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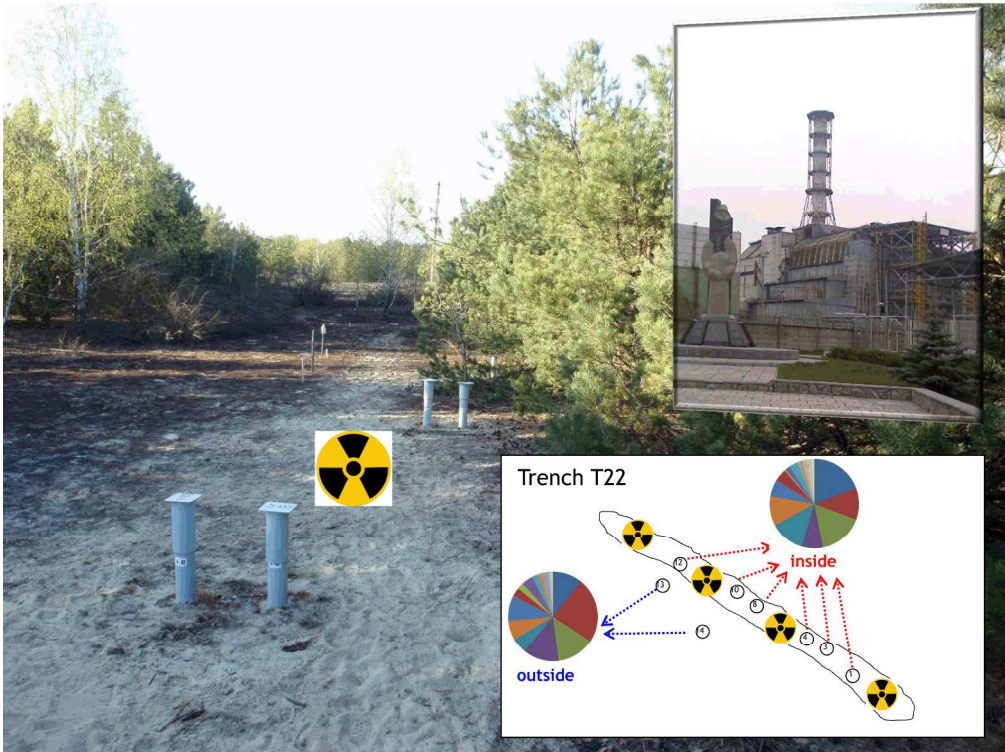
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Soil prokaryotic communities in Chernobyl waste disposal trench T22 are modulated by organic matter and radionuclide contamination.

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Running title: Prokaryotic diversity in Chernobyl soils

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1 **Abstract**

2 After the Chernobyl nuclear power plant accident in 1986, contaminated soils,
3 vegetation from the Red Forest, and other radioactive debris were buried within trenches. In
4 this area, trench T22 has long been a pilot site for the study of radionuclide migration in soil.
5 Here, we used 454 pyrosequencing of 16S rRNA genes to obtain a comprehensive view of the
6 bacterial and archaeal diversity in soils collected inside and in the vicinity of the trench T22
7 and to investigate the impact of radioactive waste disposal on prokaryotic communities. A
8 remarkably high abundance of *Chloroflexi* and AD3 was detected in all soil samples from this
9 area. Our statistical analysis revealed profound changes in community composition at the
10 phylum and OTUs levels and higher diversity in the trench soils as compared to the outside.
11 Our results demonstrate that the total absorbed dose rate by cell and, to a lesser extent the
12 organic matter content of the trench, are the principal variables influencing prokaryotic
13 assemblages. We identified specific phylotypes affiliated to the phyla *Crenarchaeota*,
14 *Acidobacteria*, AD3, *Chloroflexi*, *Proteobacteria*, *Verrucomicrobia* and WPS-2, which were
15 unique for the trench soils.

17 **Introduction**

18 In the past decade, uranium mining, military activities and nuclear power plant
19 accidents have introduced anthropogenic radioactive contaminants into the environment (Hu
20 2008). The Chernobyl nuclear power plant disaster led to the release of $13\,650 \times 10^{18}$
21 Becquerel (Bq) (Martin-Garin *et al* 2012) and contributed significantly to the dispersal of
22 many radioactive isotopes in the environment such as ^{137}Cs , ^{90}Sr , ^{60}Co , ^{154}Eu , ^{241}Am ,
23 $^{234,235,236}\text{U}$ and $^{239,240,241}\text{Pu}$, exhibiting different kinds of radiation (some are pure α emitters
24 whereas others emit β - and/or γ -radiation). Due to their intrinsic properties (*i.e.* radioactive
25 decay half-life, volatility and mobility), most of these radionuclides (RNs) are highly

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3 26 persistent in the terrestrial environment. Radiations emitted by RNs present either in the
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5 27 environment or accumulated inside organisms are able to ionize atoms or molecules, with
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7 28 potential consequent damage to living cells. Level of such biological detriment is linked to the
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9 29 energy deposited into organisms exposed to ionizing radiation, under the consensual
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11 30 assumption of additive effects. Thirty years after the accident, ^{137}Cs is the main contributor to
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13 31 the current ambient external gamma dose rate in the Chernobyl exclusion zone. However the
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15 32 proper assessment of the intensity of radiological exposure leading to observed effects
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17 33 requires to consider not only the ambient external dose rates (as often reported in most
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19 34 studies), but the total dose rates to which living organisms are exposed by including all
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21 35 exposure pathways and all RNs whatever their emission types (Garnier-Laplace *et al* 2015).
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25 36 Soil microorganisms and RNs display complex relationships with each other. On the
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27 37 one hand, bacteria can interact with RNs *via* multiple mechanisms including bioreduction,
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29 38 biomineralization, bioaccumulation or biosorption (Lloyd and Macaskie 2002; Merroun and
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31 39 Selenska-Pobell 2008; Newsome, Morris and Lloyd 2014; Theodorakopoulos *et al* 2015). On
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33 40 the other hand, RNs may exert radio- and chemo-toxic effects on bacteria, thus influencing
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35 41 the structure and activity of microbial communities. Many previous studies have explored the
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37 42 molecular diversity of bacterial communities in various natural or contaminated sites
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39 43 containing radionuclides such as uranium (Selenska-Pobell *et al.* 2001; Rastogi *et al.* 2010;
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41 44 Islam and Sar 2011; Kumar *et al.* 2013; Radeva *et al.* 2013; Yan, Luo and Zhao 2016),
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43 45 uranium and ^{99}Tc (Fields *et al.* 2005; Akob, Mills and Kostka 2007; Barns *et al.* 2007;
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45 46 Waldron *et al.* 2009) and ^{137}Cs and ^{99}Tc (Fredrickson *et al.* 2004). Diverse and complex
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47 47 bacterial assemblages have been evidenced in these habitats and although site-specific
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49 48 composition of bacterial communities is observed, *Proteobacteria* and *Acidobacteria* are
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51 49 frequently well-represented. Several studies highlighted significant changes of bacterial
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53 50 communities upon RNs exposure (Geissler and Selenska-Pobell 2005; Akob, Mills and
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51 Kostka 2007, Rastogi *et al.* 2010; Mondani *et al.* 2011, Islam, Paul and Sar. 2014, Yan, Luo
52 and Zhao 2016).

53 In the case of Chernobyl, very few studies have been performed, most of which were based on
54 cultivation approaches. These studies have shown a decrease in bacterial diversity under
55 conditions of radioactive contamination/irradiation (Romanovskaya *et al.* 1998, 1999;
56 Romanovskaya, Rokitko and Malashenko 2000; Zavilgelsky *et al.* 1998; Czirják *et al.* 2010).

57 In 2011, the first study based on molecular approaches of sunlight-adapted biofilm microbial
58 communities exposed to different ambient radiation levels (0.35 – 25 µSv/h) was reported
59 from the Chernobyl area (Ragon *et al.* 2011). Although the diversity was similar in irradiated
60 and non-irradiated UV-adapted communities, an increase in the mutation level within some
61 Operational Taxonomic Units (OTUs) was correlated with ambient radiation. Niedrée *et al.*
62 (2013) revealed a shift in bacterial population in soil microcosms upon three-month exposure
63 to Chernobyl-like contamination with ⁹⁰Sr and ¹³⁷Cs.

64 Atmospheric fallout from the CNPP accident resulted in radioactive contamination of
65 large regions. One of the most impacted areas, located in the immediate vicinity of the nuclear
66 power plant, is the so-called Red Forest where the pine trees died upon exposure to extreme
67 radiation doses. Shortly after the CNPP accident, clean-up wastes from the Red Forest and
68 other radioactively contaminated debris were buried in a large network of trenches near the
69 CNPP, leading to highly contaminated environmental locations. After the clean-up operations,
70 the trenches were covered with a thick layer of sand and planted with pine saplings, which are
71 now reclaiming the area. One of these trenches (trench T22) has been studied since 1999, as a
72 model of RN migration in the environment (Martin-Garin 2012). In our previous study
73 (Chapon *et al.* 2012), we identified the presence of complex bacterial communities in
74 contaminated soil and control samples from this site using a community fingerprint method

(DGGE). However, this method did not reveal any dominant community fingerprint related to RN content.

The goals of the present study were to explore composition and diversity of soil prokaryotic communities in this understudied environment and to characterize the impact of the trench conditions (RN contamination and high organic matter content) on these communities. We hypothesized that soil prokaryotic communities inside and outside of trench T22 would differ, in particular upon radiation exposure. Therefore, we used high-throughput pyrosequencing of 16S rRNA genes to compare the prokaryotic communities in soil samples exhibiting contrasted RN contamination levels, collected inside and in the vicinity of the trench T22.

Materials and Methods

Sample collection, isolation of culturable bacteria and DNA extraction

Sampling, soil analyses, isolation of culturable bacteria and DNA extraction procedures were previously described (Chapon *et al.* 2012). Briefly, sandy soil samples containing various RN contents were collected between 50 – 60 cm depth from nine different positions in the area of the RN-contaminated trench T22, located in the Chernobyl exclusion zone (51°23'N, 30°04'E; Bugai *et al.* 2005). In April and October 2009, RN-contaminated soil samples (numbers 1, 3, 4, 8, 10 and 12) were collected inside the trench, along with low-contamination controls (numbers 13, 14 and 20) collected in the vicinity (Figure 1). The soil samples were processed at Chernobyl within 18 hours after sampling. In April, environmental DNA was extracted from a single subsample of each soil sample, whereas in October environmental DNA was extracted from triplicate subsamples (designated as a, b and c), after homogenization of the soil. DNA was typically extracted from 1g of soil using the PowerSoil™ DNA isolation kit (MO Bio; USA) and stored at -20°C. Culturable bacteria were

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3 100 recovered from soil samples in aerobic conditions on non-selective media agar plates (tryptic
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5 101 soy broth and AEM1 medium, see Chapon *et al* 2012).
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7 102
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9 103 *PCR amplification and pyrosequencing*
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11 104 For the pyrosequencing analyses, amplicons from the V4 region of the 16S rRNA
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13 105 genes were generated by PCR using the universal primer set 530f (5'-
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15 106 GTGCCAGCMGCNGCGG-3'; Dowd *et al.* 2008) and 802r (5'-
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17 107 TACNVGGGTATCTAATCC-3'; Claesson *et al.* 2009). The coverage of this primer set was
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19 108 89.1% for Bacteria and 54.1% for Archaea with no mismatch and reached 96.8% for Bacteria
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21 109 and 96.5% for Archaea with 2 mismatches as predicted with the Testprime tool of SILVA
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23 110 (Klindworth *et al.* 2013). PCR amplifications were performed in triplicate in 50-µl reactions
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25 111 consisting of: 30 – 50 ng of template DNA, 0.6 µM of forward and reverse primers, 0.2 mM
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27 112 dNTPs, 2 mM MgCl₂, 1.25 U of Go Taq® Hot start polymerase (Promega; USA), and 1 X
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29 113 reaction buffer. For PCR, the thermocycler was programmed for an initial denaturation at
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31 114 94°C for 5 min followed by 30 cycles of amplification (94°C for 30 s, 55°C for 30 s, and
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33 115 72°C for 45 s) and a final extension at 72°C for 5 min. Triplicate samples were pooled before
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35 116 purification of the amplicons. DNA from each amplicon (500 ng) was sent separately for
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37 117 sequencing by GATC (Konstanz, Germany) using a Roche 454 GS-FLX system (Titanium
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39 118 Chemistry). An adaptor and sample-specific 4-bp Molecular Identifier tag (MID) were added
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41 119 to each amplicon by an additional PCR step before mixing and pyrosequencing. Raw reads
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43 120 are available at the NCBI Sequence Read Archive (accession number PRJNA241298).
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52 122 *Processing of pyrosequencing data*
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54 123 The raw dataset (.sff files from two half-plates) contained 825 622 reads and was analyzed
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56 124 with QIIME version 1.8 (Caporaso *et al.* 2010a) with standard parameters. Overall sequences
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with quality score average < 25, short sequences (< 200 bp) and sequences containing mismatches as well as homopolymers were removed. We conducted a denoising of chimeras and the multiplex reads were assigned to corresponding sample according to their barcode, and we carried out Operational Taxonomic Unit (OTU) picking with Uclust (Edgar, 2010). Representative sequences of the OTUs were aligned using the PyNAST algorithm with a minimum percent of 80% (Caporaso *et al.* 2010b). These OTUs were taxonomically classified using RDP method (Wang 2007) and Greengenes database (DeSantis *et al.* 2006). The taxonomic position of bacteria and archaea was characterized using taxa summary QIIME scripts until genus level (L6). Alpha diversity parameters (number of observed OTUs, qualitative Chao1 index, Faith's Phylogenetic Diversity (PD_{whole}) and Shannon index) were calculated with a fixed number of 16 182 randomly picked reads for each sample. Beta-diversity was calculated using Bray-Curtis and unweighted and weighted UniFrac metrics (Lozupone, 2005). PCoA plots were visualized with Emperor (Vazquez-Baeza, 2013).

The representation of cultured bacteria (described in Chapon *et al.* 2012) in the soil bacterial community was assessed by clustering the 16S sequences derived from these bacteria and the 454 reads in OTUs at 97% sequence similarity.

Analysis of soil physico-chemical parameters

For both sampling dates, pH, water content (WC) and NaOH-extractable organic carbon (C_{org(NaOH)}) were analyzed as described in Chapon *et al.* (2012). Briefly, soil pH was measured in a 1:5 soil:water suspension, and 2 g of soil were mixed with 10 mL of distilled water and shaken for 30 min. The pH was measured after decanting and 2 hours of atmosphere equilibration. WC was determined by drying 10 g of soil at 105°C for 24 h. C_{org(NaOH)} was determined by dissolving 0.15 g of soil samples in 1.5 mL of 0.1 M NaOH for 15 h. After decanting and centrifugation, the organic carbon concentration in the supernatant

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was determined by spectrometry (absorbance measurement at 280 nm).

The ¹³⁷Cs concentrations in the soil samples were measured by gamma spectrometry. In addition, ²⁴¹Am, ¹⁵⁴Eu and ⁶⁰Co concentrations were measured in October 2009 by gamma spectrometry, whereas actinide concentrations (²³⁴U, ²³⁵U, ²³⁶U, ²³²Th, ²³⁹Pu, ²⁴⁰Pu and ²⁴¹Pu) were measured in April 2009 by ICP-MS after dry “ashing” and mineralization of the soil samples (Chapon *et al.* 2012). ⁹⁰Sr concentrations were derived from ¹³⁷Cs content assuming a constant ratio of 1.87 between ¹³⁷Cs and ⁹⁰Sr concentrations (Kashparov *et al.* 2001). All RN contents were significantly correlated with ¹³⁷Cs content (Figure S1) except ²³²Th whose concentration is unvarying close to its natural value in soil (Table S1). Thus, constant ratios between ¹³⁷Cs and each RN were used to derive the concentrations of ²⁴¹Am, ¹⁵⁴Eu, ⁶⁰Co, ²³⁴U, ²³⁵U, ²³⁶U, ²³⁹Pu, ²⁴⁰Pu and ²⁴¹Pu when they were missing or below the detection limits (Table S1). When not measured, the ²³²Th concentration was considered equal to the mean of ²³²Th concentrations in all soil samples.

Estimation of the total absorbed dose rate by prokaryotic cell

In the absence of any method to measure the total absorbed dose rates (TADR) by prokaryotic cell, TADR values were calculated based on soil RN activities. For this, two irradiation pathways were considered in the calculation: the external irradiation from the radio-contaminated habitat of bacteria and archaea (*i.e.* the soil) and the internal irradiation resulting from the internalization of RN in the cells (Garnier-Laplace *et al.* 2015).

The external and internal dose rates were calculated at each sampling point and for each sampling date by multiplying the Dose Conversion Coefficient (DCC) for each RN by the measured RN concentration at this sampling point or in the cells, respectively. The RN concentration inside the cells was considered in equilibrium with the soil RN concentration (conservatively assuming a concentration ratio of 1 for each RN) in the absence of any other

information. External and internal DCCs (Table S2A) were calculated for each RN with the EDEN software version 2.3 (Beaugelin-Seiller *et al.* 2006), assuming a different biological effectiveness for the different types of radiation (applied weighting factors were 10 for α -radiation, 3 for low β -radiation, and 1 for other β -radiation and γ -radiation) (Pröhl *et al.* 2003). The prokaryotic cells were described as a sphere with a 1- μ m diameter, living in an infinitely extended and uniformly contaminated soil (compositions are provided in Table S2B).

Statistical analyses

Non-parametric tests (Kruskal-Wallis) were calculated using QIIME (Caporaso *et al.* 2010a). Redundancy Analysis was performed with R software (R Core Team 2011) on the OTUs and phylum abundance data with the Principal Component Analysis on Instrumental Variables (PCAIV) function of the ade4 package (Dray and Dufour, 2007). Heatmap was constructed with the gplots package of R (Bolker *et al.* 2011).

Results

Overall prokaryotic diversity in the Chernobyl soils

This study focused on the prokaryotic communities in 36 soil samples collected inside and outside the RN-contaminated trench T22 at nine different positions in April and October 2009 (Figure 1). The pH values ranged from 4.4 to 6.1 and soil water content ranged from 2.2% to 7.3% (Table 1). The NaOH extractable organic C content ($C_{org(NaOH)}$) was within the same range for all samples (average 2.25 ± 0.83 g kg⁻¹), except for samples 4, 13, and 14 which were lower in content (average 0.35 ± 0.30 g kg⁻¹). Soils collected within the trench (sample numbers 1, 3, 4, 8, 10 and 12) were contaminated with RNs (¹³⁷Cs activity ranging from 61 – 750 Bq g⁻¹), whereas soils collected in the vicinity of trench T22 (sample numbers 13, 14 and

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20) exhibited low RN levels (^{137}Cs ranging from 0.35 – 1.50 Bq g⁻¹; Table 1). Three soil subsamples from October 2009 (designated as a, b and c) were used for DNA extraction in order to assess the reproducibility of the experiment. After quality filtering, we obtained 753 703 reads with a read length average of 255 bp, ranging from 16 182 – 23 686 reads in the 36 samples (Table 1). From the reads, 8 167 OTUs at 3% genetic distance were identified and after normalization of the dataset to 16 182 reads per sample and deletion of singletons, 7 663 OTUs were identified with OTU counts varying between 633 – 2 120 per sample (Table 1). The rarefaction curves based on PD whole tree, Chao1 and Shannon estimators tended to reach an asymptote (Figure S2). The Good's estimator of coverage varied between 95 – 98% across the samples, indicating that the depth of sequencing was nearly sufficient to detect most of the dominant OTUs (Table 1).

Of the reads, 95.7% were affiliated with Bacteria and 4.3% were affiliated with Archaea. The most consistently detected bacterial phyla (relative abundance > 1%) across all samples were *Proteobacteria*, *Acidobacteria*, AD3, *Planctomycetes*, *Chloroflexi*, *Verrucomicrobia* and *Actinobacteria*, with an uneven distribution across the samples (Figure 1). Combined, these seven groups accounted for 83.3% of the sequences. Twenty-seven additional bacterial phyla were detected as well as three archaeal phyla.

Trench T22 hosts specific microbiota

The rarefaction curves calculated using different estimators revealed a higher apparent species richness in almost all samples collected in the trench, as compared to the samples collected outside of the trench (Table 1). Accordingly, mean Chao1 richness and Shannon diversity indices were significantly higher in samples collected inside as opposed to outside the trench ($p < 0.05$; Figure S2).

Principal Coordinates Analysis (PCoA) based on bacterial and archaeal OTUs and performed with Bray-Curtis, Unweighted and Weighted Unifrac methods all showed a clear separation between the communities inside and outside the trench (Figure 2). The first principal components of the PCoA accounted for 20 – 40% of the variation between the samples, depending on the method used. Some divergence between samples taken at the same location in April and in October could be observed, particularly for samples collected within the trench. By contrast, high consistency between replicates (labeled as ‘a’, ‘b’ or ‘c’) was observed for each soil sample collected in October 2009, indicating high reproducibility of the experimental procedure.

Impact of long-term exposure to radioactive contaminated wastes on prokaryotic microbiota

To examine how the prokaryotic microbiota was affected by radioactivity, the TADR was calculated for each soil sample. On average, estimated TADR by cell were two orders of magnitude higher in trench soils than in samples collected outside of the trench; the maximum TADR was *ca.* 150 mGy h⁻¹ in sample 8 in October 2009, whereas the lowest value was 0.1 mGy h⁻¹ in samples 13 and 20 in April and October 2009, respectively (Table 1). Internal absorbed dose rates contributed to only 3% of the estimated TADR by cell (Table S3A and B) which is mainly due to the small size of prokaryotic cells. Actinides were the primary contributors to the estimated TADR by cell, and ²³⁹Pu and ²⁴⁰Pu were responsible for 80 – 96% of the estimated TADR by cell (Table S3A and B). Since the PCoA analysis was able to confirm the reproducibility of the experiment, the triplicates performed in October were averaged in order to create a single dataset and to facilitate depiction of the data. A redundancy analysis (RDA) coupled to a permutation test (randtest function) was used to test whether the community composition at the different sampling sites was related to the physico-chemical properties of the soil samples (Ramette 2007). The four physico-chemical variables

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included in the analysis (TADR, $C_{org}(NaOH)$, water content, and pH) significantly explained 43% of the variation of the bacterial and archaeal communities ($r^2 = 0.43$, $p = 0.001$ based on 999 permutations). The first two axes of the RDA explain 85% of the variance due to the soil physico-chemical properties, 71% of which is accounted for by the first axis, which correlated primarily with TADR ($r = -0.90$), $C_{org}(NaOH)$ ($r = -0.84$) and water content ($r = -0.76$) (Figure 3). Using the RDA function in the ‘vegan’ package to separately analyze the effect of each variable indicates that TADR was the principal variable to influence the prokaryotic communities at the OTU-level ($p < 0.05$).

The same analysis at the phylum level confirmed the effect of the environmental variables on the bacterial communities since 52 % of the variation of the prokaryotic communities at the phylum level were explained by the environmental variables ($r^2 = 0.523$, $p = 0.001$ based on 999 permutations). The first two axes of the RDA explained 92 % of the variance, among which 74 % is explained by the first axis which correlated mainly with TADR ($r = -0.89$) and to a lesser extent with $C_{org}(NaOH)$ ($r = -0.69$) and water content ($r = -0.66$) (data not shown). The analysis of the effect of each variable separately showed that the principal variable influencing the prokaryotic communities at the phylum level were TADR ($p < 0.05$) and $C_{org}(NaOH)$ ($p < 0.1$).

Primary differences between prokaryotic microbiota of the trench and surrounding soils

Non-parametric tests (Kruskal-Wallis) were used to determine the significance of differences in prokaryotic diversity between samples and to identify the taxa representative of either the trench or the surrounding soils. This analysis revealed that among the 7 663 OTUs identified, 1 781 OTUs displayed significant changes in abundance between the highly contaminated trench soils and the low-contamination surrounding soils ($p < 0.05$). Specifically, 973 OTUs were significantly more abundant in the trench soils than in the

274 surrounding soils, whereas 808 OTUs were significantly more abundant in the surrounding
275 soils than in the trench soils. Furthermore, these 1 781 OTUs accounted for 50% of the total
276 prokaryotic microbiota in the trench and for 67% of the total prokaryotic microbiota in the
277 surrounding soils, and could be classified into 26 bacterial phyla and three archaeal phyla.

278 To further explore the primary differences between microbiota of the trench soils and
279 surrounding soils, a heatmap was constructed with the most abundant OTUs (> 1% relative
280 abundance in at least one sample; see Table S4 for details) differing significantly between the
281 trench and the surrounding soil microbiota (Figure 4). The surrounding soils were
282 characterized by 20 abundant OTUs affiliated with the phyla *Crenarchaeota* (NRP-J order),
283 *Acidobacteria* (Ellin6513, 32-20, DS-18 orders), *Actinobacteria* (*Mycobacterium* genus and
284 MB-A2-108 class), AD3 (ABS-6 and JG37-AG-4 classes), *Gemmatimonadetes* (Gemm-1
285 class), *Verrucomicrobia* (DA101 genus) and WPS-2 (Figure 4). On the other hand, the trench
286 prokaryotic community was characterized by 19 abundant OTUs. These OTUs were affiliated
287 with the phyla *Crenarchaeota* (SAGMA-X family), *Acidobacteria* (11-24, Ellin6513, DS-18
288 orders), AD3 (ABS-6 and JG37-AG-4 classes), *Chloroflexi* (*Ktedonobacteraceae* family),
289 *Proteobacteria* (*Bradyrhizobium*, *Rhodoplanes* and *Burkholderia* genera, SC-I-84 order and
290 *Sinobacteraceae* family), *Verrucomicrobia* (auto67-4W family) and WPS-2 (Figure 4). Within
291 the Archaea, the representative sequence of OTU 1143180 (*Crenarchaeota* (SAGMA-X))
292 exhibited 100% sequence similarity with *Candidatus Nitrosotalea devanaterrea*, an acidophilic
293 ammonia oxidizer (Lehtovirta-Morley *et al.* 2011), as well as sequences detected in acid water
294 from a gold mine (AB050208; Takai *et al.* 2001). The OTUs specific to the trench did not
295 have any cultured representatives within the Bacteria, with the exception of *Bradyrhizobium*,
296 *Rhodoplanes*, *Burkholderia* and *Sinobacteraceae*.

297 Relationships with other environments were investigated by performing a BLAST
298 search of the non-redundant database, using representative sequences of the 19 OTUs specific

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299 to the trench soils. Interestingly, among the closest neighbors (98 – 100% sequence
300 similarity), we found sequences derived not only from a wide range of forest soils (data not
301 shown) but also from cold environments (alpine tundra, boreal wetlands and forest, Finland,
302 Himalayan mountains, Alaska, Iceland, Greenland, Antarctic habitats, the Arctic), acidic
303 environments (acid water, acid mine drainage, acidic fen soil, acidic subalpine forest, acidic
304 wetland), volcanic soils (Kasatochi Island in Alaska, a lava forest soil in Korea, Hawaiian
305 volcanic deposit), RN- and heavy metal-contaminated soils, sediment and water samples
306 (neptunium-contaminated sediments, soil samples of uranium mining waste pile, acid water
307 from gold and potassium mines, lead-contaminated forest soil) as shown in Table S5. The
308 representative sequence of OTU 573135, which is affiliated with *Bradyrhizobium*, was
309 reported to have a 100% sequence similarity with many sequences, including a bacterium
310 cultured from dust samples from the International Space Station (ISS) (LT617088; Mora *et al.*
311 2016).

312
313 *Representation of cultured isolates in the prokaryotic community*

314 We previously characterized a collection of 303 isolates recovered from Chernobyl
315 soil samples by 16S rDNA partial sequencing (Chapon *et al.* 2012). Here, we analyzed
316 whether the 284 isolates with available appropriate sequence data (*i.e.* the V4 domain of the
317 16S rDNA) were detected in the pyrosequencing dataset, and if they represent an abundant
318 taxon in the prokaryotic community. In total, 206 sequences derived from isolates exhibited at
319 least 97% sequence similarity with a pyrotag, falling within 47 OTUs of the pyrosequencing
320 dataset. 183 isolates fall within 42 OTUs that represented a very minor fraction of the
321 community across all samples, since altogether they accounted for less than 0.2% of the total.
322 By contrast, 23 isolates fall within 5 well-represented OTUs (maximum relative abundance
323 ranging from 0.71 – 6.97%): OTUs 125947, 156722, 196652, and 4057260, all affiliated to

324 *Burkholderia* spp. and OTU 573135, affiliated to *Bradyrhizobium* spp. (Table S6). The
325 relative abundance of OTUs 4057260 and 573135 reached 6.97% and 2.90%, respectively, in
326 the most contaminated soil (750 Bq/g of ^{137}Cs), which was collected in October 2009 at
327 sampling point 3 (sample 3O; Tables 1 and S6). Furthermore, OTUs 125947 and 573135 are
328 characteristic of the trench prokaryotic community.

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330 Discussion

331 The current work explored the diversity of soil prokaryotes that have been evolving
332 since more than two decades in RN-contaminated soils inside and outside of the trench T22,
333 located within the Chernobyl exclusion zone. The presence of complex prokaryotic
334 communities in contaminated soil and control samples from this site was revealed by a
335 genetic fingerprint method (DGGE) in our previous study (Chapon *et al.* 2012). We observed
336 that apparent diversity in the trench soils was similar to that observed in the surrounding soils
337 on DGGE. However, this approach did not uncover any dominant community fingerprint
338 clearly related to RN content, nor did it provide any direct taxonomic information. Here, we
339 revisited this previous work to address these issues using 454 pyrosequencing of 16S rRNA
340 genes. We surveyed the prokaryotic diversity of this understudied environment, and we
341 assessed the effects of RN contamination and $\text{C}_{\text{org}}(\text{NaOH})$ content on soil microbiota using a
342 comparative analysis with low-contamination soils collected outside of the trench in the same
343 area. The ^{137}Cs content in the soil samples collected outside of the trench is consistent with
344 worldwide upper background levels of ^{137}Cs due to Chernobyl fallout (*e.g.* in France: from
345 0.01 - 0.1 Bq g $^{-1}$ in upper soils, max value in hotspots: 10 Bq g $^{-1}$; IRSN, 2016). In addition,
346 Michel *et al.* (2015) reported several values for Ukraine (from 0.3 mBq g $^{-1}$ to 6.6 Bq g $^{-1}$)
347 outside of the Chernobyl exclusion zone.

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348 The seven dominant bacterial phyla in the Chernobyl soils were *Proteobacteria*,
349 *Acidobacteria*, AD3, *Planctomycetes*, *Chloroflexi*, *Verrucomicrobia* and *Actinobacteria*.
350 These phyla are commonly encountered as the dominant taxa in soils (Janssen 2006; Rastogi
351 *et al.* 2009). Here, the abundance of *Chloroflexi* (mean relative abundance 9.4%, ranging from
352 2.4 – 16.1%) and AD3 (mean relative abundance 14.8%, ranging from 3.3 – 39.0%) was high,
353 both inside and outside the trench. On a global scale, this is the most noticeable feature of
354 Chernobyl soil prokaryotic communities. This high abundance is not an artefact linked to
355 DNA extraction or PCR amplification, since other soil samples processed in the same
356 experimental run did not exhibit the same profile (data not shown). High abundance of
357 *Chloroflexi*/AD3 has been described in cold environments (Costello and Schmidt 2006; Taş *et*
358 *al.* 2014; Hultman *et al.* 2015; Frey *et al.* 2016). Members of *Chloroflexi*/AD3 are reported to
359 predominate following acute gamma irradiation of soils (McNamara *et al.* 2007; El-Sayed and
360 Ghanem 2009) and have been detected in natural uranium ores (Mondani *et al.* 2011) and in
361 uranium-contaminated sites (Selenska-Pobell 2002; Barns *et al.* 2007; Hug *et al.* 2013). It has
362 been proposed that degradation of plant polymers is widespread across this phylum (Hug *et*
363 *al.* 2013). Thus, members of this phylum are well-adapted to cold environments; they are
364 potentially able to degrade lignocellulosic material; and they can tolerate uranium and/or its
365 associated radioactivity. The high abundance of *Chloroflexi*/AD3 members both inside and
366 outside trench T22 is coherent, taking into account the facts that Chernobyl upper soils are
367 partly frozen during winter (Bixio *et al.* 2002), that wood debris were buried in the trench,
368 and that bacteria and archaea are exposed to different levels of irradiation and contamination
369 whether they are inside or outside of the trench. Nevertheless, when examining the results in
370 greater detail, we noted that specific phylotypes within *Chloroflexi*/AD3 were selected by the
371 soil trench. Indeed, the abundance of seven OTUs affiliated with classes ABS-6 and JG37-
372 AG-4 was dramatically reduced in the trench, whereas three OTUs from the same classes

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3 373 were strongly enriched in the trench, suggesting that these phylotypes may have distinct
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5 374 ecological niches and physiological characteristics such as tolerance to RNs exposure.
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7 375 Our detailed comparative analysis of soils collected inside and outside the trench has
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9 376 revealed several specific patterns of soil prokaryotic assemblages. First, the Chao1 richness
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11 377 and Shannon diversity indices were significantly higher inside the trench as compared to the
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13 378 samples collected outside of the trench. On average, 1 589 observed OTUs were detected in
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15 379 the trench soils, whereas 1 103 OTUs were detected in the surrounding soils, indicating a
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17 380 positive effect of trench conditions on diversity. This had not been evidenced in our previous
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19 381 study, probably because of the limitation of DGGE to resolve complex profiles. Second, the
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21 382 PCoA ordination demonstrated significant differences between the prokaryotic communities
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23 383 from the trench and the surrounding area. When the trenches were dug, large amounts of
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25 384 highly irradiated and contaminated wood debris were buried, providing a considerable input
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27 385 of RNs as well as organic matter into this oligotrophic environment. The introduction of
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29 386 organic matter is expected to enhance diversity either by stimulating heterotrophic
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31 387 microorganisms which were already present as minor species, or by introducing new species
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33 388 from outside of the site. For instance, Field *et al.* (2010) showed that bacterial diversity was
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35 389 significantly enhanced upon cellulosic waste burial in the soil, with a corresponding shift in
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37 390 OTU number from approximately 750 to 1 600. By contrast, RN contamination is expected to
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39 391 have a negative impact on microbial communities due to radiotoxicity. According to Field *et*
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41 392 *al.* (2010), the impact of organic matter in the trench should have been larger than that
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43 393 actually observed in this study. We thus hypothesized that the presence of RNs
44
45 394 counterbalanced the positive effect of lignocellulosic material on prokaryotic diversity in the
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47 395 trench, which would suggest that the RNs remaining in the trench still exert a negative impact
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49 396 on the prokaryotic communities. This hypothesis is supported by the statistical analysis of the
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51 397 data, since the RDA coupled to a permutation test revealed that the principal variable, among
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all measured variables, influencing the prokaryotic communities at the OTU and phylum level was TADR and to a lesser extent $C_{org}(NaOH)$ when the analysis was performed at the phylum level. However, we could not exclude that other confounding factors not monitored during our study might also have influenced the prokaryotic communities composition.

808 OTUs were significantly enriched in the prokaryotic community outside of the trench, including 20 abundant OTUs. These abundant OTUs were affiliated to different phyla and do not have any cultured representatives, with the exception of *Mycobacterium*. Members of these phylotypes probably correspond to RN-sensitive species and/or oligotrophic microorganisms. We also identified specific OTUs that distinguished the trench microbiota from surrounding microbiota, indicating that specific phylotypes were selected by the trench soils. Close relationships with a wide range of cold environments and acidic soils were revealed, as well as volcanic soils or soils that have been subjected to fire, showing that traces of past events are still detectable in the trench prokaryotic community. In addition, relationships with radioactive environments were shown for five OTUs specific to the trench soils. Thus, members of the phylotypes that were selected by the trench conditions, in particular by high TADR, may have the capacity to survive under stressful conditions. These phylotypes are distributed among several phyla, illustrating that adaptation to life in radioactive environment is widespread in prokaryotes.

A limited number of reads from the 454 dataset identified as *Deinococcus* were detected in the soil samples, representing a very minor fraction of the total abundance (0.004 – 0.084%; mean relative abundance 0.017%). However, we failed to confirm the presence of this bacterium in Chernobyl soils using specific primers (Theodorakopoulos *et al.* 2013), suggesting that the very low relative abundance is the consequence of sequencing errors or PCR bias. Furthermore, *Deinococcus*-related species were not identified among the cultured bacteria retrieved from the soil samples (Chapon *et al.* 2012). Taken together, these results

strongly suggest that *Deinococcus* are either absent or are in extremely low abundance in this environment. This may be related to the high moisture content of Chernobyl soils, whereas *Deinococcus* members have been shown to be well-adapted to dry or low-moisture soils (Fredrickson *et al.* 2004; Chanal *et al.* 2006; Theodorakopoulos *et al.* 2013; Li *et al.* 2015).

Finally, the identification of representatives of two abundant OTUs typical of trench microbiota (OTU 125947 and 573135) in our culture collection has yielded some interesting results. These isolates, related to the genera *Bradyrhizobium* and *Burkholderia*, represent appropriate models for assessing the impact of long-term RN exposure on bacteria. Future studies could focus on these isolates to help determine the mechanisms of adaptation to this very unusual ecological niche.

Conclusion

In this study, we demonstrated that prokaryotic communities from Chernobyl disposal trench T22, as well as samples collected in the proximity, share common traits with prokaryotic assemblages from cold environments on a global scale although a significant difference in diversity and community composition could be observed between the trench and its proximal environment. Our results indicate that the introduction of contaminated lignocellulosic material within trench T22 has shaped prokaryotic communities by modifying species composition and abundance. In this regard, TADR was the principal variable to influence the prokaryotic communities while still exerting a negative effect on prokaryotic diversity. We identified specialized phylotypes that have successfully colonized the trench, two of which have cultured representatives in our culture collection. Further work, such as shotgun metagenome sequencing and experiments on these bacterial isolates, may be helpful in determining the function of bacterial communities in this ecosystem.

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Soil sample	Sampling location	¹³⁷ Cs (Bq g ⁻¹)	pH	C _{org} (NaOH) (g kg ⁻¹)	Water content (%)	TADR (mGy h ⁻¹)	Subsample	number of high-quality reads	Observed OTUs	Chao1	Shannon diversity index	Good's estimator of coverage
1 _A	inside	420	5.1	2.56	5.8	83		21 279	1 806	2 702	8.6	0.96
1 _O	inside	420	4.8	2.61	7.3	86	a,b,c	19 818±1 094	2 087±42	3 030±78	9.3±0.1	0.95±0.00
3 _A	inside	300	5.6	1.48	5.7	28		18 822	1 628	2 568	8.4	0.96
3 _O	inside	750	5	1.77	5.1	153	a,b,c	20 827±1 469	1 622±36	2 433±29	8.0±0.2	0.96±0.00
4 _A	inside	61	5.4	0.49	3.5	9		23 083	1 454	2 197	8.3	0.96
4 _O	inside	140	4.8	0.88	5.1	29	a,b,c	20 931±489	1 478±47	2 324±51	8.4±0.2	0.96±0.00
8 _A	inside	640	5.2	4.12	6.2	137		22 159	1 353	2 102	8.1	0.97
8 _O	inside	670	4.5	2.93	3.6	137	a,b,c	23 603±90	1 498±24	2 278±54	8.4±0.1	0.96±0.00
10 _A	inside	290	4.7	2.15	4.9	90		23 613	1 333	2 054	7.9	0.97
10 _O	inside	710	4.9	2.77	5.2	145	a,b,c	21 544±2 042	1 524±27	2 275±11	8.5±0.1	0.96±0.00
12 _A	inside	260	5.4	1.16	3.7	47		19 690	1 585	2 300	8.7	0.96
12 _O	inside	370	4.4	1.48	4.8	76	a,b,c	21 515±1 521	1 452±4	2 277±82	8.3±0.0	0.96±0.00
13 _A	outside	0.37	6.1	0.16	2.2	0.1		22 966	1 145	1 755	6.9	0.97
13 _O	outside	1.5	4.3	0.12	2.7	0.3	a,b,c	17 761±1 626	639±10	977±60	5.8±0.1	0.98±0.00
14 _A	outside	0.91	6.1	0.1	2.5	0.4		22 291	969	1 355	7.2	0.98
14 _O	outside	0.79	4.8	0.34	4.1	0.2	a,b,c	20 118±1 308	1 167±16	1 684±36	7.8±0.1	0.97±0.00
20 _A	outside	0.35	5.4	1.99	4.7	0.3		23 354	1 424	2 108	8.2	0.97
20 _O	outside	0.54	4.5	1.96	5.1	0.1	a,b,c	19 364±1 469	1 427±55	2 160±144	8.0±0.1	0.97±0.01

Table 1: Primary soil characteristics of samples collected inside and outside of trench T22. The table comprises soil physico-chemical parameters (from Chapon *et al.* 2012), total absorbed dose rates (TADR) by cell, OTU counts and estimators of alpha-diversity. In the soil sample name, “A” stands for April and “O” for October.

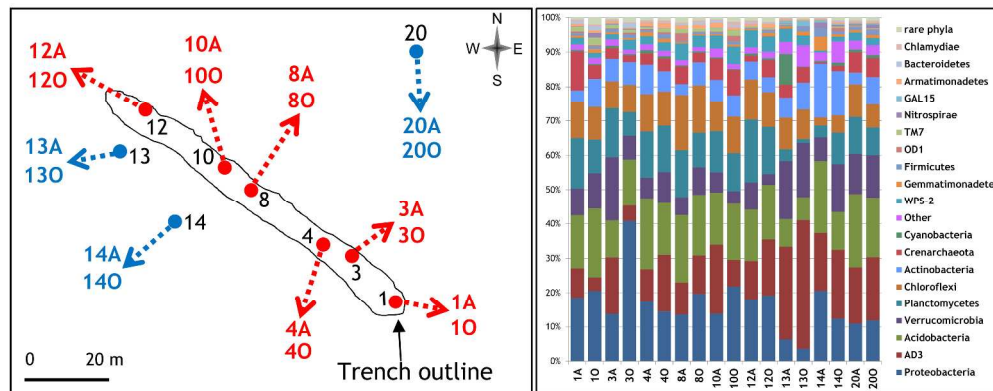
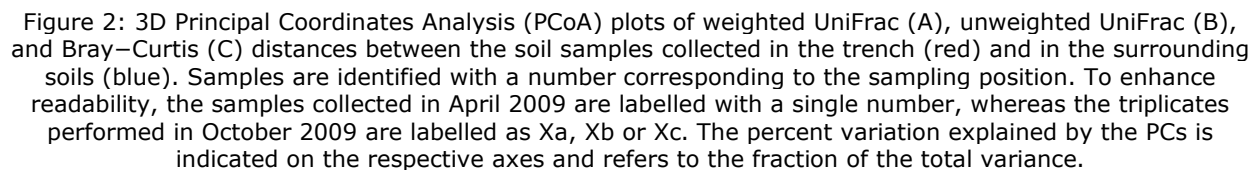


Figure 1: Location of the sampling points (left) and taxonomic profiles of the microbial communities (right), for soil samples collected in April (-A) and October (-O) 2009. The triplicate samples performed in October were combined.

1363x539mm (72 x 72 DPI)



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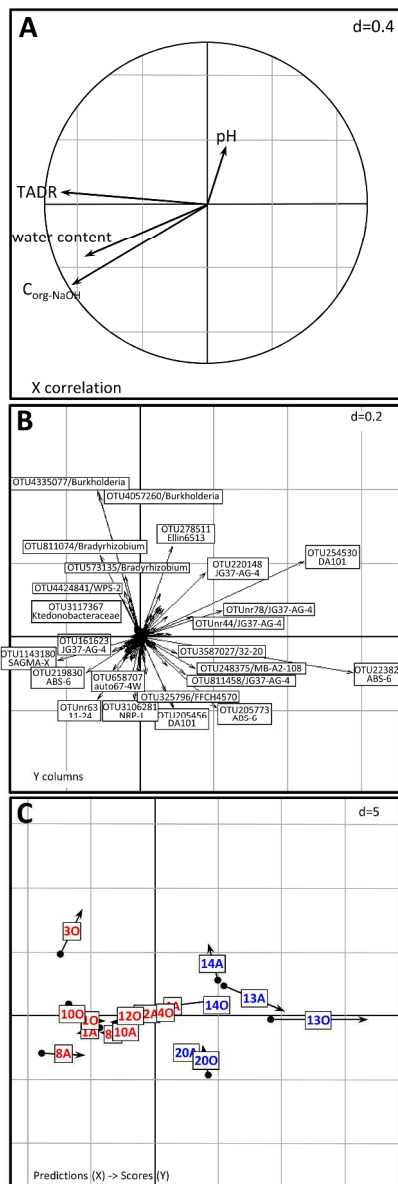


Figure 3: Multivariate redundancy analysis diagrams illustrating the relationship between the OTU-level community structure from soil samples collected in April (labelled with A) and October (labelled with O) 2009 and environmental parameters (TADR, Corg(NaOH), pH, and water content). A) correlation of physico-chemical explanatory variables with the first two axes of the PCAIV.; B) OTU scores. Only OTUs with the highest loadings are labelled with their ID number and lowest taxonomic rank. C) joint display of the position of the sampling sites by averaging (points) and by regression (arrowheads). Red: samples from the trench; blue: samples from the surrounding area.

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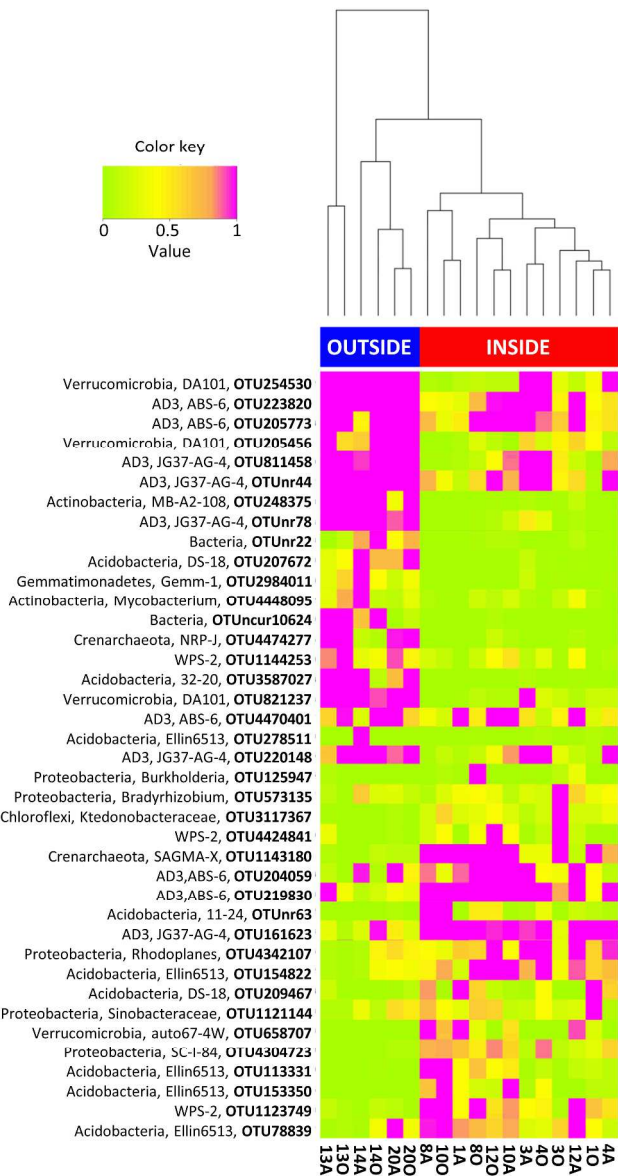


Figure 4: Cluster analysis of the soil samples and the corresponding heat map constructed with abundant OTUs (>1% relative abundance in at least one soil sample) differing significantly between the trench and the surrounding soil microbiota. Red: samples from the trench; blue: samples from the surrounding area. The highest and lowest taxonomic affiliations are indicated for each OTU.

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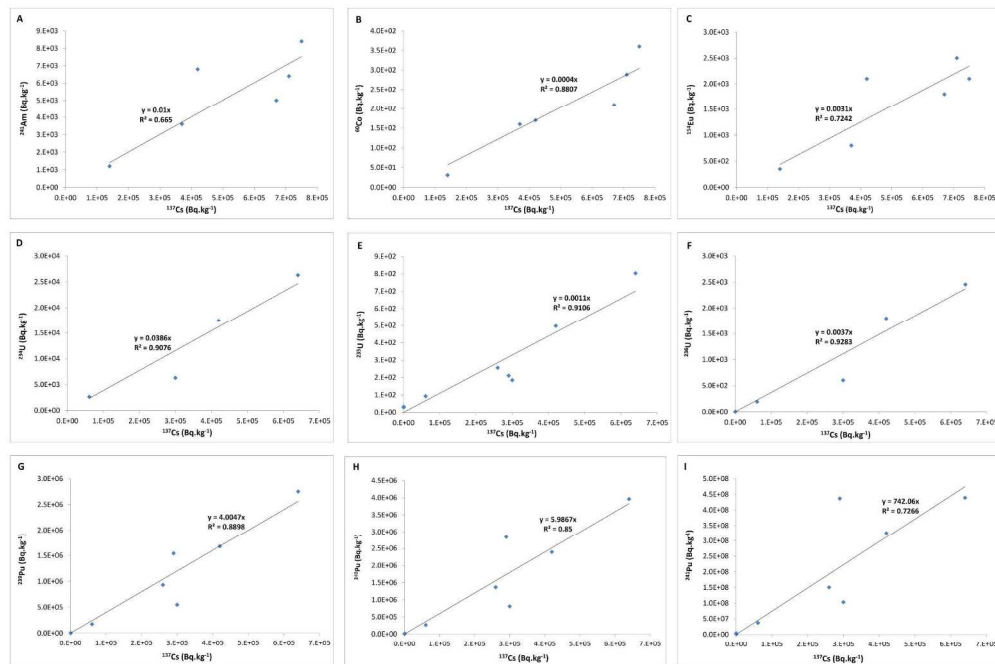


Figure S1: Relationship between measured concentrations (Bq.kg $^{-1}$) of ^{137}Cs and A) ^{241}Am , B) ^{61}Co , C) ^{154}Eu , D) ^{234}U , E) ^{235}U , F) ^{236}U , G) ^{239}Pu , H) ^{240}Pu and I) ^{241}Pu , at different soil sampling sites.

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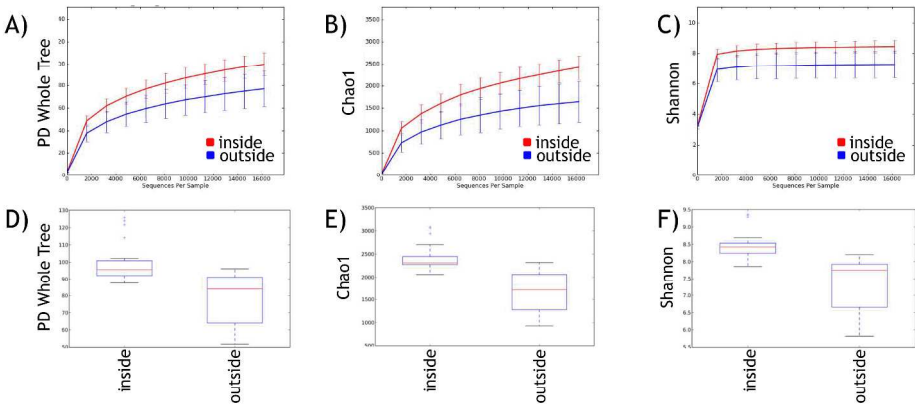


Figure S2 : Alpha rarefaction plots for Phylogenetic Distance Whole Tree (A), Chao1 (B) and Shannon (C) indices computed with the soil samples collected inside the trench (red) and outside (blue). The corresponding distance boxplots are shown in D), E) and F) respectively. The error bars show the standard error of the mean diversity.

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Table S1: Radionuclide concentrations (Bq.kg⁻¹) at the different soil sampling sites.

Soil sample	Soil radionuclide concentration (Bq.kg ⁻¹) ¹											
	¹³⁷ Cs		⁹⁰ Sr		²⁴¹ Am		⁶⁰ Co		¹⁵⁴ Eu		²³⁴ U	
	Apr 09	Oct 09	Apr 09	Oct 09	Apr 09	Oct 09	Apr 09	Oct 09	Apr 09	Oct 09	Apr 09	Oct 09
1	4.20E+05	4.20E+05	2.25E+05	2.25E+05	4.21E+03	6.80E+03	1.70E+02	1.70E+02	1.31E+03	2.10E+03	1.75E+04	1.62E+04
3	3.00E+05	7.50E+05	1.60E+05	4.01E+05	3.01E+03	8.40E+03	1.22E+02	3.60E+02	9.39E+02	2.10E+03	6.28E+03	2.89E+04
4	6.10E+04	1.40E+05	3.26E+04	7.49E+04	6.12E+02	1.20E+03	2.48E+01	3.10E+01	1.91E+02	3.50E+02	2.65E+03	5.40E+03
8	6.40E+05	6.70E+05	3.42E+05	3.58E+05	6.42E+03	5.00E+03	2.60E+02	2.10E+02	2.00E+03	1.80E+03	2.63E+04	2.58E+04
10	2.90E+05	7.10E+05	1.55E+05	3.80E+05	2.91E+03	6.40E+03	1.18E+02	2.88E+02	9.07E+02	2.50E+03	1.12E+04	2.74E+04
12	2.60E+05	3.70E+05	1.39E+05	1.98E+05	2.61E+03	3.60E+03	1.06E+02	1.60E+02	8.13E+02	8.00E+02	1.00E+04	1.43E+04
13	3.70E+02	1.50E+03	1.98E+02	8.02E+02	3.71E+00	1.51E+01	1.50E-01	6.09E-01	1.16E+00	4.69E+00	1.43E+01	5.78E+01
14	9.10E+02	7.90E+02	4.87E+02	4.22E+02	9.13E+00	7.93E+00	3.69E-01	3.21E-01	2.85E+00	2.47E+00	3.51E+01	3.05E+01
20	3.50E+02	5.40E+02	1.87E+02	2.89E+02	3.51E+00	5.42E+00	1.42E-01	2.19E-01	1.10E+00	1.69E+00	1.35E+01	2.08E+01

Soil sample	Soil radionuclide concentration (Bq.kg ⁻¹) ¹											
	²³⁵ U		²³⁶ U		²³² Th		²³⁹ Pu		²⁴⁰ Pu		²⁴¹ Pu	
	Apr 09	Oct 09	Apr 09	Oct 09	Apr 09	Oct 09	Apr 09	Oct 09	Apr 09	Oct 09	Apr 09	Oct 09
1	4.96E+02	4.60E+02	1.80E+03	1.56E+03	1.54E+03	1.55E+03	1.68E+06	1.68E+06	2.39E+06	2.51E+06	3.26E+08	3.12E+08
3	1.84E+02	8.22E+02	6.02E+02	2.78E+03	1.75E+03	1.55E+03	5.49E+05	3.00E+06	8.09E+05	4.49E+06	1.03E+08	5.57E+08
4	9.25E+01	1.53E+02	1.92E+02	5.19E+02	1.44E+03	1.55E+03	1.73E+05	5.61E+05	2.66E+05	8.38E+05	3.72E+07	1.04E+08
8	8.04E+02	7.34E+02	2.45E+03	2.48E+03	1.65E+03	1.55E+03	2.75E+06	2.68E+06	3.96E+06	4.01E+06	4.39E+08	4.97E+08
10	2.10E+02	7.78E+02	1.07E+03	2.63E+03	1.69E+03	1.55E+03	1.54E+06	2.84E+06	2.84E+06	4.25E+06	4.37E+08	5.27E+08
12	2.55E+02	4.05E+02	9.64E+02	1.37E+03	1.46E+03	1.55E+03	9.30E+05	1.48E+06	1.37E+06	2.22E+06	1.50E+08	2.75E+08
13	2.84E+01	1.64E+00	1.37E+00	5.56E+00	1.43E+03	1.55E+03	1.48E+03	6.01E+03	2.22E+03	8.98E+03	2.75E+05	1.11E+06
14	2.98E+01	8.66E-01	3.37E+00	2.93E+00	1.25E+03	1.55E+03	3.38E+03	3.16E+03	1.47E+04	4.73E+03	1.34E+06	5.86E+05
20	3.20E+01	5.92E-01	2.18E+00	2.00E+00	1.78E+03	1.55E+03	1.40E+03	2.16E+03	8.58E+03	3.23E+03	3.91E+06	4.01E+05

¹ Values in italics were derived from the ¹³⁷Cs content.

Table S2: Parameters used for Total Dose Rate calculation with EDEN.

A: Internal and external Dose Conversion Coefficients (DCC) ($\mu\text{Gy h}^{-1}$ per Bq kg^{-1}) for prokaryotic cell.

DCC	¹³⁷ Cs+	²⁴¹ Am	⁶⁰ Co	¹⁵⁴ Eu	⁹⁰ Sr+	²³⁴ U	²³⁵ U+	²³⁶ U	²³² Th	²³⁹ Pu	²⁴⁰ Pu	²⁴¹ Pu
Internal	4.92E-06	1.98E-04	8.33E-06	8.08E-06	5.75E-07	1.95E-04	2.71E-04	2.03E-04	2.20E-04	1.87E-04	1.85E-04	6.58E-06
external	4.03E-05	2.00E-02	7.29E-05	5.67E-05	5.89E-05	1.78E-02	1.56E-02	1.60E-02	1.37E-02	1.96E-02	1.96E-02	4.54E-07

B: Soil and bacteria composition (% of total mass).

Element	C	H	O	N	P	S	Ca	Na	K	Mg	Al	Si	Fe
Bacteria	53.1	6.2	28.3	12.4									
Soil	0.18	0.9602	50.73	0.03001	0.11	0.11	3.261	2.39	2.31	2.11	7.401	26.17	4.241

Table S3: A) Internal absorbed dose rate ($\mu\text{Gy}\cdot\text{h}^{-1}$)

Soil sample	Internal absorbed dose rate ($\mu\text{Gy}\cdot\text{h}^{-1}$)											
	¹³⁷ Cs		⁹⁰ Sr		²⁴¹ Am		⁶⁰ Co		¹⁵⁴ Eu		²³⁴ U	
	Apr 09	Oct 09	Apr 09	Oct 09	Apr 09	Oct 09	Apr 09	Oct 09	Apr 09	Oct 09	Apr 09	Oct 09
1	2.06E+00	2.06E+00	1.29E-01	1.29E-01	8.36E-01	1.35E+00	1.42E-03	1.42E-03	1.06E-02	1.70E-02	3.41E+00	3.16E+00
3	1.47E+00	3.69E+00	9.22E-02	2.31E-01	5.97E-01	1.67E+00	1.01E-03	3.00E-03	7.59E-03	1.70E-02	1.23E+00	5.65E+00
4	3.00E-01	6.88E-01	1.88E-02	4.30E-02	1.21E-01	2.38E-01	2.06E-04	2.58E-04	1.54E-03	2.83E-03	5.17E-01	1.05E+00
8	3.15E+00	3.29E+00	1.97E-01	2.06E-01	1.27E+00	9.92E-01	2.16E-03	1.75E-03	1.62E-02	1.46E-02	5.14E+00	5.05E+00
10	1.43E+00	3.49E+00	8.92E-02	2.18E-01	5.77E-01	1.27E+00	9.81E-04	2.40E-03	7.33E-03	2.02E-02	2.19E+00	5.35E+00
12	1.28E+00	1.82E+00	7.99E-02	1.14E-01	5.18E-01	7.14E-01	8.79E-04	1.33E-03	6.58E-03	6.47E-03	1.96E+00	2.79E+00
13	1.82E-03	7.37E-03	1.14E-04	4.61E-04	7.36E-04	2.99E-03	1.25E-06	5.07E-06	9.36E-06	3.79E-05	2.79E-03	1.13E-02
14	4.47E-03	3.88E-03	2.80E-04	2.43E-04	1.81E-03	1.57E-03	3.08E-06	2.67E-06	2.30E-05	2.00E-05	6.86E-03	5.95E-03
20	1.72E-03	2.65E-03	1.08E-04	1.66E-04	6.97E-04	1.07E-03	1.18E-06	1.83E-06	8.85E-06	1.37E-05	2.64E-03	4.07E-03

Soil sample	Internal absorbed dose rate ($\mu\text{Gy}\cdot\text{h}^{-1}$)											
	²³⁵ U		²³⁸ U		²³² Th		²³⁹ Pu		²⁴⁰ Pu		²⁴¹ Pu	
	Apr 09	Oct 09	Apr 09	Oct 09	Apr 09	Oct 09	Apr 09	Oct 09	Apr 09	Oct 09	Apr 09	Oct 09
1	1.34E-01	1.25E-01	3.66E-01	3.16E-01	3.39E-01	3.42E-01	3.14E+02	3.15E+02	4.43E+02	4.65E+02	2.14E+03	2.05E+03
3	4.99E-02	2.23E-01	1.22E-01	5.65E-01	3.84E-01	3.42E-01	1.03E+02	5.62E+02	1.50E+02	8.31E+02	6.80E+02	3.66E+03
4	2.51E-02	4.15E-02	3.91E-02	1.05E-01	3.16E-01	3.42E-01	3.24E+01	1.05E+02	4.93E+01	1.55E+02	2.45E+02	6.84E+02
8	2.18E-01	1.99E-01	4.99E-01	5.05E-01	3.62E-01	3.42E-01	5.15E+02	5.02E+02	7.33E+02	7.42E+02	2.89E+03	3.27E+03
10	5.69E-02	2.11E-01	2.19E-01	5.35E-01	3.71E-01	3.42E-01	2.88E+02	5.32E+02	5.25E+02	7.86E+02	2.88E+03	3.47E+03
12	6.91E-02	1.10E-01	1.96E-01	2.79E-01	3.21E-01	3.42E-01	1.74E+02	2.77E+02	2.53E+02	4.10E+02	9.90E+02	1.81E+03
13	7.70E-03	4.45E-04	2.79E-04	1.13E-03	3.15E-01	3.42E-01	2.77E-01	1.12E+00	4.10E-01	1.66E+00	1.81E+00	7.33E+00
14	8.08E-03	2.34E-04	6.86E-04	5.95E-04	2.75E-01	3.42E-01	6.32E-01	5.92E-01	2.72E+00	8.75E-01	8.80E+00	3.86E+00
20	8.66E-03	1.60E-04	4.43E-04	4.07E-04	3.91E-01	3.42E-01	2.62E-01	4.05E-01	1.59E+00	5.98E-01	2.58E+01	2.64E+00

Table S3: B) External absorbed dose rate ($\mu\text{Gy}\cdot\text{h}^{-1}$)

Soil sample	External absorbed dose rate ($\mu\text{Gy}\cdot\text{h}^{-1}$)											
	¹³⁷ Cs		⁹⁰ Sr		²⁴¹ Am		⁶⁰ Co		¹⁵⁴ Eu		²³⁴ U	
	Apr 09	Oct 09	Apr 09	Oct 09	Apr 09	Oct 09	Apr 09	Oct 09	Apr 09	Oct 09	Apr 09	Oct 09
1	1.69E+01	1.69E+01	1.32E+01	1.32E+01	8.41E+01	1.36E+02	1.24E-02	1.24E-02	7.45E-02	1.19E-01	3.11E+02	2.89E+02
3	1.21E+01	3.02E+01	9.45E+00	2.36E+01	6.01E+01	1.68E+02	8.88E-03	2.63E-02	5.32E-02	1.19E-01	1.12E+02	5.16E+02
4	2.46E+00	5.64E+00	1.92E+00	4.41E+00	1.22E+01	2.40E+01	1.81E-03	2.26E-03	1.08E-02	1.98E-02	4.72E+01	9.63E+01
8	2.58E+01	2.70E+01	2.01E+01	2.11E+01	1.28E+02	9.98E+01	1.89E-02	1.53E-02	1.13E-01	1.02E-01	4.69E+02	4.61E+02
10	1.17E+01	2.86E+01	9.13E+00	2.24E+01	5.81E+01	1.28E+02	8.58E-03	2.10E-02	5.14E-02	1.42E-01	1.99E+02	4.88E+02
12	1.05E+01	1.49E+01	8.19E+00	1.16E+01	5.21E+01	7.19E+01	7.70E-03	1.17E-02	4.61E-02	4.53E-02	1.79E+02	2.54E+02
13	1.49E-02	6.04E-02	1.16E-02	4.72E-02	7.41E-02	3.00E-01	1.10E-05	4.44E-05	6.56E-05	2.66E-04	2.54E-01	1.03E+00
14	3.67E-02	3.18E-02	2.87E-02	2.49E-02	1.82E-01	1.58E-01	2.69E-05	2.34E-05	1.61E-04	1.40E-04	6.26E-01	5.43E-01
20	1.41E-02	2.18E-02	1.10E-02	1.70E-02	7.01E-02	1.08E-01	1.04E-05	1.60E-05	6.21E-05	9.57E-05	2.41E-01	3.71E-01

Soil sample	External absorbed dose rate ($\mu\text{Gy}\cdot\text{h}^{-1}$)											
	²³⁵ U		²³⁸ U		²³² Th		²³⁹ Pu		²⁴⁰ Pu		²⁴¹ Pu	
	Apr 09	Oct 09	Apr 09	Oct 09	Apr 09	Oct 09	Apr 09	Oct 09	Apr 09	Oct 09	Apr 09	Oct 09
1	7.75E+00	7.19E+00	2.88E+01	2.49E+01	2.11E+01	2.13E+01	3.29E+04	3.30E+04	4.70E+04	4.93E+04	1.48E+02	1.42E+02
3	2.88E+00	1.28E+01	9.63E+00	4.45E+01	2.39E+01	2.13E+01	1.08E+04	5.89E+04	1.59E+04	8.81E+04	4.69E+01	2.53E+02
4	1.44E+00	2.40E+00	3.07E+00	8.30E+00	1.97E+01	2.13E+01	3.40E+03	1.10E+04	5.23E+03	1.64E+04	1.69E+01	4.72E+01
8	1.25E+01	1.15E+01	3.93E+01	3.97E+01	2.26E+01	2.13E+01	5.40E+04	5.27E+04	7.78E+04	7.87E+04	2.00E+02	2.26E+02
10	3.28E+00	1.21E+01	1.72E+01	4.21E+01	2.31E+01	2.13E+01	3.02E+04	5.58E+04	5.56E+04	8.34E+04	1.98E+02	2.39E+02
12	3.98E+00	6.33E+00	1.54E+01	2.19E+01	2.00E+01	2.13E+01	1.83E+04	2.91E+04	2.68E+04	4.35E+04	6.83E+01	1.25E+02
13	4.44E-01	2.57E-02	2.19E-02	8.89E-02	1.96E+01	2.13E+01	2.91E+01	1.18E+02	4.35E+01	1.76E+02	1.25E-01	5.06E-01
14	4.66E-01	1.35E-02	5.40E-02	4.68E-02	1.71E+01	2.13E+01	6.63E+01	6.21E+01	2.88E+02	9.28E+01	6.07E-01	2.66E-01
20	4.99E-01	9.24E-03	3.49E-02	3.20E-02	2.44E+01	2.13E+01	2.75E+01	4.24E+01	1.68E+02	6.34E+01	1.78E+00	1.82E-01

Table S4 : taxonomic affiliation and distribution of OTUs with (i) a relative abundance significantly different between the trench and the surrounding soils and (ii) with >1% abundance in at least one soil sample. The trench soil samples and specific OTUs are highlighted in red. The surrounding soil samples and specific OTUs are highlighted in blue.

#OTU ID	taxonomy	1A	1O	3A	3O	4A	4O	8A	8O	10A	10O	12A	12O	13A	13O	14A	14O	20A	20O
1143180	Archaea;Crenarchaeota;Thaumarchaeota;Cenarchaeales;SAGMA-X	7.30	2.07	0.31	1.05	0.77	0.31	1.90	1.02	2.58	4.90	0.12	1.77	0.04	0.01	0.00	0.14	0.09	0.08
nrOTU63	Bacteria;Acidobacteria;[Chloracidobacteria];11-24	0.00	0.11	0.04	0.01	0.06	0.32	5.16	0.50	0.03	1.03	0.17	0.48	0.00	0.00	0.01	0.00	0.05	0.02
219830	Bacteria;AD3;ABS-6	2.01	0.42	1.12	0.78	1.47	2.36	4.07	4.65	3.48	3.37	2.48	3.18	1.15	0.37	0.10	0.20	0.36	0.28
204059	Bacteria;AD3;ABS-6	0.89	0.42	1.90	0.34	0.09	0.43	0.86	1.50	3.62	0.32	1.94	3.87	0.12	0.02	0.96	0.08	1.10	0.47
125947	Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia	0.04	0.01	0.03	0.09	0.01	0.02	0.00	3.43	0.00	0.06	0.33	0.01	0.07	0.00	0.02	0.02	0.01	0.00
161623	Bacteria;AD3;JG37-AG-4	3.21	1.47	0.94	0.27	1.27	1.59	1.31	1.05	1.68	1.85	1.02	0.95	0.29	0.07	0.26	0.98	0.48	0.24
573135	Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Bradyrhizobiaceae;Bradyrhizobium	0.38	0.33	0.23	2.90	0.44	0.19	0.08	0.43	0.10	0.26	0.60	0.32	0.10	0.02	0.65	0.25	0.26	0.15
658707	Bacteria;Verrucomicrobia;[Pedosphaerae];[Pedosphaerales];auto67_4W	1.71	0.25	0.01	0.00	0.00	0.00	2.32	0.29	0.55	0.70	0.91	0.02	0.00	0.00	0.00	0.00	0.00	0.00
4424841	Bacteria;WPS-2	0.01	0.01	0.03	2.22	0.02	0.14	0.20	0.35	0.44	0.42	0.16	1.03	0.34	0.00	0.00	0.00	0.05	0.01
4383662	Bacteria;Planctomycetes;Planctomycetia;Gemmatales;Gemmataceae	1.34	0.80	0.85	0.20	1.52	2.22	1.95	0.84	0.90	0.43	0.36	0.68	0.13	0.00	0.08	0.38	0.32	0.43
3117367	Bacteria;Chloroflexi;Ktedonobacteria;Ktedonobacterales;Ktedonobacteraceae	0.26	0.21	0.09	1.93	0.31	0.19	0.19	0.29	0.25	0.61	0.20	0.40	0.03	0.00	0.00	0.00	0.07	0.00
154822	Bacteria;Acidobacteria;DA052;Ellin6513	0.20	0.60	0.75	0.37	0.70	1.05	0.46	1.69	1.39	0.80	0.91	1.44	0.07	0.03	0.05	0.37	0.41	0.35
1123749	Bacteria;WPS-2	0.44	0.68	0.38	0.08	0.53	0.46	1.52	1.02	0.83	1.51	1.35	0.55	0.13	0.05	0.01	0.15	0.10	0.04
209467	Bacteria;Acidobacteria;jiii1-8;DS-18	1.49	1.01	0.03	0.01	0.62	0.40	0.83	0.59	0.06	0.03	0.03	0.14	0.17	0.00	0.00	0.00	0.03	0.09
153350	Bacteria;Acidobacteria;DA052;Ellin6513	0.40	0.10	0.05	0.01	0.08	0.40	0.69	0.20	1.16	1.43	0.03	0.17	0.00	0.00	0.00	0.00	0.00	0.00
4342107	Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Hyphomicrobiaceae;Rhodoplanes	0.63	0.62	1.12	0.29	0.96	1.19	0.40	0.60	0.44	0.31	1.30	1.10	0.07	0.04	0.05	0.46	0.57	0.46
78839	Bacteria;Acidobacteria;DA052;Ellin6513	0.83	0.41	0.23	0.61	0.18	0.28	0.97	0.72	0.81	1.29	1.15	0.61	0.03	0.05	0.04	0.35	1.17	0.37
1121144	Bacteria;Proteobacteria;Gammaproteobacteria;Xanthomonadales;Sinobacteraceae	0.29	1.07	0.16	0.22	0.05	0.41	0.73	0.65	0.13	0.44	0.26	0.62	0.07	0.08	0.31	0.11	0.57	0.50
113331	Bacteria;Acidobacteria;DA052;Ellin6513	0.40	0.19	0.03	0.03	0.11	0.13	1.01	0.70	0.50	1.00	0.12	0.41	0.01	0.00	0.00	0.03	0.02	0.00
223820	Bacteria;AD3;ABS-6	0.30	0.43	3.30	0.46	0.55	3.18	0.42	0.73	2.08	0.37	1.11	0.98	9.84	17.07	3.18	3.83	4.24	5.35
254530	Bacteria;Verrucomicrobia;[Spartobacteria];[Chthoniobacterales];[Chthoniobacteraceae];DA101	0.09	0.36	2.85	0.28	1.44	1.91	0.05	0.12	0.08	0.04	0.14	0.09	7.70	10.88	4.04	5.55	1.65	1.99
278511	Bacteria;Acidobacteria;DA052;Ellin6513	0.02	0.05	0.04	0.02	0.03	0.08	0.02	0.03	0.01	0.02	0.04	0.02	0.00	0.00	8.07	0.01	0.03	0.01
205773	Bacteria;AD3;ABS-6	0.47	0.45	2.62	0.64	0.54	0.87	0.72	1.01	4.03	0.31	1.85	2.05	5.25	5.92	0.47	1.59	3.97	3.62
220148	Bacteria;AD3;JG37-AG-4	0.07	0.06	1.27	0.30	1.47	3.07	0.04	0.14	0.82	0.12	0.15	0.35	0.67	1.95	5.89	3.52	0.88	1.35
nrOTU78	Bacteria;AD3;JG37-AG-4	0.00	0.00	0.49	0.02	0.01	0.30	0.00	0.04	0.17	0.00	0.05	0.07	3.88	4.96	2.32	3.14	0.90	1.28
nrOTU44	Bacteria;AD3;JG37-AG-4	0.12	0.06	1.98	0.47	2.06	1.24	0.77	0.58	0.77	0.37	0.58	2.40	2.56	3.52	1.38	2.25	1.07	1.26
248375	Bacteria;Actinobacteria;MB-A2-108	0.00	0.00	0.06	0.03	0.00	0.00	0.00	0.00	0.00	0.01	0.12	0.03	1.48	3.18	1.48	2.01	0.35	2.90
811458	Bacteria;AD3;JG37-AG-4	0.05	0.03	1.12	0.49	0.80	1.99	0.04	0.12	0.88	0.04	0.18	0.39	2.00	1.60	0.94	1.62	1.87	3.11
2984011	Bacteria;Gemmatimonadetes;Gemm-1	0.02	0.00	0.17	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.27	0.61	3.07	0.38	0.17	0.35
205456	Bacteria;Verrucomicrobia;[Spartobacteria];[Chthoniobacterales];[Chthoniobacteraceae];DA101	0.05	0.39	0.60	0.44	0.10	0.20	0.01	0.26	0.00	0.01	0.59	0.10	1.13	0.55	0.64	2.24	3.01	2.95
4448095	Bacteria;Actinobacteria;Actinobacteria;Actinomycetales;Mycobacteriaceae;Mycobacterium	0.01	0.08	0.08	0.14	0.00	0.02	0.05	0.19	0.22	0.02	0.35	0.09	0.30	0.78	2.87	0.15	0.10	0.27
207672	Bacteria;Acidobacteria;jiii1-8;DS-18	0.00	0.00	0.14	0.02	0.01	0.04	0.00	0.00	0.00	0.00	0.04	0.00	0.27	0.42	2.65	0.71	0.73	1.01
3587027	Bacteria;Acidobacteria;jiii1-8;32-20	0.00	0.00	0.08	0.00	0.02	0.02	0.00	0.00	0.00	0.00	0.04	0.00	2.50	1.51	1.36	0.08	0.70	2.17
4474277	Archaea;Crenarchaeota;MBGA;NRP-J	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.08	0.16	1.11	2.11	0.13	0.07	0.98	1.18
821237	Bacteria;Verrucomicrobia;[Spartobacteria];[Chthoniobacterales];[Chthoniobacteraceae];DA101	0.03	0.16	1.05	0.16	0.16	0.25	0.02	0.10	0.04	0.01	0.05	0.04	2.03	1.49	1.18	0.91	1.04	1.01
4470401	Bacteria;AD3;ABS-6	1.07	0.24	0.53	0.46	0.49	0.14	0.37	0.51	1.79	0.22	1.26	1.10	0.61	0.99	0.23	1.86	1.06	0.59
nrOTU22	Bacteria	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.04	0.06	0.00	0.09	0.07	0.02	0.13	0.79	1.60	0.42	0.74
ncurOTU10624	Bacteria	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.05	1.55	1.51	0.67	1.44	0.03	0.15
1144253	Bacteria;WPS-2	0.00	0.00	0.11	0.04	0.08	0.27	0.20	0.19	0.54	0.15	0.43	0.38	0.85	1.20	0.29	0.37	0.92	0.47

OTU ID	Taxonomy	Closest relatives	identity	Origin			
1143180	Archaea;Crenarchaeota;Thaumarchaeota;Cenarchaeales;SAGMA-X	AB050208	100%	acid water from gold mines, South Africa			
OTUnr63	Bacteria;Acidobacteria;[Chloracidobacteria];11-24	KP915812	99%	soil after volcanic eruption on Kasatochi Island, Alaska			
209467	Bacteria;Acidobacteria;iii1-8;DS-18	KF974114	99%	permafrost-affected soils of Northeast Greenland			
153350	Bacteria;Acidobacteria;DA052;Ellin6513	EF221418	99%	Antarctic terrestrial habitats			
		HQ322990	98%	acid mine drainage in a long abandoned mining site			
		FJ570382	98%	alpine tundra soils			
		GU393466	98%	anoxic neptunium-containing sediments			
154822	Bacteria;Acidobacteria;DA052;Ellin6513	JF300958	100%	boreal forest soil			
		EU849210	100%	acidic subalpine forest and fire-induced grassland,Taiwan			
		FJ625362	100%	lead contamination of boreal pine forest			
78839	Bacteria;Acidobacteria;DA052;Ellin6513	AJ536864	100%	soil samples of uranium wastes			
		DQ450707	99%	alpine tundra wet meadow soil			
		GU393453	99%	anoxic neptunium-containing sediments			
		GU047460	99%	active layer and permafrost in an acidic wetland, Arctic			
		FJ466151	99%	Hawaiian volcanic deposit			
		FJ570548	99%	alpine tundra soils			
		GQ287472	99%	Pindari glacier, Himalayan mountain ranges, India			
113331	Bacteria;Acidobacteria;DA052;Ellin6513	KP905379	100%	soil after volcanic eruption on Kasatochi Island, Alaska			
219830	Bacteria;AD3;ABS-6	KP906936	100%	soil after volcanic eruption on Kasatochi Island, Alaska			
		FJ570325	100%	alpine tundra soils			
		HM065752	99%	Dama glacier, Switzerland			
204059	Bacteria;AD3;ABS-6	JF833936	100%	potassium mine soil, China			
		AB821068	100%	lava forest soil, Korea			
161623	Bacteria;AD3;JG37-AG-4	JX869474	100%	acid sulfate soil, Finland			
		DQ450737	100%	alpine tundra wet meadow soil			
		KP906130	100%	soil after volcanic eruption on Kasatochi Island, Alaska			
		HG324327	100%	cellulose degradation in acidic fen soil			
		FJ625337	100%	lead contamination of boreal pine forest			
573135	Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Bradyrhizobiaceae;Bradyrhizobium	LT617088	100%	dust samples from the Russian part of the ISS			
4304723	Bacteria;Proteobacteria;Betaproteobacteria;SC-I-84	GU393494	100%	anoxic neptunium-containing sediments			
1121144	Bacteria;Proteobacteria;Gammaproteobacteria; Xanthomonadales;Sinobacteraceae	KT018626	100%	Cr(VI)-contaminated Soils			
		KF800867	100%	Himalayan Mountains			
658707	Bacteria;Verrucomicrobia;[Pedosphaerae];[Pedosphaerales]; auto67_4W	KU221979	99%	Hengill Valley, Iceland			
		AB364854	99%	boreal oligotrophic peat wetlands			
		FJ466366	99%	Hawaiian volcanic deposit			
		KF863914	99%	acidic soil near an acid mine drainage, China			
4424841	Bacteria;WPS-2	GQ329594	100%	Pindari glacier, Himalayan mountain ranges			
1123749	Bacteria;WPS-2	KC562917	100%	soil, Sergyemla Mountains, southeast Tibet			
		GU393463	100%	anoxic neptunium-containing sediments			
		FJ570155	100%	alpine tundra soils			
		EU421867	100%	Lahaul-Spiti Valley of The Indian Himalayas			
		KP905215	100%	soil after volcanic eruption on Kasatochi Island, Alaska			

Table S6: distribution and taxonomic affiliation of the 5 most abundant OTUs having a cultured representative. The OTUs specific of the trench are highlighted in red.

OTU ID	1A	1O	3A	3O	4A	4O	8A	8O	10A	10O	12A	12O	13A	13O	14A	14O	20A	20O	taxonomic affiliation
4057260	0.06	0.01	0.26	6.97	0.00	0.01	0.01	0.01	0.24	0.73	0.57	1.28	0.00	0.00	0.66	0.00	0.33	0.41	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia
125947	0.04	0.01	0.03	0.09	0.01	0.02	0.01	3.43	0.00	0.06	0.33	0.01	0.07	0.00	0.02	0.02	0.01	0.00	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia
573135	0.38	0.33	0.23	2.90	0.44	0.20	0.08	0.44	0.10	0.26	0.60	0.32	0.11	0.02	0.65	0.25	0.26	0.15	Proteobacteria;Alphaproteobacteria;Rhizobiales;Bradyrhizobiaceae;Bradyrhizobium
156722	0.00	0.00	0.02	0.05	0.00	0.00	0.00	0.08	0.04	0.04	0.25	0.07	0.01	0.00	0.77	0.36	0.12	0.21	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia
196652	0.03	0.00	0.62	0.21	0.00	0.00	0.00	0.27	0.06	0.71	0.13	0.04	0.02	0.00	0.05	0.17	0.02	0.05	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia