water

Supplementary information

Linking Stoichiometric Organic Carbon–Nitrogen Relationships to planktonic Cyanobacteria and Subsurface Methane Maximum in Deep Freshwater Lakes

Santona Khatun [1,*], Tomoya Iwata [2,*], Hisaya Kojima [3], Yoshiki Ikarashi [2], Kana Yamanami [2], Daichi Imazawa [1], Tanaka Kenta [4], Ryuichiro Shinohara [5] and Hiromi Saito [6]

[1] Integrated Graduate School of Medicine, Engineering, and Agricultural Sciences, University of Yamanashi, 4-4-37 Takeda, Kofu 400-8510, Japan; g17lr002@yamanashi.ac.jp
[2] Faculty of Life and Environmental Sciences, University of Yamanashi, 4-4-37 Takeda, Kofu 400-8510, Japan; yoshiki.ikarashi@gmail.com (Y.I.); L14EV033@yamanashi.ac.jp (K.Y.)
[3] Institute of Low Temperature Science, Hokkaido University, Kita-19, Nishi-8, Kita-ku, Sapporo 060-0819, Japan; kojimah@pop.lowtem.hokudai.ac.jp
[4] Sugadaira Research Station, Mountain Science Center, University of Tsukuba, 1278-294 Sugadaira-kogen, Ueda 386-2204, Japan; kenta@sugadaira.tsukuba.ac.jp
[5] National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba 305-8506, Japan; r-shino@nies.go.jp
[6] Department of Marine Biology and Sciences, Tokai University, 1-1 1-Chome 5-Jo Minami-sawa, Minami-ku, Sapporo, 005-8601, Japan; saitou@tsc.u-tokai.ac.jp
* Correspondence: santona.khatun@gmail.com (S.K.); tiwata@yamanashi.ac.jp (T.I.)

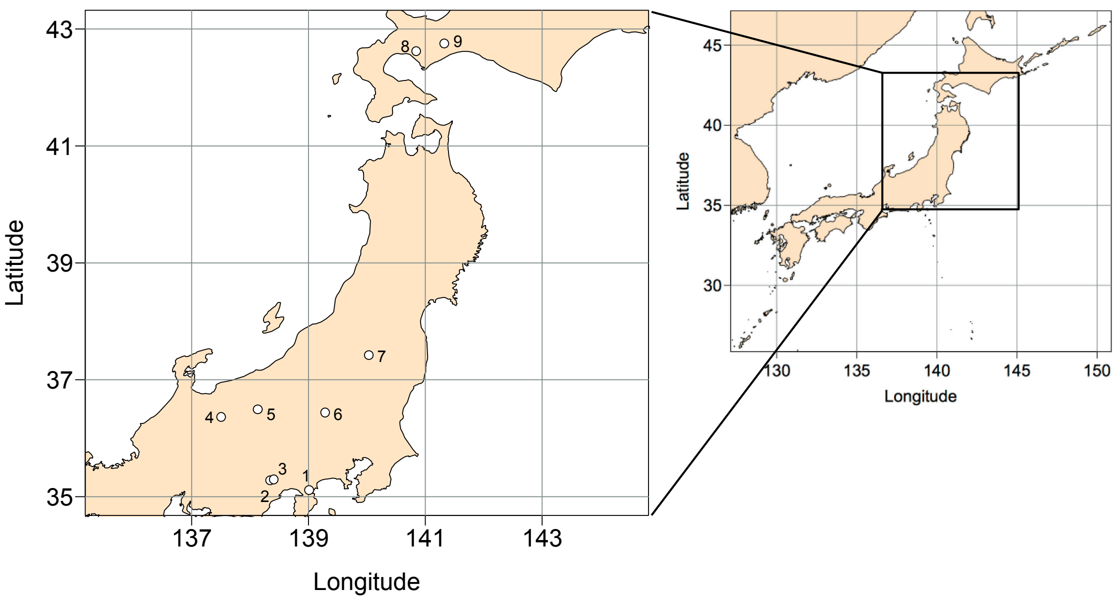

Figure S1. Geographic location of nine study lakes in Japan. (1) Lake Ashinoko, (2) Lake Motosu, (3) Lake Saiko, (4) Lake Aoki, (5) Lake Nojiri, (6) Lake Chuzenji, (7) Lake Hibara, (8) Lake Toya, (9) Lake Shikotsu.

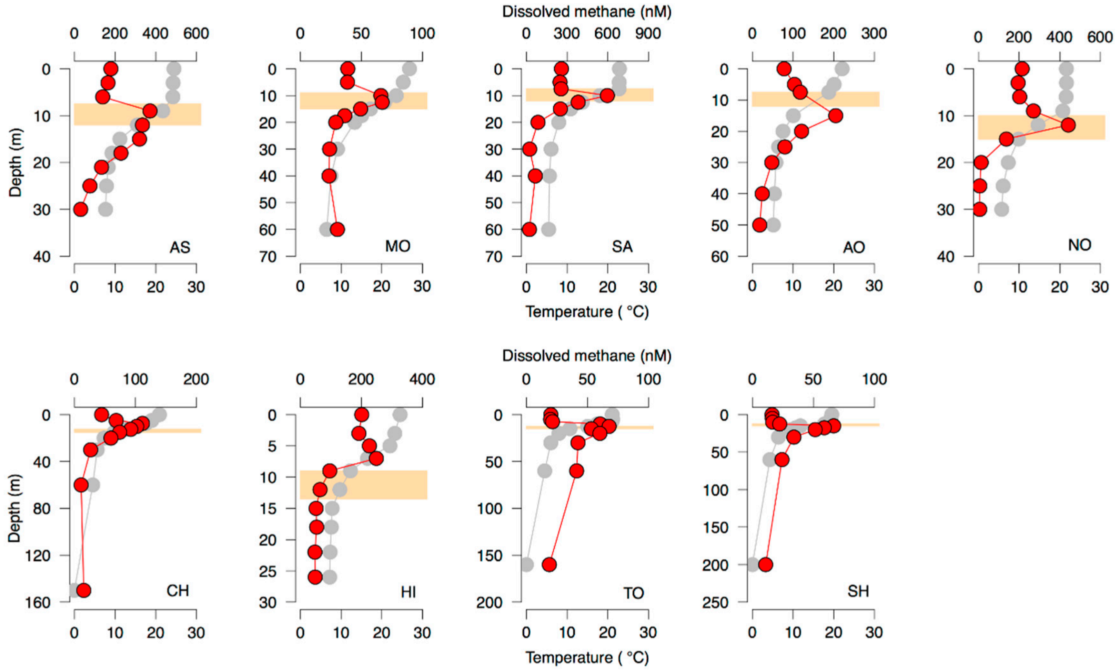

Figure S2. Vertical profiles of dissolved CH_4 concentration (nM, red circles) and water temperature (°C, gray circles) in nine study lakes during the period from July to September in 2016–2017. AS: Lake Ashinoko, MO: Lake Motosu, SA: Lake Saiko, AO: Lake Aoki, NO: Lake Nojiri, CH: Lake Chuzenji, HI: Lake Hibara, TO: Lake Toya, SH: Lake Shikotsu. Shaded areas denote the range of the thermocline.

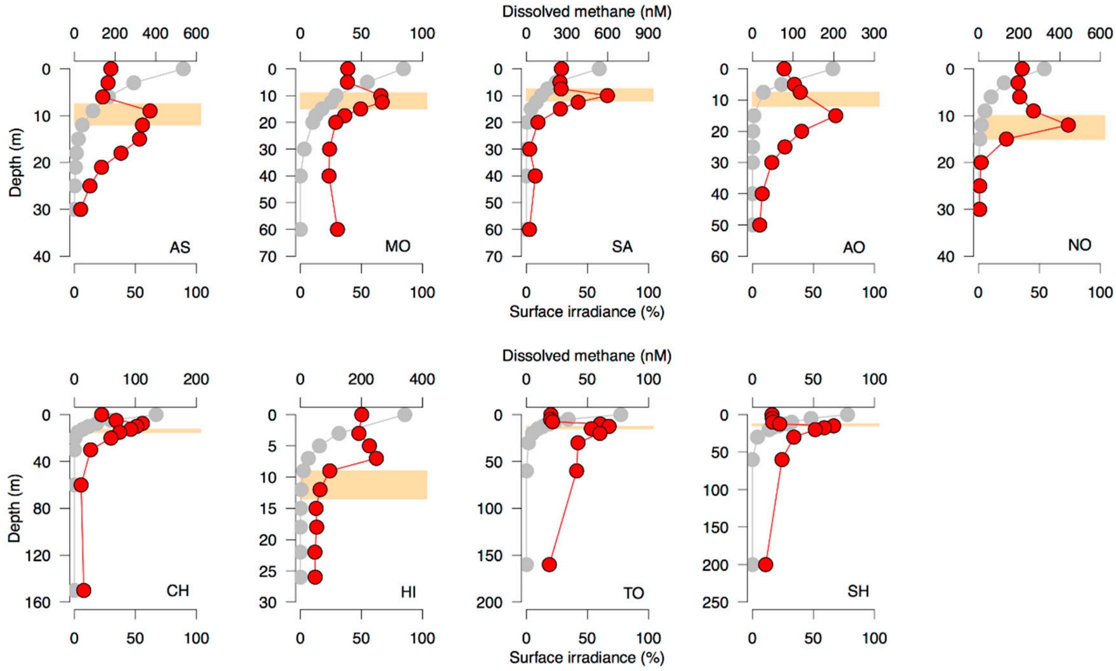

Figure S3. Vertical profiles of dissolved CH_4 concentration (nM, red circles) and the percentage of surface irradiance (%, gray circles) in nine study lakes during the period from July to September in 2016–2017. See legend of Figure S2 for the abbreviation of lake names and the descriptions of shaded areas.

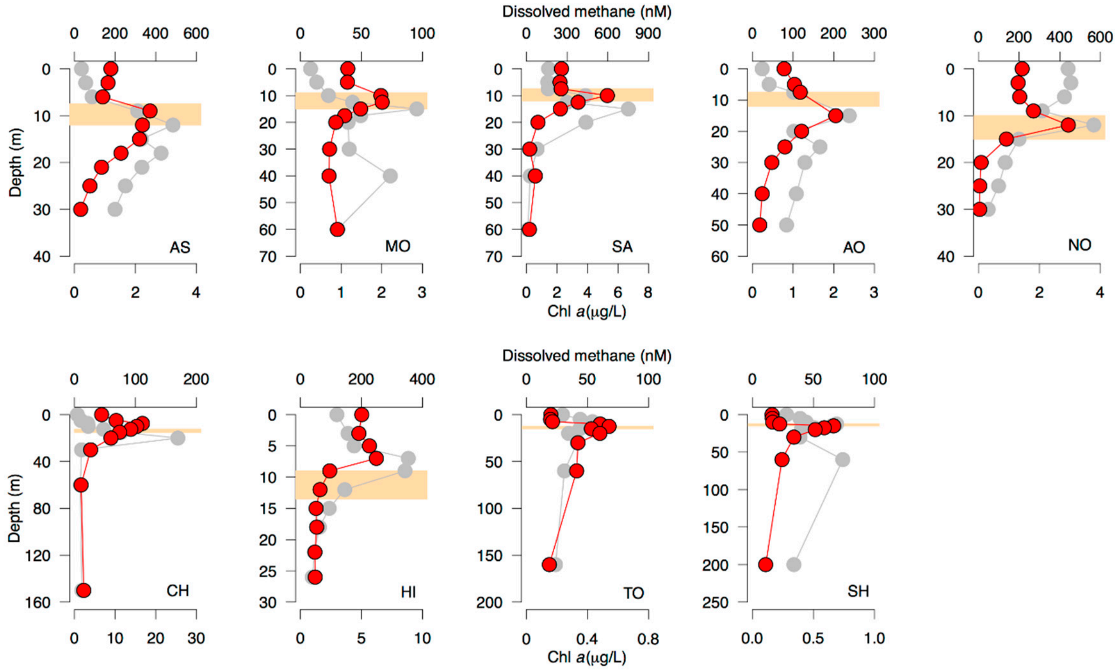

Figure S4. Vertical profiles of dissolved CH_4 (nM, red circles) and chlorophyll *a* (µg/L, gray circles) concentrations in nine study lakes during the period from July to September in 2016–2017. See legend of Figure S2 for the abbreviation of lake names and the descriptions of shaded areas.

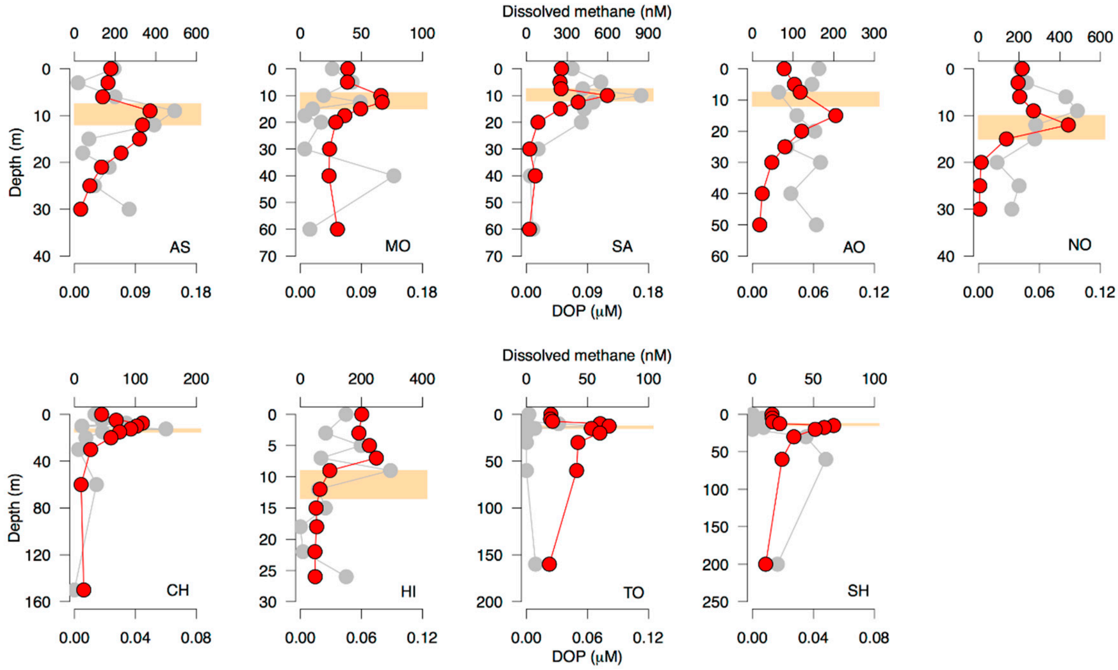

Figure S5. Vertical profiles of dissolved CH_4 (nM, red circles) and DOP (μM, gray circles) concentrations in nine study lakes during the period from July to September in 2016–2017. See legend of Figure S2 for the abbreviation of lake names and the descriptions of shaded areas.

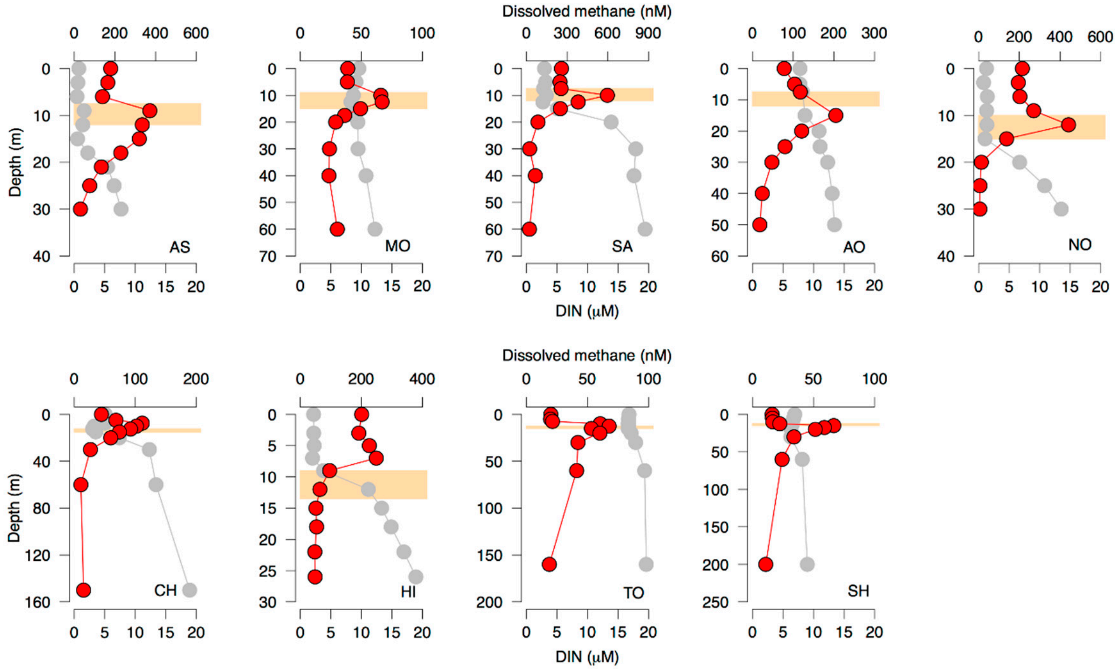

Figure S6. Vertical profiles of dissolved CH_4 (nM, red circles) and DIN (µM, gray circles) concentrations in nine study lakes during the period from July to September in 2016–2017. See legend of Figure S2 for the abbreviation of lake names and the descriptions of shaded areas.

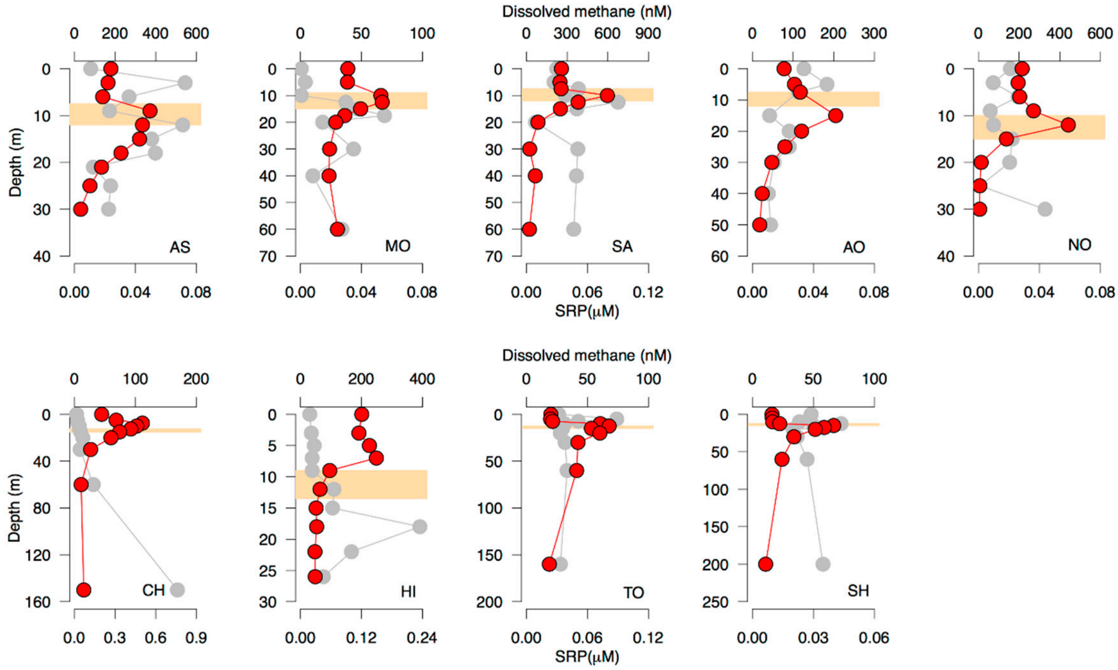

Figure S7. Vertical profiles of dissolved CH_4 (nM, red circles) and SRP (µM, gray circles) concentrations in nine study lakes during the period from July to September in 2016–2017. See legend of Figure S2 for the abbreviation of lake names and the descriptions of shaded areas.

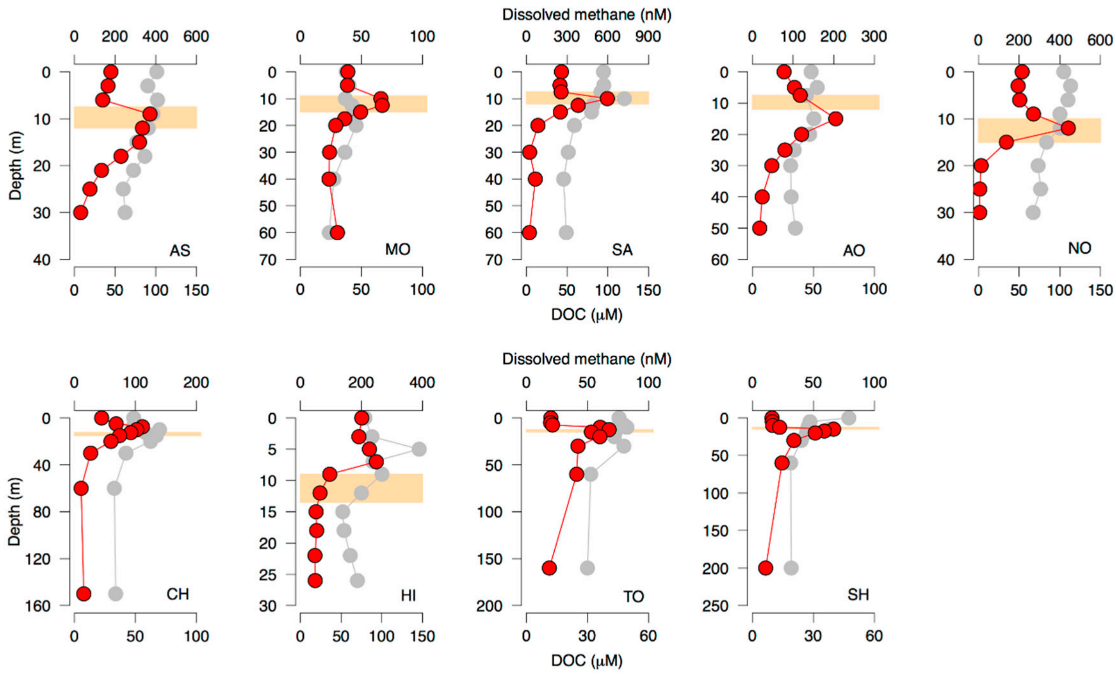

Figure S8. Vertical profiles of dissolved CH_4 (nM, red circles) and DOC (µM, gray circles) concentrations in nine study lakes during the period from July to September in 2016–2017. See legend of Figure S2 for the abbreviation of lake names and the descriptions of shaded areas.

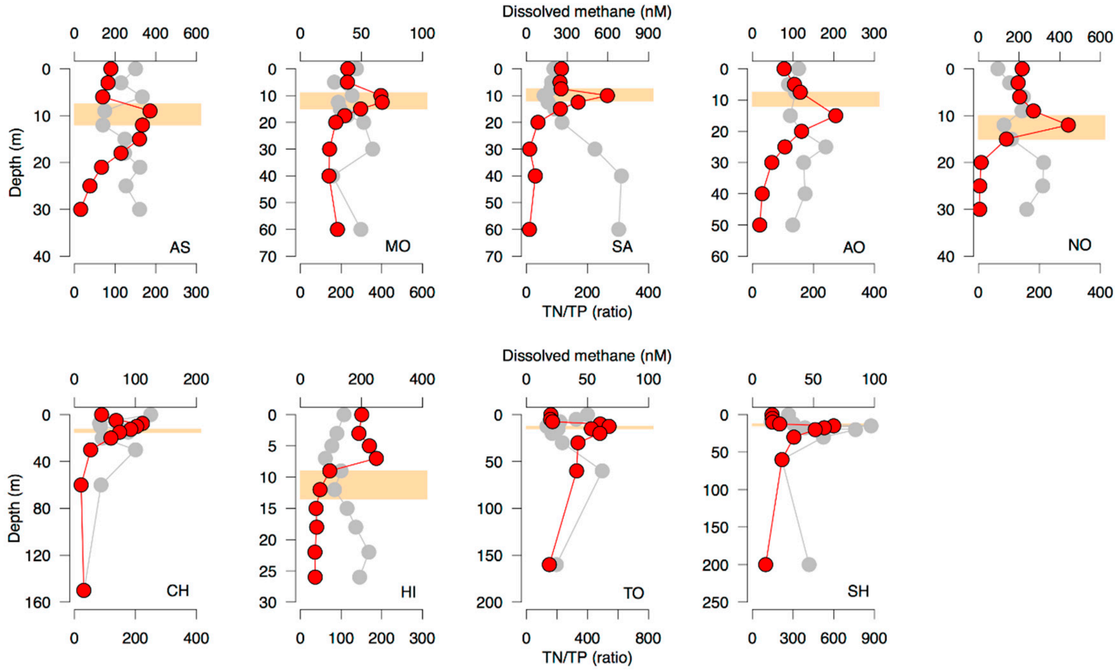

Figure S9. Vertical profiles of dissolved CH_4 concentration (nM, red circles) and TN/TP (ratio, gray circles) concentrations in nine study lakes during the period from July to September in 2016–2017. See legend of Figure S2 for the abbreviation of lake names and the descriptions of shaded areas.

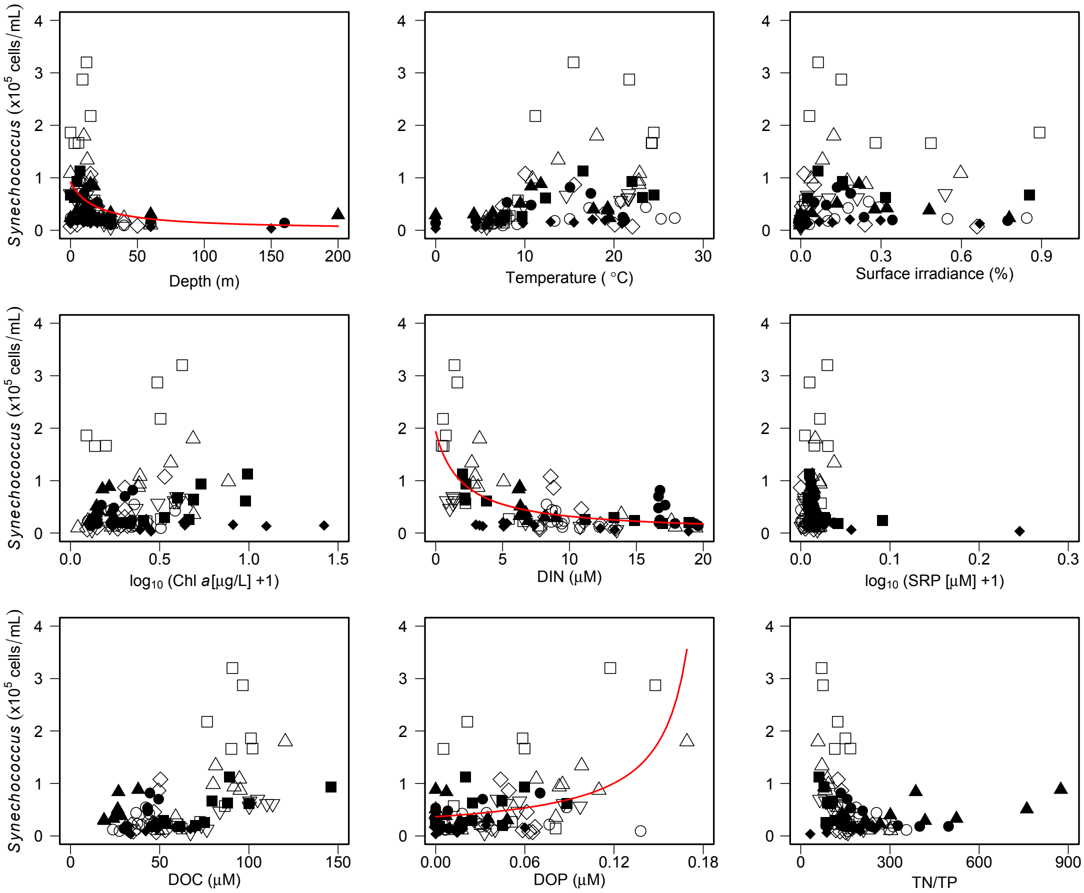

Figure S10. Relationships between *Synechococcus* cell density (cells/mL) and lake physicochemical variables in study lakes. Univariate regression lines were shown for the explanatory variables that were retained in the best model of GLMs with gamma distribution of response variable. See the legend of Figure S10 for symbol description.

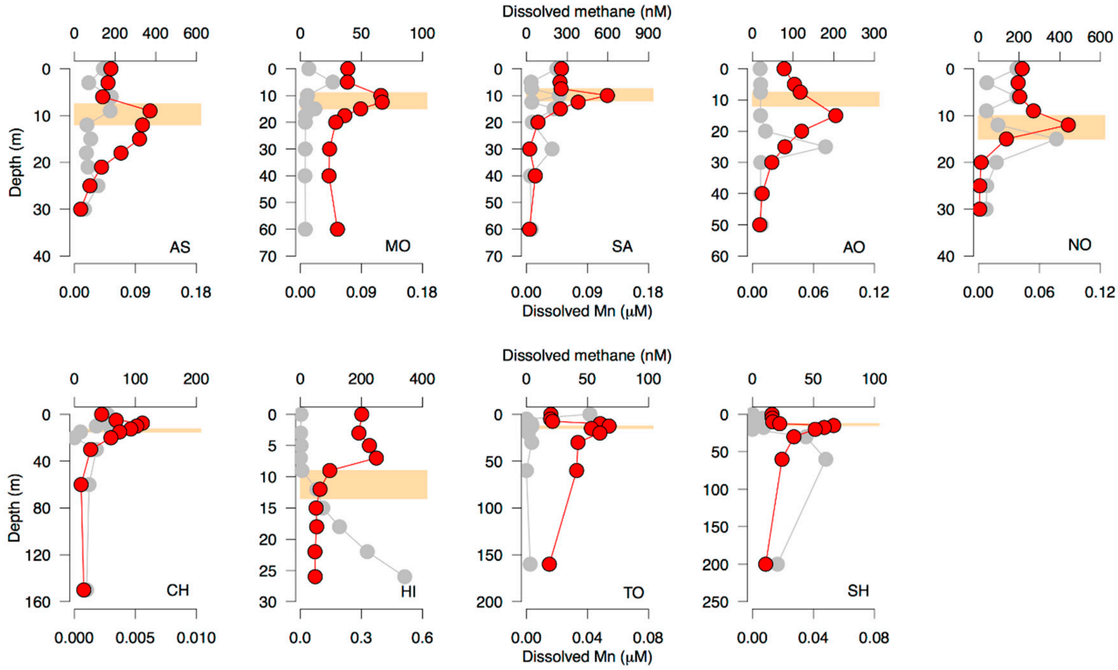

Figure S11. Vertical profiles of dissolved CH_4 concentration (nM, red circles) and Mn (µM, gray circles) concentrations in nine study lakes during the period from July to September in 2016–2017. See legend of Figure S2 for the abbreviation of lake names and the descriptions of shaded areas.

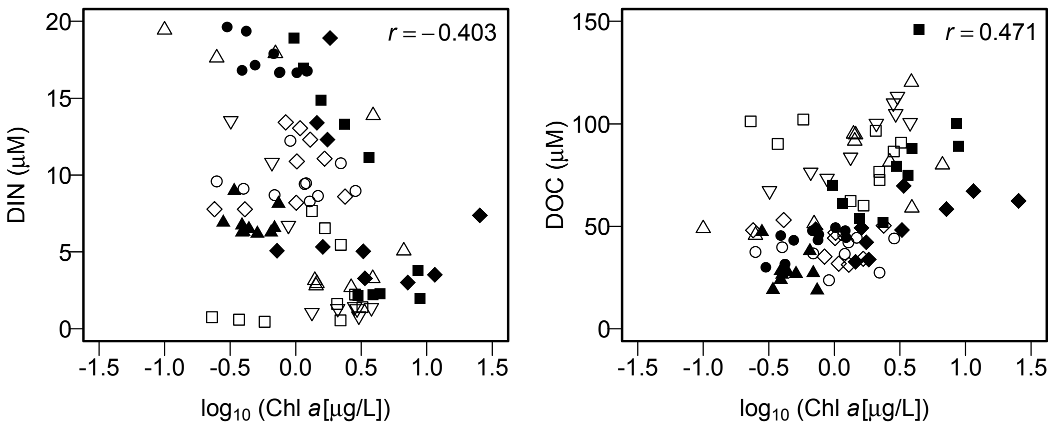

Figure S12. Relationships between phytoplankton biomass ($\log_{10}$ (Chl *a*), μg/L) and two variables (DIN and DOC, μM) in study lakes. □: Lake Ashinoko, ○: Lake Motosu, △: Lake Saiko, ◇: Lake Aoki, ▽: Lake Nojiri, ◆: Lake Chuzenji, ■: Lake Hibara, ●: Lake Toya, ▲: Lake Shikotsu.

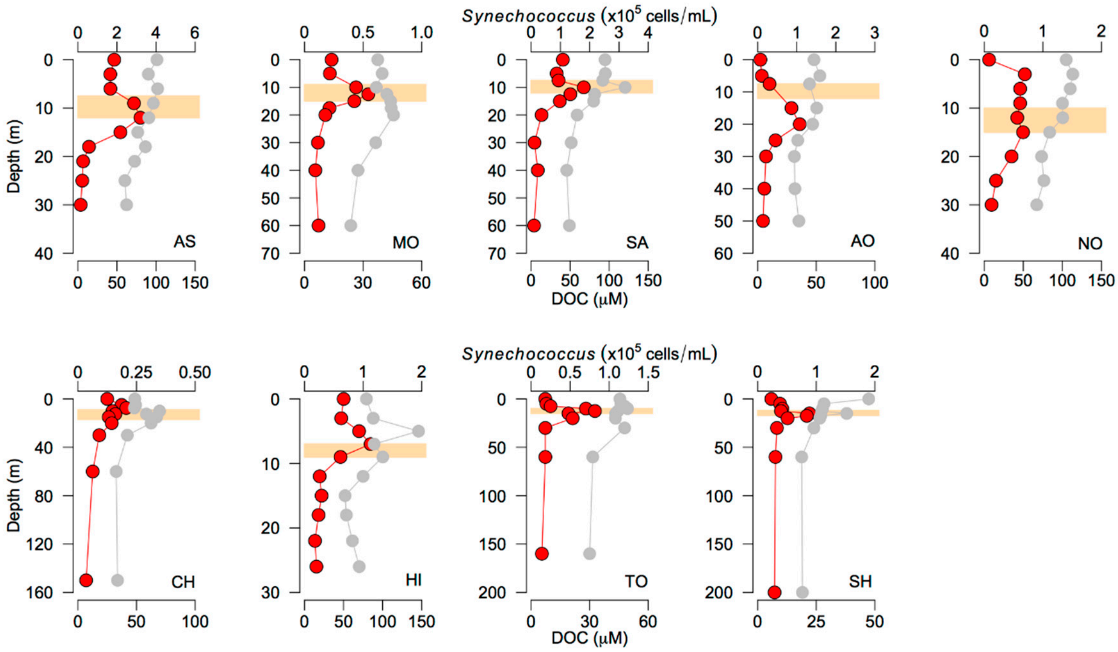

Figure S13. Vertical distribution of *Synechococcus* density (red circles) in relation to the DOC profile (gray circles) in the study lakes. Shaded areas denote the range of the thermocline. See legend of Figure S2 for the abbreviation of lake names.

Table S1. Watershed and lake morphological variables of the nine study lakes in Japan.

No.	Lake	Date	Origin	Catchment area (km^2)*	Elevation (m)	Annual precipitation (mm)†	Surface area (km^2)*	Volume (km^3)*	Mean depth (m)‡	Maximum depth (m)*	Average water residence time (year)*
1	Ashinoko	2016/8/5	Caldera	15.9	724	1,010	7.0	0.18	26	44	4.50
2	Motosu	2016/8/10	Dammed	24.6	901	1,470	4.7	0.33	70	122	6.50
3	Saiko	2016/9/3	Dammed	34.4	902	1,470	2.1	0.082	39	73	1.60
4	Aoki	2016/9/9	Tectonic	7.3	822	1,760	1.7	0.054	29	58	0.53
5	Nojiri	2016/9/29	Dammed	12.9	654	2,100	4.5	0.096	21.	39	2.00
6	Chuzenji	2017/7/12	Dammed	119.7	1,267	1,920	11.6	1.13	97	163	5.90
7	Hibara	2017/8/10	Dammed	106.4	831	1,370	10.8	0.13	12	31	0.82
8	Toya	2017/9/6	Caldera	101.6	90	930	70.4	8.19	116	180	9.00
9	Shikotsu	2017/9/8	Caldera	144.5	252	1,490	78.8	19.5	248	363	43.00

*Values determined by literature [1,2]. †Values obtained from nearby weather station for each lake. ‡Values calculated by dividing lake volume by surface area.

Table S2. Water quality variables of the nine study lakes in Japan.

No	Lake	Trophic status	Secchi depth (m)	Water temperature (°C)	pH	DOC (μM)	DIN (μM)	TN (μM)	SRP (μM)	DOP (μM)	TP (μM)	TN/TP (ratio)	Mn (μM)	Chl *a* (μg/L)
1	Ashinoko	Oligotrophic	9.2	27.9	8.4	83.9	2.7	19.3	0.038	0.058	0.165	117	0.030	1.68
2	Motosu	Ultra-oligotrophic	14.0	29.8	8.1	37.8	9.5	25.7	0.021	0.046	0.113	227	0.014	1.24
3	Saiko	Oligotrophic	8.5	23.2	8.5	76.8	8.9	23.3	0.044	0.073	0.204	113	0.022	2.24
4	Aoki	Oligotrophic	5.9	23.0	7.8	42.5	10.2	21.3	0.021	0.051	0.143	149	0.018	1.10
5	Nojiri	Oligotrophic	5.4	20.8	7.4	93.6	4.5	21.1	0.021	0.062	0.182	116	0.025	1.84
6	Chuzenji	Mesotrophic	11.5	26.5	8.7	51.2	7.7	28.4	0.118	0.017	0.305	93	0.002	5.81
7	Hibara	Mesotrophic	5.8	25.6	7.2	81.5	8.8	28.5	0.062	0.033	0.267	107	0.124	3.84
8	Toya	Ultra-oligotrophic	14.5	22.2	7.7	42.9	17.4	33.3	0.042	0.012	0.142	234	0.006	0.72
9	Shikotsu	Ultra-oligotrophic	15.2	24.8	8.0	28.4	6.9	17.2	0.029	0.011	0.053	322	0.002	0.48

Average of the measurement values shown.

Table S3. Water quality variables of tributary streams for study lakes.

No	Lake	Tributary	Discharge (m^3/s)	Water temperature (°C)	CH_4 (nM)	DOC (μM)	DIN (μM)	TN (μM)	SRP (μM)	DOP (μM)	TP (μM)	Mn (μM)	TN/TP (ratio)
1	Ashinoko	Myojin	NA	21.8	1,320.8	42.9	29.8	41.9	2.14	0.00	0.97	0.07	43
2	Saiko	Honzawa	0.018	0.01821.8	15.8	32.3	28.3	40.5	0.25	0.12	0.60	0.01	67
3	Nojiri	Denkuro	0.12	17.9	90.5	233.8	43.0	64.2	0.25	0.00	0.45	0.02	141
4	Nojiri	Sugakawa	0.012	18.2	232.4	189.4	87.3	136.2	1.75	0.25	8.94	0.03	15
5	Nojiri	Terazaki	0.026	16.5	9.1	177.2	18.3	62.6	0.75	1.03	4.85	0.01	13
6	Chuzenji	Jigoku	1.94	21.5	8.8	42.2	13.4	24.6	0.33	0.00	0.06	0.01	436
7	Hibara	Oowasezawa	0.152	18.2	9.5	92.4	16.3	32.9	0.30	0.00	0.06	0.00	586
8	Hibara	Azuma	0.90	17.5	7.0	136.3	12.2	88.9	0.11	0.00	0.04	0.00	2383
9	Hibara	Nagai	0.63	16.5	12.7	100.3	7.1	33.1	0.11	0.00	0.08	0.01	423
10	Hibara	Ookawairikawa	0.89	17.7	11.5	228.0	6.8	62.9	0.19	0.02	0.21	0.00	306
11	Hibara	Aizu	0.16	16.2	16.1	68.0	9.3	52.7	0.21	0.04	0.12	0.00	449
12	Hibara	Oshizawa	0.26	16.3	10.5	50.7	11.9	70.4	0.19	0.04	0.07	0.00	1076
13	Hibara	Shimizuzawa	0.50	15.2	103.0	56.2	18.6	48.3	0.04	0.21	0.12	0.02	418
14	Toya	Sobetsu	0.28	14.7	9.3	77.7	15.5	34.8	0.14	0.27	0.12	0.01	302
15	Toya	Aka	0.13	16.1	57.0	13.9	4.2	62.4	0.79	0.00	0.54	0.12	116

16	Toya	Ookawa	0.12	14.4	8.2	34.2	14.9	37.9	0.86	0.00	0.64	0.04	59
17	Toya	Horoto	0.019	17.1	7.7	49.0	13.9	60.5	0.12	0.00	0.92	0.05	66
18	Toya	Osaru	NA	19.0	18.8	225.1	16.0	121.2	0.08	0.05	0.46	0.01	263
19	Toya	Poromoi	0.009	15.9	8.0	41.0	13	124.8	1.10	0.00	0.77	0.18	161
20	Toya	Nishiki	0.060	16.9	48.2	113.1	105.0	168.4	1.32	0.00	1.67	0.04	101
21	Toya	Toya	0.036	18.7	47.2	109.5	96.6	170.4	0.17	0.62	1.38	0.01	123
22	Shikotsu	Bifue	0.75	18.1	11.3	412.9	17.8	116.6	0.17	0.11	0.56	0.00	207
23	Shikotsu	Ninaru	0.052	14.2	6.0	274.4	17.2	76.9	0.18	0.00	0.36	0.02	215

Table S4. The CARD-FISH probes used in the study.

Probes	Sequence (5' - 3')	Target organisms	FA (%)*	Permeabilization	References
EUB338	GCT GCC TCC CGT AGG AGT	Bacteria	35	Lysozyme	Amann et al. 1990 [3]
Mg84	CCA CTC GTC AGC GCC CGA	Type I methanotrophs	20	Lysozyme + Achromopeptidase	Eller and Frenzel 2001 [4]
Mg705	CTG GTG TTC CTT CAG ATC	Type I methanotrophs	20	Lysozyme + Achromopeptidase	Eller and Frenzel 2001 [4]
405_Syn	AGA GGC CTT CAT CCC TCA	*Synechococcus*	60	Lysozyme	West et al. 2001 [5]
ARCH915	GTG CTC CCC CGC CAA TTC CT	Archaea	35	Lysozyme + Achromopeptidase	Stahl and Amann 1991 [6]

* Formamide concentration in CARD-FISH hybridization buffer.

Table S5. Relative importance for limnological explanatory variables used in the generalized linear models (GLMs) with a gamma error distribution and an inverse link function for each response variable. Relative importance was evaluated by the sum of Akaike weights (*w*) for each model (CH_4, DIN, DOP and TN/TP).

	Akaike weights (*w*) of response variables			
Explanatory variables	CH_4	DIN	DOP+1	TN/TP
Depth	0.99	0.9	0.30	0.41
Water temperature	0.76	-	-	-
Percentage of surface irradiance	0.63	0.99	0.38	0.40
$\log_{10}$ (Chl *a* + 1)	0.30	0.99	0.33	1.00
DIN	1.00	-	-	-
$\log_{10}$ (SRP + 1)	0.29	0.71	0.67	0.95
DOC	0.37	1.00	1.00	1.00
DOP	0.98	-	-	-
TN/TP	0.90	-	-	-
TN	-	-	0.48	-
TP	-	0.96	-	-

Table S6. Results of the generalized linear models (GLM) assessing the univariate relationship between environmental variables and dissolved CH_4 concentrations (as shown in Figure 2), the univariate relationship between DOC and chemical variables (Figure 3), and the univariate relationship between lake environmental variables and the peak subsurface maximum (SMM) in nine study lakes (Figure 7). GLMs were constructed with a gamma error distribution and an inverse link function for each variable ($1/y = ax + b$), except for the variables with an asterisk whose relationships were estimated with GLMs with a Gaussian error and an identity link function ($y = ax + b$).

Response variable y	Explanatory variables x	Coefficient a (SE)	Intercept b (SE)	Null deviance	Residual deviance	%deviance explained
CH_4	Depth	0.000377 (0.000087)	0.00471 0.00108)	88.68	64.65	27.1
CH_4	Water temperature	-0.000526 (0.000147)	0.0179 (0.0029)	88.68	74.90	18.4
CH_4	Surface irradiance	-0.0000156 (0.000043)	0.00963 (0.00132)	88.68	88.54	0.2
CH_4	DIN	0.00156 (0.00018)	0.00189 (0.00070)	88.68	41.19	53.6
CH_4	DOP	-0.0780 (0.0108)	0.0142 (0.0014)	88.68	66.54	25.0
CH_4	TN/TP	0.0000763 (0.0000110)	-0.000680 (0.00118)	88.68	55.63	37.3

DIN	DOC	0.00238 (0.00040)	0.00213 (0.0175)	59.45	41.44	30.3
DOP	DOC	-0.000598 (0.000118)	0.998 (0.008)	0.109	0.084	22.9
TN/TP	DOC	0.0000924 (0.0000130)	0.000684 (0.000600)	31.92	17.92	43.9
Peak SMM*	DOC	6.03 (0.77)	-205.94 (59.95)	309571	32038	89.7
Peak SMM	DIN	0.00111 (0.00050)	0.000694 (0.00145)	6.50	2.62	59.7
Peak SMM*	DOP	2201.44 (892.22)	84.05 (77.24)	309571	165572	46.5
Peak SMM	TN/TP	0.0000344 (0.0000191)	0.000861 (0.00183)	6.50	3.47	46.6
Peak SMM*	*Synechococcus*	0.00140 (0.000703)	84.62 (90.85)	309571	198007	36.0

Table S7. Relative abundance (%) of phytoplanktonic algae in the epilimnion, metalimnion and hypolTable S6. Relative abundance (%) of phytoplanktonic algae in the epilimnion, metalimnion and hypolimnion of study lakes.

Lake	Cryptophyceae			Dinophyceae			Chrysophyceae			Bacillariophyceae			Euglenophyceae			Chlorophyceae		
	Epi	Meta	Hypo	Epi	Meta	Hypo	Epi	Meta	Hypo	Epi	Meta	Hypo	Epi	Meta	Hypo	Epi	Meta	Hypo
Ashinoko	1.9	30.7	7.6	1.9	29.8	4.9	1.9	1.0	8.9	59.6	37.6	76.0	0.0	0.0	0.0	34.6	1.0	2.7
Motosu	3.5	23.2	9.5	2.1	23.2	7.8	0.0	0.0	6.0	38.9	49.1	65.5	0.0	0.0	0.0	55.6	4.5	11.2
Saiko	4.8	3.5	18.4	4.1	3.5	14.3	0.0	0.0	0.0	15.0	16.7	59.2	0.0	0.0	0.0	76.2	76.3	8.2
Aoki	22.0	3.7	0.0	19.5	2.6	0.0	1.2	33.9	0.0	31.7	57.7	96.7	0.0	0.0	0.0	25.6	2.1	3.3
Nojiri	24.5	13.3	6.7	12.2	13.3	6.7	4.1	5.7	13.3	20.4	40.0	63.3	2.0	0.0	10.0	36.7	27.6	0.0
Chuzenji	1.2	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	13.1	47.6	54.2	0.0	0.0	0.0	85.7	51.9	45.8
Hibara	4.3	10.1	2.2	6.7	0.5	4.4	3.0	0.5	0.0	39.0	46.4	64.4	0.0	0.0	2.2	47.0	42.4	26.7
Toya	3.6	10.5	0.0	53.6	26.3	0.0	10.7	5.3	0.0	14.3	21.1	93.3	0.0	0.0	0.0	17.9	36.8	6.7
Shikotsu	0.5	0.1	0.9	0.5	0.2	0.9	0.0	0.0	0.9	96.3	99.3	97.4	0.0	0.0	0.0	2.7	0.3	0.0

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