Environmental Science: Processes & Impacts - Supplementary Material

Regulation of ammonium loss under contrasting upwelling conditions: sensitivity of Feammox to environmental drivers

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▪ Supporting Methods

▪ *Anammox rates measured through isotopic tracer incubations.*

Potential anammox rates were determined through a slurry-based ^{15}N isotope-tracing experiment [1,2]. The slurries were prepared as indicated for Feammox (See section 2.4 *"Isotopic tracer incubations"* in "*Material and Methods"*). Afterward, a $^{15}NO_3^-$ (99.5 atom%, ^{15}N) stock solution was added to each vial, yielding a final concentration of 100 μM ^{15}N. Subsequently, 200 μL of saturated $ZnCl_2$ solution was added to half of the vials with the objective of halting the reactions and establishing the initial samples. The remaining half of the slurries was further incubated for 8 h and treated as the final samples. The concentrations of $^{29}N_2$ and $^{30}N_2$ in the incubation vials were determined by a GasBench + Precon gas concentration system interfaced with a Delta V Plus Isotope-Ratio Mass Spectrometer (IRMS, Thermo-Scientific) at UC Davis Stable Isotope Facilities. The $^{29}N_2$ and $^{30}N_2$ production rates were calculated by the difference in $^{29}N_2$ and $^{30}N_2$ concentrations between the final and initial samples [3,4]. Then, the anammox rates were calculated based on Eq. (1).

$$R_A = (P_{29} - P_{30} \text{ x } (1\text{-}F_N) \text{ x } F_N^{-1}) \rho_s \text{ x } h_s \qquad \text{(Eq. 1)}$$

where R_A (mg · N m^{-2} · d^{-1}) represents the potential anammox rates. The P_{30} and P_{29} indicate the total $_{30}N_2$ and $^{29}N_2$ production rates (μg N · g^{-1} · d^{-1}), respectively, and F_N is the fraction of ^{15}N in NO_3^-, estimated from the concentrations of added $^{15}NO_3^-$ and residual NO_3^- in the incubation slurries. The ρ_s and h_s refer to the density (g · cm^{-3}) and depth (5 cm) of sediments.

▪ *Contribution of Feammox to total NH_4^+ loss*

According to the reaction stoichiometry of Feammox and Anammox, 2 mol of NH_4^+ are required to yield 1 mol of N_2 through Feammox, while Anammox requires 1 mol of NH_4^+ to generate 1 mol of N_2 through Anammox [1,5]. Consequently, the contributions of both processes to NH_4^+ loss were calculated based on the potential rates of both processes, as outlined in the following equations:

$$C_{Fe} = \frac{R_{Fe}}{R_{Fe} + R_A} \cdot 100 \qquad \text{(Eq. 2)}$$

$$C_A = \frac{R_A}{R_A + R_{Fe}} \cdot 100 \qquad \text{(Eq. 3)}$$

where C_{Fe} and C_A represent the contribution of Feammox and Anammox to NH_4^+ loss, while R_{Fe} and R_A denote the potential rates of Feammox and Anammox, respectively.

▪ Supporting results in sediment characteristics distribution

The PERMANOVA results indicated that moisture significantly differed among stations ($P < 0.001$; $r^2 = 0.44$) and between bottom types ($P < 0.001$; $r^2 = 0.45$), but not between upwelling conditions (Table S3). The highest mean moisture value (86%) was recorded in 3S. The Station factor notably accounted for the greatest degree of variation ($P < 0.001$; $r^2 = 0.88$) in granulometry (Table S3), with 1B exhibiting the highest proportion of sand (82–91%) and the lowest (21-28%) in station 3.

Significant differences ($P < 0.001$) in the NH_4^+ concentration were also found in the interactions of Upwelling-conditions × Station ($r^2 = 0.12$), Station × Bottom-type ($r^2 = 0.08$), and Upwelling-conditions × Station × Bottom-type ($r^2 = 0.07$). Urea levels in the sediments ranged from 0.09 ± 0.02 to 0.58 ± 0.06 μg N · g^{-1}. The effects of Station ($P < 0.001$; $r^2 = 0.44$) and Bottom-type ($P < 0.001$; $r^2 = 0.15$) on the urea concentration were observed. The PERMANOVA also revealed that the interactions between both factors significantly affected the distribution of urea ($P < 0.001$; $r^2 = 0.21$), with a more pronounced effect in seagrass sediments (pairwise test: $P < 0.05$).

Upwelling-conditions ($P < 0.05$), Bottom-type ($P < 0.001$), and the interaction of Upwelling-conditions × Station ($P < 0.05$) contributed 6%, 15%, and 7% to the total variation of SOC, respectively. The C:N ratio was different between upwelling conditions ($P < 0.001$; $r^2 = 0.26$) and bottom types ($P < 0.05$; $r^2 = 0.09$), with significant interaction of both factors ($P < 0.05$; r2 = 0.08). Although the CCHO concentrations in vegetated sediments (382.6 ± 146.8 μg C · g^{-1}) were consistently higher (pairwise test: $P < 0.001$) than those in bare bottoms (108.1 ± 24.3 μg C · g^{-1}) across all stations and both upwelling conditions, the most minor differences were noted at station 1 during HB (Table S2). In addition, MPB varied significantly among stations ($P < 0.001$; $r^2 = 0.37$) with a significant interaction of Station × Upwelling-conditions ($P < 0.05$; $r^2 = 0.16$).

Tables:

Table S1. Thermal cycling conditions and protocols used in the qPCR. All reactions* were performed in triplicate.

Target gene	Primer set	Sequence (5'-3')	Thermal profile*	Efficiencies	Reference
16S rRNA *Geobacteraceae*	Geo564F	AAGCGTTGTTCGGAWTTAT	40x(95˚C - 30 s, 57˚C - 30 s, 72˚C - 30 s)	95-110 %	6
	Geo840R	GGCACTGCAGGGGTCAATA			
16S rRNA *Acidomicrobiaceae* A6	Acm342F	GCAATGGGGGAAACCCTGAC	40x(95˚C - 30 s, 57˚C - 30 s, 72˚C - 30 s)	113-118 %	7
	Acm439R	ACCGTCAATTTCGTCCCTGC			

***Thermal conditions started at 95ºC for 5 min and finished at 72ºC for 5 min. Reactions finished with a melting curve starting at 60ºC and increasing by 1ºC until 95ºC to verify the amplicon specificity.**

Table S2. Sediment characteristics along Bahía de San Quintin under low BEUTI (LB) and high BEUTI (HB) conditions.

Stations	Moisture (%)		Granulometry (% sand)		pH (NBS)		[a]SOC (mg C g^{-1})		[b]CHO (µg C g^{-1})		C:N (mol C : mol N)		[c]MPB (µg C g^{-1})		NH_4^+ (µg N g^{-1})		Urea (µg N g^{-1})		[d]NO_x^- (µg N g^{-1})		[e]Fe(III) (mg Fe g^{-1})		[f]Mn(IV) (mg Mn g^{-1})	
	LB	HB	LB	HB	LB	HB	LB	HB	LB	HB	LB	HB	LB	HB	LB	HB	LB	HB	LB	HB	LB	HB	LB	HB
1B	35.53 (3.67)	42.50 (3.12)	90.75 (0.62)	81.70 (4.71)	8.01 (0.02)	7.85 (0.01)	3.10 (0.06)	5.43 (0.38)	72.08 (6.05)	109.06 (19.53)	10.05 (0.04)	7.31 (0.84)	202.36 (35.97)	309.24 (33.58)	4.01 (0.28)	9.16 (0.73)	0.09 (0.02)	0.21 (0.03)	0.35 (0.03)	1.06 (0.21)	2.95 (0.53)	3.46 (0.69)	0.09 (0.02)	0.11 (0.01)
1S	61.36 (1.78)	65.08 (4.65)	63.19 (0.85)	71.41 (3.68)	8.04 (0.02)	7.89 (0.03)	6.73 (0.98)	8.55 (0.32)	299.01 (9.39)	216.64 (21.43)	10.89 (0.47)	12.02 (0.86)	-	-	9.75 (0.98)	11.67 (2.09)	0.14 (0.06)	0.13 (0.04)	0.26 (0.02)	0.84 (0.08)	2.40 (0.28)	1.43 (0.76)	0.12 (0.02)	0.10 (0.01)
2B	50.29 (0.50)	49.88 (5.29)	68.01 (4.28)	74.60 (2.31)	8.06 (0.04)	8.01 (0.02)	7.49 (1.34)	5.91 (0.85)	126.47 (9.81)	121.88 (14.97)	11.44 (1.13)	8.69 (0.33)	250.35 (41.96)	269.23 (27.64)	6.10 (0.64)	6.59 (1.71)	0.20 (0.02)	0.25 (0.03)	0.11 (0.02)	0.62 (0.15)	4.97 (0.56)	4.42 (0.68)	0.15 (0.02)	0.14 (0.03)
2S	72.57 (1.67)	71.21 (0.69	55.84 (3.45)	63.06 (1.34)	8.08 (0.05)	7.95 (0.03)	9.90 (1.14)	10.05 (0.55)	320.78 (22.47)	274. 06 (38.79)	10.27 (0.18)	9.77 (0.53)	-	-	18.89 (0.26)	16.84 (1.49)	0.31 (0.05)	0.26 (0.04)	0.38 (0.03)	0.59 (0.07)	4.84 (1.10)	4.19 (0.52)	0.15 (0.02)	0.17 (0.04)
3B	68.11 (2.27)	67.23 (6.24)	25.12 (1.77)	21.29 (1.63)	8.19 (0.05)	8.21 (0.02)	10. 47 (0.80)	11.50 (1.50)	115.60 (4.69)	104.38 (6.85)	11.63 (0.71)	9.51 (0.76)	107.00 (30.41)	120.19 (10.80)	5.28 (0.72)	10.33 (0.95)	0.22 (0.02)	0.25 (0.04)	0.25 (0.05)	0.32 (0.07)	4.07 (0.38)	3.82 (0.79)	0.21 (0.04)	0.19 (0.01)
3S	83.03 (0.77)	86.06 (1.38)	28.23 (2.06)	23.81 (3.02)	8.15 (0.03)	8.16 (0.04)	18.50 (1.44)	22.83 (1.27)	535.15 (51.36)	604.80 (42.27)	10.63 (0.71)	10.57 (0.63)	-	-	16.02 (3.70)	25.25 (4.33)	0.58 (0.06)	0.47 (0.02)	0.37 (0.13)	0.41 (0.05)	4.37 (0.46)	4.75 (0.63)	0.18 (0.03)	0.15 (0.04)

Note: [a]SOC: sediment organic carbon; [b]CCHO: soluble carbohydrates; [c]MPB: microphytobenthos biomass; [d]NO_x^-: nitrate + nitrite; [e]Fe(III): microbiologically reducible iron; [f]Mn(IV): microbiologically reducible manganese.

Table S3. Results of three-way PERMANOVA tests for differences in sediment characteristics among Upwelling conditions (UC), stations (St), and bottom type (BT).

Source	*Df*	Moisture		Granulometry		pH		SOC		CCHO		C:N		MPB		NH_4^+		Urea		NO_x^-		Fe(III)		Mn(IV)	
		F	R^2	*F*	R^2	*F*	R^2	*F*	R^2	*F*	R^2	*F*	R^2	*F*	R^2	*F*	R^2	*F*	R^2	*F*	R^2	*F*	R^2	*F*	R^2
UC	1	1.3	0.01	0.5	0.00	**30.3***	0.20	**12.3****	0.06	0.0	0.00	**16.4****	0.26	3.2	0.06	**50.9****	0.16	0.2	0.00	**41.6****	0.37	0.4	0.00	0.1	0.00
St	2	**65.7****	0.44	**387.8****	0.88	**41.9****	0.56	**59.4****	0.58	3.1	0.06	1.5	0.05	**12.6****	0.32	2.8	0.04	**40.0****	0.44	**9.2***	0.16	**12.6****	0.45	**21.3****	0.43
BT	1	**134.8****	0.45	**28.9****	0.03	0.0	0.00	**30.5****	0.15	**390.8****	0.47	**5.5***	0.09	-	-	**230.7****	0.47	**26.3****	0.15	0.2	0.00	0.4	0.01	0.2	0.00
UCxSt	2	0.9	0.01	3.4	0.01	**3.7***	0.05	**8.0***	0.07	1.3	0.00	2.5	0.07	**8.8***	0.17	**28.6****	0.12	1.6	0.01	**10.1****	0.18	0.3	0.01	1.6	0.03
UCxBT	1	0.0	0.00	2.9	0.01	0.1	0.00	1.1	0.00	0.2	0.00	**4.8***	0.08	-	-	2.3	0.01	**8.8***	0.06	2.0	0.02	0.3	0.01	4.3	0.06
StxBT	2	1.4	0.01	**14.8****	0.03	0.6	0.01	1.4	0.01	**45.0****	0.26	2.1	0.07	-	-	**19.3****	0.08	**18.3****	0.21	2.9	0.05	2.1	0.10	3.4	0.05
UCxStxBT	2	0.4	0.00	3.2	0.00	1.7	0.02	0.6	0.01	**9.3****	0.14	0.7	0.02	-	-	**14.5****	0.07	0.1	0.00	0.6	0.01	0.9	0.03	2.8	0.09
Residuals	24		0.08		0.04		0.16		0.12		0.07		0.37		0.45		0.05		0.13		0.21		0.39		0.34
Pairwise post-hoc comparisons																									
UC		-		-		LB > HB		HB > LB		-		LB > HB		-		HB > LB		-		HB > LB		-		-	
St		3 > 2 > 1		1 > 2 > 3		3 > 2 > 1		3 > 2 > 1		-		-		1 = 2 > 3		-		3 > 2 > 1		1 = 2 > 3		3 = 2 > 1		3 = 2 > 1	
BT		S > B		B > S		-		S > B		S > B		S > B		-		S > B		S > B		-		-		-	

Note: LB: Low BEUTI; HB: High BEUTI; B: Bare sediments; S: Seagrass sediments; * and ** denote statistical significance at $P < 0.05$ and $P < 0.001$, respectively.

Table S4. Results of three-way PERMANOVA tests for differences in dissimilatory Fe(III) reduction rates (FeRR) and Feammox rates among upwelling condition (UC), stations (St), and bottom type (BT).

Source	*df*	FeRR		Feammox	
		F	R^2	*F*	R^2
UC	1	0.1	0.0	0.4	0.01
St	2	**21.3****	**0.42**	**37.8****	**0.34**
BT	1	0.2	0.01	0.7	0.02
UCxSt	2	1.6	0.03	1.8	0.03
UCxBT	1	**5.8***	**0.07**	**8.2****	**0.10**
StxBT	2	**7.4****	**0.12**	**17.4****	**0.25**
UCxStxBT	2	2.3	0.03	**6.5***	**0.08**
Residuals	24		0.32		0.17
UC		-		-	
St		3 = 2 > 1		3 = 2 > 1	
BT		-		-	

Note: LB: Low BEUTI; HB: High BEUTI; B: Bare sediments; S: Seagrass sediments; * and ** indicate statistical significance at $P < 0.05$ and $P < 0.001$, respectively.

Table S5. Results of Monte Carlo permutation tests employed to analyze the environmental characteristics that contributed to the RDA models (Fig. 3).

Environmental characteristics	Bare sediments		Seagrass sediments	
	Explains rate	*p-value*	Explains rate	*p-value*
BEUTI	**12.7**	**0.009**	**10.8**	**0.017**
CCHO	**15.1**	**0.002**	**9.2**	**0.025**
C:N	1.5	0.539	2.0	0.611
d_m	**7.9**	**0.046**	**8.5**	**0.040**
Fe(III)	**16.7**	**0.001**	**22.1**	**0.001**
MPB	**10.2**	**0.020**	**-**	**-**
Mn(IV)	**8.4**	**0.031**	4.5	0.198
μ_m	4.3	0.138	3.8	0.257
NH_4^+	**9.0**	**0.027**	5.7	0.072
NO_x^-	**8.1**	**0.034**	**6.9**	**0.048**
pH	1.6	0.642	1.3	0.805
φ_s	3.0	0.295	0.8	0.911
SOC	2.3	0.470	**15.9**	**0.001**
T	**11.7**	**0.015**	**7.2**	**0.045**
Urea	**14.2**	**0.006**	**11.6**	**0.012**

Note: BEUTI: Biologically Effective Upwelling Transport Index (mmol $NO_3^- \cdot m^{-1} \cdot s^{-1}$); CCHO: soluble carbohydrates (µg C $\cdot g^{-1}$); d_m: station distance from the inlet (km); Fe(III): microbiologically reducible iron (mg Fe $\cdot g^{-1}$); MPB: microphytobenthos biomass (µg C $\cdot g^{-1}$); Mn(IV): microbiologically reducible manganese (mg Mn $\cdot g^{-1}$); μ_m: moisture (%); NO_x^-: nitrate + nitrite (µg N $\cdot g^{-1}$); φ_s: granulometry as proportion of sand (%); SOC: sediment organic carbon (µg C $\cdot g^{-1}$); T: temperature (°C). Bold letters imply statistical significance at the $p < 0.05$ level.

- **Figures:**

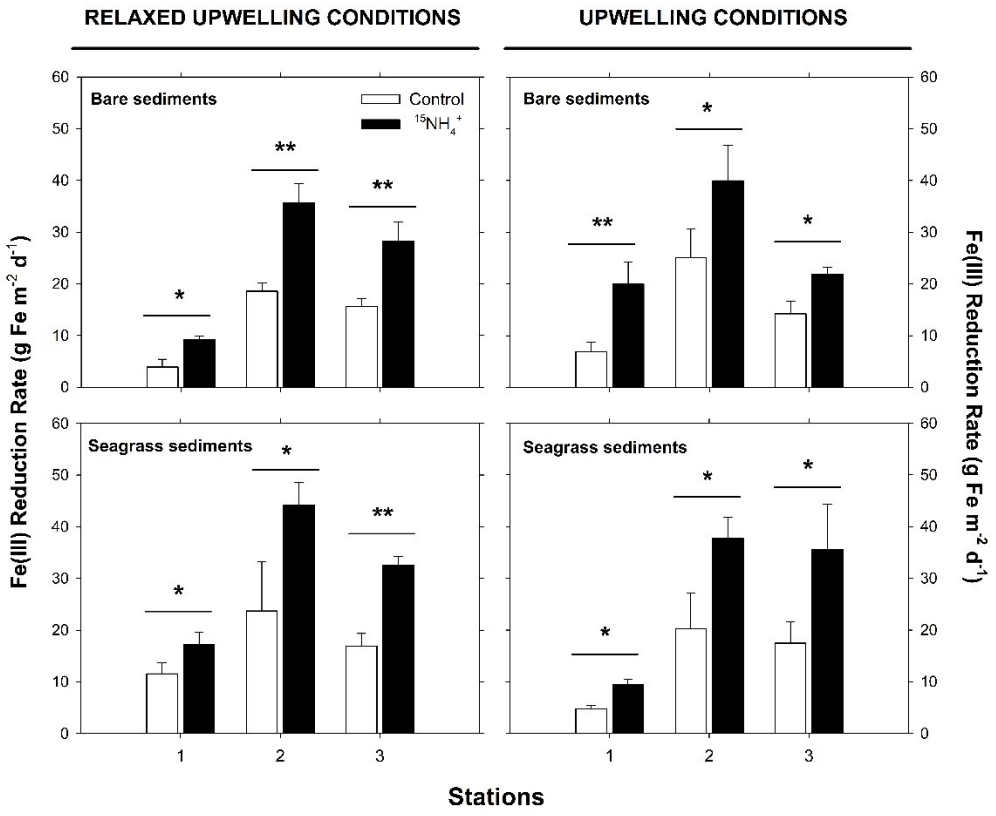

Figure S1. Fe(III) reduction rates (g Fe · m^{-2} · d^{-1}) measured through isotope tracer incubations in both bare and seagrass sediments from Bahia de San Quintin, under upwelling-relaxed and upwelling conditions. Asterisks above the horizontal line denote statistically significant differences (* at the level 0.05 and ** at the level 0.001) between treatments (control and $^{15}NH_4^+$ addition), as determined by the t-student test. Values are the means and error bars represent standard errors (n=3).

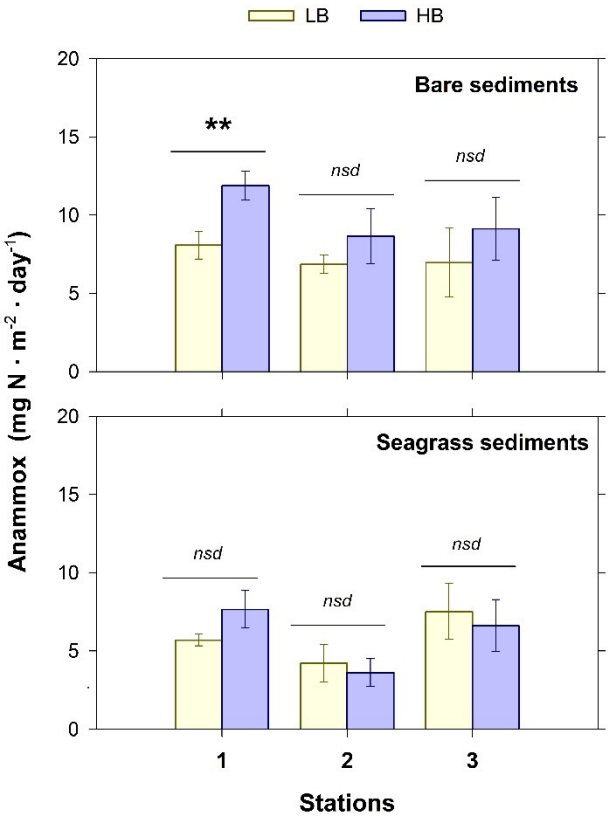

Figure S2. Seasonal changes on potential Anammox rates measured through isotope tracer incubations in bare and seagrass sediments from Bahia de San Quintin. Asterisks above the horizontal line denote statistically significant differences (* at 0.05 and ** at 0.001 level, respectively) between upwelling and relaxed upwelling conditions (n=3).

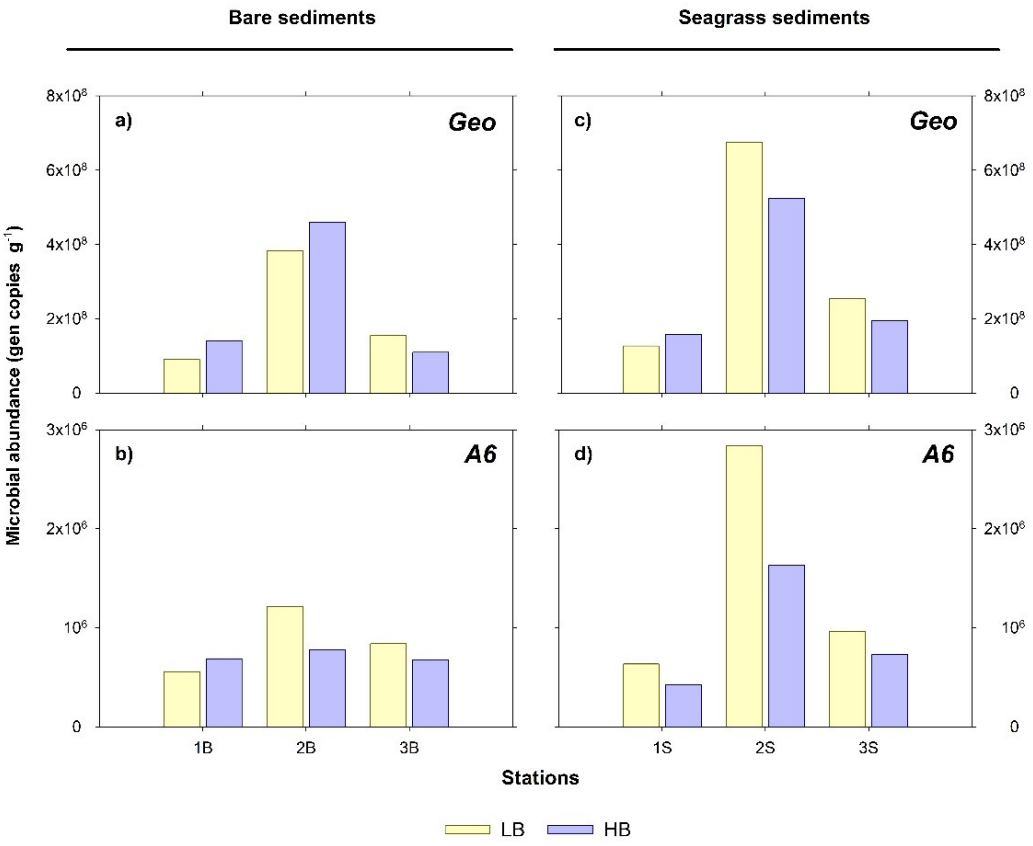

Figure S3. Gene copy numbers of *Geobacteraceae* spp (*Geo*) and *Acidimicrobiaceae* A6 spp quantified through qPCR in bare and seagrass sediments under relaxed (LB) and favourable (HB) upwelling conditions.

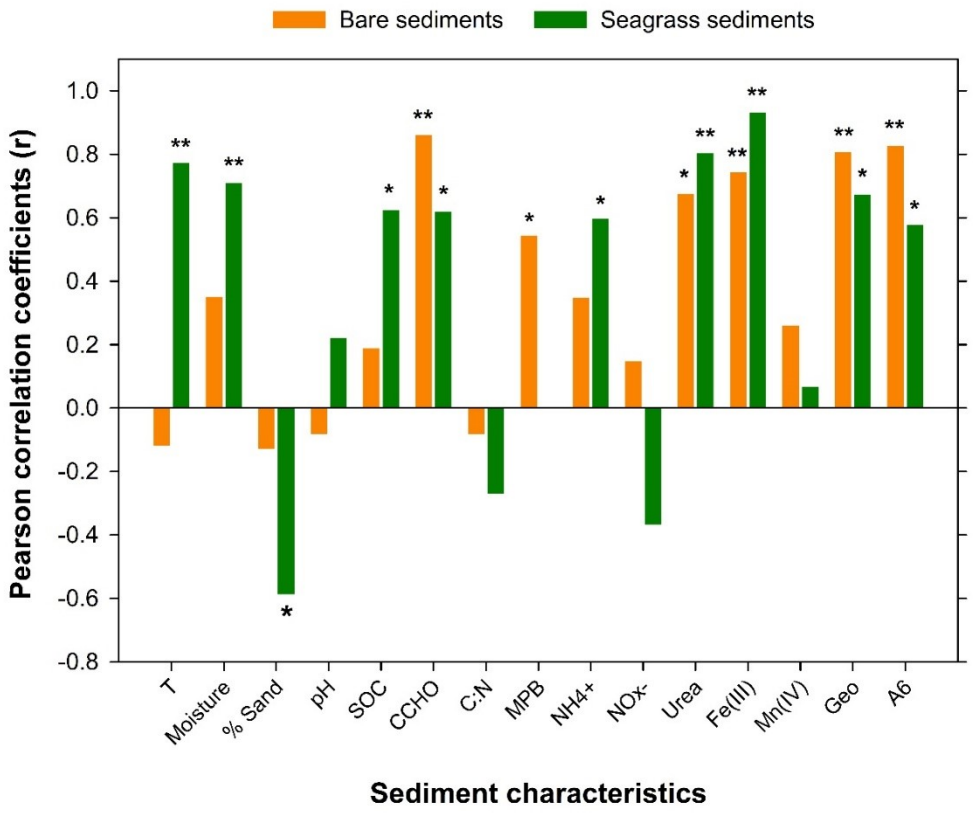

Figure S4. The relationship between Feammox rates and sediment characteristics in Bahia de San Quintin. * and ** denote statistically significant differences $P < 0.05$ and $P < 0.001$, respectively.

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