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Climate, decay, and the death of the coal forests

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After death, most of the biological carbon in organisms (C_{org}) is returned to the atmosphere as CO_2, through the respiration of decomposers and detritivores, or by combustion. However, the balance between these processes is not perfect, and when productivity exceeds decomposition, carbon sequestration results. An unparalleled interval of carbon sequestration in Earth's history occurred during the Late Carboniferous (Pennsylvanian) and Permian Periods (ca. 323-252 Mya), when arborescent vascular plants related to living club mosses (Lycophytes), ferns (Monilophytes), horsetails (Equisetophytes) and seed plants (Spermatophytes) formed extensive forests in coastal wetlands. On their death, these plants became buried in sediments, where they transformed into peat, lignite, and, finally, coal.

The botanical origin of coal is not disputed, but the causal factors that determined the rate of C_{org} sequestration and that limited the extent of coal forests are matters of debate. One explanation is that abiotic factors were solely responsible for shifts in rates of C_{org} burial. Under this view, the high rate of carbon sequestration during the Permo-Carboniferous was caused by unusually widespread mire environments with anoxic, waterlogged conditions, which inhibited decay, and contractions in coal forests were caused by climatic shifts toward drier conditions.

An alternative hypothesis, proposed by Robinson, introduced, in addition to geological and environmental factors, the concept that biological interactions among organisms might also be important in coal formation. Specifically, the dramatic accumulation of C_{org} in the Permo-Carboniferous occurred in part because the fungi that are able to efficiently decompose lignin (a recalcitrant, heterogeneous plant polymer) had yet to evolve and diversify. Robinson also suggested that coal-age spore-bearing plants had an unusually high lignin content, compared to the seed plants that would eventually replace them as dominant forest trees. This hypothesis was based on a limited fossil record of fungi, with liberal extrapolation to extant taxa. Here, we evaluate the Robinson hypothesis by considering the diversity and evolution of fungal decay

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mechanisms, which can now be addressed through comparative genomics, as well as the evolution of plant cell walls (PCWs). Historical inferences based on living species must also be reconcilable with the paleobotanical record, and ultimately should both inform and be tested by increasingly sophisticated Earth System models.

PCW decay in contemporary ecosystems

The major structural components of PCWs include lignin, cellulose, and hemicellulose. Lignin forms a dense matrix that protects the carbohydrates from enzymatic attack, but cellulose itself is also resistant to decay, particularly in its highly ordered crystalline form. Wood, which is a major pool of terrestrial organic carbon, is heavily lignified secondary xylem, but lignin is also present in primary xylem, sclerenchyma (including fibers), and periderm (protective tissue of woody stems and roots, including "bark"), which also contains the waxy polymer suberin. Cellulose occurs in cyanobacteria and is widespread in eukaryotes, whereas lignin is restricted to land plants (and possibly red algae). Thus, PCWs are complex mixtures of ancient and more recently-evolved components that collectively present a formidable barrier to microbial attack.

Although there are many agents both biotic and abiotic that attack PCWs, fungi are the major decomposers in terrestrial ecosystems. In aquatic systems, detritus and wood may also be attacked by fungi, but as sediments accumulate and oxygen levels decrease, bacterial degradation becomes the primary factor influencing decomposition. Fungal wood decay is classified into three general types: white rot, brown rot and soft rot (Figure 1). White rot and brown rot fungi (e.g., polypores, crust fungi, and other Basidiomycota) are the dominant decomposers of woody substrates (particularly in temperate ecosystems). White rot fungi are able to degrade all PCW components, including lignin; some species degrade all components sequentially, whereas others have a more selective attack, degrading lignin and hemicellulose preferentially and leaving pockets of degraded wood filled with cellulose.

White rot has been studied intensively, particularly in the model system *Phanerochaete chrysosporium*, because of its potential industrial applications. The best-characterized lignin-degrading enzymes are class II fungal peroxidases, including manganese-, lignin- and versatile peroxidases, which are derived from non-ligninolytic “generic peroxidases”. Other enzymes with demonstrated or potential ligninolytic activity include dye decolorizing peroxidases (DyP), heme-thiolate peroxidases (HTP), and multicopper oxidases (laccases). White rot fungi also produce diverse Carbohydrate-Active enzymes (CAZymes) acting on crystalline cellulose, including cellobiohydrolases, lytic polysaccharide monooxygenases (LPMO), and endoglucanases. Other CAZymes of white rot fungi digest amorphous cellulose, hemicellulose, pectin and other

substrates. Most litter-decomposing basidiomycetes have a similar, albeit somewhat reduced, repertoire of decay enzymes.

Brown rot fungi produce a diffuse attack that causes rapid depolymerization of cellulose via oxidative mechanisms, but lignin is not appreciably degraded. In advanced stages, the decayed residue has a friable texture and consists primarily of chemically modified lignin. Brown rot fungi lack ligninolytic class II peroxidases and have reduced complements of CAZymes, particularly cellobiohydrolases, compared to white rot fungi. The white rot and brown rot categories adequately describe most wood decaying basidiomycetes, but some taxa cannot be differentiated clearly into these groups based on their degradative enzymes, and it has been suggested that there is a continuum of decay modes that falls between the two groups.

The term soft rot was first coined for an unusual decay mode found in waterlogged woods caused by Ascomycota. Although originally found in aquatic systems, soft rot is the dominant type of decay in extreme terrestrial environments, such as dry deserts and polar regions, and is frequently found in tropical forest ecosystems. Two forms of soft rot are recognized. In Type I soft rot, hyphae penetrate the secondary walls of wood cells and produce cavities; in advanced stages, little of the secondary wall layers are left, most of the cellulose is degraded, and remaining material consists primarily of lignin. In Type II soft rot, an erosion of the secondary cell wall occurs that is somewhat similar to decay by simultaneous white rot fungi, but degradation is localized. In both Type I and II soft rot, the middle lamella between cells is not degraded. The mechanisms of soft rot by ascomycetes, and their overall contribution to lignocellulose decay, are not as well understood as those of white rot and brown rot basidiomycetes.

Phylogenomics and evolution of PCW decay in fungi

Comparative genomic analyses focused on Basidomycota has elucidated the evolution of decay modes. A study of the evolution of 27 gene families encoding CAZymes and decay-related oxidoreductases in 31 fungal genomes showed that most of the gene families were broadly distributed across Ascomycota and Basidiomycota, but ligninolytic class II peroxidases were restricted to white rot Agaricomycetes (mushroom-forming Basidiomycota). Gene tree/species tree reconciliation analyses suggested that diversification of these ligninolytic enzymes occurred early in the evolution of Agaricomycetes, along with expansions in about eight to ten other, older groups of decay-related enzymes (e.g., DyP, HTP, laccase, LPMO, and various CAZymes), suggesting a general elaboration of the PCW-decay apparatus. The polyphyletic evolution of brown rot fungi was associated with parallel reductions in both lignin- and cellulose-degrading

enzymes. Expanded sampling of genomes has improved resolution of the origin of white rot. The oldest lineages of Agaricomycetes (Cantharellales and Sebacinales), and all other Basidiomycota, lack ligninolytic class II peroxidases, which are first reconstructed in the common ancestor of Auriculariales, a group of jelly fungi including “wood ear” mushrooms, and other more derived Agaricomycetes (Figure 2A).

Bayesian relaxed molecular clock analyses have suggested that the Auriculariales lineage arose at about 290 Ma, but the 95% highest posterior density (hpd) interval of ages ranged from 222 to 372 Ma. The common ancestor of Ascomycota and Basidiomycota was estimated at 660 Ma (95% hpd, 500-810 Ma). These age estimates are potentially sensitive to the choice of fossils used to calibrate the molecular clock, and they have considerable uncertainty. Nevertheless, the general picture emerging suggests that the ability to degrade cellulose and hemicellulose dates back at least to the Cambrian Period, and was present in the ancestor of Ascomycota and Basidiomycota, but ligninolytic systems comparable to modern white rot arose more recently within the Agaricomycetes, possibly during the Permo-Carboniferous.

Paleobotanical evidence for origins of lignin, wood, and decay

Fossil evidence suggests that lignified plant tissues evolved long before white rot in Agaricomycetes. The first direct fossil evidence of tracheids (the basic cell type of both primary and secondary xylem) comes from Early Devonian rocks (411-419 Ma), but the record of dispersed spores suggests that vascular plants may have evolved by the latter part of the Ordovician Period (444-450 Ma). Chemical analysis of the oldest fossil tracheids does not provide an unambiguous signal for lignin, because diagenesis has altered their chemical structure, but preferential preservation of tracheids indicates that they were chemically distinct and more robust than most other tissues. Moreover, lignin is present in tracheids of all living vascular plants, so it is most likely that it was present in the earliest tracheids. Early vascular plants were small, simple, and herbaceous, but by the Middle Devonian (393–383 Ma) forests containing trees exceeding 8 m in height had evolved.

Coal-swamps were formed from the Carboniferous into the Late Permian and contained five major tree groups that differed widely in morphology and ecological tolerances. The dominant plants of wet to flooded environments of the early Pennsylvanian were tree lycopods, which grew to 30 m in height and could exceed 1 m in diameter at the base. Tree lycopods had massive periderms, but unlike woody trees today, secondary xylem accounted for only a small proportion of stem diameter. In periodically drier environments, woody shrubs or small trees

related to conifers (*Cordaites*) predominated. Marattialean tree ferns, medullosan pteridosperms (seed plants resembling modern tree ferns), and *Calamites* (arborescent relatives of modern horsetails) were also significant components of coal-swamp forests. A variety of smaller ferns and seed plants formed ground cover, vines, and lianas.

All plants contributed to the formation of coal, but their relative proportions and the importance of particular organs and tissues systems varied on both temporal and spatial scales. Tree lycopods were the major contributors to peat during the Early Pennsylvanian of Euramerica (60% - >80%) and periderm was their most abundant tissue, making up 20% to 45% of peat biomass; other contributing tissues and organs included leaves, wood, and spores. The woody *Cordaites* attained dominance in some eastern Mid Pennsylvanian coals in the USA, whereas the non-woody marattialean tree ferns predominated in Late Pennsylvanian coals of Euramerica. The plants and organ systems from which these coals were derived were distinct from those of Late Cretaceous and Cenozoic coals, which had larger inputs from wood and seeds.

The abundance of periderm in coal suggests that it was decay resistant and relatively impervious, but aspects of its chemistry are poorly understood. Diagenesis alters the original chemical structure of polysaccharides and polyphenols, so analytical approaches must draw inferences from the breakdown products detected. Independent analyses by pyrolysis-gas chromatography and x-ray photoelectron emission spectromicroscopy (X-PEEM) show that periderm residues are a mixture of aliphatic and aromatic carbon. X-PEEM indicates an elevated aliphatic component and an aromatic content no greater than other tissues, arguing against the presence of lignin. These signals are consistent with but not diagnostic of suberin, which is a major component of cork in periderms of extant plants.

Fossil fungi are not as well documented as fossil plants, and the fossil record of decay is particularly limited. The earliest evidence of alteration of tracheid cell walls by fungi comes from petrified progymnosperm wood in the Upper Devonian (360 Ma). This is suggestive of some capacity to decompose lignin, but the taxonomic identity of the fungi is unknown. Fossils resembling modern white pocket rot occur in seed ferns from the Permian (260 Ma) and gymnosperms from the Triassic (230 Ma). Thus, the fossil record suggests that white rot was probably widespread by the beginning of the Mesozoic Era, and possibly much earlier.

Earth System modeling of C_{org} sequestration and historical shifts in coal formation

Coal formation begins in peat-forming wetlands and mires. When plants are buried rapidly, or when the environment is anoxic, a combination of dehydration and carbon enrichment

(carbonization) transforms lignin and other recalcitrant organics to lignite, subbituminous, and finally bituminous coals. The entire process takes hundreds of millions of years, but the lignite stage can be reached after only about 1 million years. Coal formation has played a major role in the sequestration of C_{org} over geological time (Figure 2B), resulting in a net output of carbon from the surface system and a net oxygenation of the environment, as the sequestered carbon avoids the back-reaction with oxygen (respiration).

It is possible to estimate the worldwide rate of organic carbon (C_{org}) sequestration over geological time by analysing the relative abundance and carbon content of coal basin sediments, marine shales, and non-marine clastics. Results of such analyses suggest an approximate doubling of the C_{org} burial rate over the duration of the Carboniferous period, and a slow return to near-modern values during the Permian (Figure 2C).

An independent method for estimating C_{org} sequestration rates utilizes the isotopic record of marine carbonates (i.e., $\delta^{13}C$). Burial of large amounts of isotopically-depleted organic carbon (e.g., ^{12}C enriched coal) drives the isotopic ratio of carbon stored in the atmosphere and oceans towards heavier values. For this reason, periods of high carbonate $\delta^{13}C$ are taken to reflect times of increased C_{org} burial. Box models of global carbon cycle processes can be used to 'invert' the $\delta^{13}C$ signal to quantify the required rate of C_{org} sequestration, using a technique called isotope mass balance. Reconstructions of C_{org} burial rates suggest a similar rise over the Carboniferous and decline over the Permian as the rock abundance method (Figure 2C).

A third approach to estimating historical C_{org}, embodied in the COPSE (Carbon Oxygen Phosphorus Sulphur Evolution) model, relies on forwards modeling of coupled carbon and nutrient cycles. The dynamics of the COPSE model are governed by internal processes, rather than geological data such as isotopes or rock abundances. The results are consistent with those based on rock abundances and carbon isotopes. However, the model fails to reproduce geochemical data for the Permo-Carboniferous unless it is 'forced' by an increased rate of carbon sequestration. Such forwards modelling approaches allow for the investigation of evolutionary changes in the biotic environment, although many processes, including explicit representation of organic matter production and decay, are not currently included

Conclusions and future research

The historical pattern of C_{org} sequestration is the net result of biotic and abiotic processes operating over intracellular to global scales. Extrapolating from contemporary ecosystems, it is reasonable to suggest that the origin of white rot impacted the rate of decomposition in early forest communities, and that this could have affected coal formation. Genome-based molecular

clock estimates are consistent with the view that the evolution of white rot fungi was a contributing factor in the decline in C_{org} burial at the end of the Permo-Carboniferous, as suggested by Robinson. However, there are numerous sources of ambiguity regarding the Robinson hypothesis, beginning with uncertainty in age estimates for white rot fungi. Expanded sampling of fungal genomes has increased confidence that ligninolytic class II peroxidases evolved early in Agaricomycetes, around the time of divergence of Auriculariales, but the absolute age of this node is not resolved with precision. Additional fossils would enable much-needed independent estimates of the age of origin of white rot.

Much of the discussion on carbon sequestration has focused on lignin, but other plant polymers, particularly suberin, cutin, and sporopollenin, appear to have been important components of coal. A few reports have suggested that certain ascomycetes and basidiomycetes possess cutinases (carbohydrate esterase family CE5) that can degrade suberin. Sequence comparisons suggest that ascomycete and basidiomycete CE5 genes are homologous, implying that suberin decay could be very ancient, but additional genome-based studies are required to understand the diversity, functional biology, and evolution of these and other potentially important enzymes. Ultimately, a holistic approach to the evolution of decay is needed, assessing the synergistic actions of diverse fungal enzymes on all PCW components. In this context, it is important to note that the early evolution of Agaricomycetes was marked by expansions in multiple decay-related gene families, not only class II peroxidases.

Functional and evolutionary genomic studies in fungi can make predictions about patterns of decay that should be observable in the paleobotanical record. However, despite the abundance of permineralised woods in museum collections, wood decay in Paleozoic ecosystems is poorly documented. Systematic surveys focusing on the nature of the fungal evidence and its impact on tracheid cell walls are also needed to test genome-based inferences. Such surveys must have sufficient sampling to assess prevalence, not merely presence, of different decay types, including soft-rot, which is potentially plesiomorphic for Ascomycota and Basidiomycota. The high lignin content of Late Paleozoic ecosystems proposed by Robinson is a conjecture that seems increasingly unlikely (based on geochemical analyses of periderm) and which also requires further critical analysis.

Current Earth System models confirm that global coal deposition increased greatly during the Carboniferous, fell during the Permian, and has subsequently proceeded at a much reduced rate, but they are unable to elucidate the mechanisms responsible for the apparent rise and fall in C_{org} sequestration. Of the current approaches, the COPSE model's dynamic biosphere component has the most potential for extension to include the evolution of fungal

decay capabilities. As understanding of decay in paleoecosystems improves, it should become possible to use a forwards modeling approach to resolve the factors that have modulated C_{org} sequestration over geologic time and that caused the demise of the Permo-Carboniferous coal swamp ecosystems.

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Figure 1. Characteristics of fungal wood degradation.

Scanning electron micrographs (A-D) of transverse wood sections showing white rot (A and D), brown rot (B) and soft rot (C). White rot causes a thinning of cell walls and an erosion that removes all cell wall layers resulting in complete degradation of some cells (A). Diffuse attack caused by brown rot fungi leaves a residue of degraded cells that consist primarily of lignin with little cellulose remaining (B). Type I soft rot produces numerous small cavities formed in the secondary cell walls (C). Selective attack by a white pocket rot fungus results in delignified cells where the middle lamella between cells is degraded but the cellulose-rich secondary wall remains (D, used with permission Annu. Rev. Phytopathol. *29*, 381-403). Tangential section of contemporary wood showing white pockets of cellulose left after decay (E). Transverse section of fossil *Araucarioxylon* wood from the Triassic Period with white pocket rot (F). Bar = 10 µm in A to D, 2 cm in E and F.

Figure 2. Fungal evolution (A), abundance of coal basin sediments over the Phanerozoic (B) and model predictions for the rate of C_{org} burial (C).
Tree figure (A) summarizes multiple phylogenomic analyses with overlapping sampling. Black and gray triangles indicate clades with or without homologous ligninolytic class II peroxidases (respectively), and red bars indicate 95% hpd intervals on age estimates from Bayesian molecular clock analyses (where applicable). The majority of coal reserves were deposited during the Carboniferous and Permian periods, but coal formation is an ongoing process (B). Coal basin sediments have been found throughout the Mesozoic and Cenozoic, right up to the present day, although the abundances are substantially lower than during the Permo-Carboniferous. Analyses using the rock abundance model (BC89, dashed blue line), isotope mass balance (GEOCARBSULF, solid blue line), and forwards modelling (COPSE, green line) yield similar predictions of C_{org} burial rates.

Figure 1

Click here to download Figure Fig1.tif

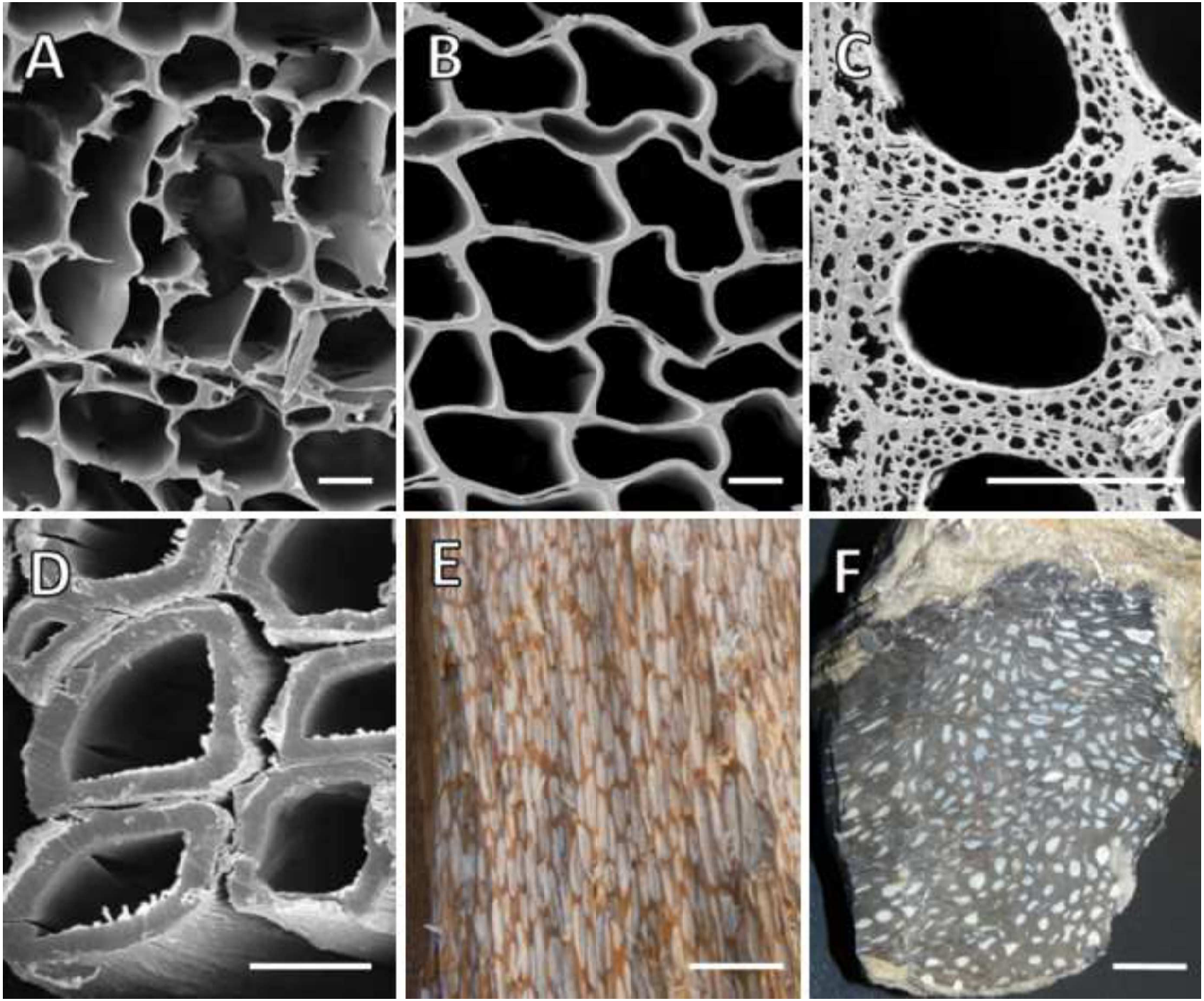
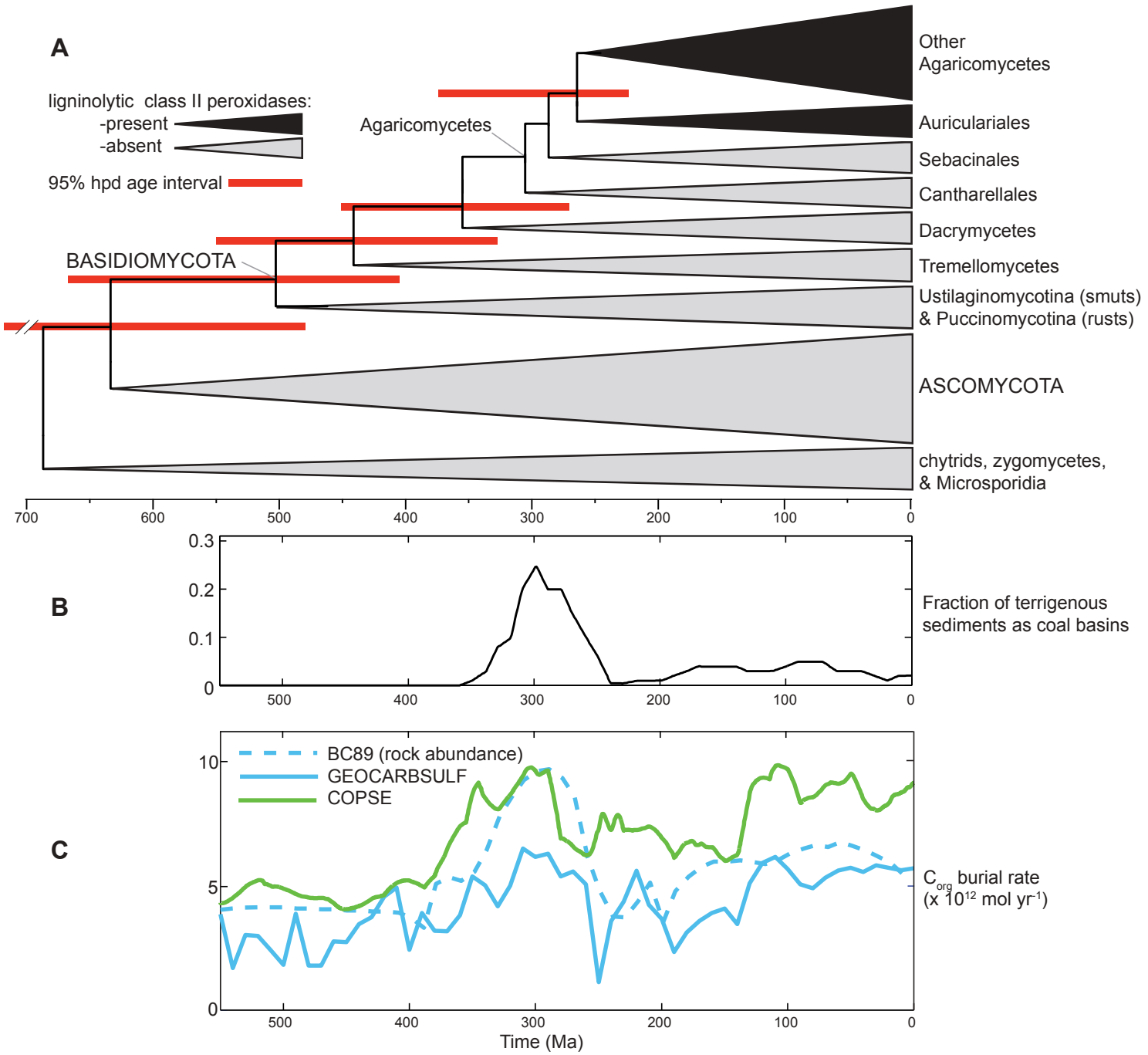


Figure 2


A
ligninolytic class II peroxidases:
-present
-absent
95% hpd age interval
Agaricomycetes
BASIDIOMYCOTA
Other Agaricomycetes
Auriculariales
Sebacinales
Cantharellales
Dacrymycetes
Tremellomycetes
Ustilaginomycotina (smuts) & Puccinomycotina (rusts)
ASCOMYCOTA
chytrids, zygomycetes, & Microsporidia
700
600
500
400
300
200
100
0
B
0.3
0.2
0.1
0
Fraction of terrigenous sediments as coal basins
C
BC89 (rock abundance)
GEOCARBSULF
COPSE
10
5
0
C_{org} burial rate (x 10^{12} mol yr^{-1})
Time (Ma)