Supplementary Information.

METHODS.

PCR. For each set of primers, ~ 1 ng of cDNA was subjected to PCR in triplicate using the following cycling conditions: initial denaturation at 94°C (4 min), followed by 30 cycles of denaturation at 94°C (1 min), annealing at the optimal temperature for each primer pair (1 min) (SOM Table 1), primer extension at 72°C (1.5 min), followed by a final extension step at 72°C for 20 min. PCRs contained 2 mM $MgCl_2$ (Invitrogen), 200 μM each deoxynucleotide triphosphate (Eppendorf, Hamburg, Germany), 0.5 μM forward and reverse primer (Integrated DNA Technologies, Coralville, IA), 0.4 mg ml^{-1} molecular-grade bovine serum albumin (Roche, Indianapolis, IN) and 0.25 units Taq DNA polymerase (Invitrogen) in a final reaction volume of 50 μL. An equal volume of each replicate reaction was pooled and purified using a QIAquick PCR Purification Kit (Qiagen, Valencia, CA).

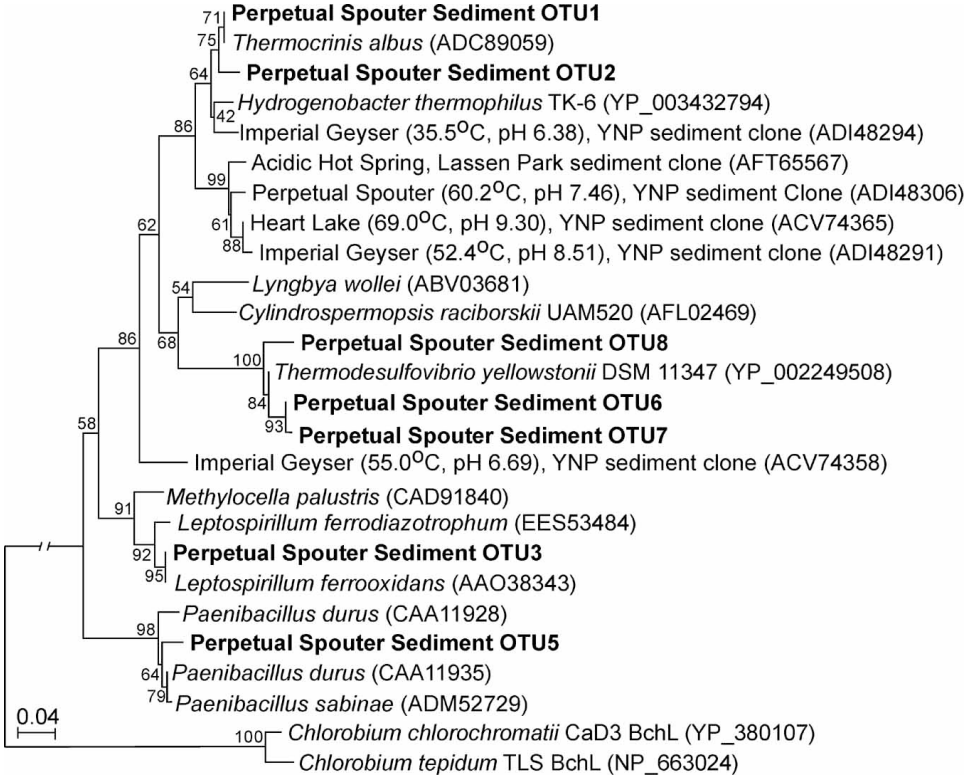
Quantitative PCR (qPCR) of 16S rRNA Genes. Reactions were performed in triplicate in optically pure 0.5 mL PCR tubes (Qiagen, Valencia, CA), with 1 ng of total DNA quantified using a Qubit DNA Assay kit (Molecular Probes) with 500 nM forward and reverse primer (SOM Table 1). Assays were amended with molecular-grade bovine serum albumin to a final concentration of 0.4 mg ml^{-1} (Roche, Indianapolis, IN) in a final reaction volume of 20 μL using the following cycling conditions: initial denaturation (95°C for 10 min) followed by 40 cycles of denaturation (95°C for 10s), annealing (55°C for 10 s), and extension (72°C for 20 s). Reactions and specificity was verified by melt curve analysis. Standard curves relating template copy number to threshold qPCR amplification signal (SOM Table 2) were generated using plasmid DNA generated from the cloning of bacterial and archaeal 16S rRNA genes previously generated

from PS as previously described (Boyd et al, 2011). Template abundances reported reflect the average and standard deviation of three replicate q-PCR assays for each gene.

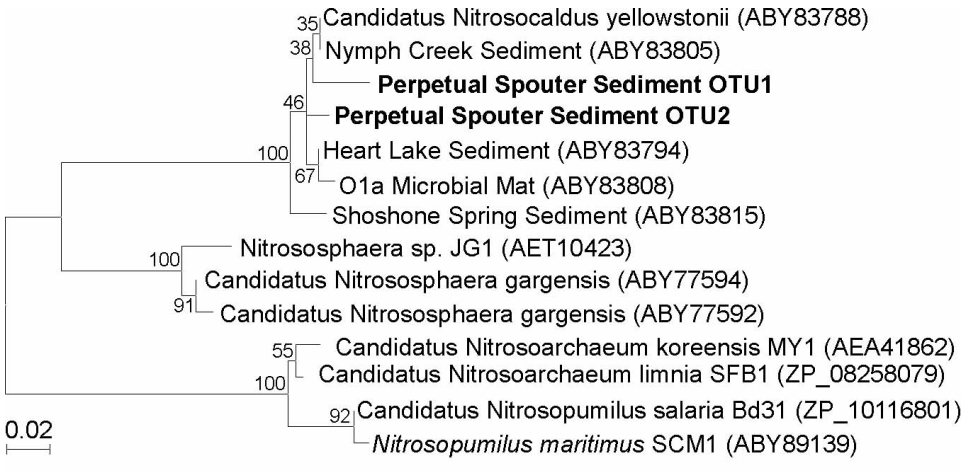
Quantitative reverse transcription-PCR (qRT-PCR). Reactions were performed in triplicate in optically pure 0.5 mL PCR tubes (Qiagen, Valencia, CA), with 10 ng of total RNA using the Qubit RNA Assay kit (Molecular Probes) with 500 nM forward and reverse primer (SOM Table 1), in a final reaction volume of 20 μL using the following cycling conditions: reverse transcription at 48°C (30 min) followed by initial activation of the DNA polymerase at 95°C (10 min) followed by 40 cycles of denaturation at 95°C (15 s), annealing and extension (1 min) (see SOM Table 1 for primers and optimal annealing temperature). Specificity of the assays was verified by melt curve analysis. Template abundances reported reflect the average and standard deviation of three replicate qRT-PCR assays for each gene. Fold change, used to compared two values of transcript abundance, was determined as previously described (1). All fold changes reported had P value < 0.05 and were considered significant.

SSU cDNA sequencing. Raw libraries were trimmed, filtered for quality and length, and all ambiguous base calls were removed using Mothur (ver. 1.25.1) (2). Unique sequences were aligned to the SILVA bacterial or archaeal databases and sequences that started or ended before defined positions in the alignment that were met by 75% of total sequences, were removed. The resulting sequences were pre-clustered in Mothur to remove amplification and sequencing errors. Chimeras were detected using UCHIME (3) and were removed. Operational taxonomic units (OTUs) were assigned at a sequence similarity of 0.03 using the furthest-neighbor method within Mothur. Sequences were classified using the Bayesian classifier and the RDP database using Mothur and were then manually verified with BLASTn (SOM Tables 5 & 6).

Phylogenetic Analysis. A representative sequence for each NifH and archaeal AmoA protein OTU (defined at 0.03 sequence identities) was aligned with ClustalX (ver. 2.0) (4) specifying default gap extension and opening settings. The evolutionary history of NifH and archaeal AmoA was inferred by Neighbor Joining method with 100 bootstrap replicates (SOM Figs. 1 & 2). The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site (5).



Supplemental Figure 1. Phyogenetic reconstruction of NifH deduced amino acid sequences recovered from Perpetual Spouter. Sequence designations correspond with those presented in Supplemental Table 3.



Supplemental Figure 2. Phyogenetic reconstruction of AmoA deduced amino acid sequences recovered from Perpetual Spouter. Sequence designations correspond with those presented in Supplemental Table 4.

Supplemental Table 1. Primers, primer sequences, annealing temperatures and corresponding references for the primers used in the present study.

Target	Primer	Tm (ºC)	Reference[a]
Archaeal 16S	344F (5'-ACGGGGYGCAGCAGGCGCGA-3')	55.0	Boyd et al. 2007
	915R (5'-GTGCTCCCCCGCCAATTCCT-3')		
Bacterial 16S	1100F (5'-YAACGAGCGCAACCC-3')	55.0	Boyd et al. 2007
	1492R (5'-GGTTACCTTGTTACGACTT-3')		
nifH	nifH-119F (5'-THGTHGGYTGYGAYCCNAARGCNGAYTC-3')	60.9	Hamilton et al. 2011
	nifH-471R and (5'- GGHGARATGATGGCNMTSTAYGCNGCNAA-3')		
Archaeal *amoA*	Arch-amoAF (5'-STAATGGTCTGGCTTAGACG-3')	53.0	Francis et al. 2011
	Arch-AmoAR (5'-GCGGCCATCCATCTGTATGT-3')		
Bacterial *amoA*	amoA1F (5'-GGGGTTTCTACTGGTGGT-3′)	53.0	Hoshino et al. 2001
	amoA2R (5′-CCCCTCKGSAAAGCCTTCTTC-3′)		

[a] References 6-9

Supplemental Table 2. Dynamic range and detection limit of qPCR and qRT-PCR assays.

qPCR		
Target[a]	Dynamic Range	Detection Limit
Archaeal 16S	8.9×10^1 to 4.6×10^6 ($R^2 = 0.997$)	11 copies
Bacterial 16S	8.9×10^1 to 4.6×10^6 ($R^2 = 0.997$)	8 copies
qRT-PCR		
Target[a]	Dynamic Range	Detection Limit
Archaeal 16S	1.3×10^2 to 2.6×10^7 ($R^2 = 0.997$)	9 copies
Bacterial 16S	6.6×10^1 to 2.3×10^6 ($R^2 = 0.997$)	8 copies
nifH	8.6×10^1 to 9.3×10^6 ($R^2 = 0.997$)	10 copies
Archaeal *amoA*	1.1×10^2 to 1.6×10^7 ($R^2 = 0.997$)	11 copies
Bacterial *amoA*	1.1×10^2 to 1.6×10^7 ($R^2 = 0.997$)	9 copies

[a]Primers for each assay target are listed in Supp. Table 1.

Supplemental Table 3. Clone frequencies, accession numbers, and affiliations of *nifH* transcripts recovered in the present study.

Frequency[a]	Designation	Accession Number	Closest Affiliated Sequence[b]	Phylum[b]	% Identity[b]	% Similarity[b]
44	PS nifH-1	KC254651	*Thermocrinis albus*	Aquificae	100	100
5	PS nifH-2	KC254652	*Thermocrinis albus*	Aquificae	97	98
3	PS nifH-3	KC254653	*Leptospirillum ferrooxidans*	Nitrospirae	97	98
2	PS nifH-5	KC254654	*Paenibacillus azotofixans*	Firmicutes	97	97
26	PS nifH-6	KC254655	*Thermodesulfovibrio yellowstonii*	Nitrospirae	98	98
1	PS nifH-7	KC254656	*Thermodesulfovibrio yellowstonii*	Nitrospirae	96	98
1	PS nifH-8	KC254657	*Thermodesulfovibrio yellowstonii*	Nitrospirae	98	98

[a]The frequency of the clone recovered in the present study.

[b]Closest cultured representative, phylum, genus, % sequence identity and similarity as determined using tBLASTn.

Supplemental Table 4. Clone frequencies, accession numbers, and affiliations of *amoA* transcripts recovered in the present study.

Frequency[a]	Designation	Accession Number	Closest Affiliated Sequence[b]	Phylum[b]	% Identity[b]	% Similarity[b]
106	PS amoA-1	KC254659	*Candidatus* Nitrosocaldus yellowstonii	Thaumarchaeota	97	99
5	PS amoA-2	KC254660	*Candidatus* Nitrosocaldus yellowstonii	Thaumarchaeota	99	99

[a]The frequency of the clone recovered in the present study.

[b]Closest cultured representative, phylum, genus, % sequence identity and similarity as determined using tBLASTn.

Supplemental Table 5. OTU frequencies, accession numbers, and affiliations of Archaeal 16S rRNA transcripts recovered in the present study.

Frequency[a]	Designation	Accession Number	Genus[b]	Phylum[b]
973	PS Archael 16S-1	KC254661	Candidatus Nitrosocaldus yellowstonii	Crenarchaeota
2108	PS Archael 16S-2	KC254662	*Vulcanisaeta*	Crenarchaeota
8	PS Archael 16S-3	KC254663	*Pyrobaculum*	Crenarchaeota
4	PS Archael 16S-4	KC254664	*Pyrobaculum*	Crenarchaeota
1	PS Archael 16S-5	KC254665	*Methanocella*	Euryarchaeota

[a]The frequency of the clone recovered in the present study.

[b]Closest cultured representative, phylum, genus, % sequence identity and similarity as determined using tBLASTn.

Supplemental Table 6. OTU frequencies, accession numbers, and affiliations of Bacterial 16S rRNA transcripts recovered in the present study.

Frequency[a]	Designation	Accession Number	Genus[b]	Phylum[b]
4	PS Bacterial 16S-1	KC254666	*Sulfobacillus*	Firmicutes
463	PS Bacterial 16S-2	KC254667	*Hydrogenobaculum*	Aquificae
120	PS Bacterial 16S-3	KC254668	*Rhodanobacter*	Proteobacteria
2243	PS Bacterial 16S-4	KC254669	*Thermotoga*	Thermotogae
326	PS Bacterial 16S-5	KC254670	*Thiomonas*	Proteobacteria
2	PS Bacterial 16S-6	KC254671	*Sulfobacillus*	Firmicutes
2	PS Bacterial 16S-7	KC254672	*Acidiphilium*	Proteobacteria
2	PS Bacterial 16S-8	KC254673	*Acidithiobacillus*	Proteobacteria
5	PS Bacterial 16S-9	KC254674	*Rhodanobacter*	Proteobacteria
1	PS Bacterial 16S-10	KC254675	*Azotobacter*	Proteobacteria
6	PS Bacterial 16S-11	KC254676	*Aciditerrimonas*	Actinobacteria
1	PS Bacterial 16S-12	KC254677	*Sulfobacillus*	Firmicutes
2	PS Bacterial 16S-13	KC254678	*Acidicaldus*	Proteobacteria
3	PS Bacterial 16S-14	KC254679	*Desulfurella*	Proteobacteria
1	PS Bacterial 16S-15	KC254680	*Acidimicrobium*	Actinobacteria
1	PS Bacterial 16S-16	KC254681	*Thermodesulfobium*	Firmicutes
2552	PS Bacterial 16S-17	KC254682	*Thermocrinis*	Aquificae
1	PS Bacterial 16S-18	KC254683	*Paenibacillus*	Firmicutes
1	PS Bacterial 16S-19	KC254684	*Marinobacter*	Proteobacteria
166	PS Bacterial 16S-20	KC254685	*Thermotoga*	Thermotogae
1598	PS Bacterial 16S-21	KC254686	*Thermus*	Deinococcus-Thermus
1	PS Bacterial 16S-22	KC254687	*Thermanaerothrix*	Chloroflexi
146	PS Bacterial 16S-23	KC254688	*Thermodesulfovibrio*	Nitrospira
2	PS Bacterial 16S-24	KC254689	*Dictyoglomus*	Dictyoglomi
3	PS Bacterial 16S-25	KC254690	*Stenotrophomonas*	Proteobacteria
1	PS Bacterial 16S-26	KC254691	*Thermomicrobium*	Chloroflexi
1	PS Bacterial 16S-27	KC254692	*Fervidicola*	Firmicutes

[a]The frequency of the clone recovered in the present study.

[b]Closest cultured representative, phylum, genus, % sequence identity and similarity as determined using tBLASTn.

Supplemental Table 7. Rates of acetylene reduction in *Azotobacter vinelandii*.

Treatment	Acetylene Reduction Rate (nmol C_2H_4 gdm^{-1} h^{-1}[a,b])
Biological	327 ± 11.1
NH_4^+[c]	BDL
ATU[d]	331 ± 9.5
NH_4^+ & ATU	BDL

[a]Acetylene reduction values represent the average and standard deviation of assays performed in triplicate

[b]BDL, below detection limit of 50 C_2H_4 pmol gdm^{-1} $hour^{-1}$

[b]Ammonia added as NH_4Cl (1 mM final concentration)

[c]ATU (allylthiourea) (1 mM final concentration)

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