Resident bacterial populations mediating biogeochemical dynamics in a Uranium roll front deposit

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Abstract

Background: Uranium-mineralized sandy aquifer, planned for a mining by in situ recovery (U ISR), harbors a reservoir of bacterial life that may influence the biogeochemical cycles surrounding the Uranium roll front deposits. Since microorganisms are likely to play an important role at all stages of U ISR, a better knowledge of the resident bacteria before any ISR actuations is essential to face environmental quality assessment. The focus here was made on the characterization of the resident microbiome of an aquifer surrounding uranium roll-front deposit that is part of an ISR facility project at Zoovch Ovoo (Mongolia).

Water samples were collected following the natural redox zonation inherited in the aquifer, including the native mineralized orebody, and both the upstream and downstream compartments.

Results: An imposed chemical zonation for all sensitive redox elements through the roll-front system was observed in all water samples. High-throughput sequencing showed that the bacterial community structure was shaped by the redox gradient and the oxygen availability. Several interesting bacteria were observed including sulphate-reducing (e.g. Desulfovibrio, Nitrospira), iron-reducing (e.g. Gallionella, Sideroxydans) iron-oxidizing (e.g. Rhodobacter, Albidiferax, Ferribacterium), and nitrate-reducing bacteria (e.g. Pseudomonas, Aquabacterium), which may be also involved in metal reduction (e.g. Desulfovibrio, Ferribacterium, Pseudomonas, Albidiferax, Caulobacter, Zooglea). The taxa residing in each aquifer compartment followed a strong redox zonation differentiation, although as a whole the population of each water section seems to define an ecologically functional ecosystem containing suitable microorganisms that are probably prone to promote the remediation of the acidified aquifer by natural attenuation. Co-occurrence patterns confirmed a strong correlations among the bacterial genera suggesting either a shared and preferred environmental conditions or the performance of functions similar or
complementary to each other.

Conclusions Assessing the composition, and structure of the resident bacterial communities is a prerequisite for understanding the natural attenuation and predicting bacterial input in the improvement of ISR efficiency.

Background

Naturally occurring Uranium (U) is used in several industrial applications [1]. Mining of this uranium has been performed by several techniques, among which open pit or underground mines were the most common during the early years of uranium extractions. The use of each approach actually depends on the localization of the ore and also on the uranium content that must be between 0.1% and 0.2% [2]. From low grade ores (<0.1%), mining of uranium is based on utilizing In Situ Recovery (U ISR), one of the most economical strategies that accounts for almost half of the world’s uranium production [3,4]. This strategy consists in recovering uranium by circulating acidic or alkaline extraction solution into the mineralized aquifer, using injection and recovery wells, through which the uranium-bearing solution is pumped to the surface for further processing [5]. The type of solution used as leaching reactant is mainly dependent on the mineralogical (carbonate content) and geochemical properties of the host rock. Such mining technique is mainly applied to sandstone-hosted roll-front uranium deposits localized in confined aquifers preferably below the water table [6]. The ore deposits occur within a redox barrier in a diffuse boundary delineating a reducing domain enriched in organic matter and sulfides on the down-gradient side (downstream compartment), and an oxidizing domain where sediments are usually oxidized on the up-gradient side (upstream compartment). Uranium being insoluble under reducing conditions, precipitates in this environment as uraninite and coffinite [6–8].

ISR is considered beneficial for many advantages involving no impact on the landscape, no
de-watering of groundwater system above or around the deposit and a minimum distortion of the hydrological/environmental system [3]. However, the main challenges with this technology are 1) the incapacity to recover all of the U in the ore zone, and most importantly, 2) the complications in restoring groundwater to baseline conditions and the mined host rock to chemically reducing conditions able to immobilize any residual U or other contaminants. During ISR mining, acidification of groundwater could lead to mineral dissolution and heavy metal/radionuclide mobilization, which have the potential to leach into the environment contaminating adjacent aquifers [9,10]. These potential aquifer contaminations call for the urgent need to develop in situ remediation technologies for the removal of uranium released [11,12]. As the main focus is succeeding in the re-establishment of water pre-existing conditions, restoration of the mined aquifer following ISR mining is typically conducted by using groundwater sweep, reverse osmosis, reducing agents (such as H$_2$S), bioremediation (stimulating metal reducing bacteria) and/or natural attenuation (NA) [5,13–15]. The latter can be envisaged as a possible remediation strategy, and in fact, while considered as the prime groundwater restoration technique, it is often chosen as a cost- and labour-efficient strategy in many ISR operations [3]. NA is commonly used to reduce the concentration of dissolved contaminants by different processes including physical (e.g.: dilution), chemical (e.g.: precipitation), physico-chemical (e.g.: sorption, ion exchange), and microbial (e.g.: reduction, biomineralization) processes [10,13].

One point of U ISR that has remained largely overlooked, and yet might help mitigate some of the issues mentioned above as well as improve ISR efficiency, is the monitoring of the naturally occurring aquifer microbiome. It is well known that bacteria are key organisms in many elemental biogeochemical cycles on earth (e.g. carbon, nitrogen, and sulphur cycles), and strongly influence the mobility of a wide range of metals (e.g., iron,
manganese, copper and U) [16]. Under ISR conditions, the presence of key bacteria could partially govern the return to the pristine original biogeochemical conditions. Indeed, they could reduce and re-precipitate contaminants to an insoluble form through natural metabolic pathways while increasing their proliferation by using several amended electron donors [17,18]. Hence, the microbiome of the native aquifer may very likely play an important role at all stages of U ISR. Bioremediation may thus be considered as an alternative for biorestitution of post-ISR aquifer due mainly to several advantages such as shorter restoration periods, lower costs and high potential for a more effective restoration [15]. In fact, this strategy has been tested in many aquifer systems with some success [12,17,19]. Many field studies have demonstrated that bacterial mediation is often required to enzymatically catalyze uranium reduction in natural environmental systems even in the presence of Fe(II) and sulfide (i.e., abiotic uranium reduction is negligible). From this concept, bioremediation may help the system to return to pre-ISR redox conditions characterized by a very low dissolved oxygen that thermodynamically favours low contaminant concentrations. However, data on water chemistry and bacterial community composition in roll-front context is relatively rare in the literature [20,21]. Water chemistry in ISR-prone aquifers is mainly governed by classical water/rock equilibria (solubility, sorption), while redox conditions are induced by biogeochemical reactions involving both water/rock equilibria and bacterial activities, especially when redox contrasts are noticed and the water temperature is below 100 °C. Therefore it is necessary to accurately determine hydrological, chemical, and especially, bacterial composition relative to original groundwater and their potential under roll-front relevant conditions to influence the constituent concentrations before ISR actuations. All of these point to the importance of assessing the presence of bacterial communities, prior mining operations, in order to verify to what extent the hydrochemistry is governed by such key
bacterial players, leading in turn to the verification of natural attenuation or bioremediation hypotheses.

In this study, a deep 16S rRNA gene sequencing was conducted to characterize the bacterial communities present within groundwater collected from a uranium roll-front deposit planned to be mined by acidic ISR in Mongolia (Zoovch Ovoo) (Fig. 1). Beside the description of abundance, diversity, distribution, and co-occurrence of bacteria present in the aquifer zones, accurate analyses of the water geochemical characteristics were performed. While distinct abiotic features may be sufficiently recovered by groundwater monitoring, this study points out the importance of naturally occurring microorganisms and their potential to neutralize the acid employed at the ISR site. Thus, the complete description of the groundwater bacterial structure helps to improve the understanding of the natural attenuation process, and to predict the efficacy of remediation strategies. Finally, this will also enable a better understanding of the influence of the acidification process on the water bacterial community and consequently on the biogeochemical cycling, allowing thus an effective evaluation and prevention of environmental risks at such U ISR sites.

Results

Water chemistry

The electrical balance was assessed for every sample. All of them exhibit values lower than 5% aiming at using the total analyses for comparison and discussion of the results. Chemical analyses of the different water samples involved in this study are represented in Table 1; complete water chemistry analyses are presented in Table SI-1 (Additional file 1). Concentrations of several trace elements (PO$_4$, Al, Co, Cu, Ni, Pb, V, Zn, Re, W) are below detection limits in all studied samples (Table SI-1) and are therefore not discussed here.
All the waters exhibit Na + K—Cl—SO₄ facies, with a relatively high electrical conductivity EC (ranging from 3.79 to 6.81 mS.cm⁻¹) and circumneutral pH values.

Two grids of lecture regarding the water chemistry can be proposed and superimposed: i) the one governed by the contrast in salinity, mainly the Na and Cl concentrations, with higher values observed downstream the roll front [22,23]. This trend may be explained by the hydrogeology of the aquifer typical of an arid endorheic basin, as described earlier by Grizard et al. [24]; ii) the second is driven by the roll front chemistry inducing a redox contrast as usually proposed in such geochemical context, even if such literature is often limited by the lack of data [25]. Here, a focus will be made on this second aspect. As the salinity gradient tends to fade the redox contrast (Fig. 2), a correction using EC was applied to the reported concentrations (see Supporting information S1).

As expected in such geochemical system, a strong contrast in Eh was observed according to the location of the sampling point. Hence, an oxidizing signature characterized the samples PZOV_0013, and PZOV_0001 (ORP values reaching 379.1 mV/SHE), which were the only oxygenated samples (6.73 and 3.81 mg/L). The water in this aquifer compartment comes into contact with the mineralized zone (samples MOZO_0007, MOZO_0009, and PTZO_0001) with ORP values varying between +69.9 and +172.3 mV/SHE. Lower values (–9.4, 3.8 and –14 mV/SHE) were observed downstream in the reduced part of the aquifer (samples PZOV_0024, PZOV_0021 and PZOV_0022 respectively). This zonation can be also extended to pH and TIC as slightly alkaline pH values (8.15–8.17) jointly with higher TIC concentrations (6.6 mmol/L) were observed for all the samples from the reduced zone of the aquifer (PZOV_0021_1, PZOV_0022_1 and PZOV_0024_1), while circumneutral pH values (7.60) and lower TIC (~3.5 mmol/L) were found for the other samples. Such variations could be explained by the activity of sulphate reducing bacteria (SRB) known to
favour the reduction of sulphate concurrently to the oxidation of organic carbon (higher values observed in the mineralized and reduced parts of the aquifer, up to 3.67 mg/L for MOZO_0007) into carbonate and sulphide according to the overall simplified reaction, where redox conditions are here controlled by the solubility of ferric hydroxide (Fe(OH)₃(s)) and pyrite (FeS₂(s)), respectively, upstream and downstream the roll-front.

$$4 \text{CH}_2\text{O} + \text{Fe(OH)}_3(s) + 2 \text{SO}_4^{2-} + \frac{1}{4} \text{O}_2 \rightarrow 4 \text{HCO}_3^- + \text{FeS}_2(s) + 7/2 \text{H}_2\text{O}$$

Eq. (1)

Where CH₂O stands for the reactive dissolved organic carbon.

The chemical zonation through the roll-front system has been also observed for other sensitive redox elements. Higher concentration in NO₃ was observed in the oxidized compartment, while in the reduced part of the aquifer higher concentrations in As, Mo and NH₄, known to have higher solubility in reducing conditions, were observed. The same behavior for Se, Fe, and Mn was observed with higher concentrations in the ore area. Even if U is known to be more mobile in oxidizing conditions, higher concentrations were observed in the mineralized compartment jointly with ²²⁶Ra and Ba. The latter is known to control the solubility of ²²⁶Ra according to the solid solution (Ba, Ra)SO₄ within barite ([26] and references therein). Even though the normalized concentrations used for Fig. 2 are corrected regarding to the salinity gradient in order to enhance the distribution of redox sensitive elements, a contrast in the concentrations of major cations (Na, Ca, Mg, K), silica and other alkali/alkaline earth metals (Sr, Li) was observed. This may be interpreted globally as a consequence of two main mechanisms of water-rock interactions governing the groundwater chemistry: on one hand, the increase of carbonate and pH inherited from the sulphate-reduction reaction (see Eq. (1)) and, on the other hand, the affinity of cations towards clay minerals through sorption processes [27–29].
Concentrations of major anions (Cl, SO_4) are therefore also varying in the same way with respect to the electrical balance.

**Richness and distribution of the bacterial communities**

Alpha-diversity analysis revealed no significant differences in bacterial richness of the water samples regardless of the metrics used (Table 2). Chao1 richness estimator and observed species index (Sobs) indicated high and comparable bacterial richness in the communities of all water samples. As seen in the table, the samples have many sequences, which were also confirmed by Shannon, Simpson and the Goods coverage indexes. Water samples displayed high richness and had similar alpha-diversities. According to Simpson index, all taxa were distributed equitatively in the bacterial communities of samples MOZO_0007, MOZO_0009, and PZOV_0013 with an average ranging from 0.59 to 0.67, while in the other samples, more than one taxon were dominating the communities with a Simpson index ranging from 0.80 to 0.86. Rarefaction curves showed that sufficient sequencing depth was reached to well represent the bacterial community of all water samples, although not enough to adequately detect all phylotypes in the libraries (Fig. S1).

Beta-diversity revealed significant differences in the bacterial community structure and abundance. Water samples formed three distinct clusters in accordance with the redox zonation on the principal coordinate analysis (PCoA = Multidimensional scaling, MDS) plot of the bacterial community composition (using OTU abundance) of all tested wells (Fig. 3). Accordingly, the analysis of the oxidized (PZOV_0013, and PZOV_0001), the mineralized (MOZO_0007, MOZO_0009, and PTZO_0001) and the reduced (PZOV_0024, PZOV_0021, and PZOV_0022) samples indicated that they were much more similar to one another in terms of bacterial abundance, which revealed significant clustering of replicates by water
sample as well as by water physico-chemical properties (redox, O₂, etc).

Microbiome composition

A total of 969,344 bacterial 16S rRNA gene sequences were recovered for all water samples, which were distributed in a mean of 39,065 for PZOV_0013, 20,583 for PZOV_0001, 47,441 for MOZO_0007, 44,419 for MOZO_0009, 22,708 for PTZO_0001, 40,693 for PZOV_0024, 38,910 for PZOV_0021, and 34,883 for PZOV_0022. These sequences were used for community analyses by QIIME. OTUs were assigned by clustering sequences with over 97% sequence identity. A number of 1,165 OTUs were identified indicating high microbial diversity. These OTUs were distributed in Phyla (99.99% of phylotypes), Class (99.76% of phylotypes), Order (99.39% of phylotypes), Family (98.34% of phylotypes), and Genera (86.40% of phylotypes). Among the 30 phyla determined in the water samples, Proteobacteria, Actinobacteria, and Bacteroidetes were the most abundant in the different water samples (Table 3; Additional file 1 S3). Some other rare phyla were also obtained such as Acidobacteria, Candidate_division_OP3, Candidate_division_WS3, Elusimicrobia, and Synergistetes, which were detected in proportions below 1% (Fig. S2).

At genus level, identification of the bacterial groups revealed the presence of 340 different genera in the bacterial communities (Fig. 4). Among the oxidized water samples (the upstream waters), PZOV_0013 was dominated by unclassified genera of Sporichthyaceae (48.64%), followed by Polaromonas (31.80%), Fluviiicola (4.72%), Flavobacterium (4.24%), Aquabacterium (3.90%), Bdellovibrio (2.06%), and Pseudomonas (1.18%), among others with an occurrence of below 1%. Unlike the latter, in sample PZOV_0001 more genera with an occurrence of >1% were observed, being the most dominant genus Pseudomonas (30.97%) followed by unclassified members of
Sporichthyaceae (22.04%), unclassified genera of Oxalobacteraceae (14.19%), Polaromonas (9.32%), C1-B045 (3.22%), Rhodobacter (2.81%), Flavobacterium (2.37%), Aquabacterium (2.13%), unclassified members of Sphingomonadaceae (2.03%), Emticia (1.99%), unclassified members of Rhodobacteraceae (1.83%), and Parvibaculum (1.10%). Similar bacterial composition characterized the mineralized samples. MOZO_0007 was dominated by Flavobacterium (61.81%), followed by Pseudomonas (14.40%), Aquabacterium (10.70%), unclassified Sporichthyaceae (4.63%), Rhodobacter (2.68%), unclassified genera from CHAB-XI–27 (1.19%), and unclassified genera from Oxalobacteraceae (1.18%), among others with an occurrence of <1.0 %. In sample MOZO_0009, the most dominant genus was Aquabacterium (50.08%) followed by Pseudomonas (36.06%), Flavobacterium (2.72%), Methylotenera (2.16%), Polaromonas (1.61%), Hydrogenophaga (1.15%), and unclassified genera of Sporichthyaceae (1.08%), among others with an occurrence of less than 1%. In sample PTZO_0001, the most dominant genus was Pseudomonas (67.69%) followed by Microbacterium (16.77%), Methylotenera (2.24%), unclassified members of Rhodobacteraceae (1.99%), unclassified members of Rhodocyclaceae (1.30%), and unclassified members of Sphingomonadales (1.03%).

A few differences in the bacterial community composition and structure were observed in the downstream waters (reduced water samples). Sample PZOV_0024 was dominated by the genus Legionella (40.92%) followed by Hydrogenophaga (25.86%), Flavobacterium (8.79%), Aquabacterium (7.16%), Pseudomonas (2.84%), unclassified members of Rhodobacteraceae (1.85%), Rhodobacter (1.83%), Lutibacter (1.47%), Hyphomonas (1.43%), Owenweeksia (1.23%), Coxiella (1.22%), among others. While in sample PZOV_0021, the most dominant genus was Hydrogenophaga (31.39%) followed by Aquabacterium (23.66%), Flavobacterium (17.77%), Rhodobacter (5.16%), Pseudomonas
(4.85%), *Brevundimonas* (3.51%), *Owenweeksia* (1.99%), and *Lutibacter* (1.35%), among others. Finally, in sample PZOV_0022 the most dominant genus was *Pseudomonas* (56.34%) followed by *Hydrogenophaga* (11.62%), *Rhodobacter* (10.35%), *Flavobacterium* (3.70%), *Algoriphagus* (2.03%), *Planctomyces* (2.02%), unclassified members of *Saprospiraceae* (1.47%), *Pirulella* (1.44%), unclassified members of *Pseudomonadaceae* (1.34%), unclassified members of *Rhodobacteraceae* (1.28%), and *Lutibacter* (1.19%), among others.

**Bacterial diversity analyses and statistics**

To further identify the similarity in abundance between the bacterial communities, a heatmap was performed based on the relative abundance of the genera with an average abundance of > 0.5% in at least one sample, which were defined as dominants. Abundance of 50 major genera present in the 21 water samples (3 replicates from each) were illustrated in Fig. 5. Major genera with occupancies of > 80% of all samples and average abundance of > 0.05% were defined as common genera in the present study. Among these 50 major genera, 13 belonging mainly to the phyla *Proteobacteria* and *Bacteroidetes* were considered as common genera including *Pseudomonas*, *Rhodobacter*, *Methylotenera*, *Hydrogenophaga*, *Caulobacter*, *Brevundimonas*, *Flavobacterium*, *Aquabacterium*, *Methylibium*, unclassified *Comamonadaceae*, unclassified *Rhodobacteraceae*, and *Albidiferax*.

Similarity of percentages analysis (SIMPER) was used to determine the relative contribution of each individual genus to the dissimilarity between the three water clusters. The average Bray-Curtis dissimilarity and the contribution of each genus to the total dissimilarity between communities in the different zonation waters (Mineralized, Oxidized, and Reduced) were calculated. The top major genera responsible for the microbial community difference (>98% contribution to cumulative dissimilarity) was summarized in
Table 4. Among them, *Sporichthyaceae* had the largest dissimilarity contribution (14.72%), followed by *Pseudomonas* (14.66%), *Flavobacterium* (13.85%), *Aquabacterium* (12.62%), *Hydrogenophaga* (11.15%), *Polaromonas* (8.46%), *Legionella* (6.78%), *Oxalobacteraceae* (3.04%), *Rhodobacter* (2.44%), and *Fluviicola* (1.1%).

Statistical analysis using One-way ANOVA test (p < 0.05; n = 3), which determine whether individual OTUs differ significantly in abundance and/or prevalence between two groups of data, showed that between the mineralized and the upstream water samples twelve taxa were statistically the most significant at confidence level of 95% (p-value of ≤0.05) (Additional file 2). These are unclassified *Sporichthyaceae*, *Polaromonas*, *Fluviicola*, *Methylotenera*, *Bdellovibrio*, *Devosia*, *Sphingobium*, NKB5, *Ferribacterium*, *Denitratisoma*, *Candidate_division_WS3*, *Hirschia*, *Pseudolabrys*, being *Sporichthyaceae* and *Ferribacterium* statistically highly significant with a p-value of ≤0.001 (which means less than one in a thousand chance of being wrong). While comparing the upstream and downstream water samples, 28 genera (*Hydrogenophaga*, unclassified *Sporichthyaceae*, *Polaromonas*, *Lutibacter*, *Rhodobacter*, *Oxalobacteraceae*, *Fluviicola*, *Owenweeksia*, *Methyloversatilis*, among others) were the most significant taxa at the confidence level of 95% (p-value of ≤0.05), being *Hydrogenophaga*, unclassified *Sporichthyaceae*, *Polaromonas*, and *Lutibacter* highly significant genera with a p-value of ≤0.001.

Considering the downstream and mineralized water samples, 28 genera (*Hydrogenophaga*, *Rhodobacter*, *Rhodobacteraceae*, *Methylotenera*, *Lutibacter*, *Owenweeksia*, *Hyphomonas*, *Phyllobacteriaceae*, *Caulobacteraceae*, *Opitutus*, *Percludibaca*, *Limnobacter*, *Azoarcus*, *Solimonadaceae*, *Ferribacterium*, among others) were the most significant taxa at the confidence level of 95% (p-value of ≤0.05), being *Hydrogenophaga*, *Lutibacter*, and *Ferribacterium* highly significant genera with a p-value of ≤0.001.

To further investigate the taxonomic distribution and significantly differential dominant
clades in the water microbiomes, LDA effect Size (LefSe) method was performed to compare and detect the abundance of groups at each taxonomic level and determine taxa involving significant differences among the different water samples (upstream, orebody, and downstream compartments). Fig. 6 depicts cladogram that visualize all detected significant taxa from domain to genus level in each water sample, being the reduced (PZOV_0024, PZOV_0021, and PZOV_0024) and mineralized (MOZO_0009, and PTZO_0001) waters with more significant taxa than the others (Fig. S3).

Correlations between bacterial genera and groundwater chemistry

The constrained multivariable canonical correspondence analysis (CCA) was used to examine and visualize the relationship between the target major genera composition and the environmental factors (i.e.: pH, Eh, sulphate, nitrate, U, Fe, Mn, Mo, etc) (Fig. 7). Both the first and second axes were positively correlated with high concentrations of O\textsubscript{2}, NO\textsubscript{3}, Eh, Sr, Mg, Ca, and negatively correlated with U, \textsuperscript{226}Ra, and Fe, while the first axis was negatively correlated with Se and Si and the second axis negatively correlated with SO\textsubscript{4}, Cl, Na, C, pH, DIC, DOC, Mn, Mo, As, and Ba. Regarding the bacterial community, results from the CCA analysis shows that three clusters of major groups of genera (>1%), sharing specific oxidation and reduction activities are formed. The first cluster representing oxidation conditions and neutral pH, included strictly aerobic heterotrophic organisms typically found in oxidative habitats, such as *Polaromonas*, *Sporichthyaceae*, *Fluviicola*, *Bdellovibrio*, *Oxalobacteraceae*, *Alteromonadaceae* C1-B045, *Emticicia*, and *Sphingomonadaceae*. These genera were positively correlated with high concentrations of O\textsubscript{2}, NO\textsubscript{3}, Mg, Ca and Sr. The second cluster harboured genera representing reductive conditions and neutral pH as well as high concentrations of U, Fe, \textsuperscript{266}Ra, and Si. These representatives include *Pseudomonas*, *Methylotenera*, *Planctomyces*, *Pseudomonadaceae*,
Pirellula, Microbacterium, Rhodocyclaceae, Saprospiraceae, and Sphingomonadales. The third cluster harboured genera such as Owenweeksia, Hydrogenophaga, Lutibacter, Flavobacterium, Brevundimonas, Aquabacterium, Parvibaculum, Rhodobacter, Rhodobacteraceae, CHAB-XI–27, Legionella, Coxiella, and Algoriphagus that were positively correlated with slightly higher pH values and high conductivity, reductive conditions and high concentrations of SO$_4$, Na, Cl, Mn, Mo, As, Ba, DOC, and DIC.

Co-occurrence and co-exclusion patterns among bacterial taxa and environmental parameters

The co-occurrence and co-exclusion patterns among the bacterial communities and the environmental factors were explored using network analysis based on strong correlations ($\rho > 0.6$ or $\rho < -0.6$). A total of 53 nodes (39 bacterial taxa and 14 environmental parameters) and 235 edges were represented in Fig. 8. Topological characterization commonly used in network analysis were calculated to describe the complex network of interrelationship among bacterial taxa and environmental parameters (Additional file 1 S4). Based on the modularity classification and compared to a random association, the network could be divided into 12 major modules, which are clusters of nodes interacting more among themselves than with others. The four largest modules, I, II, III, and IV, were occupied by 10, 8, 7, and 7 of the 53 total vertices, respectively. The co-occurring bacterial taxa and environmental parameters of each module are summarized in Table SI-2 (Additional file 1). In contrast, the co-exclusion pattern of the bacterial taxa and the environmental parameters showed the significantly negative correlations containing 27 nodes (15 bacterial taxa and 12 environmental factors) and 72 edges (Fig. S4).

Discussion

Microbiome of subsurface waters likely contain thousands of different species, which can
profoundly impact global biogeochemical cycles of elements and water quality. In an ISR site this impact can additionally be influenced by the mining and post-mining (remediation) processes. To better understand the subsurface chemistry related to uranium roll-front deposits, and to develop an effective post-ISR monitoring program, geochemical and bacterial composition relative to the original groundwater and their potential influence on the constituent concentrations, need to be accurately determined before actuations in the U ISR mine. In this study, the unmined roll-front uranium deposit is located at about 200 m below ground surface in a typical sandstone host rock within a confined aquifer. There, a redox front of oxidized groundwater (upstream) are permanently reacting with unaltered reduced groundwater (downstream). The groundwater within the upstream compartments of the aquifer oxidized samples PZOV_0013 and PZOV_0001) were characterized by high concentration of O₂ (oxic conditions), positive ORP (oxidizing conditions), and the highest concentrations of nitrate, Mg, and Ca. These conditions are commonly linked to the proliferation of aerobic heterotrophic bacteria that use oxygen (for aerobic respiration) as an available terminal electron acceptor for energy generation. When oxygen runs out, aerobic denitrifiers are forced to conduct an aerobic respiratory process in which nitrate, present in these upstream waters with high concentrations, is used as terminal electron acceptor and converted gradually to N₂ using nitrate reductase (Nar or Nao), nitrite reductase (Nir), nitric oxide reductase (Nor), and nitrous oxide reductase (Nos) [30]. Pseudomonas, unclassified Sporichthyaceae, Polaromonas, Rhodobacter, Flavobacterium, Aquabacterium, unclassified Oxalobacteriaceae, unclassified Rhodobacteraceae and Fluviiicola were dominating the bacterial community in this compartment of the aquifer. Adapted to these oxic and oxidizing conditions, some of them are also interesting for their unusual abilities, like the storage in Polaromonas of all three common types of prokaryotic cellular reserve
materials (PHA, polyglucose and polyphosphate granules), a characteristic that makes them important for their use in bioremediation at sites contaminated by polycyclic aromatic hydrocarbons (PAHs) [31]. Interesting is also the metabolization by *Pseudomonas* of chemical pollutants in the environment, resulting in their suitability for bioremediation processes of many compounds including heavy metals and radionuclides (e.g. uranium) [32–34], polycyclic aromatic hydrocarbons, organic solvents (e.g., toluene), carbon tetrachloride, carbazole, and a variety of simple aromatic organic compounds [35,36]. *Rhodobacter*, an iron-oxidizing bacterium who is able to utilize Fe(II) as an electron donor for iron oxidation in the periplasm [37], was found among the dominant bacteria in these waters. In turn, the produced biogenic Fe(III) mineral phases would play a major role in the reduction of U(VI) under anoxic conditions [38]. Besides, the presence here of members of the family *Alteromonadaceae*, collecting diverse sets of *Gammaproteobacteria*, mostly marine in origin and requiring sodium to grow [39], was enhanced but not unexpected due to the high concentrations of Na and Cl characterizing these water samples. Lastly, members of the family *Sphingomonadaceae* also accounted among the dominant bacteria in the upstream compartments. These organisms are free living and widely distributed in nature [40], being aerobic with a strictly respiratory type of metabolism where oxygen is used as terminal electron acceptor and providing in this section of the aquifer their known ability to degrade some aromatic compounds, in addition to their known ability for biomineralization of U(VI) as U phosphate mineral phases [41], all of which makes them of interest to environmental remediation [42]. The mineralized sections of the aquifer encompass the roll-front ore deposits, which are planned to be mined via ISR technology by solubilisation of the ore using an acidic leach solution. The groundwater here within the intermediate compartment of the aquifer (samples: MOZO_0007, MOZO_0009 and PTZO_0001) were characterized by a very low
concentration of O$_2$ (anoxic conditions), positive Eh and higher DOC and DIC concentrations than in the upstream groundwater. These conditions favour the proliferation of facultative aerobic to strictly anaerobic microorganisms, which grow in such microaerophilic waters. Genera like *Flavobacterium*, *Pseudomonas* and *Aquabacterium*, the dominant genera here, occur more frequently and abundantly in this type of water samples. According to the literature, these genera have a widespread occurrence in water environments, where they decompose organic matter [43,44]. On the other hand, high concentrations of nitrate, sulphate, and Fe were found in this section of the aquifer. These compounds promote the presence of iron-reducing bacteria like *Desulfovibrio* and *Ferribacterium*, iron-oxidizers like *Rhodobacter* and denitrifying bacteria such as *Pseudomonas*. In fact, both of these latter microorganisms (*Pseudomonas* and *Rhodobacter*) are known to use periplasmic nitrate reductase as the major player for energy generation process through the mechanism of dissimilatory aerobic nitrate reduction [37,45]. Furthermore, although in low abundance, many iron-reducing bacteria were detected in these water samples including *Albidiferax*, *Ferribacterium*, *Pedomicrobium*, and *Desulfovibrio* (some of them are also metal-reducing bacteria), while members of the genera *Aquabacterium*, *Rhodobacter*, and *Gallionella* dominated the iron oxidizers group. In fact, neutrophilic Fe-oxidizing bacteria are rather associated with subsurface groundwater where reducing Fe$^{2+}$-containing water flows from anoxic to oxic conditions. Here, the low O$_2$ concentrations retard abiotic Fe oxidation, allowing thus certain specialized Fe oxidizers (e.g., *Gallionella*, and *Rhodobacter*) to grow [46]. In these mineralized samples, Fe, nitrate, sulphate, and heavy metals/radionuclides such as Mn, Mo, Se, and U were present with considerable concentrations in comparison to the rest of the samples. These elements are used to be multiple electron acceptors, as NO$_3^-$, Mn(IV),
Fe(III), U(VI), and \( \text{SO}_4^{2-} \) in many natural environments are used by microbes typically in sequence of energy yield, playing an important role in the biogeochemistry of the water. For example, according to Elias et al. [47], *Desulfovibrio vulgaris* showed utilization of Fe(III) first, followed by U(VI), and finally sulphate in a competition experiment [47]. In the field, nitrate has been shown to be reduced prior to the U(VI) reduction, which often occurs simultaneously with Fe(III) reduction and prior to sulphate reduction [48,49]. The high concentration of sulphate present in these water samples induced the presence of sulphate-reducing bacteria including *Desulfovibrio, Desulfurivibrio,* and *Nitrospira,* among others. Although it is common to attribute the major role in U(VI) reduction, for example, to the most dominant organisms in the groundwater during uranium bioremediation, in reality a minor components of the community may be sufficiently abundant to account for and play an important role in U(VI) reduction. *Desulfovibrio* or *Acidovorax* are among the low abundant bacteria found in these intermediate compartments and could be most likely the most important microorganisms involved in metal reduction including U(VI) reduction [50]. Nevertheless, the number of these specialized bacteria may increase in the groundwater during mining period and after the acidification of the waters, due to the stimulation of acidophilic bacteria like *Acidovorax* or mixotrophs that have the ability to live in a wide range of environmental conditions by using alternative electron acceptors, such as nitrate, potentially contributing to the acidification of their habitat in absence of oxygen [51]. Na and Cl were found with high concentrations in these mineralized waters providing then the occurrence of many halotolerant and marine microorganisms such as *Flavobacterium* and *Aquabacterium.* In addition, Sun et al [52] found that *Flavobacterium* had a relatively high abundance in a watershed heavily contaminated by an active antimony (Sb) in an acid mine in Southwest China. In samples MOZO_0009
and PTZO_0001, genera such as *Methylotenera* and *Hydrogenophaga* were abundant. *Methylotenera* are methylotroph organisms that can oxidize methylamine (one-carbon compound) as a single source of carbon, energy and nitrogen, while *Hydrogenophaga* is another interesting microorganism for its capacity to oxidize hydrogen as an energy source and to utilise CO$_2$ as a carbon source. In this genus, firstly, the oxidative carbohydrate metabolism is either achieved with oxygen as a terminal electron acceptor or by heterotrophic denitrification of nitrate [53], which is likely to occur in these water samples lacking O$_2$. Secondly, while it is not clear if these H$_2$-oxidizing bacteria use abiotically produced H$_2$ or biotically produced H$_2$ (through fermentative or photosynthesizing activity), the presence however in all sampled waters of typical H$_2$-producing bacteria (e.g. *Cyanobacteria*, members of the *Clostridia* class, *Enterobacteriales*, *Bacillales*, or *Thermotogales*) in extremely low relative abundance, may suggest the use of an abiotic source of H$_2$. Oxidation of hydrogen is considered to be potentially linked to the reduction of a range of electron acceptors, including radionuclides [54].

Contrary to the intermediate compartment, the most downstream sections of the aquifer (reduced samples PZOV_0024, PZOV_0021, and PZOV_0022), being anoxic and with reducing conditions (negative ORP), were characterized by the highest concentrations of DIC, sulphate, Na, Cl, K, Mn, and Mo, while minimal concentrations of nitrate, Mg, Ca, and uranium were obtained. The bacterial community here were very similar to the previous one with some exceptions. Shared between the three replicates of this compartment were genera such as *Flavobacterium*, *Aquabacterium*, *Pseudomonas*, *Rhodobacter*, *Hydrogenophaga*, *Lutibacter*, *Owenweeksia* and unclassified members of *Rhodobacteraceae*. In this downstream groundwater, new genera emerged such as
*Lutibacter* that is an interesting microorganism characterized by the capacity to adapt to environments with different oxygen affinities enabling them to thrive in microaerophilic to aerophilic conditions, which is further indicated by the production of diverse ferredoxin utilizing enzymes [55]. According to Le Moine-Bauer et al [55], some members of this genus display the complete pathway for denitrification in addition to oxygen respiration and nitrate reduction to nitrite, which were confirmed under microaerobic conditions. Interestingly, these bacteria are characterized by sulfide:quinone oxidoreductase (SQR) involved in the sulphur metabolism, which may play an important role in sulphide detoxification in an environment with high sulphide concentration. Besides, strong activity for alkaline and acid phosphatase is characteristic of these bacteria, which is a plus considering their possible application in bioremediation of radionuclides such as uranium [55]. Some interesting bacteria such as *Planctomyces*, *Pirellula*, *Algoriphagus*, and unclassified members of the family *Saprospiraceae* seem to widely co-occur in these samples. The first two belong to the phylum *Planctomycetes*, which are in general intriguing because they are the only free-living bacteria known to lack peptidoglycan in their cell walls; they are instead stabilized by the protein sacculus with disulfide bonds [56]. *Planctomyces*, a marine bacterium found in various habitats around the world (e.g. freshwater, saltwater, acid bog water), owns a relatively large genome that is thought to be necessary for the adaptation to changing environmental conditions [56]. In *Planctomyces*, as well as in the rest of the planctomycetes, “anammox” metabolism, a process in which ammonia is oxidized by nitrate to nitrogen gas yielding energy under anaerobic conditions, is mainly conducted. The other genus of this order, *Pirellula*, is in addition an interesting microorganism since it has two orders of magnitude more sulfatase genes, used in sulphur scavenging and for the effective assessment of sulphated compounds as an energy source [57]. In the water column, *Pirellula* are able to survive
under low oxygen conditions, since anoxic conditions force them to switch to heterolactic acid fermentation or pathways involving formaldehyde conversion, if not to support growth then at least to allow basic metabolism maintenance [57]. The genus *Algoriphagus*, a marine psychrotolerant and moderately halotolerant bacteria, requires nearly seawater concentration of NaCl for their growth [58]. Thus their high detection here is not surprising since high concentrations of Na and Cl were characterizing this water samples. Lastly, the family *Saprospiraceae* can exhibit large variation in cell length with axenic conditions and some of them show the ability for the hydrolysis and utilization of complex carbon sources. Such complex carbon utilization role is demonstrated *in situ* for activated sludge wastewater treatment systems where these organisms are frequently observed in abundance [59]. Once more in these water samples, the high concentration of sulphate probably induce the presence of sulphate-reducing bacteria, such as *Desulfovibrio*, although they are present in low abundance.

In general, in all three compartments, correlations with environmental parameters revealed putative associations among the associated genera, and particular environmental parameters. This finding could support the results of CCA between bacterial communities and groundwater geochemistry from another perspective. For instance, the positive correlations between SO₄ and *Rhodobacteraceae*, *Hydrogenophaga*, *Lutibacter*, and *Bacteroidetes WCHB1–32* were also observed by CCA analysis perhaps indicating that the increase in sulphate concentration may promote the proliferation of these genera. In the same way, the negative correlations between DOC and *Phenylobacterium*, *Pseudospirillum*, *Emticicia*, *Sphingomonadaceae*, *Parvibaculum*, and *Alteromonadaceae C1-B045* may indicate that the increase in the groundwater in the organic matter was likely to reduce the abundance of these bacterial genera. These consistent results obtained by CCA and network analyses were also commonly observed for the rest of the environmental factors-
genus associations, including the negative correlations between U and *Lutibacter*, between
NO$_3$ and *Hydrogenophaga*, and between ORP and *Comamonadaceae / Hydrogenophaga*, the
positive correlations between U and *Methyloptera / Sphingomonadales / Pseudomonas / Microbacterium / Rhodocyclaceae* as well as the positive correlations between O$_2$ and *Emticicia / Sphingomonadaceae / Pseudospirillum / Alteromonadaceae C1-B045*. These co-
occurrence patterns among the bacterial genera were derived from either genera sharing
similar ecological niches or a specific traits among them. In this case, strong ecological
associations evolving as a cluster of robust co-occurrence correlations were observed. For
example, the co-occurrence pattern among *Methyloptera, Sphingomonadales, Pseudomonas, Microbacterium, and Rhodocyclaceae* might result from their sharing similar
ecological niches, since all of them could use Fe and U and they are competitors from this
point of view. Meanwhile, *Emticicia, Sphingomonadaceae, Pseudospirillum, Phenylbacterium, Oxalobacteraceae, Parvibaculum, and Alteromonadaceae C1-B045* co-
occurred in presence of oxygen in a strong positive correlations since all of them are
strictly aerobic genera and they shared similar ecological preferences. The co-exclusion
patterns among different genera as well as with environmental parameters might instead
reveal niches preferences for the specific genera or competition. On the other hand, there
were several co-occurrence relationships among known bacterial genera and some that
have been very poorly studied such as Candidate_division_OD1 (in Module XI), Candidatus-captivus (in Module V), *ThiotrichalesCHAB-XI-27* (in Module VI), *Alteromonadaceae C1-
B045* (in Module II), and *Bacteroidetes WCHB1–31* (in Module I). This type of patterns may
provide interesting clues that these distinct organisms are closely connected ecologically,
although the nature of these possible interactions is still unclear. From all of this, we
suggest that some of the co-occurrence patterns may reveal associations of bacteria
performing functions similar or complementary to each other, while others may co-occur
due to shared and preferred environmental conditions. Thus, the co-occurrence and co-exclusion patterns will improve our understanding of ecological niches and help to gain clues on ecologically bound microorganisms under these ISR relevant conditions. Finally, our study demonstrates that 1) NA as a part of ISR remediation strategy requires a proof that the aquifer down-gradient of mining operations is able to reduce U and that 2) the *in situ* microbial communities through their metabolic versatility are prone to promote such metal reductions predicting and improving ISR efficiency. The next important step in our investigations will be to determine the microbial metabolic activities associated with the distinct aquifer compartments. Studying microbial processes enhanced or induced by ISR mining may significantly contribute to better predict remediation efficacy and even prevent operational failure. Future work should focus on monitoring and assessing the effects of acidification on the diversity and activity of these microorganisms, which is critical for the correct evaluation of the potential and efficacy of either natural attenuation or active bioremediation strategies, and definitely for the determination and mitigation of the environmental risks at the ISR sites.

**Conclusions**

In summary, this study was aimed to highlight how pre-mining distribution of the bacterial communities resident in the different groundwater compartments surrounding roll-front deposit can provide insights not only into the removal of U from groundwater, but also direct implications for the optimization of the ISR process. In terms of microbial communities, such background data and understanding of the natural system are a prerequisite to predicting microbial assistance in ISR operations as well as in further developing bioremediation strategies. In the mineralized zones, bacteria would be impacted by environmental influences in the oxic/anoxic boundary resulting from the acid solution injections. Significant population change would probably occur upon such
intervention at the ISR mining site, with a large decrease in both bacterial cell number and diversity, as was seen in a previous study performed in Kazakhstan ISR mine after acid injection [20]. Although, more acidophilic bacteria and other mixotrophs similar to those abundant in an acid mine drainage, and detected in these pristine waters with low abundance (e.g. Acidovorax), would be stimulated and the redox-zonation would be enhanced with additional facultative anaerobic and acidophilic microorganisms, present now with a low abundance. These conditions may be suitable for the natural attenuation of ISR mining through the reduction of metals/metalloids, sulphate, and other elements. The elucidation of the overall bacterial diversity and the chemistry of these groundwater underline the first step in the vast swath of U ISR context that remains to be explored for the correct understanding of the natural attenuation process.

Methods

Site description and sample collection

The studied site is located in the Gobi desert of Mongolia, Zoovch Ovoo in Sainshand region, whose resource is estimated in excess of 50,000 tons of uranium and where sandstone is the main host rock of the uranium found at this site (Fig. 1). Since 2015, this region of Mongolia is known with recoverable uranium resources producing, through ISR mining, about 2 % of the world uranium resources. The Zoovch Ovoo deposits are mineralized roll-front extended on approx. 10 km long and consist of a massive sub-tabular sandstone deposits, which are irregular, elongated lenticular bodies parallel to the depositional trend [60].

Groundwater was sampled from August to November 2016 with a special emphasis on the localization of each piezometer related to the U orebody (see Fig. 1). Two sampling campaigns were performed: 1) for the geochemical analysis and 2) for the bacterial
diversity study. As the hydraulic gradient is very low (~ m/y) [24], we made the assumption that both sampling campaigns are representative of the same in situ conditions. Sampling was performed accordingly to the best standards. Hence the samples were taken after properly purging the volume of piezometer several times in order to 1) ensure representativeness of the water samples, but also to 2) benefit from this operation to rinse the submersible groundwater pump. Groundwater samples were pumped at the depths of ~100–200 m below the surface in the K2Ss2 aquifer, in the monitoring wells of the Zoovch Ovoo ISR project mine with the use of submersible groundwater pump (Grundfos #MP1 and #SQ7).

Water samples dedicated to the microbiome study were collected in sterile autoclaved bottles (Carboy 10 L Round, VWR International), carried and transported to the laboratory at 4 °C using standard procedures, and immediately processed upon arrival.

**Water chemistry measurements**

Physico-chemical parameters were measured on site with several dedicated probes (pH and T°: WTW IDS pH SenTix® 940–3; Conductivity: WTW TeTracon® 925–3; Eh: WTW SensoLyt® ORP 900-P; dissolved O₂: WTW IDS FDO® 925–3) connected to a previously calibrated portable multi-parameter instruments (WTW #3430). Sampling was achieved on a derivation to avoid any contamination when physico-chemical parameters were stable.

All the filtered samples were analyzed for cations, traces, U, ²²⁶Ra, anions, Total Inorganic Carbon (TIC) and Total Organic Carbon (TOC). Samples were acidified down to pH 2 with ultrapure HNO₃ for cations/traces/radioactivity measurements and with ultrapure H₂SO₄ for TOC analyses. Last, a special care was devoted to analyze NO₃⁻ and NH₄⁺ concentrations on field to prevent any oxidation of N species. Measurements were performed by spectrophotometry (HACH LANGE DR3900) according to the methods Hach
Lange LCK 303–304 and LCK 339–340. Anions (Cl\(^-\), F\(^-\), Br\(^-\)) were determined by ionic chromatography (DIONEX ICS 5000—DX 500) and in the case of SO\(_4^2-\) and PO\(_4^{3-}\) by photometry (Thermofisher AQUAKEM 250). Major cations (Na, K, Ca, Mg) were analyzed by Flam Atomic Absorption Spectrometry (AGILENT AA240). Silicium and trace elements (Al, As, Ba, Co, Cu, Fe, Li, Mn, Mo, Ni, Pb, \(^{226}\)Ra, Re, Se, Sr, U, V, W and Zn) were analyzed by ICP-AES (VARIAN 720ES) and ICP-MS (THERMO ELECTRON X7). TIC and TOC were also quantified (Shimadzu TOC VCPN).

Concentrating microorganisms for DNA extraction

A sterile 45 mm Polysulfone (PSF) Filter Funnel (NALGENE, Rochester, NY) was connected directly to a sterile neck-screwed receiving bottle, with a filter holder between the funnel and the receiving bottle. One and a half liters of water from each sample was vacuum filtered through autoclaved, polycarbonate, hydrophilic Isopore\textsuperscript{TM} membrane filters (Millipore): first through 0.4-µm pore size and then through 0.22-µm pore size filters with a 47-mm of diameter. Then, the filters were placed separately in 5 mL-tubes and stored at –20 °C until analyses were performed. Detailed workflow for the pre-treatment of the studied water samples is summarized in Fig. S5.

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from the biomass retained on the filters (kept at—20 °C), joining both pore-size filters. Three replicates of each water sample were performed. Each filter was cut in 4 pieces and each piece aseptically placed in a 2-mL sterile screw-cap tube containing glass beads of different diameters. One mL of lysis buffer (100 mM Tris-HCl [pH 8.0], 100 mM EDTA [pH 8.0], 100 mM NaCl, 1% polyvinylpyrrolidone [PVP], and 2% SDS), 24 µL freshly made lysozyme (10 mg/mL), and 2 µL proteinase K (20 mg/mL) were added to each tube. The tubes were vigorously shaken for 2 min in vortex and then
mechanical lysis of the cells was performed twice by using a FastPrep® FP120 (at a speed of 5.5 m/s for 45 s) with intermittent cooling on ice for 5 min. The tubes were incubated at 37 °C for 30 min and then at 60 °C for another 30 min and subsequently centrifuged at 14,000 × g for 5 min at room temperature. The upper (aqueous) phase was then collected in new 15-mL Falcon tubes and mixed by gently inverting with one volume of phenol:chloroform:isoamyl alcohol (25:24:1, pH 8) and then centrifuged at 1500 × g for 10 min at 4 °C to separate the aqueous and organic phases. The upper (aqueous) phase was washed with one volume of phenol:chloroform (1:1) and centrifuged at the same conditions. DNA was precipitated by adding 1/10 volume of sodium acetate (3 M, pH 5) and one volume of isopropanol, incubating for 1 hour at -80 °C and centrifuging for 30 min at 5,000 × g at 4 °C. Subsequently, the obtained DNA was dissolved in Tris (5 nM, pH 8.5)-TE buffer (10 mM Tris-HCl [pH 8.0] and 1 mM EDTA) previously heated at 65 °C. Extracted DNAs were stored at -20 °C until all sample extractions were completed. The concentrations of extracted DNA were determined using Qubit Fluorometer 3.0 [61].

Bacterial diversity analysis was performed by means of high-throughput amplicon sequencing using 250 bp paired end sequencing chemistry (MiSeq Illumina). Total DNA of each sample was amplified using the 16S rRNA gene primers 341F and 785R [62] targeting the hypervariable V3-V4 regions, suitable for detecting a wide range of bacterial taxa. PCR amplification and Illumina libraries were constructed and sequenced at LGC Genomics, GMBH, Berlin, Germany (http://www.lgcgroup.com/).

Bioinformatics analysis

The paired-end reads were first quality controlled and combined using PandaSeq [63], and then renamed using BESPOKE software (SeqSuite, http://bioware.soton.ac.uk). Then, the resulting read data were analyzed through the QIIME v1.8 pipeline [64]. OTUs were identified using UCLUST [65] and classified using the SILVA 119 QIIME 16S database [66].
Clustered and annotated OTUs were finally analyzed in Explicet 2.10.5 [67]. Rarefaction curves were determined using the VEGAN v2.4-6 package in R v. 3.4.3 environment [68].

**Statistical analysis**

Species richness was measured through the use of alpha-diversity metrics (Chao1, Shannon diversity index, and observed species) in Explicet and R software. Beta-diversity, the similarity between the identities of taxa and their abundances by water sample, was assessed using Bray-Curtis distances (weighted UniFrac distances) measured in QIIME and PAST3 v. 3.18 [69] and visualized the output with principal coordinate analysis (PCoA). Heatmap was constructed for the visualization of specific differences in community composition using the heatmap.2 function in the R gplots v2.11.0 package on log-normalized abundance data. At the genus level, heatmap included only taxa at ≥ 1% relative abundance in all samples/replicates. Additionally, canonical correspondence analysis (CCA), and similarity of percentages analysis (SIMPER) were performed using PAST3 software. One-way ANOVA test was conducted to look for significant differences in the alpha diversity (p <0.05) between the samples (α < 0.05), followed by the Tukey post hoc test using SPSS v. 20.0 (SPSS, Inc., Chicago, IL, USA). Additionally, linear discriminate analysis (LDA) of effect size (LEfSe) was performed to identify among the significant bacterial taxa those differentially represented between the water samples at all taxonomic levels. LeFSe method uses the Kruskal-Wallis test to identify features with significant differences in the abundance, and groups with a significant abundance difference (i.e., “biomarkers”). Only taxa meeting a LDA significance threshold ≥ 2 and p <0.05 were adopted [70]. Network analyses were conducted in the R environment using the VEGAN package [68] and only strong Pearson’s correlations (ρ > 0.6 or ρ < −0.6) were considered. Network visualization and modularization were carried out on the interactive platform of CYTOSCAPE [71].
Declarations

Ethics approval and consent to participate
Not applicable

Availability of data
All raw sequences used in this study are available in the sequence read archive (SRA) at NCBI database under the accession number PRJNA553521.

Competing interests
The authors declare no competing interests

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Authors’ contributions
M. L. M. and M. D. conceived the concept and led this project. M. L. M., M. D., and F. J. designed the experimental setup. V. S., P. G., and M. D. set up and managed the sample collection. I. S. C. was involved in the acquisition of the concentrated samples. F. J. performed all laboratory work. F. J. and C. P. P. analyzed the data and created the graphs and figures. F. J. and M. D. were major contributors in writing the manuscript with critical input from M. L. M and P. G. All authors read and approved the final version of the manuscript.

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Tables

|                | pH  | ORP | Cond | Na  | Ca   | Mg   | K   | SO4 |
|----------------|-----|-----|------|-----|------|------|-----|-----|
| PZOV_0013      | 7.67| 379.1| 4.26 | 801 | 72.1 | 41.1 | 4.6 | 721 |
| PZOV_0001      | 7.76| 354.6| 3.79 | 735 | 67.7 | 37.1 | 4.72| 580 |
| MOZO_0007      | 7.71| 172.3| 4.34 | 835 | 65.3 | 36.7 | 5.31| 682 |
| MOZO_0009      | 7.69| 166.2| 3.98 | 767 | 60.6 | 34.4 | 5.09| 620 |
| PTZO_0001      | 7.6 | 69.9 | 3.81 | 738 | 54.7 | 31.7 | 4.78| 576 |
| PZOV_0024      | 8.15| -9.4 | 6.81 | 1580| 39.2 | 24.9 | 7.01| 1235|
| PZOV_0021      | 8.16| 3.8  | 4.65 | 1030| 17.4 | 10.6 | 3.71| 823 |
| PZOV_0022      | 8.17| -14  | 5.38 | 1200| 18.9 | 12.8 | 4.61| 894 |

Table 1: Chemistry of the water samples. All elements are presented in mg/L, ORP and conductivity (Cond) are respectively expressed in mV/SHE and mS/cm

Table 2: Inference statistics at genus level of the different water samples. The species richness (Taxa richness, CHAO1, ACE), evenness (Pielou’s evenness, Simpson 1-D), and diversity (Shannon_H, Fisher’salpha) indexes were calculated with a 3% distance cutoff
| SAMPLES   | Individuals | Taxa richness | CHAO1   | ACE      | Pielou’s evenness | Simpson_1-D | Shannon H |
|-----------|-------------|---------------|---------|----------|-------------------|-------------|-----------|
| PZOV_0001 | 62,190      | 336           | 533.1   | 581.5    | 0.40              | 0.8292      | 2.315     |
| PZOV_0013 | 117,397     | 299           | 661.5   | 793.3    | 0.26              | 0.6572      | 1.506     |
| MOZO_0007 | 143,037     | 442           | 1362    | 1436     | 0.25              | 0.5928      | 1.519     |
| MOZO_0009 | 133,865     | 616           | 1656    | 1689     | 0.27              | 0.6758      | 1.718     |
| PTZO_0001 | 69,067      | 309           | 346.6   | 375.4    | 0.38              | 0.7985      | 2.167     |
| PZOV_0021 | 118,708     | 478           | 1237    | 1366     | 0.42              | 0.8627      | 2.583     |
| PZOV_0022 | 105,217     | 264           | 450.2   | 425.9    | 0.44              | 0.8204      | 2.43      |
| PZOV_0024 | 123,862     | 359           | 633     | 627      | 0.42              | 0.8593      | 2.484     |

. TIC: total inorganic carbon. TOC: total organic carbon.

Table 3: List of the phyla and their contribution (in %) to the bacterial community
| OTU_Name                  | MOZO_0007 | MOZO_0009 | PTZO_0001 | PZOV_0001 | PZOV_001 |
|--------------------------|-----------|-----------|-----------|-----------|-----------|
| Proteobacteria           | 32.57     | 95.59     | 80.27     | 71.70     |           |
| Bacteroidetes            | 62.67     | 3.12      | 0.70      | 5.37      |           |
| Actinobacteria           | 4.69      | 1.14      | 17.08     | 22.19     |           |
| Planctomycetes           | 0.00      | 0.00      | 0.06      | 0.02      |           |
| Gemmatimonadetes         | 0.00      | 0.00      | 0.11      | 0.00      |           |
| Candidate_division_OD1   | 0.01      | 0.02      | 0.34      | 0.08      |           |
| Firmicutes               | 0.00      | 0.02      | 0.97      | 0.08      |           |
| Verrucomicrobia          | 0.00      | 0.04      | 0.02      | 0.04      |           |
| Candidate_division_TM7   | 0.00      | 0.00      | 0.01      | 0.15      |           |
| Chlorobi                 | 0.00      | 0.01      | 0.04      | 0.01      |           |
| Candidate_division_BRC1  | 0.00      | 0.00      | 0.00      | 0.05      |           |
| Nitrospirae              | 0.00      | 0.01      | 0.09      | 0.00      |           |
| Chlamydiae               | 0.00      | 0.00      | 0.00      | 0.19      |           |
| Tenericutes              | 0.00      | 0.00      | 0.00      | 0.00      |           |
| Chloroflexi              | 0.01      | 0.03      | 0.04      | 0.01      |           |
| Fusobacteria             | 0.00      | 0.00      | 0.09      | 0.01      |           |
| Unclassified_Bacteria    | 0.01      | 0.00      | 0.04      | 0.07      |           |
| TM6                      | 0.00      | 0.00      | 0.00      | 0.01      |           |
| Acidobacteria            | 0.00      | 0.00      | 0.00      | 0.01      |           |
| BHI80-139                | 0.00      | 0.00      | 0.00      | 0.00      |           |
| Candidate_division_OP3   | 0.00      | 0.00      | 0.03      | 0.00      |           |
| BD1-5                    | 0.00      | 0.00      | 0.00      | 0.00      |           |
| Candidate_division_WS3   | 0.01      | 0.01      | 0.01      | 0.00      |           |
| Cyanobacteria            | 0.02      | 0.00      | 0.00      | 0.00      |           |
| Deinococcus-Thermus      | 0.00      | 0.00      | 0.00      | 0.00      |           |
| Elusimicrobia            | 0.00      | 0.00      | 0.06      | 0.00      |           |
| Spirochaetae             | 0.00      | 0.00      | 0.00      | 0.00      |           |
| Synergistetes            | 0.00      | 0.00      | 0.01      | 0.02      |           |
| TA06                     | 0.00      | 0.00      | 0.02      | 0.00      |           |

**Table:** SIMPER analysis of bacterial community dissimilarity (>98% of contribution to cumulative dissimilarity) of the three natural redox zonation waters.
| Genus/Microbial Phyla       | (%) 1 | (%) 2 | (%) 3 |  (%) |
|-----------------------------|-------|-------|-------|------|
| Sporichthyaceae             | 11.22 | 14.72 | 14.72 | 2.85 |
| Pseudomonas                 | 11.18 | 14.66 | 29.38 | 25.3 |
| Flavobacterium             | 10.56 | 13.85 | 43.24 | 32.3 |
| Aquabacterium              | 9.623 | 12.62 | 55.86 | 30.4 |
| Hydrogenophaga              | 8.5   | 11.15 | 67.01 | 0.768 |
| Polaromonas                 | 6.448 | 8.459 | 75.47 | 0.832 |
| Legionella                  | 5.167 | 6.778 | 82.24 | 0.233 |
| Oxalobacteraceae           | 2.319 | 3.042 | 85.29 | 0.718 |
| Rhodobacter                 | 1.859 | 2.438 | 87.72 | 1.4  |
| Fluviicola                  | 0.8349| 1.095 | 88.82 | 0.0449|
| C1-B045                    | 0.5064| 0.6642| 89.48 | 0.0112|
| Lutibacter                  | 0.501 | 0.6571| 90.14 | 0.00187|
| Rhodobacteraceae           | 0.5009| 0.657 | 90.8  | 0.141 |
| Brevundimonas              | 0.478 | 0.6271| 91.42 | 0.143 |
| Owenweeksia                | 0.4295| 0.5634| 91.99 | 0.00322|
| Methylotenera              | 0.3495| 0.4585| 92.45 | 1.17  |
| Algoriphagus               | 0.3279| 0.4301| 92.88 | 0.281 |
| Bdellovibrio               | 0.3242| 0.4252| 93.3  | 0.000374|
| Sphingomonadaceae          | 0.3178| 0.4169| 93.72 | 0.0007 |
| Emticia                    | 0.3154| 0.4137| 94.13 | 0.0248|
| Planctomyces               | 0.2624| 0.3442| 94.48 | 0    |
| Hyphomonas                 | 0.2491| 0.3268| 94.8  | 0.00435|
| Parvibaculum               | 0.2264| 0.297 | 95.1  | 0.00914|
| Saprospiraceae            | 0.2211| 0.29  | 95.39 | 0.0243|
| CHAB-XI-27                 | 0.1967| 0.258 | 95.65 | 0.595 |
| WCH81-32                  | 0.1893| 0.2484| 95.9  | 0.208 |
| Pirellula                  | 0.1803| 0.2365| 96.13 | 0    |
| Pseudomonadaceae           | 0.1713| 0.2247| 96.36 | 0.0276|
| Candidatus_Captivus        | 0.1709| 0.2242| 96.58 | 0.484 |
| Coxiella                   | 0.1541| 0.2022| 96.78 | 0    |
| Phenyllobacterium          | 0.1476| 0.1936| 96.98 | 0.000748|
| Microbacterium             | 0.1326| 0.1739| 97.15 | 0.0224|
| Comamonadaceae             | 0.1288| 0.1689| 97.32 | 0.247 |
| Pseudospirillum            | 0.1165| 0.1528| 97.47 | 0.00035|
| Candidate_division_OD1     | 0.1062| 0.1393| 97.61 | 0.0157|
| Caulobacter                | 0.1022| 0.1341| 97.75 | 0.466 |
|          |          |          |          |          |
|----------|----------|----------|----------|----------|
| Sulfuritalea | 0.1017   | 0.1334   | 97.88    | 0.0946   |
| Methylibium | 0.09204  | 0.1207   | 98       | 0.181    |

**Figures**

![Map of the area with geological features and sampling locations.](image-url)
Figure 1

Map A showing location of principal sandstone-hosted uranium districts of the South-East Mongolia, and operating ISR mines (Modified from Hocquet, 2016).

Map B showing the location of each piezometer within the K2Ss2 aquifer sampled during this study (X, Y = WGS84 coordinates, UTM zone 49N, in m).

Figure 2

Representation (heatmap) of the water chemistry through the different compartments of the roll front. Concentrations were corrected for the salinity gradient and normalized (see Additional file 1).
Figure 3

PCoA plot showing the relationship between the bacterial population structures of the water samples-(based on Bray Curtis index).
Figure 4

Taxonomic distribution of water bacterial community at genus level
Figure 5

Heatmap based on the relative abundance of the genera with an average abundance of > 0.1% in at least one sample.
LEfSe analysis of microbial abundance with significant difference from phyla to genus level detected in the water samples collected from the different compartments of the roll-front deposit.
A canonical correspondence analysis plot reveals the relationships between the target bacterial genera and the geochemical parameters.
Figure 8

Network analysis revealing the co-occurrence patterns among bacterial taxa and environmental parameters. The nodes are colored according to modularity class. A connection represents a strong correlations based on Pearson's correlation coefficient ($\rho$ of $>0.6$). The size of each node is proportional to the number of connections, i.e., the degree.

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