Supplementary Information (SI) for Environmental Science: Nano.

Supplementary Information for:

Montmorillonite loaded nano calcium peroxide as an effective material to mitigate methane emission in shallow lakes

Shiming Fan [a, b], Tong Li [a, b], Xuexin Han [a, b], Yu Gu [a, b], Biao Li [a, *], Qinglong L. Wu [a, b, c, d, e, *]

*

[a] Key Laboratory of Lake and Watershed, Science for Water Security, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing 210008, China

[b] University of Chinese Academy of Sciences, Nanjing 211100, China

[c] Center for Evolution and Conservation Biology, Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou), Guangzhou 511458, China

[d] Sino-Danish Centre for Science and Education, University of Chinese Academy of Sciences, Beijing 100039, China

[e] Fuxianhu Research Station for Plateau Deep Lake Ecosystem, Chinese Academy of Sciences, Chengjiang 652500, China

[*]Corresponding author: Biao Li (libiao@niglas.ac.cn) and Qinglong L. Wu (qlwu@niglas.ac.cn)

This file includes:

Supplementary results

Table S1 to S2

Figures S1 to S7

Supplementary results

In 16S rRNA gene amplicon sequencing, we obtained 125593-248590 high-quality sequences from 10 samples and 17515 OTUs by clustering 97% similarity. After addition of nanoCaO_2@MMT, the α-diversity indices of microbial community (Chao1, Shannon_e) in surface sediments were lower than the control (Fig. S6), but there was no significant difference (p>0.05). There were no significant differences in microbial community structure and composition at the phylum level (Fig.S7a, p>0.05), and further, based on relative abundance, the top ten most abundant genera were selected to analyze the prokaryotic microbial community composition, and the top ten species belonged to bacteria. They are *Sva0081_sediment_group*, *UTCFX1*, *LD29*, *Nitrospira*, *Luteolibacter*, *OLB12*, *Odorifustis*, *Dechloromonas* and *Candidatus_Competibacter*, *Ellin6067*, respectively (Fig. S7b). The top three bacterial species in the additive group were *Sva0081_sediment_group* (11.01 ± 1.11%), *UTCFX1* (7.54 ± 1.07%) and *LD29* (5.30 ± 1.56%), while the top three species in the control were *Sva0081_sediment_group* (10.84 ± 2.56%), *Luteolibacter* (6.20 ± 3.56%) and *UTCFX1LD* (5.05 ± 0.93%). The results of Anosim analysis showed that the microbial community composition of the sediments was significantly different from the control after nanoCaO_2@MMT addition (p<0.05).

We obtained 65241-354362 high quality sequences related to methanogenic functional gene (*mcrA*) and 90351-181034 high quality sequences for the methanotrophic functional gene (pmoA) from 10 samples. It was found that the addition of nanoCaO_2@MMT increased Chao1 richness and Shannon_e diversity index of methanogens and methanotrophs simultaneously (Fig. S6), but the difference from the control was still not significant (p>0.05).

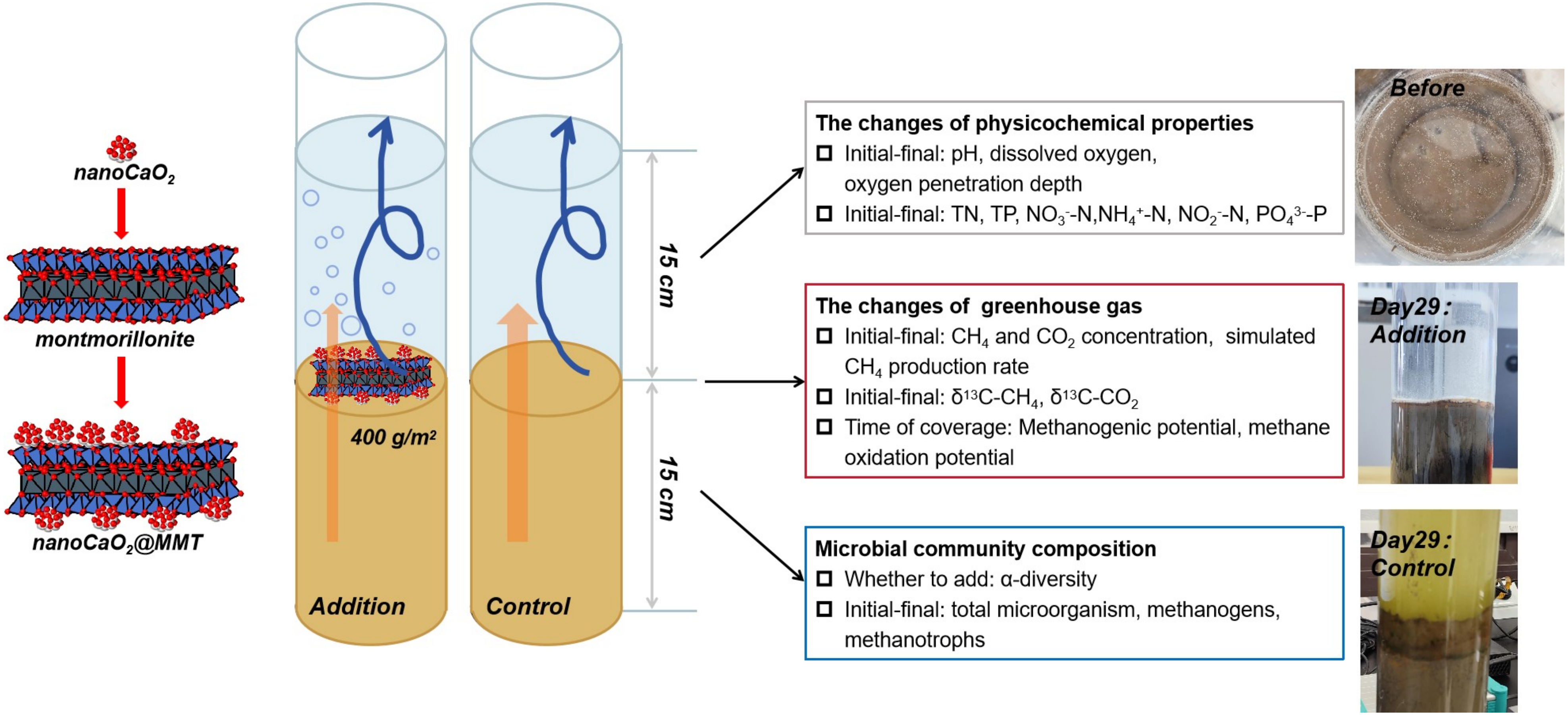
Table. S1 Basic physicochemical properties of sediments in the sediment core incubation experiment.

	TN (g/kg)	TC (%)	TOC (%)	TP (g/kg)	C/N
Mean ± Std.	0.260 ± 0.08	2.90 ± 0.95	2.08 ± 0.57	0.99 ± 0.15	8.219 ± 1.73

Table. S2 Basic physicochemical properties of lake water in the sediment core incubation experiment.

	SAL (‰)	pH	NO_3^--N (mg/L)	NO_2^--N (mg/L)	NH_3^--N (mg/L)	PO_4^{3-}-P (mg/L)
Mean ± Std.	0.16 ± 0.03	8.34 ± 0.42	0.556 ± 0.433	0.029 ± 0.021	0.121 ± 0.135	0.0032 ± 0.0043
	DTP (mg/L)	DTN (mg/L)	TP (mg/L)	TN (mg/L)	Chl.a (μg/L)	
Mean ± Std.	0.024 ± 0.010	0.945 ± 0.490	0.078 ± 0.029	1.41 ± 0.417	19.98 ± 11.87	

Fig. S1 Summary of the sediment core incubation experimental design to quantify the effect of nanoCaO_2@MMT addition in the reduction of CH_4 in sediment.



The changes of physicochemical properties

- ☐ Initial-final: pH, dissolved oxygen, oxygen penetration depth
- ☐ Initial-final: TN, TP, NO_3^--N, NH_4^+-N, NO_2^--N, PO_4^{3-}-P

The changes of greenhouse gas

- ☐ Initial-final: CH_4 and CO_2 concentration, simulated CH_4 production rate
- ☐ Initial-final: $\delta^{13}C$-CH_4, $\delta^{13}C$-CO_2
- ☐ Time of coverage: Methanogenic potential, methane oxidation potential

Microbial community composition

- ☐ Whether to add: α-diversity
- ☐ Initial-final: total microorganism, methanogens, methanotrophs

Fig. S2 Differences in CH_4 and CO_2 concentrations water column between the additive and control groups.

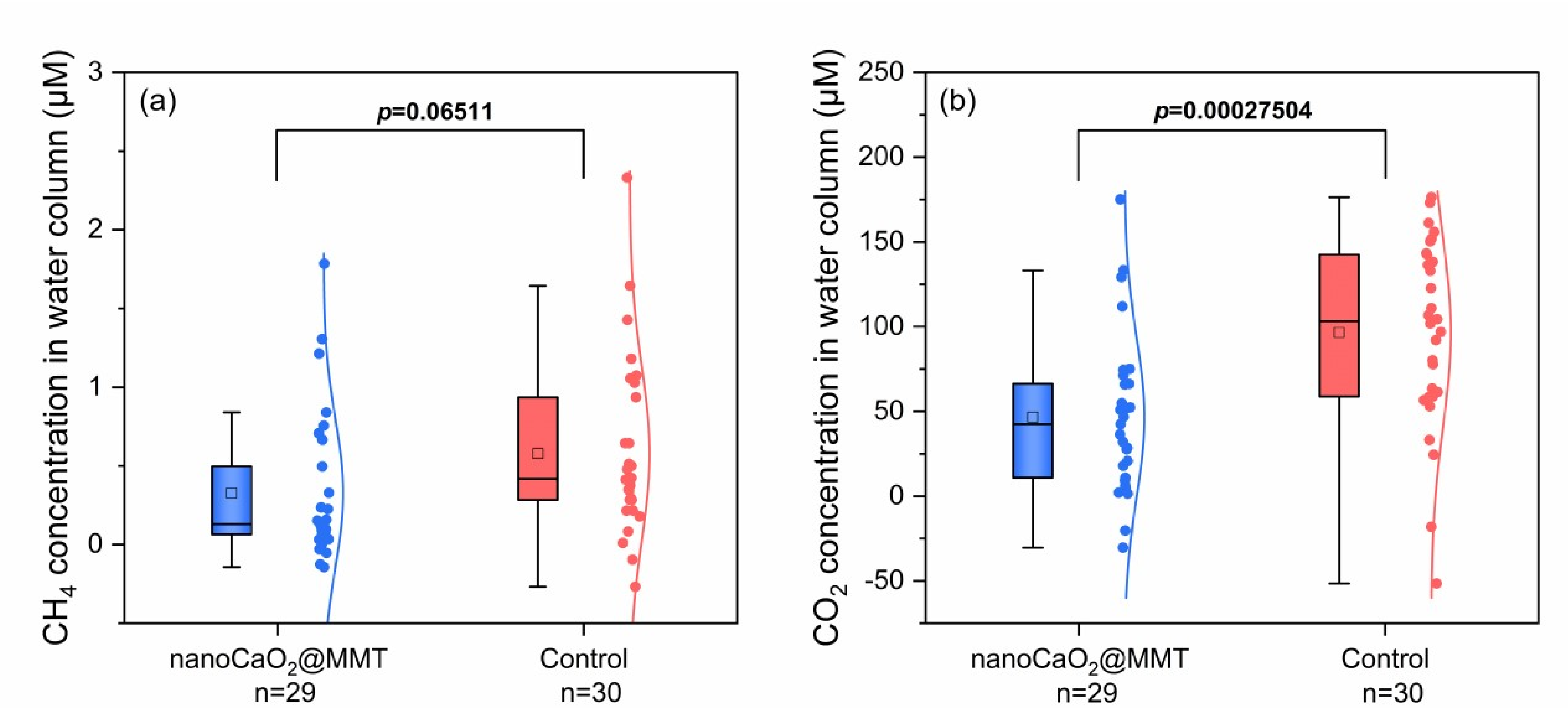


Fig. S3 The relationship between the actual concentration and the CH_4 concentration predicted by PROFILE model.

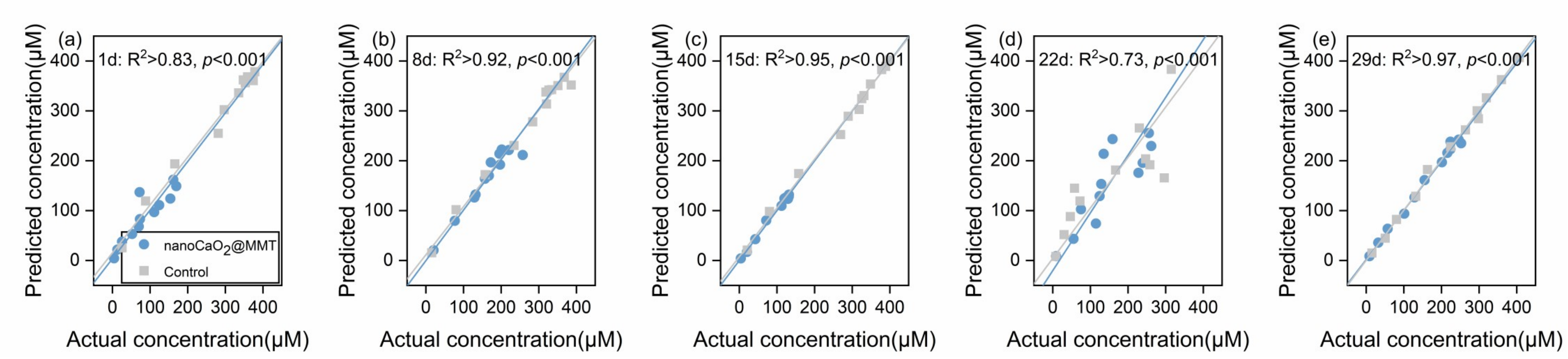


Fig. S4 Distribution of storage CO_2 along sediment depth. **(a)** pre-addition, **(b)** 1d, **(c)** 8d, **(d)** 15d, **(e)** 22d, **(f)** 29d.

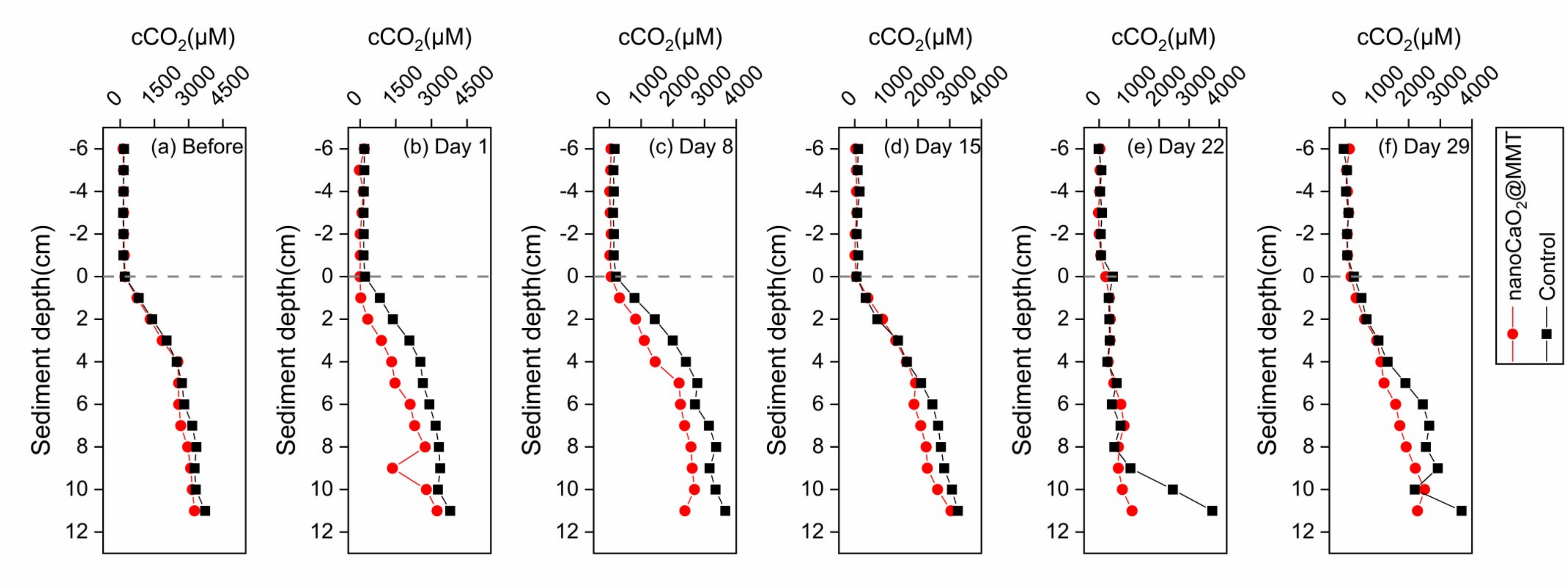


Fig. S5 Distribution of δ^{13}C-CH$_4$ and δ^{13}C-CO$_2$ along sediment depth, **(a)**pre-addition, **(b)**1d, **(c)**8d, **(d)**15d, **(e)**22d, **(f)**29d, where the solid and dashed lines represent δ^{13}C-CH$_4$ and δ^{13}C-CO$_2$, respectively, and the red and blue represent nanoCaO$_2$@MMT and Control, respectively.

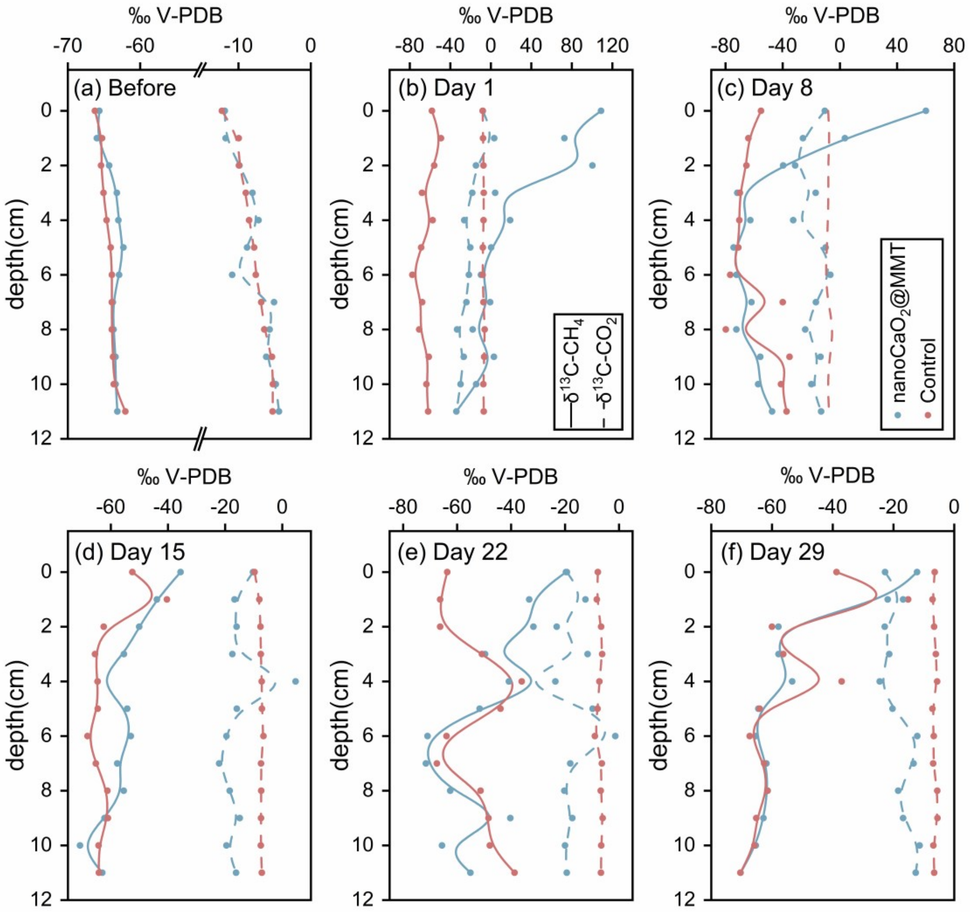

‰ V-PDB
-70
-60
-10
0
(a) Before
depth(cm)
0
2
4
6
8
10
12
‰ V-PDB
-80
-40
0
40
80
120
(b) Day 1
δ13C-CH4
δ13C-CO2
‰ V-PDB
-80
-40
0
40
80
(c) Day 8
nanoCaO2@MMT
Control
‰ V-PDB
-60
-40
-20
0
(d) Day 15
‰ V-PDB
-80
-60
-40
-20
0
(e) Day 22
‰ V-PDB
-80
-60
-40
-20
0
(f) Day 29

Fig. S6. Microbial community diversity index, **(a)**Chao1, **(b)**Shannon_e, **(c)**Simpson.

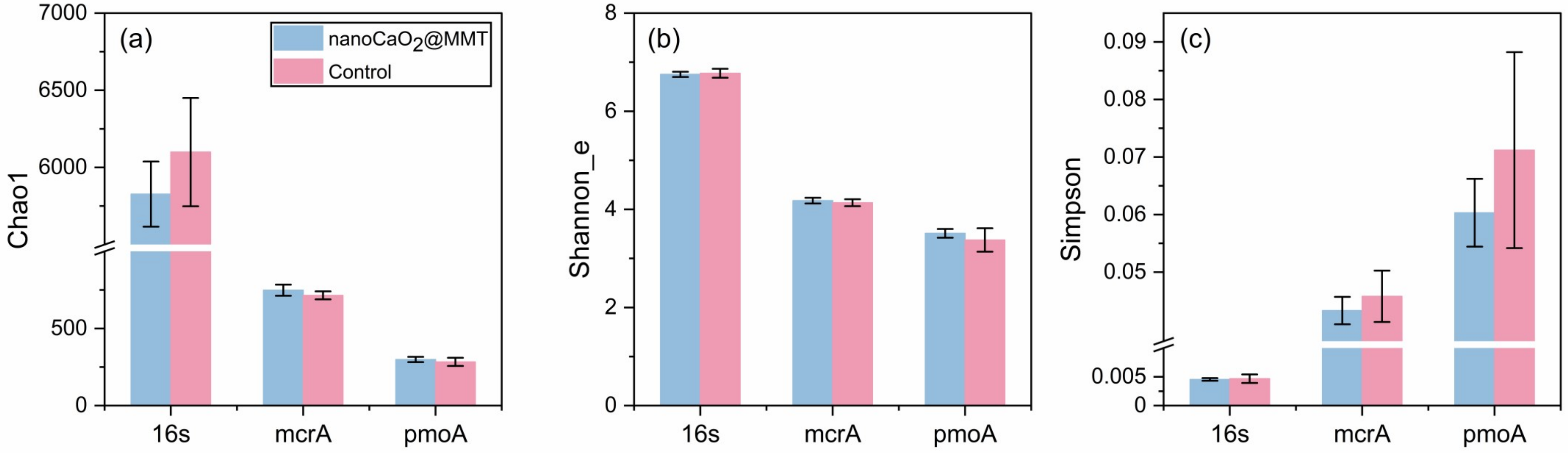


Fig. S7. Relative abundance of the prokaryotic microbial community at the (a) phylum level and (b) genus level.