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Competitive interactions between methane- and ammonia-oxidizing bacteria modulate carbon and nitrogen cycling in paddy soil

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Supplemental Material for

Competitive interactions between methane- and ammonia oxidizing bacteria modulate carbon and nitrogen cycling in paddy soil

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Table S1. The scenario of SIP microcosm construction over the course of 19 days of incubation

Treatment	^{13}C-CH_4	^{13}C-Urea	^{13}C-CH_4+Urea	^{12}C-CH_4+Urea	^{13}C-CH_4	^{13}C-Urea	^{13}C-CH_4+Urea	^{12}C-CH_4+Urea	^{13}C-CH_4	^{13}C-Urea	^{13}C-CH_4+Urea	^{12}C-CH_4+Urea
	CH_4 added (ppmv)*				Urea added (μg N g^{-1} *d.w.s.*)*				CO_2 added (ppmv)*			
Day-0 -18:00pm†	9460	---	9322	9035	---	100	100	100	---	50000	50000	50000
Day-5- 8:00‡	The destructive sampling performed and the remaining microcosms were flushed with pressurized synthetic air (20% O_2, 80% N_2)											
Day-5-18:00 pm	6114	---	7770	6821	---	100	100	100	---	50000	50000	50000
Day-7-18:00 pm	7946	---	8020	6362	---	---	---	---	---	---	---	---
Day-8-20:00 pm	8355	---	8018	8482	---	---	---	---	---	---	---	---
Day-9-18:00 pm	---	---	6755	7067	---	---	---	---	---	---	---	---
Day-10-10:00am	---	---	6201	6718	---	---	---	---	---	---	---	---
Day-10-18:00 pm	---	---	9766	9552	---	---	---	---	---	---	---	---
Day-11-10:00am	---	---	9113	9164	---	---	---	---	---	---	---	---
Day-11-20:00 pm	---	---	9229	9541	---	---	---	---	---	---	---	---
Day-12-10:00am‡	The microcosms were flushed with pressurized synthetic air (20% O_2, 80% N_2).											
Day-12-21:00pm	18947	---	16081	15720	---	100	100	100	---	50000	50000	50000
Day-14-11:00am	---	---	11724	14355	---	---	---	---	---	---	---	---
Day-14-21:00pm	---	---	11762	12491	---	---	---	---	---	---	---	---
Day-15-11:00pm	---	---	8678	10431	---	---	---	---	---	---	---	---
Day-15-21:00pm	---	---	9591	11225	---	---	---	---	---	---	---	---
Day-16-11:00pm	---	---	17923	18598	---	---	---	---	---	---	---	---
Day-16-21:00pm	---	---	18190	17706	---	---	---	---	---	---	---	---
Day-17-11:00am	---	---	8792	10788	---	---	---	---	---	---	---	---
Day-17-21:00pm	---	---	17533	15901	---	---	---	---	---	---	---	---
Day-18-11:00am	---	---	10286	10430	---	---	---	---	---	---	---	---
Day-18-21:00pm	---	---	11928	12638	---	---	---	---	---	---	---	---
Day-19-11:00am ‖	The destructive sampling performed.											

* The amount of substrate added to microcosms. The ^{13}C and ^{12}C-substrates were used for labeled and control microcosms, respectively.

† The timing of substrate added to microcosms, and the numbers in brackets indicate the time of day.

‡ The date of SIP microcosms were flushed with pressurized synthetic air (20%O_2, 80%N_2), and subsequently amended with fresh substrate.

--- No substrate added

Table S2. Primers and PCR conditions used in this study

Primer name	primer sequence(5'-3')	Targeted gene	Thermal Profile	Molecular analysis	Reference
515F	CCAGCMGCCGCGG	16S rRNA gene	95℃,3.0min;30×(95℃,30s; 55℃, 30s;72℃, 45s);72℃,10min	Pyrosequencing	(Xia et al., 2011)
907R	CCGTCAATTCMTTTRAGTTT				
A189F	GGNGACTGGGACTTCTGG	*pmoA* gene	95℃,3.0min;40×(95℃,10s; 55℃, 30s;72℃, 30s; 80℃ 5s; with plate read); melt curve 65℃ to 95℃, incremental0.5℃, 0:05+plate read	Real-time PCR	(Costello and Lidstrom, 1999; Holmes et al., 1995)
mb661r	CCGGMGCAACGTCYTTACC		95℃,3.0min;30×(95℃,30s; 55℃, 30s;72℃, 45s); 72℃,10min	Pyrosequencing	
amoA-1F	GGGGTTTCTACTGGTGGT	bacterial *amoA* gene	95℃,3.0min;40×(95℃,10s; 55℃, 30s;72℃, 30s; with plate read); melt curve 65℃ to 95℃, incremental 0.5℃, 0:05+plate read	Real-time PCR	(Rotthauwe et al., 1997)
amoA-2R	CCCCTCGGGAAAGCCTTCTTC		95℃,3.0min;30×(95℃,30s; 55℃, 30s;72℃, 45s); 72℃,10min	Pyrosequncing	
Arch-amoAF	STAATGGTCTGGCTTAGACG	Archaeal *amoA* gene	95℃,10.0min;40×(95℃,30s; 55℃, 45s;72℃, 30s;82℃ 15s with plate read); melt curve 65℃ to 95℃, incremental 1.0℃, 0:05+plate read	Real-time PCR	(Francis et al., 2005)
Arch-amoAR	GCGGCCATCCATCTGTATGT				

Table S3. Pyrosequencing summary of the total microbial communities in SIP microcosms using the universal primers 515F-907R of the total 16S rRNA genes

Treatment*		Pyrosequencing reads number†				
		High-quality read number	Bacteria	Archaea	MOB	AOB
Zero time	Zero Time-R1	9519	9218(96.8%)	290(3.05%)	28 (0.29%)	22 (0.23%)
	Zero Time-R2	9110	8775(96.3%)	327(3.59)	26 (0.29%)	16 (0.18%)
	Zero Time-R3	9369	9082(96.9%)	276(2.95)	24 (0.26%)	22 (0.23%)
Day-5	^{13}C-CH_4-R1	7758	7593(97.9%)	160(2.06%)	1252 (16.1%)	12 (0.15%)
	^{13}C-CH_4-R2	8630	8381(97.1%)	244(2.83%)	1273 (13.5%)	16 (0.19%)
	^{13}C-CH_4-R3	8829	8626(97.7%)	202(2.29%)	1192 (13.5%)	18 (0.20%)
	^{13}C-Urea-R1	7803	7525(96.4%)	273(3.50%)	31 (0.40%)	10 (0.13%)
	^{13}C-Urea-R2	7807	7607(97.4%)	199(2.55%)	13 (0.17%)	26 (0.33%)
	^{13}C-Urea-R3	6541	6372(97.4%)	167(2.55%)	17 (0.26%)	24 (0.37%)
	^{13}C-CH_4+Urea-R1	7431	7290(98.1%)	138(1.86%)	1637 (22.0%)	15 (0.20%)
	^{13}C-CH_4+Urea-R2	8372	8210(98.1%)	157(1.88%)	1633 (19.5%)	30 (0.36%)
	^{13}C-CH_4+Urea-R3	7568	7435(98.2%)	129(1.70%)	1559 (20.6%)	22 (0.29%)
	^{12}C-CH_4+Urea-R1	6995	6826(97.6%)	166(2.37%)	1109 (15.9%)	9 (0.13%)
	^{12}C-CH_4+Urea-R2	8083	7944(98.3%)	133(1.65%)	1782 (22.1%)	12 (0.15%)
	^{12}C-CH_4+Urea-R3	7809	7648(97.9%)	157(2.01%)	1433 (18.4%)	11 (0.14%)
Day-19	^{13}C-CH_4-R1	10104	9821(97.2%)	274(2.71%)	640 (6.33%)	18 (0.18%)
	^{13}C-CH_4-R2	41172	40101(97.4%)	1045(2.54%)	3330 (8.09%)	86 (0.21%)
	^{13}C-CH_4-R3	41230	40426(98.0%)	788(1.91%)	3235 (7.85%)	104 (0.25%)
	^{13}C-Urea-R1	8294	7907(95.3%)	375(4.52%)	23 (0.28%)	26 (0.31%)
	^{13}C-Urea-R2	31675	30450(96.1%)	1205(3.80%)	110 (0.35%)	125 (0.39%)
	13C-Urea-R3	44313	43117(97.3%)	1181(2.67%)	129 (0.29%)	149 (0.34%)
	^{13}C-CH_4+Urea-R1	10370	10245(98.8%)	122(1.18%)	2961 (28.6%)	12 (0.12%)
	^{13}C-CH_4+Urea-R2	7309	7190(98.4%)	118(1.61%)	1963 (26.9%)	7 (0.10%)
	^{13}C-CH_4+Urea-R3	6494	6402(98.6%)	91(1.40%)	1955 (30.1%)	15 (0.23%)
	^{12}C-CH_4+Urea-R1	9485	9299(98.0%)	183(1.93%)	2672 (28.2%)	13 (0.14%)
	^{12}C-CH_4+Urea-R2	7695	7595(98.7%)	96(1.25%)	2129 (27.7%)	9 (0.12%)
	^{12}C-CH_4+Urea-R3	6663	6573(98.6%)	85(1.28%)	1750 (26.3%)	13 (0.20%)
	Average	12831				
	Total reads	346428	337658	8581		

*: The designation of R1 to R3 represents triplicate microcosm incubations.

†: The value in parentheses represents the percentage of the targeted 16S rRNA phylotype reads to total 16S rRNA gene sequence reads in each microcosm.

--- Not detected

Table S4. Pyrosequencing summary of the total microbial communities in the fractionated DNA by isopycnic centrifugation of total DNA extracted from SIP microcosms using the universal primers 515F-907R of the total 16S rRNA genes

DNA gradient fraction*	High-quality reads number							
	Day-5				Day-19			
	^{13}C-CH_4	^{13}C-Urea	^{13}C-CH_4+Urea	^{12}C-CH_4+Urea	^{13}C-CH_4	^{13}C-Urea	^{13}C-CH_4+Urea	^{12}C-CH_4+Urea
Fraction-13	39	107	4240	4593	4488	1384	5859	6410
Fraction-12	5134	4677	3861	5387	7446	3318	8093	8171
Fraction-11	4318	4658	3615	6492	6046	6916	7277	5441
Fraction-10	3227	4531	4941	5556	3682	6224	2472	6090
Fraction-9	4647	4710	5583	6323	6149	6102	4542	3867
Fraction-8	6195	5239	5534	4925	4108	6825	7147	8140
Fraction-7	8080	6620	3796	7488	5079	5527	6684	7118
Fraction-6	5889	7060	3736	1925	2570	3087	6374	4740
Fraction-5	7270	3306	6278	481	2134	5643	5804	5725
Fraction-4	9622	427	195	931	402	4526	7471	9873
Fraction-3	---	---	---	---	5568	5644	4424	1835
Average	5442	4134	4178	4410	4334	5018	6013	6128
Subtotal	54421	41335	41779	44101	47672	55196	66147	67410
Total	418061							

*: indicates DNA gradient fractions with different buoyant densities, and the smaller the number, the heavier the fractionated DNA.

--- Not determined.

Table S5. Pyrosequencing summary of *pmoA* and *amoA* genes in the total DNA extract from SIP microcosms and in the ^{13}C-DNA fractions after isopycnic centrifugation of total DNA using primer pairs A189F-mb661r and amoA1F-2R, respectively.

Organisms*	Replicate	Day-0	Day-19					
			^{13}C-CH_4		^{13}C-Urea		^{13}C-CH_4+Urea	
		Total DNA†	Total DNA†	^{13}C-DNA‡	^{13}C-DNA	^{13}C-DNA	Total DNA†	^{13}C-DNA
pmoA genes of MOB	R1	4295	8244		---		1106	
	R2	3129	5297	367	---	---	5074	6877
	R3	---	5509		---		6073	
amoA genes of AOB	R1	5484	---		7572		2728	
	R2	472	---	---	10656	472	3262	1115
	R3	6261	---		4832		4449	

* MOB and AOB represent methane-oxidizing bacteria and ammonia-oxidizing bacteria, respectively.

† indicates that pyrosequencing was performed on the total DNA extract from the ^{13}C-labeled microcosms.

‡ indicates that pyrosequencing was performed on the ^{13}C-DNA fraction after ultracentrifugation of total DNA extract.

---Not determined

. **Table S6.** The estimated budget of carbon and nitrogen assimilation by methanotrophs and ammonia oxidizers in microcosms at day 19

Treatment [a]	μmol CH_4-C/microcosm [b]			μg urea-N/microcosm		
	CH_4 consumed	CO_2 produced	CO_2 assimilated by methanotrophs	Assimilation of urea-N by methanotrophs [c]	Nitrate produced from urea-N by ammonia oxidizers	Urea-N recovery
^{13}C-CH_4+Urea-R1	1111	730.9	380.5 (34.2%)	1332 (74.0%)	364.2 (20.2%)	94.2%
^{13}C-CH_4+Urea-R2	1081	688.8	392.4 (36.3%)	1373 (76.3%)	339.6 (18.9%)	95.2%
^{13}C-CH_4+Urea-R3	1030	746.3	283.9 (27.6%)	994 (55.9%)	372.4 (20.69%)	76.6%
Average	1074±41.1	722.0±29.8	352.3±59.5 (32.7%±4.54%)	1234±208.4 (68.7%±11.2%)	358.7±17.1 (19.9%±0.93%)	88.7±10.5%

[a] The designation of R1 to R3 represents incubation of triplicate microcosms.

[b] The amount of CH_4 consumed was calculated as the net difference in CH_4 concentration between day 0 and day 19. The amount of CO_2 produced was estimated in a similar way. Assuming that all CH_4 consumed were converted to CO_2, the amount of CO_2 assimilated by methanotrophs could be calculated as the net difference between the consumed CH_4 and the produced CO_2 at day 19 as previously described (Whalen et al., 1990).

[c] For every mole of assimilated carbon 0.25 moles of nitrogen have to be taken up (Bodelier and Laanbroek, 2004).

Figure S1. Methane consumption in soil microcosms over an incubation period of 19 days. Methane consumption is expressed as the percentage of the methane concentrations left in the headspace of the microcosms relative to the initial methane concentration in the microcosms in the absence (a) and presence (b) of urea nitrogen. The numbers above the columns denote the initial concentration (ppmv) immediately after the methane additions.

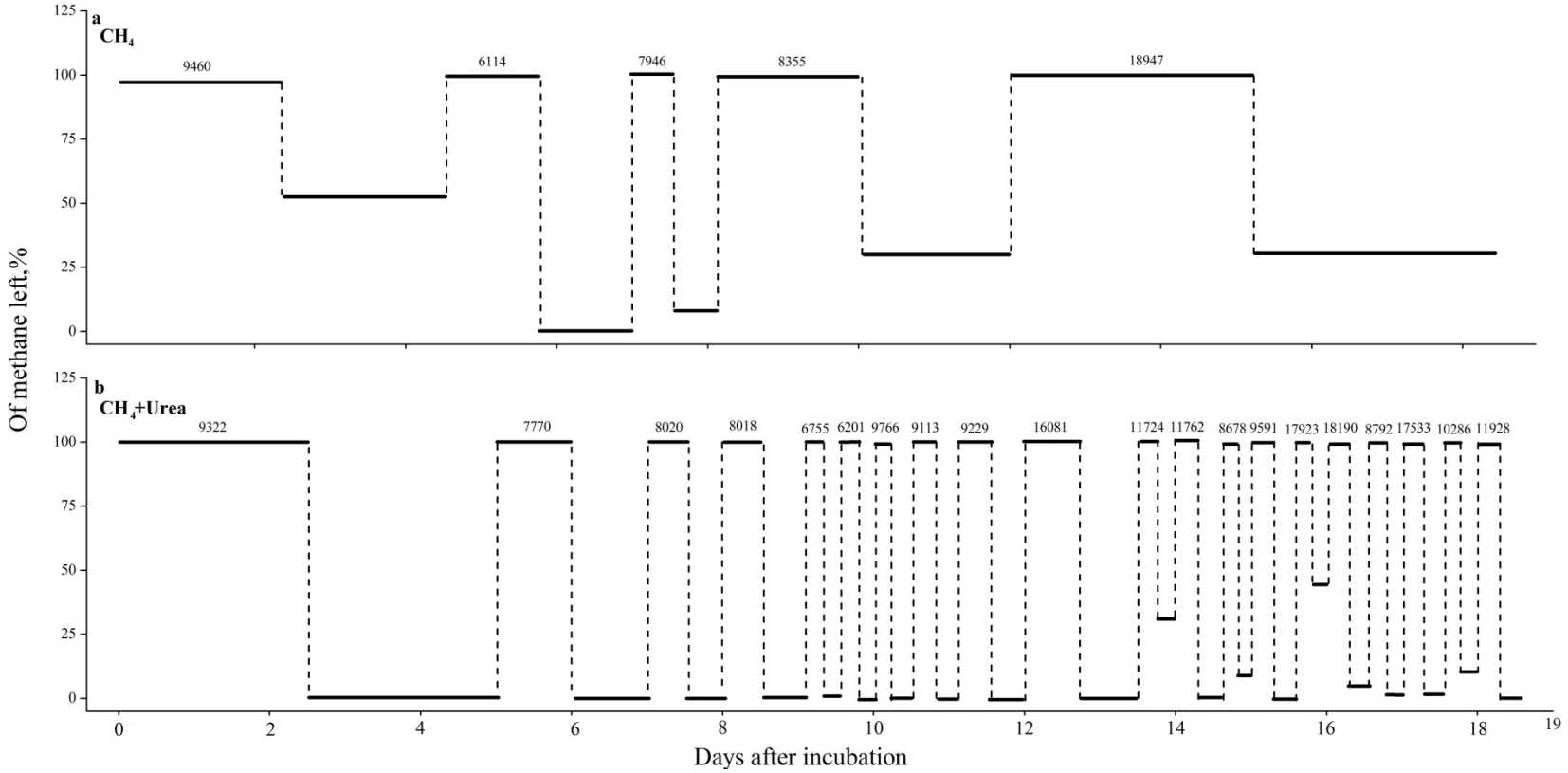


Figure S2. Changes in NH_4^+-N and NO_3^--N content in soil microcosms incubated with urea or CH_4+Urea over the course of 19 days of incubation. The error bars represent standard deviation of the triplicate microcosms, while for the soil microcosms of CH_4+Urea treatment 6 replicates were used including both ^{12}C-control and ^{13}C-labeled treatments.

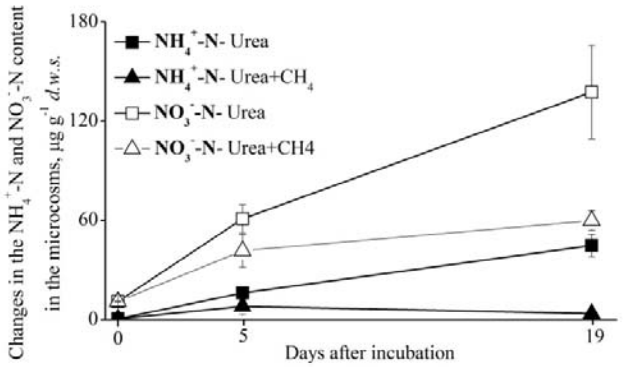


Figure S3. Quantitative distribution of *pmoA* gene copy numbers (a), *amoA* gene copy numbers of Bacteria (b) and Archaea (c) in total DNA from microcosms after incubation for 5 and 19 days. The error bars represent standard deviations of the triplicate microcosms. The different letters above the columns indicate a significant difference ($P<0.05$) using analysis of variance.

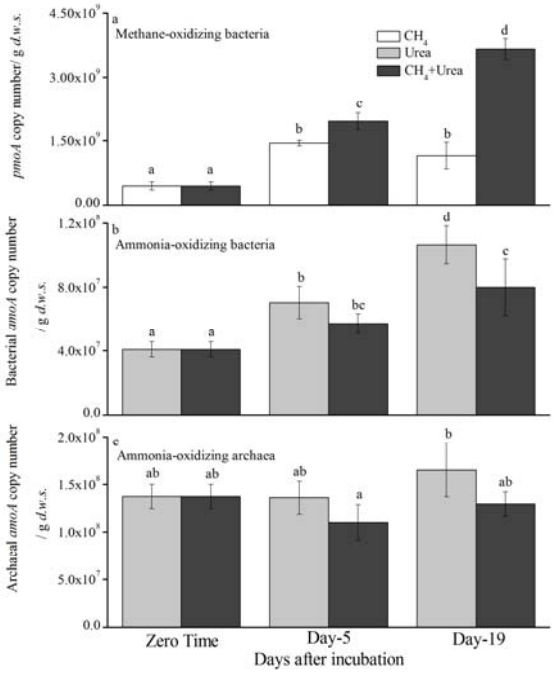


Figure S4. The effect of methane on ammonia-oxidizing archaea (AOA) in soil microcosms incubated for 19 days. The relative frequency is expressed as the percentage of the targeted reads to the total 16S rRNA gene sequences reads in soil sample. The error bars represent standard deviation of the triplicate microcosms, while for the soil microcosms of CH_4+Urea treatment 6 replicates were used including both ^{12}C-control and ^{13}C-labeled treatments. The different letters above the columns indicate a significant difference ($P<0.05$) using analysis of variance.

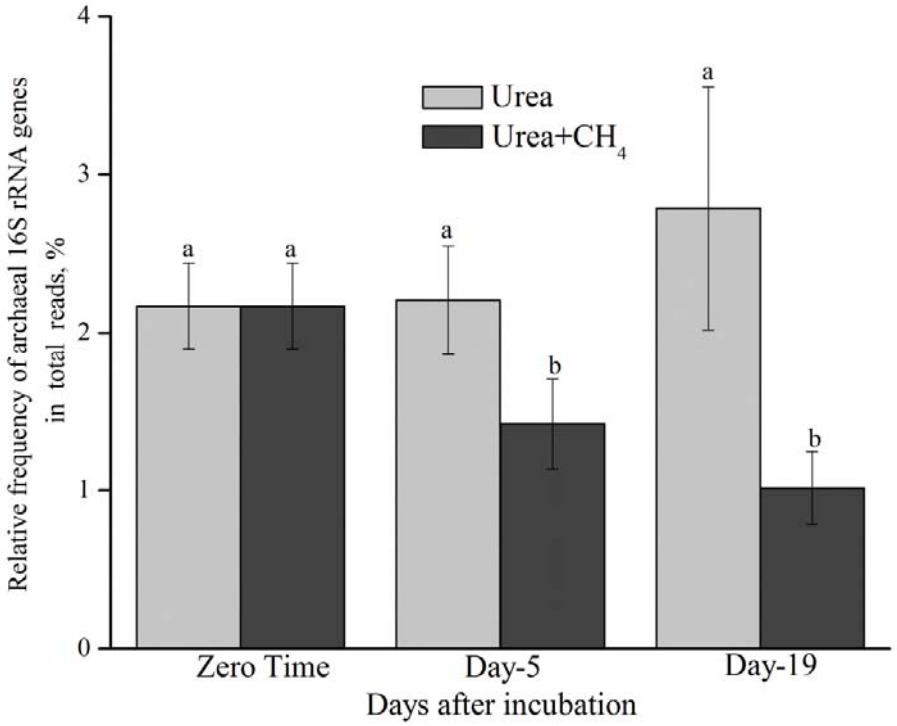


Figure S5. Phylogenetic tree showing the relationship of methane-oxidizing bacterial 16S rRNA gene (a) and *pmoA* gene (b) sequences in soil microcosms to those deposited in the GenBank. Pyrosequencing reads of methanotrophic 16S rRNA genes and *pmoA* genes were used from triplicate microcosms at day 0 and day 19, and representative sequences were chosen for analysis. The designation of CH_4+Urea-OTU-1-38.5%-(616) indicates that OTU-1 containing 616 sequences with identity of >97% comprised 38.5% of methanotrophic 16S rRNA gene sequences in ^{13}C-CH_4+Urea treatment after incubation for 19 days. CH_4-OTU-1-69.8%-(3844) indicates that OTU-1 containing 3844 sequences with identity of >87% comprised 69.8% of *pmoA* gene sequences in ^{13}C-CH_4 treatment after incubation for 19 days. One representative sequence was extracted using mothur software package for tree construction. The scale bar represents nucleotide acid substitution percentage.

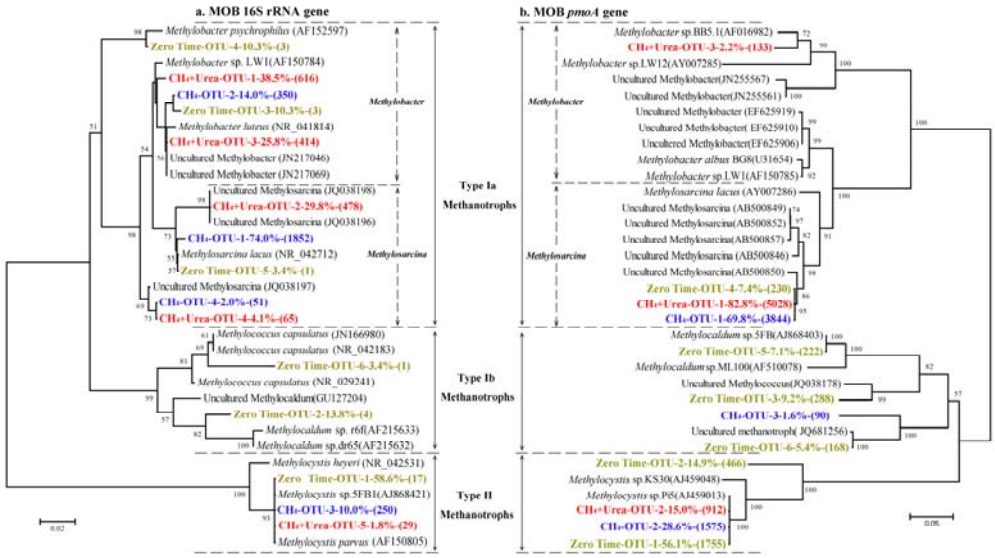


Figure S6. Phylogenetic tree showing the relationship of ammonia-oxidizing bacterial 16S rRNA gene (a) and *amoA* gene (b) sequences in soil microcosms to those deposited in the GenBank. Pyrosequencing reads of AOB 16S rRNA genes and *amoA* genes were used from triplicate microcosms at day 0 and day 19. As for 16S rRNA genes, all AOB sequence reads were retrieved for analysis using mother software package, and only representative *amoA* gene reads were included for clarity to construct phylogenetic tree. The designation of Urea+CH_4-OTU-1-52.3%-(2326) indicates that OTU-1 containing 2326 sequences with identity of >97% comprised 52.3% of ammonia-oxidizing bacterial *amoA* gene sequences in ^{13}C-Urea+CH_4 treatment after incubation for 19 days, and one representative sequence was extracted using mothur software package for tree construction. The scale bar represents nucleotide acid substitution percentage.

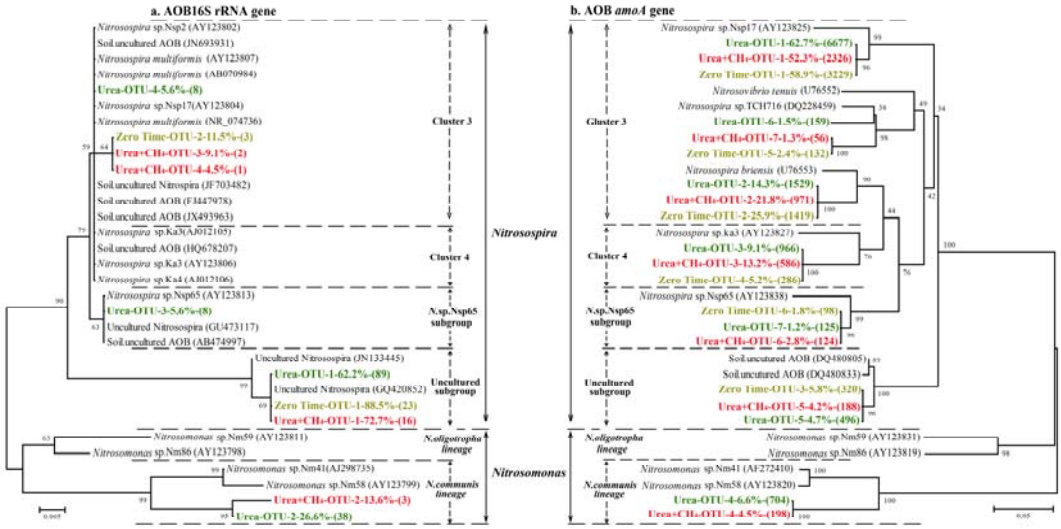


Figure S7. Quantitative distribution of *pmoA* gene copy numbers (a,b), *amoA* gene copy numbers of *Bacteria* (c,d) and *Archaea* (e,f) across the entire buoyant density gradient of the fractionated DNA from SIP microcosms after incubation for 5 and 19 days. The normalized data are the ratio of gene copy number in each DNA gradient fraction to the maximum quantities for each treatment. The error bars represent standard deviations of the duplicate microcosms.

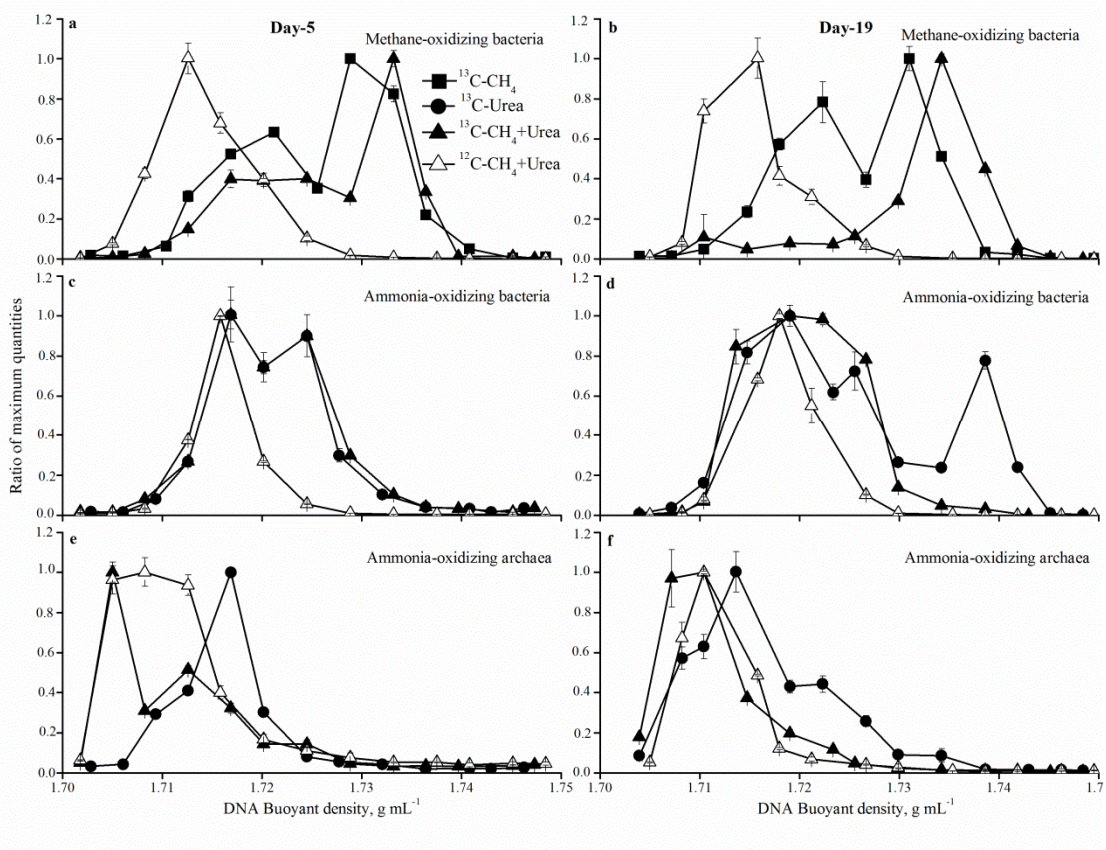
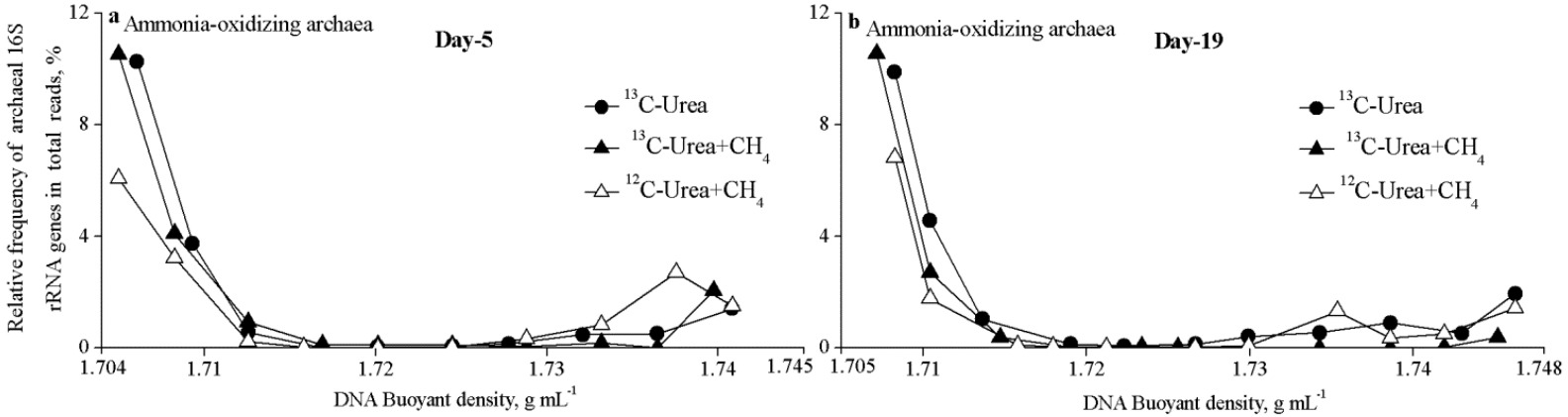


Figure S8. Relative frequency of the archaeal 16S rRNA gene sequences reads in DNA gradient fractions with a buoyant density gradient isolated from SIP microcosms after incubation for 5 and 19 days. The frequency is expressed as the percentage of the targeted archaeal reads to the total 16S rRNA gene sequences reads in each DNA gradient fraction.



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