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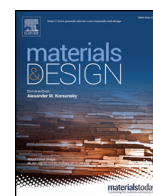
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Fabrication factors influencing mechanical, moisture- and water-related properties of mycelium-based composites

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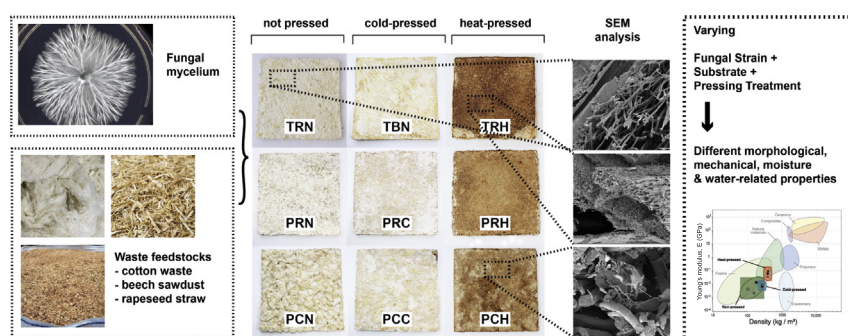
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HIGHLIGHTS

- The type of fungus influences homogeneity of mycelium composite materials and thickness of the fungal skin.
- The fungal skin impacts mechanical behaviour of the mycelium composite material and improves water resistance.
- Straw-based materials are stiffer and less moisture-resistant than cotton-based mycelium composites.
- Heat pressing improves homogeneity, strength and stiffness of mycelium composite material.
- Heat-pressed mycelium materials have similar density and elastic modulus as natural materials (e.g. cork and wood).

GRAPHICAL ABSTRACT



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ABSTRACT

Mycelium-based composites result from the growth of filamentous fungi on organic materials such as agricultural waste streams. These novel biomaterials represent a promising alternative for product design and manufacturing both in terms of sustainable manufacturing processes and circular lifespan. This study shows that their morphology, density, tensile and flexural strength, as well as their moisture- and water-uptake properties can be tuned by varying type of substrate (straw, sawdust, cotton), fungal species (*Pleurotus ostreatus* vs. *Trametes multicolor*) and processing technique (no pressing or cold or heat pressing). The fungal species impacts colonization level and the thickness of the air-exposed mycelium called fungal skin. Colonization level and skin thickness as well as the type of substrate determine the stiffness and water resistance of the materials. Moreover, it is shown that heat pressing improves homogeneity, strength and stiffness of the materials shifting their performance from foam-like to cork- and wood-like. Together, these results demonstrate that by changing the fabrication process, differences in performance of mycelium materials can be achieved. This highlights the possibility to produce a range of mycelium-based composites. In fact, it is the first time mycelium composites have been described with natural material properties.

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1. Introduction

One of the challenges of our society is the transition towards a sustainable economy. To this end, the use of non-renewable resources has to be reduced for the production of materials and consumer products [1–4]. Renewable mycelium-based materials have the potential to contribute to the new economy by replacing petroleum-based products such as plastics. These bio-based products could for instance be used as thermal and acoustic insulation [5,6], and packaging [7].

So far, mycelium-based materials have been produced mainly from mushroom forming fungi. These fungi are known for their ability to colonize large areas in nature. For example, single individuals of the genus *Armillaria* have been identified that had colonized ≥ 1000 ha of soil, making them the largest organisms on earth [8,9]. Moreover, mushroom forming fungi are known for their ability to degrade lignocellulosic waste streams such as sawdust and straw. Like other fungi, they colonize their substrate by means of 2–10 μm -wide filamentous cells called hyphae. These hyphae form a three-dimensional network by growing at their tips and by branching subapically. This mycelium secretes enzymes that convert polymers in the substrate into breakdown products that can be taken up to serve as nutrients. As a result, the organic material is being degraded in time, while being replaced by fungal biomass on and within substrate particles. At a certain moment, hyphae grow out of the substrate into the air creating a fluffy or compact layer covering the substrate. This compact layer is also known as fungal skin.

Pure and composite fungal materials are distinguished [10]. Pure fungal materials are the result of complete degradation of the substrate or are obtained by removing the fungal skin from the substrate. The properties of pure mycelium materials depend on the substrate, the type of fungus, and its growth conditions [11–13] as well as post-processing. *Pleurotus ostreatus* mycelium material grown on cellulose is more stiff when compared to that of *Ganoderma lucidum*, while addition of dextrose to the cellulose based substrate makes both fungal materials more elastic [12]. Even a single gene can affect the material properties of the mycelium. The mycelium of a *Schizophyllum commune* strain in which the hydrophobin gene *sc3* is inactivated [14] has a 3–4-fold higher maximum tensile strength when compared to the wild-type [13]. This is caused by increased mycelium density. On top of this, the mycelium of the Δsc3 deletion strain retains more water when compared to the wild type strain [13]. This is explained by the fact that the encoded protein coats aerial hyphae with a hydrophobic coating [15]. Environmental growth conditions also impact *S. commune* mycelium properties [13]. Its maximum tensile strength ranges between 5.1 and 9.6 MPa depending whether this fungus is grown in the light or in the dark at ambient or 7% CO_2 . Together, mechanical properties of wild type and Δsc3 mycelium of *S. commune* are similar to those of natural materials and thermoplastics, respectively.

During colonization of the substrate, fungal growth can be stopped by drying and/or heating the material. By drying, the fungus is preserved in a 'hibernated' state, allowing the fungus to restart growth when moisture conditions become favourable again. Instead, heating will kill the fungus. Drying and/or heating of the substrate at some stage during colonization will result in mycelium-based composites. So far, composite materials have been shown to exhibit properties similar to expanded polystyrene or other foams [5,6,16,17]. The mycelium matrix in the composite dominates the soft compression at small strain, while the organic substrate particles cause rapid stiffening at higher strain. The mycelium composite shows the Mullins effect under cyclic conditions (i.e. the stress–strain curve depends on the maximum loading previously encountered) [17]. Studies indicate that, like pure mycelium, properties of the mycelium composites depend on the fungus, substrate, growth conditions, and processing of the material, as well as its additives [18]. The effect of the substrate on mycelium composite material is illustrated by differences in acoustic properties [5]. For instance, cotton bur fibre was a relatively low performer when compared to other feed stocks. Still, it showed 70–75% acoustic absorption at a

peak frequency of 1000 Hz, suggesting that the source of the feedstock is quite flexible when implementing a design for mycelium based acoustic absorption panels. The impact of growth conditions was reported by Yang et al. [6]. Densely packed substrate resulted in higher dry density, elastic moduli, and compressive strength when compared to loosely packed substrate. Time to inactivation of the fungus had a small impact on the dry density and thermal conductivity, but showed a negative impact on the elastic moduli and a positive impact on the compressive strength. On the other hand, addition of natural fibre improved both the elastic moduli and compressive strength.

Here, we addressed whether non-foam type of mycelium composite materials can be obtained by varying the type of fungus, substrate, and processing of the material. Heat pressing resulted in mycelium composite material with density, elastic modulus and flexural strength similar to that of natural materials like wood and cork.

2. Materials and methods

2.1. Strains and culture conditions

Trametes multicolor (*Trametes ochracea*) (Mycelia BVBA M9915) and *Pleurotus ostreatus* (SPOPO Sylvan 195) were grown for 14 days by CNC Exotic Mushrooms (Gennep, The Netherlands) on beech sawdust and 1–3 cm rapeseed straw (Gedizo trading BV, Netherlands) and by Mogu on non-woven low-quality cotton fibre (proprietary information of Mogu, Lombardy, Italy). Rapeseed straw and beech sawdust were supplemented with bran (CNC Exotic Mushrooms) and had a final humidity of 65–70%, while non-woven low-quality cotton fibres had a final humidity of 55%. In all cases, autoclavable bags (SacO2, Belgium) with filter size XL were filled with 3 kg substrate, sterilized and inoculated with spawn of *T. multicolor* or *P. ostreatus* (Mycelia, Belgium).

2.2. Fabrication conditions

Plastic thermo-formed moulds ($34 \times 34 \times 4$ cm, PET-G) were filled with pre-grown substrate. The material was hand-pressed to distribute the substrate as uniform as possible and covered with perforated cellophane foil (0.35 μm , standard commercial PPI). The fungus was allowed to grow further at 25 °C for 14 days in the dark. In order to achieve a homogeneous colonization at both sides, plates were demoulded and kept at the same conditions for 10 more days in opposite orientation to extend the growth on the side that was previously in contact with the mould. Heat (150 °C) or cold (20 °C) pressing was performed with a CE-certified mechanical multi-plate press (Vigevano, Italy) for 20 min at $F < 30$ kN. Materials exposed to heat pressing were cooled at room temperature, whereas non-pressed or cold-pressed materials were dried at environmental conditions for 24–48 h.

2.3. Specimen preparation

Specimens were cut from two different plates by manual vertical sawing. They were cut in dog-bone shape for tensile tests (155×35 mm, neck 75×22 mm), in rectangular shape for flexural testing (155×34 mm) and as square specimens (45×45 mm) for moisture and water absorption tests. Specimen dimensions were measured before testing, showing minimal dimensional variation (e.g. 155.5 ± 0.85 mm $\times$ 28 ± 0.9 mm $\times$ 13 ± 0.75 mm for TRN). Prior to testing, materials were dried at 80 °C for 24 h. Tests were performed within 30 min after the drying treatment at 22 °C and 50% humidity.

2.4. Morphological analysis

All samples were analysed by visual inspection, while TRN, TRH and PCH materials were also subjected to light microscopy (Nikon SMZ25, Japan) and cryo scanning electron microscopy (cryo-SEM). In the latter case, materials were cut into small squares (5×5 mm) with a scalpel

Table 1
Materials tested in this study.

Material type ^a	Fungus	Substrate	Pressing
TRN	<i>T. multicolor</i>	Rapeseed straw	Non-pressed
TBN	<i>T. multicolor</i>	Beech sawdust	Non-pressed
TRH	<i>T. multicolor</i>	Rapeseed straw	Heat-pressed
PRN	<i>P. ostreatus</i>	Rapeseed straw	Non-pressed
PRC	<i>P. ostreatus</i>	Rapeseed straw	Cold-pressed
PRH	<i>P. ostreatus</i>	Rapeseed straw	Heat-pressed
PCN	<i>P. ostreatus</i>	Cotton	Non-pressed
PCC	<i>P. ostreatus</i>	Cotton	Cold-pressed
PCH	<i>P. ostreatus</i>	Cotton	Heat-pressed

^a The material types are coded using the initials of each fabrication variable, e.g. **TRN** represents **T**rametes, **R**apeseed straw, **N**on-pressed.

and attached to a 10 mm $\varnothing$ copper cup with a 2 mm piece of Scotch tape. Samples were snap-frozen with liquid nitrogen and transferred to an Oxford CT1500 Cryostation attached to a JEOL 5600LV scanning electron microscope (JEOL, Tokyo, Japan). Specimens were sublimated at -85°C to remove ice and sputter-coated with gold for 2 min before acquiring micrographs at an acceleration voltage of 5 kV.

2.5. Density measurements

Density was calculated from the weight after drying and the volume of each specimen prepared for tensile and flexural tests.

2.6. Mechanical tests

Ten specimens of each material were tested for tensile and bending behaviour. Tests were performed with a Zwick/Roell Z010 universal

testing machine (Ulm, Germany) using an elongation rate of 2 mm/min and a maximum force of 1 kN. Flexural tests were performed in a three-point bending setup with the same machine using a cross-head speed of 2 mm/min and clamp support distance of 80 mm. Data were analysed to obtain stress-strain plots, tensile and flexural strength, and the elastic and flexural modulus.

2.7. Moisture exposure

Five square specimens were tested for each material to determine water uptake at 40°C at a relative humidity (RH) of 60% and 80%. After drying until constant mass (m_0) and measuring thickness at three different points, specimens were placed on a grid in a moisture chamber (Espec SH-660, Michigan, US) exposing both sides of the material to the selected temperature and moisture conditions. Weight was measured every 10 min within the first hour of testing and after 2, 4, 24, 48, 96 and 192 h. Weight increase was plotted against the square root of time. Thickness of specimens was measured at the start of the experiment and after 4 and 192 h to record expansion of volume.

2.8. Water absorption

Square specimens were tested in triplo to determine the water uptake when placed on top of water. Specimens were placed in containers filled with distilled water maintained at $23 \pm 1^{\circ}\text{C}$ and weight was measured after 1, 2, 4, 24, 48, 96, and 192 h. For each measurement, samples were removed from the water surface, manually removing the superficial water with filter paper, and weighed within 1 min after removal from the water.

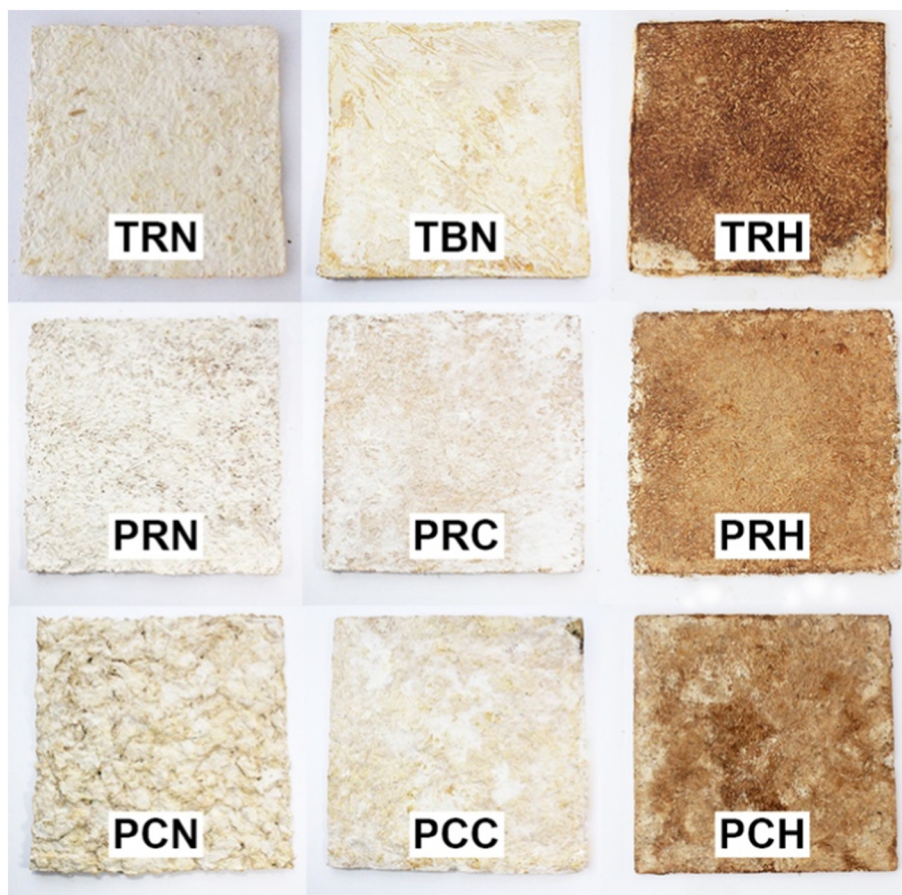


Fig. 1. Materials resulting from growth of *T. multicolor* on sawdust (TBN) and straw with (TRH) or without (TRN) heat pressing and growth of *P. ostreatus* on cotton with heat pressing (PCH), cold pressing (PCC) and without pressing (PCN) and on straw with heat pressing (PRH), cold pressing (PRC) and without pressing (PRN).

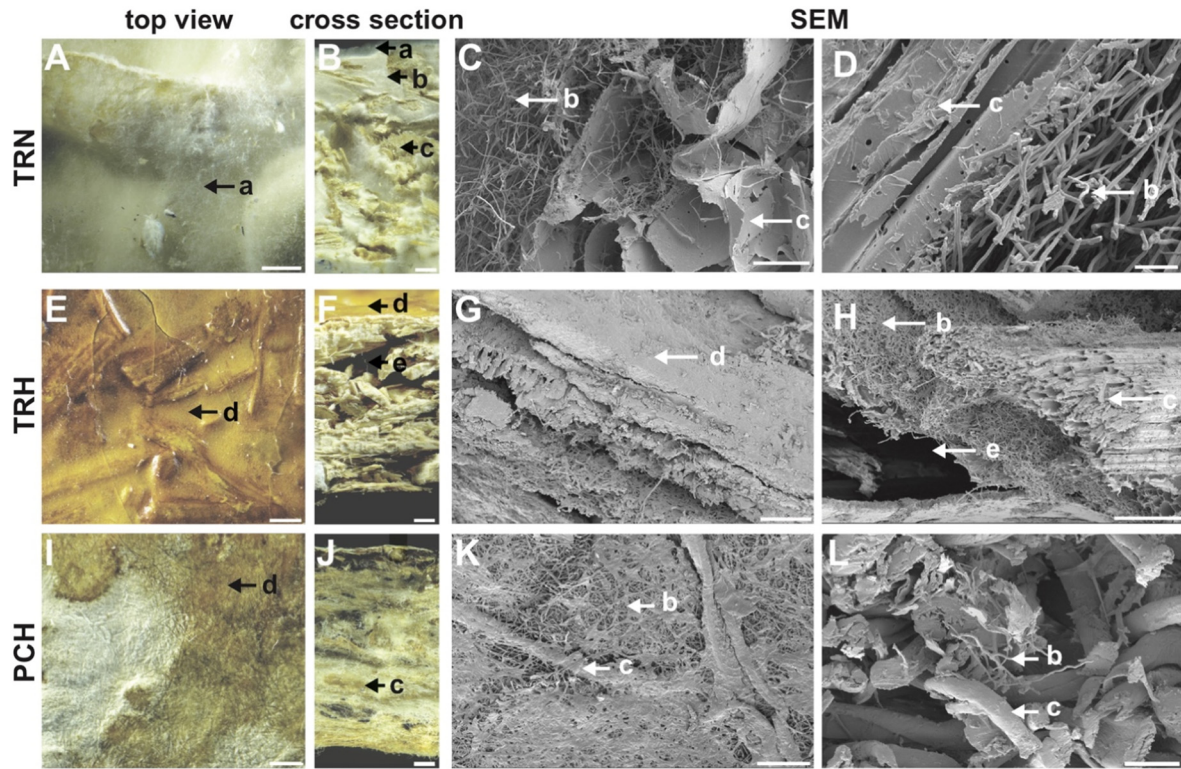


Fig. 2. Stereomicroscopy and cryo-SEM images of TRN (A–D), TRH (E–H) and PCH (I–L). Arrows indicate aerial hyphae (a), mycelium (b), substrate (c), fused hyphae (d) and air-voids (e). Scale bars represent 1 mm (A, B, E, F, I, J), 100 μ m (H), 50 μ m (C, G, K), 20 μ m (L) and 10 μ m (D).

2.9. Thermogravimetric analysis

Thermogravimetric analysis was performed with a TGA Q50 (TA Instruments, New Castle, DE, USA). Measurements were performed with biological duplicates of 25 mg of mycelium in a platinum pan using an air flow of 100 mL/min. Temperature increased from 20 to 600 $^{\circ}$ C with a rate of 10 $^{\circ}$ C/min.

2.10. Statistical analysis

Statistical analysis was performed with the software package IBM SPSS statistics 22.0 (IBM Corporation, Armonk, New York). Welch's unequal variances *t*-tests were performed followed by a Games-Howell post hoc test ($p \leq 0.05$) for tensile and flexural strength experiments and for density and water absorption experiments. Final weight increase of a given material after exposure to 60% and 80% RH was analysed using independent sample *t*-tests ($p \leq 0.05$).

3. Results and discussion

3.1. Morphological analysis

The nine materials that were produced (Table 1) presented different visual characteristics (Fig. 1). The non-pressed (TRN, TBN, PCN, PRN) and cold-pressed (PCC, PRC) materials showed the same colour and texture. However, the non-pressed TRN, PCN, and PRN materials looked soft and foamy and appeared flexible, while the non-pressed material TBN and the cold-pressed materials PCC and PRC appeared tougher at visual inspection. Thus, sawdust resulted in a more dense appearance when compared to straw and cotton fibres in the case of non-pressed materials. *T. multicolor* grown on rapeseed straw (TRN) formed a soft, velvety skin at the substrate surface and had an elastic, foam-like appearance. The equivalent material of *P. ostreatus* (PRN) showed a rough skin and more rigid appearance. Light microscopy of cross sections of TRN showed that fungal colonization was more dense close to air-exposed sides of

Table 2

Overview of properties ($\pm$ SEM) of mycelium-based composites. Letters indicate statistically significant differences with the materials with the same lettering (Games-Howell, $p \leq 0.05$, $n \geq 4$).

Material	Density (g/cm ³)	Thickness (mm)	Tensile strength (MPa)	Elastic modulus (MPa)	Elongation at break (%)	Flexural strength (MPa)	Flexural modulus (MPa)
TRN (a)	0.10 $\pm$ 0.01 ^{b,c,e-i}	13.9 $\pm$ 0.4 ^{g,h}	0.04 $\pm$ 0.01 ^{d,g-i}	4 $\pm$ 0.4 ^{a-i}	4.7 $\pm$ 0.9 ^{b,e-i}	0.22 $\pm$ 0.07 ^g	3 $\pm$ 1.6 ^{f-i}
TBN (b)	0.17 $\pm$ 0.01 ^{a-i}	15.2 $\pm$ 0.3 ^{g-i}	0.05 $\pm$ 0.01 ^{d,f-i}	13 $\pm$ 0.5 ^{a-c,g,i}	1.5 $\pm$ 0.3 ^a	0.29 $\pm$ 0.02 ^{c,d,g}	9 $\pm$ 1.4 ^{c,d,f-i}
PCN* (c)	0.13 $\pm$ 0.01 ^{a,b,e-i}	11.5 $\pm$ 0.3 ^h	n/a*	n/a*	n/a*	0.05 $\pm$ 0.01 ^{b,e-i}	1 $\pm$ 0.2 ^{b,f-i}
PRN (d)	0.13 $\pm$ 0.01 ^{b,e-i}	11.2 $\pm$ 0.1 ^{g,h}	0.01 $\pm$ 0.00 ^{a-i}	2 $\pm$ 0.3 ^{a-i}	2.8 $\pm$ 0.4 ^{f,g,i}	0.06 $\pm$ 0.01 ^{b,e-i}	1 $\pm$ 0.4 ^{b,f-i}
PCC (e)	0.24 $\pm$ 0.01 ^{a-d,g-i}	11.7 $\pm$ 0.1 ^{g-i}	0.03 $\pm$ 0.00 ^{d,g-i}	6 $\pm$ 0.3 ^{a-d,g-i}	1.4 $\pm$ 0.2 ^{a,i}	0.24 $\pm$ 0.03 ^{c,d,g}	12 $\pm$ 3.3 ^{g,i}
PRC (f)	0.24 $\pm$ 0.01 ^{a-d,g-i}	11.6 $\pm$ 0.1 ^{g-i}	0.03 $\pm$ 0.00 ^{b,d,g-i}	9 $\pm$ 1.2 ^{a,d,g-i}	0.8 $\pm$ 0.1 ^{a,d}	0.21 $\pm$ 0.01 ^{c,d,g}	15 $\pm$ 1.1 ^{a-d,g,i}
TRH (g)	0.35 $\pm$ 0.01 ^{a-f}	8.8 $\pm$ 0.1 ^{a,b,d-h}	0.15 $\pm$ 0.01 ^{a-f}	59 $\pm$ 6.8 ^{a-f}	0.9 $\pm$ 0.1 ^{a,d}	0.86 $\pm$ 0.06 ^{a-f}	80 $\pm$ 7.9 ^{a-h}
PCH (h)	0.35 $\pm$ 0.02 ^{a-f}	8.0 $\pm$ 0.0 ^{a-i}	0.13 $\pm$ 0.02 ^{a-f}	35 $\pm$ 6.5 ^{a,c-f,i}	1.6 $\pm$ 0.3 ^a	0.62 $\pm$ 0.11 ^{c,d}	34 $\pm$ 5.5 ^{a-d,g}
PRH (i)	0.39 $\pm$ 0.01 ^{a-f}	9.5 $\pm$ 0.0 ^{b,e,f,h}	0.24 $\pm$ 0.03 ^{a-f}	97 $\pm$ 9.0 ^{a-f,h}	0.7 $\pm$ 0.1 ^{a,d,e}	0.87 $\pm$ 0.14 ^d	72 $\pm$ 6.6 ^{a-f}

* Properties of material PCN could not be determined, as specimens failed at clamping.

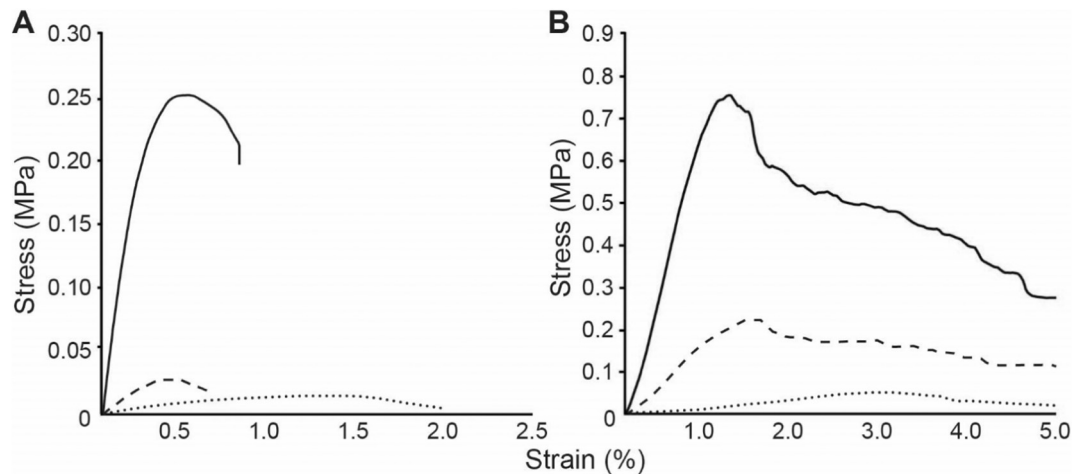


Fig. 3. Tensile (A) and bending (B) tests of *P. ostreatus* grown on rapeseed straw without pressing (dotted line), and cold (striped line) or hot (solid line) pressing.

the material when compared to the material centre (Fig. 2B). Hyphae had fully colonized the space in between the straw particles at the outer part of the material but they had not extensively penetrated the organic material as shown by cryo-SEM (Fig. 2C, D).

Heat-pressed *T. multicolor* and *P. ostreatus* materials (TRH, PCH and PRH) resembled natural composites like fibreboard by sight (Fig. 1). These samples showed chromatic variation, ranging from white to brown. The browning of the material is most likely caused by Maillard reactions involving sugars and proteins present in the fungal cell walls and the plant material. Alternatively, it may be caused by caramelization of plant and fungal sugars or by pyrolysis of organic material [19]. Light microscopy showed that browning of TRH had mainly occurred in the fungal skin (Fig. 2F), possibly explained by the low water content of this part of the material [19].

In all cases, individual hyphae within the mycelium were less clearly visible after heat pressing (2C, D, G, H, K, L), probably due to gluing of hyphae and substrate together. Heat treated materials appeared also more dense when compared to the non-pressed materials but still hyphae were less numerous in the material centre. The reduced presence of hyphae in the centre of the materials, even after heat pressing, is expected to impact the strength of the material. Hyphal abundance in the centre of the materials may be improved by forced air flow through the substrate during colonization, thus increasing oxygen levels in the material centre. Alternatively, the time of colonization may be increased.

3.2. Density

Density of the mycelium composite materials ranged from 0.10 to 0.39 g/cm³ (Table 2). Non-pressed materials (TRN, TBN, PCN, PRN) had a density of 0.10 to 0.17 g/cm³, similar to those (0.06 to 0.22 g/cm³) of other non-pressed mycelium composites [7]. The sawdust-based material (TBN) had the highest density among the non-pressed samples (Table 2). Cold pressing (PCC, PRC) increased density 2-fold, while heat pressing (TRH, PCH, PRH) resulted in a >3-fold density increase. Moreover, heat pressing resulted in a lower density variation across specimens with a standard error of 5–7.5% contrasting 10–18% in the case of non-pressed and cold-pressed materials (Table 2). Heat-pressing also resulted in more even thickness within the same specimen. For instance, variation decreased from 4.5% (PCN) to 2.3% (PCC) to 0.5% (PCH). Together, mycelium-based materials are lighter when compared to other wood composites such as medium-density fiberboard (0.50–1.00 g/cm³) and oriented strand board (OSB) wood composite (0.55–0.70 g/cm³) [20].

3.3. Mechanical properties

The nine mycelium composites showed different behaviour when subjected to tensile stress, particularly depending on the pressing treatment but not on substrate or fungus used (Table 2). Heat-pressed samples had significant higher tensile strength and elastic modulus when compared to their corresponding cold-pressed and non-pressed samples, while also cold-pressed *Pleurotus* rapeseed (PRC) samples had higher tensile strength and elastic modulus compared to non-pressed *Pleurotus* rapeseed material (PRN). Heat-pressed *P. ostreatus* rapeseed straw material (PRH) was stiff and strong but brittle, as shown by its steep curve, high tensile strength (0.24 MPa) and relatively low rupture strain (0.7%) (Table 2; Fig. 3A). Cold pressing of the same substrate-fungus combination (PRC) resulted in a material with much lower stiffness and tensile stress, but similar rupture strain. The non-pressed sample (PRN) on the other hand had a very low stiffness (2 MPa) and tensile strength (0.01 MPa) but took $\pm 4.7\%$ strain before failing completely (Table 2; Fig. 3A). Elongation at break decreased to a similar extent after pressing *P. ostreatus* material with or without heat, irrespective of the substrate. However, cold- and heat-pressed *P. ostreatus* materials

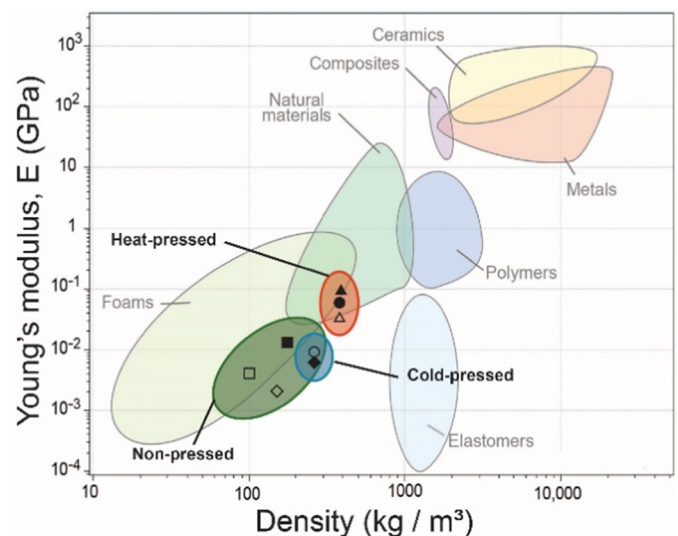


Fig. 4. Material family chart of the Young's modulus (GPa) vs density (kg/m³). Non-, cold-, and heat-pressed materials form clusters within foam- (TRN □, TBN ■, PRN ◇, PCC ◆, PRC ○), and natural-like materials (TRH ●, PCH △, PRH ▲). Source: Figure adapted from [21].

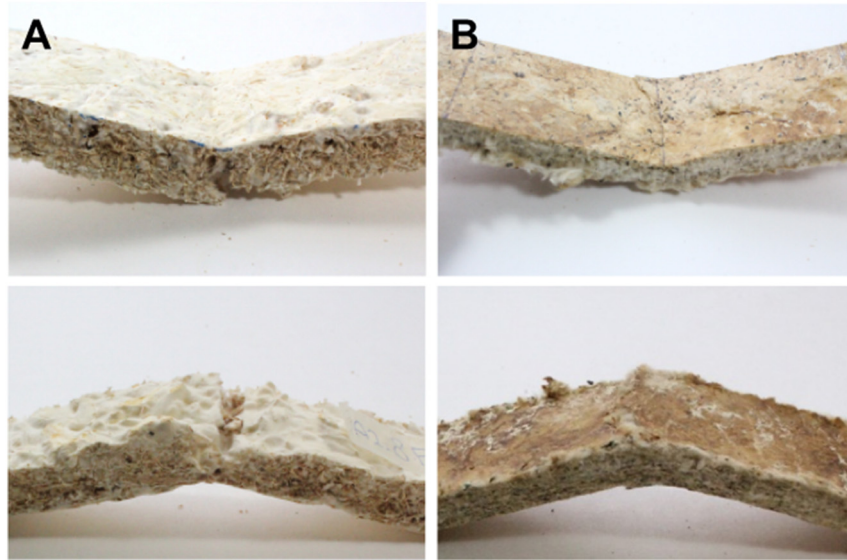


Fig. 5. Non-pressed *T. multicolor*-straw material (TRN) (A) and heat-pressed *P. ostreatus*-cotton material (PCH) (B) under flexural stress leaves the top side intact while the bottom side is broken.

grown on cotton (PCC, PCH) showed a higher elongation at break than straw-based materials (PRC, PRH). Together, the density and the Young's modulus of mycelium materials are similar to those of natural materials and foams (Fig. 4).

Similar trends in stiffness and maximum stress were seen during three-point bending. The flexural strength increased from non-pressed to cold-pressed and hot-pressed. The non-pressed materials presented flexural strengths varying from 0.05 to 0.29 MPa (Table 2) and flexural moduli from 1 to 9 MPa (Table 2). The ultimate rupture strain upon bending was larger for all three processing conditions when compared to those obtained with the tensile measurements (Fig. 3). This is explained by the fact that the fungal skin of the materials was more resistant to bending when compared to the substrate part (Fig. 5). Together, our data extend the range in flexural strengths and moduli that can be obtained with non-pressed fungal materials. Non-pressed *Ganoderma*-cotton plant biomass materials were reported to have bending strengths in the range of 7–26 kPa [7], while flexural moduli of 66–72 MPa were found in the case of cotton or hemp based mycelium materials [16].

3.4. Moisture exposure and water immersion

Weight and thickness of mycelial materials was measured before and after exposure to 60% and 80% RH. Statistical analysis showed no general increase in weight except for PRC and PRH placed at 80% RH. However, a trend was observed with all mycelium materials gaining most weight within the first 2 h of moisture exposure and reaching

saturation (M_{∞}) in approximately 12 h. Saturation time and overall weight increase of all mycelium materials was higher in samples exposed to 80% RH compared to 60% RH. They showed a 7.57–11.63% and a 3.15–8.22% final weight increase at 40 °C, respectively. Overall, cotton-based materials showed a lower final weight increase (3.15–5.80% [60% RH] and 7.57–8.12% [80% RH]) compared to rapeseed straw based materials (3.87–8.22% [60% RH] and 10.00–10.96% [80% RH]) (Table 3).

TRH and PRH materials showed an increase in thickness at 80% RH but not 60% RH (Table 3). Conversely, thickness of PRN, PCC and PRC had increased at 60% RH but not at 80% RH. The latter may be due to collapse of material due to higher water content. The expansion analysis is particularly relevant for the choice of mycelium-based composites for applications where the materials should be sandwiched between others, as for example is the case with insulation materials in the building industry.

Placing materials on top of water resulted in increased weight for all mycelial materials. TBN showed the lowest water uptake when the mycelium materials were placed on water (Table 3). This is likely explained by the water repellent fungal skin of *T. versicolor* formed under this condition. In contrast, TRN and PCN showed the highest water uptake. Thus, there was no relation between water absorption and the type of fungus, substrate, or pressing condition used. Other *Pleurotus*-based mycelium composites grown on grain fibres were reported to absorb up to 278% water over a maximum of 24 h [22], while cotton-based mycelium composites from an undocumented fungus absorbed 198% water after 168 h immersion.

Table 3

Weight increase and thickness expansion at saturation of mycelium-based composites when exposed to RH 60% and 80% at 40 °C and after placing the material on water for 192 h. Asterisks indicate statistical significant differences (t -test, $p \leq 0.05$, $n \geq 3$) and letters indicate statistical significant differences with corresponding materials (Games-Howell, $p \leq 0.05$, $n = 3$).

Material	Weight increase 60% RH (%)	Weight increase 80% RH (%)	Thickness expansion 60% RH (%)	Thickness expansion 80% RH (%)	Weight increase after placing material on water (%)
TRN (a)	8.22 ± 0.25	10.44 ± 0.17	0.29 ± 1.19	5.43 ± 1.38	436 ± 73
TBN (b)	5.71 ± 0.12	11.63 ± 0.10	6.72 ± 2.81	−0.47 ± 1.21	43 ± 5 ^{d,i}
PCN (c)	3.15 ± 0.14	7.57 ± 0.14	18.74 ± 4.16	0.57 ± 2.62	508 ± 76
PRN (d)	3.87 ± 0.32	10.26 ± 0.31	24.24 ± 4.61*	−5.13 ± 1.64	279 ± 2 ^{b,e,i}
PCC (e)	3.74 ± 0.18	7.84 ± 0.20	22.12 ± 1.07*	−2.96 ± 1.08	238 ± 1 ^{b,d,h}
PRC (f)	4.94 ± 0.30	10.00 ± 0.10*	21.06 ± 1.68*	0.96 ± 0.26	262 ± 8 ^b
TRH (g)	7.26 ± 0.09	10.96 ± 0.20	0.93 ± 0.41	1.98 ± 0.78*	246 ± 8 ^b
PCH (h)	5.80 ± 0.11	8.12 ± 0.30	−1.93 ± 2.12	−0.14 ± 1.64	281 ± 5 ^{b,e,i}
PRH (i)	7.09 ± 0.27	10.92 ± 0.26*	0.97 ± 0.57	2.5 ± 0.56*	239 ± 3 ^{b,d,h}

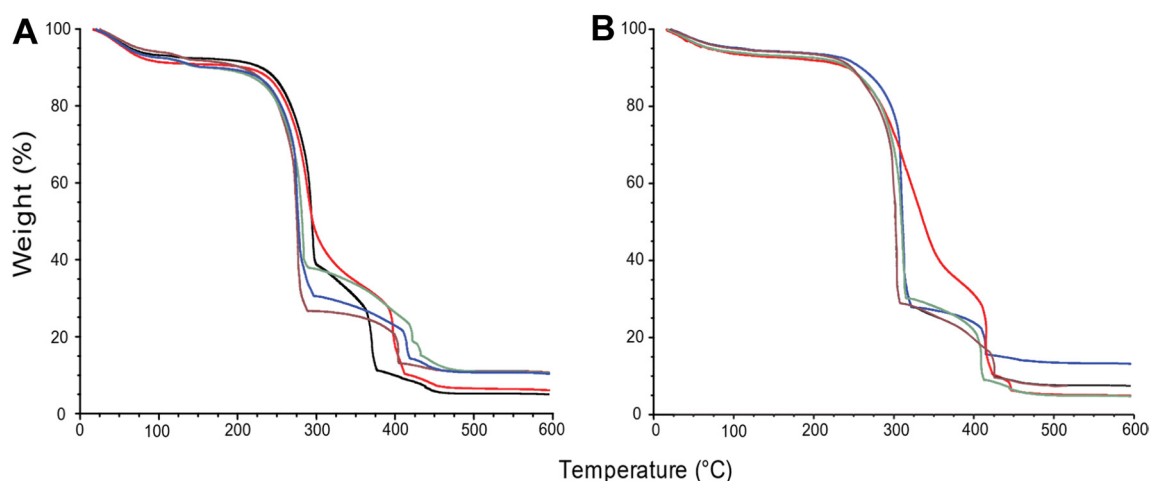


Fig. 6. TGA analysis of mycelial materials with rapeseed straw as substrate (A) or cotton as substrate (B). Decrease in weight % was measured while increasing temperature. The line in blue, green, brown and black represent PRH, TRH, TRN and PRC, respectively (A), while the lines in blue, green, and brown represent PCN, PCH, and PCC, respectively (B). Red lines represent the uncolonized substrates. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.5. Thermogravimetric analysis of mycelial materials

Thermogravimetric analysis showed similar degradation patterns for mycelial materials grown on rapeseed straw, cotton or beech sawdust as substrate. Water content was higher for samples grown on rapeseed straw (7.6–9.6%) than on cotton (5.8–7.2%) as shown by the weight loss at 100 °C (Fig. 6). Initial degradation temperature was ± 225 °C for rapeseed straw and ± 242 °C for cotton materials. Pressing or the type of fungus did not impact the degradation profiles. Yet, uncolonized substrates had a slower decrease in weight when compared to the colonized substrates. Possibly, fungal colonization makes the substrate more accessible for thermal degradation.

4. Conclusions

Visual appearance, density, mechanical properties and water-absorbing behaviour was assessed of a range of mycelium based composites that were obtained by varying the type of fungus, substrate and pressing conditions. *P. ostreatus* and *T. versicolor* colonized the substrate but also formed a fungal skin at the substrate–air interface. The skin of *T. multicolor* was thicker than that of *P. ostreatus*. In general, the non-pressed and cold-pressed materials had a whitish, velvety appearance with a foam like structure. In contrast, the heat-pressed materials were compact and had a brown appearance. The latter is probably due to Maillard reactions, caramelization, and/or pyrolysis of organic material [19]. The mycelium materials had a density of 0.10 to 0.39 g/cm being lighter than for instance medium-density fibreboard (0.50–1.00 g/cm) and oriented strand board (OSB) (0.55–0.70 g/cm) wood composites [20]. Saw dust resulted in a higher density than cotton fibres or straw as a substrate, while cold- and heat-pressing increased density 2- and >3-fold, respectively. Moreover, heat pressing reduced density and thickness variation between and within the samples.

Pressing, but not the type of substrate or fungus, impacted the tensile strength and elasticity modulus of the mycelium materials. Tensile strength and elasticity modulus of heat-pressed materials were higher when compared to the corresponding cold-pressed and non-pressed materials. A similar trend was observed during three-point bending. The flexural strength increased from non-pressed to cold-pressed and hot-pressed. The flexural strain needed to break the samples was higher than those needed during the tensile measurements. This is explained by the fact that the fungal skin is more elastic than the colonized substrate and therefore breaks at higher strain when compared to the substrate part of the material.

There was no overall relation between water absorption and the type of fungus, substrate, or pressing condition used to produce the material. TRH and PRH materials showed an increase in thickness at 80% RH but not 60% RH, while thickness of PRN, PCC and PRC had increased at 60% RH but not 80% RH. The latter may be due to collapse of material due to higher water content. Saturation time of mycelium materials as well as their overall weight increase was higher in samples exposed to 80% RH. They showed a 3.15–8.22% and a 7.57–11.63% final weight increase at 40 °C when exposed to 60% and 80% RH, respectively.

Thermogravimetric analysis showed similar degradation patterns for mycelial materials grown on rapeseed straw, cotton or beech sawdust as substrate. Moreover, pressing or the type of fungus did not impact the degradation profiles. Yet, uncolonized substrates showed slower decrease in weight when compared to colonized substrates. Possibly, fungal colonization makes the substrate more accessible for thermal degradation. In addition we found that the water content of samples grown on rapeseed straw (7.6–9.6%) was higher than on cotton (5.8–7.2%) and that the initial degradation temperature of rapeseed straw based materials was lower (± 225 °C) than that of cotton based materials (± 242 °C).

Based on density and elastic modulus, mycelium-based composites produced in this study qualify as foam-like and natural materials [21] (Fig. 4). Heat pressing shifted mycelium composites from foam-like performance to cork and wood-like performance. Further improvement of the mycelium materials may be obtained by promoting colonization in the central part of the substrate. It was shown that colonization by *P. ostreatus* and *T. versicolor* was much higher at the outer parts of the substrates. Improved colonization in the central part of the materials may be accomplished by forcing air through the feedstock during colonization, thus increasing oxygen levels in the material centre. Alternatively, the time of colonization may be increased.

CRediT authorship contribution statement

Freek V.W. Appels: Methodology, Data curation, Formal analysis, Visualization, Writing - review & editing. **Serena Camere:** Methodology, Data curation, Formal analysis, Visualization, Writing - review & editing. **Maurizio Montalti:** Conceptualization, Methodology. **Elvin Karana:** Supervision. **Kaspar M.B. Jansen:** Supervision. **Jan Dijksterhuis:** Methodology, Visualization, Formal analysis. **Pauline Krijgsheld:** Methodology, Writing - original draft. **Han A.B. Wösten:** Supervision, Writing - review & editing.

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Data availability

The raw/processed data required to reproduce these findings cannot be shared at this time as the data also forms part of an ongoing study. Data will be presumably available for sharing before submission.

Declaration of interest

SC and MM are employees of the company Mogu. Utrecht University has a collaboration agreement with Mogu for development of mycelium materials. The funding body had no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

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