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Influence of Fungal Mycelium on Desiccation Cracking in Expansive Soils

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ABSTRACT

This study investigates the potential of fungal mycelium to mitigate desiccation cracking in expansive soils, a common issue caused by evaporation-induced volumetric shrinkage, which negatively impacts the stability of geotechnical structures. We hypothesize that the engineered growth of *Pleurotus ostreatus* mycelia, a 3D network of moisture-regulating biofibers could reduce or remediate cracks in clayey soils. Fungal slurry was applied either on the soil surface or mixed uniformly with clayey soil and subjected to controlled drying for 120 hours. Air temperature, humidity, water content changes, and crack propagation were monitored. The results showed that soil samples mixed with fungal slurry retained a higher *amount* of their initial water content, recorded a lower evaporation rate, delayed crack appearance, and reduced the overall crack length per unit area, compared to the untreated and surface-treated samples. These findings suggest that fungal mycelium offers a promising solution for controlling soil desiccation cracking.

Keywords: fungal mycelium, desiccation cracking, expansive soil, unsaturated soil

INTRODUCTION

Expansive soils, prevalent in arid and semi-arid regions, pose significant challenges to geotechnical engineering (Zapata, 2024). The mischaracterization of their volume change behavior leads to substantial annual economic losses, with damage to civil infrastructure in the United States alone estimated in the multi-billion-dollar range (Rosenbalm & Zapata, 2017). These soils pose significant challenges for pavements and various civil infrastructures due to their clay-water interactions. Expansive soils are characterized by their propensity to swell and shrink, primarily attributed to the presence of montmorillonite clay minerals (Agarwal & Sachan, 2024). This behavior leads to a cyclical pattern of expansion during periods of moisture ingress, such as precipitation, followed by contraction and crack formation during dry periods. The resultant soil instability can cause substantial distress to structures built upon these soils, necessitating specialized geotechnical approaches.

In response to global concerns regarding ecological degradation and resource scarcity, there is a growing emphasis on developing environmentally conscious solutions and materials based on renewable resources. Researchers are increasingly turning to bio-mediated and bioinspired approaches for the design and engineering of advanced materials to address critical global challenges (Attias et al., 2021; Elsacker, Vandelook, et al., 2023). This paradigm shift has led to the exploration of various innovative methods to mitigate geotechnical issues, including those related to expansive soils and desiccation cracking. Several promising approaches have been investigated by researchers, including microbial/enzyme-induced calcium carbonate precipitation, fiber reinforcement techniques, lime stabilization, and vegetation-based solutions. These methods, among others, are being evaluated for their sustainability and efficacy in preventing desiccation cracking in soils (Agarwal & Sachan, 2024; Firouzi et al., n.d.; Li et al., 2024; Qi et al., 2023; Xu et al., 2021)..

Recent research has highlighted the potential of fungal mycelium as a potentially sustainable and environmentally friendly solution to various geotechnical and geoenvironmental challenges (Elsacker, Zhang, et al., 2023; Geoffrey Michael Gadd, 2018; Islam et al., 2018; Jones et al., 2021; Mountassir et al., n.d.; Peng et al., 2023; Serish Manan, 2022; Vandelook et al., 2021). The effectiveness of fungal mycelium in modifying soil shear strength through the formation of interconnected hyphae networks has been demonstrated by Lim et al. (2020) and Salifu & Mountassir (2019). These networks bind loose sand particles, via their biochemical secretions, alter soil hydrophobicity and hydraulic properties (Salifu & El Mountassir, 2021; Salifu et al., 2022; Lim et al., 2024; Park et al., 2024).

This study addresses a gap in the literature regarding the use of fungal mycelium to mitigate desiccation cracking in expansive soils. While extensive research has been conducted in related areas, the application of fungal mycelium in this specific context remains largely unexplored. Our research seeks to fill this knowledge gap through an experimental exploration of crack propagation in soil samples with and without fungal mycelium treatment. Gravimetry, digital photography/microscopy and image processing using ImageJ software were employed for quantitative analysis, providing data on the effects of fungal mycelium on moisture changes, desiccation crack formation and propagation.

MATERIALS AND METHODS

Soil. The laboratory tests were conducted on a low plasticity clayey sand with 16.4% passing the #200 sieve. The index properties of the soil are listed in Table 1.

Table 1. Index properties of the tested soil.

Specific Gravity	Moisture Content (%)	Dry Density (g/cm3)	Void Ratio	Liquid Limit (%)	Plastic Limit (%)	Plasticity Index	%Sand	USCS
2.7	12.5	1.97	0.37	29	20	9	79.5	SC

Fungus. WC 1016 white *P. ostreatus* fungus was supplied as a live culture on potato dextrose agar by the Mushroom Spawn Lab, Department of Plant Pathology and Environmental Microbiology, Pennsylvania State University.

Preparation of Fungi Slurry. Liquid culture of *P. ostreatus* was prepared using 24 g/L of Potato Dextrose Broth (PDB). The medium was sterilized via autoclaving at 121°C for 15 minutes and cooled under sterile conditions in a biosafety cabinet. A total of 100 mL of sterilized PDB was transferred into 250 mL Erlenmeyer flasks and inoculated with 200 mg of *P. ostreatus* mycelium scraped off from an agar plate culture. The flasks were incubated in the dark at 25°C with agitation at 150 rpm for 7 days. After the incubation period, the mycelium biomass and remaining (spent) media were homogenized using a waring blender at low speed to minimize cell wall damage. The resulting homogenate was used as a fungal slurry inoculant for mycelium growth in soil specimens. Aseptic conditions were maintained throughout to minimize contamination.

Specimen Preparation. The soil was airdried, crushed and sieved through a 0.75mm sieve. In this study, 50% (w/w) of a liquid additive (either fungal mycelium slurry or water) was added to the soil (dry weight = 200g). Two approaches were used to induce desiccation cracking and soil treatment: mixing and surface spraying. In the mixed approach, the fungal-treated specimens were mixed with the soil and lightly compacted into a 150 mm diameter petri dish to achieve a density of 0.75 g/cm^3. The second approach involves compacting and levelling dry soil in the petri dish before applying fungal mycelium slurry to the soil surface by spraying or pouring. This simulates field conditions for hydraulically applied soil treatments, such as overland flow or precipitation leading to infiltration in expansive soil. Untreated or control specimens were prepared using the same procedure (mixed and surface spraying) but tap water was used as the liquid additive instead of fungal slurry.

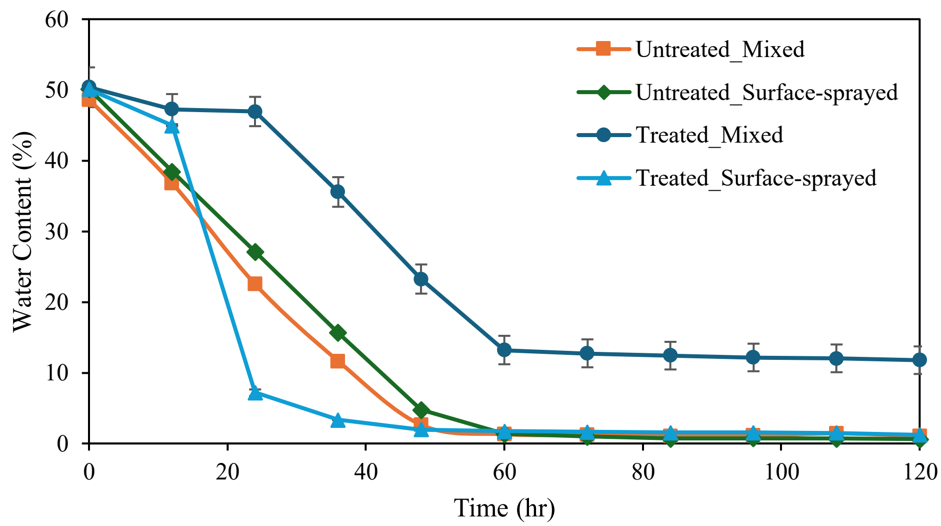
Experimental Procedure. To evaluate soil sample evaporation rates during desiccation, specimens were placed on electronic balances accurate to 0.1g. High-resolution images were captured using a Technaxx timelapse camera (full HD, TX-164) at one-minute intervals over 120 hours, with ambient temperature maintained between 23-28°C for consistency across all samples. Quantitative analysis of desiccation crack characteristics, including total crack length, and maximum width was performed using Fiji ImageJ software. Figure 1 illustrates the schematic representation of the test setup. All experiments were conducted in duplicates and the average values were used for computations.

Figure 1. Schematic representation of the test setup

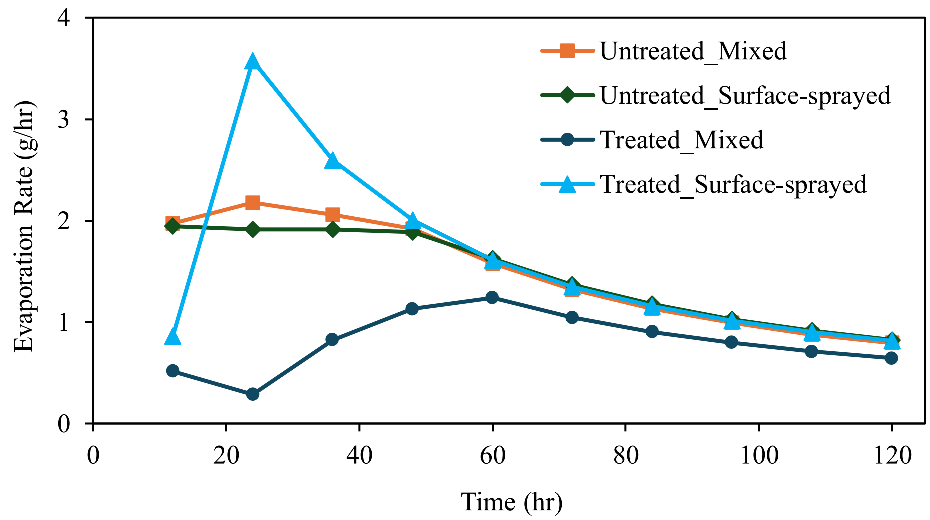
The Microstructural observation of samples before and after crack formation and fungal growth was conducted using a KEYENCE VHX-7000 digital microscope system.

RESULTS AND DISCUSSION

Influence of fungal mycelium on water content and evaporation rate. Figure 2a presents the change in water content over time for both treated and untreated soil samples subjected to controlled drying over 120 hours. The initial gravimetric water content of all samples was ≅ 50%. The untreated samples, both mixed and surface-sprayed, showed a rapid decrease in water content, reaching near zero after 60 hours of drying. The *treated_mixed* samples exhibited the most significant moisture retention, stabilizing at approximately 12% of their initial water content after 60 hours and maintaining this moisture until the end of the experiment. The treated surface-sprayed samples lost water quicker than the untreated samples, with a sharp drop in water content during the first 24 hours, reaching near-zero levels by the 60th hour. Being slightly viscous, the slurry applied to the soil surface most likely experienced minimal infiltration into the soil, with mycelium growth barely beginning within the soil in the first 24 hours before the slurry started drying from the surface.



(a)



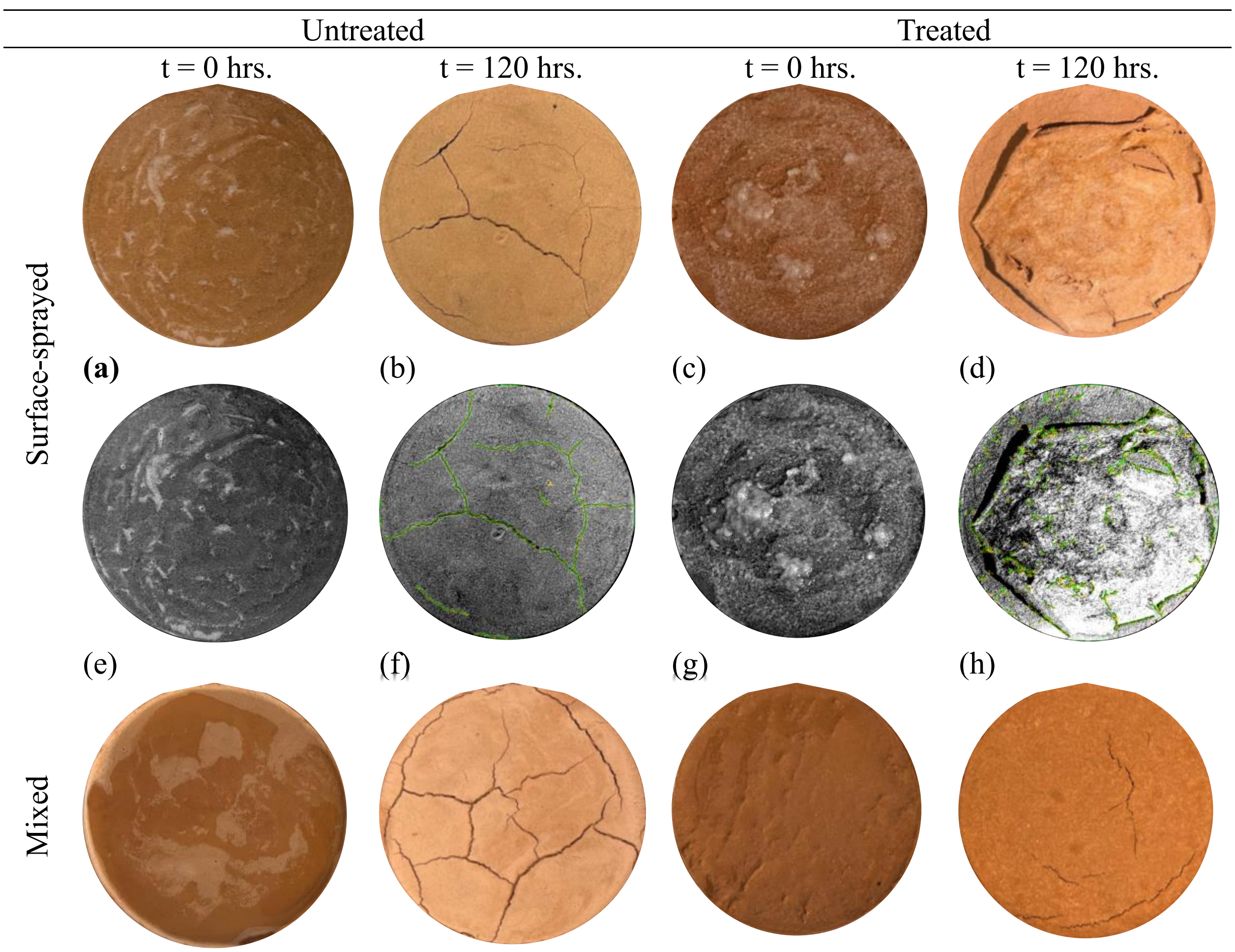
(b)

Figure 2. Relationship between the (a) water content and (b) evaporation rate with time

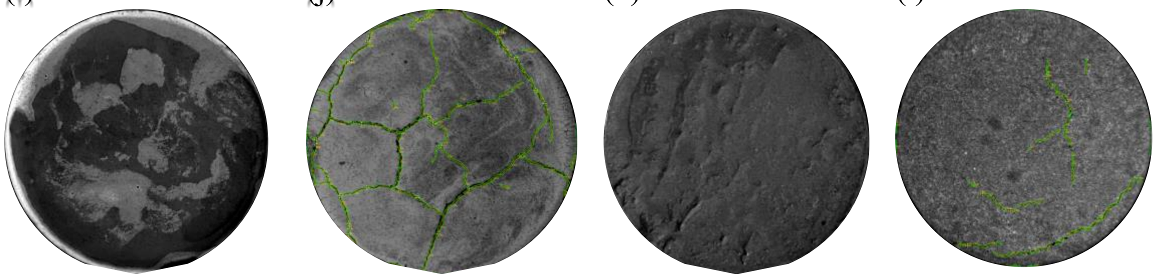
At the 120-hour mark, soil samples mixed with fungal slurry exhibited higher water retention, maintaining a water content of 11.8%. In contrast, the water content of samples mixed with water, those with fungal slurry applied to the surface, and those with water sprayed on the surface was 1.1%, 1.2%, and 0.6%, respectively. The enhanced water retention in fungal slurry-mixed samples can be attributed to the formation of dense, mycelial networks within the soil matrix compared to the surficial mat-like growth observed in the surface sprayed samples, where the mycelium dried and shrank before it could sufficiently develop into the soil, leaving parts of the soil surface exposed to evaporation. Living mycelia bio-fibers, characterized by a high surface-to-volume ratio, are reported to retain up to 60% water in their cells (Elsacker et al., 2019). They absorb and retain moisture for cellular transport, and as they fill soil pores (as observed in the treated-mixed samples), they contribute to the overall moisture retention within the soil.

Figure 2b shows the evaporation rates over time for the same treated and untreated samples. The *treated_surface-sprayed* sample exhibited the highest initial evaporation rate, peaking sharply above 3.5 g/hr around 20 hours, before declining steadily down to 0.8 g/hr at the end of the experiment. The *treated_mixed* samples, however, showed a more stable and consistently lower evaporation rate throughout the experiment but between 20 and 60 hours, the evaporation rate slightly increased because fungal mycelium struggled to grow within the soil layer due to the smaller pores spaces in the clay soil. Both untreated samples exhibited similar evaporation patterns, with their rates almost constant at around 2.0 g/hr across the first 50 hours of drying, before gradually decreasing as water is lost from the soil pores. The initial reduction in evaporation rate for the *treated_mixed* samples within the first 24hrs, followed by the consistently lower evaporation rate, may be attributed to moisture absorption by the mycelium for growth activities and consequent soil desaturation, which reduced the available moisture for evaporation. In the *treated_surface-sprayed* sample, most of the water was lost before the fungal slurry could infiltrate and establish mycelium growth within the soil. These results indicate a reduction in evaporation rate in the *treated_mixed* sample, while the *treated_surface-sprayed* sample exhibited rapid initial moisture loss. This also suggests that fungal treatment could contribute to water retention and lower evaporation rates when uniformly mixed into the soil rather than when applied via surface spraying.

Crack characterization and evolution in fungal-treated and untreated samples. Time-lapse images of the samples were downloaded, and crack length (total surface crack length per unit area) and width (maximum crack width) were quantified using a plugin in Fiji ImageJ software. Figures 3a-p present representative experimental and analyzed images of the samples at t = 0 hours and t = 120 hours, marking the beginning and end of the experiments. Crack features for the *treated_surface-sprayed* samples could not be accurately visualized or quantified. The fungal slurry dried up within 24 hours, forming a shrunken mycelial mat that obstructed the visibility of any cracks within the soil (Figures 3d and 3h). Peeling off the mat would cause disturbances and introduce artifacts; therefore, crack features for these samples are neither presented nor discussed. At 120 hours, the *untreated_surface sprayed* and *untreated_mixed* samples exhibited significant cracking, characterized by an extensive network of prominent fissures indicating greater shrinkage and desiccation compared to the *treated_mixed* samples. This visual analysis highlights the differential impacts of fungal treatment on soil desiccation and cracking patterns, with fungal treatment notably reducing the extent of desiccation cracking in the expansive soils tested in this study.



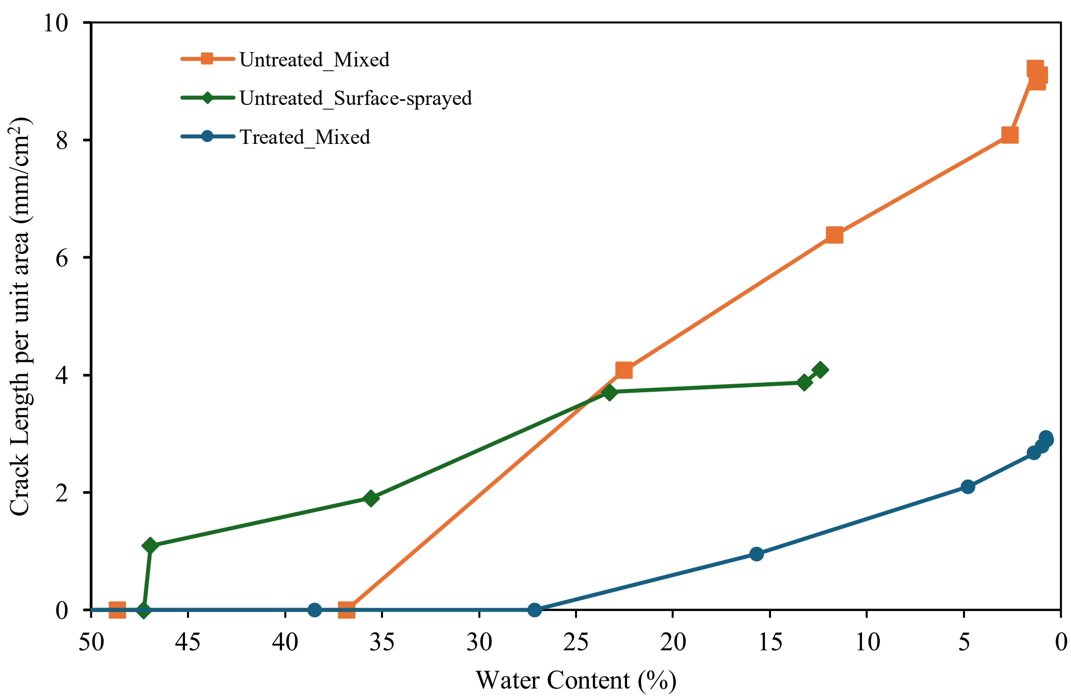
(i) (j) (k) (l)



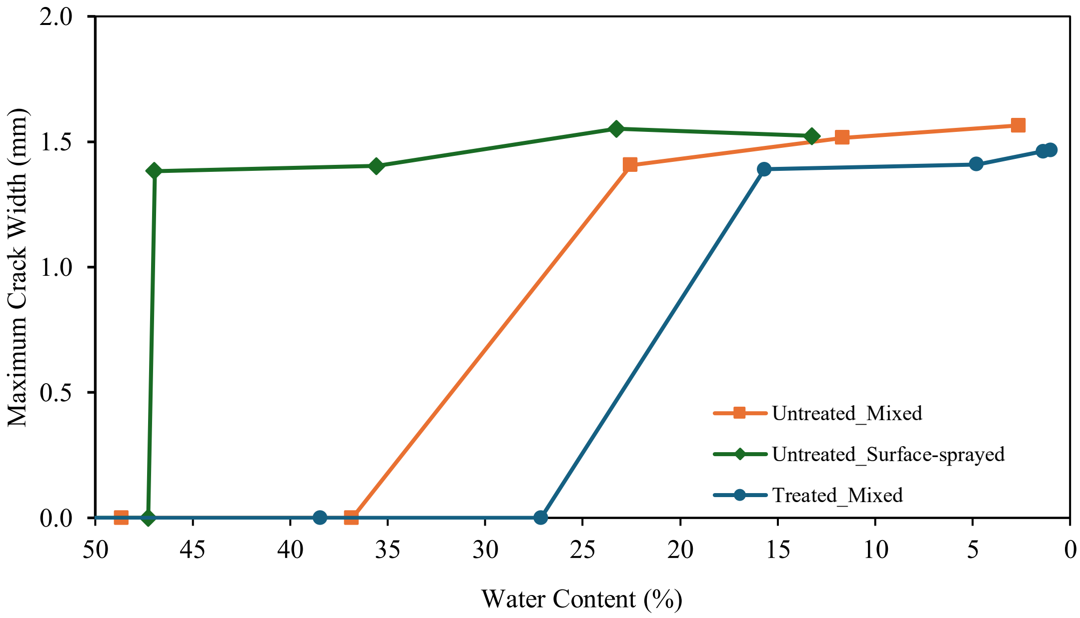
(m) (n) (o) (p)

Figure 3. Experimental and analyzed images of the respective treatment approaches for treated and untreated samples at t = 0 hours and t = 120 hours

Figure 4a presents crack length per unit area plotted against water content for three different treatments: *untreated_mixed, untreated_surface-sprayed, and treated_mixed*. Crack initiation is caused by moisture evaporation from the soil samples. As the water content decreased, the *untreated_mixed* sample showed a sharp increase in crack length, particularly when the water content fell below 35%. By the end of the experiment, with water content reaching about 1.2%, the crack length reached its maximum value of around 9 mm/cm², indicating significant cracking. The *untreated_surface-sprayed* sample showed a similar trend but with slightly lower crack lengths compared to the mixed sample, and an earlier appearance of cracks. The *treated_mixed* sample demonstrated a much better performance, with delayed crack initiation (water content of 27%) and crack length remaining minimal even as water content dropped below 5%, reaching about 2.9 mm/cm² at its peak.



(a)

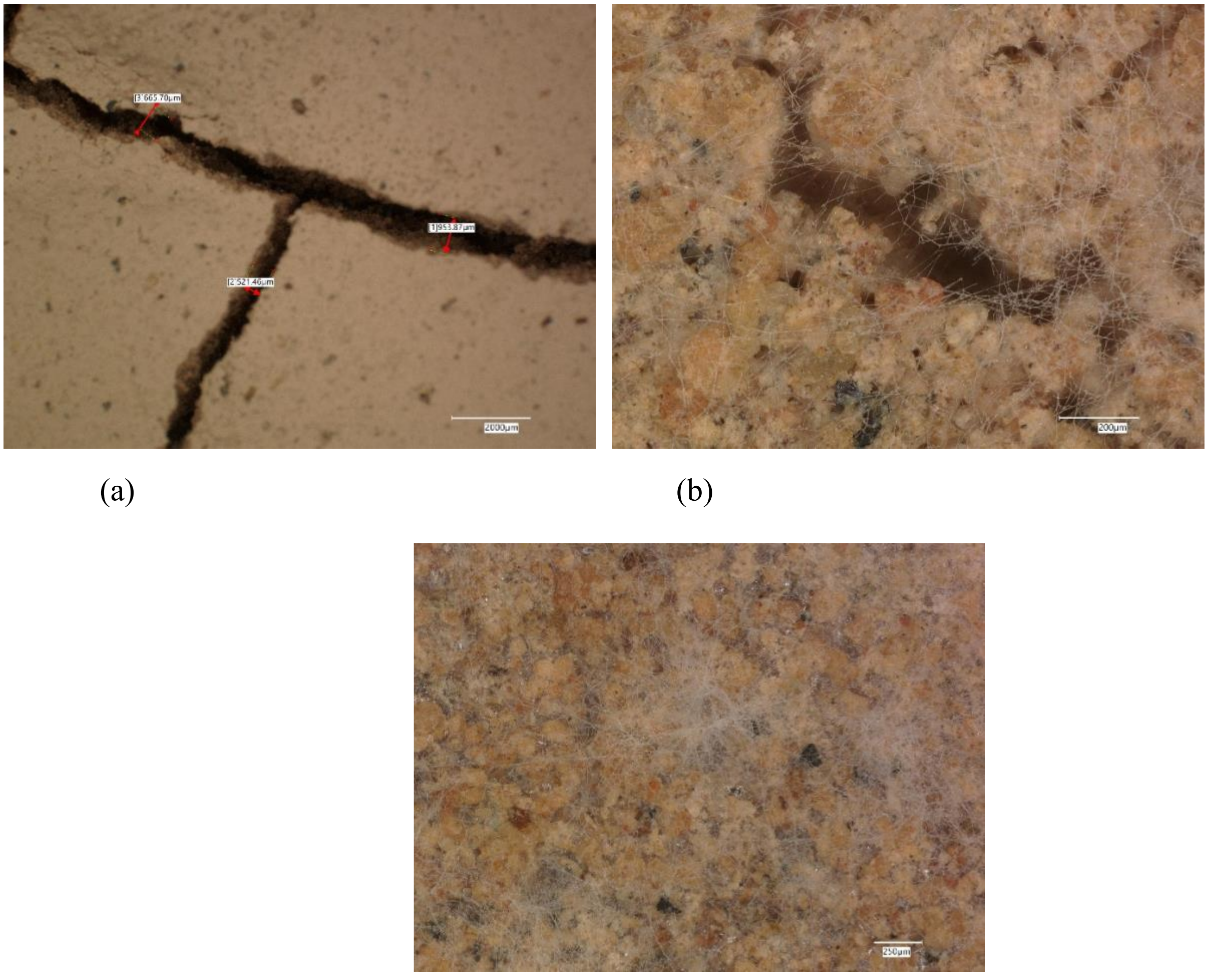


(b)

Figure 4. Crack evolution and characteristics for treated and untreated samples: (a) Crack length per unit area versus water content, and (b) Maximum crack width at varying moisture contents.

Similarly, Figure 4b shows that the untreated samples exhibited earlier crack widening as water content decreased. The maximum crack width for the *untreated_mixed* sample reaches about ~1.6 mm, while the *untreated_surface-sprayed* sample remains slightly lower at around 1.5 mm. On the other hand, the *treated_mixed* sample shows relatively smaller crack widths, with a maximum width of around 1.4 mm, at the lowest water content levels. Notably, this sample delayed crack initiation and maintains narrower cracks across the entire range of water content compared to the untreated samples. The growth of fungal mycelium in expansive clayey sand tested in this study demonstrates enhanced moisture retention, lower evaporation rates, and fewer, narrower cracks ultimately reducing desiccation cracking compared to untreated samples. A close-up view of the narrow cracks in a fungal-treated sample (not presented here) reveals a mycelium network growing across the fissure, partially filling the cracks. This suggests the potential capability of mycelium to heal emerging desiccation cracks when grown in such soils. The fibrous nature of fungal mycelium presents a promising, nature-mediated solution for mitigating desiccation cracking in soils.

Microstructural observations. Distinct morphological differences were observed in the untreated and mycelium-treated expansive soil samples a week after the experiments were completed. Figure 5a shows a profile of randomly selected desiccation cracks in the untreated soil, with measured crack widths ranging between 521-954 µm. In the *treated-mixed* sample (Figure 5b) thread-like filaments were observed filling up and growing across desiccation cracks, showing potential for crack healing. A portion of the surface-sprayed treatment (Figure 5c) showed a mycelial layer covering the soil surface with no visible crack formation.



(a) (b) (c)

Figure 5. Microstructural characterization of (a) untreated sample (b) Treated mixed sample and (c) Treated surface sprayed sample.

CONCLUSION

This study demonstrates the effectiveness of fungal mycelium in mitigating desiccation cracking and enhancing water retention in expansive soils. Soil samples mixed with fungal slurry recorded less evaporation rates and retained 11% of their water content after 120 hours of drying, compared to 1.1% for untreated samples. The treated mixed samples exhibited delayed crack formation, with narrower cracks and a lower total crack length per area, indicating an overall reduction in cracking due to fungal treatment. Further studies will focus on the hydraulic properties of the fungal-treated expansive soils, specifically the soil-water characteristic curve and unsaturated hydraulic conductivity functions, as well as volume change characteristics. These properties are essential for accurately predicting the behavior of fungal-treated unsaturated expansive soils.

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