

Chemical nature of the 3.4 Ga Strelley Pool microfossils

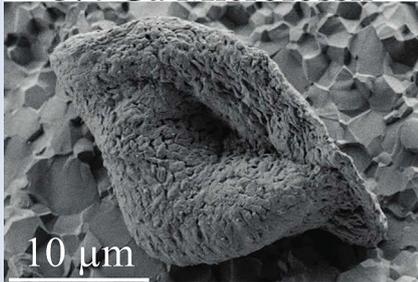
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Abstract

3.4 Ga Microfossil



The biogenicity of putative traces of life found in early-Archean rocks is strongly debated. To date, only equivocal lines of evidence have been reported, which has prevented a full consensus from emerging. Here we report elemental and molecular data from individual organic microfossils preserved within the 3.4 billion-year-old cherts of the Strelley Pool Formation, Western Australia. The present results support the growing body of evidence advocating their biogenicity, promoting them as the oldest known authentic organic microfossils. These microfossils consist of nitrogen- and oxygen- rich organic molecules that have been only slightly degraded despite experiencing temperatures of ~300 °C. Such molecular preservation emphasises the palaeobiological potential of the Earth's oldest geological record, whilst providing a promising window into the early biosphere.

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Letter

Recent studies have suggested that the biosphere had emerged approximately 4.0 billion years ago (Bell *et al.*, 2015; Dodd *et al.*, 2017), proliferating and evolving to a 'sophisticated' state by 3.7 Ga (Nutman *et al.*, 2016; Hassenkam *et al.*, 2017). These conclusions, however, are based on equivocal lines of evidence (e.g., ¹³C-depleted carbonaceous materials and/or mineral structures) that equally could have been the product of abiotic processes (Grotzinger and Rothman, 1996; Garcia-Ruiz *et al.*, 2003; McCollom and Seewald, 2006). The absence of indisputable traces of life in Earth's earliest rocks, specifically the paucity of authentic organic microfossils with biogenically and geologically consistent molecular structures, has prevented the community from reaching a consensus.

Unambiguous identification of organic microfossils in the early geological record has always been challenging (Schopf, 1975; Knoll, 2015). Quite illustrative is the long-standing debate about the origin of filamentous microstructures from the 3.5 Ga cherts of the Apex Basalt Formation (Pilbara Craton, Australia). There, the disputed microstructures have been interpreted as either remnants of Earth's oldest cellular life (Schopf and Packer, 1987; Ueno *et al.*, 2001; Schopf *et al.*, 2002, 2018) or as abiotic organo-mineral structures (Brasier *et al.*, 2002; Marshall *et al.*, 2011; Wacey *et al.*, 2016). Isotopic investigation aside (Ueno *et al.*, 2001; Schopf *et al.*, 2018), molecular appraisal of the biogenicity of the Apex microstructures has proven inconclusive, hindered because the analyses were performed on bulk powdered samples rather

than targeting the microstructures themselves (De Gregorio *et al.*, 2009).

To date, the oldest commonly accepted organic microfossils were found within the sedimentary cherts of the 3.4 Ga Strelley Pool Formation from the Panorama greenstone belt (Pilbara Craton, Australia). These cherts are thought to have formed through the crystallisation of colloidal silica that was deposited in a shallow water marine environment, with some hydrothermal inputs (Sugitani *et al.*, 2013). Since their formation, these cherts have only undergone low grade metamorphism (prehnite-pumpellyite to lower greenschist facies; Lepot *et al.*, 2013; Sugitani *et al.*, 2013).

The Strelley Pool microfossils display morphologically diverse microstructures (Wacey *et al.*, 2011a; Sugitani *et al.*, 2013), featuring ¹³C-depleted carbon (Lepot *et al.*, 2013), and are sometimes associated with pyrites that exhibit sulphur isotope signatures consistent with a biological origin (Wacey *et al.*, 2011b). Yet, as for the Apex cherts cited above, the only existing molecular insight into the Strelley Pool organic matter was obtained from powdered material rather than directly from the microfossils (De Gregorio *et al.*, 2011).

Here, following the methodology adopted for earlier studies (Benzerara *et al.*, 2005; Bernard *et al.*, 2007; Lepot *et al.*, 2008), we exploited advanced microscopy and spectroscopy techniques including Raman microspectroscopy, focused ion beam (FIB), scanning and transmission electron microscopy (SEM and TEM) and scanning transmission X-ray microscopy (STXM) coupled with X-ray absorption near edge structure (XANES) spectroscopy, to conduct spatially-resolved

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molecular investigations of the Strelley Pool microfossils at the submicrometre scale. N/C atomic ratio values were estimated following the methodology and calibration outlined in Alleen *et al.* (2015) and successfully used in Alleen *et al.* (2016a, 2017).

Consistent with previous observations of thin sections (Lepot *et al.*, 2013; Sugitani *et al.*, 2013; Fig. 1b, h), SEM observations reveal the presence of lenticular and film-like organic microfossils present on freshly fractured faces of the PAN1-1A chert sample (Fig. 1d–g, i). Raman data collected from each of the individual microfossils demonstrate the poorly ordered nature of the organic material that typifies these microfossils (Fig. 1j). Application of the RSCM (Raman Spectroscopy of

Carbonaceous Material) geothermometer (Beysac *et al.*, 2002; Lahfid *et al.*, 2010) indicates that the Strelley Pool microfossils experienced peak temperatures of approximately 300 °C, which is in good agreement with regional metamorphic histories (Wacey *et al.*, 2011a; Lepot *et al.*, 2013; Sugitani *et al.*, 2013). Transmission electron microscopy conducted on FIB foils demonstrates that, even at the nanometre scale, the organic matter is intimately associated with quartz and clays, as well as crystalline chlorides or sulphate minerals (Fig. 2). Assimilated, these observations confirm the syngenetic nature of the Strelley Pool microfossils, testifying that they were entombed within the chert at the time of its formation.

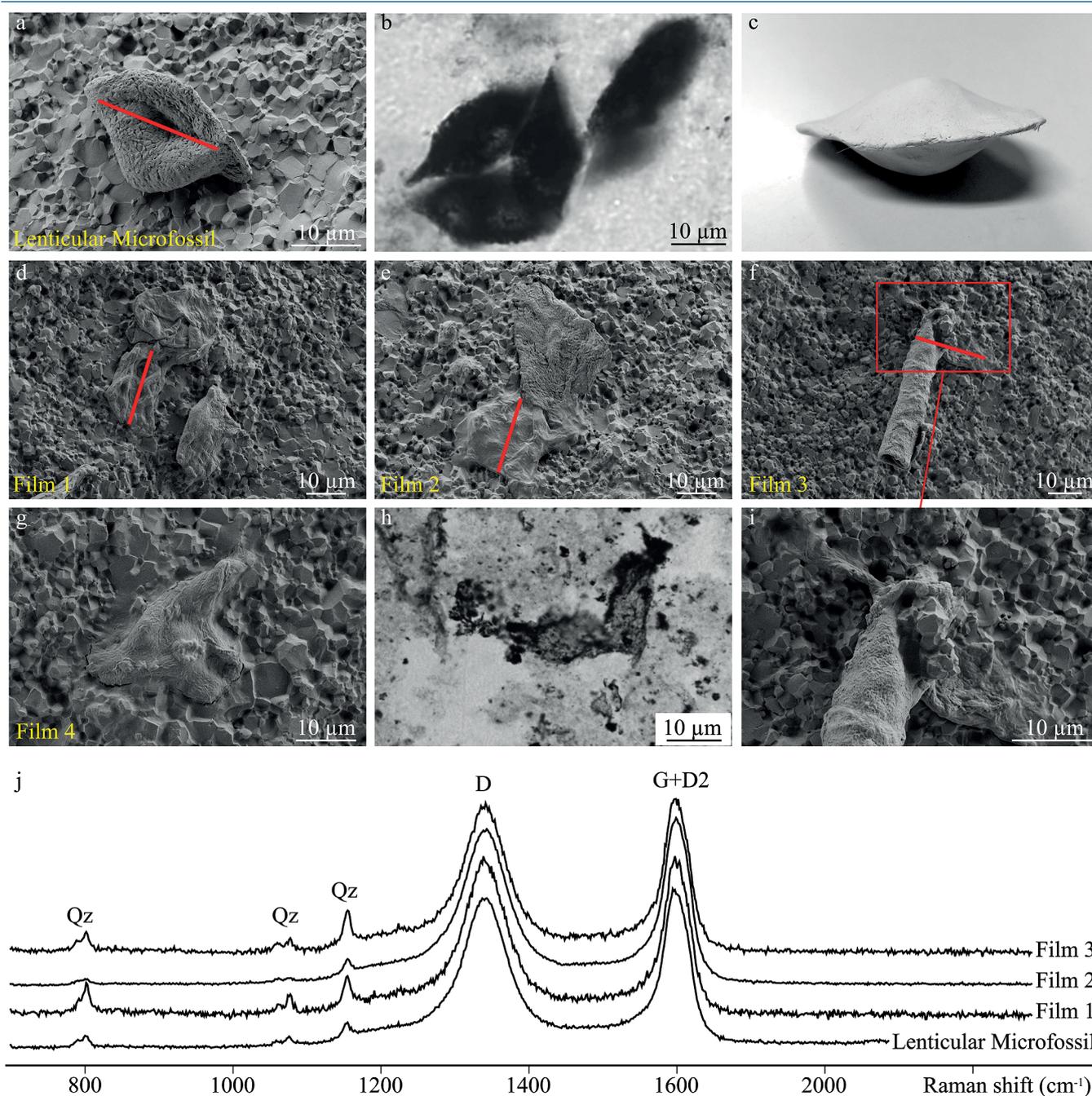


Figure 1 Optical photomicrographs, SEM images and Raman spectra of the 3.4 Ga Strelley Pool organic microfossils observed on a freshly fractured chert fragment. (a) SEM image of a lenticular Strelley Pool microfossil, (b) optical photomicrograph of Strelley Pool lenticular microfossils (Sugitani *et al.*, 2013) and (c) model morphological reconstruction in clay. (d–g) SEM images of Strelley Pool film-like microfossils, (h) optical photomicrograph of a film-like Strelley Pool microfossil (Sugitani *et al.*, 2013) and (i) an enlargement of the film-like microfossil shown in f that looks like a tubular structure. Red lines indicate where the FIB sections were extracted for TEM and STXM analyses (Film 1–4). (j) Raman spectra of the Strelley Pool organic microfossils. These spectra are typical of disordered carbon having experienced 300 °C, with the D and composite G + D2 bands displaying similar intensities. Qz corresponds to quartz.

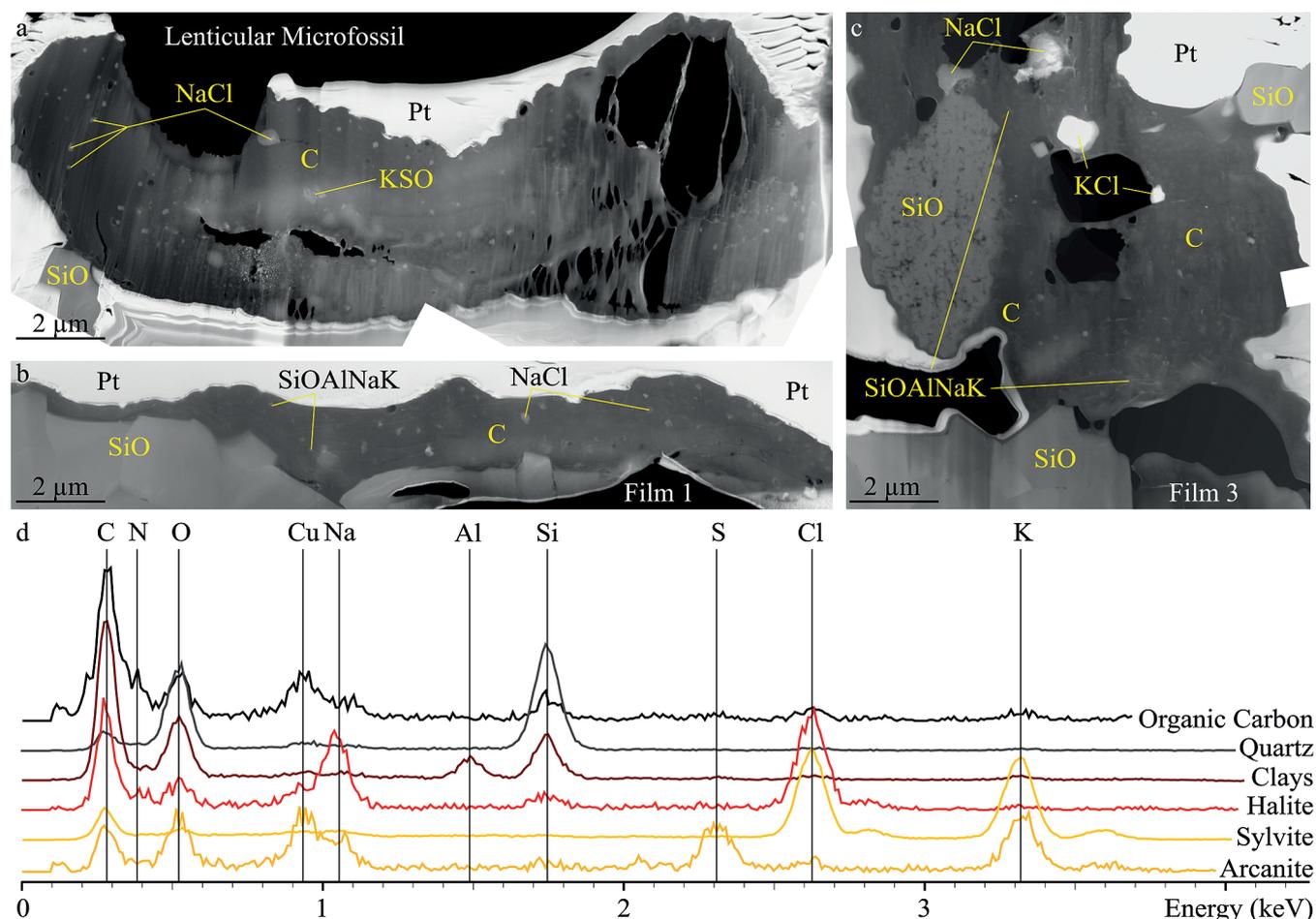


Figure 2 TEM analyses of the Strelley Pool organic microfossils. STEM image of the lenticular microfossil (a) shown in Figure 1a and of the two film-like microfossils (b–c) shown in Figure 1d and 1f, respectively. Organic carbon (which appears darker than mineral phases) is closely associated with micro-quartz, clays and crystals of chlorides and sulphates. The main chemical elements are indicated. (d) TEM-EDX spectra of the organic microfossils and the associated mineral phases.

Spatially-resolved chemical investigation exploiting STXM reveal that the Strelley Pool microfossils possess very high N/C values (0.24 ± 0.02 ; Fig. 3a). Although the microfossils are associated with potassium and calcium nitrates, these phases are only present in trace amounts and do not significantly contribute to the estimated N/C values (*cf.* Supplementary Information). The XANES spectra of the Strelley Pool microfossils resemble those reported from the best preserved microfossils found in the 1.9 Ga Gunflint cherts (Fig. 3b–c; Alleon *et al.*, 2016a). Here, the C-XANES spectra display multiple peaks: the intense peak at energies ranging between 288.2 and 288.6 eV, likely reflects a mixed contribution from amide ($(R_1, R_2)N-C=O$), carboxylic (COOH), ester (COOR) and/or acetal (C(OR)₂) groups. The peak at 289.5 eV signals the presence of hydroxyl (OH) groups. The peak at 286.7 eV reveals the presence of imine (C=N), nitrile (C≡N), carbonyl (C=O) and/or phenolic (Ar–OH) groups. Whereas, the much smaller peak centred at 285.1 eV signals the presence of aromatic and/or olefinic groups (De Gregorio *et al.*, 2011; Alleon *et al.*, 2017). Similarly, the N-XANES spectra display a broad absorption feature at 401.4 eV, which is related to the presence of amide, imine and/or nitrile groups. The peak at 399.9 eV and shoulder around 398.8 eV indicate the presence of nitrogen within aromatic moieties (Leinweber *et al.*, 2007; De Gregorio *et al.*, 2011; Alleon *et al.*, 2017). The two absorption features at 401.7 and 405.4 eV can be attributed to the presence of trace quantities of potassium and/or calcium nitrates (Leinweber *et al.*, 2007; Alleon *et al.*, 2016a). Altogether, consistent with previous claims (Lepot *et al.*, 2013; Sugitani *et al.*, 2013), the

spatially resolved chemical information reported here supports the biogenicity of the Strelley Pool microfossils, advocating them as the oldest currently known authentic microfossils.

The general palaeobiological perception is that organic diagenesis results in a convergence in the composition of organic materials from different sources, reducing the utility of chemical composition for discriminating between taxa (Briggs and McMahon, 2016). Yet, the lenticular and film-like Strelley Pool microfossils do not produce identical XANES spectra. The film-like microfossils contain slightly more abundant carboxylic, ester and/or acetal groups and fewer amide groups than their lenticular counterpart (Fig. 3). Recent laboratory experiments have demonstrated that thermal degradation of chemically distinct organic precursors can lead to chemically distinct residues (Alleon *et al.*, 2017). The spectral differences between the lenticular and film-like microfossils may thus signal differences in the initial molecular composition of organic material now composing the Strelley Pool microfossils. The data reported here therefore suggest inheritance from slight, yet morphotype-specific, differences in the initial proportions of proteins, sugars and lipids of the Strelley Pool microfossils.

The Strelley Pool microfossils investigated here exhibit high N/C values and contain amide functional groups despite a 3.4 Gyr geological history and heating episodes up to 300 °C, *i.e.* conditions that normally lead to intense chemical degradation (Bernard and Horsfield, 2014; Briggs and Summons, 2014). Such a high level of molecular preservation was achieved likely because of their early silicification. A similar mechanism was proposed for the chemically well preserved 1.9 Ga Gunflint

microfossils that underwent temperatures up to 150–170 °C (Alleon *et al.*, 2016a). Consistently, recent laboratory experiments demonstrated that early entombment in colloidal silica significantly reduces thermally induced molecular degradation of microorganisms, even if the silica ends up being completely converted into thermally stable quartz (Alleon *et al.*, 2016b).

Surprisingly, the 3.4 Ga Strelley Pool microfossils are better preserved than the 1.9 Ga Gunflint microfossils having experienced temperatures of about 200–220 °C (Alleon *et al.*, 2016a). These chemically more degraded Gunflint microfossils have much lower N/C values and do not contain any detectable amide groups (Alleon *et al.*, 2016a). This difference may be related to differences in porosity of the chert matrices. Cherts have essentially no measurable permeability (Knauth, 2005), which generally excludes the circulation of oxygenated diagenetic fluids which would impair molecular information. This

was the case for the Strelley Pool chert, as supported by the absence of secondary minerals, but not for the most hydrothermally altered Gunflint cherts, as evidenced by the presence of iron oxides that likely formed during diagenesis (Shapiro and Konhauser, 2015; Alleon *et al.*, 2016a). Assimilating these observations, we speculate that timing and severity of permeability loss may be more critical than temperature for the molecular preservation of organic microfossils.

The present study demonstrates that ancient organic microfossils may exhibit a high level of chemical preservation in appropriate settings independent of a long and complex geological history. These findings highlight the potential of the oldest geological record to offer a unique window into the Earth's juvenile biosphere and, eventually, to place constraints on the timing, tempo and trajectory of its evolution.

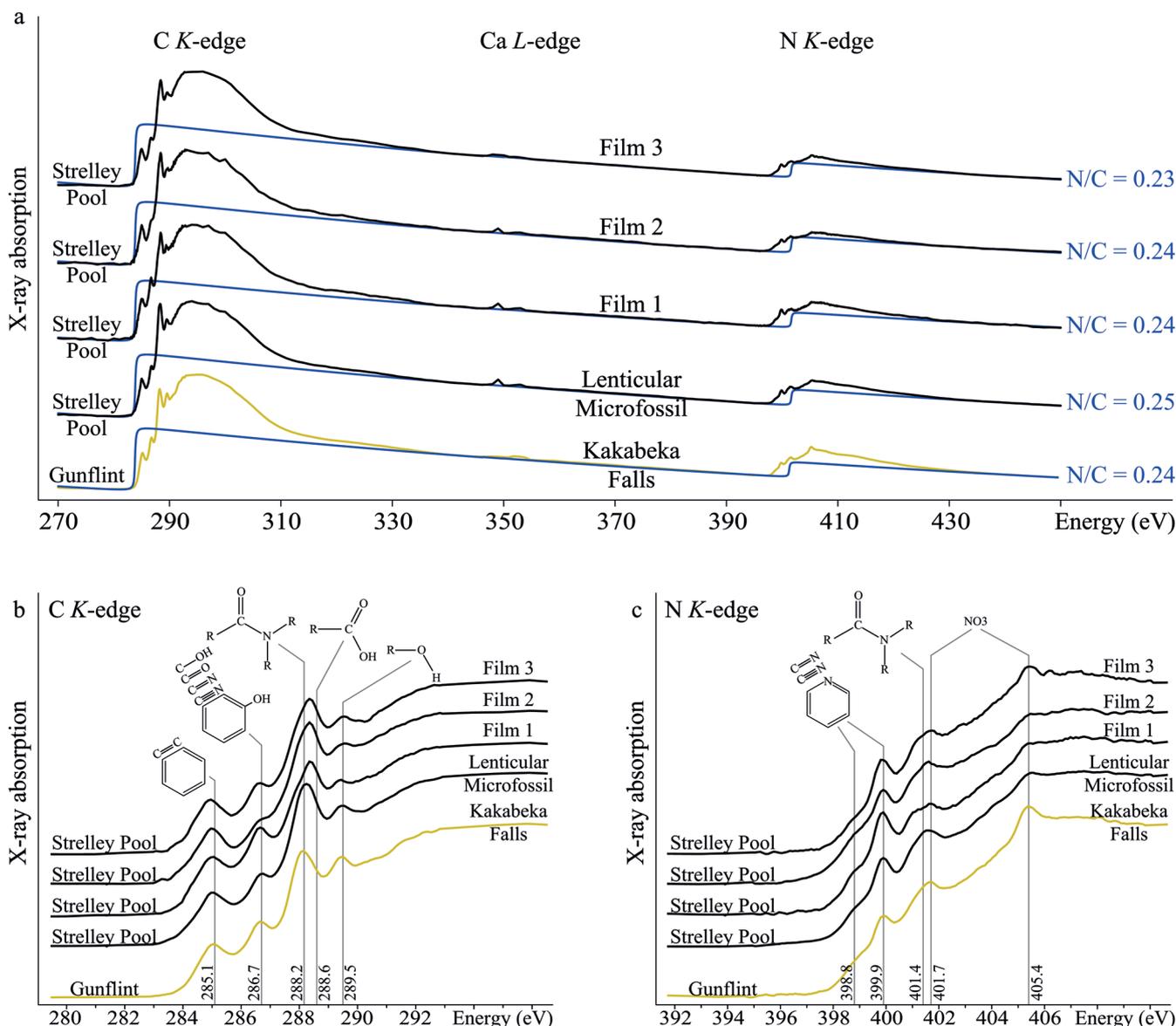


Figure 3 X-ray absorption analyses of the Strelley Pool organic microfossils shown in Figures 1 and 2 and corresponding N/C values. (a) X-ray absorption near edge structure (XANES) spectra at the carbon K-edge and (b) nitrogen K-edge. (c) Diagnostic absorption features include: 285.1 eV for aromatic/olefinic groups, 286.7 eV for imine/nitrile/carbonyl/phenol groups, 288.2 eV for amide groups, 288.6 eV for carboxyl/ester/acetal groups, 289.4 eV for hydroxyl groups, 398.8–399.9 eV - imine/nitrile/aromatic groups, 401.4 eV for amide groups, 401.7 and 405.4 eV for potassium and/or calcium nitrates. A spectrum of a 1.9 Ga Gunflint microfossil (Alleon *et al.*, 2016a) is shown for comparison.

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Author Contributions

JA, SB and FR conceived and designed the research. JA and SB performed the SEM experiments. JA, SB and OB performed the Raman spectroscopy experiments. CLG performed the TEM experiments. JA, SB and CLG performed the STXM-based XANES experiments, interpreted the data and wrote the manuscript with contributions from OB, KS and FR.

Additional Information

Supplementary Information accompanies this letter at <http://www.geochemicalperspectivesletters.org/article1817>.



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