The Impact of NaOH, CaO, and $Ca^{2+} + HCO_3^-$ ~~$HCO_3^- + Ca^{+2}$~~ Addition on PIC and POC Formation in Los Angeles Harbor Waters

Rucha P. Wani[1], Devan L. Roper[1], Ria Agrawal[2], Esther J. Lim[1], Nick E. Rollins[1], and William M. Berelson[1]

[1]Department of Earth Sciences, University of Southern California, Los Angeles, 90089, United States

[2]Rosenstiel School of Marine, Atmospheric & Earth Science, University of Miami, Miami, 33149, United States

Correspondence: Rucha P. Wani (rwani@usc.edu)

Abstract

Negative CO_2 emission technologies such as ocean alkalinity enhancement ~~(OAE)~~, in tandem with emissions reduction are necessary to keep the climate system below a critical tipping point. While the biogeochemical consequences of alkalinity enhancement ~~OAE~~ remain poorly constrained, field deployment is accelerating in the commercial sector, leaving questions of ecosystem impact in the wake. In this study we conduct alkalinity perturbation experiments to capture the resultant impact to the organic carbon and calcium carbonate pools. We quantify shifts in dissolved/particulate inorganic ~~(DIC, PIC)~~ and organic ~~(DOC, POC)~~ carbon after the addition of three alkalinity sources – NaOH, CaO, and $CaCl_2$ + $NaHCO_3$ ~~+ $CaCl_2$~~ (to simulate ~~dissolved~~ accelerated weathering of limestone). These experiments are conducted with coastal sea water with enhancements of +500 and +~~-~~1000 µmol kg^{-1} alkalinity, and incubated in situ, to elucidate the~~ir~~ impact over ~~the short term,~~ 0-4 days. ^{13}C isotope spikes were added to trace carbon~~C~~ partitioning throughout the experiment~~.~~ ~~Select experiments~~ and a few bottle experiments ~~are also~~were completely isolated from the light via bottle shading to assess impacts on a majority heterotrophic ecosystem. Despite a wide range in the initial ambient particulate carbon (both organic and inorganic) ~~With most treatments, there is~~there was no statistically significant ~~$CaCO_3$~~ calcium carbonate precipitation or change in the organic carbon partitioning after a perturbation of +500 µmol kg^{-1} alkalinity in the form of NaOH, CaO or $CaCl_2$ + $NaHCO_3$. An increase of alkalinity by 1000 µmol kg^{-1} after CaO addition resulted in the statistically significant decrease of particulate organic carbon and dissolved oxygen relative to the control. To understand how a decrease in the particulate organic carbon may impact downstream trophic

Formatted: Indent: First line: 0.5"

transfers, future studies should resolve the observed changes in particulate organic carbon by characterizing biomass within various particle class sizes with further filtration, microscopy, flow cytometry, and 'omics analyses. ~~after OAE nor changes in organic matter production or consumption, relative to the untreated controls. One exception is when CaO addition enriches the water by 1000 µmol kg^{-1}, here we find a significant decline in POC production. ^{13}C isotope spikes were added to trace C partitioning throughout the experiment. The seawater used in these experiments had a wide range of initial ambient PIC and POC, and within this wide range, a +500 µmol kg^{-1} alkalinity in the form of NaOH, CaO or $NaHCO_3$ + $CaCl_2$ addition did not change the production or consumption of carbon in these waters.~~

1. Introduction

To mitigate the impact of excess atmospheric carbon dioxide (CO_2), humans need to reduce CO_2 emissions and remove CO_2 from the atmosphere. According to the Intergovermental Panel on Climate Change~~IPCC~~, in the 8 years since the Paris Agreement, global efforts to reduce greenhouse gas emissions are still not on track to avoid surpassing the 1.5 °C threshold of temperature increase by the end of the century (UNFCC, 2022). Since reducing CO_2 emissions may not be sufficient to meet the 2015 Paris Agreement Goals (National Academies of Sciences, 2022), strategies for marine Carbon Dioxide Removal (mCDR) are being investigated to aid in the uptake and storage of CO_2. The Earth has balanced its own carbon storage and acidity on geological timescales (i.e., 100-1000 ka) through mineral weathering and mineral precipitation (Berner et al., 1983; Berner and Berner, 1997; Holland, 1978; Penman et al., 2020; Walker et al., 1981). While there may be more than enough calcium carbonate ($CaCO_3$) in ocean sediments to neutralize all anthropogenic CO_2 (Archer, 1996; Sulpis et al., 2018), this recovery could take >10,000 years (Zachos et al., 2005) given slow dissolution kinetics and ocean mixing rates. Ocean Alkalinity Enhancement (OAE) is a mCDR method that can potentially speed up the process of oceanic CO_2 uptake for human relevant timescales. A thorough assessment of the scientific background and considerations for various forms of OAE has been presented in Renforth & Henderson (2017). The proposed risks and co-benefits of increasing surface ocean alkalinity based on different deployment strategies have been theorized in Bach et al. (2019).

Formatted: Indent: First line: 0"

There are still many technical challenges to overcome before ~~Ocean Alkalinity Enhancement~~OAE can be scaled to remove gigatons of CO_2 (Eisaman et al., 2023; Renforth, 2025). The impacts of different alkaline feedstocks and concentrations on ocean chemistry and the ecosystem are yet to be completely understood. ~~We need to understand what will happen to ocean chemistry and ecosystems if certain types and amounts of alkalinity enter the ocean purposefully.~~ The successful long-term sequestration of CO_2 relies on many factors – the rate of air-sea gas exchange, the loss of alkalinity to re-precipitation of carbonate minerals or even the loss of an unequilibrated plume as it subducts below the surface (Lunstrum et al., 2025). Increasing alkalinity could change the cycling of inorganic carbon in the ocean by increasing the likelihood of ~~calcium carbonate~~$CaCO_3$ precipitation and thereby affecting the associated organic carbon cycling pumps (Falkowski et al., 2000; Ridgwell and Zeebe, 2005; Riebesell et al., 2000; Zondervan et al., 2001). Limiting secondary precipitation of $CaCO_3$ is ~~-~~important for OAE because there is ~~re~~release of CO_2 from the precipitation of $CaCO_3$ (Eq. 1) – which reduces the efficiency of OAE.

Formatted: Subscript

$$Ca^{+2}{}^{2+} + 2 + \boldsymbol{HCO_3^-} \leftrightarrow \boldsymbol{CO_2} + H_2O + \boldsymbol{CaCO_3} \quad (1)$$

Another potential outcome of OAE is for organic matter to be ballasted to the deep ocean by aggregating on newly formed particulate inorganic carbon (PIC) (Riebesell et al., 2009). The natural global production of particulate organic carbon (POC) is known to be 4-10 times higher than PIC production (Barker et al., 2006); although this ratio is highly variable in time and space (Berelson et al., 2007; Dunne et al., 2007). Renforth & Henderson, (2017) hypothesized that if the POC production remains higher than PIC production even under OAE, then the CO_2 emissions from $CaCO_3$ production by marine calcifiers could be offset. This highlights the need for us to assess whether~~n~~ enhanced calcification after OAE will contribute to CO_2 production or if it might further sequester organic carbon by enhancing POC production or by aggregating and ballasting POC via enhanced PIC (Bach et al., 2019).

Formatted: Indent: First line: 0"

Given the variety of compounds available to increase alkalinity (Renforth and Henderson, 2017), their composition and deployment style will determine the magnitude of change in Omega (Ω = the saturation state) and likelihood of $CaCO_3$ precipitation. This applies to both the near

field impacts at the point of injection and the far field impacts after the alkalinity has been dispersed. Hereafter, we shall reference Ω in terms of $\Omega_{aragonite}$ as this is the morphotype that precipitates more readily in present-day seawater (Morse et al., 2007). Many studies have observed increased carbonate mineral precipitation, as demonstrated by the loss of alkalinity (TA) and dissolved inorganic carbon (DIC) after the addition of sodium hydroxide (NaOH), quicklime/slaked lime (CaO/ $Ca(OH)_2$), and even sodium carbonate (Na_2CO_3). Precipitation was observed in both short term ~~Thus far, some studies have characterized instances of short term~~ (<5 days) (Hartmann et al., 2023; Hashim et al., 2025; Moras et al., 2022; Subhas et al., 2022), and longer term (~20 days) perturbation experiments at a range Ωs (Moras et al., 2022, 2024; Paul et al., 2024; Ringham et al., 2024; Suitner et al., 2024) ~~increased carbonate mineral precipitation by showing the loss of alkalinity and DIC following OAE~~. ~~These examples come from natural waters around Spain, North Pacific, North Atlantic gyres, and Australian coastal waters.~~ Yet, ~~In Norway, in water between 10–15°C one maximum threshold for NaOH addition before the onset of secondary precipitation has been reported at ~ 1500 µmol kg^{-1} ($\Omega_{aragonite}$ ~ 22) after which dilution of the water mass must occur within 15 days. In the same study, $NaHCO_3$/Na_2CO_3 addition was stable up to $\Omega_{aragonite}$ ~ 20, with no observed precipitation without dilution for 20 days . Yet, in the Sargasso Sea, in water around 27°C secondary precipitation was observed even at an addition of 500 µmol kg^{-1} NaOH ($\Omega_{aragonite}$ ~ 11) within 5 days . With grain feedstock added to Australian waters at 21°C, secondary precipitation was not observed for up to 25 days only after increasing TA by 250 µmol kg^{-1} ($\Omega_{aragonite}$ ~ 5) with $Ca(OH)_2$.~~ OAE experiments conducted with cultures of coccolithophores ~~report negligible~~have noted no change~~s~~ to calcification rates after additions of bicarbonate and calcium ($Ca^{2+} + HCO_3^-$) ~~raise $\Omega_{aragonite}$ to 15~~ (Gately et al., 2023). The experimental conditions of observed $CaCO_3$ precipitation will be discussed to provide further context to the results presented in this study.~~These prior experiments leave ambiguity in extrapolating the precipitation thresholds of various feedstocks to waters in other regions.~~

Formatted: Subscript

Formatted: Indent: First line: 0"

These examples of existing perturbation experiments come from natural waters around Spain, North Pacific, North Atlantic gyres, and Australian coastal waters. To this ~~steadily~~ rapidly growing body of research, we present the changes of ~~particulate~~ organic and inorganic carbon

inventory and partioning during 4-day OAE experiments conducted using coastal California waters from Los Angeles (LA) Harbor. The waters offshore from Los Angeles are richly characterized by decades of regional studies (https://dornsife.usc.edu/spot/publications-and-projects/, https://calcofi.org/publications/peer-reviewed-publications/). Yet waters from within LA Harbor contain blends of offshore ecosystems modified by nearshore processes. In our study, we use three different approaches to~~of~~ elevat~~e~~ing~~ing~~ $\Omega_{aragonite}$ by: (i) increasing pH (using two different bases) and (ii) adding Ca^{2+} and HCO_3^- to simulate accelerated weathering of limestone~~adding Ca^{+2} coupled with increasing the total dissolved inorganic carbon (DIC)~~. In constraining the resultant changes to the amount of PIC, POC and DOC production, we conduct a carbon budget analysis that helps define how alkalinity may change the natural cycling of organic and inorganic carbon.

2. Study Area & Methods

2.1 Study Area

With the exception of some short-~~-~~term experiments conducted on Santa Catalina Island at the USC Dornsife Wrigley Marine Science Center (33° 26′ 40.52″ N, 118° 28′ 47.787″ W), all other experiments were conducted with water collected from ~1 m water depth in Los Angeles Harbor, at AltaSea Marine Center (33° 43′ 33.64″ N, 118° 16′ 11.999″ W). This region exchanges seawater with the Southern California Bight, which is a part of the California Current System. The microbial community structure in the Port of Los Angeles in the summer is dominated by diatoms, pico-eukaryotes, and heterotrophic bacteria. Dinoflagellates, Synechococcus, and photosynthetic nanoplankton are in less abundance. In the fall, the carbon biomass shifts to the following in order of abundance: Bacteria > Picoeukaryotes > Synechococcus > Diatoms ~ Pico nanoplankton > Dinoflagellates; these proportionally decrease in the winter when Diatoms become the dominant species again (Bialonski et al., 2016; Caron et al., 2017; Connell et al., 2017). Regardless of the season, the carbon biomass is higher in the Port compared to the costal San Pedro Ocean Time series station (~17 km offshore) or in the marine protected area near Santa Catalina Island (~45 km offshore) (Connell et al., 2017).

Alkalinity perturbation experiments were conducted with 5-L, Thermo Scientific Nalgene PETG bottles (total volume is ~6L with no headspace) suspended 1 m below the water surface off a dock at AltaSea. Surface seawater conditions (~~salinity,~~ temperature~~,~~ and sunlight exposure)

during each experiment are recorded in Table 1. Temperature (°C) and Light Intensity (lux) were measured with HOBO™ Temperature and Light Loggers, and the lux was converted to photosynthetic photon flux density (µmol photons m^{-2} s^{-1}) by multiplying lux with the standard conversion factor of sunlight (0.0185). Salinity of the control waters from all experiments were roughly 35 ± 0.5 ppt; salinity was measured with a refractometer.~~Salinity was measured with a refractometer (units reported in ppt).~~

Table 1. Ambient Sea Surface Conditions during each Experimental Deployment.

	Start Date	Sea Surface Temperature (°C)	Light (µmol m^{-2} s^{-1})
$CaCl_2$ + $NaHCO_3$ (+500 µmol kg^{-1})	06-24-24	16.4 – 22.3	0 – 540
	07-15-24	17.4 – 24.5	0 – 605
NaOH (+500 µmol kg^{-1})	06-17-24	17.2* - 21.1*	0 – 640
	07-22-24	15.8 – 20.8	0 – 605
CaO (+500 µmol kg^{-1})	11-18-24	13.7* - 14.7*	0 – 400
	01-27-25	13.5 – 20.8	0 – 470
CaO (+1000 µmol kg^{-1})	11-04-24	14.1 – 22.01	0 – 620

*Data taken from *NOAA Tides & Currents* from Station #9410840 Santa Monica, CA. All other data measured from the AltaSea dock side water at 1 m water depth.

Formatted Table

2.2 Alkalinity Types

In this study we induce an alkalinity perturbation that simulates two different methods of OAE at two target enrichments, (1) Base Addition: where a strong base is delivered to the surface of the ocean and is intended to increase oceanic uptake of atmospheric CO_2 by disrupting the pH~~/charge~~ balance. To simulate this, we use two common bases, ~~Sodium Hydroxide (~~NaOH~~,~~ (J. T. Baker, New Jersey. ACS Grade, Lot. No.: 99057~~J. T. Baker, New Jersey~~), and ~~Lime (~~CaO~~,~~ (Sigma-Aldrich, Missouri. Reagent Grade, Batch No.: MKCS6828). The other method considered here is (2) accelerated weathering of limestone (AWL) (Dong et al., 2025; Kheshgi, 1995; Rau and Caldeira, 1999) where atmospheric CO_2 and water are pumped through a

limestone reactor to neutralize the CO_2. The outflow water is enriched in the byproducts of this reaction (Eq. 2) – Ca^{2+} and HCO_3^- ions. In this method the CO_2 is neutralized before it enters the seawater, as opposed to being neutralized in-situ during air-sea gas exchange with the base addition method. We mimic AWL with the addition of Calcium Chloride ($CaCl_2$, Sigma-Aldrich, Missouri. Assay ≥ 97%, Batch No.: SLCL2754) and Sodium Bicarbonate ($NaHCO_3$, Sigma-Aldrich, Missouri. ACS Grade, Batch No.: SLCM3421) with a molar ratio of 1:2 for Ca^{2+}:HCO_3^- (Eq. 3).

$$\boldsymbol{CO_2} + H_2O + \boldsymbol{CaCO_3} \leftrightarrow Ca^{2+}\,2 + \boldsymbol{HCO_3^-} \quad (2)$$

$$2\,Na\boldsymbol{HCO_3} + \boldsymbol{Ca}Cl_2 \leftrightarrow \boldsymbol{Ca^{2+}}\,2 + \boldsymbol{HCO_3^-} + \boldsymbol{2Na^+} + \boldsymbol{2Cl^-} \quad (3)$~~the product of limestone weathering, purposeful weathering is called accelerated weathering of limestone, AWL . When this occurs with seawater, the products are enriched in Ca^{+2} ion and HCO_3^- ions. We mimic AWL with the addition of Calcium Chloride ($CaCl_2$, Sigma-Aldrich, Missouri) and Sodium Bicarbonate ($NaHCO_3$, Sigma-Aldrich, Missouri) with a molar ratio of Ca:HCO_3^- of 1:2.~~

~~S~~To achieve~~ince we were most concerned with assessing the impact of alkalinity at~~ specific dosages/concentrations~~, we wanted~~ the alkalinity needed to be fully dissolved, thus ~~and~~ we avoided introducing any particles into the incubations. Also, aqueous alkalinity delivery is currently considered a favorable delivery method for OAE projects (Cai and Jiao, 2022; Savoie et al., 2025). To compare our results with other published regional studies (Bach et al., 2024; Ferderer et al., 2022; Kousoulas et al., 2025; Subhas et al., 2022) and evaluate impacts above current instrument detection limits (See Section 3.1.1), we chose target enrichments of ~500 and ~1000 µmol kg^{-1}. We created dissolved stock solutions of each alkalinity type (NaOH, CaO, $CaCl_2$, $NaHCO_3$) with minimal exposure to the atmosphere. A solid salt or base was precisely weighed, transferred to a pre-weighed volumetric flask, the flask was filled with Milli-Q water, and no headspace remained. After all solids were dissolved, the solution was siphoned through a 0.2 µm GF/F filter into an evacuated gas impermeable foil bag (Supelco, Pennsylvania) for storage. We took subsamples for TA and DIC (measurement details in Section 2.5)~~)~~ to calculate the final concentrations of the stock solutions and precisely dosed our incubations according to these measurements to achieve the desired alkalinity enrichments (30 mL of 0.94M NaOH (+

500 µmol kg^{-1}), 30 mL 0.05M $CaCl_2$ + 0.12M $NaHCO_3$ (+ 500 µmol kg^{-1}), 100mL of 0.016M (+ 500 µmol kg^{-1}), and 160 mL of 0.018M CaO (+1000 µmol kg^{-1})) ~~desired~~. ~~In all CaO experiments, the DIC was immediately lowered (by <2%) due to an expected dilution by the lower salinity stock solution (we add 100-160 mL of stock depending on the desired concentration).~~ Alkalinity was measured with an open-system Gran titration method at 25 °C (details in (Lunstrum and Berelson, 2022; Subhas et al., 2015)) and no precipitation was observed in the storage containers (stored at 4 °C until analysis). As the solubility of CaO in water is less than that of the other chemicals used in this study, a larger volume of a weaker CaO stock was required to make the alkalinity additions. Hence, an immediate drop in DIC (by <2%) due to the expected dilution by the stock solution is observed in all CaO experiments.

To increase the sensitivity of our measurements for the carbon budget analysis, we added 1-2mL of a ^{13}C tracer in the form of $NaH^{13}CO_3$ (Sigma Aldrich, Missouri. CAS No.: 87081-58-1, Assay = 99%, Lot No.: MBBC0208V) to all controls and treatments in five experiments: 07-15-24, 07-22-24, 11-04-24, 11-18-24, and 01-27-25. The tracer solution (~0.015M) was prepared similarly to the stock solutions discussed above. The tracer only changed the alkalinity by 2 µmol kg^{-1} but increased the $\delta^{13}DIC$ signal to 80-200 ‰ (precise start values are included in the associated dataset (Wani et al., 2026)).

Formatted: Indent: First line: 0"

2.3 Experimental Set Up

There is long standing discourse on whether bottle experiments mimic natural conditions enough to be useful to answer questions of "ecosystem impact" (Daehler and Strong, 1996; Fraser and Keddy, 1997; Giesy, 1980; Ives et al., 1996; Lawton et al., 1993; Stachowski-Haberkorn et al., 2008). There are many experimental design factors that can influence the community assemblage, such as: material used for the structure, microcosm volume, filling mechanism, mixing, replication, carbonate chemistry, exclusion of larger organisms, and wall surface growth (Riebesell et al., 2010). In perturbation experiments conducted with small volume microcosms (< 10L) it is recommended to limit the timescale of study to days-weeks if the goal is to understand the impact of the perturbation on microbial or plankton assemblages (Parsons, 1982; Riebesell et al., 2010). The purpose is to limit unwanted effects such as biofouling on the walls of the enclosure, nutrient limitation, excess grazing or changes due to lack of predators- these are commonly referred to as "bottle effects". Our aim was to compare multiple alkalinity

sources, thus we opted for a replication method that incorporated sacrificial bottle timepoints over the span of four days (Iglesias-Rodríguez et al. 2023). We maintained a closed system experiment (with no headspace) to constrain the carbon budget by eliminating gas exchange as a potential variable for this study. This can be an analog for water that may temporarily lose contact with the atmosphere, e.g. when waters mix deeper into surface ocean or for times when gas exchange is minimal. ~~Since the chapter on laboratory and mesocosm experimental design written by in State of the Planet's "Guide to Best Practices in Ocean Alkalinity Enhancement Research" details how different styles of lab experiments are very useful we follow their recommendations. We determined the best design to answer our questions for this study were sacrificial replication 4-day bottle incubation experiments. We intended to maintain a closed system experiment to constrain the carbon budget by eliminating gas exchange as a potential variable for this study. This can be an analog for water that may temporarily lose contact with the atmosphere, e.g. when waters mix deeper into surface ocean or for times when gas exchange is minimal.~~

We collected seawater for each experimental treatment by pumping from 1 m below the surface from a dock at AltaSea, in the Los Angeles Harbor. The water passed through a 250 µm mesh (to remove large grazers) (Carter et al., 2005; de la Broise and Palenik, 2007) and ~~the~~ 22 bottles were filled in a 'round robin' manner to promote homogeneity between bottles filled at different times (filling occurred in ~~~20 bottles were filled~~ < 20 mins). Once all bottles were full, with no headspace, half were randomly selected to receive a dosage of alkalinity (NaOH, CaO, or $CaCl_2$ + $NaHCO_3$). Bottles were opened, 50-150 mL of seawater was removed; stock water was pulled out of the stock solution foil bags into volumetric syringes and injected into the bottles. Any remaining headspace was once again filled with seawater; bottles were sealed and then inverted five times before being tied to the dock. All treatment bottles were given a dose of alkalinity on Day 0, some were immediately sampled, others left in the water column to experience natural changes in temperature and light condition until their sampling day. Only one treatment experiment was conducted per week; hence, we can only compare each alkalinity type to its respective control (no addition of alkalinity). Sampling occurred on days 0, 2, and 4 with three bottle replicates per time point.

We also filled and added alkalinity to 2 bottles that were incubated devoid of light as well as 2 dark controls (wrapped in black tape, See Fig. 1). Results and discussion of all dark bottle experiments appear in the Supplemental Information. ~~All treatment bottles were given a dose of alkalinity on Day 0, some were immediately sampled, others left in the water column to experience natural changes in temperature and light condition until their sampling day. While dosing the bottles, we added a ^{13}C tracer (controls and treatments) in the form of NaH^{13}CO$_3$ (Sigma Aldrich, Missouri). This spike only changed the alkalinity by 2 µmol kg^{-1} but increased the δ^{13}DIC signal to a couple hundred per mil – the purpose of this was to increase the sensitivity of our measurements for our carbon budget analysis.~~

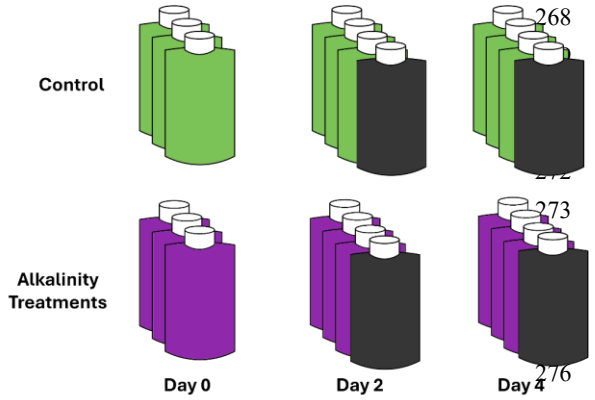


Figure 1. Experimental Set-up. Triplicate bottles shown for each timepoint. Controls are shown in green, alkalinity amended bottles shown in purple, and bottles with light restriction are shown in black.

2.4 Sample Processing

Bottles pulled out of the ocean were transported back to the lab (on ice) on their sampling day and weighed to track water that was used for sub-sampling. Bottles were gently inverted 5 times and then opened to measure temperature (°C) with a probe and dissolved oxygen (µmol kg^{-1}) with PreSens Optical Oxygen Sensor Spots that were attached to each bottle. The oxygen measurements were corrected to the mean temperature recorded by the in situ HOBO™ Loggers. To avoid sampling from the surface layer of water in contact with the air, a ~6 inch piece of Tygon® F-4040-A attached to a 50 mL syringe via a one-way valve was inserted into bottom of the sample bottle. The first 15 mL of sample water was used to rinse the syringe, filter and needle that would be used to collect carbonate chemistry samples, ensuring no bubbles entered the

sample storage containers as recommended by standard operating procedures (Dickson et al., 2007). Through subsequent sampling, 250 mL of sample water was filtered through a 25 mm 0.7 µm glass fiber filter (Whatmann GF/F) (~15 mins/bottle for filtration and subsampling). A portion of the filtrate was collected for ~~dissolved inorganic carbon~~5mL (duplicates of DIC (injected into septum-top of pre-evacuated exetainers)~~of 5 mL)~~, alkalinity (15 mL Falcon tubes with no headspace), nutrients (15 mL with no headspace), dissolved organic carbon – DOC (40 mL pre-combusted amber glass septa vials), and 50 mL for archival purposes. We note that there could be some contamination in DOC samples because we used Tygon® to pull the sample into the syringe (Dickson et al., 2007); however, this would affect the controls and treatments in the same manner. The GF/F filter was ~~was~~ wrapped in aluminum foil and placed in a freezer (-20 °C) for later Chl a analysis (< a month) (using a Turner Trilogy Fluorometer and the EPA SOP LG405.v11 for 'Chlorophyll-A Non-Acidification Method' (U.S. EPA, 2021)). One liter was filtered for future genomic analyses not discussed in this paper, on Supor 0.2 µm 47 mm PES filters, and stored at -80 °C. The remaining (4.5 L) seawater was filtered (detailed in Section 2.5.2) for particulate organic and inorganic carbon.

2.5 Measurements for Carbon Budget

A carbon budget for each bottle was created by assessing the total concentration of carbon in the dissolved and particulate pools. Carbonate system parameters were derived from ~~Total Alkalinity~~TA and DIC measurements (discussed below) using CO2Sys.v3 (Pierrot et al., 2021) (Parameters: pH Scale = Total Scale (mol per kg-SW), CO_2 Constants = (Dickson and Millero, 1987), KSO_4 = (Dickson, 1990), KF = (Perez and Fraga, 1987), Boron = (Lee et al., 2010)). $\Omega_{aragonite}$ values for treatments with added Ca^{+2} (CaO & $[Ca^{2+}]$ + $[HCO_3^-]$ Experiments~~&~~ ~~Dissolved Limestone Experiments~~) were calculated by multiplying the $[CO_3^{2-}]$ output from CO2Sys and $[Ca^{+2}]$ accounting for the increased Ca^{2+}. All calculations use K_{sp}' for Aragonite in seawater ($K_{sp}' = 10^{-6.19}$). All other $\Omega_{aragonite}$ values derived from CO2Sys scale $[Ca^{+2}]$ to salinity.

Formatted: Superscript

2.5.1 Dissolved Carbon Phases

Samples for Dissolved Inorganic Carbon (DIC) were collected in pre-evacuated 12 mL borosilicate glass Labco Exetainers® ~~Labco Exetainers~~ and immediately placed at 4 °C for

storage until analysis (< 1 week). For analysis, samples were acidified with 2 mL of 10 % H_3PO_4 (Spectrum Chemicals, Gardena, California) and the evolved CO_2 was analyzed by a Picarro G2121-i Cavity Ring Down Spectrometer (as detailed in (Subhas et al., 2015)). Optical grade calcite, with a known ^{13}C value (2.47 ‰) was used to create an eight-point standard curve (ppmCO_2 per mg of $CaCO_3$) for each run and a certified reference material (CRM-Batch 214) generated by Andrew Dickson's Lab at SIO, UCSD was used to correct all the final DIC concentrations (4 CRM replicates included per run). The total CO_2 standard curve produced with the Automate Sample Delivery System + Picarro CRDS had an $R^2 = 0.9999 \pm 0.0001$ and slope variability = 1.5 % for all runs in this dataset. Replicate CRM samples provided a measure of precision, ± 20 µmol kg^{-1}. The isotopic values of replicate standards were within ± 0.2 ‰.

Samples for Dissolved Organic Carbon (DOC) were collected in 40 mL pre-combusted amber glass vials, acidified to 0.1 % v/v with concentrated hydrochloric acid (ACS grade, Supelco, Massachusetts), and placed in cold storage (4 °C) until analysis. The samples were run on a Shimadzu TOC-L Analyzer (NPOC method; Injection volume = 150 µL; No. of Injections: Best 4 of 5; Max Variance of Replicates: 2.00 %). Within a run the standard curve had an $R^2 = 0.9998 \pm 0.0002$, between runs the reproducibility of the slope had 16.5 % error. Samples were initially purged with CO_2 -free compressed air to remove inorganic carbon, combusted in a furnace via catalytic oxidation, and the generated CO_2 was measured by an infrared gas analysis. Standards for organic carbon were made with Oxalic Acid (dried at 70 °C for 2 hr), and CRMs from the Hansell Lab (University of Miami, RSMAS) were used to correct final concentrations (DSR Batch 22 Lot # 10-22) with a replicate precision of ± 10 µmol kg^{-1}.

2.5.2 Particulate Carbon Phases

Roughly 4.5 L of seawater was filtered on 1-2 filters (0.7 µm 47mm Whatman GF/F) for particulate inorganic carbon (PIC) and particulate organic carbon (POC) analyses. After filtration, ~10 mL of buffered DI water (NaOH added to Milli-Q, pH 9) was used to rinse any residual seawater, and then filters were placed in the oven to dry overnight (60 °C). Filters were hole punched (diameter = duplicates of 6-12.5 mm); the hole punched fractions (2-8 % of total filtered area) were wrapped in tin foil capsules (9 x 5 mm) for combustion in a CosTech Elemental Analyzer – which is connected to the Picarro for CO_2 and $^{12}C/^{13}C$ analysis. This analysis of Total Carbon had a reproducibility of ± 1 %. The remaining fraction of the filters

were rolled and placed into Labco Exetainers® – these samples were acidified with 5 mL 10 % H_3PO_4 and the evolved CO_2 measured similarly to DIC described above. Particulate Organic Carbon (POC) was calculated by subtracting the PIC (µgC L^{-1}) concentration from the Total Carbon (µgC L^{-1}) calculated for the whole filtered area.

2.6 Data Visualization and Statistical Analysis

Statistical analysis and data visualization was conducted in R and R Studio version 4.5.1. Means and standard deviations for each treatment by time point were generated using the data_summary function. Triplicate values were averaged, but data points that were found outside of three standard deviations from the mean were flagged as outliers and not included in the statistical analysis (Pukelsheim, 1994). The dataset was analyzed with two statistical approaches: (i) To test whether the effect of alkalinity addition had a statistically significant impact on PIC, POC, DOC and Chl a over time, we used a linear model with an interaction term and assessed if the p-value on the interaction term (i.e., the difference between the slopes of the control and the treatment) was statistically significant (significance threshold $\alpha = 0.05$) (results summarized Table 4). (ii) Since the previous method assumes linear relationships and normally distributed data (which may apply to some but not all variables) we also used a nonparametric two way ANOVA (Analysis of Variance) – Scheirer Ray Hare test – to assess the impact of alkalinity addition on the Control and Treatment groups (significance threshold $\alpha = 0.05$) (results summarized in Table 5). The implications of these tests and any statistically significant responses are summarized in the context of ecological variability in the Results and Discussion sections.

3. Results

3.1 Simulating AWL with $[Ca^{2+}]$ and $[HCO_3^-]$ ~~$CaCl_2 + NaHCO_3$~~ Addition

3.1.1 ~~Observing~~ Short Term Impacts at Extremely Low Alkalinity Additions

~~With the intent to~~We intended to recreate an alkalinity impact condition that resembles extremely high dilution of ~~bicarbonate~~ AWL after a few hours~~,~~. ~~we conducted an experiment where the presumed~~an example outflow of alkalinity from a shipping vessel fitted with an AWL reactor would be about 3000-~~of~~ 5000 µmol kg^{-1} (Dong et al., 2025). As the ship's wake would dilute the outflow, we chose to dilute a stock (~4754 µmol kg^{-1}) by different proportion; 1:150 (40 ~~ml~~ mL of stock), 1:300 (20 mL of stock), and 1:600 (10 mL of stock)~~.~~ (Table 2) based on

dilution calculations presented by Dong et al., (2025) (inspired by (Caserini et al., 2021; Chou, 1996)). These incubations that lasted 3 hours ~~and~~ were conducted on Santa Catalina Island

As seen in Table 2, additions at what could be realistic deployment concentrations after several hours of mixing result in changes that are extremely close to replicate error and instrument measurement error. In the least diluted sample, the seawater alkalinity changes from 2223 ± 6~~2~~ µmol kg^{-1} to 2233 ± 5~~2~~ µmol kg^{-1} (the expected increase would be 2248 µmol kg^{-1}) but there is no~~t a~~ statistically significant impact on the concentrations of ~~particulate inorganic~~PIC or ~~organic carbon~~POC ($p > 0.05$). Since we saw no impact on PIC or POC at such small changes in alkalinity, and these additions were at the lower limit of our measurement capabilities, ~~(at what could be realistic deployment concentrations after several hours of mixing),~~ we focused the rest of the paper on experiments that perturb the alkalinity by larger amounts (25-50 %) and for longer durations (2-4 days). All the following experiments were conducted with water collected and incubated at AltaSea in LA Harbor.

Table 2. Summary of low concentration alkalinity amendments.

Treatment	Target ΔTA (µmol kg^{-1})	Measured ΔTA (µmol kg^{-1})	Target ΔDIC (µmol kg^{-1})	Measured ΔDIC (µmol kg^{-1})	Δ $\Omega_{aragonite}$	ΔpCO_2 (µatm)	ΔPIC (µg L^{-1})	ΔPOC (µg L^{-1})
1:600	12	9.3 ± 8	12	11.1 ± 20	-0.01 ± 0.02	9 ± 8	-0.3 ± 0.2	0.6 ± 9.7
1:300	16	11.5 ± 5	16	21.8 ± 17	-0.09 ± 0.02	38 ± 8	-0.4 ± 0	-1.5 ± 6.7
1:150	25	10.4 ± 5	25	24.9 ± 22	-0.12 ± 0.06	51 ± 21	-0.1 ± 0.3	12.2 ± 20.5

~~Treatment~~	~~Alkalinity (µmol kg^{-1})~~	~~DIC (µmol kg^{-1})~~	~~$\Omega_{aragonite}$~~	~~pCO_2 (µatm)~~	~~PIC (µg L^{-1})~~	~~POC (µg L^{-1})~~
~~Control~~	~~2223 ± 2~~	~~2000 ± 1~~	~~2.53 ± 0.02~~	~~585 ± 5~~	~~7.1 ± 0.2~~	~~68.4 ± 7.7~~
~~1:600~~	~~2232 ± 5~~	~~2010 ± 3~~	~~2.57 ± 0.08~~	~~580 ± 20~~	~~6.8 ± 0.2~~	~~69.0 ± 9.7~~
~~1:300~~	~~2234 ± 2~~	~~2025 ± 25~~	~~2.46 ± 0.2~~	~~625 ± 90~~	~~6.7 ± 0~~	~~66.9 ± 6.7~~
~~1:150~~	~~2233 ± 2~~	~~2030 ± 6~~	~~2.40 ± 0.06~~	~~640 ± 25~~	~~7.0 ± 0.3~~	~~80.6 ± 20.5~~

The control seawater had an alkalinity = 2223 ± 6 µmol kg^{-1}, DIC = 2015 ± 21 µmol kg^{-1}, $\Omega_{aragonite}$ = 2.41 ± 0.02, pCO_2 = 536 ± 4 µatm, PIC = 7.1 ± 0.2 µg L^{-1}, and POC = 68.4 ± 7.7 µg L^{-1}. The treatments are shown as dilution ratios~~ – i.e., the 1:600 treatments received 1 part of a stock alkalinity (5000 µmol kg^{-1}) per 600 parts of seawater~~. Expected TA and DIC have been calculated by only assuming [HCO_3^-] has increased. The reported error is standard deviations between triplicate bottles (n = 3).

Formatted: Font: 11 pt, Not Italic, Font color: Auto

Formatted: Font: 11 pt, Not Italic, Font color: Auto

Formatted: Font color: Auto

Formatted: Font color: Auto

Formatted: Font color: Auto

Formatted: Font color: Auto

Formatted: Font: 11 pt, Not Italic, Font color: Auto

~~3.1.2 [Ca^{+2}] and [HCO_3^-] addition: Alkalinity Enhancement ~500 µmol kg^{-1}~~

~~Table 3. We performed two calcium and bicarbonate addition experiments on 24 June 2024 and 15 July 2024. Alkalinity added in the form of HCO_3^- increases both alkalinity and DIC in a 1:1 ratio – hence, the change in pH relative to the Control is minimal for this treatment (Fig. 2). Over the 4 day incubation, this treatment resulted in no statistically significant difference in the production of PIC ($p > 0.05$, ANOVA and Linear Regression method, see Table 4 & 5), the $\Omega_{aragonite}$ only increases by 0.2 units in both experiments (Table 3). POC in the control (untreated) seawater triples by the end of the experiment (Fig. 3), but there is no statistical difference between the POC or DOC change in concentration of the control vs. the treatment condition for both experiments ($p > 0.05$, ANOVA and Linear Regression method). Chl a also shows that changes in concentration over time were similar in both the control and the dissolved limestone treatment.~~

~~Table 3.~~ **Initial Concentrations of Biogeochemical Parameters at Day 0 (Control) of 4-day Experiments**

	Start Date	PIC µgC L^{-1}	POC µgC L^{-1}	CHL µg L^{-1}	DOC µmol kg^{-1}	Control $\Omega_{aragonite}$	Treatment $\Omega_{aragonite}$
$CaCl_2$ + $NaHCO_3$ (+500 µmol kg^{-1})	06-24-24	3.90 ± 1.15	278 ± 20	2.37 ± 0.25	*NA*	2.02 ± 0.06	2.28 ± 0.05
	07-15-24	2.51 ± 0.30	209 ± 18	0.60 ± 0.21	117 ± 15	1.88 ± 0.07	2.12 ± 0.05
NaOH (+500 µmol kg^{-1})	06-17-24	0.95 ± 0.21	366 ± 33	1.82 ± 0.32	*NA*	2.28 ± 0.02	8.21 ± 0.04
	07-22-24	1.16 ± 1.02	369 ± 26	1.91 ± 0.40	96.1 ± 12.8	1.92 ± 0.04	7.63 ± 0.07
CaO (+500 µmol kg^{-1})	11-18-24	100.0 ± 49.3	171 ± 48	0.09 ± 0.02	29.2 ± 1.15	1.47 ± 0.02	7.97 ± 0.23
	01-27-25	28.4 ± 4.53	279 ± 83	0.56 ± 0.07	66.2 ± 2.16	1.23 ± 0.14	8.63 ± 0.16
CaO (+1000 µmol kg^{-1})	11-04-24	4.83 ± 0.79	218 ± 25	0.76 ± 0.07	65.2 ± 1.59	1.81 ± 0.04	13.73 ± 0.09

3.1.2 [Ca^{+2}] and [HCO_3^-] addition: Alkalinity Enhancement~500 µmol kg^{-1}

Formatted: Font: 12 pt, Italic
Formatted: Normal, Line spacing: single
Formatted: Font: Bold
Formatted: Font: Bold
Formatted: Font: Bold

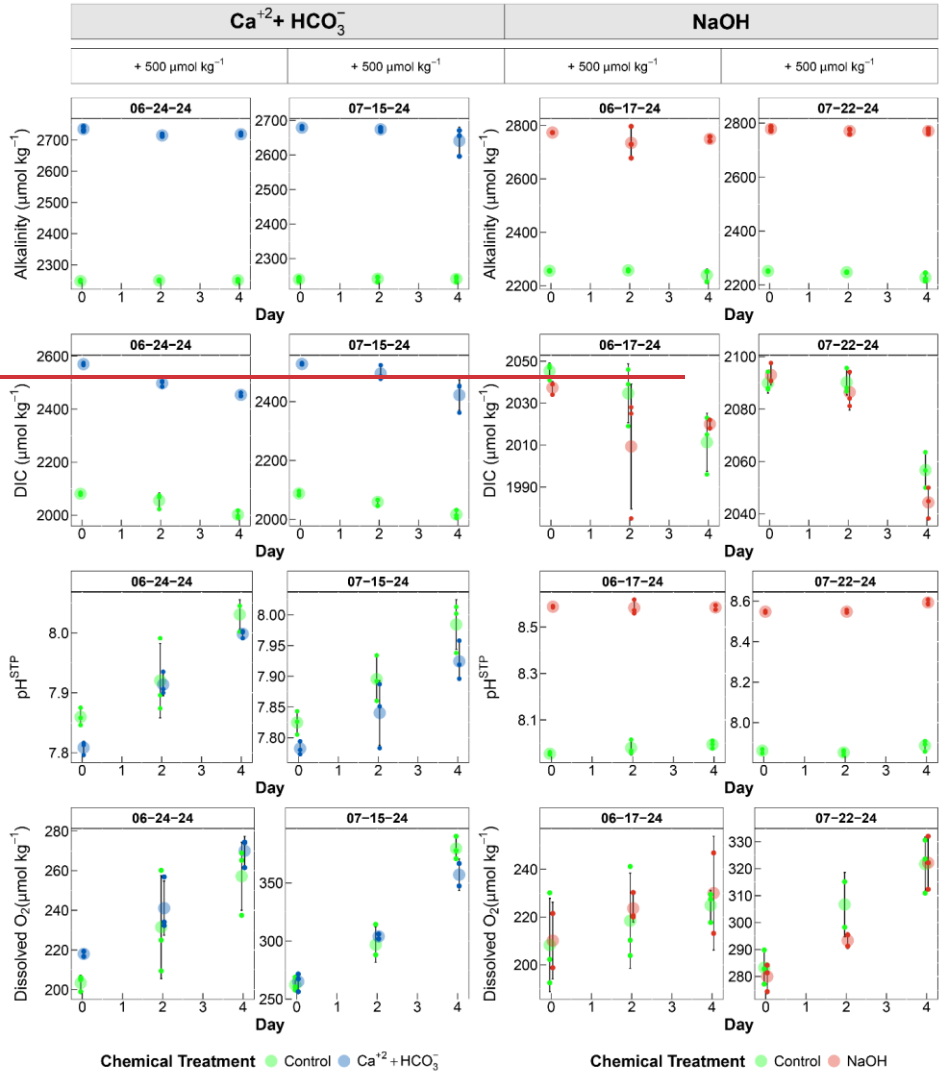
We performed two [Ca^{2+}] and [HCO_3^-] ~~calcium and bicarbonate~~ addition experiments on 24 June 2024 and 15 July 2024 which each lasted four days. Alkalinity added in the form of HCO_3^- increases both alkalinity and DIC in a 1:1 ratio – hence, the change in pH relative to the Control is minimal for this treatment (Fig. 2). Over the ~~4 day~~ incubation, this treatment resulted in no statistically significant difference in the production of PIC ($p > 0.05$, ANOVA and Linear Regression method, see Table 4 & 5), the $\Omega_{aragonite}$ only increased~~s~~ by 0.2 units in both experiments (Table 3). Chl a shows that changes in concentration over time were similar in both the control and the treatment. The [Ca^{2+}] + [HCO_3^-] addition experiments intentionally showed the largest increase within DIC pool compared to all other alkalinity enhancements. There was a concurrent increase in both organic carbon pools of POC and DOC, but it was not statistically dissimilar from the changes within the control condition ($p > 0.05$, ANOVA and Linear Regression method). The partitioning of carbon between the inorganic and organic pools is further discussed in Section 3.4.2 Carbon Partitioning.

~~POC in the control (untreated) seawater triples by the end of the experiment (Fig. 3), but there is no statistical difference between the POC or DOC change in concentration of the control vs. the treatment condition for both experiments ($p > 0.05$, ANOVA and Linear Regression method). Chl a also shows that changes in concentration over time were similar in both the control and the dissolved limestone treatment.~~

Formatted: Font: Not Bold

3.2 NaOH addition: Alkalinity Enhancement ~500 µmol kg^{-1}

~~Sodium hydroxide~~NaOH additions occurred on 17 June 2024 and 22 July 2024. The addition of NaOH increases alkalinity but not DIC which results in a large change in $\Omega_{aragonite}$ from ~2 to 8.21 ± 0.04 (06-17-24; pH increases 0.63) and 7.63 ± 0.07 (07-22-24; pH increases 0.71). When compared to the control condition, the changes in PIC, POC, DOC, and Chl a in the treatment conditions are also not statistically significant (Fig. 3) via either of two statistical analyses ($p > 0.05$, ANOVA and Linear Regression methods). As with the [Ca^{+2}] and [HCO_3^-] addition, there are certain points in time where the controls diverge from the treatments, but there are no consistent trends across multiple variables (i.e., no indication that one group (control/treatment) is consistently higher/lower through time).


Ca+2+ HCO3−
NaOH
+ 500 µmol kg−1
06–24–24
07–15–24
06–17–24
07–22–24
Alkalinity (µmol kg−1)
DIC (µmol kg−1)
pHSTP
Dissolved O2(µmol kg−1)
Day
Chemical Treatment Control Ca+2+HCO3−
Chemical Treatment Control NaOH

443

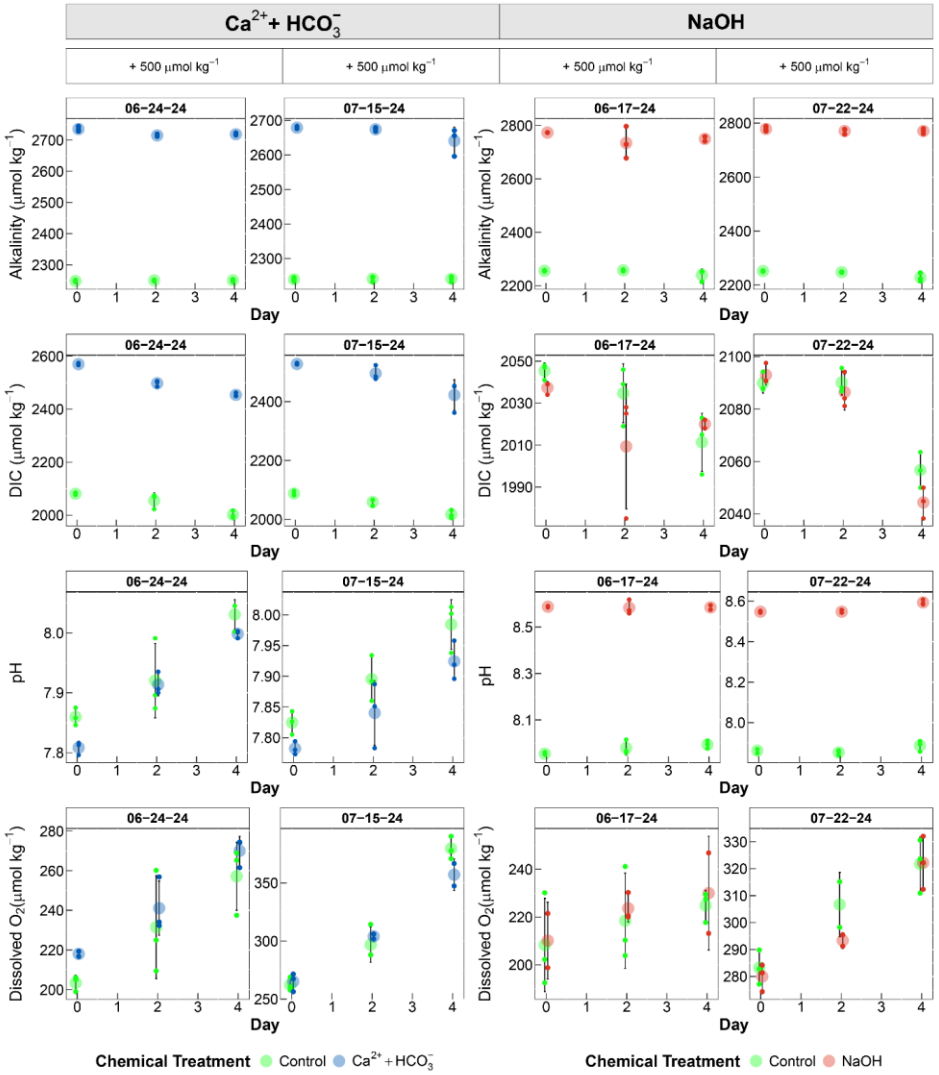
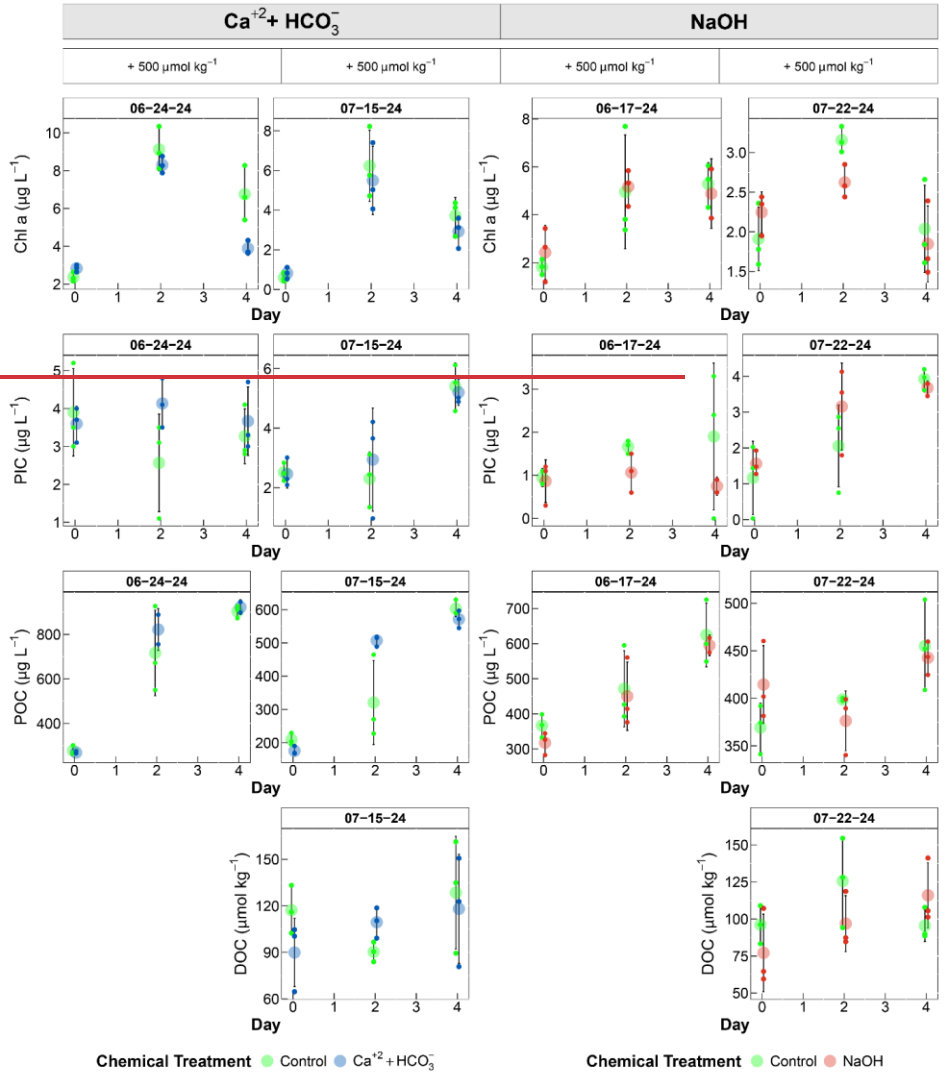


Figure 2. ~~Summary of~~ **Water Chemistry from (NaOH and** $[Ca^{2+}] + [HCO_3^-]$ **addition** ~~$NaHCO_3$ + $CaCl_2$~~**) Amendment Experiments.** Panels are grouped by the alkalinity treatment they received: Controls (green), NaOH (red) and $CaCl_2$ + $NaHCO_3$ (blue). The start date of the experiment is listed for reference to differentiate between duplicate experiments. The four variables displayed here are measured Alkalinity (µmol kg^{-1}), measured Dissolved Inorganic Carbon (DIC, ~~)~~ (µmol kg^{-1}), pH calculated from CO2Sys.v3,

and Ddissolved Ooxygen (O_2, µmol kg^{-1}). Each plot shows the average of the treatment per timepoint (n = 3), and the error bars are the standard deviation between the triplicate bottles. Smaller symbols show individual bottles.

Formatted: Subscript


Ca+2 + HCO3−
NaOH
+ 500 µmol kg−1
06–24–24
07–15–24
06–17–24
07–22–24
Chl a (µg L−1)
PIC (µg L−1)
POC (µg L−1)
DOC (µmol kg−1)
Day
Chemical Treatment
Control
Ca+2 + HCO3−
NaOH

453

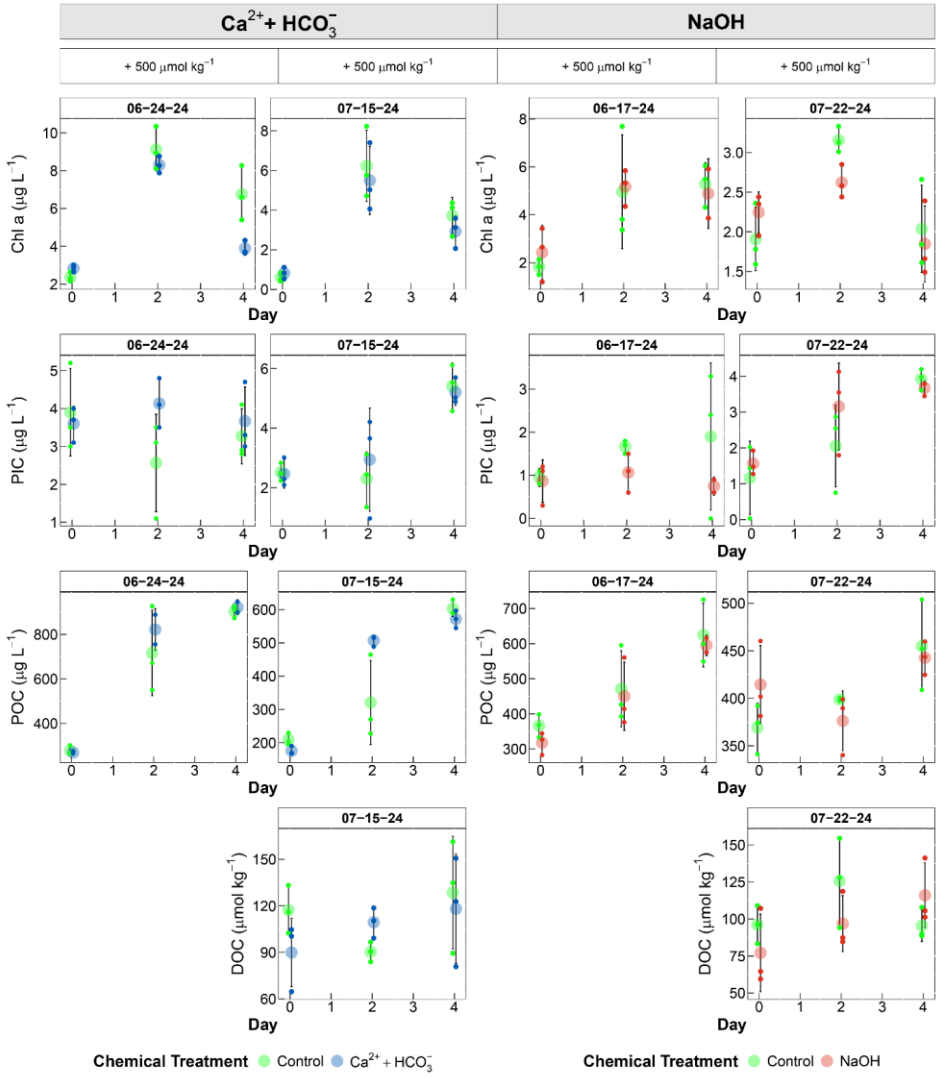


Figure 3. Four Day Timeseries of Biogeochemical Response to Different Alkalinity Enhancements. Smaller symbols show individual bottles; larger symbols show the mean. Error bars are the standard deviation between replicates (n = 3). The start date of the experiment and the amount of alkalinity added are included as headers for reference. The variables displayed are Chlorophyll a (Chl a, µg L^{-1}), ~~PIC~~

Particulate Inorganic Carbon (PIC, µg L^{-1}), ~~POC~~ Particulate Organic Carbon (POC, µg L^{-1}), and ~~DOC~~ Dissolved Organic Carbon (DOC, µmol kg^{-1}).

3.3 CaO Additions

3.3.1 CaO addition: Alkalinity Enhancement ~1000 µmol kg^{-1} (4 November 2024)

We raised the $\Omega_{aragonite}$ value, with ~~The~~ CaO addition, ~~experiment raised the Omega value~~ above the threshold for spontaneous precipitation of ~~calcium carbonate~~$CaCO_3$ in seawater ($\Omega_{aragonite} > 12$, $\Omega_{calcite} > 19$ (Marion et al., 2009; Morse and He, 1993)) according to the references cited. Increasing TA by 1000 µmol kg^{-1} ~~umol/kg~~ raised the $\Omega_{aragonite}$ from 1.81 ± 0.04 to 13.73 ± 0.09 and pH was raised from 7.85 ± 0.01 to 8.91 ± 0.01 (error is standard deviation between replicate bottles). Yet in these incubations, we see no statistically significant increase in PIC either immediately after CaO addition or throughout the 4-day experiment ($p > 0.05$, nonparametric ANOVA and linear analysis, Table 4 & 5). We also see no statistically significant divergence between the Control bottles and CaO bottles in DOC or Chl a. ~~(nonparametric ANOVA and linear analysis, $p > 0.05$).~~ However, the O_2 and POC trends are statistically different than the Control after CaO addition ($p < 0.05$ with linear analysis, Table 4). This is the only experiment where we see a statistically significant impact of CaO addition with a 95% confidence interval. There is production of O_2 ~~=is~~ 70 ± 20 µmol kg^{-1} over 4 days in the Control, compared to no change in measured O_2 in the CaO treatment. In the Control, the POC production increases by 180 ± 35 µgC L^{-1} (~ 15 ± 3 µmol kg^{-1}) compared to only a 70 ± 40 µgC L^{-1} (~ 5.8 ± 3.6 µmol kg^{-1}) increase in the treated waters. Further implications of these parameters are discussed in Section 4.2.2.

3.3.2 CaO addition: Alkalinity Enhancement ~500 µmol kg^{-1} with Higher Ambient PIC (18 November 2024)

~~Twice we added CaO to~~We conducted two CaO addition experiments to enhance alkalinity by 500 µmol kg^{-1}, similar to the amount of enhanced alkalinity in the [Ca^{+2}] + [HCO_3^-] and NaOH treatments. In the 18 November 2024 experiment, ~~ambient~~ PIC concentrations in the harbor water were ~20 times higher than the ambient PIC (5±5 µgC L^{-1}) of the experiments described above (Fig. 3 & Fig. 5). We used this opportunity as an analog to study an alkalinity addition with greater suspended solids – i.e., greater nucleation sites for secondary precipitation

Formatted: Not Superscript/ Subscript

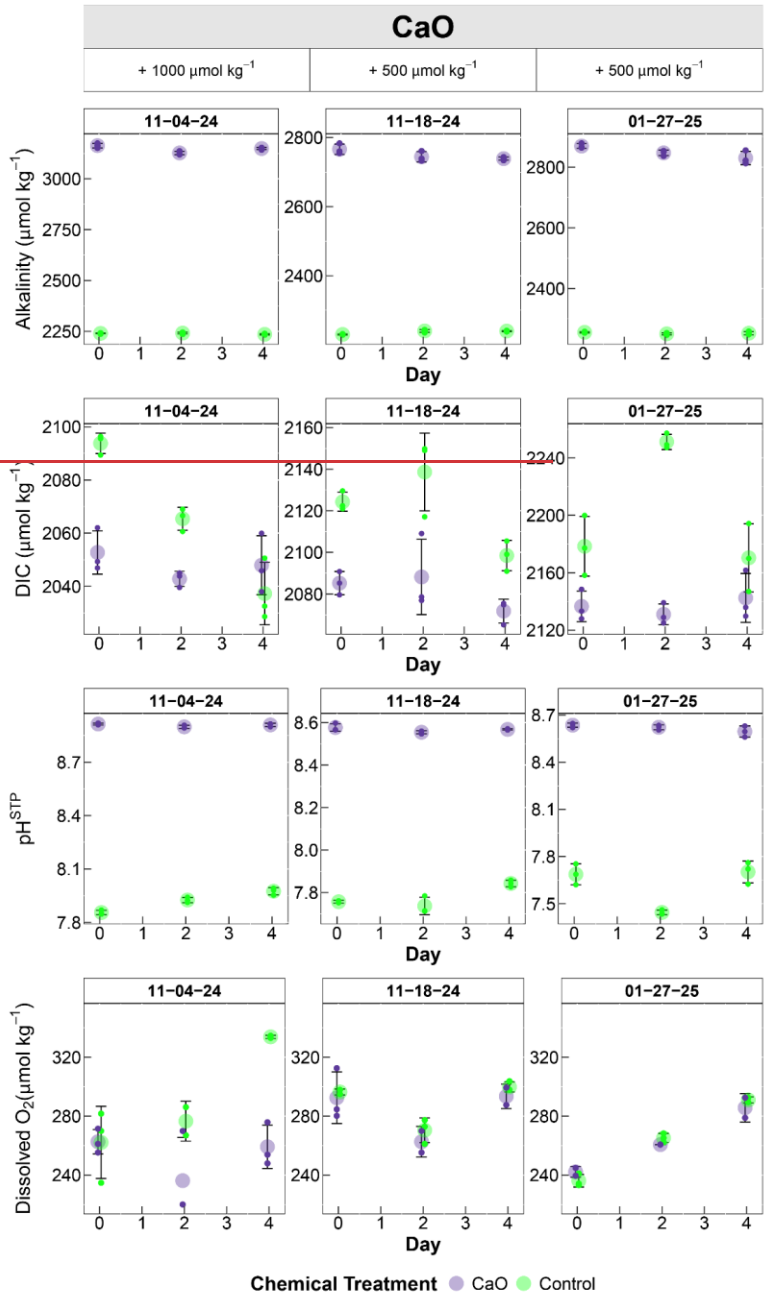
Formatted: Subscript

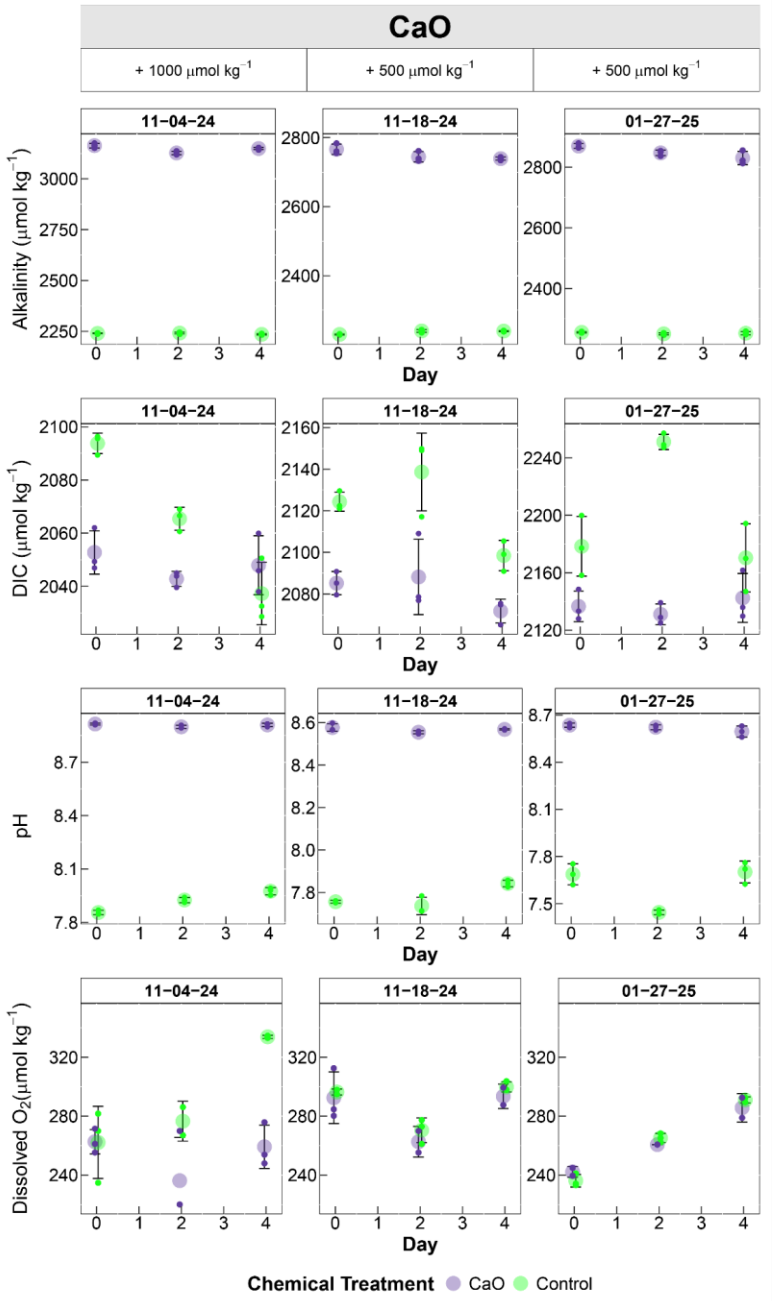
Formatted: Font: Not Bold

~~– in a natural seawater environment~~. The CaO addition raised pH from 7.75 ± 0.01 to 8.57 ± 0.01, and $\Omega_{aragonite}$ from 1.47 ± 0.02 to 7.97 ± 0.23. We saw no increase in PIC formation in the treatment compared to the control. Instead, we observed a loss of PIC occurring over time in both the control and treated bottles. Chl a, DOC, and dissolved O_2 show no statistically significant changes over time from the alkalinity addition; POC in the Control samples is higher than the treatment (Fig. 5), however, Day 0 variability negates any statistically significant impact.

3.3.3 CaO Addition: Alkalinity Enhancement ~500 µmol kg^{-1} (27 January 2025)

We replicated the previous experiment with the goal to capture a lower ambient PIC signal, but in this experiment the average starting PIC was 6-10 times the normal background (30-50 µgC L^{-1} compared to ~5 µgC L^{-1}). Upon CaO addition the system experienced a pH increase of 0.95 units (pH_f = 8.63 ± 0.01), and $\Omega_{aragonite}$ increased from 1.23 ± 0.14 to 8.63 ± 0.16. While there were samples in which the values of PIC concentration in the treatment samples averaged higher than the Controls, the overall 4-day trends are not statistically significant ~~(*p* > *0.05*, ANOVA and Linear Regression methods)~~. The Control averages 1 µg L^{-1} Chl a higher than the treatment and almost 100 µgC L^{-1} POC higher than the treatment by Day 4. Yet, due to high variability between bottles, the differences in POC and DOC between the controls and treatment are not statistically different when tested with nonparametric ANOVA or linear analysis. Of note, the differences in Chl a overtime are statistically significant when analyzed with a linear regression analysis and a more lenient threshold for significance (Table 4, $p = 0.08$, $\alpha = 0.1$) but are not significant with a nonparametric ANOVA.

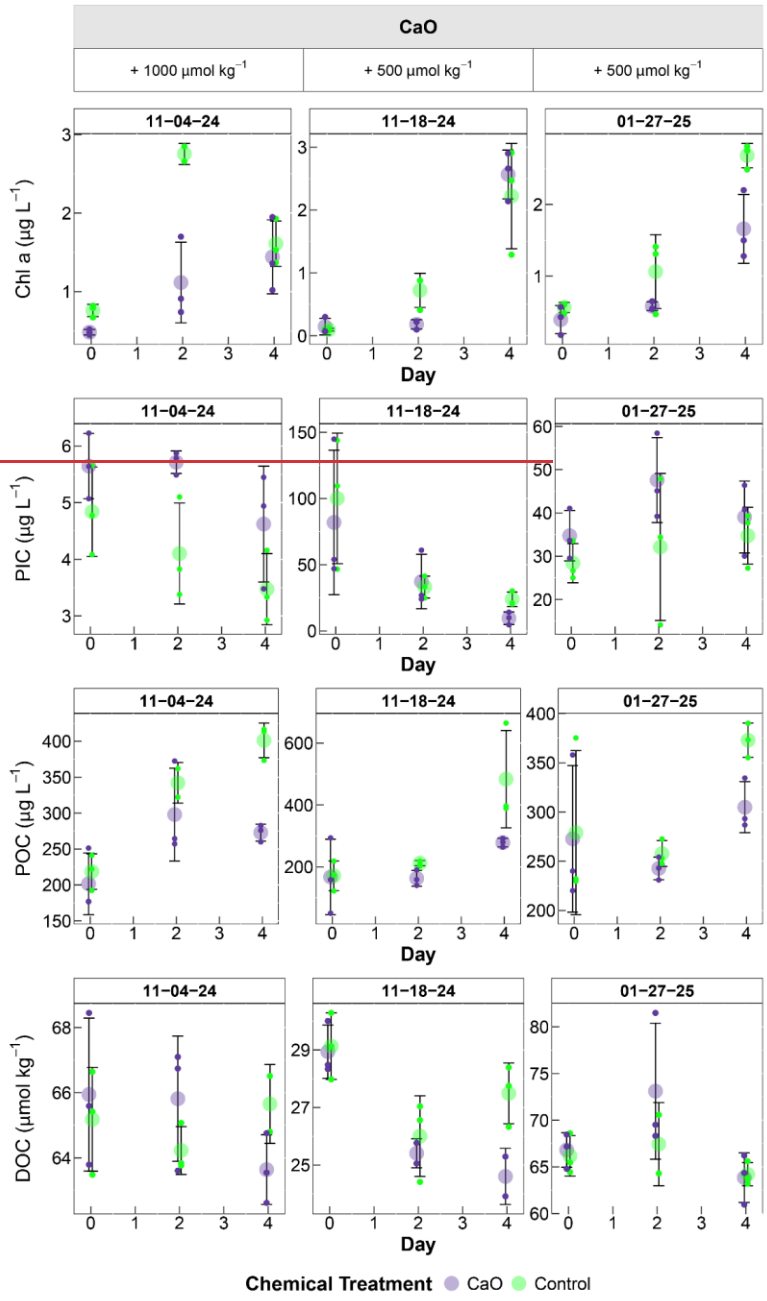

CaO
+ 1000 µmol kg⁻¹
+ 500 µmol kg⁻¹
+ 500 µmol kg⁻¹
11–04–24
11–18–24
01–27–25
Alkalinity (µmol kg⁻¹)
DIC (µmol kg⁻¹)
pH^STP
Dissolved O2(µmol kg⁻¹)
Day
Chemical Treatment
CaO
Control


CaO
+ 1000 µmol kg⁻¹
+ 500 µmol kg⁻¹
+ 500 µmol kg⁻¹
11–04–24
11–18–24
01–27–25
Alkalinity (µmol kg⁻¹)
DIC (µmol kg⁻¹)
pH
Dissolved O2(µmol kg⁻¹)
Day
Chemical Treatment
CaO
Control

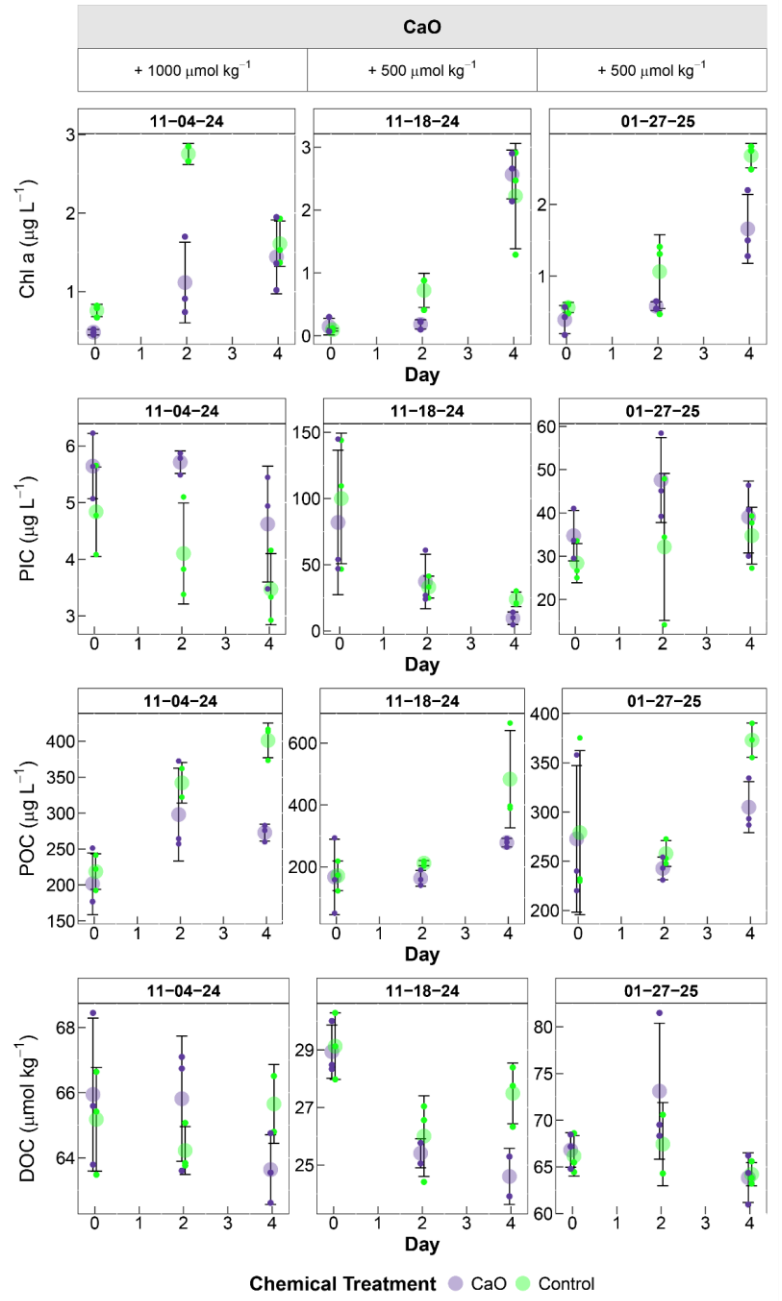
511

Figure 4. ~~**Summary of**~~ **Water Chemistry from (CaO) Amendment Experiments.** Panels are grouped by the alkalinity treatment they received: Controls (green) and CaO (purple). The start date of the experiment is listed for reference to differentiate between duplicate experiments. The four variables displayed here are measured Alkalinity (µmol kg^{-1}), measured Dissolved Inorganic Carbon (DIC, ~~) (~~µmol kg^{-1}), pH calculated from CO2Sys.v3, and D~~d~~issolved O~~o~~xygen (O_2, µmol kg^{-1}). Each plot shows the average of the treatment per timepoint (n = 3), and the error bars are the standard deviation between the triplicate bottles. Smaller symbols show individual bottles. ~~As mentioned in the methods section, DIC is immediately lowered on Day 0 due to an expected dilution by the addition of the CaO stock.~~

Formatted: Subscript


CaO
+ 1000 µmol kg⁻¹
+ 500 µmol kg⁻¹
+ 500 µmol kg⁻¹
11–04–24
11–18–24
01–27–25
Chl a (µg L⁻¹)
PIC (µg L⁻¹)
POC (µg L⁻¹)
DOC (µmol kg⁻¹)
Day
Chemical Treatment
CaO
Control

520


CaO
+ 1000 μmol kg⁻¹
+ 500 μmol kg⁻¹
+ 500 μmol kg⁻¹
11–04–24
11–18–24
01–27–25
Chl a (μg L⁻¹)
PIC (μg L⁻¹)
POC (μg L⁻¹)
DOC (μmol kg⁻¹)
Day
Chemical Treatment
CaO
Control

521

Figure 5. Four Day Timeseries of Biogeochemical Response to Different Alkalinity Enhancements. Smaller symbols show individual bottles, large symbol shows mean. Error bars are the standard deviation between replicates (n = 3). The start date of the experiment and the amount of alkalinity added are included as headers for reference. The variables displayed are Chlorophyll ~~l~~ a (Chl a, µg L^{-1}), ~~PIC~~ Particulate Inorganic Carbon (PIC, µg L^{-1}), ~~POC~~ Particulate Organic Carbon (POC, µg L^{-1}), and ~~DOC~~ Dissolved Organic Carbon (DOC, µmol kg^{-1}).

Table 4. Summary of Linear Model Analysis by Experiment.

	Experiment Number	[ALK Added]	Variable	Control Slope ± se	Treatment Slope ± se	*P-value* Interaction Term	Significance
06-17-24	NaOH	500	PIC	0.23 ± 0.17	-0.02 ± 0.17	0.33	FALSE
	NaOH	500	POC	64.6 ± 14.5	69.3 ± 16.1	0.83	FALSE
	NaOH	500	CHL	0.87 ± 0.29	0.67 ± 0.33	0.67	FALSE
	NaOH	500	O_2	4.14 ± 2.97	4.96 ±3.65	0.86	FALSE
07-22-24	NaOH	500	PIC	0.69 ± 0.17	0.53 ± 0.17	0.50	FALSE
	NaOH	500	POC	21.4 ± 7.25	7.03 ± 7.25	0.18	FALSE
	NaOH	500	DOC	-0.16 ± 4.63	9.72 ± 4.63	0.15	FALSE
	NaOH	500	CHL	0.03 ± 0.12	-0.10 ± 0.12	0.46	FALSE
	NaOH	500	O_2	9.61 ± 1.65	10.5 ± 1.65	0.69	FALSE
06-24-24	Ca^{+2} + HCO_3^-	500	PIC	-0.16 ± 0.19	0.02 ± 0.19	0.53	FALSE
	Ca^{+2} + HCO_3^-	500	POC	156 ± 25	170 ± 28.6	0.73	FALSE
	Ca^{+2} + HCO_3^-	500	CHL	1.10 ± 0.54	0.26 ± 0.54	0.29	FALSE
	Ca^{+2} + HCO_3^-	500	O_2	13.5 ± 2.82	13.1 ±3.12	0.93	FALSE
07-15-24	Ca^{+2} + HCO_3^-	500	PIC	0.72 ± 0.22	0.68 ±0.22	0.91	FALSE
	Ca^{+2} + HCO_3^-	500	POC	98.4 ± 16	98.9 ± 16	0.98	FALSE
	Ca^{+2} + HCO_3^-	500	DOC	2.83 ± 5.18	7.05 ± 5.18	0.57	FALSE
	Ca^{+2} + HCO_3^-	500	CHL	0.78 ± 0.47	0.53 ± 0.47	0.71	FALSE
	Ca^{+2} + HCO_3^-	500	O_2	29.3 ± 2.81	22.8 ± 3.12	0.14	FALSE
11-18-24	CaO	500	PIC	-19.0 ± 6.39	-18.1 ± 6.39	0.92	FALSE
	CaO	500	POC	52.7 ± 14.4	27.8 ± 12.9	0.22	FALSE
	CaO	500	DOC	-0.41 ± 2.9	-1.12 ± 0.32	0.13	FALSE
	CaO	500	CHL	0.53 ± 0.12	0.61 ± 0.21	0.68	FALSE
	CaO	500	O_2	1.92 ± 4.13	-0.64 ± 4.14	0.86	FALSE
01-27-25	CaO	500	PIC	1.58 ± 1.99	1.08 ± 1.99	0.86	FALSE
	CaO	500	POC	23.5 ± 11.1	8.03 ± 11.1	0.34	FALSE
	CaO	500	DOC	-0.49 ± 0.53	-0.74 ± 0.53	0.74	FALSE
	CaO	500	CHL	0.53 ± 0.08	0.32 ± 0.08	0.08*	FALSE
	CaO	500	O_2	13.8 ± 0.97	10.9 ± 1.07	NA	*n <3*
11-04-24	CaO	1000	PIC	-0.34 ± 0.15	-0.26 ± 0.15	0.68	FALSE
	CaO	1000	POC	45.7 ± 8.85	17.8 ± 8.85	**0.04**	**TRUE**
	CaO	1000	DOC	0.07 ± 0.36	-0.58 ± 0.32	0.20	FALSE

	CaO	1000	CHL	0.21 ± 0.13	0.24 ± 0.13	0.88	FALSE
	CaO	1000	O_2	17.2 ± 4.86	-0.86 ± 4.37	**0.01**	**TRUE**

Table shows the slope ± the standard error around the slope of the Control and Treatment groups by the variable being analyzed. The p-value is shown, and the test result indicating statistical significance is shown when $p < 0.05$. The * marks a relaxed significance threshold ($\alpha = 0.1$). TRUE OR FALSE indicates whether the interaction is statistically significant or not. Bold for emphasis. NA written where there are less than three data points per time point for control and treatment.

Table 5. Summary of Two-Way Non-parametric Analysis of Variance (ANOVA) by Experiment.

	Experiment Number	[ALK Added]	Variable	*P-value* Interaction Term	Significance
06-17-24	NaOH	500	PIC	0.55	FALSE
	NaOH	500	POC	0.88	FALSE
	NaOH	500	CHL	0.88	FALSE
	NaOH	500	O_2	0.93	FALSE
07-22-24	NaOH	500	PIC	0.52	FALSE
	NaOH	500	POC	0.22	FALSE
	NaOH	500	DOC	0.24	FALSE
	NaOH	500	CHL	0.44	FALSE
	NaOH	500	O_2	0.86	FALSE
06-24-24	$Ca^{+2}{}^{2+} + HCO_3^-$	500	PIC	0.39	FALSE
	$Ca^{+2}{}^{2+} + HCO_3^-$	500	POC	0.94	FALSE
	$Ca^{+2}{}^{2+} + HCO_3^-$	500	CHL	0.56	FALSE
	$Ca^{+2}{}^{2+} + HCO_3^-$	500	O_2	0.95	FALSE
07-15-24	$Ca^{+2}{}^{2+} + HCO_3^-$	500	PIC	0.89	FALSE
	$Ca^{+2}{}^{2+} + HCO_3^-$	500	POC	0.53	FALSE
	$Ca^{+2}{}^{2+} + HCO_3^-$	500	DOC	0.18	FALSE
	$Ca^{+2}{}^{2+} + HCO_3^-$	500	CHL	0.82	FALSE
	$Ca^{+2}{}^{2+} + HCO_3^-$	500	O_2	0.83	FALSE
11-18-24	CaO	500	PIC	0.74	FALSE
	CaO	500	POC	0.66	FALSE
	CaO	500	DOC	0.55	FALSE
	CaO	500	CHL	0.71	FALSE
	CaO	500	O_2	0.98	FALSE
01-27-25	CaO	500	PIC	0.90	FALSE
	CaO	500	POC	0.86	FALSE
	CaO	500	DOC	0.78	FALSE
	CaO	500	CHL	0.97	FALSE
	CaO	500	O_2	0.74	FALSE

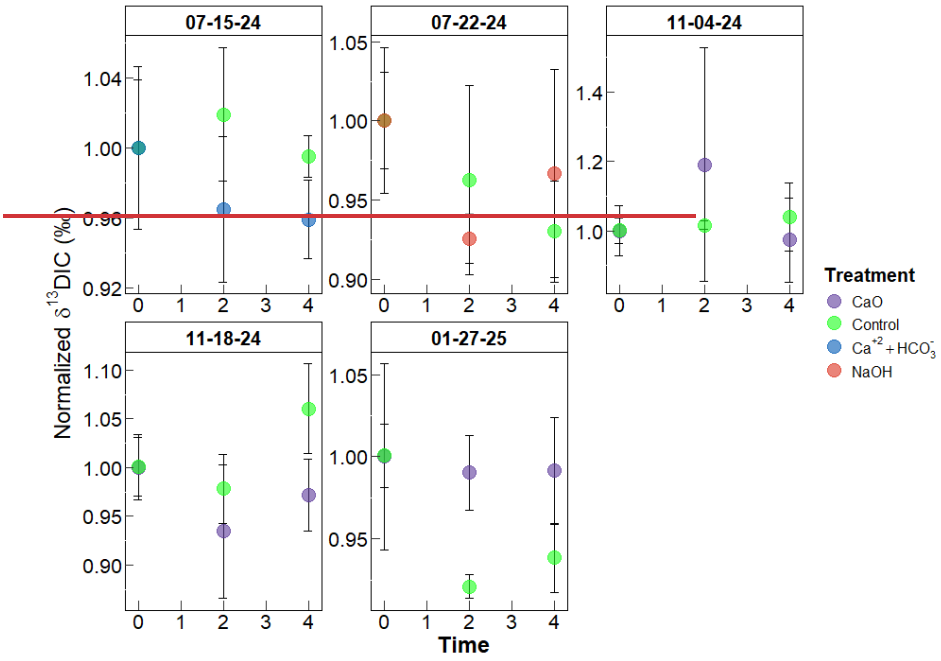
11-04-24	CaO	1000	PIC	0.72	FALSE
	CaO	1000	POC	0.64	FALSE
	CaO	1000	DOC	0.15	FALSE
	CaO	1000	CHL	0.54	FALSE
	CaO	1000	O_2	0.34	FALSE

The Scheirer Ray Hare test is used for this analysis. The interaction between treatment (Control vs. Treatment) condition and time on PIC, POC, Chl a, DOC, and dissolved O_2 is summarized by the p-value. TRUE indicates whether the interaction is statistically significant with an ($\alpha = 0.05$), FALSE shows an insignificant impact on the measured "Variable" column.

3.4 Carbon Budgets

3.4.1 Carbon Isotopes to ~~track~~ Verify Estimates of Respiration

Organic carbon is subject to net increases due to production and decreases attributed to respiration. We added a ^{13}C isotope spike to controls and treatment bottles to assess if an alkalinity addition would change ~~the rate of~~ organic matter remineralization; the remineralized organic matter would add unspiked DIC into a spiked DIC pool. ~~and therefore change the isotopic signature of the residual seawater.~~ The isotope addition only increased DIC by 2 µmol kg^{-1}, ~~but as this was a separate added stock~~ the starting isotopic $\delta^{13}DIC$ varied by bottle. Hence to compare across various days and treatments, we divided the measured $\delta^{13}DIC$ value from each bottle by the average $\delta^{13}DIC$ on Day 0 Control or Treatment value. An increase in organic matter remineralization would make the pool of $\delta^{13}DIC$ lighter. The effect of treatment on $\delta^{13}DIC$ was tested with a two-factor ANOVA where the variables 'Experiment Day' and 'Treatment' were taken as factors. The interaction of these factors results in a $p > 0.05$ for all experiments. Hence, there were no consistent and sustained changes in the $\delta^{13}DIC$ pool of the treatment bottles when compared to the control bottles (~~seawater isotopes shown in~~ Fig. 6). There was also no decrease in the $\delta^{13}DIC$ values due to invasion of CO_2 even in the base addition experiments, because there was no headspace in the bottles, i.e. this isotope tracer demonstrated that a closed system was maintained.


07-15-24
07-22-24
11-04-24
11-18-24
01-27-25
Normalized δ^{13}DIC (‰)
Time
Treatment
CaO
Control
$Ca^{+2} + HCO_3^-$
NaOH

561

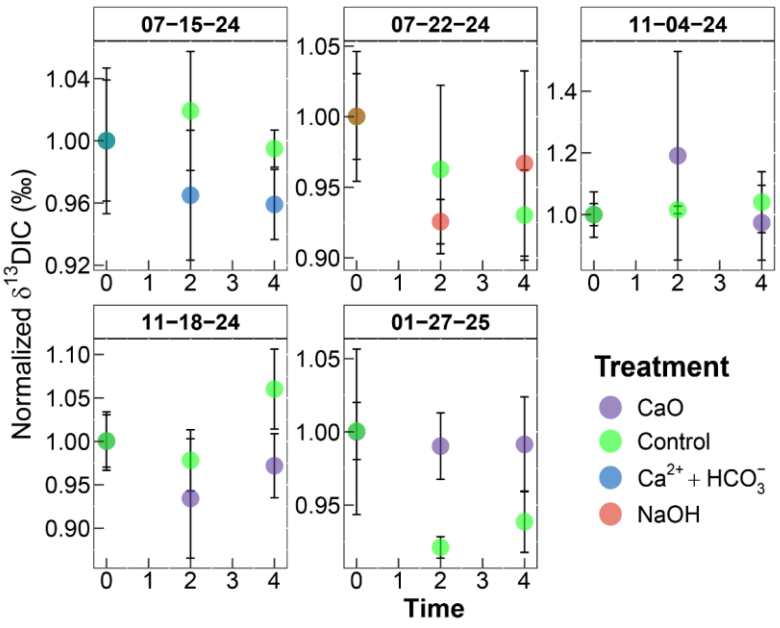
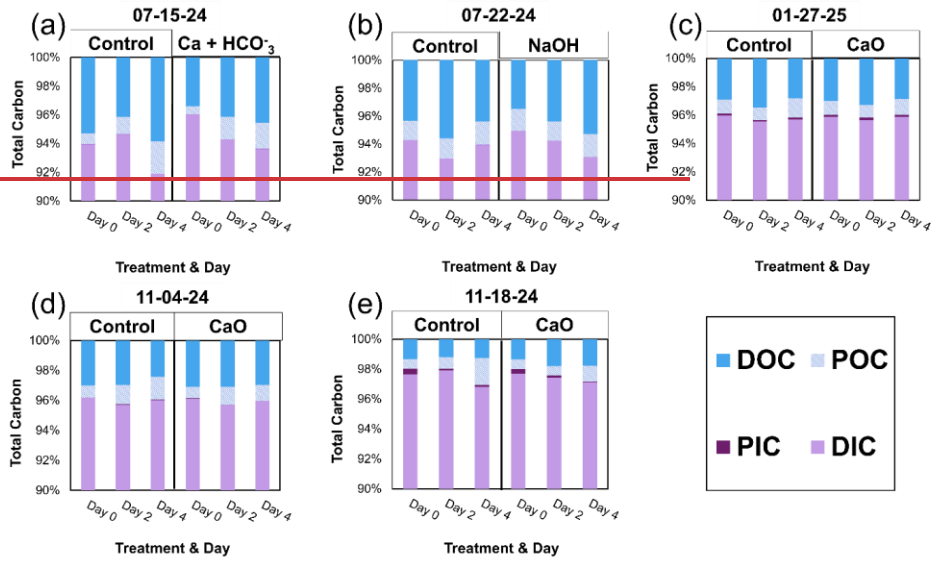


Figure 6. Normalized Seawater (DIC) $\delta^{13}C$ ‰ over Time in all Experiments. Panel (07-15-24) + 500 µmol kg^{-1} $CaCl_2$ ~~+&~~ $NaHCO_3$, (07-22-24) + 500 µmol kg^{-1} NaOH, (11-04-24) + 1000 µmol kg^{-1} CaO, (11-18-24) + 500 µmol kg^{-1} CaO with higher ambient PIC, and (01-27-25) + 500 µmol kg^{-1} CaO. Each data point has an n = 3, and error bars are the standard deviation of the averaged values. Data was 'normalized' by dividing each measured value with the initial starting $\delta^{13}C$ value of the DIC ~~after all bottles were given the isotope spike~~ on Day 0.

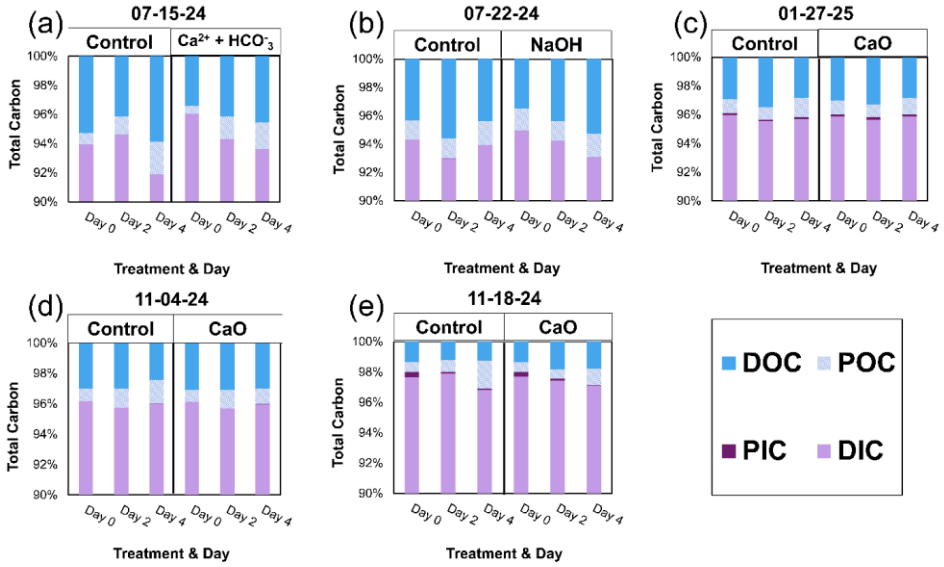
3.4.2 Carbon Partitioning

The purpose of conducting closed system experiments was to better understand and constrain the partitioning of carbon between various pools after increasing the amount of ~~of carbon in the dissolved inorganic phase and/or by adjusting~~ alkalinity ~~with a strong base~~. Here we present the distribution of carbon in various pools (DIC, DOC, PIC, POC) and track the movement of carbon over the span of four days (Fig. 7). These distributions are generated from the mean measured values of triplicate bottles at each time point. In our experiments~~general~~,

~95 % of the carbon exists as DIC, ~3.5 % is stored as DOC, ~ 1.2 % is found in the POC pool, and < 0.1 % in the PIC pool. The non-DIC pool is highly variable across the "Day 0" starting conditions of all experiments (summarized in Table 3). In many experiments, t~~T~~he addition of alkalinity does not change carbon distribution ~~in most of these experiments~~relative to the controls (Fig. 7). The decrease in %DIC (Panel a & b, Fig. 7) is matched by an increase of either %POC or %DOC, ~~. The measured DOC values do not show any statistical differences between the treatment and control conditions~~which is expected from photosynthesis and organic matter cycling.



585



586

587 **Figure 7. Total Carbon Budget for All Experiments.** Panel (a) + 500 µmol kg^{-1} $CaCl_2$ ~~+&~~ $NaHCO_3$, (b)
588 + 500 µmol kg^{-1} NaOH, (c) + 500 µmol kg^{-1} CaO, (d) + 1000 µmol kg^{-1} CaO, and (e) + 500 µmol kg^{-1}

CaO with higher (ambient) particles of inorganic carbon. Bar plots are an average of 3 bottles per timepoint. Plots show changes in distribution of carbon over the duration of the experiment between treatments and controls. The total amount of C will always = 100%.

We can use a mass-balance approach to check if the carbon pools change over time. For example, one assumption may be that decline in DIC is compensated by increase in POC. In this case, one would expect a 1:1 relationship between ΔDIC and ΔPOC (Fig. 8a). However, the Ca^{2+} + HCO_3^- treatments fall furthest from this line, which means these experiments show a deficit of POC production relative to DIC drawdown.

~~We compared the total measured DIC decrease with the amount of POC increase over 4 days to check if our measured carbon pools are stoichiometrically consistent with one another and also to see how DOC production may account for some of the DIC drawdown. The Ca^{+2} + HCO_3^- treatments fall furthest from a 1:1 relationship (Fig. 8 (a)), which means these experiments show a deficit of POC production relative to DIC drawdown.~~To account for DIC lost through other processes, we considered the formation of $CaCO_3$ as a DIC sink, assuming 1 mole of PIC growth is equivalent to 1 mole of DIC consumed. Our data indicate that the DIC lost to the precipitation of ~~calcium carbonate~~$CaCO_3$ is < 1 µmol kg^{-1} over the span of 4 days; this is a trivial amount. We also considered ~~, and~~ CO_2 los~~t~~s from the ~~minimal~~ permeability of the PETG bottles. ~~A could be at most 3 µmol kg^{-1} day^{-1} (~~assuming that CO_2 permeability for PETG is 125 cc-mil $inch^{-2}$ day^{-1}, wall thickness = 0.76 mm, surface area ~ 300 $inch^2$ and the ΔpCO_2 gradient between water inside the bottle vs. outside in the ocean = 300 µatm, the maximum amount of CO_2 that could diffuse out of the bottle would be 3 µmol kg^{-1} day^{-1}~~)~~. Therefore, only ~13 µmol kg^{-1} DIC could potentially be lost to $CaCO_3$ precipitation or diffusion through the bottles' walls, and the ~~Figure 8 (a) shows that the~~ 50-100 µmol kg^{-1} decrease in DIC in Figure 8(a) must result from ~~lead to greater~~ production of POC and DOC.

As an additional test of carbon partitioning, we can check to see whether these experiments fall within Redfield stoichiometries by comparing the total drawdown of DIC with the change in dissolved oxygen (Fig. 8 (b)). A range of DIC:O_2 ratios are~~is~~ considered. The Ca^{2+} + HCO_3^- ~~calcium + bicarbonate~~ experiments and one of their controls fall furthest from the range of Redfield ratios. The same Ca^{+2}~~2+~~ + HCO_3^- experiments that show a deficit in POC also show a

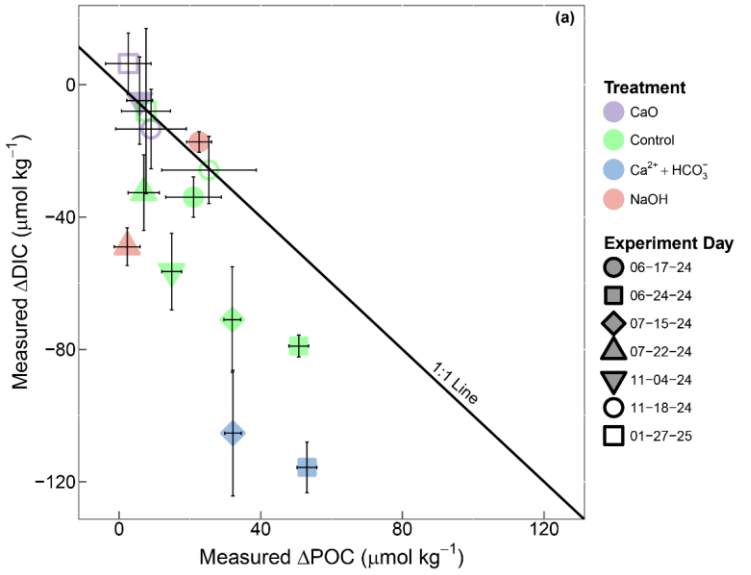
Formatted: Subscript

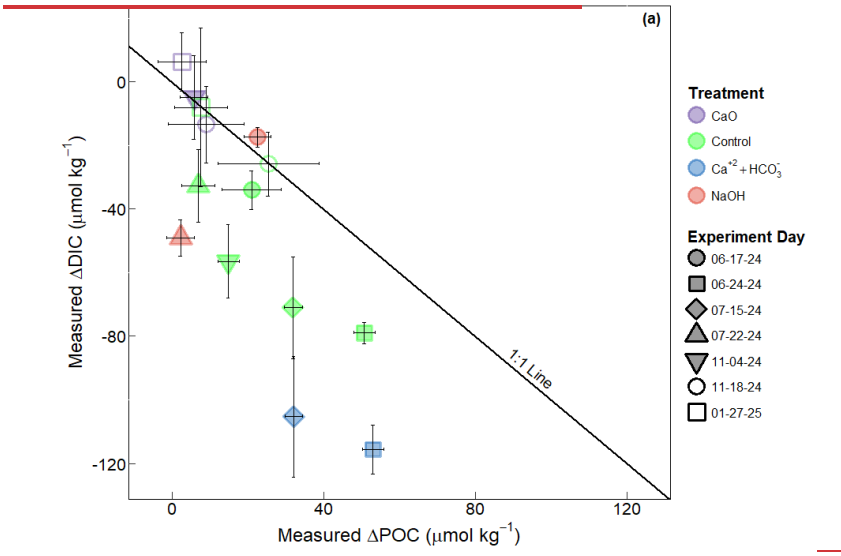
Formatted: Subscript

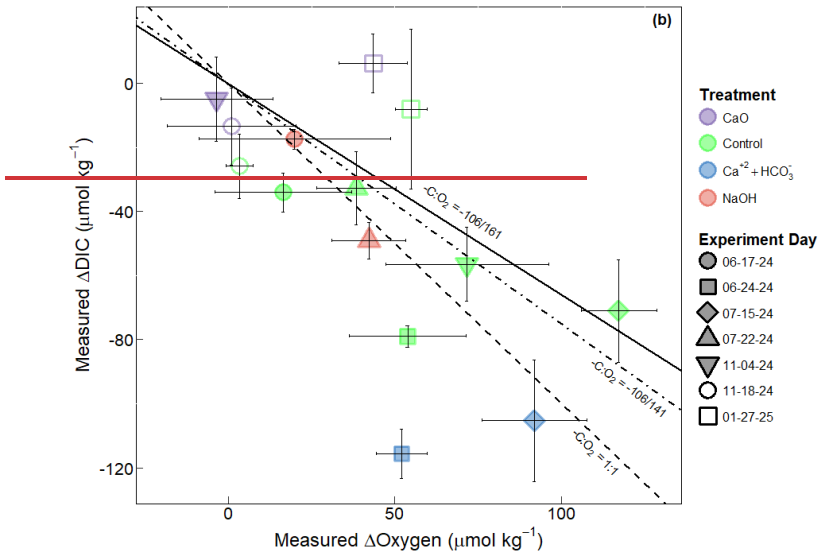
Formatted: Subscript

Formatted: Subscript

deficit in dissolved oxygen ~~than what~~which would be expected if DIC consumption were producing DOC if the POC was being remineralized.







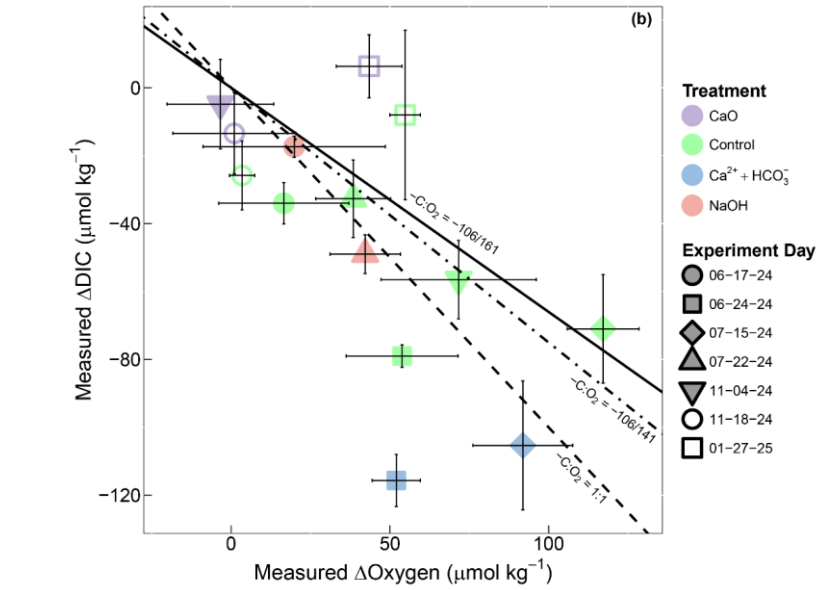


Figure 8. Comparison of Stoichiometric Changes in DIC, Dissolved O_2, and POC in terms of the Redfield Ratio. (a) Cross plot of measured decrease of DIC versus the measured increase of POC; solid line is the 1:1 line. Points that fall below the 1:1 line are thought to have increased DOC. (b) Cross plot of

Formatted: Line spacing: 1.5 lines

measured decrease of DIC versus measured production of dissolved oxygen. Plotted lines show the slopes of various Redfield Ratios: solid line (-C:O_2 = -106:161), dot-dash line (-C:O_2 = -106:141), and dashed line (-C:O_2 = -106:-106 i.e. a 1:1 ratio). Ratios taken from (Anderson, 1995). All points in (a) and (b) have a n = 3, and the delta is calculated as the difference between the average of Day 0 and Day 4 bottle values. Error bars are propagated standard deviations of averaged values used to generate the delta values.

4. Discussion ~~— Relevance to OAE~~

If alkalinity is added to the ocean, we would need to know the effects of different minerals/alkalinity sources – i.e., to test if one ~~is more harmful than another~~produces rapid changes to the ocean ecosystem than another. Thus, we conducted standardized experiments with different feedstocks that raised alkalinity by the same threshold (+500 ~~µmol kg^{-1}~~ and +1000 µmol kg^{-1}). ~~The base addition method for~~ OAE functions on the principle of raising pH which will promote uptake of atmospheric CO_2. One of the simulations we tested was the addition of [Ca^{2+}] + [HCO_3^-] which represents the products of accelerated weathering of limestone (Dong et al., 2025) yet does not drastically raise pH like NaOH or CaO addition. CaO addition increases both pH and the [Ca^{2+}], hence the resultant change in $\Omega_{aragonite}$ is higher than the change in $\Omega_{aragonite}$ after NaOH addition. ~~The properties of each alkalinity source (NaOH, CaO, and simulated dissolved limestone – $NaHCO_3$ + $CaCl_2$) created dissimilar carbonate chemistry regimes in the experiments.~~ Here we discuss how these different regimes influenced abiotic ~~side~~ effects — such as precipitation of minerals and biotic ~~side~~ effects such as organic matter cycling.

Formatted: Not Superscript/ Subscript

4.1 The Absence of Secondary $CaCO_3$ Precipitation ~~of $CaCO_3$~~

The likelihood of observing mineral precipitation after OAE is controlled by several factors including supersaturation of the seawater with respect to a particular mineral, nucleation sites, temperature, time, and even interference from catalytic or inhibitor ions (Berner, 1975; Marion et al., 2009; Moras et al., 2024; Morse et al., 2007; Morse and He, 1993; Mucci and Morse, 1983; Naviaux et al., 2019; Nguyen Dang et al., 2017, Suitner et al., 2025). Here we summarize similar studies that used NaOH, $NaHCO_3$, and CaO to find the minimum thresholds for precipitation of $CaCO_3$ after OAE (detailed experimental conditions are included in Supplemental Table 1 & 2).

Several other studies that conducted similar manipulations of alkalinity additions to natural seawater (initial $\Omega_{aragonite}$ = 1-3) have reported no observed precipitation of $CaCO_3$ (Hartmann et al., 2023; Moras et al., 2022; Ringham et al., 2024; Suitner et al., 2024). Of these, experiments conducted with $NaHCO_3$ + Na_2CO_3 or NaOH have shown stable seawater alkalinity up to $\Omega_{aragonite} \approx$ 15-19 for at least four days and longer (~20 days) in closed systems (Hartmann et al., 2023; Moras et al., 2022; Suitner et al., 2024). In an open system that was allowed to equilibrate with atmospheric CO_2, no precipitation was observed after NaOH addition up to $\Omega_{aragonite} \approx$ 27 for at least 20 days (Ringham et al., 2024). At present, we are the only study to use CaO in an aqueous form. The one study that does not see $CaCO_3$ precipitation but uses solid CaO and $Ca(OH)_2$ feedstocks to add alkalinity, only raised alkalinity by 250 µmol kg^{-1} (raising $\Omega_{aragonite}$ from 2 to 5), after which higher additions resulted in precipitation (Moras et al., 2022).

In these same experimental conditions discussed above, the threshold for $CaCO_3$ was surpassed after $\Omega_{aragonite}$ increased, temperature increased, or the mineral induction period where alkalinity is stable had ended. In some cases, precipitation was favorable due to the presence of nucleation sites (Hartmann et al., 2023; Hashim et al., 2025; Moras et al., 2022, 2024; Ringham et al., 2024; Suitner et al., 2024; Varliero et al., 2024). These are all parameters that vary in a given oceanic environment. With $NaHCO_3$ + Na_2CO_3 additions or NaOH additions where no solid feedstock was used, $CaCO_3$ precipitated as early as 1 day when $\Omega_{aragonite} > 30$ (Hartmann et al., 2023; Ringham et al., 2024; Suitner et al., 2024) or after 20 days when $\Omega_{aragonite}$ exceeded 15 (Hartmann et al., 2023; Suitner et al., 2024). At warmer surface temperatures (27°C) precipitation started at $\Omega_{aragonite} \approx$ 10 (NaOH addition) within hours in a closed system (Hashim et al., 2025). In the experiments where nucleation sites were present, precipitation could be observed as low as $\Omega_{aragonite} \sim$ 7 with CaO and $Ca(OH)_2$ (Moras et al., 2022; Varliero et al., 2024). ~~(Chen et al., 2005; Marion et al., 2009; Wolf et al., 2008)~~

As our alkalinity additions only raised $\Omega_{aragonite}$ to 2 (for $[Ca^{2+}]$ + $[HCO_3^-]$ addition) and $\Omega_{aragonite}$ to 7-8 (NaOH) for 4 days, which is within the range of no observed precipitation of other studies, we believe our results are consistent with existing literature. With our CaO addition we raised $\Omega_{aragonite}$ to 8-14, and we expected $CaCO_3$ as our saturation values were above that of Moras et al. (2022). However, our experimental conditions did not have mineral feedstock like Moras et al. (2022), which could explain the lack of precipitation. Given our results, OAE

Formatted: Indent: First line: 0.5"

practioners in LA Harbour, perhaps analogous to other near-shore oceanic regions, could expect no $CaCO_3$ precipitation with NaOH, $[Ca^{2+}] + [HCO_3^-]$, or CaO addition at 500 µmol kg^{-1} for at least four days if the alkalinity is added in an aqueous form. Thus far it is clear that the thresholds for heterogeneous precipitation (i.e., relevant if using mineral feedstock) versus pseudo-homogeneous precipitation (i.e., relevant if primarily using aqueous solutions for OAE in natural seawater) (Chen et al., 2005; Marion et al., 2009; Wolf et al., 2008) must be different, and may vary with different temperature, salinity, or even organic matter content. Future studies could focus on comparing these precipitation thresholds by systematically changing parameters that influence the induction period before mineral precipitation. ~~The base addition method for OAE functions on the principle of raising pH which will promote uptake of atmospheric CO_2. Adding dissolved limestone does not drastically raise pH like NaOH or CaO addition. CaO addition increases both pH and the $[Ca^{+2}]$, hence the resultant change in $\Omega_{aragonite}$ is higher than the change in $\Omega_{aragonite}$ after NaOH addition. Yet, even with $\Omega_{aragonite}$ ~ 14 (CaO + 1000 µmol kg^{-1}) – which is above the threshold for pseudo-homogenous precipitation in seawater according to Morse and He (T = 25 °C) we do not see any statistically significant secondary precipitation in these experiments.~~

~~Our results are consistent with other studies conducting similar manipulations to find the minimum thresholds of secondary precipitation of $CaCO_3$. $NaHCO_3$ + Na_2CO_3 additions, below $\Omega_{aragonite}$ < 15 without Ca^{+2} increase, resulted in no precipitation in seawater in the absence of particles (0.2 µm filtered) or with small particles (55 µm filtered) . In the case of NaOH addition experiments, immediate brucite ($Mg(OH)_2$) precipitation was observed in multiple studies , but has been shown to quickly redissolve. 'Runaway' $CaCO_3$ has been observed immediately after NaOH addition at $\Omega_{aragonite}$ > 25 (T = 11-20 °C or after 24 hours at $\Omega_{aragonite}$ > 15 (T = 11-23 °C,). Hashim et al. (2025) find $CaCO_3$ production after a +500 µmol kg^{-1} TA enhancement, but their resultant $\Omega_{aragonite}$ = 11. In the range that is comparable to our study ($\Omega_{aragonite}$ = 7.63 with NaOH), no $CaCO_3$ formation was observed for up to 20 days .~~

~~We expected to see increased PIC formation with the CaO addition experiments ($\Omega_{aragonite}$ = 8.5, 8.6, 13.7) because observed $CaCO_3$ precipitation after hydrated lime addition around $\Omega_{aragonite}$ = 7, but their experimental conditions allowed for heterogenous precipitation~~

~~(precipitation on mineral phases), whereas our experimental set-up would have primarily allowed for pseudo-homogenous precipitation (precipitation on colloids or organic materials) and it seems that this threshold must be higher than $\Omega_{aragonite}$ = 14 when using an aqueous medium to increase alkalinity.~~

~~Thus far it is clear that the thresholds for heterogeneous precipitation (i.e., relevant if using mineral feedstock) versus pseudo-homogeneous precipitation (i.e., relevant if primarily using aqueous solutions for OAE in natural seawater) must be different, and may vary with different temperature, salinity, or even organic matter content . When considering a broader impact, increasing alkalinity with NaOH, CaO or even $CaCl_2$ + $NaHCO_3$ at 500-1000 µmol kg^{-1} did not significantly increase $CaCO_3$ production in waters from LA Harbor with very different ambient PIC concentrations.~~

4.2 Impact of Alkalinity on Organic Matter Cycling

4.2.1 ~~Neutral~~ Minimal Impacts of NaOH and $[Ca^{2+}]$ and $[HCO_3^-]$ ~~Dissolved Limestone~~ Addition

The Port of Los Angeles has a dynamic seasonal cycle that directly influences the carbon standing stock – driven by the seasonally dominant organisms within the water. The organic carbon~~C~~ biomass is highest in Spring at 300 µgC L^{-1}, followed by 150 µgC L^{-1} in Summer, 100 µgC L^{-1} in Fall, and 80 µgC L^{-1} in the Winter (Connell et al., 2017). There is also seasonality in the types of organisms that are creating biomass and are thus responding to the alkalinity addition during our experiments.

~~-~~The NaOH (starting [POC] = 200 µgC L^{-1}) and $[Ca^{2+}]$ and $[HCO_3^-]$ ~~dissolved Limestone~~ (starting [POC] = 400 µgC L^{-1}) experiments were conducted in the Summer, when Diatoms make up more than half of the particulate biomass, followed by Picoeukaryotes in the LA Port region (Connell et al., 2017). The lack of impact on POC or Chl a production between the controls and treatments after NaOH and $[Ca^{2+}]$ + $[HCO_3^-]$ addition (+500 µmol kg^{-1} , pH 7.77 – 8.62) in this study is congruent with results seen from other Diatom specific studies ~~The lack of a negative impact on primary production after NaOH and dissolved limestone addition (pH < 8.55) in this study is congruent with results seen from other Diatom specific studies~~ (Gately et al., 2023; Oberlander et al., 2025) and natural community studies (Guo et al., 2025) where the

resultant change in POC production was also negligible. Other studies have seen a decrease in Diatom concentration once the alkalinity addition has increased pH beyond 8.55 (Kousoulas et al., 2025; Oberlander et al., 2025) and note a decrease in Picoeukaryote concentration after NaOH addition (Ferderer et al., 2022; Kousoulas et al., 2025; Oberlander et al., 2025). In this study, we do not have evidence to say if the species compositions shifted, however, these alkalinity additions did not statistically alter the POC concentrations when compared to the control (Fig. 3). Federer et al. (2022) saw POC decline after alkalinity addition, which they attributed ~~this~~ to a possible alteration in the composition of the diatom community and increased heterotrophic bacterial activity in their alkalinity (NaOH and $NaHCO_3$) treatments.

Heterotrophic bacteria continuously recycle organic matter derived from primary production and thus will be affected by processes changing carbon biomass (Dittmar et al., 2021; Santinelli et al., 2024; Soong et al., 2020). Some of our results suggest that NaOH addition could change PIC and DOC production in waters incubated in the dark ~~to simulate decoupling from the euphotic zone~~ (See Supplemental Information), but this is not observed in the dark CaO or $[Ca^{2+}]$ + $[HCO_3^-]$ additions.~~,~~ In the non-shaded bottles of the $[Ca^{2+}]$ + $[HCO_3^-]$ experiments, there appear to be substantial amounts of DOC production, but statistically we cannot say this is a result of the +500 µmol kg^{-1} alkalinity enhancement. The observed heterogeneity may simply be caused by when the water was sampled. These results, when considered with existing literature suggest that more OAE impact studies should focus on changes in heterotrophic bacteria, metabolic reactions that utilize $[HCO_3^-]$, and recycling of DOC.~~We also do not see substantial evidence from direct measurements to suggest that calcium and bicarbonate addition increases the production of DOC above the control samples, however, there appear to be times in the water mass sampled when DOC production is more prevalent.~~

The pH in LA Harbor waters has a natural measured variability of 7.57-8.46 (Lyons and Birosik, 2007). Under the conditions of our incubations, the natural microbial populations may be acclimated to pH shifts of the order generated by the NaOH and $[Ca^{2+}]$ + $[HCO_3^-]$ additions (+500 µmol kg^{-1}, pH 7.77 – 8.62) in these experiments. It is not uncommon for coastal environments to experience pH oscillations of 1 or more pH units due to tidal fluctuations or metabolic processes such as photosynthesis and respiration. Hinga, (2002) reviewed 21 studies that assess the impact of pH on the growth rates of coastal marine phytoplankton. Some species

were able to thrive at a wide pH range, but others were extremely sensitive to changes of <0.5 pH units. In LA Harbor there are also natural alkalinity changes experienced by the ecosystems, and therefore these water column ecosystems may be able to handle some extent of OAE. However, determining "safe" thresholds for OAE is a nuanced ecological and societal issue that requires further scientific consensus. ~~Of note is the natural variability of pH in LA Harbor waters which has been previously measured within range 7.57-8.46 . Under the conditions of our incubations, the natural microbial populations may be acclimated to pH shifts of the order generated by NaOH or HCO_3^- in these experiments (pH in Treatments < 8.55). but we encourage further experiments for more conclusive results.~~

4.2.2 Potential Impact of CaO Addition

The impacts to Chl a and POC from the CaO addition when compared to the unamended conditions ~~are inconsistent across the experiments conducted in this study~~show different outcomes. Of the two experiments conducted around +500 µmol kg^{-1}, one (01-27-25) shows that Chl a was statistically lower. This trend was not seen in Chl a in the experiment conducted at +1000 µmol kg^{-1} (11-04-24). ~~One experiment had a statistically significant impact to Chl a, which occurs at + 500 µmol kg^{-1} but not + 1000 µmol kg^{-1}, indicating that this result did not reproduce.~~ POC production was~~is~~ reduced by 100 µgC L^{-1} after 4-days from the + 500 µmol kg^{-1} CaO additions (but the trend is not statistically significant due to high initial variability). POC production is reduced by 200 µgC L^{-1} after the 1000 µmol kg^{-1} addition, *which is statistically significant*. This coincides with statistically different (lower) dissolved oxygen after a 1000 µmol kg^{-1} CaO addition as compared to the control. The impacts of CaO addition at +500 µmol kg^{-1} may not be statistically significant on LA Harbor organic matter cycling, but at + 1000 µmol kg^{-1} the~~se results~~ impacts do become statistically significant.

Other studies that test the impact of ocean liming – either simulated by CaO, $Ca(OH)_2$, or even NaOH + $CaCl_2$ addition – have seen minimal effects on growth rates, grazing rate, and community composition when the resultant change has been below pH 9 (de Castro et al., 2025; Traboni et al., 2025). In our study, the starting pH of the seawater is around 7.68-7.85 and the

CaO additions bring this pH to 8.57-8.91 (+ 500-1000 µmol kg^{-1} experiments). ~~When the CaO~~Several studies, that raised ~~addition raises~~ pH ~~>~~ ~~above~~ 9 with CaO, ~~a couple of studies~~ have shown a decrease in organism quality (i.e., nutrient composition) and growth rate (Bhaumik et al., 2025) or decreased motility and even increased mortality ~~depending on the exposure time to elevated pH~~ (Camatti et al., 2024; Pedersen and Hansen, 2003). CaO addition, that reduces pCO2 below ~100 µatm, ~~(in our study this is observed only during CaO + 1000 µmol kg^{-1}),~~ could cause issues in primary production from the CO_2 limitation. This has been shown in coccolithophore ~~performance~~ growth (Bach et al., 2011; Faucher et al., 2025; Riebesell et al., 1993; Sett et al., 2014) and ~~some~~ for other marine phytoplankton (Hansen, 2002; Pedersen and Hansen, 2003). These results draw attention to how reorganization of community composition (with smaller phytoplankton that are better adapted to lower pCO_2 availability (Chrachri et al., 2018; Flynn et al., 2012; Wolf-Gladrow and Riebesell, 1997)) could result from alkalinity additions, This in term could reduce the transfer efficiency of POM to the deeper ocean (Boyd and Newton, 1995). In this study we do not incorporate a characterization of size classes, but future alkalinity enhancement studies should also try to incorporate an analysis of changes in particle size class, among a suite of other ecological impacts (Marx et al., 2025 Preprint).

5. Conclusions

Regional studies that systematically explore the impacts on biomass transfer but also characterize the changing biomass will be highly informative when considering the impacts of OAE on carbon partitioning. In our LA Harbor waters, sometimes POC increased or decreased over 4-day incubations. However, there was a consistent pattern of less POC production in CaO added treatment bottles –lower by 100-200 µgC L^{-1} compared to control samples; as was statistically lower at $\Omega_{aragonite}$ = 14. Future studies should resolve changes in POC particle class size and leverage 'omics tools to understand what parts of the organic matter cycle may be impacted by CaO addition. Studies should also draw attention to the impacts of increased alkalinity in regions where the amended water mass sinks below the euphotic zone and heterotrophic reactions may dominate carbon cycling.

Our experiments show that even factors that should increase the likelihood of $CaCO_3$ precipitation – supersaturation & greater nucleation sites – did not lead to $CaCO_3$ precipitation

over a four-day incubation period at $\Omega_{aragonite} \leq 14$ in closed but photosynthetically active systems. Additionally, Chlorophyll as a proxy for the community of phytoplankton, did not show sensitivity to any of the three alkalinity types at an addition threshold of +500 µmol kg^{-1}.

There may be other environmental parameters that influence PIC and POC production in concert with alkalinity enhancement. We recommend conducting longer duration experiments across a wide range of waters and ecosystems, especially regions that may experience smaller shifts in alkalinity. However, natural ecosystems that do experience more variability in alkalinity may be resilient to some OAE intervention.~~When considering the impacts of various minerals used to simulate OAE on carbon partitioning in a natural marine plankton community, we see no overall trends that could be statistically significant suggesting secondary precipitation of $CaCO_3$. Our experiments show that even factors that should increase the likelihood of $CaCO_3$ precipitation – supersaturation & greater nucleation sites – will not always result in precipitation. In the case of LA Harbor waters, there may be microbial communities that are not sensitive nor responsive to the amount of alkalinity added in these experiments. There also may be other environmental parameters that influence POC and PIC production more than alkalinity enhancement. Conducting more experiments across a wide range of waters and ecosystems is recommended.~~

~~Chl a did not always reliably track organic matter production and should not be used as a proxy for POC. In our waters, sometimes POC increased and sometimes it decreased over 4-day incubations. However, there was a consistent pattern of less POC production in CaO added treatment bottles – lower by 100-200 µgC L^{-1} compared to control samples. From our work, CaO additions are potentially harmful to the production of POC – but we encourage further studies on changes in particle class size, and studies that may leverage 'omics tools to understand what parts of the organic matter cycle may be impacted by CaO addition.~~

~~We do not see statistically significant changes in production of particulate and dissolved organic matter after NaOH and Dissolved Limestone alkalinity addition as compared to control waters (untreated). Future studies should draw attention to the impacts of increased alkalinity in regions where the amended water mass is lost from the euphotic zone, where primarily heterotrophic reactions occur. This may require expanding our understanding of downstream trophic level concerns of OAE.~~

Formatted: Indent: First line: 0.5"

Formatted: Indent: First line: 0"

Data Availability

Data are publicly available at https://doi.org/10.5281/zenodo.20059856.~~https://github.com/ruchap-wani/Wani-et-al-OAE-Carbon-Partioning~~

Author Contributions

RW and WB designed the experiments with consultation from NR. RW conducted these experiments with the help of DR, RA, and EJ. RW was responsible for sample analysis, data curation, and formal statistical analysis with consultation from NR and WB. Funding was acquired by WB. The manuscript was written and edited by RW with contributions from WB.

Competing Interests

WB is the co-founder of Calcarea, Inc. The authors declare that they have no other competing interests.

Acknowledgements

We would like to thank the USC Wrigley Institute of Environment and Sustainability, AltaSea, and Calcarea (Pierre Forin), for access to their laboratory and dock resources. We acknowledge our presence on the ancestral and unceded territory of the Kizh (Gabrieleño) people, and we pay our respect to the members and elders of these communities. We also ~~would like to~~ thank Doug Capone, Myrna Jacobson, Sarah Feakins, and the Department of Environmental Engineering at the University of Southern California for access to their instruments to make Chl-a and dissolved organic carbon measurements. Additionally, we thank the two anonymous reviewers and the associate editor, Perran Cook, for their efforts in reviewing this manuscript.

Financial Support

This research was conducted while RW was a recipient of the Wrigley Institute Graduate Fellowship in 2023. The research was funded by the Lott Innovation Award and the Faculty Innovation Award (to WB) through the USC Wrigley Institute for Environmental Studies (WIES). We acknowledge an NSF-REU award to USC WIES for support of RA (2024) and a Zinsmeyer Summer Research Award to EJM (2023) through grant no. OCE-2244583.

899

900 **References**

901 Anderson, L. A.: On the hydrogen and oxygen content of marine phytoplankton, Deep-Sea
902 Research Part I, 42, 1675–1680, https://doi.org/10.1016/0967-0637(95)00072-E, 1995.

903 Archer, D. E.: An atlas of the distribution of calcium carbonate in sediments of the deep sea,
904 Global Biogeochem. Cycles, 10, 159–174, https://doi.org/10.1029/95GB03016, 1996.

905 Bach, L. T., Riebesell, U., and Schulz, K. G.: Distinguishing between the effects of ocean
906 acidification and ocean carbonation in the coccolithophore *Emiliania huxleyi*, Limnol.
907 Oceanogr., 56, 2040–2050, https://doi.org/10.4319/lo.2011.56.6.2040, 2011.

908 Bach, L. T., Gill, S. J., Rickaby, R. E. M., Gore, S., and Renforth, P.: CO2 Removal With
909 Enhanced Weathering and Ocean Alkalinity Enhancement: Potential Risks and Co-benefits for
910 Marine Pelagic Ecosystems, Frontiers in Climate, 1, https://doi.org/10.3389/fclim.2019.00007,
911 2019.

912 Bach, L. T., Ferderer, A. J., LaRoche, J., and Schulz, K. G.: Technical note: Ocean Alkalinity
913 Enhancement Pelagic Impact Intercomparison Project (OAEPIIP), Biogeosciences, 21, 3665–
914 3676, https://doi.org/10.5194/bg-21-3665-2024, 2024.

915 Barker, S., Archer, D., Booth, L., Elderfield, H., Henderiks, J., and Rickaby, R. E. M.: Globally
916 increased pelagic carbonate production during the Mid-Brunhes dissolution interval and the CO2
917 paradox of MIS 11, Quat. Sci. Rev., 25, 3278–3293,
918 https://doi.org/10.1016/j.quascirev.2006.07.018, 2006.

919 Berelson, W. M., Balch, W. M., Najjar, R., Feely, R. A., Sabine, C., and Lee, K.: Relating
920 estimates of CaCO3 production, export, and dissolution in the water column to measurements of
921 CaCO3 rain into sediment traps and dissolution on the sea floor:A revised global carbonate
922 budget, Global Biogeochem. Cycles, 21, 1–15, https://doi.org/10.1029/2006GB002803, 2007.

923 Berner, R. A.: The role of magnesium in the crystal growth of calcite and aragonite from sea
924 water, Geochim. Cosmochim. Acta, 39, 489–504, https://doi.org/10.1016/0016-7037(75)90102-
925 7, 1975.

926 Berner, R. A. and Berner, E. K.: Silicate Weathering and Climate, in: Tectonic Uplift and Climate
927 Change, Springer US, Boston, MA, 353–365, https://doi.org/10.1007/978-1-4615-5935-1_15,
928 1997.

929 Berner, R. A., Lasaga, A. C., and Garrels, R. M.: The carbonate-silicate geochemical cycle and
930 its effect on atmospheric carbon dioxide over the past 100 million years, Am. J. Sci., 283, 641–
931 683, https://doi.org/10.2475/ajs.283.7.641, 1983.

932 Bhaumik, A., Faucher, G., Henning, M., Meunier, C. L., and Boersma, M.: Prey dynamics as a
933 buffer: enhancing copepod resilience to ocean alkalinity enhancement, Environmental Research
934 Letters , 20, https://doi.org/10.1088/1748-9326/adaa8c, 2025.

Bialonski, S., Caron, D. A., Schloen, J., Feudel, U., Kantz, H., and Moorthi, S. D.: Phytoplankton dynamics in the Southern California Bight indicate a complex mixture of transport and biology, J. Plankton Res., 38, 1077–1091, https://doi.org/10.1093/plankt/fbv122, 2016.

Boyd, P. and Newton, P.: Evidence of the potential influence of planktonic community structure on the interannual variability of particulate organic carbon flux, Deep Sea Research Part I: Oceanographic Research Papers, 42, 619–639, https://doi.org/10.1016/0967-0637(95)00017-Z, 1995.

Cai, W.-J. and Jiao, N.: Wastewater alkalinity addition as a novel approach for ocean negative carbon emissions, The Innovation, 3, 100272, https://doi.org/10.1016/j.xinn.2022.100272, 2022.

Camatti, E., Valsecchi, S., Caserini, S., Barbaccia, E., Santinelli, C., Basso, D., and Azzellino, A.: Short-term impact assessment of ocean liming: A copepod exposure test, Mar. Pollut. Bull., 198, 115833, https://doi.org/10.1016/j.marpolbul.2023.115833, 2024.

Caron, D. A., Connell, P. E., Schaffner, R. A., Schnetzer, A., Fuhrman, J. A., Countway, P. D., and Kim, D. Y.: Planktonic food web structure at a coastal time-series site: I. Partitioning of microbial abundances and carbon biomass, Deep Sea Research Part I: Oceanographic Research Papers, 121, 14–29, https://doi.org/10.1016/j.dsr.2016.12.013, 2017.

Carter, C. M., Ross, A. H., Schiel, D. R., Howard-Williams, C., and Hayden, B.: In situ microcosm experiments on the influence of nitrate and light on phytoplankton community composition, J. Exp. Mar. Biol. Ecol., 326, 1–13, https://doi.org/10.1016/j.jembe.2005.05.006, 2005.

Caserini, S., Pagano, D., Campo, F., Abbà, A., De Marco, S., Righi, D., Renforth, P., and Grosso, M.: Potential of Maritime Transport for Ocean Liming and Atmospheric CO2 Removal, Frontiers in Climate, 3, 1–18, https://doi.org/10.3389/fclim.2021.575900, 2021.

de Castro, I., Ribeiro, S. C., Louvado, A., Gomes, N. C. M., Cachão, M., Brito de Azevedo, E., and Barcelos e Ramos, J.: Ocean liming effect on a North Atlantic microbial community: changes in composition and rates, Front. Mar. Sci., 12, https://doi.org/10.3389/fmars.2025.1602158, 2025.

Chen, T., Neville, A., and Yuan, M.: Calcium carbonate scale formation—assessing the initial stages of precipitation and deposition, J. Pet. Sci. Eng., 46, 185–194, https://doi.org/10.1016/j.petrol.2004.12.004, 2005.

Chou, H.-T.: On the dilution of liquid waste in ship's wake, J. Mar. Sci. Technol., 149–154, https://doi.org/10.1126/science.155.3770.1621, 1996.

Chrachri, A., Hopkinson, B. M., Flynn, K., Brownlee, C., and Wheeler, G. L.: Dynamic changes in carbonate chemistry in the microenvironment around single marine phytoplankton cells, Nat. Commun., 9, 74, https://doi.org/10.1038/s41467-017-02426-y, 2018.

Connell, P. E., Campbell, V., Gellene, A. G., Hu, S. K., and Caron, D. A.: Planktonic food web structure at a coastal time-series site: II. Spatiotemporal variability of microbial trophic activities,

Deep Sea Research Part I: Oceanographic Research Papers, 121, 210–223, https://doi.org/10.1016/j.dsr.2017.01.007, 2017.

Daehler, C. C. and Strong, D. R.: Can You Bottle Nature? The Roles of Microcosms in Ecological Research, Ecology, 77, 663–664, https://doi.org/10.2307/2265487, 1996.

Dickson, A. G.: Thermodynamics of the dissociation of boric acid in synthetic seawater from 273.15 to 318.15 K, Deep Sea Research Part A. Oceanographic Research Papers, 37, 755–766, https://doi.org/10.1016/0198-0149(90)90004-F, 1990.

Dickson, A. G. and Millero, F. J.: A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media, Deep Sea Research Part A. Oceanographic Research Papers, 34, 1733–1743, https://doi.org/10.1016/0198-0149(87)90021-5, 1987.

Dickson, A. G. ., Sabine, C. L. ., and Christian, J. Robert.: Guide to best practices for ocean CO_2 measurements, North Pacific Marine Science Organization, 2007.

Dittmar, T., Lennartz, S. T., Buck-Wiese, H., Hansell, D. A., Santinelli, C., Vanni, C., Blasius, B., and Hehemann, J.-H.: Enigmatic persistence of dissolved organic matter in the ocean, Nat. Rev. Earth Environ., 2, 570–583, https://doi.org/10.1038/s43017-021-00183-7, 2021.

Dong, S., Berelson, W. M., Forin, P., Gutierrez, M., Carroll, D., Menemenlis, D., Kyi, A. Y., and Adkins, J. F.: Potential of CO_2 sequestration through accelerated weathering of limestone on ships, Sci. Adv., 11, https://doi.org/10.1126/sciadv.adr7250, 2025.

Dunne, J. P., Sarmiento, J. L., and Gnanadesikan, A.: A synthesis of global particle export from the surface ocean and cycling through the ocean interior and on the seafloor, Global Biogeochem. Cycles, 21, https://doi.org/10.1029/2006GB002907, 2007.

Eisaman, M. D., Geilert, S., Renforth, P., Bastianini, L., Campbell, J., Dale, A. W., Foteinis, S., Grasse, P., Hawrot, O., Löscher, C. R., Rau, G. H., and Rønning, J.: Assessing the technical aspects of ocean-alkalinity-enhancement approaches, State of the Planet, 2-oae2023, 1–29, https://doi.org/10.5194/sp-2-oae2023-3-2023, 2023.

Falkowski, P., Scholes, R. J., Boyle, E., Canadell, J., Canfield, D., Elser, J., Gruber, N., Hibbard, K., Högberg, P., Linder, S., Mackenzie, F. T., Moore III, B., Pedersen, T., Rosenthal, Y., Seitzinger, S., Smetacek, V., and Steffen, W.: The Global Carbon Cycle: A Test of Our Knowledge of Earth as a System, Science (1979)., 290, 291–296, https://doi.org/10.1126/science.290.5490.291, 2000.

Faucher, G., Haunost, M., Paul, A. J., Tietz, A. U. C., and Riebesell, U.: Growth response of *Emiliania huxleyi* to ocean alkalinity enhancement, Biogeosciences, 22, 405–415, https://doi.org/10.5194/bg-22-405-2025, 2025.

Ferderer, A., Chase, Z., Kennedy, F., Schulz, K. G., and Bach, L. T.: Assessing the influence of ocean alkalinity enhancement on a coastal phytoplankton community, Biogeosciences, 19, 5375–5399, https://doi.org/10.5194/bg-19-5375-2022, 2022.

Flynn, K. J., Blackford, J. C., Baird, M. E., Raven, J. A., Clark, D. R., Beardall, J., Brownlee, C., Fabian, H., and Wheeler, G. L.: Changes in pH at the exterior surface of plankton with ocean acidification, Nat. Clim. Chang., 2, 510–513, https://doi.org/10.1038/nclimate1489, 2012.

Fraser, L. H. and Keddy, P.: The role of experimental microcosms in ecological research, Trends Ecol. Evol., 12, 478–481, https://doi.org/10.1016/S0169-5347(97)01220-2, 1997.

Gately, J. A., Kim, S. M., Jin, B., Brzezinski, M. A., and Iglesias-Rodriguez, M. D.: Coccolithophores and diatoms resilient to ocean alkalinity enhancement: A glimpse of hope?, Sci. Adv., 9, https://doi.org/10.1126/sciadv.adg6066, 2023.

Giesy, J. P. .: Microcosms in ecological research : selected papers from a symposium held at Augusta, Georgia, November 8-10, 1978, Technical Information Center, U.S. Dept. of Energy ; Available from National Technical Information Service, U.S. Dept. of Commerce, 1110 pp., 1980.

Guo, J. A., Strzepek, R. F., Yuan, Z., Swadling, K. M., Townsend, A. T., Achterberg, E. P., Browning, T. J., and Bach, L. T.: Effects of ocean alkalinity enhancement on plankton in the Equatorial Pacific, Commun. Earth Environ., 6, https://doi.org/10.1038/s43247-025-02248-7, 2025.

Hansen, P.: Effect of high pH on the growth and survival of marine phytoplankton: implications for species succession, Aquatic Microbial Ecology, 28, 279–288, https://doi.org/10.3354/ame028279, 2002.

Hartmann, J., Suitner, N., Lim, C., Schneider, J., Marín-Samper, L., Arístegui, J., Renforth, P., Taucher, J., and Riebesell, U.: Stability of alkalinity in ocean alkalinity enhancement (OAE) approaches - consequences for durability of CO2storage, Biogeosciences, 20, 781–802, https://doi.org/10.5194/bg-20-781-2023, 2023.

Hashim, M. S., Marx, L., Klein, F., Dean, C. L., Burdige, E., Hayden, M., McCorkle, D. C., and Subhas, A. V.: Mineral formation during shipboard ocean alkalinity enhancement experiments in the North Atlantic, Biogeosciences, 22, 7149–7165, https://doi.org/10.5194/bg-22-7149-2025, 2025.

Hinga, K.: Effects of pH on coastal marine phytoplankton, Mar. Ecol. Prog. Ser., 238, 281–300, https://doi.org/10.3354/meps238281, 2002.

Holland, H. D. .: The chemistry of the atmosphere and oceans, Wiley, 351 pp., 1978.

Iglesias-Rodríguez, M. D., Rickaby, R. E. M., Singh, A., and Gately, J. A.: Laboratory experiments in ocean alkalinity enhancement research, State of the Planet, 2-oae2023, 1–18, https://doi.org/10.5194/sp-2-oae2023-5-2023, 2023.

Ives, A. R., Foufopoulos, J., Klopfer, E. D., Klug, J. L., and Palmer, T. M.: Bottle or Big-Scale Studies: How do we do Ecology?, Ecology, 77, 681–685, https://doi.org/10.2307/2265491, 1996.

Kheshgi, H. S.: Sequestering atmospheric carbon dioxide by increasing ocean alkalinity, Energy, 20, 915–922, https://doi.org/10.1016/0360-5442(95)00035-F, 1995.

Kousoulas, K., Ferderer, A., Eriksen, R., and Bach, L. T.: Winners and losers under hydroxide-based ocean alkalinity enhancement in a Tasmanian plankton community, J. Phycol., https://doi.org/10.1111/jpy.70052, 2025.

de la Broise, D. and Palenik, B.: Immersed in situ microcosms: A tool for the assessment of pollution impact on phytoplankton, J. Exp. Mar. Biol. Ecol., 341, 274–281, https://doi.org/10.1016/J.JEMBE.2006.10.045, 2007.

Lawton, J. H., Naeem, S., Woodfin, R. M., Brown, V. K., Gange, A., Godfray, H. J. C., Heads, P. A., Lawler, S., Magda, D., Thomas, C. D., Thompson, L. J., and Young, S.: The Ecotron: a controlled environmental facility for the investigation of population and ecosystem processes, Philos. Trans. R. Soc. Lond. B Biol. Sci., 341, 181–194, https://doi.org/10.1098/rstb.1993.0102, 1993.

Lee, K., Kim, T.-W., Byrne, R. H., Millero, F. J., Feely, R. A., and Liu, Y.-M.: The universal ratio of boron to chlorinity for the North Pacific and North Atlantic oceans, Geochim. Cosmochim. Acta, 74, 1801–1811, https://doi.org/10.1016/j.gca.2009.12.027, 2010.

Lunstrum, A. and Berelson, W.: CaCO3 dissolution in carbonate-poor shelf sands increases with ocean acidification and porewater residence time, Geochim. Cosmochim. Acta, 329, 168–184, https://doi.org/10.1016/j.gca.2022.04.031, 2022.

Lunstrum, A., Keller, D., Hartmann, J., Moras, C., and Wilcox, J.: A framework for understanding efficiency losses of Ocean Alkalinity Enhancement, in: Goldschmidt 2025 Conference, 2025.

Lyons, M. J. and Birosik, S.: WATER QUALITY IN THE DOMINGUEZ CHANNEL AND LOS ANGELES/LONG BEACH HARBOR WATERSHED MANAGEMENT AREA , 2007.

Marion, G. M., Millero, F. J., and Feistel, R.: Precipitation of solid phase calcium carbonates and their effect on application of seawater SA-T-P models, Ocean Science, 5, 285–291, https://doi.org/10.5194/os-5-285-2009, 2009.

Marx, L., Rheuban, J., McCorkle, D., Murray, C., Guo, Y., Wang, Z., Michel, A., Chen, K., Kim, H., and Subhas, A.: Development of the ecological activity index as an integrative ecosystem assessment and monitoring asset for ocean alkalinity enhancement, https://doi.org/10.21203/rs.3.rs-6371725/v1, 28 May 2025.

Moras, C. A., Bach, L. T., Cyronak, T., Joannes-Boyau, R., and Schulz, K. G.: Ocean alkalinity enhancement - avoiding runaway CaCO3 precipitation during quick and hydrated lime dissolution, Biogeosciences, 19, 3537–3557, https://doi.org/10.5194/bg-19-3537-2022, 2022.

Moras, C. A., Cyronak, T., Bach, L. T., Joannes-Boyau, R., and Schulz, K. G.: Effects of grain size and seawater salinity on magnesium hydroxide dissolution and secondary calcium carbonate precipitation kinetics: implications for ocean alkalinity enhancement, Biogeosciences, 21, 3463–3475, https://doi.org/10.5194/bg-21-3463-2024, 2024.

Morse, J. W. and He, S.: Influences of T, S and PCO2 on the pseudo-homogeneous precipitation of CaCO3 from seawater: implications for whiting formation, Mar. Chem., 41, 291–297, https://doi.org/10.1016/0304-4203(93)90261-L, 1993.

Morse, J. W., Arvidson, R. S., and Lüttge, A.: Calcium Carbonate Formation and Dissolution, Chem. Rev., 107, 342–381, https://doi.org/10.1021/cr050358j, 2007.

Mucci, A. and Morse, J. W.: The incorporation of Mg2+ and Sr2+ into calcite overgrowths: influences of growth rate and solution composition, Geochim. Cosmochim. Acta, 47, 217–233, https://doi.org/10.1016/0016-7037(83)90135-7, 1983.

National Academies of Sciences, E. and M.: A Research Strategy for Ocean-based Carbon Dioxide Removal and Sequestration, National Academies Press, Washington, DC, 1–308 pp., https://doi.org/10.17226/26278, 2022.

Naviaux, J. D., Subhas, A. V., Dong, S., Rollins, N. E., Liu, X., Byrne, R. H., Berelson, W. M., and Adkins, J. F.: Calcite dissolution rates in seawater: Lab vs. in-situ measurements and inhibition by organic matter, Mar. Chem., 215, 103684, https://doi.org/10.1016/j.marchem.2019.103684, 2019.

Nguyen Dang, D., Gascoin, S., Zanibellato, A., G. Da Silva, C., Lemoine, M., Riffault, B., Sabot, R., Jeannin, M., Chateigner, D., and Gil, O.: Role of Brucite Dissolution in Calcium Carbonate Precipitation from Artificial and Natural Seawaters, Cryst. Growth Des., 17, 1502–1513, https://doi.org/10.1021/acs.cgd.6b01305, 2017.

Oberlander, J. L., Burke, M. E., London, C. A., and Macintyre, H. L.: Assessing the impacts of simulated ocean alkalinity enhancement on viability and growth of nearshore species of phytoplankton, Biogeosciences, 22, 499–512, https://doi.org/10.5194/bg-22-499-2025, 2025.

Parsons, T. R.: The Future of Controlled Ecosystem Enclosure Experiments, in: Marine mesocosms: Biological and Chemical Research in Experimental Ecosystems, edited by: Grice, G. D. and Reeve, M. R., Springer-Verlag, New York, 411–418, 1982.

Paul, A. J., Haunost, M., Goldenberg, S. U., Hartmann, J., Sánchez, N., Schneider, J., Suitner, N., and Riebesell, U.: Ocean alkalinity enhancement in an open ocean ecosystem: Biogeochemical responses and carbon storage durability, EGUsphere, 2024, 1–31, https://doi.org/10.5194/egusphere-2024-417, 2024.

Pedersen, F. and Hansen, P.: Effects of high pH on a natural marine planktonic community, Mar. Ecol. Prog. Ser., 260, 19–31, https://doi.org/10.3354/meps260019, 2003.

Penman, D. E., Caves Rugenstein, J. K., Ibarra, D. E., and Winnick, M. J.: Silicate weathering as a feedback and forcing in Earth's climate and carbon cycle, Earth. Sci. Rev., 209, 103298, https://doi.org/10.1016/j.earscirev.2020.103298, 2020.

Perez, F. F. and Fraga, F.: Association constant of fluoride and hydrogen ions in seawater, Mar. Chem., 21, 161–168, https://doi.org/10.1016/0304-4203(87)90036-3, 1987.

Pierrot, D., Epitalon, J. M., Orr, J. C., Lewis, E., and Wallace, D. W. R.: MS Excel program developed for CO2 system calculations – version 3.0, 2021.

Pukelsheim, F.: The Three Sigma Rule, Am. Stat., 48, 88–91, https://doi.org/10.1080/00031305.1994.10476030, 1994.

Rau, G. H. and Caldeira, K.: Enhanced carbonate dissolution: A means of sequestering waste CO2 as ocean bicarbonate, Energy Convers. Manag., 40, 1803–1813, https://doi.org/10.1016/S0196-8904(99)00071-0, 1999.

Renforth, P.: Perspective: The European Union is not prepared for geochemical carbon dioxide removal*, Nº, 37–72 pp., 2025.

Renforth, P. and Henderson, G.: Assessing ocean alkalinity for carbon sequestration, Reviews of Geophysics, 55, 636–674, https://doi.org/10.1002/2016RG000533, 2017.

Ridgwell, A. and Zeebe, R.: The role of the global carbonate cycle in the regulation and evolution of the Earth system, Earth Planet. Sci. Lett., 234, 299–315, https://doi.org/10.1016/j.epsl.2005.03.006, 2005.

Riebesell, U., Wolf-Gladrow, D. A., and Smetacek, V.: Carbon dioxide limitation of marine phytoplankton growth rates, Nature, 361, 249–251, https://doi.org/10.1038/361249a0, 1993.

Riebesell, U., Zondervan, I., Rost, B., Tortell, P. D., Zeebe, R. E., and Morel, F. M. M.: Reduced calcification of marine plankton in response to increased atmospheric CO2, Nature, 407, 364–367, https://doi.org/10.1038/35030078, 2000.

Riebesell, U., Körtzinger, A., and Oschlies, A.: Sensitivities of marine carbon fluxes to ocean change, Proceedings of the National Academy of Sciences, 106, 20602–20609, https://doi.org/10.1073/pnas.0813291106, 2009.

Riebesell, U., Lee, K., Nejstgaard, J. C., and Fisheries, I.: Pelagic mesocosms Part 2 : Experimental design of perturbation experiments Pelagic mesocosms, 2010.

Ringham, M. C., Hirtle, N., Shaw, C., Lu, X., Herndon, J., Carter, B. R., and Eisaman, M. D.: An assessment of ocean alkalinity enhancement using aqueous hydroxides: kinetics, efficiency, and precipitation thresholds, Biogeosciences, 21, 3551–3570, https://doi.org/10.5194/bg-21-3551-2024, 2024.

Santinelli, C., Valsecchi, S., Retelletti Brogi, S., Bachi, G., Checcucci, G., Guerrazzi, M., Camatti, E., Caserini, S., Azzellino, A., and Basso, D.: Ocean liming effects on dissolved organic matter dynamics, Biogeosciences, 21, 5131–5141, https://doi.org/10.5194/bg-21-5131-2024, 2024.

Savoie, A. M., Ringham, M., Torres Sanchez, C., Carter, B. R., Dougherty, S., Feely, R. A., Hegeman, D., Herndon, J., Khangaonkar, T., Loretz, J., Minck, T., Pelman, T., Premathilake, L., Subban, C., Vance, J., and Ward, N. D.: Novel field trial for ocean alkalinity enhancement using electrochemically derived aqueous alkalinity, Frontiers in Environmental Engineering, 4, https://doi.org/10.3389/fenve.2025.1641277, 2025.

Sett, S., Bach, L. T., Schulz, K. G., Koch-Klavsen, S., Lebrato, M., and Riebesell, U.: Temperature Modulates Coccolithophorid Sensitivity of Growth, Photosynthesis and Calcification to Increasing Seawater pCO2, PLoS One, 9, e88308, https://doi.org/10.1371/journal.pone.0088308, 2014.

Soong, J. L., Fuchslueger, L., Marañon-Jimenez, S., Torn, M. S., Janssens, I. A., Penuelas, J., and Richter, A.: Microbial carbon limitation: The need for integrating microorganisms into our understanding of ecosystem carbon cycling, Glob. Chang. Biol., 26, 1953–1961, https://doi.org/10.1111/gcb.14962, 2020.

Stachowski-Haberkorn, S., Becker, B., Marie, D., Haberkorn, H., Coroller, L., and de la Broise, D.: Impact of Roundup on the marine microbial community, as shown by an in situ microcosm experiment, Aquatic Toxicology, 89, 232–241, https://doi.org/10.1016/j.aquatox.2008.07.004, 2008.

Subhas, A. V., Rollins, N. E., Berelson, W. M., Dong, S., Erez, J., and Adkins, J. F.: A novel determination of calcite dissolution kinetics in seawater, Geochim. Cosmochim. Acta, 170, 51–68, https://doi.org/10.1016/j.gca.2015.08.011, 2015.

Subhas, A. V., Marx, L., Reynolds, S., Flohr, A., Mawji, E. W., Brown, P. J., and Cael, B. B.: Microbial ecosystem responses to alkalinity enhancement in the North Atlantic Subtropical Gyre, Frontiers in Climate, 4, https://doi.org/10.3389/fclim.2022.784997, 2022.

Suitner, N., Faucher, G., Lim, C., Schneider, J., Moras, C. A., Riebesell, U., and Hartmann, J.: Ocean alkalinity enhancement approaches and the predictability of runaway precipitation processes: results of an experimental study to determine critical alkalinity ranges for safe and sustainable application scenarios, Biogeosciences, 21, 4587–4604, https://doi.org/10.5194/bg-21-4587-2024, 2024.

Sulpis, O., Boudreau, B. P., Mucci, A., Jenkins, C., Trossman, D. S., Arbic, B. K., and Key, R. M.: Current CaCO3 dissolution at the seafloor caused by anthropogenic CO2, Proc. Natl. Acad. Sci. U. S. A., 115, 11700–11705, https://doi.org/10.1073/pnas.1804250115, 2018.

Traboni, C., Nocera, A. C., Romano, F., Courboulès, J., Chantzaras, C., Magiopoulos, I., Varliero, S., Basso, D., and Pitta, P.: Plankton do not care: Minimal effects of ocean liming on plankton growth and grazing in the Eastern Mediterranean, Limnol. Oceanogr., https://doi.org/10.1002/lno.70136, 2025.

(UNFCC) United Nations Framework Convention on Climate Change: NDC Synthesis Report, 2022.

U.S. EPA: Standard Operating Procedure for In Vitro Determination of Chlorophyll a in Freshwater Phytoplankton by Fluorescence LG405, 2021.

Varliero, S., Buono, A., Caserini, S., Raos, G., and Macchi, P.: Chemical Aspect of Ocean Liming for CO_2 Removal: Dissolution Kinetics of Calcium Hydroxide in Seawater, ACS Engineering Au, 4, 422–431, https://doi.org/10.1021/acsengineeringau.4c00008, 2024.

Walker, J. C. G., Hays, P. B., and Kasting, J. F.: A negative feedback mechanism for the long-term stabilization of Earth's surface temperature, J. Geophys. Res. Oceans, 86, 9776–9782, https://doi.org/10.1029/JC086iC10p09776, 1981.

Wani, R. P., Roper, D. L., Agrawal, R., Lim, E. J., Rollins, N. E., and Berelson, W. M.: Dataset for "The Impact of NaOH, CaO, and Ca2+ + HCO_3^- Addition on PIC and POC Formation in Los Angeles Harbor Waters," https://doi.org/https://doi.org/10.5281/zenodo.20059856, 19 February 2026.

Wolf, S. E., Leiterer, J., Kappl, M., Emmerling, F., and Tremel, W.: Early Homogenous Amorphous Precursor Stages of Calcium Carbonate and Subsequent Crystal Growth in Levitated Droplets, J. Am. Chem. Soc., 130, 12342–12347, https://doi.org/10.1021/ja800984y, 2008.

Wolf-Gladrow, D. and Riebesell, U.: Diffusion and reactions in the vicinity of plankton: A refined model for inorganic carbon transport, Mar. Chem., 59, 17–34, https://doi.org/10.1016/S0304-4203(97)00069-8, 1997.

Zachos, J. C., Röhl, U., Schellenberg, S. A., Sluijs, A., Hodell, D. A., Kelly, D. C., Thomas, E., Nicolo, M., Raffi, I., Lourens, L. J., McCarren, H., and Kroon, D.: Rapid Acidification of the Ocean During the Paleocene-Eocene Thermal Maximum, Science (1979)., 308, 1611–1615, https://doi.org/10.1126/science.1109004, 2005.

Zondervan, I., Zeebe, R. E., Rost, B., and Riebesell, U.: Decreasing marine biogenic calcification: A negative feedback on rising atmospheric $p\,CO_2$, Global Biogeochem. Cycles, 15, 507–516, https://doi.org/10.1029/2000GB001321, 2001.