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Differing effects of four building materials on viable bacterial communities and VOCs

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ABSTRACT

Indoor environmental quality is a paramount concern among architects. Exposure to VOCs and microorganisms impacts occupant health, yet the role of materials on these exposures remains poorly understood. In this study, we placed four material types in individual microcosms to test whether material type influences bacterial community structure and VOC emission. We used culture-independent methods to characterize bacterial communities and TD-GC-MS to measure VOC emission. We found that viable bacterial communities had different patterns of abundance, diversity, and composition, in comparison with total (viable plus dead cells) bacterial communities. Examining viable bacteria only, Earth had the highest abundance and diversity, unique community composition, and overall negative VOC emission. Timber had the lowest bacterial abundance, composition similar to Gypsum and Concrete, and the highest VOC emission rate. Our research provides further evidence that architects' decisions about building materials can influence chemical and microbial exposures indoors.

1. Introduction

Architects strive to design buildings that protect and promote occupant health, while also achieving other sustainability targets. Several standards, such as Leadership in Energy and Environmental Design (LEED), WELL Building Standard, Living Building Challenge (LBC), and Fitwel provide guidelines for architectural factors with known impacts to human health, such as indoor environmental quality (IEQ) (Allen et al., 2015). IEQ encompasses the provision of adequate daylight, proper ventilation rates, and limits to chemical or biological contaminant exposure (Heidari et al., 2016), which are of particular interest. For instance, the International Living Future Institute has created a "Red List" of chemicals and materials that should be avoided to achieve LBC status; antimicrobials, formaldehyde, and volatile organic compounds (VOCs) are just a few of the compounds included in the list.

Despite the generally negative perception of VOCs, recent studies have suggested that positive health effects may arise from exposure to biogenic VOCs associated with natural elements, such as soil and timber (Azuma et al., 2016; Matsubara and Kawai, 2014). A substantive body of literature attests to reductions in stress, cortisol levels, and heart rate, as well as increases in activity of natural killer cells associated with inhalation of plant-produced terpenes, a class of VOCs (Antonelli et al., 2019; Matsubara and Kawai, 2014; Ikei et al., 2016; Cho et al., 2017). In addition, several studies have examined potential health effects of short-term exposure to high levels (up to 18 mg/m^3) of VOCs emitted from pine wood and found no negative health effects (Gminski et al., 2011; Nore et al., 2017), though in one of the studies participants perceived a somewhat "pleasant" woody odor that was likely a result of terpene emission. It is also important to note that terpenes are highly reactive and, while exposure to terpenes themselves may not constitute a health issue, oxidation reactions involving terpenes can generate byproducts that are irritating to the respiratory system (Nazaroff and Weschler, 2004; Wolkoff et al., 2000). On the other hand, formaldehyde emission from adhesives used in some engineered wood products, such as

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cross-laminated timber (CLT), as well as from the wood itself, may also because for concern (Stenson et al., 2019; Knowles et al., 2011; Alapieti et al., 2020). Finally, while some materials may emit VOCs, others may be able to sequester them (Niedermayer et al., 2013; Won et al., 2001).

Building materials also provide a habitat for microorganisms, particularly when moisture accumulates. Intrinsic properties of building materials, such as hygroscopicity and porosity, may promote or inhibit growth of microorganisms (Gadd, 2017). For example, Hoang et al. (2010) demonstrated that organic matter and equilibrium moisture content control different materials' susceptibility to fungal growth, which can have negative impacts to human health (Hoang et al., 2010). However, even in the absence of moist conditions, diverse bacteria, fungi, and other microorganisms are ubiquitous on and within building materials and are generally neutral with regard to human health, while some can even be beneficial (Horve et al., 2020). A number of studies have suggested that exposure to diverse microbiota during childhood is critical for proper immune system development (Ege et al., 2011; Lehtimäki et al., 2018; Stein et al., 2016; von Hertzen and Haahtela, 2006; Blum et al., 2019).

In addition to fostering occupant well-being, buildings should aim for low embodied energy and greenhouse gas emissions in response to climate change. A life cycle assessment comparing climate change impacts of high-rise mass timber versus reinforced concrete buildings estimated a 34–84% better performance of mass timber (Skullestad et al., 2016). Selection of structural and finish materials is a decision that impacts both embodied energy and greenhouse gas emissions, however, the choice is fraught with complexity and much of the necessary information is missing. Among architects, wood is generally perceived as a more environmentally sustainable and healthy material than steel or concrete (Conroy et al., 2019; Laguarda Mallo and Espinoza, 2015) and exposure to natural materials, such as wood, has been recognized to have health benefits (Fell, 2010; Kotradyova et al., 2019; Augustin and Fell, 2015; Burnard and Kutnar, 2015; Nyrud et al., 2010; Sakuragawa et al., 2005; Zhang et al., 2017).

In this study, we investigated biological and chemical emissions of four different materials ranging in sustainability values, as perceived by architects. We hypothesized that: A) when isolated from the influence of human occupants, viable microbial communities inhabiting different materials will diverge over time, due to ecological selection processes; B) each material has a unique VOC profile, which is associated with microbial community composition; and C) a subset of microbial taxa are differentially abundant on particular materials, depending on whether environmental conditions of the material surface fosters or inhibits growth and survival. The results will provide a preliminary foundation for evidence-based material selection to control occupants' exposure to microorganisms inhabiting the fabric of our built environments.

2. Methods

2.1. Study overview

We examined bacterial community succession on the surfaces of four different building material types over a 39-day period. The materials used were: painted gypsum board over light-frame stud wall (Gypsum); cross-laminated timber using Douglas Fir (*Pseudotsuga menziesii*) coated with linseed oil (Timber); earthen plaster over strawbale (Earth); and concrete block (Concrete). The gypsum board was painted with white indoor latex paint, which did not contain any microbial inhibitor ingredients. Concrete block, gypsum board, and indoor paint were purchased from Jerry's Home Improvement Center in Springfield, Oregon. The CLT was donated from Oregon State University Department of Forestry. The earthen plaster was fabricated and donated by the Maitreya EcoVillage in Eugene, Oregon. All building material blocks were approximately 20 × 40 × 25 cm in dimension. We used environmental scanning electron microscopy (ESEM) imaging to create magnified images of materials' surface microtopography (Fig. 1). Five replicates of each material type were passively inoculated with ambient microorganisms by being placed in an occupied laboratory space for a 13-week period from January 23, 2018 to April 30, 2018. Immediately following inoculation, three blocks of each material were individually placed in sealed chambers (microcosms). The remaining two blocks of each material remained outside in the larger occupied laboratory space for the duration of the experiment.

Microcosms were constructed from large plastic storage bins,

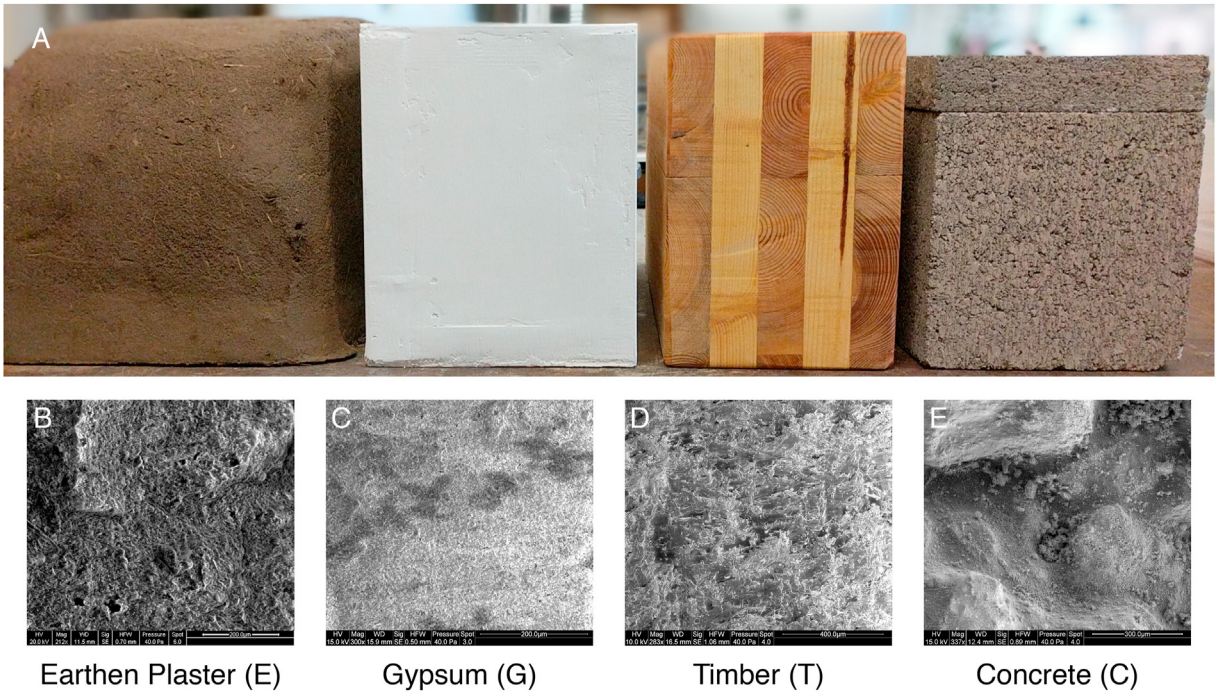

Fig. 1. One replicate of actual material blocks shown above corresponding ESEM images for: Earth at 212 × magnification, Gypsum at 300 × magnification, Timber at 283 × magnification, and Concrete at 337 × magnification.

approximately 137.6 L in volume. Two 12.7 cm diameter ports were cut into the front of each bin to attach nitrile chemistry gloves (ULINE S-19714-L) for sample collection. A 10.2 cm diameter port on the back of each microcosm introduced filtered air through a ventilation duct network. The ventilation system was calibrated to maintain positive pressure inside the microcosms to prevent contamination with external laboratory air. Ports were sealed with polyvinyl chloride (PVC) or acrylonitrile butadiene styrene (ABS) gaskets and silicon sealant to maintain integrity of the sampling area. The air inside each chamber was flushed with filtered air at an exchange rate of 44 air changes per hour (ACH). A 16 × 20 Varicel RF/C Plus SAAFOxi MERV 15 filter removed particulate matter from incoming laboratory air (Fig. 2). The filter contains a 50/50 blend of 60% activated carbon and proprietary activated alumina impregnated with potassium permanganate (KMn_4), which is purported to control gaseous contamination. Prior to placement of the study materials, microcosm boxes were cleaned with 70% ethanol, flushed with filtered air, and sealed overnight to establish a sterile environment. HOBO dataloggers (Onset UX100) were used to measure temperature (◦C), relative humidity (RH; %), and light intensity (lux; lumens/m^2) within each microcosm. Data were logged in 5-min intervals throughout the sampling period.

2.2. Bacterial sampling and analysis

Sample collection began on April 30 (Day 0) with the five material block sets in the original laboratory inoculation area, which provided a baseline bacterial community. Material blocks were sampled with nylon-flocked swabs (Copan Diagnostics) saturated with a 1 × phosphate-buffered saline (PBS) solution on undisturbed, uniformly segmented 5 cm horizontal and vertical segments. The swabbed area was sampled from right to left over the course of the experiment, ensuring that sample areas did not overlap. After baseline sampling, material block sets #1, #3 and #4 were moved into individual microcosms, while block sets #2 and #5 remained in the occupied laboratory space for the duration of the sampling period to serve as controls. Following the same protocols as baseline sample collection on Day 0, one sample was collected from each block on Days 1, 2, 4, 8, 16, 32, and 39.

2.3. Genomic material preparation

Samples were vortexed briefly to homogenize, then swab tips were removed from their tubes, leaving the 1 × PBS containing genomic material from the swabs. Each sample was divided into two equal aliquots. One aliquot was treated with propidium monoazide (PMA), a dye that infiltrates dead or damaged cells through disrupted cell membranes and binds to DNA, preventing polymerase chain reaction. The use of PMA provides the ability to quantify only viable cells in each sample, thus permitting comparisons between the amount of total versus viable (i.e., those with intact cell membranes) bacterial DNA in the samples (Fittipaldi et al., 2012). A 10 μL aliquot of 2.5 mM PMA solution was added to each sample (final concentration of 25 μM), incubated in the dark for 10 min, vortexed briefly, and incubated in blue light from a PMA-Lite LED Photolysis Device (OPE Biotechnology Co., Ltd., #PT-H18A) according to the manufacturer's protocol. Following treatment with PMA, samples were randomized within PMA-treated and untreated groups and DNA extractions were performed using DNeasy Power Lyzer PowerSoil Kit (Qiagen, #12855-100) following manufacturer's protocol.

2.4. Quantitative PCR

Absolute abundance of bacteria was measured using real-time quantitative polymerase chain reaction (qPCR) using protocols described

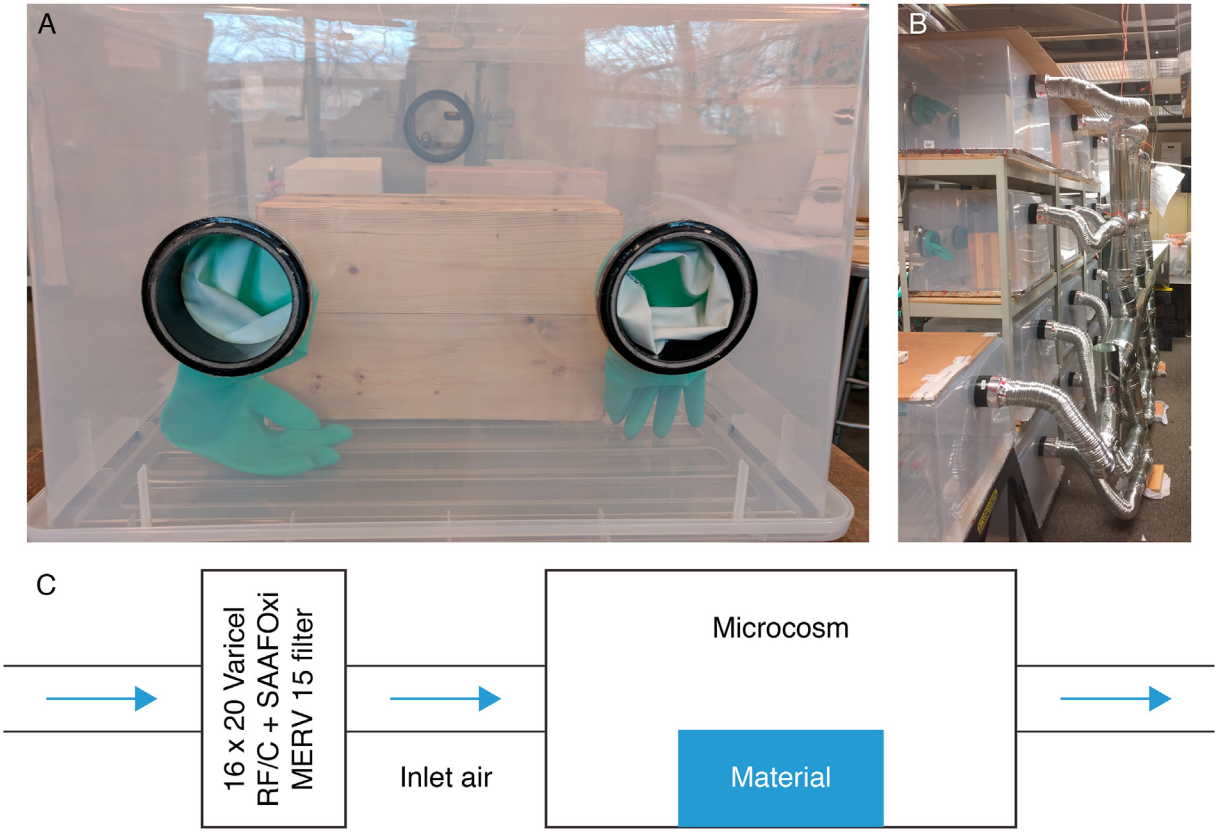

Fig. 2. (A) Individual microcosm, (B) ventilation ducting for the microcosm array, and (C) diagram of the filtration system used in the experiment.

previously (Fahimipour et al., 2018), with the exception of using 25 μL reaction volumes instead of 50 μL. Briefly, qPCR was performed using Total Bacteria F SYBR Primer 5′-GTGSTGCAYGGYTGTCGTCA-3′ and Total Bacteria R SYBR Primer 5′-ACGTCRTCCMCACCTTCCTC-3′. Each 25 μL reaction volume comprised 2.5 μL PowerUp SYBR Green PCR Mastermix (Applied Biosystems), 1 μL forward primer, 1 μL reverse primer, 8 μL PCR-grade water, and 2.5 μL DNA template diluted 1:10. An Eppendorf epMotion 5075 robot prepared the plates. We used the following thermocycling conditions: initial denaturation for 2 min at 50 °C, 2 min at 95 °C; 40 cycles of 15 s at 95 °C, 15 s at 60 °C, and 60 s at 72 °C; followed by a melt curve in the range of 60 °C to 95 °C. Standard curves were generated using serial dilutions of a synthetic 167 bp gBlocks gene fragments with known gene sequence copy numbers. All reactions were run in triplicate, including positive, negative, and no-template controls. Although exact numbers of bacterial cells cannot be quantified, due to disparity in gene copies per cell among different bacterial taxa, we assume that higher numbers of gene copies indicate higher numbers of viable cells.

2.5. 16S amplicon sequencing

Bacterial diversity and composition were characterized using PCR to amplify the V3–V4 hypervariable region of the bacterial 16S rRNA gene using 319f–806r primers (Caporaso et al., 2012). Thermocycling conditions for genomic DNA amplification were: maintain temperature at 98 °C for 5 min, followed by 35 cycles of {98 °C for 15 s, 58 °C for 30 s, and 72 °C for 30 s}, ending with 72 °C for 2 min. Resulting amplicons were quantified using Quant-iT dsDNA High Sensitivity Assay Kit on a Molecular Devices SpectraMax M5E Microplate Reader and pooled to 40 ng DNA per sample. Primer and PCR reagents were removed using Omega Bio-Tek Mag-Bind RxnPure Plus beads (Invitrogen), and the clean amplicon pool was sequenced at the University of Oregon's Genomics and Cell Characterization Core Facility. High-throughput sequencing on the Illumina MiSeq 2 × 300 PE platform was used to generate raw metabarcode data. We used the `dada2` package (Callahan et al., 2016) in R for trimming, quality filtering, and assigning taxonomy. Samples were sequenced across two separate runs; the first run comprised untreated samples and the second comprised PMA-treated samples. Since sequencing bias is known to be an issue (Song et al., 2018), we avoided direct quantitative comparisons between the two runs, treating each as a wholly separate dataset. Due to poor quality reverse reads in the first run, we used only the forward reads and trimmed to 250 base pairs, with maxEE set to 8. For the second run, we again used only forward reads, which were trimmed to 120 base pairs with maxEE set at 8. Because of the shorter trimmed read lengths, taxonomic identification of PMA-treated samples was limited to family-level resolution.

We used decontam (Davis et al., 2018) to identify and remove likely laboratory contaminants from the 170 experimental samples, based on five negative controls (one extraction control, four PCR controls). Under the default settings, a total of 1737 reads belonging to 12 ASVs were filtered out as potential contaminants (additional details in Table S2). In addition, almost all samples contained chloroplast and mitochondrial sequences, as would be expected using V3–V4 primers, which are known to co-amplify plastids (Beckers et al., 2016). In untreated samples, non-target plastids represented a low proportion of total reads (mean = 0.14). However, in our PMA-treated samples this proportion rose to dominance (mean = 0.66), with some samples comprised upwards of 90% chloroplasts. This may indicating that chloroplasts are quite robust to PMA treatment, similar to recent findings that some bacterial taxa are more resilient to PMA treatment while others are more susceptible (Wang et al., 2021). To address the issue of differing sample depths after quality filtering, including removal of chloroplast and mitochondrial sequences, we used the variance-stabilizing transformation in DESeq2 rather than rarefying, which discards a large amount of useable high-quality data (McMurdie and Holmes, 2014). We thoroughly examined alpha diversity and community composition plots and did not find low-count samples as outliers in any of the downstream analyses.

2.6. VOC sampling and analysis

VOCs were collected by glass sorbent tubes (PerkinElmer #N9307008) packed with 180 mg of Carbotrap B followed by 70 mg of Carboxen 1000 (Pankow et al., 1998) using a portable sampling pump (Universal PCXR8, SKC Inc., USA) to draw in microcosm air. The sorbent tubes were conditioned prior to sampling and sealed with stainless steel Swagelok endcaps fitted with PTFE ferrules. The sealed tubes were stored in plastic resealable bags at −6 °C prior to sample collection. Sampling and analysis of sorbent tubes occurred within a month after conditioning.

The VOC sampling was performed over three consecutive days spanning Days 37–39 of the experiment (Table S1). On the first day of VOC sampling, air from two of the three microcosm-housed replicates of each material, a control box, field blank, lab air, and inlet air were collected. The control box was an empty microcosm identical to those used for the sample material blocks, to identify VOCs emitted by the microcosms themselves. Field blanks were used to assess VOC contamination from the sampling activities (e.g., contamination from the researchers hand, transport, storage). Inlet air samples were used to determine initial concentration (C_0) of identified VOCs directly after filtration and calculate emission rate from the materials. Lab air samples represent incoming air prior to filtration, identifying compounds present in the external space, in the event that microcosms were not fully sealed, and demonstrating the effectiveness of the cleaning system. On the second day of VOC sampling, corresponding to Day 37 of the experiment, we sampled, in duplicate, the microcosms containing the final replicates for each material, which provided a true duplicate for quality assurance and quality control (QA/QC) on the sampling method, rather than variance across the materials. Again, samples were collected for the control, field blank, lab air, and inlet air. On the third day, single samples of microcosm air for two of the three replicates across all materials were collected, as on the first day of VOC sampling, but with reduced air exchange rate (AER) in each chamber to elevate chamber VOC concentrations. Control, field blank, lab air, and inlet air samples were also collected.

The sampling was performed at a flow rate of 50 mL/min for 60 min with a total sample volume of 3 L for each sample. Two pumps were used for these experiments and the flow of each pump was measured each day (average of 15 measurements for each pump) using a primary flow calibrator (Gilian Gilibrator 2). After sampling, the sorbent tubes were capped and stored in two plastic resealable bags at −4 °C until analysis.

The samples were analyzed using an Absorption/Thermal Desorption (ATD) instrument (PerkinElmer Turbo Matrix 650) connected to a gas chromatograph (model 7890 A, Agilent Technologies) with a DB-VRX column (60 m length × 0.25 mm i.d. × 1.4 μm film thickness, Agilent J&W) coupled to a mass selective detector (model 5975 C, Agilent Technologies). Each sample was desorbed at 300 °C for 10 min and all compounds were concentrated into a cold trap at −30 °C. Samples were then injected in a split/splitless injector maintained at 180 °C. The injector was in split mode with a split flow of 2.76 mL/min. Helium was used as the carrier gas at a constant flow of 0.92 mL/min. The oven temperature started at 45 °C for 10 min, then increased by 12 °C/min until reaching 190 °C, after which it was maintained isothermal for 2 min. The temperature was raised again at 6 °C/min until reaching 240 °C, kept isothermal for 5 min, and finally decreased at a rate of 10 °C/min until reaching 210 °C. The mas spectrometry (MS) conditions were: transfer line at 230 °C, ion source at 250 °C and EI voltage at 70 eV. Data were recorded in full scan mode (m/z range: 34-400 amu).

Compounds were identified on the basis of their mass spectra and the injection of standards. The mass spectra were compared with those from two database: National Institute of Standards and Technology (NIST) Mass Spectral Database 2008 (NIST08) and W8N08 library (John Wiley & Sons, Inc., USA). Quantification was achieved with five-point external calibration using a TO-15 gas mixture containing a representative mix of

VOCs (65 component) from Linde (Alpha, NJ, USA) certified to ± 5% accuracy allowing for the identification and quantification of compounds. To verify thermodesorption and analysis efficiency and to obtain relative concentrations for those compounds lacking standards, four internal standards were also injected in each sample.

For the quantification, field blanks were removed from all the samples (materials, control chamber, inlet air and lab air); inlet air was also removed from the control chamber prior to removing control chamber from the sample (materials) in order to obtain VOCs only emitted by the materials and the microorganisms inhabiting them. To calculate emission rates, we assumed steady state conditions in the microcosms, as they were in operation for over one month. For the last day at a low flow rate, the sampling was performed 15 h after changing the flow rate, when near-steady state conditions could be assumed based on chamber dynamics considering the lower air exchange rate and an assumed constant emission rate from the material and associated microorganisms. A mass balance written on each chamber, assuming constant chamber volume, flow rate during a given sampling period, and emission rate is shown in Equation (1):

$$dC/dt = \lambda C_0 - \lambda C + E/V \quad (1)$$

where:

V = Volume of the chamber minus volume of the material (m^3)
C = Concentration of the compound in the chamber (μg/m^3)
C_0 = Concentration of the compound in inlet air (μg/m^3)
Q = Flow rate (m^3/h)
λ = Exchange rate (h^{-1}) = Q/V
E = Emission rate (μg/h)

In the case of a steady state, $dC/dt = 0$ and Equation (1) becomes:

$$0 = \lambda C_0 - \lambda C + E/V \quad (2)$$

From Equation (2) we obtain the emission rate with Equation (3):

$$E = \lambda (C - C_0)^* V \quad (3)$$

2.7. Statistical analysis

We used the R statistical computing environment for all preprocessing and analyses, particularly packages `dada2`, `phyloseq`, `DESeq2`, `ggplot2`, and `vegan` (see Supplementary Information for a list of all packages used). Environmental conditions were summarized by material block and sampling day, and ANOVA was used to test for associations between temperature, relative humidity, or light intensity and sampling day. To test for associations between VOC concentration and material type, we performed a pairwise *t*-test between Timber and all other materials combined, since exploratory visualization indicated that Concrete, Earth, and Gypsum had similar concentration values and our sample sizes were small. Two-way ANOVAs with Tukey's HSD were used to evaluate differences in bacterial abundance and alpha diversity, estimated using the Shannon index, for material type, location (microcosm, laboratory), and sampling day. We used the Kendall Rank Correlation Coefficient to assess correlations between abundance and alpha diversity and environmental conditions (relative humidity, temperature, light intensity). For all beta diversity visualizations and analyses, we used the variance stabilizing transformation function in `DESeq2`, which adjusts for variation in dispersion due to differing sample sizes (Love et al., 2014). We specified a model design that transformed raw count data while controlling for sampling day and material type. Overall patterns in beta diversity were visualized using principal coordinates analysis (PCoA) ordination of Morisita-Horn distances. To test for associations between bacterial community composition and material type or environmental factors we used permutational multivariate analysis of variance (PERMANOVA (Anderson, 2017);), as implemented in the adonis function of the `vegan` package (Oksanen et al., 2018), with values obtained using type III sums of squares with 9999 permutations of residuals under a reduced model. Finally, generalized linear models (GLMs) based on the negative binomial distribution were executed using `DESeq2` to determine which ASVs contributed to differences in community structure across material types. To perform this test, we split up the dataset pairwise so that every combination was tested separately. All statistical analyses used a significance level of $P < 0.05$ and were adjusted for multiple testing using Bonferroni correction.

3. Results & discussion

3.1. Environmental conditions in microcosms and office space

We monitored temperature, relative humidity, and light individually for each microcosm and for the laboratory space that housed material blocks #2 and #5. Overall, there was little difference in environmental conditions among the different locations. The outer laboratory space tended to have slightly higher temperatures, slightly lower relative humidity, and 1–2 degrees of magnitude higher light intensity (Table S3 and Figs. S1–S3). Temperature and relative humidity were both associated with sampling day (ANOVA: F = 124, $P < 0.005$, $Df_{temp} = 7$, $Df_{resid} = 104$; F = 395, $P < 0.005$, $Df_{RH} = 7$, $Df_{resid} = 104$, respectively), while lighting intensity was not (F = 0.51, $P = 1.00$, $Df_{lux} = 7$, $Df_{resid} = 104$).

3.2. Timber differed from other materials in VOC concentration, emission rate, and profile

In total, 46 compounds were identified by TD-GC-MS and total concentration levels of these VOCs in the 36 samples that passed quality control ranged from 18 to 118 μg/m^3 (Fig. 3). All samples, regardless of material type and including inlet and lab air, contained high quantities of acetone, ethanol and methyl ethyl ketone (MEK) in roughly equal proportion, which suggests that they may have been present in the incoming air and were not removed by the filtration system. Timber samples had substantially higher total identified VOC concentration than other materials (*t*-test: t = −2.9, $P = 0.02$, Df = 6.9) and were characterized by many compounds not generally present in samples from other materials (Fig. 4 and Fig. S4). These were primarily terpenes, which are a class of organic compounds produced by a variety of plants, especially conifers. They often have a strong odor and may play roles in protecting the plants against herbivores, pathogens, and environmental stressors (Niinemets and Monson, 2013). In this study, terpene concentrations in Timber samples ranged from 13 to 73 μg/m^3. Gypsum samples also emitted α-pinene (a type of terpene), but in smaller quantities, possibly due to the wood framework underlying the Gypsum boards. Terpenes are also known to be emitted by some microorganisms. In addition, it is important to note that, despite using air filters purported to remove VOCs, air inlet VOC levels were quite high and may be a source of uncertainty in the concentration values. The emission rates calculated below and corrected for air inlet contamination may provide a clearer picture of VOC emissions from each material.

Total emission rates of identified VOCs had the same trend as their concentration levels, although there was considerable variation among material blocks (Fig. 5). Materials can be sources or sinks for VOCs due to interactions occurring at their surfaces. Only Timber had consistently positive overall emission rates of identified VOCs, whereas all other materials had negative overall emission rates. This indicates that Concrete, Gypsum, and Earth acted as net sinks for certain VOCs, with Earth acting as the most efficient sink. Different pore sizes may create potential for capillary condensation of water, which will cause absorption since some VOCs are more or less soluble in liquid water (Bouilly et al., 2006). Thus, the earth's capacity to absorb VOCs may be, in part, due to its greater hygroscopic potential. Overall, all materials most readily removed acetone from air; notably, acetone is miscible in water and may

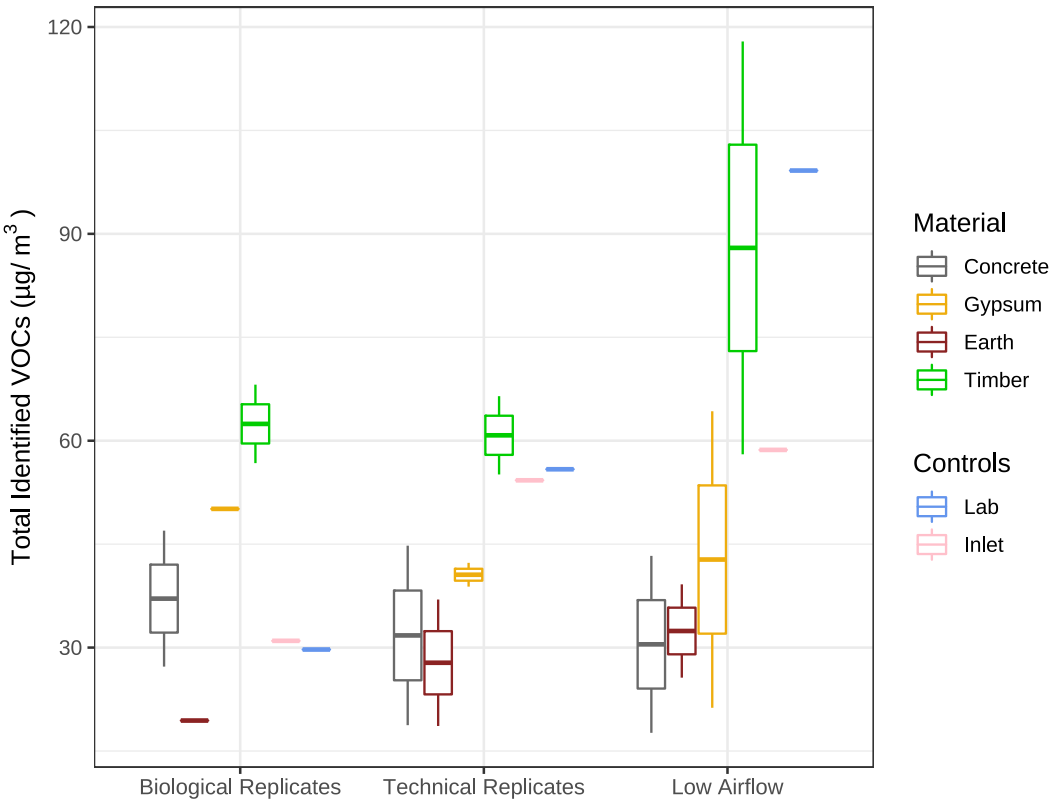

Fig. 3. VOC concentration levels for different material types, as well as inlet and lab controls. Two blocks of each material were tested in the first group (Biological Replicates); two replicates of the same material blocks were tested in the second group (Technical Replicates); in the third group, the blocks from the first group were tested again, but under low airflow rates (Low Airflow). See Table S1 for additional sampling details. The y-axis shows total concentration for all identified VOCs in µg/m^3.

be effectively uptaken into material pore water.

Timber primarily emitted terpenes, ranging from 36 to 148 µg/h. Of note, terpenes have been suggested to contribute to the low survival rates on wood of several pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli* (Coughenour, 2009; da Costa et al., 2008; Greatorex et al., 2011; Vainio-Kaila et al., 2017; Pailhoriés et al., 2017). They have also been shown to have anti-cancer, anti-inflammatory, immune-activating, antioxidant, and antimicrobial properties (Son et al., 2013; Ikei et al., 2016; Munir et al., 2019). For softwoods, including pine and fir, which are commonly used in the production of CLT, the majority (70-90%) of VOC emissions are terpenes (Pohleven et al., 2019). The terpene emission and concentration values we observed are in line with other work, though we found no other studies using CLT made from Douglas Fir. Normalizing for exposed surface area of the CLT (0.38 m^2) during tests, we report a terpene flux of 96–388 µg/m^2/h. This range is higher than a prior study which reported total terpene emissions from Douglas Fir particleboard of 32 µg/m^2/h (Baumann and Batterman, 1999). Our value is similar to that reported for Pine particleboard, for which Baumann et al. report a terpene emission factor of 284 µg/m^2/h (Baumann and Batterman, 1999). Notably, our study investigated samples of CLT, we suggest that the difference in measured monoterpene emission factors across Douglas Fir particleboard and Douglas Fir CLT may result from differences in processing of material and/or resins and binders used in manufacture.

The conditions (i.e., terpene levels in microcosms) in our experiment were consistent with studies done at larger, realistic scale. For example, one study found terpene concentrations in a model room constructed with Norway Spruce CLT ranged from 11 to 65 µg/m^3 (Höllbacher et al., 2014). In an unrelated but similar experiment, terpene concentrations ranged from 50 to 120 µg/m^3 in a Spruce CLT test room, but had concentration levels from 985 to 1580 µg/m^3 when Pine CLT was used (Nore et al., 2017). In the same study, occupant exposure to terpene concentrations up to 18 mg/m^3 did not result in any physical irritation symptoms. On the other hand, terpenes have structural characteristics that make them highly reactive with other compounds potentially present in indoor air, such as ozone (Calogirou et al., 1999); the products of such transformations may be less benign towards human health (Wolkoff et al., 2000).

We also note that some VOCs found in the samples are known to be emitted by bacteria and fungi, thus it may not be possible to confidently apportion the emissions observed here to the microbial communities or the materials themselves.

3.3. Overview of total versus viable bacterial community structure

After removing contaminants, positive and negative controls, and samples with fewer than 100 reads, we observed 12,402,745 total reads from the 71 untreated samples remaining after quality control (henceforth referred to as 'total bacterial community') and 3,507,414 total reads from the 89 PMA-treated samples remaining after quality control (henceforth 'viable bacterial community'), representing 28,815 and 3799 different amplicon sequence variants (ASVs), respectively. Proteobacteria (58.7%), Actinobacteria (22.3%), Firmicutes (8.5%), and Bacteroidetes (4.43%) were the most abundant phyla in the total bacterial community. These phyla were also the most abundant in the viable bacterial community, although with different relative abundances—-Proteobacteria (80.6%), Actinobacteria (8.05%), Firmicutes (8.69%), and Bacteroidetes (0.591%). Relative abundance of Proteobacteria was markedly higher in the viable versus total community, while relative abundances of most other phyla were lower, indicating that a higher proportion of Proteobacteria were intact and viable at the time of sampling. Notably, Actinobacteria and Bacteriodetes had a low proportion of

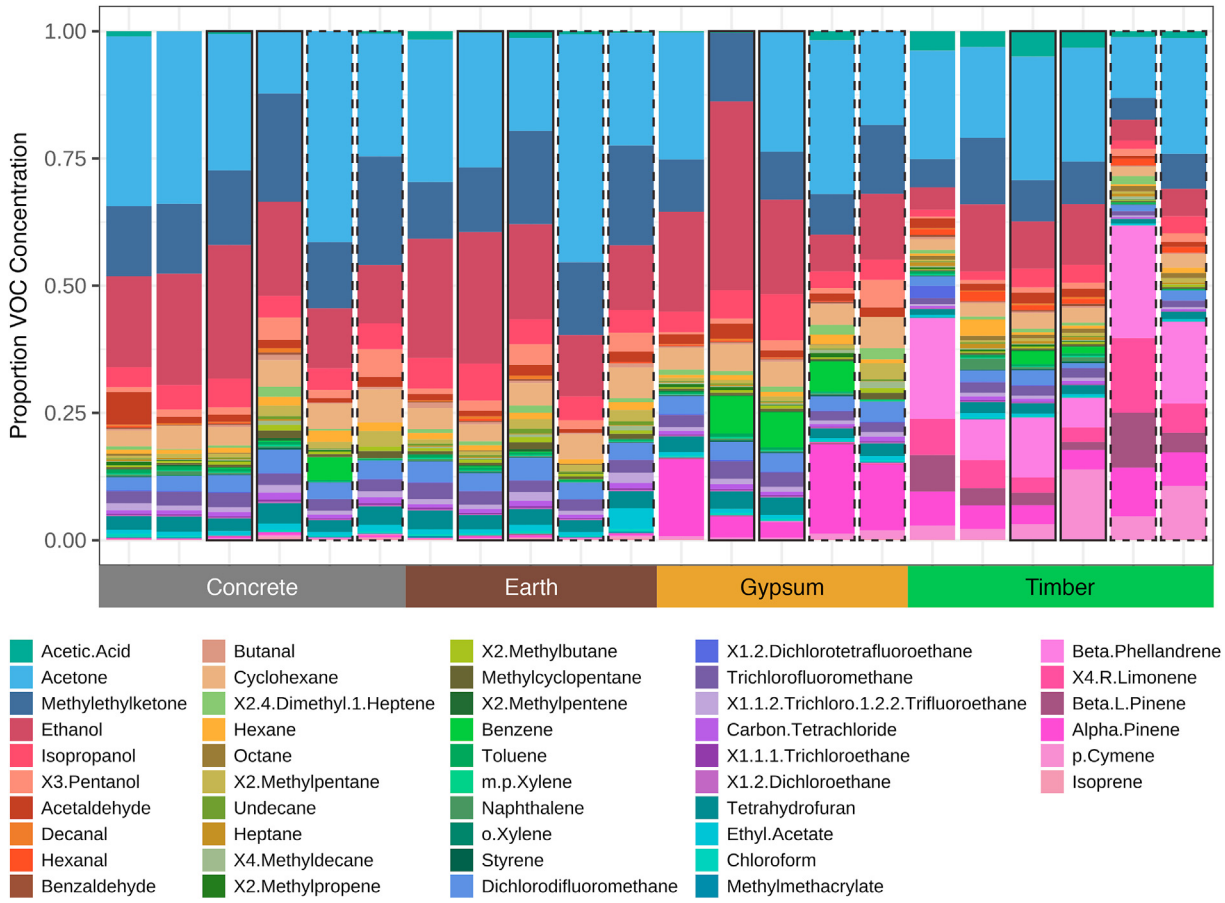

Fig. 4. VOC profiles for different material types. Concentration values have been normalized to show the percentage of each identified VOC compound (indicated by colors of stacked bars). Samples are arranged by material type, as indicated by labels below the plot. Samples collected under low airflow conditions have a dashed outline, and technical replicates for each material have a solid black outline. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

viable to total cells. These differences between viable and total community composition align with a number of other studies reporting that relic DNA comprises a substantial fraction of overall bacterial DNA present in a given environment, which can lead to different conclusions than when analyzing only viable cells (Ni et al., 2020; Carini et al., 2016).

The single most abundant ASV observed in the total bacterial community was *Pseudomonas* sp., comprising 9.3% of reads. It was hyper abundant in one Earth sample and observed to a lesser degree of abundance in a handful of samples across all material types. In the viable community, the most abundant ASV was a member of bacterial family Enterobacteriaceae that could not be identified to the genus level; it comprised 22% of viable bacterial DNA and was primarily observed in Earth samples (Fig. S5).

3.4. PMA treatment revealed different patterns of abundance, diversity, and composition for total versus viable bacterial communities

We observed substantially higher bacterial gene copy abundances in the total bacterial community compared with the viable community (*t*-test: $t = 18$, $P < 0.005$, $Df = 114$), though they displayed contrasting patterns over time (Fig. 6). Viable cells decreased substantially during the first week of the study, while the number of total cells remained relatively constant. Later in the experiment, the total number of cells began to decrease, while viable cells began exhibiting an upward trend. Our results agreed with a similar recent study, which reported a drop-off in viable gene copy abundance after material samples were placed in microcosms (Hu et al., 2019). However, there were several methodological differences that prompt caution in comparing the two studies: 1) Hu et al. used a mock community of only five bacterial taxa, while we used passive deposition to inoculate samples with hundreds of bacterial taxa and 2) their study lasted seven days, in contrast with our 39-day experiment. Thus, their study did not observe a bounce-back effect in viable bacterial abundance, which only occurred after Day 8 in our experiment.

We hypothesize that at the beginning of the study, all materials had a similar abundance of microorganisms at the surface due to their recent inoculation in the occupied laboratory space. Over time, some members of the microbial communities died off due to lack of water or energy sources, as well as a potentially hostile environment (chemicals from substrate, competition, other environmental conditions). Eventually, the taxa that survived became dominant and proliferated, being released from competition pressure and also having new energy sources (dead microbial cells). We were unable to test this hypothesis rigorously in this study, as we quantified absolute abundance for the entire bacterial community using universal 16S primers, rather than quantifying individual taxa. However, from our community composition data (Fig. 7), we did indeed find hints that support this idea. For example, genus *Blastococcus*—a member of the family Geodermatophilaceae, increased in relative abundance only on Concrete blocks over the course of the experiment. In contrast, Earth blocks were dominated by members of genus *Pantoea* in the first few days of the experiment, but by Day 8 they were largely displaced by a more diverse community, including genera *Gaiella*, *Nitrobacter*, and *Bacillus*; on Earth block #3, this community was later overwhelmed by genus *Pseudomonas*. However, this interpretation is speculative, since our method of collecting samples from new areas on the material blocks each time, which was intended to avoid destructive sampling of the microbial communities, may have confounded spatial

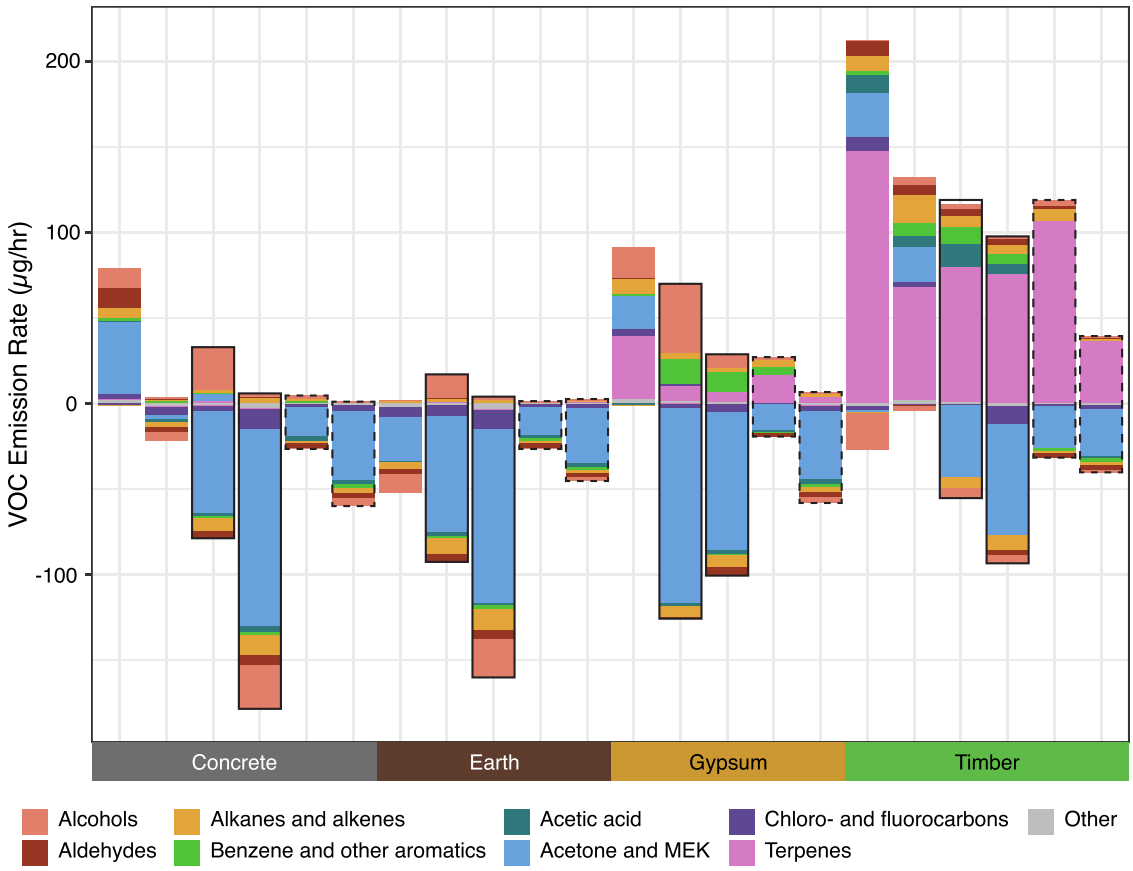

Fig. 5. Emission rates of different VOC groups. Samples are arranged by material type, as indicated by labels below the plot. Samples collected under low airflow conditions have a dashed outline, and technical replicates for each material have a solid black outline.

and temporal effects.

In terms of composition, the most abundant bacterial genera observed in the total community were *Blastococcus*, *Escherischia*, *Pantoea*, *Paracoccus*, *Pseudarthrobacter*, *Pseudomonas*, and *Sphingomonas* (Fig. 7A). Twelve out of the top 25 most abundant genera from the total community were also in the top 25 for the viable community, though the relative abundances of some of these taxa (e.g., *Escherischia*, *Paenibacillus*) were dramatically different. Some genera, including *Corynebacterium*, *Pseudarthrobacter*, *Staphylococcus*, and *Streptococcus*, were only in the top 25 for the total community, suggesting that they may have originated from the relic DNA pool. Notably among these, *Staphylococcus* and *Streptococcus* are typically human-associated and thus may not be well-adapted to survive on building materials. On the other hand, *Methylobacterium*, *Tepidimonas*, and *Thermoactinomyces* were only in the top 25 for the viable community (Fig. 7B), possibly indicating that they are highly represented in the viable, but not the relic, fraction of the community. Some of the most abundant bacterial genera we observed, including *Bacillus* and *Pseudomonas*, can exert antagonist effects against wood decay fungi and have been used as biocontrol agents on timber products (Susi et al., 2011). Finally, we reiterate that our non-destructive sampling methodology may explain, in part, the lack of a clear pattern in composition by material type or sampling day. It is possible that different areas of the material blocks harbored distinct communities due to stochastic processes occurring during the inoculation period and over the course of the experiment itself.

In aggregate, the total bacterial community had 80% greater mean Shannon diversity than the viable community (Fig. S6; *t*-test: $t = -10$, $P < 0.005$, Df = 142). This finding is in contrast to previously published literature suggesting that relic, or nonviable, bacterial DNA degrades at a constant rate in the environment regardless of taxonomy and, therefore, has little effect on diversity measures (Lennon et al., 2018).

Further testing to assess the influence of the exterior environment, sampling day, and material type was performed for the viable bacterial community only, since we were most interested in the effects of these factors on living and potentially metabolically active bacteria.

3.5. Earth and Timber materials had divergent patterns of viable bacterial abundance and diversity

Material type was associated with abundance of viable bacteria (Fig. 6; ANOVA: F = 23, $P < 0.005$, Df = 3), a relationship that was due to significantly higher abundances on Earth blocks in comparison with Gypsum (Tukey's HSD: $P < 0.005$), Timber ($P < 0.005$), and Concrete ($P < 0.005$), and lower abundance on Timber in comparison with Concrete ($P = 0.04$). Material type was also associated with taxonomic diversity of the viable bacterial communities (Fig. 8; ANOVA: F = 6.8, $P < 0.005$, Df = 3). Again, Earth had significantly greater diversity than Gypsum, (Tukey's HSD: $P < 0.005$), Timber ($P = 0.03$), and Concrete ($P = 0.03$). No other significant differences in alpha diversity were observed. These results may be explained by a number of discrete, though possibly synergistic, factors:

1. **Hygroscopicity**: Earth absorbs and retains higher levels of moisture than other materials, possibly permitting survival of more taxonomic groups in higher numbers (Viitanen et al., 2009; Stephens, 2016).
2. **Adaptation**: Microbial generalists may be better-adapted to survival on this surface, as soil is known to harbor vast abundance and diversity of microorganisms, whereas Timber produces various chemical compounds, such as terpenes and formaldehyde, which can inhibit microbial growth and survival (Chen et al., 2016; Scheffer, 1966).

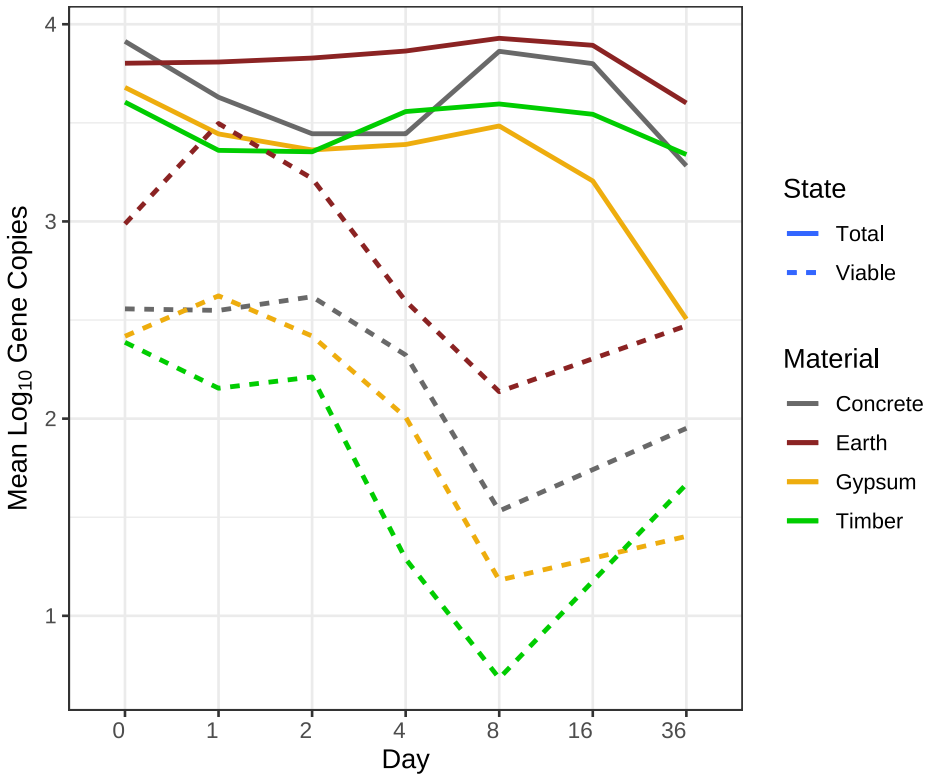

Fig. 6. Average abundances across all samples, colored by material type. Viability status indicated by solid (total) and dashed (viable) lines.

Another possibility is that the lignin component of timber may be responsible for the low biomass and diversity found on CLT, since lignin is also known to have antimicrobial properties (Alzagameem et al., 2019).

3. **Sampling efficiency**: Swabs likely picked up more biomass from Earth blocks than other materials, because the earthen plaster was not fixed and swabs were notably dirty after collection. In particular, microorganisms are known to enter wood pores, where they become unrecoverable by swabbing the surface, explaining the low abundances of Timber samples. For example, one study found that after a 2-h drying period, more than 90% of cells inoculated onto wood surfaces could not be recovered by vigorous rinsing (Abrishami et al., 1994), while another found that bacteria could not be recovered after only 3–10 min (Ak et al., 1994). Numerous other studies provide similar evidence, although the effect may depend on the species of wood and whether it is coated or not (Aviat et al., 2016; Boersig and Cliver, 2010; Boursillon and Riethmüller, 2007; da Costa et al., 2008; Hedge, 2015; Koch et al., 2002; Milling et al., 2005a; Milling et al., 2005b; Moore et al., 2007; Pailhoriés et al., 2017; Schönwälder et al., 2002; Vainio-Kaila et al., 2011; Vainio-Kaila et al., 2017).

Other studies that have used microcosms to investigate the effects of different building materials on microbial communities had similar results. That is, some types of materials supported bacterial survival and growth better than others—a pattern possible related to material surface pH, hygroscopicity, and/or presence of chemical compounds (Lax et al., 2019; Hu et al., 2019).

Interestingly, neither abundance nor diversity of viable cells were significantly associated with the location of blocks inside sealed microcosms versus exposed to the occupied laboratory space (ANOVA: $F = 1.3$, $P = 0.25$, $Df = 1$; $F = 1.2$, $P = 0.28$, $Df = 1$, respectively). However, we did observe an effect of sampling day on abundance (ANOVA: $F = 20$, $P < 0.005$, $Df = 5$), but not diversity (ANOVA: $F = 1.6$, $P = 0.16$, $Df = 7$).

We tested for effects of environmental conditions (relative humidity, temperature, light intensity) separately, since they tended to be correlated with sampling day, location, or both. Relative humidity showed a negative correlation with abundance of the viable bacterial community (Fig. 9A; Kendall Rank Correlation: $\tau = -5.2$, $P < 0.005$), but a positive correlation with alpha diversity (Fig. 9B; Kendall Rank Correlation: $\tau = 2.7$, $P = 0.02$). We found no significant correlations with temperature or light intensity for either abundance or alpha diversity.

Other published studies have reported a complex relationship between bacterial survival and relative humidity (Tang, 2009), with many studies finding that bacterial survival in the air and on surfaces is high at very low and very high RH levels but decreases in the intermediate range, although the effect varies by bacterial taxa (Dunklin and Puck, 1948; Turner and Salmonsen, 1973; Mcdade and Hall, 1964; Kramer and Assadian, 2014; McEldowney and Fletcher, 1988). Several particularly relevant studies demonstrated that substrate hygroscopicity and air relative humidity jointly influence survival rates and metabolic activity of microbes living at the surface-air interface. Specifically, they found that survival rates were higher at 30% RH than at 70% RH, and that microbes were more metabolically active on hygroscopic surfaces (bentonite clay) under high RH than under low RH or on non-hygroscopic surfaces under any RH conditions (Stone et al., 2016a, 2016b). Although our study agrees with the published literature, we also note that we cannot distinguish between the effects of relative humidity

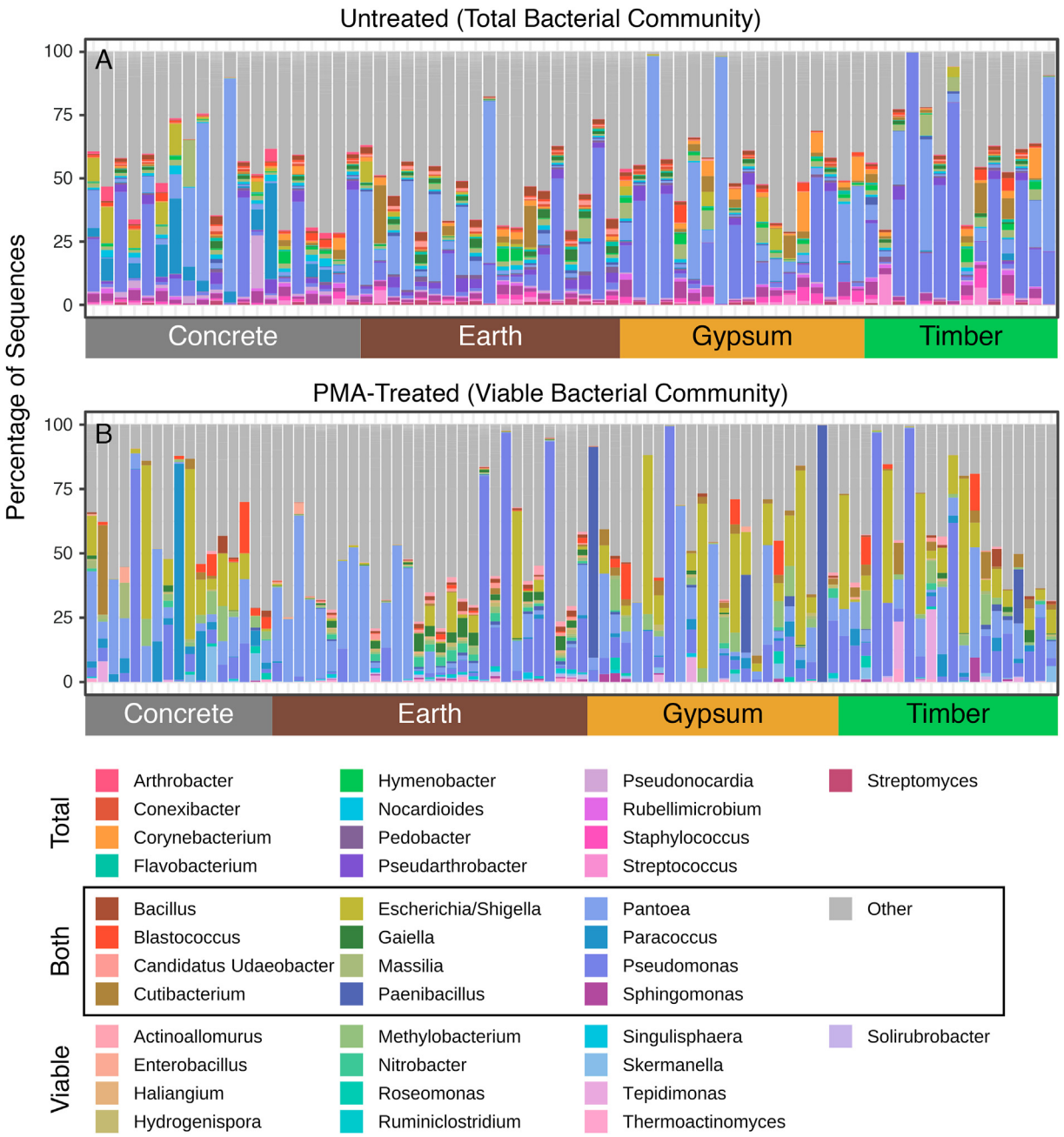

Fig. 7. Top 25 most abundant taxa across all samples representing the total bacterial community (A) and those representing the viable bacterial community (B). Genera shown inside black box in the legend were in the top 25 for both sample groups.

and those of sampling day, given that they covaried.

3.6. *Viable bacterial communities inhabiting earth are dissimilar to communities on other materials*

As previously noted, composition differed substantially between viable and total bacterial communities, explaining 23% of the variation among all samples (PERMANOVA: $P < 0.005$, $Df_{var} = 1$, $Df_{resid} = 158$). For the viable community only, we found that material type explained 18% of the variation (PERMANOVA: $P < 0.005$, $Df_{var} = 3$, $Df_{resid} = 77$), although this effect was due entirely to the difference between Earth and all other materials (Fig. 10). When we removed Earth samples from the analysis, there was no longer any significant effect of material type. Similarly, sampling day had an important effect when we analyzed all materials together ($R^2 = 0.1$, $P < 0.005$, $Df_{var} = 7$, $Df_{resid} = 77$), but the effect disappeared when Earth samples were removed from the analysis. These results suggest that, in terms of community composition, the majority of variation in surface microbes of different material types is driven by the unique properties of earthen plaster as a habitat for microorganisms. This is unsurprising, given that soil is known to host enormous prokaryotic (bacteria and archaea) abundance and diversity, estimated at up to 20×10^9 cells per cubic centimeter, representing 100–9000 different taxa (Bardgett and van der Putten, 2014). Additionally, several other factors may have influenced the bacterial communities from Earth samples: 1) the clay and cut straw used in the earthen plaster mix could have been an initial source of microbial taxa beyond those present in the passive inoculant (i.e., laboratory air), and 2) soil bacterial communities are known to differ substantially across very small spatial scales, e.g., centimeters (O'Brien et al., 2016; Vos et al., 2013), so samples taken later in the study may have different communities than earlier samples due to

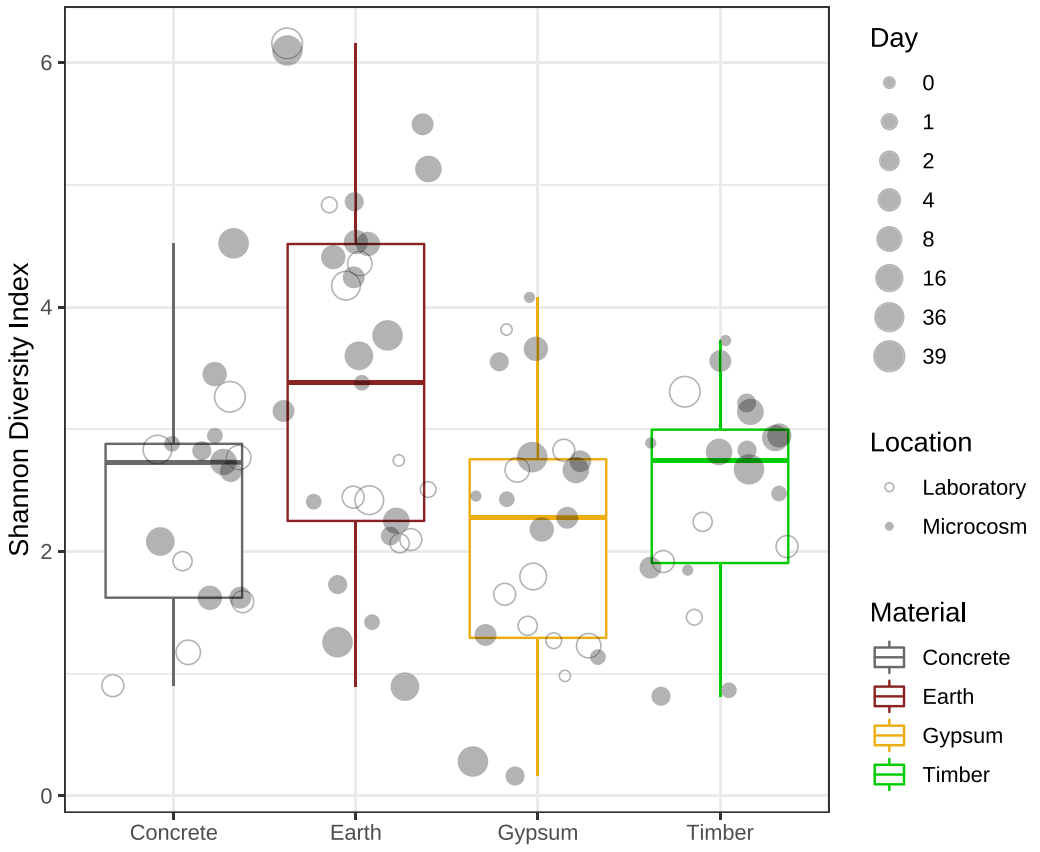

Fig. 8. Alpha diversity of samples from the four different materials. Hollow circles represent samples from materials housed in individual microcosms, while filled circles represent samples from materials in the occupied laboratory. Circle size indicates sampling day.

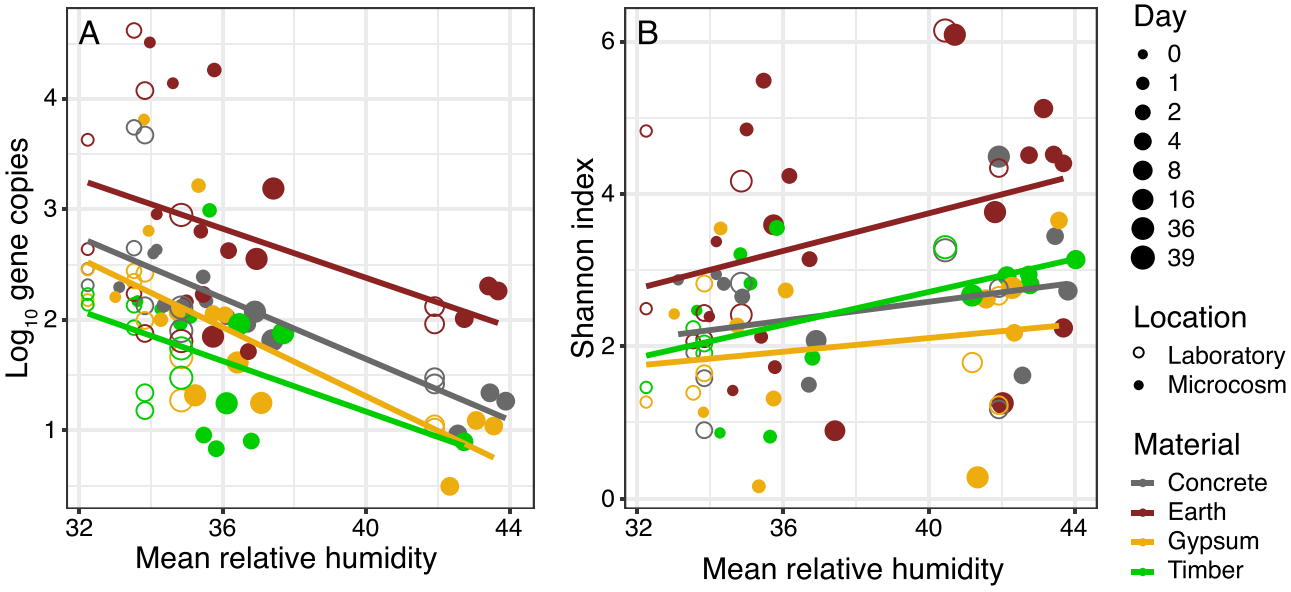

Fig. 9. Scatterplots showing relationships of viable bacterial abundance (A) and alpha diversity (B) with relative humidity. Point and line colors indicate material type, hollow versus filled circles indicate whether materials were inside microcosms or exposed to the occupied laboratory, and point size indicates sampling day. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

either temporal or spatial variation.

We also noted that beta-diversity was not influenced by location inside the microcosms versus exposed to the occupied laboratory (PERMANOVA: $R^2 = 0.01$, $P = 0.12$, $Df_{var} = 1$, $Df_{resid} = 77$). Other studies have found that surface location within a room—a proxy for type of contact with occupants—is the strongest driver of community

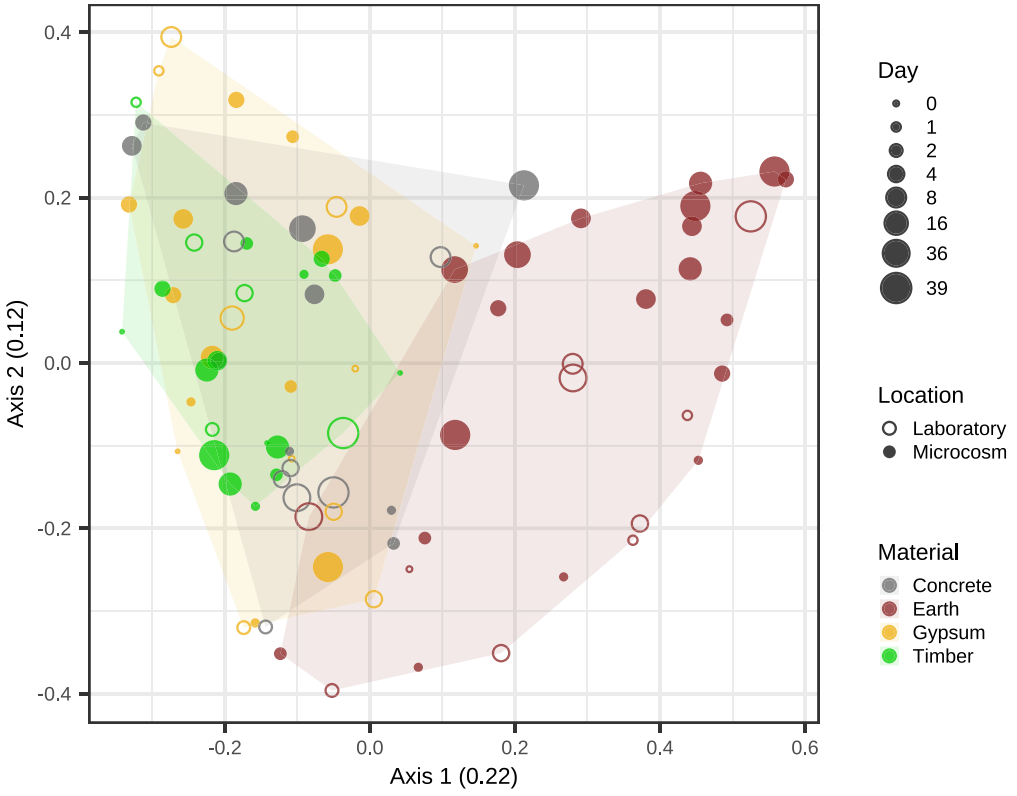

Fig. 10. PCoA ordination showing difference between viable bacterial communities from different materials and sampling days. Color of points indicates material type, size indicates sampling day, and hollow vs. filled circles indicate location. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

composition for high-touch surfaces, such as floors, desks, walls, chairs (Chase et al., 2016; Meadow et al., 2014). Our results suggest that for materials that do *not* experience frequent physical contact with occupants (like those in our study), the material itself may be a stronger driver of microbial community structure.

3.7. Many bacterial taxa were enriched on earth material blocks

Few bacterial taxa were preferentially associated with non-Earth materials, but many with Earth (Fig. 11 and Figs. S7–S10), which explains why only Earth samples clustered discretely in the PCoA ordination plot. Of these differentially abundant taxa, we noted several of interest. First, a member of the genus *Ralstonia* was enriched in Timber—although in this study we were unable to identify ASVs at the species level, *R. solanacearum* has been observed in conifers affected by pine wilt disease (Proença et al., 2017). Additionally, *Paenibacillus* was enriched on painted Gypsum, agreeing with other work that has found *Paenibacillus* species on gypsum boards (Knudsen et al., 2017), while *Enterobacter* and *Paracoccus* were both enriched on Concrete. *Enterobacter* has been used in microbially-treated concrete to increase strength. Lastly, 37 ASVs, including members of soil-associated genera *Gaiella*, *Mycobacterium*, and *Bacillus*, were always enriched on Earth materials, regardless of which other material it was compared against. In another study of earthen building material, *Bacillus* was noted as highly prevalent across all samples, suggesting a strong ability to survive on soil substrates (Simons et al., 2020). The same study found surprisingly few human-associated microbial taxa, despite collecting the samples from occupied earthen buildings, which may indicate that raw earth is a hostile environment for human-associated microorganisms. Despite the fact that humans have lived in caves throughout recorded history and even now over 30% of the global population lives in earthen homes (Minke, 2009), few have investigated the potential health implications of earth as a building material. Though there is a lack of evidence in the building materials and construction literature, research in landscape architecture has begun to show how exposure to natural materials, such as soil, in the building environment can impact human skin and gut microbiota (Nurminen et al., 2018; Hui et al., 2019; Roslund et al., 2020).

4. Conclusion

There is growing interest in buildings that attain high sustainability and health-promotion standards. Decisions regarding structural and finish material selection can have a profound impact on both concerns over building lifetimes. This study provided initial evidence that microbial communities on indoor surfaces, as well as airborne VOCs, can be affected by material type. Of the materials investigated in this study, the two that are typically perceived as more environmentally sustainable (Earth and Timber) also appeared to have possible human health implications, albeit for quite different reasons. Earth had the highest bacterial abundance and diversity of any material, bacterial community composition that differed substantially from other materials, and was a net sink for VOCs. Thus, in a context where exposure to high microbial diversity and low VOC concentrations is desirable, earthen plaster may be considered as an appealing material choice. Timber, on the other hand, had the lowest bacterial abundance and diversity, did not have a notably unique bacterial community composition, and had the highest concentration and emission of VOCs, largely due to high terpene levels. These qualities may be more appropriate in settings where exposure to high levels of bacterial abundance and diversity are to be avoided. Concrete was most similar to Earth, in terms of VOC composition, while Gypsum was similar to Timber in both bacterial and VOC composition. Future studies exploring the potential health effects of microbial and VOC

Fig. 11. Heatmap of all differentially abundant bacterial genera. Cell coloring indicates taxon relative abundance, coloring of y-axis labels indicates in which material type each genus was enriched, and coloring of bar above x-axis labels indicates from which material type each sample was collected. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

communities associated with different building materials would be of great value.

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Declaration of competing interest

The authors report no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dibe.2021.100055.

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