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Vibrations from the crypt: Investigating the possibility of vibrational communication in burying beetles

Taina Conrad[1] | **Louise Roberts**[2] | **Sandra Steiger**[1] | **Marie Ringlein**[1]

[1]Evolutionary Animal Ecology, University of Bayreuth, Bayreuth, Germany

[2]Department of Earth, Ocean and Ecological Sciences, School of Environmental Sciences, University of Liverpool, Liverpool, UK

Correspondence
Taina Conrad, Evolutionary Animal Ecology, University of Bayreuth, Universitätsstrasse 30, 95440 Bayreuth, Germany.
Email: taina.conrad@uni-bayreuth.de

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Abstract

Communication is fundamental in the animal kingdom, essential to interactions such as mating, defense, and parental care. Vibrational communication has often been overlooked in the past, but in recent decades, it has become clear that insects use substrate vibrations as a communication signal. In burying beetles of the genus *Nicrophorus*, which are known for their biparental brood care, both parents stridulate. Spending a considerable period of their lives underground, it is very likely the beetles utilize vibrations as part of their communication system. As playback experiments are challenging with this species, this study looked at the physical propagation of the signal of *Nicrophorus vespilloides* Herbst (Coleoptera: Siliphidae) through three soil types, as well as behavior, to see whether vibrational communication is possible. The aims were to determine: (1) whether the soils used in the laboratory compare to soil from the field, (2) whether the distance of propagation is enough for the range the beetles cover during brood care, (3) whether the two sexes show a difference in stridulation likelihood, (4) whether propagation of defensive signals differs from brood care signals, and (5) whether we can determine a behavior during stridulations that shows a clear and useable reaction to the signal. We manipulated beetles to induce stridulation and then used laser Doppler vibrometers to record the signals using three substrates and various distances, alongside behavioral observations. We showed that the three substrates tested, peat, coconut coir, and forest soil, displayed differences in terms of vibrational propagation, and that burying beetle stridulation signals can be transmitted up to about 25 cm in the soil. We also showed that the location where the animals stridulate exerts a significant influence on the total duration and number of stridulations. Overall, vibrational communication is in principle conceivable in this species, as the signals are transmitted far enough in the natural substrate to allow complex communication, opening possibilities for vibrational communication during this biparental brood care.

KEYWORDS
amplitude, biotremology, brood care, Coleoptera, frequency, *Nicrophorus vespilloides*, Siliphidae, soil types, stridulation, vibrational communication

INTRODUCTION

Communication is a fundamental part of animal behavior and an essential prerequisite to many animal interactions. Animals communicate all around us, all the time, in a diverse range of ways (Bradbury & Vehrencamp, 2011). As a result, it is especially important in social contexts, where interactions can be complex and need suitable information transfer. This can lead to various communication channels being used in different species and even multimodal communication (Bradbury & Vehrencamp, 2011). For example, courtship of fruit flies is based on both chemical, vibrational, and visual communication (Fabre et al., 2012; Greenspan & Ferveur, 2000; Tauber & Eberl, 2003). As soon as a male sees a conspecific female, he identifies her and moves toward her (Greenspan & Ferveur, 2000). In addition, the female perceives acoustic/vibrational, as well as chemical, signals from the male working in synergy to induce mating (Rybak et al., 2002).

Vibrational communication refers to the use of the mechanical signal carried by the substrate, which is perceived via specific perceptual organs (Hill & Wessel, 2016; Roberts & Wessel, 2023). To perceive vibration signals, most insects have vibration sensors in their legs. In the Coleoptera and Diptera groups, however, no special vibration sensors have yet been discovered in the appendages (Hill et al., 2019). However, there is no doubt that all insects have mechanoreceptors and are thus capable of perceiving vibration signals (Cocroft et al., 2014). In the past, vibrational communication has often been overlooked as an integral part of animal communication (Hill et al., 2019, 2022; Roberts & Wessel, 2023; Virant-Doberlet et al., 2023). However, in recent decades, it has become clear that many insects use the vibrations of the substrate for communication. It is estimated that 600 000 insect species rely on this form of communication, with at least 150 000 of these species using it as the only form of communication (Cocroft et al., 2014; Hill, 2008; Virant-Doberlet et al., 2023).

Vibrational signals have been associated with a variety of animal behaviors and in various contexts. One interesting example comes from eusocial *Polistes* wasps, in which larvae perceive substrate vibrations produced by the adults and react with behavioral changes, making this clear adult–larvae communication (Pepiciello et al., 2018). Vibrational signals are generated by various behaviors, but mainly by tremulation, drumming, or stridulation (Hill et al., 2022). In stridulation, sounds are produced by rubbing body parts together (Virant-Doberlet et al., 2023). Such a stridulation event produces a multimodal signal, both air-borne and substrate-borne, plus visual and chemical cues, potentially, through the same action (Hill et al., 2019). For example, terrestrial crustaceans produce a measurable acoustic and vibrational signal (Popper et al., 2001). In many species, it is not yet clear whether both the sound in the air and the vibrations generated on the substrate are used as signals (Cocroft et al., 2014).

In beetles of the genus *Nicrophorus*, which are known for their biparental brood care, both parents stridulate (Pukowski, 1933). Burying beetles "chirp" during the entire brood care and it has already been shown that these stridulations have a significant impact on offspring survival and weight gain (Conrad et al., 2022, 2024; Hall et al., 2015). The stridulations are produced when the plectrum (stridulatory edge) strikes downward against the pars stridens (stridulatory bars) (Pukowski, 1933; Schumacher, 1973). The pars stridens are located caudally on the fourth abdominal segment, whereas the plectra are located on the underside of the elytra (Pukowski, 1933).

The reproduction in burying beetles is well researched (Bartlett, 1988; Eggert & Müller, 1997; Pukowski, 1933; Scott, 1998; Steiger, 2015). Provided a suitable carcass of a small mammal or bird is encountered, the couple buries the carcass and subsequently, the carcass is stripped of its fur or feathers and rolled into a ball (Pukowski, 1933). The underground chamber holding the ball, also called a crypt (Bartlett, 1988), is where most of the brood care takes place. The female then begins laying eggs in the immediate vicinity (Pukowski, 1933), the larvae develop on/in the carcass, and leave 7–9 days after hatching (Fetherston et al., 1994; Trumbo, 2006). All the while, parents provide elaborate brood care in feeding the offspring, conserving the carcass, and defending against predators or competitors (Eggert & Müller, 1997; Pukowski, 1933; Smiseth et al., 2003; Steiger, 2015). Therefore, via this mode of reproduction, it can be concluded that the beetles spend a considerable period of their lives underground, making it very likely that they at least use vibrations as part of their communication. The stridulations of *Nicrophorus* are audible to the human ear aboveground, but for insects to communicate effectively with acoustic signals, they must be large relative to the wavelength of the transmitted signal. Thus, smaller animals would need to generate signals at higher frequencies, which would result in attenuation or degradation of the signal as it propagates (Virant-Doberlet & Čokl, 2004). As a result, the substrate-borne signals occur more often in smaller animals, which represent the most cost-effective and far-reaching signals (Virant-Doberlet & Čokl, 2004). Hence, given the subterranean lifestyle of the signalers, could the stridulations of the beetles also be substrate-borne? This could make burying beetles vibrational communicators, and potentially open the possibility of vibrations playing a role in their brood care. Certainly, soil-dwelling invertebrates have already been shown to communicate and sense their surroundings via vibrations in the soil (Catania, 2008; Fielde & Parker, 1904; Gogala et al., 1974; Kojima et al., 2012).

Nicrophorus can reproduce in a range of soil substrates, including forest soils, peat, coconut fiber, and sand (Eggert & Müller, 1997; Scott, 1998; Capodeanu-Nägler et al., 2016; Keppner et al., 2023), and typically occupies a highly variable subterranean environment. This is particularly important given that some substrates propagate vibrations more efficiently than others (Brownell & van Leo, 2001; Elias et al., 2010; Mortimer, 2017), and given that soils are a complex mix of materials and composites, making biotremological studies challenging (Elias et al., 2004; Mankin, 2022). Soils are not quiet environments, and eavesdropping on belowground signals and cues occurs frequently (Kojima et al., 2012; Mankin, 2022; Virant-Doberlet et al., 2023; Wenninger et al., 2009). Should vibrations be used in *Nicrophorus*, and should the type of soil make a difference, it could be hypothesized that signaling would be more useful in particular substrates, thus, possibly influencing search behaviors or even population distributions. In turn, this could have an impact on our studies in the laboratory,

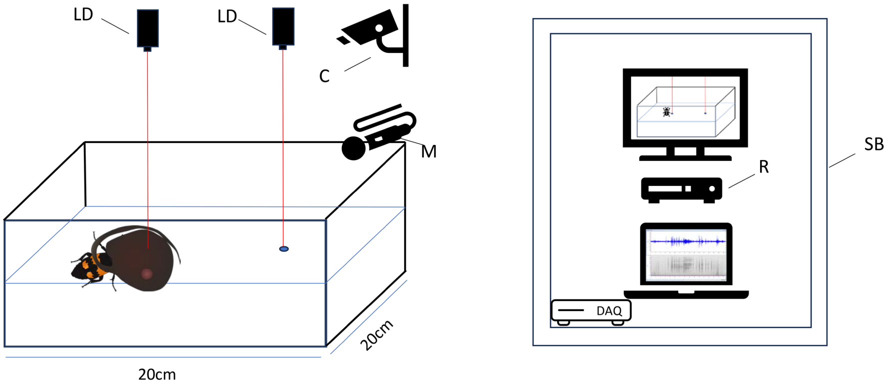


FIGURE 1 Experimental setup for burying beetle recordings. (left) Experimental arena with two laser Doppler vibrometers (LD), a camera (C), and a microphone (M). (right) Experimental arena, data acquisition (DAQ), recorder (R), and laptop computer in a soundproof box (SB).

where we use either peat or coconut coir, exclusively for logistical reasons. Additionally, the carcass itself provides a different type of substrate entirely, and to date, it is unclear whether stridulations on the carcass are transmitted to the surrounding substrate.

To address whether vibrational communication is possible, or relevant, to the beetles, here, we focused on the propagation of the stridulation signal through substrates (soils), with the aims to determine: (1) whether the soils used in the laboratory compare to natural soil from the forest in terms of their propagation of a signal; (2) the distance of signal propagation, to see whether the radius is sufficient for communication during brood care; and (3) whether defensive signals differ from brood care signals in their propagation. Additionally, in an attempt to establish a method to induce stridulations, which could be used for future experiments, we wanted to know: (4) whether the two sexes show a difference in stridulation likelihood, and (5) whether the larvae come up for feeding as a response to parental stridulations, making it a clear response to the signal. By answering the above, we aimed to understand whether vibrational communication is possible and likely in *Nicrophorus*. We expected propagation differences between the three substrates, but not between defensive and brood care signals. In addition, we assumed that sex would not exert a serious influence on stridulation, as both sexes have been shown to stridulate during brood care (Darwin, 1871; Pukowski, 1933). Furthermore, we expected to observe that larvae surface as a result of the stridulation signal, to be subsequently fed by their mother. A specific response on the part of the larvae could be used as part of playback manipulations in the future and thus allow further tests to clarify the question of acoustic or vibratory communication.

MATERIALS AND METHODS

Origin and husbandry of the breeding animals

The burying beetles (*Nicrophorus vespilloides* Herbst) (Coleoptera: Siliphidae) used were sixth-generation descendants of beetles collected from carrion-baited pitfall traps. Adult *N. vespilloides* were caught in a forest near Bayreuth, Germany (49°55′18.192″ N, 11°34′19.9488″ E). All beetles are maintained in environmentally controlled chambers at 20°C, 60% relative humidity, and L16:D8 photoperiod (Rubarth Apparate, Laatzen, Germany). Groups of up to five adults of the same sex and family were kept in plastic containers (10 × 10 × 6 cm) filled with moist coconut coir. To ensure optimal outbreeding, KINSHIPPER software v.1.0, (kinshipper.com; Conrad, Bayreuth, Germany) was used to calculate optimal mating pairs for each new generation. Beetles were fed whole fly larvae, *Lucilia sericata* (Meigen) (Diptera: Calliphoridae), ad libitum 2× per week. At the time of the experiments, beetles were virgin and 20–30 days of age.

In burying beetles, despite biparental care, there is task allocation, with the female mostly feeding the offspring and the male defending the carcass, although both parents can undertake any role (Fetherston et al., 1990; Trumbo, 2006). Hence, all experiments utilized the females for comparability (except for the experiment testing the role of sex). For brood care experiments, one female along with one male and one mouse carcass (frostfutter.de; B.A.F. Group, Thalmässing, Germany) were placed in a plastic box (11.5 × 11.5 × 5.5 cm) filled with forest topsoil originating from the Studentenwald, where beetles were caught (Bayreuth, Germany). Subsequently, these boxes were kept in climate chambers at 20°C and steady darkness, as beetles at this stage would be underground. At the time of the experiments, the larvae were 24 and 48 h old, respectively, which is a time right in the middle of brood care, where most feeding and care would occur (Capodeanu-Nägler, 2018; Smiseth et al., 2003).

General experimental setup

The following setup was used for all experiments measuring vibrational signals (Figure 1). Substrate-borne (soil) vibrations were recorded using a laser Doppler vibrometer (LDV) setup (Polytec Laser Vibrometers PDV-100; Polytec, Waldbronn, Germany; 0–22 kHz, 20 mm s^{-1}) directed at a nail (2.9 cm long, fully pushed into the soil with the head in line with the soil surface), with reflective foil on the exposed nail head surface (0.5 × 0.5 cm).

As a reference, stridulatory sounds were recorded using a directional microphone (AKG CGN 99 HS; Thoman, Treppendorf, Germany; 70–18 000 Hz, 125 dB SPL max) positioned close to the beetle. Signals were digitized through a multichannel calibrated data acquisition device (TASCAM Celesonic US-20×20 interface; Tascam, Tokyo, Japan; 44.1 kHz, 16-bit resolution) and a laptop computer both of which were encased in a soundproof box to minimize background noise from the equipment. Digitized signals were viewed and analyzed within RAVEN PRO v.1.4 sound analysis software (Cornell Lab of Ornithology, Ithaca, NY, USA).

Additionally, the parameters water content of the soil (%), pronotum width (mm), temperature (°C), and humidity (% r.h.) were documented for all experiments.

Transmission of the signal through three types of soil

Boxes (20 × 20 × 8.7 cm) were filled by loosely pouring in one of three substrates: peat (Floratorf; Floragard, Oldenburg, Germany), coconut fiber humus (TropicShop, Nordhorn, Germany), and forest soil (topsoil collected from the forest where we also set up our pitfall traps) to a depth of 5.5 cm (Table S1). The *N. vespilloides* females (*n* = 20, randomly selected) were held with tweezers (standard pattern forceps; Fine Science Tools, Heidelberg, Germany) on the surface of each of the three substrates to trigger stridulation. This method means that playback of the signal is not required, as it can be instantaneously produced by manipulation of the beetles. Using numerous individuals meant that intraspecific variation was included in our experiments across the entire signal range of the beetles. The distances between the beetle and the laser beams were 8 cm (LDV 1) and 16 cm (LDV 2) throughout the experiment.

For the analysis of the sound recordings, the .WAV files were assessed within RAVEN PRO v.1.6.4 (Yang, 2023) using the measurement tool. Ten pulses per individual, per test were selected and analyzed with respect to the parameter peak amplitude (U), where "U" are the Raven relative amplitude units (Figure S1). Although preliminary frequency measurements of pulses were undertaken, due to the short duration of pulses, the frequency calculations were not dependable.

Transmission distance

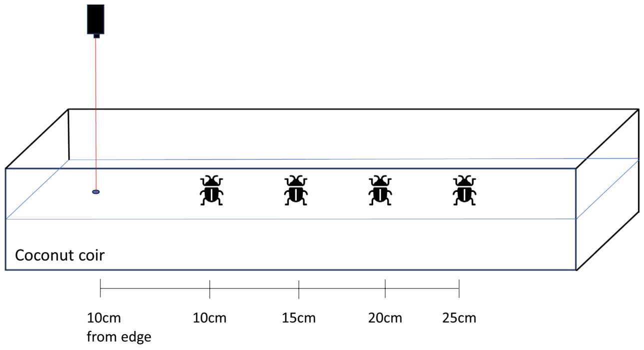


FIGURE 2 Distance measurements to evaluate how far the vibrational signal travels in soil. The laser was pointed at a nail inserted into the soil with the top on the surface and covered with reflective tape. The burying beetle was placed at four distances away from the nail and induced to stridulate. The soil depth was 5 cm and the size of the box was 56.5 × 36.5 × 20.5 cm.

We then investigated signal propagation in more detail, to establish whether the distance the signal travels through the substrate is sufficient to be used as a communication signal during brood care. To do this, we measured the signal attenuation at four distances from the beetle, focusing on coconut coir, which is more homogenous and showed no significant difference to the forest soil in the previous experiment. We used a large box (56.5 × 36.5 × 20.5 cm) with a nail for recording on one side (approx. 10 cm from the edge of the box). The beetle was held with tweezers (see above) at four distances from the nail (10, 15, 20, and 25 cm). The coir soil depth was 5 cm (Figure 2).

For the analysis of the sound recordings, the .WAV files were assessed within RAVEN PRO v.1.6.4 (Yang, 2023) using the measurement tool. A series of pulses per individual, per test were selected and analyzed with respect to the parameters U (see above), and peak frequency (Hz).

Signals and behavior during brood care

To analyze the stridulation signal of a nurturing female in forest soil, breeding was induced by placing a mouse carcass (20 ± 2.5 g) in a box together with a sixth-generation female and male. After 96 h, the carcass, parents and larvae were transferred to a recording box (20 × 20 × 8.7 cm) filled with 5.5 cm of forest soil. Metal nails (2.9 cm long) covered with reflective foil were pushed into the soil at 2 cm and 14 cm distance from the carcass to provide recording points. Beetles (*n* = 15 tested separately) and larvae were given a 30 min acclimation period prior to recordings. A camera (CMOS TVI; Sony, Minato, Japan) with a recorder (Lupus Electronics, Landau, Germany) was positioned above the beetles to record behavior for later analysis.

To investigate the role of the larvae and carcass in signal propagation, in a separate test, LDV measurements were taken from the mouse itself (with reflective foil on the carcass body surface), plus at 5 cm distance (with a nail in the soil). In total, eight female beetles were recorded together with their 48-h-old larvae (*n* = 10–20), plus the carcass for approximately 1 h each.

The behavior of the beetles and larvae was analyzed from the videos using the program BORIS v. 8.14 (Friard & Gamba, 2016), which is a behavioral tracking program. The following behaviors were recorded: stridulation on top of the carcass, stridulation away from the carcass, stridulation in the carcass, beetles in the carcass without stridulation, and larval behavior (did larvae come up for feeding or not).

The role of sex

To see whether one of the sexes was more likely to elicit stridulations after manipulation, 20 families were randomly selected from sixth-generation beetles. From each family, one male and one female were directly compared (total $n = 40$). The beetles were manipulated 3× for 5 s using tweezers (standard pattern forceps; Fine Science Tools) by squeezing or poking them. Subsequently, it was recorded each time if they reacted with stridulations or not.

Statistical analysis

For statistical analysis, peak amplitude and, where possible, peak frequency were used for the defense signals. Furthermore, the signals during brood care were analyzed for peak amplitude. The effect of location of the female on stridulations was analyzed in terms of the number and duration of stridulations. The influence of sex on the number of stridulations was also considered.

Data analysis was performed in R Studio v.2023.09.0 (R Core Team, 2020) using R v.4.2.3. (R Core Team, 2009) and the packages "multcomp" v.1.4–23 (Hothorn et al., 2008), "car" v.3.1–2 (Fox & Weisberg, 2019), and "Lme4" v.1.1–32 (Bates et al., 2015).

Differences in substrates were tested in a generalized linear model (GLM) with gamma distribution and subsequent ANOVA () function in R to extract F- and p-values. For this, peak amplitude was used as response variable. Distance, substrate, size (pronotum width), and temperature were set as fixed factors:

$$\text{Response} = \text{distance} + \text{substrate} + \text{size} + \text{temperature}.$$

We arrived at this model after using multiple models, including a full generalized linear mixed model (GLMM) with individual identifiers (ID) as random factor; after subsequent model reduction we compared Akaike information criterion to arrive at the above model.

For the analysis of signal propagation over distance, we correlated peak amplitude and distance using Pearson's product–moment correlation. In addition, to analyze the effect of temperature and r.h. on peak amplitude and peak frequency over distance, we used a GLMM with subsequent ANOVA () function in R to extract F- and p-values. For this, peak amplitude/peak frequency was used as response variable and r.h. and temperature was set as fixed factors, with ID as a random factor.

$$\text{Response} = \text{distance} + \text{humidity} + \text{temperature} + (1 | \text{ID}).$$

To analyze the signals emitted during brood care, differences in total duration, average duration, and number of stridulations across sites of stridulation were tested using a GLMM followed by ANOVA () function in R to extract F- and p-values. For this, total duration, average duration, and number of stridulations were set as response variables. Carcass weight, size, and location of the female were used as fixed variables and ID was set as a random factor to account for repeated measures. When there was evidence of significant effects, a Tukey test was performed to determine which means differed from each other.

$$\text{Response(total duration, average duration, number of stridulations)} \\ = \text{carcass weight} + \text{size} + \text{location of the female} + (1 | \text{ID}).$$

To test whether males and females stridulated the same number of times, we used a GLMM with number of stridulations as response variable and an interaction term for sex × size as well as ID as a random factor to account for repeated measures.

Residuals of all linear models were checked visually based on standard residual plots and by plotting residuals against predictors. Residuals of GLMs were checked using DHARMa v.0.4.6 (Hartig, 2017). Additional dispersion parameters were fitted to GLMs where necessary.

The figure displaying the interaction between size and sex was produced using the packages interactions v.1.1.50 (Long, 2019) and ggplot2 v.3.4.0 (Wickham, 2016). The program SigmaPlot v.14.5 (Systat Software, San Jose, CA, USA) was used to build all other graphs and PowerPoint was used to build schematics of the setups.

RESULTS

Transmission and soil type

The burying beetle signal peak amplitude was significantly higher in peat than in coconut coir and forest soil (GLM: $F_{2,1177} = 147.142$, $p < 0.001$; Figure 3). The other parameters (temperature, size, and distance) also had a significant effect (GLM: temperature: $F_{1,1122} = 3.464$, $p < 0.05$; size: $F_{1,19} = 2.591$, $p < 0.05$; distance: $F_{1,1176} = 69.3127$, $p < 0.05$).

Transmission distance

There was a negative correlation between burying beetle signal distance and amplitude (Pearson's correlation: $t = -4.267$, df = 40, $p < 0.05$; $r = -0.56$; Figure 4). At a distance of 20 cm to the stridulating beetle, the amplitude approached background levels. Temperature and humidity had no effect on this correlation (GLMM: temperature: $F_{1,38} = 2.1559$, $p > 0.05$; humidity: $F_{1,38} = 2.4469$, $p > 0.05$).

There was also no significant correlation between distance and peak frequency (Pearson's correlation: $t = 0.99$,

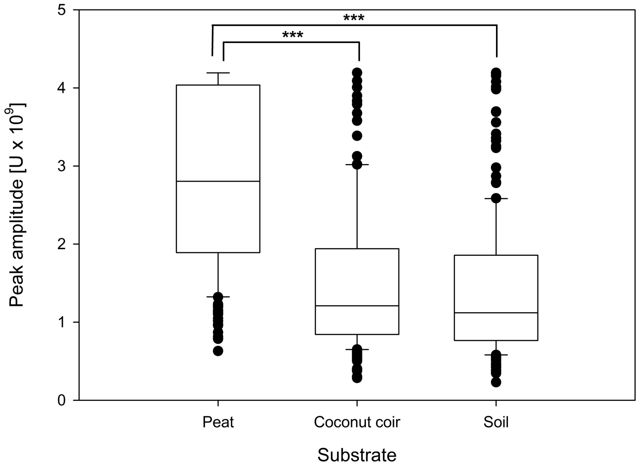


FIGURE 3 Boxplots showing peak amplitude (measured in U which is proportional to the sound pressure) of burying beetle vibrational signals in three types of soil at 8 cm ($n = 20$). The boxplots show the median value and the first and third quartile (upper and lower box). The whiskers show 1.5× the interquartile range. Outliers are shown with circles. Asterisks indicate significant differences (generalized linear model: ***$p < 0.001$).

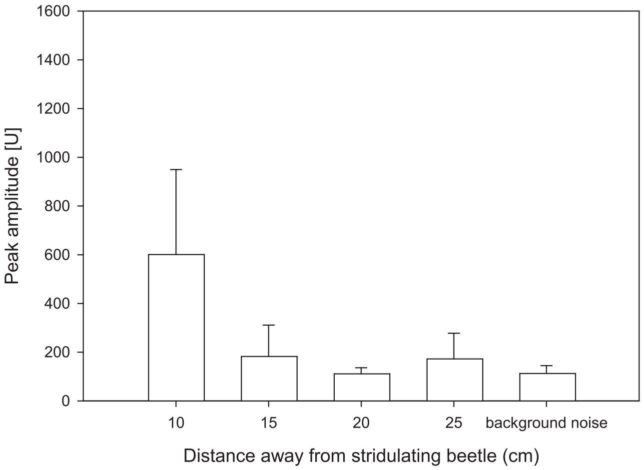


FIGURE 4 Mean (±SD) peak amplitude (measured in U which is proportional to the sound pressure) of burying beetle vibrational signals at four distances and average background noise at 10 cm away from the stridulating beetle ($n = 12$).

df = 39, $p > 0.05$; $r = 0.16$) and no significant difference in peak frequency between any of the distances from the stridulating beetle (GLMM: distance: $F_{1,37} = 1.248$, $p > 0.05$; temperature: $F_{1,37} = 0.105$, $p > 0.0.5$; humidity: $F_{1,37} = 0.503$, $p > 0.0.5$).

The role of sex

There was no significant difference in the number of times a manipulated beetle reacted with stridulations between the sexes (GLMM: $\chi^2 = 0.17$, df = 1, $p = 0.68$). However, there was an interaction between sex and body size in the number of reactions with stridulations (GLMM: $\chi^2 = 6.32$, df = 1, $p < 0.05$), with increasing stridulations with size in females and decreasing stridulations with size in males. Estimations of the interaction between size and sex show opposite curves (Figure 5).

Signals and behavior during brood care

Signals produced by adult burying beetles after artificial manipulation were visually similar to signals produced during brood care, in terms of temporal pattern, frequency, and amplitude (Figure S1). There was a significant drop in peak amplitude between the measurement 2 and 14 cm away from the carcass (GLMM: $F_{1,260} = 98.90$, $p < 0.001$; Figure 6A). Physical parameters (temperature, size, and water content) had no significant effects (GLMM: temperature: $F_{1,10} = 0.4654$, $p > 0.05$; size: $F_{1,10} = 0.1432$, $p > 0.05$; water content: $F_{1,10} = 0.3955$, $p > 0.05$). Peak amplitude was higher when recording at 5 cm from the carcass than directly (0 cm) at the carcass (GLM: $F_{1,152} = 276.19$, $p < 0.001$; Figure 6B).

Both the total duration and the number of stridulation events were significantly affected by the location of stridulation. The total duration of stridulations by the female, when the location of the female was "away from carcass," was significantly longer than when the female was "on carcass" or "inside the carcass" (GLMM: $F_{5,15} = 6.06$, $p < 0.001$; Figure 7A). Likewise, the number of stridulations when the female was "away from the carcass" was significantly higher than when "inside the carcass" (GLMM: $F_{5,15} = 5.095$, $p < 0.01$; Figure 7B). Regarding larvae, no coming up for feeding was observed as a result of the stridulation signal.

DISCUSSION

We showed that the three substrates tested, peat, coconut coir, and forest soil, affected the amplitude of burying beetle stridulations to varying degrees. In addition, we found that the stridulation signals in the earth were detectable to about 20 cm with the LDV. We also demonstrated that the location where the animals stridulate exerts a significant influence on the total duration and number of stridulations. Finally, we showed that although there was no influence of sex alone on stridulation responses, there is an interaction of sex and size as to how likely an individual is to respond with stridulations. Bigger females and smaller males are more likely to respond with stridulations.

Although a reduction in vibrational amplitude across distance is to be expected (Elias & Mason, 2014), little is known about substrate-dependent effects for the particular substrates relevant to burying beetles. Whereas the amplitude of the signal at 16 cm distance is similar for coconut coir and forest soil, amplitude is much higher for peat. That is, peat attenuated the signal less than the other substrates, including their natural habitat of forest soil. Similar studies with spiders have shown

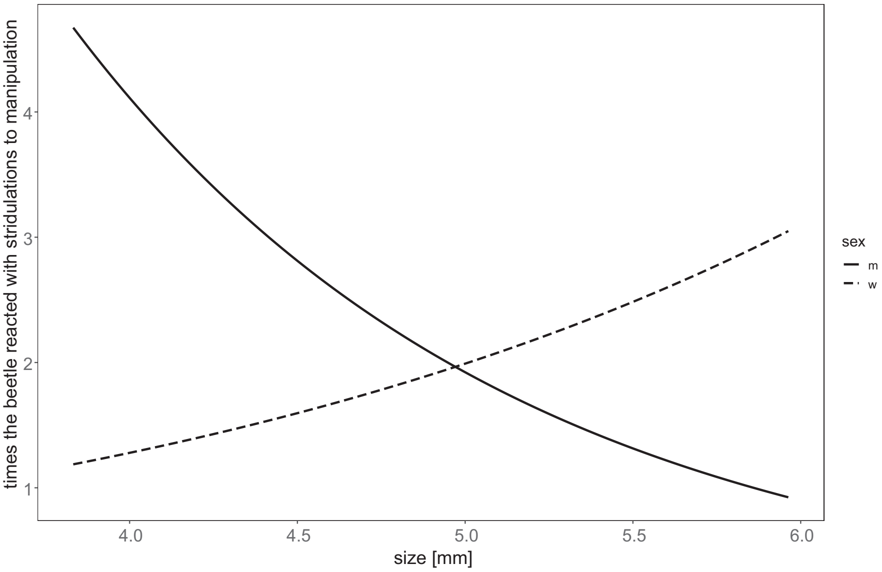


FIGURE 5 Estimated number of stridulation responses when male and female burying beetles of different sizes (mm) were manipulated with tweezers. Figure produced with the package "interaction" in R.

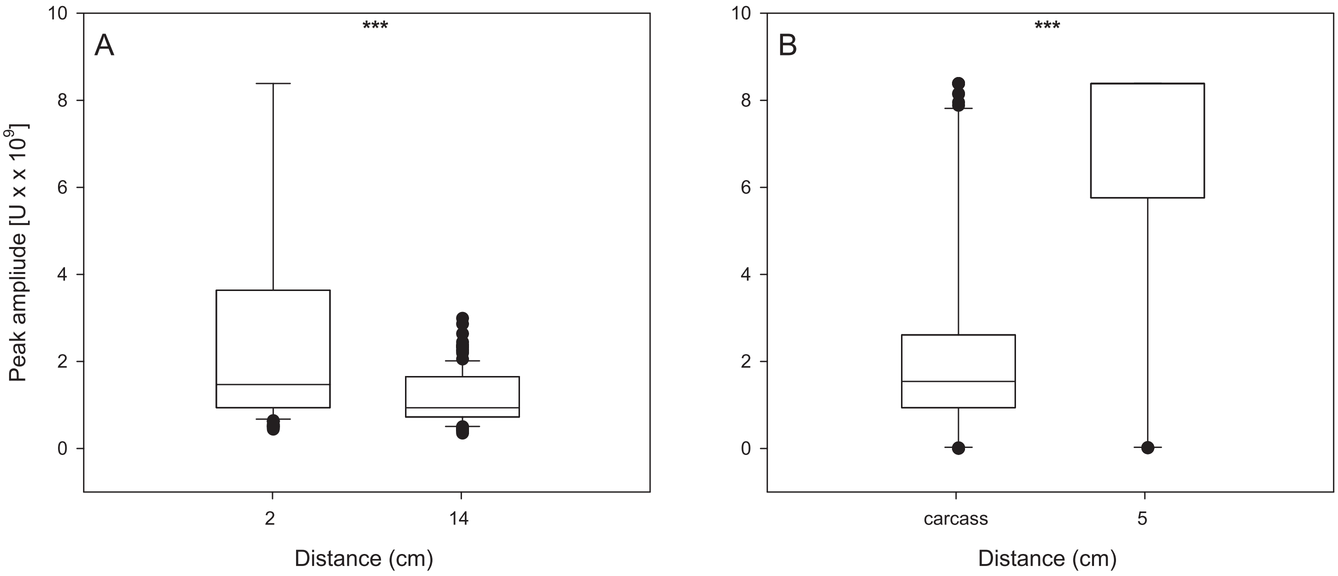
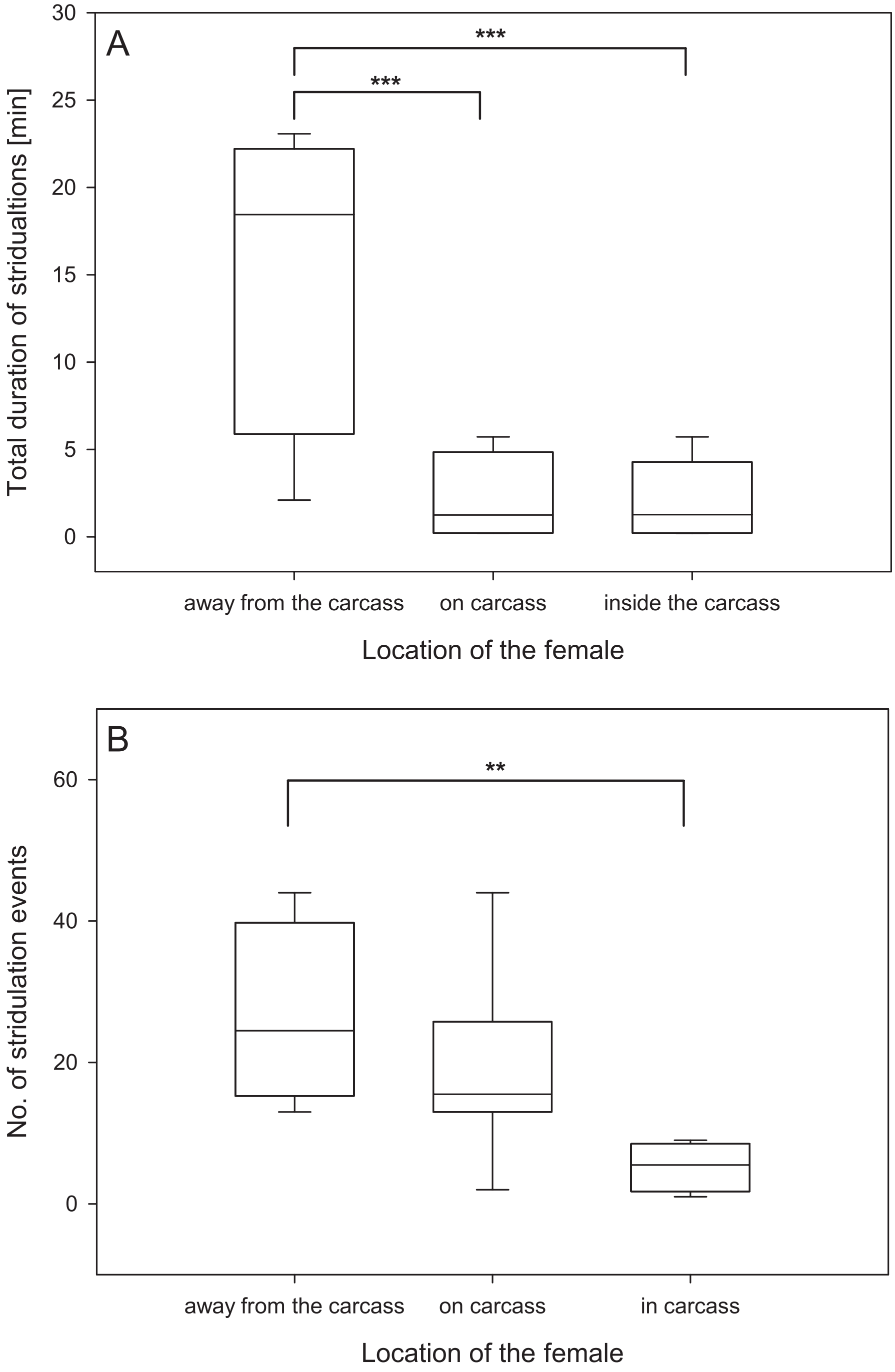


FIGURE 6 Boxplot showing peak amplitude (measured in U which is proportional to the sound pressure) of a burying beetle brood care signal at (A) 2 and 14 cm away from the beetles and (B) recorded directly from the carcass (0 cm) and at 5 cm away from the carcass, measured in forest soil. (A) 138 and (B) 80 repeated measures taken from eight individuals. The boxplots show the median value and the first and third quartile (upper and lower box). The whiskers show 1.5× the interquartile range. Outliers are shown with circles. Asterisks indicate significant differences (generalized linear model: $^{***}p < 0.001$).

that substrates have various properties and, thus, affect signal transmission (Elias et al., 2010; Magal et al., 2000). For example, the drumming signals of *Schizocosa stridulans* (Stratton) are best transmitted over leaf litter so that reproductive success is increased when this substrate is used. With other tested substrates, such as red clay or pine litter, signal transmission was less favorable (Elias et al., 2010). Effects of substrate on signal transmission have been found in numerous other species with a variety of substrates (Elias & Mason, 2014). With peat having the highest humus content and bulk density (Table S1), the difference in attenuation might be based on those parameters. Further in-depth analysis of soil parameters and signal attenuation would be needed to establish which parameter is most important in attenuation. Our findings have repercussions for future laboratory tests, which must choose a substrate matched to the beetle's natural signaling substrate, as unlike other insects where the substrate can be replaced by an artificial one for vibrational tests, these particular beetles require belowground conditions for their natural behavior. Additionally, previous studies on the southern green stink bug, *Nezara viridula* (Linnaeus), have shown that not only is the substrate crucial in signal propagation and individuals specifically choose it (Čokl, 2008; Elias & Mason, 2014; Virant-Doberlet & Čokl, 2004), but using an

FIGURE 7 Boxplots showing (A) total duration of stridulation (min) and (B) number of stridulation events of burying beetle females, depending on where the female was located ($n = 8$). The boxplots show the median value and the first and third quartile (upper and lower box). The whiskers show 1.5× the interquartile range. Outliers are shown with circles. Asterisks indicate significant differences (generalized linear model: $^{**}p < 0.01$, $^{***}p < 0.001$).



artificial substrate can skew results in an unnatural way (Miklas et al., 2001). We conclude that coconut coir is still suitable to represent natural conditions of propagation for burying beetles, but if the aim is to increase the signal propagation, then another substrate, such as peat, should be used.

As in Elias et al. (2010), in the course of our study, we only considered the soil type at a broadscale level, but other factors play a role, such as moisture, density, stiffness, and particle size (Elias & Mason, 2014; Mortimer, 2017). However, with soil being considered one of the most complicated biomaterials on earth (Young & Crawford, 2004), this might prove challenging. Regardless, future experiments should investigate the differences in signal propagation according to these physical parameters additionally (Mankin, 2022).

The maximum distance the vibrational signal can travel is crucial for brood care. Elias et al. (2010) also showed that the drum signal of *S. stridulans* became weaker with increasing distance; therefore, it is important to determine at which distances signals are still detectable, to evaluate whether they are suitable as a means of communication. Burying beetles lay their eggs in the substrate surrounding the carcass (Capodeanu-Nägler, 2018; Pukowski, 1933) and larvae can use the parent's stridulatory signals while finding the carcass (Niemitz, 1972;

Niemitz & Krampe, 1972). Additionally, parents (predominantly the males) patrol around the carcass (Fetherston et al., 1994; Trumbo, 2006), making signal propagation over distances relevant to both larvae and adults.

The effective range, or active space (Marten & Marler, 1977; Morton, 1975), of a vibrational signal is the area within which the signal is detectable by the receiver (Mazzoni et al., 2014; Šturm et al., 2019). Two components describing this are the amplitude of the signal at source, and the attenuation rate through the medium (Brenowitz, 1982). In this case, we found the signal was detectable (by the LDV sensor) at a distance up to 20 cm. Previous literature has shown that the area covered by a burying beetle couple is usually about 5–10 cm^3 (Pukowski, 1933). Although the sensitivity of this species is unknown, given the proximity of the beetles to the stridulations, we can hypothesize that these beetles are moving within the active space of the signal.

Our results when using the mouse carcass clearly deviate from all the other results. Here, the maximum amplitude on the carcass was lower than at 5 cm distance. From this result follows the possibility that the carcass amplifies the signal, as it is transferred to the substrate. There are several examples in the literature of animals using their environment to boost their signals (Daws et al., 1996; Herberstein & Tso, 2012; Holland et al., 1998; Lugli, 2013; Mortimer et al., 2016; Polajnar et al., 2012). In honeybees, for example, it was shown that they specifically seek out particular areas of the comb with a small open cell, which facilitates transmission of substrate-borne signals, leading to the recruitment of 3× more workers. This finding opens up new possibilities for the role of the carcass in the communication mode of burying beetles and should be investigated in more detail in the future. It is conceivable that carcass manipulation and carcass choice are also influenced by transmission properties.

In addition, the location of the parents while signaling during brood care significantly affected the total duration and number of stridulations. The female stridulated away from the carcass most frequently and for the longest duration. One possible explanation could be that the female was out of range of the larvae (in a tactile and visual sense) and thus stridulated to maintain contact with her larvae, which needs further tests to confirm.

While stridulation is clearly a predominant part of brood care (Conrad et al., 2022, 2024; Hall et al., 2015), our results did not provide evidence for a specific reaction of the larvae as a result of the stridulation signal. Further experiments need to ascertain whether such a reaction is present, perhaps on a finer scale than we were able to look at. We believe an experiment using scan sampling and simultaneous recordings could result in identifying a reaction of larvae to stridulations (Martin & Bateson, 1986). Direct observations are often clearer, if more difficult and time consuming, because larvae are hard to observe while feeding inside the carcass—anecdotal evidence seems to suggest that larvae do come up for feeding after stridulation from the mother (MA Prang, S Steiger & K. Streller, pers. comm.). Should such a response be observed, the question of which form of communication is present could possibly be resolved.

In order to prove that vibrational rather than acoustic signals are indeed used within the genus *Nicrophorus*, playback experiments could be performed (Hebets, 2005). That is, acoustic and vibrational replications of stridulations could be played back to the subjects and the response observed. If an expected response occurs to a solely vibrational stimulus, then vibrational communication is present, otherwise acoustic communication may be involved. However, for this particular species, no clearly observable behavior has been identified in response to naturally occurring chirps; therefore, playback experiments presumably would not yield useful results, although we know that stridulations have a strong impact on offspring (Conrad et al., 2022; Hall et al., 2015; Niemitz & Krampe, 1972). Larval reactions to stridulations could be a crucial first step for such playback experiments.

Finally, we show that bigger females and smaller males are more likely to produce stridulation after artificial manipulation. As the signal emitted after manipulation is thought to be a defensive signal to startle the predator into dropping the beetle (Buchler et al., 1981), we expected all beetles to respond similarly, regardless of size or sex. However, it has recently been shown that even taking into account the bigger elytra area, bigger males still show relatively more aposematic coloring (Lindstedt et al., 2019). Therefore, bigger males might make use of their coloring in defense and smaller males might use the stridulation defense signal. Additionally, there might also be behavioral differences in defense, which might account for these results. In future studies, both sex and size should always be accounted for.

Communication is particularly useful for social understanding during brood care. Both between offspring and parents and between the two parents, exchange is essential for optimizing biparental brood care (Conrad et al., 2024). For example, studies have shown that nymphs and parents of the tree locust *Umbonia crassicornis* (Amyot & Serville) communicate via vibrational communication. The nymphs produce synchronized signals to the mother to communicate the presence of a predator and thus trigger the mother's defensive behavior (Cocroft, 1999, 2001; Cocroft et al., 2000). Adult–larval communication is also known from *Polistes* wasps, in which larvae change their begging behavior due to the vibrational signals of the adults (Pepiciello et al., 2018). However, to date, vibrational communication during brood care has been demonstrated in very few subsocial species and the specific functions of most signals are still unclear.

Through our study, we were able to show that the transmission of burying beetle stridulation signals in soil depends on several factors, such as distance or substrate. Nevertheless, vibration communication is in principle conceivable in this case, as the signals are transmitted far enough in the natural substrate to allow complex communication. If this is the case, burying beetles would be one of the few subsocial species in which vibrational communication during biparental brood care has been demonstrated. However, the crucial missing aspect here is whether larvae respond to the signals, as that will define whether the communication is present or not (Bradbury & Vehrencamp, 2011). Playback experiments are a crucial next step in tandem with more detailed behavioral observations.

AUTHOR CONTRIBUTIONS

Taina Conrad: Conceptualization; data curation; formal analysis; funding acquisition; investigation; project administration; resources; supervision; visualization; writing – original draft; writing – review and editing. **Louise Roberts:** Conceptualization; writing – review and editing. **Sandra Steiger:** Resources; supervision; writing – review and editing. **Marie Ringlein:** Formal analysis; investigation; visualization; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

All data and code are available on github (https://github.com/TainaConrad/Conrad-et-al-2024).

ORCID

Taina Conrad https://orcid.org/0000-0002-0115-498X

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. An example of a microphone recording of a burying beetle defensive signal (for better visibility) with each part of the signal marked.

Table S1. Soil parameters of the three soil types tested.

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