

Supplementary Materials

Distribution of nitrogen-cycling genes in an oxygen-depleted cyclonic eddy in the Alfonso Basin, Gulf of California

Ramiro Ramos-de la Cruz^{A,B}, Silvia Pajares^{B,C}, Martín Merino-Ibarra^B, María Adela Monreal-Gómez^B and Erik Coria-Monter^B

^APosgrado en Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Circuito exterior s/n, Ciudad Universitaria, Mexico City, 04510, Mexico.

^BUnidad Académica de Ecología y Biodiversidad Acuática, Instituto de Ciencias del Mar y Limnología (ICML), Circuito exterior s/n, Ciudad Universitaria, Universidad Nacional Autónoma de México, Mexico City, 04510, Mexico.

^CCorresponding author. Email: spajares@cmarl.unam.mx

Table S1. qPCR primers, thermal cycling and reaction conditions used in this study

Gene	Primers	Sequence (5'-3')	Cycling conditions	Reaction conditions	Efficiency	Reference
Archaeal <i>amoA</i>	Arch-amoAF	STAATGGTCTGGCTTAGACG	95°C 5 min, 40 x (95°C 45 s, 53°C 1 min, 72° 1 min), 72°C 5 min	0.2 µM primers, 0.3 µg/µl BSA	84%	Francis <i>et al.</i> (2005)
	Arch-amoAR	GCGGCCATCCATCTGTATGT				
Bacterial <i>amoA</i>	amoA1F	GGGGTTTCTACTGGTGGT	95°C 5 min, 40 x (95°C 45 s, 55°C 45 s, 72° 45 s), 72°C 5 min	0.3 µM primers	111.35%	Rotthauwe <i>et al.</i> (1997)
	amoA2R	CCCCTCKGSAAAGCCTTCTTC				
<i>nirS</i>	nirS-cd3aF	G TSAACG TSAAGGARACSGG	Touchdown (-1°C/cycle) 95°C 5 min, 6 x (95°C 30 s, 62°C 20 s, 72°C 40 s), 35 x (95°C 30 s, 60°C 20 s, 72°C 40 s), 72°C 5 min	0.3 µM primers, 0.3 µg/µl BSA	82%	Michotey <i>et al.</i> (2000)
	nirS-R3cd	GASTTCGGRTGSGTCTTGA				
<i>nirK</i>	nirK-876F	ATYGGCGGVAYGGCGA	Touchdown (-1°C/cycle) 95°C 5 min, 6 x (95°C 15 s, 65°C 30 s, 72°C 30 s), 35 x (95°C 15 s, 60°C 30 s, 72°C 30 s), 72°C 5 min	0.5 µM primers, 0.3 µg/µl BSA	96%	Henry <i>et al.</i> (2004)
	nirK-1040R	GCCTCGATCAGRTTTRTGGTT				
<i>nrfA</i>	nrfAf2aw nrfAR1	CARTGYCAYGTBGARTA TWNGGCATRTGRCARTC	95°C 5 min, 40 x (95°C 15 s, 52°C 30 s, 72°C 30 s), 72°C 5 min	0.5 µM primers, 0.2 µg/µl BSA	93.47%	Mohan <i>et al.</i> (2004), Welsh <i>et al.</i> (2014)
<i>hzo</i>	hzoF1	TGTGCATGGTCAATTGAAAG	95°C 5 min, 40 x (95°C 45 s, 53°C 1 min, 72°C 1 min), 72°C 5 min	0.4 µM primers, 0.4 µg/µl BSA	88.36%	Li <i>et al.</i> (2010)
	hzoR1	CAACCTCTTCWGCAGGTGCATG				

Table S2. Multiple linear regression models and their parameters for the N cycling genes

Significance codes: *** = 0.001, ** = 0.01, * = 0.05, . = 0.1.

Gene	Linear models				
<i>A-amoA</i>	lm (A-amoA ~ Oxygen + NO3)				
<i>B-amoA</i>	lm (B-amoA ~ Oxygen + NO2)				
<i>nirK</i>	lm (nirK ~ Oxygen + Chlorophyll + NO3)				
<i>nirS</i>	lm (nirS ~ Salinity + NO3)				
<i>nrfA</i>	lm (nrfA ~ Chlorophyll + NH4 + NO3)				
<i>hzo</i>	lm (hzo ~ Chlorophyll + NH4 + NO3)				

Coefficients	Estimate	Std. Error	t value	Pr(> t)	vif
A-amoA					
Intercept	9.35132	0.48887	19.128	2.52E-14***	
Oxygen	0.15871	0.07966	1.992	0.0602 .	1.75753
NO ₃ ⁻	0.14927	0.10739	1.39	0.1798	1.75753
B-amoA					
Intercept	1.4727	0.6357	2.316	0.0313*	
Oxygen	0.3439	0.1421	2.42	0.0252*	1.04273
NO ₂ ⁻	-8.6419	3.7636	-2.296	0.0326*	1.04273
nirK					
Intercept	5.6996	0.7304	7.804	0.000000242***	
Oxygen	-0.3507	0.136	-2.579	0.01838*	2.404638
Chlorophyll	-0.8957	0.3061	-2.927	0.00866**	1.4009
NO ₃ ⁻	0.6143	0.162	3.792	0.00123**	1.87807
nirS					
Intercept	121.6614	26.7753	4.544	0.000198***	
Salinity	-33.1173	7.4669	-4.435	0.000254***	1.01473
NO ₃ ⁻	0.2307	0.0648	3.559	0.001965**	1.01473
nrfA					
Intercept	2.0502	0.6635	3.09	0.00602**	
Chlorophyll	-3.6969	0.4627	-7.99	1.71E07***	1.0322
NH ₄ ⁺	1.9819	0.5269	3.762	0.00132**	1.0285
NO ₃ ⁻	1.3724	0.2135	6.426	3.67E06***	1.04834
hzo					
Intercept	2.0432	0.7658	2.668	0.015204*	
Chlorophyll	-3.9209	0.5341	-7.341	5.86E-7***	1.0322
NH ₄ ⁺	2.4124	0.6082	3.967	0.000827***	1.0285
NO ₃ ⁻	1.5256	0.2465	6.189	6.03E-6***	1.04834

Gene	Residuals					Residual	Mult.	Adj.	F-	p-
	Min	1Q	Median	3Q	Max	std.	R ²	R ²	statistic	value
						20 d.f.				
A-amoA	-0.83204	-0.2905	-0.0682	0.2206	0.7726	0.4402	0.166	0.0826	1.99	0.1638
B-amoA	-1.7464	-0.713	-0.2175	0.6764	2.2665	1.02	0.316	0.2481	4.629	0.0223
nirK	-0.8509	-0.6006	0.1562	0.4334	1.052	0.6424	0.842	0.8171	33.76	8.13e ⁻⁸
nirS	-0.6903	-0.2242	-0.0411	0.1808	0.6452	0.3496	0.647	0.6118	18.34	2.99e ⁻⁵
nrfA	-1.53598	-0.8664	-0.05	0.7162	1.9986	1.133	0.89	0.8726	51.23	2.69e ⁻⁹
hzo	-1.7465	-0.9536	0.1803	0.7046	2.8353	1.308	0.881	0.8617	46.68	5.85e ⁻⁹

Shapiro-Wilk normality test

Gene	W	p-value
A-amoA	0.95872	0.4379
B-amoA	0.94847	0.2719
nirK	0.92372	0.08008
nirS	0.9665	0.6059
nrfA	0.9383	0.1652
hzo	0.94595	0.2407

Supplementary Figures

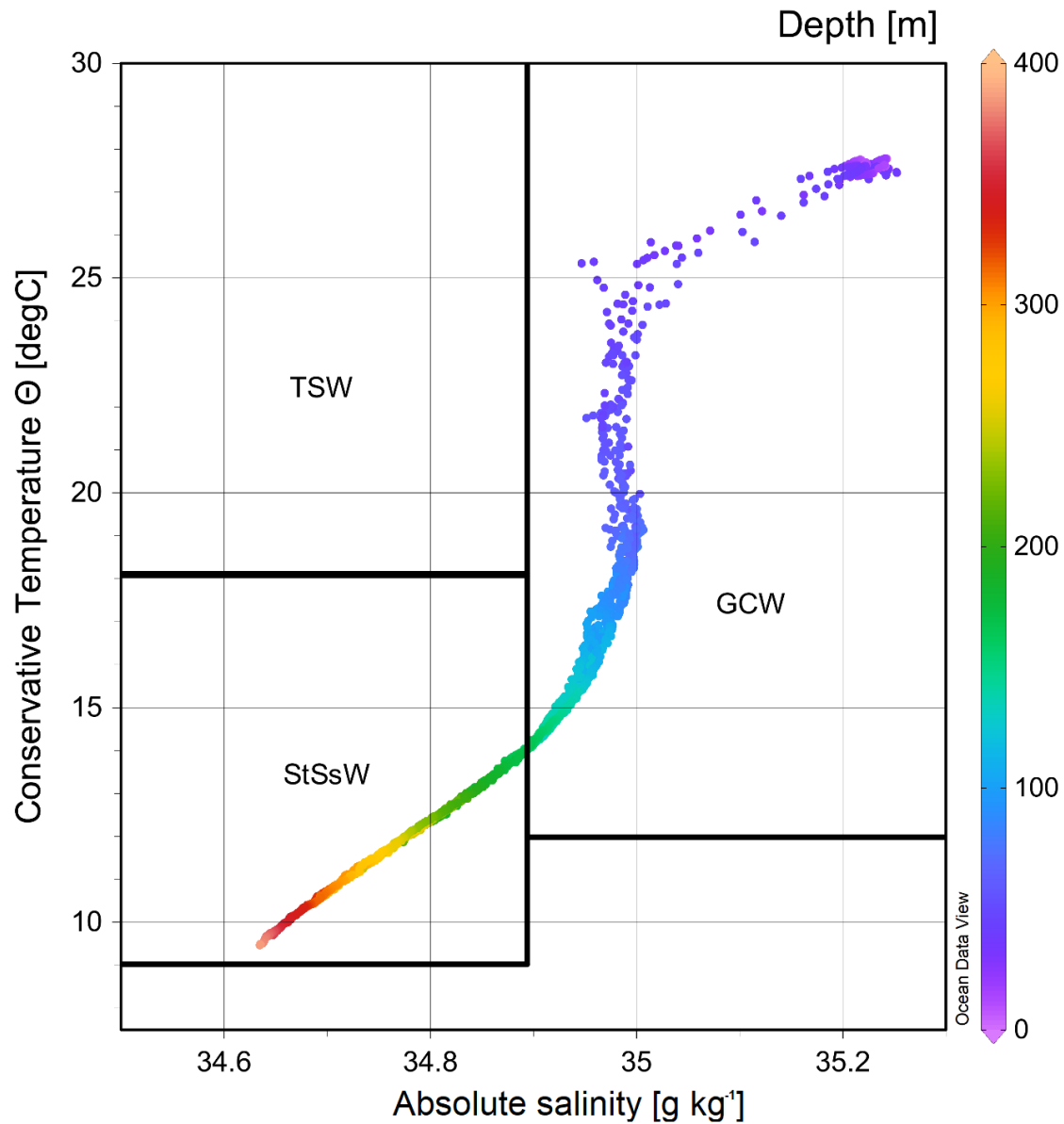


Fig. S1. T-S diagram of the water masses in the Bay of La Paz. TSW, Tropical Surface Water; GCW, Gulf of California Water, StSsW, Subtropical Subsurface Water.

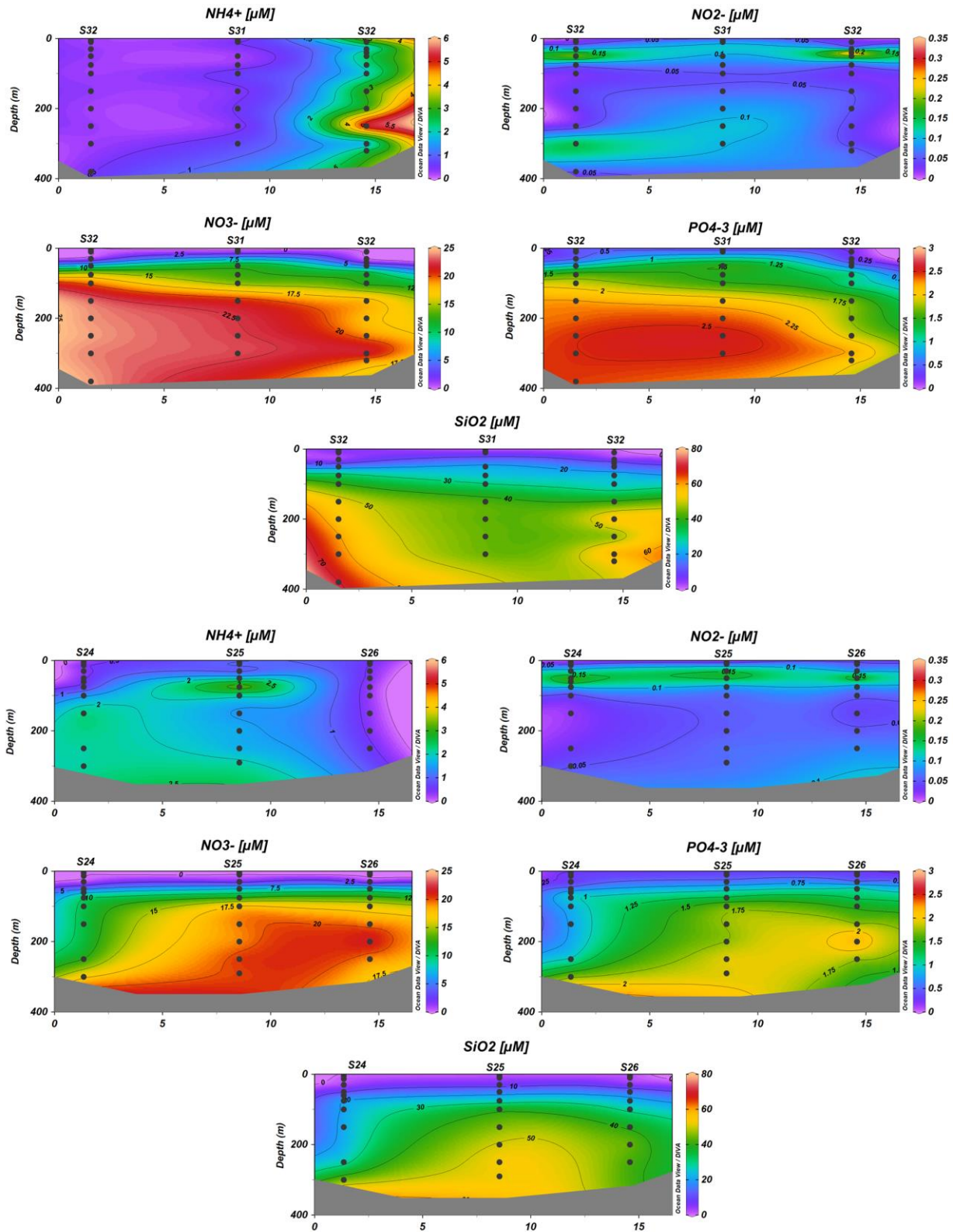


Fig. S2. Depth profiles of ammonium, nitrite, nitrate, phosphate, and silicon in the northern (up) and southern (down) sections.

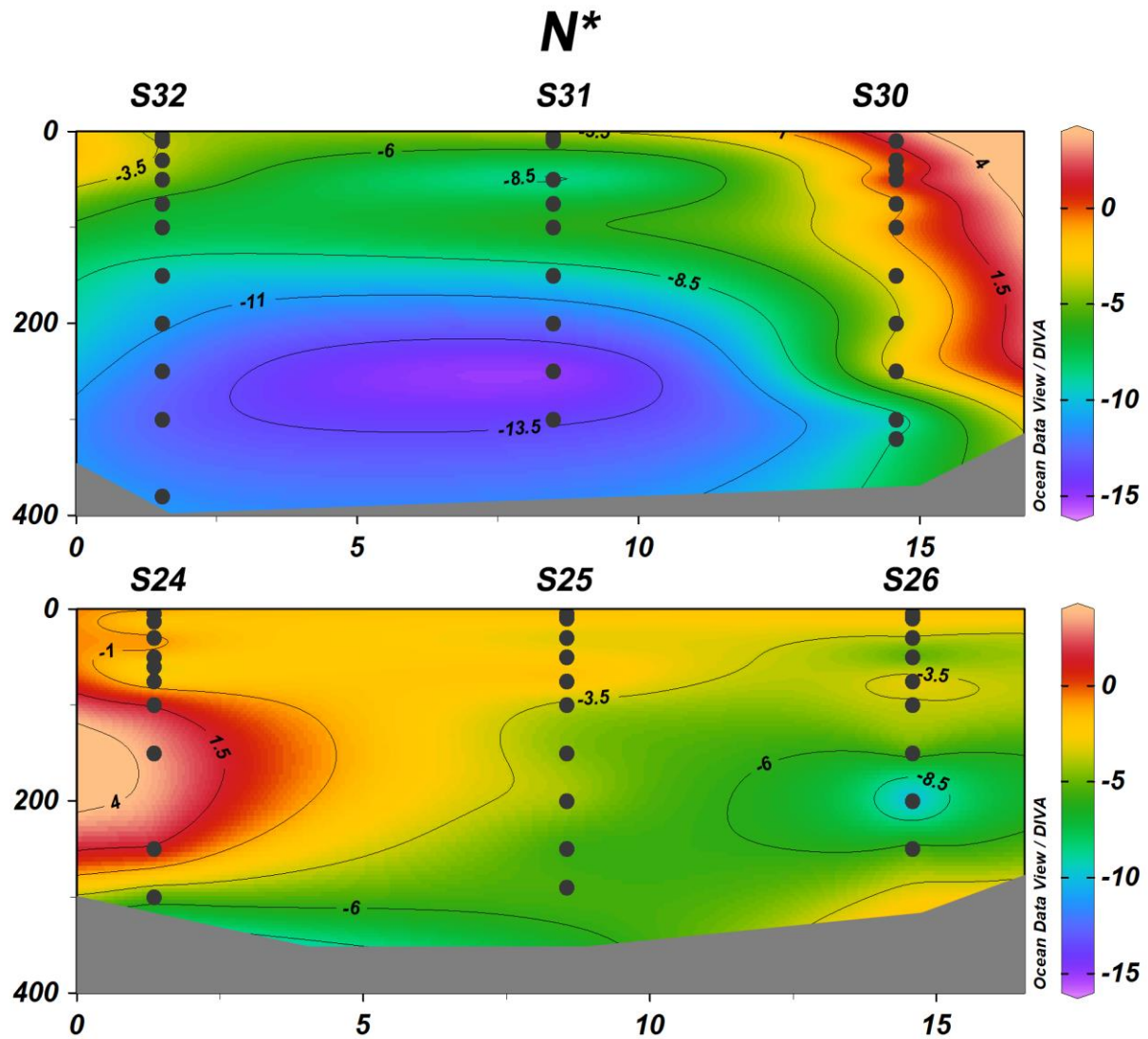


Fig. S3. Nitrogen deficit (N^*) in the northern (up) and southern (down) sections.