

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:**

**Main comments:**

**Referee #2:** The main weak point of this study is methodological. Authors consider bacteria absent in the fraction  $< 0.45\mu\text{m}$ , but they can actually be comprised between  $0.45\text{-}0.2\mu\text{m}$ . According to this, half of the total APA in your study is found in the dissolved fraction (53.4%, L235) where a huge amount of bacteria are still present after filtration. I suggest authors to take this comment into account and modify results and discussion sections accordingly.

**Response:** Yes, we agree with you that the fraction  $< 0.45\mu\text{m}$  contains some pico-bacteria between  $0.45\text{-}0.2\mu\text{m}$  and some picophytoplankton. The fraction  $< 0.45\mu\text{m}$  can't be called as the dissolved fraction though many articles named it as the dissolved fraction. We changed it as the picoplankton/dissolved fraction.

**Referee #2:** Hypothesis in this study are missing. Please, supply them at the end of the introduction section.

**Response:** Agreed and revised (please see L70-72).

**Referee #2:** All environmental parameters analyzed in this study were retained, after MonteCarlo's permutation test, in the RDA which is quite surprising. I was really confused after reading P10 L200-202 where authors state that permutation permitted to determine the significance of canonical axes. Could you please clarify this?

**Response:** Thanks for your comment and sorry for our unclear expression.

The environmental parameters listed in Table 1 were proved correlated with APA fractions. But the parameters such as total nitrogen (TN), nitrate nitrogen ( $\text{NO}_3\text{-N}$ ) and ammonium nitrogen ( $\text{NH}_4\text{-N}$ ) were proved not correlated with APA, so these parameters were excluded after MonteCarlo's permutation test. That is why all environmental parameters analyzed in Table 1 were retained, after MonteCarlo's permutation test.

The RDA analysis in our study was done in strict accordance with the steps described in the book "Multivariate analysis of ecological data using CANOCO". It was illustrated that the permutation tests can be used to test virtually any relationship. To illustrate its logic, the permutation tests are used for testing the significance of a regression model.

**References:** Lepš J, Šmilauer P. Multivariate analysis of ecological data using CANOCO[M]. Cambridge university press, 2003.

**Referee #2:** Second, I suggest authors to remove APA total from the RDA since it will covaries with APA in fractions.

**Response:** Agreed and revised (please see L229).

**Referee #2:** Third, is there a sense in discussing about correlations between APA and environmental parameters showing such a low “r-values”? In my opinion, the correlations presented in Figure 6 and 8 should be removed.

**Response:** The source of alkaline phosphatase is complicated. It may excrete from phytoplankton, bacteria, zooplankton, sediment and so on. As to the phytoplankton, the species-specific result in the related environmental parameters to the alkaline phosphatase differed. Besides, the previous studies proved the correlation relationship between APA and SRP emerged within a certain threshold. These uncertainties lead to the correlations between APA and environmental parameters showing such a low “r-values”. Although the “r-values” was low, the results of correlated analysis and RDA analysis were consistent. So both the correlations presented in Figure 6 and 8 and the RDA analysis in Figure 7 can prove the interaction between APA and the environmental parameters.

**Referee #2:** Fourth, is there a sense in checking for chl-a and APA<0.45μm correlations when you already know that algae are not present in this fraction?

**Response:** Yes, you are right. This part was deleted.

**Referee #2:** Finally, standard errors over means are not present in Figure 2. Please supply.

**Response:** Agreed and revised (please see L152).

**Referee #2:** I suggest authors to include phytoplankton community composition (i. e. diversity indices) in RDA in order to reinforce discussion in P13 L253-277. I would also appreciate a deeper discussion in spatial differences in APA in the Xiaojiang River. Why APA decrease downstream in the estuary zone?

**Response:** Thanks for your good suggestion. The species-specific of phytoplankton excreting alkaline phosphatase was meaningful and interesting. We conducted the research that focus on the relationship between phytoplankton community composition and alkaline phosphatase, and found some interesting results (unpublished). This study focuses on the spatio-temporal heterogeneity of alkaline phosphatase activity. So it is difficult for us to explain the meaningful topic mentioned above explicitly. As to the APA decrease downstream in the estuary zone, it results from the higher algal cell density in midstream than in estuary that has been illustrated in P13 (L307-310).

#### **Minor comments:**

**Referee #2:** The amount of replicates analyzed for each of the biological parameters (i. e. APa, chl-a...) measured has not been specified in the methods section.

**Response:** Agreed and revised (please see L92).

**Referee #2:** Be consistent through your MS on: APA or APase? Check APA units ( molPNP L-1min-1)

**Response:** Thanks for your comments. APA is different from APase. APA means alkaline phosphatase activity (L13), and APase means alkaline phosphatase (L32).

**Referee #2:** L27-27. This statement is contradictory according to what you described above

(L22-27).

**Response:** This study came to a conclusion that phytoplankton communities determine the spatio-temporal heterogeneity of alkaline phosphatase activity (L23-24). Besides this, water temperature was proved to be positively correlated with APA and water level (WL) were negatively correlated with APA (L27-28). So it was concluded that spatio-temporal heterogeneity of APA was also related to water temperature and hydrodynamics.

**Referee #2:** L32-32. Not only cell surfaces but also freely diffusible enzymes (See Burns et al. 2013)

**Response:** Agreed and revised (please see L34).

**Referee #2:** L54-55 and L59-60. Repetition.

**Response:** Agreed and revised

**Referee #2:** L 57-59. A reference is missing.

**Response:** Agreed and revised (please see L61).

**Referee #2:** L68-70. Not clear, rephrase please.

**Response:** Agreed and revised (please see L70-72).

**Referee #2:** L 96. Correct.

**Response:** Agreed and revised (please see L108).

**Referee #2:** L122-123. Supply reference here, please.

**Response:** Agreed and revised (please see L134).

**Referee #2:** Figure 2. Remove lines indicating seasons, they are confusing. Use dotted line, at least, for Water Level, this will improve lecture.

**Response:** Agreed and revised (please see L152).

**Referee #2:** Show ANOVA P-values in a separate Table for improving clarity of results.

**Response:** All the P-values listed in this study were less than 0.05, some of them were even less than 0.01. So the correlation analysis results were reliable. It is sufficient to prove the relationship between APA and the environment parameters.

**Referee #2:** L158. This sentence is not correct since cyanophyta are dominant in April.

**Response:** Agreed and revised (please see L174).

**Referee #2:** L247-248. This statement is wrong. Reconsider it after reading main comments described above.

**Response:** Agreed and revised (please see L247-253).

**Referee #2:** L270-271. Rephrase, please.

**Response:** Agreed and revised (please see L275-279).