Response to the comments from Community Comment 1

We sincerely thank Ian Jenkinson for his valuable comments. Our manuscript has been revised according to the comments and our responses to the comments are as follows. For clarity, the comments are reproduced in blue, authors' responses are in black and changes in the manuscript are in red.

A very interesting and important paper!

However, quoting Lines 190--219, "Several studies have found that phytoplankton blooms can result in the formation of mucus on the water surface, which is typically an excessive accumulation of extracellular polysaccharides (Ternon et al., 2024; Medina-Pérez et al., 2021). In contract, this can increase the viscosity of SML and potentially enhance its surface tension (Jenkinson and Sun, 2010). From day 1 to day 5, the rapid increase in the surface tension of SML samples appears to be related to the rise in their saccharide concentration (see in Fig. 6a).".

As the authors say, mucus, secreted by organisms such as phytoplankton, consists of polymers can indeed increase viscosity of seawater. However, it tends to reduce surface tension below the value for "pure" (i. e. organics-free) seawater about 74 mN.m-1, not enhance (increase) it. As shown in the authors' Fig. 6d, the surface tension of SML water remained consistently less than that of subsurface water (SSW) by about 0.5 to 1 mN.m-1, consistent with enrichment in the SML. The much lower values at the beginning of the experiment remain enigmatic to me, unless they might have been caused by some tiny contaminant by a surfactant molecule such as detergent, often present on the surface of new apparatus. The subsequent increase could then have represented the incorporation of such a surfactant into other organic matter in the experiment, or its conversion or utilization by organisms present. I think this small issue does not affect the validity of the rest of the presentation.

Author Reply

Thank you for your comment. We agree with your view that, compared to pure seawater, the presence of organic matter generally reduces surface tension. Since the sea surface microlayer (SML) typically contains higher concentrations of organic matter than subsurface water (SSW), the surface tension of SML was consistently lower than that of SSW in our experiments.

We can rule out the possibility of these compounds originating from our homemade SSA simulation tank. On the one hand, the new equipment was thoroughly scrubbed with a brush and rinsed multiple times with both tap and deionized water before the next SSA experiment. Even if contaminants remained, they are unlikely to have such a significant impact on the surface tension of SML. On the other hand, the surface tension of SML showed a gradual increase over the first five days. If these organic compounds originated from the equipment surfaces, we would expect the surface tension of the SML to rapidly return to its “original” state during the second SSA experiment.

Another possibility is that the surface-active organic matter may already exist in the coastal seawater’s microlayer. To investigate this further, we analyzed the mass spectrometry data. Figure 1 shows the base peak chromatograms for three samples: SSW and SML sample on Day 1 and SML sample on Day 9. We observed a prominent peak (Peak 1) between elution times of 21.1 and 21.9 minutes in the SML on Day 1, significantly higher than in the seawater on Day 1 and SML on Day 9. Results in Figure 2(a) indicate that the ion at m/z 221.0813 is the primary contributor to Peak 1, with an assigned molecular formula of $C_{12}H_{13}O_4$ (error = 1.1 ppm). The results in Figure 2(b) show that the primary signal intensities in the secondary mass spectrometry fragments of the ion at m/z 221.0813 originate from m/z 177.0913 and 144.0964. These characteristic ions match those observed in the mass spectrum of diethyl phthalate (DEP) standard in the NIST Standard Reference Database (Figure 3). Therefore, Peak 1 can be primarily attributed to DEP. DEP is a commonly used plasticizer, and high concentrations (in the range of mg L^{-1} or mg kg^{-1}) have been detected in various aquatic environments (Gani and Kazmi, 2016; Lu et al., 2023; Liang et al., 2024). Figure 1 reveals that high concentrations of DEP signal was present only in the Day 1 SML samples, while signals in the Day 1 seawater samples were very low. This could be due to DEP's low solubility in water and hydrophobic nature, which makes it significantly enriched in the SML. The DEP signal in the Day 9 SML sample was also low, likely due to reduced concentrations from biosorption or transformation processes (Gao and Chi, 2015; Liang et al., 2024). We further examined the relationship between DEP concentration and surface tension in artificial seawater (Figure 4). Even at extremely low concentrations, DEP can significantly reduce surface tension. For example, a DEP concentration of 2 μM can reduce surface tension to the initial SML value of 65.84 ± 0.36 mN m^{-1}, which is significantly lower than DOC concentration in the SML at that time. Therefore, the presence of DEP in the SML at the start of the experiment was a significant factor contributing to its low surface tension.

We consider that DEP was present in the seawater from the outset of the experiment, likely originating from coastal pollution or being introduced during seawater transport. SML consists of an extremely thin layer at the water's surface, ranging from 1 to 1000 μm, occupying a negligible fraction of the total seawater volume. Although DEP exhibited strong mass spectrometry signals in the day 1 SML sample, its signal in SSW sample on Day 1 were very low (Figure 1). This suggests that the concentrations of DEP in the seawater used in our study were actually quite low. As a result, we no longer consider its impact on phytoplankton blooms.

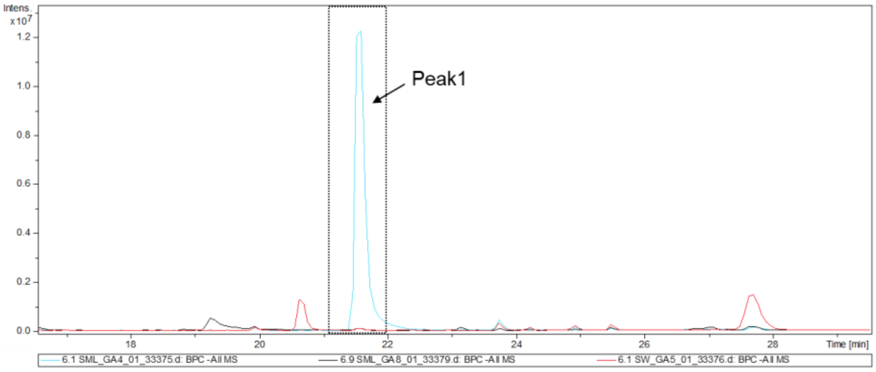

Figure 1. Base peak chromatogram for three samples: SML sample on day 1(blue line), seawater sample on day 1(red line), SML sample on day 9 (black line).

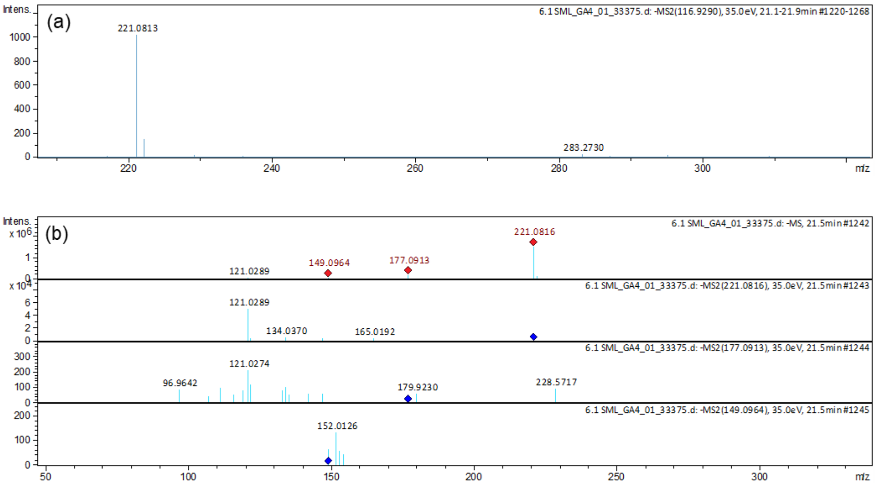

Figure 2. The primary contributing ion of Peak 1 and its secondary mass spectrometry fragments.

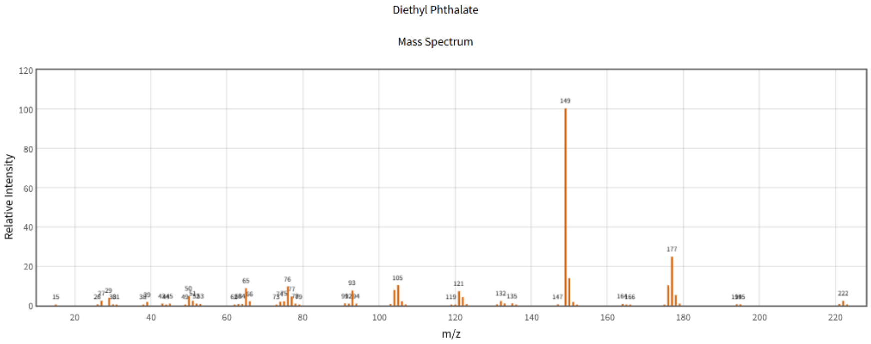

Figure 3. Standard spectrum of diethyl phthalate from NIST Standard Reference Database 69: NIST Chemistry WebBook (https://webbook.nist.gov/chemistry). Note that the standard spectrum employs electron ionization, whereas we utilize an electrospray ionization source. Nevertheless, certain characteristic ions from the standard spectrum remain useful for our identification.

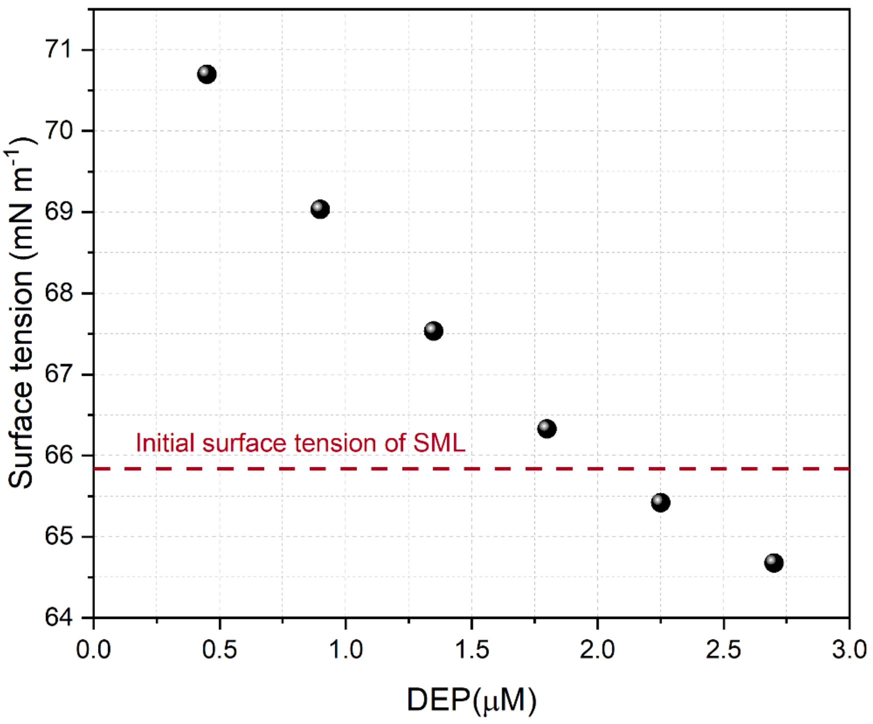

Figure 4. The relationship between different concentrations of DEP and the surface tension of artificial seawater.

We have revised the previous description in the manuscript.

Page 7, lines 186-196

As shown in Figure 2d, the surface tension of SML at the start of the experiment was measured to be 65.84 ± 0.36 mN m^{-1}, which exceeded our expectations. Using both primary and secondary mass spectrometry, we detected diethyl phthalate in the SML on Day 1. As common plasticizer, it is often found in coastal seawater and accumulates in SML due to its low solubility and hydrophobic nature (Lu et al., 2023), significantly reducing surface tension even at low concentrations (Figure S8). However, no diethyl phthalate was detected in bulk seawater on Day 1, which suggests that they likely do not influence phytoplankton blooms in bulk seawater. Detailed mass spectrometry analysis can be found in the Supplement. The surface tension of the SML increased rapidly from Day 1 to Day 5, possibly due to the rapid increase in DOC concentration in the SML during phytoplankton growth (Figure 3). Organic matter secreted by microorganisms can significantly affect the physical properties of the SML (Jenkinson and Sun, 2010; Ternon et al., 2024), which may partially mitigate the low surface tension observed at the beginning. Furthermore, the rapid increase in surface tension may also be linked to the biosorption of diethyl phthalate or transformation by marine microorganisms (Liang et al., 2024; Gao and Chi, 2015).

We added the following in the Supplement.

S5. Identification of phthalate esters in initial SML samples through mass spectrometry

Figure S7(a) shows the base peak chromatograms for three samples: SSW and SML on Day 1 and SML on Day 9. We observed a prominent peak (Peak 1) between elution times of 21.1 and 21.9 minutes in the SML on Day 1, significantly higher than in the seawater on Day 1 and SML on Day 9. Results in Figure S7(b) indicate that the ion at m/z 221.0813 is the primary contributor to Peak 1, with an assigned molecular formula of $C_{12}H_{13}O_4$ (error = 1.1 ppm). The results in Figure S7(c) show that the primary signal intensities in the secondary mass spectrometry fragments of the ion at m/z 221.0813 originate from m/z 177.0913 and 144.0964. These characteristic ions match those observed in the mass spectrum of diethyl phthalate (DEP) standard in the NIST Standard Reference Database (Figure S7(d)). Therefore, Peak 1 can be primarily attributed to DEP. DEP is a commonly used plasticizer, and high concentrations (in the range of mg L^{-1} or mg kg^{-1}) have been detected in various aquatic environments (Gani and Kazmi, 2016; Lu et al., 2023; Liang et al., 2024). Figure 1 reveals that high concentrations of DEP signal was present only in the Day 1 SML, while signals in the Day 1 seawater were very low. This could be due to DEP's low solubility in water and hydrophobic nature, which makes it significantly enriched in SML. The DEP signal in the Day 9 SML was also low, likely due to reduced concentrations from biosorption or transformation processes (Gao and Chi, 2015; Liang et al., 2024). We further examined the relationship between DEP concentration and surface tension in artificial seawater (Figure S8). Even at extremely low concentrations, DEP can significantly reduce surface tension. For example, a DEP concentration of 2 μM can reduce surface tension to the initial SML value of 65.84 ± 0.36 mN m^{-1}, which is significantly lower than DOC concentration in the SML at that time. Therefore, the presence of DEP in the SML at the start of the experiment was a significant factor contributing to its low surface tension. Therefore, the presence of DEP in the SML sample at the start of the experiment was a significant factor contributing to its low surface tension.

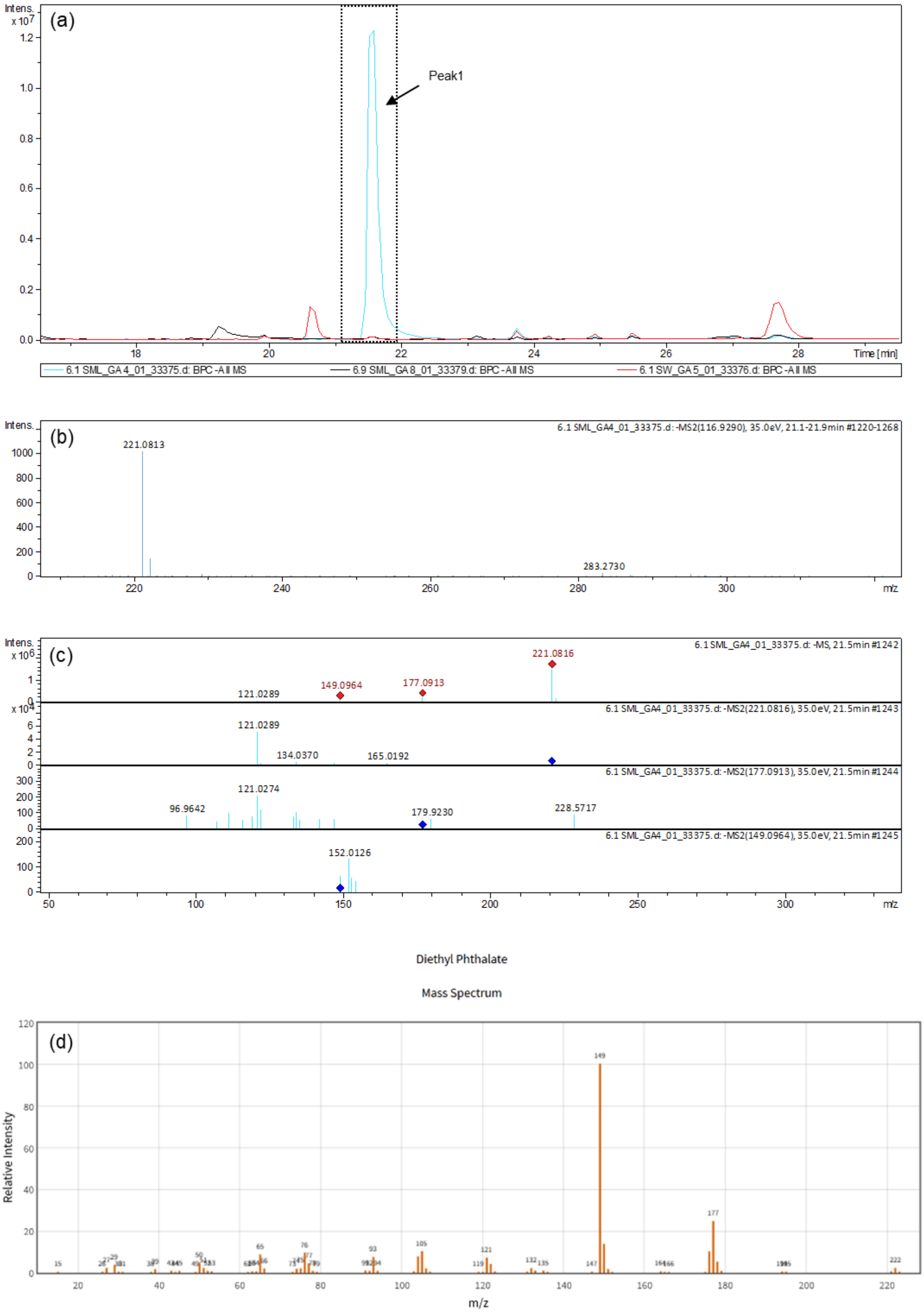

Figure S7. Identification of phthalate esters in initial SML samples through mass spectrometry. (a) Base peak chromatogram for three samples: SML on Day 1(blue line), seawater on Day 1(red line), SML on Day 9 (black line); (b) Primary contributing ion of Peak 1 and its secondary mass spectrometry fragments; (c) Standard spectrum of diethyl phthalate from NIST Standard Reference Database 69: NIST Chemistry WebBook (https://webbook.nist.gov/chemistry). Note that the standard spectrum employs electron ionization, whereas we utilize an electrospray ionization source. Nevertheless, certain characteristic ions from the standard spectrum remain useful for our identification.

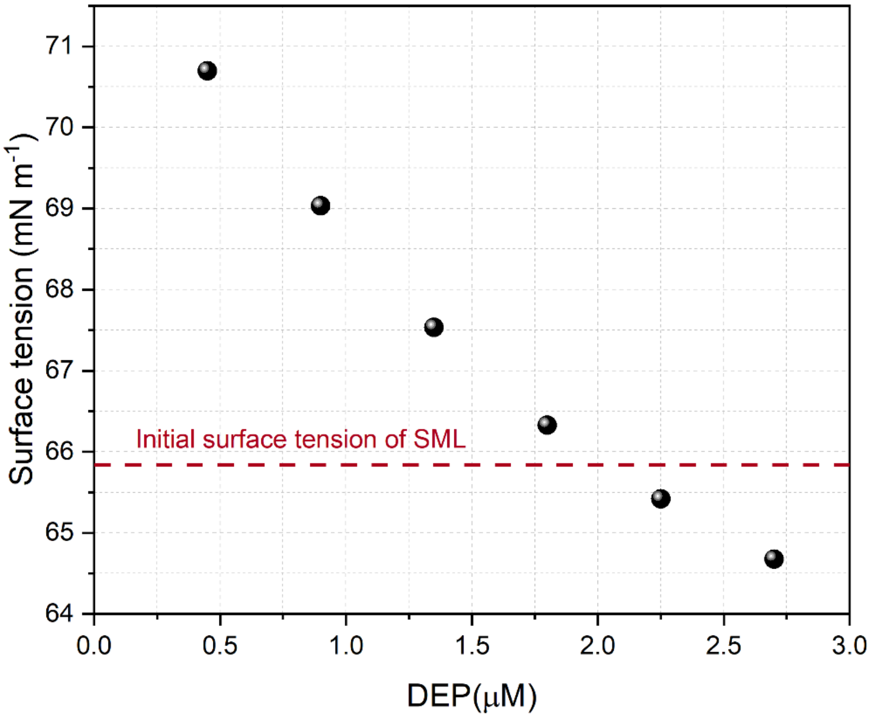

Figure S8. Relationship between different concentrations of DEP and the surface tension of artificial seawater.

References

Gani, K. M. and Kazmi, A. A.: Phthalate contamination in aquatic environment: A critical review of the process factors that influence their removal in conventional and advanced wastewater treatment, Critical Reviews in Environmental Science and Technology, 46, 1402-1439, https://doi.org/10.1080/10643389.2016.1245552, 2016.

Gao, J. and Chi, J.: Biodegradation of phthalate acid esters by different marine microalgal species, Mar. Pollut. Bull., 99, 70-75, https://doi.org/10.1016/j.marpolbul.2015.07.061, 2015.

Jenkinson, I. R. and Sun, J.: Rheological properties of natural waters with regard to plankton thin layers. A short review, J. Mar. Syst., 83, 287-297, https://doi.org/10.1016/j.jmarsys.2010.04.004, 2010.

Liang, J., Ji, X., Feng, X., Su, P., Xu, W., Zhang, Q., Ren, Z., Li, Y., Zhu, Q., Qu, G., and Liu, R.: Phthalate acid esters: A review of aquatic environmental occurrence and their interactions with plants, J. Hazard. Mater., 470, 134187, https://doi.org/10.1016/j.jhazmat.2024.134187, 2024.

Lu, M., Jones, S., McKinney, M., Kandow, A., Donahoe, R., Cobb Faulk, B., Chen, S., and Lu, Y.: Assessment of phthalic acid esters plasticizers in sediments of coastal Alabama, USA: Occurrence, source, and ecological risk, Sci. Total Environ., 897, 165345, https://doi.org/10.1016/j.scitotenv.2023.165345, 2023.

Ternon, E., Dinasquet, J., Cancelada, L., Rico, B., Moore, A., Trytten, E., Prather, K. A., Gerwick, W. H., and Lemée, R.: Sea-Air Transfer of Ostreopsis Phycotoxins Is Driven by the Chemical Diversity of the Particulate Fraction in the Surface Microlayer, Environ. Sci. Technol., 58, 18969-18979, https://doi.org/10.1021/acs.est.4c06691, 2024.