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Research Letter

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Community structure and functional genes in radionuclide contaminated soils in Chernobyl and Fukushima

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One sentence summary: Prokaryotic community structure in soils from Chernobyl and Fukushima with high levels of radionuclide contamination clustered apart from soils with low levels of radionuclides.

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ABSTRACT

Chernobyl and Fukushima were subjected to radionuclide (RN) contamination that has led to environmental problems. In order to explore the ability of microorganisms to survive in these environments, we used a combined 16S rRNA and metagenomic approach to describe the prokaryotic community structure and metabolic potential over a gradient of RN concentrations (^{137}Cs 1680–0.4 and ^{90}Sr 209.1–1.9 kBq kg^{-1}) in soil samples. The taxonomic results showed that samples with low ^{137}Cs content (37.8–0.4 kBq kg^{-1}) from Fukushima and Chernobyl clustered together. In order to determine the effect of soil chemical parameters such as organic carbon (OC), Cesium-137 (^{137}Cs) and Strontium-90 (^{90}Sr) on the functional potential of microbial communities, multiple predictor model analysis using piecewiseSEM was carried out on Chernobyl soil metagenomes. The model identified 46 genes that were correlated to these parameters of which most have previously been described as mechanisms used by microorganisms under stress conditions. This study provides a baseline taxonomic and metagenomic dataset for Fukushima and Chernobyl, respectively, including physical and chemical characteristics. Our results pave the way for evaluating the possible RN selective pressure that might contribute to shaping microbial community structure and their functions in contaminated soils.

Keywords: 137Cs; 90Sr; radionuclides; Fukushima; Chernobyl; metagenomics; 16S rRNA

INTRODUCTION

Chernobyl and Fukushima regions were exposed to significant radionuclide (RN) pollution by fission products due to system breakdowns at their respective nuclear power stations. Considerable ecosystem studies on the effects of the sudden release of RNs were performed at the Chernobyl site, but only a few on microorganisms and the results have been contradictory.

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Chapon *et al.* (2012) and Ragon *et al.* (2011) both found that the microbial diversity of contaminated and uncontaminated soils was similar, suggesting that RN pollution did not lead to extinction of bacterial diversity. Other studies on Chernobyl reported a decrease in bacterial diversity in contaminated soils and isolated ultraviolet and H_2O_2-resistant bacteria (Romanovskaia *et al.* 1998, 1999; Zavilgelsky *et al.* 1998; Romanovskaia, Rokitko and Malashenko 2000).

A study on the gut microbiome of bank voles from Chernobyl concluded that radiation-associated bacteria had distinct inferred functional profiles, including pathways involved in degradation, assimilation and transport of carbohydrates, xenobiotics biodegradation and DNA repair (Lavrinienko *et al.* 2018). Radiation was also suggested as a selective pressure for bacteria that facilitates survival in certain environments (Ruiz-González *et al.* 2016). Microorganisms might be involved in the fate of RNs through the bioreduction of uranium (U) from a soluble to a nonsoluble immobile form, U(VI) to U(IV) (Lovley *et al.* 1991), the biosorption of U and other potentially toxic metals on bacterial membranes (Merroun *et al.* 2005), the bioaccumulation of U inside bacteria (Merroun *et al.* 2001) and the bioprecipitation of U(VI)-phosphate phases (Beazley *et al.* 2007). Most of the key microbial interactions with RNs have been studied using U as a model and little is known on the response of microorganism to ^{137}Cs and ^{90}Sr, which are major radioactive products and analogs to potassium and calcium. Theodorakopoulos *et al.* (2017) suggested that the RNs remaining in Chernobyl soil still exert an impact on prokaryotic communities. Our metagenomic approach can provide a deeper view on the genes prokaryotic communities use to overcome radiation stress conditions in soils. These metagenomic and amplicon sequencing techniques have been used to describe prokaryotic communities in metal-contaminated environments (Valenzuela *et al.* 2006; Hemme *et al.* 2010; Kumar *et al.* 2013; Gołębiewski *et al.* 2014; Yan, Luo and Zhao 2016) and should provide insight into prokaryotic community structure and functioning in RN exposed soils.

In this study, we focused on the prokaryotic community from contaminated soils in Chernobyl and Fukushima. We used a combined taxonomic and metagenomic approach to explore prokaryotic community structure and metabolic potential. We expected that the genes affected by RN and OC are related to radiation stress response, such as DNA repair, RN internalization and efflux decontamination (Makarova *et al.* 2001; Valls and Delorenzo 2002).

MATERIALS AND METHODS

Sample collection

Chernobyl soils were collected from Trench 22 (Fig. 1A) in September 2015, previously described by Bugai *et al.* (2005). The site is located 2.5 km from the Chernobyl Nuclear Power Plant and contains clean-up waste from the Red Forest and other radioactively contaminated debris generated by emergency decontamination actions from 1986 to 1987. The samples ($n = 16$, 5 replicates each a–e) were selected based on the relative cps/s activity of the soil to study community structure and gene diversity along a gradient of RN activity (Supporting Information—soil screening). Soils samples were collected from different depths (0.5 to 3, 5 m) using a standard agrochemical sampler with a cylindrical corer ($l = 6$ cm, $h = 10$ cm) and stored in sterile tubes for on-site DNA extraction (Mobio DNA extraction kit), and measurements of pH, water content, ^{137}Cs^{137}Cs, ^{90}Sr, ^{154}Eu, ^{241}Am and organic carbon (OC). Fukushima soils were collected 40 km away from the reactor in July 2014 (Fig 1. B). Two types of forest soil were sampled: a forest with mature cedars 'MC' 35 years old ($n = 3$, with 1 or 2 replicates) and another with young cedars 'YC' 15 years old ($n = 3$, with 1, 2 or 3 replicates); the first 40 cm were sampled with four replicates. These sites were described by Coppin *et al.* (2016).

RN measurements, pH, water content and organic carbon

Gamma-spectrometry of the soil samples (120 g) was performed using a low-background gamma-spectrometer with multichannel analyzer ASPEC-927 (software GammaVision 32) equipped with a passive shield and a semiconducting detector of pure germanium GEM-30 185, (EG&G ORTEC, USA) for ^{137}Cs, ^{154}Eu and ^{241}Am activity. The activity of ^{90}Sr soil samples (10 subsamples of 10 g each) after radiochemical separation from solutions was measured using scintillation beta-gamma-spectrometer SEB-01–150 or SEB-01–70 (Research and Production Enterprise 'AKP'—'Atom Komplex Prylad', Ukraine). The pH was measured with a 1:5 soil:water suspension. Two grams of soil samples were combined in a polypropylene tube with 10 mL of 18 MX water. Suspensions were shaken for 30 min, decanted and left for 2 h for atmosphere equilibration, and the pH of the supernatant was measured. To determine water content (W°), a 10 g soil sample was oven-dried at 105°C until the weight remained constant, and the change in weight was calculated. Soil NaOH-extractable organic carbon (Corg(NaOH)) was measured based on the International Humic Substances Society (IHSS) protocol as described in Coppin, Chabroullet and Martin-Garin (2006). A 0.15 g soil sample was mixed with 1.5 mL of NaOH (0.1 mol L^{-1}) and shaken overnight (Coppin, Chabroullet and Martin-Garin 2006; Chapon *et al.* 2012). After centrifugation (10 min at 10 000 g), the solution was removed, and the absorbance was measure at 280 nm (A280) (BioPhotometer, Eppendorf, Germany). The absorbance at 280 nm (A280) was calibrated to total organic C (mg L^{-1}) with humic acid solutions of known concentrations (3–60 mg L^{-1}).

16S rRNA gene sequencing

We used the Illumina workflow adapted as described in Masy *et al.* (2016) for the sequencing of 16SrRNA gene amplicons (V3 region) on the MiSeq system, Illumina, San Diego, California, 92122, USA (http://web.uri.edu/gsc/files/16s-metagenomic-library-prep-guide-15044223-b.pdf). The putative chimeric sequences were identified with ChimeraSlayer using the same Silva 108 reference set and discarded. The remaining sequences were used for closed reference OTU-picking at the 0.97 threshold with UCLUST (Edgar 2010). Resulting tables were standardized and the OTUs that represented <0.3% in a sample were discarded. The data set was compared and visualized using the R software (standard functions). The data are stored on the NCBI Sequence Read Archive (SRA) with all their attributes and details. SRA accession: PRJNA494853.

Metagenome sequencing

A total of 32 samples from Chernobyl were selected based on a gradient of RN concentrations for metagenomic sequencing using the MiSeq system. We used the Illumina Nextera XT DNA Sample Preparation kit, Illumina, USA following the supplier's

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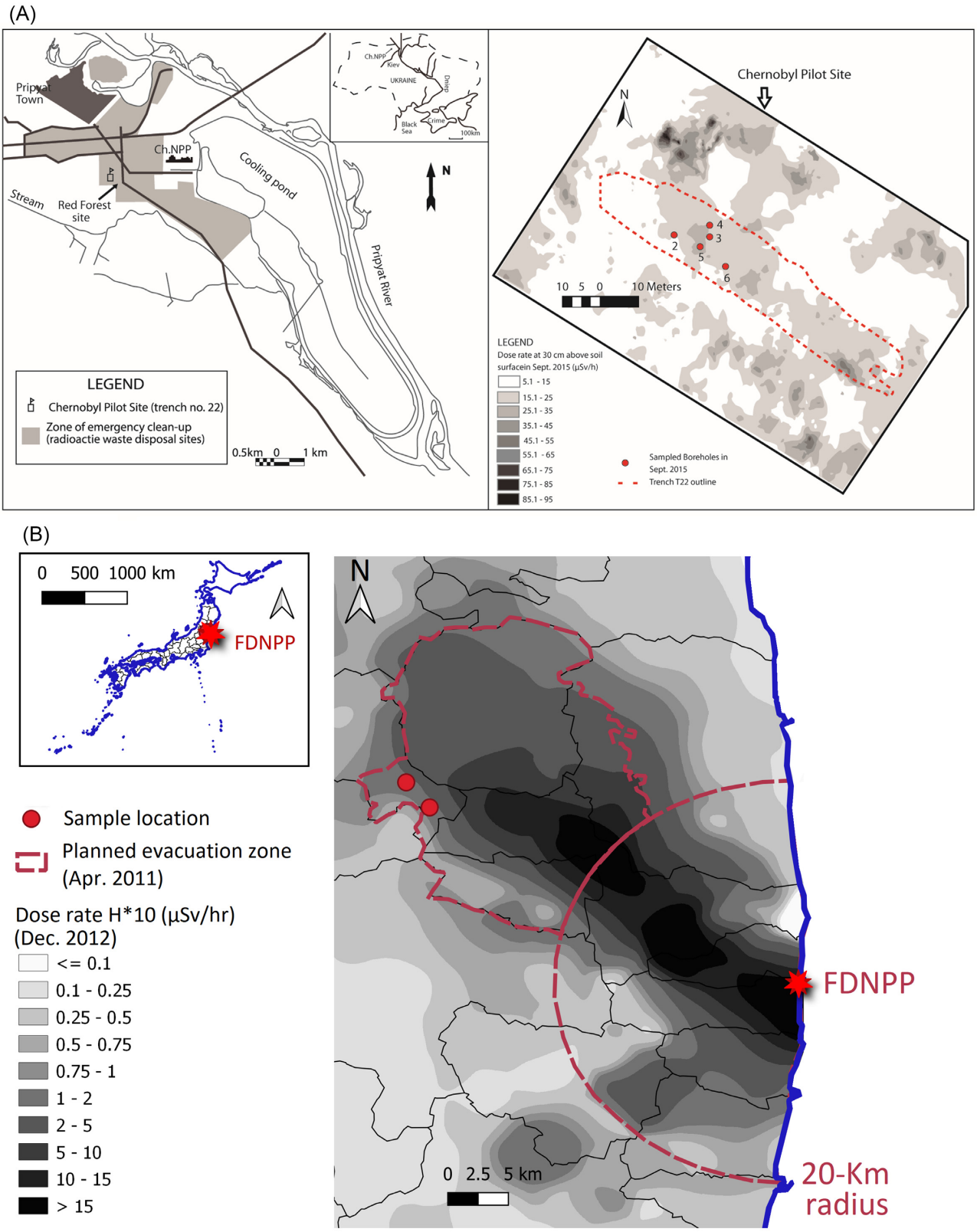


Figure 1 (A) Location trench 22 at 2,5 km from the Chernobyl nuclear power plant. (B) Fukushima soil sample location at 40 km away from the reactor, FDNPP= Fukushima-Daiichi nuclear power plant. Dose rates taken from JAEA https://emdb.jaea.go.jp/emdb/en/ . Evacuation zone perimeter taken from Fukushima prefecture website https://embd.jaea.go.jp/emdb/en/

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workflow. The sequences obtained were merged and then discarded if they contained a Q < 20 or with a length <50 bp using the UCLUST program (Edgar 2010). DIAMOND was used to align DNA sequence reads to the NCBI-NR protein reference database using the default settings (Buchfink, Xie and Huson 2015), and then the duplicate records from BLAST output file were removed, and only the 'best' records were kept (sorted by smallest e-value and then biggest % identity) using the script by Jennifer Meneghin (Supporting Information—blast script). Result summary tables were standardized and genes with relative abundancies >0.02% in a sample were analyzed. The data are stored on SRA with all their attributes and details. SRA accession: PRJNA494853.

R-analysis of taxonomic and metagenomic data

Fukushima and Chernobyl taxonomic as well as Chernobyl metagenomic dendrograms were constructed with the Pvclust package (Suzuki and Shimodaira 2006). Venn diagrams were constructed with the Venn Diagram package (Chen and Boutros 2011). The Supporting Information table shows the samples used for building the taxonomic dendrograms. Chernobyl data has 16 samples, with five replicates (a–e), which were collected from five different boreholes (2–6; Fig. A) and each borehole has at least three different depths sampled. Additionally, these were analyzed together with six Fukushima's samples, each one with 1, 2 or 3 replicates, corresponding to soils with young cedars or mature cedars. A total of 93 samples were used to create the taxonomic dendrogram.

The gene abundance outputs from the Chernobyl metagenomic dataset were used for the Chernobyl dendrogram. These tests were elaborated from a selection of the 16 samples, with 1, 2 or 3 replicates, which were collected from five different boreholes (2–6) and each borehole has at least three depths sampled (Supporting Information—metagenomic data set). The gene abundance data were then tested as response variables for ^{137}Cs, ^{90}Sr and OC with piecewiseSEM (Lefcheck 2016). This methodology allows (i) to separate the effects of correlated drivers (Grace 2006), and (ii) to account for spatial nonindependence by using sampling location as random effect in our analyses. These samples had four types of variables: OC, ^{137}Cs, ^{90}Sr, and the 208 genes found (Supporting Information—metagenomic data set). The model pathway tested was the response of ^{137}Cs and ^{90}Sr to OC predictor, and the response of each gene 'i' independently from the 208 found to ^{90}Sr and ^{137}Cs predictors. A correlated error between ^{90}Sr and ^{137}Cs that reflects the relation between these two variables, which is not causal but rather driven by another parameter, was also added. In order to validate the model, where OC is a predictor of both ^{137}Cs and ^{90}Sr, a direct separation test was performed. This test evaluates the independence of indirect pathways such as OC as a direct predictor of gene abundance. All packages are available in R.

RESULTS

Physiochemistry of Chernobyl and Fukushima sites

Chernobyl and Fukushima samples had different physicochemical characteristics. In Chernobyl, sample concentrations ranged from 4.21 to 1680 for ^{137}Cs, 1.9 to 209.1 for ^{90}Sr, 0.06 to 35 for ^{241}Am and 0.048 to 3600 for ^{154}Eu (all values in kBq kg^{-1}). Organic carbon ranged from 0.25 to 3.82 mg g^{-1}, pH ranged from 5.7 to 7.1, and depth ranged from 0.5 to 3.5 m. For Fukushima samples, we measured ^{137}Cs concentrations that ranged from 0.4 to 34.43, pH of 5.7 and 5.8, and depth from 0.03 to 0.2 m. The Supporting Information table summarizes the data collected at the sites. The strongest correlations detected in Chernobyl samples were between ^{90}Sr and ^{137}Cs (R^2 0.77), followed by OC and ^{137}Cs (R^2 0.67).

Community structure in Chernobyl and Fukushima

A similarity dendrogram using the relative abundance of the observed OTUs was constructed from Chernobyl and Fukushima samples (Fig. 2A). The replicates clustered together and the samples within each cluster shared several physicochemical characteristics. The samples in the first OTU cluster had the same OC content, the samples in the second cluster similar levels of ^{90}Sr, but not the same levels in other RNs nor in OC content. The samples belonging to the fifth cluster all shared the same low levels of RNs. The Fukushima samples clustered with Chernobyl samples that had relative low RN content. The Fukushima cluster was separated into two sub clusters that matched with the types of forest soil sampled. The Venn diagram shows that both sites share 417 OTUs (Supporting Information—supplementary material 1). Some of the OTUs found in Chernobyl and Fukushima samples were Rhodospirillales, Acetobacteraceae and Candidatus Solibacter, Acidimicrobiales, Verrumicrobia, Bryobacter, Rhizobiales, Proteobacteria, Acidobacteria (subgroups 1 and 2), Ktedonobacterales, Chloroflexi and Thermotogales. The most abundant OTUs across the samples were Actinobacteria, Acidobacteria, and Proteobacteria (Fig. 3). The Shannon diversity index for Chernobyl varied between 2.38 and 2.97 and between 3.07 and 3.6 for Fukushima. Less contaminated samples, which were taken near the surface, had a relatively higher diversity.

Chernobyl metagenomes

The metagenomic datasets (n = 32) contained an average of 13 458 sequences with an average length of 230 nucleotides. A dendrogram (Fig. 2B) was built with the annotated genes that represented >0.02% relative abundance in each sample. One cluster in the dendrogram shared the same level of organic carbon for all the samples. Another cluster shared the same level of ^{90}Sr concentrations, and yet another cluster represented samples with low levels of RNs. In clusters 2 and 3, RN concentrations were higher in samples close to the surface. The most abundant genes are listed in supplementary material 2 (Supporting Information). The samples with a high content of RNs had a higher relative abundance of genes related to transmembrane transport, metal transporters, regulation of transcription, DNA repair after damage, DNA mismatch repair and protein secretion. For the samples with low RN content, genes related to cell membrane components, multisensor signals, potassium transport, transporter activities, nucleotide catabolic processes and general carbohydrate metabolic processes were the most abundant.

Impact of organic carbon, ^{90}Sr and ^{137}Cs on gene abundance

In order to separate the effects of correlated drivers (Grace 2006), and to account for spatial nonindependence by using sampling location as a random effect in our analyses, piecewiseSEM (Lefcheck 2016) methodology was used. The path OC→^{137}Cs, and^{90}Sr → Gene 'i' (where i refers to one of the 208 genes found) was tested. The OC is closely related to RNs, therefore we set two directional hypothesis paths from OC to ^{137}Cs, and another from OC to ^{90}Sr. Both of these RNs are related to each other, hence, their correlated errors were added to the model to link

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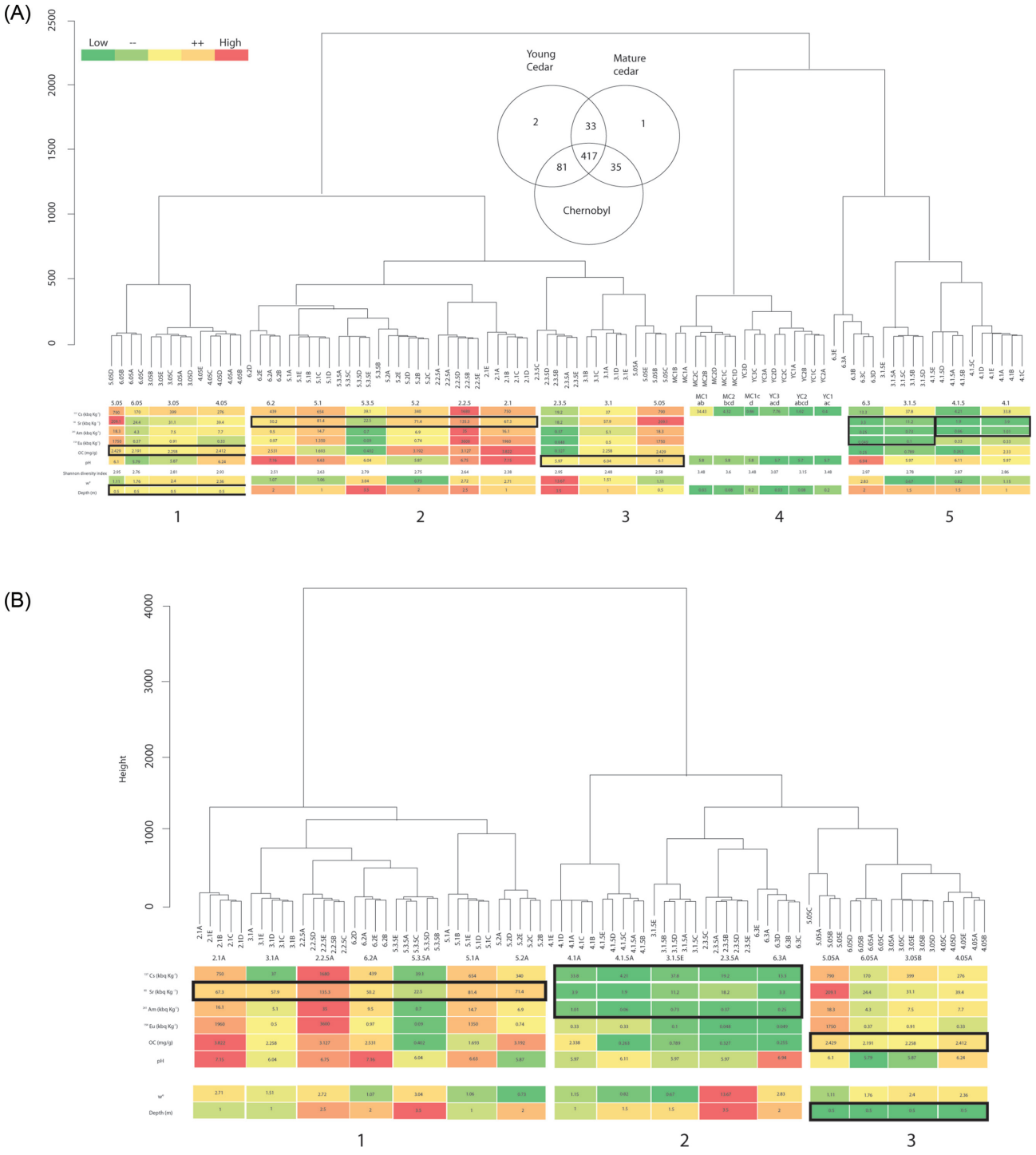


Figure 2. (A) Hierarchical clustering of Chernobyl soil samples based on the sequence similarity of the V3 region of the gene that codes for the 16S rRNA. Dendrogram was built using the OTUs for each Chernobyl and Fukushima soil sample. Physicochemical characteristics of samples are shown below the dendrogram, the concentration of radionuclides is in kBq kg^{-1}, organic carbon in mg/g and depth in meters. The color scale corresponds to the values of each parameter: the higher the redder and the lower the greener. ^{137}Cs 1680–0.4 kBq kg^{-1}, ^{90}Sr 209.1–1.9 kBq kg^{-1}, ^{241}Am 35–0.06 kBq kg^{-1}, ^{154}Eu 3600–0.048 kBq kg^{-1}, OC 3.82–0.26 mg g^{-1}, pH 7.19–5.70, Shannon diversity index 3.6–2.3, humidity (w°) 13.67–0.67 and Depth 3.50–0.03 m. Venn diagram for shared OTUs in Chernobyl and Fukushima. Physicochemical similarities within the cluster are outlined. **(B)** Hierarchical clustering of Chernobyl based on metagenomic data. Dendrogram was built using the gene abundances for each Chernobyl sample. Physicochemical characteristics of samples are shown below the dendrogram, ^{137}Cs 1680–4.21 kBq kg^{-1}, ^{90}Sr 209.1–1.9 kBq kg^{-1}, ^{241}Am 35–0.06 kBq kg^{-1}, ^{154}Eu 3600–0.048 kBq kg^{-1}, OC 3.82–0.26 mg g^{-1}, pH 7.19–5.70, humidity (w°) 13.67–0.67 and Depth 3.50–0.03 m. The color scale corresponds to the values of each parameter, the higher the redder and the lower the greener. Physicochemical similarities within the cluster are outlined.

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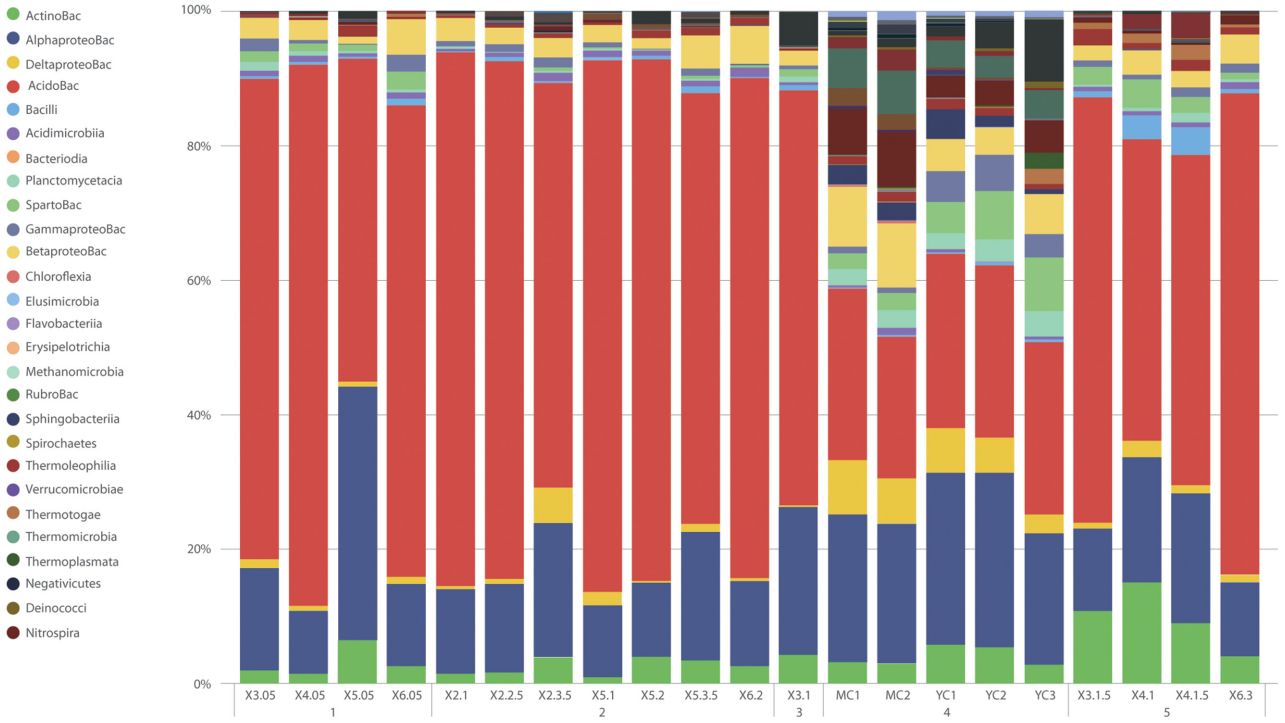


Figure 3. Taxonomic profiles of the microbial communities for Fukushima and Chernobyl based on relative abundance. The replicate samples (a–e) were combined.

them together. Our results show the positive response of ^{137}Cs (Std. Estimate 0.62***) and of ^{90}Sr (0.51**) on the OC predictor. The standard estimates found for ^{90}Sr and ^{137}Cs on gene abundance are listed in Table 1. The listed 46 genes (Table 1) correspond to the models that showed statistically significant data. Based on the modeling results, OC does not have a direct influence on the gene abundance (test of D-separation $P > 0.05$). In the model, gene abundance was mainly related to the presence of ^{90}Sr, rather than ^{137}Cs.

DISCUSSION

The results from both taxonomic and metagenomic approaches suggest that functional and community structure data cluster similarly based on soil physicochemical characteristics such as OC, medium Sr or low RNs levels. The RNs levels on the metagenomic dendrogram seem to be related to depth, with higher RN concentrations found close to the surface. This might be a result of RN pumping by plants, leading to increased surface concentrations (Bugai *et al.* 2012). The taxonomic dendrogram clustered all the samples that had low RN concentrations, including the Fukushima samples, which grouped with the low contaminated Chernobyl samples. Similar clusters were found for Chernobyl metagenomes. The relatively uncontaminated soil samples were more similar to each other than to contaminated samples. Thus, the differences in OTU and gene abundances could be due to differences in RN contamination levels among the sample groups. If these differences were due to microorganisms that are tolerant or sensitive to RNs, then the potential complexity due to other physical or chemical parameters, such as organic carbon, which is often co-correlated to RN concentrations (Szenknect 2003), would be minimized. Further research and comparison with soils that have not been exposed to RN pollution are needed to determine whether RNs could impact the community structure and their functionality as the clustering suggests.

The most detected bacterial phyla in our study are similar to those observed by Theodorakopoulos *et al.* (2017) collected from the same site. However, in our study, we found that the diversity index was higher outside the trench than inside the trench, while the opposite was reported by Theodorakopoulos *et al.* (2017). In our study, the Shannon diversity varied between 2.38 and 2.97 for the Chernobyl samples and between 3.07 and 3.6 for Fukushima. The diversity in Chernobyl was lower than in Fukushima. This might be related to spatial and chemical parameters, since Fukushima soils are less contaminated, and sampling occurred at the surface of the soil.

Many of the organisms detected in both soils have been described as tolerant to extreme environmental conditions such as Rhodospirillales, Acetobacteraceae and Candidatus Solibacter (Challacombe *et al.* 2011; Gołębiewski *et al.* 2014) as well as to metals such as Acidimicrobiales (Baker and Banfield 2003; Haferburg and Kothe 2007; Mendez, Neilson and Maier 2008). Acetobacteraceae, relatives of Rhodospirillaceae, have been studied for their capacity to reduce selenium, tellurium, rhodium and europium oxyanions (Moore and Kaplan 1994). Verrumicrobia have previously been isolated from uranium deposits (Mondani *et al.* 2011) and from numerous mercury and pesticide contaminated environments (Vishnivetskaya *et al.* 2011). Acidobacteria have been observed in Chernobyl T22, (Theodorakopoulos *et al.* 2017), as well as in uraniferous soils (Mondani *et al.* 2011). Bryobacter has been described to withstand metal-contaminated, acidic and other extreme environments contaminated with uranium (Barns *et al.* 2007). Rhizobiales and Proteobacteria have been reported in coastal areas contaminated by potentially toxic metals possibly containing strontium and cesium (Quero *et al.* 2015). The presence of these organisms would suggest some form of selection for tolerant organisms. However,

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Table 1. Genes identified by the piecewiseSEM model. The table displays the gene identifier (GI) for the NCBI data base (https://www.ncbi.nlm.nih.gov/protein/). The standard estimate for the ^{90}Sr and ^{137}Cs on gene response is also given, NS stands for not statistically significant.

GI	Definition	^{90}Sr→gene	^{137}Cs→gene
503332345	IS110 family transposase [Terriglobus saanensis]	0.5215*	NS
575457053	Amidohydrolase 3 [Gemmatirosa kalamazoonesis]	0.5215*	NS
575460631	Tetratricopeptide TPR_1 repeat-containing protein [Gemmatirosa kalamazoonesis]	0.5678***	NS
665505553	putative Peptidase S8 and S53 subtilisin kexin sedolisin [Tetrasphaera japonica T1-X7]	0.6328**	NS
522209400	response regulator [Acidobacteriaceae bacterium KBS 89]	0.4718*	NS
499841612	ABC transporter permease [Candidatus Koribacter versatilis]	0.6598**	NS
499843835	DEAD/DEAH box helicase [Candidatus Koribacter versatilis]	0.5359*	NS
94553466	serine/threonine protein kinase [Candidatus Koribacter versatilis Ellin345]	0.5856*	NS
505061075	PAS domain S-box protein [Singulisphaera acidiphila]	0.6481**	NS
522210291	DUF2029 domain-containing protein [Acidobacteriaceae bacterium KBS 96]	0.5723*	NS
499843698	phospho-N-acetylmuramoyl-pentapeptide-transferase [Candidatus Koribacter versatilis]	0.6274**	NS
517239079	hypothetical protein [Hoeflea sp. 108]	0.5177*	NS
116227723	acriflavin resistance protein [Candidatus Solibacter usitatus Ellin6076]	0.4814*	NS
500008383	ABC transporter ATP-binding protein [Candidatus Solibacter usitatus]	0.6823**	NS
504030943	sugar transporter [Granulicella mallensis]	0.5183*	NS
116226075	serine/threonine protein kinase with TPR repeats [Candidatus Solibacter usitatus Ellin6076]	0.6597**	NS
499844739	DNA-directed RNA polymerase subunit beta [Candidatus Koribacter versatilis]	0.6245**	NS
498272011	hypothetical protein [Schlesneria paludicola]	0.5544*	NS
499844359	RNA polymerase sigma factor RpoD [Candidatus Koribacter versatilis]	0.5744*	NS
522212461	hypothetical protein [Acidobacteriaceae bacterium KBS 96]	0.5603*	NS
499844136	multidrug efflux RND transporter permease subunit [Candidatus Koribacter versatilis]	0.7610**	−0.5173*
522201728	GntR family transcriptional regulator [Acidobacteriaceae bacterium KBS 83]	0.7090**	−0.4722*
769160203	2-oxoglutarate dehydrogenase E1 component [Acidobacterium ailaaui]	NS	0.4848*
499842742	hypothetical protein [Candidatus Koribacter versatilis]	0.5265*	NS
297552190	K + -transporting ATPase. B subunit [Ktedonobacter racemifer DSM 44 963]	0.5773*	NS
355340847	putative peptidase M14-like [uncultured Acidobacteria bacterium]	0.7168**	NS
94552434	multisensor signal transduction histidine kinase [Candidatus Koribacter versatilis Ellin345]	0.5161*	NS
499843492	bifunctional homocysteine S-methyltransferase/methylenetetrahydrofolate reductase [Candidatus Koribacter versatilis]	0.7991 ***	−0.5205*
671514150	magnesium-translocating P-type ATPase [Bryobacter aggregatus]	NS	0.5035*
500002211	SAM-dependent methyltransferase [Candidatus Solibacter usitatus]	NS	0.5646*
503344245	TonB-dependent receptor [Granulicella tundricola]	0.7118**	−0.5127*
522207602	tetratricopeptide repeat protein [Acidobacteriaceae bacterium KBS 89]	0.7080**	−0.4967*
522215542	efflux RND transporter permease subunit [Acidobacteriaceae bacterium KBS 96]	0.5174*	NS
504873984	hypothetical protein [Pseudomonas sp. K-62]	0.5965*	NS
575458049	peptidase M14 carboxypeptidase A [Gemmatirosa kalamazoonesis]	NS	0.5956**
499840321	outer membrane protein assembly factor BamA [Candidatus Koribacter versatilis]	0.6548**	NS
765025554	transposase [Bradyrhizobium sp. LTSPM299]	1.2768 ***	−0.7365 ***
522201791	hypothetical protein [Acidobacteriaceae bacterium KBS 83]	1.3018 ***	−0.8164 ***
94553468	serine/threonine protein kinase [Candidatus Koribacter versatilis Ellin345]	0.5620*	−0.5666*
499840205	valine–tRNA ligase [Candidatus Koribacter versatilis]	0.4994*	NS
499844447	IS110 family transposase [Candidatus Koribacter versatilis]	NS	0.5209*
499842065	Type II and III secretion system protein [Candidatus Koribacter versatilis]	0.5334*	NS
575458384	acriflavin resistance protein [Gemmatirosa kalamazoonesis]	NS	0.5321*
737373746	integrase [Edaphobacter aggregans]	0.6332**	NS
746991949	Carboxypeptidase regulatory-like domain [Pyrinomonas methylaliphatogenes]	0.8901 ***	NS
495186936	thiolase family protein [Ktedonobacter racemifer]	0.6501**	−0.4955*

Acidobacteria, Chloroflexi and Thermotogales have also been reported as sensitive to potentially toxic metals (Llanos *et al.* 2000; Chen *et al.* 2014; Li *et al.* 2015), highlighting the difficulty in drawing inferences based on taxonomy.

The metagenomic approach provided a deeper insight on the types of mechanisms that might be required for inhabiting RN exposed environments. In order to identify those whose abundance was related to RN and OC presence, we used piecewiseSEM modeling. This type of structural equation modeling can circumvent restrictions of sample size (Shipley 2000). The functions encoded by genes that were identified by the model as being correlated to RNs and OC (Table 1) were related to 'decontamination and radiation repair', 'information storage and processing', 'cellular processes' and 'signaling and metabolism'. Among the signaling related sequences, we found 'TonB-dependent receptors', which have been linked to the response to metals such as chromium, cadmium and uranium (Hu *et al.* 2005); 'tetratricopeptide repeats (TPR)' as well as 'serine/threonine protein kinase', which play a role in Deinococcus signaling (Makarova *et al.* 2001); 'PAS domains', which detect changes in the electron transport system (Taylor and Zhulin 1999) and 'response regulator', suggested as responsible for radiation tolerance (Song *et al.* 2013). Other statistically significant sequences were the 'RND protein family', involved in transfer of

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hydrophobic compounds, tolerance to potentially toxic metals, and nodulation factors (Nies 2003); the 'efflux ABC transporter inner membrane', which is related to a number of biological processes including uptake of nutrients, protection against xenobiotics and stress response (Thekkiniath, Ravirala and San Francisco 2016) and several others. Many of the functions encoded by these genes seem to be related to not only stress and metal tolerance but also radiation tolerance.

Our model identified several genes associated to toxic metal contamination (Thompson *et al*. 2010) like 'sigma factors' known to provide effective mechanisms for regulating bacterial stress responses (Boor 2006) and 'multisensor signal transduction histidine kinase' involved in a variety of responses to the extracellular environment (Pirrung 1999). The model also identified 'magnesium-translocating P-type ATPase', a large and varied family of trans membrane proteins that enable prokaryotes to withstand high concentrations of potentially toxic metals (Mathur 2017). The gene abundance of insertion sequences 'IS110', mobile elements that provide the host with adaptive strategies to withstand environmental challenges (Vandecraen *et al*. 2017), were also linked to RN concentrations by our model. Other genes included the 'outer membrane protein assembly factor BamA' involved in assembly and insertion of beta-barrel proteins into the outer membrane responsible for the motility, signaling, survival, and nutrient uptake of the bacteria; and the 'homocysteine Methyltransferase' enabling the microbial organisms to tolerate high concentrations of selenium in the environment (Ying *et al*. 2015). Our model also identified genes that have previously been shown to be highly expressed in four radiation-resistant bacteria, *Deinococcus geothermalis, Deinococcus radiodurans, Kineococcus radiotolerans and Rubrobacter xylanophilus* (Gao *et al*. 2009). These included 'putative Peptidase S8 and S53 subtilisin kexin sedolisin'; 'DEAD/DEAH box helicase' involved in information storage and processing; 'extracellular solute binding protein family 5' involved in amino acid transport and metabolism; and 'DNA-directed RNA polymerase subunit beta' involved in translation factors.

Our results suggest that prokaryotic communities in soils with high RN concentrations have functional profiles that would allow them to cope with the RN pollution. The abundance of some of the genes that encode these functions was also linked to the RN and OC concentrations in the soil. However, these results should be confirmed in further experiments with more replicates, which is one of the limitations of this study. In fact, the lack of replicates could influence the direct separation test, suggesting that the model fits (Grace, Scheiner and Schoolmaster 2015; Lefcheck 2016). The next step would be to test if increased RN exposure affected community structure and function similarly to that observed in Chernobyl soil samples under controlled laboratory conditions. It would also be interesting to distinguish the chemical and the radiological toxicity of RN since not only genes involved in DNA repair are correlated with the RN but also genes involved in metal tolerance mechanisms.

CONCLUSION

We have described the prokaryotic community present in contaminated soils from Chernobyl and Fukushima, as well as the genes that are potentially used to tolerate high radionuclide activities, such as those found in Trench 22 at Chernobyl. We observed the same sample clusters (relatively contaminated vs low contaminated samples) using either taxonomic or metagenomic approaches. The soil samples containing low levels of RNs clustered apart from those with high RN levels, which could further indicate RN impact on community structure. The effects of OC, ^{137}Cs and ^{90}Sr were tested using piecewiseSEM. Our study proposes different genes/mechanisms that are influenced by these parameters. Further research is needed to test whether RNs constitute a selective pressure that can shape prokaryotic community structure. Our findings may be helpful for designing solutions to treat fission-contaminated soil and pave the way for understanding the roles of new proteins that have yet to be related to pollution. To our knowledge, this is the first study that uses a metagenomic approach to gain insight on the prokaryotic community response to environmentally relevant levels of RN pollution.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSLE* online.

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