Differential abundance and metagenome functional composition of microbiomes suggests genetic basis for survivability of specific genera on plastic and wood surfaces in the HI-SEAS IV built environment

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SUMMARY The Hawaii Space Exploration Analog and Simulation Mission was a year-long space isolation study aimed to investigate the effects of space travel and isolation on microbial community composition. Material types used for isolated spacecraft environments have been suggested to select for microbes resistant to extreme environments and sanitation processes, thus creating uniquely resistant populations of microbes. Consideration of changes to isolated microbiomes on abiotic surfaces found within the HI-SEAS environment may provide further insight into their role in the crew's health. In this study we aim to characterize the impact that surface materials have on the diversity of microbial communities found on abiotic surfaces in the HI-SEAS environment, as well as determine if there is a difference in the prevalence of gene families that play a role in microbial survivability on these surfaces. Our results demonstrate an increased microbial diversity between plastic compared to wood surfaces and unique taxonomic community structures between the two surface materials alongside differentially abundant pathways linked to material-specific survival and metabolism.

INTRODUCTION

The Hawaii Space Exploration Analog Simulative IV (HI-SEAS IV) was a year-long confined space isolation study that investigated the effects of limited space and isolation on microbial communities, and whether these conditions would result in adverse or unexpected changes in composition in anticipation of future space exploration events (1). Since microbiomes have been shown to intimately affect and interact with human health, especially in confined environments such as space travel (2), consideration of biotic and abiotic microbiomes within the HI-SEAS IV setting may provide insight regarding potential microbial composition changes that could arise during extraterrestrial missions with similar environmental parameters.

The HI-SEAS IV mission consisted of 6 individuals within a confined dome located on the slope of the Mauna Loa volcano over a 1-year period. Data was collected via swab or wipe samples from various locations in the dome, which includes different abiotic surfaces within the habitat and samples from the skin of the 6 individuals every other week over the period of the mission. Abiotic surfaces present in the HI-SEAS IV environment included wood (e.g. kitchen counter) and plastic surfaces (e.g. toilet bowl, bedroom desk, and main room desk). A total of 186 samples including the laboratory controls were collected. Seventy-eight samples from the plastic abiotic surfaces were retrieved, along with 26 samples from the wood surfaces. Collected swab and wipe samples were stored at -80 Cº, then extracted, amplified, and sequenced for the V4 region of the 16S rRNA gene using next-generation sequencing techniques. Finally, these samples were sequenced and output in the fastq format (1).

The HI-SEAS IV dataset has previously been investigated by Manhnert *et al.*, which explored the longitudinal dynamics of microbial communities over the study period on both crew members and abiotic surfaces, as well as the interactions between them (1). An eventual "microbial homogenization" between the microbiomes of individuals and abiotic surfaces was observed by Mahnert *et al.*, and overall, they found that microbial communities on abiotic

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surfaces were relatively stable in terms of composition and diversity, whereas skin profiles of the crew were highly dynamic (1).

Li *et al.* also investigated the HI-SEAS IV dataset, focusing on the differences in microbial diversity by surface material type (3). The results obtained by Li *et al.* were in agreement with the conclusions made by Mahnert *et al.* Furthermore, Li et al found that microbial communities on plastic surfaces have significantly greater alpha diversity and are significantly different from those on wood surfaces, indicating unique microbial community structure on different abiotic surfaces.

Microbial communities and microbes that colonize abiotic surfaces in built environments (e.g., the HI-SEAS IV habitat) likely play a role in the health of inhabiting individuals (4). It has been shown that abiotic surfaces found in different environments are composed of vastly different communities, suggesting that selection based on external factors or abiotic material plays a role (5). Due to the close and constant human contact with abiotic surfaces and possible health impacts, it is important to understand the effects different abiotic surfaces will have on microbe composition.

In terms of the impacts of abiotic surfaces undergoing similar uses in human environments, there has been extensive research on differences in microbial ecology of plastic versus wooden cutting boards. A study by Ak *et al.*, comparing microbiota after inoculation of plastic and wood cutting boards, observed that wood harbours less bacteria (6). They suggest this difference is due to the rough surface and antimicrobial molecules produced by wood inhibits the growth of microbes (6). Another study by Chiu *et al.* found that *Vibrio parahaemolyticus,* a gram-negative bacterium which causes gastrointestinal illness in humans, survived better on plastic cutting boards compared to wooden boards. The authors suggest that this is due to the smooth surface of the plastic boards retaining more moisture and maintaining a favourable environment for microbes (7). Finally, a study by Gough *et al.* found that wood cutting boards harboured more *Salmonella typhimurium* compared to plastic (8). However, material type may not systematically affect all types of microbial species equally. A study by Miller *et al.* investigating *Escherichia coli* contamination after contact with raw meat products found no difference in contamination regardless of plastic or wood material (9). With regards to the HI-SEAS IV dataset, it should be noted that samples collected from the desks in the main room and bedroom were from fiberboard surfaces laminated with plastic, which results in their classification as plastic surfaces (1). This may introduce differences when compared to previous studies using purely wood or plastic surfaces.

Building on previous research, the aims of this study were to first confirm that different abiotic surface materials result in differential microbial diversity and then to further investigate the impact of wood vs. plastic surfaces on abundances of specific microbes. We hypothesized that specific microorganisms (e.g., *V. parahaemolyticus*) survive better on plastic surfaces and others (e.g., *S. typhimurium*) on wood surfaces due to intrinsic properties of the material (7,8). Clear differences in species diversity and abundance between wood and plastic surfaces would support this hypothesis. Furthermore, the presence of surface-specific metabolites or pathways would provide evidence of the genetic basis of differential colonization and survival as a result of the intrinsic properties of abiotic surface material and not other confounding variables.

We found that the two surface materials harbored microbial communities with significantly different diversities, with overall higher diversity in plastic surfaces compared to wood surfaces when phylogenetic distance was considered. Differences in the relative abundance of *Staphylococcus* and *Brevundimonas* were also found on plastic and wood surfaces, respectively. Inferred metagenomic pathway analysis further revealed surface-specific differentially abundant orthologs and pathways associated with wood or plastic molecule degradation and utilization, suggesting that the intrinsic properties of plastic or wood surfaces affects the abundance of specific microbes on both surfaces.

METHODS AND MATERIALS

The HI-SEAS IV Environment. The HI-SEAS IV environment was an isolated built environment mission operated by the University of Hawaii and funded by NASA spanning

from August 28th, 2015, to August 28th, 2016. The built environment consisted of a 1200 square foot dome at 2.5km of altitude on the slope of Mauna Loa, on the Island of Hawaii in the U.S. Six crew members were selected based on potential astronaut profiles and resided in the environment throughout the mission to carry out research work mimicking interplanetary conditions such as collecting outdoor samples in mock spacesuits, as well as household chores and physical exercise. Food was supplied to the environment via 9 total resupply events, and included dried, canned, and fermented food (1).

Cleaning of abiotic surfaces within the habitat were conducted every Sunday by the crew with Simple Green's cleaner, vacuuming or bleach-based cleaner as needed. Bokashi composting bins and composting toilets were utilized within the environments (1).

Sample collection and extraction. Samples in the HI-SEAS IV environment were collected every other week starting from Sept 4th, 2015, to August 5th 2016. Abiotic surfaces swabbed in 4 locations (Toilet bowl, kitchen floor, bedroom desk and main room desk), for a total of 111 swab samples from abiotic surfaces, while 66 biotic wipe samples were taken from crew by wipes (1).

For each abiotic surface sample, a sterilized swab was moistened with autoclaved water and swiped over a 5 × 5 cm area. Samples were then stored at -80°C until DNA extraction and analysis were carried out. Controls during each sampling session were collected by waving the swab in the air for a few seconds without contact to any surfaces (1). QIAGEN DNeasy PowerSoil Kit was used to carry out DNA extractions for these samples (QIAGEN, Hilden, Germany) (1).

Metadata associated with the HI-SEAS IV mission is available on Qiita (https://qiita.ucsd.edu/) (study ID 12858). Samples information is tracked for categories such as collection surface, collection date, and collection site.

16S rRNA gene amplicon generation and sequencing. Amplicon sequence data for the HI-SEAS IV environment was collected by Mahnert *et al.* and described in their original study. Refer to the Methods section for details such as reagent concentration, PCR cycle lengths and other experiment-specific protocols (1). The V4 region of the 16S rRNA gene was amplified using the F515-R806 primer pair with Illumina tags to capture bacterial and archaeal profiles (10). Refer to Mahnert at al. for protocol-specific PCR conditions and lengths (1). Next-generation sequencing was performed on all samples by the Core Facility Molecular Biology at the Center for Medical Research located within the Medical University Graz, Austria. DNA concentrations were normalized and indexed with barcode sequences, pooled and purified via gel cutting, then sequenced on an Illumina MiSeq instrument with the MiSeq Reagent Kit v3.

Sequence quality control. Sequences were processed using QIIME2 version 2021.4.0 (11). Demultiplexed forward sequences were truncated to 290 bases and denoised using DADA2 (12) The truncation length was chosen to remove low-quality bases at the end of sequences while capturing the entire V4 region (254 bp). The resulting amplicon sequence variants (ASVs) are exact, unique sequences theoretically each representing a unique organism. They are termed 'features' in QIIME2, which produces a representative sequences file containing the ASVs as well as a features table for further analysis. Command details for this and all subsequent QIIME2 analysis can be found in the supplemental script, script1.sh.

Phylogenetic tree and taxonomic classification. To compute alpha and beta diversity metrics including Faith's phylogenetic distance (Faith's PD) (13), unweighted (14) and weighted UniFrac (15), representative sequences were inserted by alignment into the SILVA 128 reference tree backbone using QIIME2 (16-21). Representative sequences were then classified by a Naïve Bayes trained classifier (22) based on the SILVA 138 99% OTU's reference tree (16,17), which recognizes the 250 bp V4 hypervariable region amplified by the 515F/806R primers (10).

Dataset filtering and rarefaction. The features table was filtered using QIIME2 to remove low abundance (rare) ASVs that accounted for less than 0.005% of total reads. The table was subsequently filtered to retain only the abiotic surface samples. Furthermore, mitochondrial

sequences were filtered out (23, 24). The QIIME2 diversity alpha-rarefaction plugin was used to subsample the features table at different depths, calculating alpha diversity (Shannon's diversity for our dataset (25)) to generate alpha rarefaction curves (12, 23, 24). To maximize the samples retained and the library size, a rarefaction depth of 14,710 was selected.

Alpha and beta diversity analysis. Using the filtered features table and phylogenetic tree, the QIIME2 diversity function generates the core alpha and beta diversity metrics for each sample, sorting them based on their metadata (11, 18-21, 23, 24). For phylogenetic data, we assessed Shannon's (25) and Faith's (13) alpha diversity, as well as Unweighted (14) and Weighted Unifrac (15) beta diversity metrics. Pathway abundance data was analyzed with Shannon's alpha diversity (25) and Bray Curtis beta diversity metrics.

Differential and relative abundance plots. Outputs from the QIIME2 pipeline were then exported to R and visualized using R studio using the CRAN packages tidyverse, vegan, and ape, and Bioconductor packages phyloseq and DESeq2 (26-30). Relative abundances for each ASV as classified previously were calculated in R studio. Differential and relative abundance analyses were performed on the filtered table with taxonomy information and our phylogenetic tree with DESeq2 (26). Detailed commands for the visualization of alpha and beta diversity plots and all subsequent analysis in R can be found in the supplemental R script (script2.R).

Inferring metagenomic functional profiling. Functional metagenomic profiles were inferred using a standalone installation of PICRUSt2 2.4.1 (31). In brief, PICRUSt2 allows for inference functional metagenomic contents of each sample, which can then be categorized by frequencies of KEGG pathways. The filtered ASV frequency table, with rare ASVs and mitochondrial sequences removed as described in the section "Dataset filtering and rarefaction, was inputted into PICRUSt2. HMMER 3.3.2 was used to organize the study sequences into a reference multi-sequence alignment, which was then placed in PICRUSt2's reference phylogeny using EPA-NG (20,32). PICRUSt2's reference phylogeny utilizes the Integrated Microbial Genomes (IMG) reference database (33). Following, the castor package was run for hidden state prediction of gene family abundances, with the maximum parsimony method (34, 35). The data had to be filtered such that any metabolic predictions accurately represented the sequences. Nearest-sequenced taxon index (NSTI) values are a scoring system to measure the sum of the phylogenetic distances from ASVs to their inferred closest related organism from a reference genome (42). This is evaluated through the number of base pair substitutions in the 16S rRNA gene and is also weighted by the frequency that the ASV appears. Hidden state predictions were filtered to a NSTI cutoff of under 2, since values higher indicate uncharacterized phyla or off-target sequences (31). Marker gene abundances were normalized using predicted 16S rRNA copy number for each ASV (31). KEGG orthologs (KOs) and MetaCyc pathway abundances were then outputted through PICRUSt2 (36, 37).

Differential and relative abundance between surfaces was then analyzed through STAMP version 2.1.3 (38). Significance pathways and KOs were determined using a Welch's T test, and multiple comparisons were accounted for using a Benjamini-Hochberg FDR procedure (39, 40). Pathways and KOs were filtered to only ones with a proportional relative abundance of 5 or higher. This effect size was chosen was to eliminate the inferred genes that did not have clear differences in relative abundance, as defined by the ratio between the absolute abundance between surfaces. This was also done to filter out inferred genes that were determined to be differentially abundant, only because one surface type had near zero expression and the other had minimal expression. Doing so allows for a qualitative examination on the genes that have a higher likelihood for providing a fitness benefit for microbial organisms on a specific surface type for microbial organisms on a specific surface type. Refer to supplemental scripts (picrust2_1.sh and picrust2_2.R) for specific commands and parameters for analysis of metagenomic functional profiling, interpretation, and visualization.

RESULTS

Microbiomes on plastic surfaces have significantly greater alpha diversity compared to those on wood surfaces, and beta diversity significantly differs. Since we were interested in the differences in microbiome diversity and differential microbe abundance on wood and plastic surfaces, we first carried out alpha and beta diversity analysis of wood and plastic surfaces using the QIIME2 pipeline with the methods described above to confirm the differences between the two surface materials. While the median alpha diversity score of samples collected from plastic surfaces is greater when measured in both Shannon's diversity and Faith's PD , pairwise Kruskal-Wallis test results from Shannon's diversity indicate that there is no significant difference (q-value = 0.08) (FIG. 1A). On the other hand, pairwise Kruskal-Wallis test results for Faith's PD suggests that there is a significant difference (q-value = 4 x 10^{-6}) (FIG. 1B) (13, 25). We also observed that Faith's scores are about 4-fold higher than Shannon's. Both observations together suggest that differences in diversity on the surfaces and their differences are strongly influenced by phylogenetic relatedness.

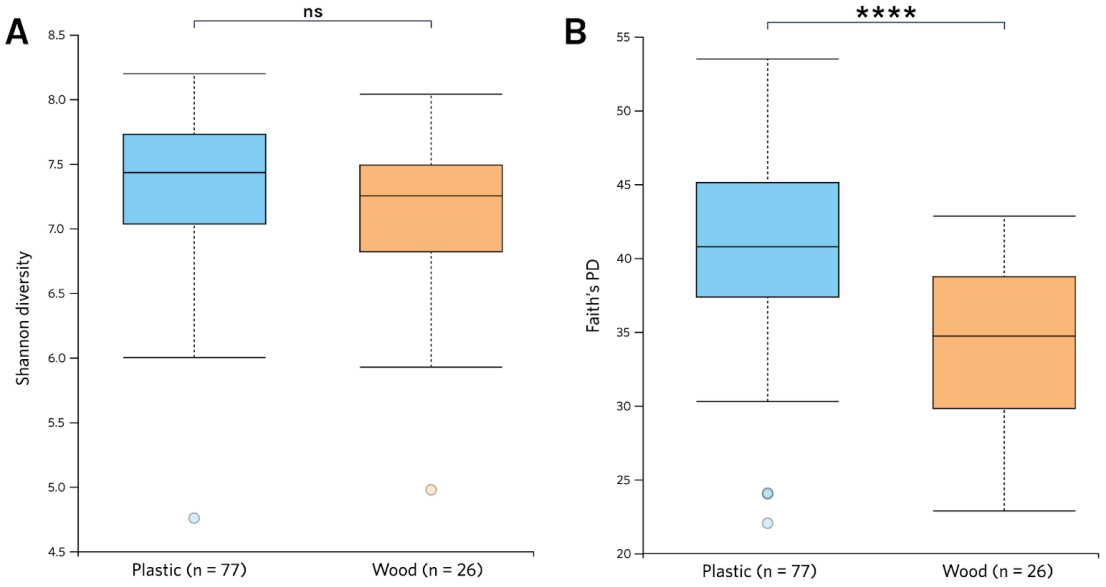

FIG. 1 Plastic microbiomes have greater alpha diversity than wood microbiomes according to Faith's phylogenetic diversity. Boxplots show the distribution of **(A)** Shannon's diversity scores in plastic (n = 77) and wood samples (n = 26). q-value = 0.08 (pairwise Kruskal-Wallis tests, α = 0.05) and **(B)** Faith's phylogenetic diversity scores in plastic (n = 77) and wood samples (n = 26). q-value = 4 x 10^{-6} (pairwise Kruskal-Wallis tests, α = 0.05). Outliers are indicated by color-coded dots. q-values less than 0.0001 are indicated by (****), and q-values greater than 0.05 are marked with ns (not significant).

Discrete clusters are observed for plastic and wood samples in both Unweighted and Weighted PCoA plots (FIG. 2). This is supported statistically by pairwise PERMANOVA test results indicating that plastic and wood surface beta diversity differs significantly in both Unweighted and Weighted Unifrac (both q-values = 0.001) (14, 15, 41). This is surprising as Weighted Unifrac considers abundances in addition to phylogenetic distance. The former of which did not yield a significant difference in alpha diversity (Shannon's). However, given that beta diversity metrics are more sophisticated, this suggests that abundance does play a role in diversity differences.

***Staphylococcus* and *Brevundimonas* are more abundant on plastic and wood surfaces, respectively.** To better understand the diversity differences observed between the two surfaces, differential abundance analysis was carried out at the genus level to elucidate which genera may be causing the observed differences in diversity on the different surfaces. A total of 45 genera were found to be differentially abundant between wood and plastic surfaces, with 32 genera being more abundant on plastic surfaces and 13 more abundant on wood surfaces (FIG. 3). These genera exhibit significant differences in relative abundance by

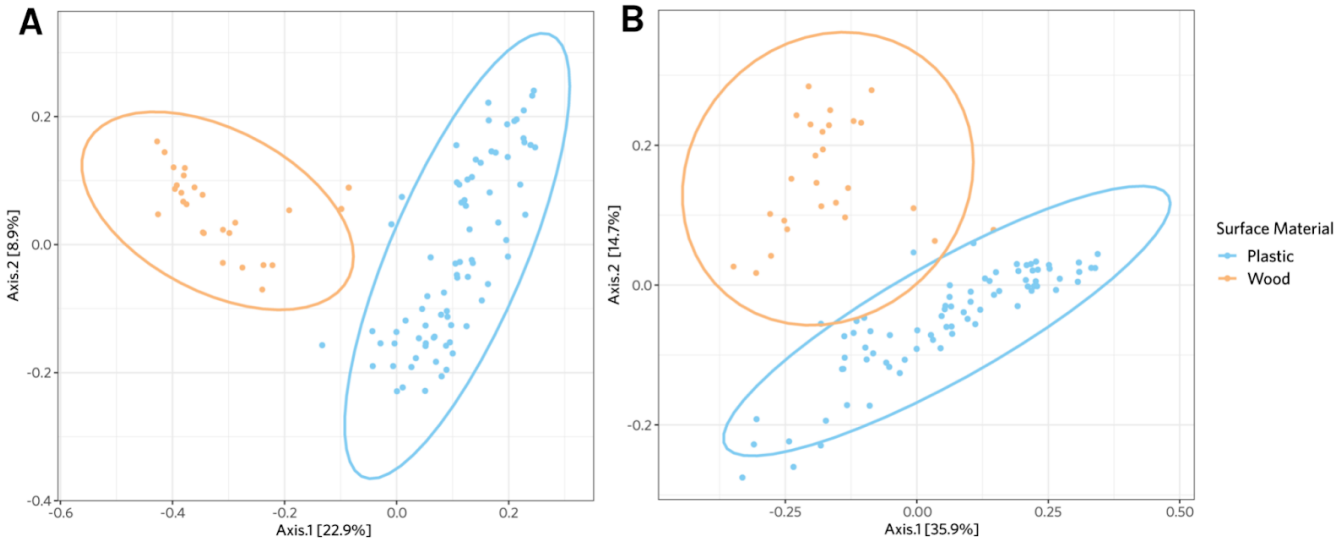

FIG. 2 Beta diversity is significantly different between plastic and wood surfaces. Principal coordinate analysis (PCoA) plots based on **(A)** Unweighted Unifrac distance. q-value = 0.001 (pairwise PERMANOVA tests, α = 0.05) and **(B)** Weighted Unifrac distance. q-value = 0.001 (pairwise PERMANOVA tests, α = 0.05). Coloured dots indicate samples; blue are plastic (n = 77) and yellow are wood samples (n = 24). Percent variance accounted for by axis 1 and 2 are displayed next to the axis label.

surface material type. The *Rothia* and *Neisseria* genera were found to be the top 2 most differentially abundant on plastic surfaces, while the *Tsukamurella* and *Methylobacterium-Methyloruburm* genera were more commonly found on wood surfaces. However, after performing relative abundance analysis on each differentially abundant genera, only *Staphylococcus* and *Brevundimonas* showed a median relative abundance higher than 5% in plastic and wood surfaces respectively (FIG 3B & 3C). Given that genera with very low relative abundance are likely due to outliers or sampling error, we will limit our discussion to these two genera.

Plastic and wood surfaces within the HI-SEAS IV built environment are predicted to have differentially abundant functional metagenomic profiles. Finally, to explore the possible genetic basis allowing for differential taxa to proliferate better on one surface material relative to another, the PICRUSt2 pipeline was executed on the 16s rRNA data for functional metagenomic profiling (34). The PICRUSt2 software package allows for prediction of functional compositions using a marker gene, such as the 16S rRNA used here. First, the data had to be filtered such that any metabolic predictions accurately represented the sequences. An NSTI value above 2 indicates uncharacterized phyla or off-target sequences that degrade the accuracy of inferring metagenomic profiling (31). 228 out of our 14021 ASVs had NSTI values above 2, resulting in the removal of these ASVs. Additionally, 100% of our samples (n=104) had NSTI scores below 0.15, suggesting that functional metagenomic predictions are holistically accurate to our ASVs within the samples.

As a preliminary step to determine if there is a difference in the functional metagenomic profiles between samples from plastic and wood surfaces, alpha and beta diversity was executed between these samples using the q2-PICRUSt2 package. A pairwise Kruskal-Wallis test for the Shannon alpha diversity data indicates that there is no significant difference in the overall amount of metabolic pathway diversity in these samples (q-value = 0.33)(FIG. 4A). Conversely, A visualization of Bray-Curtis beta diversity through PCoA plot suggests that there is distinct clustering for both plastic and wood originated samples. (Fig. 4B). To further validate the beta diversity data, a PERMANOVA significance test was executed. Pairwise PERMANOVA results indicate that there is a significant difference between the functional metagenomic profiling of samples from these two surfaces (q=0.001). Therefore, further differential abundance analysis on these data will be useful to elucidate the differences in metagenomic profiling of samples from these two surfaces.

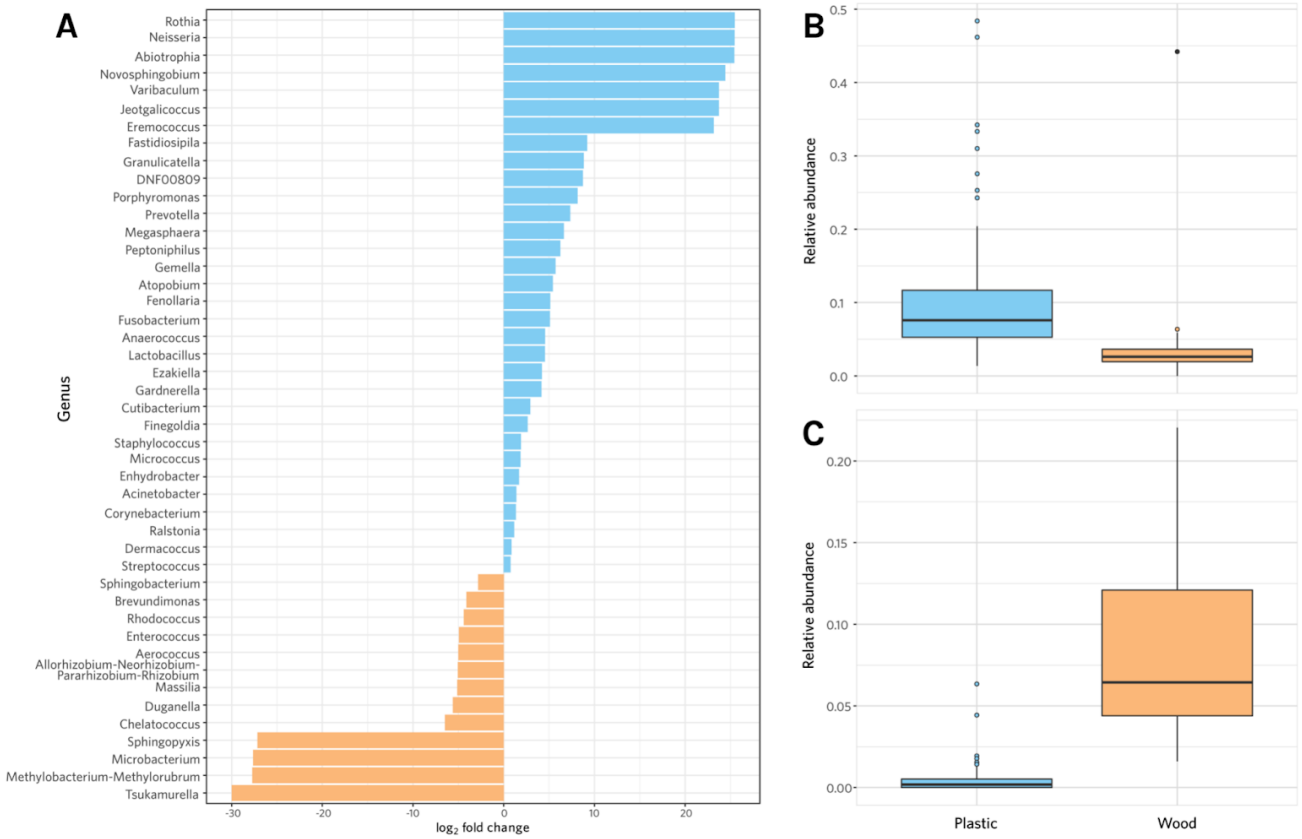

FIG. 3 Plastic and wood surfaces have differentially abundant genera. (A) Differential abundance analysis of plastic and wood surfaces at the genus level. Positive and negative log2 fold change indicates higher abundance in plastic and wood surfaces, respectively. Boxplots show the distribution of relative abundance of **(B)** *Staphylococcus* and **(C)** *Brevundimonas* on plastic (blue) and wood (orange) surfaces. Outliers are indicated by color-coded dots.

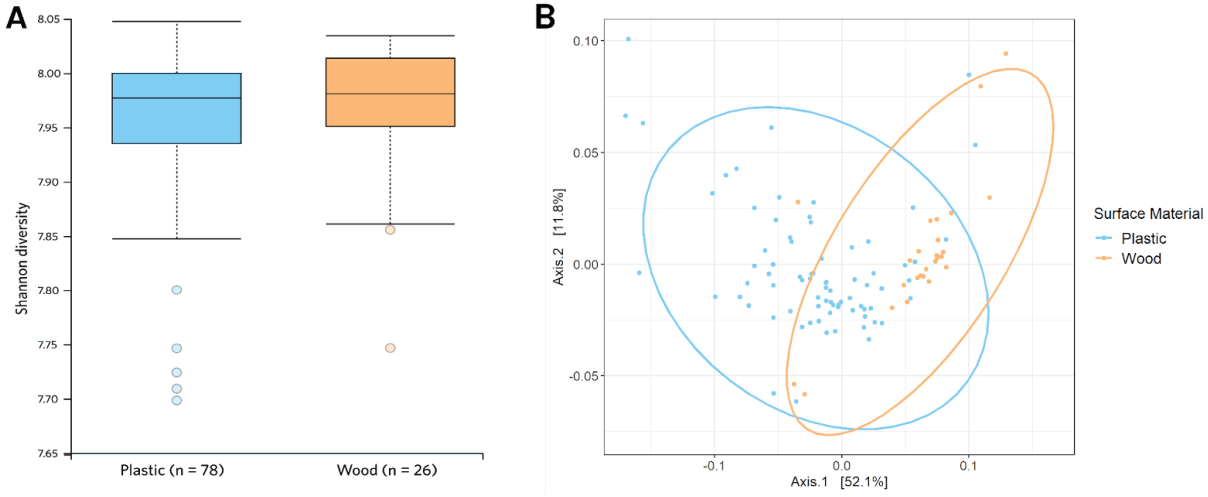

FIG. 4 Pathway abundances between microbiomes on plastic and wood surfaces do not have different alpha diversity, but do have different beta diversity. Boxplots show the distribution of **(A)** Shannon's diversity scores in plastic and wood samples. q-value = 0.33 (pairwise Kruskal-Wallis tests, $\alpha = 0.05$) Outliers are indicated by color-coded dots. **(B)** Principal coordinate analysis (PCoA) plots based on Bray-Curtis distance. q-value = 0.001 (pairwise PERMANOVA tests, $\alpha = 0.05$). Coloured dots indicate samples; blue are plastic (n = 77) and yellow are wood samples (n = 24). Percent variance accounted for by axis 1 and 2 are displayed next to the axis label.

Utilizing the full PICRUSt2 pipeline, 6481 KEGG orthologs functions (KOs), and 392 MetaCyc pathways were discovered on the abiotic surfaces. Metabolic data was then subset by sampling surface type of either plastic or wood, which allowed for differential metabolic abundance analysis using (Statistical Analysis of Metagenomic Profiles) STAMP (38). 144 and 242 metabolic pathways were significantly and differentially abundant for samples from plastic and wood samples respectively. On the same note, 2013 and 1938 KOs were significantly and differentially abundant for plastic and wood samples, respectively.

Following filtering for a high abundance effect size, as discussed in the methods section of this paper,8 KOs and 12 MetaCyc pathways, with statistical significance and a high effect size, were differentially abundant in samples on plastic surfaces (FIG. 5). In contrast, 10 KOs and 15 Metacyc pathways, with statistical significance and a high effect size, were abundant in samples on wood surfaces (FIG. 5). One interesting differentially abundant KO K07133 represented in samples from plastic surfaces had the highest effect size, yet no information on the metabolic pathway of this protein was available on the KEGG database (36, 37).

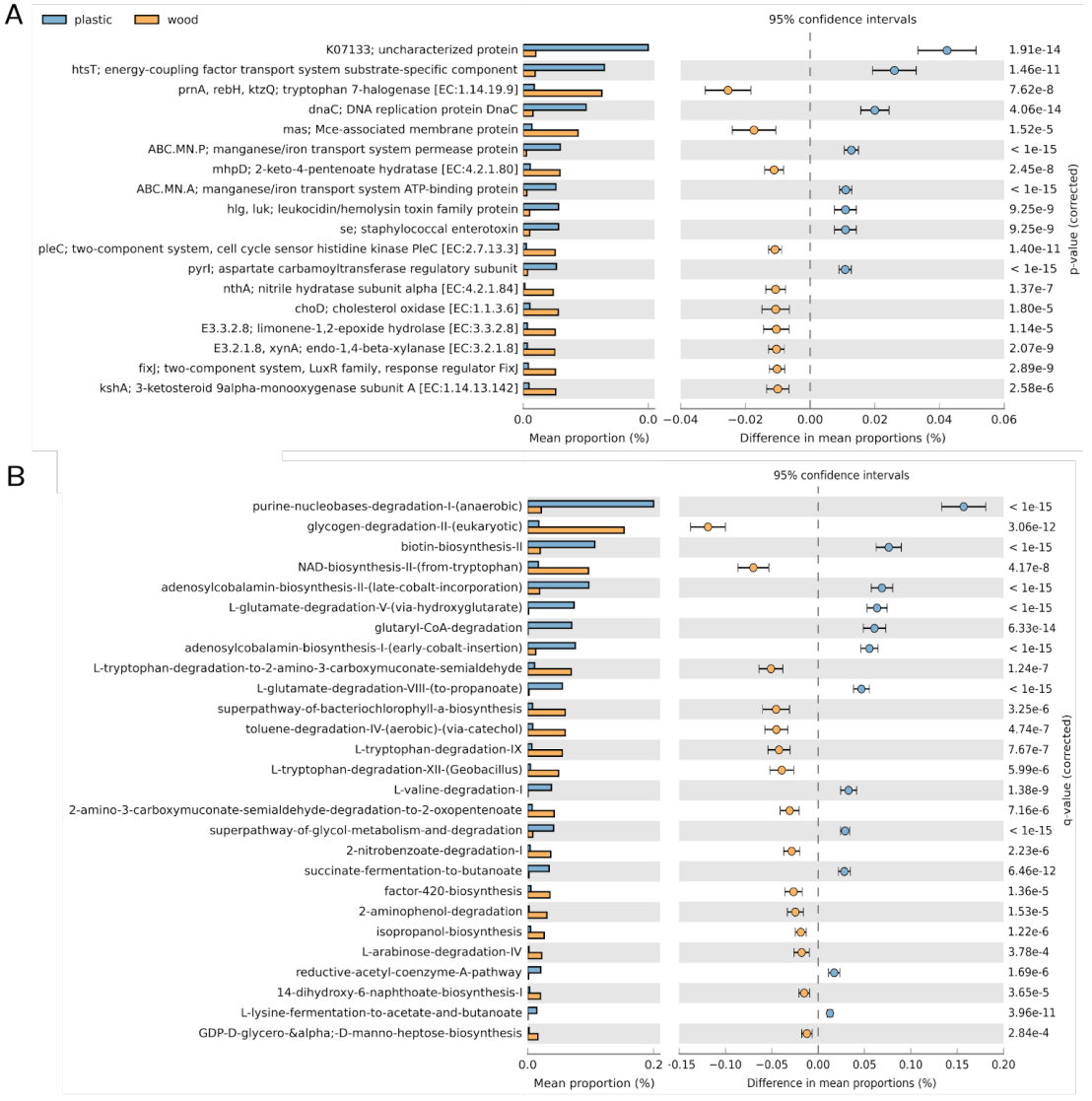

FIG. 5 Plastic and wood surfaces have differentially abundant inferred functional metagenomic profiles. STAMP extended error bar plots depicting differential abundance between wood and plastic samples. Only significant features ($q<0.05$) with an effect size > 5, determined by ratio of abundance, are shown. Significance was determined using Welch's T-test followed by Benjamini-Hochberg FDR correction. 95% CI is shown on error bars. **(A)**. Differential abundance of KEGG orthologs (KOs). **(B)**. Differential abundance of MetaCyc pathways.

DISCUSSION

Given the increasing interest in long-term space travel and settlement, earth-based models serve as a critical tool to longitudinally monitor the human microbiota in an isolated and unique environment. Microbiomes have also been demonstrated to directly be involved with the overall health of an individual (43). Therefore, investigating the microbial diversity dynamics within the highly specific and isolated HI-SEAS environment may provide further insight into the factors affecting the abundance of beneficial and harmful microbial compositions, as well as better inform surface material selection in future isolate or off-Earth missions.

By characterizing the microbial diversity and functional profiles of the microbial communities on abiotic surfaces in the HI-SEAS environment, we aimed to test our hypothesis that the intrinsic properties of abiotic surface material would impact which microbial taxa were able to colonize and survive on each given surface. We found that surface material significantly impacted diversity and microbial community structure, and that metagenomic profiling of these communities revealed differentially expressed proteins and pathways that may allow for survival under different conditions and material types, supporting our hypothesis and providing a starting point for further metagenomic analysis.

Diversity differs by surface material type. Interestingly, our analysis of alpha diversity revealed no significant difference in Shannon's diversity between plastic and wood, contrary to the previous findings by Li et al (3). This may be due to a difference in truncation length (290 vs 220 bases) or rarefaction depth (14,710 vs 20,706) (3). Our results suggest that both plastic and wood surfaces have relatively even microbial communities, with no taxa being overly dominant in either environment. However, when considering phylogenetic distance using Faith's PD, we noticed a significant difference in alpha diversity between plastic and wood surfaces (13). Differences in alpha diversity measured with Faith's PD but not Shannon diversity suggests that while microbial taxa are even, plastic surfaces harbour microbial taxa that are more distantly related (13, 25). In contrast, our beta diversity analysis was consistent with the previous findings by Li *et al.*, showing significant differences in both Weighted and Unweighted Unifrac (3). Distinct clustering of samples in both Weighted and Unweighted UniFrac analyses in addition to significant differences in Faith's PD suggests the microbial community structure differs greatly between wood and plastic surfaces (13-15).

A possible explanation for these observations is that the two surfaces provide different selective pressure due to characteristics such as moisture retention or surface texture (7), promoting survival and colonization by genetically and phenotypically unique taxa. In addition, trees have been shown to produce several different classes of compounds with antimicrobial properties in the wood, including phenols, lignans, tannins, stilbenes, flavonoids, and terpenoids (44). These antimicrobial compounds have been shown to persist even when the wood is transformed into surfaces meant for food contact (45). This indicates that wooden surfaces in the HI-SEAS kitchen may retain these antimicrobial properties and thus differentially impact the survival of resistant or susceptible microbes.

Genus level classification and differentially abundant genera on wood and plastic surfaces. Investigation of differentially abundant genera on plastic versus wood surfaces was used to further characterize these communities and their structure. Differential and relative abundance analysis was conducted at the genera level, as higher taxonomic ranks may be too broad to be able to make generalizations about functions and significance, while the species rank may be too specific to be able to observe patterns between the two surface materials and some ASVs may not be resolved to the species rank. Differentially abundant species also may not have been comprehensively evaluated and it would prove difficult to draw generalizations about the microbial communities. The genus level strikes a balance between generalizable information and specificity of that information with regards to taxa.

We found a significant fold change in the following genera on plastic surfaces: *Rothia, Neisseria, Abiotrophia, Novosphingobium, Baribaculum, Jeotgalicoccus, Eremococcus,* and *Staphylococcus*. Similarly, differential abundance of *Tsukamurella, Methylobacterium-Methylorubrum, Microbacterium*, *Sphingopyxis*, and *Brevundimonas* genera were observed on wood surfaces (FIG. 3). Given the nature of sampling, the differential abundance metric and its measurement of $\log_2$ fold change, it is possible that significant fold changes were attributed to outlier genera that are found in very small

abundances. Relative abundance analysis was performed on each differentially abundant genera to evaluate whether the difference was due to very small relative abundances. The differences observed in the differential abundance analysis were not reflected in the relative abundance analysis. This suggests that differences observed in the differential abundance analysis was due to the nature of the random sampling or outliers in the dataset (FIG. 3). Only *Staphylococcus* and *Brevundimonas* genera had median relative abundance higher than 5% in plastic and wood surfaces respectively (FIG 3).

The *Staphylococcus* genus is more abundant on plastic surfaces. The *Staphylococcus* genus is associated with many forms of human infection and are common human skin commensals. They were characterized in high abundance on the skin samples by Mahnert et al (1, 46). Members of the *Staphylococcus* genus have been shown to incite skin lesions, abscesses, gastroenteritis and on occasion, septic shock like illness. Staphylococcal enterotoxins (SEs) produced by members of the genus have been suggested to play a role in causing these symptoms as well as clinical food poisoning (47).

Given that the relative abundance of the *Staphylococcus* genus was significantly greater on plastic surfaces compared to wood, we suggest that this observation can be attributed to the low interaction of human skin contact with the kitchen floor (wood surface) (46). Mahnert et al, also suggests that the low microbial diversity associated with the kitchen floor may be due to less contact compared to the other, regularly touched surfaces (toilet bowl & desks) (1). Pinosylvin is an antimicrobial naturally produced on wood surfaces and has been demonstrated to inhibit the growth of gram-positive bacteria including *Staphylococcus aureus* (48). While it may be possible that the prevalence of the *Staphylococcus* genus on wood surfaces was not significantly abundant due to reduced contact with the crew's skin, pinosylvin may also contribute to the inhibition of growth and colonization of *Staphylococcus*. Resveratrol, a stilbene, has shown a significant inhibitory effect on *Staphylococcus aureus* in particular, which may contribute to the significantly greater abundance of *Staphylococcus* observed on plastic surfaces (49).

While relative and differential abundance analyses may provide insight into microbial structures and which genera are prevalent on either surface, the mechanisms involved with our results remain largely unknown.

The *Brevundimonas* genus is more abundant on wood surfaces. The *Brevundimonas* genus was found to show significant relative abundance on wood surfaces (50). The genus consists of non-fermenting opportunistic gram-negative bacteria. Human pathogenesis in immunocompromised individuals or those with underlying medical conditions is associated with mild bacteraemia. Some species within the genus have the ability to pass through sterilising filters, allowing them to potentially cause harmful infection if ingested, however no details were provided on how the crew drank and filtered water. *Brevundimonas* isolates have been found in multiple environments, including soils, deep sea floor sediment, activated sludge, black sand, purified water and interestingly, in the condensation water of a Russian space laboratory (50). This may suggest that the genus is resistant to current laboratory space protocols. Furthermore, literature demonstrates that the *Brevundimonas* genus is able to weather martian radiation and temperatures (51). There is also evidence to support the results that *Brevundimonas* was differentially found on wood surfaces. Silva *et al.* demonstrated that *Brevundimonas* possesses the metabolic pathways to use sawdust as a sole carbon source. These findings were consistent with a study by Gelbrich that found *Brevundimonas* in high abundance in degraded wood samples (52). While the genus has not yet been successfully cultured to systematically review their role with wood *in situ*, further investigation may provide insight into whether the intrinsic properties of wood or whether the genus has a genetic basis for survival on wood surfaces (50).

Pathways involved cofactor synthesis, metal transport, pathogenicity, nucleic acid, and amino acid degradation were more abundant on plastic surfaces. Differentially expressed various metabolic pathways and proteins were found that are involved in cofactor synthesis and metal transport. This dataset inferred the presence of htsT, which has been documented for use in iron, cofactor B12, and cation transport in plastics (53). Similarly, the ABC manganese/iron transporter systems that were differentially expressed, which could indicate that metal cations are more beneficial for survival is required in a higher amount in microbes on plastic surfaces. This aligns with the pathway abundance data, since cofactor

B12 biosynthesis is highly differentially expressed in plastics. Cofactor B12 has been previously described to be pertinent for organization and stability in a microbial community, through regulating protein synthesis and DNA regulation (54). As such, the production of vitamin B12 may provide a microbial fitness advantage in plastics, however more research will be required to determine why cofactor B12 synthesis is not required as heavily in wood surfaces.

In terms of pathogenicity, microbes on plastic surfaces were inferred to express higher levels of released toxins. This includes gamma-hemolysin and leukocidin, which are known for pore creation, lysis of leukocytes, and tissue necrosis (55, 56). Staphylococcal enterotoxin was also expressed, which is a mediator of host foodborne illness (57). This correlates with what was observed in this study since our taxonomy data display microbes from the *Staphylococcus* genus as differentially abundant on plastic surfaces. These findings suggest that the routine cleaning within a confined environment is pertinent for reducing the risk of sickness and infection through microbes found on surfaces.

Lastly, various pathways were found for degradation of amino acids and specific nucleic acids. The anaerobic purine degradation pathway in plastics was the most differentially expressed pathway found relative to wood surfaces. Additionally, glutamate and glutaryl-CoA degradation, both used in the process of catabolism of amino acids, are differentially expressed in plastics. Therefore, it can be inferred that these organic compounds are potentially more commonly found within plastic surfaces.

Pathways involved catabolism of wood-associated compounds and fungicide synthesis were more abundant on wood surfaces. One of the primary findings following analysis of differentially abundant metabolic data on wood surfaces was the robust presence of catabolic pathways related tree-derived compounds. Limonene hydrolases and beta xylanases, and arabinose degradation pathways are evident in microbial samples from wood. Limonene is a commonly found aromatic terpene in conifers, while xylan is an abundant hemicellulose found in plant cell walls (58). Arabinose is found within xylan and other hemicelluloses (59). Genes for nitrobenzene reduction and aminophenol degradation are also apparent. Nitrobenzenes are used to create aniline dye which, similar to aminophenol, are used for wood treatment (60, 61).

The presence of these compounds may explain the high level of differentially abundant pathways for aromatic compound degradation. Genes for 3-phenylpropanoate, tryptophan, cholesterol degradation, and transport are differentially abundant within wood surfaces. This indicates that the microbes on these surfaces may have a selective advantage because they can actively digest the surface they reside on. Additionally, there may be a selective advantage to being able to digest the chemical treatments done to the wood surfaces, but further investigation would be needed.

Further, genes encoding for tryptophan chlorination, for secondary metabolites, have also been inferred. This is the first step in the pathway leading to pyrrolnitrin biosynthesis (62). Pyrrolnitrin is an antifungal antibiotic that acts via interference of osmotic signal transduction (63). As a result, this may stipulate that wood surface digestion allows for a symbiotic relationship since wood surface residing prokaryotes may create fungicides to prevent opportunistic fungal pathogens from infecting a host plant or tree in areas that may be harm or detrimental to the host plant. Concurrently, nutrient uptake from the surface of the wood may simultaneously also occur.

Limitations Some limitations of the HI-SEAS IV dataset include the lack of appropriate pre-experiment baselines that would be required to further interpret the dataset. There is no catalog of pre-existing microbes in the isolated abiotic surfaces in the environments or on crew members, so it is unknown how introduction of the crew to the isolated environment may have shifted microbiota at the beginning of the mission. Fermented or microbially active foods, the compost toilet and food waste bin may also have introduced microbes to the environment prior to the start of the mission, resulting in different microbiome composition had those microbe sources not been introduced (1). This may introduce difficulties in terms of making protocol and building material recommendations for future isolation missions, as features of this specific microbiota and the differences observed between surfaces may not be generalizable to other, unknown microbiome profiles.

Limitations regarding the HI-SEAS IV environment as a model for investigating the impacts that surface materials have on microbial communities are also present in this study. Of note is the weekly cleaning schedule, which may have introduced selective pressures in the form of extinction events to abiotic surfaces. This includes selection for resistant microbes in addition to the intrinsic material properties. These selective pressures may not be present in other isolated built environment surfaces, making extrapolation of our observed differences in microbial communities problematic. In addition, the weekly sterilization extinction events may have skewed the sampling data and may not provide information about the microbial climax community on the surface. Therefore, extrapolation of our findings on non-isolated surfaces would also be questionable. Finally, the HI-SEAS IV built environment is not a perfect replica of an isolated extraterrestrial environment. Factors such as water availability, sanitary products, oxygen availability during field work, or similar discrepancies may have impacted microbial composition and diversity within the environment, making direct comparisons and conclusions regarding real interplanetary missions difficult.

Furthermore, database and version mismatches occurred between generation of the diversity metrics due to lack of computational resources and previously generated databases. Phylogenetic trees for diversity metrics were generated with the SILVA 128 release, while taxonomic classifications utilized the 138 release (16-17). Finally, PICRUSt analysis was conducted using the IMG open reference database (33). Database and version mismatches may result in some recently updated or cataloged taxa being classified differently between the different analyses.

It is important to recognize the limitations of inferring functional metagenomic profiles as well. While amplicon-based predictions are highly correlated with the functional profiles from shotgun metagenomics data, the functions indicated as significant can significantly differ (31). On another note, since this data was processed using the V4 hypervariable region of the 16S rRNA, information as detailed as strain variation cannot be inferred (31). Lastly, qualitative analysis on the functional profiles was only done on the MetaCyc pathways and KOs that fulfilled the subjective high effect size we chose, as discussed in the methods section of this paper. However, it is likely that many of the pathways and KOs that do contribute to a different functional profile were likely filtered out.

Conclusions After investigation of how wood or plastic surface materials impact the abundance of specific microbial taxa, our study suggests that the intrinsic properties of wood and plastic impact the ability of different microbial taxa to colonize and survive. Similarly, there may be a genetic basis for increased survivability of certain genera on these surfaces. Investigation into these metabolic pathways has demonstrated the potential for metabolites to confer increased microbial fitness on plastic surfaces and toxins for pathogenesis. Although wood surfaces harbour antimicrobial compounds that limit the growth of microbes, specific genera of bacteria demonstrate the ability to digest the wood surfaces.

While there are limitations of the dataset and analysis such as lack of microbial cataloging and database mismatches, this study provides a starting point for the consideration of surface building material in isolated environments and how the intrinsic properties of these materials may impact microbial communities. Future studies in isolated built environments may further break down the surface-microbiome relationship and provide insight into appropriate material selection in these scenarios.

Future Directions While this study provides a basis for how the intrinsic properties of the built environment material may affect the microbiome, future missions may benefit from further investigation of the biological impacts on microbial communities in these surfaces. Furthermore, the use of biotic skin swabs to compare microbial profiles and investigate potential overlap of genera may provide insight into biological factors such as skin contact that may affect the abiotic surface diversity.

With regards to future isolated built environment studies similar to the HI-SEAS IV mission, researchers may choose to reduce the frequency of cleaning to better understand how

intrinsic surface material selects for microbial communities without the presence of weekly extinction events being a possible confounding factor.

Additionally, the furniture in the built environment consisted of both laminated and raw wood as well as plastic surfaces (1). This study did not take this into consideration. Subsequent studies may further break down these surfaces and determine the impact of laminated wood versus raw wood versus plastic surfaces as well how the spatial location of sampled surfaces may play a role in shaping in microbial populations. Additionally, more detail on the treatment and sourcing of the wood could be useful in microbial studies. While we have demonstrated the presence of certain metabolic pathways that may contribute to increased survivability of specific genera, a link between how taxa may contribute to the presence of these pathways remains largely unknown. These answers may all provide a basis of knowledge useful in conducting future long-term space travel.

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