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Research article

In vitro microcosm study of *Morchella eximia* mycelial growth on post-wildfire burnt soil and charcoal fragments

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Abstract
This study investigates the in vitro mycelial growth dynamics of the pyrophilous mushroom *Morchella eximia* on substrates composed of burnt soil and charcoal fragments. Soil samples were collected one month after a wildfire from a *Pinus pinaster* forest on Monte Pisano (Italy). These samples were inoculated with *M. eximia* mycelium, and fungal colonization and development under controlled microcosm conditions were subsequently assessed. The chemical analysis was performed on the burnt soil to quantify macro- and micronutrients. After the mycelial growth, the charcoal structure was examined using attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy. Additionally, Scanning Electron Microscopy (SEM) was applied to visualize and confirm hyphal colonization of the charcoal. *Morchella eximia* colonization elicited significant structural alterations in charcoal structure. Spectroscopic analysis demonstrated an increased relative intensity of the primary charcoal band concomitant with a pronounced decrease in the secondary band, indicative of modifications within the charcoal structural matrix. Furthermore, reduced spectral signals observed near 1169 cm^{-1}, corresponding to C–O functional groups in lignin-derived phenolic compounds, provide evidence for chemical transformations occurring in the charcoal. These results highlight the potential involvement of pyrophilous fungi in the degradation of charcoal and emphasize the necessity for further investigations to clarify their ecological functions and long-term effects on fungal community dynamics.

Keywords
Morels, Wildfires, Pyrophilous fungi, FTIR, SEM

Introduction

Wildfires are a major ecological disturbance that significantly impact terrestrial ecosystems worldwide. Their frequency, intensity, and spatial extent have been increasing in recent decades, largely due to climate change and human activities (Cunningham et al., 2024; NASA, 2025). In fact, rising global temperatures, prolonged droughts, and altered precipitation patterns have created conditions that favor more frequent and severe wildfires. Land-use modifications associated with

agriculture, forestry, and urban expansion, coupled with practices of fire suppression and ignition, have significantly disrupted natural fire regimes. Moreover, the global movement of species has led to the formation of new ecological communities, altering fuel distribution, influencing fire patterns, and reshaping post-fire ecological processes (Bowman et al., 2011; Keeley et al., 2019; Barreiro and Díaz-Raviña, 2021).

Wildfires impact ecosystem structure and function at multiple levels, influencing biodiversity, soil composition, and recovery processes, not only through direct effects but also via complex interactions among ecosystem components (Hrelja et al., 2020; Kelly et al., 2020). The immediate consequences of wildfires include the loss of vegetation, the release of CO_2 and other greenhouse gases, and significant alterations to soil properties, all of which can negatively affect living organisms (Farid et al., 2024; Gajendiran et al., 2024). In the medium and long term, the disruption of biogeochemical cycles, and the alteration of soil biodiversity, may drive shifts in ecosystem composition, influencing species diversity and regeneration potential (Rovira et al., 2012; Keeley et al., 2019; Pellegrini et al., 2021).

Among the different ecological components affected by fire, soil plays a fundamental role in post-fire recovery. Wildfire-induced soil alterations are multifaceted, including increased erosion and nutrient loss, which can lead to land degradation and reduced soil fertility (Francos et al., 2016; Hrelja et al., 2020; Imeson et al., 1992; Farid et al., 2024). These disturbances collectively create a challenging environment for soil microbial communities, which play a critical role in nutrient cycling, organic matter decomposition, and overall soil recovery after fire events (Barreiro and Díaz-Raviña, 2021).

Of particular concern is the accumulation of aromatic and refractory carbon compounds, resistant to microbial decomposition, further alters soil chemistry and organic matter dynamics (Rovira et al., 2012; López-Martín et al., 2016; Salgado et al., 2024). Among these, charred organic matter constitutes a complex and heterogeneous mixture of carbon forms with varying degrees of aromaticity (Francioso et al., 2011; Mastrolonardo et al., 2015, 2017). Identifying and quantifying charcoal in natural environments remains challenging due to this chemical heterogeneity. Its composition and properties are influenced by multiple factors, including fire type, intensity, and duration (González-Pérez et al., 2004; Shakesby, 2011). High-severity fires, in particular, may induce partial distillation, charring, or complete oxidation of organic matter, thereby further complicating its characterization and ecological implications (Certini, 2005). Charcoal characterization is generally a laborious and costly process, underscoring the need for rapid techniques that require little to no sample preparation, especially in large-scale studies. In this context, FT-IR spectroscopy represents a promising alternative to traditional physio-chemical methods.

Fungal communities are particularly sensitive to fire-induced changes in soil properties and vegetation dynamics. The direct effects of wildfires on fungal assemblages include exposure to extreme temperatures, organic matter combustion, and alterations in soil chemistry (Barreiro and Díaz-Raviña, 2021). Indirectly, wildfires influence fungal populations by modifying plant communities, altering resource availability, and shifting competitive interactions among microbial species (Dove and Hart, 2017; Barreiro and Díaz-Raviña, 2021). While some fungi may experience declines in abundance or local extinctions, pyrophilous fungi such as those inside *Anthrocobia*, *Morchella* and *Pyronema* genera develop rapidly thanks to their ability to colonize recently burned areas in post-fire environments (Claridge et al., 2009; Reazin et al., 2016; Fox et al., 2022). These

fungi often exhibit traits that allow them to take advantage of post-fire environments, such as spore germination triggered by heat or smoke, rapid colonization of nutrient-enriched soils, and symbiotic interactions with fire-adapted plant species (Raudabaugh et al., 2020; Fox et al., 2022).

However, the exact mechanisms underlying this phenomenon remain only partially understood. Recent studies conducted on *Pyronema* species reveal their rapid colonization of post-fire environments, probably driven by their ability to metabolize aromatic compounds found in charred organic matter (Fischer et al., 2021). This ability can be attributed to the capacity of certain fungi to release a wide range of enzyme, such as laccase, that enable the acquisition of nutrients from recalcitrant molecules under specific conditions (Sinsabaugh, 2010). However, the presence of charcoal in soil can influence the activity of enzymes like phenol oxidase by altering soil pH and substrate availability, thereby affecting how these enzymes participate in organic matter decomposition and nutrient cycling (Gao et al., 2016). Genomic analyses further suggest that their success may be linked to the presence of specific genes that confer adaptations for charcoal tolerance and reproduction in fire-altered soils (Bruns et al., 2020; Fischer et al., 2021; Steindorff et al., 2022).

Species of the genus *Morchella* (true morels) exhibit a strong ecological association with fire and their ability to proliferate in fire-affected areas has drawn significant interest from mycologists and ecologists alike. These ascomycetous mushrooms exhibit a not yet fully clarified life cycle that allows them to fruit abundantly in the first year following a wildfire, taking advantage of the altered ecological conditions (Larson et al., 2016; Hughes et al., 2020). In particular, the emergence of post-fire *Morchella* is thought to be linked to changes in soil properties, the release of specific nutrients, and the reduction of competitive microbial pressure (Larson et al., 2016). Additionally, fire may play a role in breaking dormancy mechanisms in *Morchella* sclerotia, facilitating their rapid development in newly disturbed environments (Fox et al., 2022).

The present study aims to investigate the capacity of *Morchella eximia* Boud. to colonize and degrade charcoal after wildfire under laboratory conditions. Due to the complex charcoal structure, micro–Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR) spectroscopy was selected for the analyses. This technique is both fast and simple, requires no sample preparation and can be performed in situ. Additionally, Scanning Electron Microscope (SEM) was employed to visualize and confirm the colonization of charcoal by fungal hyphae. SEM provides high-resolution imaging of the charcoal surface, enabling detailed observation of fungal structures and their interaction with the substrate.

Materials and Methods

Soil sampling and study area

The soil and charcoal used in the *in vitro* degradation tests were collected in a study area located on Monte Pisano, in the municipality of Buti (Pisa, Italy). Prior to the high-severity and high-intensity wildfire that affected approximately 6 ha in September 2023, the site was covered by a forest of *Pinus pinaster* Aiton, with an understory predominantly composed of *Erica* sp., *Arbutus unedo* L., and *Ulex europaeus* L. A total of five soil and charcoal samples, each weighing 0.5 kg, were randomly collected one month after the wildfire, placed in sterile polyethylene bags, and transported to the laboratory under controlled conditions (4 °C in the dark). In the laboratory, the samples were combined and air-dried at room temperature. A part of the dried soil was subsequently sieved at 2 mm to separate the soil from the charcoal to be used in subsequent *in vitro* tests.

Physical and chemical analyses of burnt soil

The soil total carbon and nitrogen contents were determined using an elemental analyzer (CHNS-O EA 1110, Thermo Fisher Scientific). The total metal contents were measured in finely ground samples through microwave-assisted acid digestion (Milestone, Shelton, CT, USA) with suprapure HNO_3 (Carlo Erba, Italy) and 30% H_2O_2 solution (RPE, Carlo Erba, Italy). The mineralized samples were then diluted with ultrapure water, and each element was quantified using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). The pH was determined using a pH meter (Hanna Instruments, Padova, Italy). The soil organic matter (SOM) content, moisture and ash were analyzed using a TG-DTA92 instrument (SETARAM, France). A quantity of 20 mg of the soil was placed in an alumina crucible and heated from 30 to 700 °C at 10 °C min^{-1} under flow ultra-zero grade air at 130 mL min^{-1}. The SOC was estimated as weight loss from 250 to 500 °C. All analyses were performed in triplicate.

Mycelial culture and molecular characterization

The *M. eximia* strain used in this study was isolated from an exsiccated ascomata collected in May 2021 in a *Pinus nigra* J.F. Arnold, forest located in Arischia (L'Aquila province, central Apennines, Italy), which was affected by a wildfire on August 30, 2020. *Morchella eximia* mycelium was obtained from a diluted spore suspension in sterile water on potato dextrose agar (PDA, 20 g L^{-1}, DIFCO), supplemented with antibiotics (200 µg mL^{-1} of streptomycin, ampicillin, and chloramphenicol). The strain was stored at 22 ± 1 °C in the dark and renewed every month.

Molecular identification was performed by amplifying the internal transcribed spacer (ITS) region of the rDNA using the primer pair ITS1F/ITS4 (White et al., 1990; Gardes and Bruns, 1993) by applying a direct Polymerase Chain Reaction (PCR) approach that avoid DNA extraction (Iotti and Zambonelli, 2006). PCR was carried out in 50 µL reaction volumes using the Terra™ Direct PCR Polymerase Mix (TaKaRa), and conducted in a SimpliAmp thermal cycler (Thermo Fisher) under the following conditions: 98 °C for 2 min, 95 °C for 4 min, followed by 30 cycles of 94 °C for 30 s, and 56 °C for 30 s with a final extension of 7 min at 72 °C. The amplification product was sequenced, and the resulting sequence was deposited in NCBI under the accession number PV796461.

In vitro degradation study

To investigate the ability of *M. eximia* to colonize and degrade charcoal, an *in vitro* microcosm was set up. Five autoclavable polypropylene boxes with a diameter of 8 cm and a height of 6 cm, equipped with a 0.2 µm filter on the lid, were filled with 150 g of burned soil at 75% humidity, on the surface of which 15 g of charcoal fragments were placed (Fig. 1). Given the heterogeneity of the charcoal, some pieces were split into two. One of the fragments was marked with a pin and introduced into the microcosm, while its corresponding counterpart was kept as a control. The sealed boxes were autoclaved at 121 ± 1°C for 20 min and then inoculated with a 1 cm diameter plug from a 15 days-old colony. Boxes were kept in the dark at 22 ± 1 °C for 180 days.

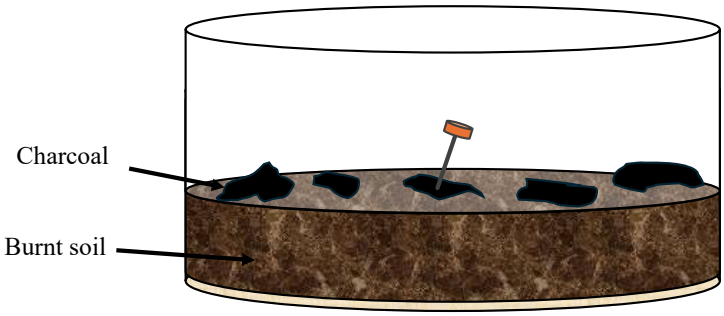

Fig. 1. – Microcosm representation for the *in vitro* test of *Morchella* mycelia growth on burnt soil and charcoal fragments.

Assessment of charcoal degradation through ATR-FTIR spectroscopy and SEM observation

After 180 days of mycelial colonization, the boxes were opened, and the pinned charcoal fragments were extracted, the superficial hyphae were removed under a dissecting microscope (Photomacroscop M400, Wild, Switzerland) and, subsequently dried in an oven at 40 ± 1 °C for 48 h, and analyzed. The ATR-FTIR spectra of colonized and the correspondent uncolonized charcoal fragments were acquired using a Bruker Tensor FT-IR spectrophotometer (Bruker, Ettlingen, Germany) equipped with a micro-ATR accessory featuring a single reflection and a 45° angle of incidence analysis (Specac Quest ATR, Specac Ltd, Orpington, Kent, UK). The spectra were recorded (100 scans per sample or background) within the spectral range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} and processed using the Grams/386 spectroscopic software (version 6.00, Galactic Industries Corporation, Salem, NH, USA). Pre-processing of the spectra included baseline correction and normalization to adjust the baseline and shift the intensity values, ensuring that the minimum absorbance was set to zero. The spectra were processed by curve fitting in the spectral region from 1800 to 600 cm^{-1}. Spectra were deconvoluted into Gaussian components, with optimal fitting parameters obtained through minimization of the reduced chi-square (χ^2) statistic. The fitting quality was confirmed by high coefficients of determination (R^2) ranging from 0.999 to 0.988, and standard errors (SE) between 0.005-0.001 indicating good concordance between experimental and modeled spectral data (Mastrolonardo et al., 2015). The identified peaks were integrated, and their areas were calculated and expressed as relative percentages. These percentages were then used to represent the relative amounts of each functional group. The spectral analysis was performed in five replicates. Since the replicated spectra exhibited a high degree of similarity; therefore, an averaged spectrum is presented in Figure 2.

Scanning Electron Microscopy (SEM) analysis was performed to assess the adhesion and growth of fungal hyphae as well as the degradation of colonized charcoal fragments. Small fresh colonized pieces of charcoal where initially fixed in a 5% glutaraldehyde solution for 24 h at 4° C. Subsequently, the samples underwent two washes of 7 min each in 0.1 M phosphate buffer, followed by a graded ethanol dehydration series consisting of two washes (7 min each) at 20%, 30%, 50%, 75%, 90%, and 100% ethanol. All washing steps were performed at 4 °C under continuous stirring.

The prepared samples were then stored in absolute ethanol (99.9%) before undergoing supercritical dehydration using liquid CO_2. Following dehydration, the samples were mounted on an aluminum stub (26 mm) and coated with gold in real time for 100 sec using an ion sputter coater (E-1010; Hitachi, Tokyo, Japan). SEM imaging of the gold-coated samples was conducted using a

Philips 515 scanning electron microscope (Philips, Amsterdam, Netherlands) with an acceleration voltage of 25.0 kV.

Results and discussion

Chemical characterization of burnt soil

The chemical and physical parameters of soil are shown in Table 1. The soil had a sandy loam texture (54% sand, 44% silt, 2.3% clay). The C and N contents, along with the C/N ratio, fell within the range reported for burnt soils in the literature (Deus et al., 2021). Specifically, the low nitrogen percentage (0.10%) is largely due to volatilization of nitrogen compounds during the fire event (Caldwell et al., 2002). Research shows that the C/N ratio in soils affected by fire generally increases due to the buildup of pyrogenic carbon (Lehmann and Joseph, 2015). This carbon form is produced through the incomplete combustion of organic matter and the partial decomposition of partially burned biomass, combined with a simultaneous reduction in soil nitrogen levels (Abney and Berhe, 2018). As a result, the accumulation of this thermally altered carbon form, which is chemically stable and resistant to decomposition, leads to higher soil C/N ratios after burning events (Agbeshie et al., 2022). This imbalance may substantially hinder plant regeneration and negatively affect the soil microbiome. Consequently, the ecosystem's capacity to regulate carbon dioxide (CO_2) emissions is markedly diminished (Delgado-Baquerizo et al., 2013; Wang et al., 2020).

Table 1 – Chemical and physical parameters of soils one month after the wildfire

Parameter	**Mean ± SD***
	(%)
C	3.29 ± 0.97
N	0.10 ± 0.02
C/N	32.9
SOC (250-500 °C)	4.5 ± 0.6
Moisture	1.1 ± 0.1
Ash	95.5 ± 3.0
pH-H_2O (1:2.5, m:v)	5.3 ± 0.2
	mg kg^{-1}
P	196 ± 33
S	155 ± 65
K	2076 ± 251
Na	339 ± 27
Ca	1900 ± 261
Mg	2142 ± 222
Fe	236 ± 20
Cu	204 ± 27
Zn	397 ± 40

*Data calculated on 3 technical replicates.

The pH was found to be acidic (pH 5.3), contrary to what has been reported in the literature, as the pH increases after fire, especially after high intensity fires, due to the deposition of ash rich in

base-cations after the combustion of plant materials (Alcañiz et al., 2018). In our case, the pH remained stable due to the geological conditions of the study area. This pattern has also been reported in previous studies, where pH levels were observed to remain unchanged following fire events (Badía et al., 2014; Fernández-Fernández et al., 2015; Fernández-García et al., 2019a, 2019b). Environmental factors such as erosion process (Fernández-García et al., 2019a, 2019b) are involved in the pH variation. The pH of soil is very important for how much of the nutrients in soil are available to plants (Rosques et al., 2013). At low pH values, phosphorus becomes a limiting nutrient factor for the complex formation with Al and Fe oxides (Abolfazli et al., 2012). Notably high concentrations of Fe, Cu, and Zn were observed (Table 1). A number of factors may be responsible for the presence of metals in post-fire soils, including low soil organic matter content, low nutrient levels and pH imbalances (Chiu et al., 2006).

Assessment of charcoal degradation and SEM observations

ATR-FTIR spectra of the charcoal fragments before (red line) and after growth of *M. eximia* mycelium (black line) are shown in Figure 2a. The spectra exhibited intense, broad absorption bands at 1585-1579 cm^{-1}, 1490-1300 cm^{-1} (broad region) and 1162 cm^{-1}. Additional medium-intensity bands appear at 874 cm^{-1}, 808 cm^{-1}, and 748 cm^{-1}. A key feature is the peak at 1699-1696 cm^{-1}, corresponding to the C=O stretching vibration of carboxylic acids. This band can be considered a marker of charcoal produced at low temperatures (about 320 °C), since the carbonyl/carboxylic stretching of lignin is resistant at this temperature(~320 °C) (Boeriu et al., 2004; Constantine et al., 2021). In addition, carbonyl/carboxylic group is detectable in charcoals subjected to biological oxidative degradation (Yao et al., 2010). These spectral features offer valuable information about the thermal history and environmental conditions experienced by the charcoal sample. Notably, the spectra are characterized by two prominent peaks near 1579 and 1359 cm^{-1}, which correspond to the stretching vibrations of aromatic rings (Fuente et al., 2003; El-Hendawy, 2006). Additionally, a distinct peak at 1162 cm^{-1} is observed, corresponding to the in-plane deformation of aromatic C-H bonds and C-O stretching in phenols, a characteristic commonly associated with lignin (Fornito et al., 2020; Puliga et al., 2022; Zuffi et al., 2023). Nevertheless, the potential influence of mineral components cannot be definitively ruled out. Other peaks at 874 cm^{-1}, 808 cm^{-1}, and 790 cm^{-1} are assigned to out-of-plane aromatic C-H bending vibrations of isolated hydrogens adjacent to aromatic systems bonds (Bornemann et al., 2008).

Following mycelial growth, significant structural changes were observed in the spectrum (Fig. 2a, black line). Specifically, the curve fitting of the analyzed region (Fig. 2b,c) offers semi-quantitative insights into the distribution of functional groups present within the charcoal. The appearance of the peak at 1699 cm^{-1} accounted for 5% in charcoal treated with *M. eximia* and 2% in the control (Fig. 2b). The carboxylation process is considered a key factor in the alteration of coal (Cohen-Ofri et al., 2006). As a result, a higher concentration of carboxyl groups suggests that coal is more prone to degradation, making it more readily transformed in the environment.

In charcoal-treated samples, the percentage area of the primary carbon band increased from 18% (1579 cm^{-1} in the control) to 22% (1585 cm^{-1}), while the secondary carbon band showed a notable decrease from 14% (1372 cm^{-1} in the control) to 10 % (1354 cm^{-1}) following *M. eximia* growth. The reduction in the intensity of the secondary band reflects structural alterations in charcoal induced by fungal activity. This interpretation is consistent with previous study on fungal interactions with carbon-based materials, including graphene and charcoal (Carniel et al., 2021).

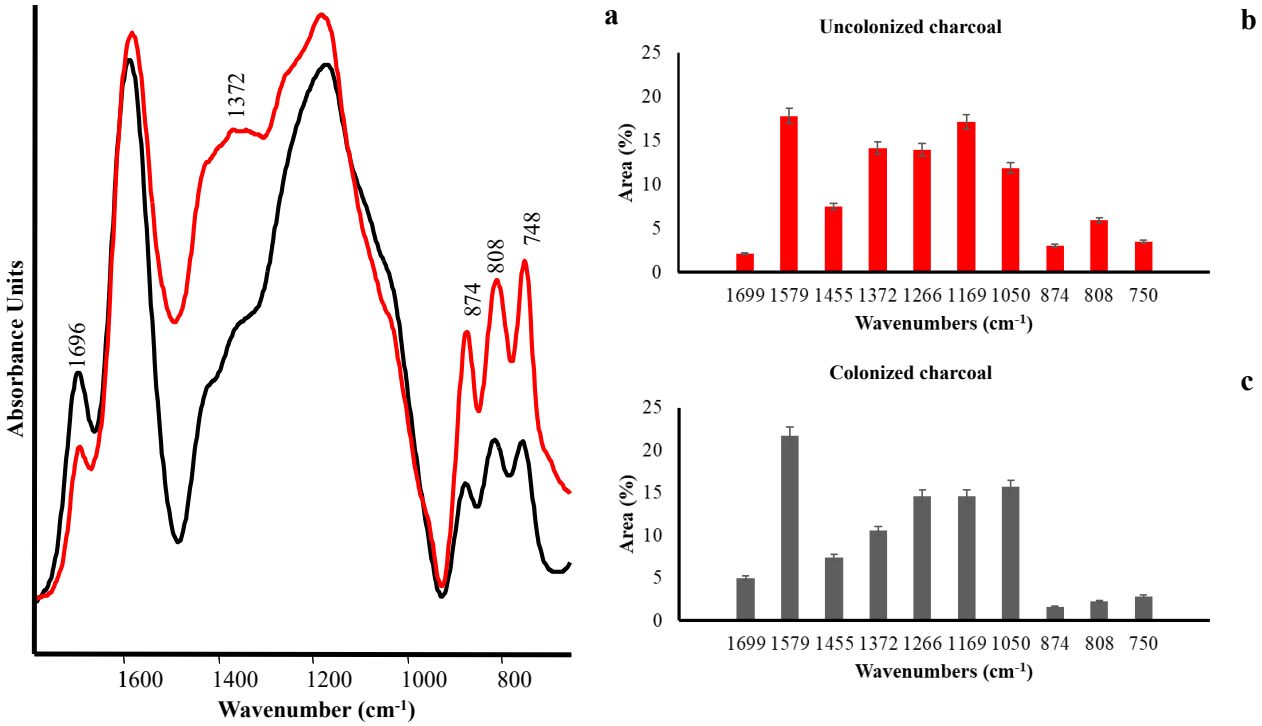

Fig. 2. – (a) ATR-FTIR spectra of uncolonized charcoal fragments (red line) and following mycelium growth (black line). (b, c) shows the percentage integration areas of the deconvoluted bands from 1700 to 600 cm^{-1}.

In particular, Carniel et al. (2021) demonstrated through Raman spectroscopy that fungal-driven oxidation or degradation processes alter the intensity of the D band (~1354 cm^{-1}), thereby indicating an increase in structural defects attributable to fungal action. In parallel, the relative areas associated with CH functional groups in aromatic rings at approximately 874 cm^{-1}, 811 cm^{-1}, and 748 cm^{-1} exhibited a marked decrease in charcoal exposed to *M. eximia*. Additionally, the spectral region around 1169 cm^{-1} showed a 14% reduction (Fig. 2), whereas those near 1266 cm^{-1} and 1030 cm^{-1} displayed only minor variations in the relative area. *Morchella eximia* colonization induces significant structural modifications in charcoal. Specifically, spectral analyses reveal an increase in the relative intensity of the primary charcoal band and a marked reduction in the secondary band, indicating alterations in the charcoal's structural matrix. These changes suggest that the charcoal becomes more susceptible to degradation following fungal colonization. Collectively, these modifications imply that *M. eximia* mycelium alters the physicochemical properties of charcoal, potentially enhancing its biodegradability and contributing to nutrient cycling in fire-affected soils (Lohberger et al., 2019).

SEM observations, performed on five charcoal fragments collected after 180 days of mycelial growth, revealed complete colonization by *M. eximia* (Fig. 3a). This fungus demonstrated the ability to adhere to and penetrate the charcoal matrix, initiating early stages of visible degradation (Fig. 3b,c). These findings are consistent with previous studies investigating the capacity of saprotrophic fungi to colonize the charred biomass (Ascough et al., 2010; Jaafar et al., 2014). Moreover, the fire-affected soil within the microcosm was also entirely colonized by *M. eximia* mycelium (data not shown), further supporting literature reports, such as those by (Filialuna and Cripps, 2021) on the ability of pyrophilous fungi to aggregate and stabilize post-fire soils.

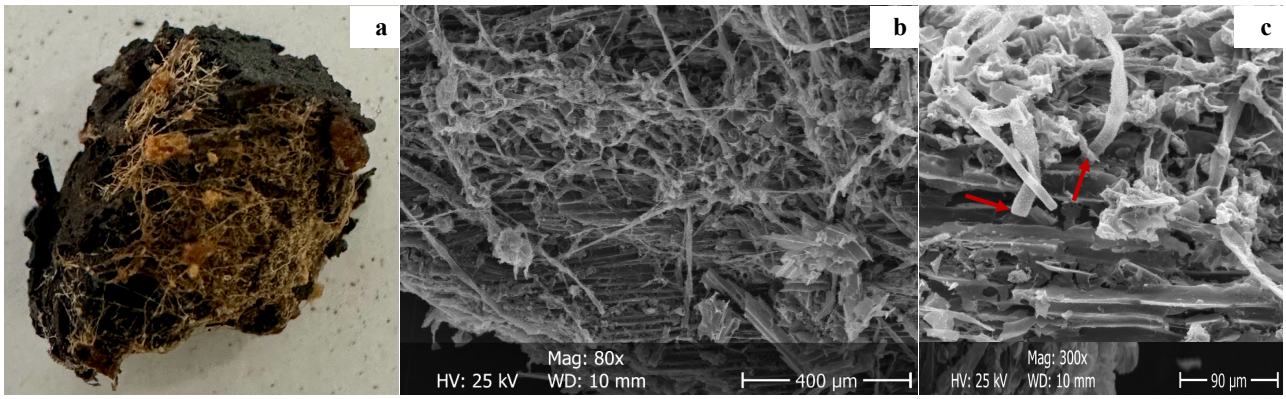

Fig. 3. – (a) Macro and (b, c) SEM microscope images of charcoal colonized fragments by *M. eximia*.

Conclusion

Our study demonstrates that *M. eximia* is capable of colonizing and actively modifying the structural and chemical properties of charcoal fragments in post-fire soils. ATR-FTIR spectroscopic analyses revealed several specific alterations induced by fungal growth: (i) the emergence and relative increase of the C=O stretching band at ~1699 cm^{-1}, reflecting carboxylation processes and the introduction of oxidation-derived functional groups; (ii) an increase in the relative intensity of the primary aromatic carbon band (from 18% to 22%), coupled with a marked reduction of the secondary carbon band (from 14% to 10%), indicative of structural reorganization within the aromatic matrix; (iii) a substantial decrease in peaks associated with CH functional groups in aromatic rings (874, 811, and 748 cm^{-1}), pointing to bond disruption and loss of aromatic integrity; and (iv) a 14% reduction in the spectral region at ~1169 cm^{-1}, linked to phenolic C–O stretching and aromatic C–H deformation. Collectively, these spectral changes provide clear evidence of fungal-driven chemical degradation of charcoal. Complementary SEM analyses confirmed complete colonization of charcoal fragments after 180 days, with hyphae adhering to and penetrating the carbon matrix, consistent with the onset of physical and chemical degradation.

Taken together, these results indicate that *M. eximia* not only colonizes but also alters the physicochemical stability of pyrogenic carbon, thereby enhancing its susceptibility to biodegradation. Such modifications have important ecological implications: by transforming charcoal into a more labile form, *M. eximia* may contribute to nutrient release, microbial succession, and the restoration of soil fertility in fire-affected ecosystems. Furthermore, the successful application of ATR-FTIR highlights the value of rapid, non-destructive techniques for monitoring charcoal transformation in complex soil matrices. Future research employing multi-omics approaches and in situ ecological trials will be essential to elucidate the molecular mechanisms underlying fungal-mediated charcoal degradation and to quantify the role of pyrophilous fungi in post-fire ecosystem resilience and nutrient cycling.

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