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A Longitudinal, Taxonomical Analysis of the Microbiome and Spatial Mapping of the Dynamics of Genera of Interest Reveals Pathogenic Spread Throughout Confined Living Quarters

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SUMMARY Space travel requires extensive research on its impacts on the astronaut microbiome and subsequent health. In this study, we explore the microbial profile of surfaces over time in an isolated, confined, and extreme (ICE) environment, which is an analog for real space stations. We used a dataset from a year-long Mars simulation mission from the Hawaii Space Exploration Analog and Simulation IV (HI-SEAS IV) mission, which contains 16S sequencing data generated from inanimate object sampling within the facility. We performed longitudinal analysis for alpha and beta diversity, taxonomic and differential abundance, and mapped out spatial representations to visualize bacterial travel. We found that beta diversity, taxonomy, and differential abundance differed between locations over time. Furthermore, we found evidence of contamination from pathogenic bacteria which showed ease in traversing the station over time. Our results suggest inconsistencies within the sanitary and disinfectant procedures of the HI-SEAS IV mission and emphasize the need for updated procedures and further examination into the viability of the HI-SEAS IV mission as an analog space station.

INTRODUCTION

Space travel is an important endeavor that impacts the astronaut's microbiome. Since the groundbreaking day of April 12, 1961 when Yuri Gagarin became the first human to enter space, space exploration has continued to be an exciting, continuously advancing field economically, philosophically, and scientifically (1). In 2021, the space sector contributed $2.8B to Canada's GDP and supported 24,190 employees (2). Since the 1970s, the Soviet space stations, the U.S. Skylab station, and various space shuttle flights have been launched, allowing for extraterrestrial human habitation and activity (1). However, space travel is known to affect the composition of the astronaut's microbiome (3). This is a concern given the role of the human microbiome in the bioconversion of nutrients, production of compounds (such as short-chain fatty acids), and mediation of resistance against various diseases (4, 5).

There is a substantial body of evidence for microbial population changes that occur on space flights, as well as the impacts these changes cause. Certain microbiota changes are transient; notably *Gamma-* and *Betaproteobacteria* abundance decreased while *Malassezia* species and Firmicutes tended to increase (6, 7). Space environments have also been found to suppress the function of innate and adaptive immune responses, including the differentiation of leukocytes, and reducing the production of type I interferons, all of which potentially contribute to microbiome shifts (8). Alterations of the microbiome are proposed to arise from the living conditions in space, particularly the impact of microgravity (the lower gravity in space compared to Earth) and the difficulty of washing skin with conventional methods (6).

Isolated, Confined, and Extreme (ICE) environments allow for more practical analogs for the study of space travel. Consequently, the importance of studying microbiota dynamics in space travel environments is emphasized. However, difficulties in microbial monitoring in true space environments due to logistic and funding constraints make research unfeasible (9). Instead, terrestrial analogues allow for more controlled and safe measures (10). These analogues, often referred to as ICE environments, are established by the National Aeronautics

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and Space Administration (NASA) and other similar entities to simulate the conditions in space (11). Notable ICEs include the Mars500, Lunar Palace 1, Antarctic Base Concordia, the Inflatable Lunar/Mars Analogous Habitat (ILMAH), and the Hawaii Space Exploration Analog and Simulation (HI-SEAS) habitat (12–17).

To ensure the well-being of astronauts, extensive studies on microbial dynamics on surfaces in ICEs have been conducted over time. A study by Schwender et al. tracked microbiome data in the air and on surfaces in the Mars500 habitat over 520 days (15). They found that microbial communities were concentrated in areas with high human traffic, such as the exterior of the toilet, the utility module, the bedroom, the community room, and the habitable module (analogous to a kitchen). While the microbiome was highly dynamic throughout the confinement period, diversity was highly influenced by the location of the sample and the duration of confinement. A study by Mayer et al. investigating changes in various surfaces of the ILMAH habitat yielded similar results (16). Over the 30-day period, the spatial diversity of microorganisms changed drastically between each time point, further addressing the highly dynamic nature of microbes in ICEs. Conversely, a study by Sun et al. on microbial air communities over 105 days in the Lunar Palace 1 revealed that while *Proteobacteria* predominated the early time points, an even distribution of *Proteobacteria, Firmicutes, Cyanobacteria, Deinococcus-Thermus,* and little *Bacteroidetes* and *Actinobacteria* was found during later time points (17). Discrepancies among these and similar studies highlight a need for further research into the microbial distribution over time on surfaces of ICEs.

Further studies are required on the HI-SEAS IV mission. The HI-SEAS habitat is a Mars and Moon exploration analog station operated by the International MoonBase Alliance located on the Mauna Loa volcano in Hawaii. It has allowed for long-duration (4 to 12 months) NASA Mars simulation studies, such as the HI-SEAS IV mission (18). Prior studies on HI-SEAS IV have found delayed longitudinal microbial homogenizations between the crews and habitat surfaces (9). Additionally, microbial analyses on the habitat's surfaces have found that plastic and wood exhibited significant differences in alpha and beta diversity, with plastic surfaces having more differentially abundant taxa (19–21). More specifically, bacteria commonly found in the human microbiome predominated the bathroom and bedroom environments, and the living room and bedroom sites had high rates of *Methylophilus* (22).

However, none of the previous studies on the HI-SEAS IV dataset have analysed the spatial evolution of microbial populations in the different living sites over time. Additionally, the lack of congruence in prior studies on microbial distribution over time in confined environments necessitates the present research. Given that human presence is often the primary contamination source on surfaces in confined environments, we examined the microbial composition from the kitchen floor, living room desk, bedroom desk, and bathroom toilet at 5 different time points in the HI-SEAS IV environment (15, 16, 23). We found that microbial composition differed between locations over time, and we discovered evidence of contamination from pathogenic bacteria and their ease of spreading between locations through the mission.

METHODS AND MATERIALS

HI-SEAS IV dataset information. The HI-SEAS IV mission, located on a dome of the Mauna Loa volcano in Hawaii, was operated by the University of Hawaii and funded by NASA (18). Six crew members (three male, three female) resided in the analog station from August 28, 2015 to August 28, 2016 with no outside contact, showers one to three times per week, and training activities performed in a mock space suit. Cleaning of the habitat occurred once per week with nine resupply events overall, with contact delays and sanitation. Microbiome samples were taken every other week with the habitat/furniture surface samples swabbed at four different locations: 1) the front part of the composting toilet bowl in the upstairs bathroom; 2) the kitchen floor in an area where dust accumulated; 3) a desk in one of the bedrooms; and 4) one desk in the main living room. Field controls were also performed for each sampling session. Mahnert et al. performed the analysis and dataset creation on which our study is based on. Mahnert's team performed DNA extraction, followed by microbial profiling based on amplicons targeting the V4 region of the 16S rRNA gene using the primer

pair F515-R806 with tags for Illumina sequencing (9). The raw amplicon data is available from the European Nucleotide Archive (accession number EMBL-EBI ERP118380).

Sequence quality control. After demultiplexing, sequences were imported into QIIME2 and corrected for sequencing errors. Sequences were then truncated to 285 bases, retaining 14,158 unique ASVs and 7,328,198 reads (24). Sequences were clustered into amplicon sequence variants (ASVs) with DADA2, as well as performing denoising, clustering, and the creation of a features table and representative sequences.

Phylogenetic tree, OTU table, and taxa generation. A phylogenetic tree, OTU table, and taxa were generated for future diversity, taxonomic metrics such as Evenness, Observed Features, Faith's PD, Shannon, and Weighted UniFrac, and differential abundance analysis. FastTree 2 was utilized to align ASVs and assess base pair differences to generate representative sequences. This was then used to produce phylogenetic trees based on relatedness and ancestral information, an OTU table, and a taxa list.

Filtration of data. The features table was filtered using QIIME 2 to include only samples taken from the surfaces, excluding sequences of mitochondria and chloroplasts as our analysis focuses on prokaryotic ASVs (24). These features tables were then binned to 5 time frames: early, mid-early, mid-late, late, and last based on collection time points, with the early phase having 6 time points and others having 5.

Alpha rarefaction. Alpha rarefaction curves were produced in QIIME2 (24). Curves and features tables were used to decide on a rarefaction depth of 14970 reads per sample. This sequencing depth was chosen to retain the maximum amount of ASVs while normalizing sequencing depth between samples.

Alpha and beta diversity. Alpha and beta diversity analysis was run with QIIME2 on features table and phylogenetic tree with the aforementioned rarefaction depth of 14970 reads per sample (24). We chose the Evenness, Observed Features, Faith's PD, and Shannon tests as our alpha diversity metrics and chose weighted UniFrac for beta diversity. PERMANOVA tests were run for statistical analysis of results (36).

Taxonomic classification and taxa barplot generation. The classifier was trained based on the Silva 138 99% OTUs reference tree at a truncation length of 285 in order to be used for the taxonomic classification of each representative sequence (37). The top 25 most abundant bacterial genera were selected based on the phyloseq object generated using the taxonomy information, OTU table, phylogenetic tree, and metadata. Taxonomic barplots were then generated for each location with the microViz package.

Differential abundance analysis. Differential abundance analysis was carried out with CRAN packages tidyverse, vegan, ape, and Bioconductor packages phyloseq and DESeq2 (38-42). All the analyses were carried out with the bedroom as the baseline and compared against the bathroom. A comparison for all genera was first carried out, and the focus was switched subsequently to genera *Gardnerella* and *Atopobium*.

Spatial modeling analysis. The 25 most abundant genera found through taxonomic classification, as well as popular bacterial pathogens, were screened in R for traversal over time. This was done using "ggplot2" heatmaps of read numbers in each location, plotted over the full duration of the experiment. With a focus primarily on pathogenic bacteria, genera with unique traversal patterns were identified and further explored using spatial modeling. The website "geogebra.org" was used to plot a coordinate system for the HI-SEAS environment, which was then translated into R and combined with read numbers using the "sf" package (40). Notably, the locations were plotted as though the microbial composition was consistent throughout the entirety of each location, not accounting for specific sampling points. The outcome was then plotted with the package "ggplot2" (41-42).

RESULTS

Alpha diversity metrics yielded no significant results. Evenness, Observed Features, Faith's PD, and Shannon tests were used to calculate alpha diversity in each location over time using the binned time points. Observed features of the living room significantly differed over time (p = 0.016, Figure 1). There were no significant changes in alpha diversity metrics in any other location analysed.

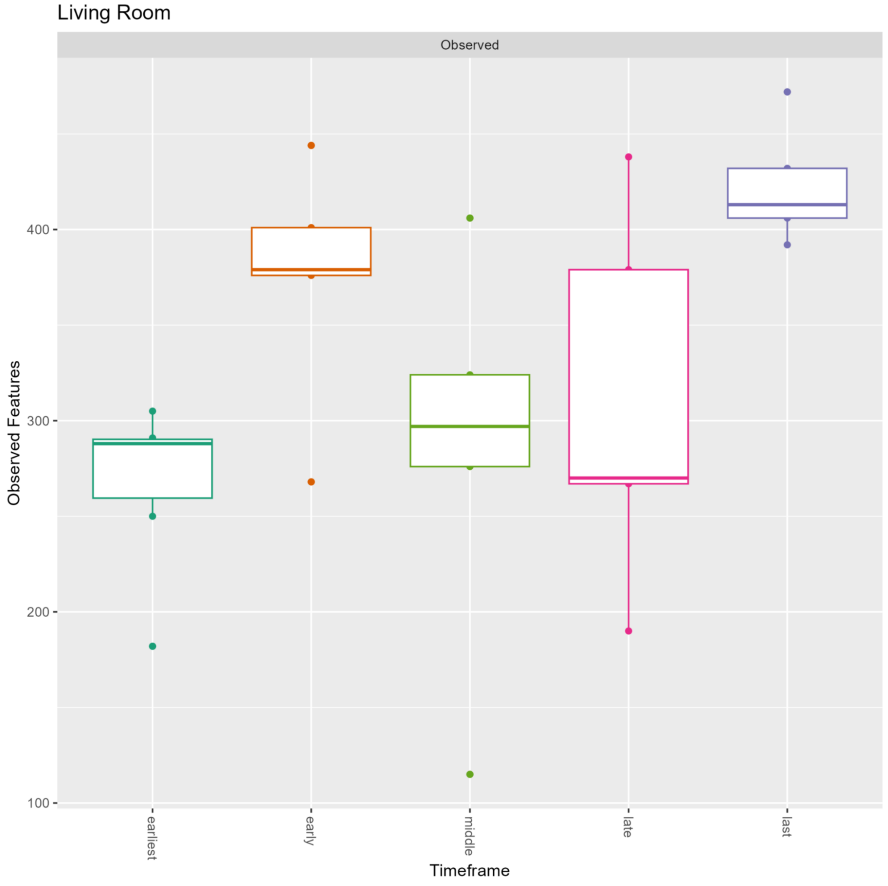

FIG. 1 Comparison of Observed Features in the living room at different stages during the HI-SEAS IV mission. Data collection dates binned into five time frames (earliest, early, middle, late, last) and PERMANOVA significance testing. p = 0.016. Each group had 5 time points, except for the earliest group, which had 6.

Weighted UniFrac Diversity was driven by location rather than time, but still changed significantly within each location over time. Analyzing the Weighted UniFrac Diversity of the communities in each location yielded significant differences depending on how the data was visualized. When the PCoA plot was grouped by location, the resulting communities, indicated by different colours, were very distinct from each other (Figure 2a). In particular, the kitchen (orange) and toilet (pink) showed almost no overlap with each other or with the bedroom (green) and living room (blue). The bedroom and living room shared a noticeable overlap in communities. When grouped by certain time periods, the resulting clusters all shared significant overlaps (Figure 2b). This was an indication that location, rather than time, was the driving factor for microbial diversity between locations in the HI-SEAS IV mission. In addition, the Weighted UniFrac Diversity changed significantly within each location over time. The microbial communities of the bedroom and toilet, in particular, had random fluctuations regardless of time (Figure 2c-d). Each location contained consistent communities at the beginning of the experiment, indicated by the smaller size of the light-coloured circles, with the exception of the kitchen.

The relative abundance of bacterial genera underwent substantial shifts across four locations over time, revealing potential genera for spatial modeling. Taxonomic analysis at the genus level revealed significant shifts in the relative abundance of certain genera at specific time points (Figure 3). Increases in *Staphylococcus, Acinetobacter, Gardnerella, and Atopobium* abundances were observed in both the bedroom and bathroom on multiple days (Figure 3a and 3b). The kitchen exhibited a significant rise in *Pseudomonas*, *Staphylococcus, Lactobacillus*, and *Lactococcus* at early time points, while the living room experienced an

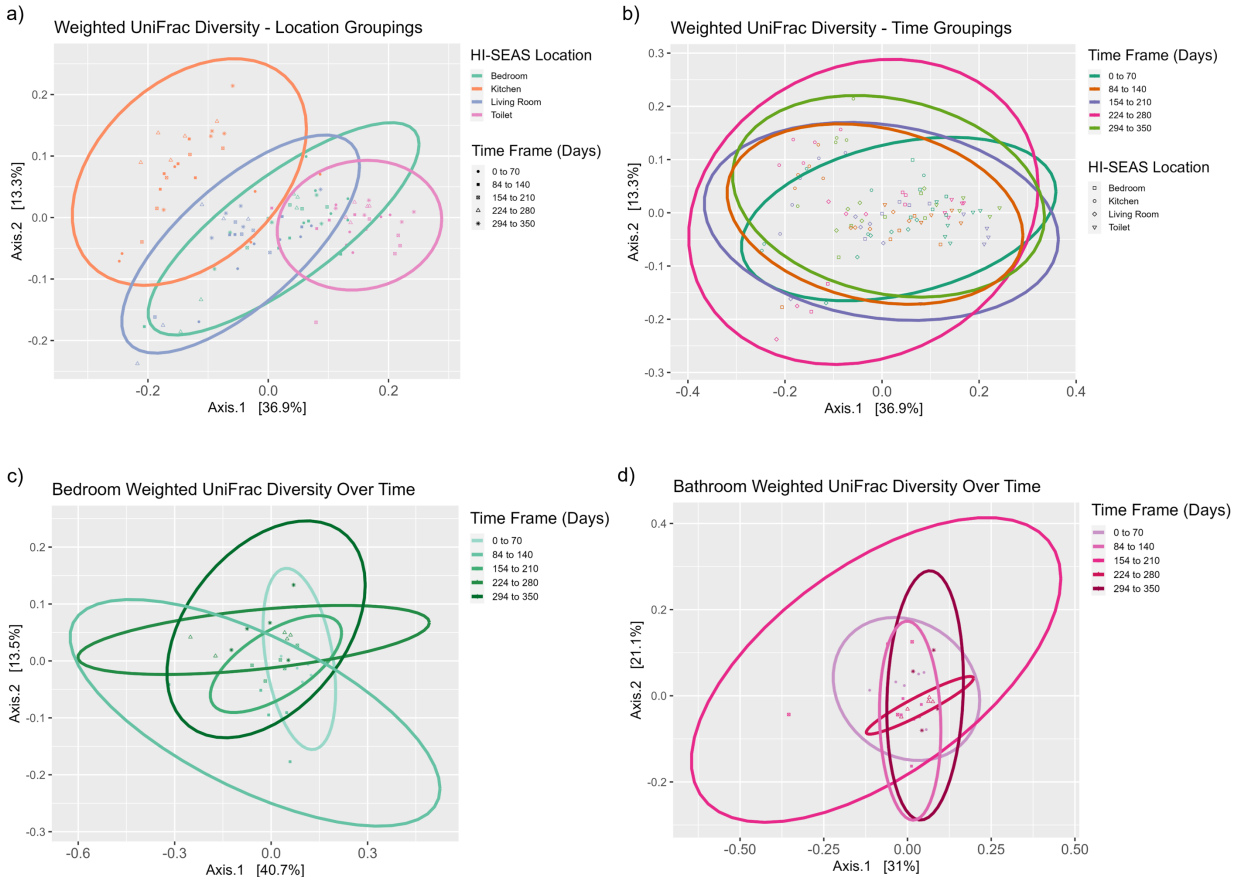

FIG. 2 Weighted UniFrac Diversity analysis of locations (living room, bedroom, kitchen, toilet) at different stages during the HI-SEAS IV mission. (a, b) PCoA plot of diversity analysis containing coloured points with regard to location (a) or time (b). p = 0.001. (c, d) PCoA plots of diversity analysis within the bedroom (c) and toilet (d). p-value = 0.008 and 0.013, respectively. PERMANOVA significance testing used for all.

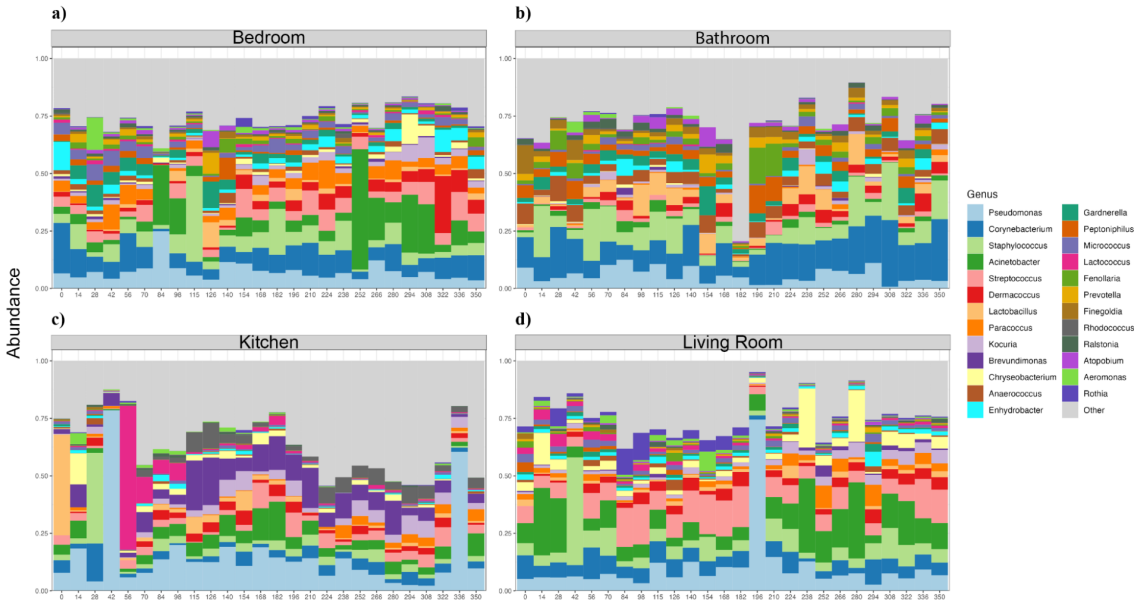

FIG. 3 The alteration of taxonomic composition over time at four locations. Taxonomic bar plots at genus level illustrating the relative abundance of bacterial genera in the bedroom (a), bathroom (b), kitchen (c), and living room (d). The top 25 most abundant genera were outlined in the legend with colours, and other genera were in gray.

increase in *Pseudomonas*, *Staphylococcus*, and *Chryseobacterium* at late time points (Figure 3c-d). Differential abundance analysis at the genus level comparing the bacterial composition in the bathroom and bedroom at two time points, early and last, underscored changes in the composition (Figure 4a-b). In the early six time points, 27 genera were more abundant in the bedroom, whereas 14 were more abundant in the bathroom (Figure 4a). Conversely, in the last five time points, 11 genera were more abundant in the bedroom, and 5 were more abundant in the bathroom (Figure 4b). Notably, genera *Gardnerella* and *Atopobium* were singled out for comparison, as changes in these two were observed in the taxonomic bar plots and both were associated with bacterial vaginosis. Differential analysis also revealed that both genera increased in the bathroom from day 154 to 210 and from day 224 to 294 (Figure 4c and 4d).

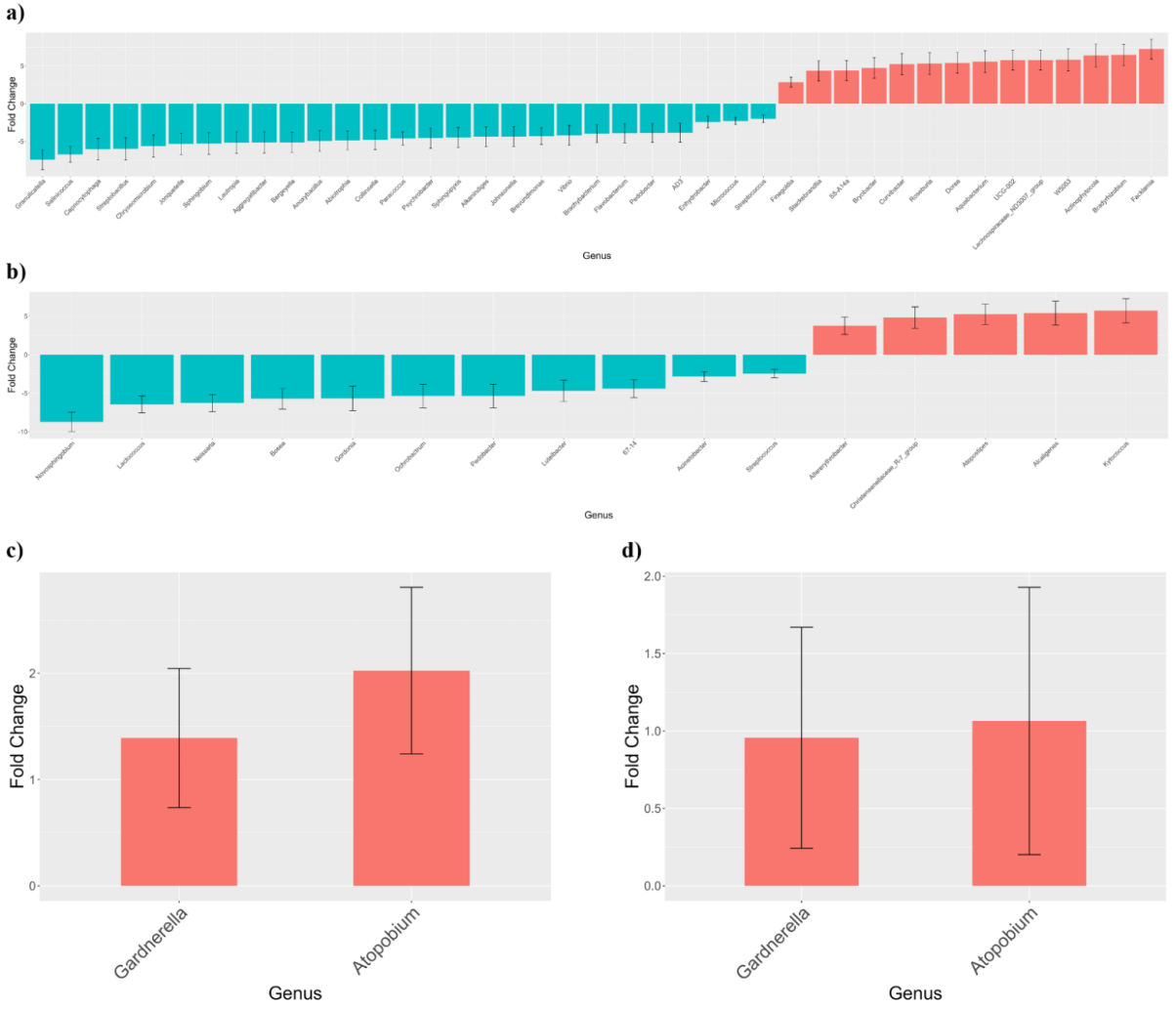

FIG. 4 DESeq analysis of the composition of microbiome of bathroom compared to bedroom at different time points. Genera that displayed a significant change ($p < 0.05$) according to the Wald test for (a) the first and (b) the last time point. The plots for (c) bedroom and (d) bathroom genera *Gardnerella* and *Atopobium* genera were shown separately. Red indicates an increase in abundance, while cyan indicates a decrease in abundance.

Bacterial genera widely spread throughout the HI-SEAS IV environment at all time points. Spatial analysis revealed multiple genera that travelled across different locations of the HI-SEAS IV environment over time (Figure 5). Genera *Aeromonas* and *Staphylococcus* spread turbulently across all locations early in the experiment, with large and fluctuating numbers of reads (Figure 5b, c). *Atopobium* and *Gardnerella* travelled throughout the top floor at all time points, with widespread travel to three locations across both floors during

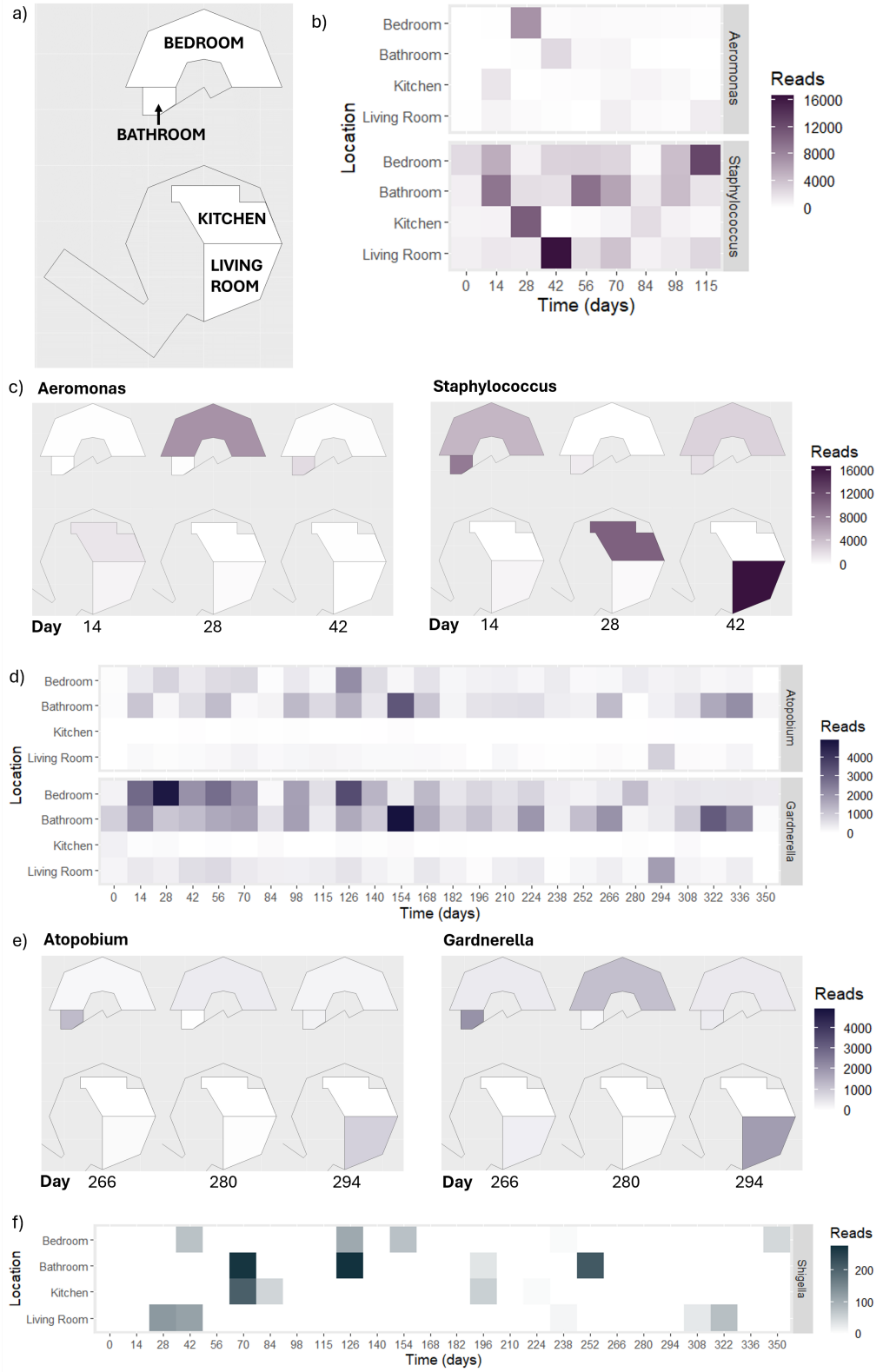

FIG. 5 Spatial mapping of bacterial genera throughout the HI-SEAS IV environment. Presence of bacterial genera, measured through read number, in different locations over time. (a) Reference of the HI-SEAS IV environmental layout as used in spatial mapping. (b, c) Heat map (days 0-115) and spatial analysis (days 14-42) of *Aeromonas* and *Staphylococcus* spread. (d, e) Heat map (days 0-350) and spatial analysis (days 266-294) of *Atopobium* and *Gardnerella* spread. (f) Heat map analysis (days 0-350) of *Shigella* spread.

later time points. These genera also had movement patterns consistent with one another (Figure 5d, e). Low amounts of *Shigella* were found emerging, disappearing, and reemerging throughout all locations at all time points, most strongly in the bathroom (Figure 5f).

DISCUSSION

Isolated, confined, and extreme (ICE) environments are necessary in order to study the extensive impacts of the space travel environment on astronauts, including their microbiomes. The HI-SEAS IV mission is one of these analog stations. The existing literature is heterogeneous and inconsistent, with a specific lack of longitudinal analysis on spatial diversity in ICEs. Our study evaluated the microbial composition of different sites within the HI-SEAS IV mission environment and visualized their travel over time.

Beta diversity is driven by location as opposed to time. Longitudinal examination of alpha and beta diversity of the HI-SEAS IV locations revealed that beta, but not alpha diversity, differed between locations (Figure 1, 2). This builds upon prior literature that found almost identical and analogous results with plastic and wood surfaces exhibiting significant alpha and beta diversity differences within the HI-SEAS IV station (19–22). Fung et al. (2022) specifically found that the living room and bedroom shared higher frequencies of shared microbial species, which is confirmed by our own findings (22). In particular, the kitchen and bathroom locations differed (Figure 2a), likely due to the unique microbial populations found in the food versus the gut as excreted in feces. This is corroborated by Mahnert et al.'s (2021) finding that the kitchen floor surface had notably low microbial diversity (9). Locations over time demonstrated consistent communities in the early periods of the analysis but fluctuated throughout with no clear patterns observed (Figure 2c-d). This mirrors findings on the Mars500 analog station (15). Thus, time is not a significant factor for microbial diversity. This is incongruent with the majority of the prior literature on the ILMAH and the ISS which found that bacterial community profiles changed over time (16, 25). It is useful to note that two prior studies on the HI-SEAS IV station found that the crew members' skin microbiome, consequently, showed a pattern of delayed longitudinal homogenization which may have influenced surface microbial composition (9, 26).

The abundance of specific resistant skin-associated genera shifted significantly over time. Taxonomic and differential abundance analysis of the top 25 taxa reveals significant shifts over time for most genera (Figure 3, 4). This corroborates previous studies on the HI-SEAS IV environment (19–22). Specifically, *Staphylococcus* and *Acinetobacter* genera show a dynamic profile. The main reservoirs of *Staphylococcus* are humans, mostly found in the skin and nasopharynx (27, 28). *Staphylococcus* acquire genetic resistance to heavy metals and antimicrobial agents, such as those used during sanitation of the HI-SEAS IV environment (28). *Acinetobacter* are skin-associated nosocomial pathogens with a high degree of resistance to drying and disinfectants, found in infected patients (29). These results agree once more with Mahnert et al.'s (2021) findings that skin-associated bacteria (*Staphylococcus, Brevundimonias, Streptococcus*) are easily traced in the bedroom and other skin-associated bacteria (*Acinetobacter*) in the living room as well as bedroom (9). This data emphasizes the importance of the exchange of microorganisms between astronauts and their environments, with similar findings in true space environments (6).

Pathogenic bacteria traverse widely throughout the HI-SEAS IV environment. Spatial analysis and visualization of select genera revealed the concerning spread of *Aeromonas, Staphylococcus* (Figure 5b, c)*, Atopobium, Gardnerella* and *Shigella* over time. Earlier time points seem to provide prime opportunities for turbulent bacterial spread for pathogenic and non-pathogenic genera alike. This is likely due to the early environment not being well established with necessary competing genera. This agrees with Schwender et al.'s (2017) study on the Mars500 station which predicted an increase in opportunistic pathogens and stress-tolerant bacteria, emphasizing the importance of continuous monitoring of the environment (15). However, widespread traversal of pathogenic bacterial genera was also seen throughout later time points, suggesting that pathogenic bacteria could widely spread at any time.

Bacteria involved in bacterial vaginosis traverse together throughout HI-SEAS IV locations. Of most notable concern, *Atopobium* and *Gardnerella*, as well as other less

abundant bacteria (*Megasphaera* and *Prevotella*), spread over time. These pathogenic bacteria are involved in bacterial vaginosis, which is the most common vaginal infection, characterized by a shift in the normal vaginal microbiota composition from *Lactobacillus*-dominated to anaerobic and facultative aerobic bacteria-dominated (30). Fung et al. (2022) similarly found the same bacteria in their study and asserted such a finding was reasonable given half the crew members were female (22). These pathogenic genera were found on the toilet, which is compatible with their association with the vaginal microbiota but also points to the bathroom as being a prime location where the microbiome is influenced. Our findings also show travel of these genera at later time points which adds to the supposition that pathogenic bacteria are particularly dangerous in their ability to quickly spread (31).

The HI-SEAS IV environment was likely contaminated with gastroenteritis-associated, soil-associated, and other bacteria. *Aeromonas* are gastrointestinal pathogens associated with gastroenteritis that are found in almost every environmental niche, while *Shigella* are likewise associated with gastroenteritis but are normally found in humans (32, 33, 34). These are intestinal pathogens that, once ingested, cause bacillary dysentery as well as diarrhea (33). They were found in the kitchen and bathroom locations where they have high infective potential if they contaminate food or dental hygiene products. Certain time points without reads for *Shigella* indicate these species may have travelled elsewhere beyond swabbed areas or, unfavorably, on a crew member. This is a likely supposition as humans are the primary reservoir of *Shigella* (34). Given that *Shigella* was first identified in the days following the supply event, this implies the pathogen may have been introduced by contamination from the external environment. It is possible supplies exposed to soil externally were not properly disinfected or handled once brought into the HI-SEAS IV station. Other sources of microorganism contamination are unclear but could include sanitary complications, the crew members' rotational cleaning of the composting toilet, or dispersal of fermenting food supplements (9).

Biocontamination aboard true spacecrafts also occurs and reflects some of the contamination found in the HI-SEAS IV environment. A review by Marra et al. (2023) found *Corynebacterium, Bacillus,* and opportunistic pathogens such as *Pseudomonas* and *Staphylococcus* onboard various space environments. Of significance in our data was the finding that three of the six bacterial pathogens from the ESKAPE list, a list of the six most virulent and antibiotic-resistant human bacterial pathogens, were present in the HI-SEAS IV mission. These include *Pseudomonas*, *Staphylococcus,* and *Acinetobacter* (31). A theory for the prevalence of these pathogenic and antibiotic-resistant bacteria in space environments is that exposure to space environments enhances the biomass and thickness of bacterial biofilms (35). Biofilms are recognized as a strategy microorganisms use to face environmental stressors, so biofilm-forming bacteria will likely resist the sanitation protocols of many space stations and prevail in the less competitive environment (35).

The results of our study show greater differences in microbial composition over locations than time, highlighting the functional specializations of each location and according contact with the crew members. It is thus especially concerning that pathogenic bacteria such as *Atopobium, Gardnerella, Shigella,* and *Aeromonas* were not only able to grow on surfaces in the station but also to traverse widely throughout the station. This points to the potential in future space missions of pathogenic bacteria to more easily spread given lower microbial competition and warrants close attention to disinfectant and sanitation protocols in such missions.

Limitations Our study included several limitations. One sample was collected per sampling day for each location, meaning no replicates were assessed. This reduces the validity of any subsequent findings as the data is far more susceptible to random outliers. Similarly, the locations of samples collected on surfaces were very particular (the anterior portion of the composting toilet bowl, a specific area of the kitchen floor, one desk in a bedroom, one desk in the main room). These measurements do not give us a full picture of the microbial composition in the HI-SEAS IV environment, or even a full picture of the furniture items being assessed. Additionally, confounding variables were not assessed, such as the impact of sanitation practices, resupply events, and baseline environmental and human microbiome compositions. The latter would be of particular use in analyzing longitudinal changes in the

microbiota in order to establish a true baseline against all subsequent findings. Particularly, the baseline health, antibiotic usage, and existing bacterial species of crew members would be helpful and may explain certain results.

An inherent limitation to the HI-SEAS IV station is that it only mimics in part a true space environment. As seen from our study, contamination from Earth microbiota is possible. The environment is also not able to replicate the ionizing radiation and microgravity effects that exist in space. Furthermore, a peer study on the HI-SEAS IV mission found that the HI-SEAS model is not very confined and is significantly different from the true ISS conditions (Anggunmulia et al., personal communication, 2023). However, even with these limitations, our study benefits from its longitudinal design, a minimal amount of confounding environmental variables, a set number of crew members, and the previously validated microbial hotspots used including desks, bedrooms, and toilet bowls (15).

Conclusions Our study aimed to assess the microbial composition, diversity, abundance, and spatial diversity over time in the space analog HI-SEAS IV mission station. We first examined the alpha and beta diversity across locations and time and found that beta diversity differed significantly across locations and not as significantly across time. We identified the most prevalent genera with taxonomic and differential abundance analysis and discovered that their frequencies shift throughout time. We then analysed the spatial distribution of select genera and found patterns of travel for certain pathogenic bacteria, most worryingly bacteria associated with bacterial vaginosis and gastroenteritis. Our results suggest that one or more contamination events occurred throughout the HI-SEAS IV mission, and that pathogenic, antibiotic-resistant bacteria are persistent and prevalent in the station. These findings warrant caution from space researchers and organizations manning future analog and real space stations, with particular attention being paid to sanitation and containment measures to prevent such pathogenic bacterial cultivation and travel.

Future Directions The dataset included skin samples that we chose to exclude from our research as we were focused on how bacteria travelled across different abiotic surfaces in the HI-SEAS environment. However, we would like to examine the taxonomy and diversity metrics of the post-isolation skin samples collected at day 400 and compare it to the baseline data at the beginning of the mission. This would allow us to track traversal of bacteria with people and perhaps determine if certain crew members were responsible for contaminations or occurrences of unique bacterial genera.

We would also like to recommend certain protocols to address the traversal of pathogenic bacteria, particularly those to help detect the pathogens and safety protocols to prevent them from spreading. When spaceflights are biocontaminated, it is usually remediated using fungistat wipes, air filtration, or disassembly and replacement of the contaminated payload (35). However, in a microgravity environment, the persistence of biofilms may be an issue due to their resilience and survivability on Earth. A possible solution is to adopt a combination of strategies including coatings, biocides, and shear stresses to delay biofilm formation for as long as possible. In addition, broad-spectrum antibiofilm treatments on abiotic surfaces and surface modifications to prevent biofilm formation could both be helpful in preventing pathogenic bacteria from spreading in the environment (35). For Shigella specifically, prevention of fecal-oral transmission would be the most effective control strategy.

During our research, we did not control for any confounding variables that may have impacted the results of the analysis. Sanitation practices and the impact of substances such as paint composition were factors present during the HI-SEAS IV mission that could have influenced our findings and should be considered in future studies to improve the robustness of any conclusions.

However, it is important to note that since our analysis was longitudinal and resulted in very detailed data, the overall impact of specific outlier events was likely decreased significantly. It is also possible for us to assess if any events relating to the confounding variables affected microbial diversity at certain time points.

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CONTRIBUTIONS

Melissa Lagace: Contributed to the dataset preparation, alpha and beta diversity analysis, and spatial modeling analysis, created spatial modeling analysis figures and edited full manuscript.
Rachel Leong: Contributed to literature review and composition of Abstract, Introduction, Methods, Discussion, Conclusion and References sections and edited full manuscript.
Soll Chi: Contributed to the dataset preparation, and alpha and beta diversity analysis including creation of figures and composition of according Methods and Results sections, composed Future Directions section, and edited full manuscript.
Qingyue Guo: Contributed to the taxonomic analysis and differential abundance analysis including creation of the figures and composition of according Methods and Results sections, and edited full manuscript.
Frank Zhang: Contributed to the taxonomic analysis and differential abundance analysis including creation of the figures and composition of according Methods and Results sections, composed Abstract and Future Directions sections, and edited full manuscript.

REFERENCES

1. **Logsdon JM**. 2023. space exploration. *Encylopedia Br*.
2. 2023. 2021 & 2022 State of the Canadian Space Sector Report - Facts and Figures 2020 & 2021. Canadian Space Agency.
3. **Voorhies AA, Mark Ott C, Mehta S, Pierson DL, Crucian BE, Feiveson A, Oubre CM, Torralba M, Moncera K, Zhang Y, Zurek E, Lorenzi HA**. 2019. Study of the impact of long-duration space missions at the International Space Station on the astronaut microbiome. *Sci Rep* **9**:9911.
4. **Young VB**. 2017. The role of the microbiome in human health and disease: an introduction for clinicians. *BMJ* **356**:j831.
5. **Nieuwdorp M, Gilijamse PW, Pai N, Kaplan LM**. 2014. Role of the microbiome in energy regulation and metabolism. *Gastroenterology* **146**:1525–1533.
6. **Tozzo P, Delicati A, Caenazzo L**. 2022. Skin microbial changes during space flights: a systematic review. *Life* **12**:1498.
7. **Sugita T, Yamazaki T, Makimura K, Cho O, Yamada S, Ohshima H, Mukai C**. 2016. Comprehensive analysis of the skin fungal microbiota of astronauts during a half-year stay at the International Space Station. *Med Mycol* **54**:232–239.
8. **Taylor P**. 2015. Impact of space flight on bacterial virulence and antibiotic susceptibility. *Infect Drug Resist* **8**:249–262.
9. **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.
10. **Terhorst A, Dowling JA**. 2022. Terrestrial analogue research to support human performance on mars: a review and bibliographic analysis. *Space Sci Technol* **2022**:2022.
11. **Golden SJ, Chang C, Kozlowski SWJ**. 2018. Teams in isolated, confined, and extreme (ICE) environments: review and integration. *J Organ Behav* **39**:701–715.
12. **Brereton NJB, Pitre FE, Gonzalez E**. 2021. Reanalysis of the Mars500 experiment reveals common gut microbiome alterations in astronauts induced by long-duration confinement. *Comput Struct Biotechnol J* **19**:2223–2235.
13. **Yang J, Hao Z, Zhang L, Fu Y, Liu H**. 2022. Surface fungal diversity and several mycotoxin-related genes' expression profiles during the Lunar Palace 365 experiment. *Microbiome* **10**:169.
14. **Zhao T, Liu G, Liu D, Yi Y, Xie B, Liu H**. 2022. Water recycle system in an artificial closed ecosystem – Lunar Palace 1: Treatment performance and microbial evolution. *Sci Total Environ* **806**:151370.
15. **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**:129.

16. **Mayer T, Blachowicz A, Probst AJ, Vaishampayan P, Checinska A, Swarmer T, De Leon P, Venkateswaran K**. 2016. Microbial succession in an inflated lunar/Mars analog habitat during a 30-day human occupation. *Microbiome* **4**:22.
17. **Sun Y, Xie B, Wang M, Dong C, Du X, Fu Y, Liu H**. 2016. Microbial community structure and succession of airborne microbes in closed artificial ecosystem. *Ecol Eng* **88**:165–176.
18. 2022. HI-SEAS: The Hawai'i Space Exploration Analog and Simulation.
19. **Li D, Ching K, Hunt WJ**. 2021. Examination into the HI-SEAS IV built environment reveals differences in the microbial diversity and composition of plastic and wood surfaces. *Undergrad J Exp Microbiol Immunol* **26**:1–10.
20. **Shen J, Chen A, Xiao K, Immanuel A**. 2023. 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. *Undergrad J Exp Microbiol Immunol* **28**:1–15.
21. **Rajkumar G, Khan A, Martens K, Park J**. 2022. Surface material and location impact microbial communities colonizing plastic and wood surfaces during the HI-SEAS IV Mission. *Undergrad J Exp Microbiol Immunol* **27**:1–9.
22. **Fung K, Ly HH, Soriano S, Song C**. 2022. Location and surface materials drive differences in microbial communities in the confined HI-SEAS IV habitat. *Undergrad J Exp Microbiol Immunol* **27**:1–13.
23. **Andersen BM**. 2019. Microbes, transmission routes and survival outside the body, p. 23–28. *In* Prevention and Control of Infections in Hospitals. Springer International Publishing, Cham.
24. **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. *Nat Biotechnol* **37**:852–857.
25. **Checinska Sielaff A, Urbaniak C, Mohan GBM, Stepanov VG, Tran Q, Wood JM, Minich J, McDonald D, Mayer T, Knight R, Karouia F, Fox GE, Venkateswaran K**. 2019. Characterization of the total and viable bacterial and fungal communities associated with the International Space Station surfaces. *Microbiome* **7**:50.
26. **Frese K, Naraina B, Pornsinsiriruk V, Shad A**. 2022. Examination into the HI-SEAS IV crew member microbiome reveals potential role of preferred interactions on microbial community structure. *Undergrad J Exp Microbiol Immunol* **8**:1–14.
27. **Jfoster T**. 2002. Staphylococcus aureus, p. 839–888. *In* Molecular Medical Microbiology. Elsevier.
28. **Seo KS, Bohach GA**. 2014. Staphylococcus aureus, p. 547–573. *In* **Doyle MP, Buchanan RL** (eds.), Food Microbiology. ASM Press, Washington, DC, USA.
29. **Towner KJ**. 2009. Acinetobacter: an old friend, but a new enemy. *J Hosp Infect* **73**:355–363.
30. **Rosca AS, Castro J, Sousa LGV, Cerca N**. 2020. *Gardnerella* and vaginal health: the truth is out there. *FEMS Microbiol Rev* **44**:73–105.
31. **Rice LB**. 2008. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. *J Infect Dis* **197**:1079–1081.
32. **Janda JM, Abbott SL**. 2010. The genus *Aeromonas*: taxonomy, pathogenicity, and infection. *Clin Microbiol Rev* **23**:35–73.
33. **Hale TL, Keusch GT**. 1996. Shigella. *In* **Baron S**, (ed.), Medical Microbiology, 4th ed. University of Texas Medical Branch at Galveston, Galveston (TX).
34. **Strockbine NA, Maurelli AT**. 2015. Shigella, p. 1–26. *In* **Whitman WB,** (ed.), Bergey's Manual of Systematics of Archaea and Bacteria, 1st ed. Wiley.
35. **Marra D, Karapantsios T, Caserta S, Secchi E, Holynska M, Labarthe S, Polizzi B, Ortega S, Kostoglou M, Lasseur C, Karapanagiotis I, Lecuyer S, Bridier A, Noirot-Gros M-F, Briandet R**. 2023. Migration of surface-associated microbial communities in spaceflight habitats. *Biofilm* **5**:100109.
36. **Anderson MJ**. 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* **26**:32–46.
37. **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.

38. R Core Team. 2021. R: A language and environment for statistical computing (v.4.0.4). https://www.R-project.org/. Retrieved 10 December 2023. {Code and/or software}.
39. **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.
40. **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 10 December 2023. {Code and/or software}.
41. **Wickham H**. 2016. *In* **Gentleman R, Hornik K, Parmigiani G** (eds.), ggplot2: Elegant graphics for data analysis, 2nd ed. Springer-Verlag, New York, NY.
42. **Arnold JB**. 2021. ggthemes: Extra themes, scales and geoms for "ggplot2" (v.4.2.4). https://CRAN.R -project.org/package=ggthemes. Retrieved 10 December 2023.