SUPPORTING INFORMATION

Impaired microbial N-acyl homoserine-lactone signalling increases plant resistance to aphids across variable abiotic and biotic environments

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Figures

Tables

Supporting Information Figure S1

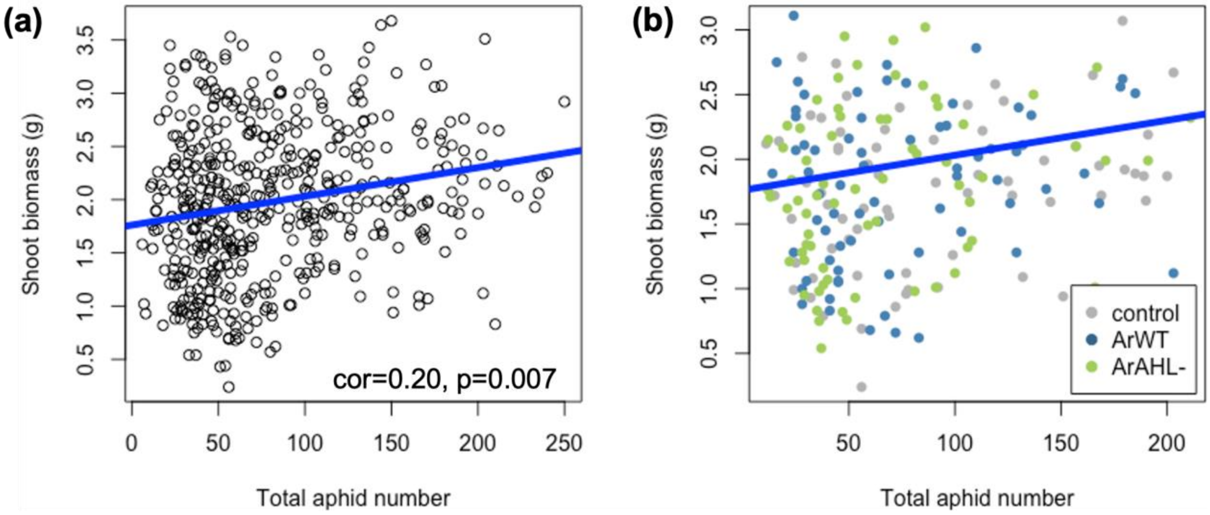

Figure S1. Correlation between the plant shoot biomass (*Hordeum vulgare)* and the aphid numbers (*Sitobion avenae*). (a) correlation without separation of the treatments by colour. (b) correlation with visualization of each bacteria treatment by colour. The correlation value was computed by Pearson's correlation coefficient.

Supporting Information Figure S2

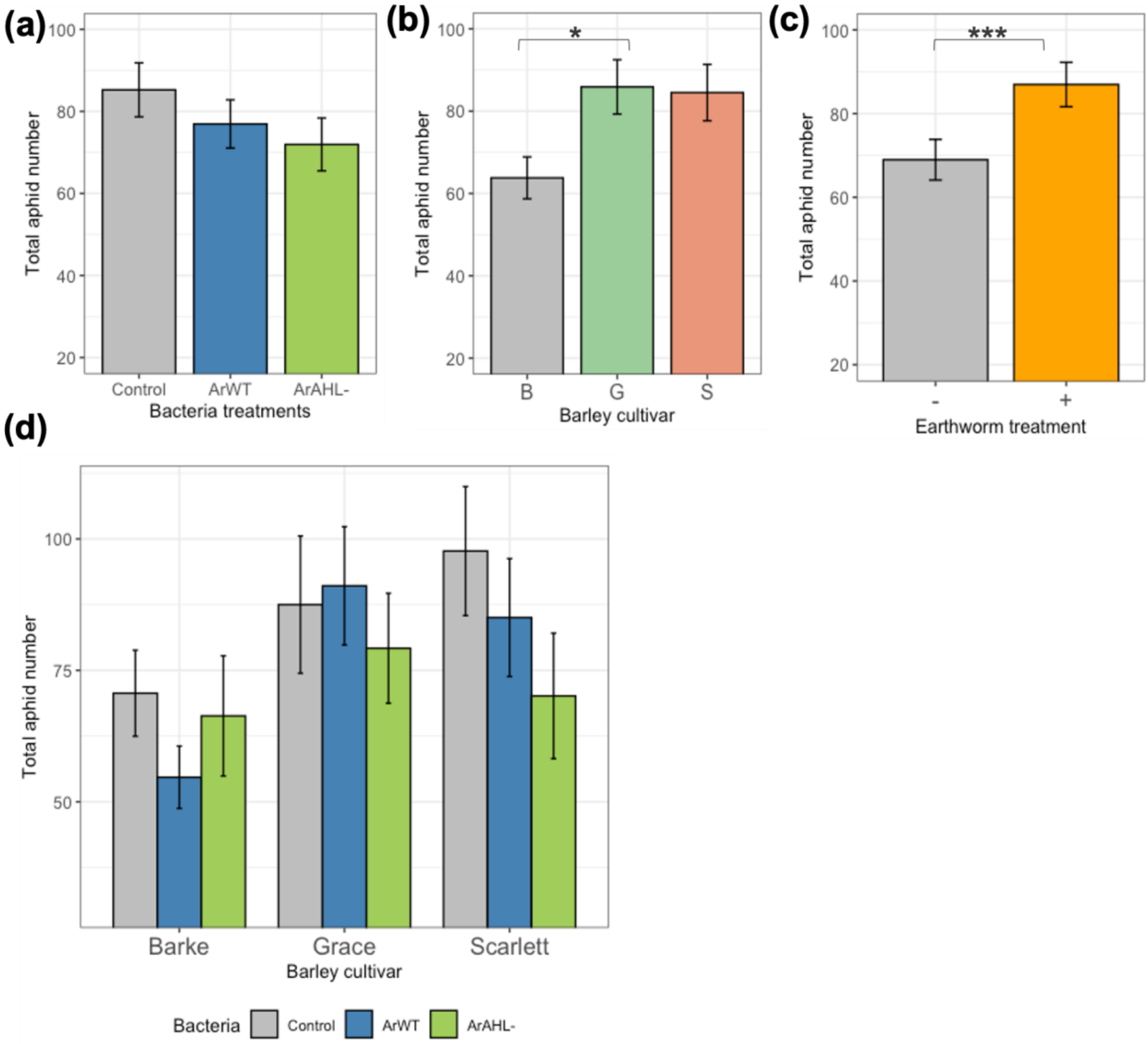

Figure S2 Effects of the main experimental factors on total aphid numbers (*Sitobion avenae*). (a) bacteria treatments effects on aphids. (b) Barley cultivar effect on aphid. (c) Earthworms' treatment effect on aphids. Combined effect of barley cultivar x bacteria on aphids. Asterisk and points on the bars of the main effect figures represent the significant difference between groups: *P*<0.0001 '***'; *P*<0.01 '*'. From computing the estimated marginal means EMMs, Tukey method, calculated with R version 4.0.5, package emmeans). Bars represent mean ± SE (n=6 replicates).

Supporting Information Figure S3

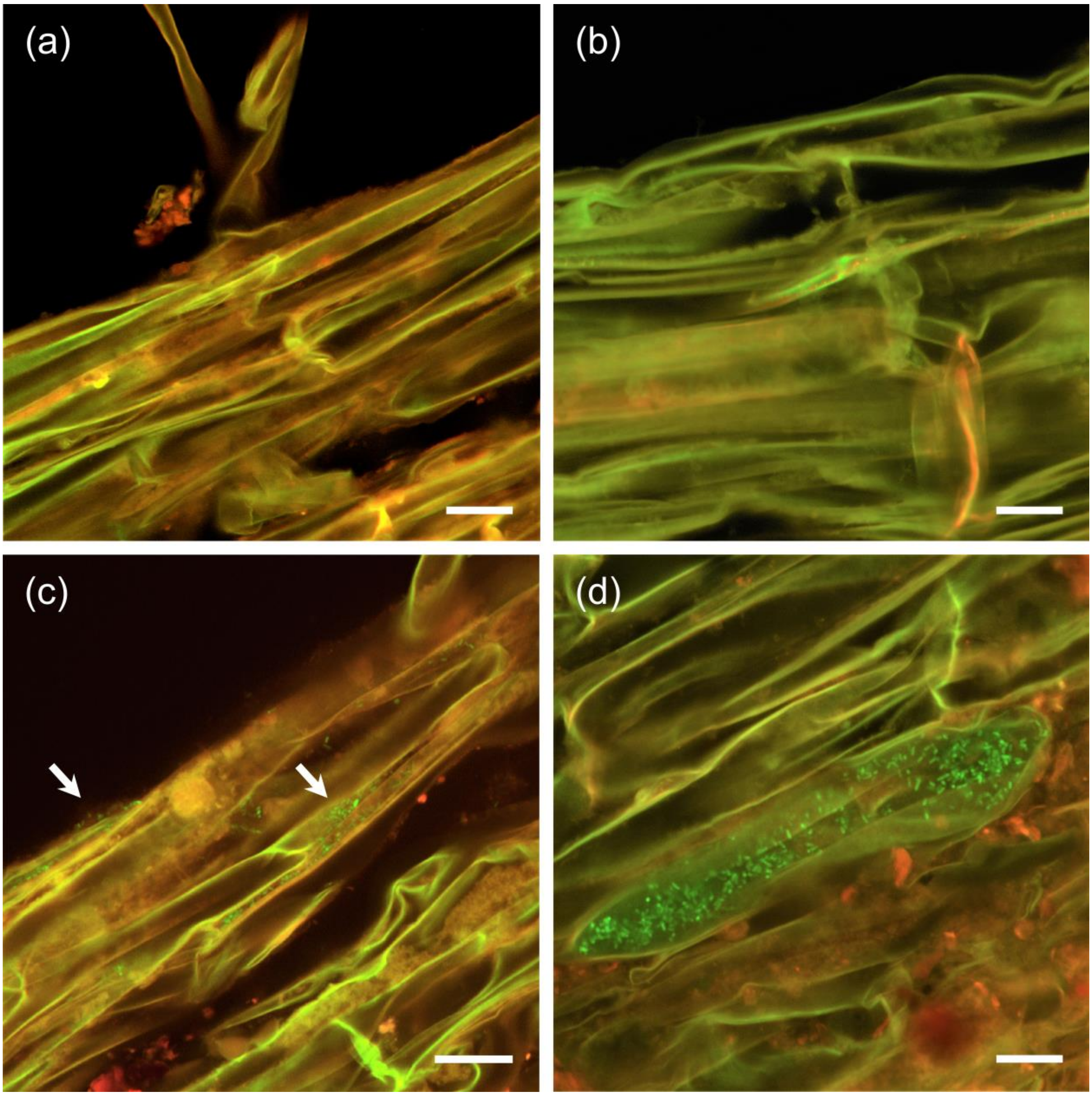

Figure S3 Confocal micrographs of *Acidovorax radicis* colonisation on barley (*Hordeum vulgare)* roots. *A. radicis* wildtype (ArWT) was not detected in the rhizosphere (a) seven and (b) ten days after inoculation. *A. radicis* AHL-negative mutant (ArAHL-) was still detected in the rhizosphere (c) seven and (d) ten days after inoculation. ArWT cells were chromosomally GFP-labeled. ArAHL- cells were GFP-labeled on plasmid level. GFP-labelled cells are shown in green, root background is shown in yellow, soil particles appear in red. Micrographs were taken from two independent time series experiments in soil. Scale bar = 10 µm.

Supporting Information Figure S4

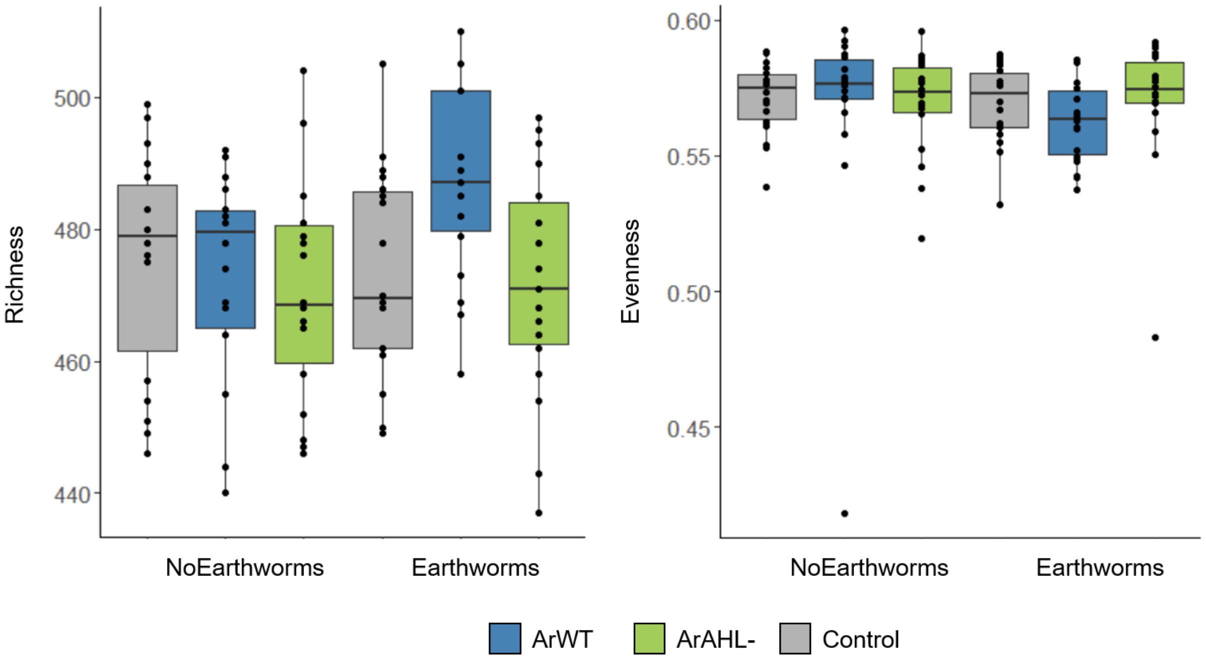

Figure S4 Diversity of the rhizosphere microbiome. (a) Species richness per bacterial treatment devided by earthworm treatment. Shown are the effects of the not inoculated control (grey), the AHL-producing strain *A. radicis* N35e (ArWT, blue) und the *A. radicis* AHL-negative mutant (ArAHL-, green), respectively. The differences were not significant. (b) Species evenness devided by earthworm treatment. Shown are the effects of the not inoculated control (grey), the AHL-producing strain *A. radicis* N35e (ArWT, blue) und the *A. radicis* AHL-negative mutant (ArAHL-, green), respectively. There was no significant variance between the treatment groups.

Supporting Information Figure S5

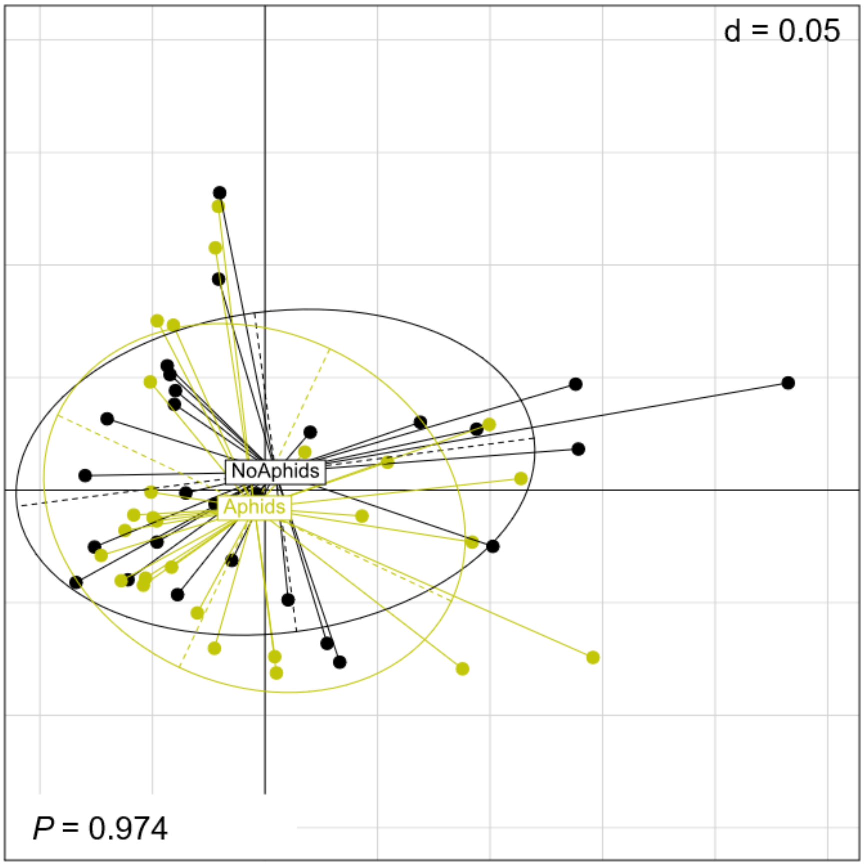

Figure S5 Microbial profiles of aphid treatments. Multi-dimensional scaling plots of microbial profiles. Samples treated with aphids (yellow) showed a similar microbial community composition as samples without aphids (black).

Supporting Information Figure S6

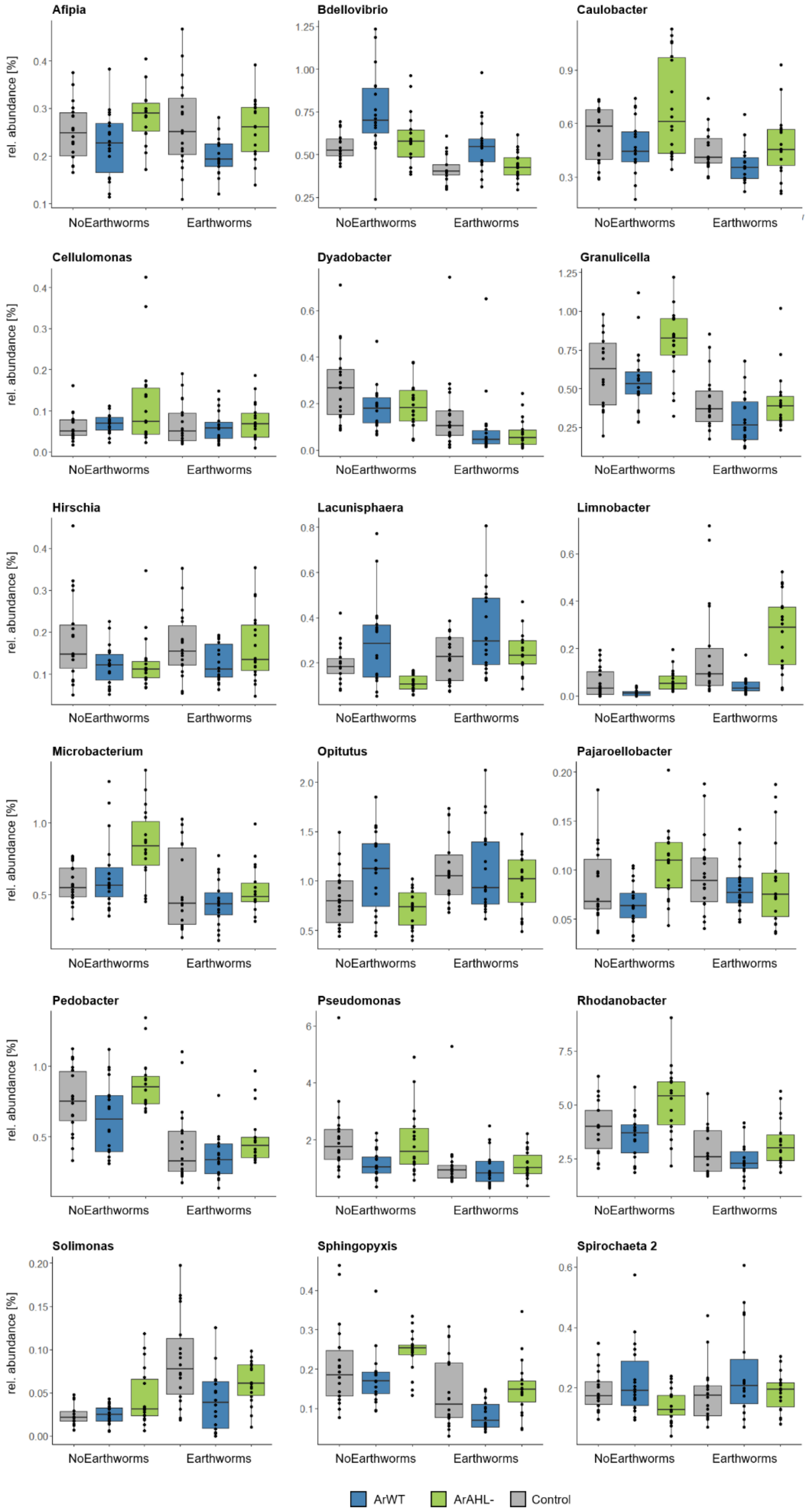

Figure S6 Relative abundances of the most changing genera. Shown are the effects of the not inoculated control (grey), the AHL-producing strain *A. radicis* N35e (ArWT, blue) und the *A. radicis* AHL-negative mutant (ArAHL-, green), devided by earthworm treatment. Genera were selected by MANOVA analysis based on their main bacterial effect.

Supporting Information Figure S7

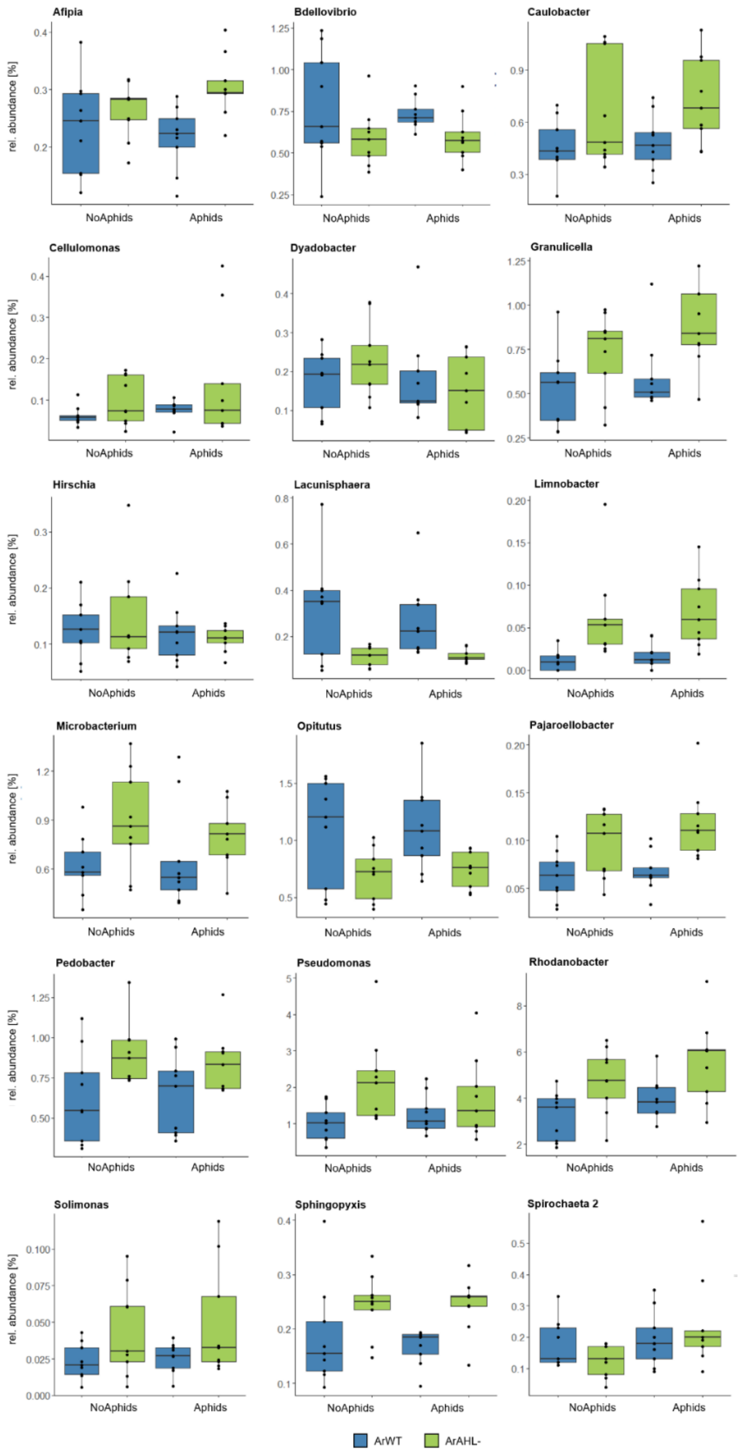

Figure S7 AHL effect of most changing genera. Shown are the relative abundances of significantly changing genera devided by aphid treatment within the NoEarthworm subset. Shown are the effects of the AHL-producing strain *A. radicis* N35e (ArWT, blue) und the *A. radicis* AHL-negative mutant (ArAHL-, green). Genera were selected by MANOVA analysis based on their main bacterial effect

Supporting Information Table S1

Table S1 Reference and target primers list used in *Hordeum vulgare* defence analysis through qPCR technique (number 1-6) and primers utilized on microbiome analysis (number 7). Sequence overhangs are underlined.

Nº	**Primer name**	**Sequence**	**Gene name annotation**	**Sequences source**	**Primer type**
1	HvEF1α F	5'-GTCATTGATGCTCCTGGTCA-3	Elongation factor	Dey *et al.*, 2014	House-keeping gene
	HvEF1α R	5'-CTGCTTCACACCAAGAGTGA-3'			
2	HvERF-like F (24530)	5´-CCGTACTTCTTCTACGAACA-3´	Ethylene-responsive factor-like transcription factor IPR016177	Dey *et al.*, 2014	Target
	HvERF-like R (24530)	5'-CGGTTCAGATCCAGATCAAA-3'			
3	HvWRKY_22 F (45055)	5'-AGAGCACTACCCGTTCTCCA-3'	WRKY TF 22 IPR003657 (DNA-binding WRKY)	Dey *et al.*, 2014	Target
	HvWRKY_22 R (45055)	5'-GACACCACCTCGTCCAACTC-3'			
4	HvPR1 F	5'-GGACTACGACTACGGCTCCA-3	Pathogenesis-related protein	Shrestha *et al.*, 2019	Target
	HvPR1 R	5'-GGCTCGTAGTTGCAGGTGAT-3			
5	HvPR17b F	5'-CGAGGTTCCTCGACTACTGC-3			Target
	HvPR17b R	5'-ATCACATTCAGCCTCCGAAC-3			
6	MLOC_10956 F	5'-GCCAGAAGCCATATCTGCAC-3	UDP-glycosyltransferase-like protein (UGT)	Han *et al.*, 2016	Target
	MLOC_10956 R	5'-GCAGAAAAACTCACCGGAGC-3			
7	Illumina 515 F	5’-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGATTAGATACCCTGGTA-3’	V4-V5 region of bacterial 16S rRNA gene	*Caporaso et al*., 2011	Target
	Illumina 806 R	5’-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCGACRRCCATGCANCACCT-3’			

Supporting Information Table S2

Table S2 Mean relative abundance of all known genera across bacterial treatments. Genera are sorted by descending mean relative abundance across all samples per treatment.

Genera	**Control**	**ArWT**	**ArAHL-**
Rhodanobacter	3.998	3.538	5.186
Devosia	3.992	3.704	3.600
Streptomyces	3.829	3.229	3.096
Cellvibrio	3.555	3.826	2.687
Cytophaga	3.430	2.814	2.720
Flavobacterium	2.623	2.066	2.759
Asticcacaulis	2.130	1.735	2.279
Rhodopseudomonas	1.625	1.597	1.956
Pseudomonas	2.033	1.144	1.939
Fluviicola	1.473	1.246	1.362
Pseudolabrys	1.185	1.453	1.208
OM27 clade	1.240	1.216	1.163
Ramlibacter	1.138	1.194	1.103
Rhodobacter	1.156	1.277	0.891
Luteolibacter	0.924	1.017	1.077
Pseudarthrobacter	0.866	0.775	1.165
Opitutus	0.828	1.095	0.722
Sporocytophaga	0.586	1.057	0.680
Pedobacter	0.752	0.637	0.870
Planctopirus	0.628	0.751	0.791
Kosakonia	1.135	0.197	0.820
Myxococcus	1.213	0.639	0.257
Microbacterium	0.573	0.641	0.850
P3OB-42	0.533	0.735	0.752
Granulicella	0.601	0.558	0.801
Bdellovibrio	0.546	0.753	0.595
Chthoniobacter	0.550	0.735	0.576
Caulobacter	0.536	0.472	0.692
Acidothermus	0.501	0.558	0.631
Massilia	0.553	0.389	0.741
Aquicella	0.574	0.755	0.275

Mesorhizobium	0.516	0.467	0.549
Mycobacterium	0.468	0.486	0.491
Edaphobaculum	0.484	0.474	0.448
Hydrogenophaga	0.473	0.441	0.395
Blyi10	0.419	0.419	0.426
Peredibacter	0.416	0.423	0.421
Parafrigoribacterium	0.390	0.390	0.465
Flavisolibacter	0.371	0.374	0.427
Pseudoxanthomonas	0.395	0.371	0.376
Novosphingobium	0.337	0.361	0.400
Mucilaginibacter	0.382	0.335	0.375
Haliangium	0.323	0.344	0.385
Flavitalea	0.268	0.287	0.316
Chitinophaga	0.223	0.185	0.460
Emticicia	0.328	0.301	0.236
Nocardioides	0.271	0.288	0.299
Pirellula	0.367	0.276	0.202
Rhodococcus	0.294	0.285	0.260
Parvibaculum	0.294	0.282	0.226
Afipia	0.252	0.225	0.284
Gemmatimonas	0.211	0.291	0.254
Brevundimonas	0.219	0.267	0.271
Prosthecobacter	0.272	0.208	0.274
Pelomonas	0.356	0.209	0.172
Stenotrophomonas	0.239	0.217	0.270
Phenylobacterium	0.257	0.223	0.213
SH-PL14	0.202	0.242	0.246
Sediminibacterium	0.206	0.281	0.189
Thermomonas	0.225	0.183	0.247
Dyadobacter	0.281	0.179	0.189
Gryllotalpicola	0.194	0.203	0.246
possible genus 04	0.103	0.312	0.226
Sphingopyxis	0.208	0.176	0.244
Isoptericola	0.167	0.200	0.257
Lacunisphaera	0.197	0.292	0.112
Dokdonella	0.180	0.177	0.220
Ciceribacter	0.210	0.224	0.132
Spirochaeta 2	0.192	0.226	0.142
Luteimonas	0.194	0.163	0.203

Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	0.161	0.255	0.134
Castellaniella	0.166	0.176	0.198
Bosea	0.166	0.192	0.166
Anaeromyxobacter	0.155	0.197	0.157
Legionella	0.185	0.215	0.093
Sphingomonas	0.156	0.151	0.184
Sulfurovum	0.147	0.208	0.133
Herpetosiphon	0.296	0.142	0.043
Hirschia	0.184	0.121	0.127
Gemmata	0.125	0.198	0.108
Azospira	0.000	0.001	0.424
IMCC26134	0.188	0.164	0.063
Rheinheimera	0.164	0.181	0.063
Occallatibacter	0.113	0.130	0.148
Duganella	0.142	0.056	0.192
Methyloversatilis	0.100	0.129	0.108
Singulisphaera	0.096	0.092	0.113
Paracoccus	0.112	0.091	0.088
Pedomicrobium	0.091	0.094	0.093
YC-ZSS-LKJ147	0.000	0.269	0.000
Burkholderia-Caballeronia-Paraburkholderia	0.065	0.065	0.135
Herminiimonas	0.084	0.081	0.097
Pajaroellobacter	0.085	0.065	0.107
Cellulomonas	0.062	0.069	0.120
Iamia	0.083	0.085	0.081
Conexibacter	0.073	0.077	0.094
Roseiarcus	0.069	0.076	0.097
Rhodopirellula	0.065	0.097	0.078
Marmoricola	0.071	0.076	0.079
Reyranella	0.074	0.077	0.059
Methylophilus	0.061	0.070	0.068
SWB02	0.076	0.076	0.041
Buchnera	0.073	0.115	0.001
Roseomonas	0.057	0.065	0.059
Roseimicrobium	0.067	0.060	0.051
Nakamurella	0.040	0.081	0.053
Bryobacter	0.055	0.058	0.060
Panacagrimonas	0.058	0.056	0.047

Deinococcus	0.046	0.080	0.031
Bacteriovorax	0.040	0.071	0.043
Rubellimicrobium	0.051	0.055	0.044
Azospirillum	0.039	0.020	0.087
Steroidobacter	0.061	0.030	0.050
Schlesneria	0.043	0.042	0.052
Limnobacter	0.058	0.014	0.065
Pseudonocardia	0.062	0.030	0.040
Ferruginibacter	0.029	0.045	0.046
Flavihumibacter	0.035	0.039	0.041
Kaistia	0.055	0.020	0.032
Kocuria	0.017	0.054	0.034
Solimonas	0.025	0.025	0.046
Sanguibacter	0.028	0.035	0.032
Phreatobacter	0.040	0.035	0.016
Pigmentiphaga	0.043	0.018	0.027
Corallococcus	0.036	0.013	0.036
Paenibacillus	0.047	0.008	0.027
Pseudoflavitalea	0.000	0.081	0.000
Pir4 lineage	0.023	0.041	0.016
OLB13	0.061	0.014	0.004
Aquabacterium	0.009	0.048	0.018
Lechevalieria	0.001	0.001	0.072
Nubsella	0.040	0.019	0.014
TM7a	0.027	0.031	0.015
Aeromicrobium	0.019	0.038	0.016
Hydrocarboniphaga	0.040	0.011	0.021
Methylobacterium-Methylorubrum	0.030	0.030	0.012
Domibacillus	0.034	0.022	0.014
Leptolyngbya PCC-6306	0.047	0.014	0.008
FFCH7168	0.005	0.043	0.022
Halomonas	0.004	0.004	0.058
Actinoplanes	0.013	0.023	0.023
Candidatus Solibacter	0.021	0.021	0.016
Taibaiella	0.009	0.012	0.038
Ketobacter	0.025	0.023	0.010
Exiguobacterium	0.014	0.041	0.001
Fimbriimonas	0.012	0.026	0.012
mle1-7	0.014	0.021	0.012

Coxiella	0.007	0.011	0.026
Sandaracinus	0.019	0.020	0.005
Dongia	0.013	0.020	0.010
Chthonobacter	0.014	0.021	0.008
Clostridium sensu stricto 1	0.000	0.000	0.035
Verrucomicrobium	0.008	0.019	0.007
Lachnoclostridium	0.001	0.001	0.028
Lacibacter	0.015	0.010	0.004
Sphaerisporangium	0.009	0.012	0.007
Sorangium	0.000	0.025	0.000
Dinghuibacter	0.008	0.012	0.004
Fibrella	0.006	0.015	0.002
Larkinella	0.015	0.005	0.004
Longimicrobium	0.006	0.007	0.010
Phaeodactylibacter	0.019	0.002	0.000
Clostridium sensu stricto 3	0.000	0.000	0.020
Terrimonas	0.001	0.000	0.017
Acinetobacter	0.004	0.003	0.009
Silvanigrella	0.003	0.009	0.004
Fontimonas	0.005	0.004	0.007
Blastocatella	0.000	0.000	0.015
Niastella	0.003	0.010	0.002
Vogesella	0.003	0.009	0.003
Lactococcus	0.000	0.014	0.001
Candidatus Chloroploca	0.000	0.000	0.012
Polyangium	0.000	0.010	0.000
Tychonema CCAP 1459-11B	0.010	0.000	0.000
Abditibacterium	0.001	0.000	0.008
Hymenobacter	0.000	0.003	0.005
Subgroup 10	0.000	0.006	0.001
Arcicella	0.006	0.001	0.000
Candidatus Ovatusbacter	0.001	0.001	0.005
Adhaeribacter	0.001	0.000	0.005
Zoogloea	0.001	0.003	0.001
Chryseolinea	0.000	0.000	0.002
Unknown	37.964	41.049	38.830