


Groundwater Microbiology of an Urban Open-Loop Ground Source Heat Pump with High Methane

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Abstract

Ground source heat pumps (GSHPs) are low-carbon alternatives to gas boilers for decarbonizing heating. Open-loop GSHP systems abstract groundwater, pass it through a heat exchanger, and return it to ground or surface water. Groundwater samples from the top and base of an abstraction and a recharge borehole of an open-loop GSHP system in Cardiff, UK were assessed, and compared to two local boreholes in the same aquifer. Groundwater samples were taken when the GSHP system was active (once) and inactive (twice) and analyzed for changes in geochemistry, viable cell counts, and microbial community (16S rRNA gene sequencing). The GSHP had a distinct geochemistry and microbial community compared to the control boreholes, and the abstraction borehole showed greater variability than the recharge borehole. The microbial community of the GSHP system showed an increase in relative abundance of genera involved in oxidation of methane and methylated compounds, of which *Methylothera* was the most abundant (up to 83.9% of 16S rRNA gene sequences). There were also changes in genera associated with nitrification (*Nitrospira*, *Nitrosomonas*) and those with potential for sulfur and iron cycling (*Rhodoferrax*). Methane concentration was analyzed after identification of methylotherophs and found that methane concentrations were up to 2855 $\mu\text{g L}^{-1}$, thus likely having had a significant impact on the bacterial communities present. Understanding the microbiology and biogeochemistry of GSHP systems provides insight into potential issues with local infrastructure and long-term system performance, and supports modeling to maximize efficient and sustainable use of the subsurface.

Introduction

The subsurface is increasingly being seen as an energy generating and storage resource, including the use of shallow groundwater for space heating and cooling. Such systems do not require a high geothermal gradient to super heat water to drive turbines, as in “traditional” geothermal power stations but rely instead on heat pumps and low enthalpy thermal transfer with the environment. The heat exchanger transfers a small proportion of

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heat from the groundwater to a separate network of “clean” water which is passed through a heat pump that concentrates the temperature to a suitable temperature (e.g., 30–80 °C) for space heating of buildings and hot water. The submersible well pump and heat pump unit are usually powered by electricity, providing 3–4 units of heat output for one unit of electrical input, and have 15–77% lower CO₂ emissions than gas boilers, which reduces to zero net CO₂ emissions once the grid is supplied with 100% renewable energy (Menegazzo et al. 2022). This renewable heating technology is likely to become dominant for domestic and non-domestic heating as the UK government is due to phase out installation of new domestic gas boilers by 2035 (BEIS and E. A. I. S. Business 2021). To reach NetZero emissions by 2050 has set a target to install 600,000 heat pumps per year by 2028. Their use is of increasing interest in urban areas to replace gas-fired heating systems and to take advantage of the “Subsurface Urban Heat Island effect,” where shallow groundwater temperatures can be several degrees higher than in non-urbanized areas (Allen et al. 2003; Benz et al. 2016; Farr et al. 2017; Patton et al. 2020). Anthropogenic factors including land cover and subsurface infrastructures, such as basements, sewers, tunnels, and landfill can all contribute to the Subsurface Urban Heat Island effect (Menberg et al. 2013; Benz et al. 2016; Bidarmaghz et al. 2019; Makasis et al. 2021).

Open-loop GSHPs abstract groundwater from one borehole, pass it through a heat exchanger to transfer heat energy over to the heat pump, then re-injected the same cooled “waste” water back into the aquifer via an injection well located several 100 m away from the abstraction well, or discharge it directly to surface water body or drain. The environmental conditions within the groundwater are likely to be perturbed, with respect to temperature, flow, and oxygen levels, each of which are key controls on microbial community composition and activity in all environments. Changes to microbial communities affect environmental processes such as geochemical cycling and corrosion of metal infrastructure, and under certain circumstances, microbial activity can also lead to clogging well screens and aquifer pore space (Burte et al. 2019).

Several laboratory and field studies have looked at the effect of temperature changes associated with geothermal energy production, or heat storage on microbial communities, but due to the diversity of geothermal energy options, these studies often cover temperature ranges that are not relevant to the limited temperature changes seen during the operation of shallow open-loop GSHPs in mid-latitudes. It has previously been observed that a 6 °C temperature increase in an oligotrophic aquifer changed the microbial community but not ecosystem functioning (Briellmann et al. 2009) and an increase from 11 °C to 25 °C showed a switch from a microbial community dominated by iron and nitrite reducing bacteria to sulfate reducers has also been observed (Jesùbek et al. 2012; Bonte et al. 2013). The authors hypothesized that changes in function may be seen in aquifers with higher organic carbon and nutrient content. In these more mesotrophic

environments, there is concern that elevated temperature could lead to increased biochemical activity that results in anoxia (Griebler et al. 2016).

Sulfate oxidizing bacteria have been reported at a range of temperatures. These organisms can coexist with sulfate-reducing prokaryotes (SRP), and may increase if electron acceptors such as molecular oxygen or nitrate are introduced into the system (Westphal et al. 2016; Würdemann et al. 2016; Lienen et al. 2017). The increase in these sulfur-cycling organisms may be particularly important as they have been associated with the corrosion of infrastructure (Ura-Bińczyk et al. 2019), especially in geothermal energy operations at elevated temperatures (Lerm et al. 2013; Westphal et al. 2016; Würdemann et al. 2016). Other microbial activity could have an influence on mineral precipitation and biofouling (Hähnlein et al. 2013) as was observed in an aquifer thermal energy storage system at the German Parliament buildings in Berlin, which took groundwater in winter with a natural temperature of 12 °C–14 °C and cooled it to 5 °C. In summer groundwater between 6 °C and 10 °C was used for air conditioning, re-injecting water at 15 °C–30 °C (Lerm et al. 2011). The aquifer thermal energy storage system was prone to clogging as a result of Fe hydroxide crusts during the summer, associated with increased sulfur-oxidizing bacteria (of the genus *Theothrix*) and iron-oxidizing bacteria (of the genus *Gallionella*) during the summer phase (Lerm et al. 2011; Vetter et al. 2012). Although some molecular analysis was carried out that detected variability in the microbial communities (Lerm et al. 2011), to the best of our knowledge, no systematic assessment of impact of heating or cooling was undertaken on the groundwater microbial communities. Iron oxidizing bacteria were also implicated as the cause of clogging in 10 out of 15 [open-loop] GSHPs surveyed in Sweden (Lindblad-Påsse 1998) in oxygenated water. In more reducing conditions sulfur oxidizing bacteria were more commonly identified.

This study investigated an open-loop GSHP (Boon et al. 2019) operating in a shallow coastal glacio-fluvial sand and gravel aquifer in Cardiff Bay, Wales, UK. Samples were collected to monitor the effect of the GSHP on microbiology and groundwater chemistry and to understand whether these could have an effect on the operation of the GSHP or the environment.

Research Method

Site Description and Sampling

The open-loop GSHP scheme is located at Grange-town Nursery School, Cardiff, Wales, UK, and it is part of a wider urban project called the “Cardiff Urban Geo-Observatory” (Patton et al. 2020). The GSHP comprises a 22-m deep abstraction borehole and a 18.6-m deep recharge borehole, both drilled into a confined Quaternary glacio-fluvial sand and gravel aquifer (Boon et al. 2019; Patton et al. 2021). The coastal setting of the study area has undergone significant changes, with land reclamation

Table 1
Summary of Sample Sites Used in This Study

Sample site	Aquifer	Borehole Depth (m)	Location (E, N)	Samples
GSHP Plant Room	Sand and Gravel	N/A	318114, 174504	Plant room
GSHP Abstraction borehole	Sand and Gravel	22.0	318104,174495	Abstraction top and Abstraction base
GSHP Recharge borehole	Sand and Gravel	18.6	318120,174502	Recharge top and Recharge base
CS317L borehole	Sand and Gravel	13.7	318139,174388	Control borehole CS317
CS241 borehole	Sand and Gravel	13.6	317980,174445	Control borehole CS241

Note: The GSHP Plant Room samples were taken prior to this study and are for water chemistry only.

and also construction of a tidal barrage in 1999. Before the tidal barrage was constructed saline water would have reached the area of the river adjacent to the GSHP site during high tides, however, it is now entirely fresh surface water. The barrage also exerts a hydraulic head boundary on the sand and gravel aquifer which has the effect of maintaining groundwater levels to a broadly similar elevation (~4.5 m above Ordnance Datum) to the impounded water behind the barrage (Heathcote et al. 2003).

A former landfill site and a gasworks site lie approximately 500 m to the west and southwest of the GSHP. The landfill site contained household waste plus 5000 m³ waste from the gasworks site (George 2006) and was producing 2500 m³ methane when monitored in 2005 (Cherrill and Phillips 2005).

Water samples were collected from the abstraction and recharge boreholes, plus two nearby control boreholes (Table 1 and Figure 1) on three dates: 31st July 2019, 29th October 2019, and 21st December 2020. In addition, groundwater chemistry data is included from the GSHP plant room, collected from the sediment filter or a sample tap on the inflow side of the system after the abstraction borehole and prior to the heat exchanger.

In July 2019, the GSHP was not in operation (i.e., water was not being pumped between abstraction and recharge boreholes). The GSHP had been turned off on 16th April 2019 and was switched back on after the summer on 10th October 2019. Therefore, at the July sampling time point, the system had not been operational for approximately 14 weeks. At the time of the October 2019 sampling, the GSHP had been operational for just under 3 weeks as it was the start of the heating season. For the December 2020 sampling, the GSHP had been turned off since 18th November 2020, and thus had not been operational for approximately 5 weeks.

Groundwater samples were collected using a submersible WASP™ 12v pump, except during operation (October 2019) when a bailer was used. For the two control boreholes (CS317 & CS241) the pump was lowered into the borehole several meters below the glacio-fluvial water table, which at time of survey lies at approximately 3.4 m depth below ground level (bgl; Heathcote et al. 2003; Farr et al. 2017). To gain a representative sample from the aquifer into which the boreholes are installed, three borehole volumes (more than 50 L) of groundwater were purged from each control borehole before sampling.

Abstracted groundwater was passed through a flow cell allowing field measurements of temperature, dissolved oxygen (DO), Eh, electrical conductivity (EC), and pH. Data was collected by separate meters, all from SevenGo pro series (Mettler Toledo®, Columbus, Ohio), and probes were from the following manufacturer's temperature (Hanna Instruments®, Woonsocket, Rhode Island), DO (InSitu®, Fort Collins, Colorado), Eh (VWR®, Radnor, Pennsylvania) and EC (Mettler Toledo®, Columbus, Ohio). For the abstraction and recharge boreholes, the top samples were collected first and at a depth of 4–5 m bgl. Before sample collection, 5 L groundwater was pumped/bailed. Sampling of the base of the boreholes was carried out at 10–15 m bgl. Before sampling the base, a further 10 L of groundwater was pumped/bailed.

All samples were transported back to the laboratory in a cool box, and stored temporarily at 4 °C before processing. Samples were processed within 24 h of collection, details are provided in the relevant section as follows.

Chemical data from previously collected samples have been included to give an indication of the range of chemistries at this site (Plant Room data). These were collected from the sample tap in the GSHP plant room, that is, a sample point physically located between the abstraction borehole and plate heat exchanger/recharge borehole. These samples were collected between November 2015 and January 2016.

Sand and gravel sediment, collected during drilling of the boreholes, was used as a starting material to investigate the microbial colonization of mineral surfaces. Details of sampling methods and results are provided in Supporting Information.

Water Chemistry

Water samples were collected after purging of boreholes as described in the previous section. Samples intended for analysis of selected anions and cations were processed at point of collection. All samples were passed through a 0.45 µm syringe filter. In addition, samples for selected cations were acidified (1% HNO₃). Analysis of selected anions was analysed using ion chromatography (IC) and cations by inductively coupled plasma mass spectrometry (ICP-MS) following methods outline in Watts et al. (2019); Marriott et al. (2020) and Watts et al. (2021).

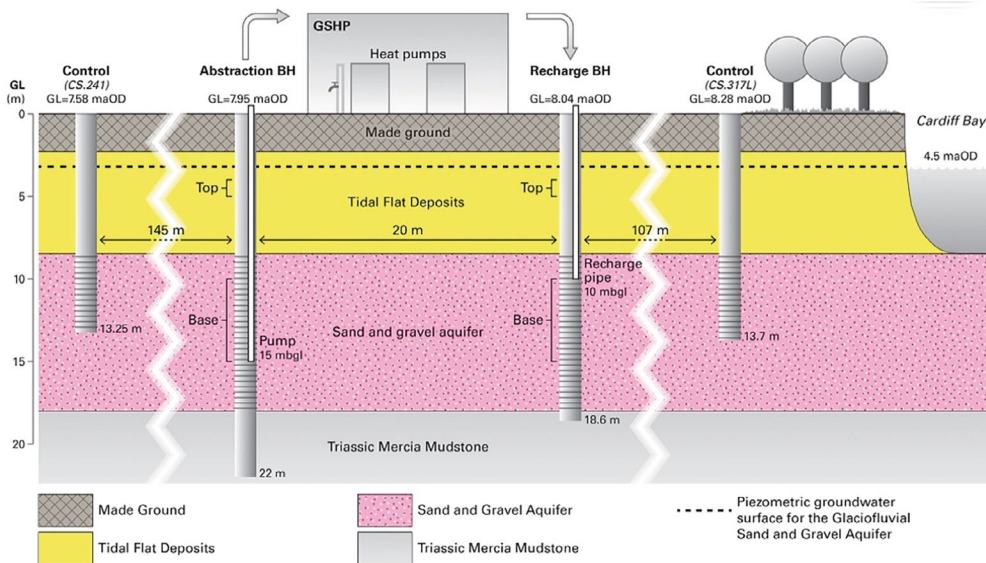
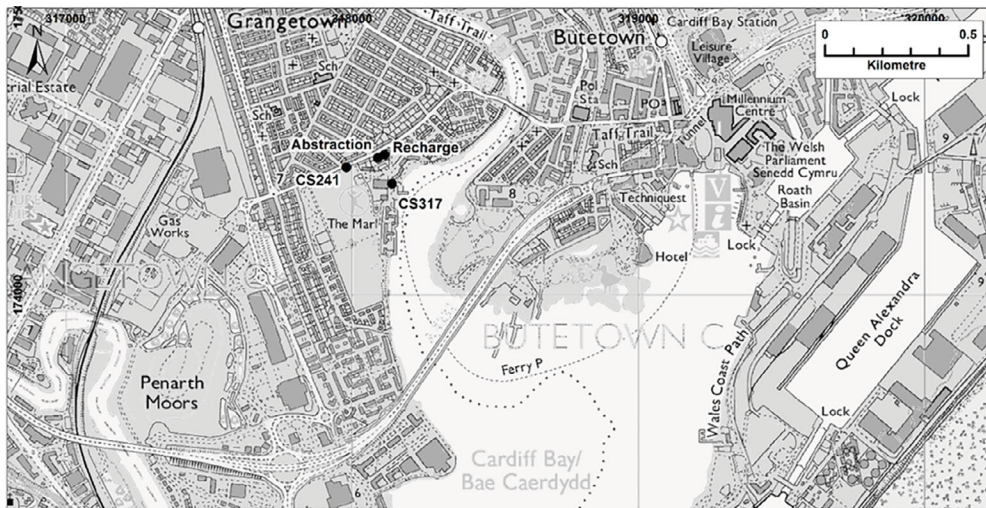


Figure 1. Map of the Cardiff Bay area and conceptual diagram of the ground source heat pump and monitoring boreholes (the latter modified from Boon et al. 2019, with permission from Elsevier).

The following analytes were determined by Dionex ICS5000 (ThermoFisher Scientific, Waltham, Massachusetts) via IC: Cl^- , SO_4^{2-} , NO_3^- , NO_2^- , Br^- , F^- , and HPO_4^{2-} using conductivity detectors. Calibration and QC standards were freshly prepared and traceable.

The following analytes were determined by ICP-MS using an 7500CX or 8900 ICP-QQQ series (Agilent, Santa Clara, California) via ICP-MS: Ca, Mg, Na, K, Si, Total P, Total S, Ba, Sr, Mn, Total Fe, Li, Be, B, Al, Ti, V, Cr, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Y, Zr, Nb, Mo, Cd, Sn, Sb, Cs, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Hf, Ta, W, Tl, Pb, Bi, Th, and U. The former instrument was for the Plant Room (2015–2016) samples only. Primary multi-element and single-element calibration standards and QC standards were conducted at the beginning and end of instrument run. All standards were certified and traceable to international standards (ISO 17034).

Additional water samples were collected into gas-tight sample containers from the December 2020 to analyze for light gas composition (CH_4 , CO_2 , and C_2H_6). Data were analyzed from all sampling locations except recharge base. Apart from the reduction in batch size, the sample collection and analysis methods were conducted as outlined in Bell et al. (2017). Pumped water samples were collected directly from borehole, prior to contact with atmosphere, in double-valved steel cylinders. The dissolved gas samples were analyzed using a headspace method on an Agilent 7820A gas chromatograph with an evacuated inlet system and a flame ionization detector.

Culture-Based Microbiology

Culture-based methods were used to quantify the total number of aerobic and anaerobic heterotrophic bacteria, as well as numbers of key microbial groups involved in

iron and sulfur cycling. Most probably number (MPN) for iron-reducing bacteria were conducted but cross-contamination meant very few results were reliable so have not been included. A 10-fold dilution series was prepared from each sample using sterile 0.9% NaCl. The detection limit for MPN-based methods was an order of magnitude lower in December 2020 samples.

Total aerobes and anaerobes were quantified using a Miles–Misra plate count (Miles et al. 1938). Eight 15 μ L drops of each dilution were spotted onto a 5% PTYG agar plate (Supplementary Information). Once the drops had dried, plates were incubated at 25 °C, anaerobic plates were incubated in an anaerobic cabinet (95% N₂/5% H₂). The number of colonies on each spot was counted after 5 and 15 days for aerobes and anaerobes, respectively.

SRP were quantified using an MPN method in triplicate. Each dilution was added to Postgate's medium (recipe in Supplementary Information) 10% (v/v) in serum bottles or centrifuge tubes and incubated in the anaerobic chamber at 25 °C with butyl stoppers fitted. Bottles, where black precipitate (presumptive of microbiologically produced FeS) formed, were considered positive for SRP. MPN counts were made after 30 days.

IOB were quantified using an MPN method in triplicate. Oxygen gradient tubes were prepared based on the method of Emerson and Moyer (1997). Approximately 1.25 mL of 32 g L⁻¹ Fe⁰ powder in MWMM media (recipe in Supporting Information) with 1% agarose was added to the bottom of a glass test tube. Once set, this layer was overlain with 3–4 mL MWMM medium in 0.15% agarose. 10 or 100 μ L of each dilution was added to each test tube ensuring inoculation along the length of the soft agarose. Test tubes were sealed with bacti-caps and grown aerobically at 25 °C. The presence of IOB was confirmed by the formation of horizontal rust-colored bands. Counts were made after 30 days.

DNA Analyses

For samples from June and October 2019, 1 L of groundwater was transported to the BGS laboratory before filtering onto sterile 0.22 μ m cellulose nitrate membranes and stored at –20 °C until DNA extraction. In December 2020, 2.5–3 L samples were filtered in the field using Sterivex membranes, and shipped in a chilled cool box to the laboratory, and stored at –20 °C until DNA extraction. The outer casing of Sterivex filters was removed using a pipe cutter and the filter cut from internal support using a sterile scalpel. Groundwater DNA was extracted directly from filter membranes using a DNeasy PowerWater DNA extraction kit (Qiagen, Germany), following the manufacturer's protocol.

16S rRNA gene analysis was conducted on DNA extracts using a nanopore sequencing platform. All products and software are produced by Oxford Nanopore Technologies (Oxford, UK) unless otherwise stated. 16S barcoding kit (SQK-RAP204) which includes the 27F and 1492R primer set, was used along with Flongle flow cells. For library preparation, manufacturer's protocols and

recommended reagents were followed with the following modifications. (1) PCR amplification was performed in triplicate and pooled before sequencing with total volume of 30 μ L and for 35–40 cycles. (2) ChargeSwitch PCR Clean up kit (Thermo Fisher Scientific, Massachusetts) was used for post-PCR DNA purification step.

Basecalling was performed on MinIT using Guppy (v4.2.3) in high accuracy mode and controlled by MinKNOW software (v20.10.6). Passed reads were analyzed using EPI2ME Fastq 16S workflow (r2021.09.09). EPI2ME Fastq 16S output was collated into different taxonomic levels by Analysis of EPI2ME 16S CVS Output workflow in EPI2ME Labs (Server v1.1.6). Clustering analysis was carried out using the nearest neighbor method with Bray–Curtis dissimilarity index using PAST v4.09 (Hammer et al. 2001). Past v4.09 was also used for principal component analysis (PCA) using a correlation matrix for geochemical data.

Potential function prediction based on 16S rRNA sequences was conducted using the pipeline described in Shen et al. (2020), but briefly as follows. Amplicon sequence variant (ASV) feature tables and representative sequences used for PICRUST2 function prediction were generated via MetONTIME (Maestri et al. 2019). The PICRUST2 full workflow was executed to sequentially achieve sequence placement, hidden-state prediction, metagenome prediction, pathway abundance inference, and finally annotation (Douglas et al. 2020). The pathways output from PICRUST2 was interrogating using inorganic nutrient metabolism and respiration classifications according to MetaCyc.org (Caspi et al. 2020).

Results

Samples were taken at three different times of the year spanning a consecutive 18-month period which also represents different levels of operation for the GSHP, with the system being on during October and off in December and July. The system was only off for a few weeks before the December sampling but for several months before the July sampling. Therefore, results are displayed in figures and tables from most to least active timepoints (i.e., October, December then July) rather than in chronological order.

Water Chemistry

The water chemistry differed between the two control boreholes. There were some changes between the different timepoints, the most noticeable variation is elevated DO in December 2020 samples in both control boreholes (Table 2). In the GSHP samples, the DO was highest in October (heat pump system on). The sulfate was also highest in October, with the exception of recharge top.

When PCA was applied to the water chemistry (ICP-MS and IC and field data), the three samples from each of the two control boreholes grouped together (Figure 2), with CS317 being more similar to the GSHP system samples than CS241 (Component 1). The recharge boreholes

Table 2
Selected Biogeochemical Parameters for Boreholes Tested

Sample	Dissolved oxygen (%)	SO ₄ ²⁻ (mg L ⁻¹)	NO ₃ ⁻ (mg L ⁻¹)	NO ₂ ⁻ (mg L ⁻¹)	HPO ₄ ²⁻ (mg L ⁻¹)	Fe (μg L ⁻¹)
Plant room filter November 2015	n.d.	1.44	<0.6	<0.1	0.758	3397
Plant room filter December 2015	n.d.	1.20	<0.6	<0.1	1.019	3981
Plant room filter January 2016	n.d.	<1	<0.6	<0.1	0.611	3986
Plant room sample tap January 2016	n.d.	1.67	<0.6	<0.1	<0.2	16
CS241 October 2019	0.04	13.4	<0.06	<0.01	35.8	714
CS241 December 2020	1.6	9.09	<0.6	<0.1	31.2	751
CS241 July 2019	0	8.40	<1.5	<0.25	34.8	716
CS317 October 2019	0.03	0.276	<0.06	<0.01	8.32	2751
CS317 December 2020	4.5	0.325	<0.15	<0.025	5.98	3048
CS317 July 2019	0	1.01	<0.6	<0.1	9.10	2849
Abstraction top October 2019	11.8	22.4	0.425	0.012	<0.02	133
Abstraction top December 2020	0.06	16.6	0.217	<0.01	<0.02	2346
Abstraction top July 2019	0.05	<0.5	0.481	<0.05	<0.1	5824
Abstraction base October 2019	5.28	13.0	0.232	<0.01	0.874	1402
Abstraction base December 2020	0.3	<0.25	<0.15	<0.025	0.054	6426
Abstraction base July 2019	0.03	<1	35.1	<0.1	<0.2	8605
Recharge top October 2019	11.9	1.49	<0.06	<0.01	0.082	2502
Recharge top December 2020	2.52	0.518	2.655	0.029	<0.05	223
Recharge top July 2019	0.02	2.45	<0.6	<0.1	<0.2	12,680
Recharge base October 2019	8.52	1.48	<0.06	<0.01	0.071	3590
Recharge base December 2020	0.3	<0.25	<0.15	<0.025	<0.05	4002
Recharge base July 2019	0.01	<1	<0.6	<0.1	<0.2	6149

Note: n.d. not determined.

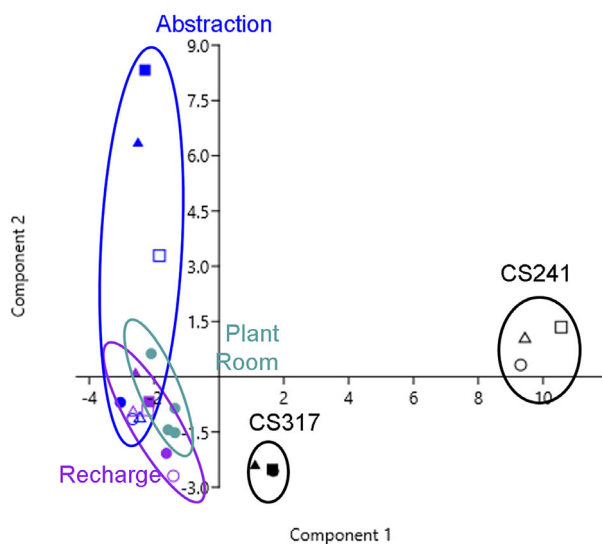


Figure 2. PCA for chemical and field data. Ovals indicate different sample locations and are to aid visualization rather than indicate a statistical significance. Squares = October (system on), triangles = December (system off), and circles = July samples (system off). For abstraction and recharge the open symbols are base and closed are top. Component 1 = 44.0% of the variance, component 2 = 19.6% of the variance. Variables consist of the following parameters: EC (μscm), pH, DO (%), temperature (°C), Ca, Mg, Na, K, Cl⁻, SO₄²⁻, Br⁻, HPO₄²⁻, F⁻, total P, total S, Si, Ba, Sr., Mn, Fe, Li, B, Al, Ti, V, Cr, Co, Ni, Cu, Zn, As, Se, Rb, Y, Zr, Mo, Sb, Cs, La, Ce, and U.

Table 3
Gas composition of Groundwater Samples from December 2020

Sample	CH ₄ (μg L ⁻¹)	C ₂ H ₆ (μg L ⁻¹)	CO ₂ (mg L ⁻¹)
CS241	2831	4	12
CS317	302	<1	6.8
Abstraction: top	1894	<1	15.2
Abstraction: base	2847	<1	15
Recharge: top	2855	<1	11.9

were a little more varied and the abstraction borehole showed the greatest variability in water chemistry, with abstraction top showing the greatest difference when the system was on (squares - October), or recently switched off (triangles—December). Total P was the greatest contributor to Component 1 and Ni (closely followed by total S, U, and sulfate) to Component 2, both positively.

In addition to the IC and ICP-MS data, samples from December were analyzed for dissolved gases (Table 3). Concentrations of methane were highest in the recharge top, abstraction base, and CS241 (2831–2855 μg L⁻¹). Methane concentrations were approximately 33% lower in the Abstraction top and an order of magnitude lower in CS317 which is located next to the river. Carbon dioxide concentration was also lowest in CS317. A small amount of ethane could be detected in CS241 (4 μg L⁻¹).

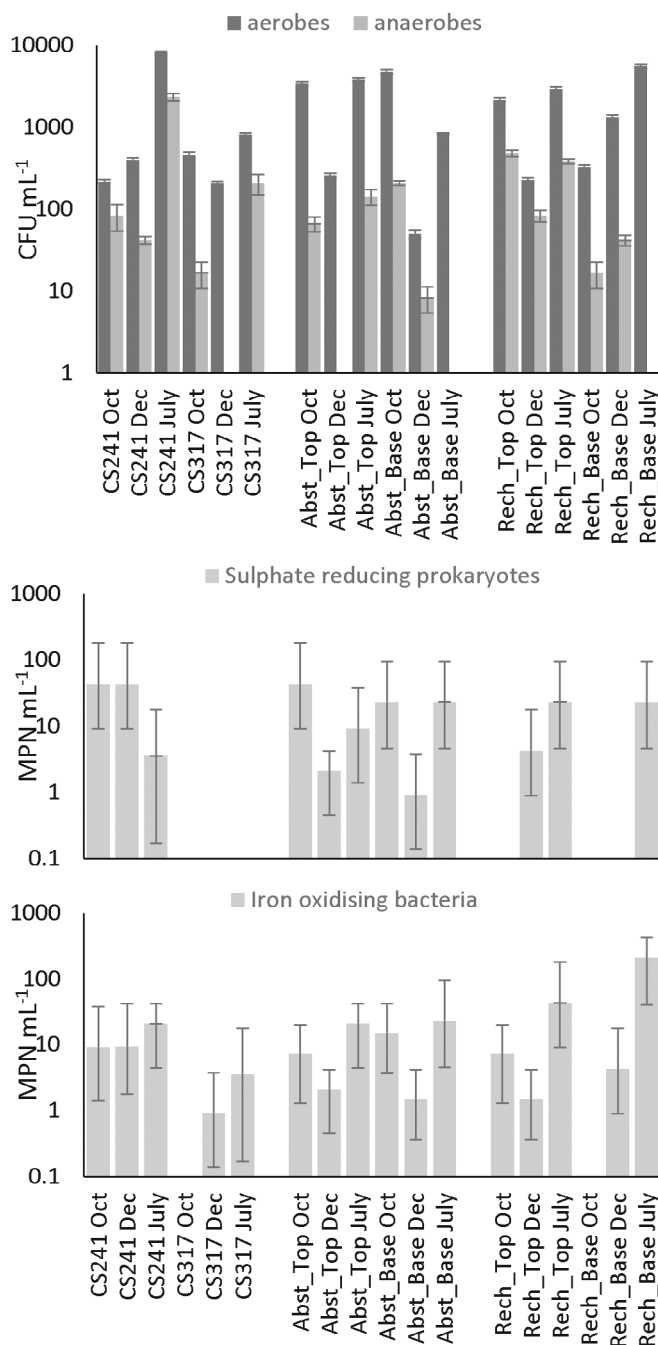


Figure 3. Viable counts for culture-based assays. *Top*: aerobic and anaerobic heterotrophs. *Middle*: sulfate-reducing prokaryotes (SRP). *Bottom*: iron-oxidizing bacteria (IOB). There is no data for CS317 October IOB. All other absences indicate MPN less than the detection limit. Error bars for CFU (heterotrophs and methanotrophs) are 1 SD and 95% confidence limits for MPN (SRP and IOB).

Microbiological Analysis—Culture-Based Assays

Aerobes were more prevalent than anaerobes in each of the samples except CS241 July 2019 and Abstraction base July 2019 (Figure 3). To understand seasonal variations in biomass, the colony-forming units (CFUs) for all six sample locations were averaged; the highest cell counts for aerobic and anaerobic heterotrophs were made in July 2019 (3.7×10^3 and 5.1×10^2 CFUs mL⁻¹ for aerobes and anaerobes, respectively), then October 2019 (1.8×10^3 and 1.5×10^2 CFUs mL⁻¹ for

aerobes and anaerobes, respectively) and were lowest in December 2020 (4.5×10^2 and 2.9×10^1 CFUs mL⁻¹ for aerobes and anaerobes, respectively). Using the same method to compare the control and abstraction and Recharge boreholes, the differences were much less and aerobes and anaerobes do not follow the same trend. Aerobes were higher in the abstraction and recharge wells (2.2×10^3 and 2.1×10^3 CFUs mL⁻¹) than in the control wells (1.7×10^3 CFUs mL⁻¹). Anaerobes were highest in control wells (4.5×10^2 CFUs mL⁻¹),

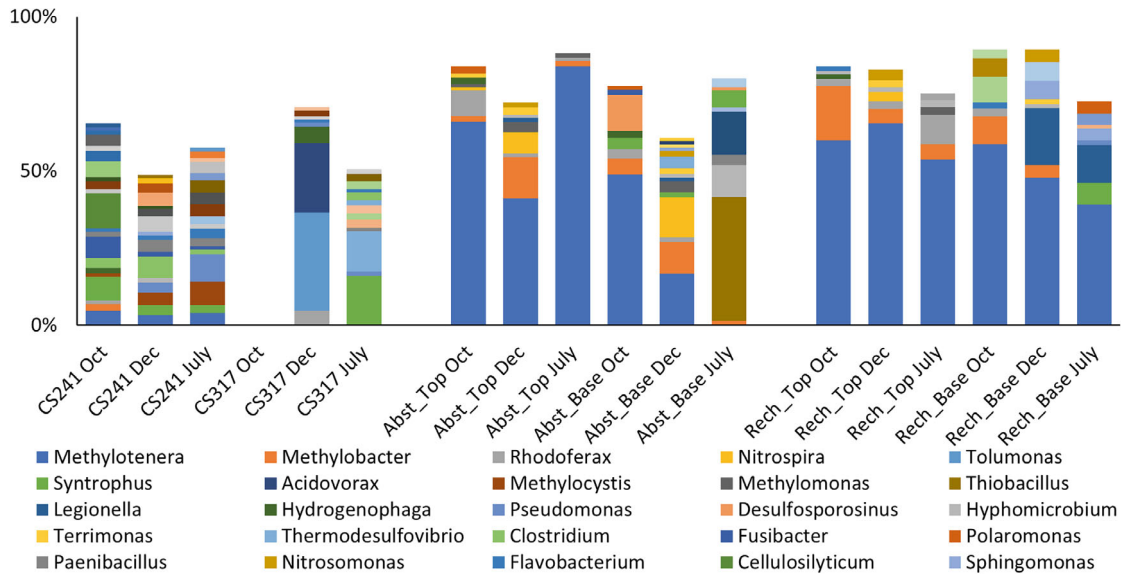


Figure 4. Microbial diversity of groundwater samples and the original material based on 16S rRNA sequencing. Genus level classification at more than 1% abundance relative to classified reads.

then recharge (1.7×10^2 CFUs mL⁻¹), and lowest in abstraction (7.0×10^1 CFUs mL⁻¹).

Culturable SRP and IOB were low in all samples with concentration of SRP never exceeding 43 MPN mL⁻¹. SRP were not detected in CS317 in any time period or in the recharge borehole in October 2019, and Recharge base in December 2020 (Figure 4). IOB cell counts were generally low, but only below detection limit in one sample (Recharge base October 2019). The Recharge borehole July 2019 had slightly higher counts where 43 MPN mL⁻¹ (top) and 210 MPN mL⁻¹ (base) were recorded.

Microbiological Analysis – 16S rRNA Gene Sequencing

Applying a minimum Q score of 7 to the data, DNA sequencing resulted in 394,302 reads. Despite repeated PCR attempts, there were only 650 passed reads for sample CS317 October 2019 so this had been removed from the analysis. Classification at Order and Genus level for the remaining samples was successful for 290,362 and 151,105 reads, respectively.

The most striking difference in bacterial communities between the samples was the high prevalence of *Methylothenera* in most of the GSHP water samples compared to the control boreholes. This genus represented up to 83.8% of classified reads (Abstraction top July 2019; Figure 4). *Methylothenera* was found in four of the six control samples but with a much lower abundance (a maximum of 6.90% in CS241 October 2019). *Methylobacter*, *Hyphomicrobium*, and *Rhodofera* were also found in at least half of the GSHP samples and rarely in the control boreholes (maxima of 17.7, 10.2 and 9.46% for each genus, respectively). The following genera were found in four of six of the control samples but rarely in GSHP: *Syntrophus*, *Pseudomonas*, *Fusibacter*, *Paenibacillus*, and *Flavobacterium* (maxima of 16.0, 8.87, 6.91, 3.94 and 2.97%,

**Table 4
Diversity Indices by Order (Shannon)**

Shannon H	October 2019	December 2020	July 2019
CS241	2.343	3.205	3.038
CS317	2.833	1.878	2.992
Abstraction top	1.370	1.978	0.994
Abstraction base	2.039	2.739	2.226
Recharge top	1.494	1.615	2.002
Recharge base	1.211	1.923	2.450

Note: Generated in PAST v4.09, samples were rarefied to 380 classified reads.

respectively). *Methylocystis* was found in all CS241 samples but nowhere else. *Methylomonas* was found in four of six samples in Abstraction borehole and only Recharge top in July 2019. Considering just the GSHP system boreholes some genera showed differences in abundance at different timepoints. *Nitrospira*, *Legionella*, *Terrimonas*, and *Nitrosomonas* were found in three of four samples from December 2020 (up to 4.30% in the Abstraction base), and only in one other sample and at lower relative abundance. *Hydrogenophaga* was found in three of four October samples but at no other timepoint. In comparison, the control samples were generally more diverse (Table 4).

Clustering analysis based on sequencing data divided the samples into two main clades (Figure 5). The control samples clustered together within the larger clade along with four inactive GSHP base samples (July 2019 and December 2020) and then two of the abstract top samples. The other clade consists of the three Recharge top samples along with Recharge base October 2019 and Abstraction top July 2019. Abstraction base October 2019 sits outside of these two clades.

66.2 49.6 33.1 16.5 0.01 n.d.

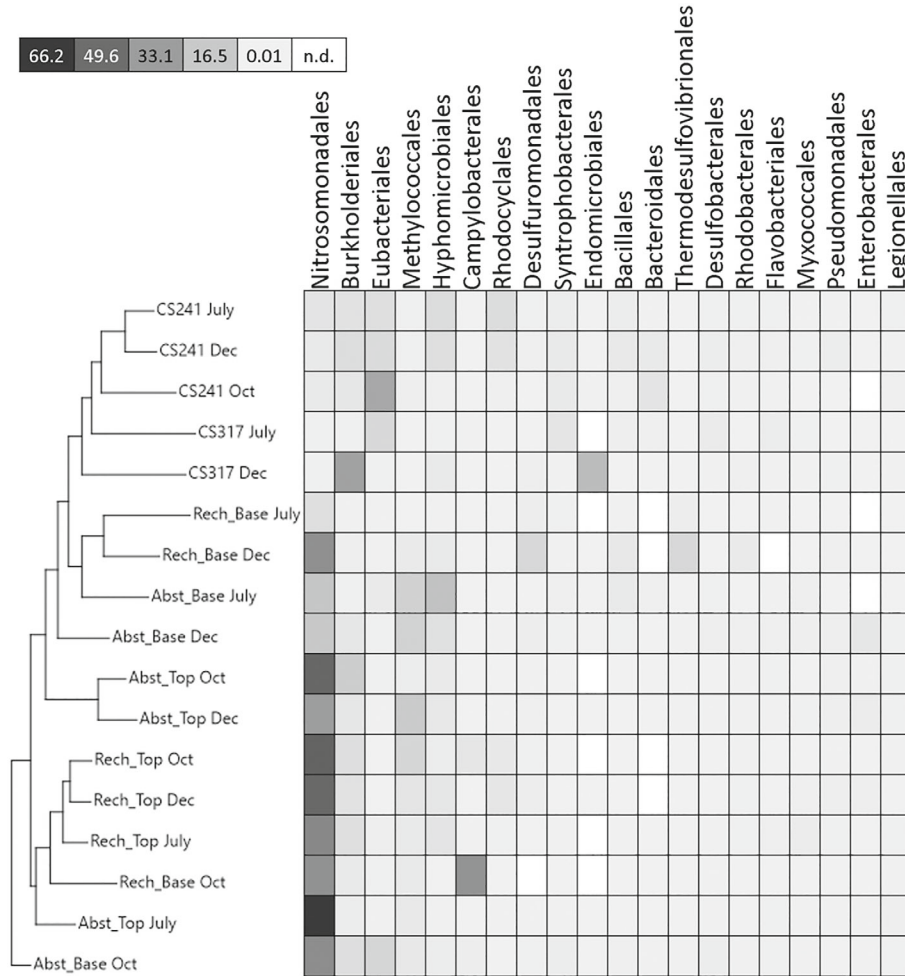


Figure 5. *Left*: nearest neighbor clustering of water samples using Bray Curtis dissimilarity index. Clustering was performed on Order level classification of 16S rRNA gene. *Right*: heatmap showing relative abundance of Orders with average abundance of more than 0.05%.

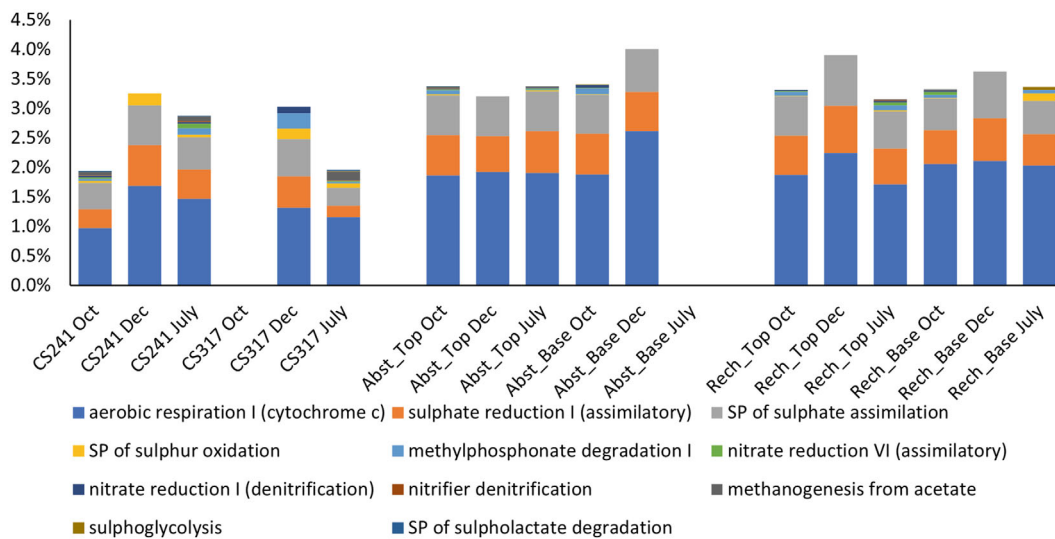


Figure 6. Potential pathways and superpathways (SP) as predicted by PICRUSt2. There were limited matches to 16S database for samples CS317 October 2019 and Abstraction base July 2019 so these were not included. Full names for “SP of sulfate assimilation and cysteine biosynthesis” and “SP of sulfur oxidation (*Acidianus ambivalens*)” were shortened in the figure legend. Percentages are abundance of selected pathway as proportion of total pathways identified in the sample.

PICRUSt2 bioinformatic software was used to provide insights into the potential functional metabolic content of the microbial community. Eleven pathways and superpathways were identified that classified as inorganic nutrient metabolism and or respirations in four or more samples (Figure 6). Aerobic respiration was found in all samples and was more abundant in the GSHP samples compared to the control boreholes. Two other pathways were found in all samples and were both involved in sulfur cycling. Two nitrate cycling pathways were identified, these were much less abundant than sulfur cycling, and were seen in 10 samples across control, abstraction, and recharge boreholes.

Discussion

Comparison of Microbiology and Chemistry in GSHP Boreholes Compared to Nearby Control Boreholes

Investigating how the operation of an open-loop GSHP can modify the groundwater chemistry and microbial community is important to understand whether microbial activity could affect the operation of the GSHP or impact the local groundwater, aquatic, and built environment. In this study, the groundwater chemistry in the GSHP system was different from and much more varied than the control boreholes, especially the Abstraction borehole. The GSHP system water chemistry more closely resembled the chemistry in monitoring borehole CS317 compared to CS241, yet the methane concentration at CS241 is more similar to abstraction base and recharge top. Analysis of methane concentration was only carried out on the last sampling date (December 2020) and thus was not included in multivariate analysis, but revealed high methane concentrations in all boreholes. The recent geological history, the history of land reclamation, former landfill and gasworks, and the construction of the tidal barrage mean neither control borehole would truly represent the undisturbed groundwater at the GSHP site. As the high methane concentrations were not identified until the end of this project, the source of methane was not investigated. The proximity of the landfill and gasworks sites may explain why all methane concentrations were high compared to the baseline values for the UK (Bell et al. 2017). Methane production from a landfill site would be consistent with the high methane:ethane ratio, and the ethane that is observed potentially arising from the hydrocarbon waste likely to be present on landfill from the gasworks waste. Although the landfill site is near to the GSHP site, it is worth noting that the predominant flows of gas from the landfill are believed to westward, toward the River Ely to the south (Cherrill and Phillips 2005), and away from the GSHP site. Alternatively, as this land was reclaimed from the bay during the construction of the barrage, the methane could be derived from underlying marine sediments. High levels of methanogenesis have been reported in sediments currently within the bay (Olivier 2016). The influence of land reclamation on methanogenesis from former marine sediments does not appear to be well studied, either at this site or elsewhere.

The control boreholes could also be distinguished from GSHP boreholes by the differences in diversity, particularly the dominance of key bacterial genera, however, the culture-based analysis showed only minor differences in viable microorganisms. Of the five genera identified by DNA sequencing as occurring more frequently in the control boreholes, two are considered to be strictly anaerobic (*Syntrophus* and *Fusibacter*), one is facultative anaerobic (*Paenibacillus*) and one contains anaerobic species (*Pseudomonas*). *Flavobacterium* is the only aerobic genus and is found in lower abundance than those capable of anaerobic metabolism. Although these genera are found in multiple control borehole samples, they do not necessarily dominate the microbial community of the control boreholes. This is reflected in the culture-based assays which showed a predominance of aerobic bacteria and the PICRUSt2 analysis which predicted aerobic metabolism to be abundant in all samples.

The GSHP samples were characterized by containing up to 83.9% *Methylothera* sequences, as well as other bacteria involved in the oxidation of methylated compounds (methylotrophs) or methane (methanotrophs) including *Methylomonas* and *Methylobacter*. Methane was found in the open-loop GSHP system boreholes and the control boreholes although the range of concentrations varied by nearly an order of magnitude (302 $\mu\text{g L}^{-1}$ in C317 to 2855 $\mu\text{g L}^{-1}$ in Recharge, top), however all recorded concentrations were high compared to UK averages (Bell et al. 2017). If methane was the main carbon source a dominance of methanotrophs over methylotrophs might be expected (Jin et al. 2020; van Grinsven et al. 2020) but *Methylothera* are known to oxidize one-carbon compounds (e.g., methylamine and methanol) and are not reported to be able to oxidize methane. Methanol oxidation is often coupled with dissimilatory nitrate reduction (Afshin et al. 2021). *Methylothera* are often associated with methanotrophs such as *Methylobacter* and *Methylomonas* in environmental samples as they facilitate different stages of oxidation of methane, and it is thought that carbon derived from oxidation of methane is passed from the methanotroph to *Methylothera* (Martineau et al. 2010; Hernandez et al. 2015; van Grinsven et al. 2020) and the presence of nitrate has been observed to promote the transfer of carbon between methylotrophs and methanotrophs (van Grinsven et al. 2020). In addition to reports of co-occurrence, metagenomic and ^{13}C tracer studies support this close relationship between species and suggest that *Methylothera* occurrence depends on active in-situ methane cycling (Afshin et al. 2021). Microcosm studies have found that *Methylothera* is more competitive under low oxygen than other methylotrophs (15 μM equivalent to 0.024% DO; Oshkin et al. 2015). In this study, *Methylothera* dominated samples at a range of oxygen saturation; from 0.01% to 11.94%. The abundance of *Methylothera* has also previously been shown to increase during water abstraction phases from boreholes and has been identified as a potential cause of aquifer clogging (Braun et al. 2016). *Hyphomicrobium* are also more abundant in the GSHP

system, they are facultative methylotrophs, and certain species are able to couple methane oxidation with nitrate reduction (Layton et al. 2000; Martineau et al. 2015), and again activity levels depend on oxygen concentration (Martineau et al. 2015). Nitrate and nitrite concentrations were generally low and below the detection limit in the control boreholes and nitrate was only quantifiable in six of the GSHP samples and nitrite in two samples. Nitrate concentration in Abstraction base July 2019 was more than 10 times higher than the other samples, and was the only GSHP sample with *Methylothermobacter* abundance less than 1%, although did have 1.4% *Methylobacter*. This is a tentative indication that *Methylothermobacter* could be responsible for nitrate reduction in the GSHP system and is limited by another factor. *Methylothermobacter mobilis* has been reported to be inhibited by high nitrate concentrations (Afshin et al. 2021). This was tested at 0.1% nitrate (about 30 times concentration in this sample), but inhibition could be possible at lower concentrations. *Rhodospirillum rubrum* is the fourth genus that was more commonly observed in GSHP system and dominated the abstraction borehole sediment (Supporting Information). *Rhodospirillum rubrum* has diverse metabolic pathways including species capable of denitrification, iron reduction, and sulfur oxidation (Finneran et al. 2003; Jin et al. 2020). The change in microbial community could be explained by the stimulation of methane and methylated compound oxidation due to the introduction of low concentrations of oxygen in the GSHP, and seen by predicted increase in aerobic respiration (predicted using PICRUSt2 analysis). This may or may not be coupled to nitrate reduction as nitrate reduction pathways were less commonly predicted and do not follow the same pattern. There may also be microbial involvement in iron and sulfur nutrient cycles, although only pathways involved in sulfur cycling were predicted.

Microorganisms associated with iron and sulfur cycling are often implicated in clogging of GSHP systems (Lindblad-Påsse 1998; Lerm et al. 2011; Vetter et al. 2012). Although there have been no significant clogging issues associated with this GSHP system (injection well water level has stayed constant over 5 years), there is some rust-colored precipitation in the recharge borehole (Figure S1). The similar concentrations of culture-based SRP in CS214 and GSHP boreholes and IOB throughout the system are consistent with the lack of clogging issues. SRP were below detection limit in CS317, although *Thermodesulfobacterium* was identified in the 16S sequence data. Known strains of *Thermodesulfobacterium* exclusively grow above 40 °C (Haouari et al. 2008), so would not be expected at the lower incubation temperature used in this study; however, Chang et al. (2001) found representatives related to *Thermodesulfobacterium yellowstonii* in an impacted aquifer at temperatures ranging from 8.4 to 14.0 °C. More generally any microorganisms capable of forming biofilms can cause clogging, several genera, including *Methylothermobacter*, are detected in the GSHP system indicating multiple risk factors that lead to GSHP clogging and risk of failure as seen in other systems (Braun et al. 2016).

Temporal Changes (System Active or Inactive)

There are limited differences in the chemistry of both control boreholes over time, whereas the Abstraction and in particular Recharge boreholes show much greater differences. This likely reflects the disturbance to hydrogeological conditions caused by pumping and re-injection of the water only 22 m away. Indeed, during GSHP-related pumping and injection periods (typically 1.5 L/s), cooled groundwater is being “recirculated” between the injection well and abstraction well due to the close well spacing (Boon et al. 2019) and this process may cause mixing between the injection and abstraction boreholes.

There is some evidence for seasonal variation in microbial activity in the control boreholes, with culturable bacteria being highest in summer sampling. The pattern is less clear for SRP and IOB, but the confidence limits on these tests, combined with missing data make it difficult to draw firm conclusions. The gravel aquifer is locally around 8–18 m bgl, and in this area and is confined by a few meters of low permeability tidal flat deposits (soft organic silty clay with occasional peat lenses) and made ground. The “Zone of Seasonal [temperature] Fluctuation” in the Cardiff Urban area varies from 7.1 to 15.5 m bgl, with the average of 9.5 m bgl (Farr et al. 2017), which correlates with the reported depth to the zone of seasonal fluctuation for CS241 of 8.5 m bgl (Farr et al. 2017). Differences were seen among the time points in the microbial community composition in both control boreholes however the clustering analysis indicates the difference is greater in CS317. There are insufficient samples to draw firm conclusions, however seasonal variation in the microbial community has previously been seen in pristine (Zhou et al. 2012) and impacted groundwaters (de Lambert et al. 2021), with rainfall (Zhou et al. 2012), and connection to surface (Bougon et al. 2012) being cited as important factors as they recharge groundwater with nutrients. Although surface connection is unlikely as the head of the water in the aquifer is slightly higher than the bay, pumping or leakage from sewers could introduce nutrients to the groundwater (Heathcote et al. 2003).

Differences among the samples taken at different time points do suggest that there is an effect on the microbial community depending on whether the GSHP system is active or inactive. Differences can also be seen in the culturable microbial community, although this may be due to seasonal differences. Although there are differences in community composition, samples tend to cluster on location (i.e., abstraction, recharge, top, or base) rather than timepoint, this is particularly clear for the recharge top samples. Additional sampling would be required to properly separate the effects of location, GSHP status, and seasonal factors.

Conclusions

It is clear that the open-loop GSHP operation had an influence on the chemistry and microbial community in

groundwater of the glacio-fluvial sand and gravel aquifer in which it is situated. The most striking finding was observation of high methane concentrations and the dominance of methane and methyl oxidizers including *Methylobacter* and *Methylomonas* and particularly *Methylothera* in the open-loop GSHP borehole samples. These genera are potentially stimulated by increases in oxygen through the operation of the pumps. There are subtler differences in the microbial community between the abstraction and recharge boreholes as well as changes related to the operational state of the GSHP (i.e., on or off). On top of these influences, some seasonal variation is seen, at least in the overall microbial biomass, as estimated by heterotrophic cell counts as observed in control boreholes. DNA sequencing indicates a microbial community that is involved in carbon, nitrogen, and sulfur cycling. The high methane concentrations make this GSHP an unusual case study, but as the use of GSHP systems becomes more widespread, it will become increasingly important to understand the impacts that they can have on groundwater chemistry and microbiology and how this could affect the operation of the GSHP. Although clogging was not an issue reported to date with this GSHP system, risk microorganisms were found. With this in mind, there is a need for more research and monitoring, especially as this study found that the GSHP was dominated by *Methylothera* species that have previously been associated with aquifer clogging.

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Data Availability Statement

The DNA sequence data and link to the geochemical data can be found under BioProject PRJNA925452 on the NCBI database

Authors' Note

The author(s) does not have any conflicts of interest or financial disclosures to report.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article. Supporting Information is generally *not* peer reviewed.

Data S1. Media Recipes: This section contains the details of the microbiology media using for the culture-based analyses.

GSHP sediment materials methods and results of 16S rRNA gene sequencing.

These sections contain the details of sand and gravel material that was incubated in the four boreholes. These sections also contain the results of these incubations.

Figure S1. Sediment housing after 17 months down hole. Left to right CS317, CS241, Abstraction, and Recharge sediments.

Figure S2. Microbial diversity of sediment traps from the base of each borehole and the starting material based on 16S rRNA gene sequencing.

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