

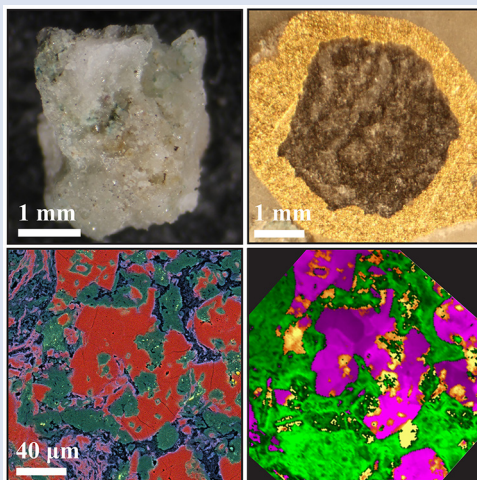
Metamorphic evolution of carbonate-hosted microbial biosignatures

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Abstract



Microbial biosignature assemblages captured within mineral substrates experience extreme pressures (P) and temperatures (T) during rock burial and metamorphism. We subjected natural microbial biofilms hosted within thermal spring carbonate to six high pressure, high temperature (HPHT) conditions spanning 500 and 800 MPa and 200 to 550 °C, to investigate the initial petrographic transformation of organic and inorganic phases. We find biogenic and amorphous silica mineralises increasingly mature organic matter (OM) as temperature and pressure increase, with OM expelled from recrystallised calcite at the highest HPHT, captured within a quartz phase. Sulfur globules associated with microbial filaments persist across all HPHT conditions in association with microbially-derived kerogen. These data demonstrate how microbial material captured within chemically-precipitated sediments petrographically evolves in high grade rocks during their first stages of transformation.

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Introduction

Our knowledge of microbiological evolution is informed by organic, morphological, and geochemical biosignatures preserved within a rock record that is destroyed or modified over geological time (Westall, 2008). Chemically-precipitated sediments, particularly carbonate and silica, host microfossils spanning much of Earth history, including in association with early evidence for microbial life on Earth (Moreau and Sharp, 2004; Westall, 2008; Wacey *et al.*, 2011; Djokic *et al.*, 2017). The petrographic relationships between microbially-derived kerogen and the inorganic matrix are important in establishing microfossil biogenicity (Brasier *et al.*, 2005; Wacey *et al.*, 2011; Foucher and Westall, 2013), in addition to understanding broader interactions of microbe-mineral systems back into the early Archean (Westall *et al.*, 2015).

Significant experimental effort has furthered our understanding of microbial biomineralisation (Westall *et al.*, 1995; Orange *et al.*, 2012, 2014; Li *et al.*, 2013a and references therein; Gaboyer *et al.*, 2017). High pressure, high temperature (HPHT) experiments are now shedding light on microfossil taphonomy during geological sequestration, with a focus on

thermal degradation of biogenic minerals (Li *et al.*, 2013b) and biomineralised cells (Li *et al.*, 2014). Experiments have demonstrated the persistence of microbiological structures such as iron oxide organominerals up to 250 °C and 140 MPa (Picard *et al.*, 2015a), and the preservation of lipids and polysaccharides within Fe-encrusted microbial cells at the same PT conditions (Picard *et al.*, 2015b). Recently, Alleen *et al.* (2016) showed that microbial entombment within silica limited the degradation of molecular biosignatures exposed to 25 MPa and 250 °C for 100 days.

As improving analytical techniques expand biosignature studies into increasingly metamorphosed terrains (Bernard *et al.*, 2007, 2010; Galvez *et al.*, 2012; Papineau *et al.*, 2019), there is a need to understand the influence of higher PT regimes (Li *et al.*, 2013a). We present an experimental investigation into the effects of HPHT on carbonate-hosted microbial biofilms from a natural thermal spring environment. We trace the first stages of petrographic evolution of geochemical and morphological sample components in response to 500 and 800 MPa and temperatures spanning 200 to 550 °C, significantly expanding the PT space previously investigated (Fig. 1a).

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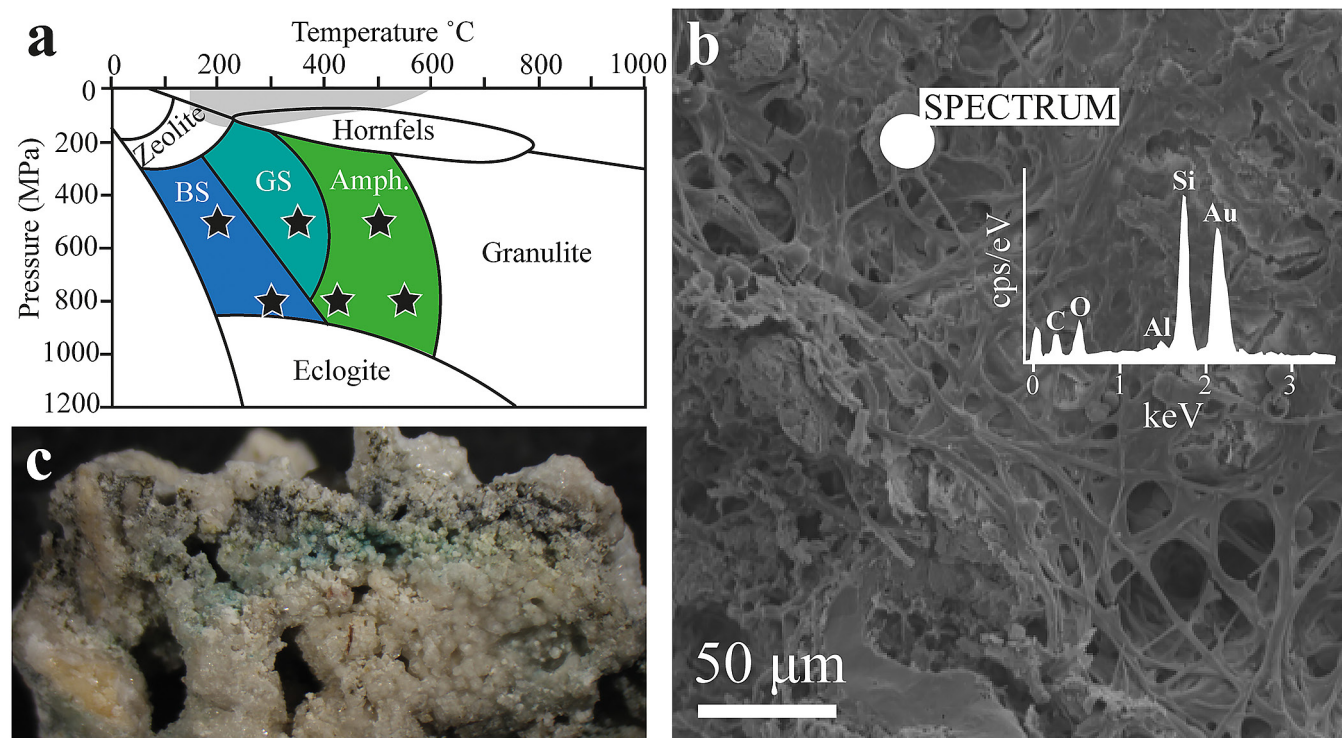


Figure 1 Starting material for experiments showing (a) Six high pressure, high temperature (HPHT) experimental conditions investigated and their corresponding metamorphic grades spanning lower and upper blueschist (BS), greenschist (GS) and amphibolite (Amph.). Grey depicts HPHT conditions investigated previously (Schiffbauer *et al.*, 2012; Li *et al.*, 2013b, 2014; Picard *et al.*, 2015a,b; Alleon *et al.*, 2016, 2017; Miot *et al.*, 2017). (b) Si-rich microbial filamentous material and coccoidal cell structures are observed under SEM in the starting material, shown in (c) by a view ~ 1 cm across; forming Si-rich biofilms around calcite grains (Fig. S-1a,b).

Sample Material

Porous carbonate precipitates containing green, orange, and grey chasmolithic microbial communities (Fig. 1c) were collected from a CO₂ thermal spring (Jotun Spring; Banks *et al.*, 1998) in Spitzbergen, Svalbard, during the Arctic Mars Analog Svalbard Expeditions from 2006–2011 (Starke *et al.*, 2013). An air dried sample was subsampled into multiple ~2 mm³ fragments for six HPHT experiments. Four additional fragments of this starting material were subjected to the same preparation and analytical techniques. Methods are described in the Supplementary Information.

Results

Visible light microscopy of polished HPHT samples reveal dark carbonised organic matter (OM) forming a compositional fabric within the calcite matrix, absent in the starting material (Fig. S-1a,c,d). Secondary electron imaging of a duplicate, acid etched 500 MPa, 350 °C sample shows mineralised biomass maintains its intact extracellular filamentous structure (Figs. 1b and S-1e) and coccoidal cell morphologies (Fig. S-1f). Intact diatom frustules are preserved at the lower PT conditions (500 MPa, 200 °C and 500 MPa, 350 °C), becoming structurally disintegrated in the higher PT experiments (500 MPa, 500 °C; 800 MPa, 300 °C and 800 MPa, 425 °C; Fig. S-1g,h).

Raman spectra of experimental sample surfaces exhibit peaks for carbonate (1100 cm⁻¹), and D1 (1360 cm⁻¹) and G (1610 cm⁻¹) carbon bands (Fig. 2; Pimenta *et al.*, 2007). An additional quartz band at 465 cm⁻¹ is observed for the three highest temperature samples (425, 500, and 550 °C), irrespective of pressure. Secondary electron imaging shows OM transitioning from amorphous biogenic material with cellular structures in the starting material to increasingly crystalline structures as

PT increases (Fig. 2). This is reflected in the changing carbon G-band centre and width, whereby temperatures of 350 °C and above produce a sharpened (more crystalline) G-band, and the increase in temperature from 200 to 550 °C also produces a peak centre shift to longer wavenumbers (Fig. S-2). Across all HPHT conditions OM maintains its original petrographic texture, either within a biofilm structure (Fig. 1c), or bound within diatom frustules (Fig. 1).

Raman spectroscopy and SEM+EDS elemental mapping of experimental sample surfaces show kerogenous material (D1 and G Raman bands) remains spatially-concurrent with the siliceous phase (Fig. 3). At the lowest PT condition (500 MPa, 200 °C), petrographic textures are largely indistinguishable from those in the starting material. At 800 MPa, 425 °C (Fig. 3d) these phases form compositional fabrics, where the organic-rich phase exists within silicified or quartz-rich fabrics, which also have elevated Fe (Fig. 4h,i). With increasing PT, the silica-organic phase becomes partitioned from the recrystallising calcite, eventually forming discrete petrographic end members at 800 MPa and 550 °C, whereby kerogen either forms an organic carbonaceous film around quartz crystals (Fig. 3e) or is captured within the quartz itself (Fig. 3f). Water degassing is observed in the carbonate matrix for samples treated at 500 MPa, 350 °C; 500 MPa, 500 °C and 800 MPa, 425 °C (Fig. S-4b,c,e), and extensive fracturing (Fig. S-4b,c,d) is observed at 500 MPa (350 °C and 500 °C) and 800 MPa (300 °C).

Finally, discrete clusters of sulfur globules 1 – 3 μm in size are observed within the starting material (Fig. 4a,b). These globules persist through all thermal conditions at 800 MPa, and at 500 MPa, 500 °C, remaining petrographically associated with the siliceous kerogen-bearing phase (Fig. 4a–g). At the highest PT condition (800 MPa, 550 °C), globules become irregular structures (Fig. 4f). Fe co-occurs within the Si phase

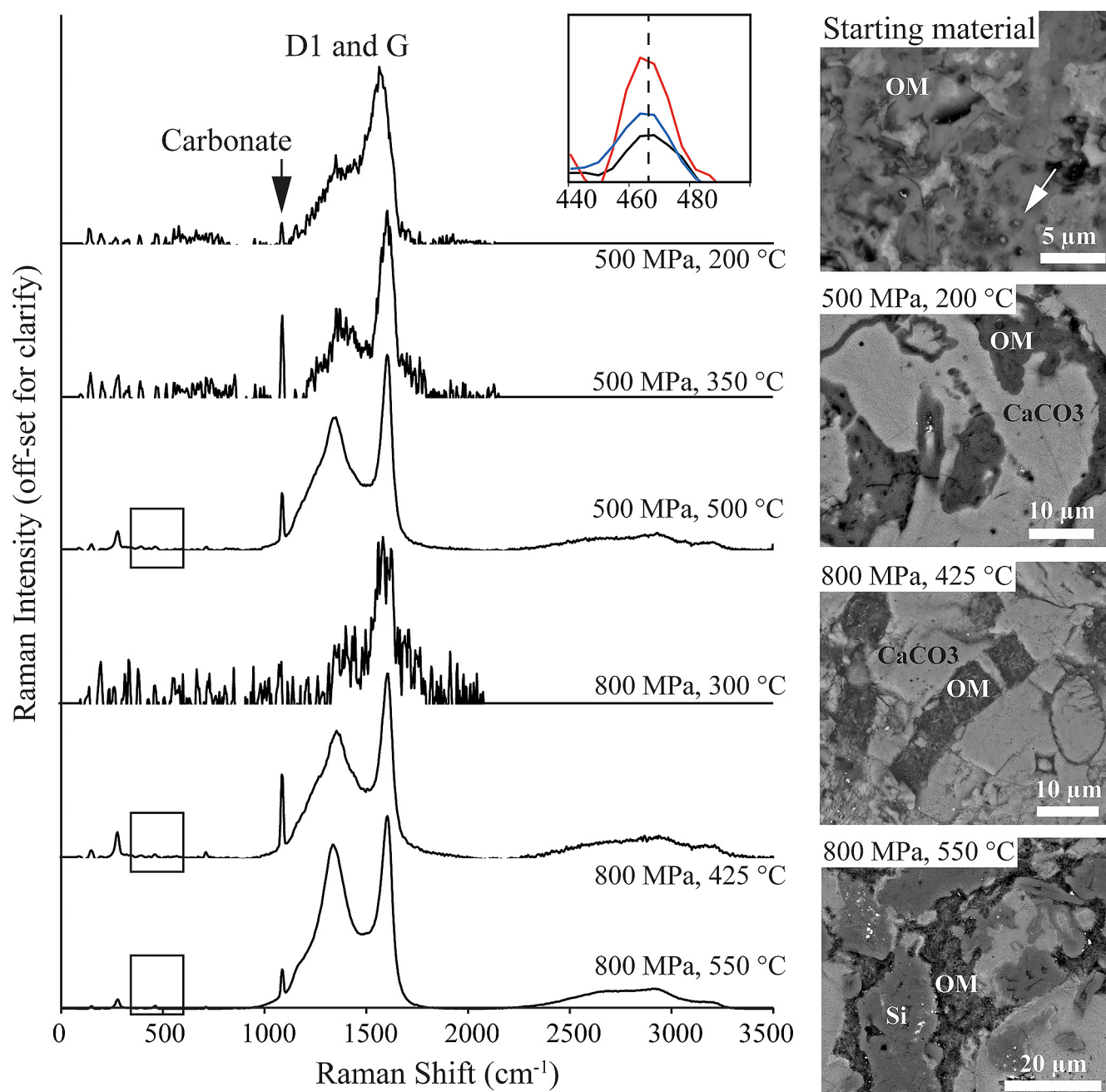


Figure 2 Evolution of D1 ($\sim 1360 \text{ cm}^{-1}$) and G ($\sim 1610 \text{ cm}^{-1}$) Raman peaks from experimental samples, and the increasing 467 cm^{-1} quartz peak at 425, 500, and 550°C (box inset); and corresponding SEM images showing the textural evolution of OM mineralisation within the carbonate (CaCO_3) matrix and siliceous (Si) phases. Individual cells in the amorphous organic matrix can be seen in the starting material (arrows). Unprocessed Raman spectra are given in Figure S-3.

for all samples, including in association with the S globules (Fig. 4h). Despite being a strong Raman scatterer, elemental sulfur was not detected by Raman spectroscopy (Fig. 2).

Discussion

Within the bounds of a closed system, mm- to micron-scale elemental (Si, S, Fe) biosignatures and microbially-derived kerogen maintain their petrographic relationship throughout initial exposure to HPHT (Fig. S-1c). *In situ* silicification of biomass occurs without petrographic disruption to the captured OM. With increasing PT, the organic-rich amorphous siliceous phase crystallises into α -quartz, and the originally porous calcite anneals into a crystalline matrix at 800 MPa, 550°C , expelling siliceous OM-bearing material. Extensive fracturing of the carbonate matrix observed at 800 MPa, 300°C may be

detrimental to preservation of biosignatures in comparison to an annealed calcite matrix, as the fractures would provide pathways for altering fluids. Disintegration of diatom frustules may be an additional source of Si as PT conditions increase.

Displacement of kerogen during diagenetic coarsening of quartz has been identified as a mechanism to explain petrographic relationships in the microfossil-bearing Gunflint chert (Moreau and Sharp, 2004; Foucher and Westall, 2013). This has implications for the longevity of biosignatures, whereby the quartz fraction resists weathering or secondary aqueous alteration, protecting captured OM (Toporski *et al.*, 2002). The preservation of OM within siliceous material is consistent with Alleon *et al.* (2016), who demonstrated the role of silica in limiting thermally-induced molecular degradation of OM in experimental samples. The transformation of Fe minerals to more stable, crystalline phases has also been shown to be conducive to the preservation of organic components (Ferris

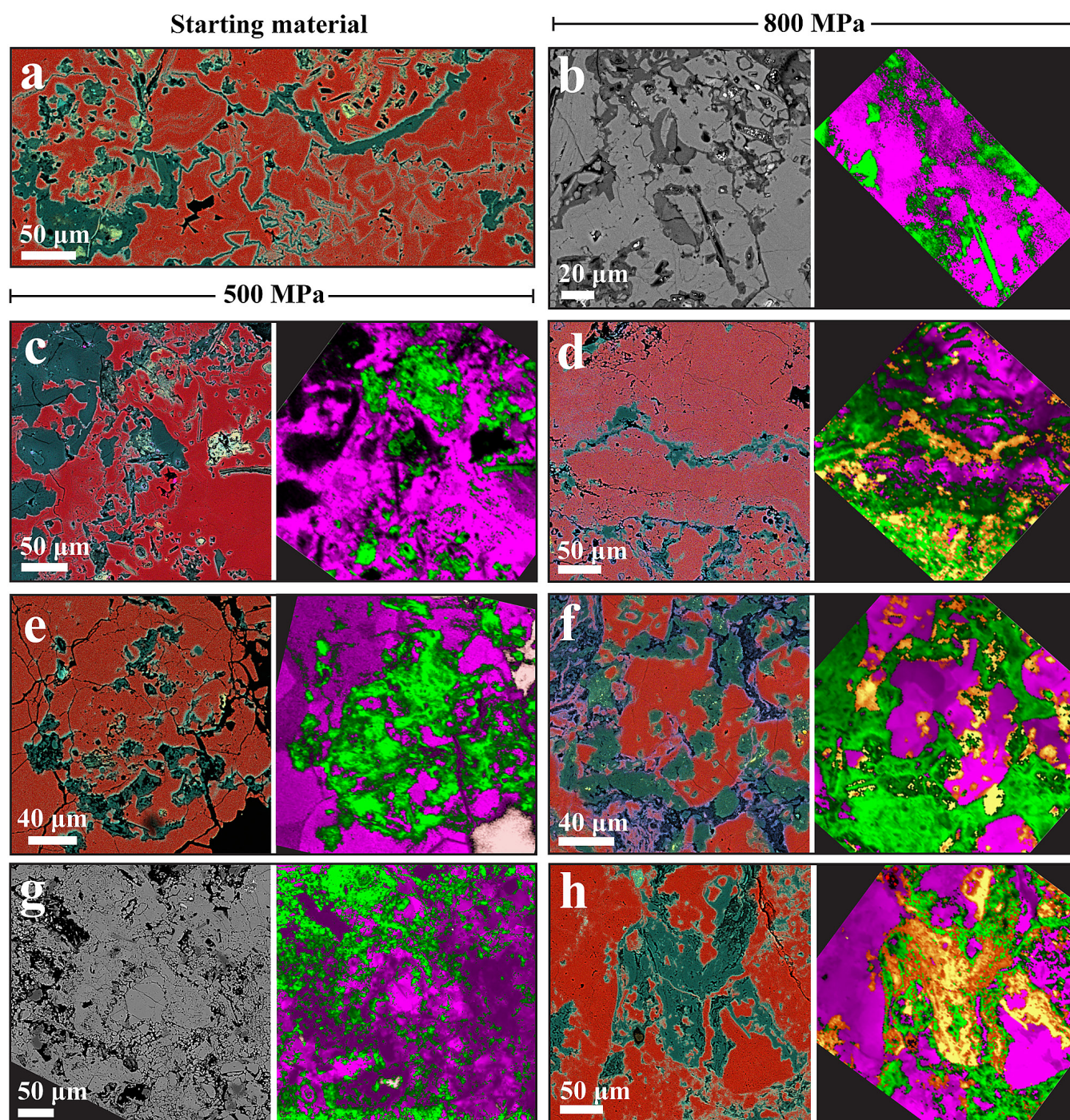


Figure 3 BSE+EDS elemental maps or plain BSE image (left), where red = Ca and green = Si, and corresponding Raman maps (right) of the same region, where fuchsia = calcite (1006 cm⁻¹ band), orange/yellow = quartz (465 cm⁻¹ band), green = carbon (combined D1 and G bands), light pink = resin, black = masked fluorescence. (a) Starting material; (b) 800 MPa, 300 °C; (c) 500 MPa, 200 °C; (d) 800 MPa, 425 °C; (e) 500 MPa, 350 °C; (f) 800 MPa, 550 °C; (g) 500 MPa, 500 °C; (h) 800 MPa, 550 °C. EDS spectra are provided in Figure S-5.

et al., 1988; Picard *et al.*, 2015a). Finally, the sulfur globules observed in the starting material and experimental samples are consistent in size and shape to intracellular sulfur globules found within filamentous sulfide oxidising bacteria (Dahl and Prange, 2006). Their preservation at the highest HPHT condition holds promise for establishing the biogenicity of similar features in filamentous microfossils (Wacey *et al.*, 2011; Bailey *et al.*, 2013) and distinguishing them from abiotic S microstructures (Cosmidis and Templeton, 2016).

While the OM Raman spectra are relatively close to those observed in the Gunflint and Draken formation (Foucher *et al.*, 2015), such experiments are inherently limited by their short duration. Even with the addition of P, Raman D1 and G

bands of experiment sample OM are broader than those from metasediments of equivalent metamorphic grade (Beyssac *et al.*, 2002), indicating they are less mature. This limitation was also observed by Li *et al.* (2014) for experiments at similar temperatures (300 °C, 600 °C) in the absence of pressure.

Conclusions

This study presents an experimental investigation into the first stages of HPHT evolution of microbial biosignatures within a natural siliceous-carbonate matrix. While thermal maturation of OM is well understood (Vandenbroucke and

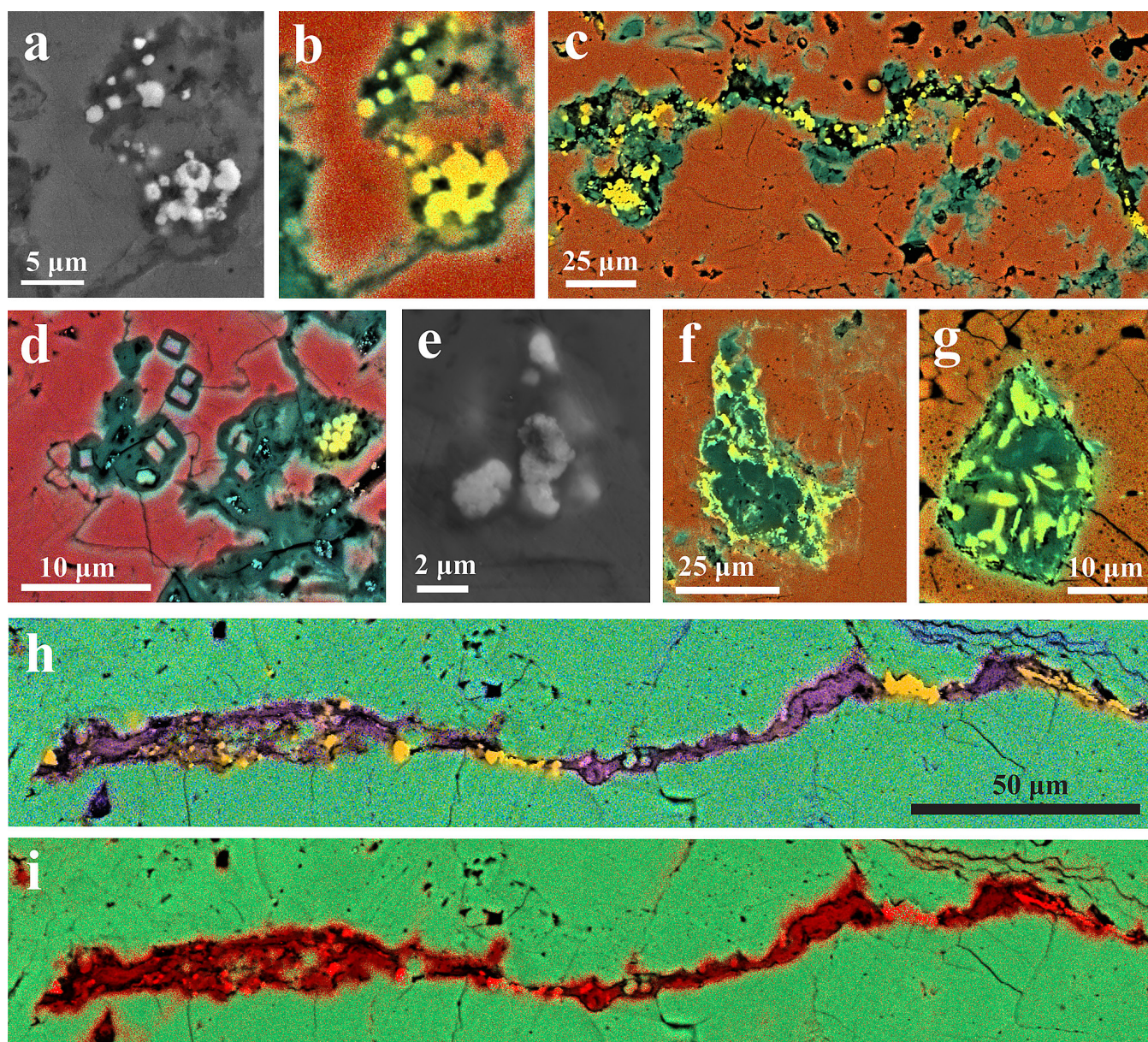


Figure 4 (a – g) BSE+EDS elemental maps (red = Ca, green = Si, yellow = S) and SEM images of sulfur globules in (a, b) Starting material; (c) 800 MPa, 425 °C; (d, e) 800 MPa, 300 °C; (f) 800 MPa, 550 °C; and (g) 500 MPa, 500 °C; (h) BSE+EDS elemental map showing the co-location of Fe (Fe = purple) with S (S = yellow) within a silica-rich (Si = red) fabric (i) in the 800 MPa, 425 °C experiment.

Largeau, 2007), we show that the early effects of temperature and pressure play an important role in the petrographic redistribution and capture of OM due to the transformation of the low temperature silica and carbonate phases. We demonstrate that exposure up to 800 MPa and 550 °C does not disrupt the petrographic relationship of captured microbial material with its inorganic host phase during the first stages of metamorphism. Future experimental work should address open system effects, including the influence of fluids on the modification of sample components, and longer duration experiments.

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Additional Information

Supplementary Information accompanies this letter at <http://www.geochemicalperspectivesletters.org/article2002>.



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References

- ALLEON, J., BERNARD, S., LE GUILLLOU, C., DAVAL, D., SKOURI-PANET, F., PONT, S., DELBES, L., ROBERT, F. (2016) Early entombment within silica minimizes the molecular degradation of microorganisms during advanced diagenesis. *Chemical Geology* 437, 98-108.
- ALLEON, J., BERNARD, S., LE GUILLLOU, C., DAVAL, D., SKOURI-PANET, F., KUGA, M., ROBERT, F. (2017) Organic molecular heterogeneities can withstand diagenesis. *Scientific Reports* 7, 1508.
- BAILEY, J.V., CORSETTI, F.A., GREENE, S.E., CROSBY, C.H., LIU, P., ORPHAN, V.J. (2013) Filamentous sulfur bacteria preserved in modern and ancient phosphatic sediments: implications for the role of oxygen and bacteria in phosphogenesis. *Geobiology* 11, 397-405.
- BANKS, D., SLETTEN, R.S., HALDORSEN, S., DALE, B., HEIM, M., SWENSEN, B. (1998) The Thermal Springs of Bockfjord, Svalbard: Occurrence and Major Ion Hydrochemistry. *Geothermics* 27, 445-467.
- BERNARD, S., BENZERARA, K., BEYSSAC, O., MNGUY, N., GUYOT, F., BROWN JR., G.E., GOFFÉ, B. (2007) Exceptional preservation of fossil plant spores in high-pressure metamorphic rocks. *Earth and Planetary Science Letters* 262, 257-272.
- BERNARD, S., BENZERARA, K., BEYSSAC, O., BROWN, G.E. (2010) Multiscale characterization of pyritized plant tissues in blueschist facies metamorphic rocks. *Geochimica Cosmochimica Acta* 74, 5054-5068.
- BEYSSAC, O., GOFFÉ, B., CHOPIN, C., ROUZAUD, J.N. (2002) Raman spectra of carbonaceous material in metasediments: a new geothermometer. *Journal of Metamorphic and Geology* 20, 859-871.
- BRASIER, M.D., GREEN, O.R., LINDSAY, J.F., MCLOUGHLIN, N., STEELE, A., STOAKES, C. (2005) Critical testing of Earth's oldest putative fossil assemblage from the ~3.5 Ga Apex chert, Chinaman Creek, Western Australia. *Precambrian Research* 140, 55-102.
- COSMIDIS, J., TEMPLETON, A.S. (2016) Self-assembly of biomorphic carbon/sulfur microstructures in sulfidic environments. *Nature Communications* 7, 12812.
- DAHL, C., PRANGE, A. (2006) Bacterial Sulfur Globules: Occurrence, Structure and Metabolism. In: Shively J.M. (Eds) *Inclusions in Prokaryotes*. Springer, Berlin, Heidelberg, 21-51.
- DJOKIC, T., VAN KRANENDONK, M., CAMPBELL, K.A., WALTER, M.R., WARD, C.R. (2017) Earliest signs of life on land preserved in ca. 3.5 Ga hot spring deposits. *Nature Communications* 8, 15263, doi: 10.1038/ncomms15263.
- FERRIS, F.G., FYFE, W.S., BEVERIDGE, T.J. (1988) Metallic ion binding by *Bacillus subtilis*: implications for the fossilization of microorganisms. *Geology* 16, 153-157.
- FOUCHER, F., WESTALL, F. (2013) Raman Imaging of Metastable Opal in Carbonaceous Microfossils of the 700–800 Ma Old Draken Formation. *Astrobiology* 13, 57-67.
- FOUCHER, F., AMMAR, M.R., WESTALL, F. (2015) Revealing the biotic origin of silicified Precambrian carbonaceous microstructures using Raman spectroscopic mapping, a potential method for the detection of microfossils on Mars. *Journal of Raman Spectroscopy* 46, 873-879.
- GABOYER, F., MILBEAU, C.L., BOHMEIER, M., SCHWENDNER, P., VANNIER, P., BEBLO-VRANESEVIC, K., RABOW, E., FOUCHER, F., GAUTRET, P., GUÉGAN, R., RICHARD, A., SAULDUBOIS, A., RICHMANN, P., PERRAS, A.K., MOISSE-LEICHTINGER, C., COCKELL, C.S., RETTBERG, P., MARTEINSSON, V., MONAGHAN, E., EHRENFREUND, P., GARCIA-DESCALZO, L., GOMEZ, F., MALKI, M., AMILS, R., CABEZAS, P., WALTER, N., WESTALL, F. (2017) Mineralization and Preservation of an extremotolerant Bacterium Isolated from an Early Mars Analog Environment. *Scientific Reports* 7, 14.
- GALVEZ, M.W., BEYSSAC, O., BENZERARA, K., BERNARD, S., MENGUY, N., COX, C.S., MARTINEZ, I., JOHNSTON, M.R., BROWN JR., G.E. (2012) Morphological preservation of carbonaceous plant fossils in blueschist metamorphic rocks from New Zealand. *Geobiology* 10, 118-129.
- LI, J., BENZERARA, K., BERNARD, S., BEYSSAC, O. (2013a) The link between biomineralization and fossilization of bacteria: Insights from field and experimental studies. *Chemical Geology* 359, 49-69.
- LI, Y.L., KONHAUSER, K.O., KAPPLER, A., HAO X.L. (2013b) Experimental low-grade alteration of biogenic magnetite indicates microbial involvement in generation of banded iron formations. *Earth and Planetary Science Letters* 361, 229-237.
- LI, J., BERNARD, S., BENZERARA, K., BEYSSAC, O., ALLARD, T., COSMIDIS, J., MOUSSOU, J. (2014) Impact of biomineralization on the preservation of microorganisms during fossilization: An experimental perspective. *Earth and Planetary Science Letters* 400, 113-122.
- MOREAU, J.W., SHARP, T.G. (2004) A Transmission Electron Microscopy Study of Silica and Kerogen Biosignatures in ~1.9 Ga Gunflint Microfossils. *Astrobiology* 4, 196-210.
- MIOT, J., BERNARD, S., BOURREAU, M., GUYOT, F., KISH, A. (2017) Experimental maturation of Archaea encrusted by Fe-phosphates. *Scientific Reports* 7, 16984.
- ORANGE, F., DISNAR, J.R., GAUTRET, P., WESTALL, F., BIENVENU, N., LOTTIER, N., PRIEUR, D. (2012) Preservation and evolution of organic matter during experimental fossilization of the hyperthermophilic archaea *Methanocaldococcus jannaschii*. *Origins of Life and Evolution of Biospheres* 42, 587-609.
- ORANGE, F., DUPONT, S., GOFF, O.L., BIENVENU, N., DISNAR, J.R., WESTALL, F., LE ROMANCER, M. (2014) Experimental fossilization of the thermophilic Gram-positive Bacterium *Geobacillus* SP7A: a long duration preservation study. *Geomicrobiology Journal* 31, 578-589.
- PAPINEAU, D., DEGREGORIO, B., SAGAR, J., THOROGATE, R., WANG, J., NITTLER, L., KILCOYNE, D.A., MARBACH, H., DROST, M., THORNTON, G. (2019) Fossil biomass preserved as graphitic carbon in a late Paleoproterozoic banded iron formation metamorphosed at more than 550 °C. *Journal of the Geological Society of London* 176, 651-668.
- PICARD, A., KAPPLER, A., SCHMID, G., QUARONI, L., OBST, M. (2015a) Experimental diagenesis of organo-mineral structures formed by microaerophilic Fe(II)-oxidizing bacteria. *Nature Communications* 6, 6277 (2015), doi: 10.1038/ncomms7277.
- PICARD, A., OBST, M., SCHMID, G., ZEITVOGEL, F., KAPPLER, A. (2015b) Limited influence of Si on the preservation of Fe mineral-encrusted microbial cells during experimental diagenesis *Geobiology* 14, 276-292.
- PIMENTA, M.A., DRESSSELHAUS, G., DRESSSELHAUS, M.S., CANCADO, L.G., JORIO, A., SAITO, R. (2007) Studying disorder in graphite-based systems by Raman spectroscopy. *Physical Chemistry Chemical Physics* 9, 1276-1291.
- SCHIFFBAUER, J. D., WALLACE, A.F., HUNTER, J.L., KOWALEWSKI, M., BODNAR, R.J., XIAO, S. (2012) Thermally induced structural and chemical alteration of organic walled microfossils: an experimental approach to understanding fossil preservation in metasediments. *Geobiology* 10, 402-423.
- STARKE, V., KIRSCHSTEIN, J., FOGEL, M.L., STEELE, A. (2013) Microbial community composition and endolith colonization at an Arctic thermal spring are driven by calcite precipitation. *Environmental Microbiology Reports* 5, 648-659, doi: 10.1111/1758-2229.12063.
- TOPORSKI, J.K., STEELE, A., WESTALL, F., THOMAS-KEPRTA, K.L., MCKAY, D.S. (2002) The simulated silicification of bacteria - new clues to the modes and timing of bacterial preservation and implications for the search for extraterrestrial microfossils. *Astrobiology* 2, 1-26.
- WACEY, D., KILBURN, M.R., SAUNDERS, M., CLIFF, J., BRASIER, M.D. (2011) Microfossils of sulphur-metabolizing cells in 3.4-billion-year-old rocks of Western Australia. *Nature Geoscience* 4, 698-702.
- WESTALL, F., BONI, L., GUERZONI, E. (1995) The experimental silicification of microorganisms. *Palaeontology* 38, 495-528.
- WESTALL, F. (2008) Morphological Biosignatures in Early Terrestrial and Extraterrestrial Materials. *Space Science Reviews* 135, 95-114.
- WESTALL, F., CAMPBELL, K.A., BRÉHÉRET, J.G., FOUCHER, F., GAUTRET, P., HUBERT, A., SORIEUL, S., GRASSINEAU, N., GUIDO, D.M. (2015) Archean (3.33 Ga) microbe-sediment systems were diverse and flourished in a hydrothermal context. *Geology* 43, 615-618.

