

Comment on bg-2022-100

Referee #1

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-RC1>, 2022

We thank referee 1 for his suggestions made online. We respond in blue below to his comments.

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 were found "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?

The water column does not seem more stable at 1.5 or 3 m after the rain events. No change was found regarding water temperature, conductivity, and oxygen concentrations which were similar up to 3 m deep after RP1 (oxygen concentration around 8.72 mg. L⁻¹, conductivity = 116.8 – 117 $\mu\text{S}\cdot\text{cm}^{-1}$) and after RP2 (oxygen concentration between 7.7 and 7.9 mg. L⁻¹ and conductivity between 101.2 and 102.48 $\mu\text{S}\cdot\text{cm}^{-1}$). However, as an abiotic factor, only the water irradiance was different and could explain a slightly different distribution of the phytoplankton abundance at 1.5 m after RP2 and 3 m after RP1. Regarding fig 8B, we did not discuss a potential vertical displacement according to the rain type, convective or stratiform, as other rain events have occurred during RP1 and RP2. We analyzed only three rain events to illustrate the monitoring strategy in this article. However, in a future study, more results and observations will help investigate the potential effect of convective or stratiform rain events on vertical lake stability.

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 referee, we performed a supplementary analysis by Multiple factor analysis (MFA) (Fig 9, below) using the abiotic lake factors (water temperature and irradiance and rain amount), phytoplankton taxa (described in Fig 8B), and the lake chemical composition (Fig 8C & D) to characterize which cyanobacteria taxa could be favored after rain events. Multiple factor analysis (MFA) is an extension of principal component analysis (PCA) tailored to handle multiple data tables that measure sets of variables collected on the same observations. Such analysis was more adapted considering our set of data. The results are presented in the pdf attached.

The MFA analysis confirms a negative correlation between the rain amount and *Microcystis*, *Merismopedia*, and *Coelomoron* abundances, also confirming the significant change of abundance between the “before” and “after” rain period reported for these species in the results (line 403). Nevertheless, as suggested by the referee, we can add this model to the results (if the ninth figure is authorized in the manuscript) as it also shows a positive correlation between the rain amount and the abundance of diatom (*Asterionella*), colonial microalgae (*Elakatothrix*), and unicellular picocyanobacteria (*Synechocystis*) abundances associated with the increase of some inorganic ions (SO₄²⁻, Ca²⁺ and NH₄⁺) and the rain amount. In any case, we will indicate the species that increase following the rain amount in reference to Fig 8B.

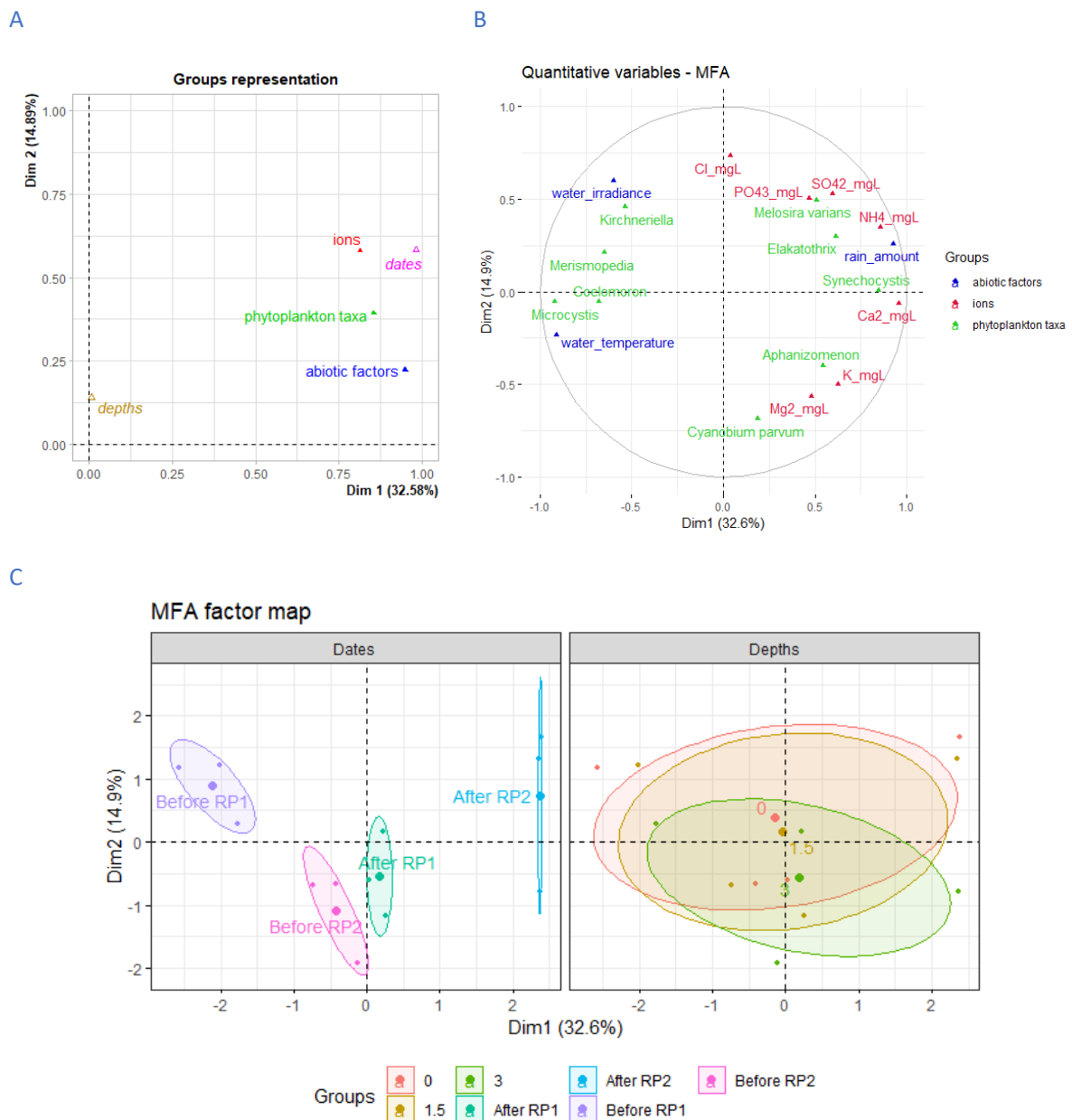


Fig. 9: Multiple factor analysis (MFA). Projection of variable groups (A), Correlation circle map of the MFA, for clarity, only variables with a \cos^2 equal to 0.45 are represented in the correlation circle. (B), individual factor map, showing the position of samples in the MFA with confidence ellipses around “depths” and “dates” variables (C).

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, WiÅniewska 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 ensure a correlation between emission and deposition fluxes in Aydat lake, we should sample airborne microalgae close to Aydat lake's surface. Unfortunately, we did not measure the emission fluxes from the Aydat lake and cannot directly link the phycocyanin detection in the rain (from one rain event, CR1) and the increase of picocyanobacteria abundance at the end of lake sampling. Nonetheless, we discuss deposition fluxes by comparing the level of photosynthetic cells collected in the rainwater with those in the lake, suggesting a very low amount of photosynthetic species introduced in the lake by the wet deposition. Moreover, we also discuss the deposition flux that can impact the diversity in the lake by introducing different species collected by the rain (lines 560-567).

I believe that certain of these aspects would enrich the discussion of the manuscript.

Moreover, it can be useful to run the analysis considering the ecology and behavior of these organisms to avoid a Simpson paradox.

The multiple factor analysis was performed, showing the same results as reported in the manuscript.

Minor comments:

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.

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.

We will modify the plots, add the material manufacturer and country and spell out the acronyms.

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.

To provide more details about the lake sampling, we will add this sentence: *"In addition to the lake monitoring, we collected lake sampling at three depths, surface, 1.5 and 3 m deep, using a Van Dorn horizontal Bottle Water Sampler (2.2L, PVC) deployed vertically with a weight to take it to the desired depth. Then water was transferred in 15 Liter Jerrican to keep the water temperature stable during transport back to the laboratory (~20 min)."*

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.

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.

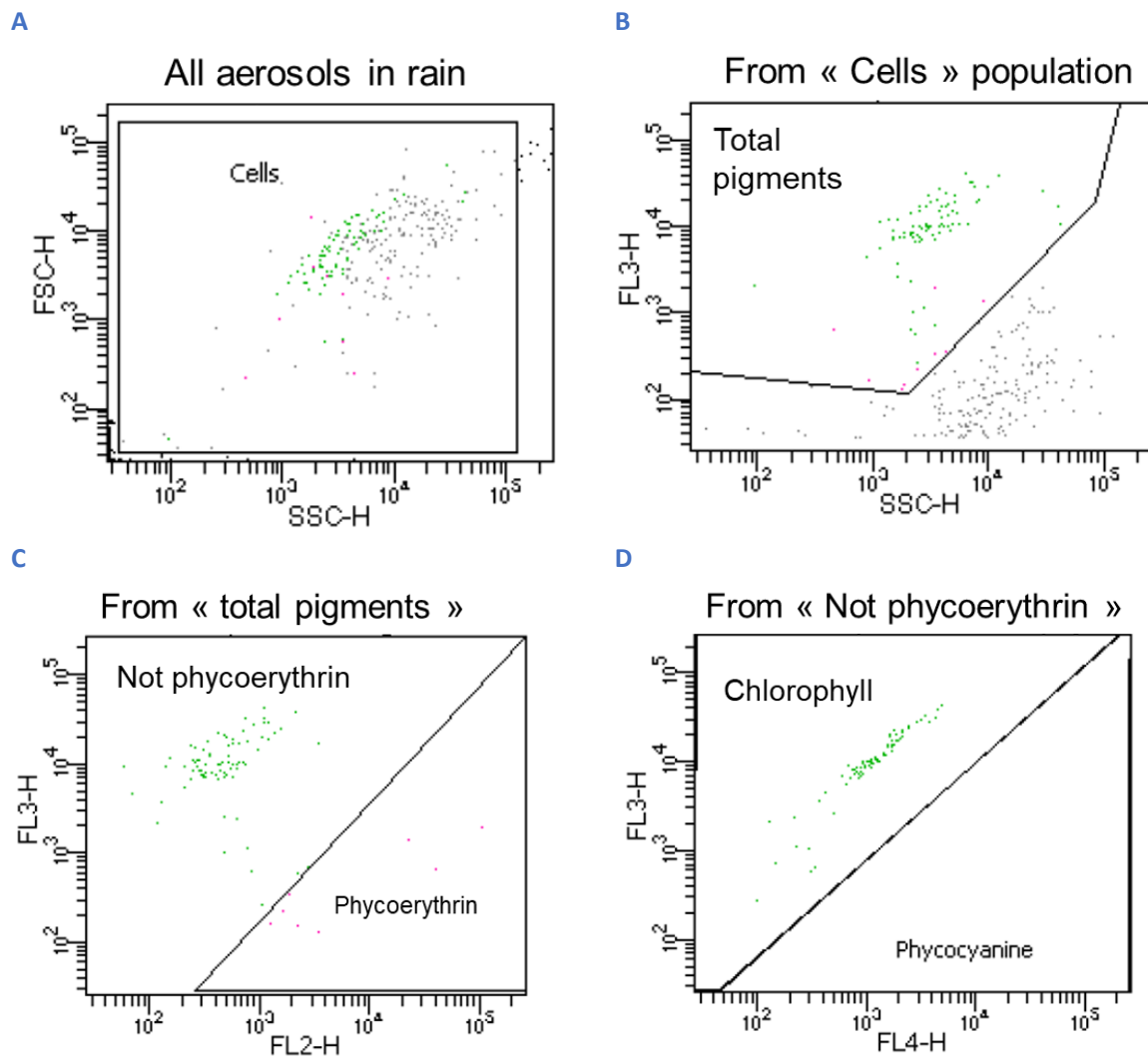
We thank the referee for pointing out an inconsistency with the pore size. The correct size of pores used will be corrected in the new version. The correct sentence is that we filtrated the lake water on a 150 μm Nylon membrane to avoid the presence of zooplankton in lake samples. The filtrate (under 150 μm) was fixed in a neutral Lugol solution (Sigma-Aldrich), and 10 ml of Lugol's iodine stock solution was added to 150 ml of lake filtrated samples for the microscopy. For the nutrient analysis, the lake water was filtrated on 0.2- μm pore-size filters Nylon membrane by rinsing the filtrate previously with 500 ml of ultra-pure Milli-Q water to avoid contamination.

The fixed samples using Lugol were stored in the dark at 4°C and were counted within the year. The lake samples filtrated under 0.2 μm were kept at -20°C until the chromatography analysis was performed within the year.

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 use conventional flow cytometry measurements with bandpass filters to detect the autofluorescence signals from photosynthetic pigments excited by the 640 nm and 488 nm lasers and collected using FL3, FL2, and FL4 detectors (670 Long Pass, 585/42, and 661/16 Band Pass filters respectively) (Dashkova et al., 2016). However, we did not use any fixators like glutaraldehyde because the count of photosynthetic cells can be underestimated (Troussellier et al., 1995). 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. The rainwaters, which could not be analyzed after 48h was discarded.

We report a different approach to quantifying the autofluorescence to measure specific pigment populations of photosynthetic cells rich in chlorophyll, phycocyanin, or phycoerythrin. An example is showed in the supplementary figure below. Indeed, we first select the photosynthetic cells population (total pigments) based on minimal chlorophyll fluorescence on the cytogram by selecting FL3 and SSC, opened on the preselected population without unwanted debris (Cells, plot A & B). Then, to distinguish the photosynthetic cells based on individual pigments autofluorescence, we create a new cytogram by selecting FL3 and FL2 channels taken from the "total pigments" population (plot C). This cytogram was divided into two: a "phycoerythrin rich population" and a not phycoerythrin-rich population. From the "NOT Phycoerythrin population," we create a new cytogram by selecting FL3 and FL4 channels divided into "chlorophyll" and "phycocyanin" populations (plot D). These selections allow us to separate the phycoerythrin population from chlorophyll or phycocyanin.



Suppl. figure: Flow cytometry based-method to quantify the autofluorescence from specific pigment from photosynthetic cells in the rain samples. Detection of all aerosols in the rain (A), the total pigments population based on minimal chlorophyll fluorescence (B), Phycoerythrin population (C), Chlorophyll, and Phycocyanin populations (C).

Figure 7A - The graphical scale for the water temperature does not cover the whole data spectrum - can the authors extend the x-axis from 6 to 20°C to include all data points?

The graph will be modified.

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 removed 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 microscope-based techniques. Please reformulate the sentence.

To clarify lines 470-475, the sentence will be rephrased as below, including new references:

“Moreover, the detection of photosynthetic cells by microscopy in rain samples is often measured on rain cultures after several days (Wiśniewska et al., 2022). However, this methodology seems inappropriate for estimating the natural environmental biodiversity because an unadapted artificial medium for the growth of all microorganisms could apply a selection pressure. Hence, we recommend using flow cytometry or microscopy-based techniques on fresh samples without fixators. Indeed, glutaraldehyde 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). »

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

A native speaker will check the English mistakes.

References:

Dashkova, V., Segev, E., Malashenkov, D., Kolter, R., Vorobjev, I., and Barteneva, N. S.: Microalgal cytometric analysis in the presence of endogenous autofluorescent pigments, *Algal Res.*, 19, 370–380, <https://doi.org/10.1016/j.algal.2016.05.013>, 2016.

Lepesteur, M., Martin, J. M., and Fleury, A.: A comparative study of different preservation methods for phytoplankton cell analysis by flow cytometry, *Mar. Ecol. Prog. Ser.*, 93, 55–63, 1993.

Troussellier, M., Courties, C., and Zettelmaier, S.: Flow Cytometric Analysis of Coastal Lagoon Bacterioplankton and Picophytoplankton: Fixation and Storage Effects, *Estuar. Coast. Shelf Sci.*, 40, 621–633, <https://doi.org/10.1006/ecss.1995.0042>, 1995.