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Local nutrient regimes determine site-specific environmental triggers of cyanobacterial and microcystin

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Dear Dr Stamm

Thank you and the two reviewers for providing insightful comments and feedback on our manuscript. We have now completed the revisions and are happy to provide this detailed point-by-point revision report along with the revised manuscript for your consideration. We have highlighted the sections in the manuscript which have been amended or re-written.

	Reviewer 1		
	Comments	**Response**	**Location in text**
1	Line 15, pag.4. A map showing the position of the lakes would be helpful.	We agree to this comment and we have included a map to show the position of the study lakes.	Fig.1
2	Line 11, pag.10. Bimonthly: I suppose this means twice a month...	We have substituted the word bimonthly with twice a month.	P7, line 152
3	Pag.12, line 9. It is possible that the positive correlation with TN:TP is not driven by nitrogen, but is simply the	We agree and have mentioned this briefly in the previous manuscript. In this revised manuscript, we	P18, line 446-448

	result of the negative correlation between cyanobacteria and TP.	have included this more upfront in section 4.	
4	Pag.15, lines 2-5. About the relationship between cyanobacteria and TP, my main concern is that none of the lakes you studied are phosphorus limited. Moreover, the absolute biomass of cyanobacteria is significantly higher in the lake with the highest TP concentration. Why do not carry out a RDA analysis with the absolute cyanobacterial biomass, instead of their relative proportion? I suspect that the relationships could be quite different...	Following on from your suggestion, we have conducted a RDA analysis with absolute cyanobacterial biomass and found a positive correlation between TP and biomass. This indicates that although the lakes were not limited in P as there were sufficient amount available, possibly the changes in P concentrations seem to drive some changes in the phytoplankton communities. The RDA analysis with absolute cyanobacterial biomass is not included in this revised manuscript as we believe that the updated analysis presented in Table 2, Figure 5-7 of this revised manuscript are sufficient to address this issue. Although all of our lakes have high concentrations of P, they still present a range of P levels, with TP in Bibra Lake being an order of magnitude higher than the other two lakes. However, we did find various correlations between P and cyanobacterial dominance and microcystin dynamics and these were different between lakes. We think that this is very interesting as it shows that even in (by definition) non-nutrient limited lakes, triggers for cyanobacteria depend on the local nutrient regime and that a generalization by only using concentrations of nutrients might not be sufficient for future management of lakes. We have included this into the discussion in our revised manuscript.	P17, Line 409-417. P18, line 437-445

5	Pag.15, lines 7-13. According to Reynolds (2006), the ability of Microcystis, the most abundant taxon in your lakes, to become dominant under P limiting conditions is not so straightforward. In general, cyanobacteria as a group can dominate under a very wide spectrum of trophic conditions, depending on the species involved and their respective growth and survival strategies	We agree to this comment and we have made this clear by changing the following sentence: "Although cyanobacteria as a group can dominate under a wide range of conditions, high phosphorus concentrations have been shown to potentially limit the ability of cyanobacteria to become dominant in the phytoplankton community (Chorus and Bartram, 1999; Reynolds et al., 2006). One reason for that is the higher grow rate of other phytoplankton compared to cyanobacteria, and, as such, their ability to utilize nutrients faster under high nutrient conditions."	P18, line 437-433
6	Pag. 15, lines 27-29. In general terms, a high TN:TP ratio does not necessarily indicate a P limitation, because the limitation depends on the absolute nutrient concentration, not on the ratio. Nutrient ratio can be quite varibale from time to time, but, considering the data on absolute concentration, phosphorus limitation seems to be an exception in these lakes.	We agree that our use of the words "nutrient limitation" or "low phosphorus concentration" were not strict enough and we have carefully edited our manuscript accordingly. For instance, we have substituted "low phosphorus availability" or "phosphorus limited conditions" with "lower relative phosphorus availability".	Example: P18, line 437 P19, line 458-462
7	Table 1. Check the range for TDP in Bibra Lake: 16.0-18.0 seems not correct respect to Mean and SD.	This has been corrected. The range should be 16.00 – 180.01	Table 1
	Reviewer 2		
	Comments	**Response**	**Location in text**

1	A few mistakes can still be found, e.g. p. 11111 line 3-5 "The management of toxic cyanobacterial blooms is one of the biggest challenges due to the variability cyanobacteria biomass and cyanotoxins". where a word is missing. Also p. 11112, line 21: "[: : :] are permanent lake" should read "[: : :] are permanent lakes". Throughout the manuscript: Ammonium is an ion should be written NH4+	We have carefully edited the manuscript and modified the following sentences: 1. "The management of toxic cyanobacterial blooms is one of the biggest challenges due to the variability in cyanobacteria biomass and cyanotoxins". 2. "Jackadder Lake and Yangebup Lake are permanent lakes……" 3. NH_4 is now written as NH_4^+ throughout the manuscript.	P3, line 59-61 P5, line 108 Throughout the manuscript
2	Our main concerns are: the text seems a bit overstated in the abstract, discussion and conclusions section, findings regarding site-specificity of environmental factors in explaining cyanobacterial dominance, and MC variation, are not absolutely novel and the paper would benefit from a more honest assessment of results in relation to previous work.	We understand the reviwer's concern and have restated some sentences such as: 1. "The findings of this study suggest that identification of site-specific environmental factors under unique local conditions might be an important strategy to enhance positive outcomes in cyanobacterial bloom control measures." 2. "These results illustrate that reducing phosphorus and iron concentrations in water bodies could potentially reduce the overall toxicity of cyanobacterial bloom, even though it might not completely prevent from the occurrence of cyanobacterial bloom." 3. "However, reducing phosphorus and iron could reduce the amount of microcystin being produced within cyanobacterial cells."	P2, Line 42-44 P19, Line 468-470 P21, 503-504
3	Some environmental factors such as temperature and pH were measured but not included in the statistical analyses although the authors mentioned them as being	We agree to this comment and we have included pH and temperature in all analyses where data was available to allow the inclusion. (see response to	

	important explanatory factors in previous studies.	editor below for more details).	
4.	Hydrological and morphological characteristics of the lakes were mentioned in the sites description but never included in the study.	We agree to this comment and we have included the lakes' characteristics to support our findings on different nutrient regimes. The following sentence was added to the revised manuscript: "Higher phosphorus, iron and nitrogen concentrations in Bibra Lake are expected as the adjacent urban areas and water birds may represent significant sources of nutrients."	P12, Line 291-293
5.	Abstract: When stating the objective in the abstract, "In this study, we investigated the site-specificity of environmental triggers for cyanobacterial bloom and cyanotoxins dynamics". The authors should use "microcystins" instead of "cyanotoxins", which is a term too broad for this study where only one type of cyanotoxins, namely the microcystins, was investigated.	We agree to this comment and the sentence was rewritten as: "In this study, we investigated the site-specificity of environmental triggers for cyanobacterial bloom and microcystin dynamics".	P2, Line 35-36
6.	Introduction: Objectives (2) and (3) described (line 10 to 13 of p. 11112) are somehow a repetition of the same objective. "Identifying the relationship between environmental factors and cyanobacterial biomass and toxin dynamics bloom in each lake" sounds to me like it is a site-specific investigation of the relationships. I don't understand what the 3rd objective adds to the previous one.	We agree to this comment and the two objectives are now combined as: "…identify the site-specific relationship between environmental factors and cyanobacterial biomass or microcystin dynamics."	P4, line 97-98
7.	Methods: The authors use a rather complicated way to test for lake-specificity in the response of	We agree that the suggested analysis could have been an easier option. Therefore, we recalculated	

	cyanobacterial biomass / toxicity, comparing the slope between 2 regression models (page 11118). It is not clear from the text what is the rationale for this particular approach, does this approach require correction for multiple hypothesis testing? In my view it could have been solved by adding lake ID as an explanatory variable in their regression models, and if significant) study its interaction with the other explanatory variables. In most cases it is fair to let all variables compete in the same model (after testing for collinearity).	the analysis with a general linear model. This does not change any of the results and findings. We have re-written the section on statistical analysis accordingly (2.3). "Site-specificity analysis was performed in General Linear Model (SPSS 17.0) to identify if the correlation between environmental variables and cyanobacterial biomass and microcystin concentration was similar in all lakes. The site-specificity was determined by the significant interaction between lake and environmental variable in predicting the variability of cyanobacteria biomass or microcystin concentration."	P11, Line 267-271
8	This study was conducted over a period of a 3 months with bi-monthly sampling in each lake resulting in a time series of 6, 4, and 6 time point in lakes Jackadder, Bibra and Yangebup, respectively. However time is not taken into account in any of the analyses nor mentioned anywhere in the manuscript. Temporal data also need to be treated in order to account for temporal autocorrelation, which has an effect on statistical analysis. If/how authors have dealt with serial autocorrelation of data in their analysis has not been mentioned in the manuscript.	In this study, sampling dates in each lakes were two weeks apart and this could have reduce the chances of autocorrelation between the data points. To ensure that autocorrelation is not an issue in our data, we have carried out sample Autocorrelation Function (ACF) and Partial Autocorrelation Function (PACF) within the SPSS. From the analysis, the autocorrelation coefficients for all parameters were within the upper and lower confidence limits. Therefore, we are confident that our data are independent from each other. We added this information and results to the manuscript: "Autocorrelation Function (ACF) and Partial	

		Autocorrelation Function (PACF) were carried within the SPSS in order to verify if autocorrelation exists between the data points. The analyses revealed that autocorrelation coefficients for all parameters were within the upper and lower confidence limits, thus suggest independency between data from each sampling date."	P10, Line 248-P11, Line 252
9	Section 3.2 Fig 1 shows the proportions of different genera in the cyanobacterial communities of the three lakes. Is the community only composed of these genera, or where there more genera present which are not shown in Figure 1? It seems strange to me that there is a diversity= 3-max 4 genera per lake. Does this figure only show the potentially toxic genera? The legend is not clear enough and this figure is confusing.	The figure only shows the proportions of potentially toxic genera. We have clarified this by re-writing the figure legend as: "Average biomass (μm^3 mL^{-1}) proportions of potentially toxic cyanobacterial genera in Jackadder, Bibra and Yangebup lakes during the study period."	Legend Figure 3
10	Section 3.5 RDA: We would like to see the % of variance explained and the results of the test of significance by permutation. The results of the RDA should be more clearly reported.	In section 3.5 of the previous section, we believe that we had included the percentage of variance explained ; we have now included further statistics to this section, such as the test of significance by permutation (F value and probability (999 permutations).	P16, Line 377-381
11	Discussion p.11123, lines 1-2: "In this study, TFe was negatively correlated to cyanobacterial fraction in	In this section, the site specificity of TFe was described based on the RDA analyses carried out on	

	Jackadder Lake, while in Bibra Lake, a positive correlation was shown between the two (Fig. 3a and b)". The authors do not specify here that these results were obtained when all lakes were combined. The authors report that in the lake-specific RDA in lake Bibra (Fig. 3b) TFe is positively correlated to the cyanobacterial fraction. This section is confusing.	each lake separately. Only the general correlation pattern presented in Table 2 was obtained from analysis on the combined dataset.	
12	p.11123, lines 2-3: These correlations illustrate the cyanobacterial ability to dominate under low phosphorus availability" Were P concentrations measured in the study lakes ever low? According to Table 1, TDP values were between 12 and 40 ug L-1 and TP was between 20 and 1150 ug L^{-1}. Therefore, I'm not sure if the P storage strategy described in this section can support the negative correlation observed study between cyanobacterial fraction and phosphorus concentration in the present. Previous studies have reported the threshold of phosphorus inducing cyanobacterial dominance being around 20-30 ugL-1 which is within the range of the results reported in the present study.	Similar to our responses to Referee #1, we agree that our use of the words "low phosphorus availability" were not strict enough and we have carefully edit our manuscript accordingly. For instance, we have substituted "low phosphorus availability" or "phosphorus limited conditions" with "lower relative phosphorus availability". Regarding to the P storage strategy, we clarified this further by changing the following sentence: "Although cyanobacteria as a group can dominate under a wide range of conditions, high phosphorus concentrations have been shown to potentially limit the ability of cyanobacteria to become dominant in the phytoplankton community (Chorus and Bartram, 1999; Reynolds et al., 2006). One reason for that is the higher grow rate of other phytoplankton compared to cyanobacteria, and, as such, their ability to utilize nutrients faster under high nutrient conditions."	P18, Line 437-441

13	Furthermore, Briand et al., 2008 is misquoted here. In their study, Briand et al. found a positive correlation between TP concentrations and Planktothrix agardhii cell density (PCA, Figure 5).	This has been corrected.	
	Editor		
	Comments	**Response**	**Location in text**
1	p. 11111, L. 1: Which are the global communities you have in mind?	We have specified the term as "water authorities around the world".	P3, Line 56-57
2	p. 11111, L. 4: … one of the biggest challenges…: based on what can you make this statement?	We restate the sentence as: "The management of toxic cyanobacterial blooms is often challenging due to the variability in cyanobacteria biomass and microcystins."	P3, Line 59-61
3	p. 11114, L. 21/22: Reichwaldt, unpublished data: The assumption of complete mixing is essential for the interpretation of the results. Therefore you have to show some of the unpublished data in the SI such that one can see that your statement on the complete mixing is actually supported by the data.	We fully agree and have included Fig 2 and associated statistical analyses to show that the water column was fully mixed. We have included information a previous study which focused solely on the mixing regime of this lake.	Figure 2
4	p. 11115, L. 11: … an algorithm…: please specify.	We have made the statement more specific as follows: "The fluorescence is used to calculate total biomass of each phytoplankton group that is expressed as	P8, Line 180-182

		chl-*a* concentration equivalents (µg chl-*a* L^{-1})."	
5	p. 11118: L. 7 – 18: I am not fully convinced by your answer to the comment by Reviewer 2. You agree that his suggestion of including the sites as an additional variable was an easier option. But you do not provide an argument why not to adopt this proposition. It seems not to be a tremendous hurdle regarding time and effort but could improve readability of the manuscript. Therefore, I suggest that you carry out the proposed analysis or provide a clear argument why not to do so. Whether the analysis is carried out in R or not is not an issue here.	We have addressed this by carrying out the analysis as suggested by reviewer 2. The new analysis does not change any of the previous results and conclusions. We have adjusted the Material and Method section (section 2.3 on data processing) accordingly. "Site-specificity analysis was performed with a General Linear Model (SPSS 17.0) to identify if the correlation between environmental variables and cyanobacterial biomass and microcystin concentration was similar in all lakes. The site-specificity was determined by the significant interaction between lake and environmental variable in predicting the variability of cyanobacteria biomass or microcystin concentration."	P11, Line 266-271
6	p. 11120/11121, sections 3.3 and 3.4: For these two sections, I share similar concerns as expressed in Review 2. One concern is the writing/presentation. For example, you state that the lakes differ regarding the correlations that are observed and refer to Table 2 (p. 11120, L. 24). This table however, does not report the site-specific correlations. Therefore, it is not appropriate. A table showing the site-specific results is missing.	In the earlier manuscript, the site-specific significant correlations were indicated by "*" in Table 2. The meaning of the symbol was added as a note at the bottom of the table. In order to clarify this issue, we have also re-written the following sentence in section 3.4: "Most of the significant correlations between environmental factors and cyanobacterial fraction, cellular microcystin concentration or extracellular microcystin fraction were different between lakes (Table 2)."	P14, Line 340

7	You also hardly present data that allow for a better understanding why the correlation structures obtained differ depending on whether one looks at the lakes individually or if pooled together. In order to improve this part of the manuscript I suggest three things: i) Complement Table 2 with the results for the single lakes ii) Include additional scatter plots that depict all data from all lakes in a single chart for each single explaining variable (e.g., cyanobacterial fraction as a function of TP). Include the correlations of the single lakes and the correlation for the pooled data set. iii) Discuss these scatter plots. The table and figures will allow for a much better understanding of what is going on. The scatter plots for example will allow for a better understanding to which degree the partially conflicting results between single-lake and pooled lake data sets can be explained by the different ranges of the explaining variables among lakes.	We agree to this comment and we have included Table 3, which shows the correlation coefficients between the environmental factors and cyanobacterial fraction, cellular microcystin concentration and extracellular microcystin fraction analyzed for each lake separately. Additionally, we also added three new figures which show lake specific and overall correlations between cyanobacterial fraction (Figure 5), cellular microcystin concentration (Figure 6) or extracellular microcystin fraction (Figure 7) and explanatory environmental variables. . The interpretation of these new figures was added to the result section (3.4) and discussion.	Table 3 Figure 5, 6, and 7 P15, Line 351-368 P17, Line 409-417
8	p. 11122, L. 1 (and elsewhere): The expression of site-specific and unique environmental triggers may be somewhat misleading. Perhaps it would be more appropriate to talk of context-dependency: one probably does not need a unique explanation at each site, but within a general explanatory concept for cyanobacterial blooms the relevance of a single environmental factor may depend on the site-specific combination of other factors. Reading your manuscript	We agree to this comment and we have restated the expression of site-specific and unique environmental triggers in some of the sentences such as: 1. “This issue has raised the question if the relevance of environmental triggers maybe depend on the site-specific combinations of environmental variables.”	P2, Line 33-36

	I have the impression that this corresponds to what you have in mind. Hence, I think it is more of a linguistic issue you may think about.	2. "The findings of this study suggest that identification of significant environmental factors under site-specific conditions might be an important strategy to enhance positive outcomes in cyanobacterial bloom control measures." 3. "This approach might not always be successful in preventing the occurrence of cyanobacterial blooms, due to the roles of physicochemical factors on cyanobacteria and microcystin variability being dependent on the site-specific combination of other factors."	P2, Line 42-44 P20, Line 488-491
9	p. 11124, L. 15 – 19: This statement seems to contradict the statement on p.11123, L. 25 – 27. Please clarify.	We agree that these two statements can lead to confusion. Therefore, we have re-written both of the statements and they are now read as: 1. "In contrast to cyanobacterial fraction, the variability of cellular microcystin concentration was positively correlated to TP, TDP, TFe, TDFe and negatively correlated with TN:TP and NH_4^+. High availability of phosphorus relative to other nutrients is required for energy and material supply in microcystin biosynthesis as microcystin production in cyanobacterial cells is an energy intensive process (Vezie et al., 2002)." 2. "If they are direct ones, our results suggest that regardless of the potentially low microcystin production, cyanobacteria may release higher fraction of microcystin into the surrounding water,	P19, Line 456-458 P20, Line 478-481.

		under lower nitrogen and phosphorus concentrations.”	
10	A further comment regarding your replies to the reviews: Response 3 to Review 2: You argue that you have not included temperature and pH into the statistical analysis because these variables did not differ among the lakes. This argument is not convincing for two reasons: i) It contradicts your own approach: Table 1 shows that TFe did not differ among the lakes. Nevertheless, you have included TFe into the analysis. ii) The fact there is no significant difference between lakes does not imply that there is no influence. I strongly suggest that you include the two variables into the analysis.	We fully agree with the editor that the fact that there is little variation in the temp and pH is not a justification for excluding the variables in the model. As such we have now included the two variables where sufficient data allows us to do so (Jackadder and Yangebup), but not Bibra Lake. Our new analysis revealed that the inclusion of pH and temperature did not largely improve the RDA model on Jackadder and Yangebup Lake. The adjusted R-square only improved from 0.70 to 0.72 and 0.70 to 0.75 in Jackadder and Yangebup Lake, respectively. Therefore, we strongly believe that the exclusion of pH and temperature might have only minor effect on the interpretation of RDA analysis in Bibra Lake. The two variables were included in RDA analysis on Jackadder and Yangebup Lakes, but not in Bibra Lake as the available data points are inadequate to run the RDA model (residual d.f<0). As we have included pH and temperature in all analyses, the following sentences were also added to the revised manuscript: 1. Surface water temperatures were between 19.9 and 28.7°C during the study period. However, the onsite measurements of surface water temperatures were dependent on the time of sampling and varied	P10, Line 244-248

		by up to 3.9°C over the course of a day. Therefore, maximum air temperature on each sampling day recorded by weather stations located nearest to the studied lakes was used as a substitute for surface water temperature in all analysis.” 2. The three lakes were significantly different in most physicochemical factors except for pH, Temp and TDFe (Table 1).	P12, Line 281-282