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The response of diazotrophs to nutrient amendment in the South China Sea and western North Pacific

Zuozhu Wen et al.

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Table S1. Quantitative PCR primers and probes used in this study.

Species/clone	Forward primer	Probe	Reverse primer	Standard clone	References
Trichodesmium	GACGAAGTATTGAAGCCAGGTTTC	CATTAAGTGTGTTGAATCTGGTGGTCCTGAGC	CGGCCAGCGCAACCTA	AY528677	Church et al., 2005a
UCYN-A1	AGCTATAACAACGTTTTATGCGTTGA	TCTGGTGGTCCTGAGCCTGGA	ACCACGACCAGCACATCCA	AF059642	Church et al., 2005a
UCYN-A2/A3	GGTTACAACAACGTTTTATGTGTTGA	TCTGGTGGTCCTGAGCCCGGA	ACCACGACCAGCACATCCA	KF806604	Thompson et al., 2014
UCYN-B	TGGTCCTGAGCCTGGAGTTG	TGTGCTGGTCGTGGTAT	TCTTCTAGGAAGTTGATGGAGGTGAT	AF299418	Church et al., 2005a
het-1	CGGTTTCCGTGGTGTACGTT	TCCGGTGGTCCTGAGCCTGGTGT	AATACCACGACCCGCACAAC	AY706898	Church et al., 2005b
24774A11	CGGTAGAGGATCTTGAGCTTGAA	AAGTGCTTAAGGTTGGCTTTGGCGACA	CACCTGACTCCACGCACTTG	EU052413	Moisander et al., 2008

Table S2. Pearson correlation coefficients (r) between paired variables including surface temperature (SST) and salinity (SSS), surface chlorophyll *a* concentration (Chl *a*), surface N_2 fixation rate (SNF), depth-integrated N_2 fixation rate (INF) and primary production (IPP), and total *nifH* gene abundance. Asterisks denote a significant correlation ($p < 0.05$).

	SSS	**Chl *a***	**D_{Nitr}**	**SNF**	**INF**	**IPP**	***nifH***
SST	0.08	0.15	0.52	0.32	0.52	-0.67*	-0.05
SSS		-0.48	0.81*	-0.22	0.30	-0.47	0.75*
Chl *a*			-0.43	0.21	-0.10	0.21	-0.52
D_{Nitr}				0.15	0.62	-0.69*	0.74*
SNF					0.68*	-0.29	-0.01
INF						-0.32	0.72*
IPP							-0.04

Table S3. Upper 150 m depth-integrated *nifH* gene abundances of six targeted diazotrophs. nd, no data.

Station	Depth-integrated *nif*H gene abundance ($\times 10^9$ copies m^{-2})						
	Trichodesmium	**UCYN-A1**	**UCYN-A2/A3**	**UCYN-B**	**het-1**	**γ-24774A11**	**Total**
$SEATS_{2016}$	2.21	0.72	0.009	2.02	0.00	1.01	5.96
$SEATS_{2018}$	6.48	0.01	0.10	0.78	1.33	3.84	12.5
S1	3.43	0.06	0.005	3.25	0.16	0.18	7.08
S2	4.01	0.02	0.007	0.24	1.52	1.16	6.96
S3	35.4	0.05	0.005	0.16	0.08	0.99	36.7
S4	21.6	0.02	0.005	0.15	0.09	0.56	22.4
SK1	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
SK2	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
K1	10.2	17.3	1.09	12.0	0.23	3.14	44.0
WP	1.73	5.63	0.23	66.0	0.42	0.80	74.8

Table S4. Intracellular particulate Fe (PFe_{intra}), total particulate Fe (PFe_{total}) and particulate organic carbon (POC) concentrations, and particulate Fe to POC ratios in surface waters of the NSCS and the Kuroshio during 2018 cruise.

Station	**PFe_{intra} (nM)**	**PFe_{total} (nM)**	**POC (μM)**	**PFe_{intra}:POC (nmol Fe μmol C^{-1})**	**PFe_{total}:POC (nmol Fe μmol C^{-1})**
$SEATS_{2018}$	1.16	1.10	2.31	0.50	0.48
S2	0.34	0.86	2.28	0.15	0.38
S3	0.70	0.71	2.70	0.26	0.26
S4	0.45	1.00	3.03	0.15	0.33
SK2	1.34	2.40	2.46	0.54	0.98
K1	0.62	0.94	2.14	0.29	0.44

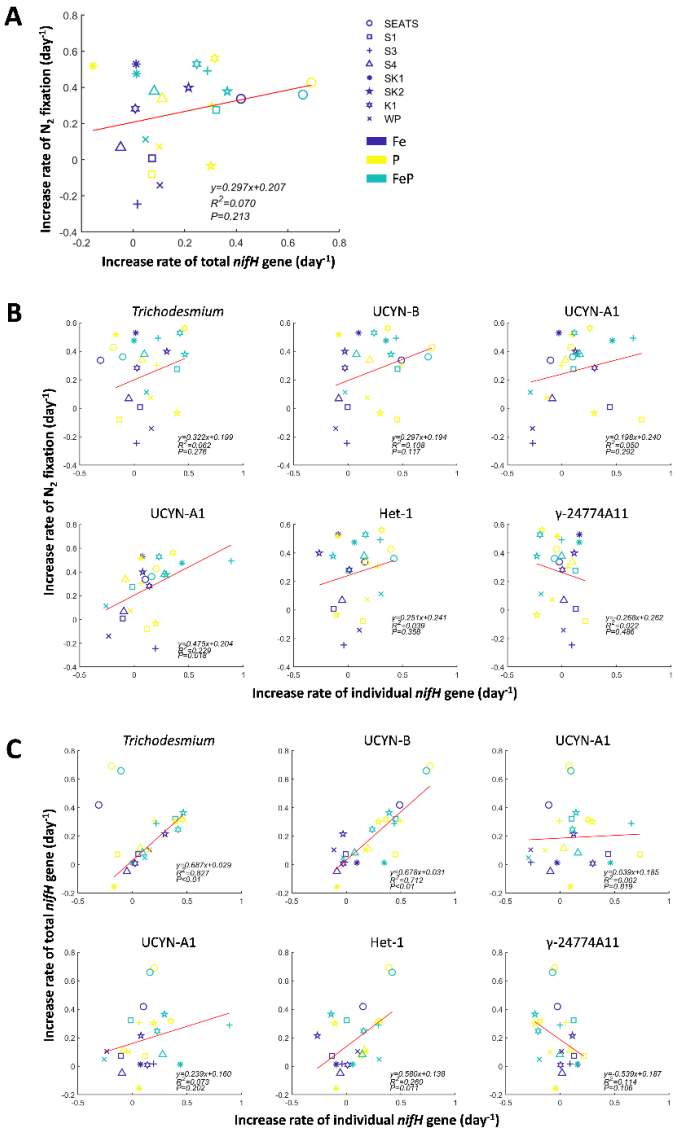

Figure S1. Correlations of response rates between (A) total *nifH* gene abundances and N_2 fixation rates, (B) individual diazotroph *nifH* gene abundances and N_2 fixation rates, and (C) individual diazotroph *nifH* gene abundances and total *nifH* gene abundances. The response rates were defined as the relatively changes of N_2 fixation rates or *nifH* abundances after nutrient addition compare to the control. The equation for the increase rate calculation is Ln(rate_ or *nifH*_treatment / rate_ or *nifH*_control)/incubation time, where time is in days.

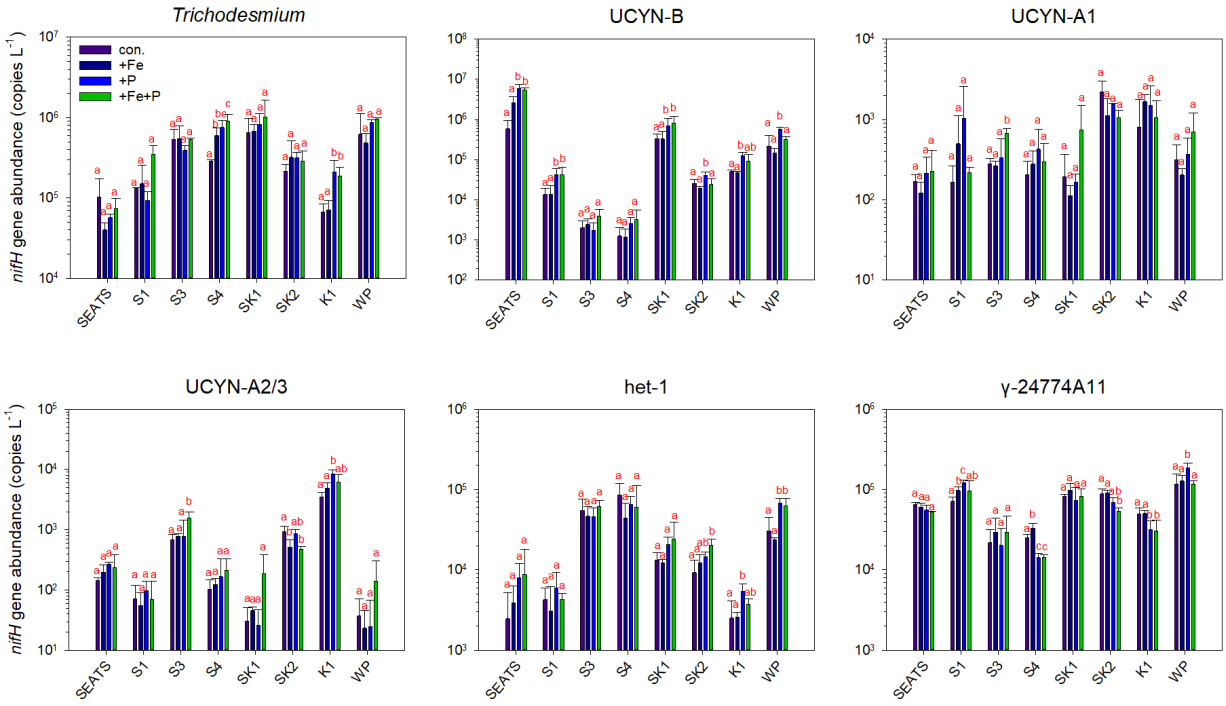

Figure S2. Responses of different diazotroph phylotypes to nutrient amendment. The data are mean + SD (n = 2 or 3). Different letters indicate statistically significant difference ($p < 0.05$) among treatments (ANOVA followed by Fisher PLSD test). Should be noted that those statistical results which were produced from only two replicates were not statistically valid (e.g., control and +Fe+P at station SEATS2016, control and +Fe+P at stations S1, +P and +Fe+P at station SK2).

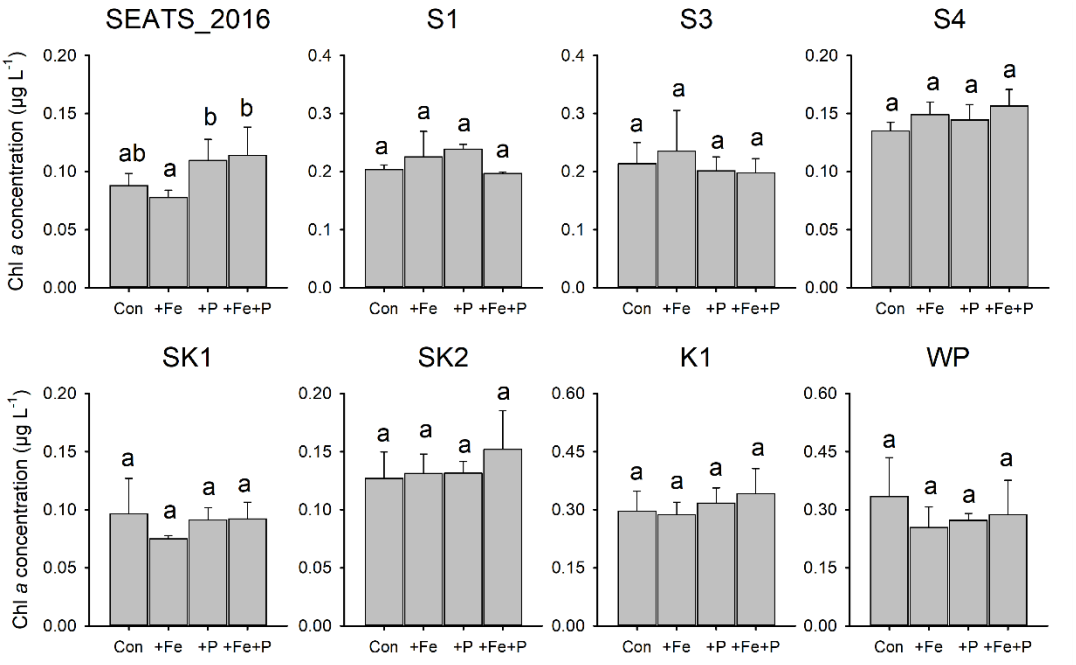

Figure S3. Response of Chl *a* concentration to nutrient amendment. Error bars represent the standard deviation of biological replicates (n = 2 or 3). Different letters above error bars indicate statistically significant differences ($p < 0.05$) between treatments (ANOVA followed by Fisher PLSD test). Note the caveat that statistical results were produced from only two replicates for control and +Fe+P at station $SEATS_{2016}$.

Parameters involved in nitrogen fixation rate calculation

Cruise	Date	Station	Long [degree East]	Lat [degree North]	Depth [m]	SST [°C]
NSCS_2016	2016/5/22	SEATS_2016	116.067	18.017	5	30.26
NSCS_2016	2016/5/22	SEATS_2016	116.067	18.017	15	30.25
NSCS_2016	2016/5/22	SEATS_2016	116.067	18.017	25	29.34
NSCS_2016	2016/5/22	SEATS_2016	116.067	18.017	50	23.31
NSCS_2016	2016/5/22	SEATS_2016	116.067	18.017	75	20.91
NSCS_2016	2016/5/22	SEATS_2016	116.067	18.017	100	19.14
NSCS_2016	2016/5/22	SEATS_2016	116.067	18.017	130	17.09
NSCS_2017	2017/7/23	S1	116.503	19.502	5	29.46
NSCS_2017	2017/7/23	S1	116.503	19.502	25	29.35
NSCS_2017	2017/7/23	S1	116.503	19.502	50	24.92
NSCS_2017	2017/7/23	S1	116.503	19.502	65	22.78
NSCS_2017	2017/7/23	S1	116.503	19.502	100	18.48
NSCS_2017	2017/7/13	SK1	120.413	21.03	5	30.45
NSCS_2017	2017/7/13	SK1	120.413	21.03	25	30.25
NSCS_2017	2017/7/13	SK1	120.413	21.03	50	29.17
NSCS_2017	2017/7/13	SK1	120.413	21.03	75	28.06
NSCS_2017	2017/7/13	SK1	120.413	21.03	100	25.17
NSCS_2017	2017/7/14	WP	123	22	5	30.86
NSCS_2017	2017/7/14	WP	123	22	25	30.7
NSCS_2017	2017/7/14	WP	123	22	50	29.8
NSCS_2017	2017/7/14	WP	123	22	75	27.32
NSCS_2017	2017/7/14	WP	123	22	100	26.37
NSCS_2018	2018/7/6	SEATS_2018	116.046	17.978	5	30.27
NSCS_2018	2018/7/6	SEATS_2018	116.046	17.978	25	29.34
NSCS_2018	2018/7/6	SEATS_2018	116.046	17.978	75	20.91
NSCS_2018	2018/7/6	SEATS_2018	116.046	17.978	88	20.05
NSCS_2018	2018/7/6	SEATS_2018	116.046	17.978	100	19.14
NSCS_2018	2018/7/6	SEATS_2018	116.046	17.978	150	16.34
NSCS_2018	2018/7/1	S2	117.94	19.57	5	29.38
NSCS_2018	2018/7/1	S2	117.94	19.57	25	29.38
NSCS_2018	2018/7/1	S2	117.94	19.57	50	27.26
NSCS_2018	2018/7/1	S2	117.94	19.57	95	20.69
NSCS_2018	2018/7/1	S2	117.94	19.57	100	20.13
NSCS_2018	2018/7/1	S2	117.94	19.57	150	16.02
NSCS_2018	2018/6/23	S3	119.34	20.86	5	28.65
NSCS_2018	2018/6/23	S3	119.34	20.86	25	27.44
NSCS_2018	2018/6/23	S3	119.34	20.86	50	25.73
NSCS_2018	2018/6/23	S3	119.34	20.86	75	21.97
NSCS_2018	2018/6/23	S3	119.34	20.86	100	19.87
NSCS_2018	2018/6/23	S3	119.34	20.86	150	17.66
NSCS_2018	2018/6/25	S4	120.05	21.54	5	29.47
NSCS_2018	2018/6/25	S4	120.05	21.54	25	29.22
NSCS_2018	2018/6/25	S4	120.05	21.54	50	27.93
NSCS_2018	2018/6/25	S4	120.05	21.54	62	26.87
NSCS_2018	2018/6/25	S4	120.05	21.54	100	23.04
NSCS_2018	2018/6/25	K1	122.5	22	5	29.1189
NSCS_2018	2018/6/25	K1	122.5	22	25	28.5271
NSCS_2018	2018/6/25	K1	122.5	22	50	26.3913
NSCS_2018	2018/6/25	K1	122.5	22	75	25.264
NSCS_2018	2018/6/25	K1	122.5	22	100	23.2653
NSCS_2018	2018/6/25	K1	122.5	22	150	22.36
NSCS_2018	2018/6/28	SK2	121.27	21.31	5	no data

SSS	Chla [µg L^{-1}]	PON_0 [µM]	15N atom% enrichment of PON_0	PON_{24h} concentration [µM]	15N atom% enrichment of PON_{24h}
33.46	0.26	no data	no data	0.38	0.3816805
33.46	0.26	no data	no data	0.37	0.3798570
33.62	0.30	no data	no data	0.33	0.3708272
34.36	0.45	no data	no data	0.31	0.3750863
34.56	0.61	no data	no data	0.64	0.3674203
34.65	0.31	no data	no data	0.17	0.3701849
34.64	no data	no data	no data	0.10	0.3741270
33.73	0.24	0.33	0.3668207	0.43	0.3767814
33.71	0.37	0.42	0.3669390	0.58	0.3686309
34.44	0.84	0.67	0.3674356	0.88	0.3683459
34.54	0.98	0.48	0.3669079	no data	no data
34.66	0.15	0.19	0.3680782	0.20	0.3677434
33.62	0.22	no data	no data	0.33	0.3943007
33.75	0.21	no data	no data	0.34	0.3799960
34.11	no data	no data	no data	0.57	0.3893380
34.32	no data	no data	no data	0.63	0.4776194
34.57	no data	no data	no data	0.66	0.3688654
34.47	0.11	0.23	0.36781801	0.37	0.382999743
34.41	0.11	0.23	0.36801961	0.24	0.440051932
34.53	0.13	0.25	0.36856696	0.28	0.438787892
34.89	0.17	0.18	0.36801961	0.21	0.467344503
34.96	0.29	0.21	0.37211872	0.24	0.373643014
33.46	0.11	0.25	0.36588618	0.27	0.376683576
33.62	0.13	0.29	0.36654193	0.29	0.376494591
34.56	0.65	0.47	0.36696012	0.42	0.367307142
34.61	0.60	0.28	0.36504468	0.30	0.366694829
34.65	0.51	0.37	0.36525123	0.25	0.366694099
34.62	0.07	0.11	0.36663827	0.10	0.368374111
33.75	0.10	0.25	0.36629562	0.26	0.385884928
33.78	0.12	0.25	0.36631459	0.32	0.380279581
33.98	0.27	0.32	0.36698311	0.36	0.367273154
34.67	0.53	0.22	0.36589932	0.30	0.366632742
34.7	0.35	0.19	0.36612192	0.22	0.367169886
34.61	0.05	0.13	0.36758702	0.10	0.367449038
33.53	0.15	0.41	0.36610039	0.56	0.371222007
33.73	0.17	0.41	0.36840695	0.29	0.385794335
34.05	0.78	0.50	0.36706959	0.48	0.3679406
34.58	0.79	0.47	0.37048536	0.66	0.371195082
34.77	0.26	0.21	0.36691706	0.21	0.366320972
34.72	0.07	0.09	0.36833251	0.09	0.366939499
33.74	0.17	0.56	0.36740275	0.43	0.371252697
33.74	0.13	nd	0.36662075	0.38	0.38311794
33.92	0.50	0.59	0.36729218	0.42	0.376236431
33.88	0.59	0.40	0.37216232	0.42	0.3759452
34.45	0.21	0.32	0.36821392	0.26	0.367758162
34.4468	0.11	0.21	0.36537165	0.28	0.368736205
34.5194	0.10	0.27	0.36547419	0.27	0.372847664
34.6763	0.12	0.28	0.36515927	0.29	0.373097594
34.7557	0.34	0.33	0.36576248	0.29	0.366441681
34.8755	0.07	0.29	0.36573036	0.27	0.365967681
34.902	0.20	0.14	0.3675166	0.14	0.366298654
no data	0.11	0.28	0.36665797	0.19	0.375381825

NFR [nmol N L^{-1} d^{-1}]	SD_{NFR} [nmol N L^{-1} d^{-1}]	NFR_LOD [nmol N L^{-1} d^{-1}]	PP [µmol C L^{-1} d^{-1}]	SD_{PP} [µmol C L^{-1} d^{-1}]
1.1		0.23	0.59	0.00
2.0	0.04	0.23	0.48	0.02
0.5	0.34	0.20	0.25	0.02
0.3		0.14	0.38	0.05
0.2	0.03	0.39	0.01	0.00
0.2	0.05	0.10	0.01	0.00
0.3	0.17	0.06	0.00	0.00
0.8	0.00	0.54	0.37	0.03
0.8	0.05	0.71	0.48	0.01
0.6	0.32	0.99	0.69	0.04
no data	no data	no data	no data	no data
0.0	0.00	0.48	0.02	0.00
10.4	0.01	0.29	0.08	0.01
3.5	0.00	0.22	0.09	0.01
5.4	0.00	0.36	0.11	0.12
2.6	0.00	0.40	0.16	0.01
0.2	0.17	0.42	0.05	0.02
1.9	0.74	0.19	0.12	0.02
3.3	0.00	0.15	0.09	0.01
7.9	0.43	0.17	0.11	0.01
4.0	0.00	0.12	0.07	0.00
0.1	0.00	0.14	0.06	0.02
1.8	0.11	0.23	0.30	0.01
2.1	0.40	0.26	0.29	0.01
0.3	0.25	0.40	0.14	0.02
0.3	0.05	0.26	0.11	0.02
0.3	0.02	0.28	0.10	0.02
0.1	0.02	0.10	0.01	0.00
3.0	0.01	0.23	0.22	0.01
2.1	0.33	0.25	0.26	0.01
0.0	0.02	0.30	0.27	0.04
0.2	0.06	0.23	0.11	0.00
0.1	0.01	0.19	0.07	0.00
0.0	0.04	0.10	0.01	0.00
2.4	1.20	0.69	0.61	0.09
5.9	1.23	0.50	0.39	0.09
0.5	0.67	0.69	1.04	0.01
0.0	0.00	0.76	1.71	0.10
0.0	0.02	0.30	0.28	0.02
0.0	0.00	0.13	0.03	0.00
1.8	0.31	0.70	0.41	0.03
6.1	0.38	0.54	0.80	0.11
4.4	1.50	0.72	0.85	0.11
0.0	0.00	0.58	0.66	0.01
0.0	0.00	0.41	0.11	0.04
0.8	0.21	0.34	0.20	0.01
1.9	0.13	0.38	0.18	0.00
2.2	0.23	0.40	0.21	0.00
0.3	0.48	0.44	0.22	0.01
0.1	0.06	0.39	0.09	0.00
0.0	0.00	0.20	0.02	0.00
2.0	0.00	0.34	0.26	0.00

Trichodesmium [copies L^{-1}]	UCYN-B [copies L^{-1}]	UCYN-A1 [copies L^{-1}]	UCYN-A2/A3 [copies L^{-1}]	het-1 [copies L^{-1}]	γ-24774A11 [copies L^{-1}]
5.57E+04	4.16E+04	6.34E+02	8.93E+02	0.00E+00	1.26E+04
6.60E+04	5.42E+04	0.00E+00	0.00E+00	0.00E+00	3.45E+04
5.68E+04	6.04E+04	8.71E+03	0.00E+00	0.00E+00	0.00E+00
0.00E+00	0.00E+00	2.23E+04	0.00E+00	0.00E+00	1.75E+03
0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	1.57E+04
0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	8.25E+03
0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	9.08E+03
1.97E+05	2.11E+05	2.94E+02	9.83E+01	1.15E+03	5.63E+03
1.98E+04	1.13E+03	4.87E+02	2.64E+01	4.83E+02	2.23E+03
9.48E+02	4.61E+02	4.91E+02	3.70E+01	5.75E+03	7.96E+02
2.31E+02	1.09E+03	5.54E+02	3.65E+01	3.23E+02	4.12E+02
2.97E+02	1.23E+03	1.17E+03	6.50E+01	4.48E+02	1.00E+03
no data	no data	no data	no data	no data	no data
no data	no data	no data	no data	no data	no data
no data	no data	no data	no data	no data	no data
no data	no data	no data	no data	no data	no data
no data	no data	no data	no data	no data	no data
1.02E+03	8.67E+05	7.80E+02	3.93E+01	9.00E+01	6.70E+03
1.19E+03	9.52E+05	3.97E+03	1.09E+03	2.76E+02	3.73E+03
4.30E+03	7.99E+05	2.62E+04	5.38E+02	1.17E+04	9.91E+03
3.28E+03	4.54E+05	1.58E+05	6.87E+03	4.67E+03	1.07E+04
1.20E+05	2.17E+04	7.52E+04	1.73E+03	3.69E+02	7.61E+03
1.76E+05	5.77E+03	1.51E+02	4.53E+02	1.80E+04	7.17E+04
1.09E+05	1.81E+04	1.67E+02	2.75E+03	2.96E+04	7.79E+04
7.46E+02	1.58E+03	8.94E+01	1.48E+01	5.76E+02	8.01E+02
2.20E+02	5.10E+02	1.68E+01	0.00E+00	1.54E-01	6.66E+02
0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
no data	no data	no data	no data	no data	no data
1.78E+05	8.25E+03	7.71E+02	2.79E+02	7.61E+04	3.79E+04
5.73E+04	3.73E+03	1.40E+02	3.71E+01	1.62E+04	1.97E+04
1.02E+03	6.28E+02	3.91E+01	3.62E+01	2.93E+02	3.98E+03
5.16E+02	3.59E+02	2.08E+02	3.35E+01	2.93E+02	3.07E+02
0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
no data	no data	no data	no data	no data	no data
8.00E+05	7.30E+02	4.24E+01	6.43E+01	1.65E+03	1.28E+04
9.07E+05	4.39E+03	5.02E+02	1.26E+02	1.29E+03	1.56E+04
9.54E+04	1.14E+03	7.85E+02	1.57E+01	6.34E+02	5.54E+03
2.63E+04	9.42E+02	8.44E+02	3.42E+01	2.70E+02	1.23E+04
0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
no data	no data	no data	no data	no data	no data
4.22E+05	2.30E+02	5.67E+01	2.96E+01	1.09E+03	1.17E+04
4.95E+05	4.45E+03	4.87E+01	1.34E+02	2.45E+03	1.11E+04
2.01E+05	1.81E+03	2.63E+02	5.39E+01	8.28E+02	4.05E+03
1.53E+04	5.33E+02	6.09E+02	2.24E+00	1.38E+02	2.53E+03
0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
1.29E+05	6.55E+04	2.30E+03	1.55E+03	2.44E+03	4.39E+04
2.23E+05	2.64E+05	8.55E+04	1.46E+04	4.53E+03	5.61E+04
1.18E+05	1.94E+05	6.09E+05	2.95E+04	2.04E+03	4.51E+04
1.18E+04	5.99E+03	5.27E+03	1.06E+02	6.17E+02	3.18E+03
8.93E+02	2.77E+03	2.39E+03	6.83E+01	1.58E+03	6.86E+02
2.11E+02	2.76E+03	8.90E+02	7.58E+01	8.16E+02	3.84E+02
1.37E+05	2.24E+04	5.72E+02	2.81E+02	1.69E+04	1.96E+04

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