Reply to Referee #2 comment made on bg-2020-300, “Cross-basin differences in the nutrient assimilation characteristics of induced phytoplankton blooms in the subtropical Pacific waters ” by Hashihama et al.

Comments to Author(s): This manuscript by Hashihama et al. describes the variation in macronutrient drawdown among Pacific Ocean surface microbial communities using deep water additions to bottle incubations. The authors present data from seven subtropical gyre sites with distinct nutrient uptake ratios, where the nutrient limiting net biological production is unknown. These observations are linked to pigment proxies for phytoplankton taxa and diatom densities to examine the role of community in nutrient drawdown ratios. The experiments yielded increased phytoplankton biomass at all sites, but varying stoichiometric ratios of uptake for DIN:PO4:Si(OH)4. I feel this manuscript strongly expands upon existing studies on the question of nitrogen, phosphorus and iron limitation in the North and South Pacific Ocean.

The data is presented in a straightforward manner and explained well. All sections are well written and figures are easily digested. I have a few concerns on assumptions made regarding phytoplankton pigments proxies and deep DOM composition, but otherwise recommend the manuscript should be accepted with minor revisions.

Our reply (in italics hereafter): Thank you for your constructive comments. We will address your comments as seen below.

General comments

1. Regarding the methods, I request that the deep water collection be clarified slightly. It is not stated if the water was filtered to remove living cells. This is of particular concern at Stations A and 2. If unfiltered seawater was used, both grazers and microbial cells could impact the conclusions at these stations. At other stations, freezing the seawater would remove this concern, but introduce additional nutrients from burst cells. The nutrient composition of cellular detritus is likely different and more bioavailable (urea, NH4, labile DOM) than deep nutrients (NO3, recalcitrant DOM).

We used the unfiltered deep water from 1500 m depth to avoid ammonium contamination from filtration process. As you have considered, there might be a potential influence of heterotrophs in the deep water on the results of incubation experiments. However, concentration of particulate organic carbon in the deep layer below 1000 m is generally less than 10% of that in the surface layer (Hebel and Karl, 2001 DSR-II 48, 1669-1695; Yamada et al., 2017 MEPS 583, 81-93) and prokaryotic abundance and production exponentially decrease with depth (Yokokawa et al., 2013 LO 58, 61-73). Furthermore, the proportion of deep water to total incubated volume (surface water + deep water) was only 2.1% as

stated in the section 2.2. The large dilution was also confirmed from T0 data of DON and DOP in the treated bottles which were not significantly different from those in the control bottles (paired t-test, $p>0.05$, Fig. A2), indicating that the influence of labile DON and DOP additions were negligible. Thus, we conclude that the influences of heterotrophs and labile DOM supply by freezing were at negligible levels compared to the large enrichments of inorganic nutrients.

2. My largest concern is directly assuming that divinyl chlorophyll A concentrations are representative of Prochlorococcus abundances. While a useful indicator, the concentration of divinyl chlorophyll a could change between sites, season, light/depth level, etc. It is very possible Prochlorococcus cells in the Eastern South Pacific have a lower density of photosynthetic pigments, especially in the summertime at the surface where these cells were collected. In addition, since Nitrogen is limited, the cells may have adapted by lowering the concentration of N-rich photopigments further. This combined effect of photo-acclimation and adaptation to low N could explain the low divinyl chlorophyll A concentrations in the South Pacific subtropical gyre. This caveat should be acknowledged in the Discussion.

We agree that seasonal/regional PAR level influences the cellular pigment quotas. In contrast, N limitation occurred not only in the South Pacific but also in the North Pacific, because ambient DIN:PO_4 ratios ($\leq$8) were much lower than the Redfield ratio (16) and subtropical particulate N:P ratio (28) (Martiny et al., 2013 Nat. Geosci. 6, 279-283). Therefore, N limitation might not be a robust reason for any seasonal/regional variations in the pigment concentrations, including DVchl a. Furthermore, we consider that pigment ratios (Tfuco:Zea (no N in either of the pigments) and DVchl a:Tchl a (both pigments contain N)) are useful for comparing the regional variations in phytoplankton composition even if seasonal/regional differences of photo-acclimation/adaptation and N-limitation occur. In the perspective of phytoplankton physiology, Tfuco and Zea play roles in light-harvesting and photoprotection, respectively (Falkowski, 2013 Aquatic Photosynthesis 2nd edn). Based on these roles, Zea content in cyanobacteria should be higher in the high-PAR South Pacific than the low-PAR North Pacific, while Tfuco content in eukaryotes should be higher in the low-PAR North Pacific than the high-PAR South Pacific. However, ambient Zea (Tfuco) concentrations were higher (lower) in the North Pacific than the South Pacific, indicating that the biomass proportion of cyanobacteria to eukaryotes was higher in the North Pacific than the South Pacific. Thus, although we will revise the statements for seasonal/regional variation in each pigment concentration, we do not revise the statements for pigment ratios and seasonal/regional variations in phytoplankton composition.

3. Regarding DOM, I had two points to consider. The authors mention more bioavailable forms of DOM that may not be present in water at 1500m. Perhaps the DOM added then would not be consumed, leading to no net changes over the incubation period. Alternatively, the balance of net uptake and

release could yield no change. This possibility should be acknowledged for silicic acid as well considering the longer incubation time and high diatom abundances at station 15.

We also interpret that DON and DOP in the deep water did not have an influence on various parameters in the incubation, because these additions were negligible due to the large dilution as mentioned above. Since the resident phytoplankton in the DIN and PO_4-depleted subtropical ocean might have a high affinity to DON and DOP, rather than DIN and PO_4, we considered the possibility that phytoplankton in the incubated bottles consume labile DON and DOP in the surface water (not in the deep water) rather than DIN and PO_4. However, such a phenomenon did not occur in the present study and the uptake and release of DON and DOP were balanced. As you suggest, the uptake and release of silicic acid likely occurred. We will add the related statement in the revised manuscript.

Specific comments

Line 47 - Changing 'alleviates temporarily ' to 'temporarily alleviates' reads a bit better.

We will revise as you have suggested.

Line 74 - 'observation' should be plural

Yes, it will be plural.

Line 74-75 - I suggest changing ', to understand them, experimental validations are required.' to ', and to understand them experimental validations are required'.

We will revise based on your suggestion.

Line 95 - See comment on methods

See our answer above.

Line 120 - To what extent does the water volume in the bottle change between T0 and the last time point? Also are collection intervals than shorter for the shorter incubation times?

At the end point, water volumes in the bottles just prior to final sampling were approximately 1.8 L, because the subsamples for nanomolar nutrients and DON/P were collected from the initial volume of 2.35 L (surface water + deep water). Also, the collection intervals were shorter for the shorter incubation times. We subsampled 5-6 times for nanomolar nutrients and 3 times for DON and DOP during the incubation periods for 48-96 h. We will add these statements in the revised manuscript.

Line 199 - 'Dominance of Prochlorococcus' not actually quantified. See comment above.

See our answer above.

Line 202 - A brief description for how the Nitzschia longissimi was identified should be included (i.e. by sight, microscope identification?).

We described the microscopic analysis of diatoms in the section 2.6. of Materials and methods.

Lines 312-315 (347) - See comment on phytoplankton proxies above.

See our answer above.

Lines 333-340 - See comment on DOM above.

See our answer above.

Line 347 - Based on uncertainty of phytoplankton composition estimates, I don't believe this phytoplankton uptake theory can be thrown out.

See our answer above.

Line 362 - Alternatively, DOP uptake and release is balanced.

We will revise based on your suggestion.

Line 396-7 - This point is very interesting and I point the authors to this short compilation reference of nitrogen fixation estimates by Bonnet et al. 2017 in (https://doi.org/10.1073/pnas.1619514114). It is possible that iron (or a trace metal) is limiting, but the microbial population does not have standing stocks of nitrogen fixers.

We will refer Bonnet et al. (2017 PNAS) in the revised manuscript.