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

Jiaying A. Guo[1,2], Robert F. Strzepek[2], Kerrie M. Swadling[1,2], Ashley T. Townsend[3], Lennart T. Bach[1]

[1]Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, Tasmania, 7000 Australia

[2]Australian Antarctic Program Partnership (AAPP), Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, Tasmania, 7000 Australia

[3]Central Science Laboratory, University of Tasmania, Sandy Bay, Tasmania, 7005 Australia

Correspondence to: Jiaying A. Guo (Jiaying.guo@utas.edu.au)

Supplements

Table S1. The GAM of biochemical parameters. The formula is the established formula of variables in R. Estimates are the estimated mean effects from different treatments on the measured parameters. The GLM 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 positive (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 P_mean value is <0.05, then the GAMs from the two compared treatments have significant differences in the overall mean values. If 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
Cyanobacteria Con. = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Slag - Control	0.236	0.839	0.962	
	Olivine - Control	5.205	<0.001	<0.001	
	Olivine - Slag	4.969	<0.001	<0.001	0.764
	Slag - Control	0.003	0.570	0.465	
	Olivine - Control	0.017	0.003	<0.001	0.897

Cryptophytes Con. = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Olivine - Slag	0.013	0.015	<0.001	
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_percent = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Slag - Control	0.094	<0.001	0.011	0.272
	Olivine - Control	0.067	0.012	0.003	
	Olivine - Slag	-0.027	0.295	0.992	
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	0.254
	Olivine - Control	-0.010	0.016	0.007	
	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	0.295
	Olivine - Control	-0.057	0.004	<0.001	
	Olivine - Slag	0.017	0.382	0.996	
	Slag - Control	-0.001	0.092	0.981	0.413
	Olivine - Control	0.000	0.235	0.957	

Pico_ percent = Treatment + s(Day, k = 10, fx = TRUE) + s(Day, by = oTreatment, k = 10, fx = TRUE)	Olivine - Slag	0.000	0.619	1.000	
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	0.670
	Olivine - Control	0.000	0.460	0.631	
	Olivine - Slag	0.000	0.652	0.635	
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	0.309
	Olivine - Control	0.000	0.977	0.160	
	Olivine - Slag	0.009	0.414	0.729	

Table S2. The GLM of trace metal data. In the content column, "Dissolved" represents the dissolved trace metal concentrations from the seawater; "Total" is the total particulate trace metal. Estimates are the estimated effects from different treatments on the concentrations of trace metals. The "Treatment" in the formula represent three conditions: "Control", "Olivine", and "Slag". Please note that, if the Family and link is "Gamma inverse", then the larger estimate values are (Treatment A – Treatment B), the Treatment B has large effects on the 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 the trace metal's concentrations.

Element	Content	Treatments comparison	Estimate	P values	Formula	GLM Family and Link	Test
Al	Dissolved	Olivine - Control	-0.002	<0.001	Concentration = Treatment + Zday + Zday2	Gamma inverse	Tukey
		Slag - Control	-0.002	<0.001			
		Slag - Olivine	0.000	0.189			
Mn	Dissolved	Olivine - Control	0.002	0.722	Concentration = Treatment + Zday + Zday2	Gamma inverse	Tukey
		Slag - Control	-0.015	<0.001			
		Slag - Olivine	-0.017	<0.001			
Fe	Dissolved	Olivine - Control	-0.003	0.237	Concentration = Treatment + Zday + Zday2	Gamma inverse	Tukey
		Slag - Control	-0.001	0.865			
		Slag - Olivine	0.002	0.502			
Ni	Dissolved	Olivine - Control	-0.031	<0.001	Concentration = Treatment + Zday + Zday2	Gamma inverse	Tukey
		Slag - Control	0.002	0.956			
		Slag - Olivine	0.033	<0.001			
Cu	Dissolved	Olivine - Control	-0.020	0.068	Concentration = Treatment + Zday + Zday2	Gamma inverse	Tukey
		Slag - Control	0.014	0.462			
		Slag - Olivine	0.034	0.003			
Zn	Dissolved	Olivine - Control	0.003	0.127	Concentration = Treatment + Zday + Zday2	Gamma inverse	Tukey
		Slag - Control	0.002	0.332			
		Slag - Olivine	-0.001	0.853			
Mn	Total	Olivine - Control	14.005	0.270			Tukey

		Slag - Control	20.178	0.067	Concentrations = 1 + Treatment + Day	Gaussian identity	
		Slag - Olivine	6.173	0.775			
Fe	Total	Olivine - Control	134.460	0.003	Concentrations = 1 + Treatment + Day	Gaussian identity	Tukey
		Slag - Control	140.605	0.002			
		Slag - Olivine	6.145	0.988			
Ni	Total	Olivine - Control	4.238	<0.001	Concentrations = 1 + Treatment + Day	Gaussian identity	Tukey
		Slag - Control	-0.040	0.999			
		Slag - Olivine	-4.278	<0.001			
Zn	Total	Olivine - Control	-2.135	0.676	Concentrations = 1 + Treatment + Day	Gaussian identity	Tukey
		Slag - Control	-0.645	0.965			
		Slag - Olivine	1.490	0.826			

Table S3. 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 "Gamm, inverse" in "glm()" function in R. The p-values were calculated from Tukey tests. If the p-values is <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	Test
	Olivine - Control	0.087	0.931	Species abundance = 1 + Treatment + Day + Day^2	Gamma inverse	Tukey
	Slag - Control	0.041	0.983			
Oithona	Slag - Olivine	-0.045	0.981			
	Olivine - Control	1.960	0.093	Species abundance = 1 + Treatment + Day + Day^2	Gamma inverse	Tukey
	Slag - Control	-0.080	0.961			
Penilia	Slag - Olivine	-2.041	0.074			
	Olivine - Control	-0.550	0.291	Species abundance = 1 + Treatment + Day + Day^2	Gamma inverse	Tukey
	Slag - Control	-0.392	0.587			
Calanoid	Slag - Olivine	0.158	0.826			
	Olivine - Control	-0.598	0.025	Species abundance = 1 + Treatment + Day + Day^2	Gamma inverse	Tukey
	Slag - Control	-0.298	0.484			
Noctiluca	Slag - Olivine	0.299	0.070			
Oikopleura	Olivine - Control	0.158	0.958		Gamma inverse	Tukey

	Slag - Control	-0.177	0.890	Species abundance = 1 + Treatment + Day + Day^2		
	Slag - Olivine	-0.335	0.786			
Shannon Diversity Index	Olivine - Control	-0.293	<0.001	Shannon Diversity Index = 1+ Treatment +Day	Gaussian identity	Tukey
	Slag - Control	-0.030	0.916			
	Slag - Olivine	0.263	0.001			

Table S4. The ratio of non-surface to total particulate trace metal concentrations. The results were average 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

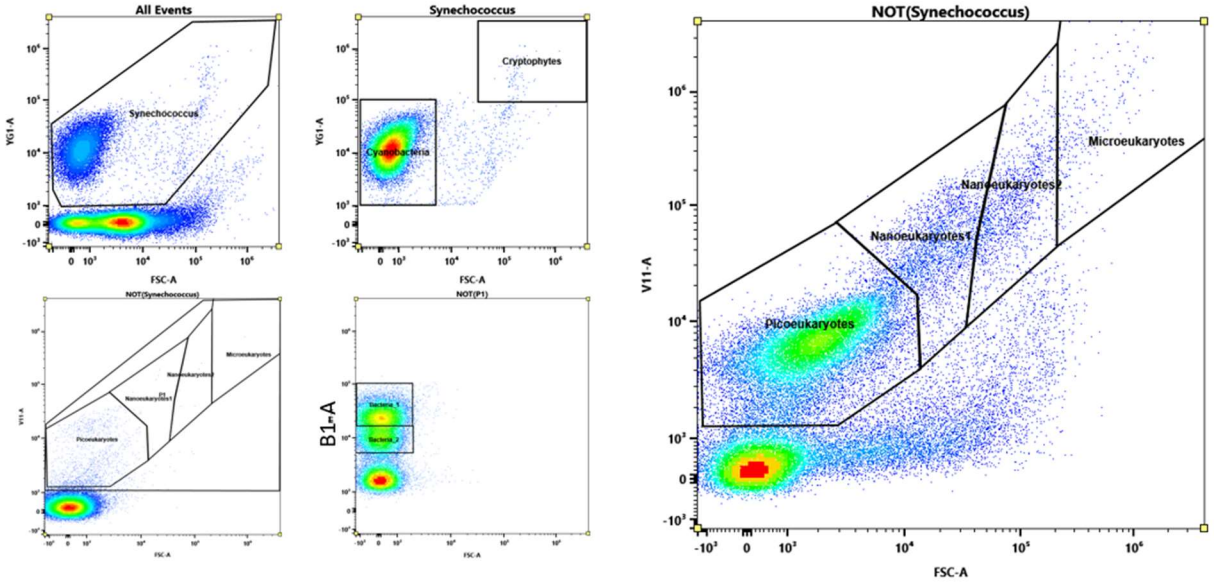
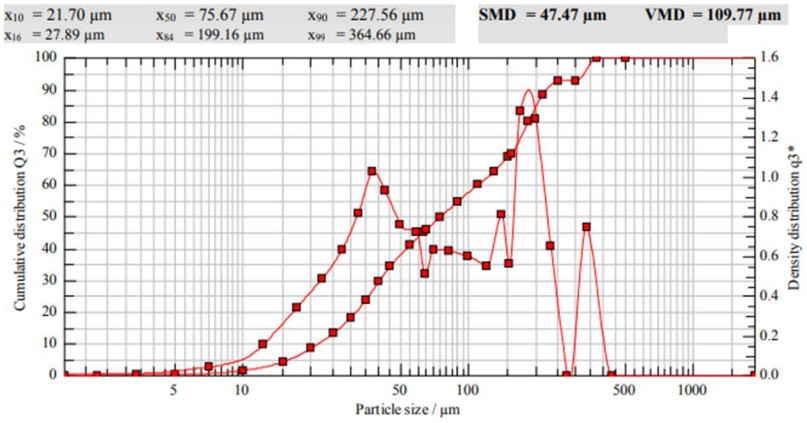


Fig. S1. The gate information of flow cytometry data.

(a)



(b)

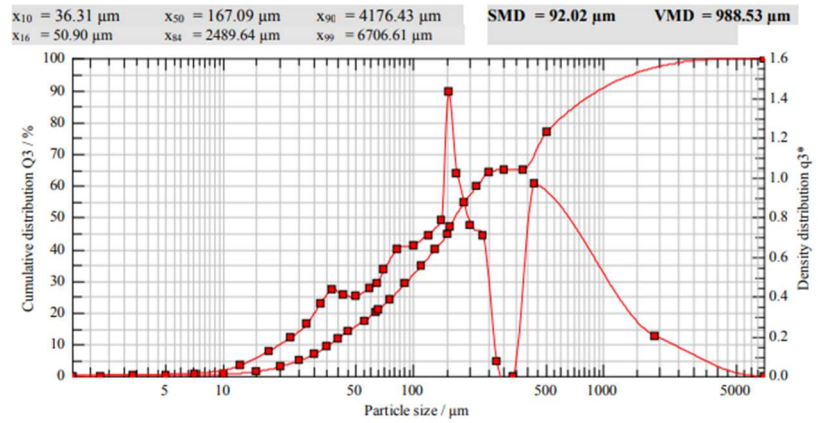


Fig. S2. The particle size spectrum of OAE materials. (a) the ground olivine minerals from Mortlake, Australia; (b) the ground slag minerals from South Australia, Australia. Cumulative distribution Q3 is the accumulative volume of the measured particles

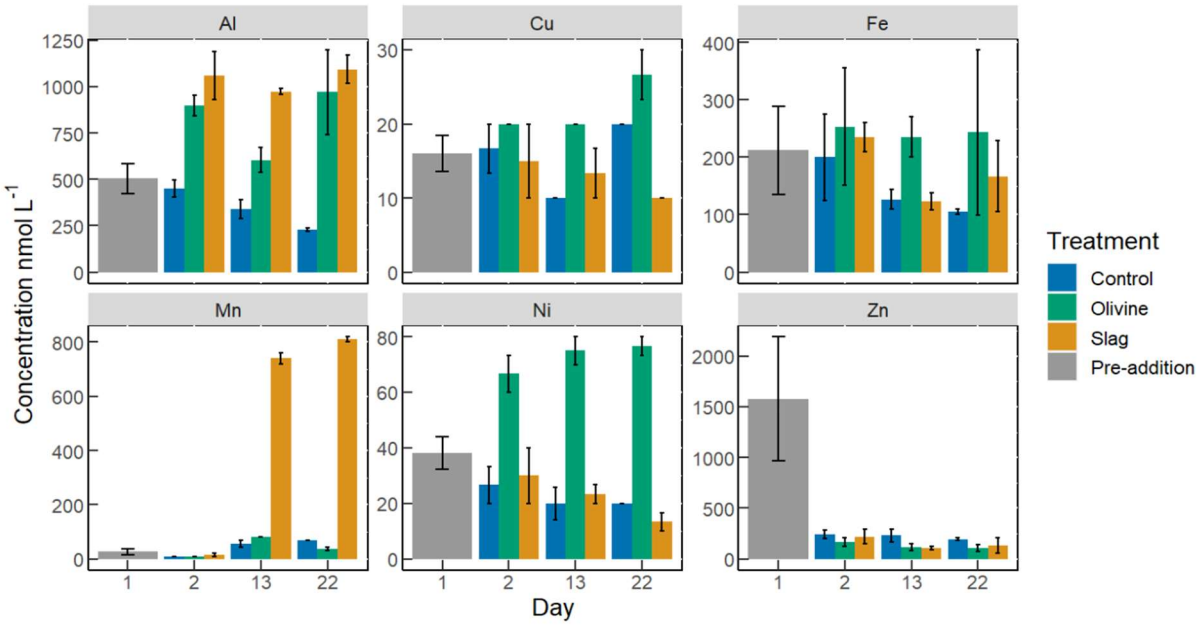


Fig. S3. Dissolved trace metal concentrations in microcosm seawater. The error bars represent the standard error from measured samples. The pre-addition data shown here represent the average of 5 microcosms before addition of slag or olivine. The data for the slag treatment on day 2, the olivine treatment 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.

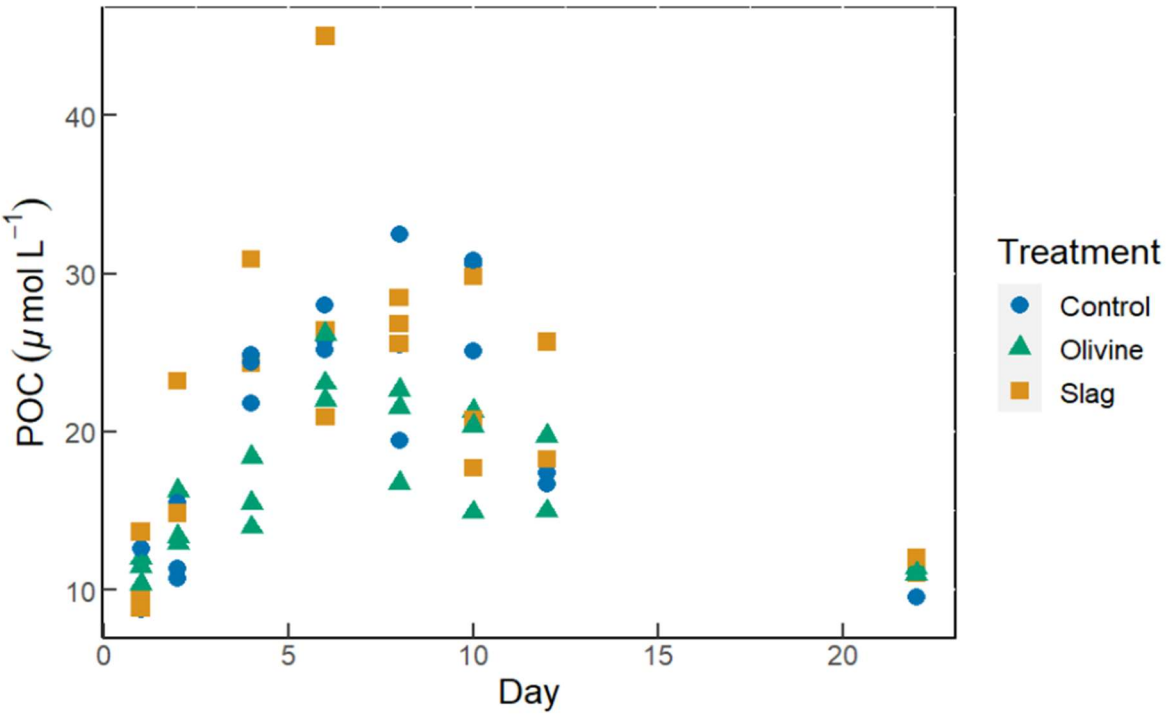


Fig. S4. The particulate organic carbon (POC) in microcosms. Each dot represents the POC in a microcosm on the sampling day. Part of the data from day 12 to day 22 were missing due to a measurement facility issue.

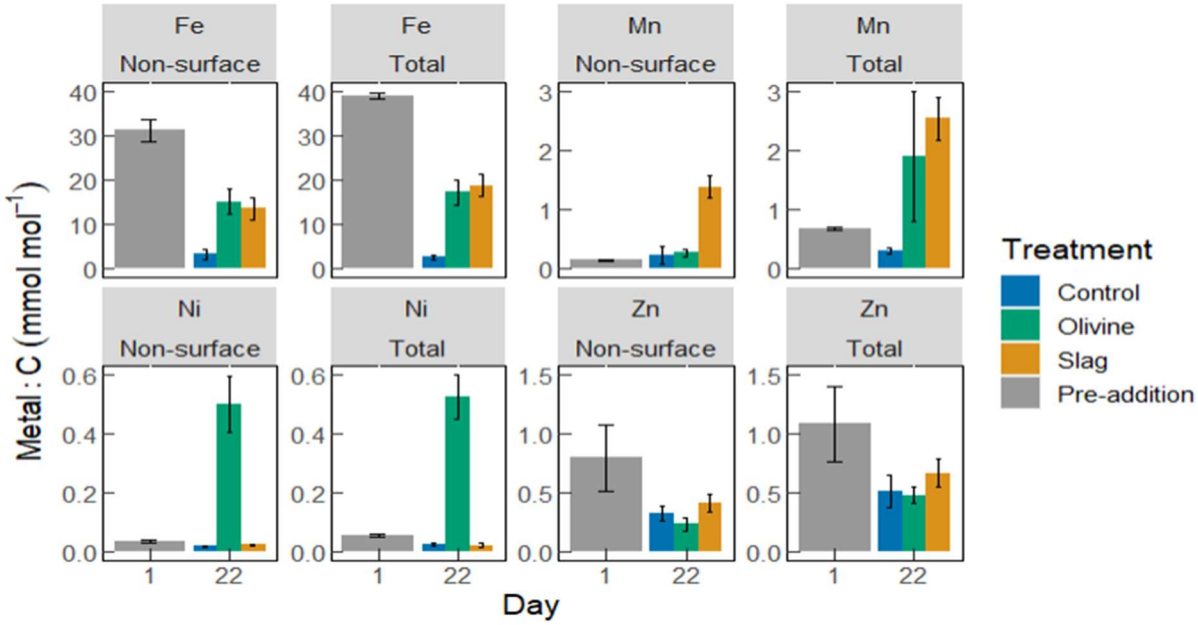


Fig. S5. Particulate trace metal concentrations normalized to particulate carbon concentrations. "Total" indicates the total particulate trace metal concentrations, and "Non-surface" indicates the particulate trace metal concentrations after oxalate wash. On day 1, n=2 for non-surface pre-addition data.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