




Significance of lignin and fungal markers in the Devonian (407 Ma) Rhynie Chert

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Abstract

The Rhynie Chert (Lower Devonian, Scotland) hosts a remarkably well-preserved early terrestrial ecosystem. Organisms including plants, fungi, arthropods, and bacteria were rapidly silicified due to inundation by silica-rich hot spring fluids. Exceptional molecular preservation has been noted by many authors, including some of the oldest evidence of lignin in the fossil record. The evolution of lignin was a critical factor in the diversification of land plants, providing structural support and defense against herbivores and microbes. However, the timing of the evolution of lignin decay processes remains unclear. Studies placing this event near the end of the Carboniferous are contradicted by evidence for fungal pathogenesis in Devonian plant fossils, including from the Rhynie Chert. We conducted organic geochemical analyses on a Rhynie Chert sample, including hydropyrolysis (HyPy) of kerogen and high-resolution mass spectrometric mapping of a thin section, to elucidate the relationship between lignin and the potential fungal marker perylene. HyPy of kerogen showed an increase in relative abundance of perylene supporting its entrapment within the silicate matrix of the chert. Lignin monomers were isolated through an alkaline oxidation process, showing a distribution dominated by H-type monomers. G- and S-type monomers were also detected, preserved by rapid silicification. Polycyclic aromatic hydrocarbons including perylene, a known marker for lignin-degrading fungi, were also concentrated in the kerogen and found to be localized within silicified plant fragments. Our results strongly link perylene in the Rhynie Chert to the activity of phytopathogenic fungi, demonstrating the importance of fungal degradation processes as far back as the Early Devonian.

KEYWORDS

exceptional preservation, fungal degradation, lignin, organic geochemistry, perylene, Rhynie Chert

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1 | INTRODUCTION

The world-renowned Lower Devonian (~407 Ma) Rhynie Chert of the Dryden Flags Formation of Aberdeenshire, Scotland, is host to a myriad of exceptionally well-preserved fossils, including bacteria, algae, plants, fungi, lichens, amoebae, arthropods, and nematodes (Garwood et al., 2020; Trewin & Kerp, 2017). Their paleontological study has made a major contribution to understanding early terrestrial ecosystems (Edwards et al., 2018; Garwood et al., 2020; Strullu-Derrien et al., 2019; Trewin & Kerp, 2017). The exceptional preservation of the fossils is a consequence of episodic inundation of the Rhynie Basin by silica-rich waters, produced by hydrothermal vents associated with an epithermal mineral system (Rice et al., 1995, 2002; Trewin, 1993; Trewin & Wilson, 2004). The timing and rate of silicification were variable (Powell et al., 2000; Trewin, 1993, 1996): some fossils show unambiguous visual evidence of decomposition prior to encapsulation, whereas others were clearly rapidly silicified alive (Remy et al., 1993; Trewin et al., 2003; Trewin & Fayers, 2016). Preservation quality varies within and between beds (Powell et al., 2000), and even within individual tissue types of a single specimen (Wellman et al., 2006). Excellent preservation of organic matter and biomolecules within the Rhynie Chert has been confirmed by several geochemical studies (e.g., Abbott et al., 2018). High abundances of carbon were correlated with morphologically intact plant tissues by electron microprobe mapping (Boyce et al., 2001). Various aliphatic, aromatic, and functional group signals have also been detected with traditional analytical approaches as well as sophisticated micro-selective analyses of isolated specimens (Abbott et al., 2018; Akinsanpe et al., 2024; Loron et al., 2023; Preston & Genge, 2010; Tewari et al., 2020).

Lignin is a recalcitrant biopolymer ubiquitous in tracheophytes, providing structure and rigidity (Weng & Chapple, 2010). It is selectively preserved in sediments over more labile components such as carbohydrates (van der Heijden & Boon, 1994) due to its large molecular size, insolubility, and heterogenous molecular structure (Hedges et al., 1985; Kirk & Farrell, 1987). Chemical and isotopic signals indicative of lignification were detected by Boyce et al. (2003) in Rhynie Chert plant fragments. This represents some of the oldest evidence of lignin in the fossil record, exceeded in chronostratigraphic age only by “lignin-like constituents” detected in Early Silurian plant fossils (Niklas & Pratt, 1980; Raven, 2018). The evolution of lignin profoundly altered the history of life on Earth and the development of the carbon cycle, allowing the wide expansion of tracheophytes on land (Kenrick & Crane, 1997). Although some authors have argued that the development of lignin increased carbon burial (Berner, 1998; Robinson, 1990), and that lignin recalcitrance and lack of biodegradation were responsible for massive coal formation during the Carboniferous (Floudas et al., 2012), this hypothesis has been disputed based on evidence of decay in fossilized plants throughout the Carboniferous and a revised assessment of the distribution of coal-bearing deposits in the Phanerozoic (Nelsen et al., 2016). A potential indicator for the fungal degradation of lignin in the Devonian is perylene, a five-ringed polycyclic aromatic hydrocarbon (PAH)

considered to be a diagenetic product of perylenequinone pigments primarily produced by such lignin-degrading fungi (Grice et al., 2009; Hanke et al., 2019; Marynowski et al., 2013; Tulipani et al., 2015). Unlike most sedimentary PAHs, perylene is not thought to be produced by combustion processes. No perylene was detected in several combustion studies of plants (Karp et al., 2020; O'Malley et al., 1997; Venkatesan & Dahl, 1989), and it has been shown to be an energetically unfavorable hydrothermal alteration product (Stein & Fahr, 1985). Perylene has been cited as evidence for fungal degradation in the Middle-Late Devonian (Grice et al., 2009; Tulipani et al., 2015); however, non-fungal sources for perylene have been proposed (e.g., Thomas & Blumer, 1964; Zdravkov et al., 2017). A recent report of perylene in Rhynie Chert did not definitively assign it to a fungal source (Akinsanpe et al., 2024) despite the abundance of fungi reported in the chert (e.g., Strullu-Derrien et al., 2023; Taylor et al., 2003).

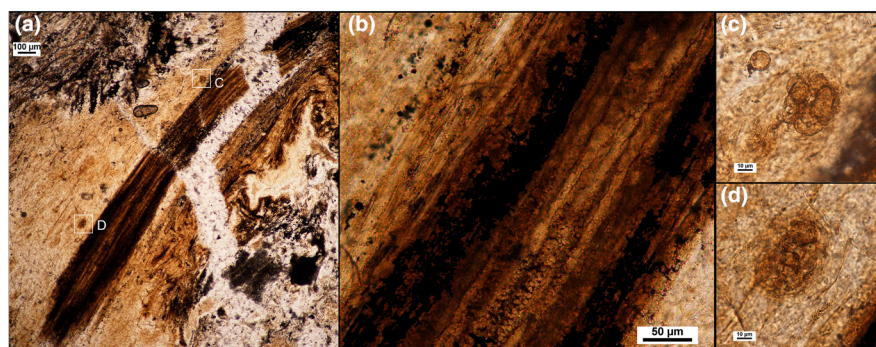
The aim of this study is to present a comprehensive organic geochemical characterization of a Rhynie Chert specimen, with a view to establishing whether or not a sample containing degraded plant matter and fungal spores contains evidence of perylene and lignin. We combine traditional organic geochemical techniques with high-resolution molecular mapping by Time-of-Flight-Secondary Ion Mass Spectrometry (ToF-SIMS). ToF-SIMS uses a primary ion beam to produce organic and inorganic secondary ions from the sample surface in situ at micrometer resolution, which are analyzed by time-of-flight mass spectrometry at high mass resolution (Thiel & Sjövall, 2014). ToF-SIMS has been used to map the organic matter in a range of ancient fossils and related deposits (e.g., Greenwalt et al., 2013; Lindgren et al., 2019; Plet et al., 2017; Siljeström et al., 2022; Tripp, 2023), including aliphatic and aromatic hydrocarbons in a Rhynie Chert plant fragment (Abbott et al., 2018). Detailed molecular analysis was undertaken using gas chromatography–mass spectrometry (GC–MS), hydrolysis (HyPy) of insoluble organic matter (kerogen), and isolation of lignin from isolated kerogen by alkaline oxidation, to confirm the association of lipid biomarkers (e.g., perylene) with a fungal source. Organic compounds were spatially mapped using ToF-SIMS to elucidate their spatial relationship with fossil plant material, with implications for understanding the mechanism of fossil and molecular preservation in the Rhynie Chert.

2 | MATERIALS AND METHODS

2.1 | Sample and preparation

A specimen of Rhynie Chert was provided by the Smithsonian National Museum of Natural History (uncatalogued specimen, Figure 1, Figures S1 and S2). Observation with a light microscope revealed fragments of partially decayed plant tissue. Some of the plant fragments showed a high degree of preservation and certain tissues (e.g., xylem) were observed. However, much of the sample comprised amorphous organic material and lacked morphological detail, reflecting observations by Trewin (1993) and Powell

FIGURE 1 (A and B) Photomicrograph of Rhynie Chert thin section in transmitted light mode, showing a plant xylem. (C and D) fungal spores identified in close proximity to the targeted plant xylem.



et al. (2000). Thus, the sample represents what Trewin (1993) described as, "...silicification of *in situ* or transported, partially decayed, litter."

Small pieces (approx. 20 g) were split from the specimen with a hammer and chisel and cleaned in an ultrasonic bath with dichloromethane (DCM) and methanol (MeOH) (9:1 v/v; 3 × 15 min) to remove surficial contamination. The cleaned pieces were powdered in a RockLabs Benchtop Ring Mill with a Zirconia 40 head. The head was pre-annealed (500°C for 2 h) before grinding, and annealed sand was ground before the sample for use as a procedural blank.

A thin section was prepared from the Rhynie Chert sample at Microanalysis Australia (Figure S2). The sample was trimmed using a clean diamond trim saw (lubricated with deionized water) and mounted onto a glass microscope slide using Renalm epoxy resin and hardener. The mounted block was trimmed using a diamond trim saw and polished to a final thickness of 30 μm using automated lapping wheels followed by 3- and 1-μm polishing cloths with Struers DiaPro and Kemet Liquid polish 3 and 1 μm suspensions.

2.2 | Soxhlet extraction

Powdered Rhynie Chert material was extracted in a Soxhlet apparatus (72 h; 9:1 v/v DCM:MeOH) in a pre-extracted cellulose thimble (Whatman). Activated copper turnings (4 M HCl) were added to the extraction to remove elemental sulfur. Extracted organic material (bitumen I) was filtered through anhydrous MgSO₄ to remove fine particles and residual water. A full procedural blank was prepared alongside the Rhynie Chert sample using the above methodology.

2.3 | Hydropyrolysis of extracted residue

Extracted rock powder (1.6 g) mixed with ammonium dioxodithiomolybdate catalyst ((NH₄)₂MoO₂S₂, 0.085 g) was pyrolyzed using a commercial hydropyrolysis apparatus (STRATA Technology LTD) following standard procedures (Love et al., 1995; Meredith et al., 2015). Briefly, the sample was heated from 25 to 250°C at 300°C min⁻¹, then from 250 to 520°C at 8°C min⁻¹ and held for 2 min. A constant pressure (15,000 kPa) and flow (5 L min⁻¹) of ultra-high purity hydrogen (Coregas) were maintained throughout thermal treatment.

The released compounds were cryogenically trapped on a silica-filled trap cooled with dry ice. Products adsorbed on the silica trap were eluted with DCM (40 mL) on a large chromatographic column; elemental sulfur was removed with activated copper turnings. The reactor and trap were cleaned between analyses by (i) 2 × 15 min ultra-sonication in 9:1 vol:vol DCM: MeOH; and (ii) sample-free HyPy treatment at 300°C min⁻¹ from 25 to 300°C then 10°C min⁻¹ to 550°C held for 5 min under constant pressure (15,000 kPa) and H₂ flow rate (5 L min⁻¹). An analyte-free, clean HyPy system was confirmed prior to analysis by pyrolyzing catalyst-loaded silica gel.

2.4 | Acid digestion and extraction of bitumen II

Extracted rock powder (~15 g) was digested with equal volumes of HF (48%) and milli-Q water in an ice bath for 1–2 h with regular shaking to dissolve silicate minerals. The sample was centrifuged, the supernatant decanted, and the remaining powder (0.22 g) was washed with water to remove residual acid and then freeze dried. Acid-digested powder was extracted in an ultrasonic bath (9:1 v/v DCM:MeOH, 3 × 15 min) to yield "bitumen II," a fraction of soluble organic matter that is occluded within the kerogen-mineral matrix (Holman et al., 2014; Nabbefeld et al., 2010). The residue consisted predominantly of insoluble macromolecular organic matter (kerogen) along with a small amount of acid-insoluble minerals.

2.5 | Column chromatography

Bitumen I, bitumen II, and hydropyrolysate were each separated into saturated, aromatic, and polar fractions using small-scale column chromatography using activated silica gel (160°C overnight, 5.5 cm × 0.5 cm i.d.). Saturated hydrocarbons were eluted with hexane, aromatic hydrocarbons eluted with 7:3 v/v hexane:DCM, and polar compounds with 1:1 v/v DCM:MeOH (4 mL each).

2.6 | Alkaline CuO oxidation of lignin

Alkaline oxidation of lignin was performed on the acid-digested kerogen powder, following an established microwave digestion procedure (Bhandari et al., 2022; Goñi & Montgomery, 2000). Briefly,

kerogen (50 mg) was loaded into a Teflon digestion vessel with finely powdered copper (II) oxide (CuO, 500 mg), ferrous ammonium sulfate ($\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$, 50 mg), and 2 N NaOH (15 mL, bubbled overnight with N_2), inside a glove bag in an N_2 atmosphere. The vessel was heated in a Milestone Start E Microwave Extraction System for 90 min at 150°C. The vessel contents were centrifuged and the solids washed with 2 N NaOH; the supernatant and wash were combined and made to pH 1 with dropwise addition of concentrated HCl. Lignin monomers were extracted from the aqueous supernatant by repeated shaking with ethyl acetate. The organic extract was evaporated to dryness under N_2 , redissolved in pyridine, and derivatized with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS) (60°C for 10 min) for GC-MS analysis.

2.7 | Gas chromatography-mass spectrometry analysis

Aromatic hydrocarbons and lignin monomers were analyzed on an Agilent 6890N gas chromatograph, fitted with an Agilent DB-5MS ultra-inert column (60 m × 0.25 mm × 0.25 μm), coupled to an Agilent 5975B MSD. 1 μL of sample was injected into a split-splitless injector operating in pulsed splitless mode (inlet temperature 280°C, 1.1 mL/min He carrier gas flow). Oven temperature was held at 40°C for 1 min, heated at 3°C/min to 325°C, then held for 30 min. Data were simultaneously collected in full scan and SIM modes, with a mass range of *m/z* 50–600. Perylene and lignin monomers were identified and quantified using authentic standards (Sigma Aldrich).

2.8 | Time-of-flight-secondary ion mass spectrometry

The Rhynie Chert thin section was prepared for analysis by rinsing briefly with ethanol. Standard compounds were prepared for analysis by dissolving (or suspending if insoluble) in DCM at 1 mg mL⁻¹ and adhering onto a 10 × 10 mm Si wafer (ProSciTech, pre-cleaned with DCM). Standards used were perylene (purchased from Sigma Aldrich) and known diagenetic products of phenanthroperylene-quinone pigments phenanthro[1,10,9,8-*opqra*]perylene-7,14-dione (PPQ) and 1,2,3,4,5,6-hexahydrophenanthro[1,10,9,8-*opqra*]perylene (HHPP) (see Wolkenstein, 2019).

Secondary ion analysis by Time-of-Flight-Secondary Ion Mass Spectrometry (ToF-SIMS) was performed using an IonTOF M6 instrument, equipped with a bismuth liquid metal ion gun and a gas cluster ion source. The area targeted was an intact plant fragment (Figure 1); an area of embedding epoxy resin was also analyzed for comparison. Directly before analysis, the targeted areas were sputter cleaned in situ to remove organic surface contaminants, using an Ar gas cluster ion beam (GCIB). The GCIB was configured for either 10 keV Ar clusters with a median of 1600 atoms, or 5 keV Ar clusters with a median of 1300 atoms, for approximately 30 s over a raster size of 500 × 500 μm for the perylene standard analysis, or

600 × 600 μm for all other analyses. The analysis of perylene was performed at high mass resolution, using a 30 keV Bi₃⁺ ion source with a pulsed beam current of approximately 0.25 pA at a raster of 100 × 100 μm. Analyses were obtained in positive and negative ion acquisition modes; however, the pure analytical standards showed the best occurrence of characteristic fragment ions in positive ion acquisition; therefore, only positive spectra are presented for this study. Positive secondary ion maps of the Rhynie Chert were obtained with a 30 keV Bi₃⁺ ion beam rastered over a 500 × 500 μm area for 30 min, with a beam current of 0.04–0.05 pA. The electron flood gun was used to improve charge compensation on the insulating mineral surface. Spectra were calibrated for identification of organic ions using organic fragments that were abundant in all spectra: C₂H₅⁺, C₃H₅⁺, C₃H₇⁺, C₄H₇⁺, and C₅H₇⁺. Calibration ions were kept consistent for all samples and standards to allow accurate spectral comparisons and identification of secondary ions.

3 | RESULTS

3.1 | Quantification of lignin monomers

Lignin is formed from the polymerization of the hydroxycinnamyl alcohols *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, to produce hydroxyphenyl (H), guaiacyl (G), and syringyl (S) lignins, respectively (Boerjan et al., 2003; Weng & Chapple, 2010). H- and G-type lignins are found across all tracheophytes, while biosynthesis of S-type lignin is largely restricted to angiosperms with some exceptions (Espineira et al., 2011; Weng et al., 2008).

Seven individual lignin monomers were identified in the Rhynie Chert and quantified by external quantification with authentic standards (Table 1). The most abundant monomers were *p*-hydroxybenzoic acid and *p*-hydroxybenzaldehyde from H-type lignin (36.5 and 28.2 μg/g total organic carbon [TOC], respectively), followed by vanillin and vanillic acid from G-type lignin (8.4 and 7.0 μg/g TOC respectively). Syringaldehyde was the only S-type lignin monomer detected and was the least abundant of the identified monomers at 3.0 μg/g TOC. H-type lignin monomers comprised 76.1% of total lignin by mass, followed by G-type lignin at 20.6% and S-type lignin at 3.3%.

TABLE 1 Lignin monomers isolated by alkaline oxidation of HF-digested Rhynie Chert and quantified using authentic standards.

| Lignin type | Species | Quantity (μg/g TOC) |
|-------------|-------------------------------|---------------------|
| H-type | <i>p</i> -hydroxybenzoic acid | 36.5 |
| | <i>p</i> -hydroxybenzaldehyde | 28.2 |
| | <i>p</i> -hydroxyacetophenone | 4.9 |
| G-type | Vanillin | 8.4 |
| | Vanillic acid | 7.0 |
| | Acetovanillone | 3.4 |
| S-type | Syringaldehyde | 3.0 |

3.2 | Polycyclic aromatic hydrocarbons

The five-ringed PAH perylene was detected in the aromatic fraction of the kerogen hydropyrolysate and tentatively identified in trace amounts in bitumen I and bitumen II (Figure 2). Also detected in all aromatic fractions were benzo[e]pyrene, benzo[a]pyrene, and isomers of benzofluoranthene (Grice et al., 2007). These highly condensed five-ringed PAHs are known markers of combustion processes (Venkatesan & Dahl, 1989) and hydrothermal activity (Stein & Fahr, 1985), or may have biological precursors in the Rhynie Chert that are as yet unknown. External quantification with an authentic perylene standard (Table 2) showed low concentrations of these PAHs in bitumen I and bitumen II (benzofluoranthenes were not quantified as the various isomers do not fully separate by GC-MS). The concentrations of all five-ringed PAHs were greatly increased in the kerogen hydropyrolysate, with perylene highly abundant (3095 $\mu\text{g/g}$ TOC). It should be noted that this result may in fact underrepresent the true concentration of perylene in the Rhynie Chert, as experiments with standard compounds have shown that perylene is unstable and degrades during hydropyrolysis (Grotheer et al., 2015). Therefore, the “true” abundance of perylene may be higher than reported in Table 2.

3.3 | ToF-SIMS

In situ mapping of organic and inorganic phases in the Rhynie Chert thin section by ToF-SIMS was applied to investigate the association of biomarkers with preserved plant fragments. An intact plant axis vasculature, including the xylem, was targeted (Figure 1, Figure S3). Silicate, represented by SiOH^+ , was highly abundant throughout the scanned area but absent within the morphology of the plant xylem (Figure 3b). The secondary ion spectrum of perylene was characterized using a pure standard, and its characteristic positive secondary ions (listed in Table 3) were tentatively identified in the chert (Figures S4 and S5). Due to the identical atomic mass and structural similarities of perylene with other five-ring PAH compounds (e.g., benzopyrenes and benzofluoranthenes), the secondary ions used here are representative of contributions from multiple five-ring PAH compounds as identified by GC-MS (Figure 2). A total abundance map of these PAH secondary ions demonstrated their localization within the plant xylem fragment (Figure 3c), supporting the derivation of PAH compounds from the fossil material itself, rather than the surrounding silica. It cannot be ruled out that a proportion of these secondary ions may come from other aromatic compounds, as they have been previously observed in TOF-SIMS analyses together with other ions indicative of aromatic fragments (e.g., Ivarsson et al., 2013; Siljeström et al., 2013), but the abundance of five-ringed PAHs in the GC-MS analyses supports the assignment of the majority of these ions to five-ringed PAHs. Characteristic ions of PPQ and HHPP were identified by analysis of authentic standards but were not detected in the chert.

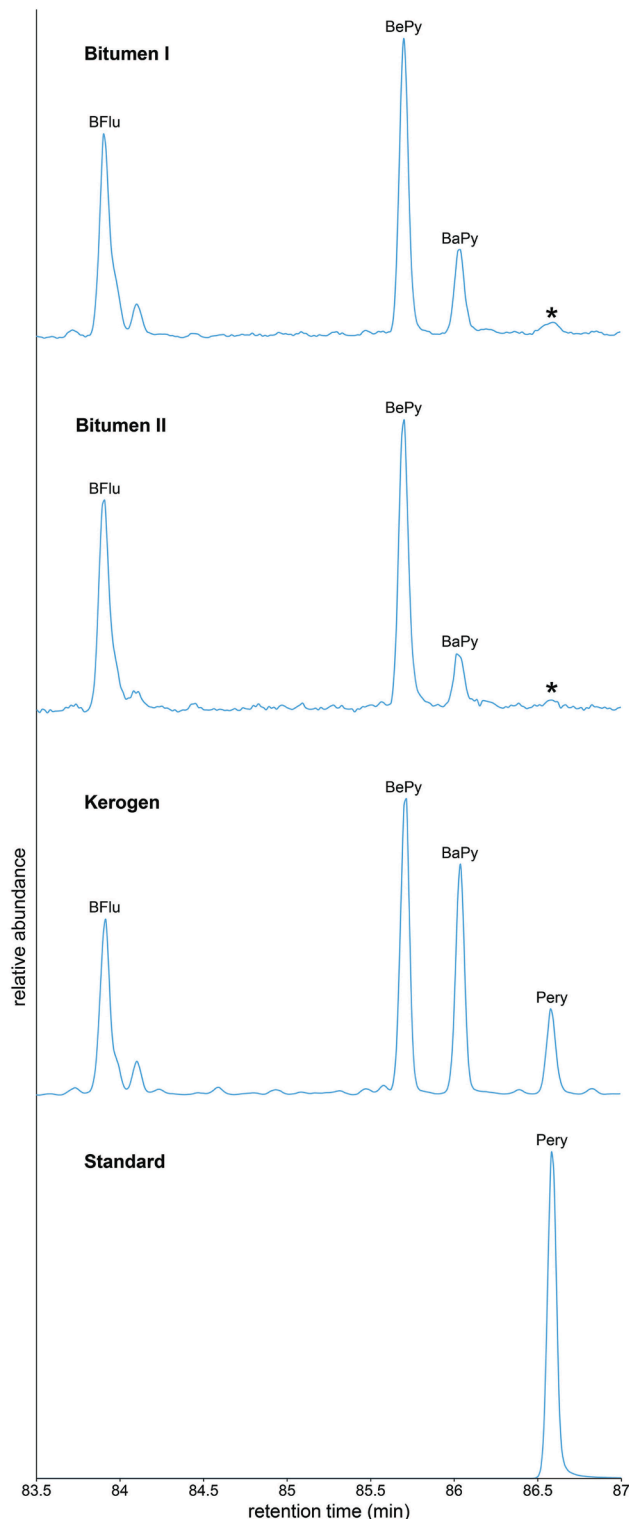


FIGURE 2 GC-MS selected ion monitoring (SIM) chromatograms of m/z 252, showing five-ringed PAHs in bitumen, bitumen II, and the kerogen hydropyrolysate. Identification of perylene in the kerogen hydropyrolysate was confirmed by comparison of retention times with an authentic standard. Perylene was tentatively identified in bitumen I and bitumen II but is below the limit of quantification and is labeled with *. BaPy, benzo[a]pyrene; BePy, benzo[e]pyrene; BFlu, benzofluoranthenes; Pery, perylene.

The contribution of the embedding epoxy resin to the organic secondary ion distribution was excluded by careful comparison of the secondary ion spectrum of the resin with that of both the specimen and the pure perylene standard. The characteristic secondary ions of the perylene standard were not observed in the secondary ion spectrum of the resin (Figure S4). Comparison of the measured secondary ion spectrum of the embedding resin of the thin section with characteristic secondary ions of epoxy (using the IONTOF spectra library) was used to select ions that were not common with the perylene standard ($C_6H_6O^+$, $C_6H_7O^+$, $C_7H_7O^+$, and $C_9H_{11}O^+$). These were tentatively identified in the thin section and were mapped across the analysis area to show that they did not contribute substantially to the organic distribution of the plant xylem (Figure 3d, Figure S6). Additionally, the observed abundance of perylene and

other five-ringed PAHs in GC-MS analysis of chert that had not been exposed to resin (Section 3.2) is further evidence that the five-ringed PAHs detected by TOF-SIMS are indigenous to the plant fragment and are not the result of contamination.

4 | DISCUSSION

4.1 | Characterization of Rhynie Chert lignin

Characterization of isolated lignin monomers shows a distribution dominated by H-type units, with lower amounts of G-type and only traces of S-type lignin detected (Table 1). Chemical signatures of lignification were first identified in the Rhynie Chert by Boyce et al. (2003) through bulk carbon isotope measurements and carbon X-ray Absorption Near Edge Spectroscopy, but these analyses did not directly detect lignin monomers. More recently an unpublished abstract reported phenols and methoxybenzenes from pyrolysis indicative of lignin monomers (Tewari et al., 2020), but the specific type of lignin was not interpreted. The present work marks the first time that individual lignin monomers from the Rhynie Chert have been identified and linked to lignin type.

Similar studies of Paleozoic lignin with which to compare these results are rare. In compression fossils of lycophytes from the Carboniferous (Westphalian), Logan and Thomas (1987) reported lignin comprised of *p*-hydroxybenzaldehyde (H-type), vanillin and acetovanillone (both G-type) with trace amounts of syringaldehyde (S-type), a similar distribution to that found in the Rhynie Chert. In contrast, recent spectroscopic and pyrolytic analyses of Early

TABLE 2 Quantification of polycyclic aromatic hydrocarbons in *m/z* 252 extracted ion chromatograms of bitumen I, bitumen II, and kerogen hydropyrolysate.

| Compound | Bitumen I ($\mu\text{g/g TOC}$) | Bitumen II ($\mu\text{g/g TOC}$) | Kerogen ($\mu\text{g/g TOC}$) |
|----------|--------------------------------------|---------------------------------------|------------------------------------|
| BePy | 2.8 | 2.3 | 10,872.2 |
| BaPy | 0.9 | 0.4 | 7820.5 |
| Pery | - | - | 3095.3 |

Note: Perylene was tentatively identified in bitumen I and bitumen II based on retention time (see Figure 2) but was below the limit of quantification.

Abbreviations: BaPy, benzo[*a*]pyrene; BePy, benzo[*e*]pyrene; Pery, perylene.

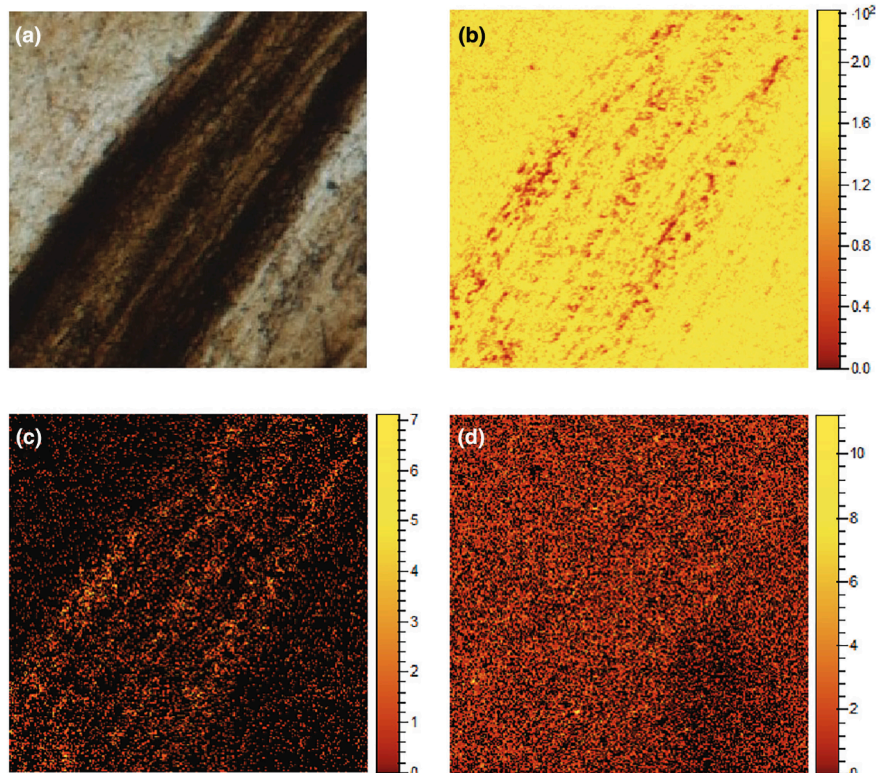


FIGURE 3 ToF-SIMS secondary ion maps of the plant xylem identified in Figure 1. (a) Transmitted light microscopy image of the scanned area; (b) Silicate matrix, represented by $SiOH^+$; (c) Sum of characteristic ions from five-ringed PAH compounds as identified by analysis of pure perylene standard; (d) Sum of diagnostic secondary ions of embedding epoxy resin. Mapped areas are $500 \times 500 \mu\text{m}$. All organic secondary ions used in maps are identified in Table 3. Color gradients of secondary ion maps represent the abundance of the displayed ions, measured as total counts for each pixel over the 30-min scan time.

TABLE 3 Characteristic and diagnostic secondary fragment ions of perylene detected in the Rhynie Chert thin section by comparison with pure standard, used to create ion map in Figure 3.

| Ion | Observed mass | Observed mass |
|------------------|---------------|---------------|
| | Standard | Sample |
| $C_{20}H_{12}^+$ | 252.095 | 252.066 |
| $C_{20}H_{13}^+$ | 253.104 | 253.073 |
| $C_{20}H_{14}^+$ | 254.107 | 254.083 |

Carboniferous seeds (Telnova et al., 2022) and Middle Devonian coals (Telnova et al., 2023) found lignin composed entirely of *p*-coumaric units (H-type lignin). The authors proposed that this represents a relict or proto-lignin unknown in modern plants but recognized that it was likely modified as a result of fossilization (Telnova et al., 2022). The non-detection of G- and S-type units in these studies is potentially due, at least in part, to the lower degree of molecular preservation in the studied samples relative to the Rhynie Chert. Fossilization and diagenesis result in modification of lignin through the loss of methoxy groups, observed by Logan and Thomas (1987) as a reduction of G-type and S-type units and an increased proportion of *p*-hydroxybenzaldehyde. Increased thermal maturation or coalification eventually leads to the conversion of all lignin units into phenols and methylated phenols (Hatcher et al., 1988), as was observed in coalified vascular plants from the Early Devonian (Emsian) which contained no guaiacolic (G-type) units (Ewbank et al., 1996). In contrast, early silicification such as that seen in the Rhynie Chert has been shown to limit the molecular degradation of organic matter during diagenesis (Alleon et al., 2016). Rhynie Chert lignin in this study contained approximately 20% G-type and 3% S-type monomers.

The presence of S-type lignin in the Rhynie Chert is unexpected as this lignin type is predominantly produced by angiosperms (e.g., Boerjan et al., 2003; Vanholme et al., 2010). There is evidence however that the synthesis of S-type lignin evolved multiple times in separate clades, including angiosperms, gymnosperms, and lycophytes (Labeeuw et al., 2015; Novo-Uzal et al., 2012; Rencoret et al., 2021). Fossil lycophytes are known in the Rhynie Chert (Hetherington & Dolan, 2019) and are a plausible source for the detected traces of S-type lignin. While the organic matter in the Rhynie Chert was assessed as being of relatively high thermal maturity based on standard organic geochemical techniques (see Appendix S1), the rapid silicification experienced by Rhynie Chert plant fragments (e.g., Powell et al., 2000; Trewin et al., 2003) has acted to preserve the structure of these monomers that would otherwise have been degraded. These results demonstrate that lignins of all three types were present in the terrestrial ecosystems of the Early Devonian.

4.2 | Perylene—evidence of phytopathogenic fungi

The presence of abundant perylene in sediments is widely considered to be indicative of the activity of phytopathogenic fungi (Grice et al., 2009; Hanke et al., 2019; Marynowski et al., 2013), based on

isotopic evidence (Grice et al., 2009; Hobbie et al., 1999; Suzuki et al., 2010) and its similar structure to perylenequinone pigments produced by such fungi (Khiralla et al., 2022; Stierle et al., 1989). Fungi in the Rhynie Chert are abundant and diverse (Krings et al., 2018; Taylor et al., 2003), and fungi-plant associations and interactions have been well documented (Krings et al., 2018; Parnell et al., 2022; Strullu-Derrien et al., 2014; Taylor & Taylor, 2000). The production of fungal perylenequinones is restricted to the phylum *Ascomycota* (Khiralla et al., 2022). While the most efficient and commonly reported lignin-degrading fungi are *Basidiomycetes* (e.g., Nelsen et al., 2016), *Ascomycota* have also been found to be important contributors to lignin degradation (Ferrari et al., 2021; Nilsson et al., 1989; Schwarze, 2007). Multiple ascomycetes have been identified in the Rhynie Chert (Krings et al., 2018; Taylor et al., 2005) including the recently described *Potteromyces asteroxylicola*, observed as a pathogen on fragments of the plant *Asteroxylon mackiei* (Strullu-Derrien et al., 2023). Fungal spores are observed in the present sample in close proximity to the analyzed plant fragment (Figure 1), and elsewhere on the slide a specimen is present closely resembling *Paleopyrenomycites devonicus* (Figure S7), an ascomycete described by Taylor et al. (2005). The attribution of perylene in the Rhynie Chert to a phytopathogenic fungal source therefore appears logical. However, a study assigning a terrigenous source to perylene without explicitly invoking fungi (Li et al., 2022) has led Akinsanpe et al. (2024) to leave the origin of perylene in the Rhynie Chert an open question. Alternative sources of perylene must therefore be considered.

Perylenequinone pigments have also been reported in aphids (Cameron et al., 1964) and in the roots of the angiosperm *Scutia myrtina* (Ayers et al., 2007), but these are unlikely contributors to the Rhynie Chert as Aphidomorpha do not appear in the fossil record until the Middle Permian (Szwedo et al., 2015) and angiosperms are only evident from the Early Cretaceous (Coiro et al., 2019). Structurally related phenanthroperylenequinone pigments have been isolated from modern and fossil crinoids (Blumer, 1962; De Riccardis et al., 1991; Wolkenstein, 2015). Although the crinoid fossil record extends to the Early Ordovician (Ausich et al., 2015; Guensburg & Sprinkle, 2001), these echinoderms are exclusively marine (Baumiller, 2008) and would not be expected in the terrestrial Rhynie Chert ecosystem, which shows no known evidence of marine influence (Trewin & Kerp, 2017). These alternative sources of perylenequinone pigments can therefore be discounted as contributors to the Rhynie Chert. An alternative explanation is the formation of perylene by combustion or hydrothermal processes. Limited studies have proposed combustion processes as the major source of perylene (Zdravkov et al., 2017); however, perylene is not reported from combustion studies of plants (Karp et al., 2020; O'Malley et al., 1997; Venkatesan & Dahl, 1989), nor is it energetically favorable for generation from high-temperature processes such as hydrothermal alteration (Stein & Fahr, 1985). The perylene found in this study is highly unlikely to have been produced by any of the above-suggested alternative sources; hence, we attribute its presence in the Rhynie Chert to a fungal source. Its abundance in the kerogen hydropyrolysate reflects the activity

of phytopathogenic fungi on plant fragments. These fungi were entrapped together with the plant fragments by silicification, and the perylenequinone pigments became incorporated into the kerogen during burial and maturation. Perylene is less thermally stable than other five-ringed PAHs (Stein & Fahr, 1985) and is subject to degradation at moderate thermal maturities (Marynowski et al., 2015); hence, its abundance in the kerogen is further evidence for the preservation of thermally labile organic molecules within the silicified plant fragments of the Rhynie Chert. Morphological preservation was accompanied by enhanced molecular preservation, as rapid silicification of plant fragments prevented extensive breakdown of organic material during burial and maturation. This is further evidenced by ToF-SIMS secondary ion mapping (Figure 3). Organic fragment ions indicative of five-ringed PAHs including perylene, which were determined not to be contamination from the embedding resin, were localized within a plant xylem. The secondary ion maps give strong evidence that these PAHs are associated with plant fragments and demonstrate that TOF-SIMS analysis can provide valuable information on the source of organic molecules in cherts. These results imply the activity of phytopathogenic fungi in the Rhynie Chert (Strullu-Derrien et al., 2023) and show that fungal degradation of lignin was an active and important process during the Early Devonian.

5 | CONCLUSIONS

To understand the origin of perylene and its relationship to lignin within the Rhynie Chert, we undertook organic geochemical analyses of extractable and insoluble organic matter (bitumen and kerogen) coupled with ToF-SIMS analysis to elucidate the spatial relationship between perylene and lignin. For the first time, lignin monomers from the Rhynie Chert were isolated and characterized, revealing a distribution composed predominantly of H-type lignin (76%) but also containing G- and S-type monomers. The rapid silicification of plant fragments acted to preserve the less stable G- and S-type monomers where they would otherwise be expected to degrade based on the age and apparent thermal maturity of the system. Five-ringed PAHs including perylene were detected from hydrolysis of kerogen and found to be closely spatially associated with silicified plant fragments. This work strongly indicates a fungal origin for perylene, supporting recent evidence of fungal pathogenesis within the Rhynie Chert.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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