



Ocean liming effects on dissolved organic matter dynamics

Chiara Santinelli¹, Silvia Valsecchi^{1,2,3}, Simona Retelletti Brogi^{1,4}, Giancarlo Bachi¹, Giovanni Checcucci¹, Mirco Guerrazzi¹, Elisa Camatti⁵, Stefano Caserini^{3,6}, Arianna Azzellino^{2,3}, and Daniela Basso^{3,7}

¹Consiglio Nazionale delle Ricerche (CNR), Istituto di Biofisica, Via Moruzzi 1, 56124 Pisa (PI), Italy

²Politecnico di Milano, Dipartimento di Ingegneria Civile ed Ambientale, Piazza Leonardo da Vinci 32, 20133 Milan (MI), Italy

³Consorzio Nazionale Interuniversitario per le Scienze del Mare (CoNISMa), Piazzale Flaminio 9, 00196 Rome (RM), Italy

⁴Sezione di Oceanografia, Istituto di Oceanografia e Geofisica Sperimentale (OGS), Via Piccard 54, 34151 Trieste (TS), Italy

⁵Consiglio Nazionale delle Ricerche (CNR), Istituto di Scienze Marine, Arsenale Tesa 104, Castello 2737/F – 30122 Venice (VE), Italy

⁶Dipartimento di Ingegneria e Architettura, Università di Parma, Parco Area delle Scienze 181/A, 43124 Parma (PR), Italy

⁷Dipartimento di Scienze dell'Ambiente e della Terra, Università degli Studi di Milano-Bicocca, Piazza della Scienza 4, 20126 Milan (MI), Italy

Correspondence: Chiara Santinelli (chiara.santinelli@ibf.cnr.it)

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Abstract. Ocean liming has gained attention as a potential solution to mitigate climate change by actively removing carbon dioxide (CO₂) from the atmosphere. The addition of hydrated lime to oceanic surface water leads to an increase in alkalinity, which in turn promotes the uptake and sequestration of atmospheric CO₂.

Despite the potential of this technique, its effects on the marine ecosystem are still far from understood, and there is currently no information on the potential impacts on the concentration and quality of dissolved organic matter (DOM), which is one of the largest, most complex and yet least understood mixtures of organic molecules on Earth.

The aim of this study is to provide the first experimental evidence about the potential effects of hydrated lime addition on DOM dynamics in the oceans by assessing changes in its concentration and optical properties (absorption and fluorescence).

To investigate the effects of liming on DOM pools with different concentrations and quality, seawater was collected from two contrasting environments: the oligotrophic Mediterranean Sea, known for its dissolved organic carbon (DOC) concentration comparable to that observed in the oceans, and the eutrophic Baltic Sea, characterized by high

DOM concentration mostly of terrestrial origin. Hydrated lime was added to both waters to reach pH values of 9 and 10.

Our findings reveal that the addition of hydrated lime has a noticeable effect on DOM dynamics in both the Mediterranean Sea and Baltic Sea, determining a reduction in DOC concentration and a change in the optical properties (absorption and fluorescence) of DOM. These effects, detectable at pH 9, become significant at pH 10 and are more pronounced in the Mediterranean Sea than in the Baltic Sea. These potential short-term effects should be considered within the context of the physicochemical properties of seawater and the seasonal variability.

1 Introduction

Oceans are a natural sink for atmospheric CO₂, having the potential to mitigate its increase and therefore the effects of climate change (Gattuso et al., 2013; Heinze et al., 2015). The massive amount of atmospheric CO₂ absorbed by the oceans in the last decades (~ 30 %–40 % of

anthropogenic emissions) is generating dramatic global-scale changes in seawater chemistry, such as decreases in pH, in carbonate concentration and in the ocean's buffering capacity (Chikamoto et al., 2023). Even if the ongoing efforts toward a global reduction in anthropogenic CO₂ emissions were rapidly intensified, the available projections highlight the need for additional strategies, such as the development of efficient ocean-based negative emission technologies (NETs) (Calvin et al., 2023; Royal Society and Royal Academy of Engineering, 2018). Some NETs are capable of not only removing atmospheric CO₂ and storing it as bicarbonate ions in the oceans but also increasing the water pH, restoring ocean buffering capacity to the pre-industrial era (Butenschön et al., 2021; Gore et al., 2019). One of these NETs is ocean alkalinity enhancement (OAE, also called artificial ocean alkalization; AOA), which relies on the dissolution of alkaline minerals such as hydrated lime (calcium hydroxide, Ca(OH)₂) into the oceans (Khesghi, 1995). Although the exact amount of hydrated lime to be released, as well as its dispersal methods, is still under debate, one of the proposals is to discharge highly concentrated slurry (lime milk) from large cargo ships, tankers and/or dedicated vessels. Caserini et al. (2021) simulated the pH dynamics within the wake of a dispersal 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 under these conditions a temporary, sharp increase in pH of about 1 unit can be observed at the discharge site and that the effects decrease moving farther from the discharge site, becoming lower than 0.2 pH units at a distance of 1400–1600 m (0.8–0.9 nautical miles).

The discharge of alkaline minerals may trigger the inorganic precipitation of calcium carbonate (CaCO₃), reducing the efficiency of the CO₂ sink and negatively affecting seawater transparency and photosynthetic rates (González and Ilyina, 2016), with possible consequences for the biogeochemical cycles and the functioning of the marine ecosystem (Camatti et al., 2024). The side effects of OAE techniques on the marine environment need to be thoroughly investigated before making any decision on their use. To the best of our knowledge, there is no information on the effects that ocean liming may have on dissolved organic matter (DOM) and its chromophoric fraction (CDOM, i.e. the light-absorbing fraction, and FDOM, i.e. its fluorescent fraction). Holding 6.6×10^{14} kg of carbon and being the most-concentrated dissolved component in the oceans (Hansell et al., 2009), every action that may modify seawater chemistry is expected to have an impact on this key component of the carbon cycle. DOM represents the main source of energy for heterotrophic prokaryotes; a change in its concentration and/or quality could therefore have a cascading effect on the functioning of the marine ecosystem.

The aim of this study is to provide the first experimental evidence about the potential effects of hydrated lime addition on DOM dynamics in the oceans by assessing changes in its concentration and optical properties (absorption and flu-

orescence). In order to investigate the impact on the DOM pool with different origins and optical properties, seawater was collected from two highly diverse environments: (1) the oligotrophic Mediterranean Sea, characterized by a dissolved organic carbon (DOC) concentration comparable to that observed in the open oceans, and (2) the eutrophic Baltic Sea, characterized by a high DOC concentration of mostly terrestrial origin.

2 Materials and methods

In order to investigate the effects of ocean liming on DOM dynamics, ultrapure Ca(OH)₂ powder was added to natural seawater, and changes in DOC concentration, absorption and fluorescence of CDOM were observed for 24 h at the laboratories of the Biophysics Institute, CNR (Pisa, Italy). 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 dispersal ship, the experiment was carried out at pH 9. Although unlikely under the 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 with high primary productivity enhanced by eutrophication; Hinga, 2002). Ca(OH)₂ was provided by UNICALCE (Sedrina (BG), Italy) and supplied as powder (Table S1 in the Supplement). Seawater was collected at Marina di Pisa, Tyrrhenian Sea, Italy (Mediterranean Sea), and in the coastal area surrounding Riga, Latvia (Baltic Sea; Table 1).

2.1 Experimental setup

In order to investigate the impact of slaked lime on chemical–physical processes affecting DOM dynamics, seawater was sterilized by filtration through a 0.2 µm pore size filter (Polycap AS36 filter capsule, Whatman, UK) using a peristaltic pump (Masterflex™ L/S™, Germany). Salinity was measured using an HI 9033 portable probe (Hanna Instruments, USA). The experiments were carried out in 2 L acid-washed polycarbonate Nalgene® bottles as follows.

1. Mediterranean Sea

- a. Treatment with filtered surface seawater enriched with Ca(OH)₂ powder to reach
 - pH 9, [Ca(OH)₂] 0.04 g L⁻¹,
 - pH 10, [Ca(OH)₂] 0.25 g L⁻¹.
- b. Control with filtered surface seawater (pH = 8.2).

2. Baltic Sea

- a. Treatment with filtered surface seawater enriched with Ca(OH)₂ powder to reach
 - pH 9, [Ca(OH)₂] 0.01 g L⁻¹,
 - pH 10, [Ca(OH)₂] 0.06 g L⁻¹.

b. Control with filtered surface seawater (pH = 8.1).

All the experiments were carried out in triplicate, and the bottles were stored in the dark and at room temperature ($22 \pm 1^\circ\text{C}$). Immediately after the addition of the $\text{Ca}(\text{OH})_2$ powder, the bottles were gently mixed. Before and after powder addition and before each sampling time, pH was measured using an edge HI2002-02 pH-meter (Hanna Instruments, USA). In the treatment at pH 9, the pH slightly decreased by 0.06 (Baltic Sea) and 0.29 (Mediterranean Sea) between 3 and 22 h after the addition (Table S2). In the treatment at pH 10, 3 h after the addition the pH decreased by 0.3 in the Mediterranean Sea, and after 22 h it decreased by 0.45 in the Mediterranean Sea and 0.26 in the Baltic Sea (Table S2).

Subsamples for DOC (40 mL), CDOM and FDOM (60 mL) analyses were collected before $\text{Ca}(\text{OH})_2$ addition and 5 min, 30 min, 3 h and 22 h after $\text{Ca}(\text{OH})_2$ addition.

The bottles were gently mixed before subsampling at 5 min, 30 min and 3 h. After 22 h, carbonate sedimentation was clearly visible at the bottom of the bottles; samples of the supernatant were therefore collected before mixing for DOC, CDOM and FDOM analyses, and an additional sample was collected after gently mixing for DOC analyses only since CDOM and FDOM would have been strongly affected by the scattering due to the suspended particles.

Samples for CDOM and FDOM analyses were brought to $\text{pH } 7.5 \pm 1.0$ with high-purity 2 M HCl, to avoid the effect of pH on DOM absorption and fluorescence, and filtered through a PES 0.2 μm pore size syringe filter (Minisart 16534K, Sartorius, Germany), to avoid the scattering due to the presence of carbonate particles in solution.

2.2 DOC

Samples for DOC analyses were acidified at pH 2 with high-purity 2 M HCl. DOC measurements were carried out with a TOC-L analyzer (Shimadzu, Japan) by high-temperature catalytic oxidation following Santinelli et al. (2015). Samples were sparged for 3 min with CO_2 -free ultrahigh-purity nitrogen to remove inorganic carbon. After a three-fold rinsing with the sample to be analyzed, 150 μL of the sample was injected into the furnace. From three to five replicate injections were performed until the analytical error was lower than 1 %. A four-point calibration curve was measured using a standard solution of potassium hydrogen phthalate in the same concentration range as the samples. The system blank was measured every day at the beginning and the end of the analyses using low-carbon water ($2\text{--}3 \mu\text{M C}$). The instrument performance was verified daily using the DOC consensus reference material (CRM; Hansell, 2005 – CRM batch no. 20/08-20, nominal concentration of $42 \pm 1 \mu\text{M}$; measured concentration $40 \pm 2 \mu\text{M}$, 76 CRM samples analyzed).

2.3 CDOM optical properties

2.3.1 Absorption

Absorption spectra were measured between 230 and 700 nm using a UV–Vis spectrophotometer (Mod-7850, Jasco, USA) with a 10 cm quartz cuvette. The absorption spectrum of Milli-Q water was subtracted from each sample spectrum. The absorption coefficient at 254 nm (a_{254}) and the spectral slope between 275 and 295 nm ($S_{275\text{--}295}$) were calculated from the absorption spectra using the ASFit tool (Omanović et al., 2019). a_{254} gives semi-quantitative information on CDOM, since primary CDOM absorption is caused by conjugated systems having an absorption peak near 254 nm (Del Vecchio and Blough, 2004; Weishaar et al., 2003). $S_{275\text{--}295}$ can be related to changes in the average aromaticity and molecular weight of the molecules in the CDOM pool (Helms et al., 2008). The absorption coefficient at 280 and 325 nm (a_{280} , a_{325}) and the spectral slope ratio (Sr, ratio between $S_{275\text{--}295}$ and $S_{350\text{--}400}$) are also reported for comparison (Table S2), as they are among the most commonly used CDOM indices in the literature.

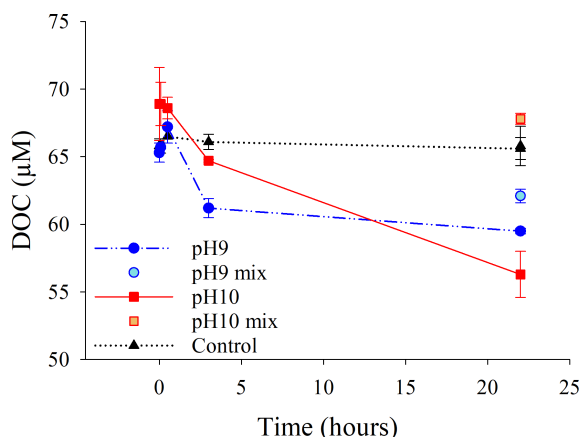
2.3.2 Fluorescence

Fluorescence excitation–emission matrices (EEMs) were recorded using an Aqualog fluorometer (Horiba-Jobin Yvon, UK) with a 1 cm quartz cuvette. Excitation ranged between 250 and 450 nm at 5 nm increments; emission was recorded between 212 and 619 nm at 3 nm increments. The EEMs were processed using the TreatEEM software (Omanović et al., 2023). EEMs were corrected for instrumental bias in excitation and emission, and Rayleigh and Raman scatter peaks were removed using the monotone cubic interpolation (shape-preserving). EEMs were normalized to the water Raman signal, dividing the fluorescence by the integrated Raman band of Milli-Q water ($\lambda_{\text{ex}} = 350 \text{ nm}$, $\lambda_{\text{em}} = 371\text{--}428 \text{ nm}$; Lawaetz and Stedmon, 2009) measured on the same day of the analyses. The fluorescence intensity is therefore reported as equivalent to water Raman units (R.U.).

Parallel factor analysis (PARAFAC) was separately applied to the Mediterranean Sea and Baltic Sea samples (45 EEMs for each experiment) using the decomposition routines of the EEMs toolbox for MATLAB software (drEEM; Murphy et al., 2013). PARAFAC validated a three-component model for both the Mediterranean Sea and the Baltic Sea (Figs. S1 and S2 in the Supplement). OpenFluor, an online database of environmental fluorescence spectra, was used as a validation tool to characterize the three components (Tables S3 and S4). OpenFluor compares excitation and emission spectra of the validated components with all the components present in the database and allows comparison of the spectra using the Tucker congruence coefficient (TCC; Murphy et al., 2014).

Table 1. Chemical and physical properties of the water from the Mediterranean and Baltic seas used for the experiment.

	Sampling date	Salinity	pH	DOC (μM)	a_{254} (m^{-1})	$S_{275-295}$ (nm^{-1})
Mediterranean Sea	Mar-22	38	8.2	66 ± 0.5	1.9	0.024
Baltic Sea	Apr-22	6	8.1	364 ± 3	24.5	0.021

**Figure 1.** Trend of DOC concentration in the Mediterranean Sea treatments at pH 9 and 10 and in the control. Error bars refer to the standard deviation among the three replicates. Please note that for some samples the error bars are smaller than the symbols and are therefore not visible.

2.4 Statistical analyses

For all parameters, differences were tested using the Kruskal–Wallis nonparametric test and were considered significant at the threshold of $p < 0.05$. All statistical analyses were performed using OriginPro version 9 (OriginLab, USA).

3 Results

3.1 Mediterranean Sea

3.1.1 DOC

In the Mediterranean Sea, 3 h after $\text{Ca}(\text{OH})_2$ addition, a $4 \mu\text{M}$ (6 %) DOC decrease was observed in both treatments (Fig. 1, Table S2). A further decrease was observed in the supernatant of the unmixed sample 22 h after the addition, with DOC reaching $59.5 \pm 0.1 \mu\text{M}$ (9 % decrease) at pH 9 and $56.3 \pm 1.7 \mu\text{M}$ (18 % decrease) at pH 10 (Table S2). It is noteworthy that such a decline was only observed in the unmixed samples, whereas no significant change was observed in the mixed samples 22 h after the addition (Fig. 1, Table S2).

3.1.2 CDOM absorption

A slight decrease in a_{254} was observed 3 h after $\text{Ca}(\text{OH})_2$ addition at both pH values (9 and 10). Interestingly, 22 h after the addition, in the supernatant of unmixed bottles, a marked decrease of 0.2 m^{-1} (10 %) and 0.4 m^{-1} (19 %) was observed (Fig. 2a, Table S2) together with an increase in $S_{275-295}$ from 0.0247 to 0.0256 nm^{-1} (4 %) and from 0.0239 to 0.0264 nm^{-1} (10 %) at pH 9 and 10, respectively (Fig. 2b, Table S2). Mixed samples were not collected for CDOM analyses since scattering due to the particles would have affected the results.

3.1.3 FDOM fluorescence

PARAFAC validated a three-component model for the Mediterranean Sea EEMs (Fig. S1, Table S3). Component one ($\lambda_{\text{Ex}}/\lambda_{\text{Em}} = 315/409 \text{ nm}$; Fig. S1a) shows spectroscopic characteristics similar to Coble's peak M ($\lambda_{\text{Ex}}/\lambda_{\text{Em}} = 312/[380]420$; Coble, 1996). The comparison with similar components in the OpenFluor database (matches with a TCC > 0.95) allowed us to characterize it as marine humic-like ($\text{C1}_{\text{Mh-Med}}$). Component two ($\lambda_{\text{Ex}}/\lambda_{\text{Em}} = 275/331 \text{ nm}$; Fig. S1b) shows spectroscopic characteristics similar to Coble's peak T ($\lambda_{\text{Ex}}/\lambda_{\text{Em}} = 275/340 \text{ nm}$; Coble, 1996). The comparison with similar components in the OpenFluor database (matches with a TCC > 0.95) allowed us to characterize it as tryptophan-like ($\text{C2}_{\text{Trp-Med}}$). Component three ($\lambda_{\text{Ex}}/\lambda_{\text{Em}} = 260/[380]456 \text{ nm}$; Fig. S1c) shows spectroscopic characteristics similar to Coble's peaks C and A ($\lambda_{\text{Ex}}/\lambda_{\text{Em}} = 350/451$ and $245/451 \text{ nm}$, respectively; Coble, 1996). The comparison with similar components in the OpenFluor database (matches with a TCC > 0.95) allowed us to characterize it as terrestrial humic-like ($\text{C3}_{\text{Th-Med}}$).

$\text{C1}_{\text{Mh-Med}}$ did not show significant changes over the incubation time at pH 9 and in the control (Fig. 3a, Table S5). At pH 10, a decrease of 0.004 R.U. (20 %) was observed 22 h after the addition. $\text{C2}_{\text{Trp-Med}}$ did not show significant changes in the treatments or the control during the incubation (Fig. 3b, Table S5). $\text{C3}_{\text{Th-Med}}$ showed variations only at pH 10 (Fig. 3c, Table S5), with a slight decrease 3 h after the addition and a significant decrease of 0.006 R.U. (26 %) at the end of the incubation (22 h).

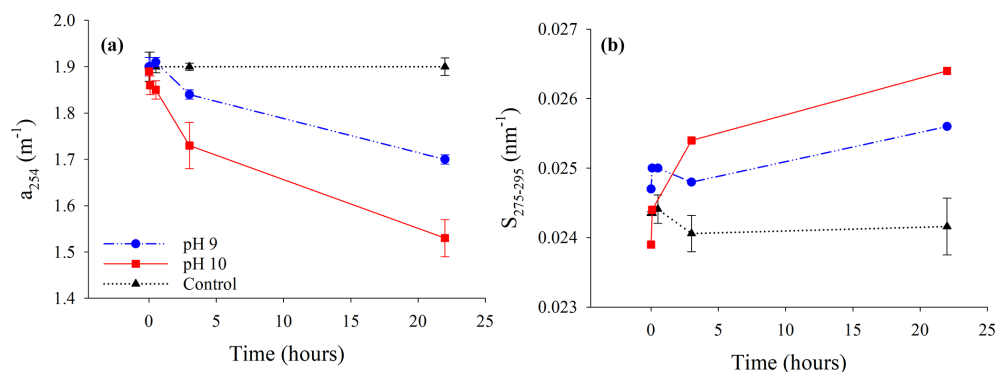


Figure 2. Trend of a_{254} (a) and $S_{275-295}$ (b) in the Mediterranean Sea treatments at pH 9 and 10 and in the control. Error bars refer to the standard deviation among the three replicates. Please note that for some samples the error bars are smaller than the symbols and are therefore not visible.

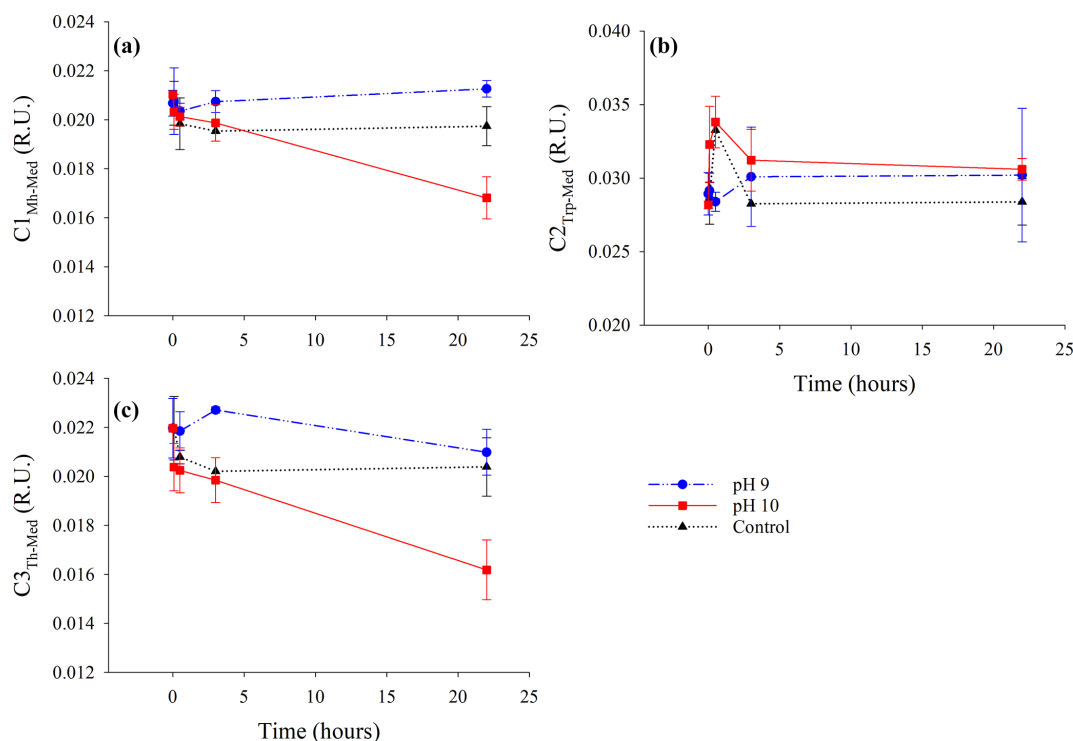


Figure 3. Trend of the fluorescent intensity of $C1_{\text{Mh-Med}}$ (a), $C2_{\text{Trp-Med}}$ (b) and $C3_{\text{Th-Med}}$ (c) in the Mediterranean Sea treatments at pH 9 and 10 and in the control. Error bars refer to the standard deviation among the three replicates.

3.2 Baltic Sea

3.2.1 DOC

In the Baltic Sea, no significant change was observed 3 h after $\text{Ca}(\text{OH})_2$ addition in both treatments (pH 9 and 10; Fig. 4, Table S2). At the end of the experiment (22 h), DOC decreased by $23 \mu\text{M}$ (6 %) at pH 10, whereas no significant change was observed at pH 9. It is noteworthy that DOC showed significant differences between the mixed and unmixed samples at

pH 10, whereas in the mixed samples DOC was similar to the control (Fig. 4, Table S2).

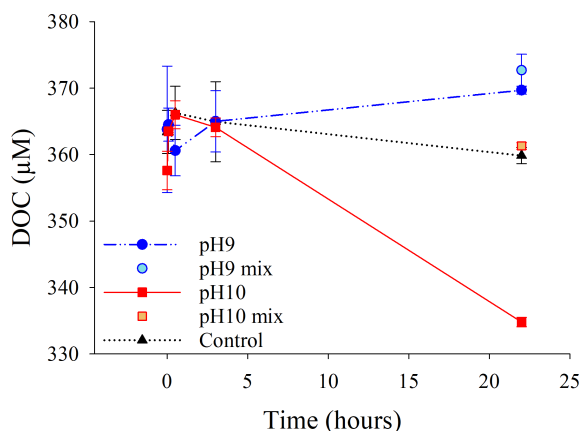


Figure 4. Trend of DOC concentration in the Baltic Sea treatments at pH9 and 10 and in the control. Error bars refer to the standard deviation among the three replicates. Please note that for some samples the error bars are smaller than the symbols and are therefore not visible.

3.2.2 CDOM absorption

In this experiment, 22 h after the addition of $\text{Ca}(\text{OH})_2$, a_{254} decreased by 0.03 m^{-1} (0.1 %) and by 3.6 m^{-1} (15 %) at pH9 and pH10, respectively (Fig. 5a). $S_{275-295}$ increased from 0.0215 to 0.0227 nm^{-1} (6 %) at pH10, whereas no significant change was observed at pH9 (Fig. 5b). The change in CDOM is therefore visible only at pH10 (Fig. 5).

3.2.3 FDOM fluorescence

PARAFAC validated a three-component model for the Baltic Sea EEMs (Fig. S2, Table S3). Component one ($\lambda_{\text{Ex}}/\lambda_{\text{Em}} = 290/400 \text{ nm}$; Fig. S2) shows spectroscopic characteristics similar to Coble's peak M ($\lambda_{\text{Ex}}/\lambda_{\text{Em}} = 12/[380]420$; Coble, 1996). The comparison with similar components in the OpenFluor database (matches with a TCC > 0.95) allowed us to characterize it as marine humic-like ($\text{C1}_{\text{Mh-Bal}}$). Component two ($\lambda_{\text{Ex}}/\lambda_{\text{Em}} = 330/452 \text{ nm}$; Fig. S2) shows spectroscopic characteristics similar to Coble's peak C ($\lambda_{\text{Ex}}/\lambda_{\text{Em}} = 350/451$; Coble, 1996). The comparison with similar components in the OpenFluor database (matches with a TCC > 0.95) allowed us to characterize it as fulvic-like ($\text{C2}_{\text{Flv-Bal}}$). Component three ($\lambda_{\text{Ex}}/\lambda_{\text{Em}} = 280/485 \text{ nm}$, Fig. S2) shows spectroscopic characteristics similar to Coble's peak A ($\lambda_{\text{Ex}}/\lambda_{\text{Em}} = 260/[380]460 \text{ nm}$; Coble, 1996). The comparison with similar components in the OpenFluor database (matches with a TCC > 0.95) allowed us to characterize it as terrestrial humic-like ($\text{C3}_{\text{Th-Bal}}$).

$\text{C1}_{\text{Mh-Bal}}$ did not show significant changes in the treatments or the control during the incubation (Fig. 6a, Table S5). $\text{C2}_{\text{Flv-Bal}}$ did not show significant changes during the incubation at pH9 and in the control, whereas a decrease of 0.03 R.U. (10 %) was observed at pH10 after 22 h (Fig. 6b,

Table S5). $\text{C3}_{\text{Th-Bal}}$ did not show significant changes during the incubation at pH9 and in the control, whereas a decrease of 0.05 R.U. (13 %) was observed at pH10 after 22 h (Fig. 6c, Table S5).

4 Discussion

Even if OAE using alkaline minerals is considered a promising tool to mitigate climate change through the sequestration and storage of atmospheric CO_2 in the ocean (DOSI, 2022), its impact on the marine ecosystem is still far from understood. To the best of our knowledge, this is the first study investigating the potential effects of OAE by hydrated lime addition on DOM dynamics, with particular regard to DOC concentration and CDOM optical properties. Given the crucial role that DOM plays in the marine ecosystem, any impact on its dynamics is expected to affect the water quality and ecosystem functioning through a cascading effect on the microbial loop and the microbial food web.

4.1 Liming impact on DOM dynamics

Our data show the potential effects of hydrated lime addition on DOM dynamics, causing a decrease in DOC concentration (Figs. 1 and 4) and a change in the optical properties of CDOM (Figs. 2, 3, 5 and 6). The decrease in a_{254} , the increase in $S_{275-295}$ (Figs. 2 and 5) and the decrease in humic-like fluorescence (Figs. 3 and 6) indicate a change in DOM quality with a shift towards molecules with lower average molecular weight and degree of aromaticity. These effects are already visible at pH9 but become relevant at pH10. Different hypotheses can explain our results:

1. DOM reacts with $\text{Ca}(\text{OH})_2$, and the largest and most-aromatic molecules are oxidized to CO_2 ;
2. the largest and most-aromatic molecules adsorb onto primary and secondary carbonate precipitates that form following the $\text{Ca}(\text{OH})_2$ addition and sink;
3. the largest and most-aromatic molecules aggregate forming polymer gels or large colloidal material and sink.

Interestingly, a significant decrease in DOC concentration was observed only in the unmixed samples at the end of the experiment (22 h after the addition, Figs. 1 and 4). DOC oxidation to CO_2 by reaction with $\text{Ca}(\text{OH})_2$ (hypothesis one) can therefore be ruled out as a possible removal mechanism. The other two hypotheses remain plausible and are supported by the available literature (Conzonno and Cirelli, 1995; Kaushal et al., 2020; Leenheer and Reddy, 2008; Pace et al., 2012).

In lake water, DOM was observed to adsorb onto carbonate particles and co-precipitate with them; the use of CaCO_3 precipitation was indeed suggested as an efficient technique

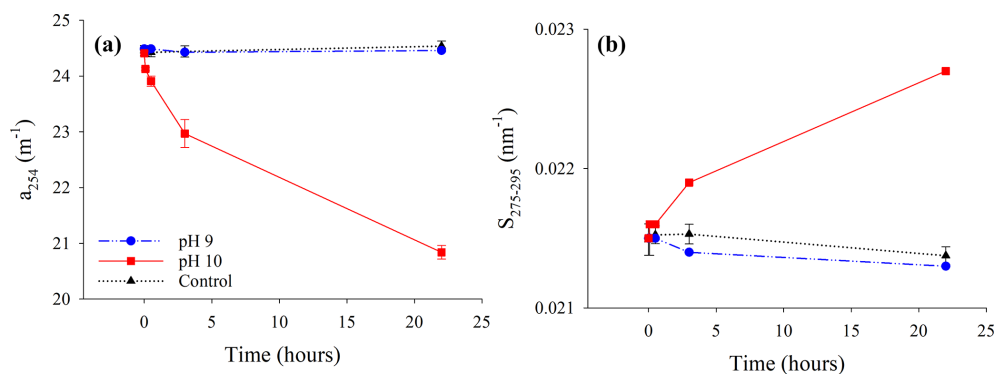


Figure 5. Trend of a_{254} (a) and $S_{275-295}$ (b) in the Baltic Sea treatments at pH 9 and 10 and in the control. Error bars refer to the standard deviation among the three replicates. Please note that for some samples the error bars are smaller than the symbols and are therefore not visible.

for DOM removal during drinking-water treatment processes (Leenheer and Reddy, 2008). The mechanism of DOM coprecipitation and/or physical incorporation into CaCO_3 is due to the formation of insoluble calcium. This hypothesis is further supported by the observation of CaCO_3 precipitation following the dissolution of hydrated lime in seawater that was enhanced by the occurrence of nucleation surfaces as particles or solid mineral phases in the solution (Moras et al., 2021).

In freshwater ponds, the high affinity of molecules with high molecular weight to adsorb onto particles like CaCO_3 was observed by Conzonno and Cirelli (1995), together with a preferential removal of high-molecular-weight humic substances during CaCO_3 crystal formation. Since humic acids have important environmental functions in controlling the pH and the bioavailability of dissolved metals (Baalousha et al., 2006), their removal may trigger a cascade effect with possible impacts on water quality. Past studies showed that pH per se can affect DOM dynamics, as DOM can undergo a fast transition from dissolved to polymer gels (Chin et al., 1998) or to large colloidal material (Pace et al., 2012) when pH switches toward more basic values ($\text{pH} > 8$ for seawater).

Among the three hypotheses mentioned above, the decrease in a_{254} observed in our experiments supports hypothesis two, suggesting that following the addition of $\text{Ca}(\text{OH})_2$, the largest and most-aromatic dissolved organic molecules adsorb to primary and secondary mineral particles and sink. This hypothesis is further supported by the high removal of the terrestrial components observed in both the Mediterranean Sea ($\text{C3}_{\text{Th-Med}}$, -26%) and the Baltic Sea ($\text{C3}_{\text{Th-Bal}}$, -13%). This observation agrees with the results of Kaushal et al. (2020), who reported a higher incorporation of the terrestrial humic substances into abiogenically precipitated aragonite then transferred within coral skeletons, with respect to marine humic substances.

4.2 Different effects on Mediterranean and Baltic waters

The Mediterranean Sea and the Baltic Sea are basins with different biogeochemical characteristics (Table 1). Our results show that DOC concentration (Figs. 1 and 4) and a_{254} (Figs. 2 and 5) are 6 and 13 times higher in the Baltic Sea than in the Mediterranean Sea (Table 1); these data are consistent with previous studies (Hoikkala et al., 2015; Santinelli, 2015; Santinelli et al., 2010). The lower $S_{275-295}$ (Table 1) and the different FDOM compositions (Figs. S1 and S2) indicate a higher percentage of terrestrial DOM in the Baltic Sea than in the Mediterranean Sea, as previously reported by Deutsch et al. (2012) and Hoikkala et al. (2015). Indeed, in the Baltic Sea, PARAFAC allowed us to characterize humic-like and fulvic-like components but not protein-like ones (Fig. S2, Table S4), while in the Mediterranean Sea, the protein-like component was identified (Fig. S1, Table S3). Protein-like compounds are usually related to in situ production, whereas fulvic-like substances mostly have a terrestrial origin. The predominance of terrestrial DOM in the Baltic Sea is due to the high freshwater input from the wide catchment area (~ 4 times as large as the sea itself) and the low seawater input from the North Sea. The Baltic Sea is also characterized by a peculiar carbonate system (Kuliński et al., 2017), exhibiting a wider range of total alkalinity (and pH) compared to the oceans. In particular, the Gulf of Riga, where the water for our experiment was collected, is characterized by a higher total alkalinity and a higher pH with respect to the rest of the Baltic Sea (Beldowski et al., 2010; Kuliński et al., 2017).

In our experiments, we observed different impacts of $\text{Ca}(\text{OH})_2$ addition in the Mediterranean and Baltic seas. In the Mediterranean Sea, a DOC decrease of 6 and 13 μM was recorded at pH 9 and 10, respectively (Table S2), indicating a net removal up to 18 % of the initial DOC (Fig. 1, Table S2). In the Baltic Sea, the maximum removal observed was 6 % at pH 10, whereas no effect was recorded at pH 9 (Fig. 4).

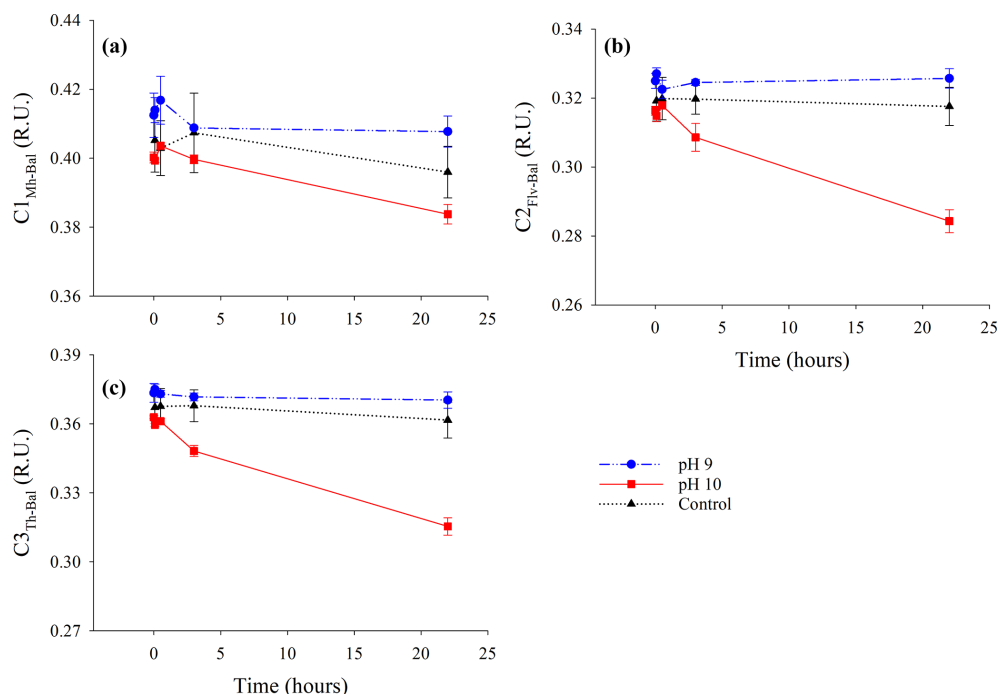


Figure 6. Trend of the fluorescent intensity of C1_{Mh-Bal} (a), C2_{Flv-Bal} (b) and C3_{Th-Bal} (c) in the Baltic Sea treatments at pH 9 and 10 and in the control. Error bars refer to the standard deviation among the three replicates. Please note that for some samples the error bars are smaller than the symbols and are therefore not visible.

Even if the salinity, markedly lower in the Baltic Sea than in the Mediterranean Sea, is probably the main driver of the less pronounced effects on DOM dynamics, we cannot exclude that the peculiar carbonate system combined with the different concentrations and quality of DOM may have influenced the lower removal rates observed in our experiment. The influence of water chemistry is already evident in the 4-times lower amount of $\text{Ca}(\text{OH})_2$ needed to reach pH 9 and 10 in the Baltic Sea than in the Mediterranean Sea. Since CaCO_3 precipitation is one of the main mechanisms explaining our results, the lower amount of $\text{Ca}(\text{OH})_2$ added in the Baltic Sea can explain the lower decrease in DOC observed in this basin compared to the Mediterranean Sea. At pH 10, the DOC removed in the Baltic Sea is higher than ($27\ \mu\text{M}$) than in the Mediterranean Sea ($11\ \mu\text{M}$) despite the lower amount of $\text{Ca}(\text{OH})_2$ added. This suggests a removal of $450\ \mu\text{mol}$ of DOC per gram of $\text{Ca}(\text{OH})_2$ added in the Baltic Sea and $44\ \mu\text{mol}$ of DOC per gram of $\text{Ca}(\text{OH})_2$ added 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 (Kaushal et al., 2020) during abiogenic precipitation of aragonite with respect to marine DOM.

It is noteworthy that DOM in the Mediterranean Sea and in the oceans shows a clear seasonal cycle, mostly attributed to the changes in temperature, water stratification and biological activity, affecting DOM concentration, optical prop-

erties and stoichiometry (Carlson and Hansell, 2015; Santinelli, 2015; Santinelli et al., 2013). Seasonality strongly affects DOM dynamics in the Baltic Sea as well, with prevalent allochthonous sources in winter and in situ production by phytoplankton in spring (Hoikkala et al., 2012; Seidel et al., 2017). Our results, combined with the observed seasonality in DOM dynamics, stress that any plans for liming-based OAE should also take the season into consideration.

4.3 Changed DOM dynamics: implication for the marine ecosystems

Our results suggest that CaCO_3 precipitation is the main driver for the sequestration of DOM from the water column. The sinking of the largest and most-complex fraction of DOM into the deep oceans could lead to different scenarios.

1. If the exported DOM is labile (i.e. it is available for microbial removal on the short temporal scale), its export would determine the following.
 - In the surface layer, the energy available for heterotrophic prokaryotes would be reduced, causing a malfunctioning of the microbial loop that could impact the energy transfer to the higher trophic levels. This process could be further enhanced if the primary production is limited by the reduced water transparency due to carbonate formation.

- The energy would be exported to the deepest layer (below the carbon compensation depth, CCD), leading to increased bacterial production in response to the labile DOM released by CaCO_3 dissolution.
2. If the exported DOM is refractory (i.e. it is not available for microbial removal on the short temporal scale), it will contribute to carbon sequestration in the deep waters.

Our results indicate the preferential removal of the humic-like fractions by CaCO_3 precipitation. Humic-like substances are thought to constitute the less-labile fraction of DOM (Bachi et al., 2023; Zigah et al., 2017), supporting carbon sequestration in the deep waters (hypothesis two) and a change in the lability of DOM in the surface waters, with an increase in the percentage of the labile fraction after CaCO_3 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. The water for the experiments was filtered through a $0.2\ \mu\text{m}$ filter and it was therefore considered sterile; in order to investigate the potential removal of DOM by microbes, we could inoculate the natural microbial community adding 10 % unfiltered water from the same site. In order to avoid artifacts from direct pH impacts on the microbial community, before the inoculum the pH should be brought to natural pH by adding HCl.

We should also take 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 in the deep waters, but it would reduce the energy available for the marine ecosystems.

5 Conclusions

This study reports the first evidence of the potential effects of OAE on DOM dynamics in two contrasting environments: the oligotrophic Mediterranean Sea, known for its low DOC concentration, and the eutrophic Baltic Sea, characterized by a high DOM concentration of mostly terrestrial origin. Our findings suggest that ocean alkalization by $\text{Ca}(\text{OH})_2$ dispersal may alter DOM dynamics and consequently have a potential impact on the entire marine ecosystem. To mitigate these effects, it is crucial to reduce the duration and intensity of pH spikes, ensuring that they remain below the safety threshold of pH 9. We stress the need to take into consideration the physicochemical properties (e.g. salinity, pH, DOM concentration and quality) of the basin and the season to efficiently manage ocean liming and mitigate the potential impacts of ocean alkalization on the DOM pool.

Although the experimental conditions used in this study were more severe than actual liming practices, where the release of $\text{Ca}(\text{OH})_2$ in the ship's wake undergoes rapid dilution that significantly reduces pH changes, our results provide new insights into the possible impacts due to physicochemical processes.

It is important to highlight that the experiments in this study were conducted using sterilized seawater, thus excluding the potential interplay of biological processes on DOM dynamics. To gain a more-comprehensive understanding of possible OAE impacts, future research should address the influence of biological processes, as well as factors like dilution rates, water mixing, and realistic durations and severities of pH peaks. Scaling up the experimental setup to mesocosms would allow for repeated additions and longer observation periods, enabling a more-accurate representation of real-world conditions.

Data availability. All the data are reported in the Supplement, Tables S2 and S5.

Supplement. The supplement related to this article is available online at: <https://doi.org/10.5194/bg-21-5131-2024-supplement>.

Author contributions. Conceptualization was performed by CS, DB and AA. CS designed and supervised the experiments, and SV, SRB, GB, GC and MG carried them out. Funding acquisition was done by SC and AA. CS prepared the paper with contributions from all co-authors.

Competing interests. The contact author has declared that none of the authors has any competing interests.

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Supplement of

Ocean liming effects on dissolved organic matter dynamics

Chiara Santinelli et al.

Correspondence to: Chiara Santinelli (chiara.santinelli@ibf.cnr.it)

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Standard Hydrated lime range	Hydrated lime properties
Chemical purity (CaO) in %	-
Equivalent Ca(OH) ₂ %	91-95
Particle size distribution and average diameter d50 (μm)	10-16
Specific surface area (m ² /g)	15-23
Bulk density (Kg/m ³)	600

Table S1: Properties of the high-purity calcium hydroxide used for the experiment. The product was provided by Unicalce (<https://www.unicalce.it/>)

Experiment	Time (hh:mn)	pH	DOC (μM)	a_{254} (m^{-1})	a_{280} (m^{-1})	a_{320} (m^{-1})	$S_{275-295}$ (10^{-3}nm^{-1})	Sr
MED SEA	00:00	8.21 ± 0.02	65.3 ± 0.5	1.90 ± 0.02	1.21 ± 0.01	0.46 ± 0.01	24.7 ± 0.4	1.58 ± 0.04
	00:05	9.01 ± 0.02	65.7 ± 0.1	1.90 ± 0.02	1.21 ± 0.02	0.46 ± 0.02	25.0 ± 0.6	1.56 ± 0.02
	00:30	9.03 ± 0.03	67.2 ± 0.9	1.91 ± 0.01	1.20 ± 0.01	0.45 ± 0.01	25.0 ± 0.7	1.63 ± 0.07
	03:00	9.05 ± 0.01	61.2 ± 0.5	1.84 ± 0.01	1.17 ± 0.01	0.44 ± 0.01	24.8 ± 0.2	1.58 ± 0.04
	22:00	8.76 ± 0.07	59.5 ± 0.1	1.70 ± 0.01	1.09 ± 0.01	0.40 ± 0.01	25.6 ± 0.5	1.54 ± 0.05
	(mix)		(62.1 ± 0.4)					
MED SEA	00:00	8.23 ± 0.01	68.9 ± 2.7	1.89 ± 0.00	1.21 ± 0.01	0.45 ± 0.00	23.9 ± 0.5	1.62 ± 0.02
	00:05	10.26 ± 0.01	68.9 ± 1.6	1.86 ± 0.02	1.19 ± 0.01	0.43 ± 0.01	24.4 ± 0.2	1.54 ± 0.02
	00:30	10.19 ± 0.04	68.6 ± 0.8	1.85 ± 0.02	1.20 ± 0.03	0.44 ± 0.01	24.6 ± 0.1	1.48 ± 0.00
	03:00	9.97 ± 0.03	64.7 ± 0.1	1.73 ± 0.05	1.10 ± 0.04	0.39 ± 0.02	25.4 ± 0.2	1.62 ± 0.04
	22:00	9.81 ± 0.01	56.3 ± 1.7	1.53 ± 0.04	0.97 ± 0.03	0.34 ± 0.04	26.4 ± 0.2	1.36 ± 0.02
	(mix)		(67.8 ± 0.4)					
MED SEA	00:00	8.22 ± 0.04	65.8 ± 0.5	1.90 ± 0.03	1.22 ± 0.02	0.47 ± 0.02	24.4 ± 0.2	1.68 ± 0.04
	00:05	8.22 ± 0.04	65.8 ± 0.5	1.90 ± 0.03	1.22 ± 0.02	0.47 ± 0.02	24.4 ± 0.2	1.68 ± 0.04
	00:30	8.21 ± 0.04	66.5 ± 0.4	1.90 ± 0.01	1.22 ± 0.00	0.46 ± 0.02	24.5 ± 0.2	1.62 ± 0.04
	03:00	8.20 ± 0.04	66.1 ± 0.6	1.90 ± 0.01	1.12 ± 0.02	0.47 ± 0.01	24.1 ± 0.3	1.65 ± 0.01
	22:00	8.13 ± 0.01	65.6 ± 0.8	1.90 ± 0.02	1.24 ± 0.01	0.46 ± 0.01	24.2 ± 0.4	1.59 ± 0.14
	(mix)							
BALTIC SEA	00:00	8.07 ± 0.02	363.8 ± 9.5	24.49 ± 0.02	16.26 ± 0.01	6.06 ± 0.01	21.5 ± 0.0	1.08 ± 0.00
	00:05	9.03 ± 0.01	364.5 ± 2.5	24.44 ± 0.03	16.23 ± 0.03	6.06 ± 0.02	21.5 ± 0.1	1.09 ± 0.00
	00:30	9.02 ± 0.00	360.6 ± 3.8	24.49 ± 0.01	16.23 ± 0.04	6.04 ± 0.05	21.5 ± 0.1	1.08 ± 0.00
	03:00	9.03 ± 0.03	365.0 ± 4.6	24.43 ± 0.02	16.22 ± 0.01	6.08 ± 0.01	21.4 ± 0.1	1.08 ± 0.01
	22:00	8.97 ± 0.01	369.7 ± 0.4	24.46 ± 0.02	16.28 ± 0.01	6.15 ± 0.01	21.3 ± 0.0	1.07 ± 0.00
	(mix)		(372.7 ± 2.4)					
BALTIC SEA	00:00	8.08 ± 0.01	357.6 ± 2.9	24.41 ± 0.04	16.17 ± 0.03	6.03 ± 0.01	21.5 ± 0.0	1.09 ± 0.00
	00:05	10.09 ± 0.04	363.5 ± 0.2	24.13 ± 0.03	15.96 ± 0.02	5.93 ± 0.02	21.6 ± 0.1	1.10 ± 0.01
	00:30	10.13 ± 0.04	366.0 ± 2.1	23.91 ± 0.09	15.82 ± 0.07	5.90 ± 0.02	21.6 ± 0.1	1.10 ± 0.00
	03:00	10.07 ± 0.03	364.1 ± 1.4	22.97 ± 0.25	15.22 ± 0.26	5.60 ± 0.08	21.9 ± 0.2	1.09 ± 0.02
	22:00	9.83 ± 0.03	334.8 ± 0.7	20.84 ± 0.12	13.71 ± 0.09	4.85 ± 0.06	22.7 ± 0.2	1.08 ± 0.01
	(mix)		(361.3 ± 0.4)					
BALTIC SEA	00:00	8.06 ± 0.01	363.4 ± 3.2	24.45 ± 0.09	16.22 ± 0.08	6.05 ± 0.01	21.5 ± 0.1	1.08 ± 0.01
	00:05	8.06 ± 0.01	363.4 ± 3.3	24.45 ± 0.10	16.22 ± 0.08	6.05 ± 0.01	21.5 ± 0.1	1.08 ± 0.01
	00:30	8.06 ± 0.06	366.3 ± 4.0	24.43 ± 0.08	16.19 ± 0.07	6.04 ± 0.02	21.5 ± 0.2	1.09 ± 0.01
	03:00	8.09 ± 0.05	364.9 ± 6.0	24.44 ± 0.10	16.21 ± 0.10	6.07 ± 0.03	21.5 ± 0.1	1.09 ± 0.02
	22:00	8.14 ± 0.09	359.8 ± 1.2	24.54 ± 0.09	16.29 ± 0.09	6.12 ± 0.04	21.4 ± 0.1	1.07 ± 0.01
	(mix)							

Table S2: pH, DOC and CDOM data (average $\pm$ standard deviation) in the Mediterranean and Baltic Sea treatments and control.

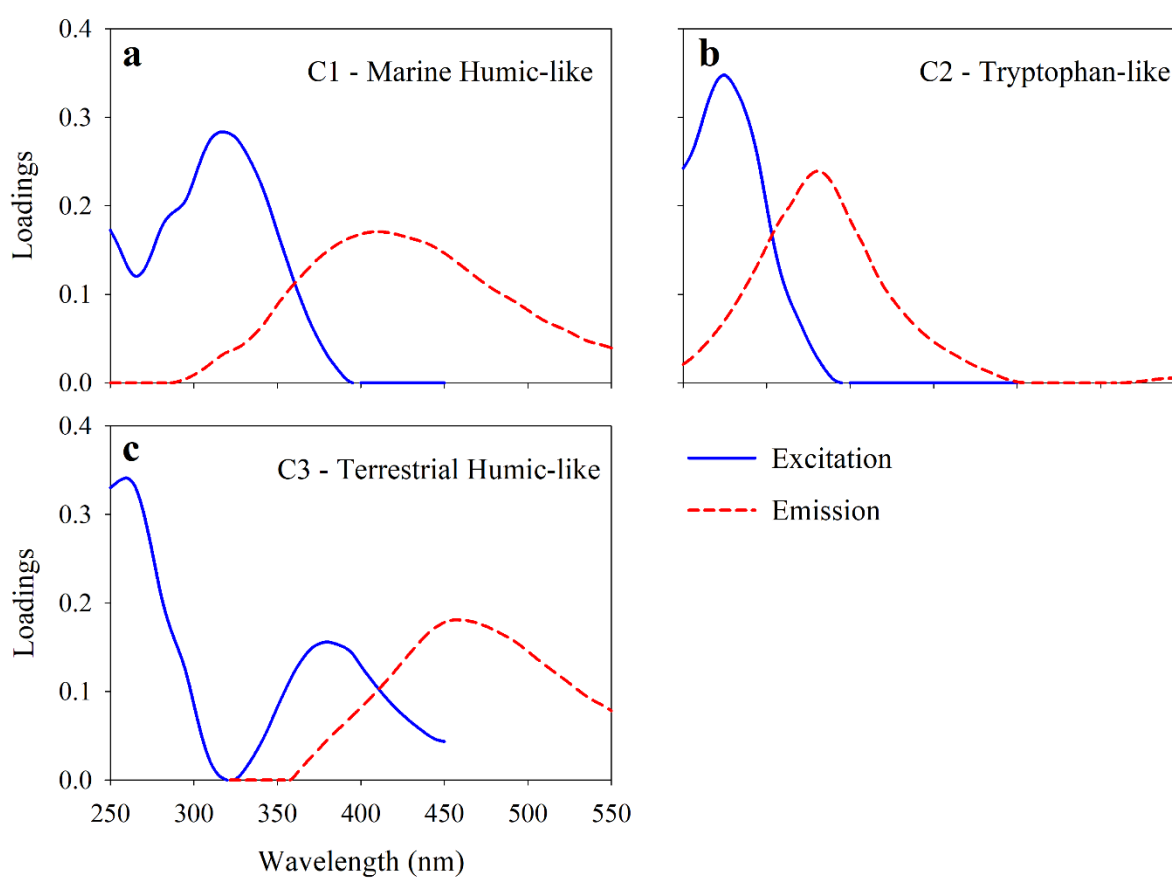


Figure S1 –Excitation and Emission spectra of the 3 components validated by the PARAFAC analysis applied to the 45 EEMs from the experiment with Mediterranean Sea water.

Components	λ_{ex} peak (nm)	λ_{em} peak (nm)	Identification	Similar components
C1_{Mh-Med}	315	409	Marine humic-like	Pitta and Zeri (2021) Chen et al. (2016) Gao and Guéguen (2018) Gao and Guéguen (2017) Kida et al. (2019) Dainard and Guéguen (2013) Lei et al. (2021)
C2_{Trp-Med}	275	331	Tryptophan-like	Cawley et al. (2012a) Wünsch and Murphy (2021) Yamashita et al. (2021) Cawley et al. (2012b) Shutova et al. (2014)
C3_{Th-Med}	260	[380] 456	Terrestrial humic-like	Pitta and Zeri (2021) Gonçalves-Araujo et al. (2015) Dainard and Guéguen (2013)

Table S3. Characterization of the PARAFAC components validated for the Mediterranean Sea. The comparison with the components in the literature was carried out by using the Openfluor database.

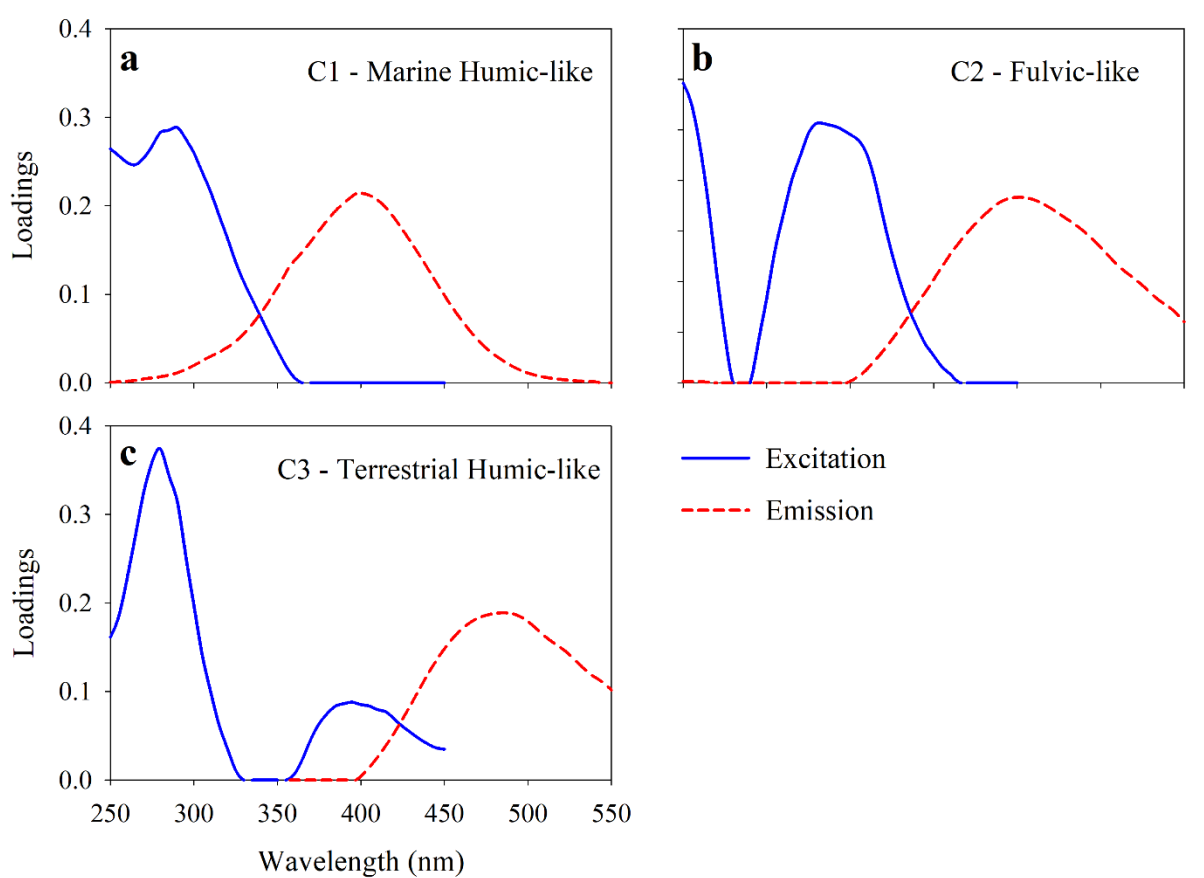


Figure S2. Excitation and Emission spectra of the 3 components validated by the PARAFAC analysis applied to the 45 EEMs from the experiment with Baltic Sea water.

Components	λ_{ex} peak (nm)	λ_{em} peak (nm)	Identification	Similar components
C1_{Mh-Bal}	290	400	Marine humic-like	Wünsch et al. (2015) Wauthy et al. (2018) Retelletti Brogi et al. (2022) Yu et al. (2015) Guéguen et al. (2015) Lapierre and Del Giorgio (2014)
C2_{Flv-Bal}	330	452	Fulvic-like	Kothawala et al. (2014) Andersson et al. (2018)
C3_{Th-Bal}	280	485	Terrestrial humic-like	Andersson et al. (2018)

Table S4. Characterization of the PARAFAC components validated for the Baltic Sea. The comparison with the components in the literature was carried out by using the Openfluor database.

Experiment	Time (hh:mn)	C1 _{Mh-Med} (R.U.)	C2 _{Trp-Med} (R.U.)	C3 _{Th-Med} (R.U.)
MEDITERRANEAN SEA	00:00	0.0207 ± 0.001	0.0289 ± 0.001	0.0220 ± 0.001
	00:05	0.0208 ± 0.001	0.0292 ± 0.001	0.0220 ± 0.001
	00:30	0.0204 ± 0.000	0.0284 ± 0.001	0.0218 ± 0.001
	03:00	0.0207 ± 0.000	0.0301 ± 0.003	0.0227 ± 0.000
	22:00 (mix)	0.0213 ± 0.000	0.0302 ± 0.005	0.0210 ± 0.001
MEDITERRANEAN SEA	00:00	0.0210 ± 0.000	0.0282 ± 0.000	0.0220 ± 0.000
	00:05	0.0203 ± 0.001	0.0323 ± 0.003	0.0204 ± 0.001
	00:30	0.0201 ± 0.000	0.0338 ± 0.002	0.0202 ± 0.001
	03:00	0.0199 ± 0.001	0.0312 ± 0.002	0.0198 ± 0.001
	22:00 (mix)	0.0168 ± 0.001	0.0306 ± 0.001	0.0162 ± 0.001
MEDITERRANEAN SEA	00:00	0.0208 ± 0.001	0.0284 ± 0.001	0.0220 ± 0.001
	00:05	0.0207 ± 0.001	0.0286 ± 0.002	0.0220 ± 0.001
	00:30	0.0198 ± 0.001	0.0333 ± 0.001	0.0208 ± 0.000
	03:00	0.0195 ± 0.002	0.0283 ± 0.001	0.0202 ± 0.002
	22:00	0.0197 ± 0.001	0.0284 ± 0.002	0.0204 ± 0.001
Experiment	Time (hh:mn)	C1 _{Mh-Bal} (R.U.)	C2 _{Flv-Bal} (R.U.)	C3 _{Th-Bal} (R.U.)
BALTIC SEA	00:00	0.4125 ± 0.006	0.3250 ± 0.002	0.3735 ± 0.004
	00:05	0.4140 ± 0.004	0.3270 ± 0.002	0.3750 ± 0.002
	00:30	0.4168 ± 0.007	0.3225 ± 0.003	0.3731 ± 0.001
	03:00	0.4088 ± 0.001	0.3245 ± 0.001	0.3717 ± 0.002
	22:00 (mix)	0.4078 ± 0.004	0.3257 ± 0.003	0.3704 ± 0.004
BALTIC SEA	00:00	0.4002 ± 0.002	0.3163 ± 0.001	0.3629 ± 0.001
	00:05	0.3993 ± 0.001	0.3148 ± 0.002	0.3596 ± 0.001
	00:30	0.4036 ± 0.001	0.3179 ± 0.001	0.3612 ± 0.001
	03:00	0.3997 ± 0.001	0.3086 ± 0.004	0.3482 ± 0.002
	22:00 (mix)	0.3838 ± 0.03	0.2843 ± 0.003	0.3154 ± 0.004
BALTIC SEA	00:00	0.4063 ± 0.009	0.3205 ± 0.006	0.3680 ± 0.007
	00:05	0.4052 ± 0.009	0.3192 ± 0.006	0.3672 ± 0.007
	00:30	0.4029 ± 0.008	0.3199 ± 0.006	0.3676 ± 0.008
	03:00	0.4074 ± 0.012	0.3197 ± 0.004	0.3679 ± 0.007
	22:00	0.3960 ± 0.008	0.3176 ± 0.006	0.3617 ± 0.008

Table S5: FDOM components (average ± standard deviation) in the Mediterranean and Baltic Sea treatments and control

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