

We would like to thank the reviewer for their time in reading the revised version of the paper and for the positive feedback on the new version. We have responded to the minor comments below and have made the relevant edits on the manuscript.

The methods section does not describe the enzyme kinetic bioassays used to determine the V_{max} and K_m values presented in Figure 8. As these details are critical for interpreting the results, I recommend describing the assay methodology in the main text and providing the full experimental details in the supplementary materials.

We have added the following text to lines 170 to 175:

Enzyme kinetic parameters were determined at each station by incubating unfiltered surface seawater with various concentrations of the synthetic fluorogenic substrate 4-methylumbelliferyl-phosphate (MUFP, Sigma Aldrich) and measuring the change in fluorescence for 8 hours (as described by Davis et al., 2019). The maximum hydrolysis rates (V_{max}) and the half saturation constant (K_m) were determined using the Hanes-Woolf plot graphical linearization of the Michaelis-Menten equation following Duhamel et al. (2011).

2. The influence of nitrogen acquisition on the biogeography of *Prochlorococcus* and *Synechococcus* has not been comprehensively discussed. While the study describes the spatial patterns of nitrogen stress in *Prochlorococcus* and *Synechococcus*, the direct influence of this stress on their biogeographical distribution and abundance is not sufficiently explored. A more thorough discussion explicitly linking the observed N stress to the control of population dynamics is needed.

We have revised Section 3.5 (from line 611) to read as follows:

The spatial patterns in nitrogen stress biomarkers gleaned from non-targeted metaproteomics provided insight into how fixed nitrogen availability contributed to shaping the biogeography of *Prochlorococcus* and *Synechococcus* across the subtropical Atlantic. Surface ocean gradients in fixed nitrogen are established via a combination of upwelling in the eastern Atlantic (Menna et al, 2015), nitrogen fixation (Fig. 3g, Cerdan-Garcia et al., 2022) and dust deposition (Powell et al., 2015) delivering nitrate, ammonium and urea to the surface subtropical Atlantic Ocean alongside microbial demand consuming fixed nitrogen. In summer 2017, concentrations of nitrate (< 40 nM) and ammonium (< 20 nM) were relatively low across the transect (Fig. 1e and f). The lowest spectral counts of the N-stress proteins, AmtB and UrtA in *Prochlorococcus* (Fig. 4c) coincided with a region of elevated nitrogen fixation rates (Fig 3g), reflecting alleviation of N stress in *Prochlorococcus*. The combination of efficient uptake of nitrogen derived from nitrogen fixers (Caffin et al., 2018), alongside the small cell size of *Prochlorococcus* provides a strong competitive advantage under oligotrophic conditions, as observed in the North Pacific gyre (Saito et al., 2014, 2015).

Otherwise, *Prochlorococcus* was subjected to increasing N stress towards the eastern Atlantic, evidenced by eastward increases in protein biomarkers in *Prochlorococcus*, specifically P-II, ammonium transporter AmtB and urea transporter UrtA (30-70%, Fig. 4b and Table 2).

In contrast to *Prochlorococcus*, consistently elevated UrtA in *Synechococcus* suggests chronic N stress throughout the transect, with UrtA in *Synechococcus* being more than 5 times higher than for *Prochlorococcus* (Fig. 4b). This is consistent with the physiological disadvantage of *Synechococcus*, with its larger cell size and less efficient surface-area to volume ratio for nutrient acquisition (Chisholm 1992). P-II and AmtB (or NtcA) was not detected in the metaproteome of *Synechococcus*, perhaps because *Synechococcus* was 5 to 10 times less abundant in the metaproteomes compared to *Prochlorococcus*. The dominance of proteins for ammonium and urea acquisition of *Synechococcus* and *Prochlorococcus* are consistent with the premise that while marine *Synechococcus* and some *Prochlorococcus* strains have the genetic makeup to assimilate nitrate (Berube et al., 2015; Domínguez-Martín et al., 2022; Martiny et al., 2009), it accounts for < 5% of their total N demand, and instead ammonium and urea are the dominant N sources (Berthelot et al., 2019; Casey et al., 2016; Painter et al., 2008). The patterns observed align with established biogeographical trends in which *Prochlorococcus* dominates in the nutrient-deplete surface ocean due to its competitive advantage as a small cell, whereas *Synechococcus* persists in regions where fixed N is available. The proteomic data indicate that nitrogen acquisition traits are one of the key determinants of population dynamics, driving spatial partitioning between *Prochlorococcus* and *Synechococcus* and ultimately influencing primary productivity and nutrient cycling across the subtropical Atlantic.

This manuscript is part 1 in a two-part publication from a project on the prevalence of zinc and iron limitation in alkaline phosphatase in the subtropical ocean. As such, we have been advised to add a pre-title to both manuscripts and therefore the title has been edited to the following:

Proteomic and biogeochemical perspectives on cyanobacteria nutrient acquisition: Part 1: Zonal gradients in phosphorus and nitrogen acquisition and stress revealed by metaproteomes of *Prochlorococcus* and *Synechococcus*