

Enhanced Adsorptive Bioremediation of Heavy Metals (Cd²⁺, Cr⁶⁺, Pb²⁺) by Methane-Oxidizing Epipelton

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Supplementary References

Table S1. Primers and PCR conditions used in this study

Primer name	Primer sequence (5'-3')	Targeted gene	Thermal condition	Molecular analysis	Reference
515F	GTGCCAGCMGCCGCGG	universal 16S rRNA gene	95 °C, 5 min; 33× (95 °C, 30 s; 54 °C, 30 s; 72 °C, 30 s); 72 °C, 8 min	MiSeq sequencing	[1]
907R	CCGTCAATTCMTTTRAGTTT				
A189F	GGNGACTGGGACTTCTGG	<i>pmoA</i> gene	95 °C, 3 min; 35× (95 °C, 10 s; 54 °C, 30 s; 72 °C, 30 s; 80 °C, 5 s; plate read); melt curve 65 °C to 95 °C, incremental 0.5 °C, 0:05+plate read	Real-time PCR	[2,3]
mb661r	CCGGMGCAACGTCYTTACC		95 °C, 5 min; 35 95 °C, 30s; 54 °C, 30 s; 72 °C, 45 s); 72 °C, 10 min	MiSeq sequencing	

Table S2. Summary of MiSeq amplicon sequencing of 16S rRNA genes from total DNA sample

Treatment	High Quality Read Number		
	Replicate-1	Replicate-2	Replicate-3
6 ppm ¹³CH₄	46980	48980	51980
120000 ppm ¹³CH₄	54713	571157	55654
Cr+120000 ppm ¹³CH₄	23343	27124	24463
Pb+120000 ppm ¹³CH₄	55454	66328	56110
Cd+120000 ppm ¹³CH₄	43119	45264	40069

Table S3. Summary of Illumina MiSeq amplicon sequencing of *pmoA* genes from total DNA

Treatment	High Quality Read Number	
	16S rRNA gene	<i>pmoA</i> gene
120000 ppm ¹³ CH ₄	59575	17145
Cr+120000 ppm ¹³ CH ₄	21432	5600
Pb+120000 ppm ¹³ CH ₄	58616	14521
Cd+120000 ppm ¹³ CH ₄	55432	12518

Table S4 Topological properties of the co-occurrence networks of lake microbial communities

Network parameter	Cr+120000 ppm ¹³CH₄	Pb+120000 ppm ¹³CH₄	Cd+120000 ppm ¹³CH₄	120000 ppm ¹³CH₄	6 ppm ¹³CH₄
Nodes	156	256	269	287	178
Edges	2128	4228	4526	5128	2728
Average Degree	1.11	1.65	1.71	1.87	1.23
Network diameter	10	10	10	10	10
Graph density	0.0952	0.166	0.173	0.182	0.097
Modularity	0.17	0.18	0.19	0.193	0.181
Network distance	1.65	2.23	2.34	2.54	1.88

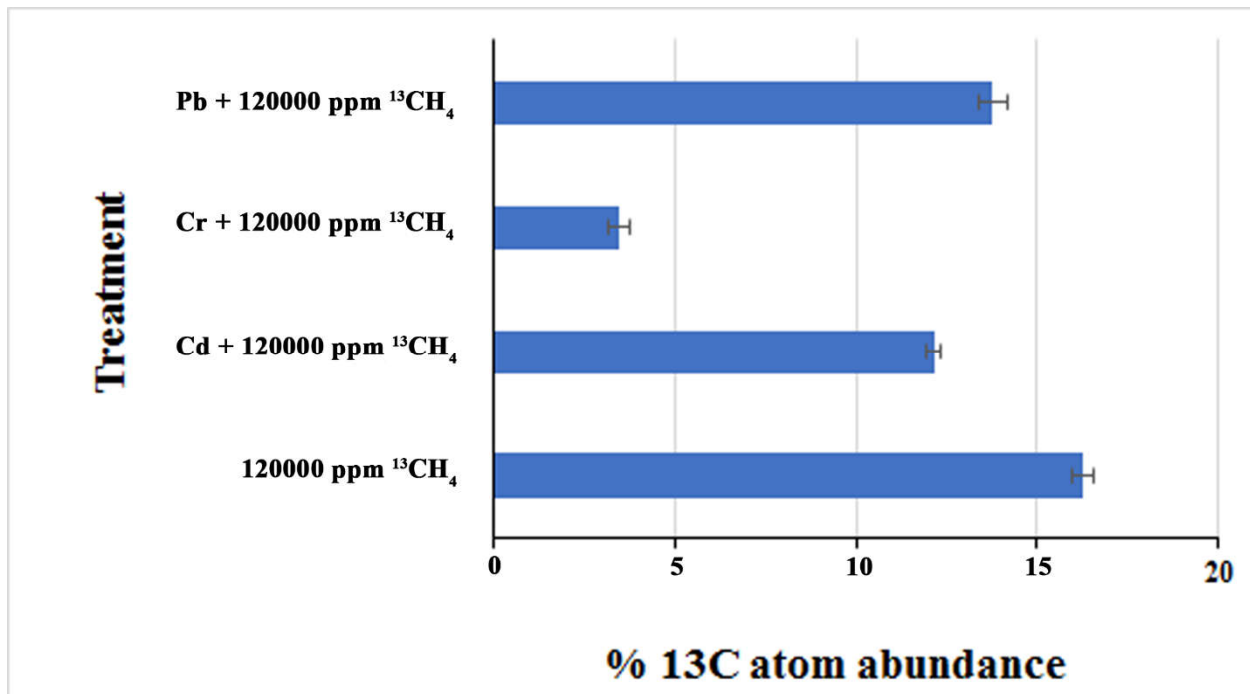


Fig S1. Percentage of ^{13}C atoms abundance accumulated by methanotrophs in Epipelon

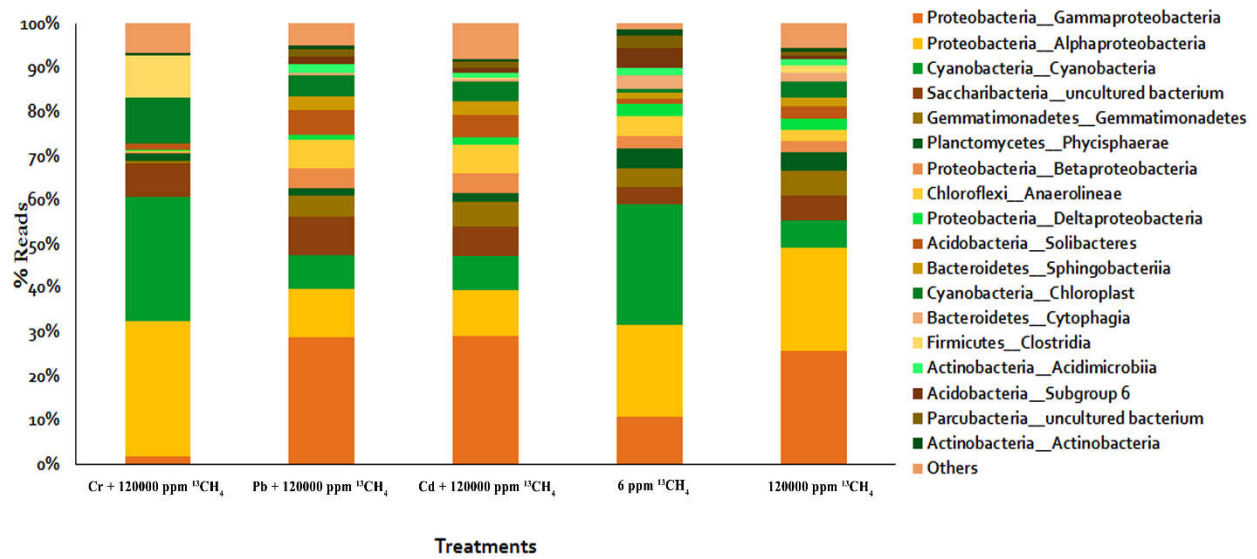


Figure S2. The changes in community composition of microbes following microcosm incubation with respective methane doses based on high throughput sequencing of 16S rRNA genes at class level.

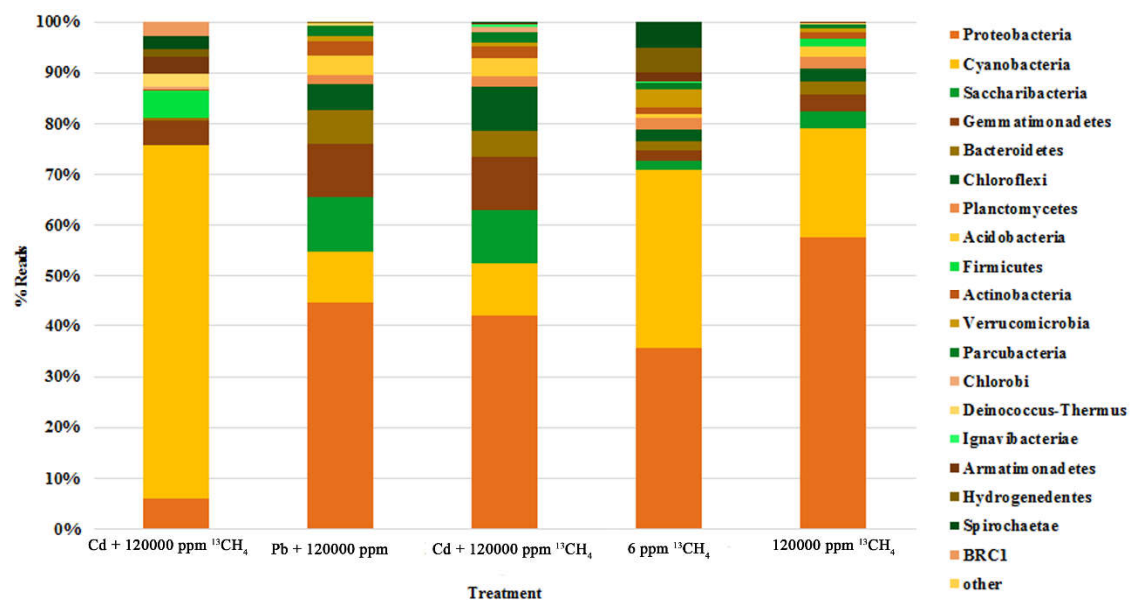


Figure S3. The changes in community composition of microbes following microcosm incubation with respective methane doses based on high throughput sequencing of 16S rRNA genes at phylum level.

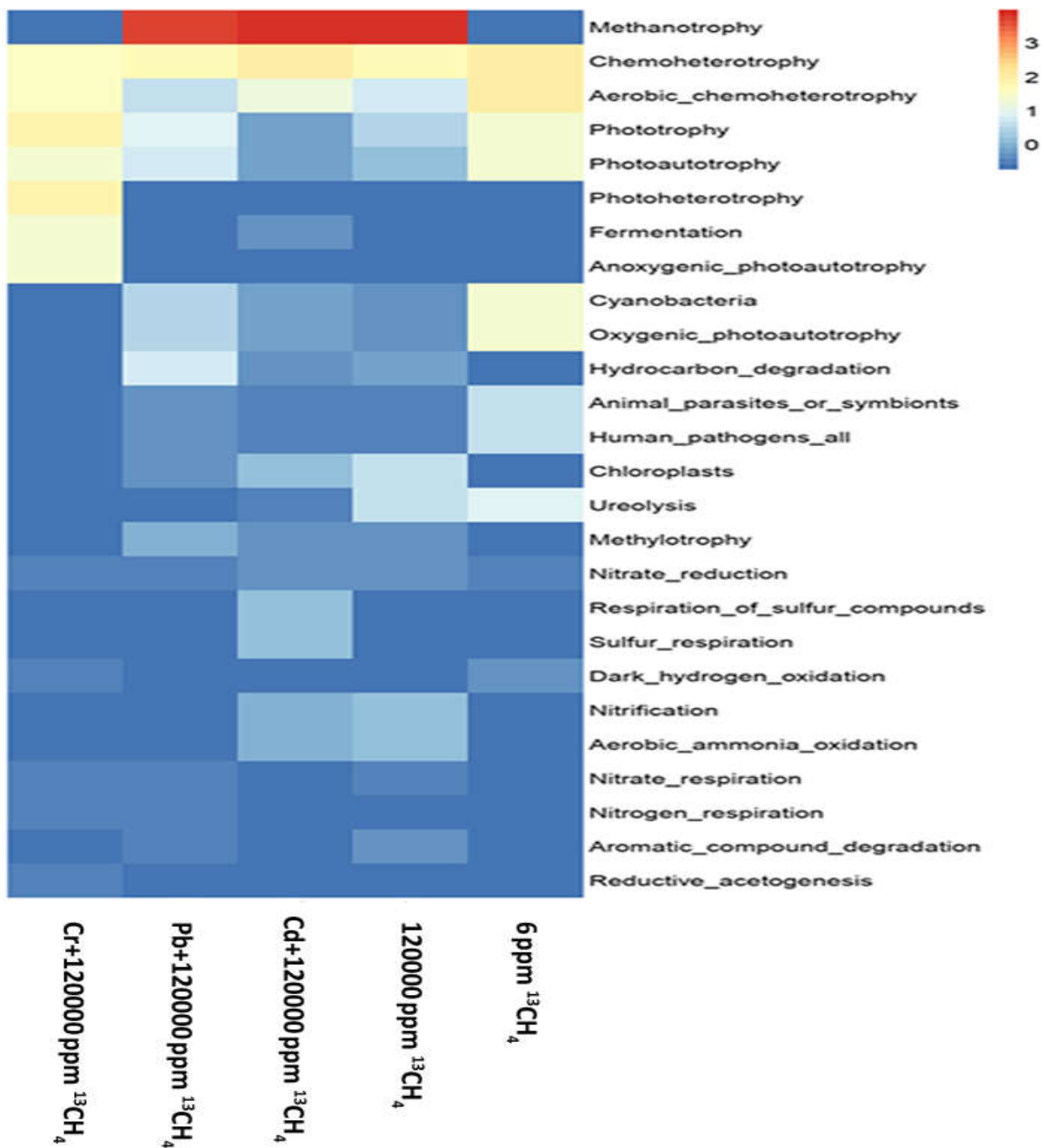


Figure S4. Functional phenat map of Epipelton under different methane and metals doses

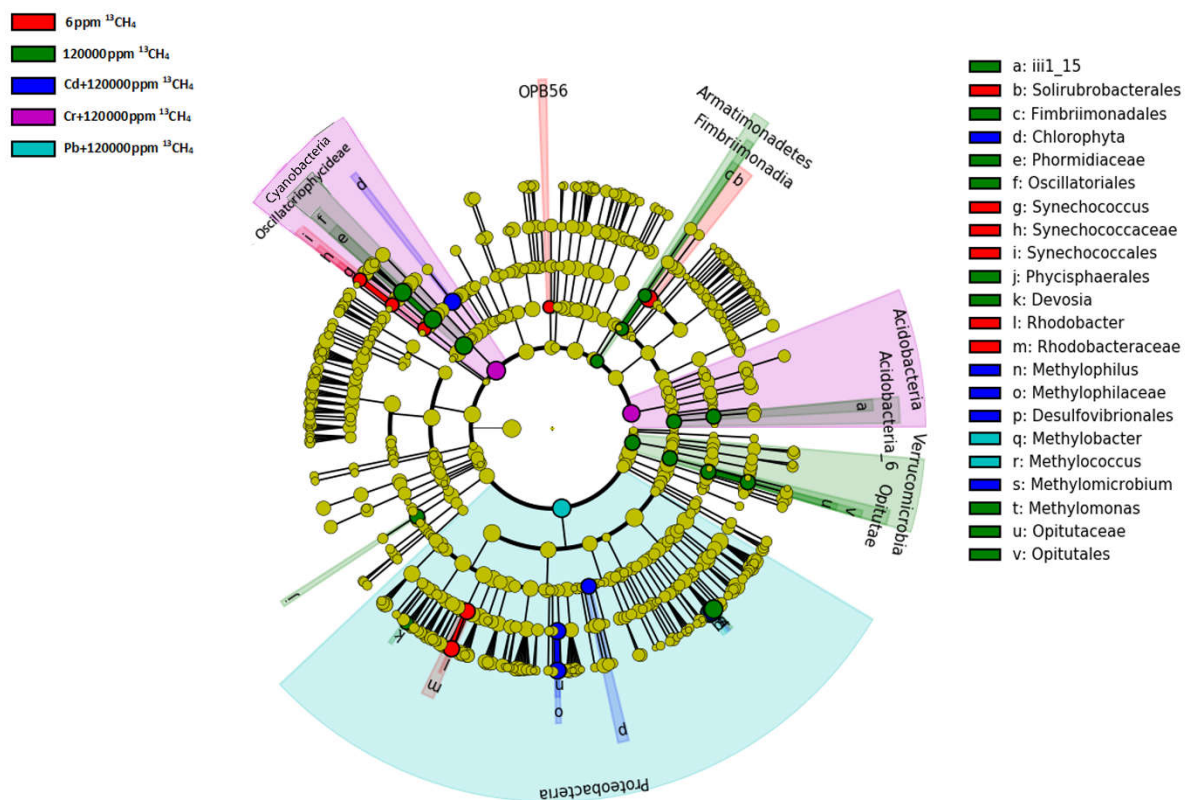


Figure S5. linear discriminant analysis effect size pipeline (LEfSe) analysis for dominant phyla, class and species.

References

1. Stubner, S. Enumeration of 16S rDNA of Desulfotomaculum lineage 1 in rice field soil by real-time PCR with SybrGreen™ detection. *Journal of Microbiological Methods* **2002**, *50*, 155-164, doi:[https://doi.org/10.1016/S0167-7012\(02\)00024-6](https://doi.org/10.1016/S0167-7012(02)00024-6).
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