Electronic Supplementary Material (ESI) for Environmental Science: Water Research & Technology.

Supporting Information

Archaeal Ammonium Oxidation Coupled with Bacterial Nitrite Oxidation in a Simulated Drinking Water Premise Plumbing System

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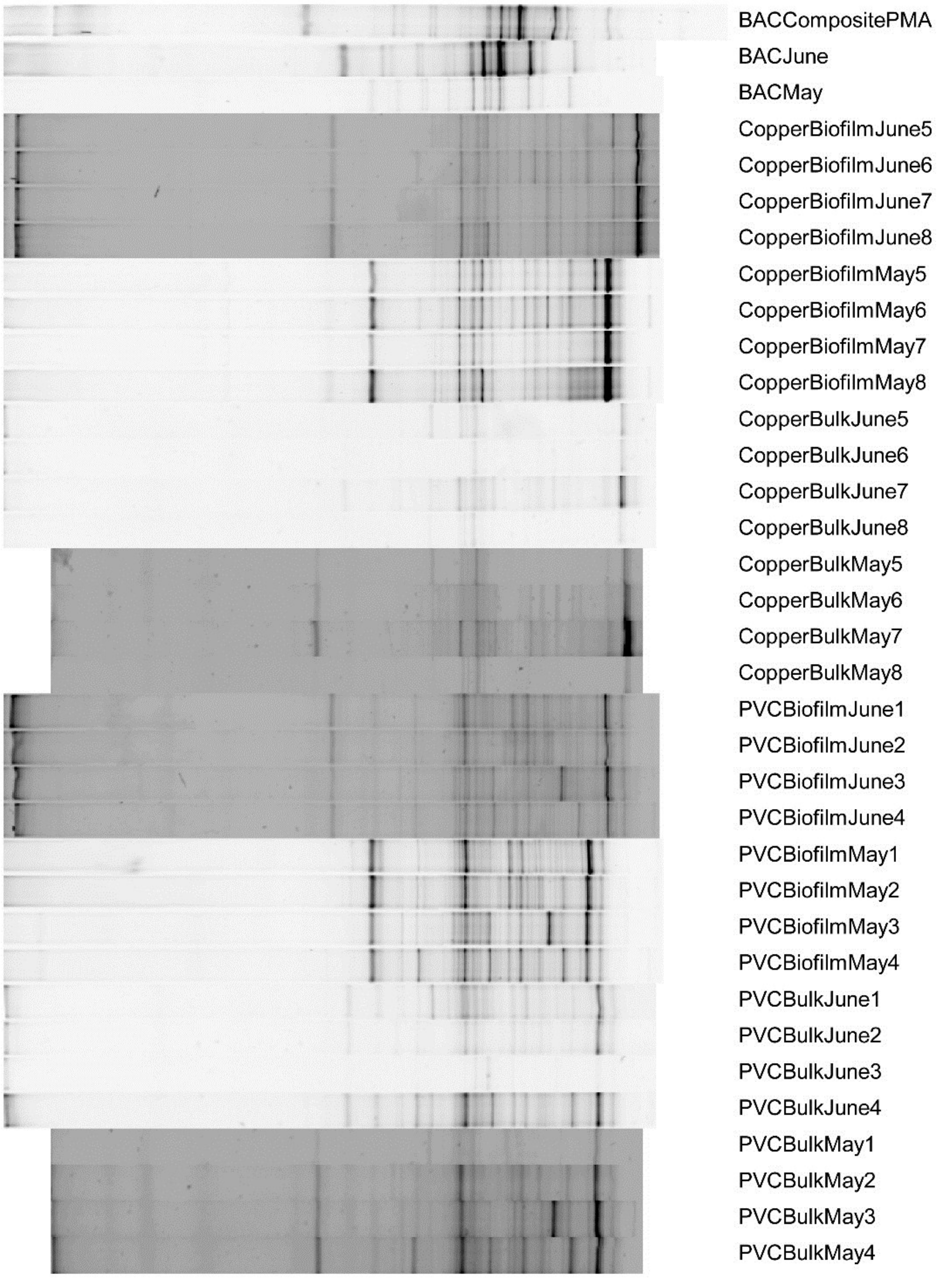
Eight pages, one figure, four tables.



Supporting Information Figure 1. DGGE from repeated samplings of the copper and PVC reactors. “Bulk” samples refer to reactor effluent, which was not further analyzed.

Table S1. Specific information and references for PCR, qPCR and fluorescence in situ hybridization methods.

Endpoint PCR

Bacterial *amoA*

PCR:

amoA1-F	5’ GGGGTTTCTACTGGTGGT 3’	Rotthauwe et al., 1997
amoA2-R	5’ CCCCTCKGSAAAGCCTTCTTC 3’	

Thermal cycling conditions:
5 min at 94°C; pause at 80°C to add polymerase; 42 cycles consisting of 60 s at 94°C, 90 s at 60°C, 90 s at 72°C; a final cycle of 10 min at 72°C.

Bacterial *nxr*A

PCR:

nxrAF	5’ CAGACCGACGTGTG CGAAAG 3’	Wertz et al., 2008
nxrAR	5’ TCCACAAGGAACGG AAGGTC 3’	

Thermal cycling conditions:
3 min at 94ºC; 35 cycles consisting of 30 s at 94ºC, 45 s at 55ºC, 45 s at 72ºC; a final cycle of 5 min at 72ºC.

Bacterial *nxr*B

PCR:

nxrBF1	5’ TACATGTGGTGGAACA 3’	Maixner, 2009
nxrBR6	5’ CGGTTCTGGTCRATCA 3’	

Thermal cycling conditions:
4 min at 95ºC; 35 cycles consisting of 40 s at 95ºC, 30 s at 56.2ºC, 60 s at 72ºC; a final cycle of 10 min at 72 ºC.

Bacterial 16S rRNA gene

PCR:

1055F	5’ATGGCTGTCGTCAGCT 3’	Ferris et al., 1996
1392R	5’ ACGGGCGGTGTGTAC 3’	

Thermal cycling conditions:
2 min at 94°C; 30 cycles of 45 s at 94°C, 45 s at 55°C, 45 s at 72°C; and a final cycle of 7 min at 72°C.

PCR-DGGE:

1055F	5’ATGGCTGTCGTCAGCT 3’	Ferris et al., 1996
1392R-GC	5’ CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCC-ACGGGCGGTGTGTAC 3’ (GC clamp underlined, Ferris et al., 1996)	

Thermal cycling conditions:
2 min at 94°C; 30 cycles of 45 s at 94°C, 45 s at 55°C, 45 s at 72°C; and a final cycle of 7 min at 72°C.

Archaeal *amoA*

PCR:

arch-amoAF	5’ STAATGGTCTGGCTTAGACG 3’	Francis et al., 2005

arch-amoAR	5’ GCGGCCATCCATCTGTATGT 3’	

Thermal cycling conditions:
95°C for 5 min; 30 cycles consisting of 94°C for 45 s, 53°C for 60 s, and 72°C for 60 s; and 72°C for 15 min.

PCR-DGGE:

Arch-amoA-for	5’ CTGAYTGGGCYTGGACATC 3’	Wuchter et al., 2006
Arch-amoA-rev-GC	5’CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCC-TTCTTCTTTGTTGCCCAGTA 3’ (GC clamp underlined, Ferris et al., 1996)	

Thermal cycling conditions:
95°C for 10 min; 50 cycles consisting of 95°C for 20 s, 53°C for 45 s, and 72°C for 60 s.

Archaeal 16S rRNA gene

PCR:

21F	5’TTCCGGTTGATCCYGCCGGA3’	DeLong, 1992
1492R	5’GGTTACCTTGTTACG ACTT3’	

Thermal cycling conditions:
30 cycles consisting of 95° C for 1.5 min, 55°C for 1.5 min, and 72° C for 1.5 min .

PCR-DGGE (screening clones):

ARC-915R	5’GTGCTCCCCCGCCAATTCCT 3’	Casamayor et al., 2000
ARC-344F-GC	5’ CGC CCG CCG CGCCCC GCG CCC GTCCCG CCG CCC CCGCCC G-ACGGGGYGCAGCAGGCGCGA 3’ (GC clamp underlined)	

Thermal cycling conditions:
94°C for 5 min; 30 cycles consisting of 94°C for 60 s, 55°C for 60 s, 72°C for 60 s; and 72°C for 9 min.

Sequencing of Amplicons

M13F	5’-TGTAAAACGACGGCCAGT-3’

Quantitative PCR (qPCR)

Bacterial 16S rRNA gene

qPCR:

338F	5’ ACTCCTACGGGAGGCAGCAG 3’	Einen et al.,2008
518R	5’ ATTACCGCGGCTGCTGG3’	

Thermal cycling conditions:
95°C for 15 min; 45 cycles of 94°C for 15 s, 61°C for 30 s, 72°C for 30 s.

Archaeal 16S rRNA gene

qPCR:

931F	5’ AGGAATTGGCGGGGGAGC A3’	Einen et al., 2008
m1100R	5’ BGGGTCTCGCTC 3’	

Thermal cycling conditions:
95°C for 15 min; 45 cycles of 94°C for 15 s, 64°C for 30 s, 72°C for 30 s.

Bacterial *amoA* gene qPCR

amoA1-F	5’ GGGGTTTCTACTGGTGGT 3’	Rotthauwe et al., 1997
amoA2-R	5’ CCCCTCKGSAAAGCCTTCTTC 3’	

Thermal cycling conditions:
95°C for 15 min; 45 cycles of 94°C for 15 s, 61°C for 30 s, 72°C for 30 s.

Archaeal *amoA* gene

qPCR:

Arch-AmoA-for	5’ CTGAYTGGGCYTGGACATC 3’	Wuchter et al., 2006
Arch-AmoA-rev	5’ TTCTTCTTTGTTGCCCAGTA 3’	

Thermal cycling conditions:
95°C for 15 min; 45 cycles of 94°C for 15 s, 64°C for 30 s, 72°C for 30 s.

Fluorescent *in-situ* Hybridization (FISH)

DOPE- EUB338	5’GCTGCCTCCCGTAGGAGT 3’	Stoecker et al., 2010 Amann et al., 1990

Targets bacteria, one Cy3 label on each end

ARCH 915	5’ GTGCTCCCCCGCCAATTCCT 3’	Stahl and Amann, 1991

Targets archaea, 5’ Cy5 label

DOPE-NONEUB	5’ACTCCTACGGGAGGCAGC 3’	Wallner et al., 1993

Negative control, non EUB, one Cy3 label on each end

NONSENSE	5’ AGAGAGAGAGAGAGAGAG 3’	Hatzenpichler et al., 2008

Negative control, nonsense, 5’ Cy5 label

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Wallner, G., Amann ,R.; Beisker, W. Optimizing fluorescent in situ hybridization with rRNA-targeted oligonucleotide probes for flow cytometric identification of microorganisms. *Cytometry*. **1993,** *14*: 136-143.

Hatzenpichler, R. E.; Lebedeva, V.; Spieck, E.; Stoecker, K.; Richter, A.; Daims, H.; Wager, M. A moderately thermophilic ammonia-oxidizing crenarchaeote from a hot springs. *Proc. National Academy of Sciences, USA* **2008**, *105* (5), 2134-2139.

Table S2. Accession numbers deposited in the NCBI database. Table and Band refer to DGGE bands sequenced and reported in Tables 1 and 2. Three bands (NA) were sequenced but not reported in the tables.

Accession	Table	Band	Description	Date Submitted
JQ406488	2	9	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406489	2	1	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406490	1	1	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406491	1	9	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406492	2	2	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406493	1	10	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406494	2	3	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406495	2	11	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406496	1	3	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406497	1	11	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406498	2	4	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406499	2	12	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406500	1	12	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406501	2	5	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406502	2	13	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406503	NA	13	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406504	2	6	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406505	2	14	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406506	1	6	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406507	1	14	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406508	2	7	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406509	2	15	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406510	1	7	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406511	NA	15	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406512	2	16	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406513	1	8	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406514		10	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406515	NA	2	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406516	1	5	bacterial DGGE band, 16S rDNA	11-Jan-12
JQ406517			archaeal 16S rDNA, 219 bp, qPCR std.	11-Jan-12
JQ406518			bacterial 16S rDNA, 190 bp, qPCR std.	11-Jan-12
JQ406519			archaeal *amoA*, 256 bp, qPCR std.	11-Jan-12
JQ406520			archaeal *amoA,* 617 bp	11-Jan-12
JQ717297			lower DGGE band, archaeal *amoA*, 254 bp	16-Jul-12
JQ717298			upper DGGE band, archaeal *amoA*, 253 bp	16-Jul-12
JQ717299			archaeal 16S rDNA clone, 710 bp	16-Jul-12

Table S3. Taxonomic identity of the bands excised from the 16S rDNA PCR-DGGE profile of the biofilm community obtained from the copper coupon. Bands 4, 13, and 15 could not be reamplified to obtain DNA sequence information.

Band number	**GenBank accession (this study)**	**Nearest taxonomically identified relatives and GenBank accession numbers**	**Identity comparisons, % similarity**
1	JQ406490	Uncultured *Acidobacterium* sp. clone EU403975	318/319 (99%)
2	JQ406515	*Sterolibacterium* strain TKU1 AM990454	318/320 (99%)
3	JQ406496	Uncultured *Gemmata sp.* clone JX114456	303/319 (95%)
5	JQ406516	Uncultured *Gemmata sp.* clone JX114456	316/319 (99%)
6	JQ406506	Uncultured *Acidobacterium* sp. clone EU403975	221/235 (94%)
7	JQ406510	*Hylemonella gracilis* strain AB539995	308/315 (98%)
8	JQ406513	Uncultured Nitrospirae clones AB252937, AB252938 Uncultured *Nitrospira* sp. clone JQ346777	320/320 (100%) 318/318 (100%)
9	JQ406491	Uncultured Bacteroidetes clone EU633829 Uncultured Chloroflexi clone EU633735	223/225 (99%) 223/225 (99%)
10	JQ406493	Uncultured Rhodospiralles clones KJ600189, KJ600316, KJ600590	315/321 (98%)
11	JQ406497	Uncultured Gemmatimonadaceae clone HQ044269, HQ042984	308/323 (95%)
12	JQ406500	Uncultured Rhodospillales clones KJ600189, KJ600316, KJ600590	293/322 (91%)
14	JQ406507	Uncultured planctomycete clones JN656770 JN656908	271/325 (83%)

Table S4. Taxonomic identity of the bands excised from the 16S rDNA PCR-DGGE profile of the biofilm community obtained from the PVC coupon. Bands 8 and 10 could not be reamplified to obtain DNA sequence information.

Band number	**GenBank accession (this study)**	**Nearest taxonomically identified relatives and GenBank accession numbers**	**Identity comparisons, % similarity**
1	JQ406489	Uncultured planctomycete clone FM945332	314/321 (98%)
2	JQ406492	Uncultured *Flavobacterium* sp. clone GU230410	313/323 (97%)
3	JQ406494	Uncultured *Nitrospira* sp. clones GQ176326, GQ421145, JQ278935, JQ278947	315/317 (99%)
4	JQ406498	Uncultured Chloroflexi clone FM 253630	287/307 (93%)
5	JQ406501	Uncultured *Nitrospira* sp. clones GQ176345, GQ452974, JQ278818, JQ278967	307/325 (94%)
6	JQ406504	Uncultured Comamonadaceae clones JN125747, JN125748, JN125752	312/319 (98%)
7	JQ406508	Uncultured Comamonadaceae clones JN125747, JN125748, JN125752	306/319 (96%)
9	JQ406488	Uncultured Desulfuromonadales clone AM935314	314/323 (97%)
11	JQ406495	Uncultured *Nitrospira* sp. clones AF351228, AJ582041, HE974818	319/322 (97%)
12	JQ406499	Uncultured *Nitrospira* sp. clones GQ176345, JQ278818, JQ278967	322/322 (100%)
13	JQ406502	Uncultured Chloroflexi clone JF707623	259/324 (80%)
14	JQ406505	Uncultured planctomycete clone EU373997	304/322 (94%)
15	JQ406509	Uncultured Verrucomicrobiales clone FJ542937	204/234 (87%)
16	JQ406512	Uncultured Chloroflexi clone EU403822	245/262 (94%)