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Nitrogen and phosphorus co-limitation of bacterial productivity and growth in the oligotrophic subtropical North Atlantic

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Abstract

Bacterial productivity and biomass are thought to be limited by dissolved organic carbon (DOC) in much of the world's oceans. However, the mixed layer of oligotrophic oceans is often depleted in dissolved inorganic nitrogen and phosphate, raising the possibility that macronutrients may also limit heterotrophic bacterial growth. We used nutrient bioassay experiments to determine whether inorganic nutrients (N, P, Fe) and/or DOC could limit bacterial productivity and biomass in the central North Atlantic during the spring of 2004 (Mar–Apr). We observed that both heterotrophic bacterial productivity and biomass were co-limited by N and P in the oligotrophic North Atlantic, and additions of labile DOC (glucose) provided no stimulation unless N and P were also added. Flow cytometry results indicated that only a small subset of large cells high in nucleic acid content were responsible for the increased productivity in the combined NP amendments. In contrast, nutrient additions elicited no net change on the dominant component of the bacterial population, composed of small cells with relatively low nucleic acid content. In the combined NP treatments the relative increase in bacterial production was greater than that measured when phytoplankton productivity was relieved of nitrogen limitation. These results suggest that N and P co-limitation in the bacterial community results in increased competition between the heterotrophic and autotrophic components of the surface communities in the Central North Atlantic Ocean, and potentially impacts the cycling of organic matter by the bacterioplankton.

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In oligotrophic oceanic surface waters, the cycling of organic matter by the bacterial community and the subsequent nutrient uptake and production by autotrophs are tightly coupled. The bacterioplankton consume labile organic carbon released by the phytoplankton (Duarte and Cebrian 1996), which in turn depend on the inorganic nutrients mineralized through consumption of the dissolved organic matter by the bacteria (Kirchman 2004). In this way, the bacteria and the phytoplankton do not compete but are dependent on each other for production.

The assumption in this scenario is that the organic matter produced by phytoplankton is rich enough in nitrogen (N) and phosphorus (P) to sustain the bacterial requirement. Bacteria typically have lower carbon : nitrogen (C : N) and carbon : phosphorus (C : P) requirements than phytoplankton (Vadstein and Olsen 1989). Therefore, either the C : N : P stoichiometry of bacterial substrates must be relatively low in order to meet bacterial N and P demands or the bacteria must utilize supplementary N and P sources to meet these demands. In coastal systems that receive enhanced nutrient loads phytoplankton release dissolved organic matter (DOM) with a low C : N (La Roche et al. 1997). However, in oligotrophic environments where inorganic nutrients are scarce (Wu et al. 2000), phytoplankton may release nutrient-poor DOM (Obernosterer and Herndl 1995). Hence, carbon substrates for the acquisition of energy might be plentiful while available nitrogen and phosphorus for building proteins and nucleic acids may be scarce. The heterotrophic bacteria may, therefore, become limited by nitrogen and phosphorus, putting them in direct competition with phytoplankton for the same nutrients.

Nitrogen is thought to be the primary limiting nutrient for phytoplankton productivity in the oligotrophic North Atlantic Ocean (Graziano et al. 1996; Moore et al. 2006). However other studies have used geochemical evidence and enzyme activity assays to suggest that P availability plays a role in controlling autotrophic productivity and community structure in the North Atlantic Ocean (Ammerman et al. 2003; Lomas et al. 2004). In oligotrophic environments where ambient nutrient concentrations are extremely low, the paradigm of a single nutrient limiting phytoplankton may not always be valid, and the autotrophic community can be co-limited by multiple nutrients (Arrigo 2005). For example, (Mills et al. 2004) showed that the diazotrophic community of the Eastern Tropical North Atlantic was co-limited by phosphorus and iron, while Davey et al. (unpubl. data) and Moore et al. (2008) found that cell division of the picophytoplankton community in the same location required addition of both N and P. Similarly, the phytoplankton community was shown to be NP co-limited in the Eastern Mediterranean during the large-scale phosphorus enrichment experiment CYCLOPS (Thingstad et al. 2005).

Several investigations have indicated that heterotrophic bacterial populations can also experience N and/or P limitation in oligotrophic systems (Cotner et al. 1997; Rivkin and Anderson 1997; Caron et al. 2000), although limitation by carbon is thought to be more common (Cherrier et al. 1996; Church et al. 2000; Carlson et al. 2002). If heterotrophic bacterial limitation by macronutrients is an emerging pattern for the oligotrophic ocean gyres, what are the implications for the biogeochemistry of the oceans?

The micronutrient iron (Fe) is also important to both prokaryotic and eukaryotic microbial communities in the oceans. Its role in limiting phytoplankton communities in high-nutrient low-chlorophyll (HNLC) regions of the oceans is definite (Boyd et al. 2007), while in the North Atlantic Moore et al. (2006) recently showed that Fe can regulate primary productivity in deep mixed layers during the spring bloom. There is relatively little evidence, however, for proximal Fe limitation in oceanic prokaryotes, except for the expected role that iron availability may play in controlling nitrogen fixation in diazotrophic cyanobacteria (Mills et al. 2004). On the basis of regular inputs of desert dust rich in Fe over the North Atlantic Ocean it has been argued that Fe concentrations in this region should exceed the requirements of diazotrophs (Kustka et al. 2003). However, even in the oceanic region receiving the highest dust deposition, recent evidence of PFe co-limitation of diazotrophic activity (Mills et al. 2004) indicates that our understanding of diazotroph Fe demands and/or the bioavailability of dissolved Fe in the oceans is incomplete.

With few exceptions, the bulk heterotrophic bacterial community does not generally appear to suffer from Fe limitation, perhaps due to the ability of this group to produce siderophores which bind inorganic Fe(III) making it more available for uptake (Rue and Bruland 1995). A direct stimulation of bacterial activity upon Fe additions has been recorded (Pakulski et al. 1996) in the Southern Ocean, and an indirect secondary stimulation of the bacterial community has been observed when the primary DOC limitation was alleviated in the same environment Church et al. (2000).

In order to address issues of microbial nutrient limitation we focused our investigations on heterotrophic bacterial communities in the oligotrophic North Atlantic where autotrophic production has previously been directly shown to be strongly N limited (Graziano et al. 1996; Mills et al. 2004; Moore et al. 2006). We examined the changes in bacterial productivity and biomass in response to additions of inorganic nutrients (N, P, Fe), alone and in combination with a labile DOC (glucose). Our results indicate that there is significant overlap in the inorganic nutrients that limit autotrophic and heterotrophic biomass in this system and consequently potential competition for the sparse resources between these groups. As such we hypothesize that macronutrient limitation of the heterotrophic bacterial community results in increased resource competition between the autotrophic and heterotrophic portions of the microbial community and that the tightly coupled production and recycling of organic matter in this environment may be affected.

Material and methods

Study site and sample collection—We conducted our experiments aboard the Transient Tracers Revisited cruise (Meteor 60/5) between 09 Mar and 13 Apr 2004 in the tropical and central North Atlantic Ocean (Fig. 1). Eight experiments were conducted during the cruise; five of which

were in oligotrophic waters ($NO_3^- < 0.03$ μmol L^{-1}, chlorophyll *a* (Chl *a*) < 0.1 μg L^{-1}; Table 1) while the remaining three were performed in waters associated with winter mixing that contained relatively high nutrient and Chl *a* concentrations and were published elsewhere (Moore et al. 2006). The present paper discusses only those experiments in the oligotrophic environments.

Surface seawater was collected between 22 : 00 and 02 : 00 using trace-metal clean techniques. Nocturnal mixing ensured that the water samples represented a mixed water column. Water was pumped while underway (ship speed $<$ 18 km h^{-1}, $\sim$ 3 m depth) from a towed fish using a polytetrafluoroethylene (PTFE) diaphragm pump (A15, Almatec) into a 60-liter carboy housed in a trace-metal clean container (class 1000) where all manipulations of the water samples took place. Water was siphoned from the carboy into randomly selected 1.2-liter acid-washed polycarbonate bottles. All initial conditions were determined from three samples randomly collected during the filling period. Under a laminar flow hood (class 100), nutrients (N, P, Fe) were randomly added alone and in all possible combinations to final concentrations of 1.0 μmol L^{-1} NH_4^+ + 1.0 μmol L^{-1} NO_3^- (combined nitrogen treatment), 0.2 μmol L^{-1} NaH_2PO_4, and 2.0 nmol L^{-1} $FeCl_3$ (Fig. 2) to triplicate bottles. The nutrient concentrations were chosen so that they were comparable to concentrations measured after deep mixing in the North Atlantic and to obtain a measurable response within the relatively short 48-h experimental period. All nutrient stocks, with the exception of the $FeCl_3$, were pretreated with Chelex to remove trace metal contamination. A separate NO_3^- treatment (1.0 μmol L^{-1}) was also carried out. The bottles were capped, sealed with a strip of Parafilm, and placed into plastic ziplok bags in on-deck incubators with circulating surface seawater. In three of the experiments (53°W, 44°W, 27°W) DOC (glucose) was added to duplicate bottles at $t = 24$ h to a final concentration of 10 μmol glucose L^{-1}, either alone or in combination with the other nutrients. The glucose addition was similar to previously conducted experiments by Carlson and Ducklow (1996) and Carlson et al. (2002). The controls in the nonDOC experiments did not receive any nutrient additions while controls for the DOC treatments received only glucose. The bottles were incubated for 48 h before the final samples were collected. We used the shortest experimental duration possible that allowed for a detectable response to nutrient additions while also minimizing bottle effects. Light was attenuated to 20% of incident surface irradiance with blue filters (Lee Filters No. 172, Lagoon Blue). Two other sets of triplicate bottles were treated exactly the same as described above but were analyzed for $^{14}CO_2$ fixation and $^{15}N_2$ fixation. The results from the $^{14}CO_2$ fixation bottles were reported in (Moore et al. 2006), while the N_2 fixation results will be reported elsewhere. In a companion paper Moore et al. (2008) describe the phytoplankton community response in these same experiments.

Subsamples were taken from the N_2 fixation set of triplicate bottles at the end of the 48-h incubation period for inorganic nutrient analysis. Nitrate and soluble reactive phosphorus (SRP) were analyzed using a nutrient autoanalyzer with a

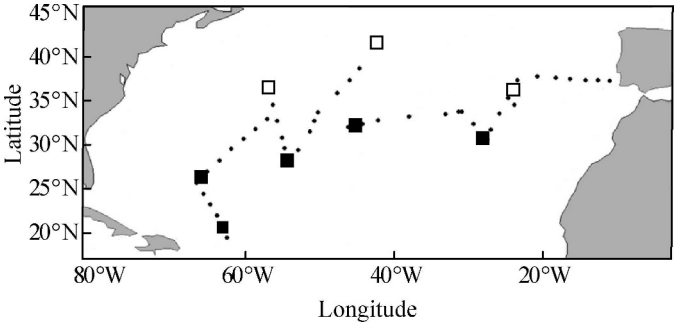

Fig. 1. Map showing the Meteor 60 cruise track. Experiments were conducted at the squares on the map and dots are stations along the cruise track. Experimental sites denoted by filled squares were oligotrophic ($NO_3^- < 0.03$ μmol L^{-1}, Chl $a <$ 0.1 μg L^{-1}) and are included in this manuscript. Experimental sites denoted by open squares were spring bloom sites ($NO_3^- >$ 0.03 μmol L^{-1}, Chl $a > 0.1$ μg L^{-1}) and are not included here.

detection limit of 0.03 μmol L^{-1} (Grasshoff et al. 1999); SRP was also determined in those treatments without added PO_4^{-3} using the MAGIC protocol described in Karl and Tien (1992) with a detection limit of 8.0 nmol L^{-1}. Ammonium was measured using the method of Holmes et al. (1999) with a detection limit of 15 nmol L^{-1}. Total dissolved Fe (DFe) was analyzed using flow-injection chemiluminescence (Bowie et al. 1998) with a detection limit of 40 pmol L^{-1}. Samples for DFe were filtered using 25-mm-diameter Gelman™ syringe filters (0.2 μm pore size, PTFE membrane). A 12-h acidification and 12-h reduction period was allowed prior to analysis of DFe, in order to ensure all DFe was converted to Fe(II).

Bacterial abundance and production—Samples for bacterial abundance measurements were collected by pipetting 1.9-mL subsamples into cryotubes containing 0.1 mL of glutaraldehyde (20%). The samples were immediately frozen at −80°C and stored frozen until flow cytometric analysis. Prior to enumeration the samples were thawed at room temperature, and analyzed on a Becton Dickinson FACSort™ flow cytometer after staining with the nucleic acid stain SYBR Green II. The heterotrophic bacterial community was distinguished from the two dominant cyanobacterial groups *Synechococcus* sp. and *Prochlorococcus* sp. based on side scatter (cell size), red (chlorophyll) and orange (phycoerytherin) fluorescence, and relative nucleic acid content (Simon et al. 1994, Marie et al. 1997). Heterotrophic bacterial abundance and estimates of their relative nucleic acid content and size were evaluated from plots of cellular side scatter versus green fluorescence using WinMDI (version 2.8, Joseph Trotter). The heterotrophic bacteria were further divided into two subgroups, small cells with low nucleic acid content, SLNA, and large cells with high nucleic acid content, LHNA (Fig. 3). Particle counts and fluorescence were calibrated against a 0.5-μm bead standard (Fluoresbrite Microparticles, Polysciences).

Bacterial productivity was measured according to Lochte et al. (1997) by incubating 10-mL of water (taken from a 100-mL subsample) with 0.185 MBq of ^{3}H-Thymidine, equivalent to an addition of 8 nmol thymidine

Table 1. Initial conditions and limiting nutrients for bioassay experiments. Mean (±SD) of triplicate samples, except for DFe where the standard error of an individual sample analyzed four times is presented.

Latitude (°N)	21°N	28°N	29°N	32°N	31°N
Longitude (°W)	62°W	64°W	53°W	44°W	27°W
Bacterial productivity	0.27	0.31	0.35	0.69	0.78
(pmol thymidine L^{-1} h^{-1})	−0.42	−0.18	−0.17	−0.17	−0.08
Bacterial abundance $\times 10^8$ L^{-1}	2.63	2.25*	3.38	2	3.99
	−0.38		−0.22	−0.06	−0.13
Chlorophyll a (μg L^{-1})	0.067	0.043	0.045	0.081	0.091
	−0.006	−0.002	−0.006	−0.004	−0.01
NH_4^+ (μmol L^{-1})	N/A	N/A	0.135	<0.025	<0.025
NO_3^- (μmol L^{-1}) †	<0.03	<0.03	<0.03	<0.03	<0.03
PO_4^{3-} (μmol L^{-1}) †	<0.010	<0.010	<0.010	<0.010	0.014
					−0.005
DFe (nmol L^{-1})	0.51	0.47	0.21	0.27	0.25
	−0.05	−0.01	−0.02	−0.03	−0.01
Limiting nutrient‡	NP	NP	NP	NP	NP

* Only one replicate was available for analysis.
† Sample concentrations below the detection limits of 0.03 μmol L^{-1} and 0.08 μmol L^{-1} for NO_3^- and PO_4^{3-}, respectively.
‡ Nutrient treatment resulting in significant increase of bacterial productivity (and abundance of the LHNA group).

L^{-1} (specific activity of 0.002 MBq $pmol^{-1}$) for approximately 6 h in the dark. At the end of the incubation, formalin (100 μL 37%) was added to stop bacterial activity. For each treatment, a poisoned control was measured to correct for nonspecific labeling. Samples were filtered onto polycarbonate filters (25-mm diameter, 0.2-μm pore size) under low vacuum (<0.2 bar) and rinsed well. Filters were placed in 10-mL scintillation vials and 4-mL scintillation cocktail was added (Lumagel Plus; Lumac SC, Groningen, The Netherlands). The samples were left for 24 h in the dark before counting on a scintillation counter. Bacterial productivity is presented here as the thymidine uptake rate. In order to compare bacterial productivity to phytoplankton productivity ($^{14}CO_2$ fixation from Moore et al. 2006) we applied a thymidine conversion factor of 1.18×10^{18} cells produced per mol thymidine incorporated (Ducklow 1983). An average cell volume of 0.04 μm^3 (Carlson and Ducklow 1996) and a carbon conversion factor of 2.5×10^{-7} μg C μm^{-3} (Fry 1988) were used to determine equivalent carbon units.

Results

Bacterial productivity and abundance—Bacterial productivity in the initial (ambient water) samples at the oligotrophic stations was highest at the two eastern-most experimental sites, decreasing by approximately 50% to the west and south (Table 1). These observations coincided with similar trends in Chl *a* concentrations. Total bacterial cell abundance was constant across the oligotrophic Central North Atlantic Ocean averaging $2.94 \times 10^8 \pm 0.35 \times 10^8$ cells L^{-1}.

Bottle effects were minimal as shown by the comparison of the initial measurements and controls measured after 48 h (Fig. 4). Across all experiments, bacterial productivity in the controls did not significantly differ from the initials (Fig. 4A, analysis of variance [ANOVA] with Tukey HSD post hoc test, $p > 0.05$). Likewise, total cell abundance of the heterotrophic bacterial community in the controls did not differ significantly from the initials (Fig. 4B; ANOVA with Tukey HSD post hoc test, $p > 0.05$). Small, though significant, differences were noted when examining bacterial groups separately. The SLNA group differed significantly in abundance between the control and initial in the experiment at 44°W, with the control having approximately 1.3-fold higher cell abundances (Fig. 4C). The abundance of the LHNA group was approximately 1.5-fold higher in the control at the 53°W site as well as the control and +DOC alone treatment at the 27°W site (Fig. 4D). These were small differences when compared to the treatment responses shown below.

At all sites, bacterial productivity was co-limited by N and P (Fig. 5A–E). Productivity was 11–35-fold greater in the +NP treatments than in the control. The combined addition of +NPFe did not significantly stimulate productivity above that of the +NP treatment. In contrast to productivity, total community abundance of heterotrophic bacteria did not significantly increase relative to the control in the +NP treatment (Fig. 5F–J).

The addition of glucose alone resulted in no significant increase in bacterial productivity, total community abundance, or group-specific abundance (Fig. 4A–D). Bacterial productivity increased 50–100-fold and bacterial abundance increased 2–3-fold compared with the control when glucose was added along with N and P. No further stimulation in productivity or abundance was recorded when labile DOC was added with NPFe, with the exception of a small increase in abundance at the 32°N station (Fig. 5I).

Bacterial productivity was also measured in the dark at two stations, with and without the combined addition of N and P, to assess if autotrophic activity resulted in enhanced bacterial production rates (Fig. 6). Unamended dark productivity was not significantly different than that measured in the control ($p > 0.05$). However, the +NP amended dark productivity was 18–60-fold higher than the control rates (natural irradiance), and equal to, or greater than in the +NP treatments with natural irradiance. At the 32°N site, the productivity in the dark +NP treatment

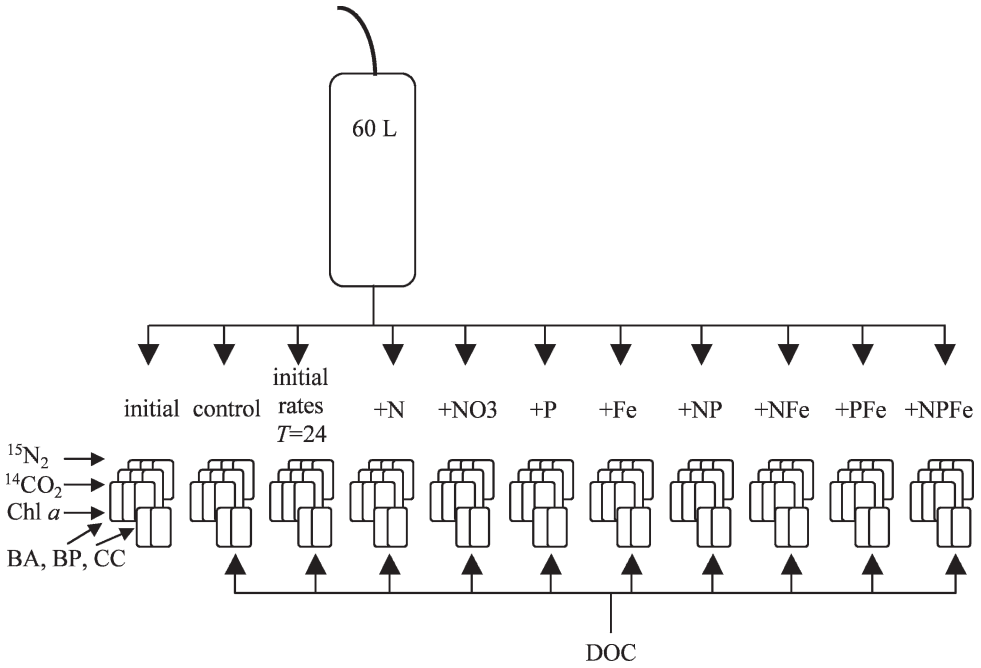

Fig. 2. Experimental design for the nutrient limitation bioassays onboard the Meteor 60 cruise. Surface seawater was collected trace-metal clean, dispensed into 1.0-liter polycarbonate bottles and nutrients (N, P, Fe) and dissolved organic carbon (DOC) were added alone and in combination. Triplicate incubations for each nutrient treatment were performed. Three full sets of triplicate bottles were carried out; one for measurements of $^{15}N_2$ fixation rates, one for $^{14}CO_2$ fixation rates, and the third for measurements of bacterial production rates (BP), bacterial abundance (BA), Chl *a* concentrations, and community composition (CC) measurements. See text for full description of design and methods.

responded approximately three times more then the +NP treatment in the light, suggesting competition between autotrophs and heterotrophs for the nutrients N and P.

At all sites the initial abundance of the LHNA group was an order of magnitude lower ($\times 10^4$) than the SLNA group ($\times 10^5$). The treatment responses in the LHNA group mirrored that of the net heterotrophic bacterial productivity changes, increasing above the control only when N and P were added together (Fig. 5K–O). No further significant increase was measured when Fe was added together with N and P. Net growth rates for the LHNA group in the +NP and +NPFe treatments averaged 0.46 d^{-1}. The SLNA heterotrophic bacterial group did not increase in biomass above the control under any nutrient amendments. In fact, the abundance in the SLNA group at times decreased below the control abundances (Fig. 5R,S,T).

The addition of labile DOC did not increase the abundance of the SLNA group. As in the −DOC treatments, the LHNA group only increased its abundance when glucose was added in combination with N and P (Fig. 5M–O). The combined NP + DOC treatment resulted in a 10–38-fold increase in cellular abundance in this group. A significant further increase in the number of LHNA cells was recorded when all nutrients (NPFe) and labile DOC were added at the 32°N site. Net growth rates of the LHNA in the NP + DOC and NPFe + DOC treatments were 2.75 and 2.97 d^{-1} respectively.

Relative changes in the heterotrophic bacterial community—The LHNA heterotrophic bacterial group comprised <20% of the initial bacterial community. This remained relatively constant in all nutrient treatments, except for the combined N and P treatments where they comprised up to 40% (Fig. 7). In the NP + DOC treatments, the LHNA group comprised 50–70% of the community.

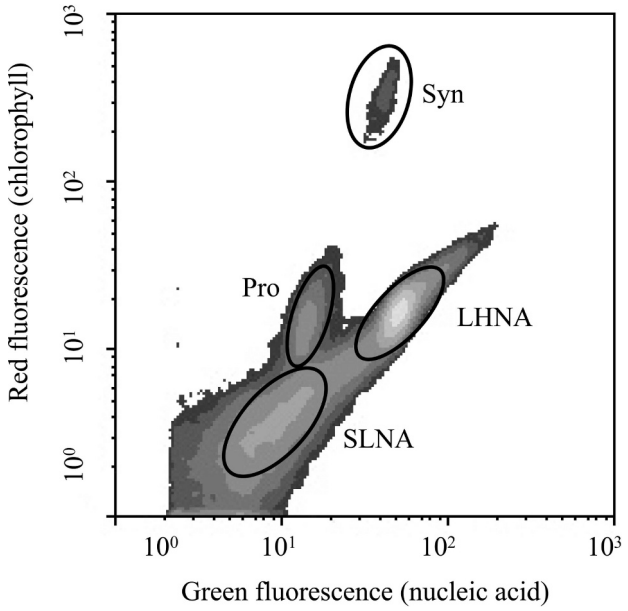

Fig. 3. Typical plot from flow cytometry analysis showing separation of the two heterotrophic bacterial groups (SLNA and LHNA), as well as the two dominant cyanobacterial groups *Prochlorococcus* and *Synechococcus*.

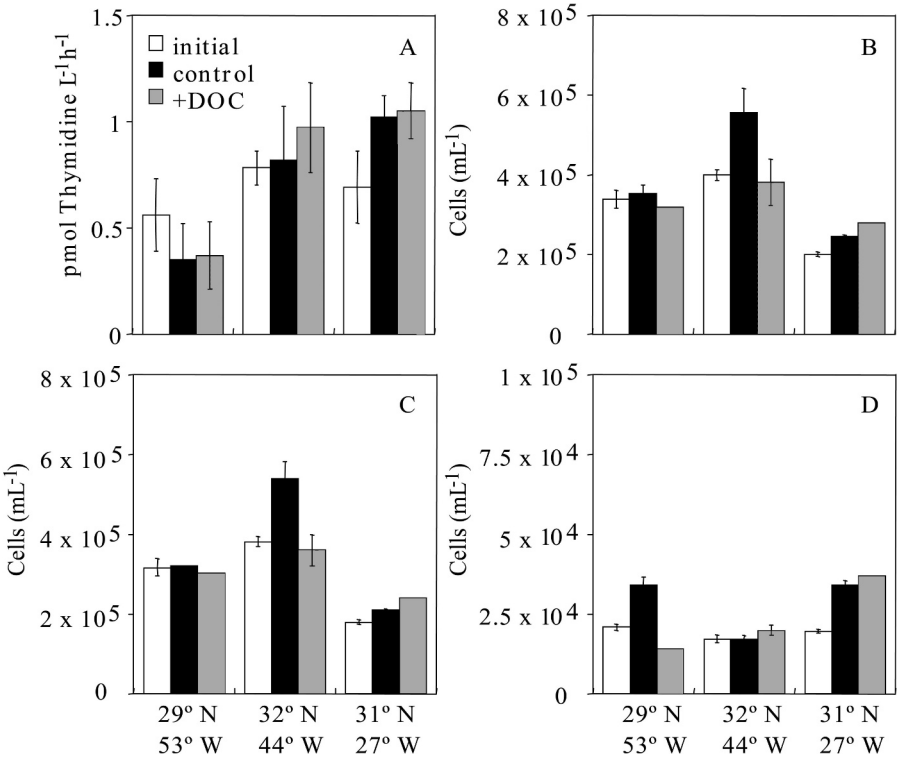

Fig. 4. Results from three experiments comparing (A) thymidine incorporation rate, (B) total heterotrophic cell abundance, (C) SLNA cell abundance, and (D) LHNA cell abundance in the initial, control, and DOC treatments. Error bars represent ±1 standard error.

Despite the low percent contribution of the LHNA group in the ambient oceanic water, it accounted for between 26–57% of the heterotrophic bacterial nucleic acid pool (Fig. 7). After 48 h, the LHNA nucleic acid pool increased by approximately 35% in the controls at the 28°N and 29°N sites, respectively, but no increase was measured at the other sites. Upon the combined addition of N and P, the LHNA nucleic acid pool ranged from 59% to 85%, a 1.5–2-fold increase. In the DOC treatments, little change was recorded in the percent composition of the nucleic acid pool relative to the −DOC treatments, with the exception of those treatments containing both N and P. In these treatments the LHNA nucleic acid pool increased to between 89–95% of the total heterotrophic bacterial nucleic acid pool.

Bacterial productivity relative to primary productivity—We estimated carbon-based bacterial production to determine the relative proportion of bacterial productivity to primary productivity. The initial rates averaged 5% ± 2% (avg. ±SE) of $^{14}CO_2$ net carbon fixation rates (Fig. 8A). This average remained statistically unchanged unless both N and P were added together. Net bacterial productivity rates then increased to 29–40% of the net CO_2 fixation rates. Assuming autotrophic productivity was not stimulated by the addition of DOC, than net bacterial productivity rates would have increased to >100% of the net CO_2 fixation rates in the NP + DOC treatments. Autotrophic fixation of CO_2 was not measured in the DOC treatments, but in vivo chlorophyll *a* fluorescence measurements were lower in the NP + DOC treatments relative to the NP treatments alone at the 31° and 32°N sites (Fig. 8B) suggesting lower Chl *a* concentrations resulted from increased competition for inorganic N and P between autotrophs and heterotrophs.

Discussion

The results of the bioassay experiments clearly demonstrated that net heterotrophic bacterial productivity and biomass in the central North Atlantic were nitrogen and phosphorus co-limited during Mar and Apr 2004. There was a greater than 10-fold increase in bacterial productivity and biomass when N and P were added together, as well as a substantial change in bacterial community composition. A further 3–10-fold increase in productivity and biomass occurred when DOC was added together with N and P, but not in any other treatment. This enhancement suggests that once NP co-limitation was relieved, net heterotrophic bacterial production became carbon (energy) limited.

The addition of Fe, alone or with N and P did not stimulate bacterial productivity or biomass. The concentrations of dissolved Fe observed during our study are consistent with the measurements of Wu and Boyle (2002), who reported surface water values for the Sargasso Sea decreasing from 0.8 nmol L^{-1} (26°N) to 0.2 nmol L^{-1} at 32°N. Additionally, dFe concentrations made near the BATS site during spring 2004, the same time and area as our study, ranged between 0.09 and 0.26 nmol L^{-1} (Sedwick et al. 2005), and are in good agreement with the values we present. If the measured initial thymidine uptake

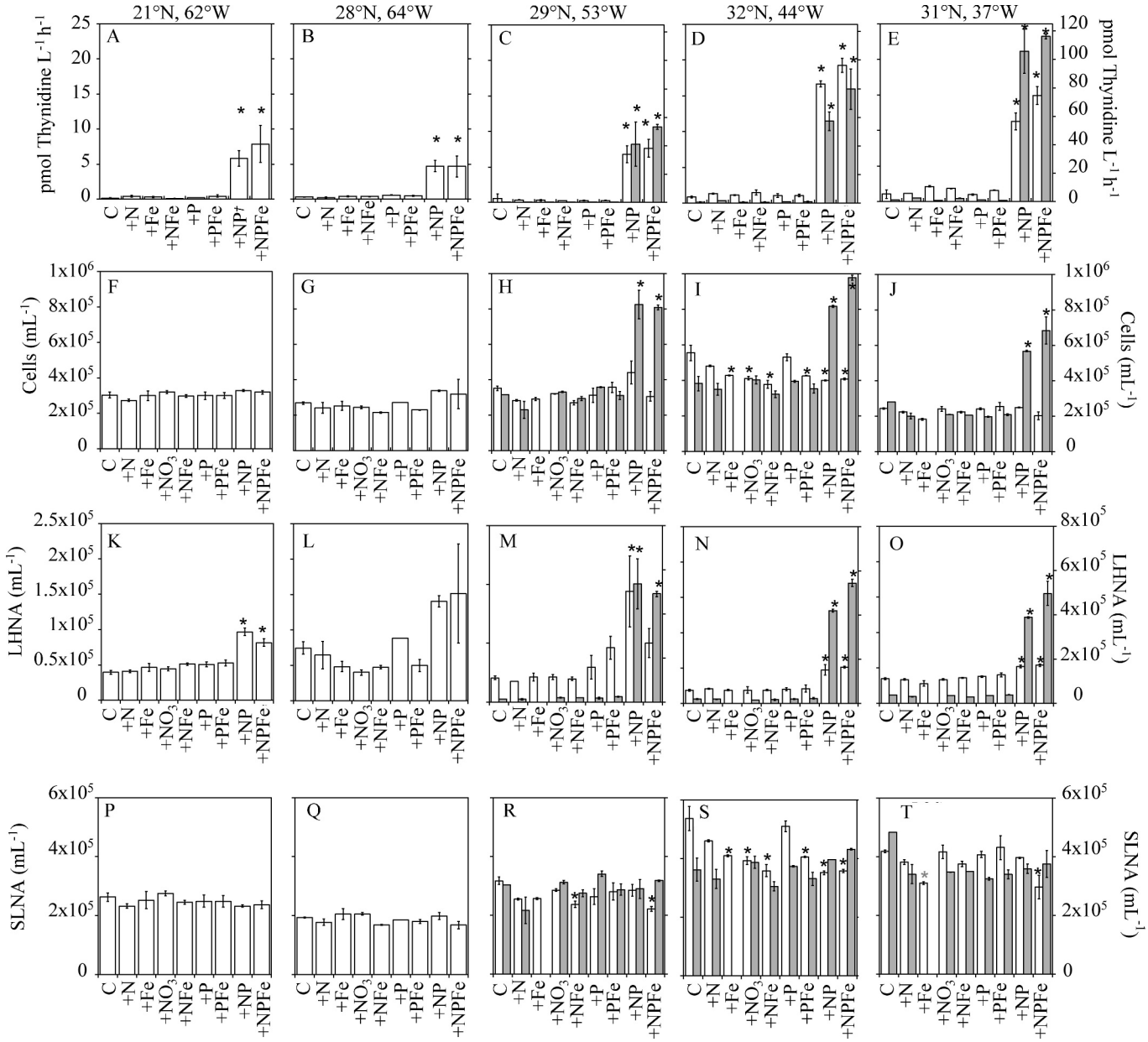

Fig. 5. Comparison of (A–E) thymidine incorporation rate, (F–J) total heterotrophic cell abundance, (K–O) LHNA cell abundance, and (P–T) SLNA cell abundance responses in the different nutrient treatments. Thymidine incorporation rates were measured between 24–48 h; all cell abundances were sampled at 48 h. Note DOC responses are shaded bars and where present correspond to the second *y*-axis. All white bars are the –DOC treatments and correspond to the primary *y*-axis. Error bars represent ±1 standard error. Asterisks (*) indicate significant difference from the control indicated ($\alpha = 0.05$).

rates are converted to equivalent carbon productivity rates (as described above) and multiplied by a Fe : C of 7.5–9.1 (pmol : μmol; Tortell et al. 1996) we calculate the Fe required to meet the initial bacterial productivity rates ranges from 0.030 to 0.130 nmol L^{-1} d^{-1}. The lowest ambient dFe concentrations measured here were approximately 0.25 nmol L^{-1}, almost 2-fold greater than needed to support heterotrophic bacterial productivity. Thus, the experimental results and simple calculation of bacterial Fe requirements suggest that Fe availability at this time of year was sufficient to meet the demand of the heterotrophic bacterial community.

The fact that both the autotrophic (Moore et al. 2008) and heterotrophic microbial communities in the present investigation were co-limited by N and P suggests there was competition for macronutrients between these two groups. Consistent with this, we recorded a noticeable decrease in the in vivo chlorophyll fluorescence between the +NP treatment and the NP + DOC treatment, presumably due to the heterotrophic bacterial community out-competing the phytoplankton community for N and P under these conditions (Fig. 8B). Our data demonstrates that the relative increase in the net heterotrophic bacterial production, when NP co-limitation was relieved, was greater than the relative

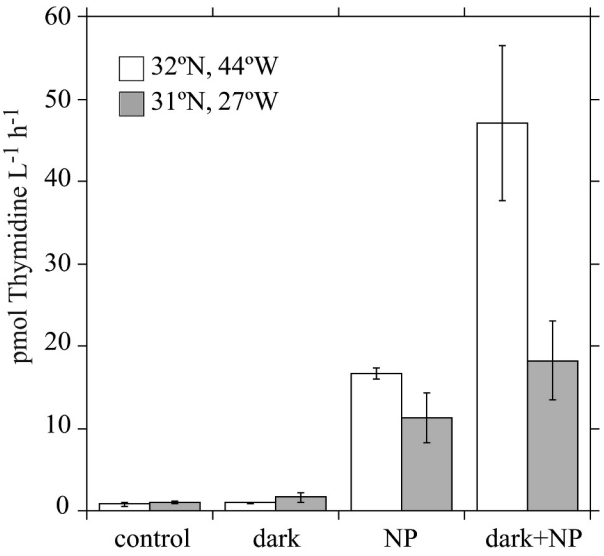

Fig. 6. Thymidine incorporation rates in the control, dark (no nutrient amendment), +NP, and dark + NP treatments at the 31°N and 32°N sites. Error bars represent ±1 standard error.

increase in phytoplankton productivity. In the present study, net heterotrophic production by bacteria as a percentage of net phytoplankton production (BP : PP) was estimated to be 5% ± 2%, similar to estimates from the BATS station in the Sargasso Sea (Steinberg et al. 2001). When only N was added, BP : PP decreased slightly as only phytoplankton productivity was stimulated. The ratio increased dramatically to 30–40% when N and P were added together. The high BP : PP under simultaneous N and P amendments are in line with the global estimate of 31% made by (Cole et al. 1998), although our bacterial production rates are net and, thus, likely underestimates. If our bacterial productivity rates are converted to bacterial carbon demand (BCD) using a bacterial growth efficiency of 14% (Carlson and Ducklow 1996), then BCD is up to four-fold higher than net primary productivity in the NP treatments.

Our results contrast with nutrient addition experiments in the Mediterranean Sea where *Synechococcus* sp. had higher maximum uptake rates and a higher affinity for inorganic phosphate than the heterotrophic bacterial community (Moutin et al. 2002). Similar to the Moutin et al. (2002) investigation, *Synechococcus* sp. responded most strongly to NP amendments in the present experiments (Moore et al. in press). However, the greater net increase in heterotrophic bacterial production relative to autotrophic production suggests that the heterotrophs were the superior competitors. One reason for this observation may have been that our experiments were conducted relatively soon after the high spring productivity period. DOC lost from the phytoplankton during the spring bloom, through exudation, grazing, and/or viral lysis, may have sustained the post–spring-bloom heterotrophic bacterial community, which was subsequently driven to NP co-limitation. These experiments support the idea that the low concentration of inorganic nutrients and proportionately higher concentration of organic matter in oligotrophic systems favors prokaryotic heterotrophs (Cotner and Biddanda 2002).

In addition, recent evidence has shown that while heterotrophic bacteria have high P demands due to membrane phospholipids, some autotrophs (e.g., *Prochlorococcus*) have high concentrations of sulpholipid membranes (Van Mooy et al. 2006) and, thus, possibly lower P demands. This would be an ecological advantage in a phosphate-depleted environment and a likely contributor to the ecological success of *Prochlorococcus* in the open ocean. However, microbes containing relatively more phospholipids, such as heterotrophic bacteria or *Synechococcus,* would likely be better at acquiring phosphate when it becomes available.

Interestingly, the separation of the heterotrophic bacterial community into two groups based on their nucleic acid content lead us to conclude that the increase in bacterial productivity after enrichment with N and P can be attributed to the response of the LHNA group, a numerically minor component of the bacterial population. The LHNA group accounted on average for <15% of the initial bacterial community and increased in abundance to >30% in response only to the +NP amendments. Thus, growth of LHNA bacteria was presumably the reason for the stimulated productivity measured in the bulk community. While the LHNA group was relatively less abundant the cells were estimated to be >5-fold larger, based on cellular side scatter and nucleic acid content, and thus formed a major proportion of the bacterial biomass. The cellular RNA content indicated that the LHNA group made up approximately 20–70% of the initial heterotrophic bacterial biomass. Under the NP amendments the RNA content rose to >50–80% of the biomass and we assume a proportionally similar amount of the productivity. Thus comparisons based on cell abundance most likely underestimate the importance of the LHNA group to the cycling of nutrients in these waters. The LHNA heterotrophic bacterial group appears adapted to maximize their growth under transient periods of high nutrient inputs, while remaining numerically a small component of the bacterial community until such a transient period arrives.

In contrast to the LHNA, net growth of the SLNA cells was not limited or co-limited by N, P, or DOC. We hypothesize that this group was a true oligotrophic prokaryote such as the SAR-11 bacterioplankton *Pelagibacter ubique* (Giovannoni et al. 2005). The SAR11 clade consistently peaks in abundance during the summer (Morris et al. 2005) when temperature and nutrient concentrations are at their seasonal highest and lowest respectively. Likewise, concentrations of inorganic N and P in the mid-latitude North Atlantic were typically low during this cruise (Table 1), but have been shown to be even lower at other times of the year (Wu et al. 2000). Members of the SAR11 clade, such as *Pelagibacter ubique,* do not respond to nutrient enrichment and are adapted to grow slowly (Giovannoni et al. 2005). Their streamlined genome and small cell size economize in P and N and the analysis of their genome indicates they are metabolically very simple. Thus, genes coding for nitrate uptake and assimilation are absent, as for *Prochlorococcus*, the main group of oligotrophic photosynthetic prokaryotes (Rocap et al. 2003). Members of the SAR-11 clade were present in the initial community at the 64°W and 27°W sites, the only

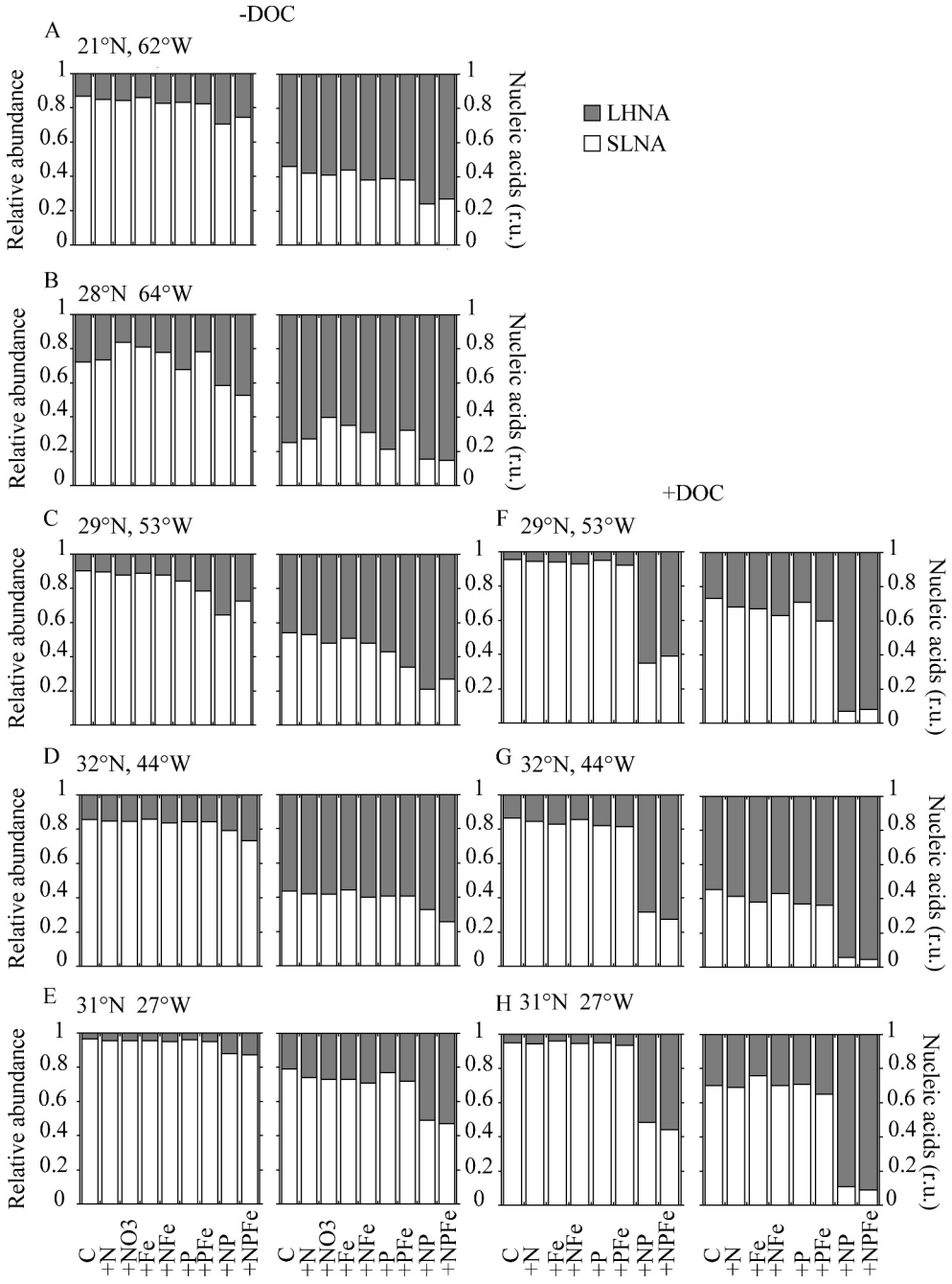

Fig. 7. Relative abundances of the two heterotrophic bacterial groups (SLNA and LHNA) and their relative contributions to the heterotrophic bacterial nucleic acid pool in the different treatments (A–E) –DOC treatments, (F–H) +DOC treatments. r.u. is relative units and is a measure of the fluorescence emitted by the stained nucleic acids. The fluorescence is proportional to the nucleic acid content per cell.

two stations for which 16S rRNA sequences are available (R. Langlois and W. Mohr pers. comm.). The lack of response by the SLNA group to nutrient addition suggests that they predominantly belong to SAR11 clade and are true oligotrophic bacteria (Giovannoni et al. 2005).

The low levels of nutrients in our study region would likely limit the largest cells most (e.g., the LHNA group) due to their lower surface-area-to-volume ratio. Moreover, the low nucleic acid content of the SLNA group implies their cellular requirement for P (and N) was low. Although we assume that the SLNA and LHNA groups in the present study are two different ecological types of bacteria, the former truly "oligotrophic" and the latter "opportunistic," we cannot rule out that these may be inactive and active components of a single group with the techniques we employed here. However, field data often indicate that different groups of bacteria account for differences in bacterial activity (Cottrell and Kirchman 2003) and that

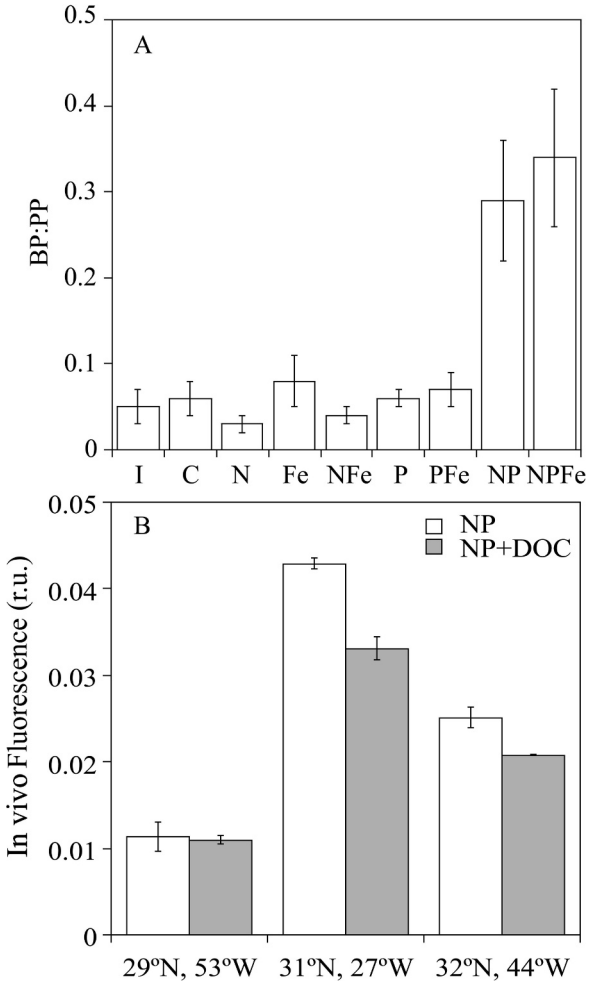

Fig. 8. A) Bacterial production (BP) as a proportion of primary production (PP). BP estimated using a thymidine conversion factor of 1.18×10^{18} cells produced per mole thymidine incorporated (Ducklow 1993). An average cell volume of 0.04 μm^3 and a carbon conversion factor of 2.5×10^{-7} μg C μm^{-3} was used to determine equivalent carbon units. PP data are from Moore et al. 2006 and Moore et al. (in press). B) Comparison of the mean (±SE) in vivo chlorophyll *a* fluorescence in the NP and NP + DOC treatments presented in relative fluorescence units (r.u.).

bacteria responding to a stimulus are clearly distinguishable from those that do not respond (Cottrell and Kirchman 2000). The presence of more opportunistic bacterial groups related to *Alteromonas, Pseudoalteromonas*, Marinobacter, Marinomonas and members of the Cytophagale clades were also detected in the initial bacterial community at these stations (R. Langlois and W. Mohr, pers. comm.). Sequenced genomes from representative members of these bacterial groups indicate that at least some have the necessary genes encoding for the assimilation of nitrate.

The consistency of the NP co-limitation across the large latitudinal (21°N–32°N) and longitudinal (62°W–27°W) gradient covered in our study suggests that net heterotrophic bacterial community productivity was NP co-limited across the oligotrophic mid-latitude waters of the North Atlantic during the period of our study. Analyses of two bacterial groups suggest that the net productivity response to inorganic nutrient addition was most likely dominated by increase in growth rate of large, nucleic-acid-rich "opportunistic" bacteria, while the smaller "oligotrophs" were unaffected. The opportunistic bacteria that were N and P co-limited are likely in direct competition with photoautotrophs for macronutrients. Such competition may interfere with the tightly coupled production and recycling of organic matter in oligotrophic oceanic surface waters (Thingstad et al. 1997). Likewise, atmospheric deposition of high N : P aerosols, a major source of new nutrients to the open ocean, would stimulate autotrophic productivity (Moore et al. 2006) and fuel the competition between autotrophs and heterotrophs for sparse nutrients. Future global warming scenarios predict increased oceanic stratification and acute nutrient limitation. Thus, understanding how nutrients constrain microbial productivity in the surface waters of oligotrophic oceans may lead to improvements in our comprehension of the role these processes play in the biogeochemistry of present and future oceans.

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