

The authors provide data on elemental ratios of organic matter from the Atlantic Ocean. The data is highly valuable, and thus EGU sphere seems suitable for this manuscript. The measurements from two different transects provide insights into both latitudinal and longitudinal variation. The authors explore the relationship between the elemental ratios and environmental factors. One major concern is the description of nutrient stress and nutrient limitation. The entire manuscript seems to rely on genomics (of *Prochlorococcus*), which does not match the nutrient limitation data based on the established method. I suggest that the authors clarify this discrepancy in the main text so that the readers are aware of this limitation. Such clarification is important because the nutrient limitation indicated by the *Prochlorococcus* only genomics analysis tends to be skewed toward P limitation, creating a misleading impression of nutrient limitation.

The primary concern brought up by this reviewer relates to how we incorporate genomic information about nutrient stress as predictors for differences in C:N:P. The reviewer argues that bioassays show that N is the primary limiting nutrient in many of the places we study whereas we observe high frequencies of P stress genes. The issue of which element limits growth and productivity in different regions is a very tough question that we do not intend on solving in this study.

We recognize that we were inconsistent in the presentation of our biomarker data and used the term limitation. This will be corrected. Instead, the combination of metagenomic biomarkers of nutrient stress and ecosystem C:N:P suggest that when ecosystems are 'stressed' by a particular nutrient, C:N:P changes. This is consistent with culture and community experiments showing that C:N:P is very sensitive to changes in nutrient availability. Independent of which element ultimately control growth, stressful conditions can affect the resulting C:N:P. We aim to more carefully delineate this argument and ensure that we are only considering stress conditions and not outright nutrient limitation. This will be done by introducing this difference between stress and limitation in the introduction, more careful use of terms in the results, and then revisit the issue in the discussion. We hope that such edits will align with how we and the reviewer view these important biological and biogeochemical controls.

L72:

Regarding P limitation, the prediction from an established method shows that it is a secondary limitation (Moore et al., 2013). It might be good to clarify that in these regions, N is the main limiting factor. The paper shows that P does not come mainly as a main limitation. A recent study shows P limitation-related genes across the ocean, but having related genes might be different than the actual limitation on organismal growth.

You are right that there is an established methodology that supports nitrogen as the primary limitation and phosphorus as the secondary. This is supported by more recent papers – e.g., Browning and Moore, 2023. It was not our intention to have the paper present phosphorus as the limiting nutrient for the region, rather that cells are stressed by phosphorus and thus altering C:P. Culture experiments show that elemental ratios are very sensitive to nutrient

stress – even in the absence of overall biomass accumulation being limited. Thus, we plan to summarize and discuss our hypothesis for how nutrient stress impact ecosystem C:N:P.

L266:

Genomics may not necessarily represent the nutrient limitation: having genes is different than the actual growth limitation. Whether the genes are used to compensate for nutrient limitation is not clear with genomics analysis. Likely because of that, the genomics and the actual limitation seem very different (compare Ustick et al., 2021 with Moore et al., 2013). I suggest that the authors explicitly state this discrepancy in the manuscript to reduce misleading impressions.

We agree and will carefully describe that biomarkers indicate that cells are ‘stressed’ by a particular element.

L269:

>93%: I suggest the authors clarify this is based on the cell count. I see that Fig. S4 has it, but clarifying this in the main text would help readers understand the number.

Rereading this section, I can understand the confusion in the percentage. There are a few places in this paragraph with a similar structure that we will clarify.

E.g., From this *Prochlorococcus* was determined to make up 93% of the community from cellular counts in the subtropical gyres and equator and contributing to over 50% of the total biomass in those same regions.

L268-273

Fig. S4 has *Synechococcus* in it, which I found valuable information. I hope that the authors describe it in the main text.

While the data we have available does include *Synechococcus* counts, we do not have corresponding genomic data. We find that the addition of *Synechococcus* might cause some confusion, as to why they are brought up when the primary focus is *Prochlorococcus*. This is still an interesting point, however. A paper by Garcia et al., 2020 uses genomics from several cruises to compare *Prochlorococcus* and *Synechococcus*. They found that they follow the same trends across the transect as each other. This will be clarified in the revised version.

Reference: Garcia, C.A., Hagstrom, G.I., Larkin, A.A., Ustick, L.J., Levin, S.A., Lomas, M.W., Martiny, A.C., 2020. Linking regional shifts in microbial genome adaptation with surface ocean biogeochemistry. *Philosophical Transactions of the Royal Society B: Biological Sciences* 375, 20190254. <https://doi.org/10.1098/rstb.2019.0254>

L280-282

As mentioned above, there is a discrepancy in nutrient limitation between the metagenomic estimate and the established methods. I suggest this point is clarified somewhere in the text. For example, the established methods show N as a key limiting factor (and P as secondary), and the result in this present paper may not represent the actual growth limitation.

We agree and will clarify that cells are 'stressed' by a particular element. See also the earlier comment for details on this point.

Fig. 4

Because the nutrient limitation is based on the metagenomics analysis, this result could be misleading. I suggest that the authors make clear the difference between the actual growth limitation and the prediction of nutrient limitation based on the metagenomics analysis. For example, Moore et al. 2013 compiled the results of nutrient incubation analysis, resulting in N as a primary limitation in the North Atlantic. Given that, this figure seems to overemphasize P limitation because it is based on metagenomics, and I suggest that the authors make clear the caveats (especially the inconsistency with the outcome of the established methods) of the metagenomics analysis somewhere in the text.

We plan to carefully discuss the role of nutrient stress vs. growth limitation and what it means for the regulation of ecosystem C:N:P. We believe this will reconcile the differences between bioassays and metagenomics and still provide important insights into differences in ocean C:N:P.

L297

Here, genes may not tell nutrient stress. For example, in culture studies, organisms with the same gene may experience various nutrient stresses regardless of genes. Genes could be a proxy, but as mentioned above, there seems to be a clear discrepancy between the established methods and estimates from genes. I suggest using terms such as "stress proxy," "stress indicator," or, more explicitly, "stress-related genes."

Now I noticed that Figure 4 uses the term "nutrient gene index." I think it is a good expression, and the term is well defined. I suggest including such a definition in the main text as well and using the term throughout the paper instead of simply saying "nutrient stress" or "nutrient limitation" because, apparently, these are different things.

We agree that there is an inconsistency with the terms we use, and that they were used interchangeably. Using the term "nutrient gene index" we can prevent confusion as to which nutrient stressor we are referring to. We also agree that in future edits we will take care to standardize the terms we use and to give them a well-defined definition that remains

consistent through the paper. Along with nutrient gene index we will also make sure that the other terms referred to will be corrected and/or defined.

L335-337

Please see the earlier comments. These may not be actual P limitations, so I suggest clarifying this a bit more. e.g., shift from N stress genes toward P stress genes.

We agree with this statement and will correct it in future edits.

L343

Similarly, stronger P limitation may not be accurate. I suggest rephrasing (see above).

We agree that the use of limitation was too broad and should be narrowed down to a more accurate statement. i.e nutrient stress shift based on the nutrient gene index.

L347 “N and P-limitation” Please see the above comments.

This will be corrected in future edits.

References

Moore CM, Mills MM, Arrigo KR, Berman-Frank I, Bopp L, Boyd PW, et al. (2013). Processes and patterns of oceanic nutrient limitation. *Nature Geoscience* **6**: 701–710.

Ustick LJ, Larkin AA, Garcia CA, Garcia NS, Brock ML, Lee JA, et al. (2021). Metagenomic analysis reveals global-scale patterns of ocean nutrient limitation. *Science* **372**: 287–291.

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