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# Plant ultrasound detection: a cost-effective method for identifying plant ultrasonic emissions

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## ABSTRACT

Plants have been observed to produce short ultrasonic emissions (UEs), and current research is focusing on developing noninvasive techniques for recording and analyzing these emissions. A standardized methodology has not been established yet; in this paper we suggest a cost-effective procedure for recording, extracting, and identifying plant UEs using only a single ultrasound microphone, a laptop computer, and open-source software.

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## KEYWORDS

Plant acoustics; plant ultrasonic emissions; airborne sound vibrations; non-invasive plant UE recording; ultrasound microphone

## Introduction

Acoustic emissions produced by plants have been studied for decades, first in the hearing range,<sup>1</sup> then also in the ultrasonic range.<sup>2–6</sup> According to the cohesion-tension model, in vascular plants the transpiration of water vapor in the leaves creates a negative pressure (tension) along the water-filled xylem conduits. This tension allows water to be carried upwards from roots to leaves against gravity. The water column tension inside the xylem conduits can be affected by multiple factors, such as the soil water content, the ambient temperature, the height of the plant, etc. If the tension in the xylem conduits becomes too high, the water column may undergo cavitation and embolism. As a result of this “air seeding” process,<sup>7</sup> a microscopic bubble may form, inducing vibrations within the adjacent water and tissues, and generating a short ultrasonic emission. Cavitation and embolism can be induced by different conditions, such as drought,<sup>6</sup> freeze-thaw events,<sup>8</sup> and biotic stress.<sup>9–11</sup> After bubble formation, further proposed mechanisms that may result in ultrasonic emissions are the abrupt regrouping of the bubble system adhering to xylem walls<sup>12</sup> and the fragmentation of bubbles inside pit chambers between neighboring xylem conduits.<sup>13</sup> The properties of ultrasonic emissions have been shown to partially depend on xylem anatomy. There is evidence that the number of UEs is related to the number of xylem conduits per unit area,<sup>14</sup> and that UEs with higher energy and longer settling time are related to xylem conduits with a larger lumen area.<sup>8,14,15</sup>

In the past, and sometimes until recently, to detect sound emissions, many studies have relied on techniques that involved the removal of bark and underlying tissues (phloem and cambium) to expose xylem conduits in woody stems, then clamping contact ultrasonic sensors in place (e.g.).<sup>8,14,16–19</sup>

In the last few years, there has been an increasing interest in noninvasive methods, employing contactless microphones for capturing plant UEs. Furthermore, attention has been extended to the investigation of non-woody species such as tomato.<sup>3,15</sup>

Understanding plant UEs holds great significance in the field of plant physiology and ecology. These emissions, often triggered by factors like drought, freeze-thaw events, and biotic stress, provide valuable insights into the physiological responses of plants to various environmental stressors. By studying UEs, a deeper understanding of the mechanisms behind plant water transport and stress responses can be gained, and potentially this could lead to improved strategies for mitigating the adverse effects of environmental challenges on plant health. Furthermore, the future development of non-invasive methods for field UE detection opens up new avenues for monitoring plant well-being in real-time, both in woody and non-woody species. Thus, this research field not only contributes to our fundamental understanding of plant biology but also has practical implications for agriculture, forestry, and environmental conservation.

The ultrasound pulses emitted by plants, as recorded by contactless microphones, are in general characterized by a very short duration (<1 ms) and a spectral peak in the near-ultrasound region typically falling within the range of 20–100 Hz.<sup>3,15</sup> Khait and colleagues used three pairs of coupled microphones to record acoustic data from tomato, tobacco, and other plants (wheat, maize, grapevine, etc.), eventually training a machine learning model to analyze these emissions.<sup>3</sup> Their findings revealed distinctive patterns in plant UEs associated with different stressors, contributing valuable insights to the understanding of plant responses to environmental factors.



**Figure 1.** Example of recording setup.

Dutta and collaborators used two independent microphones to record ultrasonic data in different directions (axial and radial) from several vascular species (*Hydrangea quercifolia*, tomato, sage, chili pepper, etc.) and developed custom MATLAB software to analyze them.<sup>15</sup> They found a correlation between the acoustic characteristics of UEs and the xylem radii, providing a noninvasive way to gather information on plant anatomy.

There is a growing interest in noninvasive study of plant UEs, but the lack of standard methodology for recording and analyzing these emissions sometimes makes it challenging to compare results across different studies.

This work describes an alternative low-cost procedure aimed at recording, extracting and identifying plant UEs in a non-anechoic setting, using a single ultrasound microphone, a laptop computer and the open-source software Audacity® (<https://www.audacityteam.org/download/>). The aim of this study, namely, is not to propose an entirely new standard technique that would replace other methods of investigating plant UEs with airborne sensors, nor to investigate the behavior of a group of plants in response to stress. Instead, it aims to provide a versatile yet robust and cost-effective alternative that can be further developed to contribute to the exploration of sounds emitted by plants.

## Materials and methods

### Pencil lead break test

Pencil lead break tests are used to generate reproducible test signals in acoustic emission applications.<sup>20</sup> We have recorded a series of pencil lead breaks on a wooden surface at increasing distances from the microphone sensor (from 1 cm to 80 cm), to evaluate sound attenuation. The recording conditions were similar to our experiments (the same room and the same time of the day, with all the electronic devices unplugged or turned off).

Our measurements show that sound levels tend to decrease depending on distance, as expected. The attenuation of the sound levels exhibits linear behavior (see Supplementary Material).

### Recording setup

All the recordings were performed using a Dodotronic Ultramic 384K BLE ultrasound microphone, which allows to record digital audio at a 384 kHz sampling rate, with a 16 bit depth. According to the Nyquist theorem, the microphone allows a maximum recordable frequency of 192 kHz (well above our needs, since we focused on the 20–100 kHz band). We mounted the microphone on an adjustable stand, to regulate the microphone position when placing it next to a plant. (Figure 1)

The microphone was connected via a USB cable to a laptop computer equipped with an open-source audio recording and editing software (Audacity®).

All the recordings were carried out in a quiet lab room. The doors and windows were closed and the electric devices inside the room were unplugged or turned off (except for the recording laptop computer) to minimize external ultrasound signals. For the same reason, the laptop power adapter was acoustically shielded under a plastic barrier. Every recording session was performed in daylight, between 11.00 AM and 2 PM, in spring (between April 4, 2023, and May 8, 2023). The microphone was placed at a distance of 1–2 cm from the closest stem and 22–23 cm from the farthest.

### Recording sessions

We performed a total of 18 digital recording sessions (Figure 2), each 30 minutes long to maintain a manageable file size during data processing (about 2.7 Gb for each file).

- 6 sessions with plants (“Plant group”).

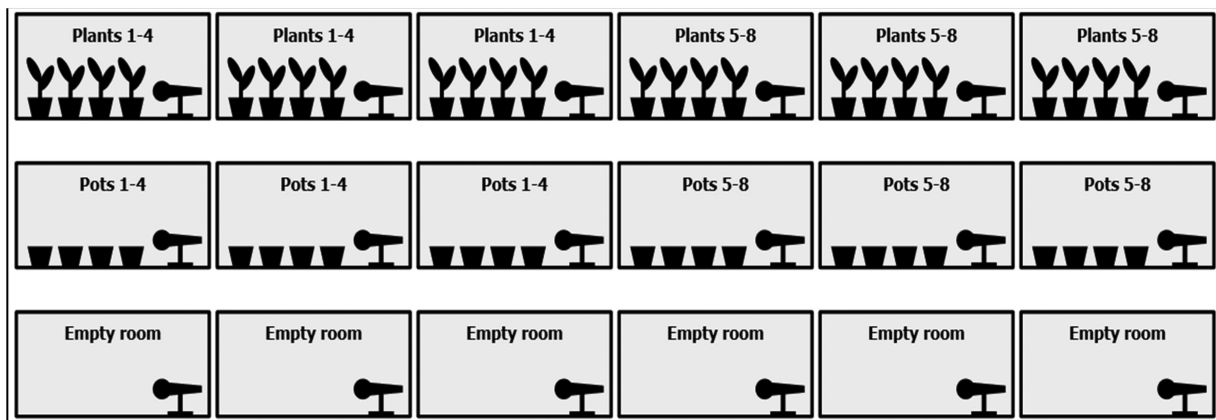
We used 8 pinto bean plants (*Phaseolus vulgaris*) divided in 2 sub-groups: plants # 1-4 and plants # 5-8 (two replicas of the same treatment). In each session we recorded one sub-group (4 plants at the same time). The plants were 36 days-old in the first sub-group and 42 days-old in the second sub-group, and the overall average stem diameter was  $4.04 \pm 0.2$  mm at the closest point to the microphone sensor. All the plants were in a vegetative growth stage and free from biotic or abiotic stress. We placed the microphone at a distance of 1–2 cm from the closest stem, without touching any part of the plant.

- 6 sessions with only soil-filled pots (“Soil group”).

To match the plant group, we set up 2 sub-groups of 4 pots each (pots # 1-4 and pots # 5-8, two replicas of the same treatment). The pots contained watered soil, but no plants. In order to check for possible UEs coming from the soil itself and from the interaction between soil and water, in each session we recorded one sub-group. (4 pots, same number as the plant group)

- 6 sessions with no pots and no plants (“Empty room group”).

We recorded the empty room in different days to check for possible external signals in the background noise.



**Figure 2.** Visual representation of the 18 recording sessions (see text for details).

### Data processing

We developed a 4-step procedure for extracting and identifying plant-emitted ultrasound pulses:

- (1) extract the ultrasound frequency bands;
- (2) identify all possible UE peaks (this step was performed in 2 iterations: first using a manual procedure, then using an automated procedure);
- (3) exclude artifacts and UE peaks that could be attributed to sources other than plants;
- (4) count the remaining acceptable peaks and perform statistical analysis on them.

The procedure is the main result of this paper and is described in detail in the “results” section.

### Statistical analysis

We performed statistical tests for the following reasons:

- Assess possible significant differences between the two iterations (manual and automatic) of step 2 in our procedure (see “results”).
- Assess if our procedure can identify plant-emitted UEs. We did this by comparing the entire plant group and two control groups, one with only pots (soil group) and the other with the empty room (empty room group).

To compare the two iterations of step 2 in our procedure (see “results”), we applied the Mann-Whitney U test (2-ways, p-value threshold = 0.05) to the number of UE peaks found with each iteration, and it resulted in no significant difference (see Table 3 and Figure 10; p-value = 0.81).

To assess if our procedure is capable of identifying plant-emitted UEs, we performed a Kruskal-Wallis test (p-value threshold = 0.05) to the 3 groups (plant group, soil group, empty room group) after excluding all artifacts and UEs that could be attributed to other sources (see Table 2 and Figure 9b). The test showed a significant difference (p-value = 0.0085). We also performed a Mann-Whitney U test (1-way, p-value threshold = 0.05) to confirm a significant difference

between the plant group and the two control groups (see Table 2 and Figure 9b; p-value 0.028).

### Results

A 4-step procedure to identify plant-emitted UEs is suggested:

- (1) extract the ultrasound frequency bands;
- (2) identify all possible UE peaks;
- (3) exclude artifacts and UE peaks that could be attributed to sources other than plants;
- (4) count the remaining acceptable peaks and perform statistical analysis on them).

Each step of the procedure is explained below.

#### 1 - extracting the frequency bands

As a first step, we removed the audible frequencies from the recorded tracks, and analyzed the whole ultrasound band from 20 kHz to 192 kHz in the time domain. However, it became quickly apparent that the background noise was far too loud to identify any UE (Figure 3). To overcome this issue, we extracted significantly smaller frequency bands, only 20 kHz wide. Since plant UEs are expected to be found in the near-ultrasound region, for each recording session we extracted 4 separate frequency bands: 20–40 kHz, 40–60 kHz, 60–80 kHz, and 80–100 kHz (Figure 4). This allowed us to easily spot potential UE peaks (Figure 5).

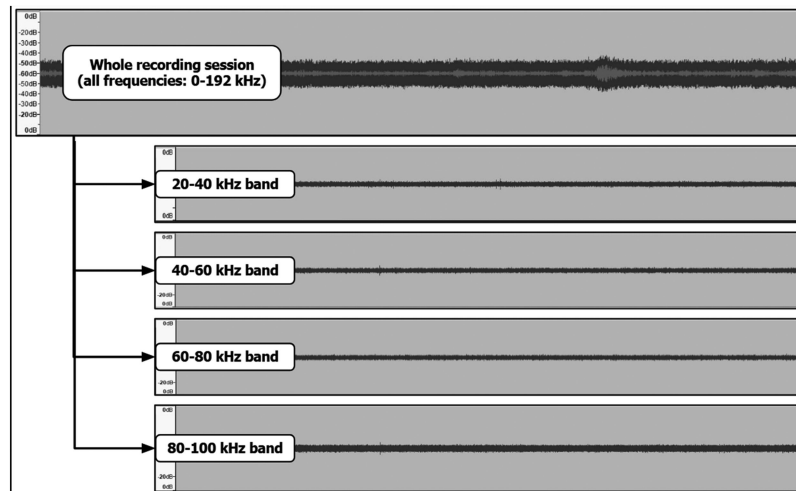
Since we extracted 4 frequency bands from every one of the 18 recording sessions, we obtained a total of 72 files, and we performed the subsequent steps on each one of those files.

#### 2 - identifying the ultrasonic emissions

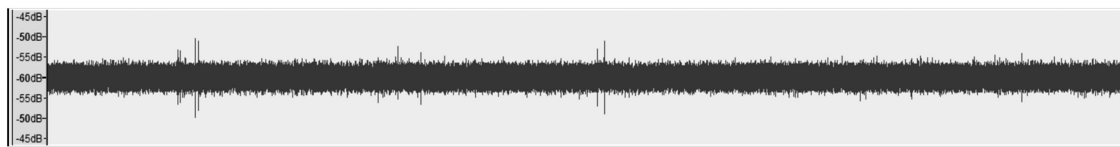
To identify the UE peaks, we developed a procedure using Audacity® commands to calculate a threshold value (corresponding to the maximum sound level of the background noise), then label as a potential UE every peak above that threshold. The procedure was applied to each one of the 72 files and went through two different iterations. The first one was slower, relying on manual steps. The second one was faster



**Figure 3.** Waveform view of the whole ultrasound band (20–192 kHz) of a recording session. The horizontal axis represents time (0–30 min.) and the vertical axis represents the sound level (dB FS). No UE peaks can be identified due to the background noise.



**Figure 4.** From each recording session, 4 ultrasonic frequency bands were extracted (20–40 kHz; 40–60 kHz; 60–80 kHz, 80–100 kHz).



**Figure 5.** Waveform view of a 20 kHz-wide band extracted from a recording session. The horizontal axis represents time (0–30 min.) and the vertical axis represents the sound level (dB FS). Potential UE peaks (vertical lines) can be easily spotted thanks to the lower background noise.

because it was fully automated. Since the outputs were slightly different, we compared the two iterations with a Mann-Whitney U test (2 ways, p-value threshold = 0.05) to check for statistically significant differences between them, and we found none (see discussion).

The first iteration (Figure 6) required the following steps:

- (1) manually select 3–5 regions with no peaks (background noise only);
- (2) manually measure the maximum sound level (dB FS) in each region;
- (3) keep only the highest (less negative) value;
- (4) add further 0.5 dB to calculate the threshold value;
- (5) automatically label all peaks above the threshold value as potential emissions.

The maximum sound level always returned values very close to each other (0.8% relative error). Therefore, in every audio track the ultrasonic background noise showed very little variation. As a consequence, the manual steps (selecting 3–5 regions with no peaks, and measuring the maximum sound level in each region) could be automated by measuring the average sound

level (Root Mean Square – RMS) of the whole track, and multiplying it by a constant value:

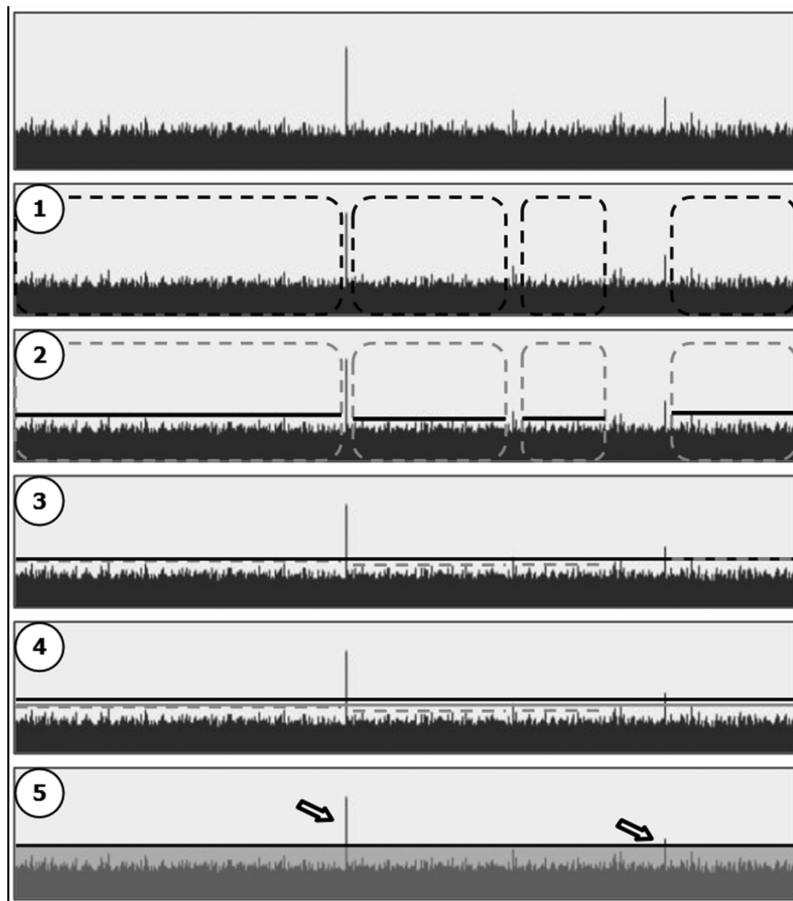
$$\text{threshold} = \text{RMS} \cdot \text{constant} + 0.5 \text{ dB}$$

In our experiments we found the constant value to be 0.7797413899 (see the “Comparison between the automatic and the manual thresholds” below).

The second iteration (Figure 7) relied on the constant value and proved to be significantly faster:

- (1) measure the average sound level (RMS) of the whole track;
- (2) multiply it by 0.7797413899;
- (3) add 0.5 dB to calculate the threshold value;
- (4) automatically label all peaks above the threshold value as potential emissions.

No significant difference was found between the manual and the automatic thresholds (see the “Comparison between the automatic and the manual thresholds” below). Therefore it is possible to use the faster automatic procedure.



**Figure 6.** Visual representation of the manual procedure used to identify potential UEs (see text). The horizontal axis represents time and the vertical axis represents the sound level (dB FS).

The 72 automatic thresholds calculated in these experiments proved to be at a distance of  $5.217 \pm 0.007$  standard deviations from the average sound level (RMS) in linear scale, therefore it is very unlikely to identify random fluctuations of the background noise as meaningful UE peaks.

### 3 - excluding non-significant peaks

After identifying all potential UE peaks, we excluded the ones that could be attributed to artifacts or sources other than plants:

- (a) artifacts produced by the “Spectral Delete” Audacity® filter, that was used to extract the frequency bands (Figure 8a);
- (b) ultrasonic peaks that occurred at the exact same time of audible sounds, and therefore likely part of those sounds (Figure 8b);
- (c) peaks made by a single anomalous sample, most likely an artifact (Figure 8c);
- (d) peaks made by a very small number of samples, resulting in a waveform with less than 3 complete oscillations, likely artifacts as well (Figure 8d). The procedure to calculate the number of wave oscillations is described below;

- (e) duplicate emissions (peaks occurring at the exact same time in multiple frequency bands, that are likely part of the same UE). (Figure 8e);
- (f) peaks representing UEs potentially coming from the soil or other sources (e.g., wi-fi access points, etc. . . .) (Figure 8f).

The features of these non-significant peaks are described below.

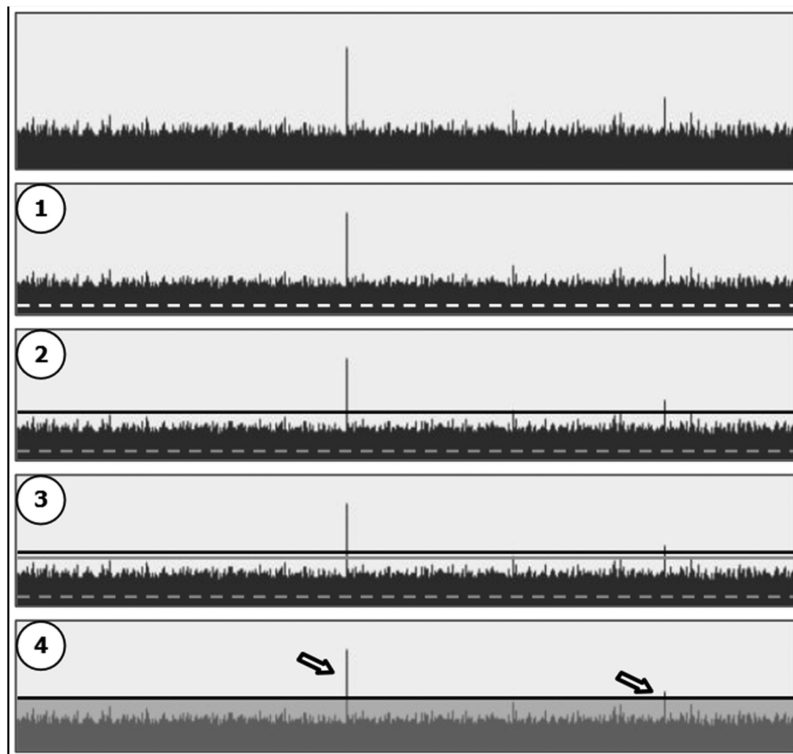
#### 3a – “spectral delete” artifacts

In Audacity®, the “Spectral Delete” filter doesn’t apply properly at the very beginning and at the very end of an audio track, leaving 7–8 ms long artifacts. This resulted in a large number of artifacts (2 for each audio file), that had to be excluded.

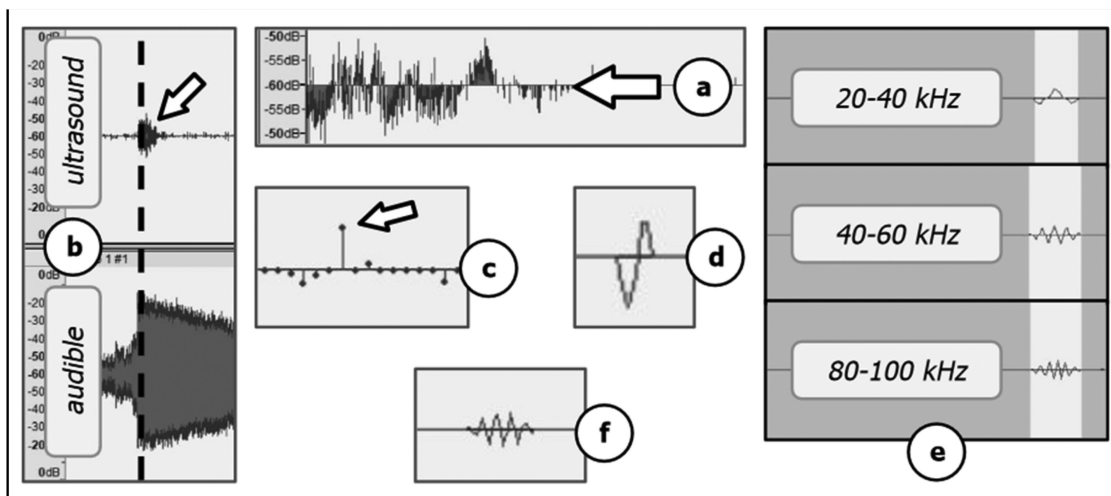
#### 3b – ultrasonic peaks aligned with audible sounds

Some ultrasonic peaks (most notably the longer ones, with duration of several ms or tens of ms) occur at the same time as audible sounds that can be seen in the waveform and/or heard in playback.

Most audible sounds could be attributed to sources different than plants (e.g. people talking in a corridor or



**Figure 7.** Visual representation of the automated procedure used to identify potential UEs (see text). The horizontal axis represents time and the vertical axis represents the sound level (dB FS).



**Figure 8.** Examples of UE peaks that were excluded for different reasons (see text).

taking the elevator, birds chirping outside the window, the laptop fan whirring), while other sounds had an uncertain origin. Since it was impossible to precisely identify audible sounds produced by plants just by listening to the recording sessions, as a precautionary measure we assumed that all audible sounds needed to be excluded. For the same reason we also assumed that any ultrasonic pulse that occurred at the exact same time of an audible sound was part of the same sound, therefore it needed to be excluded, too.

### **3c, 3d – peaks made by a single, anomalous sample and peaks with less than 3 complete oscillations**

Peaks made by a small number of samples, such as single anomalous samples or peaks with less than 3 complete oscillations, might be artifacts caused by electric events or random fluctuations of the background noise (Dodotronic company, personal communication), and therefore were excluded.

The number of oscillations in each peak was calculated as follows:

- (1) measure the peak duration as a number of samples (we did not use time units because we were computing intervals shorter than 1 ms);
- (2) plot the spectrogram of a small selection of 128 samples centered around the peak, and measure the peak frequency (Hz);
- (3) multiply the two values and divide by the sample rate (384000 samples/second).

Written as a formula:

$$\text{number of oscillations} = \text{duration} \cdot \text{peak frequency} / \text{sample rate}$$

### 3e – Duplicate emissions

Sound emissions consist of a spectrum of frequencies that may be larger than the 20 kHz-wide bands we analyzed. Therefore, we needed to consider the possibility of finding the same UE in different frequency bands. We found a few UEs that occurred at the exact same time (down to the millisecond) in more than one band, and therefore were likely part of the same sound. We considered those UEs as a single emission for counting purposes. This step allowed us to perform statistical analysis only on unique UEs rather than duplicate ones.

### 3f – UE peaks potentially coming from sources other than plants

To exclude possible emissions coming from sources other than plants, as a control we analyzed both the “soil group” and the “empty room group” recordings. We found 11 UE peaks that could not be excluded for other reasons. All these peaks occurred in the upper frequency bands (60–80 kHz and 80–100 kHz), and had a very short duration (<0.07 ms).

Such peaks obviously could not be coming from plants, because there were no plants in these recordings. Therefore, we excluded all UE peaks with similar features from every group (plant group, soil group, empty room group).

After excluding all non-significant UE peaks, the remaining ones were considered “acceptable” for statistical analysis purposes.

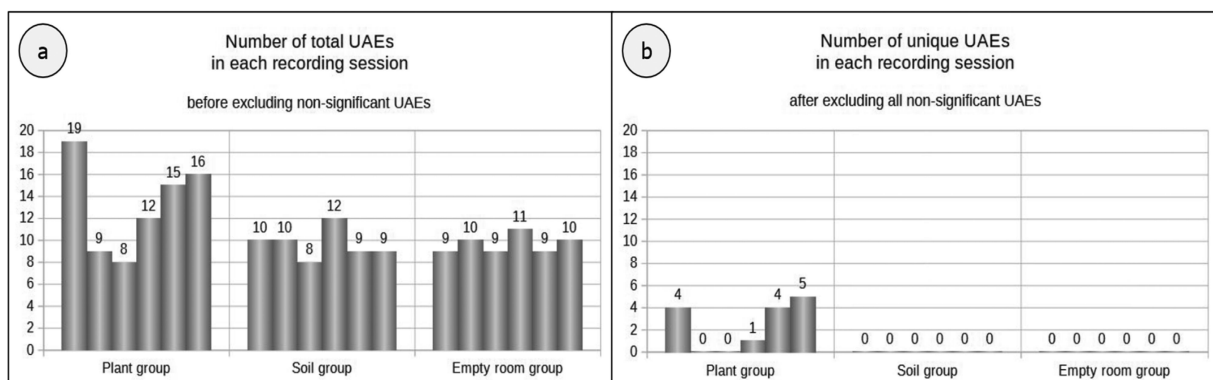
Lastly, we added together all the acceptable UEs pertaining to each recording session, because the statistical comparison between plant group, soil group and empty room group needed to be based on the 18 recording sessions, not between the 72 frequency bands (Table 1, Table 2, Figure 9).

**Table 1.** Number of unique acceptable UEs in each recording session before excluding all non-significant UEs.

Plant group		Soil group		Empty room group	
Plants #1–4	19	Pots #1–4	10	Days 1–6	9
	9		10		10
	8		8		9
Plants #5–8	12	Pots #5–8	12		11
	15		9		9
	16		9		10
Average		Average		Average	
	13.17 ± 1.74 UEs/30 min.		9.67 ± 0.56 UEs/30 min.		9.67 ± 0.33 UEs/30 min.

**Table 2.** Number of unique acceptable UEs in each recording session after excluding all non-significant UEs.

Plant group		Soil group		Empty room group	
Plants #1–4	4	Pots #1–4	0	Days 1–6	0
	0		0		0
	0		0		0
Plants #5–8	1	Pots #5–8	0		0
	4		0		0
	5		0		0
Average		Average		Average	
	2.33 ± 0.92 UEs/30 min.		0 UEs/30 min.		0 UEs/30 min.



**Figure 9.** Number of UEs in each recording session, (a) before and (b) after excluding all non-significant UEs. Each column represents one of the 18 recording sessions, and the vertical axis represents the number of UEs found in that recording session. The UEs in (b) were considered acceptable for statistical analysis purposes.



## Discussion

### Comparison between the automatic and the manual threshold procedures

The constant value needed in the automatic procedure was calculated by analyzing 31 previous recording sessions (that are not part of the experiment described in this paper). For each of the 31 recording sessions, we manually determined the threshold value and we measured the average sound level (RMS), then we reversed the formula ( $threshold = RMS \cdot constant + 0.5 \text{ dB}$ ) to calculate the constant value. The average of the 31 constant values was 0.7797413899.

This allowed us to focus back on the recordings described in this paper and compare the two iterations (manual and automatic) of the procedure. After applying both iterations on each one of the 72 files, we found a total of 195 automatic peaks and 194 manual peaks. The manual iteration found an average of  $2.69 \pm 0.17$  peaks per file, while the automatic iteration found an average of  $2.71 \pm 0.17$  peaks per file (Table 3).

65 out of 72 times the two iterations identified the same peaks; 4 out of 72 times the automatic procedure identified 1 more peak than the manual one; and 3 out of 72 times the manual procedure identified 1 more peak than the automatic one (Figure 10). While some variability between the two iterations can be expected, they never differed by more than 1 peak, and the average difference is very small ( $0.01 \pm 0.04$  peaks per file). This shows that the two iterations yield very similar results. To compare the two iterations, we performed a 2-ways Mann-Whitney U test (p-value threshold = 0.05), and we found a p-value of 0.81. Therefore the two iterations yielded no statistically significant difference. Overall, the automatic procedure, which is desirable because it is faster, was found to be equally or more sensitive than the manual procedure 69 out of 72 times (96%) and identified 195 peaks out of

the 198 total peaks that were found by either iteration (98%). For this reason, in the statistical analysis we used the data obtained with the automatic iteration.

The finding that the automatic procedure yielded results consistent with the manual one is promising. This suggests that the faster and less resource-intensive automatic procedure can be a viable alternative to the more labor-intensive manual approach. However, there may be situations in which the manual procedure remains essential. One such scenario is the presence of unusual or highly variable environmental conditions that could affect the acoustic characteristics of UEs. In cases where the acoustic signal is particularly noisy due to external factors, such as strong wind or ambient noise, the automatic procedure may struggle to accurately distinguish genuine UEs from background noise. In such challenging environments the expertise of human operators in manually assessing and confirming UEs can be decisive.

Therefore, while the automatic threshold procedure offers significant advantages in terms of speed and resource efficiency, there may be instances where a manual procedure remains necessary to ensure the accuracy and reliability of UE identification.

### Short- and long-timescale UEs

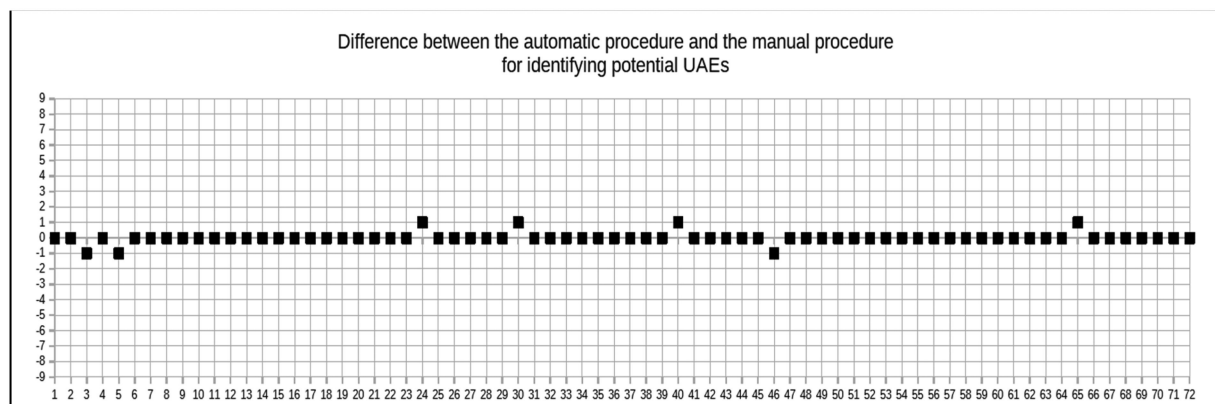
In our recordings, we found two main kinds of peaks:

- short-timescale UE peaks (duration <1 ms, Figure 11a);
- long-timescale UE peaks (duration >1 ms, up to tens of ms, Figure 11b).

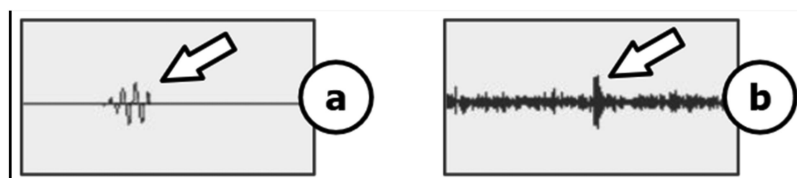
We found that all the long-timescale UE peaks perfectly lined up with audible sounds, therefore we considered them non-significant and excluded them. The observation that the only

**Table 3.** Comparison between the two iterations (manual and automatic) of the threshold procedure. Both iterations were applied on all 72 files described in this paper.

Iteration	Number of files on which the iterations was applied	Total number of peaks found across all files	Average number of peaks in each file
Manual	72	194	$2.69 \pm 0.17$
Automatic	72	195	$2.71 \pm 0.17$



**Figure 10.** Difference between the automatic procedure and the manual procedure in identifying potential UE peaks. In most of the 72 files the difference is 0, therefore the two procedures identified the same peaks. Where the difference is +1, the automatic procedure identified one more peak than the manual one. Where the difference is -1, the manual procedure identified one more peak than the automatic one. The two procedures never differed by more than one peak.



**Figure 11.** Example of (a) a short timescale UE (< 1 ms) and (b) a long timescale UE (several ms or tens of ms) at different zoom levels.

acceptable peaks were short (<1 ms) is consistent with the current literature (Tyree and Dixon 1986.<sup>3,6,15,18,21–24</sup>

## Conclusion

The described procedure utilizes a low cost ultrasound microphone, a laptop computer, and the open-source Audacity® software for recording, extracting, and identifying UEs in non-anechoic conditions. It also allows to analyze every session without having to split it in shorter chunks due to the large file size, like other studies did.<sup>25</sup> The applications of a cost-effective methodology for identifying plant UEs include, in the short term, enhancing accessibility for researchers to delve into plant signaling and communication studies, as well as monitoring plant stress. Over the long term, potential applications may be extended, and developed to noninvasive, cost-effective crop monitoring for the optimization of cultivation practices.

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