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X-ray photoelectron emission spectromicroscopic analysis of arborescent lycopsid cell wall composition and Carboniferous coal ball preservation

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ABSTRACT

Two alternative processes complicate understanding of the biochemical origins and geochemical alteration of organic matter over geologic time: selective preservation of original biopolymers and *in situ* generation of new geopolymers. One of the best constrained potential sources of bio- and geochemical information about extinct fossil plants is frequently overlooked. Permineralized anatomically preserved plant fossils allow analysis of individual cell and tissue types that have an original biochemical composition already known from living plants. The original composition of more enigmatic fossils can be constrained by geochemical comparisons to tissues of better understood fossils from the same locality. This strategy is possible using synchrotron-based techniques for submicron-scale imaging with X-rays over a range of frequencies in order to provide information concerning the relative abundance of different organic bonds with X-ray Absorption Near Edge Spectroscopy. In this study, X-ray PhotoElectron Emission spectroMicroscopy (X-PEEM) was used to analyze the tissues of *Lepidodendron*, one of the lycopsid trees that were canopy dominants of many Pennsylvanian coal swamp forests. Its periderm or bark—the single greatest biomass contributor to many Late Paleozoic coals—is found to have a greater aliphatic content and an overall greater density of organic matter than lignified wood. Because X-PEEM allows simultaneous analysis of organic matter and matrix calcite in fully mineralized fossils, this technique also has great potential for analysis of fossil preservation, including documentation of significant traces of organic matter entrained in the calcite crystal fabric that fills the cell lumens.

1. Introduction

1.1. The evolving biochemistry of coal inputs

The structural compounds of plant cell walls are primary contributors to organic sedimentary accumulations because of their abundance and decay resistance relative to other biochemical components, like nucleic acids, proteins, and secondary metabolites. All land plant cell walls are composed of cellulose and other polysaccharides. In vascular plants, lignin is selectively deposited in cell walls that provide biomechanical support (e.g. sclerenchyma and xylem). Other compounds, like suberin, sporopollenin, and some cuticular compounds, are of structural importance in specific tissues (de Leeuw and Largeau, 1993).

Lignin, because of its resistance to decay relative to polysaccharides (van der Heijden and Boon, 1994), is a reasonable biochemical starting point among plant structural compounds in attempts to reconstruct the geochemical alteration pathways that are followed as rank increases during coalification. Coal is a complex and heterogeneous mixture, but—aside from coals of specialized composition like sporinites and resinites (Teichmuller, 1989; Van Bergen et al., 1995; Cody et al., 1996; Scott, 2002)—at least half of that chemical diversity is consistent with derivation from lignin through its degradation. The alkyl-aryl backbone of lignin is disrupted early in diagenesis. As coalification progresses, the functional groups of lignin monomers are progressively lost and more complex polycyclic aromatics such as napthalenes are eventually generated (Hatcher and Clifford, 1997; Cody and Sághi-Szabó, 1999). The remaining chemical diversity that is not consistent with a lignin source has been attributed to other components of land plant biochemistry with perhaps some chemical contribution from algae growing in the swamp environment (Hatcher and Clifford, 1997).

To ensure that appropriate comparisons are made, circumscription of the geochemical pathway from plant to coal has relied on analysis of recognizable wood fragments collected from coals of different rank occurring in the same depositional/stratigraphic system (Hatcher et al., 1992; Hatcher and Clifford, 1997). Unfortunately, strata containing a related series of coal beds ranging from lignite to bituminous rank are usually confined to relatively young (Cretaceous or Cenozoic) coal basins. The great Carboniferous coal deposits are

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almost exclusively higher rank. For studies focused on understanding the geochemical processes that occur during coalification, reliance on the use of young coals is of little concern if the emphasis is on selective preservation (e.g. Tegelaar et al., 1989; de Leeuw et al., 1991; van Bergen et al., 1995; Hatcher and Clifford, 1997; Edwards et al., 1997), because most lignins are chemically similar no matter which vascular plants produced them or their geologic age. However, preservation of organic carbon derived from less resistant biomolecules through polymerization into more stable geomolecules has also been documented (Mösle et al., 1998; Briggs, 1999; Boyce et al., 2002; de Leeuw et al., 2006; Gupta et al., 2007b). Given the potential for aromatization of originally non-aromatic organic matter during thermal maturation (Cody et al., 1996; Boyce et al., 2002), many molecular compounds in coal that would appear consistent with derivation from lignin could actually be derived from non-lignin precursors.

Some investigators have commented about the remarkable preservation of delicate anatomy despite removal of the polysaccharide content of vascular cell walls (Hatcher and Clifford, 1997), while others have argued that the lignin may provide some degree of protection from degradation for the polysaccharides in these cell walls and thus enhance polysaccharide preservation potential in the lignocellulosic complex (de Leeuw and Largeau, 1993). Both possibilities may be true under differing degradation pathways. However, geochemical alteration processes that can obscure the origins of a compound from more resistant biochemical sources (like lignin) may also work to obscure origin from more labile sources (like polysaccharides). This observation suggests an important question—does the bulk biochemical input into organic accumulations shift with evolutionary changes in the taxonomic composition of floras (e.g. Collinson et al., 1994; Petersen and Nytoft, 2006).

There is little meaningful overlap—either taxonomic or anatomical—between the composition of Cretaceous/Cenozoic and Carboniferous wetland vegetation. Lignified wood comprises the bulk of the biomass of the conifer and angiosperm trees that dominate Cretaceous and Cenozoic landscapes. Before the Stephanian, few Carboniferous coals were dominated by extensively woody plants (DiMichele and Phillips, 1994). The dominant contributors of biomass to most of these coals are the now extinct arborescent lycopsids, unusual plants that possessed little wood, but produced an abundance of periderm or secondary cortex (Fig. 1). Lycopsids can constitute 60 to 80% of the biomass of most Carboniferous coals (Phillips et al., 1985; DiMichele and Phillips, 1985, 1988), and the periderm alone represents 20 to 56% of these coals (DiMichele and Phillips, 1985; DiMichele et al., 1986; DiMichele and Phillips, 1988). Since this tissue is analogous to the bark of living seed plant trees, it might be expected that its polysaccharide walls were embedded with the mixed aromatic/aliphatic suberin. However, lycopsid periderm is not homologous to the bark of seed plants and is in many ways distinct (Phillips and DiMichele, 1992). Since the periderm was rigid, dense, and resistant to herbivores (C. Labandeira, Personal Communication, 2008) and other forms of degradation (Phillips and DiMichele, 1992), its polysaccharide walls were presumably embedded with some more recalcitrant compounds, though the nature of this original biochemistry is unknown.

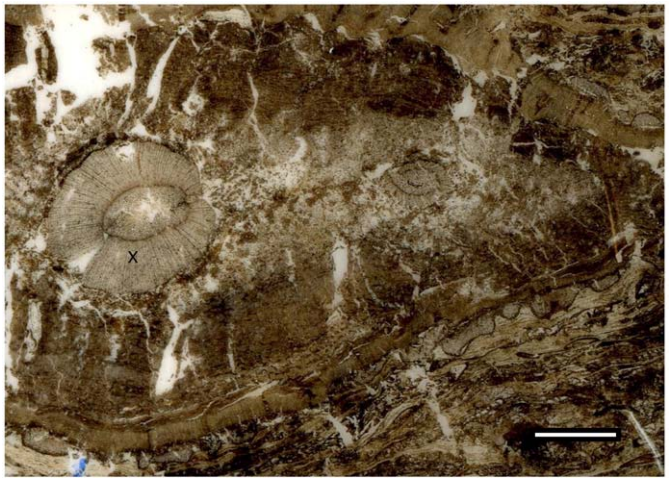

Fig. 1. Cellulose-acetate peel of *Diaphorodendron scleroticum* axis in cross section (Harvard Botanical Museum collections #60432), showing the small amount of xylem (1 cm ring toward left, labeled with X) in comparison to abundant surrounding cortical tissues (filling the majority of the image) that is common in arborescent lycopsids. Scale bar equals 5 mm.

The bulk chemistry of lycopsid periderm has been analyzed previously. In a Gas Chromatography/Mass Spectrometry (GCMS) analysis of chemical extracts from several arborescent lycopsid compression fossils, the highest aromatic content was attributed to the periderm. This finding was considered evidence for periderm lignification. A subset of analyses showing reduced aromatic content were interpreted to be analyses of the superficial leaf bases (Logan and Thomas, 1987). In later pyrolysis-GCMS analyses of lycopsid periderm, it was concluded that the aromatics were unlikely to be derived from lignin because they were secondary in abundance to long-chain aliphatics (Collinson et al., 1994). These authors considered the periderm chemistry to be more consistent with derivation from suberin or suberan (Tegelaar et al., 1995). However, the postmortem stabilization of less resistant biochemistry through polymerization that complicates interpretation of aromatics also occurs among aliphatics. The widespread occurrence of cutan in fossil plant (and animal) cuticles has been interpreted as the result of such secondary polymerization (Gupta et al., 2007a,b). Sampling accuracy is an additional concern when interpreting the results of bulk analysis of compression fossils, because there is always the possibility that the chemistries of several distinct tissue types were mixed during sample collection (e.g. sampling the periderm along with an epidermis lined with aliphatic cuticle (Collinson et al., 1994)).

1.2. XANES and micron-scale comparative chemistry of plant fossils

Since arborescent lycopsids have an unusual anatomy without modern homologue and are the largest contributors of biomass to many Carboniferous coal deposits, understanding their original cell wall biochemistry could affect our understanding of their biology, coalification pathways, and the Paleozoic carbon cycle. It is, however, unclear that such an understanding can be achieved because of the uncertain biochemical origins of geomolecules found in more thermally mature coals. Heating experiments using recent plant materials (and lower rank coal) can mimic some thermal maturation processes (Behar and Hatcher, 1995; Orem et al., 1996; Czaja et al., 2004; Gupta et al., 2007a), but cannot supply all of the answers (Behar and Hatcher, 1995; de Leeuw, 2007). An alternative approach is suggested by this study. Here, geochemical comparisons of specific cell and tissue types are confined to fossils collected from the same locality with the same diagenetic history. No matter what analytical advances may lay ahead, the chemistry of anatomically preserved fossils will remain the greatest source of information about the original biochemistry of a fossil organism. Regardless of the extent of geochemical alteration, the original composition of wood and several other tissue types is sufficiently well constrained that they can serve as points of comparison for understanding the chemical changes occurring in other tissues that lack living homologues and for which original composition is more enigmatic (Edwards et al., 1997; Boyce et al., 2003).

In addition to advances in spot analysis techniques that couple laser or ion-beam ablation of a sample with GCMS or Isotope Ratio Mass Spectrometry (Greenwood et al., 1993; House et al., 2000; Greenwood et al., 2001; Sangely et al., 2005; Jacob et al., 2007), detailed spatial mapping of bond-type distributions is available using X-ray Absorption Near Edge Spectroscopy (XANES). Across the

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electromagnetic spectrum, X-rays provide the optimal combination of spatial and chemical resolution for mapping functional group distributions in heterogeneous organic complexes (Cody et al., 1996). With increasing X-ray energy, there is a sharp rise in X-ray absorbance at the ionization edge of a particular element. Positive deviations from the rising background absorption at specific energies within this edge reflect the bond environment of the element in question. Within the carbon edge (280–305 eV), absorption peaks include 285.2 eV (C_{aryl}–C,H), 287.1 eV (C_{aryl}–O), 287.9 eV ($C_{aliphatic}$–H), 288.5 eV (carboxylate), 289.5 eV ($C_{aliphatic}$–O), and major peaks at 290.5 eV and 302 eV and minor peaks at 296 eV and 298 eV associated with calcite (Madix et al., 1988; Botto and Cody, 1994; Cody et al., 1996; Myneni, 2002; Boyce et al., 2002; De Stasio et al., 2005). Imaging at a particular frequency within this range can provide the anatomical distribution of a particular bond type.

Alternatives exist for measuring X-ray absorbance. With Scanning Transmission X-ray Microscopy (STXM), X-rays are passed through a sample with absorbance determined from the incident and transmitted intensities. This technique requires samples thin enough for adequate X-ray transmission, approximately 100 nm thick in the 280–320 eV range. To meet these sample requirements, living plant tissue can be stabilized in epoxy and ultramicrotomed directly (Boyce et al., 2004). For paleontological applications, either a demineralized 100 nm section of one or a few cell walls can be generated from a cellulose-acetate peel (Joy et al., 1956) of an acid-etched fossil surface that is subsequently epoxy embedded and ultramicrotomed (Boyce et al., 2002, 2003) or a somewhat larger and non-demineralized section can be produced by use of a focused ion-beam mill (Bernard et al., 2007). Although fundamentally a point analysis, STXM imaging can be achieved by rastering a series of point analyses. With an alternative technique, X-ray PhotoElectron Emission Microscopy (X-PEEM), X-ray absorbance is indicated by the abundance of electrons emitted from the sample surface. Thus, the need for a sample thin enough for X-ray transmission is avoided and mm-scale anatomical features can be analyzed directly with submicron-scale resolution on fully mineralized rock thick sections (Fig. 2). For X-PEEM, a negative voltage applied to the sample accelerates the emitted electrons into an electron optics system where they are focused directly to produce an image of the sample. In this study, the application of X-PEEM to permineralized fossils is explored. Focus is on the tissue-specific chemistry of arborescent lycopsid cell walls and the nature of organic matter preservation in calcite coal balls.

2. Material and methods

Intact coal balls from the bituminous Herrin No. 6 coal, Sahara mine, of Illinois (Phillips and DiMichele, 1981) were obtained from the collections of the University of Illinois at Chicago and cut on a water saw. Areas of interest within a calcite permineralization of a partially crushed *Lepidodendron* sp. axis were cut into roughly 8 mm square sections approximately 1 mm thick. These were highly polished with 0.05 μm alumina grit in water suspension. After ultrasonic cleaning in ethanol and distilled water, the sample surface was sputter coated with a conductive platinum layer 500 Å thick. Measurements were taken through smaller observation windows coated with only 10 Å of platinum (De Stasio et al., 2003; Fig. 2). This preparation allowed for analysis of the primary xylem, secondary xylem, periderm, and some thin-walled tissue of indeterminate identity peripheral to the periderm.

X-PEEM analyses were performed using SPHINX (Spectromicroscope for the PHotoelectron Imaging of Nanostructures with X-rays) on the HERMON (High Energy Resolution MONochromator, 62–1360 eV) beamline at the University of Wisconsin-Madison Synchrotron Radiation Center. Samples were analyzed at the carbon K-edge, while kept at room temperature, a potential of −20 kV, and a base pressure of 10^{-10} Torr. Images were taken between 275 and 325 eV with 0.1 to 0.5 eV step sizes. Spectra from areas of interest were obtained from stacked images through the energy range using Image J software (Abramoff et al., 2004). Baseline I_o values for normalization of spectra were attained from thick coated areas of the sample.

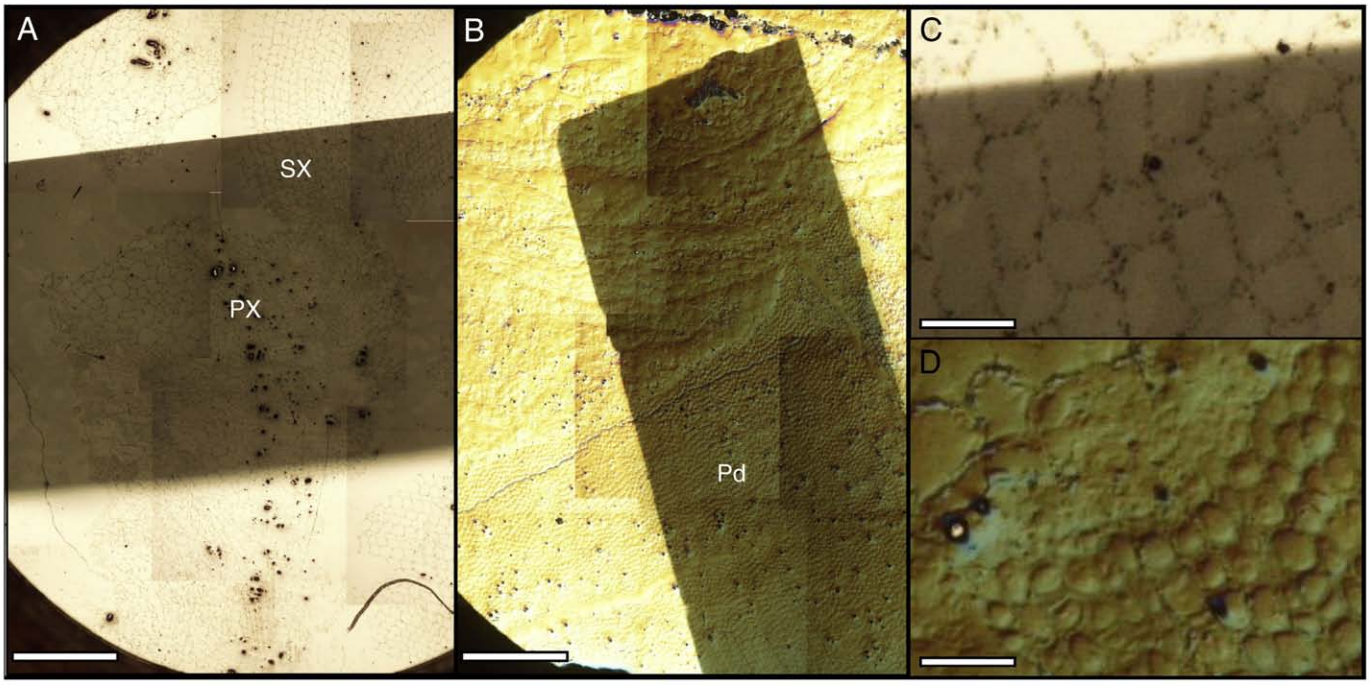

Fig. 2. Coal ball samples prepared for X-PEEM showing *Lepidodendron* primary (A) and secondary (A, C) xylem and periderm (B, D). Primary xylem (PX), secondary xylem (SX), and periderm (Pd) labeled in A and B. Lighter areas of specimen indicate thicker platinum coating and darker central rectangles are the windows of thinner coating where analyses were actually performed. Scale bars equal 1 mm in A and B and 100 μm in C and D.

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3. Results/discussion

3.1. STXM vs. X-PEEM for fossil analyses

Measurement of X-ray transmission through the sample and electron emission from a sample surface each has advantages for XANES-based fossil study. The ultramicrotoming of samples for STXM analysis allows more precise orientation and observation of specific cell structures like macerated spores and cellular debris (W. A. Taylor and C. K. Boyce, unpublished data). In addition, STXM analyses are free of the patchy shielding of organic matter by the mineral matrix that can lead to a discontinuous image of fine subcellular detail in X-PEEM (Fig. 3). However, X-PEEM dramatically improves the ability to understand complex tissues with multiple cell types because chemical information from a wide field of study encompassing many cells can be acquired simultaneously. X-PEEM also allows the analysis of organic matter–mineral interactions because no demineralization is required. However, X-PEEM must be conducted under ultrahigh vacuum in order to ensure a clear path for the electrons accelerated from the sample into the optics system, while carbon K-edge STXM only requires operation under a positive pressure of helium to ensure that X-rays are not scavenged by atmospheric CO_2. Hence, X-PEEM requires a lengthy vacuum pumping with each sample change while STXM can have a much higher sample throughput.

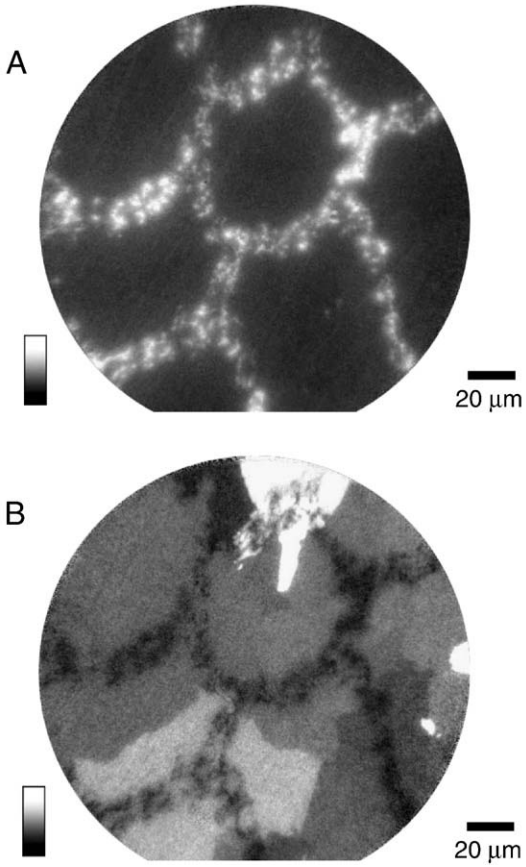

Fig. 3. X-PEEM images of *Lepidodendron* secondary xylem showing distribution of organics and calcite. A, Division map of 288.7 eV image divided by the pre-edge image, showing distribution of the C=O π* bond of carboxylate (white). B, Division map of 290.5 eV image divided by the pre-edge image, showing distribution of C=O π* bond of carbonate (intensity is sensitive to crystal orientation). Individual calcite crystals (each identified by a discrete shade of gray) infiltrate and pass through cell walls (dark in B). Because X-PEEM analyzes just the sample surface, cell walls appear to be disrupted by calcite and fragmentary even though they would be recognized as intact if viewed with transmitted light through the continuous 30 µm thickness of a thin section. Gray-scale indicated with vertical bars. Horizontal scale bars equal 20 µm.

XANES spectra from X-PEEM analysis of coal ball fossils all show less aromatic carbon than seen previously with STXM-based XANES analyses of plant fossils (Cody et al., 1996; Boyce et al., 2002, 2003). If limited to lycopsid periderm samples, low aromaticity could reflect an aliphatic original biochemistry, but X-PEEM analyses of lycopsid wood also show lower aromatic content than previous STXM analyses of other fossil woods that likely had comparable original lignin content (Boyce et al., 2002, 2004). A greater prevalence of aromatic carbon could result from beam damage induced by the analysis itself, a common cautionary concern (e.g. Ade et al., 1992; Beetz and Jacobsen, 2003; Flynn et al., 2004; Brandes et al., 2004; Metzler et al., 2008a,b; Cody et al., 2008; Bernard et al., 2009). X-PEEM on a bending magnet beamline entails much less risk of X-ray damage of the sample than undulator-based STXM. Also, the calcite matrix of a mineralized fossil sample for X-PEEM might better dissipate the potential damage caused by beam-generated ions than the embedding epoxy in a demineralized fossil sample for STXM. The platinum coating of X-PEEM samples might also afford similar protection not available to uncoated STXM samples. Even if STXM beam damage was shown to be a problem, it would not invalidate previous conclusions drawn within a comparative framework of STXM analyses that all involved similar exposure, but would instead highlight the need for caution in interpreting individual spectra in isolation. Sequential analysis with X-PEEM and STXM of the same sample would be conclusive. However, solid state ^{13}C NMR of bulk macerated Rhynie Chert organic matter shows an aromatic dominance (G. D. Cody and C. K. Boyce, unpublished data) in keeping with STXM results from the same material (Boyce et al., 2003), suggesting that STXM beam damage has not been a significant factor and the chemical differences between fossil samples reflect varying geologic history and original biology.

3.2. Fossil preservation in coal balls

X-PEEM provides the ability to distinguish organic matter and mineral matrix and investigate both of them simultaneously. As an example of the complications bypassed with X-PEEM, cell walls preserved in carbonate permineralizations that appear to be organic matter-rich in thin section under visible light may represent carbon content minima with electron microprobe mapping of elemental abundances (Scott and Collinson, 2003). Because organic matter can disrupt the fabric of matrix minerals and lower the abundance of matrix-prevalent elements (Boyce et al., 2001), an electron probe analysis indicating lower carbon content in the cell walls of a fossil preserved in carbonate could signify that organic matter either has been entirely replaced or that it is still present, but in quantities insufficient to offset the disruption of the carbon-rich calcite. Thus, other mineralogical techniques are ultimately more informative. With X-PEEM, however, X-ray absorption by calcite has two discrete major peaks at 290.5 and 302 eV. Because the absorbance at these two peaks is dependent on crystal orientation, crystal fabric can be studied in detail along with the local organic matter. As has been previously demonstrated in silicified permineralizations (Boyce et al., 2001), the calcite matrix is often seen to significantly infiltrate the cell walls, resulting in a patchy shielding of the organic matter that can disrupt the apparent continuity of cell walls. However, both organic matter and matrix calcite are distinguishable phases that can be analyzed simultaneously with the X-PEEM technique (Fig. 3).

Rather than being confined to the cell walls, chemically distinct organic matter is also found in cell cavities (Figs. 4 and 5) even though traces of lumen organic matter have not been noted in previous studies. Such organic traces may have been present, but at carbon concentrations too low to be detectable using an electron probe against a higher molecular weight matrix containing abundant calcium or silica (Boyce et al., 2001; Scott and Collinson, 2003; Boyce et al., 2007) and might simply have been washed away during the generation of the demineralized acetate peels used in previous STXM-

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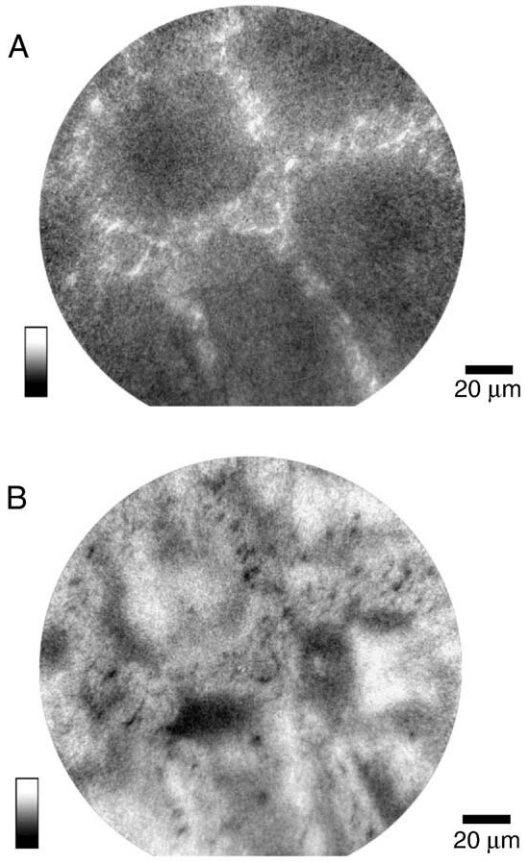

Fig. 4. X-PEEM images of *Lepidodendron* secondary xylem at, A, 286.7 eV, with absorbance reflecting either C–H σ* or C=C π* bonds and at, B, 288.7 eV showing the distribution of the C=O π* bond of carboxylate. Organic traces chemically distinct from the cell walls are present in cell lumens despite tracheids being devoid of living cell contents at maturity. Gray-scale indicated with vertical bars. Horizontal scale bars equal 20 µm.

based XANES studies of fossils (Boyce et al., 2002, 2003). Fossil preservation can be so superlative as to reveal intact organelles and cytoplasm (Millay and Eggert, 1974; Nishida et al., 2003; Hagadorn et al., 2006), but such an interpretation would not apply here where lumen organic matter is seen even in tracheids (albeit at somewhat lower concentrations), which are dead and evacuated of cell contents when functional. Imaging of this organic matter also does not reveal anatomically interpretable microstructure (Fig. 4). Rather, these traces of lumen organic matter entrained into the calcite crystal fabric presumably include microbial decay products and may represent the capture by early mineralization of an initial stage of the filling of cell cavities with colloidal organic material at the expense of the degradative loss of the cell walls as can be seen in peats and coals (Teichmuller, 1989; Scott, 2002).

X-PEEM analyses also reveal an interesting difference concerning the calcite spectra, although one that is not yet understood. Minor peaks at ~296 eV and ~298 eV, characteristic of calcite, are suppressed in the periderm and the primary xylem (Fig. 5B). These minor carbonate peaks were previously observed to be suppressed upon organic-molecule binding in calcite (Metzler et al., 2008b) and aragonite (Zhou et al., 2008) and were also suppressed within bacterial cells in a modern aragonitic microbialite even though prominent in spectra of the surrounding matrix (e.g. Fig. 4d in Benzerara et al., 2006). This variable peak suppression, thus, implies a greater mineral–organic binding in the periderm and primary xylem, compared to secondary xylem and peripheral tissue. In theory, such differences in mineral–organic interactions could be due to a number of factors: original tissue biochemistry or its decay products, organic matter abundance, or even tissue geometry and cell size and shape, whereby proximity to cell wall material is important or elongate cells act as conduits to the surrounding environment and facilitate a more open system. Given the different behavior of primary and secondary xylem, however, a unitary explanation may be unlikely and proximal causes may have been more complex or idiosyncratic. Nonetheless, the preservation over 300 Ma of the organic matter–calcite coordination involved in peak suppression is striking.

The permineralization process typically involves minerals precipitating directly on the organic matter of the incipient fossil (Leo and Barghoorn, 1976; Scott and Rex, 1985; Knoll, 1985; Scott et al., 1996; Xiao and Knoll, 1999; Briggs, 2003) and dispersed organic matter can influence crystal size and shape and stabilize energetically unfavorable phases (Benzerara et al., 2006). What specific role the original plant biochemistry, the microbial activity it fostered, and the decay products produced might have played in crystal nucleation, growth rate, and stabilization remains unclear, but such organic matter/mineral interactions are readily subject to X-PEEM investigation (e.g. De Stasio et al., 2005; Gilbert et al., 2005; Metzler et al., 2007; Zhou et al., 2008; Politi et al., 2008; Gilbert et al., 2008) and await broader tissue-specific investigation of the coal ball flora.

3.3. Organic differences

X-PEEM analyses conducted here are consistent with previous analyses of lycopsid periderm (Logan and Thomas 1987; Collinson et al. 1994) in that a mixture of aliphatic and aromatic carbon is indicated, however our within-sample comparisons indicate that the aromatic content is no more than that seen in other tissues of the same fossil and that it is specifically the elevated aliphatic content that is distinctive of the periderm (Fig. 5). The overall abundance of organic matter per unit area is also higher in the cell walls of the periderm than in those of other tissues, which is consistent with anatomical arguments for enhanced decay resistance (Phillips and DiMichele, 1992) that was presumably due to impregnation of the polysaccharide walls with a more recalcitrant biopolymer.

The prevalence of aromatic carbon in all tissues investigated suggests that this aromaticity is not exclusively derived from originally aromatic biochemistry, but reflects the cyclization of originally aliphatic compounds. However, some of the tissues investigated were undoubtedly lignified and the others have no homologues among living plants and, thus, are of unknown original composition. Because arborescent lycopsids are so far removed from the structural organization of living plants, a wider array of within-locality comparisons involving fossils with more anatomically similar living relatives and less ambiguous tissue homologies will be needed to assess what proportion of the aromaticity of Carboniferous coals might have been inherited from the original biomass.

Cell walls in the corky layers of the bark of living seed plant trees are typically infiltrated with the mixed aliphatic/aromatic polymer suberin. The elevated aliphatic content of the lycopsid periderm is at least consistent with a suberin derivation, as is its greater decay resistance and abundance of organics. However, since the lycopsid periderm has no extant homologues, a link to suberin is tenuous without broader tissue comparisons from local fossils sharing the same diagenetic history. Plants are adept at production of secondary metabolites and an original biochemistry that is now extinct is a possibility, but tissue comparison within the same coal flora with the bark of fossil seed plants, for which a high suberin contribution was likely, should help constrain the biochemical sources of the lycopsid geochemistry. At least in the Carboniferous, the aromatics that are often attributed to lignin derivation and the aliphatics that are attributed to algae and other sources might have a common source in the biochemistry of an unusual but abundant vascular plant and a far greater proportion of the original biomass may have been preserved than would be expected from lignin enrichment scenarios alone.

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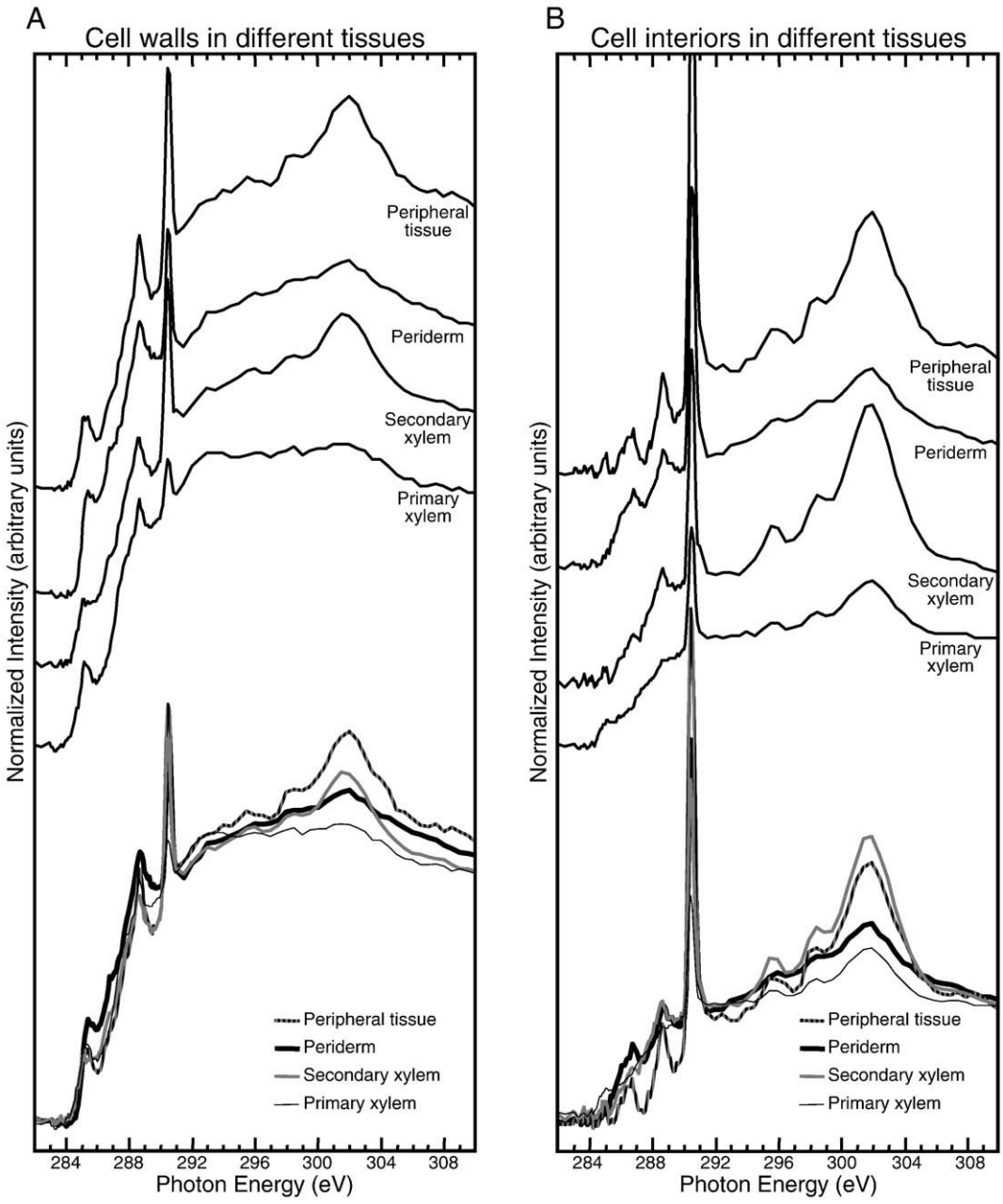

Fig. 5. X-PEEM-derived C-edge XANES spectra of *Lepidodendron* tissues focusing on cell walls and cell lumens. All analyses show peaks at 290.5 eV and 302 eV reflecting calcite. A, All cell walls show peaks at 285 eV (C=C π*) and 288.7 eV (C=O π* of carboxylate). Shoulders at 286.7 eV (either C–H σ* or C=C π*) and 287.9 eV (C–H σ*) indicate higher aliphatic content most prevalent in periderm. B, The organic matter embedded in cell lumen calcite variably includes high absorbance at 286.7 eV (either C–H σ* or C=C π*) and 288.7 eV (C=O π* of carboxylate), and lesser absorbance at 285 eV (C=C π*). Characteristic calcite peaks at 296 eV and 298 eV are suppressed in the primary xylem and periderm.

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