Response to referee #1 (our answers in blue):

General comments

The authors present an interesting manuscript about the different effects of seasonal and diel variations in a river's thermal stratification on phytoplankton community. This work is timely given the recent intensification of interest in global temperature increase and helps predicting its short/long term consequences in freshwater ecosystems. I am pleased that the authors made a valuable contribution to the field with the high-frequency data of phytoplankton community rarely seen in other studies. The authors hypothesized that river stratification would have different environmental drivers and effects on phytoplankton in the two different time scales then analyzed them separately. Interestingly, the authors found that the seasonal shifts in phytoplankton community structure were either insensitive and showed a limited response to the stratification indices. Summer cyanobacterial bloom intensity, here cell abundance and accumulation into the surface water, was positively affected by the diel variations in the stratification indices and thermocline. Based on the environmental drivers of stratification, the authors discuss the generalization of stratification events for the river system and the implementation strategy for flow management to mitigate cyanobacterial blooms. Overall, the manuscript has interesting research questions and the data collection/analysis/interpretation seem sound. However, the manuscript needs to be revised before publication. I hope the comments below can help the authors improve their manuscript.

We would like to thank Reviewer#1 for the positive and constructive review. We have carefully responded to each comment/suggestion and did our best to improve the manuscript accordingly. We clarified and simplified the paper all along: We re-wrote the methods section to make it easier for the reader to follow. The diel stratification was discussed more thoroughly. All co-authors edited the letter and the manuscript, with additional English editing by a native speaker collaborator. We believe that the paper flows better now, making it easier for the reader.

Specific comments

Section 2.2: the used thermal stability indices are all based on the vertical temperature difference (potential energy), but their calculations lack the concept of vertical mixing (mixing energy) that against the formed stratification. Prandtl number, Richardson number, or Lake number could be additionally considered when appropriate (Kirillin and Shatwell, 2016).

We admit that the stratification indices we used do not include a term for vertical mixing as mentioned by the reviewer. However, we will keep the indices as they have advantage over the suggested indices after considering two major issues written below. We will add a clarification for the choice of indices.

First, one of the purposes of the study is to scale the river stratification then test its relationship with hydrological and meteorological conditions. The indices used in the present study (RWCS, Schmidt stability, and maximum temperature gradient) do not include hydrological nor meteorological parameters in their calculation unlike the suggested indices. Also, we found that most researches on phytoplankton response against stratification in freshwater ecosystems had used these indices (Becker et al., 2008 and 2009; Cui et al., 2021 and 2022; Rocha et al., 2019). In the Discussion part, we compared our findings with other stratification cases based on these indices.

Second, thermal structure is always “subjectively” identified because an optimal solution for the threshold determination is rarely found (Zhang et al., 2014a, Chu and Fan, 2011). A number of studies have evaluated the overall thermal structure to study its response to external forces, such as air temperature, wind, and rainfall. For this purpose, the Schmidt index (Wang et al., 2019, Wang et al., 2012, Winder and Schindler, 2004), RWCS (Relative Water Column Stability) (Zhang et al., 2021b, Zhu et al., 2013), RTRM (Relative Thermal Resistance to Mixing) (Pu et al., 2020, Wetzel and Likens, 2000), and other global indexes have been widely adopted. The thermocline, which is observed as a region of sharp changes in temperature, separates the epilimnion and the hypolimnion, and significantly impacts water movement and vertical substance translocation (Hachaj and Szlapa, 2017). At present, the gradient criterion is mostly used to identify these thermal layers, as it distinguishes a region with a temperature gradient greater than a specific threshold as the thermocline, and the remaining two layers are then determined. This method is concise and practical, but the threshold is given empirically and varies from 0.2 $^{\circ}C \cdot m^{-1}$ (Yang et al., 2020a, Zhang et al., 2014a), 1.0 $^{\circ}C \cdot m^{-1}$ (Hadley et al., 2014, Wang et al., 2012), and 2.0 $^{\circ}C \cdot m^{-1}$ (Huang et al., 2016, Coloso et al., 2011) with the environment.

Section 2.3: It would improve the readability of the materials and methods section, if the different data analyses were more clearly linked with specific hypotheses which already stated in the results section.

Thank you for your instruction. Firstly, to clarify hypotheses, we have revised the aims of the study in the Introduction, as follow: “Therefore, the purpose of this study was to (i) diagnose the stratification degree and identify the stratification characteristic in the Nakdong River, (ii) examine the relationships between stratification degree with hydrometeorological variations and vertical nutrients patterns, and (iii) identify the phytoplankton changes in community composition and vertical cell distribution associated with stratification variability. We hypothesized that the river stratification would have different relationships with environmental variables and effects on phytoplankton between seasonal and diel scales, then we analyzed them separately. By including water quality and hydrometeorological parameters, mediation strategies for what? were discussed based on the results.”

Secondly, we have extensively revised the Materials and Methods to improve its readability by highlighting the hypotheses for each analysis.
As for the first aim, three thermal stratification indices were selected and calculated. To underscore this, we have revised the sentence as “To identify the existence and intensity of thermal stratification in the Nakdong River, three indices… ” (from line 91 in Section 2.2).
As for the second aim, we have revised the phrase, “To examine the correlation structure among environmental variables including the stratification indices,” for the link with principal component analyses (PCA).
As for the third aim, the sentence for the canonical correspondence analysis (CCA; replaced into RDA in the revised MS) were changed to “To delineate the effect of

significant environmental drivers including the stratification indices on the vertical structure of phytoplankton community,”.

Additionally, we have changed the subtitle for Section 3.1. in Results from “Stratification indices” to “Stratification patterns”.

Section 3.1: temporal variations in the stratification indices are investigated, but why are the authors interested in the scales of variation? What do they expect? This is one of many examples, where the formulation of a hypothesis would improve the storyline. Are the authors expecting that short-term stratification will have a different ecological mechanism or consequence from lake stratification which persists longer?

Yes, we investigate how long did the river stratification persists and compare the duration with other stratification cases in the Discussion section as written below.

“An earlier onset of thermal stratification can lead to an increase in the spring peak biomass of phytoplankton which can lower summer biomass of zooplankton (George and Taylor, 1995). Consequently, phenological change in plankton seasonality can be influenced by the change in timing of stratification (Thackeray et al., 2008; Winder and Schindler, 2004)”.

Fig 4: why are the authors presenting additional information on the thermoclines and their vertical variations? It would be easier to read if the authors formulated a hypothesis about how the diel variation of the thermoclines affect the vertical distribution of phytoplankton cell in Fig 8 and then investigate these.

Thermocline depth and its vertical migration are known to control cell density and vertical distribution of the phytoplankton (Santos et al., 2015). We revised the Introduction and Discussion.

Section 3.2: the first paragraph summarizes the changes over seasons and sites in the multiple parameters which were later analyzed against the stratification indices. From reading, it is not clear why all this information (and with the standard error of detail) is presented. Parts of the paragraph are trivial and the text could easily be reduced substantially (e.g. the two first sentences could be removed).

The research questions and corresponding results are like below.

H1. Is the river in eutrophic or physically calm status during the stratification period?: R1. Trophic status/ hydrometeorological condition

H2. Does the stratification affect the phytoplankton via vertical difference in the water quality within the water column?: R2. Kruskal Wallis test on the water quality at various water depths

The text had been shortened as written below.

“Table 2 summarizes the environmental conditions of the lower Nakdong River for the five months. Though several morphological (i.e., depth) and hydrological (i.e., flow rate and water level) parameters were site-dependent, all three sites were highly eutrophic based on nutrient concentrations and chlorophyll a concentration. All the water quality variables were not significantly different among the three water depths of 0 m, 3 m, and bottom (Kruskal Wallis test, $p > 0.05$, n=15). However, the diel survey in August showed that water temperature and chlorophyll a varied significantly with depth (Kruskal Wallis test, $p < 0.01$, n=18). Dunn post hoc tests revealed that differences between WT0, WT3, and WTB were all significant, but ChlB was only significantly different from Chl0 and Chl3 ($p < 0.05$, n=18)."

Section 3.3: relationships between phytoplankton assemblage and multiple environmental factors including the stratification indices are investigated. It is described that the diel CCA showed a positive relationship between air temperature and cyanobacterial density. The authors must draw a conclusion by combining the PCA results, which showed a strong relationship between chlorophyll a and the stratification indices.

We recognized that both PCA and CCA depicted the relationship between the stratification indices and phytoplankton abundance differently at the diel scale. We solved this by replacing CCA into RDA assuming a linear response in phytoplankton community against an environmental gradient as we assumed linear among variable relationships in PCA. RDA has been widely used to describe changes in phytoplankton community in stratified freshwater ecosystems (Becker et al., 2009; Xiao et al. 2011; Zhou et al., 2016). RDA showed a high association of cyanobacteria to RWCS. RDA maintained high model significance ($p<0.01$) and explained more variation in phytoplankton data compared to CCA by 16.8%.

Fig 8. I suggest the authors to present cyanobacterial cell density, which was used in the CCA analyses in Fig 7. This may give a reason for the different stratification-phytoplankton relationships between the PCA and CCA.

Thanks for this comment. We replaced chlorophyll a concentration into cyanobacterial cell density in Fig. 8 and overlaid it with the depths of thermoclines.

Section 4.2: 'The PCA ordinations revealed that thermal stratification is one of the most important drivers of water environments in the Nakdong River, largely accounting for their seasonal and diel variations'. What do the authors mean by this?

Each ordination axis is a linear combination of all explanatory variables. The PCA returned the ordination axes corresponding to the directions of greatest variability within the dataset (meteo-hydrological variables, water quality, and stratification indices). As the sites and seasons (or times) were ordinated along these axes, and the stratification indices had higher loading to the axes. Therefore, we concluded that 'thermal stratification is one of the most important drivers of water environments in the Nakdong River, largely accounting for their seasonal and diel variations'

Reference

Kirillin, G., & Shatwell, T. (2016). Generalized scaling of seasonal thermal stratification in lakes. Earth-Science Reviews, 161, 179-190.

Thank you for the reference.