Undergraduate Journal of Experimental Microbiology and Immunology (UJEMI) Vol. 27:1-9


# Surface material and location impact microbial communities colonizing plastic and wood surfaces during the HI-SEAS IV Mission

Garshana Rajkumar, Aneesa Khan, Kitty Martens, John Park

Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada

**SUMMARY** Microbial communities that colonize surfaces have the ability to influence human health and can cause infection and illness. This is an important factor in long-term space travel due to the confined nature of the environment and the frequent interaction between the microbiomes of the crew and surfaces. The Hawaii Space Exploration Analog and Simulation IV study examined the microbial dynamics of crew skin and surfaces on earth that mimicked the isolated and confined environment of Mars and Moon exploration missions. Fluctuations in microbial diversity were found for abiotic surfaces, but the role of surface material and location on microbial community composition had yet to be examined. In our study, we examined how microbial communities changed in relation to surface material and location and found that microbial communities on plastic and wood surfaces showed significant dissimilarities based on beta diversity analysis. From taxonomic barchart analyses, we found that microbial communities on plastic and wood surfaces had different taxonomic compositions based on surface type and location. Lastly, through assessment of differential abundance analysis at the genus level, we were able to find more differentially abundant taxa on plastic compared to wood. Our study showed that surface material and location did impact microbial community composition and could provide insight when designing environments for future space exploration missions.

## INTRODUCTION

Humanity's desire to uncover the mysteries of our solar system has led to deeper space exploration initiatives such as reaching Mars. Although technological advances have allowed for numerous exploration rovers to be sent to the Martian surface, a human crew has yet to achieve similar results. One limitation to consider before humans can make this trip is the challenge of protecting the crew from illness and infection caused by pathogenic microorganisms during long-term space travel (1). Studies have shown that the bacteria that colonize humans and the built environment have the ability to influence human health (2). This is further seen in confined settings such as space travel due to the frequent interaction between the microbiome of humans and surfaces (3). Although space stations apply strict cleaning and disinfecting procedures to keep the environment clean, this could also place pressure on microbes and select for antibiotic resistant strains that could be harmful to humans (4). Therefore, it is important to understand the microbial diversity of surfaces to identify and eliminate potentially pathogenic strains to ensure the well-being of the crew.

The Hawaii Space Exploration Analog and Simulation (HI-SEAS) IV mission provided researchers the opportunity to study the microbial dynamics in confined and isolated environments similar to that of Mars and Moon exploration missions (5). A dataset was generated (European Nucleotide Archive (ENA) accession code ERP118380) with the goal of understanding how hostile confined environments would impact the human microbiome and how microbial communities would fluctuate over time. In the HI-SEAS IV mission, six people spent 336 days of isolation in a dome located on the slopes of the Mauna Loa Volcano, and swab and wipe samples were collected from surfaces and the crew's skin (5). Mahnert *et al*. analyzed these data and found that the microbial diversity of skin increased over time while the microbial diversity of abiotic surfaces fluctuated (5). The authors, however, did not look into the trends in microbial dynamics in abiotic surfaces which led to a follow-up study done by Li *et al* (6). In their study, they were able to show that the alpha diversity is higher

**Published Online:** September 2022

**Citation:** Garshana Rajkumar, Aneesa Khan, Kitty Martens, John Park. 2022. Surface material and location impact microbial communities colonizing plastic and wood surfaces during the HI-SEAS IV Mission. UJEMI 27:1-9

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



Address correspondence to: https://jemi.microbiology.ubc.ca/

on plastic surfaces compared to wood, and that the surfaces harbored significantly different communities (6). However, they did not comprehensively look into the effect of material and location on microbial communities on abiotic surfaces in the HI-SEAS IV mission. Our research will follow up on this knowledge gap by determining how diversity and taxonomy differ between plastic and wood surfaces from different locations. Furthermore, our study will identify differentially abundant microbial taxa on these surfaces through differential abundance analysis. We hypothesize that material and location does impact bacterial composition of plastic and wood surfaces. We expect more taxa are able to colonize plastic surfaces compared to wood since wood was found to have antimicrobial properties, only taxa that are able to withstand these restraints can colonize wood (7). As well, we expect location to impact microbial communities since studies have found that the composition of indoor microbial communities was determined by location and the surrounding external environment (8).

## METHODS AND MATERIALS

**Data collection.** The dataset generated by Manhert *et al*. was used for all analyses (Accession number: ERP118380). Biweekly swab samples were taken for the abiotic surfaces in the HI-SEAS environment from four different locations: the toilet bowl, kitchen floor, bedroom desk, and main room desk (5). The kitchen floor was composed of wood, while the toilet bowl, bedroom desk, and main room desk were composed of plastic. Each sampling session had field controls performed by swabbing the air instead of the surface (5). A total of 111 swab samples were collected for DNA extraction. 16S rRNA gene amplicons were generated by targeting the V4 region of the 16S rRNA gene and then amplified using the F515-R806 primer pair (5). The amplicons were sequenced on Illumina MiSeq and then demultiplexed on QIIME2 (5, 9)

**Quality control.** The demultiplexed 16S rRNA sequences were imported into QIIME2 and truncated to 292 bases using the Divisive Amplicon Denoising Algorithm 2 (DADA2) in order to remove low quality bases and retain sufficient sequence quality (phred quality score of 30) (9, 10).

**Metadata filtering and alpha rarefaction.** Following sequence quality control, the denoised sequences were used to generate a features table and representative sequences file to visualize the features through amplicon sequence variants (ASVs). The features table was then filtered using the q2-feature-table plugin to exclude all samples except for those collected from plastic and wood surfaces (9). An alpha rarefaction plot was generated and a rarefaction depth of 20,000 was selected to represent sample richness by maximizing the amount of ASV's retained while normalizing sequencing depth between samples. At this depth, a high number of samples were retained, and the majority of the samples plateaued in terms of Shannon diversity.

**Beta diversity analysis.** The q2-diversity plugin was used to generate commonly used beta diversity metrics (11). Beta diversity metrics examine diversity between samples. Assessed metrics included Weighted UniFrac, Unweighted UniFrac, and Bray-Curtis beta diversity analysis. Statistical analyses were done for the diversity metrics using pairwise PERMANOVA tests (12). Beta diversity metrics were also visualized on R using the tidyverse, phyloseq, ape, vegan, ggplot2, and ggthemes packages (13-19). All steps in the analysis are outlined in the QIIME2 and R script ("QIIME2script.txt" and "PCoA.R").

**Taxonomic classification and taxa bar plot.** After filtering, a rooted phylogenetic tree was created with the representative sequences using the q2-fragment-insertion plugin (20) and was used for all diversity analyses. The SILVA sequence database and pre-trained classifier (sklearn) was used to assign taxonomy (22-24). The taxonomy.qza file and features table rarified at 20,000 were used to generate taxonomy barcharts at the genus level. All steps in the analysis are outlined in the QIIME2 script ("QIIME2script.txt").

**Differential and relative abundance analysis in R.** The filtered metadata was imported from QIIME 2 into RStudio using the phyloseq package (17). Low abundant features (features representing less than 0.005% of total sequencing reads) as well as mitochondrial and chloroplast sequences were removed, and differential and relative abundance analysis was completed using DESeq2 (25). Pairwise Kruskal-Wallis tests were performed for the statistical analysis of relative abundance plots (26). Differential abundance comparisons were performed between wood and plastic samples, regardless of location, as well as between the one wood and 3 plastic surface sampling locations individually. Venn diagrams were constructed using the VennDiagram library to compare the differential abundant taxa from the three plastic surfaces (27). All steps in the analysis are outlined in the R script ("DiffAbund.R").

**Heatmap analysis in R.** The differential abundance data generated in DESeq2 were also used to construct a heatmap of the samples based on the 20 most variant taxa. The workflow used to generate this heatmap was adapted from the DESeq2 package tutorial (25). All steps in the analysis are outlined in the R script ("Heatmap.R").

## RESULTS

**Beta diversity analysis shows significant dissimilarities between microbiomes on plastic and wood surfaces.** Analysis of principal coordinate analysis (PCoA) plots based on Weighted UniFrac distance showed wood and plastic surfaces harbored distinct microbial communities (Figure 1). Comparison of Weighted UniFrac distance between the two materials showed that this difference in clustering was significant (q = 0.001).

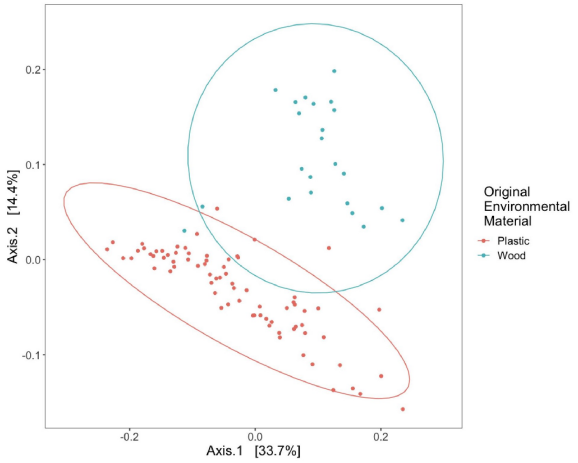


**FIG. 1 Plastic and wood samples had significant differences in beta diversity in terms of Weighted UniFrac distance.** Principal coordinate analysis (PCoA) plot of Weighted UniFrac distance between plastic (n = 75) and wood (n = 23) surfaces shows distinct clustering based on surface material. The ellipses represent the estimated 95% confidence intervals of the samples, and are added to better visualize the clustering between surface materials. This difference in clustering is statistically significant (q = 0.001) (pairwise PERMANOVA test, $\alpha = 0.05$).

**Plastic and wood samples cluster based on surface type and location.** We further employed Weighted UniFrac to identify distinct sample clustering based on location. Analysis of PCoA plots based on Weighted UniFrac distance showed that plastic surfaces form clusters based on location and that this difference in clustering was significant between all three plastic locations (q = 0.001) (Figure 2). The clusters for the bedroom and main room plastic samples seemed to overlap more closely, while the toilet bowl samples appeared to cluster slightly away from the other plastics. This result was supported through heatmap analysis which showed strong grouping of samples according to material and some sub-grouping of samples according to location (Figure S1).

**Plastic surfaces had significantly more differentially abundant taxa compared to wood surfaces.** Analysis of taxonomic composition at the genus level showed that higher frequency of *Brevundimonas* was observed in wood samples compared to plastic (Figure 3). In contrast, a higher frequency of *Staphylococcus, Corynebacterium*, and *Acinetobacter* are observed in plastic samples in comparison to wood samples. When examining the differences between plastic samples at different locations, there was a higher frequency of *Brevundimonas* in toilet bowl samples, while the main room and bedroom samples had higher frequencies of *Acinetobacter.*

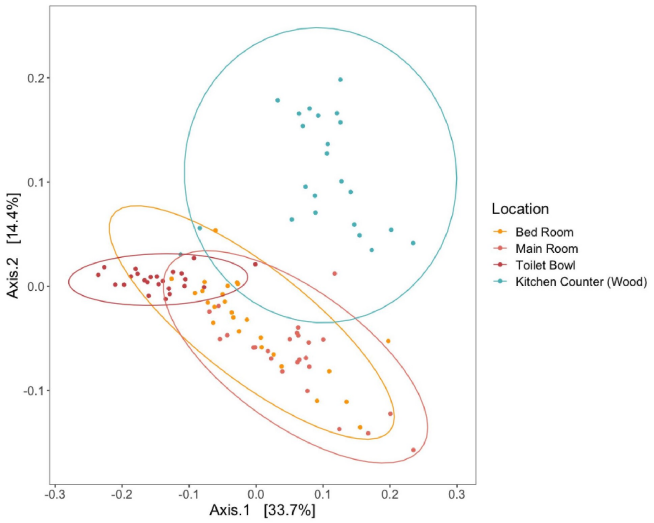


**FIG. 2 Beta diversity in terms of Weighted UniFrac distances showed clustering of samples based on material and location.** Principal coordinate analysis (PCoA) plot of Weighted UniFrac distance where colors indicate different sample locations: Bedroom (n = 26), Main Room (n = 25), Toilet Bowl (n = 24), and Kitchen Counter (n = 23). The ellipses represent the estimated 95% confidence intervals of the samples and are added to better visualize the clustering between surface locations. All pairs of clusters are statistically significant (pairwise PERMANOVA, $\alpha = 0.05$) ($q < 0.001$).

Assessment of differential abundance at the genus level revealed that there were more differentially abundant taxa on plastic compared to wood (Figure 4). This result was seen for each plastic sample location with the exception of the toilet bowl where there were an equal number of differentially abundant taxa in comparison to the wood kitchen counter (Figure S2). Upon further examination of 41 differentially abundant taxa, two genera with high relative abundance were found to be potentially pathogenic to humans (Figure S3). Relative abundance analysis showed that *Brevundimonas* had significantly higher relative abundance in wood compared to plastic ($q = 6.6x10^{-11}$) (Figure 5A). In contrast, *Acinetobacter* had significantly higher relative abundance in plastic compared to wood ($q = 4.8x10^{-5}$) (Figure 5B).

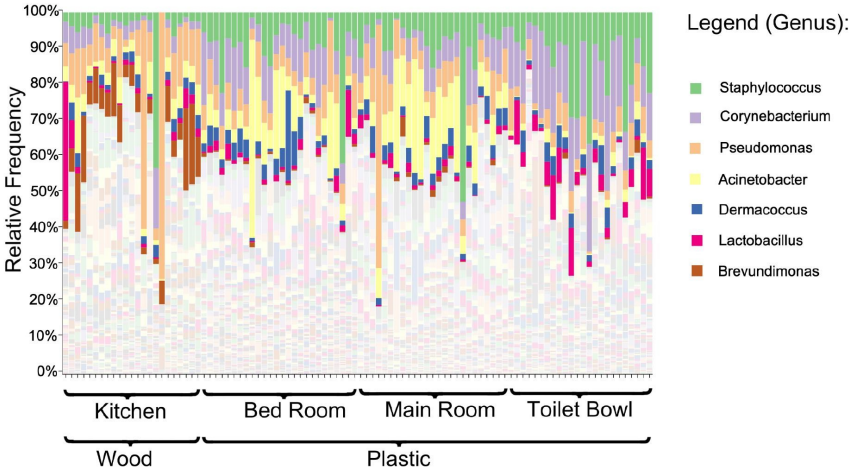


**FIG. 3 Taxonomic composition of samples varies with surface type and location.** Taxonomic barcharts showing the percentage relative frequency of microorganisms at the genus level for the different surface materials and locations. Colors indicate different genera. Only the top seven most abundant genera were outlined in the legend.

## DISCUSSION

In this study, our aim was to understand how microbial communities change in relation to surface material type (plastic and wood) and surface location (bedroom, main room, washroom, and kitchen) during the HI-SEAS IV mission using the dataset generated by Mahnert *et al*. (5). In support of our hypothesis, we found that surface material type and location had an impact on microbial composition.

**Surface material and location impact the beta diversity of microbial communities.** The surfaces of plastic and wood samples taken from different locations in the HI-SEAS built environment were found to have significant dissimilarities in microbial communities. Examination of principal coordinate analysis (PCoA) plots based on Weighted UniFrac distance showed distinct clustering by surface material and location (Figure 1 and 2). The Weighted UniFrac metric was highlighted in this study because it considers both phylogenetic

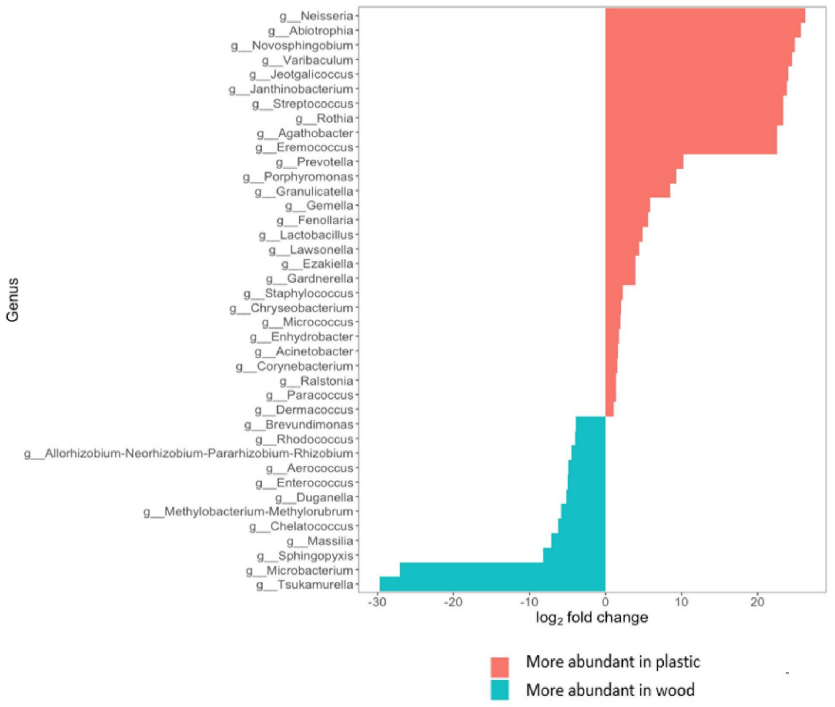


**FIG. 4 More differentially abundant taxa were found on plastic surfaces compared to wood.** Differential abundance analysis for wood and plastic samples at the genus level. The bars represent log2 fold change.

distance and abundance in its analysis of diversity, whereas the other metrics focus only on one, or neither. The phylogenetic distance (or relatedness) between species ties into the analysis of taxonomic differences between surfaces, while abundance relates to the discussion on differential and relative abundance analyses. However, consistent with previous literature, distinct clusters were also observed in Unweighted UniFrac plots (Figure S4 and S5) (6). These differences in beta diversity could be partially due to differences in the material properties of plastic and wood, limiting which bacteria are able to colonize these surfaces (7). One notable limitation of the study was that wood and plastic samples were taken from different locations in the HI-SEAS built environment. Therefore, this difference in beta diversity could also be due to differences in location, in addition to surface material.

Despite being in different locations, plastic samples taken from the bedroom desk and main room desk were found to cluster more closely together than to the plastic sample taken from the toilet bowl (Figure 2). This could be due to the fact that similar activities are

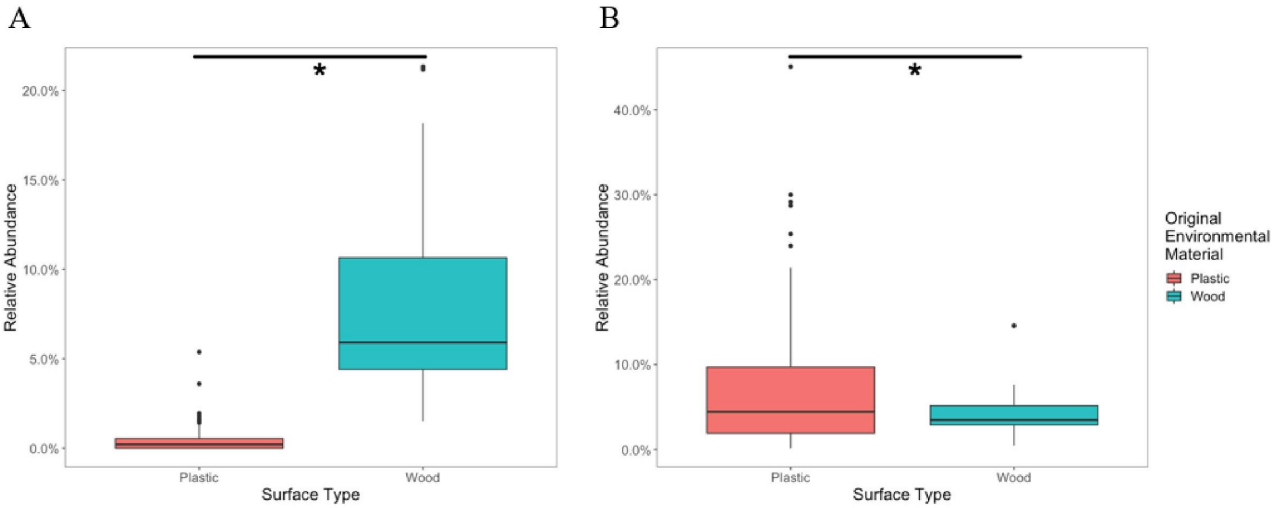


**FIG. 5 *Brevundimonas* showed higher relative abundance in wood and *Acinetobacter* showed higher relative abundance in plastic.** Boxplots comparing relative abundance of *Brevundimonas* (A) and *Acinetobacter* (B) between plastic (red) and wood surfaces (blue). Significance was determined by Kruskal wallis pairwise test (A, q = 6.6x10-11) (B, q = 4.8x10-5).

performed on both bed room and main room desks, while very different activities are performed on the toilet. Plastic laminate fiberboard was used to make the bed room and main room desks, while the toilet bowl material was composed of a different, high–density plastic, which could also have an impact on beta diversity (5). These differences in beta diversity support our hypothesis that surface material and location impact the bacterial composition of plastic and wood surfaces.

**Taxonomic composition of microbial communities varied based on surface material and location.** Analysis of taxonomic composition at the genus level showed distinct differences between plastic and wood surfaces (Figure 3). A higher frequency of *Brevundimonas* was found on wood samples, which is consistent with findings in previous literature (28). In contrast, *Staphylococcus*, *Corynebacteria* and *Acinetobacter* were found in higher frequencies on plastic. Wood surfaces have been found to contain antimicrobial compounds of different chemical classes (6, 7, 29). Specifically, the antimicrobial Pinosylvin is derived from wooden material, and was found to impede the growth of *Staphylococcus* aureus (30). Thus, relatively low frequency of the *Staphylococcus* genus in the wood samples as compared to the plastic samples could be due to the presence of Pinosylvin.

Variation in taxonomic composition was also found between surface locations for the plastic samples. The toilet bowl samples had a higher frequency of *Brevundimonas*, while the bedroom and main room samples had a higher frequency of *Acinetobacter* (Figure 5). Previous literature has shown that *Brevundimonas* constitutes a major portion of biofilms extracted from toilet bowl samples (31). It was also found that *Acinetobacter* can survive much longer on dry surfaces than those containing moisture (32). Liquids likely come into contact with the toilet bowl more frequently due to the water in the bowl and the activities performed on the toilet, which could be a reason as to why *Acinetobacter* frequency was found to be lower in the toilet bowl sample.

**Plastic surfaces had significantly more differentially abundant taxa compared to wood surfaces.** Consistent with our hypothesis, assessment of differential abundance samples revealed that there are more differentially abundant taxa on plastic compared to wood for most plastic samples (Figure 4), with the exception of the toilet bowl sample, which had an equal number of differentially abundant taxa as the wood kitchen counter (Figure S2). As discussed earlier, the antimicrobial properties of wood is likely one of the reasons for fewer differentially abundant taxa being found on wooden surfaces (7).

Relative abundance analysis showed that *Brevundimonas* had higher relative abundance in wood (Figure 5A) while *Acinetobacter* had higher relative abundance in plastic (Figure 5B). The high relative abundance of *Acinetobacter* on the frequently used plastic desks in the main room and bedroom could be a point of clinical concern. This is because certain species of *Acinetobacter* (such as *A. baumannii*) have been found to cause infection in humans, such as bacteremia, urinary tract infections (UTIs), secondary meningitis, infective endocarditis, and wound and burn infections (33). *A. baumannii* was also found to easily develop resistance to multiple broad-spectrum antibiotics and disinfectants, making its presence on common surfaces even more concerning (34, 35). Similarly, some strains of *Brevundimonas* (such as *B. diminuta* and *B. vesicularis*) are opportunistic pathogens and have been found to cause infection to people with underlying medical conditions (36). Therefore, determining which species of *Acinetobacter* and *Brevundimonas* are present on plastic and wood surfaces respectively could be a component of a future study on this dataset.

**Limitations** In the dataset supplied by Mahnert *et al*. plastic samples were taken from 3 different locations, while wood samples were only gathered from one location (5). To have a better understanding of the differences between microbial communities found on plastic and wood surfaces, it would be beneficial to have more sampling locations, particularly for the wood surface. Additionally, to better understand the effect of only surface material (and not location) on microbial communities, at least one sample from both plastic and wood surfaces should be taken from the same location in the HI-SEAS IV built environment. Otherwise, it is difficult to differentiate between differences due to material, and differences due to location.

Another key difference in the plastic samples is the composition of the plastic used to build these surfaces. The toilet bowl was made of high-density plastic, while the desks in the main room and bedroom were made of plastic laminated fiberboard (5). The taxonomic composition of bacterial communities have been found to vary based on the composition of the plastic, so this difference introduces another confounding variable (6, 37). Additionally, the wooden kitchen table was described as being covered with a coating of paint (5). Some paints have been found to contain nanomaterials that exhibit antimicrobial properties to prevent biodeterioration of the painted surfaces (6, 38). Because the type of paint used on the wooden kitchen table was not shared, it is possible that certain bacterial populations that are normally found on wood were excluded from the wood samples due to the additional antimicrobial properties of the paint.

**Conclusions** Our study explored how microbial communities change in relation to surface material type and surface location during the HI-SEAS IV mission. From beta diversity analysis of Weighted UniFrac PCoA plots, analysis of taxonomic composition and differential abundance analysis, we found distinct differences between microbial communities based on surface material (plastic and wood) and location (kitchen table, bed room desk, main room desk and toilet bowl). We also found *Acinetobacter* to be in higher relative abundance in plastic samples and *Brevundimonas* in wood samples, which could possibly serve as a health risk. Overall, our findings suggest that both surface material and location have an impact on the composition of microbial communities living on these surfaces and provide reasons for further research into health risks associated with using certain surface materials that should be taken into consideration when designing environments for space travel.

**Future directions** When looking at the differences between microbial communities from different surface materials and locations, we chose to look at the entirety of the dataset for plastic and wood samples, rather than focusing on certain timepoints. It is possible that certain surface materials or locations promote a faster or more gradual change over time. A longitudinal analysis of samples taken from different timepoints during the mission could reveal information on how the microbial communities change over the course of the HI-SEAS IV mission.

*Acinetobacter* and *Brevundimona*s were found to be higher in relative abundance in plastic and wood samples, respectively. Both of these genera contain species that are capable of causing infection in humans. Differential abundance analysis at taxonomic level 7 (species) would highlight which specific species within these genera are differentially abundant on plastic and wood surfaces, and address this potential risk. It would also reveal species belonging to other genera that may also be of clinical significance. This knowledge could influence which materials are selected for designing environments for future space exploration missions.

## ACKNOWLEDGEMENTS

We would like to thank Manhert *et al*. for providing the dataset which was used for this study, as well as the MICB 447 teaching team (Dr. Evelyn Sun, Dr. Stevan Hallam, Dr. Stephan Koenig, Zakhar Krekhno, and Stefanie Sternagel) for their endless support and mentorship throughout this study. Lastly, we would like to thank the University of British Columbia for providing the resources and funding to make this project possible.

## CONTRIBUTIONS

GR contributed to the analysis of beta diversity, taxonomy barcharts and differential abundance graphs. Composed abstract, introduction, methods, results and figure captions. Contributed to the editing of this manuscript. AK contributed to the analysis of beta diversity, taxonomy barcharts and differential abundance graphs. Composed the discussion, study limitations, conclusion, future studies and figure captions. Contributed to the results section and editing of this manuscript. KM contributed to the analysis of beta diversity, taxonomy barcharts and differential abundance graphs. Generated most figures. Contributed to the figure captions. JP contributed to the analysis of beta diversity, taxonomy barcharts and differential abundance graphs. Generated some figures, contributed to the figure captions and formatted references.

## REFERENCES


1. **Schwendner P, Mahnert A, Koskinen K, Moissl-Eichinger C, Barczyk S, Wirth R, Berg G, Rettberg P**. 2017. Preparing for the crewed Mars Journey: Microbiota dynamics in the confined mars500 habitat during simulated Mars flight and landing. Microbiome. **5**.
2. **Lax S, Smith DP, Hampton-Marcell J, Owens SM, Handley KM, Scott NM, Gibbons SM, Larsen P, Shogan BD, Weiss S, Metcalf JL, Ursell LK, Vázquez-Baeza Y, Van Treuren W, Hasan NA, Gibson MK, Colwell R, Dantas G, Knight R, Gilbert JA**. 2014. Longitudinal analysis of microbial interaction between humans and the indoor environment. Science. **345**:1048–1052.
3. **Lang JM, Coil DA, Neches RY, Brown WE, Cavalier D, Severance M, Hampton-Marcell JT, Gilbert JA, Eisen JA.** 2017. A microbial survey of the International Space Station (ISS). PeerJ. **5.**
4. **Mora M, Mahnert A, Koskinen K, Pausan MR, Oberauner-Wappis L, Krause R, Perras AK, Gorkiewicz G, Berg G, Moissl-Eichinger C.** 2016. Microorganisms in confined habitats: Microbial monitoring and control of intensive care units, operating rooms, cleanrooms and the International Space Station. Front Microbiol. **7**:1573.
5. **Mahnert A, Verseux C, Schwendner P, Koskinen K, Kumpitsch C, Blohs M, Wink L, Brunner D, Goessler T, Billi D, Moissl-Eichinger C.** 2021. Microbiome dynamics during the HI-SEAS IV mission, and implications for future crewed missions beyond Earth. Microbiome. **9**:27.
6. **Li D, Ching K, Hunt W**. 2021. Examination into the hi-seas IV built environment reveals differences in the microbial diversity and composition of plastic and wood surfaces. Undergraduate Journal of Experimental Microbiology and Immunology. **26.**
7. **Aviat F, Gerhards C, Rodriguez-Jerez J, Michel V, Bayon IL, Ismail R, Federighi M.** 2016. Microbial safety of wood in contact with food: A review. Comprehensive Reviews in Food Science and Food Safety. **15**:491–505.1.
8. **Bennett JW, Allen J, Cox-Ganser J, Gilbert J, Gold D, Green J, Haas C, Hernandez M, Holt R, Latanision R, LEvin H, Loftness V, Nelson K, Peccia J, Persily A, Zhou J**. 2017. Microbiomes of the Built Environment: A Research Agenda for Indoor Microbiology, Human Health, and Buildings. National Academies Press. Washington, D.C.
9. **Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MGI, Lee J, Ley R, Liu Y-X, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S,van der Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG**. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology. **37**:852–857.
10. **Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP**. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods. **13**:581-583.
11. **Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, Huttley GA, Gregory Caporaso J**. 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome. **6**:90.
12. **Anderson MJ**. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology **26**:32–46.
13. **R Core Team**. 2021. R: A language and environment for statistical computing (v.4.0.4). https://www.R-project.org/. Retrieved 15 April 2021. {Code and/or software}
14. **Wickham H, Averick M, Bryan J, Chang W, McGowan LD, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen TL, Miller E, Bache SM, Müller K, Ooms J, Robinson D, Seidel DP, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H**. 2019. Welcome to the Tidyverse. J Open Source Softw. **4:**1686.
15. **Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H**. 2020. vegan: Community ecology package (v.2.5-7). https://CRAN.R-project.org/package=vegan. Retrieved 15 April 2021. {Code and/or software.}
16. **Paradis E, Schliep K**. 2019. Ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics. **35**:526–528.
17. **McMurdie PJ, Holmes S.** 2013. phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLOS ONE. **8**:e61217.
18. **Wickham H**. 2016. In Gentleman R, Hornik K, Parmigiani G (ed), ggplot2: Elegant graphics for data analysis, 2nd ed. Springer-Verlag, New York, NY.

19. **Arnold JB**. 2021. ggthemes: Extra themes, scales and geoms for "ggplot2" (v.4.2.4). https://CRAN.R -project.org/package=ggthemes. Retrieved 15 April 2021. {Code and/or software.}.
20. **Janssen S, McDonald D, Gonzalez A, Navas-Molina JA, Jiang L, Xu ZZ, Winker K, Kado DM, Orwoll E, Manary M, Mirarab S, Knight R**. 2018. Phylogenetic placement of exact amplicon sequences improves associations with clinical information. mSystems **3**.
21. **Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Pep**
22. **lies J, Glöckner FO**. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research. **41**:D590–D596.
23. **Yilmaz P, Parfrey LW, Yarza P, Gerken J, Pruesse E, Quast C, Schweer T, Peplies J, Ludwig W, Glöckner FO**. 2014. The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks. Nucleic Acids Research. **42**:D643–D648.
24. **Glöckner FO, Yilmaz P, Quast C, Gerken J, Beccati A, Ciuprina A, Bruns G, Yarza P, Peplies J, Westram R, Ludwig W**. 2017. 25 years of serving the community with ribosomal RNA gene reference databases and tools. Journal of Biotechnology. **261**:169–176.
25. **Love MI, Huber W, Anders S**. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. **15**:550.
26. **Kruskal WH, Wallis WA.** 1952. Use of ranks in one-criterion variance analysis. J Am Stat Assoc **47**:583–621.
27. **Chen H, Boutros PC**. 2011. VennDiagram: a package for the generation of highly-customizable Venn and Euler diagrams in R. BMC Bioinformatics **12**.
28. **Proença DN, Francisco R, Kublik S, Schöler A, Vestergaard G, Schloter M, Morais PV**. 2017. The Microbiome of Endophytic, Wood Colonizing Bacteria from Pine Trees as Affected by Pine Wilt Disease. Sci Rep. **7**:4205.
29. **Pearce RB.** 1996. Antimicrobial defenses in the wood of living trees. New Phytol. **132**:203–233.
30. **Välimaa A-L, Honkalampi-Hämäläinen U, Pietarinen S, Willför S, Holmbom B, von Wright A**. 2007. Antimicrobial and cytotoxic knotwood extracts and related pure compounds and their effects on food-associated microorganisms. Int J Food Microbiol. **115**:235–243.
31. **Mori M, Gomi M, Matsumune N, Niizeki K, Sakagami Y**. 2013. Biofilm-Forming Activity of Bacteria Isolated from Toilet Bowl Biofilms and the Bactericidal Activity of Disinfectants against The Isolates. Biocontrol Sci. **18**:129–135.
32. **Jawad A, Seifert H, Snelling AM, Heritage J, Hawkey PM**. 1998. Survival of *Acinetobacter baumannii* on Dry Surfaces: Comparison of Outbreak and Sporadic Isolates. J Clin Microbiol. **36**:1938–1941.
33. **Dent Lemuel L, Marshall DR, Pratap S, Hulette RB**. 2010. Multidrug resistant Acinetobacter baumannii: a descriptive study in a city hospital. BMC Infect Dis. **10**:196.
34. **Hu Q, Hu Z, Li J, Tian B, Xu H, Li, J**. 2011. Detection of OXA-type carbapenemases and integrons among carbapenem-resistant Acinetobactor baumannii in a Teaching Hospital in China. J Basic Microbiol. **51**:467–472.
35. **Fournier PE, Richet H, Weinstein RA.** 2006. The Epidemiology and Control of Acinetobacter baumannii in Health Care Facilities. Clinical Infectious Diseases. **42**:692–699.
36. **Ryan MP, Pembroke JT**. 2018. *Brevundimonas* spp: Emerging global opportunistic pathogens. Virulence. **9**:480–493.
37. **Pinto M, Langer TM, Hüffer T, Hofmann T, Herndl GJ**. 2019. The composition of bacterial communities associated with plastic biofilms differs between different polymers and stages of biofilm succession. PLoS ONE. **14**:e0217165.
38. **Ganguli P, Chaudhuri S**. 2021. Nanomaterials in antimicrobial paints and coatings to prevent biodegradation of man-made surfaces: A review. Materials Today: Proceedings.