

Supplementary Information for

Silicate biomineralization of sediment- and carbonate-hosted anaerobic methane-oxidizing consortia and implications for microbial fossil preservation

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This PDF file includes:

Figures S1 to S7
Legends for Datasets S1 to S4

Other supplementary materials for this manuscript include the following:

Datasets S1 to S4

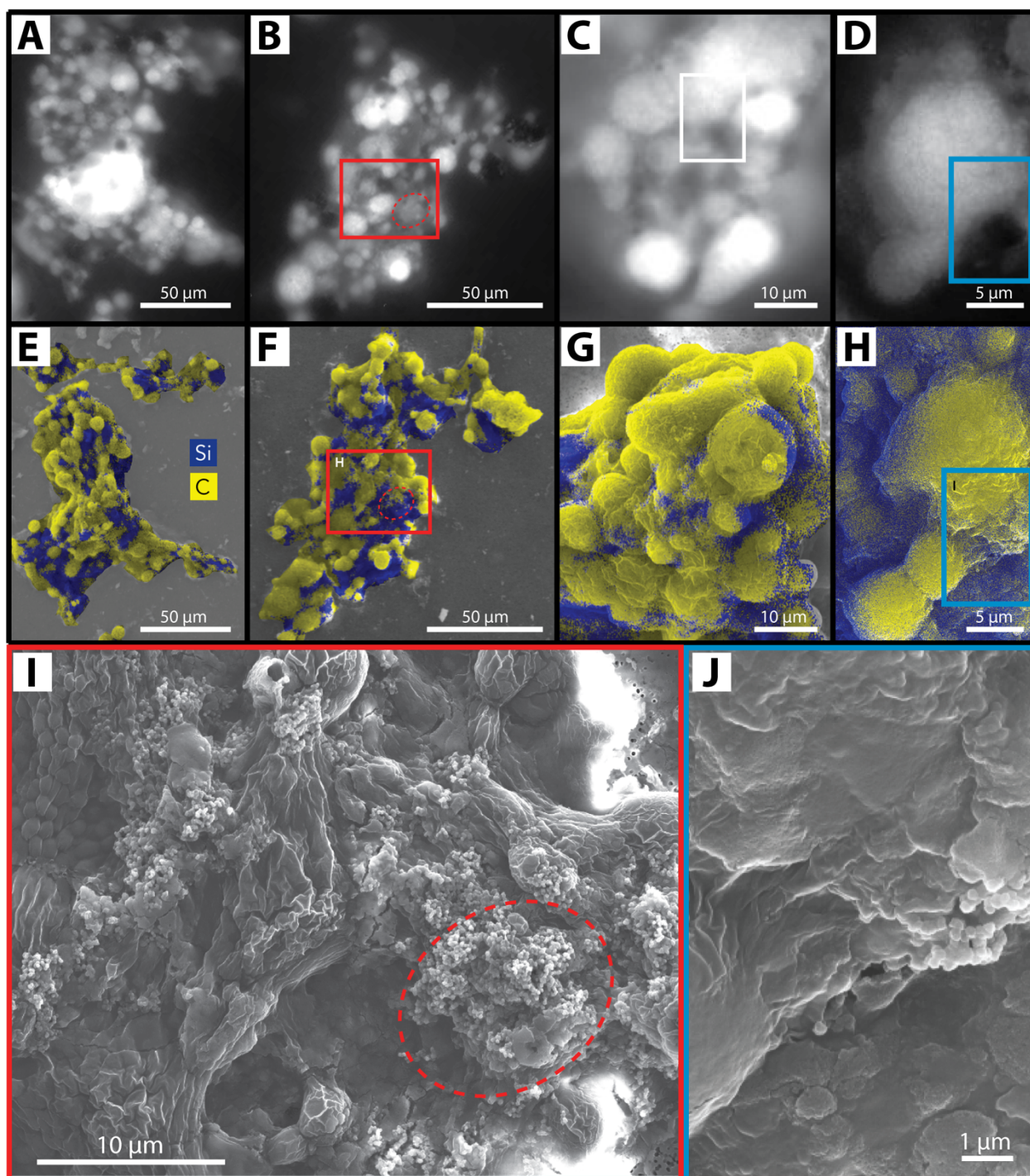


Figure S1 | Correlative epifluorescence microscopy and EDS analysis of ANME-SRB consortia clusters from sediment-free AOM enrichment cultures. **A-D)** DAPI-stained consortia clusters at different magnifications showing the gross morphology of the clusters. **(E-H)** Corresponding SEM-EDS data documenting the spatial proximity of Si-rich phases (blue) with carbon-rich AOM consortia (yellow). **I-J)** SEM images of silica rich spherules at 2 magnifications. **I)** Enlargement

of the red box area in B and F, where a region enriched in silica nanoparticles is highlighted with dashed red oval. **J)** Enlargement of the blue box area in D and H, illustrating Si-rich silicates surrounding an ANME-SRB consortium.

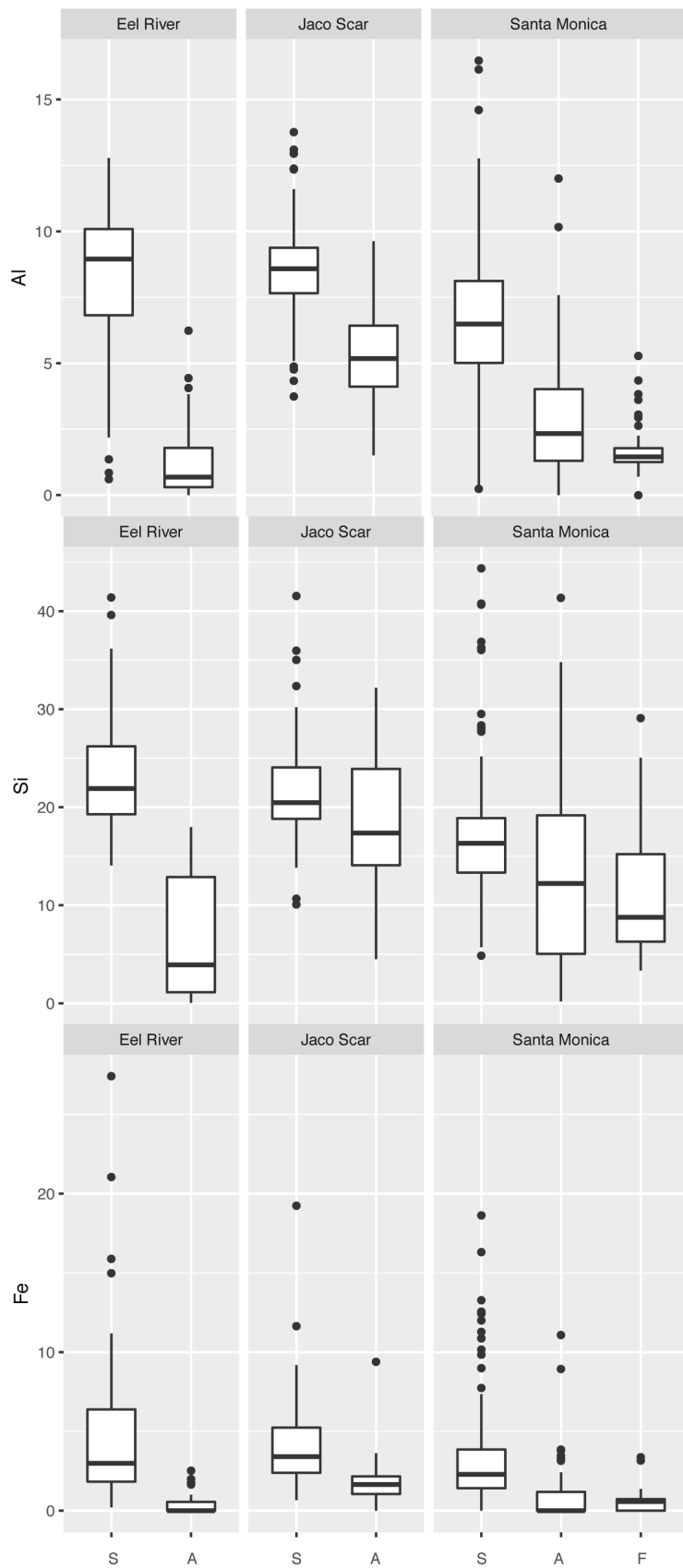


Figure S2 | EDS compositional data showing the difference in aluminum, silica, and iron in Si-rich phases attached to ANME-SRB consortia recovered directly from methane seep sediments (letter A) or grown under sediment-free conditions (letter F), compared with the composition of the source sediment (letter S). Details on sampling locations can be found in Methods.

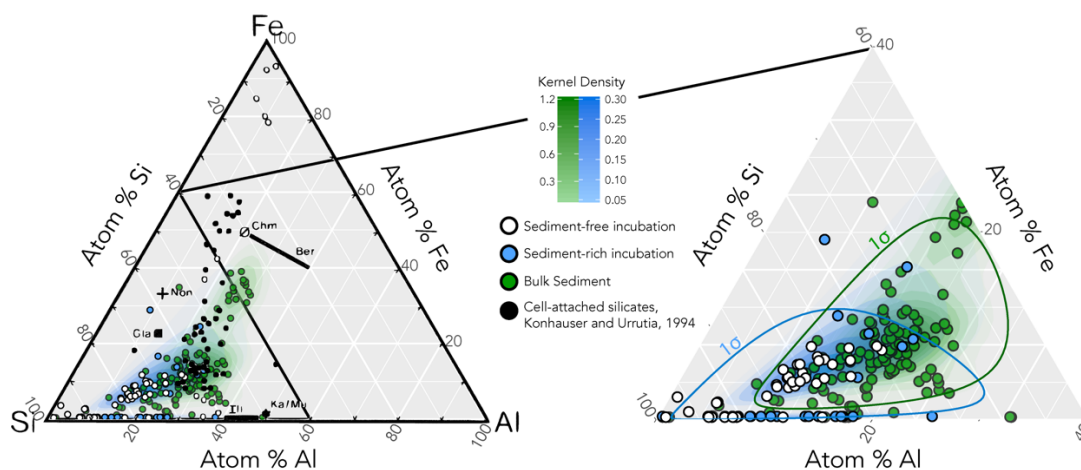


Figure S3 | Fe-Al-Si ternary plot of EDS compositional data for Si-rich phases attached to AOM consortia sourced directly from Santa Monica Basin methane seep sediments or from sediment-free AOM consortia enrichment cultures, plotted alongside reported compositions of silicates attached to bacterial cell walls.

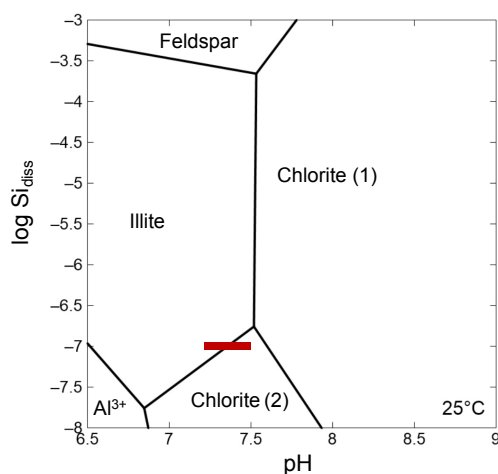


Figure S4 | Predicted mineral stability diagram for silicate mineral formations in the experimental solution composition with only illite and smectite included as clay minerals. Feldspar refers to albite; Chlorite(1) and Chlorite(2) refers to chamosite and clinocllore, respectively; and the red bar corresponds to ICP-MS measurement of [Si] in artificial seawater

media from the sediment-free AOM enrichments. Crystalline or amorphous silica precipitation would require higher dissolved silica and lower pH values.

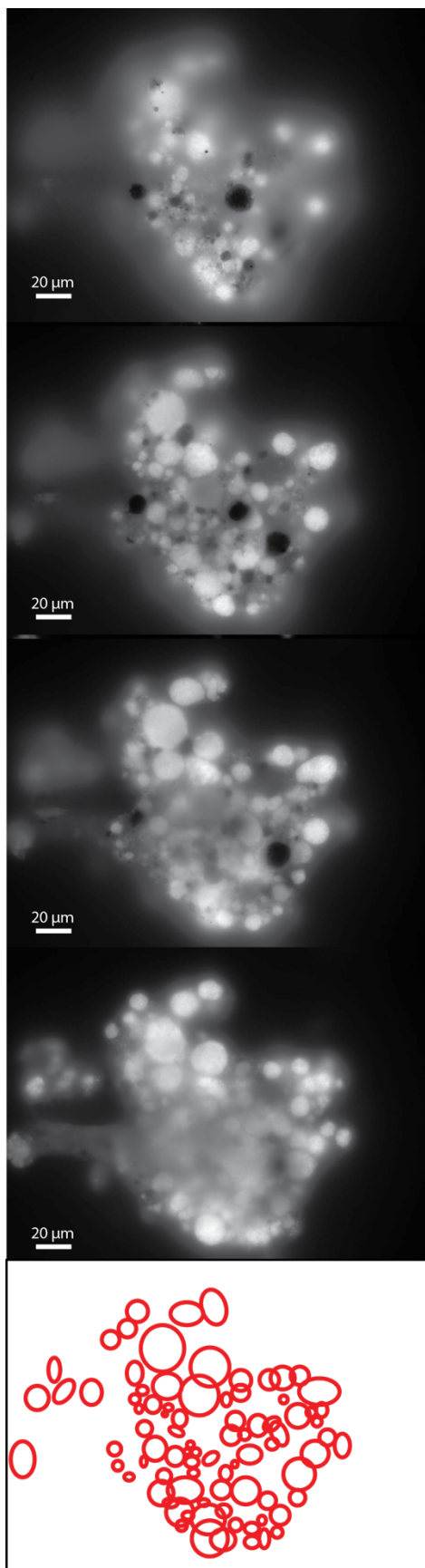


Figure S5 | Epifluorescence microscopy images of a DAPI-stained large cluster of multiple ANME-SRB consortia from a sediment-free AOM enrichment culture, and selected regions of interest (ROI) of consortia outlines (in red) shown in the lower panel. These ROIs illustrate the method used for estimating consortia numbers as an input for the growth model shown in Fig. 3b.

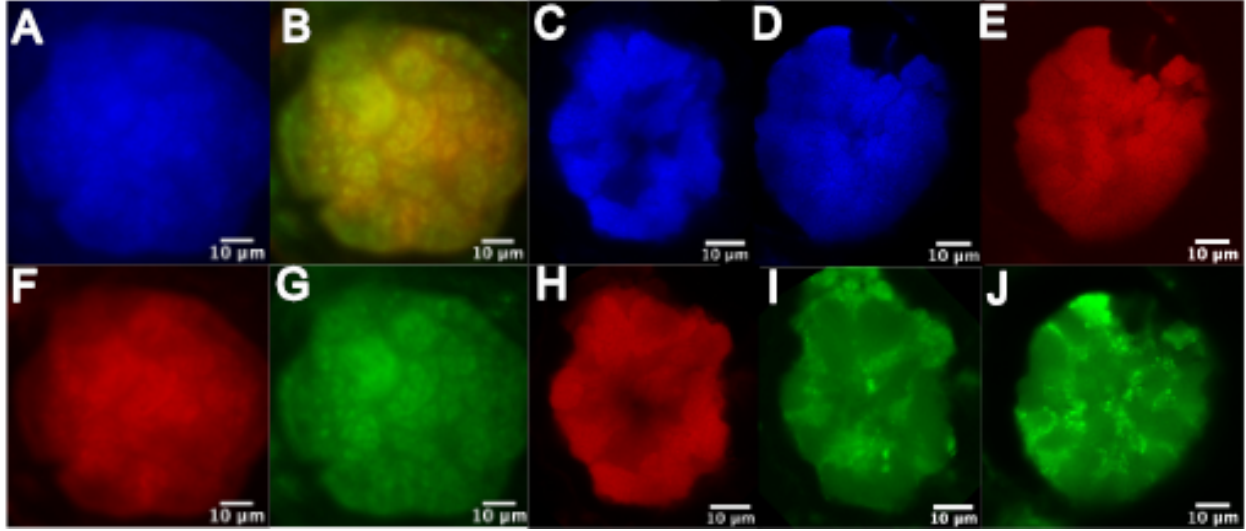


Figure S6 | Individual epifluorescence microscopy pictures of the ANME-2/SRB consortium in seep carbonate from Fig. 5, stained with **A, C, D**) DAPI for DNA, **E, F, H**) ANME-2 (ANME-2-712(73)) specific FISH probe, **G, I, J**) SRB (DSS658(4)) specific probe, and **B**) Both ANME-2 and SRB specific probes (enlargement of large consortia in Fig. 5a). **A, B, F** and **G** correspond to the consortia in Fig 5a; **C, H**, and **I** correspond to the consortia in Fig. 5d; and **D, E** and **J** correspond to the consortia in Fig. 5g.

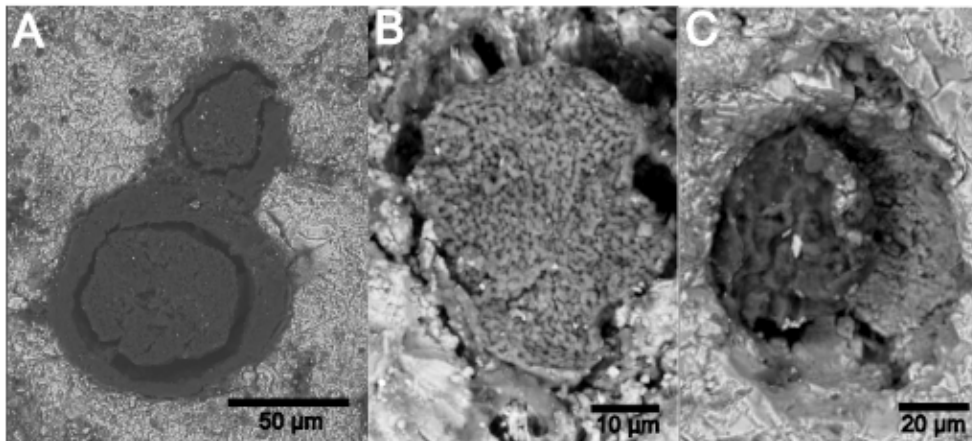


Figure S7 | Scanning electron microscopy (SEM) pictures of the ANME-2/SRB consortia shown in Fig. 5. **A**) Consortia from Fig. 5a, **B**) Consortia from Fig. 5d, and **C**) Consortia from Fig. 5g.

Dataset S1. Elemental composition of Si-rich rings surrounding ANME2-SRB aggregates. The different spectra correspond to the points shown in Figs. 4 and 5.

Dataset S2. Supplementary Excel data file with Al:Si ratios for different basins and treatments, utilized to make the plots in Fig. 3a.

Dataset S3. Supplementary Excel data file with (Mg+Al+Fe):Si ratios for different basins and treatments, utilized to make the plots in Fig. 3a.

Dataset S4. Supplementary Excel data file with the concentrations of relevant elements in sediment-free ANME-SRB incubations, utilized to make the plots in Fig. 3c.