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Genetic linkage of soil carbon pools and microbial functions in subtropical freshwater wetlands in response to experimental warming

Supplementary Information

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A. MATERIALS AND METHODS

SI-1: *Microcosm configuration*

A microcosm setup (Fig. S1) for simulating climate warming at a minute-scale under both daily and seasonal scenarios was developed for this study by using independently monitored water-bath jackets. Microcosms consisted of four major components: a storage section, a heating section, a water circulation section, and a real-time control section. The storage section was composed of two stainless steel incubation boxes: one for the present-day ambient temperature treatment (Control), and the other for the +5^{o}C-increased temperature treatment (Warmed). The real-time control section was composed of a computer (HP a6315cn), a custom-built controller (TZ2008, Jiaxing China), digital temperature probes (NB 407-25a, China), and lab-designed software (C^{++} language). The temperature probes, heater, and pump were programmed through the custom-built controller by the computer. With the help of the software, the temperatures in both incubation boxes were continuously recorded and differences in the box temperatures were compared by digital probes at two-minute intervals. The temperature difference between the two incubation boxes was set at 5^{o}C±1^{o}C. The custom-built controller simultaneously turned the heater and pump on or off when the temperature difference was less than 4^{o}C or more than 6^{o}C, respectively. Except for the computer and the controller, the rest of the microcosm components were set up outdoors in May 2008. This novel microcosm offers a high resolution temperature comparison, repeatability, and the capability for simulating more realistic warming conditions.

B. SUPPORTING TABLES

Table S1 Description and basic properties of wetland sites for this study

Wetland ID	County	Latitude and longitude	Main wetland use	Annual mean water depth, m	Annual mean flow rate, m min^{-1}	Dominant macrophytes
YaTang riverine wetland (YT)	TongXiang	120°29'13"E, 30°43'15"N	Mixed use	0.80	1.02	*Trapa bispinosa, Alternanthera philoxeroides, Trapa* spp, *Arundo dona*, *Arundo donax*
XiaZhuhu (XZ)	DeQing	120°02'54"E, 30°31'28"N	Tourism and aquaculture	1.50	0.12	*Trapa bispinosa, Alternanthera philoxeroides, Trapa* spp, *Arundo dona*, *Arundo donax*
XiXi national wetland park (XX)	HangZhou City	120°03'59"E, 30°16'23"N	Tourism	0.85	0.10	*Phragmites communis, Trapa spp, Acorus calamus, Sagittaria sagittifolia, Miscanthus floridulus*

Table S2 Overall microbial diversities and detected functional gene shifts by GeoChip analysis for samples collected from YT, XZ and XX wetland columns in the microcosm experiment under warmed and control treatments (Control: ambient temperature; Warmed: ambient temperature +5^{o}C). Values in parentheses are percentages.

Wetland columns		Genes		Simpson's		Total no. of genes detected
		Overlap	Uniqueness	Diversity Index (*1/D*)	*Evenness* (E_H)	
YT	C	10860 (*67.3*)	891 (*6.98*)	4218	0.33	12771
	W		1454 (*10.2*)	3948	0.28	14216
XZ	C	6600 (*54.8*)[a]	163 (*2.17*)	2010	0.27	7499
	W		518 (*4.64*)	3380	0.30	11156
XX	C	8502 (*63.3*)	415 (*4.09*)	2639	0.26	10147
	W		731 (*6.20*)	2895	0.25	11785
p-value		---	**0.033**	0.449	0.707	0.086

57 **Table S3** Top 20 genes with the highest normalized signal intensities involved in carbon degradation for starch, hemicellulose, cellulose, chitin
58 and lignin degradation detected in warmed and control samples from tested wetland columns in the microcosm experiment (Control: ambient
59 temperature; Warmed: ambient temperature + 5^{o}C). All data are expressed as mean, with standard deviation in parentheses. The *p*-values are
60 from Student's *t*-tests on the difference between warming and control treatments. Significant (< 0.05) and marginally significant (< 0.1) *p*-values
61 are in bold.

Gene information	Functional category	**Control**	**Warmed**	*p*-value
133778506 (uncultured bacterium)	Chitin	15.97 (8.24)	11.75 (3.93)	0.144
209503326 (Burkholderia sp. H160)	Cellulose	2.86 (0.95)	7.06 (1.55)	**0.007**
9105753 (Xylella fastidiosa 9a5c)	Chitin	7.94 (5.42)	6.66 (5.65)	**0.074**
40714547 (Trametes versicolor)	Lignin	3.23 (1.61)	6.38 (2.97)	**0.047**
262195882 (Haliangium ochraceum DSM 14365)	Starch	3.09 (2.10)	6.34 (3.66)	**0.035**
239930964 (Streptomyces ghanaensis ATCC 14672)	Chitin	2.32 (1.43)	5.65 (3.40)	**0.075**
256769453 (Streptomyces sp. C)	Lignin	1.71 (0.58)	5.40 (4.67)	0.130
29160339 (Botryosphaeria rhodina)	Cellulose	2.38 (0.92)	4.75 (1.18)	**0.007**
209516215 (Burkholderia sp. H160)	Cellulose	3.36 (1.34)	4.74 (0.14)	0.123
76876845 (Pseudoalteromonas haloplanktis TAC125)	Lignin	2.51 (0.23)	4.72 (4.43)	0.230
240278619 (Ajellomyces capsulatus H143)	Chitin	2.73 (0.96)	4.62 (2.70)	0.111
145304813 (Salinispora tropica CNB-440)	Cellulose	5.75 (1.54)	4.44 (0.29)	0.163
21929222 (Phanerochaete sordida)	Lignin	2.96 (1.80)	4.42 (2.16)	**0.061**
88814399 (marine actinobacterium PHSC20C1)	Hemicellulose	1.69 (0.58)	4.37 (1.61)	**0.070**
239982330 (Streptomyces albus J1074)	Chitin	3.72 (1.36)	4.23 (0.80)	0.217
256686144 (Jonesia denitrificans DSM 20603)	Chitin	2.16 (0.58)	4.01 (1.97)	0.160
115353435 (Chaetomium globosum)	Chitin	3.21 (2.02)	3.47 (2.85)	0.325
170654540 (Methylobacterium radiotolerans JCM 2831)	Starch	2.20 (1.44)	3.11 (1.80)	0.101
269923210 (Brevundimonas subvibrioides ATCC 15264)	Starch	1.33 (0.94)	3.04 (2.55)	0.119
239928121 (Streptomyces ghanaensis ATCC 14672)	Cellulose	2.11 (2.02)	2.98 (1.04)	0.267

C. SUPPORTING FIGURES

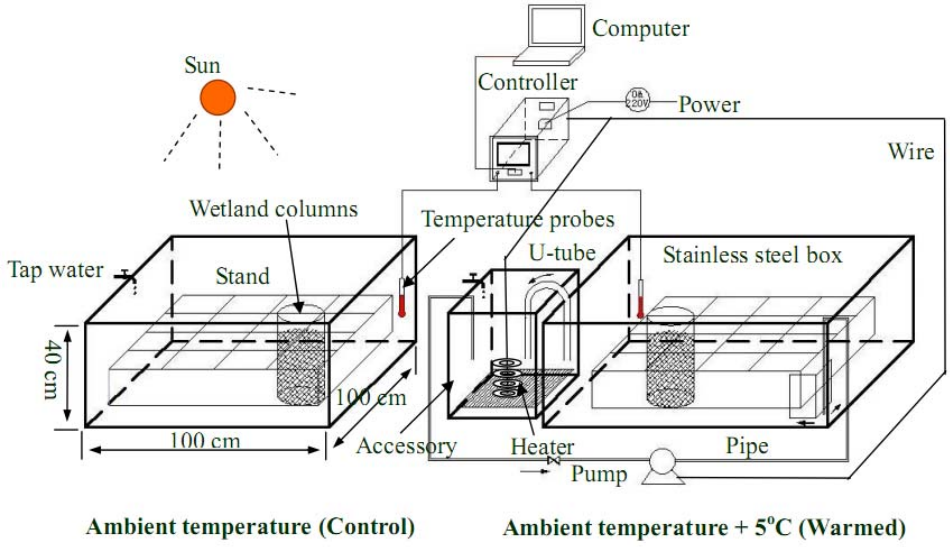

Fig. S1 Schematic of the experimental wetland microcosm system developed using independently monitored water-bath jackets under the current climate condition (Left: ambient temperature, control) and the simulated climate warming condition (Right: ambient temperature + 5^{o}C, warmed).

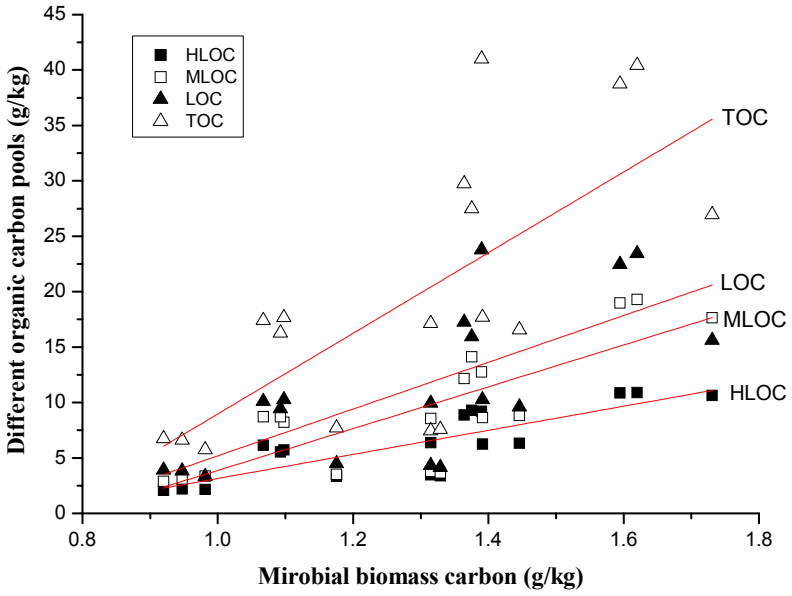

Fig. S2 Linear correlations ($p < 0.01$) between microbial biomass carbon (MBC) and soil organic carbon pools, including total organic carbon (TOC) and labile organic carbon pools (HLOC, MLOC, and LOC) for tested subtropical wetland soils in the microcosm experiment. The linear regression model for MBC and TOC is y = 36.4x - 27.4, adj. R^2 = 0.474; MBC and LOC: y = 21.1x – 15.9, adj. R^2 = 0.471; MBC and MLOC: y = 18.9x – 15.0, adj. R^2 = 0.623; MBC and HLOC: y = 10.9x – 7.7, adj. R^2 = 0.663.

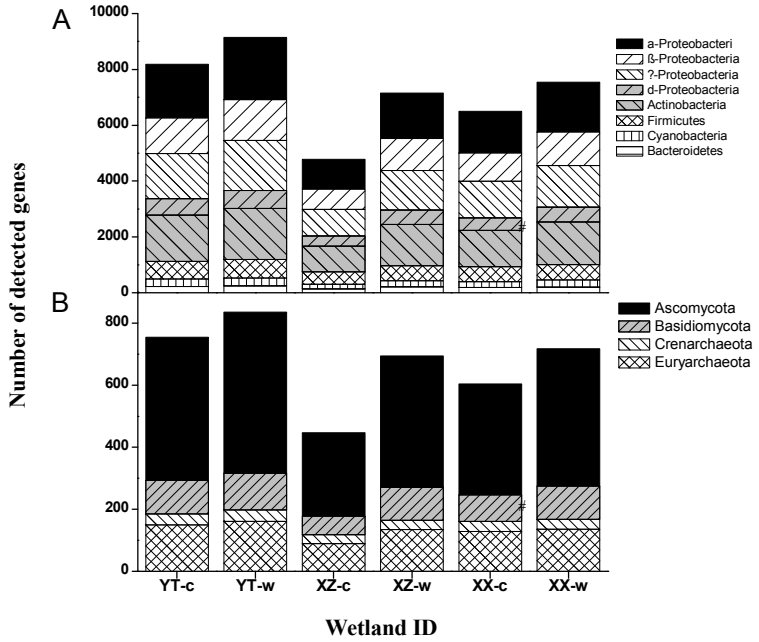

Fig. S3 Microbial community structure features of main phyla for (A) bacteria and (B) fungi and archaea as indicated by detected gene numbers under warmed and control treatments (Control: ambient temperature; Warmed: ambient temperature +5^{o}C). α-, β-, γ-, δ-Proteobacteria, Actinobacteria, Firmicutes and Cyanobacteria are phyla of bacteria; Ascomycota and Basidiomycota are phyla of fungi, and Crenarchaeota and Euryarchaeota are phyla of archaea.

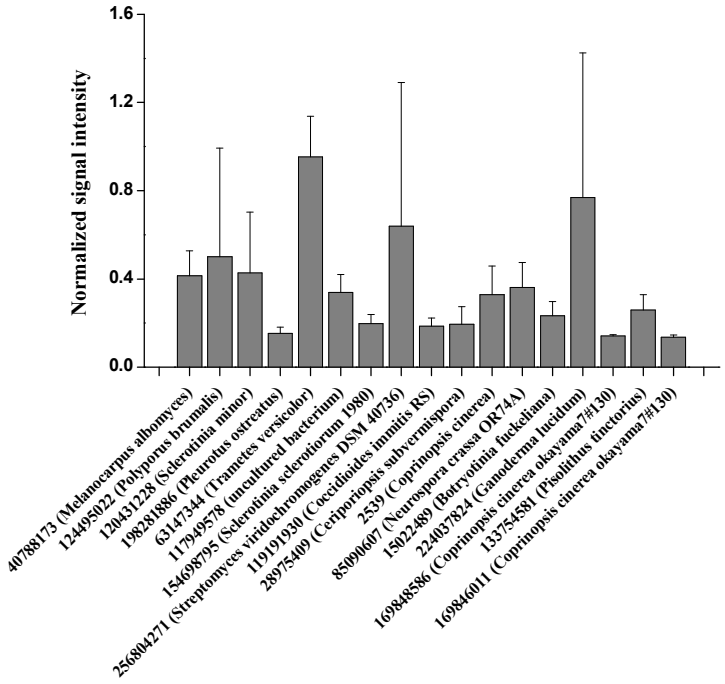

Fig. S4 Gene sequences responsible for lignin degradation involved in carbon cycling were detected in warmed samples but were absent in control samples. The signal intensity for each sequence was the average of the total signal intensity from all warmed samples. Error bars are + 1 standard deviation. Gene number on the x-axis is the protein ID number for each gene as listed in the GenBank database.