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 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 ¹³C and ¹²C-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% O₂, 80% N₂), 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°C,3.0min;30×(95°C,30s; 55°C, 30s;72°C, 45s);72°C,10min	Pyrosequencing	(Xia et al., 2011)
907R	CCGTCAATTCMTTTRAGTTT				
A189F	GGN GAC TGG GAC TTC TGG	<i>pmoA</i> gene	95°C,3.0min;40×(95°C,10s; 55°C, 30s;72°C, 30s; 80°C 5s; with plate read); melt curve 65°C to 95°C, incremental0.5°C, 0:05+plate read	Real-time PCR/Pyrosequencing	(Costello and Lidstrom, 1999; Holmes et al., 1995)
mb661r	CCG GMG CAA CGT CYT TAC C				
amoA-1F	GGGGTTTCTACTGGTGGT	bacterial <i>amoA</i> gene	95°C,3.0min;40×(95°C,10s; 55°C, 30s;72°C, 30s; with plate read); melt curve 65°C to 95°C, incremental 0.5°C, 0:05+plate read	Real-time PCR/Pyrosequencing	(Rotthauwe et al., 1997)
amoA-2R	CCCCTCGGGAAAGCCTTCTTC				
Arch-amoAF	STAATGGTCTGGCTTAGACG	Archaeal <i>amoA</i> gene	95°C,10.0min;40×(95°C,30s; 55°C, 45s;72°C, 30s;82°C 15s with plate read); melt curve 65°C to 95°C, incremental 1.0°C, 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

		Pyrosequencing reads number†				
Treatment*		High-quality read number	Methane oxidizing bacteria	Methanol oxidizing bacteria	Ammonia oxidizing bacteria	Nitrite oxidizing bacteria
Zero time	Zero Time-R1	9519	28 (0.29%)	---	22 (0.23%)	81 (0.85%)
	Zero Time-R2	9110	26 (0.29%)	---	16 (0.18%)	74 (0.81%)
	Zero Time-R3	9369	24 (0.26%)	---	22 (0.23%)	99 (1.06%)
Day-5	¹³ C-CH ₄ -R1	7758	1252 (16.1%)	1 (0.01%)	12 (0.15%)	64 (0.82%)
	¹³ C-CH ₄ -R2	8630	1273 (13.5%)	1 (0.01%)	16 (0.19%)	55 (0.64%)
	¹³ C-CH ₄ -R3	8829	1192 (13.5%)	---	18 (0.20%)	50 (0.57%)
	¹³ C-Urea-R1	7803	31 (0.40%)	---	10 (0.13%)	80 (1.03%)
	¹³ C-Urea-R2	7807	13 (0.17%)	2 (0.03%)	26 (0.33%)	108 (1.38%)
	¹³ C-Urea-R3	6541	17 (0.26%)	1 (0.02%)	24 (0.37%)	88 (1.35%)
	¹³ C-CH ₄ +Urea-R1	7431	1637 (22.0%)	---	15 (0.20%)	60 (0.81%)
	¹³ C-CH ₄ +Urea-R2	8372	1633 (19.5%)	1 (0.01%)	30 (0.36%)	88 (1.05%)
	¹³ C-CH ₄ +Urea-R3	7568	1559 (20.6%)	4 (0.05%)	22 (0.29%)	47 (0.62%)
	¹² C-CH ₄ +Urea-R1	6995	1109 (15.9%)	---	9 (0.13%)	67 (0.96%)
	¹² C-CH ₄ +Urea-R2	8083	1782 (22.1%)	3 (0.04%)	12 (0.15%)	55 (0.68%)
	¹² C-CH ₄ +Urea-R3	7809	1433 (18.4%)	2 (0.03%)	11 (0.14%)	63 (0.81%)
Day-19	¹³ C-CH ₄ -R1	10104	640 (6.33%)	---	18 (0.18%)	67 (0.66%)
	¹³ C-CH ₄ -R2	41172	3330 (8.09%)	18 (0.04%)	86 (0.21%)	274 (0.67%)
	¹³ C-CH ₄ -R3	41230	3235 (7.85%)	1 (0.00%)	104 (0.25%)	305 (0.74%)
	¹³ C-Urea-R1	8294	23 (0.28%)	---	26 (0.31%)	121 (1.46%)
	¹³ C-Urea-R2	31675	110 (0.35%)	2 (0.01%)	125 (0.39%)	465 (1.47%)
	¹³ C-Urea-R3	44313	129 (0.29%)	1 (0.00%)	149 (0.34%)	587 (1.32%)
	¹³ C-CH ₄ +Urea-R1	10370	2961 (28.6%)	465 (4.48%)	12 (0.12%)	34 (0.33%)
	¹³ C-CH ₄ +Urea-R2	7309	1963 (26.9%)	238 (3.26%)	7 (0.10%)	33 (0.45%)
	¹³ C-CH ₄ +Urea-R3	6494	1955 (30.1%)	231 (3.56%)	15 (0.23%)	21 (0.32%)
	¹² C-CH ₄ +Urea-R1	9485	2672 (28.2%)	163 (1.72%)	13 (0.14%)	51 (0.54%)
	¹² C-CH ₄ +Urea-R2	7695	2129 (27.7%)	121 (1.57%)	9 (0.12%)	33 (0.43%)
	¹² C-CH ₄ +Urea-R3	6663	1750 (26.3%)	132 (1.98%)	13 (0.20%)	30 (0.45%)
Average		12831				
Total reads		346428				

*: The designation 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

7 **Table S4.** Pyrosequencing summary of the total microbial communities in the fractionated DNA by isopycnic centrifugation of total DNA
8 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							

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

10 --- Not determined.

11 **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
 12 isopycnic centrifugation of total DNA using primer pairs A189F-mb661r and amoA1F-2R, respectively.

Organisms*	Replicate	Day-19						
		Day-0	$^{13}\text{C-CH}_4$		$^{13}\text{C-Urea}$		$^{13}\text{C-CH}_4\text{+Urea}$	
		Total DNA†	Total DNA†	$^{13}\text{C-DNA}‡$	$^{13}\text{C-DNA}$	$^{13}\text{C-DNA}$	Total DNA†	$^{13}\text{C-DNA}$
<i>pmoA</i> genes of MOB	R1	2788	6046		---		2728	
	R2	2242	4138	331	---	---	3262	5991
	R3	---	4200		---		4704	
<i>amoA</i> genes of AOB	R1	5484	---		7572		2728	
	R2	472	---	---	10656	472	3262	1115
	R3	6261	---		4832		4449	

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

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

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

16 ---Not determined

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

Treatment ^a	$\mu\text{mol CH}_4\text{-C/microcosm}^b$			$\mu\text{g urea-N/microcosms}$		
	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 $\pm$ 41.1	722.0 $\pm$ 29.8	352.3 $\pm$ 59.5 (32.7% $\pm$ 4.54%)	1234 $\pm$ 208.4 (68.7% $\pm$ 11.2%)	358.7 $\pm$ 17.1 (19.9% $\pm$ 0.93%)	88.7 $\pm$ 10.5%

^aThe designation R1 to R3 represents incubation of triplicate microcosms.

^bThe amount of CH₄ consumed was calculated as the net difference in CH₄ concentration between day 0 and day 19. The amount of CO₂ produced was estimated in a similar way. Assuming that all CH₄ consumed were converted to CO₂, the amount of CO₂ assimilated by methanotrophs could be calculated as the net difference between the consumed CH₄ and the produced CO₂ at day 19 as previously described (Whalen et al., 1990).

^cFor every mole of assimilated carbon 0.25 moles of nitrogen have to be taken up (Bodelier and Laanbroek, 2004; Bodelier and Laanbroek, 2004).

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

Figure S2. 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 CH₄+Urea treatment 6 replicates were used including both ¹²C-control and ¹³C-labeled treatments. The different letters above the columns indicate a significant difference ($P<0.05$) using analysis of variance.

13

Figure S3. 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 CH₄+Urea-OTU-1-38%-(616) indicates that OTU-1 containing 616 sequences with identity of >97% comprised 38% of methanotrophic 16S rRNA gene sequences in ¹³C-CH₄+Urea 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.

24

Figure S4. 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 Urea+CH₄-OTU-1-52%-(2326) indicates that OTU-1 containing 2326 sequences with identity of >97% comprised 52% of ammonia-oxidizing bacterial *amoA* gene sequences in ¹³C-Urea+CH₄ 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.

38 **Figure S5.** Quantitative distribution of *pmoA* gene copy numbers (a,b), *amoA* gene
 39 copy numbers of *Bacteria* (c,d) and *Archaea* (e,f) across the entire buoyant density
 40 gradient of the fractionated DNA from SIP microcosms after incubation for 5 and 19
 41 days. The normalized data are the ratio of gene copy number in each DNA gradient
 42 fraction to the maximum quantities for each treatment. The error bars represent
 43 standard deviations of the duplicate microcosms.

44

45 **Figure S6.** Relative frequency of the archaeal 16S rRNA gene sequences reads in DNA gradient fractions with a buoyant density gradient
 46 isolated from SIP microcosms after incubation for 5 and 19 days. The frequency is expressed as the percentage of the targeted archaeal reads to
 47 the total 16S rRNA gene sequences reads in each DNA gradient fraction.

49 **Figure S7.** Phylogenetic tree of *pmoA* genes for methane-oxidizing bacteria (a) and *amoA* genes for ammonia-oxidizing bacteria (b) in the
50 ^{13}C -DNA ‘heavy’ fraction from the labeled microcosm after incubation for 19 days. The designation CH₄-HF-OTU-1-(272)-82% indicates that
51 OTU-1 containing 272 reads with sequence identity of >97% comprised 82% of *pmoA* gene sequences retrieved from the ‘HF’ fraction in
52 microcosms amended with ^{13}C -CH₄ for incubation after 19 days, and one representative sequence was extracted using mothur software package
53 for tree construction. The scale bar represents nucleotide acid substitution percentage.

54

Figure S8. Phylogenetic tree showing the relationship of the high-throughput sequence reads of *Undibacterium* (a) and *Pseudomonas* (b) in the ¹³C-labeled ‘heavy’ DNA fractions (HF) to those deposited in the GenBank. The designation of CH₄+Urea-HF -OTU-1-13‰-(172) indicates that OTU-1 contains 172 sequences associated with *Undibacterium* comprising 13‰ of 16S rRNA gene sequence reads in the ‘heavy’ DNA fractions with identity of >97%, and one representative sequence was extracted using mothur software package for tree construction. The scale bar represents nucleotide acid substitution percentage.

63

64 **Reference**

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