Supplementary Materials for

Rhizosphere *Streptomyces* confers dual-mode resistance to root-knot nematodes through nematicidal metabolites and JA-mediated immunity in maize

Zheng *et al*.

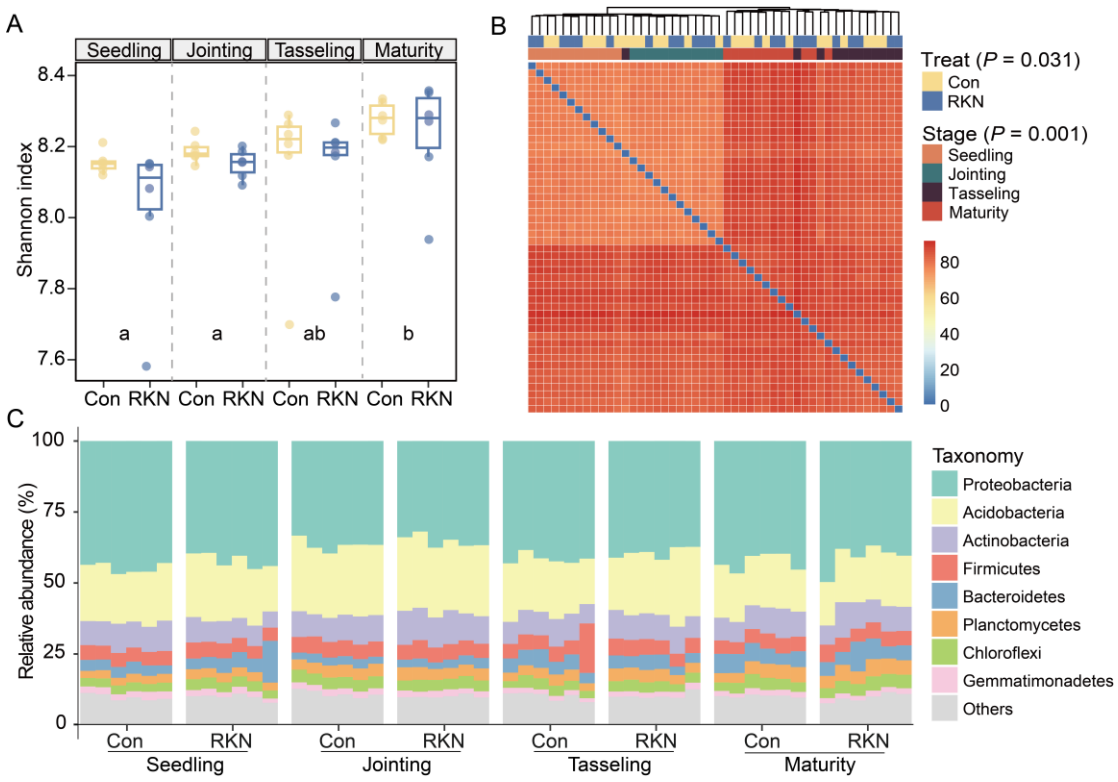
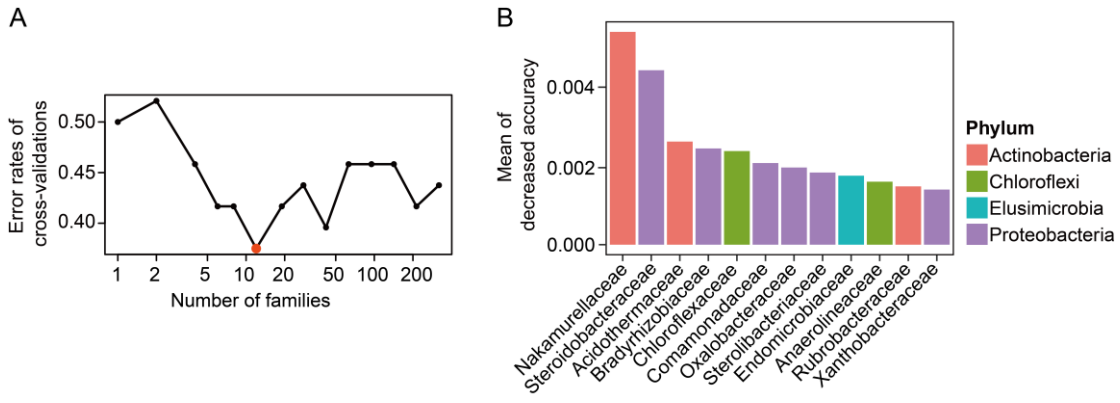


Fig. S1 Composition of the rhizosphere microbiome across maize developmental stages. (A) Shannon diversity index of rhizosphere bacterial communities in healthy (Con) and RKN-infected (RKN) maize plants at the seedling, jointing, tasseling, and maturity stages (mean ± SD; $n = 6$). Boxes represent the interquartile range (IQR; 25th to 75th percentiles), and individual data points denote biological replicates. Different letters indicate statistically significant differences between developmental stages (Kruskal–Wallis test; $P < 0.05$). "n.s." indicates there was not significant between Con and RKN treatments (Wilcoxon test). **(B)** Heatmap based on Euclidean distance illustrating differences in rhizosphere bacterial community composition between Con and RKN treatments across the four maize growth stages. Each square represents an individual replicate. 999 permutational multivariate analysis of variance (PERMANOVA, adonis) was used to analysis the significance in developmental stages and treat. **(C)** Relative abundance (%) of the top eight bacterial phyla in the rhizosphere across all maize developmental stages.



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826 **Fig. S2 Key rhizosphere bacterial families identified from healthy and RKN-**
827 **infected maize across the full growth period by Random Forest. (A)** Number of
828 bacterial families selected by Random Forest as key discriminators between healthy
829 (Con) and RKN-infected (RKN) treatments across the full growth period (n = 12). **(B)**
830 Model classification accuracy of Random Forest based on the selected 12 bacterial
831 families.

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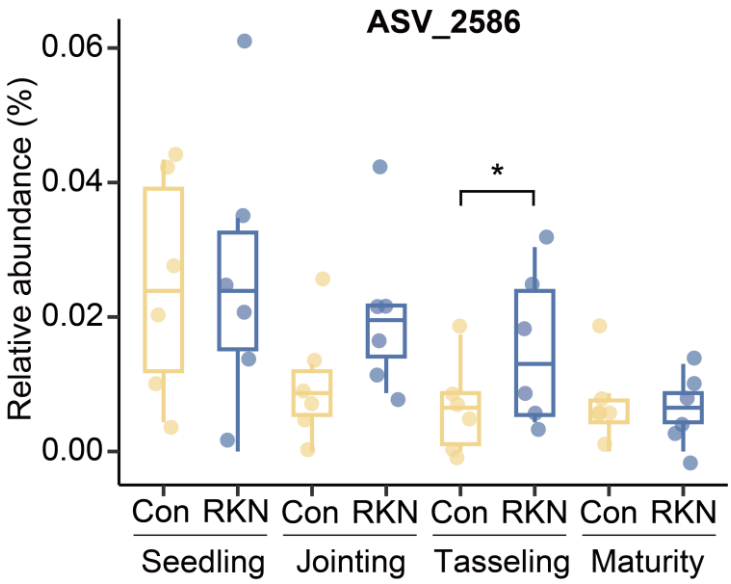

Fig. S3 Relative abundance of ASV_2586 in healthy and RKN-infected maize rhizospheres across four developmental stages. Relative abundance (%) of ASV_2586 in the rhizosphere of healthy (Con) and RKN -infected (RKN) maize at the seedling, jointing, tasseling, and maturity stages (mean ± SD, $n = 6$). Asterisks indicate statistically significant differences between treatments at each stage (two-sided Welch's t-test; $P < 0.05$).

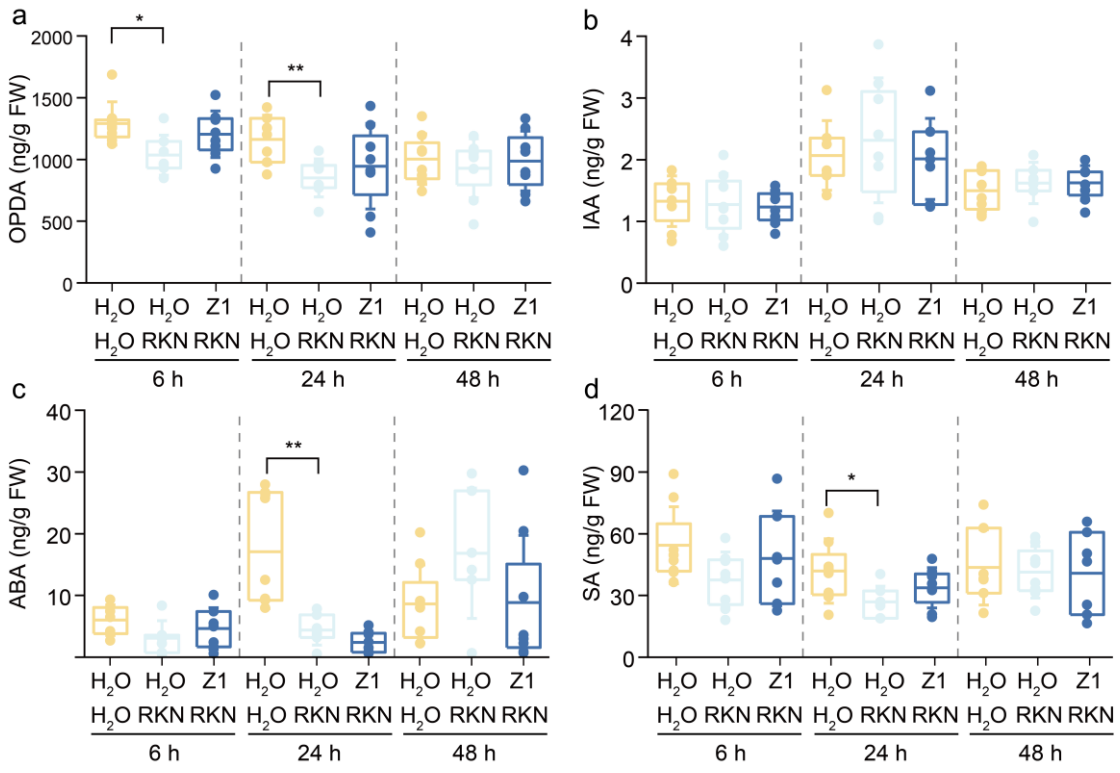

Fig. S4 Phytohormone dynamics in maize roots following RKN infection under Z1 inoculation. (A–D) Concentrations of phytohormones measured at 6, 24, and 48 hours after RKN infection in maize roots inoculated with Z1: (A) 12-oxo-phytodienoic acid (OPDA), (B) indole-3-acetic acid (IAA), (C) abscisic acid (ABA), and (D) salicylic acid (SA) (mean ± SD, n = 6–8). Boxes represent the interquartile range (IQR; 25th to 75th percentiles), and data points indicate individual biological replicates. Asterisks denote statistically significant differences between treatments (two-sided Student's t-test; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

Genus	Difference in mean proportions (%)	Mean proportion (%) (Control)	Mean proportion (%) (RKN-infected)	statistic	p.value	parameter	conf.low	conf.high	method	alternative
Magnetospirillum	0.8391	3.1366	2.2975	4.4665	0.0022	7.8514	0.4045	1.2738	Welch Two Sample t-test	two.sided
Gaiella	0.6917	2.3518	1.6601	3.8901	0.0033	9.5684	0.2931	1.0904	Welch Two Sample t-test	two.sided
Caballeronia	0.4757	1.5826	1.1069	4.7994	0.0012	8.2787	0.2485	0.7030	Welch Two Sample t-test	two.sided
Solimonas	0.3577	0.8220	0.4642	2.8423	0.0249	7.0152	0.0603	0.6552	Welch Two Sample t-test	two.sided
Arenimonas	0.3323	0.8205	0.4882	4.1338	0.0048	6.7044	0.1405	0.5241	Welch Two Sample t-test	two.sided
Paraburkholderia	0.2673	1.0713	0.8041	2.8258	0.0208	8.5878	0.0517	0.4828	Welch Two Sample t-test	two.sided
Haliangium	0.2645	0.6495	0.3850	5.4466	0.0003	9.4925	0.1555	0.3735	Welch Two Sample t-test	two.sided
Methyloversatilis	0.2627	1.2374	0.9748	2.9186	0.0189	8.1855	0.0559	0.4694	Welch Two Sample t-test	two.sided
Delftia	0.1614	0.2028	0.0414	4.8385	0.0037	5.4858	0.0779	0.2450	Welch Two Sample t-test	two.sided
Nocardioides	0.1604	0.4547	0.2943	3.4634	0.0063	9.7330	0.0568	0.2640	Welch Two Sample t-test	two.sided
Actinomarinicola	0.1204	0.6236	0.5032	2.6219	0.0264	9.5658	0.0175	0.2234	Welch Two Sample t-test	two.sided
Chondromyces	0.1196	0.2405	0.1209	2.6056	0.0460	5.2144	0.0030	0.2361	Welch Two Sample t-test	two.sided
Racemicystis	0.1110	0.2716	0.1607	8.7019	0.0001	7.0115	0.0808	0.1411	Welch Two Sample t-test	two.sided
Solirubrobacter	0.1081	0.2892	0.1811	3.2732	0.0109	8.2347	0.0323	0.1839	Welch Two Sample t-test	two.sided
Panacagrimonas	0.1065	0.2607	0.1541	2.7446	0.0276	7.3039	0.0155	0.1976	Welch Two Sample t-test	two.sided
Marmoricola	0.0942	0.2581	0.1639	4.8397	0.0018	7.1120	0.0483	0.1400	Welch Two Sample t-test	two.sided
Sorangium	0.0820	0.1078	0.0258	4.7130	0.0015	7.9690	0.0418	0.1221	Welch Two Sample t-test	two.sided
Kofleria	0.0741	0.1435	0.0694	4.7614	0.0031	6.0434	0.0361	0.1121	Welch Two Sample t-test	two.sided
Azospirillum	0.0603	0.1435	0.0832	4.5427	0.0011	9.8604	0.0307	0.0899	Welch Two Sample t-test	two.sided
Blastococcus	0.0600	0.1527	0.0927	2.8701	0.0170	9.8170	0.0133	0.1067	Welch Two Sample t-test	two.sided
Nocardia	0.0520	0.0839	0.0319	3.3393	0.0089	8.8373	0.0167	0.0874	Welch Two Sample t-test	two.sided
Byssovorax	0.0375	0.0754	0.0378	3.2138	0.0116	8.3654	0.0108	0.0643	Welch Two Sample t-test	two.sided
Ferrovibrio	0.0232	0.0452	0.0220	4.7210	0.0030	6.2166	0.0113	0.0352	Welch Two Sample t-test	two.sided
Enhygromyxa	0.0221	0.0605	0.0384	2.6587	0.0364	6.2022	0.0019	0.0423	Welch Two Sample t-test	two.sided
Microbacterium	0.0201	0.0404	0.0203	2.4357	0.0411	7.9372	0.0010	0.0392	Welch Two Sample t-test	two.sided

Conexibacter	0.0165	0.0230	0.0065	3.1414	0.0181	6.4671	0.0039	0.0292	Welch Two Sample t-test	two.sided
Ornithinibacter	0.0154	0.0304	0.0150	2.6740	0.0239	9.7250	0.0025	0.0283	Welch Two Sample t-test	two.sided
Simplicispira	0.0144	0.0315	0.0171	2.4700	0.0474	6.1563	0.0002	0.0287	Welch Two Sample t-test	two.sided
Methylophilus	-0.0107	0.0118	0.0225	-3.1984	0.0100	9.5942	-0.0182	-0.0032	Welch Two Sample t-test	two.sided
Acidiferrimicrobium	-0.0138	0.0047	0.0185	-2.6943	0.0365	5.8977	-0.0264	-0.0012	Welch Two Sample t-test	two.sided
Tellurimicrobium	-0.0166	0.0409	0.0575	-2.7543	0.0203	9.9967	-0.0300	-0.0032	Welch Two Sample t-test	two.sided
Luteitalea	-0.0230	0.0367	0.0598	-2.7201	0.0249	8.4781	-0.0424	-0.0037	Welch Two Sample t-test	two.sided
Geofilum	-0.0278	0.0253	0.0531	-2.4357	0.0383	8.7741	-0.0537	-0.0019	Welch Two Sample t-test	two.sided
Sediminibacterium	-0.0602	0.0453	0.1055	-2.4428	0.0498	6.0756	-0.1202	-0.0001	Welch Two Sample t-test	two.sided
Rhizorhapis	-0.0621	0.1427	0.2048	-2.3217	0.0471	8.4501	-0.1232	-0.0010	Welch Two Sample t-test	two.sided
Hyphomonas	-0.0652	0.1074	0.1726	-4.1071	0.0021	9.9576	-0.1006	-0.0298	Welch Two Sample t-test	two.sided
Afifella	-0.0699	0.1089	0.1787	-2.6451	0.0283	8.3810	-0.1303	-0.0094	Welch Two Sample t-test	two.sided
Lacipirellula	-0.0855	0.1671	0.2526	-3.1196	0.0128	8.7264	-0.1477	-0.0232	Welch Two Sample t-test	two.sided
Thermomarinilinea	-0.0855	0.3838	0.4693	-2.8325	0.0229	7.7219	-0.1555	-0.0154	Welch Two Sample t-test	two.sided
Bradyrhizobium	-0.0929	0.2671	0.3600	-2.9079	0.0184	8.4994	-0.1658	-0.0200	Welch Two Sample t-test	two.sided
Aggregatilinea	-0.0944	0.2585	0.3529	-2.9467	0.0151	9.6801	-0.1660	-0.0227	Welch Two Sample t-test	two.sided
Litorilinea	-0.1486	0.3668	0.5154	-3.1399	0.0113	9.3858	-0.2550	-0.0422	Welch Two Sample t-test	two.sided
Acidibacter	-0.1695	1.0494	1.2190	-2.7076	0.0222	9.8992	-0.3092	-0.0298	Welch Two Sample t-test	two.sided
Ferruginibacter	-0.2425	0.3062	0.5487	-4.8863	0.0011	8.2166	-0.3564	-0.1286	Welch Two Sample t-test	two.sided
Terrimonas	-0.2947	0.4724	0.7671	-5.0506	0.0008	8.6678	-0.4274	-0.1619	Welch Two Sample t-test	two.sided
Rhodoplanes	-0.2994	0.8112	1.1105	-3.4185	0.0066	10.0000	-0.4945	-0.1042	Welch Two Sample t-test	two.sided
Sphingomonas	-0.7029	1.6831	2.3860	-2.4194	0.0441	7.4530	-1.3815	-0.0243	Welch Two Sample t-test	two.sided

Table S1 Differential genus between the healthy (Con) and RKN-infected (RKN) maize rhizosphere at the seedling stage by STAMP analysis.

Genus	Difference in mean proportions (%)	Mean proportion (%) (Control)	Mean proportion (%) (RKN-infected)	statistic	p.value	parameter	conf.low	conf.high	method	alternative
Nitrospira	0.8095	2.8511	2.0417	2.6339	0.0339	6.9560	0.0818	1.5371	Welch Two Sample t-test	two.sided
Limnobacter	0.0906	0.2794	0.1888	2.9691	0.0184	7.7928	0.0199	0.1612	Welch Two Sample t-test	two.sided
Minicystis	0.0394	0.0722	0.0328	3.9976	0.0063	6.3967	0.0157	0.0632	Welch Two Sample t-test	two.sided
Rhizorhapis	0.0362	0.0994	0.0631	2.9186	0.0194	7.9738	0.0076	0.0648	Welch Two Sample t-test	two.sided
Nitrosospira	0.0357	0.2088	0.1731	3.1386	0.0106	9.9798	0.0103	0.0610	Welch Two Sample t-test	two.sided
Niveibacterium	0.0199	0.0564	0.0365	2.9533	0.0181	8.0756	0.0044	0.0355	Welch Two Sample t-test	two.sided
Desulfofundulus	0.0178	0.0278	0.0100	4.9357	0.0008	9.1833	0.0097	0.0259	Welch Two Sample t-test	two.sided
Allosphingosinicella	0.0156	0.0273	0.0118	2.8256	0.0181	9.9339	0.0033	0.0278	Welch Two Sample t-test	two.sided
Delftia	0.0122	0.0267	0.0145	3.1416	0.0105	9.9455	0.0035	0.0208	Welch Two Sample t-test	two.sided
Paradevosia	0.0121	0.0132	0.0011	2.5635	0.0458	5.5453	0.0003	0.0239	Welch Two Sample t-test	two.sided
Polaromonas	0.0061	0.0079	0.0017	2.3433	0.0499	7.3415	0.0000	0.0123	Welch Two Sample t-test	two.sided
Spiribacter	-0.0197	0.0253	0.0450	-2.4065	0.0377	9.6675	-0.0380	-0.0014	Welch Two Sample t-test	two.sided
Nonomuraea	-0.0273	0.0183	0.0456	-2.5914	0.0349	7.2266	-0.0520	-0.0025	Welch Two Sample t-test	two.sided
Chloroflexus	-0.0306	0.0661	0.0966	-2.7363	0.0307	6.6228	-0.0573	-0.0038	Welch Two Sample t-test	two.sided
Acidiferrimicrobium	-0.0369	0.0296	0.0665	-2.6058	0.0315	7.9429	-0.0696	-0.0042	Welch Two Sample t-test	two.sided
Desulfomonile	-0.0443	0.2004	0.2448	-2.7899	0.0193	9.8738	-0.0798	-0.0089	Welch Two Sample t-test	two.sided
Niastella	-0.0676	0.0935	0.1611	-3.4506	0.0127	6.2576	-0.1151	-0.0202	Welch Two Sample t-test	two.sided
Steroidobacter	-0.1066	0.5254	0.6320	-3.0888	0.0115	9.9630	-0.1835	-0.0297	Welch Two Sample t-test	two.sided
Actinomarinicola	-0.1221	0.6241	0.7462	-2.7786	0.0226	8.5287	-0.2224	-0.0219	Welch Two Sample t-test	two.sided

Table S2 Differential genus between the healthy (Con) and RKN-infected (RKN) maize rhizosphere at the jointing stage by STAMP analysis.