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1 **A microbial role in the construction of Mono Lake carbonate chimneys?**

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26 **Running title: Mono Lake carbonate chimneys**

27

28 **ABSTRACT**

29 Lacustrine carbonate chimneys are striking, metre-scale constructions. If these were
30 bio-influenced constructions, they could be priority targets in the search for early and
31 extraterrestrial microbial life. However, there are questions over whether such
32 chimneys are built on a geobiological framework, or are solely abiotic
33 geomorphological features produced by mixing of lake and spring waters. Here we
34 use correlative microscopy to show that microbes were living around Pleistocene
35 Mono Lake carbonate chimneys during their growth. A plausible interpretation, in
36 line with some recent works by others on other lacustrine carbonates, is that benthic

37 cyanobacteria and their associated extracellular organic material (EOM) formed
38 tubular biofilms around rising sub-lacustrine spring vent waters, binding calcium ions
39 and trapping and binding detrital silicate sediment. Decay of these biofilms would
40 locally have increased calcium and carbonate ion activity, inducing calcite
41 precipitation on and around the biofilms. Early manganese carbonate mineralization
42 was directly associated with cell walls, potentially related to microbial activity though
43 the precise mechanism remains to be elucidated. Much of the calcite crystal growth
44 was likely abiotic, and no strong evidence for either authigenic silicate growth or a
45 clay mineral precursor framework was observed. Nevertheless it seems likely that the
46 biofilms provided initial sites for calcite nucleation and encouraged the primary
47 organized crystal growth. We suggest that the nano, micro and macro scale fabrics of
48 these Pleistocene Mono Lake chimneys were affected by the presence of centimeter-
49 thick tubular and vertically-stacked calcifying microbial mats. Such carbonate
50 chimneys represent a promising macro-scale target in the exploration for ancient or
51 extra-terrestrial life.

52

53 **INTRODUCTION**

54 Distinguishing biologically-influenced sedimentary rock structures from abiotic ones
55 in the field, or when selecting high priority targets from remote images, is a key
56 challenge in the search for early and extra-terrestrial life. There are few recognisable
57 millimetre to decimetre-scale structures identifiable as definitively 'microbial' in
58 outcrop. Free-standing chimneys composed of carbonate, sulphate or silicate minerals,
59 if requiring the influence of organisms to develop, provide a potential set of targets
60 for terrestrial geological and astrobiological investigation (e.g., Walter and Des
61 Marais, 1993).

62

63 Mono Lake, California, is a globally important site for studying potential
64 biogeochemical processes creating ‘tufa’ limestone chimney constructions around
65 sub-lacustrine vents. It is renowned for its Pleistocene to 20th century vegetation-
66 encrusting tufa, found aligned along faults associated with springs, and boulder-
67 encrusting tufa sheets on the lake margins (Russell, 1889; Dunn, 1953). These
68 impressive, tower-like structures are only the most recent phase of lacustrine
69 carbonate deposition that has been occurring sporadically within Mono Lake at least
70 since the last glaciation (Wang et al., 2014). Although together these features are
71 considered an archetypal carbonate-precipitating hyperalkaline lacustrine environment
72 (Della Porta, 2015), their depositional mechanisms are surprisingly little studied. This
73 is in part because no active carbonate mineral precipitation has been reported since
74 sporadic events in the 1980s and early 1990s, when ikaite ($\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$) and
75 gaylussite ($\text{Na}_2\text{Ca}(\text{CO}_3)_2 \cdot 5\text{H}_2\text{O}$) were reportedly forming where spring waters mixed
76 with lake waters along the southern shore of the lake (Stine, 1987; Bischoff et al.,
77 1993).

78

79 Both geochemical and geobiological models have been put forward in an attempt to
80 explain voluminous past tufa formation in Mono Lake. In the purely geochemically
81 driven models, it is postulated that calcium carbonate precipitation was caused by
82 mixing of carbonate-rich, high pH lake water with Ca-rich spring water (Dunn, 1953;
83 Cloud and Lajoie, 1980; Rieger, 1992). Similar models have recently been invoked to
84 help explain sublacustrine chimneys of the Afar Rift (Dekov et al., 2014). A popular
85 geochemical model for Mono Lake contends that the dominant primary-formed
86 carbonate mineralogy is ikaite (Bischoff et al., 1993), and that in most cases this later

87 recrystallizes to gaylussite (Bischoff et al., 1993) or calcite (Council and Bennett,
88 1993). Mound and chimney morphologies in these geochemical models are explained
89 by mineral precipitation from upward rising, low-density sub-lacustrine plumes of
90 spring waters. However, such chimneys have not actually been demonstrated to
91 spontaneously form under sterile conditions, and turbulent mixing zones between
92 water masses may produce powder-like mineral precipitates in the water column
93 (consistent with the ‘milky white’ spring waters observed by Stine, 1987) rather than
94 coherent benthic constructions. Macro-crystalline, benthic sheets of calcite in other
95 contexts require the presence of a benthic biofilm (cf. Pedley et al., 2009).

96

97 Early geobiological models were based on observations of microbes or algae at sites
98 of active tufa growth that were inferred to have influenced carbonate precipitation
99 (Scholl and Taft, 1964). Given the enormous dissolved inorganic carbon (DIC) pool
100 in Mono Lake and high pH such that the majority of this DIC is present as carbonate,
101 postulated photosynthetic effects on carbonate mineral deposition are likely to be
102 negligible (cf. Arp et al., 2001). Stable carbon isotopes of Mono Lake tufa samples
103 record temporal variations in the bulk lake water DIC that relate to changes in
104 plankton productivity and burial (Li and Ku, 1997). However, no study has yet
105 reported any local $\delta^{13}\text{C}_{\text{calcite}}$ enrichment relative to lake average that could be
106 attributed to photosynthetic CO_2 uptake at the site of carbonate precipitation. Instead,
107 a more logical microbial mechanism for carbonate mineral formation is via binding of
108 calcium by the copious amounts of extra-cellular organic material (EOM) produced
109 by cyanobacteria (Emeis et al., 1987), with calcification taking place during or
110 following calcium release on heterotrophic EOM breakdown (Arp et al., 1998; 1999;
111 2001; Dupraz et al., 2004). We follow others including Dupraz et al. (2013) in using

112 the broader term EOM rather than EPS (extracellular polymeric substances) here
113 because EOM encompasses all organic matter external to the cell, including low
114 molecular weight organic carbon, not just the larger EPS molecules.

115

116 An alternative geobiological model for carbonate chimney growth in Mono Lake
117 might be calcite replacement of a microbially-precipitated clay precursor, as has been
118 proposed for thrombolitic microbial carbonates of Lake Clifton, Australia (Burne et
119 al., 2014) and dolomitic microbialites of Great Salt Lake, USA (Pace et al., 2016).

120 Pertinent to this model was a report of potential microbial mediation of magnesium
121 silicate growth within calcite-cemented clastic lake sediment (locally known as ‘sand
122 tufa’; Cloud and Lajoie, 1980) on the southern shore of Mono Lake (Souza-Egipsy et
123 al., 2005). In these lake sands, however, no evidence for calcite precipitation around
124 living or decaying microbes was found (Souza-Egipsy et al., 2005).

125

126 Geomorphologically, mound and column structures are well known to arise under the
127 influence of photosynthetic (cf. stromatolites and thrombolites) and chemosynthetic
128 (black and white smoker) microbial activity, with the biofilm providing a focus for
129 mineral growth and directly promoting benthic carbonate mineral sheet formation
130 (Kempe et al., 1991; Reid, et al., 2000; Bosak et al., 2012; Petroff et al., 2013).

131 Here we provide new *in situ* macro- to nano-scale evidence for the participation of
132 microbes in the construction of Mono Lake chimneys, and argue that the location and
133 morphology of the Mono Lake tufa chimneys is a result of a complex interplay
134 between lake dynamics (faults, venting of sub-lacustrine springs, lake chemistry) and
135 the benthic microbial communities within the lake.

136

137 **METHODS**

138 **Fieldwork and sample processing**

139 Fieldwork around Mono Lake was undertaken in October 2014. Samples were taken
140 with permission of the Mono Lake State Natural Tufa Reserve following their
141 guidelines and under their supervision. It was a requirement of the permit that only
142 the minimum required number of naturally broken samples should be taken. Several
143 different occurrences of tufa carbonate rocks were examined around the lake, but the
144 most interesting were Pleistocene chimney structures close to Mono City (Fig. 1, Fig.
145 2A) that are the focus of this article. Possible microbial influence on construction of
146 these chimneys was noted in the field (Fig. 2), based on mat-like sheets that connected
147 chimney pipes (Fig. 2B). The locations of the chimneys as determined by GPS are
148 linearly arranged, presumably along a fault. Indeed faults mapped by Jennings et al.
149 (1977) run parallel to the line of the Pleistocene chimneys (Fig. 1). Further laboratory
150 work was aimed at determining whether the central chimney pipes were solely abiotic
151 or microbially influenced in origin. For this, a representative (naturally broken)
152 sample that was clearly from the centre of a chimney was sent to VU University
153 Amsterdam. Pipe material was cut up and pieces were consolidated by impregnation
154 with blue-stained epoxy resin so that several thin-sections could be made. Very high
155 abundance of organic filaments was apparent in each of these pipe wall thin-sections
156 (described and illustrated further below). A large number (>10) of thin sections of this
157 pipe material were optically examined and then sent to the Centre for Microscopy,
158 Characterisation and Analysis at the University of Western Australia. One
159 representative thin-section was selected for NanoSIMS, FIB-SEM and TEM analyses
160 to test (1) whether the filaments were genuine microfossils, and (2) to determine

161 spatial relationships between cell walls and minerals, which might help to determine
162 whether these purported microbes influenced chimney growth form.

163

164 **XRD analysis**

165 Three sub-samples of the same specimen from which the thin-sections were produced
166 were crushed for XRD analysis at the University of Hull. X-ray powder diffraction
167 data were collected from ground samples mounted in stainless steel sample holders.
168 Analysis was performed on a PANalytical Empyrean X-ray diffractometer (XRD)
169 operating in Bragg-Brentano geometry using copper $K\alpha_1$ radiation ($\lambda = 1.540546$
170 Å), and a PIXEL detector. Each data set was the sum of three identical data
171 collections with $4 \leq 2\theta /^\circ \leq 100$, a step size of 0.02626° , and counting time 1140
172 s per step.

173

174 **Biomarker analysis**

175 Biomarker analysis was conducted on a further off-cut of the Pleistocene tufa
176 chimney specimen used for XRD analysis and microscopy. This was done in the
177 organic geochemistry laboratory at the University of Aberdeen. The sample was first
178 cleaned with Dichloromethane. It was then crushed and Soxhlet extracted using a
179 Dichloromethane/Methanol mixture (93:7). The extract was analysed on an Agilent
180 6890GC with an Agilent 5975MS. The GC column was a 30m long * 0.25mm i.d. *
181 0.25um film thickness GC-5 column. The gas chromatography temperature
182 programme was 60°C for 2 minutes, heating at 20°C per minute up to 120°C , then at
183 4°C per minute up to 290°C , and holding for 27.5 minutes.

184

185 **Focussed ion beam (FIB) preparation of TEM samples**

186 Prior to FIB preparation, resin-embedded polished geological thin sections were
187 examined by optical microscopy, using *Zeiss Axioskop 2 and Leica DM2500M*
188 microscopes, plus scanning electron microscopy (SEM), using a *FEI Verios XHR*
189 SEM, in order to gain an understanding of filament distributions and morphologies,
190 and to select the most appropriate targets for detailed study. A dual-beam FIB system
191 (*FEI Helios NanoLab G3 CX*) at the Centre for Microscopy, Characterisation and
192 Analysis, University of Western Australia, was then used to prepare ultrathin TEM
193 wafers from the thin sections coated with c. 20 nm of gold. Electron beam imaging
194 within the dual beam FIB was used to identify previously mapped microstructures of
195 interest in the thin sections allowing site-specific TEM samples to be prepared. The
196 TEM sections were prepared by a series of steps involving different ion beam
197 energies and currents (see Wacey et al., 2011 for details); after initial thinning to c. 1
198 μm the wafers were extracted using an *in-situ* micromanipulator and welded onto
199 *PELCO* FIB-lift-out Cu TEM grids. Final thinning to c. 150 nm was then done *in situ*
200 on the grid using lower beam currents. FIB preparation of TEM sections allows
201 features below the surface of the thin sections to be targeted, thus eliminating the risk
202 of surface contamination producing artefacts. Distinction between the epoxy resin
203 used in sample preparation and other organic materials was possible via NanoSIMS
204 ion mapping (see below).

205

206 **TEM analysis of FIB-milled wafers**

207 TEM data were obtained using a *FEI Titan G2 80-200* TEM/STEM with *ChemiSTEM*
208 *Technology* operating at 200 kV, located within the Centre for Microscopy,
209 Characterisation and Analysis, University of Western Australia. Data obtained
210 included bright-field TEM images, HAADF (high angle annular dark-field) STEM

211 images, EDS (*ChemiSTEM*) maps, and selected area electron diffraction patterns for
212 mineral identification.

213

214 **SEM-EDS**

215 SEM-EDS was performed on the *FEI Helios Nanolab G3 CX* instrument at the
216 Centre for Microscopy, Characterisation and Analysis, University of Western
217 Australia which is equipped with an *Oxford Instruments X-Max 80* energy dispersive
218 X-ray spectroscopy (EDS) system and *Oxford Instruments AZtec 3.0* nano-analysis
219 software. All analyses were performed on FIB-milled faces below and perpendicular
220 to the surface of the thin sections to avoid surface contamination.

221

222 **NanoSIMS ion mapping**

223 NanoSIMS ion mapping was performed using a *CAMECA NanoSIMS 50* at the
224 Centre for Microscopy, Characterisation and Analysis, University of Western
225 Australia, with instrument parameters optimized as described in Wacey et al., 2011.
226 Analysis areas varied from 22 x 22 μm up to 35 x 35 μm , at a resolution of 256 x 256
227 pixels (each pixel measuring between 86 nm and 137 nm, depending on the size of the
228 area imaged). Dwell times were 30 ms per pixel with a beam current of c. 1.3 pA
229 ($D1=3$). Secondary ions mapped were $^{16}\text{O}^-$, $^{24}\text{C}_2^-$, $^{12}\text{C}^{14}\text{N}^-$, $^{28}\text{Si}^-$, $^{24}\text{Mg}^{16}\text{O}^-$ and
230 $^{56}\text{Fe}^{16}\text{O}^-$, and charge compensation was achieved by using the electron flood gun. In
231 all cases, regions c. 2-5 μm larger than the intended analysis area were pre-sputtered
232 with the primary ion beam (using c. 17 pA beam current; $D1=1$) to $> 5 \times 10^{16}$
233 ions/ cm^2 in order to remove surface contamination, implant Cs^+ ions and reach a
234 steady-state of ion emission. NanoSIMS data presented were produced in one session,
235 but to enable measurement of six different ions with the *CAMECA NanoSIMS 50* each

236 area was analysed twice, with one detector retuned between analyses. For each area,
237 analysis one was O, C, CN, Si, FeO, and analysis two was O, C, CN, MgO, FeO.
238 Differences in the relative intensities of the $^{24}\text{C}_2^-$ versus $^{12}\text{C}^{14}\text{N}^-$ maps are partly due to
239 the higher ionization potential of the secondary ion $^{12}\text{C}^{14}\text{N}^-$; here we report only the
240 $^{12}\text{C}^{14}\text{N}^-$ maps because of the higher secondary ion yield and the fact that $^{24}\text{C}_2^-$ is also
241 found in the carbonate minerals surrounding the organic material. $^{12}\text{C}^{14}\text{N}^-/^{24}\text{C}_2^-$ ratios
242 were used to identify epoxy resin introduced during sample preparation based on
243 previous data showing that the epoxy possesses significantly lower $^{12}\text{C}^{14}\text{N}^-/^{24}\text{C}_2^-$ than
244 that of biological cell walls or potential extracellular organic material (Wacey et al.,
245 2010).

246

247 **RESULTS**

248 Our study aimed to determine whether Mono Lake chimneys are purely physico-
249 chemical constructions, or whether microbes influenced their development. We focus
250 on chimneys found north of the present lake at Mono City, on a Pleistocene lake
251 terrace (Zimmerman et al., 2011) at around 2065 m altitude, extending along a 2.25
252 km long N to NNE oriented line interpreted as a fault trace (Fig. 1). Latitudes and
253 longitudes of the studied chimneys are provided as supplementary information. The
254 late Pleistocene Mono City chimneys reported and described here are older than the
255 more commonly studied and illustrated mounds of presumed Holocene and younger
256 age found close to the modern shoreline in the south east (South Tufa) and north east
257 (Boardwalk area) of the lake (Fig. 1). A sample of a Holocene fallen block from the
258 Lee Vining area on the southwest shore of the modern lake was also analysed by
259 XRD for comparison to the Pleistocene materials.

260

261 The Mono City chimneys vary from c.3 to 4 m in height, and from c. 1.5 to 3 m in
262 width (Fig. 2A). Internally the chimneys are constructed of stacks of numerous
263 calcitic cones or pipes, each 30 to 60 cm in height and around 3 cm in width (Figs.
264 2A-C). Each pipe has a central 1 cm-sized void or conduit (Figs. 2B, 3A, 3B). The
265 outsides of these pipes are commonly coated in botryoids of calcite (confirmed by
266 XRD) that range from 0.5 to 3 cm across (Fig. 2C). Sub-horizontal 15 cm-thick
267 laminated calcitic sheets were observed between the pipes, binding them together
268 (Fig. 2B). Each chimney was found rooted in calcite-cemented lake sediment ('sand
269 tufa'). Externally, the chimneys were encrusted in 20 to 50 cm thick blankets of
270 centimetre-scale mesh-like networks of euhedral pseudomorphs after the low
271 temperature hydrated CaCO_3 mineral ikaite, locally known as 'thinolite tufa' (Russell,
272 1889; Shearman et al., 1989) (Figs. 2A, 2D). Some of these pseudomorphs after ikaite
273 are found on individual pipes that make up the chimneys, but only on outer pipe
274 surfaces, which would have been exposed to lake water after the chimney had formed.
275 Individual pseudomorphs of ikaite crystals in these 'thinolite' blankets mostly
276 measured around 5 cm in length, and crystals clustered together to form rosettes (Fig.
277 2D).

278

279 **Petrography of chimney pipes**

280 The walls of the c. 3 cm wide chimney pipes are mostly 0.5 to 1 cm thick, constructed
281 of columns of calcite that grew radially around the c. 1 cm wide central conduit (Fig.
282 3A-C). In places the calcite crystal columns that make up the pipe walls were
283 cemented together, particularly towards the outside of the pipes. In zones closer to
284 pipe conduits, however, the microsparry calcite columns were separated from each
285 other by elongated voids running parallel to the column long axis (Fig. 3A-B). Calcite

286 crystal microspar columns may also branch and fan outwards resulting in millimetre
287 to centimetre scale shrub-like morphologies (Fig. 3D). When cut perpendicular to the
288 crystal growth axes these radiating calcite fans appeared sub-circular. Between some
289 of the pipes, fissure fills of clastic sediment were present.

290

291 **Microfossils within chimney pipes**

292 Clusters of filaments, observed in thin-sections under the optical microscope, are a
293 major component of the chimneys: they are ubiquitous within the columnar to shrub-
294 like calcite growths that make up the pipe walls (Fig. 4A-C), with many thousands of
295 specimens in a single thin section. Many of these filamentous structures were found
296 rather randomly oriented, though others were oriented approximately perpendicular to
297 outward-radiating sub-crystal boundaries (Fig. 4C).

298

299 Filaments may be divided into two main types, found together in the same thin-
300 sections, occupying the same niche. The most common (Type 1) have diameters in the
301 range of c. 0.8 to 8 μm with modal peaks at c. 1 μm and c. 2.5 μm ($n > 500$) (Fig. 4D,
302 E, H). Type 1 filaments tend to be dark in colour, suggesting high organic content and
303 poorly mineralised interiors. The Type 1 assemblage is dominated by empty sheaths
304 (Fig. 4A, H), although occasionally trichomes, some with putative segmentation, can
305 be recognised (Fig. 4D). Type 2 filaments have diameters in the range of c. 10 to
306 14.5 μm ($n > 100$), and comprise well-preserved trichomes with clear interior
307 mineralisation. Trichome segmentation is present in almost all cases (Fig. 4B, E-G)
308 and potential remains of cell contents are sometimes observed (Fig. 4G). Type 2
309 filament sheaths are either completely mineralised or were absent. Coccoids were also
310 identified, ranging from 6.8 to 15 μm diameter ($n = 25$), but these were much rarer

311 than filaments in the thin-sections examined (Fig. 4I). The morphologies of the
312 filaments, including their size, segmentation, and cases of trichomes inside sheaths,
313 are consistent with their being fossils of cyanobacteria, as are found within modern
314 Mono Lake spring systems (Kulp et al., 2008). Biomarker data reinforces this
315 interpretation (see below and Fig. 5). The microfossils are almost all in the inclusion-
316 rich calcite of the chimney pipe walls, being rare to absent in the optically transparent
317 calcitic pseudomorph after ikaite overgrowths of the thinolite blanket (Fig. 3E).

318

319 **Tufa and microfossil chemistry**

320 Bulk rock mineralogy was determined by XRD, with GC-MS for biomarkers of
321 organic carbon entombed within the rock. The chemistry and ultrastructure of the
322 filamentous microfossils and surrounding minerals were further investigated using a
323 combination of nano-scale secondary ion mass spectrometry (NanoSIMS), scanning
324 electron microscopy (SEM) and transmission electron microscopy (TEM).

325

326 The XRD data showed ~99% of chimney pipe rock to be calcite with an average
327 magnesium content of 8.1(1) %. The remaining ~1% constituent of the rock was
328 quartz (Fig. S1A-C). The Pleistocene Mono City chimneys are therefore significantly
329 different in mineralogy from the younger, aragonitic, Holocene tufa mounds (Fig.
330 S1D).

331

332 The hydrocarbon lipids obtained from a solvent extract of the Mono City chimney
333 specimen (Fig. 5) are similar to those of Arp et al. (1999) reported for Pyramid Lake
334 and also to those found in endolithic and mat-forming cyanobacteria reported by
335 Parnell et al. (2007). Typical features found in these stressed environments include a

336 predominance of *n*-C₁₅ to C₁₈ *n*-alkanes with a maximum at C₁₇, plus abundant
337 monomethylalkanes and hydrocarbon hopenes such as diploptene (Fig. 5). More
338 generally it has been known for some time (Han et al. 1968; Thiel et al. 1997) that
339 abundant *n*-C₁₇ and monomethylalkanes (Shiea, 1990), can be found in photosynthetic
340 cyanobacteria. This is entirely consistent with the specimen petrography.

341

342 Filaments identified in the chimney pipe wall thin-sections are highlighted
343 particularly well in carbon and nitrogen NanoSIMS ion maps, with trichome
344 segmentation in Type 2 filaments notable in several cases (Fig. 6). Carbon and
345 nitrogen are also frequently found with a very patchy distribution in places outside the
346 trichomes (Figs. 6-9) and are here interpreted as degraded extracellular organic
347 material (EOM) from cyanobacterial sheaths and biofilms. This preserved 'EOM' can
348 be distinguished in the NanoSIMS data from potential epoxy resin contamination as
349 the latter lacks nitrogen, so its ¹²C¹⁴N⁻²⁴C₂⁻ ratio is significantly less than the
350 ¹²C¹⁴N⁻²⁴C₂⁻ ratios for cell walls or potential EOM (Fig. S2). Alternatively this
351 organic carbon could be interpreted as escaped cell contents or degrading cell
352 walls, but an EOM interpretation is the most parsimonious explanation.

353 NanoSIMS, SEM-EDS and TEM-EDS maps of Si show abundant silicate nanograins
354 surrounding many of the Type 1 and Type 2 filaments, spatially associated with the C
355 and N (detected by NanoSIMS and/or TEM) that can be interpreted as fossilised EOM
356 (Fig. 6B-C, E-F, H-I; Fig. 7C, I-L; Fig. 8B; Fig 9). These silicates have rather variable
357 compositions and include (in order of abundance) quartz, K-Na-rich aluminosilicate
358 (cf. alkali feldspar), plus Fe and Mg rich aluminosilicates (cf. chlorite group) (Fig. 8-
359 9). Silicates were only exceptionally rarely found in the interior of the filaments, and
360 in these few cases they were very close to the cell wall. In most observed cases

361 filament interiors were filled solely with calcite. In one examined Type 2 filament, a
362 Mn-Fe-rich carbonate mineral was observed within and just exterior to a cell wall
363 (Fig. 7A, E-F), while in one examined Type 1 filament a Mn-Ti-Fe-rich carbonate
364 was observed partially replacing the filament wall (Fig. 9).

365

366 A rare sub-population of Si-rich grains were found in close association with organic
367 cell wall material; these comprise SiC and are laboratory contamination, having been
368 introduced during polishing of the thin sections. These can easily be discriminated
369 from true silicate minerals by their characteristic Si and C chemistry and lack of other
370 elements (e.g., O) found in silicate minerals (Fig. 6, white grains), and have been
371 eliminated from further discussions.

372

373 **3D microfossil morphology and mineral distribution**

374 A three-dimensional reconstructions of a Type 2 fossil filament was produced from
375 stacked SEM-BSE images captured during sequential focussed ion beam milling (see
376 materials and methods). The reconstruction, correlated with SEM-EDS and
377 NanoSIMS ion maps, reveals carbonaceous cell walls and cell dividing cross walls of
378 a filamentous cyanobacterium (Fig. 7A-C, black; Fig. 7D-L, brown). Some internal
379 cell contents are remarkably fossilised, preserved as organic carbon structures encased
380 in calcite (Fig. 7D-L, green). The reconstruction emphasises that the interior of the
381 cells are preserved entirely in calcite and lack inclusions of other grains. In one region
382 an external cell wall is directly associated with an Mn-Fe-rich carbonate mineral (Fig.
383 7E-F, H-L, blue). Silicate minerals are again shown to be spatially associated with
384 partially degraded EOM surrounding the cells (Fig. 7C, I-L, silicates in purple, EOM
385 in yellow).

386

387 **DISCUSSION**

388 **Temporal environmental changes**

389 Some aspects of the chimneys appear to be entirely abiotic in origin: for example, the
390 thinolite around the outside of the chimney and in places on the outsides of the central
391 pipes very likely formed via recrystallization of ikaite to calcite. This process is
392 accompanied by a significant volume decrease, explaining the extreme porosity of the
393 mesh-like thinolite networks (Shearman et al., 1989). That the ikaite formed a blanket
394 covering of the chimneys suggests a change in environmental conditions, probably
395 including a drop in lake water temperature, at some time following chimney
396 development (Shearman et al., 1989). Suggestions of temporal environmental changes
397 affecting carbonate mineral fabric are entirely consistent with the early observations
398 of Russell (1889) who first noted that thinolite was only found at higher elevations
399 around the lake.

400

401 A further environmental change is apparent in our new data: the dominance of high-
402 Mg calcite within the Pleistocene deposits of the Mono City chimneys contrasts with
403 aragonite (Fig. S1) and Mg-silicate (Souza-Egipsy et al., 2005) mineralogy of the
404 younger Holocene mounds. Unfortunately, it is not possible for us to conclusively
405 demonstrate co-occurrence of Mg-Si phases and aragonite because the samples we
406 analysed were free of this material, and Souza-Egipsy et al (2005) did not present
407 crystallographic data. Rather, they assumed that Ca, O, C phases identified by EDS
408 were low-Mg calcite: it seems likely that this was in fact aragonite. Calcite solubility
409 is known to increase as fluid Mg/Ca ratios are raised (Davis et al., 2000), such that
410 rising Mg/Ca in the lake water sometime after the Mono City chimneys had formed

411 could itself cause a change in the precipitating carbonate mineral from Pleistocene
412 calcite to Holocene aragonite. Mg/Ca in spring and runoff waters within the Mono
413 region range from 0.04 to 1, whereas lake waters are always >1 (Table 1) indicating
414 that Ca is consumed selectively over Mg. This scavenging of Ca will be inversely
415 proportional to the alkalinity (due to combined influence on the saturation product),
416 and therefore likely inversely proportional to the lake level via dilution. Enhanced
417 scavenging of Ca relative to Mg during lake lowstands will raise Mg/Ca during these
418 time intervals, favouring aragonite precipitation. Reduced incorporation of Mg into
419 aragonite due to very low $D_{\text{Mg-aragonite}}$ (Wassenburg et al., 2016) will cause a further
420 rise in the lake water Mg concentration, ultimately triggering precipitation of non-
421 carbonate Mg phases alongside aragonite. Hence, highstand high-Mg calcite
422 deposition and lowstand aragonite + Mg-silicate precipitation can be seen as an
423 inherent, thermodynamically controlled behaviour of Mono Lake, and similar hyper-
424 alkaline systems.

425

426 In contrast to the outer thinolite blanket, there is no petrographic evidence of any
427 recrystallization of the Pleistocene calcitic chimney pipe walls (Fig. 3). It is in these
428 un-recrystallised and earlier formed calcite pipe walls that filamentous inclusions are
429 found.

430

431 **Inclusions in the calcite**

432 Biomarkers, NanoSIMS, SEM- and TEM-EDS elemental maps show the filamentous
433 structures examined in pipe walls are clearly carbonaceous microfossils, while these
434 techniques combined with light microscopy observations, together show the detailed

435 spatial relationships between these fossil cyanobacterial filaments and calcite and
436 silicate minerals.

437

438 Several mechanisms are plausible to explain the observed arrangement of silicates
439 around filaments. First is an abiotic hypothesis, in which the microbes had no effect
440 on silicate mineral distribution. However, here we might expect that any partially
441 decayed cells would contain silicate grains that had been washed inside, and this was
442 not observed. Rather, it seems that the organic matter that we interpret as cell walls
443 and EOM was spatially linked to the locations of the silicate grains (e.g., Fig. 7). This
444 leaves us with two hypotheses to assess: 1) that silicates could have been precipitated
445 *in situ* via the metabolic activity of microbes (cf. Burne et al., 2014); or 2) they could
446 be fine detrital grains that have been trapped and bound by microbial EOM (cf. Reid
447 et al., 2000).

448

449 **Microbial precipitation of silicate precursors versus detrital silicate grains**

450 Authigenic microbial precipitation of silicates has recently been reported in a number
451 of alkaline lakes (e.g., Lake Satonda (Arp et al., 2003); Lake Clifton (Caselmann,
452 2005; Burne et al., 2014); Mexican Crater Lakes (Zeyen et al., 2015); Great Salt Lake
453 (Pace et al., 2016); Lake Thetis (Caselmann, 2005; Wacey et al., 2017)), where it has
454 been suggested that local increases of pH during oxygenic photosynthesis favour the
455 precipitation of Mg-Si phases in and around cyanobacterial sheaths and in webs of
456 EOM (e.g., Pace et al., 2016). In this scenario, carbonate minerals only precipitate
457 (and often replace Mg-Si phases) at a later stage when the activities of CO₃ and Ca
458 rise during heterotrophic degradation of cyanobacteria and their associated EOM.
459 However, the chemistry and distribution of silicates in those aforementioned lake

460 deposits are rather different to that observed in the calcitic Pleistocene Mono City
461 chimneys. Electron diffraction patterns of individual silicates around the Mono City
462 chimney filaments revealed compositions compatible with chlorite and feldspar (Fig.
463 9), while bulk-rock XRD also indicates minor quartz (Fig. S1). Few of the silicate
464 grains examined contained abundant magnesium and none were pure Mg-Si phases
465 such as the stevensite and kerolite phases previously reported from other alkaline
466 lakes (Burne et al., 2014; Zeyen et al., 2015). Intriguingly, the lack of Mg-Si phases
467 around the Pleistocene Mono chimney microbes is a notable difference from Mg-Si
468 mineralised EOM reported from sand-cementing tufa of the modern Mono Lake
469 shoreline (Souza-Egipsy et al., 2005).

470

471 It seems most likely that most if not all of the Pleistocene Mono City chimney
472 silicates were detrital grains. In support of this interpretation, we note that many
473 contained Al, which is often cited to indicate a detrital sediment component (e.g.,
474 Koning et al., 2002), and alkali feldspars cannot have formed *in situ* under lakewater
475 pressures. Furthermore, the very patchy isolated pattern of grain distribution and their
476 angular shapes (Figs. 6, 8-9) are in stark contrast to the generally massive nature of
477 Mg-Si precipitates found replicating entire cyanobacterial sheaths or large volumes of
478 thrombolites (Caselmann, 2005; Burne et al., 2014; Wacey et al., 2017) in other
479 alkaline lakes such as Lake Clifton and Lake Thetis in Western Australia.

480

481 While some authigenic chemical control of silicate precipitation by microbes, for
482 example by attraction of cations including Al, K and Si to negatively charged
483 compounds including uronic acids within the EOM (cf. Drews and Weckesser, 1982;
484 Saunders et al., 2014), cannot be ruled out, the mechanism that best explains the

485 morphologies, compositions and distribution of silicates in the Mono Lake chimneys
486 is via trapping and binding by microbial EOM (cf. Reid et al., 2000).

487

488 In summary, there is no clear evidence that the individual and rather isolated silicate
489 grains surrounding cyanobacterial filaments in the Mono Lake chimneys once formed
490 a substantial and coherent template that could have acted as a precursor to the
491 carbonate that is the dominant chimney constituent. That exceptionally preserved
492 fossil microbial filaments are found in chimney calcite that is Mg-Si poor
493 demonstrates that precursor silicate matrices are not prerequisites for cellular
494 preservation of microbial life in alkaline lakes. Rather, formation of calcite in the
495 Pleistocene Mono Lake (and elsewhere?) instead of coupled aragonite and amorphous
496 Mg-Si phases seen in the Holocene Mono Lake mounds may dominantly reflect lower
497 Mg/Ca ratios of the precipitating lake waters. Rising water levels in Mono Lake
498 resulting in falling Mg/Ca ratios would likely cause the system to revert to
499 precipitation of calcite, rather than aragonite and amorphous Mg-silicates.

500

501 **Intracellular carbonate minerals**

502 Carbonate minerals dominate the pipes making up the Mono Lake chimneys and on
503 the microscale are found both intracellularly and extracellularly in the examined
504 groups of filamentous microfossils. Intracellular calcification of cyanobacteria was
505 recently described from modern lacustrine microbialites of Lake Alchichica, Mexico
506 (Couradeau et al., 2012). This took the form of spherical granules of benstonite
507 $(\text{Sr}_1\text{Ba}_{2.7}\text{Mg}_{1.4}\text{C}_{10.9})\text{Ca}_6\text{Mg}(\text{CO}_3)_{13}$, averaging 270 nm in diameter that may have
508 nucleated on carboxysomes. The authors of that study suggested that excess alkalinity
509 produced during photosynthesis was trapped inside the cell by active precipitation of

510 benstonite and not exported beyond the cell wall. However, the mineral fill of the
511 fossil cyanobacteria of Mono Lake is calcite, which is neither rich in barium nor
512 strontium, and no evidence for spherical granules was observed. There is some
513 evidence of cell contents preserved within the cyanobacteria (Fig. 7) but this takes the
514 form of patchy degraded organic material and this likely represents the least labile
515 intracellular material. The cause(s) and timing of carbonate mineral growth within
516 such cells remains to be elucidated, though here could simply have been post-mortem
517 infiltration of the cell by a calcite-supersaturated fluid.

518

519 Manganese (+/- Fe and Ti) carbonate is rare but when present is an amorphous phase
520 based on electron diffraction, and distinctly associated with the cell wall itself (Fig.
521 7A, Fig. 9). This was true of both Type 1 and Type 2 filaments. Bacterial cell walls
522 are known to selectively accumulate Mn from surface waters (Konhauser et al., 1993).
523 Association of Mn with the cell wall and not just the EOM suggests the mineralisation
524 process involved more than a simple attraction of Mn cations to a negatively charged
525 surface. The difference in Eh-pH conditions between the inside of the cell and mixed
526 vent and lake waters outside the cell wall could explain this, as Mn^{2+} would be soluble
527 inside the relatively low pH cell, but Mn carbonates would precipitate out at the redox
528 boundary with high pH lake conditions (cf. Davison, 1993). This suggests that the
529 rather rare Mn carbonates could have formed early.

530

531 **Biological influences on chimney growth**

532 The majority of the calcite that makes up the chimneys developed outside the
533 microbial filaments. By binding calcium the EOM will initially have inhibited calcite
534 crystal formation, but on degradation this calcium will have been released (cf. Arp et

535 al., 1999; 2001), and critically, tubular sheets of EOM from microbes that inhabited
536 the zones around the rising vent waters will have been preferential sites of calcite
537 crystal nucleation. Once calcite crystals had formed in the EOM of these
538 mucilaginous, cohesive and quickly mineralised tubular sheets, there would be a
539 lower activation energy to deposit more mass on these developing crystals than to
540 generate new nuclei in the mixing water masses. Consequently, even if further
541 chimney wall crystal growth operated via a largely abiotic process, this latter
542 deposition could still be considered bio-influenced due to the control on location from
543 the presence of biofilm. It is tempting to speculate that the chimney form owes its
544 origins in part to the presence of a biofilm. The rising chimney would present a stable
545 substrate for microbial colonisation around likely the nutrient-rich (Table 1) and
546 relatively well illuminated waters. The result could be that tubular biofilm growth
547 around rising spring waters leads to the formation of carbonate chimney pipes, and in
548 turn chimney growth assists biofilm development around the rising spring waters.
549 Hence, the Mono City calcitic lacustrine chimneys seem broadly similar to other
550 lacustrine hot-spring carbonates (Arp et al., 1999), volcanic crater lake carbonates
551 (Arp et al., 2003; 2012; Kazmierczak et al., 2011), tufa barrages (Emeis et al., 1987),
552 chimney-like giant (40 m high) aragonitic microbialites of Lake Van (Kempe et al.,
553 1991), simpler structured carbonate mounds of other alkaline lakes like those of Inner
554 Mongolia (Arp et al., 1998) and the Ries Crater, Germany (Pache et al., 2001), as well
555 as siliceous lacustrine hydrothermal chimneys like those of Lake Taupo, New Zealand
556 (Jones et al., 2007) in that the *location* of the calcitic chimneys has a primary abiotic
557 control (fault controlled rising spring waters), but chimney *morphology* may well be
558 microbially mediated. Calcitic tufa chimney systems could therefore be added to the

559 list of macroscale products of local environmental chemistry, physics and
560 microbiology, with the biota exerting a strong control on fabric.
561
562 Support for microbial mediation of tufa chimneys comes from simpler structured tufa
563 mounds like those of nearby Pyramid Lake (Arp et al., 1999b) and Searle’s Lake,
564 California (Guo and Chafetz, 2012), where the mounds contain a macroscopic
565 columnar stromatolite component plus microscopic calcite cemented spheres, chains
566 of beads, rods, and filaments that strongly resemble bacteria (Guo and Chafetz, 2012).
567 These observations led Guo and Chafetz (2012) to conclude that microbially induced
568 calcification was the predominant process creating these deposits but they were
569 unable to demonstrate a role for EOM. Similar observations come from the alkaline
570 Lake Alchichica in Mexico where tubular chimney-like carbonate deposits (with
571 central conduits similar to the Mono Lake material described herein) occur side-by-
572 side with nodular and domal structures, all of which were interpreted as microbialites,
573 with some preserving remnants of filamentous and coccoid cyanobacteria
574 (Kazmierczak et al., 2011). These authors inferred that primary carbonate
575 mineralization was nucleated within EOM secreted by cyanobacterial biofilms.

576

577 **CONCLUSIONS**

578 **Summary model for chimney growth**

579 Our results show that the Mono City chimney pipes are packed full of fossil microbial
580 filaments, with rare coccoids. These might be interpreted as centimetre-thick tubular
581 (“rolled up”) and vertically stacked calcified microbial mats. Evidently there was
582 strong growth of a cyanobacterial biofilm around rising spring waters in the
583 Pleistocene Mono Lake, and these microbes influenced tufa chimney fabric

584 development at least at the nano- and microscales. Just exterior to the preserved
585 microbial cells, quartz and aluminosilicate grains are best taken as evidence that
586 ‘sticky’ EOM produced by cyanobacteria (and potentially other microbes) trapped and
587 bound some clastic lake sediment grains. We cannot rule out that some of the
588 aluminosilicates surrounding these filaments could have formed authigenically within
589 the EOM (cf. Pace et al., 2016), although the chemistry of these grains, together with
590 their angular shapes and isolated context in which several grains ‘float’ in a calcite
591 matrix, indicates these silicate grains are mostly detrital. A key finding is that Mg-Si
592 phases were absent here, so this phase cannot be seen as a prerequisite for exquisite
593 fossilisation of microbes in alkaline water settings (cf Souza-Egipsy et al., 2005;
594 Burne et al., 2014). Instead it seems likely that the calcitic nature of these Pleistocene
595 chimneys reflects a low lake water Mg/Ca ratio, arising from dilution during phases of
596 high lake level. Holocene aragonitic mounds coupled to Mg-Si phases (Souza-Egipsy
597 et al., 2005) can then be explained by a rise in the lake water Mg/Ca ratio through
598 time, and are a predictable feature of the thermodynamics of this lake and any similar
599 system.

600

601 Fossilisation of the microbes was likely related to the microbial EOM (cf. Arp et al.,
602 1999) in that acidic extracellular substances will have stripped calcium from the rising
603 vent waters, favouring calcite nucleation and subsequent organised calcite crystal
604 growth at the vent site. Calcite mineralisation of the EOM and intracellular
605 calcification were likely *post-mortem* processes (cf. Arp et al., 2001, 2012).
606 Adsorption of Mn to the organic carbon of the cell wall could also have happened
607 *post-mortem*, but petrography suggests this was an early process.

608

609 Summation of the evidence shows Pleistocene fossil tufa chimneys of Mono Lake are
610 not solely the result of abiotic mixing between calcium-rich spring waters and alkaline
611 lake waters (cf. Council and Bennet, 1993), although they were arranged linearly
612 along an apparent fault line, showing that water chemistry and tectonics controlled
613 chimney locations if not their fabrics. Rather, chimney fabric development was
614 influenced at least at the nano- to micro-scales by microbes that colonised the fertile
615 and relatively nutrient-rich vent sites. These findings have direct applicability to the
616 search for ancient and extraterrestrial microbial life.

617

618

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632

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835 *Earth Science* **3**, 64.

836

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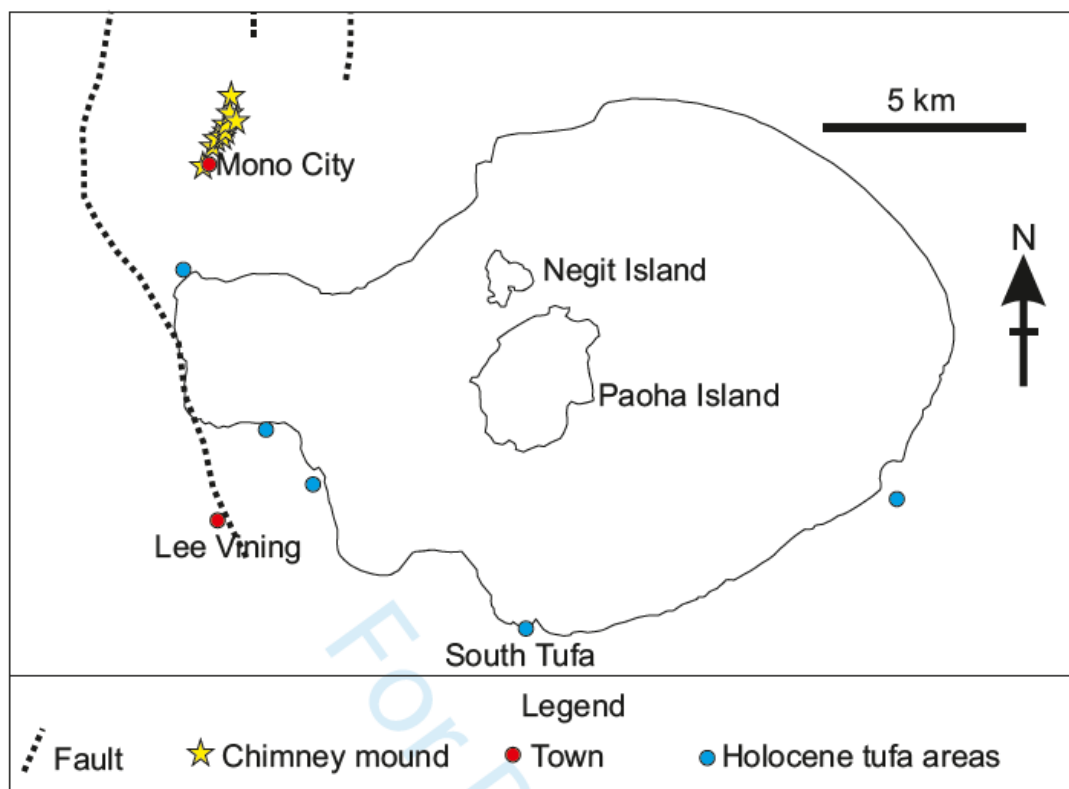
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839 Lake, California. *Geological Society of America Bulletin* **123**, 2320-2334.

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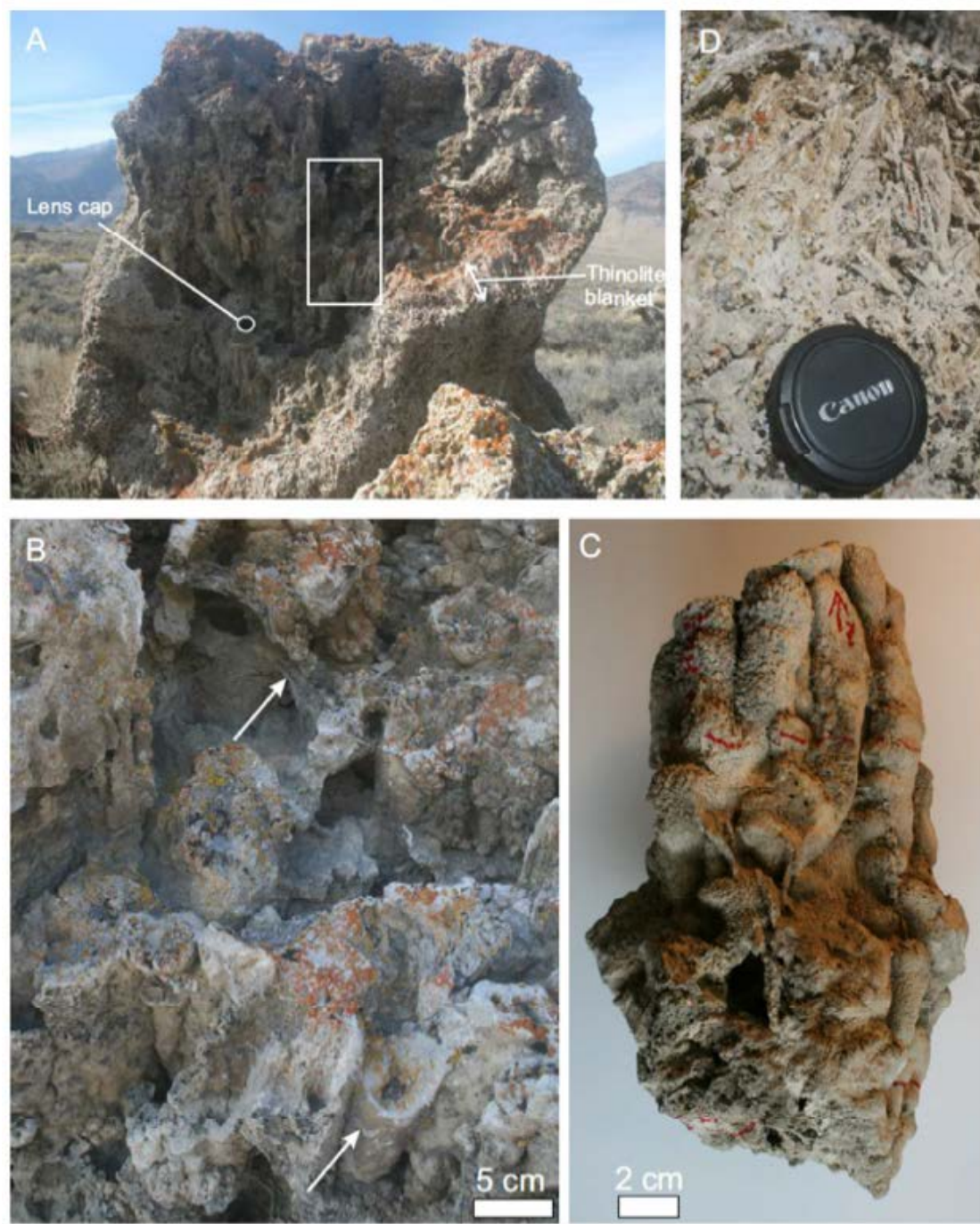
842 **FIGURE CAPTIONS**



843

844 **Fig. 1** Map of Mono Lake, California. Pleistocene chimney mounds of the Mono City
845 area described in this article are yellow stars in the northwest corner. Other areas
846 known for the more widely reported Holocene tufa carbonates are shown as blue
847 circles. Towns of Lee Vining and Mono City are shown as red circles. Scale bar is 5
848 km.

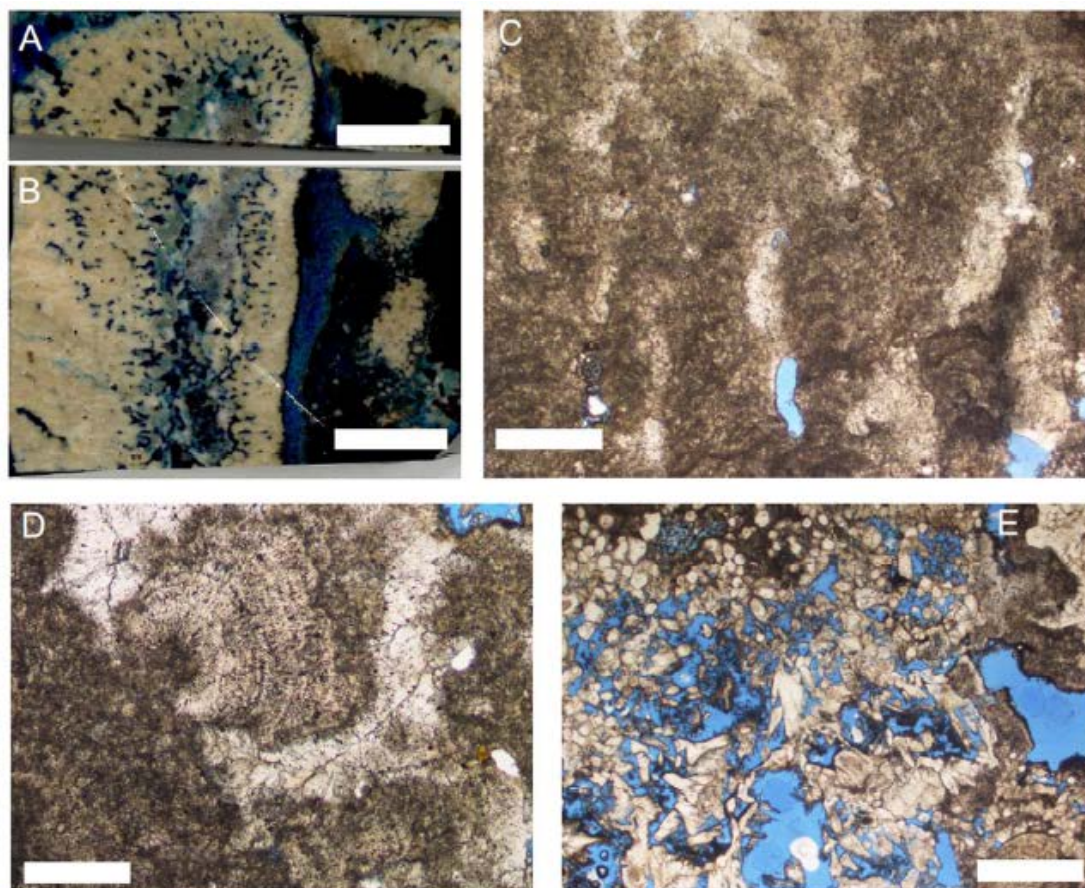
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850

851 **Fig. 2** Field images of Mono City tufa chimneys. (A) One of the chimneys standing
852 erect on former lake floor sediments and coated in a blanket of “thinolite”
853 (pseudomorphs after ikaite, labelled). Lens cap for scale is circled. (B) Close up of
854 pipes in the chimney from the boxed area in (A). Lower arrow points to the exterior of
855 one of the cylindrical pipes that makes up the chimney structure, and upper arrow
856 points to calcitic mat-like structure bridging between pipes. (C) Specimen of pipes on

857 which further micro-scale analyses were undertaken. (D) Close-up of thinolite blanket
858 on the exterior of one of the chimneys, showing these pseudomorphs after ikaite have
859 a very different appearance from the underlying chimney construction illustrated in
860 (B) and (C).
861



862
863 **Fig. 3** Petrography of Mono City chimneys. (A) Transverse cut and (B) longitudinal
864 cut through one cylindrical chimney pipe. The specimen was impregnated with blue
865 resin, so blue areas were void space in the rock. Axis of the pipe is the (blue resin
866 impregnated) vertical cavity to the left of centre of (B). Space between pipes is also
867 filled with blue resin. (C) Thin-section of pipe wall under plane polarised light
868 showing it comprises columns of laminated micritic calcite separated by partially
869 spar-filled voids (white where spar filled, blue resin where empty). (D) A shrub-like

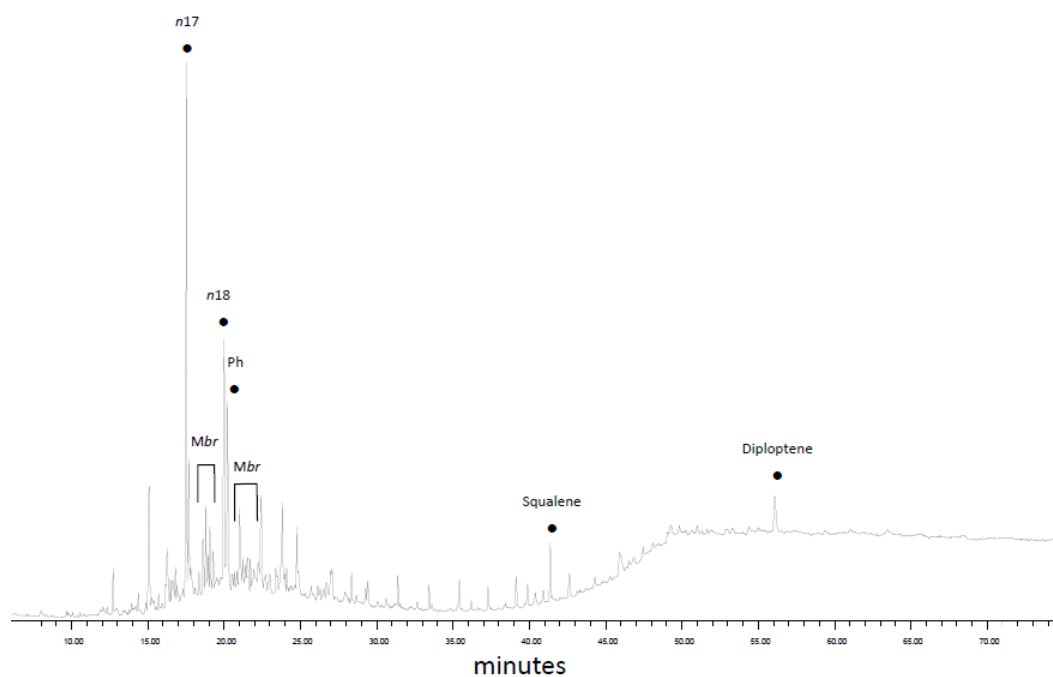
870 calcite microspar crystal fan, surrounded by clear white spar and nucleated on darker
871 micrite. The micrite and shrub-like fan in particular are full of dark inclusions
872 (microbial filaments). (E) Inclusion-free crystals best interpreted as pseudomorphs
873 after euhedral ikaite (Shearman et al., 1989), now calcite, are distinctively different
874 from the pipe wall material. The high porosity results from the significant volume
875 change on transformation from ikaite to calcite. Scale bars are 10 mm for (A-B), 1
876 mm for (C, E) and 500 μm for (D).
877



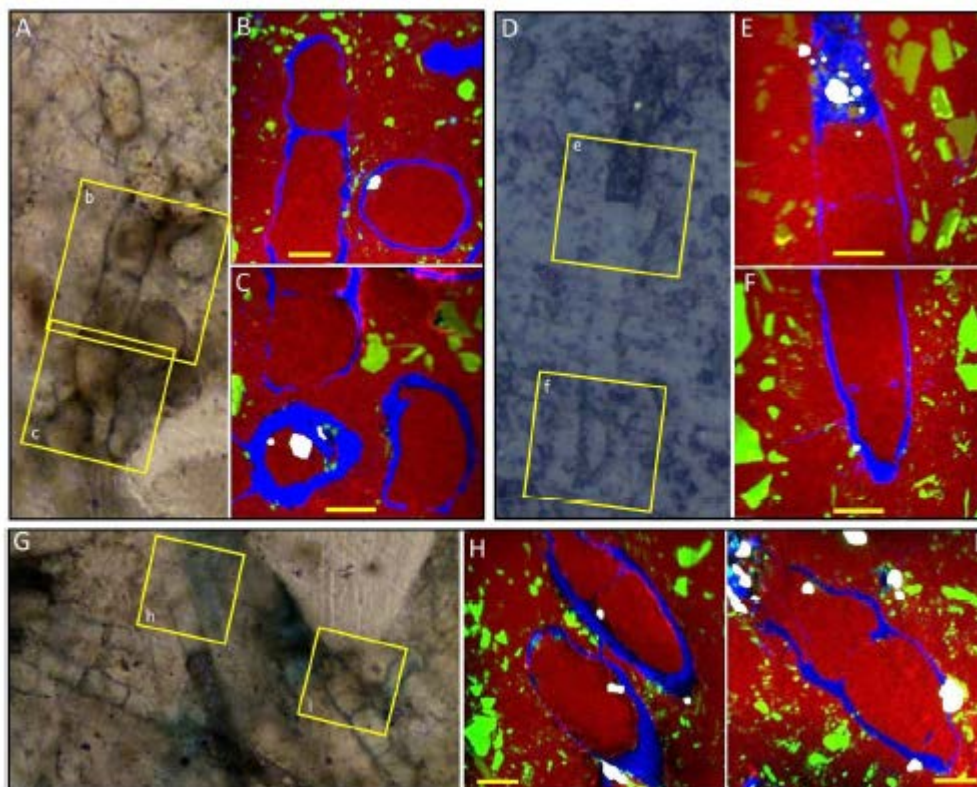
878

879 **Fig. 4** Fossilised bacteria within Mono Lake tufa chimneys. (A) Multiple type 1
880 filaments. (B) Cluster of approximately aligned segmented type 2 filaments. (C) Type
881 1 filaments aligned perpendicular to outward-radiating sub-crystal boundaries. (D)
882 Single Type 1 filament showing putative segmentation. (E) A type 1 and type 2
883 filament side by side. (F) Type 2 filament showing clear segmentation and potentially

884 partially surrounded by a mineralised sheath. (G) Type 2 filament showing
885 segmentation and potential remnants of cell contents. (H) Typical type 1 empty
886 sheaths. (I) Typical coccoid bacterium. Scale bars are 50 μm for (A-C) and 10 μm for
887 (D-I). Dashed black lines indicate transition between images taken at different focal
888 depths.
889



890
891 **Fig. 5** An 85 + 189 m/z ion chromatogram for a Mono City chimney pipe specimen.
892 $n17 = C_{17}$ n -alkane, Ph = phytane, Mbr = methylbranched alkane. This is consistent
893 with the presence of cyanobacteria entombed within the Mono City chimney pipes.
894



895

896 **Fig. 6** NanoSIMS analysis of Mono Lake tufa cyanobacteria and associated minerals.

897 Transmitted (A,G) and reflected light (D) images of several segmented filamentous

898 cyanobacteria from a Mono Lake tufa chimney pipe. In each case the areas analysed

899 by NanoSIMS are highlighted by the yellow boxes. (B-C; E-F; H-I) Four colour

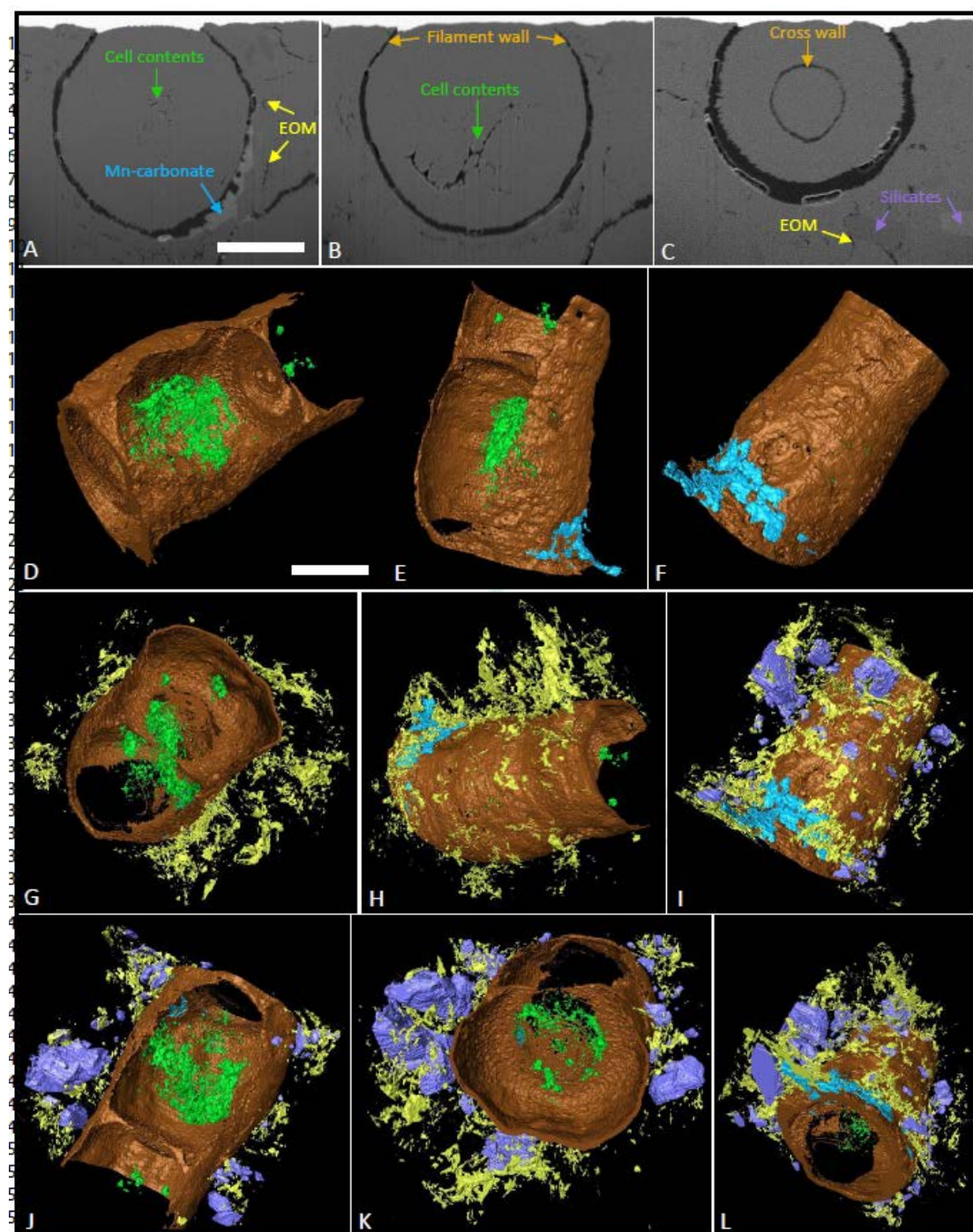
900 overlays of NanoSIMS ion images where blue represents organic material, green

901 represents silicate grains, red represents calcite, and white represents contaminant SiC

902 grains from polishing. Silicate grains are found surrounding, but very rarely within,

903 the cyanobacteria. Scale bars are 5 μm for all NanoSIMS images.

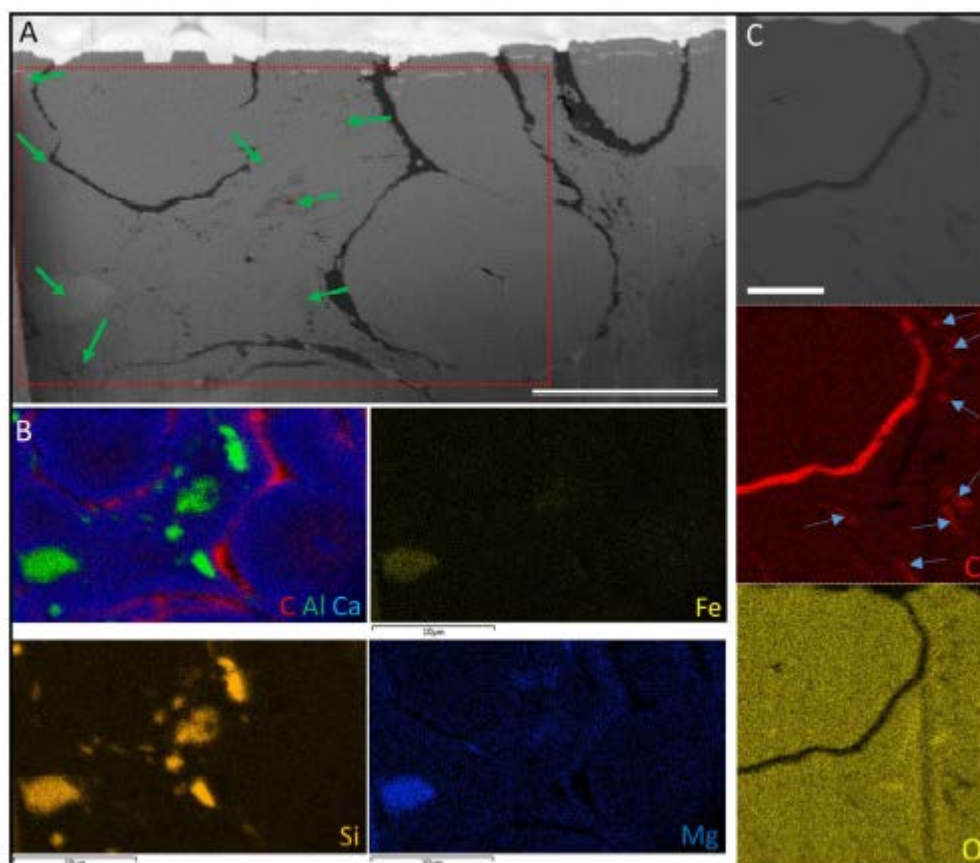
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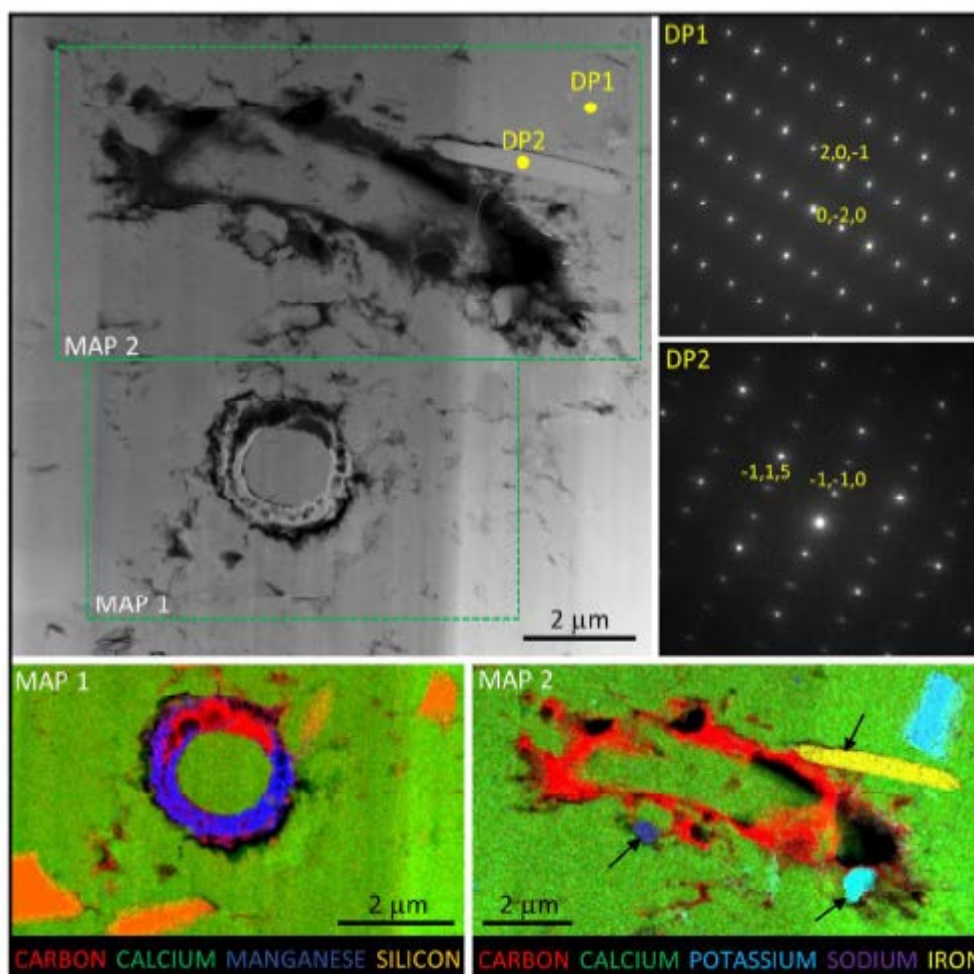
906 **Fig. 7** Three-dimensional analysis of fossilised cyanobacteria from Mono Lake tufa
907 chimney pipes. (A-C) SEM-BSE images of a series of cross sections through a
908 fossilised cyanobacterium. The different components of the cyanobacterium and
909 associated minerals are labelled (based on correlative evidence from EDS and
910 NanoSIMS maps), and the uniform mid-grey material making up most of the field of
911 view is calcite. (D-L) 3D reconstructions of the cyanobacterium and associated

912 minerals. Filament walls and cross walls are shown in brown, organic cell contents in
913 green, inferred extracellular polymeric substances (EOM) in yellow, silicate minerals
914 in purple, and Mn-carbonate in blue. The remainder of each field of view (here made
915 transparent black) is calcite. In reconstructions (D-F) only cell walls, organic cell
916 contents and the Mn-carbonate mineral are shown for clarity; in (G-H) the EOM has
917 been added to the reconstruction; while in (I-L) all components are shown,
918 demonstrating the close spatial association of silicate minerals and EOM. Scale bar in
919 (A) is 5 μm and applies to (A-C), scale bar in (D) is also 5 μm and applies to (D-L).
920



921
922 **Fig. 8** SEM-EDS analysis of the distribution of silicates and organic carbon around
923 Mono Lake tufa cyanobacteria. (A) SEM-BSE image of a cross section through at
924 least three Type 2 cyanobacteria. Note organic cell walls (black) and wispy

925 extracellular organic material (EOM; also black). Red box shows area analysed in (B)
926 and green arrows point to examples of silicates. (B) SEM-EDS elemental maps of
927 carbon, aluminium and calcium (shown as RGB 3 colour overlay), plus iron, silicon
928 and magnesium showing the close association of organic material and various silicate
929 grains. Scale bars are 10 μm . (C) SEM-BSE image of a further Type 2
930 cyanobacterium with SEM-EDS elemental maps of carbon (red) and oxygen (yellow);
931 both the cell wall and the wispy black material seen in the BSE image have elevated
932 carbon contents (arrows) compared to the background carbonate mineral. Scale bar is
933 2 μm .
934



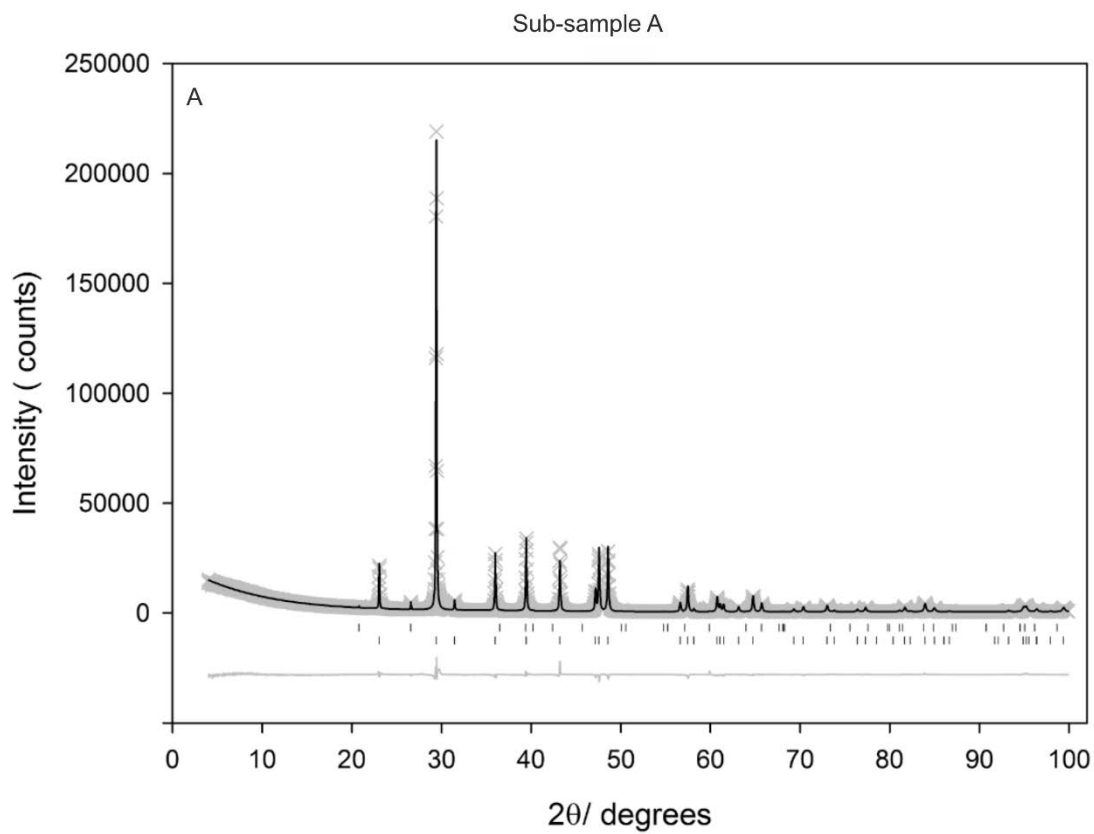
935

936 **Fig. 9** Chemistry of Type 1 filaments and surrounding minerals. High angle annular
 937 dark field scanning TEM image showing longitudinal and transverse sections
 938 respectively through two Type 1 filaments. The TEM-EDS elemental maps (bottom
 939 row) show that the filament walls and some extracellular material retain a
 940 carbonaceous composition (red). Some void space is present (black in these elemental
 941 maps) and this is not filled with epoxy resin. The lower filament is partly mineralised
 942 by an amorphous Mn-rich carbonate (dark blue; also containing minor Ti and Fe).
 943 Angular detrital aluminosilicate grains are closely associated with the filaments and
 944 some are attached to the outer walls or extracellular organics of the upper filament
 945 (black arrows). Aluminosilicate grains are of variable composition with Na-K-rich
 946 varieties plus Fe-Mg-rich varieties. Selected area electron diffraction patterns show
 947 that the Na-K-rich grains are alkali feldspar (DP1 is consistent with anorthoclase
 948 viewed down the [1,0,2] axis), while the Fe-Mg-rich grains are chlorite (DP2 is
 949 consistent with clinocllore viewed down the [-5,5,-2] axis).
 950

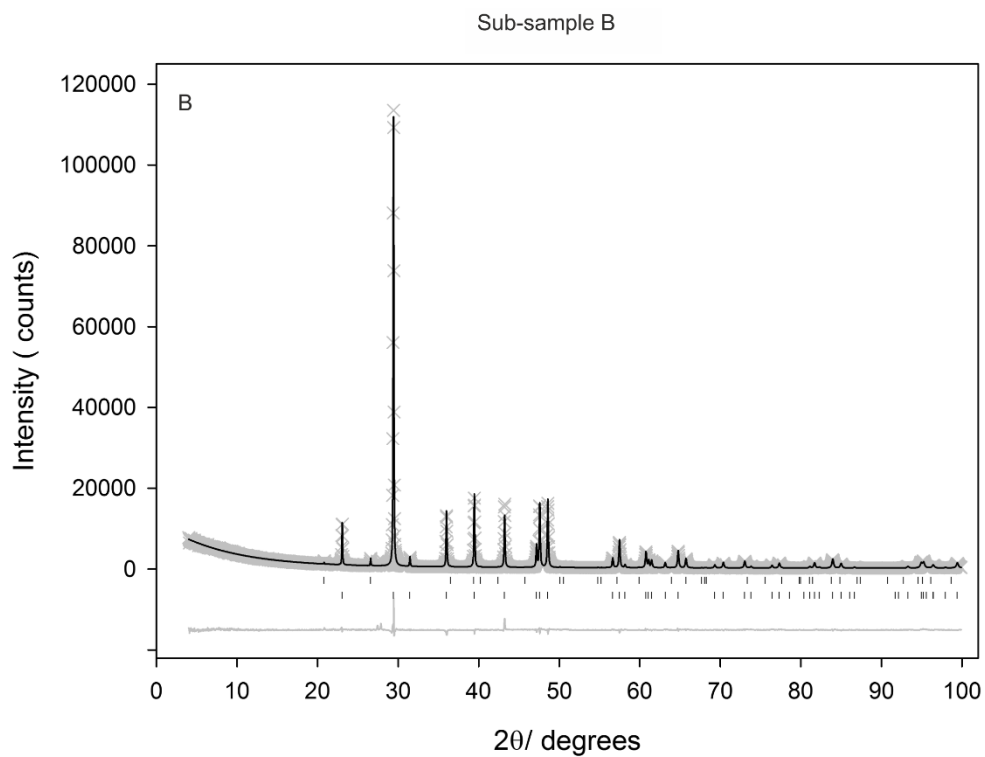
Sample	Al	Ba	Ca	Cu	Fe	K	Li	Mg	Mn	Na	S	Si	Sr
Dechambeau Hot spring	0.01 9	0.01 0	11.567	0.00 2	0.041	6.860	0.232	2.460	0.01 6	324.78 9	31.326	18.26 9	0.16 2
Lakeside Spring	0.08 4	0.00 1	2.238	0.00 2	0.036	17.584	0.254	0.587	0.00 1	189.07 2	17.976	5.272	0.00 5
Lakewater at island	0.17 4	0.00 7	3.316	0.00 3	0.064	1434.739	11.85 5	12.86 1	0.01 2		1656.13 8	4.917	0.02 1
Lakewater at tower	0.04 6	0.00 7	3.025	0.00 4	0.065	1383.753	11.65 3	12.58 5	0.01 1		1704.60 2	5.092	0.01 7
Lakewater site 2	0.19 8	0.00 8	3.378	0.00 5	0.066	1461.272	12.06 9	12.94 6	0.01 2		1659.95 5	4.995	0.02 1
Lee Vining Creek	0.04 6	0.01 1	7.916	0.00 4	0.124	0.964	0.000	0.565	0.00 7	2.911	2.171	2.308	0.04 0
Mill Creek	0.11 6	0.02 1	8.526	0.01 1	0.226	3.466	0.000	0.857	0.01 1	9.287	3.843	3.093	0.03 8
Naval Beach spring	0.07 0	0.37 2	86.349	0.00 3	1.178	29.358	2.237	57.63 4	1.07 0	305.56 1	12.326	32.43 7	0.47 3
Rush creek	0.02 5	0.00 6	6.707	0.00 2	0.101	0.567	0.000	0.655	0.00 5	3.056	1.618	2.011	0.03 0
Simon Spring 2	0.24 9	0.02 7	32.969	0.00 3	0.467	2.442	0.136	18.71 4	0.06 4	28.648	2.577	19.28 2	0.19 0
Simon Spring 3	0.03 4	0.01 6	22.401	0.00 0	0.045	7.964	0.221	22.58 0	0.00 5	40.587	3.974	18.25 3	0.17 3
South Tufa lakeside Spring	0.03 1	0.29 2	111.94 3	0.00 0	8.462	32.005	1.441	45.90 1	1.99 4	207.52 8	15.698	31.00 1	0.53 7
Warm spring near Dechambeau	0.07 5	0.02 2	17.041	0.00 3	0.187	2.017	0.000	0.680	0.02 2	4.892	4.265	1.397	0.05 2

951

952 **Table 1** –Trace element concentrations of waters found in and around Mono
953 Lake, California in October 2014
954

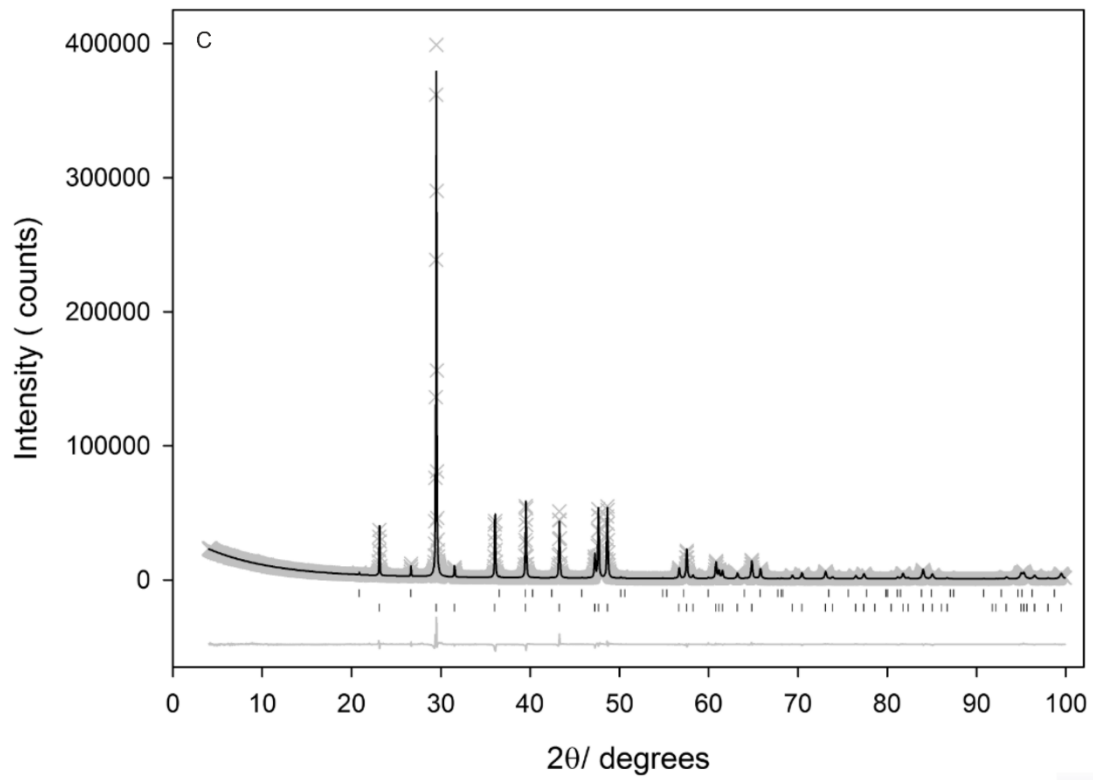


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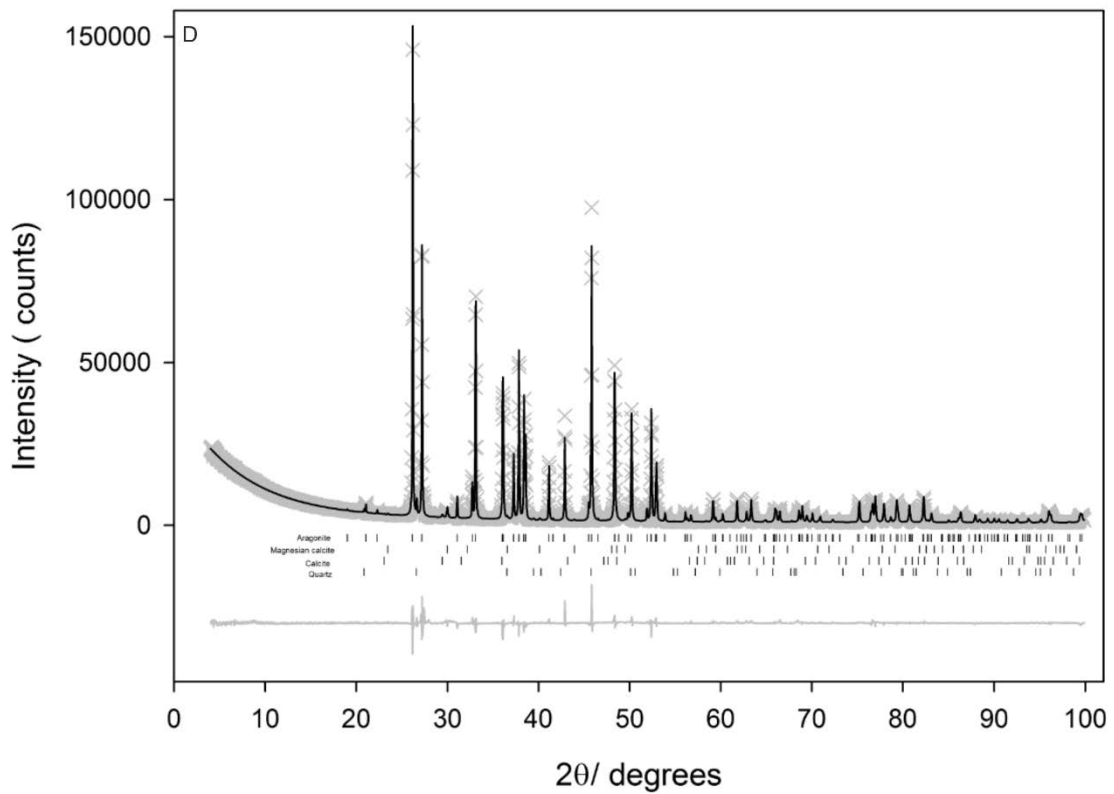
Sub-sample C



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Sample D



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960

961 **Fig. S1** Final Rietveld refinement profiles for XRD data from Mono Lake tufa
962 samples. Pleistocene chimney pipe samples (A-C) and Holocene tufa from Lee Vining
963 on the south shore of the modern lake (D) are shown. Plots show observed (grey
964 crosses), calculated (solid black line), and difference (grey line) X-ray powder
965 diffraction profiles for Mono Lake samples at room temperature. The upper tick
966 marks in A, B and C indicate positions of allowed reflections from the $K\alpha_1$ diffraction
967 from silica; the lower set mark allowed reflections from magnesian calcite. The three
968 independent patterns A, B and C are three different sub-samples from the same
969 chimney pipe specimen. Their average composition is 98.99(5) % magnesian calcite,
970 1.01(5)% quartz. The upper tick marks in (D) indicate positions of allowed reflections
971 from the $K\alpha_1$ diffraction from aragonite (highest tick marks); magnesian calcite;
972 calcite; and quartz (lowest tick marks). Holocene tufa from Lee Vining (D) contains
973 the following crystalline constituents by weight:

974 Aragonite 97.15(9) %;

975 Mg-calcite 1.50(11) % (Composition fixed at $Mg_{0.12}Ca_{0.88}CO_3$);

976 Calcite 0.42(4) %;

977 Quartz 0.93(5) %/.

978

979

980

981 **Magnesian calcite** space group R-3m, refined unit cell parameters

982 **Sub-sample A**

983 $a = b = 4.97864(7) \text{ \AA}$ $c = 17.0214(3) \text{ \AA}$ $\gamma = 120^\circ$ $V = 365.382(12) \text{ \AA}^3$

984 **Sub-sample B**

985 $a = b = 4.97800(6) \text{ \AA}$ $c = 17.0186(3) \text{ \AA}$ $\gamma = 120^\circ$ $V = 365.228(11) \text{ \AA}^3$

986 **Sub-sample C**

987 $a = b = 4.97813(5) \text{ \AA}$ $c = 17.0172(2) \text{ \AA}$ $\gamma = 120^\circ$ $V = 365.216(10) \text{ \AA}^3$

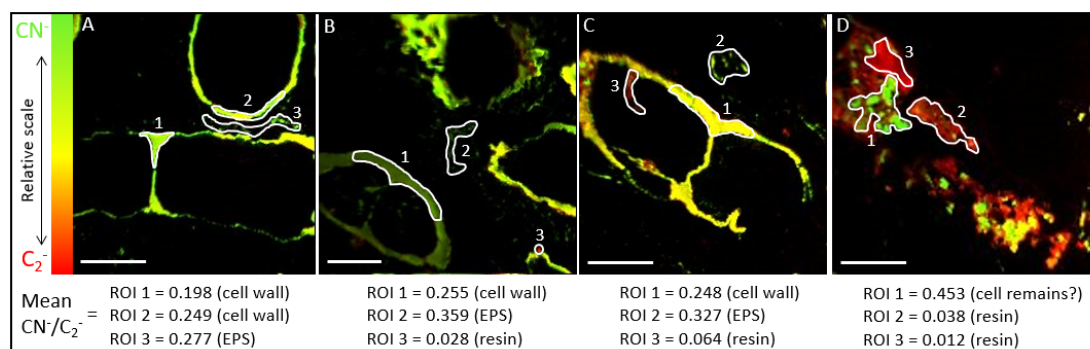
988 **Sample D**

989 $a = b = 4.9072(14) \text{ \AA}$ $c = 16.678(6) \text{ \AA}$ $\gamma = 120^\circ$ $V = 347.82(10) \text{ \AA}^3$

990

991 **Alpha quartz** space group P3₂21, refined unit cell parameters

992	Sub-sample A				
993	a = b = 4.9134(11) Å	c = 5.406(2) Å	$\gamma = 120^\circ$	V = 113.03(2) Å ³	
994	Sub-sample B				
995	a = b = 4.9123(11) Å	c = 5.408(2) Å	$\gamma = 120^\circ$	V = 113.03(2) Å ³	
996	Sub-sample C				
997	a = b = 4.97813(5) Å	c = 5.4056(13) Å	$\gamma = 120^\circ$	V = 113.032(14) Å ³	
998	Sample D.				
999	a = b = 4.9122(14) Å	c = 5.406(3) Å	$\gamma = 120^\circ$	V = 112.98(3) Å ³	
1000					
1001	Aragonite space group Pmcn, refined unit cell parameters				
1002	Sample D				
1003	a = 4.966617(5) Å	b = 7.96480(8) Å	c = 5.74961(6) Å	V = 227.423(5) Å ³	
1004					
1005	Calcite space group R-3m, refined unit cell parameters				
1006	Sample D				
1007	a = b = 4.984(5) Å	c = 17.009(19) Å	$\gamma = 120^\circ$	V = 366.0(4) Å ³	
1008					
1009					
1010	(Sub-sample A) Rietveld refinement details				
1011	Magnesian calcite 99.13(3)% by weight. (Refined Mg content 7.5(8) %)				
1012	Quartz 0.87(3)%				
1013	Quality of fit indicators: wRp = 0.0657 R(F ²) = 0.0534				
1014					
1015	(Sub-sample B) Rietveld refinement details				
1016	Magnesian calcite 99.08(3)% by weight. (Refined Mg content 7.4(8) %)				
1017	Quartz 0.92(3)%				
1018	Quality of fit indicators: wRp = 0.0662 R(F ²) = 0.0502				
1019					
1020	(Sub-sample C) Rietveld refinement details				
1021	Magnesian calcite 98.76(3)% by weight. Refined Mg content 9.4(7) %)				
1022	Quartz 1.24(3)%				
1023	Quality of fit indicators: wRp = 0.0581 R(F ²) = 0.0506				
1024					
1025	(Sample D) Rietveld refinement details				
1026	Quality of fit indicators: wRp = 0.0579 R(F ²) = 0.0542				
1027					
1028	$wRp = \left\{ \frac{\sum w_i (y_i(obs) - y_i(calc))^2}{\sum w_i (y_i(obs))^2} \right\}^{\frac{1}{2}} \text{ and } R(F^2) = \frac{\sum_{hkl} (F_{obs}^2 - F_{calc}^2)}{\sum_{hkl} F_{obs}^2}$				
1029					
1030					
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1032					
1033					
1034					



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1037 **Fig. S2** NanoSIMS CN^-/C_2^- maps demonstrating differential CN^-/C_2^- in cellular and
 1038 extracellular indigenous organics, plus epoxy resin. (A) Portions of two type 2
 1039 filaments (ROI 1 and ROI 2), plus patchy extracellular organics interpreted as EOM
 1040 (ROI 3) with relatively higher CN^-/C_2^- . (B-C) Examples of Type 2 filaments (ROI 1),
 1041 patchy EOM (ROI 2), plus zones of porosity within the thin section inferred to be
 1042 infilled by epoxy resin (ROI 3). Note how the resin has c. 5 to 13 times lower CN^-/C_2^-
 1043 than the EOM. (D) A degraded type 2 filament that has left a partial hole at the
 1044 surface of the thin section. The majority of this is infilled by resin with very low CN^-
 1045 $/\text{C}_2^-$ (ROI 2 and 3), while some remnants of the cellular material are also likely
 1046 present (ROI 1). Note that these maps are not quantitative, so the colour scale only
 1047 permits comparison of features within the same map, not with features across other
 1048 maps. Nevertheless, the same pattern emerges in all maps, with epoxy resin having
 1049 significantly lower CN^-/C_2^- than either the filament walls or the inferred EOM. Scale
 1050 bars are 5 μm .

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