

Case Study

Groundwater microbiology of an urban open-loop ground source heat pump with high methane

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carbon, nitrogen and sulphur cycles and alter bacterial communities*

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Abstract

Ground source heat pumps (GSHPs) are low carbon alternatives to gas boilers for decarbonising 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 analysed 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 *Methylothermobacter* 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 sulphur and iron cycling (*Rhodospirillum rubrum*). Methane concentration was analysed after identification of methylothermotrophs and found that methane concentrations were up to 2855 $\mu\text{g L}^{-1}$, 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 support modelling to maximise 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 heat from the groundwater to a separate network of 'clean' water which is passed through a heat pump which 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 three to four units of heat output for one unit of electrical input, and has 15 to 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 2021) and to reach NetZero emissions by 2050 and 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-urbanised areas (Farr et al. 2017; Allen, Milenic, and Sikora 2003; Benz et al. 2016; 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 (Benz et al. 2016; Bidarmaghz et al. 2019; Makasis et al. 2021; Menberg et al. 2013).

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 100m away from the abstraction well, or discharge it direct 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 (Briemann et al. 2009) and an increase from 11-25°C showed a switch from a microbial community dominated by iron and nitrite reducing bacteria to sulphate reducers has also been observed (Bonte et al. 2013; Jesušek, Grandel, and Dahmke 2012). The authors hypothesised 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). Sulphate oxidising bacteria have been reported at a range of temperatures. These organisms can coexist with sulphate reducing prokaryotes (SRP), and may increase if electron acceptors such as molecular oxygen or nitrate are introduced into the system (Lienen et al. 2017; Westphal et al. 2016; Würdemann et al. 2016). The increase in these sulphur cycling organisms may be particularly important as they have been associated with 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 to 14 °C and cooled it to 5 °C. In summer groundwater between 6 and 10 °C was used for air conditioning, re-injecting water at 15-30 °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 sulphur oxidising bacteria (of the genus *Theothrix*) and iron oxidising 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 oxidising 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 sulphur oxidising 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 ground source heat pump scheme is located at Grangetown 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 of a 22m 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 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, Lewis, and Sutton 2003).

A former landfill site and a gasworks site lie approximately 500 m to the west and south-west 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.

Table 1. Summary of sample sites used in this study. The GSHP Plant Room samples were taken prior to this study and are for water chemistry only.

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

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 in 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 three 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, thus had not been operational for approximately five weeks.

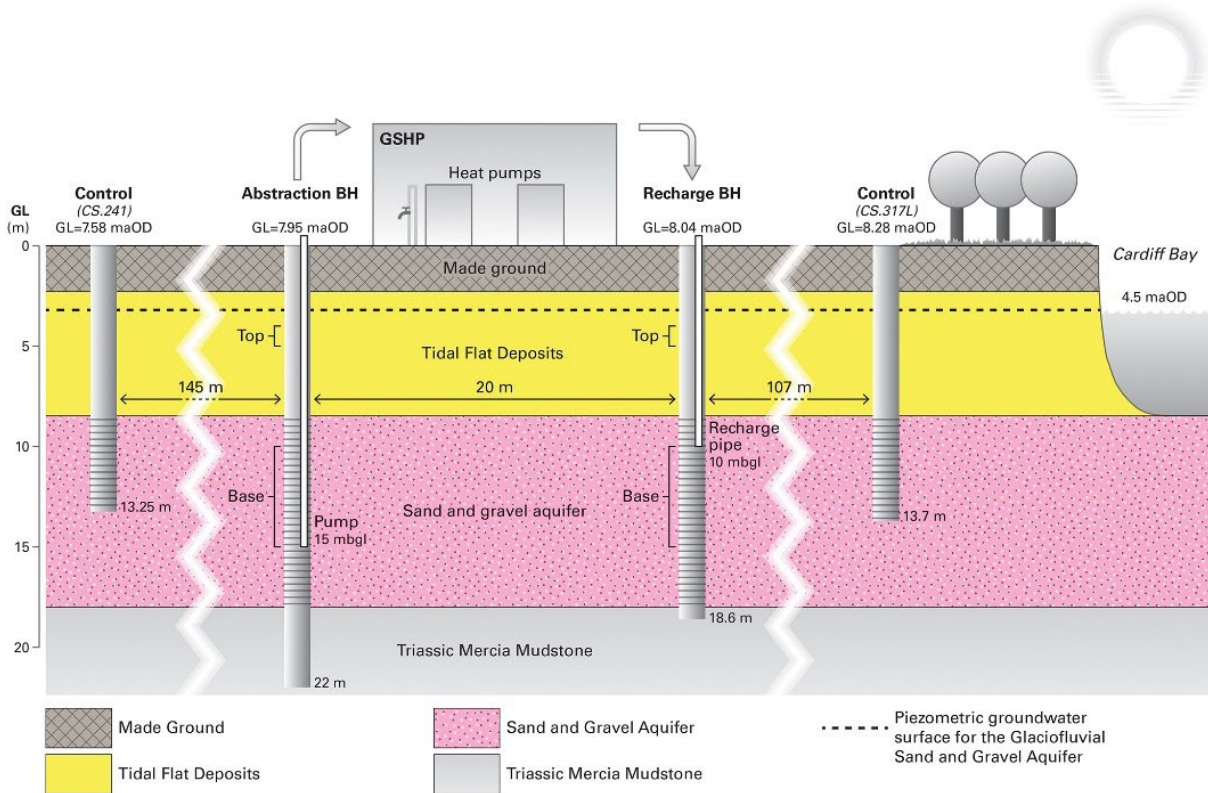


Figure 1. Map of the Cardiff Bay area and conceptual diagram of the ground source heat pump and monitoring boreholes (the latter modified from Boon et al 2019, with permission from Elsevier).

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 metres 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 (>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, DO, Eh, EC and pH. Data was collected by separate meters, all from SevenGo pro series (Mettler Toledo®, Columbus, OH, USA) and probes were from the following manufacturers temperature (Hanna Instruments®, Woonsocket, RI, USA), DO (InSitu®, Fort Collins, CO, USA), Eh (VWR®, Radnor, PA, USA) and EC (Mettler Toledo®, Columbus, OH, USA). For the abstraction and recharge boreholes, the top samples were collected first at a depth of 4-5 m bgl. Before sample collection 5L groundwater was pumped/bailed. Sampling the base of the boreholes was carried out at 10-15 m bgl. Before sampling the base, a further 10 L 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 hours of collection, details are provided in the relevant section below.

Chemical data from previously collected samples have been included to give an indication of the range of chemistries at this site (Historical GSHP data). These were collected from the sample tap in the GSHP plant room, i.e. 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 colonisation of mineral surfaces. Details of sampling methods and results are provided in Supplementary Material.

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 and acidified (1% HNO_3). Analysis of selected anions using ion chromatography (IC) and cations by inductively couple plasma mass spectrometry (ICP-MS) (Marriott et al. 2020; Watts et al. 2021; Watts et al. 2019). The following analytes were determined by IC using Dionex ICS5000 (ThermoFisher Scientific, Waltham, MA, USA) 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, CA, USA) 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 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 sampling to analyse for light gas composition (CH_4 , CO_2 and C_2H_6). Data was analysed from all sampling locations except Recharge base. Apart from the reduction in batch size, sample collection and analysis 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 analysed using a headspace method on an Agilent 7820A gas chromatograph with an evacuated inlet system and a flame ionisation 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 sulphur 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 ten-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, Misra, and Irwin 1938). Eight 15 μ l drops of each dilution were spotted onto a 5% PTYG agar plate (Table S1). 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 were 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 Table S1) 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 (Supplementary Material) 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. Presence of IOB was confirmed by the formation of horizontal rust coloured bands. Counts were made after 30 days.

DNA analyses

For samples from June and October 2019, 1L 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, 2.5-3L 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, MA, USA) 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 analysed using EPI2ME

Fastq 16S workflow (r2021.09.09). EPI2ME Fastq 16S output was collated into different taxonomic levels by Analysis of EPI2ME 16CVS Output workflow in EPI2ME Labs (Server v1.1.6). Clustering analysis was carried out using the nearest neighbour method with Bray-Curtis dissimilarity index using PAST v4.09 (Hammer, Harper, and Ryan 2001). Past v4.09 was also used for principal component analysis (PCA), correlation matrix for geochemical data.

Potential function prediction based on 16S 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 MetONTIIME (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 represent 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 boreholes (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 noticeably variation is elevated

dissolved oxygen (DO) in December 2020 samples in both control boreholes (Table 2).

In the GSHP samples, the dissolved oxygen was highest in October (heat pump system on). The sulphate was also highest in October, with the exception of Recharge top.

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 Nov 2015	n.d.	1.44	<0.6	<0.1	0.758	3397
Plant room filter Dec 2015	n.d.	1.20	<0.6	<0.1	1.019	3981
Plant room filter Jan 2016	n.d.	<1	<0.6	<0.1	0.611	3986
Plant room sample tap Jan 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 Oct. 2019	11.8	22.4	0.425	0.012	<0.02	133
Abstraction top Dec. 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 Oct. 2019	5.28	13.0	0.232	<0.01	0.874	1402
Abstraction base Dec. 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	12680

Recharge base October 2019	8.52	1.48	<0.06	<0.01	0.071	3590
Recharge base Dec. 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

n.d. not determined

When principal component analysis 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 were a little more varied and the greatest the abstraction borehole showed 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 sulphate) to Component 2, both positively.

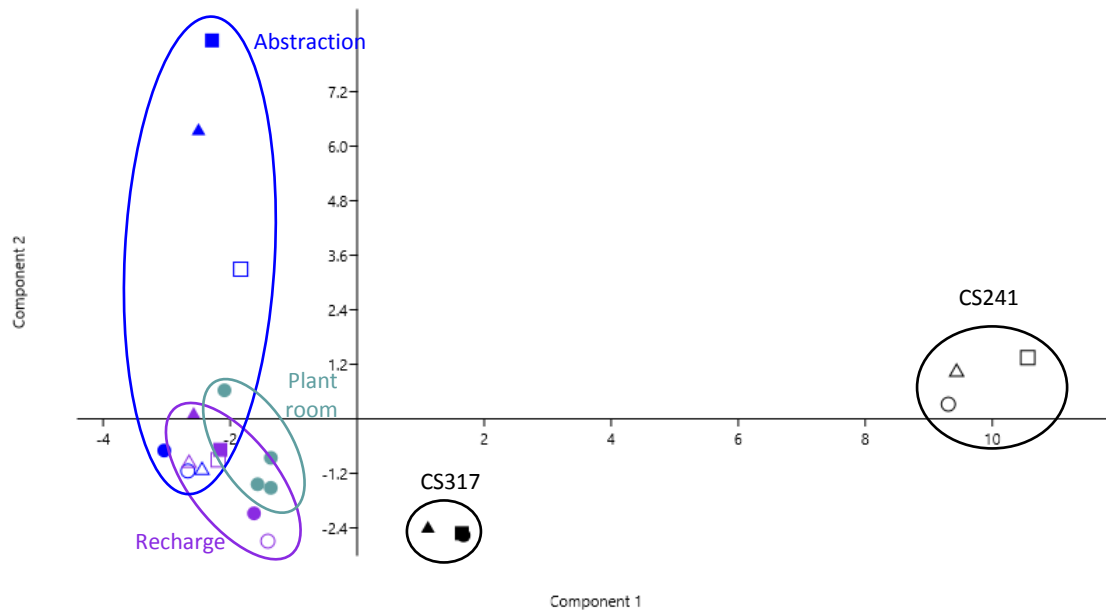


Figure 2. PCA for chemical and field data. Ovals indicate different sample locations and are to aid visualisation rather than indicate a statistical significance. Squares = October (system on), triangles = December (system off) and circles = July

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 (%), temp ($^{\circ}\text{C}$), Ca, Mg, Na, K, Cl, SO_4^{2-} , Br $^{-}$, HPO_4^{2-} , 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.

In addition to the IC and ICP-MS data, samples from December were analysed for dissolved gases (Table 3). Concentrations of methane were highest in the Recharge top, Abstraction base and CS241 (2831-2855 $\mu\text{g L}^{-1}$). 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 $\mu\text{g L}^{-1}$).

Table 3. Gas composition of groundwater samples from December 2020

Sample	CH ₄ ($\mu\text{g L}^{-1}$)	C ₂ H ₆ ($\mu\text{g L}^{-1}$)	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

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 the control wells (1.7×10^3 CFUs mL⁻¹). Anaerobes were highest in control wells (4.5×10^2 CFUs mL⁻¹), then Recharge (1.7×10^2 CFUs mL⁻¹) and lowest in Abstraction (7.0×10^1 CFUs mL⁻¹).

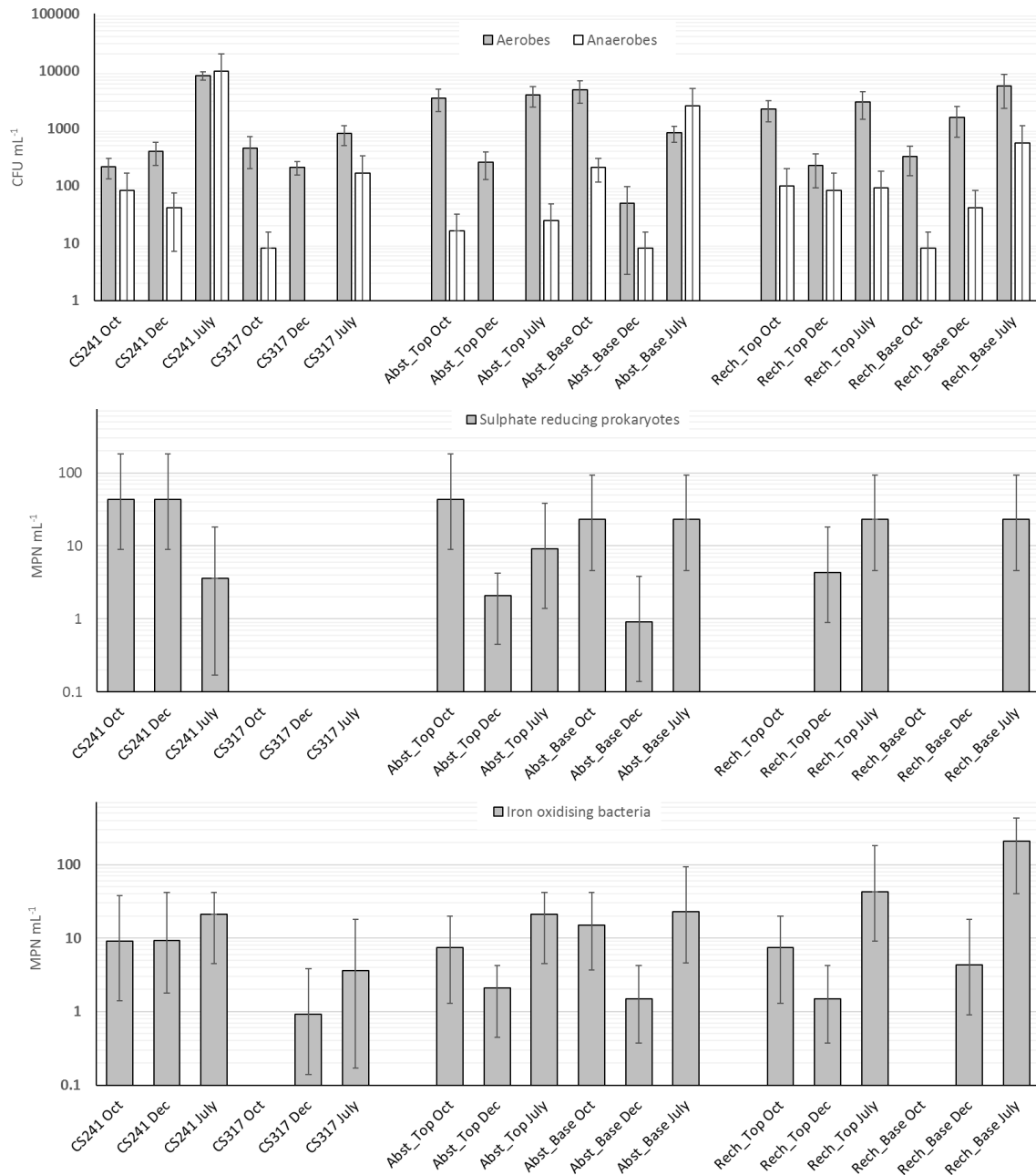


Figure 3. Viable counts for culture based assays. Top: aerobic and anaerobic heterotrophs. Bottom: sulphate reducing prokaryotes (SRP), iron oxidising bacteria (IOB) and methanotrophs. There is no data for CS317 October IOB. All other

absences indicate MPN less than detection limit. Error bars for CFU (heterotrophs and methanotrophs) are 1 SD and 95% confidence limits for MPN (SRP and IOB).

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 3). 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 394302. 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 290362 and 151105 reads, respectively.

The most striking difference in bacterial communities between the samples was the high prevalence of *Methylothera* 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). *Methylothera* 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 *Rhodospirillum* 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%, 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 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).

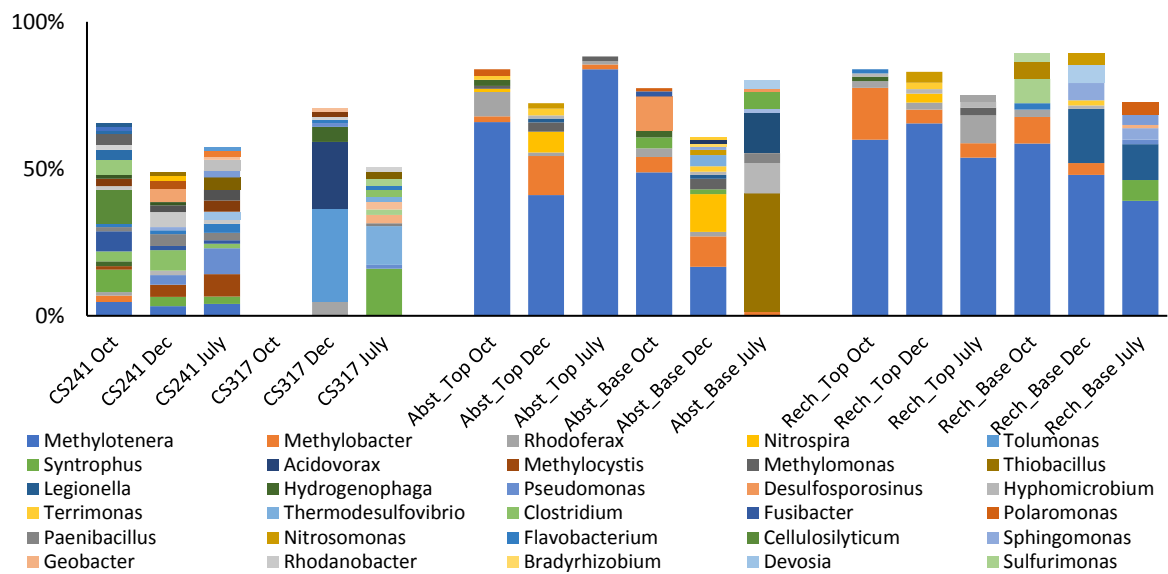


Figure 4. Microbial diversity of groundwater samples and the original material based on 16S rRNA sequencing. Genus level classification at >1% abundance relative to classified reads.

Table 4. Diversity indices by order (Shannon). Generated in PAST v4.09, samples were rarefied to 380 classified reads.

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

Clustering analysis based on sequencing data divided the samples into two main clades (Figure 5). The control samples forming clustering 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.

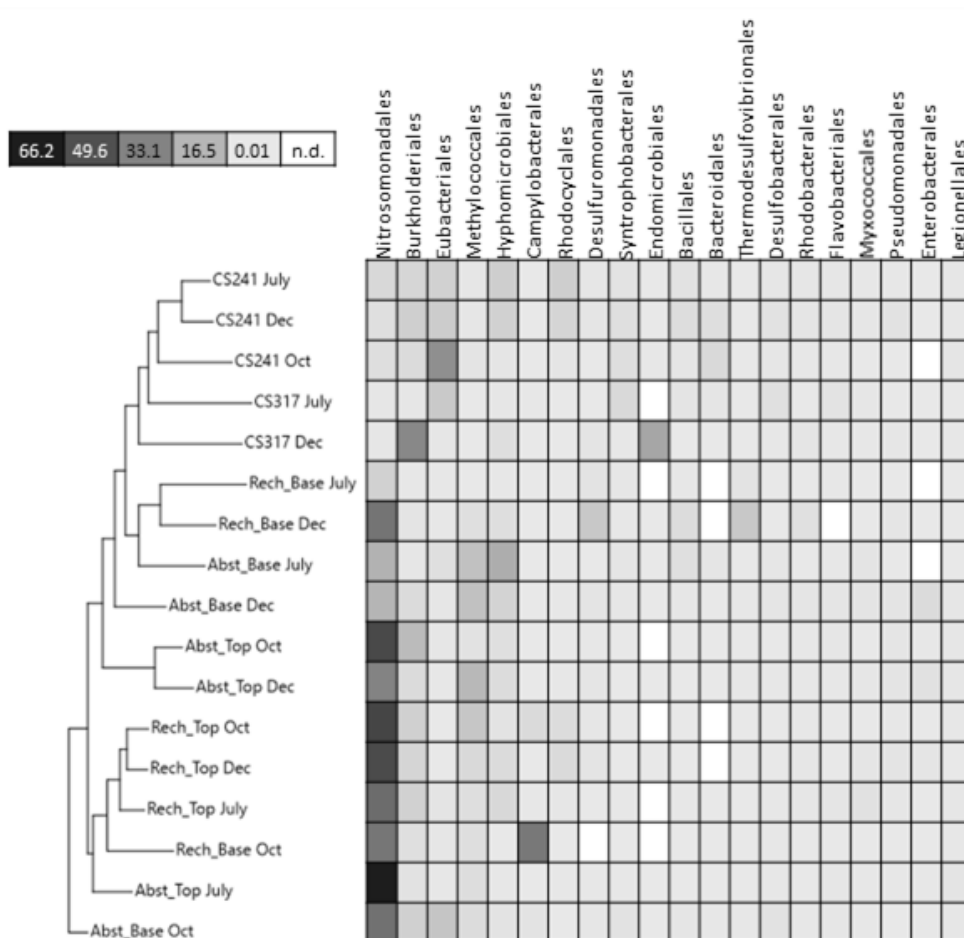


Figure 5. Left: nearest neighbour clustering of water samples using Bray Curtis dissimilarity index. Clustering was performed on Order level classification of 16s rRNA gene. Right: heatmap showing relative abundance of Orders with average abundance >0.05%.

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 sulphur cycling. Two nitrate cycling pathways were identified, these were much less abundant than sulphur cycling, and were seen in ten samples across control, abstraction and recharge boreholes.

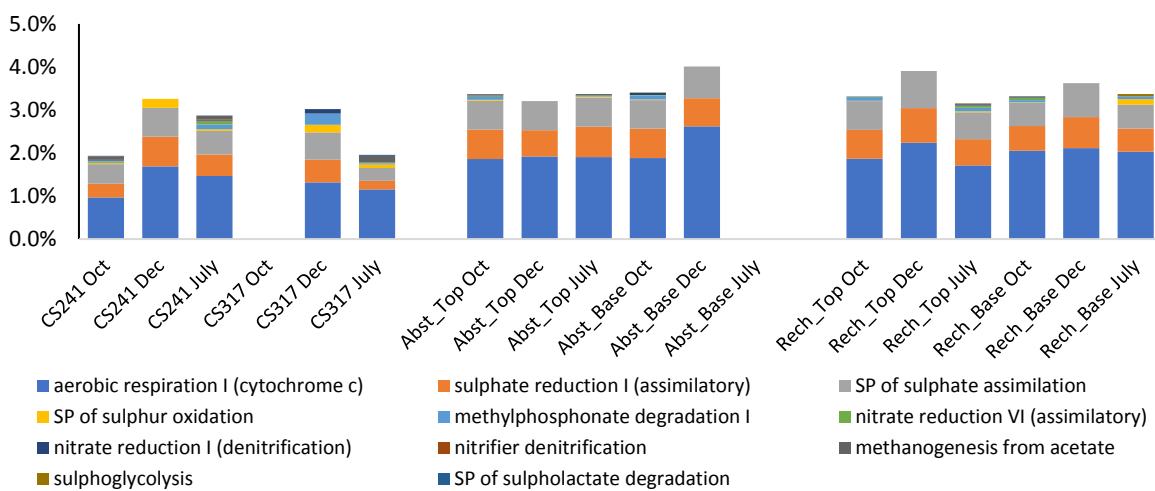


Figure 6. Potential pathways and superpathways (SP) as predicted by PICRUSt2. There were limited matches to 16S database for samples CS317 October 2019 and Abstraction base July 2019 so these were not included. Full names for 'SP of sulphate assimilation and cysteine biosynthesis' and 'SP of sulphur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

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 to 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, towards 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

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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 characterised 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 the recharge borehole, top) 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 (van Grinsven et al. 2020; Jin et al. 2020) but *Methylothera* are known to oxidise one-carbon compounds (e.g. methylamine and methanol) and are not reported to be able to oxidise methane. Methanol oxidation is often coupled to dissimilatory nitrate reduction (Afshin, Delherbe, and Kalyuzhnaya 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* (van Grinsven et al. 2020; Hernandez et al. 2015; Martineau, Whyte, and Greer 2010) 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 ¹³C tracer studies support this close relationship between species and suggests that *Methylothera* occurrence depends on active *in-situ* methane cycling (Afshin, Delherbe, and Kalyuzhnaya 2021). Microcosm studies have found that *Methylothera* is more competitive under low oxygen than other methylotrophs (15µM 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 able to couple methane oxidation with nitrate reduction (Martineau, Mauffrey, and Villemur 2015; Layton et al. 2000), and again activity levels depend on oxygen concentration (Martineau, Mauffrey, and Villemur 2015). Nitrate and nitrite concentrations were generally low and below detection limit in the control boreholes. 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 *Methylothera* abundance less than 1%, although did have 1.4% *Methylobacter*. This is a tentative indication that *Methylothera* could be responsible for nitrate reduction in the GSHP system and is limited by another factor. *Methylothera mobilis* has been reported to be inhibited by high nitrate concentrations (Afshin et al 2015). This was tested at 0.1% nitrate (about 30 times concentration in this sample), but inhibition could be

possible at lower concentrations. *Rhodoferrax* is the fourth genus that was more commonly observed in GSHP system and dominated the abstraction borehole sediment (Supplementary Material). *Rhodoferrax* has diverse metabolic pathways including species capable of denitrification, iron reduction and sulphur oxidation (Jin et al. 2020; Finneran, Johnsen, and Lovley 2003). 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 of iron and sulphur nutrient cycles, although only pathways involved in sulphur cycling were predicted.

Microorganisms associated with iron and sulphur cycling are often implicated in clogging of GSHP systems (Lerm et al. 2011; Vetter et al. 2012; Lindblad-Påsse 1998). 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-coloured precipitation in the recharge borehole (Supplementary Material Figure S1). The similar concentrations of culture-based SRP CS214 GSHP boreholes and IOB throughout the system are consistent with the lack of clogging issues. SRP were below detection limit in CS317, although *Thermodesulfovibrio* was identified in the 16S sequence data. Known strains of *Thermodesulfovibrio* 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 *Thermodesulfovibrio 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 *Methylothera*, 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 22m 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 well.

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 7.1-15.5 m bgl, with the average of 9.5m bgl (Farr et al. 2017), which correlates with the reported depth to the zone of seasonal fluctuation for CS241 of 8.5m 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 indicate the difference is greater in CS317. There are insufficient samples to draw firm conclusions, however seasonal variation in the microbial community have previously been seen in

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pristine (Zhou, Kellermann, and Griebler 2012) and impacted groundwaters (de Lambert et al. 2021), with rainfall (Zhou, Kellermann, and Griebler 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, Lewis, and Sutton 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 oxidisers including *Methylobacter* and *Methylomonas* and particularly *Methylothermobacter* in 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

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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 sulphur 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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Supporting Information

Additional supporting information can be found in the online version of this article:

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.

Please note: 'Supporting Information' is generally not peer reviewed. Wiley is not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author.

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Figure Captions

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)..... 8

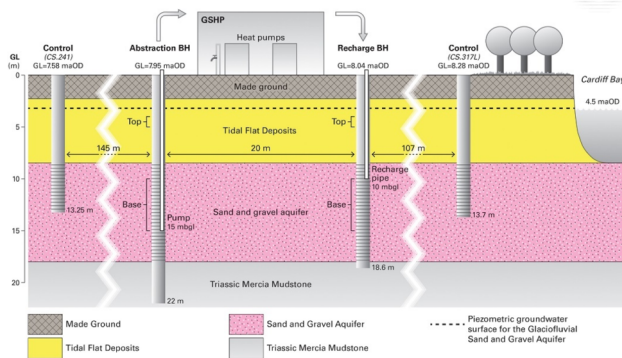
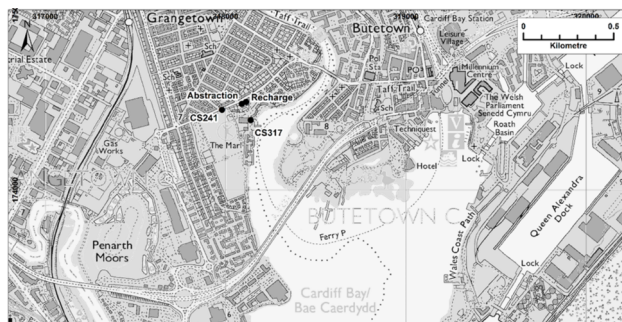
Figure 2. PCA for chemical and field data. Ovals indicate different sample locations and are to aid visualisation rather than indicate a statistical significance. Squares = October (system on), triangles = December (system off) and circles = July samples (system off). For abstraction and recharge the 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 (%), temp ($^{\circ}\text{C}$), 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..... 15

Figure 3. Viable counts for culture based assays. Top: aerobic and anaerobic heterotrophs. Bottom: sulphate reducing prokaryotes (SRP), iron oxidising bacteria (IOB) and methanotrophs. There is no data for CS317 October IOB. All other absences indicate MPN less than detection limit. Error bars for CFU (heterotrophs and methanotrophs) are 1 SD and 95% confidence limits for MPN (SRP and IOB).17

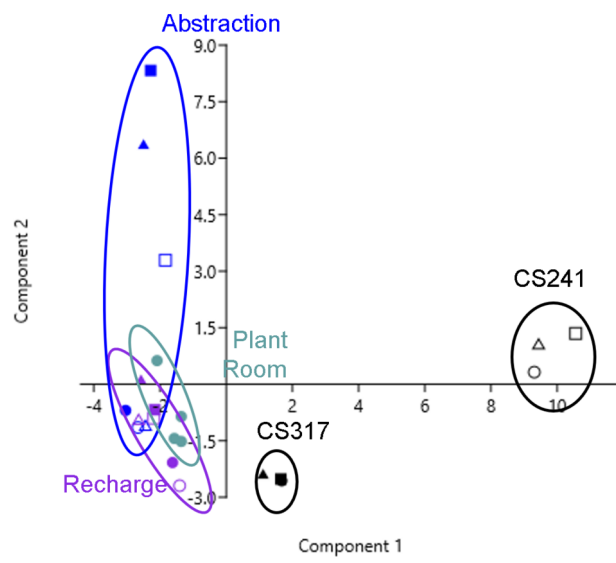
Figure 4. Microbial diversity of groundwater samples and the original material based on 16S rRNA sequencing. Genus level classification at >1% abundance relative to classified reads.....19

Figure 5. Left: nearest neighbour 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 >0.05%.20

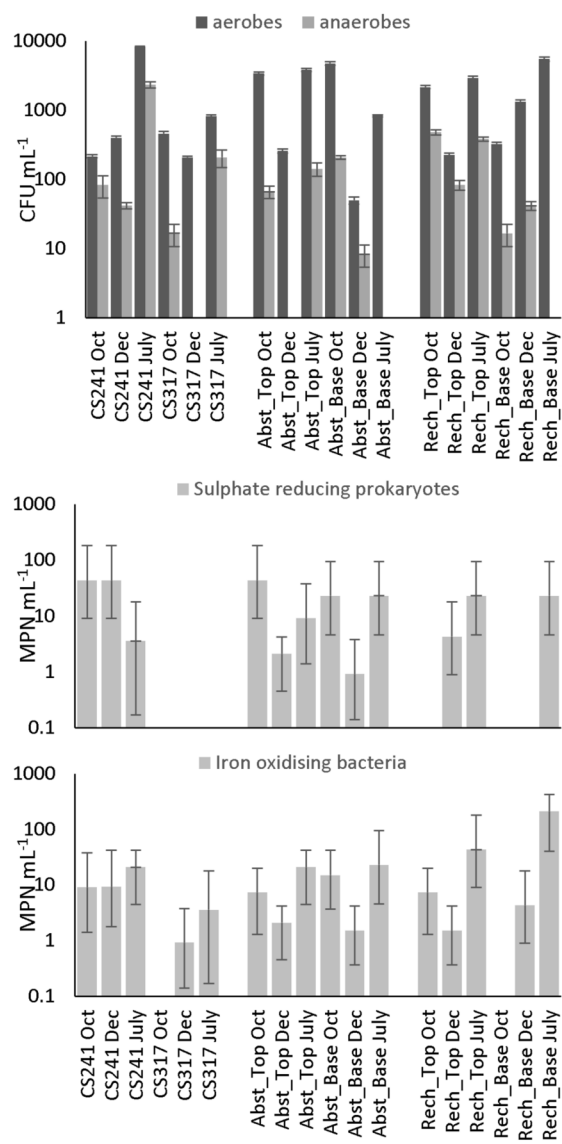
Figure 6. Potential pathways and superpathways (SP) as predicted by PICRUSt2. There were limited matches to 16S database for samples CS317 October 2019 and Abstraction base July 2019 so these were not included. Full names for 'SP of sulphate assimilation and cysteine biosynthesis' and 'SP of sulphur oxidation (*Acidianus ambivalens*)' were shortened in the figure legend. Percentages are abundance of selected pathway as proportion of total pathways identified in the sample.21



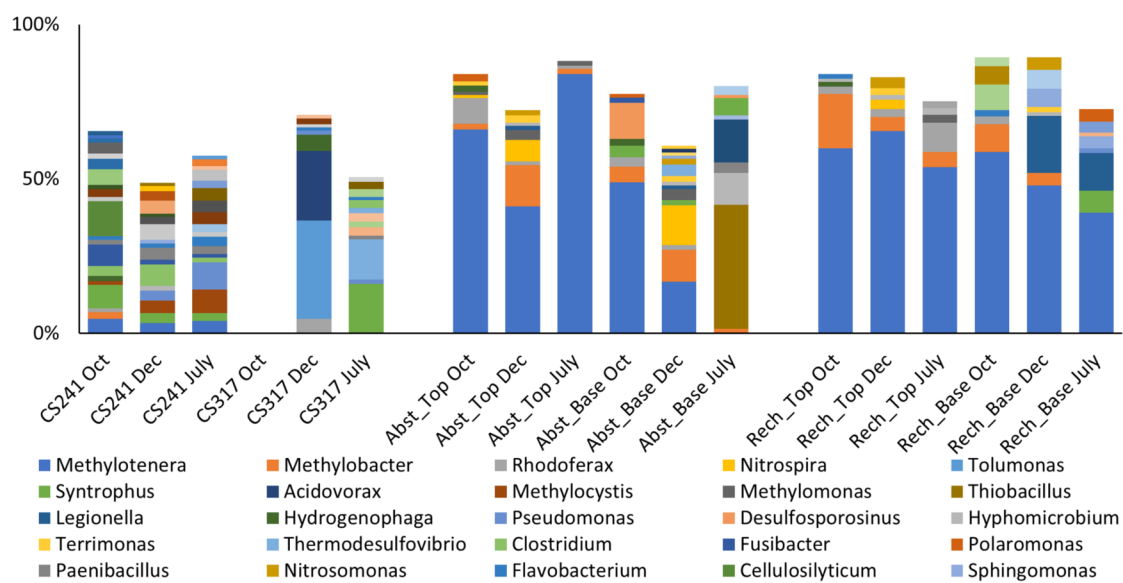
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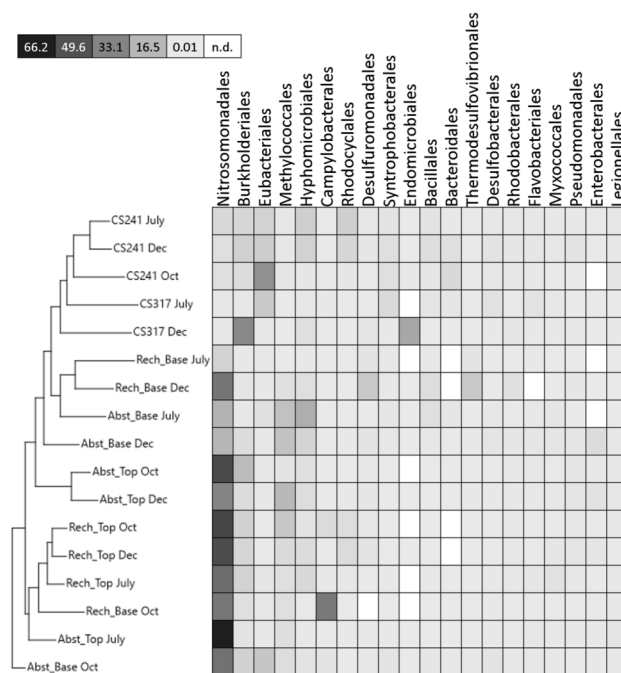
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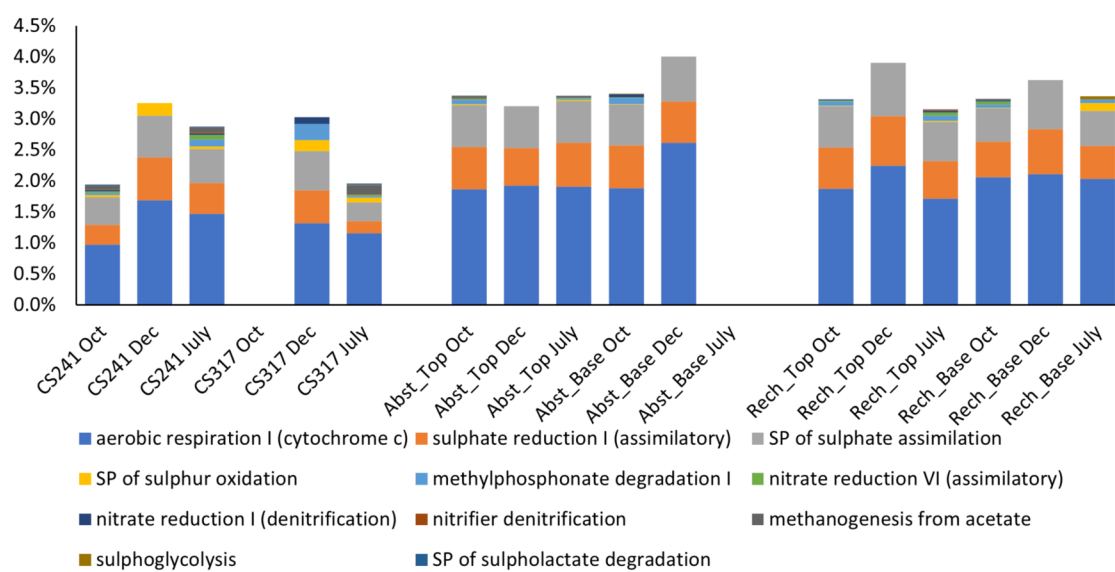
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GWAT_13291_Figure4.tif



GWAT_13291_Figure5.tif



GWAT_13291_Figure6.tif