Assessment of bacterial dependence on marine primary production along a northern latitudinal gradient

Eric Fouilland[1], Emilie Le Floc'h[1], Debra Brennan[2], Elanor M Bell[2,3], Sian L. Lordsmith[2,4], Sharon McNeill[2], Elaine Mitchell[2], Tim D Brand[2], E. Elena García-Martín[5], and Raymond J.G. Leakey[2]

[1]MARBEC Univ Montpellier, CNRS, IFREMER, IRD, Sète, France.[2] Scottish Association for Marine Science, Scottish Marine Institute, Oban, Argyll, PA37 1QA, UK. [3] Australian Antarctic Division, 203 Channel Highway, Tasmania 7050, Australia.[4] Cardiff University, School of Earth and Ocean Sciences, 1.74B/3.01, Main Building, Park Place, Cardiff, CF10 3AT, UK.[5] Centre for Ocean and Atmospheric Sciences, School of Environmental Sciences, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, UK. Correspondence and requests for materials should be addressed to E.F. (email: eric.fouilland@cnrs.fr)

Abstract

Recent observations in polar marine waters have shown that a large fraction of primary production may be lost to respiration by planktonic bacteria due to very low bacterial growth efficiencies in cold waters. Here we report that sea temperature may be a key factor influencing the interaction between bacteria and primary production in North Atlantic and Arctic waters, suggesting that low primary production rates could not sustain bacterial carbon demand in the coldest Arctic waters. The use of freshly produced phytoplankton exudate by bacteria in early- and mid-summer was assessed, together with the bacterial uptake of dissolved inorganic nitrogen (DIN= nitrate and ammonium), in surface waters along a latitudinal gradient from the North Sea to the Arctic sea ice. Bacterial production was independent of the low primary production measured in the coldest waters. Under these conditions, heterotrophic bacteria can consume a large fraction of DIN and N-rich organic matter, making them strong contributors to N fluxes in these waters.

Keywords: Carbon coupling, Arctic waters, Phytoplankton and bacteria interactions, Nitrogen fluxes

Introduction

Marine oceanic carbon and nitrogen cycles are driven primarily by the combined activities of phototrophic and heterotrophic micro-organisms. Within surface waters, phytoplankton cells (phototrophic bacteria and protists) take up dissolved inorganic carbon (DIC) and nitrogen (DIN) to support their growth. Zooplankton (herbivorous protists and metazoans) graze on this phytoplankton, channelling the carbon and nitrogen, relatively efficiently, into larger particulate material which may eventually sink to the seabed as part of the oceanic biological carbon pump (Fenchel 1988). Some carbon, however, is released by phytoplankton as dissolved organic carbon (DOC) which may be consumed by the bacterioplankton (heterotrophic prokaryotes, for simplicity referred to as "bacteria" in this article). The DOC consumed by bacteria is used for production of organic matter for growth (bacterial production BP) and respiration (bacterial respiration BR). At low bacterial growth efficiencies (BGE = BP/BP+BR) only a small fraction is used for growth. This alternative carbon pathway supports a complex, microbial-dominated food web, which is less efficient at packaging carbon and nitrogen into larger particulate material (Azam *et al.* 1983; Fenchel 1988). The coupling of bacterial activity, via DOC, to phytoplankton activity, is, therefore, an important factor influencing the efficiency of marine food webs and carbon cycling.

Hoppe *et al.* (2002) observed that the interaction between phytoplankton and bacteria activities seems to be closely related to the meridional water temperature gradient in the Atlantic Ocean. These authors demonstrated that the ratio of bacterial carbon demand (BCD = BP+BR) to total phytoplankton primary production (TPP= particulate + dissolved) was positively correlated with the water temperature; this ratio being 1:10 in cold (10-15°C) North Atlantic waters and >1:1 in warmer (23-27°C) tropical waters. However, in polar waters a large fraction of the primary production may be lost to respiration by bacteria due to a very low BGE of about 7% (Kirchman *et al.* 2009; Ortega-Retuerta *et al.* 2012; Nguyen *et al.* 2012). Direct comparisons between bacterial respiration and production and primary production in Canadian and Norwegian Arctic waters have indicated that more carbon was respired than could be accounted for by primary production (Kirchman *et al.* 2009, Nguyen *et al.* 2012, Poulton *et al.* 2016) during spring and summer. This suggests that bacteria may also use sources of organic matter other than phytoplankton derived organic carbon (e.g. riverine organic matter and ice algae, Rich *et al.* 1997; Bussmann 1999; Dyda *et al.* 2009) during the growing seasons. Further, if the BCD is greater than TPP in such waters, then a high requirement for nitrogen may be necessary to support bacterial growth, and this may lead to an increase in competition for DIN between bacteria and phytoplankton. Indeed, in Arctic waters heterotrophic bacteria can take up a substantial part of the DIN pool (Allen *et al.* 2002; Fouilland *et al.* 2007), while urea excretion and nutrient released through viral infection may be an important source of nitrogen for phytoplankton (Harrison *et al.* 1985, Shelford and Suttle 2018).

In our study, we investigated the phytoplankton-bacterial carbon coupling, and the contribution of bacteria to DIN uptake rates, in natural plankton communities collected along a south-north gradient from warm northern temperate waters to cold Arctic ice-covered surface waters in early and mid-summer. We provide observational evidence to support the hypotheses that (i) bacterial carbon consumption is independent of freshly produced primary production in waters where PP is low (i.e. low productive waters), (ii) bacteria take up a significant fraction of the DIN in these low productive waters, and (iii) DIN represents only a small fraction of bacterial nitrogen requirements in these low productive waters.

Materials and Methods

Study sites. The surveys were undertaken between 3rd and 16th August 2008, and between 14th June and 10th July 2010, as part of the UK ICECHASER I and II research cruises on the RSS *James Clark Ross* (cruises JR210 and JR219). In total sixteen stations were sampled in the North Sea, Norwegian Sea and Greenland Sea, including coastal and offshore waters to the west and north of the Svalbard archipelago (Fig. 1 and Table S1). In total 4 stations were sampled in 2008. One station was in the Fram Strait, two in offshore waters to the north of Svalbard (one of them ice-covered and the other one in the ice-edge), and one station in Rijfjorden on the north coast of Svalbard. Twelve stations were sampled in 2010. Six stations, were in offshore waters along a latitudinal transect from the UK to Svalbard while another six stations were in offshore waters of the Fram Strait to the west of Svalbard (including one ice-covered station overlying the Greenland Shelf, two ice-covered stations located in the northern Fram Strait and three open water stations). The sampling locations were influenced by various water masses: (i) the transect stations by the Baltic Sea outflow (Kempe and Pegler 1991) and the Norwegian Current (Swift 1986) (ii) northern and west Fram Strait, and north Svalbard stations by the relatively warm West Spitsbergen Current which penetrates into the Arctic region and mixes with colder waters influenced by melting ice; (iii) the eastern Greenland Shelf station by cold waters from the southward flowing East Greenland Current (Rudels *et al.* 2005); (iv) the Rijfjorden station by cold waters from Arctic water masses (Ambrose *et al.* 2006; Wallace *et al.* 2010).

Sampling. Temperature and salinity measurements were undertaken at each station using Sea-Bird Electronics SBE 911 and SBE 917 series CTD profilers, with salinity sensors calibrated during each cruise. Duplicate 10 litre water samples were collected from surface waters (2-10m depth) using Niskin sampling bottles and gently screened through a 200 µm mesh to remove the metazoan zooplankton community. Each sample was then processed for the determination of phytoplankton-bacterial C coupling, bacterial DIN uptake, and associated chemical variables, as outlined below.

Measurement methods. Phytoplankton-bacterial C coupling was determined i) indirectly by paired comparisons of the freshly produced phytoplankton dissolved organic carbon (DOC exudate) against the bacterial carbon demand (BCD) and ii) directly by measuring the bacterial uptake of freshly produced phytoplankton DOC exudates. The phytoplanktonic uptake of dissolved inorganic carbon (DIC), the phytoplanktonic production of DOC exudate and the heterotrophic bacterial uptake of the phytoplanktonic DOC exudate were measured using ^{14}C tracer size-fractionation experiments with an initial addition of ^{14}C-DIC at trace concentration. The use of radioactive ^{14}C was preferred to stable ^{13}C because ^{14}C is a very sensitive tracer allowing short-term incubation, low microbial activity assessment and C-labelled dissolved organic carbon measurements. The ^{14}C uptake measured on the 0.8 µm filters represents the ^{14}C-DIC incorporated into phytoplankton biomass. The <0.8 µm filtrate collected on the 0.2 µm filters represents the ^{14}C incorporated into the heterotrophic bacterial biomass through the bacterial use of freshly release phytoplanktonic ^{14}C-DOC exudates. The < 0.2 µm filtrate represents ^{14}C that was fixed by phytoplankton and released as ^{14}C-DOC (see section **Size-fractionated DIC uptake**).

Bacterial carbon demand was calculated from measurements of bacterial production and respiration, and also estimated indirectly because respiration measurements were not possible in sea-ice influenced waters, from measurements of bacterial production converted to carbon demand using assumed bacterial growth efficiencies (see section **Bacterial production, respiration and carbon demand**).

The bacterial contribution to total DIN uptake was calculated from measurements of size-fractionated ^{15}N-labelled DIN uptake in which the ^{15}N uptake by the <0.8 µm size fraction was considered to be predominantly due to bacterial uptake of DIN, while ^{15}N uptake by the >0.8 µm size fraction was attributed to phytoplankton (see section **Size-fractionated DIN uptake**). For our study, the bacterial uptake of ^{15}N-labelled DON released by phytoplankton was considered to be negligible during the measurement period.

The abundance of phototrophic and heterotrophic picoplankton was determined by flow cytometry in order to estimate the contribution of bacteria to the <0.8 µm plankton size fraction (see section **Bacterial abundance**). In addition, the potential use of diverse organic substrates by the bacterial community was estimated by microplate-based enzyme assays in order to relate the degree of phytoplankton-bacterial C coupling observed to potential bacterial metabolic diversity (see section **Potential bacterial metabolic diversity**).

Size-fractionated DIC uptake. The incorporation of dissolved inorganic carbon into the dissolved and particulate fractions (0.8 µm and 0.2 µm) was measured in duplicates using additions of 0.7 MBq (20 µCi) of $NaH[^{14}C]O_3$ (Steemann Nielsen 1952) and 6-7h incubations. Radioactivity was measured in a

Beckman Coulter LS6500 liquid scintillation counter. No correction for dark ^{14}C uptake was performed, in order to assess the uptake of all autotrophically fixed CO_2, as high anaplerotic C fixation by phytoplankton may occur in marine surface waters (Fouilland *et al.* 2000; Fouilland *et al.* 2001), and can be enhanced under sudden darkness (Mortain-Bertrand *et al.* 1987). The dissolved inorganic carbon DIC concentrations measured in the samples (Charalampopoulou *et al.* 2008; Tynan Eithne pers comm) were used to calculate the carbon production rates using an isotopic discrimination factor of 1.05. Further experimental details are provided as supporting information S2.

An average of 90±7% of total heterotrophic bacterial cells detected by flow cytometry passed through 0.8 µm filters whereas autotrophic bacteria and picoeukaryote cells represented less than 0.5% of the cell abundance in the filtrate. We therefore considered that the ^{14}C-labelled carbon uptake rates measured in the fraction between 0.8 and 0.2 µm were due to the bacterial use of fresh ^{14}C-dissolved organic exudate derived from phytoplankton. This assessment based on size-fractionation probably underestimated the bacterial use of fresh-produced phytoplankton exudates as some large or attached bacterial cells may have been retained by the 0.8 µm filters. An underestimation of 10% to 30% was expected corresponding to the fraction of large free-living cells retained on 0.8 µm filters (10%) observed in our study, and also from the contribution of >0.8 µm fraction to total bacterial production (30%) previously observed in Svalbard coastal surface waters (Kongsfjord waters in Motegi *et al.* 2013) in June 2010.

Bacterial production, respiration and carbon demand. BP was determined in duplicates using [^{14}C]leucine and [^{3}H]thymidine amendments at 10-30 nM and 1-2 h incubations (Bell 1993; Kirchman 2001). Radioactivity incorporation was measured with a Beckman Coulter LS6500 liquid scintillation counter using corrections for counting efficiency as in Kirchman (2001) or Bell (1993). Bacterial growth (cells litre^{-1} day^{-1}) was calculated from [^{14}C] leucine incorporation assuming an isotope dilution factor of 1 or 2 (Simon and Azam 1989; Kirchman 2001) and from [3H]thymidine incorporation using conversion factors of 1 or 2 x 10^{18} cells mole^{-1} (Ducklow and Carlson 1992). BP (bacterial carbon litre^{-1} day^{-1}) was calculated using carbon conversion factors of 6.3 or 20 fg C cell^{-1} (Lee and Fuhrmann 1987; Kawasaki et al. 2011). Further experimental details are provided as supporting information S2.

Bacterial respiration (BR) was estimated in triplicates after the inoculation of Iodonitrotetrazolium salt (INT, 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium) at final concentration of 0.2 mM and 1-5 h incubations (Martinez-Garcia *et al.* 2009) at selected stations (Table 1). The *in vivo* reduction of INT was measured after filtration through 0.8 µm and onto 0.2 µm polycarbonate filters and after extraction with propanol and measuring the absorbance at 485 nm with a Beckman model DU640 Spectrophotometer. The INT values were converted into carbon units by applying a conversion equation derived from INT measurements and oxygen concentration measured at some stations from the same study sites in 2010 (Garcia-Martin et al 2014a,b) assuming a respiratory quotient of 1. We assumed that respiration rates estimated in the fraction between 0.2 µm and 0.8 µm were representative of the total free-living bacterial respiration rates although such rates were probably underestimated (see above for size fractionation limitations). Further experimental details are provided as supporting information S2.

BCD was estimated using three different methods. BCD was measured directly from the sum of the BP and BR measurements (= method A). No clear relationship between INT reduction measurements and oxygen concentration was obtained in stations with sea-ice influenced waters, preventing us to convert INT values into carbon units (Garcia-Martin et al 2014b). Therefore BCD was also calculated for all the sampled stations from BP measurements using two indirect methods: (i) the method B as the sum BP and BR calculated from BP using a value of BGE determined empirically from the relationship between BGE and chlorophyll *a* (Lopez-Urrutia and Moran 2007); iii) the method C as the sum of BP and BR calculated from an empirically-determined relationship between BR and BP (BR=3.69 $BP^{0.58}$, Robinson 2008).

Size-fractionated DIN uptake. The nitrate and ammonium uptake rates were estimated in duplicates using trace additions of $Na^{15}NO_3$ and $(^{15}NH_4)_2SO_4$ at a final concentration of 0.05 µmol L^{-1} and 6-7 h incubations. The nitrogen uptake rates of the total plankton fraction and the bacterial fraction were obtained from filtrations through Whatman GF/F filter with or without 0.8 µm pre-filtration. The ^{15}N particulate organic nitrogen (PON) was measured using a Europa Scientific ANCA mass spectrometer. Nitrogen uptake rates were calculated using the equation of Dugdale and Wilkerson (1986). The values of the ammonium uptake rates obtained during the 2008 cruise were all corrected for isotope dilution (Kanda *et al.* 1987) assuming an equivalence between NH_4 production and uptake rates, as net production of ammonium was occasionally observed during the incubation period (data not shown). Further experimental details are provided as supporting information S2.

The bacterial DIN uptake was estimated from $^{15}NO_3^-$ and $^{15}NH_4^+$ uptake rate measurements performed on 0.8 µm filtrate filtered onto GF/F filters. 40% ±10% of the bacterial cells in the <0.8 µm filtrate were not retained by the GF/F filters, as measured by flow cytometry. The ^{15}N uptake rates were therefore corrected for this loss of bacterial cells by correcting the uptake rates using the ratio between the bacterial abundance measured for the 0.8 µm filtrate and that for the GF/F filtrate. We assumed that the DIN uptake rates estimated for the <0.8 µm fraction were representative of the total bacterial DIN uptake although we probably underestimated such rates when using size-fractionation because of it omits bacterial cells larger than 0.8 µm (see above).

Bacterial abundance. The concentration of free-living bacteria was measured in duplicates using a FACSort (Becton Dickinson, Oxford, UK) flow cytometer with a 15mW 488 nm laser and standard filter set (including FL1: 530/30nm band-pass).

Potential bacterial metabolic diversity. Biolog-EcoPlate™ (Biolog Inc.) microplates containing 31 different substrates, combined with a redox dye, were used to estimate the potential metabolic diversity of natural bacterial communities from 6 stations during the 2010 cruise. Microplates were incubated at 5°C in the dark for 30 days. The degradation of substrates was detected spectrophotometrically (Garland and Mills 1991) using a Bio-Rad (model 680) microplate reader set at 590 nm. The potential metabolic diversity was expressed using the Hill index (Hill 1973) and a nitrogen use index (NUSE) was also calculated in order to assess the fraction of N-containing substrates potentially used by the bacteria communities (Sala *et al.* 2006). Further experimental details are provided as supporting information S2.

Chemical properties. From each duplicate sample, a ~40mL sub-sample was filtered through a 25 mm diameter glass fibre (Whatman GF/F) filter and the filtrates analysed, in triplicate, on ship for nitrate+nitrite, phosphate, silicate, and ammonium concentrations by colorimetric flow injection analysis using a Lachat *QuikChem 8500* flow injection autoanalyser following the manufacturers recommended methods. These methods are modified versions of the standard seawater analyses given by Grasshoff *et al.* (1983).

From each duplicate sample, a 500 mL sub-sample was filtered onto a pre-combusted (500°C, 4h) 25 mm diameter glass fibre (Whatman GF/F) filter and stored frozen at –80 °C for post-cruise analysis. The filters were dried at 60°C for 24 hours, pelletized and analysed for particulate organic carbon (POC) and nitrogen (PON) concentrations by elemental analysis using a Europa Scientific ANCA mass spectrometer.

From each duplicate sample, a 300 mL sub-sample was filtered onto a 25 mm diameter glass fibre (Whatman GF/F) filter and stored frozen at –80 °C for post-cruise analysis. Chlorophyll-*a* concentration was measured by high-performance liquid chromatography (HPLC) after extraction by sonication in 90% acetone (Mantoura and Llewellyn 1983).

Data Analyses. The Pearson coefficient was used to test correlations between physical and chemical data and biomass and rates at the sampling stations. All the tested variables were $\log_{10}$ transformed and than tested for normality and homogeneity for subsequent correlation analyses. The correlation significance was tested with and without Bonferroni correction with a significance level set at $p \leq 0.01$, n=19 using SYSTAT v.11 software.

Results and discussion

General ecosystem properties. The different water masses encountered during our study, which was undertaken in early summer (2010) and mid-summer (2008), were mainly influenced by the Atlantic waters moving northwards from UK to Svalbard where they ultimately mixed with cold Arctic waters and melting ice (Greenland shelf, ice edge stations). Surface waters in the Norwegian Sea and Kongsfjorden were considered as coastal waters receiving inputs of freshwater from land. The highest concentrations of nitrate and silicate (> 1 µM) were measured in the surface waters of the East Greenland Sea, where the highest chlorophyll *a* concentrations were also recorded (4.8 µg L^{-1}). No significant differences in physico-chemical conditions were detected between the two study periods (t-test, p>0.05). There were positive correlations between temperature, chlorophyll *a* concentration and particulate primary production (Table 1), suggesting that the observed decrease in sea-surface temperature with increasing latitude had a strong effect on phytoplankton. The lowest particulate primary production rates (< 0.1 µgC L^{-1} h^{-1}) were measured in waters below 0°C which were strongly influenced by melting ice. This agrees with some previous observations undertaken in the same area (Vaquer-Sunyer *et al.* 2013) but contrasts with other reports of high production rates in low temperature conditions (Perrette *et al.* 2011); this suggests that other factors such as light and nutrient availability can also control phytoplankton production in addition to temperature.

When comparing the different methods used (labelled ^{3}H-Thymidine or ^{14}C-Leucine) and conversion factors, bacterial production (BP) can vary within an order of magnitude with the ratio between the maximal and minimal estimation varying between 13 to 28 (Table S4). BP estimates measured using thymidine incorporation with a thymidine conversion factor of 1 (with a carbon conversion factor of 6.3 or 20) or a carbon conversion factor of 6.3 (with a thymidine conversion factor of 1 or 4) are much lower than bacterial uptake rates of phytoplankton exudate (BPP). Bacterial production cannot be lower than bacterial uptake of phytoplankton exudate, suggesting that these conversion factors for BP estimates are unrealistic. By contrast the BP estimates measured using thymidine incorporation with a thymidine conversion factor of 4 and a carbon conversion factor of 20 and estimates measured with ^{14}C-leucine incorporation and an isotope dilution factor of 2, both gave the highest estimates (Table S4). The BP estimates measured using ^{14}C-leucine incorporation and an isotope dilution factor of 1 (BP_{LEU}) provided the lowest but realistic estimates of BP. We considered these low BP estimates as the easiest bacterial carbon demand to meet by primary production. This allows us to test the robustness of the degree of uncoupling with primary production in the following discussion. In the study areas during the summer, BP may amount to more than 20% of the total phytoplankton production (TPP) in colder waters (<0°C) (Table S3), compared to <10% in warmer waters. This suggests that the bacterial use of primary production in cold waters may be substantially higher than in warmer waters. The high BP:TPP ratio in the coldest waters reflects the lower TPP in these waters as BP did not vary significantly with temperature (Table 1).

Our results contrast with the low ratios between bacterial production (BP) and particulate primary production (PPP) (< 10%) reported in cold Arctic waters using depth-integrated rates (Kirchman 2009). However, high integrated BP:PPP ratios (> 50% and even greater than 200%) have also been observed in low PPP Arctic coastal waters (Ortega-Retuerta *et al.* 2012; Iversen and Seuthe 2011; Poulton *et al.* 2016). The variability in the ratio between autotrophic and heterotrophic processes in Arctic waters seems mainly driven by differences in PPP as suggested by Ortega-Retuerta *et al.* (2012). Both temperature and

the release of dissolved organic matter affect BP in cold Arctic waters (Ortega-Retuerta *et al.* 2014). However, the higher BP:TPP ratio in colder waters in our study was mainly caused by a reduction in TPP at lower temperatures rather than a direct effect of temperature on BP. The low TPP in waters below 0°C was associated with a high dissolved primary production (DPP) as the phytoplankton production of dissolved organic carbon via exudation represented on average 52% (standard deviation of 19%) of the total primary production in our study. In contrast, DPP made up generally less than 30% of the TPP in the warmest waters (Table S3). These percentages are much higher than those averaging 15% reported in a previous study undertaken in a neighbouring geographical area during early summer 2012 (Poulton *et al. 2*016). This discrepancy may be explained by the much lower nutrient concentrations reported in our study.

Bacteria-phytoplankton carbon coupling. The dissolved PP (DPP) was compared to the bacterial carbon demand (BCD) using the lowest but realistic BP estimates (BP_{LEU}) in order to assess the degree of carbon coupling between phytoplankton and bacteria (Morán et al 2002). The DPP:BCD ratio clearly decreased with decreasing PPP (particulate PP) (insert plot in Fig. 2) for all methods of estimating BCD. The median DPP:BCD ratio was 20% (11% and 38% upper and lower quartiles, n=18), for all BCD estimation methods combined, in cold (< 0^oC) and unproductive waters (PPP < 0.5 µgC L^{-1} h^{-1}), but 92% (48% and 171%, upper and lower quartiles, n=14) in warmer (> 4^oC) and more productive waters (PPP > 1µgC L^{-1} h^{-1}). Our results suggest that freshly produced phytoplankton exudate can largely support the bacterial carbon demand in the relatively warm and productive surface waters of the Northern Seas in summer. However, the carbon coupling between bacteria and phytoplankton is weak in cold and low productive waters during our study. Similarly, low values of PP relative to BCD have also been reported for the coastal Beaufort Sea and the Nordic Seas (Kirchman 2009; Ortega-Retuerta *et al.* 2012; Iversen and Seuthe 2011; Poulton *et al.* 2016). This is in agreement with the suggestion that BCD can exceed DPP in coastal environments and/or in low productive ecosystems (Moran *et al.* 2002, Fouilland and Mostajir 2010, 2011), where bacteria may have used other sources of carbon to fulfil their requirement (such as semi-labile dissolved organic matter or organic matter from the land and sea ice in our study).

Comparison between BCD and DPP rates do not provide measurements of actual use of phytoplanktonic exudates by bacteria for their growth. We, therefore, measured the bacterial uptake rates of phytoplanktonic exudates (BPP) using ^{14}C- dissolved inorganic carbon (DIC) isotope tracking experiments at the same time as measurements of bacterial production (BP). After an initial addition of ^{14}C-DIC, the ^{14}C uptake measured in the small size fraction (between 0.2 µm and 0.8 µm), represents the heterotrophic bacterial uptake of ^{14}C-DOC released by phytoplankton (i.e. BPP). Therefore, the comparison between BPP and BP may be considered as a proxy of the direct and immediate C-dependence of bacteria upon phytoplankton (Fouilland and Mostajir 2010). This assumes that the <0.8 µm size fraction used in our study represented most of the heterotrophic bacterial community. If this is not the case, the bacterial contribution to carbon and nitrogen uptake could be underestimated by 10-30% (corresponding to the fraction of large free-living bacteria cells retained on 0.8 µm filter relative to total bacterial cell abundance, see Material and Methods section).

The BPP:BP ratio was less than 50% in low productive cold waters during our study (Fig. 2). If BPP had been underestimated by 30%, this conclusion would still be valid. This clearly confirms that the carbon coupling between bacteria and primary production was weak in cold, low productive coastal waters in summer, and the bacteria obtained much of their carbon from sources other than fresh phytoplankton exudates. In contrast, the bacterial uptake of phytoplanktonic exudates represented more than 50% of BP in the most productive, warmer waters (Fig. 2), confirming the high phytoplankton bacteria-phytoplankton carbon coupling in such waters during our study. This shift from bacterial independence to bacterial dependence on carbon from phytoplanktonic exudates along the gradient of increasing primary production observed during our study may explain the positive relationship between the bacterial growth efficiency and the primary production reported using data from the same time and locations (Garcia-Martin *et al.* 2014a).

Our results support previous reports that bacteria in Arctic coastal waters, such as Beaufort Sea and Nordic Seas, may be consuming organic matter from sources other than phytoplankton, such as rivers (Ortega-Retuerta *et al.* 2012; Poulton *et al.* 2016), and explain the net heterotrophy reported for some other Arctic locations and times (Cottrell *et al.* 2006; Kirchman 2009; Nguyen *et al.* 2012; Ortega-Retuerta *et al.* 2012; Iversen and Seuthe 2011). Bacteria may also use recycled carbon released by viral lysis and predation, especially in areas or during periods of high grazing pressure (Fouilland *et al.* 2014). This hypothesis is supported by the high contribution of organisms larger than 0.8 µm to the total respiration (65-86%) in Arctic sea ice stations reported for the same time and locations (García-Martín *et al*. 2014b). Our study suggests that a large fraction of organic carbon flow may be channelled through heterotrophic bacteria during the summer period, reducing the transfer of energy to the upper trophic levels and, in turn, reducing new production and carbon export in these waters, as reported for a pelagic marine system (Berglund *et al.* 2007).

Bacterial contribution to nitrogen fluxes. In our study bacteria were found to contribute more than 20% of the total inorganic nitrogen DIN uptake (Fig. 3a) in low PPP waters (< 1µgC L^{-1} h^{-1}). This confirms previous results from other geographical areas (reviewed by Fouilland *et al.* 2007) suggesting that bacteria may outcompete phytoplankton for DIN in low PP waters. The N requirement of bacteria biomass, with a median biomass C:N ratio of 4.7, was significantly greater than that of phytoplankton, with a median biomass C:N ratio of 11.5 (Fig. 4). However, the C:N ratio of the substrate uptake, estimated using labelled C and N substrates ($^{14}CO2$, ^{14}C-Leucine, $^{15}NO3$+$^{15}NH4$) was not significantly different between bacteria and phytoplankton (Fig. 4). The C:N ratio of bacterial uptake was slightly higher (median of 7.2) than the bacterial biomass C:N ratio, suggesting that bacteria may take up N from other sources than DIN. During our study, ammonium was mainly taken up by bacteria (60±25%) while similar proportions of ammonium and nitrate were taken up by phytoplankton. The uptake of both ammonium and nitrate supported about 70% (median) of bacterial N production (converted from BP expressed in µgN L^{-1} h^{-1} using a molar C:N ratio of 5). The lowest DIN contribution to bacterial production was measured in cold (<0°C) and low productive waters (<0.1µgC L^{-1} h^{-1}) (Fig. 3b). In these low PPP waters, bacteria may use other N sources for growth, such as the nitrogen fraction of the organic matter released by the phytoplankton (Fig. 3b), or the fraction associated with allochthonous sources or decomposing living materials. This hypotheses is supported by the cultivable

fraction of the bacterial community collected during our study from cold (<0°C) and low productive (<0.1µgC L^{-1} h^{-1}) waters being able to use more diverse BIOLOG substrates (Hill index) and N-containing substrates (NUSE index) such as the diamine putrescine, compared with the community from warmer (>3°C) and productive (>1µgC L^{-1} h^{-1}) waters (Fig. 3b inset). Substrates for sustaining bacterial production in cold, low productive waters could include older, recalcitrant organic compounds from phytoplankton and freshwater inputs, suggesting a diverse bacterial community using a wide range of substrates. This is supported by the greater ability of bacteria to hydrolyse polysaccharides in cold Arctic waters than in warmer Atlantic waters during the early summer (Piontek *et al.* 2014).

The strength of the coupling between bacteria and primary production in Arctic waters in summer does not appear to be an effect of temperature on bacteria as previously suggested (Hoppe *et al.* 2002; Kirchman 2009) but is determined mainly by the level of primary production and associated exudation rates. As PPP and chlorophyll *a* biomass were strongly correlated during our study (Table 1), primary production appeared to be more sensitive to factors influencing the phytoplankton biomass (e.g. grazing rate) than the phytoplankton activity *per se*. This is supported by the lack of strong correlation observed between sea temperature and the biomass-specific primary production ($p = 0.06$, Pearson correlation with Bonferroni correction). This may be representative of an Arctic post-bloom situation. Therefore any environmental or biological factors that reduce primary production may result in a shift towards bacteria using substrates other than the phytoplankton exudates to sustain their growth. A similar observation was previously reported in temperate coastal waters (Trottet *et al.* 2016). Under these conditions bacteria can take up a large fraction of DIN and N-rich organic matter, making them strong contributors to N fluxes.

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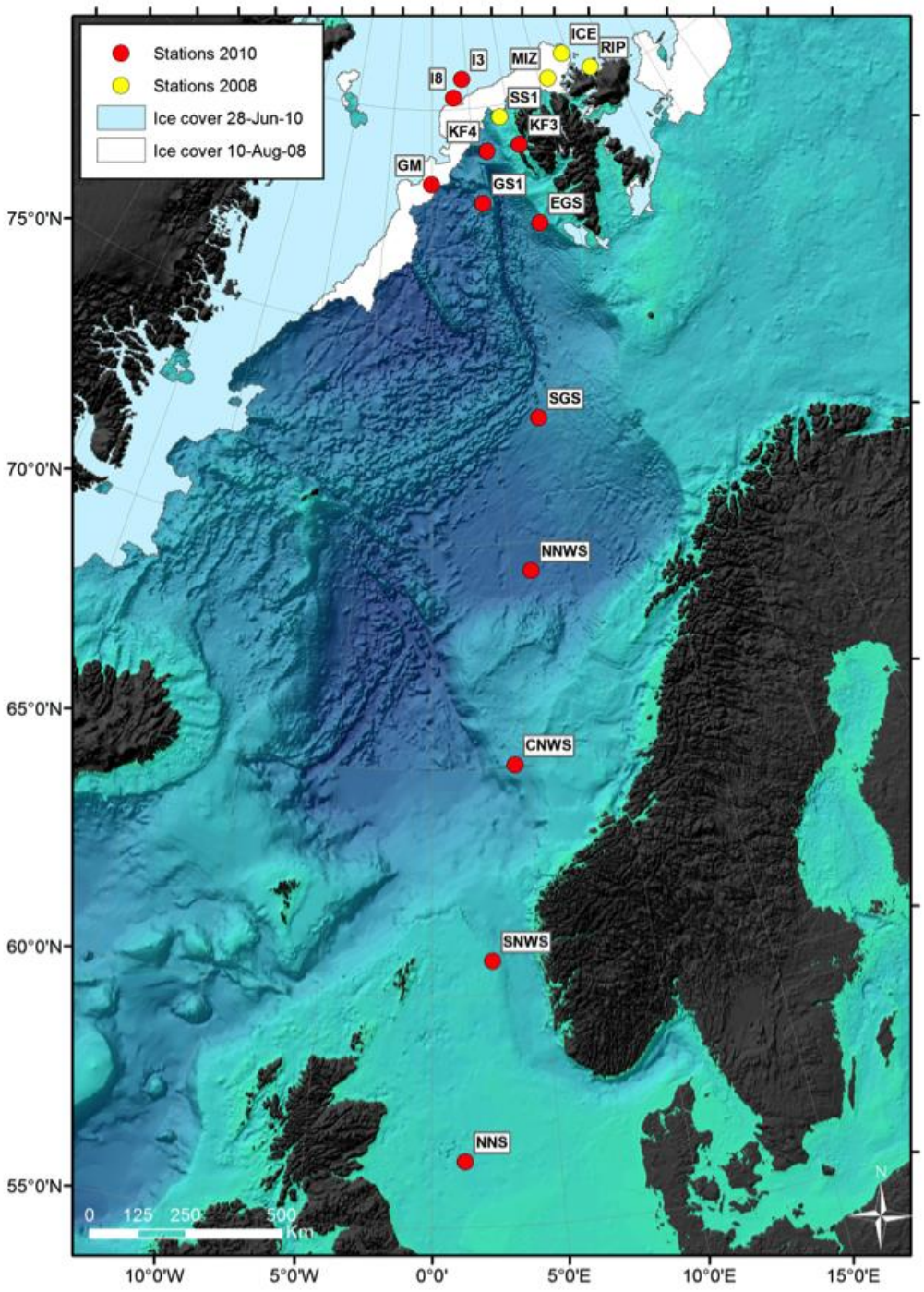

Figure 1. Sampling locations from the North Sea to the Arctic. The bathymetry is reproduced from the GEBCO Digital Atlas published by the British Oceanographic Data Centre on behalf of IOC and IHO, 2003. Sea ice cover is obtained from the National Snow and Ice Data centre, NSIDC MASI-NH (http://nsidc.org/data/G02186). See Table S1 for details of the sampling stations and their acronyms.

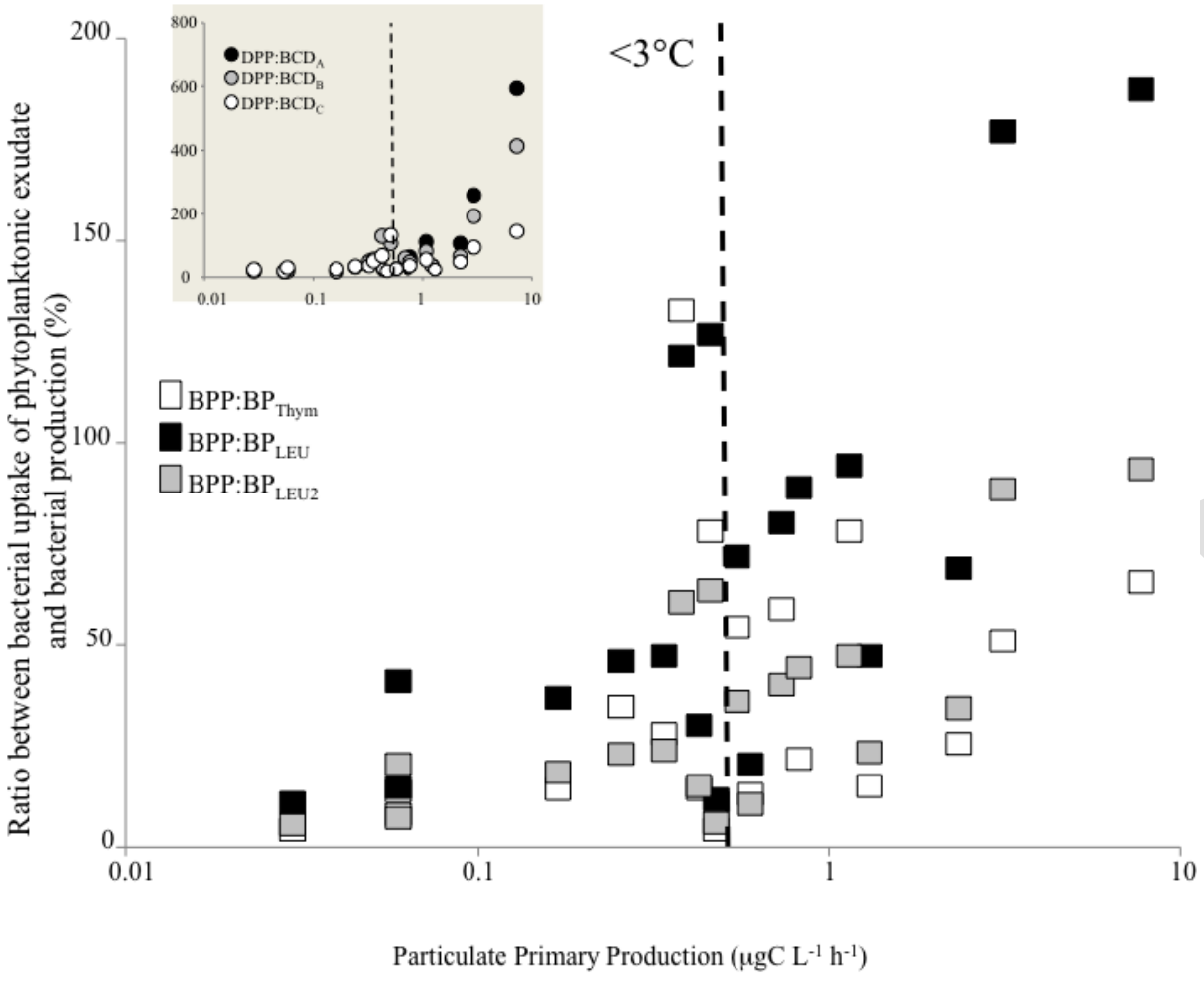

Figure 2. Bacterial C coupling with phytoplankton along a primary production gradient. The bacterial size fraction (%) of primary production (BPP) supporting bacterial production plotted against the rate of particulate primary production measured in the surface waters sampled from North Sea to Greenland waters. The fraction (%) of dissolved primary production (DPP) supporting bacterial carbon demand (BCD), calculated using different BDC methods, is shown in the box plotted against the particulate primary production rates. Bacterial production was measured by ^{14}C-Leucine incorporation assuming a dilution isotope factor of 1 (BP_{LEU}) or 2 (BP_{LEU2}), or by thymidine incorporation with a thymidine conversion factor of 4 and a carbon conversion factor of 20 (BP_{THYM}). BCD_A : BCD calculated according to the method A using the bacterial respiration measured by *in vivo* INT reduction. BCD_B: BCD calculated according to the method B using the bacterial growth efficiency calculated from chlorophyll a concentration using the equation in Lopez-Urrutia & Moran 2007. BCD_C: BCD calculated according to the method C using the bacterial respiration calculated from BP_{LEU} dilution 1 and using the equation in Robinson (2008). Colder water samples (< 3°C) are on the left side of the broken line.

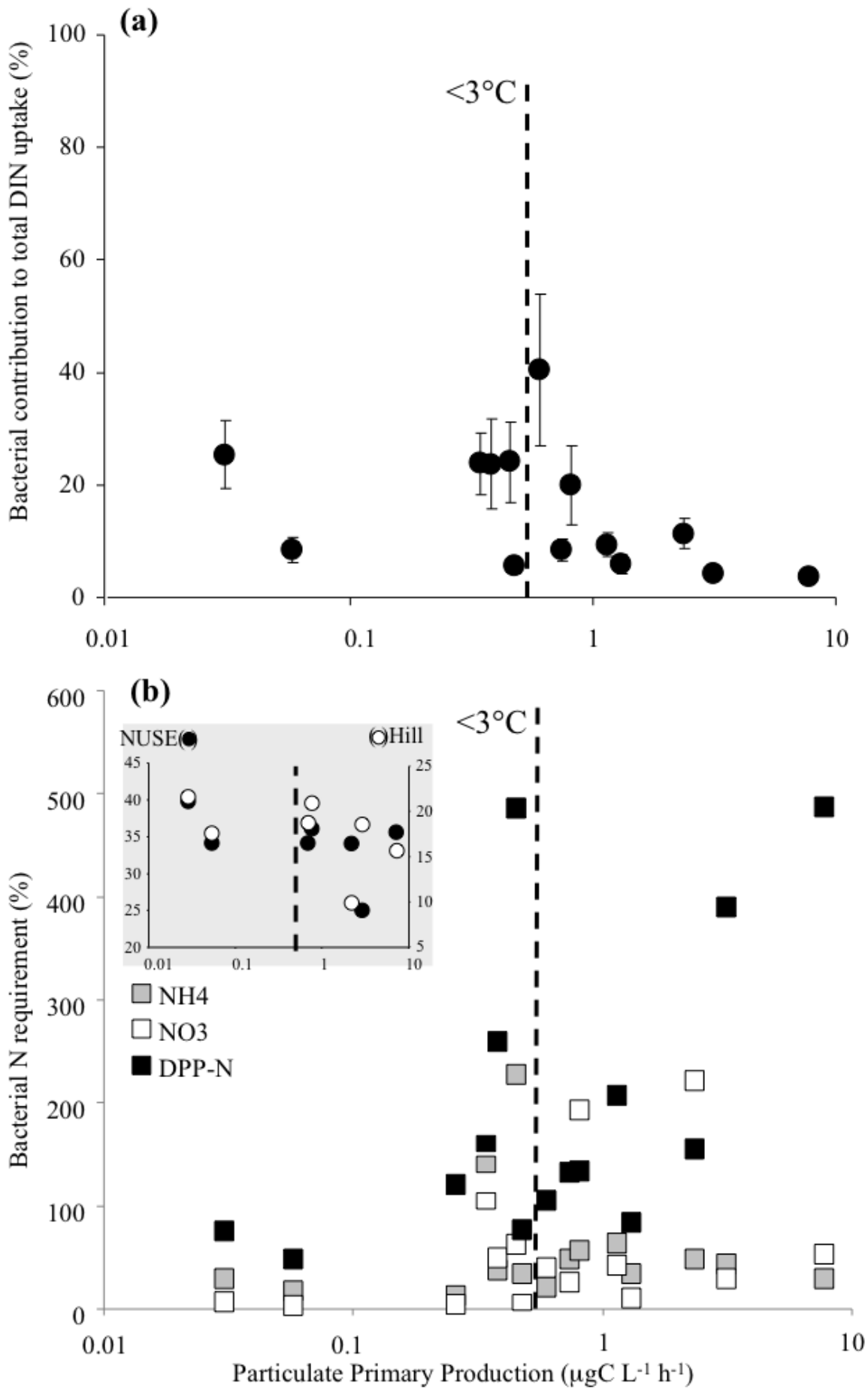

Figure 3. Bacterial DIN fluxes along a primary production gradient. (a) The bacterial contribution to total DIN (NH_4^+ + NO_3^-) uptake rates and (b) the bacterial uptake of NH_4^+ and NO_3^-, and the fraction of DPP converted in N (DPP-N) using a C:N ratio of 11, supporting the bacterial N production (computed from BP_{LEU} and using a C:N molar ratio of 5) plotted against the rate of particulate primary production measured in the surface waters sampled from North Sea to Greenland waters. The various BIOLOG organic substrates used by the bacterial community are plotted in the box and expressed as the percentage of N-containing substrate used (NUSE Index) and the Hill diversity Index. Colder water samples (< 3°C) are on the left side of the broken line.

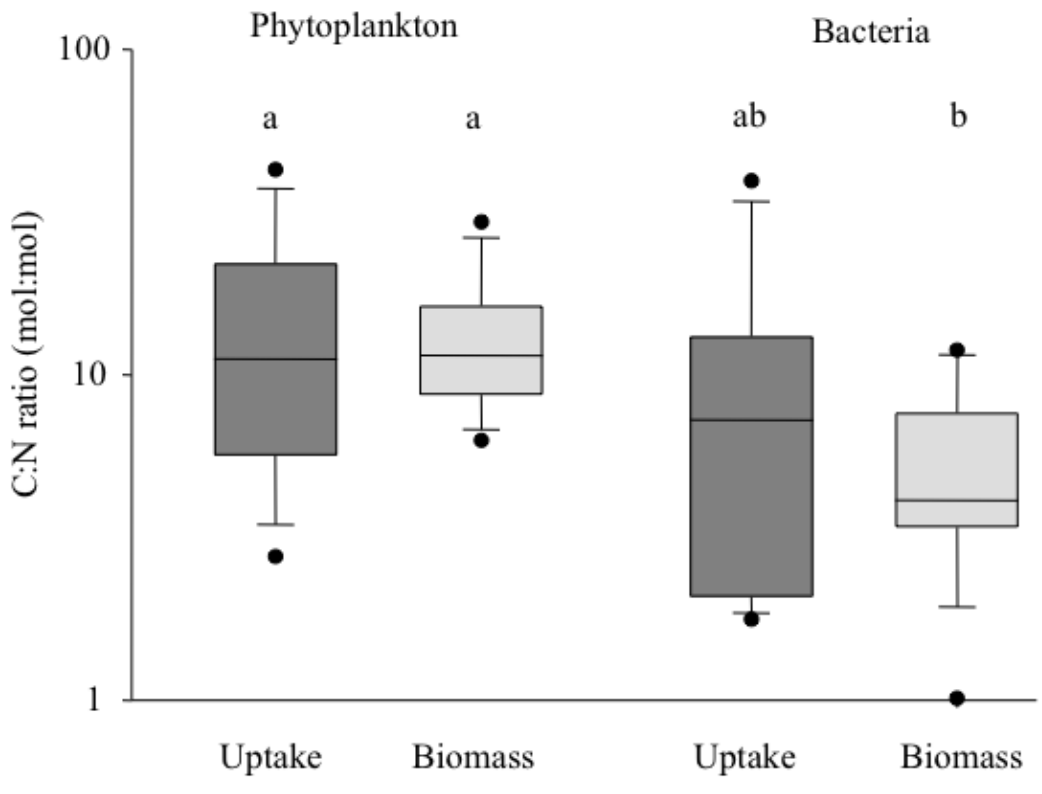

Figure 4. Bacteria and phytoplankton C and N requirements. The carbon:nitrogen ratio (mol:mol) of substrate uptake and biomass for phytoplankton and bacteria measured using incorporation of ^{14}C-Leucine, ^{14}CO2 and $^{15}NH_4$ and $^{15}NO_3$, and size-fractionation for particulate organic carbon and nitrogen measurements. The lowest boundary of the boxes indicates the lower quartile, the line within the boxes marks the median, and the upper boundary of the boxes indicates the upper quartile. Lines above and below the boxes indicate the upper and lower deciles, respectively. Dots represent outliers.

Table 1. Correlation matrix of variables from North Sea to Greenland waters.

Pearson correlations were performed on log10-transformed variables. The correlation significance was tested with (dark values) and without (greyed values) Bonferroni correction with a significance level set at $p \leq 0.01$, n=19. TEMP: sea temperature, SAL: salinity, DIC, dissolved inorganic carbon concentration , PO4: phosphate, SiO4: silicate, NH4: ammonia, NO3: nitrate, $POCt_{ot}$: total particiulate organic carbon concentration, PON_{tot}: Total particulate organic nitrogen concentration, CHLa: Chlorolophyll *a* concentration, BACT: free-living bacteria abundance, $BacNO3_{up}$: bacterial nitrate uptake rate,$BacNH4_{up}$: bacterial ammonia uptake rate, $PhyNO3_{up}$: Phytoplanktonic nitrate uptake rate, $PhyNH4_{up}$: Phytoplanktonic ammonia uptake rate, PPP: Particulate primary production rate, BPP: bacterial uptake rate of phytoplanktonic exudate, DPP: dissolved primary production rate, BP_{Thy}: bacterial production using ^{3}H-Thymidine, BP_{Leu}: bacterial production using ^{14}C-Leucine

	TEMP	SAL	DIC	PO4	SIO4	NH4	NO3	POC_{tot}	PON_{tot}	CHL*a*	BACT	Bac $NO3_{UP}$	Bac $NH4_{UP}$	Phy $NO3_{UP}$	Phy $NH4_{UP}$	PPP	BPP	DPP	BP_{Thy}	BP_{Leu}
TEMP	1																			
SAL	0.81	1.00																		
DIC	0.60	0.56	1.00																	
PO4	-0.08	0.08	0.27	1.00																
SIO4	0.28	0.32	0.53	0.76	1.00															
NH4	-0.17	0.06	-0.22	0.22	0.18	1.00														
NO3	0.26	0.41	0.44	0.69	0.88	0.21	1.00													
POC_{tot}	0.28	0.18	0.27	0.38	0.61	0.44	0.39	1.00												
PON_{tot}	0.07	0.23	0.14	0.33	0.58	-0.09	0.58	0.20	1.00											
CHL*a*	0.61	0.53	0.47	0.13	0.61	0.11	0.41	0.80	0.40	1.00										
BACT	0.27	-0.02	0.40	-0.01	0.25	-0.02	0.26	0.11	-0.10	0.16	1.00									
Bac $NO3_{UP}$	0.54	0.60	0.52	0.47	0.72	0.01	0.88	0.28	0.56	0.42	0.32	1.00								
Bac $NH4_{UP}$	0.37	0.43	0.57	0.30	0.55	0.39	0.53	0.57	0.35	0.48	0.39	0.58	1.00							
Phy $NO3_{UP}$	0.52	0.61	0.45	0.37	0.69	0.13	0.62	0.64	0.62	0.78	0.04	0.69	0.64	1.00						
Phy $NH4_{UP}$	0.43	0.31	0.50	0.17	0.55	0.10	0.34	0.69	0.42	0.75	0.39	0.34	0.65	0.80	1.00					
PPP	0.64	0.54	0.49	0.10	0.59	0.06	0.45	0.68	0.40	0.94	0.24	0.54	0.50	0.84	0.74	1.00				
BPP	0.56	0.44	0.37	0.01	0.53	0.03	0.37	0.59	0.44	0.90	0.20	0.41	0.29	0.72	0.64	0.95	1.00			
DPP	0.54	0.45	0.37	-0.06	0.34	-0.11	0.23	0.37	0.34	0.73	0.13	0.40	0.11	0.72	0.37	0.81	0.88	1.00		
BP_{Thy}	0.32	0.44	0.36	0.35	0.52	0.06	0.42	0.39	0.40	0.56	0.03	0.41	0.37	0.77	0.71	0.68	0.58	0.43	1.00	
BP_{Leu}	0.47	0.52	0.43	0.09	0.28	-0.22	0.23	0.30	0.35	0.56	-0.06	0.36	0.20	0.73	0.63	0.66	0.58	0.55	0.85	1.00