



# OPEN Spatial and temporal variation in vibroscape composition in two grassland habitats

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Substrate-borne vibrational signaling is one of the oldest and taxonomically widespread forms of animal communication. For many animals the natural vibrational environment (vibroscape) is an essential source of information underlying their behavioral decisions; however, the structure and dynamics of vibroscape composition of the natural habitats are largely unexplored. We studied vibroscape composition in a eutrophic lowland hay meadow and a sub-Mediterranean dry karst grassland. The results obtained by simultaneous, multi-channel recordings showed that vibroscape composition changes throughout the day and from day to day, differs between plant species, depends on the spatial position of the plant within the habitat and is also influenced by environmental parameters. The results highlight the unpredictability and high variability of the vibrational environment encountered by plant-dwelling insects, and the complexity in implementing biodiversity monitoring based on vibroscape composition.

**Keywords** Biotremology, Ecotremology, Vibroscape, Vibrational communication, Vibrational community, Environmental factors

Vibrational communication (i.e. communication by substrate-borne mechanical waves) is one of the oldest and most widespread forms of communication<sup>1–5</sup>. Vibrational behavior is prevalent in arthropods<sup>1,4–7</sup>, is common also in vertebrates<sup>8–11</sup> and is not only associated with intraspecific communication, but also with prey detection and predator avoidance<sup>12</sup>. The emergence of biotremology as a discipline studying vibrational behavior<sup>13,14</sup> increased the interest in this communication channel in a wider research community. However, our understanding of the evolutionary processes shaping vibrational behavior is limited by the lack of available information on the natural vibrational environment in which these behaviors take place.

Vibroscape has been defined as “the collection of biological, geophysical and anthropogenic vibrations emanating from a given environment creating unique vibrational patterns across a variety of spatial and temporal scales”<sup>15,16</sup>. Its importance as a source of essential information underlying arthropod behavioral decisions (e.g. mate finding, prey detection, predator avoidance) that are crucial for their reproduction and survival has only recently been recognized<sup>3,5,15–19</sup>. The structure and dynamics of the vibroscape composition of natural habitats are important, on the one hand to understand the selection pressures influencing the evolution of vibrational communication, and on the other hand to allow us to assess biodiversity and ecosystem processes<sup>20–23</sup>.

The vibroscape composition associated with the biological component reflects vibrational community (i.e. an association of vibrationally signaling species sharing the same habitat over a defined time)<sup>15,16</sup>. The selection pressures that arise within the vibrational community result from masking interference and the ‘cocktail-party problem’<sup>12,15,16,24</sup>. While it has been shown that members of a vibrational community avoid interference by short-term adjustment of signaling activity<sup>16,18</sup>, the extent of variation in the vibroscape composition experienced by plant-dwelling arthropods within the same habitat is unknown.

At the time when the critical insect decline has been universally recognized<sup>25–27</sup>, insect monitoring has been recognized as one of the priorities in biodiversity assessment<sup>21,28,29</sup>. While terrestrial acoustic monitoring includes only those insect groups that emit air-borne sounds (e.g. orthopterans and cicadas), studying the ecological significance of the vibroscape (i.e. ecotremology) offers a non-invasive approach to monitor most insects<sup>20,30</sup>. However, to date, the number of vibroscape studies focused on arthropod communities is low<sup>16–19</sup>, and we lack a basic understanding of vibroscape dynamics needed to develop an appropriate sampling design for ecotremological monitoring (e.g. diel and seasonal variations, the extent of spatial and temporal variation within the habitat).

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Here, we studied spatial and temporal variation of the vibroscape composition in two grassland habitats, a lowland eutrophic hay meadow and a sub-Mediterranean dry grassland. While plants are the most common substrate for vibrationally signaling insects<sup>4</sup>, grasslands are important terrestrial ecosystems in which insects are one of the dominant animal groups by number and species richness<sup>31</sup>. Our aims were (a) to investigate in each habitat the extent of variation in vibroscape composition among plants of different species growing in the sunny and shady areas of the meadow, and (b) to provide more detailed information on activity patterns within vibrational community. By addressing these fundamental aspects in biotremology and ecotremology research, our goal was to improve our understanding of the information available in the natural vibrational environment.

## Materials and methods

### Study sites

The field work was carried out in two different grassland habitats in Slovenia: a lowland eutrophic hay meadow in the Ljubljana Moors (Ljubljansko Barje) near Bistra (N 45°56'42.40"; E 14°20'09.21") and an eastern sub-Mediterranean dry karst grassland near Krkavče (N 45°28'37.30"; E 13°42'27.1"). The consent of the landowners was obtained in advance for work at these sites. We chose these sites, because of low levels of anthropogenic vibrational noise and because the meadow vegetation at both sites was not affected by agricultural practices. There is a local road with low-traffic (up to ten passing cars between 8:00 and 18:00) approximately 30 m from the edge of each site; although low amplitude traffic vibrational noise was occasionally discernible in the recordings, we did not observe any effect on signaling activity during supervising the recordings in the field.

In Bistra, the recordings were carried out on an area of around 300 m<sup>2</sup> on which there were seven mature high-trunk apple trees (*Malus domestica*). The meadow was mowed in the previous autumn. The site is surrounded by areas that are mowed more frequently, grazed by cattle or used as an allotment (Supplementary Fig. S1). None of the activities mentioned occurred during the recordings.

The study site in Krkavče was more than 400 m<sup>2</sup> in size and was surrounded by smoke bushes (*Cotinus coggygria*) and oaks (*Quercus pubescens*). As the meadow is a stony karst grassland, it is not mowed (Supplementary Fig. S1).

### Field recordings

Regarding the general practical and technical issues of vibroscape field recordings we followed the published guidelines<sup>15,16</sup> as described here below.

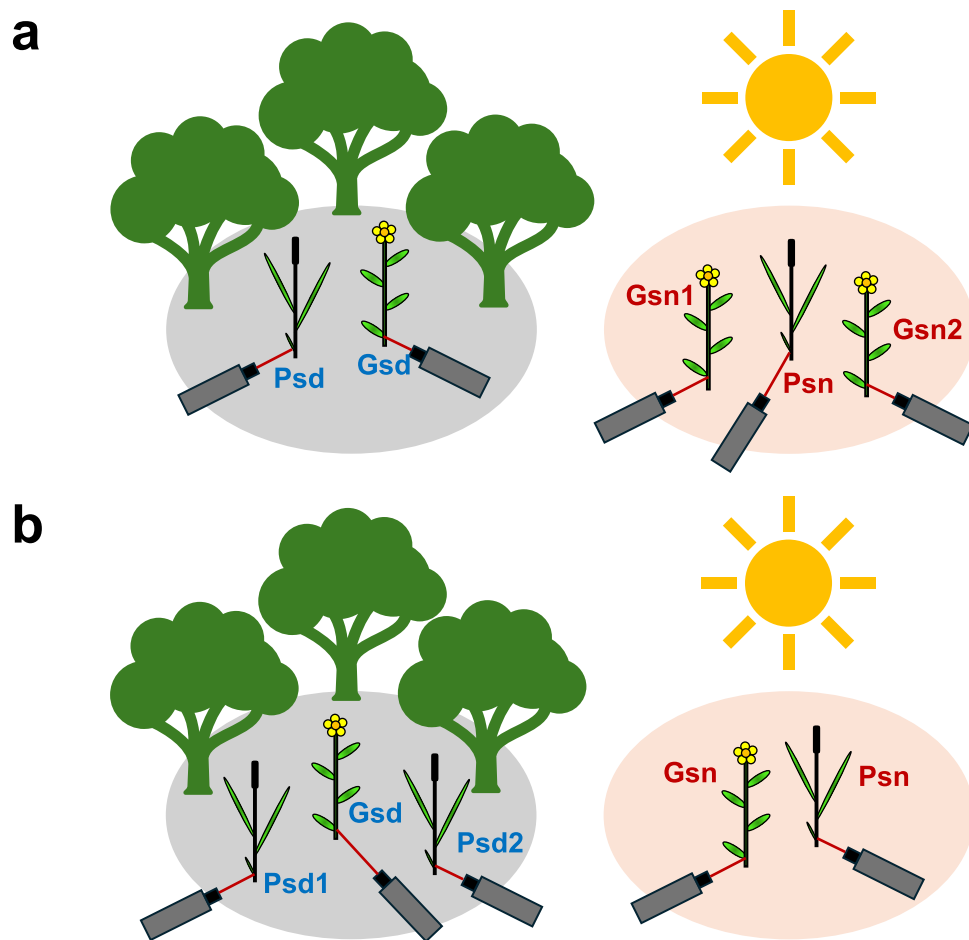
The vibroscape was recorded simultaneously with five portable laser Doppler vibrometers (five PDV 100 in Bistra and four PDV 100 and one VibroGo® in Krkavče, all Polytec, Germany). For all recordings, the vibrometers were set to 20 mm s<sup>-1</sup> V<sup>-1</sup>. The recordings were digitized and stored in a laptop using a multi-channel field recorder (Zoom f8n) (Zoom, USA) and Raven Pro 1.4 software (Cornell Lab of Ornithology, Ithaca, USA) with 44,100 Hz sampling rate and 16-bit resolution. The recorded files were automatically saved in WAV format in Bistra and Krkavče every 8 and 5 min, respectively. To avoid overheating, the PDV 100 laser vibrometers were preferably placed in a tree shade, or if this was not possible, under a 3 × 3 m portable gazebo, while the VibroGo® did not need a protection from direct sun exposure. The recording station with a multi-channel recorder, a laptop and batteries was also set-up in the tree shade or under a gazebo.

The site in Bistra has access to the power grid and the equipment was connected directly to the power grid via an extension cable. In Krkavče, the equipment was powered by a battery (Ritar DG12-100 gel rechargeable battery, Hengyang Ritar Power Co., LTD.). A full charge of this battery allowed complete autonomy for nine hours, and during the 10-h recordings we had to switch to another battery of the same brand. The Polytec VibroGo® laser vibrometer has its own portable battery that can power the laser for 5 h, after which the vibrometer was connected to the battery.

During each recording session, we also recorded the ambient temperature and wind velocity at the site. We used the Almemo 2590 data logger system with the FHAD46-C2 digital sensor (both Ahlborn GmbH, Germany) for temperature (range -20 to +60 °C with minimum accuracy ±0.7 °C) in the 'Sun' area of the meadow (see below). To simultaneously record the temperature in the 'Shade' area, we used a HOBO data logger (range -20 to +70 °C with minimum accuracy ±0.5 °C) (Onset Computer Corp., Pocasset, MA). Both temperature sensors were placed on the ground in the vegetation between the recorded plants in each area. To record wind speed, we used the Almemo 2590 data logger system with the FVA615 2 digital sensor (Ahlborn GmbH, Germany). The sensor was placed 85 cm above the ground in the sunny area but in the middle between two recording areas. The data was automatically stored every 10 s (Supplementary Table S1).

The recordings in Bistra were carried out from July 9 to July 13, 2022, while recordings in Krkavče took place between July 15 and July 17, 2023. In both locations, we attempted to conduct vibroscape recordings on three consecutive days; however, due to rain and strong wind, we were unable to carry out the recordings in Bistra on July 10 and 11. On each day, recordings started at 8:00 and continued until 18:00.

Within the sites, we selected two recording areas (Fig. 1). The 'Sun' area was exposed to the sun during the entire recording period, while the 'Shade' area was under the tree canopy. In each of these areas, the vibroscape was recorded from hedge bedstraw (*Gallium mollugo*) and grass (Poaceae). We chose these plants because they were found at both sites and in both recording areas (see below). The 'Sun' and 'Shade' areas were more than 3 m apart, while the individual plants in these areas were more than 35 cm apart to avoid recording the same signal from two positions simultaneously. In Bistra, we included two *Gallium* plants (Gsn1, Gsn2) and one grass (Psn) in the recordings in the 'Sun' area, while in the 'Shade' the recordings were carried out on one *Gallium* (Gsd) and one grass (Psd). Although we intended to duplicate Bistra's recording arrangement in Krkavče, the extreme heat wave limited the availability of *Gallium* plants at the site. Therefore, in the 'Sun' area we recorded from two plants, a *Gallium* (Gsn) and a grass (Psn). The 'Shade' area included two grass plants (Psd1, Psd2) and one *Gallium* (Gsd). Additional information on the plants is provided in the Supplementary Table S2. Since the



**Fig. 1.** Schematic presentation of the vibroscope recording set-up in the ‘Sun’ and ‘Shade’ areas in (a) Bistra and (b) Krkavče. Gsn – *Gallium* plants growing in the ‘Sun’ area, Psn – *Poaceae* plants growing in the ‘Sun’ area. Gsd – *Gallium* plants growing in the ‘Shade’ area, Psd – *Poaceae* plants growing in the ‘Shade’ area.

vegetation in the grassland is dense and interconnected, all plants were also connected to neighboring plants via touching leaves and stems.

Because the top of the plant is more exposed to the wind, we recorded the vibroscope closer to the ground to ensure stable recordings<sup>15,16</sup>. We focused the laser beam on a small piece of reflective tape attached to a plant stem to increase and thus improve the signal-to-noise ratio.

### Quantification and statistical analysis

First, we scanned and manually annotated the vibrational events associated with animal vibrational communication (e.g. vibrational signals) in all recordings (300 h = 2 sites, 3 days, 5 plants, 10 h per day). We listened to the recordings as many times as necessary and visualized them simultaneously as spectrograms in Raven 1.6<sup>16</sup>. In total, we annotated 18,000 vibrational events. To facilitate the comparisons, we excluded the periods of high amplitude vibrational noise induced by wind and periods of equipment malfunction (e.g. overheating, power failure). Consequently, we took into account in all recordings a continuous 464-min interval from 08:40 to 16:24 in Bistra (i.e. 58 8-min intervals) and a 440-min interval from 10:30 to 17:50 in Krkavče (i.e. 88 5-min intervals), giving a total of 116 h and 110 h for Bistra and Krkavče, respectively. Within these time periods, we annotated 14,700 vibrational events, 12,500 in Bistra and 2200 in Krkavče.

We assigned all annotated signals to vibrational signal types (VST) and one of four general categories based on their distinct temporal and spectral properties: pulse (P), harmonic signals (F), train (T) or complex (C)<sup>16,30</sup> (Supplementary Fig. S2). Vibrational signals used in communication are species- and sex-specific; however, in the absence of reference libraries, the majority of recorded signals cannot be identified to the species level<sup>15–18,20,30</sup>. The main criteria we relied on for the VST assignment are the general signal structure and composition as well as the temporal and spectral characteristics. To ensure consistent identification and classification, we cross-referenced the VSTs with the existing library of vibrational signals at the National Institute of Biology (Ljubljana, Slovenia) that contains multiple examples of each VST recorded in the field (including at different locations when available) and in the laboratory (when the species was identified). The signals that had not been previously recorded were integrated into the library for future reference. The VST assignment is a consensus based on currently available information.

From the sections of annotated 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 chosen period)<sup>15,16</sup>, and VST abundance (= temporal occupancy of the vibroscape), calculated by summing the durations of individual VSTs in the recorded files<sup>16</sup>. The 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 plant, we used sample-based accumulation curves obtained with R package *vegan*<sup>16,32</sup>, where the VST richness in each recorded file (8 and 5 min in Bistra and Krkavče, respectively) included in the analyses corresponds to one sample.

Statistical analyses were carried out using R version 4.0.2<sup>33</sup> within the RStudio interface<sup>34</sup>. Data visualization was performed using the “ggplot2” package<sup>35</sup>.

Differences in vibroscape structure can result from differences in VST composition and VST richness, as well as from differences in the abundance of these VSTs. To compare vibroscape structure across days, plants and ‘Sun’ and ‘Shade’ areas, we composed two community matrices: the abundance matrix with values (cumulative durations) for each VST on each day, where the abundance value was 0 for VSTs that were not recorded on a given day (Supplementary Tables S3, S5), and the presence-absence matrix with values 1 (present) and 0 (absent). Differences/similarities were 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*<sup>16,32</sup>. For statistical assessment we applied Analysis of similarities (ANOSIM) using Bray–Curtis distances to test whether differences between groups were statistically significant<sup>36–38</sup>.

To model the relationship between VST abundance and environmental variables, we used a Tweedie generalized linear model (GLM) with a log-link function using the “glmmTMB” package<sup>39</sup>. We refined the model using backward selection.

$$VST\ abundance \sim Area * poly(Temperature, 2) + Area * Wind + VST\ Richness$$

Fixed effects in the model included “area” (categorical variable with levels “Shade” and “Sun”), “temperature”, “wind”, and “VST richness”. We included interaction terms between “area” and both, “temperature” and “wind” to examine whether the effects of these environmental variables differed between sunny and shady conditions.

As it has been previously shown that high temperatures have negative effect on the vibrational signaling, indicating a nonlinear relationship<sup>17,40,41</sup>, we included a second-degree polynomial term for “temperature” using the  $poly(Temperature, 2)$  function. This function generates orthogonal polynomials, which are uncorrelated with each other.  $Poly(Temperature, 2)$ , breaks the temperature into two components, a primary (“linear”) effect and a secondary (“quadratic”) effect. This allowed us to better model the predicted curvilinear relationship between temperature and VST abundance.

Model selection was guided by the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC), comparing models with and without interaction terms and polynomial effects. The final model was selected based on the lowest AIC and BIC values, indicating the best balance between model fit and complexity. The inclusion of the polynomial term for “temperature” and its interaction with “area” significantly improved the model.

Before fitting the final model, we checked for multicollinearity among predictors by calculating the variance inflation factors (VIFs). Variables with high VIFs were scrutinized, but none exceeded the acceptable threshold, so all were retained in the model. Model assumptions were verified using diagnostic plots generated with the “DHARMa” package<sup>42</sup>, assessing for residual patterns, overdispersion, and zero-inflation.

The VST richness and abundance in the vibroscape recorded in Krkavče were too low to allow detailed analyses of daily vibroscape dynamics (see Results).

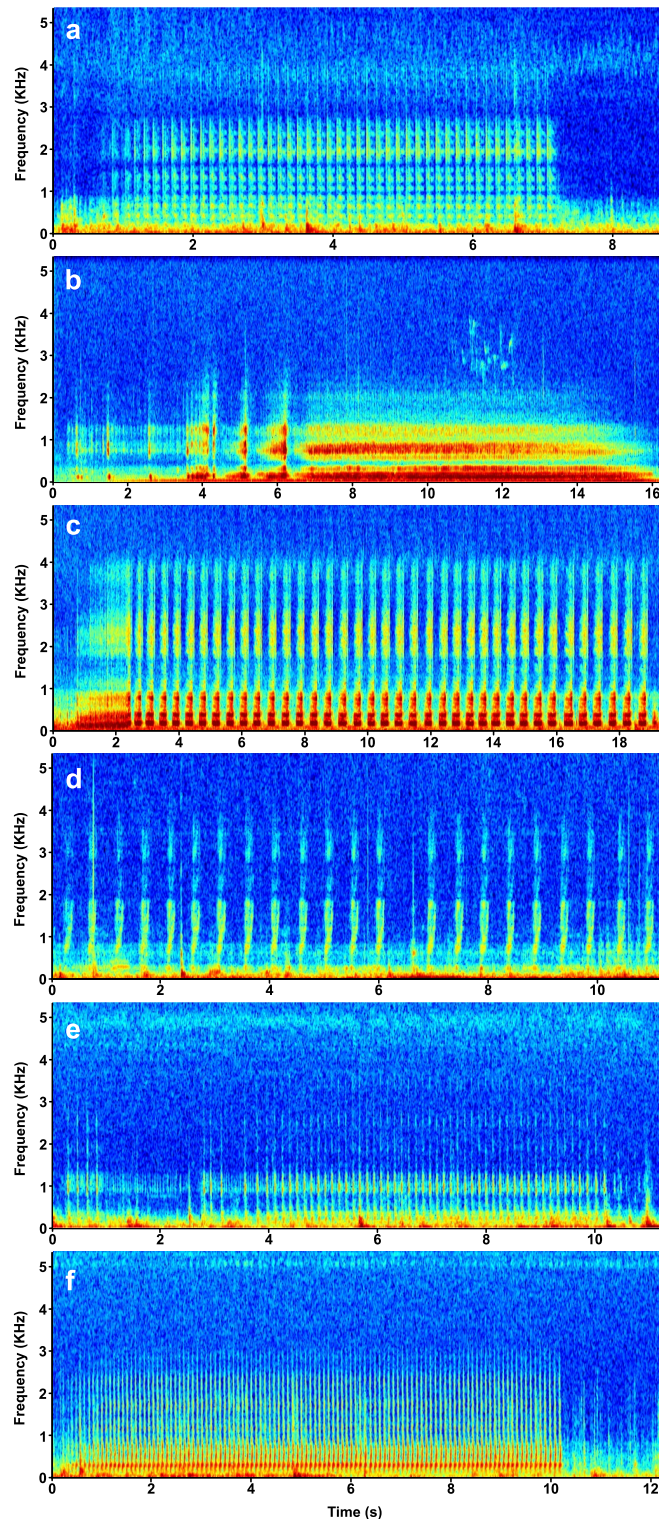
## Results

### Lowland eutrophic hay meadow in Bistra

In general, the vibroscape recorded in Bistra was rich in species-specific vibrational signals (Supplementary Table S2). Within the 8-min recorded files we determined up to 6 different VSTs. Over a period of three days, we encountered 38 VSTs; however, we could reliably identify only the vibrational signals of 13 hemipteran species: *Philaenus spumarius* (Aphrophoridae), *Anoscopus serratulae* (Cicadellidae), *Anoscopus albiger* (Cicadellidae), *Aphrodes makarovi* (Cicadellidae), *Aphrodes bicincta* Dragonja (Cicadellidae), *Graphocraerus ventralis* (Cicadellidae), *Orientus ishidae* (Cicadellidae), *Megophthalmus scanicus* (Cicadellidae), *Streptanus aemulans* (Cicadellidae), *Cicadella viridis* (Cicadellidae), *Euscelis incisus* (Cicadellidae), *Dicranotropis hamata* (Delphacidae), *Graphosoma italicum* (Pentatomidae). One VST was also attributed to the vibrational component of a song of an unknown grasshopper species. Most VSTs were assigned to train and complex categories (19 and 12 VSTs, respectively), while harmonic and pulse VSTs were rare (4 and 3 VSTs, respectively) (Supplementary Table S3).

According to VST abundance the vibroscape was dominated, regardless of the plant species and recording area, by male calls of the three leafhopper species, *A. serratulae* (VST C2), *A. makarovi* (VST C1) and *A. bicincta* Dragonja (VST T1), which contributed 49.5%, 24.5% and 10%, respectively, to the overall time occupancy of the vibroscape (Fig. 2a–c; Supplementary Table S3). Vibrational signals of these three species were dominant on all individual plants, where their cumulative overall abundance ranged between 53 and 92.5%. In particular, on the plants in the shade the overall abundance of vibrational signals of *A. serratulae* reached 52.7% on grass and 63.5% on *Gallium*. Nevertheless, the vibroscape recorded on a single plant included on average 16 VSTs (SD = ± 2) each day, while the richness varied between 23 and 27 VSTs in a three-day period (Supplementary Table S4). On a single day, VST richness on individual plants reached between 50 and 76% of the total daily VST richness recorded, while the richness over a three-day period varied between 60 and 71% (Supplementary Table S4). The





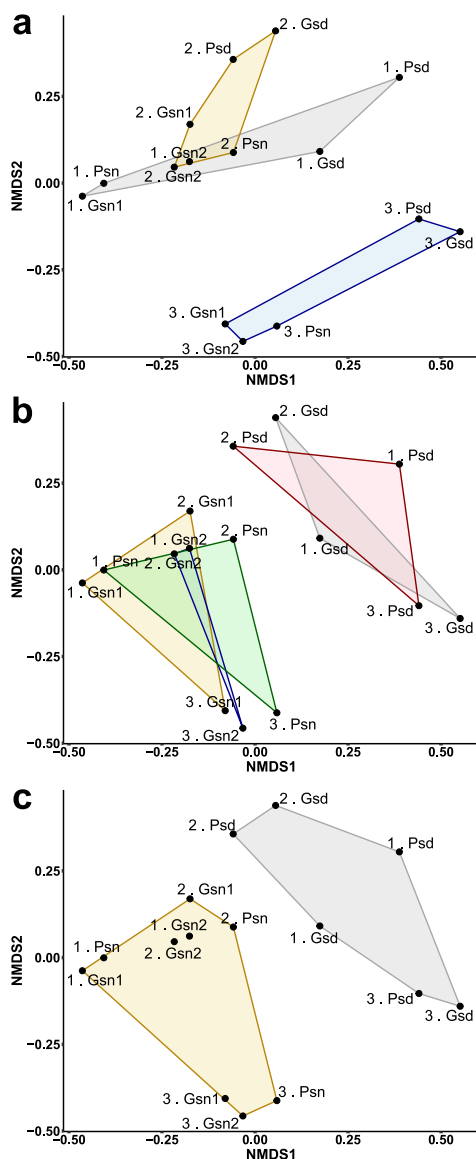
**Fig. 2.** Spectrograms of the representative examples of dominant VSTs encountered in Bistra (**a–d**) and Krkavče (**e,f**). (**a**) *Anoscopus serratulae*; (**b**) *Aphrodes makarovi*; (**c**) *Aphrodes bicincta* Dragonja, (**d**) VST T40, the fourth most abundant vibrational signal in Bistra; (**e**) VST T51; (**f**) VST T16. The latter three VSTs were emitted by unknown species. All examples were recorded in the field and spectrograms were generated in Raven pro 1.6 with Hamming window of 1027 samples with 50% overlap.

calculated VST richness on individual plants never reached the plateau, neither on one day nor in the three-day recording period (Supplementary Figs. S3, S4).

However, looking at the number of 8-min intervals in which the individual VSTs were recorded, reveals different dominance patterns (Supplementary Fig. S5). On plants in the shade, only the male advertisement calls of *A. serratulae* consistently dominated also by their presence on each day, whereas the male calls of *A. bicincta* Dragonja and VST T40 (Fig. 2d) were consistently present in fewer than half of the intervals. On plants growing in the sun, the four most abundant vibrational signals only rarely dominated also by their presence throughout the day.

The vibroscape composition differed significantly between the recording days when the VST richness and composition were taken into account (NMDS, ANOSIM,  $p = 0.0006$ ) (Fig. 3a, Supplementary Fig. S6). On each day, more than half of the VSTs (52–60%) were recorded in both areas, and more than 75% of the VSTs were found on both plant species (Supplementary Table S3).

While differences in VST composition and VST richness did not reveal significant differences between plants (NMDS, ANOSIM,  $p = 0.125$ ) (Fig. 3b), the vibroscape properties differed significantly also between plants when VST abundance was included, (NMDS, ANOSIM,  $p = 0.0017$ ) (Supplementary Fig. S6). Looking at the individual plants, the recorded vibroscape was characterized by high VST turnover. Only about one third (33.3–39.1%) of



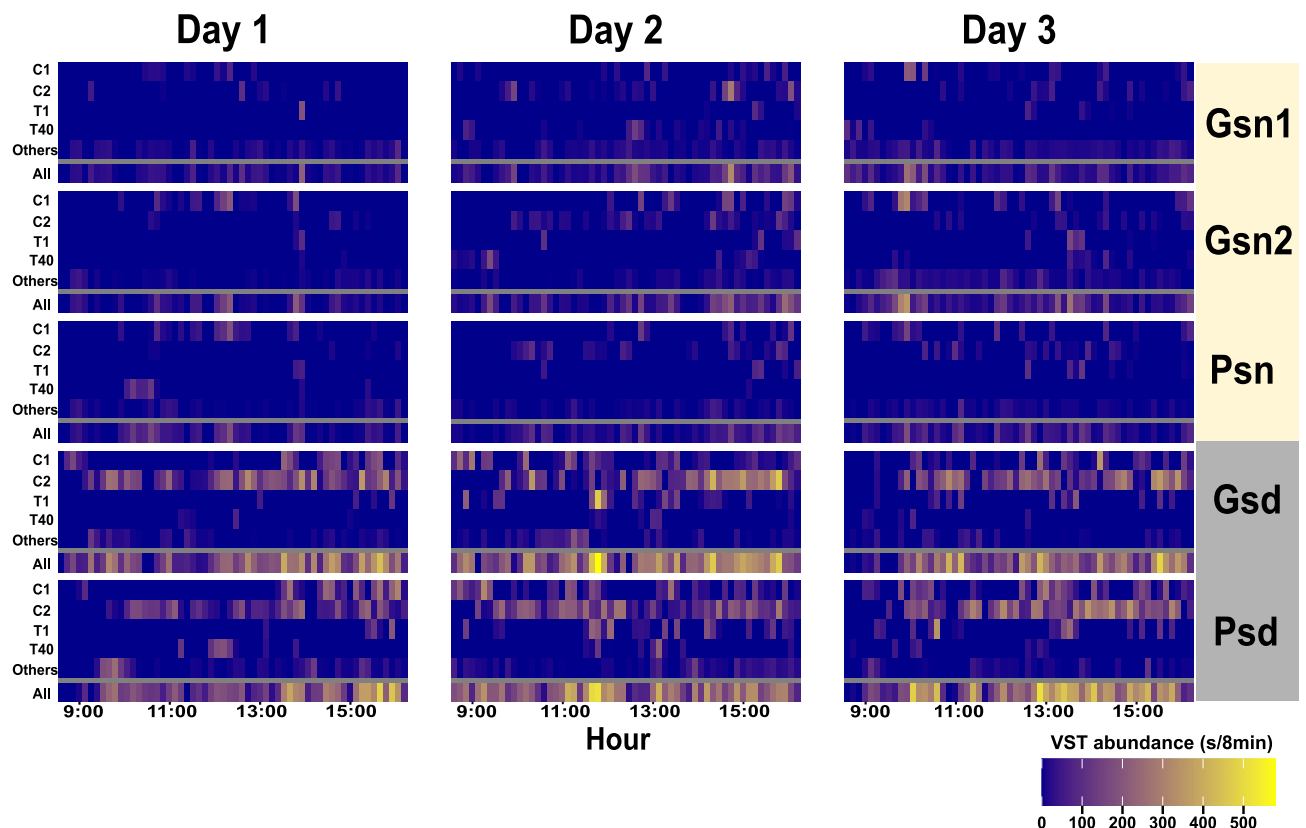
**Fig. 3.** Differences in vibroscape composition in Bistra between days (a), plants (b) and ‘Sun’ and ‘Shade’ areas (c). Nonmetric multidimensional scaling (NMDS) analysis based on VST presence-absence matrix (Supplementary Table S3). (a)  $k = 3$ , stress value = 0.076,  $p = 0.0006$ ; (b)  $k = 3$ , stress value = 0.076,  $p = 0.125$ ; (c)  $k = 3$ , stress value = 0.076,  $p = 0.0007$ . Gsn1, Gsn2 – *Gallium* plants growing the sunny area; Psn – grass plant growing in the sunny area; Gsd – *Gallium* plant growing the shade area; Psd – grass plant growing in the shade area. The numbers 1, 2 and 3 in front of the plant code indicate the recording day.

VSTs were found on the same plant on all three days, and between 42 and 48% of VSTs were recorded only on one day. Eight VSTs were recorded only on one plant species; however, with one exception, these VSTs were found on only one plant on a single day (Supplementary Table S3).

We observed large differences in vibroscape composition between ‘Sun’ and ‘Shade’ areas (NMDS,  $p = 0.0007$ ;  $p = 0.0003$ ) (Fig. 3c, Supplementary Fig. S6), which were due to a significantly higher abundance of vibrations on plants growing in shade and VSTs that occurred only in one area (Supplementary Fig. S7 Supplementary Table S3). Six and seven VSTs were recorded only in the ‘Sun’ or ‘Shade’ area, respectively. Two VSTs, T10 and C16, the former of which was assigned to a grasshopper song, were consistently associated with the sunny area on all three days, whereas most of the area-specific VSTs were recorded on only one plant on a single day (Supplementary Table S3). Male calls of *A. serratulae* contributed most to the high VST abundance on the shade-growing plants (Fig. 4, Supplementary Table S3).

The time occupancy of the vibroscape and the abundance of individual VSTs were fluctuating throughout the day (Fig. 4). The dominance in the abundance of male calls of *A. makarovi*, *A. bicincta* Dragoja and *A. serratulae* on the plants growing in the sunny area resulted from prominent activity peaks of these VSTs that occurred seemingly randomly throughout the day. In these plants, the peaks in the time occupancy of the vibroscape were often associated with an activity peak of a single dominant VST. In contrast, in the shaded area, the abundance of *A. serratulae* vibrational signals was high throughout the day and the individual peaks of dominant VSTs were often clustered together, although their peaks rarely overlapped within the same 8 min recording intervals. Despite VST dominance, abundance was positively correlated with the VST richness (Table 1, Supplementary Figs. S8–S12).

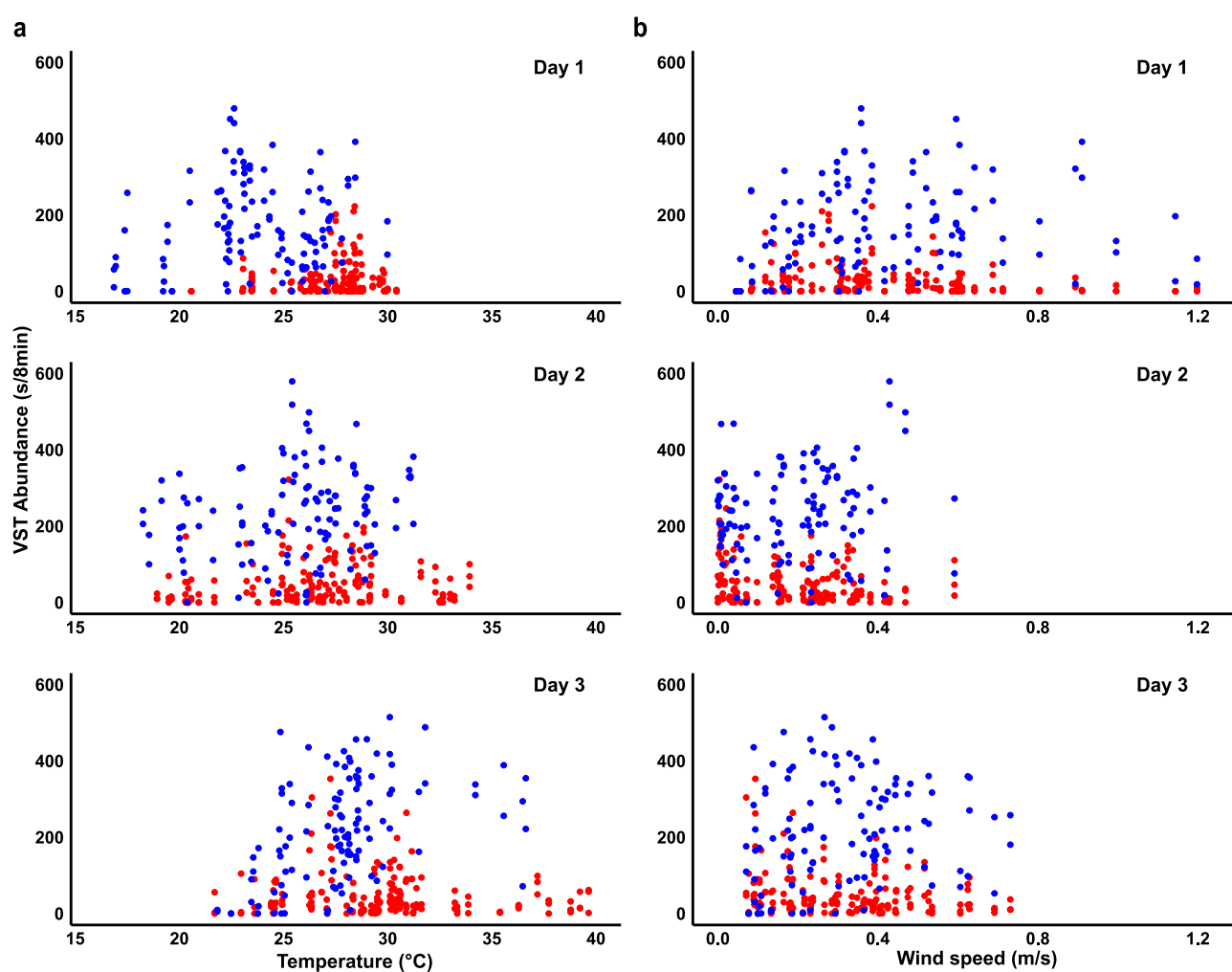
The VST abundance was significantly influenced by environmental parameters, which differed from day to day and between the ‘Sun’ and ‘Shade’ areas (Fig. 5, Table 1, Supplementary Fig. S13, Supplementary Table 1). Up to 30 °C, ambient temperature had a significant positive effect on signaling activity in both areas, while VST abundance decreased at higher temperatures in the afternoon of the second and third day, with the effect on signaling activity being stronger on the plants in the sunny area (Fig. 5a, Table 1, Supplementary Figs. S8–S12). For all plants, VST abundance was negatively influenced by wind speeds above 0.4 m/s, which occurred in the afternoon of the first and third day (Fig. 5b, Supplementary Figs. S8–S12, S13). As with temperature, a stronger negative effect of the wind speed on signaling activity was observed for plants in the sunny area. As the negative effects of environmental parameters were mainly observed in the afternoon, the general daily activity pattern



**Fig. 4.** Daily dynamics of the vibroscape composition on plants in Bistra. Variation in cumulative VST abundance (All) and abundance of the four dominant VSTs in 8-min intervals. C1—*Aphrodes makarovi*, C2—*Anoscopus serratula*, T1—*Aphrodes bicincta* Dragonja, T40—unknown species. Gsn1, Gsn2 – *Gallium* plants growing the sunny area; Psn – grass plant growing in the sunny area; Gsd—*Gallium* plant growing the shade area; Psd – grass plant growing in the shade area.

	Estimate	Std. error	z value	p-value
Intercept	4.607	0.089	51.64	<0.001
Area	−1.144	0.101	−11.38	<0.001
VST richness	0.297	0.022	16.60	<0.001
Poly (Temp, 2) <sup>&amp;</sup>	5.491	1.191	4.61	<0.001
Poly (Temp, 2) <sup>#</sup>	−0.453	1.256	−0.36	0.718
Wind	−0.026	0.169	−0.15	0.879
Area*poly (Temperature, 2)1	−0.549	2.124	−0.26	0.796
Area*poly (Temperature, 2)2	−6.651	1.900	−3.50	<0.001
Area*wind	−1.936	0.308	−6.29	<0.001

**Table 1.** Estimated regression parameters, standard errors, z and p values for the Tweedie GLM testing the effect of VST richness, recording areas, temperature and wind speed. <sup>&</sup>Poly(Temperature, 2)1 – linear predictor of interaction between temperature and VST abundance. <sup>#</sup>Poly(Temperature, 2)2 – quadratic predictor of interaction between temperature and VST abundance.



**Fig. 5.** Scatterplots showing the effect of ambient temperature (a) and wind speed (b) on VST abundance on plants in the 'Sun' area (red dots) and 'Shade' area (blue dots) in Bistra. VST abundance is shown as a cumulative abundance in 8-min intervals, while temperature and wind speed are represented as average values in those intervals.



differed between the plants in the two areas. On both plants in the shade, the VST abundance was significantly higher in the afternoon (e.g., after 12:30), than in the morning, whereas we did not observe such a difference in signaling activity in the sunny area (Supplementary Fig. S14).

### Karst grassland in Krkavče

In contrast to the vibroscape in the eutrophic lowland meadow in Bistra, the VST richness and abundance in the Karst stony grassland was low (Supplementary Table S5). The main reason for this may be the extremely high temperature during the field recordings (see Discussion). Ambient temperatures registered at Krkavče regularly exceeded 40 °C, reaching values up to 46°C and 41°C in the sunny and shady areas, respectively, and strong wind gusts (> 3 m/s) also occurred on each day (Supplementary Table S1). Consequently, the VST richness and abundance were too low to allow a meaningful, more detailed analysis of the daily dynamics of vibroscape composition.

Over a three-day period, we recorded only 13 VSTs, and the cumulative three-day abundance was almost 15 times lower than in Bistra (Supplementary Tables S3, S5). On any given day, the vibroscape recorded on a single plant contained on average only 4 VSTs ( $SD = \pm 2$ ), while over a three-day period the richness on individual plants varied ranged from 5 to 10 VSTs (Supplementary Table S4). None of the three VSTs dominant in Bistra were present in Krkavče, and of the seven VSTs found at both sites, two were attributed to *P. spumarius* and *G. italicum* (Supplementary Table S5). We were unable to identify other VSTs recorded. As in Bistra, most VSTs were assigned to the train and complex categories (6 and 5 VSTs, respectively), while we registered one VST each for the harmonic and pulse category. On individual days, the calculated VST richness approached or even reached the plateau on individual plants (Supplementary Fig. S15); however, we also registered VSTs on the third day that had not previously occurred (Supplementary Table S5). VST richness was noticeably lower on the plants growing in the sunny area.

Two dominant vibrational signals in Krkavče were assigned to VSTs T51 and T16, the latter of which was also recorded in Bistra (Fig. 2e, f), contributing overall 53.7 and 22.9% to VST abundance, respectively (Supplementary Table S3). VST T51 was dominant on plants growing in shade, where its overall cumulative abundance ranged from 50.8 to 78.6%, while VST T16 was dominant in the sunny area, where its overall cumulative abundance was 67.9% and 90.9% on Psn and Gsn, respectively.

As in Bistra, the vibroscape properties in Krkavče differed significantly between plants (NMDS, ANOSIM,  $p = 0.004$ ) (Fig. 6b, Suppl. Fig. S16) and the sunny and shade area (NMDS, ANOSIM,  $p = 0.0003$ ) (Fig. 6c, Supplementary Fig. S16); however, in contrast to Bistra, the results did not show differences between recordings days (NMDS, ANOSIM,  $p = 0.944$ ) (Fig. 6a, Supplementary Fig. S16). Higher VST richness was found on all plants growing in the shade; however, the VST abundance was higher on the grasses and lower on *Gallium* than on the plants of the same species growing in the sunny area (Supplementary Table S5). Seven VSTs were encountered in only one area, most of them on only one plant on a single day (Supplementary Table S5). Among the six VSTs recorded only in the shade were also vibrational signals of *P. spumarius* and *G. italicum*.

## Discussion

The results of our study highlight the extent and importance of spatial and temporal variation in vibroscape composition in a meadow habitat, as well as the effects of environmental parameters on signaling activity. As shown in previous vibroscape studies<sup>15–18</sup>, the vibroscape was rich in species-specific vibrational signals, and the screening of more than 7 h of continuous daily recordings provided detailed information on activity patterns within the vibrational community. Our study demonstrates the unpredictability and high variability of grassland vibroscape. The vibroscape detected on the plant by a receiver (an insect or a sensor used in ecotremological monitoring) depends on the temporal occurrence of vibrational signals within the vibroscape and on the spatial position of the plant in the habitat and is also influenced by the microclimate.

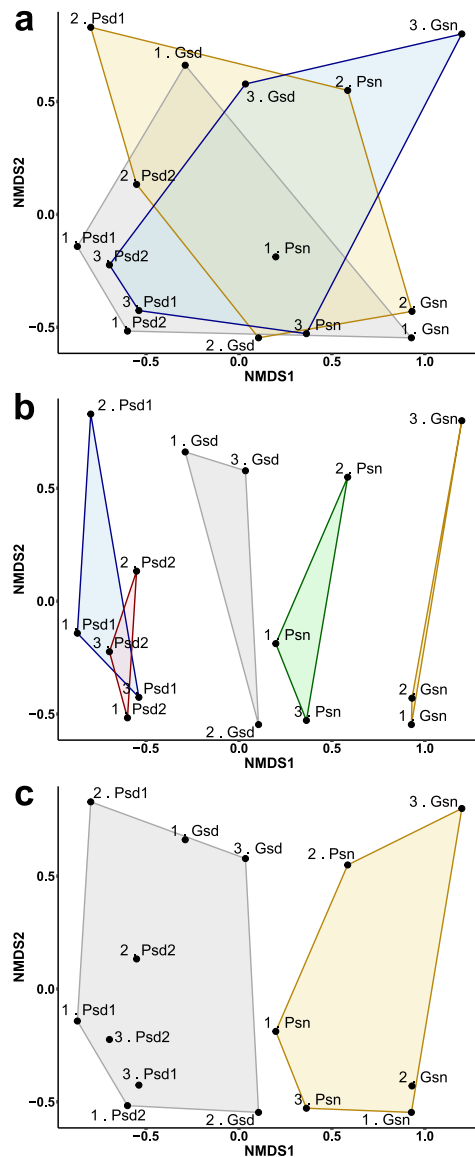
### Variation in vibroscape composition

Our results show that plant-dwelling insects living in a meadow encounter unpredictable and highly variable vibrational environment, in which the vibroscape composition changes throughout the day and from day to day, differs between plant species and also depends on the spatial position of the plant within the meadow.

The vibroscape complexity results on the one hand from the complexity of the habitat itself, which contains dense vegetation of randomly distributed plants with different geometries and heterogeneous plant substrates. In such a habitat, the area where the amplitude of vibrational signals is above the detection threshold extends beyond the plant on which an insect is calling<sup>43,44</sup>, however, the size and shape of the active space is unpredictable<sup>1,15,16,20</sup>. As shown in the present study, grasslands also include areas with different microclimates, which also affect signaling activity (see discussion below).

On the other hand, the vibroscape complexity arises from the random spatial distribution of the signalers within the meadow, as well as spatial and temporal unpredictability of the occurrence of the VSTs within the vibroscape. The vibroscape in Bistra was characterized by prominent peaks in the abundance of male advertisement calls of the dominant species; however, we did not observe consistent daily species-specific patterns of signaling activity. The current study did not include continuous 24-h recordings, which may be necessary to reveal such patterns. A pilot analysis of such recordings showed that the peak daily abundance of male advertisement calls of *A. makarovi*, *A. bicincta* Dragonja and *A. serratulae* was covered in the present study, whereas *A. albiger* (VST C6) which was only rarely encountered in our recordings, appears to be active mainly during the night and around sunrise and sunset<sup>30</sup>.

The abundance peaks of individual VSTs appearing randomly on the plants are likely associated with mate searching behavior of many plant-dwelling insects in a meadow vibrational community. When searching for the female, males move randomly through the habitat and emit advertisement calls from different positions to



**Fig. 6.** Differences in vibroscape composition in Krkavče between days (a), plants (b) and ‘Sun’ and ‘Shade’ areas (c). NMDS analysis based on VST abundance matrix (Supplementary Table S5). (a)  $k=3$ , stress value = 0.078,  $p=0.944$ ; (b)  $k=3$ , stress value = 0.078,  $p=0.0004$ . Partial overlap between the vibroscape composition is observed only between both grass plants growing in the shade. (c)  $k=3$ , stress value = 0.078,  $p=0.0003$ . Gsn—*Gallium* plant growing the sunny area; Psn – grass plant growing in the sunny area, Gsd—*Gallium* plant growing the shade area; Psd1, Psd2 – grass plants growing in the shade area. The numbers 1, 2 and 3 in front of the plant code indicate the recording day.

trigger a reply from stationary females (i.e. ‘call-fly’ strategy)<sup>45–47</sup>. Although individual species may be strictly host plant specific, their signals are not restricted to their host plants, as they are transmitted to neighboring plants.

Our results suggest that the individual experience of the vibrational environment of plant-dwelling insects living in grasslands may also depend on their sex-specific mate searching behavior. While a stationary female perching on the plant during recordings would experience the daily VST richness and abundance as determined in this study (i.e. 13–19 VSTs per recording daily period), a mobile male moving from one plant to another might at each position primarily encounter the dominant VSTs, especially if the presence and abundance dominance of these vibrational signals is high throughout the day. Information on the movement of individual insects within the grassland habitat during mate search is lacking and we do not know the size of the area that males include in their ‘call-fly’ search, whether they revisit the same plant during the day, or whether they potentially establish the signaling territories.

Although the analyses for Krkavče revealed differences in vibroscape composition between plants, a more detailed interpretation is difficult due to the low VST richness and abundance at this site. At present, it is not

clear, whether such low vibroscape complexity is characteristic of a karst grassland in midsummer and to what extent it was affected by the extreme environmental conditions encountered during the recordings.

### The influence of environmental parameters

Our results show that signaling activity in grassland is strongly influenced by environmental parameters. In particular, high ambient temperatures above 35 °C had negative effect on VST abundance. Temperature affects mating activity associated with the emission of vibrational signals<sup>41</sup>. Male signaling activity generally peaks at intermediate temperatures because males take on the costs of energetically demanding production of advertising signals, as well as energetic costs linked to locomotion associated with locating the stationary female<sup>17,40,41,48–51</sup>. Such activity pattern was observed also in Bistra, where in dominant VSTs the peak abundance associated with the emission of male advertisement calls was predominantly observed at temperatures between 22 and 30 °C. In contrast, ambient temperatures in Krkavče were most likely high enough to induce lethal heat stress in plant-dwelling insects<sup>49</sup>.

The local temperatures experienced by the insects perching on the plants were most likely different from the registered ambient temperatures<sup>49</sup>. In the vegetation of the rich eutrophic meadow, plants with different heights and very different geometries grow close together and are intertwined, creating many micro-areas with different microclimates. Microclimate differences are likely to be more extreme in the sunny areas, where the leaves and stems at the top of the plants are constantly exposed to the sun, while the areas close to the ground are protected from a direct sun exposure by overlapping leaves and stems. The signaling males could escape heat stress either by moving closer to the ground to the areas with lower temperatures, and also higher humidity, or by moving to a shady area or shifting their activity to the night period, when temperatures may be more favorable. In addition to the lethal temperatures, the dry vegetation in Krkavče could also provide a nutritionally low-quality habitat for plant-dwelling insects, and combination of these two factors could lead to insect escape from this habitat.

Besides temperature, vibrational noise induced by wind is another important environmental parameter affecting vibrational signaling<sup>1,17,24,52–55</sup>. In both grassland habitats included in the current study, wind-induced vibrations contributed near constant vibrational noise to the vibroscape and the recordings over the time frame used here showed no predictable daily pattern of wind speed. Instead, wind conditions varied substantially from day to day and showed unpredictable fluctuations over shorter time periods. Consistent with other field and laboratory studies<sup>52–55</sup>, signaling activity in Bistra was low during the time intervals including wind gusts of higher velocities. Our results show that the negative effect of wind is not prominent in the 'Shade' area, most likely because it was shielded from wind by trees. However, in addition to wind speed, the wind induced noise experienced by the plant-dwelling insects in the grasslands depends also on the height and geometry of the resident plant, its connections and anchoring with neighboring plants, and the position of the insect on the plant. The signalers perching at the top of a tall, isolated plant or at the bottom of a short plant growing in a dense thicket would most likely perceive wind induced noise of different amplitudes and spectral characteristics.

Avoiding signaling at times when interference from abiotic or biotic vibrational noise is high appears to be a common strategy to increase the probability of successful communication<sup>1,16,17,19,24,52–56</sup>. It has been suggested that insects could avoid noise interference also by moving to the wind protected areas<sup>53,54</sup>. In general, our study shows that the vibroscape composition on individual plants is crucially influenced by their spatial position within the habitat, and suggests that in grasslands the permanently shaded areas provide more favorable microclimate conditions for plant-dwelling insects by protecting them from heat stress and wind-induced vibrational noise. However, we do not know whether the higher VST abundance registered in shade is also associated with a higher insect density in this area. To gain a more comprehensive insight into the effects of abiotic factors on vibroscape composition, future studies should also include areas where plants are exposed to the sun only for a limited time of the day.

### Characterization of the vibrational community

The grassland vibrational communities include numerous arthropod species, especially from the hemipteran groups Auchenorrhyncha (e.g. leafhoppers, planthoppers, treehoppers, spittlebugs) and Heteroptera (e.g. shield bugs, plant bugs, leaf-footed bugs). In Bistra, we were able to identify one third of the species in the registered vibrational community, and substantially less in Krkavče, despite the low VST richness at this site. The characterization of the entire meadow vibrational community is not yet feasible. The main problem is the lack of a comprehensive, verified public library of vibrational signals, which prevents the identification of signals not encountered previously<sup>15–18,20</sup>. Although the VSTs included in our own signal library at the National Institute of Biology allowed us to assign the recorded VSTs to spittlebug, leafhopper and planthopper male advertisement calls and stink bug female advertisement call, the unidentified VSTs do not necessarily belong to separate species. Advertisement calls are the most common vibrational signals recorded in the vibroscape, as they are emitted by the partner that is vibrationally more active during the initial stage of the mate searching sequence (e.g. emitting signals spontaneously and often continuously)<sup>15–18,20,52,57</sup>. However, the complete species-specific vibrational repertoires can be quite extensive and include several male and female signals associated with different stages of the mating sequence, including rivalry<sup>58–62</sup>. Furthermore, species-specific duet structures can be highly variable<sup>63</sup>, and a temporally coordinated male–female duet composed of structurally different sex-specific signals may be assigned to an unknown complex VST if it is not recognized as a duet. In the absence of a publicly accessible reference library, targeted laboratory recordings of species collected at the studied field sites are the most obvious way to avoid overestimating the number of species forming vibrational community. However, such an approach is time consuming and within the short time frame of a seasonal signaling activity may still overlook less numerous and rarer species.

A reference library composed of laboratory recordings also has some limitations. When recording vibroscape in dense and tangled vegetation, the spatial position of the signalers in relation to the recording point is not

known. Consequently, in a complex natural situation, the registered signals may bear little resemblance to the signals recorded in the laboratory, due to unpredictable degradation of vibrational signals during transmission over an unknown distance and different plant substrates<sup>15</sup>. Although the spectral and temporal parameters of vibrational signals are species-specific, they are temperature-dependent<sup>48,64,65</sup>. While laboratory recordings are usually carried out at the room temperature, the ambient and surface temperature in the field can vary greatly throughout the day<sup>16–18,48,49,65</sup>. Moreover, the signalers perching on a single plant can inhabit microclimates with temperatures differing for more than 10 °C<sup>48,49</sup> and some care should be taken when attributing field recorded vibrational signals with similar structure to a new VST or different species.

### Ecotremological monitoring in grassland habitat

Overall, our results also highlight the difficulties that can arise when conducting ecotremological studies. Biodiversity assessments should take into account the high variability and unpredictable changes in vibroscape composition over short distances, and surveys should include several plants as well as areas with different environmental conditions. This highlights the need for more affordable recording devices than laser vibrometers<sup>15,16,18</sup>, and other less expensive vibration sensors are already available<sup>66</sup>. Based on the available data, it is not possible to estimate how long the vibration recording devices should be deployed to capture species richness, mainly because the periods of signaling activity peaks of individual species are unpredictable, often relatively short (10–20 min) and the duration of many VSTs is less than 15 s. As shown here, the VST and probably also the species richness were higher than determined in three days with more than 7 h of continuous daily recordings. While long-term 24-h recordings are essential to obtain basic information on vibroscape composition<sup>20</sup>, the effort required to manually analyze a large number of recordings is enormous. Recently, computational methods have been successfully used to automatically classify and identify vibrational signals in field vibroscape recordings<sup>30</sup>. However, the availability of a common reference library and annotated datasets of vibroscape recordings is crucial for the implementation of automatic detection in ecotremological monitoring.

To summarize, our results show that even continuous grassland contains microclimate areas where temperature and wind-induced environmental noise are sufficiently diverse that the vibroscape composition varies considerably over short distances. Changes in global climate include more frequent and longer heat waves and, depending on the region, an increase in the number of days with strong winds. In such a scenario, the mating behavior of vibrationally signaling species may be affected not only by lethal temperatures but also by higher levels of vibrational noise in the environment. Although our results suggest that shaded areas may provide more favorable microclimate conditions, it is important to note that sustained ambient temperatures high enough to induce lethal heat stress in plant-dwelling insects will most likely have a negative impact across the entire grassland habitat. Ultimately, this may lead not only to changes in insect communities, but also to reduced richness and complexity of the vibroscape. At this stage, it is important to document vibrosapes not only in habitats dominated by anthropogenic vibrational noise<sup>19</sup>, but also in undisturbed environments to obtain baseline information that will allow us to assess future changes in the environment<sup>20</sup>.

### Data availability

All data generated or analyzed during this study, except recorded WAV files, are included in this published article and its supplementary information files. Sample audio files of all recorded VSTs are available as a Zenodo dataset<sup>67</sup> (<https://doi.org/10.5281/zenodo.15097634>). Due to the large file sizes, full recorded files are available from the corresponding author per reasonable request.

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## Author contributions

All authors conceived the study, reviewed drafts and approved the final version of the paper; J.J.L.D. and M.V.D. designed the study, J.J.L.D. carried out the field work, screened the recordings, analyzed the data and prepared figures and tables; R.Š. and J.P. helped with the field work and provided technical support, M.V.D. supervised all aspects of the work and wrote the first draft of the paper.

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## Declarations

## Competing interests

The authors declare no competing interests.

## Additional information

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