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Supplementary material

Re-evaluation of the diversity and distribution of diazotrophs in the South China Sea by pyrosequencing the *nifH* gene

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Table S1. Primers in the nested PCR protocol

The primers nifH3 and nifH4 were used in the first-round PCR reaction (Zehr and Turner 2001). The underlined sequences were the barcodes. There were 27 different barcode sequences designed for the 27 samples. The adapter sequences are on the left of the barcodes. The sequences in bold were the nifH1 and nifH2 primers (Zehr and Turner 2001)

The first-round primers of nested PCR	Primer sequences
nifH3	ATRTTRTTNGCNGCRTA
nifH4	TTYTAYGGNAARGGNGG
The second-round primers of nested PCR	**Primer sequences**
nifH1_P09SU-U	CGTATCGCCTCCCTCGCGCCATCAGAGACTCGACG**TGYGAYCCNAARGCNGA**
nifH1_T09SU-U	CGTATCGCCTCCCTCGCGCCATCAGAGTACGAGAG**TGYGAYCCNAARGCNGA**
nifH1_T09SU-M	CGTATCGCCTCCCTCGCGCCATCAGAGTACTACTA**TGYGAYCCNAARGCNGA**
nifH1_T09SU-L	CGTATCGCCTCCCTCGCGCCATCAGAGTAGACGTC**TGYGAYCCNAARGCNGA**
nifH1_L09SU-U	CGTATCGCCTCCCTCGCGCCATCAGAGTCGTACAC**TGYGAYCCNAARGCNGA**
nifH1_C09SU-U	CGTATCGCCTCCCTCGCGCCATCAGAGTGTAGTAG**TGYGAYCCNAARGCNGA**
nifH1_P10SU-U	CGTATCGCCTCCCTCGCGCCATCAGATAGTATACG**TGYGAYCCNAARGCNGA**
nifH1_T10SU-U	CGTATCGCCTCCCTCGCGCCATCAGCAGTACGTAC**TGYGAYCCNAARGCNGA**
nifH1_T10SU-M	CGTATCGCCTCCCTCGCGCCATCAGCGACGACGCG**TGYGAYCCNAARGCNGA**
nifH1_T10SU-L	CGTATCGCCTCCCTCGCGCCATCAGCGACGAGTAC**TGYGAYCCNAARGCNGA**
nifH1_L10SU-U	CGTATCGCCTCCCTCGCGCCATCAGCGATACTACG**TGYGAYCCNAARGCNGA**
nifH1_C10SU-U	CGTATCGCCTCCCTCGCGCCATCAGCGTACGTCGA**TGYGAYCCNAARGCNGA**
nifH1_P10SP-U	CGTATCGCCTCCCTCGCGCCATCAGCTACTCGTAG**TGYGAYCCNAARGCNGA**
nifH1_T10SP-U	CGTATCGCCTCCCTCGCGCCATCAGGTACAGTACG**TGYGAYCCNAARGCNGA**
nifH1_T10SP-M	CGTATCGCCTCCCTCGCGCCATCAGGTCGTACGTA**TGYGAYCCNAARGCNGA**
nifH1_T10SP-L	CGTATCGCCTCCCTCGCGCCATCAGGTGTACGACG**TGYGAYCCNAARGCNGA**
nifH1_L10SP-U	CGTATCGCCTCCCTCGCGCCATCAGACACAGTGAG**TGYGAYCCNAARGCNGA**
nifH1_C10SP-U	CGTATCGCCTCCCTCGCGCCATCAGACACTCATAC**TGYGAYCCNAARGCNGA**
nifH1_B10SP-U	CGTATCGCCTCCCTCGCGCCATCAGACAGACTATA**TGYGAYCCNAARGCNGA**
nifH1_B10SP-M	CGTATCGCCTCCCTCGCGCCATCAGACAGACAGCG**TGYGAYCCNAARGCNGA**
nifH1_B10SP-L	CGTATCGCCTCCCTCGCGCCATCAGACAGAGACTC**TGYGAYCCNAARGCNGA**
nifH1_N10SP-U	CGTATCGCCTCCCTCGCGCCATCAGACAGCTCGTG**TGYGAYCCNAARGCNGA**
nifH1_O10SP-U	CGTATCGCCTCCCTCGCGCCATCAGACAGTGTCGA**TGYGAYCCNAARGCNGA**
nifH1_C07SU-U	CGTATCGCCTCCCTCGCGCCATCAGACGAGCGCGC**TGYGAYCCNAARGCNGA**
nifH1_C07SU-M	CGTATCGCCTCCCTCGCGCCATCAGACGATGAGTG**TGYGAYCCNAARGCNGA**
nifH1_C07SU-L	CGTATCGCCTCCCTCGCGCCATCAGACGCGAGAGA**TGYGAYCCNAARGCNGA**
nifH1_T07SU-U	CGTATCGCCTCCCTCGCGCCATCAGACGCTCTCTC**TGYGAYCCNAARGCNGA**
nifH2	CTATGCGCCTTGCCAGCCCGCTCAG**ADNGCCATCATYTCNCC**

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Table S2. The abundance-based Sorenson similarity index based on the 96% cutoff protein identity

Indices larger than 70% are coloured yellow; sites that were quite different from others are shown in red

	C07su-L	C07su-M	C07su-U	C09su-U	C10sp-U	C10su-U	L09su-U	L10sp-U	L10su-U	S10sp-U	O10sp-U	P09su-U	P10sp-U	P10su-U	B10sp-L	B10sp-M	B10sp-U	T07su-U	T09su-L	T09su-M	T09su-U	T10sp-L	T10sp-M	T10sp-U	T10su-L	T10su-M
C07su-M	0.83842																									
C07su-U	0.17524	0.49598																								
C09su-U	0.9853	0.45831	0.9793																							
C10sp-U	0.86619	0.46136	0.968	0.94736																						
C10su-U	0.83623	0.45787	0.9565	0.98191	0.9505																					
L09su-U	0.23193	0.43852	0.9799	0.99698	0.9492	0.978																				
L10sp-U	0.17816	0.42972	0.9585	0.99914	0.9537	0.9814	1																			
L10su-U	0.87875	0.42976	0.8829	0.94501	0.8816	0.9717	0.9252	0.9308																		
S10sp-U	0.85796	0.91014	0.6799	0.99352	0.9802	0.9535	0.9736	0.9704	0.9138																	
O10sp-U	0.98249	0.52696	0.8332	0.99424	0.9904	0.963	0.9841	0.9668	0.9086	0.99047																
P09su-U	0.8097	0.43179	0.9505	0.99103	0.9364	0.9833	0.9705	0.9521	0.8949	0.6811	0.83191															
P10sp-U	0.93018	0.87897	0.8244	0.6195	0.849	0.659	0.6181	0.6065	0.6278	0.97442	0.9829	0.58337														
P10su-U	0.44838	0.43202	0.9795	0.99498	0.9357	0.9858	0.9964	0.9725	0.903	0.6835	0.82835	0.98052	0.5959													
B10sp-L	0.83229	0.40958	0.7869	0.78134	0.6147	0.6091	0.772	0.3924	0.7054	0.88125	1	0.07177	0.5819	0.2722												
B10sp-M	0.74049	0.52342	0.0144	0.00037	0.1378	0.0443	0.0012	0.0213	0.1635	0.52129	0.26504	0.03008	0.5911	0.0002	0.0683											
B10sp-U	0.22966	0.43147	0.9437	0.99597	0.9413	0.9868	0.9946	0.9943	0.9931	0.99094	0.98665	0.95145	0.6316	0.9696	0.2135	0.07748										
T07su-U	0.1733	0.44555	0.9801	0.9918	0.9804	0.9712	0.9922	0.975	0.8999	0.68385	0.83219	0.97566	0.8135	0.9948	0.1621	0.0108	0.9658									
T09su-L	0.35728	0.34205	0.1407	0.08488	0.0999	0.2227	0.2576	0.3395	0.307	0.08861	0.08425	0.39312	0.1754	0.0372	0.0582	0.23867	0.0613	0.01226								
T09su-M	0.49173	0.41089	0.8286	0.77761	0.6186	0.6157	0.8164	0.3676	0.7081	0.8949	0.90527	0.02121	0.591	0.2778	0.9232	0.00217	0.2171	0.16385	0.0359							
T09su-U	0.85184	0.44188	0.9752	0.99532	0.9433	0.9788	0.9941	0.994	0.9244	0.97076	0.98327	0.98195	0.6147	0.9919	0.8188	0.00358	0.9923	0.99	0.0925	0.83545						
T10sp-L	0.24598	0.42634	0.9487	0.99011	0.9388	0.9713	0.9876	0.9878	0.9157	0.95812	0.96894	0.96136	0.595	0.9783	0.0823	0.02378	0.9925	0.97498	0.0649	0.08284	0.98684					
T10sp-M	0.81642	0.82138	0.2582	0.23116	0.3207	0.3893	0.1613	0.1604	0.42	0.8726	0.4254	0.12743	0.9453	0.1066	0.1537	0.95978	0.6009	0.25775	0.3196	0.13454	0.17912	0.1718				
T10sp-U	0.79958	0.45222	0.9385	0.99395	0.9352	0.995	0.9594	0.9663	0.9662	0.97938	1	0.98127	0.6216	0.9624	0.2033	0.09124	0.966	0.95765	0.2224	0.1225	0.98226	0.9649	0.43606			
T10su-L	0.06986	0.05142	0.0336	0.27959	0.224	0.2064	0.2429	0.2565	0.4824	0.17812	0.16557	0.17542	0.0473	0.1237	0.0006	0.03052	0.1893	0.03588	0.2582	0.03846	0.15138	0.1937	0.07517	0.2223		
T10su-M	0.85048	0.42691	0.0355	0.76322	0.615	0.6129	0.0392	0.036	0.7281	0.8891	0.89603	0.07191	0.5814	0.0312	0.9593	0.09339	0.0346	0.02887	0.2446	0.03956	0.81971	0.0276	0.16622	0.2048	0.0171	
T10su-U	0.18312	0.43833	0.9699	0.98091	0.916	0.9553	0.9872	0.9525	0.9015	0.96582	0.97377	0.9125	0.6157	0.9553	0.9216	0.00801	0.9463	0.95008	0.0127	0.9754	0.97562	0.9272	0.15699	0.9206	0.0272	0.03867

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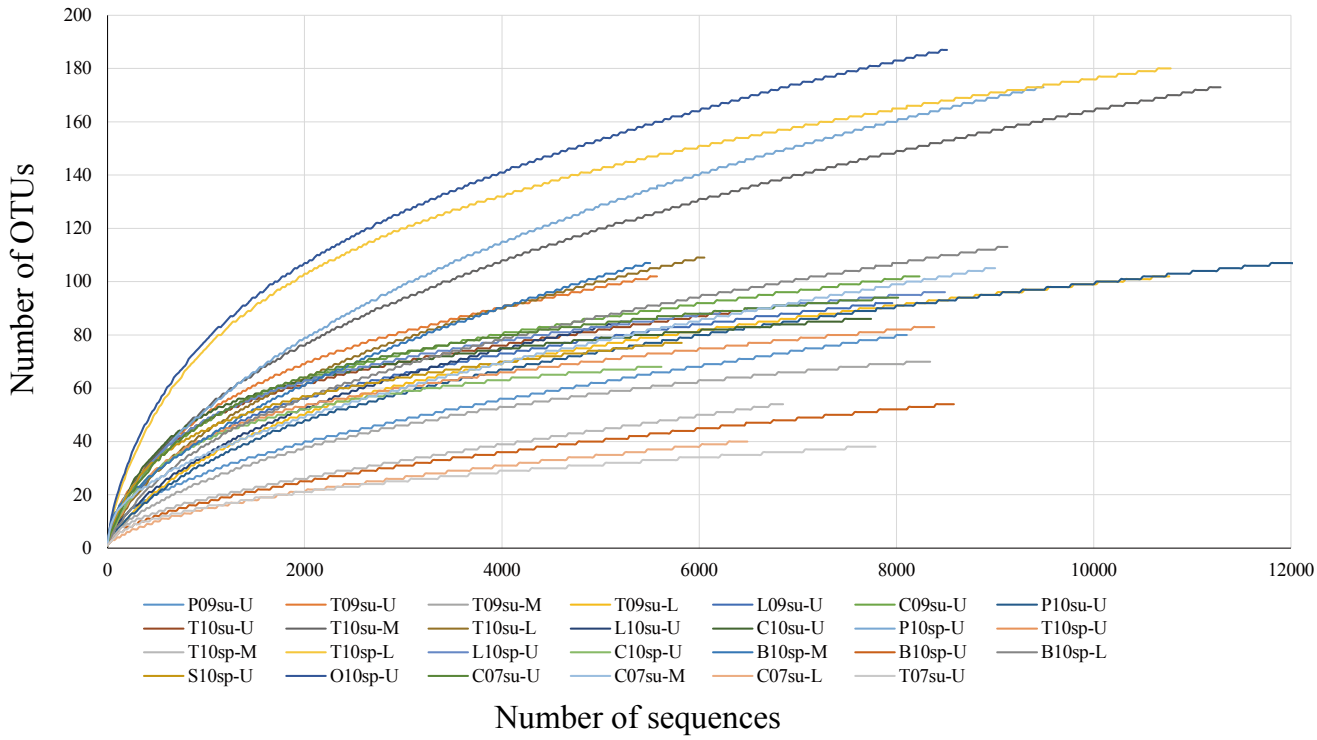

Fig. S1. Rarefaction curves of OTUs clustered at 96% protein identity across different environment samples.

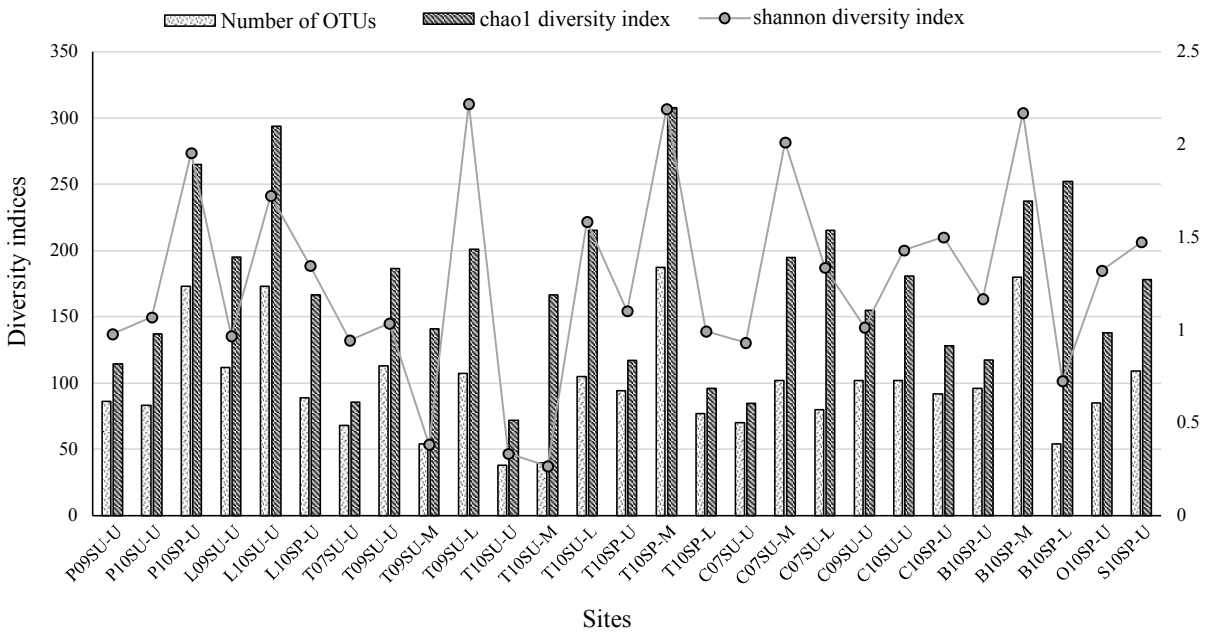

Fig. S2. The diversity index of diazotrophs shared 96% *nifH* amino acid identity. The diversity indices were calculated on the basis of 5289 subsampled sequences.

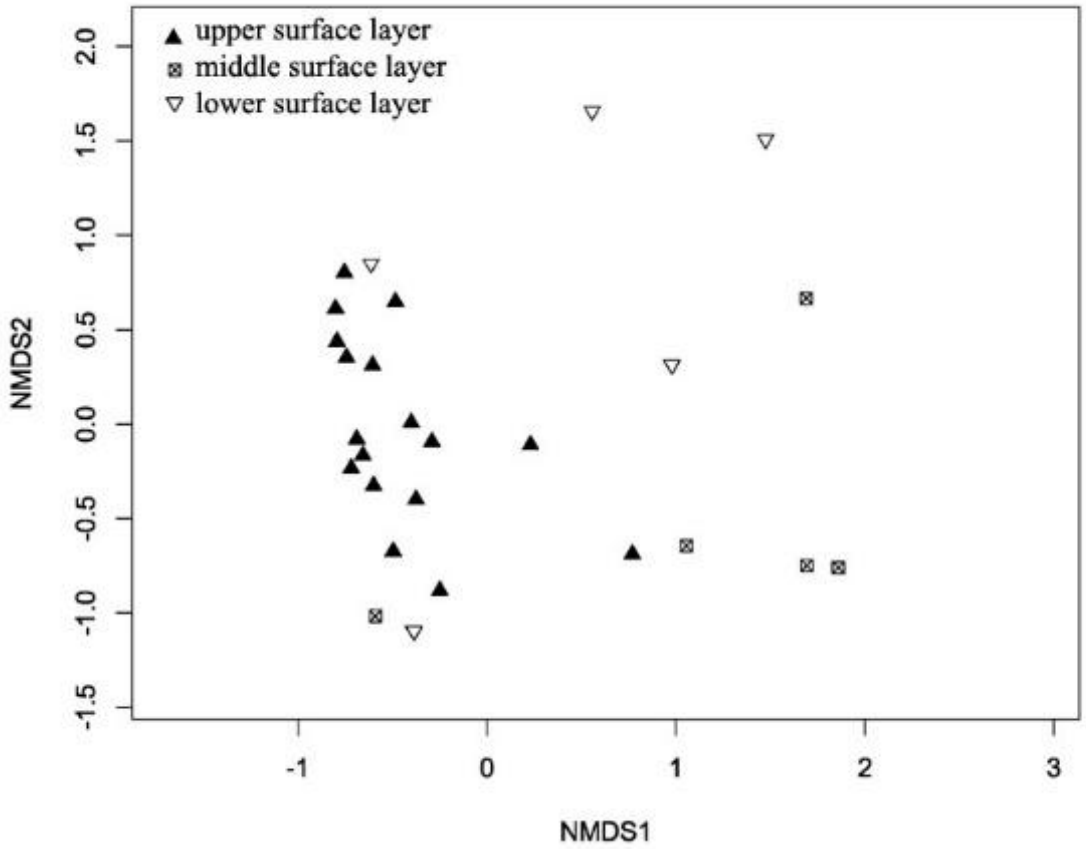

Fig. S3. Non-metric Multidimensional Scaling analysis for the composition of diazotrophs. The analysis was based on the composition of the diazotrophs with 96% *nifH* protein similarity cutoff in each sample.

Reference

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