New Phytologist

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Article title: Provenance Legacies Override Species Effects in Shaping Oak Rhizosphere Microbiomes and Metabolomes

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The following Supporting Information is available for this article:

Fig. S1: Illustration of the oak (*Quercus spp*.) rhizosphere sampling procedure. The photograph shows the extraction of the soil and root samples taken in February 2023.

Fig. S2: Rarefaction curves for 16S and ITS sequencings from oak (*Quercus spp*.) rhizosphere samples. The plots show rarefaction curves drawn from the unnormalized abundance table resulting from the DADA2 pipeline for (a) 16S sequencing and (b) Internal Transcribed Spacer (ITS) sequencing. Abbreviations: ASV, Amplicon Sequence Variant.

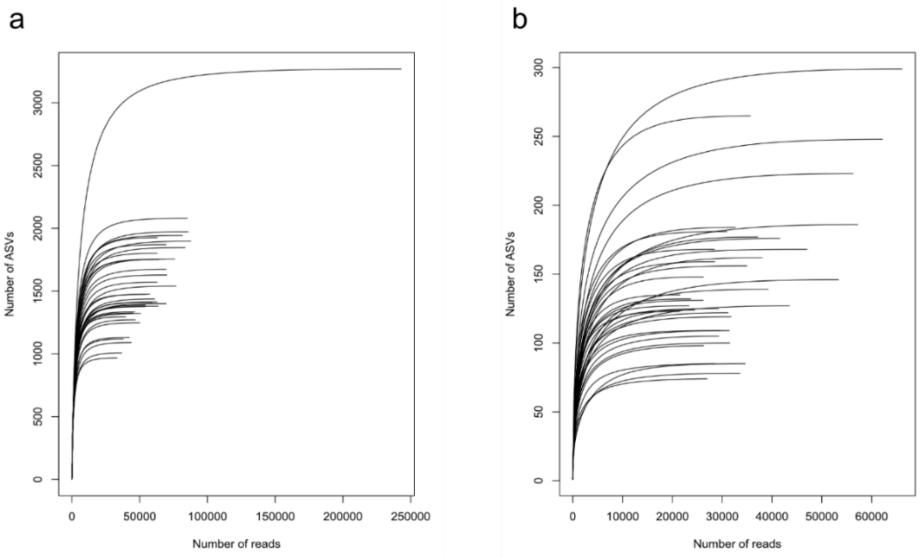

Fig. S3: Bacterial and fungal community composition of oak (*Quercus spp.*) rhizosphere samples at phylum and family level. Shown are the relative abundance of the most prominent prokaryotic phyla (a), bacterial families (b), fungal phyla (c), and fungal families (d) across species and origins (upper Rhine basin, URB; north German lowland, NGL).

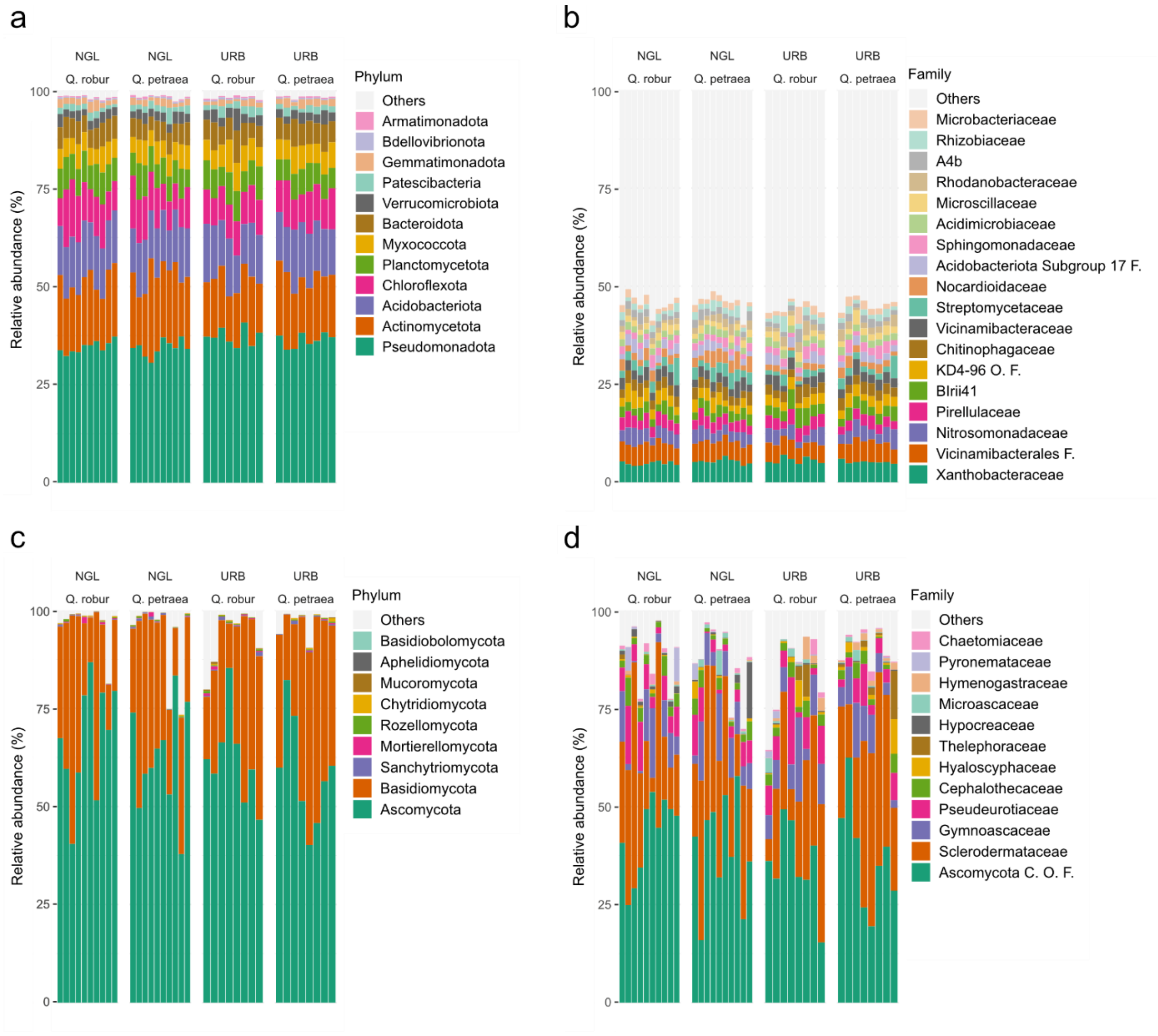

Fig. S4: Fungal guild classification of Internal Transcribed Spacer (ITS) reads from oak (*Quercus spp.*) rhizosphere samples. The bar plots display the relative abundance of fungal guilds split by origin site (URB; NGL) and tree species. Classification was performed using FunGuild. Guilds with a total read amount of less than 1000 were excluded from the visualization. The guild assignment with the highest likelihood was chosen for each Amplicon Sequence Variant (ASV). Abbreviations: URB, upper Rhine basin; NGL, north German lowland.

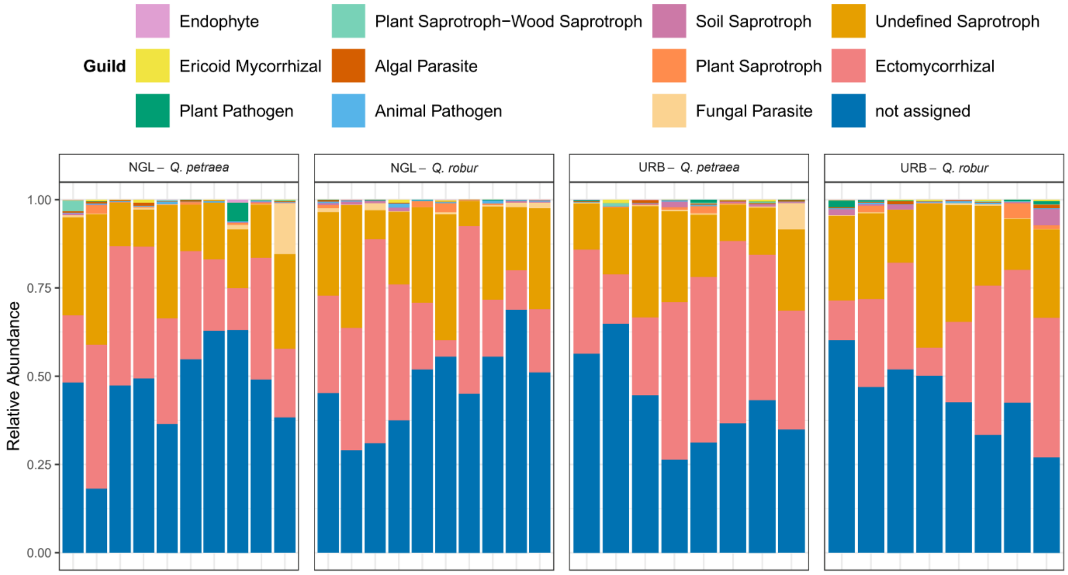

Fig. S5: Abundance of key metabolites identified from oak (*Quercus spp*.) rhizosphere samples. The plot shows metabolites identified through Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) based on specific thresholds (VIP > 1, log-FC > 1, p-value ⩽ 0.05). Metabolite data were log10-transformed and Pareto-scaled. Abbreviations: VIP, Variable Importance in Projection; log-FC, log-fold change.

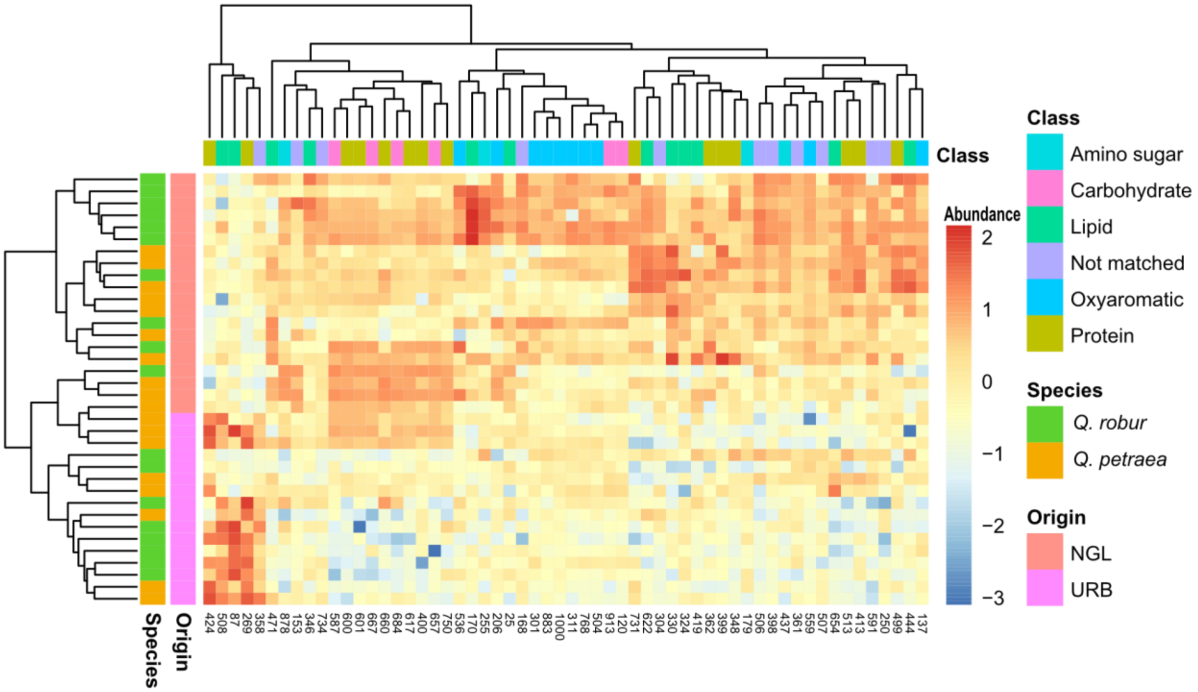

Fig. S6: Spearman correlation of microbial Shannon diversity and intrinsic water use efficiency (iWUE) from oak (*Quercus spp.*) rhizosphere samples. The plots show the relationship across different origins (URB; NGL) and sites. The Spearman rank correlation coefficient (ρ) and exact p-values are indicated within the plots. Solid lines indicate statistically significant correlations (p ≤ 0.05), while dotted lines represent non-significant trends. Abbreviations: URB, upper Rhine basin; NGL, north German lowland.

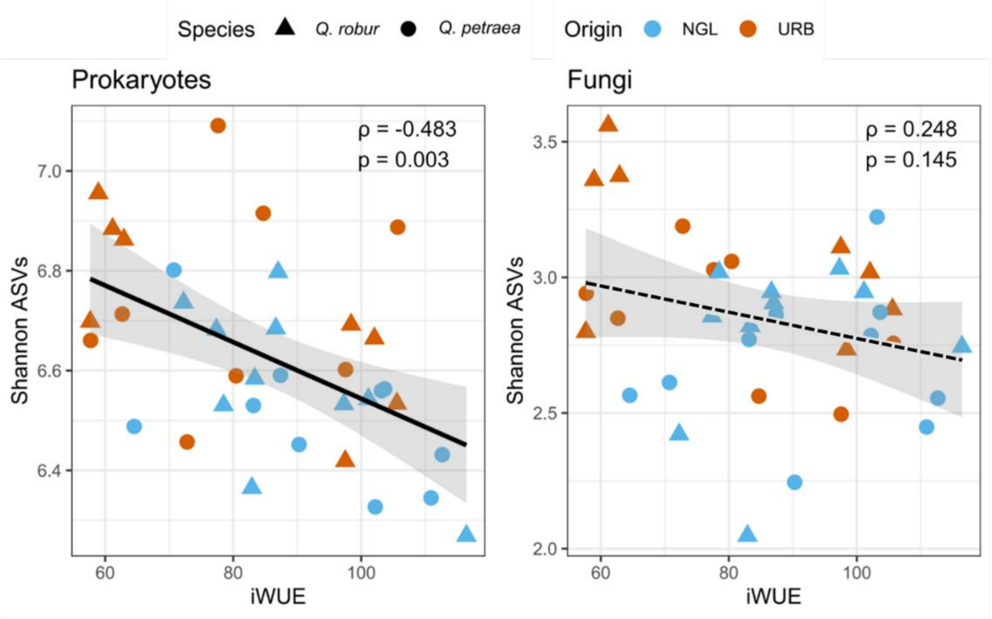

Fig. S7: Spearman correlation of metabolite Shannon diversity with prokaryotic and fungal Shannon diversity indices from oak (*Quercus spp*.) rhizosphere samples. The panels display the correlation for the prokaryotic microbiome (left) and the fungal microbiome (right). The Spearman rank correlation coefficient (rho) and exact p-values denoted in the plots refer to whole dataset tests. The correlation of prokaryotic microbiome and metabolome diversity was also significant for the upper Rhine basin (URB) data subset (rho = -0.57, p = 0.0249). Solid lines indicate statistically significant results (p ≤ 0.05), while dotted lines represent non-significant trends.

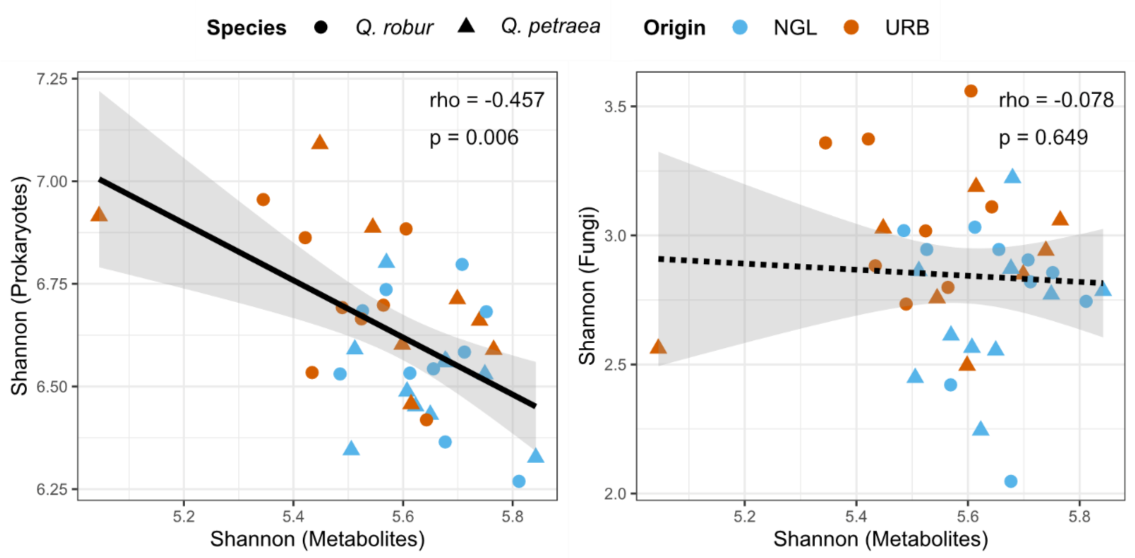

Table S1: Origin climatic information for upper Rhine basin (URB) and north German lowlands (NGL). Only environmental parameters relevant to drought tolerance in *Q. robur* and *Q. petraea* are shown (Nosenko et al., 2025).

	URB	**NGL**
Latitude of the population origin site	49,014	54,144
Longitude of the population origin site	8,156	13,072
ID the German Weather Service (DWD) station nearest to the population origin site	4177	6199
Water holding capacity of soil in up to 1 m of effective rooting depth of vegetation period (mm)	307,15	254,74
Soil water available for plants in the effective rooting depth in the vegetation period (mm)	170,13	137,24
Mean annual precipitation (mm)	803	657
Mean annual temperature (°C)	11,2	8,9
Mean precipitation of vegetation period (mm)	485	413
Mean temperature of vegetation period (°C)	16,9	14
Mean precipitation of the driest month in Germany (mm)	49	36

Table S2: Soil properties at the common garden for growing *Quercus spp*. (standard container substrate).

Characteristic	**per m^3 / value**
white peat	60 %
mixed peat	20 %
wood fiber	15 %
cocopeat	5 %
wet clay	75 kg
PG mix 14-16-18 (Yara, Brunsbüttel, Germany)	1.2 kg
Radigen micronutrient slow-release fertilizer	50 g
soil surfactant	500 ml
pH target value	5.8
Structure	coarse

Table S3: Leaf carbon isotope ratios ($\delta^{13}C$) and calculated intrinsic water-use efficiency (iWUE) for the used *Quercus spp*. trees, split by origin (*upper Rhine basin (URB) and north German lowlands (NGL)) and species.*

treeID	Origin	Species	Fraction	d13C	iWUE
2_01	NGL	*Quercus robur*	solid	-27.15	90.08
2_01	NGL	*Quercus robur*	soluble	-27.44	86.66
2_03	NGL	*Quercus petraea*	solid	-25.9	104.99
2_03	NGL	*Quercus petraea*	soluble	-26.01	103.66
2_04	NGL	*Quercus petraea*	solid	-26.52	97.57
2_04	NGL	*Quercus petraea*	soluble	-26.05	103.16
2_07	NGL	*Quercus robur*	solid	-25.33	111.72
2_07	NGL	*Quercus robur*	soluble	-24.93	116.45
2_09	NGL	*Quercus petraea*	solid	-26.32	99.96
2_09	NGL	*Quercus petraea*	soluble	-26.13	102.21
2_101	NGL	*Quercus petraea*	solid	-28.91	69.02
2_101	NGL	*Quercus petraea*	soluble	-29.29	64.53
2_19	NGL	*Quercus petraea*	solid	-29.09	66.92
2_19	NGL	*Quercus petraea*	soluble	-28.77	70.75
2_20	NGL	*Quercus robur*	solid	-27.2	89.44
2_20	NGL	*Quercus robur*	soluble	-27.41	87.03
2_25	NGL	*Quercus robur*	solid	-29.08	67.01
2_25	NGL	*Quercus robur*	soluble	-28.65	72.24
2_28	NGL	*Quercus robur*	solid	-28.03	79.59
2_28	NGL	*Quercus robur*	soluble	-28.12	78.49
2_32	NGL	*Quercus robur*	solid	-28.12	78.54
2_32	NGL	*Quercus robur*	soluble	-27.71	83.37
2_33	NGL	*Quercus robur*	solid	-28.82	70.12
2_33	NGL	*Quercus robur*	soluble	-28.21	77.38
2_35	NGL	*Quercus robur*	solid	-27.27	88.69
2_35	NGL	*Quercus robur*	soluble	-26.54	97.29
2_44	NGL	*Quercus robur*	solid	-28.36	75.6
2_44	NGL	*Quercus robur*	soluble	-27.75	82.92
2_57	NGL	*Quercus petraea*	solid	-28.9	69.25
2_57	NGL	*Quercus petraea*	soluble	-27.73	83.16
2_63	NGL	*Quercus robur*	solid	-26.93	92.65
2_63	NGL	*Quercus robur*	soluble	-26.22	101.13
2_70	NGL	*Quercus petraea*	solid	-28.41	75

2_70	NGL	*Quercus petraea*	soluble	-27.13	90.3
2_81	NGL	*Quercus petraea*	solid	-25.84	105.64
2_81	NGL	*Quercus petraea*	soluble	-25.4	110.92
2_92	NGL	*Quercus petraea*	solid	-25.83	105.73
2_92	NGL	*Quercus petraea*	soluble	-25.25	112.66
2_93	NGL	*Quercus petraea*	solid	-28.49	74.11
2_93	NGL	*Quercus petraea*	soluble	-27.38	87.39
7_08	URB	*Quercus robur*	solid	-29.53	61.7
7_08	URB	*Quercus robur*	soluble	-29.86	57.66
7_09	URB	*Quercus petraea*	solid	-29.03	67.64
7_09	URB	*Quercus petraea*	soluble	-28.6	72.78
7_12	URB	*Quercus petraea*	solid	-27.19	89.6
7_12	URB	*Quercus petraea*	soluble	-26.52	97.57
7_28	URB	*Quercus robur*	solid	-29.78	58.69
7_28	URB	*Quercus robur*	soluble	-29.76	58.93
7_31	URB	*Quercus petraea*	soluble	-29.45	62.62
7_31	URB	*Quercus petraea*	solid	-29.82	58.18
7_37	URB	*Quercus petraea*	solid	-28.44	74.69
7_37	URB	*Quercus petraea*	soluble	-27.96	80.42
7_40	URB	*Quercus petraea*	solid	-29.41	63.1
7_40	URB	*Quercus petraea*	soluble	-29.86	57.71
7_48	URB	*Quercus robur*	solid	-29.71	59.5
7_48	URB	*Quercus robur*	soluble	-29.57	61.16
7_51	URB	*Quercus robur*	solid	-26.62	96.44
7_51	URB	*Quercus robur*	soluble	-26.45	98.41
7_52	URB	*Quercus robur*	solid	-26.49	97.92
7_52	URB	*Quercus robur*	soluble	-25.85	105.58
7_53	URB	*Quercus petraea*	solid	-28.31	76.24
7_53	URB	*Quercus petraea*	soluble	-28.19	77.67
7_64	URB	*Quercus robur*	solid	-26.85	93.59
7_64	URB	*Quercus robur*	soluble	-26.53	97.51
7_67	URB	*Quercus robur*	solid	-26.3	100.24
7_67	URB	*Quercus robur*	soluble	-26.14	102.07
7_79	URB	*Quercus petraea*	solid	-27.68	83.76
7_79	URB	*Quercus petraea*	soluble	-27.6	84.71
7_80	URB	*Quercus petraea*	solid	-27.09	90.79
7_80	URB	*Quercus petraea*	soluble	-25.83	105.77

7_83	URB	*Quercus robur*	solid	-29.36	63.75
7_83	URB	*Quercus robur*	soluble	-29.42	62.92

Table S4: Number of 16S rRNA gene reads retained at each step of the DADA2 pipeline for oak (*Quercus spp.*) rhizosphere samples.

Sample	**Input**	**Passed filter**	**Denoised F**	**Denoised R**	**Merged**	**Non chimeric**	**Passed total [%]**
1	95609	79096	72680	74535	61233	60932	63,73
2	103407	85008	79222	80490	69328	69045	66,77
3	114382	94742	87879	89367	74975	74574	65,2
4	105008	84055	78768	79850	68892	68386	65,12
5	118082	99291	91750	94084	78504	77750	65,84
6	99625	84657	77708	79635	65099	64443	64,69
7	90310	73307	68358	69332	59218	58824	65,14
8	80798	64409	59412	60305	49823	49626	61,42
9	137163	112692	105932	107552	93197	92773	67,64
10	132776	99073	93394	94415	79600	78860	59,39
11	106710	89889	82816	85269	70645	69757	65,37
12	69999	56571	52350	53121	44141	43883	62,69
13	84088	71021	65520	66838	55343	55155	65,59
14	80991	69038	62998	64728	52325	51915	64,1
15	61056	52030	46722	48702	38911	38827	63,59
16	83448	68637	63137	64906	53502	53069	63,6
17	79375	66479	60610	62750	50383	49874	62,83
18	59880	47719	43867	44798	37300	37172	62,08
19	67541	54602	50537	51258	42647	42591	63,06
20	143994	92847	86225	88485	73203	72751	50,52
21	70983	57683	53068	53897	44415	44272	62,37
22	134698	108752	101993	103045	88416	87461	64,93
23	123603	101229	95485	96806	84597	83715	67,73
24	93327	71744	66516	67505	55710	55608	59,58
25	98433	80319	74650	75518	64248	63903	64,92
26	104632	84761	78334	79469	67049	66633	63,68

27	143383	116422	110340	111245	98837	98139	68,45
28	76286	61227	56650	57400	47583	47455	62,21
29	409869	297612	284160	287540	253354	247768	60,45
30	103986	86442	81094	82338	72000	71459	68,72
31	118926	97542	91804	92602	80029	79649	66,97
32	132539	107807	100557	102156	86343	85969	64,86
33	127169	104181	98424	99283	87870	86842	68,29
34	104700	84829	78860	79886	67949	67621	64,59
35	105733	88195	82041	83386	70710	70363	66,55
36	73448	58457	54369	55499	47864	47663	64,89

Table S5: Number of Internal Transcribed Spacer (ITS) reads retained at each step of the DADA2 pipeline for oak (*Quercus spp.*) rhizosphere samples.

Sample	**Input**	**Passed filter**	**Denoised F**	**Denoised R**	**Merged**	**Non Chimeric**	**Passed total [%]**
1	62276	32456	32117	32022	29444	28511	45,78
2	56286	29492	29325	29321	27818	26985	47,94
3	56488	28658	28352	28340	27307	26253	46,48
4	59129	31172	30911	30851	30313	29287	49,53
5	77178	39420	39169	39174	38172	33588	43,52
6	55631	28025	27729	27667	26560	26229	47,15
7	42675	22709	22398	22372	21877	21509	50,4
8	52221	26971	26574	26606	25952	24439	46,8
9	54902	25752	25369	25267	24342	23674	43,12
10	59150	30332	29981	29923	29302	28446	48,09
11	66948	33858	33289	33347	32339	30928	46,2
12	95102	45471	45148	45132	44031	43515	45,76
13	78361	40842	40424	40439	39761	38028	48,53
14	71146	39092	38688	38676	37645	37146	52,21
15	75813	41677	41330	41324	40524	39220	51,73
16	75130	38009	37764	37719	36818	34606	46,06
17	67859	31801	31589	31501	31063	28561	42,09

18	89875	47234	46741	46808	44789	41560	46,24
19	109665	57717	57342	57329	56565	53332	48,63
20	121614	63293	62826	62824	61823	57222	47,05
21	66160	34377	33999	34077	33546	31529	47,66
22	63946	35515	35092	35068	34229	32666	51,08
23	65527	34886	34639	34546	33523	31792	48,52
24	45295	23330	22966	22881	22279	21330	47,09
25	52098	25762	25450	25416	24899	23339	44,8
26	71352	38447	37815	37717	36515	35664	49,98
27	66470	34578	34311	34360	33735	31419	47,27
28	67907	36654	36186	36157	35205	34931	51,44
29	55127	29691	29322	29308	28583	26180	47,49
30	92955	50112	49629	49613	48173	47025	50,59
31	63037	34206	33863	33888	33338	31181	49,46
32	67643	32206	31960	31945	31390	29286	43,29
33	66072	32441	32143	32088	31581	29610	44,81
34	126974	66168	65638	65574	64104	62191	48,98
35	130886	70876	70283	70113	68298	66101	50,5
36	110947	61045	60558	60573	58267	56265	50,71

Table S6: Summary of statistical test results for microbial and metabolomic diversity and composition. Reported are the test statistics (H for Scheirer-Ray-Hare, Pseudo−F for PERMANOVA, F for ANOVA, W for Wilcoxon, Q2 for OPLS-DA, and ρ for Spearman rank correlations), degrees of freedom (df), and p-values.

Variable	**Predictor**	**Test**	**Test Statistic**	**Degrees of Freedom (df)**	**p-value**
Prokaryotic Alpha Diversity					
Richness	Origin	Scheirer-Ray-Hare	$H = 6.65$	1	0.01

Shannon Index	Origin	Scheirer-Ray-Hare	*H = 8.21*	*1*	0.004
Shannon Index (*Q. robur*)	Origin	Wilcoxon	*W = 11*		0.009
Fungal Alpha Diversity					
Shannon Index	Origin	Scheirer-Ray-Hare	H = 4.41	1	0.036
Pielou Evenness	Origin	Scheirer-Ray-Hare	H = 6.01	1	0.014
Prokaryotic Beta Diversity					
Community Composition	Origin	PERMANOVA	Pseudo-F = 1.47	[1, 28]	0.024
Community Composition	iWUE	PERMANOVA	Pseudo-F = 1.43	[1, 28]	0.036
Fungal Beta Diversity					
Community Composition	Origin:iWUE	PERMANOVA	*Pseudo-F = 1.70*	[1, 28]	0.014
Metabolome					
Richness	Origin	ANOVA	F = 6.06	[1, 32]	0.02
Shannon Index	Origin	ANOVA	F = 6.01	[1, 32]	0.02
Pielou Evenness	Species	ANOVA	F = 28.72	[1, 32]	< 0.001
Global Profile	Origin	OPLS-DA	Q^2 = 0.648		0.05
Correlations with iWUE					
Prokaryotic Richness	iWUE	Spearman	ρ= -0.50	34	0.002

Prokaryotic Shannon	iWUE	Spearman	ρ = -0.60	34	0.002
Fungal Evenness	iWUE	Spearman	ρ = -0.46	34	0.005

Table S7: Taxonomic classification of Amplicon Sequence Variants (ASVs) removed as contaminants from the oak (*Quercus spp.*) rhizosphere dataset.

Contaminant nr	**1**	**2**	**3**	**4**
Kingdom	Bacteria	Bacteria	Bacteria	Archaea
Phylum	Cyanobacteriota	Actinomycetota	Chloroflexota	Halobacteriota
Class	Cyanobacteriia	Actinobacteria	Anaerolineae	Methanosarcinia
Order	Chloroplast	Micrococcales	Aggregatilineales	Methanosarcinales
Family	NA	Cellulomonadaceae	Aggregatilineaceae	Methanosarcinaceae
Genus	NA	Actinotalea	Aggregatilinea	Methanosarcina
Species	NA	NA	NA	NA

Table S8: Permutational Multivariate Analysis of Variance (PERMANOVA) results based on Bray-Curtis dissimilarities. The model tested the fixed effects of origin, species, intrinsic water use efficiency (iWUE), and their interactions on the prokaryotic and fungal community composition of *Quercus spp*. rhizosphere samples. Statistical significance was determined using Type I sum of squares with 999 permutations.

	Prokaryotes		**Fungi**	
	R^2	**p-value**	**R^2**	**p-value**
Origin	0.04	0.024*	0.04	0.077
Species	0.03	0.662	0.03	0.493
iWUE	0.04	0.036*	0.03	0.508
Origin:Species	0.03	0.468	0.04	0.105
Origin:iWUE	0.03	0.296	0.05	0.014*
Species:iWUE	0.03	0.202	0.03	0.237
Origin:Species:iWUE	0.03	0.201	0.03	0.572

Table S9: Key metabolites identified in the oak (*Quercus spp*.) rhizosphere through Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) for factor origin. Key metabolites have VIP (Variable Importance in Projection) score > 1, logFC > 1 and p-value <= 0.05). Positive Log_2FC indicates enrichment at origin upper Rhine basin (URB), negative Log2FC indicates enrichment at origin north German lowlands (NGL). “NA” corresponds to “no assigned name”.

Nr	**VIP score**	**Log_2FC**	**Adjusted p-value**	**Class**	**Assigned name**
513	4.34	-3.33	2.24E-04	Protein	NA
506	4.29	-3.53	3.98E-05	Not Matched	NA
269	3.84	6.20	3.61E-03	Protein	NA
622	3.78	-4.15	3.82E-04	Lipid	NA
444	3.60	-3.84	5.69E-03	Lipid	NA
654	3.59	-1.37	5.11E-03	Lipid	NA
508	3.55	3.81	5.11E-03	Lipid	NA
559	3.49	-2.69	1.45E-03	Oxyaromatic Compound	Ellagic Acid
731	3.48	-3.77	9.49E-04	Protein	NA
330	3.37	-5.39	3.82E-04	Lipid	NA
87	3.32	5.35	3.52E-02	Lipid	NA
591	3.31	-2.53	3.61E-03	Not Matched	NA
424	3.21	5.14	4.46E-03	Protein	NA
601	3.08	-1.86	9.77E-03	Protein	NA
667	3.06	-1.86	6.42E-03	Carbohydrate	beta-Lactose
437	3.04	-2.40	1.45E-03	Amino Sugar	NA
170	3.00	-5.71	1.26E-02	Lipid	NA
587	2.96	-1.90	6.32E-03	Carbohydrate	1,6-Anhydro-β-D-glucose
348	2.97	-2.31	7.90E-04	Protein	NA
499	2.96	-3.56	6.42E-03	Protein	NA
600	2.91	-1.84	5.08E-03	Protein	NA
250	2.88	-2.34	9.74E-03	Not Matched	NA
362	2.87	-2.70	7.90E-04	Protein	NA
657	2.79	-1.90	9.74E-03	Carbohydrate	Raffinose

413	2.77	-2.39	5.11E-03	Protein	NA
304	2.75	-2.86	1.45E-03	Not Matched	NA
399	2.68	-3.08	3.82E-04	Protein	NA
684	2.64	-1.45	2.51E-02	Carbohydrate	NA
398	2.56	-2.07	5.82E-04	Not Matched	NA
255	2.53	-2.65	2.29E-02	Amino Sugar	NA
168	2.32	-2.50	4.34E-02	Not Matched	NA
419	2.25	-2.14	1.75E-02	Lipid	NA
617	2.24	-1.53	9.52E-03	Protein	NA
536	2.22	-3.01	3.51E-02	Oxyaromatic Compound	NA
878	2.19	-1.54	4.95E-02	Amino Sugar	NA
206	2.16	-2.82	1.95E-02	Oxyaromatic Compound	NA
660	2.14	-1.51	4.71E-02	Protein	Arginine
768	2.14	-1.50	2.46E-02	Oxyaromatic Compound	3-Furoic acid
507	2.12	-1.10	8.32E-03	Not Matched	NA
504	2.10	-1.33	2.72E-03	Oxyaromatic Compound	NA
311	2.10	-1.72	2.84E-02	Oxyaromatic Compound	NA
361	2.08	-1.23	1.09E-02	Not Matched	NA
400	2.04	-1.62	4.86E-02	Protein	NA
471	2.03	-1.92	4.33E-02	Lipid	NA
137	2.01	-2.10	4.33E-02	Oxyaromatic Compound	NA
346	2.00	-1.92	2.21E-02	Lipid	NA
750	1.20	-1.28	2.51E-02	Protein	NA
913	1.98	-1.55	3.06E-02	Carbohydrate	NA
324	1.95	-2.18	2.69E-02	Lipid	NA
358	1.89	1.58	1.67E-02	Not Matched	NA
883	1.76	-1.07	1.40E-02	Oxyaromatic Compound	NA
179	1.75	-1.02	4.33E-02	Amino Sugar	NA
25	1.73	-1.41	3.65E-02	Lipid	NA
120	1.72	-1.12	2.15E-02	Carbohydrate	NA

301	1.70	-1.29	4.34E-02	Oxyaromatic Compound	NA
1000	1.70	-1.10	4.00E-02	Oxyaromatic Compound	NA
153	1.63	-1.47	4.58E-03	Not Matched	NA
734	1.61	-1.11	2.51E-02	Not Matched	NA