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Influence of environmental conditions on the growth of *Pleurotus ostreatus* in sand

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ABSTRACT

Pleurotus ostreatus, a saprotrophic fungus, has been proposed for the remediation of organic contaminants in soils and more recently for modifying the hydraulic and mechanical behaviour of granular soils. The in situ performance of fungal-based biotechnologies will be controlled by the fungal growth and associated biochemical activity that can be achieved in soil. In this study, the influence of environmental conditions (temperature, degree of saturation), substrate type (lignocellulose and spent coffee grounds) and concentration on the mycelium growth of *P. ostreatus* in sand are investigated. Furthermore, the evolution of growth/survival indicators (respiration, ergosterol concentration) and enzymatic activity (laccase, manganese peroxidase) are investigated. Temperature was shown to have a strong influence on the growth of *P.ostreatus* in sand: growth was observed to be delayed at low temperatures (e.g. 5 °C), whereas growth was prevented at high temperatures (e.g. 35 °C). No growth was observed at very low degrees of saturation (S_r = 0% and 1.2%), indicating there is a critical water content required to support *P.ostreatus* growth. Within the mid-range of water contents tested radially, growth of *P.*ostreatus was similar. However, growth under saturated soil conditions was restricted to the air-water atmosphere due to the requirement for oxygen availability. Low substrate concentrations (1%–5%) resulted in high radial growth of *P.ostreatus*, whereas increasing substrate content further acted to reduce radial growth, but visual observations indicated that fungal biomass density increased. These results are important for understanding the feasibility of *P.ostreatus* growth under specific site conditions and for the design of successful treatment strategies.

1. Introduction

In an effort to develop more sustainable ground improvement techniques, there has been significant research activity in recent years investigating the potential role of microorganisms in geotechnical engineering (DeJong et al., 2014; El Mountassir et al., 2018; Mitchell and Santamarina, 2005). Studies have largely focused on the use of bacteria, for (i) microbially induced carbonate precipitation (MICP) (e.g. see review papers by DeJong et al., 2014; El Mountassir et al., 2018; Fu et al., 2023; Terzis and Laloui, 2019), (ii) biogas production (e.g. He et al., 2013; He and Chu, 2014; Pham et al., 2018; Wang et al., 2023) and (iii) bioclogging (e.g. Blauw et al., 2009; Lambert et al., 2010; Seki et al., 2005). Of these biogeochemical processes by far the most studied to date is that of microbially induced carbonate precipitation (MICP). Studies have shown treatment of granular soils via MICP can increase soil shear strength, enhance stiffness, mitigate soil liquefaction, reduce hydraulic conductivity and reduce soil erodibility (e.g. Al Qabany & Soga, 2013; Fu et al., 2023; DeJong et al., 2006; Li et al., 2023, 2024; Salifu et al., 2016; Whiffin et al., 2007; Van Paassen et al., 2010; Wang et al., 2023). MICP is triggered either by injection of a particular strain of bacteria (typically *Sporosarcina pasteurii)* into the soil or by stimulation of native bacteria already existing within the soil (e.g.

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Burbank et al., 2013; Gomez et al., 2017). However, fungi account for up to 75% of the microbial biomass in soils (Wick and Harms, 2018) and yet their potential with respect to ground engineering has remained relatively unexplored until recently (El Mountassir et al., 2018; Gou & Li, 2024; Lim et al., 2020; Lim et al., 2023; El Mountassir, 2021; Salifu and El Mountassir, 2019; Salifu and El Mountassir, 2021; Salifu et al., 2022; Park et al., 2023; Zhang et al., 2022). Geoenvironmental engineers, on the other hand, have long recognised the potential of fungi to remediate environmental organic pollutants in the field of study known as *mycoremediation* (e.g. Murphy and Perry, 1984; Bumpus et al., 1985).

Pleurotus ostreatus (*P. ostreatus*), a saprotrophic fungus, whose fruiting body is commonly known as the 'oyster mushroom', is the second most cultivated edible mushroom in the world (Sánchez, 2010). *P. ostreatus* secretes extracellular ligninolytic enzymes (including lignin peroxidase, manganese peroxidase, versatile peroxidase and laccase), which facilitates the degradation of wood.

In mycoremediation research, *P. ostreatus* has been shown to successfully degrade organic contaminants in soils, including polycyclic aromatic hydrocarbons and polychlorinated biphenyls (Bezalel et al., 1996; Mohammadi-Sichani et al., 2019; Purnomo et al., 2010; Zebulun et al., 2012). It is the ligninolytic enzymes that are directly responsible for the degradation and transformation of pollutants (Golan-Rozen et al., 2015; Lang et al., 1998; Rodríguez et al., 2004). Sustained in situ enzymatic activity is critical for successful bioremediation in the field (Lang et al., 1998).

Within geotechnical engineering, recent research has shown that the growth of *P. ostreatus* in sand modifies the solid-liquid contact angle, inducing soil water repellency (Salifu and El Mountassir, 2021, see hydrophobic nature of sand following growth of *P.ostreatus* in Fig. 1(b)). Furthermore, growth of *P.ostreatus* alters soil water retention behaviour, delays and reduces water infiltration and lowers hydraulic conductivity (Salifu et al., 2022). As well as modifying hydraulic behaviour, *P. ostreatus* growth in the form of fungal mycelium can contribute to binding of soil particles (see Fig. 1(a)), leading to an increase in soil cohesion (Lim et al., 2023; Salifu and El Mountassir, 2019). This binding of soil particles within the mesh of the fungal mycelium combined with induced hydrophobicity can significantly reduce soil erodibility (Salifu, 2019; Zhang et al. 2022). Salifu and El Mountassir (2021) and Salifu et al. (2022) proposed based on these modifications to soil behaviour that fungal treatment could have potential as a treatment for granular soils in slopes/hillsides where failures are triggered in response to wetting as a means of reducing infiltration, thereby maintaining higher levels of suction and associated shear strength, without enhancing soil erosion. *P.ostreatus* has also been proposed for the creation of lightweight sand-mycelium soil with beneficial damping charcteristics suitable for use as seismic buffers (Gou and Li, 2024). These results demonstrate the potential for *P. ostreatus* to be used as a bio-based technology within a geotechnical engineering context to modify granular soil behaviour.

Previous studies have investigated the growth of *Pleurotus* species in laboratory culture media (e.g. potatoe dextrose, malt extract, yeast extract) and/or on various substrates (e.g. rice straw, wheat straw, saw dust, coffee grounds, soybean) with the focus on determining optimal conditions for mushroom cultivation and/or production of enzymes for application in food, medicinal and biotechnological industries (Badu et al., 2011; Carrasco Cabrera, 2018; Das et al., 2015; Hoa and Wang, 2015; Mikiashvili et al., 2006; Patel et al., 2009; Shah et al., 2004). More recently, studies have investigated the growth of *P. ostreatus* for the production of mycelium composites for a range of potential applications, including architectural materials, (e.g. use in insulation panels) and for food packaging (e.g. Haneef et al., 2017; Houette et al., 2022). These applications allow for growth in tightly controlled conditions, and require only a source of fungal inoculant, nutrition source(s) (i.e. substrate) as well as moisture and oxygen. Whereas, in order for *P.ostreatus* to be practically deployed in geotechnical engineering for soil improvement, growth must occur in situ within the pore space of the soil, and under environmental conditions which will be dictated by the site weather conditions prior to and during fungal inoculation and fungal growth phases. Some studies have investigated the influence of environmental conditions on the growth of other saprotrophic fungi in soils (e.g. *Stropharia caerulea, Phanerochaete velutina, Rhizoctonia solani)* (e.g. Donnelly & Boddy, 1997; Harris, 2003; Otten, 1999) but the growth behaviour of *P.ostreatus* in soils has not yet been systematically investigated.

The performance of fungal biotechnologies for ground improvement and/or bioremediation of contaminated soils will be controlled by the fungal growth that can be achieved in soil (i.e. extent of mycelium

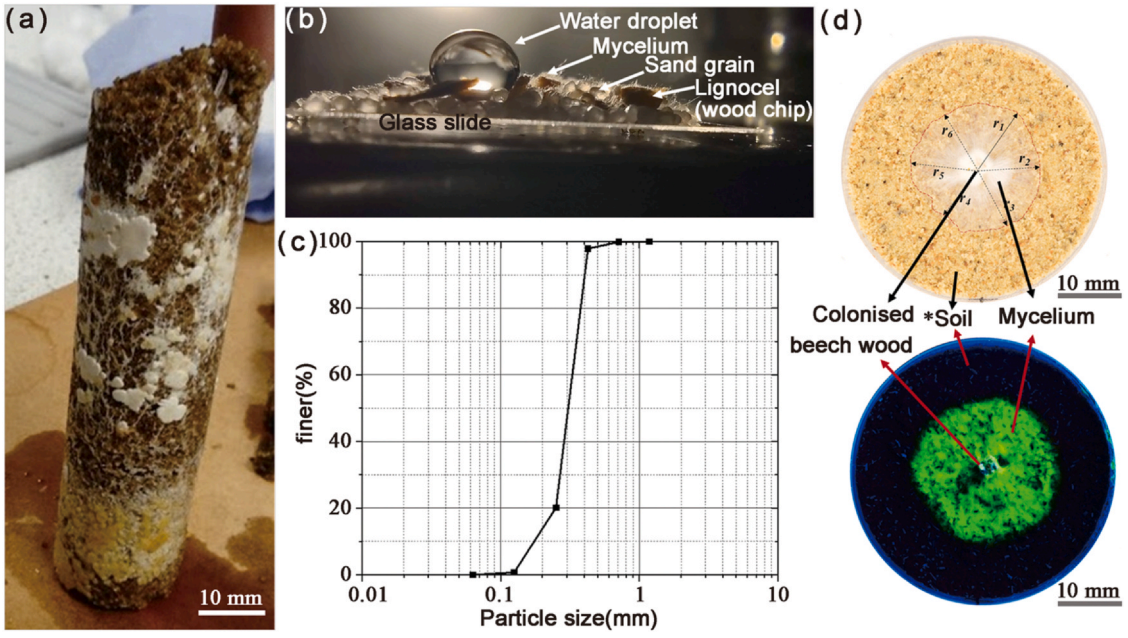

Fig. 1. *P. ostreatus* growth in sand contributes to (a) particle binding and (b) induces water repellency; (c) Particle size distribution curve for uniformly graded medium sand used in this study; (d) Mycelium growing radially from the colonised beech wood inoculant in a 90 mm diameter petri-dish with measurement of average radius of mycelium growth: $[(r_1 + \ldots. + r_6)/6]$ mm unstained (top) compared with stained specimen using FDA (bottom).

growth, morphology of mycelium-soil interactions, amount of fungal biomass) and its sustained biochemical activity. The specific objectives of this study were to investigate in sand: (1) the influence of environmental conditions (temperature, degree of saturation), substrate type and concentration on the mycelium growth of *P. ostreatus*; (2) to determine the evolution of growth/survival indicators (respiration, ergosterol concentrations) and enzymatic activity (laccase, manganese peroxidase) over a 4-week period of *P. ostreatus* growth and (3) to observe via scanning electron microscopy, the morphology of *P. ostreatus* hyphae -sand grain interactions.

2. Methodology

2.1. Experimental design and set up

Table 1 presents details of the four experiments conducted in this study to address the aforementioned objectives. Experiment 1 (SLP-T) investigated the influence of temperature on the extent of mycelium growth in sand amended with lignocellulose. Experiment 2 (SLP-S_r) investigated the influence of degree of saturation on mycelium growth. Experiment 3 (SP-SUB) investigated the influence of substrate concentration on mycelium growth at varying concentrations between 1%–15% for sand amended with (i) lignocellulose and (ii) spent coffee grounds. In Experiment 4, changes in physico-chemical and biochemical parameters were monitored over a 4-week period for specimens: (i) Sand (S), (ii) Sand and lignocellulose (SL), (iii) Sand inoculated with *P. ostreatus* (SP) and (iv) Sand and lignocellulose inoculated with *P.ostreatus* (SLP).

2.2. Materials

2.2.1. Soil

Uniformly graded medium sand with a particle size distribution curve, as shown in Fig. 1(c), was used for this study. The sand was sterilised via autoclaving prior to inoculation to study the growth of *P. ostreatus* in the absence of other soil microorganisms. Two organic substrates were investigated: lignocellulose and spent coffee grounds. Lignocellulose of size 0.5–1 mm (Grade HB 500 – 1000) was sourced from J. Rettenmaier & Söhne GmbH. Table 2 shows the elemental/biochemical composition of lignocellulose. The main components are lignin, cellulose and hemicellulose as is typical for decaying organic matter in natural soil, and it has a Carbon/Nitrogen (C/N) ratio of ~450.

Spent coffee grounds (SCG): a by-product from brewing coffee were obtained from *Nourish*®, the University of Strathclyde catering services. SCG typically has a C/N of between 17:1 to 20:1 (Ballesteros et al., 2014; Campos-Vega et al., 2015). SCG was selected in this study since it is readily available as a 'waste' material and could be a low-cost substrate for growth of fungi in field applications.

2.2.2. Fungal strains and inoculants

Table 1 provides details of the respective strains of *P. ostreatus* used for each test component, as well as the type of inoculant and composition of specimens. For Experiments 1 to 3, colonised beech wood was used to inoculate specimens, whereas in Experiment 4, specimens were inoculated using fungal spore/hyphal suspensions. The inoculants were prepared as follows:

2.2.2.1. Colonised beech wood inoculant. Colonised beech wood inoculants were prepared based on the method outlined in Donnelly and Boddy (1997). Cubes of beech wood (1 cm^3), supplied from Timbercut4u UK, were autoclaved at 121 °C for 20 min. These were then aseptically placed in 500 mL conical flasks containing 7-day old cultures of *P. ostreatus* (strain DSM No. 3344) grown on malt extract agar. The culture was incubated in the dark at 25 °C for 14 days to allow for colonisation of the cubes. The inoculum was obtained by taking out a colonised beech block and scraping off any excess mycelium that was attached to it prior to placing it on a sand specimen.

2.2.2.2. Fungal spore and hyphal suspension. The fungal spore/hyphal suspension was prepared using M2191 spawn (millet grains colonised by mycelium of *P. ostreatus*) obtained from GroCycle UK. 10 g of spawn was placed in a 500 mL conical flask. 100 mL of deionised water was added, and the mixture was shaken vigorously, manually, for 10 min. The flask containing the mixture was then placed on a shaker at 150 r/min for 20 min. Thereafter, the vigorous manual shaking was repeated for 5 min to facilitate detachment of mycelium from the grains into the water. A sieve with 2 mm mesh size was used to separate any millet grains from the liquid. The resulting filtrate formed the fungal spore/hyphal suspension.

2.2.3. Preparation of specimens

Sand, lignocellulose and spent coffee grounds were sterlised via autoclaving at 121 °C for 20 min. Specimens were prepared using 20 g of sand mixed with varying amounts of substrates and liquid (water or fungal spore/hyphal suspension), as shown in Table 1. For Experiments 1 to 3, specimens were inoculated by placing a single colonised beech wood inoculum centrally on the sand surface in the petri dish, while untreated controls were prepared in a similar manner but with no beech wood inoculum added. For Experiment 4, treated specimens included the addition of spore/hyphal suspension whereas deionised water was added to the untreated (control) specimens (i.e., Exp 4, S and SL). In all experiments, the mixture of sand, substrate and liquid additives were placed in a 90 mm diameter petri dish and gently compacted to provide a level surface. All specimens were prepared in triplicates.

2.3. Methods

2.3.1. Measurement of mycelium radius (Experiments 1 – 3)

Multicellular fungi grow as hyphae, tubular structures with diameters between 2–7 μm (Lehmann et al., 2019) and lengths from microns to several metres (Islam et al., 2017). Hyphae form complex networks as fungi explore their environment in search of nutrients, branching out and anastomosing, forming collectively a three-dimensional structure known as the mycelium. In this study, mycelium growth was monitored via time lapse photography using a DSLR camera set at an aperture of f/5.6 and ISO-100. Photographs were imported into ImageJ (opensource) Fiji software (Schindelin et al., 2012), and six measurements of the mycelium radius were made for each specimen and the average radius of mycelium growth determined as shown in Fig. 1(d) (top). In order to validate this procedure, the average mycelium radius was also measured after staining of specimens with Fluorescein Diacetate, FDA (a cell viability stain that indicates the presence of living fungi in a medium (Stahl and Parkin, 1996). FDA was prepared according to the protocol published in the Cold Spring Harbour protocols (Anonymous, 2008). 3 mL of the staining solution was sprayed onto the specimens using a fine mist spray bottle, thereafter specimens were left to stand for 4 h at room temperature to allow the stain to permeate the hyphae. Images of the stained mycelium in the specimens were then captured by placing them under ultraviolet (UV) light and imported into ImageJ software to estimate average radius of mycelium growth. This check was carried out for 3 specimens (each with triplicates).

Fig. 1(d) presents images of a specimen 6 days after inoculation (top) unstained, and (bottom) the same specimen after FDA-staining. The unstained specimen shows visible mycelium growth in the form of a white cotton-like mass of hyphae, radially extending from the colonised beech wood inoculant (Fig. 1(d) top). Staining with FDA and imaging under UV light provided a more enhanced delineation of the visual extent of mycelium growth (in green) markedly differentiated from the rest of the specimen (shown in black) where there was no mycelium growth (Fig. 1(d) bottom). For this specimen, the average

Table 1
Experimental details including respective fungal strains, specimen composition, test conditions and variables measured for each experimental component.

Experimen	Ref.	Fungal strain	Inoculant	Sand (g)	Temperature (°C)	Liquid Content′ (%)	Degree of saturation, S_r′ (%)	Substrate	Substrate Contentˆ (%)	Measured Variables	Time of measurements after inoculation (days)
1	SLP-T	Jacquin ex Fr. Kummer, (DSM No. 3344)	Colonised beech wood	20	5, 10, 15, 20, 25, 30, 35	11.1	13.6	Ligno-cellulose	5	Mycelium radius	3, 6, 9, 12
2	SLP-S_r		Colonised beech wood	20	25	0, 1, 3.1, 5.3, 11.1, 17.7, 25, 42.9, 66.7, 100	0, 1.2, 3.8, 6.5, 13.6, 21.6, 30.5, 52.3, 81.4, 100	Ligno-cellulose	5	Mycelium radius	4, 12
3	SP-SUB		Colonised beech wood	20	25	11.1	13 − 16	Ligno-cellulose Spent Coffee Grounds	1, 3, 5, 10, 15	Mycelium radius	3, 6
4	S	No fungal inoculation	-	21	25	11.1	13.3	-	0	pH	0, 7, 14, 28
	SL		-	20	25	11.1	13.6	Ligno-cellulose	5	EC	
	SP	M2191 (GroCycle UK)	Spore/hyphal suspension	21	25	11.1	13.3	-	0	Laccase activity Manganese	
	SLP		Spore/hyphal suspension	20	25	11.1	13.6	Ligno-cellulose	5	Peroxidase activity Organic carbon Total Nitrogen Respiration Ergosterol SEM*	

Abbreviations: S – Sand; SL – Sand & lignocellulose; SLP – Sand, Lignocellulose & *P.ostreatus,* T – temperature, S_r – degree of saturation, SUB - substrate.
′For experiments 1-3, liquid was deionised water, for experiment 4 deionised water was added to S and SL specimens, whereas fungal spore/hyphal suspension was added to SP and SLP specimens.
ˆSubstrate content is defined as (mass of substrate/mass of sand) ×100.
*Scanning electron microscopy, SEM was performed at the end of the study

Table 2
Physico-chemical characteristics of Lignocel and spent coffee grounds.

Physical and chemical properties	Lignocellulose	Spent coffee grounds
Particle density (g/cm^3)	1.49	0.91^
Cellulose (%)	35*	30.58'
Hemicellulose (%)	29*	20.20'
Lignin (%)	28*	17.92'
Ash (%)	0.5*	1.71 ± 0.05'
Carbon (%)	45*	47.9 ± 0.15'
Nitrogen (%)	0.1*	2.01 ± 0.074'
Carbon/Nitrogen ratio	450	23.83'

* Imran, et al., 2016; 'Silva et al., 2018; ^Nakilcioğlu-Taş and Ötleş, 2019

radius of mycelium growth estimated for treated-unstained (top) was 21 ± 0.9 mm and for treated-stained (bottom) was 22 ± 0.1, implying that the difference in methods used to determine the average radius of mycelium growth was negligible. Therefore, the average radius of mycelium for the specimens reported in Experiment 1 were determined without any staining. This was deemed to be preferable as FDA has been reported to be less effective in staining older or less metabolically-active fungal hyphae (Stahl and Parkin, 1996); and not staining the specimens allowed different timepoints to be measured on the same individual specimen.

2.3.2. Measurment of chemical and biochemical parameters (Experiment 4)

In Experiment 4, samples were air-dried overnight before performing chemical and biochemical analyses. Table 3 provides a summary of the parameters measured and the corresponding test methods used. Details of the test procedures are provided in the following subsections.

2.3.2.1. Basal respiration. Basal respiration is a measure of the carbon dioxide (CO_2) released from a sample. It is released as a result of decomposition of organic matter and plant litter by soil microbes and due to plant respiration in the roots and respiration of soil fauna such as fungi. Basal respiration was determined according to the method presented in Piotrowska et al. (2006) using the alkali absorption method. Twenty-five grammes of an air-dried sample was placed in a 1 L glass jar. The water content was adjusted to 50% of water holding capacity (WHC, i.e. the total amount of water that can be adsorbed by the sample). A single plastic vial containing 10 mL of 0.5 mol/L NaOH, was placed inside the glass jar and served as an absorption collector for CO_2 produced by the system. After 24 h of incubation at 25 °C, 5 mL of 0.5 mol/L $BaCl_2$ was added to the NaOH and then titrated with 0.05 mol/L HCl. The amount of CO_2 was calculated as follow:

$$CO_2\ (\mu g \cdot kg^{-1} \cdot h^{-1}) = \left(\frac{(V_b - V_s) \times c_{HCl} \times 22}{M_d * t} \right) \times 1000 \quad (1)$$

where V_b is the volume of HCl used for the titration of the blank (mL); V_s is volume of HCl used for the titration of the sample (mL); c_{HCl} is the molar concentration of HCl (mol/L); 22 is the equivalent mass of CO_2, M_d is the dry mass of the sample in kg; t is the incubation time in hours; 1000 is a conversion factor from mg to μg of CO_2. The procedure was repeated at 0, 7, 14 and 28 days and the basal respiration was expressed as the quantity of CO_2 produced by 1 kg of air-dried sample in 1 h (μg·kg^{-1}· h^{-1}).

2.3.2.2. Ergosterol extraction and analysis. Ergosterol is a fungus specific lipid and bio-marker. Ergosterol concentration is used here as an indicator of fungal biomass. In this study, a rapid technique for the determination of ergosterol concentration via physical disruption was used (Gong et al., 2001). Acid-washed glass beads (10 g, sized 212–1180 μm diameter) were introduced into a 50 mL scintillator vial containing 10 g of specimen (sand/organic substrate/water/mycelium). 15 mL of methanol was added. The mixture was vortexed for 10 s and placed on a shaker set at a speed of 3200 r/min for 1 h. Thereafter, the mixture was left to stand for 15 min. 5 mL aliquots of supernatant were transferred into a 15 mL centrifuge tube and subjected to centrifugation for 10 min at a speed of 1100 r/min and temperature of 5 °C. 3 mL of the supernatant was then filtered through a 0.2 μm syringe filter and 1 mL of the filtrate was transferred into a 1.5 mL vial. This portion of the extract was loaded into the auto sampler of the High-Performance Liquid Chromatography (HPLC) for analysis.

Ergosterol analysis was carried out with Agilent® 1100 Series using a Phenomenex C-18 RP column (250 mm × 4.6 mm, 4 μm) and a diode-array detector. Methanol (100%) was the mobile phase and the flow rate was set on 1 mL/min, UV detector wavelength was set at 282 nm. The sample injection volume was 20 μL. Linear calibration curves were determined for ergosterol standards prepared using pure ergosterol (Sigma Aldrich). Five different concentrations were used and linearity was checked for each day of specimen analysis. Parameters from the linearity plots were used for converting the peak areas (milli-absorbance unit-minutes or mAU*min) to ergosterol concentrations (μg/L) for the test specimens.

2.3.2.3. Enzymatic activities. Enzymes produced by *P.ostreatus* are investigated in this study as indicators of the evolution of fungal biochemical activity over time. The two enzymes investigated were laccase and manganese peroxidase, both of which contribute to lignin decomposition.

2.3.2.3.1. Laccase activity. The laccase activity was determined according to Diez et al. (2006) using 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). ABTS is a chemical compound used to assess the reaction kinetics of specific enzymes including laccase. The method is based on the spectrophotometer measurement of the green colour developed by the enzymatic oxidation of ABTS. Briefly, 1 g of moist sample was weighed and incubated for 30 min at 25 ± 1 ºC with 4 mL of ABTS 0.15 mmol/L prepared in sodium acetate buffer 0.1 mol/L, pH 5.0. To stop the reaction, samples were immediately centrifuged at 4000 r/min for 5 min at 4 °C and filtered with Whatman filter paper 42. Absorbance was then measured using a spectrophotometer at 420 nm and the laccase activity expressed as $ABTS^+$ mmol·g^{-1}·h^{-1}.

2.3.2.3.2. Manganese peroxidase. Manganese peroxidase activity was determined according to Bach et al. (2013) and Paszczyński et al.

Table 3
Physico-chemical parameters measured.

Parameter	Method	Reference
pH	pH meter (1:5 soil-water suspension)	Di Rauso Simeone et al., 2018, 2020
Electrical Conductivity (EC)	Conductivity meter (1:2.5 soil-water suspension)	
Total organic Carbon (TOC)	Dichromate oxidation titration method	Sparks et al., 1996
Total Nitrogen (TN)	Kjeldahl method	Sparks et al., 1996
Respiration	Determination of CO_2 produced	Piotrowska et al., 2006
Laccase activity	Spectrophotometry	Diez et al., 2006
Manganese activity	Spectrophotometry	Bach, 2013; Paszczyński et al., 1988
Ergosterol concentration	High Performance Liquid Chromatography	Gong et al., 2001

(1988), with some modifications. Briefly, 1 g of moist sample was incubated with 3.2 mL of 2,6-Dimethoxyphenol (2,6-DMP) 0.1 mmol/L prepared in sodium tartrate buffer 0.1 mol/L, pH 5.0 and 0.4 mL of 1 mmol/L manganese sulphate, was added. In order to start the reaction 0.4 mL of 1 mmol/L of H_2O_2, was added. Absorbance was then measured using a spectrophotometer at 568 nm after 3 h at 25 ± 1 ºC and the manganese peroxidase activity was expressed as 2,6-DMP mmol·g^{-1}·h^{-1}.

2.3.2.4. Scanning electron microscopy. After a growth period of 28 days in the incubator at 25 °C, a set of replicate samples (Experiment 4 - S, SP, SLP) were collected for observation using Scanning Electron Microscopy. Due to an unforeseen delay in availability of SEM equipment, samples were stored at –20 °C to prevent further growth until SEM was carried out at 15 weeks after storage. Images were captured using a Jeol JSM5310 SEM device, which enables three-dimensional imaging using a secondary electron detector and topographical and compositional imaging using Back Scattered Electrons. Specimens were gold-coated before performing SEM imaging.

3. Results

3.1. Influence of environmental conditions on mycelium growth

3.1.1. Influence of temperature (Experiment 1)

Fig. 2(a) presents the cumulative radius of the *P.ostreatus* mycelium (based on an average of 3 specimens) for specimens incubated at temperatures between 5 °C and 35 °C from 3 to 12 days after inoculation. Growth at 5 °C was not recorded until 12 days after inoculation, whereas at 10 °C and 15 °C no growth was recorded until 6 days. No growth was recorded at 35 °C throughout the period of observation. The radius of the mycelium increased both with growth duration and with increasing temperature up to 25 °C, attaining the maximum value of 37.78 mm for specimens incubated at 25 °C after 12 days of growth. Above 25 °C there was a marked decline in growth with the greatest extent of the mycelium achieved equal to 26.33 mm at 30 °C.

Fig. 2(b) presents the mycelium growth rates from 3 to 12 days after inoculation. At lower temperatures (5–20 °C) the highest growth rate was exhibited later in the study (i.e. between 6–12 days), whereas at higher temperatures (25–30 °C) the highest growth rate was exhibited between 3–6 days. The maximum growth rate was exhibited at 25 °C in the period 3–6 days (5.4 mm/day). The marked decline in growth rates observed in Fig. 2(b) beyond 6 days after inoculation may be attributed to boundary conditions. For example, for the condition of 25 °C at Day 9 the radius of mycelium growth was already > 35 mm and the maximum radius of the petri dish was 45 mm. According to Boddy et al. (2009), some fungal species forage for resources by first deploying thin peripheral exploratory networks of hyphae as a less aggregated growing front ensuring transport and growth efficiency. It is possible that individual hyphae in this case may have already reached the edge of the petri-dish and upon encountering the 'obstruction' (boundary), initiated a morphological response 'communicated' across the highly coordinated hyphal network (Crowther et al., 2012) leading to a reduction in growth rate. Individual exploratory hyphae may not have been sufficiently visible via the DSLR camera and image analysis approach used.

Although the radial mycelial growth increased with temperature (up to 25 °C), fungal biomass density (i.e. hyphae present per unit area on soil surface) was visually observed to decrease with increasing temperature from 10 to 30 °C (based on observations made on Day 12 after inoculation; see Supplementary information Fig. S1). Fungal biomass

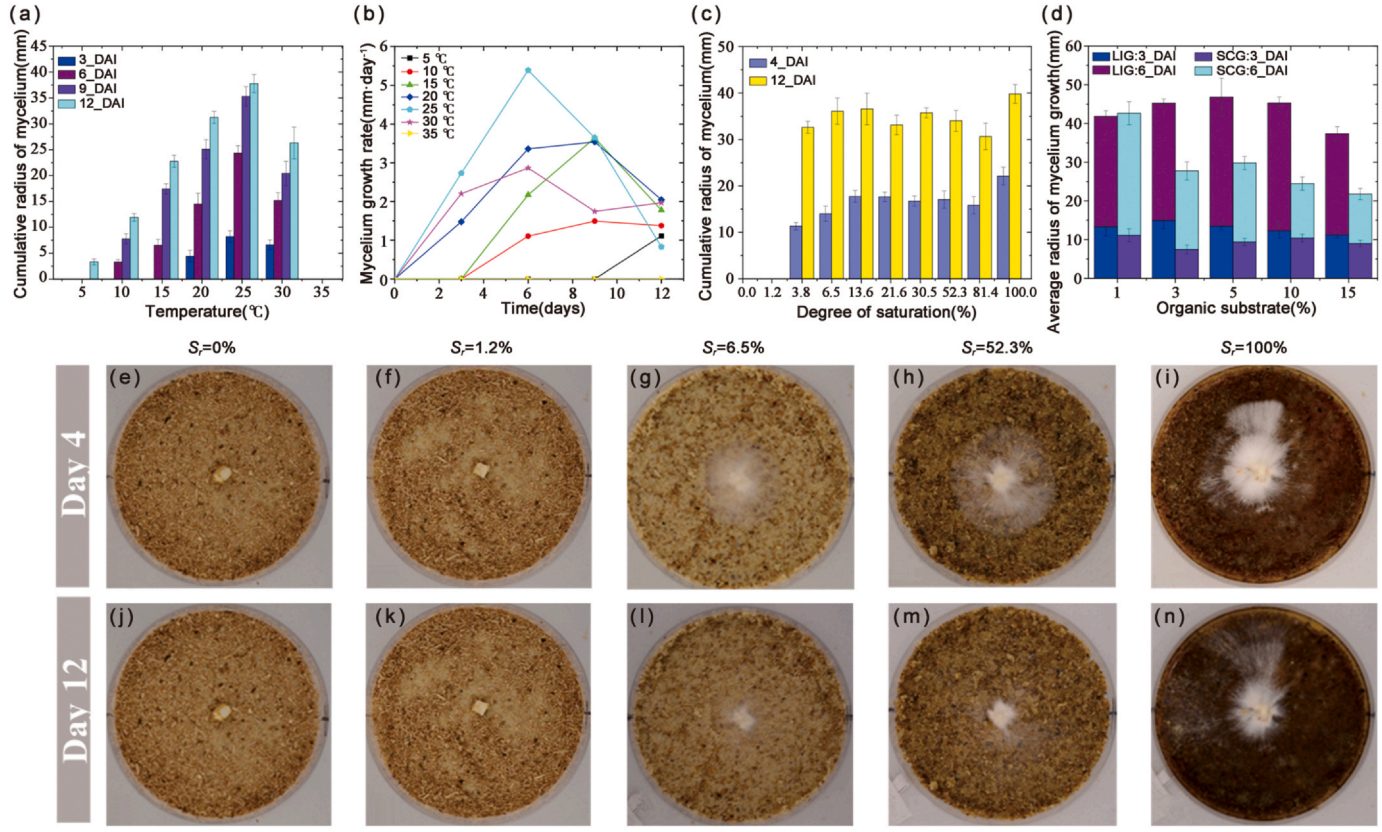

Fig. 2. (a) Cumulative radius of mycelium growth at different temperatures 3–12 days after inoculation; (b) Mycelium growth rate for temperatures between 5–35 °C; (c) Cumulative radius of mycelium growth at different degrees of saturation for 4 and 12 days after inoculation (DAI); (d) Stacked column plots of average radius of mycelium growth at varied amounts of lignocel (LIG) and spent coffee grounds (SCG) observed on the 3rd and 6th day after inoculation. Mycelia of P. ostreatus growing in sand 4 days and 12 days after inoculation at degrees of saturation of (e & j) 0%, (f & k) 1.2%, (g & l) 6.5%, (h & m) 52.3% and (i & n) 100%.

density was also observed to decrease over time for specimens incubated at 20 °C and 25 °C (see supplementary information, Fig. S1, e.g. compare days 3, 9 and 12 at 25 °C), with the mycelia transitioning from dense diffuse networks of fine hyphae to networks characterised by less dense (i.e. less visible), mycelia. This is similar to observations of mycelial morphological response of cord-forming saprotrophs (e.g., *Phanerochaete velutina*) to depleting resources reported in the literature where the mycelial network is reorganised to optimise nutrient transport in nutrient depleted environments, resulting in less dense mycelia systems (Bebber, 2007; Donnelly et al., 1995).

3.1.2. Influence of degree of saturation (Experiment 2)

Fig. 2(c) presents the cumulative radius of mycelium at 4 and 12 days after inoculation for specimens prepared at different initial degree of saturation (all incubated at 25 °C and prepared with 5% lignocellulose, Experiment 2, Table 1). No growth was observed at degrees of saturation (S_r) of 0% and 1% indicating there is a critical water content required to support fungal growth.

Within the range 3%–14% the mycelium radius increased with increasing degree of saturation. Increasing the S_r further appears to slightly reduce the extent of growth, but with similar growth achieved within range S_r = 20%–80%. The maximum radius of mycelium growth was recorded under fully saturated conditions,i.e. S_r= 100%. At this S_r, no growth was observed within the sand, instead, mycelium growth was observed only to occur at the air-water surface (see Fig. 2(i) and (n)) with hyphae not penetrating the sand.

It was also observed that the fungal biomass density appeared to be homogeneous and characterised by a diffuse dense mycelium on the 4th day after inoculation (Fig. 2(g–i) and Supplementary information Fig. S2). Whereas by day 12 after inoculation, the mycelia appeared to reduce in density (i.e.'thin out') with hyphae becoming translucent (see Fig. 2(l–n)) and Supplementary information Fig. S2).

3.1.3. Influence of substrate concentration (Experiment 3)

Fig. 2(d) presents a stacked plot of the radius of mycelium growth 3 days after inoculation and the total mycelium radius after 6 days for specimens (a) amended with lignocellulose (LIG) and (b) spent coffee grounds (SCG) at concentrations between 1%–15%. At 1% substrate concentration, after 6 days similar mycelium growth was achieved for both lignocellulose and spent coffee grounds amended specimens. At substrate concentrations above 1% lignocellulose consistently exhibited a greater mycelium radius after 3 days and after 6 days compared to specimens amended with spent coffee grounds. Mycelium radius after 6 days was similar between 3%–10%, however increasing the lignocellulose amendment further to 15% resulted in reduced radius of mycelium but greater mycelium density was observed (Supplementary information Fig. S3). The SCG specimens showed a more clear trend of reduction in mycelium radius with increasing susbtrate concentration in the range 5%–15%). This is likely due to the fact that with an abundance of nutrients in close proximity, homogeneous in their distribution, the mycelium of *P. ostreatus* preferred to grow locally, finding no need to deploy hyphae further away from the inoculant in search of resources. Species exhibiting this kind of behaviour are described as 'short-range foragers' (Boddy et al., 2009). This behaviour is supported by the visually observed increase in fungal biomass density with increasing SCG concentration (Supplementary information, Fig. S3). Indeed, the fungal biomass density appears to be higher overall for the SCG specimens compared to the LIG specimens for a given substrate content. This suggests that in the SCG specimens, *P. ostreatus* grew more intensively locally, perhaps due to the lower Carbon/nitrogen ratio and SCG being more readily degraded (compared to lignocellulose), both contributing to the lower mycelium radius as observed in the SCG specimens at susbtrate contents > 1%.

3.2. Physico-chemical and biochemical changes due to the growth of mycelium (Experiment 4)

The environmental and substrate variables in Experiment 4 were selected on the basis of the results from experiment 1, 2 and 3 (i.e. optimum growth, observed at T= 25 °C, S_r=13.6% and at 5% lignocellulose content). The key objective here was to investigate physiochemical changes occurring in the sand and survival and activity of *P.osteratus* over a 4-week period.

3.2.1. pH, electrical conductivity (EC), total organic carbon (TOC) and total nitrogen (TN)

Table 4 presents the pH, EC, TOC and TN measurements for each specimen on the day of inoculation (Day 0) and then at 7, 14 and 28 days after inoculation. At each timepoint the highest pH was observed in the sand only specimens (S), whereas the lowest pH was consistently measured at every timepoint in the Sand-Lignocellulose-*Pleurotus ostreatus* (SLP) specimens, lower than that measured in the Sand-*Pleurotus ostreatus* (SP) specimens alone. Electrical conductivity on the other hand was lowest (~0.04 $dS{\cdot}m^{-1}$) in the sand only specimens (S) and highest

Table 4
pH, EC, TOC and TN observed for specimens from 0 – 28 days after inoculation.

Specimen	pH	EC ($dS{\cdot}m^{-1}$)	TOC ($g{\cdot}kg^{-1}$)	TN ($g{\cdot}kg^{-1}$)
Day 0				
S	7.15 ± 0.07	0.03 ± 0.01	00.19 ± 0.00	0.00 ± 0.01
SL	6.11 ± 0.03	0.15 ± 0.00	27.13 ± 0.22	0.14 ± 0.01
SP	6.83 ± 0.04	0.13 ± 0.00	00.53 ± 0.07	0.07 ± 0.01
SLP	5.89 ± 0.04	0.23 ± 0.00	26.89 ± 0.41	0.19 ± 0.00
Day 7				
S	7.73 ± 0.11	0.04 ± 0.01	00.10 ± 0.14	0.00 ± 0.01
SL	6.42 ± 0.06	0.15 ± 0.02	26.88 ± 0.58	0.14 ± 0.01
SP	7.24 ± 0.03	0.14 ± 0.00	00.24 ± 0.07	0.07 ± 0.01
SLP	5.78 ± 0.02	0.25 ± 0.02	26.77 ± 0.14	0.20 ± 0.01
Day 14				
S	7.31 ± 0.08	0.03 ± 0.00	00.29 ± 0.00	0.00 ± 0.01
SL	6.59 ± 0.01	0.14 ± 0.02	27.14 ± 0.46	0.14 ± 0.01
SP	7.06 ± 0.00	0.15 ± 0.01	00.34 ± 0.07	0.07 ± 0.01
SLP	5.76 ± 0.08	0.24 ± 0.02	27.00 ± 0.14	0.19 ± 0.01
Day 28				
S	7.46 ± 0.00	0.04 ± 0.00	00.27 ± 0.33	0.00 ± 0.01
SL	6.66 ± 0.01	0.16 ± 0.01	26.75 ± 0.36	0.14 ± 0.01
SP	7.29 ± 0.05	0.15 ± 0.01	00.18 ± 0.07	0.07 ± 0.01
SLP	5.76 ± 0.06	0.25 ± 0.01	26.85 ± 0.72	0.20 ± 0.02

S – Sand; SL – Sand & lignocellulose; SP – Sand & *P. ostreatus;* SLP – Sand, lignocellulose & *P.ostreatus*

(~0.25 $dS \cdot m^{-1}$) in the SLP specimens at every timepoint. pH and EC remain fairly constant in the SLP specimens over the 4-week period.

As expected, TOC for the control (sand only) and the SP specimens were close to 0 $g \cdot kg^{-1}$ while the the Sand-Lignocellulose specimens (SL) and SLP specimens each had TOC content of ~27 $g \cdot kg^{-1}$ which remained almost constant from day 0 to day 28.

Results for TN imply that lignocellulose and inoculation with *P. ostreatus* each contributed to an increase in TN from 0 $g \cdot kg^{-1}$ in the control to 0.14 ± 0.01 and 0.07 ± 0.01 in the SL and SP specimens respectively and these values remained constant across the 28-day period of observation. TN was highest in the SLP specimens and again remained constant over the 28-day period.

3.2.2. Growth indicators: Ergosterol concentration and respiration

Fig. 3(a) and (b) present the ergosterol concentration and basal respiration rate up to 28 days after inoculation. As expected, ergosterol concentration is absent in the sand only (S) specimens. A low ergosterol concentration was determined in the sand and lignocellulose (SL) specimens, yet SL specimens showed zero basal respiration (Fig. 3(b)), this indicates possible contamination of the SL specimens from airborne fungal spores during the study, i.e. spores were present but not active and, hence no respiration was detected.

Both increasing ergosterol concentration and basal respiration rate with time was only observed in the specimens comprising sand, lignocellulose and *P. ostreatus* (SLP) specimens indicating that the presence of lignocellulose (i.e. a carbon and nitrogen source) is necessary for continued growth and viability of *P.ostreatus*. Whereas in the sand and *P. ostreatus* (SP) specimens the highest ergosterol concentration was observed on the day of inoculation and thereafter decreased and the basal respiration rate at 28 days was only around 15% of that measured in the SLP specimens.

3.2.3. Enzymatic activity: laccase and manganese peroxidase

Fig. 3(c) presents the laccase activity measured in the corresponding S, SL, SP and SLP specimens on the day of inoculation and 7, 14 and 28 days after inoculation. As expected laccase activity is low in the uninoculated control specimens (S and SL) and activity is much higher in the inoculated specimens SP and SLP. Although in the SP specimen where no substrate (i.e. carbon/nitrogen source) was present laccase activity decreased steadily with time after inoculation. Whereas in the inoculated specimen with lignocellulose (SLP), laccase activity was initially low on the day of inoculation and was significantly higher by 7 days and thereafter remained at a similar level up to 28 days.

Fig. 3(d) presents the manganese peroxidase activity on the day of inoculation and 7, 14 and 28 days after inoculation. As for laccase activity, the uninoculated controls exhibited low manganese peroxidase activity, but not zero activity. Considerably higher activity was observed in the inoculated specimens (SP and SLP). As observed for laccase activity, in the SP specimens the manganese peroxidase activity decreased overtime after inoculation. Whereas in the SLP specimens manganese peroxidase activity continued to increase over the 28-day growth period.

For both laccase activity and managanese peroxidase activity the Day 0 activity of the SLP specimens was considerably lower than that of the SP specimens, this is likely due to the *P.ostreatus* adjusting to the presence of and colonising a new substrate in the SLP specimens, lignocellulose, as the fungus was originally grown on millet grains. In the SP specimens no new substrate is present in Day 0, therefore the fungus is continuing to use residual nutrients in the spore/hyphal suspension, but with no new substrate present activity decays after Day 0.

3.2.4. Pearson correlation analysis

A Pearson correlation analysis was conducted on the physico-chemical and biochemical data obtained in Experiment 4 data. The Pearson

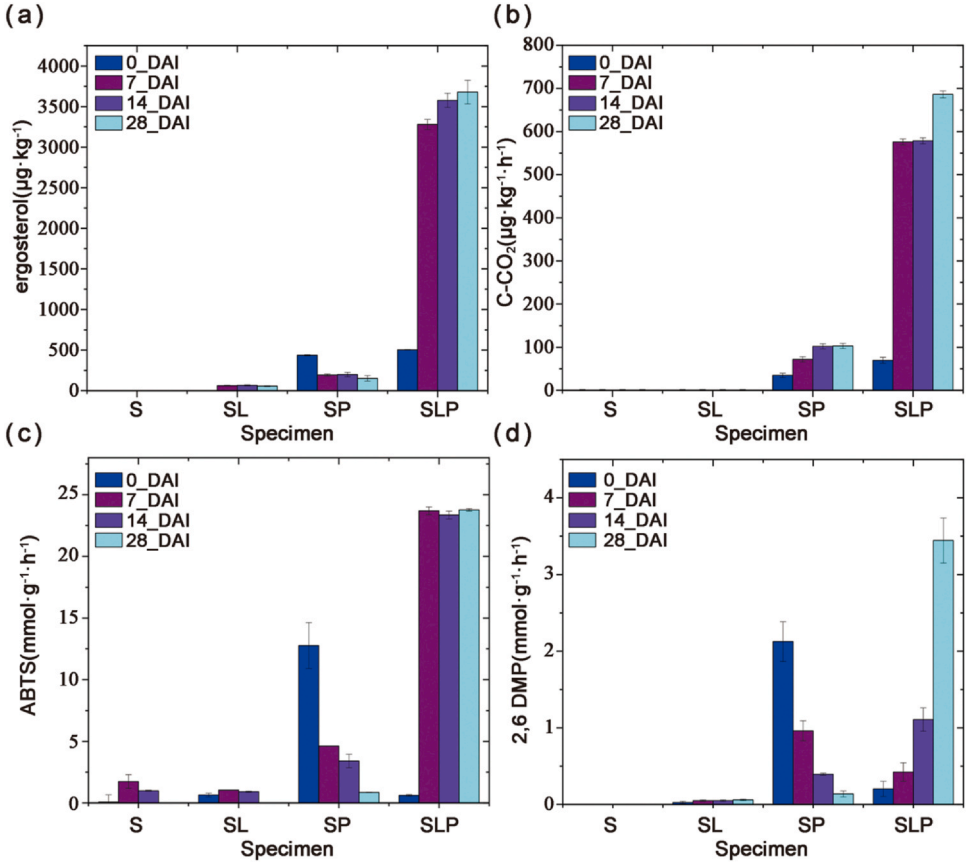

Fig. 3. (a) Ergosterol concentration; (b) Basal respiration rate; (c) Laccase activity; (d) Manganese peroxidase activity measured on 0, 7, 14 and 28 days after inoculation.

Table 5
Pearson correlation coefficients between physico-chemical and biochemical variables.

Variables	pH	EC	TOC	TN	Laccase activity	Mn peroxidase activity	Basal respiration	Ergosterol conc.
pH	**1**							
EC	**–0.875**	**1**						
TOC	**–0.880**	**0.727**	**1**					
TN	**–0.944**	**0.948**	**0.903**	**1**				
Laccase activity	–0.430	0.461	0.348	0.454	**1**			
Mn peroxidase activity	–0.391	0.465	0.119	0.363	**0.721**	**1**		
Basal respiration	–0.371	0.422	0.429	0.421	**0.939**	**0.640**	**1**	
Ergosterol conc.	–0.313	0.421	0.480	0.444	**0.956**	**0.630**	**0.990**	**1**

Bold values denote statistical significance at the $p < 0.05$ level

correlation coefficients between each of the variables are presented in Table 5. pH shows a strong negative linear correlation with the other physico-chemical parameters EC, TOC and TN (all < -0.85). EC, TOC, TN show strong positive linear correlations with each other (all > 0.70). The physico-chemical variables (pH,EC,TOC,TN) are not correlated with the biochemical variables (Laccase activity, Managanese Peroxidase activity, Basal respiration and Ergosterol concentration). The biochemical variables (Laccase activity, Managanese Peroxidase activity, Basal respiration and Ergosterol concentration) show strong positive correlations with each other (all > 0.60), with the strongest positive correlations observed between Laccase activity and Basal respiration (~ 0.939), Laccase activity and Ergosterol conc. (~ 0.956) and Basal respiration and Ergosterol conc. (~ 0.990). Considering that ergosterol concentration is used as an indicator of living fungal biomass (Stahl and Parkin, 1996), laccase activity or basal respiration may also be useful parameters for monitoring fungal growth in soils.

3.3. Scanning electron microscopy

Fig. 4 presents SEM images of the S, SP, SLP specimens. It is clear in Fig. 4(a) that the uninoculated sand specimens (S) comprise of clean separated sand grains. The rough surface of a sand grain is visible in Fig. 4(b). In the Sand-*Pleurotus* (SP) specimens fungal hyphae are evident spanning between sand grains (Fig. 4(c) and (d)) and hyphae are observed adhering to grain surfaces (Fig. 4(e) and (f)). In the Sand-Lignocellulose-*P.ostreatus* (SLP) specimens fungal hyphae are clearly visible and are in close physical association with lignocellulose particles (Fig. 4(g) and (h)). The mass of hyphae appears to be enmeshing sand particles as shown in Fig. 4(g). There appears to be a higher density of fungal hyphae present in the SLP specimens compared to the SP specimens (e.g. compare Fig. 4(c) and (g)). This supports the biochemical results obtained which showed higher ergosterol concentration, higher basal respiration rates and higher enzymatic activity in the SLP specimens.

4. Discussion

4.1. Implications for practical applications

Cellular processes required for fungal growth are slowed down at low temperatures (0–5 °C) (Kubicek et al. 2007), this is evident in our results as *P. ostreatus* mycelium growth was not obvious until the 12th day in sand at 5 °C. Whereas no growth was recorded at 35 °C throughout the period of observation and fastest growth rates were observed at $T = 25$ °C. These results indicate that *P. osteratus* is a mesophile, defined as growing between 5–35 °C with optimum growth between 20–30 °C (Dix & Webster, 1995). Only thermophiles with specially adapted mechanisms for dealing with elevated temperatures can maintain cellular integrity and support growth at higher temperatures (Kubicek et al. 2007). These results are similar to what has been found for the growth of other species of saprotrophic fungi in soils (*Phanerochaete velutina* and *Stropharia caerula*) (Donnelly and Boddy, 1997). Donnelly and Boddy (1997) observed no growth at 30 °C for these species, indicating that *P.ostreatus* is less sensitive to temperature than these species.

> **Implication 1:** Temperature should be considered at the earliest possible stage to ensure fungal treatment at a given site is indeed likely to be feasible (to rule out deployment at sites with extreme low and high soil temepratures). The site specific monthly temperature variation should be a key piece of information used to design the timing of a *P. ostreatus* based geotechnical or geo-environmental treatment. For example, in the UK *P. ostreatus* initial inoculation and growth phase wouldn't be recommended for during Winter months when soil temperatures are likely to be < 10 °C, but rather could take place during Spring, Summer and Autumn months when soil temperatures are higher (depending on site specific data).

Mycelia growth was recorded for degrees of saturation within the range ~3.8%–100%. The results here indicate that as long as some moisture is present ($S_r > 1.2\%$), the radial growth of *P. ostreatus* is not very sensitive to the soil water content, with similar growth achieved over a wide range of degree of saturation in a uniformly-graded medium sand (14%–80%). However, at $S_r = 100\%$ growth occurred predominantly at the air-water interface. This is due to the preference for growth in high oxygen availability environments in order to maintain aerobic activity. Moisture content and its distribution strongly influences gas transport rates in the soil, with oxygen diffusion 10^4 times higher in air than in water (Young and Ritz, 2000). Indeed, the soil air permeability has been shown to strongly influence the growth behaviour of other saprotrophic fungi (*Rhizoctonia solani*) (Otten et al., 1999).

> **Implication 2:** *P. ostreatus* growth in soils is feasible under a range of saturation conditions, as long as some moisture is present. The degree of saturation of soil in the field is of course variable in response to infiltration, water table variations and groundwater flow. *P. ostreatus* penetration with depth into soils will be more successful in partially saturated soils, whereas surface films of mycelium could be created in saturated soils.

It was confirmed that for *P. ostreatus* a substrate (nutrient source) proving carbon and nitrogen is required for hyphal and cellular activities to ensure growth, survival and sustained enzymatic activity. Low substrate concentrations (1%–5%) resulted in high radial growth of *P. ostreatus*, increasing substrate content further (for both lignocellulose and spent coffee grounds) reduced radial growth, but visual observations indicated that the mycelia were denser (i.e. fungal biomass density increased).

> **Implication 3:** This indicates that the design of fungal treatment strategies should be tailored in terms of the inoculation and nutrient provision strategies in order to balance the desired lateral coverage and the fungal biomass density to be achieved within the soil, to reach target treatment levels for contamination remediation or modifications to hydraulic and/or mechanical behaviour. E.g. if

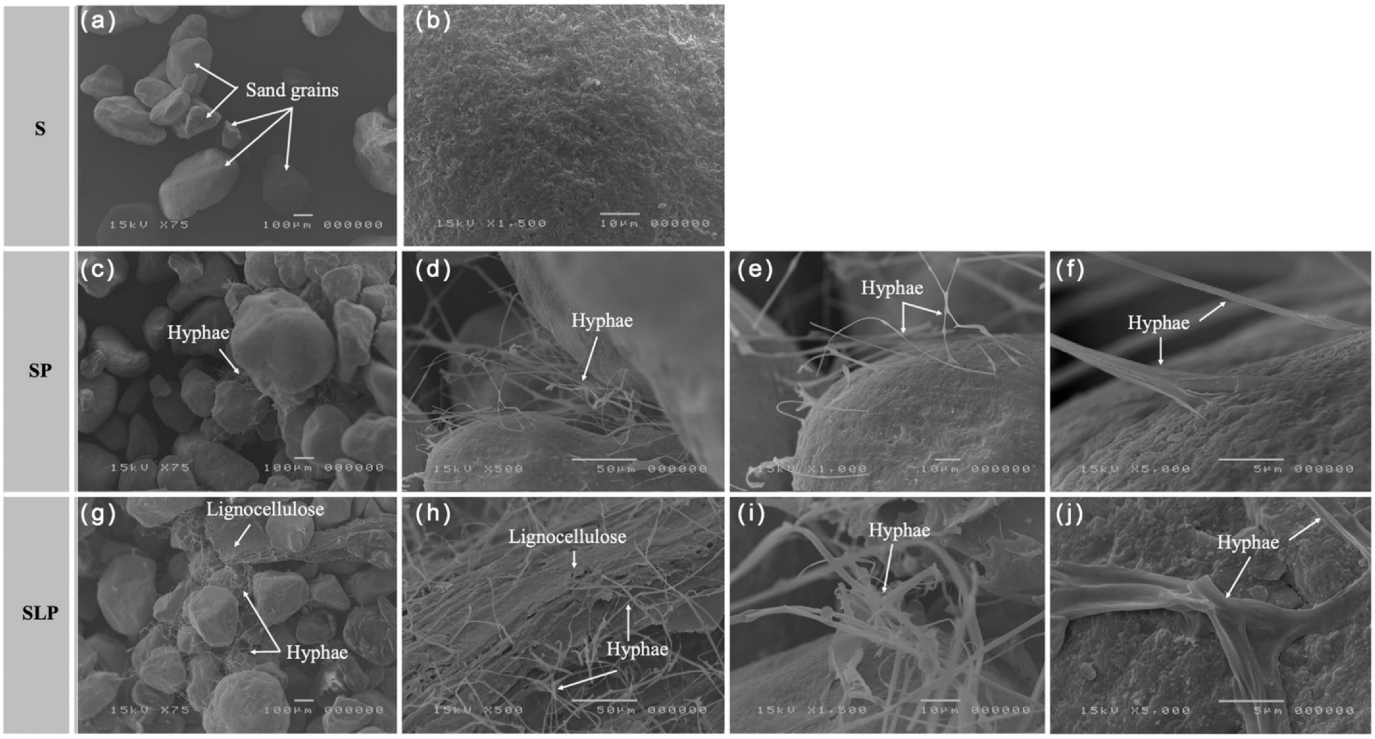

Fig. 4. Scanning Electron Microscope images of (a,b) Sand (S) specimens, (c–f) Sand-P.ostreatus (SP) specimens and (g–j)Sand – Lignocellulose-P.ostreatus (SLP) specimens.

more distributed growth is desired over a soil volume it may be beneficial to use lower percentages of nutrient amendments but with corresponding lesser effects on soil mechanical behaviour. Whereas on the other hand if denser growth is desired, with corresponding greater modifications to soil behaviour higher percentages of nutrients could be supplied, but with the trade off that fungal inoculation may be required at more frequent distances.

4.2. Limitations and future work

One limitation of this study is that the growth of *P. ostreatus* has been assessed here based on visual observations of the mycelium radius at the soil surface, which permitted subsequent timepoint measurements to be taken (non-destructively) on the same specimen. The specimens prepared in this study were shallow in depth (4 mm) and so didn't permit evaluation of growth with depth into the soil. This study acts as a first step into understanding the influence of environmental conditions on *P. ostreatus* growth in soil. Future studies should investigate growth with depth into the soil. The soil considered here was a uniformly-graded medium sand, future studies should also investigate the influence of soil composition and chemistry on *P. ostreatus* growth.

In this study the lignocellulose used had a relatively large particle size (0.5–1.00 mm), and Experiment 4 shows that over the short duration of the experiments (up to 1 month) much of the substrate remained unused (TOC and TN values remained constant in SLP specimens). The SEM images (Fig. 4) show a close association between lignocellulose and fungal hyphae on the substrate surface. Further studies are required to understand how particle size and surface area of the organic substrate influences *P. ostreatus* growth in terms of mycelial extent and fungal biomass density and ultimately how this in turn influences soil hydro-mechanical properties or mycoremediation efficiency.

Experiment 4 data shows excellent correlation between ergosterol concentration (an indicator of living fungal biomass) and basal respiration and laccase activity for *P.ostreatus* growth in sand amended with lignocellulose. This indicates that basal respiration and laccase activity may be useful variables for monitoring of *P. ostreatus* growth in the laboratory and potentially in the field, this should be investigated further.

4.3. Deployment and applications of fungal-based geotechnologies

Although research into fungal-based technologies for ground improvement remains at an early stage, it is useful at this stage to highlight how they might compare to bacteria-based technologies in terms of their deployment and also possible applications. One fundamental difference, considering bioaugmentation strategies is that fungi do not require a continuous water phase in order to be transported within the pore space of soils. Fungal hyphae can grow across air-water interfaces, extending across air filled pores, and infilling soil pores (Harms et al., 2011). This is in contrast to bacteria that cannot across air gaps and require to be transported within a liquid phase, (i.e. for bioaugmentation strategies bacteria are typically injected within a liquid suspension). Furthermore, saprotrophic fungi can forage for nutrients, with long-range foragers foraging over several metres (Donnelly & Boddy, 2001), and fungi have also been shown to grow to massive sizes in natural environments, with individual organisms measured up to 10 km^2 in size and > 1900 years old in North America (Ferguson et al., 2003). These indicate that fungal-based geotechnologies may be beneficial for deployment over large areas (e.g. slopes/hillslopes) and have the potential to require fewer injection/inoculation points than bacterially-based systems. Furthermore, fungal treatment is likely to be inexpensive particularly if the nutrient source supplied is a waste material (e.g. spent coffee grounds as used in this study) and could be carried out in a similar manner to hydroseeding. Given the aerobic nature of basidiomycota fungi, it is anticipated that geotechnical applications where such treatments would be most promising would be in surface/near-surface applications, e.g. for erosion control, for controlling/regulating moisture content and soil suction.

In terms of hydraulic and mechanical behaviour, to date the (limited) research indicates that *Pleurotus ostreatus* treatment of sand can result in an increase of cohesion from 0 kPa to several kPas (3–4 kPa) as

determined in direct shear tests (Salifu & El Mountassir, 2019) with Unconfined Compressive Strength values of up to 73 kPa reported for nutrient rich specimens (Lim et al., 2023). The transition from cohesionless to low cohesion soils is an important one, and supports applications related to erosion control. These values are much lower than values reported for MICP treated soils, with cohesion values up to 725 kPa (Van Paassen, 2009) and UCS values up to 18 MPa (Li et al., 2018) reported for heavily cemented sands. Yet, these technologies should not be viewed as being in competition but rather fungal-based technologies should be viewed as a potential additional nature-based technology in the tool kit of ground engineering contractors. Indeed, the induced hydrophobicity from fungal treatment lends itself to different applications than typically proposed for MICP, (for example hydraulic barriers to provide temporary infiltration control). Furthermore, it should be noted that enhanced strength, erosion resistance and durability of fungal-treated soils could be provided via induced fungal biomineralisation which has remained relatively unexplored to date. Fang et al. (2018) report a UCS value of 1800 kPa for a fungal treated sand (fungal species: *Penicillium chrysogenum)*.

5. Conclusions

The main conclusions of this paper are:

- Temperature has a strong influence on the growth of *P. ostreatus* in sand: at low temperatures (e.g. 5 °C) growth was delayed, whereas at high temperatures (e.g. 35 °C) growth was prevented. This influence is important for understanding the feasibility of *P. ostreatus* growth at particular site locations and for designing suitable inoculation strategies (e.g. seasonal dependent inoculations).
- Within a mid-range of water contents tested radial growth of *P. ostreatus* was similar. However, no growth was observed at very low water contents ($S_r = 0$ and 1.2%) indicating there is a critical water content required to support *P. ostreatus* growth. Growth under saturated conditions ($S_r = 100\%$) was restricted to the air-water atmosphere due to the requirement for oxygen availability. This has implications for how *P. ostreatus* treatment could be deployed in situ, e.g. penetration into soils will require partially saturated conditions, whereas surface films could be created in saturated soils.
- Low substrate concentrations (1%–5%) resulted in high radial growth of *P. ostreatus*, increasing substrate content further (for both lignocellulose and spent coffee grounds) reduced radial growth, but visual observations indicated that fungal biomass density increased. This indicates that the design of fungal treatment strategies should be tailored in terms of the inoculation and nutrient provision strategies in order to balance the desired lateral coverage and the fungal biomass density to be achieved within the soil, to reach target treatment levels for contamination remediation or modifications to hydraulic/mechanical behaviour.

CRediT authorship contribution statement

Emmanuel Salifu: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Giuseppe Di Russo:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Giacomo Russo:** Supervision, Methodology, Investigation, Conceptualization. **Maria A. Rao:** Supervision, Methodology, Conceptualization. **Gianfranco Urciuoli:** Supervision, Project administration, Funding acquisition. **Grainne El Mountassir:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary information associated with this article can be found in the online version at doi:10.1016/j.bgtech.2024.100137. Data associated with this publication are openly available from the University of Strathclyde KnowledgeBase at https://doi.org/10.15129/83d3eba5-173f-4435-ab2f-22ffe18bd0dd.

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