AUDITORY DISPLAY OF SPATIAL MICROBIOME DIFFERENCES BASED ON BIOLOGICAL CLASSIFICATION

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ABSTRACT

Understanding the spatial heterogeneity of microbial communities is crucial for microbial ecology. While visualizations are common, sonification offers a complementary approach, leveraging human auditory perception. Existing microbiome sonification research primarily focuses on representing raw sequence data or overall presence, overlooking spatial differences in community composition. This paper introduces an auditory display system that sonifies microbiome data based on biological classification (Class and Genus), emphasizing these variations. Taxonomic information is mapped to musical parameters, including pitch and Frequency Modulation/Phase Modulation (FM/PM) synthesis. Sampling locations are associated with interactive virtual record playing. A controlled, auditory-only experiment showed participants could reliably distinguish between microbial environments based solely on the sonified representations, performing significantly above chance. This demonstrates that carefully designed sonification can effectively communicate complex scientific data, specifically nuanced spatial variations in microbial communities. This work contributes to microbiome sonification and auditory display, promoting public understanding and providing new tools for data exploration.

1. INTRODUCTION

The Earth's microbial communities are extraordinarily diverse and play critical roles in global ecosystems. Advances in analysis of environmental samples have revolutionized our ability to study these previously hidden microbial worlds, providing unprecedented insights into microbial ecology and function [1, 2]. However, despite their fundamental importance, public awareness and understanding of microorganisms, particularly their spatial distribution, remain limited [3].

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While visual representations of microbiome data (e.g., compositional bar charts) effectively communicate microbial diversity to a certain extent [4], they often fall short in conveying the crucial concept of spatial heterogeneity – the fact that different microorganisms inhabit different locations [5]. Understanding this spatial variation is essential for a complete picture of microbial ecology [2]. This study focuses on sonifying these differences in microbial composition between discrete sampling sites, rather than implementing audio spatialization techniques related to the physical layout of a single site.

Sonification, the use of non-speech audio to represent data, offers a complementary approach to visualization [6]. Sound possesses unique characteristics that can engage human sensibilities and facilitate the understanding of complex, multidimensional data [7, 8]. Prior research demonstrates that sonification can effectively represent biological sequence data, revealing subtle patterns that might be difficult to discern visually [9, 10]. These studies highlight the potential of sonification as a powerful tool for data analysis, education, and public engagement with science [11].

However, much existing microbiome sonification research focuses on representing raw sequence data or demonstrating the presence of microorganisms, rather than explicitly highlighting spatial differences in community composition

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[12]. This paper addresses this gap by proposing a novel auditory display system that sonifies microbiome data based on biological classification, specifically designed to emphasize spatial variations [13].

2. RELATED WORK

This research builds upon and extends prior work in three main areas: environmental DNA and microbial ecology, sonification of biological and sequence data, and auditory information processing and perception of complex data.

2.1. Environmental DNA and Microbial Ecology

The field of analysis of environmental samples has rapidly advanced, providing powerful tools for studying microbial communities in diverse environments [5]. These methods allow researchers to detect and identify microorganisms without direct culturing, as shown in soil and marine microbial studies [2]. Such analyses have revealed the vast diversity of microbial life, underscoring the need to understand microbial spatial distribution as a crucial aspect of ecology [1].

2.2. Sonification of Biological and Sequence Data

A growing body of research explores sonification for representing biological data, including Deoxyribonucleic Acid (DNA)/Ribonucleic Acid (RNA) sequences, gene expression data, protein structures, and microbiome data [9, 16]. Early work focused on mapping sequence data to musical parameters for pattern recognition [10]. More recent studies investigate not only data analysis but also public engagement and educational tools, such as systems that sonify human microbiome data [11]. However, these systems primarily represent the composition of individual samples, rather than rather than explicitly highlighting spatial differences by employing microbial classification composition to emphasize spatial diversity[13].Auditory Information Processing & Perception of Complex Data

Effective auditory display design requires careful consideration of human auditory perception and cognition [6]. Studies from psychoacoustics and Human-Computer Interaction (HCI) have identified key principles for mapping data features to auditory parameters [17]. For example, humans can detect patterns in auditory data even when the data are complex or multidimensional [7, 18]. This ability can be leveraged to communicate intricate scientific information, including the subtle nuances of microbial ecology [19].

2.3. Research Gap and Contribution

While previous approaches demonstrate sonification’s power for communicating the existence of microbiota [9, 11], a gap remains regarding sonification optimized for conveying spatial variations in microbial communities. The contribution of this work is a sonification approach specifically designed to highlight spatial distribution differences, moving beyond

Figure 1. The auditory display system for microbiome sonification.

simply representing microbial presence. By mapping taxonomic information (rather than raw sequences) to sound, we create a more intuitive auditory experience of spatial heterogeneity [20]. This can facilitate deeper insights into microbial ecosystems for both scientific and public audiences.

3. SYSTEM DESIGN AND IMPLEMENTATION

This paper proposes a novel approach to intuitively communicate the invisible information of spatial microbiome distribution by linking biological classification information with the familiar experience of playing records. The core concept is to draw an analogy between:

- Microbial Sampling: Swiping a cotton swab across a surface.
- Record Playing: Dragging a needle across a record's grooves.

By allowing users to "play" records corresponding to biological classification information derived from microbiome samples from different locations, the system creates an auditory experience of spatial microbiome differences. The system comprises several key components. Microbiome data were obtained from samples collected at six distinct locations within and around Civic Creative Base Tokyo (CCBT): carpet in a back room, the back of a stainless steel trash can lid, a staircase brick, wallpaper at the venue entrance, a stuffed animal at the reception desk, and the leaves of a courtyard plant. Data processing involved DNA extraction, Polymerase Chain Reaction (PCR) amplification, and sequencing of these samples. Taxonomic classification (Class and Genus) was performed using vitcomic2, and the top 20 microbial entities with the highest PCR read counts were selected for each location. The user interface consists of custom-designed records, each representing a sampling location and equipped with reacTIVision tags for identification. The hardware includes a custom-built record player featuring a stepper motor controlled by an Arduino Uno. Software components include Processing for tag recognition and communication with Arduino, and MaxMSP for sound generation. Finally, a visual display presents synchronized environmental footage and microbial waveform data. Figure 1 shows the system architecture.

3.1. System Overview

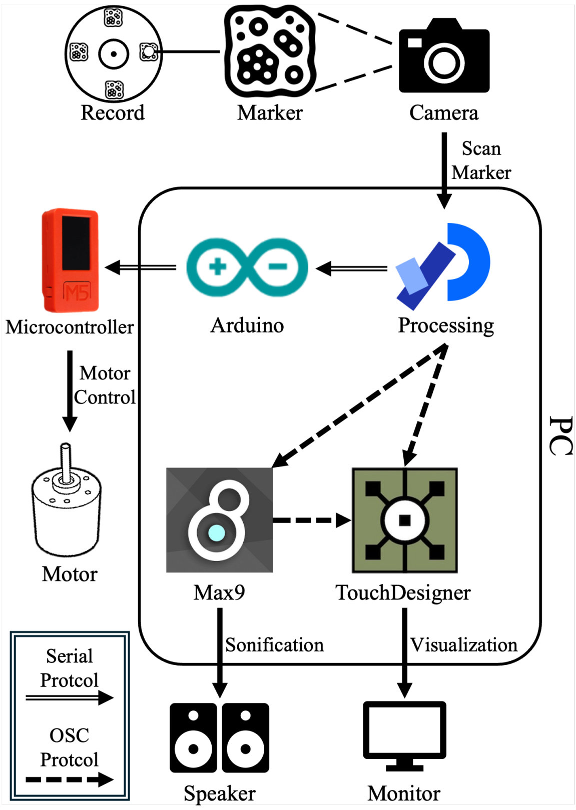

Figure 2. Data flow diagram of the auditory display system. The system processes microbiome data and maps it to auditory parameters via a custom sonification algorithm. User interaction triggers sound generation and synchronized visual output.

3.2. Data Collection and Processing

Samples were collected on August 31, 2024. Sterile cotton swabs wiped each surface for 3 minutes. Samples were sent to Genome Lead; DNA was extracted, PCR and sequencing were performed. vitcomic2 classified genes into Class and Genus. Top 20 microbial entities (highest PCR reads) per location were extracted.

3.3. Sonification Mapping

The sonification mapping utilizes the Class and Genus levels of the microorganisms identified in the samples. Data from the swab samples were organized into lists. The primary goal is taxonomic sonification, rather than a direct translation of nucleobase sequences into musical notes.To map these categorical taxonomic ranks to numerical sound parameters, a systematic indexing process was employed. First, for the mapping of Class to the FM/PM algorithms, the taxonomic classification software, vitcomic2, identified Class names from the entire dataset. The data from the six sampling locations (1. carpet, 2. back of a stainless steel trash can lid, 3. staircase brick, 4. wallpaper at the venue entrance, 5. stuffed animal at the reception desk, and 6. leaves of a courtyard plant) were ordered according to their collection sequence. Within each sampling location, the identified taxa were already sorted by their PCR read counts in descending order. These ordered datasets from all six locations were then concatenated into a single, comprehensive list, respecting the collection sequence of the locations. This consolidated list was processed sequentially. A unique integer index, starting from 1, was assigned to each distinct Class name as it first appeared in this list. If a Class name had already been encountered and assigned an index, that existing index was used. This integer index, ranging from 1 to 11 (corresponding to the number of unique Classes or capped at 11 if more Classes were present), directly corresponded to one of the 11 FM/PM algorithms described below, determining the timbre for microorganisms belonging to that Class.

Similarly, for the mapping of Genus to pitch, the top 20 microbial entities names were selected for each sample based on the highest PCR read counts. The data from the six sampling locations were ordered according to their collection sequence, with taxa within each location sorted by PCR read counts. These were concatenated into a single list. A unique integer index, starting from 1, was assigned to each distinct Genus name as it first appeared in this consolidated list. If a Genus name had already been assigned an index, that existing index was used. This Genus index then determined the specific pitch. The index value generates a frequency based on the 12-tone equal temperament chromatic scale, starting from C3. For example, an index of 1 would map to C3, an index of 2 to C#3, an index of 3 to D3, and so on. If the number of unique Genus indices exceeded 12, the mapping would cycle through octaves (e.g., index 13 would map to C4, index 14 to C#4, and so forth).

The temporal organization of the sound was also data-driven. PCR read counts influenced the amplitude envelope of each sonified microorganism; higher PCR read counts resulted in a stronger attack and a longer decay. The pacing of onsets, or the time until the next microorganism in the sequence was sonified, was also linked to PCR read counts, with higher counts leading to a longer overall duration for that sound event. Each microorganism from the list of top 20 microbial entities for a given sample was sonified sequentially, one by one, in the order determined by their PCR read counts (highest first). Within a given listening period for a sample, the probability of a specific microorganism's sound being triggered again was proportional to its PCR read count, creating a sense of varying presence for more or less abundant taxa. A subtle reverberation effect was applied to the final audio output purely for auditory smoothness, to prevent an overly abrupt or clipped sound, and does not represent any spatial data. No specific audio spatialization techniques linked to the data were implemented.

The sonification process is structured into four conceptual layers: data input, data communication, a voicing program, and sound generation modules. These layers, described in more detail in Section 3.5, work together to translate the taxonomic data, now indexed numerically and with temporal parameters defined, into sound.

Taxonomic information is mapped to sound parameters as follows: the Genus Index determines pitch, with the index value generating a frequency based on the 12-tone equal temperament chromatic scale starting from C3; the Class Index selects an FM/PM algorithm, producing a distinct timbre and also determining modulation indices within the voicing programs; and PCR Read Counts are used to determine tempo, amplitude envelopes, and playback probability.

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The 11 FM/PM algorithms are:

1. Basic FM Synthesis: One modulator, one carrier in a simple frequency modulation structure.
2. Feedback FM: One modulator with internal feedback path, one carrier. The feedback affects the modulator's own signal before it modulates the carrier as well as the signal folder that the carrier goes though.
3. Cascaded Dual Modulator FM: Two modulators in cascade structure with one carrier. The modulators' base frequencies are derived from fractional multiplication of the carrier frequency.
4. Hierarchical Cascade FM: Two modulators in cascade with one carrier. The second modulator's frequency is calculated by multiplying the primary modulator with the modulation index and a fractional multiplier of the carrier frequency.
5. Envelope-Controlled Phase Modulation: Two modulators and one carrier in a phase modulation structure. The input envelope modifies the phases of both modulators and the phasor.
6. Karplus-Strong FM String: One modulator, one carrier with phase modulation, utilizing a feedback delay line in a Karplus-Strong string synthesis configuration.
7. Triple Cascade FM: Three modulators in cascade structure with one carrier, implementing both phase modulation and frequency modulation techniques.
8. Dual Carrier Filtered FM: Two carriers with two feedback delay lines, combining phase modulation and amplitude modulation, processed through a ladder filter.
9. Envelope-Modulated FM: Two modulators, one carrier where an envelope affects phase modulation on the modulators. The modulators provide both amplitude and frequency modulation to the carrier.
10. Cross FM with Down-sampling: Cross frequency modulation structure where two oscillators mutually modulate each other through delay lines and sample-and-hold for down sampling. One oscillator provides additional amplitude modulation to the summed signal.
11. Feedback Cascade FM: Three modulators in cascade structure with one carrier. Each modulator applies both phase and frequency modulation to the next oscillator, with the third modulator feeding back to the primary modulator in a closed loop.

3.4. Sound Design Rationale

The sound design aims to represent phylogenetic relationships and spatial differences through sound. By transforming microorganism characteristics into sounds, the design seeks to make users aware of microscopic co-inhabitants. FM/PM synthesis was chosen for its ability to produce a wide range of complex tones with relatively simple organization. The algorithms can produce diverse timbres.

3.5. Implementation Details

The system is implemented using a combination of Processing, Arduino, and MaxMSP. The core sonification engine is built within MaxMSP, and it is structured into four layers:

1. Top Layer (Data Input): This layer handles the input of data from **Comma-Separated Values (CSV)** files. These files contain the taxonomic information (Class and Genus) for the top 20 microbial entities from each sampling location. Data are parsed and organized for use by the subsequent layers.
2. Second Layer (Data Communication): This layer manages communication between MaxMSP and other software components. It receives data from the top layer and transmits it to the voicing program (layer 3). It also handles communication with Processing, which is used for recognizing the reacTIVision tags on the custom-designed records, and with any external visual display software.
3. Third Layer (Voicing Program): This layer contains the core logic for mapping the taxonomic data to sound parameters, as described in Section 3.3. It receives the Class and Genus indices from the second layer. Based on these values, it selects the appropriate FM/PM algorithm, sets the pitch based on the Genus index.
4. Fourth Layer (Sound Generation): This layer comprises the 11 FM/PM synthesis algorithms. These modules receive control parameters from the voicing program (layer 3) and generate the actual audio output. Each algorithm produces a distinct timbre, representing a different microbial Class.

Class and Genus information were used for the sonification. As an example, index "1" might be assigned to Actinobacteria (Class) and index "1" to Geodermatophilus (Genus), while index "2" could be assigned to Blastococcus (Genus) within the same Class. This indexing scheme allows each microorganism to be represented by a two-dimensional vector: (Class, Genus). The Genus (represented on the x-axis in this conceptualization) is mapped to pitch, while the Class (represented on the y-axis) is mapped to the FM algorithm type. The real-time conversion from biological data to sound is implemented using MaxMSP. We refer to the supplementary materials[1] for generated sound.

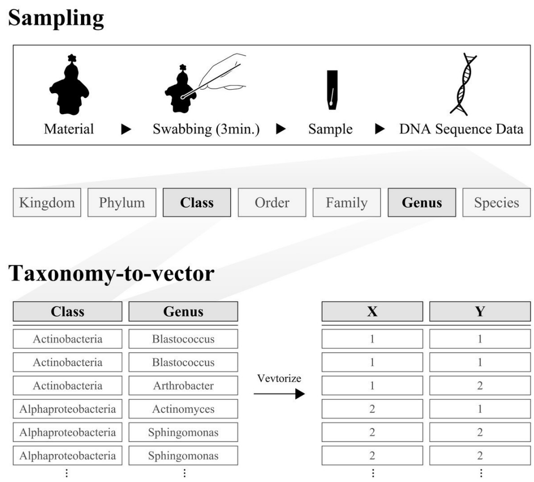

Figure 3. Data processing pipeline from environmental sampling to taxonomic vectorization. Samples are collected via swabbing, followed by DNA sequencing. Taxonomic classification is performed to identify Class and Genus. These taxonomic identifiers are then converted into a two-dimensional vector representation for sonification.

[1] https://drive.google.com/drive/folders/1dZR4QXff84XAuNhWC1ycyvXg7tPaERxg?usp=sharing

3.6. Record and Interface Design

The record surfaces are designed to enhance the connection with sound both visually and tactilely. Materials mimicking the sampling locations (carpet, metal, brick, paper, cloth, plant) are affixed to the record surfaces. This provides a tangible link between the abstract sound and the physical environment from which the samples were collected. The needle arm is designed with a small human figure holding a cotton swab, visually reinforcing the analogy between microbial sampling and record playing. We refer to the supplementary video[1] for record and interface design.

4. EVALUATION EXPERIMENT

To evaluate the sonification system's effectiveness in conveying differences between microbial communities, a discrimination experiment was conducted using only auditory stimuli. Visual/tactile cues were excluded to isolate the sonification mapping's impact.

4.1. Methods

The experiment employed a two-alternative forced-choice (2AFC) design to assess participants' ability to discriminate between auditory representations of microbial communities sampled from six distinct locations (Environments 1-6). These locations, previously described, were used to generate the auditory stimuli via the sonification mapping detailed in Section 3.3.

The experiment was conducted online and unsupervised. Participants were recruited, and they completed the experiment using their own computer and audio equipment. Critically, participants were instructed to rely solely on auditory information, with no visual aids or contextual cues provided, ensuring that any discrimination performance was attributable to the sonification mapping alone.

A custom JavaScript program was developed to manage the experiment, ensuring that each participant received a unique, randomized set of trials. The program automatically generated 12 trials for each participant. These trials were equally divided into two types: "Same Environment" trials and "Different Environment" trials.

For the "Same Environment" trials, the program randomly selected two distinct 12-second segments from the 30-second sound file representing a single environment. This process was repeated for each of the six environments, resulting in six "Same Environment" trials. For the "Different Environment" trials, the program randomly selected two 12-second segments, each from a *different* environment's 30-second sound file. This generated six "Different Environment" trials, ensuring a balanced representation of environment pairs.

Each trial presented two auditory segments sequentially. A 2-second silent interval separated the two segments. Thus, each trial had a total duration of 26 seconds (12s segment + 2s silence + 12s segment).

Participants (n=22; 11 male, 11 female, average age 24.7 years) were tasked with listening to the two segments in each trial and judging whether they originated from the same environment or different environments. Their responses were recorded on a 5-point Likert scale: 1 (Definitely Different), 2 (Probably Different), 3 (Not Sure), 4 (Probably Same), and 5 (Definitely Same). The total experiment duration was approximately 7 minutes.

4.2. Results

The overall mean accuracy across all trials and participants was 70.09%. This was significantly above chance performance (50%), as determined by a two-tailed t-test ($t(21) = 11.45$, $p < 0.001$).

Analysis of accuracy based on trial type revealed that participants were more accurate on "Different Environment" trials (72.68%) than on "Same Environment" trials (67.50%).

Within-environment discrimination, assessed by the "Same Environment" trials, varied considerably across the six environments. Environment 3 exhibited the highest internal consistency, with participants correctly identifying it as "same" in 81.82% of trials. Conversely, Environment 2 showed the lowest internal consistency, with an accuracy of only 45.45%.

Between-environment discrimination, assessed by the "Different Environment" trials, also showed significant variation. The most difficult discrimination was between Environment 1 and Environment 3 (25.00% accuracy). The easiest discriminations were between Environment 2 and Environment 6, and between Environment 4 and Environment 6, both achieving 100% accuracy. Environment 6 was generally easily distinguishable from other environments, with accuracy ranging from 58.34% to 100% across different pairings.

4.3. Discussion

The results of the discrimination experiment provide strong evidence that participants were able to distinguish between the auditory representations of microbial communities from different locations significantly above chance level. This finding supports the central hypothesis that the sonification mapping, by translating taxonomic information (Class and Genus) into distinct auditory parameters (pitch and FM/PM algorithm), effectively conveys information about the spatial differences in microbial community composition.

Several key observations emerge from the data. Firstly, the low internal consistency observed for Environment 2 (45.45% accuracy on "same" trials) suggests that the auditory representation of this environment was not particularly distinctive or consistent. This may be due to a lack of dominant microbial taxa in Environment 2, resulting in a less characteristic sound profile. Conversely, Environment 3 exhibited high internal consistency (81.82% accuracy on "same" trials), indicating a more distinct and recognizable auditory signature, likely reflecting a more homogenous or uniquely structured microbial community.

Environment 6, on the other hand, was generally the most distinguishable environment, particularly from Environments 2 and 4. This distinctiveness likely reflects a unique microbial community composition in Environment 6, which was effectively captured and conveyed by the sonification mapping.

The overall pattern of discrimination accuracies suggests that the six environments are not uniformly distributed within the perceptual space created by the sonification. Some environments, like 1 and 3, appear to be perceptually closer, while others, like 6 and the remaining environments, are more distinct. This non-uniformity is likely a reflection of the underlying complexity of the microbial data itself, and it suggests that the sonification is preserving, at least to some extent, the inherent structure of the data.

Furthermore, the variations in discrimination accuracy between different environment pairs imply that participants were likely utilizing multiple auditory features, derived from the mapping of Class and Genus to pitch and timbre, to make their judgments. It is unlikely that a single perceptual dimension could account for the observed pattern of results. This suggests that the sonification is creating a multi-dimensional auditory space, allowing for nuanced distinctions between the different microbial environments.

4.4. Participant Feedback

Qualitative feedback from participants provided further insights into the perceptual effects of the sonification. Several participants reported perceiving subtle, yet distinguishable, differences between the auditory representations of the different environments. One participant described these differences as variations in "texture" or "feeling," suggesting that the sonification mapping created nuanced auditory distinctions, even when clear-cut identification was challenging.

Furthermore, the sonification elicited personal recollections related to microbiology in some participants. One participant, for instance, reported that the sounds evoked memories of their experience in a food hygiene laboratory, specifically recalling the process of collecting and culturing microbial samples. This suggests that the auditory display, despite its abstract nature, was able to connect with participants' prior knowledge and experiences, creating a link between the sonified data and real-world phenomena. This capacity to evoke relevant associations is a potentially valuable aspect of the sonification approach, as it may facilitate learning and engagement with the underlying scientific concepts.

5. DISCUSSION AND FUTURE WORK

This paper presented a novel auditory display system and an experimental evaluation demonstrating that spatial microbiome differences can be communicated effectively via sonification. The interactive interface and the auditory-only experiment confirm that users can discern differences based solely on the sonified data.

The results provide strong evidence supporting the core hypothesis: participants performed significantly above chance in discriminating between microbial environments based on their auditory representations. This indicates that the sonification mapping, translating taxonomic information (Class and Genus) into auditory parameters (pitch and FM/PM algorithm), successfully conveys information about differences in microbial community composition. The varying distinguishability between environments, and the participant feedback, suggest a complex, multi-dimensional perceptual space is created by the sonification. Some environments (like 1 and 3) are perceptually close, while others (like 6) are more distinct. This non-uniformity reflects the underlying complexity of the microbial data itself. The participant feedback, highlighting subtle textural differences and the evocation of memories related to microbiology, suggests that the sonification taps into meaningful auditory features, potentially relating to past experiences.

It is important to clarify the scope of this research in light of the term "spatial." The "spatial" aspect addressed in this paper refers to the differences in microbial community composition observed across distinct, sampled locations, rather than the acoustic spatialization of sound within the auditory display itself. The primary aim of this study was to develop and evaluate a sonification method capable of representing these site-specific microbial variations effectively through sound alone, as demonstrated by our auditory-only experiment. The interactive record-player interface, while a core component of the overall system designed for user engagement, serves primarily as a means to present and interact with these sonifications of distinct locations. The effectiveness of the underlying sonification algorithm in conveying the targeted information (i.e., differences between microbial profiles of separate environments) was the main focus of the reported evaluation. This paper concentrates on the validity of the sonification method itself for representing spatial heterogeneity in microbiome data. The broader utility and impact of the interactive installation, including its potential for public engagement and education, represent an interesting avenue for continued investigation in future work.

This study has several limitations that suggest avenues for future research. More comprehensive user studies are needed. These studies should investigate the learnability of sonification mapping, examining how quickly users can form associations between the auditory features and the underlying microbial data. It would also be valuable to assess the impact of musical training on performance, as prior musical experience may influence a user's ability to discern subtle differences in the sonified representations. Additionally, future studies should evaluate the system's effectiveness for specific tasks, such as identifying particular microbial patterns or comparing multiple environments.

Beyond user studies, future work should explore the use of expanded datasets, including data from a wider range of environments and time points, to assess the scalability and generalizability of the sonification approach. The investigation of alternative sonification techniques, beyond FM/PM synthesis, could also reveal new and potentially more effective ways to represent the complexities of microbiome data. The parameter mapping could also be refined.

The current system integrates the auditory display with visual representations (environmental footage and waveform data) and haptic feedback (tactile materials on the record surfaces). While these additional modalities were included to create a richer and more engaging user experience, mirroring the act of physical sampling, their specific impact was not directly assessed in this study. Future research should, therefore, investigate the individual and combined contributions of each modality. It is possible that visual or haptic cues could either enhance or interfere with the processing of auditory information, and a careful investigation of these multimodal interactions is warranted. This could involve systematically manipulating the presence or absence of each modality and measuring the effects on discrimination performance, subjective experience, and learning outcomes.

6. CONCLUSION

This paper introduced a novel approach to sonifying spatial microbiome differences, using a biological classification-based mapping and an interactive interface. A controlled auditory-only experiment demonstrated effectiveness: participants reliably distinguished between environments based solely on sonified representations, significantly above chance. This provides strong evidence that carefully designed sonification can communicate complex scientific data, specifically spatial variations in microbial communities. This

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work contributes to microbiome sonification and auditory display, promoting public understanding and providing researchers with new tools. The findings, limitations, and future directions lay a foundation for continued investigation into sonification's potential to reveal the hidden complexities of the microbial world.

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