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

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ABSTRACT

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

1. 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

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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.

2. 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-L 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-μm filtered seawater to 4 parts 0.2-μm 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 μM. 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 (±0.05 °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 min 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 FACSort™ (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 Rohlf, 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.

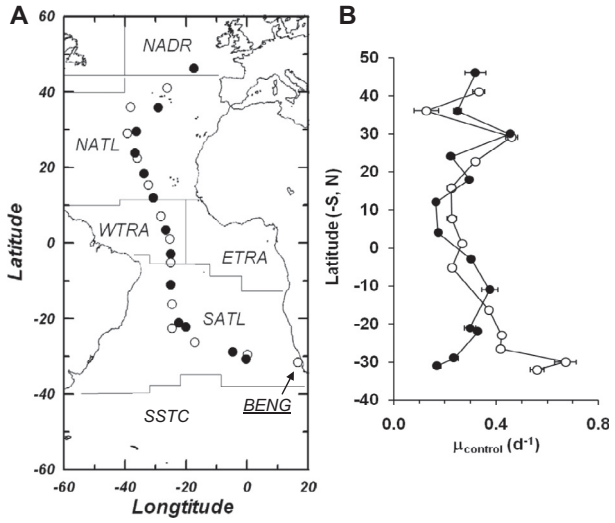

Fig. 1. (A) Sampling locations during May–June 2005 (AMT16; open circles) and October–November (AMT17; filled circles) and the biogeochemical province boundaries for the North Atlantic Drift (NADR), North Atlantic Tropical Gyral (NATL), Western Tropical Atlantic (WTRA), Eastern Tropical Atlantic (ETRA), 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).

3. 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 0.11–0.33 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 0.13–0.67 d^{-1} and 0.16–0.45 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

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