Molecular identification of fungi microfossils in a Neoproterozoic shale rock.

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ABSTRACT

Precambrian fossils of fungi are sparse and the knowledge of their early evolution as well as the role they played in the colonization of land surface are limited. Here, we report the discovery of fungi fossils in a dolomitic shale from the 810-715 Myr old Mbuji-Mayi Supergroup (MMS), Democratic Republic of Congo (DRC). Syngenetically preserved in a transitional, subaerially-exposed paleoenvironment, these carbonaceous filaments of ~5 µm in width exhibit low frequency septation (pseudosepta), and high-angle branching that can form dense interconnected mycelium-like structures. Using an array of microscopic (SEM, TEM and confocal laser scanning fluorescence microscopy) and spectroscopic techniques (Raman, FTIR and XANES), we demonstrated the presence of vestigial chitin in these fossil filaments and document the eukaryotic nature of their precursor. Based on those combined evidences, these fossil filaments and mycelium-like structures are identified as remnants of fungal networks and represent the oldest, molecularly-identified remains of Fungi.

INTRODUCTION

Fungi are an eukaryotic kingdom that performs critical roles in the soil ecosystem(1). By forming vast microscopic filamentous networks -mycelium- in symbiosis with the roots of most plants - mycorrhiza-, fungi can enhance rock weathering(2) and help the nutrient supply of plants particularly in young, poorly-evolved soils. Because of these abilities, ancestral fungi were crucial partner of the first phototrophs that colonized land surfaces(3). Although, these associations are acknowledged as a requirement of terrestrial invasion, the timing of this evolutionary transition is largely unknown(4).

The Rhynie cherts (407 Ma)(5) with their superbly-preserved fungi are considered a milestone of fungal fossil record and early colonization of land. However, despite their Devonian age, fungal remains in Rhynie chert display a remarkable diversity(6) including members of Chytridiomycota, Blastocladiomycota, Glomeromycota, Mucoromycotina and Ascomycota. Accordingly, molecular clock studies have placed the divergence of the main groups of Fungi within the Meso-Neoproterozoic (4). The terrestrialization of fungi dates from sometimes between the Ordovician (443-485 Ma) and <800 Ma while the earliest obligate biotrophic Glomeromycota fossils date from 455-460 Ma(4). The large uncertainty in the timing of fungal evolution and their transition to land essentially stems from the scarcity and the ambiguous nature of the Precambrian fungi fossils which are notoriously difficult to distinguish from prokaryotic remains. A number of Precambrian remains (e.g., Caudosphaera, Hurionospora, Shuiyousphaeridium, Horodyskia, Tappania)(7-8), lichen-like
fossils from ~ 600 Ma as well as, filament fragments and spores from 0.9-1 Ga in Arctic Canada (10), mycelium-like structures found at Lakhanda group (11) or even older structure found in 2.4-billion- year-old basalt (12) were inferred to be of fungal nature, yet their conclusive attribution to Fungi remains problematic.

RESULTS

Geological setting

Here, we report the presence of filamentous networks (Fig. 1 and 2 and Fig. S1) attributed to Fungi in dolomitic shale rock of the MMS in the Sankuru-Mbuji-Mayi-Lomami-Lovoy Basin (SMMLL), south-central of Democratic Republic of Congo (13) (Sup. Fig S2). The filamentous fossils were identified in a thin section cut in BIIc8 level (Sup. Fig. S2) from a depth of 118.2 m in the Kanshi B13 drillcore sample BK457b, core 118/4. The fungal networks were found embedded (Fig.1F) in a homogenous mineral matrix preserved as cylindric filaments - as clearly visible in Figure 1C and 2. This '3-D' preservation results from an early dolomitic cementation (possibly due to schizohaline conditions (14), which hindered significant compaction during burial (Fig S3 and "Paleoenvironment and petrography of BIIc subgroup" in Suppl. Materials for details). This BIIc8 dolomitic shale is a member of the BII Group of the Mbuji-Mayi Supergroup (MMS) that dominantly consist of shaley and stromatolitic dolostones with dolomitic shale interbeds. The lower BII group (i.e., BIIa-BIIb) accumulated on a marine outer-to-inner ramp while the paleoenvironment of the upper BII (BIIc-BIIid Fig. S2) evolved periodically towards coastal, lagoon, perennial lacustrine pond with the deposition of a well preserved fossil assemblage (15) and the development of cyanobacterial mats and stratiform stromatolites (16). The transition to terrestrial paleoenvironments in the upper BIIc is further evidenced by (i) the presence of coated pisoids and vadose/meteoric cements related to subaerial exposures (Fig. S3) and (ii) a depleted, uniform light REE concentrations reflecting riverine water and continental sediment inputs (17) (Supplementary text “Paleoenvironment and petrography of BIIc subgroup”). The MMS deposition timeframe is constrained (13) with an Ar-Ar age of 882.2 ± 9 Ma on a dolerite sill at the BI/BII group contact (Fig S2). Within BIIc, the δ13C of carbonate rocks presents a large positive-to-negative excursion (±7‰) coeval to the Bitter Springs δ13C anomaly at ca 810 Ma while the age of overlaying Kabele-Kabenga conglomerate is estimated at 715 Ma (18) (Sup. Fig S2). Therefore, the estimated age of the fossiliferous dolomitic shales from BIIc8 is between ca 810 Ma and 715 Ma.

Microfossil morphology

We document dark, non translucide, cylindrical filaments typically between 3.5 µm to 11.5 µm in width (Fig. 1, 2 and Fig. S1), extending over several hundreds of microns in length (> 1 mm of
cumulated length in Fig 1D). These filaments sometimes evolve into dense interconnected networks of ~ 500 µm in diameter (Fig. 1B). In these mycelium-like structures, filaments exhibit multiple order, high-angle branching and possibly anastomosing filaments (Fig. 1 and 2 - Fig. S1), common features of fungal networks yet rare for prokaryotes. The width of filaments for extant and fossil fungi can easily range from 2 to >20 µm (19). Thus, the size of the fossil filaments observed here fits well with fungal dimensions. However, size alone cannot be a reliable criterion to distinguish fungal from bacterial remains as prokaryotes also form filaments with large dimensions. A previous study (15) of rocks from the Bie-BIIc6 interval reported putative diversified prokaryotic and eukaryotic assemblages typical for the Tonian period (1000-720 Ma) with abundant sphaeromorph and acanthomorph acritarchs. In the BIIc8 unit, three putative fungal species - *Eomycetopsis septata*, *Eomycetopsis cylindrica* and *Eomycetopsis rugosa* - were documented (20) (depth interval between 118.4 – 122.90 m i.e., 20 cm above our fossiliferous rocks at 118.2 m). They were described as cylindrical filaments aggregated in groups and parenchyma-like masses with septate filaments. Interestingly, at a later stage, those fossils first attributed to Fungi (20) were re-assigned to tubular sheaths of cyanobacteria (21). Thus, in order to unequivocally determine whether the filamentous networks that we describe here represent Neoproterozoic fungal or cyanobacterial remains, an in-depth chemical characterization is required.

**Chitin staining**

One of the major constituent of fungal cell wall is chitin, a biopolymer of β 1-4 bonded N-acetyl-glucosamine, which is abundant in fungi and more generally in animal taxa (i.e., ciliates, arthropods, chrysophytes, diatoms), yet absent in prokaryotic organisms such as Actinomycetes (which can also form mycelium-like structures). As such, chitin alone cannot be used as unique defining trait of fungi, however the chitin-producing organisms listed above are morphologically distinct from our fossils in that they do not form mycelial networks, the presence of chitin within our filaments would be a strong argument for a fungal affinity. Chitin can be preserved throughout geological time. It has been detected in insects in Oligocene shale (22), in fungi preserved in Cretaceous amber (23) and it was even found in a 505 million-year old marine sponge from the Burgess shale (24). Those discoveries support the idea that the main control of chitin preservation is not age but rather the environment, with organic matter-rich sediment being ideal for preservation (22). Here, we document the presence of chitin in the fossil filaments by staining with wheat germ agglutinin conjugated with fluorescein isothiocyanate (WGA-FITC), a highly specific dye of N-acetyl glucosamine trimers (19). WGA-FITC has been widely used to discriminate between fungal hyphae and filamentous prokaryotes in soils, sediments and rocks, as well as in cyanobacteria-fungus endosymbiosis (i.e., *Geosiphon-Nostoc*) and has been also applied as a fungal marker in Eocene basalt (19) and in Cretaceous
We used confocal laser scanning fluorescence microscopy, and observed that the WGA-FITC binds to extensive portions of the mycelial structure (Fig. 2). In the mycelium, WGA-FITC was detected in the cell wall of filaments which appear cylindrical (rounded cross section of filament in Fig 2C), suggesting a preservation in 3 dimensions of the remains. A few septa were stained as well (Fig. 2C-F), and on some instances what appear to be ‘incomplete septa’ (pseudosepta) that do not fully separate adjacent cells (Fig 3C and insert 3F). Confocal views also allow observing some putative anastomosis of fungal filaments (Fig 2C). In contrast to our observations in filamentous networks, staining was negative when WGA-FITC was exposed to inclusion of “non-fungal” organic matter in BIIc6 shale indicating that WGA-FITC maintained its high-specificity binding when exposed to ancient, mature organic matter (Fig. S4). Considering the fungus-like morphology, the co-localization/binding of the WGA-FITC with/to cell wall/septa and its specific affinity for chitin, we assert that the staining can be used as a clear indicator of chitinous remnants. Simultaneously, the detection of vestigial chitin in these filamentous fossils is a strong indication of their fungal origin.

Organic matter ultrastructure in fossil filament

Raman micro-spectroscopy on filaments (Fig. 3) revealed two broad peaks at ~1350 cm\(^{-1}\) (D) and at ~1600 cm\(^{-1}\) (G) typically associated with low structural order, amorphous carbonaceous matter (25). Deconvolution of those two spectral features provided D1, D3, D4 and G-bands (Fig. 3E) which are characteristic of organic matter that experienced advanced diagenesis or low-grade metamorphism(26). We calculated the peak temperature of the fossil filament (and of the dolomitic shale organic matter away from mycelium/filament structure) to range between 150°C-250°C (Fig. 3) which is also in line with the Raman-derived temperature calculated for asphaltite inclusions in various BIIc dolostone (27). Note that the dissolution of fungal hypha and chitin were shown both to start at much higher temperature ~250 – 300°C and is complete at 380-390°C (28). Here, considering the peak temperature experienced by the fossil filaments, it is likely that they retain some characteristics or structural compounds (e.g., chitin) that can be diagnostic of the original microorganism.

Recently, the Raman 1350 to 1600 cm\(^{-1}\) intensity ratio - I(1350)/I(1600) – which was previously used to estimate the structural order of carbonaceous matter(29) was combined with the FTIR branching index of aliphatic chains, R 3/2 (derived from the 2800-3000 cm\(^{-1}\) spectral regions). The idea is to use the I(1350)/I(1600) versus R 3/2 trends to discriminate between prokaryotic and eukaryotic fossils (29)(30)(31). Eukaryotic cells differs widely from the prokaryotic ones in terms of their cell structure and chemical compositions (i.e. different membranes, cell wall, organelles) which result in different taphonomic behaviors and distinctive I(1350)/I(1600) versus R3/2 trends(29)(30). Synchrotron-based (SR)-µFTIR spectra (Fig. 4) acquired on the fossil filaments revealed a strong aliphatic signal with an
average branching index R3/2 of 0.34 ± 0.1. This rather low R3/2 value indicates the presence of long straight-chains aliphatic molecules and fits reasonably well with known R3/2 values for extant eukaryotic groups (31) including fungi (Fig. 5). When compared with I(1350)/I(1600) versus R3/2 signatures of known fossils of eukaryotic and prokaryotic microorganisms, our fossilized filaments with a I(1350)/I(1600) ratio ~ 0.6 ± 0.06 fall well in the eukaryotic trend and clearly differ from a prokaryotic signature (Fig. 5). This combined Raman-µFTIR signature suggests an eukaryotic precursor for the organic matter of the fossil filament. This result, together with the morphology, dimensions and the presence of chitin remnants further support the idea of a fungal origin for those fossil filaments.

**Chemical characterization and molecular signature**

Beyond the R3/2 ratio, the SR-µFTIR data also revealed additional important information about the functional groups present in the fossil filaments (Fig. 4 – Table S1 for detailed assignments). First, the kerogenization of the fossil filaments is evidenced by aromatic bands at 1597 cm\(^{-1}\) (v C=C), 3070 cm\(^{-1}\) (v C-H) and 1270 cm\(^{-1}\) (C-O-C in aromatic ether/phenol groups) and by the strong aliphatic signal with peaks at 2960 cm\(^{-1}\) (v\(_{as}\) CH\(_3\)), 2920 cm\(^{-1}\) (v\(_{as}\) CH\(_2\)), 2875 cm\(^{-1}\) (v\(_s\) CH\(_2\)), 2850 cm\(^{-1}\) (v\(_s\) CH\(_3\)), and at lower wavenumber 1458 cm\(^{-1}\), 1408 cm\(^{-1}\) (δ\(_{as}\) and δ\(_s\) in CH\(_2\)) and finally at 727 cm\(^{-1}\), the latter being indicative of (CH\(_2\))\(_n\), polymethylene chains with n>4. As kerogen matures, aromaticity tends to increase relative to the aliphatic character with fully aromatic, pure graphite being the ultimate end member of this process. Here, the transformation into graphite is far from complete as the aliphatic vibrations are still preeminent. As for the formation of those long aliphatic chains, it has been extensively studied in fossil and thermally-matured cuticle of arthropods (22) which are made of a similar protein-chitin complex than fungal cell wall. With time, this structural assemblage is altered by the breaking of ester, amide and glycosidic bonds -hence producing free aliphatic groups and unsaturated carbon which then polymerize into long polymethylene chains (32)(33). Aliphatic molecules associated with vestigial chitin would then create a hydrophobic, protective barrier against microbial or aqueous thermolytic degradation and may explain the excellent preservation of these organic remains (34)(32). In our case, even though the aliphatic signal is strong, the degradation of this protein-chitin complex into aliphatic compounds appears incomplete as we found sharp vibrations characteristic of amide at 3286, 3070 cm\(^{-1}\) and especially at 1651 and 1540 cm\(^{-1}\) which are assigned respectively to amide A (v N-H), amide B and amide I (v C=O), amide II (δ N-H). As for carbohydrates (usually between 950-1200 cm\(^{-1}\)), they are depleted however we were still able to detect a pyranose peak (v C-C and v\(_{as}\) C-O) at 1170 cm\(^{-1}\). The fact that, in addition to the aromatic and aliphatic vibrations, amide and pyranose peaks are present is a testimony of the exceptional preservation of those filamentous remains. So far, the oldest fossil exhibiting organic remains other
than kerogens was a *Vauxia gracilenta* sponge (505 Ma) in which vestigial chitin were identified (24).

Our SR-µFTIR data for the fossil filament is remarkably close to those reported for *Vauxia gracilenta* (Fig. S5 and Table S1 for respective peak positions) which supports the notion that vestiges of chitin are present (in line with the positive WGA-FITC staining). SR-µFTIR spectra of the fossil filament differ, however, from pure chitin (Fig. S5). This is to be expected, because the precursor organic matter (i.e., fungal cell walls) of the fossil filaments contained not only chitin but also large amounts of phospholipids, proteins and glucans. The latter together with the carbohydrate moieties of chitin were likely preferentially degraded and thus it appears logical to find carbohydrate bands to be almost absent in the SR-µFTIR spectra of the fossil filament (apart from pyranose vibrations at 1171 cm⁻¹ - Fig. 4 and Fig. S5). With respect to amide I and II vibrations, there is good match with those of pure chitin - usually 1650-1660 cm⁻¹ and 1550-1560 cm⁻¹ respectively. All together, those SR-µFTIR data revealed that the fossil filament is made of partially kerogenized organic matter which exhibit vibration bands consistent with vestigial ‘chitin’ – hence supporting our previous positive WGA-FTIC staining essay.

**Carbon and nitrogen speciation**

Ultrathin sections of filaments were sampled perpendicularly across filament by focused ion beam milling (FIB; see Fig. 1D for localization and Fig. S6 for detailed views) and analyzed by X-ray absorption near edge spectroscopy (XANES) at the carbon and nitrogen K edge. Synchrotron XANES analyses is an excellent non-destructive and direct method for quantifying speciation and bonding in organic molecules in organic-rich fossils. Recently, XANES was used to detect chitin remnant in *Vauxia gracilenta* (24) and to show that organic matter compositional heterogeneity (e.g., prokaryotic vs. eukaryotic microorganism) can be maintained throughout pressure and temperature typically encountered during diagenesis (up to 250°C and 250 bars). Here, through deconvolution of our C-XANES (Fig. 6 and Fig. S7), in the fossil filament we detected two characteristic peaks of α-chitin at 285.1 eV (C=C 1s-π* transition in aromatic carbon) and at 288.6 eV (1s-π* transition of amide carbonyl and C-N bonds). Compared to pure chitin, the strengthening of the aromatic carbon peak (285.1 eV) and the occurrence of two additional features in the filament spectra (286.7 eV C=O/C=N bonds and 287.5 eV for aliphatic carbon) are consistent with the SR-µFTIR results and illustrate the incomplete microbial degradation, the subsequent dehydration/heating processes of the advanced diagenesis and the resulting kerogenization of the fossil filament. For instance, the presence of a ketone peak (286.7 eV C=O/C=N, Fig. 6 and Fig. S7) indicates the dehydration/heating of polysaccharide moieties in chitin and glucans of the fungal cell wall(33). The selective degradation and the ensuing burial experienced by the fossil filament are also evidenced by the low levels of N in the fossil filaments: C:N ratio varies from 50 to 199 versus 5-30 in fresh fungi. Still, our N-XANES data
confirmed the presence of amygdal N with a peak at 401.3 eV that corresponds to the main spectral feature of chitin on the nitrogen K-edge (Fig. 6A). Other peaks are also present in the N-XANES, at 398.7 and 399.7 eV, which are associated with pyridine and its derivatives. Those compounds were previously detected in Carboniferous and Silurian arthropod cuticles and were shown to form when chitinous tissues were subjected, experimentally, to temperatures and pressures typical of advanced diagenesis. Interestingly, those pyridine peaks (398.7 and 399.7 eV) in addition to the amide peak (401.3 eV) were also found in the N-XANES of fungal hypha from the lichen Parmelia saxatalis (Fig. 6B). When compared to other modern fungal species, the C- and N-XANES of our fossil filaments exhibit the same trend than when it is compared with chitin, i.e., more intense aromatic/olefinic and imine/ketone/phenolic peaks (at 285.1/398.8-399.9 eV and 286-287.2 eV) and reduced amide/carboxylic peaks (at 288.6 eV/401.3 eV). Equivalent C- and N-XANES trends were observed in a recent experimental study simulating burial-induced maturation of various modern microorganisms during advanced diagenesis. This difference in C-XANES is particularly marked for Aspergillus fumigatus, but also visible C-XANES of Paxillus involutus and for Parmelia saxatalis although to a lesser extent (Fig. 6A). Those two fungal species exhibit significant aromatic/olefinic peak and generally have C-XANES spectra close to the one of our fossil filaments with common absorption peaks centered at ~285 eV, 286.7 eV and 288.6 eV. As an element of comparison, cyanobacteria Gloeobacter violaceus have distinctive C- and N-XANES with sharp amide peaks at 288.1 eV and 401.3 eV, a moderate aromatic peak at 285 eV and a shoulder for aldehydes at 289.4 eV which differ markedly from fungal and fossil filament XANES spectra (Fig. 6). Although not directly diagnostic, the fossil filaments exhibit C- and N-XANES spectral features consistent with the presence of vestigial chitin, and also share some similarities with modern fungal species.

**Syngeneticity of the fossil filaments**

The question naturally arises as to when these filamentous networks formed relative to their host rocks? We did not detect any living fungal cells or fresh fungal organic matter which precludes recent contamination. Rather we showed that filamentous organic matter has undergone partial kerogenization as a result of advanced diagenesis which suggests burial over geological timescales. We also found that all organic matter found in the BI-II groups of MMS i.e., asphaltite, dolomitic shale organic matter (in the vicinity of filamentous networks), fossil assemblages including our fossil filaments exhibit the same range of Raman-peak temperature of 150-250°C indicating a common thermal exposure. Further, in several ultrathin FIB section, we detected paragenetic jarosite (KFe^{3+}{(OH)}_{6}{(SO}_{4})_{2}, quartz around the filament (Fig. S6). Alunite (KAl_{3}{(SO}_{4})_{2}{(OH)}_{6}) was also identified within fossil filaments (not shown). Those phases are likely related to evaporate-leaching fluid circulation induced by the Lufilian orogen (~560-480 Ma). This important compressional event is
known to have been the cause of large sulphur-mineralization events in the Katangan Basin (SE of SMMLL Basin) and also that such mineralization was related to thermochemical (280-390°C) sulfate reduction of evaporite-leaching fluid (38). This upward fluid migration in the adjacent SMMLL Basin is evidenced by anhydrite (CaSO₄) in faults in the BIIb/d as well as by gypsum in Ble2 (16). This fluid circulation would explain the occurrence of jarosite/alunite as well the thermal maturation (150-250°C) of the fossil filaments. Thus, based on paragenetic mineralization alone, a minimum Cambrian-Ordovician age can be inferred. Furthermore, the fungal filaments were not restricted to cracks or voids and not related to tunneling – a known feature of fungal endolithic colonization. In addition, previous study (20) which sampled in close vicinity to our dolomitic shale in the BIIc8 level also described similar cylindrical, septated filaments (obtained as palynomorph via acidic maceration). Thus, we consider the fossil filament described here as syngenetic with the host dolomitic shale rock formed ca 810 – 715 million-year ago.

DISCUSSION
We report the presence of ancient fungal filaments and mycelium-like structures preserved in a Neoproterozoic dolomitic shale (ca 810 and 715 Ma). This discovery proves definitely that filaments, found earlier in (20) which were later reassessed as cyanobacterial tubular sheath, were in fact remains of fungal origin. As such, this extends the record of fungi fossils by more than 250 million-years with respect to previously reported oldest fungi fossil dating from the mid-Ordovician (460-55 Ma). Considering that our study merged several complementary, in situ and spatially-resolved molecular identifications by confocal µRaman (Figure 3), synchrotron-µFTIR (Figure 4, 5 and S5 and Table S1), staining (Fig. 2) and µXANES (Figure 6), we contend that the identification of chitin biosignatures in our samples is, by far, more comprehensive than the evidence reported (10) recently where only µFTIR was used. In our study, confocal laser scanning fluorescence microscopy was instrumental to demonstrate the presence of vestigial chitin and the occurrence of key fungal features such as septa and anastomosing filaments in those mycelium-like structures. Taking advantage of the unique sensitivity and spatial resolution of synchrotron-based µFTIR, XANES and µRaman spectroscopy, the present study revealed that the molecular biosignature of the filamentous fossils have been exceptionally preserved despite their 715-810 million-years long geological history. We believe that with the early cementation and burial in a shale rock relatively rich in organic matter (presumably preventing oxidative degradation) and a rather low peak-temperature (150-250°C), the fossil filaments have benefited from a very favorable preservation context over timescales. This exceptional preservation made possible a whole range of chemical characterizations.
Even though, the width of the filament (Fig. 1E) is coherent with either fungal or filamentous prokaryotic precursor, we could infer that these remains had most likely an eukaryotic origin by combining SR-μFTIR- with μRaman spectral signatures (Fig. 5). We further showed that despite a kerogenization process evidenced by intense aromatic signals and low C:N ratio (50-199), the fossil filaments exhibit SR-μFTIR, C- and N-XANES spectra sharing significant similarities with modern fungal species and chitinous fossil such as *Vauxia gracilenta* and that they are not similar to typical prokaryotic microbes. We could even detect amide functional groups (C- and N-XANES absorption at 288.2 eV, N-XANES at 401.3 eV and FTIR bands at 1651 and 1540 cm⁻¹) which were likely to be involved in the cell-wall protein-chitin complex synthesized by the once living fungi – hence confirming our positive WGA-FITC staining of vestigial chitin by confocal laser scanning fluorescence microscopy. Based on the combined evidences of presence of vestigial chitin, eukaryotic nature, syngenecity, size, morphology, the filaments and mycelium-like structures are identified as the fossilized remnants of fungal networks.

In our view, the observed septation of the hypha does not indicate a dikaryan affinity which appears unlikely considering the Neoproterozoic age of the remains and the much later timing of Dikarya evolution (Basidiomycota and Ascomycota). Instead, the low frequency of the septation and also the presence of incomplete septa (pseudosepta) in the fossil filaments resembles more to Blastocladiomycota hypha (39). This basal phylum regroups parasitic or saprotroph fungal species and are found in both aquatic and terrestrial environments. As such, considering the very shallow, periodically subaerial-exposed, lacustrine nature of the upper BIIc paleoenvironment where the fungal remains were discovered, one may hypothesize that those fungi were in associations (either parasitic or symbiotic) or decomposers of an early photosynthetic community in transition towards terrestrial life (40). Indeed, ephemeral ponds, with their frequent wet-drying cycles (e.g. along the shores) might have been favorable environments for physical interactions between fungi and algae. Those early associations with photosynthetic organisms were presumably restricted to free living, and possibly lichenized, green algae or cyanobacteria(9) which may have represented a primitive form of cryptogamic cover(41). Although no such symbiotic relationships have been observed directly here, it is worth noting that the upper BIIc shale beds, in addition to fungal remains, exhibit an abundant and diverse assemblage of microfossils including 11 eukaryotic, acanthomorph, presumably algal- species(15). As such, our study represents the oldest, documented fungi to date and push well into the Neoproterozoic the possibility that fungi helped to colonize land surface, almost 300 millions year prior to the first evidence of land plants. Overall, our discoveries also lend support to previous assumptions regarding the role of fungi in land colonization and by extension on the evolution of Earth’s biogeochemical cycles(40)(42). Neoproterozoic soil development driven by early terrestrial biota has generated increasing amounts of pedogenic clay minerals that helped to
stabilize organic matter and enhanced carbon burial, which, in turn, contributed to the stepwise oxygenation of the late Precambrian.

MATERIALS AND METHODS

The dolomitic shale sample (BK457b, 118.2 m, core 118/4) from BIIc8 was cut to produce three petrographic, non covered thin sections of which one showed five areas where mycelia were apparent. The sections were studied using light microscopy (LM), scanning electron microscopy (SEM), chitin staining with WGA-FITC with fluorescent microscopy, synchrotron-radiation-µFTIR (SR-µFTIR). Furthermore, nine FIB foils were sampled in order to be analyzed by synchrotron-X-ray Absorption Near Edge Structure (XANES) spectroscopy and by analytical Transmission Electron Microscopy.

Scanning Electron microscopy

The images in Fig. 1, Fig. S1 and S6A were obtained with an Ultra55 Field Emission Gun-SEM (Zeiss), operated at 2.00 kV accelerating voltage. The samples were uncoated.

Confocal Raman Imaging Spectroscopy

Raman spectra and images were collected using a Witec α-Scanning Near-Field Optical Microscope customized to incorporate confocal Raman spectroscopic imaging. The excitation source is a frequency-doubled solid-state YAG laser (532 nm) operating between 0.3 and 1 mW output power (dependent on objective), as measured at the sample using a laser power meter. Objective lenses used included a x100 LWD and a x20 LWD with a 50 μm optical fiber acting as the confocal pin hole. Spectra were collected on a Peltier-cooled Andor EMCCD chip, after passing through a f/4 300mm focal length imaging spectrometer typically using a 600 lines/mm grating. The lateral resolution of the instrument is as small as 360 nm in air when using the x100 LWD objective, with a focal plane depth of ~800 nm.

Typically 2D imaging and single spectra modes were used during this study. Single spectra mode allows the acquisition of a spectrum from a single spot on the target. Average spectra are produced typically using integration times of 30 seconds per accumulation and 10 accumulations to allow verification of weak spectral features. Target areas were identified on the thin section in reflected light. The height and width of the field of interest within the light microscopy image were then measured and divided by the lateral resolution of the lens being used, to give the number of pixels per line. The instrument then takes a Raman spectrum (0 – 3600 cm⁻¹ using the 600 lines mm⁻¹ grating) at each pixel using an integration time of between 1 s per pixel.

A cosmic ray reduction routine was used to reduce the effects of stray radiation on Raman images, as was image thresholding to reject isolated bright pixels. Fluorescence effects were inhibited by the use of specific peak fitting in place of spectral area sums and by the confocal optics used in the
instrument. The effects of interfering peaks were removed by phase masking routines based on multiple peak fits as compared to standardized mineral spectra. This produces an average spectrum over the number of pixels chosen in the area of interest. Peak center maps were produced by using a Gaussian fit to the G band and then confirmed by taking representative spectra from the range of peak centers as seen in Fig 3.

**Confocal laser scanning fluorescence microscopy**

The incubation with WGA-FITC lasted for 1 h. The concentration of the WGA-FITC in the incubation medium was 80 µg.mL⁻¹ WGA-FITC in 10 mM of phosphate buffer at pH 7.8. After incubation the samples were thoroughly washed with MilliQ water. Confocal fluorescence imaging was realized on a Zeiss LSM-7 10 confocal microscope using both Zeiss x2 0/0.8 PlanApochromat and Zeiss x63/1.4 PlanApochromat objectives and with specific excitation using a 488nm Argon ion laser. Fluorescence detection was realized using a 500-550 BP emission filter and the Zeiss Airyscan GaAsP detector in order to increase signal/noise ratio and resolution. Images were acquired using Zeiss ZenBlack software (Zeiss, Oberkochen, Germany). Fluorescence images of the BK233 sample were done on a Zeiss AxioObserver Z1 widefield microscope using a Zeiss x20/0.8 PlanApochromat objective and a 493nm LED excitation. Detection was done on a Hamamatsu Flash4.0 sCMOS Camera with a 1.5 second exposure time. Image adjustments (i.e. brightness and contrast) and Z projections were done using Fiji software (43).

**Synchrotron-radiation-µFTIR**

The µFTIR measurements were all done at the beamline B22 at Diamond Light Source (UK). All measurements were conducted in transmission mode with the samples -fragments of filamentous network- manually deposited on CaF₂ slides. We used the Hyperion 3000 SR-µFTIR setup to analyze our sample with the x36 objective. Usually, 512 or 1024 scans were accumulated at a resolution of 4 cm⁻¹ between 500-4000 cm⁻¹ (later cut between 1000 and 3500 cm⁻¹) and generally with a 10×10 µm aperture. Spectra were then baseline-corrected using the concave rubber band routine in OPUS-Spectroscopy software (Bruker).

**Ion milling by Focus Ion Beam (FIB)**

Nine ultrathin foils, typically 15 x 7 x 0.15 µm, were sampled from various filamentous networks using a FIB single beam instrument (FEI FIB 200 TEM) at the GFZ Potsdam following the procedure described in (44). This ion milling procedure maintains textural and chemical integrity even in the case of sensitive materials. Milling was conducted at low-gallium ion currents in order to minimize gallium implantation or redeposition of the sputtered materials on the sample surface and to prevent significant changes in the speciation of complex carbon-based polymer.

**X-ray Absorption Near Edge Structure (XANES) spectroscopy**
All XANES measurements were all done at the I08-SXM beamline at the Diamond Light Source (UK).

Collection of XANES spectra at the C (from 280 to 310 eV) and N (from 395 to 420 eV) K-edges were performed on all nine FIB foils. Using a Fresnel zone plate monochromatic X-rays was focused on the sample. To collect 2D images the sample was raster-scanned and the transmitted x-rays was detected with a photo diode at a fixed energy with a spot size of 50 nm using a dwell time of 3 msec per energy step per pixel. Combining the images from each energy steps (0.1 eV in fine regions or 0.5 eV in pre- or post-edge) produced 3D image “stacks” of x-ray absorption of the FIB foils after the normalization with the incident x-ray intensity. All subsequent XANES data processing was performed by MANTiS (45) and deconvolution was done using MagicPlot Pro.

**High Resolution Transmission Electron Microscopy**

Transmission electron microscopy was performed on FIB foils at the GFZ Potsdam using a FEI Tecnai G2 F20 X-Twin microscope equipped with a Gatan imaging filter 5GIF Tridiem) for acquisition of energy-filtered images, an EDAY X-Ray analyser with an ultrathin window, and a Fischione high-angle annular dark field detector (HAADF) allowing imaging (by Z-contrast) in the scanning transmission electron microscopic mode (STEM). The system was operated at 200 kV with an FEG electron source.

Energy dispersive X-ray analysis (EDX) in scanning transmission mode (STEM) was applied. Electron energy-loss spectroscopy (EELS) analysis was used to derive C/N ratio values in FIB foil.

**SUPPLEMENTARY MATERIALS**

Supplementary text: Paleoenvironments of the BIIc subgroup

Fig. S1: High-resolution SEM micrographs of the mycelial networks

Fig. S2: Geological map of SMLL basin and synthetic stratigraphic and chemostratigraphic column of the MMS.

Fig. S3: Optical micrographs of petrographic thin sections cut at the depth or in close vicinity of the fossiliferous sediment in BIIc8 (15) (BK 457b, 118.2 m) showing subaerial exposure feature and the little to no compaction of fossiliferous dolomitic shale.

Fig. S4: Fluorescence micrograph of thin sections in BIIc6 shale bed (controls of WGA-FITC staining specificity)

Fig. S5: Comparison of µFTIR spectra of fossil filaments, cyanobacteria, extant fungi, fossil sponge *Vauxia gracilenta* and α-chitin.

Fig. S6: SEM, TEM (HAADF) micrographs and elemental (STEM-EDS) and mineralogical analysis (SAED) on FIB foil #4984 sampled collected fossil filament and associated paragenetic mineral phases.

Fig. S7: Deconvolution of the C-XANES edge for fossil filament FIB foil #4984.

Table S1: Assignment table for µFTIR spectra for fossil filaments, α-chitin and *Vauxia gracilenta*. 


Table S2: Peaks positions and possible molecular functional groups detected in XANES at the C and N 1s edges.
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Data availability: All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to the paper may be requested from the authors. Correspondence and requests should be addressed to S.B.

Contributions
S.B. directed the research and wrote the manuscript. R.W. did all TEM-related analysis, imaging and interpretation. A. Sc. did the ion milling. S.B and L.G.B. acquired and interpreted the µFTIR dataset. C. C. performed the WGA-staining. T.A., M.K.A, S.B., L.G.B and A. St. acquired, data-processed and interpreted XANES data. A. St. did the Raman analysis. F.D. and A.P. did the stratigraphy and sedimentological analysis. All authors discussed the results and commented on the manuscript.

Competing Interest: The authors declare no competing interests.
Figure 1: Textures and structures observed via light (LM) or scanning electron microscopy (SEM) of a thin section from the fossiliferous dolomitic shale rock from BIIc8 (Mbuji-Mayi Supergroup). (A) Composite image of LM views of dark, non-translucid, interconnected filaments forming part of a large mycelium-like structure covering ~0.2 mm² detailed in Suppl. Fig. S1 (as well as other mycelium-like structures). Note that the area shown in (B) was exposed to WGA-FITC in Fig. 2. (C) SEM micrograph illustrating the presence of cells (~20 to 25 µm in length) regularly spaced by putative septa or pseudo-septa (white arrows) and showing putative anastomoses (red arrow). (D) LM image of a mycelium network of dark-colored filaments with Y and T-branching (red arrows); black arrow marks the position of an FIB foil #4984 cut across a filament in the thin section. Insert shows a portion of mycelial network composed of dark, non-translucid filaments branching at right angle. (E)
Histogram showing the width frequency distribution of filaments with the solid line depicting the median at 5.5 µm (the dashed line as 1\textsuperscript{st} and 3\textsuperscript{rd} quartile at 4.5 µm and 6.7 µm). (F) illustrates the encrusted aspect of these fungal networks and the absence of tunneling patterns suggesting the syngenicity of these remains.
**Figure 2**: Confocal laser scanning fluorescence microscopy using WGA-FITC of mycelium-like structure illustrated in Fig. 1. Overview image in A shows the absence of large natural auto-fluorescence (i.e., without WGA-FITC) of the thin section. In contrast, with WGA-FITC labeling, the same area exhibits filamentous, mycelium-like structure is visible in B (top section). C-F are high-resolution confocal fluorescence views of the mycelium-like structures. The WGA-FITC binds specifically on the cell-wall of the fossil filaments which appears cylindrical (as evidenced by rounded cross section of filaments). Several septa (s) are also stained. Note in C that the arrows highlight septa present in putative anastomosing filaments (a) illustrated in Fig 1A (arrows – image are mirrored as confocal microscope is inverted). Inserts in F illustrate septa (ps) with perforation or ‘bulged’ which resembles to pseudosepta (39). Scale bars in image C-F are 10 µm.
Figure 3: (A) Reflected light microscopy of a filamentous network in Fig 1A. (B) G band intensity map of the filament shown in A. (C) Representative spectra showing the D and G bands analyzed (marked ‘D’ at ~1350 cm$^{-1}$ and ‘G’ ~1600 cm$^{-1}$ on the spectra) from the areas marked by crosses in B). The coloration of the spectra corresponds to the color of the crosses seen in B. (D) G band peak center map from the same area (i.e., B) that shows significant variations in the G band peak center across the filamentous network. (E) Representative Raman spectrum illustrating the deconvolution in D1-, D3-, D4-, D5-, and G-bands used to calculate diagenetic peak-temperature and the determination of the parameter I(1350)/I(1600). The thermal maturation of the fossilized filament was derived according to the FWHM-D1 geothermometer relationship in (26).
Figure 4: Synchrotron-µFTIR (average over 31 10 by 10 µm areas) of fragments of fossilized filaments illustrated in Fig 1E. Assignments of absorption bands and vibration modes (δ = deformation; ν = stretching; s = symmetric; as = asymmetric) are indicated in parentheses (see Suppl. Table 2 for full details). Note the strong contributions of amide functional groups (at 1650, 1540 and 3286 cm⁻¹) and aliphatic CH₂ and CH₃ moieties in the 2800-3000 cm⁻¹ region which is also evidenced by the presence of 727 cm⁻¹ peak indicating long \((CH_2)_n\) chains with \(n \geq 4\). (Insert) Detail of the 2800-3000 cm⁻¹ spectral region used to calculate the branching index (i.e. \(R_3/2\) - the relative ratio of CH₃ to CH₂ carbon molecules) based on the intensity of their respective asymmetric stretching.
Figure 5: (A) Plots of the µFTIR parameter $R^{3/2}$ versus Raman parameter $I(1350)/I(1600)$ of fossilized filament from Mbuji-Mayi Supergroup (red) and comparison to eukaryotic (green) and prokaryotic (blue) fossils from previous studies (31) (29) (30). Within the Rhynie chert plant fossils, several regions were measured including cell wall, epiderm, protoplasm and extracellular carbon. Our fossilized filaments fall close to the protoplasm of plants. The colored areas provide possible discrimination between Eukaryotes and Prokaryotes fossils based on their respective organic matter $R^{3/2}$ and $I(1350)/I(1600)$. The average values with standard deviations are plotted. (B) Box and whisker plot of the R 3/2 ratio of a range of modern organisms spanning across the three domain of life (data for Eukaryotes, Prokaryotes and Archaea are from (46) (47). R 3/2 ratio quantifies the extent of branching and length of aliphatic moieties of the precursor membrane and can be used as a domain-specific proxy. As fossilization proceeds, it seems that the R3/2 values tend to decline (47) which also seem to be the case here for fungi. *Note that ‘Extant fungi’ values were calculated from FTIR spectra (48) (49) (50) (51) of Rhizopus sp., Motierella alpina, Mucor circinelloides, Paxillus involutus, Aspergillus nidulans, Neurospora sp., Penicillium glabrum and Umbelopsis isabellina.
Figure 6: Compilation of C-XANES spectra for a range of modern fungi (Paxillus involutus, Aspergillus fumigatus), fungal hypha in lichen (Parmelia saxatilis), cyanoabacteria (Gloeobacter violaceus)[36], α-chitin and fossil filament (FIB foil #4984 - Suppl. Fig. S7 for detailed views). The colored bands denote the energy range for which various C functional groups are expected (see in Suppl. Table 1). For the fossilized filament, the main peaks are centered at 285.1 eV (aromatic C=C), 286.7 (ketone and phenol C) and a shoulder in amide and carboxylic energy bands (288-288.7 eV). A complete
deconvolution of the C-edge for fossilized filament was performed (Fig S6 in Suppl. Info). Note that for *P. involutus*, the XANES was acquired on FIB section at the interface between fungi and biotite, the double peak between 297-300 eV are thus absorption peaks for potassium. (B) N-XANES spectra of fossilized filament, fungal hypha in *Parmelia saxatalis* and α-chitin(32). The fossilized filament exhibits spectral features at 398.7 (imine), at 399.8 eV related to pyridine(35) and a shoulder at 401.3 eV which corresponds well with the main spectral feature of the α-chitin (N 1s-3p/σ* transition in amide). Note that all those features are visible in the spectra of *P. saxatalis*. 


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Supplementary materials
Paleoenvironment and petrography of the BIIc subgroup

A full description of paleoenvironment combining (micro)facies analysis and detailed geochemistry of Mbuji-Mayi Supergroup is available in (52) (16). Briefly, the carbonate rocks from lower to upper BIIc were deposited from stromatolitic bioherms in mid ramp, marine evaporative inner ramp and lacustrine environments. The rocks formed are stromatolitic dolostone in the lower BIIc while the upper BIIc is made of shaly dolostone interbedded with shales.

The lower part of the BIIc subgroup displays negative δ13C values (Suppl. Fig. S2), low Sr/Ca ratios, elevated Sr and Mn concentrations and 87Sr/86Sr isotopic ratios between 0.7062 and 0.7082 which typically indicates marine conditions. In addition, those rocks have discrete seawater REE + Y distributions, probably due to the high degree of interaction with reduced dolomitizing fluids, with a weak light REE depletion ([Nd/Yb]paas = 1.05), positive La anomaly ([La/La*]paas=1.19), weak Gd enrichment ([Gd/Gd*]paas=1.11) and positive Y anomaly. No significant Ce anomaly was found with an average ([Ce/Ce*]paas = 1.08 (Ce⁴⁺ is oxidized to Ce⁶⁺ under oxidizing conditions resulting in a negative Ce anomaly i.e., ([Ce/Ce*]paas< 0.9)). Overall, in the lower BIIc stromatolitic dolostone were deposited under primary marine conditions subjected to evaporitic reflux.

The upper part of the BIIc subgroup displays δ13C variations from negative to positive values (Suppl. Fig. S2) towards the top, without any δ18O variations and high Mn concentrations which suggests an evolution from marine to evaporitic conditions. The host rocks were periodically emerged as shows desiccation cracks, coated pisoids from aggregation of stromatoclasts and reworked ooids, and vadose or meteoric cements. The shaley dolostones are characterized by enrichments of REE concentration and show a LREE depletion ([Nd/Yb]paas = 0.69, a slight MREE depletion ([Nd/Dy]paas=0.54, and positive La and Eu anomalies - ([La/La*]paas=1.26 and ([Eu/Eu*]paas= 1.08–1.20- and a moderate to high Y/Ho ratios. No significant Ce anomaly is encountered with an average ([Ce/Ce*]paas = 0.95. In addition with REE+Y distribution, high 87Sr/86Sr ratios in shaly dolostones revealed that upper BIIc carbonates settled out from seawater influenced by episodic river water influx (17).

BIIc8 has been deposited in lacustrine paleoenvironment corresponding to a shallow closed-lake, bordered by cyanobacterial organisms, and fringed by restricted pools as evidenced by dolomitic shales. BIIc8 recorded a sea-level regressive highstand, which was marked by a relative and progressive fall of sea level in a low rate of subsidence (16). Emersion features such as calcretes, desiccation cracks, coated pisoids from aggregation of stromatoclasts and reworked ooids, and vadose or meteoric cements developed on the dried margin of the paleolake (16). Climate periodically changed to humid and warm conditions, which were marked by a relative short-term shut down of production of carbonate and the river-influenced deposition of shales and “sandy” coarse-grained dolostones in the lake. Freshwater altered host rocks initiating precipitation of vadose or meteoric cements (16) (52) as in our fossiliferous-shale BIIc8 where we can observe emersion features represented by vadose or meteoric cements (Fig. S3C – black arrow) that marked a deposition upon desiccation on clotted stromatolitic sediments.

Detailed description of thin sections (Figure S3) from rocks at the same depth and in close vicinity of our fossiliferous beds revealed that the fossils were found in dolomitic shales (overlying dolostone and dolomudstone) in which an early dolomitic cementation took place before burial. This cementation is possibly due to schizohaline conditions coupled to anoxic microbial respiration of organic matter, which create a favorable environment (high alkalinity and elevated pH of porewater)
for pre-compactional growth of interstitial dolomite \(^{(14)}\). Once cemented, these sediments were not subjected to significant additional compaction or diagenetic alterations during burial. This is supported by the fact that, contacts between grains are rare, there are no tangential, concavo-convex or sutured contacts and no truncated or flattened grains are visible. Further, there are no deformations or broken micrite envelopes in peloids or oolites, we observed no mechanical rearrangements or overpacking of grains and no stylolites (see new Figure S3 in Suppl. Materials showing samples BK457a and BK458 from depth of 118.2 m and 118 m, from the cores 118/4 and 118/7 respectively). In contrast, we could often observe diagenetic features related to burial and cementation. These include: (i) thinned and wispy laminations/seams of organic matter-rich sediment (Figure S3A); (ii) burial cements (new Figure S3B in Suppl. Materials); and (iii) multi-stage dolomitization features. All these observations confirm an early cementation followed by burial without substantial compaction of the sediments where the fungal fossils were found.
**Figure S1:** A-C High-resolution SEM micrographs of the mycelial network in Fig. 1A with detailed views of anastomosing filaments (a arrows), ‘Y’ (b arrow) and high angle ‘T’ (c arrows) (D) Stitched LM micrographs showing the density of branching in the fungal network.
Figure S2: (A) Synthetic stratigraphic column of the Mbuji-Mayi Supergroup in the Democratic Republic of Congo combined with composite chemostratigraphic-isotope profiles (data from (17)). Note the presence of the $\delta^{13}$C anomaly ($\pm 7\%$) in the carbonate rocks in the lower BIIc subgroup coeval to the Bitter spring anomaly (52). On top of the BIIc subgroup, the Kabele-Kabenga conglomerate is stratigraphically-equivalent to with the Grand Conglomérat in Katanga (>715 Ma) (52). Note the evolution of $^{87}$Sr/$^{86}$Sr ratio which become increasingly radiogenic (i.e., enriched in $^{87}$Sr).
from BIIb to the upper BIIc. (B) Detail stratigraphic log of BIIc subgroup where the fossiliferous shale bed was found (BIIc8) located at a depth of 128.1 m from the surface (i.e., 292 m from the base of Kanshi B13 core). (C) Sketched geological map of the Sankuru–Mbuji-Mayi–Lomami–Lovoy Basin (SMLL) in the southern central margin of the Congo Craton.
Figure S3: Optical micrographs of petrographic thin sections cut at the depth or in close vicinity of the fossiliferous sediment in BIlc8 (15) (BK457b, 118.2 m). (A) BK457a (118.2 m) shows a dolobindstone overlied by dolomudstone and dolomitic shale from which the fossiliferous thin section was extracted (BK457b). (B) BK458 (118 m) illustrates a level of dolomudstone and stromatolite-derived peloidal dolopackstone. (C) BK457 (118.1 m) is a dolomitic bindstone consisting of rounded to irregular anastomosing patches of stromatolite margin clots, selvages and ‘grumeaux’ with larger peloids in tubular fenestrae filled by drusy white dolomite (blue arrow). Black arrow in (C) denote of thin, bladed irregular cements and fine grained, pendant beard-like cements (gray), which indicate near evaporitic and vadose environment typically found in perennial lake with subjected to semi-arid and warm conditions. Note that in all three thin sections, compaction is not significant as cementation (dolomitic, in white) took place during early diagenesis. Scale bar is 1 mm (C) and 500 µm in (A) and (B).
Figure S4: Fluorescence micrographic views of shale thin sections of BIIc6 with large amounts of organic matter inclusions in veins and also as globules. A-C show the complete lack of natural autofluorescence (i.e., without WGA-FITC labeling) of the organic matter (dark veins-like and globular areas) while the mineral matrix exhibit some limited autofluorescence (dark to light green). D-F views illustrate roughly the same areas after exposure to WGA-FITC labeling. The clear absence of fluorescence of the ancient organic matter when exposed to WGA-FITC demonstrates the high-specificity binding of our chitin-staining method.
Figure S5: µFTIR spectra of fossil filaments, cyanobacteria (*Calothrix* sp.) (53), extant fungi (*Rhizopus* sp. and *Mortiriella alpina*) (48) (49), fossil sponge *Vauxia gracilenta* (24) and reference α-chitin from shrimp. The spectrum of the fossil filament is the average of over 31 10x10 µm areas. Dotted vertical lines and numbers refer to peak assignation for the fossil filament in Suppl. Table 1 which also compiles the equivalent peak position in α-chitin and *Vauxia gracilenta* fossils.
Figure S6: (A) SEM micrograph of branching fungal filament (colored in red) with the position where the FIB foil was cut (white rectangle). (B) High-angle annular dark-field (HAADF) imaging in scanning transmission electron microscopy (STEM) of the FIB foil #4984 (thickness ~150 nm) with the position of areas C, E and D marked. The white layer on top of the FIB foil is a protective platinum layer deposited during ion milling process. (C) Detailed STEM view of FIB section showing in dark gray the upper portion of the filament where pores (in black) as well as quartz infillings are visible (red square shows areas where elemental analysis by Energy Dispersive X-ray Spectroscopy (EDS) was done). (D) Detailed view of lower portion of filament encrusted by jarosite detected by selected area electron diffraction (SAED, insert) and by EDS analysis (area in red square). Note that the Ga signal in the EDS spectra is a consequence of implanting due to ion milling process. (E) Porous, vesicular portion of a filament (lower red square) in which mixture of alunite, jarosite or quartz are associated with the organic matrix (top red square).
Figure S7: Deconvolution of the C-XANES edge for fossil filament. The deconvolution procedure applied to the C1s edge involved a background subtraction extrapolated from a linear regression over 280-283 eV energy range. Gaussians functions with a fixed half-width and at half maximum (0.4 eV) and energy position have been used for the deconvolution.
| Band # | Fungi Filaments | Vauxia gracile * | α-chitin | Assignment of functional groups in filament | References |
|-------|----------------|-----------------|-----------|---------------------------------------------|-------------|
| 1     | 3268           | 3289            | 3267      | $\nu\text{ N-H in amide A}$                | a           |
| 2     | 3070           | -               | 3108      | $\nu\text{ C-H in aromatic/amide B}$       | g           |
| 3     | 2960           | 2956            | 2961      | $\nu_{\text{CH}_2}$ in aliphatic chains    | a           |
| 4     | 2920           | 2922            | 2934      | $\nu_{\text{CH}_2}$ in methylene groups; $\nu\text{ C-H in chitin (COCH}_3)$ | a           |
| 5     | 2850/2873 (sh) | 2852            | 2875-2889 | $\nu_{\text{CH}_2}$ and $\text{CH}_3$ respectively | b           |
| 6     | 1738           | -               | 1738      | $\nu\text{C}=\text{O in ester groups in aliphatic chains}$ | a           |
| 7     | 1651           | 1653            | 1657/2624 | $\nu\text{C}=\text{O in amide I}$         | c, d, e     |
| 8     | 1597 (sl)      | -               | -         | $\nu\text{C=C in aromatic groups}$         | f           |
| 9     | 1540           | 1545            | 1559      | $\delta\text{ N-H and }\nu\text{C-N in amide II}$ | a, c        |
| 10    | 1458           | 1466/1454       | -         | $\delta_{\text{CH}}$ in aliphatic chain    | b, f        |
| 11    | 1408           | 1413            | 1416      | $\delta\text{ C-H in pyranose groups, }\delta_{\text{CH}}$ in aliphatic chains | b, f, g     |
| 12    | 1319 (wk)      | -               | 1311      | $\nu_{\text{C-N, N-H in amide III}}$       | g           |
| 13    | 1270 (wk)      | -               | 1238      | Amide III ($\delta\text{ N-H and C-N deformation}, \nu$ in aromatic ether (C-O-C), phenolic (C-O) and ester (C=O-C)) | g           |
| 14    | 1171           | 1170            | 1156      | Pyranose $\nu\text{C-C}$ and $\nu_{\text{C-C}}$ oxygen bridge | b           |
| 15    | 1106-1108      | -               | -         | Si-O-Si                                      | c           |
| 16    | 1045/1088      | -               | 1036/1072 | Aliphatic ether C-O-C and alcohol C-O stretching | g           |
|      | 727            | -               | -         | Polymethylene chains ($n \geq 4$) rocking   | g           |

**Table S1:** μFTIR assignments based on the following references

- (a) (49)
- (b) (24)
- (c) (53)
- (d) (54)
- (e) (46)
- (f) (55)
- (g) (56)
Table S2: Peaks detected in XANES at the C and N 1s edges and used for the deconvolution in Fig. S8.

The assignment, range and position of the peaks were derived from (35) (36) (57) (58).