

Dear Editors and Reviewer,

Thank you very much for your positive and constructive comments on our manuscript. We have carefully made corrections according to the comments, we hope it could meet with approval. Please see the attached point-by-point responses and the tracked change version of manuscript for your further evaluation.

### **Response to Reviewer's comments:**

#### **General Comments:**

**Referee #1:** First, the authors purport that since APA on size fractions  $>3.0\mu\text{m}$  is greater than that on  $0.45\text{--}3\mu\text{m}$ , phytoplankton are the main source of APA. There is a wealth of emerging information showing that many (if not all) phytoplankton cells have a host heterotrophic bacteria inhabiting or in close association with cells, making these types of measurements difficult to assign to individual cells alone. Further, many phytoplankton exist in the  $1\text{--}3\mu\text{m}$  size range. At best, the study can show distributions of bulk APA across different size fractions. To assign them to phytoplankton or bacteria requires additional analysis (likely coupled genetic probes and/or ELF). Lastly, to call the  $<0.45\mu\text{m}$  “dissolved” seem suspect as well as many bacteria can slip through a  $0.45\mu\text{m}$  filter and there are likely significant populations of heterotrophic bacteria inhabiting this size fractioned water.

**Response:** Yes, we admit the methods of coupled genetic probes and ELF are more accurate than the method of filtration, we will use the two methods to verify our results in future. We know the size fractionation by filtration is never completely absolute (i.e., overlapping size), it is still widely accepted in the field of aquatic ecology because of the limitation of great amounts of water samples and the equipment in situ (Zhou et al., 2016; Wang et al., 2015). The same method on the size fractionation by filtration was used in some previous studies (Cao et al., 2010; Song et al., 2009), this method still provides useful information on the major microorganisms possibly contributing to APA. In this manuscript we used this method according to the previous references and we purport that since APA on size fractions  $>3.0\mu\text{m}$  is greater than that on  $0.45\text{--}3\mu\text{m}$ , phytoplankton are the main source of APA. We mentioned the main source of APA originated from phytoplankton, but phytoplankton not the only one source. We don't ignore the contribution of the host heterotrophic bacteria and overlapping size. For the  $<0.45\mu\text{m}$  “dissolved” one, we also declare the dissolved is the main type not the only one type. We provided the previous studies in the following:

Xiuyun Cao, Chunlei Song, Yiyong Zhou. Limitations of using extracellular alkaline phosphatase activities as a general indicator for describing P deficiency of phytoplankton in Chinese shallow lakes. *J Appl Phycol*, 2010, 22:33–41.

Song Chunlei, Cao Xiuyun, Zhou Yiyong. Fluctuation of size-fractionated alkaline phosphatase after bloom disappearance in two shallow ponds. *Fresenius environmental bulletin*, 2009, 18(6):982–988.

Jian Zhou . Boqiang Qin . Céline Casenave . Xiaoxia Han. Effects of turbulence on alkaline phosphatase activity of phytoplankton and bacterioplankton in Lake Taihu. *Hydrobiologia*, 2016, 765:197–207.

Peifang Wand, Lingxiao Ren, Chao Wand, Jin Qian, Jun Hou. Presence and patterns of

alkaline phosphatase activity and phosphorus cycling in natural riparian zones under changing nutrient conditions. J. Limnol., 2015,74(1): 155-168.

**Referee #1:** Secondly, there is a timing issue of when samples were retrieved and when they were analyzed. The methods seem to indicate that samples were collected and then 24 hours later, analyzed. Depending on how the water was stored (which was not indicated in the methods) many of the physiological and biological parameters which were measured (such as chlorophyll, TP, SRP, and COD, and APA) will have dramatically changed in that timeframe. Therefore, what is observed at 24 h post collection will not reflect in situ conditions. Therefore, any conclusions based upon these methods and assumptions are difficult to interpret.

**Response:** Thanks for your comment and sorry for our unclear expression. We have rewritten this section and some information was provided in detail. The water samples for APA test were filtered immediately after collection in situ, the filters were put into a portable refrigerator at 0 °C and analyzed within 24 h. In order to avoid the physiological and biological parameters changed dramatically, all water samples for the other parameters measurement were also stored in a portable refrigerator at 0 °C after collected. Therefore, the parameters can reflect in situ conditions. So the conclusions based upon these methods are reliable (See L92-96).

#### **Specific Comments:**

**Referee #1:** APA method is the same as Wang et al., perhaps the authors should acknowledge that.

**Response:** Sorry for our carelessness. We have added the part in the *Acknowledgements* (please see line L337).

**Referee #1:** In the discussion section, it seems the authors suggest that the dominant cyanobacteria was *Microcystis*. Since this organism exists in colonial form, how were these counted? Further, supporting points above, colonies of *Microcystis* are inhabited by a host of other organisms including heterotrophic bacteria and in some cases diatoms. Therefore, when the authors correlate bulk APA to cyanophyta when cyanophyta dominate the community, they inadvertently neglect an important complexity to these communities.

**Response:** Yes, you are right. One difficulty we encountered in the phytoplankton counts was caused by the *Microcystis* colonies. In order to calculate the biomass of phytoplankton as accurately as possible, a rapid, high-speed blending method for disrupting colonies of *Microcystis aeruginosa* to single cells in preparation for cell counts was employed. This sample preparation method was proved rapid and convenient for counting *M. aeruginosa* and associated organisms (Tamar and Arcangela, 1987). Therefore, the result of the correlation analysis based on the bulk APA to cyanophyta was reliable.

On the other hand, Classically, colonies of *Microcystis* are inhabited by a host of other organisms including heterotrophic bacteria and in some cases diatoms. In this study, you can find that when *Microcystis* colonies dominated the community, the relative abundance of diatom is very low (Fig.4). As the heterotrophic bacteria, water samples were filtered after the strong oscillation, many heterotrophic bacteria was peeled by the shearing force. Due to the heterotrophic bacteria and diatom is not the major contributor, we think the bulk APA to cyanophyta when cyanophyta

dominate the community.

**The related references:** Tamar Zohary, Arcangela M. Pais Madeira. Counting natural populations of microcystis aeruginosa: a simple method for colony disruption into single cells and its effect on cell counts of other species. J. Limnol. SOC. sth. Afr. 1987,13(2):75-77.

**Referee #1:** Fig 8 – Although I have no way to disprove the authors, based upon this plot it seems suspect that  $APA_{>3.0\mu m}$  would have a significant positive relationship with cell density

**Response:** Yes, what you said is correct. We check the data again and find there is no significant relationship between  $APA_{>3.0\mu m}$  and cell density. This result indicated the species-specific of the phytoplankton excreting alkaline phosphatase.

#### **Technical Corrections:**

**Referee #1:** Line 12 – “investigation was” should be “investigations were”

**Response:** Agreed and revised (please see L13).

**Referee #1:** Lines 18 to 19 – “Cyanophta” and “Bacillariophyta” are not “species” but phyla

**Response:** Agreed and revised (please see L19).

**Referee #1:** Line 31 – add a “a” between “hydrolyze” and “broad”

**Response:** Agreed and revised (please see L32).

**Referee #1:** Lines 44 to 46 – I don’t believe Nausch says this at all.

**Response:** Agreed and revised (please see L44-47).

**Referee #1:** Line 81 – How were water samples stored between sample time and analysis 24 hours later?

**Response:** In order to avoid the physiological and biological parameters changed dramatically, the samples were stored in portable refrigerator after collected, all samples were analyzed within 24 h.

**Referee #1:** Line 96 – there seems to be a problem with the PDF here as some of the methods appear outside of the margins

**Response:** Agreed and revised (please see L107).

**Referee #1:** Line 258 – or more likely, increased concentrations of SRP

**Response:** Thanks for your comment. According to your suggestion, we have rewritten this sentence. The increase concentration of SRP isn’t contradictory to the decrease of algal cell density of phytoplankton. SRP is the bioavailable form of phosphorus that the phytoplankton can uptake directly. As the cell density decreased in winter, the concentrations of SRP increased. So the low  $APA_{>3.0\mu m}$  in winter may result from the low algal cell density of phytoplankton and the increased concentrations of SRP in parallel.