Calcitic nanofibres in soils and caves: a putative fungal contribution to carbonatogenesis

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Abstract: The origin of soil mineralized nanofibres remains controversial. It is attributed to either biogenic factors or physicochemical processes. Scanning electron microscope and transmission electron microscope observations show that nanofibres could originate from the breakdown of fungal hyphae, especially its cell wall. It is hypothesized that during the decay of organic matter, cell wall microfibrils are released in the soil where they are exposed to mineralizing pore fluids, leading to their calcitic pseudomorphosis and/or are used as a template for calcite precipitation. When associated with needle fibre calcite bundles, nanofibres could indicate the relict of an organic sheath in which calcite has precipitated. This paper emphasizes the important roles of both organic matter and fungi in carbonatogenesis, and consequently in the soil carbon cycle.

Natural nanofibres have been observed in various environments such as subtropical and temperate soils (Verrecchia & Verrecchia 1994; Cailleau et al. 2005) and cave deposits such as moonmilk (Borsato et al. 2000; Cañaveras et al. 2006). They are often associated with Needle Fibre Calcite (NFC; Verrecchia & Verrecchia 1994; Borsato et al. 2000). The aim of this study is to provide new insight into the processes at the origin of nanofibres.

In order to differentiate between organic and mineral nanofibres, the term ‘organic nanofibre’ will be used for nanofibres whose organic nature has been determined by analytical methods. The term ‘mineral nanofibre’ will be used for: (i) nanofibres observed in scanning electron microscopy (SEM), in absence of specific labelling (see ‘Materials and methods’ section); and (ii) nanofibres diffracting the electron beam under transmission electron microscopy (TEM). The term nanofibre alone refers only to a shape or an object and therefore used for morphological descriptions.

Previous work on mineral nanofibres

Since 1980, many authors have reported mineral nanofibres from various environments (Table 1). The four following authors have specifically observed organized structures related to mineral nanofibres: (i) filamentous, ramified, microscopic structure composed by a dense nanofibre scaffolding (Borsato et al. 2000; Richter et al. 2008); (ii) a straight macro-structural alignment (3–5 μm wide and >70 μm long) of unordered nanofibres observed close to an organic filament (possibly actinomycetes, cyanobacteria, or fungi; Benzerara et al. 2003); and (iii) 3 μm wide and >50 μm long filaments interpreted as ‘calcified filaments with needles in grain-coating needle mat’ have also been observed by Jones & Ng (1988).

Filamentous organisms and structures in soils and caves

Filamentous organisms living within the soil or in caves must be heterotrophic organisms. Algae and cyanobacteria are photosynthetic organisms and thus are present only at the soil surface or in rock fractures near a light source. Indeed, in mineral substrates that are far away from any light, these organisms are absent due to the lack of their energy source. Accordingly, filamentous fabrics present in these environments could be fungi, filamentous bacteria (in soils and caves mostly actinomycetes), and roots (Paul & Clark 1996; Gobat et al. 2003). Taking into account their sizes and morphologies summarized in Table 2, fungi are the most suitable organisms associated with nanofibres and NFC.
Table 1. Review of nanofibres in the literature

<table>
<thead>
<tr>
<th>Authors</th>
<th>Nomenclature</th>
<th>Geological setting</th>
<th>Interpretation/context</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pouget &amp; Rambaud</td>
<td>Calcite 'en bâtonnets'</td>
<td>Soil with calcareous crust</td>
<td>Mesh of monocrystalline calcite crystals</td>
</tr>
<tr>
<td>Vergès et al.</td>
<td>Small needle-shaped crystals</td>
<td>Calcareous soils</td>
<td>Tangled crystals</td>
</tr>
<tr>
<td>Ducloux et al.</td>
<td>Calcite 'en bâtonnets'</td>
<td>Developed on scree slope</td>
<td>Covering larger needle-fibre calcite crystals</td>
</tr>
<tr>
<td>Phillips &amp; Self</td>
<td>Micro-rods</td>
<td>Pedogenic calcrete</td>
<td>Interpreted as calcified rod-shaped bacteria</td>
</tr>
<tr>
<td>Phillips et al.</td>
<td>Submicron size rods</td>
<td>Pedogenic calcrete</td>
<td>Interpreted as calcified rod-shaped bacteria</td>
</tr>
<tr>
<td>Jones &amp; Ng</td>
<td>Needles</td>
<td>Rhizolith from the Pleistocene Ironshore Formation</td>
<td>Calcified filaments coated with needles (i.e. nanofibres)</td>
</tr>
<tr>
<td>Verrecchia &amp; Verrecchia</td>
<td>Micro-rods</td>
<td>Quaternary calcretes, Israel</td>
<td>Disordered mesh</td>
</tr>
<tr>
<td>Loisy et al.</td>
<td>Micro-rods</td>
<td>Carbonate paleosol in scree deposits</td>
<td>Mineralized threadlike and bacilliform bacteria</td>
</tr>
<tr>
<td>Borsato et al.</td>
<td>Nanofibres</td>
<td>Moonmilk (cave deposits)</td>
<td>Probably abiogenic precipitation</td>
</tr>
<tr>
<td>Benzerara et al.</td>
<td>Nanobacteria-like rods</td>
<td>At the surface of the Tataouine meteorite</td>
<td>Straight micro-alignment of nanofibres; possible organic origin</td>
</tr>
<tr>
<td>Cailleau et al.</td>
<td>Micro-rods</td>
<td>Orthox soils</td>
<td>Observed on burnt oxalate crystals embedded in tree tissues</td>
</tr>
<tr>
<td>Jeong &amp; Chun</td>
<td>Nanofibre calcite</td>
<td>Aerosols coming from loess plateau and desert</td>
<td>–</td>
</tr>
<tr>
<td>Richter et al.</td>
<td>Nanofibres</td>
<td>Moonmilk (cave deposits)</td>
<td>–</td>
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</table>

Note: Review of nanofibres present in soils and caves: nomenclature, occurrence and interpretation in the literature.

Fungal presence and activity in soil and caves

Fungi are present in large amounts in soils. As an example, one metre square of fertile soil can contain a 10 000-km long fungal network (Gobat et al. 2003). About 80% of land plant species are colonized by arbuscular mycorrhizal fungi (endomycorrhiza), and around 3% of phanerogam species are colonized by ectomycorrhizal fungi (EcM), especially plants with a large distribution at a global scale (Pinaceae, Fagaceae). In soils, a vertical distribution can be distinguished regarding fungal type in terms of their ecology. Organic layers are mostly colonized by saprophytic fungi, whereas mineral layers are colonized by EcM fungi (van Schöll et al. 2008). The latter has been demonstrated as being a significant agent of mineral weathering of ecosystem-wide importance (van Schöll et al. 2008).

The mycelial network is able to efficiently translocate nutrients in solution from one place to another (Gobat et al. 2003). Basidiomycetes, and among them EcM fungi, are able to build structures named fungal strands that can extend meters away from the roots (Finlay & Söderström 1992; van Schöll et al. 2008). Thus, the presence of mycorrhized roots, fungal hyphae and strands in deep mineral layers or in caves is not surprising. Canadell et al. (1996) showed an average rooting depth of $4.6 \, \text{m} \pm 0.5 \, \text{m}$, with a maximum depth of $7.0 \, \text{m} \pm 1.2 \, \text{m}$ for trees. In their review, only the root itself is taken into account. Considering the mycorrhiza, it can considerably extend the root network (Timonen & Marschner 2006). Observations of roots at depths below $2-3 \, \text{m}$ in caves have also been observed (Canadell et al. 1996). Jasinska et al. (1996) demonstrated that root mats could be the sole source of food for faunal communities in an Australian cave.

Cave geomicrobiology

Caves are nutrient-limited environments due to the absence of light that prevents primary production through photosynthesis, contrary to other common environments on Earth. Thus, in terms of presence of life, this kind of environment can be considered
as ‘extreme’. On the other hand, physicochemical parameters tend to be buffered and constant throughout the year (e.g. mild temperature normally equals MAST (Mean Annual Surface Temperature) and fairly high humidity). These extreme but constant conditions allow the presence of underground ecosystems, which may or may not be connected to aboveground energy-sources (Jasinska et al. 1996; Sarbu et al. 1996). Prokaryotes and fungi are the most common organisms that can be encountered in caves, and their link in speleothem formation is often proposed and debated. Moonmilk is a common speleothem mineral, and its biological or physico-chemical origin has long been discussed. Today most of the theories involve microbial mediation in its formation, but the exact role that microorganisms play, whether it is bacterial or fungal, is still being discussed (Gradzinski et al. 1997; Northup & Lavoie 2001; Cañaveras et al. 2006; Barton & Northup 2007). In caves, moonmilk is more likely to be present in the vicinity of soils (Gradzinski, pers. comm.). This increases the probability of cave access for roots and their fungal associates, and consequently their involvement in the genesis of moonmilk.

### Table 2. Characteristics of filamentous organisms in soils and caves

<table>
<thead>
<tr>
<th>Organism</th>
<th>Diameters</th>
<th>Morphologies/structures</th>
<th>Cell wall morphology</th>
<th>Cell wall biochemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungi</td>
<td>3–6 μm on average</td>
<td>Hyphae with or without septum, more or less ramified. Bundles of differentiated hyphae forming linear organs, fungal strands</td>
<td>Thick-walled (up to 1 μm) and thin-walled (100–200 nm) hyphae</td>
<td>Two layers: a fibrillar component with chitin and β-glucans and an amorphous component with glycoproteins specific to taxonomic groups</td>
</tr>
<tr>
<td></td>
<td>1 μm (min)–30 μm (max)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Strands: 0.02</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>-&gt; 1 mm</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Actinomycetes</td>
<td>0.5–1 μm in average, max</td>
<td>Ramified mycelium, sometimes fragmented</td>
<td>Wall 20–80 nm thick</td>
<td>Gram positives bacteria with one homogenous layer of peptidoglycan (murein). Four types of peptidoglycans depending on genus</td>
</tr>
<tr>
<td></td>
<td>2 μm in some genus</td>
<td></td>
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<tr>
<td>Fine roots</td>
<td>&lt;2–0.2 mm</td>
<td>Ramified structure composed, at a micromorphological level, of complex arrangements of linear vessels</td>
<td>Highly variable in thickness, from 0.1 μm in young cells to 100 μm in mature cells</td>
<td>Primary wall: network of fibrous cellulose and hemicellulose embedded in a matrix of pectin. Secondary wall: only in mature cells, can contain lignin</td>
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<td></td>
<td>Single conducting vessel</td>
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<tr>
<td></td>
<td>10–30 μm</td>
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</tbody>
</table>

**Note:** Review of the different filamentous organisms in soils synthesising characteristics such as filament diameter and morphology and their cell wall morphology and biochemistry. References from Jones 1970; Dix & Webster 1995; Paul & Clark 1996; Bouma et al. 2001; Carlile et al. 2001; Pregitzer et al. 2002; Prescott et al. 2005; Coleman et al. 2004; Pessoni et al. 2005; Hishi 2007.

### Fungal strands and hyphae ultrastructure

#### Fungal hyphae size

Single fungal hyphae diameter is highly variable, depending on the taxonomic position, environmental conditions, age, and function of hyphae. Nevertheless, for functional hyphae, an average diameter of 3–6 μm is found in the literature (Dix & Webster 1995; Carlile et al. 2001). Non-functional hyphae, such as those from the cortex of fungal strands, can have a diameter of 1 μm, with an inner diameter of <0.5 μm.

#### Fungal strands

Basically, a fungal strand is a bundle of juxtaposed linear hyphae. They are organs produced by fungi to explore their environment and to translocate nutrients from one place to another. They have the ability to extend over long distances, that is up to 30 m. In addition, macromorphologically, they exhibit a variable diameter, depending mostly on their age and remoteness from nutrient sources. This diameter ranges from a few μm up to 4 mm.
in some wood decaying species (Thompson 1984). The structure of the fungal strand is composed of: (i) an outer layer (the cortex) composed of a thick layer of narrow thick-walled multisepate dead hyphae (average diameter of 1 μm); and (ii) an inner layer (the medulla) composed of a few linear wide thin-walled sparsely septate living hyphae (average diameter of 6–10 μm). The latter seems to be less resistant to hydrostatic pressure (Watkinson 1979) and thus is more rapidly exposed to decay processes than hyphae with a thick melanized wall in the outer layer. The inner part often collapses, leaving fungal strands composed of a thick layer of narrow hyphae with empty wide channels in the middle (Watkinson 1984). The cortex of the fungal strands makes it an impermeable organ where fluids can be bidirectionally translocated (Watkinson 1979; Dix & Webster 1995).

Composition and structure of the fungal cell wall

The thickness of the cell wall also shows great variability depending on physiological processes and the function of hyphae. Single hyphae have an average cell wall thickness of 150 nm (Jones 1970; Beckett et al. 1974; Farkas 1979; Ruiz-Herrera et al. 1996; Pessoni et al. 2005). On the other hand, hyphae from the cortex of a fungal strand can exhibit very thick cell walls, up to 500 nm. The wall thickening can even occlude the hypha lumen (Watkinson 1984). Moreover, the walls of these hyphae often present a high degree of melanization, which is also a factor in impermeabilization (Paul & Clark 1996).

The fungal cell wall can be described by two main types of materials, an outer layer composed of amorphous material (mainly mannanproteins), and an inner layer composed of fibrous material, chitin and β-glucans (Burnett 1979; Ruiz-Herrera 1992; Bowman & Free 2006). Chitin is a polymer of a polysaccharide, N-acetyl-glucosamine. It is present in the form of long microfibrils, sometimes over 1 μm, with a diameter of 10–25 nm. It is located in the innermost part of the wall, arranged as an intertwined mesh embedded in an amorphous matrix (Aronson & Preston 1960; Carlile et al. 2001). β-glucans are homopolysaccharides of glucose. In the fungal wall, it is present either as β(1-3) glucan or in a lesser amount as β(1-6) glucan. They are found in greater amounts than chitin (Carlile et al. 2001; Farkas 2003). Figure 1 shows a sketch of the fungal cell wall.

![Fig. 1. Sketch of the fungal cell wall (modified from Latgé 2003). Note the fibrous layer composed by chitin and β-glucan fibres and the amorphous layer. In order to give an orientation to this sketch, the plasma membrane of the fungal cell has been represented by the phospholipids bilayer.](image-url)
Cell wall material can be a significant part of the resistant organic matter in soils. Depending on physico-chemical parameters, enzymatic degradation of polymerous substances from the cell wall may or may not be possible (Paul & Clark 1996; Coleman et al. 2004).

Materials and methods
Secondary carbonate accumulations have been sampled at four sites: (i) in the mineral layers (calcic horizon of a calcisol; IUSS Working Group WRB 2006) at a quarry near Villiers (Swiss Jura Mountains, 47°04′N, 6°59′E), as well as (ii) at an outcrop near Ainsa (Spain, 42°21′N; 0°04′E); (iii) in the narrow entrance of a cave near les Cornettes de Bises (Swiss Alps, 46°19′N, 16°48′E); and (iv) in the vers chez le Brandt cave, inside a wide chamber at 100 metres from the entrance (Swiss Jura Mountains, 46°56′N, 6°28′E). At the first two sites (soils developed on scree slopes), samples exhibit two different morphologies directly visible in the outcrop: (i) cotton-ball-like NFC that accumulates in the soil pores; and (ii) coatings on grains and centimetric to decimetric cryoclasts. When wet, these coatings constitute a plastic paste, which becomes pulverulent when dry. At the third and fourth sites (caves), only moonmilk deposits were sampled in the form of a wet plastic wall coating, up to 30 cm thick. In addition to crystals, fungal strands associated with different macroscopic morphologies of NFC have been sampled for electron microscope observations. Strands have been taken from cotton-ball-like NFC, associated with rock fragment coatings, or free in the soil pores. All samples were collected using polypropylene tweezers and stored in sterilized 50 mL tubes at low temperature.

Bulk samples were analysed by X-ray diffraction (XRD) using a Scintag diffractometer in order to determine their mineralogical nature. In each sample, quartz powder was added in order to normalize and compare samples with each other.

In order to detect organic from mineral material, prior to observations, each macromorphological sample was stained using a 4% osmium aqueous solution from a modified Pearson et al. (2004) protocol. However, using this labelling method, the presence of the organic matter can be determined but not its nature. Samples were gold-coated (10 nm) and observed using a Phillips ESEM-FEG XL30 Field Emission Gun Scanning Electron Microscope (FEG-SEM). Osmium staining was detected with an EDAX Energy Dispersive Spectrometer (EDS) coupled to the electron microscope. With natural non-flat samples and absence of a standard, EDS spectra only give qualitative information.

In order to check possible artifacts due to high-vacuum, some representative samples were observed using XL30 SEM in its LTSEM cryo mode (Low-temperature SEM). Some fungal strands and coatings were embedded in an epoxy resin, and ultrathin sections were performed using a Reichert Ultracut S (Leica) microtome with a diamond knife. Ultrathin sections (200–220 nm thick) were observed using a Phillips CM-200 Transmission Electron Microscope (TEM) with a voltage of 200 kV. Crystal properties were determined using microdiffraction.

Results
XRD analysis of the three types of samples (cotton-ball-like structures, coatings on grains, and moonmilk) shows that their mineralogy is calcitic in nature (Fig. 2a–c). Moreover, the absence of shift (expected in presence of Mg in the crystal lattice) after normalization with quartz powder characterizes the presence of low magnesium calcite (LMC) at these sites.

SEM observations of soil samples show recursive associations between NFC, unidentified nanofibres, and fungal strands. NFC is characterized by a width between 0.5–2.0 μm and a length <100 μm. Some epitactic growths are present but no important development is observed (no big euhedral crystals due to epitactic cementation). The NFC is present either as random meshes, or as bundles, 3–30 μm in diameter (average 10 μm; Fig. 3a). This microscopic feature constitutes the macroscopic cotton-ball-like structures. Bundles contain some nanofibres, occasionally associated with amorphous matter assumed to be an organic veil (Fig. 3b). These nanofibres are rarely observed on NFC when the latter are randomly oriented and/or strongly modified by epitactic growth. At a microscopic scale, coatings from soil grains exhibit various amounts of NFC and nanofibres, in which needles are less represented than in the cotton-ball-like morphology. NFC often shows random orientations, whereas nanofibres are packed in clusters. This morphology shows great similarity with microstructures of moonmilk samples, in which NFC is even less represented.

SEM measurements show that nanofibres are up to 6 μm long (the shortest is 0.2 μm long) with an average width of 78.6 nm (standard deviation of 22.5 nm, based on 106 measurements; Fig. 3c). They are characterized by a high flexibility (as mentioned by Borsato et al. 2000), leading to spectacular curvature (Fig. 3c). They appear smooth under TEM. Two kinds of structures are observed: (i) a randomly-oriented framework of nanofibres, in which widespread putative organic veils and calcitic micro-aggregates are present; these meshes are
Fig. 2. X-ray diffractogramme showing the low-magnesium calcite nature of (a) cotton-ball like NFC from the Swiss Jura Mountains, (b) coatings on blocks from Swiss Jura Mountains and (c) moomilk from the Vers chez le Brandt cave in the Swiss Jura Mountains samples. Dotted lines correspond to the main peaks of low-magnesium calcite (CaCO$_3$), the other peaks mainly correspond to quartz (quartz added prior to analysis to allow peak correction).
often associated with other components (i.e. NFC, fungal strands, hyphae, etc.) as observed in moonmilk deposits (Fig. 3d); and (ii) organized structures of nanofibres (Fig. 4), either as small pieces that have apparently undergone a breakdown, or as a tubular/circular microscopic network (Fig. 4a, c). The term organized refers to a non-random distribution of nanofibres, whatever their nature. These networks are composed by intertwined nanofibres oriented in two main directions (Fig. 4b, d).

Another main component is frequently observed associated with soil samples: macroscopic, brown organic filaments, identified as fungal strands. Their average diameter can reach 100 μm and they are composed of the two typical mycelial strand structures, an external part made of several narrow fungal hyphae with a thick cell wall and an inner part characterized by a few wide thin-walled hyphae, which often lack in our observations due to their ability to be rapidly decayed (Fig. 5a, b). Nanofibres are abundant all along the macroscopic filament where fungal strands seem to break down.

A cross-section of a fungal hypha shows that the fungal wall is composed of two layers, an inner part composed of fibrous material and an external part composed of an amorphous material (Fig. 5c, d). From these observations, it is obvious that there is an intimate relationship between the hyphae and the nanofibres (Fig. 5c, d). Optical observations, hydrochloric acid tests on moonmilk, as well as TEM microdiffractions (Borsato et al. 2000) indicate that the nanofibres are mineral in nature. In order to test this hypothesis, in-situ analyses were performed to distinguish organic from mineral matter using osmium labelling with EDS control on samples. The osmium stains only organic matter and not mineral material (Pearson et al. 2004). Osmium peaks indicate that non-organized frameworks composed of only nanofibres do not

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**Fig. 3.** (a–d) SEM photomicrographs. (a) Bundle of NFC present in a sample from the Spanish site of cotton-ball-like NFC associated with a fungal strand (observed macroscopically). (b) Bundle of NFC covered by putative organic veils. Some organic nanofibres are also present. Sample from a grain coating associated with a fungal strand and some cotton-ball like NFC. (c) Close-up of an organized mesh composed by nanofibres (moonmilk, Swiss Alps). Preferential orientations of nanofibres are shown by the crossed double-headed arrows. Some nanofibres are curved (arrows). This characteristic indicates a contact-deformation. (d) Mesh composed by randomly-oriented nanofibres associated with NFC (white arrows) and putative organic veils (black arrows). Swiss Jura Mountains, coatings on block.
contain organic material (Fig. 6a, b) and organized meshes do contain organic matter (Fig. 6c, d).

Discussion

The presence of nanofibres in various vadose environments has been widely observed. Their origin is either attributed to a biogenic factor (‘probably rod-shaped calcified bacteria’ Phillips & Self 1987; Ould Mohamed & Bruand 1994; ‘microrod attributed to bacteria or nuclei in gel’ Verrecchia & Verrecchia 1994; Loisy et al. 1999) or physicochemical processes ['precipitation from pore filling fluids', sometimes associated with organic filaments, that is, nanofibres ‘cannot be fully attributed to direct organic activity’ (Jones & Ng 1988); ‘nanofibres show microstructures that are typical of inorganic, crystalline material’ (Borsato et al. 2000)]. Therefore, the origin of terrestrial accumulations of nanofibres remains controversial.

The nanofibres discussed in this paper are similar to those described in Phillips & Self (1987), based on their size and flexibility. It is important to note that they identified the organic filaments associated with nanofibres as fungi, and the same conclusion is drawn in this study, based on their size and morphology, as well as presence of macrostructures.
such as mycelial strands (Fig. 5a, b). Two layers are visible during the breakdown of the hyphal cell wall (Fig. 5d). It is known that the inner wall layer is composed of a hard microfibril framework (theoretically 10–25 nm in diameter, Carlile et al. 2001) made of chitin and β-(1-3), β-(1-6) glucan. Based on this fact and the recognition of the organic nature of some nanofibres (Fig. 6c, d), the organized meshes of nanofibres are considered as the result of a slightly destructive decay of the fungal fibrous cell wall material. At this stage, the organized meshes are interpreted as the first step in the breakdown of the fungal hyphae cell wall, whereas the non-organized mats represent the ultimate state of decay and reworking of this organic matter. In other words, the nanofibres could be interpreted as organic in origin and being the result of an incomplete decaying of fungal matter. During early diagenesis, their calcitic pseudomorphosis (Cailleau 2005; Cailleau et al. 2005) and/or their role as a template for calcite precipitation results from the release of the nanofibres in the soil environment, followed by their exposure to mineralizing pore filling fluids. This could explain why non-organized meshes (interpreted as the oldest decay product) are often composed of nanofibres of calcite, due to the longer exposure time to soil fluids. To conclude, the release of nanofibres may represent a partly destructive decay of the fibrous cell wall material.

Moreover, this interpretation has an important implication for NFC origin. The observation of NFC inside organic sleeves and the presence of small mats of nanofibres on bundles of NFC (Fig. 5d (star); Cailleau et al. 2009) suggest a large contribution of organic matter for their genesis.
Fig. 6. (a) and (c) SEM view of samples. The black window shows the area analysed. (b) and (d): EDS spectra, dotted lines correspond to Au peaks (samples coated with gold for SEM observations) and Os is the osmium labelling. (a, b) Analysis performed on a dense unorganized mesh of nanofibres (sample from coatings on a block). Note the absence of an osmium peak on the spectrum in the grey box due to the purely mineral nature of nanofibres. (c, d) Analysis performed on a dense non-random mesh of nanofibres (sample from a coating on a block associated with a decaying fungal strand). Note the presence of osmium peaks on the spectrum due to the organic nature of nanofibres.
Fig. 7. Hypothetical sketch recapitulating the potential processes of fungal organic matter decay and mineralization of cell wall fibrous material. This model is based on observations, analysis, and interpretations given in this paper. The first stage starts with a fungal hyphae or a group of hyphae forming a fungal strand. The cell wall is constituted by two main layers, an amorphous one (on the top) and a fibrous one (at the bottom; for more details see Fig. 1). It is assumed that the cell wall fibrous material is not decayed at the same rate than the amorphous material. When released in the soil microenvironment, these nanofibres could act as template for calcite nucleation, eventually leading to calcitic pseudomorphosis. The ultimate step is represented by possible reworking due to various processes (bioturbation, water movements, cryoturbation, etc.). Nanofibres are often associated with other calcitic features, such as NFC, leading to the complex microfabric observed in soil and cave deposits. O.M., organic matter.
As first noted by Phillips & Self (1987), NFC bundles could be the first step in the distribution of NFC in soil pores. Indeed, with the collapse of bundles due to various processes, such as weathering and bioturbation, would lead to a random distribution of NFC (mesh). Nanofibres on bundles would then be a relict of the organic sheath (assumed to be fungal in origin). The presence of mycelial strands is critical to understand the origin of the bundles. Strands and bundles are both organized as a tubular structure (Figs 3a & 5b) composed of sub-parallel components. They have similar diameters: 2–30 μm on average for the bundle and 8–80 μm for the whole fungal strand. But the outer layer of the mycelial strand is usually wide and often represents between a third to a half of the strand section. Thus, only the inner diameter should be considered in this case. The outer layer is composed of hyphae with thick cell walls. Their central hole is probably too small to contain any NFC, whereas the inner part of the strand contains wider hyphae that would have enough room to allow the formation of a crystal such as a needle.

One of the most important elements for fungal growth is calcium. Indeed, it is implicated in the apical growth control. Nevertheless, Ca2+ is considered as toxic when present in high concentrations (Gadd 1993). Consequently, its concentration within the fungal cell, and especially in the apex, must be under strict control of the organism in order to allow proper growth (Jackson & Heath 1993). Under hydrous stress conditions, the concentration of calcium could reach a high level, close to saturation. As it has been suggested for metal-oxalate (Whitney 1989; Gadd 1999), fungi could induce the precipitation of carbonate, possibly leading to a decrease of their internal calcium content (Gadd 2007). This process is documented for bacteria (Simkiss 1986; Schultz-Lam et al. 1996; Barton & Northup 2007). The inner layer of the fungal cell wall is composed by a large amount of chitin known to be a good template for calcite precipitation (Manoli et al. 1997). Consequently, nucleation of calcite crystals inside the inner functional hyphae from mycelial strands constitutes a serious hypothesis. The role of fungal hyphae as a crystal nucleation enhancer has already been suggested in the past (Went 1969; Northup & Lavoie 2001; Gadd 2007). Any other cell wall fibrous material or polymeric substance, for example β (1-3) glucan or a glycoprotein, may have the same effect (Burazerovic et al. 2007; Shen et al. 2007). To conclude, all our observations are recapitulated in a step-by-step hypothetical model (Fig. 7), showing the potential relationships between fungal organic matter and calcium carbonate precipitation.

Conclusion
Considering previous hypotheses on the origin of nanofibres (i.e. biogenic or purely physico-chemical), the results presented here indicate that nanofibres could also originate from the breakdown of fungal hyphae, especially their cell walls. During the decay of organic matter, microfibrils such as chitin or β (1-3) glucan, are released from the inner layer of the fungal cell wall. When these organic nanofibres are exposed to mineralizing pore fluids, they could undergo calcitic pseudomorphosis and/or be used as templates for calcitic precipitation. In the case of NFC bundles, which have an intimate relationship with nanofibres, these nano-features could indicate the relict of an organic sheath. As interpreted by Phillips & Self (1987), the implication of fungal strands in the genesis of NFC is now better supported. In other words: bundles could be the ultimate remains of the presence of a fungal strand. This hypothesis emphasizes the important role of organic matter in carbonatogenesis as well as the fundamental role of fungi in the terrestrial carbon cycle.

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