

Experimental investigation of the influence of the growth of saprotrophic fungi (*Pleurotus ostreatus*) on the aggregate stability of a silty sand

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Abstract. Granular soils are highly susceptible to erosion, a process that may contribute to slope instability. For example, gully erosion promotes preferential flow and toe erosion, eventually leading to undercutting and slope failure. Given that soil erodibility is correlated with soil aggregate stability, here we use the slaking test as an indicator of the loss of soil structure and ultimately erodibility upon wetting. The overall aim of this research is to experimentally study the influence of the growth of saprotrophic fungus *Pleurotus ostreatus* (*P. ostreatus*), on the aggregate stability of a silty sand. Two main factors were investigated in this study: (i) the amount of fungal biomass inoculant and (ii) the concentration of nutrients (malt extract broth) supplied to the soil. The results demonstrate that *P. ostreatus* growth can enhance the aggregate stability of silty sand. Based on visual observations, the slaking index and UV microscopy, these preliminary results indicate that aggregate stability is positively related to the amount of fungal hyphae in the soil, which is influenced both by the initial amount of fungal biomass added into the soil and the nutrient concentration supplied.

1 Introduction

Granular soils are highly susceptible to erosion, a process that may contribute to slope instability. For example, gully erosion promotes preferential flow and toe erosion, eventually leading to undercutting and slope failure [1]. Conventional measures to mitigate slope instability can be implemented for the case where the hazard is restricted to a localised area and are typically require high inputs of energy (e.g. reprofiling slopes) or involve materials which have a high carbon footprint (e.g. steel and cement used in barrier systems). Given that the construction sector accounts for more than one third of global carbon emissions [2], there has been a sustained effort in geotechnical engineering research over the last twenty years towards developing biological interventions for soil improvement in order to reduce/replace the use of conventional ground improvement approaches [3].

Biological ground improvement methods include microbially-induced carbonate precipitation [4], the use of plant roots for mechanical and hydrological reinforcement [5] and soil treatment via the growth of saprotrophic fungi [6]. Among these methods, the fungal treatment method is a new emerging avenue because of the beneficial effect on slope stability via the modification of the hydraulic and mechanical behaviour of the soil. Salifu & El Mountassir [6] found that the growth of *Pleurotus ostreatus* in sands induces extreme water repellency (contact angles greater than 110°) and Salifu *et al.* [7] demonstrated reduced and delayed infiltration of water into *P. ostreatus* treated sand. Further, the growth of *P. ostreatus* and *Rhizopus* species has been shown to result in binding of soil particles leading to enhanced

strength and reduced erodibility [8-10]. Such modifications are beneficial to mitigate rainfall-induced instability of slopes composed of granular soils. Reducing water infiltration helps to maintain higher soil suction in the subsoil where the failure surface may develop. While the fungal-induced mechanical reinforcement of the top layer prevents its erosion, which might otherwise be expected due to ponded infiltration generated by the induced soil hydrophobicity.

Given that soil erodibility is correlated with soil aggregate stability [11], the slaking test can be used as an indicator of the loss of soil structure and ultimately erodibility upon wetting. The slaking test is commonly used due to its simplicity and speed [12].

The overall aim of this research is to investigate experimentally the influence of the growth of the saprotrophic fungus *Pleurotus ostreatus* on the aggregate stability of a silty sand. Two main factors were investigated in this study: (i) the amount of fungal inoculant and (ii) the concentration of nutrients (malt extract broth) supplied to the soil.

2 Materials and methods

2.1 Soil

The soil tested was a silty sand, prepared by mixing 74% sand and 26% silt. The particle size distribution is shown in Figure 1. The silty sand has a coefficient of uniformity of 28.5 and coefficient of curvature of 6.9 following the method of BS EN ISO 14688-2:2018. The optimum moisture content for this material is 9% and the maximum

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dry density is 1.86 g/cm³ following the British Standard (BS EN 13286-4:2021).

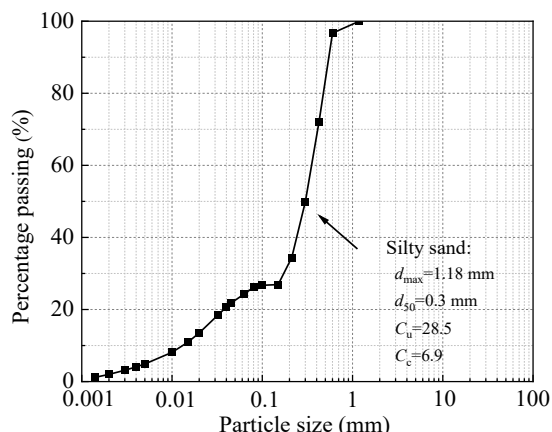


Fig. 1. Particle size distribution of silty sand.

2.2 Preparation of fungal suspension

The fungal species used in this study was *Pleurotus ostreatus*. This fungal culture was provided by the School of Biosciences at the University of Cardiff. The fungal inoculant used in this research was obtained through a three-step process. The first step consisted of growing the fungus on a petri dish filled with malt extract agar for 7-days, i.e. until the mycelium covered the entire surface area of the petri dish. The second step involved the preparation of a culture of the fungi in malt extraction broth. A sterilised cork borer with a 10 mm inner diameter was used to take fungal culture ‘plugs’ from the petri dishes with the pre-grown fungal mycelium. Six plugs were added to a flask containing 600 mL of sterilised malt extract broth (20g/L). It is worth noting that a 1L capacity flask was used in this step along with a magnetic stirrer to ensure sufficient aeration during fungal growth. The flask was then wrapped with aluminium foil to keep the culture in the dark and incubated at 20°C for 7 days. The third step was to harvest the fungal biomass. The fungal biomass was harvested by centrifuging with a Relative Centrifugal Force (RCF) of 12,857 for 7 min and a decline of 1 min. The harvested fungal pellet was then mixed with a sterile 50mM NaCl solution at a ratio of 1g of biomass per 1ml of saline solution. Two fungal suspension contents (i.e. mass of fungal suspension/mass of dry solids) were used in the preparation of the specimens, 2% and 4% respectively. Thus providing either 1 g (F1) or 2g (F2) of fungal biomass as inoculant per specimen.

Nutrients were supplied to the soil in the form of autoclaved malt extract broth liquid. Three malt extract nutrient (N) concentrations were prepared at 25g/l, 50g/l and 100g/l. The liquid nutrient media was autoclaved prior to mixing with the soil.

In this study, six cases were tested, to investigate the influence of the fungal suspension content as well as nutrient concentration on the slaking behaviour of fungal treated silty sand (Table 1). For each specimen the nutrient content (i.e. mass of nutrient liquid / dry mass of silty sand) was held constant at 2.4% while the concentration of the liquid nutrient media was varied. All

specimens were prepared with the same initial liquid content (7.5%). It should be noted that the liquid content calculated includes the total mass of fungal suspension, deionised (DI) water and liquid broth divided by the dry mass of silty sand (100g). To achieve the same moisture content with the varying concentration of fungal biomass, we adjusted the amount of DI water added. In the control sample (Untreated) no fungal suspension or nutrient media was added.

The soil specimens were prepared in a split acrylic mould 36mm diameter and 72mm height and lightly compacted to achieve a dry density of 1.2 g/cm³. The specimens were placed in an incubator for 100 days, with a constant relative humidity of 80% and temperature of 23°C. The details of the specimens used in the experiment are summarised in Table 1.

These specimens were tested via unconfined compression testing (not discussed in this paper) and, after failure, soil was sampled from each of the specimens for the slaking test.

Table 1. Test specimen details.

Specimen	Fungal suspension content (%)	Nutrient media conc. (g/l)	Nutrient content (%)	DI-water content (%)	Total liquid content
Untreated	0	0	0	7.5	7.5
F1N25	2	25	2.4	3.1	7.5
F1N50	2	50	2.4	3.1	7.5
F1N100	2	100	2.4	3.1	7.5
F2N25	4	25	2.4	1.1	7.5
F2N50	4	50	2.4	1.1	7.5

F stands for fungal suspension; *N* stands for nutrient. Note the fungal suspension is made up of half fungal biomass and half saline water; nutrient content = mass of nutrient liquid / dry mass of silty sand.

2.3 Slaking test

Ten grams of each soil sample were collected and placed in a petri dish (90 mm diameter). Subsequently, 5 mL of DI water was added via a pipette to the base of the petri dish next to (but not directly on) the soil sample, to avoid any effect of the impact of the droplet on aggregate breakdown. Photographs were taken before the test began ($t = 0$ min) and after adding DI water at $t = 1$ min, 5 min, 60 min, 1440 min (24 hours) and 5760 min (4 days). Image analysis of the photographs was carried out using the open source software ImageJ. The images were cropped, colour-quantized, and converted into binary images for further processing. At each timepoint, the area of the water including soil particles was calculated using the embed function measurement to determine the number of pixels with a gray value (intensity) of 255. Note clear

water (without soil particles) is not detected using this process. The workflow for the image processing is shown in Figure 2. After that, the slaking index was calculated as the orthographic projected area of the soil and water at time t , divided by the initial orthographic projected area of the soil.

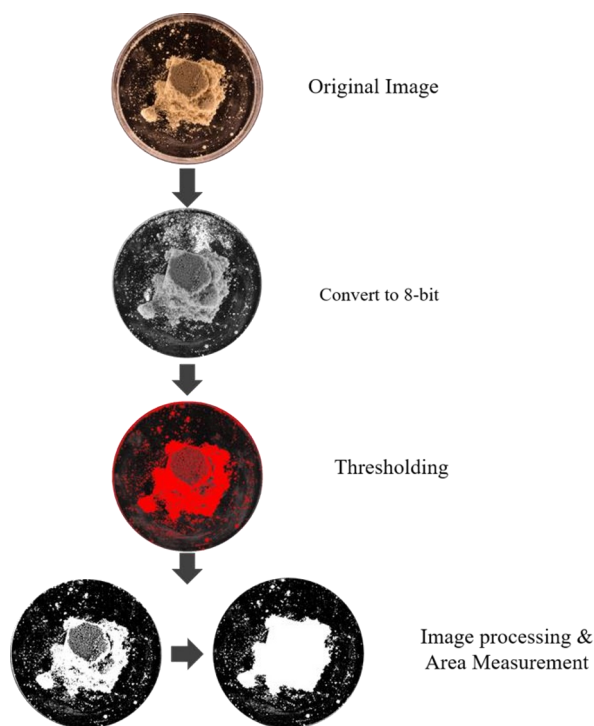


Fig. 2. Image processing diagram for calculating slaking index.

2.4 Optical microscopy

After the slake test, the specimens were heated overnight at 60°C. The specimens were then imaged in a Nikon optical microscope, using a UV lamp with a wavelength of 365 nm and a FL2 filter. Chitin a major component of the fungal cell wall is known to fluoresce at this wavelength [13, 14].

3 Results

Figure 3 shows the process of soil slaking. Slaking of the untreated control sample occurred immediately after deionised water was added to the petri dish. Complete disintegration of the specimen occurred with the silty sand spreading from the centre toward the edges of the petri dish. Most of the disintegration occurred within the first minute after contact with water. In contrast, for the fungal treated specimens, some aggregates remained intact and visible throughout the duration of the test, showing a resistance to disintegration. As shown in Figure 3, the majority of disintegration occurred in all cases within the first minute. By comparing the image at $t=4$ day with that at $t=0$ min in Figure 3, it is evident that the soil structure was better maintained in the specimens prepared with higher amounts of initial fungal biomass inoculant (F2N25, F2N50).

Specimen	0 min	1 min	60 min	4 days
Untreated				
F1N25				
F1N50				
F1N100				
F2N25				
F2N50				

Fig. 3. Images of soil slaking processes. The petri dish in each image has a diameter of 90mm.

Figure 4 (a) and (b) show the variation of slaking index with time for specimens treated with 1g and 2g of fungal biomass inoculant respectively, compared to the untreated control sample. It can be observed that, for all curves, the slaking index increases markedly between 0 and 1 minute. After this initial period, the slaking index increases at much slower rate. The fungal-treated specimens all exhibited lower slaking indices than the untreated control up to $t = 1$ day. Although, with prolonged exposure to water, the slaking index of the fungal treated specimens tends towards that of the control specimen.

Figure 4(c) presents the relationship between slaking index and nutrient broth concentration. At lower concentrations, the addition of fungal biomass appears to have only a minor effect on the slaking index of the treated soil samples. As the nutrient levels increase, fungal hyphae growth becomes more extensive, thereby improving the soil's resistance to erosion—reflected in the lower slaking index. However, it should be noted that there is a lot of variability observed in the results, particularly with F1N50 showing a higher slaking index than the control sample at the end of the test. This result does not reflect the visual observation that some aggregates remain intact in F1N50, whereas they do not in the control (untreated soil). This is a limitation of the use of the slaking index to assess aggregate stability [15]. Note there will also be variability due to fungal growth, fungal growth will not be homogenous throughout an individual specimen. The heterogeneity within specimens will be investigated in future studies.

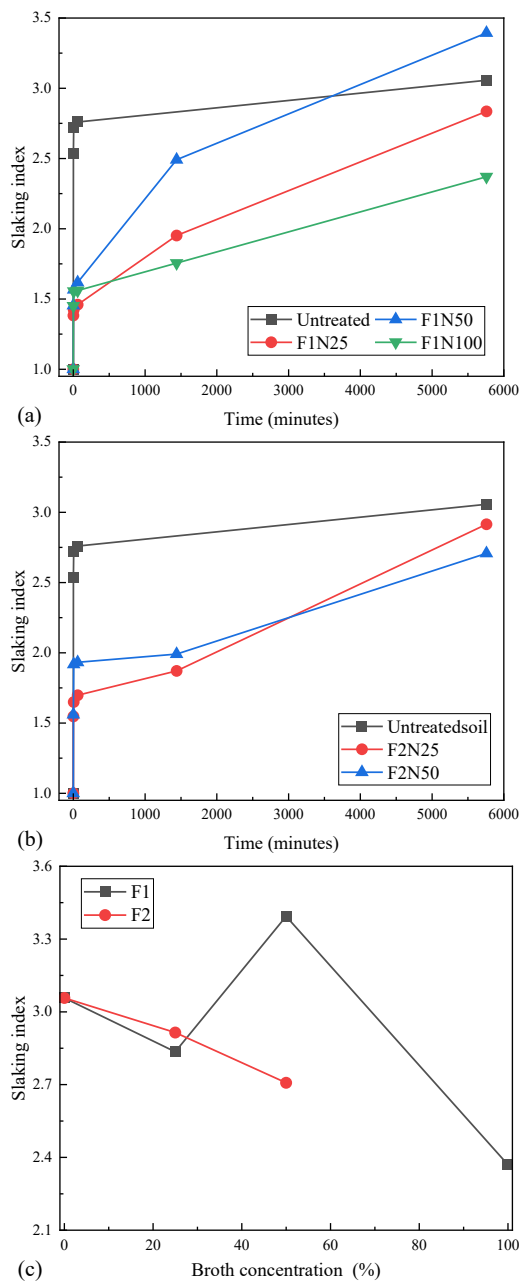


Fig. 4. (a) the variation of slaking index with time for F1 group; (b) the variation of slaking index with time for F2 group; (c) The variation of slaking index with broth concentration.

Figure 5 presents the microscopy photographs. It can be observed that in the untreated case, the image is essentially black and there is an absence of blue light. This is indicative of a lack of chitin in the specimen due to an absence of fungal hyphae. In contrast, the shining blue light and, in some cases, distinct filaments can be observed in the other specimens inoculated with fungal suspension, indicating the presence of fungal hyphae. Also, there is a clear difference in the amount of fungal hyphae: the smallest amount of fungal hyphae is observed in the fungal-treated specimen F1N25. By comparing F1N25 with F1N50 and F1N100, it appears that increasing the nutrient concentration enhances the amount of fungal growth. Furthermore, by comparing F1N25 and

F2N25, it appears that increasing the fungal biomass in the inoculant also increases the amount of the fungal growth. F2N50 and F1N100 had the lowest slaking values at t=4 day and, in these specimens, distinct fungal hyphae (i.e. filaments) stretching between grains are visible. It appears that the soil aggregate stability positively correlates with the amount of fungal hyphae present in the soil.

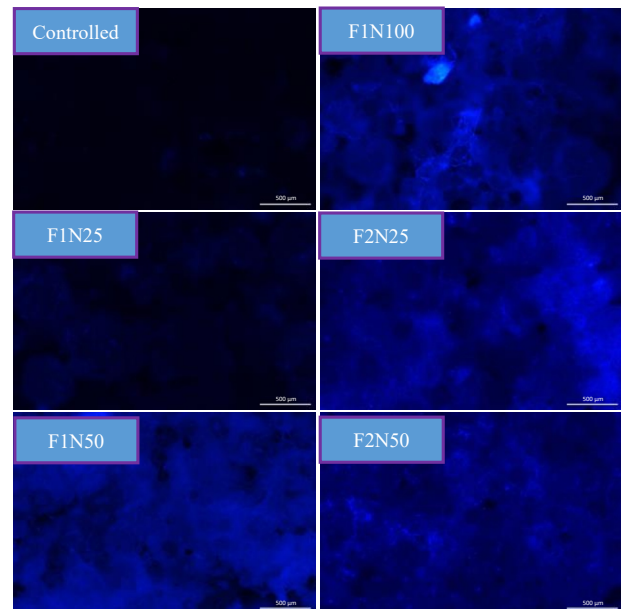


Fig. 5. UV- microscopy photographs for control specimen and fungal treated specimens, F1 group and F2 group.

4 Conclusions

This research has explored the soil aggregate stability of silty sand treated with *P. ostreatus* saprotrophic fungi under different inoculation conditions, including different amounts of fungal biomass inoculant and nutrient concentration. The research has demonstrated that *P. ostreatus* growth can enhance the aggregate stability of silty sand. Based on visual observations, slaking index, and UV microscopy, we have demonstrated that aggregate stability is positively related to the amount of fungal hyphae in the soil, which is influenced both by the initial amount of fungal biomass added to the soil and the concentration of nutrients supplied, although fungal hyphae/biomass should be quantified in future experiments. Note, the slaking index should be used with caution and complemented/replaced with other measures for assessing soil aggregate stability, including for example the mass of water stable aggregates [13]. These preliminary results support the potential of saprotrophic fungal treatment as a means of enhancing soil aggregate stability and ultimately reducing the erodibility of granular soils with potential application to enhancing the stability of granular slopes.

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