Supporting Information

Zhang et al. 10.1073/pnas.1004947107

DNAS Nd



Fig. S1. Abundance of archaeal (*A*) and bacterial (*B*) amoA genes in microcosms incubated with ambient or 5% CO_2 concentrations for 28 d. Data plotted are mean values and SEs from triplicate microcosms destructively sampled at each time point. The same letter above different bars indicates no significant difference (P > 0.05).



Fig. 52. Analysis of 21 clones from CrenamoA23f-616r gene PCR products comigrating with band positions 1 (green) and 2 (orange) in Fig. 4. Nine are from this study (prefixed with "HBD" for high buoyant density), six from a previous study (1), and six from a third study (prefixed with "TS" for total soil) and all are derived from Craibstone pH 7.5 soil incubated under similar conditions (30 °C, 28–30 d). (A) PCR products were amplified from genomic DNA recovered from two CsCl fractions with a buoyant density of approximately 1.725 g mL⁻¹ or from total soil genomic DNA and cloned into a T-vector (1) before screening by DGGE. Dates describe sampling and initiation of incubation. (*B*) Maximum likelihood analysis (486 aligned nucleotide positions, GTR correction, invariable plus four γ -rates, 100 bootstrap replicates) of the 21 clone sequences together with reference sequences [clone name (accession no.)]. (Scale bar: 0.1 changes per nucleotide position.) Tree was rooted with sequences from "thermophilic AOA" lineage.

1. Offre P, Prosser JI, Nicol GW (2009) Growth of ammonia-oxidizing archaea in soil microcosms is inhibited by acetylene. FEMS Microbiol Ecol 70:99–108.



Fig. S3. Analysis of total archaeal community 16S rRNA genes amplified from recovered fractions of genomic DNA after isopycnic ultracentrifugation. (*A*) DGGE analysis of archaeal 16S rRNA genes from total genomic DNA and individual cloned sequences, amplified using nested PCR amplification with primers A109f/Ar9r and rSAF/PARCH519r (see ref. 1 for cycling conditions and primer references within) in 12 fractions of DNA recovered from CSCI ultracentrifugation gradients. Total genomic DNA was extracted from microcosms incubated with ¹²C- or ¹³C-CO₂ for 28 d and five selected individual A109f/Ar9r cloned sequences were recovered from two fractions with a buoyant density of approximately 1.725 g mL⁻¹. Band doublets are produced as a result of primer degeneracies. (*B*) Maximum likelihood analysis (685 aligned nucleotide positions, GTR correction, invariable plus four γ -rates, 100 bootstrap replicates) of the five clone sequences together with reference sequences [clone name (accession no.)]. (Scale bar: 0.1 changes per nucleotide position.) Tree was rooted with sequences from "thermophilic AOA" lineage.

1. Nicol GW, Tscherko D, Chang L, Hammesfahr U, Prosser JI (2006) Crenarchaeal community assembly and microdiversity in developing soils at two sites associated with deglaciation. Environ Microbiol 8:1382–1393.



Fig. S4. Maximum likelihood analysis of derived protein sequences (118 aligned amino acid positions, JTT correction, invariable plus four γ -rates, 100 bootstrap replicates) of 20 randomly selected cloned sequences from *hcd* gene qPCR products from one day-28 incubation sample. All sequences were placed within one of two clusters, both of which were placed within a lineage together with sequences from *N. maritimus* and *C. symbiosum* and metagenomic scaffolds obtained in the Global Ocean Sampling (GOS) survey (1) and identified as being of assumed archaeal origin (2). (Scale bar: 0.1 changes per nucleotide position.)

1. Rusch DB, et al. (2007) The Sorcerer II Global Ocean Sampling expedition: Northwest Atlantic through eastern tropical Pacific. PLoS Biol 5:e77.

2. Ettema TJ, Andersson SG (2008) A 3-hydroxypropionate/4-hydroxybutyrate autotrophic carbon dioxide assimilation pathway in Archaea. Science 321:342.

SAND SAL