

Insights into nitrogen fixation below the euphotic zone: trials in an oligotrophic marginal sea and global compilation

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Supplemental information

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Table S1. Statistical information for log-transformed linear regressions of aphotic N₂ fixation (ANF) and particulate nitrogen (PN) concentration, ANF and specific N₂ fixation rate (SNFR), SNFR and PN concentration in different study areas, calculated via SPSS Statistics. *n*: sample size; *r*: correlation coefficient; *F*: *F* value for *F*-test, *p*: significance; SW: *Shapiro-Wilk* test for residual normality. Asterisks represent significant regressions (*p* < 0.05).

Location	<i>n</i>	log ₁₀ (ANF) vs. log ₁₀ (PN)				log ₁₀ (ANF) vs. log ₁₀ (SNFR)				log ₁₀ (SNFR) vs. log ₁₀ (PN)			
		<i>r</i>	<i>F</i>	<i>p</i>	SW	<i>r</i>	<i>F</i>	<i>p</i>	SW	<i>r</i>	<i>F</i>	<i>p</i>	SW
Southern California Bight (Hamersley et al., 2011)	14	0.35	1.71	0.22	0.42	0.95	121.90	*0.00	0.08	0.62	7.37	*0.02	0.42
Eastern Tropical South Pacific (Bonnet et al., 2013; Löscher et al., 2016)	33	0.47	8.98	*0.01	0.04	0.94	234.44	*0.00	0.00	0.19	1.17	0.29	0.15
Mediterranean Sea (Benavides et al., 2016)	10	0.35	1.10	0.33	0.07	0.49	2.47	0.16	0.95	0.65	5.90	*0.04	0.07
Western Tropical South Pacific (Benavides et al., 2018)	59	0.06	0.23	0.64	0.00	0.82	117.32	*0.00	0.73	0.62	36.02	*0.00	0.00
Eastern Tropical North Pacific (Selden et al., 2019)	8	0.61	3.52	0.11	0.05	0.89	23.20	*0.00	0.79	0.23	0.35	0.58	0.14
South China Sea (This study)	11	0.73	10.28	*0.01	0.77	0.96	95.02	*0.00	0.80	0.51	3.21	0.11	0.52

Table S2. Amino acid addition effects on ANF below the euphotic zone.

Location	Depth	DOM Addition Type	Final	Effect on ANF	Reference
			Concentration ($\mu\text{M C}$)		
Gulf of Aqaba	160-720 m	Amino Acid Mixture (Sigma A9906)	0.5	1.5-2 fold increase	Rahav et al., 2013
Eastern Tropical South Pacific	OMZ core (between 140- 475 m)	Amino Acid (20 % leucine, 23 % glutamic acid, 56 % alanine)	4	4-7 fold increase	Bonnet et al., 2013
Gulf of Aqaba	160 m	DCMU+Amino Acid Mixture (Sigma A9906)	0.5	2-4 fold increase	Rahav et al., 2015
Solomon seas	300-400 m	20 % leucine, 23 % glutamic acid, 56 % alanine	4.03	0-2 fold increase	Benavides et al., 2015
Bismarck Seas	300-400 m	20 % leucine, 23 % glutamic acid, 56 % alanine	4.03	0-2 fold increase	Benavides et al., 2015
Eastern Tropical North Pacific	135-205 m	20 amino acid mixture (Cambridge Isotope)	40	Decrease to increase	Selden et al., 2019
Eastern Tropical North Pacific	500-2000 m	20 amino acid mixture (Cambridge Isotope)	40	0 to decreased	Selden et al., 2019
South China Sea	200-855m	17 % glutamic acid, 50 % glycine, 33 % alanine	0.2; 0.4; 2; 10; 20	Decrease to around 3 fold increase	This Study

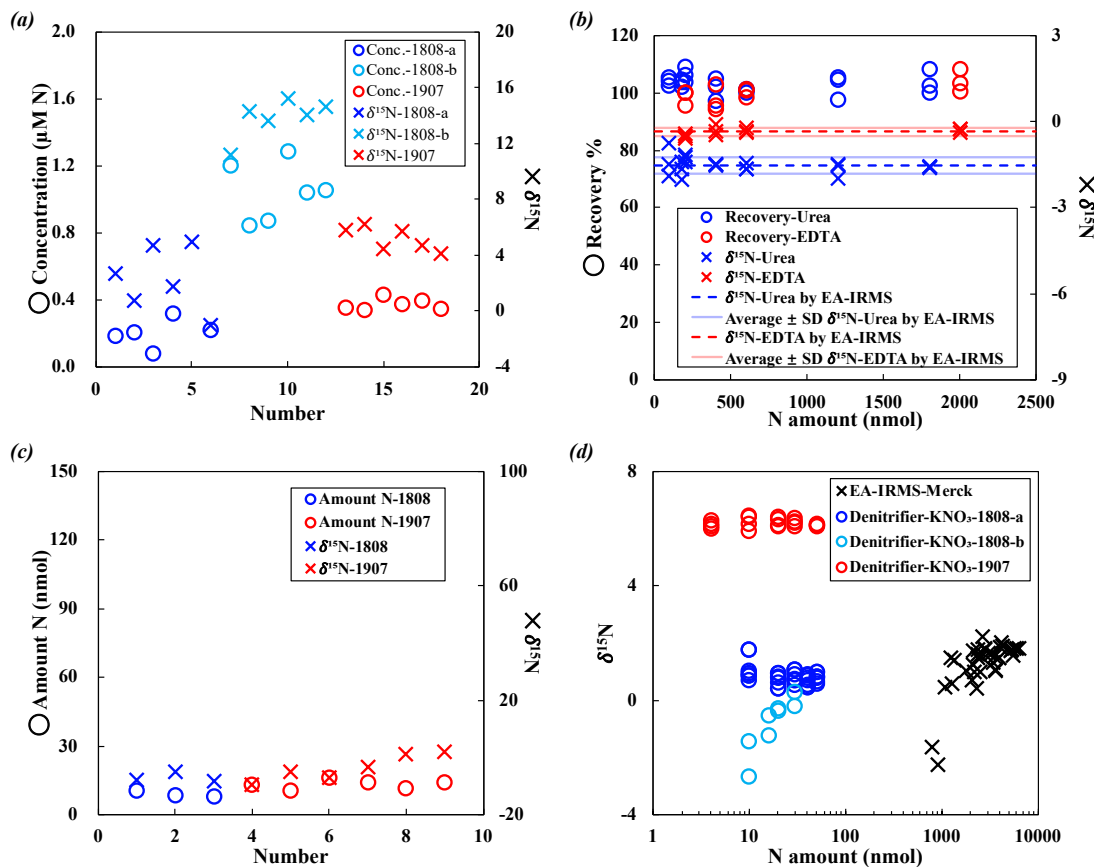


Figure S1. (a) Concentrations (circles) and isotopic composition (crosses) of blanks of alkaline persulfate oxidation method. 3 batches are shown in different colours (blue, red and green). (b) Recovery rates (circles) and isotopic composition (crosses) of known amounts of urea (blue) and EDTA (red). Blue and red dotted lines represent the $\delta^{15}\text{N}$ value of urea and EDTA obtained via EA-IRMS, respectively. (c) Nitrogen amount (circles) and isotopic composition (crosses) of filter blanks (25 mm GF75) in August 2018 (blue) and July 2019 (red) cruises. (d) N amount needed to attain stable $\delta^{15}\text{N}$ value via EA-IRMS (crosses) and denitrifier method (circles). For EA-IRMS, Acetanilide from Merck were used and KNO_3 (XiLong SCIENTIFIC) was used for denitrifier method. Circles in different colours represent different batches of analysis.

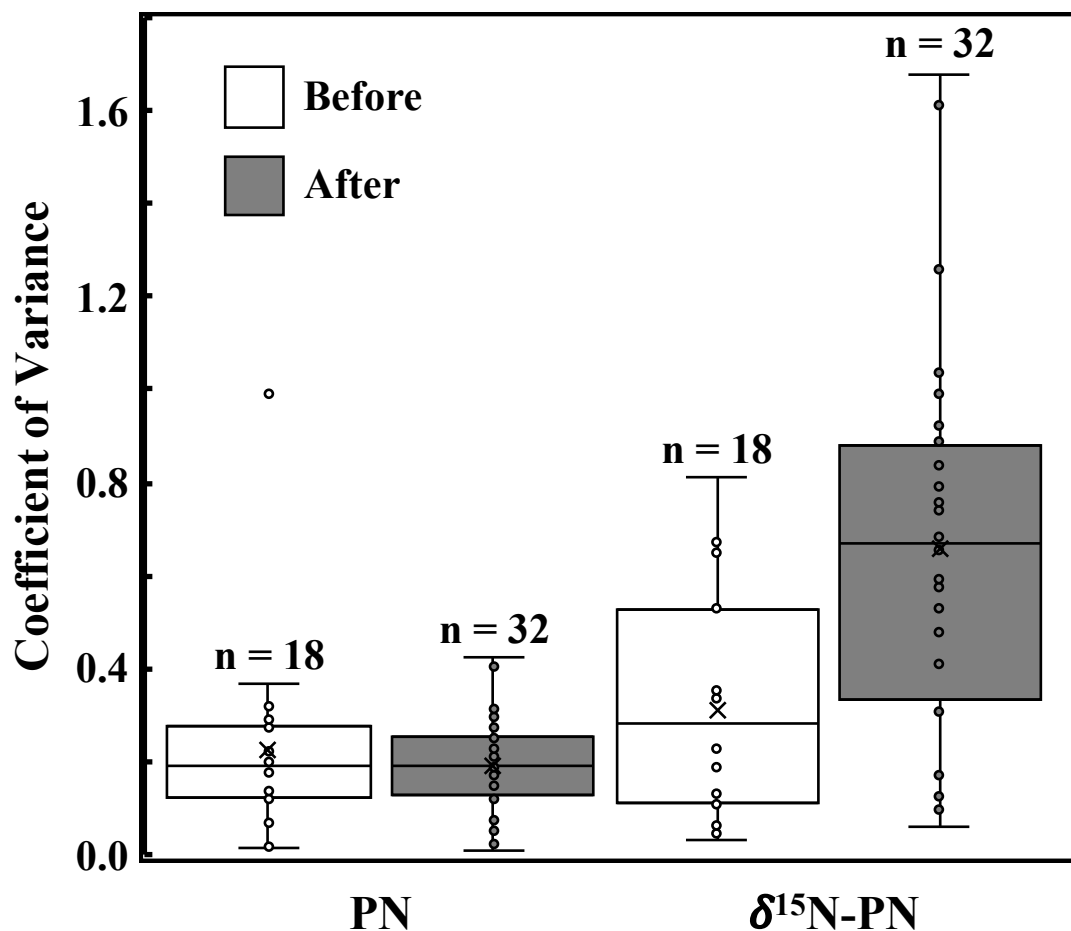


Figure S2. Coefficient of variance (CV) of PN concentration and $\delta^{15}\text{N-PN}$ in replicates before and after $^{15}\text{N}_2$ tracer incubation. Boxes represent the 25–75 % quantile range, with the center line depicting the median (50 % quantile); bars encompass data points within 1.5× the interquartile range. CVs are shown as points. Crosses represent mean CV values in each group.

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