Review ms essd-2023-13

Shao et al. present an update of the diazotroph database published in 2012
https://essd.copernicus.org/articles/4/47/2012/

The new version adds up data published between 2012 and 2023, including volumetric and depth-integrated N_2 fixation rates, diazotroph microscope counts and nifH gene counts. This new version also discusses microscope-nifH count comparisons. While this update is valuable for the community as a tool for comparison and contextualization of diazotrophy studies, it fails to account for many diazotrophy studies published between 2012 and 2023. The text has several misinterpretations that need correction. The new version also includes N_2 fixation rates proxied with other methods (ARA). I think this is a major problem, since these rates are not currently solidly comparable and downplay the robustness of the database. The manuscript also eliminates nifH gene counts from non-cyanobacterial diazotrophs (NCDs), which is another major issue since NCDs are considered to be outnumber cyanobacterial diazotrophs in the ocean. Finally, the diazotroph microscopy count versus nifH gene count conversion discussion does not seem appropriate here, since very few of the papers listed have compared these approaches on a same given sample, and the issue has been discussed thoroughly in other publications by specialists.
In all, while I acknowledge the effort and usefulness of this manuscript, I advise major revisions as detailed in the comments below.

L28: N2 gas is not inert to diazotrophs.
L31: The balance between N loss/gains in the ETSP has been widely demonstrated to be false in several publications after that of Deutsch et al., see for example (Knapp et al. 2016; Bonnet et al. 2017).

L35: Only cyanobacterial diazotrophs can be confidently counted by microscopy.

L36: "NifH gene copies"

L40: This issue has been thoroughly discussed in (Gradoville et al. 2022), validating the use of nifH gene counts as a means to quantify diazotrophs.

L42-47: Other sources of unbalance should be briefly mentioned here.

L50: Diazotroph activity was there before, it is our notion of them that increases, the data available.

L56-57: I don't think that the dataset assembled here covers enough studies comparing microscopy and nifH based comparisons, and I strongly recommend removing this sentence and section 4.2 from the manuscript.

L61: The N_2 fixation rates from Tang et al. 2019 are based on an ARA-$15N_2$ fixation comparison including only 8 datapoints. This is not robust enough to provide a reliable comparison and downplays the robustness of the 15N2-based rates dataset collected here. I strongly recommend removing these from the database and derived basin-scale and global calculations. These may be mentioned as discussion and the Tang paper cited, but not included for quantitative purposes.

L72: Removing NCDs is an error in my opinion. NCDs have recurrently been shown to be dominant in the ocean (Farnelid et al. 2011; Delmont et al. 2018, 2021; Riemann, Farnelid, and Steward 2010) and may impact N cycling decisively (Riemann et al. 2022; Turk-Kubo et al. 2022). I strongly recommend that any nifH gene counts of NCDs are added. The previous database included Gamma A and Cluster III. I don't see a solid reason to remove NCDs from the database at this stage, as evidence of their importance increases.

L82: Group-specific N_2 fixation rates can only be estimated using single-cell approaches. I'm not sure what approach was followed here to derive specific rates, but these can certainly not be estimated with the data

collected here. I would rather recommend the authors to collect all *Trichodesmium,* UCYN-B, DDAs and UCYN-A single-cell rates published, which would be very helpful for the community. See for instance (Foster, Sztejrenszus, and Kuypers 2013; Foster et al. 2011; Benavides et al. 2017; Bonnet et al. 2016; Filella et al. 2022; Krupke et al. 2015; K. Harding et al. 2018; Mills et al. 2020; K. J. Harding et al. 2022; Benavides et al. 2022).

Tables 2 and 4 : Many studies are missing in this table, some include (Benavides et al. 2014, 2021; Saulia et al. 2020; Henke et al. 2018; Bonnet et al. 2018; Gradoville et al. 2017; Moreira-Coello et al. 2017; Wilson et al. 2019). Also, in the table some studies are listed as not including counts of some diazotrophs, which needs correction (e.g. Bombar 2011 and Bonnet 2015, 2019 did have qPCR counts). Please revise all these publications thoroughly and correct accordingly.

L104: The ARA method is rarely used nowadays.

L106-107: The ARA to N_2 fixation ratio is highly variable (Mulholland et al. 2006; Benavides et al. 2011; Wilson et al. 2012)

L110: Many other factors affect this difference, including acetylene gas impurity, Bunsen dissolution coefficient, etc.

L112: This is not true. The 15N2 method is much more sensitive, does not require biomass preconcentration (biomass is concentrated during filtration, after the incubation), and requires longer incubations for enough tracer to be detectable in biomass. ARA is usually done in 3-4 h incubations and requires biomass pre-concentration to reach detectable signal (Staal et al. 2007; Benavides et al. 2011).

L120: Wannicke et al. say the opposite of Mohr and Grosskopf.

L123: What White et al. say is that the bubble release method is the most reliable and recommended by the diazotroph research community, with the elimination of rate underestimation benefits overcoming the very unlikely burdens of contamination. This should be corrected in L274-275 as well.

L150: There are 4 UCYN-A sublineages (Farnelid et al. 2016).

L328: UCYN-A has been found in symbiosis with other eukaryotic algae (Zehr et al. 2016)

L370: The first version of the database included all the authors that had contributed to its construction with their seagoing expeditions, laboratory analyses and publications. I humbly find it sad and somewhat unfair that this is not the case in this update.

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