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Evaluating the Influence of Fungal Growth on Soil Water Repellency and Soil Aggregation for Biogeotechnical Slope Stabilisation Applications

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ABSTRACT

Many slope failures globally are triggered by heavy rainfall, and the corresponding loss in shear strength as water infiltrates into initially partially saturated slopes. Here, we explore the potential use of saprotrophic fungi, in particular *Pleurotus ostreatus* (*P. ostreatus)* for biogeotechnical slope stabilisation. *P. ostreatus* has been previously shown to enhance water repellency, reduce water infiltration and hydraulic conductivity and improve resistance to erosion in sterile sands (Salifu, 2019; Salifu and El Mountassir, 2021; Salifu et al, 2022). Such modifications to soil behaviour could be beneficial in the treatment of granular slopes, to reduce water infiltration and thus enhance runoff without enhancing soil erosion.

In this study we investigated *P. ostreatus* growth in soils that were initially sterile and non-sterile to understand the influence of the pre-existing microbial community. Three soils were investigated compost, sand and a silty clay. *P. ostreatus* growth and its influence on water repellency and aggregate size distribution was measured over an 8 day period. Our results for the silty-clay indicate that it is possible for *P. ostreatus* growth to occur in non-sterile soil, with corresponding desirable modifications to soil properties, and at a similar level of modification to that achieved in sterile conditions, even within the short timeframe of this study (8 days).

INTRODUCTION

Many slope failures globally are triggered by heavy rainfall, and the corresponding loss in shear strength as water infiltrates into initially partially saturated slopes. Methods to modify water infiltration using chemically-based water repellent soils have been proposed in the literature. Yet, if infiltration is reduced and runoff enhanced there is the potential to increase soil erosion (Zheng et al., 2017, 2019). Here, we explore the potential use of saprotrophic fungi, in particular *Pleurotus ostreatus* (*P. ostreatus)* for biogeotechnical slope stabilisation.

P. ostreatus, commonly known as the oyster mushroom, is a saprotrophic fungus that thrives on decaying wood (Ghafoor and Niazi, 2024). As a decomposer, *P. ostreatus* plays a vital role in forest ecosystems by breaking down dead wood and releasing nutrients back into the soil, which can benefit plant growth (Liu et al., 2022). *P. ostreatus* breaks down organic matter through a process called decomposition (Pavlik and Pavlik, 2013). Its mycelial network, a vast network of fungal filaments, secretes enzymes that can break down complex organic compounds like cellulose and lignin, which are found in plant cell walls (Manan et al., 2021).

P. ostreatus growth in sand has been previously shown to induce soil water repellency (Salifu & El Mountassir, 2021) via the production of hydrophobins. This increased hydrophobicity can contribute to delayed and reduced water infiltration into the soil (Salifu et al., 2022), which could be advantageous in areas prone to slope failures triggered by heavy rainfall. Furthermore, *P. ostreatus* is a filamentous fungus, and forms an intricate network of hyphae known as the mycelium as it grows. When grown in soil the formation of this hyphal network can act to bind soil particles together, and ultimately has been shown to improve the resistance of sands to erosion (Salifu, 2019). The aforementioned studies all investigated *P. ostreatus* growth in sterile sand. In order to understand the potential to deploy *P. ostreatus* as a biotechnology in field conditions we here explore *P. ostreatus* growth in three different soil compositions and under both sterile and non-sterile conditions. We measured mycelium growth over time and its influence on soil properties, namely soil water repellency and soil aggregate distribution. Soil water repellency is assessed via the simple low-cost method (water drop penetration test) and is primarily used in this study as an indicator of fungal-induced changes to the soil hydraulic behaviour. Furthermore, soil aggregate stability is used here as a simple low-cost method for assessing changes to soil erodibility (Le Bissonnais, 1996). Hydraulic behaviour (i.e. water infiltration) and soil erodibility are important factors in determining the stability of slopes. The main objective of this study is to understand the ability of *P. ostreatus* to grow in non-sterile soils with pre-existing microbial communities and assess the soil modifications that can be induced in non-sterile conditions.

MATERIALS AND METHODS

Soil.

Three different soil types were used in this experiment: (1) Compost, (2) Sand and (3) a silty-clay of intermediate plasticity. The silty-clay was sampled from an embankment along the Humber Estuary close to the village of Thorngumbald, in east England, UK.

In this study, for the sterile soil specimens, the soils were sterilised by autoclaving at 121°C for 20 minutes to eliminate pre-existing microbial communities. In the non-sterile specimens, no sterilisation of the soil took place. By comparing these two conditions, we can observe the influence of the existing microbial community on *P. ostreatus* growth.

30 grams of each sterilised or non-sterilised soil was weighed and placed in individual 90 mm diameter Petri dishes. The soil in each dish was adjusted to achieve a target moisture content of 10% (w/w). This was achieved by adding sterile distilled water and thoroughly mixing the soil to ensure even distribution of moisture. 5% (w/w) of sterile beechwood chips, with a maximum dimension of 3 mm, was added to each Petri dish containing the prepared soil. Beechwood chips were included to serve as a carbon source for fungal growth within the soil.

Fungal Inoculation.

P. ostreatus strain was acquired from the collection of Richard Wright at the University of Cardiff. To prepare for the experiments, *P. ostreatus* was sub-cultured and grown on malt extract agar in an incubator at 25°C for 7 days. Subsequently, 20% glycerol was added to sterile cryovials containing the *P. ostreatus* culture. These vials were then placed in a controlled-rate freezing container and gradually cooled to -80°C for long-term storage and future use.

Beechwood cubes with a diameter of 1 cm were sterilised using autoclaving at 121°C for 20 minutes. *P. ostreatus* was grown on malt extract agar plates for seven days at 25°C in an incubator. Following this initial growth period, the sterilised beechwood cubes were placed on the

surface of the malt extract agar plates covered by the *P. ostreatus* mycelium. The inoculated plates were then incubated for an additional 7 days to allow for fungal colonisation of the beechwood cubes. After the 7-day colonisation period, excess mycelium growing on the surface of the beechwood cubes was carefully removed using sterile techniques. These pre-colonised beechwood cubes served as the inoculum source for the soil specimens in the petri dishes.

A single pre-colonised beechwood cubes, inoculated with *P. ostreatus*, was placed in the centre of each Petri dish containing the prepared soil and substrate mixture with 10% moisture content. The prepared specimens were then incubated at 25°C for 8 days. Over this incubation period the radius of the mycelium growth was measured on Days 1, 3, 4, 7, and 8. Since the mycelium does not necessarily grow evenly in all directions, four measurements were taken per specimen at each timepoint and averaged to get a more accurate representation of the mycelium growth radius.

Water Drop Penetration Time.

Water repellency was assessed using the water drop penetration time (WDPT) test (Doerr, 1998). A single droplet of 5 μL deionized (DI) water was carefully placed on the surface of each soil specimen. The time required for complete infiltration of the water droplet was recorded, with a maximum observation period of 24 hours. This was conducted 5 times for each specimen, at 5 locations extending radially from the centre of the petri dish to the edge.

Soil Aggregate Distribution.

Harvested soil samples were placed on a stack of sieves with the following mesh sizes: 2 mm, 1.18 mm, 600 microns, 212 microns, 106 microns, and 63 microns. The sieve stack was then submerged in a solution of deionized (DI) water. The stack was raised and lowered by 3 cm for 30 cycles, at a rate of one stroke per two seconds. The remaining soil on each sieve was dried and weighed, and the results were compared to control samples to assess changes to soil aggregate distribution as a result of *P. ostreatus* growth.

RESULTS AND DISCUSSION

Mycelium Growth

Figure 1 presents an example of the radial growth of *P. ostreatus* mycelium. The specimen shown here is sterilised Humber soil (silty-clay) amended with 5% beechwood with growth shown for up to 7 days. After Day 1, there is only dense localised mycelium growth (mycelium visible in white) in the vicinity of the beechwood inoculum. By Day 3 radial expansion of the mycelium into the soil is clearly visible and by Day 7, the mycelium has fully colonised the soil. As shown in Figure 1 (e.g. Day 1 and Day 3), the mycelium's growth was initially more pronounced in certain directions (towards the bottom right of the images). This unevenness likely stems from variations in the amount of mycelium on the beechwood cube's surface, even after careful removal of excess mycelium before inoculation.

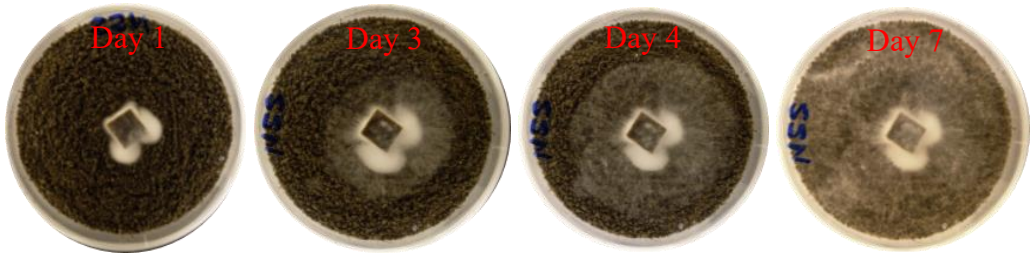

Figure 1. Mycelium growth on Humber soil with 5% beechwood as substrate in sterile condition.

The radius of *P. ostreatus* mycelium growth was measured over an 8-day growth period in the three different soil types (compost, sand, and Humber soil) for both the sterilised and non-sterile soil conditions and these results are presented in Figure 2.

The results reveal clear differences in growth patterns between the sterile and non-sterile soil conditions across all three soil types. For the compost and sand specimens, considerably more growth was observed in the sterile soil conditions, with the radius of growth reaching the edge of the petri dishes by Day 7 for both soils. For the compost and sand specimens, the growth pattern in the sterile condition was uniform, similar to what was observed on day 7 of the Humber soil specimens (Figure 1). In non-sterile soil conditions, the mycelium growth was slower and ultimately reached a maximum extent by Day 4 in the compost and Day 7 in the sand, likely due to competition with other microorganisms present.

While, in the Humber soil, more growth was still observed in the sterile condition, in the non-sterile conditions the mycelium was continuously expanding without slowing down as was observed in the non-sterile Compost and Sand conditions. The sterile condition in all cases facilitated more rapid and expansive growth of *P. ostreatus*, likely due to the absence of competing organisms that would otherwise inhibit or slow fungal colonisation.

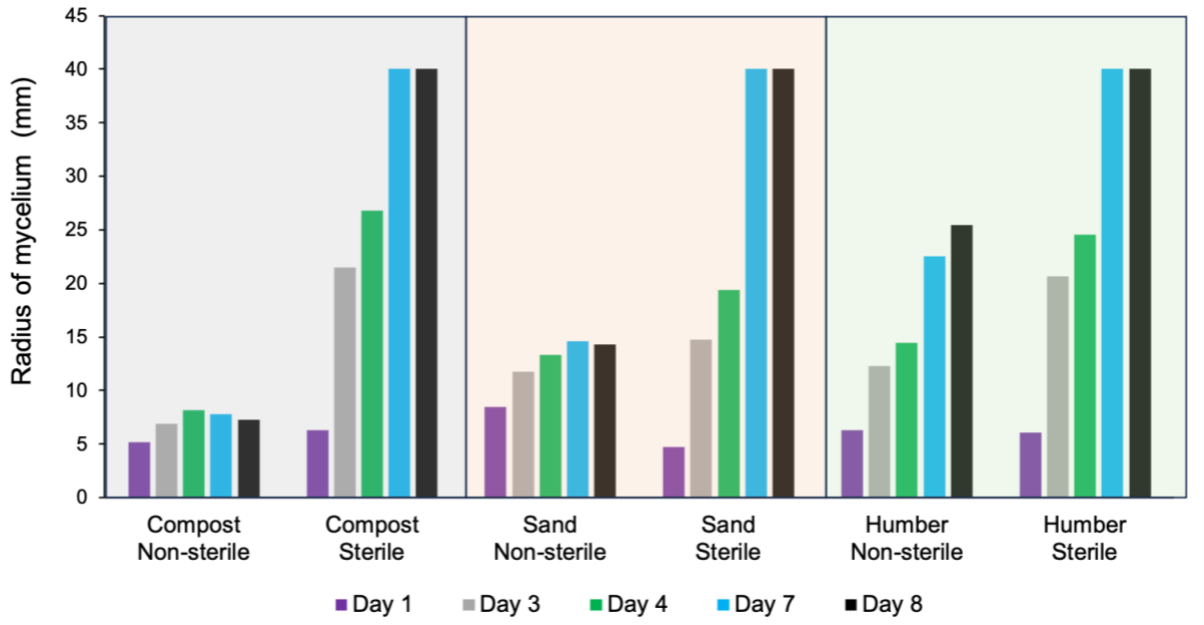

Figure 2. Radius of mycelium growth of *P. ostreatus* in initially sterile and non-sterile compost, sand and Humber soil.

Water Repellency.

Figure 3 presents the results of the water droplet penetration tests (WDPT) conducted on three soil types: compost, sand, and Humber soil, under both sterile and non-sterile conditions after 8 days of *P. Ostreatus* growth, as well as control groups. The y-axis represents the water drop penetration time in seconds on a logarithmic scale, ranging from hydrophilic (penetration of water droplet in <5s) to extreme water repellency (water droplet penetration time > 3,600 s). Water repellency classification here is based on Doerr et al., (2006).

All of the controls, with the exception of the non-sterile compost, exhibited hydrophilic behaviour. Whereas the non-sterile compost control exhibited slight water repellency. After 8 days of *P. ostreatus* growth in the initially sterile soils (Compost, Sand, Humber), all specimens exhibited extreme water repellency, with water droplets not penetrating into the specimens even after 24hrs (86,400s). In the non-sterile soil specimens, after *P. ostreatus* growth water repellency was observed to range from severe (non-sterile compost) to extreme (Sand and Humber). However, the non-sterile specimens exhibited greater variability in the water drop penetration time. Considering that five WDPT measurements were taken along a radius from the centre to the edge of each petri dish, the greater variability is reflective of the more limited mycelium growth observed in the non-sterile specimens compared to that in the initially sterile soils as shown in Figure 2.

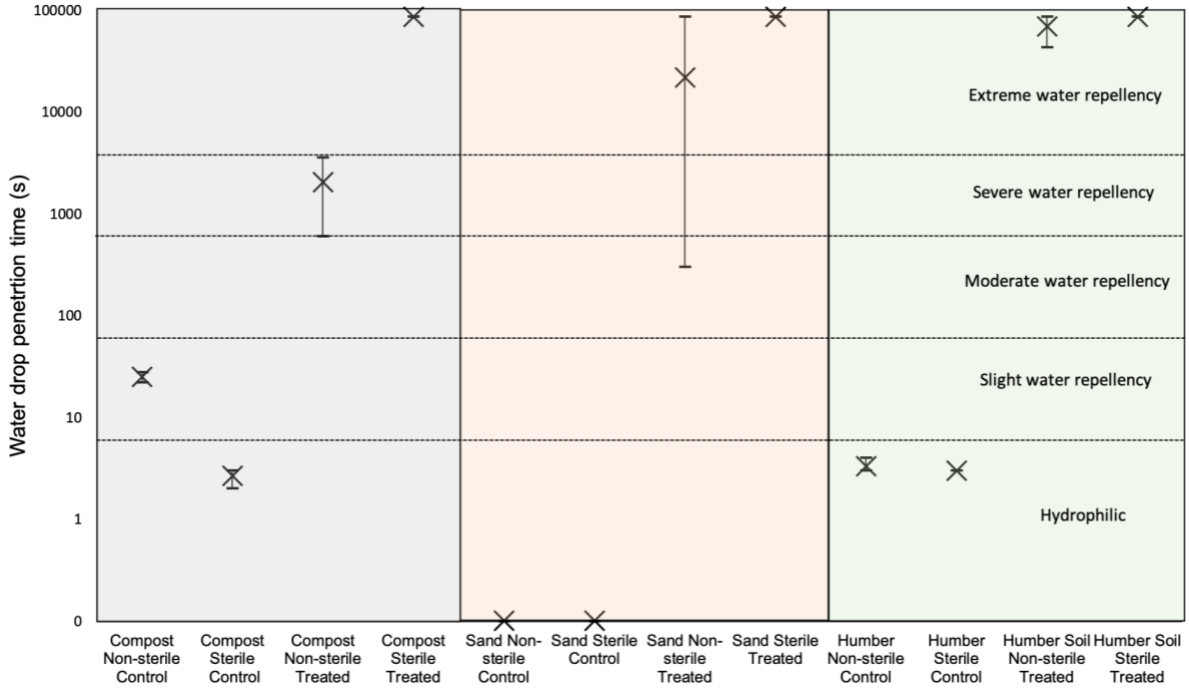

Figure 3. Water droplet penetration time for non-sterile and sterile compost, sand and Humber soil after 8 days of *P. ostreatus* growth (n=5, the mean value is presented alongside the minimum and maximum WDPT determined for each specimen).

Soil Aggregation

The soil aggregate distribution as shown in Figure 4 was determined for compost, sand, and Humber soil, for both initially sterile and non-sterile conditions after 8 days of *P. ostreatus* growth.

The x-axis represents the aggregate size in millimeters (mm), and the y-axis shows the percentage of soil passing through a sieve of the corresponding size.

The results for compost show clear differences between the control (black solid line), non-sterile (grey solid line), and sterile conditions (black dashed line). In particular there is a shift in the aggregate size distribution, with a reduction in the % of soil passing sieve sizes in the range 600μm to 2mm range, this is most noticeable for the specimens where *P. ostreatus* was grown in initially sterile soil, although a small shift is also detectable for the non-sterile condition. This indicates that *P.* ostreatus growth has resulted in the binding of soil particles together to form larger aggregates than are present in the control specimen.

Similar behaviour was observed for the sand specimens, with only a very slight change in aggregate size distribution observed at the larger aggregate sizes (1.18mm-2mm) for the non-sterile sand. Whereas a small shift towards larger aggregate sizes was observed in the sterile sand condition across a wider range of sizes (212μm – 2mm).

For the Humber soil, both the sterile and non-sterile conditions, showed a shift towards lower percentage of soil passing, indicating the formation of larger stable aggregates in the specimens treated with *P. ostreatus* (across the size range tested 63μm-2mm). The sterile condition exhibited larger aggregates compared to the non-sterile condition in the range 106μm -212μm. This increase in aggregate size in sterile conditions aligns with the trend observed in compost and sand, where the absence of microbial competition seemed to contribute to enhanced *P. ostreatus* growth (Figure 2) and ultimately the formation of larger aggregates.

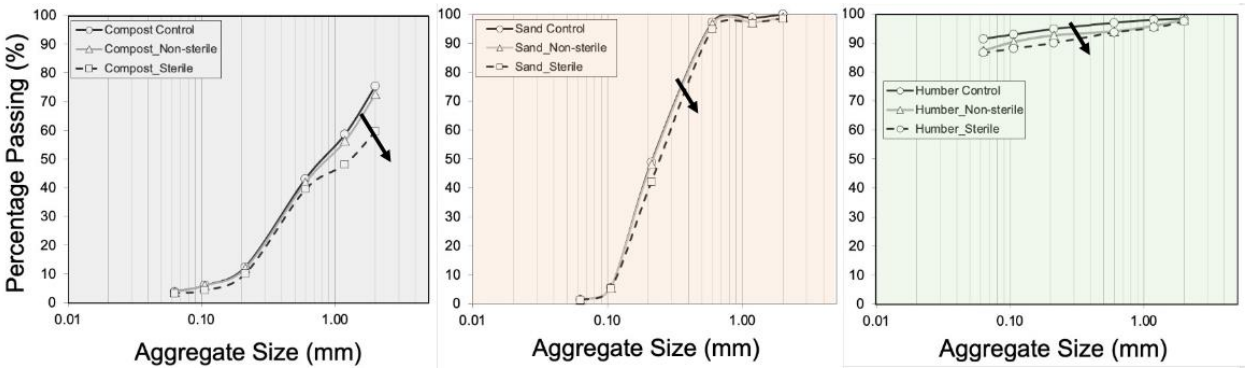

Figure 4. Soil aggregate size distributions for compost, sand and Humber soil after 8 days of *P. ostreatus* growth.

DISCUSSION

The results presented in Figure 2 show that greater extent of *P. ostreatus* growth was observed in the initially sterile soil conditions compared to the non-sterile soil conditions for all the soil types studied (compost, sand, silty-clay). In the sterile conditions there are a number of factors which may be contributing to the enhanced growth compared to the non-sterile conditions, namely: sterilisation by autoclaving will eliminate microorganisms from the soil, thus removing the potential for competition between pre-existing soil microorganisms and the *P. ostreatus* which has been introduced to the soils. However, there is also another factor at play here. Sterilisation by autoclaving has an influence on soil chemical and physical properties, modifying the availability of soil macronutrients (Lotrario et al., 1995; McNamara et al., 2003; Hu et al., 2019). This suggests

the possibility that some of the enhanced growth observed in the sterile conditions is not only due to a lack of microbial competition but also due to differences in the soil chemistry in which growth is occurring. Future studies targeted at understanding the effect of existing microorganisms on the growth of fungal inoculants in soil should consider other sterilisation methods which can induce total sterilisation while also minimising changes to soil chemical and physical properties, for example gamma (γ-) irradiation (McNamara et al., 2003). Finally, the particle size distribution of the soils used in the study could have played a role in the growth patterns of the fungus. Larger particles, like those in sand, have larger pores that allow better oxygen circulation and water drainage. It has previously been reported that fungi preferably grow in large air-filled pores (Erktan et al., 2020). Smaller particles, like those in clay, have smaller pores that can retain water at higher suctions compared to coarse-grained soils. Pore size distributions may therefore influence mycelium growth, although this should be investigated further.

CONCLUSION

This study investigated the growth of *P. ostreatus* mycelium on different soil types (compost, sand and silty-clay) under both sterile and non-sterile conditions. Greater mycelial growth was observed in sterile conditions across all soil types, indicating that the absence of microbial competition allowed for more efficient colonisation by *P. ostreatus*. Although this enhanced growth may also have been in part due to changes in soil chemistry induced by autoclaving.

The water droplet penetration time results reveal that *P. ostreatus* treatment can significantly increase the hydrophobicity of soil, changing it from hydrophilic to severe or extremely water repellent after 8 days of growth in both sterile and non-sterile soils. This suggests that *P. ostreatus* growth may influence soil water infiltration and retention properties for a range of soil types, this has previously only been shown for sterile sands (Salifu and El Mountassir., 2021; Salifu et al., 2022). The aggregate size distribution tests indicate that 8 days of *P. ostreatus* growth can lead to the formation of larger stable aggregates, this was more pronounced in the sterile soil conditions. The formation of larger aggregates is indicative of the binding capability of the fungal hyphal network, and suggests the application of *P. ostreatus* as a means of enhancing soil erosion resistance, again this has only been shown previously for sterile sands (Salifu, 2019).

Of the three soil types tested, in non-sterile conditions *P. growth* was greatest in the silty-clay (Humber soil) with an associated greater influence on the soil behaviour (water repellency and aggregate size distribution). The silty-clay results indicate that it is possible for *P. ostreatus* growth to occur in non-sterile soil, with corresponding desirable modifications to soil behaviour, and at a similar level of modification to that achieved in sterile conditions, even within the short timeframe investigated here (8 days). These results lend support to the potential of deploying fungal treatment in field (non-sterile) conditions for hydraulic control and erosion mitigation applications.

Future research should investigate the interactions between *P. ostreatus* and existing soil microorganisms over the longer term and their influence on soil properties.

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