The lens of the Sonic Holobiont. A perspective on acoustic influence on microbial communities and its application as an additional layer to the holobiont concept.

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Abstract

When studying micro and macro biomes in the quest for a more general understanding, we can hardly escape from a holistic perspective. At first, symbiosis was demonstrated to be a ubiquitous phenomenon in living cells, shaping evolutionary patterns across species at very different scales. The "holobiont" concept gains a central role in modern biology. The observation of the complex inter- and intra-specific interactions among organisms living in the same ecological niche, becomes itself an object of study. Stemming from Bernie Krause's "acoustic niche" hypothesis, we extend his observations on stratification and interaction of bioacoustics stimuli to include microorganisms as an integral part of any ecosystem, highlighting interactions at the acoustic level. A mechanosensitive element set is evolutionary conserved, suggesting mechanical perception as an important feature for (micro)organisms thriving and survival. We propose the concept of "*sonic holobiont*" to include all sonic interactions in order to tackle the complexity of all relationships occurring within an ecosystem at all scales. Informed by the current developments in microbial acoustics and recording techniques, we highlight open questions in need of being addressed to get a broader understanding on this young discipline. We advance a novel viewpoint on reported evolutionary conserved mechanoperception, inviting further exploration of this abundant and unexplored, to use Feynman's words, "space at the bottom".

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Glossary

- Ecoacoustics: study of the interrelationships sound-mediated between organisms in an ecosystem. This term includes bioacoustics and soundscape ecology [1,]
- Acoustic ecology / sonic ecology/ soundscape ecology: discipline that studies the relationship between living beings and their environment mediated through sound. These terms will be here considered as synonyms
- Bioacoustics: the investigation of sound production, dispersion and reception in living organisms
- Microbial acoustics: the branch of bioacoustics focused on microorganisms para-acoustic phenomena, such as mechanoception
- Acoustic signal: mechanical waves, pressure waves, sonic waves
- Communication: process of information / data exchange among entities that share a common perception system
- Holobiont: The terms holobiont (the collection of host and associated microbial cells) and hologenome (the sum of genetic material from all the species part of the holobiont, including of viral vectors) unify microbial symbiosis into the structure, function, and evolution of macroorganisms. We may see the holobiont as a fractal system not scale-dependent.
- Labophony: soundscape component originated by the overlapping of electromechanical instruments. It is a subclass of anthrophony and biophony, emerging specifically in the bio-laboratory and production facilities that exploit microbes. It virtually influences all species cultivated in the laboratory.

1. The acoustic dimension of ecological niches

The ecological niche is a fundamental ecological concept, describing both the range of conditions needed for species' persistence and its ecological role in the ecosystem[2]. It includes all the interactions of the species it comprises with the biotic and abiotic environment; it has helped ecology and evolutionary biology to address the complexity of multispecies interactions occurring within a niche. Amongst some examples of such interaction we find competition, mutualism, symbiosis etc. Interestingly, symbiosis appears to play a role in community dynamics, for its implication in shaping evolutionary conserved relationships among organisms.

Symbiosis is a widespread form of ecological interaction on which life relies on, despite the early beliefs of ecology and evolutionary biology scholars who thought of it as an exceptional event[3]. One recent framework, proposed to explain the variety and interdependence of symbiotic multispecies niches is the concept of *holobiont*[4], defined as the assemblage of different species forming a discrete ecological unit. The more we understand multicellular life organisation, the less we can distinguish among all the life forms involved in this complex and multifaceted meta-organism, the holobiont.

The ecological niche, shaped by interspecies and intraspecies interactions can be understood in terms of holobiont, and hologenome, the collective genome of eukaryotes and associated microorganisms[5]. We want to bring attention to one biologically relevant aspect that is often overlooked in ecological niches, which is sound, intended as a means of communication amongst species at different scales.

Although studies focusing on sound effects on ecosystems are increasing[6], our understanding of the role of sound amongst living beings is still quite fragmented. The idea that sound participates in biological processes in the biosphere is quite recent, noticeably progressing from the studies of Bernie Krause on acoustic ecology. Krause proposed the *acoustic niches theory*, expanding the concept of ecological niches[7] by adding acoustic observations of ecosystems to conventional ecological indicators. Krause stated that in ecosystems the range of sounds emitted by each species tends to not overlap, as to maximize the possibility of sharing information on a peculiar and unique range of frequency in a defined time. The breakthrough hypothesis came from his sound studies in the tropical rainforest in Borneo, one of the oldest, most pristine and stable acoustic environments on Earth[8]. At the equator, days and seasons have alternated with incredible regularity for millions of years. Species that populate such soundscape have had millions of years to stabilize around this cyclicity, tuning their unique vocalization and relating it to the environment's peculiar characteristics.

Dawn after dusk, across long periods of time, animals have sung their mating songs, communicated predator positions, used sound to orient themselves, and evolved alongside each other. The sum of acoustic sources in an environment can be conceptualised as soundscape. It is generally conceived to include the sound of all sources that can be heard in that place or space by a human listener[9]. Krause noticed a differentiation of sounds emitted across the acoustic spectrum in a defined and limited range of frequencies in the audible range. Moving away from the original setting, i.e. the tropical rainforest, toward more anthropic environments, he noted that soundscapes present a variety of biotic and abiotic sources, and went further into identifying three acoustic components of ecosystems: *geophony*, *biophony* and *anthrophony* (see section 2). These seem to describe the entirety of acoustic sources we can distinguish in the environment as they interact with and shape ecosystems[8]. Although this view broadened the understanding of the biological interrelations that are sound-mediated in nature, it only considered the section of the acoustic spectrum that is audible to our species (indicatively 20 to 20000 Hertz, see Fig. 1).

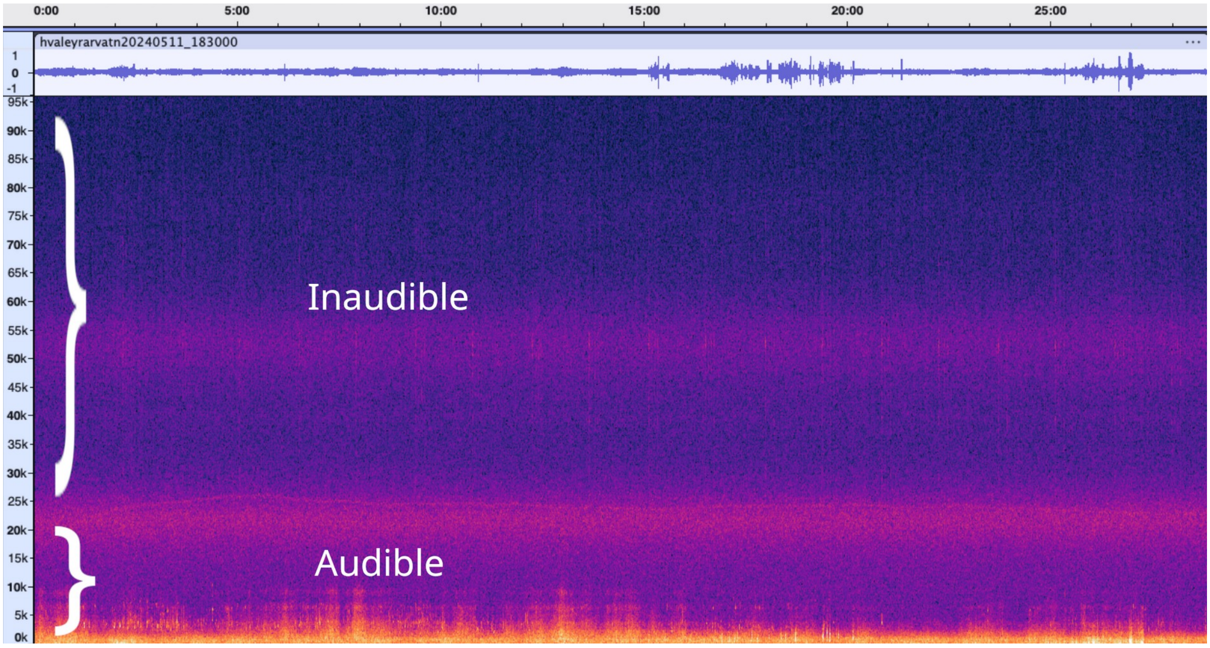

Figure 1. Open field Spectrogram. *Example of spectrogram, from an audio recording taken near the lake Hvaleyrarvatn (Hafnarfjörður, Iceland) in May 2024. In the top view the recording is represented in the time domain as a waveform. In the bottom view the recording is represented in the dimensions of time (x axis), frequency (y axis) and intensity (brightness). It is evident how a large portion of the audio spectrum recorded, spanning from 0 to 96 kHz, occupies the ultrasonic (above 20 kHz) and infrasonic (below 20 Hz) range of the spectrum (not highlighted in the picture) which are inaudible to humans; the inaudible portion is nonetheless, populated by events, in this case mainly the ultrasonic harmonic bands of birds singing. Recorded with a μMoth 1.0.1 (sample rate 192 kHz). Visualised in Audacity 3.7.1*

All sounds above and below this range, respectively *ultrasounds* and *infrasounds*, were hence cut off from early acoustic ecology observations. Today we know that many species of animals and plants emit and receive sounds in the ultrasonic range (chiropterans, dogs, aquatic mammals, and so do insects etc.[10]); some animals (e.g. elephants and mysticeti) can also detect and produce infrasonic signals. Indeed, animals have developed various filters in both the visual and acoustic domains, allowing them to perceive only the frequency bands relevant to their needs. In general, according to allometric relationships[11], an animal's acoustic emission and reception capabilities (i.e. inner ear measurements in sharks[12]) are proportional to its body size[13]. The correlation between organism size and acoustic range of interaction interestingly overlaps with the concept of sound-mediated resonance at a single-cell level, discussed further in sections 4 and 6.
Hence the need to reframe acoustic ecology to include such processes, which largely elude human perception but are nonetheless pervasive in the biosphere around us.
Much like the initial observations on visible light missed to identify its place within the whole spectrum of electromagnetic radiation, the initial studies of soundscapes, centered around human perception in the audible range, have now become insufficient to describe and understand a holistic soundscape ecology. We aim to arouse an interest about the phenomena that are left

unheard to us, especially at a microscopic level, but nonetheless participate in all acoustic environments.
The spectrogram reported in Fig. 1 is an example of how limited our ability to perceive the acoustic environment truly is; the natural world, however, spans across much broader horizons. The aspects beyond our sensory range remain, for the most part, unknown to us.
These considerations may influence the definition of the acoustic niche and the complex communication occurring among its inhabitants from macroscopic scale towards the bottom.

2. Sound classes present into an environment

Soundscapes studies can be of help in many research fields and practices: urban planning, sound quality, natural reserves monitoring, music, to name a few. The unicity of each soundscape is constituted by the nature of its sonic sources and attempting to recognise its components can be useful. As mentioned above, one way to do so is considering soundscapes as composed of three types of sources: *biophony, geophony,* and *anthrophony*[14] (see fig. 2).

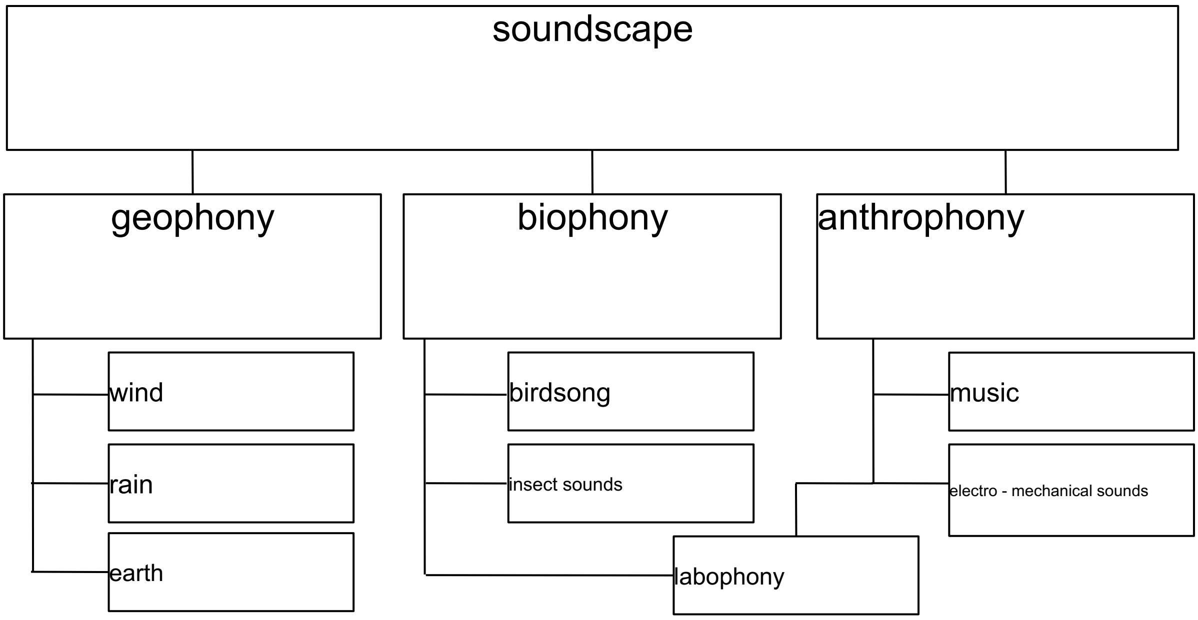

Figure 2. Soundscape components. *A scheme summarizing a rough taxonomy of soundscape classes. Labophony, a concept that we illustrate in the text, is placed at the intersection of biophony and anthrophony. These categories can be referred to in trying to picture the interrelation between different sources within a soundscape.*

Each of them points at sources that are respectively biological in origin and non-human; or generated by physical processes such as wind, rain or the earth itself; or lastly, introduced by human activity, including music and electro-mechanical sounds. The complexity of the latter makes it possible to distinguish a variety of sources, as many manmade devices emit sound outside the human audible spectrum[15] (e.g. welders, drillers, washers[16], centrifuges). Notably, some anthrophonic sources (spanning into ultrasonic range) are so distinguishable that they are exploited as a marker of machinery functionality: in fact, sonic feature recognition, enhanced using Recurring Neural Network, is adopted to drive preventive maintenance, *i.e.* of peristaltic pumps, based on real-time analysis of the audio spectrum[17].

It is interesting to pinpoint the overlapping of anthrophonic sources in environments where microbial cultures are grown, and / or biomaterials harvested. Such environments include for example biology laboratories, pharmaceutical factories etc. These acoustic niches raise a peculiar issue, which is evaluating the impact of anthrophonic sources on the microbial acoustic niche. Given the relevance of acoustics niche on species physiology and evolution, we propose the novel term *labophony* to indicate the sum of acoustics signals present and embedded into a laboratory (or another setting where microbial cultures are maintained).
Our preliminary observations suggest labophonic sources may include audible as well as inaudible (ultra- and infra-sonic) sounds (see fig. 3).

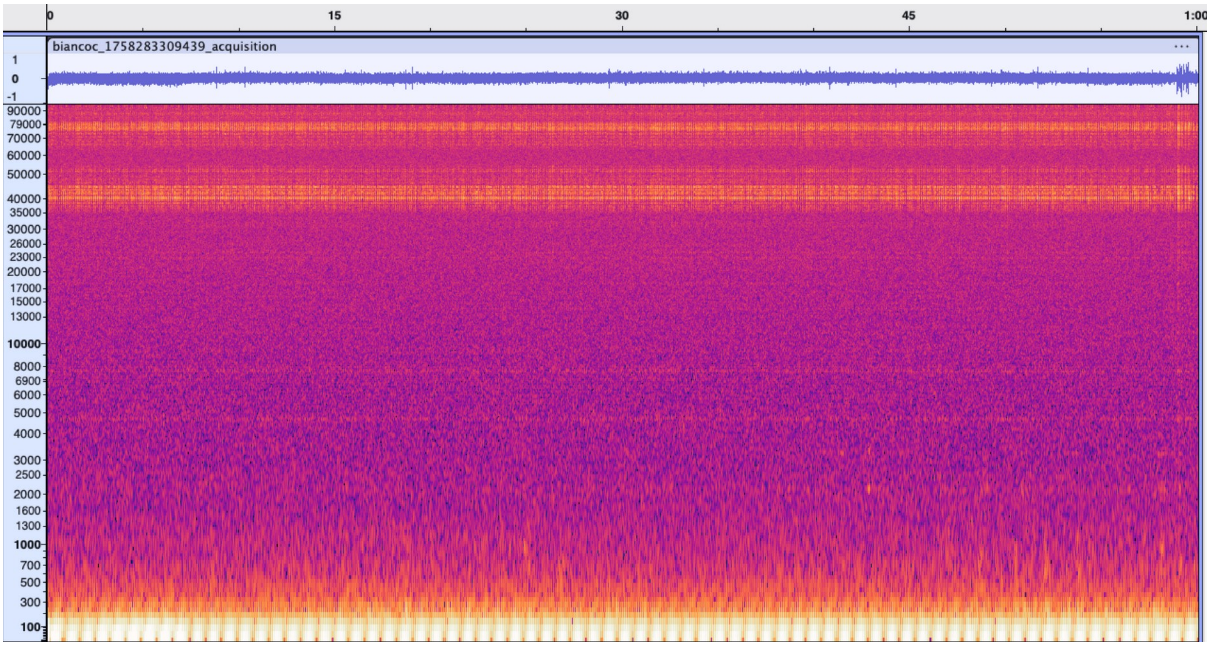

Figure 3. Labophony. *Spectrogram of a recording by Mariano José Guillen at Tor Vergata University of Rome, which represents an example of labophonic sources. The audio was recorded using a Nauta AS-1 hydrophone placed inside a 1L glass becker containing only water, with no additional sound sources directed at it aside from the background noise of the laboratory. In this spectrogram, visualized in Audacity 3.7.1, there are clearly visible bands in the ultrasonic range (at about 40 kHz and 79 kHz) that are ascribable to environmental labophonic sources inside the research lab.*

The most likely hypothesis is that these sounds come from electronic apparata that are normally found in a biolaboratory environment (fridges, mixers, lights etc.).

Soundscape studies in the ecological scope constitute *acoustic ecology,* which examines the sound-mediated interaction between elements in an ecosystem. Within this particular context we postulate on the holistic nature of sound as a mechanoreception-based communication medium in biological systems. Visualising the interconnectedness in which species at all scales participate in the biophonic soundscapes on earth can constitute a source of profound reflection (fig. 4). It helps focus our attention towards the human impact on the inaudible part of the acoustic spectrum; it also remarks on the ubiquity of evolutionary preserved behaviours and features, which allow production and detection of sound at a cellular level.

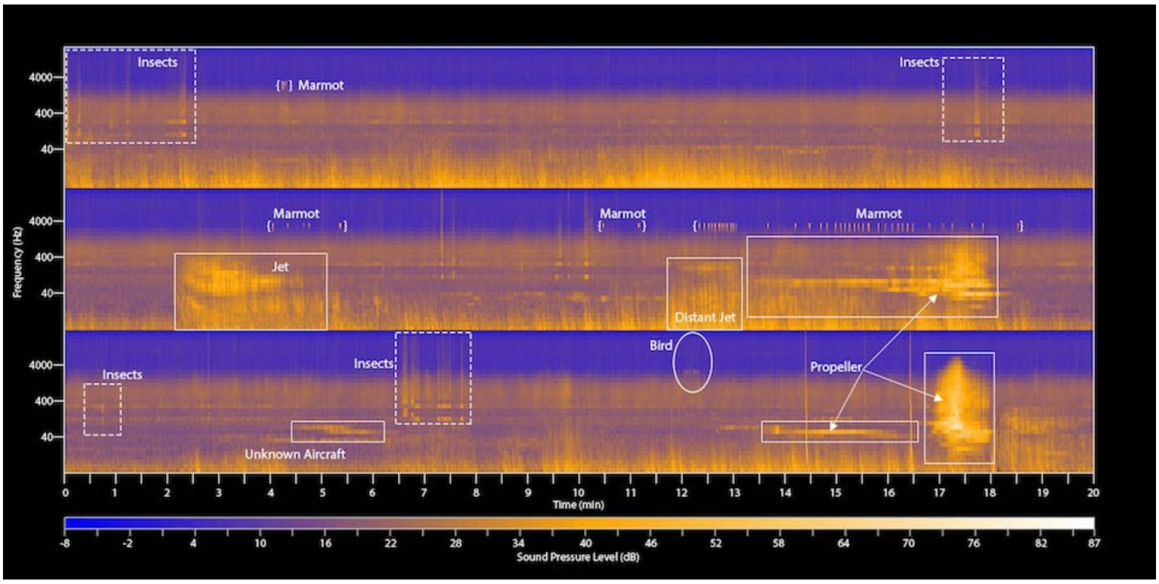

Figure 4. Components of a soundscape. *Mount Rainier Spectrogram (all credits to National Park Service, USA). Spectrogram displaying 60 minutes of sounds from Ptarmigan Ridge, Mount Rainier National Park in 2013. Each of the three rows displays 20 minutes of acoustical data just as introduced in fig. 1. Each type of sound in this acoustic niche has its own unique signature, allowing us to recognise the individual sound sources detected (here mainly biophonic and anthrophonic). The range of the sounds here represented spans between approximately 0-40 kHz..*

Notably, the three components of soundscapes mentioned above also provide a solid analysis tool to think of soundscapes as ecological units, in which the evolutionary selected, inter- and intraspecific interactions between elements within it will be highly interdependent with the specific features of the ecosystem (such as air or water pressure, density, noisiness etc.) and the acoustic phenomena that take place under such conditions.

These considerations triggered a wider-angle perspective on acoustic environments, one that keeps into account holistic factors in the description of a soundscape. Amongst these factors we find the heavy impact of anthropic activities on almost each ecosystem. Human presence and anthrophonic sources, including inaudible infrasonic and ultrasonic pollution produced by our tools and technologies, can have a disruptive effect on the pre-existing soundscapes[18]. These sources may interfere with communication methods and channels evolved through millions of years in the natural soundscape. An example is the interference of low pitched sounds, the most common into the anthrophony group, on the reproductive success of birds[19]. Human noise negatively impacts diversity and density of bird communities associated with that habitat and related soundscape[20]). Indeed, human sound and noise are considered factors shaping bird diversity of a defined habitat[21]. Another pertinent example involves marine mammals: anthropogenic noise, such as ship traffic, military sonar, and industrial activities, can affect cetacean communication. Some cetacean species alter their vocalizations, including shifting their frequency ranges, to avoid being masked by noise pollution. This behavioral adaptation is a response to the increasing levels of underwater noise, which can interfere with their ability to communicate, navigate, mate and hunt[22].

Despite some studies taking into consideration the effects of ultrasounds on humans[23] and animal health[24], most existing regulations solely focus on working environments[25]. Regulations governing noise pollution in the natural environment do not concern portions of the spectrum inaudible to humans in terms of amplitude or frequency, unless there is evidence of their impact on animals. This limitation underlies the need of a holistic approach when thinking of novel guidelines that are aimed at preserving the ecosystem as a whole.

We mentioned some cases in which anthrophonic sources are documented to be disruptive in acoustic niches. It becomes evident that anthrophony occupies a huge section of the acoustic spectrum across a wide range of frequencies, varying from infrasonic to ultrasonic ranges; this problem is becoming more evident as regulation is starting to include ecoacoustics concerns. Anthropogenic noise pollution significance is underlined by its influence on national and international laws for habitat conservation. Urban areas are among the most affected biomes, indeed we find directive on aerial noise from United Nations[26]-[27]. Recently, underwater noise gathered a central role as demonstrated by the agreement on guidelines from International Maritime Organization[28] and the European strategy framework directive[29] that fixed noise thresholds to preserve marine life.

3. Bioacoustics and current tools

3.1 Definition and applications

Bioacoustics is a cross-disciplinary science that combines biology and acoustics (physics); usually it refers to the investigation of sound production, dispersion and reception in animals[30]. This involves the neurophysiological and anatomical basis of sound production and detection, and the relation of acoustic signals to the medium they disperse through. Findings based on bioacoustics, provide clues about the evolution of acoustic mechanisms, and from that, the evolution of animals that employ them[31]. Bioacoustics is not only a theoretical science, but finds application in modern ecology by using sound technologies to record, store and analyze large collections of animal communication data. Field recording is a now consolidated technique to assess biodiversity of an ecosystem[32]. Numerous parameters have been developed to correlate biodiversity with acoustic complexity in natural environments. A detailed explanation of these practices goes beyond the scope of this paper; however, it is worth mentioning a few parameters used in bioacoustic analysis. Amongst them, Acoustic Complexity Index (ACI) that measures temporal variability in signals, useful for identifying species-rich habitats; Bioacoustic Diversity Index (BDI), that combines frequency and intensity to distinguish animal communities (e.g., birds vs. insects) and Spectral Entropy Index (H), which evaluates the "disorder" of the sound spectrum and correlates entropy with biodiversity [33]. Some well known examples of open-source tools for analyzing these indices include *soundecology* package in R and PAMGuard, mainly used for marine mammals.

3.2 Ecological applications

Bioacoustic monitoring is based on passive and active recording devices, somehow automated in the most up-to-date scenarios. The frequency recording range is chosen according to the species we are going to study. According to the Nyquist-Shannon sampling theorem, the bandwidth of a signal must be less than or equal to half the sampling frequency (Nyquist frequency), *e.g.* if the aim is to record bird vocalizations that reach maximum audible frequencies of 20 kHz, the sampling frequency must be at least 40 kHz. Beaked whales (*Ziphiidae*) can emit sounds up to 200 kHz, requiring a sampling rate of at least 400 kHz to avoid aliasing and accurately capture their ultrasonic signals.

Many international projects are based on automatic recognition systems powered by AI.[34]
A promising application of autonomous or AI-supported acoustics monitoring is species identification[35]. Other applications span from the study of ecology and animal behaviour[36]-[37], species monitoring to assist land species conservation[38]. Bioacoustics underwater study led to its application to assess underwater biodiversity, to monitor habitat health and fish species location[39]. A novel bioacoustics application is its usage as a tool to enrich fish communities and to be integrated for efficient active management to counteract habitat loss in coral reefs[40].

3.3 Methods and instrumentation

The first recording of natural sound began in 1889 with Ludwig Koch and a bird [41] [42]. Koch's work, focused on birds, will set the stage for the universal audio capture model of single-species. From that moment on, audio recording systems evolved year after year; one of such major shifts was the technological transition from tape-based recording systems to today's digital systems.
Sensor technology has also been improved, moving from condenser microphones, ribbon microphones, dynamic microphones to modern MEMS (micro-electromechanical systems). In marine environments, the most commonly used sensors are piezoelectric, but laser-based hydrophones have also been proposed and developed[43].
Digitization has improved audio quality and, most importantly, enabled processing in digital format. The technology has evolved from CDs with 44.1 kHz sampling to today's multichannel 32-bit systems. Recording dynamics have also seen unimaginable improvements, increasing from 94 dB (16-bit) to 144 dB (24-bit) and up to 1500 dB for 32-bit floating point. As points of comparison, the amplitude of a whisper is about 20 dB, while the noise of a jet taking off is 130 dB at a distance of 100 meters.
For wildlife applications, many autonomous recording systems have been developed, ranging from stereo audio formats such as the Song meter (Wildlife Acoustics) up to professional-grade autonomous recording systems with 4 simultaneous sampling channels at 32-bit resolution and support for complex scheduling.
In marine environments, hydrophones with piezoelectric sensors are used for audio-range recordings as the nRADAR-mk2, or high-sample-rate systems for both audio and ultrasonic recordings.

4. Communication into the acoustic niche: bioacoustics as a tool for comprehension

Thanks to classical methods we humans were able to study sound communication and then to observe it through an evolutionary lens. By definition, communication is a process of information (signal) exchange between a sender and a receiver through a common medium[44].

Sound, *i.e.* mechanical, acoustic wave, propagates via local compression and rarefactions of particles into elastic media. Sound speed is determined by wavelength and frequency, but influenced by many factors such as temperature, salinity and pressure[45] and travels about five times faster in water than air (respectively ~1530 m/s and ~340 m/s).

Current literature on animal communication is focused on air, as the most characterised fluid medium. In humans, as an example, mechanisms of sound transduction and perception are well documented and consolidated, with dedicated organs and physiology. On the other hand, molecular mechanoception, which appears more central in a microbial acoustics setting, is a more recent molecular biology branch[46] which is starting to gather academic interest: the 2022 Nobel Prize for medicine awarded for discovery of human mechanoreceptor, PIEZO 1. This ion channel is devoted to transduce mechanical cues[47] and represents a good instance of a novel discovery in the field.

4.1 Aerial sonic communication

Among the oldest animals, insects have roamed the planet for over 400 million years. One of the ways insects communicate is through vibrations, a behavior that has been observed to be quite pivotal[48]. Moreover, the presence of five defense mechanisms based on acoustic emission (stridulation, percussion, tymbalation, tremulation, and forced air) underlines the relevance of vibration as a medium for communication and survival, affecting fitness of insects[49].
The jumping spider, famously responsive to visual stimuli, can not only perceive sound, but also respond to sounds with specific frequency and activate neural response as a reaction to these sound bands[50].
Aerial communication has been characterized in such a detail that it allows to recognise and classify pristine environments based on their sonic footprint, in which we find peculiar acoustic niches. Vocalization of living organisms occurs in distinct frequency bands of the acoustics spectrum, and more interestingly following a circadian cycle[51]. A sophisticated example of sound usage comes from moths, where 52 genera are reported to produce ultrasound in order to disturb the established echolocation mechanism that bats use for feeding; this mechanism is also evolutionarily widespread[52].

There are other curious documented cases where vibration is used as a means of communication and interaction at the interspecies level. In an aerial environment, bees' pollination is influenced by their capability of emitting sounds. By sonication, bees induce more efficient pollen release from apical pores of the flower they enclose, and hence influence the reaching of pollen to the stigma of another flower[53]. In simpler terms, sound produced by bees can influence the efficacy of plant species spreading and reproduction.

Aside from some serendipitous encounters, sound-based studies seem to be mainly focused and restricted to the human field. Many frameworks which are centered on a human listener are now recognised as too narrow, and redesigned to include other species: this seems an inevitable conclusion given the discovery of ancient, evolutionary conserved audiovocal phenomena. A fitting example is the Lombard effect, which describes the involuntary tendency of speakers to increase their vocal effort when speaking in loud environments. It may have emerged around 450 million years ago underlying the soundscape-induced adaptation mechanisms of its inhabitants. Interestingly, the Lombard effect was recently demonstrated to apply to organisms never thought of before as bats, whales and songbirds[54].

We believe expanding human-centered theories of sound, embedding them within the framework of holobiont biology, can help us understand and visualize the complex and various examples here mentioned within a single comprehensive continuum. In the next sections, we examine further biophonic forms of communication in other media and at other scales.

4.2 Underwater sonic communication

Sound plays a privileged role for communication and survival in marine ecosystems. With scarce sight efficacy at high depths due to light scattering, and ineffective smell due to slow water diffusion, species rely on communication at a mechanical level[55]. Take for instance the *Alpheidae*: it is a caridean snapping shrimp of 3-5 cm length, that possesses a claw dedicated to producing a loud sound (reaching 218 dB intensity) that functions as defense, predation, intraspecific communication and as disruption from predators. This loud emission can interfere with human developed sonars.

Marine mammals as well, have developed complex sound production systems. In particular, odontocetes—toothed cetaceans—emit sounds for echolocation, hunting, and social interactions. Many bioacoustics studies focus on odontocetes, as animals capable of producing rapid bursts of high-frequency sounds, defined clicks. Clicks were assigned to echolocation functions, but recently it was found to correlate with feeding grounds, reproduction and individual recognition, working as a dialect. Recently, new sound emission methods have been discovered in humpback whales, which can produce two sounds simultaneously or generate continuous sounds by recirculating air through specialized cavities[56].

These recent findings indicate a novel role for sound, not only to perceive the environment but also the self and its origin. As discussed in section 3, technical recording advancements allowed to propose novel theories in this field of study.

4.3. Forms of microbial communication

4.3.1 **Sonic signaling**

Innovative technologies, exemplified by the development of piezoelectric elements of precision manufacturing and application, have allowed the development of methods to hear, detect, and hence analyze regions of the sound spectrum farther away from the range of interest in animals. Precision microphones, such as infrasonic and ultrasonic hydrophones, allow to extend the sound spectrum comprising human-inaudible sounds. The possibility of measuring sounds in a range as wide as possible may allow us to better grasp the acoustic and mechanoceptive relationships between species that populate an ecosystem, as we encounter patterns in the acoustic niches.

An interesting recent research from Khait and colleagues, shows that plants that are stressed (by water shortage, cutting or viral infection) are capable of emitting airborne ultrasounds[57]. The capability of detecting these sounds opens new fields of investigation and questions about the amount of data and information exchanged sonically at an inter- and intra- specific scale.
Focusing on microbes, a pivotal research explored the response and the production of sounds in a bacterial community (*Bacillus carboniphilus*) grown on an agar plate[58]. This research showed, for the first time, the presence of sonic data into microbial cultures growing on solid medium, indicating how pressure signals contribute to microbial growth and possibly taking part in communication within microbial cultures.

To sum up, physical signals display faster propagation than chemical ones, since they are less affected by diffusion, hence providing a functional mechanism when rapid cell response is required (as in the case of a stress onset). Beside traveling through different media, physical signals can propagate across the cell envelope, being transmitted to intracellular protein matrices and directly signalling to intracellular targets to modulate their activity, hence rendering membrane receptors virtually unnecessary. This mechanism constitutes a rapid transmission pathway that could provide cells a response advantage.

4.3.2 **Chemo-perception vs physical-perception**

The most renowned and studied form of microbial communication is the chemical one, with the chemosensory system as the most complex, detailed and specialized reported signal transduction way in bacteria and archaea[59].

If we compare chemical and physical signals in an underwater environment, sound may not need dedicated cellular structures for its production (since the cell may shrink and create vibrations), nor for its detection (since the cell membrane may act as a resonant element [see section 5.1], thus allowing for vibration detection, with lower energy demand than a dedicated receptor/structure).
Physical signals display faster propagation than chemical ones, since they are less affected by diffusion, hence providing a functional mechanism when rapid cell response is required (as in the case of a stress onset). Beside traveling through different media, physical signals can propagate across the cell envelope, being transmitted to intracellular protein matrices and directly signalling to intracellular targets to modulate their activity, bypassing even receptors on cell membranes. This mechanism constitutes a rapid transmission pathway that could provide cells a response advantage.
If we crunch the numbers, it appears that sound signaling communication can be favoured underwater, where sound speed is between 1450 and 1570 ms^{-1}, while it is 340 ms^{-1} in air, approximately five times slower. At the same time, the self-diffusion coefficient of pure water is $2.3 \cdot 10^{-9}$ $m^2 \cdot s^{-1}$ at 25 °C and $1.3 \cdot 10^{-9}$ $m^2 \cdot s^{-1}$ at 4 °C, approximable to the size of a small signaling molecule in a water solution. The distance that physical signals, *i.e.* sound, can travel depends on intensity, frequency (mainly evident on porous media) and on temperature of the medium[55].

The lack of diffusion constraints and the rapid propagation of the signal also make physical signaling ideal for cell–cell communication over long distances. Furthermore, the possibility of transduction of intracellular motions to the outer environment in the form of sound, supports the idea of cell sound emission. Reguera postulates that this may carry data regarding cell metabolic status and modulate other cell metabolism features or details [61]
We think these observations open the possibility of intra/inter-species and domain sonic communication (fig. 5).

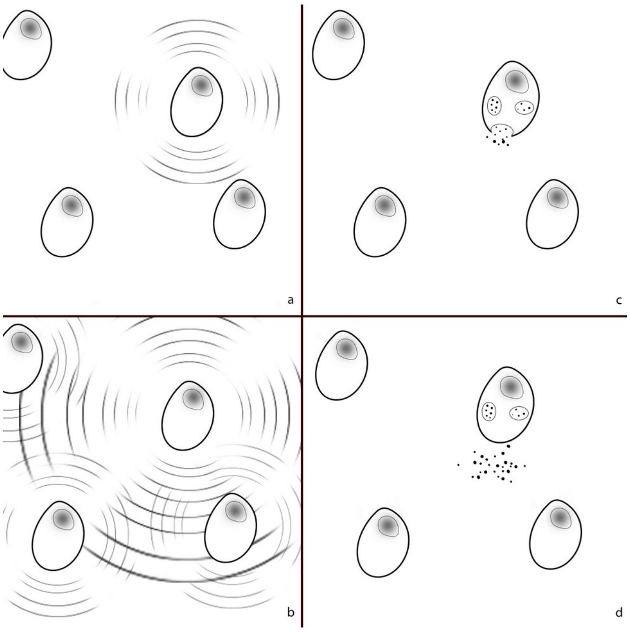

Figure 5. Comparison of mechanoception and chemoception in the same time interval. *This picture illustrates the modality in which mechanical (physical) signalling might be better suitable for cell-cell communication rather than chemical signalling, being the former propagating faster in the same medium, and not being bound to passive diffusion like the latter. In quadrant* a, *a cell membrane starts vibrating, propagating mechanical waves that reach several other cells in quadrant* b *in a small amount of time* Δt. *Quadrant* c *and* d *depict a chemical signalling process happening in the same* Δt. *In quadrant* c, *a cell releases a vesicle containing chemical substances (eg. neurotransmitters); in the same time it takes a mechanical wave from a cell membrane to reach several others, the cloud of molecules released from a cell membrane has not reached any cell yet in quadrant* d. *This simplified picture assumes a stationary position for every cell involved, however similar premises may apply to real-life scenarios.*

We may even advance a bolder speculation - the existence of a microscopic communication system, *i.e.* information / data sharing, that may have been developed as an ancestral analogue of the macroscopic ones (*e.g.* human language) and that this may influence species distribution and evolution.

4.4 Microbial acoustics

We know microbes can live at different heights along the water column and have evolved diverse survival mechanisms to withstand extreme conditions they face. If we think of adaptation to pressure in the media, for instance, we see that cells evolved biochemical adaptations to thrive at different pressure gradients. Within the populations that live at the bottom of bodies of water (*benthos*), we find *eurybathic* and *stenobathic* organisms. The former possess a wide range of tolerance to pressure change and are capable of living in deep and shallow water; the latter are more fragile and only capable of living at a limited pressure range[60]. These aspects may influence the sound class (in terms of frequency, intensity and rate) a cell can perceive and respond to, if we consider a transduction chain a form of response.

Norris and Hyland postulated that sonic microbial intercellular communication may involve coherent collective vibrational modes that could reach enzyme activities and gene expression[61]. In accordance, we hypothesize intra and interspecific communication among microbes and that their communication can be probed via a holistic bioacoustic approach. It would be fascinating to find functions for sound as an inducer of response that affects other species than ours in a sonic holobiont: as sonicating bees do for pollen, so microbes may work (in an evolutionary perspective) for macroalgae or coral reefs. However, the technical challenges associated with probing microbial physical signaling networks at the intensities and time scales required have long limited this field of research[55]. More work is needed to explore the hypothesis that physical signals act as carriers of specific information at a microscopic scale. Progress in this field can only advance as technical barriers are overcome, jointly with the required curiosity needed to ask uncommon questions, without shying away from the answers.

The current adoption of microbial bioacoustics focuses on a single-cell scale: being microbial cultures heterogeneous (in terms of metabolic state, ultrastructure and life stage), their physical properties at an individual or molecular level are often masked in a colony. Associated microorganisms, mainly bacteria, are regarded as contaminants; hence, highly complex and time-demanding methods have been developed to establish axenic cultures[62]. However, recent studies show cultures have unique relationships with their associated microbiome selecting their associated communities[63]. Take microalgae as an example: their associated microbial community has been shown to promote growth and advantages to the host culture (the microalgae themselves). In particular, associated communities confer resistance to antagonists[64], increase the biomass productivity, and favor the accumulation of highly valuable compounds[65]. Indeed, novel culturing methods rely on the establishment of functional co-cultures and consortia[66], even at industrial scale[67].

Probing acoustics at a single-cell scale is considered, although technically difficult, virtually advantageous; we argue instead that sonic (holistic) properties should be observed as they occur in nature - rarely at a single-cell scale, and more often in multi-cellular networks.

In light of this, we coined the term “sonic holobiont” to refer to the entirety of species in the same acoustic niche that they interact and evolve with. In other words, the sonic holobiont can be seen as a framework that considers the entirety of sonic interrelationships occurring in a given acoustic niche, not just from a human perspective (which characterizes the definition of soundscape). Ultimately, the sonic holobiont can be represented as a holistic soundscape, not inherently anthropocentric. In a way, this idea builds on top of the concept of soundscape, adding a functional aspect of reciprocation to the interactions amongst micro- and macro- organisms parts of it.

Under this lens, the previously introduced soundscape, according to Schafer’s definition, is examined in relation to two aspects: *who* hears the soundscape (listener, human or non-human) and *what* signals are heard within it, even at the microscale. The sonic holobiont addresses both these aspects under a non-human centered perspective; it provides a framework to apply and understand the observations that followed the laying out of acoustic ecology. In the next section we examine mechanoception in more detail and explain its role in probing and understanding the sonic holobiont.

5. Mechanoreceptors' role in the microbioacoustic niche

Animal[68] and plant[69] mechanosensing is a complex multifactorial process. Looking into microbes, we find reported mechanosensitive elements as a hallmark of evolutionary conserved mechanoperception[70].

Mechanobiology studies cell response to physical forces and related response mechanisms.

Mechanosensing can occur at different levels. Firstly, plasma membranes are directly exposed to external forces, with pressure waves that influence biological structures in peculiar ways. For instance, membrane deformation may induce a change in tension and lipid packing and recruit curvature-sensing proteins. This event can influence the dynamics and activity or gating of mechanoreceptors (many of which are transmembrane proteins)[71]. The sum of these events converts mechanical signals into biochemical stimuli.

5.1 Evolutionary conserved mechanosensitive elements

Discovered via the application of patch clamp in spheroplasts,[72] microbial mechanoreceptors are highly studied nowadays. Currently two bacterial mechanosensitive families have been identified in the model organism of *E. coli*: MscL and MscS. The MscL-ancestor hypothesis states that the duplication of the MscL gene (occurred before the separation of Bacteria and Archea) is supposed to have led to the evolution of MS channels in other phyla. This hypothesis is based on the high level of sequence identity of MscL transmembrane residues found throughout the sequence of the members of the MscMJ channel family, confirming conservation in cell walled-eukarya[73-74].

The MscS family contains the channels MscK and YbdG, that alongside YnaI, YbiO or YjeP constitute the seven channels present in *E. coli*[75]. Microbial mechanosensitive elements combine sensor and effector functions in a single molecule, offering simple effective events while transducing outer physical forces to intracellular elements. Protein database searches show members of these two families are present in the majority of bacterial groups and even Archea[76-77]. These findings indicate mechanosensation is universal, probably evolved due to ancestral cell needs such as osmotic force detection, solute and water concentration. Hence, mechanoreceptors in bacteria are characterized to act as shock valves allowing them to survive in highly dynamic environments[78].

Alongside the reported presence of evolutionary conserved mechanoreceptors, localized at the cell wall and cell membrane, we find organelle-localized mechanoreceptors as the Transient Receptor Potential family (TRP), reported among the basic and necessary components that facilitated the evolution of complex physiology. The TRP is a highly diverse protein superfamily. TRP is structured as an ion channel sharing six transmembrane segments with varying cation selectivity, and it is gated by a variety of stimuli (mechanical, thermal and chemical). Just in animals, we find 9 families[79], with TRP widely expressed among eukaryotic taxa[80].

Some observations report the effect of external physical forces on organelles and their capability of responding by perceiving, modulating and signalling. Organelles' membrane may be deformed, with changes in lipid packing state or alteration in intraluminal solvent flows through organelles. In particular, endoplasmic reticulum have been reported to present mechanosensitive channels into the membrane, while mitochondria have been observed to alter their fission and fusion process by mechanical forces; Golgi apparati appear to be directly involved in mechanosensing[81].

Interestingly, we find cytoskeleton elements having properties that allow them to transmit internal and external physical forces, participating in mechanotransduction. The most prominent candidate that may contribute to mechanosensing is actin, surrounding nucleus and Golgi apparatus, regulating mitochondrial fission and interconnecting intracellular structures by anchoring them to microtubules and cell membranes or cell wall. The cytoskeleton can transduce physical forces throughout what we may think of as a resonant cell[82]. Interestingly, actin dynamics mediates cell response to repetitive pressure stimuli regulating the cell's viscoelastics properties[83].

Lastly, the nucleus is emerging as a core mechanosensor apparatus. It is in fact tight to the endoplasmatic reticulum and in contact with cytoskeletal elements: nuclear mechanotransduction allows the cell to adapt growth and physiology according to the surrounding physical stimuli[84]. Nucleus can be deformed by mechanical cues via the activity of surrounding actin filaments[85], with reports on chromatin conformation and gene expression modulation[86].
A comprehensive theory that tries to solve the complex framework of cellular elements and their implications in mechanosensing is the tensegrity model. According to this model, there are fixed elements (such as microtubules) that may act as supporting elements for stretchable elements (such as actin[87]). Tensegrity is a fascinating model that underlines how complex and relevant it is for a cell to precisely detect and transduce mechanical signals.

To sum up, if we look at sound as a pressure wave propagating by oscillations through the medium where cells thrive, we find cell structures adapted to perceive and transduce such signals. In light of this, we may hypothesize there is a yet-to-be-discovered process of information exchange taking place underwater. In this context bioacoustics data may play a fundamental role, having provided a framework to study sound-mediated interaction in acoustic niches. Microbial acoustics can offer a comprehension of these phenomena by focusing on mechanosensitive elements, which ultimately are microscopic transducers at a cellular level.

Notably, scientific literature documents that mechanosensing abilities extend across living organisms at all scales; we hypothesise that even unicellular organisms, such as (cyano)bacteria, diatoms, microalgae and yeasts participate in this interspecies sonic dialogue. Indeed, mechanosensing is an evolutionary conserved mark, which suggests a role in sound as a possible carrier of information even between cells.

6. Bioacoustics of the sonic holobiont: from nature to laboratory

6.1 The Sonic Holobiont

The conservation of mechanosensitive structures at a cellular level suggests that the ability of interacting with mechanical signals by reception and emission may be evolutionarily conserved. This observation alone has huge implications: if mechanosensing is an ability that evolved at first thanks to structural properties of the cell, then it is as omnipresent as life itself. We are accustomed to think of sound as something we can *listen to*, meaning process via an auditory cortex into auditory images; this complex ability, however, evolved starting from the same molecular mechanisms and same mechanoceptive structures that we share with microbes. Even our auditory system ultimately relies on cilia-mediated mechanoception in the cochlea to transduce and modulate mechanical waves received by the eardrum.

According to the presence of reported evolutionary conserved pressure wave-sensitive elements, the definition of bioacoustics, currently mammal-centered, should be enlarged and reframed so as to include microorganisms, in what we propose to define as ''microbial acoustics''. Since diversity of bioacoustic sources is a hallmark of biodiversity[88], microbial acoustics must be taken into consideration and preserved to ensure comprehensive biorestoration plans.

Sonic holobiont is a term we coined to describe this conceptual superimposition.
It describes the whole of sonic inter-relationships that occur within the holobiont matrix, advancing the idea that micro- and macro-organisms within the holobiont might have evolved in the same mechano-acoustic environment. This would imply that ultrasonic, audible and infrasonic communication, intended as exchange of signals, has evolved concurrently in a symbiotic way and is hence quite ubiquitous. Such a paradigm invites us to consider anthropic influence in sonic environments as broader and more pervasive as it has been so far argued. For instance, probing microbial cultures' sound niches can be addressed by taking into consideration the culture itself may represent a sonic holobiont: a functional entity in which interspecies interactions should be kept into consideration as an integral part of the process. Such observations can be effectively put into practice while probing microbial cultures if the idea of the holobiont is adapted to fit with the acoustic niche being considered.

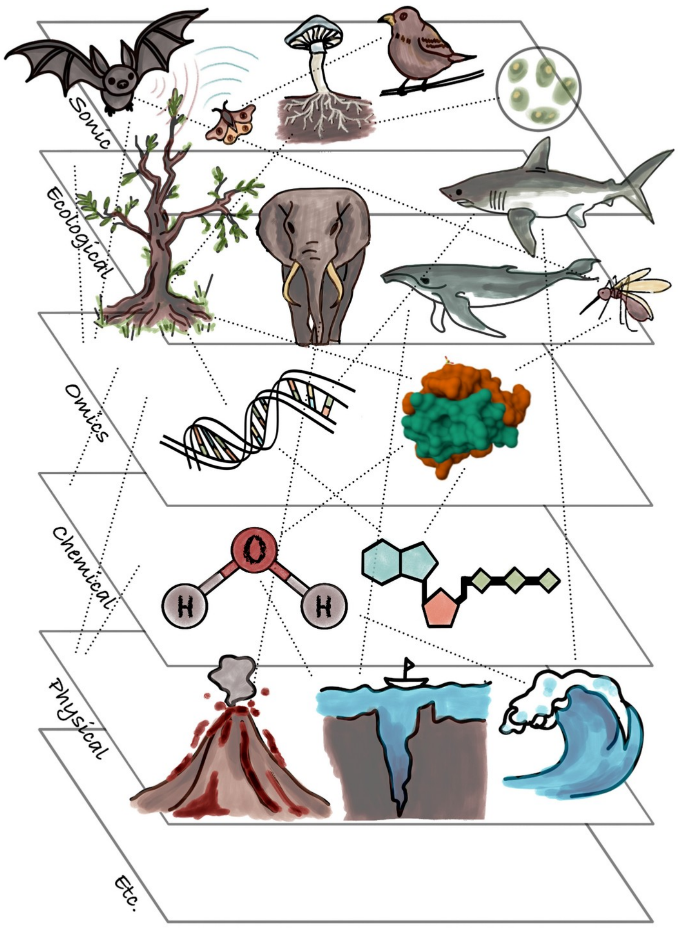

Figure 6. Proposed analysis planes, or lenses, to address components of the holobiont. This image, realised by the artist Váli Andreis, depicts the lenses through which an ecosystem/holobiont may be analysed. From top to bottom, the sonic lens, the one mainly addressed in this paper, where all interactions are mediated by mechanical pressure waves. Below is the ecological lens, referring to the ecosystem and evolutionary concepts of natural sciences; then the omics lens, this integrates the novel discipline trying to understand a system response by studying the sum of the responses occurring in a target class of molecules, i.e DNA, RNA or proteins; the chemical lens, where atomic interactions are exploited to interpret phenomena; the physical lens, the basic one where all the processes occur and can be interpreted; the last plane leaves space for future lenses to adapt to more a comprehensive understanding of an ecosystem/holobiont. Intra-level interactions are omitted for clarity.

6.2 Nature and laboratory

The concept of the holobiont provides a crucial framework for understanding how living organisms are enmeshed in multispecies interactions, which are in nature quite dynamic. The very relationship between hosts and associated species in a holobiont presents a fluctuating character that becomes more evident in natural ecosystems than, for instance, in the laboratory. The majority of labs do not fully meet axenic conditions (although they may attempt to). We argue the quest for axenic conditions may be misleading and limiting a broader comprehension of cellular interactions with the environment. The current understanding of how the relationship between uni-specific cultures, objects of study, and their associated species, displays how the latter is of extreme importance - perhaps even more than the former. In a non-axenic context, the holobiont provides the right framework to notice the numerous multispecies interactions occurring at a microscopic level. Purity of cultures is hence only motivated by maintaining and multiplying specific strains, but does not offer an optimal simulation of the natural ecosystems, for which the lens of the holobiont would instead suit best.

However, when we consider holobionts not just as biological entities but as sonic ecologies, we gain a new perspective on their structural and functional complexity. Sound—both as a physical vibration and a communicative medium—plays a fundamental role in how organisms within a holobiont interact. These sonic exchanges are not merely incidental but are deeply embedded in the metabolic, regulatory, and sensory processes that sustain the holobiont as an integrated system.

The examples provided so far help us clarify in how many ways microbial acoustics connect to macro-bioacoustics. We think holobiont biology is now overlapping to bioacoustics and showing the amount and variety of sonic interactions occurring from a cellular scale to a macroscopic scale.

6.3 Putative additional evolutionary factors

In the laboratory, the production needs have led to peculiar conditions that impose two artificial selection factors, bringing additional forces to evolutionary ones:

- one based on *strains used:* among the most reported examples, there is one model organism adopted as the first ever biotechnology, the yeast *Saccharomyce cerevisiae.* This strain has been exploited since the Egyptians for fermenting goods, and later selected for modern brewing processes. Another model organism is the bacterium *Escherichia coli,* renowned for the capability of heterologous protein expression. These examples show that human selection interfered with natural selection by favouring certain strains over others.
- one based on the *type of stimuli* occurring: strains are propagated by setting defined biotic and abiotic factors such as nutrients, irradiance or shaking levels, and they differ from the sum of *stimuli* a microorganism finds within its natural environment

The sum of these two factors can result in the propagation of strains exhibiting certain traits more effectively than others, as much as inhibiting growth of certain species, or induce certain behaviors

or outputs that emerge *via* mechanostimulation. These considerations result in a question: does *labophony* (as we could call the subtype of bio-anthrophony that occurs in a biolaboratory environment) contribute to the set of artificial stimuli shaping cell culture's selection?
Laboratory investigations, aligned to the sonic holobiont concept, may aim to uncover whether and how microbial life forms respond to mechanically induced vibrations, particularly in relation to ecosystem dynamics and symbiosis.
In more detail, we may refer to two different lines of research. One line has focused on how directed sound waves can modify bacterial growth rates or alter quorum sensing mechanisms (the chemical signaling process by which bacteria coordinate behavior based on population density)[89]. Another line focused on the influence of substrate morphological properties on cell development[90] that were demonstrated to influence even cell differentiation. Following the principle according to which cell fate can be affected by mechanical forces, we can hypothesize the acoustic niche in a laboratory may play a role in this complex process.
These examples hint to how the perspective of the sonic holobiont can reveal evolutionary aspects that can find purpose just as much on the field than in the lab.

6.4 Microbial sound ecologies

Experimental work in bioacoustics suggests that sonic interactions within holobionts might not only be an emergent property, but a functional driver of their biological organization.
We postulate that microbial cells' sound detection may be influenced by cell morphology and cell ultrastructure. These two characteristics may influence sonic wave passage and perception: depending on its frequency and intensity, a wave may be differentially perceived by cells with a diverse morphology, ultrastructure and habitus. The underlying theory is that cells evolved sound perception apparata that trigger a cellular response, ensuring culture thriving in the natural environment. Sound-based technologies are currently adopted for instance in bioproductions assisted by sound technologies and tools[91]. Bioproductions are processes where cells or cell structures produce compounds of interest; bioacoustics is exploited to increase biomass production[92] or high value molecule yield[93]. The industrial exploitation of biomass may trigger the need of a ''microbial ethics'' that addresses the limits and extent of using such technologies; for instance, by the assumption that harvesting bioprocesses should serve human purposes.

6.5 Implications for ecological resilience

If the sonic holobiont is indeed a viable framework, it has profound implications for evolutionary theory and ecosystem resilience. It challenges the widespread assumption that chemical and genetic factors are the main or sole drivers of symbiotic adaptation, proposing instead that mechanical wave communication may contribute to the evolutionary pressure shaping holobiont communities. Could microbial populations within a host "tune" their biological responses to the mechanical conditions of their environment? Could environmental noise pollution disrupt these delicate sonic interactions, leading to dysbiosis in microbial ecosystems? And if so, to which extent? These questions remain largely unexplored, but they open new pathways for understanding how sound influences the evolutionary and functional dynamics of multispecies life.

The development of an accurate experimental setup or methodologies that take into consideration the sum of cues involved in the study of the holobiont, *i.e.* light, pressure / mechanical waves (the sonic layer), genes, chemical gradients, competitors etc. will allow the interested research communities (evolutionary biologists, bioacoustic professionals, biotechnologists, microbiologists, astrobiologists, physicists, creative researchers) to actively engage in facing these novel questions (see fig.6)

7. Conclusion and future prospects

In this paper we introduced the sonic holobiont, a novel concept we propose to address the complexity in acoustic ecology in the broadest way possible .
Here we propose the novel concept of the sonic holobiont, which develops from the framework of holobiont biology towards the relationships and influences occurring in a defined soundscape, from micro- to macro-scopic scale. In doing so, we expand the concept of soundscape towards human non-audible frequencies, hence considering the entirety of sound sources.

We first referred to the concept of acoustic niche, and presented the three sound classes Bernie Krause identified within human-audible soundscapes (biophony, geophony, anthrophony). We then introduced current methods and tools in bioacoustics and their application in acoustic ecologies, especially to understand intra- and interspecies acoustic communication techniques. In thinking of bioacoustics and their application in the acoustic niche, we included a focus on microbial acoustics as well. In particular, we reflected on the role of mechanoception in the microbial acoustic niche, an evolutionary conserved trait that suggests sound might have shaped life at all scales from the very beginning. These considerations were channeled into the concept of sonic holobiont, which we can synthesize as “the entirety of mechanoacoustic influences within an environment, including microbial acoustic sources and receivers”.

The sonic holobiont helps us address the complexity of sonic interactions occurring within the biosphere, and is a useful lens to contemplate the wide influence of anthropic activities on the soundscapes we inhabit. We think the holobiont concept can be included into the WHO’s *One Health* concept, that takes into consideration the health of human civilisation and the state of the broader natural system on which it depends, among which microbes are gaining a central role.
In orienting our perspective and sensibility towards such aspects, a finer field of bioacoustics studies could emerge; one that touches many practical applications such as sampling, observations, measurements and could inform the adoption of certain species as model organisms. Nevertheless, we want to move the attention towards approaches that are integrative, synergic, and non-invasive towards the environment they work with. The sonic holobiont has to be first and foremost be *defined* as a theoretical concept and refined in an operative framework, before its evidence can be recognised across fields of studies.

In conclusion, there are many open questions and hypotheses arising from these considerations (some of such questions are outlined in the text, for the community of readers to try to think of and answer to). One is certainly whether bacteria could, in fact, be using some form of sonic or

mechanoceptive language. Such an idea might seem far fetched, but one must approach the microscopic world with curiosity and ingenuity. Whether such language exists or not, why not attempt *listening* to it, given the current status of bioacoustics technologies?
As Bernie Krause stated in his groundbreaking paper, the acoustics niche: "We are beginning to learn that the isolated voice of a song bird cannot give us very much useful information. It is the acoustical fabric into which that song is woven that offers up an elixir of formidable intelligence that enlightens us about ourselves, our past, and the very creatures we have logged to know so well".
It might be purely speculative, and the mechano- and sonic signaling might be simply passive physical phenomena with no symbolic equivalence for the cell. But through the lenses of the sonic holobiont, a multi- and inter-species acoustic network at multiple scales could be conceived and recognised -or even disproven, in nature. Regardless of the outcome, the curiosity to inquire in the acoustic niche through this lens can reward us with further understanding of the world around us.

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