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Highlights

The natural vibrational environment was recorded from plants using laser vibrometer

Hay meadow vibroscape was rich in species-specific vibrational signals

Vibroscape composition changed throughout the seasons

Species avoided interference by dividing vibrational space on a fine temporal scale

Article

Hay meadow vibroscape and interactions within insect vibrational community

Rok Šturm,[1,2] Behare Rexhepi,[1,2] Juan José López Díez,[1,2] Andrej Blejec,[1] Jernej Polajnar,[1] Jérôme Sueur,[3] and Meta Virant-Doberlet[1,4,*]

SUMMARY

Our experiences shape our knowledge and understanding of the world around us. The natural vibrational environment (vibroscape) is hidden to human senses but is nevertheless perceived and exploited by the majority of animals. Here, we show that the vibroscape recorded on plants in a temperate hay meadow is a dynamic low-frequency world, rich in species-specific vibrational signals. The overall vibroscape composition changed throughout the season and also depended on the plant species, as well as on the spatial position of individual plants within the meadow. Within the studied community, vibrationally signaling species sharing this communication channel avoided interference primarily by partitioning vibrational space on a fine temporal scale. The vibroscape is a reliable source of information in the environment and expands our understanding of ecological and evolutionary processes.

INTRODUCTION

In their natural habitats, animals continuously encounter different types of signals and cues from which they obtain information crucial for their survival and reproduction (Conchou et al., 2019; Virant-Doberlet et al., 2019). The environment shapes animals' signals and communication strategies directly via its physical properties that affect signal transmission and reception, as well as indirectly by interactions with other species sharing the habitat, which can result in interference and exploitation of signals (Endler, 1993).

Air-borne sounds (i.e., air-borne mechanical waves) are ubiquitous in terrestrial environments, and acoustic signals are considered one of the most efficient forms of communication (Wilkins et al., 2013). In recent years, with the emerging fields of soundscape ecology (Pijanowski et al., 2011b) and ecoacoustics (Sueur and Farina, 2015), the number of studies describing the structure of natural soundscapes and interspecific acoustic interactions has increased rapidly (Servick, 2014; Ruppé et al., 2015; Burivalova et al., 2019; Farina and Reid, 2020; Scarpelli et al., 2020) and obtained information has been applied to habitat assessment (Rankin and Axel, 2017; Elise et al., 2020), community ecology (Gasc et al., 2013; Dodgin et al., 2020), and conservation biology (Krause and Farina, 2016; Sueur et al., 2019) among others. Humans' reliance on vocal communication and perception of other sounds in the environment has focused our attention on the soundscape, to the extent that we have largely overlooked the ecological importance and context of substrate-borne mechanical signaling (i.e., vibrational communication). Considering that arthropods, which primarily rely on vibrational communication and substrate vibrations to gather information from the environment (Cocroft and Rodríguez, 2005; Virant-Doberlet et al., 2019), are essential for the functioning of ecosystems (Wilson, 1987; Prather et al., 2013; Risch et al., 2018), eavesdropping only on the air-borne sound domain of ecosystems (Servick, 2014) misses a crucial portion of the available information.

The recent emergence of biotremology (see Table 1) as a new scientific discipline (Hill and Wessel, 2016) has underscored the importance of vibrational communication. Although it is now recognized that vibrational signaling is one of the oldest and most widespread forms of animal communication (Cocroft et al., 2014), the complexity of the natural vibrational environment (vibroscape), where this communication takes place and evolves, has not been studied. How species which rely on substrate-borne vibrations interact at the level of a natural community and partition the vibrational channel is virtually unexplored.

While with terrestrial soundscapes we are usually quantifying what we experience ourselves (Pijanowski et al., 2011b; Servick, 2014), the main challenge in studying the vibroscape is that we need to understand its role in

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Table 1. Glossary of less common terms used in the present study

Definition	Reference
Biotremology: 'Study of mechanical communication by surface-borne waves'. Because biotremology studies are not limited to intraspecific communication, original definition was upgraded to 'study of biological interactions guided by surface-borne mechanical waves'.	Hill and Wessel (2016) Hill et al. (2019)
Surface-borne waves: Substrate-borne mechanical waves occurring at the boundary between two media (solid/air, solid/water), also known as boundary vibrations. Particles oscillate perpendicular to the direction of wave travel. Surface-borne waves are the most important types of waves for animal communication.	Hill (2008) Hill (2009) Hill and Wessel (2016)
Vibroscape: Natural vibrational environment composed of biological vibrations (vibrational signals emitted in animal intraspecific communication, incidental vibrations induced as a by-product of other animal activity, vibrational components of animal air-borne sounds), geophysical vibrations (induced by wind, rain, thunder, running water) and anthropogenic vibrations that result from human activity (traffic, machinery). Vibrations from these sources combine to create a characteristic vibrational pattern across a variety of spatial and temporal scales.	Šturm et al. (2019)
Vibroscape components: Biological vibrations, geophysical vibrations, anthropogenic vibrations	Šturm et al. (2019)
Vibrational community: Association of vibrationally signaling species sharing the same habitat at a particular time	Šturm et al. (2019)
Vibrational event: shift in vibration frequency over background vibrational noise and characterized by distinct temporal and spectral properties.	this study
Vibrational signal: vibrational event with specific and predictable temporal and spectral characteristics involved in transfer of information from the sender to the receiver and affects the behavior of the latter.	this study
Active space of vibrational signals (i.e., effective range): The area in which the signal amplitude is sufficiently above the amplitude of the detection threshold of potential receivers to have an effect on their behavior.	Mazzoni et al. (2014) Šturm et al. (2019)
VST: vibrational signal type. In the absence of comprehensive public reference libraries of vibrational signals, recorded vibrational signals were classified by their distinct temporal and spectral properties and assigned to VSTs.	this study
VST richness: The number of different VSTs in the recording	this study
VST abundance (i.e., time occupancy of vibrational space): The sum of durations of individual VSTs in the recording.	this study

ecosystems primarily from the perspective of the animals which rely on information provided by substrate vibrations hidden to our senses (Narins et al., 2018). In contrast to terrestrial acoustic communication, humans do not intuitively recognize the challenges that animals which rely on substrate-borne mechanical communication face in their environment. Thus, the first fundamental question that we addressed was how complex is the vibroscape (i.e., which components and sources contribute to the natural vibrational environment)? We studied the vibroscape in a eutrophic Central European hay meadow, using laser vibrometry to regularly record vibrations from focal plants for six months, between May and October, 2017. We focused on plants in a hay meadow community for two main reasons: (a) vibrational communication is ubiquitous among insects (Cocroft and Rodríguez, 2005), which are by number and species richness one of the dominant animal groups in grasslands (Joern and Laws, 2013), and (b) plants are the most common substrate used by insects to transmit their vibrational signals (Cocroft and Rodríguez, 2005). In the next step, we addressed the question as to how vibrationally signaling species shared the vibrational communication space. As is the case for acoustically communicating species, for members of the vibrational community (see Table 1), the signal transmission channel is an ecological resource shared by species signaling at the same time and place (Schmidt and Balakrishnan, 2015). Masking interference from overlapping heterospecific signals impairs the detection and recognition of relevant conspecific signals. Signallers can reduce interference by partitioning the signal space in time and/or frequency domains (Jain et al., 2014; Schmidt et al., 2013; Schmidt and Balakrishnan, 2015). Here, we analyzed the temporal and spectral composition of recorded vibrational signals to determine if observed signal overlap patterns differed from those expected by chance.

RESULTS AND DISCUSSION

General characteristics of recorded meadow vibroscape

Recorded vibrational events (see Table 1) resulted from geophysical (i.e., abiotic), biological (i.e., biotic), and anthropogenic sources. However, we focused our attention primarily on the biological component,

which included species-specific vibrational signals used in intraspecific communication, incidental vibrations induced as a by-product of other animal activities, as well as substrate-borne components of air-borne sounds produced by animals (Figure 1).

A general characteristic of the hay meadow vibroscape was that in contrast to a terrestrial soundscape, the natural vibrational environment was predominantly a low-frequency (<2000 Hz) domain, where vibrations induced in plants by wind of low velocity (<0.2 m/s) provided nearly constant background vibrational noise in the frequency range below 200 Hz (Figure 1). On each recording day, the vibroscape also included vibrational events originating from biological sources, which usually stood out from abiotic noise not by their amplitude but primarily in the frequency domain (Figures 1 and S6). While the spectral characteristics of these events associated with insect communication spanned a frequency range between 70 and 7000 Hz, most of the signal energy was focused into the frequency range below 1000 Hz, except for the leafhopper *Megophthalmus scanicus*, which produced signals with dominant frequencies above 1500 Hz (Figures 1C and 2C). In contrast to vibrational signals, vibrational components of bird songs appeared at frequencies above 3000 Hz (Figure 1B).

Currently, there is no direct information as to which vibroscape components are detected by insect members of a vibrational community. All insects possess a variety of mechanoreceptors to detect substrate vibrations; however, knowledge of the frequency sensitivity of insect vibration receptors is limited to a handful of species, mostly orthopterans, which primarily rely on air-borne sound communication (reviewed in the studies by Čokl et al., 2006; Lakes-Harlan and Strauβ, 2014; Stritih and Čokl, 2014). In most of our recordings, the frequency range below 50 Hz contained the most energy (Figure 1). The measured amplitude of this pervasive low-frequency abiotic vibrational noise was in the range of 10^{-6} m/s, which is still below the currently determined threshold values of insect vibration receptors at these frequencies (Lakes-Harlan and Strauβ, 2014). The amplitudes of vibrational signals measured at signal dominant frequencies (100-500 Hz) ranged between 10^{-5} and 10^{-7} m/s and, according to the frequency tuning characteristics of vibroreceptors, these signals were sufficiently high to be detected by insects (Lakes-Harlan and Strauβ, 2014). The same was true for the vibrational signals of *M. scanicus*, with their dominant frequency around 3000 Hz. The amplitudes of the vibrational components of bird songs were lower, in the range of 10^{-7} m/s, at or below the threshold of detection of insects' vibration receptors, and most likely not detected.

Seasonal changes in vibroscape composition

Our approach gave an insight into the dynamics of the vibroscape composition at different temporal scales, from seasonal changes to diel variation. The time occupancy of the vibrational channel was highest in May and July (Figure 1A). However, in May, the majority of recorded biotic vibrations were from bird songs (Figure 1B), whereas in July, the abundance of biotic vibrations in the vibroscape reflected communication among vibrationally signaling species (Figure 1C, Videos S1, S2, and S3).

Vibrational signals are characterized by distinct, species-specific temporal and spectral properties (Cocroft and Rodríguez, 2005) (Figure 2C, Videos S4, S5, S6, and S7). Owing to the lack of comprehensive public reference libraries of vibrational signals (Šturm et al., 2019), the majority of the recorded vibrational signals could not be identified to the species level and so were primarily assigned to vibrational signal types (VSTs). Throughout the season, we identified a total of 53 VSTs, of which 13 were attributable to known insect species (see Table S1). The most common communication pattern in species relying on vibrational signaling is that males spontaneously emit advertisement calls that trigger the emission of a female reply (Bailey, 2003; Derlink et al., 2014; Gordon et al., 2017); therefore, the most commonly encountered vibrational signals in the field are likely to be male calls. From the existing recordings stored at the library of vibrational signals at the National Institute of Biology (Ljubljana, Slovenia), we identified the male calls of the following species: Hemiptera, Aphrophoridae: *Philaenus spumarius*; Hemiptera, Cicadellidae: *Anoscopus serratuale*, *Aphrodes makarovi*, *A. bicincta* Dragonja, *Arthaldeus striifrons*; *Cicadella viridis*, *Empoasca vitis*, *Megophthalmus scanicus*; *Streptanus aemulans*; Hemiptera, Delphacidae: *Dicranotropis hamata*; *Javesella dubia*; *Megadelphax sordidula*, *Ribautodelphax albostriatus*. We also identified the female reply of *A. makarovi*.

According to a signal's temporal and spectral structure, we assigned VSTs to one of four general categories: (a) pulse: vibrational signals composed of short, single pulses lasting less than 0.2 s with either broadband or harmonic frequency structure; (b) harmonic signals: vibrational signals composed of pulses longer than 0.2 s and with clear harmonic frequency structure; (c) train: vibrational signals composed of
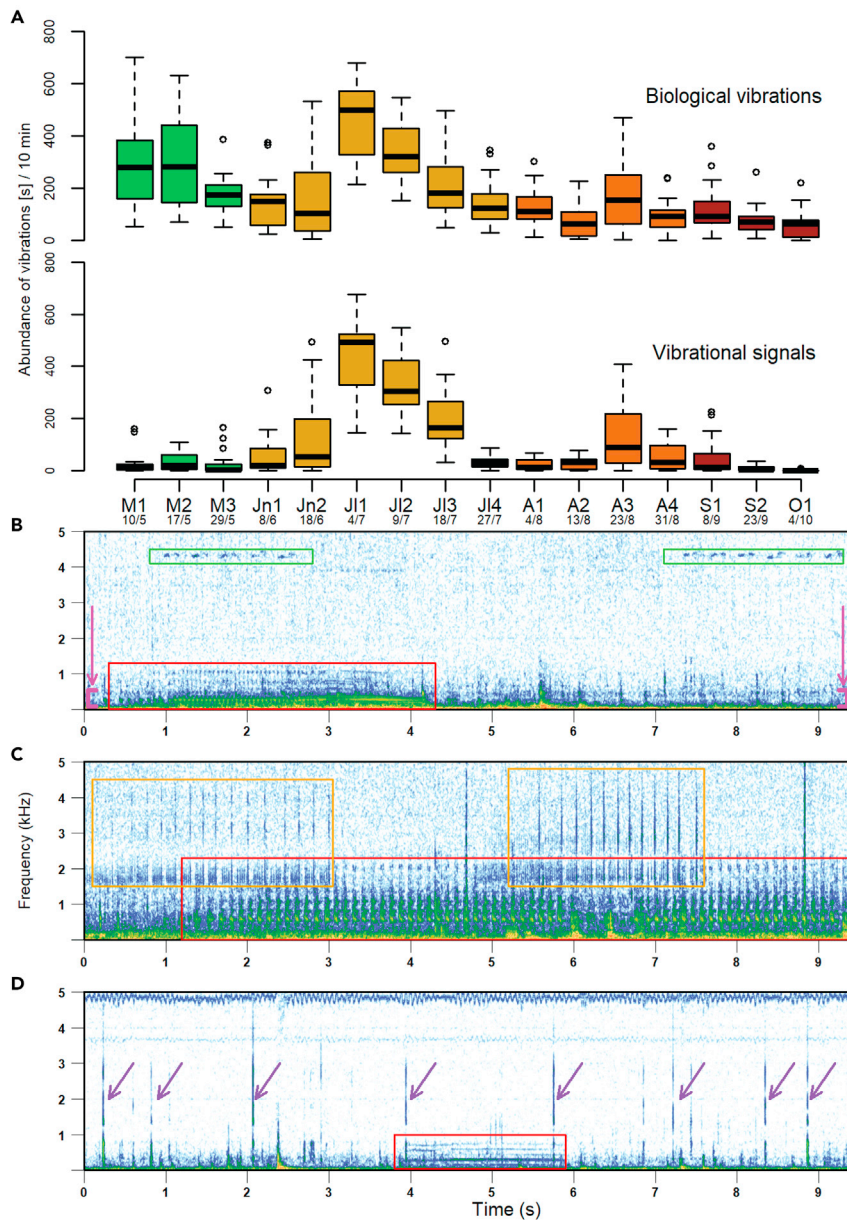
Figure 1. Seasonal changes in the biotic component of a hay meadow vibroscape

(A) Box and whisker plot for each day (above: all biological vibrations [vibrational signals, incidental vibrations, substrate-borne components of animal air-borne sounds]; below: vibrational signals) is based on 18 points, each representing a cumulative duration of biological vibrations/vibrational signals per 10 min (i.e., duration of individual recorded files). Plots (green: spring; yellow: early summer; red: late summer; brown: autumn) show the median (thick line), the 25-75% interquartile range (boxes), the lowest and the highest data points still within 1.5 times the interquartile range (whiskers), and outliers (circles).

(B–D) Visualisations of the recorded vibroscape in the form of spectrograms. (B) Vibroscape recorded in spring (May 10, 2017; Movie File S1). Red markers and arrows indicate low-frequency vibrational noise induced by wind, while orange and red frames mark vibrational components of a bird song and vibrational calls of male *Dicranotropis hamata* (Hemiptera, Delphacidae), respectively. (C) Vibroscape recorded in early summer (July 9, 2017; Movie File S2), which includes vibrational calls of male *Megophthalmus scanicus* and *Anoscopus serratulae* (Hemiptera, Cicadellidae) (orange and red frames, respectively). (D) Vibroscape recorded in autumn (September 23, 2017; Movie File S3) with vibrational signal of unknown species (red frame). Arrows indicate incidental biotic vibrations, such as insect movements.
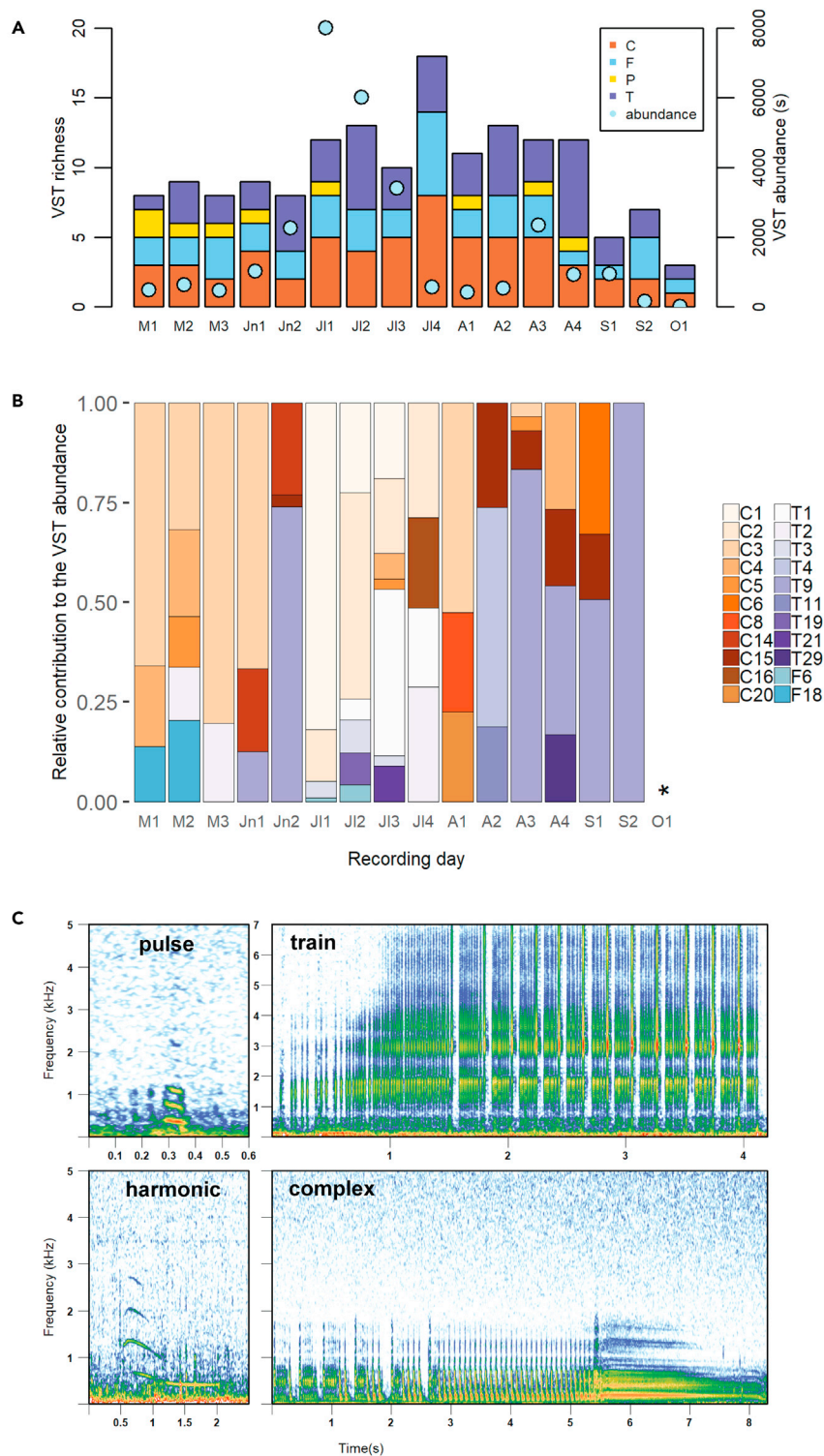
Figure 2. Contribution of different vibrational signal types (VSTs) and VST categories to vibroscape composition

(A) Time occupancy of vibroscape (VST abundance) on a particular day was not correlated with the number of different VSTs recorded on each day (VST richness). Columns show the number of different VSTs in each signal category (yellow: pulse (P); blue: harmonic (F); purple: train (T); orange: complex (C)), while circles indicate the cumulative duration of vibrational signals recorded at each day.

Figure 2. Continued

(B) Relative contribution of different VSTs to the time occupancy of vibroscape. Each VST is represented by a different color: orange, VSTs in complex category; purple: VSTs in train category; blue: VSTs in harmonic category. Only VSTs with cumulative duration of over 1 min during 180 min recording are included. * In October, none of the three recorded VSTs exceeded 1 min cumulative duration.

(C) Spectrograms of an example of each of the four signal categories (Movie Files S4-S7). Train and complex vibrational signal belong to *Megophthalmus scanicus* and of *Dicranotropis hamata*, respectively, while pulse and harmonic signals were emitted by unknown species. All spectrograms were generated in seewave (R package) with a Hanning window of 1500 samples with 80% overlap.

regularly repeated pulses or harmonic signals; (d) complex: vibrational signals composed of at least two of the previous categories (Figure 2C, Movie Files S4-S7). The pulse category was the least, and the train the most diverse category (see Table S1). The time occupancy of vibrational space was not correlated with the VST richness recorded on a particular recording day (Kendall's correlation coefficient, τ = 0.35) (Figure 2A). The vibroscape was often dominated by only a few VSTs, and signals in the train and complex categories contributed more to the time occupancy of the vibrational communication channel than pulse and harmonic signals (Figure 2B).

The hay meadow vibroscape was complex and characterized by high VST turnover. On each recording day, the analyzed vibroscape included on average 10 VSTs (SD = $\pm$ 4). However, the accumulation curves of VSTs rarely reached a plateau, suggesting that the signal richness experienced by a receiver was higher and could on a single day reach more than 20 different VSTs (see Figure S1). Twenty-eight percent of recorded VSTs were encountered only on one day, and consecutive weekly recordings shared from 15 to 46% of VSTs. On all recording days, except two (September 8 and October 4), we encountered new VSTs not recorded previously. Because of the multivoltine life cycles of some insect species encountered in our study, their vibrational signals appeared repeatedly over the course of the year (see Table S1). Nevertheless, the overall vibroscape composition changed significantly throughout the seasons (Figures 3A and S2).

Consecutive daily recordings during the period of the highest vibrational activity at the beginning of July revealed higher consistency of vibroscape composition than across weeks (see Table S2). Over a 3-day period we identified 24 VSTs, and recordings on consecutive days shared 54-85% VSTs. Nevertheless, during this period, 11 VSTs were recorded only on one day each. The calculated signal richness for this period approached 30 VSTs, but did not reach a plateau (see Figure S3).

Diel variation in vibroscape composition

Continuous 24-h recordings during the period of the highest occupancy of a vibrational channel at the beginning of July revealed diel variation in signaling activity, with peak activity between 1 and 5 pm and with only a few unidentified vibrational events occurring at night (see Figure 4A). Such an activity pattern seems to be primarily regulated by temperature (Figure 4B), likely because in insects the processes associated with the production of mechanical signals are temperature dependent (Sanborn, 2006). While some studies suggest that peak daily vibrational signaling activity coincides with low wind levels (McNett et al., 2010; Velilla et al., 2020), at our field site, wind velocity generally followed the same pattern as signaling activity (Figure 4C).

Spatial differences in vibroscape composition

The high VST turnover represents a dynamic meadow arthropod community, coupled with substantial changes in the meadow vibroscape composition over short distances. Simultaneous recordings from hammer sedge (*Carex hirta*) and hedge bedstraw (*Gallium mollugo*) plants growing in proximity in different parts of the meadow revealed that a receiver's individual experience of a vibroscape not only depended on the resident plant species (Figures 3B and S2) but can also depend on the spatial position of individual plants within the meadow (Figure 3C). Spatial differences in vibroscape composition resulted from unique VSTs recorded only in one plant species and in the abundance of recorded vibrations (see Table S3). In hay meadows, where vegetation is dense and closely interconnected (see Figure S5), the active space of vibrational signals is not limited to the plant on which an animal is signaling but extends to neighboring plants connected by roots and touching leaves and even across a several-centimeter-wide air gap between overlapping leaves (Čokl and Virant-Doberlet, 2003; Eriksson et al., 2011; Mazzoni et al., 2014; Gordon et al., 2019). A random distribution of plants within the grassland habitat, together with differences in plant geometries and heterogeneity of plant substrates that strongly affect damping and selective frequency
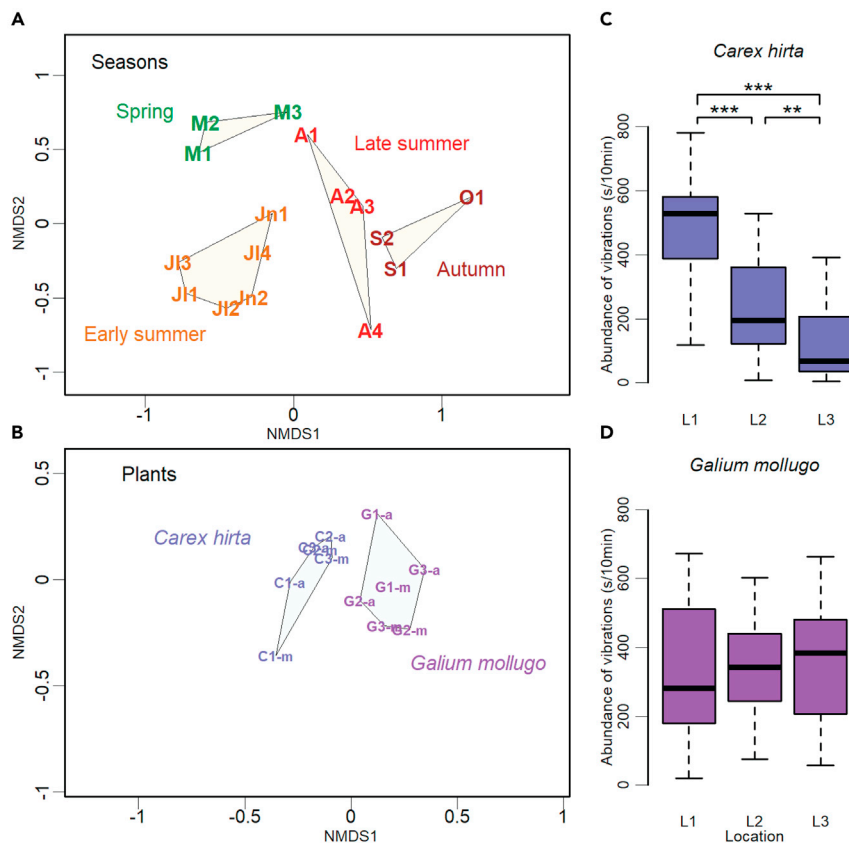
Figure 3. Differences in vibroscape composition

(A) Differences in vibroscape composition throughout the seasons. Nonmetric multidimensional scaling (nMDS) analysis based on VST presence-absence matrix (Table S1) (k = 3, stress value = 0.10).

(B) Differences in vibroscape composition recorded simultaneously on *Carex hirta* (C) and *Galium mollugo* (G) (nMDS, VST presence-absence matrix, k = 3, stress value = 0.05) (Table S3). Numbers 1, 2, and 3 indicate the location of the plant within the meadow and 'a' and 'm' the afternoon and morning recording session, respectively.

(C) Differences in vibroscape composition (VST abundance) between *C. hirta* plants growing at different positions (L1, L2, and L3) in the same meadow. Box and whisker plot for each location is based on 24 points, each representing a cumulative duration of vibrational signals per 10 min (i.e., duration of individual recorded files) and shows the median (black line), the 25-75% interquartile range (boxes), the lowest and the highest data points still within 1.5 times the interquartile range (whiskers), and outliers (circles). Pairwise comparison, Wilcoxon rank-sum test for multiple comparisons with Bonferroni corrections, ***p < 0.001, **p < 0.01.

(D) Similar analysis for *G. mollugo* found no differences.

filtering, result in unpredictable size and shape of the active space of vibrational signals (Cocroft and Rodríguez, 2005; Šturm et al., 2019; Virant-Doberlet et al., 2019).

Partitioning of the vibrational communication channel

During the peak daily signaling activity in early summer, the VST overlap in time and frequency domains was significantly smaller than it would be by chance (Figures 5C and S4), suggesting that species avoid interference by partitioning the vibrational communication channel. Within the studied community, the main observed strategy appeared to be dividing the communication space in time because the majority of recorded vibrational signals occupied the same dominant frequency range, between 50 and 1000 Hz (Figure 5B). The only exceptions were vibrational signals of *M. scanicus*, which at dominant frequencies showed almost no frequency overlap with other co-occurring signals (Figure 5B), but which overlapped substantially more often with other signals in time (Figure 5D).

With the lack of comparative data, it is unclear whether such overwhelming reliance on partitioning on a fine temporal scale (over seconds to minutes) is an evolutionarily established strategy to reduce interference
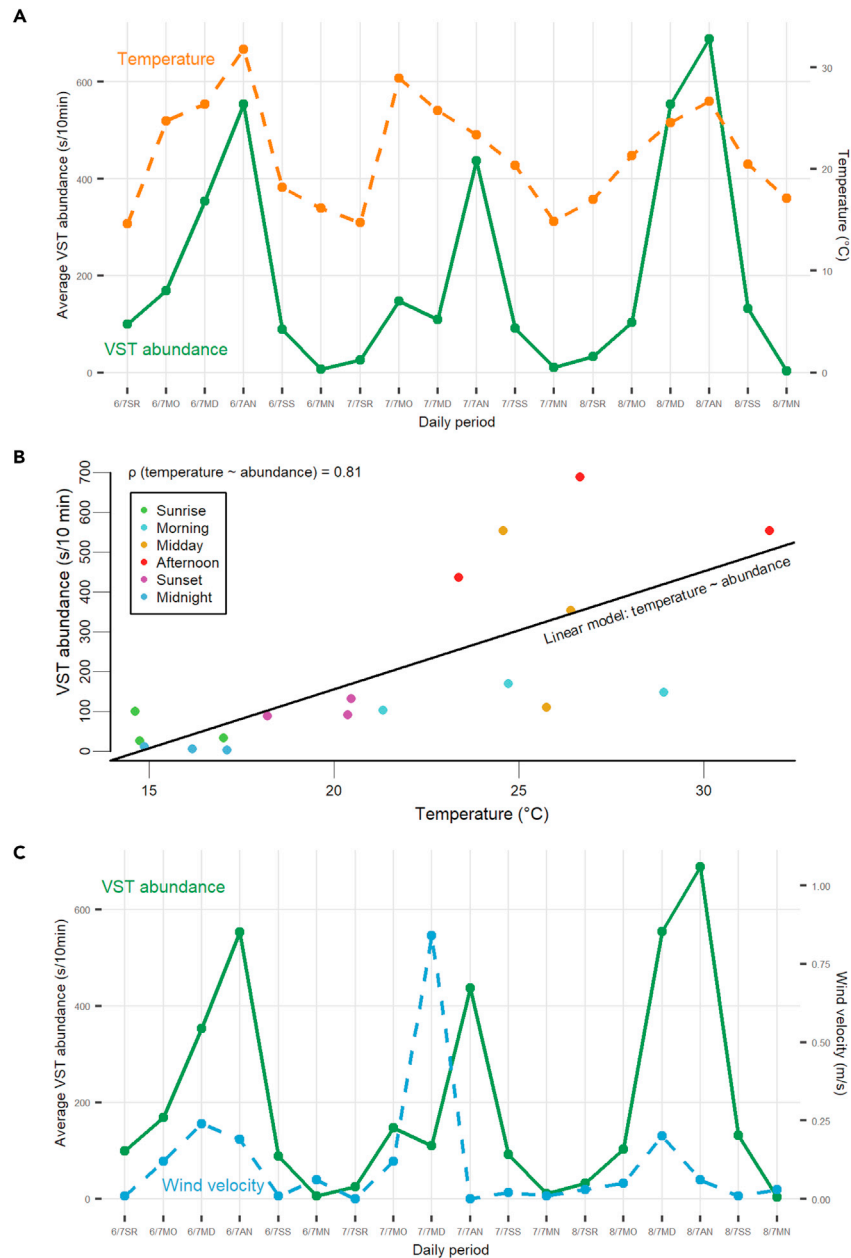
Figure 4. Diel variation in vibrational signaling activity

(A) Diel variation in temperature (orange dashed line) and VST abundance (green line) obtained during continuous 24-h recordings in the period July 6-8, 2017. Evaluated were the following daily periods: SR, sunrise, 05:00h - 05:30h; MO, morning, 09:00h - 09:30h; MD, midday, 12:50h - 13:20h; AN, afternoon, 16:40h - 17:10h; SS, sunset, 20:40h - 21:10h; MN, midnight: 0:50h–1.20h. Temperature was recorded every 10 s and is represented as average temperature in each 30-min period. VST abundance is shown as average cumulative duration of vibrational signals per 10 min (i.e., duration of individual recorded files) in each 30-min period.

(B) Correlation between VST abundance and temperature. Spearman's correlation coefficient $\rho = 0.81$ ($p < 0.001$).

(C) Diel variation in wind velocity (blue dashed line) and VST abundance (green line) obtained during continuous 24-h recordings. Wind speed was recorded every 10 s and is represented as average wind speed in each 30-min period. VST abundance is shown as in (A). Morning and midday periods on July 7 were windy, and this is reflected in low VST abundance. While this may represent a short-term negative effect of wind on signaling activity, it may also be a result of high-amplitude broadband abiotic noise preventing reliable annotations of vibrational signals.
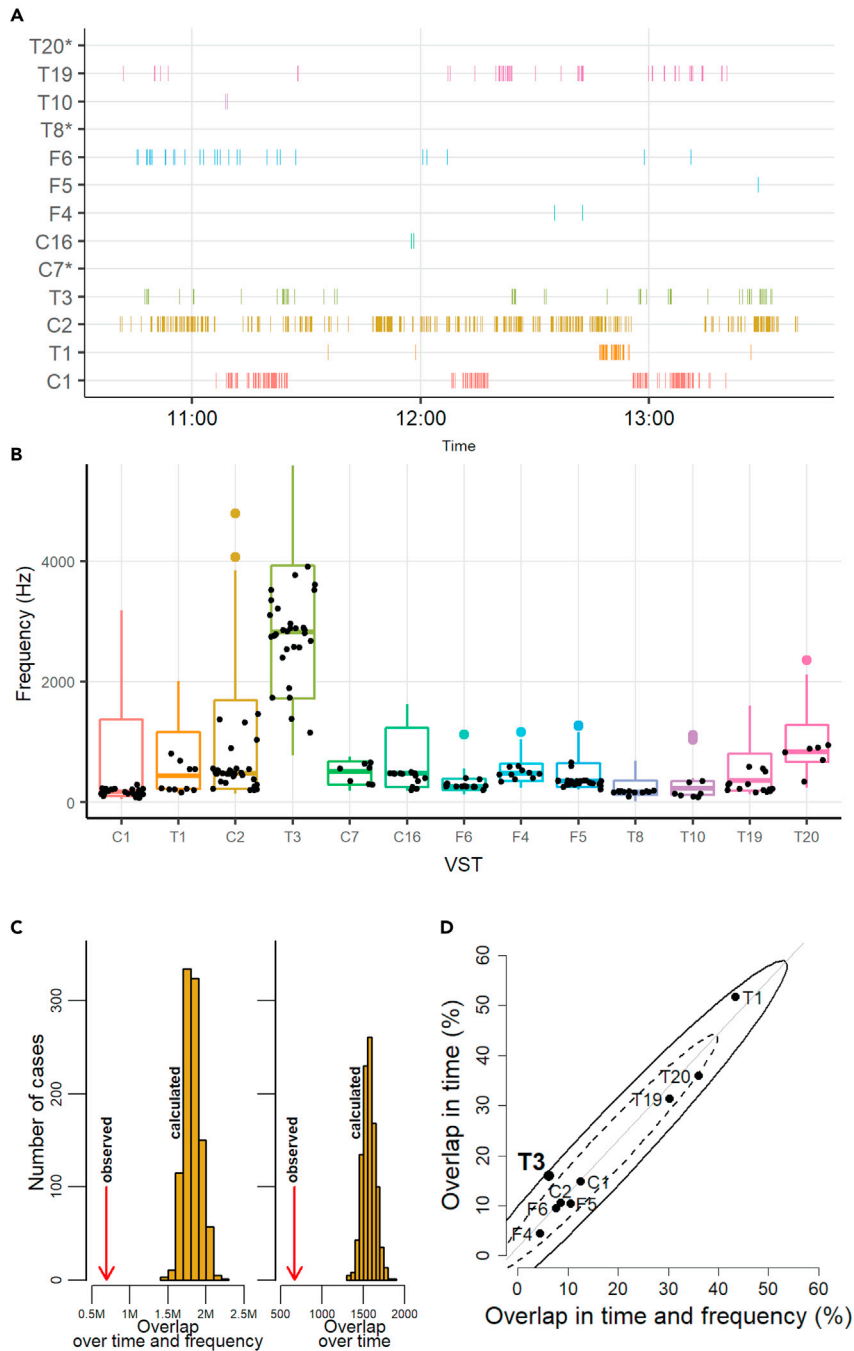
Figure 5. Partitioning of the vibrational communication channel

(A) Temporal distribution of VSTs recorded on July 9, 2017. The duration of VTSs marked with* is too short to be shown on the timeline.

(B) Frequency characteristics of recorded VSTs. Box and whisker plots show the complete frequency range, while black dots represent distribution of their dominant frequencies with points jittered horizontally. Plots show the median (thick line), the 25-75% interquartile range (boxes), the lowest and the highest data points still within 1.5 times the interquartile range (whiskers), and outliers (colored circles).

(C) Frequency histograms showing expected random overlap of VSTs. Left: overlap in time and frequency domain; right: overlap in time domain only. Red arrows indicate the observed level of overlap. Permutation tests: p = 0.

Figure 5. *Continued*
(D) Linear relation among the observed overlap of VSTs in time and frequency (x axis) and time only (y axis). Ellipses show 68 and 95% prediction regions (dashed and solid lines, respectively) based on Mahalanobis distance. For T3 (=*Megophthalmus scanicus*), the overlap in time is higher than expected according to the value of its overlap in time and frequency and is located on the outer edge of the 95% prediction region (Mahalanobis distance 2.19, p = 0.09).

among members of vibrational communities in temperate hay meadow habitats, or potentially a signature of disturbed communities altered by human activity (Pijanowski et al., 2011a; Sueur et al., 2019). Adaptation of signals to a specific vibrational community is likely to be constrained by convergent selection on signals which ensure reliable transfer of information in a particular habitat because selection pressures imposed by the filtering properties of the plants and pervasive low-frequency abiotic vibrational noise are channelling the signal frequencies into a relatively narrow frequency range between 50 and 5000 Hz (Cocroft and Rodríguez, 2005), regardless of whether the signals have narrowband, harmonic, or broadband characteristics. Even in this frequency range, plants impose unpredictable changes onto the spectral properties of the signal (Michelsen et al., 1982), thus likely rendering relatively small frequency differences as unreliable parameters for signal recognition. Our results suggest that a meadow vibrational community may be inherently too dynamic and the vibroscape too variable and unpredictable for species to effectively partition vibrational space in the frequency domain because this requires evolutionary changes in signal production mechanisms, as well as matching frequency tuning of the receptors (Pijanowski et al., 2011a). Taking into account the temperature dependence of signaling activity, which to some extent precludes diel partitioning, a behavioral strategy to avoid signaling at times with high background vibrational noise (abiotic and biotic) appears to be the best solution. Furthermore, the signal amplitude oscillates during transmission through plants; thus, increasing the amplitude of the emitted signals does not necessarily result in a better signal-to-noise ratio at the location of the receiver (Michelsen et al., 1982).

Importance of vibroscape for ecological studies

The vibroscape includes information important not only for species using vibrational signals in their intraspecific communication but for all animals able to detect vibrational signals or cues in their environment (Virant-Doberlet et al., 2019). As such, the vibroscape is a crucial element of ecological processes and ecosystem dynamics. In an environment that is rapidly changing owing to human activity, climate change, and invasive species (Sueur et al., 2019; Wagner, 2020), this vibratory world to which humans are essentially 'deaf' is also likely to be changing in unknown ways, with potentially crucial effects on arthropod communities. While the current study focused on a site with low levels of anthropogenic vibrational noise, the effects of this pollutant are likely to impose further constraints on interactions within invertebrate communities (Morley et al., 2014; Raboin and Elias, 2019).

Limitations of the study

While the emergence of biotremology (Hill and Wessel, 2016) put vibrational behavior into research focus, this study extends the focus to the ecological context of vibrational communication. Without any other published vibroscape studies, the conclusions are limited to the vibrational community found at our study site and should be, in the future, used as a guideline for comparative studies in different habitats. Owing to the large number of recorded files and the large number of vibrational events in them, it was not possible to annotate entire recordings. Although so far computational methods for automated classification and identification of vibrational signals in the field recordings have not been developed and/or tested, such approach should, in the future, enable to screen and analyze a large amount of recordings. In recent years, affordable autonomous sound recorders have become an important tool in soundscape ecology and ecoacoustics (Gibb et al., 2019). The availability of costly laser vibrometers limited the possibility of working simultaneously at different locations. While much cheaper sensors for recording plant-borne vibrations (Nieri et al., 2021) that have also been used to record vibrational signals in the field (Narins et al., 1992; Lewis et al., 2001; Cocroft, 2003) are available, our own tests in a hay meadow habitat showed that they are not suitable to record vibroscape on small herbaceous plants with thin and soft stems. Although insect vibrational signals are species and sex specific (e.g., Derlink et al., 2014), in the absence of comprehensive publicly available reference libraries of vibrational signals, care should be taken not to overestimate the number of species forming a vibrational community. The richness of vibrational repertoire differs among species, and regional vibrational dialects have also been described (Šturm et al., 2019). Temporal parameters of vibrational signals are also temperature dependent (Brandt et al., 2018), as well as subject to unpredictable degradation in the frequency domain during the transmission through the plants (Michelsen et al., 1982). We opted to assign all vibrational signals to 'vibrational signal

types' first and then link VSTs to the individual species, in case they matched the recordings in our own signal library. Under windy conditions encountered between 12:50h and 13:20h on July 7, it was not possible to un-equivocally determine a short-term negative effect of wind on vibrational signaling because wind gusts of higher velocities (>2.5 m/s) resulted in high-amplitude abiotic vibrational noise up to 5 kHz, thus overlapping the whole frequency range of biotic vibrations. Substrate vibrations induced by strong wind gusts are characterized by large short-term amplitude variations; however, it was not possible to reliably annotate vibrational events in the recordings and to determine whether biological vibrations were masked by noise or signaling activity had stopped. Weekly recordings carried out to determine the seasonal dynamics of the vibroscape composition were done under comparable conditions on calm days with average wind speeds below 0.25. m/s and wind gusts below 1.5 m/s. Although VST overlap analyses consistently show that when the time occupancy of the vibrational communication channel is high, the temporal distribution of VSTs within the vibroscape is not random, we have no direct information on the spatial position of the emitters of the VSTs included in the VST overlap analyses in relation to the recording point, nor whether they were located in each other's signal active space (i.e., whether they could affect each other's signaling behavior).

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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  - Annotation of vibrational signals
  - VST richness and VST abundance
  - Differences in vibroscape composition
  - VST overlap

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2021.103070.

AUTHOR CONTRIBUTIONS

R.Š., J.P., and M.V.D. conceived and designed the study; R.Š. and B.R. carried out the field work; R.Š., B.R., and J.J.L.D. screened the recordings; R.Š, B.R., and J.S. analyzed data; A.B. wrote the R code for VST overlap analyses; R.Š. and M.V.D. prepared figures and wrote the manuscript with edits from all authors; M.V.D. supervised all aspects of the work.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
Raven Pro 1.5	Cornell Lab of Ornithology	https://ravensoundsoftware.com/software/raven-pro/
Sinus Samurai 2.6.2	SINUS Messtechnik GmbH	https://sinus-leipzig.de/de/produkte/software/samurai
Seewave	Sueur et al. (2008)	http://rug.mnhn.fr/seewave/
R programming language	R Core Team	https://www.R-project.org/
vegan R package	Oksanen et al., 2017	https://github.com/vegandevs/vegan
VST overlap calculation algorithm	this paper	https://github.com/NIB-SI/VSToverlap
Audio files of all VSTs	this paper	https://oblak.nib.si/index.php/s/IYm2msH52vKZwjh

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to the lead contact and will be fulfilled by the lead contact, Meta Virant-Doberlet (meta.virant-doberlet@nib.si).

Materials availability

This study did not generate new unique reagents or other new materials.

Data and code availability

- Data: Audio files of all VSTs are available at: https://oblak.nib.si/index.php/s/IYm2msH52vKZwjh.

- Code generated during this study has been deposited at: https://github.com/NIB-SI/VSToverlap

METHOD DETAILS

Study site

Our field study was carried out in a eutrophic lowland hay meadow located in Ljubljana Moors, Slovenia (N 45°56′42.40′′; E14°20′09.21″). This site was chosen because the owners allowed us to control the agricultural practices in this meadow, and the vegetation was not mowed until late autumn (mid-October) when seasonal observations ended. The focal area measured approximately 400 m^2 and included 10 apple trees. On the south, west, and north borders of the study area, the grass was cut more often, while on the eastern side it was grazed by cattle. In July 2017 we carried out a botanical survey at the site and the list of plant species is shown in Table S4.

Vibroscape and field recordings

Regarding the general practical and technical issues associated with field recordings we followed recently published guidelines (Šturm et al., 2019).

To follow the seasonal changes in vibroscape composition, we carried out field recordings on 16 days from May 10 to October 4, 2017 (see Table S1). We attempted to obtain recordings once a week; however, in order to carry out recordings under comparable conditions, we had to choose sunny and calm days. Although the equipment was constantly overseen, heavy rain, thunderstorms, and strong wind prevented fieldwork during several weeks, so the recordings were carried out on the following days: May 10 (M1), May 17 (M2), May 29 (M3), June 8 (Jn1), June 18 (Jn2), July 4 (Jl1), July 9 (Jl2), July 19 (Jl3), July 27 (Jl4), August 4 (A1), August, 13 (A2), August, 23 (A3), August 31 (A4), September 8 (S1), September 23 (S2), October 4 (O1).

On each day, the vibroscape was recorded for four hours during the period between 10:00h and 15:00h. The vibroscape was recorded with a portable laser Doppler vibrometer (Polytec PDV 100) and stored in

a laptop computer using an external sound card (Sound Blaster SBX) and Raven Pro 1.5 software at the sampling rate 44 kHz and 16-bit resolution. Sound files in.wav format were automatically saved every 10 min in order to keep audio file size small enough for more convenient analyses. To obtain information on the natural amplitudes of recorded vibroscape components, on July 9 we recorded vibrations with a calibrated SINUS Soundbook Quadro system with Samurai 2.6.2 software, which measured the absolute amplitude of recorded signals (as velocity in mm/s). All equipment was placed under a canopy to avoid exposure to sun and overheating in summer months, and to provide protection from rain. The site had access to the power grid and electricity was directly brought to the equipment via extension cable.

Plants on which the vibroscape was recorded were growing about 1.5 m away from the laser vibrometer. On May 10 we chose a clover (*Trifolium pratense*) as the focal plant. The same plant was used in all subsequent recordings until July 18, when this particular plant died out and we had to switch to the neighboring plants. Because there were no other clover plants available at our study area, on July 27 and August 13 the vibroscape was recorded from hedge bedstraw (*Galium mollugo*), on August 4 from germander speedwell (*Veronica chamaedrys*), on August 23, 31 and September 8 from ground-ivy (*Glechoma hederacea*) and on September 23 and October 4 from hammer sedge (*Carex hirta*). We focused the laser beam on a small piece of reflective tape (weight 3-6 mg) that was placed on a plant stem in order to increase the reflectance and thus improve the signal-to-noise ratio. Because the top of the plant was more exposed to the wind, we recorded the vibroscape closer to the ground to ensure stable recordings (see Figure S5).

To follow diurnal changes in the natural vibrational environment, we recorded the vibroscape continuously for 24 h on four successive days from July 5 to July 8, but excluded the first day from further analyses, as it contained many rainy and windy periods (see Table S2). Wind gusts of higher velocities (>2.5 m/s) resulted in high amplitude abiotic vibrational noise up to 5 kHz, thus overlapping the whole frequency range of biotic vibrations. Under such conditions it was not possible to detect vibrational events in the recordings and to determine whether biological vibrations were masked by noise or signaling activity had stopped.

To study the differences in vibroscape composition within the meadow, we carried out field recordings at the same meadow in the period between July 7-14, 2018 (see Table S3). The vibroscape was recorded simultaneously with two laser vibrometers from hedge bedstraw (*Galium mollugo*) and hammer sedge (*Carex hirta*). Within the meadow we chose three sites at around 5 m from each other, where plants of these two species were growing in close proximity. Because the active space of vibrational signals in a hay meadow habitat was not known, we chose plants that were growing around 50 cm apart in order to avoid simultaneously recording the same signal from two positions. Recordings were carried out as described above and were divided into two periods: the first from 10:00h to 14:00h (morning session) and the second from 14:00h to 18:00h (afternoon session). We rotated the recordings among six plants and two daily sessions, so that ultimately each plant was recorded in both daily periods.

Other data collected in the field

Throughout each recording session we also recorded with the sensors positioned near the recording point in the vegetation (see Figure S5) the following environmental parameters: temperature, humidity, pressure, illumination, and wind speed at the site. We used the Ahlborn Almemo 2590 data logger system with the following sensors: Ahlborn FHAD46-C2 digital sensor (temperature, humidity, atmospheric pressure) with ranges: −20 to +60°C, 5 to 98% RH and 700 to 1100 mbar, with minimal accuracy of ±0.7°C, ±4% RH and ±2.5 mbar respectively; FLA 623 x for illumination and FVA615 2 for wind speed. Data were automatically stored every 10 s.

QUANTIFICATION AND STATISTICAL ANALYSIS

Annotation of vibrational signals

The first step in the analyses of vibroscape recordings was manual annotation of recorded vibrations. We listened to the files as many times as necessary and, because vibrational events originating from biological sources stand out from abiotic noise primarily in the frequency domain (see Figure S6), we simultaneously visualized the recordings as spectrograms in Raven 1.5.

Due to the large number of recorded files (1032 10-min sound files, altogether 172 h of recordings), we screened only a sub-set of recorded audio files. We marked the vibrational events in time and frequency domain (see Figure S6) and we annotated 13,680 vibrational events assigned to biological sources.

To describe seasonal changes in vibroscape composition, we annotated a continuous 3-h interval for each recording session, thus excluding the first hour in order to avoid potential negative effects of setting up the equipment on vibrational activity. In addition, we annotated 3 h from each day of the recordings on successive days in July. For the latter, we took into account the intervals 11:00h–12:00h, 14:00h–15:00h and 17:00h–18:00h.

To follow diurnal changes in the natural vibrational environment, we divided each day into six periods according to the sun cycle: sunrise, morning, midday, afternoon, sunset, and midnight. Sunrise and sunset times for Ljubljana for that period of the year were obtained from the web page https://www.timeanddate.com/sun/slovenia/ljubljana. Midnight and midday were determined as midpoints between the sunrise and sunset, morning between sunset and midday, and afternoon between midday and sunset. To determine diel variation in signaling activity, we took into account 30-min intervals at the beginning of each daily period (sunrise: 05:00h–05:30h; morning: 09:00h–09:30h; midday: 12:50h–13:20h; afternoon: 16:40h-17:10h; sunset: 20:40h-21:10h; midnight: 00:50h-01:20h).

To study the differences in vibroscape composition within the meadow, we annotated a continuous 2-h section of each recording session. The main obstacle in characterizing the vibroscape was a lack of comprehensive public reference libraries of vibrational signals (Šturm et al., 2019; Frommolt et al., 2019). It is currently estimated that around 200,000 insect species rely on vibrational signaling (Cocroft and Rodríguez, 2005). Vibrational signals used in communication are species- and sex-specific; however, due to enormous diversity and the huge number of species relying on vibrational communication, the information on the vibrational repertoire of the great majority of species encountered in the field is not available. Moreover, due to their small size and dense meadow vegetation, during the field recordings we were not able to see the insects emitting vibrational signals. Consequently, the majority of recorded vibrational signals could not be identified to the species level, and so were primarily assigned to vibrational signal types (VST) by their distinct temporal and spectral properties. Because the recordings were screened by several people (R.Š., J.J.L.D. and B.R), we crosschecked the annotations in order to ensure consistent identification and classification.

VST richness and VST abundance

From the screened recordings, we obtained two variables to describe the biological component of the vibroscape and vibrational community: VST richness (the number of different VSTs in the recording) (Desjonquères et al., 2015) and VST abundance (= time occupancy of vibrational space), calculated by summing up the durations of individual VSTs in the recording. Many VSTs were composed of repetitive pulses or units (e.g. Figure 2C) and when their duty cycle (the ratio between the pulse duration and signal duration) was less than 50%, we took into account individual pulses and not the overall duration of the signal. Contribution of each VST to the occupancy of the channel was expressed as a percentage. To estimate the richness of the vibrational community on each date, we used sample-based accumulation curves obtained with R package *vegan* (Oksanen et al., 2017; Desjonquères et al., 2015), where VST richness in each 10-min sound file of annotated 3-h sequence equals one sample. We used this method to calculate the expected VST richness because samples were successive in time (Oksanen, 2015).

Differences in vibroscape composition

To compare the vibroscape structure across seasons, we arbitrarily divided the recording dates into four periods: spring (May, 1, 2, 3); early summer: (June 1, 2 and July 1, 2, 3, 4), late summer (August 1, 2, 3, 4) and autumn (September 1, 2, October 1). We built two community matrices; the abundance matrix with values (cumulative durations) for each VST on each day, where for VSTs not recorded on a particular day, the abundance value was 0 (see Table S1) and the presence-absence matrix with values 1 (present) and 0 (absence). The effect of seasons on communities was estimated by exploring the first three dimensions of a non-metric multidimensional scaling analysis (nMDS). This reduction analysis was done in R environment with the package *vegan* (Oksanen et al., 2017).

nMDS was also used to compare the vibroscape structure recorded on *C. hirta* and *G. mollugo* (Table S3). Among many multivariate statistical methods, nMDS was chosen because it uses rank orders instead of distances, so it is more suitable for community matrices in which many 0 values appear (Oksanen, 2018).

VST overlap

To investigate how the members of a hay meadow vibrational community share vibrational communication space, we calculated VST overlap, both in time only and combined time-frequency domain in recordings obtained in early summer period, when the time occupancy of vibrational channel was high.

Simultaneous channel use in time domain was estimated by sum of overlapped signal time intervals, measured in seconds. Simultaneous channel use in time-frequency domain was estimated by the sum of overlapped time-frequency rectangles (see Figure S6), measured in arbitrary units. To test the hypothesis of coordinated partitioning of vibrational space, we compared observed overlaps with the distribution of overlaps obtained from 1000 simulations with permuted (randomized) signal onsets which mimics the null hypothesis situation of the non-coordinated use of vibrational space. Due to the intricacies of unknown coordination parameters, the permutation test outperform parametric testing.

For the recording of July 9, 2017 we used a similar protocol to calculate overlap between individual VSTs and expressed this value in percentage of each VST that is overlapped. For detection of a relation between the percentage overlaps in time and time-frequency domains, Mahalanobis prediction regions (Krzanowski, 1988) were constructed. Mahalanobis distance is a way of expressing the distance from the center of multivariate distribution and can detect cases that deviate more than expected. From this analysis we excluded VSTs C7, C16, T8 and T10, because these signals never overlapped with others and also their contribution to the time occupancy of vibrational channel was less than 30s during 180 min recording.