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Supplemental information

Bacterial transfer from *Pristionchus entomophagus* nematodes to the invasive ant *Myrmica rubra* and the potential for colony mortality in coastal Maine

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Supplemental Material

Supplemental Tables

Table S1: Bacterial morphotypes isolated from the digestive tract and external cuticle of *Pristionchus entomophagus* nematodes and the hemolymph of nematode-exposed *Galleria mellonella* larvae, Related to Table 2 and Figure 1. Originally printed as Table 1 in Dumont, 2011.

Cadaver	Isolate	Colony appearance
Cultured from external cuticle of nematodes		
MGH F2	1	large, round, cream colored. Wavy/bumpy surface texture.
MGH F2	2	small, irregularly shaped, resembles a fried egg. Very clear on the outside, slightly yellowish cloudy tint in center, striated edges
MGH F2	3	small, cloudy in middle with more solid off-white color around outside. Round with slightly wavy edges.
MGH F2	4	small, round with smooth edges. General cloudy white color. Shows some signs of having a lighter color in the middle like isolate 3, but hemispheric shape suggests otherwise.
MGH F2	5	completely irregular. Cloudy. Generally ovular in shape with irregular edges and raised portions all over. Almost looks like a clovered-tongue.
MGH F2	6	irregular shape, heart like with wavy edges. Egg-like appearance with coloration different in the middle. Possible resemblance to isolate 2 but this colony is much thicker and has more color.
MGH F2	7	small, round with slightly wavy edges. General cloudy white color. Shows some signs of having a fried egg appearance. Smooth surface
MGH E3	1	white, crystalline. Varies in size. Raises off the surface of the plate. Looks icy in a way
MGH E3	2	irregular in shape, slightly round with an invagination. Appears to have an egg-like appearance. Resembles isolates from MGH with egg appearance (2,7)
MGH E3	3	small, round with smooth edges. General cloudy white color. Very much resembles isolate MGH F2 #4
MGH E3	4	small, round with slightly wavy edges. Lighter white color. May be a younger version of isolate 3 from this set.
MGH E3	5	medium/large. Fried egg appearance. Cream color.
COA C8	1	large, round, cream colored. Wavy/bumpy surface texture. A virtual identical match to MGH F2 #1
COA C8	2	small, round, clearer that isolates 1 or 2 from MGH E3.
COA C8	3	yellow, round, slightly irregular edges, wavy surface

Cadaver	Isolate	Colony appearance
COA C8	4	cream colored, round with wavy edges, no apparent discoloration, slightly wavy color.
COA C8	5	Small, round with smoother edges. Wavy surface appearance. Slightly transparent in middle, with solid color around outside, resembles MGH F2 #3
COA C8	6	big, white, round, smooth edges, no discoloration, resembles MGH E3 #3 and MGH F2 #4
COA C8	7	stringy, white, no crystalline appearance. Smooth looking with stringy appearance
COA C8	8	fungi? Fuzzy/hairy. Round conical colonies.
COA C7	1	small, irregularly shaped, resembles a fried egg. Very clear on the outside, slightly yellowish cloudy tint in center, striated edges. Resembles MGH F2 #2
COA C7	2	Left side of photo, small round, clear-ish appearance, smooth edges and surface. Resembles COA C8 #2
COA C7	3	Right side of photo, small round with slightly wavy edges. No overall discoloration. Resembles MGH E3 #4.
COA C7	4	mix between the bigger, white smooth/round colonies and the smaller, clearer smooth colonies (see COA C8 #6 and COA C7 #2)
COA C7	5	white, crystalline. Varies in size. Raises off the surface of the plate. Looks icy in a way. Closely resembles MGH E3 #1
COA C7	6	large, round, cream colored. Wavy/bumpy surface texture. A virtual identical match to MGH F2 #1
COA C7	7	yellow, round, slightly irregular edges, wavy surface. Similar to COA C8 #3
COA C7	8	small, clear with some cloudy white coloration. Smooth surface with a raised donut in the middle of the colony. Dissimilar to all other colonies.
Cultured from nematode digestive tract		
MGH E3	1	small, round, white color with slightly wavy edges. Similar color to big, white and round colonies such as COA C8 #6
MGH E3	2	small, round, cloudy white color with no discoloration.
COA B6	1 (Small)	small, round, no apparent color. After further analysis and streaking it appears that this may have been a raised water droplet or extra agar.
COA D5	Int 1	irregular shape most likely caused by wet plate. Edges do seem to wavy though. White color, no discoloration
COA D5	Int 2	somewhat round. Edges are very odd. They are irregular and appear to have bumps in them. Creamy color.
MGH D3	Int 3	creamy white large colony with a small white dot in the middle.

Cadaver	Isolate	Colony appearance
COA B6	2 (Big)	egg-shaped with raised circle in the slender part of the colony. After looking at the picture under closer scrutiny, it appears that this larger ovular colony may consist of two or three circular colonies. One appears to be a larger white colony which is at the right. another looks like the raised donut colony COA C7 #8.
MGH E3	Rep 1	small white colonies. Appear to be somewhat "bumpy" looking." round with irregular edges.
Cultured from hemolymph of *G. mellonella* larvae co-cultured with nematodes		
COA 1		No Notes, colony present
COA 2		Smooth
COA 2		Fuzzy
COA 4		No Notes, colony present
COA 5		Dark
COA 5		Light
COA 6		No Notes, colony present
MGH 6		Raised, White, Big
MGH 6		Flat, Yellow, Odd Edge

Table S2. Summary of unique bacterial isolates from the digestive tract and external cuticle of *Pristionchus entomophagus* nematodes and the hemolymph of nematode-exposed *Galleria mellonella* larvae, Related to Table 2 and Figure 1.

Source	Cadaver	Bacterial isolates morphotypes	Selected for molecular analysis
Pristionchus entomophagus nematode external cuticle	COA C8	8	8
	COA C7	8	0
	MGH F2	7	7
	MGH E3	5	0
Pristionchus entomophagus nematode digestive tract	COA B6	2	1
	COA D5	2	2
	MGH D3	1	1
	MGH E3	2	2
Hemolymph of *Galleria mellonella* waxworm larvae co-cultured with *Pristionchus entomophagus* nematodes	All COA	7	7
	All MGH	2	2
	Total	**45**	**32**

Supplemental Figures

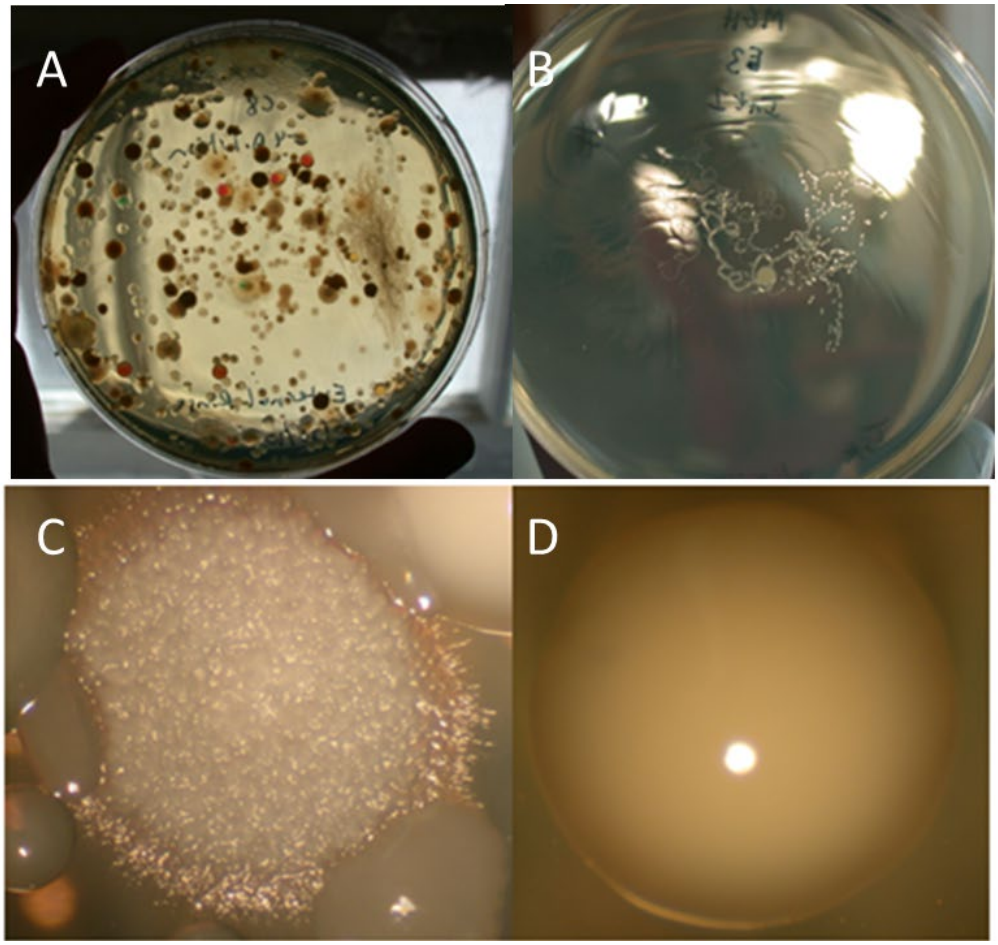

Figure S1. Bacterial colonies isolated from *Pristionchus entomophagus* nematodes collected from collapsed *Myrmica rubra* nests in Maine, Related to Table 2 and Figure 1. A) Original 10^{-4} dilution from external cuticle of nematodes from sample COA-C8 plated on TSA. B) Bacteria cultured from excrement of surface-sterilized nematodes from sample MGH-E3. C) MGH-E3-1 and D) MGH-E3-2 1 isolated from the external cuticle of nematodes after 72 hours of colony growth, both at 40X magnification. Plates are 90 mm in diameter.

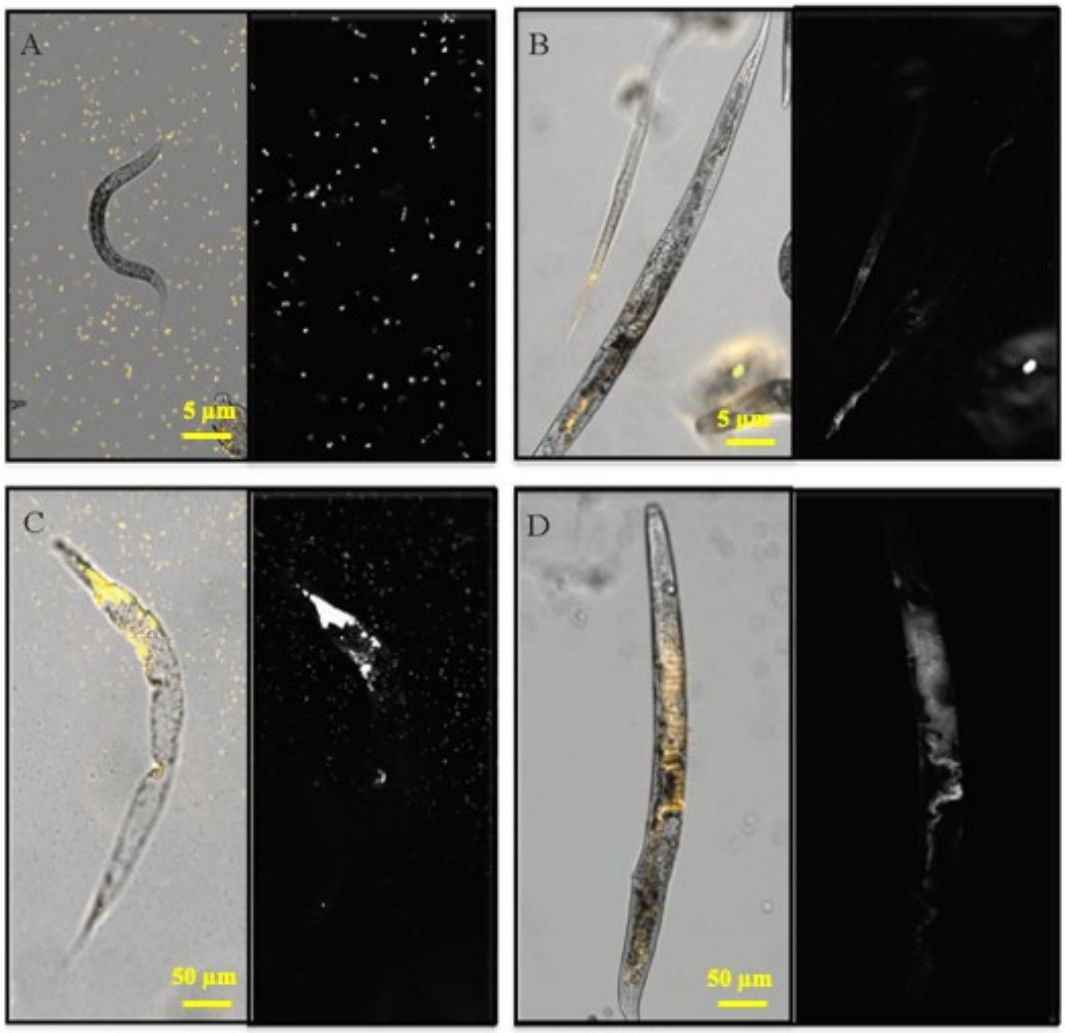

Figure S2. ***Pristionchus entomophagus*** **nematodes in co-culture with red fluorescent protein (RFP)-labelled A-C)** ***Pseudomonas aeruginosa*** **strain PA14 or D)** ***Escherichia coli strain*** **HB101, Related to Figure 3.** Each panel shows the same image with brightfield and fluorescence microscopy (left) and fluorescent microscopy only (right). A) Juvenile nematode with no fluorescence. B) Juvenile nematode with external fluorescence (smaller individual) next to a larger adult nematode with internal fluorescence. C) Juvenile nematode with external fluorescence. D) Adult nematode with internal fluorescence. Nematodes in panels A-C were exposed to RFP-labeled *P. aeruginosa* and the nematode in panel D was exposed to RFP-labeled *E. coli*. All panels are at 100X magnification. Adult nematodes from these locations ranged from 568 – 780 µm in length, mean 668 µm, and 37 – 62 µm in width, mean 41 µm.

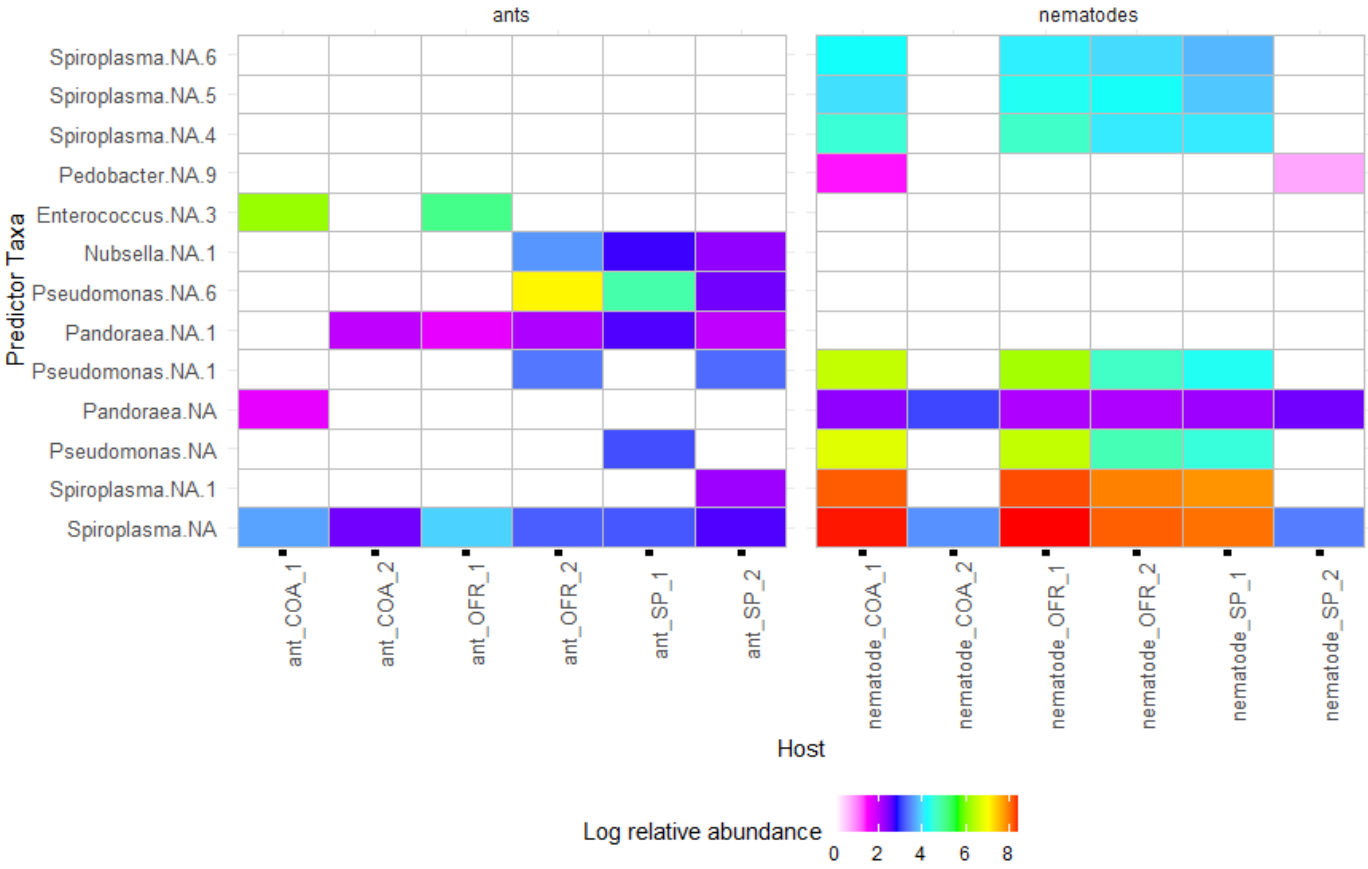

Figure S3. Abundance of taxa identified as important community members associated with *Myrmica rubra* ant and *Pristionchus entomophagus* nematode bacterial communities from three field sites on Mount Desert Island, Maine, Related to Figures 6 and 7. Field sites on Mount Desert Island, Maine include Eden St. South (COA), Old Farm Road (OFR), and Sports Park (SP). Samples are numbered by the transfer set. Random forest model accuracy was 77%.

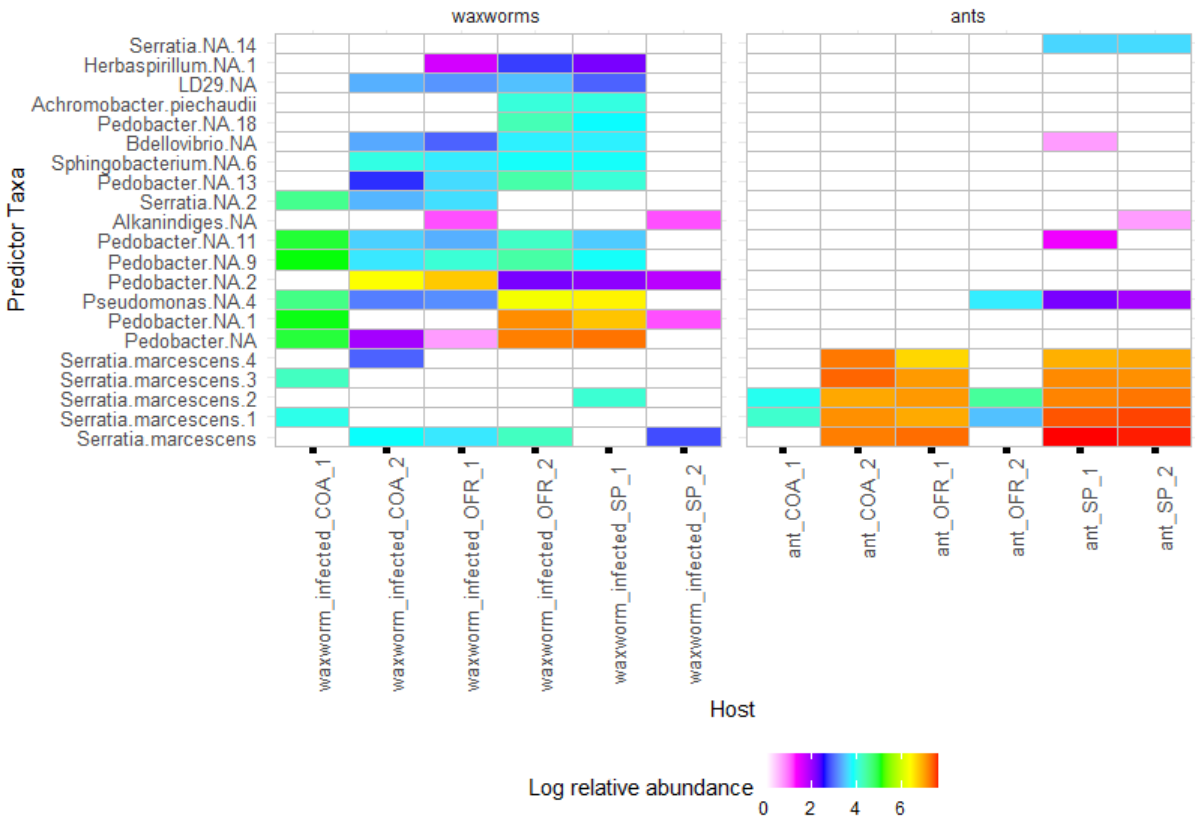

Figure S4. Abundance of taxa identified as important community members associated with *Pristionchus entomophagus*-nematode-infected *Galleria mellonella* waxworm larvae and field-collected *Myrmica rubra* ants from three sites on Mount Desert Island, Maine, Related to Figures 6 and 7. Field sites on Mount Desert Island, Maine include Eden St. South (COA), Old Farm Road (OFR), and Sports Park (SP). Samples are numbered by the transfer set. Random forest model accuracy was 75%.

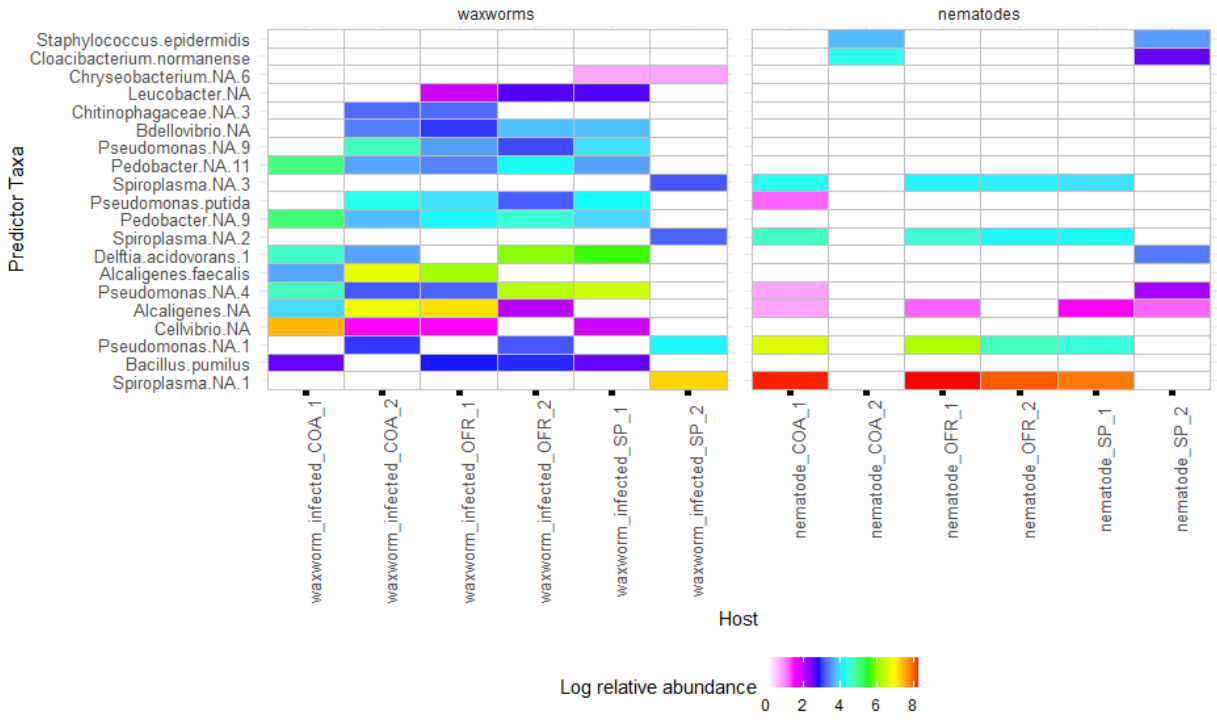

Figure S5. Abundance of taxa identified as important community members associated with field-collected *Pristionchus entomophagus* nematodes and nematode-infected *Galleria mellonella* waxworm larvae, Related to Figures 6 and 7. Field sites on Mount Desert Island, Maine include Eden St. South (COA), Old Farm Road (OFR), and Sports Park (SP). Samples are numbered by the transfer set. Random forest model accuracy was 83%.

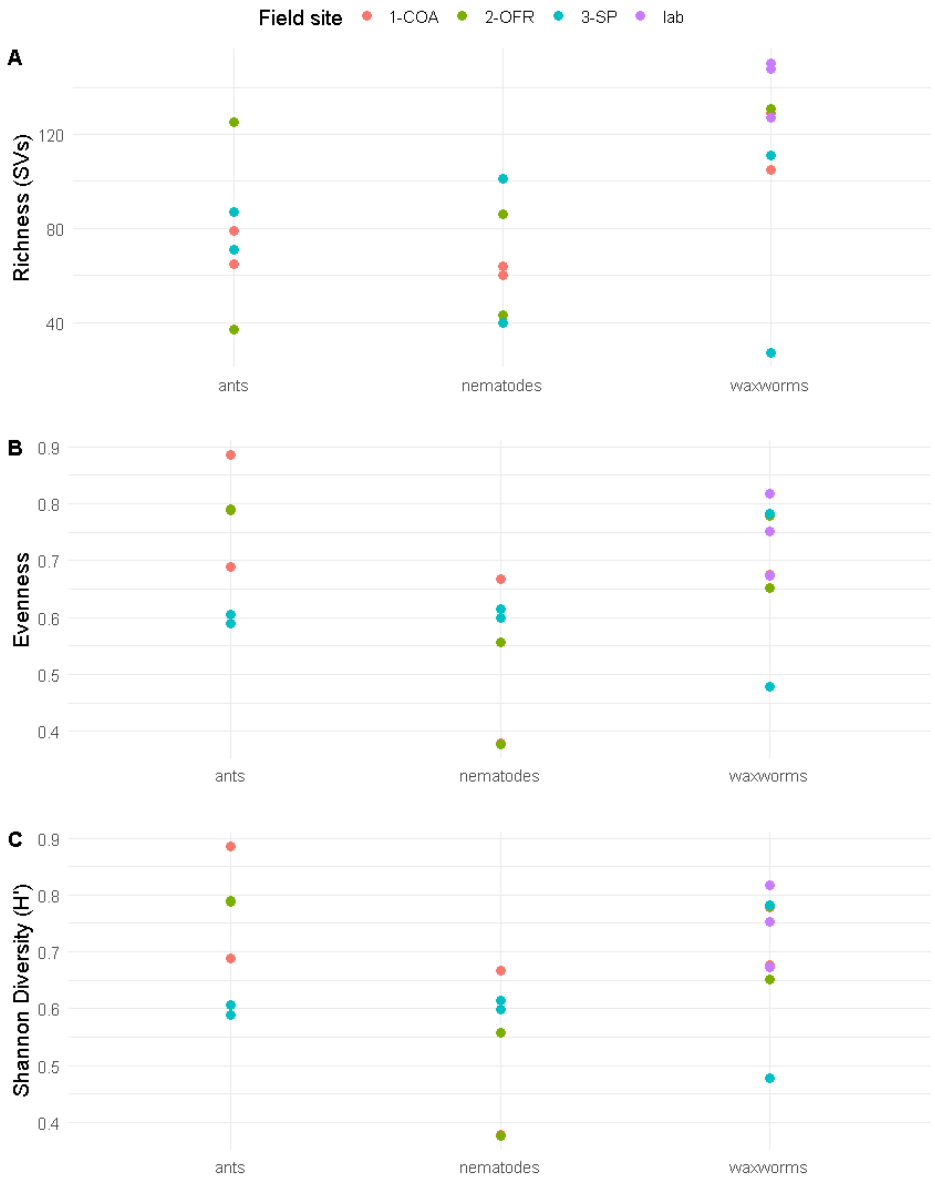

Figure S6. Alpha diversity metrics for field collected *Myrmica rubra* ants and *Pristionchus entomophagus* nematodes, and nematode-infected *Galleria mellonella* waxworm larvae, Related to Figures 4 and 5. Field sites on Mount Desert Island, Maine include Eden St. South (COA), Old Farm Road (OFR), and Sports Park (SP), or laboratory for control larvae.

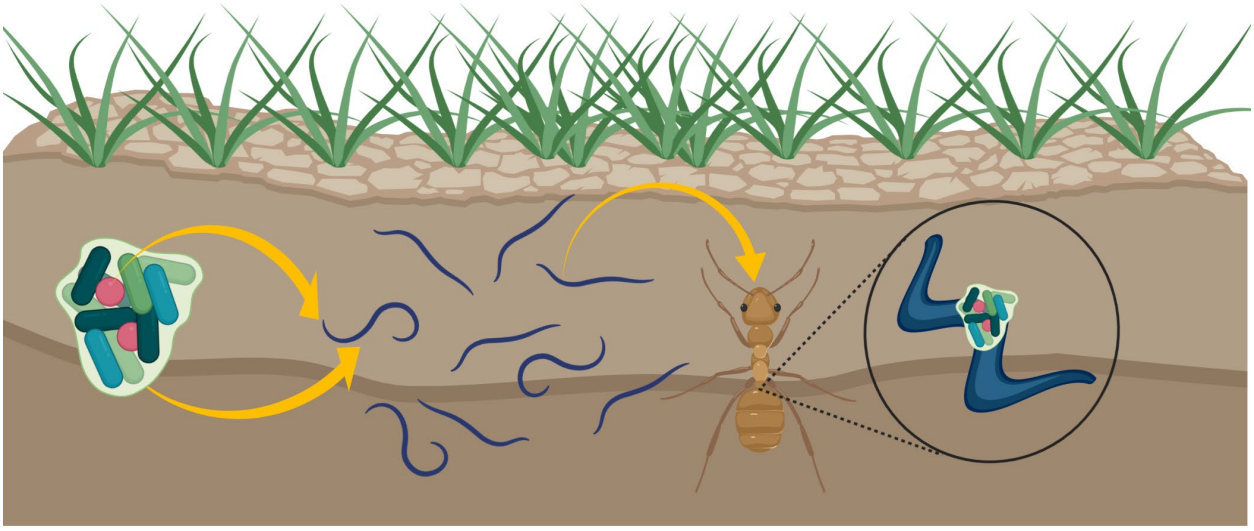

Figure S7 Schematic of the theoretical model of mortality in *Myrmica rubra* ants via vectoring of environmental bacteria by *Pristionchus entomophagus* nematodes, Related to Figures 4 and 5.

Figure S8: White trap containing a *Galleria mellonella* larva cadaver for the collection of emerging nematodes, Related to Figure 2. Waxworm larvae are approximately 1.9 cm in length.

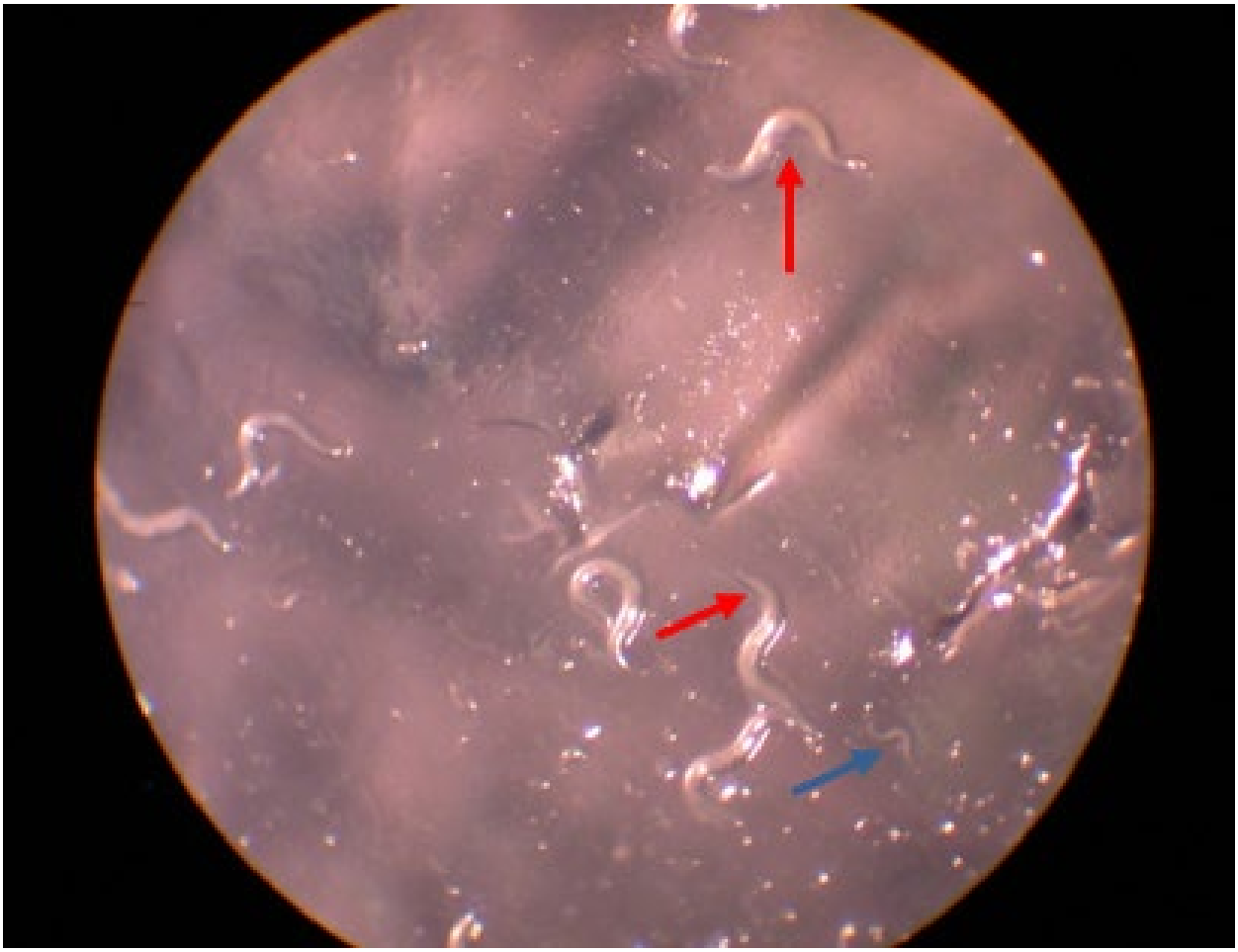

Figure S9: Adult and juvenile *Pristionchus entomophagus* nematodes on NGM. Red arrows indicate adults and the blue arrow indicates juveniles, Related to Figures 2 and 3. Adult nematodes from these locations ranged from 568 – 780 µm in length, mean 668 µm, and 37 – 62 µm in width, mean 41 µm.

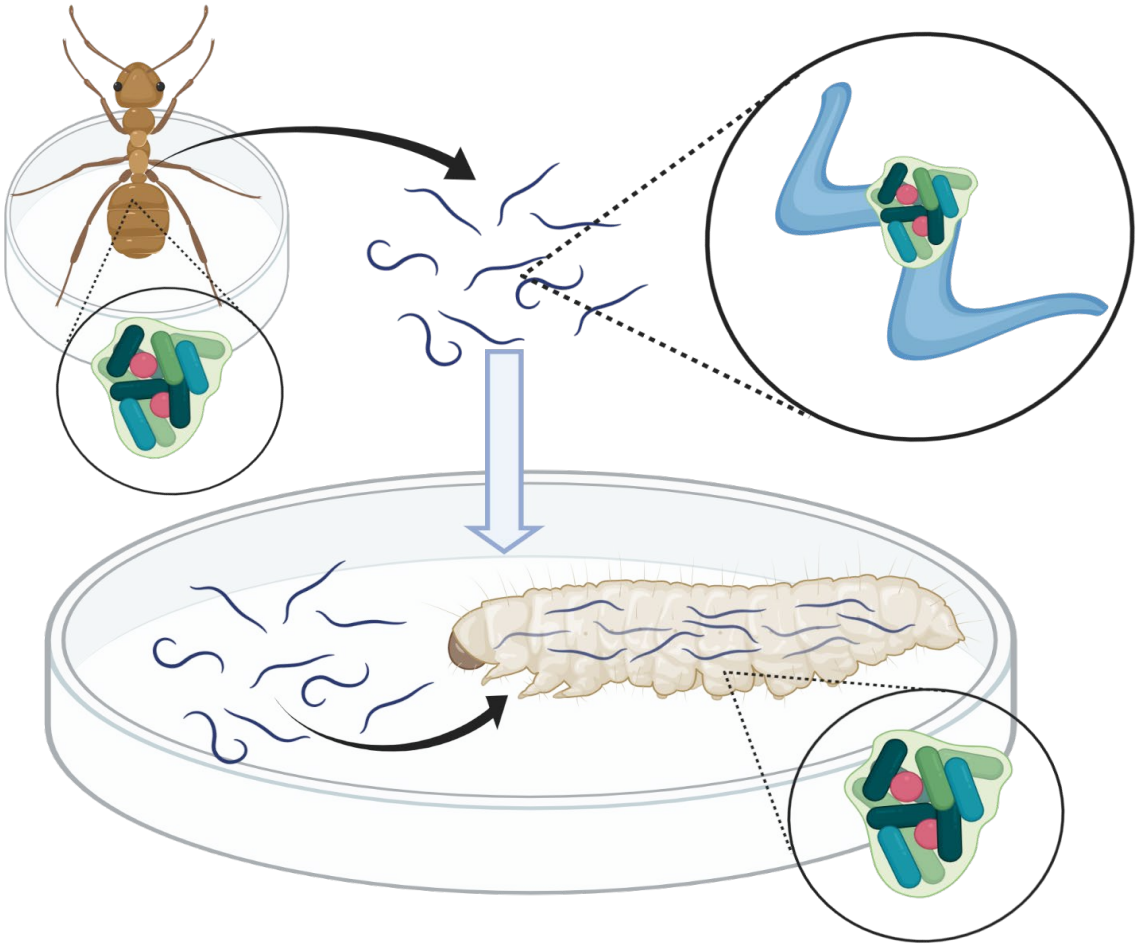

Figure S10 Schematic of the experimental design to assess bacterial community similarity in *Myrmica rubra* ants, *Pristionchus entomophagus* nematodes, and nematode-exposed *Galleria mellonella* larvae, Related to Figures 4 and 5.