



*Supplement of*

## **Picoplanktonic methane production in eutrophic surface waters**

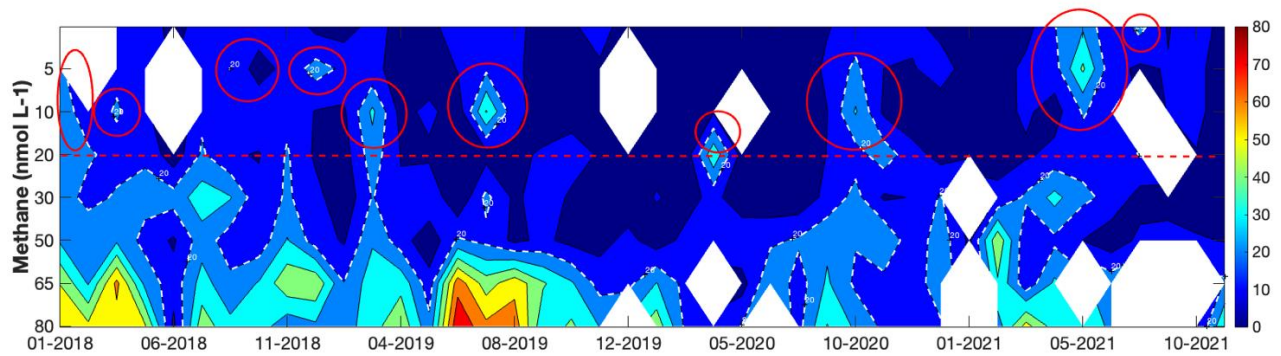
**Sandy E. Tenorio and Laura Farías**

*Correspondence to:* Laura Farías ([laura.farias@udec.cl](mailto:laura.farias@udec.cl))

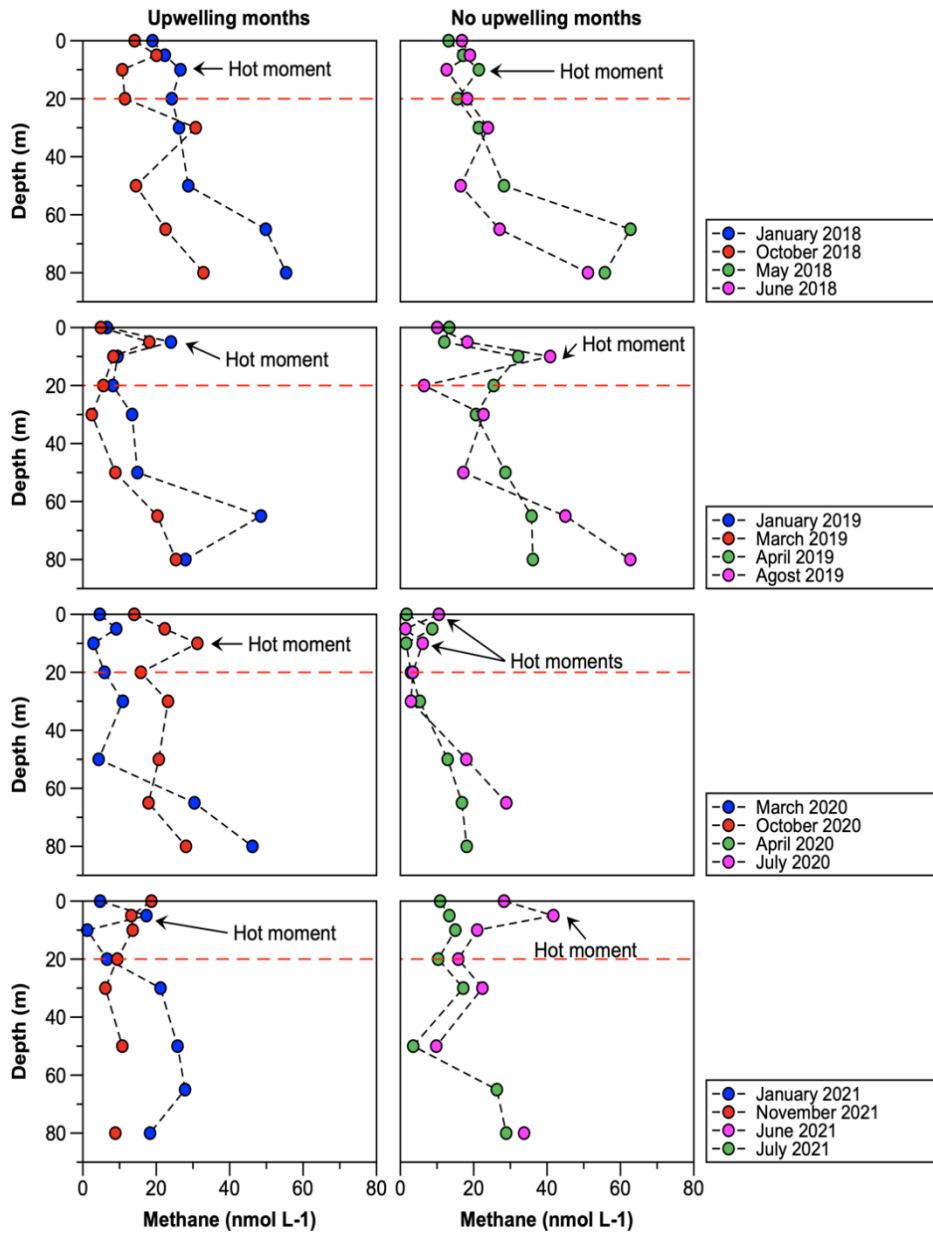
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**Figures:**

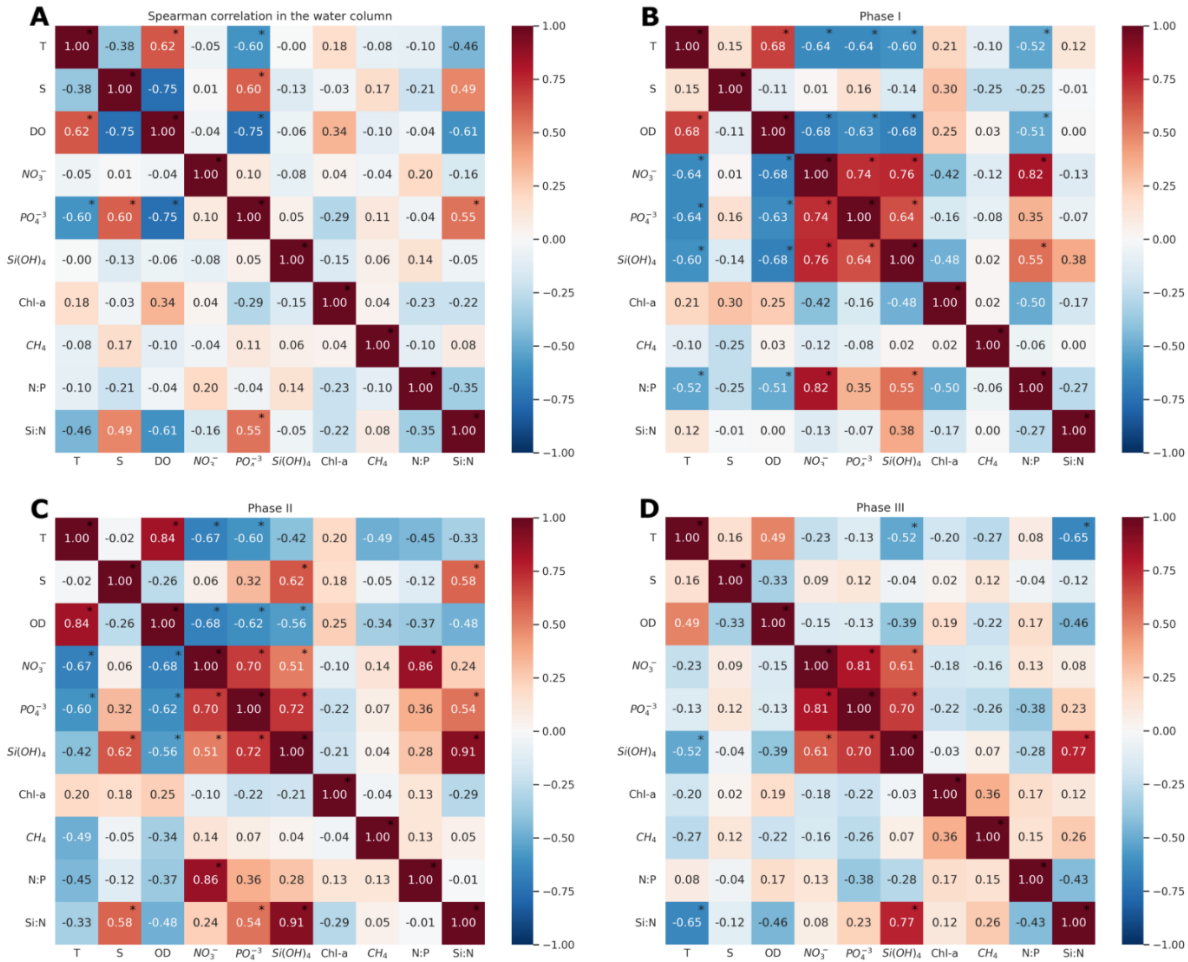
**Figure S1.** Time series of vertical distribution of dissolved methane (nM) showing CH<sub>4</sub>' hot spots in red ellipses in the surface layer at central Chile upwelling platform (ST18). It was during January 2018 to December 2021. Red dotted line indicates a depth of 20m. 0-20m generally oxygenated. White spaces indicate Not a Number (NaN).



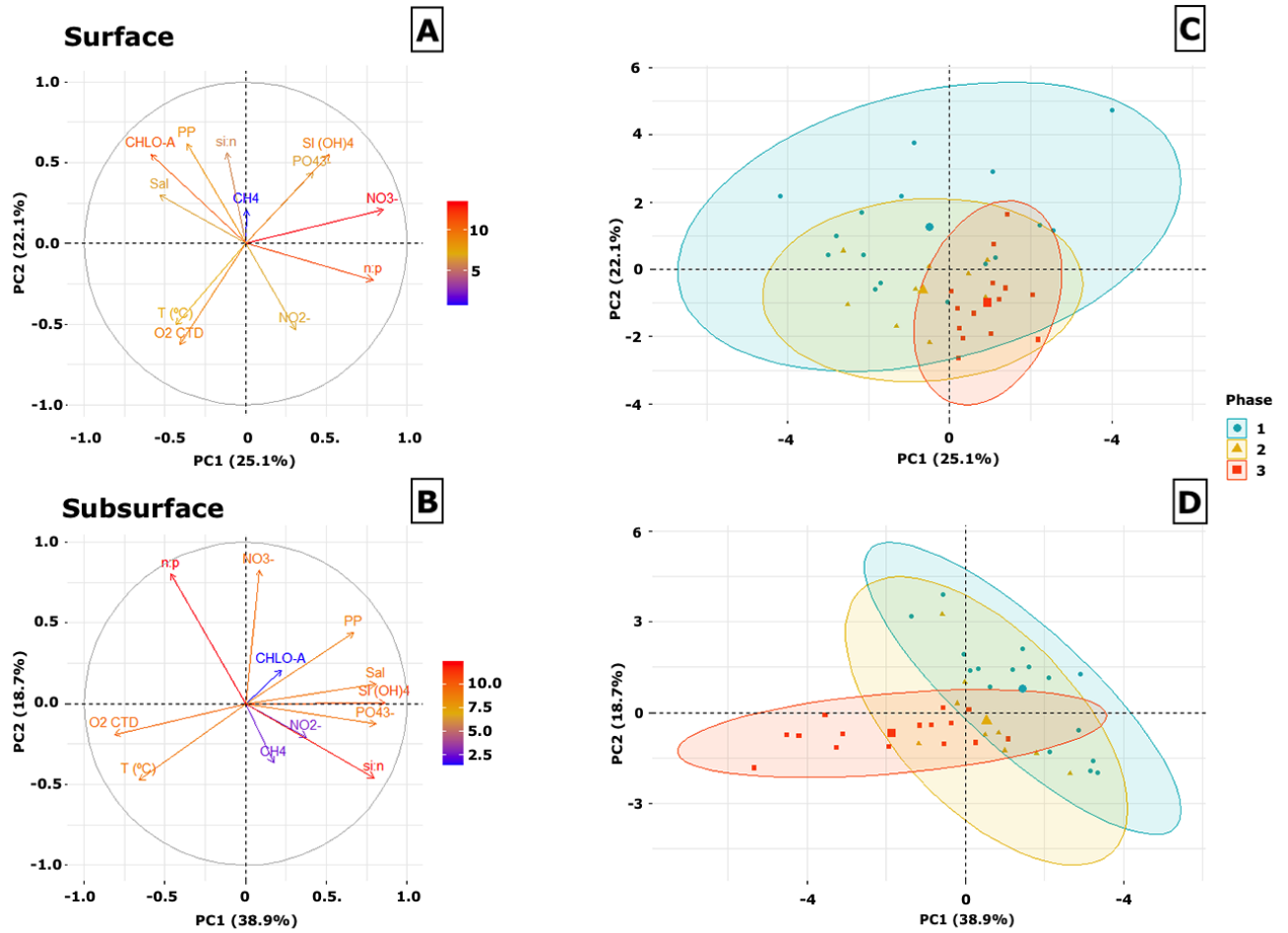
**Figure S2.** Vertical distribution of dissolved methane (nM) at central Chile upwelling platform (ST18), in two periods of upwelling and non-upwelling during 2018 and 2021. This shows some months where hot moments are observed in the oxygenated surface layer (0 - 20m). Depth of 20m is in red dotted line.



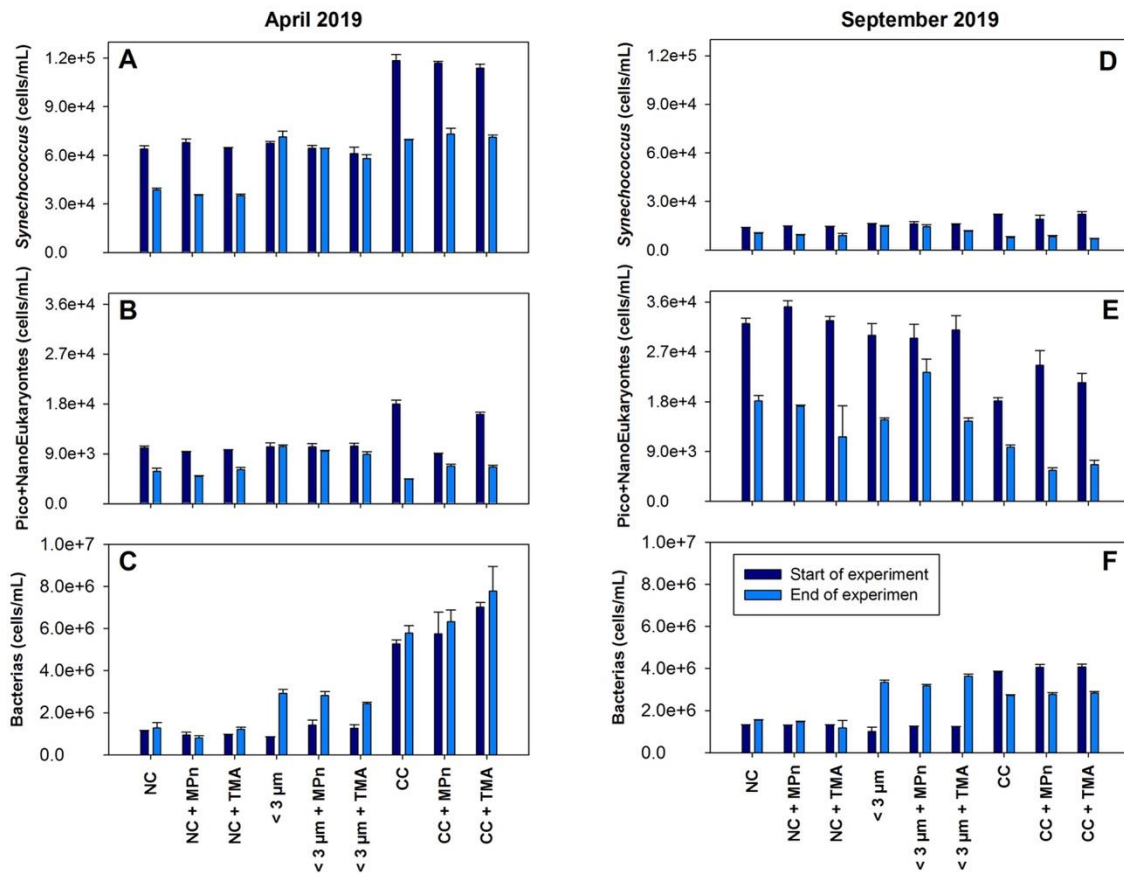
**Figure S3.** Spearman correlations matrix between biochemical variables at A. all the water column and at surface, and B. Phase I, C. Phase II and D. Phase III at the surface layer (20m). Values on the red and blue color scale represent positive and negative values of the Spearman correlation (rho). Variables are: T: temperature, S: salinity, DO: dissolved oxygen;  $\text{NO}_3^-$ ; nitrate;  $\text{PO}_4^{3-}$ : phosphate;  $\text{Si(OH)}_4$ : silicic acid; Chl-a, chlorophyll-a;  $\text{CH}_4$ : methane; N:P ratio and Si:N ratio. \* represents a significant correlation below or equal to 0.05.



**Figure S4.** Principal Component Analysis (PCA) showing the distribution of vectors and clusters made for each phase of productivity in the surface (0-20 m; A and C) and subsurface (21-80 m; B and D) of the upwelling zone of central Chile.



**Figure S5.** Abundance of *Synechococcus sp.* (cell mL<sup>-1</sup>), pico + nano eukaryotes (cell mL<sup>-1</sup>), and bacteria (cell mL<sup>-1</sup>) at the beginning (dark blue) and at the end (light blue) of the microcosm experiments with the addition of methylated substrates (MPn: methyl phosphonic acid and TMA: trimethylamine) performed with three planktonic communities (NC: natural planktonic community; <3 μm: picoplankton and CC: picoplankton concentrate) under oxygenated conditions in April (A, B, and C) and September 2019 (D, E, and F), corresponding to Phase II and I, respectively.



**Tables:**

**Table S1.** Initial concentrations of biogeochemical variables in methane cycling experiments with the following treatments: NC: natural planktonic community; <3  $\mu\text{m}$ : picoplanktonic fraction and <0.2  $\mu\text{m}$ : femtoplanktonic fraction; and with the addition of methylated substrate (MPn: methyl phosphonic acid and TMA: trimethylamine) conducted in Phase I (December 2018 and January), Phase II (March), and Phase III (May 2019).

Month	Treatments ( $\mu\text{m}$ )	CH <sub>4</sub> (nM)	DO ( $\mu\text{M}$ )	Chl-a ( $\text{mg m}^{-3}$ )	DOC ( $\mu\text{M}$ )	NO <sub>3</sub> <sup>-</sup> ( $\mu\text{M}$ )	NO <sub>2</sub> <sup>-</sup> ( $\mu\text{M}$ )	PO <sub>4</sub> <sup>3-</sup> ( $\mu\text{M}$ )	Si(OH) <sub>4</sub> ( $\mu\text{M}$ )
Dec	CN	2.21	274.67	43.81	101.12	1.55	0.14	0.77	0.30
	<3	6.98	273.32	6.50	127.10	1.31	0.13	0.66	0.07
	<0.2	3.88	279.55	-	124.26	2.29	0.14	0.79	0.47
Jan	CN	13.77	185.48	15.70	158.82	16.18	0.33	1.62	4.21
	<3	5.01	235.28	2.39	98.36	15.86	0.35	1.69	3.94
	<0.2	3.46	259.79	-	123.60	17.46	0.36	1.91	5.53
Mar	<3 Ctrl	8.65	187.81	1.39	73.94	6.61	0.60	1.54	5.29
	<3+MPn	22.74	218.23	0.44	88.73	5.92	0.56	1.53	4.56
May	<3 Ctrl	17.41	275.27	0.46	166.68	9.71	0.30	1.15	3.15
	<3+MPn	16.25	268.94	0.41	123.06	13.45	0.40	1.25	4.24
	<3+TMA	18.15	268.74	0.43	149.62	13.69	0.43	1.37	4.49

**Table S2.** Net rates of methane cycling (net CH<sub>4</sub> accumulation and/or consumption; nmol L<sup>-1</sup> h<sup>-1</sup>) under different treatments: NC: natural planktonic community and <3 μm: picoplanktonic fraction; and with the addition of methylated substrates as MPn (methyl phosphonic acid) and TMA (trimethylamine) in Phase I (December 2018 and January), Phase II (March), and Phase III (May 2019).

Month	Treatments (μm)	Total	Light	Dark
Dec	CN	0.10 ± 0.04	0.10 ± 0.06	0.08 ± 0.27
	<3	0.3 ± 0.07	0.11 ± 0.11	0.59 ± 0.13 (*)
Jan	CN	-0.41 ± 0.16	-0.28 ± 0.41	-0.04 ± 0.1
	<3	-0.46 ± 0.17	-0.41 ± 0.5	-0.28 ± 0.24
Mar	<3 Ctrl	0.16 ± 0.11	-0.51 ± 0.23	0.28 ± 0.10 (*)
	<3+MPn	0.05 ± 0.29	-0.99 ± 0.09	-0.71 ± 0.18
May	<3 Ctrl	-0.14 ± 0.09	-0.25 ± 0.12	-0.13 ± 0.33
	<3+MPn	0.17 ± 0.22	0.19 ± 0.33	1.02 ± 0.53 (*)
	<3+TMA	-0.01 ± 0.16	0.21 ± 0.60 (*)	0.25 ± 0.08 (*)



**Table S3.** Initial concentrations of biogeochemical variables in long-term microcosm experiment with the addition of methylated substrates such as MPn (methyl phosphonic acid) and TMA (trimethylamine) conducted over three planktonic communities (NC: natural plankton community; <3  $\mu\text{m}$ : picoplankton and CC: picoplankton concentrate) in Phase II (April 2019) and Phase I (September 2019).

Month	Treatments	CH <sub>4</sub>	DO	Chl-a	DOC	NO <sub>3</sub> <sup>-</sup>	NO <sub>2</sub> <sup>-</sup>	PO <sub>4</sub> <sup>3-</sup>	Si(OH) <sub>4</sub>
	( $\mu\text{m}$ )	(nM)	( $\mu\text{M}$ )	( $\text{mg m}^{-3}$ )	( $\mu\text{M}$ )	( $\mu\text{M}$ )	( $\mu\text{M}$ )	( $\mu\text{M}$ )	( $\mu\text{M}$ )
Apr	NC Ctrl	25.13	223.35	8.48	97.27	15.35	0.74	1.95	3.72
	NC + MPn	25.13	211.44	8.47	90.76	14.40	0.71	1.93	4.04
	NC TMA	25.13	202.06	7.36	94.81	13.16	0.62	1.75	3.15
	<3 Ctrl	25.09	209.11	0.39	115.27	13.32	0.66	1.74	3.84
	<3 + MPn	25.06	217.01	0.16	117.97	13.66	0.65	1.73	3.91
	<3 + TMA	25.05	220.72	0.88	118.02	13.69	0.73	1.93	3.69
	CC Ctrl	23.71	273.65	1.76	118.98	14.49	0.43	1.84	4.69
	CC + MPn	24.37	264.15	1.19	156.74	11.76	0.35	1.51	3.90
	CC + TMA	21.05	270.68	1.64	142.50	10.76	0.37	1.47	3.51
Sep	NC Ctrl	21.13	210.03	1.31	162.65	12.44	0.23	2.08	12.09
	NC + MPn	20.43	175.84	1.16	100.93	14.95	0.24	1.68	11.35
	NC TMA	20.33	189.53	1.02	179.15	11.92	0.23	1.73	9.12
	<3 Ctrl	20.08	201.42	0.39	151.80	13.81	0.23	1.85	8.69
	<3 + MPn	20.33	209.76	0.36	102.51	12.89	0.23	1.97	8.63
	<3 + TMA	20.73	192.07	0.35	104.45	12.27	0.15	1.56	8.22
	CC Ctrl	-	194.99	0.85	107.82	13.51	0.11	1.84	9.53
	CC + MPn	-	178.55	0.80	122.47	13.10	0.07	1.77	11.36
	CC + TMA	-	155.93	0.83	174.24	13.59	0.09	1.48	7.91

**Table S4.** Net rates of methane cycling (net accumulation and/or consumption;  $\text{nmol L}^{-1} \text{h}^{-1}$ ) under different treatments: NC: natural planktonic community;  $<3 \mu\text{m}$ : picoplanktonic fraction and CC: picoplanktonic concentrated community; and the addition of methylated substrates such as MPn (methyl phosphonic acid) and TMA (trimethylamine), conducted in Phase II (April 2019) and Phase I (September 2019).

Treatments ( $\mu\text{m}$ )	Phase II			Phase III		
	Total	Light	Dark	Total	Light	Dark
NC Ctrl	$-0.18 \pm 0.04$	$-0.19 \pm 0.06$	$-0.14 \pm 0.01$	$0.02 \pm 0.02$	$0.03 \pm 0.03$	$-0.02 \pm 0.02$
NC + MPn	$-0.04 \pm 0.05$	$-0.06 \pm -0.01$	$0.07 \pm -0.02$	$-0.03 \pm -0.35$	$-0.02 \pm 0.03$	$-0.11 \pm 0.02$
NC TMA	$-0.23 \pm 0.07$	$-0.24 \pm -0.05$	$-0.13 \pm 0.03$	$-0.04 \pm -0.46$	$-0.03 \pm 0.03$	$-0.11 \pm 0.02$
$<3$ Ctrl	$-0.02 \pm 0.06$	$-0.03 \pm -0.01$	$0.08 \pm -0.01$	$-0.01 \pm -0.09$	$0.00 \pm 0.03$	$-0.07 \pm 0.02$
$<3$ + MPn	$-0.17 \pm 0.04$	$-0.18 \pm -0.06$	$-0.12 \pm 0.03$	$0.00 \pm -0.03$	$0.01 \pm 0.03$	$-0.06 \pm 0.02$
$<3$ + TMA	$0.11 \pm 0.03$	$0.11 \pm -3.23$	$0.14 \pm -0.02$	$0.01 \pm 0.18$	$0.02 \pm 0.03$	$-0.03 \pm 0.02$
CC Ctrl	$-0.07 \pm 0.03$	$-0.08 \pm -0.62$	$-0.02 \pm 0.02$	$-0.02 \pm -0.31$	$-0.03 \pm 0.03$	$0.02 \pm 0.01$
CC + MPn	$0.10 \pm 0.02$	$0.10 \pm -0.17$	$0.12 \pm -0.25$	$-0.07 \pm -0.53$	$-0.09 \pm 0.04$	$0.03 \pm 0.01$
CC + TMA	$0.07 \pm 0.02$	$0.07 \pm 0.01$	$0.06 \pm 0.04$	$0.01 \pm 0.08$	$-0.02 \pm 0.03$	$0.09 \pm 0.03$