Elemental composition of natural populations of key microbial groups in Atlantic waters

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Summary

Intracellular carbon (C), nitrogen (N) and phosphorus (P) content of marine phytoplankton and bacterioplankton can vary according to cell requirements or physiological acclimation to growth under nutrient limited conditions. Although such variation in macronutrient content is well known for cultured organisms, there is a dearth of data from natural populations that reside under a range of environmental conditions. Here, we compare C, N and P content of *Synechococcus*, *Prochlorococcus,* low-nucleic acid (LNA)-containing bacteria and small plastidic protists inhabiting surface waters of the North and South subtropical gyres and the Equatorial Region of the Atlantic Ocean. Whilst intracellular C:N ratios ranged between 3.5-6, i.e., below the Redfield ratio of 6.6, all the C:P and N:P ratios were up to ten times higher than the corresponding Redfield ratio of 106 and 16, respectively, reaching and in some cases exceeding maximum values reported in the literature. Similar C:P or N:P ratios in areas with different concentrations of inorganic phosphorus suggests that this is not just a response to the prevailing environmental conditions but an indication of the extremely low P content of these oceanic microbes.

Introduction

Carbon (C), nitrogen (N) and phosphorus (P) are major elements that constitute the building blocks and transfer the metabolic energy required by all living cells for maintenance, growth and reproduction. Whereas C is found in most organic molecules, N is abundant in proteins, nucleic acids, osmolytes and, in the case of marine phototrophs also in pigments such as chlorophylls *a*, *b*, *c*, and phycobilins. P, on the other hand, is abundant in nucleic acids (RNA and DNA) and phospholipids. In 1958, based on a large set of environmental measurements, Alfred C. Redfield formulated the concept that in marine plankton the elements C, N and P are in a constant molar ratio of 106:16:1 (i.e. the Redfield ratio) based on the average elemental composition of marine organisms (Goldman *et al.*, 1979). Since then these ratios have been used, amongst other things, to assess the physiological status of photosynthetic organisms, where deviations from Redfield would indicate growth limitation due to low nutrient availability (reviewed in Geider and La Roche, 2002).

Although some environmental data is available for heterotrophic marine bacteria (Fagerbakke et al., 1996; Kuipers et al., 2000; Gundersen et al., 2002; Heldal et al., 2012), the elemental composition of oceanic photosynthetic organisms (both eukaryotic and prokaryotic), and hence their Redfield ratio, has mainly been determined from cultures grown under nutrient-replete or nutrient-deplete conditions (e.g. see Verity et al., 1992; Geider and La Roche, 2002; Bertilsson et al., 2003; Heldal et al., 2003; Ho et al., 2003; Veldhuis et al., 2005 and references therein). Estimates of C content for environmental samples are usually obtained by using a conversion factor to cell volume (e.g. see Zubkov et al., 2000; Worden et al., 2004; Grob et al., 2007), but to our knowledge no direct measurements have ever been published. Thus, little is still known of the elemental composition of natural marine microbial cells.

We focus here on determining cellular elemental stoichiometry of key marine microbial groups: i) cyanobacteria of the genera *Prochlorococcus* and *Synechococcus* responsible for a major fraction of primary production (Li et al., 1994; Jardillier et al., 2010), ii) plastidic protists that can dominate the photosynthetic carbon biomass and contribute significantly to total primary production (Jardillier et al., 2010; Grob et al., 2011) and graze upon bacterioplankton at rates comparable to those of aplastidic protists (Hartmann et al., 2012) and, iii) low nucleic acid content (LNA) bacterioplankton mainly comprising SAR11, a ubiquitous clade in the marine environment that numerically dominates bacterioplankton (Morris et al., 2002; Mary et al., 2008; Gómez-Pereira et al., 2012).

We chose four regions of the Atlantic Ocean with different nutrient, chlorophyll *a* and primary production levels (Poulton et al., 2006) to compare the elemental composition of individual cells (see Table S1 for the number of cells analysed in each case) from the above mentioned groups using X-ray microanalysis in the transmission electron microscope (TEM) (Norland et al., 1995). This approach allowed us to measure *in situ* elemental composition of environmentally relevant groups that have few representatives in culture and to evaluate the influence of the environment, compared to group-specific variability, in driving their intracellular C:N:P stoichiometry.

Results

The cells analysed in the present work were collected during two different cruises across the Atlantic Ocean, AMT18 and AMT19, that took place in October-November 2008 and 2009, respectively, between the United Kingdom and Chile. Environmental conditions from the regions sampled are summarised in Table 1. Briefly, chlorophyll *a* concentrations at the surface were very low during AMT18, i.e., ≤ 0.02 mg m^{-3}, the macronutrients being below the detection limit at all locations for inorganic nitrogen and in the Northern Gyre (NG) for

inorganic phosphate (Pi) with the latter increasing towards the Southern Gyre (SG) to a maximum value of 0.13 µmol l^{-1}. For samples taken at depth, chlorophyll *a* and nutrient concentrations were higher than at the surface in the northern part of the NG (NGI), whereas in the southern part of the NG (NGII) Pi was still below the detection limit (NGII seems to have more relaxed P dynamics, i.e. a longer turnover time than NGI). These two distinct NG provinces were defined based on clear differences in *Synechococcus* abundance (Zubkov et al., 2000) and the biogeography of *Synechococcus* clades observed in dot blot hybridisation and metagenomics data (see methods; see Zwirglmaier et al 2007; Ostrowski and Scanlan, *unpublished data*). Surface chlorophyll *a*, as well as inorganic nitrogen concentrations, were higher during AMT19 than AMT18 in both NGI and SG. During AMT19 Pi concentrations were higher in NGI than in the SG compared to AMT18, reaching a maximum of 0.03 mg m^{-3} in the latter (Table 1).

Intracellular C, N and P content.

Surface samples. During AMT18, *Prochlorococcus* intracellular C and N content ranged on average between ~10-70 and 1.5-18 fg $cell^{-1}$, respectively, increasing from north to south (Fig. 1a-b) following the trend in mean cell volume (Fig. 1d). *Synechococcus* C and N content, on the other hand, averaged between ~50-95 and 10-20 fg $cell^{-1}$, respectively (Fig. 1a-b), the former element and cell volume being also higher in EQ and SG than in the NG (Fig. 1a and d). With the exception of NGII, however, no significant differences in their N content were found between regions ($p > 0.05$). The average C content registered for LNA cells increased from ~8 fg $cell^{-1}$ in NGI to ~15 fg $cell^{-1}$ in NGII and EQ ($p < 0.05$; Fig. 1a) in agreement with the trend observed in cell volume (Fig. 1d). The intracellular N content for this group was approximately 2 fg $cell^{-1}$ in all of the above regions, with a statistically significant difference observed only between NGII and EQ ($p < 0.05$).

In terms of P content, a minimum of 0.03-0.05 fg cell^{-1} was registered in NGII for *Prochlorococcus* and LNA bacteria and in EQ for the latter, with no significant differences observed between these three populations ($p > 0.05$; Fig. 1c). In NGI and EQ ($p > 0.05$) *Prochlorococcus* cells contained on average ~0.13 fg of P, reaching a maximum of ~0.4 fg cell^{-1} in SG which was comparable with the maximum registered for *Synechococcus* cells in both NGI and SG ($p > 0.05$; Fig. 1c). It is worth noting that although *Synechococcus* cells were on average significantly larger than *Prochlorococcus* in SG ($p < 0.01$; Fig. 1d), no significant differences in C, N or P content were found between the two cyanobacteria at this station ($p > 0.05$). Overall, *Prochlorococcus* P content tended to increase from north to south whereas that of *Synechococcus* was highest at the northern- and southern-most stations, reaching only ~0.26 fg cell^{-1} in NGII and EQ ($p > 0.05$; Fig. 1c). LNA bacteria maximum P content, on the other hand, was registered in NGI and was not significantly different from that of *Prochlorococcus* at the same station ($p > 0.05$; Fig. 1c).

C, N and P elemental content measured for small plastidic protists in NGI during AMT19 were significantly higher than for any other population analysed ($p < 0.001$; Fig. 1a-c), as expected from their larger cell volume ($p < 0.001$; Fig. 1d). *Prochlorococcus* sampled in NGI during the same cruise had on average ~6.8, 1.8 and 0.03 fg of C, N and P per cell (Table S2), with C and P content being significantly lower ($p < 0.01$) and N content being not significantly different ($p > 0.05$) from what was measured during AMT18. *Prochlorococcus* cells were also significantly smaller than observed during AMT18 ($p < 0.001$). *Synechococcus* sampled in the SG during AMT19, on the other hand, had significantly higher C and N content (94 and 28 fg cell^{-1}, respectively; $p < 0.01$) and significantly lower P content (0.26 fg cell^{-1}; $p < 0.001$) than the same population sampled during AMT18, even if no significant differences were found between their average cell volume ($p > 0.05$).

Deep samples. All cyanobacterial cells sampled at depth in NGI (142m) and/or NGII (127m) during AMT18 had significantly higher C and N content than cells samples in surface waters ($p < 0.05$). Indeed, a three- and two-fold increase in their elemental content was observed in the case of *Prochlorococcus* and *Synechococcus*, respectively (Table S2). No differences were found, however, in intracellular P content of surface and deep populations sampled in the Northern Gyre ($p > 0.05$; Table S2). Also, although deep *Prochlorococcus* cells were significantly larger than at the surface in NGI ($p < 0.01$), this was not the case in NGII ($p > 0.05$; Table S2).

Finally, it is worth noticing that when considering the entire dataset there is a clear positive relationship between cell volume and average C, N and P content, the latter being the weakest of the three ($R^2 = 0.73$; Fig. S1), despite the large cell to cell variability observed in some cases within a given population (Fig. S2).

Molar C:N, C:P and N:P ratios. Most of the C:N ratios measured here, including both AMT18 and AMT19 cruises, as well as surface and deep waters, were lower than the established Redfield ratio of 6.6 (Fig. 2a; Table 2). C:P and N:P ratios, on the other hand, were all above Redfield values, i.e., above 106 and 16, respectively (Fig. 2b-c).

Surface samples. During AMT18, *Prochlorococcus* and *Synechococcus* C:N ratios varied from 4.0-7.3 and 3.6-5.5, respectively, with the lowest values being recorded in NGI. With the exception of a *Prochlorococcus* C:N ratio maximum in NGII, the C:N ratio for both cyanobacteria was quite homogeneous across the different sampled regions (Fig. 2a). For LNA bacteria, on the other hand, the C:N ratio varied from 5-6 in the Northern Gyre to a maximum of 16 in EQ (Fig. 2a).

In general, C:P ratios tended to increase from NGI to EQ where maxima of ~880, 1000 and 1430 were recorded for *Synechococcus, Prochlorococcus* and LNA bacteria, respectively

(Fig. 2b). The same trend was observed for the N:P ratios of cyanobacteria, with *Synechococcus* and *Prochlorococcus* reaching ~180 and 200 in the EQ region, respectively (Fig. 2c). LNA bacteria, however, presented a N:P ratio of ~80 in both NGI and EQ and a maximum of ~190 in NGII (Fig. 2c). Finally, the C:P and N:P ratios registered in SG for cyanobacteria were closer to those observed in NGI than any other region (Fig. 2b and c).

In the case of small plastidic protists sampled during AMT19, the C:N, C:P and N:P ratios reached ~5, 640 and 128, respectively. Compared to AMT18, the *Prochlorococcus* population sampled in NGI during this cruise showed higher C:N, C:P and N:P ratios, with the latter two reaching ~690 and 150, respectively (Table 2). *Synechococcus* sampled in SG during AMT19, on the other hand, showed similar C:N but higher C:P and N:P ratios than during AMT18.

Deep samples. Surface C:N ratios were higher than at depth for both cyanobacterial genera in NGII, but not for *Prochlorococcus* in NGI (Table 2). C:P and N:P ratios, on the other hand, were always higher at depth. The largest difference between surface and deep populations was observed for *Prochlorococcus* in NGI, where C:P and N:P experienced a ~4- and 3-fold increase, respectively. Finally, when comparing deep cyanobacteria populations *Prochlorococcus* had higher C:N, C:P and N:P ratios than *Synechococcus* (Table 2).

Discussion

Here we present data on the C, N and P content of natural *Prochlorococcus*, *Synechococcus*, LNA bacteria and small plastidic protists cells sampled in four different regions of the Atlantic Ocean. Overall, our results show that the intracellular C, N and P content of these groups vary between regions and with depth, following a general trend in cell volume. In some cases, however, cells of similar size show significant differences in elemental content, e.g., *Prochlorococcus* and *Synechococcus* in the SG, implying a certain degree of plasticity.

Undoubtedly, the most striking finding in this dataset is the very low intracellular P content in most of the cells analysed. Indeed, in five samples the average P content for LNA bacteria and *Prochlorococcus* was considerably lower than the theoretical values of 0.13 and 0.17fg $cell^{-1}$, respectively, expected from their genome size alone, i.e., 1.3 Mb (Giovannoni et al., 2005) for *Pelagibacter ubique* (SAR11 clade, the main component of LNA bacteria; Mary et al., 2008; Gómez-Pereira et al., 2012) and 1.64 Mb (Kettler et al., 2007) for *Prochlorococcus*. To rule out a methodological problem we examined individual background spectra and corroborated that their P signal was similar across all samples, implying that a higher background signal would not explain the extremely low P values registered for the organisms mentioned above. The fact that we used an internal standard, i.e., purposefully calibrated beads, further supports the idea of these extreme values not being related to any methodological errors associated with the x-ray microanalysis technique.

It is also possible that the extremely low P content recorded for LNA bacteria (NGII and EQ) and *Prochlorococcus* (NGII at the surface and at depth and NGI during AMT19) was the result of particles on grids being wrongly identified as intact cells under the microscope, especially given these cells are the smallest and potentially most fragile ones. Whilst cell damage or lack of viability cannot be completely ruled out, several lines of evidence suggest against this possibility. Firstly, cells were sorted on the basis of their DNA content by using a nucleic acid stain (see Experimental procedures) so these cells clearly possess DNA and hence should be intact. Furthermore, under the microscope these cells looked essentially the same as those cells clearly possessing sufficient P to account for known genome sizes (see Fig. S3). Finally, the C and N content of these cells was within previously published ranges (Fagerbakke et al., 1996). Hence, it is possible that there are open ocean representatives of these populations with genome sizes even smaller than those established for cultured representatives, although further genomes would need to be sequenced to address this issue.

197 Regardless of the above, our dataset clearly shows that open-ocean picocyanobacteria, LNA
198 bacteria and small plastidic protists possess an intracellular P content at a strict minimum
199 level to support life and growth. For instance, *Synechococcus* P content in NGI and SG is
200 comparable to that of the same group grown in culture under P-limited conditions (Bertilsson
201 et al., 2003). The same is true for *Prochlorococcus* in SG (Bertilsson et al., 2003), although
202 similar values have also been reported for cultures grown under P-replete conditions (Heldal
203 et al., 2003). In NGII and EQ, however, both groups have lower intracellular P content than
204 previously reported. LNA bacteria, on the other hand, have barely enough P to account for
205 their whole genome in NGI and not even that in NGII and EQ (see above). Because surface
206 Pi concentrations were below the detection limit at most stations, we cannot deduce whether
207 the P content of cyanobacteria or LNA bacteria is related to the latter or not (Fig. 1c; Table
208 1).

209 Since the genetic diversity of picocyanobacterial populations is high (Fuller et al., 2003;
210 Johnson et al., 2006; Martiny et al., 2007; Mazard et al., 2012) many discrete strains are
211 likely to be present in the cyanobacteria sorted populations. Hence, differences in average P
212 content observed for *Synechococcus* cells of similar size sampled in the various regions (i.e.,
213 NGI and NGII or EQ and SG; Fig. 1c-d) might be due to inter-strain differences in
214 polyphosphate content (Heldal et al., 2003; Mazard et al., 2012). For *Prochlorococcus*, in the
215 EQ and SG regions, the P content of membrane lipids may be responsible for this variation in
216 P content since in the oligotrophic open ocean *Prochlorococcus* is known to synthesize lipids
217 that contain sulphur and sugar instead of phosphate (i.e., sulfoquinovosyldiacylglycerol; Van
218 Mooy et al., 2006). Furthermore, physiological responses in terms of P content in lipids have
219 been reported for microcosm experiments amended with both Pi and inorganic nitrogen in the
220 Mediterranean Sea (Popendorf et al., 2011). The above could also account for the lack of
221 significant differences in P content between surface and deep *Prochlorococcus* populations

222 but higher at depth for *Synechococcus* (Van Mooy et al., 2006), which would also be
223 consistent with observations that *Prochlorococcus* are more tolerant of low Pi conditions than
224 *Synechococcus* (Moutin et al., 2002; Fuller et al., 2005). The lower P content observed in
225 *Synechococcus* cells of comparable size ($p > 0.05$) sorted in the SG during AMT19 compared
226 to AMT18, may also be attributed to differences in environmental Pi concentration (Table 1).
227 Given that the SG is usually Pi-replete, the above further emphasises the idea of plasticity of
228 elemental content to adapt to lower Pi availability.

229 To our knowledge, this is the first report of direct measurements of intracellular P content in
230 natural picoplanktonic plastidic protist cells. Given that the sorted plastidic protist population
231 contained members of different classes (Grob et al., 2011), the average P content for this
232 group in NGI during AMT19 would correspond to an organism with a genome size of 26 Mb.
233 This is consistent with genome sizes ranging from 21 to 26Mb reported for eukaryotic
234 picophytoplankton such as *Micromonas sp* (Worden et al., 2009) and picoprymnesiophytes
235 (the latter estimated from gene content; Cuvelier et al, 2010), respectively. As for
236 cyanobacteria then, most of their P content can be allocated to nucleic acids suggesting that in
237 the open ocean these organisms, by necessity, maintain a strict P minimum. Plastidic protists
238 presumably achieve this low P content by reducing their Pi demand elsewhere in the cell, as
239 has been shown for eukaryotic phytoplankton grown under Pi-limiting conditions (Geider and
240 La Roche, 2002), including substituting phospholipids by non-phosphorus membrane lipids
241 (Van Mooy et al., 2009).

242 Considering all sampled stations, the intracellular C and N content recorded here for
243 cyanobacteria was within previously reported ranges for cultured representatives (Bertilsson
244 et al., 2003 and references therein; Heldal et al., 2003). Although *Synechococcus* C content
245 was lower than reported by Heldal et al. (2003), it was comparable with values reported by
246 Bertilsson et al. (2003) for cultures and by Grob et al. (2007) for open-ocean populations.

247 Interestingly, these groups' average cell volume and C content was higher where the
248 environmental Pi concentration was above the detection limit, even if nitrate + nitrite
249 concentrations remained undetectable, i.e., EQ and SG (Fig. 1b-c; Table 1). The latter
250 suggests that Pi availability could play an important role in determining cyanobacterial cell
251 size and elemental content. The fact that only *Prochlorococcus* had a lower N content in the
252 Northern Gyre supports the idea that this genus has different N requirements to
253 *Synechococcus* (Heldal et al., 2003). The C content for small plastidic protists sampled in the
254 NGI during AMT19 is, on the other hand, within the range of values expected for these
255 organisms (see Worden et al., 2004; Grob et al., 2007). If we only consider the smaller cells
256 used by Verity et al. (1992) to establish a direct relationship between cellular C and N content
257 for different nano- and picophytoplanktonic groups (including *Synechococcus*), N
258 concentration in these cells is also close to the values predicted from C.

259 In deeper waters of the NG, C and N content of *Synechococcus* and *Prochlorococcus* was
260 higher than at the surface (Table 2), presumably a result of the high C and N content of
261 chlorophyll *a* and accessory pigments that are present in larger amount in cells from these
262 low-light environments. Similarly, the high productivity reflected by higher chlorophyll *a*
263 levels and *Synechococcus* abundance in the SG during AMT19 compared to AMT18 (Table
264 1) would account for the greater intracellular C and N content recorded for this
265 cyanobacterium.

266 **Molar ratios.** The Redfield ratio of 106:16:1 for C:N:P has long been used as a reference to
267 assess the physiological status of marine phytoplankton (e.g., Geider and La Roche, 2002;
268 Bertilsson et al., 2003). Recently, it has been suggested that it is the difference in elemental
269 stoichiometry between species that allows for niche creation and the maintenance of
270 biodiversity (Göthlich and Oschlies, 2012). We compare below C:N, C:P and N:P ratios of
271 natural microbial populations sampled in different regions of the Atlantic Ocean to evaluate

the influence of the environment, compared to group-specific variability, in driving their element stoichiometry.

Considering our entire data set (i.e., four different groups spanning 17 samples), the only C:N ratio that was not within previously published average ranges was that of LNA bacteria in the EQ region, i.e., ~16 (Fig. 5a). Although this ratio may seem high, values up to ~20 have been estimated from C production coupled with N and P consumption measurements in N+P amended bio-essay experiments in the DCM of the subtropical North Atlantic Ocean (Kuipers et al., 2000). The rest of our C:N ratios for LNA bacteria fall, however, within the range of 1.1-12 established for heterotrophic bacteria from cultures or natural populations grown under different conditions (Kuipers et al., 2000; Gundersen et al 2002; Vrede et al., 2002; Heldal et al., 2012). This plasticity in bacterioplankton elemental ratios is thought to be a strategy to overcome, at least partly, periods of nutrient limitation in oligotrophic regions (Kuipers et al., 2000).

C:N ratios reported for various cultured phytoplankton (including cyanobacteria) grown under different nutrient conditions range between 3-17 (Geider and La Roche, 2002; Ho et al., 2003; Veldhuis et al., 2005 and references therein), where values higher than 12 would indicate a drop in protein content below about 25% of cell biomass (Geider and La Roche, 2002). Despite this large variability, however, the C:N ratio is generally very close to the Redfield value of 6.6. According to the literature mentioned above, our C:N values are consistent with organisms grown under nutrient-replete conditions. In contrast C:P and N:P ratios tell a very different story.

Previously reported C:P ratios vary between 73 and 787 for cyanobacterial cultures grown under normal and P-limited conditions, respectively (Heldal et al., 2003, Bertilsson et al., 2003). For different phytoplankton groups (including cyanobacteria) grown under nutrient-

296 replete conditions this range extends from 27-200 (Geider and La Roche, 2002; Ho et al.,
297 2003). Conversely, average C:P ratios for heterotrophic bacteria range between 15 and 180
298 (Gundersen et al., 2002; Kuipers et al., 2002; Vrede et al., 2002; Heldal et al., 2012), with a
299 couple of exceptionally high values (up to 310) matching the C:N outliers (see above,
300 Kuipers et al., 2002). Interestingly, whereas in NGI, NGII and SG we found C:P ratios that
301 were within published ranges, in EQ, where the surface Pi concentration was higher
302 compared to NGI and NGII (Table 1), C:P ratios exceeded the maximum reported for P-
303 limited conditions (Fig. 5b). The EQ region being generally more productive than the gyres
304 (Poulton et al., 2006), would impart a strong competition for resources which could explain
305 the extremely high C:P ratios measured there. The above could also explain the increase in
306 C:P ratio observed between NGI and NGII, the latter having a higher chlorophyll *a*
307 concentration (i.e., higher photosynthetic biomass) despite Pi being below the detection limit
308 in both regions.

309 Although most of our N:P data falls within previously published ranges, the values recorded
310 here for LNA bacteria in the NGII province, for both cyanobacteria in the EQ region
311 (AMT18) and for *Synechococcus* in SG during AMT19 all exceed the highest published
312 ratios (Fig. 5). N:P ratios from cultured marine cyanobacteria grown under nutrient-replete
313 conditions vary between 8 and 43 (Bertilsson et al., 2003; Heldal et al., 2003). In contrast,
314 during Pi-deplete growth this ratio increases drastically to between 62-109 (Bertilsson et al.,
315 2003). Values as low as 5 or up to 19 have also been reported for other phytoplankton groups
316 during nutrient-replete growth (Geider and La Roche, 2002), with an optimal ratio of 11-133
317 estimated for different nanophytoplankton species using models, where the highest values are
318 associated with nutrient limited growth (see Klausmeier et al., 2004). Although average
319 values for heterotrophic bacteria are between 11 and 13 (Kuipers et al., 2000; Heldal et al.,
320 2012), extreme values of 1 and 25 have previously been estimated (Kuipers et al., 2000).

321 Considering all of the above, our highest N:P ratios, i.e., above 100, suggest severe P-
322 limitation for several open ocean marine microbial populations (Geider and La Roche, 2002).

323 It has been previously shown that C:P and N:P ratios tend to be lower in fast- rather than in
324 slow- growing phytoplankton, especially under P-limited conditions, approaching Redfield
325 only at high growth rate (Goldman et al., 1979). More recently, this influence of growth rate
326 on elemental stoichiometry has been attributed to the need of different taxa to maintain high
327 numbers of P-rich ribosomes to ensure protein synthesis, (Elser et al., 2000). Interestingly,
328 the high element to P ratios found in the present work support this idea as phytoplankton
329 growth rates reported for the nutrient-poor picophytoplankton-dominated regions of the
330 Atlantic Ocean sampled here are very low (0.2 d^{-1}; Marañón et al., 2000). Furthermore, the
331 differences observed between surface and deep samples are also consistent with
332 cyanobacteria growth rates decreasing with light availability (Vaulot et al., 1995; Moore et
333 al., 1995), i.e., being lower at depth. It has also been shown that plants, animals, algae, yeast
334 and bacteria exposed to cold contain more P and N, the latter to a lesser extent in bacteria
335 (Woods et al., 2003). Although the difference in temperature between surface and deep
336 waters (i.e., lower at depth) could have an influence on the increase observed in element to P
337 ratios, it is difficult, because of our limited dataset, to assess whether this was the case for
338 latitudinal differences as well.

339 In summary, we found that C, N and P content in *Prochlorococcus*, *Synechococcus*, LNA
340 bacteria and small plastidic protists show different degrees of homeostasis and/or plasticity
341 that seems to be group- and/or element-specific and can vary between regions and depth.
342 Although average C:N ratios are close to Redfield and close to those registered for different
343 groups grown under nutrient-replete conditions according to the literature (see above), the
344 observed C:P and N:P ratios are consistent with severe Pi-limitation. Similar C:N or N:P
345 ratios in areas with different concentrations of Pi suggest these low levels of P are not just a

346 physiological response to the prevailing environmental conditions. Rather, the above agrees
347 with previous work suggesting that differences in elemental composition represent
348 evolutionary differences in cell requirements and the ability to take up and store these
349 elements, rather than environmental or culture conditions (Quigg et al., 2003; Ho et al.,
350 2003). Noteworthy though, is that despite potential P-limitation the cells analysed here are
351 still highly active, undertaking photosynthesis (Marañón et al., 2001; Grob et al., 2011),
352 acquiring amino acids and P (Zubkov et al., 2007b; Mary et al., 2008; Gómez-Pereira et al.,
353 2012) or grazing, as is the case for plastidic protists (Hartmann et al., 2012), which suggests
354 that they have adapted to the impoverished environmental conditions by lowering their
355 nutrient requirements, especially for P.

356 **Experimental procedures**

357 Samples were collected in the Northern Gyre (NG), the Equatorial Region (EQ) and the
358 Southern Gyre (SG) of the Atlantic Ocean within the framework of the Atlantic Meridional
359 Transect program (www.amt-uk.org) during AMT18 cruise (October-November 2008) on
360 board the Royal Research Ship (RRS) *James Clark Ross*. Additionally, three samples were
361 collected during AMT19 (October-November 2009) on board the RRS *James Cook* (Table 1).
362 These different regions of the Atlantic Ocean have been described previously (Hartmann et
363 al. 2012). In the present work we further divided the Northern Gyre into two distinct
364 provinces, NGI and NGII, based on clear changes in the biogeography of *Synechococcus*
365 clades observed in dot blot hybridisation and metagenomics data (see Zwirglmaier et al 2007;
366 Ostrowski and Scanlan, *unpublished data*), with surface phosphate concentrations in NGI
367 being recurrently depleted (Zubkov et al., 2007a).

368 In each case, 10 to 15 L of surface (≤ 30 m) or deep water (127 and 142 m) was collected at
369 the local solar noon using Niskin bottles mounted on a metallic frame conductivity-

370 temperature-fluorescence-depth (CTD) profiler (Sea-Bird 9/11 *plus*). Seawater samples were
371 pre-filtered through 100 µm nylon mesh into a darkened container and the 100-0.2 µm
372 plankton fraction concentrated on board by gently pumping (peristaltic pump, Watson
373 Marlow 323S/D, UK) water at a flow rate of 90-100 ml min^{-1} through a CellTrap^{TM} cartridge
374 (MEM-TEQ Ventures Ltd., UK). All samples were immediately flash frozen in liquid
375 nitrogen, without adding any fixative, and stored at -80°C until sorting. All samples were
376 concentrated and flash frozen within 1h of the CTD coming onboard.

377 A small volume (~300 µl) was carefully scraped off each frozen sample, thawed at room
378 temperature and stained with SYBR-Green I dye (see Zubkov et al. 2007b). *Prochlorococcus,*
379 *Synechococcus*, low nucleic acid content (LNA) bacteria and small plastidic protists (Plast-S)
380 were identified based on their flow cytometry autofluorescence, nucleic acid content and
381 scattering (Zubkov et al., 2007b) and sorted using a MoFlo flow cytometer (Dako
382 Cytomation, UK). Sorted cells (1×10^3-2×10^5) were centrifuged for 10min at 1700g and
383 20°C (5417R Eppendorf centrifuge equipped with a swing-out rotor) onto 100-mesh
384 aluminium electron microscope grids (Agar Scientific Ltd, UK) previously coated with
385 formvar and carbon.

386 Previous data had shown that no major loss of P was detected in x-ray microanalysis of
387 frozen/thawed cells (Heldal, Erichsen and Norland, *unpublished data*) though we cannot
388 exclude that small fractions of soluble P might be lost from bacterial and cyanobacterial cells.
389 Moreover, when analysing fresh samples, Larsen et al. (2008) showed no major differences in
390 P-leakage between fixed and unfixed *Synechococcus* cells. Furthermore, after a single freeze
391 (-80°C)-thaw cycle the viability of soil bacteria decreases by only 15% (Crisler et al., 2012).
392 Given the above, plus the long time involved in the preparation and analysis of each sample,
393 and that samples could not be processed immediately, freezing and thawing without using
394 fixatives that can be slightly acidic was considered the optimal processing pipeline.

To maximise the number of cells collected on each microscope grid we placed the latter at the bottom of a female luer sealing plug of the same diameter (Altec Products Ltd, UK) trimmed to fit inside a 500 µl Eppendorf tube (Eppendorf UK Limited) and fitted a 2 ml syringe (Becton Dickinson) onto it so that the grid rested between the plug and the syringe. We then placed the ensemble inside the Eppendorf tube and cut the syringe end to ~1.5 cm long to fit the centrifuge. The sorted cells were placed inside the syringe end for centrifugation. X-ray microanalyses were performed at the Laboratory for Electron Microscopy of the Faculty of Mathematics and Natural Sciences, University of Bergen, Norway, using the same instrument and settings as described in Heldal et al (2012), except for the tilt angle which here was 13° to maximise the amount of X-rays hitting the detector.

X-ray spectra were obtained from (1) individual cells chosen with extreme care to ensure that they appeared intact under the microscope, i.e., showed no morphological damage (see Fig. S3), and from (2) an equivalent area of the formvar and carbon coat of the grids to subtract the background from the cell signal. For each cell we made sure to choose as background an area with the same characteristics to the one where the cell was resting on. By targeting intact cells we believe we maximise the probability of analysing viable cells. After each analysis we also checked for cell drift, i.e., cells changing position while being swiped by the X-ray due to instability of the formvar and carbon coat. When this rarely occurred we either re-analysed the cells or did not consider them at all.

Our dataset includes the intracellular carbon (C), nitrogen (N) and phosphorus (P) content in fg per cell for the microbial groups mentioned above, sampled at the depths and locations indicated in Table 1. In each case between 8 and 34 cells were analysed (Table S1). Average intracellular C, N and P content for each population were calculated after removing outliers, i.e., values that were more than 2 standard deviations away from the mean. Differences in elemental composition were assessed by applying a t-test after a F-test variance analysis

using the R statistical programme (www.r-project.org). Dissolved inorganic nitrogen (DIN) and phosphorus (DIP) concentrations were determined on surface samples only using a 5 channel Bran and Luebbe AAIII, segmented flow autoanalyser within 3 h of sample collection, using analytical protocols detailed in Woodward and Rees (2001).

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575 Table 1. Summary of samples collected during AMT18 in the Northern Gyre (provinces NGI and NGII), Equatorial Region (EQ) and Southern
576 Gyre (SG) from surface waters (Surf ≤ 30m), at depth (Deep) and at the deep chlorophyll maximum (DCM). Chlorophyll *a* (Chl *a*), temperature
577 (Temp) and salinity (Sal) values are also included. The abundance (x 10^3 cells ml^{-1}) of *Prochlorococcus* (*Proc*), *Synechococcus* (*Syn*), LNA
578 bacteria and total plastidic protists (Plast) and cell area (μm^2 and standard error, SE, in brackets) is also reported when available. (-) indicates
579 that no data is available for the corresponding cruise, station, and/or group.

580

	Region	Location	Depth (m)	Chl *a* (mg m^{-3})	Temp (°C)	Sal	Nitrate + Nitrite (µmol l^{-1})	Phosphate (µmol l^{-1})	*Proc* x10^3 cells ml^{-1} µm^2 (SE)	*Syn* x10^3 cells ml^{-1} µm^2 (SE)	LNA bacteria x10^3 cells ml^{-1} µm^2 (SE)	Plast x10^3 cells ml^{-1} µm^2 (SE)
AMT18	NGI	32.4900°N; 31.7102°W	Surf	0.01	25.3	37.2	<0.02	<0.02	72 0.47 (0.04)	1.4 0.81 (0.06)	- 0.32 (0.03)	0.6 -
			Deep (142m)	0.04	18.2	36.6	2.21	0.11	1.0 0.62 (0.06)	0 -	- -	0.3 -
	NGII	21.6732°N; 39. 5964°W	Surf	0.02	26.5	37.6	<0.02	<0.02	85 0.36 (0.02)	1.2 0.64 (0.06)	- 0.43 (0.04)	0.8 -
			DCM (127m)	0.1	22.2	37.3	0.12	<0.02	20 0.41 (0.03)	0 0.79 (0.08)	-	1.2 -
	EQ	7.6627°N; 30.0594°W	Surf	0.02	29	34.9	<0.02	0.04	40 0.68 (0.06)	2.4 0.97 (0.08)	- 0.52 (0.04)	1.2 -
	SG	20.2833°S; 25.0011°W	Surf	0.01	24.3	37.2	<0.02	0.13	11 0.73 (0.04)	0.4 1.07 (0.06)	- -	0.8 -
AMT19	NGI	37.1015°N; 26.4877°W	Surf	0.02	21.9	36.3	< 0.1	0.03	194 0.26 (0.03)	7.4 -	- -	1.1 3.77 (0.55)
	SG	22.8538°S; 30.8655°W	Surf	0.03	28.6	33.7	< 0.1	< 0.01	101 -	2.2 1.07 (0.07)	-	1.0 -

581

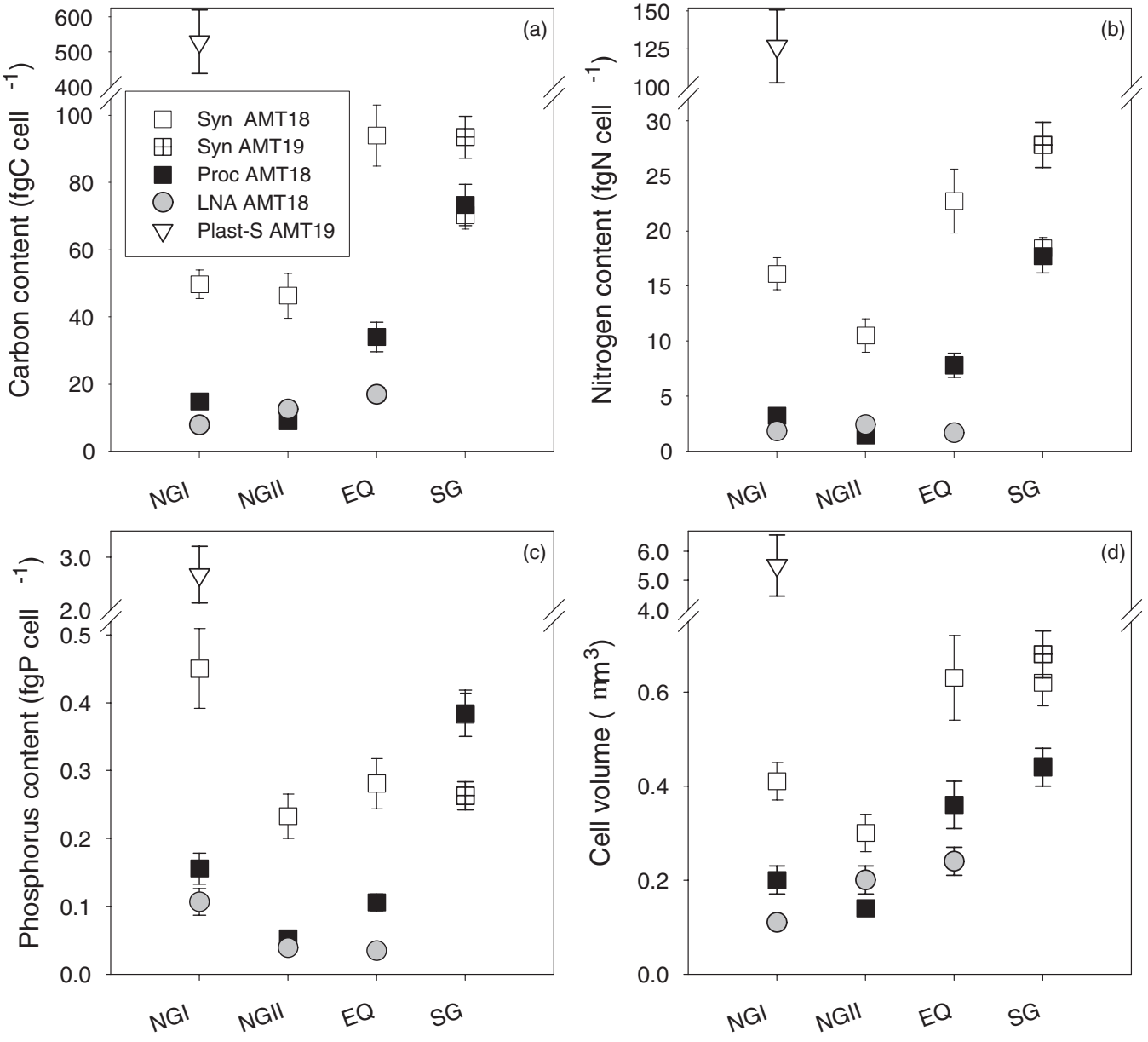
582 Table 2. Average (± standard error) carbon to nitrogen (C:N), carbon to phosphorus (C:P) and nitrogen to phosphorus (N:P) molar ratios for
583 surface (Surf) and deep *Prochlorococcus* (*Proc*) and *Synechococcus* (*Syn*) sampled in the Northern Gyre (provinces NGI and NGII). Only one
584 sample from AMT19 is included. The remainder are from AMT18.

	Proc NGI			*Proc* NGII		*Syn* NGII	
(Redfield)	Surf	Surf (AMT19)	Deep (142m)	Surf	Deep (127m)	Surf	Deep (127m)
C:N (6.625:1)	4.04 ± 0.18	4.57 ± 0.33	5.83 ± 0.15	7.28 ± 0.34	5.62 ± 0.25	5.48 ± 0.36	4.41 ± 0.20
C:P (106:1)	277 ± 43	692 ± 138	1073 ± 153	737 ± 187	1150 ± 238	465 ± 39	621 ± 41
N:P (16:1)	75 ± 10	155 ± 31	185 ± 25	99 ± 20	206 ± 41	100 ± 11	135 ± 11

Figure legends

Fig. 1. Average intracellular carbon (a), nitrogen (b) and phosphorus (c) content (fg $cell^{-1}$, error bars represent standard error) for surface *Synechococcus* (*Syn*), *Prochlorococcus* (*Proc*), LNA bacteria (LNA) and small plastidic protists (Plast-S) populations sampled in surface waters of the Northern Gyre (provinces NGI and NGII), Equatorial Region (EQ) and Southern Gyre (SG) during AMT18 and AMT19.

Fig. 2. Average carbon to nitrogen (C:N; a), carbon to phosphorus (C:P; b) and nitrogen to phosphorus (N:P; c) molar ratios for *Synechococcus* (*Syn*), *Prochlorococcus* (*Proc*), LNA bacteria (LNA) and small plastidic protists (Plast-S) sampled in surface waters from the Northern Gyre (provinces NGI and NGII), Equatorial Region (EQ) and Southern Gyre (SG) during AMT18 and AMT19 (only Plast-S). The dashed line indicates the Redfield ratio.



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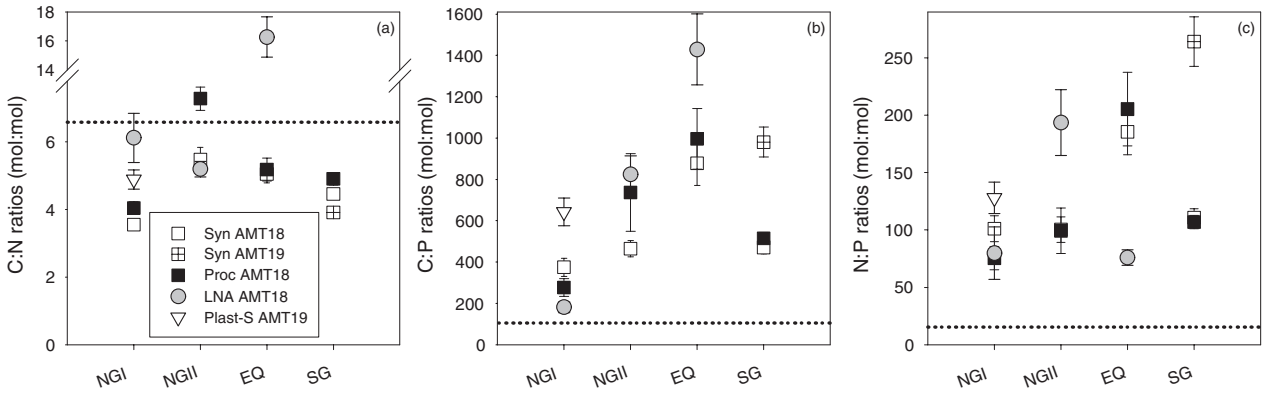

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