

Spatiotemporal Dynamics of the Barley Rhizosphere Prokaryotic Community Following Repeated Inoculation with *Ensifer meliloti* Rm2011 in a Three-Year Field Study

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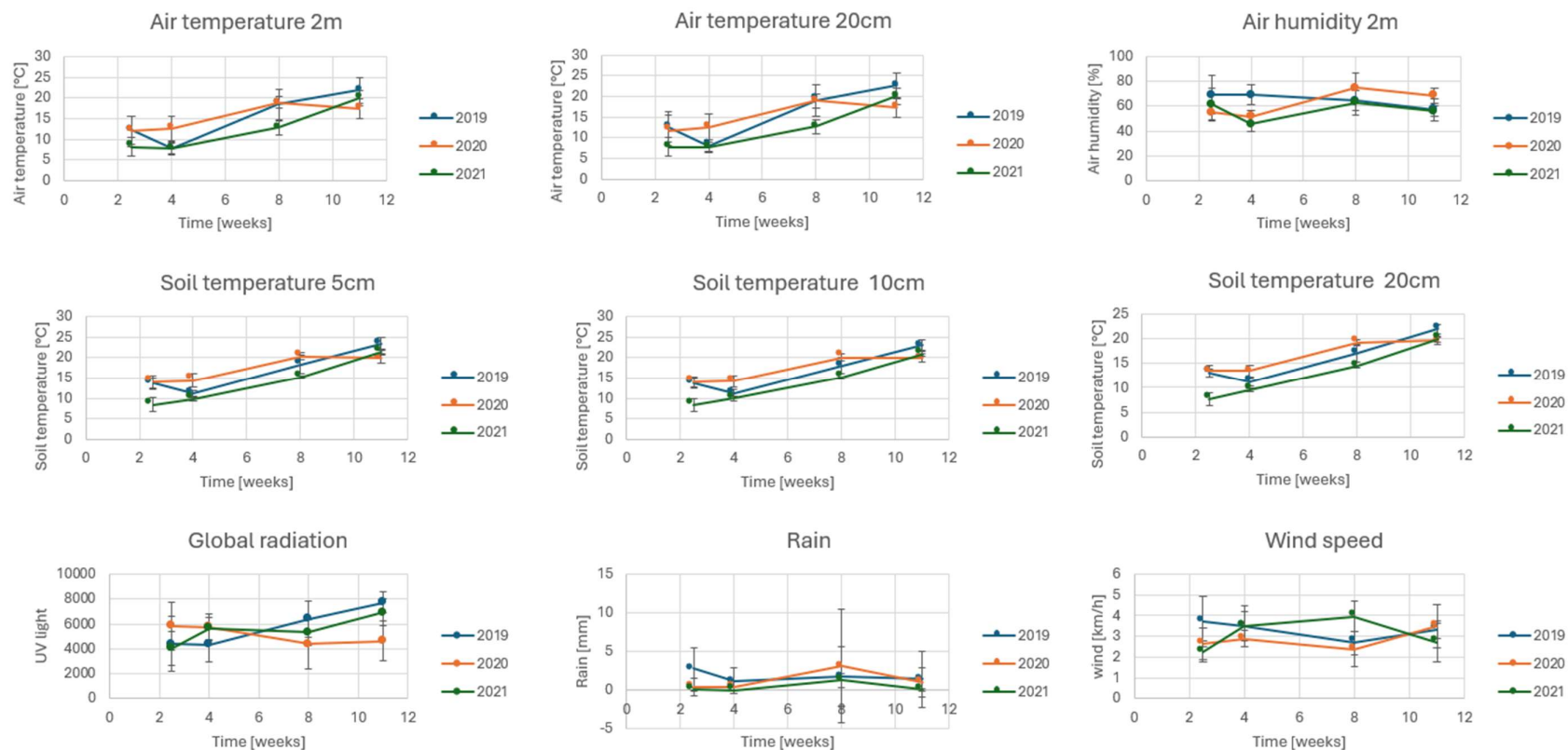
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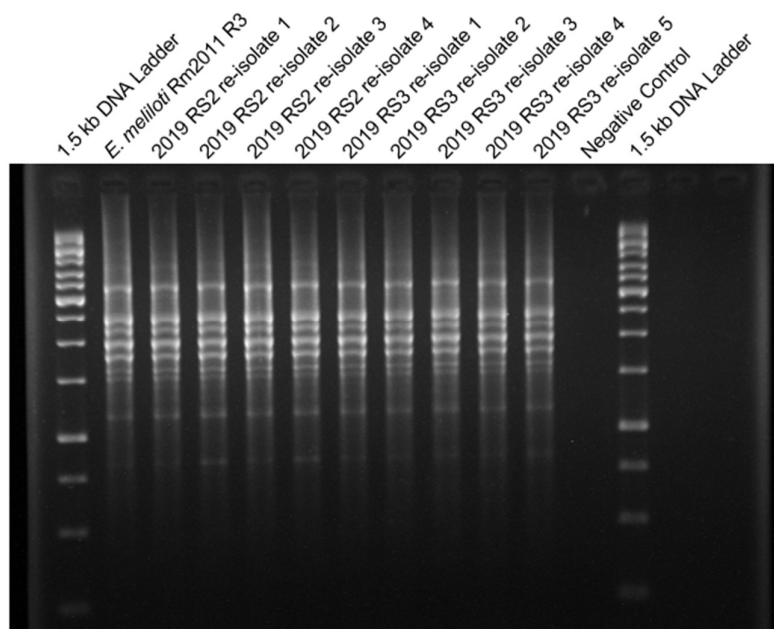
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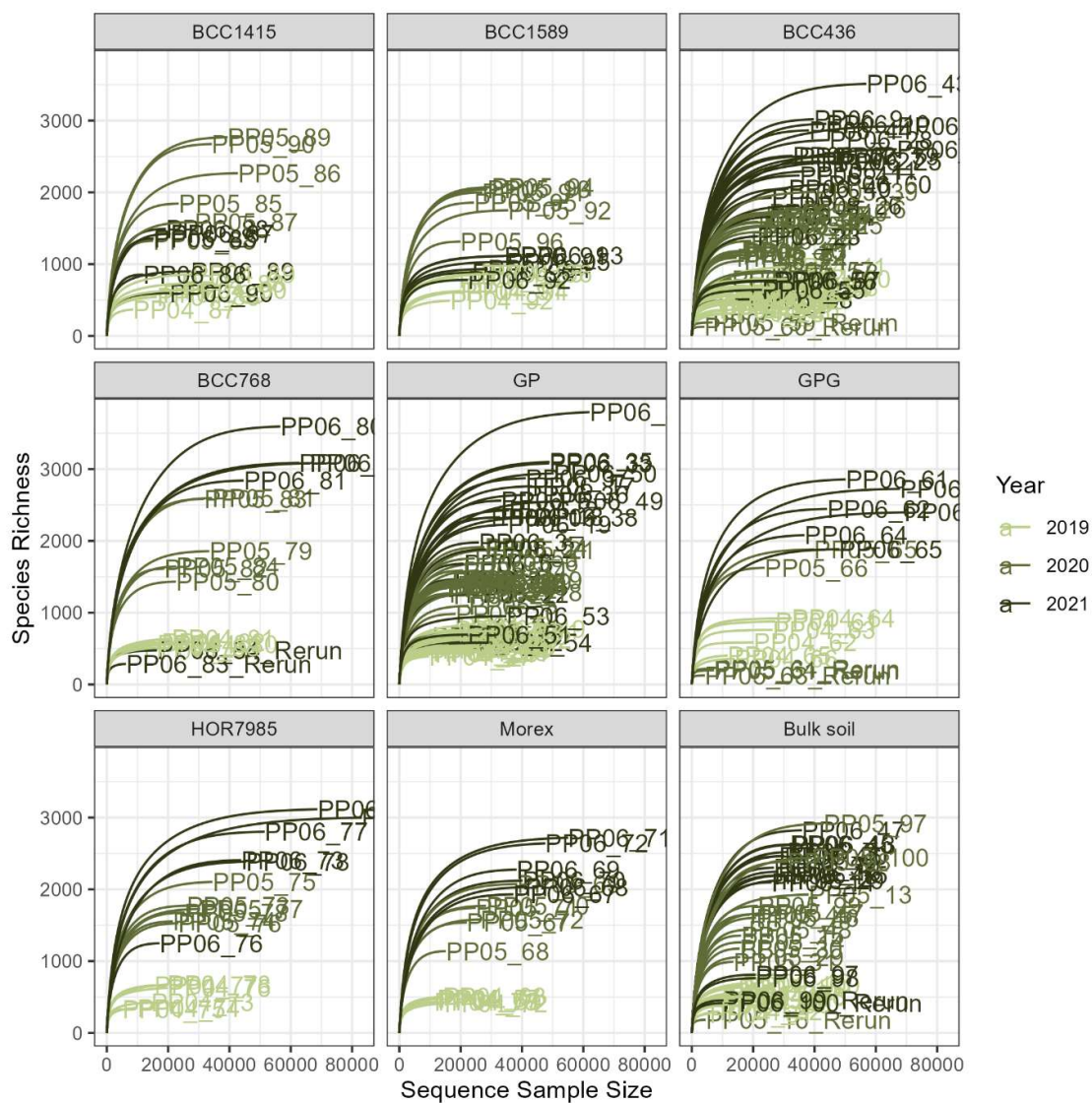
Supplementary Material



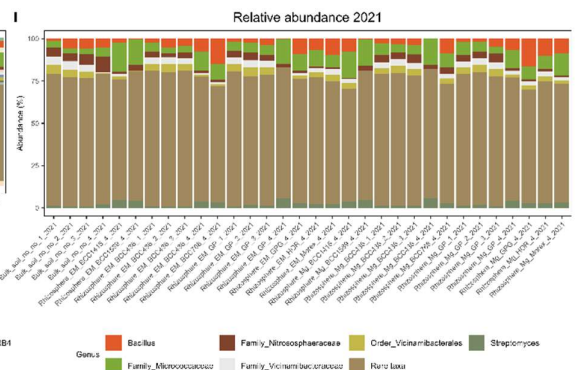
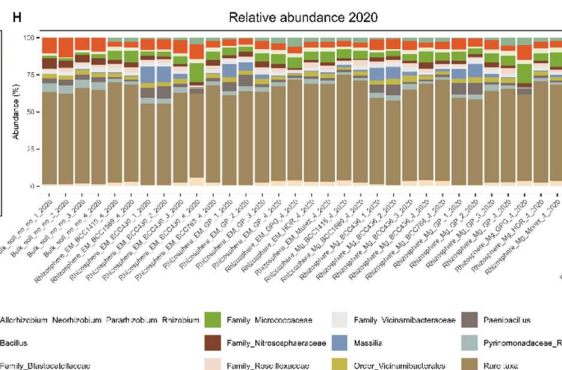
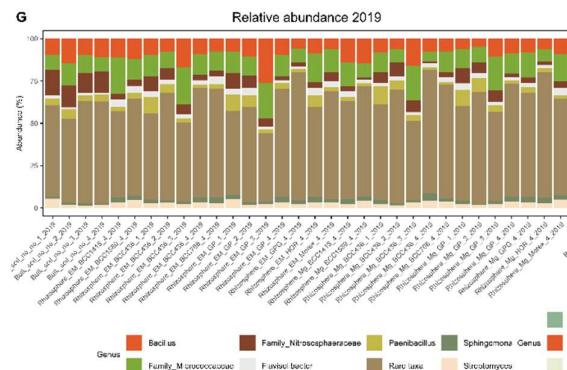
Supplementary Figure 1 Environmental metadata of the three consecutive years (soil and air temperature, humidity, radiation, rainfall, and wind). Every dot represents the averaged value of the daily temperature ranging from the rhizosphere sampling day (RS1-RS4) to 6 days before. Error bars represent the standard deviation.



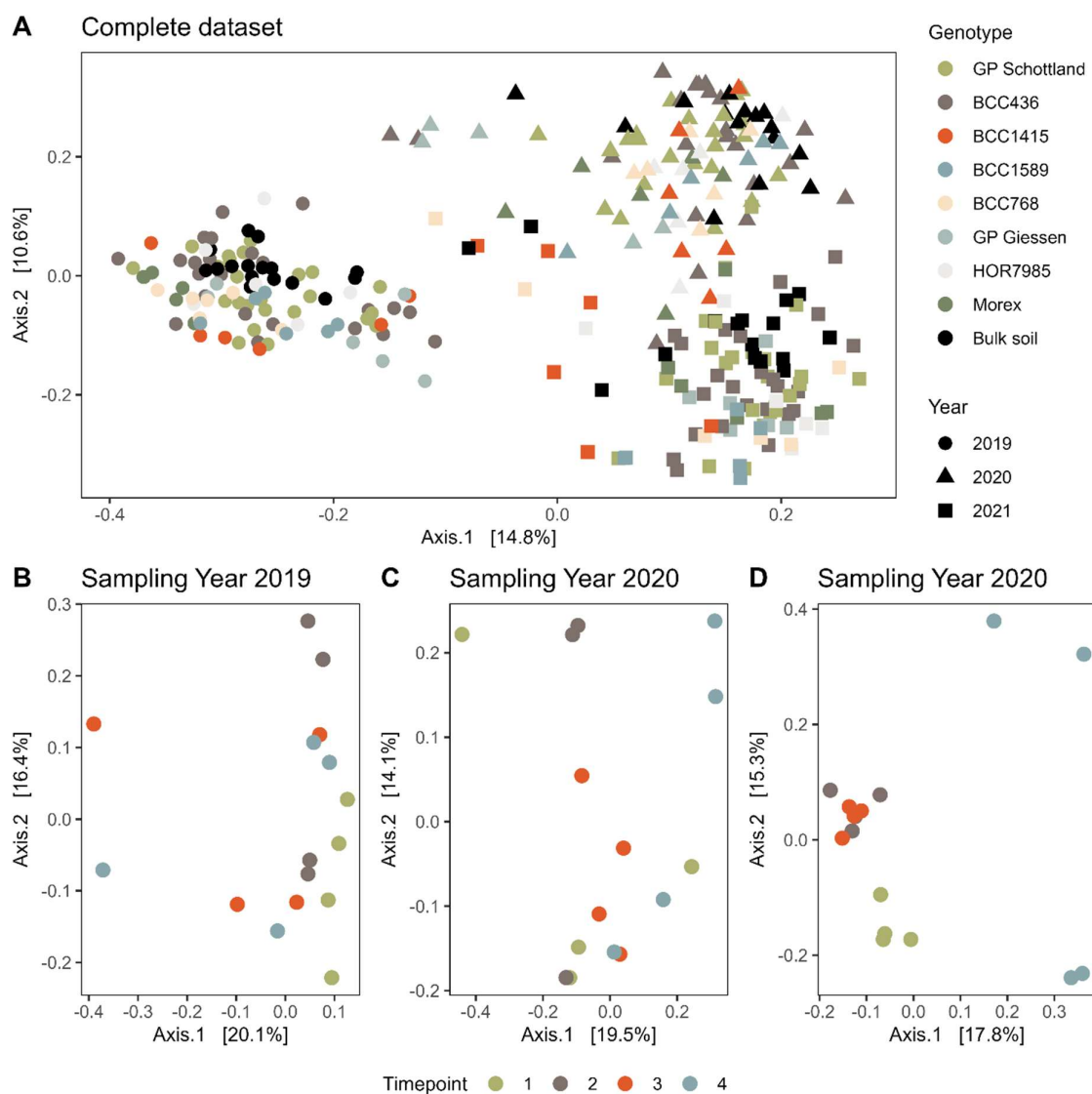
Supplementary Figure 2 Representative BOX Fingerprint of re-isolated *Ensifer meliloti* Rm2011 colonies of the second and third rhizosphere sampling (RS2 and RS3) in 2019. The molecular fingerprint is compared to the original *E. meliloti* Rm2011 strain used for inoculation.



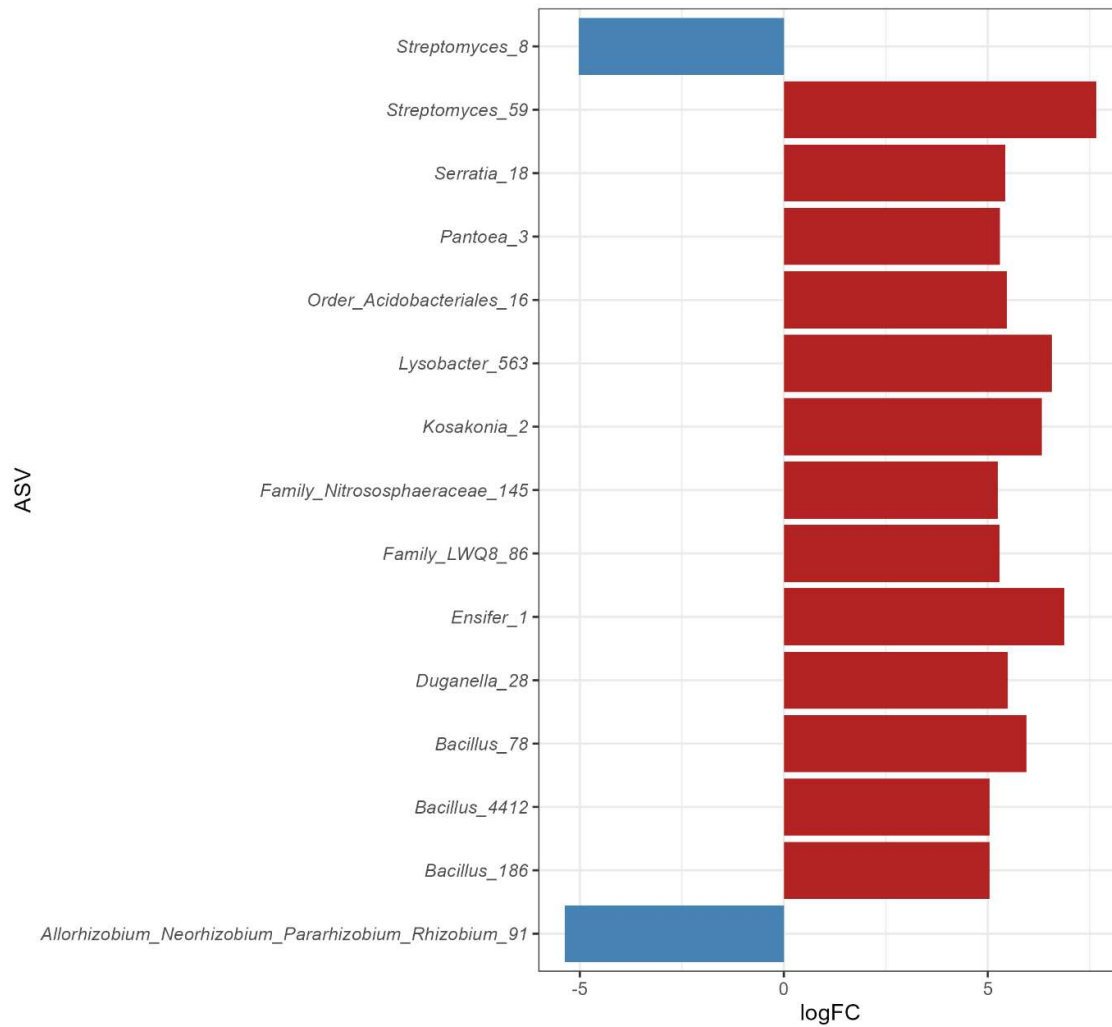
Supplementary Figure 3 Rarefaction curves of 16S rRNA gene amplicon data of barley rhizosphere and bulk soil samples of eight different genotypes from three consecutive years (2019, 2020, 2021). GP = Golden Promise Scotland; GPG = Golden Promise Gießen.



Supplementary Figure 4 Relative abundances of taxa averaged over all samples of the respective years (A, D, G) of 2019, (B, E, H) of 2020, (C, F, I) of 2021. An abundance cut-off was performed at 5% for the phylum level, and 2% for both family and genus level (subsequently categorized as rare taxa).



Supplementary Figure 5 Principal coordinates analysis (PCoA) of prokaryotic community composition across years and treatments. (A) PCoA of the complete dataset, integrating all samples from 2019 to 2021 of both rhizosphere and bulk soil. (B-D) PCoA of the three different sampling years separately for bulk soil samples.



Supplementary Figure 6 Differential abundance of Amplicon Sequence Variants (ASVs) following *Ensifer meliloti* Rm2011 application, expressed as log fold change (logFC) and determined using edgeR. The analysis was performed on a subset of samples from both barley genotypes, Golden Promise and BCC436, with data pooled across all three sampling years and all four rhizosphere sampling time points. Shown are ASVs that were significantly enriched (positive logFC) or depleted (negative logFC) in response to EM Rm2011 application. ASVs were filtered to include only those with $p < 0.03$ and $|\logFC| > 5$.

Supplementary Table 1 Daily air and soil temperature at different heights or depths, for the three consecutive sampling years between sowing and the first rhizosphere sampling. A cold period was detected for 2019 and 2021 (red box and bold letters), whereas in 2020, no temperature drop was detected.

		Air temperature 2m [°C]	Air temperature 20cm [°C]	Soil temperature 5cm [°C]	Soil temperature 10cm [°C]	Soil temperature 20cm [°C]
Sowing 2019	10.04.2019	5.29	5.75	9.00	9.39	9.30
	11.04.2019	4.80	5.26	8.35	8.74	8.72
	12.04.2019	2.99	3.34	7.27	7.74	7.85
	13.04.2019	3.04	3.37	6.91	7.21	7.21
	14.04.2019	2.96	2.93	6.19	6.55	6.64
	15.04.2019	8.03	8.55	7.65	7.50	6.94
	16.04.2019	9.13	9.71	8.35	8.30	7.76
	17.04.2019	11.87	12.36	9.38	9.25	8.55
	18.04.2019	13.64	13.71	10.50	10.33	9.48
	19.04.2019	15.06	15.27	11.55	11.30	10.37
	20.04.2019	13.84	13.85	12.11	11.97	11.12
	21.04.2019	13.38	13.55	12.30	12.25	11.51
	22.04.2019	13.77	14.07	12.56	12.54	11.85
	23.04.2019	10.33	10.80	11.56	12.11	11.92
	24.04.2019	16.96	17.90	14.10	13.75	12.74
	25.04.2019	16.70	17.35	15.37	14.97	13.86
	26.04.2019	12.79	12.91	14.78	14.82	14.18
	27.04.2019	11.21	11.72	14.53	14.38	13.75
Rhizosphere sampling 1	28.04.2019	9.35	9.66	13.36	13.53	13.21
	29.04.2019	8.39	8.13	11.70	12.09	12.12
Sowing 2020	15.04.2020	9.04	8.67	10.47	10.55	10.21
	16.04.2020	12.32	11.88	11.24	11.10	10.47
	17.04.2020	12.21	11.99	12.20	12.01	11.25
	18.04.2020	13.09	13.30	12.66	12.53	11.81
	19.04.2020	9.90	10.14	12.32	12.45	12.00
	20.04.2020	10.24	10.61	12.08	12.20	11.81
	21.04.2020	11.58	11.99	12.44	12.42	11.93
	22.04.2020	13.17	13.63	13.15	12.99	12.30
	23.04.2020	13.77	13.63	13.90	13.67	12.86
	24.04.2020	13.57	13.41	14.27	14.05	13.30
	25.04.2020	11.26	11.12	14.21	14.11	13.56
	26.04.2020	11.72	11.30	14.11	13.96	13.36
	27.04.2020	14.26	14.13	14.86	14.54	13.71
	28.04.2020	14.02	13.76	15.21	14.98	14.21
	29.04.2020	13.88	13.59	15.43	15.24	14.51
	30.04.2020	12.48	12.30	14.55	14.58	14.14
	01.05.2020	11.77	11.63	14.08	14.21	13.81
	02.05.2020	9.52	9.24	12.82	13.18	13.12

	03.05.2020	11.06	10.80	12.75	12.85	12.56
	04.05.2020	10.40	10.04	12.68	12.82	12.51
	05.05.2020	8.70	8.44	12.13	12.31	12.12
	06.05.2020	10.52	10.51	12.58	12.47	12.04
	07.05.2020	11.82	11.77	13.56	13.34	12.67
	08.05.2020	14.53	14.29	14.81	14.45	13.53
	09.05.2020	15.91	15.82	15.84	15.44	14.41
	10.05.2020	15.73	15.50	15.93	15.75	14.95
Rhizosphere sampling 1	11.05.2020	7.13	6.63	13.45	14.18	14.30
	12.05.2020	7.82	7.67	11.85	12.30	12.47
Sowing 2021	30.03.2021	14.06	13.08	9.87	9.69	8.76
	31.03.2021	14.62	13.72	10.37	10.20	9.31
	01.04.2021	11.82	11.27	10.91	10.83	10.06
	02.04.2021	6.36	6.16	9.73	10.02	9.75
	03.04.2021	7.35	6.89	8.81	9.05	8.82
	04.04.2021	5.35	4.93	7.80	8.21	8.16
	05.04.2021	2.97	2.58	6.43	7.07	7.26
	06.04.2021	1.72	1.37	4.98	5.52	5.78
	07.04.2021	2.07	1.72	4.78	5.16	5.28
	08.04.2021	3.62	3.21	4.81	5.04	4.97
	09.04.2021	7.71	7.15	5.86	5.70	5.23
	10.04.2021	6.64	6.28	6.70	6.68	6.21
	11.04.2021	7.44	7.18	7.76	7.61	6.97
	12.04.2021	4.87	4.64	7.08	7.20	6.89
	13.04.2021	4.46	4.14	6.46	6.70	6.52
	14.04.2021	4.92	4.62	6.45	6.58	6.33
	15.04.2021	5.53	5.05	6.66	6.78	6.48
	16.04.2021	7.73	7.50	7.52	7.41	6.86
	17.04.2021	8.46	8.00	8.06	7.95	7.39
	18.04.2021	9.44	9.17	9.39	9.09	8.28
Rhizosphere sampling 1	19.04.2021	9.78	9.34	10.12	9.90	9.10
	20.04.2021	11.50	10.96	10.71	10.36	9.51

Supplementary Table 2 PERMANOVA of barley rhizosphere samples, from three consecutive years (2019, 2020, 2021) analyzed together. The samples consist of eight different genotypes and four different rhizosphere sampling time points, inoculated with either EM Rm2011 or control (ctrl).

PERMANOVA	R²	<i>p</i> =
Year (all samples)	0.22	0.001
Genotype (all samples)	0.07	0.001
Rhizosphere sampling time point (all samples)	0.03	0.001
EM Rm2011 inoculation (all samples)	0.003	0.048
Year:Genotype	0.08	0.001
Year:RS timepoint	0.04	0.001
Year:EM inoculation	0.005	0.073
Genotype:RS timepoint	0.02	0.001
Genotype:EM inoculation	0.02	0.004
RS timepoint:EM inoculation	0.005	0.593
Year:Genotype:RS timepoint	0.03	0.003
Year:Genotype:EM inoculation	0.04	0.006
Year:RS timepoint:EM inoculation	0.01	0.651
Genotype:RS timepoint:EM inoculation	0.006	0.571
Year:Genotype:RS timepoint:EM inoculation	0.01	0.670

Supplementary Table 3 Shannon diversity index of barley rhizosphere and bulk soil samples, from 2019. The samples consist of eight different genotypes and four different rhizosphere sampling (RS) time points, inoculated with either EM Rm2011 (EM) or control (Mg). GP: Golden Promise; GPG: Golden Promise Gießen.

Sample name	Shannon	Treatment	RS	Genotype	Replicate
Rhizosphere_Mg_GP_1_2019_1	5.095121	Mg	1	GP	1
Rhizosphere_Mg_GP_1_2019_2	5.820543	Mg	1	GP	2
Rhizosphere_Mg_GP_1_2019_3	5.36146	Mg	1	GP	3
Rhizosphere_Mg_GP_2_2019_1	5.153365	Mg	2	GP	1
Rhizosphere_Mg_GP_2_2019_2	4.691707	Mg	2	GP	2
Rhizosphere_Mg_GP_2_2019_3	5.109432	Mg	2	GP	3

Rhizosphere_Mg_GP_3_2019_1	5.108134	Mg	3	GP	1
Rhizosphere_Mg_GP_3_2019_2	4.963668	Mg	3	GP	2
Rhizosphere_Mg_GP_3_2019_3	4.78971	Mg	3	GP	3
Rhizosphere_Mg_GP_4_2019_1	5.288696	Mg	4	GP	1
Rhizosphere_Mg_GP_4_2019_2	5.37814	Mg	4	GP	2
Rhizosphere_Mg_GP_4_2019_3	5.394811	Mg	4	GP	3
Rhizosphere_EM_GP_1_2019_1	5.265478	EM	1	GP	1
Rhizosphere_EM_GP_1_2019_2	5.019586	EM	1	GP	2
Rhizosphere_EM_GP_1_2019_3	5.245612	EM	1	GP	3
Rhizosphere_EM_GP_2_2019_1	5.275517	EM	2	GP	1
Rhizosphere_EM_GP_2_2019_2	5.24207	EM	2	GP	2
Rhizosphere_EM_GP_2_2019_3	5.602394	EM	2	GP	3
Rhizosphere_EM_GP_3_2019_1	4.503078	EM	3	GP	1
Rhizosphere_EM_GP_3_2019_2	4.283815	EM	3	GP	2
Rhizosphere_EM_GP_3_2019_3	4.682631	EM	3	GP	3
Rhizosphere_EM_GP_4_2019_1	5.502098	EM	4	GP	1
Rhizosphere_EM_GP_4_2019_2	4.886923	EM	4	GP	2
Rhizosphere_EM_GP_4_2019_3	5.347861	EM	4	GP	3
Rhizosphere_Mg_BCC436_1_2019_1	5.006226	Mg	1	BCC436	1
Rhizosphere_Mg_BCC436_1_2019_2	5.463793	Mg	1	BCC436	2
Rhizosphere_Mg_BCC436_1_2019_3	5.011165	Mg	1	BCC436	3
Rhizosphere_Mg_BCC436_2_2019_1	5.368763	Mg	2	BCC436	1
Rhizosphere_Mg_BCC436_2_2019_2	5.402947	Mg	2	BCC436	2
Rhizosphere_Mg_BCC436_2_2019_3	4.804917	Mg	2	BCC436	3
Rhizosphere_Mg_BCC436_3_2019_1	4.689426	Mg	3	BCC436	1
Rhizosphere_Mg_BCC436_3_2019_2	4.852466	Mg	3	BCC436	2
Rhizosphere_Mg_BCC436_4_2019_1	5.665806	Mg	4	BCC436	1
Rhizosphere_Mg_BCC436_4_2019_2	5.693312	Mg	4	BCC436	2
Rhizosphere_Mg_BCC436_4_2019_3	5.686732	Mg	4	BCC436	3
Rhizosphere_EM_BCC436_1_2019_1	5.527423	EM	1	BCC436	1
Rhizosphere_EM_BCC436_1_2019_2	4.665393	EM	1	BCC436	2
Rhizosphere_EM_BCC436_1_2019_3	5.120279	EM	1	BCC436	3
Rhizosphere_EM_BCC436_2_2019_1	5.389748	EM	2	BCC436	1
Rhizosphere_EM_BCC436_2_2019_2	5.139331	EM	2	BCC436	2
Rhizosphere_EM_BCC436_2_2019_3	5.2988	EM	2	BCC436	3
Rhizosphere_EM_BCC436_3_2019_1	5.008255	EM	3	BCC436	1
Rhizosphere_EM_BCC436_3_2019_2	3.918595	EM	3	BCC436	2
Rhizosphere_EM_BCC436_3_2019_3	5.048533	EM	3	BCC436	3
Rhizosphere_EM_BCC436_4_2019_1	5.473953	EM	4	BCC436	1
Rhizosphere_EM_BCC436_4_2019_2	4.944766	EM	4	BCC436	2
Rhizosphere_EM_BCC436_4_2019_3	5.859113	EM	4	BCC436	3
Rhizosphere_Mg_GPG_4_2019_1	5.883944	Mg	4	GPG	1
Rhizosphere_Mg_GPG_4_2019_2	4.98453	Mg	4	GPG	2
Rhizosphere_Mg_GPG_4_2019_3	4.606227	Mg	4	GPG	3
Rhizosphere_Mg_HOR_4_2019_1	5.509697	Mg	4	HOR7985	1
Rhizosphere_Mg_HOR_4_2019_2	5.564973	Mg	4	HOR7985	2

Rhizosphere_Mg_HOR_4_2019_3	5.492258	Mg	4	HOR7985	3
Rhizosphere_Mg_Morex_4_2019_1	5.088977	Mg	4	Morex	1
Rhizosphere_Mg_Morex_4_2019_2	5.017278	Mg	4	Morex	2
Rhizosphere_Mg_Morex_4_2019_3	4.718509	Mg	4	Morex	3
Rhizosphere_Mg_BCC768_4_2019_1	5.338409	Mg	4	BCC768	1
Rhizosphere_Mg_BCC768_4_2019_2	5.049247	Mg	4	BCC768	2
Rhizosphere_Mg_BCC768_4_2019_3	5.24155	Mg	4	BCC768	3
Rhizosphere_Mg_BCC1415_4_2019_1	5.238111	Mg	4	BCC1415	1
Rhizosphere_Mg_BCC1415_4_2019_2	5.431642	Mg	4	BCC1415	2
Rhizosphere_Mg_BCC1415_4_2019_3	5.104459	Mg	4	BCC1415	3
Rhizosphere_Mg_BCC1589_4_2019_1	4.99131	Mg	4	BCC1589	1
Rhizosphere_Mg_BCC1589_4_2019_2	5.274881	Mg	4	BCC1589	2
Rhizosphere_Mg_BCC1589_4_2019_3	5.694104	Mg	4	BCC1589	3
Rhizosphere_EM_GPG_4_2019_1	5.862831	EM	4	GPG	1
Rhizosphere_EM_GPG_4_2019_2	5.567396	EM	4	GPG	2
Rhizosphere_EM_GPG_4_2019_3	5.729312	EM	4	GPG	3
Rhizosphere_EM_HOR_4_2019_1	5.076173	EM	4	HOR7985	1
Rhizosphere_EM_HOR_4_2019_2	4.802017	EM	4	HOR7985	2
Rhizosphere_EM_HOR_4_2019_3	4.976171	EM	4	HOR7985	3
Rhizosphere_EM_Morex_4_2019_1	5.211859	EM	4	Morex	1
Rhizosphere_EM_Morex_4_2019_2	5.287964	EM	4	Morex	2
Rhizosphere_EM_Morex_4_2019_3	4.801187	EM	4	Morex	3
Rhizosphere_EM_BCC768_4_2019_1	5.114426	EM	4	BCC768	1
Rhizosphere_EM_BCC768_4_2019_2	5.230685	EM	4	BCC768	2
Rhizosphere_EM_BCC768_4_2019_3	5.345545	EM	4	BCC768	3
Rhizosphere_EM_BCC1415_4_2019_1	5.062196	EM	4	BCC1415	1
Rhizosphere_EM_BCC1415_4_2019_2	4.963608	EM	4	BCC1415	2
Rhizosphere_EM_BCC1415_4_2019_3	4.691022	EM	4	BCC1415	3
Rhizosphere_EM_BCC1589_4_2019_1	5.293123	EM	4	BCC1589	1
Rhizosphere_EM_BCC1589_4_2019_2	4.915056	EM	4	BCC1589	2
Rhizosphere_EM_BCC1589_4_2019_3	5.57875	EM	4	BCC1589	3
Bulk_soil_1_2019_1	5.36582		1		1
Bulk_soil_1_2019_2	5.188026		1		2
Bulk_soil_1_2019_3	5.469944		1		3
Bulk_soil_1_2019_4	5.082747		1		4
Bulk_soil_2_2019_1	5.337849		2		1
Bulk_soil_2_2019_2	4.913905		2		2
Bulk_soil_2_2019_3	4.740894		2		3
Bulk_soil_2_2019_4	4.683848		2		4
Bulk_soil_3_2019_1	5.078756		3		1
Bulk_soil_3_2019_2	5.349658		3		2
Bulk_soil_3_2019_3	4.973802		3		3
Bulk_soil_3_2019_4	5.3071		3		4
Bulk_soil_4_2019_1	5.147093		4		1
Bulk_soil_4_2019_2	5.35339		4		2
Bulk_soil_4_2019_3	4.96675		4		3

Bulk_soil_4_2019_4	5.525876		4		4
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Supplementary Table 4 Results of Type II Wald chi-square tests from generalized linear mixed-effects models assessing the effects of *Ensifer meliloti* Rm2011 inoculation (treatment), and barley genotypes at rhizosphere sampling time point 4 in sampling year 2019.

	Chisq	Df	Pr(>Chisq)
Treatment	0.913077	1	0.339299
Genotype	16.91196	7	0.017973
Treatment:Genotype	16.51122	7	0.020835

Supplementary Table 5 Pairwise comparisons of estimated marginal means for the effect of the *Ensifer meliloti* Rm2011 inoculation treatment compared to the control across barley genotypes at rhizosphere sampling time point 4 in 2019. SE: standard error; GP: Golden Promise Scotland; GPG: Golden Promise Gießen.

Genotype	Estimate	SE	p-value
BCC1415	-0.35246	0.223673	0.125562
BCC1589	-0.05779	0.223673	0.79789
BCC436	-0.25601	0.223673	0.261444
BCC768	0.020483	0.223673	0.927643
GP	-0.10825	0.223673	0.63191
GPG	0.561613	0.223673	0.017663
HOR7985	-0.57086	0.223673	0.016037
Morex	0.158749	0.223673	0.483348

Supplementary Table 6 Shannon diversity index of barley rhizosphere and bulk soil samples, from 2020. The samples consist of eight different genotypes and four different rhizosphere sampling (RS) time points, inoculated with either EM Rm2011 (EM) or control (Mg). GP: Golden Promise; GPG: Golden Promise Gießen.

Sample name	Shannon	Treatment	RS	Genotype	Replicate
Rhizosphere_Mg_GP_1_2020_1	6.475202	Mg	1	GP	1
Rhizosphere_Mg_GP_1_2020_2	5.574605	Mg	1	GP	2
Rhizosphere_Mg_GP_1_2020_3	6.545772	Mg	1	GP	3
Rhizosphere_Mg_GP_2_2020_1	5.801752	Mg	2	GP	1
Rhizosphere_Mg_GP_2_2020_2	6.204924	Mg	2	GP	2
Rhizosphere_Mg_GP_2_2020_3	6.539563	Mg	2	GP	3
Rhizosphere_Mg_GP_3_2020_1	6.393736	Mg	3	GP	1
Rhizosphere_Mg_GP_3_2020_2	6.41414	Mg	3	GP	2
Rhizosphere_Mg_GP_3_2020_3	6.271364	Mg	3	GP	3
Rhizosphere_Mg_GP_4_2020_1	6.36046	Mg	4	GP	1
Rhizosphere_Mg_GP_4_2020_2	6.426919	Mg	4	GP	2
Rhizosphere_EM_GP_1_2020_1	6.408809	EM	1	GP	1

Rhizosphere_EM_GP_1_2020_2	5.842273	EM	1	GP	2
Rhizosphere_EM_GP_1_2020_3	6.628141	EM	1	GP	3
Rhizosphere_EM_GP_2_2020_1	5.475274	EM	2	GP	1
Rhizosphere_EM_GP_2_2020_2	6.449744	EM	2	GP	2
Rhizosphere_EM_GP_2_2020_3	6.322628	EM	2	GP	3
Rhizosphere_EM_GP_3_2020_1	6.551968	EM	3	GP	1
Rhizosphere_EM_GP_3_2020_2	6.340559	EM	3	GP	2
Rhizosphere_EM_GP_3_2020_3	5.727272	EM	3	GP	3
Rhizosphere_EM_GP_4_2020_1	6.278228	EM	4	GP	1
Rhizosphere_EM_GP_4_2020_2	6.539624	EM	4	GP	2
Rhizosphere_EM_GP_4_2020_3	6.684124	EM	4	GP	3
Rhizosphere_Mg_BCC436_1_2020_1	6.61401	Mg	1	BCC436	1
Rhizosphere_Mg_BCC436_1_2020_2	6.359949	Mg	1	BCC436	2
Rhizosphere_Mg_BCC436_1_2020_3	6.163868	Mg	1	BCC436	3
Rhizosphere_Mg_BCC436_2_2020_1	6.308707	Mg	2	BCC436	1
Rhizosphere_Mg_BCC436_2_2020_2	6.130564	Mg	2	BCC436	2
Rhizosphere_Mg_BCC436_2_2020_3	6.525657	Mg	2	BCC436	3
Rhizosphere_Mg_BCC436_3_2020_1	6.821272	Mg	3	BCC436	1
Rhizosphere_Mg_BCC436_3_2020_2	6.643266	Mg	3	BCC436	2
Rhizosphere_Mg_BCC436_3_2020_3	6.577356	Mg	3	BCC436	3
Rhizosphere_Mg_BCC436_4_2020_1	6.60091	Mg	4	BCC436	1
Rhizosphere_Mg_BCC436_4_2020_2	6.583656	Mg	4	BCC436	2
Rhizosphere_Mg_BCC436_4_2020_3	5.773918	Mg	4	BCC436	3
Rhizosphere_EM_BCC436_1_2020_1	6.238184	EM	1	BCC436	1
Rhizosphere_EM_BCC436_1_2020_2	6.404684	EM	1	BCC436	2
Rhizosphere_EM_BCC436_1_2020_3	6.249593	EM	1	BCC436	3
Rhizosphere_EM_BCC436_2_2020_1	6.648828	EM	2	BCC436	1
Rhizosphere_EM_BCC436_2_2020_2	6.214127	EM	2	BCC436	2
Rhizosphere_EM_BCC436_2_2020_3	5.932283	EM	2	BCC436	3
Rhizosphere_EM_BCC436_3_2020_1	6.204894	EM	3	BCC436	1
Rhizosphere_EM_BCC436_3_2020_2	6.505246	EM	3	BCC436	2
Rhizosphere_EM_BCC436_3_2020_3	6.436064	EM	3	BCC436	3
Rhizosphere_EM_BCC436_4_2020_1	5.784701	EM	4	BCC436	1
Rhizosphere_EM_BCC436_4_2020_2	4.633055	EM	4	BCC436	2
Rhizosphere_EM_BCC436_4_2020_3	4.319894	EM	4	BCC436	3
Rhizosphere_Mg_GPG_4_2020_1	4.688208	Mg	4	GPG	1
Rhizosphere_Mg_GPG_4_2020_3	4.307139	Mg	4	GPG	3
Rhizosphere_Mg_HOR_4_2020_1	6.7349	Mg	4	HOR7985	1
Rhizosphere_Mg_HOR_4_2020_2	6.555787	Mg	4	HOR7985	2
Rhizosphere_Mg_HOR_4_2020_3	6.812408	Mg	4	HOR7985	3
Rhizosphere_Mg_Morex_4_2020_1	6.427215	Mg	4	Morex	1
Rhizosphere_Mg_Morex_4_2020_2	6.17311	Mg	4	Morex	2
Rhizosphere_Mg_Morex_4_2020_3	6.49898	Mg	4	Morex	3
Rhizosphere_Mg_BCC768_4_2020_1	6.585318	Mg	4	BCC768	1
Rhizosphere_Mg_BCC768_4_2020_2	6.481981	Mg	4	BCC768	2
Rhizosphere_Mg_BCC768_4_2020_3	6.869118	Mg	4	BCC768	3

Rhizosphere_Mg_BCC1415_4_2020_1	6.713606	Mg	4	BCC1415	1
Rhizosphere_Mg_BCC1415_4_2020_2	6.782237	Mg	4	BCC1415	2
Rhizosphere_Mg_BCC1415_4_2020_3	6.554714	Mg	4	BCC1415	3
Rhizosphere_Mg_BCC1589_4_2020_1	6.807948	Mg	4	BCC1589	1
Rhizosphere_Mg_BCC1589_4_2020_2	6.529979	Mg	4	BCC1589	2
Rhizosphere_Mg_BCC1589_4_2020_3	6.80414	Mg	4	BCC1589	3
Rhizosphere_EM_GPG_4_2020_1	4.863454	EM	4	GPG	1
Rhizosphere_EM_GPG_4_2020_2	6.386956	EM	4	GPG	2
Rhizosphere_EM_GPG_4_2020_3	6.456167	EM	4	GPG	3
Rhizosphere_EM_HOR_4_2020_1	6.578144	EM	4	HOR7985	1
Rhizosphere_EM_HOR_4_2020_2	6.695819	EM	4	HOR7985	2
Rhizosphere_EM_HOR_4_2020_3	6.507179	EM	4	HOR7985	3
Rhizosphere_EM_Morex_4_2020_1	6.633339	EM	4	Morex	1
Rhizosphere_EM_Morex_4_2020_2	6.520434	EM	4	Morex	2
Rhizosphere_EM_Morex_4_2020_3	6.575086	EM	4	Morex	3
Rhizosphere_EM_BCC768_4_2020_1	6.577573	EM	4	BCC768	1
Rhizosphere_EM_BCC768_4_2020_2	7.046296	EM	4	BCC768	2
Rhizosphere_EM_BCC768_4_2020_3	6.470531	EM	4	BCC768	3
Rhizosphere_EM_BCC1415_4_2020_1	6.518306	EM	4	BCC1415	1
Rhizosphere_EM_BCC1415_4_2020_2	7.036911	EM	4	BCC1415	2
Rhizosphere_EM_BCC1415_4_2020_3	6.888922	EM	4	BCC1415	3
Rhizosphere_EM_BCC1589_4_2020_1	6.815206	EM	4	BCC1589	1
Rhizosphere_EM_BCC1589_4_2020_2	6.703883	EM	4	BCC1589	2
Rhizosphere_EM_BCC1589_4_2020_3	6.057109	EM	4	BCC1589	3
Bulk_soil_1_2020_1	6.727169		1		1
Bulk_soil_1_2020_2	6.332969		1		2
Bulk_soil_1_2020_3	6.39102		1		3
Bulk_soil_1_2020_4	4.658729		1		4
Bulk_soil_2_2020_1	6.167122		2		1
Bulk_soil_2_2020_2	6.275508		2		2
Bulk_soil_2_2020_3	6.091558		2		3
Bulk_soil_3_2020_1	6.638418		3		1
Bulk_soil_3_2020_2	6.5824		3		2
Bulk_soil_3_2020_3	6.567088		3		3
Bulk_soil_3_2020_4	6.459965		3		4
Bulk_soil_4_2020_1	7.045151		4		1
Bulk_soil_4_2020_2	6.805706		4		2
Bulk_soil_4_2020_3	6.63061		4		3
Bulk_soil_4_2020_4	6.757721		4		4

Supplementary Table 7 Results of Type II Wald chi-square tests from generalized linear mixed-effects models assessing the effects of *Ensifer meliloti* Rm2011 inoculation (treatment), and barley genotypes at rhizosphere sampling time point 4 in sampling year 2020.

	Chisq	Df	Pr(>Chisq)
Treatment	0.037453206	1	0.846545238

Genotype	30.25794076	7	8.52E-05
Treatment:Genotype	15.4258974	7	0.030911468

Supplementary Table 8 Pairwise comparisons of estimated marginal means for the effect of the *Ensifer meliloti* Rm2011 inoculation treatment compared to the control across barley genotypes at rhizosphere sampling time point 4 in 2020. SE: standard error; GP: Golden Promise Scotland; GPG: Golden Promise Gießen.

Genotype	Estimate	SE	p-value
BCC1415	-0.00295	0.003425	0.388969
BCC1589	0.004715	0.003498	0.177787
BCC436	0.015069	0.005301	0.004472
BCC768	-0.00143	0.003471	0.679341
GP	-0.0034	0.004055	0.401206
GPG	-0.01251	0.006705	0.062057
HOR7985	0.002079	0.003479	0.550206
Morex	-0.00558	0.003582	0.119285

Supplementary Table 9 Shannon diversity index of barley rhizosphere and bulk soil samples, from 2021. The samples consist of eight different genotypes and four different rhizosphere sampling (RS) time points, inoculated with either EM Rm2011 (EM) or control (Mg). GP: Golden Promise; GPG: Golden Promise Gießen.

Sample name	Shannon	Treatment	RS	Genotype	Replicate
Rhizosphere_Mg_GP_1_2021_1	6.788624	Mg	1	GP	1
Rhizosphere_Mg_GP_1_2021_2	6.687987	Mg	1	GP	2
Rhizosphere_Mg_GP_1_2021_3	6.722876	Mg	1	GP	3
Rhizosphere_Mg_GP_2_2021_1	6.9589	Mg	2	GP	1
Rhizosphere_Mg_GP_2_2021_2	6.82243	Mg	2	GP	2
Rhizosphere_Mg_GP_2_2021_3	6.858202	Mg	2	GP	3
Rhizosphere_Mg_GP_3_2021_1	7.072576	Mg	3	GP	1
Rhizosphere_Mg_GP_3_2021_2	7.38297	Mg	3	GP	2
Rhizosphere_Mg_GP_3_2021_3	7.028238	Mg	3	GP	3
Rhizosphere_Mg_GP_4_2021_1	6.380642	Mg	4	GP	1
Rhizosphere_Mg_GP_4_2021_2	6.850761	Mg	4	GP	2
Rhizosphere_Mg_GP_4_2021_3	5.5782	Mg	4	GP	3
Rhizosphere_EM_GP_1_2021_1	6.507442	EM	1	GP	1
Rhizosphere_EM_GP_1_2021_2	6.933333	EM	1	GP	2
Rhizosphere_EM_GP_1_2021_3	6.578825	EM	1	GP	3
Rhizosphere_EM_GP_2_2021_1	6.457189	EM	2	GP	1
Rhizosphere_EM_GP_2_2021_2	6.647077	EM	2	GP	2
Rhizosphere_EM_GP_2_2021_3	6.344169	EM	2	GP	3
Rhizosphere_EM_GP_3_2021_1	6.963459	EM	3	GP	1
Rhizosphere_EM_GP_3_2021_2	7.11246	EM	3	GP	2
Rhizosphere_EM_GP_3_2021_3	6.889015	EM	3	GP	3
Rhizosphere_EM_GP_4_2021_1	5.44333	EM	4	GP	1

Rhizosphere_EM_GP_4_2021_2	5.794693	EM	4	GP	2
Rhizosphere_EM_GP_4_2021_3	5.407447	EM	4	GP	3
Rhizosphere_Mg_BCC436_1_2021_1	6.87713	Mg	1	BCC436	1
Rhizosphere_Mg_BCC436_1_2021_2	6.553911	Mg	1	BCC436	2
Rhizosphere_Mg_BCC436_1_2021_3	7.020685	Mg	1	BCC436	3
Rhizosphere_Mg_BCC436_2_2021_1	6.373	Mg	2	BCC436	1
Rhizosphere_Mg_BCC436_2_2021_2	6.547216	Mg	2	BCC436	2
Rhizosphere_Mg_BCC436_2_2021_3	7.033622	Mg	2	BCC436	3
Rhizosphere_Mg_BCC436_3_2021_1	6.961358	Mg	3	BCC436	1
Rhizosphere_Mg_BCC436_3_2021_2	6.776172	Mg	3	BCC436	2
Rhizosphere_Mg_BCC436_3_2021_3	6.758155	Mg	3	BCC436	3
Rhizosphere_Mg_BCC436_4_2021_1	5.468308	Mg	4	BCC436	1
Rhizosphere_Mg_BCC436_4_2021_2	5.630984	Mg	4	BCC436	2
Rhizosphere_Mg_BCC436_4_2021_3	5.590707	Mg	4	BCC436	3
Rhizosphere_EM_BCC436_1_2021_1	7.00261	EM	1	BCC436	1
Rhizosphere_EM_BCC436_1_2021_2	6.82817	EM	1	BCC436	2
Rhizosphere_EM_BCC436_1_2021_3	6.974982	EM	1	BCC436	3
Rhizosphere_EM_BCC436_2_2021_1	6.698604	EM	2	BCC436	1
Rhizosphere_EM_BCC436_2_2021_2	6.963128	EM	2	BCC436	2
Rhizosphere_EM_BCC436_2_2021_3	7.065985	EM	2	BCC436	3
Rhizosphere_EM_BCC436_3_2021_1	7.131649	EM	3	BCC436	1
Rhizosphere_EM_BCC436_3_2021_2	7.264587	EM	3	BCC436	2
Rhizosphere_EM_BCC436_3_2021_3	7.123703	EM	3	BCC436	3
Rhizosphere_EM_BCC436_4_2021_1	5.392263	EM	4	BCC436	1
Rhizosphere_EM_BCC436_4_2021_2	6.426034	EM	4	BCC436	2
Rhizosphere_EM_BCC436_4_2021_3	6.393879	EM	4	BCC436	3
Rhizosphere_Mg_GPG_4_2021_1	6.797863	Mg	4	GPG	1
Rhizosphere_Mg_GPG_4_2021_2	6.550755	Mg	4	GPG	2
Rhizosphere_Mg_GPG_4_2021_3	5.622832	Mg	4	GPG	3
Rhizosphere_Mg_HOR_4_2021_1	6.244519	Mg	4	HOR7985	1
Rhizosphere_Mg_HOR_4_2021_2	7.148211	Mg	4	HOR7985	2
Rhizosphere_Mg_HOR_4_2021_3	6.686	Mg	4	HOR7985	3
Rhizosphere_Mg_Morex_4_2021_1	6.17237	Mg	4	Morex	1
Rhizosphere_Mg_Morex_4_2021_2	6.260999	Mg	4	Morex	2
Rhizosphere_Mg_Morex_4_2021_3	6.275155	Mg	4	Morex	3
Rhizosphere_Mg_BCC768_4_2021_1	6.868477	Mg	4	BCC768	1
Rhizosphere_Mg_BCC768_4_2021_2	6.863384	Mg	4	BCC768	2
Rhizosphere_Mg_BCC768_4_2021_3	6.6938	Mg	4	BCC768	3
Rhizosphere_Mg_BCC1415_4_2021_1	6.216564	Mg	4	BCC1415	1
Rhizosphere_Mg_BCC1415_4_2021_2	5.506762	Mg	4	BCC1415	2
Rhizosphere_Mg_BCC1415_4_2021_3	5.926063	Mg	4	BCC1415	3
Rhizosphere_Mg_BCC1589_4_2021_1	5.886486	Mg	4	BCC1589	1
Rhizosphere_Mg_BCC1589_4_2021_2	5.394429	Mg	4	BCC1589	2
Rhizosphere_Mg_BCC1589_4_2021_3	5.977836	Mg	4	BCC1589	3
Rhizosphere_EM_GPG_4_2021_1	6.421957	EM	4	GPG	1
Rhizosphere_EM_GPG_4_2021_2	5.826762	EM	4	GPG	2

Rhizosphere_EM_GPG_4_2021_3	6.329928	EM	4	GPG	3
Rhizosphere_EM_HOR_4_2021_1	6.17658	EM	4	HOR7985	1
Rhizosphere_EM_HOR_4_2021_2	6.867163	EM	4	HOR7985	2
Rhizosphere_EM_HOR_4_2021_3	6.610028	EM	4	HOR7985	3
Rhizosphere_EM_Morex_4_2021_1	6.225248	EM	4	Morex	1
Rhizosphere_EM_Morex_4_2021_2	7.000872	EM	4	Morex	2
Rhizosphere_EM_Morex_4_2021_3	6.898115	EM	4	Morex	3
Rhizosphere_EM_BCC768_4_2021_1	6.7406	EM	4	BCC768	1
Rhizosphere_EM_BCC768_4_2021_2	4.85873	EM	4	BCC768	2
Rhizosphere_EM_BCC768_4_2021_3	5.274442	EM	4	BCC768	3
Rhizosphere_EM_BCC1415_4_2021_1	6.308601	EM	4	BCC1415	1
Rhizosphere_EM_BCC1415_4_2021_2	5.800651	EM	4	BCC1415	2
Rhizosphere_EM_BCC1415_4_2021_3	4.932916	EM	4	BCC1415	3
Rhizosphere_EM_BCC1589_4_2021_1	5.752933	EM	4	BCC1589	1
Rhizosphere_EM_BCC1589_4_2021_2	5.75337	EM	4	BCC1589	2
Rhizosphere_EM_BCC1589_4_2021_3	5.594934	EM	4	BCC1589	3
Bulk_soil_1_2021_1	7.051135		1		1
Bulk_soil_1_2021_2	6.814906		1		2
Bulk_soil_1_2021_3	6.833095		1		3
Bulk_soil_1_2021_4	6.854063		1		4
Bulk_soil_2_2021_1	6.907152		2		1
Bulk_soil_2_2021_2	6.982875		2		2
Bulk_soil_2_2021_3	7.006931		2		3
Bulk_soil_2_2021_4	6.88693		2		4
Bulk_soil_3_2021_1	6.984511		3		1
Bulk_soil_3_2021_2	6.957997		3		2
Bulk_soil_3_2021_3	7.041769		3		3
Bulk_soil_3_2021_4	6.819944		3		4
Bulk_soil_4_2021_1	5.814121		4		1
Bulk_soil_4_2021_2	5.709804		4		2
Bulk_soil_4_2021_3	5.505718		4		3
Bulk_soil_4_2021_4	5.367349		4		4

Supplementary Table 10 Results of Type II Wald chi-square tests from generalized linear mixed-effects models assessing the effects of *Ensifer meliloti* Rm2011 inoculation (treatment), and barley genotypes at rhizosphere sampling time point 4 in sampling year 2021.

	Chisq	Df	Pr(>Chisq)
Treatment	1.805378465	1	0.179063622
Genotype	22.05966421	7	0.002480936
Treatment:Genotype	15.16995932	7	0.033881451

Supplementary Table 11 Pairwise comparisons of estimated marginal means for the effect of the *Ensifer meliloti* Rm2011 inoculation treatment compared to the control across barley genotypes at rhizosphere sampling time point 4 in 2021. SE: standard error; GP: Golden Promise Scotland; GPG: Golden Promise Gießen.

Genotype	Estimate	SE	p-value
BCC1415	-0.20241	0.382471	0.600581
BCC1589	-0.05251	0.382471	0.891734
BCC436	0.507392	0.382471	0.194713
BCC768	-1.18396	0.385597	0.004357
GP	-0.72138	0.382471	0.069063
GPG	-0.13093	0.382471	0.734499
HOR7985	-0.14165	0.382471	0.713732
Morex	0.471903	0.382471	0.226911

Supplementary Table 12 PERMANOVA of barley rhizosphere samples, from three consecutive years (2019, 2020, 2021) of eight different genotypes at rhizosphere sampling 4 (all genotypes), and inoculated with either EM Rm2011 or control (ctrl).

Genotype - 2019	0.12	0.001
Genotype - 2020	0.1	0.001
Genotype - 2021	0.12	0.001
RS4 - genotype - 2019	0.24	0.001
RS4 - genotype - 2020	0.19	0.003
RS4 - genotype - 2021	0.23	0.001
RS4 - inoculation - 2019	0.04	0.008
RS4 - inoculation - 2020	0.02	0.742
RS4 - inoculation - 2021	0.02	0.423
RS4 - genotype - Ctrl - 2019	0.4	0.001
RS4 - genotype - Ctrl - 2020	0.4	0.003
RS4 - genotype - Ctrl - 2021	0.43	0.001

Supplementary Table 13 Results of Type II Wald chi-square tests from generalized linear mixed-effects models assessing the effects of *Ensifer meliloti* Rm2011 inoculation (treatment), and barley genotypes across the four rhizosphere sampling time points (timepoint) in sampling year 2019.

	Chisq	Df	Pr(>Chisq)
Treatment	1.276513968	1	0.258548212
Genotype	0.21838237	1	0.640275245
Timepoint	34.47909663	3	1.57E-07
Treatment:Genotype	0.003400179	1	0.953500864
Treatment:Timepoint	5.60927217	3	0.132247065

Genotype:Timepoint	3.095126411	3	0.377189828
Treatment:Genotype:Timepoint	2.165872221	3	0.538701723

Supplementary Table 14 Pairwise comparisons of estimated marginal means for the effect of the *Ensifer meliloti* Rm2011 inoculation treatment compared to the control across the barley genotypes Golden Promise and BCC436 at four rhizosphere timepoints during the vegetation period in 2019. SE: standard error; GP: Golden Promise Scotland.

Genotype	Timepoint	estimate	SE	p.value
BCC436	1	-0.05603	0.241372	0.818067
GP	1	-0.24882	0.241372	0.311137
BCC436	2	0.083751	0.241372	0.731113
GP	2	0.388492	0.241372	0.118336
BCC436	3	-0.12647	0.272631	0.646148
GP	3	-0.464	0.241372	0.064437
BCC436	4	-0.25601	0.241372	0.297612
GP	4	-0.10825	0.241372	0.657127

Supplementary Table 15 Results of Type II Wald chi-square tests from generalized linear mixed-effects models assessing the effects of *Ensifer meliloti* Rm2011 inoculation (treatment), and barley genotypes across the four rhizosphere sampling time points (timepoint) in sampling year 2020.

	Chisq	Df	Pr(>Chisq)
Treatment	1.05082959	1	0.305316102
Genotype	0.581267737	1	0.445815837
Timepoint	0.484830624	1	0.486242352
Treatment:Genotype	0.89371825	1	0.344471683
Treatment:Timepoint	0.359588557	1	0.548734829
Genotype:Timepoint	1.659907538	1	0.197615808
Treatment:Genotype:Timepoint	0.397472784	1	0.528397311

Supplementary Table 16 Pairwise comparisons of estimated marginal means for the effect of the *Ensifer meliloti* Rm2011 inoculation treatment compared to the control across the barley genotypes Golden Promise and BCC436 at four rhizosphere timepoints during the vegetation period in 2019. SE: standard error; GP: Golden Promise Scotland.

Genotype	Timepoint	estimate	SE	p.value
BCC436	1	0.002016	0.005584	0.718035

GP	1	-0.00235	0.005674	0.678959
BCC436	2	0.001503	0.00563	0.789494
GP	2	0.002505	0.005776	0.664565
BCC436	3	0.006862	0.005426	0.20598
GP	3	0.004036	0.005641	0.47431
BCC436	4	0.017591	0.008889	0.047818
GP	4	-0.00264	0.006199	0.669974

Supplementary Table 17 Results of Type II Wald chi-square tests from generalized linear mixed-effects models assessing the effects of *Ensifer meliloti* Rm2011 inoculation (treatment), and barley genotypes across the four rhizosphere sampling time points (timepoint) in sampling year 2021.

	Chisq	Df	Pr(>Chisq)
Treatment	1.276513968	1	0.258548212
Genotype	0.21838237	1	0.640275245
Timepoint	34.47909663	3	1.57E-07
Treatment:Genotype	0.003400179	1	0.953500864
Treatment:Timepoint	5.60927217	3	0.132247065
Genotype:Timepoint	3.095126411	3	0.377189828
Treatment:Genotype:Timepoint	2.165872221	3	0.538701723

Supplementary Table 18 Pairwise comparisons of estimated marginal means for the effect of the *Ensifer meliloti* Rm2011 inoculation treatment compared to the control across the barley genotypes Golden Promise and BCC436 at four rhizosphere timepoints during the vegetation period in 2021. SE: standard error; GP: Golden Promise Scotland.

Genotype	Timepoint	estimate	SE	p.value
BCC436	1	-0.00249	0.004074	0.540551
GP	1	0.001334	0.004186	0.749985
BCC436	2	-0.00562	0.004142	0.175159
GP	2	0.008902	0.004208	0.034381
BCC436	3	-0.00697	0.004012	0.082467
GP	3	0.003455	0.003969	0.384095
BCC436	4	-0.01502	0.00484	0.001911
GP	4	0.020734	0.004778	1.42810566240099e-05

Supplementary Table 19 PERMANOVA of barley rhizosphere samples, from three consecutive years (2019, 2020, 2021) sampled as time series (four different rhizosphere sampling (RS) time points for two genotypes Golden Promise and BCC436), and inoculated with either EM Rm2011 or control (ctrl).

RS time point - 2019	0.21	0.001
RS time point - 2020	0.14	0.001
RS time point - 2021	0.16	0.001
Inoculation - 2019	0.02	0.02
Inoculation - 2020	0.01	0.108
Inoculation - 2021	0.01	0.24
Time series - RS time point - 2019	0.3	0.001
Time series - inoculation - 2019	0.03	0.013
Time series - genotypes - 2019	0.02	0.184
Time series - RS time point - 2020	0.16	0.001
Time series - inoculation - 2020	0.03	0.026
Time series - genotypes - 2020	0.03	0.071
Time series - RS time point - 2021	0.22	0.001
Time series - inoculation - 2021	0.02	0.218
Time series - genotypes - 2021	0.02	0.379
Golden Promise - sampling year	0.26	0.001
Golden Promise - RS time point	0.09	0.001
Golden Promise - inoculation	0.01	0.057
Golden Promise- RS time point - 2019	0.36	0.001
Golden Promise- RS time point - 2020	0.21	0.001
Golden Promise- RS time point - 2021	0.29	0.001
Golden Promise- inoculation - 2019	0.08	0.009
Golden Promise- inoculation - 2020	0.04	0.561
Golden Promise- inoculation - 2021	0.05	0.057
BCC436 - sampling year	0.27	0.001
BCC436 - RS time point	0.1	0.001
BCC436 - inoculation	0.02	0.04
BCC436 - RS time point - 2019	0.35	0.001
BCC436 - RS time point - 2020	0.25	0.001

BCC436 - RS time point - 2021	0.26	0.001
BCC436 - inoculation - 2019	0.05	0.054
BCC436 - inoculation - 2020	0.08	0.002
BCC436 - inoculation - 2021	0.05	0.07