

Reviewer 1

Review of the manuscript "Buoyancy and polarity driven accumulation of dissolved organic matter in the sea surface microlayer during a phytoplankton bloom" by Zobelein et al.

The authors investigated the evolution of the sea surface microlayer (SML) during an artificially induced phytoplankton bloom in a mesocosm experiment. Using this approach, they aimed to identify the major film-forming organic matter, assess the influence of polarity and organic matter buoyancy, and evaluate the role of photodegradation.

There are too many abbreviations used throughout the manuscript. I suggest minimizing their use, and in particular reconsidering the abbreviation "MF" for molecular formula.

We agree with the reviewer's assessment. Accordingly, throughout the manuscript, we will revise our abbreviation scheme to make the manuscript easier to follow.

In light of the growing emphasis on Open Science, I do not think the manuscript should be published unless the raw data are made openly available, either through a public repository or in the Supplementary Materials.

All data will be made available in PANGAEA, and in the revised manuscript, we will provide the corresponding DOI.

L 60 - ... the OM composition ...

Thank you for pointing that out. L 60 will be updated accordingly.

L 76 - ... in the SML...

Thank you for pointing that out. L 76 will be updated accordingly.

L 155 – What was the final concentration of HgCl₂ in the sample?

L155 will be changed to: "All nutrient samples were poisoned immediately with a saturated mercury chloride (HgCl₂) solution (0.02% of the sample volume), and stored at 4 °C for further analysis. "

L 350 – 364 – It was not easy for me to follow the text and Fig. 4. I suggest providing more detailed connections between the text and the sub-figures. For example, the sentence: "In contrast, increasing SML compartmentalisation was noticed for the intensity weighted molecular lability boundary (H/C > 1.5, MLB_{wL}), the humic-like fluorescent DOM (FDOM), and finally the w.a. of carbohydrate-, lipid- and protein-like fractions of the DOM pool (Fig. 4 b, h, i, k, Tab. 2)." should be written as follows: In contrast, increasing SML compartmentalisation was noticed for the intensity weighted molecular lability boundary (H/C > 1.5, MLB_{wL}) (Fig. 4b) , the humic-like fluorescent DOM (FDOM) (Fig. 4?), and finally the w.a. of carbohydrate-, lipid- and protein-like fractions of the DOM pool (Fig. 4 x, y, z, respectively, Tab. 2).

We agree that our sentence structure can be improved in some places. We will do our best to reword nested sentences to improve the readability.

L 350 "In contrast, increasing SML compartmentalisation was noticed for the intensity weighted molecular lability boundary (H/C > 1.5, MLB_{wL}) (Fig. 4 b), the humic-like fluorescent DOM (FDOM) (Fig. 4 k), and finally the weighed average of carbohydrate-, lipid-like and protein-derived fractions of the DOM pool (Fig. 4 h, i, j, k, respectively Tab. S3). The compartmentalisation was distinct and consistent despite a concurrent overall increase in the ULW (e.g. in the MLB_{wL}, Fig. 4 b), or overall decreases in both, SML and ULW (e.g. FDOM, Fig. 4 k). Specifically, in the SML, the sum of the normalised intensities of laminarin-derivative formulas increased abruptly in the SML during the bloom phase (Fig. 5). Furthermore, these formulas showed a diurnal trend, where samples taken before sunrise had higher laminarin-like signal intensities than those taken in the afternoon. "

L 354 – The term “highly unsaturated formula” is not defined anywhere in the manuscript. Please clarify what is meant by this term (e.g., does it refer to compounds with more than a certain number of double bonds?).

Thank you for pointing this out. We are defining the highly unsaturated compound class by the weighted average of compounds with a modified aromaticity index equal to or above 0.50 and a H/C ratio above 1.5 ($AI_{mod} \leq 0.50$ and $H/C < 1.5$). That includes phenols such as soil-derived products of lignin degradation (Seidel et al., 2014 <https://doi.org/10.1016/j.gca.2014.05.038>). We will add this definition in the Method section (L 238).

L 365-367 – This sentence should be revised for clarity.: While both parameters increased in the SML after the bloom, the latter decreased during the pre-bloom phase, reaching the lowest values in the middle of the bloom phase.

The development of the SPE-DOC/SPE-DON ratio (Fig. S4 a) will be incorporated into paragraph L328, which describes bulk parameters with increased compartmentalisation. The O/H plot (Fig. S4 b) will be removed from the supplementary material. The O/H trend is less conclusive and does not significantly contribute to our line of argument.

The paragraph will be changed to: “DOC concentrations and the DOC/DON ratio in the SML compartmentalised in tandem with the increase of Chla during the bloom phase (Fig. 3 a,d; Tab. 1). Throughout the bloom, SML DOC was enriched compared to ULW, despite an overall increase in the ULW DOC (EF, Fig. 3 e). This enrichment increased drastically during the bloom and the post-bloom phase. Both SML and ULW maintained high DOC concentrations after the bloom, despite Chla levels dropping in the post-bloom phase. The SPE-DOC/SPE-DON ratio remained stable and within the same range between the ULW and SML during the pre-bloom and bloom stages (Fig. S4). As the bloom progressed, the ratio increased abruptly in the SML, leading to compartmentalisation as Chla levels dropped. This compartmentalisation grew during the post-bloom phase, even though the ULW SPE-DOC/SPE-DON ratio also increased. In the SML, the ratio reached its highest point midway through the post-bloom period and then decreased, eventually aligning with the ULW level.”

L 369 – Where are these data presented? So, it should be written: The DOC extraction efficiency (EE) (Table 1). According to Table 1, EE is consistently at least 10% lower in the SML than in the ULW, rather than 20%.

As suggested by the reviewer, the sentence (L369) should read as: "The DOC extraction efficiency (Tab. 1) was consistently at least 10% lower in the SML than in the ULW samples and decreased during the bloom phase in both the SML and ULW, before returning to pre-bloom levels during the post-bloom phase."

L 371 – The term “permeating DOC” is not explained in the main text. This definition should be provided here rather than only in the table caption.

As suggested by the reviewer, we have added a description in the method section and a short reminder in the results. Like "the DOC-fraction from the sample that is not retained by the PPL-cartridge was collected and will be hereafter referred to as permeated DOC". (L172, L371, L456)

L 373 – EE is mentioned only 3 times in the text so I suggest to use full name.

As suggested by the reviewer, we spell out extraction efficiency in the revised manuscript (L368, L528).

L 380 – Fig. 4: I would appreciate it if all abbreviations were defined in the figure captions, as repeatedly searching the text for their meanings is time-consuming and tiring.

Thank you for pointing this out. We checked all figure captions and have explained all abbreviations in the revised manuscript.

Also, there is missing y-axis names for 4a, 4c and 4l.

Thank you for this comment. We point out that 4 a, 4 c, and 4 l depict molecular indices that, by definition, are unitless. In the headline, we already define which index is depicted; therefore, no further information is required to accurately show the relative index abundances.

L 434-435_... decreased significantly in the SML...when or where?

Here, we refer to the relative change in protein band intensity, as shown in Fig. S5 b. Although the absolute protein band intensity slightly increases (Fig. 4 j), its relative contribution to the overall spectral intensity IC decreases during the post-bloom period (Fig. S5 b). The opposite trend occurs simply because the increase in other signal contributions (i.e., the carbohydrate signal) is more pronounced, so the relative protein contribution decreases despite a marginal absolute increase.

L 453 – lipids also may contribute to high DOC/DON ratio

Thank you for your suggestion. We conducted a literature review on lipid contributions to *in situ* produced biomass in late bloom stages. Although lipids are known to account for a high fraction of carbon fixation ($23 \pm 11\%$, Becker et al., 2018, <https://doi.org/10.1038/s41467-018-07346-z>), there is no evidence that this is also true for the DOC fraction, which we investigate in our study. In the DOC fraction, only low levels of fatty acids (3%; Kattner et al., 1983, [https://doi.org/10.1016/0304-4203\(83\)90039-7](https://doi.org/10.1016/0304-4203(83)90039-7)) or lipids (<1%; Mannino & Harvey, 2002, <https://ntrs.nasa.gov/citations/20030020914>) have been reported. Furthermore, Engel et al. (2002, <https://doi.org/10.4319/lo.2002.47.3.0753>) found that after nitrate depletion during post-bloom stages, a large portion of carbon is directed into polysaccharides with a C:N ratio of around 20. This suggests that lipid contributions to the DOC/DON ratio merely account for the observed discrepancies. Note that this may vary between POC and dry-mass lipid fractions, with lipid content potentially reaching 25% (H. Rai et al., 1997, <https://doi.org/10.1046/j.1365-2427.1997.00227.x>; Becker et al., 2018, <https://doi.org/10.1038/s41467-018-07346-z>).

We included this in the discussion in the revised version (L452): “We detected a significant accumulation of up to 600 μM DOC in the SML, along with an elevated DOC/DON ratio after the bloom (Fig. 3) that exceeded the Redfield ratio. This higher DOC/DON ratio is likely mainly due to *in situ* carbohydrate production, with a smaller contribution from lipid biosynthesis (Hammer and Kattner, 1986, <https://doi.org/10.3354/meps031035>; Mannino & Harvey, 2002; Van Den Meersche et al., 2004, <https://doi.org/10.4319/lo.2004.49.3.0862>).”

L 459 – Did the authors consider OM photochemical degradation, which often produces oxidized species as an initial step? This may be important particularly for the SML.

Thank you for your suggestion. However, analysing oxidised compounds as you proposed is not feasible because we only calculated weighted averages of the O/C ratio. While those reactions could happen, the specific photo-degraded compounds might not influence the overall O/C ratio of the sample.

Valderrama et al. (2025, <https://doi.org/10.5194/bg-23-1965-2026>), in the same special issue, explore the photochemical formation and breakdown of carbonyl compounds and examine the oxidation capacity in both ambient SML and underlying water (ULW). However, Fourier-transform ion-cyclotron mass spectrometry (FT-ICR-MS) cannot identify specific structural motifs such as carbonylic functionalities.

In our research, we used photodegradation indicators like the loss of photosensitive compounds (Bercovici et al., 2023, <https://doi.org/10.1021/acs.est.3c05929>), the breakdown of humic-like FDOM (Moran et al., 2000, <https://doi.org/10.4319/lo.2000.45.6.1254>), and the reduction of aromatic compounds (Stubbins and Dittmar, 2015, <https://doi.org/10.1016/j.marchem.2015.06.020>).

L 467 - S5).

Thank you for pointing that out. L467 has been updated.

L 486 - ...in microalgae...

Thank you for pointing that out. L486 has been updated.

L 499 - C. Closterium

Thank you for pointing that out. L499 has been updated.

L 534 – 535 – I suggest that the authors reconsider this statement. Specifically, alternative explanations should be considered, such as the possibility that POM had already been degraded to CO₂. It is well established that the turnover time of fresh carbohydrates is very short. Harvey et al. (1995; [https://doi.org/10.1016/0016-7037\(95\)00217-N](https://doi.org/10.1016/0016-7037(95)00217-N)) showed that turnover times among particulate pools are shortest for carbohydrates, ranging from approximately 10.7 days for diatom and cyanobacterial carbohydrates. Furthermore, Becker et al. (2020; doi:10.1073/pnas.1917001117) measured hydrolysis rates of 0.1–34 nmol L⁻¹ h⁻¹, indicating rapid enzymatic degradation of laminarin in the ocean. This comment is particularly relevant given that the mesocosm experiment lasted 33 days, with at least 10 days following bloom decay.

Good point. Unfortunately, we do not have data on the POM concentrations in the SML due to sample limitations. It is definitely a limitation of our study that it ends 10 days after the bloom. To gain better insight into degradation and remineralisation, a longer observation of the post-bloom phase would have been highly informative.

What we detect are molecular fragments that actually indicate the degradation of laminarin, as our fragments are all below 1000 Da. We argue that these are degradation products of longer-chain laminarin.

Additionally, Aluwihare & Repeta (1999, <https://doi.org/10.3354/meps186105>) argue that phytoplankton exudates, including E. huxleyi DOC in sea surface water, consist of structurally related and biosynthetically derived acyl oligosaccharides that might persist after more labile organic matter has been degraded. So, it might be that the "laminarin-derivative" fraction we are detecting is a residue or degradation product formed by the breakdown of those oligosaccharides, converting them in situ into more bio-accessible, short-chain, low-molecular-weight (LMW) oligosaccharides.

To summarise, the degradation of laminarin may not directly correspond to the degradation of the LMW carbohydrate-like DOM we detected, as we lack information on the POM in the SML.

L 585 – Fig. 4I

Thank you for pointing that out. L 585 has been updated accordingly.

L 599-600 – I do not understand this sentence: “... are more affected than ULW in natural environments where light penetration into the water column is limited to depths < 1 m.” as light typically penetrates much deeper than 1 m in seas and oceans.

The concept was that a strong coupling exists between ULW and SML due to the small basin size, limiting our ability to detect distinct photodegradation effects in these water layers. Nonetheless, in nature, such effects could still occur because the SML is consistently exposed to intense irradiation, while the ULW might mix with deeper water layers. Secchi disk measurements in 2016 (<https://doi.pangaea.de/10.1594/PANGAEA.858721>) near Jade Bay indicated that the disc was not visible below 1 meter, implying minimal light penetration beyond shallow depths of a few meters. The sentence in L598 will be removed.

L 606, 608, 610, 611 – please write properly *E. hux.* and *C. clos.*

Thank you for pointing that out. L 606, 608, 610 and 611 have been updated accordingly.

Reviewer 2

General comments:

This manuscript presents a very nice coupling of data from DOC and DN analyses, SPE-extraction efficiencies, and negative ionization ESI FT-ICR-MS and ATR-FTIR characterization of SPE extracted DOM to investigate DOM in the surface microlayer and in bulk underlying water during a mesocosm experiment mimicking a North Atlantic phytoplankton bloom. It should be of interest to BG readers and is generally well-written and presented. There are, however, a few areas that the authors should clarify their discussions and perhaps modify their presentation of the data. I would also encourage them to add more information about the biological succession in their mesocosm, if they have that information. Knowing what the bacterial response to the blooms was (even just in cell counts per L) and whether there was significant zooplankton grazing would add additional context for their results.

Thank you for your comments!

Bibi et al. (2025, <https://doi.org/10.5194/bg-22-7563-2025>) described the setup and development of many biogeochemical parameters in this Mesocosm study. During the bloom, a distinct surface slick with complete surfactant coverage of the air–sea interface created a biofilm-like habitat in the SML, leading to increased bacterial cell abundance. The bacterial community utilised amino acids as the preferred carbon source, followed by carbohydrates in both water layers. During the post-bloom phase, a clear slick or biofilm was observed, accompanied “by higher bacterial cell counts in both water layers (Fig. 7a), indicating a microbial response to elevated DOC concentrations” (Bibi et al., 2025, <https://doi.org/10.5194/bg-22-7563-2025>). But we decided not to include this figure into our manuscript.

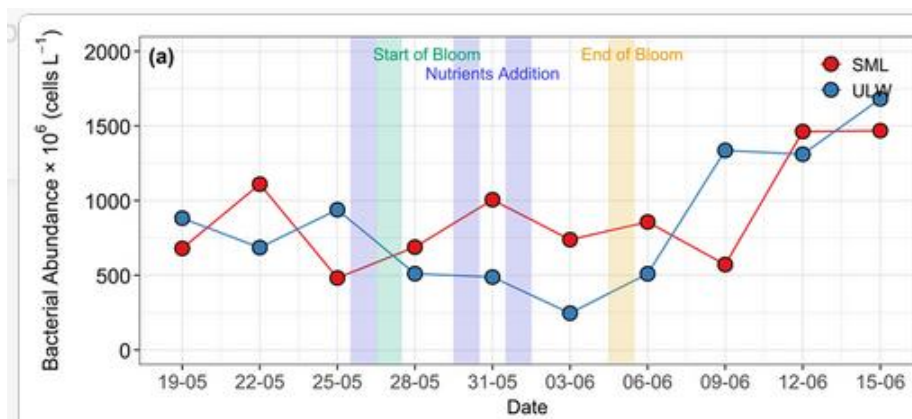


Fig. 1: Bacterial abundance during the Mesocosm experiment, figure from Bibi et al., 2025.

In the discussion, we will write (L471): “This observation also aligns with increased cell count during the post-bloom phase detected by Bibi et al. (2025) during the same mesocosm experiment. They also reported a shift in microbial substrate utilisation towards carbohydrates during the later stages of the bloom, especially in the SML (Fig. 7 a and f in Bibi et al., 2025). “

Indeed, we have FlowCam data that detected Vorticellidae in the SML throughout the whole Mesocosm and Copepoden only in the SML in the post-bloom phase. This is based on unpublished FlowCam data insufficient for statistical claims. The controlled conditions of mesocosms lack the natural variability of the ocean system (e.g., hydrodynamics and grazing that control phytoplankton blooms and bacterial populations), potentially leading to the artificial selection of microbial communities.

My first general concern is that the authors need to highlight earlier in the paper that SPE-extraction fractionates against carbohydrates and proteins; carbohydrates in particular are not well retained by the resin they use. Negative ionization ESI-MS also has some blind spots as it has low carbohydrate and protein ionization efficiencies. The authors do mention both these points later in the manuscript but they should explicitly discuss these issues in the introduction. Since their data, even with the suppressed carbohydrate response, shows carbohydrates to be important, I wonder if the authors might include in their discussion some of the ultrafiltration-of-DOM literature, where the isolated higher molecular weight DOM was often considered mainly carbohydrate and protein material (early work by Repeta and Aluwihare might be interesting in this regard).

We think this is an excellent suggestion. We adapt the introduction in L77: "However, FT-ICR-MS studies focusing on SML-DOM remain limited, and the technique has faced criticism because it does not fully reveal chemical structures (Merder et al., 2020). Additionally, PPL extraction has been shown to fractionate carbohydrates (Raeke et al., 2016). Furthermore, peptides and carbohydrates inherently exhibit poor ionisation efficiency in negative (peptides; Marija Nišavić et al., 2017, <https://doi.org/10.1021/acs.analchem.6b04466>; Konermann & Douglas, 1998, [https://doi.org/10.1016/S1044-0305\(98\)00103-2](https://doi.org/10.1016/S1044-0305(98)00103-2)) or overall ESI (carbohydrates; Tacker & Schug, 2018, <https://doi.org/10.1002/rcm.8155>). Carbohydrates are particularly underrepresented in FT-ICR-MS due to their low surface activity and lack of easily deprotonable chemical groups. During the traditional ESI ionisation process, the droplet surface is mainly occupied by more surface-active species (such as peptides) that ionise more effectively, while hydrophilic sugars are confined inside and experience suppression, (Bahr et al., 1997, <https://doi.org/10.1021/ac970624w>)."

We will mention in the discussion L 476: “Carbohydrates exhibit low recovery rates with PPL-SPE (Raeke et al., 2016), have poor ionisation efficiency with ESI (Tacker & Schug, 2018, Tacker & Schug, 2018, <https://doi.org/10.1002/rcm.8155>), and, furthermore, FT-ICR-MS detection relies solely on sum formulas for molecular identification (Merder et al., 2020). This, along with the inability to distinguish structural isomers, generally impedes the clear detection of carbohydrate compounds using untargeted ESI-FT-ICR-MS. Despite these limitations, it is especially noteworthy that we observed a selective accumulation of [...]”

And we will mention in the discussion L494: “With our analytical window of up to 1000 Da, we close the gap towards the usually used size-fragmentation by dialysis and ultrafiltration (>1000 Da; Aluwihare et al., 1997, <https://doi.org/10.1038/387166a0>). Our approach allows us to look for either building blocks or degradation products of oligosaccharides. Combined with data gained from dialysis, this might yield better insights into the formation and degradation of polysaccharides. Our relatively low-molecular-weight fraction (<1000 Da) might be of importance because it is more bioavailable. Our results indicate that SML studies in particular could benefit from a combined LMW- and HMW-analysis.”

My second concern involves the FTIR data and discussion. FTIR response factors vary considerably depending upon the dipole moment changes of the functional groups being measured. That is why C-H and aromatic bond signals are low relative to C=O and C-O-C in organic compounds even when there are plenty of the C-H and aromatic groups present. Because of this, FTIR is inherently a qualitative characterization tool, though I do think looking for changes in response of different functional groups is valid. I do wonder about normalizing FTIR response to carbon content of the initial sample, rather than total FTIR signal. Most papers I have read have used total signal as a way to adjust for the fact that loading the same amount of sample reproducibly and to the same sample thickness each time is very hard to do in an ATR cell (or in a KBr pellet, for that matter).

Lines 265-280. Usually this is addressed by normalizing to total (background corrected) FTIR signal response, as depending upon analyst, humidity, cleanliness of cell, variable surfactant levels and sample heterogeneity, reproducibility is variable, even for replicate aliquots of the same sample. Following up on this theme, can you give examples of how variable your replicate analyses are: 1. initially, 2. upon normalization to total response, and 3. with your carbon rescaling technique? Perhaps this could be added to the supplemental section?

Please find the attached document named 'FTIR_normalization_details.docx' for a detailed response. This file relates to this question and to your point 5 (see below).

Additional more specific concerns follow:

1. In lines 112-113, what was used as inoculant or did you just rely on regrowth from cells that survived the initial water treatments?

We relied solely on regrowth from cells that survived the initial bay water treatments. We adapted the paragraph L110 to: "SURF was filled with natural seawater from the nearby Jade Bay (53° 28' 42" N, 8° 12' 15" E) to replicate natural conditions. Additionally, the seawater was filtered, skimmed, and UV-treated at the beginning of the study to further reduce particle concentration and biological activity. The late spring period was chosen to ensure a low initial phytoplankton abundance in the original seawater. This approach was selected to examine the regrowth of surviving phytoplankton cells after the initial water treatments, simulating a native microbial community starting with almost no bioproduction or pre-existing bioproduction products. To induce and maintain the phytoplankton bloom, inorganic nitrogen, phosphorus, and silicate were added on May 26 May 31, and June 01, 2023. Detailed information can be found in Bibi et al. (2025)."

2. In lines 129-130, do you have any idea how much surface-active material you lost in filtering for DOC in both the SML and the ULW? Does this differ with the different filter types you used for processing samples for, say, DOC and DN relative to SPE-extraction?

Unfortunately, we lack information on surfactant levels in filtered versus non-filtered samples, and we were unable to quantify the filter-bound DOC fraction. We observed that the concentrations and extraction efficiencies of the dissolved fraction were within the same range for our two filter types, indicating no measurable difference between the membrane types (GF/F and PC) or pore sizes (0.7 and 0.2 μm).

3. Line 155, was this mercury (I) or mercury (II) chloride?

We adapted L155 to: "All nutrient samples were poisoned immediately with a saturated mercury chloride (HgCl_2) solution (0.02% of the sample volume) and stored at 4 °C for further analysis. "

4. Lines 177-178. I really like that you calculated extraction efficiency!

Thank you! Actually, we noticed that in our experiment, the extraction efficiency changed during the experiment - a clear first indication of a changing DOM composition!

5. (Comment moved up, see above)

Please, as described above, find the answer to this question in the attached document file named 'FTIR_normalization_details.docx' for a detailed response.

6. Lines 363-364. I don't really see this trend for the ULW in Figure 5, and there is a strong and opposite trend for the SML that you don't mention here.

L363 was rewritten as: "Specifically, in the SML, the sum of the normalised intensities of laminarin-derivative formulas increased suddenly during the bloom phase (Fig. 5). In the SML the laminarin-like formulas exhibited a diurnal pattern, with higher signal intensities in samples taken before sunrise compared to those taken in the afternoon. In contrast, the ULW only showed sporadic appearances of the laminarin-like formulas, with relative intensities remaining below or just slightly above the method detection limit throughout the study, more similar to the pre-bloom conditions. "

7. Lines 365-368. Why spend so much time talking about O/H? The DOC-DON trend also looks interesting (maybe even more interesting) and isn't described at all.

The development of the SPE-DOC/SPE-DON ratio (Fig. S4 a) will be incorporated into paragraph L328, which describes bulk parameters with increased compartmentalisation. The O/H plot (Fig. S4 b) will be removed from the supplementary material. The O/H trend is less conclusive and does not significantly contribute to our line of argument.

The paragraph will be changed to: "DOC concentrations and the DOC/DON ratio in the SML compartmentalised in tandem with the increase of Chla during the bloom phase (Fig. 3 a,d; Tab. 1). Throughout the bloom, SML DOC was enriched compared to ULW, despite an overall increase in the ULW DOC (EF, Fig. 3 e). This enrichment increased drastically during the bloom and the post-bloom phase. Both SML and ULW maintained high DOC concentrations after the bloom, despite Chla levels dropping in the post-bloom phase. The SPE-DOC/SPE-DON ratio remained stable and within the same range between the ULW and SML during the pre-bloom and bloom stages (Fig. S4). As the bloom progressed, the ratio increased abruptly in the SML, leading to compartmentalisation as Chla levels dropped. This compartmentalisation grew during the post-bloom phase, even though the ULW SPE-

DOC/SPE-DON ratio also increased. In the SML, the ratio reached its highest point midway through the post-bloom period and then decreased, eventually aligning with the ULW level.”

8. Line 442. *You are not studying the low molecular weight fraction but only the fraction retained on an SPE filter (mainly a polarity separation).*

The PPL cartridge resin has a 150 Å (15 nm) pore size, so it could also retain HMW DOM if it interacts with the PPL functional groups. However, FTICRMS has a mass window of 100-1000 Da (single ionisation), which corresponds to LMW. Therefore, FT-ICR-MS primarily detects LMW compounds.