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Grazing mortality as a controlling factor in the uncultured non-cyanobacterial diazotroph (Gamma A) around the Kuroshio region

Takuya Sato et al.

Correspondence to: Takuya Sato (takusato@scl.kyoto-u.ac.jp)

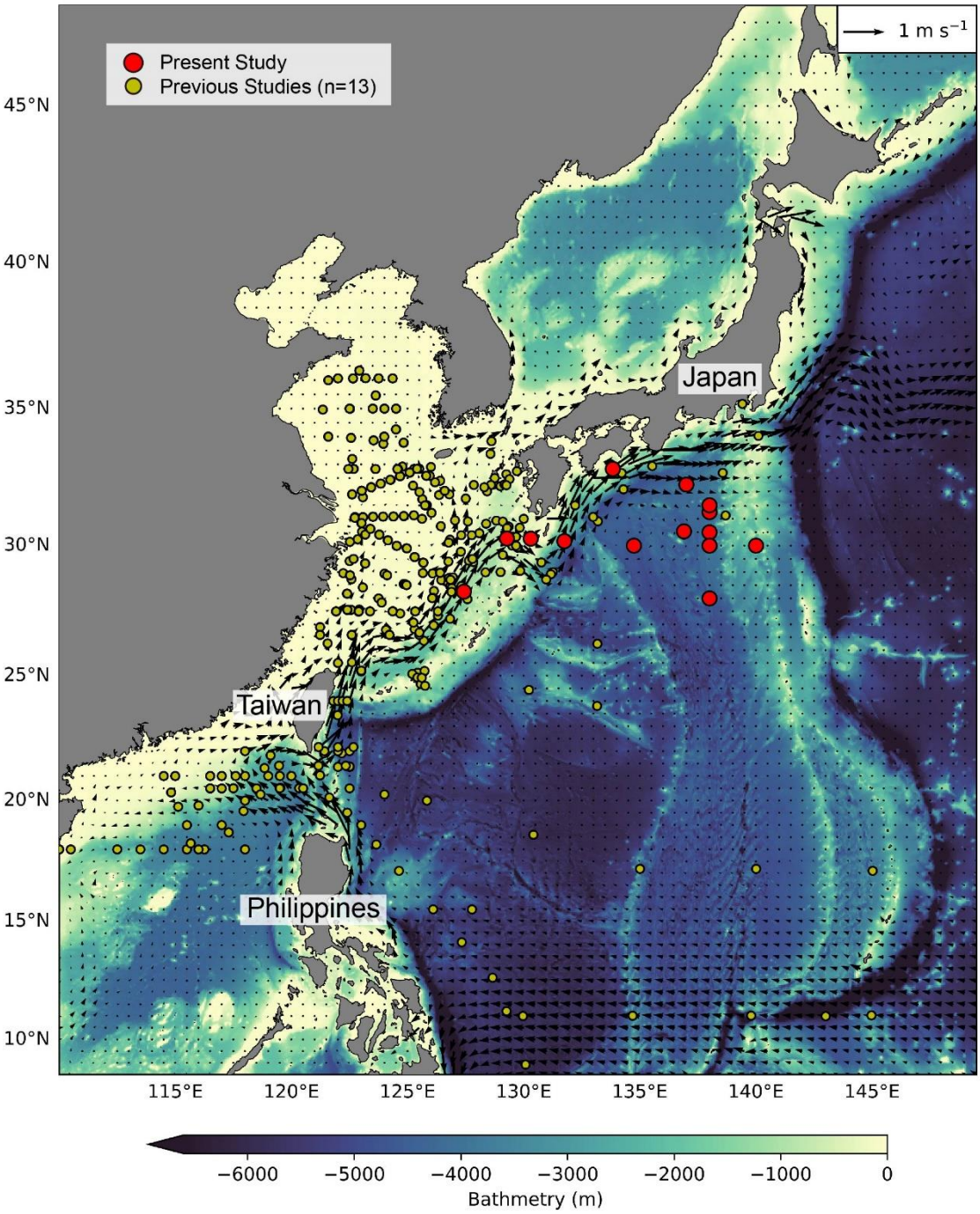

Figure S1. Sampling stations of this study and 13 previous studies that examined diazotroph abundance with microscopic or *nifH* qPCR analysis around the Kuroshio region. Previous studies around the Kuroshio region (Chang et al., 2000; Chen et al., 2009; Chen et al., 2019; Chen et al., 2011; Jiang et al., 2018; Jiang et al., 2023; Liu et al., 2020; Marumo and Nagasawa, 1976; Shiozaki et al., 2014b; Shiozaki et al., 2015b; Shiozaki et al., 2018; Wen et al., 2022a; Wu et al., 2018) were derived from global oceanic diazotroph database version 2 (Shao et al., 2023) with one additional reference (Cheung et al., 2019). Arrows represent climatological means of current directions and velocities. The bottom depth (m) is indicated as coloured contours.

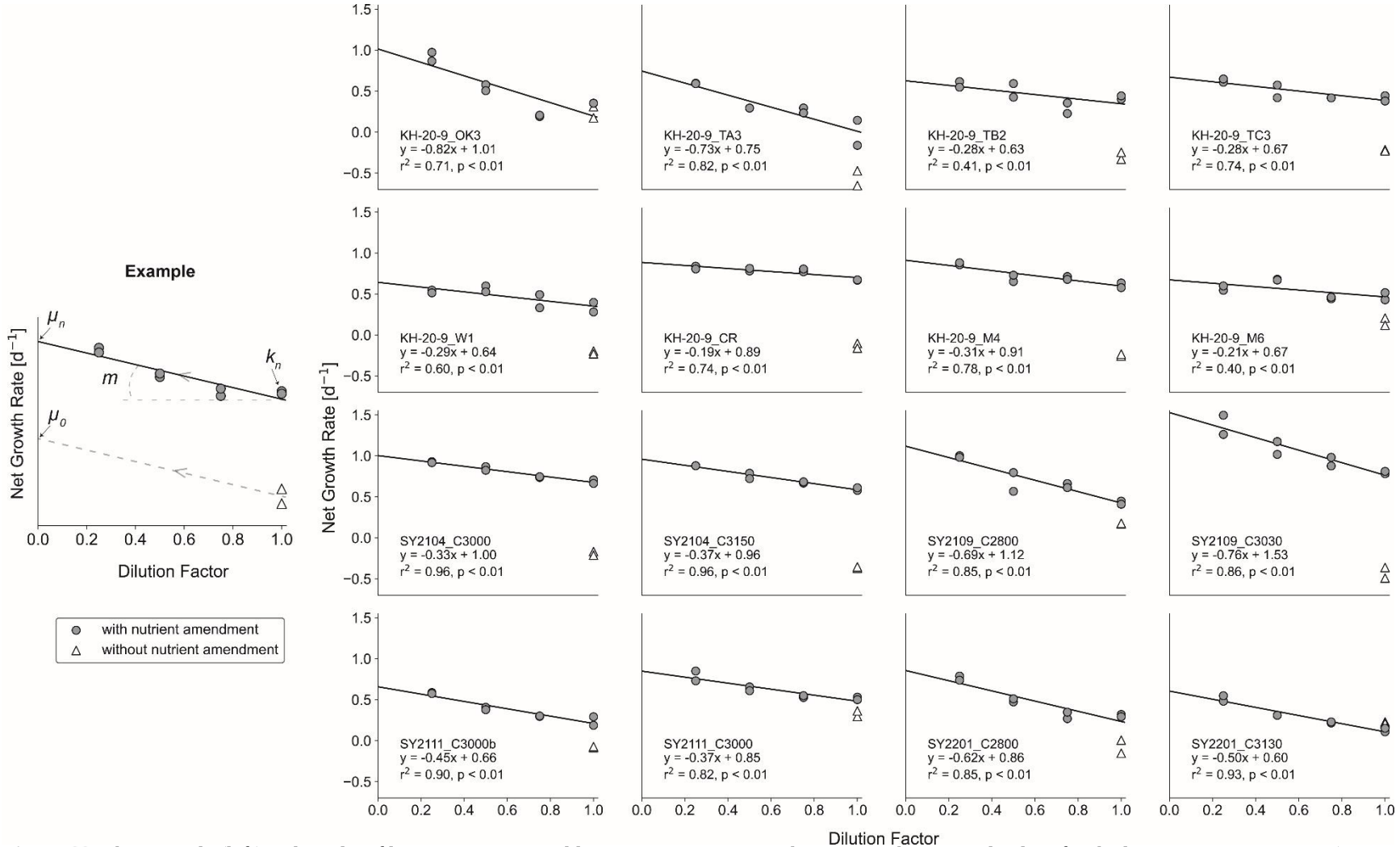

Figure S2. The example (left) and results of linear regression in dilution experiments at each station with statistical values for the least-square regression. (μ_n: nutrient-amended instantaneous rate. m: mortality rate by microzooplankton grazing. μ_0: instantaneous rate without nutrient-amendment. k_n: nutrient-amended net growth rates)

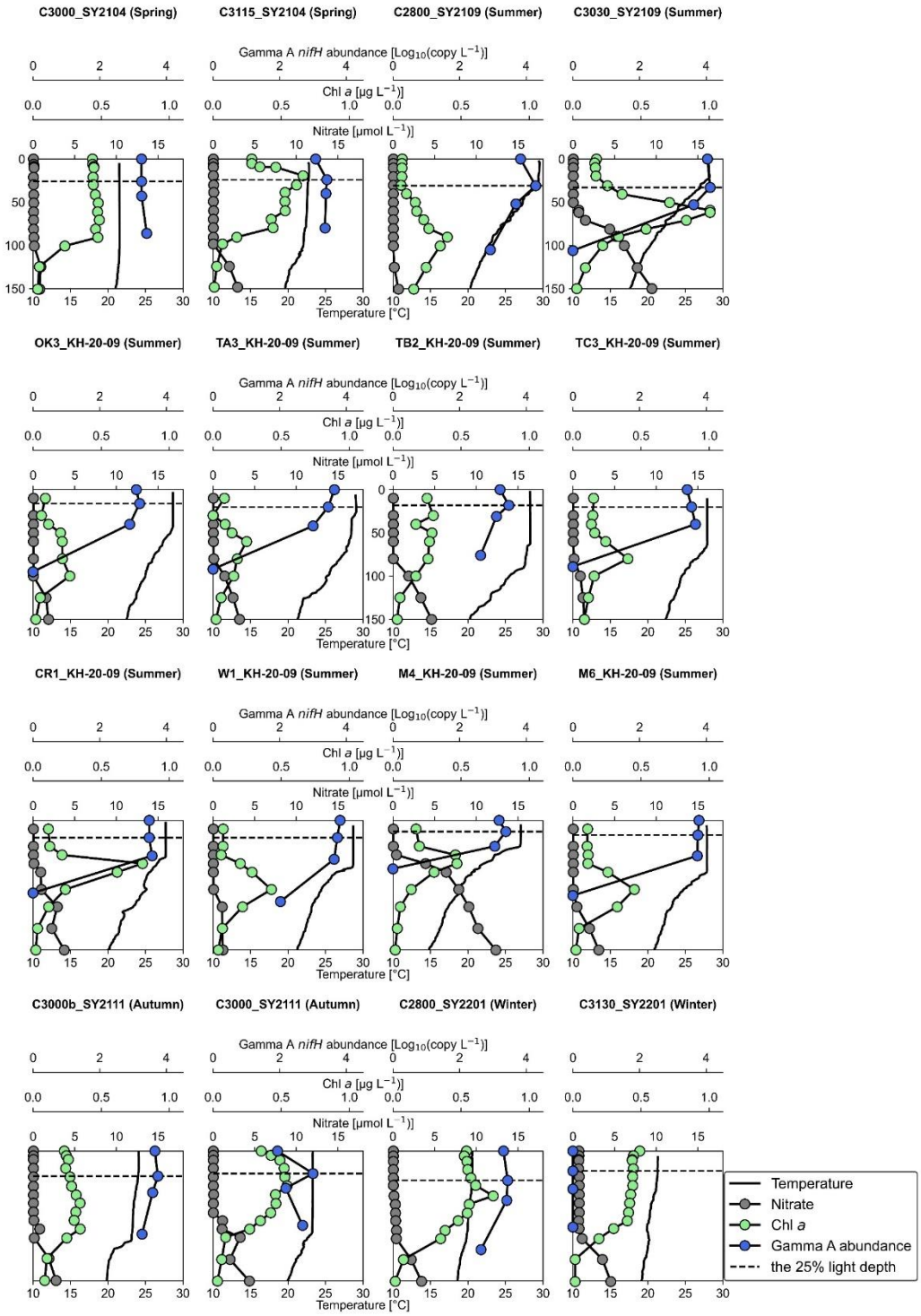

Figure S3. Vertical profiles of temperature, nitrate concentration, Chl *a* concentration, and Gamma A *nifH* abundance in the Kuroshio region during each cruise. The black dashed horizontal lines indicate the 25 % light depth in which dilution experiments were performed.

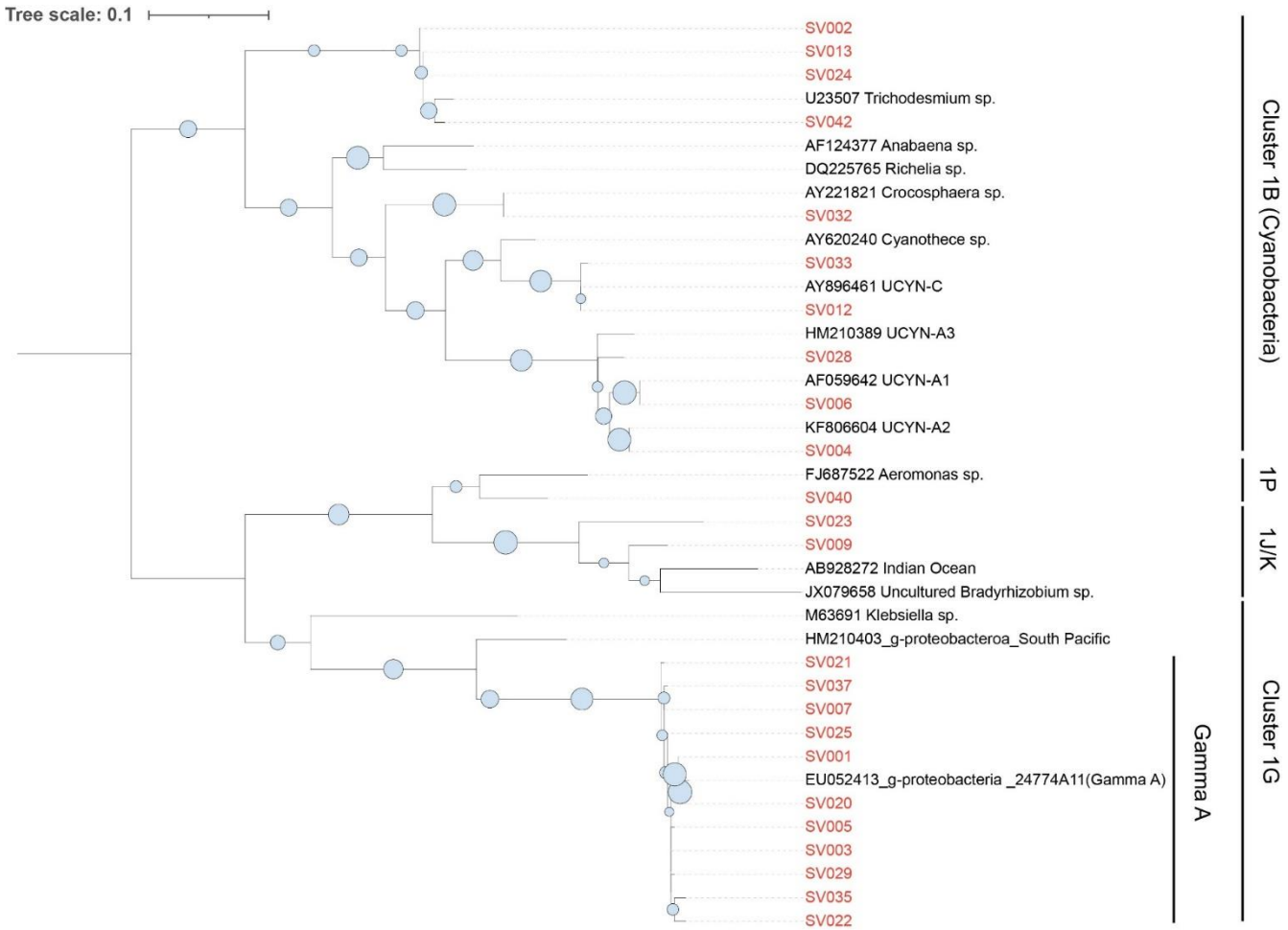

Figure S4. Maximum likelihood phylogenetic tree of *nifH* gene sequences. The 26 representative amplicon sequence variants (SVs) in this study are shown in red. Canonical *nifH* clusters according to Zehr et al. (2003) are displayed at the left of each clade with the cluster names. The areas of the blue circles are proportional to the Bootstrap value (> 50%) determined from 1000 iterations. The tree was produced using the Interactive Tree of Life (http://itol.embl.de/; Letunic & Bork, 2019).

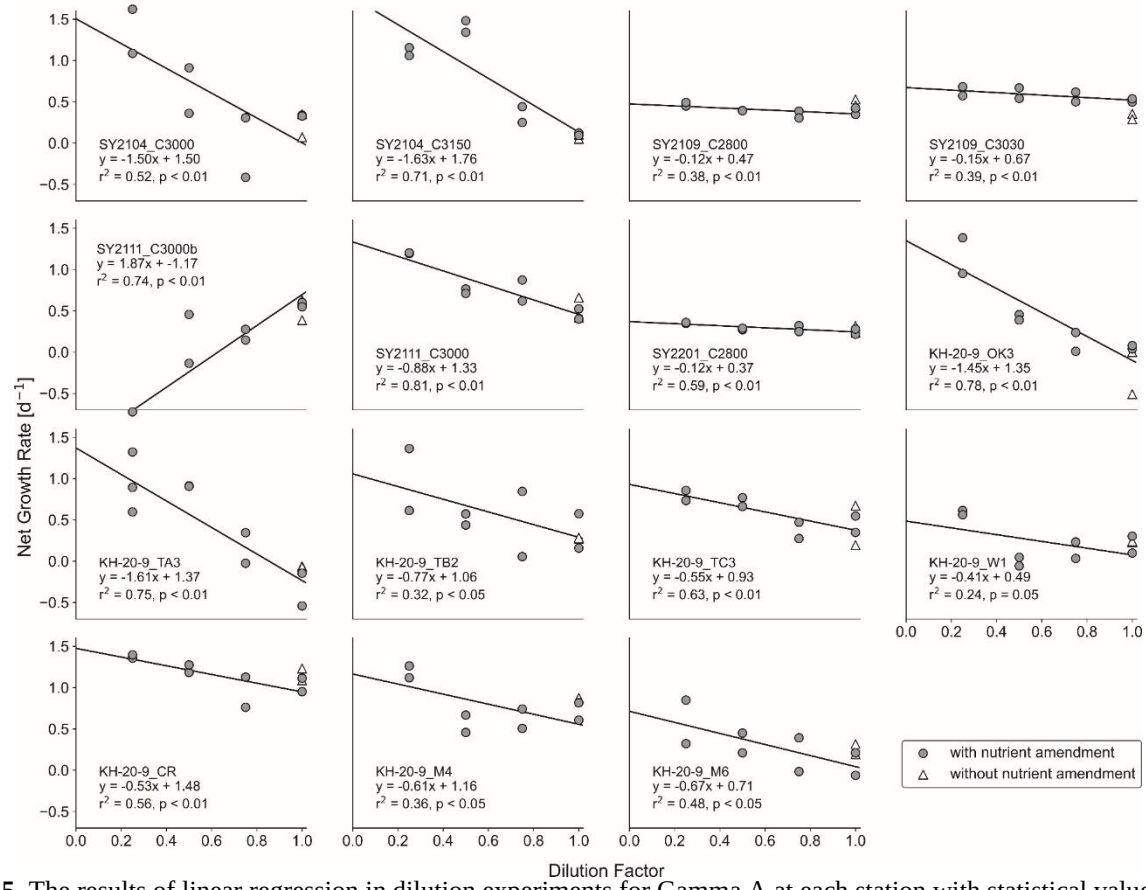

Figure S5. The results of linear regression in dilution experiments for Gamma A at each station with statistical values for the least-square regression.

Figure S6. The paired scattered plots of Gamma A, each water property, and the parameters derived from the dilution experiments for Gamma A.

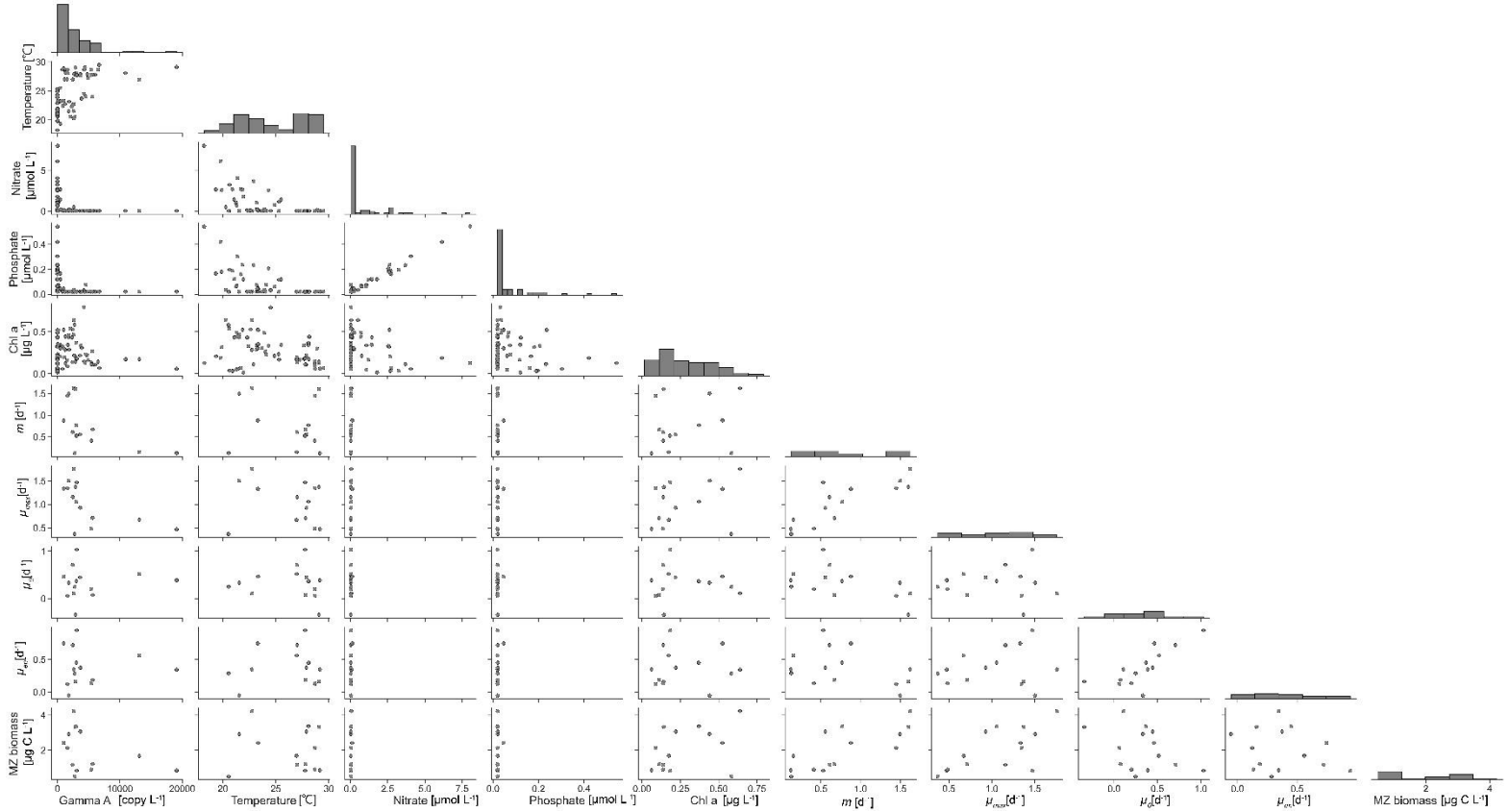

Table S1 Summary of parameters derived from the dilution experiments (μ_n: nutrient-amended instantaneous rate. m: mortality rate by microzooplankton grazing. μ_0: instantaneous rate without nutrient-amendment. k_n: nutrient-amended net growth rates) for the whole phytoplankton community and Gamma A.

Cruise	Station	The whole phytoplankton community				Gamma A			
		μ_n (d^{-1})	m (d^{-1})	μ_0 (d^{-1})	k_n (d^{-1})	μ_n (d^{-1})	m (d^{-1})	μ_0 (d^{-1})	k_n (d^{-1})
SY2104	C3000	1.00	0.33	0.14	0.68	1.50	1.50	1.83	–0.05
SY2104	C3115	0.96	0.37	0.01	0.59	1.76	1.63	1.73	0.35
SY2109	C2800	1.12	0.69	0.86	0.43	0.47	0.12	0.65	0.35
SY2109	C3030	1.53	0.76	0.33	0.76	0.67	0.15	0.67	0.56
KH-20-9	OK3	1.01	0.82	1.06	0.20	1.35	1.45	1.51	0.12
KH-20-9	TA3	0.75	0.73	0.17	0.01	1.37	1.61	1.26	0.16
KH-20-9	TB2	0.63	0.28	-0.01	0.35	1.06	0.77	1.13	0.45
KH-20-9	TC3	0.67	0.28	0.06	0.39	0.93	0.55	1.00	0.37
KH-20-9	W1	0.64	0.29	0.08	0.35	0.49	0.41	0.76	0.13
KH-20-9	CR1	0.89	0.19	0.06	0.70	1.48	0.53	1.56	0.94
KH-20-9	M4	0.91	0.31	0.55	0.60	1.16	0.61	1.24	0.62
KH-20-9	M6	0.67	0.21	0.48	0.46	0.71	0.67	0.74	0.19
SY2111	C3000b	0.66	0.45	0.37	0.21	–[a]	–[a]	–[a]	0.21
SY2111	C3000	0.85	0.37	0.7	0.48	1.33	0.88	1.34	0.75
SY2201	C2800	0.86	0.62	0.55	0.23	0.37	0.12	0.37	0.29
SY2201	C3130	0.60	0.50	0.72	0.11	No Data[b]	No Data[b]	No Data[b]	No Data[b]

[a] Since the slope of regression line was positive, the grazing mortality rate was not determined.

[b] Since the Gamma A *nifH* was undetectable at the beginning of the incubation, rates could not be determined.

Table S2 Summary of previous reports of apparent growth rate with nutrient amendment (μ_{en}) of Gamma A based on *nifH* qPCR. N: nitrogen, Fe: iron, P: phosphate, DOC: dissolved organic carbon

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μ_{en} (d^{-1})	Location	Environmental Perturbation(s)	Reference
up to 0.52	South Pacific Ocean	N, P, Fe, DOC, Fe/P, N/P, N/Fe, N/P/Fe/DOC	Moisander et al., (2012)
–0.91–1.07	Southwest Pacific Ocean	P	Turk-Kubo et al., (2015)
–0.05–0.94	Kuroshio region	Fe/P/ N	This study