Bioremediation potential of bacterial species from a contaminated aquifer undergoing intrinsic remediation

A contaminated aquifer and bioremediation potential of bacterial species

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Abstract

As a mean to understand bacterial species involved in an ongoing bioremediation of a confined aquifer contaminated by a municipal solid waste landfill, culture-dependent and fluorescence microscopic techniques were used. Water samples from the contaminated aquifer and a background aquifer were extracted and subjected to chemical, bacteriological and microscopic analysis. Eighty-seven bacterial species were isolated, representing four phyla: *Actinobacteria* (25.3%), *Bacteroidetes* (16.1%), *Firmicutes* (3.4%) and *Proteobacteria* (55.2%). Among the *Proteobacteria* were *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria*, in order of increasing abundance. A clear distinction between uncontaminated and contaminated groundwater was observed. Water samples from the uncontaminated groundwater had both low cell density and low species richness, as is expected of oligotrophic aquifers. On the other hand, water samples from the contaminated groundwater had higher cell density and species richness. The highest species richness was recorded from the distal well. The difference observed between the uncontaminated and contaminated groundwater samples highlights the influence of the landfill leachate on the microbial ecology. Majority of the species detected in the contaminated groundwater represented taxa frequently recovered from contaminated environments, with 47% of these having documented bioremediation potential either at species or at genus level. It is likely that the landfill leachate promoted a community of mostly heterotrophic culturable bacteria, as comparison between direct microscopic and plate counts seems to suggest so.

Introduction

Groundwater is the main source of freshwater for drinking, agriculture and industry in many places globally [1, 2], but faces serious pollution challenges worldwide [3]. Among the

human activities that have caused severe damage to groundwater resources is landfill. All over the world, landfills have served as the ultimate destination for municipal solid wastes [4], and continue to do so [5]. In Norway, there was little recycling of wastes until the late 1990s and most of the wastes from households and industries were deposited in municipal solid waste landfills with no provision for treatment of the resultant leachate. Revdalen Landfill represents one such historic site and was active from 1974 to 1997, which led to the contamination of Revdalen Aquifer.

Several strategies exist to reclaim contaminated aquifers. They are broadly categorised as natural and artificial. The latter, which includes the conventional pump and treat (P&T) are faster, but require a major economic input for operation and maintenance [6]. Natural attenuation such as *in situ* bioremediation on the other hand, offers inexpensive, eco-friendly yet efficient remedies [7, 8]. In addition, unlike P&T, *in situ* bioremediation does not generate secondary wastes. It is the most widely accessed in *in situ* remediation of groundwater [2]. However, *in situ* bioremediation, particularly intrinsic bioremediation is slow and the groundwater remains polluted for long, although it may be speeded up by amended bioremediation [7].

Traditionally, groundwater bioremediation has been demonstrated empirically by measuring geochemical parameters. Over the years, however, it has become apparent that studying microbial community composition in addition to geochemical measurements offers a more complete picture of bioremediation. In order to make inferences about bioremediation and effectively manage the processes, a survey to establish which microorganisms are responsible is necessary [9-11]. Both past and recent studies have been conducted on microbiomes of contaminated aquifers [12-20]. Nonetheless, this area still requires more elucidation [21, 22]. Bioremediation of hydrocarbon-polluted aquifers is well documented in the literature [14, 16,

19, 20, 23-25], while there is a dearth of studies on landfill-leachate contaminations. The bias may reflect high-profile cases of hydrocarbon pollutions and the potential health hazard presented by the concomitant xenobiotics, which are often toxic, mutagenic and carcinogenic [7]. Moreover, hydrocarbons, at least are, easily degraded in the environment and their compositions are less complex than effluents emanating from landfills. The complicated attenuation processes in landfill-leachate-impacted groundwater makes assessments of bioremediation processes a more difficult and less attractive venture, which therefore receives less attention than it deserves.

In the present study, we studied microbial diversity of a landfill-leachate-contaminated confined aquifer. The aquifer is undergoing intrinsic bioremediation and as a mean to understand which microbes may be responsible for the bioremediation, we isolated and characterised bacterial species from the aquifer. Parallel to this, we conducted direct microscopic count as a check for bias from culture-dependent method. High throughput sequencing is also being undertaken and is the subject of a future manuscript.

Materials and methods

Groundwater sampling and chemical analysis

Groundwater sampling is described elsewhere (Abiriga et al., unpublished). pH and electrical conductivity were determined in the field using pH-110 meter (VWR International) and Elite CTS Tester (Thermo Scientific, Singapore), respectively. Dissolved oxygen was determined using the Winkler method. Alkalinity was measured upon arrival at the laboratory using Mettler DL25 Titrator (Mettler Toledo, Switzerland). Major ions (sodium, potassium, calcium, magnesium, chloride, nitrate and sulphate) were determined using Ion Chromatography DIONX ICS-1100 (Thermo Scientific, USA). Total nitrogen and total

organic carbon were determined using FIAlyzer-1000 (FIAlab, USA) and TOC Fusion (Teledyne Tekmar, USA), respectively. Iron and manganese were measured using AAnalyst-400 (PerkinElmer, USA).

Bacteriology

Direct microscopic count

Water samples were collected in sterile 350 ml PETE bottles (VWR, UK). 4.5 ml water samples were fixed with 2.5% phosphate-buffered glutaraldehyde and stained with 5 µg/ml 4′,6-diamidino-2-phenylindole (DAPI) [26]. The stained cells were filtered onto 0.2 µm black polycarbonate Nuclepore membrane filters (Sigma-Aldrich, Germany), transferred onto microscope slides and overlaid with antifade mountant oil (Citifluor AF87, EMS, PA, USA). Cells were enumerated under × 100 oil objective using Olympus IX70 fluorescence microscope (Tokyo, Japan). Ten fields were counted and the average count was used to estimate bacterial density using the formula:

Bacteria (Cells/ml) = $(N \times A_t)/(V_f \times A_g \times d)$,

where N = average number of cells, A_t = effective area of the filter paper, V_f = volume of water sample filtered, A_g = area of the counting grid, and d = dilution factor [26]. No observable cells were found in our blanks and therefore correcting for background noise due to contamination was not necessary.

Evaluation of growth media

Serial dilutions of up to 10^6 of representative water samples were prepared. 100 µl of the dilutions were spread on nutrient agar, plate count agar and full-strength, half-strength, one-

111 third-strength and one-fourth-strength tryptic soy agar in triplicate. Inoculated plates were
112 incubated at 15 ℃ for >5 days.

113 Sulphate-reducing medium was prepared following method by Ňancucheo, Rowe (27), with
114 modification as follow; no addition of trace elements and pH adjusted to 6.5. Sulphide-
115 oxidising medium was prepared according to Gregorich and Carter (28). Lastly, iron-
116 oxidising medium was prepared according to Holanda, Hedrich (29), again adjusting to pH
117 6.5. The media were inoculated with 100 µl of water samples. Plates for sulphide-oxidising
118 were incubated at least one week, while sulphate-reducing and iron-oxidising plates were
119 incubated for at least four weeks. Sulphide-oxidising and iron-oxidising plates were incubated
120 under aerobic condition, while sulphate-reducing plates were incubated under anaerobic
121 condition.

122 Colony counting

123 Aerobes were counted following incubation under aerobic condition. Anaerobes were
124 enumerated in a parallel setup following incubation under anaerobic condition using GasPak
125 with EZ Anaerobe Container System (BD, USA). Two plates from two dilutions of each
126 sample were counted and the average was reported as colony forming units per ml.

127 Based on observable colony morphologies such as shape, elevation, margin, size, and colour,
128 etc., colonies covering the full diversity of colony morphologies were picked and purified by
129 repeated streaking and incubation until pure cultures were obtained. Pure cultures were then
130 subjected to wet field microscopy at × 1,000 (Olympus, CX22LEDRFS1, China) to observe
131 motility and cell shape, gram staining, oxidase activity, and catalase activity [30]. Strains
132 were stored at -70 ℃ in nutrient broth (Sigma, Switzerland) supplemented with 25% glycerol.

133 **16S rRNA sequencing and strain identification**

134 Approximately 5.0 µl of each pure culture was suspended in 750 µl PBS and centrifuged at
135 10,000 × g for 3 minutes. The supernatant discarded and the cell pellet stored at -20 ℃ prior
136 to DNA extraction. Frozen cell pellets were allowed to thaw and 2.5 µl of the suspension was
137 re-suspended in 100 µl of PCR-grade distilled water. The tubes were incubated at 99 ℃ for 10
138 minutes and then centrifuged at 10,000 × g for 3 minutes. The DNA in the supernatant was
139 quantified using Qubit® Flourometer 3.0 (Life Technologies, Malaysia).

140 A universal primer set (341f/926r) [31, 32] was used to amplify the 16S rRNA gene region
141 V3-V5. The PCR reaction was carried out in a 50 µl reaction mixture containing 10-50 ng
142 template DNA; 1X Perfecta SYBR Green PCR FastMix (Quanta Biosciences); 300 nM
143 forward and reverse primer (341f/926r, Invitrogen). Amplification was performed in a Real-
144 Time PCR format using the StepOne instrument (Applied Biosystems, Foster City, CA, USA)
145 with the following temperature profiles: initial holding at 95 ℃ for 10 min; 45 cycles of
146 denaturing (95 ℃, 15 sec), annealing (50 ℃, 30 sec) and extension (72 ℃, 2 min). A melting
147 curve was performed over the temperature range 60-95 ℃.

148 PCR product clean-up was conducted using one-step enzymatic EXO STAR solution (GE
149 Healthcare Illustra ExoProStar, UK) following manufacturer's instructions. Cycle Sequencing
150 was performed using BigDye Terminator v1.1 Sequencing Kit (ThermoFisher Scientific)
151 according to manufacturer's instructions. For clean-up of sequencing products, both ethanol
152 precipitation and BigDye XTerminator Purification Kit (ThermoFisher scientific) were
153 assessed. The former yielded better results and was subsequently preferred to the latter.
154 Purified Cycle Sequencing products were analysed using Genetic Analyser 3130xl from

155 Applied Biosystems. The 16S rRNA gene sequences of the 87 isolates have been deposited in
156 GenBank under Accession No. MT348616-MT348702.

157 **Data analysis and statistics**

158 Chromatogram files were processed using ChromasPro v2.1.8. Species identification was
159 conducted by BLAST search in NCBI database. Reference sequences of the species were
160 retrieved from GenBank and used to construct a phylogenetic tree, constructed using Mega X
161 [33].

162 Water chemistry and cell count data were analysed using R [34]. To compare water chemistry
163 between the background value and the contaminated water samples, one-tailed Wilcox signed
164 rank test was used, as the majority of the variables showed non-normal distribution. One-
165 tailed paired t-test was used for within-sample and overall comparison between plate and
166 microscopic counts. Comparison between aerobic and anaerobic counts was also done using
167 one-tailed paired t-test. Some of the data pairs for t-test were $\log_{10}$-transformed to conform to
168 normal distribution. Data distribution for normality was assessed graphically using histograms
169 and by Shapiro-Wilk normality test. Statistical significance was inferred at alpha = 0.05.

170 # Results

171 ## Groundwater chemistry

172 Concentrations of most of the solutes in the groundwater have decreased greatly compared to
173 previous data (Abiriga et al., unpublished), but sulphate and nitrate continue to be leached at
174 higher amounts. Despite the decrease, most of the solutes still occur at levels significantly
175 above that of the background value (Table 1). Concentrations of the solutes decreased along
176 the groundwater flow path from R1 through R2 to R4, (henceforth referred to proximal,

intermediate and distal wells, respectively). Similarity of water chemistry was greatest between the proximal and intermediate wells.

Table 1. Characteristics of the groundwater chemistry measured between 2018 and 2019. Values from R0 were used as a benchmark against which values of R1, R2 and R4 were compared. All units are in mg/l, except for pH (pH units), conductivity (µS/cm) and alkalinity (mM).

	Mean	**Range (min & max)**		
	R0 (N=4)	R1 (N=20)	R2 (N=16)	R4 (N=12)
pH	4.9	6.0-7.7 [s]	5.9-7.1 [s]	5.6-6.2 [s]
Conductivity	35	145-251 [s]	102-220 [s]	78-190 [s]
Dissolved oxygen	4.14	0.20-3.42 [s]	0.59-4.79 [s]	1.14-9.12 [n]
Sodium	1.74	4.52-6.91 [s]	4.96-5.63 [s]	3.99-5.69 [s]
Potassium	0.48	5.00-7.52 [s]	4.81-7.49 [s]	6.38-9.92 [s]
Calcium	1.95	24.67-39.18 [s]	17.79-27.44 [s]	10.66-21.74 [s]
Magnesium	0.50	1.80-2.84 [s]	2.69-3.43 [s]	2.68-3.66 [s]
Ammonium	0.00 [a]	0.00-1.91 [s, a]	0.00-0.57 [s, a]	0.00-0.41 [s, a]
Iron	0.02 [d]	0.02-0.02 [d]	0.02-0.02 [d]	0.02-0.09 [n]
Manganese	0.04	0.04-0.65 [s]	0.03-0.31 [s]	0.01-1.42 [s]
Alkalinity	0.05	0.71-1.92 [s]	0.58-1.66 [s]	0.49-1.33 [s]
Sulphate	2.78	8.38-24.62 [s]	7.35-11.86 [s]	5.71-10.85 [s]
Nitrate (as Nitrogen)	1.51	1.26-4.83 [s]	0.38-3.71 [n]	1.28-3.95 [s]
Chloride	2.02	3.37-7.44 [s]	4.34-8.45 [s]	3.62-6.30 [s]
Total nitrogen	1.50	1.62-4.54 [s]	0.84-3.48 [s]	1.20-3.41 [s]

Total organic carbon	4.06	2.68-5.51 [n]	2.90-6.80 [n]	1.82-6.31 [n]

N = number of samples

[s] Values are significantly higher ($P < 0.05$) than the background value, except dissolved oxygen which is lower than the background value.

[n] non-significant difference ($P > 0.05$)

[a] values below the limit of detection were treated as zero.

[d] Values are half the limit of detection (50 ppb).

Cell density and bacterial species

Selection of growth condition

Bacterial growth was examined on tryptic soy agar, plate count agar and nutrient agar. Among these three nutrient media, tryptic soy agar gave the highest number of bacterial colonies. Best growth was obtained with half-strength medium and was thus selected for the isolation of bacteria. The cell density estimate from the plate count was in the range 1×10^2 - 3.2×10^5 (aerobic incubation) and 0 - 2.4×10^5 (anaerobic incubation) cfu/ml. Despite the comparable maximum counts from the two growth conditions, anaerobic plate counts were significantly lower than aerobic plate counts ($t = 3.628$, $df = 37$, $P < 0.05$). Isolates recovered under anaerobic condition were all facultative anaerobes; the same strains dominated under both aerobic and anaerobic conditions, however, some strains were isolated only under anaerobic condition, even though they were facultative anaerobes. Such strains would have been missed if only aerobic incubation was carryout. Wells in the contamination plume had higher plate counts than the background well and the distal well had lower plate counts compared to the intermediate and proximal wells (Fig 1).

204

205 **Fig 1.** Bacterial cell density in the groundwater samples estimated from aerobic plate count
206 (PC) and direct microscopic count (MC), error bars are mean + standard error. R0 is the
207 background well located in a nearby uncontaminated aquifer upstream of the landfill, while the
208 multilevel wells (R1 – R4) are located downstream of the landfill in the contaminated aquifer
209 along groundwater flow direction.

210 Selective culture for sulphate-reducing, sulphide-oxidising and iron-oxidising bacteria was
211 also attempted. Growth was observed in sulphate-reducing and sulphide-oxidising conditions,
212 although strains isolated under sulphate-reducing condition were non-sulphidogenic. No
213 growth was observed on iron-oxidising medium, except fungal mycelium and yeast.

214 Direct microscopic count

215 Microscopic counts were in the range 7×10^3 - 3.5×10^5 cells/ml. Cell morphologies observed
216 under fluorescence microscopy included small rods, long large rods, coccobacilli and vibrios.
217 Small rods were most frequently encountered. Water samples from the proximal and
218 intermediate wells apparently were dominated by short rods, vibrios and elongated narrow
219 rods, some occurring in chains. The distal well on the other hand, did not exhibit
220 predominance of any specific cell types, an observation that agrees with the more diverse
221 colony morphology observed in samples from this well.

222 There was a tendency of decreasing cell density with depth in the proximal well, while the
223 intermediate well showed an increase with depth. The distal well which showed very low
224 plate counts gave higher microscopic count. Within-sample comparison indicates that
225 microscopic counts were in all but one case higher than plate counts (Fig 1), but only

226 significantly so in 4 of the 13 samples ($P < 0.05$). Overall, microscopic count was higher than
227 plate count ($t = 6.935$, $df = 51$, $P < 0.05$) (S1 Fig).

228 Species composition

229 Small subunit rRNA gene sequencing of pure isolates revealed higher species richness in the
230 wells located in the contamination plume than in the background well (Fig 2). The distal well
231 had the highest species richness. Class- and family-level classifications are provided in S2 and
232 S3 Fig. The dominant phylum in the aquifer was *Proteobacteria*, representing 55.2% of the
233 total isolates. *Gamma (γ)-proteobacteria* and *Beta (β)-proteobacteria* were detected in all the
234 wells, while *Alpha (α)-proteobacteria* were detected only in the proximal and intermediate
235 wells (S2 Fig). Phylum *Bacteroidetes* appears to be enriched in the first two levels of the
236 proximal well. Forty-six genera and 87 species were identified (Fig 2, S1 Table and S4 Fig).
237 Genus *Pseudomonas* was the most abundant taxon (S5 Fig).

238

239 **Fig 2.** Bacterial genera recovered from the groundwater samples. Species in R0 represents
240 biodiversity of the uncontaminated aquifer located upstream of the landfill. Species
241 composition of the multilevel wells R1-R4 show biodiversity of the contaminated aquifer at
242 the proximal, intermediate and distal positions, respectively.

243 Of the eighty-seven species isolated, 7 were found only in the background aquifer, 77 found
244 only in the contaminated aquifer and three were found in both. These three species were
245 *Rhodoferax ferrireducens* and *Pseudomonas* sp. (2) detected in the background and distal
246 wells, and *Rugamonas* sp. detected in the background, intermediate and distal wells (S1
247 Table). As shown in Fig 3, 28 species were unique to the distal well. No single species was
248 found in all the four wells, although two genera (*Pseudomonas* and *Janthinobacterium*) were

common. Among the wells located in the contamination plume, only two species i.e. *Pseudomonas veronii* and *Rhodococcus degradans* were found in all of them. The proximal-intermediate was the most similar pair of wells, with 10 species in common.

Fig 3. Venn diagram showing relationships among the groundwater samples at genus-level classification **(A)** and species-level classification **(B)**. R0 is the background well located upstream of the landfill in a nearby aquifer, while R1, R2 and R4 are wells located downstream of the landfill in the contamination plume along the groundwater flow direction.

Discussion

Cell density and species richness

Cell density estimates with plate count were generally lower than microscopic count, which is consistent with the literature [28, 35]. However, this difference was not much greater than a factor of two in most cases, and only in four of the thirteen samples was the difference significant. This indicates that in most samples, a large proportion of the microbial population is culturable. Presumably, the nutrients in the landfill leachate favour the growth of culturable heterotrophic microorganisms. Microscopic examination of the water samples from the proximal and intermediate wells showed a limited diversity of cell types, which is consistent with the plate cultures, which were in most cases dominated by colonies of a few species: *Pseudomonas veronii*, *Rhodococcus degradans*, *Pedobacter, Brevundimonas* and *Polaromonas*.

The background aquifer has a low nutrient content and both the cell density and the microbial diversity are low, although comparison of microscopic and plate counts suggest that a fairly

271 large proportion of the population is non-culturable. The contaminated aquifer, on the other
272 hand, is nutrient-rich and supports a denser and more diverse population. A low cell density
273 was also encountered at the deepest level of the proximal well (R105), although this location
274 is not nutrient-deficient. In this case, the low cell density is probably due to filtering effects of
275 the sediment, which, at this level is expected to contain much fine-grained material from the
276 till layer.

277 Groundwater quality was found to improve with distance from the landfill (Table 1), and this
278 was accompanied by an increase in biodiversity from proximal to distal wells. This may be
279 analogous to increase in diversity and community stability as leachate becomes less
280 contaminated over time [11]. We suggest that close to the landfill, only species resistant to the
281 toxic effects of the leachate are able to survive and grow, while at more distant locations,
282 toxic pollutants become attenuated by biodegradation, precipitation, sorption and dilution,
283 allowing the growth of more sensitive species. This might explain the observation in Fig 3,
284 where only 2 species were shared among the proximal, intermediate and distal wells, 10
285 species shared between the proximal and intermediate wells, and 28 species exclusive to the
286 distal well. This also suggests that the conditions prevailing in the proximal and intermediate
287 wells are relatively similar (which is reflected by the chemistry), thereby selecting for similar
288 taxa. Additional supporting information can be accessed from the online supplementary
289 material (S6 Fig).

290 Species composition and their bioremediation potential

291 Phylum *Actinobacteria*

292 The *Actinobacteria* in the genus *Arthrobacter* have been investigated for their potential in
293 biodegradation of xenobiotics and other harmful compounds [36]. Six known species and two

294 unidentified species of *Arthrobacter* were detected in Revdalen samples. The "*A. oxydans*
295 group" (*Pseudarthrobacter phenanthrenivorans, P. defluvii, P. sulfonivorans* and *P.*
296 *siccitolerans*), "*A. psychrolactophilus* group" (*A. livingstonensis*), and the "*A. globiformis*
297 group" (*A. globiformis*). *P. defluvii* is a 4-chlorophenol degrading microorganism [37], *P.*
298 *phenanthrenivorans* and *A. globiformis* are phenanthrene degrading bacteria [38, 39], and *P.*
299 *sulfonivorans* is dimethylsulfone degrading [40]. *P. siccitolerans,* and *A. livingstonensis* have
300 no known bioremediation potentials, except biogeochemical cycling, e.g. sulphate reduction
301 by *A. livingstonensis* and nitrate reduction by *P. siccitolerans* (S2 Table). Congeners of
302 polycyclic aromatic hydrocarbons (PAHs) have been detected in the contaminated
303 groundwater, albeit in minute concentrations (Abiriga et al., unpublished). This could have
304 contributed to the establishment of the *Arthrobacter* species with specialised metabolic
305 potentials.

306 Members of genus *Rhodococcus* (family *Nocardiaceae*) are widely distributed in various
307 types of environments including both pristine and hydrocarbon-contaminated soil, marine
308 sediments, sludge, freshwater and saltwater [41, 42]. The presence of aerobic alkane-
309 degrading *alkB* gene in almost every member of the genus have made *Rhodococcus*
310 biotechnologically valuable microorganisms [41]. Among the *Rhodococcus* isolated from
311 Revdalen Aquifer was *R. erythropolis*. This species possesses remarkable bioremediation
312 potential, being capable of degrading a wide range of halogenated hydrocarbons including
313 polychlorobiphenyls [36, 43], diesel oil, *normal*-, *cis*- and *cyclo*paraffins and aromatic
314 compounds e.g. naphthalene [44, 45]. Another *Rhodococcus* species recovered was *R.*
315 *degradans,* a species capable of degrading several complex organic compounds including
316 polychlorinated biphenyls (PCBs) and PAHs [42]. This was the dominant species in phylum
317 *Actinobacteria* and was recovered from all the wells in the contaminated aquifer. Other

318 species included *R. sovatensis*, so far with no reported bioremediation ability, but by the fact
319 that the type species was characterised based on *alkB* gene [41] is an indication of its potential
320 in biodegradation.

321 Other members of *Actinobacteria* recovered belong to family *Microbacteriaceae*, comprising
322 of *Cryobacterium psychrotolerans, C. articum*, *Microbacterium hydrocarbonoxydans*, *M.*
323 *maritypicum*, *Microtericola viridarii, Curtobacterium flaccumfaciens*, *Plantibacter auratus*,
324 and *Leifsonia* spp. Bioremediation capabilities include degradation of alkane by
325 *Cryobacterium*, toluene by *Plantibacter* [46], and crude-oil by *M. hydrocarbonoxydans* and
326 *Leifsonia* sp. [47, 48].

327 Family *Nocardioidaceae* and *Streptomycetaceae* within clade *Actinobacteria* are each
328 represented by a single species of respectively, *Aeromicrobium fastidiosum* and *Streptomyces*
329 sp. No bioremediation potential have been described for either species yet. The latter might
330 influence microbial interaction since the genus is a renowned biocin producer.

331 Phylum *Bacteroidetes*

332 Phylum *Bacteroidetes* is represented by *Sphingobacteriia*, *Cytophagia* and *Flavobacteria*.
333 Members of *Bacteroidetes* may be strict or facultative anaerobes with hydrolytic and
334 fermentative metabolism [11], but so far the known hydrocarbon degraders are aerobes [49].

335 Seven species of the genus *Pedobacter* (*Sphingobacteriia*) were recovered. These included *P.*
336 *cryoconitis*, *P. africana*, *P. quisquiliarum*, *P. lusitanus*, *P. kyunghensis*, *P. panaciterae*, and
337 *P. trunci*. The strains were originally isolated from respectively, alpine glacier cryoconite
338 [50]; soil and activated sludge [51]; activated sludge [52]; deactivated uranium mine sludge
339 [53]; soil of ginseng field [54]; soil [55]; and tree trunk [56]. *P. cryoconitis* was found to be
340 capable of degrading diesel oil [50], a feature that is of importance in bioremediation. The fact

341 that some of the isolates were discovered from sludge suggests that they are capable of
342 biodegradation.

343 Members of class *Flavobacteria* detected included *F. aquidurense*, *Flavobacterium* sp.,
344 *Cryseobacterium lactis* and *C. viscerum*. Strains of genus *Flavobacterium* are capable of
345 degrading biomacromolecules [57], e.g. biphenyl and phenanthrene [58]. The type species of
346 *Cryseobacterium lactis* and *C. viscerum* were originally isolated from milk and diseased fish
347 respectively; to our knowledge this is the first report of their isolation from environmental
348 samples. Class *Cytophagia* comprised of two species of *Dyadobacter hamtensis* and
349 *Dyadobacter sediminis*, neither of which have documented bioremediation potentials.

350 Phylum *Proteobacteria*

351 Of the three classes of *Proteobacteria* found, *β-proteobacteria* was only represented by order
352 *Burkholderiales*, while *α-* and *γ-proteobacteria* were composed of three and four orders,
353 respectively: *Caulobacterales*, *Rhizobiales*, *Sphingomonadales, Aeromonadales*,
354 *Enterobacterales*, *Pseudomonadales* and *Xanthomonadales*.

355 Although members of *β-proteobacteria* may grow more slowly than pseudomonads, they may
356 be more abundant and efficient degraders in natural environments [59]. This is consistent with
357 our finding that order *Burkholderiales* was more abundant (40%) than *Pseudomonadales*
358 (23%). Its members constitute those with more versatile catabolic potentials than
359 *Pseudomonadales*. Members of family *Burkholderiaceae* with biotechnological relevance
360 were *Paraburkholderia xenovorans* and *Burkholderia pseudomultivorans*, the latter being
361 recovered from the background well. *P. xenovorans* is a PCB-degrading bacterium originally
362 isolated from PCB-contaminated landfill soil [60], and so far the best-known aerobic PCB
363 degrader [61]. However, PCBs have not been detected at Revdalen (Abiriga et al.,

364 unpublished). *B. pseudomultivorans* might be speculated to involve in biodegradation, given
365 that it is a member of the renowned *Burkholderia cepacia* complex [62]. Although such
366 degradation would only involve naturally occurring organic matter.

367 The number of taxa from family *Comamonadaceae* was greater than from *Burkholderiaceae.*
368 It included genus *Polaromonas,* which frequently inhabits cold oligotrophic environments and
369 was represented by four species: *P. naphthalenivorans, P. aquatica, P. jejuensis* and *P.*
370 *vacuolata*. Bioremediation potentials include degradation of biphenyl and benzoate by *P.*
371 *naphthalenivorans* [63, 64] and pyrene by *P. vacuolata* [65]. Another genus was
372 *Janthinobacterium*, represented by *J. lividum*, *J. svalbardensis*, and *J. agaricidamnosum*. The
373 last species has been found associated with a consortium involved in degradation of
374 phenanthrene [39]. Still among members of *Comamonadaceae* is *Simplicispira psychrophila*,
375 a species originally recovered from wastewater [66], which suggests its ability to biodegrade.
376 Lastly, *Variovorax boronicumulans* is a species known to bioaccumulate boron [67], but quite
377 recently, a *Variovorax* sp. was found to be responsible for biodegradation of benzene in a
378 coal-tar contaminated aquifer [68].

379 Isolates of *Pseudomonas* (*γ-proteobacteria*) included *P. silesiensis, P. veronii, P. brenneri, P.*
380 *migulae, P. koreensis, P. frederiksbergensis, P. salomonii* and *Pseudomonas* spp. *P.*
381 *frederiksbergensis,* and *P. salomonii* were recovered from the background well. Member of
382 the genus *Pseudomonas* are ubiquitous bacteria inhabiting a variety of environmental niches,
383 and are metabolically versatile microorganisms capable of utilising a wide range of simple, as
384 well as complex organic compounds [69, 70]. Genome analysis of *P. silesiensis* has revealed
385 bioremediation potential [70], while *P. veronii* is naphthalene and pentachlorophenol
386 degrading [71, 72]. *P. frederiksbergensis* is phenanthrene degrading [73], which illustrates the
387 intrinsic potential of native microorganisms to decontaminate environments, as the species

388 was recovered from the upstream background well. The other *Pseudomonas* species are
389 normal freshwater microbes, except *P. salomonii*, which is a plant pathogen.

390 *Tolumonas* sp., a member of order *Aeromonadales* in *γ-proteobacteria* was also recovered
391 from the water samples. Its closest relative *T. auensis* can transform phenylalanine, phenyl-
392 acetate, phenyl-lactate and phenyl-pyruvate to toluene, and transform tyrosine to phenol [74].

393 Members of order *Xanthomonadales* recovered included *Luteibacter rhizovicinus*,
394 *Rhodanobacter denitrificans* and *Rhodanobacter* sp. Bioremediation potential include
395 phenanthrene degradation by an unidentified member of *Luteibacter* [75], while *R.*
396 *denitrificans* as the name implies, could be involved in denitrification processes in the aquifer.
397 Another study [76] reported *Rhodanobacter*-enriched microbial community in a metal-
398 contaminated aquifer, implying high resistance to metal toxicity by members of the genus.
399 Further, unidentified members of *Rhodanobacter* have been recovered from a
400 Benzo[a]pyrene-degrading consortium [77]. Still within order *Xanthomonadales*, genus
401 *Stenotrophomonas* was represented by four species. These included the clinically relevant and
402 pyrene degrading *S. maltophilia* [78], *S. rhizophila* with antifungal activity [79], *S.*
403 *tumulicola*, and unidentified *Stenotrophomonas* sp.

404 Order *Enterobacterales* included *Klebsiella aerogenes*, *Rouxiella badensis*, *Ewingella*
405 *americana*, *Erwinia billingiae* and *Rahnella woolbedingensis*. While scientific interest in this
406 order chiefly focuses on its medical significance, unidentified members of *Rahnella* and
407 *Klebsiella* have been implicated in bioremediation of respectively, naphthalene [80] and crude
408 oil [81].

409 Class *α-proteobacteria* were represented by species in four genera: *Brevundimonas*
410 *mediterranea*, *Methylobacterium, Sphingomonas*, and *Phyllobacterium myrsinacearum*. *B.*

411 *mediterranea* is naphthalene degrading [71]. *Methylobacterium* sp. have been implied in
412 trichloroethene bioremediation [82]. *Sphingomonas* was represented by *S. echinoides* and an
413 unidentified *Sphingomonas* sp. which closely resembles *S. faeni*. Bioremediation potential of
414 genus *Sphingomonas* include degradation of trichloroethene [82], phenanthrene [39] and
415 dibenzofuran [83]. *P. myrsinacearum* has no known bioremediation potential, but may be
416 relevant for nutrient cycling particularly nitrogen and sulphur.

417 Phylum *Firmicutes*

418 The phylum *Firmicutes* was represented by three species; *Exiguobacterium acetylicum,*
419 *Staphylococcus warneri* and *Sporosarcina aquimarina*. *S. warneri* has been implicated in
420 degradation of diesel oil [84], while *E. acetylicum* as the name suggests degrades metabolic
421 intermediate products such as acetate. No literature is available on bioremediation potential of
422 *S. aquimarina*.

423 Biogeochemical transformation

424 Attempt to cultivate iron oxidising bacteria was unsuccessful, but a potential iron reducer,
425 *Rhodoferax ferrireducens* was recovered on nutrient medium. This species is also capable of
426 respiring on manganese and nitrate [85]. While iron levels were very low and often below the
427 limit of detection, manganese and nitrate were measurable. The maximum manganese
428 coincided with the presences of *R. ferrireducens* and principal component analysis showed a
429 positive correlation between this species and manganese (S6 Fig). This suggests that the
430 species is actively involved in biogeochemical cycling of manganese, and possibly nitrate.

431 For sulphur cycling, growth was observed under sulphide-oxidising and sulphate-reducing
432 conditions. Growth of sulphide-oxidising bacteria, such as *B. mediterranea*, which showed

433 high abundance in wells with higher sulphate concentration, is suggestive of *in situ* sulphide
434 oxidation. With two exceptions, all sulphate-reducing bacteria were isolated from the
435 proximal and intermediate wells. The low dissolved oxygen levels in these wells (<1 mg/L)
436 would be conducive for sulphate reduction, although the low levels of TOC may limit its
437 extent. Additional information on sulphur and nitrogen cycling potential of the strains is given
438 in S2 Table.

439 Strains with other nutrient cycling potentials included; *Herbaspirillum autotrophicum*, a
440 bacterium capable of using carbon dioxide as a sole carbon source [86], *Herminiimonas*
441 *arsenicoxydans*, a well-known biogeochemical cycler that transforms arsenite to arsenate
442 [87].

443 Conclusion

444 This study shows that nearly half of the culturable bacterial strains from a landfill-
445 contaminated confined aquifer had bioremediation potential. Preliminary results (Khan,
446 unpublished) suggest that some of these strains have the ability to grow on low density
447 polyethylene. Comparison of microscopic cell counts and plate counts indicated that the
448 strains in the most contaminated parts of the aquifer were mostly culturable heterotrophs,
449 while a higher proportion of non-culturable strains was present in the distal well, where
450 contamination was most attenuated. Biodiversity was lowest in the uncontaminated well,
451 moderate in the proximal and intermediate wells and highest in the distal well. The higher
452 biodiversity in the distal wells is possibly as a result of plentiful residual nutrients and low
453 levels of toxic contaminants. Each well had a distinctive bacterial flora, but the proximal and
454 intermediate wells seemed to be ecologically related as they had more taxa in common. There
455 was a clear difference between the flora of the contaminated wells and the uncontaminated

well. A more comprehensive characterisation of species by DNA metabarcoding is currently underway.

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References

1. Mays D, Scheibe T. Groundwater Contamination, Subsurface Processes, and Remediation Methods: Overview of the Special Issue of Water on Groundwater Contamination and Remediation. Multidisciplinary Digital Publishing Institute; 2018.

2. O'Connor D, Hou D, Ok YS, Song Y, Sarmah AK, Li X, et al. Sustainable in situ remediation of recalcitrant organic pollutants in groundwater with controlled release materials: A review. Journal of Controlled Release. 2018;283:200-13.

3. Pous N, Balaguer MD, Colprim J, Puig S. Opportunities for groundwater microbial electro-remediation. Microbial biotechnology. 2018;11(1):119-35.

4. Reinhard M, Barker JF, Goodman NL. Occurrence and distribution of organic chemicals in two landfill leachate plumes. Environmental Science & Technology. 1984;18(12):953-61.

5. Eggen T, Moeder M, Arukwe A. Municipal landfill leachates: a significant source for new and emerging pollutants. Science of the Total Environment. 2010;408(21):5147-57.

6. Hyldegaard BH, Jakobsen R, Weeth EB, Overheu ND, Gent DB, Ottosen LM. Challenges in electrochemical remediation of chlorinated solvents in natural groundwater aquifer settings. Journal of hazardous materials. 2019;368:680-8.

7. Logeshwaran P, Megharaj M, Chadalavada S, Bowman M, Naidu R. Petroleum hydrocarbons (PH) in groundwater aquifers: An overview of environmental fate, toxicity, microbial degradation and risk-based remediation approaches. Environmental technology & innovation. 2018;10:175-93.

8. Nunes OC, Lopes AR, Manaia CM. Microbial degradation of the herbicide molinate by defined cultures and in the environment. Applied microbiology and biotechnology. 2013;97(24):10275-91.

9. Dlugonski J. Microbial biodegradation : from omics to function and application. Norfolk: Caister Academic Press; 2016.

10. Alfreider A, Vogt C, Babel W. Microbial diversity in an in situ reactor system treating onochlorobenzene contaminated groundwater as revealed by 16S ribosomal DNA analysis. Systematic and Applied Microbiology. 2002;25(2):232-40.

11. Köchling T, Sanz JL, Gavazza S, Florencio L. Analysis of microbial community structure and composition in leachates from a young landfill by 454 pyrosequencing. Applied microbiology and biotechnology. 2015;99(13):5657-68.

12. Anantharaman K, Brown CT, Hug LA, Sharon I, Castelle CJ, Probst AJ, et al. Thousands of microbial genomes shed light on interconnected biogeochemical processes in an aquifer system. Nature communications. 2016;7.

13. Cozzarelli IM, Suflita JM, Ulrich GA, Harris SH, Scholl MA, Schlottmann JL, et al. Geochemical and microbiological methods for evaluating anaerobic processes in an aquifer contaminated by landfill leachate. Environmental science & technology. 2000;34(18):4025-33.

14. Harvey RW, Smith RL, George L. Effect of organic contamination upon microbial distributions and heterotrophic uptake in a Cape Cod, Mass., aquifer. Applied and Environmental Microbiology. 1984;48(6):1197-202.

15. Hug LA, Thomas BC, Brown CT, Frischkorn KR, Williams KH, Tringe SG, et al. Aquifer environment selects for microbial species cohorts in sediment and groundwater. The ISME journal. 2015;9(8):1846-56.

16. Kleikemper J, Pombo SA, Schroth MH, Sigler WV, Pesaro M, Zeyer J. Activity and diversity of methanogens in a petroleum hydrocarbon-contaminated aquifer. Applied and environmental microbiology. 2005;71(1):149-58.

17. Ludvigsen L, Albrechtsen H-J, Ringelberg D, Ekelund F, Christensen T. Distribution and composition of microbial populations in a landfill leachate contaminated aquifer (Grindsted, Denmark). Microbial Ecology. 1999;37(3):197-207.

18. Röling W, Van Breukelen B, Braster M, Goeltom M, Groen J, Van Verseveld H. Analysis of microbial communities in a landfill leachate polluted aquifer using a new method for anaerobic physiological profiling and 16S rDNA based fingerprinting. Microbial Ecology. 2000;40(3):177-88.

19. Tischer K, Zeder M, Klug R, Pernthaler J, Schattenhofer M, Harms H, et al. Fluorescence in situ hybridization (CARD-FISH) of microorganisms in hydrocarbon contaminated aquifer sediment samples. Systematic and applied microbiology. 2012;35(8):526-32.

20. Watanabe K, Kodama Y, Hamamura N, Kaku N. Diversity, abundance, and activity of archaeal populations in oil-contaminated groundwater accumulated at the bottom of an underground crude oil storage cavity. Applied and environmental microbiology. 2002;68(8):3899-907.

21. Meckenstock RU, Elsner M, Griebler C, Lueders T, Stumpp C, Aamand J, et al. Biodegradation: updating the concepts of control for microbial cleanup in contaminated aquifers. ACS Publications; 2015.

526 22. He Z, Zhang P, Wu L, Rocha AM, Tu Q, Shi Z, et al. Microbial functional gene diversity
527 predicts groundwater contamination and ecosystem functioning. MBio.
528 2018;9(1):e02435-17.

529 23. Dojka MA, Hugenholtz P, Haack SK, Pace NR. Microbial diversity in a hydrocarbon-and
530 chlorinated-solvent-contaminated aquifer undergoing intrinsic bioremediation. Applied
531 and Environmental Microbiology. 1998;64(10):3869-77.

532 24. Pickup R, Rhodes G, Alamillo M, Mallinson H, Thornton S, Lerner D. Microbiological
533 analysis of multi-level borehole samples from a contaminated groundwater system.
534 Journal of Contaminant Hydrology. 2001;53(3-4):269-84.

535 25. Rooney-Varga JN, Anderson RT, Fraga JL, Ringelberg D, Lovley DR. Microbial
536 communities associated with anaerobic benzene degradation in a petroleum-contaminated
537 aquifer. Applied and Environmental Microbiology. 1999;65(7):3056-63.

538 26. Kepner R, Pratt JR. Use of fluorochromes for direct enumeration of total bacteria in
539 environmental samples: past and present. Microbiol Mol Biol Rev. 1994;58(4):603-15.

540 27. Ňancucheo I, Rowe OF, Hedrich S, Johnson DB. Solid and liquid media for isolating and
541 cultivating acidophilic and acid-tolerant sulfate-reducing bacteria. FEMS microbiology
542 letters. 2016;363(10).

543 28. Gregorich EG, Carter MR. Soil sampling and methods of analysis: CRC press; 2007.

544 29. Holanda R, Hedrich S, Ňancucheo I, Oliveira G, Grail BM, Johnson DB. Isolation and
545 characterisation of mineral-oxidising "Acidibacillus" spp. from mine sites and
546 geothermal environments in different global locations. Research in microbiology.
547 2016;167(7):613-23.

548 30. Csuros M, Csuros C, Ver K. Microbiological examination of water and wastewater. Boca
549 Raton, Fla: Lewis Publishers; 1999.

550 31. Lane DJ, Pace B, Olsen GJ, Stahl DA, Sogin ML, Pace NR. Rapid determination of 16S
551 ribosomal RNA sequences for phylogenetic analyses. Proceedings of the National
552 Academy of Sciences. 1985;82(20):6955-9.

553 32. Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, et al. Evaluation of
554 general 16S ribosomal RNA gene PCR primers for classical and next-generation
555 sequencing-based diversity studies. Nucleic acids research. 2013;41(1):e1-e.

556 33. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary
557 genetics analysis across computing platforms. Molecular biology and evolution.
558 2018;35(6):1547-9.

559 34. R-Core-Team. R: A language and environment for statistical computing. R Foundation
560 for Statistical Computing. Vienna, Austria: https://www.R-project.org/; 2019.

561 35. Muyzer G, Smalla K. Application of denaturing gradient gel electrophoresis (DGGE) and
562 temperature gradient gel electrophoresis (TGGE) in microbial ecology. Antonie van
563 Leeuwenhoek. 1998;73(1):127-41.

564 36. Goodfellow M, Garrity GM, Bergey DH. Bergey's manual of systematic bacteriology :
565 Vol. 5 Pt. B : The Actinobacteria. 2nd ed. ed. New York: Springer; 2012.

566 37. Kim KK, Lee KC, Oh H-M, Kim MJ, Eom MK, Lee J-S. Arthrobacter defluvii sp. nov.,
567 4-chlorophenol-degrading bacteria isolated from sewage. International journal of
568 systematic and evolutionary microbiology. 2008;58(8):1916-21.

569 38. Kallimanis A, Kavakiotis K, Perisynakis A, Spröer C, Pukall R, Drainas C, et al.
570 Arthrobacter phenanthrenivorans sp. nov., to accommodate the phenanthrene-degrading
571 bacterium Arthrobacter sp. strain Sphe3. International journal of systematic and
572 evolutionary microbiology. 2009;59(2):275-9.

573 39. Bodour AA, Wang JM, Brusseau ML, Maier RM. Temporal change in culturable
574 phenanthrene degraders in response to long-term exposure to phenanthrene in a soil
575 column system. Environmental Microbiology. 2003;5(10):888-95.

576 40. Borodina E, Kelly DP, Schumann P, Rainey FA, Ward-Rainey NL, Wood AP. Enzymes
577 of dimethylsulfone metabolism and the phylogenetic characterization of the facultative
578 methylotrophs Arthrobacter sulfonivorans sp. nov., Arthrobacter methylotrophus sp.
579 nov., and Hyphomicrobium sulfonivorans sp. nov. Archives of microbiology.
580 2002;177(2):173-83.

581 41. Táncsics A, Máthé I, Benedek T, Tóth EM, Atasayar E, Spröer C, et al. Rhodococcus
582 sovatensis sp. nov., an actinomycete isolated from the hypersaline and heliothermal Lake
583 Ursu. International journal of systematic and evolutionary microbiology. 2017;67(2):190-
584 6.

585 42. Švec P, Černohlávková J, Busse H-J, Vojtková H, Pantu R, Cnockaert M, et al.
586 Classification of strain CCM 4446T as Rhodococcus degradans sp. nov. International
587 journal of systematic and evolutionary microbiology. 2015;65(12):4381-7.

588 43. Armfield SJ, Sallis PJ, Baker PB, Bull AT, Hardman DJ. Dehalogenation of haloalkanes
589 byRhodococcus erythropolis Y2. Biodegradation. 1995;6(3):237-46.

590 44. Laczi K, Kis Á, Horváth B, Maróti G, Hegedüs B, Perei K, et al. Metabolic responses of
591 Rhodococcus erythropolis PR4 grown on diesel oil and various hydrocarbons. Applied
592 microbiology and biotechnology. 2015;99(22):9745-59.

593 45. Meyer S, Moser R, Neef A, Stahl U, Kämpfer P. Differential detection of key enzymes
594 of polyaromatic-hydrocarbon-degrading bacteria using PCR and gene probes.
595 Microbiology. 1999;145(7):1731-41.

596 46. Giudice AL, Casella P, Caruso C, Mangano S, Bruni V, De Domenico M, et al.
597 Occurrence and characterization of psychrotolerant hydrocarbon-oxidizing bacteria from
598 surface seawater along the Victoria Land coast (Antarctica). Polar biology.
599 2010;33(7):929-43.

600 47. Schippers A, Bosecker K, Spröer C, Schumann P. Microbacterium oleivorans sp. nov.
601 and Microbacterium hydrocarbonoxydans sp. nov., novel crude-oil-degrading Gram-
602 positive bacteria. International journal of systematic and evolutionary microbiology.
603 2005;55(2):655-60.

604 48. Dashti N, Khanafer M, El-Nemr I, Sorkhoh N, Ali N, Radwan S. The potential of oil-
605 utilizing bacterial consortia associated with legume root nodules for cleaning oily soils.
606 Chemosphere. 2009;74(10):1354-9.

607 49. Prince RC, Amande TJ, McGenity TJ. Prokaryotic hydrocarbon degraders. Taxonomy,
608 Genomics and Ecophysiology of Hydrocarbon-Degrading Microbes. 2018:1-41.

609 50. Margesin R, Spröer C, Schumann P, Schinner F. Pedobacter cryoconitis sp. nov., a
610 facultative psychrophile from alpine glacier cryoconite. International Journal of
611 Systematic and Evolutionary Microbiology. 2003;53(5):1291-6.

612 51. Steyn P, Segers P, Vancanneyt M, Sandra P, Kersters K, Joubert J. Classification of
613 heparinolytic bacteria into a new genus, Pedobacter, comprising four species: Pedobacter
614 heparinus comb. nov., Pedobacter piscium comb. nov., Pedobacter africanus sp. nov. and
615 Pedobacter saltans sp. nov. proposal of the family Sphingobacteriaceae fam. nov.
616 International Journal of Systematic and Evolutionary Microbiology. 1998;48(1):165-77.

617 52. Zhang B, Liu Z-Q, Zheng Y-G. Pedobacter quisquiliarum sp. nov., isolated from activated
618 sludge. International journal of systematic and evolutionary microbiology.
619 2017;68(1):438-42.

620 53. Covas C, Caetano T, Cruz A, Santos T, Dias L, Klein G, et al. Pedobacter lusitanus sp.
621 nov., isolated from sludge of a deactivated uranium mine. International journal of
622 systematic and evolutionary microbiology. 2017;67(5):1339-48.

623 54. Yang J-E, Shin J-Y, Park S-Y, Mavlonov GT, Yi E-J, Lee E-H, et al. Pedobacter
624 kyungheensis sp. nov., with ginsenoside converting activity. The Journal of general and
625 applied microbiology. 2012;58(4):309-16.

626 55. Yoon M-H, Ten LN, Im W-T, Lee S-T. Pedobacter panaciterrae sp. nov., isolated from
627 soil in South Korea. International journal of systematic and evolutionary microbiology.
628 2007;57(2):381-6.

629 56. Du J, Singh H, Ngo HT, Won K-H, Kim K-Y, Yi T-H. Pedobacter daejeonensis sp. nov.
630 and Pedobacter trunci sp. nov., isolated from an ancient tree trunk, and emended
631 description of the genus Pedobacter. International journal of systematic and evolutionary
632 microbiology. 2015;65(4):1241-6.

633 57. Jean-François B, John PB. Bergey's Manual of Systematic Bacteriology (Genus
634 *Flavobacterium)*. 2nd ed ed. New York: Springer; 2011.

635 58. Stucki G, Alexander M. Role of dissolution rate and solubility in biodegradation of
636 aromatic compounds. Appl Environ Microbiol. 1987;53(2):292-7.

637 59. Tan WA, Parales RE. Hydrocarbon Degradation by Betaproteobacteria. Taxonomy,
638 Genomics and Ecophysiology of Hydrocarbon-Degrading Microbes. 2019:1-18.

639 60. Goris J, De Vos P, Caballero-Mellado J, Park J, Falsen E, Quensen III JF, et al.
640 Classification of the biphenyl-and polychlorinated biphenyl-degrading strain LB400T
641 and relatives as Burkholderia xenovorans sp. nov. International journal of systematic and
642 evolutionary microbiology. 2004;54(5):1677-81.

643 61. Chain PS, Denef VJ, Konstantinidis KT, Vergez LM, Agulló L, Reyes VL, et al.
644 Burkholderia xenovorans LB400 harbors a multi-replicon, 9.73-Mbp genome shaped for
645 versatility. Proceedings of the National Academy of Sciences. 2006;103(42):15280-7.

646 62. Peeters C, Zlosnik JE, Spilker T, Hird TJ, LiPuma JJ, Vandamme P. Burkholderia
647 pseudomultivorans sp. nov., a novel Burkholderia cepacia complex species from human
648 respiratory samples and the rhizosphere. Systematic and applied microbiology.
649 2013;36(7):483-9.

650 63. Jeon CO, Park W, Ghiorse WC, Madsen EL. Polaromonas naphthalenivorans sp. nov., a
651 naphthalene-degrading bacterium from naphthalene-contaminated sediment.
652 International journal of systematic and evolutionary microbiology. 2004;54(1):93-7.

653 64. Yagi JM, Sims D, Brettin T, Bruce D, Madsen EL. The genome of Polaromonas
654 naphthalenivorans strain CJ2, isolated from coal tar-contaminated sediment, reveals
655 physiological and metabolic versatility and evolution through extensive horizontal gene
656 transfer. Environmental microbiology. 2009;11(9):2253-70.

657 65. Eriksson M, Dalhammar G, Mohn WW. Bacterial growth and biofilm production on
658 pyrene. FEMS microbiology ecology. 2002;40(1):21-7.

659 66. Grabovich M, Gavrish E, Kuever J, Lysenko AM, Podkopaeva D, Dubinina G. Proposal
660 of Giesbergeria voronezhensis gen. nov., sp. nov. and G. kuznetsovii sp. nov. and
661 reclassification of [Aquaspirillum] anulus,[A.] sinuosum and [A.] giesbergeri as
662 Giesbergeria anulus comb. nov., G. sinuosa comb. nov. and G. giesbergeri comb. nov.,
663 and [Aquaspirillum] metamorphum and [A.] psychrophilum as Simplicispira
664 metamorpha gen. nov., comb. nov. and S. psychrophila comb. nov. International journal
665 of systematic and evolutionary microbiology. 2006;56(3):569-76.

666 67. Miwa H, Ahmed I, Yoon J, Yokota A, Fujiwara T. Variovorax boronicumulans sp. nov.,
667 a boron-accumulating bacterium isolated from soil. International journal of systematic
668 and evolutionary microbiology. 2008;58(1):286-9.

669 68. Posman KM, DeRito CM, Madsen EL. Benzene degradation by a Variovorax species
670 within a coal tar-contaminated groundwater microbial community. Appl Environ
671 Microbiol. 2017;83(4):e02658-16.

672 69. Baïda N, Yazourh A, Singer E, Izard D. Pseudomonas brenneri sp. nov., a new species
673 isolated from natural mineral waters. Research in microbiology. 2001;152(5):493-502.

674 70. Kaminski MA, Furmanczyk EM, Sobczak A, Dziembowski A, Lipinski L. Pseudomonas
675 silesiensis sp. nov. strain A3T isolated from a biological pesticide sewage treatment plant
676 and analysis of the complete genome sequence. Systematic and applied microbiology.
677 2018;41(1):13-22.

678 71. Martirani-Von Abercron SM, Marín P, Solsona-Ferraz M, Castañeda-Cataña MA,
679 Marqués S. Naphthalene biodegradation under oxygen-limiting conditions: community
680 dynamics and the relevance of biofilm-forming capacity. Microbial biotechnology.
681 2017;10(6):1781-96.

72. Nam I-H, Chang Y-S, Hong H-B, Lee Y-E. A novel catabolic activity of Pseudomonas veronii in biotransformation of pentachlorophenol. Applied Microbiology and Biotechnology. 2003;62(2-3):284-90.

73. Andersen SM, Johnsen K, Sørensen J, Nielsen P, Jacobsen CS. Pseudomonas frederiksbergensis sp. nov., isolated from soil at a coal gasification site. International Journal of Systematic and Evolutionary Microbiology. 2000;50(6):1957-64.

74. Fischer-Romero C, Tindall B, Jüttner F. Tolumonas auensis gen. nov., sp. nov., a toluene-producing bacterium from anoxic sediments of a freshwater lake. International Journal of Systematic and Evolutionary Microbiology. 1996;46(1):183-8.

75. Muangchinda C, Pansri R, Wongwongsee W, Pinyakong O. Assessment of polycyclic aromatic hydrocarbon biodegradation potential in mangrove sediment from D on H oi L ot, S amut S ongkram P rovince, T hailand. Journal of applied microbiology. 2013;114(5):1311-24.

76. Carlson HK, Price MN, Callaghan M, Aaring A, Chakraborty R, Liu H, et al. The selective pressures on the microbial community in a metal-contaminated aquifer. The ISME journal. 2019;13(4):937.

77. Kanaly RA, Harayama S, Watanabe K. Rhodanobacter sp. strain BPC1 in a benzo [a] pyrene-mineralizing bacterial consortium. Appl Environ Microbiol. 2002;68(12):5826-33.

78. Juhasz AL, Stanley G, Britz M. Microbial degradation and detoxification of high molecular weight polycyclic aromatic hydrocarbons by Stenotrophomonas maltophilia strain VUN 10,003. Letters in Applied Microbiology. 2000;30(5):396-401.

79. Wolf A, Fritze A, Hagemann M, Berg G. Stenotrophomonas rhizophila sp. nov., a novel plant-associated bacterium with antifungal properties. International journal of systematic and evolutionary microbiology. 2002;52(6):1937-44.

80. Ma Y, Wang L, Shao Z. Pseudomonas, the dominant polycyclic aromatic hydrocarbon-degrading bacteria isolated from Antarctic soils and the role of large plasmids in horizontal gene transfer. Environmental Microbiology. 2006;8(3):455-65.

81. Odokuma L, Dickson A. Bioremediation of a crude oil polluted tropical rain forest soil. Global Journal of Environmental Sciences. 2003;2(1):29-40.

82. Connon SA, Tovanabootr A, Dolan M, Vergin K, Giovannoni SJ, Semprini L. Bacterial community composition determined by culture-independent and-dependent methods during propane-stimulated bioremediation in trichloroethene-contaminated groundwater. Environmental Microbiology. 2005;7(2):165-78.

83. Halden RU, Halden BG, Dwyer DF. Removal of Dibenzofuran, Dibenzo-p-Dioxin, and 2-Chlorodibenzo-p-Dioxin from Soils Inoculated withSphingomonas sp. Strain RW1. Appl Environ Microbiol. 1999;65(5):2246-9.

84. Saadoun I, Al-Akhras M-A, Abu-Ashour J. Bacterial degradation of hydrocarbons as evidenced by respirometric analysis. Microbios. 1999;100(395):19-25.

721 85. Finneran KT, Johnsen CV, Lovley DR. Rhodoferax ferrireducens sp. nov., a
722 psychrotolerant, facultatively anaerobic bacterium that oxidizes acetate with the reduction
723 of Fe (III). International Journal of Systematic and Evolutionary Microbiology.
724 2003;53(3):669-73.

725 86. Aragno M, SCHLEGEL HG. Aquaspirillum autotrophicum, a new species of hydrogen-
726 oxidizing, facultatively autotrophic bacteria. International Journal of Systematic and
727 Evolutionary Microbiology. 1978;28(1):112-6.

728 87. Muller D, Simeonova DD, Riegel P, Mangenot S, Koechler S, Lievremont D, et al.
729 Herminiimonas arsenicoxydans sp. nov., a metalloresistant bacterium. International
730 journal of systematic and evolutionary microbiology. 2006;56(8):1765-9.

731 Supporting information

732 **S1 Fig**. Variation in mean bacterial cell count between microscopic and plate counts. Error bars
733 are mean + standard error.

734 **S2 Fig.** Bacterial class-level taxonomic group detected from the water samples. R1, R2 and R4
735 are sampling wells located in the leachate plume, with the corresponding multilevel in each
736 well. R0 is a reference well

737 **S3 Fig.** Bacterial family taxonomic group detected from the water samples. R1, R2 and R4
738 are sampling wells located in the leachate plume, while R0 is a background well.

739 **S4 Fig.** Maximum Likelihood tree as implemented in MEGA X based on 16S rRNA gene
740 sequences of bacterial isolates, using Generalised Time Reversible Model. MSA was performed
741 using muscle in MEGA X with default settings. Values at the nodes depicts bootstrap values
742 (%) based on 1000 replications, those >50 are shown.

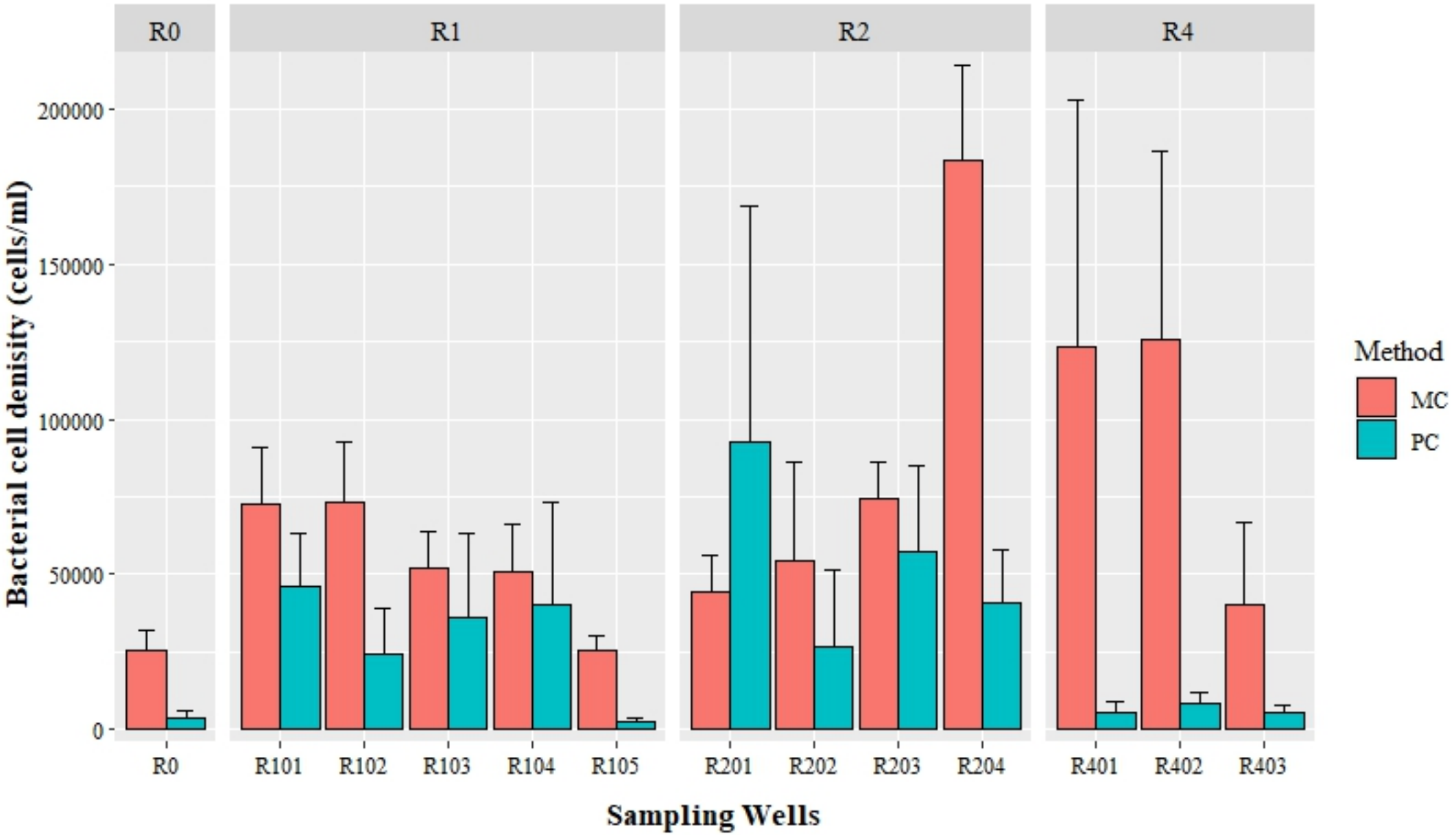
743 **S5 Fig.** Abundance of bacterial genera recovered from the water samples. A total of forty-six
744 genera were recovered.

745 **S6 Fig**. PCA of site score as the gradient behind species abundance and water chemistry
746 variation. The two axes explained 41.7% of the observed variation in the species abundance.

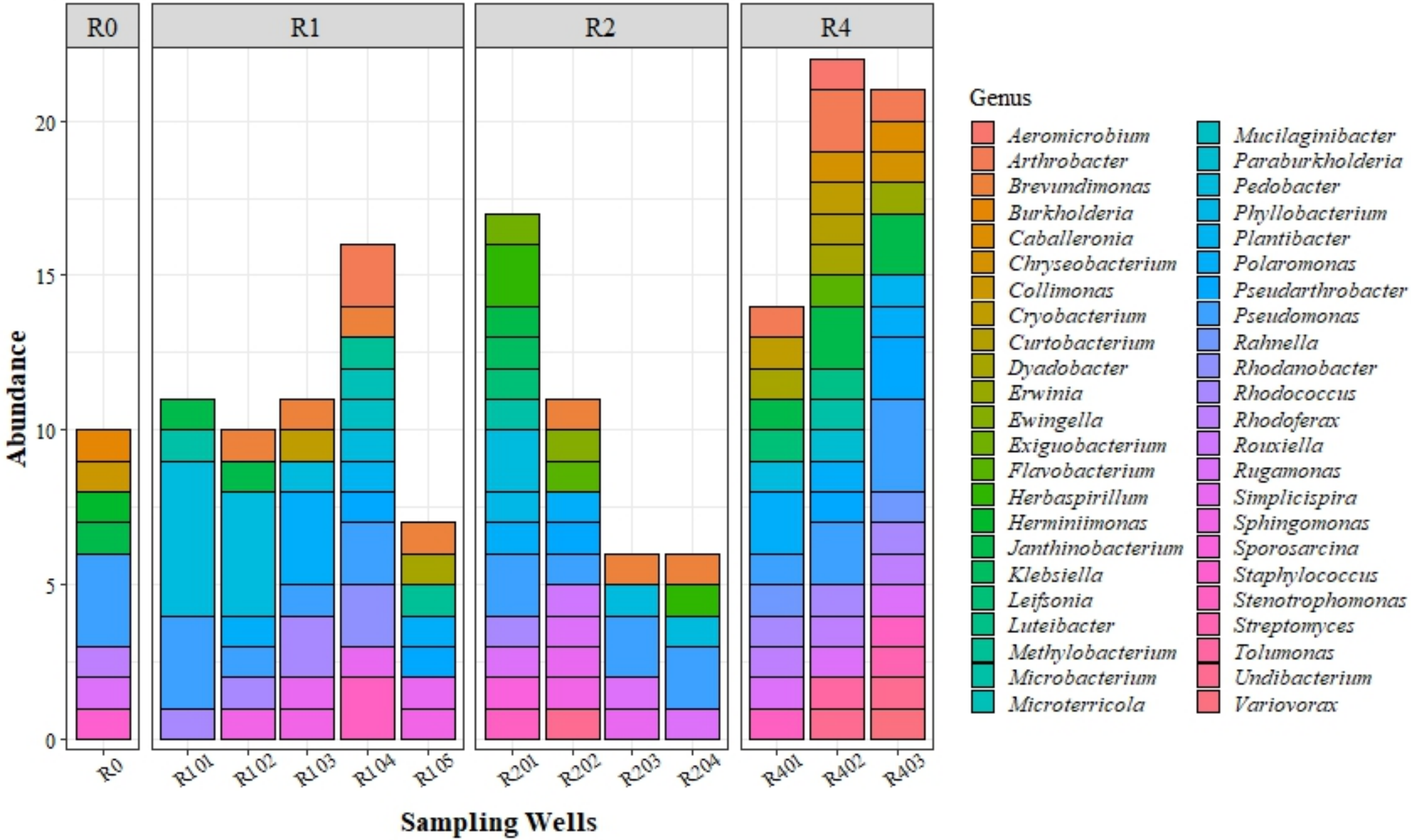
747 **Supplementary table captions**

748 **S1 Table.** Bacterial species isolated from Revdalen groundwater samples

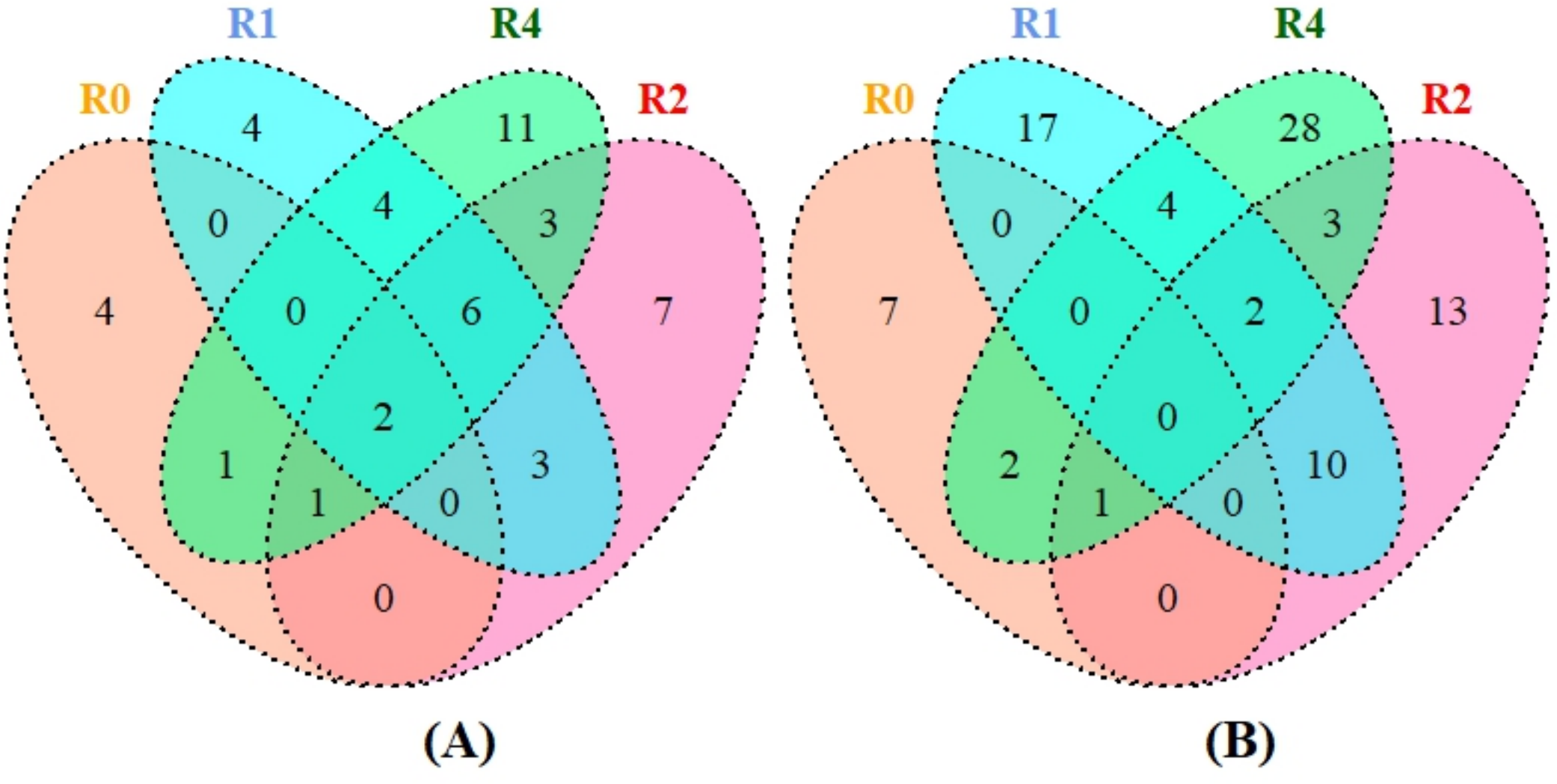
749 **S2 Table.** Biogeochemical cycling (nitrogen and sulphur) capabilities of the bacterial species
750 represented in Revdalen Aquifer.


R0
R1
R2
R4
200000
150000
100000
50000
0
Bacterial cell denisity (cells/ml)
R0
R101
R102
R103
R104
R105
R201
R202
R203
R204
R401
R402
R403
Sampling Wells
Method
MC
PC

Figure


R0
R1
R2
R4
Abundance
0
5
10
15
20
R0
R101
R102
R103
R104
R105
R201
R202
R203
R204
R401
R402
R403
Sampling Wells
Genus
Aeromicrobium
Arthrobacter
Brevundimonas
Burkholderia
Caballeronia
Chryseobacterium
Collimonas
Cryobacterium
Curtobacterium
Dyadobacter
Erwinia
Ewingella
Exiguobacterium
Flavobacterium
Herbaspirillum
Herminiimonas
Janthinobacterium
Klebsiella
Leifsonia
Luteibacter
Methylobacterium
Microbacterium
Microterricola
Mucilaginibacter
Paraburkholderia
Pedobacter
Phyllobacterium
Plantibacter
Polaromonas
Pseudarthrobacter
Pseudomonas
Rahnella
Rhodanobacter
Rhodococcus
Rhodoferax
Rouxiella
Rugamonas
Simplicispira
Sphingomonas
Sporosarcina
Staphylococcus
Stenotrophomonas
Streptomyces
Tolumonas
Undibacterium
Variovorax

Figure


R1
R4
R0
R2
4
11
0
4
3
4
0
6
7
2
1
3
1
0
0
(A)
R1
R4
R0
R2
17
28
0
4
3
7
0
2
13
0
2
10
1
0
0
(B)

Figure