

Influence of Ocean Alkalinity Enhancement with Olivine or Steel Slag on a Coastal Plankton Community in Tasmania

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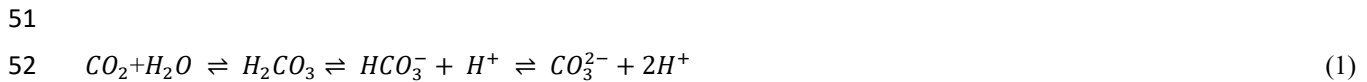
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Abstract. Ocean alkalinity enhancement (OAE) aims to increase atmospheric CO₂ sequestration in the oceans through the acceleration of chemical rock weathering. This could be achieved by grinding rocks containing alkaline minerals and adding the rock powder to the surface ocean where it dissolves and chemically locks CO₂ in seawater as bicarbonate. However, CO₂ sequestration during dissolution coincides with the release of potentially bio-active chemicals and may induce side effects. Here, we used 53 L microcosms to test how coastal plankton communities from Tasmania respond to OAE with olivine (mainly Mg₂SiO₄) or steel slag (mainly CaO and Ca(OH)₂) as alkalinity sources. Three microcosms were left unperturbed and served as a control, three were enriched with olivine powder (1.9 g L⁻¹), and three with steel slag powder (0.038 g L⁻¹). Olivine and steel slag powders were of similar grain size. Olivine was added in a higher amount than the steel slag ~~since previous tests evidenced that it would have released less with the aim to compensate for the lower efficiency of olivine to deliver~~ alkalinity over the 3-week experiment. Phytoplankton and zooplankton community responses as well as some biogeochemical parameters were monitored. Olivine and steel slag additions increased total alkalinity by 29 μmol kg⁻¹ and 361 μmol kg⁻¹ respectively, which corresponds to a theoretical increase of 0.9 % and 14.8 % of the seawater storage capacity for atmospheric CO₂. Olivine and steel slag released silicate nutrients into the seawater, but steel slag released considerably more and also significant amounts of phosphate. After 21 days, no significant difference was found in dissolved iron concentrations (>100 nmol L⁻¹) in the treatments and the control. The slag addition increased dissolved manganese concentrations (771 nmol L⁻¹), while olivine increased dissolved nickel concentrations (37 nmol L⁻¹). There was no significant difference in total chlorophyll *a* concentrations between the treatments and the control, likely due to nitrogen limitation of the phytoplankton community. However, flow cytometry results indicated an increase in the cellular abundance of several smaller (~<20 μm) phytoplankton groups in the olivine treatment. The abundance of larger phytoplankton (~>20 μm) decreased much more in the control than in the treatments after day 10. Furthermore, the maximum quantum yields of photosystem II (F_v/F_m) were higher in slag and olivine treatments, suggesting that mineral additions increased photosynthetic performance. The zooplankton community composition was also affected with the most notable changes being observed in the dinoflagellate *Noctiluca scintillans* and the appendicularian *Oikopleura* sp. in the olivine treatment. Overall, steel slag is much more efficient for CO₂ removal with OAE than olivine and appears to induce less change in the plankton community when relating the CO₂ removal potential to the level of environmental impact that was observed here.

37 1 Introduction

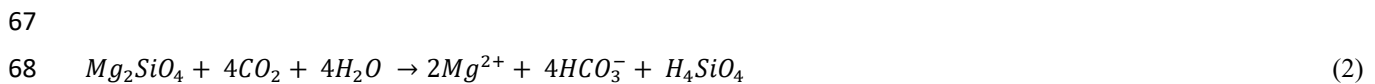
38 Keeping global warming below 2 °C requires immediate emissions reduction. Additionally, between 450-1100 Gigatonnes
39 of carbon dioxide (CO₂) need to be removed from the atmosphere by 2100 (Smith et al., 2023). This could be achieved
40 with a portfolio of terrestrial and marine Carbon Dioxide Removal (CDR) methods. Ocean alkalinity enhancement (OAE)
41 is a marine CDR method that could theoretically contribute significantly to the global CDR portfolio (Ilyina et al., 2013;
42 Feng et al., 2017; Lenton et al., 2018).

43
44 Alkalinity is generated naturally when rock weathers and it has control on the ocean's chemical capacity to store CO₂
45 (Schuiling and Krijgsman, 2006). Natural rock weathering is currently responsible for about 0.5 Gt of atmospheric CO₂
46 sequestration every year (Renforth and Henderson, 2017). The idea behind OAE is to accelerate natural rock weathering
47 by extracting calcium- or magnesium-rich rocks, such as olivine, pulverizing them, and spreading them onto the sea surface
48 to increase chemical weathering rates (Hartmann et al., 2013). The weathering (i.e., dissolution) of these alkaline minerals
49 will consume protons (H⁺), which shifts the carbonate chemistry equilibrium in seawater from CO₂ towards increasing
50 bicarbonate (HCO₃⁻) and carbonate ion (CO₃²⁻) concentrations:



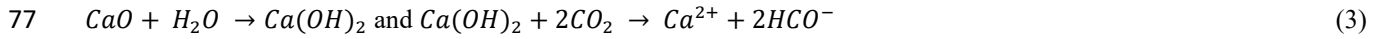
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54 thereby making new space for atmospheric CO₂ to be dissolved in seawater and permanently stored. Previous model studies
55 have shown that OAE can mitigate climate change significantly by increasing the oceanic uptake of CO₂ from the
56 atmosphere (Kohler et al., 2010; Paquay and Zeebe, 2013; Keller et al., 2014; Lenton et al., 2018). For example, the study
57 by Burt et al. (2021) suggested that the total global mean dissolved inorganic carbon (DIC) inventories would increase by
58 156 GtC after total alkalinity is enhanced at a rate of 0.25 Pmol year⁻¹ in 75-year simulations.

59
60 There are a variety of alkaline minerals that could be used for OAE. A widely considered naturally occurring mineral is
61 forsterite, a (Mg₂SiO₄)-rich olivine. This type of olivine is abundant in ultramafic rock such as dunite, constituting at least
62 88 % of the rock composition (Ackerman et al., 2009; Su et al., 2016). Olivine occurs in the Earth's crust but is more
63 abundant in the upper mantle. There are at least several billion tons of olivine resources on Earth (Caserini et al., 2022).
64 However, the extraction of olivine in 2017 was only around 8.4 Mt year⁻¹ (Reichl et al., 2018), which is about two orders
65 of magnitude below the mass needed for climate-relevant OAE with olivine (Caserini et al., 2022). The net reaction for
66 CO₂ sequestration with Mg₂SiO₄ is:



68
69
70 Another potential OAE source material is steel slag (Renforth, 2019), a by-product of steel manufacturing. During steel
71 manufacturing, high-purity calcium oxide (CaO) is used to improve the quality of the steel through accumulation of
72 unwanted materials such as sulphur and phosphorus. Steel slag mainly contains CaO, SiO₂, Al₂O₃, Fe₂O₃, MgO, and MnO
73 (Kourounis et al., 2007), and the chemical composition can vary depending on the manufacturing process (Wang et al.,
74 2011). Due to the presence of CaO and potentially other alkaline components, steel slag can increase alkalinity when
75 dissolved in seawater. The chemical reaction for CO₂ sequestration with CaO is:

76



78

79 Some of the steel slag that is produced during steel manufacturing is further used (e.g., for road construction and civil
80 engineering) but in some countries like China, 70.5 % of steel slag is left unused and stored in dumps (Guo et al., 2018).
81 In 2016, more than 300 million tons of steel slag was not used effectively, thereby occupying the land and raising
82 environmental concerns (Guo et al., 2018). The effective alkaline composition, availability, and relatively low cost of the
83 raw materials make olivine and steel slag potential source materials for OAE.

84

85 To assess whether OAE is viable, it needs to be understood how its application may affect marine biota such as plankton
86 and the biogeochemical fluxes they drive. Some data on the effects of OAE with sodium hydroxide (NaOH) on plankton
87 communities have recently been published (Ferderer et al., 2022; Subhas et al., 2022), but to the best of our knowledge, no
88 such data are available for olivine- and/or slag-based OAE. Chemical perturbations via olivine and slag should be like
89 those by NaOH in that they increase seawater pH and shift the carbonate chemistry equilibrium (see Eq. 1). However, there
90 would be additional chemical perturbations because minerals contain a variety of potentially bioactive elements that are
91 released into the environment when they dissolve in seawater (Bach et al., 2019). One particular concern is that natural and
92 anthropogenic minerals such as olivine and steel slag are rich in bioactive metals that are usually scarce in the ocean, such
93 as iron (Fe), copper (Cu), nickel (Ni), manganese (Mn), zinc (Zn), cadmium (Cd), and chromium (Cr). Many of these trace
94 metals are essential micronutrients for phytoplankton growth (Sunda, 2000; Sunda, 2012), such as being co-factors for
95 various metalloenzymes (summarized by Twining and Baines, 2013). It is possible that the addition of alkaline minerals
96 may benefit phytoplankton by providing trace metals currently limiting phytoplankton growth (Falkowski, 1994; Basu and
97 Mackey, 2018). For instance, the addition of Fe is well known to stimulate phytoplankton blooms in those vast ocean
98 regions where Fe levels limit growth (Boyd et al., 2007; Moore et al., 2013). However, some trace metals can also inhibit
99 phytoplankton growth, and different phytoplankton species have different requirements and tolerances for trace metals
100 (Sunda, 2012) so the addition of trace metals via OAE may change phytoplankton community composition.

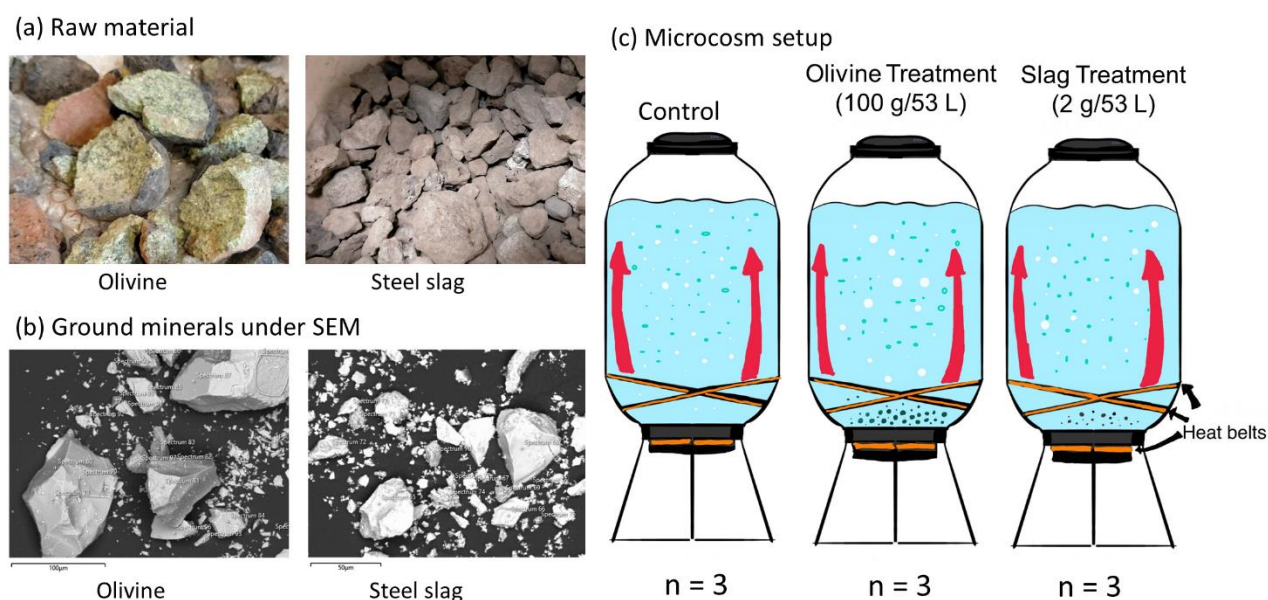
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102 Here, we describe a microcosm experiment with coastal Tasmanian plankton communities that was used to investigate: (1)
103 how effectively OAE via the application of finely ground olivine and steel slag could sequester atmospheric CO₂, and (2)
104 if/how olivine and steel slag additions affect various components of the plankton community.

105

106 **2 Methodology**

107 **2.1 Microcosm setup**



108

109 **Fig. 1.** Experimental design and alkalinity sources. (a) Raw materials used as alkalinity sources: olivine (left) and steel slag (right).
110 Olivine and steel slag were originally larger than 20 mm. (b) Ground minerals observed with a scanning electron microscope (SEM). (c)
111 Microcosm setup: each microcosm enclosed ~ 53 L of surface seawater with natural plankton communities. Olivine and steel slag
112 treatments and the control were kept in a temperature-controlled room and two heat belts were attached to the bottom of each microcosm
113 to create convective circulation.

114

115 We used nine 53 L transparent Kegland® Fermzilla conical unitank fermenters (polyethylene terephthalate) (Fig. 1) as
116 microcosms to incubate natural plankton communities. All microcosms were prewashed with hydrochloric acid (10 % v/v)
117 and rinsed five times with 18.2 MΩ Milli-Q water. Seawater with coastal plankton communities was collected at Battery
118 Point, Tasmania (42.892°S, 147.337°E) within 2 hours by lowering the microcosms into the ocean with a crane and filling
119 them in a manner similar to a Niskin bottle, as described in detail in Ferderer et al. (2022). A sieve with a mesh size of 2
120 mm was attached to the top and bottom of the microcosms during filling to avoid the entrapment of large and patchily
121 distributed organisms in the microcosms. The enclosed seawater weight was initially between 52.35-54.70 kg. After
122 seawater collection, filled microcosms were immediately transported back to the Institute for Marine and Antarctic Studies
123 (University of Tasmania) on a truck and transferred within 75 min into a temperature-controlled room set to 7.5-8 °C. Two
124 heat belts were attached to the bottom of each microcosm to induce a convective mixing current (Ferderer et al., 2022).
125 Seawater temperature inside the microcosms was about 13.5 °C due to the heating effects of the heat belts and was the
126 same as the sampled region. LED light strips were used to provide an average light intensity of 236 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$
127 (ranging from 208 to 267 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) with a daily light-dark cycle of 10:14 hours. The light intensity was the
128 average light intensity in each microcosm measured with a LICOR light meter at 0.15 m depth within the microcosm.
129 Microcosms positioned in the temperature-controlled room were shuffled anti-clockwise every day to ensure similar light

130 intensity for each microcosm throughout the experiment. Treatments were established 24 hours after collecting the seawater.
131 The total alkalinity released per amount of mineral powder added was much higher for the slag powder than the olivine
132 powder in our preliminary test trials. So, three microcosms were enriched with 100 g of olivine powder, three microcosms
133 with 2 g of steel slag powder, while the remaining three microcosms were left unperturbed and served as controls.

134

135

136 **2.2 Preparation of olivine and steel slag powder**

137 The olivine rocks were provided by Moyne Shire Council who sourced the mineral from a quarry in Mortlake, Victoria,
138 Australia. The Basic Oxygen Slag (hereafter referred to as “slag”) was provided by Bradley Mansell who sourced the
139 material from Liberty Primary Steel Whyalla Steelworks in Whyalla, South Australia, Australia. Upon delivery, the olivine
140 rocks were 40-80 mm in diameter, and slag aggregates were 20-50 mm in diameter. These were crushed to smaller than 10
141 mm pieces using a hydraulic crusher. The crushed material was further ground with a ring mill with a chrome milling pot.
142 Afterwards, finely-ground samples were sieved to get samples with 150 ~ 250 μm grain size. The sieved olivine and slag
143 grains were inspected for their appearance and elemental composition using a Hitachi SU-70 analytical field emission
144 scanning electron microscope (SEM), and energy dispersive spectrometers (Central Science Laboratory (CSL), University
145 of Tasmania). Grain size spectra were determined with a Sympatec QICPIC particle size analyser LIXCELL (CSL,
146 University of Tasmania).

147

148 **2.3 Seawater sampling**

149 Seawater was transferred with a peristaltic pump from the microcosms at a depth of about 0.15 m into 1 L acid-washed
150 sampling bottles (LDPE) using an acid-washed silicon tube. Seawater in these bottles was then subsampled for dissolved
151 trace metal samples, filtrations, Fast Repetition Rate fluorometry (FRRf), and flow cytometry analysis. Samples for
152 nutrients and total alkalinity (TA) were transferred using the same pump but through a silicone tube into 80 mL HDPE
153 bottles. Total alkalinity and macronutrient samples were filtered during this process through a 0.2 μm nylon filter attached
154 to the silicone tube to remove all particles and organisms $> 0.2 \mu\text{m}$.

155

156 **2.4 Salinity, nutrients, carbonate chemistry, and trace metal analysis**

157 Salinity was measured before and at the end of the experiment using a HACH HQ40d portable meter. The pH_T (total scale)
158 and temperatures were measured daily (2-3 hours after the onset of the light period) using a pH meter (914
159 pH/Conductometer Metrohm). We recorded voltages and temperature from the pH meter and calibrated the pH_T at original
160 temperature at sampled time using the certified reference material (CRM) Tris buffer following the method described in
161 SOP6a by Dickson et al. (2007). Briefly, the standard buffer’s pH and voltage at different temperature gradients were
162 recorded, and temperature vs. voltage polynomial regression data were generated for calculating calibrated pH values (pH_T)
163 (refer to Eq. 3 in SOP6a of Dickson et al. (2007)). The regression could then be used to obtain a CRM pH value for each
164 temperature and to calibrate the pH measured in the microcosms to the total pH scale.

165

166 Total alkalinity was sampled every four days. It was measured in duplicate using a Metrohm 862 Compact Titrosampler
167 coupled with an Aquatrode Plus with PT1000 temperature sensor following the SOP3b open-cell titration protocol
168 described in Dickson et al. (2007). Filtered TA samples were stored at 8 °C for a maximum of 23 days before measurement.
169 Titration curves were evaluated using the “calculate” script within PyCO2sys by Humphreys et al. (2022). The carbon
170 chemistry equilibrium was calculated with the R package “seacarb” Gattuso et al. (2023) from pH_T , TA, phosphate, silicate,
171 temperature, and salinities using stoichiometric equilibrium constants from Lueker et al. (2000). Dissolved macronutrients
172 were measured every second day using standard spectrophotometric methods developed by Hansen and Koroleff (1999)
173 on the day the samples were taken from the microcosms.

174

175 Dissolved trace metal concentrations were measured four times during the experiment: a few hours before olivine and slag
176 were added, a few hours after these minerals were added on day 2, near the middle of the experiment on day 13, and at the
177 end of the experiment on day 22. Sixty mL of seawater was collected using an acid-washed 60 mL syringe, and the seawater
178 was filtered through 25 mm diameter 0.2 μm pore size polycarbonate filters. Unfortunately, we did not notice that 0.2 μm
179 pore size nylon filters (acid washed) were used during sampling on days 1 and 2 so we refiltered these seawater samples
180 again using 0.2 μm pore size polycarbonate filters after one month. All seawater samples were diluted approximately 20-
181 fold by weight using Milli-Q water (18.2 $\text{M}\Omega\cdot\text{cm}$ grade) and acidified using 1 % ultrapure HCl. These samples were
182 analysed using Sector Field Inductively Coupled Plasma Mass Spectrometry (SF-ICP-MS) employing multiple resolution
183 settings to overcome major spectral interferences. Due to the presence of abundant major metal ions in our samples, such
184 as Na and Mg, natural open-ocean seawater from the Southern Ocean with very low trace metal concentrations was diluted
185 20 times with Milli-Q water and used as a representative blank. The same Southern Ocean seawater was enriched with
186 different gradients of trace metal standards to calculate the samples’ trace metal concentrations. Five of the total 36 samples
187 had abnormal trace metal concentrations, and 2 of them were from day 1. We considered values as outliers using the
188 interquartile range (IQR) criterion on pre-addition data, and if values are more than 10 times higher than replicates, they
189 are also considered as outliers. These samples containing outliers were excluded from the data analysis (Table S1.). The
190 major likely source of these metal contaminations is sampling in the temperature control room, where precautions were
191 insufficiently implemented.

192

193 **2.5 Particulate matter and plankton community analysis**

194 Chlorophyll *a* was sampled every second day by filtering the seawater through glass fibre filters (GF/F, pore size = 0.7 μm ,
195 diameter = 25 mm), and filters were stored in 15 mL polypropylene tubes wrapped with aluminium foil and stored at -80 °C
196 for 50-70 days before measurement. Each filter was immersed in 10 mL 100 % methanol for 18-20 h to extract chlorophyll
197 from phytoplankton and these samples were analysed on a Turner fluorometer (Model 10-AU) following the method
198 described by Evans et al. (1987).

199

200 Phytoplankton flow cytometry samples were fixed with 40 μL of a mixture of formaldehyde-hexamine (18 %:10 % v/w)
201 added to 1400 μL of seawater sample. All bacteria samples (700 μL) were fixed with 14 μL glutaraldehyde (Electron-
202 microscope grade, 25 %). After mixing samples with fixatives, samples were stored for 25 minutes at 10 °C, then flash-
203 frozen in liquid nitrogen, and stored at -80 °C until measurement 83-86 days later. Directly before the measurement,
204 samples were thawed at 37 °C. Bacteria samples were stained with SYBR green I (diluted in dimethylsulfoxide) at a final

205 ratio of 1:10000 (SYBR Green I: sample).

206

207 A Cytex Aurora flow cytometer (Cytex Biosciences) was used to quantify the abundance of fluorescing particles such as
208 phytoplankton or stained bacteria. Phytoplankton groups were distinguished based on their fluorescence signal intensity of
209 different laser excitation/emission wavelength combinations and forward scatter (FSC). The yellow-green laser (centre
210 wavelength: 577 nm), in combination with FSC signal strength, was used to separate cyanobacteria and cryptophytes from
211 other phytoplankton. The violet laser (centre wavelength: 664 nm) in combination with FSC was used to distinguish
212 picoeukaryotes, nanoeukaryotes, and microphytoplankton. The blue laser (centre wavelength: 508 nm) in combination with
213 FSC was used to distinguish bacteria from other living (i.e., DNA-containing) particles (Fig. S. 1).

214

215 The biovolume of each classified flow cytometry phytoplankton type was calculated using the equation:

216

$$217 \text{ Biovolume} = \text{Cell number count} \times \left(\frac{\text{FSC}}{10248}\right)^{2.14} \quad (4)$$

218

219 where biovolume is the biovolume of the phytoplankton (μm^3), cell number is the cell count per mL of sample, and the
220 FSC is the forward scatter signal value from the flow cytometry. This equation is calculated based on the relationship
221 between biovolume and FSC for different phytoplankton species (Selfe, 2022). The biovolume of each phytoplankton type
222 was then divided by the total biovolume of all phytoplankton type to calculate the biovolume proportion of each
223 phytoplankton type (Biovolume prop.). This derived value was used to estimate the phytoplankton composition in each
224 microcosm.

225

226 Phytoplankton photosynthetic performance was estimated from the rapid light curves measured with an FRRf (FastOcean
227 Sensor FRRf3, Chelsea Instruments Group) every second day following the protocol adapted from Schallenberg et al.
228 (2020). Samples were kept in the dark for 20 minutes before the measurement and then added to the FRR fluorometry
229 cuvette, which was temperature-controlled at 13.5 °C. Filtered natural seawater was used for blank correction. A channel
230 with three light wavelengths (450, 530, and 624 nm) was used in each acquisition sequence. At least 10 acquisitions were
231 measured for each sample. The maximum electron transport rate (ETR_{max}), initial slope of the rapid light curve (α), and the
232 light-saturation parameter (E_k) were calculated using the equation described by Platt et al. (1980) without photoinhibition:

233

$$234 \text{ ETR} = \text{ETR}_{\text{max}} \left[1 - e^{-\frac{\alpha E}{\text{ETR}_{\text{max}}}}\right] \quad (5)$$

235

236 These parameters together with the maximum quantum yield of PSII (F_v/F_m) were used to compare the photosynthetic
237 performance of the phytoplankton communities in different microcosms.

238

239 Seawater was sampled before the treatment and at the end of the experiment for particulate trace metal concentrations.
240 Samples of 100 mL were filtered through an acid-cleaned polycarbonate filter (25 mm diameter, 0.8 μm pore size) and
241 placed in an acid-cleaned polypropylene filter holder in a trace metal-clean laminar flow bench. The filters were washed
242 with the EDTA-oxalate reagent (1.4 mL) twice (8 min total) and rinsed with chelexed NaCl solution (0.6 mol L⁻¹ with 2.38
243 mmol L⁻¹ of HCO₃⁻, pH=8.2) 10 times (1.5 mL aliquots) (Tovar-Sanchez et al., 2003; Tang and Morel, 2006). Filters were
244 stored in acid-washed well plates at -20 °C before analysis. The digestion process followed the method reported by Bowie

245 et al. (2010). Briefly, all samples and triplicate certified reference materials plankton standards (50 mg/vial) were digested
246 in a mixture of strong ultrapure acids (750 μL 12 mol L^{-1} HCl, 250 μL 40 % HF, 250 μL 14 mol L^{-1} HNO_3) in 15 mL Teflon
247 perfluoroalkoxy (PFA) vials on a 95 °C hot plate for 12 h in a fume hood. They were then dry evaporated for 4 h and re-
248 suspended in 10 % v-v ultrapure HNO_3 . All prepared solutions had indium as internal standard added to a final
249 concentration of 10 $\mu\text{g L}^{-1}$. Three pre-mixed multi-element standard solutions (MISA) were prepared as external calibration
250 standards.

251

252 Particulate organic carbon (POC) was sampled by filtering 100 mL of seawater from each microcosm. Glass fibre filters
253 (Whatman GF/F, pore size = 0.7 μm , diameter = 13 mm) were pre-combusted at 400 °C for 6 h. Filters were stored at -20 °C
254 before measurement. Samples were treated via fuming with 2N HCl to remove carbonates overnight and dried in the oven
255 for 4h. Finally, filters were folded into silver cups and stored in a desiccator until analysis. Samples were analysed for
256 carbon with a Thermo Finnigan EA 1112 Series Flash Elemental Analyser (CSL, University of Tasmania).

257

258 Biogenic silica (BSi) concentrations were analysed every 4 days by filtering 100 mL of seawater from each microcosm.
259 Mixed Cellulose Ester (MCE) membrane filters (diameter = 25 mm, pore size = 0.8 μm) were used for BSi samples. BSi
260 filters were placed in a plastic petri dish and stored at -20 °C before measurement. Filters were processed using the hot
261 NaOH digestion method of Nelson et al. (1989). The final solution was measured using the same process as the dissolved
262 silicate (see section 2.4).

263

264 A self-made plastic zooplankton net (20 mm height and 15 mm width) with a 210 μm mesh size was acid-washed first and
265 then used to collect zooplankton from microcosms before mineral addition on day 2, near the middle (day 13), and at the
266 end of the experiment (day 23). Samples were stored in 10 % formalin seawater solutions and kept at room temperature
267 until measurements. Zooplankton were quantified and identified under a Leica M165C microscope fitted with a Canon 5D
268 camera. The number of zooplankton from one mini-trawl in each collection was converted to the unit of individual L^{-1} and
269 used for data analysis. The diversity of zooplankton communities was estimated with the Shannon Diversity Index (H)
270 calculated as:

271

$$272 \quad H = -\sum(pi \times \ln(pi)) \quad (6)$$

273

274 where pi is the proportion of the entire zooplankton community made up of individual species abundance, and ln is the
275 natural logarithm.

276

277

278 **2.6 Statistic analysis**

279 R studio was used for data analyses. Generalized additive models (GAMs) from the package “mgcv” were fitted to the data
280 to predict the changes over time. The GAMs all shared the same equations:

281

$$282 \quad Y = s(\text{Day}), \quad (7)$$

283

284 in which Y presents the dependent variable and s(Day) is the smooth term of the day of the experiment. Another GAM was
285 used to detect significant differences between treatments and the control:

$$287 \quad Y = Treatment + s(Day) + s(Day, by = oTreatment) \quad (8)$$

288
289 In this equation, the variable “Treatment” includes three conditions: “Control”, “Slag” and “Olivine”; while “oTreatment”
290 is the ordered factor of the variable “Treatment” which allowed us to compare the GAMs smooth terms from different
291 treatments and the control (Simpson, 2017).

292
293 When comparing GAMs, P-means represent the p-value obtained from comparing two GAMs, such as the control and the
294 olivine treatment. If P-means is below 0.05, it indicates that the mean values of the two GAMs exhibit significant
295 differences over the course of the experiment. Conversely, if P-means is equal to or greater than 0.05, it suggests that the
296 two GAMs have similar mean values. In contrast, P-smooths represents the p-value derived from comparing the smooth
297 terms of two GAMs. If P-smooths is below 0.05, it indicates that the two GAMs demonstrate significantly different trends
298 in their change over time.

299
300 For the analysis of trace metal concentrations and zooplankton abundance, Generalized Linear Models (GLMs) from the
301 'stats' package were fitted to the data to determine significant differences between treatments and the control. The selection
302 of specific GLMs was based on the distribution of the raw data. One GLM equation is

$$304 \quad Y = Treatment + \frac{Day}{22} + \left(\frac{Day}{22}\right)^2 \quad (9)$$

305
306 with family = Gamma, where Y represents the measured parameter (abundance of a zooplankton species and dissolved
307 trace metal concentrations); treatment is the conditions (“Control”, “Slag” and “Olivine”); and Day represents the day of
308 the experiment. The other GLM equation,

$$310 \quad Y = Treatment + Day \quad (10)$$

311
312 with family = Gaussian, was employed for particulate trace metal data and the Shannon Diversity Index. To compare the
313 contribution of the three treatments on the measured parameters, Tukey's significant difference test was conducted on the
314 GLMs using the 'glht' function.

315

316 **3. Results**

317 **3.1 Elemental composition and grain size of the finely-ground minerals**

318 SEM analysis revealed the approximate elemental composition of olivine and slag powder (Table 1). Based on this analysis
319 the olivine composition resembles the Mg-rich olivine mineral “forsterite” (Mg₂SiO₄). The particle size spectrum of olivine
320 powder is shown in detail in Fig. S2. Roughly 69 % of the olivine particles, when measured by volume, fell within the

321 diameter range of 35 - 300 μm . Additionally, SEM analysis revealed high levels of Ca and O in the slag, indicative of the
 322 considerable $\text{Ca}(\text{OH})_2$ and CaO content of the powder (Table 1; please note that H cannot be measured with the applied
 323 method). The particle size measurement (Fig. S2) showed that 78 % of the ground slag particles were between 35 - 300
 324 μm .

325

326 **Table 1.** The weight percentage of elements from two minerals. Unit: wt %.

Element	O	Ca	Mn	Si	Mg	Fe	Al	Ti	Cr	Ni
Olivine	39.9	0.4		19.9	26.4	13.0	1.0			0.8
Steel slag	41.9	36.0	7.0	6.5	4.3	3.7	3.4	1.7	1.6	

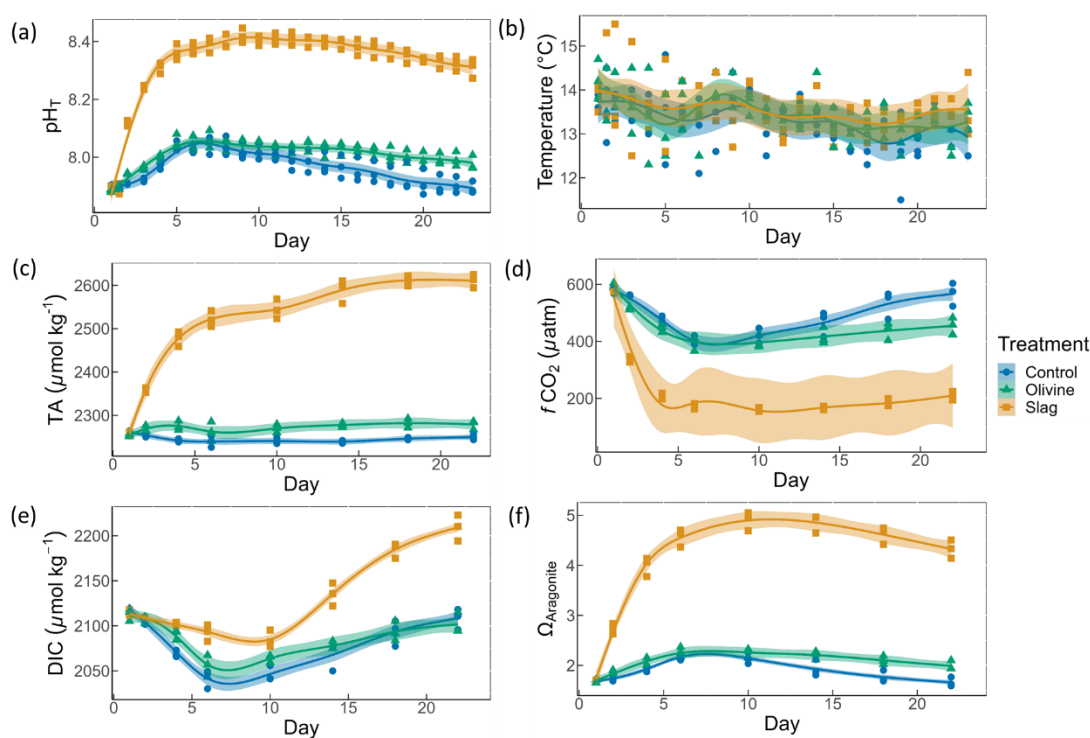
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328

329 3.2 Physical and chemical conditions over the course of the experiment.

330 On day 2 of the experiment, when olivine particles were introduced into the microcosms, the smallest fraction of the powder
 331 remained suspended, causing the seawater to become highly turbid for several days. The resulting milky appearance of the
 332 seawater eventually faded over a period of approximately five days, and by day 5, the turbidity had visually become like
 333 the slag treatment and the control. This effect was not anticipated, and as a result, we decided to investigate its impact on
 334 light intensity. To do so, a test was conducted after the main experiment in which olivine powder was added to a microcosm
 335 identical to those used in the experiment, and light intensity was measured daily at a depth of 0.15 m. The results showed
 336 that the addition of olivine caused an initial reduction in light intensity of 18.5 % at 15 mins after addition, which declined
 337 to 7.4 %, 3.7 %, 3.7 % and 0 % after 1, 2, 3, and 4 days, respectively. These findings indicate that olivine additions can
 338 significantly affect the light environment in the microcosms, whereas no such effect was observed in the slag treatment.

339



340

341 **Fig. 2.** Carbonate chemistry conditions. The temporal development of (a) pH_T , (b) temperature, (c) total alkalinity (TA), (d) CO_2 fugacity

342 ($f\text{CO}_2$) computed at *in situ* temperature and atmospheric pressure, (e) dissolved inorganic carbon (DIC), and (f) aragonite saturation state
343 ($\Omega_{\text{aragonite}}$). The dots represent the raw data ($n=3$ for each treatment per sampling time), and the fitted curve is the generalized additive
344 model (GAM). The shading represents the 95 % confidence interval of the fitted GAM.

345

346 The pH_T of all microcosms increased from day 1 to day 5 (Fig. 2a). This was due to photosynthetic CO_2 drawdown in the
347 control or photosynthetic CO_2 drawdown in combination with alkalinity release from minerals in the treatments. During
348 the peak of the bloom, pH_T was 8.037 ± 0.010 in the control (average values \pm standard error), 8.054 ± 0.014 in the olivine
349 treatment and 8.411 ± 0.015 in the slag treatment. The pH_T was significantly higher in the slag than the olivine treatment
350 and the control throughout the experiment (control and olivine pH_T were not significantly different). The pH_T on day 23 of
351 the control, olivine, and slag treatments were 7.893 ± 0.012 , 7.978 ± 0.015 , and 8.309 ± 0.019 , respectively. The temperature
352 inside of the microcosms varied between replicates, which may have added noise in the biological response data. However,
353 on average there was no statistically significant difference between control/treatments during the experiment.

354

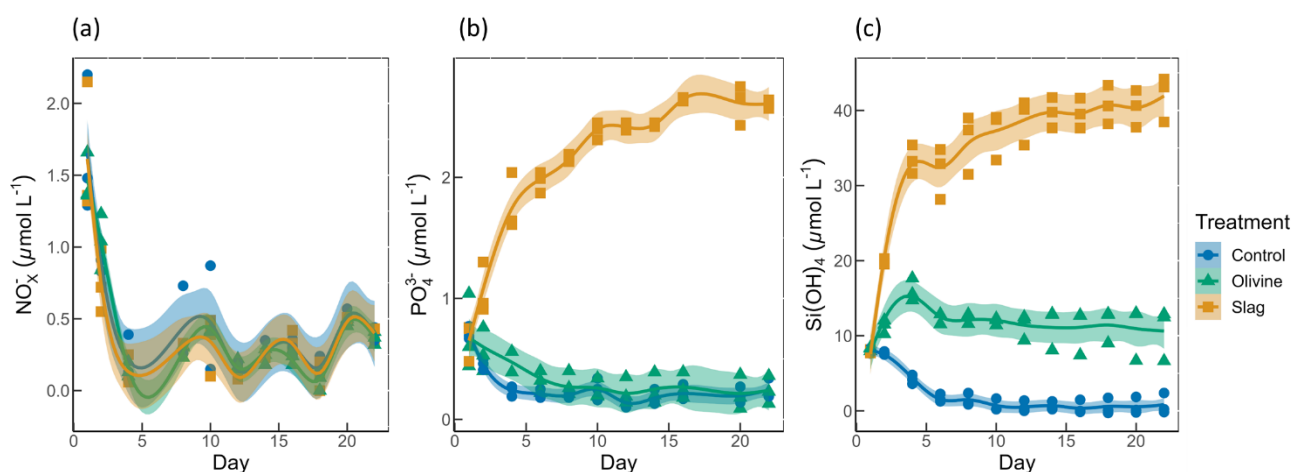
355 In our data analysis, all the fitted GAMs from the treatments and the control exhibited significant differences in pH_T from
356 each other, as evidenced by the p-values of both P-means and P-smooths being smaller than 0.001. For detailed results of
357 the GAM p-values, please refer to Table S2.

358

359 Total alkalinity increased marginally from 2255 ± 2 to $2262 \pm 13 \mu\text{mol kg}^{-1}$ within the first 6 days after olivine addition
360 while it increased more substantially from 2259 ± 1 to $2522 \pm 11 \mu\text{mol kg}^{-1}$ in the same time span in the slag treatment (Fig.
361 2c). The TA in the control decreased from $2261 \pm 2 \mu\text{mol kg}^{-1}$ to $2240 \pm 7 \mu\text{mol kg}^{-1}$ from day 1 to day 6 but remained
362 stable thereafter. The TA reached $2279 \pm 6 \mu\text{mol kg}^{-1}$ in the olivine treatment and $2611 \pm 9 \mu\text{mol kg}^{-1}$ in the slag treatment
363 on day 22. The slag treatment reached a significantly higher TA than the olivine treatment and the control (P-smooths <
364 0.001). The mean TA from GAM in olivine treatment was higher than the control (P-means < 0.001).

365

366 The CO_2 fugacity ($f\text{CO}_2$) computed at *in situ* temperature and atmospheric pressure decreased continuously in the first 6
367 days in all microcosms (Fig. 2d). Then it increased again in the control and olivine treatments while staying lower in the
368 slag treatment (P-means and P-smooths ≤ 0.001 between either treatment or the control). Dissolved inorganic carbon (Fig.
369 2e) and the aragonite saturation state ($\Omega_{\text{aragonite}}$; Fig. 2f) revealed a similar trend over the course of the experiment in the
370 control and the olivine treatment. In contrast, the slag treatment had higher DIC and $\Omega_{\text{aragonite}}$ values throughout the
371 experiment (P-means < 0.001).



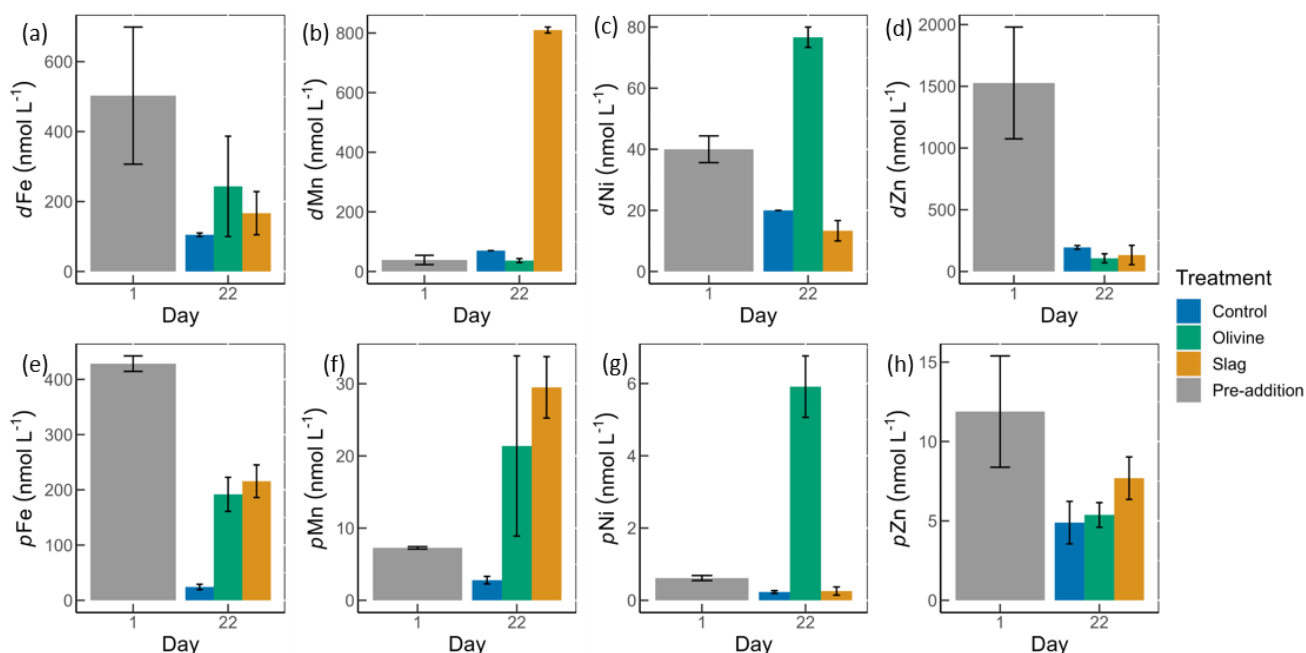
372

373 **Fig. 3.** Macronutrients concentrations over the course of the study. (a) Nitrate and nitrite concentrations. (b) Phosphate concentrations.
 374 (c) Silicic acid concentrations. The dots represent the raw data ($n=3$ for each treatment per collection), and the fitted curve is the
 375 generalized additive model.

376

377 Initial nitrate and nitrite (NO_x^-), phosphate (PO_4^{3-}), and silicic acid ($\text{Si}(\text{OH})_4$) concentrations were 1.58 ± 0.12 , 0.69 ± 0.59 ,
 378 and $8.04 \pm 0.10 \mu\text{mol L}^{-1}$, respectively (Fig. 3). NO_x^- declined rapidly in all microcosms once the experiment had
 379 commenced to values below $0.5 \mu\text{mol L}^{-1}$ and no significant difference was detected between treatments and control (P -
 380 smooths >0.05 ; Fig. 3a). In both the olivine treatment and the control, the PO_4^{3-} concentration decreased in the first six
 381 days (Fig. 3b). In the slag treatment, PO_4^{3-} increased to a maximum of $2.65 \pm 0.01 \mu\text{mol L}^{-1}$, which was significantly higher
 382 than in the olivine treatment and the control (P -means <0.001). The $\text{Si}(\text{OH})_4$ concentration increased to a maximum of
 383 $15.99 \pm 0.87 \mu\text{mol L}^{-1}$ in the olivine treatment, increased to a maximum of $41.92 \pm 1.75 \mu\text{mol L}^{-1}$ in the slag treatment, but
 384 decreased below the detection limit in the control (Fig. 3c). Significant differences were observed in the development of
 385 $\text{Si}(\text{OH})_4$ between all treatments and the control (Table S2).

386



387

388

389 **Fig. 4.** Dissolved and particulate trace metal concentrations in microcosm seawater. (a)-(d) are dissolved trace metal concentrations, and
 390 (e)-(h) are total particulate trace metal concentrations. The error bars represent the standard error from measured samples. The pre-
 391 addition data shown in (a)-(d) represent the average of 7 microcosms before addition of slag or olivine. The data for the control on day
 392 22 in (a)-(d) and for the pre-addition on day 1 in (e)-(h) were based on two of three microcosm replicates. The remaining data were based
 393 on all three microcosm replicates.

394

395 The dissolved trace metal concentrations measured from microcosms are presented in Fig. S3. While the mass of olivine
 396 added to the microcosms was 50-fold greater than in steel slag (100 g vs 2 g), it's noteworthy that the variation in dissolved
 397 trace metal concentrations between the two treatments were much smaller than 50 folds. After 21 days of experiment, the
 398 treatments showed an increase in dissolved Al concentrations from 920 ± 286 to $970 \pm 228 \text{ nmol L}^{-1}$ in olivine treatment,
 399 and from 920 ± 286 to $1093 \pm 77 \text{ nmol L}^{-1}$ in slag treatment, while in the control dissolved Al decreased to $230 \pm 10 \text{ nmol}$

400 L⁻¹ (Fig. S3). The fitted GLMs were compared, and the p-value revealed how much influence a treatment had on the
401 dissolved metal concentrations (Table S3). The results indicate that the slag and olivine additions led to significantly higher
402 Al concentrations than in the control (p-values < 0.05), but no significant difference was found between the two treatments
403 (p-value = 0.189). The Cu concentration in the olivine on day 22 was significantly higher than the slag treatment and the
404 control (p-value < 0.05) (Fig. S3). The addition of olivine and slag released some dissolved Fe, but overall, the concentration
405 of Fe did not differ significantly between treatments (Fig. 4a, Table S3). The slag released a substantial amount of dissolved
406 Mn (maximum 810 ± 10 nmol L⁻¹ on day 22) (Fig. 4b), leading to significantly higher concentrations than in the olivine
407 treatment and the control (p-values < 0.001). A significant amount of dissolved Ni (maximum 77 ± 3 nmol L⁻¹ on day 22)
408 was released from the olivine powder (p-values < 0.001) (Fig. 4c). The initial concentration of dissolved Zn in seawater
409 was much higher than on day 22 in all microcosms, and no significant difference in Zn concentrations was found between
410 the treatments and the control.

411

412 Particulate concentrations of some trace metals also differed between treatments. The total particulate Fe decreased in all
413 microcosms on day 22 comparing with the pre-addition level, but both mineral addition treatments had higher particulate
414 Fe concentrations than the control (Fig. 4e). The addition of slag elevated particulate Mn concentrations to a level higher
415 than the pre-addition and the control on day 22 (Fig. 4f), while the addition of olivine increased the particulate Ni
416 concentrations to a level higher than the slag, the control, and the pre-addition (Fig. 4g). The particulate Zn concentrations
417 in general decreased by the end of the experiment (Fig. 4h), and no significant differences were found between the
418 treatments and the control.

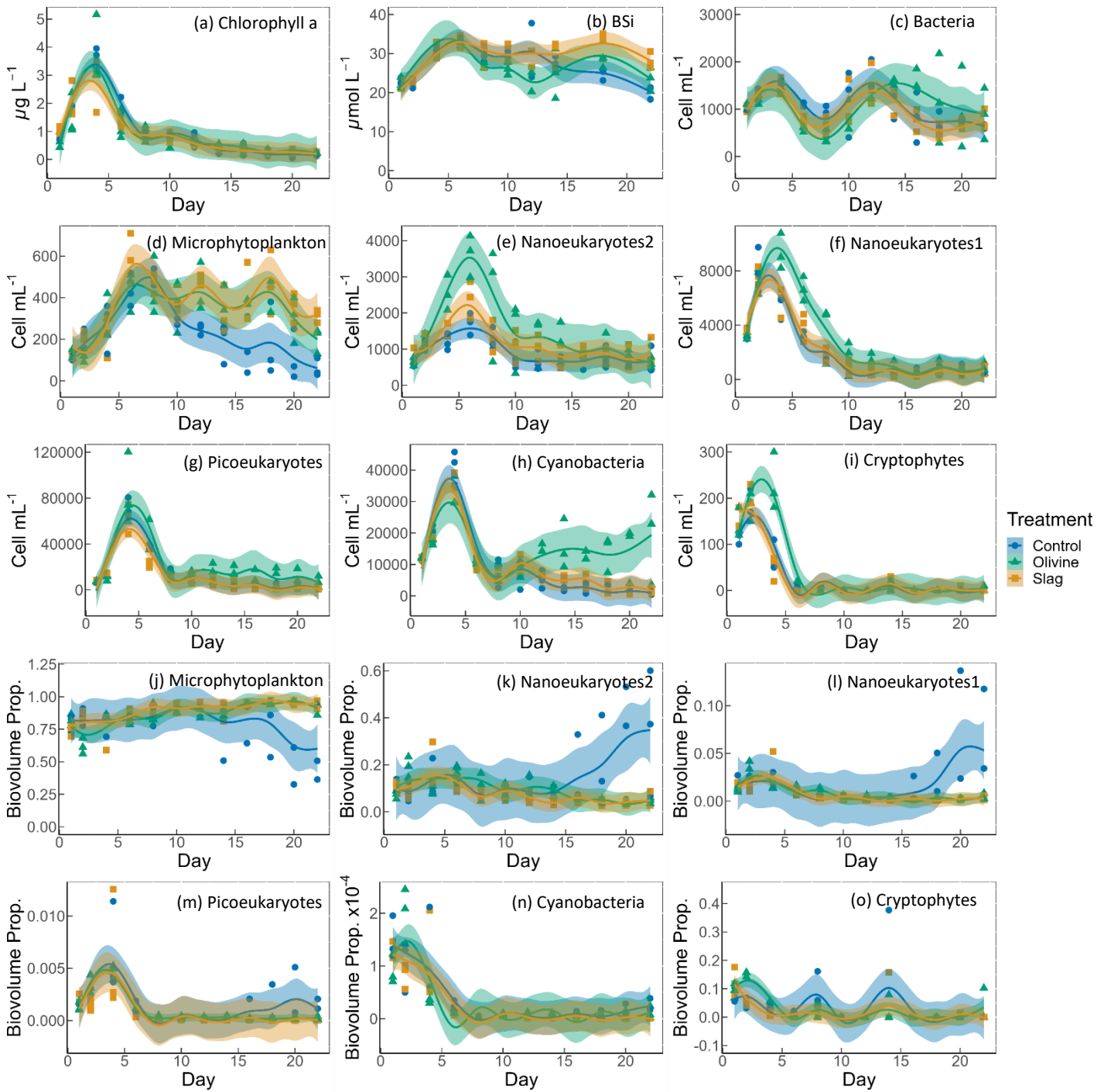
419

420 The POC on day 1 and day 22 from all microcosms were very similar, 10.99 ± 0.58 and 11.03 ± 0.41 μmol L⁻¹ respectively
421 (Fig. S4) so the metal:POC results were consistent with the particulate trace metal results (Fig. 4 e-h). In general, the non-
422 surface metal:POC are positively correlated with the total metal:POC ratios (Fig. S5). The ratio of non-surface to total
423 particulate trace metal concentrations is summarized in Table S5. Both non-surface and total Fe concentrations decreased
424 in microcosms on day 22 compared with the pre-addition level. Iron:POC ratios were significantly higher in the treatments
425 than in the control on day 22 (p-values < 0.05. Table S3), and there was no significant difference between mineral addition
426 treatments. The non-surface to total Fe:POC ratios were > 0.94 in all microcosms on both day 1 and day 22. The total and
427 non-surface Mn:POC ratio was the highest in the slag treatment. These ratios were higher than the pre-addition level and
428 the control at the end of the experiment. The total particulate Ni concentrations in the olivine treatment were significantly
429 higher than before olivine addition. The olivine treatment led to a >22-fold higher Ni:POC ratio compared to the other two
430 treatments (p-value < 0.001).

431

432

433



435
 436 **Fig. 5.** Temporal development of chlorophyll a concentration (chl-a), BSi, and different eukaryotic and bacterial plankton groups as
 437 determined with flow cytometry. (a) chlorophyll a; (b) BSi; cell concentrations of (c) heterotrophic bacteria, (d) microphytoplankton, (e)
 438 nanoeukaryotes2, (f) nanoeukaryotes1 (g) picoeukaryotes, (h) cyanobacteria, and (i) cryptophytes; biovolume proportion of (j)
 439 microphytoplankton, (k) nanoeukaryotes2, (l) nanoeukaryotes1 (m) picoeukaryotes, (n) cyanobacteria, and (o) cryptophytes. The figure
 440 data points represent the raw data, and the fitted curve is the generalized additive model. The shaded area represents the 95 % confidence
 441 interval.

442

443 The chl-a concentration in all microcosms increased from day 1 to day 4 from $1 \mu\text{g L}^{-1}$ to $3\text{-}4 \mu\text{g L}^{-1}$ (Fig. 5a). The chl-a
444 concentration then decreased rapidly from day 4 to day 8, then continued to decrease, though more slowly, to $<0.3 \mu\text{g L}^{-1}$
445 until the end of the experiment. The GAMs of chl-a did not show any difference between treatments and the control (both
446 P-means and P-smooths >0.05 , see Table S2).

447

448 The BSi concentration increased from day 1 to day 6 in all microcosms (Fig. 5b). In the olivine treatments, BSi
449 concentrations decreased slightly after the peak until day 12 but then increased again. In the slag treatment, BSi
450 concentrations remained relatively stable after the initial phytoplankton bloom. In contrast, BSi concentration decreased
451 continuously in the control after the initial peak. Olivine particles suspended in seawater after the mineral addition (see
452 section 3.2) partially ended up on BSi filters during filtration. This led to extremely high BSi measurements on days 2 and
453 4 that were removed from Fig. 5b. Without these outliers, the mean of fitted BSi GAM in the olivine treatment was lower
454 than the control and the slag treatment (Table S2), and the slag treatment had the highest average BSi over the course of
455 the experiment. Overall, the BSi trends in the two treatments were similar (P-smooths = 0.269), and both were significantly
456 different from the control (P-smooths <0.05).

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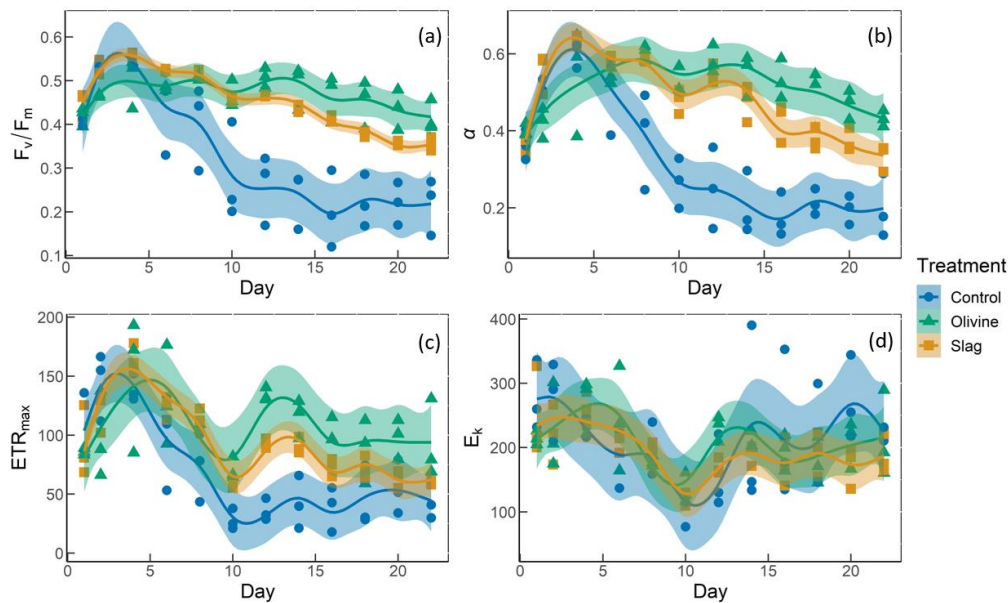
458 The development of the phytoplankton community composition showed significant differences between the treatments and
459 the control. In general, most phytoplankton groups exhibited similar patterns to chl-a, with peak cell numbers occurring on
460 day 4 (Fig. 5f-i) apart from microphytoplankton and nanoeukaryotes2 which had the peak delayed for 1-2 days (Fig. 5d-
461 e). Please be aware that flow cytometers may not capture some large and chain-forming phytoplankton. After reaching
462 peak values during the bloom, phytoplankton abundance generally decreased steadily. Microphytoplankton displayed
463 similar trends to the results for BSi. Before day 10, all microcosms had similar microphytoplankton abundances (Fig. 5d).
464 However, in the control, microphytoplankton abundance declined continuously and at a faster rate compared to the two
465 treatments (P-smooths values <0.03). From day 2 to day 6, the abundance of nanoeukaryotes1, nanoeukaryotes2,
466 picoeukaryotes, and cryophytes was higher in the olivine treatment compared to the slag treatment and the control. After
467 day 8, their abundance in the olivine treatment decreased to a similar level as the slag treatment and the control. Notably,
468 there were few significant differences observed between the slag treatment and the control in terms of the abundances of
469 nanoeukaryotes1, nanoeukaryotes2, picoeukaryotes, cyanobacteria, and cryptophytes throughout the experiment. In the
470 olivine treatment, cyanobacteria experienced a second bloom after day 10, which was significantly different from the other
471 two groups (P-smooths <0.01). Heterotrophic bacteria exhibited an increase and decline pattern following the
472 phytoplankton bloom until day 8 (Fig. 5c). Subsequently, bacteria abundance increased again, reaching a second peak
473 during days 12-14, followed by a decline until the end of the experiment. The decline in bacteria abundance was slower in
474 the olivine treatment, although no significant differences were detected between treatments (Table S2).

475

476 Among all the microcosms, microphytoplankton consistently accounted for the largest proportion of biovolume. From the
477 perspective of biovolume proportion, the mineral addition mainly influenced the microphytoplankton and nanoeukaryotes.
478 The control had similar phytoplankton biovolume distribution as the treatments from day 1 to day 15, but after that the
479 proportion of microphytoplankton biovolume decreased to a level significantly lower than the treatments. In the control
480 treatment, the proportion of nanoeukaryotes' biovolume increased as the proportion of microphytoplankton decreased. The
481 biovolume of picoeukaryotes, cyanobacteria and cryptophytes increased during the phytoplankton bloom and then
482 decreased drastically after the bloom. There were no significant differences in biovolume proportion observed for

483 picoeukaryotes, cyanobacteria and cryptophytes between the treatments and the control.

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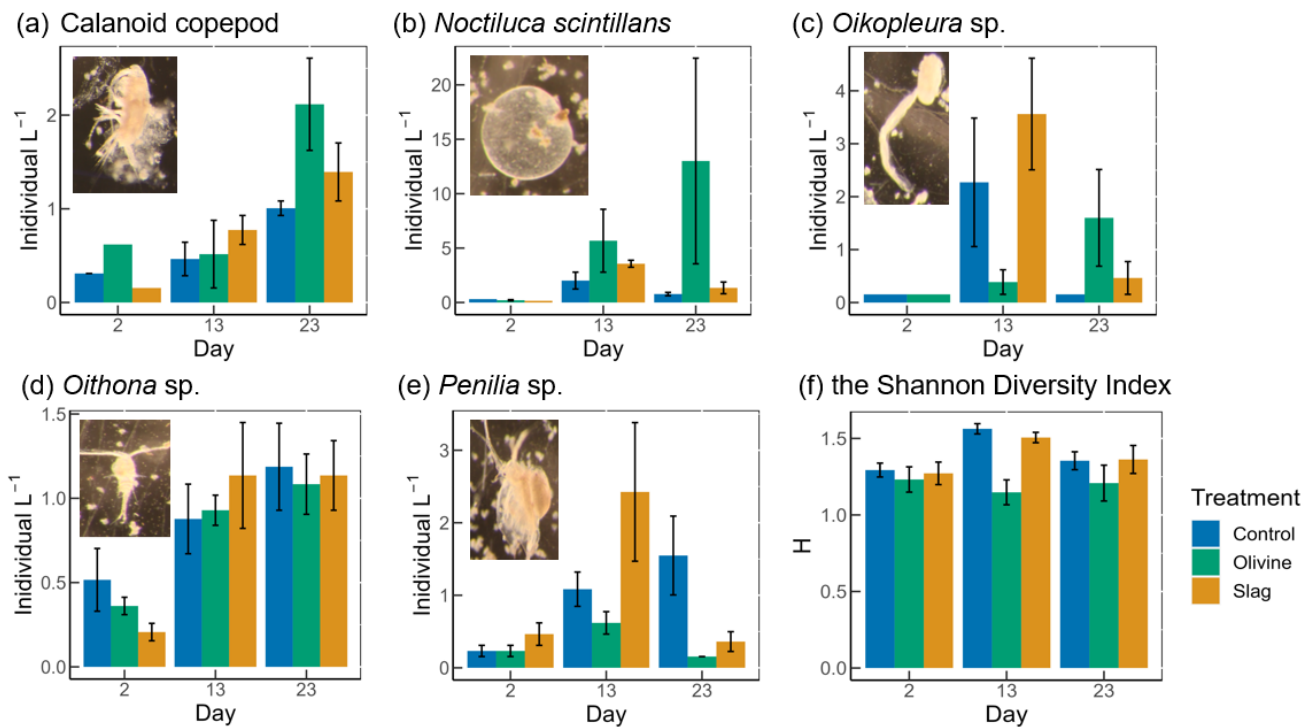
486 **Fig. 6.** The photosynthetic performance of the phytoplankton community. (a) F_v/F_m , the maximum quantum yield of photosynthesis II.
487 (b) α , the initial slope of the rapid light curves. (c) ETR_{max} is the maximum electron transport rate, the maximum potential photosynthetic
488 rate. (d) E_k is light-saturation parameter, Unit: $\mu\text{mol photons m}^{-2} \text{s}^{-1}$.

489

490 The temporal development of F_v/F_m , α , ETR_{max} , and E_k is illustrated in Fig. 6. The F_v/F_m values of the phytoplankton
491 community were approximately 0.42 ± 0.01 and increased to levels > 0.5 during the peak of the phytoplankton bloom on
492 day 4 (Fig. 6a). Following the bloom, F_v/F_m values dropped below 0.3 in the control. However, the decline in F_v/F_m after
493 the bloom was less pronounced in the two mineral addition treatments with the olivine treatment maintaining higher F_v/F_m
494 values than the slag treatment (P-smooths < 0.05). At the end of the experiment, F_v/F_m was 0.22 ± 0.04 in the control, 0.35
495 ± 0.01 in the slag treatment, and 0.42 ± 0.02 in the olivine treatment. The temporal development of α aligned with the
496 patterns observed for F_v/F_m (compare Fig. 6a and 6b). The maximum values of ETR_{max} were observed on day 4 in the
497 control and the slag treatment, while in the olivine treatment, it occurred on day 5 (Fig. 6c). Subsequently, ETR_{max}
498 continuously decreased until day 10 and then stabilized until the end of the experiment. However, ETR_{max} exhibited a
499 subsequent increase in the mineral treatments around day 12. The ETR_{max} values were higher in the mineral treatments
500 compared to the control group (P-means < 0.001 , Table S2). The parameter E_k decreased from $246 \pm 17 \mu\text{mol photons m}^{-2}$
501 s^{-1} on day 1 to $121 \pm 7 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ on day 10, and then it increased again to approximately $200 \mu\text{mol photons m}^{-2}$
502 s^{-1} by the end of the experiment (Fig. 6d). The change in E_k did not exhibit significant differences between the treatments
503 and the control (both P-means and P-smooths > 0.05).

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Fig. 7. The dominant zooplankton abundance and community diversity from different treatments. Abundance of dominant zooplankton in microcosms: (a) calanoid copepod; (b) *Noctiluca scintillans*; (c) *Oikopleura* sp.; (d) *Oithona* sp.; (e) *Penilia* sp.; and (f) the Shannon diversity index (H) of different treatments and the control. Error bars represent the standard error calculated from three microcosm replicates. Photographs of each zooplankton group are shown on the corresponding graphs.

Thirteen zooplankton taxonomic groups were identified in the microcosms. The dominant taxa were the appendicularian *Oikopleura* sp., the cyclopoid copepod *Oithona* sp., the cladoceran *Penilia* sp., the heterotrophic dinoflagellate *Noctiluca scintillans* and several calanoid copepods including *Acartia* sp., *Paracalanus* sp. and *Gladioferens* sp. The larvae and eggs of *Oikopleura*, *Penilia* and copepod were also observed under the microscope. In general, higher zooplankton numbers were observed after the bloom on day 13 (Fig. 7). The abundance of calanoid copepods and *Oithona* sp. increased after day 2 (Fig. 7a, d), and there was no significant difference between treatments and the control (p-values >0.05, Table S4). The abundance of *N. scintillans* increased significantly more in the olivine treatment than in the control and the slag treatment, with highest abundance of 13 ± 9 individual L^{-1} observed in the olivine treatment on the last day (Fig. 7b). The abundance of *Oikopleura* in the control and the slag treatment was higher than the olivine treatment on day 13 but was higher in the olivine treatment on day 22 (Fig. 7c). A higher abundance of *Penilia* sp. was found in the slag treatment on day 13 and in the control on day 23 (Fig. 7e). Due to the patchy distribution of zooplankton, these data have large standard errors and only the differences in the numbers of *N. scintillans* in the olivine treatment were statistically significantly different from the slag treatment and the control (p-value <0.05, Table S4).

Considering the control and slag treatment, the Shannon Diversity Index (H) increased from day 2 to day 13 and declined on day 23, while in the olivine treatment, H was lower on day 13 than on day 2 and day 23 (Fig. 7f). The GLMs revealed that the olivine treatment had significantly lower H on day 13 than the control and the slag treatment (p-values <0.001). There were no significant differences in H between the control and the slag treatment (Table S4). The addition of olivine decreased the zooplankton community's diversity. This is mainly driven by distinct trends observed in the abundance of *Oikopleura* sp., *Penilia* sp., and *N. scintillans* (Fig. 7).

532

533 4. Discussion

534 4.1 CO₂ removal potential of slag and olivine

535 The slag powder created significantly higher CO₂ removal potential than the olivine powder over the course of the study.
536 Ca(OH)₂ and CaO in slag and Mg₂SiO₄ in olivine are likely to be the main functional minerals driving the measured
537 alkalinity enhancement. Total alkalinity increased by 361 μmol kg⁻¹ in the slag treatment while it increased by only 29
538 μmol kg⁻¹ in the olivine treatment, equivalent to a potential increase in marine inorganic carbon by 14.7 and 0.9% within
539 3 weeks of their application. When normalizing these alkalinity increases to the same material weight, 1 g of slag would
540 release 9626 μmol TA while 1 g of olivine would release 16 μmol TA. Thus, over 3 weeks of experimental incubation, slag
541 is ~600-fold more efficient in releasing alkalinity for particles of this size class (please note that particle size spectra of
542 olivine and slag were similar but not identical; Fig. S1). We can also use these values to make a rough estimate of how
543 much CO₂ these two minerals could potentially sequester. One mole of alkalinity from olivine and slag can sequester
544 approximately 0.85 mole of CO₂. Thus, one tonne of slag and olivine powder as used here could sequester 360 and 0.6 kg,
545 respectively, within 3 weeks. It is likely that optimization of particle size and application method may lead to higher
546 efficiencies. Nevertheless, the slag showed potential as an OAE source mineral, even when applied as relatively coarse
547 powder in this experiment.

548

549 4.2 Environmental implications of slag and olivine additions

550 The amount of olivine and slag powder added to the treatments differed significantly (100 g of olivine powder were added
551 while only 2 g of slag powder were added to the 53 L microcosms). Our rationale for these different mass additions was to
552 yield somewhat similar amounts of detectable alkalinity enhancement in the dissolved phase, since we already knew from
553 tests before the experiment that slag elevates alkalinity faster than olivine. However, olivine was less efficient in releasing
554 alkalinity than we had anticipated so that even a 50-fold higher addition of olivine (in mass) did not compensate for this
555 difference. As such, our experiments are associated with an “apples and oranges issue” in that our perturbation with
556 minerals and associated OAE differs. ~~We note that an adjusted addition of minerals depending on the alkalinity
557 enhancement rate would be consistent with what OAE practitioners may do under real-world conditions. Presumably, OAE
558 deployments may have to adjust the amounts of minerals to detect alkalinity enhancement in the dissolved phase for
559 verification purposes. Nevertheless, to account for this “apples and oranges issue”, the following discussion mainly
560 relates the observed environmental effects with the alkalinity enhancement achieved over the course of the study.~~

561 4.2.1. OAE effects on phytoplankton physiology and community

562 Previous research has hypothesised that OAE-induced changes in seawater carbonate chemistry could delay phytoplankton
563 bloom formation due to reductions in seawater pCO₂ in the aftermath of an OAE deployment (Bach et al., 2019). The build-
564 up of chlorophyll *a* concentration as observed here was indistinguishable between treatments and the control, suggesting
565 no effect of slag- or olivine-based OAE on phytoplankton bloom dynamics under these experimental settings. A lack of
566 bloom delay due to carbonate chemistry is unsurprising for the olivine treatment where the release of alkalinity was small

567 (29 $\mu\text{mol kg}^{-1}$ alkalinity release), but somewhat more surprising in the slag treatment where alkalinity was quite rapidly
568 increased by 361 $\mu\text{mol kg}^{-1}$. However, the release was still lower than in a very similar study by Ferderer et al., (2022)
569 where alkalinity was increased by 500 $\mu\text{mol kg}^{-1}$ using sodium hydroxide and even there they did not observe a bloom
570 delay. Based on this very limited evidence, it seems that bloom delays do not occur consistently under OAE within the
571 alkalinity ranges tested in this study.

572

573 The nutrient data show that the phytoplankton community was most likely N-limited after day 4 so that the release of
574 Si(OH)_4 from olivine and Si(OH)_4 and PO_4^{3-} from slag did not stimulate a further increase in chlorophyll-*a* concentration
575 in the treatments. The development of BSi concentrations is indicative of the prevalence of diatoms in the microcosms but
576 differences between treatments and the control were small. The release of Si(OH)_4 through olivine and slag will most likely
577 benefit diatoms but this fertilization effect did not manifest in this specific experiment because N was limiting diatom
578 growth. However, when new N is supplied then diatoms will likely take a bigger share of the limiting N pool when olivine
579 or slag are used for OAE, as has been shown in Si(OH)_4 manipulation experiments in and outside the context of OAE
580 research (Egge and Jacobsen, 1997; Ferderer et al., 2023). In the case of slag, the release of PO_4^{3-} will likely be another
581 driver that affects plankton productivity and community composition. As for Si(OH)_4 , however, the effect of additional
582 PO_4^{3-} did likely not materialise in this experiment because PO_4^{3-} was not limiting over the course of the study. However, in
583 ecosystems where PO_4^{3-} is a limiting resource, the application of slag could enhance productivity with associated benefits
584 for higher trophic levels. In contrast, excessive applications of slag and concomitant PO_4^{3-} release could also pose a risk of
585 eutrophication. Future studies may need to investigate what the most sustainable dose of OAE via olivine and/or slag
586 applications could be and the suitable regions for application.

587

588 The flow cytometry results further revealed the change in phytoplankton community composition. Both the olivine and
589 slag treatments sustained higher microphytoplankton abundances after the peak of the phytoplankton bloom. This trend is
590 consistent with higher F_v/F_m values in the treatments than in the control so that it is tempting to assume that
591 photophysiological fitness gain measured with the FRRf led to higher competitiveness of microphytoplankton in the
592 community. Indeed, calculations of the contribution of different phytoplankton groups to total biovolume based on flow
593 cytometry indicate that microphytoplankton were predominantly contributing to the phytoplankton community biovolume
594 so that the responses measured by the FRRf were probably to a large extent driven by this group.

595

596 Apart from the increased microphytoplankton abundance, for the slag treatment, other phytoplankton groups distinguished
597 with flow cytometry did not deviate considerably from the control. The olivine addition, however, triggered more
598 pronounced shifts in the phytoplankton community. In particular, the nanoeukaryotes (roughly between 2-20 μm),
599 picoeukaryotes and the cryptophytes showed relatively higher abundance during the peak of the phytoplankton bloom, and
600 the abundance of cyanobacteria was higher after the bloom. We speculate that this shift following olivine treatment may
601 be attributable to a top-down effect from the decrease in zooplankton grazing effects in microcosms, which will be
602 discussed in section 4.2.2.

603

604 The measurement of photophysiological parameters revealed that the phytoplankton had generally better photosynthetic
605 performance in the slag and olivine treatments than in the control, especially after the phytoplankton bloom. During the
606 first 5 days, the changes in phytoplankton photosynthetic performance were indistinguishable between the control and the

607 slag treatment, while the values of α , ETR_{max} and F_v/F_m were lower in olivine treatment. At this time all microcosms had
608 similar health because of the relatively high NO_x^- concentrations and Fe supply (around 500 nmol L^{-1}), but the suspended
609 particles in the olivine treatment may have led to artifacts in the measuring of photophysiology by FRRf. Scattering and/or
610 absorption of light by suspended olivine particles is the most parsimonious explanation for the simultaneous depression in
611 α , ETR_{max} and F_v/F_m . After day 5, the F_v/F_m , α and ETR_{max} values decreased significantly faster in the control than in the
612 treatments, and to values lower than the initial condition. A decrease of F_v/F_m is commonly associated with physiological
613 stress, such as nutrient limitation, and high light stress (Bhagooli, et al., 2021), with Fe limitation causing a more
614 pronounced decline in F_v/F_m than nitrogen limitation (Gorbunov, et al., 2021). The ETR_{max} , which represents the maximum
615 electron transport rate, has also been shown to be negatively affected when phytoplankton experience nitrogen or Fe
616 limitation (Kolber et al., 1994; Gorbunov & Falkowski 2021). Furthermore, the change in photosynthesis performance
617 after day 10 was suspected to be driven by the microphytoplankton because the decrease of F_v/F_m , α , and ETR_{max} in the
618 control was coupled with the decrease in microphytoplankton abundance while the other phytoplankton groups were in
619 low abundance as in the mineral addition treatments, and the microphytoplankton contributed significantly (75 %) to
620 community biovolume. All microcosms were similarly NO_x^- limited from day 5 onward (Fig. 3) so that N-limitation is
621 unlikely to explain different trends in photophysiological parameters between the control and OAE treatments. Trace metals,
622 especially Fe, released through slag and olivine additions could potentially explain these differences.

623

624 Several of the trace metals released from slag and olivine are required for photosynthesis. For example, Fe is required for
625 many proteins functioning in photosynthesis, such as cytochromes, ferredoxin, and superoxide dismutase (SOD) (Twining
626 and Baines, 2013), and the addition of Fe can stimulate the growth of phytoplankton (Sunda and Huntsman, 1997) and
627 increase F_v/F_m (Behrenfeld et al., 2006). The dissolved and particulate Fe concentrations were higher in mineral addition
628 treatments than in the control indicating potentially more Fe available to sustain phytoplankton photosynthesis. While this
629 explanation is intriguing for the observed trends in photophysiology, it remains unclear why such strong differences
630 occurred between mineral addition and control treatments despite dissolved Fe concentrations of $\sim 500\text{ nmol L}^{-1}$ at the end
631 of the experiment in the control. In Fe-limited ocean regions, dissolved Fe is at least two orders of magnitude lower, and
632 the enhancement of Fe to $\sim 1.5\text{ nmol L}^{-1}$ can induce major phytoplankton blooms and relieve photophysiological stress (De
633 Baar et al., 2005). It is possible that these coastal phytoplankton species have higher Fe requirements than those from the
634 open ocean where Fe is limiting (Strzepek and Harrison, 2004). Our findings suggest that Fe perturbations may not only
635 be relevant for low Fe open ocean regions but could also be relevant for coastal ocean locations.

636

637 Alternatively, the addition of Mn, Ni and other trace metals from mineral addition may have benefited photosynthesis.
638 Manganese is required for the water-splitting reaction of photosystem II (Armstrong, 2008), and both Mn and Ni are
639 common bioactive trace metals for SODs in marine phytoplankton. The noxious superoxide anion radical (O_2^-) generated
640 from aerobic respiration and oxygenic photosynthesis could be harmful to phytoplankton physiology, and SOD removes
641 O_2^- , thus improving photosynthesis (Wafar et al., 1995; Wolfe-Simon et al., 2005). This is consistent with our
642 photosynthetic measurements. Interestingly, although the amounts and types of trace metals released from the slag and
643 olivine powders were different, they led to relatively similar F_v/F_m values with only slightly higher F_v/F_m in the olivine
644 than the slag treatment from days 10-21. Over this time, these trace metal additions could have fertilized different
645 phytoplankton species (Pausch et al., 2019; Balaguer et al., 2022; Guo et al., 2022) possibly because different
646 phytoplankton could have different trace metal requirements, such as for SOD. For example, cyanobacteria have NiSOD,

647 diatoms have MnSOD, dinoflagellates have both FeSOD and MnSOD (Wolfe-Simon et al., 2005). Another explanation is
648 that phytoplankton in the control were limited by bicarbonate while the treatments had sufficient bicarbonate from added
649 minerals. However, we were unable to determine the species-level changes in the phytoplankton community, and hence
650 whether these trace metals, individually or combined, could account for the observed phytoplankton community
651 photosynthetic performance.

652

653 **4.2.2. OAE impacts on the zooplankton community**

654 Slag-based OAE did not significantly influence the zooplankton community composition while olivine-based OAE induced
655 some statistically significant effects, including a lower Shannon diversity. The increase in *N. scintillans* abundance and the
656 decrease in *Penilia* sp. and *Oikopleura* sp. in the olivine treatment indicate that the zooplankton response to OAE can vary
657 among different zooplankton types.

658

659 The observed lower abundance of *Oikopleura* sp. on day 13 in the olivine treatment may indicate a temporary suppression
660 or a slower growth rate of this zooplankton species in response to the olivine addition. This could be attributed to the
661 potential effects of olivine on the availability of essential nutrients or changes in the physicochemical environment of the
662 water. However, the subsequent increase in *Oikopleura* sp. abundance by day 22 suggests that the growth of this species
663 may have recovered or accelerated in the olivine treatment, leading to a higher abundance compared to the slag treatment
664 and the control on day 22. As discussed in section 4.2.1, reduced *Oikopleura* sp. abundance was unlikely due to reduced
665 food availability since phytoplankton within the preferred edible size spectrum, such as cyanobacteria and nanoeukaryotes,
666 were even more abundant in the olivine treatment. Instead, we hypothesize it to be an effect of the suspended olivine
667 particles that occurred for approximately the first 5 days of the study that were so plentiful that they turned the enclosed
668 seawater milky and may have clogged the mucous feeding mesh of *Oikopleura* sp. (Lombard et al., 2011).

669

670 The abundance of *Penilia* sp. and *Oikopleura* sp. was lower in the olivine treatment than the other two groups throughout
671 the experiment while the abundance of *N. scintillans* was consistently higher. The second bloom of cyanobacteria in olivine
672 is potentially the results of decreased predators, like *Penilia* sp. and *Oikopleura* sp.. We cannot provide a particularly
673 convincing hypothesis about what specifically drove these in these zooplankton species, although it is tempting to speculate
674 that suspended particles present in the olivine treatment at the beginning may have played a role also for those organisms
675 since this was the only apparent systematic difference to the control and slag treatment. The proliferation of *N. scintillans*
676 can be problematic since heterotrophic dinoflagellate blooms can regulate phytoplankton communities, cause toxicity to
677 aquatic fish, and create a hypoxic sub-surface zone (Baliarsingh et al., 2016; Zhang et al., 2020; Al-Azri et al., 2007),
678 although a bloom of *N. scintillans* in southeast Australia only induced ichthyotoxicity when the cell concentration reached
679 2,000,000 cells L⁻¹ (Hallegraeff et al., 2019). For comparison, we observed a maximum of 32 cells L⁻¹ in one microcosm
680 replicate of the olivine treatment.

681

682 In comparison to olivine, steel slag seemed to have less potential to affect zooplankton community composition. The
683 abundance of all groups of phytoplankton, apart from microphytoplankton after day 10, was similar in the slag treatment
684 and the control through the experiment. This is probably because the amount of slag powder added in the treatment was
685 much less than the olivine powder resulting in fewer physical particle perturbations to zooplankton. In addition, the

686 chemistry perturbations such as enhanced alkalinity concentration and various dissolved trace metals, especially Mn, from
687 the slag powder did not seem to have a notable direct influence on zooplankton abundance over the three-week period.
688 Even though we did not observe drastic changes in zooplankton abundance during the experiment, considering there was
689 higher microphytoplankton abundance in the slag treatment after day 10, slag powder may benefit some zooplankton
690 especially those who feed on large phytoplankton on a longer time scale.
691

692 **4.2.3. Dissolved trace metal accumulation in seawater and its environmental implications**

693 The addition of olivine and slag as OAE source minerals released trace metals into the seawater, predominantly Al, Fe, Ni,
694 and Cu (olivine) as well as Al, Fe, and Mn (slag). The maximum measured concentrations for dissolved Al, Fe, Ni, Cu, and
695 Mn were 1093, 253, 77, 27, and 810 nmol L⁻¹, respectively. The threshold values for drinking water with health or aesthetic
696 considerations by the Australian Drinking Water Guidelines for Al, Fe, Ni, Cu, and Mn are 7400, 5360, 340, 15600, and
697 1800 nmol L⁻¹, respectively (NRMMC, 2022). All dissolved trace metal concentrations measured herein are well below
698 these health and aesthetic threshold values. In natural freshwater sources, the concentrations of Al, Fe, Ni, Cu and Mn are
699 generally less than 44000, 71400, 510, 156, and 25400 nmol L⁻¹ (NRMMC, 2022). Although these natural water data were
700 primarily derived from rivers and streams, they serve as valuable references for evaluating trace metal release in our
701 experiment. Thus, mineral additions to the microcosms as simulated here did not increase thresholds for any of the
702 measured trace metals beyond those that are considered safe for drinking water quality, and they were within the trace
703 metal concentration range in natural water. However, while these guidelines on drinking water provide a good starting point
704 on how to quantify what OAE perturbation could be considered “safe” and “unsafe” with regards to trace metals, it must
705 be recognized that seawater is not drinking water and that critical thresholds may be different in the latter.
706

707 The release of trace metals from OAE materials is considered to have relatively strong effects on biology, particularly in
708 the open ocean where trace metals usually occur in lower concentrations. For example, oceanic Al, Fe, Ni, and Mn
709 concentrations are about 2, 0.5, 8, and 0.3 nmol L⁻¹ (Bruland and Lohan, 2003; Sohrin and Bruland, 2011). Previous
710 research on OAE-associated trace metal impacts on individual phytoplankton species grown in laboratory environments
711 has shown that concentration thresholds beyond which trace metal induces negative effects on fitness likely differ between
712 species (Guo et al., 2022; Hutchins et al., 2023; Xin et al., 2023). Indeed, our experiment with plankton communities
713 provides further support that several components of the planktonic food web are affected by OAE. However, our experiment
714 does not allow determining whether observed effects were primarily invoked by carbonate chemistry, macronutrient (P and
715 Si), or trace metal perturbations. Thus, dedicated experiments isolating the impact of these factors on plankton will be
716 required in the future.

717 **4.2.4. Particulate trace metal accumulation in seawater and its environmental implications**

718 The Derwent Estuary (where we collected our plankton communities) was highly metal polluted due to industrial practice
719 (Macleod and Coughanowr, 2019). Both our dissolved and particulate trace metal data indicated high background metal
720 concentrations, especially for Fe and Zn. Furthermore, the metal:POC ratios found here are higher than reported for open
721 ocean studies or lab cultures. For example, the Fe:POC can vary from 2-136 $\mu\text{mol mol}^{-1}$ depending on the cultured
722 phytoplankton species and the environmental dissolved Fe concentration (Kulkarni et al., 2006; Sunda and Huntsman,
723 1995; King et al., 2012; Boyd et al., 2015). In our results the Fe:POC values ranged from 1200 to 39 000 $\mu\text{mol mol}^{-1}$, which

724 may be due to the particulate trace metal richness of the Derwent Estuary (control) and/or the addition of lithogenic particles
725 (slag and olivine treatment). The presence of abiotic particulate metal sources creates challenges to quantify metal quotas
726 and then to evaluate metal accumulation effects on biological organisms.

727

728 Our study reveals that the added minerals enriched the particulate trace metal pools to various degrees. Consistent with the
729 dissolved trace metal data, the slag treatment was enriched with particulate Fe and Mn while the olivine treatment was
730 enriched with particulate Fe and Ni. The enhanced particulate Ni and Mn concentrations were higher than before mineral
731 additions and the control levels. This is in line with previous research which indicates a positive correlation between
732 particulate and dissolved trace metal concentrations (Gaulier et al., 2019).

733

734 Based on the amounts released through OAE as simulated herein, it appears that Ni and Mn have the highest potential to
735 cause toxicity in certain marine organisms (Jakimska et al., 2011). These trace metals have the potential to accumulate in
736 marine organisms over time (bioaccumulation effects), and their increased concentrations in the food chain can lead to
737 adverse effects on the health and well-being of organisms at higher trophic levels (biomagnification effects). One crucial
738 next step will be to investigate whether the enhanced dissolved/particulate trace metal will affect higher trophic levels to
739 estimate the environmental risks of OAE on other marine organisms.

740

741 **5 Conclusions**

742 Our study aimed to assess the environmental impacts of two ground OAE minerals, olivine and steel slag, on coastal
743 plankton communities. Both minerals released alkalinity, leading to an elevation in pH_T . However, the addition of steel
744 slag exhibited significantly higher efficiency in elevating alkalinity compared to olivine.

745

746 Approximately 1.9 g L^{-1} of olivine powder were added in the olivine treatments, leading to a $29 \text{ } \mu\text{mol kg}^{-1}$ increase in
747 alkalinity and increased concentrations of Si(OH)_4 and trace metals (Fe and Ni). Compared to this relatively modest
748 increase of alkalinity and associated CO_2 removal potential, the impacts on the plankton community appeared to be
749 relatively pronounced. Thus, although our experiment ran for only 3 weeks, and olivine powder may slowly release more
750 alkalinity, the short-term response monitored here suggests that the immediate climatic benefit is relatively small compared
751 to a relatively pronounced environmental effect.

752

753 Only 0.038 g L^{-1} of slag were added to the treatment but this led to an alkalinity enhancement of $361 \text{ } \mu\text{mol kg}^{-1}$ and the
754 increased concentrations of macronutrients (P and Si) and trace metals (Mn and Fe) additions as well as changes in
755 carbonate chemistry. Although limited environmental impacts were observed from the slag treatment in our experiment,
756 some aspects require further study. For example, the pronounced release of P could cause eutrophication and the relatively
757 rapid increase in pH may be a detrimental aspect if organisms cannot acclimate fast enough. Furthermore, it is essential to
758 consider that the composition of steel slag can vary depending on the source factory (Wang et al., 2011; Proctor et al.,
759 2000), which may affect the efficiency of carbon removal and change the trace metal perturbation. Nevertheless, just based
760 on our experiment, the comparison between the immediate climatic benefit and environmental effect appears to be more
761 favourable for slag than olivine.

762

763 Based on our findings, it can be concluded that steel slag powder exhibited fewer environmental impacts on plankton
764 communities compared to olivine powder relative to its capacity for alkalinity enhancement. The results highlight the
765 importance of carefully assessing the environmental consequences of using specific OAE minerals, particularly when
766 considering their potential effects on plankton communities.

767

768 **Data availability.** Data are available in the Institute for Marine and Antarctic Studies (IMAS) data catalogue, University
769 of Tasmania (UTAS) (<https://doi.org/10.25959/X6FH-9K15>, Guo, J., & Bach, L. (2023).).

770

771 **Author contributions.** LTB, RFS, KMS and JAG designed the experiments and JAG carried them out. LTB, RFS and
772 KMS supervised the study. ATT analysed the dissolved/particulate trace metal samples. JAG conducted statistical analyses.
773 JAG prepared the manuscript with contributions from all authors.

774

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776

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