Supplementary Information: Iron availability modulates bacteria carbon cycling in the mesopelagic

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Introduction

Table S1 presents an overview of the incubations including depth, duration, and treatments applied in each set of experiments. Table S2 provides an overview of the siderophore data measured for each experiment and the estimated siderophore production from each treatment. Table S3 is an overview of Fe:C quotas of heterotrophic bacteria to date. Figure S1 is an overview of biological response for each individual experiment and depth that is summarized main text boxplots of Figure 2. Figure S2 is an overview size distribution and biomass response from small and large bacteria data derived from flow cytometry measurements. Figure S3 shows the particulate Fe and P data across all experiments. Figure S4 shows the total carbon demand and bacteria growth efficiency across all experiments and treatments where carbon substrates were added. Figure S5 is a comparison of multiple independent ways of calculating Fe quotas presented in main text Figure 6. Figure S6 displays the macronutrient and dissolved organic carbon measurements from each experiment.

Experiment	Cruise	Depth (duration)	Carbon Added	Treatments
60 μM Organic Carbon: Subtropical Gyre	Gradients 4	200 m (48 h) 250 m (72 h) 450 m (48 h)	60 μM Organic Carbon (10 μM glucose)	Control +Fe (1nM Fe^{57}) +Glucose +Fe+Glucose
60 μM Organic Carbon: Equator	Gradients 4	100 m (72 h) 300 m (92 h)	60 μM Organic Carbon (10 μM glucose)	Control +Fe (1nM Fe^{57}) +Glucose +Fe+Glucose
10 μM Organic Carbon: Equator	Gradients 5	95 m (48 h) 295 m (56 h)	10 μM Organic Carbon (1.67 μM glucose and glucosamine)	Control +Fe (1nM Fe^{57}) +Glucose +Fe+Glucose +Glucosamine +Fe+Glucosamine

Table S1. Incubation overview. Presented is a table the summarizes the number of depths, incubation time, organic carbon added, and number of treatments for each experiment spanning the Gradients 4 and 5 cruises.

Incubation	Depth	C Addition (µM)	Treatment	[Siderophore] (pM)	Bacteria cells (cells µL-1)	Cell stdev.	Siderophore quota (amol cell-1)
Gradients 4: Grye	200	-	in-situ	3.7	124	na	0.0296
Gradients 4: Grye	200	0	Control	8.6	127	3	0.0676
Gradients 4: Grye	200	0	Fe+	6.5	130	2	0.0500
Gradients 4: Grye	200	60	Glucose+	7.1	138	8	0.0511
Gradients 4: Grye	200	60	Fe+Glucose+	4.7	381	157	0.0123
Gradients 4: Grye	250	-	in-situ	4.9	95	na	0.0511
Gradients 4: Grye	250	0	Control	11.7	97	4	0.1196
Gradients 4: Grye	250	0	Fe+	6.3	98	2	0.0646
Gradients 4: Grye	250	60	Glucose+	2078.4	3171	430	0.6554
Gradients 4: Grye	250	60	Fe+Glucose+	341.9	4727	57	0.0723
Gradients 4: Grye	450	-	in-situ	8.4	61	na	0.1386
Gradients 4: Grye	450	0	Control	8.7	62	5	0.1412
Gradients 4: Grye	450	0	Fe+	2.6	66	2	0.0393
Gradients 4: Grye	450	60	Glucose+	2.9	83	16	0.0350
Gradients 4: Grye	450	60	Fe+Glucose+	144.1	2110	1365	0.0683
Gradients 4: Equator	100	-	in-situ	4.5	310	na	0.0145
Gradients 4: Equator	100	0	Control	3.7	547	101	0.0067
Gradients 4: Equator	100	0	Fe+	2.6	450	32	0.0058
Gradients 4: Equator	100	60	Glucose+	44.2	1274	482	0.0347
Gradients 4: Equator	100	60	Fe+Glucose+	97.3	4375	131	0.0222
Gradients 4: Equator	300	-	in-situ	3.9	152	na	0.0257
Gradients 4: Equator	300	0	Control	40.5	199	6	0.2042
Gradients 4: Equator	300	0	Fe+	6.8	191	11	0.0356
Gradients 4: Equator	300	60	Glucose+	1166.0	1759	319	0.6630
Gradients 4: Equator	300	60	Fe+Glucose+	181.5	4270	41	0.0425
Gradients 5: Equator	95	-	in-situ	21.9	292	na	0.0750
Gradients 5: Equator	95	0	Control	9.9	418	164	0.0237
Gradients 5: Equator	95	0	Fe+	17.4	423	116	0.0410
Gradients 5: Equator	95	10	Glucose+	53.2	1105	405	0.0482
Gradients 5: Equator	95	10	Fe+Glucose+	6.5	1249	187	0.0052
Gradients 5: Equator	95	10	Glucosamine+	7.7	369	92	0.0208
Gradients 5: Equator	95	10	Fe+Glucosamine+	8.9	661	432	0.0134
Gradients 5: Equator	295	-	in-situ	18.1	177	na	0.1023
Gradients 5: Equator	295	0	Control	11.2	330	150	0.0338
Gradients 5: Equator	295	0	Fe+	12.7	252	34	0.0503
Gradients 5: Equator	295	10	Glucose+	31.7	1200	108	0.0265
Gradients 5: Equator	295	10	Fe+Glucose+	9.0	993	66	0.0091
Gradients 5: Equator	295	10	Glucosamine+	7.5	232	42	0.0323
Gradients 5: Equator	295	10	Fe+Glucosamine+	17.6	242	44	0.0729

Table S2. Total siderophores and siderophore quotas. This table presents the dissolved siderophore concentrations measured in each experiment. Each siderophore sample was pooled from the treatment triplicates to form a single sample for each treatment. The cell abundances represent the average from the triplicate measurements from each treatment.

Source	Fe:C (μmol:mol)	Notes
Bundy and Manck et al. 2024	88 – 711	*Radiogenic ^{55}Fe uptake rates from microbial communitas using different Fe forms at 300 m depth station ALOHA for ~1 cell generation. Considered a shot-term uptake quota.*
Tortell et al. 1996	7.52 ± 1.65	*Radiogenic ^{55}Fe internalization ratio of five culture isolates from the Sargasso Sea under Fe-limited conditions* for *>8 cell generations. Considered a biomass quota.*
Fourquez et al. 2014	0.43 ± 0.1	*Radiogenic ^{55}Fe internalization ratio of oceanic strain of Alteramonas maceodii under Fe-deficient conditions for >8 cell generations. Considered a biomass quota.*
Fourquez et al. 2014	16.1 ± 2.3	*Radiogenic ^{55}Fe internalization ratio of oceanic strain of Alteramonas maceodii under Fe-replete conditions for >8 cell generations. Considered a biomass quota.*
Fourquez et al. 2014	0.56 ± 0.19	*Radiogenic ^{55}Fe internalization ratio of coastal strain of Alteramonas macleodii Fe-deficient conditions for >8 cell generations. Considered a biomass quota.*
Fourquez et al. 2014	141 ± 24.7	*Radiogenic ^{55}Fe internalization ratio of coastal strain of Alteramonas macleodii Fe-replete conditions for >8 cell generations. Considered a biomass quota.*
Mazzotta et al. 2021	83–84	*Bulk measurement from culture of Psuedoalteramonas strain with an accompanying metalloproteome measurement. Considered a biomass quota.*
This study	5.7–3215	*Represents the minimum and maximum measurements estimate from biogenic Fe measurements and flow cytometry C biomass. Considered a biomass quota.*

Table S3. Summary table of Fe quotas of heterotrophic bacteria. The existing Fe quotas of marine heterotrophic bacteria in the literature. We include the broad range observed from this study as a comparison.

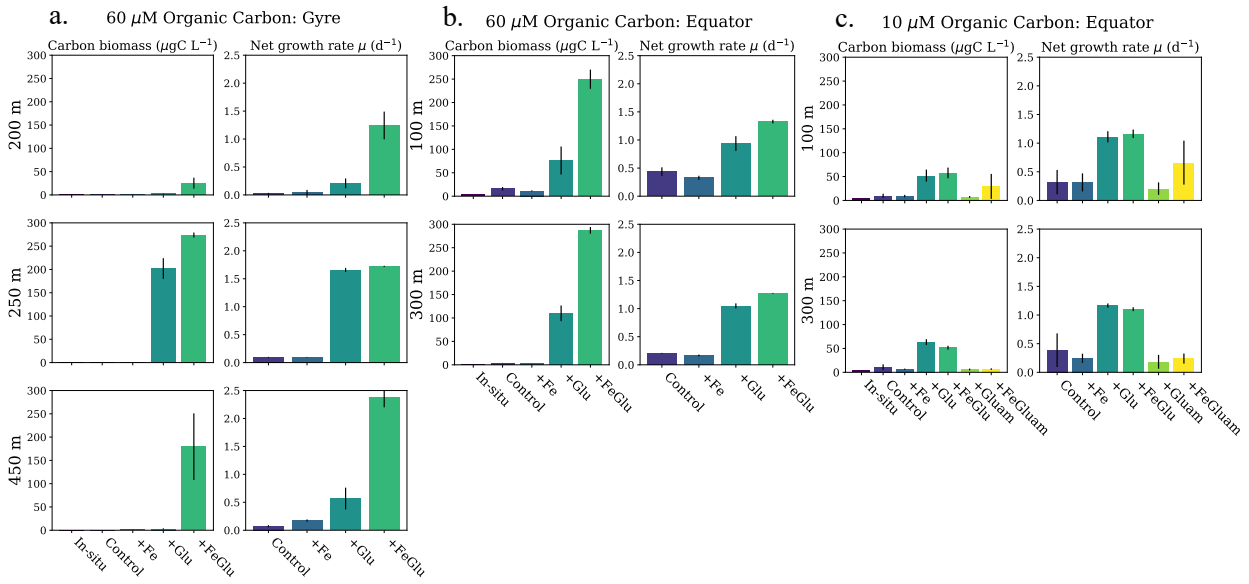

Figure S1. **Overview of the biological response in each experiment.** For each experiment incorporated in Figure 2 of the main, the biological response is presented for each treatment and depth. Depicted is the carbon biomass (μgC L^{-1}) estimated via flow cytometry (see Methods) and net growth rate (ln(C-biomass$_{final}$/C-biomass$_{in\text{-}situ}$)/time(days)) for (a) 60 μM organic carbon: gyre, (b) 60 μM organic carbon: equator, and (c) 10 μM organic carbon: equator. Error bars represent the standard deviation of triplicate treatment conditions.

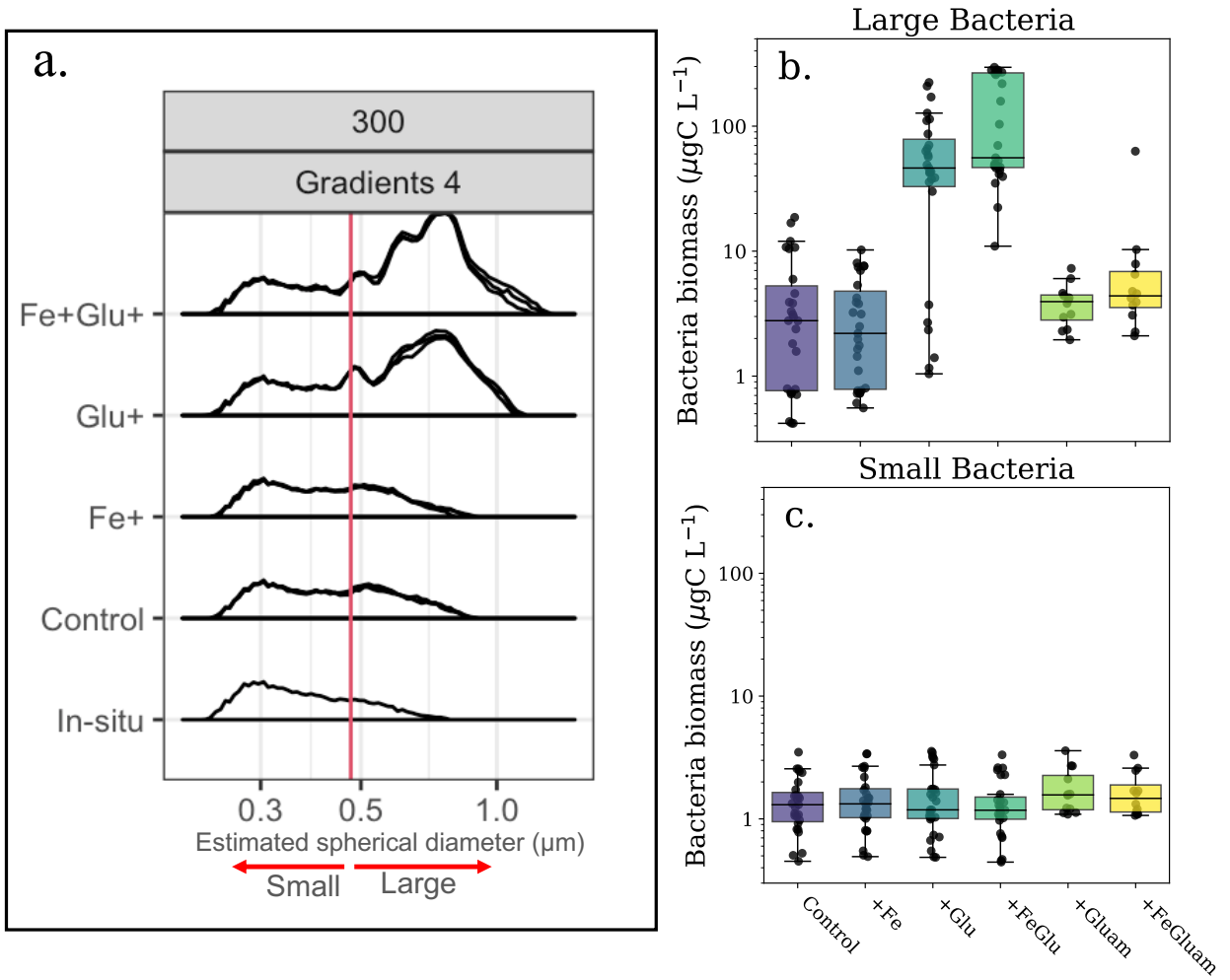

Figure S2. **Size response of bacteria to incubation conditions.** (a) Displayed is an example of the size distribution of bacteria cells from the flow cytometry samples from one incubation experiment (60 µM organic carbon, 300 m, equator). A threshold of 0.475 µm (red line) was used as a size cutoff between small and large bacteria distributions. The carbon biomass estimates for the large (b) and small (c) size classes are presented across all experiments.

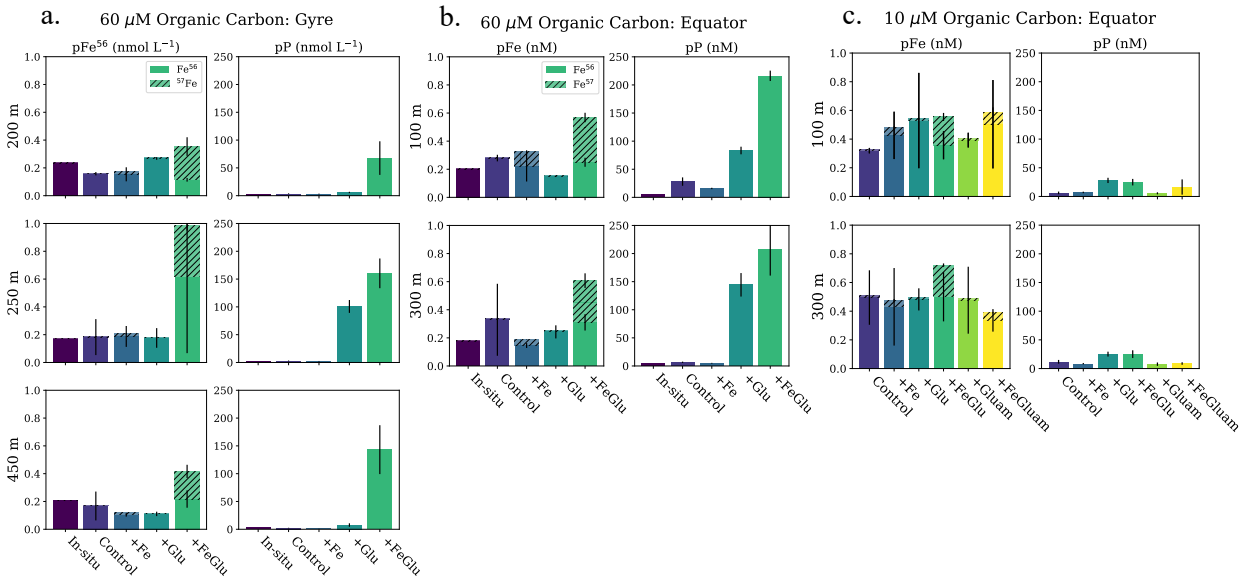

Figure S3. **Biogenic Fe and P from Gradients 4 and 5 incubations.** Presented is the particle data collected at the termination of each incubation experiment for the (a) 60 μM organic carbon: gyre, (b) 60 μM organic carbon: equator, and the (c) 10 μM organic carbon: equator experiments. Total particulate Fe (pFe; left column) represents the cumulative measurements of natural Fe^{56} and the added Fe^{57} (hatched area) used to trace biomass accumulation. Particulate phosphorous (pP) is presented in the right column as a macromolecular tracer of biomass.

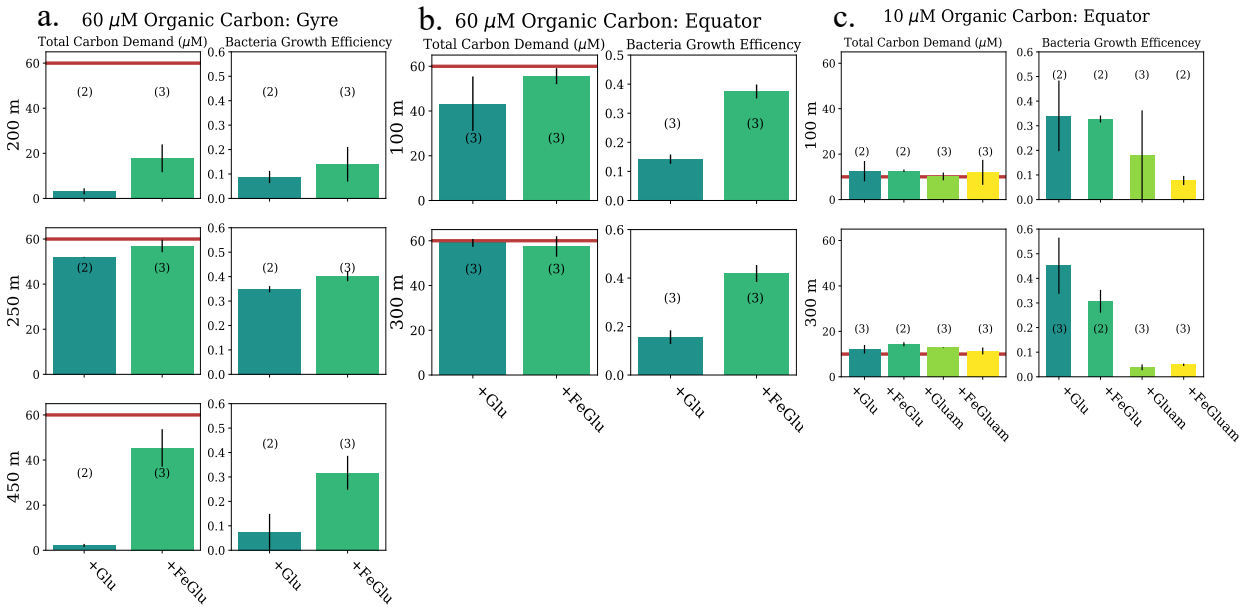

Figure S4. **Total Carbon Demand and Bacteria Growth Efficiency for the Gradients 4 and 5 incubations.** Presented is the estimated total carbon demand and bacteria growth efficiency for each carbon amended treatment for each incubation in this study from the (a) 60 μM organic carbon" gyre , (b) 60 μM organic carbon: equator, and (c) 10 μM organic carbon: equator experiments. Total carbon demand is presented for each treatment with a carbon addition, the concentration of each spike (60 μM or 10 μM) for each incubation is represented by the red lines. The bacteria growth efficiency is the fraction of total carbon demand converted to biomass. *If a sample had a calculated BGE higher than expected from natural marine bacteria (>0.8) it was removed from the average presented, likely indicating contamination of the final DOC sample.

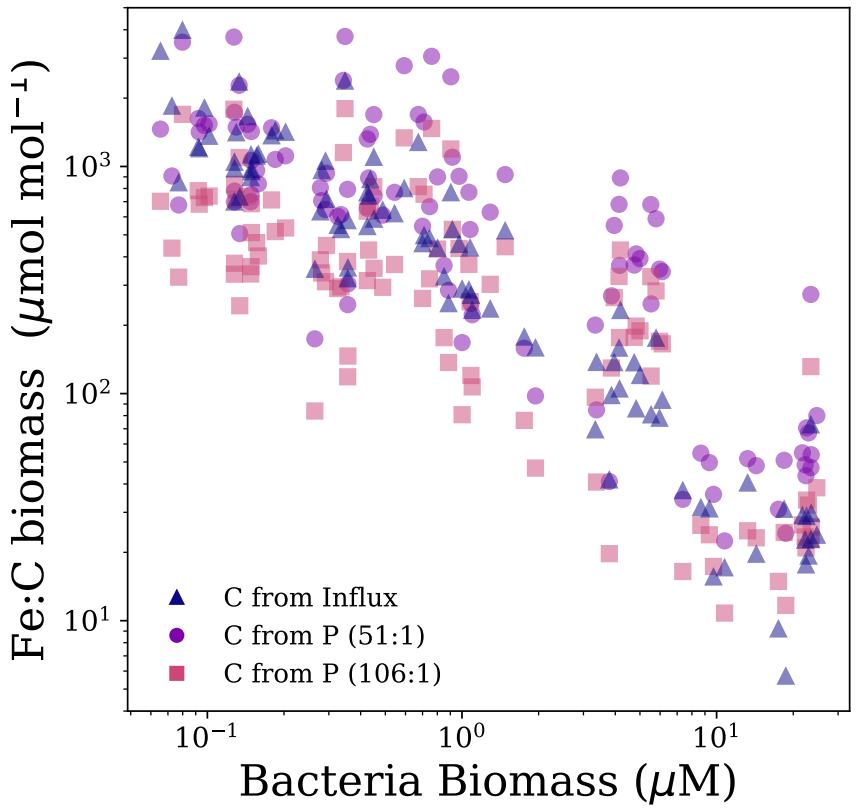

Figure S5. **Comparison of Fe quotas against bacteria biomass estimates.** To confirm the relationship displayed in Figure 4a is not an artifact in the redundant use of Carbon biomass estimates for the Fe:C quotas we estimated them independently from particulate P values for comparison. The graph depicts the Fe:C quotas calculated from flow cytometry carbon biomass estimates (blue triangles), Fe:C from particulate P assuming a C:P ratio of 51:1 (purple circles) used in Mazzotta et a.l 2020, and a C:P ratio assuming Redfield ratio of 106:1 (burgundy squares). These two different estimates of bacteria biomass (particulate P and flow cytometry C biomass) show strong overlap in the observed relationship and provide additional support for the interpretation.

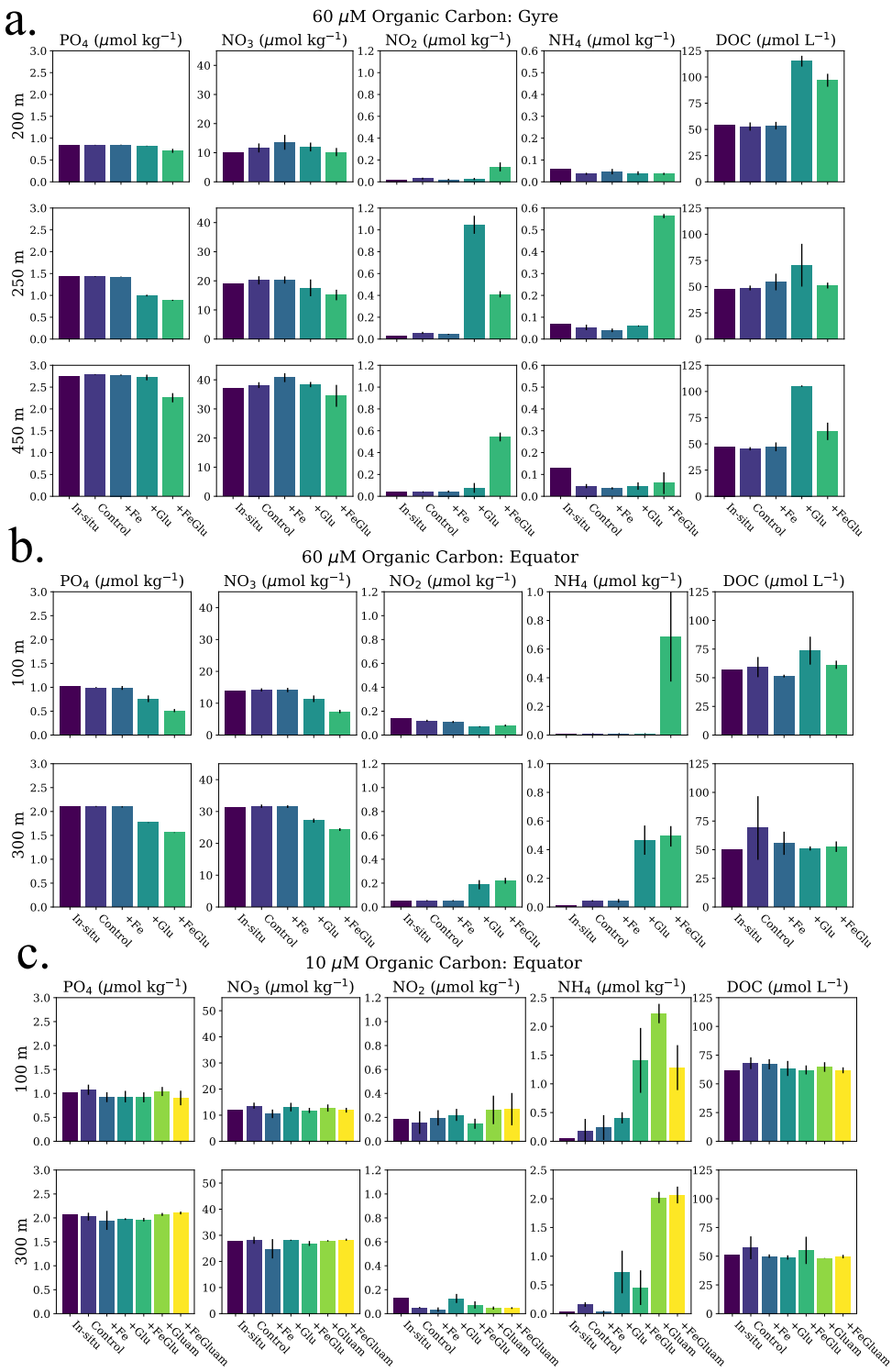

Figure S6. Macronutrient and Dissolved Organic Carbon (DOC) concentrations for the Gradients 4 and 5 incubations. In this series of panel plots is presented the macronutrient data for dissolved phosphate (first column), nitrate (second column), nitrite (third column), ammonia (fourth column) and DOC (fifth column) for the experiments conducted at each of the three stations and carbon loads.