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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.

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Response to Anonymous Referee #1

General comments

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.

Answer: We acknowledge the reviewer for their positive comments. In this study, taxa referred as "colonial picocyanobacteria" by the reviewer were found with microscopy counting, and due to their relatively low contribution (generally <2% of total biomass) they were assigned to "non-N_2-fixing cyanobacteria", and thus not further discussed in the submitted manuscript (Fig. 2). In the revised version of our manuscript, we have added information related to cyanobacteria composition and their biomass: "*Non-filamentous colonial cyanobacteria, such as Aphanocapsa spp., Aphanothece spp., Merismopedia spp. and Cyanodictyon spp. exhibited low biomass (< 2% of total) except in June, when their contribution reached 12% at the northern site (Fig. 2). Picocyanobacteria were not detected during the study period at either site.*" *(line 207-210)*.

In Zilius et al. 2020, sequences were attributed to picocyanobacteria (not referring here as "colonial picocyanobacteria"). However, a volume of 50 to 70 ml was extracted for further sequencing and only few reads were assigned to picocyanobacteria. This means that picocyanobacteria were rare in this study and that they would not be detected by methods allowing quantification such as flow cytometry or epifluorescence microscopy. Both approaches are complementary and not contradictory since DNA methods can detect rare taxa but do not allow quantification yet.

I am also a bit concerned about the method you use for measuring N_2-fixation 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 N_2-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.

Answer: Regarding the issue of hourly vs. daily rates of fixation, we agree with the reviewer's point that rates are likely to vary on a diel cycle (being lower at night). Therefore our diel incubations conducted under natural (outdoor) light conditions are more suitably expressed as daily rates than hourly rates since they are representative of both light and dark cycles. In the revised manuscript, we present daily values in figures and text.

With regards to methodology, we agree that there has been some debate about using the bubble method for N_2 fixation measurements (Mohr et al., 2010; Großkopf et al., 2012; White et al., 2020), but recent work (Wannicke et al., 2018) demonstrated that underestimation of rates is negligible (<1%) for incubations lasting 12–24 h. In the submitted version we have argued our choice for incubation duration: "*As the isotopic equilibration takes up to several hours (Mohr et al., 2010), we incubated the samples for 24 h, thus minimizing equilibration effects (Mulholland et al., 2012; Wannicke et al., 2018." (line 136-138)*. Eventually, our used technique avoids to have low labelling

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Fig. 1. Responses to Reviewer comments

Spatiotemporal patterns of N_2 fixation in coastal waters derived from rate measurements and remote sensing

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Abstract. Coastal lagoons are important sites for nitrogen (N) removal via sediment burial and denitrification. Blooms of heterocystous cyanobacteria may diminish N retention as dinitrogen (N_2) fixation offsets atmospheric losses via denitrification. We measured N_2 fixation in the Curonian Lagoon, Europe's largest coastal lagoon, to better understand the factors controlling N_2 fixation in the context of seasonal changes in phytoplankton community composition and external N inputs. Temporal patterns in N_2 fixation were primarily determined by the abundance of heterocystous cyanobacteria, mainly *Aphanizomenon flosaquae*, which became abundant after the decline in riverine nitrate inputs associated with snowmelt. Heterocystous cyanobacteria dominated the summer phytoplankton community resulting in strong correlations between chlorophyll-a (Chl-a) and N_2 fixation. We used regression models relating N_2 fixation to Chl-a, along with remote sensing-based estimates of Chl-a to derive lagoon-scale estimates of N_2 fixation. N_2 fixation by pelagic cyanobacteria was found to be a significant component of the lagoon's N budget based on comparisons to previously derived fluxes associated with riverine inputs, sediment-water exchange and losses via denitrification. To our knowledge, this is the first study to derive ecosystem-scale estimates of N_2 fixation by combining remote sensing of Chl-a with empirical models relating N_2 fixation rates to Chl-a.

Fig. 2. Revised manuscript