

Influence of Tree Vegetation on Soil Microbial Communities in Temperate Forests and their Potential as a Proactive Indicator of Vegetation Shift due to Climate Change

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Supplementary Figures



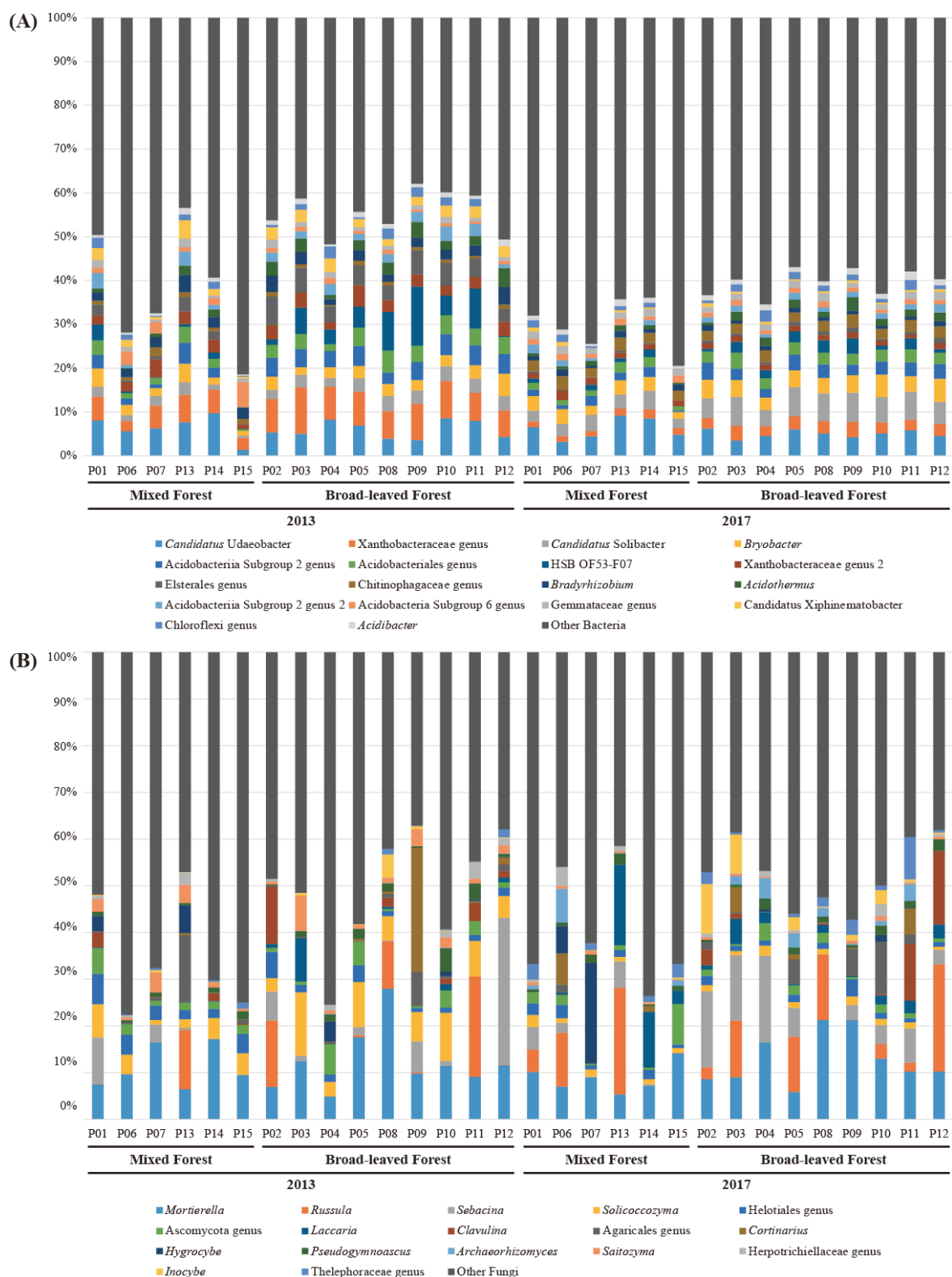


Figure S2. Relative abundances of the bacterial (A) and fungal (B) communities in forest soils presented at the genus level. Bacterial and fungal genera above 1% relative abundance were presented.

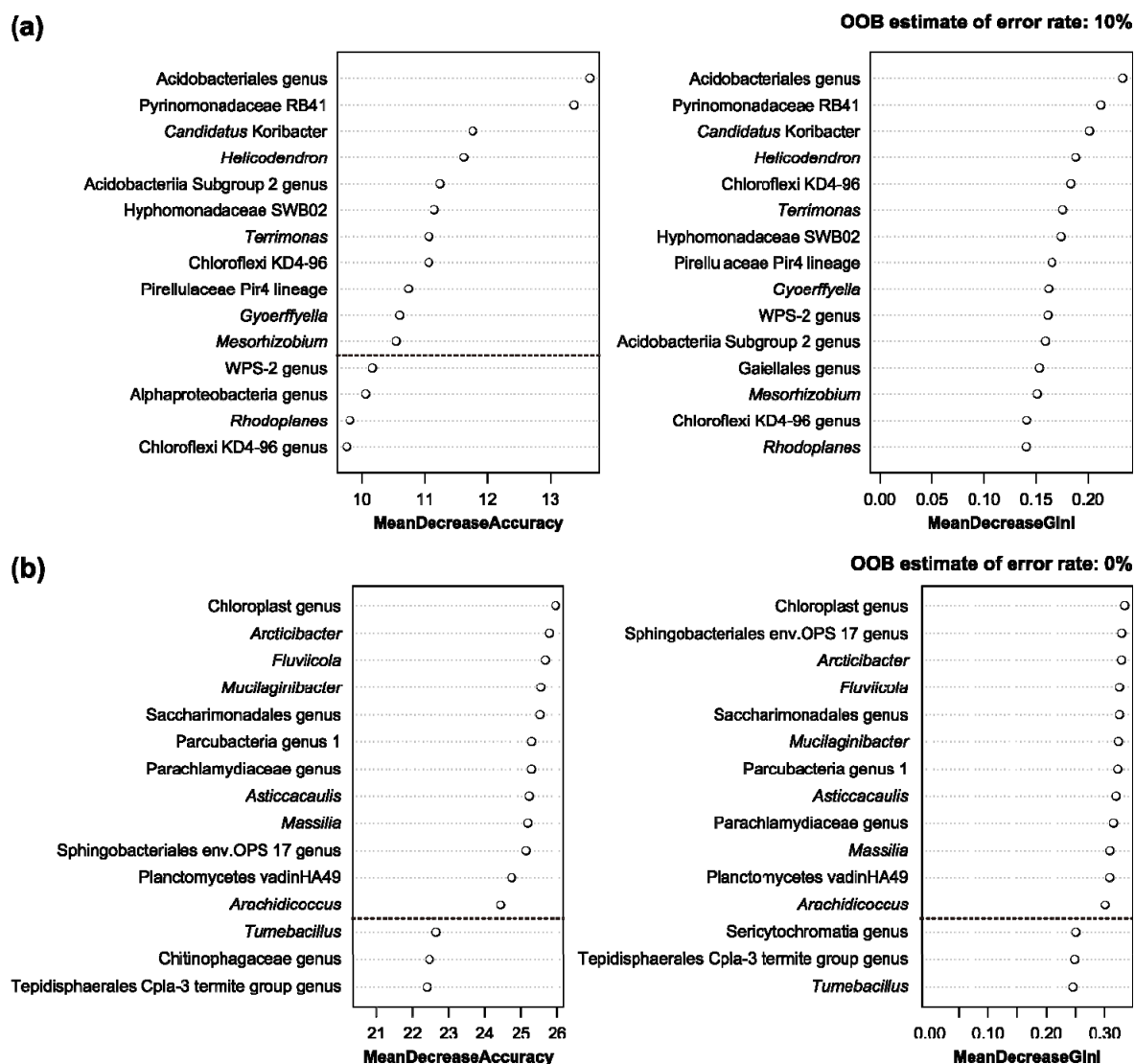


Figure S3. Importance plot of randomForest analysis using Odaesan forest soil microbial abundance by (a) forest vegetation type and (b) sampling year. Out of bag (OOB) estimate is a score that assesses the prediction performance of the model. In this study, the genera above the bold dotted line were considered the key taxa.

Supplementary Tables

Table S1. Summarized results of high-throughput sequencing by Illumina MiSeq platform.

Year	Target region	Location	Total bases ¹	Assembled Read Count ²	GC ³ (%)	Q20 ⁴ (%)	Q30 ⁵ (%)	Clustered Read Count
2013	16S	P01	45,472,154	105545	56.57	97.35	91.45	28361
		P02	38,450,051	89629	56.55	97.35	91.42	28646
		P03	43,480,449	101278	56.80	97.50	91.89	32077
		P04	45,296,257	104946	56.72	97.39	91.38	31299
		P05	44,744,064	104224	56.44	97.37	91.49	33250
		P06	40,775,358	95042	56.30	97.16	90.95	22602
		P07	41,496,958	95447	56.15	97.49	91.73	24018
		P08	47,624,329	110958	56.94	97.49	91.76	35971
		P09	43,415,265	101434	57.19	97.44	91.55	30589
		P10	43,708,775	101730	56.81	97.43	91.66	31921
		P11	50,714,281	118059	56.78	97.51	91.86	36346
		P12	40,266,522	93930	56.29	97.48	91.68	28308
		P13	45,989,504	106290	56.26	97.56	92.04	33516
		P14	44,458,565	102459	56.62	97.42	91.56	28169
		P15	42,077,950	97279	56.46	97.43	91.65	24779
	ITS	P01	44,192,535	127502	47.54	98.77	95.00	93979
		P02	41,269,246	133984	47.66	98.88	95.53	99742
		P03	50,177,164	149374	46.32	98.97	95.87	117374
		P04	51,278,727	145760	46.83	98.99	95.90	115214
		P05	45,875,331	137694	47.68	98.90	95.41	101968
		P06	46,741,689	144432	48.66	98.94	95.53	106300

		P07	45,192,459	130645	47.05	98.97	95.75	99966
		P08	34,066,803	101196	45.73	98.51	94.00	69593
		P09	46,454,565	131521	45.60	99.04	96.07	104815
		P10	37,468,116	114759	47.16	98.99	95.83	84725
		P11	46,357,151	130595	48.43	98.87	95.50	100731
		P12	39,991,583	116284	47.28	98.95	95.78	87492
		P13	45,261,391	129681	46.58	98.86	95.43	96683
		P14	34,268,391	109240	47.38	98.94	95.62	81481
		P15	39,587,827	121548	48.73	98.97	95.65	90806
2017	16S	P01	32,204,834	73423	55.31	99.24	96.87	30794
		P02	21,392,708	48782	55.29	99.25	96.92	18786
		P03	22,488,859	51635	55.71	99.20	96.69	19416
		P04	21,607,644	49446	55.48	99.22	96.77	19272
		P05	18,597,118	42558	55.84	99.19	96.61	16848
		P06	24,023,362	54985	54.98	99.19	96.65	21392
		P07	28,017,043	63941	54.93	99.21	96.79	24463
		P08	23,493,805	53673	55.79	99.22	96.80	21136
		P09	25,100,180	57617	55.77	99.21	96.75	23221
		P10	21,377,975	49021	55.22	99.20	96.70	19733
		P11	26,048,994	59642	55.80	99.20	96.70	23034
		P12	23,008,079	52601	55.59	99.19	96.66	21017
		P13	19,619,750	44708	55.34	99.22	96.80	16897
		P14	30,690,670	70215	55.51	99.12	96.43	27571
		P15	31,304,987	71186	55.08	99.24	96.88	27980
	ITS	P01	38,092,731	124013	48.27	99.71	98.65	105266

P02	26,500,724	80295	47.03	99.70	98.66	65203
P03	28,825,238	93396	45.75	99.68	98.59	74333
P04	39,409,372	117342	47.08	99.67	98.49	95500
P05	41,296,869	125009	46.62	99.69	98.60	91885
P06	23,872,024	82451	46.92	99.71	98.67	67095
P07	34,013,324	98596	47.52	99.61	98.21	75952
P08	27,726,089	87217	46.86	99.68	98.59	72787
P09	31,742,846	98330	46.45	99.70	98.67	80971
P10	34,178,667	96402	46.65	99.68	98.59	76613
P11	38,005,190	114626	46.88	99.68	98.60	95183
P12	34,797,498	94447	47.59	99.64	98.37	76623
P13	29,370,320	80709	46.94	99.70	98.65	67072
P14	24,414,574	106036	47.19	99.69	98.53	88656
P15	25,600,121	79994	47.81	99.72	98.71	65410

¹ The total number of bases in reads identified

² The total number of sequence reads

³ The GC percentage in sequence reads

⁴ The percentage of bases in which the phred score is above 20

⁵ The percentage of bases in which the phred score is above 30

Table S2. Summarized results of indicator species analysis by forest type and sampling year. Microbial genera above 0.9 and 0.8 IndVal are presented, respectively.

Taxa	Group	IndVal	p-value	Freq
by forest type				
<i>Terrimonas</i>	Mixed forest	0.943	0.001	18
Ilumatobacteraceae CL500-29 marine group	Mixed forest	0.914	0.001	19
Gaiellales genus	Mixed forest	0.913	0.001	20
<i>Helicodendron</i>	Mixed forest	0.898	0.001	15
Chloroflexi KD4-96 genus	Mixed forest	0.888	0.001	20
Hyphomonadaceae SWB02	Mixed forest	0.884	0.001	18
<i>Flavobacterium</i>	Mixed forest	0.868	0.001	30
Ilumatobacteraceae genus	Mixed forest	0.867	0.001	24
<i>Rhodoplanes</i>	Mixed forest	0.864	0.002	24
Pyrinomonadaceae RB41	Mixed forest	0.859	0.001	30
Acidobacteriia Subgroup 2 genus	Broad-leaved forest	0.858	0.001	27
Nitrosomonadaceae IS-44	Mixed forest	0.851	0.001	20
Ktedonobacterales JG30-KF-AS9 genus	Broad-leaved forest	0.844	0.001	18
Sandaracinaceae genus	Mixed forest	0.840	0.001	18
Pirellulaceae Pir4 lineage	Mixed forest	0.833	0.002	25
Spizellomycetales genus	Mixed forest	0.826	0.002	20
WPS-2 genus	Broad-leaved forest	0.826	0.001	26
<i>Mesorhizobium</i>	Mixed forest	0.826	0.001	16
Nitrosomonadaceae mle1-7	Mixed forest	0.822	0.001	26
Sphingomonadaceae genus	Mixed forest	0.819	0.001	19
Microscillaceae genus	Mixed forest	0.819	0.001	28
<i>Gyoeffya</i>	Broad-leaved forest	0.819	0.001	26
<i>Penicillium</i>	Mixed forest	0.816	0.001	30
<i>Candidatus</i> Koribacter	Broad-leaved forest	0.815	0.001	28
Ktedonobacteraceae HSB OF53-F07	Broad-leaved forest	0.814	0.003	28
Chaetothyriales genus	Mixed forest	0.814	0.004	28
<i>Tausonia</i>	Mixed forest	0.813	0.032	26
Sordariomycetes genus	Mixed forest	0.810	0.02	30
Rhizophydiales genus	Mixed forest	0.803	0.006	23
Rozellomycota genus	Broad-leaved forest	0.802	0.001	29
<i>Pirellula</i>	Mixed forest	0.801	0.001	24
by sampling year				
<i>Fluviicola</i>	2017	1.000	0.001	17
<i>Arcticibacter</i>	2017	1.000	0.001	17
Parachlamydiaceae genus	2017	0.991	0.001	24
<i>Arachidicoccus</i>	2017	0.989	0.001	18
<i>Asticcacaulis</i>	2017	0.985	0.001	18
Sphingobacterales env.OPS 17 genus	2017	0.980	0.001	26
<i>Lacunisphaera</i>	2017	0.977	0.001	20
<i>Mucilaginibacter</i>	2017	0.967	0.001	31
<i>Massilia</i>	2017	0.957	0.001	28
Chloroplast genus	2017	0.956	0.001	23
Planctomycetes vadinHA49 genus	2017	0.933	0.001	27
<i>Archaeorhizomyces</i>	2017	0.929	0.001	28
<i>Tumebacillus</i>	2017	0.920	0.001	19
<i>Cenococcum</i>	2017	0.919	0.001	21
Thaumarchaeota Group 1.1c genus	2017	0.913	0.001	26
<i>Glomus</i>	2017	0.908	0.001	23
<i>Mucor</i>	2017	0.905	0.001	29

Table S3. Contributions of microbial genera to the predicted functional abundance by forest vegetation type. Genera with a (relative contribution in mixed forest / relative contribution in broad-leaved forest) of less than 1.5 were excluded. Genera with a weighted contribution of above 0.1% are presented.

Microbial taxa	Relative contribution ¹		Weighted contribution ²
	Broad-leaved	Mixed	
Nitrate reduction I (denitrification) pathway			
<i>Flavobacterium</i>	0.36	1.09	20.86%
<i>Pedomicrobium</i>	0.41	0.99	15.93%
Gemmatimonadaceae genus	0.68	1.11	15.20%
<i>Terrimonas</i>	0.11	0.67	8.45%
<i>Nitrospira</i>	0.22	0.66	7.54%
Microscillaceae genus	0.16	0.62	6.94%
Nitrosomonadaceae MND1	0.26	0.59	5.36%
Nitrosomonadaceae mle1-7	0.04	0.41	3.25%
<i>Mycobacterium</i>	0.25	0.42	2.17%
<i>Ferruginibacter</i>	0.09	0.33	2.00%
<i>Pseudomonas</i>	0.06	0.28	1.52%
<i>Hyphomicrobium</i>	0.00	0.27	1.41%
Pyrinomonadaceae RB41	0.02	0.25	1.24%
<i>Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium</i>	0.09	0.25	1.04%
<i>Arenimonas</i>	0.02	0.21	0.86%
Blastocatellaceae genus	0.09	0.23	0.84%
<i>Lacunisphaera</i>	0.08	0.19	0.57%
Rhizobiales genus	0.00	0.16	0.49%
<i>Duganella</i>	0.00	0.14	0.40%
Roseiflexaceae genus	0.03	0.15	0.40%
Nitrosomonadaceae IS-44	0.09	0.16	0.33%
Blastocatellaceae genus	0.00	0.12	0.26%
<i>Oryzihumus</i>	0.09	0.14	0.21%
<i>Arcticibacter</i>	0.08	0.13	0.20%
Nitrosomonadaceae genus	0.00	0.10	0.19%
Burkholderiaceae genus	0.01	0.10	0.18%
<i>Mesorhizobium</i>	0.03	0.10	0.17%
<i>Chryseolinea</i>	0.00	0.09	0.16%
<i>Polaromonas</i>	0.01	0.08	0.12%
<i>Niastella</i>	0.01	0.08	0.12%
Betaproteobacteriales SC-I-84 genus	0.00	0.07	0.11%
Chitinophagales genus	0.05	0.09	0.10%

¹ Value calculated by multiplying the relative abundance of the microbial genus by the genome function count generated by PICRUSt2 analysis.

² Value calculated by multiplying (relative contribution in mixed forest - relative contribution in broad-leaved forest) by the total relative contribution.

Table S4. Contributions of microbial genera to the predicted functional abundance by sampling year. Genera with a (relative contribution in 2017 / relative contribution in 2013) ratio of less than 3 were excluded. Genera with a weighted contribution of above 0.1% are presented.

Microbial taxa	Relative contribution ¹		Weighted contribution ²
	2013	2017	
Formaldehyde assimilation II (RuMP) pathway			
<i>Mucilaginibacter</i>	0.02	1.02	14.39%
<i>Candidatus</i> Udaeobacter	0.23	0.88	10.06%
Acidobacteriales genus	0.20	0.83	9.12%
Chitinophagaceae genus	0.13	0.75	7.64%
<i>Bryobacter</i>	0.11	0.67	6.12%
<i>Candidatus</i> Solibacter	0.11	0.66	5.96%
Saccharimonadales genus	0.06	0.64	5.72%
Saccharimonadales genus 2	0.09	0.57	4.35%
Tepidisphaerales WD2101 genus	0.07	0.47	2.95%
Micropepsaceae genus	0.09	0.42	2.33%
<i>Flavobacterium</i>	0.04	0.40	2.21%
Parcubacteria genus	0.02	0.36	1.83%
Acidobacteriia Subgroup 2 genus	0.09	0.35	1.60%
Tepidisphaerales WD2101 genus 2	0.03	0.32	1.43%
<i>Acidothermus</i>	0.09	0.32	1.32%
<i>Gemmatimonas</i>	0.03	0.29	1.19%
WPS-2 genus	0.03	0.29	1.14%
<i>Haliangium</i>	0.06	0.29	1.11%
Acidobacteriia Subgroup 2 genus 2	0.08	0.27	0.95%
Acidobacteriales genus 2	0.05	0.25	0.87%
Sphingomonas env.OPS 17 genus	0.00	0.25	0.87%
Chloroflexi AD3 genus	0.05	0.25	0.86%
Pedosphaeraceae ADurb.Bin063-1	0.02	0.23	0.73%
<i>Acidibacter</i>	0.05	0.22	0.64%
<i>Burkholderia-Caballeronia-Paraburkholderia</i>	0.05	0.22	0.64%
Gemmataceae genus	0.04	0.22	0.63%
Pedosphaeraceae genus	0.03	0.21	0.61%
<i>Pseudomonas</i>	0.02	0.20	0.53%
Pirellulaceae genus	0.06	0.20	0.51%
<i>Candidatus</i> Koribacter	0.03	0.19	0.51%
<i>Ferruginibacter</i>	0.03	0.18	0.43%
<i>Sphingomonas</i>	0.02	0.18	0.43%
<i>Nitrospira</i>	0.06	0.18	0.42%
Burkholderiaceae genus	0.04	0.18	0.42%
Thaumarchaeota Group 1.1c genus	0.00	0.17	0.40%
<i>Granulicella</i>	0.02	0.16	0.35%
<i>Rhodanobacter</i>	0.02	0.15	0.31%
<i>Bdellovibrio</i>	0.02	0.15	0.29%
<i>Reyranella</i>	0.04	0.15	0.29%
Microscillaceae genus	0.01	0.14	0.28%
Phycisphaeraceae SM1A02	0.01	0.14	0.27%
Ktedonobacteria B12-WMSP1 genus	0.00	0.13	0.23%
Acidobacteriales genus 3	0.01	0.13	0.22%
<i>Chthonomonas</i>	0.01	0.12	0.21%
Planctomycetes vadinHA49 genus	0.01	0.12	0.19%
<i>Massilia</i>	0.01	0.12	0.19%
Acetobacteraceae genus	0.03	0.12	0.18%

Caulobacteraceae genus	0.02	0.11	0.16%
Tepidisphaerales CPLa-3 genus	0.01	0.10	0.15%
Obscuribacteriales genus	0.01	0.10	0.15%
<i>Lacunisphaera</i>	0.00	0.10	0.15%
Ktedonobacteriales JG30-KF-AS9 genus	0.01	0.10	0.14%
Nitrosomonadaceae MND1	0.02	0.10	0.14%
Elasterales genus	0.02	0.10	0.14%
Acidobacteriales genus 4	0.03	0.10	0.12%
Chloroflexi TK10 genus	0.02	0.09	0.12%
Gammaproteobacteria WD260 genus	0.01	0.09	0.12%
Ktedonobacteraceae genus	0.02	0.09	0.11%
<i>Candidatus</i> Nomurabacteria genus	0.00	0.09	0.11%
Ktedonobacteraceae JG30a-KF-32	0.02	0.09	0.10%
<i>Opitutus</i>	0.01	0.09	0.10%
Formaldehyde oxidation I pathway			
<i>Candidatus</i> Udaeobacter	0.22	0.87	15.52%
Acidobacteriales genus	0.19	0.78	12.50%
<i>Mucilaginibacter</i>	0.01	0.68	10.20%
Chitinophagaceae genus	0.09	0.56	6.64%
<i>Bryobacter</i>	0.09	0.56	6.60%
<i>Candidatus</i> Solibacter	0.08	0.53	5.92%
<i>Granulicella</i>	0.05	0.39	3.29%
Saccharimonadales genus	0.04	0.38	3.19%
Tepidisphaerales WD2101 genus	0.05	0.36	2.78%
Saccharimonadales genus 2	0.06	0.36	2.73%
<i>Acidothermus</i>	0.09	0.32	2.03%
Pedosphaeraceae ADurb.Bin063-1	0.03	0.29	1.80%
Acidobacteriia Subgroup 2 genus	0.07	0.29	1.68%
<i>Flavobacterium</i>	0.02	0.28	1.66%
Pedosphaeraceae genus	0.03	0.26	1.46%
Tepidisphaerales WD2101 genus 2	0.02	0.25	1.39%
Acidobacteriia Subgroup 2 genus 2	0.07	0.25	1.31%
<i>Haliangium</i>	0.04	0.22	1.03%
Acidobacteriales genus 2	0.04	0.22	1.01%
Gemmataceae genus	0.04	0.22	0.99%
<i>Pseudomonas</i>	0.02	0.21	0.98%
Micropepsaceae genus	0.04	0.20	0.83%
<i>Sphingomonas</i>	0.02	0.19	0.75%
<i>Burkholderia-Caballeronia-Paraburkholderia</i>	0.04	0.19	0.73%
<i>Acidibacter</i>	0.04	0.18	0.65%
<i>Candidatus</i> Koribacter	0.03	0.17	0.60%
<i>Gemmatimonas</i>	0.02	0.16	0.52%
Pirellulaceae genus	0.05	0.15	0.48%
<i>Rhodanobacter</i>	0.02	0.14	0.41%
Microscillaceae genus	0.01	0.14	0.40%
<i>Nitrospira</i>	0.04	0.14	0.37%
Sphingobacteriales env.OPS 17 genus	0.00	0.13	0.35%
<i>Ferruginibacter</i>	0.03	0.13	0.35%
Acetobacteraceae genus	0.03	0.13	0.33%
Acidobacteriales genus 3	0.01	0.12	0.29%
<i>Lacunisphaera</i>	0.00	0.11	0.28%
Gemmatimonadaceae genus	0.04	0.12	0.27%
Burkholderiaceae genus	0.02	0.11	0.27%
Phycisphaeraceae SM1A02	0.01	0.11	0.25%

<i>Opitutus</i>	0.01	0.11	0.25%
Gammaproteobacteria WD260 genus	0.01	0.10	0.24%
Ktedonobacteria B12-WMSP1 genus	0.00	0.10	0.23%
Acidobacteriales genus 4	0.03	0.10	0.19%
<i>Nocardioides</i>	0.03	0.10	0.19%
Parcubacteria genus	0.00	0.09	0.19%
Chloroflexi TK10 genus	0.02	0.09	0.18%
WPS-2 genus	0.01	0.09	0.18%
<i>Pseudonocardia</i>	0.02	0.09	0.17%
Acidobacteriales genus 5	0.03	0.09	0.16%
<i>Massilia</i>	0.01	0.08	0.15%
Caulobacteraceae genus	0.02	0.08	0.15%
<i>Bacillus</i>	0.01	0.08	0.14%
Tepidisphaerales Cpla-3 genus	0.01	0.08	0.14%
Ktedonobacteria JG30-KF-AS9 genus	0.01	0.08	0.14%
<i>Occallatibacter</i>	0.01	0.07	0.11%
<i>Edaphobacter</i>	0.02	0.07	0.11%
Acidobacteriia Subgroup 2 genus 3	0.01	0.07	0.11%
<i>Dyella</i>	0.00	0.07	0.11%
Ktedonobacteraceae genus	0.01	0.07	0.11%
Sphingomonadaceae genus	0.02	0.07	0.11%
Blastocatellaceae JGI 0001001-H03	0.02	0.07	0.10%
Nitrosomonadaceae Ellin6067	0.02	0.07	0.10%
Ktedonobacteraceae JG30a-KF-32	0.01	0.07	0.10%
<i>Pedospaera</i>	0.01	0.07	0.10%

¹ Value calculated by multiplying the relative abundance of the microbial genus by the genome function count generated by PICRUSt2 analysis.

² Value calculated by multiplying (relative contribution in 2017 - relative contribution in 2013) by the total relative contribution.

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