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Interactive comment on "Spatiotemporal patterns of N_2 fixation in coastal waters derived from rate measurements and remote sensing" *by* Mindaugas Zilius et al.

Anonymous Referee #1

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The manuscript has an interesting dataset where the authors combine in situ measurement with satellite imaging to estimate areal nitrogen fixation with the benefit of reducing bias due to patchiness of cyanobacteria blooms. I have however a few concerns and questions to the authors to address. I therefore suggest a revision before considering it for publication.

Something that was surprising to me was how come you didn't find any picocyanobacteria? In Zilius et al. 2020 I interpret it as you had about 20% of the community during summer? Also in Klawonn et al. 2016, colonial picocyanobacterial comprise ca. 5-10% of the cyanobacterial community in terms of carbon. It seems like you sampled

on similar locations, maybe even at the same time, as in Zilius et al. 2020 so this needs an explanation. If it has to do with method differences, it needs to be explained or the statement of no picocyanobacterial removed and refer to previous study.

I am also a bit concerned about the method you use for measuring N2-fixtaion with injection of gas rather than pre-dissolved. I think this might cause an underestimation. Also the fact that you run 24 h incubations probably lead to underestimations of N2-fixation per h since they do less in the night when its dark (1.8 times less; Klawonn et al. 2016). I think a potential underestimation should be discussed and rates presented as per day since this is what you measure.

The theory of underestimation is further supported by that you have 1000-3000 ug cyanobacterial C per L and 120-200 nmol N2 fixation per h as compared to Klawonn 2016 where 100 ug cyanobacteria per L performed 80 nmol N2 fixation per h. Why do you think you have so low rates as compared to your biomass? Can it be P limitation?

What effects do you think the fact that cyanobacteria only comprised about up to 36 or 86% of the phytoplankton fraction has for your correlations with chlorophyll and further areal estimates of N2-fixation? I guess it must be very variable over the year how well your method can be applied? I think you should discuss this bias further.

More specific questions are listed below:

Line 33, comma after algae.

Line 39, I think you need to name this 2020a since it the first one appearing?

Line 45-46, when they are dead I guess? Maybe clarify that this is when they are detritus on the bottom.

Line 52, any references to the patchiness? Maybe Rolff et al. 2007?

Line 100, triplicates of samples or sampled from the same flask?

Line 109, maybe the Whatman and pore size should be on line 100 when first men-

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tioned?

Line 120, I think it would be good to provide heterocysts per number of vegetative cells as well since they change in density over the season (Svedén et al. 2015).

Line 128, are not many picocyanobacterial smaller than 3 um? Is this what is commonly used for picocyanobacterial? Did you use any certain settings on the flow cytometer to determine picocyanobacteria, for example a cyano-specific filter? Did you use Sybr? Were they in the same sample as the heterotrophic bacteria or on its own? I am asking this since I am surprised that you did not see any, while you did in Zilius et al. 2020.

Line 133, does this mean that the flasks were top-filled without air during incubation? Did you shake/turn the flasks something to help with the mixing?

Line 137, in what way would pre-prepared isotopically enriched water be a risk of contamination? Contamination of what?

Line 139, I think its risk of underestimating rates when having flasks totally covered, 1% of light is still light and therefore it would have been more appropriate to have them covered instead. This would be good to mention in the results/discussion.

Line 148-150, did you measure the final labelled concentration in the flask or is this only an estimate from calculations? In case you only estimated the added concentration this can be a bias for your later rate calculations.

Line 153, how deep can you "see" with the satellites?

Line 181, why linear regression and not correlations? Don't you expect both of them to be interdependent rather than one dependent?

Line 189, please indicate in text and in figure legends when Chl is derived from in situ extractions and when from satellites.

Line 193, who are the non N2 fixing cyanobacteria if you did not have any picocyanobatceria? For example in November in Figure 2a?

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Lines 201-202, this surprises me, no picocyanobacterial at all? Is this common here? In Zilius et al. 2020 you had at least about 20% of the biomass?

Line 205, among how many samples? Do you mean micrograms on the y-axes, please use a proper "micro" symbol for this.

Line 209, please explain the clustered numbers (months?) in the figure. Maybe also add a legend title including what the symbols are (biomass?).

Line 228, among how many samples?

Figure 5, I think it is better to show N2-fixation as per day since this is what you measured and since night-time is lower (See Klawonn et al. 2016 for night rates where they show the double rate at day time).

Figure 5b, is this low rates maybe related to lack of light?

Line 236, how does number of heterocysts relates to total biomass/number of vegetative cells of cyanobacteria?

Line 237, please abbreviate to A. flosaque throughout the manuscript except at first place mentioned.

Figure 6, do you mean "per ml" with per mil? How come there is so many heterocysts in November in the southern station but almost no N2 fixation nor cyanobacteria biomass at that time? In contrast the highest number of both N2 fixation and heterocysts numbers correlates for the northern station. This needs to be discussed. Also, It would be good to also have heterocysts per filaments/vegetative cells here to see how it changes over the season (Aphanizomenon heterocyst density varies with season; Svedén et al. 2015).

Line 248, I think you have light limited N2 fixation? 1% of surface light can still be 10-20 um photons, which can be sufficient for carbon fixation.

Lines 247-251, did you use correlations or regressions? If this is the regression models

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you later use this must be clear.

Fig. 7 Clarify that the chl a from in situ extractions? Is this data from the whole year or only from the summer? For example if this is the whole year where is November value surface layer Chl of 250 ug L-1 but with no N2-fixation.

Line 259-260, can you provide the equation you used for these estimates?

Lines 260-261, how can you use this relationship when it was not significant, then there is no relationship?

Line 263, values of what?

Line 282, if you have ten times more Aphanizomenon you maybe should also have higher N2 fixation rates? This needs to be discussed and refer to data, for example Klawonn et al. 2016. Were they limited by something?

Line 298-300, but cyanobacteria did not dominate all the time and never close to 100%? How does this affect the results? When they are less then 50% of the Chl community is this not overestimating the N2-fixation rates? This needs to be discussed. The bottom layer had a lot of other organisms contributing to chl biomass.

Lines 309 and below, can you also put these areal N2-fixation estimates into perspective to other studies for the region? For example, Klawonn et al. 2016 and Olofsson et al. 2020 as well as references there in.

Line 343, how can the heterocyst frequency be so high without cyanobacteria biomass being high?

You need to discuss problems with N2 fixation from covered flasks and still standing flasks with gas injections somewhere in the discussion. “Caveats” with this study.

Line 500, Please change to Riemann.

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