

Abstract

1. Host-parasite interactions shape ecosystems by driving species dynamics, yet their influence is typically considered in terms of host mortality rather than the chemical environments generated during infection and decomposition.
2. Here, we show that parasitic infection actively reconfigures the chemical phenotype of decomposing hosts, reshaping ecological interactions through volatile-mediated signaling. Using soil-dwelling parasites -entomopathogenic nematodes (EPNs) and their symbiotic bacteria- as a model system, we integrate behavioral assays, comprehensive volatilomic characterization, and CO₂ quantification across three insect orders and two EPN species over four post-mortem intervals.
3. We demonstrate that infection generates a distinct and conserved volatile signature dominated by carboxylic acids, sulfur-containing compounds, and unsaturated hydrocarbons, accompanied by elevated CO₂ emissions.
4. These infection-derived blends elicit contrasting behavioral responses: they repel non-natural hosts while consistently attracting natural hosts, indicating that infected cadavers function as chemically structured information hubs. In contrast, frozen-killed insects exhibit fermentation-type profiles, and bacteria-only treatments produce simplified alkane-dominated blends, demonstrating that the full chemical phenotype emerges from the nematode-bacteria complex.
5. Our findings reveal parasitic infection as a process that reorganizes decomposition chemistry with measurable behavioral consequences, uncovering a previously underappreciated mechanism by which parasites structure soil food webs and influence multitrophic interactions.

Key words: Volatile Organic Compounds (VOCs), GC-MS, Nematode, Cadaver Decomposition, Soil Interactions, Chemical Ecology.

1. INTRODUCTION

Host-parasite interactions are key drivers of ecological dynamics, traditionally understood through their effects on host survival and population regulation (Weisskopf et al., 2021). However, beyond mortality, parasites can modify host traits in ways that influence interactions with other organisms, generating cascading effects across trophic levels (Lopes, 2022). In soil ecosystems, where visual cues are limited or absent, these interactions are principle mediated by physical and chemical signals that structure movement, interactions, and community assembly, in spatially complex below-ground environments (Wenke et al., 2010). Volatile cues play a central role in processes such as host-parasite interactions, scavenger deterrence, and microbial succession (Weisskopf et al., 2021). Growing evidence indicates that organisms respond not only to direct infection but also to the risk of parasitism, adjusting behavior and physiology in ways that reshape species interactions even in the absence of parasite contact (Foltan and Puza, 2009; Lopes, 2022; Cassells et al., 2024). Despite their ecological importance, the cues emitted by parasitized or decaying hosts (and their evolution throughout the infection) remain poorly characterized, particularly in soil systems. Behavioral studies show that insects may avoid or be attracted to infected cadavers depending on species identity and ecological context (Zhang et al., 2019; Grunseich et al., 2021). These contrasting responses suggest that infected cadavers function as chemically complex information, simultaneously conveying cues of risk, resource availability, and microbial activity. However, the chemical mechanisms underlying these behavioral decisions remain largely unresolved. Most existing studies focused on single host species or isolated time points (Foltan and Puza, 2009; Grunseich et al., 2021; Cassells et al., 2024), leaving unanswered how infection-driven chemical cues (Volatile organic compounds [VOCs] and CO₂) change over post-mortem stages and differentially shape behavioral responses among insect taxa. Addressing this gap is essential for linking parasite-driven decomposition to broader soil ecological dynamics. Entomopathogenic nematodes (EPNs) and their bacterial symbionts provide a powerful model for studying the processes. These soil-dwelling parasites, primarily from the families: Steinernematidae and Heterorhabditidae (Nematoda: Rhabditida and Panagrolamida, respectively) (Hodda, 2022) maintain mutualistic associations with enteric bacteria of the genera *Xenorhabdus* and *Photorhabdus*, respectively (Boemare et al., 1993). EPN species differ in host-seeking behavior, infection strategies, and host range (Shapiro-Ilan and Lewis, 2024). Infective juveniles (IJs) represent the mobile, infectious stage. Upon locating a suitable host, they enter through natural

openings (spiracles, anus, or mouth) (Stock, 2019). Once inside the hemocoel, IJs release their symbiotic bacteria, which produce toxins and other molecules that induce septicemia and kill the host within 24 to 72 hours (Shapiro-Ilan and Gaugler, 2002; Stock, 2019). The bacteria also secrete fungicidal and antimicrobial compounds that delay decomposition and protect the cadaver from saprophagous and scavenging organisms (Wang et al., 2012; Tobias et al., 2017; Blanco-Pérez et al., 2019). Additionally, bacteria degrade host tissues into nutrients that sustain nematode development (Clarke, 2008). EPNs' reproductive cycle lasts approximately 6-18 days, during which multiple generations can be completed within a single cadaver. After cadaver resources are depleted, new IJs that have re-established their symbiosis emerge to seek new hosts (Stock, 2019). These nematodes navigate soil guided by diverse chemical and physical cues: they detect volatile signals emitted by attacked plant roots (e.g., (E)- β -, caryophyllene), insect-specific odorants (Rasmann et al., 2005; Turlings et al., 2012; Dillman et al., 2012; Machado and Von Reuss, 2022), CO₂ and physical cues such as vibrations and electric currents produced by insects (Torr et al., 2004; Shapiro-Ilan et al., 2012), to locate potential hosts. Also, they can modulate the volatile compounds emitted by infected cadavers to attract new hosts (Grunseich et al., 2021) and simultaneously can repel potential competitors and cadaver predators, promoting nematode survival and life cycle completion (Foltan, and Puza, 2009; Jaffuel et al., 2022). Insects can also recognize EPN's cues to avoid infection (Zuzarte-Luís and Mota, 2018). Therefore, in the parasite-host system, chemical influences between them can broaden multitrophic relationships. Recent research demonstrates that EPNs-infected cadavers emit distinctive VOC blends emphasizing the central role of chemical communication in soil ecosystems (Helms et al. 2019; Grunseich et al., 2021). These VOCs influence surrounding organisms in complex ways. Helms et al. (2019) evidenced that cues associated with infected cadavers or live EPNs can activate plant defense responses. Similarly, Grunseich et al. (2021) revealed that different EPN species emit distinct VOCs, suggesting species-specific ecological roles. However, whether these cues originate primarily from the host, nematodes, symbiotic bacteria, or their interaction remains unclear. More importantly, the temporal dynamics of VOCs and CO₂ emissions throughout the EPNs-infection cycle and their impact on putative predators or hosts remain poorly characterized. Likewise, the influence of host species and the nematode-bacteria association on VOCs and CO₂ profiles is largely unknown. Addressing these gaps requires integrating behavioral ecology with chemical analysis across the full course of EPNs-infection and decomposition process to unravel how EPNs

remodel the ecological framework around them. Here, we assess how EPN-infected cadavers shape the behavior of uninfected insects and identify the chemical signatures underlying these responses. We assessed behavioral responses using four ecologically and phylogenetically distinct insect hosts: two naturally exposed to EPNs, *Agriotes* spp. (Coleoptera: Elateridae) and *Hermetia illucens* (Diptera: Stratiomyidae) and two not naturally exposed but commonly used for EPNs rearing, *Galleria mellonella* (Lepidoptera: Pyralidae) and *Tenebrio molitor* (Coleoptera: Tenebrionidae). We first quantified attraction or avoidance of uninfected larvae in dual-choice bioassays and direct-contact bioassays. Chemical analyses (VOCs and CO₂) were performed on three species (*H. illucens*, *G. mellonella*, and *T. molitor*) infected by two EPN species (*Heterorhabditis bacteriophora* enema [Hb] and *Steinernema carpocapsae* B14 [Sc]). For these species, we characterized volatile emissions across four Post-Mortem Intervals (PMIs) spanning the entire life cycle of the EPNs within the host. Finally, we compared EPN-infected cadavers with cadavers injected with only their symbiotic bacteria to determine whether chemical modulation is driven by the symbiotic complex or by the bacteria alone. This combined behavioral and chemical analysis reveals how cues mediated interactions link parasitism, decomposition, and nutrient cycling, reinforcing the role of EPNs as ecosystem engineers rather than simple predators. Our results show that EPN infection transforms host death into a chemically structured process: through the combined activity of nematodes and symbiotic bacteria, infected cadavers emit distinct volatile and CO₂ signatures reshape insect behavior and alter ecological interactions during decomposition. This study thus uncovers an overlooked axis of host-parasite communication.

2. MATERIALS AND METHODS

2.1 Organism for experiments

Agriotes spp. were collected in spring 2024 in a crop field and a meadow owned by the University of Liège (Gembloux, Belgium; 50.565699, 4.701981). As the sites are university property and the collected species are common agricultural pests with no protection status, no specific access or collection permit was required. Click beetles (adult stage) were collected in the morning using passive traps consisting of tarpaulins covered with cut grass. Collected adults were identified in the laboratory (taxonomical and molecular identification) and belonged to the following species: *Agriotes lineatus* (PQ066666), *Agriotes sputator* (PV490812), and *Agriotes obscurus* (PP892771). Adults were separated by species and maintained in rearing boxes (32 l – 39 cm × 37 cm × 27 cm)

with a maximum of 50 individuals per box. Each box was filled with a 1:1 (v/v) mixture of potting soil (La Plaine Chassart brand; dry matter: 26 %, organic matter 20 %, pH (water) 5-6.5, electrical conductivity: 400-600 μ S/cm) and vermiculite. The substrate was maintained at 80% of its water-holding capacity (WHC). Rearing took place at room temperature ($21 \pm 1^\circ\text{C}$), and adults were fed *ad libitum* germinating organic barley (Walagri, Belgium), renewed every three weeks. After five months, wireworms (larval stage) were collected from the substrate and separated by weight. They were individually placed in 5 cm³ boxes without food and stored at 4°C for up to 4 months. One month prior to the experiments, wireworms were removed from cold storage and fed organic carrots *ad libitum* to exit dormancy. Only larvae at the L11 (14 - 16 mm) developmental stage (Sufyan et al., 2014) were selected for behavioral assays.

H. illucens larvae were obtained from a Black Soldier Fly (BSF) colony established in 2017 (Hoc et al., 2020) and maintained at the Laboratory of Functional and Evolutionary Entomology in Gembloux Agro-Bio Tech (Belgium). They were reared at a temperature of $27 \pm 1^\circ\text{C}$ and a relative humidity of $60 \pm 5\%$. Larvae were maintained in plastic boxes (35 cm $\times$ 20 cm $\times$ 10 cm) and fed *ad libitum* with chicken feed (Avene, Leuven, Belgium) and mechanically extracted flax cake (Scam, Andenne, Belgium), ground to a particle size of 0.750 mm using Pulverisette 19 grinder (Fritsch, Germany). In each box, 100 larvae were maintained until the experiments. Only L4 (aged 14-15 days) developmental stage (Purkayastha and Sarkar, 2022) were selected for behavioral assays.

G. mellonella larvae were reared following the protocol described by Vicente-Díez et al. (2021). Eggs were maintained in closed plastic boxes (31–18.5 Ø – 15cm high) equipped with a metal grid to allow airflow. Larvae were fed *ad libitum* with an artificial diet composed of a mixture of dry dog food (39%), honey (11%), glycerin (11%), water (8%), and wheat bran (31%). The rearing boxes were kept in an incubator set at 30 °C with 40% relative humidity for four weeks. Only L5-L6 (1.5 - 2 cm) developmental stages (Rahman et al., 2017) were selected for behavioral assays.

T. molitor larvae were reared according to the protocol of Laboratory of Functional Entomology (Liège University, Gembloux, Belgium) (Caparros Megido et al., 2018). Larvae were maintained in open plastic boxes (26 cm $\times$ 16 cm $\times$ 8 cm) placed at room temperature ($21 \pm 1^\circ\text{C}$) and fed *ad libitum* with a mixture of organic wheat bran and flour (70:30). Carrots were added as water supply

every two weeks. Only L11-L12 (1.5 - 2 cm) developmental staged (Park et al., 2014) were selected for behavioral assays.

2.2 Entomopathogenic nematodes

Entomopathogenic nematode (EPN) species *H. bacteriophora* (GenBank PQ199404 and PQ436960), and *S. carpocapsae* (GenBank PQ199401) were reared using last instar *G. mellonella* larvae as hosts. Ten larvae were placed in a Petri dish (5.5 cm Ø) (five and five larvae) on a filter paper containing 250 IJs of each EPN species. The nematode suspension was maintained under continuous agitation in a beaker using a rotary shaker set at 40 rpm to ensure a homogeneous distribution of nematodes throughout the solution. Three aliquots of 5 µL each were collected from the continuously mixed suspension and transferred to a counting chamber for EPN enumeration under a stereomicroscope. The number of EPNs in each aliquot was recorded, and the mean number of EPNs per 5 µL was calculated. Based on this average, the concentration of EPNs in the suspension was determined and adjusted accordingly the final expected concentration. After 48-72h, infected cadavers were transferred to a White trap system (White, 1927) to collect IJs, the time range depending on the insect and nematode species used, which emerged 14 to 15 days after infection. The harvested IJs were maintained in ventilated culture flasks (250 mL/75 cm²; Falcon®) for a maximum of one week at 21± 1°C to ensure a continuous supply of EPNs for experimental use.

2.3 Infections

For infections, all species were used except *Agriotes* spp., which were only exposed to the odor of infected *T. molitor* during the bioassays, as both are coleoptera. The different infections were scheduled so that all infected individuals reached the PMI (Post-Mortem Interval) on the same day. To achieve this, the number of IJs used per larva was adjusted for each species based on previous laboratory experiments. For *H. illucens* 500 IJs, for *G. mellonella* 50 IJs, and for *T. molitor*, 200 IJs/larvae were used, based on differences in body size and susceptibility. The infections were performed in Petri dishes of 55 mm Ø, lined with moistened filter paper. A specific number of larvae (five per dish) were placed, and the corresponding number of IJs suspended in water was pipetted directly onto the filter paper. The filter papers were moistened with a maximum of 350 µL. If more solution was needed to obtain the required number of IJs, the filter paper was first

drained using a porcelain funnel filtration, to remove the excess water. Conversely, if the addition of IJs was less than 350 μ L, the volume was topped up with tap water to reach 350 μ L. The dishes were then incubated in the dark at $21 \pm 1^\circ\text{C}$ for 48 hours to allow the nematodes to infect the hosts. After incubation, dead larvae were recovered and stored under identical conditions but in 90 mm $\varnothing$ petri dishes until used in behavioral and chemical experiments.

2.4 Bacterial isolation and injection

Bacterial isolation and identification were conducted following the protocol described by Chacón-Hurtado et al. (2024). Bacterial injections were performed with minor modifications of the method described by Loulou et al. (2023). *G. mellonella* larvae were injected with the entomopathogenic bacteria *Photorhabdus laumondii* subsp. *laumondii* (GenBank PP401695) or *Photorhabdus thracensis* (GenBank OR140086). Overnight bacterial cultures were centrifuged at $1890.515 \times g$ for 10 min, and the resulting pellets were washed repeatedly with sterile phosphate-buffered saline (PBS). Larvae were injected with a suspension containing 10^6 CFU/larva. Under a laminar flow hood, 5 μ L of bacterial suspension were injected into the abdominal spiracle using a sterile gas-tight syringe fitted with a 22S gauge, Sharp 10–12° beveled, hypodermic needle (Hamilton 700 series). After injection, larvae were maintained in sterile plastic containers in the dark at $21 \pm 1^\circ\text{C}$. Mortality was assessed at 24, 48, and 72 h post-injection (Figure 1).

2.5 Behavioral assays

Prior to behavioral assays, larvae were individually isolated in 5 cm³ plastic box filled with moistened vermiculite (w/v, 1:1). Specific starvation periods per species were applied to standardize hunger levels, optimized in previous experiments: *G. mellonella* larvae five days, *H. illucens* larvae six days and, *T. molitor* and *Agriotes* spp. larvae seven days, prior to the experiments. To minimize physiological bias, coleoptera larvae and diptera larvae exhibiting signs of molting were excluded from the experiment (Figure 1). Specifically, individuals displaying pre-molt indicators (white transverse lines), molt or post-molt marks (whitish coloration) were systematically removed, as their feeding activity is low during the molting process (Furlan, 2004).

2.5.1 Dual-choice bioassays

Attraction or repulsion to cadavers' odors was assessed using a two-branch olfactometer system, adapted from Chacon-Hurtado et al. (2023). This device consists of a straight glass tube (21 cm

long, 3.2 cm internal diameter) with openings on both ends and a central GL14 inlet for larval introduction. The tube was filled with moistened vermiculite at the same 1:1 (w/v) ratio as used for larval isolation. To accommodate species-specific movement behavior, different quantities of vermiculite were used (the quantity of vermiculite was adapted according to species in previous experiments): 20 g for *G. mellonella* and *H. illucens* assays, and 40 g for *T. molitor* and *Agriotes* spp. assays. Each end of the olfactometer was connected to a 100 mL borosilicate bottles (Duran, Belgium). For treatment conditions, bottles were filled with 28 g of moistened vermiculite, on top of which seven infected larval cadavers were placed and then covered with 20 g of moistened vermiculite. Control bottles contained only vermiculite without infected cadavers. All bottles were hermetically sealed and stored overnight in the dark at room temperature ($21 \pm 1^\circ\text{C}$) for 24 hours to allow odors diffusion. After this period, bottle caps were replaced with fine mesh to prevent larvae from accessing the cadavers, while allowing volatiles compounds to pass through. The olfactometer assembly was sealed with GL45 cap with an opening coupled with PTFE joints (BOLA, Germany). The system was left for 30 minutes to allow odor diffusion inside the olfactometer. Following odor diffusion, a single larva was introduced into the central GL14 inlet. The assay was conducted in darkness at room temperature ($21 \pm 1^\circ\text{C}$) for 1 hour. At the end of this period, the larva position was recorded. Larvae located within 3 cm from the entry point were recorded as non-responding. Each experiment involved 32 olfactometers operating simultaneously, testing all PMIs, treatment groups. Four sessions were carried out daily over a two-day period. Two independent trials were performed with new and fresh material for each insect species and for all eight conditions (including four PMIs per EPNs species) (Figure 1).

2.5.2 Contact bioassays

To assess whether insect cadavers infected by EPNs act as repellent or deterrent in a foraging context, a contact-based assay was designed using a crystallizing dish arena (18.5 cm Ø). A filter paper was placed on the bottom of each dish and 5.5 ± 0.5 mL of distilled water was added to maintain humidity, taking care to avoid air bubbles between the dish and the paper. Three grams of vermiculite were placed along the edge of the dish to form the infected zone, keeping the same shape across replicates. The vermiculite was then surrounded by seven insect cadavers (Figure 1). A single live larva was then introduced near the opposite edge, away from the infected zone. The arena was then covered with its lid. Behavioral activity was recorded using a digital infrared camera

237 (Vahoiold, FHD 1080P). Two arenas were recorded simultaneously under red light to simulate dark
238 conditions.

239 The actual trial lasted 20 minutes starting from the moment the larva was introduced into the arena.
240 During each trial, four arenas were recorded simultaneously using two cameras. In total, 12
241 replicates were conducted for each larval species and EPN species. For this test only PMI one and
242 11 were evaluated because the most contrasted result was found. To provide appropriate controls,
243 larvae were killed using liquid nitrogen. These cadavers were then placed under the same
244 conditions as the infected ones, to obtain PMIs of 1 and 11 days. 12 replicates were performed with
245 these control cadavers for each larval species. In total, 288 crystallizing dishes were analyzed,
246 corresponding to 96 hours of behavioral observation.

247 Behavioral data were collected using BORIS (Behavioral Observation Research Interactive
248 Software; v. 8.4.1). Three main behaviors were quantified over the 20-minute period: the latency
249 to enter infected zone, the total time spent in infected zone, and the number of entries into infected
250 zone (Figure 1).

251 2.6 Chemical cues

252 2.6.1 Carbon dioxide (CO₂) sampling

253 Carbon dioxide (CO₂) emissions were measured from seven insect cadavers per treatment, either
254 infected with EPNs or killed with liquid nitrogen. Measurements were conducted using a 1-L
255 airtight glass chamber equipped with four sealed GL14 ports and one GL45 port. Prior to each
256 recording, a 10-min baseline measurement was performed in the empty chamber to verify the
257 stability of ambient CO₂ concentrations in the experimental room. These measures served as
258 chamber controls. CO₂ concentrations were recorded using a CO₂ probe (GMP252, Vaisala),
259 inserted through the GL45 port. Cadavers were placed in a 50-mm diameter Petri dish and
260 introduced into the chamber, which was then hermetically sealed. After a 50-min equilibration
261 period (validated in preliminary experiments and standardized across species), the probe was
262 inserted for an additional 10 min to allow sensor stabilization, resulting in a total equilibration time
263 of 60 min. CO₂ concentrations were then recorded once per minute for 10 min. This protocol was
264 applied to all insect species and post-mortem intervals (PMIs). Baseline CO₂ levels in empty
265 chambers were measured before the introduction of any treatment, ranged from 409 to 648 ppm,
266 with two outliers at 885 ppm and 831 ppm. The mean CO₂ concentration in the chamber was 516.46

± 4.93 ppm (mean ± SEM). Due to the presence of these outliers, data recorded immediately afterward were carefully checked to verify that the probe did not take measurements with an incorrect initial calibration. No anomalies were detected. Therefore, the baseline CO₂ values were maintained in the treatment measures. Chamber measurements served as the reference level for all models (Figure 1).

2.6.2 Volatile organic compounds (VOCs) collection and identification:

VOCs emitted by insect larvae were collected using HiSorb® extraction probes (DVB/CWR/PDMS; Markes, Llantrisant, UK), which allow passive headspace sampling. For each treatment, seven larvae were placed in 20-mL glass vials (n = 5 replicates per treatment). Vials were crimp-sealed and incubated in a water bath at 30 ± 1 °C to promote VOC release. HiSorb® probes were inserted into the vial headspace and VOCs were collected for 24 hours under dark conditions. Probes were subsequently analyzed using thermal desorption gas chromatography coupled to mass spectrometry and flame ionization detection (TD-GC-MS/FID; Shimadzu, Kyoto, Japan). VOCs were desorbed at 280°C for 8 min using a thermal desorber (TD30R, Shimadzu), followed by cryofocusing at –20 °C in a glass trap. The trap was then heated to 270 °C, and analytes were injected in splitless mode onto the GC column. Compound separation was achieved using an HP-5 ms capillary column (30 m × 0.25 mm × 0.50 µm; Agilent Technologies). The oven temperature program was as follows: 40 °C initial temperature, increased to 200 °C at 5 °C/min, then to 300 °C at 10 °C/min, with a final hold of 12 min. Helium was used as the carrier gas in constant-pressure mode at 200 kPa. The MS interface temperature was set to 230 °C and the ion source temperature to 200 °C. Mass spectra were acquired over a range of 35–300 m/z with a scan time of 0.2 s. Detector voltage was set to 0.1 kV during MS tuning. VOC identification was performed by comparison of mass spectra with reference libraries (NIST, FFNSC) and supported by retention indices calculated from a homologous series of n-alkanes (C7–C30; 10 µg/mL in n-hexane). Retention indices were compared with published values. Compound identities should therefore be considered putative unless confirmed with authentic reference standards. Chromatograms were integrated using GCMSsolution software (Shimadzu). Sampling order was randomized across experimental days (Figure 1).

Replication statement

Scale of inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale
Individual larvae (within species)	Individual larva exposed to odor of infected cadavers (dual-choice bioassays)	60 ± 4 larvae per species (treatment $\times$ PMI) 4 sessions for 2 days; 2 independent trials run. Total: 2,137
Individual larvae (within species)	Individual larva exposed to cadavers (contact bioassays)	12 larvae per species $\times$ treatment $\times$ PMI
Cadaver groups (chemical CO ₂ emission)	Groups of 7 cadavers per treatment.	5 replicates $\times$ treatment $\times$ species $\times$ PMI, with independent chamber baselines. Total: 360
Cadaver groups (chemical VOCs emission)	Groups of 7 cadavers per vial.	5 replicates $\times$ treatment $\times$ species $\times$ PMI, randomized across days. Total: 360
Larval infection units	Petri dish containing 5 larvae exposed to EPNs	Multiple independent infection batches per species to synchronize PMIs.
Bacterial infection	Individual larvae are injected with bacterial suspension.	Replicated across independent batches (mortality monitored at 24, 48, 72 h).

296

297 2.7 Data analysis

298 All statistical analyses were performed using R (version 4.1.3). For **dual-choice bioassays**,
299 binomial general linear mixed models (GLMM, logit link) were first fitted for each condition to
300 determine whether the larvae were attracted to or repelled by odors from infected cadavers ("lme4"
301 package). Experimental date was included as a random effect. Corresponding binomial general
302 linear models (GLMs) without random effect were also fitted, and model performance was
303 compared using likelihood ratio test (ANOVA). Final model selection was performed using Chi-
304 square tests implemented with the drop1() function. In most cases, null models provide the best fit.
305 For **contact bioassays**, all four larval species and experimental conditions were combined for
306 analysis. Differences in the number of entries into infected zone were first assessed using a Chi-

square test. When significant, post-hoc comparisons were conducted using Holm correction. Latency data were right-skewed and were therefore log-transformed [$\log(\text{latency} + 1)$] prior to analysis. Latency to enter in the infected zone and the time spent within the zone were analyzed using linear models (lm), with larval species, condition (type of cadaver), and PMI as explanatory variables. Final models were selected using Akaike Information Criterion (AIC), computed with the drop1() function. Model assumptions were assessed graphically using residual diagnostic plots, and no major deviations from normality or homoscedasticity were detected after transformation. Post-hoc comparisons were performed using estimated marginal means (“emmeans” package) with Tukey adjustment for multiple comparisons. The number of entries into the infected zone was analyzed using a Poisson generalized linear model (GLM) (log link). Overdispersion was assessed using the formula: $\text{sum}(\text{residuals}^2) / (n - \text{length}(\text{coef}(\text{mod})))$. Post-hoc comparisons were performed using emmeans and pairs with a Tukey adjustment. For **CO₂ measurements**, one model was fitted per species, using CO₂ values from empty chambers as reference. An ANOVA was used to compare several models: a generalized linear mixed model (glmer) with a random effect (IPM | ind), a linear mixed model (lmer) with (1 | ind) (“lmerTest” package), and a simple linear model (lm). If model assumptions were checked for the selected model. If the assumptions were violated, data were log-transformed; if violations persisted, a Box-Cox transformation (using the boxcox () function from the “MASS” package) was applied to estimate the optimal transformation. In our study, this led to an inverse transformation being most appropriate. Multiple comparisons were conducted using emmeans. Temporal autocorrelation of residuals was assessed using autocorrelation functions plots (acf ()), to ensure independence of observations over time. **VOCs profiles** were analyzed using Permutational Multivariate Analysis of Variance (PERMANOVA) based on Euclidean distance matrix with 999 permutations (package «vegan») after verification of the application conditions. Pairwise comparisons were performed using Bonferroni correction. Then a Principal Component Analysis (PCA) was performed to visualize the data, and a Partial Least Squares-Discriminant Analysis (PLS-DA) was applied to discriminate PMIs based on their VOC profiles (“FactoMineR”, “factoextra”, “mixOmics” packages) (Hervé et al., 2018). Variables with a variable importance in projection (VIP) score > 50 were considered discriminant and reported in the results. Figures were generated using ggplot2.

Ethics statement. This study involved only invertebrates (insects and entomopathogenic nematodes) and bacteria. No ethical approval was required, as these organisms are not covered by

Directive 2010/63/EU on the protection of animals used for scientific purposes or by its Belgian transposition (Royal Decree of 29 May 2013). Control insects were killed by immersion in liquid nitrogen to ensure rapid death.

3. RESULTS

3.1 Behavioral assays

3.1.1 Dual choice bioassays

Insect responses split along species lines when exposed to parasite-infected cadaver cues:

Natural hosts (*Agriotes* spp. and *H. illucens*) were predominantly attracted to infected cadavers, whereas non-natural hosts (*G. mellonella* and *T. molitor*) exhibited neutral or repulsive responses depending on nematode species and PMIs. *Agriotes* spp. showed robust attraction to both Hb- and Sc-infected cadavers across decomposition stages (all $p < 0.042$). *H. illucens* was similarly attracted for Sc-infected cadavers (all $p < 0.040$), although responses were temporally structured, at later PMIs for Hb-infected cadavers (PMI11 $p = 0.001$). In contrast, *G. mellonella* and *T. molitor* displayed selective avoidance, particularly toward Sc-infected cadavers at late decomposition stages (Figure 2; Details in SI Appendix Table S1). These results suggest that infected cadavers produce context-dependent cues that drive different behavioral responses across insect species, shaped by host-parasite compatibility rather than only decomposition.

3.1.2 Contact bioassays

Hierarchical structuring of behavioral decisions around infected cadavers. Behavioral engagement with cadaver-associated zones unfolded across three sequential components: entry probability, latency to enter, and post-entry persistence, each governed by distinct statistical drivers. **Initial engagement:** species identity dominates entry probability. Entry probability differed significantly among species ($\chi^2_3 = 22.651$ $p < 0.001$, *Agriotes* spp. as reference; SI Appendix Table S2a), likewise killing method exerted a weaker but significant effect ($\chi^2_2 = 9.118$ $p = 0.010$; SI Appendix Table S2a). Interactions between these two factors were not supported ($\chi^2_6 = 11.650$ $p = 0.070$), indicating that interspecific differences were broadly maintained across infection contexts and PMIs. Subsequent comparisons revealed that *G. mellonella* entered the cadaver zone less frequently than *Agriotes* spp., *H. illucens* and *T. molitor* (all $p < 0.044$; Figure 3A). At PMI1, coleoptera (*Agriotes* spp. and *T. molitor*) discriminated among killing methods,

367 showing lower probability of entering zones with Hb-infected cadavers relative to frozen-cadavers
 368 zone (OR = 0.29 df = 273 p = 0.014 and OR = 0.20 df = 273 p < 0.001 respectively). In contrast,
 369 *G. mellonella* exhibited no early discrimination but altered entry behavior at PMI11, particularly
 370 under Sc-cadaver zone (OR = 3.72 df = 273 p = 0.029) (Details in SI Appendix Tables S2b-S2c).
 371 Thus, entry probability primarily reflects species-specific baseline thresholds, with secondary
 372 modulation by infection identity. **Decision timing:** latency reflects species-level sensitivity with
 373 localized condition effects. Latency to enter the cadaver zone also differed significantly among
 374 species (F = 22.419 p < 0.001; Details in SI Appendix Table S3a). Pairwise comparisons indicated
 375 that natural host (*Agriotes* spp. and *H. illucens*) entered more slowly than non-natural host (*G.*
 376 *mellonella* and *T. molitor*) (all p < 0.003) (SI Appendix Table S3b and Figure S1). Although no
 377 global higher-order interaction was detected, within-species contrasts revealed context-dependent
 378 modulation. At PMI1 coleoptera (*Agriotes* spp. and *T. molitor*) exhibited longer latencies toward
 379 frozen-cadavers relative to Hb-infected cadavers (all p < 0.013). At PMI11, *Agriotes* spp.
 380 maintained this pattern (p = 0.026), whereas *G. mellonella* showed shorter latency toward Sc
 381 relative to Hb-cadavers (p = 0.029) (Details in SI Appendix Table S3c). Latency therefore reflects
 382 species-specific decision thresholds that can be locally adjusted by infection identity and
 383 decomposition stage. **Permanence in cadaver zone:** persistence emerges from a three-way
 384 interaction. Time spent within the cadaver-associated zone was structured by a significant
 385 interaction, indicating that sustained behavioral commitment depends on the combined effects of
 386 species identity, killing method, and PMI (F = 2.49 p = 0.023). Within-species contrasts revealed
 387 that *Agriotes* spp. consistently spent more time in zones containing Hb-infected cadavers at both
 388 PMI1 and PMI11 (all p < 0.034; Figure 3B). In contrast, *G. mellonella* spent more time near Sc-
 389 cadavers at PMI11 (p = 0.039; Figure 3B) (Details in SI Appendix Table S4a-S4b). No other
 390 consistent persistence effects were detected. Unlike entry probability or latency, sustained presence
 391 therefore emerges from context-dependent integration of species and infection cues. **Exploratory**
 392 **instability:** alternance reflects species-level strategy. Alternation frequency was strongly species-
 393 dependent (F = 18.33 p < 0.001; Figure 3C) and further modulated by a species and killing method
 394 interaction (F = 2.58 p = 0.019) but showed no consistent PMI effect. Post-hoc comparisons
 395 revealed that *T. molitor* exhibited significantly higher alternation rates than all other species across
 396 multiple treatments. This elevated exploratory turnover was observed under frozen, Hb- and Sc-

cadavers (all $p < 0.033$; Details in SI Appendix Table S5a-S5b). Thus, alternance primarily reflects species-level exploration strategy rather than decomposition-stage effects.

3.2 Chemical cues

3.2.1 Cadavers CO₂ emissions

CO₂ emissions exhibited species- and stage-specific patterns across cadaver treatments. In *H. illucens*, emissions provided only limited and transient discrimination among treatments, with higher levels from frozen-cadavers at PMI3 and from Sc-cadavers at PMI7 (all $p < 0.001$), converging by PMI11 ($p > 0.070$) (Figure 4A). In *G. mellonella*, CO₂ acted as a dynamic, temporally structured cue: early in decomposition (PMI1-PMI3) Sc-cadavers emitted the most CO₂ (all $p < 0.001$), followed by Hb- and frozen-cadavers; by PMI7, emissions from Hb- and Sc-cadavers converged ($p = 0.748$), remaining higher than frozen-cadavers (all $p < 0.001$). By PMI11 differences largely disappeared (all $p > 0.028$), highlighting early-stage EPN-induced CO₂ spikes with changing ecological relevance across the nematode life cycle (Figure 4B). For *T. molitor* cadavers, CO₂ emissions were consistently higher in nematode-infected than in frozen-cadavers (all $p < 0.001$). Early in decomposition, Sc-cadavers were dominant emitters, followed by Hb-cadavers, while frozen-cadavers remained lowest ($p < 0.001$). At PMI7, Hb- and Sc-cadavers remained elevated relative to frozen-cadavers ($p < 0.005$), and by PMI11, emissions of infected cadavers converged ($p = 0.244$), indicating stage-dependent treatment-specific dynamics (Figure 4C; Details in SI Appendix Table S6). In *G. mellonella* and *T. molitor*, parasitized cadavers consistently producing stronger metabolic signals than frozen-cadavers, translate in highest CO₂ emissions particularly during early to intermediate decomposition. These results indicate that CO₂ dynamics are temporally structured and may serve as a stage-dependent cue for ecological interactions.

3.2.2 Cadavers' volatile emissions

Parasitized cadavers generate volatilomic fingerprints specific to each host. In total, we analyzed 180 samples (60 per insect species). For each host, a complex blend of more than 100 VOCs was identified: 122 in *H. illucens*, 143 in *G. mellonella*, and 142 in *T. molitor* across all treatments and PMIs (Details in SI Appendix Table S8-S10).

Killing method strongly shaped both the composition and temporal dynamics of cadaver-associated volatile profiles in *H. illucens*.

Frozen-cadavers exhibited a progressive chemical diversification across decomposition. Early stages (PMI1) were dominated by sulfur compounds (Dimethyl disulfide [DMDS] and Dimethyl Trisulfide [DMTS], together with alcohols. As decomposition progressed, nitrogen-containing aromatics such as indole emerged, and by late stages the profile shifted toward a more nitrogen-dominated signature, reflecting a stabilized late-decomposition state. In contrast, Hb-infected cadavers maintained a comparatively simplified volatilome. Across decomposition stages, sulfur compounds remained dominant, with limited diversification over time, indicating a persistent sulfur-biased chemical signature associated with infection. Sc-infected cadavers displayed intermediate complexity. Although sulfur compounds were consistently abundant, phenolic compounds contributed substantially at several stages, producing transient increases in chemical diversity during early and mid-decomposition. However, this diversification was not sustained into late stages. Despite consistent detection of sulfur compounds across treatments, their relative contribution was stronger and more persistent in parasitized cadavers than in frozen killed (Figure 5A). Multivariate analyses confirmed clear treatment-specific chemical trajectories (all $p < 0.028$; Details in SI Appendix, Table S7; Figure 6A). Frozen-cadavers followed a gradual diversification pathway, whereas EPN-infected cadavers exhibited constrained temporal variability, consistent with infection-driven metabolic regulation (SI Appendix, Table S7). PCA revealed structured separation among treatments and PMIs (Figure 6A), and PLS-DA identified treatment-specific discriminatory compounds (VIP scores; SI Appendix, Table S8). Early frozen decomposition was characterized by alcohols, intermediate stages by short-chain acids and ketones, and late infected cadavers by sulfur- and aromatic-derived compounds, including thioesters and substituted ketones.

Parasites impose a distinctive chemical signature on the volatilome of *G. mellonella*.

Frozen-cadavers followed a gradual expansion in chemical complexity. Early PMIs were characterized by ketone dominance, particularly 2,3-butanedione and acetoin, forming a relatively simple signature. As decomposition progressed, additional compound classes, including lactones, sulfur compounds, and alcohols, emerged, culminating in a chemically richer late-stage profile. This pattern reflects progressive diversification over time. In contrast, EPN-infected cadavers exhibited a fundamentally different trajectory. Both Hb- and Sc-cadavers were marked by strong early sulfur dominance, DMDS and DMTS. However, their subsequent trajectories diverged. Hb-cadavers

displayed pronounced mid-stage diversification, incorporating aldehydes, ketones, alcohols, aromatics, alkenes, and pyrazines. This expansion was transient: by late decomposition, the profile simplified markedly, reverting to sulfur compounds and aromatic ketones as dominant components. Sc-cadavers exhibited earlier metabolic diversification, with sulfurs as predominant but accompanied of aldehydes and ketones, present at initial stages. Although sulfur compounds declined through intermediate stages, they remained proportionally more abundant than in Hb-cadavers. By late decomposition, the volatilome shifted toward ketone and pyrazine dominance, generating a distinct ketone-driven late signature. Overall, parasite infection imposed a shared temporal structure, early sulfur dominance, mid-stage diversification, and late-stage simplification, contrasting with the volatile signature observed in frozen-cadavers (Figure 5B). Multivariate analyses confirmed structured divergence among treatments and PMIs (Figure 6B). Frozen-cadavers showed moderate temporal separation in middle PMIs (all $p < 0.050$), whereas Hb-cadavers exhibited pronounced turnover consistent with infection-driven metabolic succession (all $p < 0.032$). Sc-cadavers displayed comparatively greater temporal stability and slower change in VOC profiles (SI Appendix, Table S7). PCA illustrated these divergence-convergence dynamics, and PLS-DA identified treatment- and stage-specific discriminatory volatiles (SI Appendix, Table S9), including alcohol enrichment at intermediate PMIs for frozen-cadavers, ester signatures at early PMIs and ketones and sulfurs at late PMIs in Hb-cadavers. In Sc-cadavers alcohol and sulfurs were discriminant at early PMIs.

Parasite infection alters chemical profile in *T. molitor* decomposition, redesigning odors cues:

Frozen-cadavers followed a gradual diversification pattern. Early decomposition was characterized by alcohol- and ester-dominated profiles, with alcohol becoming particularly prominent at intermediate stages. As decomposition progressed, additional compound classes, including sulfur compounds, ketones, aromatics, and pyrazines, accumulated, resulting in a progressively more complex late-stage blend. In contrast, Hb-cadavers exhibited sulfur-enriched profiles from the onset of decomposition. Early dominance of sulfur compounds, together with aromatic hydrocarbons such as styrene, defined the initial signature. Subsequent stages showed increased incorporation of ketones and additional aromatics; however, sulfur compounds remained a persistent structural component throughout decomposition, indicating sustained sulfur-centered metabolic activity. Sc-cadavers displayed a distinct temporal organization. Sulfur and nitrogen-containing compounds dominated early stages and remained proportionally stable through

intermediate PMIs. Although transient diversification occurred with the appearance of ketones and alkanes, late decomposition was marked by simplification toward sulfur-dominated blends, primarily driven by DMDS and DMTS (Figure 5C). This pattern suggests a sustained sulfur-based metabolic regime during the parasite life cycle. Multivariate analyses confirm consistent treatment-specific chemical differentiation (Figure 6C). Frozen cadavers exhibited moderate temporal diversification with limited separation among early stages, followed by clearer divergence after intermediate decomposition ($p < 0.016$) and stabilization at late stages (SI Appendix, Table S7). In contrast, EPN-infected cadavers-maintained sulfur-dominated signatures that remained distinct from frozen treatments across PMIs (all $p < 0.010$). Hb-cadavers showed greater temporal turnover (all $p < 0.030$), whereas Sc-cadavers displayed comparatively higher stability, diverging at late decomposition (all $p < 0.011$). PCA illustrated structured divergence among treatments and stages (Figure 6C), and PLS-DA identified stage- and treatment-specific discriminatory compounds (SI Appendix, Table S10), including alcohol and ester signatures at intermediate PMIs in frozen-cadavers and persistent sulfur markers in Sc-cadavers. In different hosts, results demonstrate that parasite infection reorganizes the temporal dynamics of decomposition-derived cues.

3.2.3 Volatile emissions of cadavers infected solely with symbiont

Symbiosis generates stage-structured sulfur signatures beyond symbiont metabolism alone.

To determine whether volatile production during decomposition is driven solely by symbiotic bacteria or requires the full nematode-bacteria symbiosis, we compared volatilomes generated by two *H. bacteriophora* populations (enema and UCH32913) and by their respective symbiotic bacteria across decomposition stages (Figure 7 - 8).

Nematode population identity modulates, but does not redefine, the sulfur trajectory. Both *H. bacteriophora* populations (enema and UCH32913) followed broadly similar temporal trajectories characterized by increasing sulfur dominance at late decomposition. Early stages exhibited mixed sulfur-alcohol profiles, followed by mid-stage diversification incorporating aldehydes and ketones. By PMI11, sulfur compounds accounted for a substantial fraction of the blend (~40%), indicating convergence toward sulfur-enriched terminal profiles (Figure 7A). Despite this shared trajectory, early-stage volatilomes differed between nematode populations, reflecting population-specific metabolic dynamics. These differences persisted across intermediate stages, although late decomposition showed partial convergence toward sulfur-dominated blends. Multivariate analyses

confirmed stage- and population-dependent structuring (Figure 8A; SI Appendix, Table S7), and PLS-DA identified late-stage of decomposition discriminatory ketones specific to the UCH32913 population (SI Appendix, Table S11). Thus, nematode population identity modulates timing and composition of volatile emissions but preserves a conserved sulfur-centered developmental trajectory.

Symbiont metabolism alone does not reproduce the structured sulfur persistence. Cadavers infected solely with symbiotic bacteria (*P. laumondii* subsp. *laumondii* and *P. thracensis* exhibited early sulfur-rich profiles similar to those generated by the full complex. However, this sulfur dominance was not sustained (Figure 7B). Across subsequent PMIs, alkane compounds became increasingly prominent while sulfur compounds declined sharply, resulting in volatilomes that lacked the persistent sulfur structuring observed under nematode infection. Although bacterial species showed temporal variability (all $p < 0.022$), overall compositions remained broadly similar and did not display the pronounced stage-structured sulfur reinforcement characteristic of the nematode-bacteria complex (Figure 8B; SI Appendix Table S7). PLS-DA highlighted BHT (Butylhydroxytoluene) was discriminatory at mid- and late-stages of decomposition in the two treatments (SI Appendix Table S12). Together, these results demonstrate that bacterial metabolism alone cannot reproduce the persistent, stage-structured sulfur signatures generated by the nematode-bacteria complex. The nematode therefore appears to play an active role in shaping the temporal dynamics of decomposition-derived odor landscapes, reinforcing sulfur-centered profiles across late stages.

4. DISCUSSION

Together, these findings identify parasite-driven decomposition as a key source of ecologically relevant chemical cues, linking host-parasite interactions to behavioral decision-making. They uncovered a previously unrecognized role of the nematode-bacteria complex in shaping the chemical trajectory of host decomposition and highlight its relevance in structuring multitrophic interactions within soil systems. VOCs released by the nematode-bacteria complex act as distant dual signals in the soil, mediating both attraction and avoidance among potential insect hosts. The behavioral responses of insects to EPN-infected cadavers reflected a pattern driven by the ecological context, host resistance, and the informational content of complex volatile emissions, producing during decomposition. Soil-associated species, particularly *Agriotes* spp. and *H.*

547 *illucens*, showed a persistent attraction to infected-cadavers during decomposition, consistent with
548 their frequent exposure to EPNs (Kaya and Gaugler, 1993; Diener et al., 2009). In *Agriotes* spp.,
549 this attraction probably reflects both documented resistance to EPN infection (Campos-Herrera and
550 Gutiérrez, 2009; Morton and Garcia-del-Pino, 2017; Chacon-Hurtado et al., 2024) as well as
551 opportunistic feeding behaviors, such as carnivorism and cannibalism (Samoylova and Tiunov,
552 2017), which may favor the exploitation of cadavers despite the risk of infection. Given that visual
553 and tactile cues were excluded, it is likely that attraction is mediated by generalist chemical cues,
554 such as CO₂ (driven by microbial and nematode respiration) (Ferris et al., 1995; Yu et al., 2025),
555 which is highly attractive to soil-dwelling insects (Schumann et al., 2014; Kojima, 2015; Barsics
556 et al., 2017), and reinforced by complex mixtures of volatile nitrogen-, sulfur- compounds and
557 ketones produced during infection. In contrast, non-natural hosts, such as *G. mellonella* and *T.*
558 *molitor*, exhibited primarily stage-specific avoidance or repellency. In *G. mellonella*, avoidance of
559 *S. carpocapsae* in late stages coincided with nematodes emergence (Stock, 2019) and reflects
560 increased sensitivity to signals of advanced infection rather than specific defenses against
561 nematodes, consistent with their high susceptibility to them (Hyrsl, 2011). In *T. molitor*, repellency
562 was limited to specific PMIs, indicating mixture dependent responses rather than simple reactions
563 to sulfur compounds, which were widely present in all treatments. Early avoidance of *H.*
564 *bacteriophora* cadavers likely resulted from high bacterial loads and secondary metabolites with
565 toxic or repellent properties (Clarke, 2008; Bode, 2009; Booysen et al., 2021; Lulamba et al., 2021),
566 while late avoidance of *S. carpocapsae* cadavers coincided with sulfur-dominated signals,
567 including DMDS, which had previously been shown to repel *T. molitor* (Plata-Rueda et al., 2017).
568 Taking together, these results demonstrate that volatile compounds produced by the nematode-
569 bacteria complex act as chemical signals carrying information whose ecological meaning-risk
570 versus resource-depends on the ecology of the host, its susceptibility, and its specific
571 coevolutionary history.

572 These species-specific responses likely arise from differences in how chemical information is
573 perceived and processed by their chemosensory systems. Although *Agriotes* spp., *H. illucens*, *G.*
574 *mellonella*, and *T. molitor* belong to distinct insect orders, they share a conserved chemosensory
575 architecture composed of odorant receptors (ORs), ionotropic receptors (IRs), gustatory receptors
576 (GRs), odorant-binding proteins (OBPs), chemosensory proteins (CSPs), and sensory neuron
577 membrane proteins (SNMPs), located within sensilla on antennae, palps, and mouthparts (Jacquin-

578 Joly and Merlin, 2004; Fleischer et al., 2018; Schmidt and Benton, 2020). However, the relative
579 abundance, sensitivity, and ligand specificity of these receptor systems differ among taxa. In
580 *Agriotes* spp., volatile perception is mainly associated with basiconic sensilla on the maxillary and
581 labial palps, which mediate orientation and feeding responses (Crombie and Darrah, 1947; Barsics
582 et al., 2014). In *H. illucens* larvae, transcriptomic studies have revealed a diverse repertoire of
583 OBPs, IRs, GRs, CSPs, and SNMPs, consistent with adaptation to volatile-rich decomposing
584 substrates (Scala et al., 2018; Scieuzo et al., 2021). Similarly, *G. mellonella* and *T. molitor* possess
585 OR-, IR-, and GR-based systems that likely mediate the detection of airborne and contact-
586 associated cues, although ligand specificity remains poorly characterized in larvae (Liu et al., 2015;
587 Zhao et al., 2019; Xie et al., 2025). Consequently, insects exposed to the same volatile blend may
588 interpret these cues differently depending on receptor tuning and downstream neural processing,
589 resulting in attraction, avoidance, or exploratory behavior. Such receptor-dependent variation
590 likely contributes to the contrasting responses observed among species in the present study.

591 Contact bioassays revealed species-specific strategies in response to infected cadavers, ranging
592 from avoidance to tolerance and exploratory behavior, reflecting risk assessment and behavioral
593 commitment. When confronted with the trade-off between burrowing into a substrate containing
594 cadavers or exploring an empty zone, *G. mellonella* exhibited the lowest probability of entering
595 the cadaver zone and spent the least time there, reflecting minimal substrate engagement. Although
596 capable of burrowing under laboratory conditions, *G. mellonella* naturally forages by climbing and
597 surface exploration within beehives rather than penetrating soil (Kwadha et al., 2017), contributing
598 to its reduced interaction with cadaver-associated substrates and rapid withdrawal once contact
599 occurs. In contrast, *Agriotes* spp. and *H. illucens* exhibited significant longer entry latencies into
600 the cadaver zone than *T. molitor* and *G. mellonella*, regardless of killing method or PMIs. Being
601 soil-dwelling species naturally exposed to EPNs, these insects likely employ cautious exploratory
602 strategies when encountering potential infection risk (Lopes, 2022). Notably, delayed entry also
603 occurred with frozen-cadavers, indicating that cadaver presence alone function as warning cue
604 independently of infection (De Roode and Lefèvre, 2012). Despite initial hesitation, *H. illucens*
605 spent significantly more time in the cadaver zone than *T. molitor*, indicating higher tolerance once
606 contact is established, possibly reflecting its gregarious ecology and frequent association with
607 decomposing organic matter (Boulay et al., 2013). Entry-exit dynamics further highlighted
608 behavioral instability in *T. molitor*, exhibiting frequent alternations with rapid entries and short

stays. This pattern indicates ambivalence rather than strict avoidance, consistent with thigmotaxis, a stress-associated response involving repeated repositioning under ambiguous or aversive conditions (Street, 1968). Collectively, these contact assays demonstrate that cadavers, function not only as physical substrates but as behavioral decision points, eliciting species-specific balances between avoidance, tolerance, and exploratory conflict. Integration of both assay types reveals that insect responses to EPN cadavers are hierarchical and scale-dependent. Olfactory cues act as long-range signals that mediate orientation to potential resource patches, whereas contact cues trigger fine-scale risk assessment and behavioral commitment. For *Agriotes* spp. and *H. illucens*, attraction in olfactometers did not translate into immediate contact acceptance, followed by cautious exploration followed by prolonged persistence, consistent with their ecology. In contrast, *G. mellonella* and *T. molitor* lacked consistent attraction at a distance and exhibited avoidance or conflict upon contact, highlighting the importance of co-occurrence history in shaping cue interpretation.

CO₂ emissions reflect metabolic activity but do not predict behavioral responses. CO₂ emissions varied across host species, nematode treatments, and PMIs, reflecting differences in biological activity within cadavers but with limited predictive capacity for behavioral outcomes. Elevated CO₂ levels usually indicate ongoing metabolic processes driven by nematode reproduction, symbiotic bacterial activity, and secondary microbial colonization (Carter et al., 2007; Shapiro-Ilan et al., 2017). Host-specific traits modulated these dynamics: lipid-rich and EPN susceptible hosts such as *G. mellonella* sustained higher CO₂ outputs, whereas *H. illucens* and *T. molitor*, possessing stronger antimicrobial defenses or lower compatibility with EPNs, showed faster declines in CO₂ emissions (Shapiro-Ilan et al., 2008; Van Zyl and Malan, 2015; Franco et al., 2021). However, CO₂ levels did not consistently align with attraction or avoidance responses. *H. illucens* exhibited similar CO₂ concentrations across treatments yet were preferentially attracted to EPN-infected cadavers, whereas *G. mellonella* and *T. molitor* exhibited repulsion despite substantial CO₂ production at certain stages. Conversely, *Agriotes* spp. were consistently attracted to cadavers emitting comparable CO₂ levels.

The nematode-bacteria complex reconfigures the chemical trajectory of host decomposition. Across host species, EPN infection consistently redirected the chemical trajectory of insect decomposition, producing volatilomes that were more stable over time and enriched in sulfur-

containing compounds compared with frozen-cadavers. Rather than following the progressive diversification typical of generalized microbial decomposition, infected cadavers converged toward simplified, infection-specific odor profiles, indicating that decomposition is actively structured by the nematode-bacteria complex. This reconfiguration was evident across insect models, despite certain host-specific differences. A limitation of our experimental design is that infected insects died through nematode-bacteria complex infection, whereas control insects were killed by freezing. Consequently, some differences between infected and control cadavers may reflect not only the effects of infection but also differences associated with the mode of death and subsequent decomposition dynamics. Previous studies have shown that freezing does not completely disrupt postmortem microbial succession but can alter its temporal progression and modify the abundance of particular microbial taxa, especially at finer taxonomic levels (Ogbanga et al., 2023; Chen et al., 2025; Ren et al., 2026). Therefore, some of the divergence observed between infected and frozen-cadavers may partly reflect freezing-induced effects on decomposition. Nevertheless, the consistent emergence of infection-specific sulfur-rich volatile signatures across host species, together with the marked differences between cadavers infected with the nematode-bacteria complex and those exposed to symbiotic bacteria alone, suggests that the major patterns observed are primarily driven by parasite-mediated processes rather than by freezing effects alone.

In frozen-cadavers, volatile profiles diversified over time, consistent with classical microbial succession, whereas EPN-infected cadavers rapidly established sulfur-dominated signatures that persisted across post-mortem stages. Although sulfur compounds can arise naturally during late decomposition (Schieweck et al., 2024), their early appearance and persistence under infection indicate parasite-driven metabolic processes rather than passive decomposition. The recurrence of compounds such as DMDS, DMTS, and indole, many of which have antimicrobial, nematicidal, or repellent properties (Shan et al., 2019; Kusakabe, A. et al., 2022; Li et al., 2024), links infection, volatile production, and the behavioral responses observed.

Comparisons between nematode-bacteria complex infection and those with symbiotic bacteria alone further confirm that this volatile phenotype is an emergent property of symbiosis. Bacteria alone produced transient sulfur-rich profiles, these rapidly shifted toward alkane-dominated blends, consistent with unregulated lipid degradation (Patel et al., 2025). A limitation of this study is that

the bacterial treatment did not include an injection-only control. Therefore, some contributions of injection-induced responses cannot be excluded. However, only larvae exhibiting the characteristic red pigmentation associated with *Photorhabdus* spp. proliferation were retained in VOC analyses, suggesting that the observed volatile profiles were primarily driven by bacterial infection.

The drastic shift in the odor profile across PMIs between larvae infected with the nematode-bacteria complex and those infected with only the bacteria can be explained by the life cycle of the complex within the cadaver. After nematodes release their bacteria, the symbionts undergo a growth phase lasting approximately 24-72 hours (Wang et al., 2011). Throughout the infection cycle, nematodes feed on both the cadaver tissues and the symbiotic bacteria, likely promoting continuous renewal of the bacterial population. In addition, nematodes possess a second bacterial circle in their cuticle potentially contributing to the parasitic cycle (Ogier et al., 2023) potentially influencing the development of a specific odor profile. We may conclude that the characteristic volatile signatures observed in larvae infected with the nematode-bacteria complex are inherently dependent on the presence of the complex itself.

In conclusion, parasites can drive decomposition and reshape chemical cues and behavioral interactions in soil food webs. Parasites actively reshape decomposition, with important ecological consequences. By combining behavioral assays, cue-emission analyses across multiple insect hosts, nematode-bacteria complex show their capacity to reconfigure the chemical trajectory of host cadavers through their symbiosis, generating distinct odor landscapes that elicit species-specific attraction, avoidance, or exploratory conflict. Importantly, these chemical cues do not arise from the bacteria alone but emerge from the joint action of the nematode-bacteria action, highlighting symbiosis as a central driver of soil chemical ecology. By linking parasite-induced decomposition to insect decision-making, these results uncover a previously unrecognized mechanism by which parasites shape multitrophic interactions and information flow in soil-food webs, positioning parasite-driven cues as key mediators of belowground ecological processes.

REFERENCES

Barsics, F., Delory, B.M., Delaplace, P., Francis, F., Fauconnier, M.-L., Haubruge, É., & Verheggen, F.J. (2017). Foraging wireworms are attracted to root-produced volatile aldehydes. *Journal of Pest Science*, 90(1), 69-76. <https://doi.org/10.1007/s10340-016-0734-y>

698 Barsics, F., Haubruge, É., Francis, F., & Verheggen, F. J. (2014). The role of olfaction in
699 wireworms: A review on their foraging behavior and sensory apparatus. *Biotechnology,*
700 *Agronomy, Society and Environment BASE*, 18(4), 524–535.

701 Blanco-Pérez, R., Bueno-Pallero, F.Á., Vicente-Díez, I., Marco-Mancebón, V.S., Pérez-Moreno,
702 I., & Campos-Herrera, R. (2019). Scavenging behavior and interspecific competition decrease
703 offspring fitness of the entomopathogenic nematode *Steinernema feltiae*. *Journal of Invertebrate*
704 *Pathology*, 164, 5–15. <https://doi.org/10.1016/j.jip.2019.04.002>

705 Bode, H.B. (2009). Entomopathogenic bacteria as a source of secondary metabolites. *Current*
706 *Opinion in Chemical Biology, Molecular Diversity* 13, 224–230.
707 <https://doi.org/10.1016/j.cbpa.2009.02.037>

708 Boemare, N.E., Akhurst, R.J., & Mourant, R.G. (1993). DNA Relatedness between *Xenorhabdus*
709 spp. (Enterobacteriaceae), Symbiotic Bacteria of Entomopathogenic Nematodes, and a Proposal
710 To Transfer *Xenorhabdus luminescens* to a New Genus, *Photorhabdus* gen. nov. *International*
711 *Journal of Systematic and Evolutionary Microbiology*, 43, 249–255.
712 <https://doi.org/10.1099/00207713-43-2-249>

713 Booysen, E., Rautenbach, M., Stander, M. A., & Dicks, L. M. (2021). Profiling the production of
714 antimicrobial secondary metabolites by *Xenorhabdus khoisanæ* J194 under different culturing
715 conditions. *Frontiers in Chemistry*, 9, 626653. <https://doi.org/10.3389/fchem.2021.626653>

716 Boulay, J., Devigne, C., Gosset, D., & Charabidze, D. (2013). Evidence of active aggregation
717 behaviour in *Lucilia sericata* larvae and possible implication of a conspecific mark. *Animal*
718 *Behaviour*, 85, 1191–1197. <https://doi.org/10.1016/j.anbehav.2013.03.005>

719 Campos-Herrera, R., & Gutiérrez, C. (2009). Screening Spanish isolates of steinernematid
720 nematodes for use as biological control agents through laboratory and greenhouse microcosm
721 studies. *Journal of Invertebrate Pathology*, 100(2), 100–105.
722 <https://doi.org/10.1016/j.jip.2008.11.009>

723 Carter, D.O., Yellowlees, D., & Tibbett, M. (2007). Cadaver decomposition in terrestrial
724 ecosystems. *Naturwissenschaften*, 94, 12–24. <https://doi.org/10.1007/s00114-006-0159-1>

725 Cassells, M. D., Labaude, S., & Griffin, C. T. (2024). Beware glowing cadavers: bioluminescence
 726 of nematode symbiont *Photorhabdus* protects nematode-infected host cadavers from nocturnal
 727 scavengers. *Frontiers in Ecology and Evolution*, 11, 1264251.
 728 <https://doi.org/10.3389/fevo.2023.1264251>

729 Chacon-Hurtado, A., Ruhland, F., Drabo, S., Smeets, T., Checconi, B., Campos-Herrera, R., &
 730 Verheggen, F. J. (2024). To be a good killer: Evaluation of morphometry and nematodes-bacteria
 731 complex effect on entomopathogenic nematodes virulence against wireworms. *Journal of*
 732 *Invertebrate Pathology*, 207, 108231. <https://doi.org/10.1016/j.jip.2024.108231>

733 Chen, J., Wei, Q., Yang, F., Liu, Y., Zhao, Y., Zhang, H., Huang, X., Zeng, J., Wang, X. & Zhang,
 734 S. (2025). Unveiling the forensic potential of oral and nasal microbiota in post-mortem interval
 735 estimation. *International Journal of Molecular Sciences*, 26(7), 3432.
 736 <https://doi.org/10.3390/ijms26073432>

737 Clarke, D.J. (2008). *Photorhabdus*: a model for the analysis of pathogenicity and mutualism.
 738 *Cellular Microbiology*, 10, 2159–2167. <https://doi.org/10.1111/j.1462-5822.2008.01209.x>

739 Crombie, A. C., & Darrah, J. H. (1947). The chemoreceptors of the wireworm (*Agriotes* spp.) and
 740 the relation of activity to chemical constitution. *The Journal of Experimental Biology*, 24(1–2),
 741 95–109. <https://doi.org/10.1242/jeb.24.1-2.95>

742 De Roode, J.C., & Lefèvre, T. (2012). Behavioral Immunity in Insects. *Insects*, 3, 789–820.
 743 <https://doi.org/10.3390/insects3030789>

744 Diener, S., Zurbrugg, C., & Tockner, K. (2009). Conversion of organic material by black soldier
 745 fly larvae: establishing optimal feeding rates. *Waste management & research*, 27(6), 603-610.
 746 <https://doi.org/10.1177/0734242X09103838>

747 Dillman, A. R., Guillermin, M. L., Lee, J. H., Kim, B., Sternberg, P. W., & Hallem, E. A. (2012).
 748 Olfaction shapes host–parasite interactions in parasitic nematodes. *Proceedings of the National*
 749 *Academy of Sciences*, 109(35), E2324-E2333. <https://doi.org/10.1073/pnas.1211436109>

750 Eidt, D.C., & Thurston, G.S. (1995). Physical deterrents to infection by entomopathogenic
 751 nematodes in wireworms (Coleoptera: Elateridae) and other soil insects. *The Canadian*
 752 *Entomologist*, 127, 423–429. <https://doi.org/10.4039/Ent127423-3>

753 Ferris, H., Lau, S., & Venette, R. (1995). Population energetics of bacterial-feeding nematodes:
 754 Respiration and metabolic rates based on CO₂ production. *Soil Biology & Biochemistry*, 27, 319–
 755 330. [https://doi.org/10.1016/0038-0717\(94\)00186-5](https://doi.org/10.1016/0038-0717(94)00186-5)

756 Fleischer, J., & Krieger, J. (2018). Insect pheromone receptors—key elements in sensing
 757 intraspecific chemical signals. *Frontiers in Cellular Neuroscience*, 12, 425.
 758 <https://doi.org/10.3389/fncel.2018.00425>

759 Foltan, P., & Puza, V. (2009). To complete their life cycle, pathogenic nematode–bacteria
 760 complexes deter scavengers from feeding on their host cadaver. *Behavioural Processes*, 80, 76–79.
 761 <https://doi.org/10.1016/j.beproc.2008.09.012>

762 Franco, A., Scieuzo, C., Salvia, R., Petrone, A. M., Tafi, E., Moretta, A., & Falabella, P. (2021).
 763 Lipids from *Hermetia illucens*, an innovative and sustainable source. *Sustainability*, 13(18), 10198.
 764 <https://doi.org/10.3390/su131810198>

765 Furlan, L. (2004). The biology of *Agriotes sordidus* Illiger (Col., Elateridae). *Journal of Applied*
 766 *Entomology*, 128(9-10), 696-706. <https://doi.org/10.1111/j.1439-0418.2004.00914.x>

767 Grunseich, J. M., Aguirre, N. M., Thompson, M. N., Ali, J. G., & Helms, A. M. (2021). Chemical
 768 cues from entomopathogenic nematodes vary across three species with different foraging
 769 strategies, triggering different behavioral responses in prey and competitors. *Journal of Chemical*
 770 *Ecology*, 47(10), 822-833. <https://doi.org/10.1007/s10886-021-01304-8>

771 Helms, A.M., Ray, S., Matulis, N.L., Kuzemchak, M.C., Grisales, W., Tooker, J.F., & Ali, J.G.
 772 (2019). Chemical cues linked to risk: Cues from below-ground natural enemies enhance plant
 773 defences and influence herbivore behaviour and performance. *Functional Ecology*, 33, 798–808.
 774 <https://doi.org/10.1111/1365-2435.13297>

775 Hervé, M. R., Nicolè, F., & Lê Cao, K. A. (2018). Multivariate analysis of multiple datasets: a
 776 practical guide for chemical ecology. *Journal of Chemical Ecology*, 44(3), 215-234.
 777 <https://doi.org/10.1007/s10886-018-0932-6>

778 Hoc, B., Genva, M., Fauconnier, M. L., Lognay, G., Francis, F., & Caparros Megido, R. (2020).
 779 About lipid metabolism in *Hermetia illucens* (L. 1758): on the origin of fatty acids in prepupae.
 780 Scientific Reports, 10(1), 11916. <https://doi.org/10.1038/s41598-020-68784-8>

781 Hodda, M. (2022). Phylum Nematoda: a classification, catalogue and index of valid genera, with a
 782 census of valid species. Zootaxa, 5114, 1–289. <https://doi.org/10.11646/zootaxa.5114.1.1>

783 Hyršl, P. (2011). Pathogenicity of four entomopathogenic nematodes species to *G. mellonella*
 784 larvae. Karaelmas Fen ve Mühendislik Dergisi, 1(1), 1-6. <https://doi.org/10.7212/zkufbd.v1i1.7>

785 Jacquin-Joly, E., & Merlin, C. (2004). Insect olfactory receptors: contributions of molecular
 786 biology to chemical ecology. Journal of Chemical Ecology, 30(12), 2359-2397.
 787 <https://doi.org/10.1007/s10886-004-7941-3>

788 Jaffuel, G., Krishnamani, S., Machado, R. A., Campos-Herrera, R., & Turlings, T. C. (2022). Potent
 789 ant deterrents emitted from nematode-infected insect cadavers. Journal of Chemical Ecology,
 790 48(1), 71-78. <https://doi.org/10.1007/s10886-021-01320-8>

791 Kaya, H.K., & Gaugler, R. (1993). Entomopathogenic Nematodes. Annual Review of Entomology,
 792 1993, Vol. 38, 181-206.

793 Kojima, W. (2015). Attraction to Carbon Dioxide from Feeding Resources and Conspecific
 794 Neighbours in Larvae of the Rhinoceros Beetle *Trypoxylus dichotomus*. PLoS ONE, 10(11),
 795 e0141733. <https://doi.org/10.1371/journal.pone.0141733>

796 Kusakabe, A., Wang, C., Xu, Y. M., Molnár, I., & Stock, S. P. (2022). Selective toxicity of
 797 secondary metabolites from the entomopathogenic bacterium *Photorhabdus luminescens*
 798 *sonorensis* against selected plant parasitic nematodes of the *Tylenchina* suborder. Microbiology
 799 spectrum, 10(1), e02577-21. <https://doi.org/10.1128/spectrum.02577-21>

800 Kwadha, C. A., Ong'amo, G. O., Ndegwa, P. N., Raina, S. K., & Fombong, A. T. (2017). The
 801 biology and control of the greater wax moth, *Galleria mellonella*. Insects, 8(2), 61.
 802 <https://doi.org/10.3390/insects8020061>

803 Li, J., Wei, X., Pei, Z., Sun, J., Xi, J., Li, X., & Ruan, W. (2024). Volatile organic compounds
 804 released from entomopathogenic nematode-infected insect cadavers for the biocontrol of

805 *Meloidogyne incognita*. Pest Management Science, 80(10), 5400-5411.
806 <https://doi.org/10.1002/ps.8268>

807 Liu, S., Rao, X. J., Li, M. Y., Feng, M. F., He, M. Z., & Li, S. G. (2015). Identification of candidate
808 chemosensory genes in the antennal transcriptome of *Tenebrio molitor* (Coleoptera:
809 Tenebrionidae). Comparative Biochemistry and Physiology Part D: Genomics and Proteomics, 13,
810 44-51. <https://doi.org/10.1016/j.cbd.2015.01.004>

811 Lopes, P.C. (2022). Anticipating infection: How parasitism risk changes animal physiology.
812 Functional Ecology, 37(4), 821–830. <https://doi.org/10.1111/1365-2435.14155>.

813 Loulou, A., Mastore, M., Caramella, S., Bhat, A. H., Brivio, M. F., Machado, R. A., & Kallel, S.
814 (2023). Entomopathogenic potential of bacteria associated with soil-borne nematodes and insect
815 immune responses to their infection. PLoS One, 18(1), e0280675.
816 <https://doi.org/10.1371/journal.pone.0280675>

817 Lulamba, T. E., Green, E., & Serepa-Dlamini, M. H. (2021). *Photorhabdus* sp. ETL antimicrobial
818 properties and characterization of its secondary metabolites by gas chromatography–mass
819 spectrometry. Life, 11(8), 787. <https://doi.org/10.3390/life11080787>

820 Machado, R. A., & and Von Reuss, S. H. (2022). Chemical ecology of nematodes. Chimia, 76(11),
821 945-953. <https://doi.org/10.2533/chimia.2022.945>

822 Megido, R. C., Poelaert, C., Ernens, M., Liotta, M., Blecker, C., Danthine, S., Tyteca, E., Haubruge,
823 É., Alabi, T., Bindelle, J., & Francis, F. (2018). Effect of household cooking techniques on the
824 microbiological load and the nutritional quality of mealworms (*Tenebrio molitor* L. 1758). Food
825 Research International, 106, 503-508. <https://doi.org/10.1016/j.foodres.2018>

826 Morton, A., & Garcia-del-Pino, F. (2017). Laboratory and field evaluation of entomopathogenic
827 nematodes for control of *Agriotes obscurus* (L.) (Coleoptera: Elateridae). Journal of Applied
828 Entomology, 141(4), 241-246. <https://doi.org/10.1111/jen.12343>

829 Ogbanga, N., Nelson, A., Gino, S., Wescott, D. J., Mickleburgh, H. L., Gocha, T. P., & Procopio,
830 N. (2023). The impact of freezing on the post-mortem human microbiome. Frontiers in Ecology
831 and Evolution, 11, 1151001. <https://doi.org/10.3389/fevo.2023.1151001>

832 Ogier, J. C., Akhurst, R., Boemare, N., & Gaudriault, S. (2023). The endosymbiont and the second
833 bacterial circle of entomopathogenic nematodes. *Trends in Microbiology*, 31(6), 629-643.
834 <https://doi.org/10.1016/j.tim.2023.01.004>

835 Patel, D., Burr, W. S., Daoust, B., & Forbes, S. (2025). Identifying the transition from ante-mortem
836 to post-mortem odor in cadavers in an outdoor environment. *Forensic Science International: Synergy*, 11, 100616. <https://doi.org/10.1016/j.fsisyn.2025.100616>

838 Plata-Rueda, A., Martínez, L. C., Santos, M. H. D., Fernandes, F. L., Wilcken, C. F., Soares, M.
839 A., Serrão, J.E. & Zanuncio, J. C. (2017). Insecticidal activity of garlic essential oil and their
840 constituents against the mealworm beetle, *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae).
841 *Scientific Reports*, 7(1), 46406. <https://doi.org/10.1038/srep46406>

842 Purkayastha, D., & Sarkar, S. (2022). Sustainable waste management using black soldier fly larva:
843 a review. *International Journal of Environmental Science and Technology*, 19(12), 12701-12726.
844 <https://doi.org/10.1007/s13762-021-03524-7>

845 Rahman, A., Bharali, P., Borah, L., Bathari, M., & Taye, R. R. (2017). Post embryonic
846 development of *Galleria mellonella* L. and its management strategy. *J Entomol Zool Stud*, 5(3),
847 1523-1526. E-ISSN: 2320-7078

848 Rasmann, S., Köllner, T.G., Degenhardt, J., Hiltbold, I., Toepfer, S., Kuhlmann, U., Gershenzon,
849 J., & Turlings, T.C.J. (2005). Recruitment of entomopathogenic nematodes by insect-damaged
850 maize roots. *Nature*, 434, 732–737. <https://doi.org/10.1038/nature03451>

851 Ren, L., Wang, Z., Yang, Z., Tian, R., Du, J., Dang, Z., Li, J., Guo, X., Xia, Y., Cui, W. & Wang,
852 Y. (2026). Microbial succession and machine learning for postmortem interval estimation in
853 bromadiolone-poisoned cadavers. *AMB Express*. <https://doi.org/10.1186/s13568-026-02023-7>

854 Samoylova, E. S., & Tiunov, A. V. (2017). Flexible trophic position of polyphagous wireworms
855 (Coleoptera, Elateridae): A stable isotope study in the steppe belt of Russia. *Applied Soil Ecology*,
856 121, 74-81. <https://doi.org/10.1016/j.apsoil.2017.09.026>

857 Scala, A., Salvia, R., Pezzi, M., Nardiello, M., Scieuzo, C., Farina, D., Mancini, E., Moretta, A.,
858 Bufo, S.A., Leis, M., & Falabella, P. (2018). Investigations on molecular mechanisms and

859 morphology of structures involved in chemoreception in black soldier fly larvae *Hermetia illucens*
860 (Diptera: Stratiomyidae). In XI European Congress of Entomology.
861 <http://hdl.handle.net/11563/134419>

862 Schieweck, A., Schulz, N., Amendt, J., Birngruber, C., & Holz, F. (2024). Catch me if you can—
863 emission patterns of human bodies in relation to postmortem changes. *International Journal of*
864 *Legal Medicine*, 138(4), 1603-1620. <https://doi.org/10.1007/s00414-024-03194-3>

865 Schmidt, H. R., & Benton, R. (2020). Molecular mechanisms of olfactory detection in insects:
866 beyond receptors. *Open Biology*, 10(10). <https://doi.org/10.1098/rsob.200252>

867 Schumann, M., Patel, A., Vemmer, M., & Vidal, S. (2014). The role of carbon dioxide as an
868 orientation cue for western corn rootworm larvae within the maize root system: implications for an
869 attract-and-kill approach. *Pest Management Science*, 70, 642–650. <https://doi.org/10.1002/ps.3602>

870 Scieuzo, C., Nardiello, M., Farina, D., Scala, A., Cammack, J. A., Tomberlin, J. K., Vogel, H.,
871 Salvia, R., Persaud, K & Falabella, P. (2021). *Hermetia illucens* (L.)(Diptera: Stratiomyidae)
872 odorant binding proteins and their interactions with selected volatile organic compounds: An in
873 silico approach. *Insects*, 12(9), 814. <https://doi.org/10.3390/insects12090814>

874 Shan, S., Wang, W., Song, C., Wang, M., Sun, B., Li, Y., Fu, Y., Gu, X., Ruan, W., & Rasmann,
875 S. (2019). The symbiotic bacteria *Alcaligenes faecalis* of the entomopathogenic nematodes
876 *Oscheius* spp. exhibit potential biocontrol of plant-and entomopathogenic fungi. *Microbial*
877 *Biotechnology*, 12(3), 459-471. <https://doi.org/10.1111/1751-7915.13365>

878 Shapiro-Ilan, D. I., & Lewis, E. E. (Eds.). (2024). Entomopathogenic nematodes as biological
879 control agents. CABI Publishing, 543. <https://doi.org/10.1079/9781800620322.0000>

880 Shapiro-Ilan, D. I., Hiltbold, I., & Lewis, E. E. (2017). Nematodes. *Ecology of invertebrate*
881 *diseases*, A.E. Hajek (Ed.). 415-440. <https://doi.org/10.1002/9781119256106.ch11>

882 Shapiro-Ilan, D. I., Lewis, E. E., Campbell, J. F., & Kim-Shapiro, D. B. (2012). Directional
883 movement of entomopathogenic nematodes in response to electrical field: effects of species,
884 magnitude of voltage, and infective juvenile age. *Journal of Invertebrate Pathology*, 109(1), 34-40.
885 <https://doi.org/10.1016/j.jip.2011.09.004>

886 Shapiro-Ilan, D., & Gaugler, R. (2002). Production technology for entomopathogenic nematodes
887 and their bacterial symbionts. *Journal of Industrial Microbiology and Biotechnology*, 28, 137–146.
888 <https://doi.org/10.1038/sj/jim/7000230>

889 Shapiro-Ilan, D., Rojas, M. G., Morales-Ramos, J. A., Lewis, E. E. & Tedders, W. L. (2008).
890 Effects of host nutrition on virulence and fitness of entomopathogenic nematodes: Lipid-and
891 protein-based supplements in *Tenebrio molitor* diets. *Journal of Nematology*, 40(1), 13. PMID:
892 19259513; PMCID: PMC2586524.

893 Stock, S.P. (2019). Partners in crime: symbiont-assisted resource acquisition in *Steinernema*
894 entomopathogenic nematodes. *Current Opinion in Insect Science, Ecology*
895 *Parasites/Parasitoids/Biological control*, 32, 22–27. <https://doi.org/10.1016/j.cois.2018.10.006>

896 Street, W. R. (1968). Thigmotaxis in mealworms: Effects of barrier height and illumination.
897 *Psychonomic Science*, 11(1), 37-37. <https://doi.org/10.3758/BF03328141>

898 Sufyan, M., Neuhoff, D., & Furlan, L. (2014). Larval development of *Agriotes obscurus* under
899 laboratory and semi-natural conditions. *Bulletin of Insectology*. 67, 227–234. ISSN 1721-8861

900 Tobias, N.J., Wolff, H., Djahanschiri, B., Grundmann, F., Kronenwerth, M., Shi, Y.-M., Simonyi,
901 S., Grün, P., Shapiro-Ilan, D., Pidot, S.J., Stinear, T.P., Ebersberger, I., & Bode, H.B. (2017).
902 Natural product diversity associated with the nematode symbionts *Photorhabdus* and
903 *Xenorhabdus*. *Nature Microbiology*, 2(12), 1676-1685. [https://doi.org/10.1038/s41564-017-0039-](https://doi.org/10.1038/s41564-017-0039-9)
904 9

905 Torr, P., Heritage, S., Wilson, & M.J. (2004). Vibrations as a novel signal for host location by
906 parasitic nematodes. *International Journal for Parasitology*, 34(9), 997-999.
907 <https://doi.org/10.1016/j.ijpara.2004.05.003>

908 Turlings, T.C.J., Hiltbold, I., & Rasmann, S. (2012). The importance of root-produced volatiles as
909 foraging cues for entomopathogenic nematodes. *Plant & Soil*, 358, 51–60.
910 <https://doi.org/10.1007/s11104-012-1295-3>

911 Van, Z.C., & Malan, A.P. (2015). Cost-effective culturing of *Galleria mellonella* and *Tenebrio*
 912 *molitor* and entomopathogenic nematode production in various hosts. *African Entomology*, 23,
 913 361–375. <https://doi.org/10.10520/EJC176592>

914 Vicente-Díez, I., Blanco-Pérez, R., Chelkha, M., Puellas, M., Pou, A., & Campos-Herrera, R.
 915 (2021). Exploring the use of entomopathogenic nematodes and their symbiotic bacteria to control
 916 *Lobesia botrana*. *Insects* 12, 1033. <https://doi.org/10.3390/insects12111033>

917 Wang, Y., Fang, X., Cheng, Y., & Zhang, X. (2011). Manipulation of pH shift to enhance the
 918 growth and antibiotic activity of *Xenorhabdus nematophila*. *BioMed Research International*,
 919 2011(1), 672369. <https://doi.org/10.1155/2011/672369>

920 Weisskopf, L., Schulz, S., & Garbeva, P. (2021). Microbial volatile organic compounds in intra-
 921 kingdom and inter-kingdom interactions. *Nature Reviews Microbiology*, 19(6), 391-404.
 922 <https://doi.org/10.1038/s41579-020-00508-1>

923 Wenke, K., Kai, M., & Piechulla, B. (2010). Belowground volatiles facilitate interactions between
 924 plant roots and soil organisms. *Planta*, 231(3), 499-506. [https://doi.org/10.1007/s00425-009-1076-](https://doi.org/10.1007/s00425-009-1076-2)
 925 2

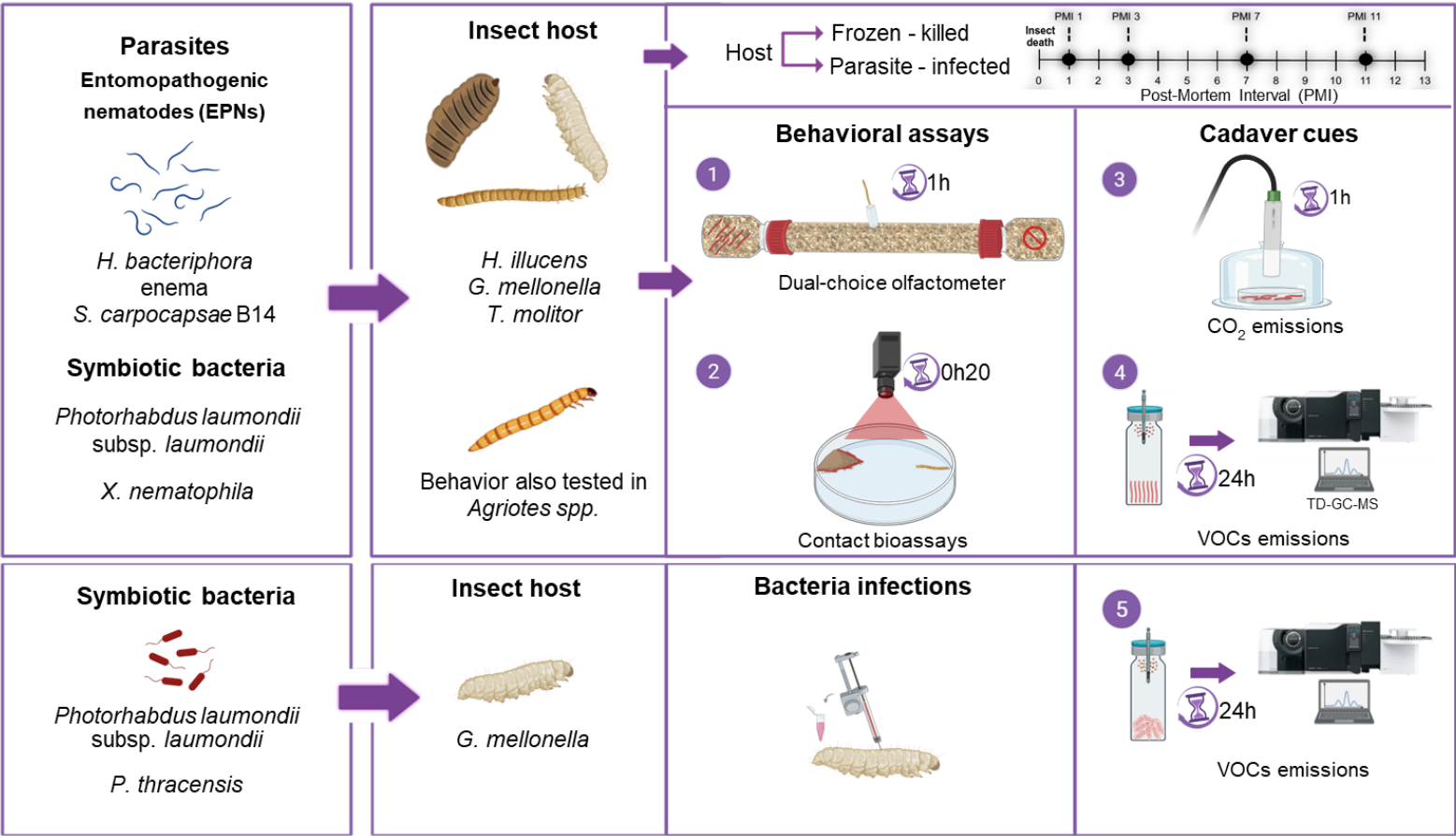
926 White, G. F. (1927). A method for obtaining infective nematode larvae from cultures. *Science*,
 927 66(1709), 302-303. <https://doi.org/10.1126/science.66.1709.302.b>

928 Xie, J., Zhang, H., Li, C., Sun, L., Wang, P., & Guo, Y. (2025). Transcriptome Analysis and
 929 Identification of Chemosensory Genes in the *Galleria mellonella* Larvae. *Insects*, 16(10), 1004.
 930 <https://doi.org/10.3390/insects16101004>

931 Yu, Y., Long, X., Lin, Y., Magura, T., Wang, S., Liao, X., Li, J., & Zhao, J. (2025). Impacts of
 932 bacterivorous nematode identity and abundances on soil greenhouse gas emissions. *Climate Smart*
 933 *Agriculture*, 2(2), 100049. <https://doi.org/10.1016/j.csag.2025.100049>

934 Zhang, X., Machado, R.A., Doan, C.V., Arce, C.C., Hu, L., & Robert, C.A. (2019).
 935 Entomopathogenic nematodes increase predation success by inducing cadaver volatiles that attract
 936 healthy herbivores. *eLife*, 8, e46668. <https://doi.org/10.7554/eLife.46668>

- 937 Zhao, H. X., Xiao, W. Y., Ji, C. H., Ren, Q., Xia, X. S., Zhang, X. F., & Huang, W. Z. (2019).
938 Candidate chemosensory genes identified from the greater wax moth, *Galleria mellonella*, through
939 a transcriptomic analysis. *Scientific Reports*, 9(1), 10032. [https://doi.org/10.1038/s41598-019-](https://doi.org/10.1038/s41598-019-46532-x)
940 46532-x
- 941 Zuzarte-Luís, V. & Mota, M.M. (2018). Parasite Sensing of Host Nutrients and Environmental
942 Cues. *Cell Host & Microbe*, 23, 749–758. <https://doi.org/10.1016/j.chom.2018.05.018>



944 **Figure 1.** Experimental framework for investigating ecological impacts of parasite-cadavers by EPNs, in insect behavior and cadaver
945 cues. Insect hosts representing distinct insect orders: Diptera (*Hermetia illucens*), Lepidoptera (*Galleria mellonella*), and Coleoptera
946 (*Tenebrio molitor* and *Agriotes* spp.), were subjected to different mortality treatments and decomposition stages. Larvae were killed by
947 liquid nitrogen (frozen) as a non-parasite control or infected with entomopathogenic nematodes (*Heterorhabditis bacteriophora* or
948 *Steinernema carpocapsae*, each associated with their respective symbiotic bacteria). Behavioral responses of uninfected larvae were

949 assessed using two complementary approaches: **(1)** Dual-choice olfactometer assays, allowing exposure to odor cues without physical
950 contact and **(2)** contact bioassays, in which larvae could interact directly with cadavers, (*Agriotes* spp. were exposed to *T. molitor*
951 cadavers). Chemical cues emitted by cadavers were quantified through infrared sensor for **(3)** CO₂ emissions and **(4)** headspace TD-
952 GC-MS analyses for Volatile Organic Compounds (VOCs). VOCs profiles emitted by insects infected with two *H. bacteriophora*
953 populations carrying distinct symbiotic bacteria and insects infected solely with the bacterial symbionts were compared to **(5)**. This
954 integrative experimental design enabled the investigation how parasite-infection, host identity, and decomposition stage shape cadaver-
955 emitted chemical cues, and their impact in soil environment.

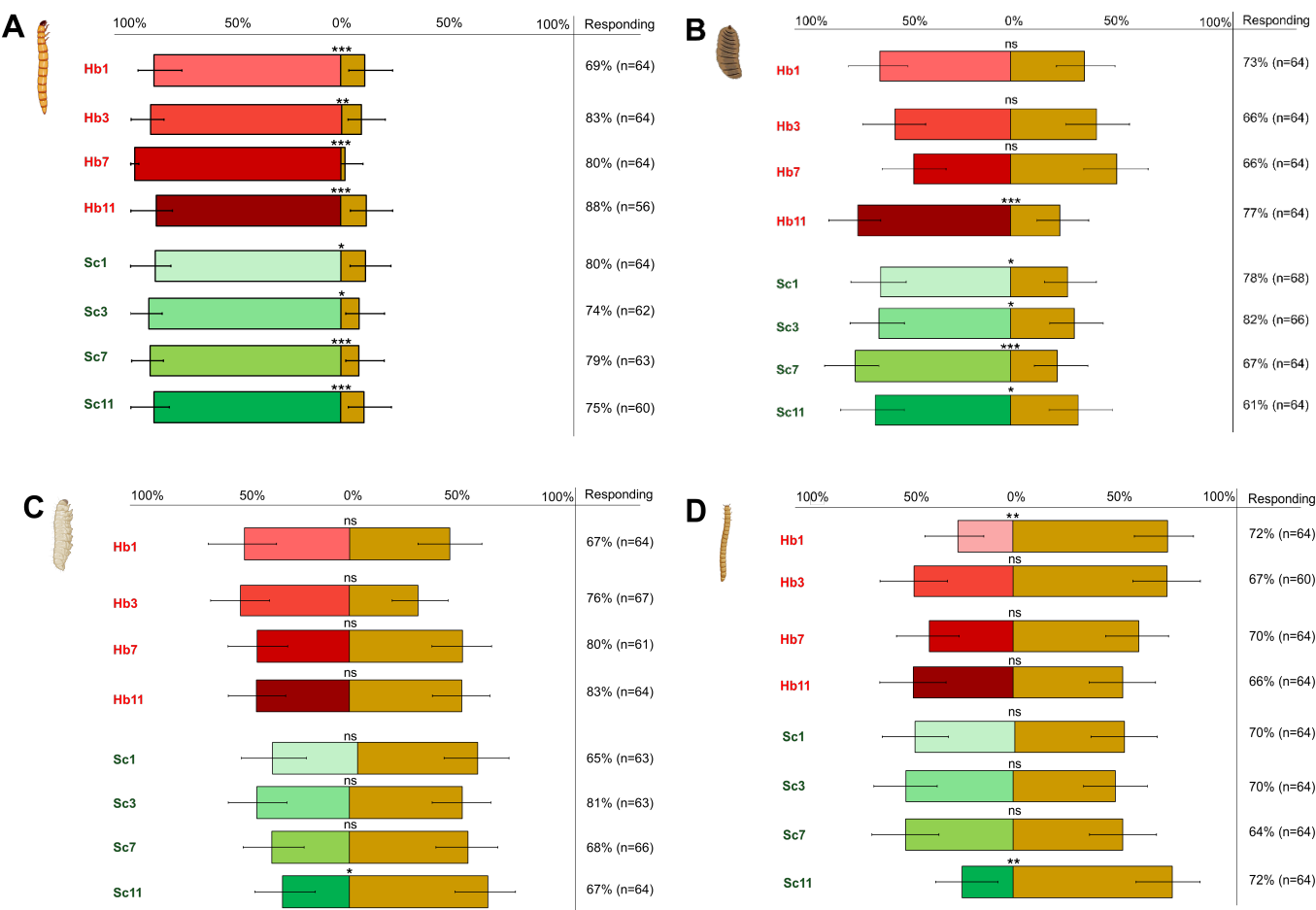


Figure 2. Percentage of individuals from four larval species reaching the olfactometer side connected to the odor of an infected insect by *Heterorhabdtis bacteriophora* (Hb) or *Steinernema carpocapsae* (Sc) at different Post-Mortem Intervals (PMIs) (1, 3, 7 and 11 days), compared to the control side, **n**, represents the total number of insects tested and the percentage the response rate in each test. **(A)** *Agriotes* spp. with *Tenebrio molitor* infected cadavers, **(B)** *T. molitor* with *T. molitor* infected cadavers, **(C)** *G. mellonella* with *G. mellonella* infected cadavers, **(D)** *Hermetia illucens* with *H. illucens* infected cadavers. Reported *p*-values correspond to the intercept significance from a generalized linear model (GLM) fitted with a binomial error distribution (and the 'brglmFit' method for (A)). For all comparisons, *n* > 33, (SI Appendix Table S1).

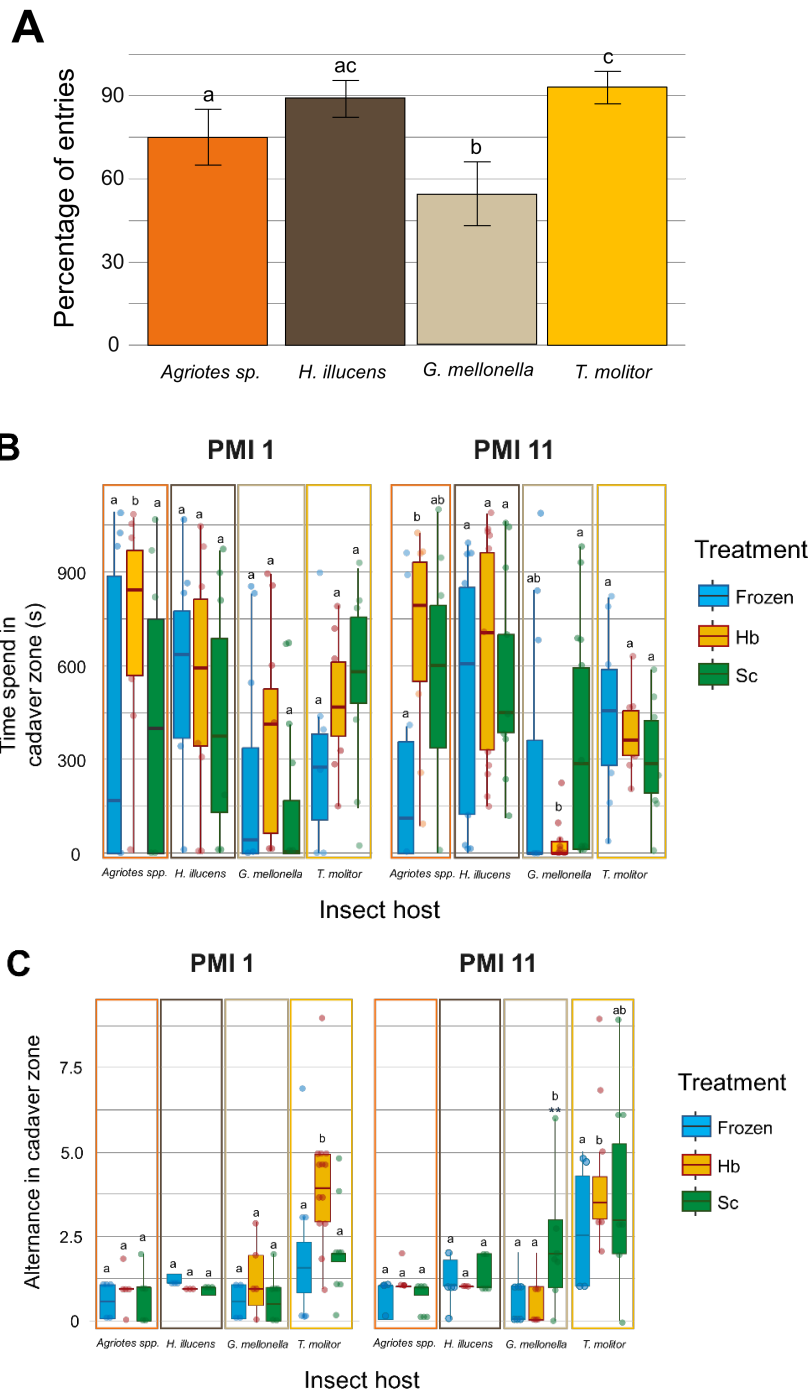
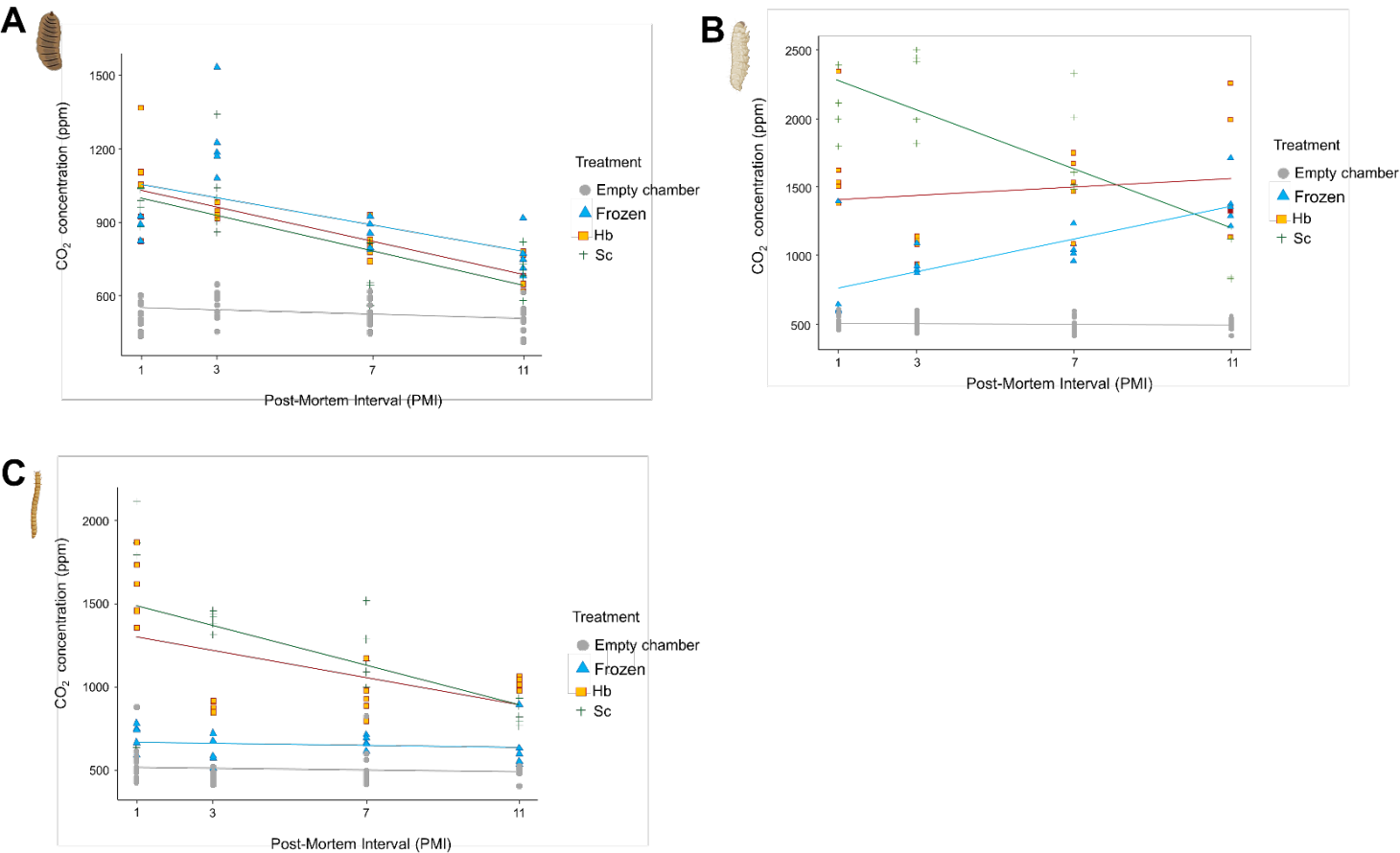


Figure 3. Behavioral responses in contact bioassays for *Agriotes* spp., *Hermetia illucens*, *Galleria mellonella*, and *Tenebrio molitor* exposed to cadavers (insects killed by liquid nitrogen -Frozen-, and EPNs-infection: *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*) and two Post-Mortem Intervals (PMI1 and PMI11). Bars show model-based estimated marginal means $\pm$ 95% confidence intervals. Different letters indicate significant differences (Tukey-adjusted pairwise comparisons, $\alpha = 0.05$; SI Appendix Tables S2a-S5b). (A) Proportion of individuals entering the

974 infected zone. **(B)** Time spent in the infected zone. **(C)** Number of entries into the infected zone.
 975 Asterisks denote significant species $\times$ killing method $\times$ PMI interactions.

976



977 **Figure 4.** CO₂ emission dynamics from insect cadavers across Post-Mortem Intervals and killing
 978 methods. CO₂ concentration (ppm) measured in cadavers of **(A)** *Hermetia illucens*; **(B)** *Galleria*
 979 *mellonella*, **(C)** *Tenebrio molitor*. Insects were killed by liquid nitrogen (Frozen) or by EPNs
 980 (*Heterorhabditis bacteriophora* or *Steinernema carpocapsae*), across four Post-Mortem Intervals
 981 (1, 3, 7, 11 days). Each point represents the mean CO₂ emission from seven larvae per treatment
 982 and PMI, with five independent replicates. Solid lines indicate model-predicted values from linear
 983 mixed-effects models including PMI, killing method, and their interaction, with larval identity as
 984 a random effect (SI Appendix Table S6).

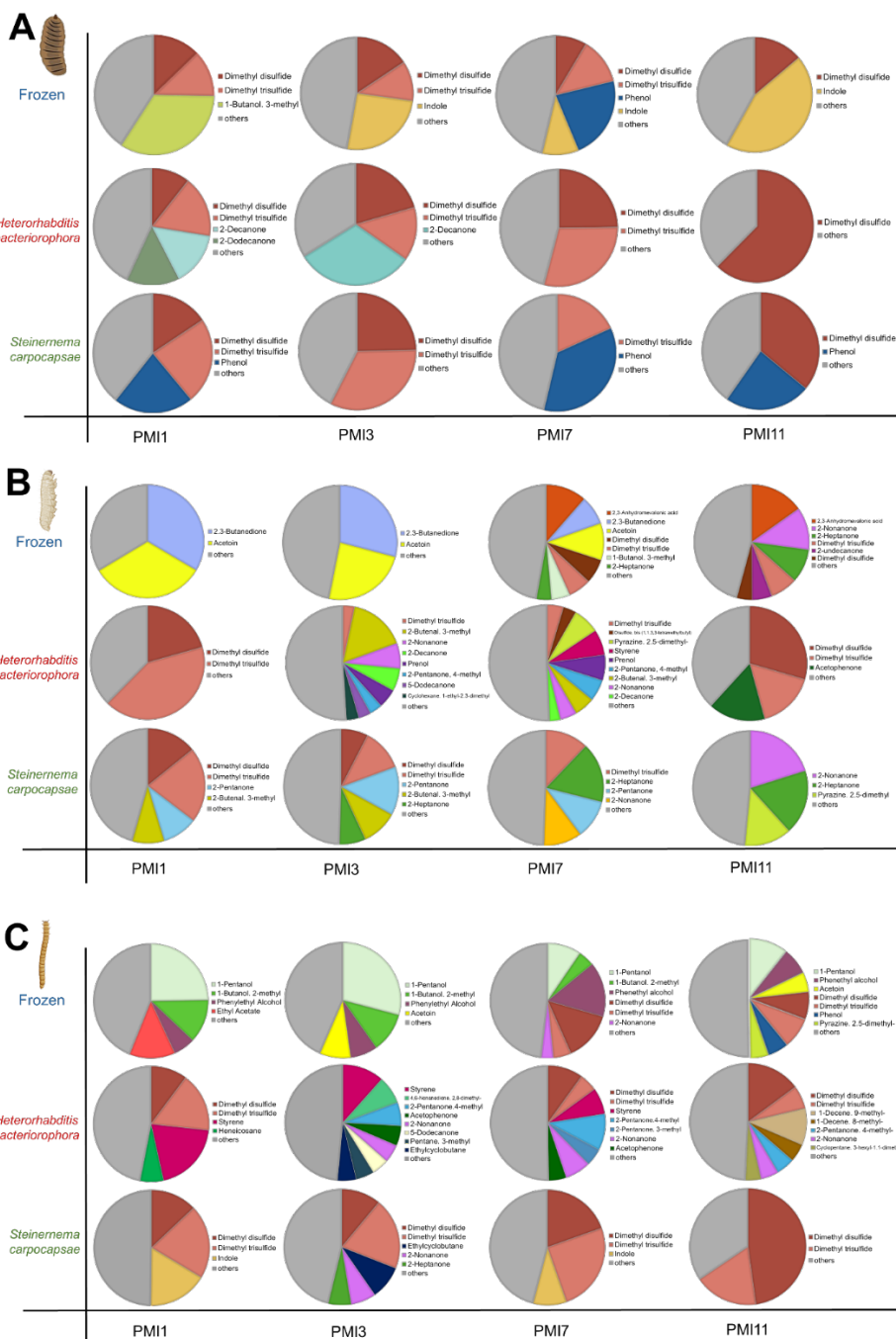
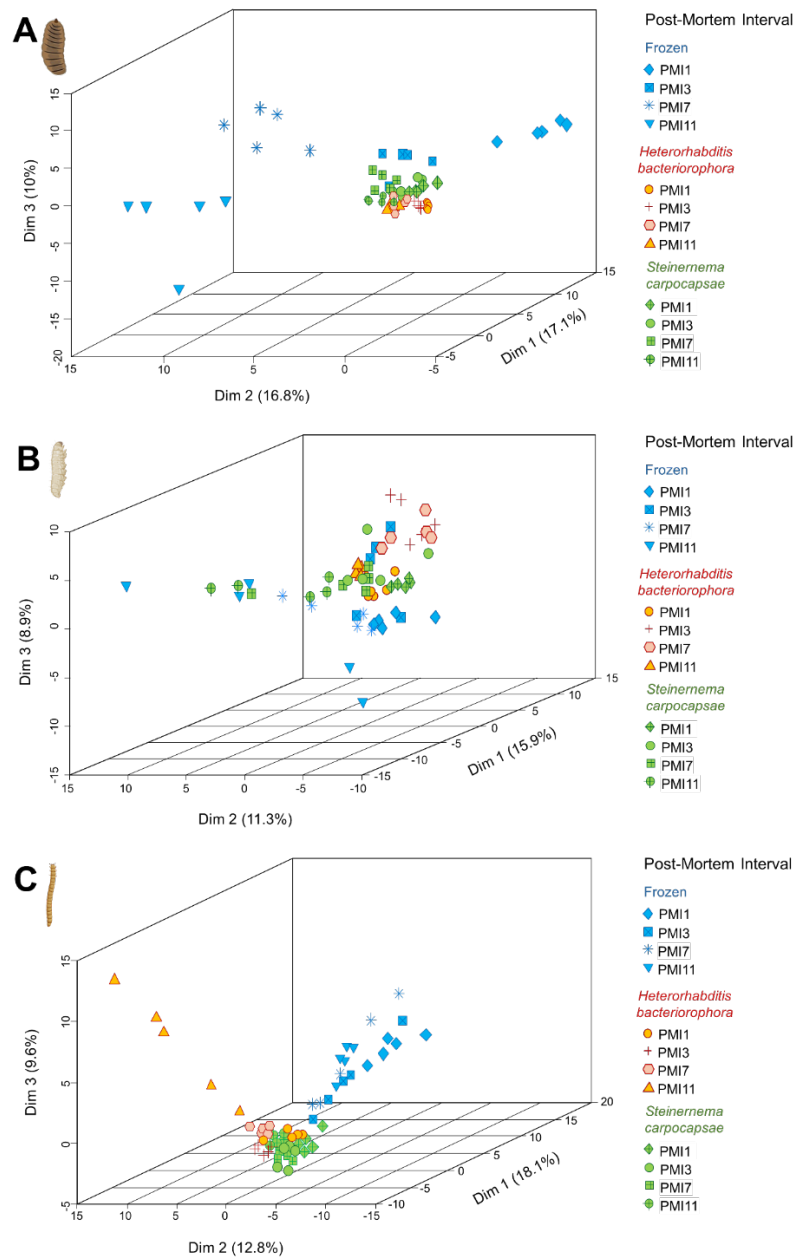


Figure 5. Relative proportions of the Volatile Organic Compounds (VOCs) contributing to each modality: killing method (Insects killed by liquid nitrogen (Frozen) or by EPNs (*Heterorhabditis bacteriophora* or *Steinernema carpocapsae*) and Post-Mortem Interval (PMI) in days (1, 3, 7 and 11). The x-axis represents the different PMIs of odor sampling, covering the duration of a complete entomopathogenic nematodes life cycle. (A) *Hermetia illucens* (B) *Galleria mellonella* and (C) *Tenebrio molitor*.



992

993 **Figure 6.** Principal Component Analysis (PCA) performed on the Volatile Organic Compounds
 994 (VOCs) profiles of insects killed by liquid nitrogen (Frozen) and EPNs: *Heterorhabditis*
 995 *bacteriophora* and *Steinernema carpocapsae*, across different Post-Mortem Intervals (PMIs) in
 996 days (1, 3, 7 and 11). **(A)** *Hermetia illucens* **(B)** *Galleria mellonella* and **(C)** *Tenebrio molitor*.
 997 PERMANOVA analysis shows that odors profiles differed significantly among treatments
 998 (*Hermetia illucens*: Df = 11, F = 10.878; $p = 0.001$; *Galleria mellonella*: PERMANOVA, Df = 11,
 999 F = 5.710; $p = 0.001$; *Tenebrio molitor*: Df = 11, F = 8.453; $p = 0.001$) (Details in SI Appendix
 1000 TableS7).

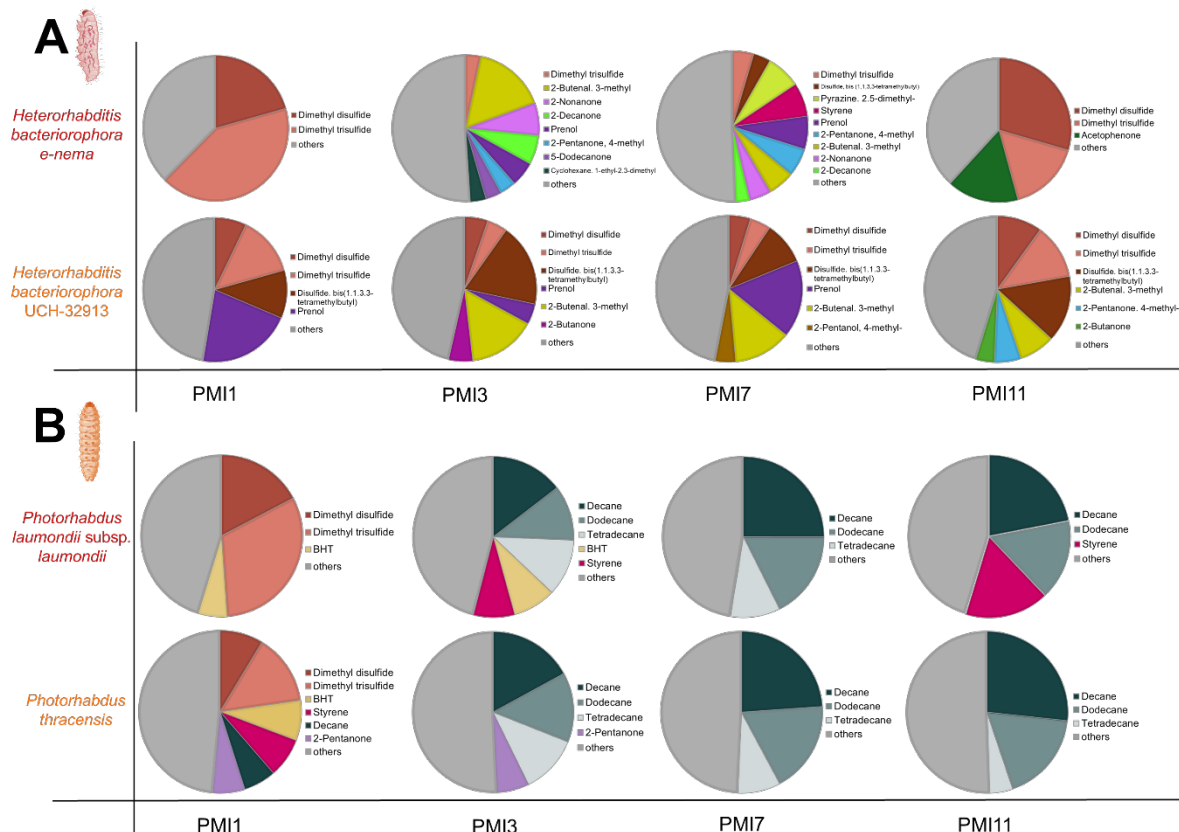


Figure 7. Relative proportions of the Volatile Organic Compounds (VOCs) contributing to each modality: killing method (A. Infected with EPN *Heterorhabditis bacteriophora* e-nema and *H. bacteriophora* UCH-32913; B. Infected with EPB *Photorhabdus laumondii* subsp. *laumondii* and *P. thracensis*) and Post-Mortem Interval (PMI) in days (1, 3, 7 and 11). The x-axis represents the different moments of odor sampling covering the duration of a complete entomopathogenic nematodes life cycle. Insect model used: *Galleria mellonella*.

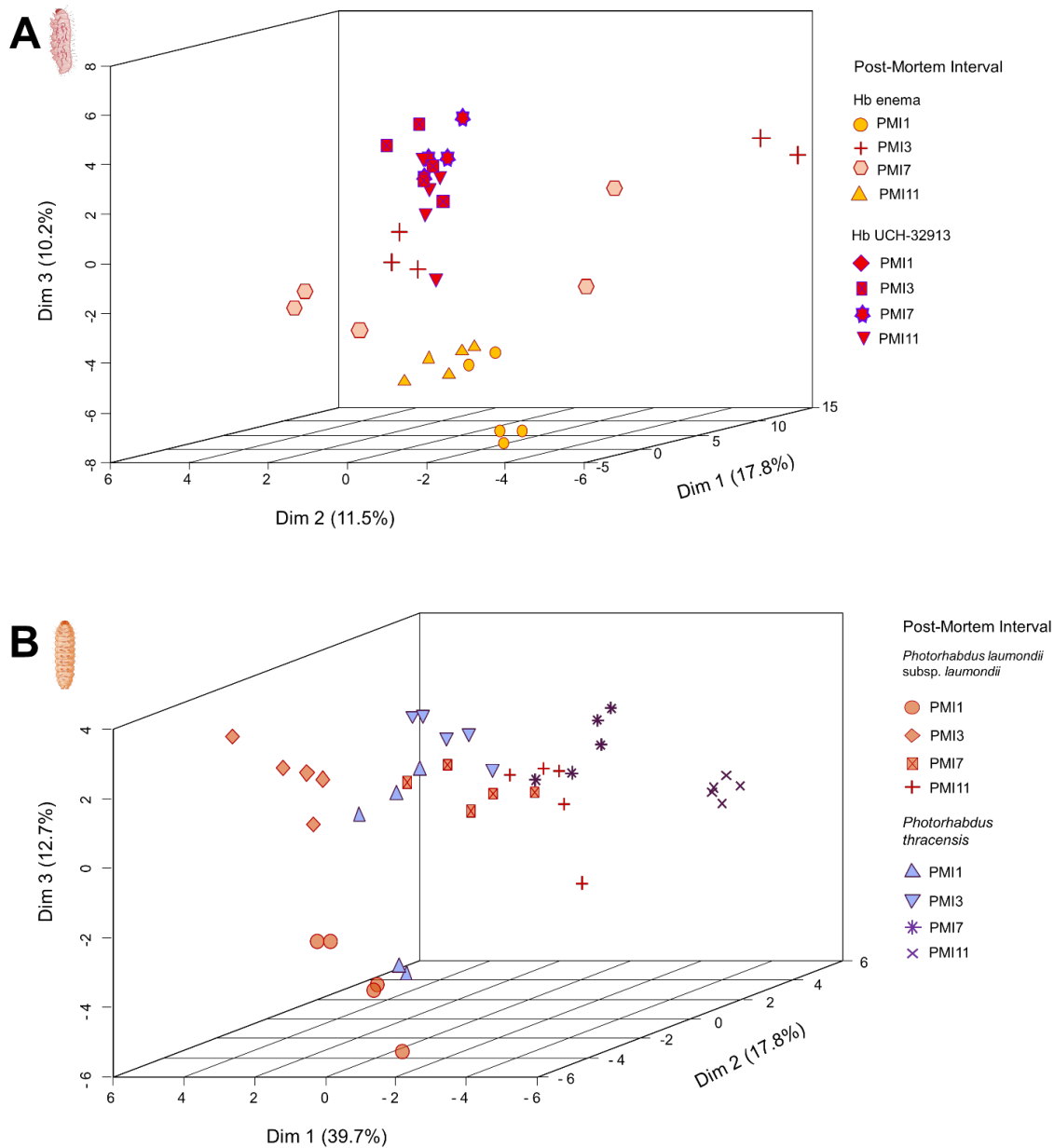


Figure 8. Principal component analysis (PCA) performed on the Volatile Organic Compounds (VOCs) profiles of insects kill by (A) Entomopathogenic nematodes: *Heterorhabditis bacteriophora* enema (Hb enema) and *H. bacteriophora* UCH-32913 (Hb UCH-32913). PERMANOVA analysis revealed differences among Post-Mortem Intervals (Df = 3, F = 9.60, $p = 0.001$), and the two populations (Df = 7, F = 6.37, $p = 0.001$). (B) Entomopathogenic bacteria: *Photorhabdus laumondii* subsp. *laumondii* and *Photorhabdus thracensis*. PERMANOVA analysis across PMIs for both bacterial treatments revealed significant differences in odor profiles (Df = 7, F = 12.033, $p = 0.001$) (Details SI Appendix Table S7).