

Supplementary Materials

Title

Full title: *Protorhabditis* nematodes and pathogen-antagonistic bacteria interactively promote plant health

Short title: *Protorhabditis* nematodes promote plant health

Authors

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Table S1. Treatment design in the fourth *in vitro* experiments.

Treatment	Combination
Ctrl	Without any bacterial strain and nematode
Art	Inoculating with test bacterial strain <i>Arthrobacter</i>
Art+Pro	Inoculating with <i>Protorhabditis</i> nematodes and test bacterial strain <i>Arthrobacter</i>
Bac	Inoculating with test bacterial strain <i>Bacillus</i>
Bac+Pro	Inoculating with <i>Protorhabditis</i> nematodes and test bacterial strain <i>Bacillus</i>
Fic	Inoculating with test bacterial strain <i>Fictibacillus</i>
Fic+Pro	Inoculating with <i>Protorhabditis</i> nematodes and test bacterial strain <i>Fictibacillus</i>
Pae	Inoculating with test bacterial strain <i>Paenibacillus</i>
Pae+Pro	Inoculating with <i>Protorhabditis</i> nematodes and test bacterial strain <i>Paenibacillus</i>
Ped	Inoculating with test bacterial strain <i>Pedobacter</i>
Ped+Pro	Inoculating with <i>Protorhabditis</i> nematodes and test bacterial strain <i>Pedobacter</i>
Phy	Inoculating with test bacterial strain <i>Phycococcus</i>
Phy+Pro	Inoculating with <i>Protorhabditis</i> nematodes and test bacterial strain <i>Phycococcus</i>
Lys	Inoculating with test bacterial strain <i>Lysinibacillus</i>
Lys+Pro	Inoculating with <i>Protorhabditis</i> nematodes and test bacterial strain <i>Lysinibacillus</i>
Mes	Inoculating with test bacterial strain <i>Mesorhizobium</i>
Mes+Pro	Inoculating with <i>Protorhabditis</i> nematodes and test bacterial strain <i>Mesorhizobium</i>
Mic	Inoculating with test bacterial strain <i>Microvirga</i>
Mic+Pro	Inoculating with <i>Protorhabditis</i> nematodes and test bacterial strain <i>Microvirga</i>

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Table S2. Treatment design in the fifth *in vitro* experiments.

Treatment	Combination
Ctrl	Without any bacterial strain and nematode
Pro	Inoculating with <i>Protorhabditis</i> nematodes
Art	Inoculating with test bacterial strain <i>Arthrobacter</i>
Art+Pro	Inoculating with <i>Protorhabditis</i> nematodes and test bacterial strain <i>Arthrobacter</i>
Bac	Inoculating with test bacterial strain <i>Bacillus</i>
Bac+Pro	Inoculating with <i>Protorhabditis</i> nematodes and test bacterial strain <i>Bacillus</i>
Fic	Inoculating with test bacterial strain <i>Fictibacillus</i>
Fic+Pro	Inoculating with <i>Protorhabditis</i> nematodes and test bacterial strain <i>Fictibacillus</i>
Pae	Inoculating with test bacterial strain <i>Paenibacillus</i>
Pae+Pro	Inoculating with <i>Protorhabditis</i> nematodes and test bacterial strain <i>Paenibacillus</i>
Ped	Inoculating with test bacterial strain <i>Pedobacter</i>
Ped+Pro	Inoculating with <i>Protorhabditis</i> nematodes and test bacterial strain <i>Pedobacter</i>
Phy	Inoculating with test bacterial strain <i>Phycococcus</i>

Phy+Pro	Inoculating with <i>Protorhabditis</i> nematodes and test bacterial strain <i>Phycococcus</i>
Lys	Inoculating with test bacterial strain <i>Lysinibacillus</i>
Lys+Pro	Inoculating with <i>Protorhabditis</i> nematodes and test bacterial strain <i>Lysinibacillus</i>
Mes	Inoculating with test bacterial strain <i>Mesorhizobium</i>
Mes+Pro	Inoculating with <i>Protorhabditis</i> nematodes and test bacterial strain <i>Mesorhizobium</i>
Mic	Inoculating with test bacterial strain <i>Microvirga</i>
Mic+Pro	Inoculating with <i>Protorhabditis</i> nematodes and test bacterial strain <i>Microvirga</i>

Table S3. Soil properties under different fertilization regimes. pH: potential of hydrogen; NO₃⁻-N: nitrate nitrogen; NH₄⁺-N: ammonium nitrogen; SOC: soil organic carbon; TN: total nitrogen; TK: total potassium; AP: available phosphorus; AK: available potassium. Data are means (±SD) (n = 3).

	pH	TOC (g/kg)	C/N	TN (g/kg)	TK (g/kg)	AP (mg/kg)	AK (mg/kg)	NH ₄ ⁺ -N (mg/kg)	NO ₃ ⁻ -N (mg/kg)
BF	7.23±0.18 a	9.20±0.04 a	7.31±0.63 a	1.27±0.12 a	5.97±0.24	71.26±10.19 a	220.67±24.12 a	1.15±0.10 a	83.38±1.94 a
CF	6.7±0.20 a	8.81±1.02 a	7.01±0.05 a	1.12±0.01 ab	6.07±0.91	67.82±3.12 a	189±14.00 ab	0.95±0.24 ab	63.12±6.68 b
Ctrl	7.14±0.20 a	7.40±0.10 a	7.88±0.32 a	0.94±0.04 b	6.27±0.61	48.67±2.79 a	156±8.04 b	0.64±0.04 b	39.33±1.23 c

Table S4. Statistical methods used and main outcomes of the *in vitro* experiments.

Experiment	Response variables	Statistical method	Test values	Degree of freedom	<i>p</i> value
<i>In vitro</i> exp 1	<i>Protorhabditis</i> number with <i>R. solanacearum</i>	ANOVA	F (121.039)	5	< 0.001
<i>In vitro</i> exp 1	<i>Protorhabditis</i> number without <i>R. solanacearum</i>	ANOVA	F (42.260)	5	< 0.001

Table S5. Statistical methods used and main outcomes of the greenhouse experiment 2.

Experiment	Response variables	Statistical method	Test values	Degree of freedom	<i>p</i> value
Greenhouse exp 2	<i>Protorhabditis</i> number	ANOVA	F (742.821)	1	< 0.001
Greenhouse exp 2	Inhibition rate to pathogen	ANOVA	F (490.330)	1	< 0.001

Table S6. Statistical methods used and main outcomes of the greenhouse experiments 3.

Experiment	Response variables	Statistical method	Test values	Degree of freedom	<i>p</i> value
Greenhouse exp 3	<i>Protorhabditis</i> density	ANOVA	F (21.651)	1	0.01
Greenhouse exp 3	<i>Bacillus</i> density	ANOVA	F (7.994)	3	0.009

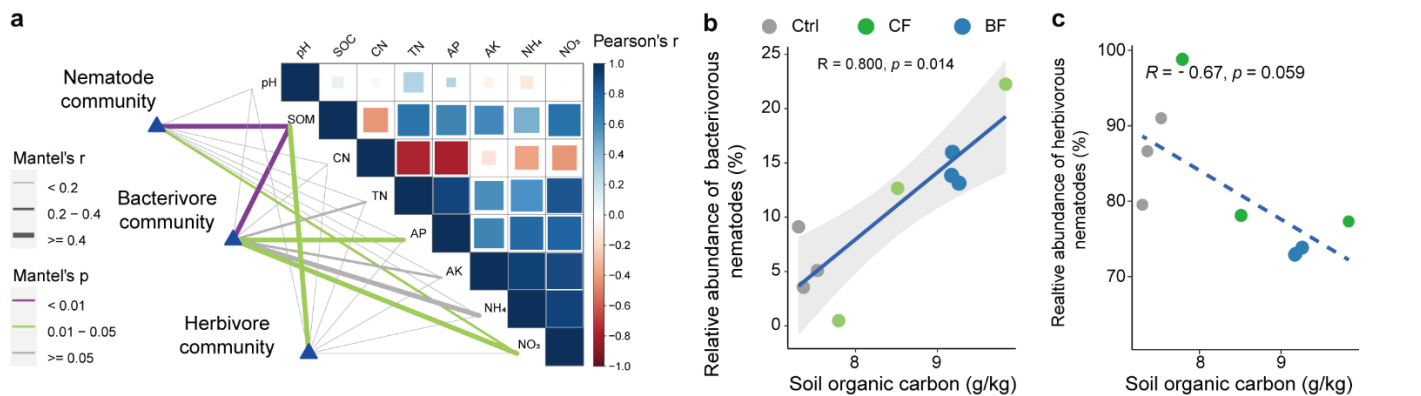


Figure S1. Effects of soil physicochemical properties on the community structure and relative abundance of different nematode trophic groups. (a) Correlation heat map of soil physicochemical properties and their correlation with nematode community. SOM: soil organic matter; CN: rate of total carbon and nitrogen; TN: soil total nitrogen; AP: available phosphorus; AK: available potassium; NH₄: ammonium nitrogen; NO₃: nitrate nitrogen. (b) Correlation between soil organic matter and relative abundance of bacterivorous nematodes in fields, gray shading denotes 95% confidence intervals. (c) Correlation between herbivorous nematodes abundance and soil organic carbon content.

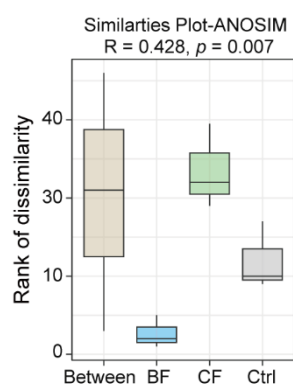


Figure S2. Analysis of similarities between different fertilization treatments. The result $R > 0$, indicating that the differences between groups were greater than the differences within groups, and the Permutation Test is used to calculate the significance.

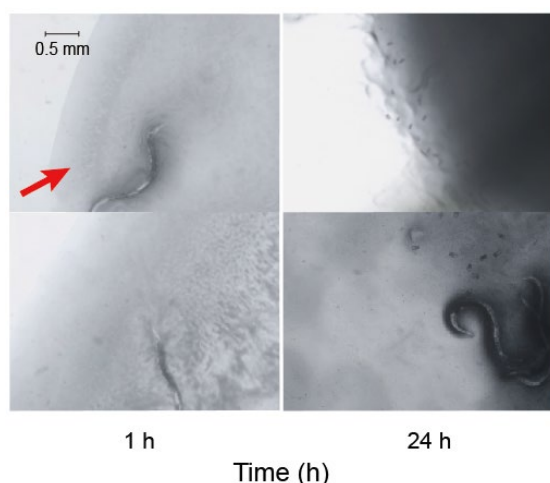


Figure S3. Tendency of nematodes to predation and reproduction within 24 hours. The red arrow indicates the movement direction of *Protorhabditis* nematodes. The two image sets demonstrate that *Protorhabditis* nematodes moved toward the growth of *R. solanacearum*, with an increasing number of nematodes observed over time.

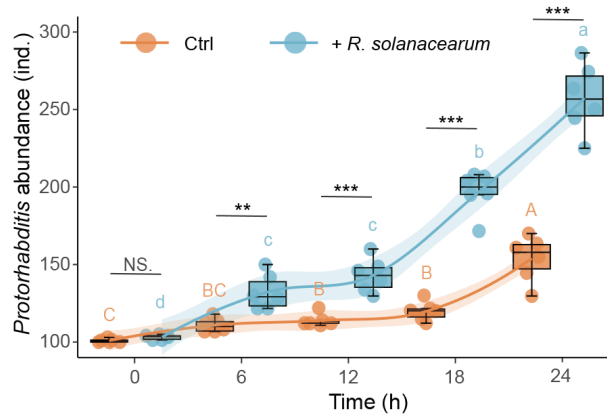


Figure S4. Increased abundance of *Protorhabditis* in nematode growth medium in 24 h. Significant differences (n = 6) in panel by two-tailed unpaired t test (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Blue lowercase letters mean Tukey test results of the differences of Ctrl treatments in 24 hours, and yellow uppercase letters mean Tukey test results of the differences of +*R. solanacearum* treatments in 24 hours.

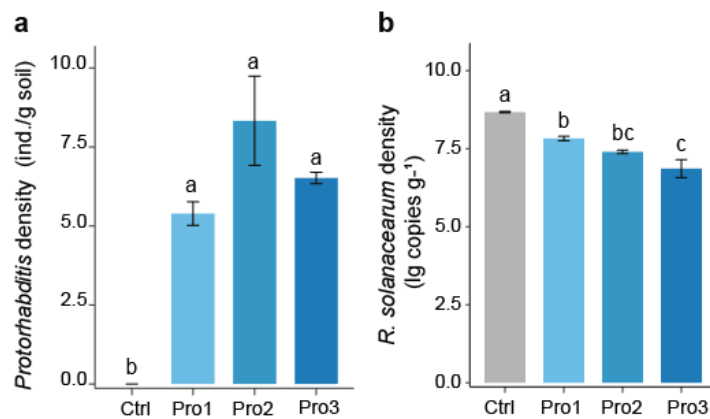


Figure S5. Effects of inoculating bacterivorous nematodes *Protorhabditis* in soils on plant health. Effects of inoculating *Protorhabditis* on (a) *Protorhabditis* abundances in pots (n = 3), (b) *R. solanacearum* density in soils. In panels a and b, data are plotted as means $\pm$ SEs, and bars with different letters indicate significant differences as defined by one-way ANOVA with Tukey's HSD test ($p < 0.05$).

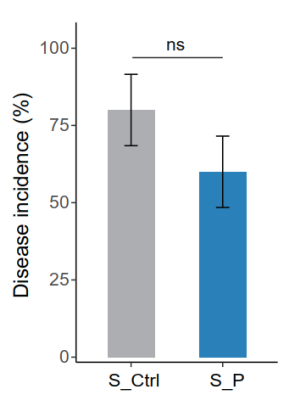


Figure S6. Effect of *Protorhabditis* on tomato bacterial wilt by directly consuming *R. solanacearum* in sterilized soils. Significant differences in panel d by two-tailed unpaired t test (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

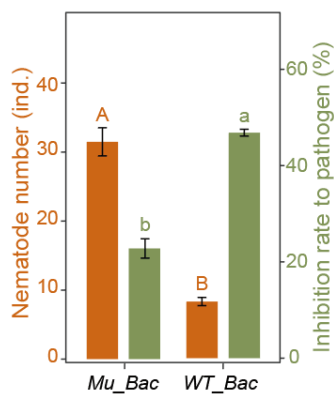


Figure S7. Antagonistic ability to *R. solanacearum* and attraction to nematode predation of *Bacillus* wild-type and mutant strains. Data are plotted as means $\pm$ SEs ($n = 6$), and bars with different letters indicate significant differences as defined by one-way ANOVA with Tukey's HSD test ($p < 0.05$).

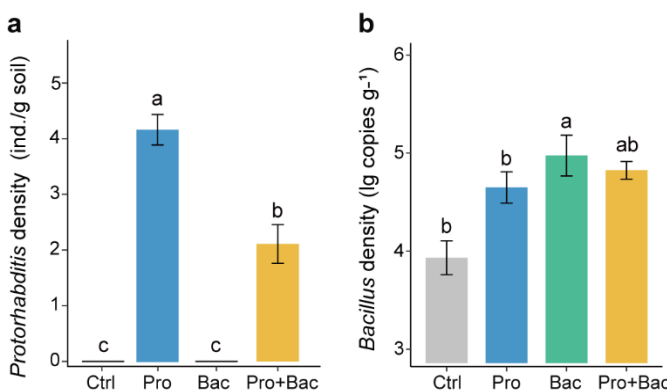


Figure S8. Effect of combinations of *Protorhabditis* and bacteria on tomato health. Pro: *Protorhabditis*, Lys: *Lysinibacillus*, Fic: *Fictibacillus*. Data are plotted as means $\pm$ SEs ($n = 6$), and bars with different letters indicate significant differences as defined by one-way ANOVA with Tukey's HSD test ($p < 0.05$).