RC2: 'Comment on egusphere-2024-625', Anonymous Referee, reply

The manuscript by Santinelli et al. tests how ocean alkalinity enhancement (OAE) affects DOM composition and concentration. They conducted an experiment with two seawater samples, in which $Ca(OH)_2$ was added to pH of 9 or 10, and they found that in both samples, there was removal of DOC and of CDOM / FDOM at the highest pH treatment. Possible reasons are discussed, and adsorption to secondary carbonate minerals is identified as the most likely explanation.

Overall, the scope of the manuscript is a bit limited in only having experimented with two different water samples. However, I think that the novelty and timeliness of the study are both very high, and the two water samples cover two extremes in terms of DOM source and composition, so I think that the results are likely to be generally representative of effects from lime addition. I think that the experiments are fundamentally robust, even though clearly more research is needed to understand the underlying mechanisms and work out the implications. But I support publishing this manuscript already, because that will motivate further work in this direction. I have a number of relatively minor suggestions for revisions as listed below.

We are grateful to the referee for her/his appreciation of our work and for having completely understood our point. We are aware of the limits of our experiments, but we really hope that this can be a stimulus for investigating in depth the effects of ocean liming on marine ecosystems, focusing also on the microbial and invisible part. We also thank the referee for the very constructive comments and for the suggestions.

Line 23: you say that DOM is "the largest … mixture of organic molecules on Earth". I suppose it depends a bit on how one wants to define individual pools, but collectively the soil organic carbon pool on land is at least twice as large as the DOC pool, so perhaps "one of the largest" would be better?

Yes, in the revised paper, we will change the sentence as suggested.

Lines 52 and 74-75: Initially you talk about enhancement by +1 pH unit and release of 10 kg/s, but then the experimental treatments are +1 and +2 pH units based on release of up to 25 kg/s, but the same reference is given for both. Maybe explain this a bit more clearly in the introduction so it doesn't come across as being inconsistent?

We thank the referee for this comment, and we apologize for not being clear. The paper by Caserini et al. (2021) does not report a release of 25 kg/s, the number (10 kg/s) reported in the introduction is correct, but we realized that the sentence was not clear. In the revised text, it can be changed as follows: "*Caserini et al., (2021) simulated the pH dynamics within the wake*

of a sparging ship releasing $Ca(OH)_2$ with an initial particle radius of 45 µm at a rate of 10 kg s^{-1}. The results of their modeling study suggest that in these conditions a temporary, sharp increase in pH of about 1 unit can be observed at the discharge site, and that the effect decreases moving far from the discharge site, becoming lower than 0.2 pH units at a distance of 1400 – 1600 m (0.8-0.9 miles)."

We will also rework the sentence in the methods as follows: "*Based on the results by Caserini et al., (2021), which suggested a sharp increase of 1 unit of pH at the discharge site of a sparging ship, the experiment was carried out at pH 9. Although unlikely under actual conditions of dilution in the open sea, an additional experiment was carried out at pH 10 because this situation may occur in coastal waters (e.g. coastal lagoons, high primary productivity enhanced by eutrophication; Hinga, 2002)*"

Reference: K.R. Hinga, 2002. Effects of pH on coastal marine phytoplankton. Mar Ecol Prog Ser 238: 281 - 300, 2002

Line 132: "The EEMs were elaborated" – better to say "processed" or "analysed".

Ok, elaborated can be replaced by processed.

Line 138-139: I don't understand well how the sample sizes come about, and especially why the sample size would be different, given that the experimental design is the same. I suggest explaining this here. Based on Section 2.1, I would expect a sample size of (2 pH treatments + 1 control) x (4 time points) x (3 replicates) = 36 samples for the CDOM/FDOM analysis.

We thank the referee for notice this mistake and we apologize for the misprint. The correct number of samples is 45 for each experiment. Time points are 5 because we considered also the t0 of the samples taken before the $Ca(OH)_2$ addition. The correct number is therefore (2 pH treatments + 1 control) x (5 time points) x (3 replicates) = 45 samples. In the revised paper, we can change the text accordingly.

Lines 247–263: This is an interesting discussion. Another study that might be relevant here is Kaushal et al. (2020), who conducted aragonite precipitation experiments with seawater and different DOM sources to investigate humic substance incorporation into coral skeletons (for transparency: I am a co-author on that study). In that case, the evidence suggested preferential incorporation of terrestrial humic substances, which I think is consistent with the results here, and more generally helps explain incorporation of FDOM into coral skeletons. The literature cited here at the moment seems to be mostly on freshwater lakes/ponds.

We really thank the referee for this comment and for suggesting this interesting paper. In the revised text, this point can be included as follows: "*Among the 3 hypotheses mentioned above, the decrease in a_{254}, observed in our experiments, supports the hypothesis 2, suggesting that, following the addition of $Ca(OH)_2$, the largest and most aromatic dissolved organic molecules adsorb to primary and secondary mineral particles and sink. This hypothesis is also supported by the higher removal of the terrestrial components in both the Mediterranean Sea ($C3_{Th\text{-}Med}$, -26%) and the Baltic Sea ($C3_{Th\text{-}Bal}$, -11%). This observation is in agreement with the results of (Kaushal et al., 2020) which observed a higher incorporation of terrestrial humic substances into abiogenically precipitated aragonite, with respect to marine-humic ones.*"

Lines 186-292: It's clear that in the Baltic sample, a smaller percentage of the initial DOC pool was removed, but I think it's important to also recognise that this represents a much larger absolute quantity of DOC removed. I didn't see data presented anywhere on the amount of CaCO3 being formed, but the first sentence here suggests that there was less CaCO3 formed in the Baltic experiment (please clarify whether you have data to show that less carbonate was formed, or whether this is an assumption). If the amount of CaCO3 that was produced in the Baltic samples was equal to or less than the amount in the Mediterranean, then that means that per mass of CaCO3 the Baltic DOM is being strongly preferentially removed compared to the DOM in the Mediterranean. This is consistent with the conclusion in Kaushal et al. (2020) that CaCO3 preferentially removes terrestrial FDOM.

Unfortunately, we did not measure the amount of $CaCO_3$ formed in the experiments, it was an assumption due to the lower amount of added $Ca(OH)_2$. However, even if the amount of $CaCO_3$ formed is unknown, in the revised paper, we can add the following paragraph taking into consideration the overall amount of DOC removed in the two experiment, together with the amount of $Ca(OH)_2$ added: "*At pH 10, the overall DOC removed in the Baltic Sea is larger (27 µM) than in the Mediterranean Sea (11 µM), despite the lower Ca(OH)2 added. This suggests a removal of 450 µmol of DOC per gram of Ca(OH)2 in the Baltic Sea, and 44 µmol of DOC per gram of Ca(OH)2 in the Mediterranean Sea. This observation can be explained by the predominance of terrestrial DOM in the Baltic Sea which was suggested to be preferentially removed during abiogenic precipitation of aragonite with respect to marine DOM (Kaushal et al., 2020).*"

Line 298: "any hypothesis of liming-based OAE" is unclear. Do you mean "any proposal" or "any plans for"?

Yes, hypothesis can be replaced by "any plans for"

Section 4.3: This section might be improved with a bit more detailed discussion. I appreciate that this will necessarily be a bit speculative, but the main change seems to be in the humic and fulvic fractions, which typically not the most highly labile parts of the DOM pool. At the same time, if the DOM is sorbed onto CaCO3 particles, that in itself might alter the bioavailability, regardless of the inherent properties of the DOM. You could therefore discuss in a few sentences which of these scenarios you think is the more probable one. Perhaps even more important would be if you could provide some thoughts on the design of experiments to test this, as it would be important to avoid artefacts from direct pH impacts on the microbial community.

Kaushal et al. (2020). Sub-annual fluorescence measurements of coral skeleton: relationship between skeletal luminescence and terrestrial humic-like substances. Coral Reefs 39:1257–1272. https://doi.org/10.1007/s00338-020-01959-x

We thank the referee for this comment and for pushing us to improve this section, even in a speculative way. In the discussion of the revised paper, we could add the following paragraph: "*Our results indicate the preferential removal of the humic-like fractions by $CaCO_3$ precipitation. Humic-like substances are considered to constitute the less labile fraction of DOM (Zigah et al. 2017; Bachi et al., 2023), supporting C sequestration in the deep waters (hypothesis 2) and a change in the lability of DOM in the surface waters, with an increase in the percentage of the labile fraction after CaCO3 formation. Even if the lability of DOM is a very complex process, depending on a large number of variables (Dittmar et al., 2021), the change in the lability of DOM could be tested in incubation experiments with natural microbial communities collected in the same area as the water used for the experiment. Since the water for the experiment is filtered through a 0.2 µm filter and it is considered sterile, so we could add unfiltered water from the same site to add the natural microbial community. In order to avoid artefacts from direct pH impacts on the microbial community, before the inoculum the pH should be brought to natural pH by adding HCl.*

It should also be taken into consideration that the adsorption of DOM onto $CaCO_3$ particles, itself might reduce the bioavailability, regardless of the inherent properties of the DOM. This process would increase the carbon sequestration into the deep waters, but it would reduce the energy available for the marine ecosystems, since it would increase DOM persistence in deep waters."