Interactive comment on "Thermocline depth and euphotic zone thickness regulate the abundance of diazotrophic cyanobacteria in Lake Tanganyika" *by* Ehrenfels et al.

Anonymous Referee #2

We thank the reviewer for commenting on our manuscript and for all the constructive feedback that helps to improve our manuscript. We have noticed that this review reiterates the points made by reviewer #1. Hence, we have concretized some of the changes in the manuscript here.

We have addressed the comments starting with "Reply:".

Comment:

This study presents a very partial view on phytoplankton of Lake Tanganyika as only large phytoplankton (>10 um) was analyzed, while it has been demonstrated more than the half of phytoplankton biomass can not be counted correctly in an inverted microscope because of it small size. The reader gets the impression that this study deals with the whole phytoplankton community, with statements like "filamentous genera Dolichospermum and Anabaenopsis, are key players under these conditions (up to 41.7 % of phytoplankton community)". This is not true because picophytoplankton, which accounts for >50% (up to 80%) of phytoplankton biomass, was totally ignored in this study. For example Fig. 2 gives the impression that phytoplankton is dominated by chlorophytes, which is not true. This is actually reinforced in the text line 207:"The phytoplankton community in Lake Tanganyika was dominated by chlorophytes, diatoms, and cyanobacteria (Fig. 2)". It is not true!.

Reply:

We acknowledge that the wording concerning the studied part of the phytoplankton community must be more precise. However, the goal of this study was to investigate

N fixing, filamentous cyanobacteria and not to analyze the diversity of the entire phytoplankton community as well as its controls. Blooms of filamentous cyanobacteria were frequently observed in Lake Tanganyika, but have been – to the best of our knowledge – never comprehensively studied. Hence, the data and analyses in the current work provide novel insight into factors potentially controlling their abundance in Lake Tanganyika, which may stimulate further research to towards understanding the future of diazotrophic cyanobacteria in an increasingly stratified lake (Verburg et al., 2003; Verburg and Hecky, 2009) as well as other deep, oligotrophic (sub)tropical water bodies.

A conservative biovolume estimate (assuming the same biovolume for cells of the smaller picocyanobacteria and filamentous cyanobacteria) based on metagenomic analyses from stations 2 and 7 (Apr/May) suggests that filamentous taxa make up ~50% of the cyanobacterial biovolume in our samples. We will present the metagenomic analyses in a forthcoming article, but data can be made available upon request for Reviewers/Editor.

Anticipated changes:
In the revised version, we make it very clear that the entire study is focused on the larger, microscopic (>10 µm) phytoplankton fraction and that we cannot make any strong assertions about either smaller fractions or about the total phytoplankton biomass in the lake. Line 207 reads now "The large fraction (>10 µm) of the phytoplankton community in Lake Tanganyika, was dominated …". At all other parts, where phytoplankton community was mentioned, we added "(>10 µm)" or "the studied/large fraction of the phytoplankton community" now. The axis labels of Fig. 2 were changed to "Abundance of >10 µm fraction …" and "Relative abundance of >10 µm fraction …". We have also updated Fig. 4 (see below).

Further, we have added a discussion of picoplankton and phycobilin pigments:
"The phycoerythrin (PE) and phycocyanin (PC) concentrations will also be influenced by picocyanobacteria, which are known to contain at least PE in Lake Tanganyika (Stenuite et al., 2009). It is important to underline here that picoplankton usually dominates the total phytoplankton abundance and biovolume in Lake Tanganyika, especially in the south of the lake and during the dry season (Descy et al., 2005, 2010;

Stenuite et al., 2009; De Wever et al., 2005). Picocyanobacteria are associated with relatively high nutrient conditions in Lake Tanganyika (e.g. Descy et al., 2005 & 2010; Stenuite et al., 2009) and decrease in abundance when filamentous cyanobacteria thrive (see Fig. 5 in Descy et al., 2010). During our surveys, changes in PC and PE concentrations were strongly correlated with the abundances of filamentous cyanobacteria ($p < 0.001$, Pearson correlation coefficients: 0.52-0.88). Thus, we have interpreted the near-surface peaks in PC and PE at stations 4-6 (Sep/Oct) as well as 2-6 (Apr/May) as coming from filamentous, diazotrophic cyanobacteria with possibly lower contributions from picocyanobacteria. The simple cell abundance-PC relationship from Kong et al. (2014) shows that *Dolichospermum* can indeed be responsible for a large fraction of PC measured in this study. Using the slope of the regression line, we estimated a PC concentration of 0.90 µg L^{-1} for the PC and *Dolichospermum* maximum (upper 25 m at station 3), representing 65 % of the measured 1.38 µg L^{-1}. By contrast, at stations without filamentous cyanobacteria, the phycobilin pigment signals must originate from *Synechococcus* and other small cyanobacteria such as *Microcystis* and *Chrococcus*. Phycocyanin concentrations were often below detection limit in the south and at least an order of magnitude lower in the north (max. 0.05 versus max. 1.67 µg L^{-1}) compared to the surface peaks associated to the presence of filamentous cyanobacteria (Fig. 1). Aside from stations 4-6 (Sep/Oct) as well as 2-6 (Apr/May), PE typically formed subsurface maxima corresponding to the chlorophyll-a peak (Fig. 1 & XXX). Noteworthy, PE occurred at concentrations of at least 0.003 µg L^{-1} at all stations including the south basin, where picocyanobacteria are known to dominate (Descy et al., 2005 & 2010; Stenuite et al., 2009). Thus, we argue that PE rather than PC represents contributions from picocyanobacteria."

Following this discussion, we have changed L. 331 to: "Our results also show that the fluorometric determination of extracted phycoerythrin and especially phycocyanin can provide a suitable proxy for filamentous cyanobacteria, when they occur in high densities."

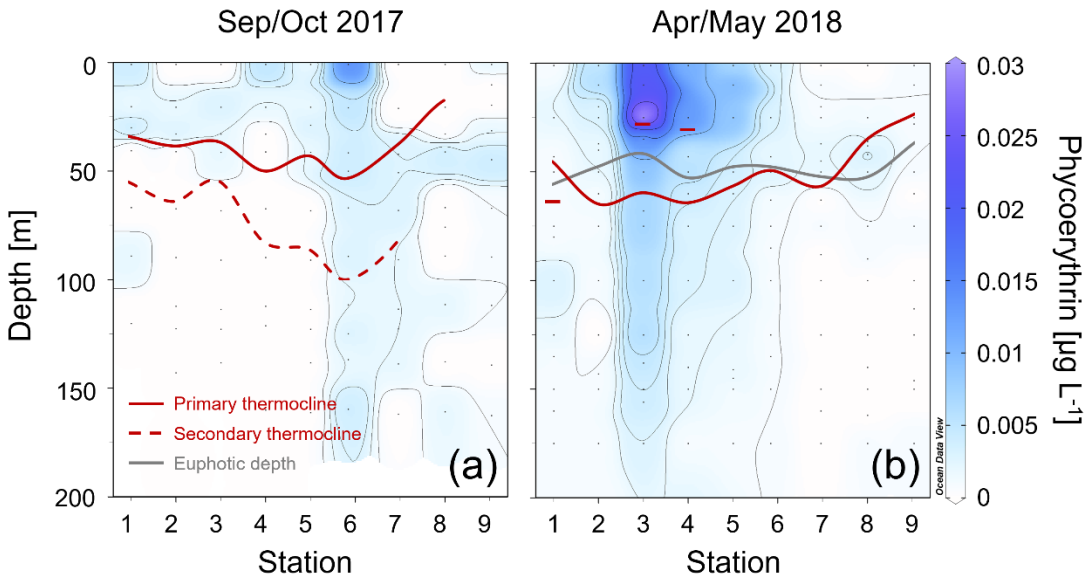


Fig. XXX: Phycoerythrin concentrations during (a) the end of the dry season 2017 (Sep/Oct) and (b) the end of the rainy season 2018 (Apr/May) in Lake Tanganyika.

Comment:

The authors focus their discussion on N limitation, but P is also a main limiting factor in such oligotrophic systems. Why N and not P? The literature on East African Great lakes suggests that P is actually the main limiting factor!

Reply:

As indicated by the reviewer, the nutrient limitation in Lake Tanganyika may be complex and spatiotemporalily variable. The seston stoichiometry and bioassay studies in this lake suggest that the nutrient supply is relatively balanced with P limitation occurring more often than N limitation (Edmond et al., 1993; Järvinen et al., 1999; North et al., 2008; Stenuite et al., 2007; De Wever et al., 2008). By contrast, the dissolved nutrient N:P ratios are typically well below Redfield ratio (Descy et al., 2010; Edmond et al., 1993). Early limnological studies have already recognized this discrepancy and argued that inputs from N fixation may potentially sustain the balanced nutrient supply (Bootsma and Hecky, 1993; Hecky, R. E., Spigel, R. H., & Coulter, 1991). Despite the potential importance, N fixation and factors controlling key diazotrophs (i.e., filamentous cyanobacteria) have not been directly studied in Lake Tanganyika.

The prevalence of N limitation during our study was supported not only by the relative N deficit, but also the fact that at 8 out of 9 stations (Apr/May) and 6 out of 9 stations (Sep/Oct), the levels of free nitrate in the surface waters were below the detection limit and more often so when the thermocline was below the euphotic zone (i.e., the supply of nitrate from deeper waters was low). In addition, we have now analyzed the seston N:P ratios, pointing towards frequent N limitation during our surveys (see below). Last but not least, phytoplankton taxa have different requirements and may therefore be limited at different nutrient levels and ratios (De Wever et al., 2008). Our study organisms (filamentous cyanobacteria) are distinguished by their capability of fixing N. Given the fact that N fixation is known to be metabolically expensive and that we found evidence for N fixation (see below) in the surface waters, we argue that phytoplankton was indeed N limited during our study.

Anticipated changes:
We are presenting now data on the seston N:P ratios that, according to Stenuite et al. (2007), are indicative of frequent phytoplankton N limitation during our survey (see below) and support our reasoning.

The respective part in the results, line 177-182 reads now as follows:
"Nitrate was the main form of DIN accessible to phytoplankton (Fig. 1), while NH_4^+ and NO_2^- remained below detection limit in the upper 120-150 m. The euphotic zone, characterized in Apr/May, varied between 35.8-54.8 m with an average of 47.3 m. Nitrate concentrations in the euphotic zone (Fig. 1 b,e) were also often below detection limit while PO_4^{3-} concentrations were relatively high (Fig. 3 & S2). The DIN-depleted euphotic zone, the N deficit (98 % of all observations) persisting throughout the water column (Fig. S2), and seston N:P ratios typically below 22 (Fig. YYY) together imply that primary productivity was likely N limited during our surveys (Guildford and Hecky, 2000; Healey and Hendzel, 1979; Stenuite et al., 2007)."

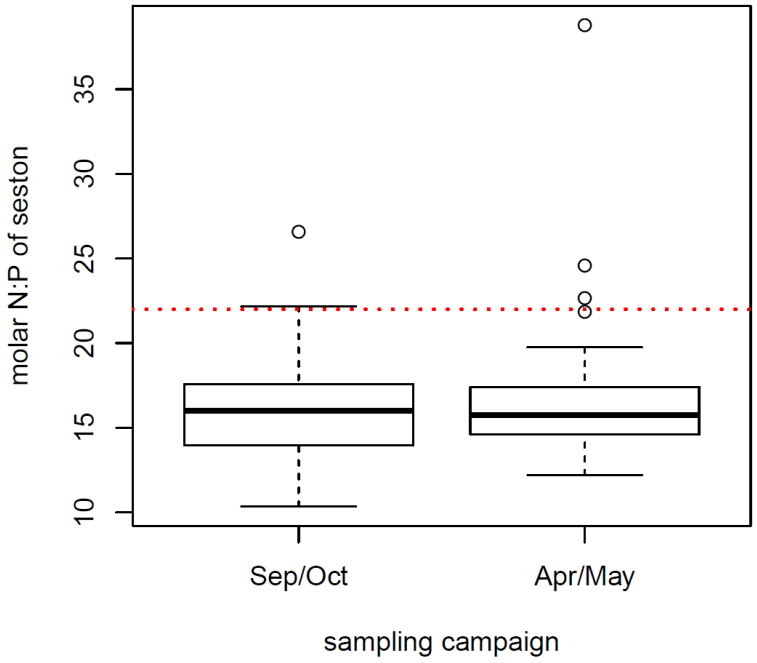


Fig. YYY: The molar N:P ratios of seston during the end of the dry season 2017 (Sep/Oct) and the end of the rainy season 2018 (Apr/May) in Lake Tanganyika. Data is shown for the upper 50 m, encompassing the euphotic zone and the deep chlorophyll maximum. The dotted red line represents the cut-off for N or P limitation defined by previous studies (Guildford and Hecky, 2000; Healey and Hendzel, 1979; Stenuite et al., 2007), with N limitation occurring at ratios <22 and P limitation at ratios >22.

Comment:

Another major fragility of this work is that the authors draw conclusions based only on circumstantial observations, linking nutrient concentration profiles with microscope observations of phytoplankton >10um. Their conclusions are not supported by any experiment nor statistical analysis. Taking into account that ecological processes in Lake Tanganyika are totally dominated by microbial compartments smaller than 10um, which were not took into account in this study, their conclusions probably do not stand.

Reply:

We fully agree with the need acknowledge the importance of picocyanobacteria in a revised version of this manuscript. The focus of this study was, however, on N fixing,

filamentous cyanobacteria (*Dolichospermum* and *Anabaenopsis*) and factors potentially regulating their abundances. The data and analyses presented in our work address this goal and support our conclusions (N fixing cyanobacteria reach higher numbers as a result of reduced NO_3^- fluxes, when free P is available).

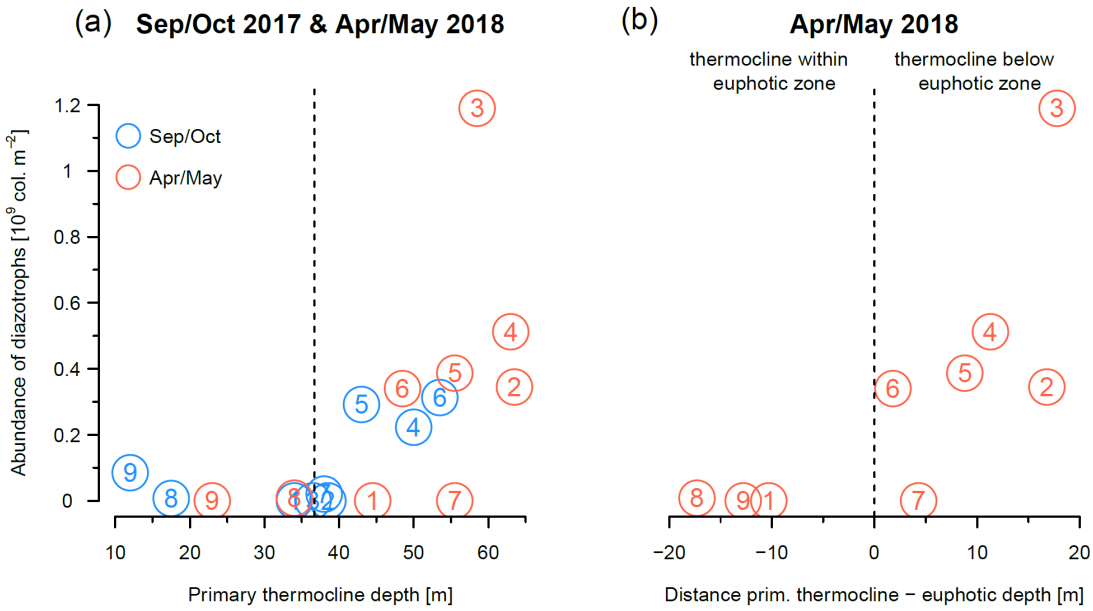
Blooms of filamentous cyanobacteria were reported from multiple phytoplankton surveys in Lake Tanganyika (Cocquyt and Vyverman, 2005; Descy et al., 2005, 2010; Hecky and Kling, 1981; Langenberg et al., 2002; Narita et al., 1986; Salonen et al., 1999; Vuorio et al., 2003). While filamentous cyanobacteria may not be the dominant component of the phytoplankton community in terms of biomass during a large part of the year, they likely are of disproportionate ecological importance when occurring in high numbers. They are the only known N fixer and thus, add freshly fixed N to the oligotrophic and highly N deficient waters of Lake Tanganyika.

The metagenomic analyses conducted during Apr/May reveal that *Synechococcus* in Lake Tanganyika do not contain any regulatory genes of the nitrogenase complex and thus, do not have the capability to fix N. On the other hand, the presence of abundant heterocysts in *Dolichospermum* colonies supports active N fixation (Fig. 5). These observations are further substantiated by $^{15-15}N_2$ incubation experiments, which yielded high maximum N fixation rates of ≥ 5 nmol N L^{-1} d^{-1} at stations where filamentous cyanobacteria were abundant. Filamentous cyanobacteria and N fixation rates were significantly correlated ($p < 0.05$, $R^2 = 0.80$). We will present the metagenomic and incubation data in a forthcoming article (data can be made available upon request for Reviewers/Editor).

We are convinced that the analysis presented in Fig. 4 is more transparent than a simplified statistical model, which does not respect the spatial autocorrelation and i.e. dependence of samples. Instead, Fig. 4 visualizes a clear pattern and the variability between individual stations. The analysis in Fig. 4 a is additionally supported by a breakpoint model estimating a mean threshold thermocline depth (36.7 m) that matches well with the average euphotic zone thickness of around 40 m (Cocquyt and Vyverman, 2005; Descy et al., 2005; Hecky et al., 1978), further supporting the results in Fig. 4 b.

Anticipated changes:

We have now removed any implications that the studied size fraction of the phytoplankton community may control the overall phytoplankton abundance or biomass (see above). To further enhancing clarity, we present the absolute instead of relative abundances of filamentous cyanobacteria in an updated version of Fig. 4 (see below). Using the total abundances no longer required treating station 9 (Sep/Oct) as an outlier in the breakpoint model due to the larger absolute changes, making the results even more convincing in our opinion. The breakpoint in panel (a) shifted only marginally, yielding the same rounded value of 36.7 m.



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