Article

Evolution of the Piauí Laterite, Brazil: Mineralogical, Geochemical and Geomicrobiological Mechanisms for Cobalt and Nickel Enrichment

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Figures

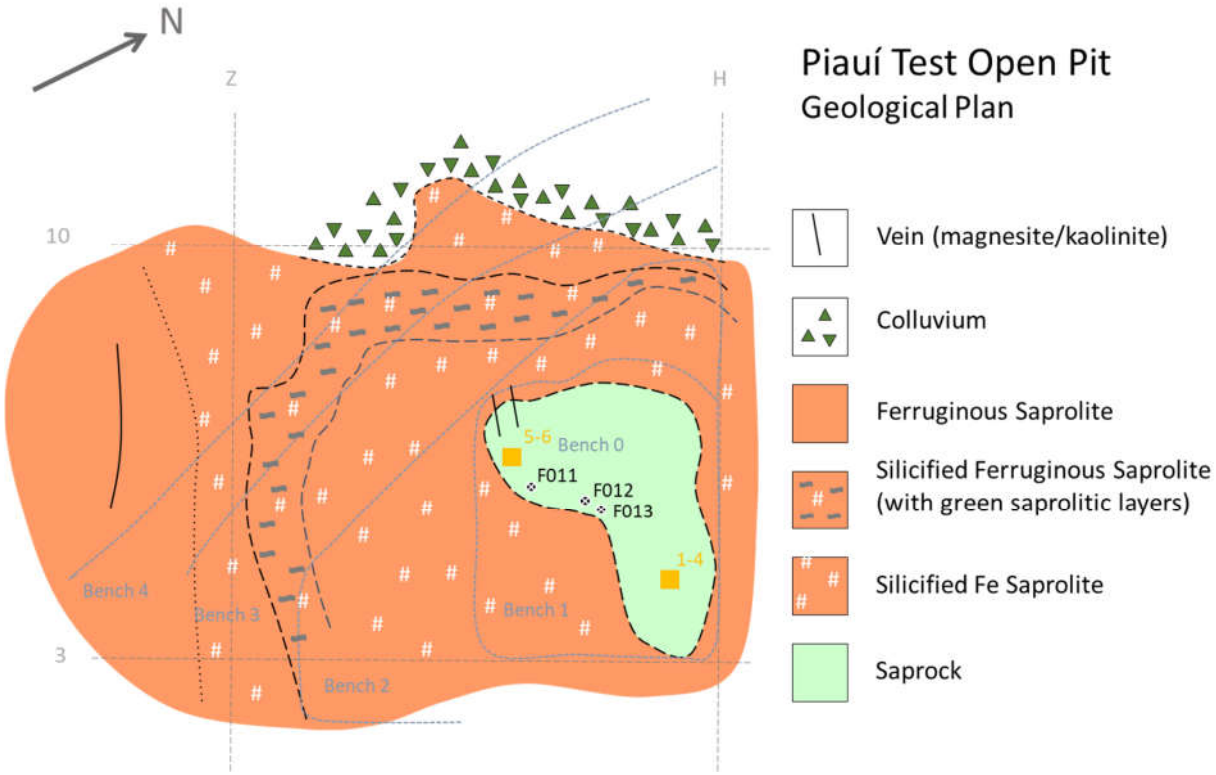

Figure S1. A schematic of the Piauí Test Pit showing a simplified general representation of the major units. Three samples from the mineralogy and geochemistry study (F011–F013) and 2 sampling sites from the microbiology study (yellow squares) are shown on the floor of Bench 0.

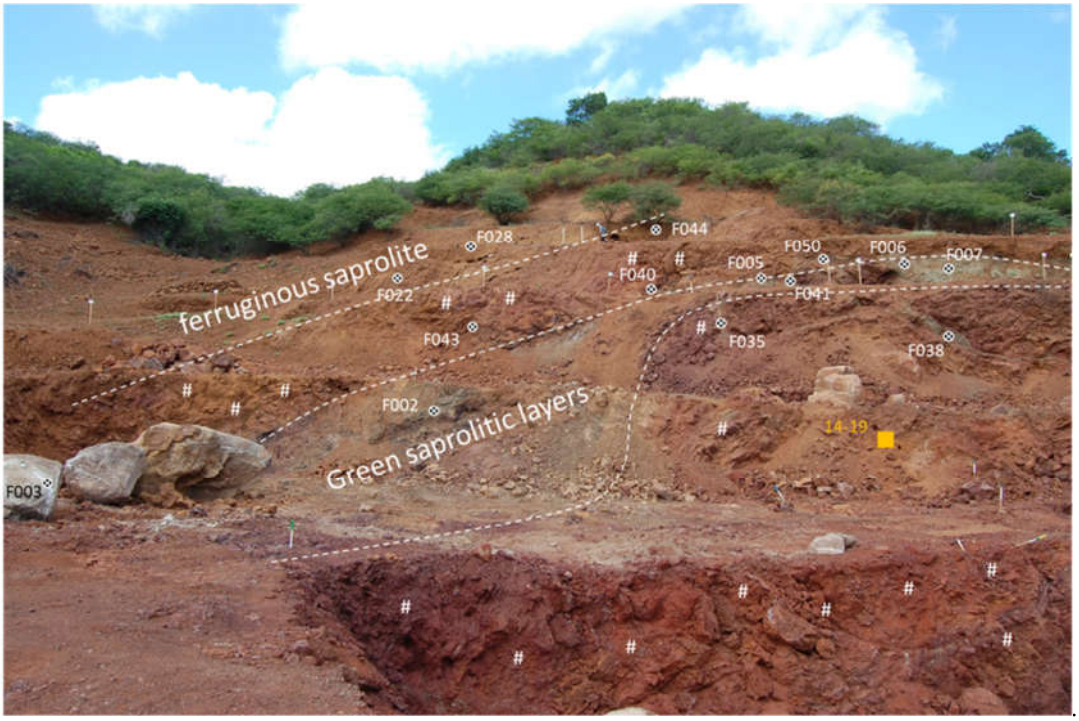

Figure S2. Sampling sites for the mineralogy and geochemistry (white circles with cross). Samples F011, F012 and F013 (green silica nodules) were collected as loose samples from Bench 0 at the base of the wall of Bench 1 (shown in Figure S1). Samples of serpentine were collected between the test pit and the processing plant. The microbiology sampling sites are shown by gold squares. The sites for samples 1-6 are shown in Figure S1). # indicates silicified Fe saprolite.

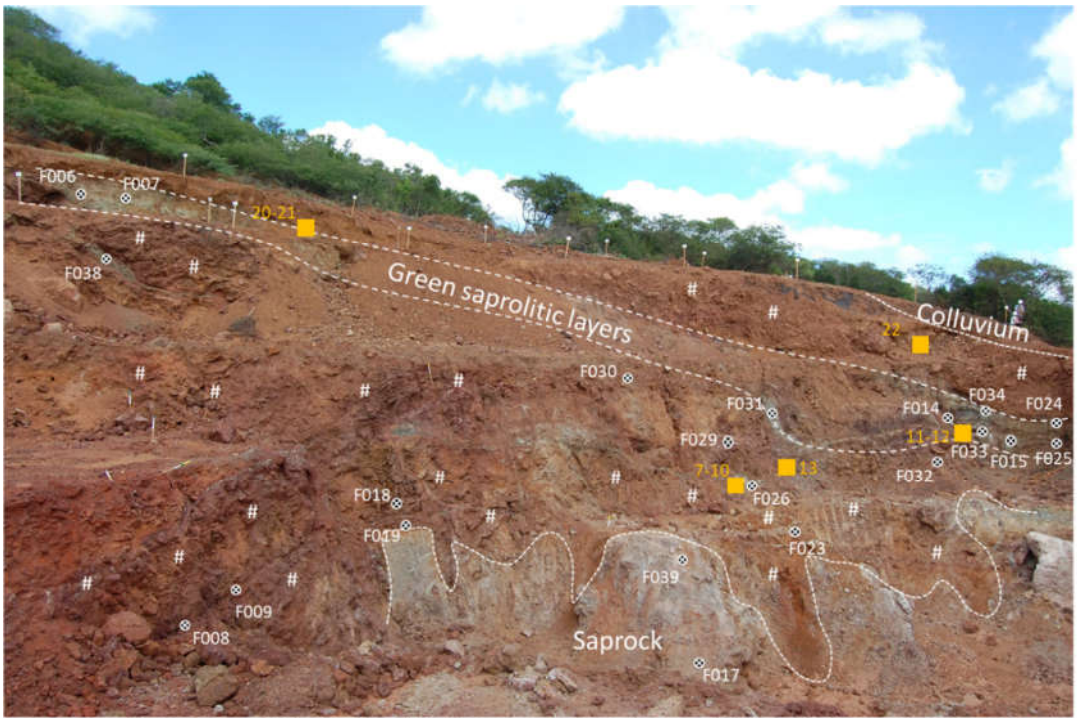

Figure S3. Sampling sites for the mineralogy and geochemistry (white circles with cross). Samples F011, F012 and F013 (green silica nodules) were collected as loose samples from Bench 0 at the base of the wall of Bench 1 (shown in Figure S1). Samples of serpentine were collected between the

test pit and the processing plant. The microbiology sampling sites are shown by gold squares. The sites for samples 1–6 are shown in Figure S1). # indicates silicified Fe saprolite.

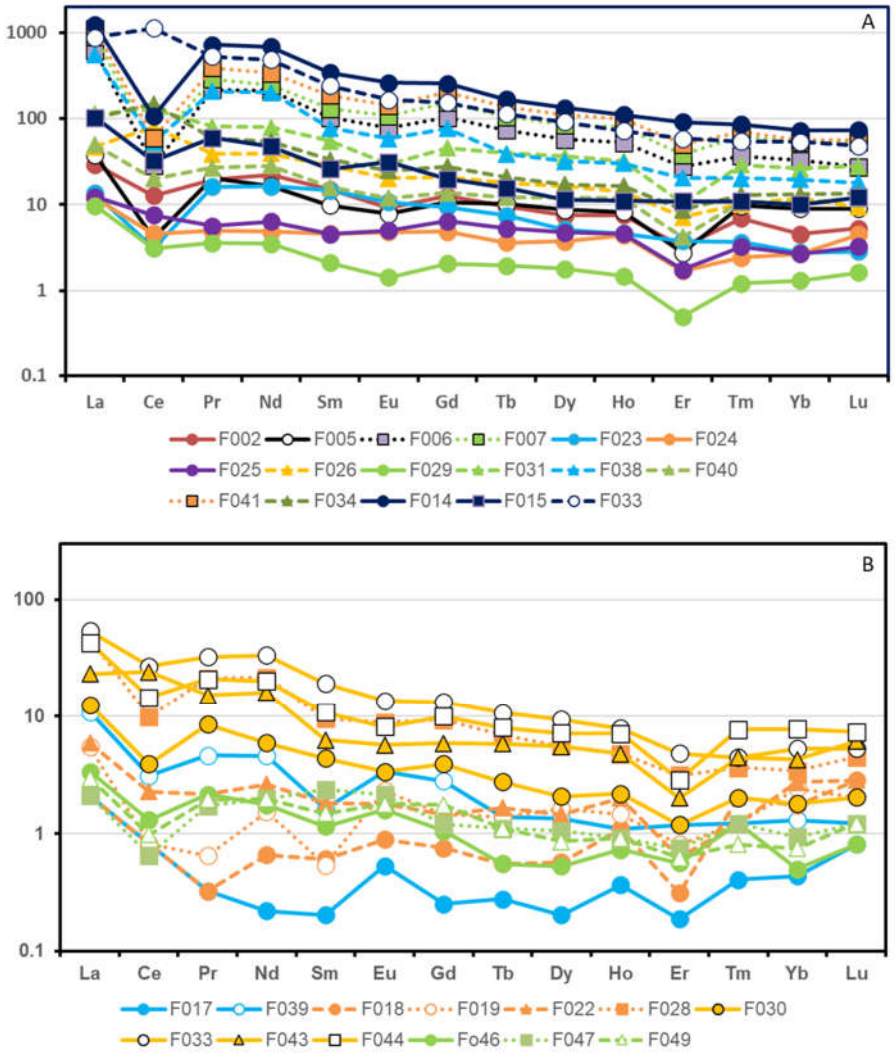

Figure S4. Chondrite normalised REE plots for (**A**) SAPMG and SAPAL (dark blue); and (**B**) serpentinite (green), saprock (light blue), SAPSILFE (gold) and SAPFE (orange). REE concentrations are normalised to the CM values of [42].

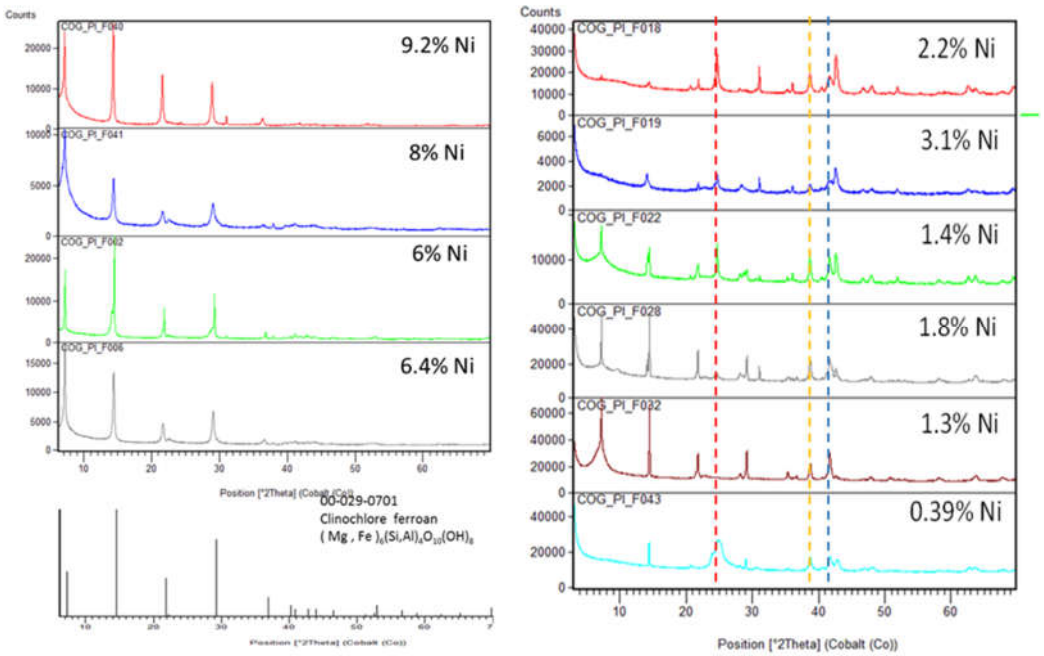

Figure S5. XRD patterns of samples with the highest Ni content (above 6wt%) on the left and with the highest content of Fe oxides on the right. Red, orange and blue dotted lines indicate the position of the main peak for goethite, hematite and maghemite, respectively.

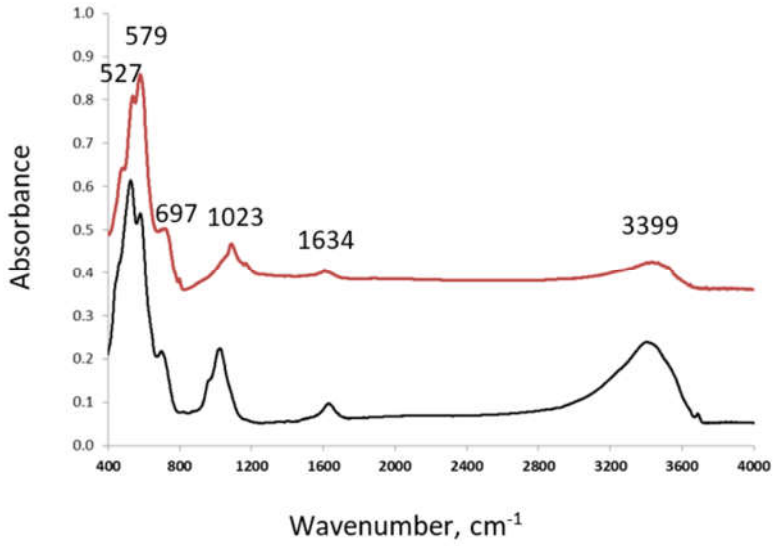

Figure S6. FTIR spectrum of sample F050 (black trace) compared with romanechite mineral standard (BM 1955,66) from the NHM mineral collection (red trace). The peak positions for the sample: 3399 cm^{-1}, 1634 cm^{-1}, 1023 cm^{-1}, 697 cm^{-1}, 579 cm^{-1}, 527 cm^{-1}.

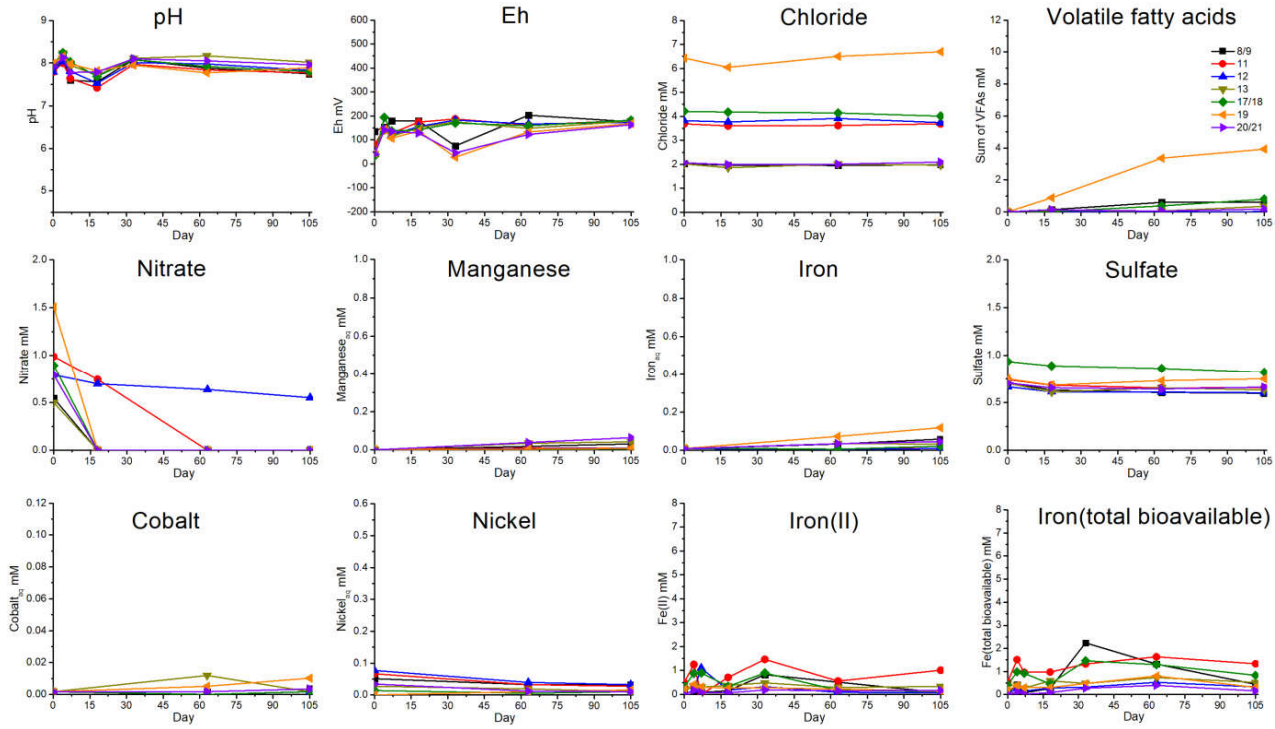

Figure S7. Glucose stimulated sediment microcosm results.

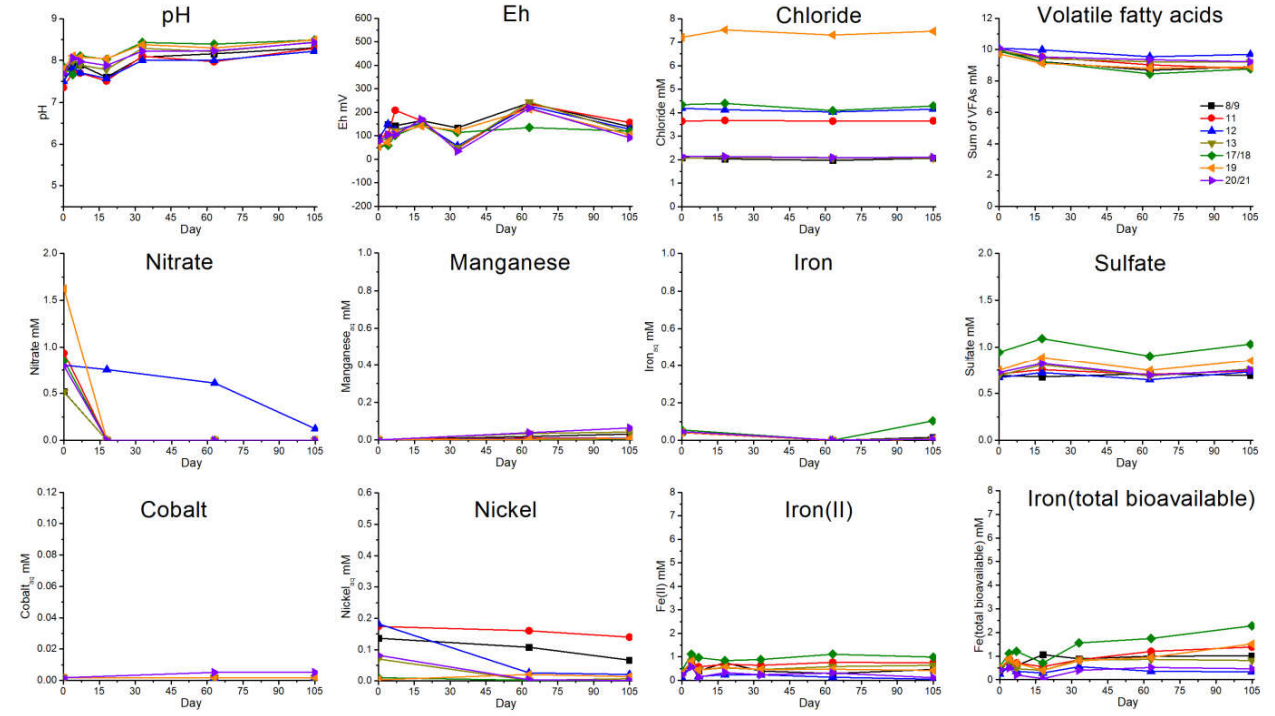

Figure S8. Acetate/lactate stimulated sediment microcosm results.

Tables

Table S1. Additional elements from the bulk chemical analyses of the samples from the Piauí laterite not presented in Table 1 of the manuscript.

(A)

		Serpentinite			Saprock		SAPFE				SAPSILFE				Green Silica Nodules					Mn Oxide
Analyte	**Unit**	**F046**	**F047**	**F049**	**F017**	**F039**	**F018**	**F019**	**F022**	**F028**	**F030**	**F032**	**F043**	**F044**	**F008**	**F009**	**F011**	**F012**	**F013**	**F050**
Cs	ppm	0.01	0.05	0.04	0.08	0.15	0.18	0.14	0.13	0.15	0.07	0.22	0.12	0.09	0.23	0.09	0.05	0.16	0.14	0.25
Ga	ppm	4.8	5.4	5.6	3.3	6.8	12	9.2	10.6	10.7	7.7	11.7	9.9	7.3	1.4	1.5	1.6	2	2.2	76.9
Hf	ppm	0.2	<0.2	<0.2	0.2	0.3	0.5	<0.2	0.8	0.2	0.2	0.2	0.4	0.2	<0.2	2.1	<0.2	0.2	0.4	<0.2
Nb	ppm	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	0.3	<0.2	0.2	<0.2	<0.2	<0.2	<0.2	1	<0.2	0.2	0.4	<0.2
Rb	ppm	1.1	0.9	1	0.4	1	1.3	1	1.4	1.3	0.7	1.4	1	0.9	0.8	0.4	0.5	1.9	1.2	2.5
Sn	ppm	1	<1	<1	<1	<1	<1	<1	<1	<1	1	<1	<1	<1	<1	4	1	1	1	<1
Sr	ppm	0.8	1.1	0.8	0.8	3.8	5.2	3.3	4	3.9	4.3	10.3	1.4	1.8	0.9	0.8	0.5	2	0.9	24.6
Ta	ppm	<0.1	<0.1	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	<0.1	<0.1	<0.1	<0.1	0.2	0.1	0.1	0.1	0.1
Th	ppm	0.07	<0.05	0.06	0.05	<0.05	0.1	0.06	0.4	0.14	0.07	0.38	<0.05	0.08	<0.05	0.12	<0.05	0.1	0.05	0.76
U	ppm	0.23	0.56	0.43	0.08	0.33	0.23	0.25	0.19	0.14	0.17	0.14	0.34	0.24	0.28	0.2	0.15	0.23	0.17	6.22
V	ppm	58	68	65	<50	<50	<50	<50	<50	<50	117	<50	<50	<50	<5	<5	<5	<5	<5	196
W	ppm	1	1	2	1	1	2	1	2	2	1	1	1	1	<1	3	3	1	2	2
Y	ppm	1.2	1.8	1.8	<0.5	3.2	3.9	3.7	2.6	13.3	3.9	11.8	7.6	12.3	1	0.4	0.2	0.3	0.4	89.8
Zr	ppm	2	2	3	4	10	9	6	24	7	3	5	9	10	3	97	3	3	14	5

Table S2. Additional elements from the bulk chemical analyses of the samples from the Piauí laterite not presented in Table 1 of the manuscript.

		SAPAL			SAPMG														magnesite	kaolinite
Analyte	**Unit**	**F014**	**F015**	**F033**	**F002**	**F005**	**F006**	**F007**	**F023**	**F024**	**F025**	**F026**	**F029**	**F031**	**F034**	**F038**	**F040**	**F041**	**F003**	**F035**
Cs	ppm	0.39	0.25	2.13	1.17	0.33	6.03	5.47	0.29	0.09	0.28	6.25	0.2	5.89	3.34	0.83	0.36	5.31	0.07	0.22
Ga	ppm	11.9	14	20.1	10.6	28.4	14.1	7.5	8.9	8.5	8.2	16.5	7.9	14.4	18.1	12	25.7	8.2	0.6	11.1
Hf	ppm	9.2	5.5	5.5	0.5	<0.2	1.1	0.6	0.3	0.2	<0.2	1.3	0.3	20.5	2.8	4.9	<0.2	1	<0.2	0.3
Nb	ppm	2.2	2.2	9.5	4.9	0.3	8.9	15	0.3	<0.2	<0.2	7.3	0.3	14.9	8.9	7.2	<0.2	17.2	<0.2	1.1
Rb	ppm	1.2	0.9	6.1	2.5	0.9	22.5	17.3	1.1	1	1.1	13.6	1.4	9.7	6.7	2.6	1.2	17.3	0.5	1
Sn	ppm	1	2	3	<1	1	8	3	1	<1	<1	1	<1	2	1	1	<1	2	<1	<1
Sr	ppm	12.5	9.9	13.5	1.6	6.5	10.1	10.8	4.2	4.7	4.6	7.9	3.5	12	12.1	3.9	5.4	11.3	2	2.5
Ta	ppm	0.6	0.2	0.8	0.4	0.1	0.2	0.2	0.1	<0.1	<0.1	<0.1	<0.1	0.2	0.1	0.5	<0.1	1.1	<0.1	<0.1
Th	ppm	39.4	4.76	45.6	0.25	0.24	1.56	0.81	0.89	<0.05	<0.05	1.31	0.42	1.78	0.99	2.71	0.21	1.93	<0.05	0.3
U	ppm	3.08	0.67	2.03	0.19	0.9	1.41	1.32	0.08	0.14	0.22	0.19	0.2	0.67	0.39	2.18	1.31	1.65	<0.05	0.37
V	ppm	14	20	78	83	30	59	42	18	<50	<50	96	<50	79	34	23	<50	28	5	<5
W	ppm	1	<1	1	1	2	2	2	<1	2	1	1	1	2	1	1	1	2	1	<1
Y	ppm	208	20	125	18.4	13.1	110	194	7.1	10.7	13.7	22.2	4.3	46.2	26.9	102	16	239	0.8	4.1
Zr	ppm	214	238	171	22	3	29	15	4	7	6	44	10	836	133	185	5	37	4	14

Table S3. Fitting parameters for the Co K-edge microfocus XAS spectra from asbolane-lithiophorite intermediates in sample F033. CN—coordination number, R—interatomic distance, σ^2—Debye–Waller factor, R-factor—Artemis quality of fit estimate [33].

			Co-O			Co-(Co, Mn)			Co-(Co, Mn) Corner Sharing Octahedra		
Site	**R-Factor**	**X**	**R(Å)**	**CN**	**σ^2**	**R(Å)**	**CN**	**σ^2**	**R(Å)**	**CN**	**σ^2**
grain 1	0.03										
Co^{3+} cluster		0.6	1.90(5)	6	0.002(6)	2.83(3)	6	0.006(5)	3.54(9)	2	0.004(9)
Co^{2+} cluster		0.4	2.09(7)	6	0.001(1)						
grain 2	0.01		1.96(1)	6	0.001(1)	2.80(1)	4	0.004(1)	3.46(4)	2	0.003(4)
grain 3	0.03		1.91(2)	6	0.002(1)	2.83(2)	6	0.006(2)			
grain 4	0.02		1.92(2)	6	0.005(1)	2.85(2)	6	0.005(1)	3.53(6)	2	0.007(8)

Table S4. Fitting parameters for the Ni K-edge microfocus XAS spectra from asbolane-lithiophorite intermediates in sample F033. CN—coordination number, R—interatomic distance, σ^2—Debye–Waller factor, R-factor—Artemis quality of fit estimate [33].

		Ni-O			Ni-Ni		
Site	**R-Factor**	**R(Å)**	**CN**	**σ^2**	**R(Å)**	**CN**	**σ^2**
grain 1	0.03	2.04(1)	6	0.008(1)	2.97(2)	2	0.008(2)
grain 2	0.03	2.06(1)	6	0.004(1)	3.08(2)	2	0.002(2)
grain 3	0.03	2.04(1)	6	0.003(1)	3.02(3)	2	0.006(3)
grain 4	0.02	2.03(1)	6	0.004(1)	3.04(3)	2	0.007(3)

Table S5. Details of samples analysed for microbial communities.

Biological Sample ID	**8**	**10**	**12**	**15**	**17**	**20**
Approximate depth below ground level	5m	5m	5m	3m	3m	1m
Lithological unit based on geochemistry	SAPSILFE	SAPSILFE	SAPMG	SAPSILFE	SAPFE	SAPSILFE
Description	Yellow black red sand & gravel, beneath green vein	Above green vein	Black magnetic rock	Red limonite	Orange limonite	Sandy soil (weathered breccia)
pH	6.16	6.54	5.05	7.39	6.99	6.54
Total organic carbon %	0.02	0.02	0.03	0.08	0.09	0.14
Inorganic carbon %	0.01	0.01	0.01	0.01	0.01	0.02
Sample type	Sediment core	Bulk sediment	Bulk sediment	Sediment core	Sediment core	Sediment core
DNA extraction kit used	PowerSoil	PowerSoil & Fast DNA	PowerSoil & Fast DNA	PowerSoil	PowerSoil	PowerSoil
Mass of sample DNA extracted from (g)	0.25	0.25 & 2.1	0.5 & 2.10	0.25	0.25	0.25
16S rRNA gene sequenced	Yes	Insufficient DNA	Yes	Yes	Yes	Yes
Number of prokaryotic OTUs	79	-	107	111	125	478
Shannon diversity at 140200 reads	4.34	-	3.28	5.29	2.90	7.38
16S rRNA gene abundance fg/g	< detection	-	< detection	< detection	1273	44.1

ITS region sequenced	Insufficient DNA	-	-	-	Yes	Yes
Number of fungal OTUs	-	-	-	-	32	21
18S rRNA gene abundance fg/g	-	-	-	-	125	167
Fe_2O_3 (%)	38.2	40.4	34.1	21.2	57.6	29.4
MnO (%)	0.54	0.60	0.38	0.25	0.67	0.61
Co (%)	0.07	0.08	0.04	0.05	0.11	0.15
Ni (%)	1.93	2.29	1.73	1.24	2.73	2.88
Cr (%)	1.88	1.45	1.31	1.03	2.16	1.04
Cu (%)	0.05	0.10	0.07	0.06	0.12	0.25
Zn (%)	0.03	0.03	0.03	0.02	0.05	0.06
Na_2O (%)	0.02	-	0.03	-	0.08	0.02
MgO (%)	10.7	11.5	12.8	6.01	10.11	8.72
Al_2O_3 (%)	4.50	3.71	5.58	2.31	3.50	8.15
SiO_2 (%)	29.8	27.4	29.4	63.5	14.1	32.5
K_2O (%)	0.01	0.01	0.01	0.02	0.01	0.04
CaO (%)	0.04	0.04	0.07	0.01	0.03	0.04
P_2O_5 (%)	0.03	0.03	0.01	0.03	0.04	0.02
SO_3 (%)	0.01	0.01	0.00	0.01	0.05	0.01
Cl (%)	0.01	-	0.05	-	0.09	0.01
TiO_2 (%)	0.07	0.06	0.04	0.06	0.08	0.26

"-"—not analysed.

Table S6. Closest phylogenetic relatives of the five most abundant prokaryotic OTUs (highlighted in yellow) in the sediment core/bulk sediment samples.

#OTU ID	Sample number / Sample depth (mbgl) / Consensus Lineage	Number of reads: 8 / 5	12 / 5	15 / 3	17 / 3	20 / 1	% of community: 8 / 5	12 / 5	15 / 3	17 / 3	20 / 1	Name of closest relative	Accession Number	% ID similarity (maximum)	Score (maximum)	Description
2	D_0__Bacteria; D_1__Proteobacteria; D_2__Gammaproteobacteria; D_3__Betaproteobacteriales; D_4__Burkholderiaceae; D_5__Burkholderia-Caballeronia-Paraburkholderia; D_6__uncultured Burkholderia sp.	5	**85408**	5	16	2	0.0	**56.1**	0.0	0.0	0.0	*Burkholderia graminis* strain C4D1M	NR_029213.2	100 (100)	457 (457)	Type strain from rhizosphere. Similarly related to other type strain and cultured *Burkholderia* spp. from the rhizosphere, and uncultured bacteria from study of hydrocarbon degrading isolates, hot springs
4	D_0__Bacteria; D_1__Proteobacteria; D_2__Gammaproteobacteria; D_3__Betaproteobacteriales; D_4__Burkholderiaceae; D_5__Massilia; D_6__uncultured beta proteobacterium	1275	10	13	**68255**	99	1.5	0.0	0.0	**54.8**	0.0	*Massilia* sp. strain UFLA01-891	KX555376.1	100 (100)	457 (457)	Rhizosphere from oxisol in the pre-Amazon region of Maranhao. Similarly related to *Massilia* spp. from freshwater lakes, high As groundwater, cave biofilms, Antarctica, vineyards, mountains, wetlands, air samples and thermal environments
6	D_0__Bacteria; D_1__Actinobacteria; D_2__Actinobacteria			5293	**7155**	3			3.7	**5.7**	0.0	Uncultured bacterium clone 7846	KU105136.1	100 (100)	457 (457)	Soil (Australian wheat & canola). Similarly related to uncultured bacteria from arid soils, rhizosphere. No close cultured relatives
7	D_0__Bacteria; D_1__Actinobacteria; D_2__Actinobacteria	1		1351	**4819**	1	0.0		0.9	**3.9**	0.0	Uncultured bacterium clone 725	KU966862.1	98 (98)	434 (434)	Rhizosphere. Similarly related to uncultured bacteria from wetland restoration, study of soil cropping systems, soils. No close cultured relatives
17	D_0__Bacteria; D_1__Proteobacteria; D_2__Gammaproteobacteria; D_3__Betaproteobacteriales; D_4__Burkholderiaceae; D_5__Noviherbaspirillum; D_6__uncultured bacterium	**24260**	**18351**	2	7	3	**29.4**	**12.1**	0.0	0.0	0.0	*Noviherbaspirillum suwonense* sp. nov	NR_133798.1	99 (99)	448 (448)	Type strain: air sample. Similarly related to *Noviherbaspirillum* spp. from clay deposits, bottled water and *Herbaspirillum* spp. from mine tailings ore. Also to study of aerobic anoxygenic phototrophs, thermal environments, glacial environments. Also closely related to *Ralstonia* spp.
21	D_0__Bacteria; D_1__Actinobacteria; D_2__Actinobacteria; D_3__Micrococcales; D_4__Micrococcaceae	**19243**		2	3	30	**23.3**		0.0	0.0	0.0	*Sinomonas notoginsengisoli* strain SYP-B575	NR_134810.1	100 (100)	457 (457)	Type strain: rhizosphere. Similarly related to *Sinomonas* spp. from study of facultatively oligotrophic soil bacteria, rock biocrusts, rhizosphere, oil shale mines, coal beds, study of silicate mineral weathering bacteria
24	D_0__Archaea; D_1__Euryarchaeota; D_2__Thermoplasmata; D_3__Methanomassiliicoccales; D_4__uncultured; D_5__uncultured archaeon; D_6__uncultured archaeon				1	**19393**				0.0	**9.4**	Uncultured bacterium clone OTU_2843	KP787684.1	99 (99)	443 (452)	Soils contaminated with heavy metals. Similarly related to uncultured bacteria from general soils, groundwater, caves, geological formations. No close cultured relatives
28	D_0__Bacteria; D_1__Cyanobacteria; D_2__Oxyphotobacteria; D_3__Chloroplast; D_4__Nannochloropsis gaditana; D_5__Nannochloropsis gaditana; D_6__Nannochloropsis gaditana	1	1	**17591**		1	0.0	0.0	**12.2**		0.0	*Nannochloropsis gaditana* chloroplast, strain RCC 504	LN735429.3	100 (100)	457 (457)	Photosynthetic eukaryote. Similarly related to *Nannochloropsis* spp. from studies of microalgae, oil/biofuel producing microalgae, aquaculture, microbial mats, rivers, sediments, Antarctic environments
44	D_0__Bacteria; D_1__Cyanobacteria; D_2__Sericytochromatia; D_3__uncultured bacterium; D_4__uncultured bacterium; D_5__uncultured bacterium; D_6__uncultured bacterium		**8826**			1		**5.8**			0.0	Uncultured bacterium clone 10370	MF014707.1	100 (100)	457 (457)	Study of wetland restoration. Similarly related to uncultured bacteria from clay rock, deep sea nodules, basalt, "lithifying microbialites"
47	D_0__Bacteria; D_1__Bacteroidetes; D_2__Bacteroidia; D_3__Sphingobacteriales; D_4__Sphingobacteriaceae; D_5__Pedobacter; D_6__uncultured bacterium	1	2	1	**9011**	1	0.0	0.0	0.0	**7.2**	0.0	*Pedobacter ginsengiterrae* strain DCY49	NR_109023.1	100 (100)	457 (457)	Type strain isolated from ginseng field soil. Similarly related to *Pedobacter* spp. from Antarctic soil, aromatic hydrocarbon degraders from Arctic soil
50	D_0__Bacteria; D_1__Verrucomicrobia; D_2__Verrucomicrobiae; D_3__Chthoniobacterales; D_4__Chthoniobacteraceae; D_5__Candidatus Udaeobacter; D_6__uncultured Prosthecobacter sp.	1		1		**8422**	0.0		0.0		**4.1**	Uncultured Spartobacteria bacterium clone F15	KX779955.1	99 (99)	452 (452)	Bacterial community after introduction sugarcane in tropical dry forest. Similarly related to uncultured bacteria from hot springs, restored wetlands, Cr(VI) contaminated soils, elevated CO_2 soils, heavy metal contaminated soils, river sediments associated with mining. Closest cultured relative is an unidentified eubacterium from the Amazon (98%, score 441, U68652.1, [113])
53	D_0__Bacteria; D_1__Proteobacteria; D_2__Deltaproteobacteria; D_3__Myxococcales; D_4__bacteriap25	2		**8882**	4		0.0		**6.2**	0.0		Uncultured bacterium clone OTU_11847	KR843131.1	92 (92)	369 (369)	No close relatives. Study of soil cropping system. Similarly related to uncultured bacteria from study of wetland restoration, Australian wheat & canola soil.

#OTU ID	Sample number	8	12	15	17	20	8	12	15	17	20	Name of closest relative	Accession Number	% ID similarity (maximum)	Score (maximum)	Description
	Sample depth (mbgl)	5	5	3	3	1	5	5	3	3	1					
	Consensus Lineage	Number of reads					% of community									
66	D_0__Bacteria; D_1__Proteobacteria; D_2__Alphaproteobacteria; D_3__Sphingomonadales; D_4__Sphingomonadaceae; D_5__Sphingomonas; D_6__Sphingomonas sp. DSV5M		**6143**		52			**4.0**		0.0		*Sphingomonas olei* strain K-1-16	NR_157757.1	100 (100)	457 (457)	Type strain: *Spirgomonas olei* that can degrade aliphatic hydrocarbons isolated from oil-contaminated soil. Similarly related to other *Spirgomonas* spp. from soil, Arctic dust, building (Lede) stone, study plant growth promoting bacteria
68	D_0__Bacteria; D_1__Proteobacteria; D_2__Gammaproteobacteria; D_3__Acidiferrobacterales; D_4__Acidiferrobacteraceae; D_5__Sulfurifustis		1	**7225**				0.0	**5.0**			Uncultured bacterium clone 795765_1	MF366392.1	98 (98)	430 (430)	Aquifer after long-term irrigation with wastewater. Similarly related to uncultured bacteria from study of bioleaching community and Fe and S cycling, study of biostimulation of nitrate-contaminated aquifer with pyrite, Fe-phyllosilicate redox cycling organisms, carbonate pools. No close cultured relatives
71	D_0__Bacteria; D_1__Proteobacteria; D_2__Alphaproteobacteria; D_3__Sphingomonadales; D_4__Sphingomonadaceae; D_5__Novosphingobium				**6428**	3				**5.2**	0.0	*Novosphingobium lindaniclasticum* LE124	NR_118312.1	100 (100)	457 (457)	Type strain: a hexacyclohexane-degrading bacterium isolated from a HCH dumpsite. Similarly related to other *Novosphingobium* spp. from soil, deep sea sediment, study of endophytes, river biofilms that degrade glyphosate, rhizosphere
73	D_0__Bacteria; D_1__Planctomycetes; D_2__Planctomycetacia; D_3__Pirellulales; D_4__Pirellulaceae; D_5__uncultured			**5825**		1			**4.1**		0.0	Uncultured bacterium clone 00868	MF005338.1	99 (99)	443 (443)	Wetland restoration. Simiarly related to uncultured bacteria from the rhizosphere, river floodplains, biochar amended soils, Cr(VI)-contaminated soils, ureolytic prokaryotes, "lithifying microbialites". No close cultured relatives
81	D_0__Bacteria; D_1__Proteobacteria; D_2__Gammaproteobacteria; D_3__Acidithiobacillales; D_4__Acidithiobacillaceae; D_5__Acidithiobacillus; D_6__Acidithiobacillus thiooxidans	**6251**		1			**7.6**		0.0			*Acidithiobacillus thiooxidans* strain ATCC 19377	NR_044920.1	100 (100)	457 (457)	Type strain: study of acidophilic bioleaching associated bacteria. Similarly related to *Acidithiobacillus ferrooxidans* type strain and *Acidithiobacillus* spp. from study of sulfur oxidising populations, copper mines, bioleaching pulp/columns
84	D_0__Bacteria; D_1__Proteobacteria; D_2__Alphaproteobacteria; D_3__Sphingomonadales; D_4__Sphingomonadaceae; D_5__Porphyrobacter; D_6__uncultured bacterium	1		**5674**	1	3	0.0		**3.9**	0.0	0.0	*Porphyrobacter neustonensis* strain DSM 9434	NR_114651.1	99 (100)	448 (457)	Type strain, a slightly thermophilic aerobic bacteriochlorophyll a-containing species. Simiarly related to *Porphyrobacter* spp. from study of algal-bacterial consortia, aerobic anoxygenic phototrophs from mountain lakes, hot springs,
98	D_0__Bacteria; D_1__Proteobacteria; D_2__Gammaproteobacteria; D_3__Betaproteobacteriales; D_4__Burkholderiaceae; D_5__Burkholderia-Caballeronia-Paraburkholderia; D_6__Burkholderia sp. Br3466		**6897**			1		**4.5**			0.0	*Burkholderia sprentiae* strain WSM5005	NR_117691.1	100 (100)	457 (457)	Type strain: root nodules. Similarly related to *Burkholderia* spp. from leaf nodules, TCE degrader, study of endophytes, rhizosphere, nitrogen-fixing bacteria
100	D_0__Bacteria; D_1__Firmicutes; D_2__Clostridia; D_3__Clostridiales; D_4__Peptococcaceae; D_5__Desulfosporosinus; D_6__uncultured bacterium	**5170**			5	1	**6.3**			0.0	0.0	*Desulfosporosinus* sp. 44a-T3a	AY082482.1	99 (99)	452 (452)	Sulfate-reducing bacteria dominated biofilm from acid mine drainage. Similarly related to *Desulfosporosinus* spp. from coastal sediments, oxidised mine wastes and uncultured bacteria from wetland restoration, rhizosphere, clay rock, algal mats, hypersaline lake, permafrost
122	D_0__Bacteria; D_1__Planctomycetes; D_2__Planctomycetacia; D_3__Gemmatales; D_4__Gemmataceae; D_5__uncultured					**3366**					**1.6**	16S rRNA amplicon fragment from a soil sample (ferralsol, Madagascar)	FQ684691.1	94 (94)	389 (394)	No close cultured or uncultured relatives. Similarly related to uncultured bacteria from forest soils, study of ureolytic prokaryotes, wetland restoration, hot springs, "lithifying microbialites"
142	D_0__Bacteria; D_1__Acidobacteria; D_2__Subgroup 6	**3526**			2	1	**4.3**			0.0	0.0	Uncultured bacterium clone KTTB3245	MG395967.1	99 (99)	446 (448)	Mixed grass prairie soils. Similarly related to uncultured bacteria from coastal saline soil rhizosphere, agricultural soils, study endophytes, study wetland denitrification, volcanic soils. No close cultured relatives.
192	D_0__Bacteria; D_1__Gemmatimonadetes; D_2__Gemmatimonadetes; D_3__Gemmatimonadales; D_4__Gemmatimonadaceae; D_5__uncultured; D_6__uncultured bacterium			785	5	**4632**			0.5	0.0	**2.2**	16S rRNA amplicon fragment from a soil sample (ferralsol, Madagascar)	FQ685425.1	99 (99)	448 (448)	Ferralsol incubation experiment. Similarly related to uncultured bacteria from elevated CO_2 soils, a hydrocarbon contaminated aquifer, agricultural soils. No close cultured relatives
231	D_0__Bacteria; D_1__Gemmatimonadetes; D_2__Gemmatimonadetes; D_3__Gemmatimonadales; D_4__Gemmatimonadaceae; D_5__uncultured				19	**3411**				0.0	**1.7**	*Gemmatimonas* sp. strain CYBC134	MK067196.1	98 (99)	434 (444)	Isolates from crude oil contaminated soil. Similarly related to uncultured bacteria from forest soil (99%, 444, KU960457.1), volcanic soils, mountain soils, Cr(VI)-contaminated soils

Table S7. Closest phylogenetic relatives of the five most abundant fungal OTUs (highlighted in yellow) in the sediment core/bulk sediment samples. Those in grey are distantly related.

#OTU ID	Sample number / Sample depth (mbgl) / Consensus Lineage	17/18	20/21	17/18	20/21	Name of closest relative (from TYPE material)	Accession Number	% ID similarity	Score	Description
		3	1	3	1					
		Number of reads		% of community						
380	k__Fungi	2142		2.4		*Rhodotorula mucilaginosa* CBS 316 ITS region	NR_073296.1	100	392	Common saprotrophic environmental yeast found in air, soil, water [114]. Can degrade PAHs [115]
1326	k__Fungi;p__Ascomycota;c__Eurotiomycetes;o__Eurotiales;f__Trichocomaceae;g__Aspergillus		2392		5.3	*Aspergillus venenatus* NRRL 13147 ITS region	NR_135448.1	100	302	Similarly related to other *Aspergillus* species. Isolated from air & soil [116]. Saprotrophic. Can live in range of pH conditions, and on high C substrates but also with limited C
1359	k__Fungi;p__Ascomycota;c__Eurotiomycetes;o__Chaetothyriales;f__Chaetothyriaceae;g__unidentified;s__unidentified_SH524252.07FU		13811		31	*Knufia karalitana* CBS 139720 ITS region	NR_145018.1	89	192	Similarly (poorly) related to other *Knufia* species
1383	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Hypocreales_fam_Incertae_sedis;g__Ilyonectria	56237		63		*Ilyonectria cyclaminicola* CBS 302.93 ITS region	NR_121495.1	97	273	Similarly related to other *Ilyonectria* species. Plant pathogen causing root disease
1435	k__Fungi;p__Ascomycota		15530		34	*Teichospora acaciae* ITS region	NR_138410.1	81	64.4	No close relatives
1436	k__Fungi;p__Ascomycota;c__Eurotiomycetes;o__Chaetothyriales;f__Chaetothyriaceae;g__unidentified;s__unidentified_SH524252.07FU		1989		4.4	*Knufia karalitana* CBS 139720 ITS region	NR_145018.1	89	183	Similarly related to other *Knufia* species
1503	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Dothideomycetidae_ord_Incertae_sedis;f__Eremomycetaceae;g__Arthrographis;s__Arthrographis_longispora_SH215555.07FU	1954		2.2		*Arthrographis longispora* UTHSC 05-3220 ITS region	NR_132009.1	99	253	Mould, isolated from air, soil, sediment, water [117]
1516	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Hypocreales_fam_Incertae_sedis;g__Ilyonectria	2846		3.2		*Ilyonectria cyclaminicola* CBS 302.93 ITS region	NR_121495.1	99	284	Similarly related to other *Ilyonectria* species. Plant pathogen causing root disease
1577	k__Fungi;p__Ascomycota;c__Dothideomycetes		3860		8.5	*Trinosporium guianense* CBS 132537 ITS region	NR_121543.1	77	87.8	No close relatives
1723	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__unidentified;g__unidentified;s__unidentified_SH182991.07FU	15233		17		*Wojnowiciella viburni* MFLUCC 120733a ITS region	NR_120266.1	89	196	Similarly related to other *Wojnowiciella* and *Septoriella* species

Methods for Microbiological Characterisation

Prokaryotes

For the five samples with adequate DNA yield (Table S5), the prokaryotic DNA was amplified using the universal 16S rRNA primers 8F and 1492R [95] and the purity of the polymerase chain reaction (PCR) products was determined by visualisation under short-wave UV light after staining with Sybersafe® and separation by electrophoresis in Tris-acetate-EDTA gel. The PCR products were cleaned up, quantified and 16S rRNA was sequenced using the Illumina MiSeq platform (Illumina, San Diego, CA, USA) targeting the V4 hyper variable region (forward primer, 515F, 5′-GTGYCAGCMGCCGCGGTAA-3′; reverse primer, 806R, 5′-GGACTACHVGGGTWTCTAAT-3′) for 2 × 250-bp paired-end sequencing (Illumina) [96, 97]. PCR amplification was performed using Roche FastStart High Fidelity PCR System (Roche Diagnostics Ltd, Burgess Hill, UK) in 50µl reactions under the following conditions: initial denaturation at 95°C for 2 min, followed by 36 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 1 min and a final extension step of 5 min at 72°C. The PCR products were purified and normalised to ~20ng each using the SequalPrep Normalization Kit (Fisher Scientific, Loughborough, UK). The PCR amplicons from all samples were pooled in equimolar ratios. The run was performed using a 4 pM sample library spiked with 4 pM PhiX to a final concentration of 10% following the method of Schloss and Kozich [98].

Raw sequences for prokaryotes were divided into samples by barcodes (up to one mismatch was permitted) using a sequencing pipeline. Quality control and trimming was performed using Cutadapt [99], FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/ (accessed on 31 August 2017)) and Sickle [100]. MiSeq error correction was performed using SPADes [101]. Forward and reverse reads were incorporated into full-length sequences with Pandaseq [102]. Chimeras were removed using ChimeraSlayer [103], and operational taxonomic units (OTUs) were generated with UPARSE [104]. OTUs were classified by Usearch [105] at the 97% similarity level, and singletons were removed. Rarefaction analysis was conducted using the original detected OTUs in Qiime [106]. OTUs detected in sequencing kit controls were removed from the dataset. The taxonomic assignment was performed by the RDP classifier [107] and Blastn was used to identify the closest GenBank matches (http://blast.ncbi.nlm.nih.gov (accessed on 31 August 2017)).

The abundance of prokaryotic DNA was determined in selected samples using quantitative PCR (qPCR). A serial dilution series was performed using a gBlock double stranded DNA gene fragment (Integrated DNA Technologies, Leuven, Belgium) covering nucleotide positions 1-570 of the 16S rRNA gene from species *Telluria mixta DSM 4832*. The standard curve for the qPCR reaction was created by plotting the C_T (Cycle threshold) values of the dilution series against the log input of DNA template. Concentrations ranging from 8.4 x 10^{-2} fg μl^{-1} to 8.4 x 10^{5} fg μl^{-1} were used to generate the standard curve. PCR amplification was performed using Brilliant II SYBR Green QPCR Master Mix (Agilent Technologies LDA UK Limited, Stockport, UK) in a 25 µl final volume containing 2 µl of sample DNA, 0.15 µM of each primer and 12.5 µl of qPCR SYBR Green Master Mix. All the amplifications were carried out in optical grade qPCR tubes on a MX3000P qPCR system with an initial step of 94 °C for 10 minutes followed by 30 cycles of 94 °C for 30 seconds, 50 °C for 30 seconds, 72 °C for 45 seconds and a dissociation curve was run between 94 °C and 50 °C to check primer specificity. Cycle threshold (C_T) was determined automatically by the instrument. All samples were analysed in triplicate and the r^2 value was 0.986.

Methods for Microbiological Characterisation

Fungi

The fungal DNA amplified from three sediment cores to represent the different depths below the 2016 ground level (Table S5) using the Internal Transcribed Spacer Region (ITS) primers ITS1F and ITS4 [108, 109] and the purity of the polymerase chain reaction (PCR) products was determined by visualisation under short-wave UV light after staining with Sybersafe® and separation by electrophoresis in Tris-acetate-EDTA gel. Two samples yielded sufficient DNA for sequencing of PCR amplicons of the ITS2 region of nuclear ribosomal DNA, conducted with the Illumina MiSeq platform (Illumina, San Diego, CA, USA) targeting the ITS2 internal transcribed spacer region between the large subunit (LSU) and the 5.8S ribosomal genes (forward primer, ITS4F, 5′-AGCCTCCGCTTATTGATATGCTTAART -3′, reverse primer, 5.8SR, 5′-AACTTTYRRCAAYGGATCWCT -3′;) [110] for 2 × 300-bp paired-end sequencing (Illumina) [96. 97]. PCR amplification was performed using Roche FastStart High Fidelity PCR System (Roche Diagnostics Ltd, Burgess Hill, UK) in 50µl reactions under the following conditions: initial denaturation at 95°C for 2 min, followed by 36 cycles of 95°C for 30 s, 56°C for 45 s, 72°C for 2 min, and a final extension step of 5 min at 72°C. The PCR products were purified and normalised to ~20 ng each using the SequalPrep Normalization Kit (Fisher Scientific, Loughborough, UK). The PCR amplicons from all samples were pooled in equimolar ratios. The run was performed using a 10 pM sample library spiked with 10 pM PhiX to a final concentration of 10% following the method of Schloss and Kozich [98].

The ITS sequencing data produced by the Miseq platform was analysed using the PIPITS automated pipeline [111]. Chimeras were removed by reference based chimera detection using UCHIME [112] in conjunction with the UNITE UCHIME reference data set. The taxonomic assignment was performed by the RDP classifier [107] using the UNITE fungal ITS reference data set. OTUs detected in sequencing kit controls were removed from the dataset.

To quantify the abundance of eukaryotic DNA the same methodology was used as for prokaryotic DNA, but covering nucleotide positions 1547-1727 of the 18S rRNA gene from species *Saccharomyces cerevisiae* strain NRRL Y-12632. Concentrations ranging from 3.3 x 101 fg μl^{-1} to 3.3 x 105 fg μl^{-1} were used to generate the standard curve. PCR amplification was performed as above, but with an initial step of 94 °C for 4 minutes followed by 30 cycles of 94 °C for 30 seconds, 55 °C for 30 seconds, 72 °C for 60 seconds and a dissociation curve was run between 94 °C and 55 °C to check primer specificity. The r^2 value was 0.983.