Response to Author comment #1 (provided in blue)

I am pleased to see that the authors have improved the manuscript according to the comments below. Which includes being open about some caveats, comparison to other studies, some methods that were missing and additional data on heterocyst frequency. However, there is still some minor adjustments I would like to suggest before moving on. See specific comments below.

Reviewer: 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.

Reviewer 1: Thank you for clarifying this in the revision of the manuscript and for looking further into this by also applying microscopy in addition to flow cytometry. Feel free to also include this extra information so that future readers do not confuse between the groups of colonial vs. free-living picocyanobacteria.

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 (percentage label should be between 5-10%) as the labelled seawater method often results in low quantities of $^{15}N_2$ gas in the water (e.g. Klawonn et al. (2015) had only 1% label in their experiment).

Reviewer 1: I am not fully sure if the last sentence about the optimum labelling ranges is within the revision or only a reply to the comment. But if it is in the text, do you have a reference for the mentioned optimum labelling percentages? I do not see any problem with having 1 % labelling when using the dilution approach (which you refer to in Klawonn et al.) since you then have this labelling in the whole flask already from the beginning, and as long as you can trace it and measure the final concentration. In case there is no suitable reference for the optimum ranges, and you want to mention it in the manuscript, I would suggest that you rephrase it to only mention that this range of percentages works well when using the bubble method and not compare to Klawonn et al. as an example of where it has not worked well(?) since I do not know if you have proof of this, and its slightly different methods. Further, I think it is great that you changed to 24 h values since this is what you measured and it includes both day and night.

The theory of underestimation is further supported by that you have 1000-3000 µg cyanobacterial C per L and 120-200 nmol N2 fixation per h as compared to Klawonn 2016 where 100 µg cyanobacteria per L performed 80 nmol N_2 fixation per h. Why do you think you have so low rates as compared to your biomass? Can it be P limitation?

Answer: We agree with the reviewer's point that P limitation may play a role in limiting N fixation in the Curonian Lagoon. In a prior study, it was shown that P additions stimulated growth rates of N_2 fixing cyanobacteria from the Curonian Lagoon (Pilkaitytė and Razinkovas, 2007*)*. Likewise, addition of P stimulated diazotrophic community resulting in elevated N_2 fixation rates (Moisander et al. 2007). We may expect that dissolved P was limiting, which constrained N_2 fixation during summer. Thus, we suppose that DIP release from sediment and higher biomass of *Aphanizomenon* and diazotrophic activity frequently observed in the end of summer (Zilius et al. 2014, 2018) are not coincidence but rather consequence of increased P availability. We have modified the Discussion to address this point "*The proliferation of heterocystous cyanobacteria in the Curonian Lagoon is favoured by P (Pilkaitytė and Razinkovas, 2007), which is released from sediments, particularly when bloom conditions result in high water column respiration and transient (night-time) depletion of oxygen (Petkuviene et al., 2016; Zilius et al., 2014). Moisander et al. (2007) demonstrated that P can enhance diazotrophic activity of heterocystous cyanobacteria in microcosms. Release of dissolved P from sediments may in turn enhance rates N_2 fixation resulting in a positive feedback for cyanobacteria bloom development."* (line 299-304)

Reviewer 1: In addition to stating that they are favoured by it you should maybe also say that this might be why they are performing compare-wise lower N2 fixation in comparison to other regions of the Baltic Sea and provide some reference to P concentrations in the lagoon during summer. Maybe they also have enough of other sources of N, such as ammonium, to support some of their needs?

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 N_2-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.

Answer: With regard to our ability to model N_2 fixation on the basis of Ch-a, we specifically address this point in the discussion: "*We benefitted from prior work deriving Chl-a estimates from satellite images and their calibration to in situ measurements (Bresciani et al., 2014), but the success of the*

approach largely relied on the fact that heterocystous cyanobacteria dominated the summer–fall phytoplankton community of the lagoon, which provided a significant correlation between N_2 fixation and in situ Chl-a. However, it would be problematic to extrapolate this approach to periods outside of cyanobacteria dominance (e.g., spring diatom bloom) or to periods when other factors (e.g., low temperature in fall) constrain N_2 fixation." (line 312-317)

References

Großkopf, T., Mohr, W., Baustian, T., Schunck, H., Gill, D., Kuypers, M.M.M., Lavik, G., Schmitz, R.A., Wallace, D.W.R., LaRoche, J.: Doubling of marine dinitrogen-fixation rates based on direct measurements. Nature, 488, 361–364, 2012

Moisander, P. H., W. Paerl, H. W., Dyble, J., and Sivonen, K.: Phosphorus limitation and diel control of nitrogen-fixing cyanobacteria in the Baltic Sea. Mar. Ecol. Prog. Ser., 345, 41–50, doi: 10.3354/meps06964, 2007.

Mulholland, M. R., Bernhardt, P. W., Blanco-Garcia, J. L., Mannino, A., Hyde, K., Mondragon, E., Turk, K., Moisander, P. H., and Zehr, J. P.: Rates of dinitrogen fixation and the abundance of diazotrophs in North American coastal waters between Cape Hatteras and Georges Bank. Limnol. Oceanogr., 57, 1067–1083, doi:10.4319/lo.2012.57.4.1067, 2012.

Pilkaitytė, R., and Razinkovas, A.: Seasonal changes in phytoplankton composition and nutrient limitation in a shallow Baltic lagoon. Boreal Environ. Res., 12, 551–559, 2007.

Wannicke, N., Benavides, M., Dalsgaard, T., Dippner, J. W., Montoya, J. P., and Voss, M.: New perspectives on nitrogen fixation measurements using $^{15}N_2$ gas. Front. Mar. Sci., 5, 120, doi:10.3389/fmars.2018.00120, 2018.

White, A.E, Granger, J., Selden, C., Gradoville, M.R., Potts, L., Bourbonnais, A., Fulweiler, R.W., Knapp, A., Mohr, W., Moisander, P.H., Tobias, C.R., Caffin, M., Wilson, S.T., Benavides, M., Bonnet, S., Mulholland, M.R., and Chang, X.B.: A critical review of the $^{15}N_2$ tracer method to measure diazotrophic production in pelagic ecosystems. Limnol. Oceanogr.-Meth. doi:10.1002/lom3.10353

Specific comments

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

Answer: we assume that respiration of living cells rather detritus cause bottom hypoxia. During summer blooms, when plankton (mainly cyanobacteria and heterotrophic bacteria) respiration exceed diffusive oxygen supply to deeper layer, benthic community eventually depletes oxygen from adjacent bottom. We have clarified sentence and it reads now "*Large blooms of living cyanobacteria are associated with high oxygen demand in the water column, which results in transient (night-time) bottom hypoxia and enhances the release of dissolved inorganic phosphorus (DIP) from sediments (Petkuviene et al., 2016; Zilius et al., 2014).*" (line 45-47)

Reviewer 1: Thank you for the explanation. Although this might be part of the problem, I think a majority of the oxygen on the bottom is consumed as they die and need to be degraded. I think you should add a sentence and a reference to this as well if you mentioned the above statement. See for example Conley et al. 2009 on Hypoxia in the Baltic Sea.

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

Answer: thanks for suggestion. We have included this reference.

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

Answer: we collected water samples in triplicates, and in the laboratory each of them were filtered

separately. Text modified: "*Triplicate water samples from each site or layer were filtered (Whatman GF/F, pore size 0.7 µm) for inorganic and organic nutrient analysis as previously described by Vybernaite-Lubiene et al. (2017).*" (line 100-101)

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

Answer: corrected accordingly.

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).

Answer: following the reviewer's suggestion, we added estimates of heterocyst frequency per cyanobacteria filament in the revised version of the manuscript, see updated Figure 6:

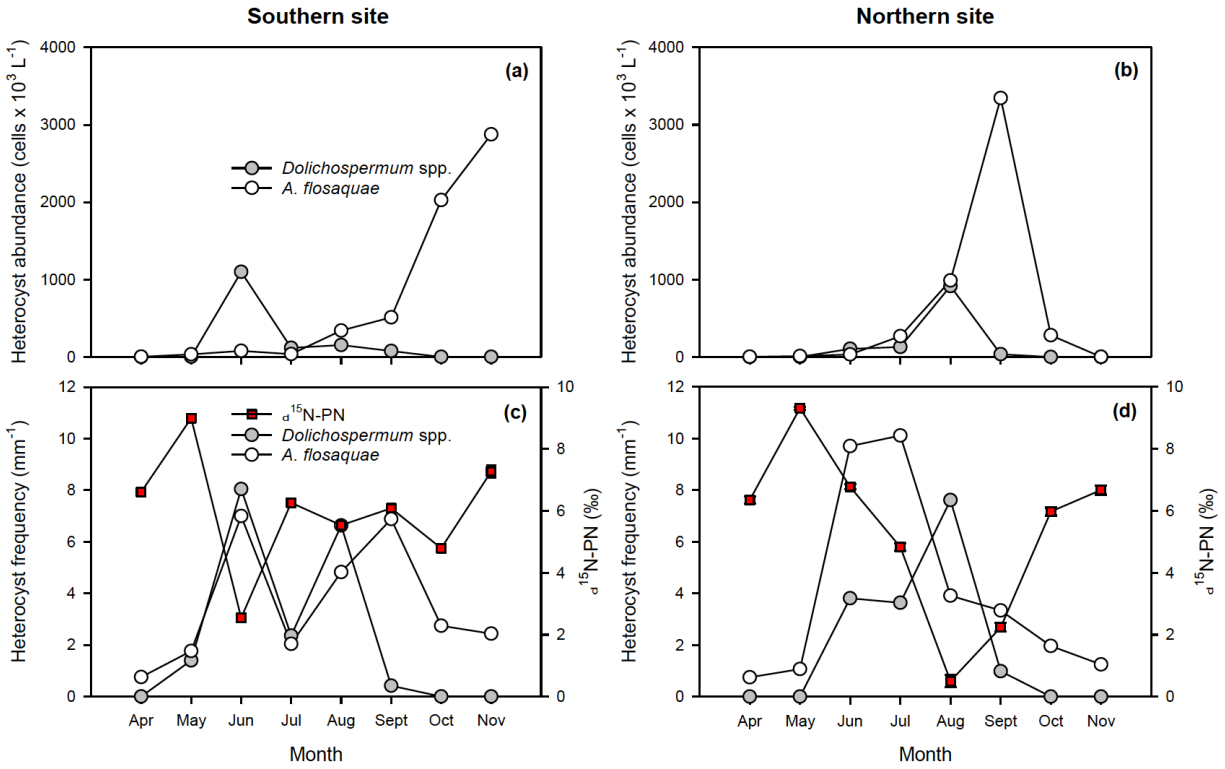

Fig. 6. Abundance of heterocysts of *Dolichospermum* spp. and *A. flosaquae* (a, b), heterocyst frequency per filament (c, d) and stable isotope composition of particulate nitrogen (δ^{15}N-PN) at southern and northern sites in the Curonian Lagoon during 2018. δ^{15}N-PN values are mean and standard error (some error bars not visible).

In the text, we have added following information:

„*The number of heterocysts (cell L^{-1}) and their frequency per millimeter of filament (mm^{-1}) was also determined.*" (line 121-122)

„*Total heterocyst frequency per filament was higher at the beginning of summer (up to 15 mm^{-1}) at both sites, and gradually declined afterwards (Fig. 6c, d).*" (line 249-250)

Reviewer 1: Thank you for including heterocysts per filaments, I think this is more informative than just heterocysts per ml. However, I think you should be more specific. In the southern site the numbers were highest in June and August/September, so actually two peaks, and in the northern sites they peaked in June/July and August depending on the species, so not only early in the summer

and declining as the sentence reads now.

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.

Answer: we kindly note the use of the term "colonial picocyanobacteria" is misleading, since "colonial picocyanobacteria" do not belong to picoplankton owing to their colony size, and need to be enumerated by methods designed for nano- and microplankton. "Colonial picocyanobacteria" refer to cyanobacteria cells of 1-3 µm size embedded in mucilaginous colonies. The colonies are most commonly over 10 µm size and not very abundant (less than 1 colony mL^{-1}) in the Baltic Sea. As the colonies are large and mucilaginous inverted microscopy after sedimentation is the preferred method for detection and quantification. Following HELCOM recommendations, the biomass is estimated by converting in biovolume-carbon each cell from the colony. These colonies have typically small abundances <1 colony mL^{-1}. A larger volume of sample is required to detect such cyanobacterial taxa. In Klawonn et al (2016), free-living picocyanobacteria have not been counted, though they are present and abundant in Baltic Proper waters (B1 or BY31 stations). They counted the "colonial picocyanobacteria" by inverted microscopy after sedimentation of 25 ml of Lugol-preserved samples. In this study, 10 to 25 ml of Lugol-preserved samples were counted by inverted microscopy and the cyanobacteria with cells <3 µm in colonies were assigned to the "non-N_2-fixing cyanobacteria" category. *Aphanocapsa* spp., *Aphanothece* spp., *Merismopedia* spp. and *Cyanodictyon* spp. were detected in low biomass (< 2% of total phytoplankton biomass) during the study period at either site, except in June when their contribution reached 12% at the northern site, as it is now specified in the text "*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)

Picocyanobacteria refer to free-living unicellular cyanobacteria with a size below 2 or 3 µm depending on the size definition chosen. And we used this original definition in the manuscript. They are free-living, belong to the picoplankton and are usually abundant (over 100 cells mL^{-1}) when present. Then can be detected and/or counted by flow cytometry or epifluorescence microscopy, methods designed to count picoplankton. By flow cytometry they are typically counted in volumes of 50-100 µL, as flow cytometry is designed to count small and abundant cells/particles.

In the present study, picocyanobacteria were counted with a flow cytometer following standard procedures. The preservation procedure, the running settings (flow rate, acquisition time, etc.) followed standard recommendations. The analyses for picocyanobacteria were performed independently from the analyses for heterotrophic bacteria. The BD Accuri C6 allows the detection of fluorescence from phycoerythrin (at 585/40 nm after excitation at 488 nm), phycocyanin (at 675/25 nm after excitation at 640 nm) and chlorophyll (>670 nm after excitation at 488 nm). During the analyses many cells showing chlorophyll fluorescence were detected but with no higher phycoerythrin or phycocyanin fluorescence over background level. Therefore, we concluded that no picocyanobacteria was detected in this study.

Reviewer 1: Thank you for your explanation and clarification.

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?

Answer: yes, the bottle was completely filled, and after injection of gas bubble was gently mixed. The missing information was added: "*The samples were filled into 500 ml transparent HDPE bottles and carefully sealed preventing formation of air bubbles. Each sample received 0.5 ml* $^{15}N_2$ *(98%*

$^{15}N_2$, Sigma-Aldrich) injected by syringe through a gas-tight septum, and then gently mixed for 10 min (Zilius et al., 2018)." (line 134-136)

Reviewer 1: Good that you added the gently mixing, I think this is important to know when reading the methods.

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

Answer: we mean that all ^{15}N label have been excreted into the surrounding waters within the incubation time is likely immediately reused and thus appears on the filters. During short-time incubations, this is in particularly relevant when proportion of excreted ^{15}N is relative close to quantities of dissolved $^{15}N_2$ gas, which can happen when using labelled seawater method water with low tracer percentage. To avoid any confusion this statement was removed from the text.

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.

Answer: we disagree with this point. Measured PAR at 2 m depth was always < 5 µmol m^{-2} s^{-1} (June–November), which is well below 1% of surface water irradiance, see added information in revised version "*Surface water samples were incubated outdoors at ambient irradiance, while samples from 2.0–3.5 m were wrapped in aluminium foil as in situ irradiance was below 1% of surface PAR at these depths (< 5 µmol m^{-2} s^{-1} in the period of June–November).*" (line 138-140). We appreciate that near-dark may not be quite the same as dark, but given the very low rates relative to surface (photic) values, we feel that this would not appreciably affect our findings. We feel that the more important methodological issue is that in these studies samples are almost always incubated at a fixed light intensity, whereas cyanobacteria mixing in the water column experience a dynamic light environment. This point is made in the Material and Methods: "*However, such fixed dark conditions is less representative to in situ conditions as cyanobacteria colonies can migrate upward to surface photic zone or use limited light for photosynthesis.*" (line 140-142) and in the Discussion: "*Our study, as well as prior work, is based on 24-h incubations, simulating conditions at a fixed depth, which may not be indicative of rates that could be sustained by diazotrophs circulating over a range of depth and light conditions*." (line 363-365)

Reviewer 1: Thank you for the clarification, I did not understand that the light was that low, good that you included the light level measured in the water.

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.

Answer: unfortunately, $^{15}N_2$ concentration was not quantified in bottles. Therefore, it may lead underestimation of rates as suggested by White et al. 2020. Though we are aware of method use, there are number of studies still published without testing $^{15}N_2$ concentration in incubated bottles. This information was added in the revised version of manuscript: "*As we have used theoretical estimation of $^{15}N_2$ gas dissolution in bottles instead quantification with membrane inlet mass spectrometer, it can result in some underestimation of rates (White et al., 2020).*" (line 153-154)

Reviewer 1: I think it is good that you included this as a potential caveat.

References

White, A.E, Granger, J., Selden, C., Gradoville, M.R., Potts, L., Bourbonnais, A., Fulweiler, R.W., Knapp, A., Mohr, W., Moisander, P.H., Tobias, C.R., Caffin, M., Wilson, S.T., Benavides, M., Bonnet,

S., Mulholland, M.R., Chang, X.B. 2020. A critical review of the $^{15}N_2$ tracer method to measure diazotrophic production in pelagic ecosystems. Limnology and Oceanography: Methods. doi:10.1002/lom3.10353

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

Answer: Optical remote sensing, i.e. the method based on passive radiometers operating in the visible and near-infrared wavelengths, is the only one which penetrates the surface of the waterbody (Robinson, 2010). The satellites can observe the water down to one optical depth, the portion of the water column where approximately 90% of the remote sensing observed signal originates (Gordon and McCluney, 1975; Werdell and Bailey, 2005). The optical depth is equivalent to the inverse of the diffuse attenuation coefficient (K_d) (Gordon and McCluney, 1975) and has also been shown to empirically relate to the Secchi disk depth (Lee et al., 2018). The range of $K_d(490)$ in Swedish coastal waters of the Baltic Sea during 2008 was 0.31–1.19 m^{-1} (Kratzer, Vinterhav, 2010). In Curonian Lagoon, estimated K_d value from daily buoy measurements was 2.7–5.7 m^{-1} during presence of cyanobacteria (2014-2015). This information was added in Material and Methods, see lines 159-163.

Reviewer 1: Thank you for the explanation.

References

Gordon, H.R., McCluney, W.R., 1975. Estimation of the depth of sunlight penetration in the sea for remote sensing. Appl. Opt. 14 (2), 413–416.
Lee, Z., Shang, S., Du, K., Wei, J., 2018. Resolving the long-standing puzzles about the observed Secchi depth relationships. Limnol. Oceanogr. https://doi.org/10.1002/lno.10940.
Kratzer, S., Vinterhav, Ch., 2010. Improvement of MERIS level 2 products in Baltic Sea coastal areas by applying the Improved Contrast between Ocean and Land processor (ICOL) – data analysis and validation. Oceanologia 52 (2), 211–236. DOI: 10.5697/oc.52-2.211
Robinson, I., 2010. Discovering the Ocean from Space. The unique applications of satellite oceanography. Springer-Verlag Berlin Heidelberg, p. 638.
Werdell, P.J., Bailey, S.W., 2005. An improved in-situ bio-optical data set for ocean color algorithm development and satellite data product validation. Remote Sens. Environ. 98 (1), 122–140.

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

Answer: while we consider that correlation coefficient represents the direction and strength of the relationship between chlorophyll and N_2 fixation rates, the regression coefficient determines the effect of chlorophyll a (independent variable) on the N_2 fixation (dependent variable), and determine the explained variation.

Reviewer 1: I guess this is fine as long as you state this as your tested hypothesis in the methods.

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

Answer: thanks for the suggestion.

Line 193, who are the non N_2 fixing cyanobacteria if you did not have any picocyanobatceria? For example in November in Figure 2a?

Answer: the main non-N_2-fxing cyanobacteria are represented by *Planktotrix agardhii* and *Microcystis* spp. This information was also added in the text: "*The non-N_2-fixing cyanobacteria were dominated by Planktotrix agardhii and Microcystis spp.*" (line 203-204)

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?

Answer: see detail answer above.

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

Answer: in the revised version, we have added information that chlorophyll a are presented as mean values and standard error (some error bars not visible) based on three replicates. Yes, on y-axis all units are in micrograms, according to the suggestions we applied proper style of "micro".

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

Answer: the information was added, and it is "*Phytoplankton biomass and community composition were generally similar between surface and bottom layers (April–September), except in October–November when the abundance of N_2-fixing cyanobacteria was greater in the surface layer (2500–3500 µg C L^{-1}) relative to the bottom layer (<100 µg C L^{-1}).*" (line 212-214). Also we have updated legend in Figure 2.

Line 228, among how many samples?

Answer: data are represented by mean values and standard error based on 3 replicates. Missing information was added to figure captions "***Figure 4****: Temporal patterns in temperature, dissolved organic carbon (a, b), dissolved and organic nitrogen (c, e), phosphorus (e, f), and DIN:DIP ratios (g, h) at southern (left panel) and northern (right panel) sites in the Curonian Lagoon during 2018 (error bars denote standard error based on 3 replicates; some not visible).*"

Figure 5, I think it is better to show N_2-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).

Answer: Thanks for the suggestion. In revised version, volumetric N_2-fixation rates are presented per day (µmol L^{-1} d^{-1}).

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

Answer: yes, these rates were measured in the dark.

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

Answer: We have plotted heterocyst abundance versus number of vegetative cells of N_2-fixing cyanobacteria, which indeed provided nice results: "*The abundance of heterocysts varied seasonally depending on the number of vegetative cells of N_2-fixing cyanobacteria (y=0.0251x+75.0, R^2=0.92) with lowest values less than 2000 cells L^{-1} and peak values exceeding 2 million cells L^{-1} in late summer.*" (line 246-248)

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

Answer: done.

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 N_2 fixation nor cyanobacteria biomass at that time? In contrast

the highest number of both N_2 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).

Answer: "per mil" means delta units that are expressed in molecules per thousand, but for convenience we have change to "‰".

In revised version, we also provided heterocyst frequency, which better corresponded to N_2 fixation dynamic. The updated Figure 6 shows that patterns in heterocyst frequency was relatively low in November coinciding to decreased N_2 fixation rates. We assume that the October-November period represents the decline of the cyanobacteria bloom. In the submitted version we discussed that "*Results from this, and a prior study (Zilius et al. 2018), show that despite a high abundance of A. flosaquae at the end of fall, heterocyst frequency, and thus N_2 fixation rates declined substantially when water temperature dropped below 15 °C. Zakrisson et al. (2014) suggested that temperature controls the enzymatic activity of nitrogenase, which directly regulates the intensity of N_2 fixation in filaments*." (line 363-367)

Reviewer 1: I am still a bit perplexed about how chl a of cyanobacteria I November at the southern site is very low, the heterocysts per filaments is very low but the heterocysts per L is very high? I understand that N2 fixation goes down with temperature, but how can it be so many heterocysts when there is no biomass?

Line 248, I think you have light limited N_2 fixation? 1% of surface light can still be 10-20 µm photons, which can be sufficient for carbon fixation.

Answer: we agree that N_2 fixation can be light limited in the turbid Curonian lagoon, but we note that the photic zone does not extend into the bottom layer (2–3.5 m depth), therefore we feel it was appropriate to incubate the bottom samples in the dark (see response to prior comment).

Lines 247-251, did you use correlations or regressions? If this is the regression models you later use this must be clear.

Answer: in present study, we have used linear regression, which later allowed to derived N_2 fixation estimates based on remote sensing Chl-a. We have reformulated the sentence to avoid confusion, and now it reads "*N_2 fixation in the surface layer was significantly ($p < 0.001$) predicted by in situ Chl-a concentration ($R^2 = 0.91$), A. flosaquae biomass ($R^2 = 0.83$) and A. flosaquae heterocysts ($R^2 = 0.88$ all $p < 0.001$; Fig. 7). Whereas N_2 fixation in the bottom layer was weakly explained by in situ Chl-a ($R^2 = 0.52$, $p = 0.07$), but not A. flosaquae biomass or heterocysts. In situ chlorophyll-specific N_2 fixation derived from regression equations (Fig. 7a, b) was considerably lower in the deeper layer (0.002 ± 0.001 µmol N μg^{-1} Chl-a d^{-1}) relative to the surface layer (0.018 ± 0.002 µmol N μg^{-1} Chl-a d^{-1}).*" (line 258-262)

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 µg L^{-1} but with no N_2-fixation.

Answer: we agree that some information is lacking. Not it reads "*Figure 7: Relationships between N_2 fixation and in situ measured Chlorophyll-a (a, b), Aphanizomenon flosaquae biomass (c, d) and their heterocysts (e, f) in surface (northern and southern sites) and bottom (southern site) layers of the Curonian Lagoon during April–September 2018."* (line 264-266)

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

Answer: equation for this estimation is already showed in Fig. 7a, but to be clear we have added this equation in brackets: "*We used the relationship between N_2 fixation and Chl-a (y = 0.018x – 0.459; Fig. 7a) for the surface layer to derive estimates of N_2 fixation for the upper water column (0–2 m).*" (line 270-271)

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

Answer: The regression is marginally significant (p = 0.08) and has a reasonable R^2 value (0.52). Therefore, we felt that this model provided the best means for estimating bottom layer N_2 fixation. We also note that the bottom layer accounts for a relative small proportion of the lagoon's volume and an even smaller proportion of N_2 fixation (since surface rates are 7x higher). Therefore, our whole-lagoon estimates of N_2 fixation are not highly sensitive to assumptions about bottom water rates.

Reviewer 1: Is this caveat explained also in the manuscript?

Line 263, values of what?

Answer: it was referred to estimated N_2 fixation rates. Now sentence reads "*The impact of the bloom on N_2 fixation can be visualized from the relatively low and uniform estimated rates throughout the lagoon during July, and the subsequent development of localized hotspots in the southern lagoon during August and September (Fig. 8).*" (line 275-276)

Line 282, if you have ten times more *Aphanizomenon* you maybe should also have higher N_2 fixation rates? This needs to be discussed and refer to data, for example Klawonn et al. 2016. Were they limited by something?

Answer: see our earlier answer.

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 N_2-fixation rates? This needs to be discussed. The bottom layer had a lot of other organisms contributing to chl biomass.

Answer: see our earlier answer.

Lines 309 and below, can you also put these areal N_2-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.

Answer: thanks for suggestion. We have put our estimates in the context of the Baltic region: "*These estimates reveal that summer N_2 fixation rates are slightly lower in the coastal site of SW Baltic (3.6 ± 2.6 µmol m^{-2} d^{-1}; Klawonn et al., 2016), but higher than those found in the Great Belt (~ 1 mmol m^{-2} d^{-1}; Bentzon-Tilia et al., 2015), Baltic Proper (0.4 ± 0.1 mmol m^{-2} d^{-1}; Klawonn et al., 2016), and Bothnian Sea (0.6 ± 0.2 mol m^{-2} d^{-1}; Olofsson et al. 2020b).*" (line 328-331)

Reviewer 1. Please use the same units across all studies so its easier for the reader to compare. Maybe you need to formulate the sentence a bit clearer: "These estimates reveal that summer N2 fixation rates are slightly lower in the Curonian Lagoon as compared to those measured at a coastal site of…"

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

Answer: Heterocyst abundance tracks cyanobacteria abundance, but in the revised version of manuscript, we also show that heterocyst frequency per filament decreases when biomass increases in November (see figure above).

You need to discuss problems with N_2 fixation from covered flasks and still standing flasks with gas injections somewhere in the discussion. “Caveats” with this study.

Answer: in the revised manuscript, we have stated that “*Our study, as well as prior work, is based on 24-h incubations, simulating conditions at a fixed depth, which may not be indicative of rates that could be sustained by diazotrophs circulating over a range of depth and light conditions.*” (line 359-361)

Technical corrections

Line 33, comma after algae.

Answer: added.

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

Answer: corrected.

Line 500, Please change to Riemann

Answer: done.