FEMS Microbiology Ecology, 2026, **102**(5), fiag036
https://doi.org/10.1093/femsec/fiag036
Published: 7 April 2026
Research article

Priority effects drive fungal and nematode emergence from insect larvae

Amaury Payelleville[1,2,*], Magdalena Warren[1], Chloe Golde[1], Devyn Sasai[1], Benny Pan[1], Patrick Cleary[1], Arizbel Gomez[1], Rubina Shrestha[1], Jean-Claude Ogier[2], Sophie Gaudriault[2], Christine Jacobs-Wagner[1,3,4,5], Tadashi Fukami[1,6,*]

[1]Department of Biology, Stanford University, Stanford, CA 94305, United States
[2]Université de Montpellier, DGIMI, Montpellier, 34090, France
[3]Sarafan ChEM-H Institute, Stanford University, Stanford, CA 94305, United States
[4]Howard Hughes Medical Institute, Stanford University, Stanford, CA 94305, United States
[5]Department of Microbiology and Immunology, Stanford University, School of Medicine, Stanford, CA 94305, United States
[6]Department of Earth System Science, Stanford University, Stanford, CA 94305, United States
*Corresponding authors. Amaury Payelleville. Université de Montpellier, DGIMI, Montpellier, 34090, France. E-mail: amaury.payelleville@umontpellier.fr
Tadashi Fukami. Departments of Biology and of Earth System Science, Stanford University, Stanford, CA 94305, United States. E-mail: fukamit@stanford.edu
Editor: Cindy H. Nakatsu

Abstract

Priority effects, in which species arrival history influences community assembly, are increasingly recognized to affect host–parasite systems. However, priority effects across disparate groups of parasitic organisms are poorly understood despite the wide range of taxonomic groups involved. In California oak woodland, we investigated how priority effects between two insect-parasitic fungi (*Metarhizium* and *Beauveria*) influenced emergence of nematodes from insect larvae. Field and laboratory results indicated that both fungi were common, but priority effects prevented them from co-emerging from the same larva. *Metarhizium*- and *Beauveria*-infected insects did not differ in the species composition of emerging nematodes, but larvae without fungal emergence had distinct nematode communities, with *Oscheius* almost always emerging without fungi. Experiments indicated that none of the commonly found nematodes (*Acrobeloides*, *Mesorhabditis*, *Oscheius*, and *Rhabditis*) were entomopathogenic, but that *Oscheius* could exclude *Beauveria* if it arrived early. This time-dependent exclusion was likely caused by a bacterium that *Oscheius* nematodes carried (*Serratia proteamaculans*). Together, these findings suggest that fungi enter insects as primary arrivers, while nematodes come as secondary arrivers to exploit fungus-killed insects, with priority effects influencing both groups. We suggest that this system is a promising natural microcosm for understanding priority effects across disparate groups in host–parasite systems.

Keywords community assembly; nematodes; entomopathogens; fungi; priority effect; bacteria

Introduction

It has been a century since Alvar Palmgren articulated the hypothesis that the order and timing of species arrival dictate how species affect one another and, consequently, which species dominate in local communities (Palmgren 1926). Known today as priority effects (Drake 1990, Chase and Leibold 2003, Fukami 2015), this historical contingency in community assembly has been observed in a variety of organisms (Stroud et al. 2024,). Among them, coinfecting parasites and pathogens are some of the organisms for which priority effects have been increasingly studied over the past decade (Karvonen et al. 2019).

In studying priority effects, natural microcosms, defined as "small contained habitats that are naturally populated by minute organisms," have played a central role in advancing basic understanding (Srivastava et al. 2004). A wide range of systems have been used as natural microcosms, and one that has been indicated only recently for their potential consists of entomopathogenic fungi in soil-dwelling insects. As suggested by Costantin et al. (2025) using fungal isolates from Brazil and *Tenebrio molitor* larvae, this system can make unique contributions to understanding host–parasite interactions when multiple parasitic species coinfect the same insect.

Costantin et al. (2025) provided evidence for priority effects between two major genera of insect-parasitic fungi, *Metarhizium* and *Beauveria*. When both were experimentally introduced, whichever genus was introduced first emerged more abundantly. However, fungi are not the only organisms that use insect larvae as resources. Soil-dwelling nematodes and their associated bacteria can also establish and emerge from dead insect larvae. Consideration of these other organisms can broaden this system's scope as a promising natural microcosm. However, few studies have docu-

Received: 13 November 2025. **Revised:** 2 April 2026. **Accepted:** 3 April 2026

mented how soil-dwelling nematodes or their associated bacteria may interact with *Metarhizium* or *Beauveria* in insect cadavers.

In this paper, we examine the possibility that entomopathogenic fungi *Metarhizium* and *Beauveria* in an insect larva influence not only each other through priority effects (by manipulating initial abundance and arrival order), as previously demonstrated, but that these priority effects influence the community of nematodes that establish and multiply in the insect cadaver. If this possibility is plausible, then that would indicate that priority effects between *Metarhizium* and *Beauveria* could have broader consequences, affecting not just their own performance, but also the community assembly of nematodes in the infected insects. To study this possibility, we conducted a year-long field survey in California oak woodland. We also did a series of laboratory coinfection experiments using the fungi, nematodes, and nematode-associated bacteria that we isolated from the field survey. Based on the findings from this study, we will propose hypotheses on how priority effects among fungi, nematodes, and bacteria influence fungal and nematode emergence from infected insects.

Materials and methods

Field survey

Study system

We conducted a year-long field survey to assess the presence of active entomopathogenic and opportunistic organisms in the soil beneath coast live oak (*Quercus agrifolia*) trees at Jasper Ridge Biological Preserve (’Ootchamin ’Ooyakma). In this preserve located in the Santa Cruz Mountains of California in the United States, adults of the acorn moth (Lepidoptera—*Cydia latiferreana*) and the filbert weevil (Coleoptera—*Curculio occidentis*) lay eggs in coast live oak acorns while acorns are still attached to the tree. The larvae of both insects hatch and grow within the acorn, and come out of the acorn after it falls to the ground in the autumn (Lewis 1992). The emerged larvae then burrow into the soil, which is the period when they can be infected by entomopathogens before they pupate and leave the soil as adults (Bruck and Walton 2007). Given their high abundance (Lewis 1992), these insects likely serve as the main resources for insect-parasitic fungi and nematodes in this preserve.

Soil sampling

Soil samples were collected from two different locations within the preserve in February, April, July, and September of 2023 and January and March of 2024. One location (hereafter called location 1; 37.4071868, −122.2344094) was on top of the ridge in *Q. agrifolia* woodland. The other location (hereafter called location 2; 37.4034912, −122.2347112) was in a small valley that had wetter and more heterogeneous woodland than location 1. At each location, three *Q. agrifolia* trees were haphazardly selected. For each tree, five soil samples, each of 10 cm diameter and a depth of 25 cm from the surface, were collected within a two-meter radius from the base of the tree. The five soil samples collected around each tree were then mixed and sieved through a 0.5-cm mesh sieve to remove rocks, roots, and leaves, and then placed in plastic bags. Once transported to the laboratory within 2 h of soil collection in the field, the three samples were mixed to make one larval trap corresponding to the respective location.

Ex situ baiting

Approximately 1 kg of soil from each location was placed inside glass boxes (17 cm wide, 22 cm long, and 6 cm deep) covered with a rubber lid pierced to allow air flow. Since larvae of the *Cydia latiferreana* moth and the *Curculio occidentis* weevil were available for collection only during the few months of the year when they come out of the acorns and burrow into the soil, we used as larval traps two surrogate species of the same order respectively: the greater wax moth (Lepidoptera—*Galleria mellonella*) and the mealworm beetle (Coleoptera—*Tenebrio molitor*). These well-studied insects were readily available in large quantities from a vendor (Carolina Biological Supply) throughout the year. They are the main larvae commonly used for live-baiting of entomopathogenic organisms from the soil (Bedding and Akhurst 1975).

However, to gain initial insights into the relevance of findings with *Galleria* and *Tenebrio* used as surrogate larvae to the understanding of insect–entomopathogen interactions in the field, we also conducted preliminary experiments where we introduced bacteria and fungi into live *Cydia* and *Curculio* larvae that came out from acorns collected at our study site, ’Ootchamin ’Ooyakma, in November-December 2024. Method details of these experiments are described below in the “In vivo competition in *Galleria*, *Cydia*, and *Curculio* larvae” section.

In addition, to test for the effects of soil moisture on fungal and nematode emergence (Fig. 1B), we conducted the baiting with soil moisture adjusted to an identical level across months. To perform this comparison, the moisture level of soil from February 2023 at each location was measured by weighing a soil sample before and after two rounds of dry autoclaving and placement in a dry cabinet for 2 days. The difference in weight was determined to estimate the amount of water in the soil for the time point of February 2023, the wettest month of the year. For each other month, the same protocol was followed, and the amount of sterile water needed to match the level of moisture observed in February 2023 was added to the soil.

Hereafter we refer to *ex-situ* baiting traps as *Galleria* or *Tenebrio* traps, depending on the larva used to bait the entomopathogenic organisms present in the soil. The goal of these *Galleria* or *Tenebrio* traps was to allow entomopathogenic organisms present in the soil sample to infest the insect larva.

For *Galleria* traps, 30 fifth-instar larvae were added to each trap, and survival monitored daily for 2 weeks. Larva and the soil were mixed each day to ensure contact between the larvae and the organisms present in the soil. When all larvae were dead or when they had been in traps for more than two weeks, all remaining larvae were removed, and a new batch of 30 larvae were added and monitored the same way. The same protocol was followed with *Tenebrio* traps but using smaller traps (∼500 g of soil in boxes that were 14 cm wide, 18 cm long, and 4.5 cm deep) and smaller batches of larvae (20 larvae).

For both *Galleria* and *Tenebrio*, each dead larvae was removed from the *Galleria/Tenebrio* traps. These cadavers were transferred to another type of traps called White traps, named after the author who invented them (White 1927). White traps allow for the entomopathogenic organisms that killed the insect larvae to emerge from the cadaver. We used them to collect and identify those

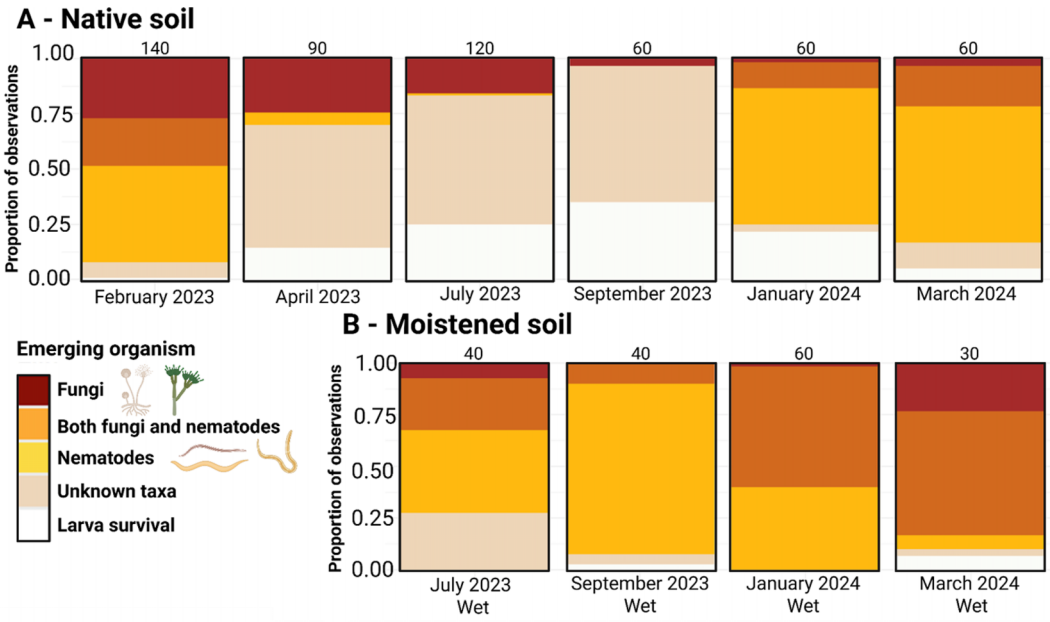

Figure 1 Organisms emerging from *Galleria* moth larvae in the field survey at Jasper Ridge Biological Preserve ('Ootchamin 'Ooyakma). Fungi and nematodes emerged from *Galleria* larvae placed in the field-collected soil throughout the survey period from February 2023 to March 2024, although the proportion of fungal, nematode, and co-emergence varied from month to month. The field collected soil was used without (A) or with (B) water added to the soil to make the soil as moist as it was in the field during the wettest month (February 2023). Each bar represents the proportion of emerging organisms for that month. The number of *Galleria* larvae tested in each month is shown above the bar corresponding to the month.

organisms and therefore labelled each one of those White traps with the larval ID corresponding to the type of larva (*Galleria* or *Tenebrio*), the month and location from where the soil was taken, and the date of larval death. The White traps contained ∼20 ml of Ringer's solution, which is a physiological salt solution, to moisten the filter on which the cadaver was deposited. The traps were then transferred to an incubator set at 25°C. After three to four weeks of incubation, larval cadavers in the White traps were checked to determine the presence of fungi, nematodes, and other organisms.

In this paper, we use the term emergence to describe an organism exiting from an insect cadaver. This term is used commonly to describe infective juveniles (IJs) of nematodes exiting a cadaver. In fungi, sporulation may be a more accurate term to describe this part of their life cycle. However, we use emergence for nematodes and fungi for consistency.

Identification of emerging fungi

When a fungus emerged from an insect cadaver in a White trap, it was re-isolated on Potato Dextrose Agar medium (PDA) plates to obtain a single morphotype. In some cases, two morphotypes could be observed on the insect cadavers. In these cases, we performed sequential re-isolations until we were able to have isolated on two individual plates, each of the morphotypes observed. The two most abundant morphotypes observed throughout this survey were identified by DNA extraction with the ExtractNAmp kit (Sigma) and amplification and sequencing of the ITS region with the primers EPF-ITS1-F and EPF-ITS4-R (Table S1). The two sequenced isolates were then used for all experiments. All other isolates corresponding to each morphotype were stored but not used in the pathogenicity assay, unless stated otherwise.

Storage of emerging nematodes

When nematodes were detected in Ringer's solution of the White traps, they were washed using tap water, sifted by a 20-μm meshed sieve, and then stored at 15°C in a cell culture flask containing fresh Ringer's solution. As many insect cadavers had more than one nematode genus emerging from them, we decided to perform amplicon sequencing to detect the full diversity of nematodes species present in this survey.

Identification of emerging nematodes

Nematode DNA extraction

For each flask containing nematodes emerging from one insect cadaver, ∼1000 infective juveniles (IJs) were taken from the flask and frozen at −80°C in Fastprep tubes. If the emergence was not dense enough, 100 μl containing as many nematodes as possible were sampled and frozen.

On the day of the DNA extraction, the tubes were heated to 80°C and centrifuged at 2000 r.c.f. for 10 min. Liquid was then removed, and the nematodes resuspended in 100 μl of the extraction solution from the ExtractNamp kit. Three 2-mm beads were added to each tube. Then, 25 μl of Tissue Prep Solution (from the above kit) and 20 μl of Proteinase K were added to each tube, and bead beating was performed for 45 s after the tubes were incubated at 55°C for 1 h. The temperature was then increased to 95°C for 3 min and 100 μl of Neutralizing Buffer were added.

Nematode amplicon library preparation

Four microlitre of the DNA extraction solution was used as DNA matrix for the PCR amplification using primers MB_ITS_APJC_F and MB_ITS_APJC_R (Table S1) at 0.5 μM final concentration each and using the red ready-Mix PCR. We performed PCR as follows: 95°C for 3 min, followed by 10 cycles of 95°C for 15 s, 52°C for 30 s,

72°C for 2 min, and 30 cycles of 95°C for 15 s, 60°C for 30 s, 72°C for 2 min, ending with 72°C for 5 min. The PCR product was then migrated on an agarose gel to verify amplification. Each PCR product showing clear amplification on the gel was kept. If no amplification was observed, the protocol was repeated.

An amplicon library was prepared for a total of 432 PCR products obtained. Each PCR product was purified using beads (Canvax, AN36, High purity clean-up magnetic beads). Ten microlitre of beads was added to each well of 96-well plates mixed by pipetting up and down ten times and left on the bench for 5 min. Plates were then transferred to a magnetic rack and the liquid removed once all beads were stuck to the side of the well. Two washes using 70% ethanol were then performed, and the plate was left to dry for 5 min. Once no ethanol could be detected, 50 μl of 55 °C DNAse-free water was added to each well. Each purified PCR product was then barcoded using the corresponding primer listed (Table S2). Five microlitre of each purified PCR product was added to a new well with 1.6 μl of premixed primers, 10 μl of red ready-Mix PCR, and 3.4 μl of water. The barcoding was performed using the following cycle: 95°C for 3 min, 8 cycles of 95°C for 30 s, 55 °C for 30 s, and 72°C for 30 s, ending with 72°C for 5 min.

Nematode DNA sequencing

After barcoding, PCR products were migrated on a gel to confirm the intensity of the band and then purified using the same protocol as above, except for the elution volume, which ranged from 20 to 60 μl, depending on the intensity on the gel (higher volume for a less intense band). The samples were then pooled together and sent to the Stanford Genomic Sequencing Service Center, where the concentration and quality of the pooled samples were analyzed with a Fragment Analyzer (Agilent, Santa Clara, CA, USA) and they were then sequenced on a MiSeq (Illumina) using a 2 × 300 cycle sequencing kit with a 15% PhiX spike-in.

Raw BCL files from the MiSeq run were demultiplexed with the Illumina bcl2fastq software, using default settings except for barcode-mismatches, which was set to 0. Index barcodes and Illumina sequencing adaptors were excluded in the resulting fastq files. The forward and reverse primers were removed from the fastq files with cutadapt (Martin 2011). The trimmed sequences were processed with DADA2 (Callahan et al. 2016), with the following modifications: in the filterAndTrim function, truncQ was 2, minLen was 50, and trimRight was 141. The quality of many of the reads deteriorated after about 160 bp, and since our reads were about 300 bp long, we trimmed the right 141 bp to remove the low-quality ends. Since we sequenced the ITS region, the amplicon reads were not long enough to span the entire gene. Therefore, the rest of the downstream analysis was completed with only the forward reads that had better quality than the reverse reads. Data are available in a BioProject (PRJNA1419355).

Nematode sequence data processing

To classify the resulting amplicon sequence variants (ASV), we built a custom BLAST database that included all ITS1, ITS2, and 18S sequences in the Nematoda phylum available in the NCBI nucleotide database. To build the database, we downloaded all the relevant sequences, deduplicated them with seqkit (Shen et al. 2016), and created a database from the deduplicated sequences with the BLAST makeblastdb tool. We then did a blastn search of our ASV sequences in our custom database with a minimum percentage identity threshold of 80 for all hits. ASVs that had no hits against our custom database were classified using a blastn of the entire NCBI nucleotide database. The hit with the highest bitscore was retained for each ASV, and taxonomic details were extracted from the hit's accession lineage. This taxonomy was combined with the DADA2 output count data and sample metadata for downstream analysis with phyloseq. For clearer and more relevant results, ASVs were aggregated at the genus level.

Principal coordinates analysis (PCoA) of nematode species composition

Binary presence/absence matrices were constructed with individual insect larvae as rows and identified nematode taxa as columns. Downstream analysis included only identified taxa, excluding non-informative and unidentifiable taxa. Our goal was to investigate whether the resulting matrices were related to other variables in our survey data, such as fungal morphotype. Therefore, treatment group (sampling time and whether the larva was *Tenebrio* or *Galleria*) and the morphology of the emerged fungus were included in the analysis, but they were not used to construct the matrices. We calculated a Jaccard dissimilarity matrix of the presence/absence ASV matrix using the vegdist function from the vegan package Oksanen et al. 2025). Principal Coordinates Analysis (PCoA) was then performed on the dissimilarity matrix with the pcoa function from the ape package (Paradis and Schliep 2019). The first two axes were extracted for visualization of sample clustering based on emerging nematode species composition.

To statistically evaluate if nematodes emerging from a single insect cadaver were related to a specific treatment group of presence of a fungus, a permutational multivariate analysis of variance (PERMANOVA) was conducted using the adonis2 function (999 permutations). This analysis tested for differences between treatment groups or morphology types based on the Jaccard dissimilarity matrix. To assess whether significant PERMANOVA results were driven by variation in group dispersion, a multivariate homogeneity of dispersion test (PERMDISP) was conducted using betadisper followed by anova.

To investigate which nematodes were emerging together and if some species were less co-emerging from one insect cadaver, we performed a cluster analysis. The optimal number of clusters of the nematode ASVs, in this case seven, was calculated by computing the within-groups sum of squares for the clusters produced by the k-means method. Hierarchical clustering analysis of the Jaccard distance matrix was conducted with the hclust function, and the result was cut into seven groups using the cutree function. The clusters were visualized with a dendrogram using the dendextend and ggdendro packages (Galili 2015, de Vries and Ripley 2024).

To aid interpretation of the main gradients of variation in nematodes emerging from the cadavers, nematode species were fitted *a posteriori* onto the ordination using the function *envfit* from the vegan package in R. Species vectors indicate the direction in which the probability of having this species emerging from the cadaver. The strength and significance of the associations were assessed using permutation tests (999 permutations) and results are presented in Table 1.

After running the PCoA, the coordinates of the first two axes for each sample were extracted and grouped depending on fungi presence/absence. The mean coordinates of each axis (centroids) for each group (*Beauveria*, *Metarhizium*, and no fungus) were then calculated and represented in the PCoA plot.

Table 1 Associations between nematode taxa and community ordination structure. Associations were assessed using permutation-based vector fitting (the envfit function in the vegan package in R). For each taxon, the strength (r^2) and significance (permutation test) of its correlation with the ordination axes were calculated. The closest fungal group was then assessed using fungal centroids.

Taxon	r2	pval	Quadrant	Closest_Fungal_Group
Acrobeloides	0.79	0.001	Lower-left	*Beauveria*
Mesorhabditis	0.47	0.001	Lower-right	*Metarhizium*
Rhabditis	0.13	0.001	Upper-right	NA
Oscheius	0.69	0.001	Upper-right	NA

Laboratory experiments

As we wanted to investigate the role of each isolated organism in our study system, we needed to start by validating each organism's pathogenicity for the insect's larvae. In other words, we sought to determine if organisms emerging from insect's cadaver were able to kill the insects by themselves, i.e. if they were entomopathogenic. For all experiments using *Galleria* larvae and unless stated otherwise, larvae that were at least 2 cm long were selected and pooled in batches of 20. Each batch was then randomly assigned to a treatment. The control was always the starting condition (10 larvae) and the end condition (10 larvae) to make sure timing didn't have an impact on the tested conditions.

Fungal pathogenicity on *Galleria* larvae

Beauveria and *Metarhizium* fungi were grown on PDA for one week or until spores covered the plate. Ten millilitre of Ringer's solution was then added on top of the plate, and the plate was gently scratched with a 1-μl loop to resuspend spores. The Ringer's solution was then transferred to a 50-ml Falcon tube, and this process repeated once. Concentration of spores was then measured using a hematocytometer. Volume was adjusted to reach an equivalent concentration for different fungi. After thorough vortexing, larvae were immersed in the spore solution (10^6 or 10^7 spores/ml) and individually put in wells in 12-well plates, and larval survival was monitored at 25°C over time. Larvae used as the control were immersed in Ringer's solution without spores. Each experiment was conducted with 20 larvae per treatment and repeated two to four times, depending on the availability of spores and larvae.

Larval survival following exposure to *Beauveria* or *Metarhizium* spores was analyzed using a binary status variable, i.e. death or survival, to compare three treatment groups: control, 10^6 spores/ml, and 10^7 spores/ml. Kaplan–Meier survival curves were constructed using the survfit() function from the survival R package. Survival probability over time was estimated separately for each concentration group within each fungal treatment (*Beauveria* and *Metarhizium*). Differences between survival curves were evaluated using the log-rank test, as implemented in ggsurvplot() from the survminer package.

To quantify the effect of fungal spore concentration on larval mortality, we fitted Cox proportional hazards models using the coxph() function. The model included spore concentration as the predictor, with 10^6 spores/ml as the reference level. Hazard ratios and 95% confidence intervals were extracted from the model summaries. A hazard ratio greater than one indicated an increased risk of death relative to the reference group, while a value less than one indicated a protective effect. Statistical significance of the model terms was assessed using Wald tests, and overall model fit was evaluated using three global tests: likelihood ratio test, Wald test, and score (log-rank) test.

Nematode pathogenicity on *Galleria* larvae

For this experiment, identified nematode emergence containing only one nematode species was used to make sure to test only this nematode pathogenicity. Concentration of nematodes in Ringer's solution was measured by counting under a dissecting microscope. A solution containing estimated 1000 nematodes/100 μl was prepared to assess pathogenicity. For inoculation, we used 2-ml Eppendorf tubes containing a piece of filter paper folded to cover the sides of the tubes, with the lid pierced to allow air flow. One *Galleria* larva from the previously prepared batches of 20, was added to each of these Eppendorf tubes and then 100 μl of the nematode solution was added to the filter paper inside the tube. Survival of the larvae was then monitored over time at 25°C, repeated for 20 independent larvae. For some nematodes, no biological replicate for each isolate was possible due to a low number of nematodes available. Results for replicates for other isolates can be found in Table S3 and correspond to a batch of 10 *Galleria* larvae each.

Interactions between fungi on *Galleria* larvae

As both *Metarhizium* and *Beauveria* fungi displayed dose-dependent pathogenicity (Fig. 3A), we manipulated spore concentration as a proxy for timing of infestation, which is an approach commonly used to study priority effects in both experiments (Eggers and Matthiessen 2013, Chappell et al. 2022) and theoretical analyses (DeMalach et al. 2021, Mohd 2022). Note that this approach to studying priority effect through manipulation of the initial abundance, as opposed to arrival order, may only capture part of the true impact of priority effects.

To study interactions between fungi, we performed a co-infection experiment adding both fungi together and monitoring which one was emerging from the cadaver. To maximize competition time and reproducibility in our experiment, we used lower spores' concentrations than in pathogenicity assay. Briefly, insect larvae were dipped into Ringer's solution with varying amounts of *Beauveria* and *Metarhizium* spores (10^5 spores/ml, 10^4 spores/ml, or no spores). Larval survival was monitored over time. Each dead larva was then transferred to a White-trap and incubated at 28°C for 2 weeks to allow fungus emergence. Fungus emergence was then identified by morphology and color (white being *Beauveria* and green *Metarhizium*). Fisher's exact tests were conducted to search for differences between the following treatment pairs of *Beauveria* / *Metarhizium*: $10^5/10^5$ vs. $10^5/10^4$, $10^5/10^5$ vs. $10^4/10^5$,

and $10^5/10^4$ vs. $10^4/10^5$.Interactions between fungi and nematodes on *Galleria* larvae.

To study interactions between fungi and nematodes, insect larvae were first dipped into Ringer's solution with *Beauveria* fungal spores and then transferred to Eppendorf tubes containing a piece of filter paper with 1000 nematodes of either *Oscheius* sp. or *Mesorhabditis* sp. Conditions tested are controls (either Ringer solution alone, Nematode alone -one condition per species- and *Beauveria* alone) or co-infestation where one nematode species is infested together with fungal spores in one larva. Each treatment was replicated at least three times independently with each 20 larvae. Larval survival was monitored over time. A Chi-squared test of independence was conducted on a contingency table of treatment conditions versus emerging organism identity to evaluate whether the distribution of emerging organisms differed significantly across co-infestation treatments.

Isolation of bacteria from crushed nematodes on agar plates

To study how nematode-associated bacteria interact with *Beauveria*, we first isolated bacteria from some of the nematodes that emerged in the field survey described above (Table S4). To this end, 1 ml of Ringer's solution containing emerged nematodes was washed using a 20-μm mesh sieve and added to an Eppendorf tube. After centrifugation at 8000 r.c.f. for 5 min, the supernatant was removed, and the nematode pellet was crushed using a sterile pellet pestle. Bacteria were then streaked on NBTA plates (Nutrient Bromothymol Blue Triphenyl Tetrazolium Chloride Agar, with the concentration of TTC ten times lower than the usual recipe to allow more bacteria to grow) using a 1-μl inoculation loop. After isolation of each colony morphology on the NBTA plates, we amplified and sequenced the *rpoB* gene (Ogier et al. 2019) of the bacteria to identify bacterium's genus and species when possible.

Interactions between fungi and bacteria on plates

Using the isolated bacteria from above, we did *in vitro* experiments to study their interactions with *Beauveria*. Fungal spores were spread vertically using a 1-μl inoculation loop on a PDA/LB plate (mix of 18.5 g of PDA with 13 g of LB agar in 1000 ml of water). After growing each bacterial isolate independently in 5 ml LB broth overnight, each isolate was streaked horizontally on the PDA/LB plate where the fungal spores had been streaked. The bacteria used in this experiment included *Enterococcus casseliflavus*, *Ochrobactrum quorumnoscens*, *Achromobacter spanius*, *Pseudomonas protegens*, *Serratia proteamaculans*, and *Serratia sp.* Three different bacterial isolates were streaked on each plate. Each species combination was conducted at least nine times. Plates were incubated for a week at 25°C. Plates were monitored over time and the growth of the fungi quantified by the development of mycelium on the plate and similarly growth of bacteria by presence of colonies or viscous streak. A time-delay experiment was conducted similarly except that we streaked *Beauveria* 24 h before streaking the bacteria on the plate. A Fisher's exact test was used to compare the different outcomes of each pairwise competition.

In vivo competition in Galleria, Cydia, and Curculio larvae

For *Galleria* larvae, *in vivo* experiments were performed as follows: *Beauveria* spores were applied to *Galleria* larvae using the same protocol as above. For bacterial injection, bacteria were first grown overnight in LB broth. Fifty microlitre of the overnight culture was then transferred to 5 ml of fresh LB broth and grown for 4 h at 25°C until it reached an optical density (600 nm) of 0.1–0.3. After washing the cells by two rounds of centrifugation (8000 r.c.f. for 5 min), dilutions were made and plated to estimate the number of viable cells injected into each larva. Injection into larvae was done using a microinjector with a foot pedal (Chemyx, model F100X). Each larva was injected with 10, 100, or 1000 bacterial cells. We did fungal spore application 24 h (F24/B) and in some cases 48 h before (F48/B) bacteria injection. We always delayed bacteria injection to allow fungal growth from the spores without giving the bacteria too big an initial advantage via direct injection. Each larva was monitored for a week, and cadavers were kept for one more week to monitor fungal emergence. When white mycelia were observed on the cadaver, the emerging organism was determined as fungi. When cadavers had no noticeable fungi, the cadaver was cut open and some of the tissue inside was streaked on NBTA plates. Bacteria resulting from this streaking had the same morphology as the injected bacteria, so we regarded bacteria to be the emerging organism. Thus, we assumed that all larvae without fungi had bacteria as the emerging organism. As no major difference in the outcome was noticed depending on bacterial concentration, the data were pooled for statistical analysis.

For the environmentally collected larvae of the filbertworm moth *Cydia latiferreana* and the acorn weevil *Curculio occidentis*, the same protocol as for *Galleria* was followed, with the following exceptions. As the number of available larvae was lower compared to *Galleria*, we could not work with triplicates and the number of larvae for each condition was adjusted as shown in Fig. S6. In addition, because *Cydia* and *Curculio* larvae were smaller than *Galleria* larvae, we lowered the volume injected to 2 ul. Controls consisted of injection of LB (resulting in death of 2 *Curculio occidentis* out of 20 and 3 *Cydia latiferreana* out of 20). In the case of co-infestation, the bacteria *Serratia proteamaculans* was injected 24 h after contact of the insect larvae with *Beauveria* spores to avoid giving too much of a head start to the bacteria with the injection. The emerging organism was identified with the same protocol as for *Galleria*. As replicates vary between conditions and the number of larvae is low, we decided to only use those data as a discussion and not a significant result.

Results

Fungal and nematode taxa showed specific associations with one another in insect larvae

Fungi and nematodes were the two major groups of organisms emerging from *Galleria* larvae in February 2023 in soil samples from location 1 (Fig. 1A). Sixty-one cadavers (44%) yielded nematodes alone, 38 (27%) yielded fungi alone, 30 (21%) had both, and 10 (7%) contained other organisms (including, but not limited to, mites, molds of various shapes and colors, and sometimes

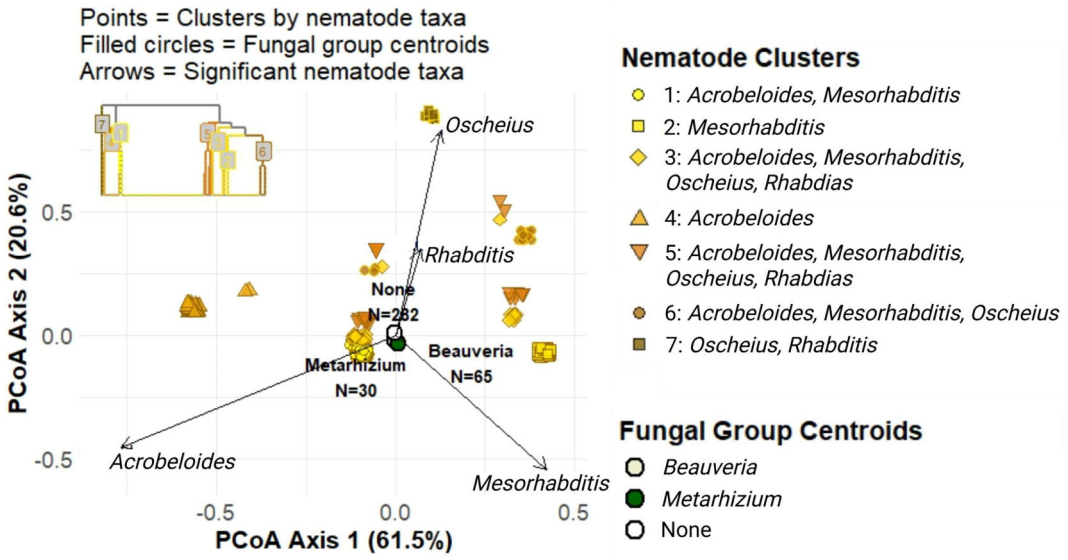

Figure 2 Principal Coordinates Analysis (PCoA) summarizing variation among the *Galleria* moth larvae tested in the field survey in the species composition of nematodes that emerged from the larvae. The PCoA plot is based on the nematode amplicon sequencing data from the field survey conducted at Jasper Ridge Biological Preserve (’Ootchamin ’Ooyakma) from February 2023 to March 2024, using the same samples as shown in Fig. 1. Each data point in the PcoA plot represents a larva tested, showing the species composition of nematodes that emerged from the larva. Each point is color-coded according to the cluster group it belongs to in the cluster analysis used to find distinct groups of nematode species composition. Dendrogram summarizing the cluster analysis is shown in the inset. Arrows indicate the contribution of major nematode taxa to each PcoA axis. In addition, the PcoA plot also show the centroids of the larval samples from which *Metarhizium* fungus emerged, those from which *Beauveria* fungus emerged, and those from which no fungus emerged (none), with the sample size for each group shown.

no organism identifiable, which could possibly mean bacteria or viruses caused insect death) (Fig. 1A). Nematode emergence declined sharply in April (five cadavers, 6%), dropped to just one cadaver in July, and disappeared in September. Fungi followed a similar change, though less pronounced.

Larval survival increased over this period, with 14% in April, 25% in July, and 35% in September (Fig. 1A). In January and March 2024, nematodes and fungi reappeared, with nematodes emerging from 37 cadavers (62%) in both months, fungi from 1 (2%) and 3 cadavers (5%) respectively, and both nematodes and fungi from 7 (12%) and 11 (18%) (Fig. 1A).

When we moistened the soil, we detected nematodes in the previously negative samples from July and September (Fig. 1B), indicating that nematodes were present but probably unable to reach and therefore emerge from larvae due to insufficient moisture. Fungal detection also increased following soil rehydration.

In fungi, two distinct morphotypes (based on the shape and color of conidiophores and conidia) were commonly observed. We sequenced the Internal Transcribed Spacer (ITS) region of the genome of one representative from each, confirming their identity as *Beauveria* (white morphotype) and *Metarhizium* (green morphotype). *Beauveria* was more frequently observed (89 cadavers, 66%) than *Metarhizium* (45 cadavers, 34%). Despite both being common, *Beauveria* and *Metarhizium* rarely co-emerged. Only two of the 132 cadavers with fungal emergence had both (Fig. S1).

Four nematode genera were commonly detected, emerging either alone or in various combinations (Fig. 2). Of these genera, *Oscheius*, *Mesorhabditis*, and *Rhabditis* are regarded as entomo-opportunists (Taboga 1981, Zhou et al. 2017, Zhang et al. 2019, Manjula et al. 2020), while *Acrobeloides* is bacterivorous (Postma-Blaauw et al. 2005). In the analysis of nematode species composition, seven distinct clusters emerged (Fig. 2). *Oscheius*, *Acrobeloides*, and *Mesorhabditis* were the primary genera characterizing nematode community structure.

When we added three fungal emergence centroids (*Beauveria*, *Metarhizium*, and no fungus) onto the PCoA summarizing nematode species composition, the centroid positions for *Beauveria*- and *Metarhizium*-emerging larvae were close to each other with no statistically significant separation (Fig. 2). Larvae with no fungal emergence clustered slightly but not significantly separately from *Beauveria*- and *Metarhizium*-emerging larvae. Using the PcoA axis, the four main nematodes genera were clustered highly significantly (P-value < 0.001) separately. While *Acrobeloides* seemed closer to the *Beauveria* centroid and *Mesorhabditis* seemed closer to the *Metarhizium* centroid, statistical tests showed no significant differences. The most interesting correlation was between *Oscheius* nematodes and the “no fungi emerging” centroid (Table 1).

The two sampling locations largely mirrored each other (Fig. S2). One difference was that fungivorous mites (included in the unknown group), likely feeding primarily on *Beauveria*, were present only at the second location. Furthermore, the other insect tested, *Tenebrio*, showed higher survival during the dry summer months (Fig. S3), but we detected similar fungal and nematode communities in *Tenebrio* and *Galleria* during the wet winter months (Fig. 1B and Fig. S4). For the rest of our study, we focused on *Galleria* larvae as they were easier to use in laboratory experiment.

Fungi were entomopathogenic, and exerted priority effects on each other

Both *Beauveria* and *Metarhizium* were pathogenic to *Galleria* larvae (Fig. 3A). When larvae were exposed to a solution

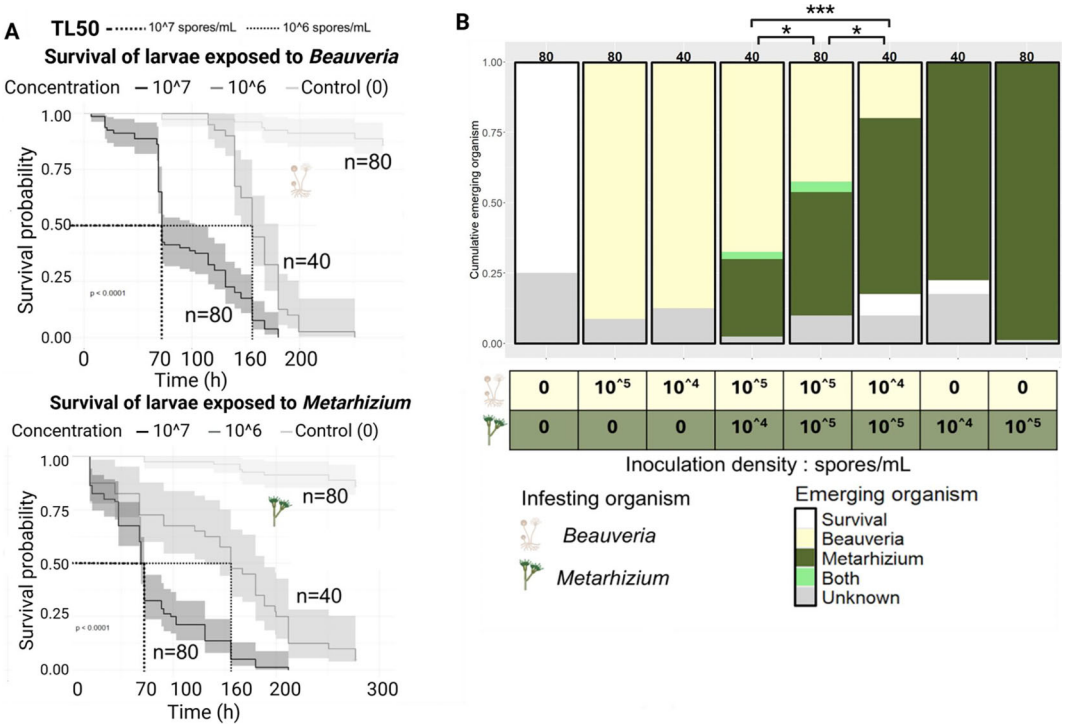

Figure 3 Survival curves of *Galleria* moth larvae that were exposed to a specific species of fungi and priority effect between fungi. Panel A shows the survival of *Galleria* larvae after exposure to two fungal species (either *Beauveria* or *Metarhizium*) at two spore concentrations (10^7 and 10^6 spores/ml), compared with a control exposed to Ringer's solution. The median lethal time (LT_{50}) for each curve is shown by dash line and displayed on *x*-axis and Kaplan–Meier survival curves are used. Panel B shows the proportion of larvae from which either species, both species, or neither emerged, at varying spore concentrations used for infection, when *Galleria* larvae were infected by either or both *Beauveria* (beige) and *Metarhizium* (green) fungi. The concentration of spore/ml used is shown in the table below the graph. Numbers above each bar indicate the number of larvae used for each infection treatment. Asterisks indicate results of the statistical comparisons performed using Fisher's exact tests, where ∗ denotes $P < 0.05$ and ∗∗∗ denotes $P < 0.001$.

containing 10^7 spores/ml, the median lethal time (LT_{50}) was ∼70 h post-infestation for both fungal species. Reducing spore concentration to 10^6 spores/ml increased LT_{50} to around 160 h. We isolated and preserved ∼200 *Beauveria* and 100 *Metarhizium* isolates from this survey. Although only a subset of these strains were tested in the assays reported here, all tested isolates were pathogenic to *Galleria*, with little variation in LT_{50} (data not shown).

As expected, inoculation with either *Beauveria* or *Metarhizium* resulted in the emergence of the same fungus from the cadaver, based on morphotype. In co-inoculation, only a single fungus emerged in most cases, with only 3% (4 out of 160) of cadavers showing co-emergence (Fig. 3B). The fungus introduced at a higher concentration was significantly more likely to dominate and emerge, indicating priority effects (Chi-square test, $P < 0.001$; Fisher Exact test for priority effect condition, P-value = 5.385e-05).

Nematodes were not entomopathogenic, but one genus, *Oscheius*, could outcompete *Beauveria* fungi

None of the nematode isolates caused significant larval mortality (Fig. 4A). All larvae survived, except in the case of *Oscheius* sp., which resulted in two deaths out of 20 trials. This overall lack of pathogenicity was unexpected, given that nematodes were among the most frequently recovered organisms in our field survey. This finding indicates that the nematodes were not entomopathogenic for the most part, despite their frequent emergence from the larvae.

In co-inoculation with *Beauveria*, *Mesorhabditis* nematodes emerged from the cadaver in only 6% of cases (10 out of 160). In contrast, co-inoculation larvae with *Beauveria* and *Oscheius* resulted in higher nematode emergence rate, with 20% of cadavers (36 out of 180) showing emergence of either nematodes alone or both nematodes and fungi (Fig. 4B).

Exclusion of *Beauveria* fungi by *Oscheius* nematodes was likely caused by associated bacteria

Sequenced bacteria from nematodes were selected for further study (bold in Table S4) based on their detection with only one nematode genus (specialist) or multiple different nematodes genus (generalists). *Serratia proteamaculans* was the only bacterial species found co-occurring only with *Oscheius* nematodes in our experiments. However, other sequenced bacteria like *Serratia* or *Pseudomonas protegens* were also potential specialists based on the literature (Ruiu et al. 2022a, Zhang et al. 2019, Ogier et al. 2020) and were therefore selected for further experiments. We used *Achromobacter spanius* (present with all nematodes) and

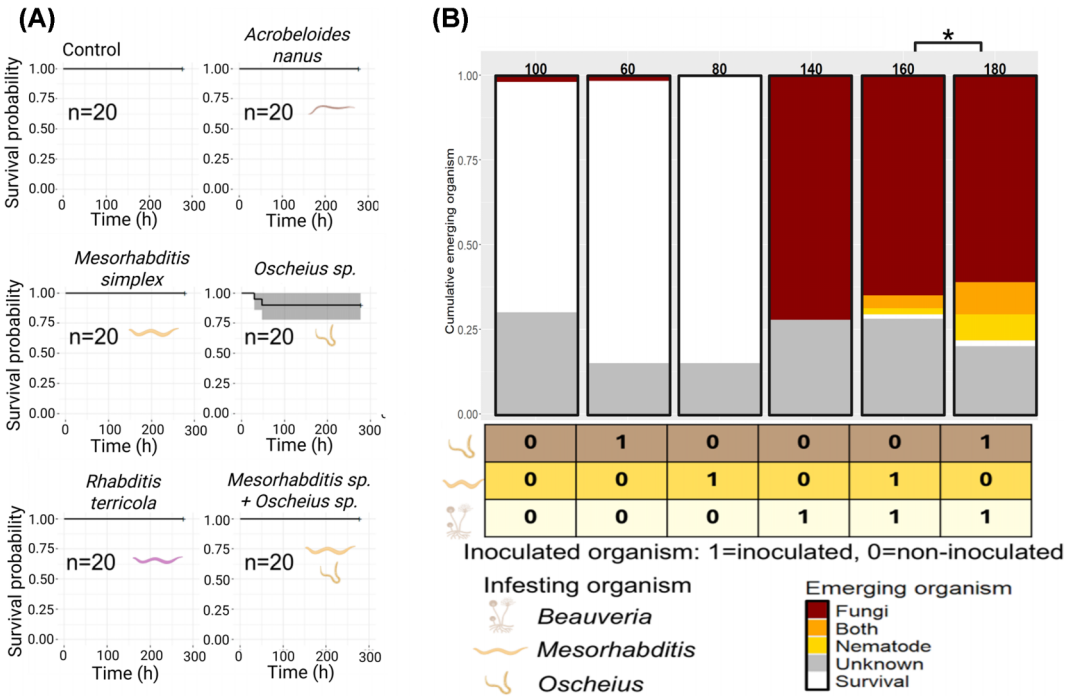

Figure 4 Experimental coinfection of *Galleria* moth larvae by fungi and nematodes. Panel A shows the survival of *Galleria* larvae exposed to different nematode species at an estimated concentration of 1000 nematodes per 100 μl of solution, Kaplan–Meier survival curves are used. Panel B shows the proportion of larvae from which the fungus, the nematode, or both emerged when they were infected with $10^{\wedge}5$ fungal spores/ml and 1000 nematodes (either *Mesorhabditis* or *Oscheius*). Statistical significance was assessed with a Chi-squared test of independence. Asterisks indicate results of statistical comparisons, where ∗ denotes $P < 0.05$.

Ochrobactrum quorumnoscens as generalists for comparison with the potential specialists. In addition, we included *Enterococcus casseliflavus* isolated from the non-infested *Galleria* larvae, as negative control.

In vitro competition of selected bacteria and fungi showed two groups of bacteria with respect to their interactions with *Beauveria*. One group, including *Enterococcus casseliflavus*, *Ochrobactrum quorumnoscens*, and *Achromobacter spanius*, rarely outcompeted *Beauveria* when introduced simultaneously with fungi, and never when *Beauveria* had a 24-h advantage. In contrast, the other group, including *Pseudomonas protegens*, *Serratia* sp., and *Serratia proteamaculans*, almost always outcompeted *Beauveria* even when the fungus had a 24-h advantage (Fig. 5A).

These *in vitro* results were consistent with *in vivo* results with *Galleria* larva. When *Enterococcus casseliflavus* and *Beauveria* were put in competition in *Galleria* larvae, only fungi emerged from the cadaver. In contrast, *Serratia proteamaculans* emerged in most cases without *Beauveria* emergence. Delaying introduction of *Serratia proteamaculans* relative to *Beauveria* led to more fungal emergence, although more than half the cadavers were still dominated by *Serratia proteamaculans* (Fig. 5B). Similar results were obtained with *Cydia* and *Curculio* larvae collected from the field (Fig. S6).

Discussion

Our results indicate that nematode species composition was not associated with the identity of the mutually exclusive fungi, *Metarhizium* or *Beauveria* (Fig. 3B), emerged from insect larvae. Nematode species composition was indistinguishable between *Metarhizium*- and *Beauveria*-emerging larvae (Fig. 2). Sometimes only the fungus emerged with no nematodes. Other times the fungus emerged with multiple nematode species (Fig. 2), but the species identity of these nematodes varied from one larva to another, with no clear distinction between *Metarhizium* and *Beauveria*-emerging larvae. The most curious case observed in this study was when nematodes emerged without any fungus (Figs 1 and 2). In these instances, the nematodes must have replaced whichever fungus initially killed the insect since no nematode species studied here could reliably establish in a larva by itself (Fig. 4A). Exploring how fungi interact with not just nematodes, but also bacteria (Fig. 5), has led us to discover that it was probably inhibition by some bacterial symbionts of the nematodes, especially *Serratia proteamaculans* in *Oscheius*, that enabled the nematodes to replace the early-arriving fungus.

Most studies investigating entomopathogenic communities have focused on *in vitro* assays using isolated and well-known pathogens (fungi, nematodes, or bacteria) against insect larvae (Eleftherianos et al. 2010, Lacey et al. 2015). Some studies have explored which organisms are present in the soil (Emelianoff et al. 2008, Noujeim et al. 2011, Araújo and Hughes 2016, Topuz et al. 2016, Depuydt et al. 2024, Zhang et al. 2024), but combined use of field survey, experimental validation and investigation of interactions explaining the observations remains rare. In our work, the field survey revealed two main groups consistently emerging from the larvae in this study system in California: fungi, primarily *Beauveria* and *Metarhizium*, and nematodes, mainly *Mesorhabditis*, *Oscheius*, and *Acrobeloides*, (Figs. 1A and 2). The two fungi rarely co-emerged (Fig. S1), whereas several nematode

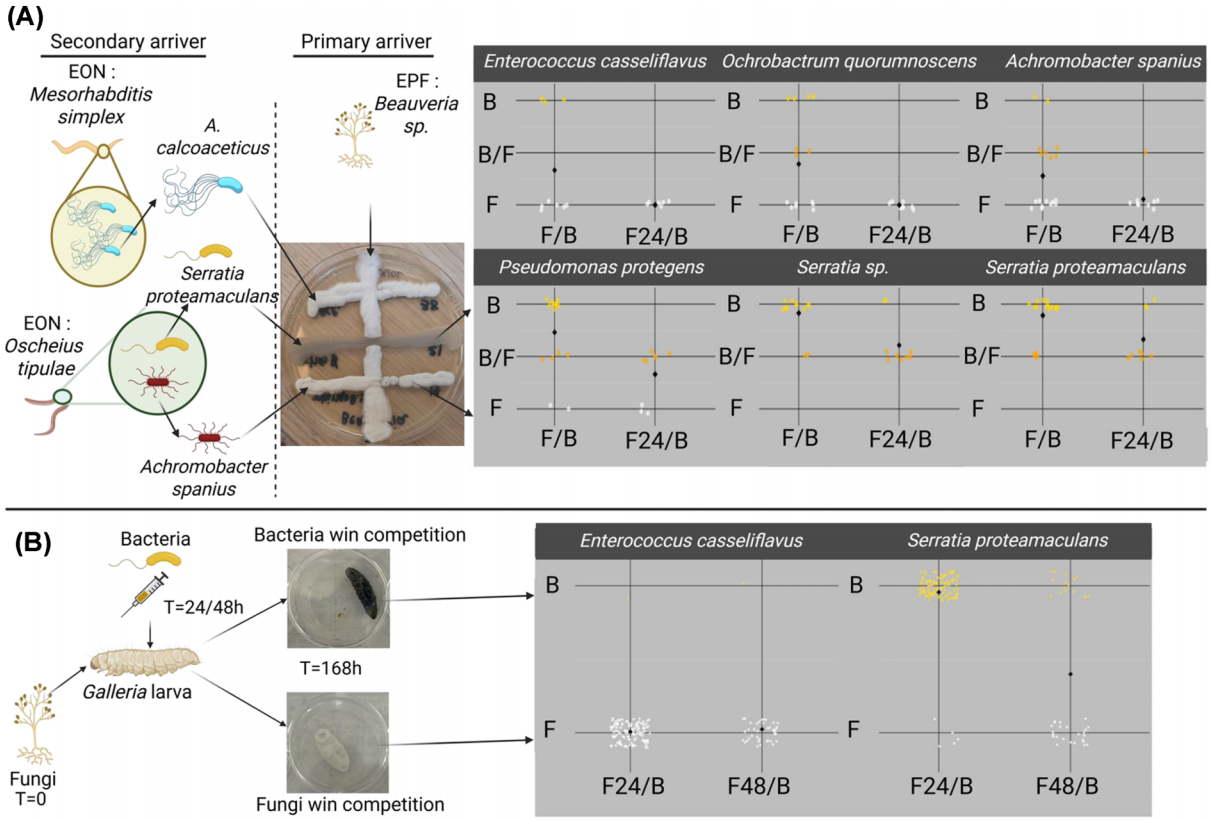

Figure 5 Experimental tests of interactions between *Beauveria* fungus and different bacterial strains isolated from *Galleria* moth larvae and the nematodes that emerged from *Galleria* larvae in the field survey. In *in vitro* experiments(A), bacteria isolated from nematodes or *Galleria* larvae were streaked on Petri dishes with *Beauveria* spores. After growth, if mycelia developed on the whole line, it was counted as the fungus winning against the bacterium (F). If only the bacterium was present, it was counted as the bacterium winning the fungus (B). If both were present, it was counted as a tie (B/F). Results are presented in plots, which also include those of the delayed experiments where the bacteria were introduced 24 h after streaking of fungal spores (F24/B). In *in vivo* experiments (B), *Galleria* larvae were put in contact with *Beauveria* spores, and either *Enterococcus casseliflavus* or *Ochrobactrum sp*. were injected to the larvae 24 or 48 h after fungal spore introduction. After larval death, emerging organism was noted (B or F). In both panels A and B, black diamonds on the graphs indicate the mean value for each treatment.

species were often observed co-emerging with a fungus (Fig. 2). Experimental validation showed that fungi were pathogenic to larvae, while nematodes alone caused little mortality (Figs 3A–4A). The discrepancy prompted us to explore whether coinfection might explain the high prevalence of nematode emergence. First, we co-infected larvae with both fungal species and confirmed that typically only one fungus would emerge, indicating mutual exclusion through priority effects (Fig. 3B), consistent with Constantin et al. (2025). As *Oscheius* nematodes were almost never co-emerging with fungi (Fig. 2) and *Beauveria* and *Mesorhabditis* were the two prevalent organisms of each group in our ecosystem (Figure S1), we performed coinfection with one fungus (*Beauveria*) and a nematode (*Mesorhabditis* or *Oscheius*), observing co-emergence in some cases, replicating field observations, but also cases where only the *Oscheius* nematode emerged, suggesting it had outcompeted the fungus (Fig. 4B).

Some nematodes have been proposed to opportunistically use cadavers killed by other pathogens (Duncan et al. 2003, Campos-Herrera et al. 2015, Blanco-Pérez et al. 2017, Blanco-Pérez et al. 2019). To our knowledge, however, this study is the first to demonstrate such behavior between nematodes and entomopathogenic fungi isolated from the same environment. Moreover, a likely explanation for this behavior is given by the finding that some bacteria associated with the nematodes could inhibit fungal growth (Fig. 5). Some of the bacteria used in our experiment were generalists (*O. quorumnoscens, A. spanius*) in the sense that they were isolated from several species of nematodes as well as *Galleria* larvae (*E. casseliflavus*). Some other bacteria were specialists in the sense that they were found only when one specific nematode was present (*Serratia proteamaculans*) or reported in the literature as specifically associated with nematodes (*Serratia* sp. and *P. protegens*) (Torres-Barragan et al. 2011, Ogier et al. 2020). Comparing how the generalists and the specialists interacted with *Beauveria*, we found no significant difference between bacteria within each group (generalists or specialists), but significant difference was observed between the two groups (Figure S5), with the specialists, but not the generalists, having the ability to inhibit fungi. This pattern suggests that only the specialist bacteria associated with specific nematode species may facilitate nematodes in establishing in insects killed by fungi.

These results were corroborated by the preliminary results with environmentally collected larvae, showing similar trends in these larvae (Figure S6). As *Cydia latiferreana* and *Curculio occidentis* larvae may have a different and more diverse microbiota compared to *Galleria*, we wondered if *Serratia proteamaculans* would still be able to show inhibition of *Beauveria* in those insects in the

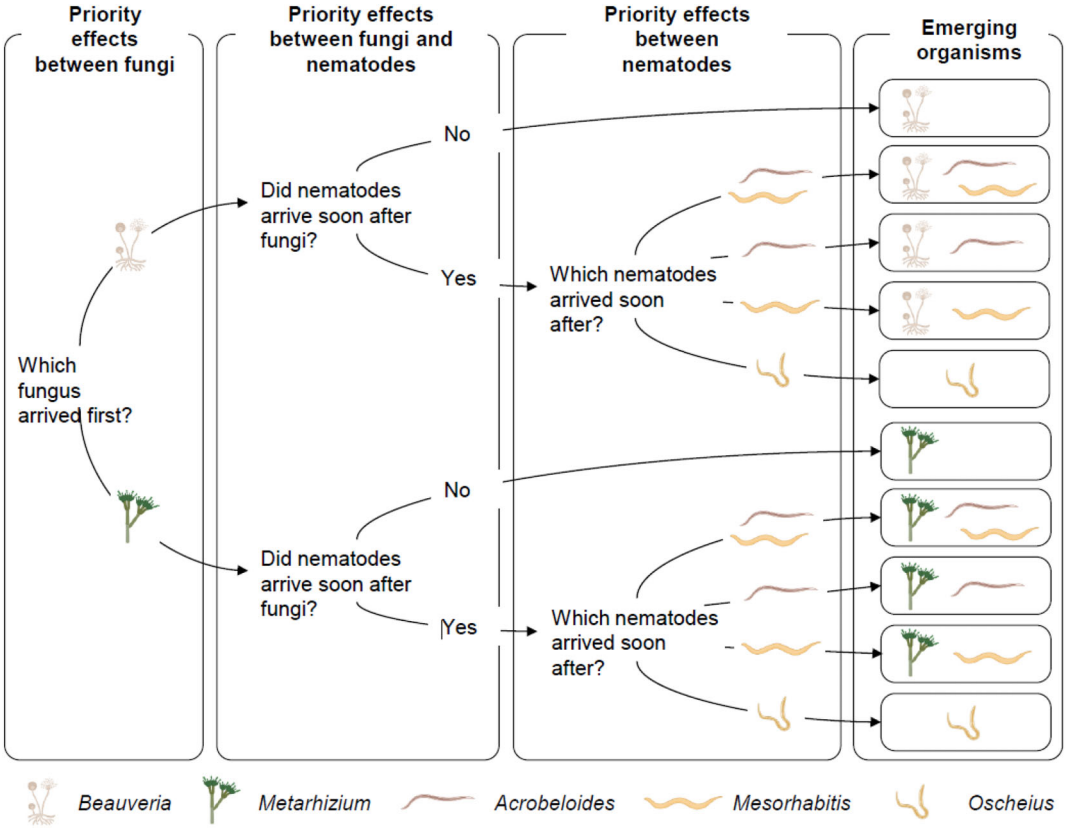

Figure 6 Hypothesis on how priority effects drive fungal and nematode emergence from insect larvae. Findings from this study suggest that fungal and nematode emergence from insect larvae is affected by priority effects among fungi, among nematodes, and among fungi and nematodes.

environment. Although our results are only preliminary at this stage, we did find inhibition of *Beauveria* emergence when *Serratia proteamaculans* was injected in both *Cydia* and *Curculio*.

Taken together, these findings lead us to classify the organisms emerging from insect larvae into two groups: fungi as primary arrivers, which are capable of killing the larvae and completing their life cycle using the cadaver, and nematodes as secondary arrivers, which cannot kill the larvae themselves but can co-emerge with, or even outcompete, the primary arrivers to exploit the cadavers, probably because of their associated bacteria. In our system, primary arrivers exclude each other due to strong priority effects, while often co-emerging with secondary arrivers. However, our results indicate that priority effects between primary and secondary arrivers can also be strong. Specifically, secondary arrivers may need to reach an insect killed by a primary arriver soon after their arrival to establish themselves in it. Otherwise, if they arrived too late, secondary arrivers would be excluded from the cadaver, as indicated by delayed introduction of secondary arrivers' symbionts (Fig. 5).

In conclusion, we suggest that the complex species interactions observed in this study make this insect-parasite system a promising natural microcosm for understanding how priority effects shape communities consisting of both primary and secondary arrivers in host–parasite systems and how their symbionts drive these priority effects (Fig. 6). The ecological assembly of many other types of biological communities, whether they consist of bacteria, fungi, plants, animals, or any combination of these, are also characterized by priority effects among primary arrivers, among secondary arrivers, and among primary and secondary arrivers. For this reason, we suggest that insect–parasite systems have the potential to inform the basic understanding of how community assembly works more broadly than currently realized.

Acknowledgements

We thank two anonymous reviewers and the members of the Community Ecology Group at Stanford University for comments. Figures were made using R and Biorender.com. CJW is an investigator of the Howard Hughes Medical Institute.

Supplementary material

Supplementary material is available at *FEMSEC* online.

Conflicts of interest

None declared.

Funding

This work was funded by the Discovery Grant from the Stanford Doerr School of Sustainability.

References

Araújo JPM, Hughes DP. Diversity of entomopathogenic fungi: which groups conquered the insect body? *Adv Genet* 2016;**94**:1–39. https://doi.org/10.1016/bs.adgen.2016.01.001.

Bedding RA, Akhurst RJ. A simple technique for the detection o f insect paristic rhabditid nematodes in soil. *Nematologica* 1975;**21**:109–10. https://doi.org/10.1163/187529275×00419.

Blanco-Pérez R, Bueno-Pallero FÁ, Neto L *et al.* Reproductive efficiency of entomopathogenic nematodes as scavengers. Are they able to fight for insect's cadavers?" *J Invertebr Pathol* 2017;**148**:1–9. https://doi.org/10.1016/j.jip.2017.05.003.

Blanco-Pérez R, Bueno-Pallero FÁ, Vicente-Díez I *et al.* Scavenging behavior and interspecific competition decrease offspring fitness of the entomopathogenic Nematode Steinernema Feltiae. *J Invertebr Pathol* 2019;**164**:5–15. https://doi.org/10.1016/j.jip.2019.04.002.

Bruck DJ, Walton VM. Susceptibility of the filbertworm (*Cydia Latiferreana*, Lepidoptera: tortricidae) and Filbert weevil (*Curculio Occidentalis*, Coleoptera: curculionidae) to entomopathogenic nematodes. *J Invertebr Pathol* 2007;**96**:93–6. https://doi.org/10.1016/j.jip.2007.02.012.

Callahan BJ, Paul J, McMurdie MJ *et al.* DADA2: high-resolution sample inference from Illumina Amplicon Data. *Nat Methods* 2016;**13**:581–3. https://doi.org/10.1038/nmeth.3869.

Campos-Herrera R, Půža V, Jaffuel G *et al.* Unraveling the intraguild competition between *Oscheius* spp. Nematodes and entomopathogenic nematodes: implications for their natural distribution in Swiss agricultural soils. *J Invertebr Pathol* 2015;**132**:216–27. https://doi.org/10.1016/j.jip.2015.10.007.

Chappell CR, Dhami MK, Bitter MC *et al.* Wide-ranging consequences of priority effects governed by an overarching factor. *eLife* 2022;**11**:e79647. https://doi.org/10.7554/eLife.79647.

Chase JM, Leibold MA. *Ecological Niches: linking Classical and Contemporary Approaches. Chicago*. University of Chicago Press. 2003. https://doi.org/10.7208/chicago/9780226101811.001.0001.

Costantin EC, Roxinol JA, Braga PF *et al.* Insect-parasitic fungi as a model system to investigate coinfections. *J Invertebr Pathol* 2025;**211**:108358. https://doi.org/10.1016/j.jip.2025.108358.

DeMalach N, Shnerb N, Fukami T. Alternative states in plant communities driven by a life-history trade-off and demographic stochasticity. *Am Nat* 2021;**198**:E27–36. https://doi.org/10.1086/714418.

Depuydt E, Ogier JC, Ali N *et al.* Combining multiple baiting cycles with digital droplet PCR optimizes description of the distribution of entomopathogenic nematodes in French maize fields. *Soil Biol Biochem* 2024;**189**:109275. https://doi.org/10.1016/j.soilbio.2023.109275.

de Vries,Ripley. 2024. *Ggdendro: create Dendrograms and Tree Diagrams Using "Ggplot2."*.https://andrie.github.io/ggdendro/authors.html

Drake JA. Communities as assembled structures: do rules govern pattern?" *Trends Ecol Evol* 1990;**5**:159–64. https://doi.org/10.1016/0169-5347(90)90223-Z.

Duncan LW, Dunn DC, Bague G *et al.* Competition between entomopathogenic and free-living bactivorous nematodes in larvae of the weevil Diaprepes Abbreviatus. *J Nematol* 2003;**35**:187–93.

Eggers SL, Matthiessen B. Initial dominance in coccolithophore communities affects community structure but does not translate into altered community functioning. *Mar Ecol Prog Ser* 2013;**473**:67–77. https://doi.org/10.3354/meps10064.

Eleftherianos I, ffrench-Constant RH, Clarke DJ *et al.* Dissecting the immune response to the entomopathogen photorhabdus. *Trends Microbiol* 2010;**18**:552–60. https://doi.org/10.1016/j.tim.2010.09.006.

Emelianoff V, Le Brun N, Pagès S *et al.* Isolation and identification of entomopathogenic nematodes and their symbiotic bacteria from Hérault and Gard (Southern France). *J Invertebr Pathol* 2008;**98**:211–7. https://doi.org/10.1016/j.jip.2008.01.006.

Fukami T. Historical contingency in Community Assembly: integrating niches, species pools, and priority effects. *Annu Rev Ecol Evol Syst* 2015;**46**:1–23. https://doi.org/10.1146/annurev-ecolsys-110411-160340.

Galili T. Dendextend: an R package for visualizing, adjusting and comparing trees of hierarchical clustering. *Bioinformatics* 2015;**31**:3718–20. https://doi.org/10.1093/bioinformatics/btv428.

Karvonen A, Jokela J, Laine A-L. Importance of sequence and timing in parasite coinfections. *Trends Parasitol* 2019;**35**:109–18. https://doi.org/10.1016/j.pt.2018.11.007.

Lacey LA, Grzywacz D, Shapiro-Ilan DI *et al.* Insect pathogens as biological control agents: back to the future. *J Invertebr Pathol* 2015;**132**:1–41. https://doi.org/10.1016/j.jip.2015.07.009.

Lewis VR. Within-tree distribution of acorns infested by Curculio Occidentis (Coleoptera: curculionidae) and Cydia Latiferreana (Lepidoptera: tortricidae) on the coast Live oak. *Environ Entomol* 1992;**21**:975–82. https://doi.org/10.1093/ee/21.5.975.

Manjula P, Lalitha K, Shivakumar MS. Diet composition has a differential effect on immune tolerance in insect larvae exposed to *mesorhabditis belari, Enterobacter hormaechei* and its metabolites. *Exp Parasitol* 2020;**208**:107802. https://doi.org/10.1016/j.exppara.2019.107802.

Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnetJournal* 2011;**17**:10–2. https://doi.org/10.14806/ej.17.1.200.

Mohd MH. Effects of dimensionality of space on the presence/absence of multiple species. *Environ Model Assess* 2022;**27**:327–42. https://doi.org/10.1007/s10666-021-09792-4.

Noujeim E, Khater C, Pages S *et al.* The first record of entomopathogenic nematodes (Rhabiditiae: steinernematidae and Heterorhabditidae) in natural ecosystems in Lebanon: a biogeographic approach in the Mediterranean region. *J Invertebr Pathol* 2011;**107**:82–5. https://doi.org/10.1016/j.jip.2011.01.004.

Ogier J-C, Pagès S, Frayssinet M *et al.* Entomopathogenic nematode-associated microbiota: from monoxenic paradigm to pathobiome. *Microbiome* 2020;**8**:25. https://doi.org/10.1186/s40168-020-00800-5.

Ogier J-C, Pagès S, Galan M *et al.* rpoB, a promising marker for analyzing the diversity of bacterial communities by amplicon sequencing. *BMC Microbiol* 2019;**19**:171. https://doi.org/10.1186/s12866-019-1546-z.

Oksanen SG, Blanchet F, Kindt R *et al.* vegan: Community Ecology Package. R package version 2.8-0 2025. https://vegandevs.r-universe.dev/vegan.

Palmgren A. Chance as an element in plant geography. *Proceedings of the International Congress of Plant Sciences*, Ithica, New York, George Banta Publishing Company, 1926, pp. 591–602.

Paradis E, Schliep K. Ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 2019;**35**:526–8. https://doi.org/10.1093/bioinformatics/bty633.

Postma-Blaauw MB, de Vries FT, de Goede RGM *et al.* Within-trophic group interactions of bacterivorous nematode species and their effects on the bacterial community and nitrogen mineralization. *Oecologia* 2005;**142**:428–39. https://doi.org/10.1007/s00442-004-1741-x.

Ruiu L, Giovanna Marche M, Mura ME *et al.* Involvement of a novel Pseudomonas protegens strain associated with entomopathogenic nematode infective juveniles in insect pathogenesis. *Pest Manage Sci* 2022a;**78**:5437–43. https://doi.org/10.1002/ps.7166.

Shen W, Le S, Li Y *et al.* SeqKit: a cross-platform and ultrafast toolkit for FASTA/Q file manipulation. *PLoS One* 2016;**11**:e0163962. https://doi.org/10.1371/journal.pone.0163962.

Srivastava DS, Kolasa J, Bengtsson J *et al.* Are natural microcosms useful model systems for ecology?" *Trends Ecol Evol* 2004;**19**:379–84. https://doi.org/10.1016/j.tree.2004.04.010.

Stroud JT, Delory BM, Barnes EM *et al.* Priority effects transcend scales and disciplines in biology. *Trends Ecol Evol* 2024;**39**:677–88. https://doi.org/10.1016/j.tree.2024.02.004.

Taboga L. *Rhabditis Terricola:* an opportunistic nematode parasite of earthworm cocoons. *J Invertebr Pathol* 1981;**38**:22–5. https://doi.org/10.1016/0022-2011(81)90029-X.

Topuz E, Erler F, Gumrukcu E. Survey of indigenous entomopathogenic fungi and evaluation of their pathogenicity against the Carmine spider mite, Tetranychus Cinnabarinus (Boisd.), and the whitefly, Bemisia Tabaci (Genn.) biotype B. *Pest Manage Sci* 2016;**72**:2273–9. https://doi.org/10.1002/ps.4266.

Torres-Barragan A, Suazo A, Buhler WG *et al.* Studies on the entomopathogenicity and bacterial associates of the Nematode Oscheius Carolinensis. *Biol Control* 2011;**59**:123–9. https://doi.org/10.1016/j.biocontrol.2011.05.020.

White GF. A method for obtaining infective nematode larvae from cultures. *Science* 1927;**66**:302–3. https://doi.org/10.1126/science.66.1709.302.b.

Zhang K, Baiocchi T, Lu D *et al.* Differentiating between scavengers and entomopathogenic nematodes: which is Oscheius Chongmingensis?" *J Invertebr Pathol* 2019;**167**:107245. https://doi.org/10.1016/j.jip.2019.107245.

Zhang W, Chen X, Eleftherianos I *et al.* Cross-talk between immunity and behavior: insights from entomopathogenic fungi and their insect hosts. *FEMS Microbiol Rev* 2024;**48**:fuae003. https://doi.org/10.1093/femsre/fuae003.

Zhou G, Yang H, Wang F *et al.* Oscheius Microvilli n. Sp. (Nematoda: rhabditidae): a facultatively pathogenic nematode from Chongming Island, China. *J Nematol* 2017;**49**:33–41. https://doi.org/10.21307/jofnem-2017-044.

Received: 13 November 2025. **Revised:** 2 April 2026. **Accepted:** 3 April 2026