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# Fungal microbiomes associated with green and non-green building materials

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ABSTRACT

Water-damaged buildings can lead to fungal growth and occupant health problems. Green building materials, derived from renewable sources, are increasingly utilized in construction and renovations. However, the question as to what fungi will grow on these green compared to non-green materials, after they get wet, has not been adequately studied. By determining what fungi grow on each type of material, the potential health risks can be more adequately assessed. In this study, we inoculated green and non-green pieces of ceiling tile, composite board, drywall, and flooring with indoor dust containing a complex mixture of naturally occurring fungi. The materials were saturated with water and incubated for two months in a controlled environment. The resulting fungal microbiomes were evaluated using ITS amplicon sequencing. Overall, the richness and diversity of the mycobiomes on each pair of green and non-green pieces were not significantly different. However, different genera dominated on each type of material. For example, *Aspergillus* spp. had the highest relative abundance on green and non-green ceiling tiles and green composite boards, but *Peniophora* spp. dominated the non-green composite board. In contrast, *Penicillium* spp. dominated green and non-green flooring samples. Green gypsum board was dominated by *Phialophora* spp. and *Stachybotrys* spp., but non-green gypsum board by *Myrothecium* spp. These data suggest that water-damaged green and non-green building materials can result in mycobiomes that are dominated by fungal genera whose member species pose different potentials for health risks.



## 1. Introduction

Green building materials are derived from recycled or renewable sources (US EPA, 2017). For example, gypsum board, composite board or ceiling tiles can be made of recycled materials. Bamboo flooring is an example of a readily renewable product compared to flooring composed of virgin wood. The building industry and homeowners are utilizing more green-building materials, which should make the built environment more sustainable (Steinemann et al., 2017). However, it is also important to determine if green products are susceptible to more or different fungal growth compared to the products they are replacing (Thatcher and Milner, 2016).

Fungal growth on moisture-damaged building materials can lead to health effects including asthma and other respiratory problems (WHO, 2009). The growth of fungi on green and non-green product pairs has been compared in three earlier studies (Hoang et al., 2010; Huang et al., 2015; Mensah-Attipoe et al., 2015). Hoang et al. (2010) inoculated green and non-green product pairs with either *Aspergillus niger* spores or by allowing the materials to be "naturally" inoculated by placing sterile pieces of each in a home and allowing airborne fungal cells to settle on each piece before testing began. The fungal growth was visually assessed by measuring the area of the surface contaminated by fungal growth. Under either inoculation method, fungal growth was comparable on green and non-green products. Huang et al. (2015) inoculated green and non-green building materials with *Aspergillus brasiliensis*

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and *Penicillium funiculosum* and found no differences in visually assessed fungal growth on each pair of materials. Mensah-Attipoe et al. (2015) used cultivation and an enzyme bioassay to compare fungal growth on green and non-green product pairs inoculated with three fungi: *Aspergillus versicolor*, *Cladosporium cladosporioides* or *Penicillium brevicompactum*. No significant differences in the growth of these three fungi on green and non-green building materials were found. However, there are about 1.5 million fungal species (Hawksworth, 2001) and testing each mold separately in such studies is not practical. Therefore, in our tests, we inoculated green and non-green materials with a complex mixture of fungi naturally occurring in indoor dust.

Cultivation has previously been used to assess fungal contamination in field samples (Hyvärinen et al., 2002). This method, however, will detect only fungi that are able to grow on the culture media used. The populations of fungi, or mycobiome, can be studied by using ITS amplicon sequencing (Schoch et al., 2012). Hoisington et al. (2014) used this technology to evaluate the complex mycobiome of a retail store. Using this technology, the identification of fungi in the indoor environment has recently provided new insights into the health effects of previously overlooked fungi, such as the fungal species placed in the genus *Cryptococcus* (Dannemiller et al., 2014). Next-generation sequencing method have also been used to analyze bacterial biomass in building materials (Adamiak et al., 2017; Laiz et al., 2011). The objective of this study was to evaluate the similarities and differences in the mycobiome developed on green compared to non-green building products.

## 2. Materials and methods

### 2.1. Selection of building materials

Based on consultation with a Leadership in Energy and Environmental Design (LEED) specialist at the U.S. Green Building Council (http://www.usgbc.org), four different types of most commonly used green and non-green building materials were chosen for this study. The green building materials included bamboo flooring (GreenFloors, Fairfax, VA, USA), wheat mineral board (Kirei, Solana Beach, CA, USA), Sheetrock gypsum board (CGC Corporation, Mississauga, Ontario, Canada) and Armstrong Acoustical ceiling tiles (Armstrong World Industries, Hilliard, OH, USA). The main components of the two first building materials are organic material (bamboo and wheat stalks), whereas sheetrock gypsum board contains up to 95% of pre-consumer recycled content and acoustical ceiling tiles which contain up to 82% recycled content which include both pre- and post-consumer waste, as well as materials including recycled newspaper, mineral wool, perlite, jute and cornstarch. The respective non-green building materials included pine hardwood flooring (BLC Hardwood Flooring, Macon, GA, USA), oriented strand particle board (LP Building Products, Binghamton, NY, USA), conventional gypsum board (Continental Building Products, Herndon, VA, USA) and conventional ceiling tile (SpectraTile, Middlebury, IN, USA).

### 2.2. Collection and preparation of inoculating dust

Indoor dust containing a complex mixture of naturally occurring fungi was used to inoculate the tested building materials. The dust was collected from five indoor locations by vacuuming floors (Filter Queen Majestic™; HMI Industries Inc., Seven Hills, OH) as previously described (Cho et al., 2006). The collected dust was pooled together and sifted using a 355-μm sieve to ensure homogeneity and exclude large particles. The resulting dust pool was then stored at −20 °C before inoculating the building materials.

### 2.3. Preparation, inoculation, and incubation of building materials

Each building material was cut into three, identical 25 $cm^2$ pieces and then gamma irradiated with a minimum dose of 25 kGray to reduce any biological contamination. Each piece was then placed in 20 mL of deionized and autoclaved water to establish a high water activity and for the ease of spreading the dust suspension. The sieved dust was suspended in 0.05% Tween 80 solution to obtain a dust concentration of 50 mg/mL and 0.5 mL of this suspension was inoculated on each building material to provide a final dust load of 1 mg/$cm^2$.

The inoculated building materials were then placed in eight different, 5.3-liter plastic containers (1 container for each of the 8 building material types) to avoid cross-contamination between building materials as previously described (Seo et al., 2008). The containers were purchased from a local hardware store and disinfected by rinsing with 70% ethanol. The containers were aerated with filter-sterilized air (pore size, 0.2 μm; GE Osmonics Inc., MN) once a day for 10 min at a flow rate of 0.53 L/min (Murtoniemi et al., 2003). Inoculated building material samples were incubated at room temperature (23 ± 1 °C) and a relative humidity of 98% (±1%) to simulate flooding situations, for two months. Two months at high humidity (95%) has been shown to be sufficiently long time for mold growth on several types of building materials (Johansson et al., 2012). The humidity was achieved by placing a saturated $K_2SO_4$ solution (150 g/liter) at the bottom of each container (Korpi et al., 1998). The temperature and humidity in each container were monitored daily using a humidity-temperature pen (Fisher Scientific Company, Pittsburgh, PA).

### 2.4. Sample preparation for genomic DNA extraction

After the 2-month incubation period, 10 mm diameter autoclaved cork-borers (Fisher Scientific) were used to scoop out approximately five to six circular pieces (thickness ~ 3 mm) of each building material. The weight of each circular piece varied from 1.5 g to 4.6 g, depending on the building material. The bores were then pooled together and placed in a sterile mortar and ground with liquid nitrogen for approximately 2 min or until a fine consistency was obtained as previously described (Ettenauer et al., 2012). The ground material was then transferred to 50 mL falcon tubes and homogenized by manually shaking the powder-like samples by hand. If not used for genomic DNA (gDNA) extraction immediately, the samples were stored in −20 °C.

Genomic DNA was extracted from each building material sample (50 mg) using the MOBIO PowerLyzer® PowerSoil® DNA isolation kit following the manufacturer's instructions (Carlsbad, California). An extract of DNA from each sample was sent to the Research and Testing Laboratory (Lubbock, Texas) for Illumina MiSeq sequencing.

### 2.5. Illumina MiSeq analysis

Research and Testing Laboratory performed the Illumina MiSeq sequencing. Forward and reverse fusion primers were used to amplify the ITS1 regions from the DNA sample. The forward primer included the (5′-3′) Illumina i5 adapter (AATGATACGGCGACCACCGAGATCTACAC), an 8-10bp barcode, a primer pad, and the ITS1F primer (CTTGGTCATTTAGAGGAAGTAA). The reverse fusion primer included the (5′-3′) Illumina i7 adapter (CAAGCAGAAGACGGCATACGAGAT), an 8-10bp barcode, a primer pad, and the unlabeled ITS2 primer (GCTGCGTTCTTCATCGATGC). The amplification was performed and visualized as previously described (Kozich et al., 2013; MacIntyre et al., 2015).

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