Author's Response:

Dear editor,

Thank you for the detailed comments on our manuscript, which have helped to improve the manuscript's cohesiveness. We considered all the comments and questions carefully and have revised the manuscript accordingly. Please find below a point-by-point response with a list of the main changes (in blue).

Reviewer #1

"The manuscript by Noirmain et al entitled "Interdisciplinary strategy to survey phytoplankton dynamics of a eutrophic lake under rain forcing: description of the instrumental set-up and first results" is well written and easy to read. The described interdisciplinary strategy provides a very useful tool to decipher the impact of atmospheric processes on lake ecology.

An enormous work has been performed on the atmospheric sciences part but the authors only quickly discuss the ecological implications related to their study. The authors also need to consider further factors related the cyanobacteria biology and ecology. This could for instance apply to Lines 400-405 and Figures 8AB.A negative effect of rain on organism abundance was mentioned for few taxa. Effect can be seen on Fig. 8B on unicellular group forming cyanobacteria (e.g. Woronichinia, Microcystis, Merismopedia...). Whereas some unicellular individual cyanobacteria (e.g. Synechocystis, Synechococcus, Pseudoanabena...) seem to undergo a positive effect. Cell distribution factor is important because it impacts the growth dynamic and survival of the species. Moreover, microalgae can move and migrate through the water column. Almost the same concentration of cells was fund "before" and "after" RP events in the system (Fig. 8A). The increase concentration at a specific depth implies displacement of the phytoplanktonic community or organismal movement (RP2 towards 1.5 m depth, RP1 towards 3 m). Apparently deeper when the rainfall was more virulent (HIR). This is not described in the results nor correlated to Fig. 8B (diversity) but quickly mentioned in the discussion. Was the water column structure more stable (nutrient, temperature, light, water agitation...) at 1.5 m depth in RP2 and at 3 m in RP1?

All the cyanobacteria taxa retrieved have different ecological requirements. In the discussion Lm and Lo codons are rapidly mentioned. A principal component analysis can further help to characterize which cyanobacteria taxa was favored under certain rain events (e.g. peculiar nutrient signature).

As suggested by the reviewer, we carried out supplementary analysis by Multiple factor analysis (MFA) (Fig. 8 B-D) using the abiotic lake factors (water temperature and irradiance and amount of rain), phytoplankton taxa (described in Fig 8A), and the chemical composition of the lake to characterize which cyanobacteria taxa were more prolific after rain events. In the result part, we add sentences confirming the negative correlation between the amount of rain and *Microcystis*, *Merismopedia*, and *Coelomoron* abundances. We consider effect of the rain events on the picocyanobacteria (*Synechocystis*& *Synechococcus*), green algae (*Elakatothrix*), and diatoms (*Asterionella* and *Melosira*) to have been positive, as there was a correlation with the rain rate, as shown by multiple factor analysis (Fig 8 B-D) (lines 448-452). We also added sentences to discuss about the displacement of the phytoplankton, especially *Microcystis*, *Coelomoron*, and *Merismopedia*, which decreased systematically following the rain events. In contrast, *Synechococcus, Synechocystis, Elakatothrix* and *Asterionella* increased following the rain events and throughout the study on the lake, suggesting they are more affected by seasonal changes than any direct impact from rain (lines 619-630).

The water column does not seem more stable at 1.5 or 3 m after the rain events, as no statistical differences were found with depth (Fig 8D).

Moreover, the differences in phytoplankton concentration and taxa diversity between "before" and "after" calls for emission/deposition fluxes (e.g. Dillon et al 2020 doi:10.1128/AEM.01850-20, Mayol et al 2014 doi: 10.3389/fmicb.2014.00557), very briefly mentioned in discussion. However this is of ecological importance. Retrieved cyanobacteria species from the investigated lakes have been reported from atmospheric samples (e.g. Sharma et al 2007 DOI: 10.1111/j.1529-8817.2007.00373.x). Also microalgae can be emitted after local disturbances such as rain (Tesson et al 2016 doi:10.1128/AEM.03333-15, WiÅ›niewska et al 2019, 2022) from station Fig 1B2 and be redeposited in local system such as station Fig 1B1 or the lake, therefore it is possible that the retrieved peak of Cr1b with highest phycocyanin value could be partly indigenous.

To jointly study emission and deposition fluxes in Lake Aydat, we should sample airborne microalgae close to the lake's surface. Unfortunately, we did not measure the emission fluxes from the lake and so cannot directly link the phycocyanin detected in the rain (from one rain event, CR1) and the increase in picocyanobacteria abundance at the end of our lake sampling campaign. Nonetheless, we discuss deposition fluxes by comparing the level of photosynthetic cells collected in the rainwater with those in the lake, suggesting that a very low number of photosynthetic species was introduced into the lake by the wet deposition. We also discuss the deposition flux, which might impact the diversity in the lake by introducing different species collected by the rain (lines 590-602).

Moreover, it can be useful to run the analysis considering the ecology and behavior of these organisms to avoid a Simpson paradox."
We thank the reviewer for this suggestion and have added the multiple factor analysis in the revised version (Fig 8 B-D) and add also new correlations between the lake temperature and the rain rate and the wind speed (Fig.7). We also add new sentences in the discussion to consider the ecology and behavior of the organisms which have different abundances after rain events during the lake campaign (lines 609-630).

Figure 1 - Please add the geographical coordinates on Fig. 1A and the cardinal directions on both Fig. 1A and Fig. 1B.
Supplement Fig. 1 - The geographical coordinates are too small to be readable.
We created new maps with geographical coordinates for Fig. 1A & B and increased the size of the geographical coordinates in the supplementary figures illustrating the retro trajectory (Suppl. Fig.2).

Lines 111 and 113 - Inform about the material manufacturer and country between brackets.
Line 121 - Please add in the text the difference of elevation between the location where the instrumental setup was installed and the lake surface.
Lines 142 and 156 - Spell out the acronyms DSD and PCB.
The missing information was added in the materials and methods section (revised manuscript, modified text).

Lines 182-184 - How was the lake sampling performed? Which parameters were investigated? Do the authors refer to Lines 114-116 in situ measurements or were lake water- and/or phytoplankton samples collected? Please describe further.

We added details about the lake sampling (lines 230-235).

Line 198 - Why was a 10 µm pore size used? In these filtrates, all microorganisms of a size inferior to 10 µm would be present (including. bacteria, cyanobacteria and other <10 µm eukaryotic microalgae), thus affecting the measurements. Moreover, -20°C storage was applied to the filtrates, conditions under which organismal cell damaging occur, releasing further nutrients in the water. Please explain further.

We thank the referee for pointing out an inconsistency with the pore size. We correct the sentences in the material and methods (lines 246 to 254 We filtrate the lake samples on a 0.2µm nylon membrane before being kept at -20°C, avoiding the release of nutrients.

Line 200 - Which Lugol's iodine solution was used (acidic vs neutral)? What was the final concentration used? How and how long before investigation were the samples stored (temperature, darkness, time)? These are important information for cross studies comparison.

We added the missing information. (line 247).

Lines 221-236 - How does the flow cytometer detection method differ from existing protocols? (e.g. Haynes et al 2020, https://www.agilent.com/cs/library/applications/application-analysis-aquatic-plankton-novocyte-5994-2112en-agilent.pdf)

We added a new figure dealing with the flow cytometry method to the supplementary material (Suppl. Fig.1) to illustrate the cytograms created for the analysis and to show how the population of photosynthetic cells were isolated based on the presence of pigments (Chlorophyll, Phycocyanin, and Phycoerythrin).

Line 470 - I disagree with the sentence. First because microalgae encompass both prokaryotic (cyanobacteria) and eukaryotic photosynthetic unicellular organisms. Second because previous studies have investigated the diversity of microalgae in wet depositions including rain. However, the methods used involved capture and growth, not rapid detection based on flow cytometry. The proposed sentence is therefore not proper, please rephrase.

Lines 473-475 - I also disagree with this sentence. One major problem with culturing is that not all organisms can grow in artificial media, therefore applying a selection pressure towards underestimating the environmental biodiversity. Another issue is that all isolated microalgae possess a biome (including bacteria). These bionts can be remove using diverse available methods. However, some microalgae need their bionts to survive. In any cases these should not impede microalgal detection using flow cytometry or microcoscope-based techniques. Please reformulate the sentence.

We modify the sentences in the discussion.

The English language and formulations need to be double checked by a native speaker, several mistakes are present in the text.

The English language was checked by a native speaker.

Reviewer #2

"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 reconciliate 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 from wind effects, but we do not have this material at our disposal and thus cannot perform this experiment nor discuss about the potential results.

To better illustrate the effect of wind on the lake temperature, as suggested by the reviewer, we added a correlation between water temperature and wind during the rain events (Fig. 7 B, D & F). We also included the Spearman correlation coefficients between the lake temperature and the rate of rainfall using the depths for the three rain events, HIR, CR1, and CR2. These relationships confirm that during HIR, the decrease in lake temperature at the surface was linked to the rain rate, and not the wind speed (no significant relationship). On the other hand, during CR1, when there was a higher wind speed, we found that the wind speed strongly impacted the lake temperature to a depth of 2.8 m. We added sentences in the results and in the discussion to describe these effects.

Although we sampled the lake as often as possible, more frequent sampling would improve our understanding of the causality between the biochemistry of the rain and biochemical composition of the lake. However, it was not our principal aim for this article, in which we wanted to illustrate a highly intensive monitoring strategy. Indeed, we present only three rain events as examples for our case study. However, two days after HIR, our results seem to show no direct link between the chemical composition of the rain and the lake's inorganic ion concentration, as they have an opposite trend after the rain events (lines 578-588). Furthermore, the phytoplankton dynamic shows a similar pattern in its assemblage, linked to the rainfall rate rather than the composition of the rain events.

The lake phytoplankton and the rain photosynthetic cells are measured with two different methods that are hard to reconciliate. 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.

The cytometry method used to detect the photosynthetic microorganisms in rainwater was developed to cover particles ranging from 0 to 30 µm in size. This methodology is well suited to species in rainwater, as they are generally under 30 µm in the atmosphere. However, the microscopy-based method is not well adapted due to the very low number of photosynthetic cells in rainwater. Hence our choice not to use this method.

On the other hand, the photosynthetic species in the lake can be composed of colonial or filamentous forms larger than 30 µm. Therefore, the flow cytometry is inappropriate and could lead to an underestimation of the concentration of colonial or filamentous species in the lake. This is why we chose to estimate the lake phytoplankton abundance by microscopy. Due to these technical issues, which we have described in more detail now in the article, we selected only the appropriate methods to detect the photosynthetic cells in rain by flow cytometry and the lake phytoplankton by microscopy.

We did not use any fixators like glutaraldehyde as it can lead to an underestimation of the count of photosynthetic cells and can alter the intensity of fluorescence according to the cell size (Troussellier et al., 1995; Lepesteur et al., 1993). Moreover, the rainwaters were kept at 4°C in the dark (no growth and cell preservation), and we performed the flow cytometry shortly after the rain events (maximum of 48h), ensuring a realistic view of the environmental signature of the samples. We precise the stored condition lines 242.

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.

We characterized the cloud-types as stratiform or convective at the beginning of the introduction, based on their physical characteristics and forecasting probabilities (lines 50-59). We rephrased the next paragraph that details the rain's impact on abiotic and biotic changes to the lake (lines 58-86). We added a new paragraph to the introduction relating to the dynamics of photosynthetic cells in the rain, their scavenging by the rain, and the potential consequences of their scavenging on a freshwater ecosystem (lines 88-98). We rephrased the main questions involved in the manuscript to correspond to our results and discussion (lines 100-117).

It was not possible to use the same methodology to estimate the concentrations of the photosynthetic cells in the rain and the phytoplankton. We cannot perform cytometry on lake samples due to the cell size larger than 3 µm, and we cannot perform microscopy on rain samples due to the low number of cells (even after concentration of the rainwater by ultrafast filtration). An example of the incompatibility of the flow

cytometry for the phytoplankton present in the lake was presented in the interactive discussion of the journal, with results on natural samples showing an underestimation of the concentration of the lake's colonial or filamentous species by flow cytometry. Due to these technical issues, we estimated the lake phytoplankton abundance by microscopy and the photosynthetic cells in the rain by flow cytometry.

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 part of the dynamics of photosynthetic cells. We developed the discussion about the link between the photosynthetic cells and the origin of the air mass (lines 520-529). We also discuss the impact of wind on the lake temperature during the period of the lake campaign (lines 563-573).

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, since we only analyzed three rain events, we think it would be premature at this stage. This will be carried out in future studies involving a higher number of rain events.

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.

This cannot be carried out because the culture will not provide us with more details about the cells found in the rainwater. Therefore, even if we used the cultures of known species to adjust cytometry parameters, we would only have 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 a culture do not include all of those found in the atmosphere (some of them are uncultivatable). In any case, the flow cytometry could not provide any more details about the lake composition of these cells as we were limited in size range to focus on the bioaerosols found in the rain and not those found in the lake.

Ions reported should be in the context of cyanobacterial biomass changes, i.e. NH4+, NO3-, and PO4-, 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

We added a paragraph mentioning the viability of the photosynthetic cells in the rain (lines 529-535) and detailed the hostile conditions facing these species in the atmosphere (UV, temperature variation, osmotic

shock, etc.). We also added the plot showing the ion concentrations in the rainwater in supplementary material (Suppl. Fig.5).

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.

Regarding the rain sampling procedure, we justified the 15-minute drought break in the rain sampling methodology by referencing a previous study showing a significant variability in the rain's chemical composition based on the air mass variability (lines 171-174). The authors of this study noted rapid changes in the atmospheric concentrations due to aerosol scavenging over a 10-minute period.

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

As recommended by the reviewer, we selected only those results that were relevant to the questions laid out in the introduction. Therefore, we discarded the plot showing phytoplankton abundance.

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).

Regarding our results, we reported that the wet deposition did not influence the lake's chemical composition, certainly due to the small volume of rain compared to the lake one (lines 578-588). However, as the literature suggested that wet depositions from the atmosphere can affect the dissolved organic carbon and nitrogen concentrations at the lake surface (line 61), we think it is interesting to make the comparison between the chemical composition of the rain and those of the lake, especially after HIR, where the lake was sampled after 2 days following the event. We also added the need to confirm these results by increasing the lake sampling at different times after the rain events (line 588).

Line 563: A discussion about phytoplankton dspersion and biogeography will greately 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.

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 modified the sentence to develop the hypothesis about the downward migration of some species after the rain events (lines 608-617). We discussed the species' tolerance to mixing and added results from the literature to explain our findings. In addition, we discussed the genera that increased after the rain events.

Best regards,

Fanny Noirmain