Undergraduate Journal of Experimental Microbiology and Immunology (UJEMI) Vol. 27:1-13

Location and surface materials drive differences in microbial communities in the confined HI-SEAS IV habitat

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SUMMARY Humans regularly interact with microbiota on abiotic surfaces promoting its growth or inhibition, which can be well characterized in confined spaces. The Hawaii Space Exploration Analog and Simulation (HI-SEAS) mission IV study examined the microbiome of a confined habitat that simulates the environment in which astronauts will live when sent out on Mars and Moon exploration missions. Indeed, differences in microbial diversity and composition was previously identified between different surface materials, but the effects of surface materials and location on bacterial taxonomic profile and abundances had yet to be explored. Using the collected data from the HI-SEAS IV environment, results showed that microbial taxa on plastic surfaces in three different locations within the habitat had highly conserved taxonomic profiles at the genus-level yet contained significantly different beta diversities and differential abundances. The few unique genera observed from each location is presumed to be due to the functional differences of each area. Notably, both the living room and bedroom compared to the bathroom had significantly higher levels of *Methylophilus*, which are facultative methanol-utilizing bacteria, possibly due to use of disinfecting wipes and hand sanitizers containing toxic methanol contaminants. Bacteria associated with the human microbiome generally dominated the bathroom and bedroom, with many significant genera being associated with the female reproductive tract. In conclusion, considerations should be given to the surface materials and locations within a confined environment when monitoring bacterial communities in enclosed environments.

INTRODUCTION

Hawaii Space Exploration Analog and Simulation IV (HI-SEAS IV) is an isolated habitat built to mimic exploration missions of Mars and the Moon. To study how space travel can influence the microbial dynamics of skin and abiotic surfaces, a study published by Mahnert et al. collected microbial data from the HI-SEAS IV mission (1). It is well known that microorganisms co-exist and interact with humans and thus will inevitably travel to space with the crew members. Therefore, it is possible that the long-term interactions of the microbiomes of humans and the surrounding environments can influence the health and safety of the crew, potentially determining the missions' success (2, 3).

In space, pathogen exposure can be especially dangerous due to increased microbial transmission, lowered human immune responses, and limited access to treatment (4). In addition, microorganisms experience selective pressures such as desiccation and lack of nutrients, which may confer microbial resistance against the unique environmental stresses in space (2, 3). As a result, it is important to regularly practice microbial screening and maintenance of the confined environments to ensure the crews' safety and to minimize biohazardous risks (5). Interestingly, a previous study conducted using the same dataset found differences in the microbial diversity and composition of plastic and wood surfaces (6). However, there remains an open question for how location of different surface materials can influence microbial community structure.

The surface material of different abiotic surfaces in confined habitats confers colonization by different microorganisms than the natural environment (7). Extra measures to maintain confined habitat could be to protect patients from infections in intensive care units or operating rooms, to ensure product quality in cleanrooms, and when confinement is necessary for survival in extreme and hostile environments like in the international space station (8). Infections can be transmitted between humans through surfaces that are frequently exposed

Published Online: September 2022

Citation: Kody Fung, Han Han Ly, Shekinah Soriano, Chaewon Song. 2022. Location and surface materials drive differences in microbial communities in the confined HI-SEAS IV habitat. UJEMI 27:1-13

Editor: Andy An and Gara Dexter, University of British Columbia

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to direct human contact despite having efficient cleaning procedures, whereas environmental microbes are more likely to be abundant in floors where there are less direct contact (8). Interaction between bacteria and the surface are known to alter their gene expression, which results in changes in the cell morphology and their behaviour (9). Bacteria use different mechanisms to sense, move, and attach to various surface types (9). Bacterial adherence can stimulate growth, optimize nutrient uptake, and form a biofilm to protect themselves from antibiotics (9). Therefore, it is important to identify the influence of surface types on the microbial communities.

During the HI-SEAS IV study (1), bacteria were sampled from various wood and plastic surfaces. Previously, a study conducted by Li et al. found that different viable species were differentially abundant on wood and plastic surfaces (6). Wood and plastic surfaces have different characteristics that may limit the growth of certain bacteria; furthermore, different surface types may receive different cleaning procedures or are lubricated by different types of chemicals (8). Desks of the bedroom and living room had plastic laminate material composed of melamine, which can have inhibitory effects on microbial growth (10). The plastic material that composes the toilet bowl is high-density polyethylene, which is also known to exert toxic effects on microorganisms (11). On the other hand, the kitchen floor was composed of painted, waterproof plywood (1). Therefore, the microbial communities on plastic surfaces are expected to have less diversity compared to wood surfaces. To expand our knowledge on the subject and to gain a deeper understanding of these relationships, this study aims to explore the effect of surface types and its interactions with humans on microbial taxa, differential abundance, and diversity.

Ultimately, two hypotheses were tested for this study. Firstly, different surface types host significantly different microbial communities. The prediction is that bacterial communities on plastic surfaces would have different bacterial profiles and abundances than those on wood surfaces. Secondly, location in an enclosed environment influence bacterial diversity and abundance. To compare the differences in bacterial composition and abundances between locations, only samples from plastic surfaces were used because wood surfaces were only sampled from one location. Given that the bathroom is closest in distance in the bedroom, the bacterial composition and abundances is expected to be comparable. Conversely, since the living room is on a different floor than the other two locations, highly differential bacterial compositions and abundances is expected to be found. The surfaces sampled in the living room and between were both from desks, which means both surfaces likely received the same biotic interactions; therefore, bacterial community composition and abundances of the living room is expected to be more similar to that of the bedroom than the bathroom.

METHODS AND MATERIALS

Study environment. The HI-SEAS IV study was performed in a confined 11-meter-diameter dome in which 6 people lived in isolation for 366 days, located at an altitude of 2.5 km above sea level on the uninhabited slopes of the Mauna Loa volcano (1). The ground floor contained the kitchen, living room, lab, and first bathroom, while the upper floor contained the bedroom and second bathroom (1). Before exiting and reentering into the habitat for extravehicular activities, crew members were required to remain in an airlock with all doors closed for 5 minutes to mimic the pressurization required to accommodate differences in air pressure on Mars (1). Additionally, the habitat received general cleaning on a weekly basis (1). Most hard surfaces were cleaned with Simple Green's cleaner, and the kitchen floor was cleaned with Comet's bleach-based powder (1). Finally, the composting toilets were maintained with Sun-Mar's Microbe Mix and Sun-Mar's compost swift, with regular manual cleaning and emptying by crew members (1).

Study system. The dataset generated by Mahnert et al. (European Nucleotide Archive (ENA) accession code ERPI118380) contains information on various microbial samples collected biweekly from different abiotic surfaces, locations, and skin surfaces throughout the 366-day mission. Swab samples of habitat surfaces at four different locations (upstairs bathroom, living room, bedroom, kitchen) were taken every other week for the entire duration of the study (1). Swab samples were taken from the following locations: front part of the composting

toilet bowl (high density plastic) in the upstairs bathroom; kitchen floor (painted, waterproof plywood) in an area where dust tended to accumulate; the desk (medium density fiberboard overlaid with plastic laminate) in one of the bedrooms; and one desk (medium density fiberboard overlaid with plastic laminate) in the living room (1). DNA was extracted from the 111 swab samples and the V4 region of the 16S rRNA gene was amplified using 515F/806R primer pair and sequenced on Illumina Sequencing Platform (1).

The metadata can be found on Qiita (https://qiita.ucsd.edu/) (study ID 12858). Surface material of the four abiotic surface locations were categorized as plastic or wood (1). The toilet bowl, bedroom desk, and living room desk had plastic surfaces, and only the kitchen floor was made of wood (1). The metadata also includes additional sample information such as the date of sampling, temperature, and CO_2 level in the habitat.

Preliminary Data Processing in QIIME 2. Using QIIME2 version 2021.11, the HI-SEAS IV metadata file was imported, and the raw sequences were demultiplexed to remove the barcode sequences using the manifest file (12). Quality control on the demultiplexed sequences was performed by truncating the sequences to 220 bases using DADA2 according to the interactive quality plot and the Phred quality scores, where low quality bases were removed, and sequencing errors were also corrected or removed (13). Then, rare amplicon sequence variants (ASVs) that account for less than 0.005% of total reads were filtered for removal. A final step involved filtering the ASVs to select for "swab" samples of abiotic surfaces based on the "collection_device" metadata category and for the removal of mitochondrial and chloroplast sequences.

An alpha rarefaction curve was generated on QIIME2 using the filtered features tables to determine the rarefaction depth (13-17). To retain the maximum number of ASVs for each of the samples while retaining at least 5 samples for each of the swab samples, rarefaction depth of 73926 reads was chosen. Based on the rarefaction curve, the optimal sequencing depth was chosen to be 10000 as it indicated the part of the curve right before a plateau is reached for all swab samples. Steps involving preliminary data processing in QIIME2 are described in Script 1.

Beta diversity analysis of microbial taxa based on surface type. To better understand if the beta diversities of microbial taxa between surface types were similar or different, several beta diversity analyses were performed. This was done using QIIME2 (12) to calculate beta diversity metrics using the dataset filtered for abiotic surfaces as mentioned above. Then, group significance box plots were generated based on Jaccard's, Bray-Curtis, Unweighted UniFrac, and Weighted UniFrac diversity analyses to compare the surface types, along with PERMANOVA analyses (18-20) (Supplemental Table 1). Weighted UniFrac results were chosen as the desired metric as it accounts for both abundance and phylogenetic distance, both of which we wanted to incorporate into our analyses in this study. The Weighted UniFrac results were further exported onto R to generate a principal coordinate analysis (PCoA) plot using the R packages tidyverse, vegan, ape, phyloseq, DESeq2, qiime2R, and ggplot2 (21-26). Statistical analyses between surface types were performed using a pairwise PERMANOVA ($\alpha = 0.05$, permutations = 999) test across all beta diversity metrics (27). Steps involving the QIIME2 beta diversity analysis are outlined in Script 1, and steps involving the PCoA plot generation in R are outlined in Script 2.

Beta diversity analysis of microbial taxa on plastic based on location. To test if surface material location influences bacteria, beta diversity metrics were performed on QIIME2 (12) using the dataset filtered only for abiotic surface samples. Box plots were generated according to the location of the plastic within the HI-SEAS IV environment and assessed based on Jaccard's, Bray-Curtis, Unweighted UniFrac, and Weighted UniFrac diversity analyses (18-20). Again, the Weighted UniFrac results were chosen as the representative beta diversity metric as it accounted for both abundance and phylogenetic distance. The Weighted UniFrac analysis results were exported to R to generate a PCoA plot in accordance with location. From this, pairwise PERMANOVAS ($\alpha = 0.05$, permutations = 999) were used to determine if there were significant differences in microbial communities between each of the three locations (bedroom, bathroom, and living room) (27). Steps involving the QIIME2 beta diversity analysis are outlined in Script #1, and steps involving the PCoA plot generation in R are outlined in Script #2.

Taxonomic and differential abundance analyses. Taxonomic analyses were performed on QIIME2 (12). We generated taxa bar plots using QIIME2View to compare the different microbial communities in plastic surface samples based on location with the genus taxonomic rank (12, 13, 17, 28). Taxonomy was assigned using a Naïve Bayes classifier with the Silva 138 99% OUT's reference, which recognizes the 16s rRNA gene amplified with the 515F/806R PCR primer pair that was used to generate the sequence data outlined in Script #1 (12, 13, 17, 28). Determination of unique and shared genera was conducted on Microsoft Excel by counting to identify whether they are unique to one specific location or if they are shared between two or all three locations around the habitat. Subsequent generation of a Venn diagram comparing how many genera was shared or unique to each location was performed on RStudio using the VennDiagram R package (28).

We used differential abundance analyses to compare samples of plastic surfaces from different locations (bedroom, bathroom, and living room). We performed these analyses on R using the following R packages: tidyverse, vegan, ape, phyloseq, DESeq2, ggplot2, and ggthemes (21-26). Following the import of the filtered taxonomy table and taxonomic classification, comparisons were made between samples of plastic surfaces from different locations (bedroom, bathroom, and living room). The relative abundances for each ASV were calculated, and only ASVs that were more abundant than 0.05% of the total abundances on plastic surfaces were considered for analysis. Comparisons were made on the genus-level, where only significant genera ($p < 0.01$) are listed. Note that this analysis used a different cutoff value for significance than the PERMANOVA tests ($p < 0.05$) above to permit analysis of genera with a higher confidence level. Steps involving differential abundance analyses are outlined in Script #2.

RESULTS

Microbial communities differ significantly by surface material in the HI-SEAS habitat. To test our first hypothesis that predicted the presence of different microbial communities on different surface materials host, microbial taxa on wood and plastic surface materials were compared. A representative boxplot comparing the two surface materials based on the Weighted UniFrac distance revealed significant differences in microbial communities between each surface type (Fig. 1A). In accordance, distinct clustering was observed from the generated PCoA plot based on these results. (Fig. 1B; Weighted UniFrac PERMANOVA: q = 0.0015).

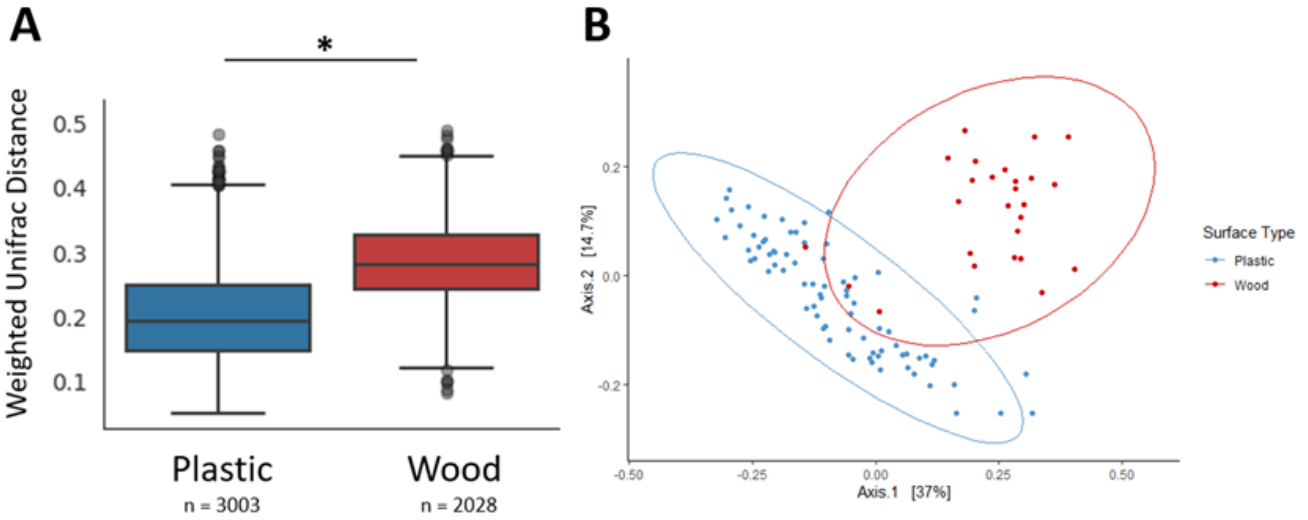

FIG. 1 Microbial communities on plastic and wood surfaces are significantly different. (A) Boxplot comparing plastic and wood based on Weighted UniFrac distance to plastic. (B) Principal coordinate analysis (PCoA) plot based on the same data used in A. (Pairwise PERMANOVA test, * indicates statistical significance. q-value = 0.0015, *n* is the number of distances calculated between each group).

Beta diversity significantly differs by location in the HI-SEAS habitat. To explore if microbial communities differ between locations, plastic surfaces in the bedroom, living room, and bathroom of the HI-SEAS habitat were compared. A PCoA plot based on Weighted UniFrac distance showed distinct clustering for all locations (Fig. 2; pseudo-F PERMANOVA: p = 0.001). Microbial communities in the living room and bathroom were furthest apart, indicating that they have higher dissimilarity in diversity than comparing the to the bedroom (Fig. 2). All three locations were found to contain significantly different microbial communities (Fig. 2).

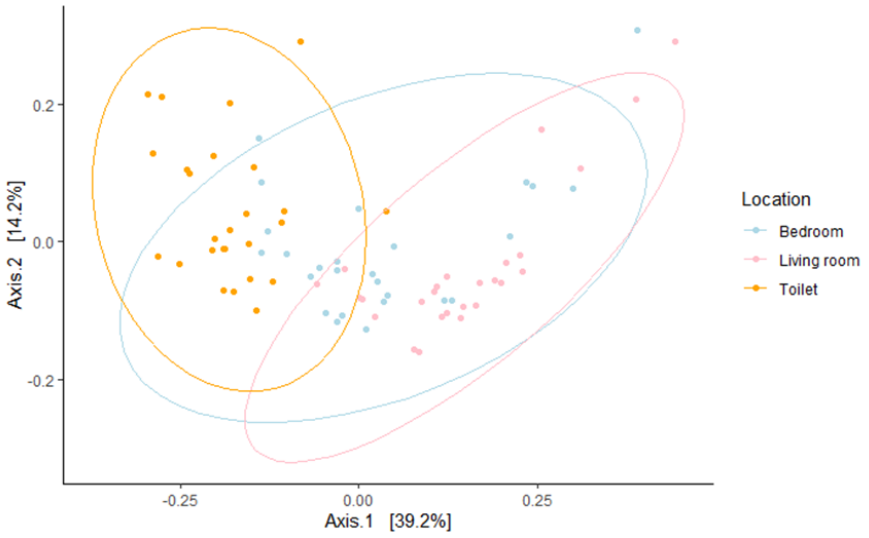

FIG. 2 Beta diversity of microbial taxa on plastic surfaces of the bedroom, living room, and bathroom are significantly different. PCoA plot based on Weighted UniFrac distance (Pairwise PERMANOVA test, q-value = 0.0012, $\alpha = 0.05$).

Unique bacterial taxa were observed in each location. To determine the degree of shared and unique taxa between different locations, we compared the microbial taxa on plastic surfaces at the genus-level between the bedroom, living room, and bathroom. A total of 250 distinct genera was observed from the collected sequences, with 206 (82.4%) of these shared between all locations. Interestingly, we observed unique genera from each location tested. The bedroom had 5 (2%) unique genera while both the bathroom and living room had 4 (1.6%) unique genera (Fig. 3). Within the bedroom, several unique genera were identified including *Thermaerobacter, Salinisphaera, Sphingobacteriacea, Dechloromonas,* and *Eubacterium siraerum* (Supplemental Table 2). *Legionella, 1174-901-12, env.OPS_17,* and *Acidiphilium* were unique to the bathroom, while *WPS-2, Rhodopseudomonas, Bdellovibrio,* and MB-A2-108 were unique to the living room (Supplemental Table 2). Each location also had overlapping genera with strictly one other location. Between the bedroom and bathroom, the bedroom and living room, and the bathroom and living room, 8 (3.2%), 17 (6.8%), and 6 (2.4%) genera were shared, respectively (Fig. 3) (Supplemental Table 2).

Bacterial abundance on plastic surfaces were significantly different across different locations. To determine differentially abundant bacteria between various locations in the HI-SEAS habitat, differential abundance analyses were performed on the genus-level for plastic surfaces of the bedroom, bathroom, and living room. From the data obtained, every location showed differentially abundant bacteria (Fig. 4). Bacteria from the living room and bedroom were most differentially abundant when compared to the bathroom (Fig. 4). Conversely, the living room and bedroom exhibited similar microbial abundance levels with only 13 differentially abundant genera (Fig. 4). In reference to the bathroom, the living room showed higher abundance of 25 bacterial genera with most notably over one-million-fold higher levels of *Methylophilus* and *Curtobacterium* and lower abundance of 24 bacterial genera at less extreme proportions (Fig. 4, Supplemental Table 3). In reference to the bathroom, the bedroom showed higher abundance of 17 bacterial genera with *Methylophilus* again being present at over a one-million-fold higher level and lower abundance of 12 bacterial genera at

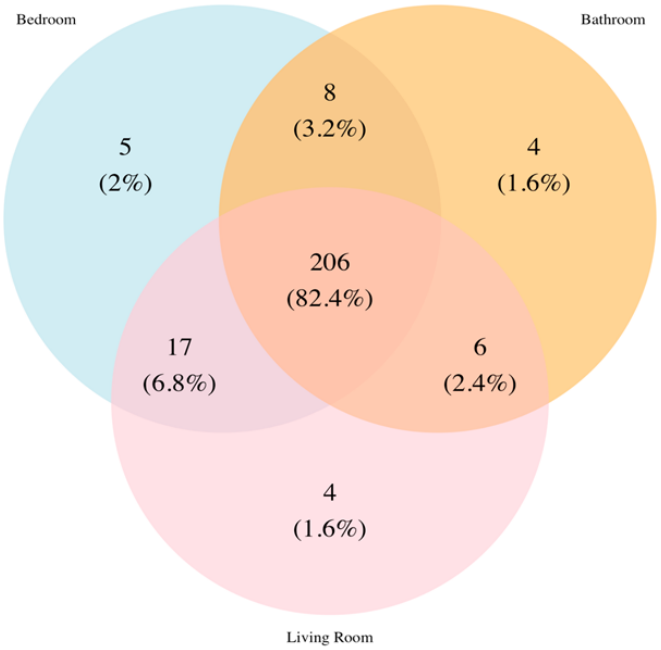

FIG. 3 Most bacterial genera are shared across plastic surfaces of the bedroom, living room, and bathroom. Venn diagram of 250 genera found in the bedroom (blue), bathroom (yellow), and living room (pink) obtained from a QIIME2 taxonomic analysis for plastic surface samples.

less extreme proportions (Fig. 4, Supplemental Table 3). When compared to the bedroom, the living room showed higher abundance of 4 bacterial genera and lower abundance of 9 bacterial genera (Supplemental Table 3). There is a total of 51 unique bacterial genera that are location-dependent (Fig. 4, Supplemental Table 3).

DISCUSSION

A major challenge associated with long-term space travel includes the potential for increased microbial transmission, selective pressure for microorganisms resistant to desiccation, and other unique environmental stressors present in a confined environment. Importantly, there may be a risk that pathogenic microorganisms are disproportionately selected for proliferation. This study intends to provide insight on the influence of abiotic factors and its interaction with biotic factors on bacterial communities in an enclosed environment. In particular, the focus is to evaluate the influence of location and surface material on bacterial communities within the confined HI-SEAS IV habitat. Through the analysis of the HI-SEAS IV microbiome amplicon dataset generated by Mahnert et al. (1), results showed that bacterial communities in the confined HI-SEAS IV habitat were dependent on surface materials and location.

Bacterial diversity on plastic and wood surfaces. Different surface materials can lead to the colonization of different microbial communities within the HI-SEAS habitat. From our results, the bacterial diversity on plastic surfaces was significantly different compared to that of the wood surface. Notably, industrial plastics are made of polymers and the chemical additives, and they can directly exert toxic effects to the microbes and impact the microbial compositions (11). Wood and plastic surfaces have different surface roughness and are composed of different organic compounds, which may result in adherence and biofilm formation of different microbial communities (29, 30). Wood is a porous material that are likely to have more surface roughness compared to plastic (29). Bhagwat et al. have found that increased surface roughness favours rapid attachment of microbes (30). Adherence plays an important role in biofilm formation, which may support the growth and survival of different microbial communities on different surface types (9). This in turn could explain the difference in microbial diversity observed on plastic compared to wood surfaces in the HI-SEAS habitat.

Bacterial diversity of plastic surfaces in different locations. Like different surface materials, the same material housed in different locations can also lead to the colonization of different microbial communities within the HI-SEAS habitat. Previous studies have observed different bacterial communities from similar surfaces found in different locations around a

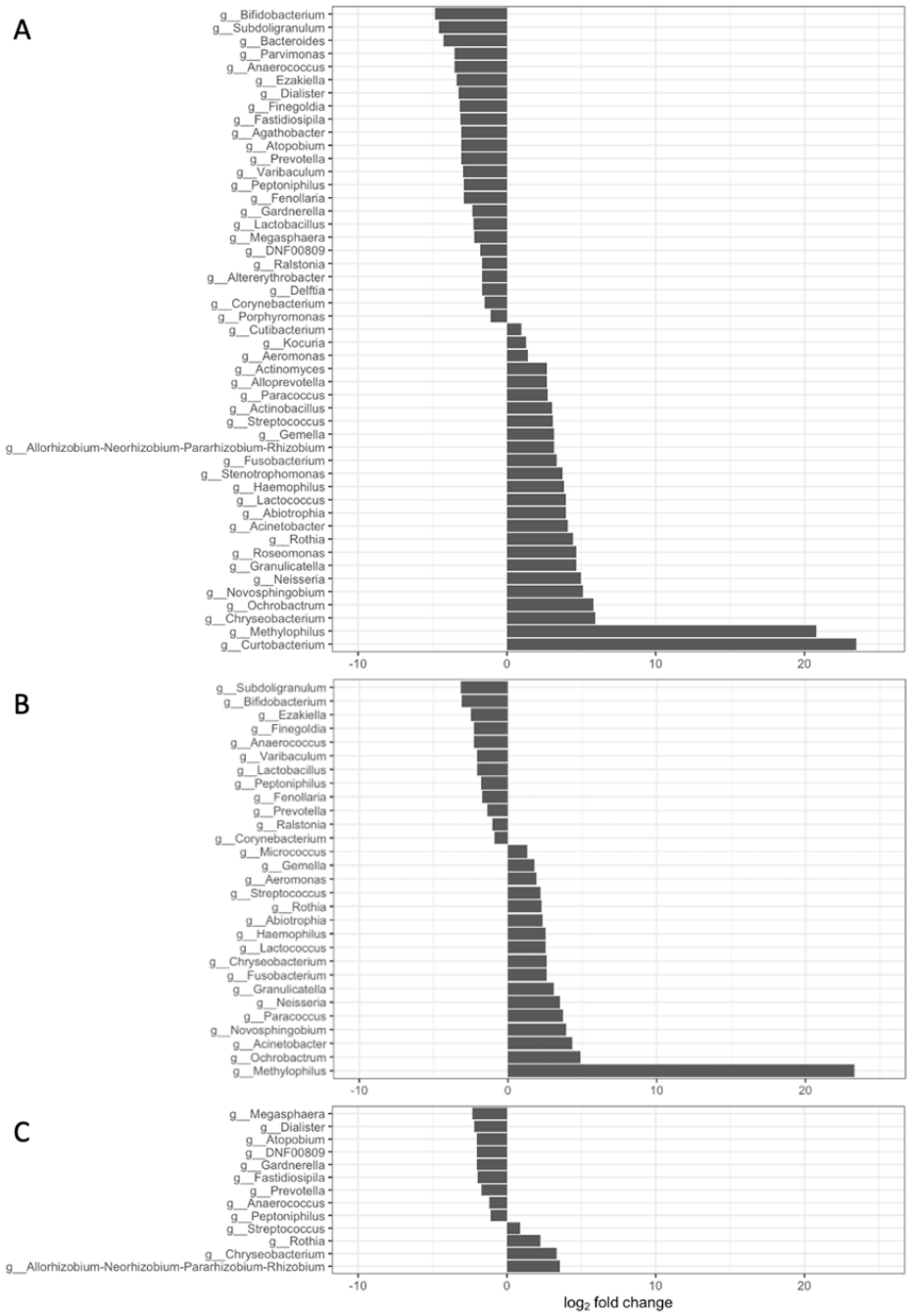

FIG. 4 Bacterial abundance on plastic surfaces within an isolated environment is dependent on location. (A) 49 differentially abundant genera in the living room compared to bathroom. (B) 29 differentially abundant genera in the bedroom compared to bathroom. (C) 13 differentially abundant genera in the living room compared to bedroom. All reported genera are significant ($p < 0.01$).

home (31). Here, beta diversity analyses demonstrated significant differences between the living room and the bathroom, while the bedroom microbial diversity had a less significant diversity relative to the bathroom or the living room. For this study, bathroom samples were exclusively collected from the second floor, which was in closer proximity to the bedrooms compared to the living room. It is possible that the greater significant difference between the microbial diversity of bathroom samples and living room samples may be due to their distances. In addition, both living room and bedroom samples were collected from desks. Thus, the similar functional purposes from these two sampling locations may have contributed to the overlap in microbial diversities for the two locations.

Bacterial profiles from different locations have slight taxonomic variations. Most of the genera present in the HI-SEAS habitat were shared between the bedroom, bathroom, and living room plastic surfaces. It has been established that various enclosed spaces such as homes or offices have distinct microbial communities (31). This is largely influenced by occupation of humans and other biotic sources that constantly spread their microbiota, which

eventually become fixed in the specific ecosystems (32). The HI-SEAS IV habitat was a confined small space with multiple occupants; thus, it is likely that bacterial transfer had occurred and was constantly being redistributed through human-to-human or human-to-surface interactions**.** This presumably resulted in circulation of the same bacterial taxa around the habitat.

Genera that overlapped between two locations were also observed from the samples, thus indicating that different locations may have some impact on bacterial growth. Genera attributed to disease and infection were seen in various locations around the dome. For instance, *Methanobrevibacter*, *Serratia, and Ignavigranum* were shared between the bedroom and the living room, while *Dolosigranulum* and *Moraxella* were shared between the bedroom and the bathroom (33-38; Centers of Disease Control and Prevention, https://www.cdc.gov/std/gonorrhea/lab/mcat.html). Many other genera such as *Carnobacterium, Actinophytocola, Xanthobacter, Terrimicrobium,* were shared between the bedroom and living room or the bedroom and the toilet as well. These are known to be present in agriculture and soil, thus presumably originated from the vegetables grown on-site for consumption and were consequently transferred by the humans in the dome (1, 37-39; List of Prokaryotic names with standing in Nomenclature, https://lpsn.dsmz.de/genus/terrimicrobium).

It is notable that a few unique taxa from each location were observed, suggesting that location can drive growth of specific bacterial genera. The unique genera in the bathroom include *Legionella,* which is known to cause Legionnaires' disease or Pontiac fever according to the Centers of Disease Control and Prevention (https://www.cdc.gov/legionella/index.html), and *Acidiphilum* which is known to survive in acidic environments. This is unsurprising as Leigonnaires' disease was reported to spread through toilet aerosols, indicating that *Legionella* is commonly found in areas like toilet bowls and toilet water (40)**.** Additionally, the presence of *Acidiphilum* in the bathroom may be attributed to regular exposure to human urine, which can be acidic as it normally ranges from a pH of 4.5 to 7.8 (41)**.** In the living room, *Rodopseudomonas,* a photosynthetic bacterium that can degrade lignin, and *Bdellovibrio*, which can prey on gram negative bacteria and is used against multi-drug resistant bacteria, were observed (42-43). In the bedroom, *Thermoaerobacter, Salinisphaera, Dechloromonas, Sphingobacteriaceae,* and *Eubacterium siraerum* were uniquely observed. The first three were unexpected since they are known to survive in specific environmental conditions like high temperatures, high salt concentrations, and areas with low oxygen levels, respectively (44-46). *Sphingobacteriaceae* has previously been found in soils and compost and likely also originated from the vegetables grown on-site (1, 47). Finally, *Eubacterium siraerum* is known to be present in the human gut, thus presumably originated from an occupant in the habitat (48). Though it is difficult to determine why some genera were only observed in specific locations despite being on the same surface type, the differences may be driven by varying functionality of the different areas around the dome. That is, occupants of the dome are more likely to shed distinctive types of bacteria in a bathroom as opposed to the living room. To add, some unique genera observed in the living room and the bathroom have yet to be cultured or characterized. Acquiring more information on these could provide more insight on why these groups of genera were exclusively growing in one location.

Bacterial abundance on plastic surfaces is location-dependent within an isolated environment. In an enclosed environment, both abiotic and biotic factors may interact affecting the colonization of bacteria in a location-dependent manner. Both the living room and bedroom contained extremely high levels of *Methylophilus* which are facultative methanol-utilizing bacteria (49). Interestingly, the cleaner used in the living room and bedroom, Simple Green's All-Purpose Cleaner (https://simplegreen.com/ingredient-disclosure/), does not contain any methanol to promote *Methylophilus* growth. However, the "astronauts" occasionally used disinfecting wipes, mainly Kirkland's Extra Large Disinfecting Wipes, in between showers along with hand sanitizers which commonly contain toxic methanol contaminants that may promote *Methylophilus* growth (50). Additionally, the living room had extremely high levels of *Curtobacterium* compared to the bathroom. *Curtobacterium* are gram-positive soil organisms and plant pathogens (51) that may have

been involuntarily brought into the living room upon re-entry after the "astronauts" regular activities outside the habitat, where vegetables were grown on-site for consumption (1).

In general, bacteria associated with the human microbiota appear to dominate the bathroom and bedroom. Compared to the other two locations, the living room alone had a significantly low abundance of bacteria associated with the human microbiota. Interestingly, many of the significant genera are associated with the female reproductive tract, such as *Megasphaera, Atopobium*, and *Gardnerella* (52-54). More specifically, these bacteria are associated with bacterial vaginosis (BV), a common vaginal disorder that is characterized by the replacement of a healthy, lactobacilli-dominated vaginal microbiota by anaerobic and facultative anaerobic bacteria (54). These results are reasonable as half of the "astronauts" were females (1). Other notable bacteria associated with the human microbiota are *Dialister, Coriobacteriales,* and *Fastidiosipila* (55-57). *Dialister* is commonly found in intestinal microbiota and has low susceptibility to antimicrobial agents like piperacillin, metronidazole, macrolides, fluoroquinolones, and rifampin (55). *Coriobacteriales* are commensal organisms that are saccharolytic and able to metabolize a wide variety of carbohydrates, producing lactate and other metabolites (56). Finally, *Fastidiosipila* is a gram-positive anerobic coccus found in the blood microbiome (57). Ultimately, bacterial abundance seemed to drive the differences in bacterial communities between locations, rather than the bacterial taxonomic profile.

Limitations The scope of our study contains limitations including the collection of data on wood from only one location and the variation of plastic compositions within each sample. In the HI-SEAS dataset generated by Mahnert *et al.* (1), wood surface types were only sampled from the kitchen. Notably, our study measured microbial community diversity between wood (N = 1) and plastic (N = 3) and as a result of the unbalanced sample locations, the wood samples in this study do not necessarily represent the microbial taxa present throughout the HI-SEAS habitat, but merely the kitchen surfaces. This in turn created another limitation such that it was not possible to delve further into other diversity metrics such as differential abundance analyses in wood, due to the low sample sizes.

Our study explored the differential abundance of microbial communities on plastic surfaces in different locations. However, these plastic samples contained different compositions including high density plastic in the bathroom and medium density fiberboard with plastic laminate in the bedroom and living room (1). As these plastics are made up of different chemical compounds, previous studies indicated that these could influence the bacterial community profile (11). Thus, the composition of plastic may be a confounding variable in our analyses.

Conclusions Our study aimed to explore the variation in microbial taxa present across several surface materials and locations within the enclosed HI-SEAS habitat. Like previous studies, wood and plastic surfaces hosted significantly different microbial communities. In terms of location, the bedroom, bathroom, and living room also hosted significantly different microbial communities. The bacterial profile was relatively consistent across plastic surfaces in different locations with only a few unique taxa, which were likely due to the various functional purposes of each room. Instead, bacterial abundance appeared to drive the differences in communities between locations. Our findings reveal new considerations to be taken when monitoring bacterial communities in enclosed habitats; however, exploration into more locations and different abiotic factors present in the HI-SEAS habitat would prove to be beneficial in further research of confined environments. Ultimately, location and surface material drive differences in bacterial communities within an enclosed environment.

Future Directions As previously mentioned in the limitations, the lack of variability in sampling locations limited the amount of representative data that could be extracted from the dataset. Particularly, not all the locations within the HI-SEAS habitat were sampled, such as the ground floor bathroom, the lab, and the airlock room (1). Sampling more locations may reveal more differences in bacterial communities which could potentially provide significant insights on how abiotic and biotic interactions influence bacterial colonization. Additionally, it would be interesting to perform further diversity analyses of wood in different locations

and compare it to plastics to better understand how surface material influences bacterial colonization. To this end, being able to identify locations associated with pathogenic bacteria and high bacterial abundance could aid in developing cleaning regimens in a location-dependent manner.

Moreover, investigating the association of pathogenic bacterial growth with specific plastic and wood compositions may be insightful. Previous studies have indicated that different surface materials have varying levels of antimicrobial properties (58) which would be interesting to investigate in the HI-SEAS habitat. As our study only encompassed the growth of bacteria on wood and two types of plastic, other surface types have yet to be studied. Future studies on the HI-SEAS habitat should try to gather as much data on different surface types and plastic composition as possible to better understand their implications within the confined environment.

With respect to other unexplored variables within the dataset, studying the effects of time and CO_2 concentrations on microbial diversity and abundance within each location could be insightful. Measuring the level of proliferation between different surface types over time may be an effective measure for creating new cleaning procedures to prevent pathogenic bacterial adhesion and growth. Additionally, the effect of CO_2 concentrations can positively or negatively affect the growth of several aerobic and anaerobic bacteria, which is yet to be studied. Therefore, the consideration of these effects could provide insight on whether these variables influence the growth permissibility of specific microbial taxa in the HI-SEAS habitat.

ACKNOWLEDGEMENTS

We would like to thank the Department of Microbiology and Immunology at the University of British Columbia for providing us with the necessary tools to conduct our research. Furthermore, we would like to thank Dr. Evelyn Sun, Zakhar Krekhno, and Emily Adamczyk for their instruction, guidance, and support throughout this project.

CONTRIBUTIONS

This study was done with the collective effort of all authors. Data analyses in QIIME2 and R were performed by all team members, each contributing to the generation of the results. The generation of the final manuscript was also completed by all authors equally.

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