Responses of keystone phytoplankton groups to olivine dissolution products and implications for carbon dioxide removal via ocean alkalinity enhancement

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Abstract

Anthropogenic greenhouse gas emissions are leading to global temperature increases, ocean acidification, and significant ecosystem impacts. Given current emissions trajectories, the IPCC calls for both the rapid abatement of CO_2 emissions and development of carbon dioxide removal (CDR) strategies that can address legacy emissions and difficult to abate emissions sources. These CDR methods must efficiently and safely sequester gigatons of atmospheric CO_2. Coastal Enhanced Weathering (CEW) via the addition of the common mineral olivine to coastal waters is one promising approach to enhance ocean alkalinity for large-scale CDR. As olivine weathers, it releases several biologically active dissolution products, including alkalinity, trace metals, and the nutrient silicate. Released trace metals can serve as micronutrients but may also be toxic at high concentrations to marine biota including phytoplankton that lie at the base of marine food webs. We grew several globally important phytoplankton functional groups under elevated concentrations of olivine dissolution products using a synthetic olivine leachate (OL) based on olivine elemental composition, and monitored their physiological and biogeochemical responses. This allowed us to determine physiological impacts and thresholds at elevated olivine leachate concentrations, in addition to individual effects of specific constituents. We found both positive and neutral responses but no evident toxic effects for two silicifying diatoms, a calcifying coccolithophore, and three cyanobacteria. In both single and competitive co-cultures, silicifiers and calcifiers benefited from olivine dissolution products like iron and silicate or enhanced alkalinity, respectively. The non-N_2-fixing picocyanobacterium could use synthetic olivine-derived iron for growth, while N_2-fixing cyanobacteria could not. However, other trace metals like nickel and cobalt supported cyanobacterial growth across both groups. Growth benefits to particular phytoplankton groups *in situ* will depend on species-specific responses and ambient concentrations of other required nutrients. Results suggest olivine dissolution products appear unlikely to cause negative effects for marine phytoplankton, even at high concentrations, and may support growth of particular taxa under some conditions. Future studies can shed light on long-term evolutionary responses to olivine exposure, and on the potential effects that marine microbes may in turn have on olivine dissolution rates and regional biogeochemistry.

Introduction

Excess anthropogenic greenhouse gas emissions are driving global changes to Earth systems and leading to simultaneous increases in sea surface temperatures, ocean acidification, and regional shifts in nutrient supplies (Bach et al., 2019). To counteract these trends and limit the average global temperature increase to 1.5-2°C, carbon dioxide removal (CDR) methods that can collectively remove and permanently store gigatons of atmospheric CO_2 ($GtCO_2$) must be developed (Renforth and Henderson, 2017). Coastal Enhanced Weathering (CEW) with olivine ($Mg_{2-x}Fe_xSiO_4$) has been proposed as an economically scalable form of ocean alkalinity enhancement (OAE), as it is a globally abundant, naturally occurring ultramafic silicate mineral (Bach et al., 2019; Beerling et al., 2021). Olivine is considered to be one of the most favorable minerals for CDR as it weathers quickly under Earth surface conditions (Renforth and Henderson, 2017; Hartmann et al., 2013; Rimstidt et al., 2012). Similar to other silicate minerals, it dissolves in water to release cations (Mg^{2+}, Fe^{2+}) and generates alkalinity (principally HCO_3^-), with up to 4 mol of CO_2 sequestered per mol of olivine [Eq. 1].

$$Mg_{2-x}Fe_xSiO_4 + 4CO_2 + 4H_2O \text{ --> } 2\text{-}xMg^{2+} + xFe^{2+} + 4HCO_3^- + H_4SiO_4 \qquad \text{[Eq. 1]}$$

Forsteritic olivine is the magnesium-rich end-member of olivine and can contain various other trace constituents. For example, olivine used in this study contains ~92% magnesium (Mg^{2+}) and ~8% ferrous iron (Fe^{2+}) along with trace amounts (<1%) of other metals such as nickel (Ni), chromium (Cr), and cobalt (Co). As olivine weathers, it releases several biologically important dissolution products into the surrounding seawater: (I) bicarbonate (HCO_3^-) and carbonate ion (CO_3^{2-}), hereafter summarized as "alkalinity"; (II) silicic acid ($Si(OH)_4$) hereafter termed silicate; (III) and a variety of trace metals including iron (Fe^{2+}, or oxidized aqueous species), nickel (Ni^{2+}), cobalt (Co^{2+}), and chromium (CrVI). These dissolution products have the potential to affect important phytoplankton functional groups like silicifying algae (diatoms), calcifying algae (coccolithophores), and cyanobacteria, which lie at the base of marine food webs and drive the biological carbon pump (Moran, 2015). Hence, it is important to understand the specific effects of these constituents on keystone phytoplankton groups, particularly at elevated concentrations to simulate large-scale CEW applications.

Significant alkalinity additions from olivine weathering can consume CO_2 from the surrounding seawater, causing a CO_2 deficit until air-sea equilibration. This shift in the carbonate system from CO_2 to HCO_3^-/CO_3^{2-} by transient, non-equilibrated OAE will affect phytoplankton functional groups differently, with some taxa being more sensitive than others. For example, it is predicted that calcifying organisms like coccolithophores may benefit from CEW due to decreases in proton concentrations (H^+) and increases in the $CaCO_3$ saturation state. Additionally, dissolving one mole of olivine leads to a one mole increase in dissolved silicate, which is an essential and often bio-limiting nutrient for silicifying organisms like diatoms, a phytoplankton group estimated to contribute up to 40% of the marine primary production

(Bertrand et al., 2012). Hence, diatoms may especially benefit from CEW applications with olivine. Additionally, diatoms are particularly noted for being dominant phytoplankton in the coastal regimes where olivine deployments are likely to take place (Field et al., 1998). While there are both planktonic and benthic species of diatoms, the latter will presumably be exposed to especially sustained and elevated levels of dissolution products when olivine is deployed in natural marine sediments. It is unknown if either group, calcifiers or silicifiers, may consistently outcompete the other following CEW with olivine (Bach et al., 2019).

Trace metals like Fe and Ni are general micronutrients required by all classes of phytoplankton, and could potentially support their growth upon fluxes into seawater from olivine weathering. In particular, dinitrogen (N_2)-fixing cyanobacteria and diatoms both have elevated Fe requirements (Hutchins and Sañudo-Wilhelmy, 2021; Hutchins and Boyd, 2016), and so may stand to benefit from increases in Fe concentrations. Although a required micronutrient at low levels, in high enough concentrations Ni may potentially negatively impact phytoplankton growth, although one recent study showed limited to no toxic effects of very high Ni concentrations (e.g. 50,000 nmol L^{-1}) for several phytoplankton taxa (Guo et al., 2022). Cobalt can also serve as a micronutrient for phytoplankton (Sunda and Huntsman, 1995; Hawco et al., 2020) but may also be toxic at high concentrations (Karthikeyan et al., 2019). However, other trace metals found with olivine such as Cr are not nutrient elements and also need to be considered in terms of their possible toxicity to phytoplankton (Flipkens et al., 2021; Frey et al., 1983).

Hence, it is important to understand the taxon-specific effects of these constituents to determine thresholds at which key phytoplankton functional groups may experience positive or negative effects. Furthermore, it is important to expose phytoplankton to elevated concentrations of olivine dissolution products simultaneously to understand what impacts may occur for large CEW applications. Exposures of organisms to concentrated olivine dissolution products also provides an "worst case scenario" benchmark, which can be compared to lower actual environmental exposures resulting from small CEW additions, slower olivine dissolution time scales, and dilution from advection. While olivine weathers relatively quickly compared to other silicate minerals (Hartmann et al., 2013), dissolution of olivine grains is gradual (i.e. years) relative to microbial physiological responses (hours), posing a challenge to test different concentrations of olivine constituents on phytoplankton physiology. To address this, we prepared a synthetic olivine leachate (OL) composed of olivine dissolution products with trace metal concentrations well over those of seawater (17-12,000 times higher), in order to represent a "worst case" scenario for a CEW project. This scenario was estimated based on the maximum expected impact of olivine weathering on the chemistry of the overlying water column. Assuming a 10 cm thick layer of pure olivine sand dissolves with a 100 year half-life dissolving into 1 meter of overlying water with a 24 hour residence time, the anticipated steady state change in the alkalinity of the overlying water column is 65 umol/kg (assuming 4 moles of alkalinity per mole olivine (Meysman and Montserrat, 2017) and 100% release to the water column). The concentrations of other components were chosen assuming stoichiometric congruent dissolution

and quantitative release to the water column as well -- a worst case scenario. Furthermore, phytoplankton were exposed to OL within a small, enclosed batch culture. We cultured 6 species representing three globally important phytoplankton functional groups: 2 diatoms (*Nitzschia, Ditylum*), 1 coccolithophore (*Emiliana*), 2 dinitrogen (N_2) fixing cyanobacteria (*Trichodesmium*, *Crocosphaera*), and 1 non-N_2 fixing picocyanobacterium (*Synechococcus*). All of these species are planktonic, with the exception of the diatom *Nitzschia* which frequently forms benthic biofilms (Yamamoto et al., 2008). Cultures were grown semi-continuously in natural seawater based modified Aquil media (Sunda et al., 2005) with OL as the only available Fe source (and Si source for diatoms). For all experiments, cultures were sampled for a basic set of core biogeochemical and physiological parameters (Fu et al., 2005, 2008; Tovar-Sanchez et al., 2003; Paasche et al., 1996). This approach allowed us to compare and contrast phytoplankton taxon-specific responses, including: **1)** physiological impacts at extremely high OL concentrations, **2)** physiological thresholds and dose responses across a range of increasing concentrations of OL, and **3)** individual effects of specific OL constituents.

Materials and Methods

Culture growth conditions and experimental set up

Six species of phytoplankton were used in these experiments, including two diatoms, *Ditylum* (centric, planktonic) and *Nitzschia* (pennate, benthic), one coccolithophore, *Emiliania huxleyi,* one picoplanktonic cyanobacterium (*Synechococcus*), and two marine dinitrogen (N_2) fixing cyanobacteria, *Trichodesmium* IMS 101 and *Crocosphaera* WH 0005. Cultures were grown in 500 mL polycarbonate flasks at 28^0C for the three cyanobacteria, and 20^0C for the diatoms and *Emiliania huxleyi*. Cool-white fluorescent light was supplied following a 12:12 light:dark cycle at an irradiance level of 150 $\mu Em^{-2}s^{-1}$. Stock cultures were grown in natural offshore seawater collected with trace metal clean methods (John et al., 2022), which was used to make modified Aquil control medium (ACM) (Sunda et al., 2005). For experiments, cultures were inoculated into the three treatments described below with the addition of 4µM phosphate (PO_4^{3-}) and 60 µM nitrate (NO_3^-). There was no nitrogen (N) added into the Aquil medium for the N_2 fixers. Iron, Cobalt, Nickel (Fe, Co and Ni, required by all species) and silicate ($SiOH_4$, required by diatoms only) were not added to the Aquil medium, except as components of the olivine leachate (see below). The background nutrient concentrations in the collected natural seawater were 1µM NO_3^-, 0.1µM PO_4^{3-} and 3µM $SiOH_4$.

Synthetic olivine leachate preparation

To simulate acute exposure of phytoplankton to elevated levels of olivine dissolution products in seawater, we prepared an artificial concentrated OL stock solution based on elemental analyses of commercial ground olivine rock (Sibelco. (2022) Technical Data - Olivine Refractory Grade

Fine. Antwerp, Belgium). For experimental exposures, this concentrated OL stock was added to seawater growth medium to yield the final concentrations shown in **Table 1**, which will be referred to throughout as a "100%" concentration of OL. Experiments examining biological effects across a dilution range (0-100%) used correspondingly lower additions of the concentrated stock. The only other components added to the OL were the major nutrients nitrate (60 µM NO_3^-) and phosphate (4 µM PO_4^{3-}). These are required nutrients for growth of phytoplankton, and were added at the same concentration to the OL and the Aquil control medium. For experiments with N_2-fixing cyanobacteria, nitrate was omitted and phosphate was the only nutrient added.

Table 1. Concentrations of added ions or compounds in serial dilutions of synthetic olivine leachate (OL, 0% to 100%) used in the phytoplankton growth experiments; and concentrations of components in the three concentrated stocks used to prepare experimental medium (1mL/L added for 100% OL). Stock C was prepared in 10 nM HCl to keep the trace metals in solution until addition.

	OL Added	Mg^{2+} (µM)	$SiOH_4$ (µM)	OH^- (µM)	Fe(II) (µM)	Ni(II) (µM)	Cr(VI) (µM)	Co(II) (µM)
Concentration added to growth medium	100%	44.9	25	100	3.36	0.13	0.12	0.006
	80%	35.9	20	80	2.7	0.10	0.10	0.005
	50%	22.5	12.3	50	1.7	0.07	0.06	0.003
	30%	13.5	7.5	30	1.0	0.04	0.04	0.002
	10%	4.5	2.5	10	0.34	0.01	0.01	0.001
	0%	0	0	0	0	0	0	0
Concentrated stock solutions (mM)		$MgCl_2$	$NaSiO_2$	NaOH	$FeCl_2$	$NiCl_2$	K_2CrO_4	$CoCl_2$
Stock A		44.9						
Stock B			25	100				
Stock C					3.36	0.13	0.12	0.006

Experimental methods

Semi-continuous culturing methods were used to achieve nearly steady-state growth. Cultures were diluted with fresh medium every 2 or 3 days, using in vivo fluorescence as a real time biomass indicator. Dilutions were calculated to bring the cultures back down to the biomass levels that were recorded after the previous day's dilution. In this way, cultures were allowed to determine their own growth rates under each set of experimental conditions, without ever nearing stationary phase, significantly depleting nutrients or self-shading (Fu et al., 2022). For all experiments, cultures were sampled for a basic set of core biomass and physiological parameters, including cell counts, CO_2 fixation, particulate organic carbon (POC), particulate organic nitrogen (PON), particulate organic phosphorus (POP) and biogenic silica (BSi, diatoms only) once steady-state growth was obtained for each growth condition (typically after 8−10

generations). Steady-state growth status was defined as no significant difference in cell- or in vivo-specific growth rates for at least 3 consecutive transfers.

There were four sets of experiments in this project:

1) Acute responses to elevated olivine leachate levels. The goal of this set of experiments was to investigate the responses of the diatoms *Nitzschia* and *Ditylum* to relatively high concentrations of olivine leachate, in order to determine acute exposure responses. To see if the leachate may have a positive or negative effect on their physiology, they were compared to their respective control cultures. There were a total of three treatments consisting of: OL (100%), ACM, and ACM with low Fe/Si (with 2 nM Fe EDTA added, and no added $SiOH_4$).

2) *Responses to a broad range of olivine leachate levels*. In these experiments, *Synechococcus*, *Crocosphaera, Ditylum,* and *Emiliania huxleyi* were grown in culture medium across a series of OL dilutions (Table 1) to determine their responses across a range of leachate concentrations, from high to very low-level exposures.

3) Fe bioavailability and Cr toxicity from olivine leachate to N_2-fixing cyanobacteria. The goal of this set of experiments was to investigate OL-derived Fe bioavailability to N_2-fixing cyanobacteria, *Trichodesmium* and *Crocosphaera*. An additional experiment was conducted to investigate potential Cr(VI) toxicity.

4) *Two species co-culture competition experiments during olivine leachate exposure*. In order to test how OL may affect co-existence and competition between the diatom *Ditylum* and the coccolithophore *Emiliania huxleyi*, a simple batch co-culture competition experiment was carried out in which the 2 species were inoculated at a 1:1 ratio (based on equivalent levels of cellular Chlorophyll a due to the large differences in their cell sizes) into 100% OL and regular ACM, and grown for 10 days until early stationary phase. In vivo fluorescence and cell counts were monitored daily. Relative abundance and growth rates of the two species were determined based on microscopic cell counts during the exponential growth phase of the mixed cultures. Biogenic silica (BSi, an indicator of diatom abundance) and particulate inorganic carbon (PIC or calcite, an indicator of coccolithophore abundance) were collected every other day in order to further determine how these two species responded to co-culture with and without leachate additions.

Analytical methods

Determination of growth rates and chlorophyll a Growth rates were determined based on both microscopic cell counts and chlorophyll a. For chlorophyll a determination, subsamples of 30 ml from each triplicate bottle were GF/F filtered, extracted in 6 ml of 90% acetone, stored overnight in the dark at −20°C, and chlorophyll a concentrations were measured fluorometrically using a

Turner 10-AU fluorometer (Welschmeyer, 1994). Specific growth rates were determined using the equation:

$$\mu = \frac{\ln\left(\frac{N_{Tfinal}}{N_{Tinitial}}\right)}{T_{final} - T_{initial}}$$

where μ is the specific growth rate (per day) and *N* is the chlorophyll a concentration at $T_{initial}$ and T_{final} (Kling et al., 2021).

Particulate C, N, P and Si. Particulate organic carbon and nitrogen (CHN) samples from all experiments were filtered (pre-combusted GF/F) and frozen for analysis using a Costech Elemental Analyzer (Hutchins et al., 2007). Samples for biogenic silica (BSi) were analyzed according to (Brzezinski, 1985). POP (particulate organic phosphorus) samples were collected onto pre-combusted 25 mm GF/F filters and analyzed as in Fu et al. 2005 (Fu et al., 2005).

Primary productivity. For all species other than the coccolithophore (see below), primary production was measured in triplicate using 24h incubations (approximating net PP) with $H^{14}CO_3$ under the appropriate experimental growth conditions for each treatment (Fu et al., 2008). CO_2 fixation rates were calculated using measured final experimental DIC concentrations and biomass. All samples for primary production were counted using a Wallac System 1400 liquid scintillation counter.

Photosynthetic and calcification rates of Emiliania huxleyi. For the coccolithophore, two 40 mL subsamples from each triplicate bottle were spiked with 0.5 μCi $NaH^{14}CO_3$. One subsample was incubated in the light and the other in the dark for 24 h. Then two sets of 20 mL aliquots from each sub- sample were filtered onto Whatman GF/F filters. The filters for photosynthetic rate determination were fumed with saturated HCl before adding scintillation cocktail fluid. Photosynthetic rate and calcification rate were calculated as described in Paasche et al. 1996 (Paasche et al., 1996).

Fe quota. Intracellular Fe content was determined by filtering culture samples onto acid-washed 0.2-μm polycarbonate filters (Millipore), and rinsing with oxalate reagent to remove extracellular trace metals (Tovar-Sanchez et al., 2003). Fe was determined with a magnetic sector-field high-resolution inductively coupled plasma mass spectrometer (ICPMS) (Element 2, Thermo) (Jiang et al., 2018; John et al., 2022).

Statistical methods. A one-way ANOVA analysis of variance was used to analyze differences between treatments using Prism 8. Differences between treatments were considered significant at $p<0.05$. Post-hoc comparisons were conducted using the Tukey's multiple comparison test to determine any pairwise differences.

Results

Diatoms

We hypothesized that diatoms may benefit from the OL products Si and Fe, as they are both required for growth and can be limiting for this group (Tréguer et al., 2018). Hence, we grew the benthic diatom *Nitzschia* across three treatments: OL alone, Aquil control medium (ACM), and ACM but with low, limiting Si and Fe concentrations (ACM-low-SF). *Nitzschia* grew and fixed carbon just as well in the OL as the ACM ($p=0.35$; $p=0.21$), while showing reduced rates in the ACM-low-SF treatment (**$p=0.02$; $p< 0.0001$; Fig. 1A, B**). Likewise, the particulate Si:C ratios demonstrated OL to be just as good a source of Si to *Nitzschia* as the ACM ($p=0.98$), and considerably better than the ACM-low-SF ($p=0.0012$; **Fig. 1C**).

Growth and CO_2 fixation rates of the planktonic diatom *Ditylum* were significantly higher in the OL treatment compared to either the ACM ($p=0.002$; $p=0.0001$) or ACM-low-Si/Fe treatments ($p=0.0002$; $p<0.0001$; **Supp. Fig. 1 A, B**), while Si:C ratios were the same ($p=0.93$; **Supp. Fig 1C**). When *Ditylum* was grown across a range of OL concentrations (i.e., a dilution series from 0% to 100% additions, where 100% corresponds to the OL treatment used in the *Nitzschia* experiment above), we observed increasing growth and CO_2 fixation rates with increasing OL concentrations, with maximum rates observed at and above 50% of the original OL (**Fig. 2A, B**). *Ditylum* particulate Si:C ratios also reached levels similar to those seen in the ACM medium in the 80-100% additions (**Fig. 2C**). Likewise, *Ditylum* cellular Fe:P ratios measured by ICP-MS were not significantly different between 100% OL and ACM treatments, suggesting the diatom could access the same amount of Fe from the precipitated Fe(III) in the OL as from the soluble (EDTA-chelated) Fe(III) in the culture medium ($p=0.56$; **Supp. Fig 2A**). These data demonstrate that even at extremely high concentrations, olivine dissolution products including trace metals are not toxic to diatoms, but instead may provide sources of the essential nutrients iron and silicate to support their growth in nutrient replete conditions.

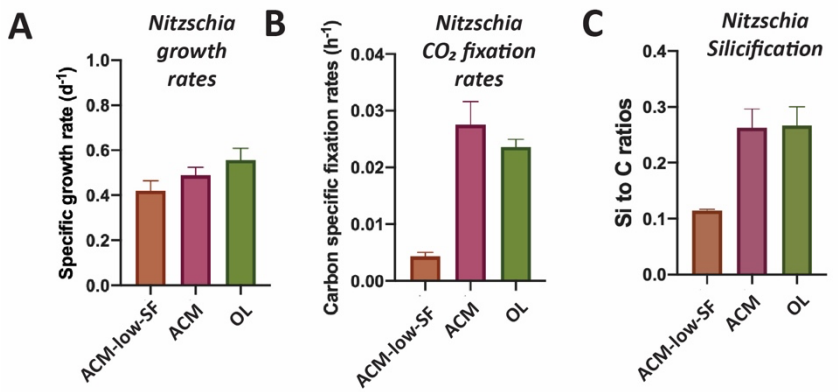

Fig 1. Effects of olivine leachate versus culture medium controls on growth and physiology of the benthic diatom *Nitzschia*. A) Cell-specific growth rates (d^{-1}), **B)** Carbon-specific fixation rates (hr^{-1}) and **C)** Si:C ratios (mol:mol) of the benthic diatom *Nitzschia* sp. Abbreviations: OL is olivine leachate, ACM is Aquil control medium, ACM-low-SF is Aquil control medium with lowered Si and Fe concentrations. Values represent the means and error bars are the standard deviations of triplicate cultures for each treatment.

Coccolithophores

It has been hypothesized that calcifying coccolithophores may benefit from an increase in alkalinity from olivine dissolution (Bach et al., 2019). In the OL dilution series, maximum growth rates for the coccolithophore *Emiliana* equivalent to those recorded in the ACM positive control medium were achieved at all added OL concentrations from 10% to 100% (**Fig. 2D**). POC production (CO_2 fixation) rates were similar at all OL levels from 30% to 100%, and were not significantly different than in the ACM treatment ($p > 0.05$, **Fig. 2E**). Particulate inorganic carbon to particulate organic carbon fixation ratios (PIC:POC production ratios) were higher at OL levels of 50-100% than in the ACM positive controls (**Fig. 2F**), possibly due to enhanced alkalinity in the high OL concentration treatments.

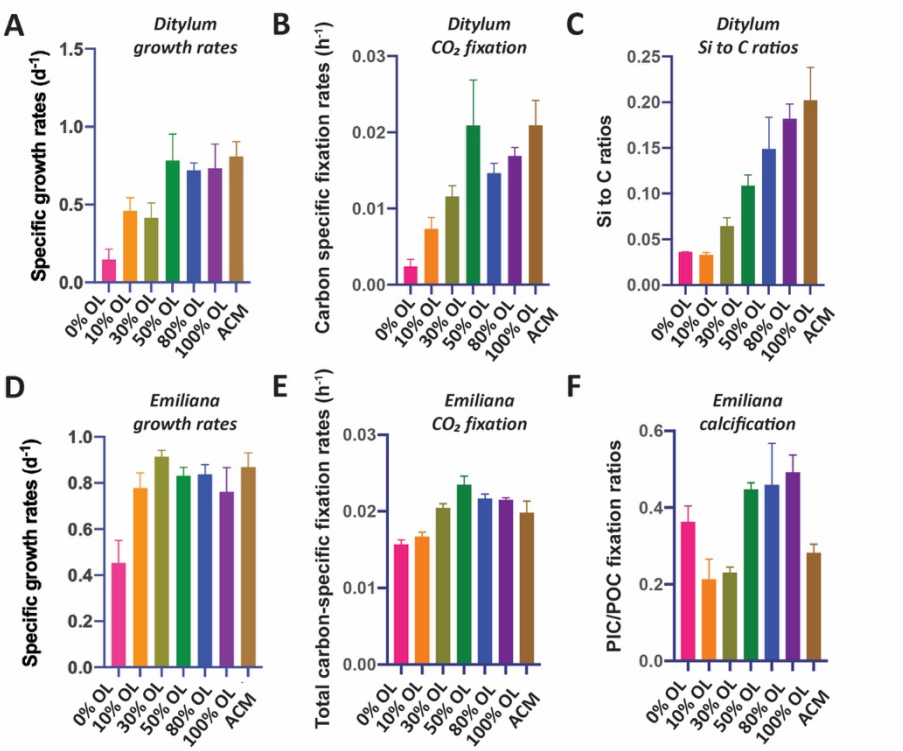

Fig 2. Dilution series of olivine leachate on growth and physiology of a marine diatom and coccolithophore. The diatom *Ditylum* sp. and the coccolithophore *Emiliana huxleyi* were grown across a range of dilutions of the olivine leachate (OL, 0-100%), and in the Aquil control medium (ACM)**.** Shown are: **A)** Cell-specific growth rates (d^{-1}), **B)** Carbon-specific fixation rates (hr^{-1}) and **C)** and Si:C ratios (mol:mol) of *Ditylum* sp.. Next, are **D)** Cell-specific growth rates (d^{-1}), **E)** Carbon-specific fixation rates (hr^{-1}), and **F)** PIC production (calcification) rates (hr^{-1}) of *Emiliana huxleyi* in the same OL and ACM treatments. Values represent the means and error bars are the standard deviations of triplicate cultures for each treatment.

An independent set of basic two-treatment experiments with the coccolithophore (ACM versus 100% OL, **Supp. Fig 3**) supported the results of the dilution series experiments shown in **Fig. 2.** *Emiliana* specific growth rates ($p=0.05$), and cellular particulate inorganic:particulate organic carbon ratios ($p= 0.07$; PIC:POC, mol:mol) were very similar in the ACM and OL treatments (**Supp. Fig. 3A**). Likewise, POC-specific fixation rates ($p=0.04$; TC h^{-1}) were slightly higher in the OL than in the ACM treatments, while PIC:POC fixation ratios were the same ($p=0.07$, **Supp. Fig. 3B**). Similar to diatoms, these data demonstrate that olivine dissolution products are also not toxic to coccolithophores and that enhanced alkalinity may support growth in nutrient replete conditions.

Cyanobacteria

Like diatoms and coccolithophores, cyanobacteria could benefit from olivine dissolution due to their relatively high Fe (Hutchins and Boyd, 2016) and Ni requirements (Dupont et al., 2008). The OL dilution series experiments using the widely distributed picocyanobacterium *Synechococcus* showed positive responses in growth rates (**Fig. 3A**) and CO_2 fixation rates (**Fig. 3B**) across the range of OL levels, similar to those of the eukaryotic algae. Both growth rates and carbon fixation rates were the same in the 100% OL treatment as in the ACM positive control treatment (p=0.94; p=0.46). ICP-MS measurements of *Synechococcus* cellular Fe:P ratios across a range of OL levels (0-100%) showed that this isolate accumulated much less Fe in the 0% OL than in the ACM treatment (p=0.02), but in all treatments with added OL, Fe:P ratios were the same as or higher than the ACM values (**Supp. Fig. 2B**). As with the eukaryotic phytoplankton, the synthetic OL provided a good source of Fe to support the growth of the picocyanobacterium.

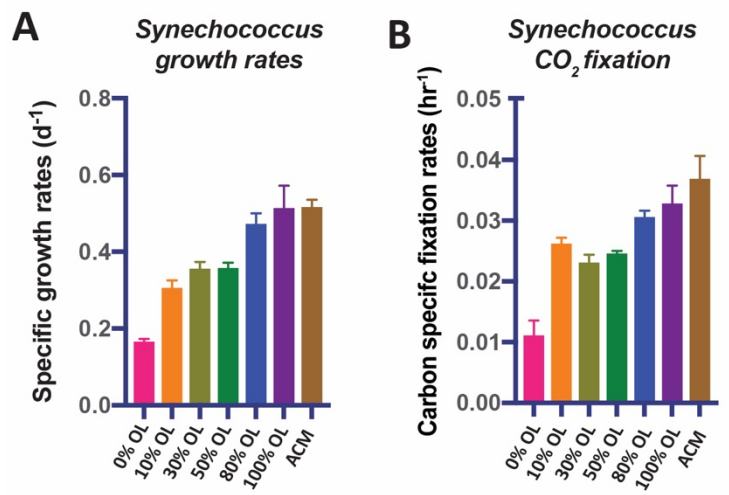

Fig 3. Dilution series of olivine leachate on growth and CO_2 fixation of a marine cyanobacterium. The unicellular picocyanobacterium *Synechococcus* was grown across a range of dilutions of the olivine leachate (OL, 0-100%), and in the Aquil control medium (ACM). Shown are: **A)** Cell-specific growth rates (d^{-1}) and **B)** Carbon-specific fixation rates (hr^{-1}). Values represent the means and error bars are the standard deviations of triplicate cultures for each treatment.

In striking contrast to the eukaryotic algae and the non-diazotrophic (i.e., non-N_2-fixing) picocyanobacterium *Synechococcus*, the N_2-fixing cyanobacterium *Trichodesmium* could not grow at any concentration of OL tested **(Fig. 4A)**. One possible explanation for this lack of growth is toxic effects by one of the trace metal components of the OL. We hypothesized that Ni and Co are less likely to be toxic, as these nutrient metals have been found to be relatively non-toxic to phytoplankton at similar environmental concentrations (Guo et al., 2022; Karthikeyan et al., 2019; Panneerselvam et al., 2018). Hence, we hypothesized that Cr toxicity should be considered as a likely possible scenario (Frey et al., 1983; Kiran et al., 2016).

Another possibility is that *Trichodesmium* did not experience toxic effects but instead was unable to access Fe from OL. This N_2-fixer requires more Fe than virtually any other phytoplankton species (Hutchins and Sañudo-Wilhelmy, 2021), and the OL was the only source of Fe provided in our experiments. Fe(II) released into seawater from olivine dissolution likely quickly oxidizes to Fe(III), which then precipitates and becomes insoluble at the elevated

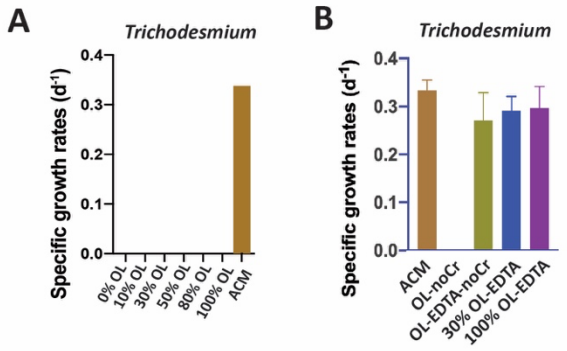

Fig 4. Effects of olivine leachate versus culture medium controls on growth of the marine N_2-fixing cyanobacterium *Trichodesmium*. Shown are **A)** Cell-specific growth rates (d^{-1}) of the colonial cyanobacterium *Trichodesmium* across a range of dilutions of the olivine leachate (OL, 0-100%) and in the Aquil control medium (ACM), and **B)** Cell-specific growth rates (d^{-1}) of *Trichodesmium* in two concentrations of OL (30% and 100%) with the synthetic metal chelator EDTA, and in OL without Cr, or OL without Cr but plus EDTA, versus ACM.

concentrations in our OL (Manck et al., 2022). This could render it biologically unavailable to the cellular Fe uptake systems of some species. We deliberately designed our OL to replicate this oxidation/precipitation process, and as expected observed visible reddish-brown amorphous Fe precipitates on the bottom of the growth flasks for all synthetic OL treatments.

Accordingly, we designed another set of experiments to test for both lack of Fe bioavailability and specific sensitivity to Cr, as has been done in previous cyanobacterial studies (Kiran et al., 2016). To do this, we formulated several variants of the olivine leachate: 1) normal OL (100% concentration), 2) OL (100% concentration) with a synthetic ligand (EDTA) that solubilizes Fe(III), and thus makes it broadly bioavailable (OL-EDTA), 3) OL (100% concentration) but with no Cr (OL-noCr), 4) and OL (100% concentration) but with no Cr and with EDTA (OL-EDTA-noCr) **(Fig. 4B**). *Trichodesmium* also could not grow in the OL medium without added Cr (OL-noCR, **Fig. 4B)**, demonstrating that the lack of growth observed in OL was not due to Cr toxicity. However, growth recovered to the same levels as in the ACM when EDTA was added (OL-EDTA) to the leachate **(**$p=0.16$; **Fig. 4B)**. This suggests that poor bioavailability of the precipitated Fe(III) was the likely cause for *Trichodesmium*'s inability to grow in the unmodified OL.

OL also inhibited the growth of the unicellular N_2-fixing cyanobacterium *Crocosphaera*, although not to the same extent as for *Trichodesmium. Crocosphaera* exhibited growth rates that were 22-44% of those in ACM across the full range of OL concentrations, but growth recovered in OL-EDTA to 76% of rates in ACM (**Fig. 5A**). Results were very similar for CO_2 fixation rates and N_2-fixation rates in OL, which were severely reduced by 64-100% (carbon fixation) and 69-88% (N_2 fixation) relative to ACM in all OL treatments, but reached maximum values of 80% and 63% of ACM treatment rates, respectively, when EDTA was added to the OL (**Fig. 5B and C**). This demonstrates that oxidized Fe from OL was not effectively utilized to support growth for either of the two N_2-fixing cyanobacteria tested, in contrast to diatoms, coccolithophores, and *Synechococcus*. However, their growth recovery after EDTA additions indicates that the other trace metals Cr, Ni, and Co in the olivine leachate were not toxic, even at extremely high concentrations. Interestingly, unlike *Trichodesmium* which either could not grow at all on OL alone or recovered fully upon EDTA additions, *Crocosphaera* could still grow at lower growth rates on OL but could not grow as fast upon EDTA additions as in ACM. Future experiments are

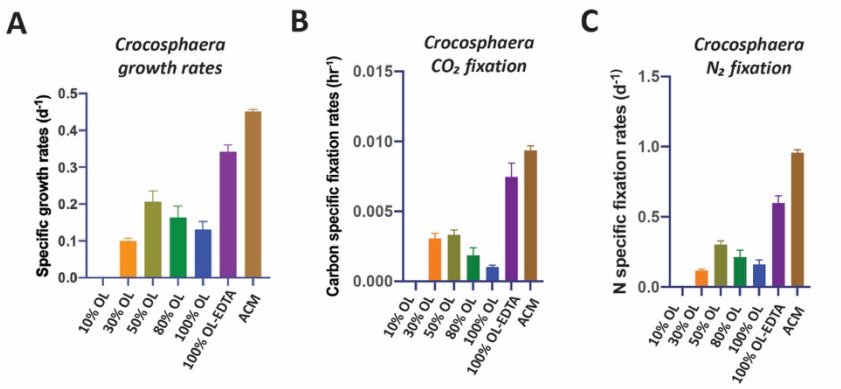

Fig 5. Effects of olivine leachate dilution series versus culture medium controls on the physiology of the marine N_2-fixing cyanobacterium *Crocosphaera*.
A) Cell-specific growth rates (d^{-1}), **B)** Carbon-specific fixation rates (hr^{-1}), and **C)** N-specific fixation rates (day^{-1}) of the unicellular cyanobacterium *Crocosphaera* grown across a range of dilutions of the olivine leachate (OL, 0-100%), in 100% OL plus EDTA (OL-EDTA), and in the Aquil control medium (ACM). Values represent the means and error bars are the standard deviations of triplicate cultures for each treatment.

needed to understand these differences in species-specific responses between these two N_2-fixers. Taken together, these data suggest that when olivine dissolves in seawater, it will likely have negligible or no effect (positive or negative) on these cyanobacteria.

Diatom/Coccolithophore Competitive Co-culture

Results of the co-culture, or competition, experiment with the diatom *Ditylum* and the coccolithophore *Emiliania huxleyi* are shown in **Fig. 6**. Unlike the semi-continuous experiments shown in the previous figures, this experiment used closed-system "batch" culturing methods in order to assess and compare effects on relative biomass accumulation by each species over time. OL (100% concentration) supported growth of both the diatom (**Fig. 6A**) and the coccolithophore (**Fig. 6B**) in mixed culture, and biomass was very similar for both species between the OL and ACM treatments throughout most of the experiment. However, cell yields were higher in the ACM at the final timepoint for the diatom ($p = 0.009$, **Fig. 6A**). Final cell counts were also higher in the ACM for the coccolithophore, but this difference was not significant ($p= 0.31$; **Fig. 6B**). Similar trends were observed when diatom biomass was estimated as biogenic silica (BSi, **Fig. 6C,** $p =0.002$) and when coccolithophore biomass was assessed as calcite or particulate inorganic carbon (PIC, **Fig. 6D,** $p = 0.04$). For the diatom, OL supported growth rates similar to those in the ACM treatment during the first half of the experiment (**Fig. 6A,C**; **Supp. Fig. 4A**). Growth rates were also similar in the OL and ACM mediums for the coccolithophore (**Fig. 6B**,**D; Supp. Fig. 4B**). Hence, both phytoplankton groups were able to grow similarly well in co-culture where each did not exhibit any strong competitive advantage over the other.

Discussion

In general, simulated olivine dissolution products did not show toxicity even at extremely high concentrations across all phytoplankton groups, consistent with other recent observations (Guo et al., 2022). Guo et al. (2022) particularly focused on exposing 11 phytoplankton groups

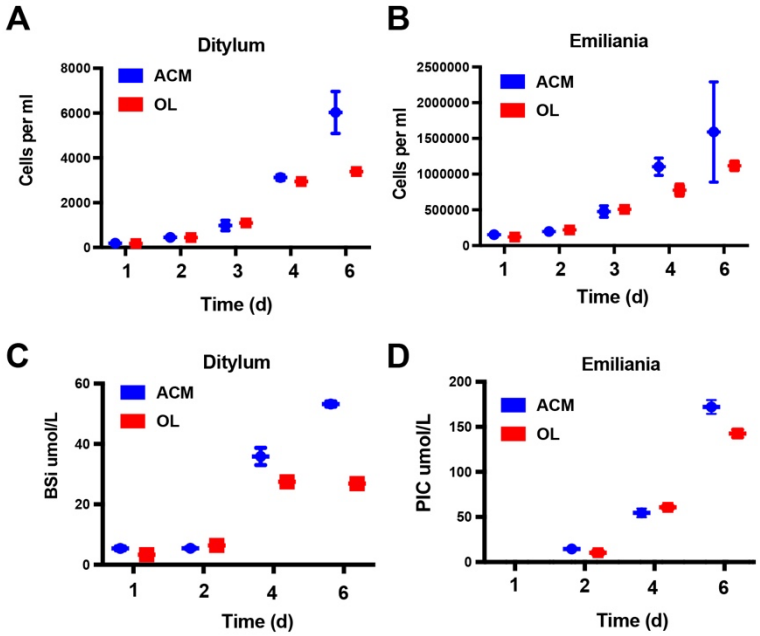

Fig. 6. Effects of olivine leachate versus culture medium controls on growth competition and biomineralization during co-culture of a diatom and a coccolithophore. Shown are 5 day growth curves (cells mL^{-1}) for **A)** the coccolithophore *Emiliana huxleyi* and **B)** the diatom *Ditylum* in mixed cultures grown in olivine leachate (OL, red symbols) and Aquil control medium (ACM, blue symbols). Also shown are **C)** Biogenic silica (BSi, µmol L^{-1}), a proxy for diatom biomass, and **D)** Calcite or particulate inorganic carbon (PIC, µmol L^{-1}), a proxy for coccolithophore biomass, in the OL and ACM treatments in the same growth competition experiment. Values represent the means and error bars are the standard deviations of triplicate cultures for each treatment.

to elevated Ni concentrations and did not observe strong effects across these taxa. Although it is unknown what chemical species of dissolved Ni primarily influence phytoplankton physiology, most studies indicate that phytoplankton primarily interact with free Ni^{2+} ions but are not particularly sensitive to the total dissolved Ni concentration (Guo et al., 2022). Guo et al. (2022) and our study used the same Ni-containing compound, $NiCl_2$, as a source of Ni^{2+}. Guo et al. also used the same base Aquil control medium as our ACM. ACM contains EDTA that binds with metal ions like Ni to improve their dissolution, which subsequently lowers the free Ni ion concentrations (e.g., Ni^{2+}) relative to the total dissolved Ni pool. Although broad negative effects of enhanced Ni concentrations were not observed across taxa, Guo observed some species-specific responses across variations in (0-100 µM) EDTA and Ni (0-50 µM) concentrations, indicating that specific phytoplankton groups are impacted differently depending on the chemical species in the total dissolved Ni pools and/or the concentration and type of organic ligands in seawater. Our results are generally consistent with their overall findings, as the phytoplankton groups tested here did not exhibit negative effects upon elevated exposure to Ni with (e.g., ACM) and without added EDTA (e.g., OL), suggesting that Ni was not toxic irrespective of the concentration of different Ni species in the dissolved pool or that of the Ni^{2+} ion. However, our experiments were not designed to test for taxon-specific differences in responses to specific Ni species or variations in EDTA concentrations in particular.

Diatoms were able to use synthetic OL-derived Si and Fe to support near-maximum growth rates and carbon fixation rates, as well as robust silica frustule development; both of these nutrients can frequently limit diatom growth in various parts of the ocean (Tréguer et al., 2018; Hutchins and Boyd, 2016). OL-derived alkalinity and iron increases also supported

coccolithophore growth, consistent with previous observations in calcifying corals (Albright et al., 2016). Similarly, the globally-distributed picocyanobacterium *Synechococcus* increased its growth and carbon fixation rates as OL concentrations increased. Although OL could not support N_2-fixing cyanobacteria due to their inability to use Fe(III), olivine dissolution products were not observed to be toxic. Their inability to use Fe(III) is a neutral effect due to other sources of bioavailable Fe in the water column (Hutchins and Boyd, 2016). Thus, these results suggest that many phytoplankton will not be negatively impacted by even high levels of elements derived from olivine dissolution, and that some olivine dissolution products may support their growth, primary productivity, and biomineralization when OL is available at high enough concentrations in certain environmental conditions. For example, it is important to note that potential growth benefits to phytoplankton *in situ* will also depend on ambient concentrations of other important nutrients, such as nitrogen (N) and phosphorus (P). Our cultures contained an abundance of other required nutrients, thus enabling phytoplankton to take advantage of particular dissolution products for growth (e.g., Si, Fe, alkalinity). However, if nutrients like N and P are primarily limiting in natural environments, then olivine dissolution products are not expected to have any growth effect. In addition, these cultures represent closed systems that do not allow olivine products to be diluted with fresh seawater. In natural settings, advection in both sediment porewaters (Reimers et al., 2004) and the water column (He and Tyka, 2022) will lead to short residence times, thereby rapidly diluting olivine dissolution products. Hence, these physical dynamics will prevent high concentrations of olivine dissolution products from accumulating in seawater in coastal systems. Thus, even the most dilute leachate treatment in this study is likely more concentrated than the anticipated concentrations of olivine dissolution products expected under field conditions.

Bach et al. (2019) (Bach et al., 2019) hypothesized that silicate, iron and nickel releases from marine applications of silicate minerals like olivine might particularly benefit diatoms and cyanobacteria, as these groups have especially high requirements for one or more of these nutrients. Thus, they expected that olivine applications might produce a "Greener" ocean. They also suggested that adding minerals derived from $CaCO_3^-$ (such as quicklime applications) would particularly favor coccolithophores, due to rapidly enhanced seawater alkalinity. This outcome would produce a "Whiter" ocean (the color of coccolithophore calcite). Although we did not test $CaCO_3^-$ derivatives, our results with synthetic OL seem to point instead to a both "Green and White" ocean, since in individual experiments diatoms, picocyanobacteria, and coccolithophores all responded positively to OL at the relatively elevated levels applied in our experiments. This conclusion is further supported by the results of our diatom/coccolithophore co-culture experiment, which showed that OL stimulated both species simultaneously rather than conferring a competitive advantage on one or the other.

Iron in olivine minerals is present as reduced Fe(II), and we added it in this form to our synthetic OL. However, when Fe(II) dissolves in oxic seawater, it quickly (within minutes) oxidizes to highly insoluble Fe(III), which almost entirely precipitates out as amorphous iron oxyhydroxides (Millero et al., 1987). Clearly, in our experiments this oxidized particulate iron

must have been available to the species that showed growth enhancement with OL, since no other iron source was provided in the seawater growth medium. Diatoms and some other eukaryotes can access precipitated Fe(III) oxyhydroxides using their well-studied reductive uptake systems (Morrissey and Bowler, 2012), or even potentially through endocytosis in some cases (Kazamia et al., 2018).

The responses of the two N_2-fixing cyanobacteria were in striking contrast to those of the other three phytoplankton groups tested. These diazotrophs were either unable to grow in our artificial OL at all (*Trichodesmium*), or could only grow to a very limited degree (*Crocosphaera*). Since olivine dissolution products support photoautotrophic growth by other phytoplankton but not diazotrophic growth, this could be taken to indicate that olivine applications might tend to drive the marine ecosystem towards nitrogen limitation, which would limit the upper bound of phytoplankton proliferation. However, our results with experimental additions of the artificial iron chelator EDTA (ethylene diamine tetra acetic acid) suggest that other mechanisms may enable iron bioavailability. For example, previous research has suggested that *Trichodesmium* cannot directly access particulate Fe(III) forms, but likely relies on bacteria residing on and in natural colonies to produce siderophores, which then solubilize particulate Fe(III) sources and make them bioavailable (Rubin et al., 2011; Lee et al., 2018). Since cultured *Trichodesmium* such as ours typically do not produce colonies, but grow instead as individual filaments of cells, cultures of this diazotroph are likely deficient in many of these iron-acquiring microbial symbionts (Rubin et al., 2011). The iron uptake systems of *Crocosphaera* have been less well-characterized, but like *Trichodesmium*, molecular studies suggest this unicellular diazotroph lacks the genetic capacity to produce endogenous siderophores (Shi et al., 2010; Yang et al., 2022). Our results show that when we add the artificial iron chelator EDTA (which substitutes for ligands produced by the missing bacteria in cultures), the synthetic OL supports near-maximum growth of both of these diazotrophs. Thus, reduced growth rates of these cyanobacteria in OL without EDTA appear to be due to severe iron limitation, not toxicity of any OL component. In our experiments the cells were forced to grow on OL as a sole source of iron, but in coastal ecosystems where olivine deployments would occur, there are typically many other natural sources of iron to support algal growth (Capone and Hutchins, 2013; Hutchins and Boyd, 2016). In nature, *Trichodesmium* is also likely to occur mostly as colonies, and so may have access to additional microbiome-provided iron, including from both naturally-occurring supplies as well as potentially from any shallow-water olivine applications. Thus, iron limitation of N_2 fixation due to olivine additions in coastal waters may not occur in the real ocean.

Growth inhibition of diazotrophs by our synthetic OL appears to be due to iron limitation, but our experiments also shed light on potential effects of other trace metals present in the formulation. Of the metals found in our synthetic OL, Ni and Co are considered nutrient elements with relatively low toxicity; in fact, the concentrations added even in our maximum dosage experiments were well below those that have been reported to be toxic to phytoplankton (Karthikeyan et al., 2019; Vink and Knops, 2023). However, Cr has the potential to be biologically problematic. Cr(III) found in olivine is relatively insoluble, so in this form it is

probably not a major source of exposure for planktonic organisms. However, if it oxidizes to Cr(VI), it becomes much more soluble, and thus more bioavailable and potentially toxic. Cr(III) oxidation is thermodynamically unfavorable, but can be facilitated by borate ions always present in seawater, or by the presence of biologically or photochemically-produced oxidants like H_2O_2 (Pettine et al., 1991), and by naturally occurring manganese oxides (Weijden and Reith, 1982). For these reasons, following the principle of "worst case scenario", we used a soluble Cr(VI) salt in our synthetic OL formulation. Despite this, we found that the presence or absence of the relatively elevated levels of dissolved Cr(VI) present in our regular synthetic OL did not make any difference to the growth of *Trichodesmium* or the other tested phytoplankton species. Particularly, because synthetic OL stimulated near-maximum growth rates in the diatoms, coccolithophore and picocyanobacterium, we presume that the Cr(VI) additions did not adversely affect these groups either.

The goal of this work was to test both extreme levels and simultaneous exposure of multiple, biologically important olivine dissolution products that could influence microbial physiology in order to identify thresholds and response curves. Accordingly, our experiments focused on determining acute effects of high concentrations of olivine dissolution products. In general, they suggest that negative impacts may be few even for large olivine deployments, given the high concentrations of tested olivine dissolution products. Because these microplankton serve as important links to higher trophic levels, these data suggest minimal long-term impacts from olivine dissolution on ecosystem services like fisheries. Future research directions may include longer term experiments with prokaryotes and natural microbial communities to expand our understanding of olivine exposure on keystone taxa that help drive biogeochemical cycling in the oceans. Similar experiments can also be conducted except with other OAE feedstocks harboring different chemical compositions and more rapid dissolution timescales (Renforth and Henderson, 2017). Future studies can also focus on determining how biological processes like photosynthesis, respiration, and organic ligand production could influence olivine dissolution kinetics and their impacts on carbon dioxide removal.

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