Meridional patterns of inorganic nutrient limitation and co-limitation of bacterial growth in the Atlantic Ocean.

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Abstract

1 Growth of heterotrophic bacteria is generally considered to be controlled by
2 temperature and the availability of organic substrates, however there is evidence that
3 bacterial growth can also be limited by the concentrations or supply rate of inorganic
4 nutrients (i.e. nitrogen, phosphorus or iron). We examined spatial and seasonal
5 patterns of organic carbon and inorganic nutrient (N and P) limitation of bacterial
6 growth along each of two meridional transects through the Atlantic Ocean, during
7 contrasting seasons. Here we used nutrient bioassays to demonstrate widespread
8 inorganic nutrient limitation and co-limitation with organic carbon in the oligotrophic
9 temperate, tropical and subtropical ocean. There were distinct seasonal and spatial
10 differences in the inorganic and organic nutrient limitation of bacterial growth, with
11 inorganic nitrogen as the primary limiting nutrient in May/June, and inorganic nitrogen

and organic carbon co-limiting growth in October/November. There was no evidence that the availability of inorganic phosphorus limited bacterial growth in the Southern Hemisphere. We propose that the patterns of nutrient-dependent bacterial growth reflect seasonal and spatial differences in aeolian inputs and the quality of dissolved organic matter, and that bacteria directly compete with autotrophs for inorganic nutrients in the oligotrophic regions of the World Ocean. The findings of this study have important implications for understanding the balance between the biological and microbial carbon pumps, and the modelling of the net metabolic balance of the Ocean in response to climate-driven changes in nutrient inputs.

Highlights

- We investigated nutrient limitation of bacterial growth in the Atlantic Ocean
- Inorganic nutrient limitation and co-limitation of bacterial growth was widespread
- Seasonal and spatial differences in the primary limiting nutrients.
- Bacteria compete with phytoplankton for inorganic nutrients in oligotrophic regions

Keywords

Nannoplankton, growth rates, nutrients (mineral), limiting factors, Atlantic Meridional Transect, Atlantic Ocean

Introduction

Heterotrophic bacteria (hereafter “bacteria”) mediate the biogeochemical cycles of many important elements (carbon (C), nitrogen (N); phosphorous (P), sulfur (S), etc), and are responsible for at least half of global oceanic respiration (del Giorgio et al., 1997; Karl, 2007). Although bacterial growth is generally controlled by temperature or the availability of energy-supplying substrates (i.e. organic carbon) (Kirchman and Rich, 1997; Pomeroy and Wiebe, 2001), in many freshwater and some ocean regions, growth is limited by the supply and availability of inorganic nutrients, such as N, P or iron (Fe) (Tortell et al., 1996; Rivkin and Anderson, 1997; Mills et al., 2008; Martínez-García et al., 2010). However, most previous nutrient bioassays were carried out under conditions where grazing-mediated mortality or the photosynthetic production of organic carbon during the incubation confounded the interpretation of the response of bacteria to the nutrient amendments (e.g. Mills et al., 2008; Martínez-García et al., 2010). The designs of these previously published experiments make it difficult to assess the extent to which inorganic rather than organic nutrients, limit bacterial growth. Here, we examined the influence of organic carbon and inorganic nitrogen and phosphorous on bacterial growth during two Atlantic Meridional Transect (AMT) expeditions (~32°S – 46°N). We show distinct seasonal and spatial differences in the nutrient limitation and co-limitation (*sensu stricto*: Seppälä et al., 1999; Elser et al., 2007; Moore et al., 2013) across five biogeochemical provinces. There was widespread inorganic N limitation and co-limitation with organic carbon in the oligotrophic Atlantic Ocean, suggesting that bacteria compete directly with phytoplankton for inorganic nitrogen, leading to the accumulation of dissolved organic carbon in surface waters. Competition between bacterial and phytoplankton for inorganic nutrients shifts the balance from the

biological carbon pump (BCP), which vertically transports phytoplankton-derived particulate organic carbon to depth, to the microbial carbon pump (MCP), which sequesters recalcitrant dissolved organic carbon (RDOC) produced mainly by the microbial food web.

Materials and methods

We investigated the spatial and seasonal patterns of bacterial growth to the additions of organic carbon (C), inorganic nitrogen (N) and inorganic phosphorous (P), using full-factorial nutrient-addition bioassays (Rivkin and Anderson, 1997). Bioassays were conducted at 26 stations, during contrasting seasons on transects between the United Kingdom and South Africa during the Atlantic Meridional Transect (AMT) programme (AMT16: 20 May to 29 June 2005, and AMT17: 15 October to 28 November 2005; Fig. 1). Water for nutrient amendment bioassays was collected approximately 1 h before sunrise from the 55% light depth using 20-litre Niskin bottles mounted on a Seabird CTD system. Chlorophyll *a* (Chl *a*), nutrient and dissolved organic carbon (DOC) concentrations were made available through the Natural Environment Research Council (www.bodc.ac.uk) and these data have been published elsewhere (Poulton et al., 2007; Mather et al., 2008; Pan et al., 2014). Modified seawater (MSW) dilution cultures (i.e. 1 part 1.0- μ m filtered seawater to 4 parts 0.2- μ m filtered seawater) were incubated in 500 ml polycarbonate bottles in the dark and at ambient temperatures. Triplicate incubation bottles were either not amended with nutrients (i.e. control) or amended with additions of organic carbon (glucose), and inorganic nitrogen (NH_4Cl) and inorganic phosphorous (Na_2HPO_4), each to a final concentration of 10 μ M. Micromolar (rather than nanomolar) concentrations of nutrients were used to avoid nutrient limitation during the

incubations, and to allow comparisons with previous studies (e.g. Caron et al., 2000). Bottles were incubated in the dark, and at ambient temperatures ($\pm 0.05^\circ\text{C}$) in temperature controlled water baths (Rivkin and Anderson, 1997).

Samples (1.8 ml) were collected at 24 h intervals for 72 h, preserved in 1% (final) paraformaldehyde for 10 minutes at room temperature, frozen in liquid nitrogen within an hour of collection and stored at -80°C until bacterial abundances were determined by flow cytometry (FCM). Samples were stained with SYBR Green 1 and analysed using standard protocols (Marie et al., 1999; Li and Dickie, 2001) using a FACSsort™ (Becton Dickinson, San Jose, CA, USA), equipped with a 488-nm argon-ion laser (Li et al., 1995). Abundances of unstained, picophytoplankton were also determined by FCM, and although picophytoplankton were detected *in situ*, they were not present in the incubations. The abundances of bacteria were also determined with Acridine Orange Direct Counts (AODC) (Hobbie et al., 1977), and bacterial biomass was determined by image analysis of AODC stained cells as described previously (Hale et al., 2006). The growth rates (μ) of heterotrophic bacteria were determined from the time-dependent changes in abundances during the linear portion of the growth curve, assuming exponential growth:

$$\frac{\ln(BA_f/BA_o)}{t}$$

Where BA_o and BA_f are the bacterial abundances at the beginning and the end of the interval of exponential growth, respectively, and t is the incubation time interval of exponential growth (in days) (Ducklow et al., 1999).

Bacterial abundances determined from flow cytometric analyses were compared to those from AODC counts using a Spearman's Rank Correlation, as the data were not normally distributed. For each experiment, the growth rates (and

residuals) were examined for normality and homogeneity of variances. Data were log-transformed where required, to ensure the assumptions of Analysis of Variance (ANOVA) were met (Sokal and Rolf, 1995). A one-way ANOVA was employed to determine the effects of nutrient amendments on mean bacterial growth rate for each bioassay. When differences were statistically significant, a post-hoc Tukey test identified homogenous subsets of treatments. All statistical analyses were conducted in SPSS 14.0.

Results

Temperature at the 55% light depth during the boreal spring (AMT16) and autumn (AMT17) ranged from 19 to 28°C and 18 to 27°C, respectively. Inorganic nutrients, DOC and Chl *a* concentrations in the mixed layer were generally low (Poulton et al., 2007; Pan et al., 2014) and typical for the regions studied (e.g. Poulton et al., 2006). Nitrate, nitrite, ammonium and phosphate concentrations at the 55% light depth were all typically < 0.03 µM, except in the southern hemisphere where phosphorus was 0.11 to 0.33 µM (Mather et al., 2008; Table 1).

Bacterial abundances determined by FCM were strongly correlated with abundances determined from AODC (Spearman's $\rho = 0.862$, $n = 50$, $p < 0.001$), however FCM-determined abundances were on average, 2-fold greater. This relationship is consistent with previous studies (e.g. Button and Robertson, 2001) and is likely due fluorescence microscopy not detecting small bacterial cells. Therefore, all reported abundances and growth rates are from FCM-determined abundances. Bacterial abundances were 2- to 3-fold higher in temperate and equatorial upwelling regions than in the subtropical gyres (Table 1). Bacterial

biomass varied from 9.2 to 20.1 fg C cell⁻¹ and there were no clear spatial or temporal trends, or significant correlations with other variables.

Growth rates of bacteria in the controls were 0.13 to 0.67 d⁻¹ and 0.16 to 0.45 d⁻¹ during AMT16 and AMT17, respectively (Fig. 1b). The latitudinal variations in growth rates were similar along both transects, except between 21 and 32°S, where the rates were up to 3-fold greater during the austral autumn (i.e. AMT16) than spring (i.e. AMT17). The higher growth rates occurred at stations where concentrations of nitrate and phosphate were higher and DOC was lower (Fig. 2). *In situ* bacterial abundance and biomass were significantly correlated with Chl *a* ($p < 0.004$). However, there were no significant relationships between bacterial growth rates in the unamended controls and *in situ* temperature, or concentrations of Chl *a*, DOC, nitrate, phosphate, or ammonia ($p > 0.16$).

Single additions of C and P significantly enhanced bacterial growth rates, relative to the controls at only 5 of the 26 stations sampled (~19%) (Table 2). In contrast, single additions of N enhanced bacterial growth up to 2.8-fold at 10 of the stations sampled (~38%). When C, N and P were added in combination (in pairs or in full combination), nutrient co-limitation occurred at 13 of stations, and sequential nutrient limitation at 18 stations (Table 1). Here, we define 'co-limitation' as a statistically significant increase in growth rate in response to the combined addition of two or more nutrients, but not to the addition of individual nutrients; for example $\mu_{\text{control}} = \mu_{\text{N}} = \mu_{\text{P}} < \mu_{\text{N+P}}$. In contrast, 'sequential limitation' occurs when a primary limiting nutrient is added in excess of the stoichiometric requirement of bacteria for that nutrient, leading to a secondary nutrient becoming growth-rate limiting; for example $\mu_{\text{control}} < \mu_{\text{N}} < \mu_{\text{N+P}} < \mu_{\text{C+N+P}}$ (Seppälä et al., 1999; Saito et al., 2008; Suggett et al., 2009).

There were spatial and seasonal variations in the responses of bacteria, with a greater frequency and magnitude of single and co-limitation of nutrients in the Southern Hemisphere compared to the Northern Hemisphere, and no evidence of P limitation in the Southern Hemisphere (Table 2, Fig. 3 and 4). N-limitation was prevalent (i.e. $\mu_{\text{control}} < \mu_{\text{N}}$ at 8 of 13 stations; Fig 3a) during AMT16, and C and N co-limitation was prevalent (i.e. $\mu_{\text{control}} = \mu_{\text{N}} = \mu_{\text{C}} < \mu_{\text{C+N}}$ at 7 of 13 stations; Fig 3b) during AMT17, particularly in the Southern Hemisphere. The concurrent additions of C and N significantly enhanced growth rates, relative to both the control (2.5- to 7-fold) and single C or N nutrient additions (1.2 to 7.5-fold) at all stations in the Southern Hemisphere (Table 2, Fig. 3). The concurrent additions of C, N and P further enhanced bacterial growth, relative to both the control (1.8- to 12.0-fold), and the single and dual nutrient amendments (by 1.1 to 10.6-fold), at 10 of 13 stations during AMT16, and 8 of 13 stations during AMT17 (Table 2, Fig. 3). The enhanced responses mainly occurred in the Northern Hemisphere and of these stations, growth was co-limited by C, N and P at three stations in the Northern Hemisphere and the remaining stations exhibited sequential nutrient limitation of N, then C and then P (Table 2).

Discussion

Although filtration may have disrupted fragile plankton such as naked flagellates, and led to the release of cellular contents that could potentially increase the DOC concentrations, it is unlikely that this affected our experiments. Firstly, the bacterial growth rates measured in the controls (Fig. 1b) were similar to those reported previously for the oligotrophic Atlantic (Zubkov et al., 2000a; Zubkov et al., 2000b). Secondly, the highest *in situ* Chl *a* concentration in this study was $0.3 \mu\text{g L}^{-1}$

and assuming that C:Chl *a* was 50 (Kirchman et al., 1993), the total amount of cellular carbon would have been ~1.3 μ M. If all available cellular carbon was released as DOC during filtration, which is unlikely, this additional contribution would have been negligible compared to the ambient DOC concentrations (56.3 to 82.3 μ M, Table 1).

While significant relationships between bacterial growth rate and temperature have been reported from meta-analyses of global aquatic (White et al., 1991) and marine (Kirchman et al., 2009) systems, representing a wide temperature range (<1.5 to 38°C), other site-specific studies have shown weak or non-significant relationships, especially when growth is assessed over a relatively narrow temperature range (reviewed by Rivkin et al., 1996). In this study we did not detect a significant correlation between bacterial growth rate and temperature. This is not surprising since firstly, the range of surface water temperatures in our study was relatively small (18 to 28°C) and bacteria were likely operating at their temperature optima (Pomeroy and Wiebe, 2001); and secondly, growth was limited (or co-limited) by inorganic and/or organic nutrients and thus temperature was not a primary factor controlling growth.

We recognize that a significant correlation between or among variables does not equate to a causal relationship. However during field studies, correlations are used to help understand the relationship among variables rather than to confirm causality. Based on the absences of significant correlations between bacterial growth and Chl *a* or DOC, we propose that bacterial growth was not limited by the availability of organic substrates in much of the oligotrophic Atlantic Ocean. This proposal is further supported by the nutrient bioassays showing organic carbon limitation of bacterial growth at only 5 of 26 stations (Table 2). While it is possible that some

bacteria were not able to utilise glucose as a substrate (Sherwood et al., 2015), it is unlikely. Glucose is commonly used as an organic carbon source in nutrient amendment experiments since glucose is considered a good low molecular weight representative of one of the major organic carbon sources in the sea (carbohydrate) and is readily used by most bacteria (Ayo et al., 2001). Moreover, unlike amino acids, glucose is not a source of organic nitrogen, which would have confounded our experimental design.

Inorganic limitation and co-limitation of bacterial growth

There was no evidence that the relatively high concentrations of NH_4^+ used in our experiments caused toxicity and affected bacterial growth (i.e. μ_{N} was never significantly lower than μ_{control}), and whilst millimolar concentrations of NH_4^+ have been shown to inhibit the growth of some marine bacteria (Taussaint et al., 1995; Kadam and Boone, 1996; Postma et al., 2002), the concentration used in this study was in the micromolar range and is typical of many coastal oceans.

Generally, bacterial growth was limited by inorganic N during AMT16 (May/June) and co-limited by organic C and inorganic N during AMT17 (October/November) (Fig. 3a). Inorganic nutrient limitation and co-limitation of growth was more widespread in the Southern than Northern Hemisphere (Fig. 3b).

There was no evidence of inorganic P limitation in the Southern Hemisphere. This may have been due to higher ambient concentrations of inorganic P compared to the Northern Hemisphere (Fig. 2b), where N_2 fixation leads to a draw-down of surface P concentrations (Reynolds et al., 2007). Overall, the spatial and seasonal differences in the nutrient limitation of bacterial growth are likely due to seasonal and spatial differences in aeolian inputs throughout the Atlantic Ocean and the resultant

quality and reactivity of dissolved organic matter (see below).

Inorganic N and P co-limitation of bacterial growth has been reported in the subtropical North Atlantic (Mills et al., 2008; Martínez-García et al., 2013). We found no evidence of N and P co-limitation, but did observe co-limitation of C, N and P, and sequential limitation of N and then P at three stations in the North Atlantic during AMT16 (Table 2). This may be because, in contrast to our study, Mills et al. (2008) and Martínez-García et al. (2013) used unmodified, whole seawater incubated in the light. Their experimental design measured bacterial growth rates in the presence of grazing mortality, where excretion by microzooplankton grazers may have provided remineralised organic and inorganic nutrients during the incubations (Sherr et al., 1986). Furthermore, it is not possible to unequivocally determine whether changes in bacterial growth were a direct response to the added nutrients, or an indirect response to changes in the supply of DOC from photosynthesizing phytoplankton (i.e. PP_{DOC}).

In addition to the experimental results presented here, a review of published nutrient amendment experiments using modified, grazer- and phytoplankton-free seawater, incubated in the dark at ambient temperatures, showed that inorganic nutrient limitation and co-limitation of bacterial growth frequently occurs in a diverse range of oceanic and coastal regions, including the Sargasso Sea, the Gulf Stream, the NW Mediterranean Sea, the Central Baltic Sea, the Caribbean Sea, and regions of the coastal and oligotrophic Pacific (Table 3). Since few previous studies used a complete factorial matrix of nutrient additions, it is generally difficult to distinguish the effects of individual nutrients, or draw general conclusions, although it is clear that inorganic nutrient limitation of bacterial growth is widespread.

The inorganic nutrient limitation and co-limitation of oceanic bacterial growth

observed in this and other studies (Tables 2, 3 and Fig. 3) can be explained by the nutrient stoichiometric of bacteria and phytoplankton. Bacteria are characterised by low C:N and C:P ratios, i.e.~4:1 and 20:1, respectively, compared to phytoplankton, i.e. 6-7:1 and 40-200:1, respectively (Donovaro, 1998). The nutrient stoichiometric ratios of phytoplankton in oligotrophic environments are at the upper end of these ranges, and laboratory studies have shown that limitation of phytoplankton growth by N and P can result in the production and release of dissolved organic matter (DOM) with even higher C:N and C:P ratios (Mykkestad, 1995; Puddu et al., 2003). Under these conditions, the N and P requirements of bacteria cannot be met from DOM alone and bacteria take up inorganic N and P to fulfil their growth demand (Donovaro, 1998).

There are several important consequences of inorganic nutrient limitation of bacterial growth on ocean carbon cycles: (1) competition with phytoplankton for nutrients and changes in the stoichiometric relationship for nutrient uptake and release; (2) alterations in the rates and patterns of carbon remineralization; and (3) impact on ocean-climate models. These are discussed below.

Competition with phytoplankton

Bacteria generally have a higher specific affinity for inorganic nutrients (Bradley et al., 2010), and as a result, they outcompete phytoplankton for inorganic P (Zubkov et al., 2007; Tambi et al., 2009) and N (Reay et al., 1999), as well as trace metal micronutrients such as Fe (Tortell et al., 1996). In this study, there were large areas of the oligotrophic temperate, subtropical and tropical Atlantic Ocean where bacteria appear to be directly competing for inorganic N and P with primary producers. The direct competition for inorganic nutrients has major implications for

biogeochemical cycling of organic carbon and for ecosystem responses to climate-related environmental changes. Firstly, the effective uptake of inorganic nutrients by bacteria may result in nutrient limitation of total primary production (PP_T), reducing the production of particulate organic carbon (POC) and the subsequent export of POC below the seasonal thermocline via the bacterial carbon pump (BCP). Secondly, where bacterial growth is limited by inorganic nutrients, the heterotrophic uptake and degradation of organic material is reduced, weakening the coupling between PP_T and community respiration (R). This may lead to both an accumulation of DOC in the surface layer (Thingstad et al., 1997; Liu et al., 2014), and a reduction in POC export via the BCP. Indeed, DOC concentrations of up to 82.3 μM were observed in the mixed layer during AMT16 and AMT17 (Table 1, Fig. 2c). Thus it appears that inorganic nutrient limitation of bacteria can lead to the accumulation of DOC, enhancing the MCP and subsequent carbon sequestration in RDOC pools (Jiao et al., 2011; Legendre et al., 2015).

The production of DOC by phytoplankton (PP_{DOC}) typically represents 10-20% of PP_T , but can be up to 40% in some oligotrophic regions (Baines and Pace, 1991; Nagata, 2000; Alonso-Sáez et al., 2008; Lopez-Sandoval et al., 2011). This DOC is an important organic substrate for bacterial growth (Cole et al., 1982; Azam et al., 1983) and may ultimately contribute to the RDOC pool, which can be important reservoir of sequestered carbon. As discussed above, the amount and quality of the DOC produced is influenced by the nutrient status and intensity of nutrient limitation of the phytoplankton (Lopez-Sandoval et al., 2011). In oligotrophic oceans, nutrient-stressed phytoplankton may release C-rich, N- and P-poor organic matter, either directly through phytoplankton extracellular release, or indirectly via the production of excreted products during inefficient micro- and macro-zooplankton grazing (Bratbak

and Thingstad, 1985; Caron et al., 2000; Thingstad et al., 2008). This extracellular release of poor quality DOM would increase the bacterial demand for inorganic nutrients and consequently, further increase the competition between bacteria and phytoplankton for inorganic nutrients. The relationship typically inferred from significant correlations between phytoplankton and bacteria, for example that bacterial activity is limited by organic carbon supplied by phytoplankton (Cole et al., 1988; Cho and Azam, 1990; Hoppe et al., 2002), may be confounded when bacteria and phytoplankton growth are constrained by availability of the same inorganic nutrients (Rivkin and Anderson, 1997). Predictive and diagnostic models which use parameterization from the regressions of bacteria vs phytoplankton parameters, or from assumptions that remineralization rates are solely a function of temperature (Parslow et al., 2013) may not be appropriate for large areas of the oligotrophic ocean where inorganic nutrient availability limits both bacterial and phytoplankton processes.

Effects of nutrient limitation on carbon remineralization

In regions where bacterial growth is limited by inorganic nutrients, the competition between phytoplankton and bacteria may alter the PP_T/R balance such that R exceeds PP_T . In addition, relative to carbon limitation, inorganic nutrient limitation of bacterial growth reduces bacterial growth efficiency (BGE) and leads to higher bacterial carbon demand and respiratory rates (Goldman, et al., 1987; Kroer, 1993; Puddu et al., 2003; Smith and Prairie, 2004; Alonso-Sàez et al., 2007; Alonso-Sàez et al., 2008, Martínez-García, 2010). When BGE is reduced, the fraction of DOC that is remineralised to CO_2 is large relative that being assimilated into bacterial biomass. Although inorganic nutrient limitation may result in lower rates of bacterial

production, the proportion of DOC that is respired would increase (due to lower the BGE), potentially leading to a higher sea-to-atmosphere flux of CO₂. These combined effects may help explain why large regions of the oligotrophic Atlantic Ocean appear to be net heterotrophic (Duarte et al., 2001; Serret et al., 2001; Robinson et al., 2002; 2006; Gist et al., 2009, Duarte et al., 2013; Ducklow and Doney 2013). Clearly the relationship between R and PP_T is complex and nonlinear, and current diagnostic and predictive models have not adequately considered the role of the microbial community in controlling this relationship.

Impact on ocean-climate models

Nutrient inputs into the surface Atlantic Ocean are predicted to change in the future climate. Most models predict both a decrease in the deposition of high N:P Saharan dust (Mahowald and Luo, 2003), currently a major source of nutrients to the subtropical North Atlantic (Baker et al., 2003, Baker et al., 2006), and an increase in ocean stratification and a concomitant reduction in nutrient supply from deep water (Behrenfeld et al., 2006, Boyce et al., 2010; IPCC 2013). Reduced dust and deep water inputs of inorganic nutrients to surface waters will further reduce primary production and nitrogen fixation. Bacterial competition with phytoplankton for inorganic nutrients may further reduce primary production and the PP_T/R ratio, reducing the export of organic carbon via the BCP and strengthening the MCP. Climate models that do not explicitly include the competition between bacteria and phytoplankton will likely underestimate the predicted weakening of the BCP and the subsequent positive feedbacks to climate.

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Fig. 1. (A) Sampling locations during May – June 2005 (AMT16; open circles) and October – November (AMT17; filled circles) and the biogeochemical province boundaries for the North Atlantic Drift (NADR), North Atlantic Tropical Gyral (NATL), Western Tropical Atlantic (WTRA), Eastern Tropical Atlantic (ETRA), South Atlantic Gyral (SATL), the Benguela Coastal Current (BENG), and the South Subtropical Convergence (SSTC) (Longhurst, 1998; Poulton et al., 2006). (B) Bacterial growth rates in the unamended, control treatments along the transects. Error bars are standard errors (n=3).

Fig. 2. (A) Ambient nitrate (NO₃), (B) phosphate (PO₄), and (C) dissolved organic carbon (DOC) concentrations observed at the 55% light depth during May – June 2005 (AMT16; open circles) and October – November (AMT17; filled circles).

Note different scales on the panels.

Fig. 3. Effect of addition of inorganic nitrogen (N; open circles), organic carbon and inorganic nitrogen (C+N; grey filled triangles), and organic carbon and inorganic nitrogen and phosphorous (C+N+P; black filled squares) normalised to the growth rates (μ) observed in the controls ($\mu_{\text{treat}}/\mu_{\text{control}}$) during (A) AMT16 and (B) AMT17. Bars are propagated standard errors from the mean of three replicate incubation bottles. The dashed line denotes where the ratio $\mu_{\text{treat}}/\mu_{\text{control}} = 1$, i.e. where the addition of nutrients had no effect on growth rate.

Table 1. Summary of *in situ* conditions for dates, locations and depths where water was collected for experiments in contrasting biogeochemical provinces, including temperature (Temp); nitrate (NO₃), phosphate (PO₄) and dissolved organic carbon (DOC) concentrations; bacterial abundance, cell biomass, and growth rate taken from the control treatments; abundances of *Synechococcus* (*Synech.*), Picoeukaryotes (Picoeuk.) and Nanophytoplankton (Nanophyto.); and chlorophyll *a* (Chl *a*) concentrations during (a) AMT16 and (b) AMT17. Although present in surface waters at most stations during both AMT16 and AMT17, the fluorescence signature of *Prochlorochoccus* was too low to allow accurate determination of their abundances by flow cytometry. Stations were classified into biogeochemical provinces (Prov) as defined by Longhurst (1998; see figure 1), based on station positions and characteristics of the Chl *a* and nutrient distributions to account for seasonal shifts in the province boundaries. Nutrient concentrations were below detection limits (ND) at some stations and DOC concentration was not available (NA) at one station.

a)

Date (2005)	Prov	Location	Depth (m)	Temp (°C)	NO ₃ (µM)	PO ₄ (µM)	DOC (µM)	Bacteria (10 ⁸ cells l ⁻¹)	Bacterial Cell Carbon (fg C cell ⁻¹)	Bacterial Growth Rate (d ⁻¹)	<i>Synech.</i> (10 ⁶ cells l ⁻¹)	Picoeuk. (10 ⁶ cells l ⁻¹)	Nanophyto. (10 ⁵ cells l ⁻¹)	Chl <i>a</i> (µg l ⁻¹)
24 June	NADR	41°08' N, 26°23' W	11	19	0.002	0.013	71.1	6.84	9.2	0.33	22.64	2.34	4.64	0.12
21 June	NATL	36°04' N, 38°21' W	12	21	0.008	0.006	65.9	5.75	9.3	0.13	3.68	0.73	2.98	0.08
17 June	NATL	29°09' N, 39°33' W	18	25	ND	0.006	76.4	3.45	9.2	0.46	4.74	0.39	1.26	0.04
15 June	NATL	22°48' N, 36°10' W	14	25	0.008	0.009	72.2	4.30	14.3	0.32	2.42	0.39	1.27	0.05
13 June	NATL	15°46' N, 32°36' W	13	25	0.057	0.025	78.8	5.07	9.7	0.22	1.79	0.60	0.82	0.09
10 June	WTRA	07°42' N, 28°41' W	7	28	ND	ND	NA	8.34	9.6	0.23	12.18	0.55	1.16	0.21
8 June	WTRA	01°10' N, 25°34' W	6.5	27.5	0.007	0.007	82.0	7.95	9.5	0.27	7.27	1.11	1.54	0.15
6 June	WTRA	05°10' S, 25°00' W	12	28	0.010	0.120	81.0	5.61	15.5	0.23	2.73	0.65	1.56	0.13
3 June	SATL	16°17' S, 24°60' W	20	25.5	0.006	0.150	77.3	4.25	18.1	0.37	0.38	0.36	0.74	0.07
1 June	SATL	22°53' S, 24°60' W	17	24.5	0.009	0.060	79.1	4.24	20.1	0.42	0.46	0.40	0.99	0.07
30 May	SATL	26°32' S, 17°14' W	15	23	0.028	0.130	71.6	4.28	13.9	0.42	0.51	0.37	0.64	0.09
26 May	SATL	29°58' S, 00°02' E	15	20.5	ND	ND	73.3	3.20	9.8	0.67	1.52	1.84	1.35	0.15
21 May	BENG	31°58' S, 16°58' E	12	19	1.670	0.330	63.8	5.57	8.8	0.56	7.26	10.19	14.27	NA

b)

Date (2005)	Prov	Location	Depth (m)	Temp. (°C)	NO ₃ (µM)	PO ₄ (µM)	DOC (µM)	Bacteria (10 ⁸ cells l ⁻¹)	Bacterial Cell Carbon (fg C cell ⁻¹)	Bacterial Growth Rate (d ⁻¹)	Synech. (10 ⁶ cells l ⁻¹)	Picoeuk. (10 ⁶ cells l ⁻¹)	Nanophyto. (10 ⁵ cells l ⁻¹)	Chl <i>a</i> (µg l ⁻¹)
19 Oct	NADR	46°23' N, 17°44' W	16	18	0.009	0.053	68.2	5.53	18.8	0.32	3.46	1.11	1.68	0.28
28 Oct	NATL	35°92' N, 29°13' W	13	23	0.006	0.014	73.9	4.36	17.0	0.25	1.71	0.44	1.53	0.17
31 Oct	NATL	29°53' N, 36°27' W	14	25	0.003	0.004	76.3	3.98	20.0	0.45	1.36	0.45	1.71	0.07
2 Nov	NATL	23°96' N, 36°78' W	16	26	0.006	0.009	70.5	4.58	16.3	0.22	1.42	0.44	1.60	0.10
4 Nov	NATL	18°38' N, 33°92' W	17	27	0.005	0.006	72.3	4.94	17.0	0.30	0.89	0.43	1.67	0.11
6 Nov	WTRA	12°07' N, 30°80' W	4	27	0.005	0.014	79.3	5.89	17.5	0.16	13.83	0.64	2.93	0.30
9 Nov	WTRA	03°48' N, 26°66' W	10	27	0.003	0.031	82.3	7.52	13.4	0.17	5.12	0.75	2.27	0.25
11 Nov	WTRA	02°89' S, 25°00' W	11	26	0.002	0.185	79.0	6.85	15.5	0.30	3.55	1.15	2.09	0.18
13 Nov	SATL	11°00' S, 25°00' W	19	26	0.006	0.207	78.4	4.52	16.0	0.37	1.05	0.76	1.64	0.08
16 Nov	SATL	21°13' S, 22°44' W	19	25	0.004	0.277	69.4	3.66	14.8	0.30	2.12	0.80	1.56	0.05
17 Nov	SATL	22°16' S, 20°16' W	20	23	0.002	0.260	73.7	3.69	14.6	0.33	1.80	0.82	1.21	0.04
20 Nov	SATL	28°85' S, 04°69' W	18	21	0.001	0.240	67.3	3.68	12.2	0.24	0.41	0.83	1.32	0.05
22 Nov	SATL	30°67' S, 00°30' W	9	19	0.003	0.220	56.3	4.78	17.6	0.17	7.06	1.08	2.63	0.10

Table 2. Summary of statistical analyses of responses of bacterial growth rates to additions of organic carbon (C) and inorganic nitrogen (N) and phosphorous (P). Stars denote where mean growth rates were significantly higher in the experimental treatments than in the controls ($p < 0.05$) and treatments are grouped into homogeneous subsets, with the number of stars indicating the strength of the response. The definitions of biogeochemical provinces (Prov.) are given in Fig. 1.

AMT16 (May - June 2005)								
Prov.	Lat.	C	N	P	C+N	C+P	N+P	C+N+P
NADR	41° 08' N							★
NAST(W)	36° 04' N							★
NAST(W)	29° 09' N		★				★	★★
NATL	22° 48' N							★
NATL	15° 46' N	★	★	★	★		★★	★★★★
WTRA	07° 42' N		★(★)		★★(★)	★	★★★	★★★★★
WTRA	01° 10' N		★		★★		★	★★★★
WTRA	05° 10' S		★		★★★		★(★)	★★
SATL	16° 17' S				★			★★
SATL	22° 53' S		★		★★		★	★★★★
SATL	26° 32' S		★		★★		★	★★★★
SATL	29° 58' S				★			★★
BENG	31° 58' S	★★	★★	★	★★★★	★★	★★	★★★★★
AMT17 (October - November 2005)								
Prov.	Lat.	C	N	P	C+N	C+P	N+P	C+N+P
NADR	46° 23' N				★			★★
NAST(W)	35° 92' N	★		★	★	★	★	★★
NAST(E)	29° 53' N					★		★★
NATL	23° 96' N			★		★	★	★★
NATL	18° 38' N							★
WTRA	12° 07' N	★	★	★	★★	★	★	★★★★
WTRA	03° 48' N		★		★		★	★★
WTRA	02° 89' S				★			★★
SATL	11° 00' S				★			★
SATL	21° 13' S				★★	★		★★
SATL	22° 16' S	★			★★	★		★★
SATL	28° 85' S				★			★
SATL	30° 67' S				★			★

Table 3. Summary of results of previous published studies on nutrient amended bacterial growth. To allow comparison among these studies and the results reported in this paper, we compiled only studies that included organic carbon (C), organic nitrogen (N_{org}), inorganic nitrogen (N) and inorganic phosphorous (P) using modified, grazer- and phytoplankton-free seawater, incubated in the dark and ambient temperature.

Location	Temp (°C)	Treatments	Full Matrix?	Limiting/ Colimiting Nutrients	Reference
Sargasso Sea	26	C, C+N _{org} , Algal lysate, N, P	No	C	Carlson and Ducklow, 1996
Sargasso Sea	28	C, N, P, C+N, C+P, C+N+P	Yes	C, P ^a	Rivkin and Anderson, 1997
Sargasso Sea	29	C, N+P	No	N+P ^b	Caron et al., 2000
Georges Bank	29	C, N+P	No	C	Caron et al., 2000
Gulf Stream	28	C, N, P, C+N, C+P, C+N+P	Yes	P	Rivkin and Anderson, 1997
Chesapeake Bay	5 - 32	C, C+N _{org} , C+N	No	No response	Shiah and Ducklow, 1994
Subtropical N. Pacific	26	C, C+N _{org} , N, C+N	No	C+N _{org} , C+N ²	Donachie et al., 2001
Coastal Pacific	20	Algal exudates, N, P ^c	No	N	Horrigan et al., 1988
W. Pacific Gyre	28	C, N+P, C+N+P, Algal exudates	No	C+N+P ^a	Liu et al., 2014
Eastern Mediterranean	26	C, C+P	No	No response	Pinhassi and Berman, 2003
NW Mediterranean	13 - 21	C, N, P, C+N, C+P, C+N+P	Yes	P	Sala et al., 2002
N. Red Sea	28	C, P, C+P	No	C	Pinhassi and Berman, 2003
Caribbean Sea	29	C, N, P, C+N, C+P, C+N+P	Yes	C, N, P ^a	Rivkin and Anderson, 1997
E. Baltic Sea	-2	C	No	No response	Autio, 1998
W. Baltic Sea	20	C+N _{org}	No	No response	Berman et al., 1994
Central Baltic Sea	20	C, N, P, C+N, C+P, C+N+P	Yes	C+P	Kuoppo et al., 2003
Greenland Sea	-1 - 3	C, N, P, C+N+P	No	C	Middelboe and Lundsgaard, 2003
Greenland & Norwegian Seas	4 - 7	C, N+P, C+N+P	No	C	Cuevas et al., 2011
Ross Sea Polynya	-2	C	No	No response	Ducklow et al., 1999
McMurdo Sound	-2	C, C+N _{org}	No	No response	Rivkin et al., unpubl.

^a Growth significantly increased in all single additions compared to control, but strongest response observed in this treatment.

^b N and P not added as sole additions, therefore unable to separate effects of individual nutrients.

^c Nutrients added sequentially in continuous culture.

Meridional patterns of inorganic nutrient limitation and co-limitation of bacterial growth in the Atlantic Ocean.

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Highlights

- We investigated nutrient limitation of bacterial growth in the Atlantic Ocean
- Inorganic nutrient limitation and co-limitation of bacterial growth was widespread
- Seasonal and spatial differences in the primary limiting nutrients.
- Bacteria compete with phytoplankton for inorganic nutrients in oligotrophic regions