

## ***Interactive comment on “Phosphate supply explains variation in nucleic acid allocation but not C : P stoichiometry in the Western North Atlantic” by A. E. Zimmerman et al.***

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We thank the reviewer for their insightful comments on our manuscript. We have taken the reviewer's concerns into consideration for the revised manuscript, and detail these changes in our responses below.

Point 1: The authors are essentially trying to test the GRH without measuring growth rate. The GRH relates elemental stoichiometry and macromolecular content through growth rate. It is unclear that growth rate differs along the environmental P supply gradient. In the North Atlantic, P-supply does not necessarily correlate with growth rate and so I am not sure I would expect to see the difference the authors expect to

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see. Perhaps N is limiting growth (there is quite a bit of evidence that shows autotrophs in the N. Atlantic subtropical Gyre are N-limited or NP co-limited) and C:P is related to N-flux.

Response: Please see response to Point 4 below, as well as response to Reviewer 1, Point 3. Work supporting N limitation or NP co-limitation has largely been done east and south of our sampling transect, in the central and eastern NASG (Mills et al., 2008; Moore et al., 2008). The biogeochemistry in these regions is distinct from the western NASG.

Mills, M.M., Moore, C.M., Langlois, R., Milne, A., Achterberg, E., Nachtigall, K., Lochte, K., Geider, R.J., La Roche, J. 2008. Nitrogen and phosphorus co-limitation of bacterial productivity and growth in the oligotrophic subtropical North Atlantic. *Limnology and Oceanography* 53(2): 824-834.

Moore, C.M., Mills, M.M., Langlois, R., Milne, A., Achterberg, E.P., La Roche, J., Geider, R.J. 2008. Relative influence of nitrogen and phosphorus availability on phytoplankton physiology and productivity in the oligotrophic sub-tropical North Atlantic Ocean. *Limnology and Oceanography* 53(1): 291-305.

Point 2: The SRP flux is calculated from the gradient of SRP concentration between 80m (or deeper) and 5m isn't it? There is no presentation of mixed layer depths or mixing rates that might convince the reader that this SRP even gets to the cells at 5m. Perhaps P turnover rates are more important than deep SRP.

Response: Please see response to Reviewer 1, Point 1. Rates of P turnover are likely also important in such a low-P system, and have been estimated previously to be on the order of < 5 hours within the surface mixed layer (Ammerman et al., 2003).

Ammerman, J.W., Hood, R.R., Case, D.A., Cotner, J.B. 2003. Phosphorus deficiency in the Atlantic: An emerging paradigm in oceanography. *Eos Trans. AGU*, 84(18): 165–170.

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Point 3: The SRP flux gradient is driven by two stations north of 32°N. South of 32°N there is little gradient. Additionally, there is little gradient in SRP or PPhos concentrations south of 32°N. However, DOP concentrations decrease >2 fold from the most southern station to 32°N. It seems like utilization of DOP in this part of the transect may be more important than flux from below.

Response: Indeed DOP is going to be important in this region. But since DOP arises from in situ biological production, the fact that it is increasing means that a) it is less labile the further north you go, b) the demand for it is lower the further N you go, or c) the production rate is higher the further N you go. We don't know which of these three is the answer or if it is some combination of all of them. Regardless, the driver behind this pattern is likely to be vertical PO4 inputs, as there does not appear to be long-range transport of DOP from the eastern North Atlantic upwelling regions (Roussenov et al., 2006; Torres-Valdes et al., 2009). Additionally, uptake of SRP by organisms in this region has been shown to be sensitive to SRP concentration while DOP uptake rates were not sensitive to DOP concentration (Casey et al., 2009). For our data, DOP was not significantly correlated with any of the biological parameters measured. We have revised the discussion to include these points (and references) related to DOP.

Roussenov, V., Williams, R.G., Mahaffey, C., Wolff, G., 2006. Does the transport of dissolved organic nutrients affect export production in the Atlantic Ocean? *Global Biogeochemical Cycles* 20, GB3002, doi:3010.1029/2005GB002510.

Torres-Valdes, S., Rousseau, V., Sanders, R.W., Reynolds, S., Pan, X., Mather, R., Landolfi, A., Wolff, G., Achterberg, E., Williams, R., 2009. Distribution of dissolved organic nutrients and their effect on export production over the Atlantic Ocean. *Global Biogeochemical Cycles* 23, GB4019, doi:4010.1029/2008GB003389.

Casey, J.R., Lomas, M.W., Michelou, V.K., Dyhrman, S.T., Orchard, E.D., Ammerman, J.W., Sylvan, J.B. 2009. Phytoplankton taxon-specific orthophosphate (Pi) and ATP utilization in the western subtropical North Atlantic. *Aquat. Microb. Ecol.*, 58: 31-44.

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Point 4: On page 16306 line 15 the authors state that both POC and DNA concentrations increased with SRP flux along the transect, suggesting greater SRP supply from deep water increased total biomass in the surface waters. This is then used as support for P- limitation of the organisms in these waters. However, this greater P supply brings with it a greater N supply (NO3 in the deeper water) and it cannot be used to indicate the limitation status of the cells. Likewise, the RNA:DNA ratio may be a potential proxy for growth rate (as the authors state). Thus the higher RNA:DNA ratio along the transect can only say that growth rate was higher but nothing about the P-limitation status of the cells (could be the N stimulating growth).

Response: We agree with the reviewer that our methods do not allow us to clearly tease apart the separate influences of N and P supplied from depth. The N:P ratio in this region of the North Atlantic has a reasonably well constrained NO3:PO4 ratio at the base of the euphotic zone, so N input will mirror P inputs by and large. However, data from cruise BV46 (same latitude stations, just sampled in 2011) shows that N:P in the upper 500m along this transect is always >16:1 and often >24:1 (see Fig. 1). The one exception is the station at ~38°N, which is Gulf Stream meander that we were passing through, and values go right back to subtropical values at the next station. These high inorganic N:P ratios indicate production may be controlled to some extent by P availability. Regardless, we have removed any wording about specific P-limitation in the discussion.

Point 5: There is a weak relationship between the RNA:DNA ratio and latitude (from Table 1,  $r^2=0.34$  and  $p = 0.59$ , not significant). If the RNA:DNA ratio were a proxy for growth rate (as the authors suggest in their discussion) this suggests there was no significant (or a small) change in growth rate across the transect. This being the case, would you expect to see a change in C:P due to RNA changes? According to the GRH I do not think you would.

Response: The reviewer brings up a valid point that the relationship between RNA:DNA and latitude is somewhat weak (linear regression results in an R-squared of 0.341 and

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P-value of 0.059). We have revised the discussion to acknowledge the relatively small change in RNA:DNA across sampling stations. The impact of RNA changes on C:P ratios really hinges on the proportion of total P bound in RNA, which is assumed by the GRH to be high under low-P growth conditions. However, the results of this and other recent studies (Zimmerman et al., 2013; Daines et al., 2014) suggest that observed patterns in P content and C:P ratios reflect other major sources of cellular P (perhaps phospholipids or storage compounds).

Zimmerman, A.E., Allison, S.D. and Martiny, A.C. 2013. Phylogenetic constraints on elemental stoichiometry and resource allocation in heterotrophic marine bacteria. *Environmental Microbiology*, doi:10.1111/1462-2920.12329.

Daines, S.J., Clark, J.R., Lenton, T.M. 2014. Multiple environmental controls on phytoplankton growth strategies determine adaptive responses of the N:P ratio. *Ecology Letters*, doi:10.1111/ele.12239.

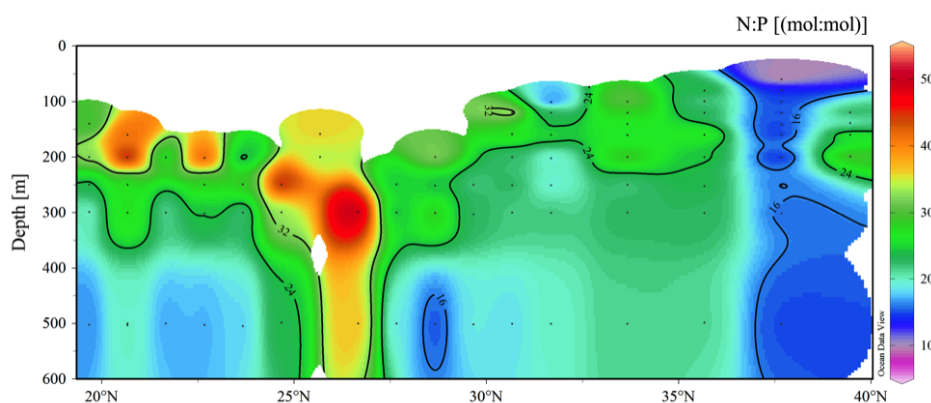
Point 6: Lastly, what is the detrital content of the particulate matter? If detrital matter is high couldn't this drive the particulate C:P variability and any impact of RNA may not be seen?

Response: Accumulated dead plankton material could mask the effect of changes in the RNA content of living plankton biomass on particulate P content and community C:P ratios. However, this does not appear to be the case in the subtropical North Atlantic, as recently suggested by an analysis of the summed contributions of flow-cytometrically sorted populations to total particulate carbon, nitrogen, and phosphorus pools (Martiny et al., 2013). These points have been added to the main text.

Martiny, A.C., Pham, C.T.A., Primeau, F.W., Vrugt, J.A., Moore, J.K., Levin, S.A., Lomas, M.W. 2013. Strong latitudinal patterns in the elemental ratios of marine plankton and organic matter. *Nature Geoscience* 6, 279–283.

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**Fig. 1.** Latitudinal contour plot of N:P molar ratios in the upper 600m of the Sargasso Sea in 2011.

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