Point-by-point response to the comments of the referee #4

(Manuscript ID bg-2014-97)

General comments of the referee #4

In their study, Zheng and co-workers investigated the competition between methane and ammonia oxidizers in paddy soil microcosms. Soil slurries were incubated with 13-C labelled CH4, 13-C urea, and 13-C-CH4 and 13-C-urea. Soils were incubated for 5 and 19 days. Methane oxidation and nitrification rates were determined and the microbial community was analyzed by qPCR and amplicon sequencing targeting the 16SrRNA and the marker genes pmoA/amoA. The authors could show a strong stimulation of methane oxidation by urea addition and on the other side, a decrease of nitrification rates by methane addition. Within the methane oxidizing community, type Ia methanotrophs were highly enriched under the tested conditions and also labelled. Within the ammonia oxidizers, Nitrosospira was most abundant; however, Nitrosomonas dominated the labelled fraction. Ammonia oxidizing archaea do not seem to play a role in this system. Furthermore, the authors describe the labelling of 16S rRNA genes affiliated to known methanol degraders, indicating the close food web between methanotrophs and methylotrophs that feed on methanol. This is an interesting topic and the authors used an appropriate experimental approach to address this question.

Reply: We than the referee for the overall positive comments.

Major comments

1. Nevertheless, the documentation of results and discussion is in my opinion not always concise and the manuscript contains too many figures and tables. This experiment contains a large dataset and not every aspect has to be discussed.

   Reply: We agree with this comment.

   A number of changes have been made to keep the results more concise and focused. For example we have deleted the discussions about type Ib (Page 3910, line 13-23), denitrification (page 3908, line 26-29; page 3909, line 1-4), *Undibacterium* (page 3911, line29-30; page 3912, line 1-3) and *Pseudomonas* (page 3913, line 2-5) in the revised manuscript.

2. However, what is missing in my opinion is the overall result of the 16S rRNA pyrosequencing (Archaea and bacteria). Of course it has

not to be discussed in detail, but it should be shown to follow the authors' argumentations and the selection of specific subsets.

Reply: The overall result of the 16S rRNA pyrosequncing has been shown in the Supplemental Table S3 in the revised version. The result revealed that bacteria were overwhelmingly dominant in total microorganism community. We have described these overall results in the revised manuscript from line 308 to line 312 on page 12 as follows.

'About 346, 000 high-quality sequence reads were obtained with an average length of 377 bp in the V3~V4 region, while about 337,000 sequences was affiliated with bacteria. Relative abundance of bacterial 16S rRNA genes in total microorganism ranged from 95.3% to 98.8% in these microcosms, which was much higher than that of archaeal 16S rRNA genes.'

Specific comments:

1. In my opinion, not all phylogenetic trees have to be shown. Information on abundance and labelling could be combined in single trees

   Reply: Corrected. We agree with this comment. Phylogenetic trees of the ^{13}C-labeled MOB 16S rRNA genes and *pmoA* genes have been presented in a single Figure 4, while ^{13}C-labeled AOB 16S rRNA gene and *amoA* gene in Figure 5 in the revised version

2. Figure S8 is not important here.

   Reply: Removed.

3. Side 3911 Lines 13-27: There are already genomes of methanotrophs published. The authors should check this. As far as I remember, sequenced type Ia methanotrophs have a single copy of the rRNA operon as well as the pmoA (excluding the very different isoenzyme)

   Reply: We agree with this comment which was raised by referee #1 as well. The incongruence has been discussed briefly as follows from line 571 to line 576 on page 21.

   'A comparison of 16S rRNA gene and *pmoA* gene sequences revealed that *Methylobacter* was detected in a higher proportion in the MOB-16S rRNA gene phylogenetic tree than in the *pmoA* gene phylogenetic tree. The incongruence might result from the bias associated with the different coverage of *pmoA* and 16S rRNA gene primers as reported previously (Costello and Lidstrom 1999).'

Title Page

1. Title:

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

2. Running Title:

Interactions between soil methane and ammonia oxidizers

3. Subject Category:

Microbial Ecology

4. Author Names:

Yan Zheng[1,2], Rong Huang[1], Baozhan Wang[1], Paul L.E. Bodelier[3], Zhongjun Jia[1*]

5. Author Affiliation

[1] State Key Laboratory of Soil and Sustainable Agriculture,
Institute of Soil Science, Chinese Academy of Sciences
Nanjing, 210008, Jiangsu Province, P.R. China

[2]University of the Chinese Academy of Sciences
Beijing 100049, People's Republic of China

[3] Netherlands Institute of Ecology
Department of Microbial Ecology
Droevendaalsesteeg 10
6708 PB, Wageningen, the Netherlands

6. Corresponding author [1,*]

Dr. Zhongjun Jia
E-mail: jia@issas.ac.cn
Tel:+86-25-86881311; Fax: +86-25-86881000

Abstract

Pure culture studies have demonstrated that methanotrophs and ammonia oxidizers can both carry out the oxidation of methane and ammonia. However, the expected interactions resulting from these similarities are poorly understood, especially in complex, natural environments. Using DNA-based stable isotope probing and pyrosequencing of 16S rRNA and functional genes, we report on biogeochemical and molecular evidence for growth stimulation of methanotrophic communities by ammonium fertilization, and that methane modulates nitrogen cycling by competitive inhibition of nitrifying communities in a rice paddy soil. Pairwise comparison between microcosms amended with CH_4, CH_4+Urea, and Urea indicated that urea fertilization stimulated methane oxidation activity by 6-fold during a 19-day incubation period, while ammonia oxidation activity was significantly suppressed in the presence of CH_4. Pyrosequencing of the total 16S rRNA genes revealed that urea amendment resulted in rapid growth of *Methylosarcina*-like type Ia MOB, and nitrifying communities appeared to be partially inhibited by methane. High-throughput sequencing of the ^{13}C-labeled DNA further revealed that methane amendment resulted in clear growth of *Methylosarcina*-related MOB while methane plus urea led to equal increase in *Methylosarcina* and *Methylobacter*-related MOB, indicating the differential growth requirements of representatives of these genera. Increase in ^{13}C-assimilation by microorganisms related to methanol oxidizers clearly indicated carbon transfer from methane oxidation to other soil microbes, which was enhanced by urea addition. The active growth of type Ia methanotrops was significantly stimulated by urea amendment, and the pronounced growth of methanol-oxidizing bacteria occurred in CH_4-treated microcosms only upon urea amendment. Methane addition partially inhibited the growth of *Nitrosospira* and *Nitrosomonas* in urea-amended microcosms, in addition of nitrite-oxidizing bacteria. These results suggest that type I methanotrophs could likely outcompete type II methane oxidizers under nitrogen-rich environment and the competitive interactions among methane and ammonia oxidizers are complicated than previously appreciated.

Key Words:

Paddy soil, methane oxidation, ammonia oxidation, microbial interactions, high-throughput pyrosequencing, DNA-SIP

Introduction

The intensive use of nitrogenous fertilizers in rice agriculture is a perquisite to meet the growing demand for food, especially since this crop feeds more than half of world's population (Galloway et al., 2008). The tight coupling between nitrogen fertilization and methane emission from rice paddy ecosystems in combination with the significant contribution of these system to the global methane emission 15 to 45% of global CH_4 budget (Bodelier, 2011) has evoked numerous studies focusing on this topic. Recent meta-analysis indicate that the increasing rice biomass by nitrogen fertilization may result in the elevated supply of readily available carbon in suport of methanogenesis, stimulating methane emission in paddy fields(Banger et al., 2012). However, opposed to this there is a strong body of evidence demonstrating stimulation of methane oxidation by ammonium-based fertilizers in rice soil, leading to reduced methane flux (Bodelier et al., 2000b). The vast amount of studies following these observations as well as possible underlying mechanisms for nitrogen regulation of methane oxidation in soils and sediments has been reviewed (Bodelier, 2011; Bodelier and Laanbroek, 2004). However, the role of interactions between methanotrophs and ammonia oxidizers and the consequences for interactions between carbon and nitrogen cycling has rarely been investigated in natural complex ecosystems (Bodelier, 2011).

Aerobic methane-oxidizing bacteria (MOB) belong to two phyla: *Proteobacteria* and *Verrucomicrobia* (Bodelier et al., 2009). Whereas proteobacterial MOB are widespread, *Verrucomicrobia* seem to be restricted to extreme environments (Dunfield et al., 2007). Aerobic proteobacterial MOB can be divided into two major groups mainly based on phylogeny being type I (*Gammaproteobacteria*) and type II (*Alphaproteobacteria*). This group assignment used to be supported by differences in biochemical, physiological and morphological properties. Based on congruent 16S rRNA and *pmoA* phylogeny, type I MOB harboring the family *Methylococcaceae* can be further divided into type Ia (including genera *Methylosarcina*, *Methylobacter*,

Methylomonas, Methylomicrobium, Methylosoma, Methylosphaera and Methylovulum) and type Ib (including genera *Methylococcus, Methylocaldum, Methylogaea, Methylobius and Methylothermus*). Type II MOB include the family *Methylocystaceae* (including genera *Methylocystis* and *Methylosinus*) and *Beijerinckiaceaea* (including genera *Methylocella, Methylocapsa and Methyloferula*) (Stein et al., 2012). The methane monooxygenase (MMO) exist either as a particulate (pMMO) or a soluble (sMMO) form. All known methanotrophs contain pMMO except *Methylocella* and *Methyloferula*, while sMMO is found only in a few species (Hanson and Hanson, 1996; Lipscomb, 1994). Methanotrops coverts CH_4 to methanol, which can be utilized by methanol-oxidzing bacteria as carbon and energy source. The known soil-retrieved methanol-oxidizing bacteria was with high diversity, however, most of them are faculative methylotrophic, indicating the capability to utilize alternative carbon substrate(Kolb 2009). The family *Methylophilaceae* is the known obligate methylotrophs that use methanol as the sole source of carbon and energy (Bratina et al 1992, He et al 2012).Nitrifying bacteria use ammonia monooxygenase (AMO) for oxidation of their primary growth substrate. Though the AMO gene was thought to be unique to ammonia-oxidizing bacteria, the discovery of ammonia-oxidzing archaea (AOA) has suggested important role of archaeal nitrification in the global nitrogen cycle (Lu and Jia, 2013; Venter et al., 2004). However, until now the relative contribution of AOB and AOA to ammonia oxidation in argricultural soil is still unclear (Prosser and Nicol, 2012; Xia et al., 2011). 16S rRNA and *amoA* gene analyses of AOB revealed that physiological group are confined to monophyletic groups whithin β- andγ-subclass of *Proteobacteria*. *Nitrosospira* and *Nitrosomonas* form a grouping within β-subclass and *Nitrosococcus* is affiliated with γ-subclass (Purkhold et al 2000, Purkhold et al 2003). Enormous diversity of AOA based on 16S rRNA and *amoA* gene has been suggested, and four major lineages have been displayed, inclusing *Nitrososphaera* cluster, *Nitrosopumilus* cluster, *Nitrosotalea* cluster, and *Nitrosocaldus* cluster (Pester et al 2012, Stahl and de la Torre 2012). The conversion of nitrite into nitrate is caused by nitrite-oxidizing bacteria (NOB). NOB are composed of four genera, including *Nitrobacter,*

115 *Nitrococcus*, *Nitrospina* and *Nitrospira*, which were assigned to the α-proteobacteria,
116 γ-proteobacteria, δ-proteobacteria and phylum *Nitrospirae*, respectively (Bock and
117 Wagner 2006).

118 The key enzymes methane monooxygenase (MMO) in methanotrophs and ammonia
119 monooxygenase in ammonia oxidizers are evolutionarily linked (Holmes et al., 1995),
120 leading to functional similarities enabling both methanotrophs and ammonia oxidizers
121 to oxidize both methane and ammonia (Jones and Morita, 1983; O'Neill and
122 Wilkinson, 1977). Pure culture studies demonstrated that methane can act as a
123 competitive inhibitor for ammonia oxidizers, and ammonia inhibits the growth and
124 activity of methanotrophs (Bedard and Knowles, 1989; Stein et al., 2012). Next to this,
125 both MOB as well as AOB have to deal with toxic intermediates (hydroxylamine in
126 case of MOB and methanol in case of AOB) (Stein et al., 2012). At the microbial
127 community level, however, the growth of methanotrophs might be nitrogen-limited
128 and nitrogen fertilization might relieve methane oxidizers from nutrient constraint
129 (Bodelier et al., 2000b). At the same time ammonia oxidizers and subsequent
130 nitrification may be inhibited by the methanotrophic N-assimilation. However, the
131 research focus of methane effect on nitrification in natural complex ecosystems is
132 poor, which is in sharp contrast with a large number of studies executed to elucidate
133 effect of nitrogenous fertilizers on methane oxidation. Moreover, the lack of
134 knowledge on this topic is even more evident taking the yet unknown role of AOA in
135 interactions with MOB into account. DNA-based stable isotope probing (DNA-SIP) is
136 generally used to link the metabolisms of ^{13}C-labeled substrates with growing
137 microbial communities in the environment. DNA-SIP has been employed to identify
138 the active methanotrophs (Dumont et al., 2011) and ammonia oxidizers in soils (Jia
139 and Conrad, 2009; Lu and Jia, 2013; Xia et al., 2011). The combined use of stable
140 isotope labeling and high throughput pyrosequencing is a powerful combination of
141 approaches that offers great opportunities in elucidating interaction between MOB
142 and AOB/AOA, because both groups can easily and specifically be labeled using
143 $^{13}CH_4$ (Bodelier et al., 2013; Bodelier et al., 2012) and $^{13}CO_2$ (Jia and Conrad, 2009).

However, studies that assessed both functional groups in interaction with each other are missing.

The interactions between methane- and ammonia-oxidizers are linked to methane-nitrogen cycle in light of climate change. However, the effects of nitrogen on methane oxidation are complicated and contradictory results are often reported. Therefore, the microbial populations and functional dynamics of methane- and ammonia oxidizers were investigated in microcosms incubated with CH_4, urea and CH_4+urea in a paddy soil using culture-independent techniques.

Materials and Methods

Site description and soil sampling

The paddy soil was collected from Yangzhou City (119°42′0″E, 32°35′5″N) of Jiangsu province, one of the major regions for rice production in China. The soil was silt clay and classified as Calcaric Glevsols. The field has a history of rice cultivation for more than 50 years. Soil sampling was performed at 0-15 cm depth by steel cores with three replicates. Soil maximum water holding capacity (WHC) was 55%, and the soil samples were homogenized by passing through a 2-mm meshed sieve. The resulting soil samples were kept at 40% maximum water holding capacity in fridge until use. Soil characteristics are as follows: 15 g total organic C kg^{-1}, 1.59 g total N kg^{-1}, 1.23 g total P kg^{-1} and pH 7.4 determined with water to soil ratio at 2.5.

DNA-SIP microcosms

Four treatments were performed including ^{13}C-CH_4-labeled microcosms (incubated with ^{13}C-CH_4), ^{13}C-Urea-labeled microcosms (incubated with ^{13}C-Urea and ^{13}C-CO_2), ^{13}C-CH_4+Urea-labeled microcosms (incubated with ^{13}C-CH_4, ^{13}C-Urea and ^{13}C-CO_2) and ^{12}C-CH_4+Urea control microcosm (incubated with ^{12}C-CH_4, ^{12}C-Urea and ^{12}C-CO_2). The hydrolysis of ^{13}C-labeled urea was employed to generate ammonia and ^{13}C-CO_2 in support of autotrophic nitrifying communities in soil as previously reported (Lu and Jia, 2013). Pairwise comparison among the treatments of ^{13}C-CH_4,

^{13}C-CH_4+Urea, and ^{13}C-Urea was used to assess the effect of urea fertilization on methane oxidation activity and MOB community composition, and the role of methane on ammonia oxidation activity and AOB/AOA community composition. The soil microcosm with ^{12}C-CH_4+Urea amendment was performed as control treatment for the labeled SIP microcosms.

Microcosms for stable-isotope probing incubations were constructed in triplicate by adding approximately 7.30 g fresh soil (equivalent to 6.0 g dry weight of soil, i.e., *d.w.s.*) to 120 mL serum bottles capped with black butyl stoppers for incubation at 28° C in the dark for 19 days. To increase the labeling efficacy of targeted microorganisms, the pre-incubation of soil at 40% maximum water-holding capacity (WHC) was performed for 14 days to reduce the amount of soil-respired ^{12}C-CO_2 (Jia and Conrad, 2009; Xia et al., 2011). The ^{13}C-CH_4-labeled microcosms and ^{13}C-CH_4+Urea-labeled microcosms were injected with $^{13}CH_4$ (99 atom %^{13}C, Sigma-Aldrich Co., St Louis, MO, USA) to reach 9000 ppmv (Table S1). Meanwhile, ^{13}C-Urea fertilization of 100 μg urea-N/g. *d.w.s.* with 5% $^{13}CO_2$ (99 atoms %^{13}C, Sigma-Aldrich Co., St Louis, MO, USA) was performed for ^{13}C-Urea-labeled microcosms and for ^{13}C-CH_4+Urea-labeled microcosms as previously described (Jia and Conrad, 2009). As for ^{13}C-CH_4-labeled microcosms, the distilled water instead of urea was added. SIP control microcosms were established in triplicate by addition of the unlabeled CH_4, urea and CO_2 instead of ^{13}C-substrate. CH_4 and CO_2 concentrations were measured every few hours depending on the rate of methane consumption by gas chromatography (Shimadzu GC12-A, Japan) as previously described (Zhu et al., 2010). After more than 90% of CH_4 was consumed, the headspace was flushed with pressurized synthetic air (20% O_2, 80% N_2) for 1 min to maintain oxic conditions before ^{13}C-labeled or unlabeled substrate was renewed, to reach about ~10000 ppmv CH_4 and/or 100 μg urea-N/g. *d.w.s.* plus 5% CO_2. Due to strong methane oxidation in microcosms amended with ^{13}C-CH_4+Urea treatment (Fig. S1), methane addition was regularly repeated, in addition to urea and CO_2 substrates. The scenario of SIP microcosm construction was detailed in supplemental Table S1. The destructive sampling was performed in

triplicate after incubation of SIP microcosms for 0, 5 and 19 days. Soil samples were immediately frozen at -20^{o} C until further use. For SIP microcosm amended with urea, approximately 3 g of fresh soil was removed from each of triplicate microcosms. The rest of the soil was homogenized with 15 mL of 2M KCl by shaking at 200 rpm for 60 min., and then passed through filter paper for determination of NH_4^+-N and NO_3^--N using a Skalar SAN Plus segmented flow analyzer (Skalar, Inc., Breda, Netherlands).

DNA extraction and Isopycnic centrifugation

The total DNA from 0.5 g soil (fresh weight) of each microcosm was extracted using the FastDNA spin kit for soil (MP Biomedicals, Cleveland, OH, USA), according to the manufacturer's instruction. Soil DNA quality and quantity were observed by a Nanodrop ND-1000UV-Vis Spectrophotometer (NanoDropTechnoloqies ,Wilmington, DE, USA), and soil DNA was stored at -20℃.

For each treatment, density gradient centrifugation of total DNA was performed to separate the ^{13}C-labeled DNA from ^{12}C-DNA as previously described in detail (Jia and Conrad, 2009; Xia et al., 2011).. In brief, approximately 2.0 μg DNA was mixed well with CsCl stock solution to achieve an initial CsCl buoyant density of 1.725 g ml^{-1} using gradient buffer (pH 8.0; 100 mMTris-HCl; 100 mMKCl; 1.0 mM EDTA). The mixture was ultra-centrifuged in a 5.1 mL Beckman polyallomer ultracentrifuge tube by using a Vti65.2 vertical rotor (Beckman Coulter, Inc., Palo Alto, CA, USA) at 177,000 g for 44 hours at 20^{o} C. A NE-1000 single syringe pump (New Era Pump Systems, Inc., Farmingdale, NY, USA) with a precisely controlled flow rate of 0.38 ml/min was used to fractionate DNA by displacing the gradient medium with sterile water from the top. Fourteen or fifteen DNA fractions were obtained with equal volumes of about 340 μL, and a 65 μL aliquot was used for refractive index measurement using an AR200 digital hand-held refractometer (Reichert Inc., Buffalo, NY, USA). The CsCl medium was removed by PEG precipitation (polyethylene glycol 6000), and the DNA pellet was further purified with 70% ethanol. The fractionated DNA was then dissolved in 30μL sterile water for downstream analysis.

Real-time quantitative PCR of total and fractionated DNA

Real-time quantitative analysis of the *pmoA* gene in total DNA and in each buoyant density of DNA gradient fraction was performed to determine the growth and efficacy of ^{13}C incorporation into the genomic DNA of MOB communities on a CFX96 Optical Real-Time Detection System (Bio-Rad, Laboratories Inc., Hercules, CA, USA)，respectively. The growth and labeling of AOB and AOA communities was assessed by real-time quantitative PCR of bacterial and archaeal *amoA* genes, respectively (Lu and Jia, 2013). The primers and PCR conditions were described in Supplementary Table S2. The reactions was performed in a 20 μL mixture containing 10.0 μL SYBR Premix Ex Taq (Takara, Dalian), 0.5μM each primer, and 1μL of DNA template. The amplification efficiencies were 93%~103% obtained with R^2 values of 99.1%~99.9%.

Pyrosequencing of 16S rRNA genes at the whole community level

Pyrosequencing of the total 16S rRNA genes was performed in triplicate microcosms (Table S3) and in the fractionated DNA from fraction-3 to 13 of each treatment (Table S4) using the universal primers 515F/907R with primer adaptors, key sequence, and tag sequence as previously described (Lu and Jia, 2013). Tag sequences were used to barcode the PCR amplicons, and PCR conditions and primers were described in Supplementary Table S2. 50 μL PCR reaction mixture containing 45μL L^{-1} Platinum PCR SuperMix (Invitrogen, Shanghai, China), a 200 nM final concentration of each primer, and 2 μL template DNAwas performed and the amplicons were purified and visualized on 1.8% agarose gels. The purified PCR products were determined by a Nanodrop ND-1000 UV-Vis Spectrophotometer. Pyrosequencing was performed on a Roche 454 GS FLX Titanium sequencer (Roche Diagnostics Corporation, Branford, CT, USA). The read was trimmed to generate high-quality sequences using mothur software (Schloss et al., 2009). Taxonomic assignment of the high-quality sequence reads were obtained by RDP Multi Classifier with a confidence threshold of 50% (Wang et al., 2007). The MOB-like and AOB-like 16S rRNA gene sequences were

extracted and clustered into operational taxonomic unit (OTU) at 97% sequence identify cut-off using mothur software package. One representative sequence of each OTU was then used for phylogenetic analysis.

Pyrosequencing of *amoA* and *pmoA* genes from total DNA and ^{13}C-labeled DNA

The *pmoA* gene for MOB and bacterial *amoA* gene for AOB were also analyzed using high-throughput pyrosequencing of the total DNA and ^{13}C-labeled DNA in the ^{13}C-labeled microcosms at day 0 and day 19 (Table S5). PCR primer pairs were A189F/mb661r for *pmoA* gene (Costello and Lidstrom, 1999; Holmes et al., 1995), and amoA-1F/amoA-2R for bacterial *amoA* gene (Rotthauwe et al., 1997), respectively (Table S2). The functional genes were amplified using total DNA extract from triplicate microcosms for each treatment. The 'heavy' DNA fraction showed the highest relative abundance of AOB and MOB 16S rRNA genes was used as the ^{13}C-DNA for pyrosequencing of functional genes. PCR was performed in a 50 µL PCR reaction mixture containing 45µL L^{-1} Platinum PCR SuperMix (Invitrogen, Shanghai, China), a 200 nM final concentration of each primer, and 2 µL template PCR products were gel purified and sent for pyrosequencing on a Roche 454 GS FLX Titanium sequencer (Roche Diagnostics Corporation, Branford, CT, USA). Raw sequences were imported into mothur software (Schloss et al 2009) for quality check, aligment and phylogenetic tree construction. High quality sequences (e.g. read legth longer than 200bp, average quantity score more than 25, without ambiguous base calls) were excluded from further analysis. Pyrosequencing of *pmoA* gene yield about 36 000 high quality sequence reads with an average legth of 482bp, while about 47 000 bacterial *amoA* gene were generated with an average length of 469bp (Table S5). *pmoA* gene sequences and bacterial *amoA* gene sequences were clustered into operational taxonomic unit at 87% (Degelmann et al 2010) and 97% sequence identity cut-off, respectively. One representative sequence was ten used from each OTU for phylogenetic analysis.

Statistical Analysis

Effect of urea or CH_4 on measured parameters was tested using one-way analysis of variance analysis (ANOVA). Prior to ANOVA analysis these data were tested for normality (plots of SD versus means) and for homogeneity of variances (Levene's test). All analyses were performed using SPSS Statistics soft package version 16.0.

Accession number of nucleotide sequences

The pyrosequencing reads have been deposited at DNA Data Bank of Japan (DDBJ) with accession numbers DRA001245 and DRA001247 for the 16S rRNA genes and functional genes (bacterial *amoA* and *pmoA*), respectively.

Results

Microbial oxidation of methane and ammonia

Methane oxidation activity was assessed by determining the amount of methane consumed in soil microcosms over the incubation course of 19 days, and the strong capacity of methane oxidation was observed in the paddy soil tested (Fig. S1). It is estimated that 4.01 and 32.4 μmol CH_4 g^{-1} *d.w.s* were oxidized in soil microcosms after incubation with CH_4 for 5 and 19 days, respectively (Fig. 1a). Urea fertilization significantly stimulated methane oxidation activity by 2- and 6-fold at day 5 and 19, respectively (Fig. 1a). Soil nitrification activity was determined as the increase of soil nitrate concentrations during incubation of microcosms for 19 days. Soil nitrate content significantly increased from 11.1 μg NO_3^--N /g *d.w.s* in urea-amended microcosms at day 0, to 61.0 and 137.6 μg NO_3^--N /g *d.w.s.* at 5 and 19 days, respectively (Fig. 1b, Fig.S2). The presence of CH_4 in the headspace of urea-amended microcosms significantly inhibited production of soil nitrate at day 19, although statistically significant inhibition was not observed at day 5 (Fig. 1b, Fig.S2).

High-throughput fingerprinting of the total microbial communities was performed by pyrosequencing of the total 16S rRNA genes in SIP microcosms over the 19 days incubation period (Table S3). About 346, 000 high-quality sequence reads were

obtained with an average length of 377 bp in the V3~V4 region, while about 337,000 sequences was affiliated with bacteria. Relative abundance of bacterial 16S rRNA genes in total microorganism was ranged from 95.3% to 98.8% in these microcosms, which was much higher than that of archaeal 16S rRNA genes (Table S3). Methanotrophic 16S rRNA genes comprised only 0.28% of total microbial communities in paddy soil tested (Fig. 1c). However, methane oxidation led to a remarkable increase of MOB-like 16S rRNA genes up to 27.9% of the total microbial communities during SIP microcosm incubations (Fig. 1c). Interestingly, methanotrophic proportions appeared to show a decreasing trend with prolonged incubation of microcosms amended only with CH_4 from 14.8% at day 5 to 7.42% to day 19. Nonetheless, urea addition resulted in higher abundance of methanotroph-like 16S rRNA gene sequences up to 19.8% and 27.9% at day 5 and day 19, respectively, representing 1.3- and 4-fold increase relative to CH_4-amended microcosms (Fig. 1c). The population size of MOB community determined by real-time PCR of *pmoA* genes (Fig. S3a) showed the similar result with 16S rRNA pyrosequencing analysis. The copy number of *pmoA* genes increased significantly from 4.44×10^8 copies g^{-1} *d.w.s.* at day 0 to 1.45×10^9copies g^{-1} *d.w.s.* and 1.66×10^9 copies g^{-1} *d.w.s.* in the microcosms incubated with CH_4 for 5 and 19, respectively. Urea addition led to 1.35 and 3.16 times more *pmoA* genes than that in only CH_4-incubated microcosms at day 5 and day 19, respectively. The family *Methylophilaceae*, using methanol as sole source of carbon and energy (Devries et al., 1990; He et al., 2012), was methanol-oxidizing bacteria analyzed in our study. Similar trend was observed for 16S rRNA gene sequences affiliated with methanol-oxidizing bacteria (Fig. 1e), the relative abundance of which was 150-fold higher in soil microcosms with CH_4+Urea treatment (2.76%) than that in CH_4-amended microcosms (0.02%) at day 19.

AOB 16S rRNA gene sequences comprised only a tiny fraction of the total microbial communities during a 19-day incubation period (Fig. 1d). The relative abundance increased significantly in urea-amended microcosms from 0.21% at day 0 to 0.35% at day 19. The presence of CH_4 significantly suppressed the proportional increase of

AOB-like 16S rRNA gene reads leading to a relative frequency down to 0.15% at day 19 (Fig. 1d). The copies of bacterial *amoA* gene detected by real-time PCR increased from 4.08×10^{7}copies g^{-1} *d.w.s.* at day 0 to 1.06×10^{8}copies g^{-1} *d.w.s.* at day 19 in the microcosms incubated with urea (Fig. S3b). The increasement also was observed in the urea+CH_4 treatment, however, the presence of CH_4 resulted in 1.33-fold decrease relative to only urea-amended microcosms after incubation for 19 days. This indicated that CH_4 partially inhibited the growth of AOB. Similar results were observed for soil nitrite-oxidizing bacteria (NOB). For instance, the relative abundance of NOB 16S rRNA gene sequences in total microbial community increased significantly from 0.91% at day 0 to 1.42% at day 19 in the urea-amended microcosms, while soil microcosms with Urea+CH_4 displayed a relative abundance as low as 0.42% at day 19 (Fig. 1f). As for AOA, there was no significant change in relative abundances upon urea fertilization during SIP microcosm incubation, although the decreasing trend was observed in the presence of CH_4 (Fig. S4). The similar result was also detected by the real-time PCR of archaeal *amoA* gene (Fig. S3c).

High-throughput fingerprinting of functional guilds against the total communities

The 16S rRNA genes affiliated with MOB and AOB were selected for phylogenetic analysis from the total pyrosequencing reads in soil microcosms, after incubation for 5 and 19 days, following the additions of methane and/or urea. Phylogenetic analysis revealed a remarkable shift of MOB community structure based on both 16S rRNA gene (Fig. S5a) and *pmoA* genes (Fig. S5b). Though type II methanotrophs dominate MOB communities in background soil at day 0, the consumption of CH_4 in soil microcosms led to a drastic increase in relative abundance of type Ia methanotrophic 16S rRNA gene sequences in the total 16S rRNA gene sequences from 0.09% at day 0 to 14.4% at day 5 (Fig. 2a). Interestingly, type II methanotroph-like 16S rRNA genes stayed at very low proportions in the total microbial community during the entire incubation period, whereas significant increase was observed from 0.12% at day 0 to 0.55% at day 19. Urea fertilization further stimulated the relative abundance of type Ia

methanotrophs reaching 1.3 and 4 times higher in the CH_4+Urea-amended microcosms than that in the microcosms amended only with CH_4 at day 5 and day 19, respectively. However, urea nitrogen appeared to have no effect on the relative abundance of type II methanotrophs. Similar results were obtained by pyrosequencing analysis of *pmoA* genes (Fig. S5b). Phylogenetic analysis of *pmoA* genes indicated that type Ia *pmoA* sequences were stimulated from 7.4% at day 0 to 69.8% of total methanotrophic communities after incubation with CH_4 for 19 days. Urea addition further stimulated the proportion of type Ia methanotroph *pmoA* gene sequences to a greater extent up to 84.7%.

AOB communities were exclusively dominated by *Nitrosospira*-like 16S rRNA gene sequences at day-0, and none of 16S rRNA gene sequences could be assigned to *Nitrosomonas* (Fig. S6a). However, the relative abundance of *Nitrosomonas*-like 16S rRNA genes rose to 0.04% and 0.06% of the total microbial communities in urea-amended microcosms after incubation for 5 and 19 days, respectively (Fig. 2b). CH_4 addition resulted in lower abundance of *Nitrosomonas*-like 16S rRNA genes in the total microbial communities at day 5 and day 19, representing 2- and 3-fold decrease relative to that in urea-amended microcosms (Fig. 2b). The relative abundance of *Nitrosospira*-like AOB was stimulated by urea fertilization, but partially inhibited in the presence of CH_4 (Fig. 2b). These results were further verified by phylogenetic analysis of the *amoA* pyrosequencing reads (Fig. S6b). For instance, none of *amoA* gene sequences was affiliated with *Nitrosomonas* in background soil at day 0, whereas 7% of *amoA* gene sequences were affiliated with *Nitrosomonas* at day 19 in the urea-amended microcosms.

Stable isotope probing of active methanotrophs and ammonia oxidizers

The incorporation of ^{13}C-label into nucleic acid of active microbial communities in complex soil was analyzed by isopycnic centrifugation of total DNA extracted from SIP microcosms. The fractionated DNA over the entire density range of a given gradient was further assessed by pyrosequencing of the total 16S rRNA gene. About

418,000 high-quality reads were generated with an average length of 356 bp in the V3~V4 region of the 16S rRNA gene (Table S4), while about 409,000 sequences were affiliated with bacteria. Pyrosequencing the relative abundance of microbial guilds as a function of the buoyant density of the DNA gradient indicated that MOB and AOB were ^{13}C-labeled to different extents. The relative abundance of 16S rRNA gene sequences of methanotrophs was exceptionally high up to 90% of the total 16S rRNA gene sequences in the 'heavy' DNA fractions from the labeled microcosms, suggesting strong labeling of methanotrophic communities in soils after incubation for 5 (Fig.3a) and 19 days (Fig.3b). This was further supported by quantitative analysis of *pmoA* gene copies reaching the peak in the 'heavy' DNA fractions from the labeled microcosms, while the highest number was observed in the 'light' DNA fractions for the ^{12}C-control treatment (Fig. S7). In addition, the relative abundance of 16S rRNA gene sequences affiliated with methanol-oxidizing bacteria was apparently higher in the 'heavy' DNA fractions from the labeled microcosms (^{13}C-CH_4 and ^{13}C-CH_4+Urea) than those in the control treatments (^{12}C-CH_4+Urea), despite the relatively low proportion of ~0.20% at day 5 (Fig. 3c). The prolonged incubation for 19 days increased the proportion of methanol-oxidizing bacteria significantly up to 11.0% of the total 16S rRNA gene sequences in the ^{13}C-DNA from the labeled soil microcosms amended both with CH_4 and Urea, but not in the labeled microcosms that received only CH_4 (Fig. 3d).

The 16S rRNA gene sequences of AOB were highly enriched in 'heavy' DNA fractions from the labeled microcosm amended only with urea at day 5 (Fig. 3e) and day 19 (Fig.3f), but not the CH_4+Urea treatment during the 19-day incubation period. For instance, up to 5.73% of total 16S rRNA gene sequences in the 'heavy' DNA fractions could be assigned to AOB for ^{13}C-Urea treatment, while only 0.33% of the total 16S rRNA gene sequences in the ^{13}C-Urea+CH_4 treatments were related to AOB at day 19 (Fig. 3f). Similar results were obtained for nitrite-oxidizing bacteria (Fig.3g and Fig.3h). The relative abundance of NOB in the 'heavy' DNA fractions was significantly higher in microcosms with ^{13}C-urea than ^{13}C-Urea+CH_4 treatment,

423 implying a much greater degree of labeling of NOB cells in ^{13}C-Urea treatments
424 during active nitrification. Furthermore, it is noteworthy that no significant
425 enrichment of archaeal 16S rRNA gene sequences occurred in the 'heavy' DNA
426 fractions from the labeled microcosms (Fig. S8).

427 Phylogenetic analysis of the ^{13}C-labeled 16S rRNA genes demonstrated that active
428 MOB were affiliated with Type Ia (*Methylobacter*- and *Methylosarcina-like*) and
429 *Methylocystis*-related type II methanotrophs, while type Ib methanotrophic sequences
430 were not detected during active methane oxidation (Fig. 4a). Active ammonia
431 oxidizers were phylogenetically assigned to distinctly different phylotypes including
432 the *Nitrosospira* clusters and the *Nitrosomonas communis* lineage on the basis of
433 ^{13}C-16S rRNA gene analysis (Fig. 5a). DNA-SIP demonstrated remarkable
434 community shifts of methanotrophs and ammonia oxidizers during the 19-day
435 incubation period (Fig. 6). Type Ia-like MOB accounted for 89% of the ^{13}C-labeled
436 methanotrophic 16S rRNA sequences in CH_4-amended microcosms at day 19, while
437 up to 98% of the active methanotrophs could be assigned to Type Ia MOB in soil
438 microcosms amended with both CH_4 and urea (Fig. 6a). This was further supported by
439 pyrosequencing analysis of *pmoA* genes in the ^{13}C- DNA (Fig. 4b). For instance,
440 85.0% of *pmoA* genes were affiliated to type Ia MOB in CH_4-amended microcosms at
441 day 19, whereas all *pmoA* sequences were detected exclusively as type Ia MOB in the
442 microcosms amended with both CH_4 and urea. As for ammonia oxidizers, the relative
443 abundance of *Nitrosomonas*-like 16S rRNA genes was as high as 88.2% of the
444 ^{13}C-labeled AOB communities in microcosms after incubation with urea for 5 days
445 (Fig. 6b). However, the presence of CH_4 resulted in lower proportions of
446 *Nitrosomonas*-like 16S rRNA genes, represented by 1.6 and 1.3 times lower than that
447 in urea-amended microcosms at day 5 and day 19, respectively. Pyrosequencing of
448 *amoA* genes in the ^{13}C-DNA lend further support for the suppression of
449 *Nitrosomonas*-like AOB since it decreased from 21% to 2% of active AOB
450 communities upon by CH_4 addition (Fig. 5b).

Discussion

The interaction between methane and nitrogen has been identified as one of the major gaps in carbon-nitrogen cycle interactions (Gardenas et al., 2011; Gärdenäs et al., 2011). There are many possible feedbacks to climate change through effects on methane and N_2O emissions and eutrophication of soils and sediments as a consequence of interactions between methane- and ammonia oxidizers. The inhibition of mineral nitrogen on methane consumption has been demonstrated from numerous studies, however, ammonium-based fertilization was observed to stimulate methane consumption in rice paddies (Bodelier and Laanbroek, 2004). Mechanistically, there is still a poor understanding of nitrogen effects on methane cycling and vice versa. Elucidation of these mechanisms is of utmost importance to obtain comprehensive understanding of the nature of the effects of e.g. climate change on the release of major greenhouse gases from various ecosystems.

Due to the enzymatic similarity of methane and ammonia monooxygenase, methane and ammonia-oxidizers can oxidize methane as well as ammonia (Bodelier and Frenzel, 1999; Oneill and Wilkinson, 1977; Stein et al., 2012). However, methane oxidizers do not gain energy out of the oxidation of ammonia while ammonia oxidizers do not grow on methane (Stein et al., 2012). Moreover, mineral nitrogen is essential for biomass formation, especially for those methanotrophs lacking the ability to fix molecular nitrogen (Semrau et al., 2010). The latter indicates that next to direct enzymatic effects, interactions at the level of competition for N will play an important role in this matter, especially in high methane environments where ammonia oxidizers will face enzymatic as well as competitive stress, with respect to which sparse information is available.

The pre-incubation was performed to increase the labeling efficiency of targeted microorganisms because the dilution of $^{13}CO_2$ by soil-respired $^{12}CO_2$ could be decreased significantly as reported previously (Jia and Conrad 2009, Xia et al 2011). No apparent changes of ammonia oxidizer communities were observed during a

4-week pre-incubation without ammonium fertilization, significant shift of AOB communities occurred in the ammonium-amended soils (Jia and Conrad 2009). The nitrogenous fertilization of paddy field in this study is about 250 kg N ha^{-1}, which is equivalent to 107 µg N g^{-1}*d.w.s*, assuming an effective soil depth of 20 cm. In addition, methane concentrations of 900 to 15000 µL L^{-1} were generally detected in paddy soil during rice-growing season (Nouchi et al 1990, Nouchi et al 1994). Therefore, the microcosms were incubated with 100 µg urea-N g^{-1}*d.w.s.* and 10000 µL L^{-1} methane to extrapolate the microbial interactions between methane- and ammonia-oxidation under field conditions. It suggests that microcosms might represent largely what is occurring under *in situ* conditions, although it could not reproduce the physiochemical and biological conditions in field. For instance, it also has been reported that the results of microcosm incubations remained largely consistent with population dynamics of methanotrophic communities in field (Eller et al 2005).

In our study, it is demonstrated that urea fertilization significantly stimulated methane oxidation activity and growth of MOB. Growth and activity of ammonia oxidizers was partially inhibited in the presence of CH_4. It is obvious that competitive inhibition of the methane monooxygenase did not occur in our microcosms. The ratio of N-CH_4 is approximately 0.11 (assuming all urea is converted to ammonium). In other studies ratios of up to 200 (Bodelier et al., 2000b) did not lead to inhibition. Hence, it is safe to conclude that the ammonium formed out of urea or the subsequently produced nitrate acted as nitrogen source for biomass generation of MOB. The decreased NH_4^+-N concentrations corresponded with the increased NO_3^--N concentrations via nitrification only in the microcosms without methane amendment. Addition of methane to microcosms led to lower recovery of mineral N (Table 1), despite the equal addition of urea (Table S1), suggesting that part of consumed ammonia was not oxidized to nitrate via nitrification or part of the nitrate disappeared. We deduce that the consumed ammonia, which was not involved in ammonia oxidation, may be assimilated as a nitrogen nutrient for cell growth of MOB. Assuming that for oxidation of every mol CH_4-C, 0.25 mol N has to be assimilated by MOB (Bodelier and Laanbroek, 2004), the amount of N-assimilated can be calculated using a 70:30

510 ratio of respiration of CH_4 vs assimilation. This calculation shows that of the total
511 amount of urea added 69% was assimilated by MOB, while 20% was nitrified (Table
512 S6). The fate of unaccounted remaining nitrogen (11%) need further experiment to
513 investigate.

514 Our results even demonstrate the dependency of the MOB on sufficient N-availability.
515 The relative abundance of both 16S rRNA and *pmoA* genes decreased when
516 incubating with methane only, demonstrating loss of activity and growth potential
517 when N is limiting. A similar result was obtained in microcosms planted with rice
518 (Bodelier et al., 2000a), where MOB even lost their potential for oxidizing methane.
519 However, adding ammonium to these inactive communities led to immediate
520 re-activation of oxidation (Bodelier et al., 2000a), indicating that N-limitation is not
521 only inhibiting growth but also regulated methane consumption enzyme machinery.
522 This inactivation and rapid re-activation of methane oxidation has even been
523 demonstrated on field scale in rice paddies (Dan et al., 2001; Kruger and Frenzel,
524 2003). It has been proposed that nitrogen fixation may deplete reducing equivalents
525 leading to lowering and even cessation of methane oxidation (Bodelier and Laanbroek,
526 2004; Dan et al., 2001). This suggests that under conditions of high methane and low
527 N availability, there is a niche for methanotrophy where they seem to overwhelmingly
528 outcompete nitrifying communities. Nitrifiers can operate in the absence of
529 competition with MOB, which may be inactivated due to energy-depletion as the
530 result of N_2-fixation. Hence, this points to niche differentiation or avoidance strategies
531 of the nitrifiers.

532 It is obvious that only a subset of the MOB profit substantially from the combined
533 addition of methane and urea-N. Although type II MOB increase in relative
534 abundance of 16S rRNA gene sequences in total microbial community with the
535 addition of methane they do not profit from the addition of urea, but are also not
536 affected by it. Addition of ammonium to rice soil has been demonstrated to inhibit
537 type II MOB (Mohanty et al., 2006). This is obviously not the case in our study where

538 the rapid growth of type Ia MOB keeps ammonium N-low. The growth of type II
539 MOB is apparently independent of the N-availability suggesting that they can rely on
540 N_2-fixation only. ^{13}C-labeled methanotrophic 16S rRNA gene sequences are closely
541 affiliated with *Methylocystis parvus* OBBP, which possesses nitrogenase and capable
542 of nitrogen fixing (Murrell and Dalton 1983). This suggested that these *Methylocystis*
543 *parvus*-like type II may respond under N-limited conditions in our study. Next to this,
544 the presence of highly active type I MOB did not prevent the growth of type II.
545 However, significant growth of type II MOB only occurs after 19 days of incubation
546 suggesting that either lower growth rates as compared to type I or dependency of type
547 II MOB on the activity of type I. The former is indeed the case as was demonstrated
548 in wetland soil microcosms (Steenbergh et al., 2010) while the latter maybe the result
549 from the fact that type II MOB may use CO_2 (Yang et al., 2013) as their main
550 C-source for assimilation (Matsen et al., 2013). Labelled CO_2 in the microcosms can
551 only be formed by methane oxidation carried by type Ia in the early stages of the
552 experiment. Another explanation may be succession of MOB, with type II MOB
553 increasing in number when type I MOB are getting limited by N (Krause et al., 2010).

554 The strong stimulation of type Ia MOB upon methane application alone and in
555 combination with urea-N application has been observed frequently in rice soils but
556 also in other environments, reflecting their competitive life-strategy as reviewed and
557 synthesized (Ho et al., 2013). The most responsive MOB species in high methane
558 habitats seem to be *Methylobacter* species (Krause et al., 2012). Our experiments
559 show that *Methylosarcina* species are clearly the most responsive without addition of
560 urea. This is in contrast with the niche differentiation observed at high spatial
561 resolution in rice soil microcosms (Reim et al., 2012). The presence of
562 *Methylosarcina* related MOB in the surface layer of thin layer microcosms and not in
563 the methane-oxygen interface, implying that *Methylosarcina* thrives under
564 low-methane ('oligotrophic') conditions, in contrast to *Methylobacter* which
565 dominates the zone of high methane flux. However, remarkably, in our experiments
566 *Methylosarcina* clearly is dominant at high methane supply but is replaced partly by

Methylobacter when urea-N is added. This might be attributed to competition for methane, nitrogen, or even oxygen. A similar result was observed in SIP analyses of lake sediment microcosms using a metagenomic approach (Beck et al., 2013). Hence, we speculate that observations by Reim et al (Reim et al., 2012) may also be explained by weak competitive abilities of *Methylosarcina* instead of being restricted to low methane habitats. A comparison of 16S rRNA gene and *pmoA* gene sequences revealed that *Methylobacter* was detected in a higher proportion in the MOB-16S rRNA gene phylogenetic tree than in the *pmoA* gene phylogenetic tree. The incongruence might result from the bias associated with the different coverage of *pmoA* and 16S rRNA gene primers as reported previously (Costello and Lidstrom 1999).

Interestingly, the significant increase of obligate methane-oxidizer *Methylophilaceae* was observed in the microcosms incubated with CH_4 plus urea. The occurrence 16S rRNA of these sequences in the 'heavy' DNA fractions indicates that these *Methylophilaceae* assimilated methane derived carbon. Cross feeding of methylotrophs by methanotrophs releasing methanol has been demonstrated before (Antony et al., 2010; Beck et al., 2013; He et al., 2012; Noll et al., 2008). The direct mechanism for this cross feeding and what compound actually is exchanged have not been elucidated yet. We can add another component to this body of unsolved mechanisms which is the strong stimulation of methylotrophs upon urea fertilization, thereby linking the nitrogen and the carbon cycle. It is very likely that the enhanced methane consumption and growth of methanotrophs leads to higher availability of methanol. However, we can not exclude that urea has stimulatory effect on the methylotrophs directly. We also speculate that the active removal of methanol by the methylotrophs is beneficial to methanotrophs given the toxic nature of the compound. However, this would be subject of further study. Interesting is this link between nitrogen and cross-feeding of methanotrophic metabolites by other microorganism, possibly creating novel niches e.g. more methane-driven carbon substrate, lower-toxic environment for methanotrophs in soil.

Our results revealed that the presence of CH_4 in microcosms partially inhibited the nitrification activity in the paddy soil tested. Physiologically, the enzymatic similarity of ammonia-oxidizers and MOB may result in ammonia oxidation by MOB (Bodelier and Frenzel, 1999), leading to reduced availability of ammonia for ammonia oxidizers. However, previous studies showed that MOB had lower affinity for ammonia than for CH_4 (Banger et al., 2012; Bedard and Knowles, 1989; Yang et al., 2011). Moreover, it has been proposed that ammonia oxidation by MOB occurred only when the ratio of ammonia to CH_4 is higher than 30 in soils (Banger et al., 2012; Bodelier and Laanbroek, 2004; Yang et al., 2011). The molecular ratio of ammonia to CH_4 was about 0.11 in our study, thus the suppression of ammonia oxidizers growth and activity in the presence of CH_4 may not be explained by ammonia oxidation by MOB. Furthermore, a large part of the applied N disappeared in the presence of CH_4, and presumably assimilated by MOB. This explanation seems plausible for the suppression of methane on ammonia oxidation and the growth of ammonia oxidizers. In the meantime, it remains elusive about the toxic effect of intermediates substance during methane oxidation on nitrifying communities. For example, methanol may inhibit the growth of AOA and AOB communities, and we detected no archaeal *amoA* genes and 16S rRNA genes. The possibility of heterotrophic AOA lifestyle could also not be excluded (Ingalls et al., 2006; Stahl and de la Torre, 2012).

The genus *Nitrosospira* was the dominant AOB in the native soil, being consistent with general observations that *Nitrosospira* are ubiquitous in upland soils as important members of nitrifying population (Hastings et al., 1997; Stephen et al., 1996). In our study, the apparent growth of *Nitrosospira* was observed in the microcosms amended with urea-N, and the cluster 3 was the dominant active *Nitrosospira* group. It has been reported that *Nitrosospira* cluster 3 was the dominant AOB group in a number of neutral soil receiving nitrogen fertilization (Bruns et al., 1999; Mendum et al., 1999). Intriguingly, methane addition suppressed the growth of *Nitrosospira*, and AOB within the cluster 3 appeared to be inhibited to a greater extent than those of cluster 4. It has been proposed that the reduced ammonia supply may select for the cluster 4

625 populations (Kowalchuk and Stephen, 2001). In the presence of methane, the growth
626 of methanotrophs was significantly stimulated and methanotrophic N assimilation
627 could have likely led to the depletion of ammonium in support of nitrification activity.
628 It was noteworthy that none of 16S rRNA and *amoA* genes were affiliated with
629 *Nitrosomonas* in the native soil at day zero. The growth of *Nitrosomonas* was
630 stimulated to a much greater extent than that of *Nitrosospira* in urea-amended
631 microcosms, but *Nitrosomonas* appeared to be suppressed more significantly than
632 *Nitrosopira*. This might be explained by the fact that hat *Nitrosomonas* are markedly
633 responsive to ammonia input (Hastings et al., 1997). Similar to methanotrophic
634 communities, the proportion of *Nitrosospira* in AOB community detected by 16S
635 rRNA gene sequences was lower than that detected by *amoA* gene. It could be in part
636 attributed to the variation of *amoA* copy numbers among different AOB. For instance,
637 the species *N. briensis* and *N. europaea* have two copies of *amoA* genes and *N. tenuis*
638 contained three identical *amoA* genes (Norton et al., 1996; Sayavedra-Soto et al.,
639 1998).

640 The abilities to catalyze the hydrolysis of urea to yield ammonia can be observed in a
641 wide range of microorganisms possessing urease activity (Mobley and Hausinger
642 1989). Some methanotrophs have been identified with the ability of urea hydrolysis
643 (Boden et al 2011, Khmelenina et al 2013), however, the ^{13}C-labeled active
644 methanotrophs on the basis of 16S rRNA gene (Fig.4a) and *pmoA* gene (Fig.4b) were
645 phylogenetically distinctly different with these known ureolytic methanotrophs.
646 However, the ^{13}C-labeled AOB (Fig.5) showed high sequence similarity with
647 ureolytic *Nitrosomonas nitrosa* and *Nitrosomonas oligotrophs*. This indicates the
648 potential of hydrolyzing urea in these active ammonia-oxidizing bacteria. It was
649 estimated that 30%~50% of ammonia could be released from hydrolysis of urea by
650 AOB in batch culture (Pommerening-Roser and Koops 2005). This suggests that
651 ammonia oxidizers may have to compete for the ammonia released into environment
652 with other ammonia-utilizing microorganisms such as methanotrophs, intensifying the
653 competition for nitrogen between AOB and MOB. It is noteworthy that there was no
654 report about the ureolysis of AOA in non-acid soils.

Taken together, the results of this study demonstrate the stimulation of methane consumption and growth of MOB by urea and the subsequent suppression of nitrifier growth and activity. Only a sub-set of the MOB profited from the urea addition, with *Methylobacter* species responding the most vigorous, showing that urea addition gives rise to niche differentiation in MOB communities. In addition, our results revealed the cross-feeding of methane-derived carbon in the soil system upon urea fertilization, indicating urea might play an important role in carbon cycle through the microbial food web processing carbon from methane oxidation in paddy soil. Assimilation of N possibly might provide mechanistic mechanisms for inhibition of ammonia oxidizers by methane addition. Therefore, we speculated that competition for nitrogen between methane- and ammonia-oxidizers play a dominant role in microbial interactions in our study, which is of help toward predictive understandings of carbon and nitrogen cycle in complex environment.

Acknowledgments

This work was financially supported by the National Science Foundation of China (41090281), the Ministry of Science and Technology of China (2010DFA22770), and the Distinguished Young Scholar Programme of Jiangsu Province (BK2012048). The authors thank our lab colleagues for helpful discussion.

Reference

Antony, C. P., Kumaresan, D., Ferrando, L., Boden, R., Moussard, H., Fernandez Scavino, A., Shouche, Y. S., and Murrell, J. C.: Active methylotrophs in the sediments of Lonar Lake, a saline and alkaline ecosystem formed by meteor impact, ISME J., 4,1470-1480,2010.

Banger, K., Tian, H.,and Lu, C.: Do nitrogen fertilizers stimulate or inhibit methane emissions from rice fields?,Glob Change Biol., 18,3259-3267,2012.

Beck, D. A. C., Kalyuzhnaya, M. G., Malfatti, S., Tringe, S. G., Glavina Del Rio, T., Ivanova, N., Lidstrom, M. E., and Chistoserdova, L.: A metagenomic insight into freshwater methane-utilizing communities and evidence for cooperation between the Methylococcaceae and the Methylophilaceae, PeerJ., 1, e23,doi:10.7717/peerj.23,2013.

Bedard, C. and Knowles, R.: Physiology, biochemistry, and specific inhibitors of CH4,

686 NH_4^+, and CO oxidation by methanotrophs and nitrifiers, Microbiol. Rev., 53,
687 68-84,1989.
688 Betlach, M.R. and Tiedje, J.M.: Kinetic explanation for accumulation of nitrite, nitric
689 oxide, and nitrous oxide during bacterial denitrificationt. Appl. Environ.
690 Microbiol.,42,1074-1084, 1981.
691 Bock, E, Wagner M. (2006). Oxidation of inorganic nitrogen compounds as an energy
692 source. In: Dworkin M,Falkow S (eds). The Prokaryotes. Springer Verlag: New
693 York, pp 457–495，2006..
694 Bodelier P.L. and Laanbroek, H.J: Nitrogen as a regulatory factor of methane
695 oxidation in soils and sediments, FEMS Microbiol. Ecol., 47, 265-277, 2004.
696 Bodelier, P. L. E. and Frenzel, P.: Contribution of methanotrophic and nitrifying
697 bacteria to CH_4 and NH_4^+ oxidation in the rhizosphere of rice plants as determined
698 by new methods of discrimination, Appl. Environ. Microbiol., 65, 1826-1833,
699 1999.
700 Bodelier, P. L. E., Bär-Gilissen, M.-J., Meima-Franke, M., and Hordijk, K.: Structural
701 and functional response of methane-consuming microbial communities to different
702 flooding regimes in riparian soils, Ecol. Evol., 2, 106-127, 2012.
703 Bodelier, P. L. E., Gillisen, M.-J. B., Hordijk, K., Damste, J. S. S., Rijpstra, W. I. C.,
704 Geenevasen, J. A. J., and Dunfield, P. F.: A reanalysis of phospholipid fatty acids as
705 ecological biomarkers for methanotrophic bacteria, ISMEJ., 3, 606-617, 2009.
706 Bodelier, P. L. E., Hahn, A. P., Arth, I. R., and Frenzel, P.: Effects of ammonium-based
707 fertilisation on microbial processes involved in methane emission from soils
708 planted with rice, Biogeochem., 51, 225-257, 2000a.
709 Bodelier, P. L. E., Roslev, P., Henckel, T., and Frenzel, P.: Stimulation by
710 ammonium-based fertilizers of methane oxidation in soil around rice roots, Nature,
711 403, 421-424, 2000b.
712 Bodelier, P. L. E.: Interactions between nitrogenous fertilizers and methane cycling in
713 wetland and upland soils, Curr. Opin. Environ. Sustainability, 3, 379-388, 2011.
714 Bodelier, P. L., Meima-Franke, M., Hordijk, C. A., Steenbergh, A. K., Hefting, M. M.,
715 Bodrossy, L., von Bergen, M., and Seifert, J.: Microbial minorities modulate
716 methane consumption through niche partitioning, ISMEJ., 7, 2214-2228, 2013.
717 Boden, R., Cunliffe, M., Scanlan, J., Moussard, H., Kits, K.D., Klotz, M.G. *et al:*
718 Complete Genome Sequence of the Aerobic Marine Methanotroph Methylomonas
719 methanica MC09, J. Bacteriol., 193: 7001-7002, 2011.
720 Bratina, B.J., Brusseau, G.A., Hanson, R.S: Use of 16S ribosomal-RNA analysis to
721 investigate phylogeny of methylotrophic bacteria, Int. J. Syst. Bacteriol., 42,
722 645-648,1992.
723 Bruns, M. A., Stephen, J. R., Kowalchuk, G. A., Prosser, J. I., and Paul, E. A.:
724 Comparative diversity of ammonia oxidizer 16S rRNA gene sequences in native,
725 tilled, and successional soils, Appl. Environ. Microbiol., 65, 2994-3000, 1999.
726 Costello, A.M. and Lidstrom, M.E.,: Molecular characterization of functional and
727 phylogenetic genes from natural populations of methanotrophs in lake sediments,

728 Appl. Environ. Microbiol., 65, 5066-5074, 1999.
729 Dan, J. G., Kruger, M., Frenzel, P., and Conrad, R.: Effect of a late season urea
730 fertilization on methane emission from a rice field in Italy, Agr. Ecosys. Environ.,
731 83,191-199, 2001.
732 Degelmann, D.M., Borken, W., Drake, H.L., Kolb, S.:Different atmospheric
733 methane-oxidizing communities in European beech and Norway spruce soils, Appl.
734 Environ. Microbiol. 76, 3228-3235,2010.
735 Devries, G.E., Kues, U., and Stahl, U.: Physiology and genetics of methylotrophic
736 bacteria. FEMS Microbiol. Rev., 75,57-101, 1990.
737 Dumont, M. G., Pommerenke, B., Casper, P., and Conrad, R.: DNA-, rRNA- and
738 mRNA-based stable isotope probing of aerobic methanotrophs in lake
739 sediment,Environ. Microbiol.,13,1153-1167, 2011.
740 Dunfield, P. F., Yuryev, A., Senin, P., Smirnova, A. V., Stott, M. B., Hou, S., Ly, B.,
741 Saw, J. H., Zhou, Z., Ren, Y., Wang, J., Mountain, B. W., Crowe, M. A., Weatherby,
742 T. M., Bodelier, P. L. E., Liesack, W., Feng, L., Wang, L., and Alam, M.: Methane
743 oxidation by an extremely acidophilic bacterium of the phylum Verrucomicrobia,
744 Nature, 450, 879-882, 2007.
745 Eller, G., Kruger, M., Frenze,l P.: Comparing field and microcosm experiments: a case
746 study on methano- and methylo-trophic bacteria in paddy soil, Fems Microb. Ecol.,
747 51, 279-291,2005.
748 Galloway, J. N., Townsend, A. R., Erisman, J. W., Bekunda, M., Cai, Z., Freney, J. R.,
749 Martinelli, L. A., Seitzinger, S. P., and Sutton, M. A.: Transformation of the
750 nitrogen cycle: Recent trends, questions, and potential solutions, Science, 320,
751 889-892, 2008.
752 Gärdenäs, A. I., Ågren, G. I., Bird, J. A., Clarholm, M., Hallin, S., Ineson, P., Kätterer,
753 T., Knicker, H., Nilsson, S. I., Näsholm, T., Ogle, S., Paustian, K., Persson, T., and
754 Stendahl, J.: Knowledge gaps in soil carbon and nitrogen interactions - From
755 molecular to global scale, Soil Biol. Biochem., 43, 702-717, 2011.
756 Hanson, R.S., and Hanson, T.E.: Methanotrophic bacteria, Microbiol. Rev.,
757 60,439-471, 1996.
758 Hastings, R. C., Ceccherini, M. T., Miclaus, N., Saunders, J. R., Bazzicalupo, M., and
759 McCarthy, A. J.: Direct molecular biological analysis of ammonia oxidising
760 bacteria populations in cultivated soil plots treated with swine manure, FEMS
761 Microbiol. Ecol., 23, 45-54, 1997.
762 He, R., Wooller, M. J., Pohlman, J. W., Catranis, C., Quensen, J., Tiedje, J. M., and
763 Leigh, M. B.: Identification of functionally active aerobic methanotrophs in
764 sediments from an arctic lake using stable isotope probing, Environ. Microbiol.,
765 14,1403-1419, 2012.
766 Ho, A., Kerckhof, F.-M., Luke, C., Reim, A., Krause, S., Boon, N., and Bodelier, P. L.
767 E.: Conceptualizing functional traits and ecological characteristics of
768 methane-oxidizing bacteria as life strategies, Environ. Microbiol. Rep., 5, 335-345,
769 2013.

770 Ho, A., Lueke, C., Cao, Z., and Frenzel, P.: Ageing well: methane oxidation and
771 methane oxidizing bacteria along a chronosequence of 2000 years, Environ.
772 Microbiol. Rep., 3, 738-743, 2011.
773 Holmes, A. J., Costello, A., Lidstrom, M. E., and Murrell, J. C.: Evidence that
774 particulate methane monoxygenase and ammonia monooxygenase may be
775 evolutionarily related, FEMS Microbiol. Lett., 132, 203-208, 1995.
776 Ingalls, A. E., Shah, S. R., Hansman, R. L., Aluwihare, L. I., Santos, G. M., Druffel, E.
777 R., and Pearson, A.: Quantifying archaeal community autotrophy in the
778 mesopelagic ocean using natural radiocarbon, Proc. Natl. Acad. Sci .U S A., 103,
779 6442-6447, 2006.
780 Jia, Z. and Conrad, R.: Bacteria rather than Archaea dominate microbial ammonia
781 oxidation in an agricultural soil, Environ. Microbiol., 11, 1658-1671, 2009.
782 Jones, R. D. and Morita, R. Y.: Methane Oxidation by Nitrosococcus oceanus and
783 Nitrosomonas europaea, Appl. Environ. Microbiol., 45, 401-410, 1983.
784 Khmelenina, V.N., Beck, D.A.C., Munk, C., Davenport, K., Daligault, H., Erkkila, T.
785 *et al*: Draft Genome Sequence of *Methylomicrobium buryatense* Strain 5G, a
786 Haloalkaline-Tolerant Methanotrophic Bacterium, Genome announcements, 4,
787 2169-8287, 2013.
788 Kolb, S.: Aerobic methanol-oxidizing bacteria in soil, *Fems Microbiology Letters*, 300,
789 1-10, 2009.
790 Kowalchuk, G. A. and Stephen, J. R.: Ammonia-oxidizing bacteria: A Model for
791 Molecular Microbial Ecology, Ann. Rev. Microbiol., 55, 485-529, 2001.
792 Krause, S., Lueke, C., and Frenzel, P.: Methane source strength and energy flow shape
793 methanotrophic communities in oxygen-methane counter-gradients, Environ.
794 Microbiol. Rep., 4, 203-208, 2012.
795 Krause, S., Lueke, C., and Frenzel, P.: Succession of methanotrophs in
796 oxygen-methane counter-gradients of flooded rice paddies, ISME J., 4, 1603-1607,
797 2010.
798 Kruger, M. and Frenzel, P.: Effects of N-fertilisation on CH4 oxidation and production,
799 and consequences for CH4 emissions from microcosms and rice fields, Glob.
800 Change Biol., 9, 773-784, 2003.
801 Lipscomb, J. D.: Biochemistry of the soluble methane monooxygenase, Annu. Rev.
802 Microbiol., 48, 371-399, 1994.
803 Lu, L. and Jia, Z.: Urease gene-containing Archaea dominate autotrophic ammonia
804 oxidation in two acid soils, Environ. Microbiol., 15, 1795-1809, 2013.
805 Matsen, J. B., Yang, S., Stein, L. Y., Beck, D., and Kalyuzhnaya, M. G.: Global
806 Molecular Analyses of Methane Metabolism in Methanotrophic
807 Alphaproteobacterium, Methylosinus trichosporium OB3b. Part I: Transcriptomic
808 Study, Front. Microbiol., 4, 40-40, 2013.
809 Mendum, T. A., Sockett, R. E., and Hirsch, P. R.: Use of molecular and isotopic
810 techniques to monitor the response of autotrophic ammonia-oxidizing populations
811 of the beta subdivision of the class Proteobacteria in arable soils to nitrogen

812 fertilizer, Appl. Environ. Microbiol., 65, 4155-4162, 1999.
813 Mobley, H., Hausinger, R.: Microbial ureases: significance, regulation, and molecular
814 characterization, Microbiol. Res., 53, 85-108, 1989..
815 Modolo, L. V., Augusto, O., Almeida, I. M. G., Magalhaes, J. R., and Salgado, I.:
816 Nitrite as the major source of nitric oxide production by Arabidopsis thaliana in
817 response to Pseudomonas syringae, FEBS Lett., 579, 3814-3820, 2005.
818 Mohanty, S. R., Bodelier, P. L. E., Floris, V., and Conrad, R.: Differential effects of
819 nitrogenous fertilizers on methane-consuming microbes in rice field and forest soils,
820 Appl. Environ. Microbiol., 72, 1346-1354, 2006.
821 Murrell, J.C., Dalton, H.: Nitrogen-fixation in obligate methanotrophs, J. Gen.
822 Microbiol., 129, 3481-3486,1983.
823
824 Noll, M., Frenzel, P., and Conrad, R.: Selective stimulation of type I methanotrophs in
825 a rice paddy soil by urea fertilization revealed by RNA-based stable isotope
826 probing, FEMS Microbiol. Ecol., 65, 125-132, 2008.
827 Nouchi, I., Mariko, S., Aoki, K.: Mechanism of methane transport from the
828 rhizosphere to the atmosphere through rice plants, Plant Physiol., 94, 59-66, 1990.
829 Nouchi, I., Hosono, T., Aoki, K., Minami, K.: Seasonal-variation in methane flux
830 from rice paddies associated with methane concentration in soil-water, rice biomass
831 and temperature, and its modeling, Plant Soil, 161, 195-208, 1994.
832 Norton, J. M., Low, J. M., and Martin, G.: The gene encoding ammonia
833 monooxygenase subunit A exists in three nearly identical copies in Nitrosospira sp
834 NpAV, FEMS Microbiol. Lett., 139, 181-188, 1996.
835 O'Neill, G. G. and Wilkinson, J. F.: Oxidation of ammonia by methane-oxidizing
836 bacteria and the effects of ammonia on methane oxidation, J. Gen. Microbiol., 100,
837 407-412, 1977.
838 Oneill, J. G. and Wilkinson, J. F.: Oxidation of ammonia by methane-oxidizing
839 bacteria and effects of ammonia on methane oxidation, J. Gen. Microbiol., 100,
840 407-412, 1977.
841 Pester, M., Rattei, T., Flechl, S., Grongroft, A., Richter, A., Overmann, J., *et al* :
842 amoA-based consensus phylogeny of ammonia-oxidizing archaea and deep
843 sequencing of amoA genes from soils of four different geographic regions. Environ
844 Microbiol, 2012, 14, 525-539.
845 Pommerening-Roser, A., Koops, H.P.: Environmental pH as an important factor for
846 the distribution of urease positive ammonia-oxidizing bacteria, Microbiol. Res.,
847 160, 27-35, 2005.
848 Purkhold, U., Pommerening-Roser, A., Juretschko, S., Schmid, M.C., Koops, H.P.,
849 Wagner, M.: Phylogeny of all recognized species of ammonia oxidizers based on
850 comparative 16S rRNA and amoA sequence analysis: Implications for molecular
851 diversity surveys, Appl. Environ. Microbiol., 66, 5368-5382, 2000.
852 Purkhold, U., Wagner, M., Timmermann, G., Pommerening-Roser, A., Koops, H.P.:
853 16S rRNA and amoA-based phylogeny of 12 novel betaproteobacterial
854 ammonia-oxidizing isolates: extension of the dataset and proposal of a new lineage

within the nitrosomonads, Int. J. Syst. Evol. Micr., 53, 1485-1494, 2003.

Prosser, J. I. and Nicol, G. W.: Archaeal and bacterial ammonia-oxidisers in soil: the quest for niche specialisation and differentiation, Trends Microbiol., 20, 523-531, 2012.

Reim, A., Lueke, C., Krause, S., Pratscher, J., and Frenzel, P.: One millimetre makes the difference: high-resolution analysis of methane-oxidizing bacteria and their specific activity at the oxic-anoxic interface in a flooded paddy soil, ISME J., 6, 2128-2139, 2012.

Rinaldo, S., Brunori, M., and Cutruzzola, F.: Nitrite controls the release of nitric oxide in Pseudomonas aeruginosa cd(1) nitrite reductase, Biochem. Biophys. Res. Commun., 363, 662-666, 2007.

Rotthauwe, J. H., Witzel, K. P., and Liesack, W.: The ammonia monooxygenase structural gene amoA as a functional marker: Molecular fine-scale analysis of natural ammonia-oxidizing populations, Appl. Environ. Microbiol., 63, 4704-4712, 1997.

Sayavedra-Soto, L. A., Hommes, N. G., Alzerreca, J. J., Arp, D. J., Norton, J. M., and Klotz, M. G.: Transcription of the amoC, amoA and amoB genes in Nitrosomonas europaea and Nitrosospira sp, NpAV, FEMS Microbiol. Lett., 167, 81-88, 1998.

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., Lesniewski, R. A., Oakley, B. B., Parks, D. H., Robinson, C. J., Sahl, J. W., Stres, B., Thallinger, G. G., Van Horn, D. J., and Weber, C. F.: Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities, Appl. Environ. Microbiol., 75, 7537-7541, 2009.

Semrau, J. D., DiSpirito, A. A., and Yoon, S.: Methanotrophs and copper, FEMS Microbiol. Rev., 34, 496-531, 2010.

Stahl, D. A. and de la Torre, J. R.: Physiology and diversity of ammonia-oxidizing archaea, Annu. Rev. Microbiol., 66, 83-101, 2012.

Steenbergh, A. K., Meima, M. M., Kamst, M., and Bodelier, P. L. E.: Biphasic kinetics of a methanotrophic community is a combination of growth and increased activity per cell, FEMS Microbiol. Ecol., 71, 12-22, 2010.

Stein, L. Y., Roy, R., and Dunfield, P. F.: Aerobic methanotrophy and nitrification: processes and connections, in: Encyclopedia of life sciences (eLS.), John Wiley & Sons Ltd, Chichester, 1-11, doi: 10.1002/9780470015902.a0022213, 2012. 2012.

Stephen, J. R., McCaig, A. E., Smith, Z., Prosser, J. I., and Embley, T. M.: Molecular diversity of soil and marine 16S rRNA gene sequences related to beta-subgroup ammonia-oxidizing bacteria, Appl. Environ. Microbiol., 62, 4147-4154, 1996.

Venter, J. C., Remington, K., Heidelberg, J. F., Halpern, A. L., Rusch, D., Eisen, J. A., Wu, D., Paulsen, I., Nelson, K. E., and Nelson, W.: Environmental genome shotgun sequencing of the Sargasso Sea, Science, 304, 66-74, 2004.

Wang, Q., Garrity, G. M., Tiedje, J. M., and Cole, J. R.: Naive Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy, Appl.

Environ. Microbiol., 73, 5261-5267, 2007.

Xia, W., Zhang, C., Zeng, X., Feng, Y., Weng, J., Lin, X., Zhu, J., Xiong, Z., Xu, J., Cai, Z., and Jia, Z.: Autotrophic growth of nitrifying community in an agricultural soil, ISME J., 5, 1226-1236, 2011.

Yang, N., Lu, F., He, P., and Shao, L.: Response of methanotrophs and methane oxidation on ammonium application in landfill soils, Appl. Microbiol. Biotech., 92, 1073-1082, 2011.

Yang, S., Matsen, J. B., Konopka, M., Green-Saxena, A., Clubb, J., Sadilek, M., Orphan, V. J., Beck, D., and Kalyuzhanaya, M. G.: Global moelcuar analyses of methane metabolism in methanotrophic alphaprotebacterium, *Methylosinus Trichosporium* OB3b. Part II. Metabolomics and 13C-labelling study, Front. Microbiol., 4, 70, doi: 10.3389/fmicb.2013.00070, 2013.

Zhu, R. B., Liu, Y. S., Xu, H., Huang, T., Sun, J. J., Ma, E. D., and Sun, L. G.: Carbon dioxide and methane fluxes in the littoral zones of two lakes, east Antarctica, Atmos. Environ., 44, 304-311, 2010.

Table 1. Changes in pH, moisture content, NH_4^+-N and NO_3^--N content in soil microcosms over the course of 19 days of incubation

Treatments	pH[a]	Moisture (%)[b]	NH_4^+-N(μg/g *d.w.s*) [c]	NO_3^--N(μg/g *d.w.s*) [c]
Zero Time	7.39±0.04	19.4±0.42	0.51±0.10	11.1±0.31
Day-5-CH_4	7.53±0.01	26.1±0.16	0.47±0.33	0.90±0.35
Day-5-Urea	7.35±0.06	25.5±0.51	16.1±3.81	61.0±8.62
Day-5-CH_4+Urea	7.37±0.12	24.8±1.31	8.01±4.66	41.6±9.87
Day-19-CH_4	7.54±0.03	28.3±1.89	0.78±0.12	0.41±0.49
Day-19-Urea	7.27±0.30	30.5±1.85	44.8±6.69	137.6±28.3
Day-19-CH_4+Urea	6.85±0.09	28.6±2.03	3.66±1.56	59.9±6.01

[a]pH was determined using a ratio of H_2O to soil as 2.5 (v/w). The mean ± standard deviation of triplicate microcosms was given for each treatment.

[b]The mean ± standard deviation of triplicate microcosms was given for each treatment.

[c]The mean ± standard deviation of triplicate microcosms was given for each treatment, while for the CH_4+Urea treatment 6 replicates were used including both ^{12}C-control and ^{13}C-labeled treatments.

Figure 1. Interactions between microbial methane and ammonia oxidation in a paddy soil. The left panel shows urea effect on methane oxidation activity (a), methane-oxidizing bacteria (c) and methanol-oxidizing bacteria (e). The right panel refers to methane effect on ammonia oxidation activity (b), ammonia-oxidizing bacteria (d) and nitrite-oxidizing bacteria (f) in soil microcosms after incubation for 5 and 19 days. The amount of methane consumed was used to assess methane oxidation activity and soil nitrate production was used to evaluate ammonia oxidation activity. The total microbial communities were pyrosequenced using universal primers of the 16S rRNA gene. The relative frequency is expressed as the percentage of the targeted 16S rRNA genes to the total 16S rRNA reads for each soil sample. The error bars represent standard deviations of the triplicate microcosms, while for the 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.

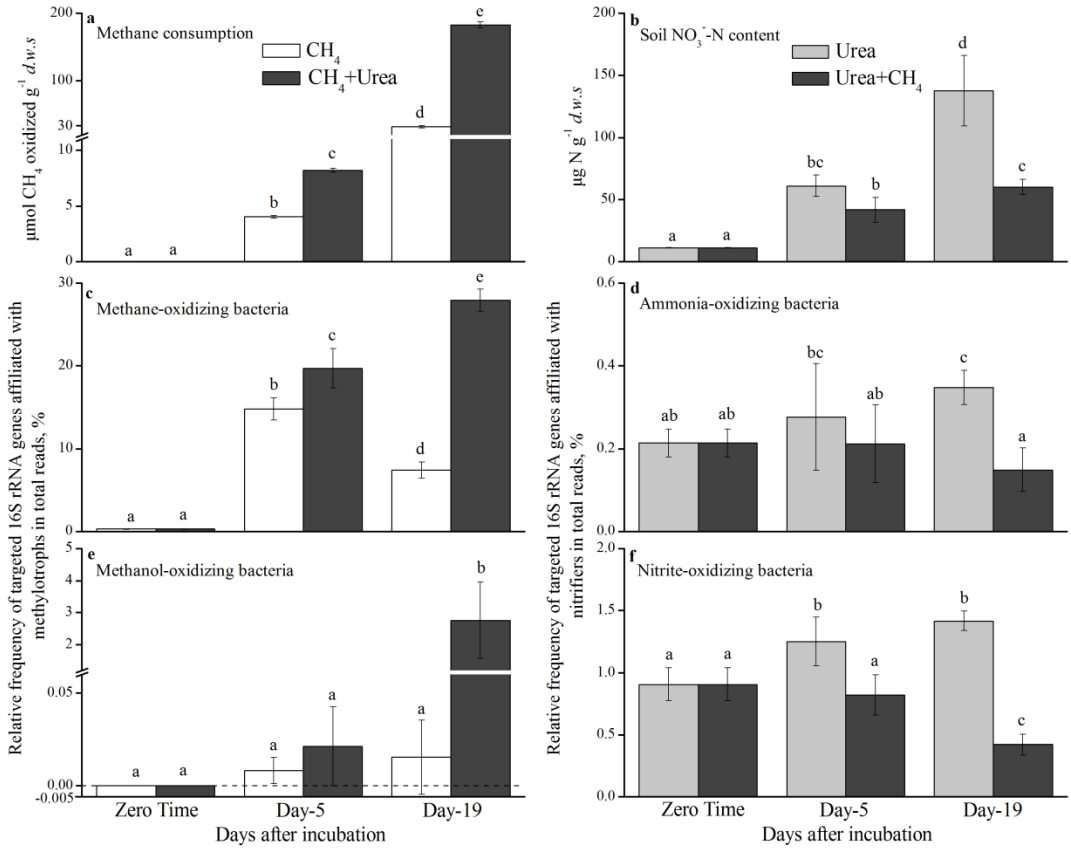


Figure 2. Change in relative abundance of methane-oxidizing bacteria (a) and ammonia-oxidizing bacteria (b) in soil microcosms incubated for 5 and 19 days. The relative abundance of type Ia, type II methanotrophs, *Nitrosospira* and *Nitrosomonas* are expressed as the targeted 16S rRNA gene to total 16S rRNA gene reads in soil microcosms incubated with CH_4, urea and CH_4+Urea. The error bars represent standard deviation of the triplicate microcosms, while for the 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.

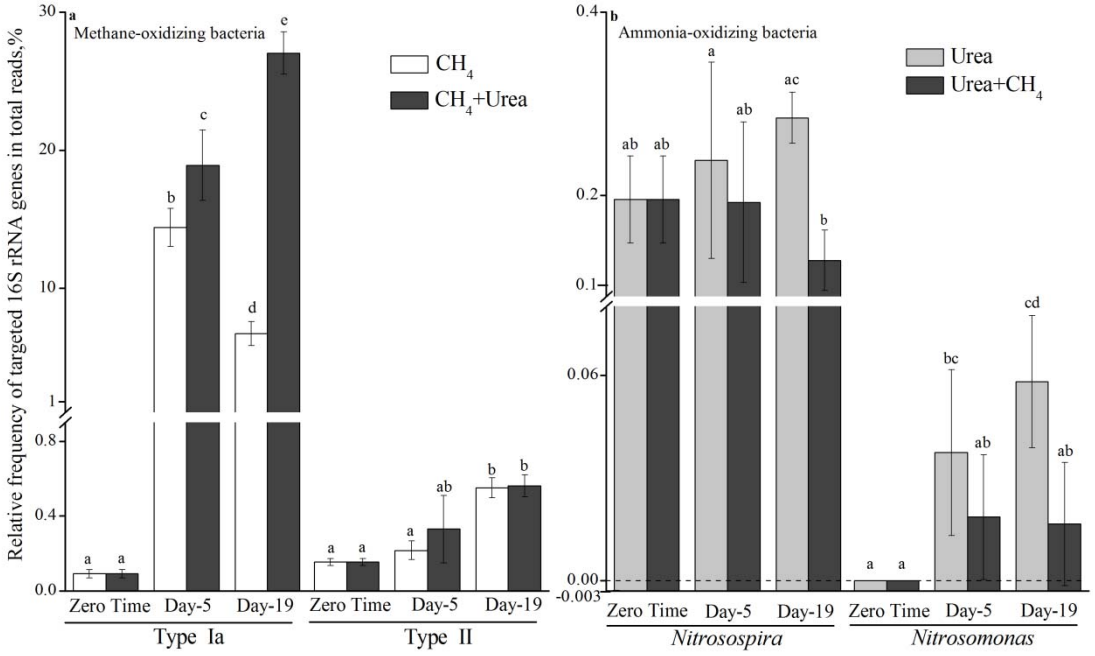


Figure 3. Relative frequency of the 16S rRNA gene sequences affiliated with methane-oxidizing bacteria (a, b), methanol-oxidizing bacteria (c, d), ammonia-oxidizing bacteria (e, f) and nitrite-oxidizing bacteria (g, h) across the buoyant density gradient of DNA fractions from the ^{13}C-labeled and ^{12}C-control microcosms after incubation for 5 and 19 days. ^{13}C-CH_4 refers to microcosm incubation with $^{13}CH_4$ for labeling of methane-metabolizing communities, and ^{13}C-Urea represents incubation with ^{13}C-Urea plus $^{13}CO_2$ for labeling of nitrifying communities. The relative frequency is expressed as the percentage of the targeted 16S rRNA genes to total 16S rRNA reads in each DNA gradient fraction.

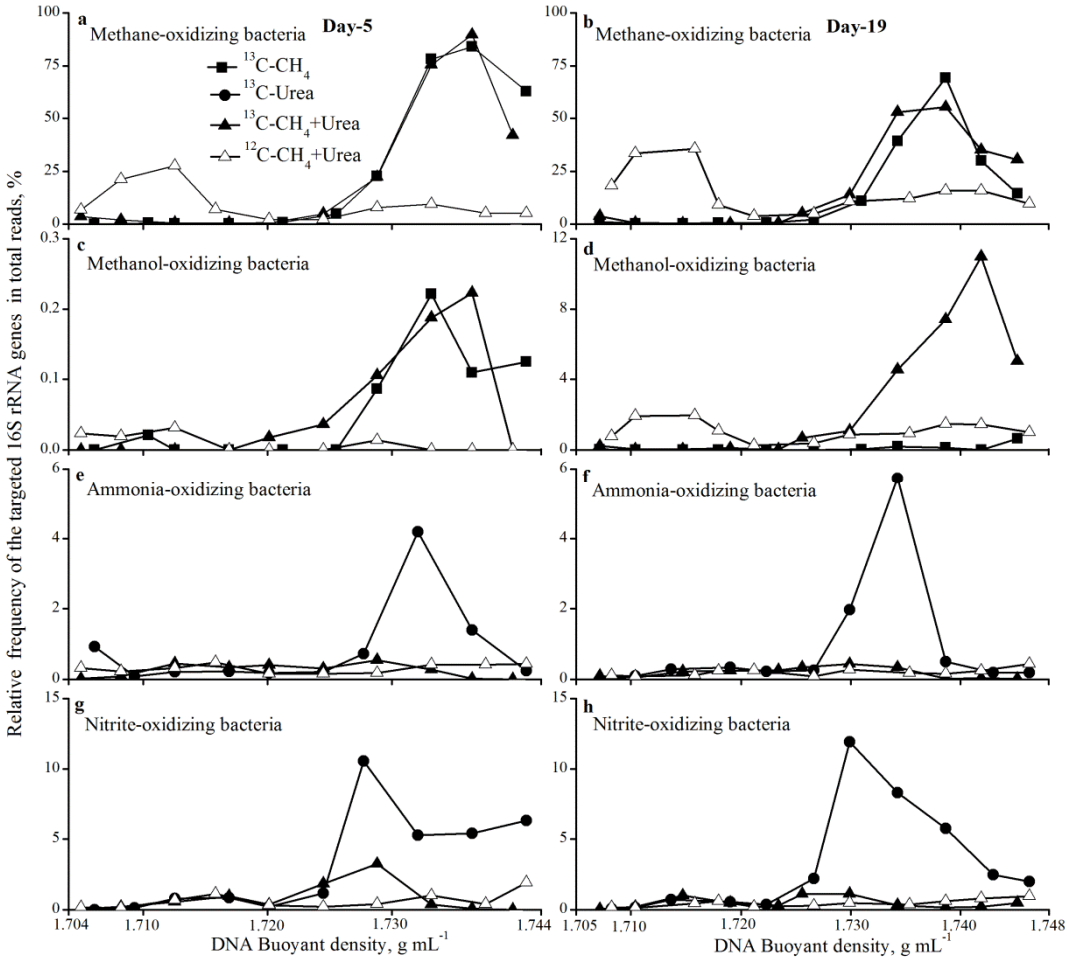


Figure 4. Phylogenetic tree of the ^{13}C-labeled 16S rRNA genes affiliated with methane-oxidizing bacteria (a) and *pmoA* genes (b) from the labeled microcosm after incubation for 19 days. The designations of CH_4 represent soil microcosms incubated with ^{13}C-CH_4, and the designation of CH_4+Urea denotes incubation with ^{13}C-CH_4 plus ^{13}C-Urea. CH_4-HF-OTU-1-(1068)-72.3%indicates that OTU-1 contained 1068 reads with sequence identity of >97%, accounting for 72.3% of the total methanotroph-like 16S rRNA genes in the 'heavy DNA fraction' from the labeled microcosms. One representative sequence was extracted using mothur software package for tree construction. The scale bar represents nucleotide acid substitution percentage.

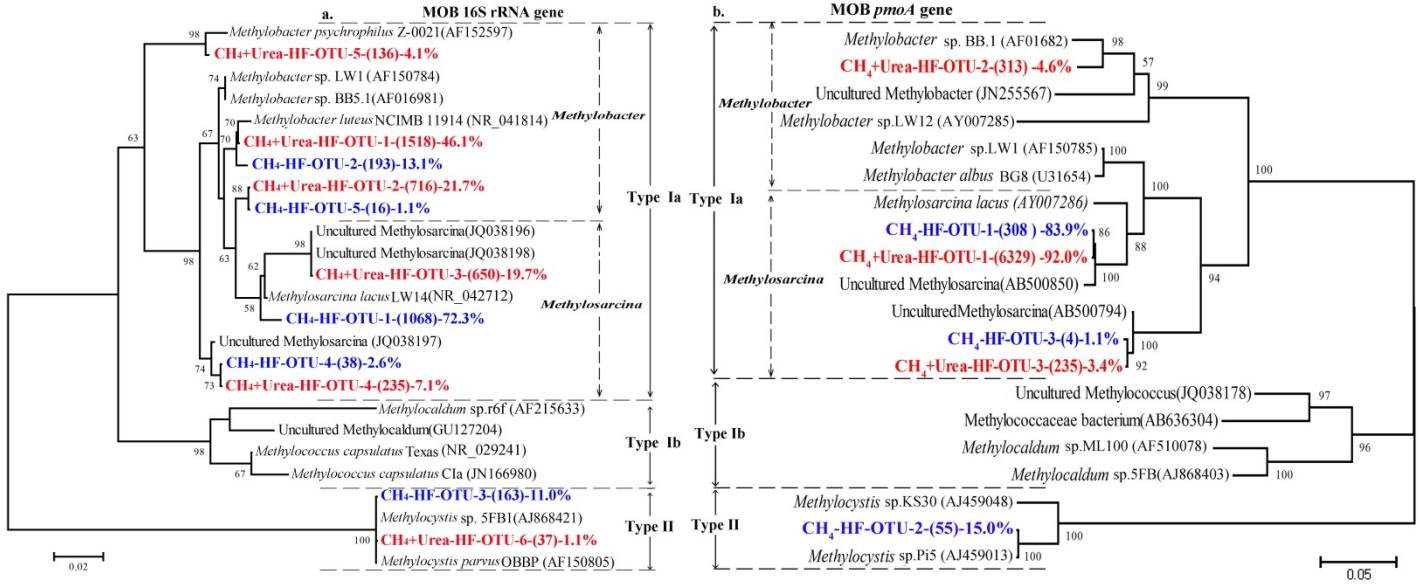


Figure 5. Phylogenetic tree of the ^{13}C-labeled 16S rRNA genes affiliated with ammonia-oxidizing bacteria (a) and bacteria *amoA* genes (b) from the labeled microcosm after incubation for 19 days. The designations of Urea represent soil microcosms incubated with ^{13}C-Urea plus ^{13}C-CO_2, and the designation of Urea+CH_4 denotes incubation with ^{13}C-CH_4 and ^{13}C-Urea plus ^{13}C-CO_2. Urea-HF-OTU-1-(134)-81.7%indicates that OTU-1 contained 134 reads with sequence identity of >97%, accounting for 81.7% of the total AOB-like 16S rRNA genes in the 'heavy DNA fraction' from the labeled microcosms. One representative sequence was extracted using mothur software package for tree construction. The scale bar represents nucleotide acid substitution percentage.

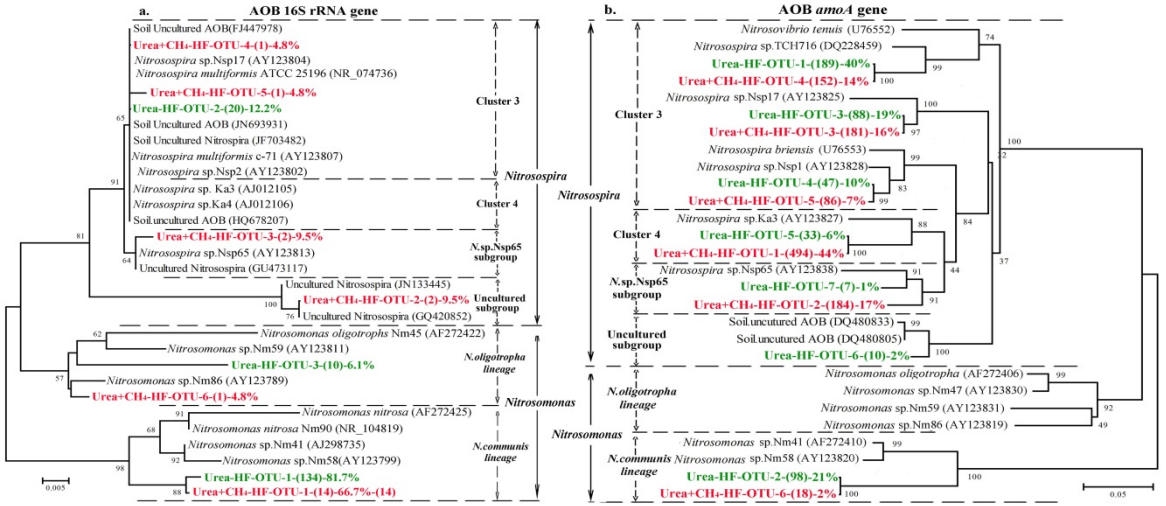
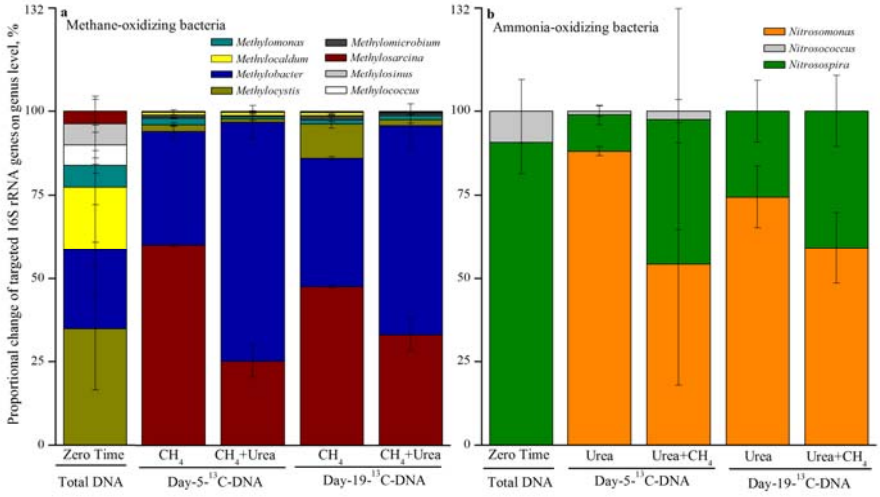


Figure 6. Percent changes of bacterial phylotypes affiliated with methane-oxidizing bacteria (a) and ammonia-oxidizing bacteria (b) in the ^{13}C-DNA fractions from the labeled microcosm after incubation for 5 and 19 days. The designation of CH_4+Urea represents soil microcosms incubated with ^{13}C-CH_4 and ^{13}C-Urea plus ^{13}C-CO_2, and the designation Day-5-^{13}C-DNA denotes the ^{13}C-labeled methanotrophic communities in the 'heavy' DNA fractions after isopycnic centrifugation of the total DNA extracted from microcosms after incubation with the labeled substrates for 5 days. The percentage of different phylotypes is expressed as the targeted 16S rRNA gene reads to the total 16S rRNA gene reads affiliated with methane-oxidizing bacteria and ammonia-oxidizing bacteria in duplicate.



Supplemental Material for

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

Yan Zheng[1,2], Rong Huang[1], Baozhan Wang[1], Paul L.E. Bodelier[3], Zhongjun Jia[1*]

Author Affiliation

[1] State Key Laboratory of Soil and Sustainable Agriculture
Institute of Soil Science, Chinese Academy of Sciences
Nanjing, 210008, Jiangsu Province, China

[2] University of the Chinese Academy of Sciences,
Beijing, 100049, China

[3] Netherlands Institute of Ecology,
Department of Microbial Ecology,
Droevendaalsesteeg 10,
6708 PB, Wageningen, the Netherlands

Corresponding author*

Zhongjun Jia
E-mail: jia@issas.ac.cn
Institute of Soil Science, Chinese Academy of Sciences, China

This file includes:

Supplemental Table S1 to S6

Supplemental Figure S1 to S8

Supplemental Reference

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 ^{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	GGN GAC TGG GAC TTC TGG	*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	CCG GMG CAA CGT CYT TAC C		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	3616	5297	384	---	---	5074	7159
	R3	---	5878		---		6303	
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/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±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 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, 2004Bodelier 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 microcosns 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.

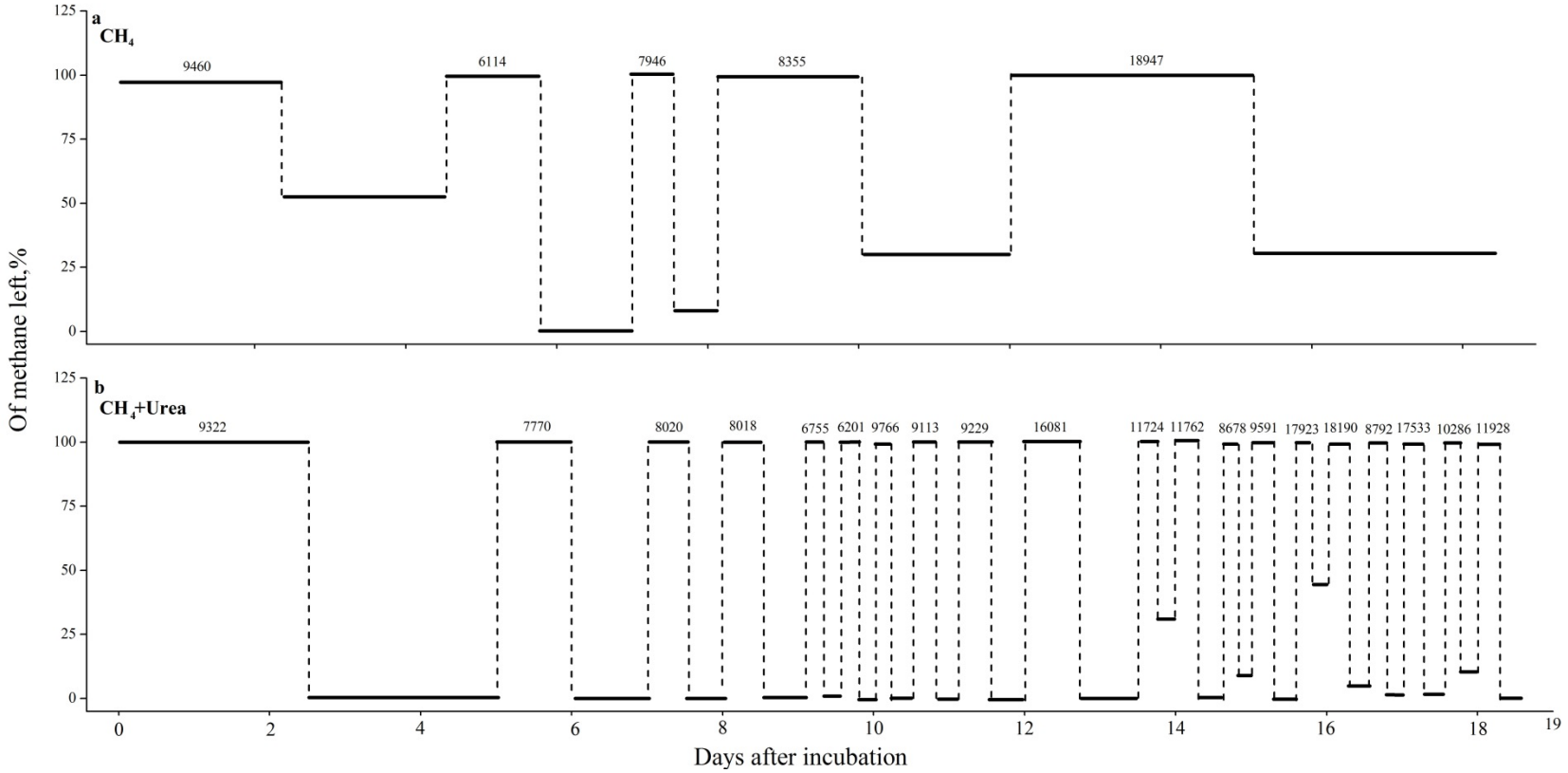


Figure S2. Changes in NH_4^+-N and NO_3^--N content in soil microcosms incubated with urea with or without CH_4 over the course of 19 days of incubation

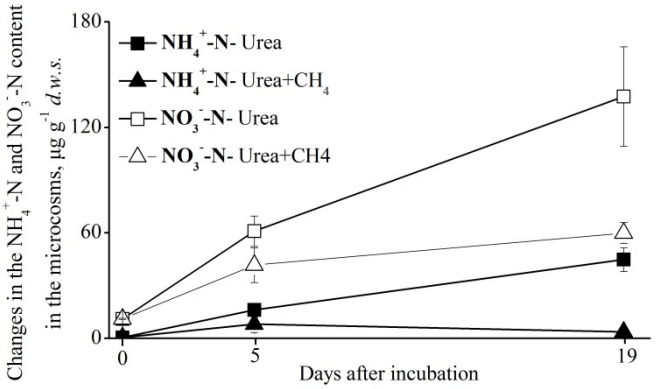


Figure S3. Quantitative distribution of pmoA gene copy numbers (a), amoA gene copy numbers of Bacteria (b) and Archaea (c) in total DNA from microcosms after incubation for 5 and 19 days. The error bars represent standard deviations of the triplicate microcosms. The different letters above the columns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

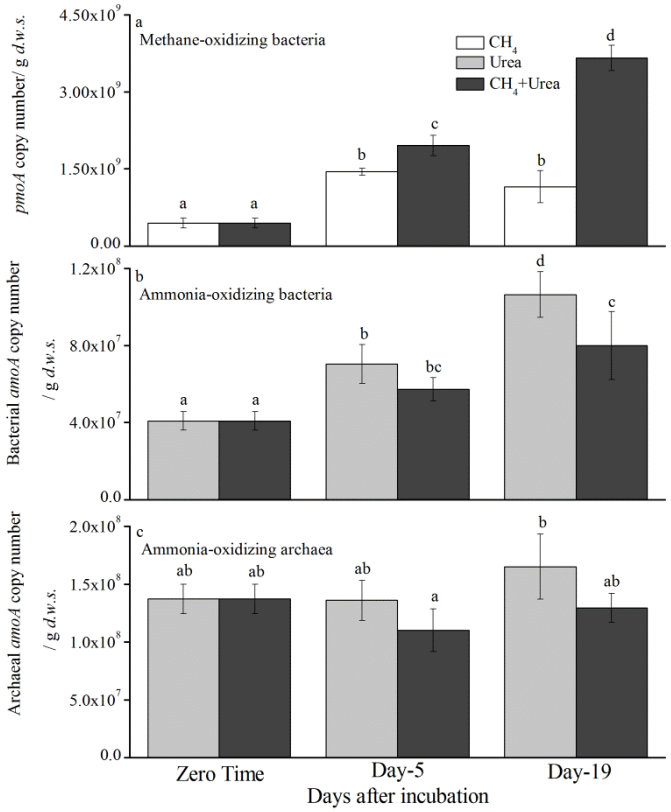


Figure S4. The effect of methane on ammonia-oxidizing archaea (AOA) in soil microcosms incubated for 19 days. The relative frequency is expressed as the percentage of the targeted reads to the total 16S rRNA gene sequences reads in soil sample.The error bars represent standard deviation of the triplicate 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.

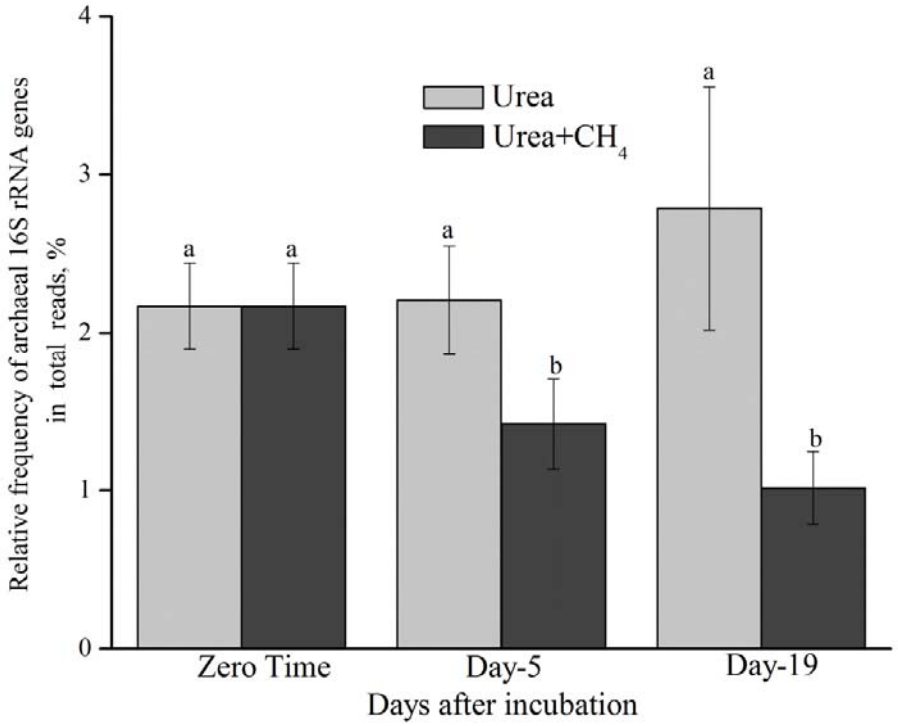


Figure S5. Phylogenetic tree showing the relationship of methane-oxidizing bacterial 16S rRNA gene (a) and *pmoA* gene (b) sequences in soil microcosms to those deposited in the GenBank. Pyrosequencing reads of methanotrophic 16S rRNA genes and *pmoA* genes were used from triplicate microcosms at day 0 and day 19, and representative sequences were chosen for analysis. The designation of CH_4+Urea-OTU-1-38%-(616) indicates that OTU-1 containing 616 sequences with identity of >97% comprised 38% 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 616 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.

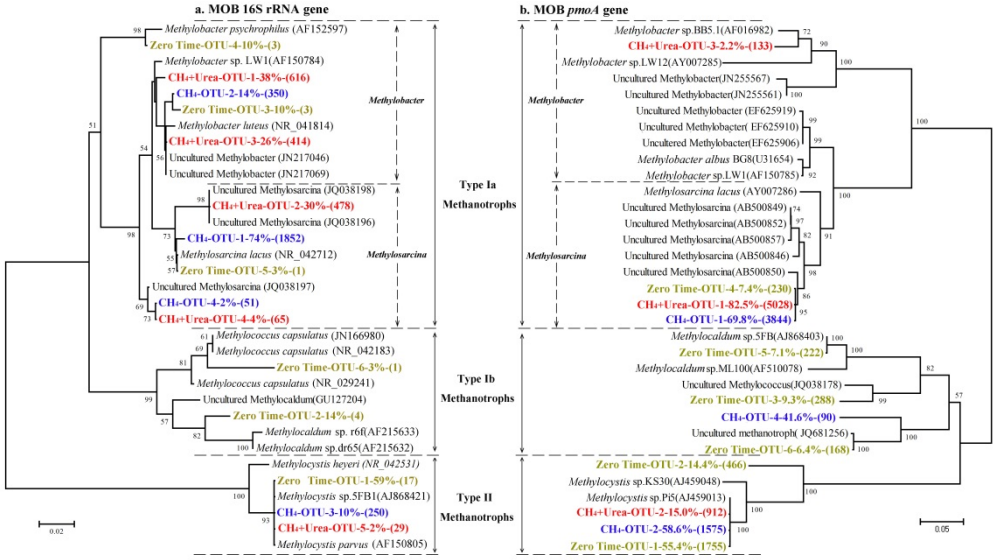


Figure S6. Phylogenetic tree showing the relationship of ammonia-oxidizing bacterial 16S rRNA gene (a) and *amoA* gene (b) sequences in soil microcosms to those deposited in the GenBank. Pyrosequencing reads of AOB 16S rRNA genes and *amoA* genes were used from triplicate microcosms at day 0 and day 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%-(2326) indicates that OTU-1 containing 2326 sequences with identity of >97% comprised 52% 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 constrution. The scale bar represents nucleotide acid substitution percentage.

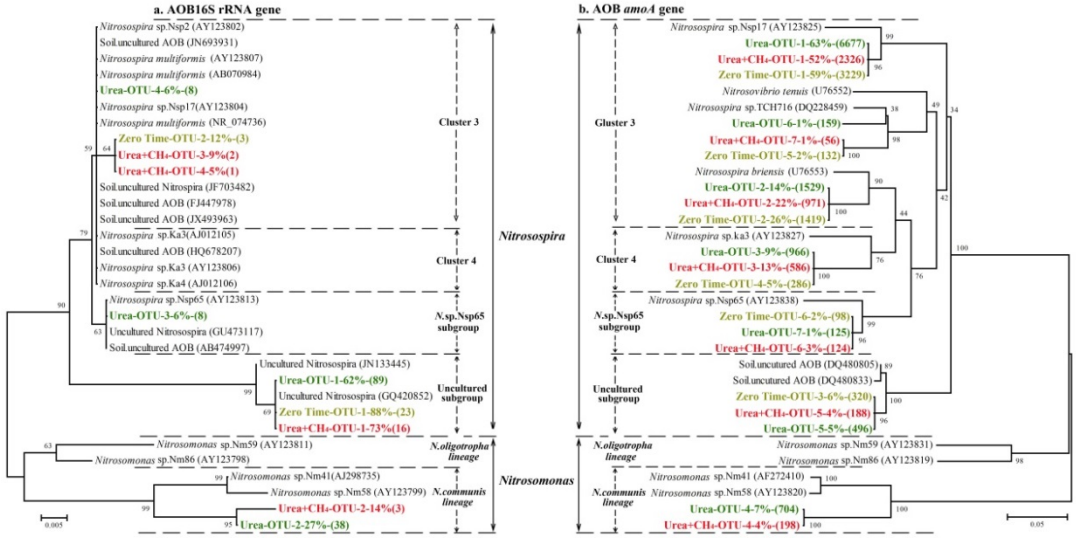


Figure S7. Quantitative distribution of *pmoA* gene copy numbers (a,b), *amoA* gene copy numbers of *Bacteria* (c,d) and *Archaea* (e,f) across the entire buoyant density gradient of the fractionated DNA from SIP microcosms after incubation for 5 and 19 days. The normalized data are the ratio of gene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

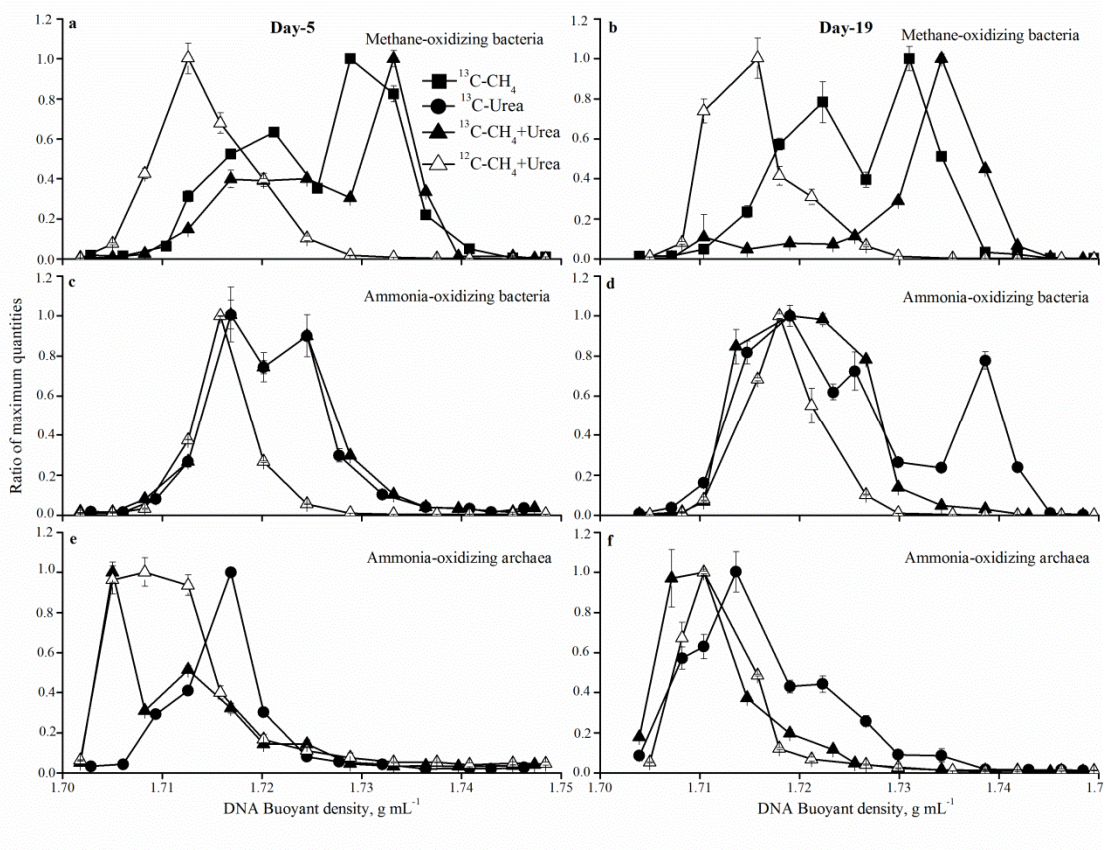
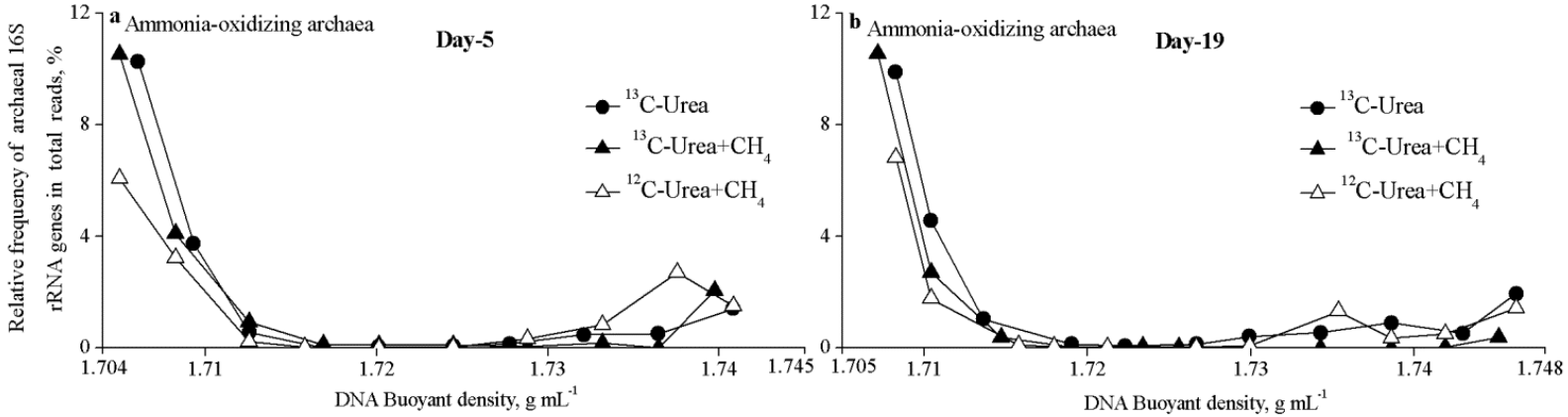


Figure S8. Relative frequency of the archaeal 16S rRNA gene sequences reads in DNA gradient fractions with a buoyant density gradient isolated from SIP microcosms after incubation for 5 and 19 days. The frequency is expressed as the percentage of the targeted archaeal reads to the total 16S rRNA gene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



Reference

Bodelier P.L. and Laanbroek, H.J: Nitrogen as a regulatory factor of methane oxidation in soils and sediments, FEMS Microbiol. Ecol., 47, 265-277, 2004.

Costello, A.M. and Lidstrom, M.E.,: Molecular characterization of functional and phylogenetic genes from natural populations of methanotrophs in lake sediments, Appl. Environ. Microbiol., 65, 5066-5074, 1999.

Francis, C. A., Roberts, K. J., Beman, J. M., Santoro, A. E., and Oakley, B. B.: Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean, Proceedings of the National Academy of Sciences of the United States of America, 102, 14683-14688, 2005.

Holmes, A. J., Costello, A., Lidstrom, M. E., and Murrell, J. C.: Evidence that particulate methane monoxygenase and ammonia monooxygenase may be evolutionarily related, FEMS Microbiol. Lett., 132, 203-208, 1995.

Rotthauwe, J. H., Witzel, K. P., and Liesack, W.: The ammonia monooxygenase structural gene amoA as a functional marker: Molecular fine-scale analysis of natural ammonia-oxidizing populations, Appl. Environ. Microbiol., 63, 4704-4712, 1997.

Whalen, S. C., Reeburgh, W. S., and Sandbeck, K. A.: Rapid Methane Oxidation in a Landfill Cover Soil, Appl. Environ. Microbiol., 56, 3405-3411, 1990.

Xia, W., Zhang, C., Zeng, X., Feng, Y., Weng, J., Lin, X., Zhu, J., Xiong, Z., Xu, J., Cai, Z., and Jia, Z.: Autotrophic growth of nitrifying community in an agricultural soil, ISME J., 5, 1226-1236, 2011.