Growing Indoor Environmental Infrastructure: Designing for Microbial Diversity with Implications for Pollutant Metabolism and Human Health

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Data Availability

The data that support the findings of this study are openly available in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) data repository at https://www.ncbi.nlm.nih.gov/sra/PRJNA1016375 ; BioProject ID: PRJNA1016375.

Connections References

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Abstract

Urban inhabitants spend upwards of 90% of their time indoors where building design and mechanical air-handling systems negatively impact air quality, microbiome diversity, and health outcomes. Urban bioremediation infrastructure designed to improve indoor environmental quality by drawing air through photosynthesizing plants and metabolically diverse rhizospheres have been investigated since the 1960s, however in-depth analysis of the potential impacts on indoor environments is required: (1) although recent evidence has illustrated human microbiome alteration and associated health benefits related to exposure to green wall systems, the mechanism(s) of diversification have not yet been established, (2) microbial metabolism and airborne chemical dynamics are extraordinarily complex and hypotheses pertaining to rhizosphere microorganisms metabolizing pollutants require more attention. To explore these areas, we applied a shotgun metagenomic approach to quantify microbial diversity and establish preliminary metabolic profiles within active green wall modules spanning a range of growth media and plant selections. Results indicate that fundamental design decisions, including hydroponic vs. organic growth media, support rhizosphere microbiomes with distinct diversity and metabolic profiles which could impact system performance. The described relationships indicate fundamental green infrastructure design represents an opportunity to "grow" indoor microbial diversity and metabolisms with potential benefits for human pollutant exposure and health outcomes.

Keywords: Urban green infrastructure, Bioremediation, Microbiome, Air Quality

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Introduction

Indoor Air Quality, Microbiome Diversity and Human Health Impacts

Global urbanization and modern building design lead to indoor spaces that contribute to intractable indoor environmental quality (IEQ) problems, including both airborne pollutants and deteriorated microbial diversity linked to negative impacts to human health outcomes. Numerous studies are published every year establishing connections between the diversity and ecology of microbial communities (microbiomes) associated with human organs and living spaces with measures of human health. Studies describing the disruptions in the compositions of both air and microbiome quality caused by urbanization have found both correlational and causal relationships with human health outcomes, yet many of these relationships are not yet mechanistically understood (Mankiewicz et al. 2021). Poor indoor air quality (IAQ) has measurable impacts to human health as levels of carbon dioxide (CO_2) (Jacobson et al. 2019; Satish et al. 2012; Vehvilainen et al. 2016), volatile organic compounds (VOCs) (Ataei et al. 2023; Halios et al. 2022) and other pollutants (Kumar et al. 2023) increase. Symptoms of chronic exposure to poor air quality specifically include both transient symptoms such as impacts to cognitive function (e.g. headaches and difficulty concentrating) (Halios et al. 2022; Jacobson et al. 2019; Kumar et al. 2023), to chronic, serious symptoms such as inflammation (Jacobson et al. 2019), respiratory issues (Halios et al. 2022; Jacobson et al. 2019; Kumar et al. 2023), allergy (Halios et al. 2022), asthma (Ataei et al. 2023; Kumar et al. 2023) and cancer (Ataei et al. 2023; Halios et al. 2022; Kumar et al. 2023) in both developing and developed nations (Sundell 2004).

Urban environmental microbiome patterns, characteristically displaying low diversity metrics, have also been correlated with negative impacts to human health metrics. Reductions in the diversity of urban microbiomes (both human and indoor surfaces) in comparison to outdoor and rural or exurban microbiomes have been linked to significant negative human health outcomes including atopic skin conditions and allergies (Haahtela et al. 2015; Hanski et al. 2012), asthma (Haahtela et al. 2015; Sharma and Gilbert 2018; Stephens et al. 2019), obesity (Pechal et al. 2018; Sharma and Gilbert 2018; Stefano et al. 2018), cancer (Winglee et al. 2017), even depression (Chen et al. 2021; Naseribafrouei et al. 2014) as well as the transmission of potential pathogens (Kembel et al. 2014; Meadow et al. 2014; Ruiz-Calderon et al. 2016). True causes of

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this decline in microbial diversity is difficult to study, although it has been proposed that it may be related to reduced contact of urban inhabitants with soil and plant-associated microbial ecosystems (Blum et al. 2019). Although many studies report co-relationships between negative health outcomes and reduced environmental microbiome diversity rather than causal mechanisms, studies are beginning to indicate that enhancing urban inhabitant contact with soil and plant-associated microbiomes may reverse these impacts and have measurable benefits to metrics of immune health and regulation (Roslund et al. 2022; Roslund et al. 2020; Soininen et al. 2022).

Urban Building Design and Human Exposure

Interdisciplinary research continues to illustrate the limitations of heating ventilation and air conditioning (HVAC) systems upon which urban indoor air quality (IAQ) is increasingly dependent. Certain acute IAQ problems, such as elevated CO_2 levels, can be alleviated and the long-term health outcomes of occupants supported by increasing HVAC system ventilation rates (Milton et al. 2000), however this strategy is energetically intensive (Administration 2018; Rackes and Waring 2017). In addition, although well-maintained HVAC systems keep increasingly air-tight buildings habitable, HVAC systems ventilate indoor spaces under the assumption that outdoor air contains fewer pollutants. This is often not the case within dense urban areas, where ventilation of indoor spaces with outdoor air can create complex mixtures of indoor- and outdoor-sourced pollutants within interior spaces (Jose and Perez-Camanyo 2023; Rosbach et al. 2016; Wong et al. 2005). In addition, HVAC system performance is impacted by age, design, and maintenance, leading to disproportionate inhabitant exposures to pollutants depending on factors such as building typology, social disparity, and access (Kheirbek et al. 2016; Menicovich et al. 2014; Menicovich et al. 2012; Nazaroff and Cass 1989; Rackes and Waring 2017; Rosenthal et al. 2014; Shmool et al. 2015). Finally, although conventional building HVAC units are designed to increase IAQ through the filtration of airborne pollutants, studies indicate they simultaneously reduce indoor microbial diversity (Kembel et al. 2012).

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Urban Bioremediation Infrastructure and Indoor Microbiome Diversity

Substantial developments have recently been published pertaining to environmental microbiome impacts in three experimental studies reporting causal relationships between urban design interventions and measured benefits to human microbiome diversity and metrics of immune health (Roslund et al. 2022; Roslund et al. 2020; Soininen et al. 2022). One study in particular implemented active air flow through plant-based infrastructure (a "green wall", see Supplemental Figure 1) as the design intervention intended to increase human microbiome diversity and human health metrics (Soininen et al. 2022). Although a prevailing hypothesis within the field of indoor urban bioremediation infrastructure is that active air flow through plant-based systems could contain beneficial microorganisms, diversify indoor microbiomes, inoculate human microbiomes, and support measurable benefits to human health, Soininen et al. (2022) is the first study to have measured and reported upon such an impact. While the mechanisms of such findings must be confirmed by future work, the results have exciting implications for how indoor environmental conditions might be shaped through the design of plant-based air bioremediation systems.

Indoor Green Infrastructure May Shape Indoor Air Quality and Microbiome Diversity

Urban bioremediation infrastructure systems designed to move indoor air past healthy photosynthesizing plant leaves and through diverse metabolically active root-associated microbial "rhizosphere" ecosystems have been studied as a strategy to improve indoor environmental quality since the 1960s (Matheson et al. 2023). Although this body of research has developed substantially in recent years, the peer-reviewed literature is still far from reaching a consensus on many issues. In particular, the hypothesis that plant root-associated microorganisms could metabolize airborne chemicals at a rate that might alter human exposure to pollutants and change health outcomes rests upon multiple assumptions, many of which have not yet been sufficiently scrutinized. Simultaneously, however, the body of literature outlining the multitude of negative impacts inhabitable urban spaces can have on human health through poor air and microbiome quality grows every year, and urban bioremediation infrastructure is still often proposed as a solution to a myriad of environmental quality challenges (Aydogan and Cerone 2020; Han and Ruan 2020; Matheson et al. 2023; Pettit et al. 2020). The urgency is

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understandable. Since 2018, more than half of the world population lives in cities (55%), and this proportion is projected to surpass 65% by 2050 (Wilmoth 2019). Within this context, humans living in urbanized areas spend upwards of 90% of their time indoors (Klepeis et al. 2001). These trends together indicate that in less than 30 years, any detrimental health impacts of common indoor urban design factors could influence the health of two thirds of the earth's population. Even though this proportion does not take socioeconomic differences or worsening conditions in environmental justice areas into consideration, the sheer number of individuals involved is staggering.

With these challenges in mind, many urban bioremediation system designs have been tested under different environmental conditions in order to evaluate their capacity for indoor air remediation, namely by quantifying their ability to reduce CO_2 (Dominici et al. 2021; Mankiewicz et al. 2022), to sequester particulate matter (Han and Ruan 2020; Matheson et al. 2023), and even to metabolize chemical pollutants such as volatile organic compounds (VOCs) (Han and Ruan 2020; Matheson et al. 2023). From a microbiome perspective, as discussed, although human microbiome diversification from exposure to indoor urban bioremediation infrastructure has often been hypothesized, impacts to the human skin microbiome and related benefits to measures of immune health were not established until 2022 (Soininen et al. 2022). Unfortunately, Soininen et al. (2022) did not sample the green infrastructure system's microbiome and thus cannot trace the origin of the species responsible for the increased skin microbiome diversity, or eliminate the hypothesis that changes to air quality (such as VOC composition (Abis et al. 2020; Yuan et al. 2017)) as a potential mechanism in altering the composition of human bacterial communities. Correlational survey studies of indoor microbiomes around passive indoor plants have also reported increased environmental microbiome diversity, and similarly did not sample the "source" microbiomes (Dockx et al. 2022). Taken together, these studies support hypotheses that urban bioremediation infrastructure could diversify indoor and human microbiomes with measurable benefits to human health outcomes, however the mechanism of diversification requires examination.

If the evidence for human microbiome diversification through exposure to plant-based indoor design can be verified and deepened, this relationship has exciting implications for shaping our indoor spaces and exposures through green infrastructure system design with human health

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outcomes in mind. Previously, many human microbiome diversification intervention studies have focused on applications-based approaches (e.g. supplements, fecal transplants, diet) (Hitch et al. 2022). In contrast, the potential to shape human microbiomes and immune function through exposure to beneficial environmental communities is an increasingly popular topic in the literature (Stanhope et al. 2022; Stanhope and Weinstein 2023). This emerging evidence has exciting implications for the application of urban bioremediation infrastructure indoors, especially if more causal relationships and mechanisms of microbiome diversification and health benefits (Roslund et al. 2022; Roslund et al. 2020) are reported.

Challenges in the Complex Chemical and Metabolic Dynamics of Urban Bioremediation Infrastructure

One of the foundational hypotheses within the field of urban bioremediation infrastructure is that plant root-associated microorganisms metabolize airborne chemicals indoors, which is often closely followed by the hypotheses that the rate at which these metabolisms occur could benefit human exposure and ultimately health outcomes (Matheson et al. 2023; Wolverton et al. 1989). Many studies that test these hypotheses in the laboratory use either a specific VOC, common choices including benzene and toluene (Matheson et al. 2023; Paull et al. 2019), and/or total VOCs (TVOCs) (Irga et al. 2019), and compare airborne concentrations up and downstream from an active green system. Unfortunately, although useful models, such experimental approaches do not necessarily relate to “real world” air quality chemistry dynamics at play or “en vivo” human exposure to airborne chemicals for two reasons: (1) Anthropogenic pollutant dynamics and chemistries are enormously complex and cannot be captured with a single variable (Lewis 2018), and (2) Both plants and microorganisms produce biogenic VOCs (BVOCs) (Ameye et al. 2018) such as isoprene which have been implicated in thousands of interactions with hundreds of intermediate species within the atmosphere (Lewis 2018), which could further complicate the measurement of indoor airborne chemical mixtures. Thus far, few studies have utilized methods that can identify diverse airborne chemicals as well as parse anthropogenic VOCs and BVOCs (Morgan et al. 2022), although as such analyses become increasingly available, this need will likely be filled.

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Finally, while the above-mentioned studies hypothesize that alterations in VOC concentrations are due to metabolic activity of the rhizosphere, none have reported genomic evidence for the presence of the appropriate metabolic pathways that could degrade the pollutants in question. Two studies measured an increase in "potential VOC-utilizing families" with 16S rRNA gene amplification and sequencing (Mikkonen et al. 2018; Russell et al. 2014), however this species classification analysis does not allow for insights into the potential for community metabolism.

Study Contribution: Metagenomic Analysis to Establish Foundational Design Criteria of Urban Bioremediation Infrastructure

This study uses shotgun sequencing and metagenomic analysis to characterize a range of urban bioremediation infrastructure designs to determine how foundational system criteria such as growth media design or plant species selection may influence microbial diversity and the metabolic potential of the system. The results are then discussed in the context of large-scale installations and their potential to shape indoor microbiome diversity and air quality at the scale of human exposure.

Methods

Indoor Green Infrastructure System Overview

The surveyed urban bioremediation infrastructure systems are described in detail in Mankiewicz et al. (2022). The bioremediation infrastructure systems were assembled three months before sampling was completed, allowing plants and microorganisms to develop within the experimental room. Eighteen unique plant/growth media pairs were sampled: Three growth media options, and six plant options. The three growth media were (1) a mineral expanded clay hydroponic media mixed with activated carbon (HAC), (2) an identical hydroponic media mixed with biochar (HBC), and (3) a commercially available organics-based growth media GaiaSoil™ containing internal organic fertilizer sources (GAIA). The plant species utilized were *Epipremnum aureum* ("golden pothos"), *Brassica narinosa* ("tatsoi"), and *Oxalis stricta* ("wood sorrel"). Tatsoi and sorrel were sprouted in peat moss plugs from seed, pothos was propagated in identical plugs from cuttings. The seedlings were planted in the 6 different groupings in each

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growth media: (1) one pothos, (2) three pothos, (3) three tatsoi, (4) three sorrel, (5) one of each, and (6) no plants, empty plugs only. Plant-growth media configurations were set up for which each of the six plant groupings were planted in each of the three media and replicated three times, resulting in 18 unique configurations and a total of 54 pots. The 54 individual 20 cm diameter pots were placed in one of three sets of shelves. Each set of shelves supported 3 of 9 laser-cut acrylic planters with a fan on one end. Each set of shelves was outfitted with a gravity-fed irrigation system on a timer. Each planter supported 6 pots and was lit from the shelf above. A visualization of the planters, growth media, and species can be found in **Error! Reference source not found.**. More information on the lighting, watering, and plant system designs can be found in Mankiewicz et al. (2022).

Experimental and Control Room Overview

The experimental and control rooms were located on the 24th and 25th floors, respectively, of a 37-story building in the financial district of New York City. The control room was located directly above the experimental room. Both spaces were windowless, relying entirely on HVAC-ventilation and artificial lighting. In addition to researchers maintaining the green infrastructure systems, the experimental room was used by students throughout the experiment as an educational space. The control room was used by students, faculty and office staff as a meeting space and did not contain any vegetation. The dimensions and furniture configurations of the experimental room can be found in Figure 1.

Sample Collection

Isohelix™ SK-2 rayon swabs were used to sample the root-microbiomes of each pot. Swabs were pulsed within the growth media in the middle of the pot and for thirty seconds. Indoor surface samples were collected by swabbing 625 cm^2 area surfaces for 30 seconds on each side of the swab. This process was completed for surfaces within the experimental room in which the green wall modules were grown (13 samples) as well as the control conference room (8 samples). The room surface swabs contained samples of both vertical (wall) and horizontal (table) surfaces. Surface swab sites were chosen based on green infrastructure proximity and occupant-use factors in each area. Horizontal sites were chosen based on chair placement and

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proximity to occupant utility. One swab was taken from a light switch in each room, and three swabs were collected in each room from wall surfaces unlikely to be touched as a comparison. Nine vertical sites within the experimental room were chosen based on proximity to green infrastructure system fan outputs (see **Error! Reference source not found.**). Swabs were transported and stored using the ZymoBIOMICs SafeCollect™ Swab Collection kit (Zymobiomics). Field blanks were collected, transported, and processed identically to experimental samples to control for contamination.

DNA Extraction & Metagenomic Analysis

DNA Extraction & Quantification

All samples were extracted using the DNeasy PowerSoil extraction kit (Qiagen), eluted in 50 µL of the provided elution buffer, and quantified using an Invitrogen™ Qubit® 3.0 Fluorometer. 5 µL of each sample was used to quantify DNA concentrations, placing the limit of detection of the process 0.5 ng/mL. Using this process, many of the samples returned "undetectable" DNA concentrations, and those that were detectable indicated an "ultra low" sequencing approach was required (requiring as little as 1 ng of DNA as opposed to standard sequencing approaches that require >500 ng (Illumina 2022; Kelley and Gilbert 2013)). DNA concentration results are reported as total ng yields within each 50 µL sample. Percent differences in DNA yield were calculated according to the equation reported in the Supplemental Information.

Library Preparation & Sequencing

Libraries for the samples were generated with the Illumina DNA (M) Prep kit (cat#20018705) using a ¼ scale reaction volume throughout the library preparation. Shotgun sequencing was performed on an Illumina 6000 Novaseq system using an S1 cluster cartridge, a S1 flow cell, and a 300-cycle kit (Illumina, cat#20028317). Sequencing parameters included 150 paired end base-pairs with dual 10 base-pair index reads.

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Bioinformatics Analysis

Taxonomic Groups: All bioinformatics analysis was performed using the high-performance cluster at New York University. The MetaSUB CAP pipeline (Danko and Mason 2020) was used for bioinformatics analysis. All analyses utilized default settings. Raw sequenced data (pair-end reads) was processed with AdapterRemoval (v2.2.2)(Schubert et al. 2016) to remove low-quality and ambiguous base reads. We then used Bowtie2 (v2.2.3) (Langmead and Salzberg 2012) to map reads to the human reference genome (hg38, including alternate contigs). Read pairs that mapped to the human genome were discarded, read pairs where neither mate mapped to the human reference ("non-human reads") were used for further processing. For taxonomic assignment, we used the clade-based aligner MetaPhlan2 (v2.7.7) pipeline (Truong et al. 2012).

Metabolic Pathways: Non-human reads were used to characterize metabolic function using the HMP Unified Metabolic Analysis Network (HUMAnN2) (v0.11.2) (Franzosa et al. 2018), specifying DIAMOND as the alignment algorithm and UniRef90 as the target database. Pathway abundance in a sample was normalized to copies per million (CPM) and stratified using the script "humann_infer_taxonomy" provided by HUMAnN2. This process utilizes data provided by the MetaCyc database (Caspi et al. 2020). Following the HUMAnN2 analysis, the relative abundances of the identified metabolic pathways were analyzed according to the Superclass and Subclass data provided by the MetaCyc database.

Analysis

Analysis of Diversity

In an attempt to expand upon previous studies that did not report microbiome diversity metrics of indoor green infrastructure (Dockx et al. 2022; Soininen et al. 2022), this section outlines the methods behind this initial exploration of potential mechanisms and benefits of indoor and human microbiome diversification through the use of indoor urban bioremediation infrastructure. First, taxonomic groups for which there are documented relationships with metrics of either human health or urban bioremediation infrastructure system performance were collected from the literature. Species-level Shannon Diversity within the taxonomic groups of interest that were also identified within the growth media samples were then compared to those identified within surface samples in both the experimental and control rooms.

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Taxonomic Groups of Interest, *Human Health*: Previous research has reported beneficial human health outcomes related to increasing diversity of 9 taxonomic groups, including phyla such as Bacteria, Proteobacteria, Bacteroidetes, Actinobacteria and Firmicutes, and classes such as Alpha-, Beta- and Gamma-proteobacteria, found in environmental and/or human associated microbiomes (Roslund et al. 2022; Roslund et al. 2020; Soininen et al. 2022). In particular, increasing relative abundance and diversity of the genus Lactobacillus may have a significant relationship with many metrics of human health (Roslund et al. 2022; Roslund et al. 2020; Soininen et al. 2022). Simultaneously, the diversity of many of these same taxonomic groups have been found to increase in sampled indoor microbiomes in the presence of plant-based systems (Dockx et al. 2022; Mahnert et al. 2015), although as discussed the mechanism of this diversification has not yet been reported. These 9 taxonomic groups of interest with previously described roles in human health will be referred to as "human health taxa of interest".

Taxonomic Groups of Interest, *Pollutant Metabolism:* From a pollutant metabolism perspective, 5 taxonomic groups, including the genera Hydrocarboniphaga and Hyphomicrobium, and families Nevskiaceae, Patulibacteraceae, and Xanthobacteraceae, have been identified as potential VOC metabolizing microorganisms in plant-based bioremediation systems (Mikkonen et al. 2018; Russell et al. 2014). These 5 taxonomic groups of interest with previously described roles in pollutant metabolism will be referred to as "pollutant metabolism taxa of interest".

Statistical Analysis

Statistical analyses were computed in R 4.2.2 (Team 2018) with the vegan (Oksanen et al. 2019) and tidyverse (Wickham 2017) packages. Uniform Manifold Approximation and Projection (UMAP) for Dimension Reduction analysis (Mcinnes et al. 2018) was used to explore overall trends in both the diversity and metabolism results. The Shannon diversity index (Shannon 1948) was used to compare species-level diversity within taxonomic groups of interest. Normality within groups of interest for both DNA yield and taxonomic composition datasets was tested using the Shapiro-Wilks normality test. Subsequent differences in each case between normally distributed data were tested using paired sample t-Tests. Differences between datasets that did not meet normality requirements of paired sample t-Tests were tested using Mann-Whitney-Wilcoxon tests. The significance of differences in metabolic pathway relative abundances found between the three growth media were calculated using Kruskal–Wallis tests. Chi-square tests

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were used to compare the number of metabolic pathways identified within each group, as well as the number of mapped and unmapped pathways. Summary statistics as well as additional p-values for all statistical tests can be found in the Supplemental Information.

Results & Discussion

Sample Extraction Results: DNA Concentrations and Inferred Microbial Material

The DNA extraction results illustrate the common challenge of collecting microbial material in indoor environments (Kelley and Gilbert 2013; Mchugh et al. 2021) and reveal a likely difference in DNA concentrations between the three growth media. Average DNA yields were higher in the organic growth media (GAIA: 16.0 +/- 10.5 ng) than either hydroponic growth media (HAC: 10.1 +/- 7.3 ng, HBC: 7.8 +/- 2.7 ng) by 58% and 105% respectively. Due many samples falling below the limit of detection, the hydroponic datasets were not normally distributed. Differences in DNA yield distributions were only statistically significant between the GAIA and HBC samples, however repeated tests assuming “undetectable” samples are valued at the limit of detection returns significant p-values between the three different growth media: GAIA, HAC, and HBC. None of the indoor surface samples collected in either the experimental or control rooms resulted in detectable DNA yields, however they all resulted in successful libraries. Calculations and summary statistics (e.g. average reads per sample, number of samples that created libraries) are reported in the Supplemental Information document and Supplemental Table 1.

The measured differences in DNA yield between the three growth media is likely due to the inherent nature of the hydroponic versus organic growth media: more organic material (which is limited in hydroponic systems) may have supported greater microbial activity. However, although the method of collection was designed to avoid collecting bulk growth media material, the results of this study cannot rule out the possibility that the activated carbon and biochar additives to the hydroponic media may have interfered in some way with the DNA extraction protocols as these materials have been used in filters to remove environmental organic contaminants (Reungoat et al. 2010). Barring such interference, these results may be used to estimate the relative microbial material in each growth media. Previous studies have found that

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while DNA yields from soil samples are correlated with microbial cell counts, this metric overestimates both cellular abundance and diversity by as much as 55% because this approach necessarily includes extra-cellular "relic" DNA (Lee et al. 2021). Given such an over-estimation, the DNA yield results in this study should not be extrapolated to estimate cell density within the growth media samples, however they can be used to compare the relative cellular abundance between the growth media samples, assuming the proportion of "relic" DNA in each growth media is consistent. Given this, the presented growth media results indicate that the organic growth media likely contained 58-105% more microbial cells than the HAC and HBC growth media respectively. None of the surface samples produced quantifiable DNA yields, so this comparison cannot be calculated between the growth media and surface samples, however even this outcome indicates the indoor urban bioremediation infrastructure systems described here, especially those including the organic growth media, could represent a comparatively rich source for dispersing microbial material within indoor airstreams. The number and viability of this microbial material would have to be evaluated using additional methods, such as culturing.

Sequencing Controls

Negative "field blank" controls were utilized in this study to control for sample contamination during the collection and extraction process. The DNA concentrations of these samples were below the level of detection of the Qubit® 3.0 Fluorometer, and for those that yielded libraries, sequencing outcomes were inconsistent between replicates. 281 species were collectively identified within all growth media and indoor surface experimental samples. 37 of these species were also identified within the field blank samples. 2 species were identified within the field blanks that were not identified within any experimental sample. None of the field blank species were found to be ubiquitously identified in all experimental samples. In addition, the 37 species identified in both experimental and field blank samples each had an average relative abundance of 0.97% within the experimental samples (see Supplemental Information). These two findings could indicate the relative abundance of the species identified within the field blanks may have been increased due to contamination in the experimental samples, although in this case we would have expected to identify the contaminant species in all experimental samples. Alternatively, if some or all the species identified within the field blanks were in fact due to contamination and not analytic artifact as their inconsistencies might suggest (Biesbroek et al. 2012), their relative

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abundance in the experimental samples were so low they were unlikely to significantly alter results. Due to these findings, the field blank control samples were included in all subsequent metagenomic analyses together with the experimental data to test these interpretations.

One potential contributor to these outcomes were the extremely low DNA yields in the field blank samples. In the future, “negative” samples might include standardized microbial DNA of known origin such as the ZymoBIOMICS Microbial Community Standard (Zymobiomics) in order to increase the reliability of sequencing for such samples, wherein “non-standard” identified species could be considered contamination. This approach would include an additional confounding factor, unintentional non-standard DNA contamination inherent to the standard, however it might increase our ability to identify non-sample DNA and remove off-target metagenomic artifacts due to insufficient DNA.

Microbiome Diversity

Despite the number of samples with "undetectable" DNA concentrations due to the limit of detection of the DNA concentration quantification process, 86 out of the 88 total samples collected and extracted successfully produced libraries using an “ultra-low” concentration Illumina sequencing approach. Species classification-based analyses resulted in two main findings: (1) samples cluster according to growth media design rather than plant selection (see Figure 2), indicating that growth media design has a greater impact on substrate diversity than plant selection, and (2) microorganism diversity within taxonomic groups linked to outcomes of interest (such as human health and VOCs metabolism) were higher within green wall growth media samples than indoor surface samples (see Figure 3), indicating that microbes within active green-wall growth media likely support potentially beneficial microbial communities. These findings reinforce many of the hypotheses cited in the literature above by contributing evidence for the impact of design criteria on green-wall microbiomes, as well as more extensive evidence of beneficial microorganisms within active green-wall systems for both human exposure and health, as well as potential pollutant metabolism.

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Growth Media Design v. Plant Selection

Uniform Manifold Approximation and Projection (UMAP) dimension reduction analysis of all species-level relative abundances for all identified taxon indicates significant clustering by growth media rather than plant selection (see Figure 2), a result which is supported by a significant Kruskal-Wallis test ($p < 2.2$ e-16).

These results indicate that fundamental growth media design decisions, such as substrate materiality as well as chemical versus organic fertilizers, result in significantly different microbial community profiles, while plant species selection is not a significant determining factor. This result aligns with previous findings that active green-wall system performance such as reductions of CO_2 concentrations varied more substantially by differences in growth media rather than plant species selection (Mankiewicz et al. 2022). Although this outcome appears to be robust, future studies should include more replicates: the replicates within each treatment group included in this study were insufficient for successful core microbiome and discriminant species analysis which would have allowed for greater insight into species-level diversity differences between the three growth media types. It is also important to note that although plant selection did not appear to significantly influence substrate microbial composition within *this* study with *these* plant species, studies in related fields suggest that more radical differences in species selections, such as those that shape rhizosphere microbiomes through root-exudates (Stassen et al. 2021), may lead to substantially different results in microbial community outcomes. What such differences may mean for human exposure and health would require further study.

Microbial Diversity and Taxonomic Groups of Interest

As discussed, one prevailing hypothesis within the field of indoor urban bioremediation infrastructure is that active air flow through plant-based systems could contain beneficial microorganisms, diversify indoor microbiomes, inoculate human microbiomes, and support measurable benefits to human health. Within this hypothesis are many falsifiable steps, two of which will be discussed in this section: (1) indoor urban bioremediation infrastructure systems contain beneficial microorganisms, and (2) indoor urban bioremediation infrastructure systems diversify indoor spaces with beneficial microorganisms. Of the 9 human health taxa of interest, 8 were identified in many of the experimental samples, with the exception of the genus Lactobacillus, which falls within the Firmicutes Phyla. Of the 5 pollutant metabolism taxa of

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interest, only one genus, Hyphomicrobium, was identified within the growth media samples. The other four (genus Hydrocarboniphaga and families Nevskiaceae, Patulibacteraceae, and Xanthobacteraceae) were not identified in any experimental sample. The calculated species-level Shannon diversity index for each taxonomic group of interest identified in each experimental group are illustrated in Figure 3.

Beneficial Microorganisms: All three growth media designs had significantly higher species-level diversity than both the experimental and control room surface samples within 5 of the human health taxa of interest: the kingdom Bacteria, the phylum Proteobacteria, two classes (Alpha- and Beta-proteobacteria), and the genus Hyphomicrobium (see Figure 3, p-values are reported in Supplemental Table 2). Within these 5 taxonomic groups, higher species counts were identified in the growth media samples in comparison to the surface samples (see Supplemental Table 3). This corresponds to higher species level relative abundance in the latter. Results differ within the remaining 3 human health taxa of interest identified within the samples. All three growth media had statistically significantly higher species-level diversity within the phylum Actinobacteria than the experimental room samples, however only the HAC growth media samples had higher diversity metrics than the control room. The species level diversity outcomes within the phylum Bacteroidetes did not differ between the growth media and surface samples in either room. Conversely, the diversity metrics within the phylum Firmicutes indicates that the experimental and control room samples had higher species-level diversity than the growth media samples. This is to be expected as this genera is made up of bacteria that are common commensal flora to human and animal organs (e.g. skin, gut, upper respiratory tract) but are relatively rare in soil samples (Parajuli et al. 2018). In comparing the species-level Shannon diversity indices within the pollutant metabolism taxa of interest, only species within the genus Hyphomicrobium were identified within this study. Although Hyphomicrobium species were identified in all three growth media, the Shannon diversity of this genus was higher in the hydroponic (HAC & HBC) growth media.

These findings indicate that the active green-wall systems included in this study contained higher species-level diversity than the indoor surface samples within taxonomic groups that have been previously connected to both human health and VOC metabolism, however these outcomes differ by system design (i.e. growth media), as well as the taxonomic group in question.

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Diversification of Indoor Spaces: Effectively spatializing growth media microorganisms within an indoor environment may require larger-scale systems with an airflow designed synergistically with the building's mechanical systems, longer timescales to establish diversified microbiomes, or more sensitive collection techniques than were included within this study. Although the results presented thus far indicate that beneficial microorganisms *were* present within the included indoor green infrastructure systems designs, we did not find evidence that these microorganisms were spatialized throughout the experimental room. Calculated species-level diversities within the taxonomic groups of interest were not statistically distinguishable from the control room samples, with the exception of Actinobacteria and Firmicutes (see Figure 3). This is unsurprising considering the scale of the included systems and orientation of the system fans (see Figure 1). A number of hypotheses can be derived from this finding: (a) the diverse microbiomes within the growth media were not effectively scaled or spatialized within the experimental room under the tested airflow conditions; (b) the three month timeline during which the bioremediation infrastructure systems were deployed was insufficient to diversify indoor surfaces; or (c) the method of sample collection within the two rooms were not capable of collecting sufficient microbial material to identify differences. Determining which of these hypotheses might be correct would require a careful examination of green infrastructure systems in association with the building airflow design, as well as the timeline and sampling protocols required to experimentally determine if inoculation is possible.

From an indoor green infrastructure design perspective, future systems designed to optimize for indoor microbiome diversification might consider the potential impacts of airflow design on inoculation. As illustrated in Figure 1, the indoor green infrastructure systems fabricated for this experiment required a 90° turn for airflow to exit the system. Although surfaces within the green infrastructure systems were not sampled in this study, airborne microorganisms leaving each pot may have been deposited on interior surfaces of the planters before exiting the system. Designers of future systems should consider altering the orientation and amplitude airflow exiting green infrastructure systems towards airstreams or surfaces that inhabitants might directly interact with, as well as sampling the interior surfaces of plant-based systems as necessary.

From a methodological perspective, both the timeline and sampling protocols of future experiments should be carefully considered. From a timeline perspective, controlled chamber

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studies have found that surface inoculation can take up to 6 months (twice as long as the presented study) if passive (non-active air flow) systems are used (Mahnert et al. 2015). Active air flow through plant-based ecosystems may reduce this time requirement, however this hypothesis requires further study. From a sampling and DNA analysis perspective, the concerns are two-fold: studies have found that the extraction efficiencies of DNA from commercially available swabs never exceeds 50%, and more often fall between 15 and 35% (Bruijns et al. 2018), and separate studies have found that microbial material concentrations can be so low indoors that results are dominated by primarily laboratory contamination (Kim et al. 2017). Such outcomes call for a sampling and extraction protocol with greater yields and efficiencies than swab-based sampling, such as emerging methods to collect airborne biological aerosols (Basapathi Raghavendra et al. 2023), as well as a more ubiquitous use of positive and negative controls and blanks processed alongside experimental microbiome samples in future studies (Kim et al. 2017).

Potential Mechanisms

Following the discussion of beneficial diversity and indoor inoculation hypotheses, one of the numerous questions left to explore within this arena is the mechanism by which indoor spaces might be diversified and how such effects might be passed on to human microbiomes. The results of this experiment support the hypothesis that indoor urban bioremediation infrastructure systems contain beneficial microorganisms, both for human health and potential VOC metabolism, however as discussed, indoor green infrastructure system design as well as sample collection and experimental timeline protocols must be revisited in order to better understand the potential for indoor green infrastructure systems to inoculate and diversify indoor surface microbiomes. In addition, although this experimental design focused on a potential inoculation pathway from the green infrastructure systems to indoor surfaces to occupants mediated by airflow through the growth media, other potential mechanisms should also be explored. For example, indoor air stream-associated microbiomes have been studied using active filtration sampling methods (Basapathi Raghavendra et al. 2023; Luhung et al. 2021), which would represent an entirely different mechanism of exposure. In addition, although this study focused on microbiomes located within the growth media due to the intersection of microbiome diversity and environmental pollutant metabolism potential, leaf-associated microbiomes are also diverse

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(Berg et al. 2014) and may represent another potential pathway towards human microbiome inoculation with interesting implications for a human behavior-mediated inoculation mechanism.

Metabolic Potential and Implications for IAQ

As discussed, a foundational hypothesis in the field of designing urban green infrastructure to bioremediate IAQ is that plant root-associated microorganisms metabolize airborne chemicals. Although "potential VOC-utilizing families" within such systems have been identified using 16S rRNA gene amplification and sequencing (Mikkonen et al. 2018; Russell et al. 2014), this method of analysis does not allow for insights into community metabolism potential. Figure 4 illustrates the analysis of the metabolic pathways identified in the indoor urban bioremediation infrastructure systems.

Once again, samples tended to cluster more closely by growth media (Figure 4A) than by plant treatment (Supplemental Figure 3), a result which is supported by a significant Kruskal-Wallis test ($p < 2.2$ e-16). This pattern of significant differences between growth media but not between plant treatments is the most consistent finding of this experiment, with clear implications for growth media as a primary criterion in the design and development of indoor bioremediation infrastructure. Within the growth media groups, the organic media (GAIA) samples returned fewer "mapped" pathways ($p < 0.001$, Figure 4B) and more "unmapped" pathways ($p < 0.001$, Supplemental Figure 4) than the hydroponic (HAC & HBC) samples. The UMAP dimension reduction of the dataset indicates the metabolic potential of the microbial communities differ by growth media, however significant variation in air quality remediation performance driven by these differences requires further measurement in parallel with airborne chemical quantification. Figure 4C is a line range plot of pathways within subclasses whose average CPM are above 300 and differ significantly ($p < 0.05$) between the three growth media, illustrating that many of the subclasses differ in abundance between one or more of the growth media. The influence that such differences may have on pollutant metabolism performance will require future experimentation to uncover: for example, within the presented dataset the subclasses "Aromatic Compound Biosynthesis" and "Aromatic Compound Degradation" did not differ substantially between the growth media, indicating that they may have similar BVOC production and anthropogenic VOC degradation potential.

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Conclusions

The results presented support two main findings with the field of indoor air bioremediation:

(1) both the organic and hydroponic growth media associated with active green infrastructure contained increased diversity related to taxonomic groups that have demonstrated both VOC metabolism and human health benefits, whith the organic growth media contained more of this microbial material;

(2) all three growth media designs resulted in unique combinations of community-level metabolic signatures.

In order for these findings to inform design recommendations for an active bioremediation system optimized for a particular context, further study is required to assess the relationships between: (a) species and metabolic pathway diversities within additional growth media designs, (b) system airflow design and its influence on indoor microbiome inoculation mechanisms, and (c) rates of microbial metabolism and how they may interface with pollutant remediation in the context of real-world indoor airstream behaviors.

Although the presented results indicate the included growth media designs represent rich reservoirs of beneficial and significantly different microbiomes, future work is required to better understand how these differences might be utilized to shape indoor environmental quality and achieve specific remediation goals. Greater DNA yields indicate the tested organic growth media contains 58 - 105% more biological material than the hydroponic media, however this is the only metric by which one growth media clearly out-performs the others. The rest of the presented analyses must be utilized in future work to determine how growth media differences might shape system performance and human exposures: Although the diversity outcomes (Figure 2) indicate the microbial diversity within the three growth media are different, all three growth media contained diverse microorganisms with potential health benefits (Figure 3). Similarly, although the metabolism analyses illustrated unique metabolic signatures within each growth media (Figure 4), future work must determine how these differences may translate into benefits to indoor airstreams. In addition, although the outcomes of this study indicate growth media design is likely a strong driver of indoor bioremediation infrastructure microbiomes and potential performance, they must be taken within the context of the limitations of current computation and

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analyses: conservative estimates report that over 99 % of global microbial taxa may remain undiscovered (Locey and Lennon 2016), which means additional patterns within the collected dataset may be uncovered as more information becomes available. A strength of the shotgun metagenomic sequencing approach, however, is that as more species and metabolic pathways are identified, the sequences collected during this experiment can be re-analyzed according to updated databases and algorithms to characterize microbial diversity and metabolisms.

Relationships between indoor microbial diversity and metabolism, indoor air quality, human exposure, and human health outcomes remains a research frontier in understanding how urban lifestyles impact human health and wellbeing. Studies conducted in diverse contexts continue to demonstrate that urban and environmental design decisions, from window operability (Kembel et al. 2014) and material choices (Simons et al. 2020) to plant-based systems (Soininen et al. 2022), impact urban and human microbiomes, which in turn have demonstrated impacts to human health outcomes.

Although built environment design and sanitation has been influenced in many ways by the need to limit pathogens and indoor exposure to disease, the accumulating body of literature linking human microbiome diversification and health benefits with exposure to diverse environmental microbiomes supports a movement towards augmenting filtration and ventilation paradigms that limit negative exposures with supplemental systems that can improve positive exposure through diversification. This study describes relationships that indicate fundamental material design choices within indoor urban bioremediation infrastructure systems, especially organic versus hydroponic growth media design, might one day become a mechanism by which indoor microbial diversity and metabolisms could be intentionally shaped through design with potential benefits for human exposure and health in mind.

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Impact

The significance of this work supports findings that growth media should be a fundamental design consideration of indoor bioremediation infrastructure (Mankiewicz et al. 2022). The presented results establish that differences in growth media, such as organic fertilizers or biochar additives, result in significant differences in microbial diversity and metabolic pathways that could transform system performance at the scale of human exposure. Future work must develop upon these findings by

(1) Increasing the scale of bioremediation systems within indoor spaces
(2) Designing and comparing the trade-offs between system airflow direction and amplitude to spatialize beneficial growth media microorganisms within indoor spaces to improve urban microbiome abundance and diversity
(3) Quantifying the impact of different growth media microorganism metabolisms on airborne pollutant concentrations
(4) Determining if bioremediation system impacts to indoor microbiomes and airborne pollutants result in improved human exposures and long-term health outcomes.

Although many questions remain as to the specific impacts growth media design may have on indoor environmental quality and human health outcomes, this study indicates growth media design could revolutionize our approach to bioremediation system design. By raising growth media as a fundamental design criterion, future research can focus specifically on how growth media materiality, additives, and fertilizers shape complex soil-plant-microbial relationships to improve indoor air and microbiome quality, reduce mechanical system energy consumption, transform human exposure, and ultimately improve long-term health outcomes for human urban inhabitants. As such, future indoor bioremediation systems could represent significant societal benefits for dense urban areas including reduced embodied building energy costs of mechanical equipment, improved indoor environmental quality, improved exposures for indoor occupants, reduced short- and long-term negative health outcomes, increased productivity and executive function, and improved short- and long-term positive health outcomes.

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Author Contribution Statement

Phoebe Mankiewicz: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Project administration. **Chandrima Bhattacharya:** Software, Validation, Formal analysis, Data Curation, Writing - Review & Editing, Visualization. **Anna Dyson:** Conceptualization, Methodology, Resources, Writing - Review & Editing, Visualization, Supervision, Project administration, Funding acquisition. **Elizabeth Hénaff:** Conceptualization, Methodology, Validation, Resources, Data Curation, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

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Conflict of Interest Statement

Competing Interests: None

Ethics Statement

This study was conducted in accordance with the CambridgeCore publishing ethics.

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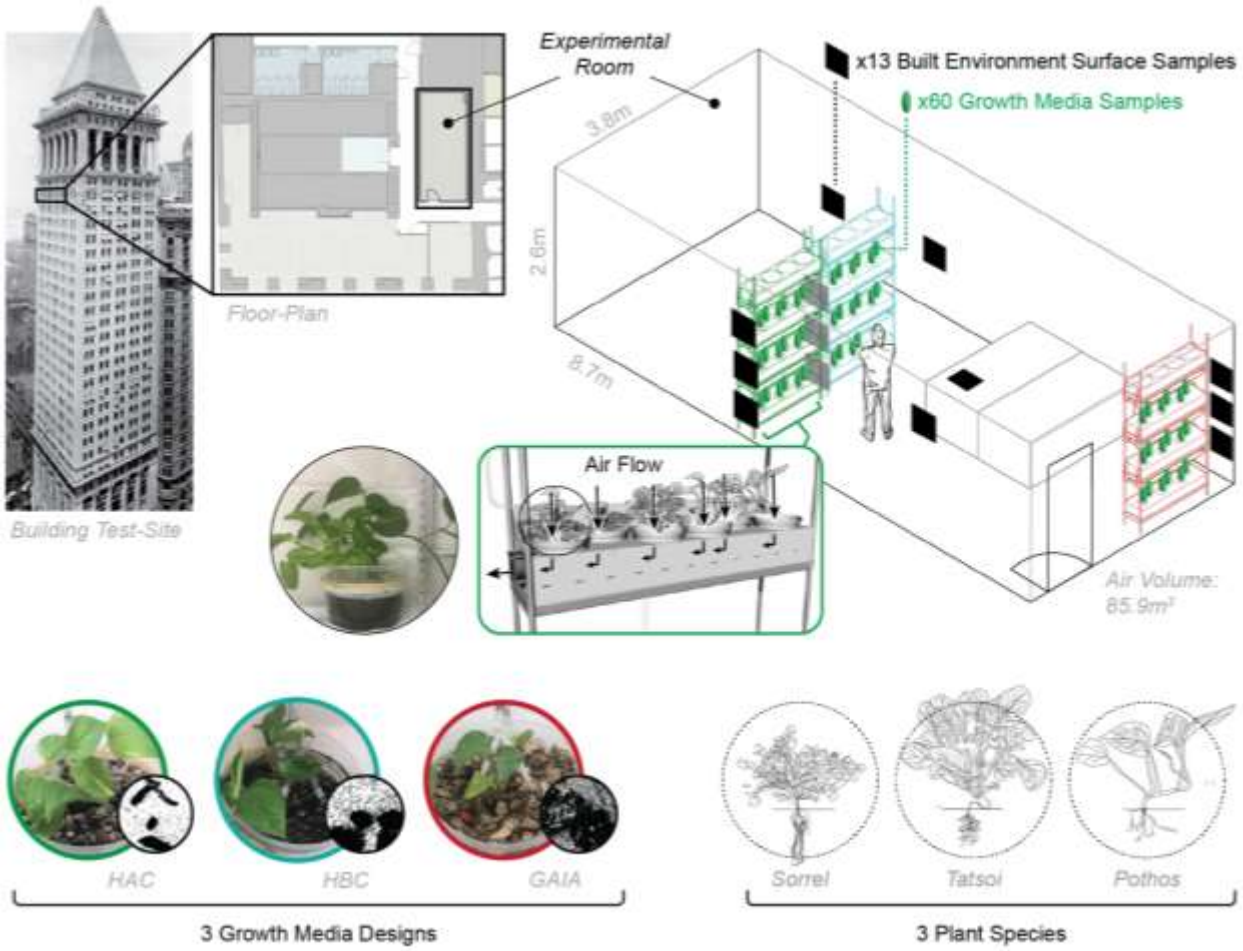

Figure 1: A depiction of the building context, floor plan, and three-dimensional representation of the experimental room. All indoor green infrastructure systems are represented, each colored according to the growth media treatment of each system. Sample collection sites, including both growth media samples (green ovals) and surface samples (black squares) are illustrated spatially throughout the experimental room model. A rendering of a single shelf system, fan, and airflow through the pots, as well as illustrations of the growth media and plant species included in individual pots are included for reference.

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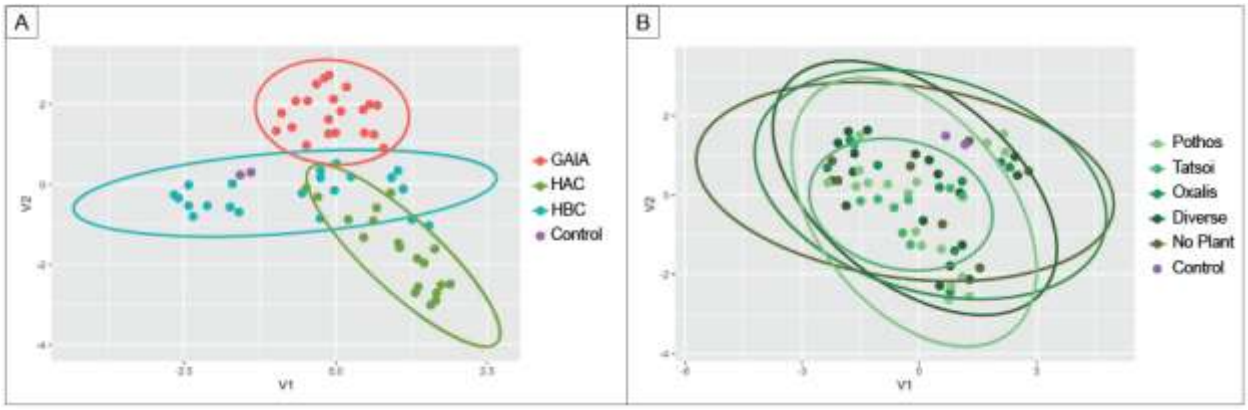

Figure 2: Dimension reduction analysis of shotgun metagenomic outcomes indicates data tend to cluster by (A) growth media design, rather than (B) plant selection. The "Control" samples in this case were the negative field blank controls. Repeat UMAP analysis excluding the field blank controls resulted in nearly identical outcomes.

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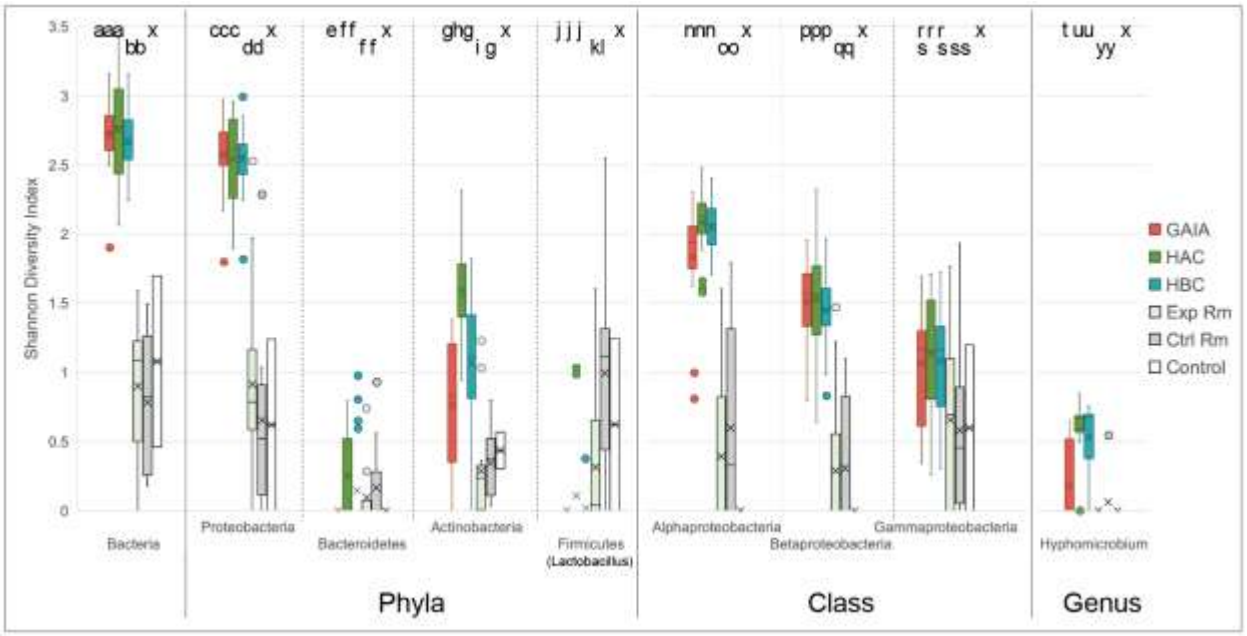

Figure 3: Species-level Shannon diversity index calculations within taxonomic groups of interest. Statistically significant differences are denoted by the letters at the top of the figure. Each letter denotes groups of variables that were not statistically significantly different within the taxonomic group in question. The statistical tests (T test or Wilcox test, dependent on independent verification of normality) and p-values can be found in Supplemental Information Table 2. Due to limited replicates, field blank "Control" samples were not statistically compared (denoted by "x"). Shannon diversity indices were not statistically compared between taxonomic groups.

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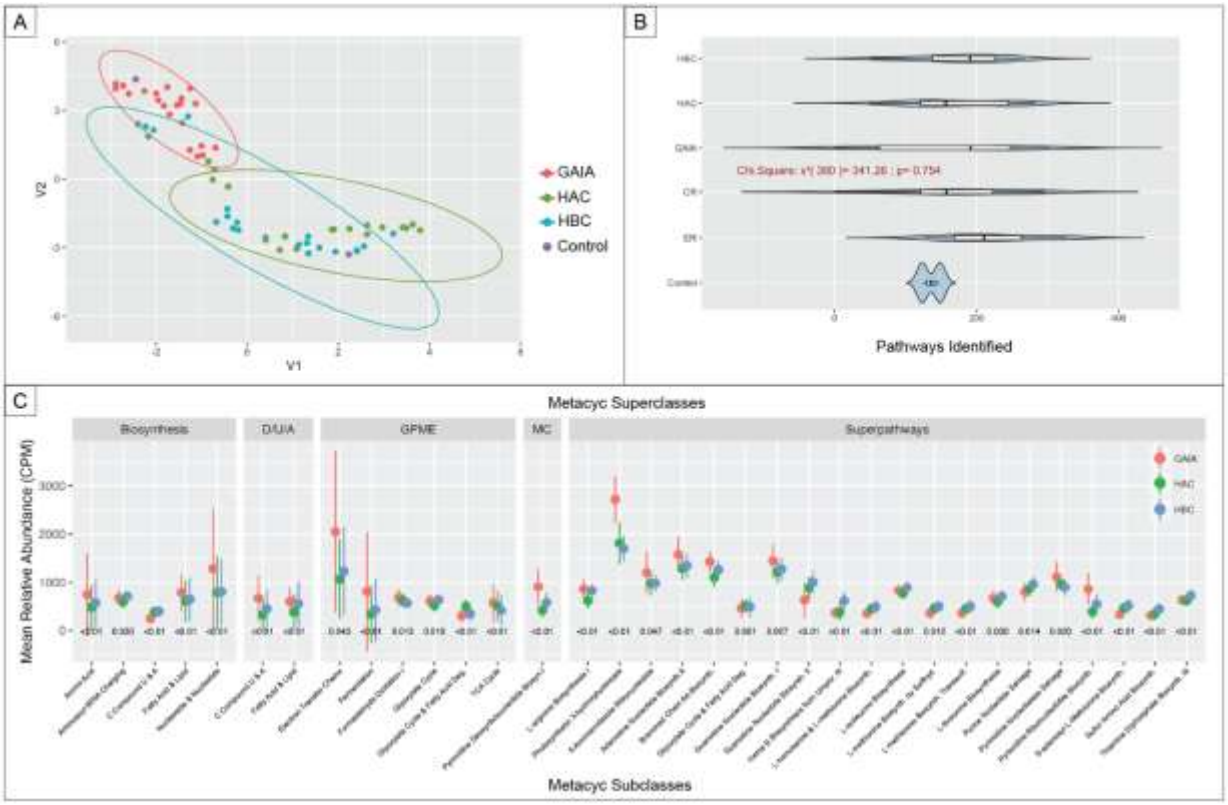

Figure 4: Analysis of the Humann2 outcomes for the sequenced samples by growth media group: (A) UMAP clustering of all identified pathways, (B) Violin plot of the number of identified pathways within each growth media and location, (C) Line range plot of metabolism subclasses that differ significantly between growth media. Kruskal-Wallis p-values are reported on the x-axis. Means and two standard deviations are shown for subclasses where all three growth media group means are over 300 CPM. Superclass abbreviations are as follows: D/U/A (Degradation, Utilization, Assimilation), GPME (Generation of Precursor Metabolites and Energy), MC (Metabolic Clusters).

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