

SUPPLEMENTARY MATERIALS AND METHODS

Title: Taxonomic and functional shifts in the beech rhizosphere microbiome across a natural soil toposequence

AUTHORS: Colin, Y.^{1,2}, Nicolitch^{1,2}O., Van Nostrand³, J.D., Zhou^{3,4,5}, J.Z., Turpault, M-P^{1,2}, Uroz, S.

^{1,2*}

¹ INRA, Université de Lorraine, UMR 1136 “Interactions Arbres Micro-organismes”, Centre INRA de Nancy, 54280 Champenoux, France

² INRA UR 1138 “Biogéochimie des Ecosystèmes Forestiers”, Centre INRA de Nancy, 54280 Champenoux, France.

³ Institute for Environmental Genomics, and Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK 73072, USA.

⁴ Earth Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA.

⁵ State Key Joint Laboratory of Environment Simulation and Pollution Control, School of Environment, Tsinghua University, Beijing 100084, China.

Running title: Beech rhizosphere microbiome

* Corresponding author: Mailing address: INRA-Université de Lorraine, Interactions Arbres Micro-organisms, UMR 1136, 54280 Champenoux, France. Phone: +33 (0)3 83 39 40 81, Fax: +33 (0)3 83 39 40 69. E-mail: uroz@nancy.inra.fr

GeoChip analysis

A total of three replicates ($n=3$) coming from each plot and each soil compartment (bulk soil and rhizosphere; n total=18) were used to perform GeoChip analyses. The gene array used (180K GeoChip 5.0) contains 167,044 distinct probes targeting functional genes involved in microbial carbon (degradation, fixation, methane), nitrogen, sulfur, and phosphorus cycling, energy metabolism, metal homeostasis, organic remediation, “Other” (phylogenetic genes and CRISPR system), secondary metabolism (e.g. antibiotic metabolism, pigments), stress responses, viruses (both bacteriophages and eukaryotic viruses), and virulence. All procedures were performed at Glomics Inc. (Norman, Oklahoma, USA). For each replicate considered, a total of 20 ng of DNA of the required quality (A260:A280 = 1.8; A260:A230 = 1.7) was amplified using the Templphi kit (GE Healthcare) with the following modifications. Spermidine (0.1 mM) and single stranded binding protein (267 ng.mL⁻¹) were added to improve the amplification efficiency (Wu et al., 2006). Reactions were scaled down to 10 μ L of sample and reaction buffers and 0.6 μ L of enzyme. Samples were amplified for 6 hr. Amplified DNA (~2 μ g) was mixed with 5.5 μ L random primers (Life Technologies, random hexamers, 3 μ g/ μ L), brought to 35 μ L with nuclease-free water, heated to 99°C for 5 min, and immediately placed on ice. The labeling master mix {15 μ L [2.5 μ L of dNTP (5 mM dAGC-TP, 2.5 mM dTTP), 0.5 μ L of Cy-3 dUTP (25 nM; GE Healthcare), 1 μ L of Klenow (imer; San Diego, CA; 40 U.mL⁻¹), 5 μ L Klenow buffer, 2.5 μ L of water]} was added and the samples were incubated at 37°C for 6 h in a thermocycler and then at 95°C for 3 min to inactivate the enzyme. After the addition of Cy3, samples were protected from the light as much as possible. Labeled DNA was cleaned using a QIAquick purification kit (Qiagen) per the manufacturer’s instructions and then dried down in a SpeedVac (45°C, 45 min; ThermoSavant). Labeled DNA was rehydrated with 27.5 μ L deionized water and then hybridization solution was added [99.4 μ L; 63.5 μ L 2X Hi-RPM hybridization buffer (Agilent), 12.7 μ L pre-prepared 10X CGH blocking agent (Agilent), 12.7 μ L formamide, 5.5 μ L Cot-1 DNA, common oligo reference standard (Liang et al., 2010), mixed well, spun to collect liquid in bottom of tube, and incubated at 95°C for 5 min. Tubes were incubated at 37°C for 30 min. Gasket slides were placed into a SureHyb chamber, the samples (120 μ L) were loaded onto the gasket slide. Samples were hybridized for 20-22 hr at 67°C. After hybridization, slides were disassembled in room temperature Wash Buffer 1 (Agilent) and then dried. GeoChips were imaged (NimbleGen MS 200 microarray scanner) as a Multi-TIFF. The GeoChip data were then extracted using the Agilent Feature Extraction program and loaded onto the GeoChip data analysis pipeline (ieg.ou.edu/microarray/). Data normalization and quality filtering were performed with multiple steps (Liang et al., 2010; Deng and He, 2014). First, the average signal intensity was calculated for each array, and the maximum average value was applied to normalize the signal intensity of samples in each array. Second, the sum of the signal intensity of samples was calculated for each array, and the

maximum sum value was applied to normalize the signal intensity of all spots in an array, which produced a normalized value for each spot in each array. Spots were scored as positive and retained if the signal-to-noise ratio [SNR = (signal mean – background mean)/background standard deviation] was ≥ 2.0 , and the coefficient of variation (CV) of the background was < 0.8 . In addition, spots with signal intensity less than 250 were discarded. Spots that were detected in only one of the biological replicates were also removed.

Deng Y, He Z. (2014). Microarray Data Analysis. In: He Z (ed) Microarrays: Current Technology, Innovations and Applications. Horizon Scientific Press: Norwich, UK).

Liang, Y.T., Z. H. He, L. Y. Wu, Y. Deng, G.H. Li, and J.-Z. Zhou. (2010). Development of a Common Oligonucleotide Reference Standard for microarray data normalization and comparison across different microbial communities. *Appl. Environ. Microbiol.* 76(4):1088-94. doi:10.1128/AEM.02749-09

Wu, L., Liu, X., Schadt, C.W., and Zhou, J. (2006) Microarray-based analysis of subnanogram quantities of microbial community DNAs by using whole-community genome amplification. *Appl Environ Microbiol* 72: 4931–4941.

Accession number

The 454 pyrosequencing data generated for this study were submitted to the Sequence Read Archive (SRA) and are available under the Bioproject ID: PRJNA270036 and accession numbers SAMN04103543- SAMN034103566. The microarray data presented here are available for download at <http://ieg.ou.edu/4download/>

Figure.S1 Principal component analysis (PCA) of the carbon substrates utilization pattern by total microbial communities. (A) PCA-ordination plots. PCA-ordination plots have been generated for the bulk soil and rhizosphere microbial communities based on their substrate utilization profile. The origins of samples are indicated as follows: filled squares, bulk soil samples from Cambisol Calcaric; open squares, rhizosphere samples from Cambisol Calcaric; filled circles, bulk soil samples from Cambisol Eutric, open circles, rhizosphere samples from Cambisol Eutric; filled triangles, bulk soil samples from Cambisol Hyperdystric; open triangles, rhizosphere samples from Cambisol Hyperdystric. Ellipses correspond to 95% confidence intervals about the mean. B) Vectors show the direction of maximum change for variables and longer arrows indicate a greater change in substrate utilization value. Substrates abbreviations used are: PAME, pyruvic acid methyl ester; tween 40, tween 40; tween 80, tween 80; a-cyclodextrin, alpha-cyclodextrin; glycogen, glycogen; D-cellubiose, D-cellubiose; a-D-Lactose, alpha-D-Lactose; BMetDGlc, beta-methyl-D-glucoside; D-xylose, D-xylose; i-Erythritol, i-Erythritol; D-Mannitol, D-Mannitol; NADG, N-Acetyl-D-Glucosamine; DGlcA, D-Glucosaminidic acid; Glc1P, Glucose 1 phosphate; GP, Glycerol phosphate; DGAGL, D-galactonique acid gamma-lactone; DGA, D-galacturonic acid; 2HBA, 2-Hydroxy-benzoic acid; 4HBA, 4-Hydroxy-benzoic acid; HbutA, Hydroxybutiric acid; Itaconic acid, Itaconic acid; ketobutyric acid, ketobutyric acid; malic acid, malic acid; arginine, arginine; asparag, asparagine; phenylala, phenylalanine; serine, serine; threonine, threonine; glutamic acid, glutamic acid; phenylethyl, phenylethylamine; putrescine, putrescine;

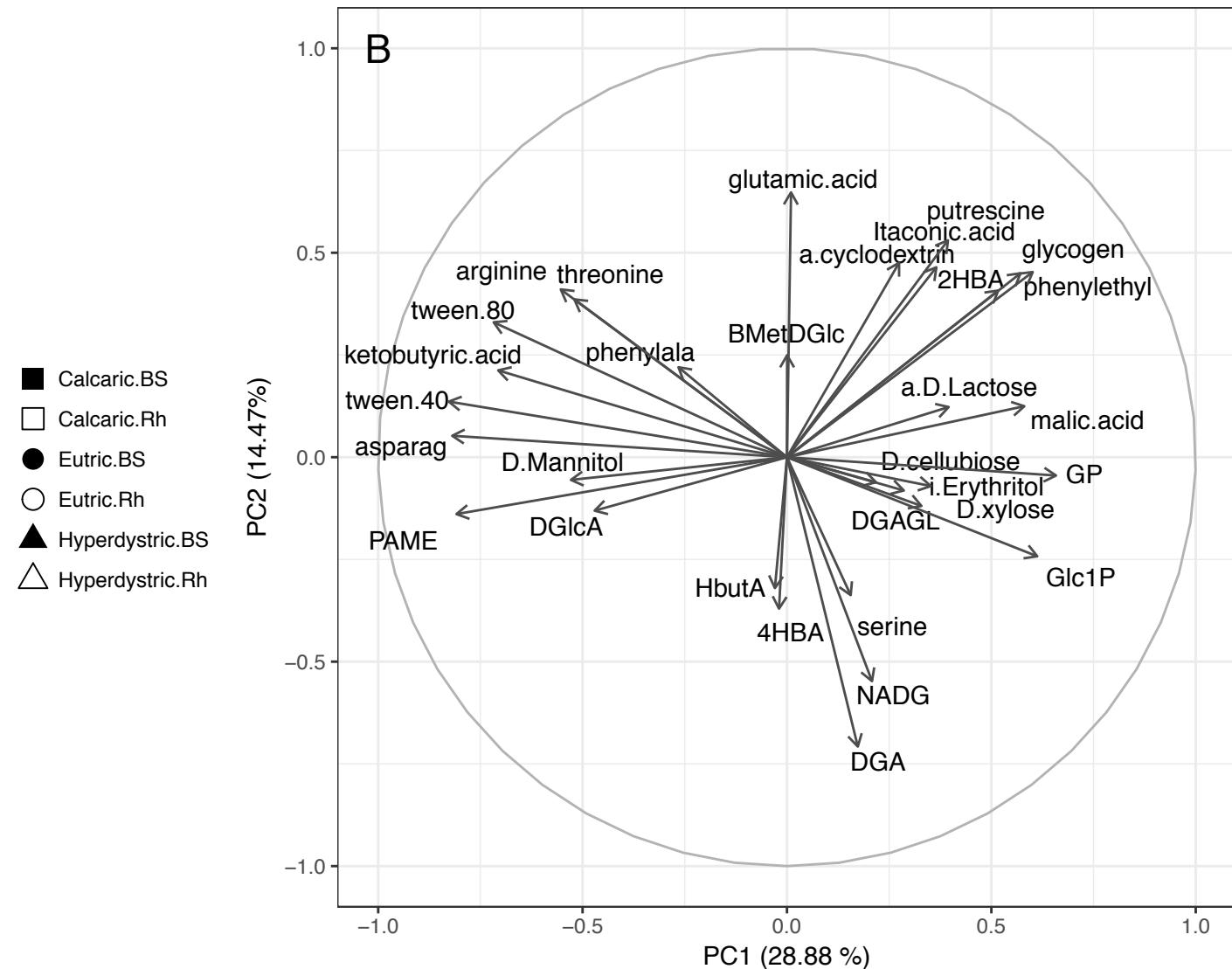
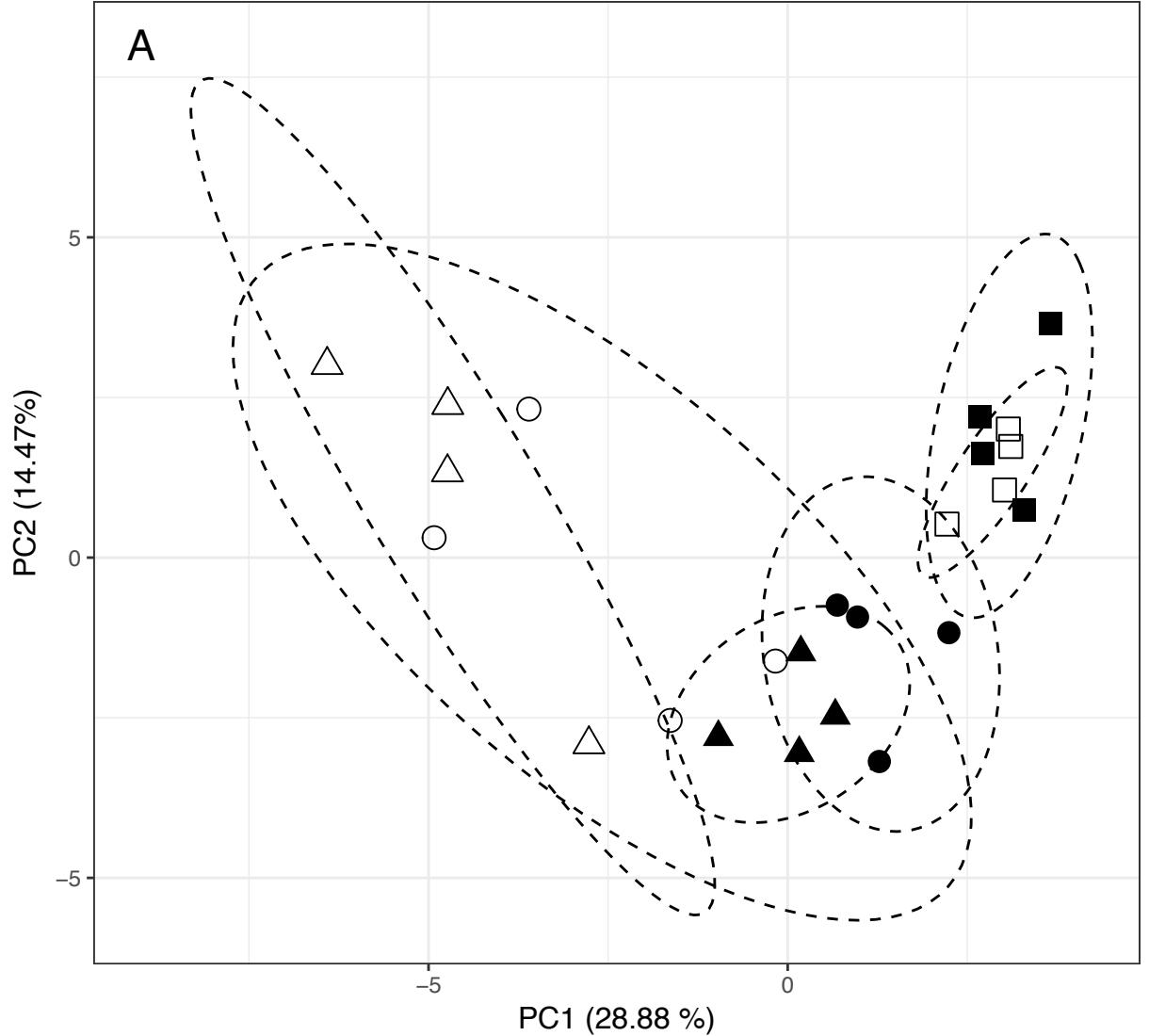


Figure.S2 Rarefaction curves indicating the number of operational taxonomic units (OTUs) at a genetic distance of 3% in Cambisol Calcaric (A), Eutric (B) and Hyperdystric (C) soils. The full and dotted lines refer to the rhizosphere and bulk soil samples, respectively. Samples are referred as follow: Calcaric.BS: Cambisol Calcaric bulk soil; Calcaric.Rh: Cambisol Calcaric rhizosphere; Eutric.BS: Cambisol Eutric bulk soil; Eutric.Rh: Cambisol Eutric rhizosphere; Hyperdystric.BS: Cambisol Hyperdystric bulk soil; Hyperdystric.Rh: Cambisol Hyperdystric rhizosphere.

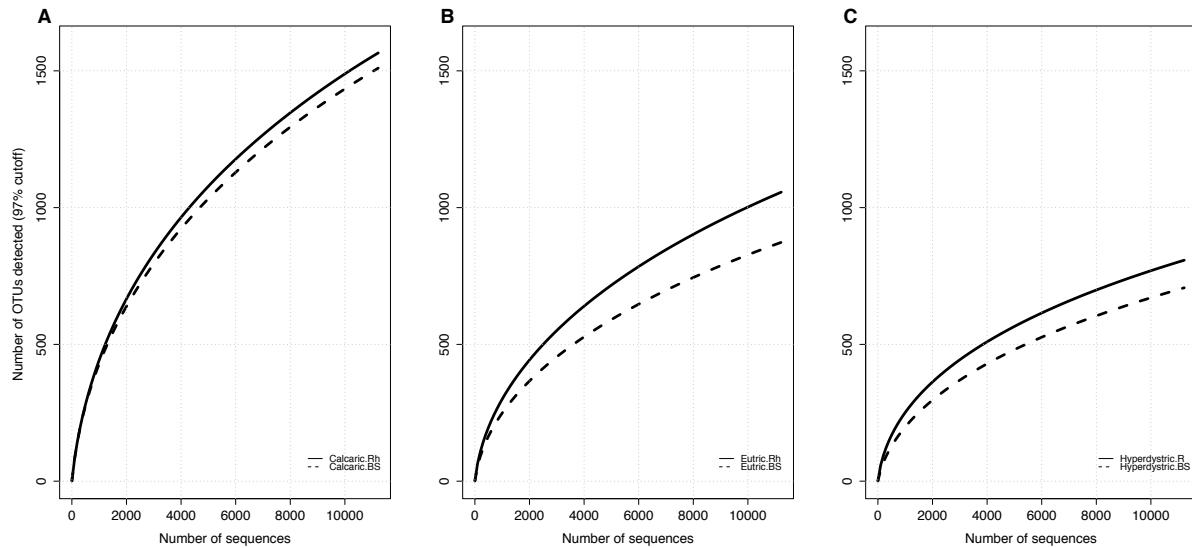


Figure S3. Shift in taxonomic diversity of bulk soil and rhizosphere-associated bacterial communities across the soil toposequence of Montiers and between bulk soil and rhizosphere compartments in each soil type. Multivariate analyses were performed at the OTU level and were conducted separately on bulk soil (BS, **Panel A**) and rhizosphere (Rh, **Panel B**) samples or on each soil types (**Calcaric samples, C**; **Eutric samples, D**; **Hyperdystric samples, E**) to determine a potential rhizosphere effect. The origins of samples are indicated as follows: filled orange squares, bulk soil samples from Calcaric; open orange squares, rhizosphere samples from Calcaric; filled green circles, bulk soil samples from Eutric, open green circles, rhizosphere samples from Eutric; filled blue triangles, bulk soil samples from Hyperdystric; open blue triangles, rhizosphere samples from Hyperdystric. Ellipses correspond to 95% confidence intervals about the mean.

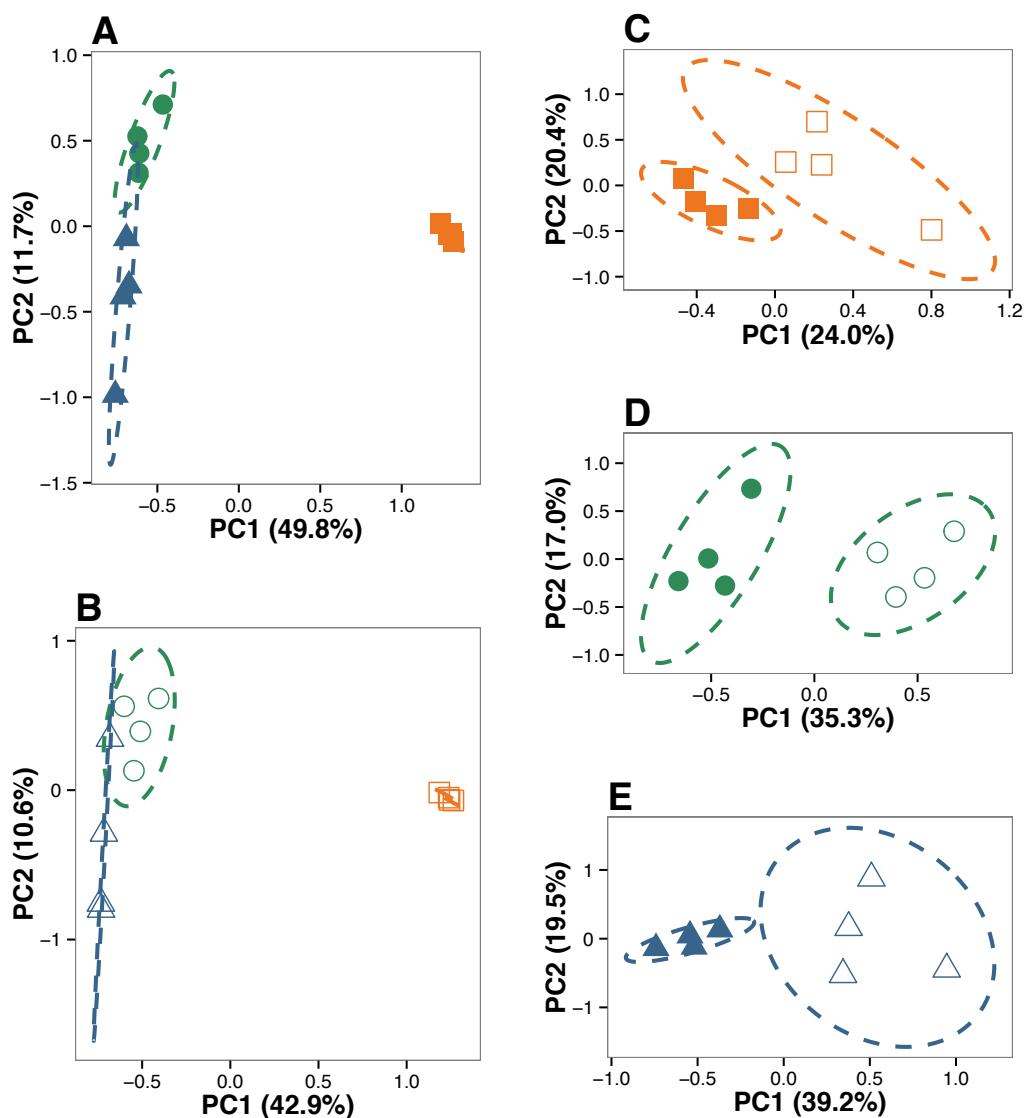


Figure.S4 Estimation of the number of functional genes shared: (A) among the bulk soil (BS) samples or the rhizosphere (Rh) samples across the toposequence and (B) between bulk soil and rhizosphere samples for each soil type.

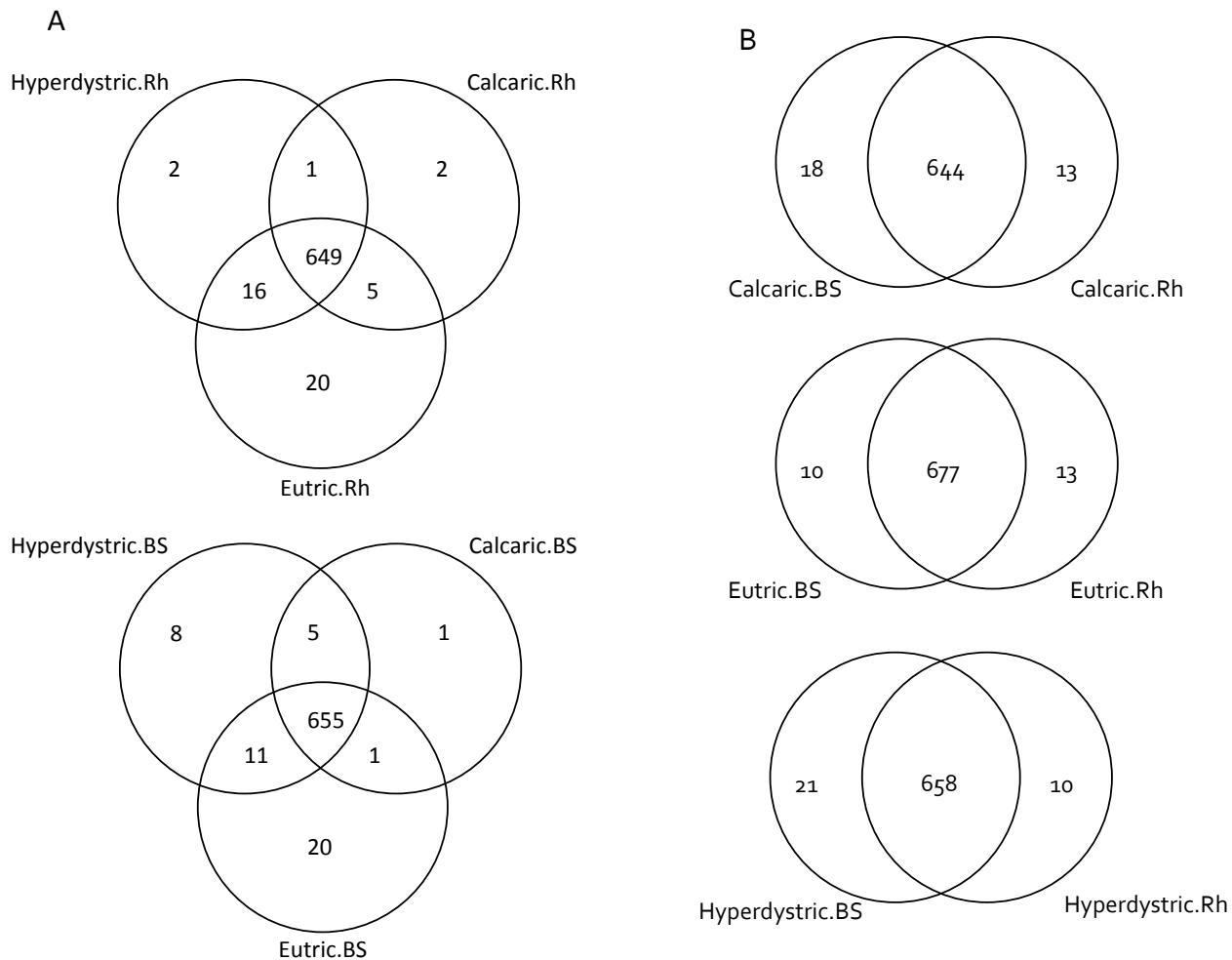


Figure.S5: Normalized signal intensity of probes detected from different gene categories. The signal intensities are the mean of detected individual probes averaged among the 3 biological replicates in the Cambisol Calcaric (A), Eutric (B) and Hyperdystric (C). All data are presented as mean (\pm Standard Error of the Mean). The significant differences among the bulk soil samples and their respective rhizosphere ($P < 0.05$) are indicated above the bars (*).

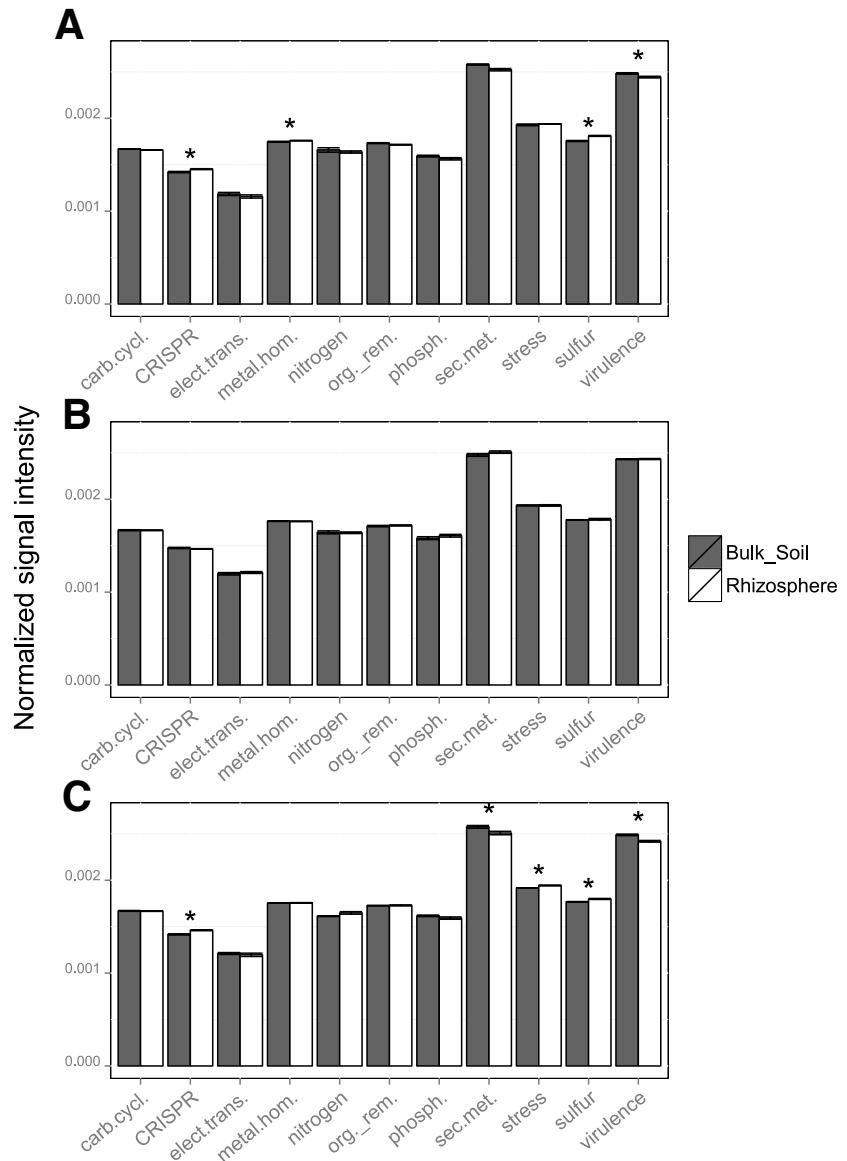


Figure.S6 Normalized signal intensity of genes involved in metal transport. The signal intensities are the mean of detected individual genes averaged among the 3 replicates from the Cambisol Calcaric (A), Eutric (B) and Hyperdystric (C). All data are presented as mean (\pm Standard Error of the Mean). The significant differences among the bulk soil (BS, grey) samples and their respective rhizosphere (R, white) are indicated by an asterisk above the bars ($p < 0.05$).

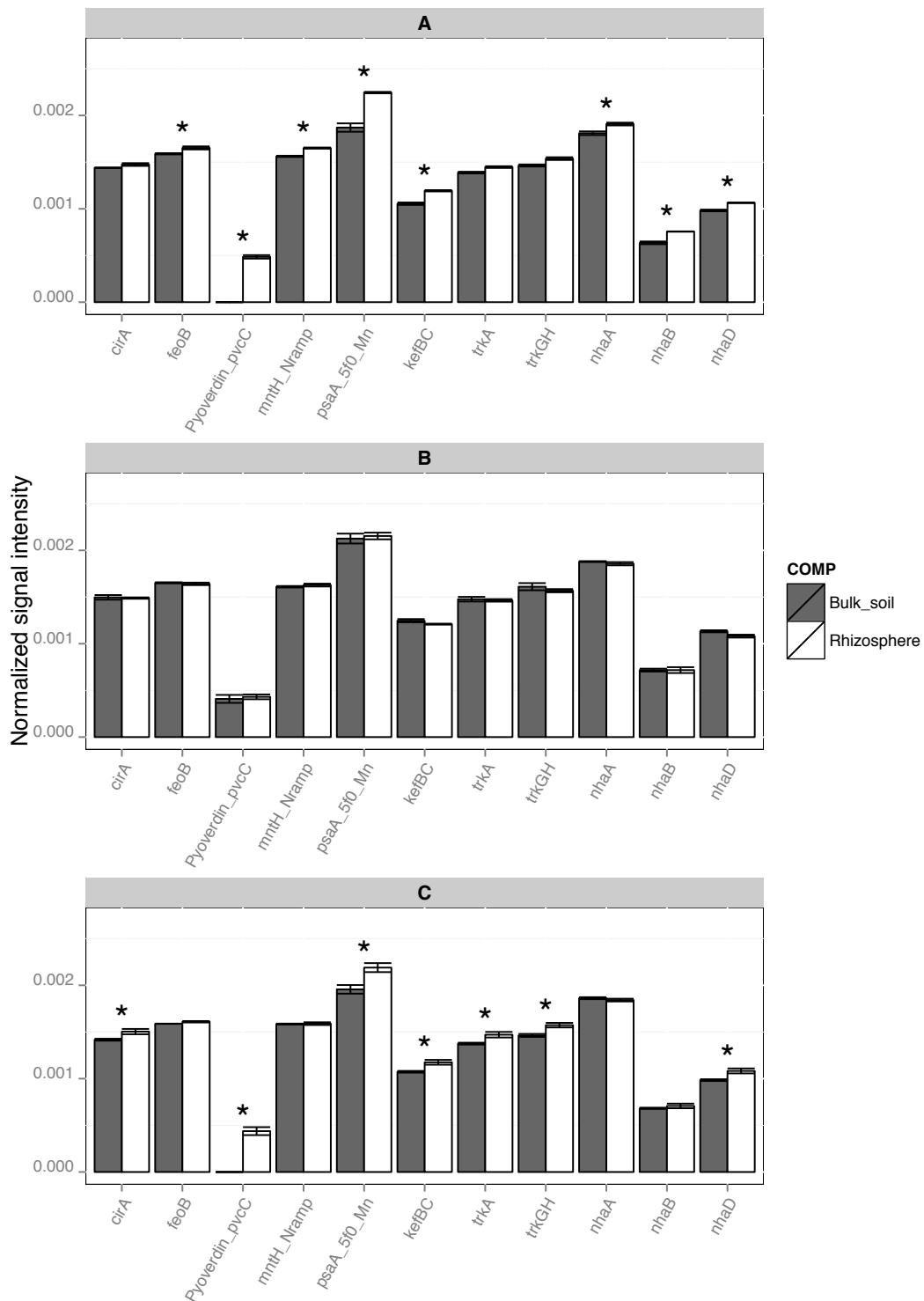


Figure S7. Heatmap analysis of genes involved in nutrient cycling. The signal intensities are the mean of detected individual probes averaged among the 3 replicates for each compartment and soil type considered. Blue indicates signal intensities above background, whereas white indicates signal intensities below background. For each heatmap, the probe code (geochip code) and the taxonomic assignation are presented and the data are organized from the probes giving the higher signal at the top to the probes giving the lower signal at the bottom. For each function, only the top 10 is presented.



Figure.S8 Normalized signal intensity of genes involved in the CRISPR system. The signal intensities are the mean of detected individual genes averaged among the 3 replicates from the Cambisol Calcaric (A), Eutric (B) and Hyperdystric (C). All data are presented as mean (\pm Standard Error of the Mean). The significant differences among the bulk soil (BS, grey) samples and their respective rhizosphere (R, white) are indicated by an asterisk above the bars ($p < 0.05$).

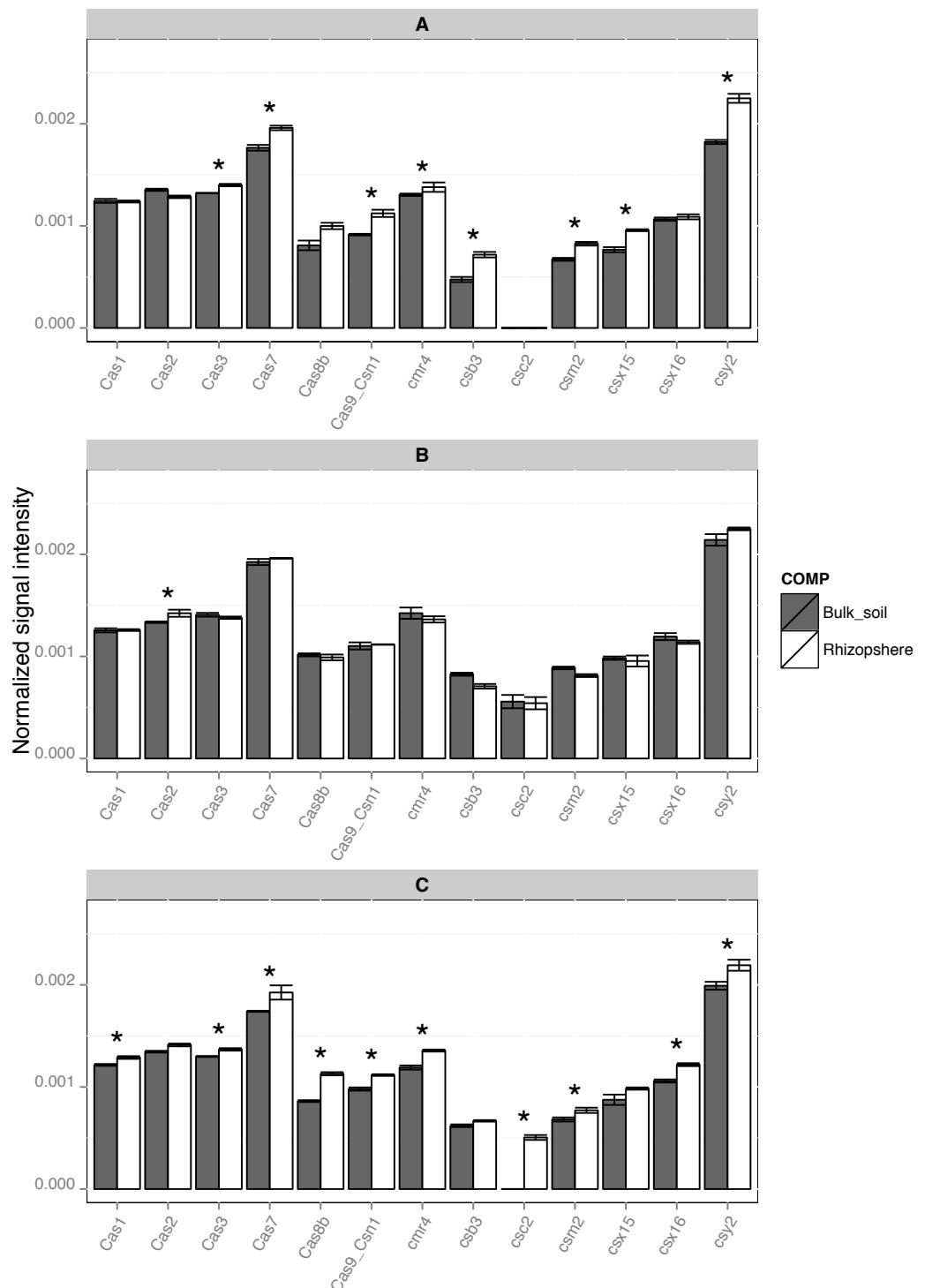


Figure S9. Heatmap analysis of genes involved in microbial interactions. The signal intensities are the mean of detected individual probes averaged among the 3 replicates for each compartment and soil type considered. Blue indicates signal intensities above background, whereas white indicates signal intensities below background. For each heatmap, the probe code (geochip code) and the taxonomic assignation are presented and the data are organized from the probes giving the higher signal at the top to the probes giving the lower signal at the bottom. For each function, only the top 10 is presented.



Figure.S10 Normalized signal intensity of genes involved in carbon degradation. The signal intensities are the mean of detected individual genes averaged among the 3 replicates from the Calcaric (A), Eutric (B) and Hyperdystric (C). All data are presented as mean (\pm Standard Error of the Mean). The significant differences among the bulk soil (BS, grey) samples and their respective rhizosphere (R, white) are indicated by an asterisk above the bars ($p < 0.05$).

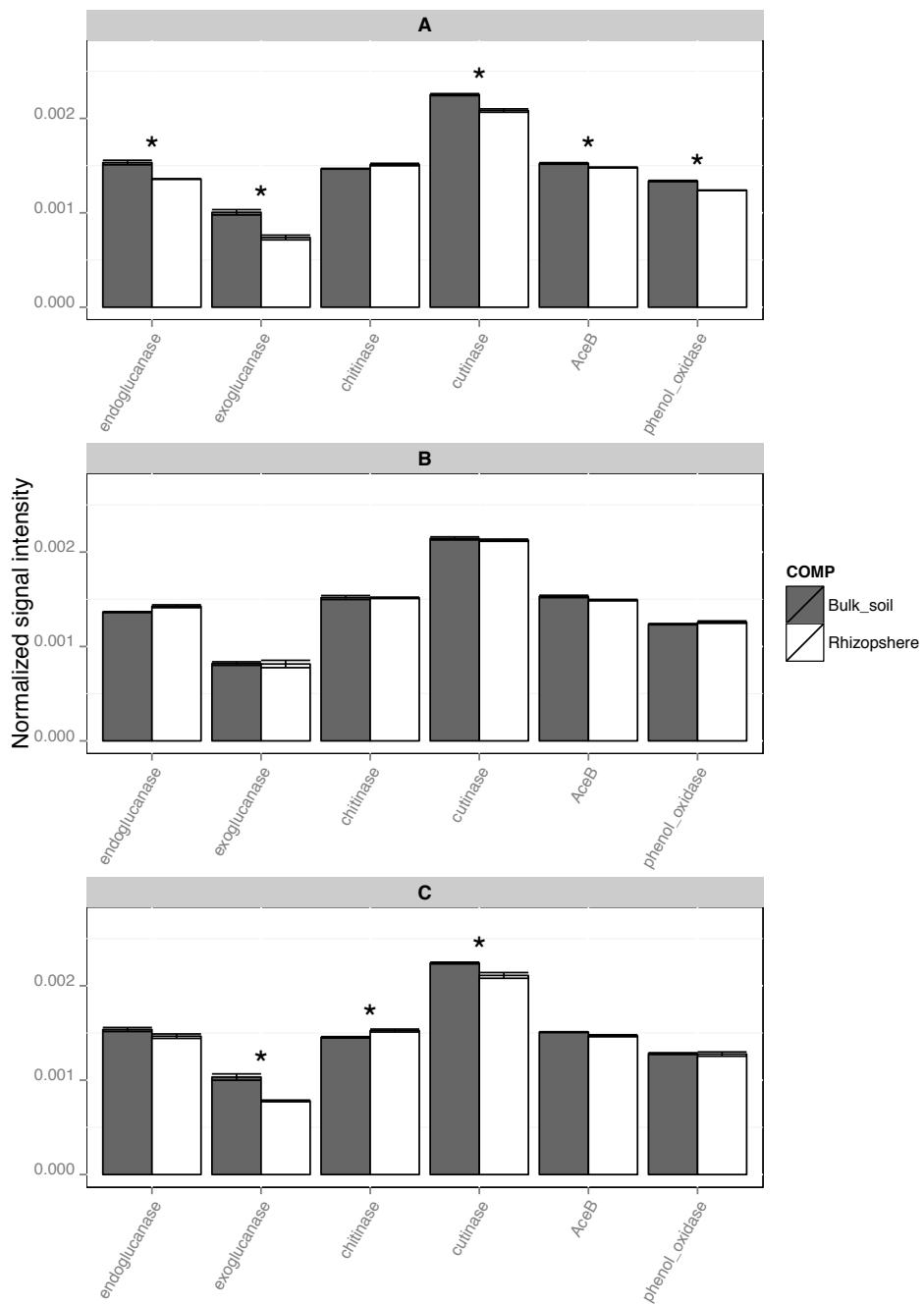


Figure S11. Heatmap analysis of genes involved in carbon cycling. The signal intensities are the mean of detected individual probes averaged among the 3 replicates for each compartment and soil type considered. Blue indicates signal intensities above background, whereas white indicates signal intensities below background. For each heatmap, the probe code (geochip code) and the taxonomic assignation are presented and the data are organized from the probes giving the higher signal at the top to the probes giving the lower signal at the bottom. For each function, only the top 10 is presented.

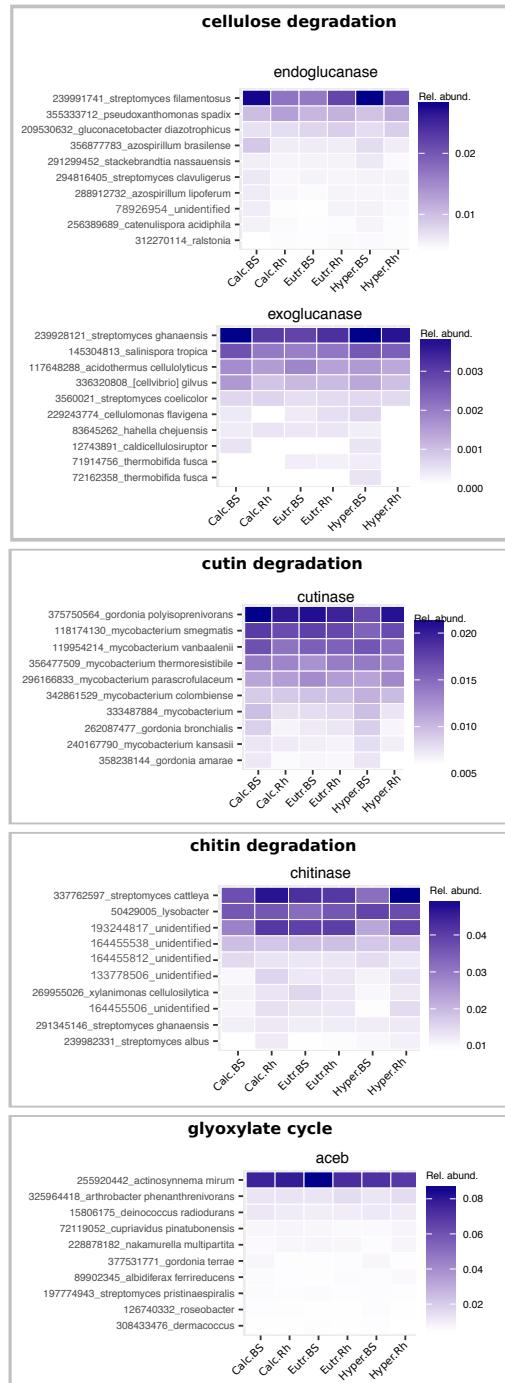


Figure S12: Partitioning of the variance in soil bacterial communities by explanatory variables (soil type and compartment). Barplots represent the variance explained by the soil variables effect, the compartment effect as well as the unexplained variance for each data measured (16S rRNA pyrosequences, Geochip functional categories and Biolog data).

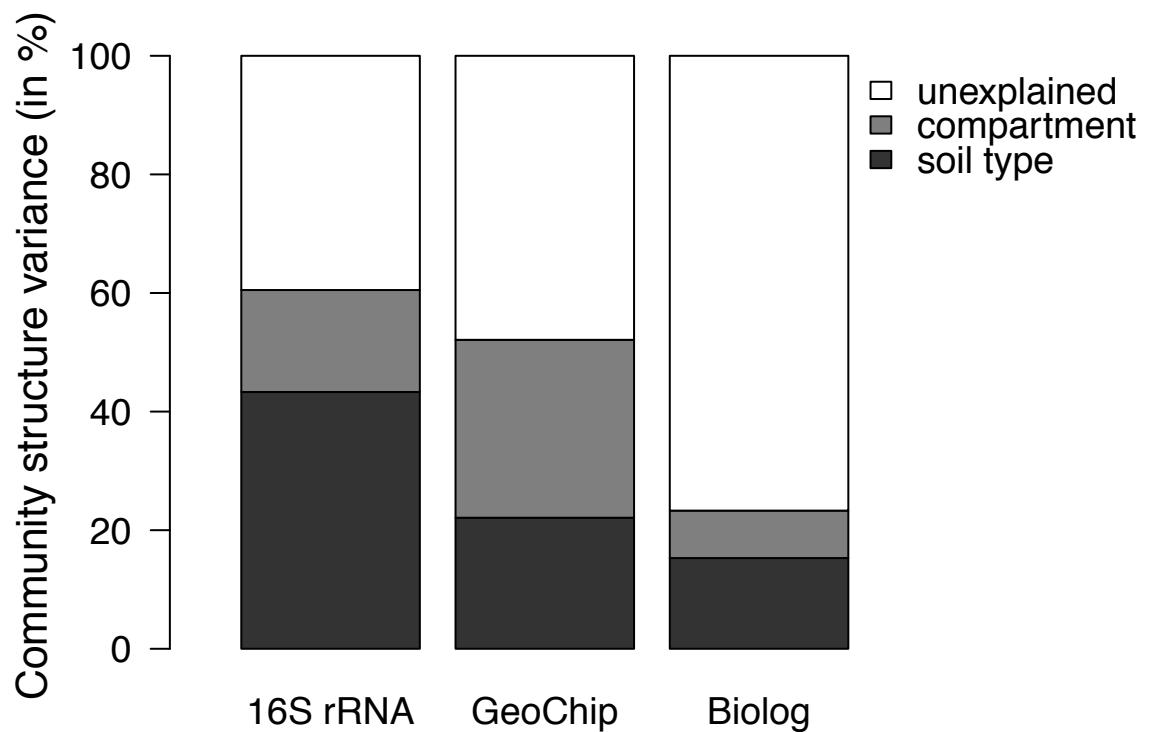


Table S1: Physico-chemical characteristics of the soil samples collected along the toposequence of Montiers. Soil analyses have been done at the Soil analysis platform of Arras (INRA, France) according to the protocols described in the material and methods section. Values are averages of 4 replicates (n=4). Several parameters measured have been abbreviated in the table as follows: Water cont., Water content; N, Total, total nitrogen; C tot, Total carbon; CEC, Cationic exchange capacity; P. Duch, Phosphorus extracted according to the Duchaufour method; P. Ols, Phosphorus extracted according to the Olsen method; OM, Organic matter; Limest., Limestone; H+, Exchangeable protons. For each soil parameter, significant differences are presented by different letters (a,b,c; P<0.05)

Soil type	pH	Water cont.	N tot	C tot	C/N	P.Duch	P.Ols	OM	Limest.
		(in %)	(g/kg)				(g/kg)		
Calcaric	7.14 ^a ± 0.03	39.65 ^a ± 1.55	4.89 ^a ± 0.40	66.88 ^a ± 6.25	13.65 ^a ± 0.19	0.34 ^a ± 0.03	0.04 ^a ± 0.00	115.63 ^a ± 10.81	7.25± 1.03
Eutric	5.01 ^b ± 0.04	16.83 ^b ± 0.77	1.46 ^b ± 0.09	21.15 ^b ± 0.54	14.63 ^a ± 0.52	0.22 ^a ± 0.02	0.01 ^a ± 0.00	36.55 ^b ± 0.93	<1
Hyperdystric	4.63 ^c ± 0.04	11.50 ^c ± 0.33	1.19 ^b ± 0.07	17.20 ^b ± 0.74	14.53 ^a ± 0.53	0.38 ^a ± 0.13	0.03 ^a ± 0.02	29.78 ^b ± 1.30	<1
Soil type	CEC	H+	Mg	Ca	K	Na	Mn	Al	Fe
					cmol+/kg				
Calcaric	48.30 ^a ± 2.04	<0.05	0.81 ^a ± 0.14	48.80 ^a ± 2.03	0.29 ^a ± 0.01	0.07 ^a ± 0.00	0.02 ^c ± 0.00	0.11 ^c ± 0.01	0.02 ^a ± 0.00
Eutric	6.45 ^b ± 0.59	0.32 ^a ± 0.01	0.25 ^b ± 0.04	2.51 ^b ± 0.61	0.10 ^b ± 0.01	0.04 ^b ± 0.00	0.12 ^b ± 0.03	3.06 ^a ± 0.22	0.03 ^a ± 0.00
Hyperdystric	2.66 ^b ± 0.16	0.33 ^a ± 0.01	0.08 ^b ± 0.01	0.26 ^b ± 0.05	0.07 ^b ± 0.01	0.01 ^c ± 0.00	0.23 ^a ± 0.03	2.17 ^b ± 0.10	0.02 ^a ± 0.00

Table S2: Estimates of the taxonomic (16S rRNA gene libraries), functional (GeoChip microarray and Biolog) bacterial diversity for bulk soil and rhizosphere samples along the toposequence of Montiers. Each value is the mean of biological replicates (\pm Standard Error of the Mean). For each indicator, significant differences are presented by different letters ($P<0.05$).

Samples	Compartment	16S rRNA gene libraries				GeoChip microarray		Biolog Ecoplate	
		No. of OTUs (0.03%)	Shannon index	Chao1 index	Coverage	No. of genes	Shannon index	No. of substrates metabolized	Shannon index
Calcaric	Bulk Soil	1510 ^a \pm 10	6.12 ^a \pm 0.02	2444 ^a \pm 19	0.94 \pm 0.001	655 ^b \pm 7.3	6.48 ^b \pm 0.01	28.50 ^a \pm 2.38	3.17 ^a \pm 0.13
Calcaric	Rhizosphere	1566 ^a \pm 44	6.16 ^a \pm 0.07	2478 ^a \pm 57	0.94 \pm 0.001	654 ^b \pm 0.3	6.48 ^b \pm 0.00	28.50 ^a \pm 1.29	3.18 ^a \pm 0.03
Eutric	Bulk Soil	881 ^b \pm 65	4.44 ^{cd} \pm 0.24	1523 ^{bc} \pm 121	0.96 \pm 0.003	681 ^a \pm 1.0	6.52 ^a \pm 0.00	29.25 ^a \pm 1.50	3.02 ^{ab} \pm 0.09
Eutric	Rhizosphere	1057 ^b \pm 66	5.18 ^b \pm 0.12	1767 ^b \pm 69	0.96 \pm 0.002	683 ^a \pm 6.8	6.53 ^a \pm 0.01	24.25 ^{ab} \pm 4.57	2.73 ^c \pm 0.21
Hyperdystric	Bulk Soil	707 ^c \pm 59	3.78 ^d \pm 0.25	1197 ^c \pm 113	0.97 \pm 0.003	674 ^{ab} \pm 3.5	6.51 ^{ab} \pm 0.01	27.00 ^{ab} \pm 1.82	2.87 ^{bc} \pm 0.06
Hyperdystric	Rhizosphere	807 ^c \pm 23	4.76 ^{bc} \pm 0.18	1299 ^c \pm 72	0.97 \pm 0.002	662 ^{ab} \pm 2.2	6.50 ^{ab} \pm 0.00	22.75 ^b \pm 1.71	2.69 ^c \pm 0.07

Table.S3: Changes in taxonomic composition along the toposequence for bulk soil and rhizosphere samples. Text in bold indicates significant differences. Samples are referred as follow: CC: Calcaric Cambisol; EC: Eutric Cambisol; HC: Hyperdystric Cambisol. The symbols ‘>’ and ‘<’ mean significantly more or less abundant and ‘=’ not significantly different.

BULK_SOIL SAMPLES			RHIZOSPHERE SAMPLES		
PHYLUM	CLASS	ORDER	PHYLUM	CLASS	ORDER
Acidobacteria HC>EC>CC	Acidobacteria_Gp1 CC>EC>HC	Acidobacteria_Gp1 HC>EC>CC	Acidobacteria HC>EC>CC	Acidobacteria_Gp1 HC>EC>CC	Acidobacteria_Gp1 HC>EC>CC
	Acidobacteria_Gp2 CC>EC>HC	Acidobacteria_Gp2 HC>EC>CC		Acidobacteria_Gp2 HC>EC>CC	Acidobacteria_Gp2 HC>EC>CC
	Acidobacteria_Gp3 EC>CC	Acidobacteria_Gp3 EC>CC		Acidobacteria_Gp3 HC>EC>CC	Acidobacteria_Gp3 HC>EC>CC
	Acidobacteria_Gp4	Acidobacteria_Gp4		Acidobacteria_Gp4	Acidobacteria_Gp4
	Acidobacteria_Gp5 CC>EC>HC	Acidobacteria_Gp5 CC>EC>HC		Acidobacteria_Gp5 CC>EC>HC	Acidobacteria_Gp5 CC>EC>HC
	Acidobacteria_Gp6	Acidobacteria_Gp6		Acidobacteria_Gp6 CC>EC>HC	Acidobacteria_Gp6 CC>EC>HC
	Acidobacteria_Gp7	Acidobacteria_Gp7		Acidobacteria_Gp7 CC>EC>HC	Acidobacteria_Gp7 CC>EC>HC
	Acidobacteria_Gp11 CC>EC>HC	Acidobacteria_Gp11 CC>EC>HC		Acidobacteria_Gp11 CC>EC>HC	Acidobacteria_Gp11 CC>EC>HC
	Acidobacteria_Gp22 CC>EC>HC	Acidobacteria_Gp22 CC>EC>HC		Acidobacteria_Gp22 CC>EC>HC	Acidobacteria_Gp22 CC>EC>HC
	Acidobacteria_Gp25 CC>EC>HC	Acidobacteria_Gp25 CC>EC>HC		Acidobacteria_Gp25 ND	Acidobacteria_Gp25 ND
Holophagae Unclassified CC>EC>HC	Holophagae	Holophagae		Holophagae	Holophagae
	Unclassified CC>EC>HC	Unclassified CC>EC>HC		Unclassified CC>EC	Unclassified CC>EC
Actinobacteria CC>EC=HC	Actinomicrobiales CC>EC>HC	Actinomycetales CC>HC	Actinobacteria	Actinobacteria	Actinobacteria
	Actinobacteria CC>EC>HC	Bifidobacteriales ND		Actinobacteria	Actinobacteriales ND
	unclassified CC>EC>HC	Solirubrobacteriales CC>EC>HC		Solirubrobacteriales CC>EC>HC	unclassified CC>HC
Bacteroidetes CC>EC=HC	Flavobacteriales CC>EC>HC	Flavobacteriales CC>EC>HC	Bacteroidetes CC>EC=HC	Flavobacteriales CC>EC>HC	Flavobacteriales CC>EC>HC
	Sphingobacteriales CC>EC>HC	Sphingobacteriales CC>EC>HC		Sphingobacteriales CC>EC unclassified CC>EC>HC	Sphingobacteriales CC>EC unclassified CC>EC>HC
	unclassified CC>EC>HC	unclassified CC>EC>HC		unclassified CC>EC>HC	unclassified CC>EC>HC
Chlamydiae	Chlamydiales	Chlamydiales	Chlamydiae	Chlamydiales	Chlamydiales
Chloroflexi CC>EC=HC	Chloroflexi CC>EC>HC	Chloroflexi CC>EC>HC	Chloroflexi CC>EC=HC	Chloroflexi CC>EC>HC	Chloroflexi CC>EC>HC
	Unclassified CC>EC=HC	Unclassified CC>EC=HC		Unclassified CC>EC=HC	Unclassified CC>EC=HC
Deinococcus	Deinococcus	Deinococcus	Deinococcus ND	Deinococcus ND	Deinococcales ND
Firmicutes CC>EC=HC	Bacillales CC>EC>HC	Bacillales CC>EC>HC	Firmicutes CC>EC=HC	Bacillales CC>EC>HC	Bacillales CC>EC>HC
	Bacilli CC>EC>HC	Lactobacillales ND		Bacilli CC>EC>HC	Lactobacillales ND
	Clostridia	Clostridia		Clostridia unclassified	Clostridia unclassified
Gemmamimotes CC>HC	Gemmamimotes CC>HC	Gemmamimotes CC>HC	Gemmamimotes CC>EC=HC	Gemmamimotes CC>EC=HC	Gemmamimotes CC>EC=HC
Nitrospira CC>EC=HC	Nitrospira CC>EC=HC	Nitrospira CC>EC=HC	Nitrospira CC>EC=HC	Nitrospira CC>EC=HC	Nitrospira CC>EC=HC
OP10	OP10	OP10	OP10 EC>CC	OP11 EC>CC	OP11 EC>CC
Planctomycetes	Planctomycetes	Planctomycetes	Planctomycetes	Planctomycetes	Planctomycetes
Proteobacteria CC>EC=HC	Alphaproteobacteria CC>HC	Caulobacteriales CC>EC=HC	Alphaproteobacteria	Rhizobiales CC>EC=HC	Rhizobiales CC>EC=HC
		Rhodobacteriales CC>EC=HC		Rhodospirillales EC>CC	Rhodospirillales EC>CC
		Rhodospirillales EC>CC		Rickettsiales CC>EC=HC	Rickettsiales CC>EC=HC
		Sphingomonadales CC>EC=HC		Sphingomonadales CC>EC=HC	Sphingomonadales CC>EC=HC
		unclassified CC>EC=HC		unclassified CC>EC=HC	unclassified CC>EC=HC
	Betaproteobacteria CC>EC=HC	Burkholderiales CC>EC=HC	Betaproteobacteria	Methylphilales CC>EC=HC	Methylphilales CC>EC=HC
		Neisseriales ND		Neisseriales ND	Neisseriales ND
		Nitrosomonadales CC>EC=HC		Rhodocyclales CC>EC=HC	Rhodocyclales CC>EC=HC
		Rhodocyclales CC>EC=HC		unclassified CC>EC=HC	unclassified CC>EC=HC
		unclassified CC>EC=HC		unclassified CC>EC=HC	unclassified CC>EC=HC
Deltaproteobacteria CC>EC=HC	Deltaproteobacteria CC>EC=HC	Bdellovibrionales CC>EC=HC	Deltaproteobacteria	Desulfobacteriales CC>EC=HC	Desulfobacteriales CC>EC=HC
		Desulfobacteriales ND		Desulfomoradales CC>EC=HC	Desulfomoradales CC>EC=HC
		Desulfomoradales CC>EC=HC		Myxococcales CC>EC=HC	Myxococcales CC>EC=HC
		Myxococcales CC>EC=HC		unclassified CC>EC=HC	unclassified CC>EC=HC
		unclassified CC>EC=HC		Xanthomonadales CC>EC=HC	Xanthomonadales CC>EC=HC
Gammaproteobacteria CC>HC	Gammaproteobacteria CC>HC	Aeromonadales CC>EC=HC	Gammaproteobacteria	Alteromonadales ND	Alteromonadales ND
		Enterobacteriales CC>EC=HC		Chromatiales ND	Chromatiales ND
Unclassified CC>EC=HC	Unclassified CC>EC=HC	Pseudomonadales CC>EC=HC	Unclassified CC>EC=HC	Enterobacteriales CC>EC=HC	Enterobacteriales CC>EC=HC
		Xanthomonadales CC>EC=HC		Pseudomonadales unclassified CC>EC=HC	Pseudomonadales unclassified CC>EC=HC
Spirochaetes ND	Spirochaetes ND	Spirochaetales ND	Spirochaetes CC>EC=HC	Spirochaetales CC>EC=HC	Spirochaetales CC>EC=HC
TM7	TM7	TM7	TM7	TM7	TM7
Verrucomicrobia CC>EC=HC	Spartobacteria CC>EC=HC	Opitutales ND	Verrucomicrobia HC>CC	Opitutales ND	Opitutales ND
		Spartobacteria CC>EC=HC		Spartobacteria	Spartobacteria
		Subdivision3 CC>HC		Subdivision3 CC>HC	Subdivision3 CC>HC
		unclassified CC>EC=HC		unclassified CC>EC=HC	unclassified CC>EC=HC
WS3 CC>EC=HC	WS3 CC>EC=HC	unclassified CC>EC=HC	WS3	WS3	WS3

Table S4 Part 1: Changes in taxonomic composition between bulk soil and rhizosphere samples for each soil type. Text in red and blue indicates a significant increase in rhizosphere and bulk soils samples, respectively. Samples are referred as follow: BS: Bulk Soil; Rh: Rhizosphere. The symbols '>' and '<' mean significantly more or less abundant and '=' not significantly different.

CALCARIC CAMBISOL				EUTRIC CAMBISOL				HYPERDYSTRIC CAMBISOL						
PHYLUM	CLASS	ORDER	FAMILY	PHYLUM	CLASS	ORDER	FAMILY	PHYLUM	CLASS	ORDER	FAMILY			
Acidobacteria BS>Rh	Gp1	Gp1	Gp1	Acidobacteria	Gp1	Gp1	Gp1	Acidobacteria	Gp1	Gp1	Gp1			
	Gp11 BS>Rh	Gp11 BS>Rh	Gp11 BS>Rh		Gp11 ND	Gp11 ND	Gp11 ND		Gp11	Gp11	Gp11			
	Gp2	Gp2	Gp2		Gp2 BS>Rh	Gp2 BS>Rh	Gp2 BS>Rh		Gp2	Gp2	Gp2			
	Gp22 BS>Rh	Gp22 BS>Rh	Gp22 BS>Rh		Gp22	Gp22	Gp22		Gp22 ND	Gp22 ND	Gp22 ND			
	Gp25 BS>Rh	Gp25 BS>Rh	Gp25 BS>Rh		Gp25	Gp25	Gp25		Gp25 ND	Gp25 ND	Gp25 ND			
	Gp3	Gp3	Gp3		Gp3	Gp3	Gp3		Gp3	Gp3	Gp3			
	Gp4	Gp4	Gp4		Gp4	Gp4	Gp4		Gp4 ND	Gp4 ND	Gp4 ND			
	Gp5 BS>Rh	Gp5 BS>Rh	Gp5 BS>Rh		Gp5	Gp5	Gp5		Gp5 BS>Rh	Gp5 BS>Rh	Gp5 BS>Rh			
Actinobacteria	Gp6	Gp6	Gp6	Actinobacteria	Gp6	Gp6	Gp6	Actinobacteria	Gp6	Gp6	Gp6			
	Gp7	Gp7	Gp7		Gp7	Gp7	Gp7		Gp7	Gp7	Gp7			
	Holophagae ND	Holophagae ND	Holophagae ND		Holophagae unclassified	Holophagae unclassified	Holophagae unclassified		Holophagae unclassified	Holophagae unclassified	Holophagae unclassified			
	Unclassified BS>Rh	Unclassified BS>Rh	Unclassified BS>Rh		Unclassified	Unclassified	Unclassified		Unclassified	Unclassified	Unclassified			
	Acidimicrobiales				Acidimicrobiales				Acidimicrobiales					
	lamellales				lamellales				lamellales					
	Acidothermaceae				Acidothermaceae				Acidothermaceae					
	Actinomycetaceae				Actinomycetaceae				Actinomycetaceae					
	Actinospiraceae				Actinospiraceae Rh>BS				Actinospiraceae Rh>BS					
	Actinomytaceae				Actinomytaceae				Actinomytaceae					
	Actinomycetomorphaceae				Actinomycetomorphaceae				Actinomycetomorphaceae					
	Catenuliporaceae				Catenuliporaceae				Catenuliporaceae					
	Cellulomonadaceae				Cellulomonadaceae				Cellulomonadaceae					
	Corynebacteriaceae				Corynebacteriaceae				Corynebacteriaceae					
	Cryptosporangiaceae				Cryptosporangiaceae				Cryptosporangiaceae					
	Geodermatophilaceae				Geodermatophilaceae				Geodermatophilaceae					
	Glycomycetaceae				Glycomycetaceae				Glycomycetaceae					
	Intrasporangiaceae				Intrasporangiaceae				Intrasporangiaceae					
	Klebsiellaceae				Klebsiellaceae				Klebsiellaceae					
	Microbacteriaceae				Microbacteriaceae				Microbacteriaceae					
	Microccocaceae				Microccocaceae				Microccocaceae					
	Micromonosporaceae Rh>BS				Micromonosporaceae Rh>BS				Micromonosporaceae Rh>BS					
	Mycobacteriaceae				Mycobacteriaceae				Mycobacteriaceae					
	Nakamurellaceae				Nakamurellaceae				Nakamurellaceae					
	Nocardiaceae				Nocardiaceae				Nocardiaceae					
	Nocardioidaceae				Nocardioidaceae				Nocardioidaceae					
	Promicromonosporaceae				Promicromonosporaceae				Promicromonosporaceae					
	Propionibacteriaceae				Propionibacteriaceae				Propionibacteriaceae					
	Pseudonocardiaceae				Pseudonocardiaceae Rh>BS				Pseudonocardiaceae Rh>BS					
	Sporichthyaceae				Sporichthyaceae				Sporichthyaceae					
	Streptomyctecaceae Rh>BS				Streptomyctecaceae Rh>BS				Streptomyctecaceae Rh>BS					
	Streptosporangiaceae				Streptosporangiaceae				Streptosporangiaceae					
	Thermomonosporaceae				Thermomonosporaceae				Thermomonosporaceae					
	unclassified				unclassified Rh>BS				unclassified Rh>BS					
Bacteroidetes BS>Rh	Bifidobacteriales				Bifidobacteriales				Bifidobacteriales					
	Sphingobacteriales				Sphingobacteriales				Sphingobacteriales					
	unclassified				unclassified				unclassified					
	Sphingobacteriaceae				Sphingobacteriaceae				Sphingobacteriaceae					
Chlamydiae	Chlamydiae	Chlamydiales	Parachlamydaceae	Chlamydiales	Chlamydiales	Chlamydiales	Parachlamydaceae	Chlamydiales	Chlamydiales	Chlamydiales	Parachlamydaceae			
Chloroflexi	Chloroflexi unclassified BS>Rh	unclassified	Chloroflexi unclassified BS>Rh	Chloroflexi unclassified BS>Rh	Chloroflexi unclassified	Chloroflexi unclassified	Chloroflexi unclassified BS>Rh							
Deinococcus	Deinococci	Deinococcales	Deinococcaceae	Deinococci	Deinococci	Deinococci	Deinococcaceae	Deinococci	Deinococci	Deinococci	Deinococcaceae			
Firmicutes	Bacilli	Bacillales	Alicyclobacillaceae ND	Firmicutes Rh>BS	Bacilli Rh>BS	Bacillales Rh>BS	Alicyclobacillaceae ND	Bacilli Rh>BS	Bacilli	Bacillales	Alicyclobacillaceae ND			
	Lactobacillales	unclassified ND	Bacillaceae		Bacillaceae	Paenibacillaceae	Bacillaceae	Bacillaceae	Bacilli	Bacillales	Bacillaceae			
	Clostridia	Clostridiales	Paenibacillaceae		Paenibacillaceae	Planococcaceae	Paenibacillaceae	Planococcaceae	Clostridia	Clostridiales	Paenibacillaceae			
	unclassified	unclassified	Staphylococcaceae		Staphylococcaceae	Staphylococcaceae	Staphylococcaceae	Staphylococcaceae	Clostridiales	Clostridiales	Staphylococcaceae			
	unclassified	unclassified	Enterococcaceae		Enterococcaceae	Enterococcaceae	Enterococcaceae	Enterococcaceae	unclassified	unclassified	Enterococcaceae			
	unclassified	unclassified	Clostridiaceae		Clostridiaceae	Gracilimonococcaceae	Clostridiaceae	Gracilimonococcaceae	Clostridiales	Clostridiales	Gracilimonococcaceae			
	unclassified	unclassified	Lachnospiraceae		Lachnospiraceae	Lachnospiraceae	Lachnospiraceae	Lachnospiraceae	unclassified	unclassified	Lachnospiraceae			
	unclassified	unclassified	Villonellaceae		Villonellaceae	Ruminococcaceae	Villonellaceae	Ruminococcaceae	unclassified	unclassified	Villonellaceae			
Gemmatimonades	Gemmatimonades	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonades	Gemmatimonades	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonades	Gemmatimonades	Gemmatimonades	Gemmatimonadaceae			
Nitrospira	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrospira	Nitrospira	Nitrosiraceae	Nitrospiraceae	Nitrospira	Nitrospira	Nitrospira	Nitrospiraceae			

Table.S4 Part 2: Changes in taxonomic composition between bulk soil and rhizosphere samples for each soil type. Text in red and blue indicates a significant increase in rhizosphere and bulk soils samples respectively. Samples are referred as follow: BS: Bulk Soil; Rh: Rhizosphere. The symbols ‘>’ and ‘<’ mean significantly more or less abundant and ‘=’ not significantly different.

CALCARIC CAMBISOL				
PHYLUM	CLASS	ORDER	FAMILY	
OP10	OP10	OP10	OP10	
Planctomycetes	Planctomycetes	Planctomycetales	Planctomycetaceae	
Proteobacteria	Alpha-proteobacteria	Caulobacterales Rh>BS	Caulobacteraceae Rh>BS	
			Hypomicrobacteraceae Rh>BS	
			unclassified	
		Rhizobiales	Beijerinckiaceae	
			Bradyrhizobiaceae	
	Beta-proteobacteria		Brucellaceae	
			Hypomicrobacteraceae	
			Methylbacteriaceae	
			Methylocystaceae	
			Phyllobacteriaceae Rh>BS	
Verrucomicrobia	Delta-proteobacteria	Rhodobacterales Rh>BS	Rhizobiaceae	
			Rhodobiaceae	
			unclassified	
		Rickettsiales	Xanthobacteraceae	
			Rhodobacteraceae Rh>BS	
	Gamma-proteobacteria		Acetobacteraceae	
			Rhodospirillaceae	
			unclassified	
			Rickettsiaceae	
			Sphingomonadaceae	
	Spirochaetes	Sphingomodales unclassified	unclassified	
			unclassified	
		unclassified BS>Rh	unclassified	
			BS>Rh	
			unclassified	
WS3	Opitutales	TM7 Rh>BS	Aeromodales	
			Alteromodales	
			Chromatiales	
		Subdivision3 unclassified	Enterobacteriales	
			Pseudomodales	
	Spartobacteria	Subdivision3 unclassified	Moraxellaceae	
			unclassified	
			Sinobacteraceae	
		Subdivision3 unclassified	Xanthomodaceae	
			unclassified	

EUTRIC CAMBISOL			
PHYLUM	CLASS	ORDER	FAMILY
OP10	OP10	genera	incertae
Planctomycetes	Planctomycetacia	ND	Planctomycetaceae ND
Proteobacteria	Alpha-proteobacteria	Caulobacterales	Caulobacteraceae
			Hypomicrobacteraceae
			unclassified
		Rhizobiales Rh>BS	Beijerinckiaceae
			Bradyrhizobiaceae
	Beta-proteobacteria		Brucellaceae ND
	Rhodobacterales	Hypomicrobacteraceae	
		Methylbacteriaceae	
		Methylocystaceae	
		Phyllobacteriaceae Rh>BS	
Verrucomicrobia	Delta-proteobacteria	Rhodospirillales	Rhizobiaceae
			Rhodobiaceae
			unclassified
		Rickettsiales	Xanthobacteraceae
			Rhodobacteraceae
	Gamma-proteobacteria		Acetobacteraceae
	Myxococcales	Rhodospirillaceae Rh>BS	
		unclassified	
		Rickettsiaceae	
		Sphingomonadaceae	
	Spirochaetes	Bdellovibrionales BS>Rh	unclassified
			BS>Rh
		Desulfobacterales	Bdellovibrionaceae
			unclassified
			Geobacteraceae
	Opitutales	Desulfovomonadales	unclassified
			Cystobacteraceae
			Hallangiacae
		TM7 Rh>BS	Kofleriaceae
			Nannocystaceae
	Spartobacteria	Opitutaceae	Polyangliaceae
			unclassified
		Subdivision3 unclassified	unclassified
			unclassified
			Xanthomodaceae
	WS3	TM7 Rh>BS	unclassified
			unclassified
		TM7 Rh>BS	Leptospiraceae ND
			Spirochaetaceae ND
			unclassified ND
WS3	Opitutales	TM7 Rh>BS	Leptospiraceae ND
			Spirochaetaceae ND
			unclassified ND
		Subdivision3 unclassified	Opitutaceae ND
			Spartobacteria Subdivision3 unclassified
	Spartobacteria	Subdivision3 unclassified	Moraxellaceae
			unclassified
		Subdivision3 unclassified	Pseudomonadaceae
			unclassified
			Xanthomodaceae Rh>BS

HYPERDYSTRIC CAMBISOL			
PHYLUM	CLASS	ORDER	FAMILY
OP10	OP10	genera	incertae
Planctomycetes	Planctomycetacia	ND	Planctomycetaceae ND
Proteobacteria	Alpha-proteobacteria	Caulobacterales	Caulobacteraceae Rh>BS
			Hypomicrobacteraceae
			unclassified
		Rhizobiales	Beijerinckiaceae Rh>BS
			Bradyrhizobiaceae Rh>BS
	Beta-proteobacteria		Brucellaceae ND
	Rhodobacterales	Hypomicrobacteraceae	
		Methylbacteriaceae	
		Methylocystaceae	
		Phyllobacteriaceae Rh>BS	
Verrucomicrobia	Delta-proteobacteria	Rhodospirillales	Rhizobiaceae
			Rhodobiaceae
			unclassified
		Rickettsiales	Xanthobacteraceae
			Rhodobacteraceae
	Gamma-proteobacteria	Sphingomonadales	Rhodospirillaceae Rh>BS
			unclassified
		Unclassified	Comamonadaceae
			Oxalobacteraceae Rh>BS
			unclassified Rh>BS
WS3	Spirochaetes	Bdellovibrionales	Bacteriovoracaceae
			Bdellovibrionaceae
		Desulfobacterales	unclassified
			Geobacteraceae
			unclassified
	Opitutales	Desulfovomonadales	Cystobacteraceae
			Hallangiacae
		TM7 Rh>BS	Kofleriaceae
			Nannocystaceae
			Polyangliaceae
WS3	Spartobacteria	Opitutaceae	Aeromonadaceae
			Alteromonadaceae
		Subdivision3 unclassified	Chromatiales
			Enterobacteriales
			Pseudomonadales
	Spartobacteria	Subdivision3 unclassified	Moraxellaceae
			unclassified
		Subdivision3 unclassified	Sinobacteraceae
			Xanthomonadaceae
			unclassified Rh>BS
WS3	Opitutales	Leptospiraceae	Aeromonadaceae ND
			Alteromonadaceae ND
		Spirochaetaceae	Chromatiales ND
			Enterobacteriales ND
			Pseudomonadaceae ND
	Spartobacteria	Spartobacteria	Moraxellaceae
			unclassified
		Subdivision3 unclassified	Pseudomonadaceae
			unclassified
			Xanthomonadaceae Rh>BS

Table S5 Relative abundance (in %) of the bacterial genera that constituted the rhizosphere/core OTUs along the soil toposequence. Each value is the mean of biological replicates (\pm Standard Error of the Mean).

Phylum	Class	Order	Family	Genus	Calcaric mean sem	Eutric mean sem	Hyperdystric mean sem
Acidobacteria	Acidobacteria_Gp2	Gp2	unclassified	unclassified	0.61 0.35	17.30 2.76	17.93 1.62
Acidobacteria	Acidobacteria_Gp3	Gp3	unclassified	unclassified	1.89 0.34	4.44 0.38	6.45 0.79
Actinobacteria	Actinobacteria	Acidimicrobiales	Acidimicrobinae	unclassified	3.97 0.47	0.37 0.13	0.58 0.07
Actinobacteria	Actinobacteria	Acidimicrobiales	Iamiaceae	Iamia	0.24 0.11	0.09 0.01	0.52 0.28
Actinobacteria	Actinobacteria	Acidimicrobiales	unclassified	unclassified	1.00 0.13	0.45 0.17	0.14 0.05
Actinobacteria	Actinobacteria	Actinomycetales	Micromonosporaceae	unclassified	0.87 0.14	0.17 0.04	0.45 0.14
Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium	23.40 6.42	15.14 2.35	7.99 1.01
Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	unclassified	2.43 0.59	0.79 0.24	0.91 0.51
Actinobacteria	Actinobacteria	Actinomycetales	Streptosporangineae	unclassified	0.12 0.05	0.16 0.04	0.17 0.04
Actinobacteria	Actinobacteria	Actinomycetales	unclassified	unclassified	0.91 0.20	17.81 2.20	20.92 2.72
Actinobacteria	Actinobacteria	Solirubrobacterales	Conexibacteraceae	unclassified	0.75 0.16	1.87 0.60	0.70 0.12
Actinobacteria	Actinobacteria	Solirubrobacterales	unclassified	unclassified	4.24 0.98	2.39 1.16	0.24 0.07
Actinobacteria	Actinobacteria	unclassified	unclassified	unclassified	4.70 0.84	3.52 0.77	2.00 0.46
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	unclassified	1.28 0.41	1.38 0.33	2.85 0.79
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Cytophagaceae	unclassified	0.56 0.06	0.46 0.17	0.47 0.14
Chloroflexi	unclassified_Chloroflexi	unclassified	unclassified	unclassified	0.52 0.11	0.08 0.03	0.18 0.07
Nitrospira	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrospira	0.33 0.05	0.34 0.06	1.50 0.08
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium	1.93 0.38	1.69 0.15	1.44 0.19
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	unclassified	0.21 0.06	0.58 0.17	1.37 0.08
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	10.45 1.88	9.75 1.44	9.23 1.17
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	unclassified	0.39 0.33	0.26 0.08	0.26 0.05
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hypomicrobiaceae	Rhodoplanes	12.26 1.79	1.04 0.11	0.52 0.11
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hypomicrobiaceae	unclassified	0.78 0.16	0.30 0.04	0.71 0.08
Proteobacteria	Alphaproteobacteria	Rhizobiales	unclassified_Rhizobiales	unclassified	0.87 0.28	1.07 0.28	1.62 0.09
Proteobacteria	Alphaproteobacteria	Rhizobiales	unclassified	unclassified	0.81 0.32	2.02 0.41	2.19 0.52
Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Pseudolabrys	0.56 0.27	0.32 0.19	0.30 0.17
Proteobacteria	Alphaproteobacteria	Rhodospirillales	unclassified	unclassified	1.41 0.58	2.07 0.25	1.45 0.26
Proteobacteria	Alphaproteobacteria	unclassified	unclassified	unclassified	7.24 1.03	1.71 0.22	1.74 0.35
Proteobacteria	Betaproteobacteria	unclassified	unclassified	unclassified	0.33 0.11	0.33 0.03	0.09 0.02
Proteobacteria	Deltaproteobacteria	Myxococcales	Cystobacterineae	unclassified	0.53 0.13	0.13 0.03	0.55 0.07
Proteobacteria	Deltaproteobacteria	Myxococcales	Sorangiineae	Polyangiaceae	1.83 0.44	0.23 0.03	0.49 0.06
Proteobacteria	Deltaproteobacteria	Myxococcales	Sorangiineae	unclassified	0.76 0.13	0.25 0.02	0.36 0.06
Proteobacteria	Deltaproteobacteria	unclassified	unclassified	unclassified	3.46 0.51	0.70 0.13	0.41 0.07
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	Steroidobacter	0.86 0.28	5.61 0.82	7.87 0.84
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	unclassified	0.82 0.39	0.87 0.22	0.10 0.03
Proteobacteria	unclassified	unclassified	unclassified	unclassified	2.43 0.54	1.96 0.48	2.53 0.63
unclassified	unclassified	unclassified	unclassified	unclassified	2.32 0.38	0.87 0.08	1.93 0.38
Verrucomicrobia	Subdivision3	Subdivision3	unclassified	unclassified	1.93 0.32	1.51 0.22	0.83 0.13

Table S6 Relative abundance (in %) of the bacterial genera that constituted the acidic rhizosphere/core OTUs in Cambisol Eutric and Hyperdystric.

Each value is the mean of biological replicates (\pm Standard Error of the Mean).

Phylum	Class	Order	Family	Genus	Eutric		Hyperdystric	
					mean	sem	mean	sem
Acidobacteria	Acidobacteria_Gp1	Gp1	unclassified	unclassified	7.1619708	1.26508868	4.27473571	0.76908305
Acidobacteria	Acidobacteria_Gp2	Gp2	unclassified	unclassified	10.7687284	1.69642531	9.65769631	2.20669787
Acidobacteria	Acidobacteria_Gp3	Gp3	unclassified	unclassified	3.92180598	0.14366937	3.75323336	0.87623791
Acidobacteria	unclassified	unclassified	unclassified	unclassified	2.29702904	0.52333814	2.01737297	0.54886993
Actinobacteria	Actinobacteria	Actinomicrobiales	lamiaceae	lamia	0.94281404	0.11548958	0.82031669	0.13818892
Actinobacteria	Actinobacteria	Actinomicrobiales	unclassified	unclassified	1.32951198	0.15983938	1.10607781	0.0470451
Actinobacteria	Actinobacteria	Actinomycetales	Actinospicaceae	Actinospica	2.19872497	0.32889383	6.45704419	2.2678257
Actinobacteria	Actinobacteria	Actinomycetales	Catenulisporeaceae	Catenulispora	0.74501019	0.26889465	0.77948347	0.16668271
Actinobacteria	Actinobacteria	Actinomycetales	Micromonosporaceae	unclassified	0.07408839	0.02259253	0.11129536	0.03648324
Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium	6.4638048	1.19515787	2.06165288	0.20265768
Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	Streptacidiphilus	2.9649012	0.40010128	7.0291416	3.50255433
Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	unclassified	0.32932238	0.09226806	0.21670263	0.11662585
Actinobacteria	Actinobacteria	Actinomycetales	unclassified	unclassified	10.6838015	1.11448578	7.77892647	0.4149742
Actinobacteria	Actinobacteria	Solirubrobacterales	Conexibacteraceae	Conexibacter	2.176975	0.51994115	0.59076219	0.10718277
Actinobacteria	Actinobacteria	Solirubrobacterales	unclassified	unclassified	1.12834373	0.46229552	0.13496295	0.01234333
Actinobacteria	Actinobacteria	unclassified	unclassified	unclassified	1.86598982	0.37060741	0.76111444	0.11055591
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	unclassified	1.53953432	0.24013384	2.27687827	0.39785909
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Cytophagaceae	unclassified	0.20321017	0.08142207	0.12867373	0.0438941
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae	unclassified	0.42013405	0.08136681	0.60118857	0.06694591
Chloroflexi	unclassified_Chloroflexi	unclassified	unclassified	unclassified	0.03359409	0.01179497	0.04358075	0.01367039
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas	0.21175362	0.03619133	0.1133986	0.03639352
Nitrospira	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrospira	0.14642928	0.02771709	0.40262733	0.05605768
OP10	OP10_genera_incertae_sedis	unclassified	unclassified	unclassified	0.03950047	0.00799948	0.05950277	0.01894935
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium	0.71866159	0.08445406	0.38543977	0.07804105
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	unclassified	0.27666577	0.07785411	0.38305028	0.05257617
Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	unclassified	0.17879214	0.02629573	0.35904672	0.1073096
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	4.05890381	0.51088834	2.52356078	0.55834465
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	unclassified	0.22283857	0.04427219	0.15402185	0.03720198
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hypomicrobiaceae	Rhodoplanes	0.44172633	0.05529381	0.14292912	0.04561979
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hypomicrobiaceae	unclassified	0.12948631	0.0221265	0.19393693	0.03695086
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Rhizobium	0.23367647	0.04889463	0.22006675	0.05441965
Proteobacteria	Alphaproteobacteria	Rhizobiales	unclassified	unclassified	2.86952085	0.23628056	2.98304448	0.96975125
Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Labrys	0.25480139	0.06688175	0.07262743	0.0155729
Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Pseudolabrys	0.15162902	0.069692308	0.0841108	0.0492838
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	unclassified	0.58017519	0.09088377	0.46613032	0.13510524
Proteobacteria	Alphaproteobacteria	Rhodospirillales	unclassified	unclassified	2.48951269	0.29920027	1.95611745	0.48003991
Proteobacteria	Alphaproteobacteria	unclassified	unclassified	unclassified	2.2069181	0.15698593	1.91379023	0.35869423
Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	0.992053	0.18199842	1.24394895	0.28202441
Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiales_incertae_sedis	unclassified	0.20401604	0.05992309	0.59756128	0.18325457
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Collimonas	0.15319064	0.04891661	0.31756524	0.14640665
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Herbaspirillum	0.01939039	0.00505322	0.058756	0.02729537
Proteobacteria	Betaproteobacteria	unclassified	unclassified	unclassified	0.25552117	0.0441768	0.07587177	0.01621753
Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	unclassified	0.57167185	0.1151067	0.76041825	0.1959481
Proteobacteria	Deltaproteobacteria	Myxococcales	unclassified	unclassified	0.20876274	0.0306421	0.32626043	0.05238568
Proteobacteria	Deltaproteobacteria	unclassified	unclassified	unclassified	0.87364326	0.14526787	0.58954662	0.14488548
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	Nevschia	0.04926228	0.02226176	0.19745547	0.03426669
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	Steroidobacter	5.44268369	0.80333849	5.38357838	1.34269212
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	unclassified	0.81742254	0.21150983	0.3287248	0.06761882
Proteobacteria	unclassified	unclassified	unclassified	unclassified	0.80212447	0.18758711	0.71780224	0.24127958
TM7	TM7_genera_incertae_sedis	unclassified	unclassified	unclassified	0.33605932	0.13612436	2.62743007	1.09875336
unclassified	unclassified	unclassified	unclassified	unclassified	15.6211728	3.11325192	23.0291592	6.49041075
Verrucomicrobia	Subdivision3	unclassified	unclassified	unclassified	1.19273931	0.17086568	0.73167955	0.17550021

Table S7: Relative distribution of the functional categories of the Geochip in phyla and major classes according to the compartment (Rhizosphere, Rh and Bulk soil, BS) and the soil type (Calcaric, Eutric and Hyperdistic).

	Relative signal expressed in %						Distribution in classes (a,b,c) according to the p values					
	Calcaric		Eutric		Hyperdistic		Calcaric		Eutric		Hyperdistic	
	BS	Rh	BS	Rh	BS	Rh	BS	Rh	BS	Rh	BS	Rh
Carbon cycling												
Actinobacteria	4.43	4.20	4.16	4.23	4.41	4.26	b	a	a	a	b	a
Alphaproteobacteria	2.30	2.27	2.31	2.26	2.30	2.27	a	a	a	a	a	a
Gammaproteobacteria	1.27	1.35	1.33	1.32	1.26	1.33	b	a	a	a	b	a
Firmicutes	0.86	0.89	0.92	0.90	0.87	0.90	b	ab	a	ab	b	ab
Betaproteobacteria	0.87	0.90	0.89	0.89	0.88	0.89	b	a	ab	ab	ab	ab
	Calcaric		Eutric		Hyperdistic							
Metal homeostasis	BS	Rh	BS	Rh	BS	Rh	Calcaric	Eutric	Hyperdistic	Calcaric	Eutric	Hyperdistic
Alphaproteobacteria	6.58	6.56	6.50	6.50	6.58	6.58	b	ab	a	a	b	b
Actinobacteria	5.26	5.13	5.06	5.09	5.30	5.07	b	a	a	a	b	a
Gammaproteobacteria	4.09	4.26	4.36	4.36	4.16	4.26	d	ab	c	ac	bd	abc
Betaproteobacteria	3.72	3.70	3.66	3.63	3.69	3.65	b	ab	ab	a	ab	ab
Firmicutes	1.40	1.53	1.56	1.53	1.38	1.55	b	a	a	a	b	a
	Calcaric		Eutric		Hyperdistic		Calcaric	Eutric	Hyperdistic	Calcaric	Eutric	Hyperdistic
Nitrogen	BS	Rh	BS	Rh	BS	Rh	BS	Rh	BS	Rh	BS	Rh
Actinobacteria	0.48	0.46	0.43	0.43	0.47	0.45	a	a	a	a	a	a
Alphaproteobacteria	0.26	0.26	0.26	0.27	0.27	0.26	a	a	a	a	a	a
Betaproteobacteria	0.26	0.25	0.26	0.26	0.26	0.26	a	a	a	a	a	a
Gammaproteobacteria	0.23	0.23	0.24	0.24	0.23	0.24	a	a	ab	b	a	ab
Firmicutes	0.10	0.12	0.12	0.12	0.10	0.12	b	a	a	a	b	a
	Calcaric		Eutric		Hyperdistic		Calcaric	Eutric	Hyperdistic	Calcaric	Eutric	Hyperdistic
Organic remediation	BS	Rh	BS	Rh	BS	Rh	BS	Rh	BS	Rh	BS	Rh
Alphaproteobacteria	2.51	2.48	2.48	2.51	2.48	2.53	ab	a	a	ab	a	b
Betaproteobacteria	1.69	1.70	1.64	1.67	1.67	1.69	a	a	b	ab	ab	a
Actinobacteria	1.41	1.32	1.32	1.33	1.42	1.33	b	a	a	a	b	a
Gammaproteobacteria	1.01	1.06	1.07	1.05	1.03	1.05	c	ab	a	ab	bc	abc
Firmicutes	0.18	0.19	0.20	0.20	0.18	0.20	c	abc	b	ab	ac	ab
	Calcaric		Eutric		Hyperdistic		Calcaric	Eutric	Hyperdistic	Calcaric	Eutric	Hyperdistic
Phosphorus	BS	Rh	BS	Rh	BS	Rh	BS	Rh	BS	Rh	BS	Rh
Alphaproteobacteria	0.48	0.47	0.48	0.47	0.49	0.47	a	a	a	a	a	a
Actinobacteria	0.47	0.45	0.44	0.46	0.48	0.46	a	a	a	a	a	a
Gammaproteobacteria	0.26	0.25	0.26	0.27	0.26	0.26	a	a	ab	b	ab	ab
Betaproteobacteria	0.20	0.20	0.20	0.20	0.21	0.20	a	a	a	a	a	a
Firmicutes	0.10	0.11	0.11	0.11	0.10	0.11	a	a	a	a	a	a

	Calcaric		Eutric		Hyperdistic	
Secondary metabolism	BS	Rh	BS	Rh	BS	Rh
Actinobacteria	1.19	1.10	1.07	1.10	1.17	1.12
Alphaproteobacteria	0.75	0.74	0.74	0.74	0.77	0.73
Gammaproteobacteria	0.27	0.28	0.29	0.28	0.27	0.28
Betaproteobacteria	0.29	0.27	0.27	0.28	0.29	0.27
Firmicutes	0.06	0.06	0.07	0.07	0.06	0.06
	Calcaric		Eutric		Hyperdistic	
Stress	BS	Rh	BS	Rh	BS	Rh
Alphaproteobacteria	4.50	4.48	4.39	4.40	4.38	4.47
Actinobacteria	3.52	3.42	3.40	3.41	3.51	3.42
Gammaproteobacteria	2.48	2.56	2.57	2.57	2.48	2.57
Betaproteobacteria	1.83	1.83	1.80	1.82	1.86	1.82
Firmicutes	0.91	0.94	0.98	0.96	0.90	0.96
	Calcaric		Eutric		Hyperdistic	
Sulfur	BS	Rh	BS	Rh	BS	Rh
Alphaproteobacteria	0.59	0.57	0.59	0.58	0.60	0.57
Gammaproteobacteria	0.38	0.40	0.40	0.39	0.39	0.39
Betaproteobacteria	0.27	0.30	0.28	0.29	0.28	0.29
Actinobacteria	0.10	0.10	0.10	0.10	0.11	0.10
Firmicutes	0.07	0.07	0.07	0.07	0.07	0.07
	Calcaric		Eutric		Hyperdistic	
Virulence	BS	Rh	BS	Rh	BS	Rh
Actinobacteria	8.52	7.95	7.89	7.88	8.46	7.92
Gammaproteobacteria	3.71	3.77	3.79	3.84	3.76	3.74
Alphaproteobacteria	3.19	3.31	3.23	3.23	3.21	3.21
Betaproteobacteria	2.50	2.46	2.46	2.47	2.54	2.44
Firmicutes	0.33	0.38	0.40	0.38	0.34	0.39
	Calcaric		Eutric		Hyperdistic	
CRISPR	BS	Rh	BS	Rh	BS	Rh
Firmicutes	0.49	0.56	0.55	0.53	0.48	0.54
Actinobacteria	0.45	0.44	0.45	0.45	0.46	0.45
Gammaproteobacteria	0.36	0.37	0.38	0.38	0.36	0.38
Alphaproteobacteria	0.29	0.29	0.29	0.29	0.28	0.29
deltaproteobacteria	0.24	0.25	0.26	0.25	0.24	0.25

	Calcaric		Eutric		Hyperdistic	
	BS	Rh	BS	Rh	BS	Rh
c	ab	a	ab	c	b	
ab	ab	ab	ab	a	b	
a	a	a	a	a	a	
b	a	ab	ab	ab	a	
a	a	a	a	a	a	
	Calcaric		Eutric		Hyperdistic	
	BS	Rh	BS	Rh	BS	Rh
a	a	a	a	a	a	a
b	a	a	a	b	a	a
b	a	a	a	b	a	a
a	a	a	a	a	a	a
a	a	a	a	a	a	a
	Calcaric		Eutric		Hyperdistic	
	BS	Rh	BS	Rh	BS	Rh
cd	ab	cd	ac	d	b	
b	a	a	ab	ab	ab	
a	a	a	a	a	a	
a	a	a	a	a	a	
b	ab	ab	ab	ab	ab	a
	Calcaric		Eutric		Hyperdistic	
	BS	Rh	BS	Rh	BS	Rh
b	a	a	a	b	a	
b	ab	ab	a	ab	b	
a	a	a	a	a	a	
bc	ab	ab	ab	c	a	
b	a	a	a	b	a	
	Calcaric		Eutric		Hyperdistic	
	BS	Rh	BS	Rh	BS	Rh
b	a	a	a	b	a	
a	a	a	a	a	a	
c	ab	a	a	bc	a	
a	a	a	a	a	a	
ab	ab	a	ab	b	ab	

Table S8 Pearson correlation between soil parameters and the relative abundance of bacterial phyla in bulk soil samples. The r-value is provided for significant correlations (**P<0.01 and ***P<0.001).

	pH	Hum	N.total	C.total	C.N	OM	Limestone	ATE	CEC	H.	Ca	K	Mg	Na	P.Duch.	P.Olsen	Mn	Al	Fe
Acidobacteria	-0.784**	-0.75**	-0.805**	-0.8**		-0.8**	-0.816**	0.914***	-0.815**	0.841***	-0.83***	-0.765**					0.911***		
Bacteroidetes	0.978***	0.974***	0.967***	0.958***		0.958***	0.929***	-0.932***	0.976***	-0.954***	0.974***	0.964***	0.904***	0.937***		-0.749**	-0.914***		
Chloroflexi	0.875***	0.884***	0.87***	0.867***		0.867***	0.808**	-0.865***	0.901***	-0.887***	0.908***	0.912***	0.865***	0.785**		-0.708**	-0.846***		
Firmicutes	0.939***	0.943***	0.951***	0.942***		0.942***	0.982***	-0.936***	0.958***	-0.951***	0.962***	0.943***	0.872***	0.854***		-0.713**	-0.918***		
Gemmatimonadetes		0.769**	0.746**							0.718**	-0.739**	0.712**	0.746**		0.816**				
Nitrospira	0.895***	0.863***	0.845***	0.823**		0.823**	0.887***	-0.93***	0.892***	-0.909***	0.901***	0.884***	0.727**	0.773**			-0.922***		
Alphaproteobacteria	0.742**	0.75**										0.753**		0.822**					
Betaproteobacteria	0.981***	0.963***	0.944***	0.934***		0.934***	0.947***	-0.957***	0.975***	-0.973***	0.979***	0.966***	0.866***	0.912***		-0.727**	-0.939***		
Deltaproteobacteria	0.954***	0.938***	0.894***	0.876***		0.875***	0.916***	-0.869***	0.938***	-0.925***	0.938***	0.955***	0.827***	0.91***		-0.789**	-0.845***		
Gammaproteobacteria	0.744**	0.754**								0.718**		0.752**		0.803**					
Proteobacteria uncl.	0.988***	0.969***	0.947***	0.932***		0.932***	0.96***	-0.945***	0.981***	-0.975***	0.983***	0.979***	0.865***	0.925***		-0.792**	-0.923***		
Acidimicrobiales	0.892***	0.88***	0.888***	0.889***		0.889***	0.857***	-0.817**	0.891***	-0.896***	0.886***	0.86***	0.813**	0.842***		-0.809**	-0.777**		
Actinomycetales	0.72**		0.732**	0.734**		0.734**								0.715**		-0.714**			
Solirubrobacteriales	0.947***	0.942***	0.947***	0.946***		0.946***	0.902***	-0.861***	0.941***	-0.935***	0.934***	0.913***	0.874***	0.924***		-0.824***	-0.827***		
Actinomycetales uncl.	0.856***	0.863***	0.861***	0.861***		0.862***	0.798**	-0.735**	0.849***	-0.827***	0.837***	0.824***	0.829***	0.847***		-0.79**			
TM7													0.718**						
Verrucomicrobia													0.71**						
Unclassified	-0.847***	-0.855***	-0.795**	-0.787**		-0.787**	-0.746**		-0.814**	0.776**	-0.8**	-0.839***	-0.796**	-0.888***		0.779**			

Table.S9 Pearson correlation between soil parameters and the substrate utilization patterns (Biolog Ecoplates). The r-value is provided for significant correlations (**P<0.01 and ***P<0.001).

	pH	Hum	N.total	C.total	C.N	OM	Limestone	ATE	CEC	H.	Ca	K	Mg	Na	P.Duch.	P.Olsen	Mn	Al	Fe
pyruvic.acid.methyl.ester		-0.706**												-0.753**		0.748**			
tween.40																			
tween.80																			
alpha.cyclodextrin	0.879***	0.889***	0.895***	0.886***		0.886***	0.935***	-0.812***	0.883***	-0.848***	0.881***	0.877***	0.834***	0.85***			-0.793**		
glycogen	0.861***	0.792**	0.738**	0.721**		0.721**	0.81***	-0.824***	0.82***	-0.876***	0.825***	0.803**	0.77**			-0.749**	-0.791**		
D.cellubiose																			
alpha.D.Lactose																			
beta.methyl.D glucoside																			
D.xylose																			
i.Erythritol																			
D.Mannitol																			
N.Acetyl.D.Glucosamine																			
D.Glucosaminidic.acid																			
Glucose.1.phosphate																			
Glycerol.phosphate																			
D.galactonique.acid.gamma.lactone																			
D.galacturonic.acid	-0.909***	-0.92***	-0.866***	-0.852***		-0.852***	-0.846***	0.713**	-0.887***	0.851***	-0.873***	-0.911***	-0.845***	-0.931***		0.903***			
X2.Hydroxy.benzoic.acid	0.841***	0.768**				0.811***	-0.775**	0.792**	-0.839***	0.797**	0.799**		0.774**			-0.738**	-0.744**		
X4.Hydroxy.benzoic.acid																			
Hydroxybutiric.acid																			
Itaconic.acid																		1***	
ketobutyric.acid																			
malic.acid																			
arginine																			
asparagine		-0.706**	-0.708**	-0.711**		-0.711**								-0.726**	-0.742**				
phenylalanine																			
serine																			
threonine																			
glutamic.acid															0.741**				
phenylethylamine																			
putrescine	0.853***	0.822***	0.849***	0.833***		0.833***	0.846***	-0.938***	0.874***	-0.887***	0.887***	0.852***	0.715**	0.715**			-0.935***		

Table S10 Pearson correlation between the bacterial diversity and the substrate utilization patterns (Biolog Ecoplates). The r-value is provided for significant correlations (**P<0.01 and ***P<0.001).

	Acidobacteria	Bacteroides	Chloroflexi	Firmicutes	Gemmatimonadetes	Nitrospira	Alphaproteobacteria	Betaproteobacteria	Deltaproteobacteria	Gammaproteobacteria	Proteobacteria uncl.	Acidimicrobiales	Actinomycetales	Solirubrobacterales	Actinomycetales uncl.	TM7	Verrucomicrobia	Unclassified
pyruvic.acid.methyl.ester		-0.562**		-0.634***	-0.611**		-0.611**		-0.562**		-0.535**		-0.548**		-0.542**		-0.542**	
tween.40				-0.649***	-0.562**							0.587**					-0.558**	
tween.80																		
alpha.cyclodextrin		0.607**		0.56**		0.588**		0.631***		0.631***		0.601**		0.555**				
glycogen	-0.734***	0.75***	0.79***	0.774***	0.722***	0.833***		0.875***		0.882***		0.881***		0.759***		0.725***		0.584**
D.cellulose																		
alpha.D.Lactose																		
beta.methyl.D.Glucoside																		
D.xylene																		
i.Erythritol																		
D.Mannitol																		
N.Acetyl.D.Glucosamine																-0.525**		0.654***
D.Glucosaminidic.acid																		
Glucose.1.phosphate																		
Glycerol.phosphate																		
D.galactonique.acid.gamma.lactone																		
D.galacturonic.acid																		
X2.Hydroxy.benzoic.acid	-0.539**	0.616**	0.568**	0.682***	0.571**	0.661***		0.651***		0.701***		0.642***		0.571**		0.704***		
X4.Hydroxy.benzoic.acid																		
Hydroxybutyric.acid																		
Itaconic.acid																		
ketobutyric.acid																		
malic.acid																		
arginine																		
asparagine		-0.567**	-0.543**	-0.664***	-0.576**			-0.523**		-0.601**		-0.535**		0.72***			0.58**	
phenylalanine																		
serine																		
threonine																		
glutamic.acid																		
phenylethylamine		0.52**	0.634***	0.659***	0.604**			0.571**		0.644***		0.599**		0.596**				
putrescine	-0.704***	0.77***	0.703***	0.774***	0.7***			0.779***		0.761***		0.787***		0.719***		0.772***		0.648***