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Influence of ocean alkalinity enhancement with olivine or steel slag on a coastal plankton community in Tasmania

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Supplements

Table S1. The dissolved trace metal concentrations in different mesocosms. Unit: nmol L^{-1}. The highlighted data points are were identified as outliers. The whole microcosm dataset of outliers were excluded from subsequent data analysis.

Day	Microcosm No.	Treatment	Al	Cu	Fe	Mn	Ni	P	Zn
1	M1	Control	390	20	240	70	30	850	3970
1	M4	Control	21760	50	72490	1190	3000	2070	3590
1	M7	Control	2330	20	1320	120	40	2530	680
1	M3	Olivine	490	10	100	10	50	390	750
1	M6	Olivine	810	20	500	20	50	650	1430
1	M9	Olivine	360	10	110	10	20	590	650
1	M2	Slag	470	20	110	20	40	370	1090
1	M5	Slag	1590	20	1140	20	50	1460	2120
1	M8	Slag	1910	30	7580	120	340	1580	500
2	M1	Control	360	10	140	10	20	670	310
2	M4	Control	500	20	350	10	20	420	260
2	M7	Control	490	20	110	10	40	400	160
2	M3	Olivine	960	20	450	10	60	380	250
2	M6	Olivine	790	20	110	10	80	760	130
2	M9	Olivine	950	20	200	10	60	390	120
2	M2	Slag	2980	50	3110	610	40	1560	550
2	M5	Slag	1190	20	210	10	40	960	290
2	M8	Slag	930	10	260	20	20	1110	150
13	M1	Control	390	10	160	30	30	520	360
13	M4	Control	240	10	110	70	10	600	170
13	M7	Control	390	10	110	70	20	470	160
13	M3	Olivine	670	20	270	80	80	520	150
13	M6	Olivine	540	20	200	80	70	730	80
13	M9	Olivine	1160	160	2160	60	80	620	230
13	M2	Slag	990	20	150	710	30	2430	140
13	M5	Slag	990	10	120	780	20	2460	110
13	M8	Slag	940	10	100	730	20	2390	80
22	M1	Control	4390	20	54020	830	2330	1110	370
22	M4	Control	220	20	100	70	20	570	210
22	M7	Control	240	20	110	70	20	500	180
22	M3	Olivine	1370	20	530	50	70	800	180
22	M6	Olivine	960	30	90	30	80	610	70
22	M9	Olivine	580	30	110	30	80	480	70
22	M2	Slag	1240	10	290	790	20	2810	290
22	M5	Slag	1060	10	110	820	10	2620	60
22	M8	Slag	980	10	100	820	10	2620	50

Table S2. The GAM of biochemical parameters. The formula used is the established formula of variables in R. Estimates are the estimated mean effects from different treatments on the measured parameters. The GAM family and link were set as "gaussian, identity" in the "glm()" function in R. If the estimate is positive (Treatment A -Treatment B >0), it means the estimated mean effects of treatment A are larger than treatment B. If the estimate is negative (Treatment A -Treatment B <0), it means the estimated mean effects of treatment A are smaller than treatment B. The "P_mean" represents the p-values of estimated means and the "P_smooths" represents the p-values of two smooth terms of GAMs (see Method 2.5). If the P mean value is <0.05, then the GAMs from the two compared treatments have significant differences in the overall mean values. If the P smooths value is <0.05, then the two GAMs from the two compared treatments have significant trends.

Formula	Treatment comparisons	Estimate	P_mean	P_smooths	R_adj
pH = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Olivine - Control	0.044	<0.001	<0.001	
	Slag - Control	0.355	<0.001	<0.001	
	Olivine - Slag	-0.311	<0.001	<0.001	0.980
Bsi Con. = Treatment + s(Day, k = 8, fx = TRUE) + s(Day, by = oTreatment, k = 7, fx = TRUE)	Slag - Control	2.015	0.010	0.012	
	Olivine - Control	-0.503	0.614	0.016	
	Olivine - Slag	-2.518	0.015	0.269	0.605
Total alkalinity Con. = Treatment + s(Day, k = 8, fx = TRUE) + s(Day, by = oTreatment, k = 8, fx = TRUE)	Slag - Control	250.188	<0.001	<0.001	
	Olivine - Control	24.702	<0.001	0.061	
	Olivine - Slag	-225.486	<0.001	<0.001	0.992
Chlorophyl-a Con. = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Slag - Control	-0.080	0.401	0.493	
	Olivine - Control	-0.048	0.617	0.922	
	Olivine - Slag	0.033	0.732	0.691	0.815
NOx- Con. = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Slag - Control	-0.056	0.165	0.993	
	Olivine - Control	-0.051	0.209	0.854	
	Olivine - Slag	0.005	0.892	0.585	0.862
PO43- Con. = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Slag - Control	1.790	<0.001	<0.001	
	Olivine - Control	0.070	0.016	0.677	
	Olivine - Slag	-1.720	<0.001	<0.001	0.984
Si(OH)4 Con. = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Slag - Control	31.681	<0.001	<0.001	
	Olivine - Control	9.278	<0.001	<0.001	
	Olivine - Slag	-22.402	<0.001	<0.001	0.982
Micro phytoplankton Con. = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Slag - Control	0.131	<0.001	0.004	
	Olivine - Control	0.095	<0.001	0.023	
	Olivine - Slag	-0.035	0.114	0.915	0.637
Nanoeukaryotes1 Con. = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Slag - Control	0.053	0.803	0.996	
	Olivine - Control	0.909	<0.001	<0.001	
	Olivine - Slag	0.857	<0.001	0.004	0.889
Nanoeukaryotes2 Con. = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Slag - Control	0.218	0.047	0.985	
	Olivine - Control	0.586	<0.001	0.002	
	Olivine - Slag	0.368	0.001	0.038	0.631
Pico eukaryotes Con. = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Slag - Control	-2.050	0.395	0.994	
	Olivine - Control	6.519	0.008	0.919	
	Olivine - Slag	8.569	<0.001	0.607	0.761
	Slag - Control	0.236	0.839	0.962	
	Olivine - Control	5.205	<0.001	<0.001	0.764

Cyanobacteria Con. = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Olivine - Slag	4.969	<0.001	<0.001	
Cryptophytes Con. = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Slag - Control	0.003	0.570	0.465	
	Olivine - Control	0.017	0.003	<0.001	
	Olivine - Slag	0.013	0.015	<0.001	0.897
Bacteria_1 /Bacteria_2 ratio = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Slag - Control	-0.256	0.109	0.777	
	Olivine - Control	0.628	<0.001	0.221	
	Olivine - Slag	0.884	<0.001	0.004	0.787
Alpha = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Slag - Control	0.154	<0.001	<0.001	
	Olivine - Control	0.183	<0.001	<0.001	
	Olivine - Slag	0.030	0.030	0.001	0.839
ETRmax = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Slag - Control	23.732	<0.001	0.094	
	Olivine - Control	35.844	<0.001	<0.001	
	Olivine - Slag	12.112	0.025	0.138	0.673
Ek = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Slag - Control	-17.806	0.141	0.474	
	Olivine - Control	-4.885	0.685	0.168	
	Olivine - Slag	12.920	0.284	0.947	0.248
Fv/Fm = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Slag - Control	0.124	<0.001	<0.001	
	Olivine - Control	0.138	<0.001	<0.001	
	Olivine - Slag	0.015	0.173	0.026	0.827
Micro_bio = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Slag - Control	827.188	<0.001	0.005	
	Olivine - Control	607.195	<0.001	0.047	
	Olivine - Slag	-219.993	0.093	0.503	0.454
Nano1_bio = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Slag - Control	0.784	0.360	0.539	
	Olivine - Control	3.745	<0.001	<0.001	
	Olivine - Slag	2.960	<0.001	0.002	0.849
Nano2_bio = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Slag - Control	26.843	0.013	0.995	
	Olivine - Control	57.495	<0.001	0.038	
	Olivine - Slag	30.652	0.005	0.050	0.661
Pico_bio = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Slag - Control	-0.249	0.307	0.936	
	Olivine - Control	0.702	0.005	<0.001	
	Olivine - Slag	0.951	<0.001	<0.001	0.789
Cyano_bio = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Slag - Control	-0.002	0.387	0.873	
	Olivine - Control	0.002	0.370	0.520	
	Olivine - Slag	0.004	0.082	0.425	0.946
Cryp_bio = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Slag - Control	1.643	0.924	0.776	
	Olivine - Control	4.556	0.792	0.650	
	Olivine - Slag	2.913	0.866	0.422	0.109
Micro_percent = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Slag - Control	0.094	<0.001	0.011	
	Olivine - Control	0.067	0.012	0.003	
	Olivine - Slag	-0.027	0.295	0.992	0.272
Nano1_percent = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Slag - Control	-0.011	0.007	0.009	
	Olivine - Control	-0.010	0.016	0.007	0.254

	Olivine - Slag	0.001	0.777	1.000	
Nano2_percent = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Slag - Control	-0.074	<0.001	0.001	
	Olivine - Control	-0.057	0.004	<0.001	
	Olivine - Slag	0.017	0.382	0.996	0.295
Pico_percent = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Slag - Control	-0.001	0.092	0.981	
	Olivine - Control	0.000	0.235	0.957	
	Olivine - Slag	0.000	0.619	1.000	0.413
Cyano_percent = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Slag - Control	0.000	0.232	1.000	
	Olivine - Control	0.000	0.460	0.631	
	Olivine - Slag	0.000	0.652	0.635	0.670
Cryp_percent = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Slag - Control	-0.009	0.427	0.602	
	Olivine - Control	0.000	0.977	0.160	
	Olivine - Slag	0.009	0.414	0.729	0.309
fCO2 = Treatment + s(Day, k = 7, fx = TRUE) + s(Day, by = oTreatment, k = 7, fx = TRUE)	Slag - Control	-247.462	<0.001	<0.001	
	Olivine - Control	-41.823	<0.001	0.001	
	Olivine - Slag	205.639	<0.001	<0.001	0.966
DIC = Treatment + s(Day, k = 7, fx = TRUE) + s(Day, by = oTreatment, k = 7, fx = TRUE)	Slag - Control	47.555	<0.001	<0.001	
	Olivine - Control	6.483	0.016	0.053	
	Olivine - Slag	-41.072	<0.001	<0.001	0.945
OmegaAragonite = Treatment + s(Day, k = 7, fx = TRUE) + s(Day, by = oTreatment, k = 7, fx = TRUE)	Slag - Control	2.083	<0.001	<0.001	
	Olivine - Control	0.174	<0.001	0.091	
	Olivine - Slag	-1.909	<0.001	<0.001	0.990

Table S3. The GLM of trace metal data. In the content column, “Dissolved” represents the dissolved trace metal concentrations in seawater; “Particulate” is the total particulate trace metal; “Total” refers to the total particulate trace metal : particulate organic carbon; “Non-surface” refers to the non-surface particulate trace metal : particulate organic carbon. Estimates are the estimated effects from different treatments on the concentrations of trace metals. The “Treatment” in the formula represents three conditions: “Control”, “Olivine”, and “Slag”. Please note that, if the Family and link is “Gamma inverse”, then the interpretation of these estimates is specific. For example, when comparing Treatment A and Treatment B ((Treatment A – Treatment B) if we observe larger estimate values for Treatment A, it implies that Treatment B exerts a greater effect on the outcome than Treatment A. The P values were calculated from Tukey tests. If the P value is <0.05, it means the two compared treatments have significant differences on trace metal concentrations.

Element	Content	Treatments comparison	Estimate	P values	Formula	GLM Family and Link
Al	Dissolved	Olivine - Control	-0.002	<0.001	Concentration = Treatment + + Day/22 + (Day/22)^2	Gamma inverse
		Slag - Control	-0.002	<0.001		
		Slag - Olivine	0.000	0.189		
Mn	Dissolved	Olivine - Control	0.002	0.722	Concentration = Treatment +	Gamma inverse
		Slag - Control	-0.015	<0.001		
		Slag - Olivine	-0.017	<0.001		

					+ Day/22 + (Day/22)^2	
Fe	Dissolved	Olivine - Control	-0.003	0.237	Concentration = Treatment + + Day/22 + (Day/22)^2	Gamma inverse
		Slag - Control	-0.001	0.865		
		Slag - Olivine	0.002	0.502		
Ni	Dissolved	Olivine - Control	-0.031	<0.001	Concentration = Treatment + + Day/22 + (Day/22)^2	Gamma inverse
		Slag - Control	0.002	0.956		
		Slag - Olivine	0.033	<0.001		
Cu	Dissolved	Olivine - Control	-0.020	0.068	Concentration = Treatment + + Day/22 + (Day/22)^2	Gamma inverse
		Slag - Control	0.014	0.462		
		Slag - Olivine	0.034	0.003		
Zn	Dissolved	Olivine - Control	0.003	0.127	Concentration = Treatment + + Day/22 + (Day/22)^2	Gamma inverse
		Slag - Control	0.002	0.332		
		Slag - Olivine	-0.001	0.853		
Mn	Particulate	Olivine - Control	14.005	0.270	Concentrations = 1 + Treatment + Day	Gaussian identity
		Slag - Control	20.178	0.067		
		Slag - Olivine	6.173	0.775		
Fe	Particulate	Olivine - Control	134.460	0.003	Concentrations = 1 + Treatment + Day	Gaussian identity
		Slag - Control	140.605	0.002		
		Slag - Olivine	6.145	0.988		
Ni	Particulate	Olivine - Control	4.238	<0.001	Concentrations = 1 + Treatment + Day	Gaussian identity
		Slag - Control	-0.040	0.999		
		Slag - Olivine	-4.278	<0.001		
Zn	Particulate	Olivine - Control	-2.135	0.676	Concentrations = 1 + Treatment + Day	Gaussian identity
		Slag - Control	-0.645	0.965		
		Slag - Olivine	1.490	0.826		
Mn	Total	Olivine - Control	1.213	0.281	Metal:C = 1 + Treatment + Day	Gaussian identity
		Slag - Control	1.713	0.080		
		Slag - Olivine	0.500	0.805		
Fe	Total	Olivine - Control	11.485	0.002	Metal:C = 1 + Treatment + Day	Gaussian identity
		Slag - Control	12.150	0.001		
		Slag - Olivine	0.665	0.980		
Ni	Total	Olivine - Control	0.375	<0.001	Metal:C = 1 + Treatment + Day	Gaussian identity
		Slag - Control	-0.006	0.998		
		Slag - Olivine	-0.381	<0.001		
Zn	Total	Olivine - Control	-0.255	0.467		

		Slag - Control	-0.127	0.827	Metal:C = 1 + Treatment + Day	Gaussian identity
		Slag - Olivine	0.128	0.826		
Mn	Non-surface	Olivine - Control	0.022	0.990	Metal:C = 1 + Treatment + Day	Gaussian identity
		Slag - Control	1.140	<0.001		
		Slag - Olivine	1.118	<0.001		
Fe	Non-surface	Olivine - Control	7.703	0.103	Metal:C = 1 + Treatment + Day	Gaussian identity
		Slag - Control	8.225	0.125		
		Slag - Olivine	0.521	0.992		
Ni	Non-surface	Olivine - Control	0.359	0.001	Metal:C = 1 + Treatment + Day	Gaussian identity
		Slag - Control	-0.056	0.876		
		Slag - Olivine	-0.414	<0.001		
Zn	Non-surface	Olivine - Control	-0.207	0.120	Metal:C = 1 + Treatment + Day	Gaussian identity
		Slag - Control	0.033	0.956		
		Slag - Olivine	0.241	0.102		

Table S4. The GLM of zooplankton abundance. Each species has its own GLM with "Treatment" and "Day" as its variables. The estimate is the estimated mean of the difference between two compared treatments. The family and link were set as "Gamma inverse" in the "glm()" function in R. The p-values were calculated from Tukey tests. If the p-values are <0.05, then the two compared treatments have significant differences in their influence on this species' abundance.

Species or Parameters	Treatment comparison	Estimate	P values	Formula	GLM Family and Link
	Olivine - Control	0.087	0.931	Species abundance = 1 + Treatment + Day/22 + (Day/22)^2	
	Slag - Control	0.041	0.983		
Oithona	Slag - Olivine	-0.045	0.981		Gamma inverse
	Olivine - Control	1.960	0.093	Species abundance = 1 + Treatment + Day/22 + (Day/22)^2	
	Slag - Control	-0.080	0.961		
Penilia	Slag - Olivine	-2.041	0.074		Gamma inverse
	Olivine - Control	-0.550	0.291	Species abundance = 1 + Treatment + Day/22 + (Day/22)^2	
	Slag - Control	-0.392	0.587		
Calanoid	Slag - Olivine	0.158	0.826		Gamma inverse
	Olivine - Control	-0.598	0.025	Species abundance = 1 + Treatment + Day/22 + (Day/22)^2	
	Slag - Control	-0.298	0.484		
Noctiluca	Slag - Olivine	0.299	0.070		Gamma inverse
	Olivine - Control	0.158	0.958		
Oikopleura	Slag - Control	-0.177	0.890		Gamma inverse

	Slag - Olivine	-0.335	0.786	Species abundance = 1 + Treatment + Day/22 + (Day/22)^2	
Shannon Diversity Index	Olivine - Control	-0.293	<0.001	Shannon Diversity Index = 1+ Treatment +Day	Gaussian identity
	Slag - Control	-0.030	0.916		
	Slag - Olivine	0.263	0.001		

Table S5. The ratio of non-surface to total particulate trace metal concentrations. The results were mean values ± standard error.

Metal	Treatment			
	Pre-addition Day 1	Control Day 22	Olivine Day 22	Slag Day 22
Fe	0.99	1.25 ± 0.15	0.94 ± 0.05	0.96 ± 0.10
Mn	0.24	0.87 ± 0.53	0.29 ± 0.13	0.72 ± 0.04
Ni	0.74	0.73 ± 0.34	1.02 ± 0.09	1.87 ± 0.46
Zn	0.86	0.77 ± 0.11	0.52 ± 0.10	0.85 ± 0.14

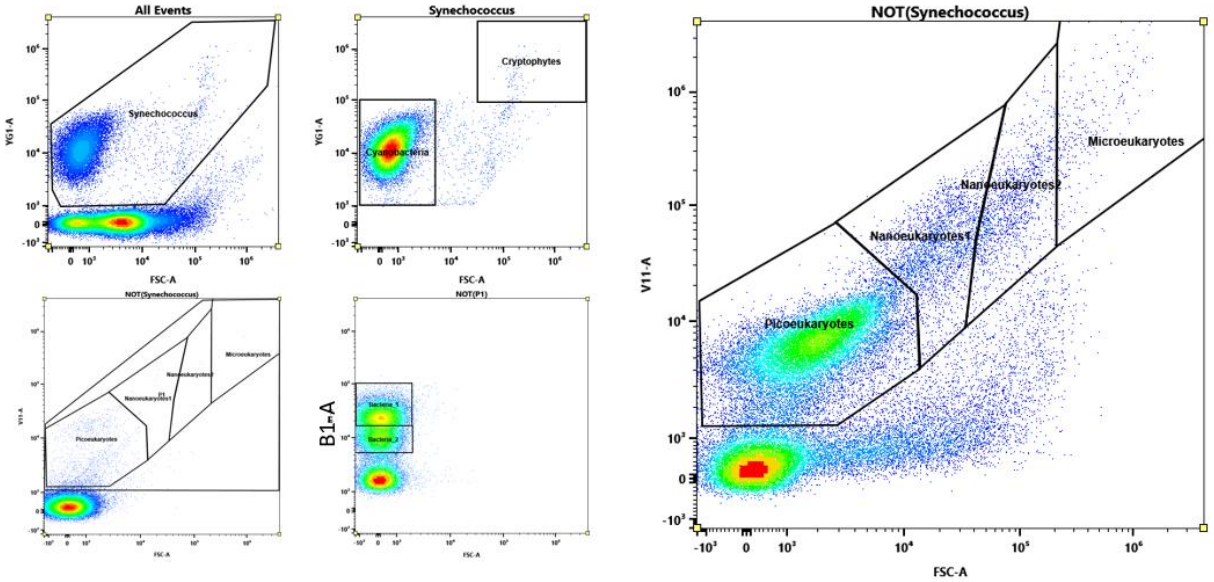
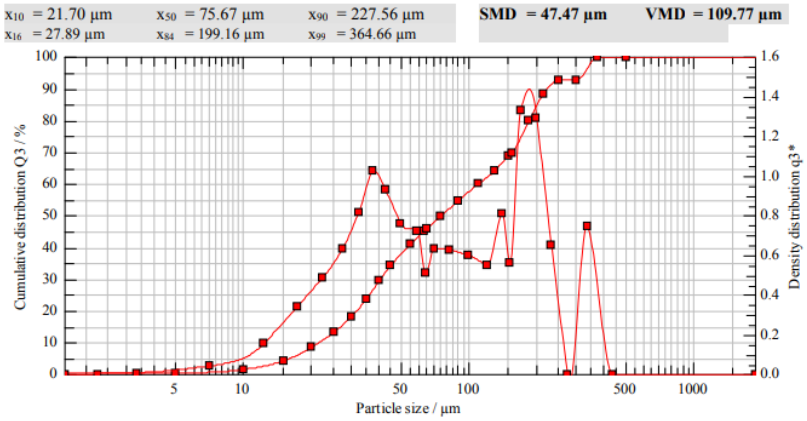


Fig. S1. The gate information of flow cytometry data.

(a)



(b)

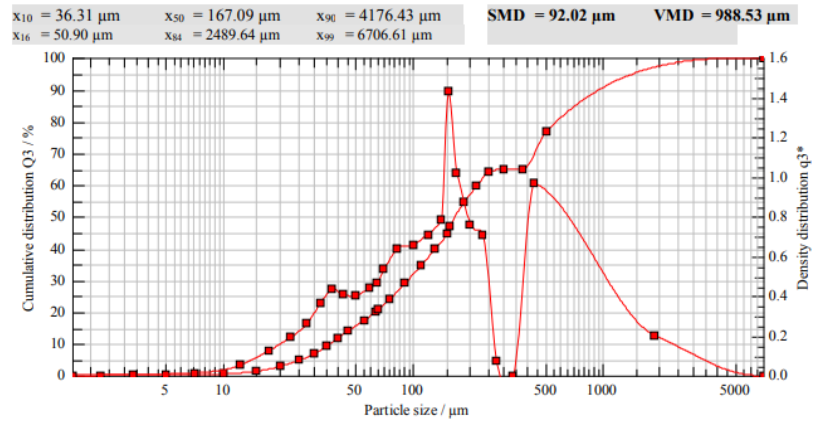


Fig. S2. The particle size spectrum of OAE materials. (a) the ground olivine minerals from Mortlake, Australia; (b) the ground slag minerals from South Australia, Australia. Cumulative distribution Q3 is the accumulative volume of the measured particles

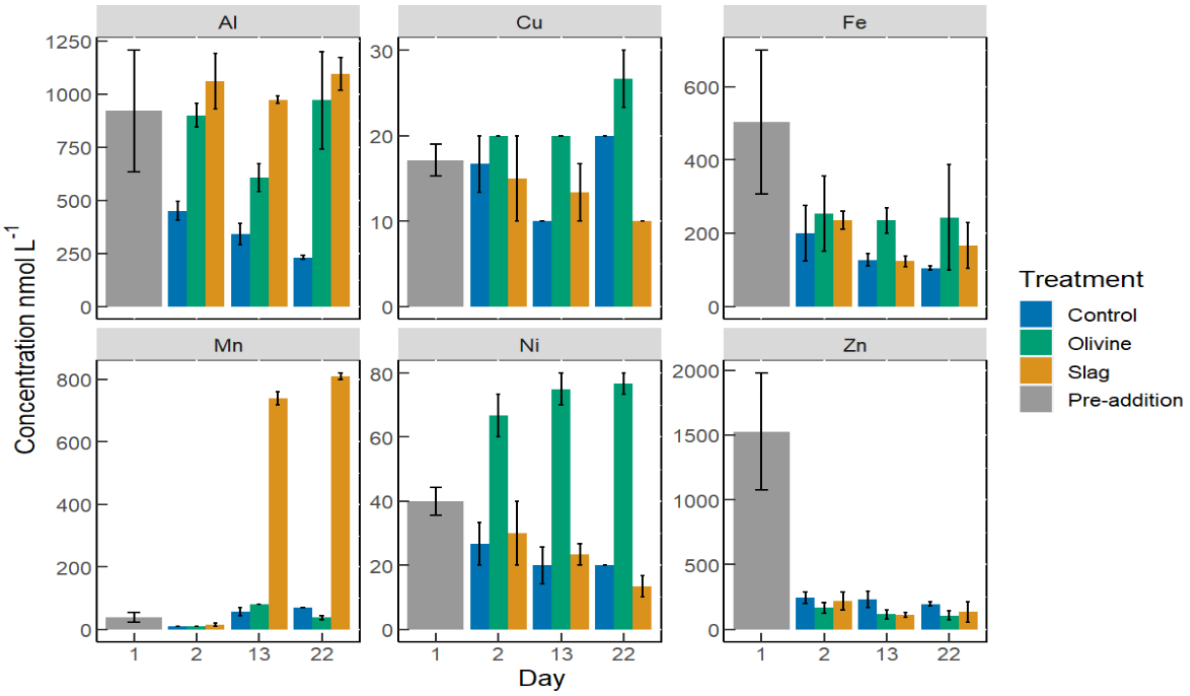


Fig. S3. Dissolved trace metal concentrations in microcosm seawater. The error bars represent the standard error from measured samples. The pre-addition data shown here represent the average of 7 microcosms before addition of slag or olivine. The data for the slag treatment on day 2, the olivine treatment on day 13, and for the control on day 22 is based on two of three microcosm replicates. The remaining data were based on all three microcosm replicates.

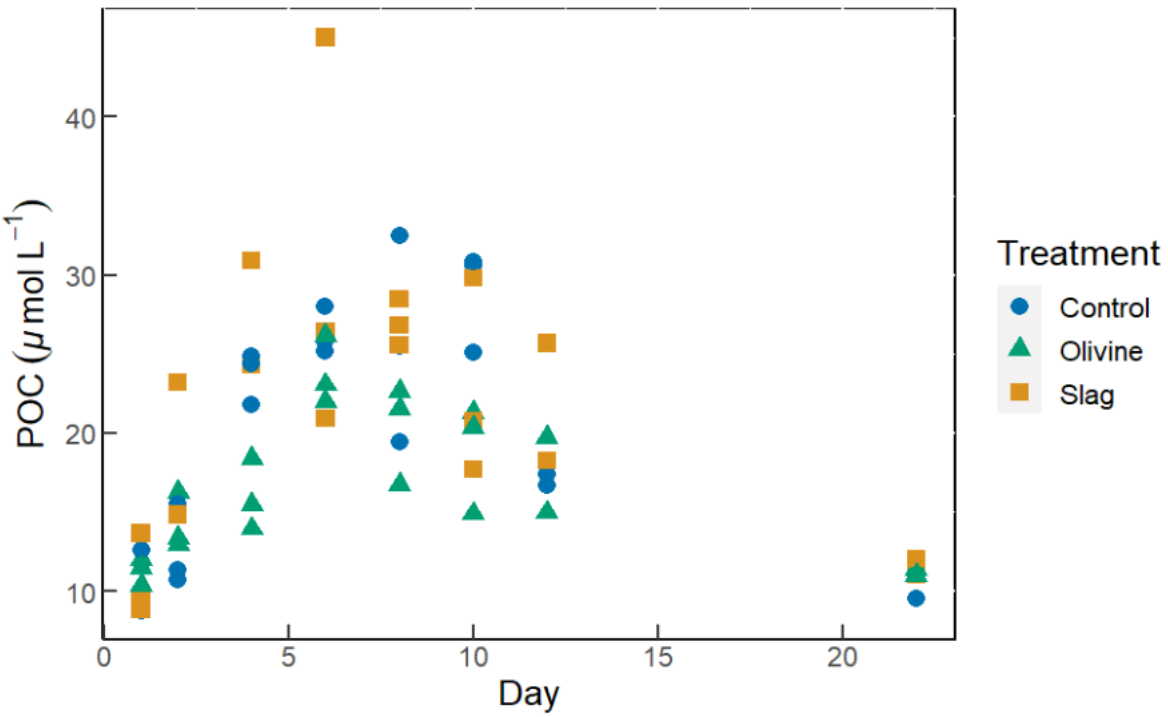


Fig. S4. The particulate organic carbon (POC) in microcosms. Each dot represents the POC in a microcosm on the sampling day. Part of the data from day 12 to day 22 are missing due to an instrument malfunction.

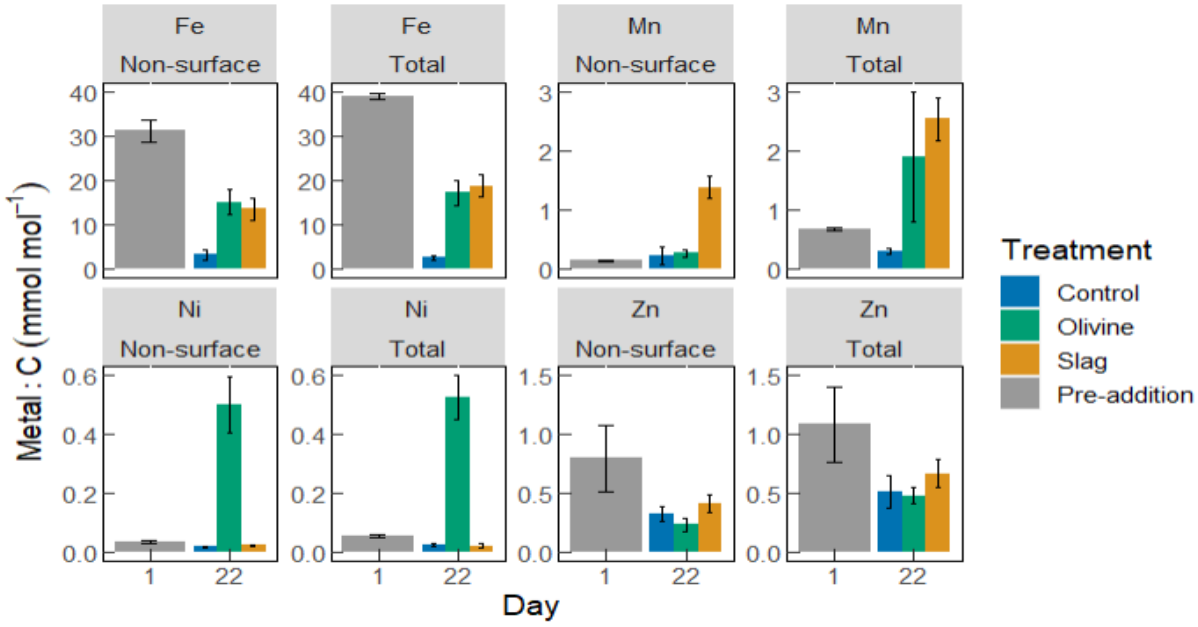


Fig. S5. Particulate trace metal concentrations normalized to particulate carbon concentrations. "Total" indicates the total particulate trace metal concentrations, and "Non-surface" indicates the particulate trace metal concentrations after oxalate wash. On day 1, n=2 for non-surface pre-addition data. For all other data presented n=3.