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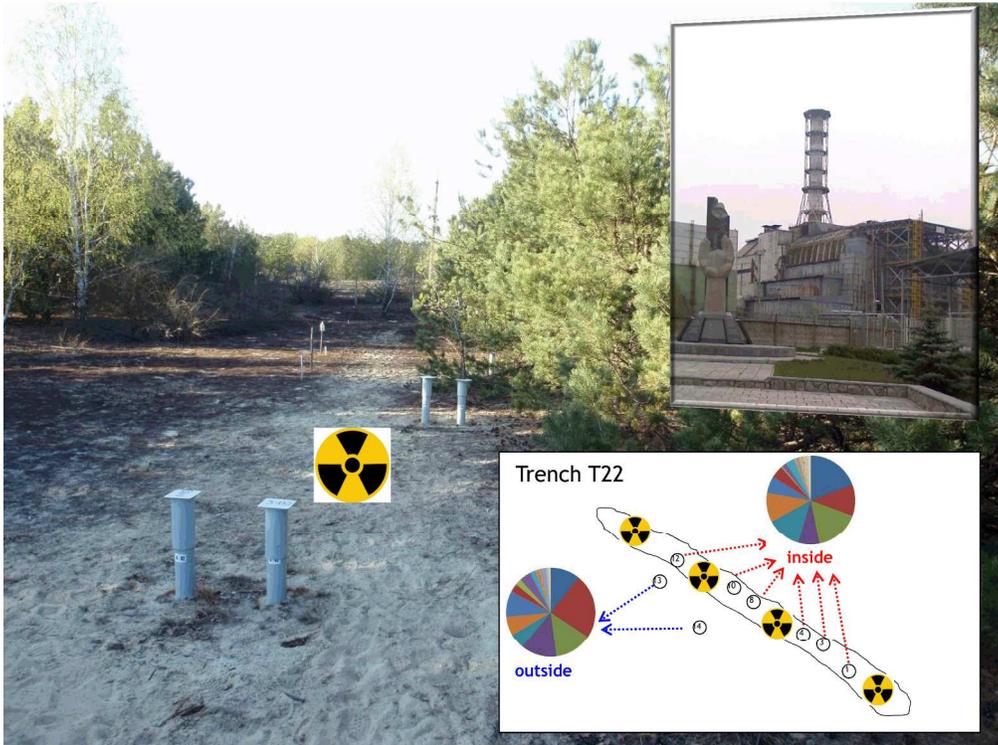
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Soil prokaryotic communities in Chernobyl waste disposal trench T22 are modulated by organic matter and radionuclide contamination.

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review

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3 **Soil prokaryotic communities in Chernobyl waste disposal trench T22 are modulated by**
4
5 **organic matter and radionuclide contamination.**
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45 **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
12
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
26
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
32
33 40 the other hand, RNs may exert radio- and chemo-toxic effects on bacteria, thus influencing
34
35 41 the structure and activity of microbial communities. Many previous studies have explored the
36
37 42 molecular diversity of bacterial communities in various natural or contaminated sites
38
39 43 containing radionuclides such as uranium (Selenska-Pobell *et al.* 2001; Rastogi *et al.* 2010;
40
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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3 51 Kostka 2007, Rastogi *et al.* 2010; Mondani *et al.* 2011, Islam, Paul and Sar. 2014, Yan, Luo
4
5 52 and Zhao 2016).
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7 53 In the case of Chernobyl, very few studies have been performed, most of which were based on
8
9 54 cultivation approaches. These studies have shown a decrease in bacterial diversity under
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11 55 conditions of radioactive contamination/irradiation (Romanovskaya *et al.* 1998, 1999;
12
13 56 Romanovskaya, Rokitko and Malashenko 2000; Zavilgelsky *et al.* 1998; Czirják *et al.* 2010).
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15
16 57 In 2011, the first study based on molecular approaches of sunlight-adapted biofilm microbial
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18 58 communities exposed to different ambient radiation levels (0.35 – 25 $\mu\text{Sv/h}$) was reported
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20 59 from the Chernobyl area (Ragon *et al.* 2011). Although the diversity was similar in irradiated
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22 60 and non-irradiated UV-adapted communities, an increase in the mutation level within some
23
24 61 Operational Taxonomic Units (OTUs) was correlated with ambient radiation. Niedrée *et al.*
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26 62 (2013) revealed a shift in bacterial population in soil microcosms upon three-month exposure
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28 63 to Chernobyl-like contamination with ^{90}Sr and ^{137}Cs .
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32 64 Atmospheric fallout from the CNPP accident resulted in radioactive contamination of
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34 65 large regions. One of the most impacted areas, located in the immediate vicinity of the nuclear
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36 66 power plant, is the so-called Red Forest where the pine trees died upon exposure to extreme
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38 67 radiation doses. Shortly after the CNPP accident, clean-up wastes from the Red Forest and
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40 68 other radioactively contaminated debris were buried in a large network of trenches near the
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42 69 CNPP, leading to highly contaminated environmental locations. After the clean-up operations,
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44 70 the trenches were covered with a thick layer of sand and planted with pine saplings, which are
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46 71 now reclaiming the area. One of these trenches (trench T22) has been studied since 1999, as a
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48 72 model of RN migration in the environment (Martin-Garin 2012). In our previous study
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50 73 (Chapon *et al.* 2012), we identified the presence of complex bacterial communities in
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52 74 contaminated soil and control samples from this site using a community fingerprint method
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3 75 (DGGE). However, this method did not reveal any dominant community fingerprint related to
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5 76 RN content.
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8 77 The goals of the present study were to explore composition and diversity of soil
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10 78 prokaryotic communities in this understudied environment and to characterize the impact of
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12 79 the trench conditions (RN contamination and high organic matter content) on these
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14 80 communities. We hypothesized that soil prokaryotic communities inside and outside of trench
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16 81 T22 would differ, in particular upon radiation exposure. Therefore, we used high-throughput
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18 82 pyrosequencing of 16S rRNA genes to compare the prokaryotic communities in soil samples
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20 83 exhibiting contrasted RN contamination levels, collected inside and in the vicinity of the
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22 84 trench T22.
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27 86 **Materials and Methods**

28 29 87 *Sample collection, isolation of culturable bacteria and DNA extraction*

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31
32 88 Sampling, soil analyses, isolation of culturable bacteria and DNA extraction
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34 89 procedures were previously described (Chapon *et al.* 2012). Briefly, sandy soil samples
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36 90 containing various RN contents were collected between 50 – 60 cm depth from nine different
37
38 91 positions in the area of the RN-contaminated trench T22, located in the Chernobyl exclusion
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40 92 zone (51°23'N,30°04'E; Bugai *et al.* 2005). In April and October 2009, RN-contaminated soil
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42 93 samples (numbers 1, 3, 4, 8, 10 and 12) were collected inside the trench, along with low-
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44 94 contamination controls (numbers 13, 14 and 20) collected in the vicinity (Figure 1). The soil
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46 95 samples were processed at Chernobyl within 18 hours after sampling. In April, environmental
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48 96 DNA was extracted from a single subsample of each soil sample, whereas in October
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50 97 environmental DNA was extracted from triplicate subsamples (designated as a, b and c), after
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52 98 homogenization of the soil. DNA was typically extracted from 1g of soil using the
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54 99 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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9
10 103 *PCR amplification and pyrosequencing*

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12 104 For the pyrosequencing analyses, amplicons from the V4 region of the 16S rRNA
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14 105 genes were generated by PCR using the universal primer set 530f (5'-
15
16 106 GTGCCAGCMGCNGCGG-3'; Dowd *et al.* 2008) and 802r (5'-
17
18 107 TACNVGGGTATCTAATCC-3'; Claesson *et al.* 2009). The coverage of this primer set was
19
20 108 89.1% for Bacteria and 54.1% for Archaea with no mismatch and reached 96.8% for Bacteria
21
22 109 and 96.5% for Archaea with 2 mismatches as predicted with the Testprime tool of SILVA
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24 110 (Klindworth *et al.* 2013). PCR amplifications were performed in triplicate in 50- μ l reactions
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26 111 consisting of: 30 – 50 ng of template DNA, 0.6 μ M of forward and reverse primers, 0.2 mM
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28 112 dNTPs, 2 mM MgCl₂, 1.25 U of Go Taq® Hot start polymerase (Promega; USA), and 1 X
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30 113 reaction buffer. For PCR, the thermocycler was programmed for an initial denaturation at
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32 114 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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34 115 72°C for 45 s) and a final extension at 72°C for 5 min. Triplicate samples were pooled before
35
36 116 purification of the amplicons. DNA from each amplicon (500 ng) was sent separately for
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38 117 sequencing by GATC (Konstanz, Germany) using a Roche 454 GS-FLX system (Titanium
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40 118 Chemistry). An adaptor and sample-specific 4-bp Molecular Identifier tag (MID) were added
41
42 119 to each amplicon by an additional PCR step before mixing and pyrosequencing. Raw reads
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44 120 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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3 125 with quality score average < 25, short sequences (< 200 bp) and sequences containing
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5 126 mismatches as well as homopolymers were removed. We conducted a denoising of chimeras
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7 127 and the multiplex reads were assigned to corresponding sample according to their barcode,
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9 128 and we carried out Operational Taxonomic Unit (OTU) picking with Uclust (Edgar, 2010).
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11 129 Representative sequences of the OTUs were aligned using the PyNAST algorithm with a
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13 130 minimum percent of 80% (Caporaso *et al.* 2010b). These OTUs were taxonomically classified
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15 131 using RDP method (Wang 2007) and Greengenes database (DeSantis *et al.* 2006). The
16
17 132 taxonomic position of bacteria and archaea was characterized using taxa summary QIIME
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19 133 scripts until genus level (L6). Alpha diversity parameters (number of observed OTUs,
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21 134 qualitative Chao1 index, Faith's Phylogenetic Diversity (PD_{whole}) and Shannon index)
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23 135 were calculated with a fixed number of 16 182 randomly picked reads for each sample. Beta-
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25 136 diversity was calculated using Bray-Curtis and unweighted and weighted UniFrac metrics
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27 137 (Lozupone, 2005). PCoA plots were visualized with Emperor (Vazquez-Baeza, 2013).

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32 138 The representation of cultured bacteria (described in Chapon *et al.* 2012) in the soil
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34 139 bacterial community was assessed by clustering the 16S sequences derived from these
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36 140 bacteria and the 454 reads in OTUs at 97% sequence similarity.

37 38 39 40 41 142 *Analysis of soil physico-chemical parameters*

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43 143 For both sampling dates, pH, water content (WC) and NaOH-extractable organic
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45 144 carbon ($C_{\text{org(NaOH)}}$) were analyzed as described in Chapon *et al.* (2012). Briefly, soil pH was
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47 145 measured in a 1:5 soil:water suspension, and 2 g of soil were mixed with 10 mL of distilled
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49 146 water and shaken for 30 min. The pH was measured after decanting and 2 hours of
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51 147 atmosphere equilibration. WC was determined by drying 10 g of soil at 105°C for 24 h.
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53 148 $C_{\text{org(NaOH)}}$ was determined by dissolving 0.15 g of soil samples in 1.5 mL of 0.1 M NaOH for
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55 149 15 h. After decanting and centrifugation, the organic carbon concentration in the supernatant

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3 150 was determined by spectrometry (absorbance measurement at 280 nm).
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5 151 The ^{137}Cs concentrations in the soil samples were measured by gamma spectrometry.
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7 152 In addition, ^{241}Am , ^{154}Eu and ^{60}Co concentrations were measured in October 2009 by gamma
8

9 153 spectrometry, whereas actinide concentrations (^{234}U , ^{235}U , ^{236}U , ^{232}Th , ^{239}Pu , ^{240}Pu and ^{241}Pu)
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11 154 were measured in April 2009 by ICP-MS after dry “ashing” and mineralization of the soil
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13 155 samples (Chapon *et al.* 2012). ^{90}Sr concentrations were derived from ^{137}Cs content assuming a
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15 156 constant ratio of 1.87 between ^{137}Cs and ^{90}Sr concentrations (Kashparov *et al.* 2001). All RN
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17 157 contents were significantly correlated with ^{137}Cs content (Figure S1) except ^{232}Th whose
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19 158 concentration is unvarying close to its natural value in soil (Table S1). Thus, constant ratios
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21 159 between ^{137}Cs and each RN were used to derive the concentrations of ^{241}Am , ^{154}Eu , ^{60}Co ,
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23 160 ^{234}U , ^{235}U , ^{236}U , ^{239}Pu , ^{240}Pu and ^{241}Pu when they were missing or below the detection limits
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25 161 (Table S1). When not measured, the ^{232}Th concentration was considered equal to the mean of
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27 162 ^{232}Th concentrations in all soil samples.
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33 164 *Estimation of the total absorbed dose rate by prokaryotic cell*

34 165 In the absence of any method to measure the total absorbed dose rates (TADR) by
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36 166 prokaryotic cell, TADR values were calculated based on soil RN activities. For this, two
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38 167 irradiation pathways were considered in the calculation: the external irradiation from the
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40 168 radio-contaminated habitat of bacteria and archaea (*i.e.* the soil) and the internal irradiation
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42 169 resulting from the internalization of RN in the cells (Garnier-Laplace *et al.* 2015).
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46 170 The external and internal dose rates were calculated at each sampling point and for
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48 171 each sampling date by multiplying the Dose Conversion Coefficient (DCC) for each RN by
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50 172 the measured RN concentration at this sampling point or in the cells, respectively. The RN
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52 173 concentration inside the cells was considered in equilibrium with the soil RN concentration
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54 174 (conservatively assuming a concentration ratio of 1 for each RN) in the absence of any other
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3 175 information. External and internal DCCs (Table S2A) were calculated for each RN with the
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5 176 EDEN software version 2.3 (Beaugelin-Seiller *et al.* 2006), assuming a different biological
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7 177 effectiveness for the different types of radiation (applied weighting factors were 10 for α -
8
9
10 178 radiation, 3 for low β -radiation, and 1 for other β -radiation and γ -radiation) (Pröhl *et al.*
11
12 179 2003). The prokaryotic cells were described as a sphere with a 1- μ m diameter, living in an
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14 180 infinitely extended and uniformly contaminated soil (compositions are provided in Table
15
16 181 S2B).
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20 21 183 *Statistical analyses*

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23 184 Non-parametric tests (Kruskal-Wallis) were calculated using QIIME (Caporaso *et al.* 2010a).
24
25 185 Redundancy Analysis was performed with R software (R Core Team 2011) on the OTUs and
26
27 186 phylum abundance data with the Principal Component Analysis on Instrumental Variables
28
29 187 (PCAIV) function of the ade4 package (Dray and Dufour, 2007). Heatmap was constructed
30
31 188 with the gplots package of R (Bolker *et al.* 2011).
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35 36 190 **Results**

37 38 191 *Overall prokaryotic diversity in the Chernobyl soils*

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41 192 This study focused on the prokaryotic communities in 36 soil samples collected inside
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43 193 and outside the RN-contaminated trench T22 at nine different positions in April and October
44
45 194 2009 (Figure 1). The pH values ranged from 4.4 to 6.1 and soil water content ranged from
46
47 195 2.2% to 7.3% (Table 1). The NaOH extractable organic C content ($C_{\text{org(NaOH)}}$) was within the
48
49 196 same range for all samples (average $2.25 \pm 0.83 \text{ g kg}^{-1}$), except for samples 4, 13, and 14 which
50
51 197 were lower in content (average $0.35 \pm 0.30 \text{ g kg}^{-1}$). Soils collected within the trench (sample
52
53 198 numbers 1, 3, 4, 8, 10 and 12) were contaminated with RNs (^{137}Cs activity ranging from 61 –
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55 199 750 Bq g^{-1}), whereas soils collected in the vicinity of trench T22 (sample numbers 13, 14 and
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1
2
3 200 20) exhibited low RN levels (^{137}Cs ranging from 0.35 – 1.50 Bq g⁻¹; Table 1). Three soil
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5 201 subsamples from October 2009 (designated as a, b and c) were used for DNA extraction in
6
7 202 order to assess the reproducibility of the experiment. After quality filtering, we obtained
8
9 203 753 703 reads with a read length average of 255 bp, ranging from 16 182 – 23 686 reads in
10
11 204 the 36 samples (Table 1). From the reads, 8 167 OTUs at 3% genetic distance were identified
12
13 205 and after normalization of the dataset to 16 182 reads per sample and deletion of singletons,
14
15 206 7 663 OTUs were identified with OTU counts varying between 633 – 2 120 per sample (Table
16
17 207 1). The rarefaction curves based on PD whole tree, Chao1 and Shannon estimators tended to
18
19 208 reach an asymptote (Figure S2). The Good's estimator of coverage varied between 95 – 98%
20
21 209 across the samples, indicating that the depth of sequencing was nearly sufficient to detect
22
23 210 most of the dominant OTUs (Table 1).

24
25 211 Of the reads, 95.7% were affiliated with Bacteria and 4.3% were affiliated with
26
27 212 Archaea. The most consistently detected bacterial phyla (relative abundance > 1%) across all
28
29 213 samples were *Proteobacteria*, *Acidobacteria*, AD3, *Planctomycetes*, *Chloroflexi*,
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31 214 *Verrucomicrobia* and *Actinobacteria*, with an uneven distribution across the samples (Figure
32
33 215 1). Combined, these seven groups accounted for 83.3% of the sequences. Twenty-seven
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35 216 additional bacterial phyla were detected as well as three archaeal phyla.
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43 218 *Trench T22 hosts specific microbiota*

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45 219 The rarefaction curves calculated using different estimators revealed a higher apparent
46
47 220 species richness in almost all samples collected in the trench, as compared to the samples
48
49 221 collected outside of the trench (Table 1). Accordingly, mean Chao1 richness and Shannon
50
51 222 diversity indices were significantly higher in samples collected inside as opposed to outside
52
53 223 the trench ($p < 0.05$; Figure S2).
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3 224 Principal Coordinates Analysis (PCoA) based on bacterial and archaeal OTUs and
4
5 225 performed with Bray-Curtis, Unweighted and Weighted Unifrac methods all showed a clear
6
7 226 separation between the communities inside and outside the trench (Figure 2). The first
8
9 227 principal components of the PCoA accounted for 20 – 40% of the variation between the
10
11 228 samples, depending on the method used. Some divergence between samples taken at the same
12
13 229 location in April and in October could be observed, particularly for samples collected within
14
15 230 the trench. By contrast, high consistency between replicates (labeled as ‘a’, ‘b’ or ‘c’) was
16
17 231 observed for each soil sample collected in October 2009, indicating high reproducibility of the
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19 232 experimental procedure.
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234 *Impact of long-term exposure to radioactive contaminated wastes on prokaryotic microbiota*

235 To examine how the prokaryotic microbiota was affected by radioactivity, the TADR
236 was calculated for each soil sample. On average, estimated TADR by cell were two orders of
237 magnitude higher in trench soils than in samples collected outside of the trench; the maximum
238 TADR was *ca.* 150 mGy h⁻¹ in sample 8 in October 2009, whereas the lowest value was 0.1
239 mGy h⁻¹ in samples 13 and 20 in April and October 2009, respectively (Table 1). Internal
240 absorbed dose rates contributed to only 3% of the estimated TADR by cell (Table S3A and B)
241 which is mainly due to the small size of prokaryotic cells. Actinides were the primary
242 contributors to the estimated TADR by cell, and ²³⁹Pu and ²⁴⁰Pu were responsible for 80 –
243 96% of the estimated TADR by cell (Table S3A and B). Since the PCoA analysis was able to
244 confirm the reproducibility of the experiment, the triplicates performed in October were
245 averaged in order to create a single dataset and to facilitate depiction of the data. A
246 redundancy analysis (RDA) coupled to a permutation test (randtest function) was used to test
247 whether the community composition at the different sampling sites was related to the physico-
248 chemical properties of the soil samples (Ramette 2007). The four physico-chemical variables

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3 249 included in the analysis (TADR, $C_{\text{org(NaOH)}}$, water content, and pH) significantly explained
4
5 250 43% of the variation of the bacterial and archaeal communities ($r^2 = 0.43$, $p = 0.001$ based on
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7 251 999 permutations). The first two axes of the RDA explain 85% of the variance due to the soil
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9 252 physico-chemical properties, 71% of which is accounted for by the first axis, which correlated
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11 253 primarily with TADR ($r = -0.90$), $C_{\text{org(NaOH)}}$ ($r = -0.84$) and water content ($r = -0.76$) (Figure
12
13 254 3). Using the RDA function in the 'vegan' package to separately analyze the effect of each
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15 255 variable indicates that TADR was the principal variable to influence the prokaryotic
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17 256 communities at the OTU-level ($p < 0.05$).

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20 257 The same analysis at the phylum level confirmed the effect of the environmental
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22 258 variables on the bacterial communities since 52 % of the variation of the prokaryotic
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24 259 communities at the phylum level were explained by the environmental variables ($r^2 = 0.523$, p
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26 260 $= 0.001$ based on 999 permutations). The first two axes of the RDA explained 92 % of the
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28 261 variance, among which 74 % is explained by the first axis which correlated mainly with
29
30 262 TADR ($r = -0.89$) and to a lesser extent with $C_{\text{org(NaOH)}}$ ($r = -0.69$) and water content ($r = -$
31
32 263 0.66) (data not shown). The analysis of the effect of each variable separately showed that the
33
34 264 principal variable influencing the prokaryotic communities at the phylum level were TADR
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36 265 ($p < 0.05$) and $C_{\text{org(NaOH)}}$ ($p < 0.1$).

266 267 *Primary differences between prokaryotic microbiota of the trench and surrounding soils*

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

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3 274 surrounding soils, whereas 808 OTUs were significantly more abundant in the surrounding
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5 275 soils than in the trench soils. Furthermore, these 1 781 OTUs accounted for 50% of the total
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7 276 prokaryotic microbiota in the trench and for 67% of the total prokaryotic microbiota in the
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10 277 surrounding soils, and could be classified into 26 bacterial phyla and three archaeal phyla.

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12 To further explore the primary differences between microbiota of the trench soils and
13
14 279 surrounding soils, a heatmap was constructed with the most abundant OTUs (> 1% relative
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16 280 abundance in at least one sample; see Table S4 for details) differing significantly between the
17
18 281 trench and the surrounding soil microbiota (Figure 4). The surrounding soils were
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20
21 282 characterized by 20 abundant OTUs affiliated with the phyla *Crenarchaeota* (NRP-J order),
22
23 283 *Acidobacteria* (Ellin6513, 32-20, DS-18 orders), *Actinobacteria* (*Mycobacterium* genus and
24
25 284 MB-A2-108 class), AD3 (ABS-6 and JG37-AG-4 classes), *Gemmatimonadetes* (Gemm-1
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27 285 class), *Verrucomicrobia* (DA101 genus) and WPS-2 (Figure 4). On the other hand, the trench
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29
30 286 prokaryotic community was characterized by 19 abundant OTUs. These OTUs were affiliated
31
32 287 with the phyla *Crenarchaeota* (SAGMA-X family), *Acidobacteria* (11-24, Ellin6513, DS-18
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34 288 orders), AD3 (ABS-6 and JG37-AG-4 classes), *Chloroflexi* (*Ktedonobacteraceae* family),
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36 289 *Proteobacteria* (*Bradyrhizobium*, *Rhodoplanes* and *Burkholderia* genera, SC-I-84 order and
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38 290 *Sinobacteraceae* family), *Verrucomicrobia* (auto67-4W family) and WPS-2 (Figure 4). Within
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40
41 291 the Archaea, the representative sequence of OTU 1143180 (*Crenarchaeota* (SAGMA-X))
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43 292 exhibited 100% sequence similarity with *Candidatus Nitrosotalea devanaterrea*, an acidophilic
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45 293 ammonia oxidizer (Lehtovirta-Morley *et al.* 2011), as well as sequences detected in acid water
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47 294 from a gold mine (AB050208; Takai *et al.* 2001). The OTUs specific to the trench did not
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49
50 295 have any cultured representatives within the Bacteria, with the exception of *Bradyrhizobium*,
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52 296 *Rhodoplanes*, *Burkholderia* and *Sinobacteraceae*.

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54 297 Relationships with other environments were investigated by performing a BLAST
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56 298 search of the non-redundant database, using representative sequences of the 19 OTUs specific

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3 299 to the trench soils. Interestingly, among the closest neighbors (98 – 100% sequence
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5 300 similarity), we found sequences derived not only from a wide range of forest soils (data not
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7 301 shown) but also from cold environments (alpine tundra, boreal wetlands and forest, Finland,
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9 302 Himalayan mountains, Alaska, Iceland, Greenland, Antarctic habitats, the Arctic), acidic
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11 303 environments (acid water, acid mine drainage, acidic fen soil, acidic subalpine forest, acidic
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13 304 wetland), volcanic soils (Kasatochi Island in Alaska, a lava forest soil in Korea, Hawaiian
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15 305 volcanic deposit), RN- and heavy metal-contaminated soils, sediment and water samples
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17 306 (neptunium-contaminated sediments, soil samples of uranium mining waste pile, acid water
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19 307 from gold and potassium mines, lead-contaminated forest soil) as shown in Table S5. The
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21 308 representative sequence of OTU 573135, which is affiliated with *Bradyrhizobium*, was
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23 309 reported to have a 100% sequence similarity with many sequences, including a bacterium
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25 310 cultured from dust samples from the International Space Station (ISS) (LT617088; Mora *et al.*
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27 311 2016).
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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

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3 324 *Burkholderia* spp. and OTU 573135, affiliated to *Bradyrhizobium* spp. (Table S6). The
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5 325 relative abundance of OTUs 4057260 and 573135 reached 6.97% and 2.90%, respectively, in
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7 326 the most contaminated soil (750 Bq/g of ¹³⁷Cs), which was collected in October 2009 at
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9 327 sampling point 3 (sample 3O; Tables 1 and S6). Furthermore, OTUs 125947 and 573135 are
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11 328 characteristic of the trench prokaryotic community.
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16 330 **Discussion**

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18 331 The current work explored the diversity of soil prokaryotes that have been evolving
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20 332 since more than two decades in RN-contaminated soils inside and outside of the trench T22,
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22 333 located within the Chernobyl exclusion zone. The presence of complex prokaryotic
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24 334 communities in contaminated soil and control samples from this site was revealed by a
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26 335 genetic fingerprint method (DGGE) in our previous study (Chapon *et al.* 2012). We observed
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28 336 that apparent diversity in the trench soils was similar to that observed in the surrounding soils
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30 337 on DGGE. However, this approach did not uncover any dominant community fingerprint
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32 338 clearly related to RN content, nor did it provide any direct taxonomic information. Here, we
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34 339 revisited this previous work to address these issues using 454 pyrosequencing of 16S rRNA
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36 340 genes. We surveyed the prokaryotic diversity of this understudied environment, and we
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38 341 assessed the effects of RN contamination and C_{org(NaOH)} content on soil microbiota using a
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40 342 comparative analysis with low-contamination soils collected outside of the trench in the same
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42 343 area. The ¹³⁷Cs content in the soil samples collected outside of the trench is consistent with
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44 344 worldwide upper background levels of ¹³⁷Cs due to Chernobyl fallout (*e.g.* in France: from
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46 345 0.01 - 0.1 Bq g⁻¹ in upper soils, max value in hotspots: 10 Bq g⁻¹; IRSN, 2016). In addition,
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48 346 Michel *et al.* (2015) reported several values for Ukraine (from 0.3 mBq g⁻¹ to 6.6 Bq g⁻¹)
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50 347 outside of the Chernobyl exclusion zone.
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3 348 The seven dominant bacterial phyla in the Chernobyl soils were *Proteobacteria*,
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5 349 *Acidobacteria*, AD3, *Planctomycetes*, *Chloroflexi*, *Verrucomicrobia* and *Actinobacteria*.
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7 350 These phyla are commonly encountered as the dominant taxa in soils (Janssen 2006; Rastogi
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9 351 *et al.* 2009). Here, the abundance of *Chloroflexi* (mean relative abundance 9.4%, ranging from
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11 352 2.4 – 16.1%) and AD3 (mean relative abundance 14.8%, ranging from 3.3 – 39.0%) was high,
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13 353 both inside and outside the trench. On a global scale, this is the most noticeable feature of
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15 354 Chernobyl soil prokaryotic communities. This high abundance is not an artefact linked to
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17 355 DNA extraction or PCR amplification, since other soil samples processed in the same
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19 356 experimental run did not exhibit the same profile (data not shown). High abundance of
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21 357 *Chloroflexi*/AD3 has been described in cold environments (Costello and Schmidt 2006; Taş *et*
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23 358 *al.* 2014; Hultman *et al.* 2015; Frey *et al.* 2016). Members of *Chloroflexi*/AD3 are reported to
24
25 359 predominate following acute gamma irradiation of soils (McNamara *et al.* 2007; El-Sayed and
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27 360 Ghanem 2009) and have been detected in natural uranium ores (Mondani *et al.* 2011) and in
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29 361 uranium-contaminated sites (Selenska-Pobell 2002; Barns *et al.* 2007; Hug *et al.* 2013). It has
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31 362 been proposed that degradation of plant polymers is widespread across this phylum (Hug *et*
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33 363 *al.* 2013). Thus, members of this phylum are well-adapted to cold environments; they are
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35 364 potentially able to degrade lignocellulosic material; and they can tolerate uranium and/or its
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37 365 associated radioactivity. The high abundance of *Chloroflexi*/AD3 members both inside and
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39 366 outside trench T22 is coherent, taking into account the facts that Chernobyl upper soils are
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41 367 partly frozen during winter (Bixio *et al.* 2002), that wood debris were buried in the trench,
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43 368 and that bacteria and archaea are exposed to different levels of irradiation and contamination
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45 369 whether they are inside or outside of the trench. Nevertheless, when examining the results in
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47 370 greater detail, we noted that specific phylotypes within *Chloroflexi*/AD3 were selected by the
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49 371 soil trench. Indeed, the abundance of seven OTUs affiliated with classes ABS-6 and JG37-
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51 372 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
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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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3 398 all measured variables, influencing the prokaryotic communities at the OTU and phylum level
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5 399 was TADR and to a lesser extent $C_{\text{org(NaOH)}}$ when the analysis was performed at the phylum
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7 400 level. However, we could not exclude that other confounding factors not monitored during our
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9 401 study might also have influenced the prokaryotic communities composition.

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11 402 808 OTUs were significantly enriched in the prokaryotic community outside of the
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13 403 trench, including 20 abundant OTUs. These abundant OTUs were affiliated to different phyla
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15 404 and do not have any cultured representatives, with the exception of *Mycobacterium*. Members
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17 405 of these phylotypes probably correspond to RN-sensitive species and/or oligotrophic
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19 406 microorganisms. We also identified specific OTUs that distinguished the trench microbiota
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21 407 from surrounding microbiota, indicating that specific phylotypes were selected by the trench
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23 408 soils. Close relationships with a wide range of cold environments and acidic soils were
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25 409 revealed, as well as volcanic soils or soils that have been subjected to fire, showing that traces
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27 410 of past events are still detectable in the trench prokaryotic community. In addition,
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29 411 relationships with radioactive environments were shown for five OTUs specific to the trench
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31 412 soils. Thus, members of the phylotypes that were selected by the trench conditions, in
32
33 413 particular by high TADR, may have the capacity to survive under stressful conditions. These
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35 414 phylotypes are distributed among several phyla, illustrating that adaptation to life in
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37 415 radioactive environment is widespread in prokaryotes.

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39 416 A limited number of reads from the 454 dataset identified as *Deinococcus* were
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41 417 detected in the soil samples, representing a very minor fraction of the total abundance (0.004
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43 418 – 0.084%; mean relative abundance 0.017%). However, we failed to confirm the presence of
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45 419 this bacterium in Chernobyl soils using specific primers (Theodorakopoulos *et al.* 2013),
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47 420 suggesting that the very low relative abundance is the consequence of sequencing errors or
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49 421 PCR bias. Furthermore, *Deinococcus*-related species were not identified among the cultured
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51 422 bacteria retrieved from the soil samples (Chapon *et al.* 2012). Taken together, these results

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3 423 strongly suggest that *Deinococcus* are either absent or are in extremely low abundance in this
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5 424 environment. This may be related to the high moisture content of Chernobyl soils, whereas
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7 425 *Deinococcus* members have been shown to be well-adapted to dry or low-moisture soils
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9 426 (Fredrickson *et al.* 2004; Chanal *et al.* 2006; Theodorakopoulos *et al.* 2013; Li *et al.* 2015).

11 427 Finally, the identification of representatives of two abundant OTUs typical of trench
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13 428 microbiota (OTU 125947 and 573135) in our culture collection has yielded some interesting
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15 429 results. These isolates, related to the genera *Bradyrhizobium* and *Burkholderia*, represent
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17 430 appropriate models for assessing the impact of long-term RN exposure on bacteria. Future
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19 431 studies could focus on these isolates to help determine the mechanisms of adaptation to this
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21 432 very unusual ecological niche.
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27 434 **Conclusion**

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29 435 In this study, we demonstrated that prokaryotic communities from Chernobyl disposal
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31 436 trench T22, as well as samples collected in the proximity, share common traits with
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33 437 prokaryotic assemblages from cold environments on a global scale although a significant
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35 438 difference in diversity and community composition could be observed between the trench and
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37 439 its proximal environment. Our results indicate that the introduction of contaminated
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39 440 lignocellulosic material within trench T22 has shaped prokaryotic communities by modifying
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41 441 species composition and abundance. In this regard, TADR was the principal variable to
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43 442 influence the prokaryotic communities while still exerting a negative effect on prokaryotic
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45 443 diversity. We identified specialized phylotypes that have successfully colonized the trench,
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47 444 two of which have cultured representatives in our culture collection. Further work, such as
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49 445 shotgun metagenome sequencing and experiments on these bacterial isolates, may be helpful
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51 446 in determining the function of bacterial communities in this ecosystem.
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17
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24
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29 460 **References**

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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.

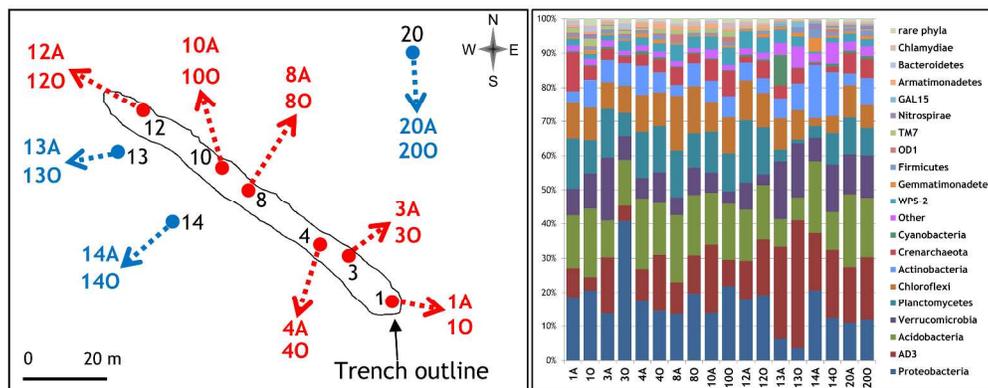


Figure 1: Location of the sampling points (left) and taxonomic profiles of the microbial communities (right), for soil samples collected in April (-A) and October (-O) 2009. The triplicate samples performed in October were combined.

1363x539mm (72 x 72 DPI)

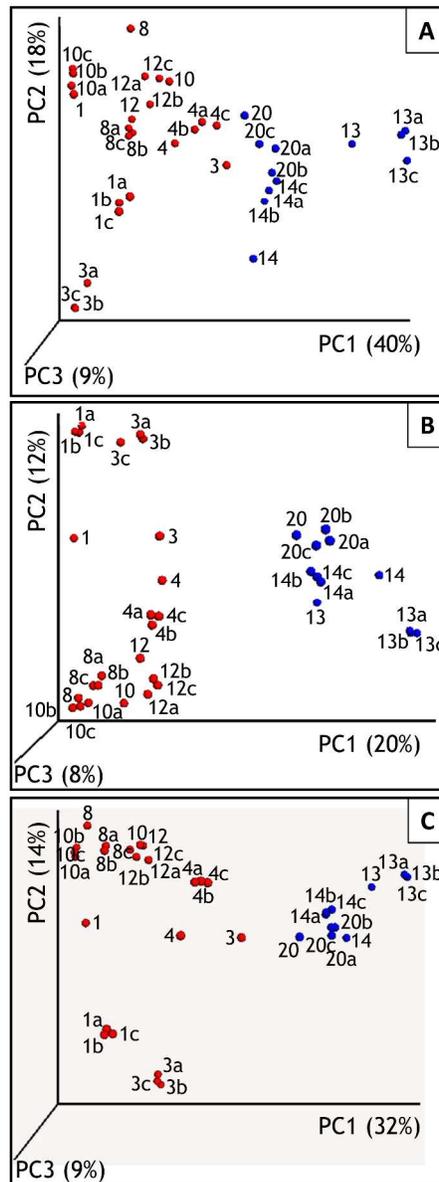


Figure 2: 3D Principal Coordinates Analysis (PCoA) plots of weighted UniFrac (A), unweighted UniFrac (B), and Bray–Curtis (C) distances between the soil samples collected in the trench (red) and in the surrounding soils (blue). Samples are identified with a number corresponding to the sampling position. To enhance readability, the samples collected in April 2009 are labelled with a single number, whereas the triplicates performed in October 2009 are labelled as Xa, Xb or Xc. The percent variation explained by the PCs is indicated on the respective axes and refers to the fraction of the total variance.

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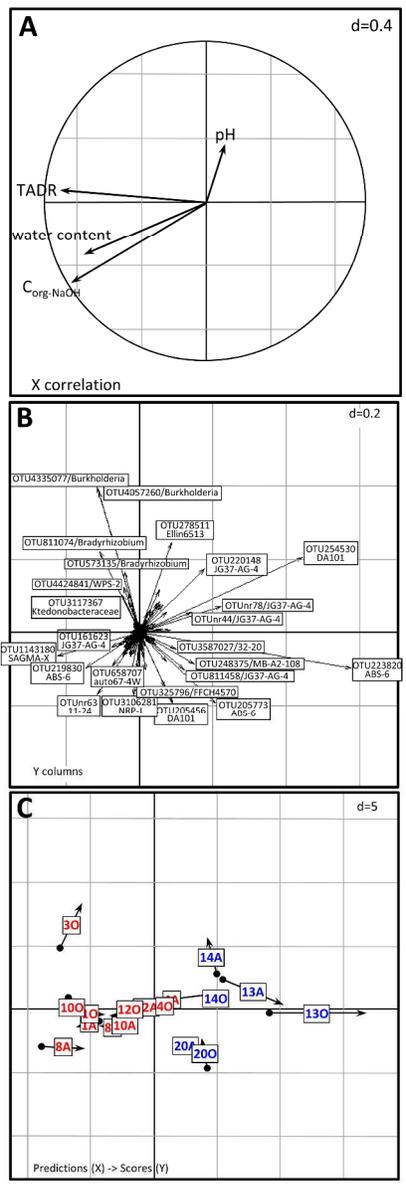


Figure 3: Multivariate redundancy analysis diagrams illustrating the relationship between the OTU-level community structure from soil samples collected in April (labelled with A) and October (labelled with O) 2009 and environmental parameters (TADR, Corg(NaOH), pH, and water content). A) correlation 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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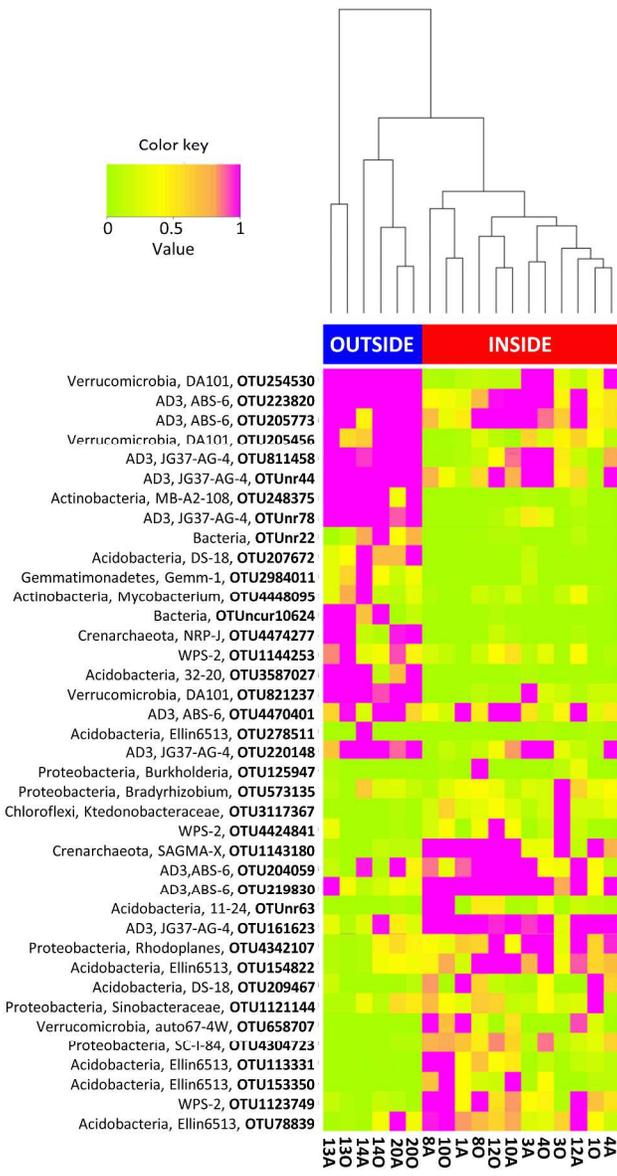


Figure 4: Cluster analysis of the soil samples and the corresponding heat map constructed with abundant OTUs (>1% relative abundance in at least one soil sample) differing significantly between the trench and the surrounding soil microbiota. Red: samples from the trench; blue: samples from the surrounding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

808x1519mm (72 x 72 DPI)

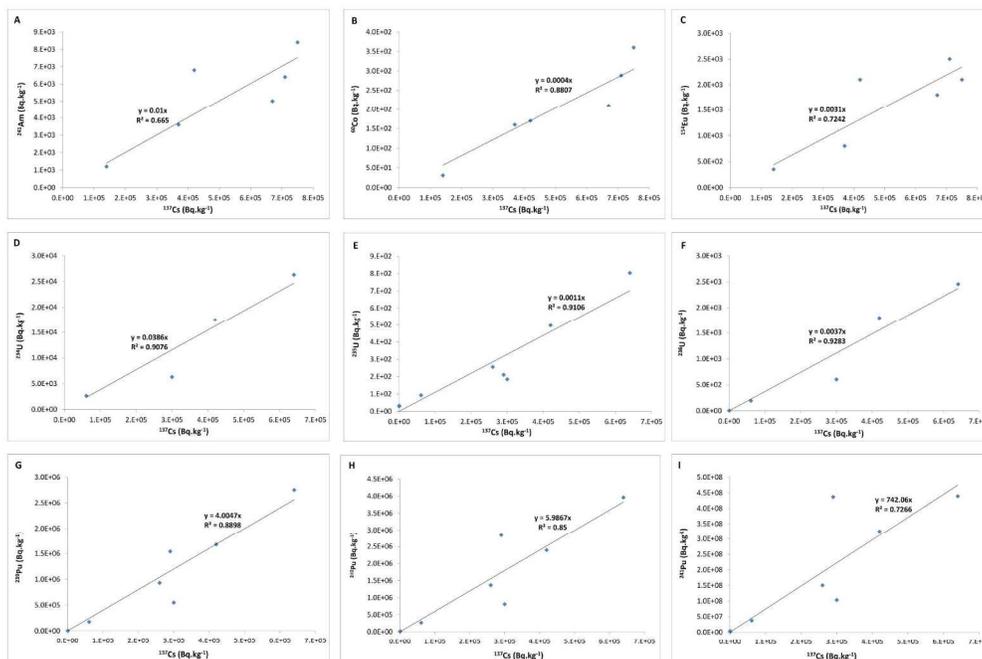


Figure S1: Relationship between measured concentrations (Bq.kg⁻¹) of ¹³⁷Cs and A) ²⁴¹Am, B) ⁶¹Co, C) ¹⁵⁴Eu, D) ²³⁴U, E) ²³⁵U, F) ²³⁶U, G) ²³⁹Pu, H) ²⁴⁰Pu and I) ²⁴¹Pu, at different soil sampling sites.

2116x1587mm (72 x 72 DPI)

review

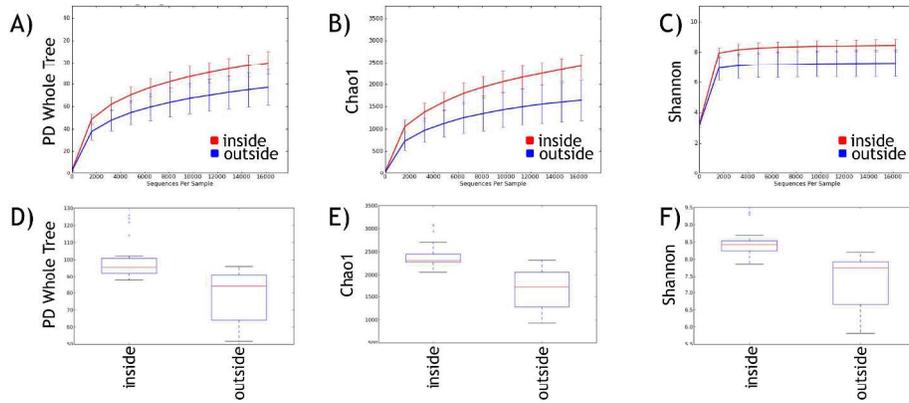


Figure S2 : Alpha rarefaction plots for Phylogenetic Distance Whole Tree (A), Chao1 (B) and Shannon (C) indices computed with the soil samples collected inside the trench (red) and outside (blue). The corresponding distance boxplots are shown in D), E) and F) respectively. The error bars show the standard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

2116x1587mm (72 x 72 DPI)

review

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.

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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;Beta proteobacteria;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;Alpha proteobacteria;Rhizobiales;Bradyrhizobia ceae;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;Alpha proteobacteria;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
nreOTU22	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

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

For Peer Review