

## Comment on bg-2022-100

### Referee #2

Referee comment on "Interdisciplinary strategy to survey phytoplankton dynamics of a eutrophic lake under rain forcing: description of the instrumental set-up and first results" by Fanny Noirmain et al., Biogeosciences Discuss., <https://doi.org/10.5194/bg-2022-100-RC2>, 2022

We thank the referee for his suggestions made online and on the pdf. We respond in blue below to his comments (our answers to the major comments of the pdf have been added at the end of the text).

The study title "Interdisciplinary strategy to survey phytoplankton dynamics of a eutrophic lake under rain forcing description of the instrumental set-up and first results" by Noirmain et al. aims to define the fine scale effect of rain and the carried algal particles on a lake physiochemistry and phytoplankton community. They combine methodology from meteorological sciences analyzing cloud structure and origin and raindrop algal cytometry, with characterization of the water column properties and phytoplankton microscopy enumeration, in an innovative approach that aims to reconcile recent findings of rain algal cell deposition (Dillon et al. 2020, Wisniewska et al. 2022, both cited thoroughly in the manuscript), with traditional works that explore the relationship between rain events and lake biogeochemistry, such as de Eyto et al. 2016 (DOI: 10.5268/IW-6.4.875). As far known, no efforts have been made to analyze the lake surface and the rain for both chemical composition and photosynthetic organisms.

The study has the potential to provide great insight into rain events effects in lake phytoplankton, short term surface stoichiometry and water column temperature changes. The evaluation of the photosynthetic organisms suspended in rain drops in comparison with the lake phytoplankton can provide great insight into the dispersal rate and mechanisms of the different phytoplankton organisms which is still a poorly understood subject. The inclusion of a phycocyanin channel (also with the chlorophyll channel provides a unique opportunity, together with the real time evaluation of the effects of rain on the physiochemistry of the lake, can provide a great insight on the effects of rain events on the phytoplankton community. Although the title of the article alludes to a presentation of experimental setup and first results, the listed objectives, rationale behind the analyses and discussion aim for a much definite style of work.

Unfortunately, the measurements provided seem disconnected. The water sampling times are too far away to assign any causality of the chemical and community changes to rain events and not wind induced or upstream inputs (which seems the case given the steady decrease of temperature in CR1). Mesocosm experiments could have helped isolate the rain effects from the basin and wind effects.

The suggestion to use a mesocosm is interesting and would help isolate rain to wind effects, but we do not have this material at our disposal and cannot perform this experiment. For this article, we focus on a case study of three rain events to illustrate a monitoring strategy of the atmosphere and lake. As we analyzed the rain impact on the lake stratification at a fine scale in real-time, we were able to report a causality between the rain intensity and the lake temperature. To better show the wind effect on the lake temperature, as suggested by the reviewer, we added a correlation between the water temperature and the wind during the rain events. So, during HIR, associated with low wind speed, we report an immediate

decrease in the diurnal surface water temperature. The correlation between the water temperature at the lake surface and the wind was positive but not significant ( $r=0.8$ ,  $p\text{-value} = 0.3333$ ). In contrast, the correlation with the rain intensity was significantly negative ( $r=-0.7$ ,  $p\text{-value}=1.2 \cdot 10^{-06}$ ), suggesting the predominant water temperature decrease due to the rain amount at the surface.

On the other hand, during CR1 associated with a higher wind speed, the correlation between the water temperature and the rain intensity was lower ( $r=-0.24$ ,  $p\text{-value}=1.3 \cdot 10^{-03}$ ), whereas those obtained with the wind speed was significantly negative ( $r=-0.63$ ,  $p\text{-value} = 2.1 \cdot 10^{-03}$ ) until 3 m deep. Moreover, as the wind was higher during RP2, it could be argued that it accelerated the decrease of the lake temperature by mixing the water column during this period and weakening the lake stratification strength. This analysis and correlations with the wind could be added to the manuscript to show better the relative contribution of wind and rain in water temperature as the wind events.

Although we sampled the lake as soon as possible, we agree that a more frequent sampling would increase the understanding of the causality between biochemical rain and lake biochemical composition. Nevertheless, for this article, it was not the principal aim as we wanted to illustrate a strategy with high monitoring. Indeed, we present only three rain events as an example for the case study that does not allow us to connect precisely the rain and lake biochemical composition

The lake phytoplankton and the rain photosynthetic cells are measured with two different methods that are hard to reconcile. The rain cytometry does not seem to fix the cells with glutaraldehyde like Dillon et al. 2020, so the cells present in rain might be over or underestimated by growth or death in the rain collector chamber.

Overall, this work provides the first attempt to measure the rain effect on a lake on the fine scale. It can be improved by a bigger connection between the variables and timescales used to measure them and the questions set in the introduction. To properly answer said questions I suggest performing cytometry on the lake surface (and/or microscopy on rain samples), isolate the effects from rain and watershed inputs using mesocosm enclosures, and shortening the sampling time after the rain event. Although the main missing link is the disconnection of methods for phytoplankton analysis for the lake and for the rain, with comparative 2d cytograms of known cultures/species or lake samples, the two datasets can be made compatible. Regarding the manuscript structure, the introduction and the discussion need higher cohesiveness and streamlining, and I suggest that it undergoes severe rewriting. The results' figures could also benefit from trimming down to the ones specifically pertaining to the questions set.

The cytometry method used to detect the photosynthetic microorganisms in the rainwater was developed to cover the size of particles ranging from 0 to 30  $\mu\text{m}$ . This methodology is well adapted for species in rainwater, as their size commonly found is under 30  $\mu\text{m}$  in the atmosphere. On the contrary, the microscopy-based method is not well adapted due to the very low number of photosynthetic cells in rainwater. Moreover, in the literature, we found that some authors let the cells grow for 30 days before estimating the diversity, which leads to underestimating the diversity as all species cannot grow in an artificial medium (Wiśniewska et al., 2019). So we decided not to use this method.

On the contrary, the photosynthetic lake species can be composed of colonial or filamentous forms larger than 30  $\mu\text{m}$ . Therefore, the flow cytometry is inappropriate and could lead to underestimating the

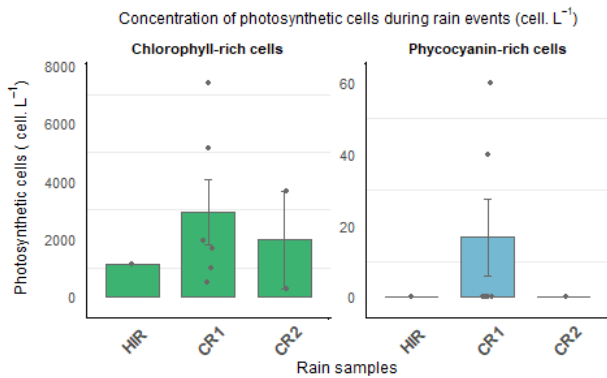
concentration of the lake's colonial or filamentous species. It is why we estimated the lake phytoplankton abundance by microscopy.

However, we also measured the phytoplankton by flow cytometry, using the same recorded parameters developed for the rainwater. An example is presented in the table below, showing a comparison between rain and lake samples measured by flow cytometry. The flow cytometry based-method detects small cyanobacteria (like *Synechococcus* and *Synechocystis*), which grow especially after RP2 (plot B, suppl. fig). On the contrary, the microscopy counts show that many cyanobacteria were present before RP1 and RP2 (plot C, suppl. fig), which were not detected by the flow cytometry based-method (plot B), corresponding to colonial cyanobacteria (*Microcystis*, *Merismopedia*...) (Fig 8B).

Although the sonication could help dissociate the colonial species, it could also damage cyanobacteria or flagellate cells, which will not be detected by flow cytometry. Moreover, chlorophyll a is not the best proxy to assess environmental diversity as the increase in chlorophyll a can be due to a higher proportion of large species (Felip and Catalan, 2000; Blottière et al., 2017).

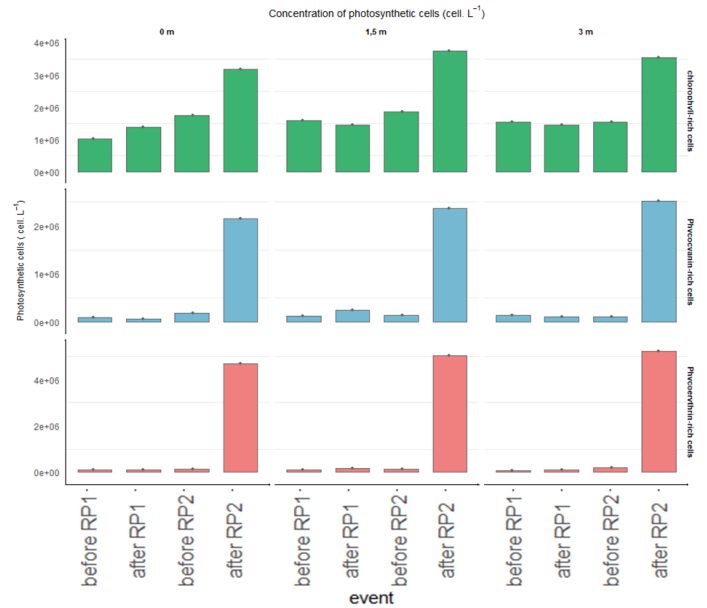
Due to these technical issues and more clarity in this article, we select only the appropriate methods to detect the rain's photosynthetic cells by flow cytometry and the lake phytoplankton by microscopy.

**A**  
Quantification of the photosynthetic cells from the pigment's fluorescence measured by flow cytometry on rain samples

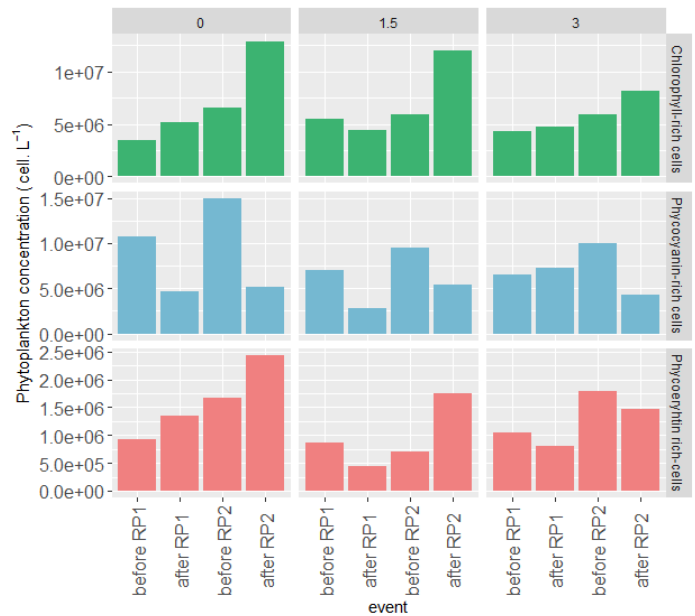


**C**  
The microscopy could not be performed on rain samples due to the low number of photosynthetic cells

**B**  
Quantification of the photosynthetic cells from the pigment's fluorescence measured by flow cytometry on lake samples.



**D**  
Quantification of the phytoplankton abundance by microscopy on lake samples (species were grouped by pigments type)



Suppl. Figure: Comparison of two methods, the flow cytometry and microscopy-based methods for the quantification of photosynthetic cells in lake and rain samples

The air mass analysis does not provide additional support to the questions set out in the introduction apart from a brief mention in the discussion about how CR rain was from the lower cloud system and not the higher one of marine origin. They discuss solar radiation and rain effects on the water column thermal structure, while avoiding wind effects, which is usually the dominant factor determining short term mixing changes (as clearly seen in the CR event).

In our view, it is essential to maintain the air mass analysis as it could explain one part of the dynamics of photosynthetic cells. However, with only the three rain events presented here, it is complicated to conclude about the origin of photosynthetic cells according to the air mass origin (from the sea or the continental, results are controversial on the topic), and more rain events analysis is necessary. Nevertheless, we aim to illustrate a strategy to investigate the potential link, and it seems crucial to keep such analyses in our article. Thus, we will improve the introduction to present this information more clearly and to improve the link between the questions set in the introduction and the discussion referring to the dynamic of photosynthetic cells in the rain. Likewise, we will add more bibliography citations related to the dynamic of these cells and will also add the following sentence in the introduction:

*“We illustrate a strategy to monitor the dynamic of photosynthetic cells in the rain by characterizing the cloud and rain physical properties, the meteorological variables, and the air mass origin.”*

The discussion will also benefit in a cloud type-oriented organization, with sections for HIR and CR going each from the cloud source, the rain physiochemistry up to the lake chemistry changes and finally phytoplankton changes, instead of partitioning into their methodological counterparts. The text contains minor errors and will greatly benefit by proofreading by a native English speaker.

The cloud-type-oriented organization could be interesting. However, with only three rain events analyzed, we think it is premature at this stage as we did not perform a climatology study with a higher number of rain events. Nevertheless, this will be performed in fore coming studies.

With the current state of this work, it might be worth considering partitioning the work in two:

Analyzing the rain drops cytometry (and/or microscopy) and its relationship with the different sources and characteristics of the rain clouds, with additional 2d cytograms of representative samples of the lake or cultures to known in greater detail the composition of said cells. Optimally, cytometry of the lake surface before and after the event should be performed, but the timing should be precise to avoid changes due to algal migration.

It cannot be done that way because the culture will not give us more detail about the cells found in the rainwater (see the answer on the cytometry method above). Therefore, even if we used the culture of known species to adjust cytometry parameters, we have only information about the pigment types and the cell densities, which do not allow species identification. Moreover, 2d cytograms of known cultures/species or lake samples could not be compared with rain samples as species in culture do not represent all those found in the atmosphere (some of them are uncultivable). In any case, the flow cytometry could not give greater detail on the lake composition of said cells as we were limited in a size range to focus on the bioaerosols found in the rain and not those found in the lake.

Report on the lake phytoplankton and physiochemistry changes before and after different precipitation events but including a further discussion on wind effects and watershed inputs until a decoupling is achieved with mesocosm deployments.

Because we did not have a mesocosm at our disposal for comparisons, we cannot argue about the wind effects and watershed inputs.

Ions reported should be in the context of cyanobacterial biomass changes, i.e.  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , and  $\text{PO}_4^-$ , and a point should be made of the viability of the rain droplets milieu as a growth media for the airborne cyanobacteria, while the other ones (that are used as fingerprints to identify the origin of the clouds) can be just mentioned in the text or in supplementary figures.

The atmospheric conditions are stressful for these species (UV, temperature variation, osmotic shock...). However, as mentioned in the literature, they could be viable, so maybe the nutrient composition favors their viability. We did not point out the viability of the rain droplets media because the rainwater is a temporary environment for the cells. Indeed, the photosynthetic cells can be scavenged in the cloud "long-range transport in the air mass" or washed out below the cloud from the air column. It is similar to ions. Hence, we did not know if their origin came from the cloud or the air and how long they stayed in this environment.

Specific comments included inline in the annotated pdf are enumerated here:

PDF comment for line 183: This 15 minute drought break seems very arbitrary and specifically to obtain 2 rain periods from what should be a single long one. Better support and justification is needed for this choices.

We agree that the two rain periods could be viewed as belonging to a larger rain event.

However, our goal is to study the possible high temporal links between rain and its origin and characteristics and its effect our lake water composition. Thus, it seems important to separate the different phases within a rain event as distinct periods, not only as a function of dynamics but also composition. In our example, it appears that the two separate periods actually show slightly different air mass origins, thus reinforcing our choice to select a drought period equivalent to the best interval to ensure a new bottle change between samples.

Line 202: Justify this normalization procedure.

The abundances of species (Fig8 A) were transformed into relative abundances to counter the heterogeneity in the number.

Fig 8A: Phytoplankton abundance should be avoided when talking about the whole community since it includes disparate taxa like *Synechocystis* (2  $\mu\text{m}$  unicellular) and *Microcystis* (500+  $\mu\text{m}$  colonies), biovolume/biomass should be reported instead.

The biovolume/biomass is particularly interesting in the food web study, which is not the case here. So, we prefer to keep the dynamics of phytoplankton in cellular concentration.

Line 545: concentration shouldn't be compared, given that the direct mass of rain is very small in comparison to the whole water column (accounting for the watershed is a different issue).

Line 548, we reported that the wet deposition did not influence the lake's chemical composition, certainly due to the small mass of rain compared to the lake (similar to the photosynthetic cells brought by wet deposition), very low in contrast to phytoplankton cells line 563).

Line 563: A discussion about phytoplankton dispersion and biogeography will greatly enhance this section.

We did not have the relevant information about species diversity in the rain samples. Thus, we cannot discuss the correlation between photosynthetic cells in the rain and the lake. Nevertheless, we will improve the introduction with citations describing the species encountered in the atmospheric compartments, especially in the rain.

Line 573: If they were "pulled down by mixing", then the charophytes and chlorophytes would have also decreased. Seems like these groups' buoyancy mechanisms created a downward migration. Lm and Lo codon have low surface/volume ratio on an individual basis, and not high as listed here. See: Reynolds 1994 (DOI: 10.1007/BF00007405)

We thank the referee for pointing out an inconsistency with the surface-to-volume ratio, which is low for Lm and Lo species. Therefore, we will rephrase the sentences as follow:

"*Microcystis*, *Merispomedia*, and *Coelomorion* belonging to Lm and Lo codons have a low tolerance to mixing. Therefore, their decrease after the rain events could be explained by a dilution caused by their transport through the entire mixed depth. However, when temporary stratification was back after the rain, a secondary thermocline nearer the surface could help these species to quickly regulate their vertical position by buoyancy mechanisms to benefit from optimal conditions (nutrient and water irradiance)."

In order to improve the discussion on wind effects, we will add sentences to describe the species increasing after the rains in link with the rain and wind events:

On the contrary, after the rain events, we reported a shift in species composition from colonial picocyanobacteria towards unicellular picocyanobacteria (*Synechococcus* and *Synechocystis*) and also the increase of diatoms (*Asterionella* and *Melosira*) and microalgae (*Elakatothrix*). Because *Synechococcus*, *Synechocystis*, and *Asterionella* have a high surface-to-volume ratio, they can quickly grow in the mixed layer depths if there is no light limitation. Indeed, Reynolds (1994) reported that optimal division rates of these species could lead to doubling their abundance per day when there is no light limitation. As there was no light-limitation during the lake campaign (the euphotic zone, 13 m deep, exceeds those of the thermocline, 7 m), it suggests that unicellular picocyanobacteria were able to develop to become dominant after the rain events.

On the other hand, we also reported the presence of larger unicellular algae, *Closterium*, present only after RP2, and the coenobial green algae *Elakatothrix*, which increased after the rain despite its low growth rate. Hence, as RP2 was characterized by stratiform rain events and high wind speed, we suggest that the wind increased diffusivity, allowing green algae to stay longer in the water column where no light-limitation was reported. Indeed, the mixing could favor larger and heavier species to stay suspended and their development, as has already been reported in the literature (Blottière et al., 2014).

References:

Blottière, L., Rossi, M., Madricardo, F., and Hulot, F. D.: Modeling the role of wind and warming on *Microcystis aeruginosa* blooms in shallow lakes with different trophic status, *Theor. Ecol.*, 7, 35–52, <https://doi.org/10.1007/s12080-013-0196-2>, 2014.

Blottière, L., Jaffar-Bandjee, M., Jacquet, S., Millot, A., and Hulot, F. D.: Effects of mixing on the pelagic food web in shallow lakes, *Freshw. Biol.*, 62, 161–177, <https://doi.org/10.1111/fwb.12859>, 2017.

Felip, M. and Catalan, J.: The relationship between phytoplankton biovolume and chlorophyll in a deep oligotrophic lake: decoupling in their spatial and temporal maxima, *J. Plankton Res.*, 22, 91–106, <https://doi.org/10.1093/plankt/22.1.91>, 2000.

Reynolds, C. S.: The long, the short and the stalled: on the attributes of phytoplankton selected by physical mixing in lakes and rivers, *Hydrobiologia*, 289, 9–21, <https://doi.org/10.1007/BF00007405>, 1994.

Wiśniewska, K., Lewandowska, A. U., and Śliwińska-Wilczewska, S.: The importance of cyanobacteria and microalgae present in aerosols to human health and the environment – Review study, *Environ. Int.*, 131, 104964, <https://doi.org/10.1016/j.envint.2019.104964>, 2019.