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

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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 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 $NaHCO_3$ + $CaCl_2$ (to simulate dissolved limestone). These experiments are conducted with coastal sea water with enhancements of +500-1000 µmol kg^{-1} alkalinity, and incubated in situ, to elucidate their impact over the short term, 0-4 days. Select experiments are also completely isolated from the light via bottle shading. With most treatments, there is no statistically significant $CaCO_3$ precipitation 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.

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1. Introduction

To mitigate the impact of excess carbon dioxide (CO_2), humans need to reduce CO_2 emissions and remove CO_2 from the atmosphere. According to the 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. While there may be 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). Ocean Alkalinity Enhancement (OAE) is a mCDR method that can 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).

There are still many technical challenges to overcome before Ocean Alkalinity Enhancement can be scaled to remove gigatons of CO_2 (Eisaman et al., 2023; Renforth, 2025). 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 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 rerelease of CO_2 from the precipitation of $CaCO_3$ (Eq. 1) – which reduces the efficiency of OAE.

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

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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 when 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).

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 (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. Thus far, some studies have characterized instances of short term (<5 days) (Hartmann et al., 2023; Hashim et al., 2025; Subhas et al., 2022), and longer term (~20 days) (Moras et al., 2022, 2024; Paul 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. 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 (Suitner et al., 2024). 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 (Hashim et al., 2025). 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 $Ca(OH)_2$ (Moras et al., 2022). OAE experiments conducted with cultures of coccolithophores report negligible changes to calcification rates after additions of bicarbonate

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and calcium raise $\Omega_{aragonite}$ to 15 (Gately et al., 2023). These prior experiments leave ambiguity in extrapolating the precipitation thresholds of various feedstocks to waters in other regions.

To this steadily growing body of research, we present the changes of particulate organic and inorganic carbon inventory during 4-day OAE experiments conducted using coastal California waters from Los Angeles 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 of elevating $\Omega_{aragonite}$ by: (i) increasing pH (using two different bases) and (ii) 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 Catalina Island, all other experiments were conducted with water collected from 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).

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Alkalinity perturbation experiments were conducted with 5 L, Nalgene PETG bottles 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 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)	Salinity (PPT)	Light (µmol m^{-2} s^{-1})
$CaCl_2$ + $NaHCO_3$ (+500 µmol kg^{-1})	06-24-24	16.4 – 22.3	35	0 – 540
	07-15-24	17.4 – 24.5	35	0 – 605
NaOH (+500 µmol kg^{-1})	06-17-24	17.2* - 21.1*	35	0 – 640
	07-22-24	15.8 – 20.8	35	0 – 605
CaO (+500 µmol kg^{-1})	11-18-24	13.7* - 14.7*	35	0 – 400
	01-27-25	13.5 – 20.8	35	0 – 470
CaO (+1000 µmol kg^{-1})	11-04-24	14.1 – 22.01	35	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.

2.2 Alkalinity Types

In this study we induce an alkalinity perturbation that simulates two different methods of OAE, (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), and Lime (CaO, Sigma-Aldrich, Missouri). The other method considered here is (2) the product of limestone weathering, purposeful weathering is called accelerated weathering of limestone, AWL (Dong et al., 2025; Kheshgi, 1995; Rau and Caldeira, 1999). When this occurs with

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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:HCO3 of 1:2.

Since we were most concerned with assessing the impact of alkalinity at specific dosages/concentrations, we wanted the alkalinity to be fully dissolved, 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). 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 alkalinity and dissolved inorganic carbon (DIC) to calculate the final concentrations of the stock solutions and precisely dosed our incubations according to these measurements to achieve the alkalinity enrichments 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)).

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). Since the chapter on laboratory and mesocosm experimental design written by Iglesias-Rodríguez et al. (2023) 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

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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 bottles were filled in a 'round robin' manner to promote homogeneity between bottles filled at different times (~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 $CaCl_2$ + $NaHCO_3$). 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 $\delta^{13}DIC$ signal to a couple hundred per mil– the purpose of this was to increase the sensitivity of our measurements for our carbon budget analysis.

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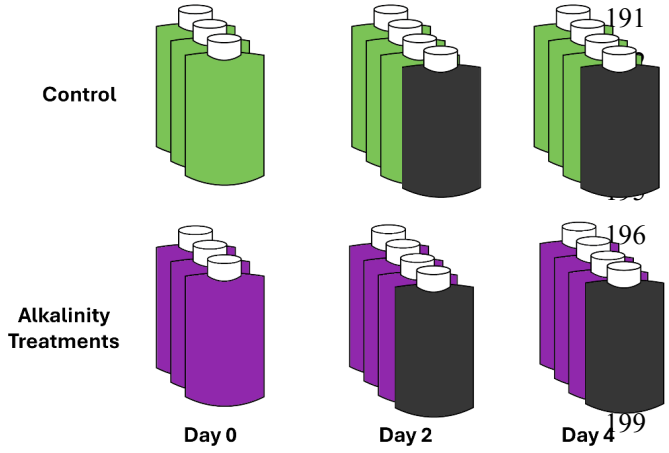

Figure 1. Experimental Set-up. Triplicate bottles shown for each timepoint. Controls are shown in green, alkalinity amended bottles shown in purple, and bottles with light restriction are shown in black.

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. The first 250 mL of sample water was filtered through a 25 mm 0.7 µm glass fiber filter (Whatmann GF/F). A portion of the filtrate was collected for dissolved inorganic carbon (duplicates of 5 mL), alkalinity (15 mL), nutrients (15 mL), dissolved organic carbon (40 mL), and 50 mL for archival purposes. This GF/F 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 and DIC measurements (discussed below) using CO2Sys.v3 (Pierrot et al., 2021)

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(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 & Dissolved Limestone Experiments) were calculated by multiplying the $[CO_3^{2-}]$ output from CO2Sys and $[Ca^{+2}]$ accounting for the increased Ca. 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.

2.5.1 Dissolved Carbon Phases

Samples for Dissolved Inorganic Carbon (DIC) were collected in pre-evacuated 12 mL 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 a standard curve (ppmCO_2 per mg of $CaCO_3$) 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. 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).

2.5.2 Particulate Carbon Phases

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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 $CaCl_2$ + $NaHCO_3$ Addition

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3.1.1 Observing Short Term Impacts at Extremely Low Alkalinity Additions

With the intent to recreate an alkalinity impact condition that resembles extremely high dilution of bicarbonate after a few hours, we conducted an experiment where the presumed outflow of alkalinity from a shipping vessel of 5000 µmol kg^{-1} was diluted by different proportions; 150, 300, and 600 x (Table 2) based on dilution calculations presented by Dong et al., (2025). These incubations that lasted 3 hours and were conducted on Catalina Island at the USC Dornsife Wrigley Marine Science Center (33° 26′ 40.52″ N, 118° 28′ 47.787″ W).

In the least diluted sample, the seawater alkalinity changes from 2223 ± 2 µmol kg^{-1} to 2233 ± 2 µmol kg^{-1} but there is not a statistically significant impact on the concentrations of particulate inorganic or organic carbon ($p > 0.05$). Since we saw no impact on PIC or POC at such small changes in alkalinity (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 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	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 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. The reported error is standard deviations between triplicate bottles (n = 3).

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

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

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303 production of PIC ($p > 0.05$, ANOVA and Linear Regression method, see Table 4 & 5), the
304 $\Omega_{aragonite}$ only increases by 0.2 units in both experiments (Table 3). POC in the control (untreated)
305 seawater triples by the end of the experiment (Fig. 3), but there is no statistical difference
306 between the POC or DOC change in concentration of the control vs. the treatment condition for
307 both experiments ($p > 0.05$, ANOVA and Linear Regression method). Chl a also shows that
308 changes in concentration over time were similar in both the control and the dissolved limestone
309 treatment.

310 **Table 3. Initial Concentrations of Biogeochemical Parameters at Day 0 (Control) of**
311 **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

312

313 **3.2 NaOH addition: Alkalinity Enhancement ~500 µmol kg^{-1}**

314 Sodium hydroxide additions occurred on 17 June 2024 and 22 July 2024. The addition of
315 NaOH increases alkalinity but not DIC which results in a large change in $\Omega_{aragonite}$ from ~2 to
316 8.21 ± 0.04 (06-17-24; pH increases 0.63) and 7.63 ± 0.07 (07-22-24; pH increases 0.71). When
317 compared to the control condition, the changes in PIC, POC, DOC, and Chl a in the treatment
318 conditions are also not statistically significant (Fig. 3) via either of two statistical analyses
319 ($p > 0.05$, ANOVA and Linear Regression methods). As with the [Ca^{+2}] and [HCO_3^-] addition,
320 there are certain points in time where the controls diverge from the treatments, but there are no
321 consistent trends across multiple variables (i.e., no indication that one group (control/treatment)
322 is consistently higher/lower through time).

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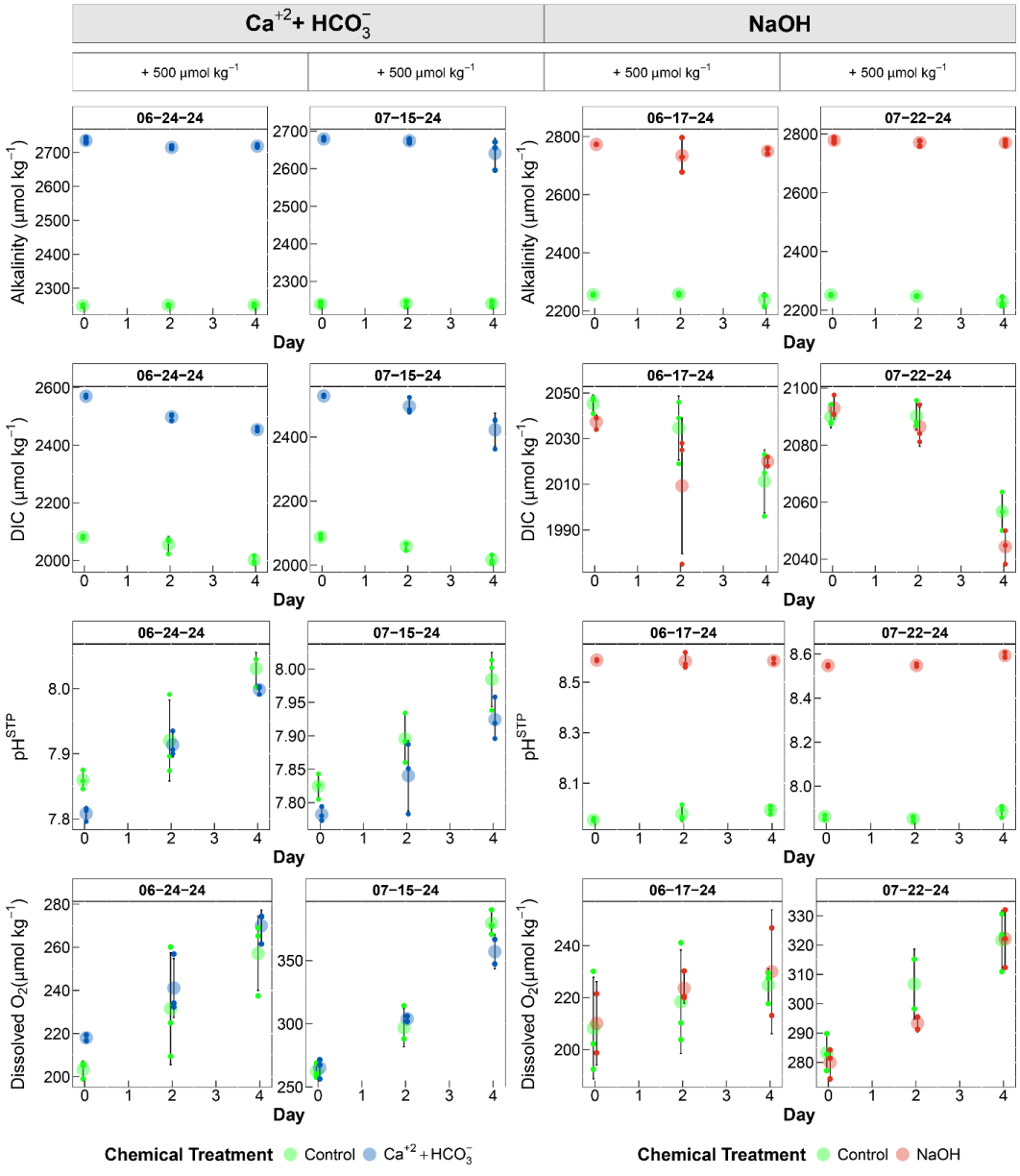

Figure 2. Summary of Water Chemistry from (NaOH and $NaHCO_3$ + $CaCl_2$) Amendment Experiments. Panels are grouped by the alkalinity treatment they received: Controls (green), NaOH (red) and $CaCl_2$ + $NaHCO_3$ (blue). The start date of the experiment is listed for reference to differentiate between 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 dissolved

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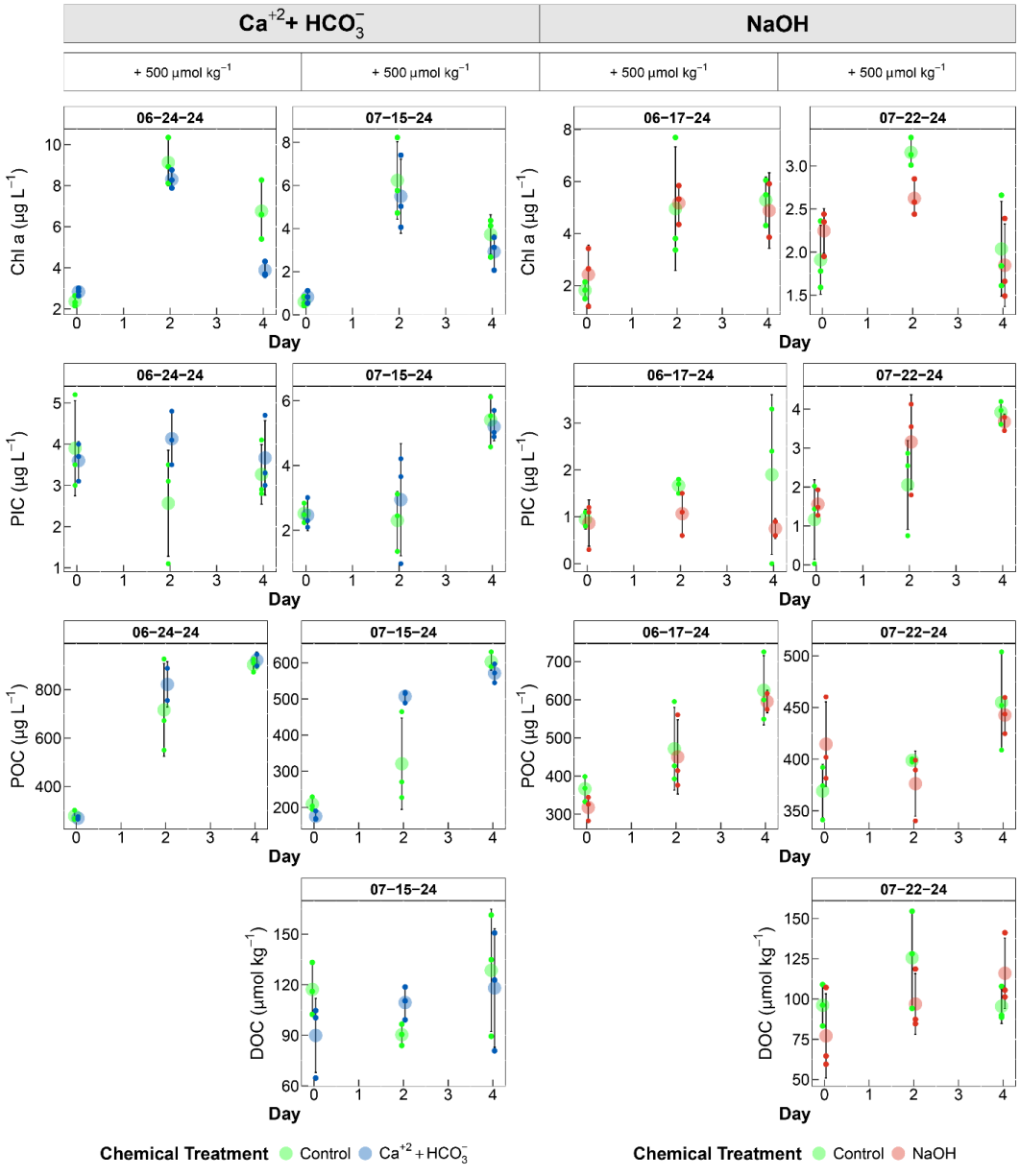
329 oxygen (µmol kg^{-1}). Each plot shows the average of the treatment per timepoint (n = 3), and the error bars
330 are the standard deviation between the triplicate bottles. Smaller symbols show individual bottles.



331

332 **Figure 3. Four Day Timeseries of Biogeochemical Response to Different Alkalinity Enhancements.**
333 Smaller symbols show individual bottles; larger symbols show the mean. Error bars are the standard

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

3.3 CaO Additions

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

The CaO experiment raised the Omega value above the threshold for spontaneous precipitation of calcium carbonate 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 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. The 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.

3.3.2 CaO addition: Alkalinity Enhancement ~500 µmol kg^{-1} with Higher Ambient PIC

Twice we added CaO 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 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 – 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

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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.

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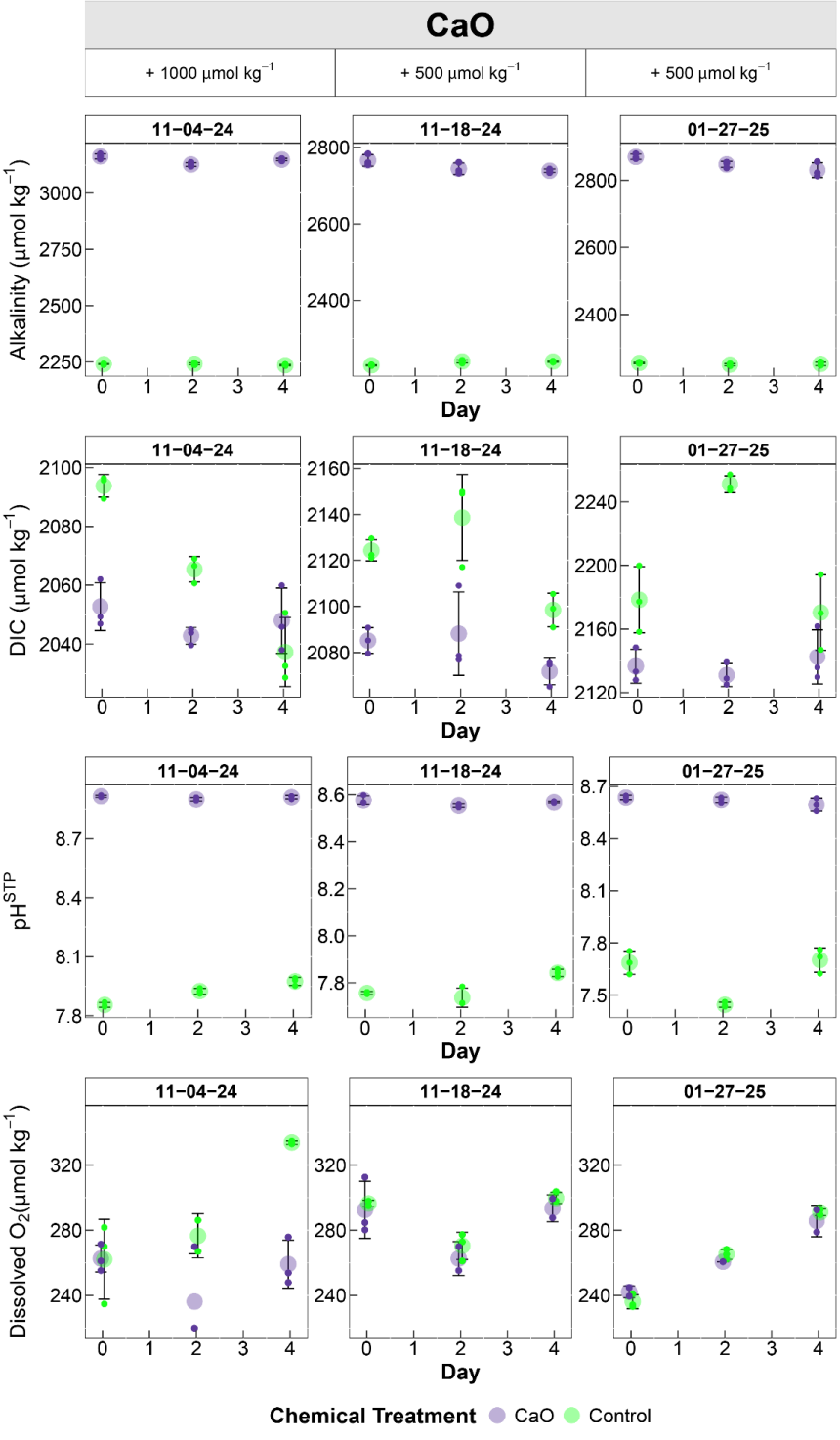

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384 **Figure 4. Summary of Water Chemistry from (CaO) Amendment Experiments.** Panels are grouped
385 by the alkalinity treatment they received: Controls (green) and CaO (purple). The start date of the
386 experiment is listed for reference to differentiate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

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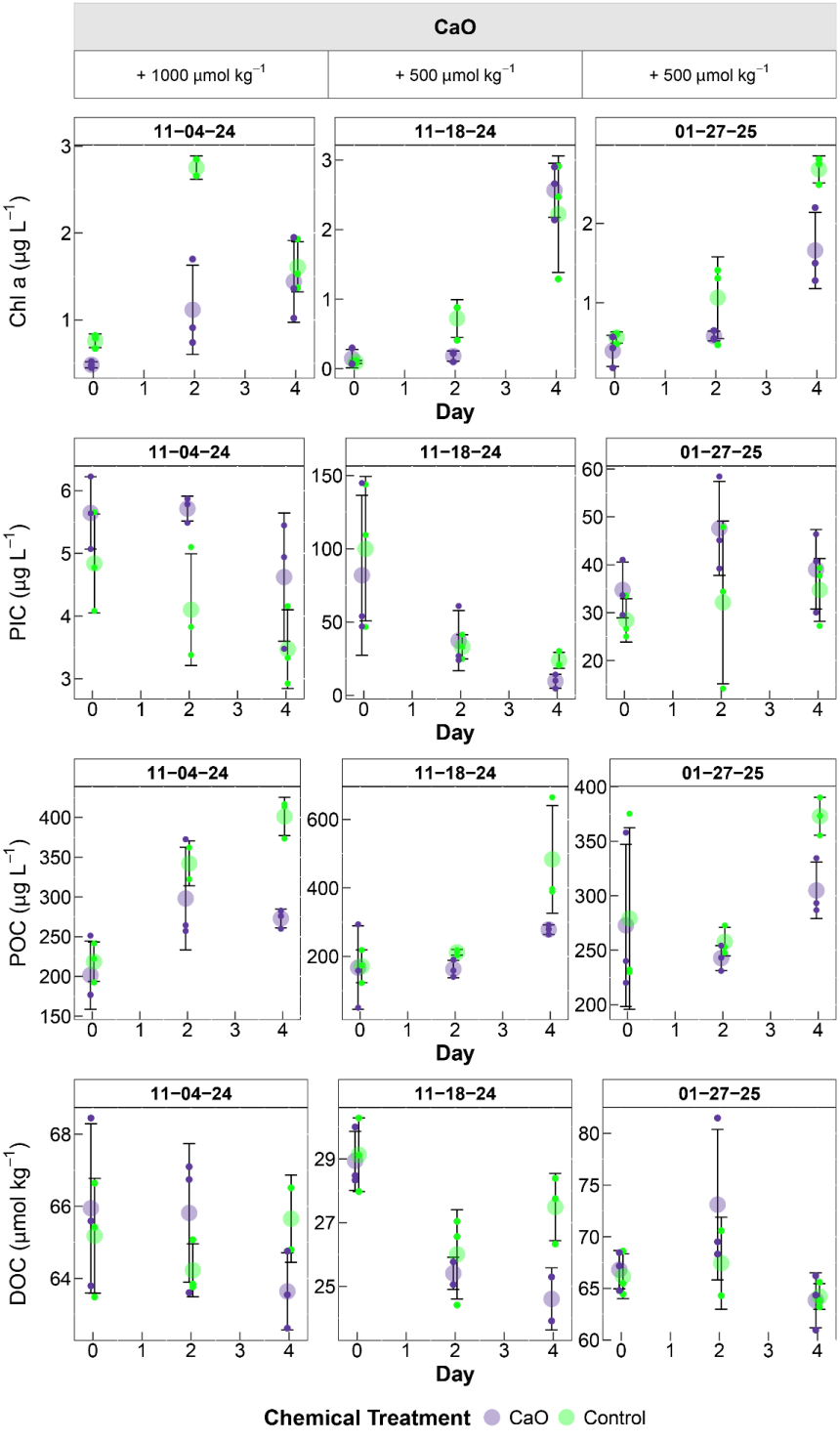

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Figure 5. Four Day Timeseries of Biogeochemical Response to Different Alkalinity Enhancements. Smaller symbols show individual bottles, large symbol shows mean. Error bars are the standard deviation between replicates (n = 3). The start date of the experiment and the amount of alkalinity added are included as headers for reference. The variables displayed are Chl-a (µg L^{-1}), PIC - Particulate Inorganic Carbon (µg L^{-1}), POC- Particulate Organic Carbon (µg L^{-1}), and DOC- Dissolved Organic Carbon (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+HCO3	500	PIC	-0.16 ± 0.19	0.02 ± 0.19	0.53	FALSE
	Ca+ HCO3	500	POC	156 ± 25	170 ± 28.6	0.73	FALSE
	Ca+ HCO3	500	CHL	1.10 ± 0.54	0.26 ± 0.54	0.29	FALSE
	Ca+ HCO3	500	O_2	13.5 ± 2.82	13.1 ±3.12	0.93	FALSE
07-15-24	Ca+ HCO3	500	PIC	0.72 ± 0.22	0.68 ±0.22	0.91	FALSE
	Ca+ HCO3	500	POC	98.4 ± 16	98.9 ± 16	0.98	FALSE
	Ca+ HCO3	500	DOC	2.83 ± 5.18	7.05 ± 5.18	0.57	FALSE
	Ca+ HCO3	500	CHL	0.78 ± 0.47	0.53 ± 0.47	0.71	FALSE
	Ca+ HCO3	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

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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+HCO3	500	PIC	0.39	FALSE
	Ca+ HCO3	500	POC	0.94	FALSE
	Ca+ HCO3	500	CHL	0.56	FALSE
	Ca+ HCO3	500	O_2	0.95	FALSE
07-15-24	Ca+ HCO3	500	PIC	0.89	FALSE
	Ca+ HCO3	500	POC	0.53	FALSE
	Ca+ HCO3	500	DOC	0.18	FALSE
	Ca+ HCO3	500	CHL	0.82	FALSE
	Ca+ HCO3	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.

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410 TRUE indicates whether the interaction is statistically significant with an ($\alpha = 0.05$), FALSE shows an
411 insignificant impact on the measured "Variable" column.

412

413 **3.4 Carbon Budgets**

414 **3.4.1 Carbon Isotopes to track Respiration**

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

430

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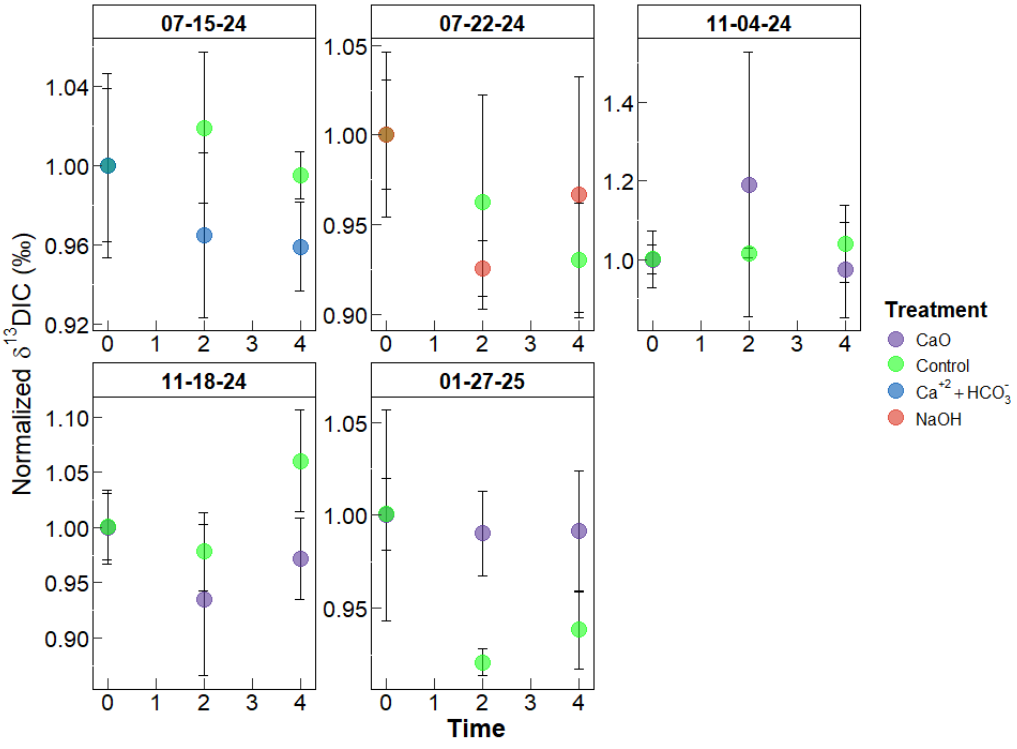

Figure 6. Normalized Seawater (DIC) $\delta^{13}C$ ‰ over Time in all Experiments. Panel (07-15-24) + 500 µmol kg^{-1} $CaCl_2$ & $NaHCO_3$, (07-22-24) + 500 µmol kg^{-1} NaOH, (11-04-24) + 1000 µmol kg^{-1} CaO, (11-18-24) + 500 µmol kg^{-1} CaO with higher ambient PIC, and (01-27-25) + 500 µmol kg^{-1} 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 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 general, ~95 % of the carbon

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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). The addition of alkalinity does not change carbon distribution in most of these experiments (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.

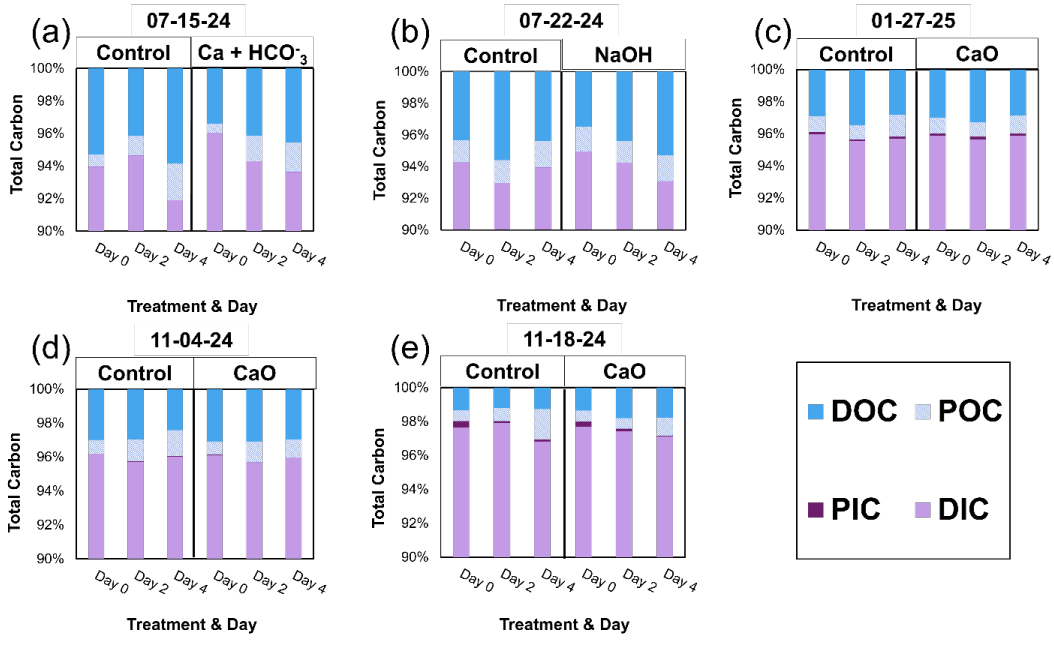

Figure 7. Total Carbon Budget for All Experiments. Panel (a) + 500 µmol kg^{-1} $CaCl_2$ & $NaHCO_3$, (b) + 500 µmol kg^{-1} NaOH, (c) + 500 µmol kg^{-1} CaO, (d) + 1000 µmol kg^{-1} CaO, and (e) + 500 µmol kg^{-1} CaO with higher (ambient) particles of inorganic carbon. Bar plots are an average of 3 bottles per timepoint. Plots show changes in distribution of carbon over the duration of the experiment between treatments and controls.

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

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experiments show a deficit of POC production relative to DIC drawdown. DIC lost to the precipitation of calcium carbonate is < 1 µmol kg^{-1} over 4 days, and CO_2 lost from the minimal permeability of the PETG bottles could be at most 3 µmol kg^{-1} day^{-1} (assuming 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). Figure 8 (a) shows that the 50-100 µmol kg^{-1} decrease in DIC must lead to greater production of 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:O2 ratios is considered. The calcium + bicarbonate experiments and one of their controls fall furthest from the range of Redfield ratios. The same Ca^{+2} + HCO_3^- experiments that show a deficit in POC also show a deficit in dissolved oxygen than what would be expected if DIC consumption were producing DOC.

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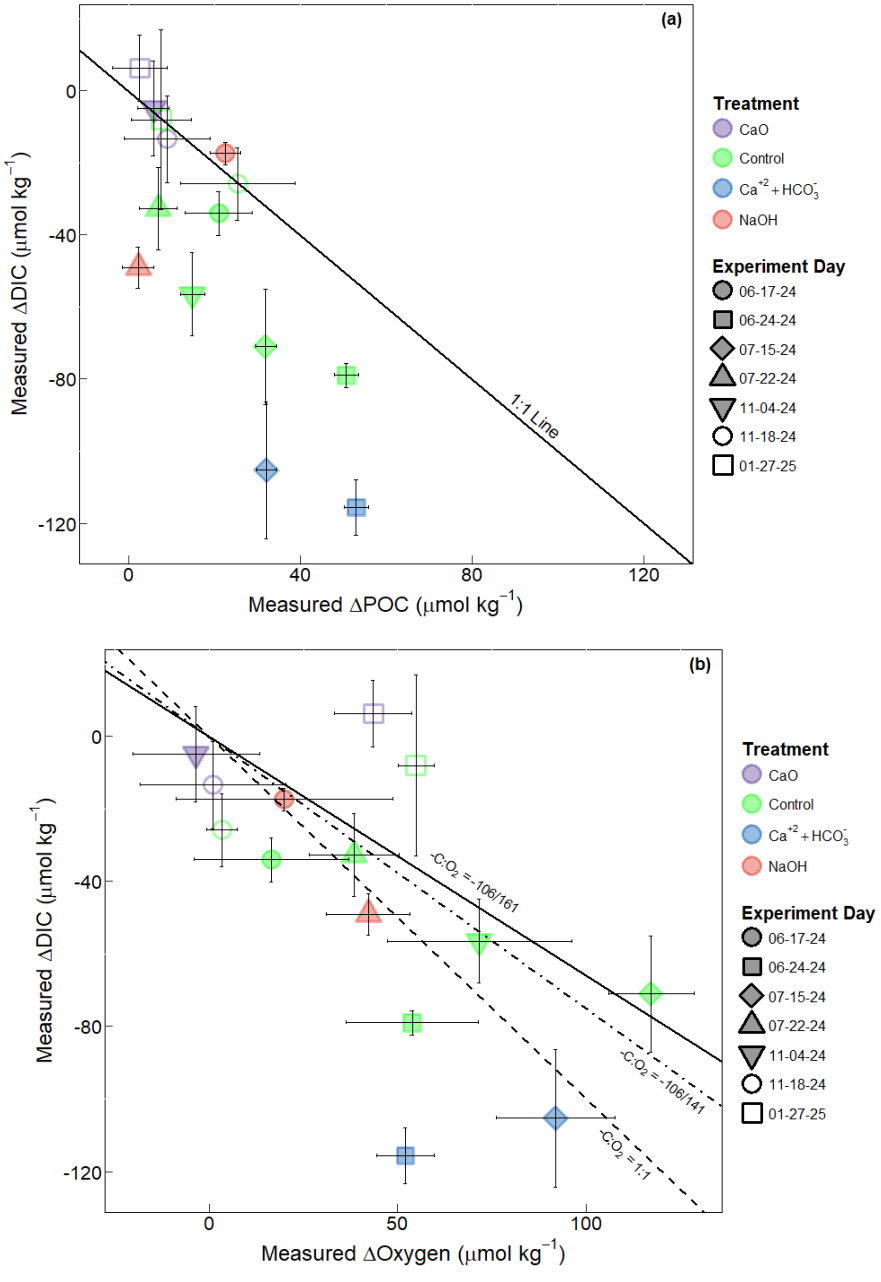

Figure 8. Comparison of Stoichiometric Changes in DIC, Dissolved O_2, and POC in terms of the Redfield Ratio. (a) Cross plot of measured decrease of DIC versus the measured increase of POC; solid line is the 1:1 line. Points that fall below the 1:1 line are thought to have increased DOC. (b) Cross plot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

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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. Thus, we conducted standardized experiments that raised alkalinity by the same threshold (+500 µmol kg^{-1}). 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.

4.1 Secondary Precipitation of $CaCO_3$

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 $\Omega_{aragonite} \sim 14$ (CaO + 1000 µmol kg^{-1}) – which is above the threshold for pseudo-homogenous precipitation in seawater according to Morse and He (T = 25 °C (Morse and He, 1993)) 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) (Hartmann et al., 2023; Moras et al., 2022; Suitner et al., 2024). In the case of NaOH addition experiments, immediate brucite ($Mg(OH)_2$) precipitation was observed in multiple studies (Bach et al., 2024; Ferderer et al., 2022; Hashim et al., 2025; Ringham et al., 2024; Suitner et al., 2024), 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 (Ringham et al., 2024; Suitner et al., 2024) or after 24 hours at $\Omega_{aragonite} > 15$ (T = 11-23 °C, (Hartmann et al., 2023; Suitner et al., 2024)). Hashim et al. (2025)

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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 (Hartmann et al., 2023; Ringham et al., 2024; Suitner et al., 2024).

We expected to see increased PIC formation with the CaO addition experiments ($\Omega_{aragonite}$ = 8.5, 8.6, 13.7) because Moras et al., (2022) 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 (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). 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 Impacts of NaOH and 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 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 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

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et al., 2017). 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, 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. 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.

Of note is the natural variability of pH in LA Harbor waters which has been previously measured within range 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 NaOH or HCO_3 in these experiments (pH in Treatments < 8.55). 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 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. 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 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}

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addition, *which is statistically significant*. This coincides with statistically lower dissolved oxygen after a 1000 µmol kg^{-1} CaO addition. 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} these results 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. When the CaO addition raises pH above 9, 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 (Bach et al., 2011; Faucher et al., 2025; Riebesell et al., 1993; Sett et al., 2014) and some 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 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

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,

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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.

Data Availability

Data are publicly available at 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.

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