

Characterizing both bacteria and fungi improves understanding of the *Arabidopsis* root microbiome

Supplementary Material.

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Fig. S1, Read counts per sample.

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Fig. S3, Key taxa in the microbiome

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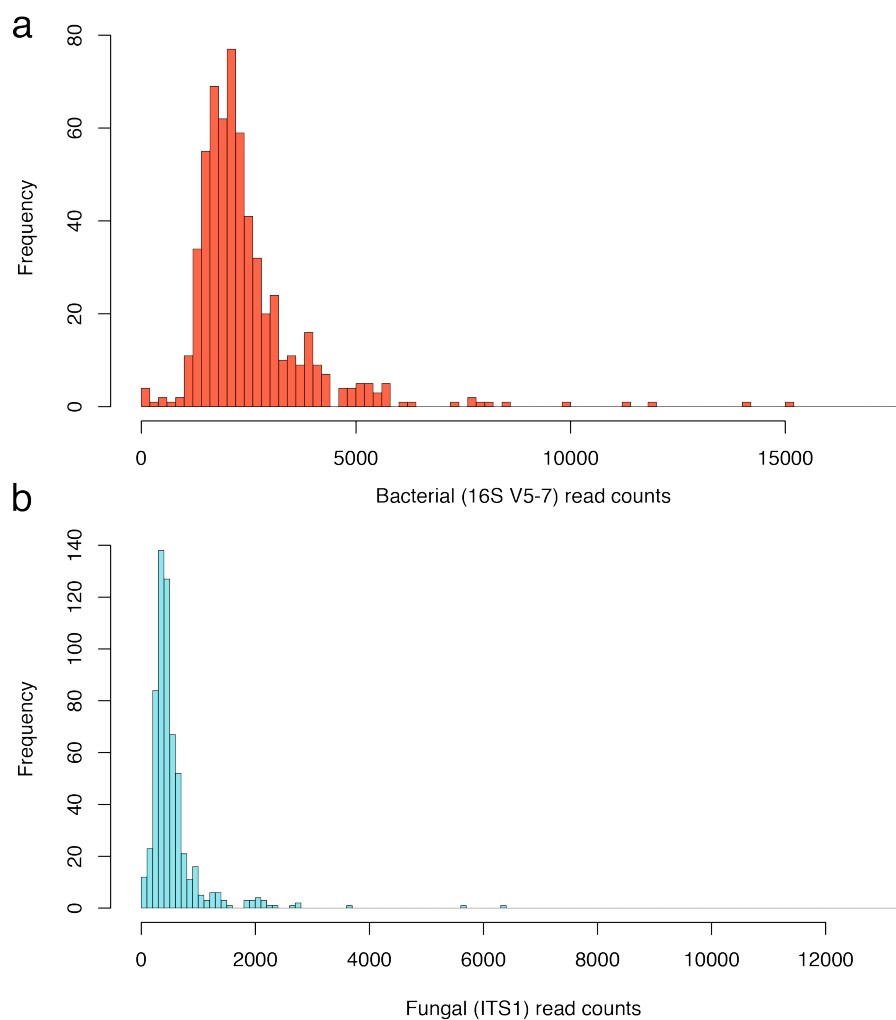
Fig. S6, GWAS of PC1 from PCA of the combined microbiome

Supplementary Tables S1 and S2:

Table S1, The list of accessions used and their collection locations

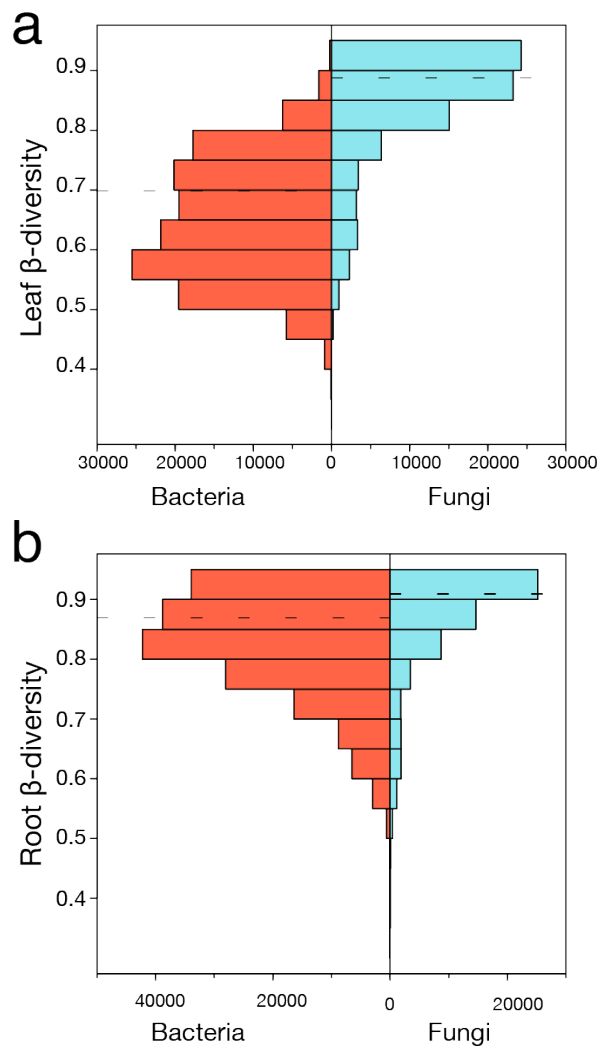
Table S2, The biological processes that underlie variation in richness in the root microbiome

Supplementary Figures



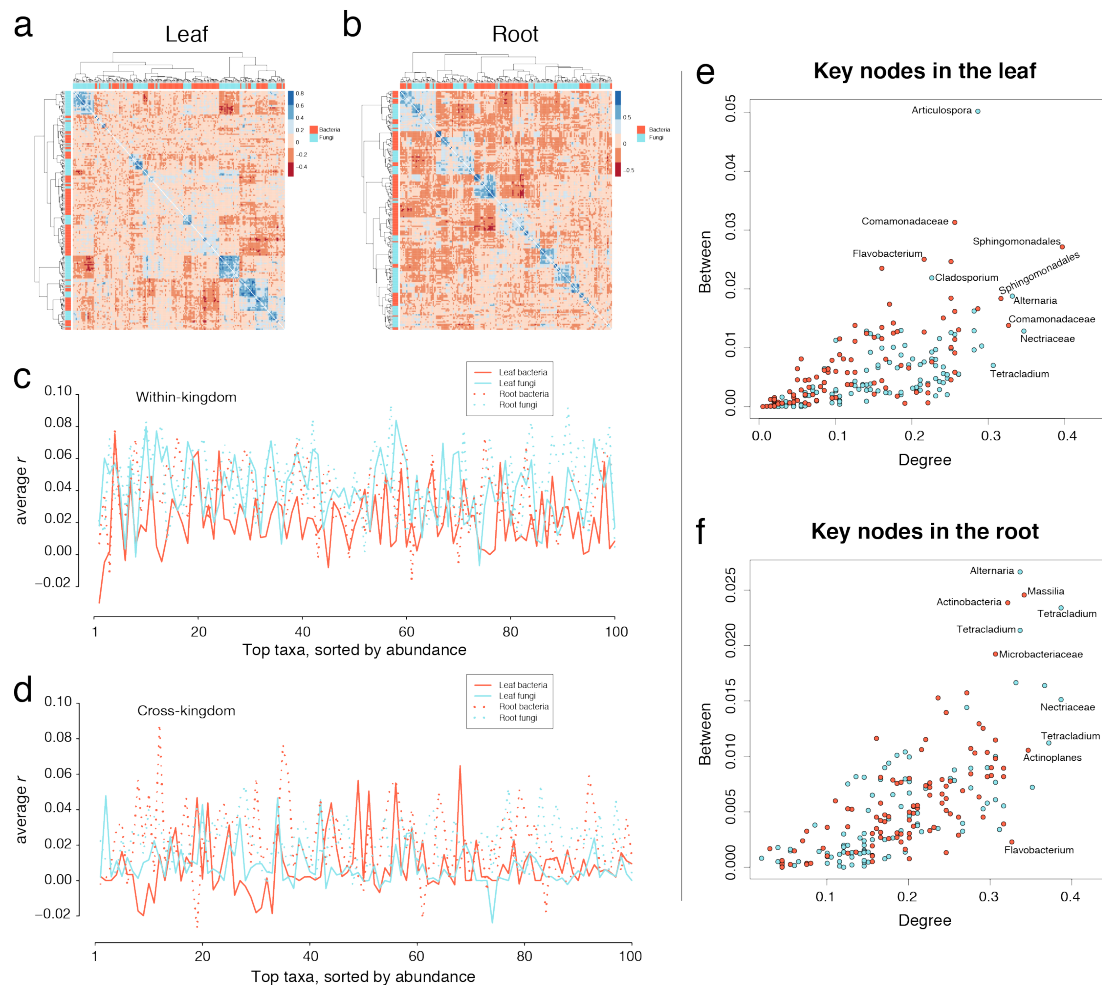
Supplementary Fig. S1. Read counts per sample

The number of reads per sample for root bacterial (a) and fungal (b) amplicon libraries.



Supplementary Fig. S2. β -diversity in the microbiome

β -diversity in both the (a) leaf and (b) root microbiome of *A. thaliana*. On average, β -diversity is higher in the root than leaf microbiome for both kingdoms.

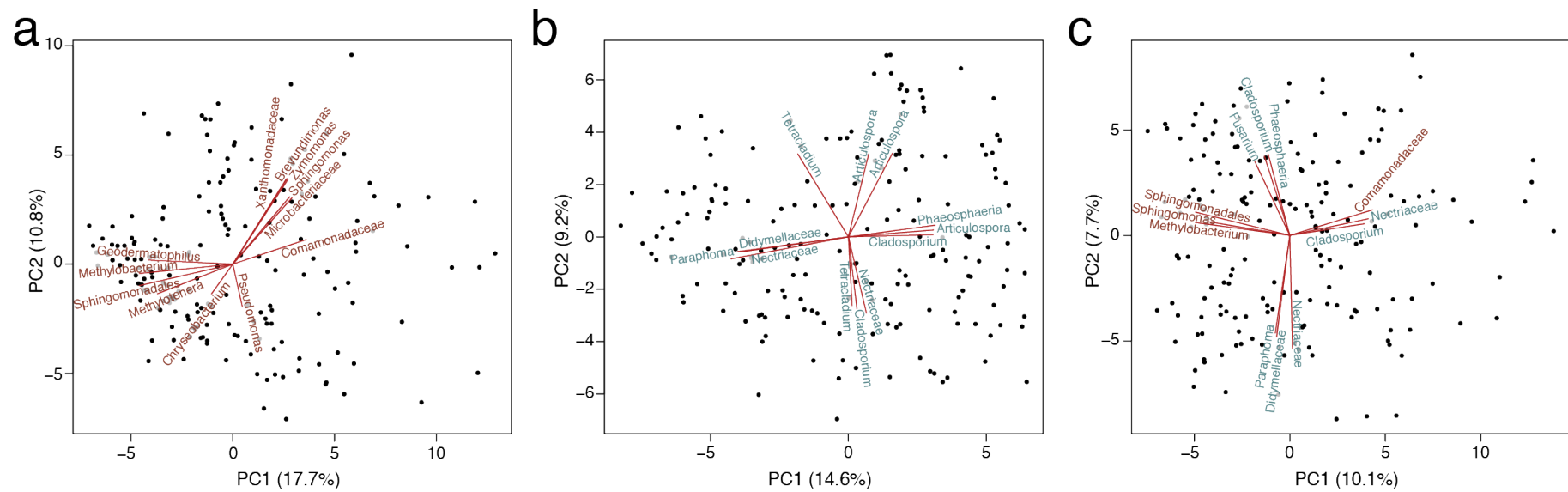


Supplementary Fig. S3. Key taxa in the microbiome

Correlations within the (a) leaf and (b) root microbiome of *Arabidopsis thaliana*. For ease of presentation and visualization, we show the top 100 taxa in each microbiome. As can be seen by the diagonal elements, the strongest correlations tend to occur among members of the same kingdom. Nevertheless, significant cross-kingdom correlations suggest that taxa within the leaf and root microbiome may interact or be shaped by similar environmental and/or host-related processes. (c) The average intra- and (d) inter-kingdom correlations for the top taxa in the plant microbiome show a slight positive skew. These Pearson correlations were calculated between each focal taxon (here, sorted based on their decreasing abundance) and their top (c) intra- or (d) inter-kingdom counterparts (that is, the comparisons are restricted to the top 100 taxa within each kingdom). (e) Network analysis helps to identify the key phylotypes in the leaf microbiome, which are shown here based on the centrality metrics *degree* and *betweenness*. (f) Influential nodes are shown for the root microbiome.

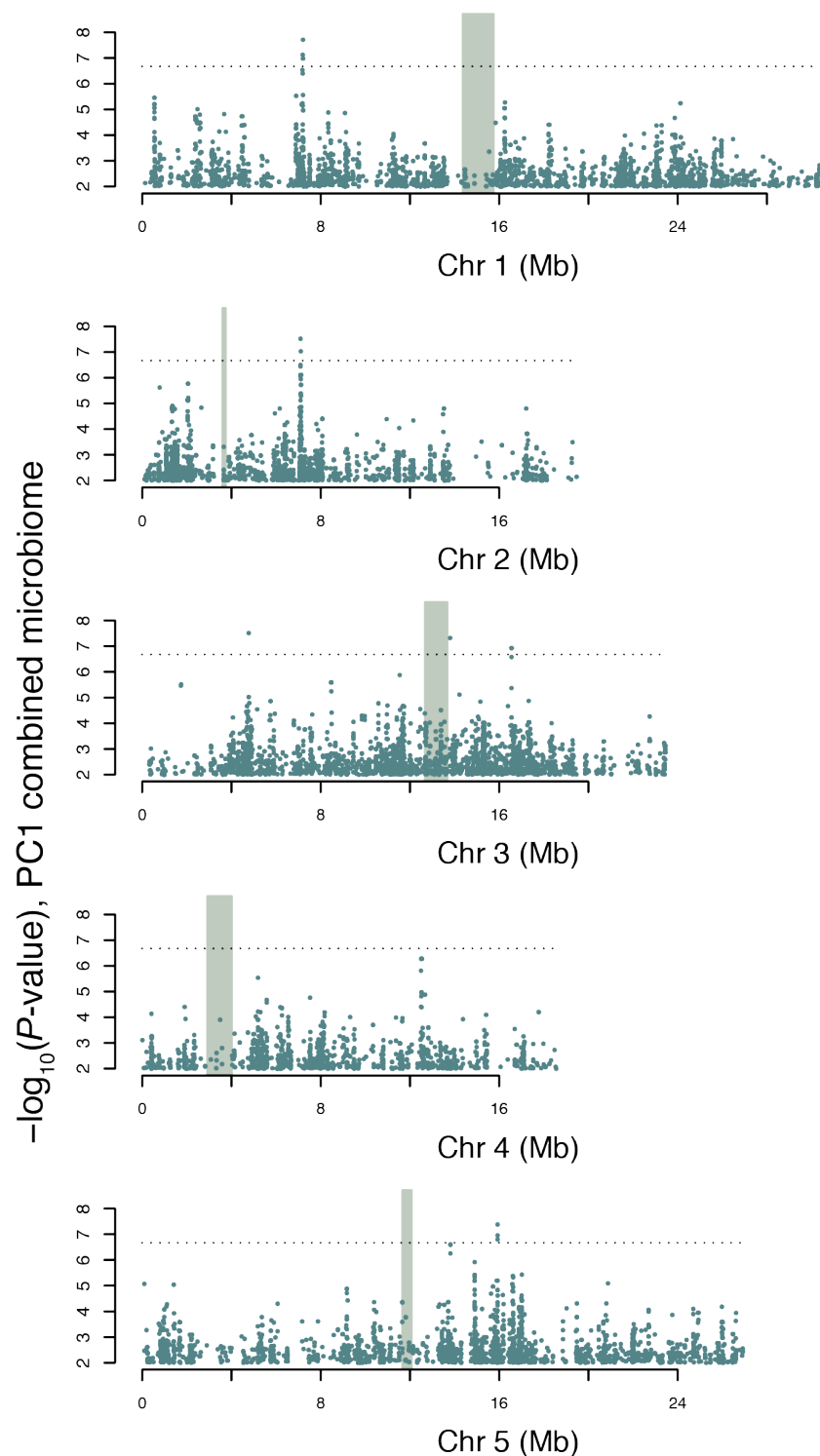
Supplementary Fig. S4. Correlations among the PCs from PCA of the microbiome

Correlation among the top 5 PCs from PCA of the leaf (a-c) and root (d-f) microbiome. PC1 and PC2 from PCA of the combined (bacteria and fungi) leaf-microbiome are correlated with PC1 from PCA of the bacterial community (a) and PC2 from PCA of the fungal community (b), respectively. (c) The correlation among the top 5 PCs from separate PCAs of the leaf bacterial and fungal community are shown. PC1 and PC2 from PCA of the combined root-microbiome are strongly correlated with PC1 (e) and PC2 (d) of the fungal and bacterial community. (f) Shown are the correlations among the top 5 PCs from PCA of the root bacterial and fungal community.



Supplementary Fig. S5. The results from PCA of the leaf microbiome

(a) A plot of principal component 1 (PC1) and PC2 from PCA of the top 100 bacteria in the leaf microbiome is shown. (b) A plot of PC1 and PC2 from PCA of the top 100 fungi in the leaf microbiome is shown. (c) A plot of PC1 and PC2 from PCA of the combined leaf microbial community, consisting of the top 100 bacteria and fungi. In each panel, the labels list the top 3 taxa whose abundances separate the samples along the axes (plotted according to their PC loadings).



Supplementary Fig. S6. GWAS of PC1 from PCA of the combined microbiome

The results from GWAS of PC1 from PCA of the combined bacterial and fungal community are shown. The candidate genes are listed in the main text. To assess genome-wide significance, a permutation approach was used that takes into account population structure. The centromeres along each of *A. thaliana*'s 5 chromosomes are shaded.

Supplementary Tables

Supplementary Table S1. The list of accessions used and their collection locations

Id	Name	Latitude	Longitude	Country
8251	Ag-0	45	1.3	France
8252	Alc-0	40.31	-3.22	Spain
8230	Algutsrum	56.68	16.5	Sweden
8253	An-1	51.2167	4.4	Belgium
8254	Ang-0	50.3	5.3	Belgium
8256	Ba1-2	56.4	12.9	Sweden
8257	Ba3-3	56.4	12.9	Sweden
8258	Ba4-1	56.4	12.9	Sweden
8259	Ba5-1	56.4	12.9	Sweden
8261	Bg-2	47.6479	-122.305	United States of America
8262	Bil-5	63.324	18.484	Sweden
8263	Bil-7	63.324	18.484	Sweden
8264	Bla-1	41.6833	2.8	Spain
8265	Blh-1	48	19	Czech Republic
8266	Boo2-1	55.86	13.51	Sweden
5837	Bor-1	49.4013	16.2326	Czech Republic
8268	Bor-4	49.4013	16.2326	Czech Republic
8269	Br-0	49.2	16.6166	Czech Republic
8231	Bro1-6	56.3	16	Sweden
8270	Bs-1	47.5	7.5	Switzerland
8271	Bu-0	50.5	9.5	Germany
8421	Buckhorn Pass	41.3599	-122.755	United States of America
8272	Bur-0	54.1	-6.2	Ireland
8274	Can-0	29.2144	-13.4811	Spain
8275	Cen-0	49	0.5	France
8276	CIBC-17	51.4083	-0.6383	United Kingdom
8277	CIBC-5	51.4083	-0.6383	United Kingdom
8278	Co	40.2077	-8.42639	Portugal
6909	Col-0			Unknown
8280	Ct-1	37.3	15	Italy
8233	Dem-4	41.1876	-87.1923	United States of America
8283	Dra3-1	55.76	14.12	Sweden
8284	Drall-1	49.4112	16.2815	Czech Republic
8285	Drall-1	49.4112	16.2815	Czech Republic
6008	Duk	49.1	16.2	Czech Republic
6009	Eden-1	62.877	18.177	Sweden
8287	Eden-2	62.877	18.177	Sweden
8288	Edi-0	55.9494	-3.16028	United Kingdom

Id	Name	Latitude	Longitude	Country
6016	Eds-1	62.9	18.4	Sweden
8289	Ei-2	50.3	6.3	Germany
8290	En-1	50	8.5	Germany
8291	Est-1	58.3	25.3	Russia
8292	Fab-2	63.0165	18.3174	Sweden
8293	Fab-4	63.0165	18.3174	Sweden
8294	Fei-0	40.5	-8.32	Portugal
8422	Fja1-1	56.06	14.29	Sweden
6024	Fly2-2	55.7509	13.3712	Sweden
8295	Ga-0	50.3	8	Germany
8296	Gd-1	53.5	10.5	Germany
8297	Ge-0	46.5	6.08	Switzerland
8298	Got-22	51.5338	9.9355	Germany
8299	Got-7	51.5338	9.9355	Germany
8300	Gr-1	47	15.5	Austria
8301	Gu-0	50.3	8	Germany
8234	Gul1-2	56.4606	15.8127	Sweden
8303	H55	49	15	Czech Republic
8304	Hi-0	52	5	Netherlands
8235	Hod	48.8	17.1	Czech Republic
8423	Hov2-1	56.1	13.74	Sweden
8306	Hov4-1	56.1	13.74	Sweden
6039	Hovdala-2	56.1	13.74	Sweden
8308	HR-10	51.4083	-0.6383	United Kingdom
8309	HR-5	51.4083	-0.6383	United Kingdom
8310	Hs-0	52.24	9.44	Germany
8236	HSm	49.33	15.76	Czech Republic
8311	In-0	47.5	11.5	Austria
8312	Is-0	50.5	7.5	Germany
8313	Jm-0	49	15	Czech Republic
8314	Ka-0	47	14	Austria
8424	Kas-2	35	77	India
8237	Kavlinge-1	55.8	13.1	Sweden
8420	Kelsterbach-4	50.0667	8.5333	Germany
8238	Kent	51.15	0.4	United Kingdom
8316	Kin-0	44.46	-85.37	United States of America
6040	Kni-1	55.66	13.4	Sweden
8317	Kno-10	41.2816	-86.621	United States of America
8318	Kno-18	41.2816	-86.621	United States of America
8239	Koln	51	7	Germany
8319	Kondara	38.48	68.49	Tajikistan
8240	Kulturen-1	55.705	13.196	Sweden

Id	Name	Latitude	Longitude	Country
8320	Kz-1	49.5	73.1	Kazakhstan
8321	Kz-13	49.5	73.1	Kazakhstan
8322	Kz-9	49.5	73.1	Kazakhstan
8323	Lc-0	57	-4	United Kingdom
8324	Ler-1	47.984	10.8719	Germany
8241	Liarum	55.9473	13.821	Sweden
8242	Lillo-1	56.1494	15.7884	Sweden
8325	Lip-0	50	19.3	Poland
8326	Lis-1	56.0328	14.775	Sweden
8222	Lis-2	56.0328	14.775	Sweden
8430	Lisse	52.25	4.5667	Netherlands
8328	LL-0	41.59	2.49	Spain
8329	Lm-2	48	0.5	France
6042	Lom1-1	56.09	13.9	Sweden
6043	Lov-1	62.801	18.079	Sweden
6046	Lov-5	62.801	18.079	Sweden
8332	Lp2-2	49.38	16.81	Czech Republic
8333	Lp2-6	49.38	16.81	Czech Republic
8334	Lu-1	55.71	13.2	Sweden
8335	Lund	55.71	13.2	Sweden
8336	Lz-0	46	3.3	France
8337	Mir-0	44	12.37	Italy
8338	Mr-0	44.15	9.65	Italy
8339	Mrk-0	49	9.3	Germany
8340	Ms-0	55.7522	37.6322	Russia
8341	Mt-0	32.34	22.46	Libya
8342	Mz-0	50.3	8.3	Germany
8429	N13	61.36	34.15	Russia
8343	Na-1	47.5	1.5	France
8246	NC-6	35	-79.18	United States of America
8344	Nd-1	50	10	Switzerland
8345	NFA-10	51.4083	-0.6383	United Kingdom
8346	NFA-8	51.4083	-0.6383	United Kingdom
8347	Nok-3	52.24	4.45	Netherlands
8348	Nw-0	50.5	8.5	Germany
6064	Nyl-2	62.9513	18.2763	Sweden
8349	Omo2-1	56.1481	15.8199	Sweden
8350	Omo2-3	56.1481	15.8155	Sweden
6074	Or-1	56.4573	16.1408	Sweden
8351	Ost-0	60.25	18.37	Sweden
8352	Oy-0	60.23	6.13	Norway
8353	Pa-1	38.07	13.22	Italy

<i>Id</i>	<i>Name</i>	<i>Latitude</i>	<i>Longitude</i>	<i>Country</i>
<i>8354</i>	<i>Per-1</i>	<i>58</i>	<i>56.3167</i>	<i>Russia</i>
<i>8355</i>	<i>Petergof</i>	<i>59</i>	<i>29</i>	<i>Russia</i>
<i>8243</i>	<i>PHW-2</i>	<i>43.7703</i>	<i>11.2547</i>	<i>Italy</i>
<i>8244</i>	<i>PHW-34</i>	<i>48.6103</i>	<i>2.3086</i>	<i>France</i>
<i>8356</i>	<i>Pi-0</i>	<i>47.04</i>	<i>10.51</i>	<i>Austria</i>
<i>8358</i>	<i>Pna-10</i>	<i>42.0945</i>	<i>-86.3253</i>	<i>United States of America</i>
<i>8359</i>	<i>Pna-17</i>	<i>42.0945</i>	<i>-86.3253</i>	<i>United States of America</i>
<i>8360</i>	<i>Pro-0</i>	<i>43.25</i>	<i>-6</i>	<i>Spain</i>
<i>8361</i>	<i>Pu2-23</i>	<i>49.42</i>	<i>16.36</i>	<i>Czech Republic</i>
<i>8362</i>	<i>Pu2-7</i>	<i>49.42</i>	<i>16.36</i>	<i>Czech Republic</i>
<i>8363</i>	<i>Pu2-8</i>	<i>49.42</i>	<i>16.36</i>	<i>Czech Republic</i>
<i>8364</i>	<i>Ra-0</i>	<i>46</i>	<i>3.3</i>	<i>France</i>
<i>8365</i>	<i>Rak-2</i>	<i>49</i>	<i>16</i>	<i>Czech Republic</i>
<i>8366</i>	<i>Rd-0</i>	<i>50.5</i>	<i>8.5</i>	<i>Germany</i>
<i>8367</i>	<i>Ren-1</i>	<i>48.5</i>	<i>-1.41</i>	<i>France</i>
<i>8368</i>	<i>Ren-11</i>	<i>48.5</i>	<i>-1.41</i>	<i>France</i>
<i>8369</i>	<i>Rev-1</i>	<i>55.6942</i>	<i>13.4504</i>	<i>Sweden</i>
<i>8370</i>	<i>Rmx-A02</i>	<i>42.036</i>	<i>-86.511</i>	<i>United States of America</i>
<i>8371</i>	<i>Rmx-A180</i>	<i>42.036</i>	<i>-86.511</i>	<i>United States of America</i>
<i>8372</i>	<i>RRS-10</i>	<i>41.5609</i>	<i>-86.4251</i>	<i>United States of America</i>
<i>8373</i>	<i>RRS-7</i>	<i>41.5609</i>	<i>-86.4251</i>	<i>United States of America</i>
<i>8374</i>	<i>Rsch-4</i>	<i>56.3</i>	<i>34</i>	<i>Russia</i>
<i>8375</i>	<i>Rubezhnoe-1</i>	<i>49</i>	<i>38.28</i>	<i>Ukraine</i>
<i>8247</i>	<i>San-2</i>	<i>56.07</i>	<i>13.74</i>	<i>Sweden</i>
<i>8376</i>	<i>Sanna-2</i>	<i>62.69</i>	<i>18</i>	<i>Sweden</i>
<i>8377</i>	<i>Santa Clara</i>	<i>37.21</i>	<i>-121.16</i>	<i>United States of America</i>
<i>8378</i>	<i>Sap-0</i>	<i>49.49</i>	<i>14.24</i>	<i>Czech Republic</i>
<i>8412</i>	<i>Sav-0</i>	<i>49.1833</i>	<i>15.8833</i>	<i>Czech Republic</i>
<i>8379</i>	<i>Se-0</i>	<i>38.3333</i>	<i>-3.53333</i>	<i>Spain</i>
<i>8245</i>	<i>Seattle-0</i>	<i>47</i>	<i>-122.2</i>	<i>United States of America</i>
<i>8380</i>	<i>Sf-1</i>	<i>41.7833</i>	<i>3.03333</i>	<i>Spain</i>
<i>8248</i>	<i>Shahdara</i>	<i>38.35</i>	<i>68.48</i>	<i>Tajikistan</i>
<i>8381</i>	<i>Sorbo</i>	<i>38.35</i>	<i>68.48</i>	<i>Tajikistan</i>
<i>8382</i>	<i>Spr1-2</i>	<i>58.4168</i>	<i>14.1612</i>	<i>Sweden</i>
<i>8383</i>	<i>Spr1-6</i>	<i>58.4168</i>	<i>14.1612</i>	<i>Sweden</i>
<i>8384</i>	<i>Sq-1</i>	<i>51.4083</i>	<i>-0.6383</i>	<i>United Kingdom</i>
<i>8385</i>	<i>Sq-8</i>	<i>51.4083</i>	<i>-0.6383</i>	<i>United Kingdom</i>
<i>8386</i>	<i>Sr:5</i>	<i>58.9</i>	<i>11.2</i>	<i>Sweden</i>
<i>8387</i>	<i>St-0</i>	<i>59</i>	<i>18</i>	<i>Sweden</i>
<i>6088</i>	<i>Stu1-1</i>	<i>56.4666</i>	<i>16.1284</i>	<i>Sweden</i>
<i>8388</i>	<i>Stw-0</i>	<i>52</i>	<i>36</i>	<i>Russia</i>
<i>8389</i>	<i>Ta-0</i>	<i>49.5</i>	<i>14.5</i>	<i>Czech Republic</i>

Id	Name	Latitude	Longitude	Country
8390	Tamm-2	60	23.5	Finland
8391	Tamm-27	60	23.5	Finland
6243	Tottarp-2	55.95	13.85	Sweden
8392	Ts-1	41.7194	2.93056	Spain
8393	Ts-5	41.7194	2.93056	Spain
8394	Tsu-1	34.43	136.31	Japan
8395	Tu-0	45	7.5	Italy
8426	Ull1-1	56.06	13.97	Sweden
8396	Ull2-3	56.0648	13.9707	Sweden
8397	Ull2-5	56.0648	13.9707	Sweden
8398	Uod-1	48.3	14.45	Austria
8428	Uod-2	48.3	14.45	Austria
8399	Uod-7	48.3	14.45	Austria
8400	Van-0	49.3	-123	Canada
8401	Var2-1	55.58	14.334	Sweden
8402	Var2-6	55.58	14.334	Sweden
9058	Vastervik	57.75	16.6333	Sweden
8249	Vimmerby	57.7	15.8	Sweden
9057	Vinslov	56.1	13.9167	Sweden
8403	Wa-1	52.3	21	Poland
8404	Wei-0	47.25	8.26	Switzerland
8405	Ws-0	52.3	30	Russia
8406	Ws-2	52.3	30	Russia
8407	Wt-5	52.3	9.3	Germany
8408	Yo-0	37.45	-119.35	United States of America
8409	Zdr-1	49.3853	16.2544	Czech Republic
8410	Zdr-6	49.3853	16.2544	Czech Republic

Table S2, The biological processes that underlie variation in richness in the root microbiome

The biological (gene ontology, GO) processes enriched in the 1% tail from GWAS of species richness in the bacterial and fungal community are shown. Storey's procedure was used to correct for multiple testing.

Kingdom	Biological process	Enrichment	FDR $q \leq 10\%$
<i>fungi</i>	translesion synthesis	77.4	0.0002
<i>fungi</i>	zeta DNA polymerase complex	77.4	0.0002
<i>fungi</i>	alpha-glucosidase activity	44.2	0.0011
<i>fungi</i>	epidermal cell differentiation	16.6	0.0025
<i>fungi</i>	structural constituent of ribosome	2.2	0.0443
<i>fungi</i>	cytosolic large ribosomal subunit	2.9	0.0536
<i>fungi</i>	DNA repair	3.1	0.0868
<i>fungi</i>	response to UV	5.4	0.0868
<i>fungi</i>	microtubule motor activity	3.2	0.0868
<i>fungi</i>	ribosome	2.2	0.0972
<i>fungi</i>	translation	2.0	0.0972
<i>fungi</i>	root epidermal cell differentiation	6.4	0.0972
<i>fungi</i>	programmed cell death	6.3	0.0972
<i>fungi</i>	cytosolic ribosome	2.2	0.0975
<i>bacteria</i>	tRNA dimethylallyltransferase activity	59.5	0.00004
<i>bacteria</i>	cell wall modification involved in multidimensional cell growth	44.2	0.0020
<i>bacteria</i>	galactose catabolic process	28.1	0.0046
<i>bacteria</i>	homoserine dehydrogenase activity	28.1	0.0046
<i>bacteria</i>	cellulase activity	22.1	0.0047
<i>bacteria</i>	phosphoglucomutase activity	22.1	0.0047
<i>bacteria</i>	cytosolic part	22.1	0.0047
<i>bacteria</i>	glucose 1-phosphate metabolic process	22.1	0.0047
<i>bacteria</i>	cytokinin biosynthetic process	12.2	0.0047

<i>bacteria</i>	tRNA processing	11.9	0.0047
<i>bacteria</i>	aspartate family amino acid biosynthetic process	18.2	0.0066
<i>bacteria</i>	detection of gravity	17.2	0.0072
<i>bacteria</i>	gametophyte development	11.0	0.0249
<i>bacteria</i>	protein import into nucleus	8.8	0.0440
<i>bacteria</i>	cotyledon vascular tissue pattern formation	8.3	0.0480
<i>bacteria</i>	starch biosynthetic process	7.5	0.0600
<i>bacteria</i>	SCF ubiquitin ligase complex	4.7	0.0793
<i>bacteria</i>	cellular response to phosphate starvation	2.9	0.0793
<i>bacteria</i>	ubiquitin-protein ligase activity	2.1	0.0793
<i>bacteria</i>	pectinesterase inhibitor activity	3.6	0.0889
<i>bacteria</i>	protein ubiquitination	3.1	0.0948