1   **Warming exacerbates the impact of nutrient enrichment on microbial functional**

2   **potentials important to the nutrient cycling in shallow lake mesocosms**

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32   **Running title**: Microbial functional response to warming

Table S1 Full names of the abbreviated enzymes/proteins/genes in the main text.

Gene category	Subcategory	Name/abbreviation of the enzyme/protein/gene	Enzyme/protein
Carbon degradation	Starch	amyA	Alpha-amylase
	Starch	amyX	Pullulanase
	Starch	apu	Amylopullulanase
	Starch	cda	Cyclomaltodextrinase
	Starch	glucoamylase	Glucoamylase
	Starch	pulA	Pullulanase
	Cellulose	cellobiase	Cellulose
	Cellulose	endoglucanase	Endoglucanase
	Cellulose	exoglucanase	Exoglucanase
	Hemicellulose	ara	Arabinofuranosidase
	Hemicellulose	mannanase	Beta-mannanase
	Hemicellulose	xylA	Xylose isomerase
	Hemicellulose	xylanase	Xylanase
	Chitin	acetylglucosaminidase	Acetylglucosaminidase
	Chitin	chitinase	Chitinase
	Chitin	endochitinase	Endochitinase
	Chitin	exochitinase	Exochitinase
	Pectin	pectinase	Pectinase
	Aromatics	AssA	Alkylsuccinate synthase
	Aromatics	camDCAB	Camphor 5-monooxygenase
	Aromatics	limEH	Limonene-1,2-epoxide hydrolase
	Aromatics	LMO	limonene monooxygenase
	Aromatics	vanA	Vanillate demethylase
	Aromatics	vdh	Aryl-aldehyde oxidase
	Lignin	phenol_oxidase	Phenol oxidase
Carbon fixation	Reductive tricarboxylic acid cycle	aclB	ATP citrate lyase
	Reductive acetyl–CoA pathway	CODH	Carbon monoxide dehydrogenase
	Wood-Ljungdahl pathway	FTHFS	Tetrahydrofolate formylase
	Calvin cycle	rubisco	RuBisCo
Methane	Methanogenesis	mcrA	Methyl coenzyme M reductase
	Methane oxidation	mmoX	Soluable methane monooxygenase
	Methane oxidation	pmoA	Particulate methane monooxygenase
Nitrogen	Ammonification	gdh	Glutamate dehydrogenase
	Ammonification	ureC	Urease
	Anammox	hzo	Hydrazine oxidoreductase
	Assimilatory N reduction	nasA	Assimilatory nitrate reductase catalytic subunit
	Assimilatory N reduction	nirA	Nitrite reductase (NAD(P)H)
	Assimilatory N reduction	nirB	Nitrite reductase (NAD(P)H)
	Denitrification	narG	Respiratory nitrate reductase alpha chain
	Denitrification	nirS	Cytochrome cd1 nitrite reductas

	Denitrification	norB	Nitric oxide reductase
	Denitrification	nosZ	Nitrous oxide reductase
	Denitrification	nirK	Copper containing nitrite reductase
	Dissimilatory N reduction	napA	Periplasmic nitrate reductase
	Dissimilatory N reduction	nrfA	Ammonia-forming cytochrome c nitrite reductase
	Nitrification	hao	Hydroxylamine oxidoreductase
	Nitrification	amoA	Ammonia monooxygenase
	Nitrogen fixation	nifH	Nitrogenase iron protein
Phosphorus	Phosphorus utilization	phytase	Phytase
	Phosphorus utilization	ppk	Polyphosphate kinase
	Phosphorus utilization	ppx	Exopolyphosphatase
Sulfur	Adenylylsulfate reductase	APS_AprA	Adenylylsulfate reductase
	Adenylylsulfate reductase	APS_AprB	Adenylylsulfate reductase
	Sulfide oxidation	fccAB	Flavocytochrome c sulfide dehydrogenase
	Sulfide oxidation	sqr	Sulfide-quinone oxidoreductase
	Sulfite reductase	CysJ	Sulfite reductase (NADPH) flavoprotein subunit alpha
	Sulfite reductase	dsrA	Dissimilatory sulfite reductase
	Sulfite reductase	dsrB	Dissimilatory sulfite reductase
	Sulfur oxidation	sox	Sulfer oxidation cycle enzymes

34 **Table S2** Permutational multivariate analysis of variance (PERMANOVA) based on

35 Jaccard/Bray-Curtis dissimilarity of overall microbial functional gene structure in

36 different warming and nutrient-enriched treatments. *: $p < 0.05$; **: $p < 0.01$.

Index	Jaccard dissimilarity			Bray-Curtis dissimilarity		
	R^2	F	p	R^2	F	p
Overall warming	0.117	2.251	0.035*	0.125	2.585	0.022*
Overall nutrients	0.116	2.286	0.024*	0.127	2.627	0.025*
Overall nutrients: overall warming	0.151	2.943	0.013*	0.168	3.466	0.01**
Residual	0.616			0.58		

37 **Table S3** Permutational multivariate analysis of variance (PERMANOVA) of

38 microbial functional gene structure of the subcategory of C, N, P, and S cycling based

39 on Jaccard/Bray-Curtis dissimilarity. *: $p < 0.05$; **: $p < 0.01$.

| Groups	Jaccard dissimilarity		Bray-Curtis dissimilarity	
	F	p	F	p
C				
Whole	3.084	0.001**	3.621	0.001**
Control vs EW	1.381	0.274	0.364	0.243
Control vs NE	1.575	0.123	1.516	0.116
Control vs NE and EW	4.535	0.025*	3.772	0.028*
EW vs NE	1.708	0.032*	1.650	0.027*
EW vs NE and EW	5.685	0.026*	4.676	0.034*
NE vs NE and EW	5.996	0.03*	4.954	0.029*
N				
Whole	3.581	0.001**	3.581	0.001**
Control vs EW	1.355	0.290	1.348	0.315
Control vs NE	1.479	0.126	1.429	0.113
Control vs NE and EW	4.551	0.026*	3.774	0.031*
EW vs NE	1.619	0.028*	1.575	0.029*
EW vs NE and EW	5.577	0.030*	4.548	0.027*
NE vs NE and EW	6.206	0.038*	5.048	0.032*
P				
Whole	3.442	0.001**	2.975	0.001**
Control vs EW	1.264	0.369	1.270	0.372
Control vs NE	1.403	0.200	1.376	0.127
Control vs NE and EW	4.564	0.031*	3.825	0.031*
EW vs NE	1.469	0.084*	1.447	0.085*
EW vs NE and EW	5.348	0.029*	4.473	0.03*
NE vs NE and EW	5.819	0.023*	4.904	0.031*
S				
Whole	3.707	0.001**	3.158	0.001**
Control vs EW	1.527	0.208	1.493	0.181
Control vs NE	1.614	0.093	1.549	0.088
Control vs NE and EW	4.641	0.029*	3.859	0.026*
EW vs NE	1.672	0.027*	1.627	0.032*
EW vs NE and EW	6.080	0.039*	4.963	0.023*
NE vs NE and EW	6.287	0.024*	5.136	0.036*

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41 **Table S4** Permutational multivariate analysis of variance (PERMANOVA) of

42 microbial functional gene structure composed of the 9619 genes under warming and

43 nutrient treatments based on Jaccard/Bray-Curtis dissimilarity. *: $p < 0.05$; **: $p <$

44 0.01.

Groups	Jaccard dissimilarity		Bray-Curtis dissimilarity	
	F	p	F	p
Whole	6.544	0.001**	9.507	0.001**
Control vs EW	2.491	0.027*	2.295	0.026*
Control vs NE	2.495	0.024*	2.265	0.030*
Control vs NE and EW	16.197	0.027*	10.459	0.032*
EW vs NE	2.077	0.028*	1.947	0.027*
EW vs NE and EW	16.941	0.028*	11.154	0.029*
NE vs NE and EW	18.549	0.025*	11.943	0.024*

45 **Table S5** Water environmental properties in response to warming and nutrient

46 enrichment in the shallow-lake mesocosms. The treatments are termed as follows:

47 ambient temperature, unenriched (control); enhanced warming (A2 + 50%),

48 unenriched (EW); ambient temperature, nutrient-enriched (NE); and enhanced

49 warming (A2 + 50%), nutrient-enriched (NE and EW).

| Index	Control	EW	NE	NE and EW
Turbidity	6.53 ± 0.98^a	5.25 ± 0.18^a	11.63 ± 7.84^a	15.33 ± 5.41^a
PO_4^{3-}-P (mg l^{-1})	0.003 ± 0.0003^a	0.003 ± 0.0006^{ac}	0.008 ± 0.001^b	0.005 ± 0.001^{ac}
NO_x^--N (mg l^{-1})	0.071 ± 0.066^a	0.0045 ± 0.0006^a	3.560 ± 0.218^b	2.290 ± 0.689^b
DOC (mg l^{-1})	1.52 ± 0.11^a	1.44 ± 0.06^a	2.42 ± 0.41^{ab}	2.76 ± 0.42^b
TOC (mg l^{-1})	1.97 ± 0.30^a	1.64 ± 0.08^a	4.11 ± 1.77^{ab}	7.68 ± 2.36^b
Chlorophyll a (μg l^{-1})	8.35 ± 4.21^a	4.77 ± 1.43^a	12.68 ± 2.82^a	340.48 ± 153.15^b
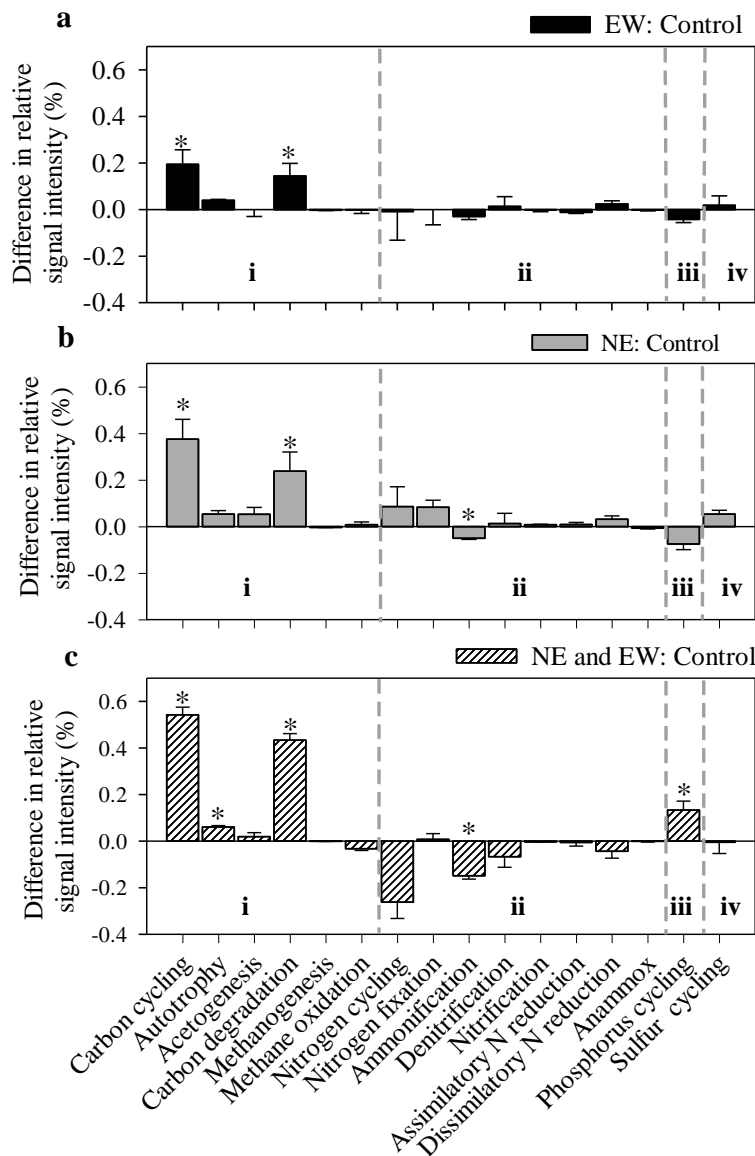
Figure S1 Differences in the microbial metabolic potentials (characterized as the relative signal intensity) involved in the biogeochemical cycling processes of carbon (i), nitrogen (ii), phosphorus (iii), and sulfur (iv). The treatments are termed as follows: ambient temperature, unenriched (control); enhanced warming (A2 + 50%), unenriched (EW); ambient temperature, nutrient-enriched (NE); and enhanced warming (A2 + 50%), nutrient-enriched (NE and EW). EW: Control (**a**), NE: Control (**b**), as well as NE and EW: Control (**c**) means the metabolic potential differences of EW with control, NE with control, NE and EW with control, respectively. *: $p < 0.05$.

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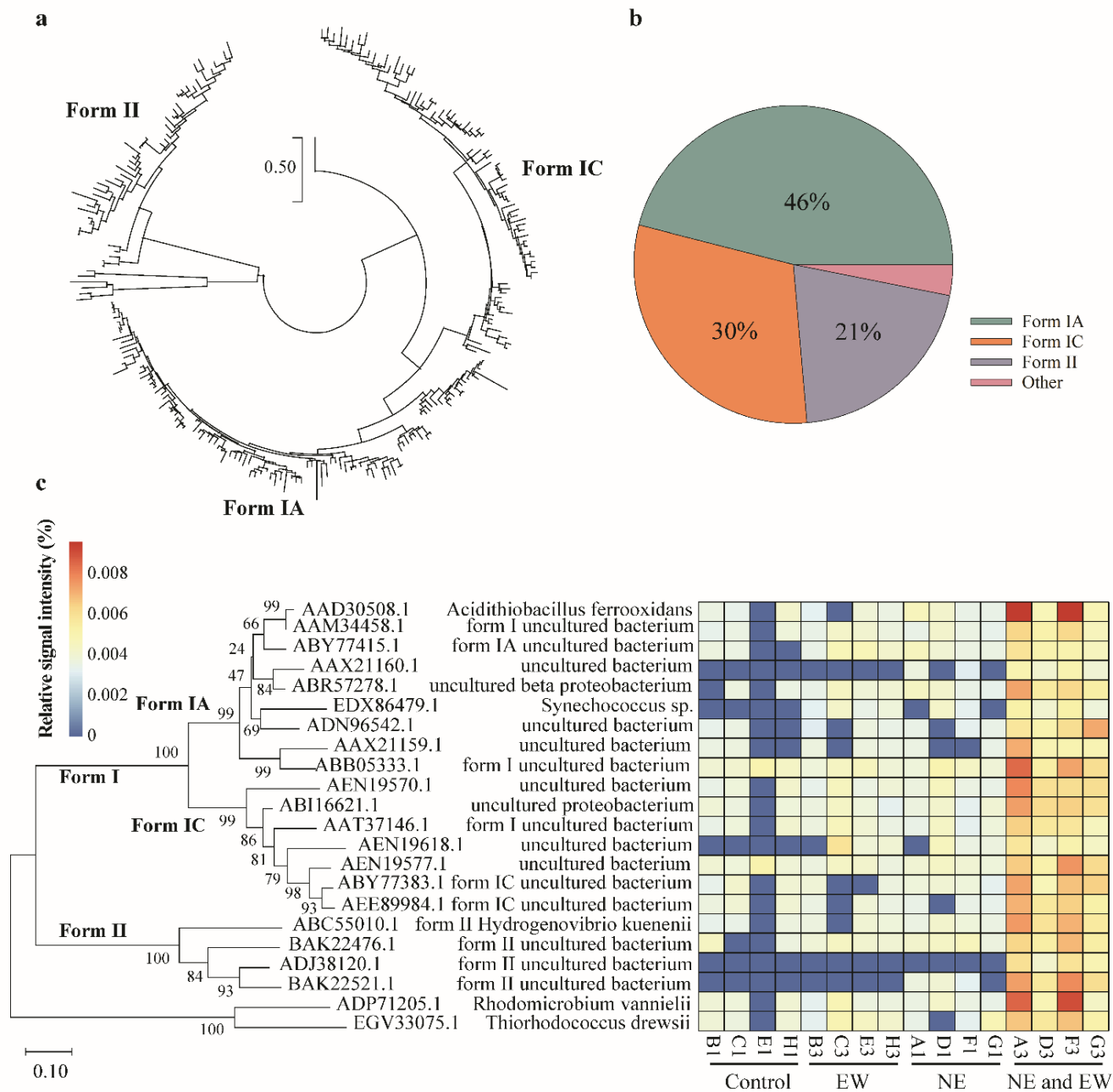
Figure S2 Rubisco genes detected in the shallow lake mesocosms. Phylogenetic tree

based on the overall rubisco large-subunit amino acid sequences obtained by GeoChip

hybridization. Tree topography and evolutionary distance are given by a

neighbor-joining method (**a**); percentages of different types of rubisco genes (**b**).

Phylogenetic tree based on the rubisco large-subunit amino acid sequences, which

differed significantly between the warming and nutrient-enriched treatments (**c**).

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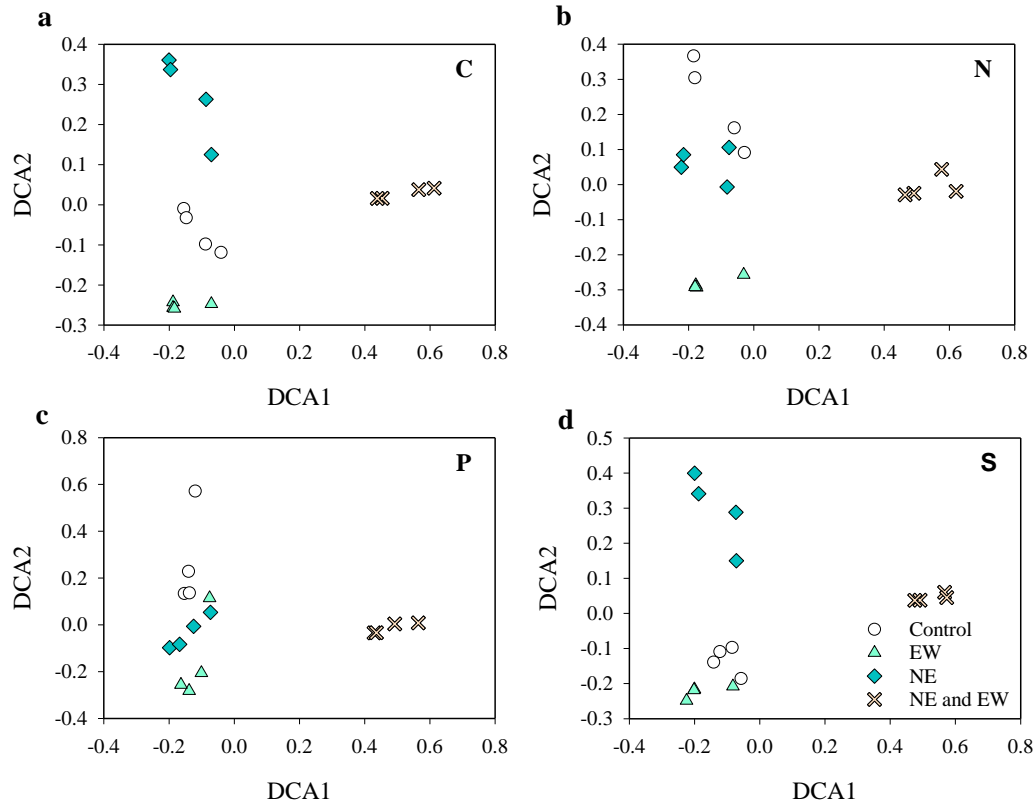
Figure S3 Microbial functional gene structure of the subcategory of C (**a**), N (**b**), P

(**c**), and S (**d**) cycling in response to warming and nutrients as identified by detrended

correspondence analysis (DCA). The treatments are termed as follows: ambient

temperature, unenriched (control); enhanced warming (A2 + 50%), unenriched (EW);

ambient temperature, nutrient-enriched (NE); and enhanced warming (A2 + 50%),

nutrient-enriched (NE and EW).

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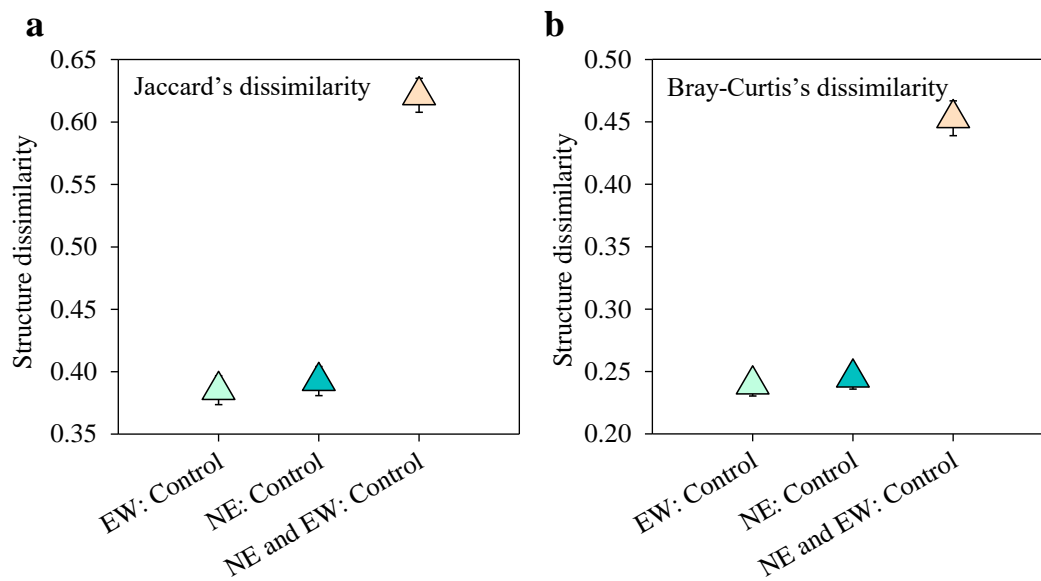
Figure S4 Structural dissimilarity of microbial functional genes composing the 9619

genes between the different warming and nutrient-enriched treatments and the

controls (**a**: based on Jaccard dissimilarity; **b**: based on Bray-Curtis dissimilarity). The

treatments are termed as follows: ambient temperature, unenriched (control);

enhanced warming (A2 + 50%), unenriched (EW); ambient temperature,

nutrient-enriched (NE); and enhanced warming (A2 + 50%), nutrient-enriched (NE

and EW). EW: Control, NE: Control, and NE and EW: Control mean the structure

dissimilarity of EW with control, NE with control, NE and EW with control,

respectively.
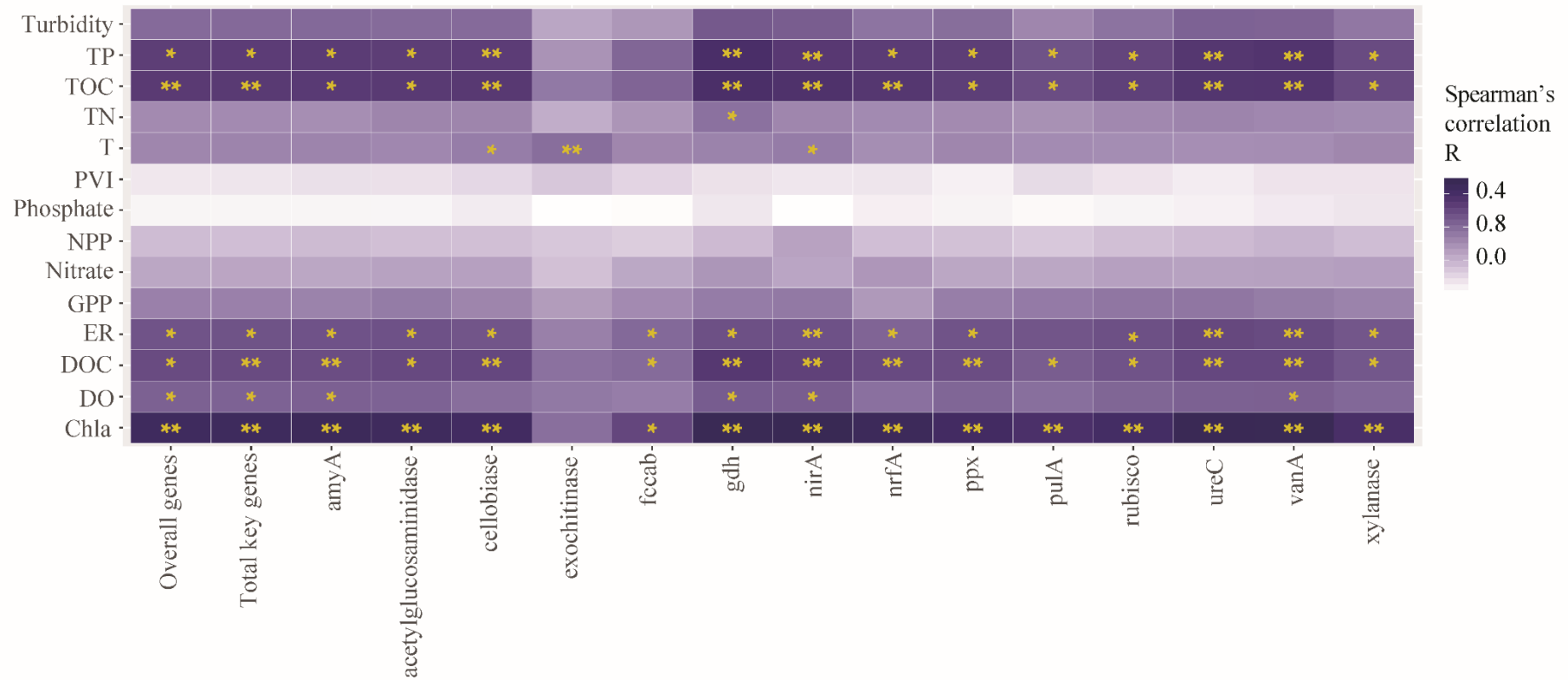
Figure S5 Spearman's correlations (R values) between transformed environmental and ecosystem metabolic variables and microbial functional gene structure (Bray-Curtis distance) as determined via Mantel tests (*: $p < 0.1$; **: $p < 0.05$).

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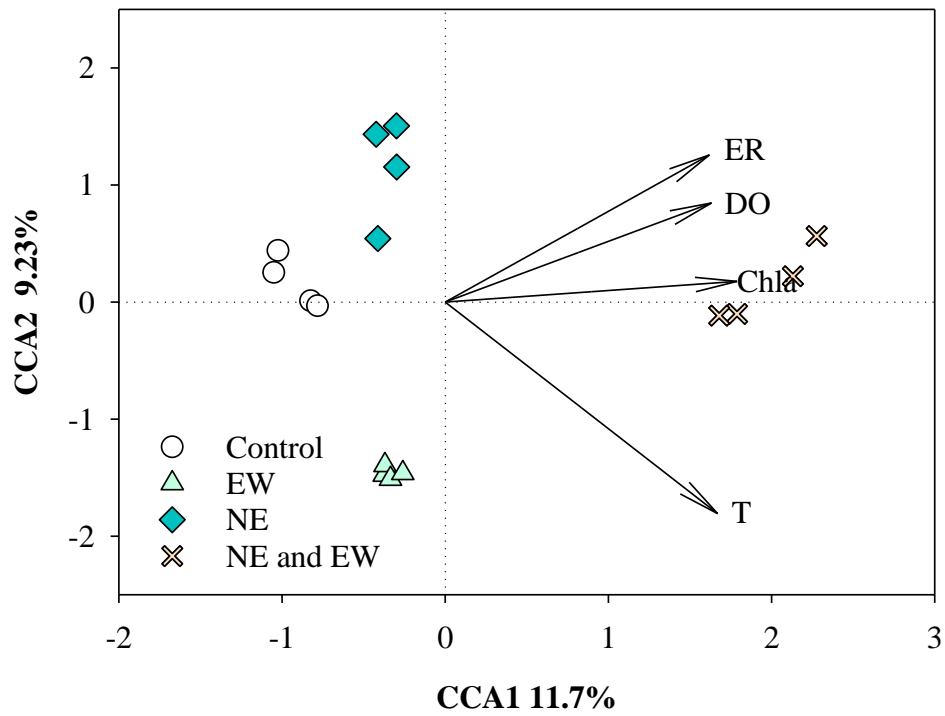
Figure S6 Canonical correlation analysis (CCA) of microbial functional gene

structure with environmental/ecosystem metabolic factors in the different warming

and nutrient-enriched treatments. Shown are factors significantly explaining the

microbial functional gene structure ($p < 0.05$). The treatments are termed as follows:

ambient temperature, unenriched (control); enhanced warming (A2 + 50%),

unenriched (EW); ambient temperature, nutrient-enriched (NE); and enhanced

warming (A2 + 50%), nutrient-enriched (NE and EW).

2.5

2.0

1.5

1.0

0.5

0.0

BPR ($\times 10^8$ cells l^{-1} h^{-1})

b

a

a

a

Control   EW   NE   NE and EW

94
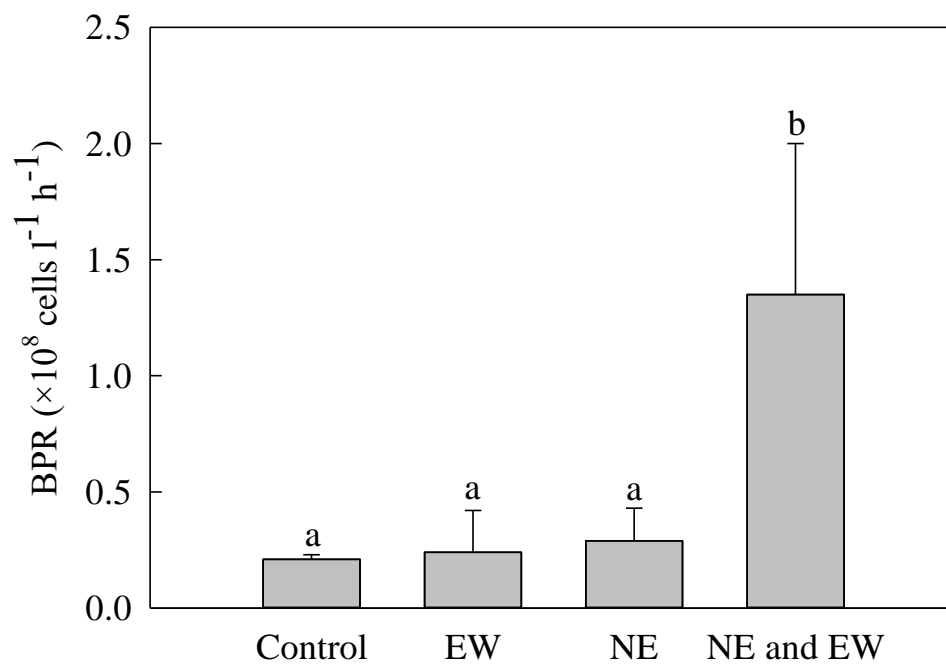
Figure S7 Bacterial production rates in the warming and nutrient-enriched treatments.

The treatments are termed as follows: ambient temperature, unenriched (control);

enhanced warming (A2 + 50%), unenriched (EW); ambient temperature,

nutrient-enriched (NE); and enhanced warming (A2 + 50%), nutrient-enriched (NE

and EW). Significant ($p < 0.05$) differences among treatments are indicated by

alphabetic letters above the bars according to post hoc comparisons.