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No ultrasounds detected from fungi when dehydrated

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ABSTRACT

Many organisms (including certain plant species) can be observed to emit sounds, potentially signifying threat alerts. Sensitivity to such sounds and vibrations may also play an important role in the lives of fungi. In this work, we explore the potential of ultrasound activity in dehydrating fungi, and discover that several species of fungi do not emit sounds (detectable with conventional instrumentation) in the frequency range of 10 kHz to 210 kHz upon dehydration. Over 5 terabytes of ultrasound recordings were collected and analysed. We conjecture that fungi interact via non-sound means, such as electrical or chemical.

1. Introduction

Plants are known to respond to stimuli in a variety of ways, including physiological change [1–4], chemical compound emission [5,6], and acoustic sounds [7]. The ability to monitor these stimuli responses using non-invasive techniques has provided valuable insight into how plants adapt to changing environments. While such techniques have been explorated for plants, they remain unexplored for fungi. An efficient method for monitoring stimuli in fungi would create a plethora of architectural [8–11] and computational [12–15] opportunities. Since the tips of plant roots are known to emit ultrasound to signify obstacles or possibly coordinate activity [16–18] and neurons may chirp with ultrasound [19,20] to transfer information in biological neural networks, there is interest in determining whether fungi or mycelium networks emit ultrasonic signals (herein termed *mycoacoustics*) when stimulated. Nonetheless, in this paper, we explore some of the difficulties associated with conducting ultrasonic recordings in practise for mycoacoustic research.

Ultrasonic signals have frequencies greater than 20 kHz, which exceeds the upper limit of human hearing. In actuality, the assumed frequency range of the signal exceeds this limit significantly. When sampling a signal, measurements are taken at regular intervals and stored in discrete arrays for computation, but the underlying continuous signal can be recovered exactly if the sample rate is at least twice the highest frequency expected and the Shannon–Nyquist sampling theorem is satisfied [21]. As a result of these two facts, ultrasonic audio recordings contain a large number of samples per recording. In this work, for instance, we analyse frequencies up to 210 kHz at a sample rate of 500 kHz (more than twice the highest frequency, satisfying the sampling theorem's conditions); this translates to a sample rate of 500,000 per second, or 1.8 billion per hour.

Ultrasound recordings, like all signal acquisitions, are susceptible to noisy measurements. Denoising algorithms have traditionally been utilised to separate signal from noise. These methods rely on prior information to separate signal from noise, such as the assumption that noise follows a different distribution from the data distribution or occupies a different frequency band.

Denoising the acquired signals is impossible without a prior model of ultrasonic mycoacoustics, including knowledge of the frequency bands they occupy. Additionally, downsampling the audio is invalid because the signal-carrying frequencies may be removed. Consequently, signal detection must be performed on the raw data.

Our initial objective was to establish a baseline for the ultrasound activity of dehydrating and already-dry hemp substrates, as well as a baseline for the recording in a silent, empty room, see section 'Recording Setup' for details. In the remaining portion of this paper, we describe our signal acquisition and analysis procedures and demonstrate that the results are inconclusive with regard to the identification of ultrasonic mycoacoustic signals.

2. Materials and methods

2.1. Toy problem: Bird chirp

To find mycoacoustic signals is to find a needle in a haystack without knowing what a needle looks like nor whether one even exists.

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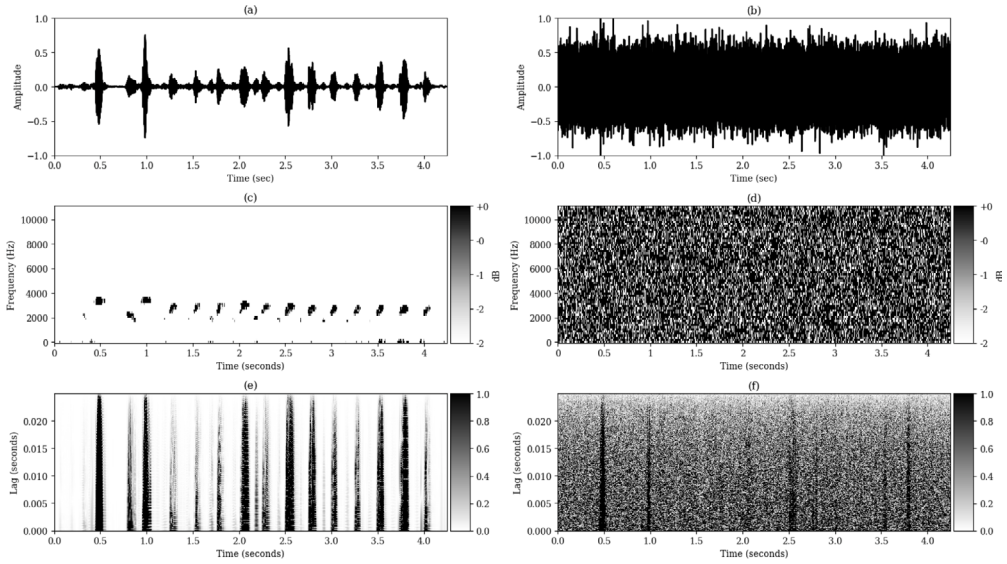


Fig. 1. Toy problem using spectral audio visualisations for bird chirp localisation. The left column is derived from a clean signal and the right is a noisy signal. (a) Clean time-domain waveform; (b) Noisy time-domain waveform; (c) short-time Fourier transform (STFT) of the clean signal; (d) STFT of the noisy signal; (e) time-lag representation of the clean signal; (f) time-lag representation of the noisy signal. Despite adjustments to the figure windowing (see associated colorbars), the STFT fails to discover the time or frequency of the noisy signal, but the time-lag representation successfully finds some chirps.

To test potential solutions, we first consider locating bird chirps in a clean and noisy environment. In Fig. 1, we show three visualisations of a clean and noisy bird chirp signal. The noisy signal x_{noisy} is simulated from the clean signal x_{clean}, where $x_{\text{noisy}} = x_{\text{clean}} + n$ and $n \sim \mathcal{N}(0, \max(x)/2)$. The first row of Fig. 1 depicts the time-domain waveform, the second depicts the short-time Fourier transform (STFT), and the third depicts a time-lag representation. This toy problem illustrates that chirping is identifiable with both the STFT and time-lag representation with the clean signal, yet in the presence of heavy noise, the STFT fails to uncover the chirp qualities and the time-lag representation reveals some chirping. We use this as our framework to analyse mycoacoustic signals where the ground-truth "clean" signal is not known a priori.

2.2. Recording setup

To record the mycelium, we designed and built a bespoke acoustically treated box to house biological samples and a microphone while allowing for injection of liquids. A render of this box is shown in Fig. 2. The internal dimensions of the enclosure were $300 \times 300 \times 270\,\text{mm}^3$ and the external dimensions were $540 \times 540 \times 440\,\text{mm}^3$. The enclosure was sound-insulated by four concentric layers to absorb externally generated sounds and dampen reflections. Firstly, the outer shell constructed from natural softwood plywood of 18 mm thickness (cut from sheet of 2440 mm length, 1220 mm width, B&Q Ltd, UK). Secondly, 50 mm thickness of stone acoustic insulation (Rockwool sound insulation slab). Thirdly, cardboard layer of 4.8 mm thickness to mitigated the possibility of particles interfering with the recordings. Fourthly, the inner lining of 15 mm thickness of polyethylene foam. Additionally, the bespoke box was partly isolated from vibration by standing on an acoustic foam flat sheet of 100 mm thickness [22].

The enclosure's cover was detachable, allowing for the replacement of fungi specimens. A small elevated platform, with slope, was constructed for the placement of the microphone in order to perform recordings, in order to have the specimen on-axis and avoid splashing when injecting liquids. The platform was attached to the enclosure's detachable cover. Biological samples were placed inside a suspended plastic pot in front of the microphone. The gap between the 'top' surface of biological samples and the front of the microphone was 10 mm.

Due to the limited availability of an acoustic room for extended periods (days), recordings were made in three locations using the bespoke acoustic box. First, inside the Esmono Sound Isolation Room [23], which has internal dimensions of 2.65 m width, 2.7 m, 2.6 m height. The recording space is a room-within-a-room arrangement, with the isolation room elevated from the concrete floor by thick rubber and sand base. The walls and floor clad with 6 cm thick, carpet. Behind the perforated steel walls is a dense layer of Rockwool. The door has a 10 cm thickness and is made of steel with a Rockwool core. An average background noise level of 15.9 dBA to 16.3 dBA was measured with digital sound level (SPL) meter (CR:162C, Cirrus Research Plc, UK). Second, an empty office with all equipment turned off (except for the recording laptop). The custom acoustic box sat on 10 cm thick foam that was placed on a freestanding table. The distance between the laptop and the custom acoustic box was greater than 1 m, and the laptop was elevated on an empty cardboard box. An average background noise level of 26.9 dBA to 27.1 dBA was measured with the same SPL meter. Third, an empty storage room. Again, the custom acoustic box sat on 10 cm thick foam that was placed on a separate table. The distance between the laptop and the custom acoustic chamber was greater than 1 m, and the laptop was elevated. An average background noise level of 23.8 dBA to 24.0 dBA was measured with the same SPL meter. All three rooms were kept locked, windows closed, lights switched off, during recordings to minimise possible background noise.

The microphone selected for recording was the M500 ultrasound microphone [24] with 500 kHz sampling frequency, 16-bit ADC resolution, and a frequency range of 10 kHz to 210 kHz. The microphone was used with the directional horn attached and biological samples on-axis with a gap of 10 mm, in order to attenuate reflections. Due to high data transfer rate, the M500 was connected to laptop (with Windows 10 operating system) using USB2 (480 Mbps max) cable. To maintain acoustic integrity a tapered rubber bung was used which constricts around the USB cables. Rock wool was used to back-fill the void behind the bung. A laptop rather than desktop computer was selected for its lower noise profile (due to cooling fan, switch mode power supply, hard drive, etc.). The computer was also placed on a vibration absorbent platform.

The software used to store the microphone recordings was BatSound® Touch Lite [25]. The primary function of the programme is the recording of ultrasonic bat sounds. In this scenario, temporary files are created at a predetermined location on the computer when a recording is created. These temporary files are then deleted when the

Fig. 2. Left: Render of the high-attenuation acoustic chamber used for recording specimens. The biological specimen is placed within a plastic pot when recording. This rendering shows the front panel removed; in reality, the lid is detachable rather than the front panel. Right: photograph with the lid partly open. The microphone cable is fed through a small hole at the top.

recording is saved and the programme is closed. In typical situations, these files do not grow massive in size. However, issues can arise when making exceptionally lengthy recordings. To address these obstacles, two custom software modifications were implemented by Pettersson Elektronik AB to enable recording for longer periods than originally envisaged. First, allowing the file to be saved to an external (large capacity) device in our case 1 TB Solid State Drive (SSD). Second, storing the recording as 4 GB files (rather than a larger number of smaller files each of 60 s maximum duration). It was not feasible to use the 'triggered recording' feature on the programme as the necessary setting level of possible fungi sound emissions was unknown. A secondary complication arises if the size of the temporary file exceeds more than half the available storage capacity of the external storage device; in this case, there is insufficient storage capacity remaining to save the data in its final format (e.g. `.wav` format). Two external 1 TB SSDs were connected to the laptop to circumvent this issue.

Recording were made for 25 h with the sound chamber empty (apart from the microphone) to determine the background noise levels.

2.3. Specimen preparation and management

The preparation of fungal specimens for acoustic recordings presented a significant challenge, as it required careful selection of both fungal species and substrates. We ultimately opted for three fungal species, *Pleurotus ostreatus* (Oyster Grey) [26], *Pleurotus columbinus* (Blue Oyster) [27], and *Hericium erinaceus* (Lion's Mane) [28], due to their widespread use, rapid growth rates, and distinct morphological features. Furthermore, we chose hemp and grain substrates for their compatibility with the selected fungal species, which could potentially influence the acoustic emissions.

Hemp fibres were prepared for the study by chopping them into shorter lengths (~20 mm to less than ~5 mm) using a high-speed, electric blender (Professional 2000 W, Homgeek, CN), as shown in Fig. 3. This process aimed to investigate the influence of fibre length on the emitted sounds during fungal growth and dehydration. In addition, we compared the acoustic emissions of unprocessed hemp to shredded hemp during dehydration, with multiple recordings taken of chopped hemp at similar moisture contents as the initial recording.

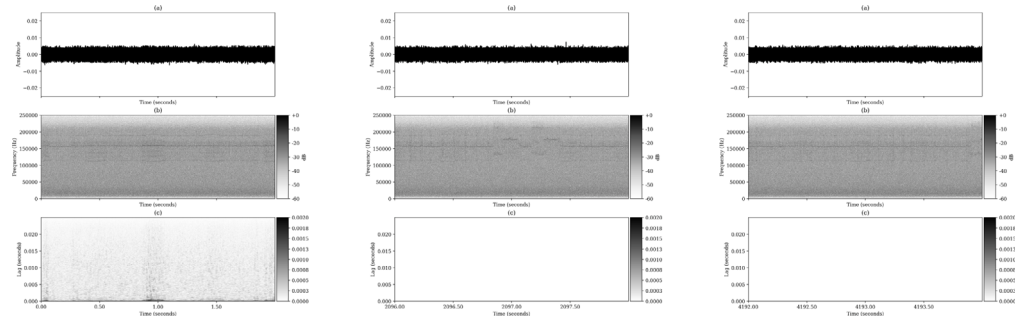
To obtain fungal samples for recordings, we grew the selected fungal species on the prepared substrates, such as hemp. It was possible to scrape mycelium off the surface of the substrate for recording, but there were concerns that the mechanical separation process could damage the mycelium and place it in a sub-optimal or stressed state without any nutrients to consume. Consequently, the data obtained from such samples may not accurately reflect the acoustic emissions of healthy mycelium.

Fig. 3. Left: original, unprocessed hemp. Right: shredded hemp via blender.

2.4. Analysis

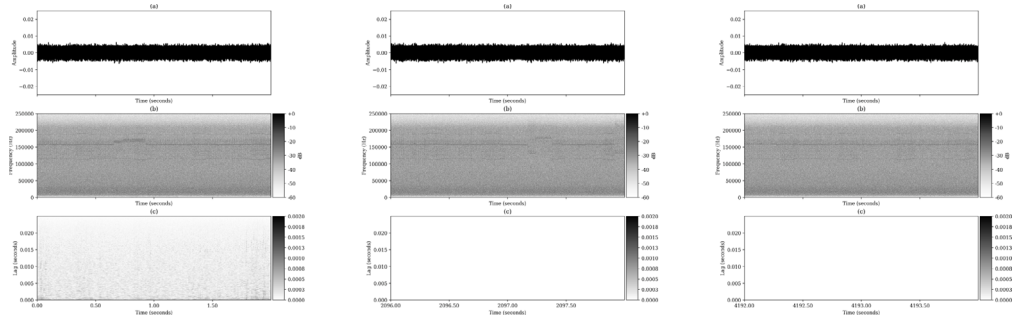
The ultrasonic recordings generated substantial amounts of data as BatSound® Touch Lite software operates in 32 bit mode recording ~60 MB per minute with M500 microphone. This equates to a storage requirement of ~4 GB h^{-1}. The 1 TB SSD has ~930 GB of usable capacity allowing ~9 d of continuous recording (without using trigger mode). Since the ultrasound recordings from each fungal species and substrate were multiple hours long at such high resolution, 5779 GB of recordings were saved for analysis in total. A larger quantity of recordings were made but only the most promising saved to conserve data storage capacity.

It was not possible to process entire sessions at once due to computational constraints. Instead, sessions were partitioned into two-second segments and each partition was processed via the `librosa` Python library [29]. Spectrograms were generated via the STFT with a 1 ms Hann window and hop size of 128 samples [30]. Audio files were normalised to $[-1, 1]$ intensity before rendering on the screen in dB. Spectrogram windowing was chosen to maximise visible signal, with a cutoff at −35 dB.



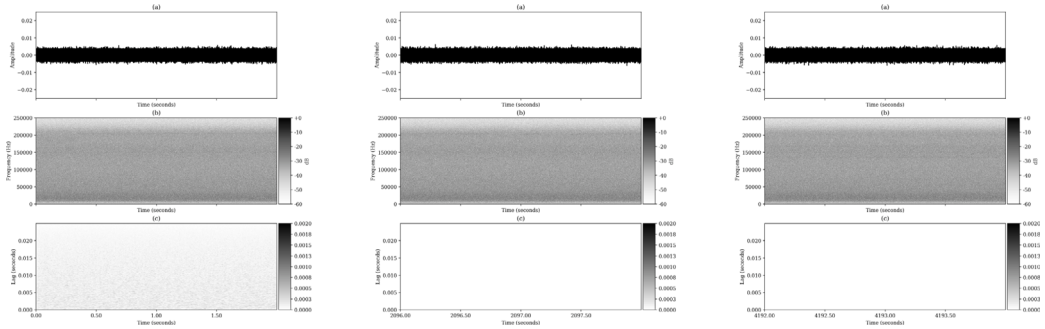
(i) A two-second window starting at 0 seconds (ii) A two-second window starting at 1048 seconds (iii) A two-second window starting at 2096 seconds

Fig. 4. Uniformly sampled time windows of the empty room acquisition 3, hour 0. (a) time-domain waveform; (b) STFT; (c) time-lag representation.



(i) A two-second window starting at 0 seconds (ii) A two-second window starting at 1048 seconds (iii) A two-second window starting at 2096 seconds

Fig. 5. Uniformly sampled time windows of Lion's Mane acquisition 1, hour 0. (a) time-domain waveform; (b) STFT; (c) time-lag representation.



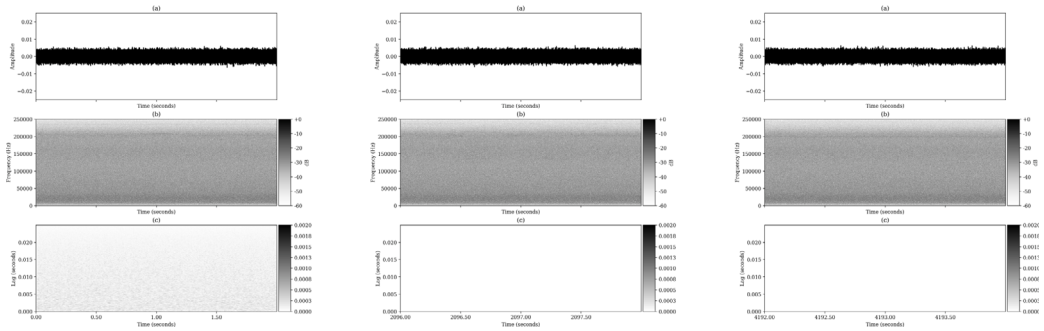
(i) A two-second window starting at 0 seconds (ii) A two-second window starting at 1048 seconds (iii) A two-second window starting at 2096 seconds

Fig. 6. Uniformly sampled time windows of Blue Oyster acquisition 1, hour 0. (a) time-domain waveform; (b) STFT; (c) time-lag representation.

Due to the absence of a ground truth, since it is unknown whether fungi emit ultrasonic signals nor the characteristics of such signals, it is not feasible to compute quantitative accuracy for the analysis. Instead, we relied exclusively on qualitative assessment. This necessitated the sub-sampling of time windows and the focus on specific time points for evaluation. Consequently, representative time windows were sampled at 0, 1048, and 2096 seconds from the start of each recording for each specimen-substrate pair. This qualitative analysis made use of the selection of STFT intensity window levels to attenuate noise and reveal the underlying signal. Consequently, an intensity windowing range of −60 to 0 dB was chosen empirically. This specific window level helped to differentiate the potentially significant signal amidst the noise (see Figs. 4–7).

2.5. Acoustic box attenuation measurement

A calibrated 40 kHz Reference Signal Generator (calibrated source) [31] rated 70 dBSPL at 250 mm was used to measure the attenuation of high-attenuation acoustic box. First, both the calibrated source and M500 microphone were placed facing each other inside the closed box. The gap between the front of the calibrated source and the front of the M500 microphone was 100 mm. The calibrated source was repeatedly switched 'on' for 5 s then 'off' for 5 s for 60 s while recording with M500

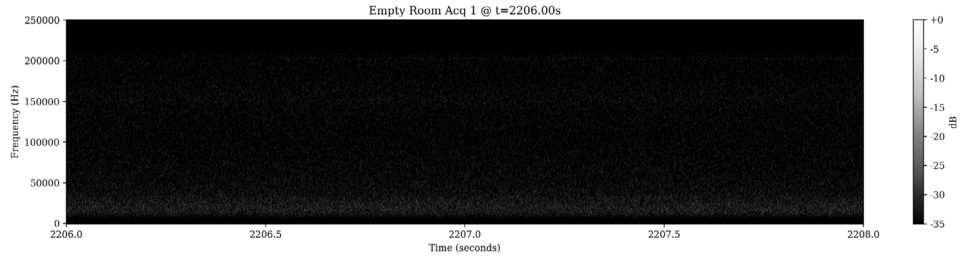


(i) A two-second window starting at 0 seconds

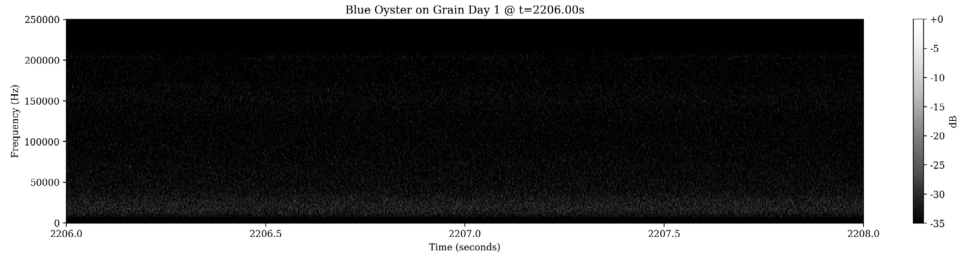
(ii) A two-second window starting at 1048 seconds

(iii) A two-second window starting at 2096 seconds

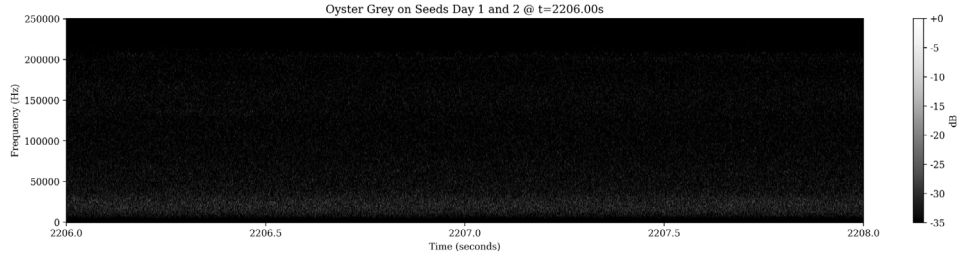
Fig. 7. Uniformly sampled time windows of Oyster Grey acquisition 1, hour 0. (a) time-domain waveform; (b) STFT; (c) time-lag representation.



(i) Empty chamber



(ii) Blue oyster



(iii) Oyster grey

Fig. 8. Representative spectrograms of an empty chamber, Blue Oyster, and Oyster Grey, respectively, shown at 2206 s. Note that the spectral characteristics are fairly empty, especially at higher frequencies, indicating a lack of signal for this duration.

microphone. Second, the calibrated source was position outside of the box while the M500 microphone remained inside close to the wall of the box. The gap between the front of the calibrated source and the front of the M500 microphone was 100 mm. The calibrated source was cycled as previously described. The recordings from the two setups were compared using analysis software (Audacity) to determine that the attenuation of the box is −43 dB at 40 kHz.

3. Results

Our findings revealed that shredded hemp produced lower acoustic emissions than unprocessed hemp when dehydrating, and notably, only wet unprocessed hemp emitted a noticeable signal. Investigation into the origin of sound waves revealed that, most likely, fast mechanical deformation of both drying hemp and drying fungi might emit similar ultrasonic signals.

We discovered conflicting results based on the spectral properties of each specimen. Some recordings indicate little or no ultrasonic emissions over many hours of dehydration. This consists of Blue Oyster and Oyster Grey, whose frequencies are comparable to those of the 'Empty Room'. These are depicted in Fig. 8. These subfigures depict only two seconds of audio, but they represent hours of recording. In contrast, Fig. 9i demonstrates that Lion's Mane emits ultrasonic chirps. As depicted in Fig. 9ii and Fig. 9iii, the contradiction arises

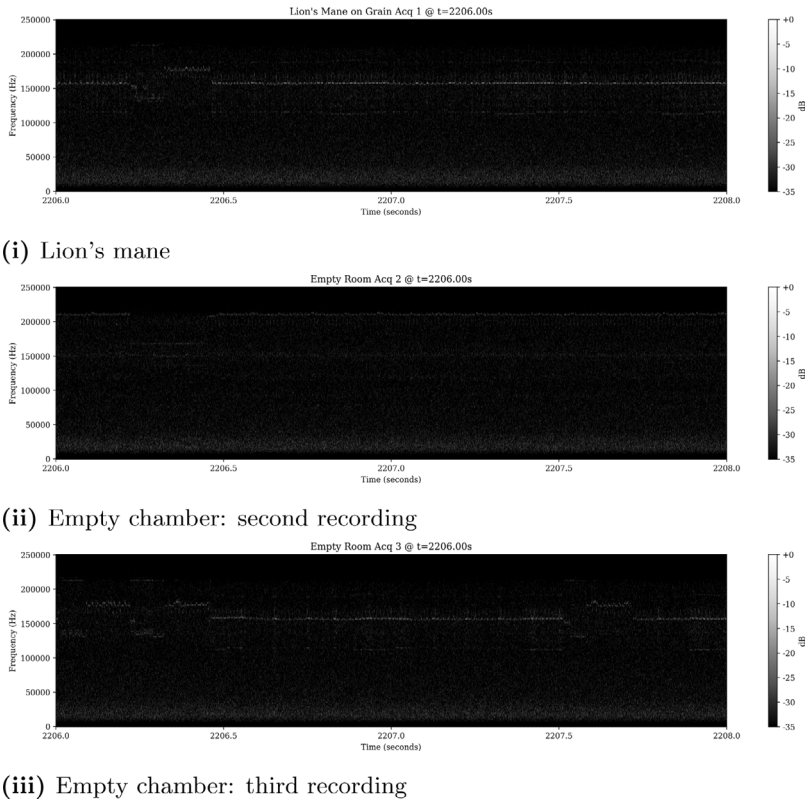


Fig. 9. Representative spectrograms of Lion's mane as well as a second and third recording of the empty chamber, respectively, shown at 2206 s. When comparing 9i to 8i, one may conclude that ultrasonic signals were emitted. However, 9ii and 9iii reveal that this type of pattern is common even when no specimens are present.

when multiple recordings of an empty chamber are considered. Despite there being no specimen in the chamber, ultrasonic chirping with characteristics similar to Lion's Mane was detected.

3.1. Discussion

Several challenges were encountered in conducting the research, in particular:

1. Minimal prior knowledge regarding ultrasonic mycoacoustic emissions has been published.
2. Due to the high sampling rate of ultrasound, audio recordings spanning multiple days quickly become 'big data'.
3. Recordings can be heterogeneous: different substrates with different species of fungi recorded in different rooms with different environmental conditions; how to harmonise all acquired signals in order to conduct analysis.

Thus, the results of our study are inconclusive in determining whether fungi emit ultrasonic signals during growth and dehydration. While some of the recorded specimens, such as Lion's Mane, demonstrated ultrasonic chirps, these chirps were also detected in empty chamber recordings. This suggests that the observed signals could be the result of environmental factors or instrumentation noise rather than the biological activity of fungi. The contradictory results point to an electronic artefact caused by prolonged microphone use, or the wood spontaneously emitting ultrasonic sounds under water stress [32–35]. Without a deeper understanding of signal emissions for each of these substrates, definitive conclusions cannot be reached. In addition, it is possible that dehydration itself does not cause ultrasonic emissions from these particular fungi, and that all present signals are noise or byproducts of ultrasonic interference.

Furthermore, our findings indicate that shredded hemp produces lower rates of acoustic emissions than unprocessed hemp during dehydration, with only wet unprocessed hemp producing a noticeable signal. The most likely origin of these sound waves appears to be the fast mechanical deformation of both drying hemp and drying fungi, which may emit similar ultrasonic signals.

Given the absence of a ground truth and the lack of quantitative analysis, our study is limited in its ability to draw definitive conclusions about fungal acoustic emissions. The qualitative nature of our analysis and the reliance on manual selection of time windows for evaluation could potentially introduce bias or overlook important data.

Future studies in this area may benefit from more extensive data collection, improved environmental controls, and the use of multiple sensors to account for potential instrumentation noise. Additionally, more advanced signal processing techniques could be employed to better distinguish between genuine biological signals and background noise. These improvements may help to provide more definitive evidence on whether fungi emit ultrasonic signals during growth and dehydration, and if so, the characteristics of these signals.

These findings suggest that fungi may exhibit other emissions, such as electrical signals [11,36–38], rather than relying on sound-based channels. Further research is necessary to determine the true nature of fungal signalling and explore the potential presence of ultrasonic emissions in different environmental conditions, fungal species, and contexts. A deeper understanding of fungal signalling could open up new architectural and computational opportunities, as well as provide valuable insights into their adaptation and response to environmental changes.

4. Conclusion

Ultrasonic emissions in dehydrating fungi within the frequency range of 10 kHz to 210 kHz were investigated. Despite collecting and analysing extensive data, we discovered that several fungal species did not emit detectable sounds during dehydration. We hypothesise that fungi interact through non-acoustic mechanisms such as electrical [37]

or chemical [39]. The findings of this study were partly inconsistent, with some recordings revealing ultrasonic chirps and others revealing no discernible emissions. Further study is recommended.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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