

Abstract:

- 1) Maybe use biological N₂ fixation and abbreviation BNF throughout the abstract and manuscript.
- 2) Why were amino acids added?
- 3) Why would the 14N amino acid signal mask 15N₂ incubations?
- 4) Change particulate nitrogen to PN since this abbreviation has already been introduced.
Line 33
- 5) What kind of relationship does PN have with aphotic BNF rates?

Introduction:

- 1) Include some citations for those studies reporting discrepancies in N budget from bottom-up measurements (line 41).
- 2) At line 45, consider introducing that the enzyme, nitrogenase, is responsible for BNF and that *nifH*-gene encodes for this enzyme. Take out “and” in “...new N to the open ocean (citation), and...”.
- 3) Line 60: start a new paragraph with “However, owing to the...”
- 4) Line 68: start a new sentence with “Thus, further investigations...”
- 5) Line 77: correct spelling for “anaerobic”. Also, check out recent publication regarding BNF rates with sinking marine particles (Chakraborty et al., 2021) – very relevant for your research. “Quantifying nitrogen fixation by heterotrophic bacteria in sinking marine particles” Chakraborty et al., 2021
- 6) Line 81-89: Maybe table 2 could be moved up to the intro and you could incorporate columns with what DOM compound was added and how the BNF rate was influenced in response to the amendment - might enhance the table.
- 7) Line 95: introduce as South China Sea once before using SCS abbreviation.
- 8) Line 100: state what different controls you used specifically.

Methods:

- 1) Fig. 1. Use SCS abbreviation for consistency since it has already been introduced.
- 2) Fig. 2. Not clear what OMD is referring to.
- 3) Table 1. Specify cruise dates for K1 and WXS stations. The control/treatment column is not super clear – what are all the other incubations considered? Also, I don’t see any specifications for the DFAA treatments here.
- 4) Section 2.4: line 180 “...representing x % of the total organic carbon...” Not certain how these % are being calculated – total organic carbon measurements of what (e.g., are these citations referencing prior measurements of TOC in the SCS)?
- 5) Lines 185 – 188 – combine with paragraph starting at line 189.
- 6) Lines 208-210 combine with previous paragraph.
- 7) Line 232: are depth integrations done using trapezoidal integration?
- 8) Table 2 would be more useful in the introduction rather than the long list of citations.

Results:

- 1) Figs. 3 and 4: Consider reducing the y-axis range so it’s easier to discern what’s happening with most of the samples. For instances where d¹⁵N is greater than 100,

consider using a broken y-axis so that you can highlight both high values and the majority of the data that falls within < 100.

- 2) OMD depth is referenced several times but haven't specified what the abbreviation stands (Oxygen Minimum Depth?) for.

Discussion:

- 1) Line 335: citations for low [PN] and low ANF rates in aphotic zone? Combine this paragraph with the next one since it's so short and interconnected.
- 2) Line 345: citations for characterizing "estuarine environments...ultraoligotrophic waters...polar waters" as "low N₂ fixation systems".
- 3) Line 348 – 349: Some citations highlighting some of these bottle effects would be good.
- 4) Paragraph 347 – 354: This section could use some extra background information and citations in general.
- 5) Line 365 - 366: You bring up sinking particles. Are you suggesting that the ANF in deep waters is particle associated? Also, do you have any explanation or prediction as to why that heterogeneity exists? Chakraborty et al. (2021) suggest from their modeling work that there's an ephemeral window for BNF to occur with sinking marine particles. Thus, linking this could give more merit to the explanation that heterogeneity of sinking particles could account for some of the heterogeneity in ANF rates.
- 6) Lines 377-379: Any speculation to why ANF rates were below detection in this study but above detection in the Mediterranean Sea?
- 7) Line 380: "Horizontally" refers to "spatially" right?
- 8) Why were parallel light incubations not carried out in the euphotic zone? This would have allowed you to determine how important diazotrophic activity by NCDs (ANF rates) are compared to cyanobacterial diazotrophic activity (light BNF rates).
- 9) Line 385 – 386: Is higher sinking flux influenced by seasonal patterns and if so, how might you expect ANF rates to be different if you were to conduct sampling in other seasons (i.e., fall, winter, or spring) than summer.
- 10) Line 387: You mention larger mean cell diameter and higher PN concentrations – any thoughts on what the community composition might be like (e.g., is it dominated by picoeukaryotes or larger, faster sinking phytoplankton like diatoms)?
- 11) Line 403: "...or ANF was limited by other factors in the SCS..." could you elaborate? Are there any studies that show DFAA assimilation is preferable to BNF?
- 12) Line 415: Could you discuss how results might have been different with just a labile C source amendment over a DFAA addition? The DFAA also provides additional labile N to the microbial community but with just a labile C addition, N would be more limiting and could potentially result in a greater stimulation of the diazotrophic community.
- 13) Line 432: Review Chakraborty et al., 2021 and incorporate their findings in your discussion – it nicely supports the points you are trying to make.
- 14) Line 462: Could be incorporated as the last sentence for the paragraph rather than a separate paragraph.
- 15) Line 467-468: "Taken together with previous reports from different oceanographic regions highlighted in Table 2, our study shows 11 of 18 sampling depths..."

- 16) Line 470: This citation (SEATS might be an overlapping station) is useful for the discussion here and brings up an important point that is currently overlooked: how do these ANF rates compare to BNF rates by cyanobacterial diazotrophs in the SCS?
Citation: Nitrogen Fixation by *Trichodesmium* and unicellular diazotrophs in the northern South China Sea and the Kuroshio in summer. Wu et al., 2018
Another citation (with SEATS as overlapping station) that could be useful to compare how ANF rates contribute to net community production at SEATS: Estimated net community production during the summertime at the SEATS time-series study site, northern South China Sea: Implications for nitrogen fixation. Chou et al., 2006
- 17) Line 477 – 479: How is particle export in the summer compared to other seasons? Could you elaborate more why higher particle export in winter would indicate your annual estimation is conservative? Also, how do your annual ANF contributions to SCS compare to other estimates of BNF contributions to the N budget in SCS (if they exist)?
- 18) Line 480: How could this be a global interpretation if it's based on one study from the Mediterranean Sea?
- 19) General discussion needs improvement and more pointed hypothesis or predictions.

Conclusions:

- 1) Line 495: You didn't have any data suggesting high particle flux was occurring – in fact, you suggest that particle flux is much higher in the winter months. You could more appropriately discuss the potential for higher integrated ANF rates than measured in this study to occur in the winter if seasonal ANF surveys were conducted in the SCS.
- 2) Paragraphs could be better organized and compiled into fewer paragraphs.