

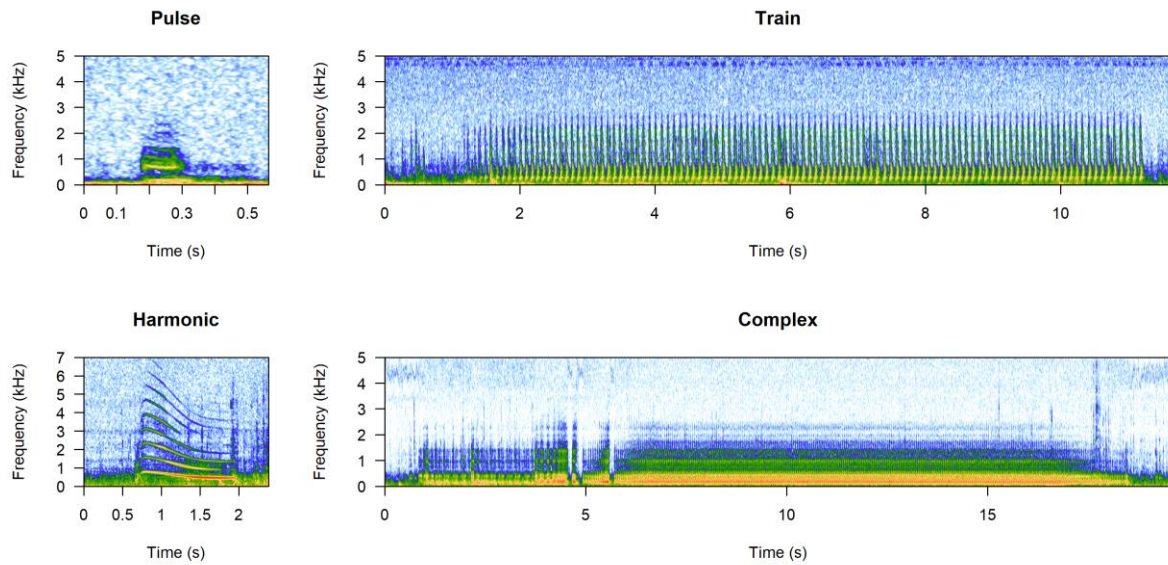
# Spatial and temporal variation in vibroscape composition in two grassland habitats

Juan José López Díez<sup>1,2</sup>, Rok Šturm<sup>1</sup>, Jernej Polajnar<sup>1</sup> and Meta Virant-Doberlet<sup>1</sup>

## SUPPLEMENTARY MATERIAL



**Supplementary Fig. S1:** (a) Field location at Bistra and (b) at Krkavče. Left: aerial views of the areas, meadows where registration of vibroscape was carried out are marked in yellow (source, Atlas okolja, ARSO). Right: vegetation and part of the set-up at each site.



**Supplementary Fig. S2:** Spectrograms of an example of each of the four general signal categories. VSTs were assigned according to their temporal and spectral structure. Pulse: vibrational signals composed of short, single pulses lasting less than 0.2 s with either broadband or harmonic frequency structure; train: vibrational signals composed of regularly repeated pulses or harmonic signals; harmonic: vibrational signals composed of pulses longer than 0.2 s and with clear harmonic frequency structure; complex: vibrational signals composed of at least two of the previous categories. Complex vibrational signal belongs to *Aphrodes makarovi*, while other signals were emitted by unknown species. All spectrograms were generated in seewave (R package) with a Hanning window of 1500 samples with 80% overlap.

**Supplementary Table S1:** Air temperature and wind velocity measured during vibroscope recordings in Bistra and Krkavče.

		Air temperature (°C)						Wind velocity (m/s)
		Sun area			Shade area			Range
		Average ± SD	min.	max.	Average ± SD	min.	max.	
Bistra	Day 1, 09. 07. 2022	27.30 ± 2.19	19.9	32.0	23.40 ± 3.30	16.8	30.0	0-3.5
	Day 2, 12. 07. 2022	26.53 ± 3.99	18.0	36.8	24.75 ± 3.23	18.3	31.2	0-1.7
	Day 3, 13. 07. 2022	29.86 ± 4.03	20.9	39.6	28.80 ± 3.90	21.0	36.6	0-2.2
Krkavče	Day 1, 15. 07. 2023	36.00 ± 3.50	29.0	43.0	34.75 ± 3.13	28.5	41.0	0-3.2
	Day 2, 16. 07. 2023	35.50 ± 4.25	27.0	44.0	31.90 ± 3.05	25.8	38.0	0-3.0
	Day 3, 17. 07. 2023	37.00 ± 4.50	28.0	46.0	31.80 ± 2.60	26.6	37.0	0-3.0

**Supplementary Table S2:** Properties of the plants used in the study. Red indicates plants growing in the sun, blue plants growing in the shade.

	Height (cm)	No. of stems
<b>Bistra</b>		
<i>Gallium</i> plant 1 (Gsn1)	30	6
<i>Gallium</i> plant 2 (Gsn 2)	25	7
Poaceae plant (Psn)	30	1
<i>Gallium</i> plant (Gsd)	18	10
Poaceae plant (Gsd)	30	1
<b>Krkavče</b>		
<i>Gallium</i> plant (Gsn)	18	5
Poaceae plant (Psn)	20	1
<i>Gallium</i> plant (Gsd)	20	5
Poaceae plant 1 (Psd 1)	30	1
Poaceae plant 2 (Psd 2)	35	1

## Supplementary Table S3

Table S3. The cumulative durations of VSTs registered from *Galium mollugo* and Poaceae plants in Bistra (abundance matrix). In the presence-absence matrix all cumulative durations were replaced with value 1.

VST	Cumulative duration (s)															Species (if identified)
	Day 1					Day 2					Day 3					
	Sun		Shade			Sun		Shade			Sun		Shade			
	Gsn1	Gsn2	Psn	Gsd	Psd	Gsn1	Gsn2	Psn	Gsd	Psd	Gsn1	Gsn2	Psn	Gsd	Psd	
C2	248,75	157,82	44,49	7392,33	4330,86	1189,41	1000,91	805,76	7636,68	6712,06	408,65	310,16	565,14	7408,31	8351,55	<i>Anoscopus serratulae</i>
C1	246,79	919,26	830,22	2045,13	2482,99	408,23	1096,52	487,69	2902,43	3058,99	826,92	1635,23	819,86	1961,54	3275,94	<i>Aphrodes makarovi</i>
T1	187,84	109,92	116,58	263,82	447,85	140,76	366,26	296,96	2122,47	1938,64	135,67	419,35	333,12	971,90	1539,96	<i>Aphrodes bicincta</i> Dragonja
T40	0	19,70	497,73	126,01	791,60	554,95	516,68	15,01	232,42	535,37	259,00	280,95	0	160,35	261,04	
C18	0	0	0	194,00	1023,46	0	0	0	0	0	373,61	499,11	28,75	5,28	0	<i>Dicranotropis hamata</i>
T26	0	0	0	9,24	67,44	0	0	0	649,60	208,61	0	0	20,75	158,03	152,10	
T3	90,14	4,72	43,62	326,96	125,56	14,87	21,46	3,49	125,29	45,28	1,88	39,01	25,55	175,85	209,14	<i>Megophthalmus scanicus</i>
T20	109,33	32,08	36,93	10,08	17,14	133,47	27,89	40,57	96,27	23,84	221,78	75,69	156,82	1,00	16,63	
C7	0,65	0	3,78	0	175,06	0	10,35	32,11	88,03	574,62	0	0	0	0	0	
T44	0	0	0	0	0	142,24	136,66	68,94	34,73	5,21	103,70	188,78	63,11	0	0	
C8	47,59	16,11	30,87	5,64	12,33	28,70	29,37	33,24	7,95	27,04	238,44	8,68	20,23	0,53	0	<i>Euscelis incisus</i>
T16	151,03	65,49	165,64	1,88	0	17,32	7,83	0	8,62	30,43	0	0	0	0	0	
C24	0	0	0	0	0	0	0	0	0	0	28,13	203,99	35,19	31,82	0,32	
T10	79,09	83,41	66,21	0	0	0	10,96	13,58	0	0	15,38	6,87	12,40	0	0	vibrational component of a grasshopper song
C16	7,29	0	3,82	0	0	131,76	7,21	72,31	0	0	24,93	22,70	3,68	0	0	
T38	70,49	73,30	19,40	1,47	0	22,76	20,56	9,93	0	2,99	12,29	4,40	22,04	0	8,62	
F2	1,75	0,35	0	22,55	42,90	108,13	1,98	3,42	42,61	0	0	12,28	0	0,61	0	<i>Graphosoma italicum</i>
P9	0	0	0	9,30	9,46	44,00	59,87	89,20	0	0,65	0	0	0	0	0	
T5	0	0	0	0	0	0	0	0	0	0	19,73	29,65	19,86	24,93	110,12	<i>Streptanus aemulans</i>
T41	38,47	0	2,65	4,98	0	28,08	0	0	7,16	0	50,30	0	7,15	0	0	
C6	99,71	0	0	0	0	0	0	0	2,21	0	0	0	0	0	0	<i>Anoscopus albiger</i> group
F5	2,63	0	0	2,31	0	0	0	0	0	0	23,32	34,19	28,48	0	0	
P1	0	0	0	8,38	0,14	0	0	8,53	3,64	0	0	0	2,27	27,89	27,06	<i>Philaenus spumarius</i>
C29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	69,20	
T43	4,14	0	8,63	0	0	0	0	0	21,26	34,35	0	0	0	0	0	
F21	0	0	0	11,40	16,11	0,82	7,62	7,18	9,14	9,33	0	0	0	0,86	2,51	
P7	0	0	0	0	0	9,26	0	44,88	0	0	0	0	0	0	0	
T7	0	18,16	31,17	0	0	0	0	0	0	0	0	0	0	0	0	
T50	0	0	0	0	0	0	0	0	0	0	0	0	0	23,77	0	
C25	0	0	0	0	0	0	0	0	0	0	0	6,00	5,78	0	0	<i>Gramphocraerus ventralis</i>
T49	0	0	0	0	0	0	0	0	11,12	0	0	0	0	0	0	
T9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8,65	
C28	0	0	0	0	0	0	0	0	0	0	0	0	0	6,73	0	<i>Orientus ishidae</i>
C10	0	0	0	0	0	6,37	0	0	0	0	0	0	0	0	0	<i>Cicadella viridis</i>
F15	0	0	0	0	5,66	0	0	0	0	0	0	0	0	0	0	
T32	0	0	0	0	0	0	0	0	3,24	1,55	0	0	0	0	0	
T8	0	0,45	0	0	3,10	0	0	0	0	0	0	0	0	0	0	
T39	0,90	0	0	0	0,70	0	0	0	0	0	0	0	0	0	0	

VSTs are listed according to their abundance, from the highest, to the lowest.

Letters C, F, P and T indicate the category to which vibrational signals were assigned. C, complex; F, harmonic; P, pulse; T, train.

Gsn1, Gsn2, Psn - *G. mollugo* and Poaceae plants in the sunny area; Gsd, Psd - *G. mollugo* and Poaceae plants in the shade area

For VSTs not registered on a particular plant or on a particular day, the cumulative duration is 0.

## Supplementary Table S4

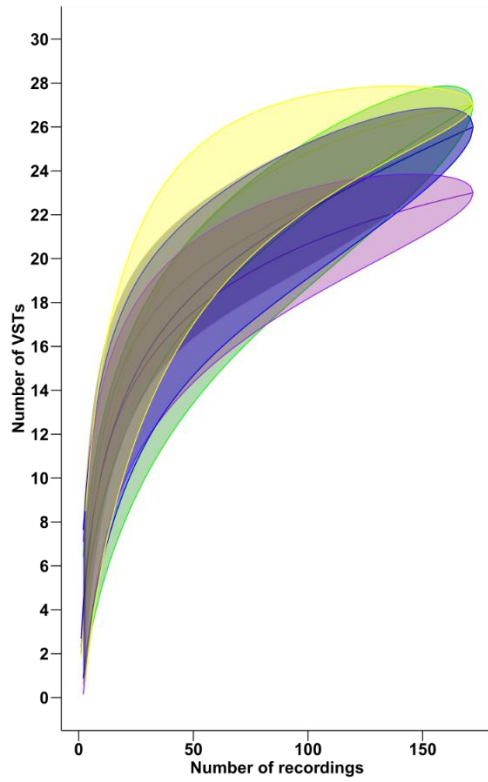
Table S4. VST richness registered from *Galium mollugo* and Poaceae plants in Bistra (a) and Krkavče (b).

**a**

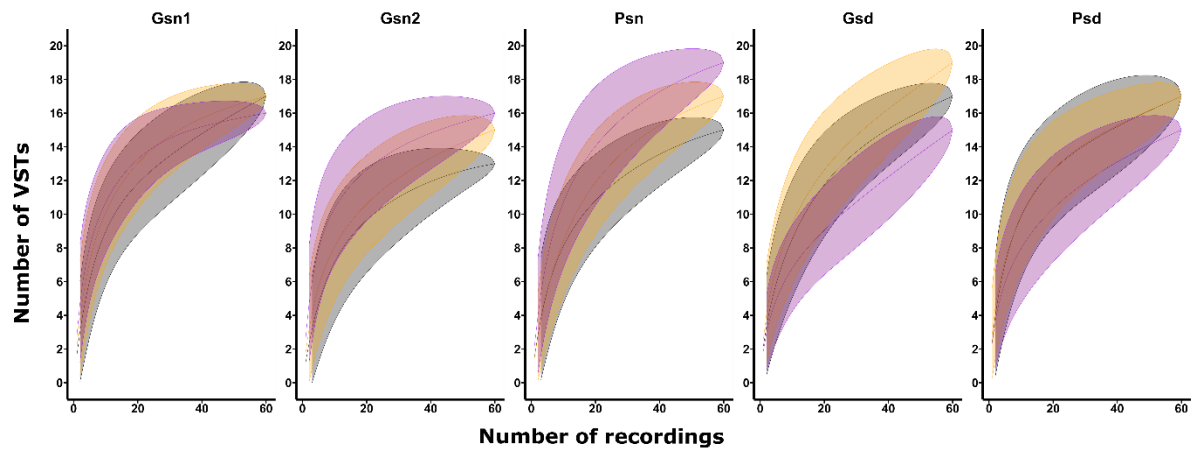
Daily VST richness														
Day 1					Day 2					Day 3				
Sun			Shade		Sun			Shade		Sun			Shade	
Gsn1	Gsn2	Psn	Gsd	Psd	Gsn1	Gsn2	Psn	Gsd	Psd	Gsn1	Gsn2	Psn	Gsd	Psd
17	13	15	17	17	17	15	17	19	17	16	16	19	15	15

**b**

Cumulative duration (s)														
Day 1					Day 2					Day 3				
Shade			Sun		Shade			Sun		Shade			Sun	
Gsd	Psd1	Psd2	Gsn	Psn	Gsd	Psd1	Psd2	Gsn	Psn	Gsd	Psd1	Psd2	Gsn	Psn
7	5	3	3	3	3	9	6	2	5	3	5	4	3	3

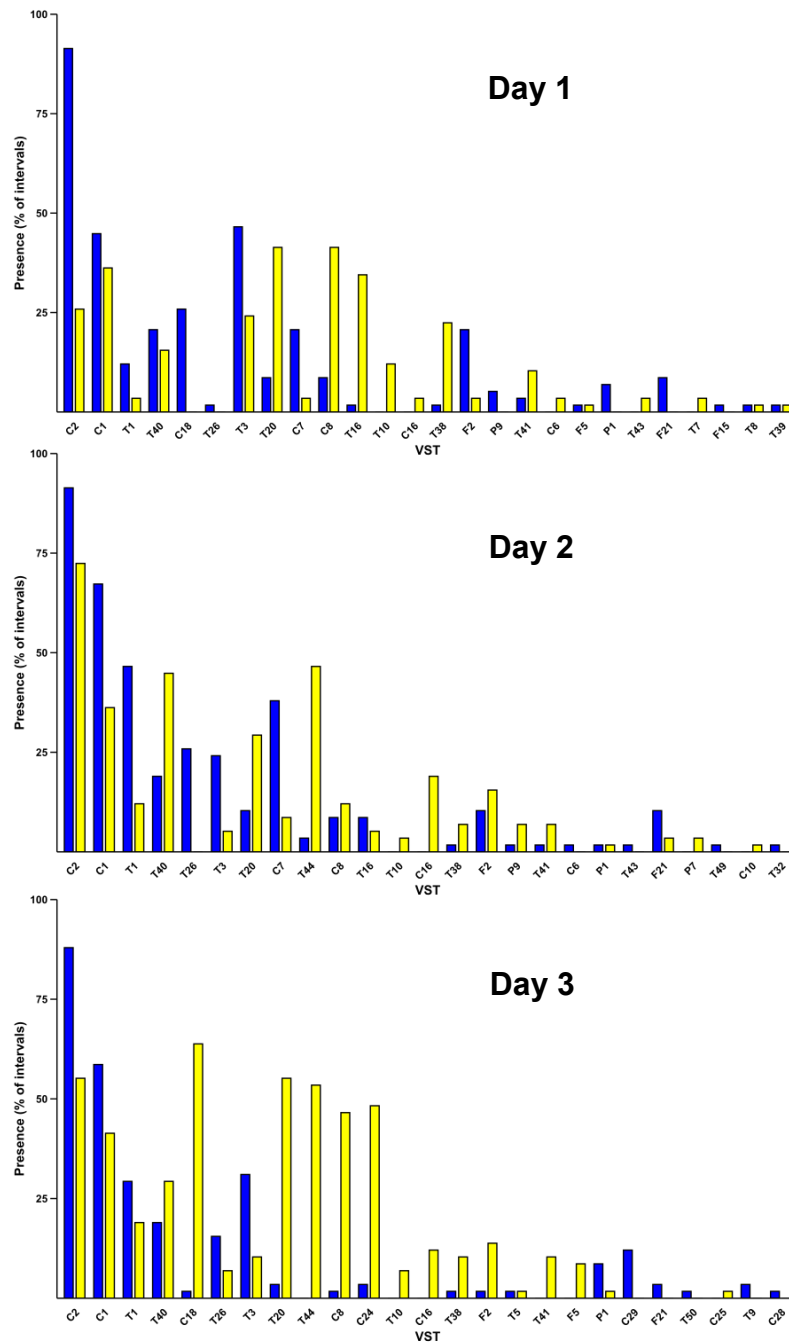


**Supplementary Fig. S3:** Accumulation curves of vibrational signal types (VST) recorded in the eutrophic lowland hey meadow at Bistra during three days of recordings. VST richness in each 8-min WAV file was a sample (174 samples). Gsn1 – grey, Gsn2 – purple, Psn – yellow, Psd – blue, Gsd – green. Coloured areas around the curve indicates 95% confidence intervals.

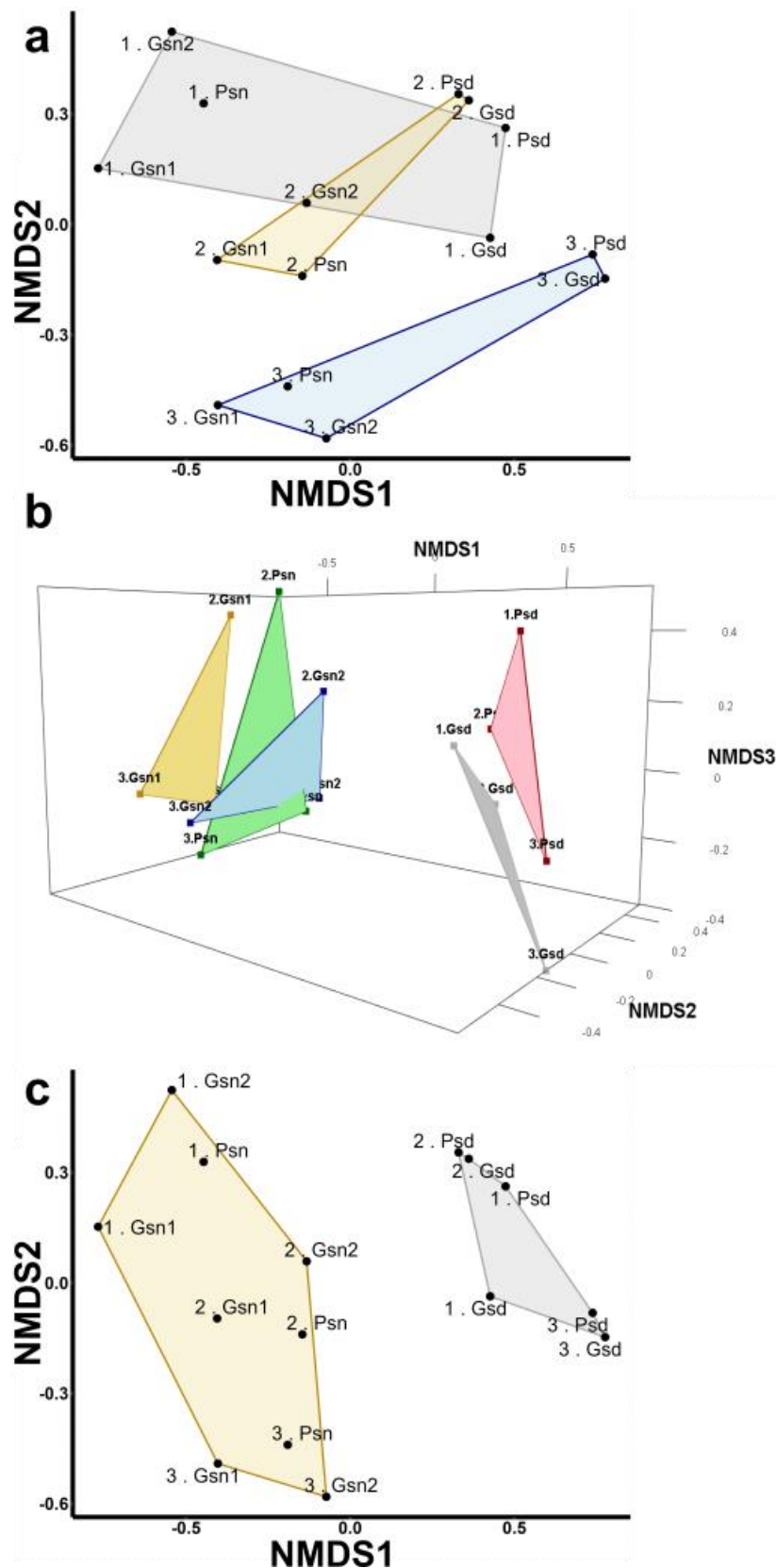


**Supplementary Fig. S4:** Accumulation curves of vibrational signal types (VST) recorded in Bistra on individual plants on individual days. VST richness in each 8-min WAV file was a sample (58 samples). Day 1 (09. 07. 2022) - grey, day 2 (12. 07. 2022)- yellow, day 3 (14. 07. 2022) – purple. Coloured areas around the curve indicate 95% confidence intervals.





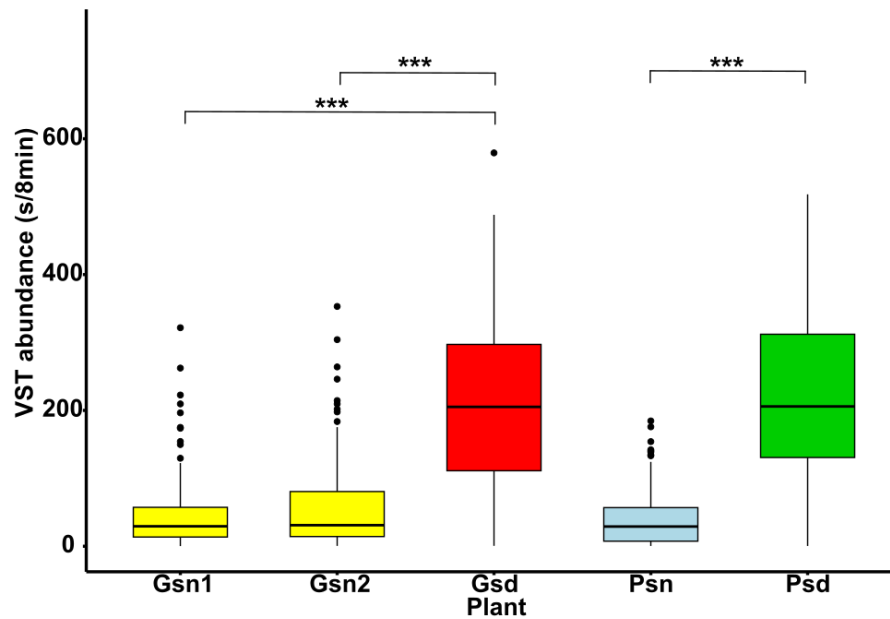
**Supplementary Fig. S5:** Presence of individual VSTs in Bistra on plants in the shade (blue columns) and in the sun (yellow columns) in 8-minute intervals on each day. VSTs are listed according to their abundance (see Supplementary Table S2).



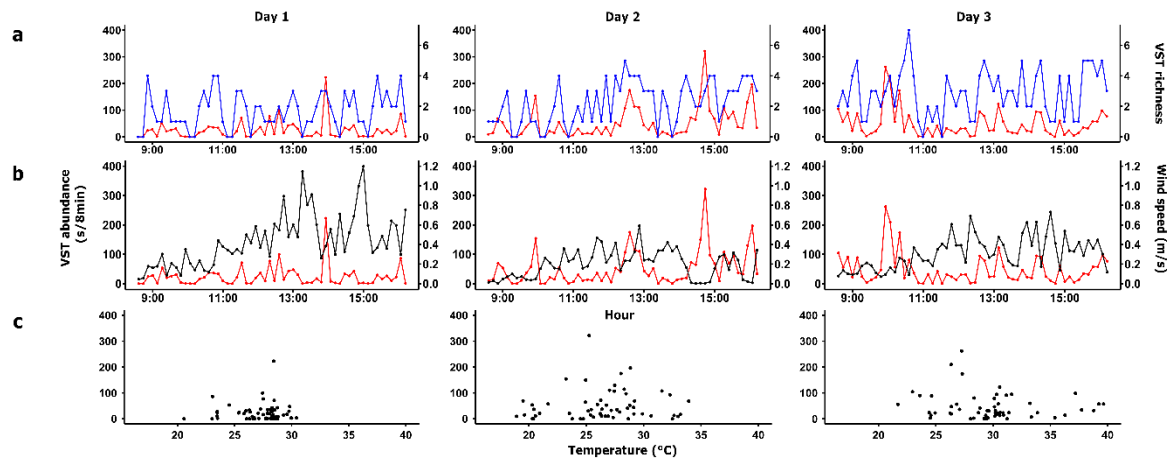
### Supplementary Figure

**S6:** Differences in vibroscape composition in Bistra between days (a), plants (b) and ‘Sun’ and ‘Shade’ areas (c).

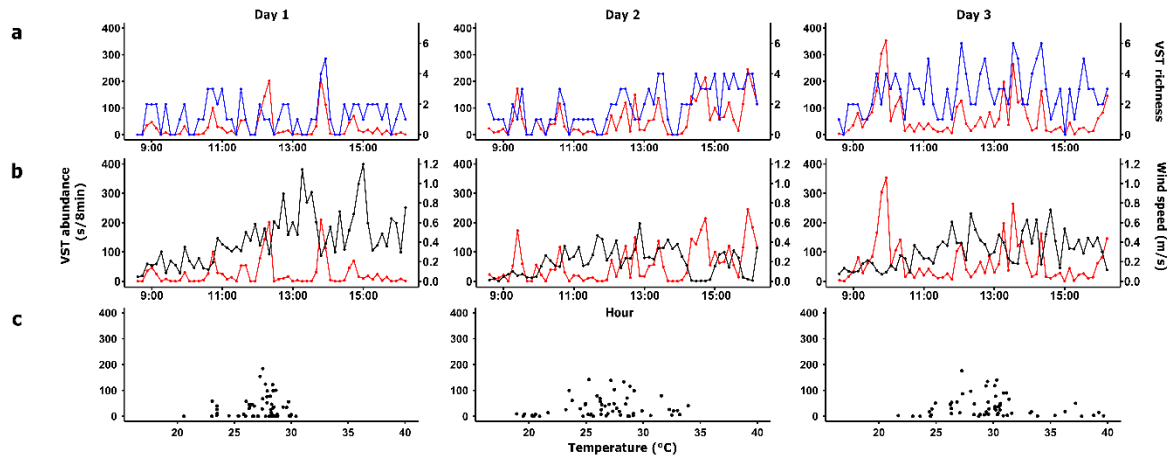
Nonmetric multidimensional scaling (nMDS) based on VST abundance matrix (Supplementary Table S2). (a)  $k = 3$ , stress value = 0.063,  $p = 0.532$ . ANOSIM and PERMANOVA indicate that the variability within the groups is higher than between groups. This results from large differences in VST abundance between plants growing in the sun and in the shade and the effect of the area drives most of the variation in signaling behavior; (b)  $k = 3$ , stress value = 0.063,  $p = 0.0017$ . There is no overlap between the vibroscape composition on *Gallium* plants. (c)  $k = 3$ , stress value = 0.063,  $p = 0.0003$ . 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.



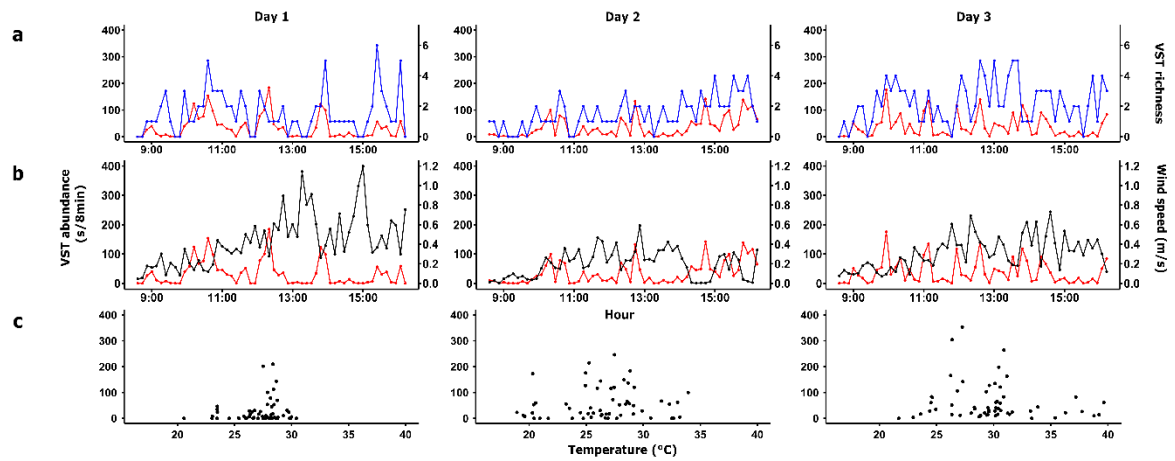
**Supplementary Fig. S7:** VST abundance on plants in Bistra growing in different areas. Gsn1, Gsn2 – *Gallium* plants growing the sunny area; Psn – grass plant growing in the sunny area; Gsd - *Gallium* plant growing the shade area; Psd – grass plant growing in the shade area. Box and whisker plot for each plant is based 174 points (58 points per day) each representing a cumulative duration of vibrational signals (abundance) per 8 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). Wilcoxon rank sum test for multiple comparisons with Bonferroni corrections, \*\*\* $P < 0.001$ .



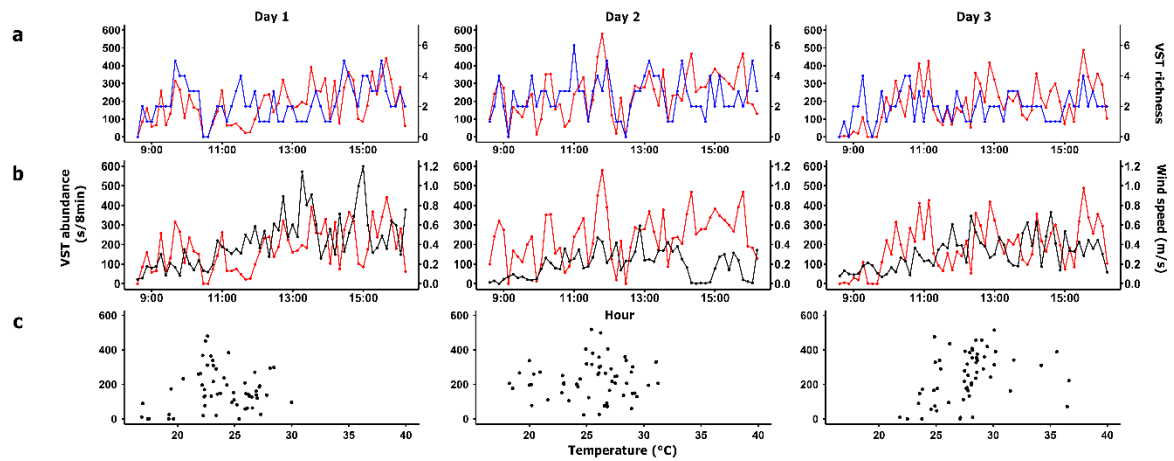
**Supplementary Fig. S8:** Daily dynamic of the vibroscape composition on the *Gallium* 1 plant growing in the ‘Sun’ area (Gsn1). **(a)** Variation in VST richness (number of VSTs) (blue) and VST abundance (red) in 8-minute intervals. **(b)** Variation in VST abundance (red) and average wind speed (black) in 8-minute intervals. **(c)** Correlation between VST abundance and average ambient temperature in 8-minute intervals.



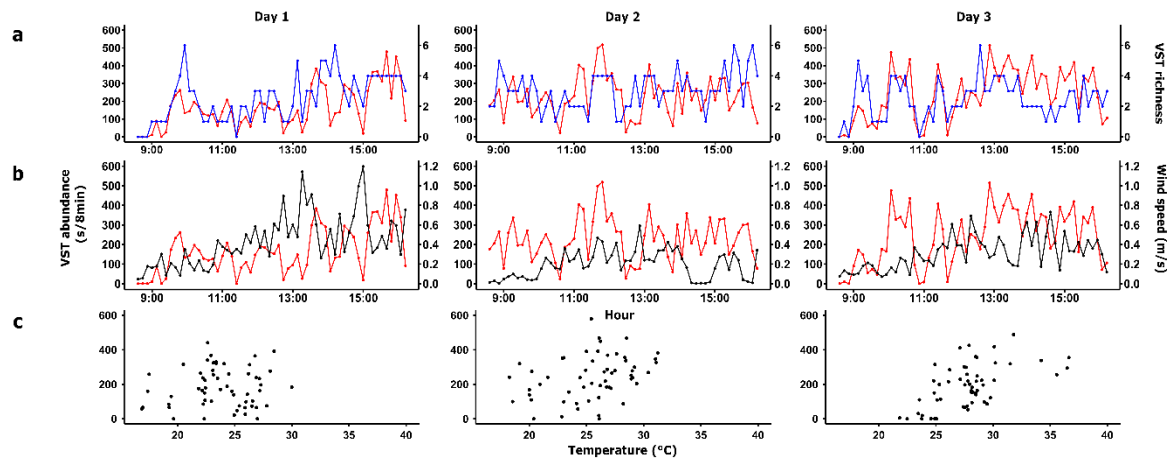
**Supplementary Fig. S9:** Daily dynamic of the vibroscape composition on the *Gallium 2* plant growing in the ‘Sun’ area (Gsn2). **(a)** Variation in VST richness (number of VSTs) (blue) and VST abundance (red) in 8-minute intervals. **(b)** Variation in VST abundance (red) and average wind speed (black) in 8-minute intervals. **(c)** Correlation between VST abundance and average ambient temperature in 8-minute intervals.



**Supplementary Fig. S10:** Daily dynamic of the vibroscape composition on the grass plant growing in the 'Sun' area (Psn). **(a)** Variation in VST richness (number of VSTs) (blue) and VST abundance (red) in 8-minute intervals. **(b)** Variation in VST abundance (red) and average wind speed (black) in 8-minute intervals. **(c)** Correlation between VST abundance and average ambient temperature in 8-minute intervals.

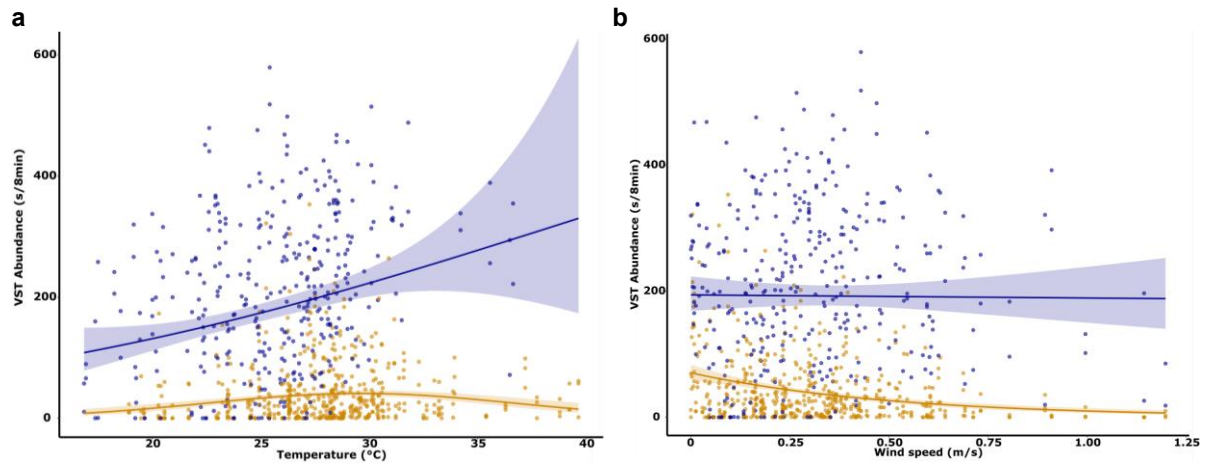


**Supplementary Fig. S11:** Daily dynamic of the vibroscape composition on the *Gallium* plant growing in the ‘Shade’ area (Gsd). **(a)** Variation in VST richness (number of VSTs) (blue) and VST abundance (red) in 8-minute intervals. **(b)** Variation in VST abundance (red) and average wind speed (black) in 8-minute intervals. **(c)** Correlation between VST abundance and average ambient temperature in 8-minute intervals.

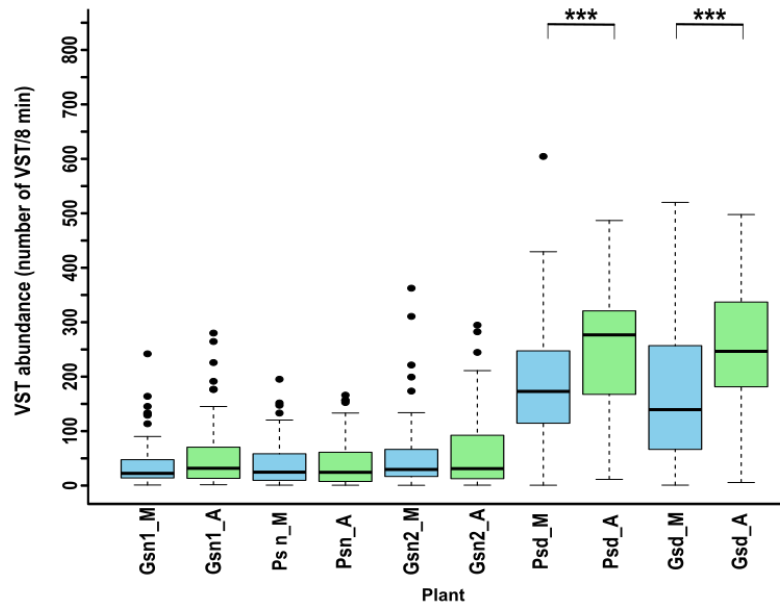


**Supplementary Fig. S12:** Daily dynamic of the vibroscape composition on the grass plant growing in the ‘Shade’ area (Psd). **(a)** Variation in VST richness (number of VSTs) (blue) and VST abundance (red) in 8-minute intervals. **(b)** Variation in VST abundance (red) and average wind speed (black) in 8-minute intervals. **(c)** Correlation between VST abundance and average ambient temperature in 8-minute intervals.





**Supplementary Fig. S13:** Tweedy GLM model predictions on relationship between VST abundance and ambient temperature (**a**) and wind speed (**b**) on plants in the ‘Sun’ area (orange dots) and ‘Shade’ area (blue dots). Blue line - predicted relationship between the temperature and VST abundance in the shade; orange line - predicted relationship between the temperature and VST abundance in the sun. Shaded areas around the lines indicate 95% confidence intervals for the predicted relationships.



**Supplementary Fig. S14:** Differences in vibroscape composition recorded on individual plants registered in the morning (M) and afternoon (A) during 3-day recordings in Bistra. Gsn1, Gsn2 – *Gallium* plants growing the sunny area; Ps n – grass plant growing in the sunny area; Gsd - *Gallium* plant growing the shade area; Psd – grass plant growing in the shade area. Individual box and whisker plot for each plant is based 87 points, each representing a cumulative duration of vibrational signals (abundance) per 8 min (i.e. duration of individual recorded files) in the morning (8:40-12:32) and afternoon (12:32-16:24). Box plot 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, \*\*\* $P < 0.001$ .

## Supplementary Table S5

**Table S5. The cumulative durations of VSTs registered from *Galium mollugo* and Poaceae plants in Krkavče (abundance matrix).**  
In the presence-absence matrix all cumulative durations were replaced with value 1.

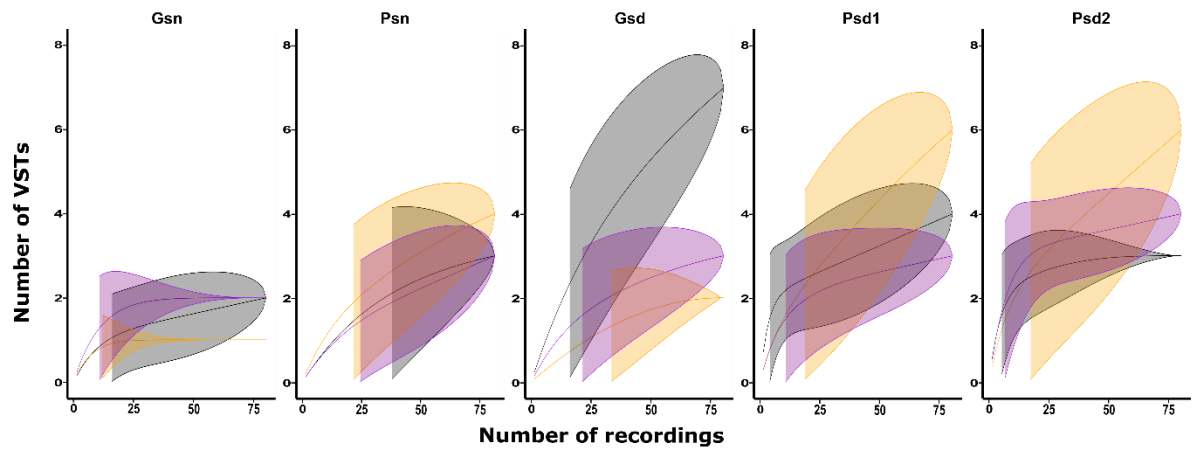
VST	Cumulative duration (s)															Species (if identified)
	Day 1					Day 2					Day 3					
	Shade			Sun		Shade			Sun		Shade			Sun		
	Gsd	Psd1	Psd2	Gsn	Psn	Gsd	Psd1	Psd2	Gsn	Psn	Gsd	Psd1	Psd2	Gsn	Psn	
T51	237,18	1216,81	856,14	15,39	4,39	97,55	3,46	254,73	0	0	0	568,75	164,84	0	7,03	<i>Philaenus spumarius</i> <i>Graphosoma italicum</i>
T16	34,86	3,77	0	368,35	27,68	37,04	4,42	11,02	578,33	138,46	32,60	0	5,81	171,76	46,93	
C24	0	127,18	85,29	0	6,39	0	138,53	118,68	0	0,60	38,43	0	91,93	0	0	
C30	9,16	31,24	204,61	0	0	41,47	8,76	79,00	6,86	17,07	0	139,10	0	0	13,40	
T52	0	0	0	0	0	0	11,29	0	0	46,52	0	0	0	74,26	0	
C31	64,79	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
T26	39,53	0	0	0	0	0	1,12	0	0	5,42	14,95	1,06	0	0	0	
T8	0	0	0	0	0	0	0	0	0	0	0	1,12	30,11	0	0	
T53	0	0	0	0	0	0	0	0	0	0	0	0	0	14,67	0	
P1	0	0	0	0	0	0	7,94	3,38	0	0	0	0	0	0	0	
F2	3,51	5,70	0	0	0	0	0,59	0,47	0	0	0	0,44	0	0	0	
C16	7,18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
C29	0	0	0	0	0	0	4,56	0	0	0	0	0	0	0	0	

VSTs are listed according to their abundance, from the highest, to the lowest.

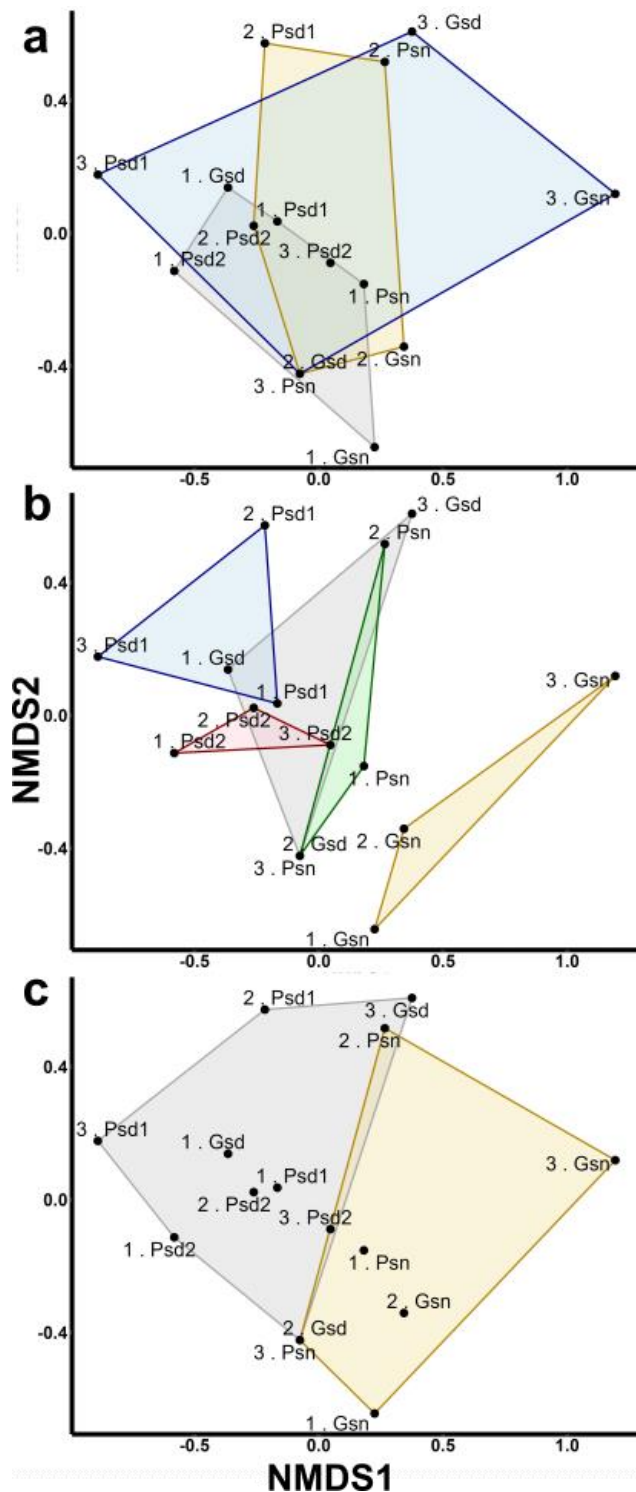
Letters C, F, P and T indicate the category to which vibrational signals were assigned. C, complex; F, harmonic; P, pulse, T, train.

Gsn1, Gsn2, Psn - *G. mollugo* and Poaceae plants in the sunny area; Gsd, Psd - *G. mollugo* and Poaceae plants in the shady area

For VSTs not registered on a particular plant or on a particular day, the cumulative duration is 0.



**Supplementary Fig. S15:** Accumulation curves of vibrational signal types (VST) recorded in the Karst grassland in Krkavče on individual plants on individual recording days. VST richness in each 5-min WAV file was a sample (87 samples per day). Day 1 (15. 07. 2023) – grey, day 2 (16. 07. 2023) – yellow, day 3 (17. 07. 2023) – purple. Coloured areas around the curves indicate 95% confidence regions.



**Supplementary Fig. S16:** Differences in vibroscape composition in Krkavče between days (a), plants (b) and 'Sun' and 'Shade' areas (c). nMDS analysis based on VST presence-absence matrix (Supplementary Table S6). (a)  $k = 3$ , stress value = 0.056,  $p = 0.473$ ; (b)  $k = 3$ , stress value = 0.056,  $p = 0.2572$ . Only vibroscape composition registered on the *Gallium* growing in the shade partially overlaps with the vibroscape compositions registered on grass plants. (c)  $k = 3$ , stress value = 0.056,  $p = 0.0535$ . 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.