reduce or neutralize the effect of low dissolved inorganic P concentrations on phytoplankton growth.

Other evidence for P limitation in the sub-tropical North Atlantic is based on geochemical arguments. Specifically, high N : P ratios exceeding the Redfield ratio of 16 : 1 in both dissolved and particulate organic pools are observed in the surface waters of the Sargasso Sea (Wu et al. 2000; Ammerman et al. 2003). Ratios of nitrate (NO_3^-) to soluble reactive P (SRP) within the thermocline in this region are also high, often greater than 30 : 1 (Fanning 1992). Inputs of nutrients from below the surface layer due to winter overturning (Michaels et al. 1994), background turbulent diffusivity (Lewis et al. 1986), or vertical transport driven by mesoscale eddies (McGillicuddy et al. 1998) thus have similarly high N : P ratios.

In a classical Redfield paradigm, where 16 mol N : 1 mol P is assumed to represent the transition from N to P limitation (Falkowski 1997; Tyrrell 1999), the high N : P ratios suggest P limitation (Ammerman et al. 2003). However, it is becoming increasingly clear that phytoplankton cellular N : P ratios and the critical ratio marking the transition between N and P limitation vary greatly between taxa and with growth conditions (Geider and La Roche 2002; Quigg et al. 2003). In particular it is possible that the optimum N : P ratio in a resource-limited environment is well in excess of 16 : 1 (Klausmeier et al. 2004). The implications of this variable biological N : P stoichiometry for global nutrient cycling remain to be fully elucidated (Arrigo 2005).

In contrast to the suggestion of proximal P limitation in the Sargasso Sea near Bermuda, experimental work in other regions of the low and mid-latitude North Atlantic has indicated proximal N limitation of CO_2 fixation and net chlorophyll synthesis (Graziano et al. 1996; Mills et al. 2004). Such direct experimental information from bioassays is not available from the regions of high inorganic N : P ratios within the Sargasso Sea. We thus performed nutrient addition bioassay experiments using trace element clean sampling to establish the nutrient(s) influencing phytoplankton physiology and limiting primary productivity on a transect across the mid-latitude North Atlantic. Biomass-independent phytoplankton physiological parameters were measured within these experiments in an attempt to circumvent weaknesses in interpretation associated with perturbation of grazing pressure (Cullen 1991; Cullen et al. 1992; Graziano et al. 1996). Such indices provide a measure of short-term physiological nutrient stress that must still be interpreted cautiously with relation to growth rate.

Here we demonstrate proximal N limitation of primary productivity and net chlorophyll accumulation consistent with results from previous bioassays elsewhere in the North Atlantic (Graziano et al. 1996; Mills et al. 2004). Data and arguments for overall N and P (hereafter NP) co-limitation of *Synechococcus*, which became the dominant component of the picophytoplankton upon relief from nutrient limitation, are also presented. It is suggested that a condition approximating NP co-limitation of some components of the autotrophic community may reconcile observations of proximate N limitation with alternative evidence for P limitation (Ammerman et al. 2003).

Methods

Eight nutrient addition bioassay experiments were performed in the central North Atlantic in March and April 2004 as part of the Meteor 60 Transient Tracers Revisited expedition (Moore et al. 2006*a*). Here we report detailed results from the five experiments initiated in waters between 21°N and 32°N where macronutrients were undetectable using standard colorimetric techniques (Fig. 1). Sampling dates and locations are presented in

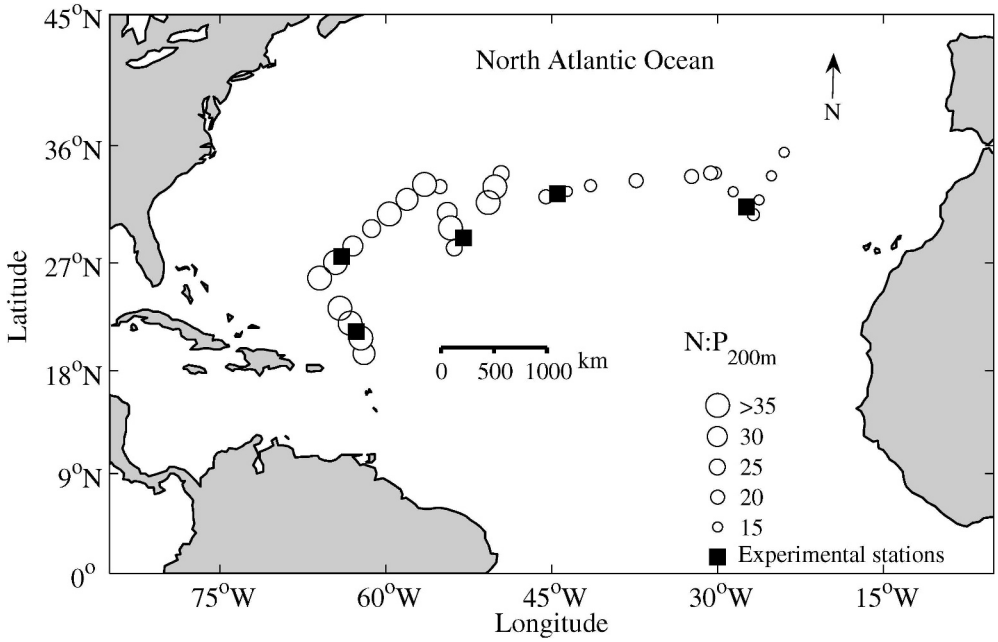

Fig. 1. Map showing the locations of nutrient enrichment bioassay experiments performed during the M60 cruise superimposed on the N : P ratio at 200 m. N : P ratios decrease from high values in the region of the Sargasso Sea to values approaching the deep-water ratio of ~14.7 : 1 in the east.

Table 1. Experiments were performed using a highly replicated fully factorial design to establish which nutrient(s) were limiting different components of the microbial community (Mills et al. 2004; Moore et al. 2006*a*).

Water sampling, bottle filling, and incubations were performed using methods described previously (Moore et al. 2006*a*). Briefly, water was collected from near surface and incubated in 1.18-liter bottles for 48 h after the addition of nutrients added alone and in combination to final concentrations of 1.0 μmol L^{-1} NH_4^+, 1.0 μmol L^{-1} NO_3^-, 0.2 μmol L^{-1} NaH_2PO_4, and 2.0 nmol L^{-1} $FeCl_3$. For the majority of treatments, 2 μmol L^{-1} N was added as 1.0 μmol L^{-1} NH_4NO_3, however, a separate treatment of 1.0 μmol L^{-1} KNO_3 added alone was also included. With the exception of the $FeCl_3$, all nutrient stocks were passed through a Chelex-100 column to remove trace metal contamination. Post-incubation measurements confirmed that the added inorganic nutrients had not been fully

Table 1. Initial conditions for bioassay experiments. Mean (±SE) of triplicate samples, except for DFe where the standard error of an individual sample analyzed four times is presented. $N:P_{200}$ indicates the N : P ratio within the thermocline at approximately 200 m below the sampling location.

	21°N 62°W	28°N 64°W	29°N 53°W	32°N 44°W	31°N 27°W
Sea surface temperature	25.4	22.7	21.4	19.6	19.7
Mixed layer depth (m)	133	67	68	129	122
NO_3((nmol L^{-1})	<30	<30	<30	<30	<30
$PO_4^{3(}$ (nmol L^{-1})	<8	<8	<8	<8	14 (3)
Total dissolved Fe (nmol L^{-1})	0.51 (0.05)	0.47 (0.05)	0.21 (0.02)	0.27 (0.01)	0.25 (0.01)
$N:P_{200}$ (mol mol^{-1})	36.2	32.3	22.6	18.6	17.6
Chlorophyll *a* (mg L^{-1})	0.067 (0.006)	0.043 (0.002)	0.045 (0.006)	0.081 (0.004)	0.09 (0.01)
Prochlorococcus (cells mL^{-1})	24,590 (5,000)	11,880 (860)	33,880 (1,220)	43,100 (2,490)	41,020 (750)
Synechococcus (cells mL^{-1})	3180 (650)	4360 (180)	8990 (580)	6840 (560)	2,970 (100)
Picoeukaryotes (cells mL^{-1})	431 (74)	325 (14)	681 (64)	392 (59)	466 (11)
Synechococcus cellular nucleic acid content (relative)	72.3 (3)	56.3 —	55.7 (0.4)	47.3 (0.2)	42.9 (0.5)

depleted. Further treatments involving the addition of Saharan aerosols and soils were also performed (Mills et al. 2004; Moore et al. 2006*a*), detailed results of which will be reported elsewhere. Trace element clean sampling and incubation techniques were strictly adhered to throughout the experimental procedure. All treatments were run in triplicate. Bottles were incubated for 48 h at 20% surface irradiance and cooled by surface seawater (Moore et al. 2006*a*). Parallel sets of triplicate incubations were run for primary production via ^{14}C incorporation and all other variables (Mills et al. 2004). Water for the initial rate measurements and state variables was collected throughout the filling of the incubation bottles.

Primary production—Phytoplankton inorganic carbon fixation was measured by the addition of 1.85 MBq ^{14}C-bicarbonate to one triplicate set of incubation bottles. Initial rates were measured on unamended bottles during the first 24 h of incubation. Carbon fixation rates in nutrient amended treatments and unamended controls were assessed during the second 24-h period. Incubations were terminated by gentle filtration onto Whatman GF/F filters. Filters were fumed with HCl for 30 minutes, then transferred to scintillation vials and 10 mL of scintillation cocktail added. Vials were left for >24 h in the dark, then counted on a Packard Tricarb liquid scintillation counter on-board ship. Total activity was assessed on 250-μL subsamples removed from initial and control bottles.

Chlorophyll a—Chlorophyll was measured in triplicate on initial water used for incubations and on sub-samples collected from all treatments at 48 h. Sub-samples of 500 mL were filtered onto GF/F filters, frozen in 1 mL of deionized water (Milli-Q, Millipore) for 3 h, then thawed with the addition of 9 mL acetone (Glover et al. 1986). After subsequent 24-h extraction in the dark, fluorescence was measured on a Turner designs 10-AU fluorometer (Welschmeyer 1994).

Analytical flow cytometry—The abundance and cellular composition of picophytoplankton was evaluated by analytical flow cytometry (AFC). Samples (2 mL) were collected and preserved in 1% gluteraldehyde, then frozen at −80°C for transport back to the laboratory (Zubkov et al. 1998). Samples were thawed at 4°C and analyzed for particle abundance, side scatter, and red (chlorophyll) and orange (phycoerytherin) auto-fluorescence using a FACSort (Becton Dickinson) flow cytometer. Data analysis was performed using WinMDI (version 2.8, Joseph Trotter). Plots of side scatter versus orange fluorescence and red fluorescence were used to discriminate and enumerate *Prochlorococcus, Synechococcus*, and picoeukaryotes. Relative changes in cellular phycoerytherin and chlorophyll for these groups were also calculated in this manner. Accurate enumeration of *Prochlorococcus* was often not possible within these oligotrophic surface waters (Zubkov et al. 1998) because of the low cellular chlorophyll as a result of acclimation to both high light and nutrient stress (*see* below). Samples were also analyzed after staining with the nucleic acid stain SYBR Green II (Molecular Probes), which binds to RNA and both single- and double-stranded DNA. Sub-samples (500 μL) were incubated for more than an hour at room temperature with 50 μL of 300 mmol L^{-1} potassium citrate and 5 μL of a 1% dilution of a commercial stock of SYBR Green II. Scatterplots of red and orange fluorescence versus side scatter and green fluorescence were used to estimate relative changes in cellular nucleic acid content (Marie et al. 1997). Particle counts and fluorescence were calibrated against a 0.5-μm bead standard (Fluoresbrite Microparticles, Polysciences).

Fast repetition rate fluorometry—Variable chlorophyll fluorescence was measured on dark adapted samples using a FASTtracka fast repetition rate fluorometer (Chelsea Scientific Instruments) using previously described protocols (Moore et al. 2006*b*). Non-linearities in instrument response were characterized using extracts of chlorophyll *a* (Chl *a*) and pigments from natural phytoplankton communities. Induction curves were averaged over 512 individual sequences to minimize error due to noise. Fluorescence transients were then fitted to a biophysical model (Kolber et al. 1998; Laney 2003; Moore et al. 2006*b*) to calculate the functional absorption cross section of photosystem II (σ_{PSII}) and the measured maximal and minimal chlorophyll fluorescence levels ($F_{m,meas}$ and $F_{o,meas}$).

Filtrates from each sample were analyzed to directly measure the background fluorescence F_b (Cullen and Davis 2003). The magnitude of background fluorescence was further estimated by regressing acetone-extracted chlorophyll from filtered samples against $F_{m,meas}$ and $F_{o,meas}$ (Fig. 2a). The calculated intercept values, indicating the background fluorescence at zero particulate chlorophyll, were highly comparable with the independent direct measurements of F_b (Fig. 2b). The background (F_b) varied little between sampling locations. For comparison, a reading of ~0.01 instrument units was obtained with Milli-Q water. Maximum (F_m) and minimum (F_o) fluorescence levels for the phytoplankton population were thus calculated as ($F_{m,meas} - F_b$) and ($F_{o,meas} - F_b$), respectively. The maximal photochemical quantum yield ($F_v : F_m$) was calculated as $(F_{m,meas} - F_{o,meas})/(F_{m,meas} - F_b)$. Failure to account for the background fluorescence, which was relatively large because of the low initial chlorophyll concentrations within the study region, would have resulted in considerable errors (40–90%) in estimates of $F_v : F_m$ (Cullen and Davis 2003).

Nutrients—Concentrations of NO_3^- and SRP were measured on-board using standard colorimetric techniques (Grasshoff et al. 1999). SRP concentrations were also measured using the MAGIC protocol (Karl and Tien 1992). For this purpose, samples (120 mL) were filtered through acid-washed 0.2-μm polycarbonate filters. then frozen and stored at −40°C. Additions of 1.0 mL of 1.0 mol L^{-1} NaOH to 100-mL subsamples induced coprecipitation of $Mg(OH)_2$ and SRP. The samples were then centrifuged (5 min at 1,000 × g) and the supernatant removed. The pellet was resuspended in 10-mL 0.1 mol L^{-1} HCl, and SRP was then determined using the standard molybdenum blue assay (Grasshoff et al 1999). Our 10-fold

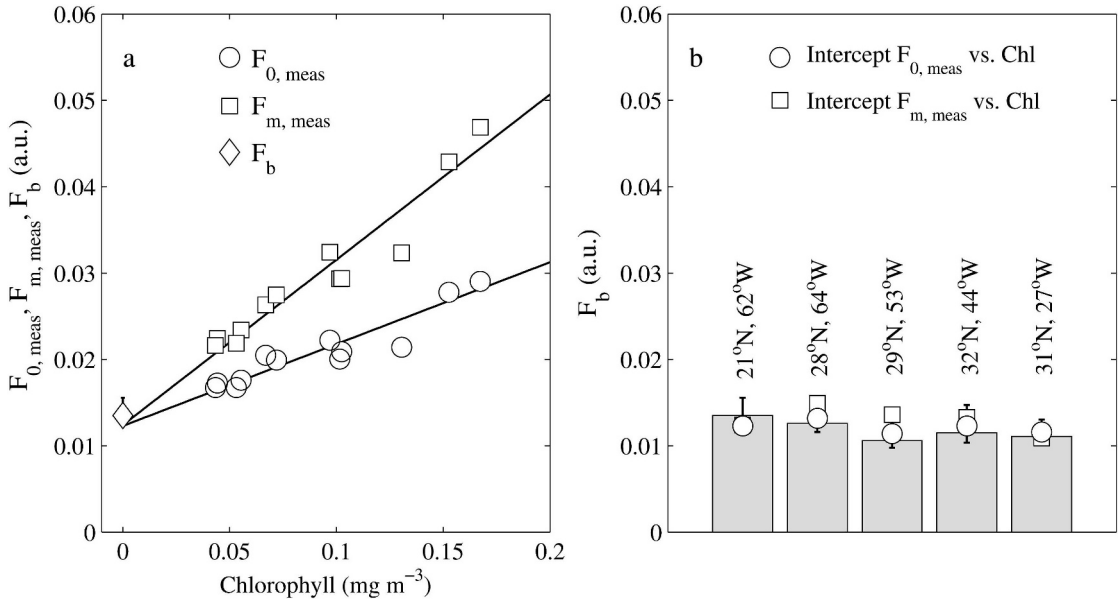

Fig. 2. Contribution of background fluorescence to measured maximal and minimal fluorescence levels. (a) $F_{o, meas}$ and $F_{m, meas}$ versus filtered chlorophyll from the experiment at 21°N, 62°W. Symbols indicate mean values ($n = 3$) from each of the treatments. Mean and standard deviation of all blank samples designated F_b ($n = 36$) also indicated. (b) Comparison of directly measured background fluorescence (bars) and estimates from regressions of $F_{m, meas}$ and $F_{o, meas}$ versus filtered chlorophyll. Error bars for measured backgrounds indicate standard deviations from all samples ($n = 36$).

concentration step resulted in a detection limit of ~8.0 nmol L^{-1}.

Total dissolved Fe (DFe) was analyzed using flow-injection chemiluminescence (Bowie et al. 1998). Samples were filtered using 25-mm-diameter Gelman syringe filters (0.2-μm pore size, polytetrafluoroethylene (PTFE) membrane). A 12-h acidification and 12-h reduction period were allowed before analysis of DFe.

Statistical analysis—Treatment means from bioassay experiments and differences between sampling locations were compared using a one-way analysis of variance (ANOVA) followed by a Tukey–Kramer means comparison test ($\alpha = 0.05$).

Results

Initial conditions—Initial nitrate concentrations were below the limit of detection (30 nmol L^{-1}) at all experimental sites (Table 1). SRP concentrations were lower than 8 nmol L^{-1} at all stations apart from the most easterly experiment (27°W, 31°N), where an initial concentration of 14 ± 3 nmol L^{-1} was measured. N : P ratios for dissolved nutrients at the thermocline ranged from >30 : 1 to <18 : 1 along a gradient from west to east (Table 1; Fig. 1). Iron concentrations were greater than 0.2 nmol L^{-1} at all locations, with highest levels (~0.5 nmol L^{-1}) observed in the Sargasso Sea to the west of the transect (Table 1). Sea surface temperature decreased from the southwest to the northeast, whereas mixed layer depths (0.5°C difference from surface) varied between 70 m and 130 m (Table 1). Initial chlorophyll concentrations (<0.1 mg Chl *a* m^{-3}) and carbon fixation rates (<0.4 mmol m^{-3} d^{-1}) were also low at all experimental sites, with chlorophyll specific production at the incubation irradiance (P*) decreasing from ~6 mol C (g Chl *a*) $^{-1}$ d^{-1} in the west to ~2–3 mol C (g Chl *a*) $^{-1}$ d^{-1} in the east. Phytoplankton abundances ranged from an estimated 1–4 × 10^4 cells mL^{-1} for the numerically dominant *Prochlorococcus* through ~3–9 × 10^3 cells mL^{-1} for *Synechococcus* to ~300–700 cells mL^{-1} for the picoeukaryotes. As mentioned above, concentrations of *Prochlorococcus* were likely to be underestimated because of a proportion of the population being indistinguishable from the baseline as a result of low cellular chlorophyll concentrations.

Analysis of climatological mixed layer depths (MLD) and ocean color data (Moore et al. 2006*a*), indicated seasonal cycles in MLD at all stations and significant seasonal cycles in surface chlorophyll at all stations excluding 21°N, 62°W (not shown). Maximum chlorophyll concentrations slightly lagged maximum MLDs in January–February. In the majority of our sampling region (Fig. 1), vertical mixing in late winter/early spring thus appears to penetrate the nutricline, resulting in the injection of nutrients into surface waters and a spring bloom. The seasonal cycle of phytoplankton pigment is thus similar to observations at the nearby Bermuda Atlantic Time-series study (BATS) (Michaels et al. 1994). Bloom magnitude increases to the north of the sampling region, presumably as a consequence of deeper mixing and, hence, enhanced nutrient input. As mentioned above, all the experiments reported here were initiated post-bloom when macronutrients were depleted and chlorophyll had decreased.

Response of the bulk phytoplankton community—The response of inorganic carbon fixation, phytoplankton

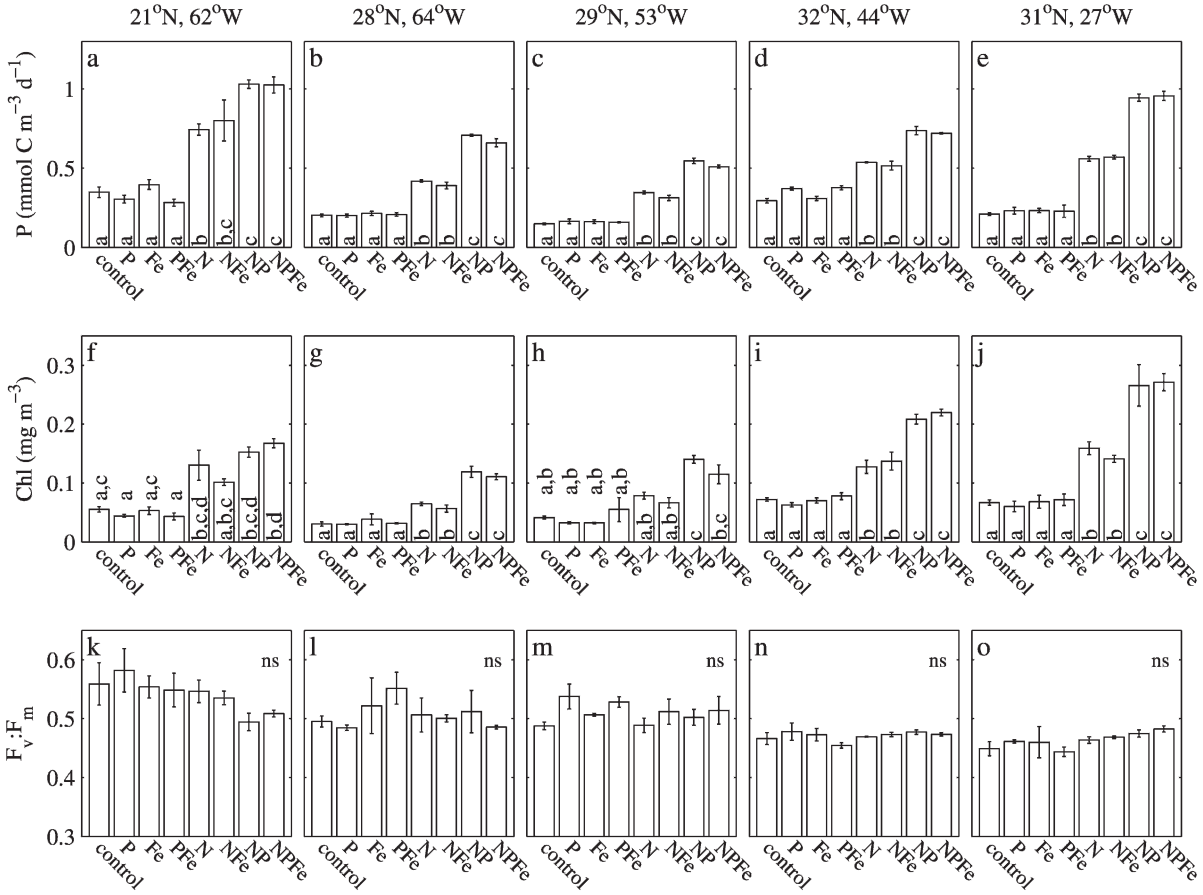

Fig. 3. Response of bulk phytoplankton community characteristics to nutrient addition. (a–e) Inorganic carbon fixation measured from 24–48 h after the addition of the indicated nutrients. (f–j) Chlorophyll concentration measured at 48 h. (k–o) Phytoplankton photochemical quantum efficiency ($F_v : F_m$) measured at 48 h. Error bars indicate standard errors. Letters indicate means that are statistically indistinguishable ($\alpha = 0.05$), with ns indicating no significant differences between treatments (ANOVA).

pigment (chlorophyll) accumulation and values of $F_v : F_m$ to the various nutrient additions were highly consistent in all the experiments (Fig. 3). No significant differences in productivity or Chl *a* were observed between control bottles and those amended with either Fe or P alone or in combination for any of the experiments (Fig. 3a–j). In contrast, addition of N alone resulted in significant (1.5–2-fold) increases in inorganic carbon fixation and Chl *a* across all experiments. Additions of P when in combination with N resulted in a further significant increase in both production and Chl *a* to levels around threefold higher than control treatments after 48 h (Fig. 3a–j). Adding Fe in combination with N or N and P did not significantly affect productivity or Chl *a*. Inorganic carbon fixation and chlorophyll concentration were highly correlated overall ($R^2 = 0.707$, $n = 50$, $p < 0.0001$).

Despite clear evidence for N limitation of phytoplankton productivity and chlorophyll concentrations, no significant differences in values of $F_v : F_m$ when corrected for blank effects were observed between treatments in any of the experiments (Fig. 3k–o). Similarly, the functional absorption cross section of photosystem II (σ_{PSII}) and both ratios of F_0 and F_m to chlorophyll ($F_0 : Chl$ and $F_m : Chl$), which have also been interpreted as being indicative of nutrient stress (Olaizola et al. 1996), were insensitive to the alleviation of N limitation. Taken across experiments, these parameters describing the efficiency of PSII light absorption and energy transfer were highly correlated, with higher $F_0 : Chl$ and σ_{PSII} associated with lower $F_v : F_m$ (Fig. 4). However, significant differences were only observed between experiments (ANOVA, $p < 0.05$) rather than within any single experiment (Figs. 3k–o, 4). For the current study, differences in PSII characteristics between natural communities therefore appeared to represent some characteristic of phytoplankton ecophysiology that was not directly related to physiological nutrient stress (Behrenfeld et al. 2006), with changes in community composition being one possibility (Moore et al. 2005*a*; Moore et al. 2006*b*).

Cellular and group specific responses—Cellular chlorophyll contents for *Prochlorococcus*, *Synechococcus*, and picoeukaryotes broadly reflected results based on bulk community chlorophyll concentrations and carbon fixation. No consistent changes in cellular chlorophyll were observed for any of the groups after the addition of P or Fe alone or in combination (Fig. 5). In contrast, cellular chlorophyll increases were consistently observed across all experiments for all three autotrophic groups after the

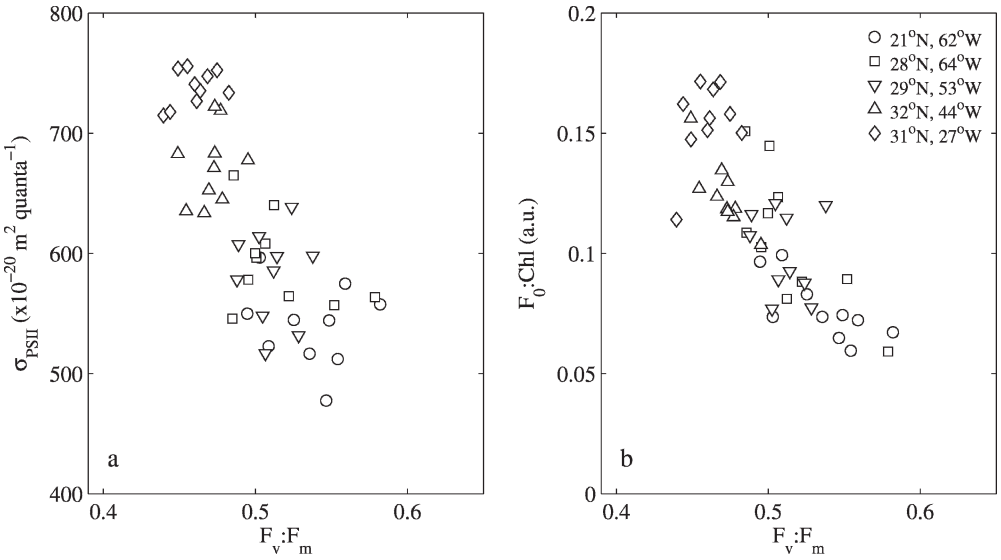

Fig. 4. Relationship between parameters describing photosystem II light absorption and photochemical efficiency taken from data collected across all treatments in all experiments. (a) Relationship of σ_{PSII} to $F_v:F_m$, $r^2 = 0.578$, $p < 0.0001$, $n = 50$. (b) Relationship of the ratio of minimal fluorescence to chlorophyll $F_0:Chl$ with $F_v:F_m$, $r^2 = 0.658$, $p < 0.0001$, $n = 50$. No significant differences ($\alpha = 0.05$) were found between initial conditions and post-incubation values regardless of the nutrient(s) added within any of the individual experiments. However, when considered across the whole data set, the ranges of parameters measured both pre- and post-incubation were statistically different between experiments, as were initial values (ANOVA, $\alpha = 0.05$).

addition of N alone or in combination with P (Fig. 5). However, differences in responses were observed between autotrophic groups.

Responses in NP treatments over that of N treatments were minor for *Prochlorococcus* with around 1.5–2-fold increases in cellular chlorophyll in both cases (Fig. 5a–e). Similarly picoeukaryote cellular chlorophyll increased by ~1.2–2-fold after the addition of N alone or in combination with P (Fig. 5k–n). The response of cellular chlorophyll was higher for *Synechococcus* with ~3–7-fold increases on the addition of N and a further significant increase to 5–15 times control values on the combined addition of N and P (Fig. 5f–j). Similarly, estimates of *Synechococcus* cellular phycoerytherin concentration in the N and NP treatments were 3–11 and 5–16 times values in the controls, respectively (Fig. 5f–j). The magnitudes of these changes were consistent with previous observations (Graziano et al. 1996).

A comparison of cellular chlorophyll concentrations between N (1 μmol L^{-1} NH_4NO_3) and NO_3^- (1 μmol L^{-1} KNO_3) treatments also indicated differences between groups. Responses of *Synechococcus* to NO_3^- additions were consistent with responses to NH_4NO_3, whereas *Prochlorococcus* cellular chlorophyll concentration showed no significant differences between controls and NO_3^- treatments (Fig. 5). Responses of picoeukaryotes to nutrient addition were generally less consistent between experiments, and differences between treatments were not as well defined as for prokaryotes (Fig. 5). This likely reflected poorer statistical confidence because of the lower number of events recorded by AFC for this numerically less abundant group and/or differences in response between taxa within the potentially more diverse assemblage represented by the picoeukaryotes. However, there was some indication that the picoeukaryotes also responded less to NO_3^- than NH_4NO_3 addition, particularly in the experiment performed at the eastern end of the transect (Fig. 5).

Low levels of chlorophyll (red) auto-fluorescence combined with leakage of SYBR Green II fluorescence into the red channel resulted in stained *Prochlorococcus* frequently being indistinguishable from heterotrophic bacteria, particularly under non-N amended conditions. However, higher levels of cellular chlorophyll and the presence of phycoerythrin enabled the separation of *Synechococcus* from heterotrophic bacteria (Marie et al. 1997). Changes in relative cellular nucleic acid content in response to the various nutrient treatments could thus be estimated. Significant increases in nucleic acid content for *Synechococcus* were typically observed within NP and NPFe treatments (Fig. 6). As SYBR Green II binds to all nucleic acids, the observed signals may have represented a combination of increased cellular DNA and/or RNA. Significant differences between experiments were also observed, with generally higher nucleic acid contents in the south and west than toward the east of the transect (Fig. 6).

Histograms of cellular nucleic acid content for *Synechococcus* only resolved single peaks for control incubations and treatments amended with N(and/or +Fe) or P(and/or Fe) alone (Fig. 6b). In contrast bimodal distributions of cellular nucleic acid content were observed in NP treatments, with peaks separated by a factor of approximately two. The combination of a relatively low number of recorded events and hence high coefficients of variation,

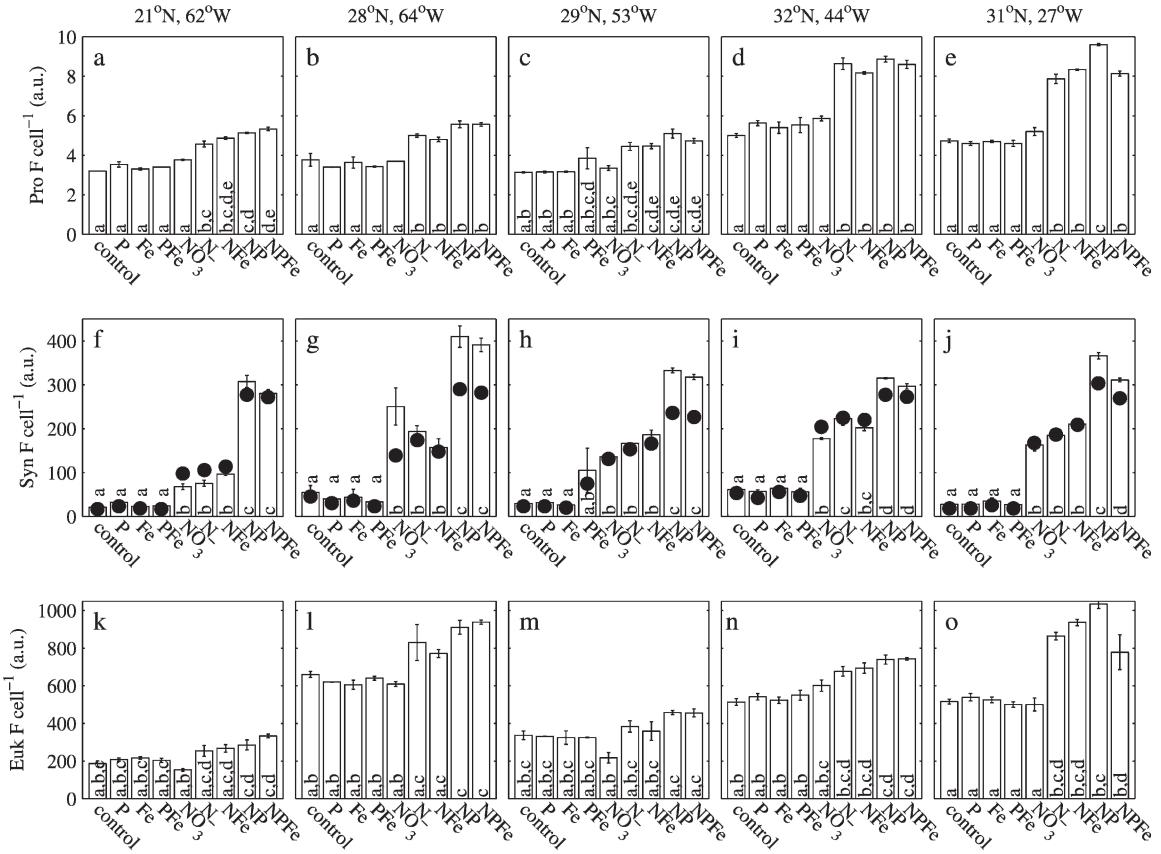

Fig. 5. Group-specific responses of fluorescence per cell as measured by AFC. (a–e) *Prochlorococcus* cellular chlorophyll fluorescence measured at 48 h after the addition of the indicated nutrients. (f–j) *Synechococcus* cellular chlorophyll fluorescence (bars) and phycoerythrin fluorescence (closed symbols). (k–o) Cellular chlorophyll fluorescence of picoeukaryotes. Error bars and letters in italics indicate standard errors and statistically indistinguishable mean values ($\alpha = 0.05$) for chlorophyll fluorescence. Statistical analysis of data for *Synechococcus* phycoerythrin fluorescence (f–j) yielded similar results (not shown). NH_4NO_3 and KNO_3 additions are labeled N and NO_3^-, respectively.

uncertainties regarding the relative contribution of RNA and DNA to the signals recorded on cells stained with SYBR Green II, and sampling only performed at a single point within the diel cycle prevented accurate cell cycle analysis (Vaulot et al. 1996; Marie et al. 1997). However, the proportion of cells in phase G_1 was estimated to have reduced from >90% under initial, control, and single additions of N(and/or Fe) and P(and/or Fe) to between 70% and 80% after NP(Fe) additions. Similar results were obtained for changes in *Synechococcus* cell cycles in the Mediterranean after the addition of P alone (Vaulot et al. 1996).

Few statistically significant changes in autotrophic cell numbers could be observed within our relatively short (48 h) experiments. As mentioned above, accurate enumeration of *Prochlorococcus* was frequently not possible under non–N-amended conditions. The addition of N, either alone or in combination with P and/or Fe, enabled more accurate counts because of the observed increase in cellular chlorophyll (Fig. 5a–e). An upper bound of at most 50% increases in *Prochlorococcus* cell abundance on N addition could thus be calculated. However, it is possible that all of this apparent increase resulted from the described artefact. No significant differences (ANOVA, $p > 0.05$) were observed between treatments for *Synechococcus* concentrations except for the experiment performed in the southwest of the transect at 21°N, 62°W. For this experiment *Synechococcus* concentrations increased around 2.7-fold from 3,170 (±650, 1 SE) cells mL^{-1} initially to 7,900 (±840) and 9,000 (±510) cells mL^{-1} for NP and NPFe treatments, respectively, compared to a range of ~4,500–5,500 cells mL^{-1} within controls and all other treatments (Fig. 7g). Some increases in picoeukaryote cell abundance were also observed within NP and NPFe treatments (Fig. 7h), however these were only significant for the 21°N, 62°W and 31°N, 27°W experiments.

Contributions of the different autotrophic groups to total community chlorophyll were calculated by multiplying cell concentrations by cellular red fluorescence (Table 2). The relative contributions of *Prochlorococcus*, *Synechococcus*, and picoeukaryotes within initial sampling water and controls indicated no tendency for dominance by any single group. In contrast, after N addition, *Synechococcus* dominated community chlorophyll (i.e., ~50% or greater) in all cases (Table 2).

Meta-analysis and comparisons of initial conditions with treatments—The gradients in initial ecosystem structure and physiology described above (Table 1) were still apparent at

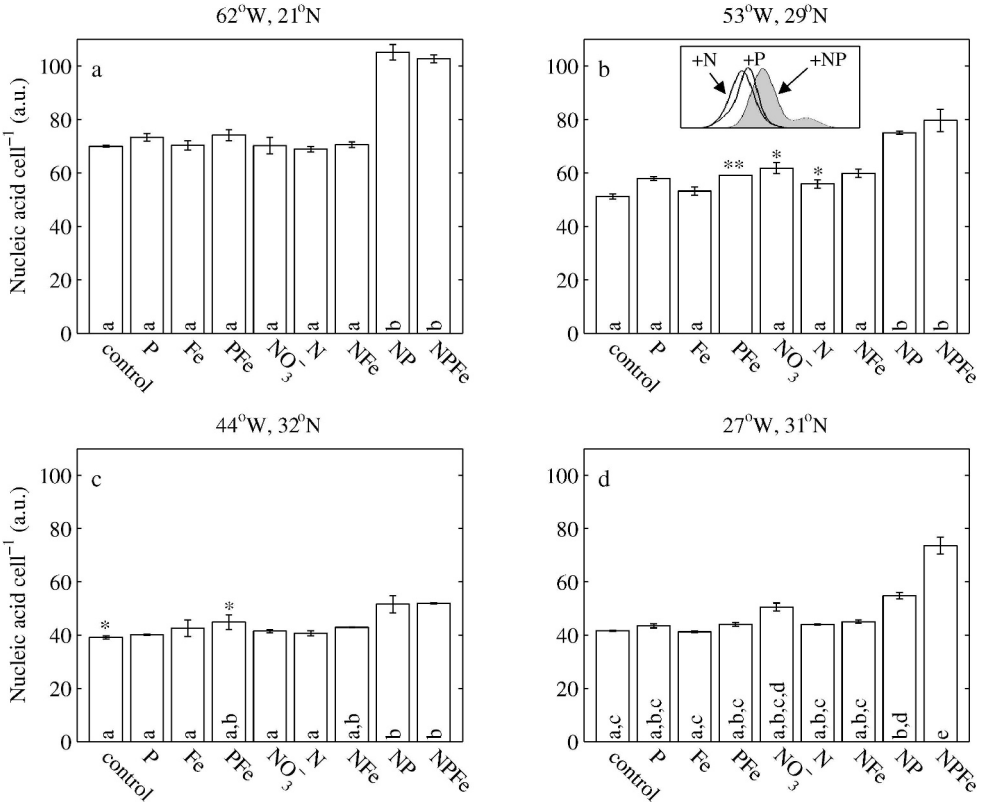

Fig. 6. Relative changes in *Synechococcus* cellular nucleic acid content. (a–d) Relative changes in nucleic acid content for four of the five experiments. Error bars and letters indicate standard errors and statistically indistinguishable mean values ($\alpha = 0.05$) respectively, $n = 3$ except for treatments indicated by * ($n = 2$) and ** ($n = 1$). (Inset in b), Smoothed histograms of *Synechococcus* cellular nucleic acid content from +N, +P, and +NP treatments as indicated.

the end of the 48-h experiments. Differences in initial chlorophyll, productivity, and community structure between sites were generally preserved within control treatments (Fig. 7; Table 2). Physiological variability including cellular chlorophyll and *Synechococcus* cellular nucleic acid content (Fig. 7), as well as PSII photophysiology (Fig. 4), was also consistent between initial conditions and control treatments. Overall, the majority of variables measured within controls were statistically indistinguishable from initial conditions. Taken together, the constancy of community structure and physiology during the short experimental duration suggests that containment effects, a potential confounding factor in the interpretation of bioassay experiments (Cullen et al. 1992), were minor. Consistent positive responses to N and NP addition in a range of variables including bulk community carbon fixation and chlorophyll (Fig. 7a,b), *Prochlorococcus* and *Synechococcus* cellular chlorophyll (Fig. 7d,e) and *Synechococcus* cellular nucleic acid (Fig. 7i) were thus observed throughout the sampling region (Fig. 1) despite variability in many of the initial values and, hence, presumably the ecosystem state.

Discussion

Limiting nutrients in the oligotrophic sub-tropical North Atlantic—No evidence for a significant effect of iron on autotrophic carbon fixation, pigment biomass, or phytoplankton physiology was found within the experiments presented here under conditions of very low macronutrients (Fig. 3). These observations contrast with the evidence obtained on the same cruise indicating periods of iron limitation during the spring bloom to the north discussed elsewhere (Moore et al. 2006*a*). For simplicity, and unless otherwise stated, in the remaining discussion the effects of macronutrient addition within our experiments are assumed to be indicative of the response both with and without further addition of Fe.

Clear evidence for macronutrient limitation was observed within every experiment. The addition of P alone resulted in no significant increases in community carbon fixation, bulk community chlorophyll concentration, or the cellular contents of chlorophyll (Figs. 3, 7). Consistent observations that the addition of N and P together resulted in larger increases in bulk chlorophyll concentration and primary productivity than N alone are open to two possible interpretations. Either these nutrients were co-limiting in situ or, during the course of our experiments, P (co-) limitation was induced (or forced) by the addition of N. The latter case implies the existence of an internal cellular P reserve or some residual bioavailable P pool in situ. Although we could not fully differentiate between all these possibilities, observations of the accumulation of cellular

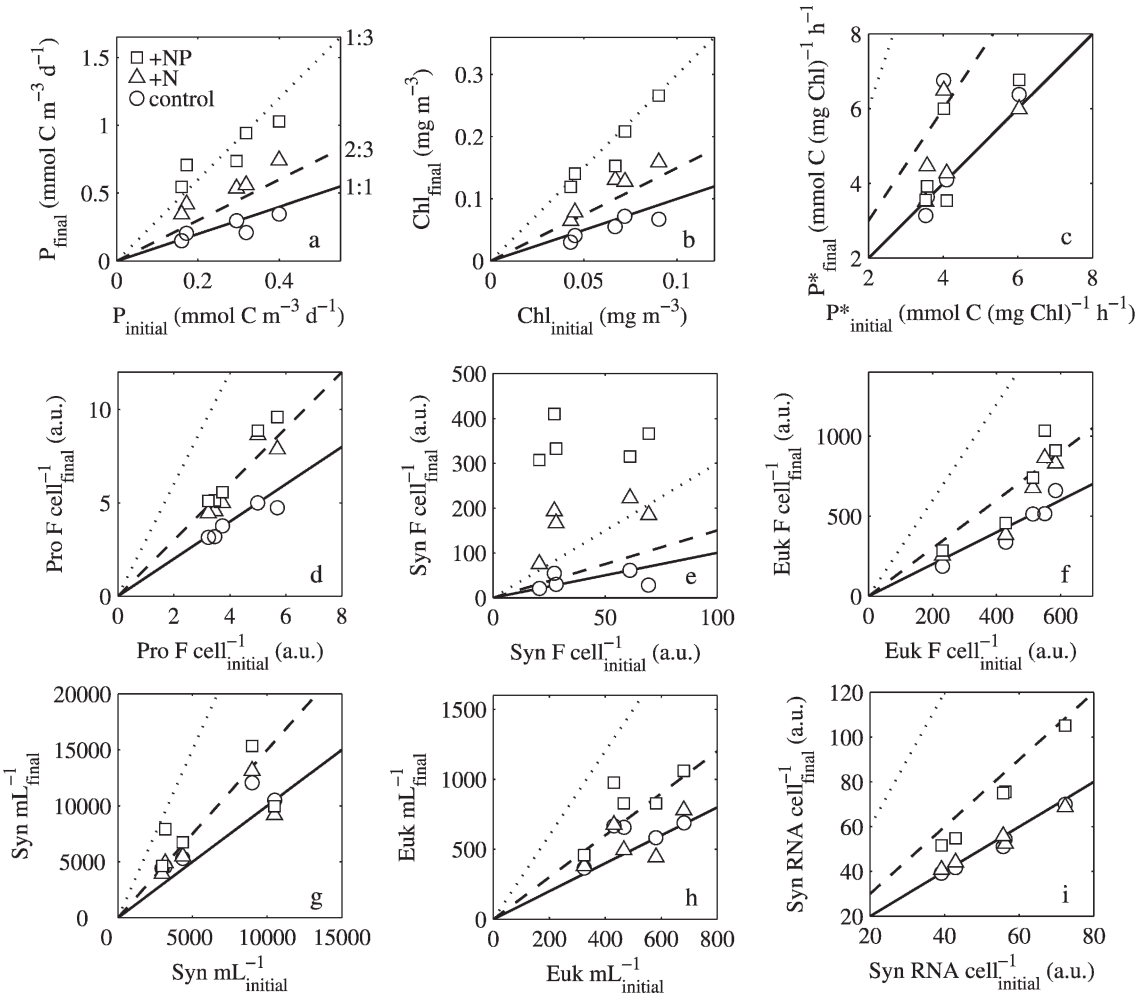

Fig. 7. Meta-analysis of experiments and comparisons of post-incubation values with initial conditions. (a) Plots of post-incubation (final) autotrophic community production measured in control, +N, and +NP treatments against initial values for all five experiments. (b) Final versus initial bulk chlorophyll concentration. (c) Final versus initial chlorophyll specific production. (d) Final versus initial *Prochlorococcus* cellular chlorophyll fluorescence. (e) Final versus initial *Synechococcus* cellular chlorophyll fluorescence. (f) Final versus initial cellular chlorophyll fluorescence for picoeukaryotes. (g) Final versus initial *Synechococcus* cell numbers. (h) Final versus initial picoeukaryote cell numbers. (i) final versus initial *Synechococcus* cellular nucleic acid content. Lines indicate ratios of post incubation values to initial conditions of 1 : 1, 2 : 3 and 1 : 3 for the indicated treatments. Symbols indicate treatment means ($n = 3$), error bars have been omitted for clarity (*see* Figs. 3, 5, and 6). All symbols and lines in (b–i) are as in (a).

pigments and nucleic acid in *Synechococcus* provided some insight.

Assuming that the observed proportionality between increases in community productivity and chlorophyll concentration (Fig. 3) were also representative of responses at the group level (Fig. 5), changes in pigmentation presumably reflected increased cellular carbon fixation. N addition alone increased the cellular contents of chlorophyll in *Prochlorococcus, Synechococcus*, and the picoeukaryote group and also increased phycoerytherin in *Synechococcus.* Such responses are consistent with the high N requirement and low P requirement for synthesis of these pigment–protein macromolecular complexes (Geider and La Roche 2002). Phycoerytherin in particular represents a significant pool of cellular N in *Synechococcus*, which has been observed to be degraded when N becomes limiting (Wyman et al. 1985). P addition with N resulted in greater increases of *Synechococcus* cellular chlorophyll and phycoerytherin content (Figs. 5, 7e). Again, this may suggest *Synechococcus* pigment synthesis was forced to P-limitation in the +N treatment. However, such limitation may not be direct because of the low P requirement for pigment–protein complexes (Geider and La Roche 2002).

For *Synechococcus,* significant increases in cellular nucleic acid content were generally only observed after the addition of N and P together (Figs. 6, 7i). Although we could not unequivocally ascribe these observations to increased DNA and/or RNA, the cellular content of both these macromolecules increases along with growth rate in *Synechococcus* (Lepp and Schmidt 1998). Further, the addition of N and P together potentially resulted in a lower proportion of *Synechococcus* cells being within G_1 at the time of sampling (Fig. 6b), suggestive of arrested cell cycles in non-NP treatments (Olson et al. 1986; Vaulot et al. 1996;

Table 2. Percent contribution of various groups to total picoautotrophic chlorophyll. Relative cellular red (chlorophyll) fluorescence multiplied by cellular abundances are expressed as percentages of the total. Values presented are mean ±SE. Groups are *Prochlorococcus* (Pro), *Synechococcus* (Syn) and picoeukaryotes (Euk).

			Treatment				
Experiment		Initial	Control	+P	+ NO_3^-	+N	+NP
21°N, 62°W	Pro	34±0.1	21±3	15±2	18±2	31±3	8±4
	Syn	26±2	34±8	47±7	67±4	47±6	83±5
	Euk	40±2	44±11	38±8	15±4	22±6	10±2
28°N, 64°W	Pro	13±1	4±2	4±1	2±1	6±0.4	2±0.2
	Syn	34±2	50±11	46±0.1	84±3	72±6	85±1
	Euk	54±2	46±13	50±1	14±2	22±6	13±1
29°N, 53°W	Pro	16±6	5±1	5±2	2±1	5±2	3±0.3
	Syn	39±5	57±6	56±5	90±7	83±4	89±1
	Euk	45±1	37±5	40±3	8±6	12±2	9±2
32°N, 44°W	Pro	26±6	23±2	25±2	15±1	17±2	10±0.4
	Syn	56±3	53±1	49±3	71±1	72±3	75±0.3
	Euk	18±4	25±1	25±2	14±2	11±1	15±1
31°N, 27°W	Pro	34±1	23±3	23±2	14±2	26±1	22±1
	Syn	30±2	20±2	20±2	53±3	47±1	52±2
	Euk	37±3	57±3	56±2	33±4	28±1	26±1

Liu et al. 1999). However, given the caveats mentioned above such a conclusion must clearly be treated with caution. Irrespectively, the cellular accumulation of at least some forms of nucleic acid was co-limited by both N and P (Fig. 6). These observations are consistent with the biochemical composition of nucleic acids, which contain significant amounts of N and P (Geider and La Roche 2002) and suggest that *Synechococcus* was co-limited by both N and P in situ.

Further, NP co-limitation of nucleic acid synthesis enables us to hypothesize indirect mechanisms for the lower cellular chlorophyll and phycoerytherin observed both in situ and after N addition alone. For example, if pigment synthesis in *Synechococcus* was transcriptionally controlled, then P-limitation may have been restricting mRNA synthesis. Alternatively, NP co-limitation of ribosomal RNA content may limit the maximum rate of protein synthesis.

In similar experiments performed in warmer (>27°C) tropical waters to the south, Davey et al. (pers. comm.) observed convincing evidence of NP co-limitation of net cell increases. For the current study the only experiment where significant changes in *Synechococcus* cell numbers were observed (21°N, 62°W) also suggested NP co-limitation of net growth. The lack of net increases in cell concentrations within our more northerly experiments (Fig. 1) may have resulted from reduced growth rates at lower temperatures (Table 1) and/or higher grazing pressure after the spring bloom. Irrespectively, in keeping with Davey et al. (pers. comm.), the data at 21°N, 62°W are again consistent with biochemical arguments as, in addition to N-rich protein and NP-rich nucleic acid synthesis, cell proliferation can require the synthesis of P-rich phospholipids (Geider and La Roche 2002), although *see* below and Van Mooy et al. (2006).

Proximal N-limitation was thus apparent in all the picophytoplankton groups measured (Fig. 5). For *Synechococcus* we further suggest that the data presented here support NP co-limitation of nucleic acid synthesis and, potentially, growth. Arrigo (2005) recently suggested a classification scheme for types of nutrient co-limitation ranging from two or more resources limiting different populations within the community to direct physiological simultaneous co-limitation. We suggest that in the central North Atlantic at the time of our study, *Synechococcus* was close to experiencing the latter type of "multi-nutrient (NP) co-limitation"(Arrigo 2005). That is, in situ concentrations of both (bio-available) N and P were depleted to levels too low for cellular uptake to maintain maximal growth rates.

Bulk community responses (Fig. 3) appeared dominated by *Synechococcus* (Fig. 5; Table 2), with the possible exception of the under-sampled larger eukaryotes (*see* below). Hence, despite proximal N limitation and no evidence for a secondary P effect on the cellular chlorophyll contents of *Prochlorococcus* and the picoeukaryotes (Fig. 5), it appears that any residual P pool in the oligotrophic sub-tropical North Atlantic during late spring is limited (Wu et al. 2000; Ammerman et al. 2003). Consequently, at the ecosystem and biogeochemical level we suggest that phytoplankton biomass (yield) was potentially restricted by a lack of both N and P (Fig. 3).

Insensitivity of $F_v : F_m$ to nutrient limitation in the oligotrophic North Atlantic—The lack of sensitivity of $F_v : F_m$ to N limitation within this study (Fig. 3k–o) is consistent with previous results from the oligotrophic North Atlantic (Graziano et al. 1996) and tropical Pacific (Behrenfeld et al. 2006). However, these results contrast with responses to alleviation of Fe stress within high-nutrient low-chlorophyll (HNLC) systems (Behrenfeld et al. 1996; Behrenfeld et al. 2006) and our observations further to the north within the spring bloom (Moore et al. 2006*a*). Behrenfeld et al. (2006) suggested that such contrasting responses of $F_v : F_m$ in different oceanographic

regions reflect fundamental differences in physiology following relief of limitation by N or Fe.

A precipitous decline in $F_v : F_m$ is typically observed in response to both N and Fe starvation in batch cultures of eukaryotes (Geider et al. 1993; Berges et al. 1996; Price 2005) and prokaryotes (Berges et al. 1996; Steglich et al. 2001). Conversely, $F_v : F_m$ is apparently less sensitive to both N (Parkhill et al. 2001) and Fe (Price 2005) limitation within steady-state cultures. Thus, the low sensitivity of $F_v : F_m$ to N limitation may indicate that in situ autotrophic populations were in a state of balanced nutrient-limited growth (Parkhill et al. 2001).

Re-supply of regenerated N and P through the microbial loop within the relatively quiescent surface waters of the macronutrient-limited oligotrophic subtropics might enable the development of balanced growth. In contrast, episodic supply of new Fe due to mixing events or atmospheric deposition may result in a greater potential for unbalanced growth within Fe-limited HNLC regions. Consequently, in addition to direct physiological arguments (Behrenfeld et al. 2006), physical and ecological processes may also contribute to observed differences in indices of nutrient stress between contrasting oceanographic regimes. Other physiological variables including σ_{PSII}, the ratio of fluorescence per unit chlorophyll, and the chlorophyll specific rate of carbon fixation, albeit at a single irradiance level, were also relatively insensitive to relief of proximal N limitation for the experimental duration (Figs. 4, 7c).

As an alternative to balanced N(P) (co-)limited growth, it could be argued that the insensitivity of $F_v : F_m$ and other variables might reflect an absence of growth rate (Blackman type) nutrient limitation. Maintenance of phytoplankton at near-maximal growth rates by efficient nutrient regeneration despite limitation of the yield of phytoplankton biomass by low nutrient supply could then be hypothesized (Goldman 1980; Cullen 1991; Cullen et al. 1992). For example, in northerly experiments, evidence for tight coupling of heterotrophic grazing to autotrophic production was provided by the lack of significant increases in autotrophic cell numbers, despite clear increases in net community carbon fixation (Figs. 3, 7). However, as discussed above, increases in cellular pigment and nucleic acid contents above in situ and control incubations on addition of N(+P) strongly suggested a degree of physiological nutrient stress.

Implications of group specific responses for community structure and function—Phytoplankton community structure can respond to changes in nutrient availability at a number of taxonomic levels. *Prochlorococcus* tends to dominate in the most oligotrophic waters with a shift to *Synechococcus*, picoeukaryotes, and larger eukaryotes as nutrient concentrations increase (Zubkov et al. 1998; Durand et al. 2001). Finer-scale genetic variation can be superimposed on these broad group-specific trends. For example, differing *Prochlorococcus* ecotypes were observed to dominate over a range of oligotrophic conditions (Johnson et al. 2006). In contrast to the majority of *Synechococcus* isolates, cultured *Prochlorococcus* strains are incapable of utilizing NO_3^- (Moore et al. 2002). The bioassay data presented here indicated that the *Prochlorococcus* ecotypes present in the studied surface waters were similarly incapable of using NO_3^- (Fig. 5).

Synechococcus demonstrated the strongest response to N addition, either as NH_4NO_3 or NO_3^- (Fig. 5). *Synechococcus* thus dominated community chlorophyll concentration after 48 h, particularly within NP or NO_3^- treatments (Table 2). Physical inputs of N from below the euphotic zone (Lewis et al. 1986; Michaels et al. 1994; McGillicuddy et al. 1998) will principally be in the form of NO_3^-. Our results are thus consistent with *Synechococcus* dominating the picoautotrophic response to such NO_3^- input (Glover et al. 1988; Durand et al. 2001). *Synechococcus* is therefore likely to contribute more to new production (Dugdale and Goering 1967) within the spring bloom than *Prochlorococcus* (Durand et al. 2001; Moore et al. 2002).

In addition to *Prochlorococcus*, picoeukaryotes also responded less in NO_3^- than in NH_4NO_3 treatments (Fig. 5). Most of the assemblage represented by the picoeukaryotes might be expected to be capable of accessing NO_3^-. Hence the lack of response may simply represent a slower induction of NO_3^- uptake or reduction compared to the rapidly responding *Synechococcus* population. The larger eukaryotes that dominate autotrophic carbon during the spring bloom in this region (Durand et al. 2001) were not evaluated during the current study. Further work investigating the response of these organisms is clearly merited.

Larger phytoplankton are expected to be at a disadvantage in nutrient-limited systems because of their lower surface area-to-volume ratio (Chisholm 1992). Consequently, the smallest organisms e.g., *Prochlorococcus*, might be expected to dominate the community. Indeed *Prochlorococcus* appears highly adapted for growth in a nutrient-impoverished environment, having a minimal genome that presumably helps to minimize both cellular N and P (Dufresne et al. 2003). Additionally *Prochlorococcus* minimizes cellular P demand further via the use of sulfolipids instead of phospholipids (Van Mooy et al. 2006). However, depending on the efficiency of recycling, it is still possible that even this likely best competitor for limiting nutrients may be growth rate limited, as the increases in *Prochlorococcus* chlorophyll fluorescence upon the addition of N alone suggest (Fig. 5); *see* also Graziano et al. (1996).

Irrespectively, larger autotrophs, including both the marginally bigger prokaryote *Synechococcus* and eukaryotes, might be expected to be physiologically limited, consistent with the data presented here (Figs. 5, 6). Additionally, when compared to *Prochlorococcus*, *Synechococcus* appears to have a reduced capacity to substitute phospholipids with sulfolipids (Van Mooy et al. 2006). In contrast, these larger organisms may be better equipped to respond rapidly under conditions where nutrients become available (Table 2), again consistent with observation (Glover et al. 1988; Zubkov et al. 1998; Durand et al. 2001). Observed differences in the degree of N or NP (co-) limitation between groups (Fig. 5) may thus have been influenced by genotypic variability in cellular N and P requirements. Differential responses of *Prochlorococcus*, *Synechococcus,* and picoeukaryotes (Fig. 5) likely contrib-

uted to the initial bulk community responses to N addition in the absence of P (Fig. 3).

Biogeochemical implications—High N : P ratios within the thermocline in the sub-tropical North Atlantic (Fig. 1) are assumed to result from high rates of N_2 fixation followed by subsequent remineralization of N-rich organic material at depth (Michaels et al. 1994; Wu et al. 2000; Hansell et al. 2004). These high rates of N_2 fixation have in turn been related to high atmospheric Fe inputs and subsequent relief of potential Fe(P) (co-)limitation of diazotrophy (Falkowski 1997; Wu et al. 2000; Mills et al. 2004). The low SRP concentrations in this region are thus suggested to be a consequence of relatively high production by diazotrophs (Wu et al. 2000). As previously pointed out (Wu et al. 2000), assuming phytoplankton N : P requirements are 16 : 1, the upward fluxes of nutrients in this region should provide N in excess of P. Such excess N should in turn result in P limitation and suppress N_2 fixation (Tyrrell 1999; Mills et al. 2004). If one accepts that the Redfield ratio of 16 : 1 is the switchover point between N and P limitation, high N_2 fixation accompanied by high dissolved and particulate N : P presents a paradox (Wu et al. 2000).

Additionally, despite the high N : P input ratios (Table 1) associated with winter convection, by the time of our study in late spring photosynthesis and chlorophyll synthesis were proximally N limited, although we cannot exclude NP co-limitation (Figs. 3, 5, 6, 7). Preferential remineralization of P over N was invoked to explain such observations (Ryther and Dunstan 1971; Wu et al. 2000). However, we suggest that plasticity within phytoplankton cellular N : P ratios (Geider and La Roche 2002) may also play a role. Comparing a theoretical model with experimental data (Klausmeier et al. 2004) suggested that higher cellular N : P ratios ($>30 : 1$) would be a selective advantage in oligotrophic conditions. Increased requirements for N-rich resource acquisition proteins, including nutrient transporters, combined with lower growth rates decreasing the requirement for relatively P-rich ribosomes, can thus be hypothesized to result in acclimation and/or adaptation toward high cellular N : P ratios (Geider and La Roche 2002; Klausmeier et al. 2004). Cellular elemental stoichiometries for the organisms dominating oligotrophic environments (Bertilsson et al. 2003) and further adaptations to minimize P requirements (Van Mooy et al. 2006) further support this suggestion.

It is thus possible that multi-nutrient (NP) co-limitation develops in the region of the current study as a consequence of biochemical co-limitation (sensu Arrigo, 2005). Specifically, during drawdown after nutrient inputs with high N : P ratios, as P approaches limiting concentrations phytoplankton may need to devote proportionally more N to the synthesis of nutrient acquisition proteins to access the increasingly scarce P resource, driving the system toward co-limitation. Such a mechanism could be hypothesized to operate at either a cellular or ecological (taxonomic) level and potentially represents an important characteristic of oligotrophic waters, governing the balance between N and P limitation.

In summary we conclude that N is the proximal limiting nutrient for chlorophyll synthesis and carbon fixation in the oligotrophic sub-tropical North Atlantic. However, our results also suggest that some phytoplankton groups and overall autotrophic community yield are limited by the availability of both N and P. Further comprehension of the causes and consequences of such NP co-limitation is likely to have important implications for our understanding of oceanic ecology and biogeochemistry.

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