

RESEARCH ARTICLE

Spatiotemporal and seasonal dynamics in the microbial communities of a landfill-leachate contaminated aquifer

Daniel Abiriga^{1,*,\dagger}, Andrew Jenkins¹, Kristian Alfsnes², Live Semb Vestgarden¹ and Harald Klempe¹

¹Faculty of Technology, Natural Sciences and Maritime Sciences, Department of Natural Sciences and Environmental Health, University of South-Eastern Norway, Gullbringvegen 36, NO-3800, Bø, Norway and

²Division for Infection Control and Environmental Health, Norwegian Institute of Public Health, PO Box 222 Skøyen, NO-0213, Oslo, Norway

*Corresponding author: Faculty of Technology, Natural Sciences and Maritime Sciences, Department of Natural Sciences and Environmental Health, University of South-Eastern Norway, Gullbringvegen 36, NO-3800, Bø, Norway. Tel: +4735575378; E-mail: daniel.abiriga@usn.no

One sentence summary: Spatiotemporal dynamics of microbial communities were discovered in a landfill-leachate contaminated aquifer.

Editor: Tillmann Lueders

^{\dagger}Daniel Abiriga, <http://orcid.org/0000-0002-7744-5759>

ABSTRACT

The microbiome of an aquifer contaminated by landfill leachate and undergoing intrinsic remediation was characterised using 16S rRNA metabarcoding. The archaeal/bacterial V3–V4 hypervariable region of the 16S rRNA gene was sequenced using Illumina MiSeq, and multivariate statistics were applied to make inferences. Results indicate that the aquifer recharge and aquifer sediment samples harbour different microbial communities compared to the groundwater samples. While *Proteobacteria* dominated both the recharge and groundwater samples, *Acidobacteria* dominated the aquifer sediment. The most abundant genera detected from the contaminated aquifer were *Polynucleobacter*, *Rhodoferrax*, *Pedobacter*, *Brevundimonas*, *Pseudomonas*, *Undibacterium*, *Sulfurifustis*, *Janthinobacterium*, *Rhodanobacter*, *Methylobacter* and *Aquabacterium*. The result also shows that the microbial communities of the groundwater varied spatially, seasonally and interannually, although the interannual variation was significant for only one of the wells. Variation partitioning analysis indicates that water chemistry and well distance are intercorrelated and they jointly accounted for most of the variation in microbial composition. This implies that the species composition and water chemistry characteristics have a similar spatial structuring, presumably caused by the landfill leachate plume. The study improves our understanding of the dynamics in subsurface microbial communities in space and time.

Keywords: microbial ecology; multivariate analysis; contaminated groundwater; aquifer sediment; aquifer recharge; municipal landfill

Received: 5 February 2021; Accepted: 15 June 2021

© The Author(s) 2021. Published by Oxford University Press on behalf of FEMS. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

INTRODUCTION

Much of the global freshwater used as potable drinking water, in agriculture and in industries comes from groundwater. In Norway, groundwater supports 15% of the total drinking water supply (Kløve et al. 2017). From biodiversity point of view, groundwater forms the largest terrestrial freshwater biome, harbouring up to 40% of the earth's freshwater prokaryotic biomass (Griebler and Lueders 2009; Griebler, Malard and Lefebure 2014), but studies on groundwater ecosystems are still scarce (Griebler et al. 2016). Despite these benefits, groundwater is subjected to frequent contamination from anthropogenic activities globally (Chapman 1996; Zaporozec and Miller 2000). In Norway, groundwater contamination from agricultural activities and landfill operations have been frequently reported (Basberg, Banks and Sæther 1998; Haarstad and Ludvigsen 2007; Haarstad and Mæhlum 2008; Kværner et al. 2014; Abiriga, Vestgarden and Klempe 2020), but industries and forestry management such as logging practices are other perturbations (Kløve et al. 2017). Landfill is still the primary municipal solid waste (MSW) disposal strategy practiced in both developing and developed countries (Mouser et al. 2005; Eggen, Moeder and Arukwe 2010; Zhang et al. 2016; Chen et al. 2017). Most of the pollution issues associated with MSW landfills relate to the leachate. Whereas newer sanitary landfills are equipped with liners, old MSW landfills represent a major source of groundwater contamination (Kjeldsen et al. 2002; Brad et al. 2013) due to lack of leachate containment systems. This makes them a potential public health concern as they may contain both legacy and emerging pollutants (Eggen, Moeder and Arukwe 2010; Lapworth et al. 2012), as well as being hotspot for antibiotic resistance (Chen et al. 2017).

The chemical composition of MSW landfill leachate can be categorised into four major components: organics, inorganics, heavy metals and xenobiotics (Kjeldsen et al. 2002), but the actual load of the individual components depends on the waste material landfilled. MSW landfills are predominantly composed of organic waste, but the story is far different in and before the 1990s, when MSW would essentially contain any waste (Christensen, Bjerg and Kjeldsen 2000). At Revdalen (the present study site), a wide range of contaminants have been detected in the groundwater (Abiriga, Vestgarden and Klempe 2020). It is widely believed that groundwater contamination from landfills can persist for decades or centuries (Bjerg et al. 2011). Such prolonged discharge of leachate into the groundwater is likely to leave an ecological footprint on the aquifer. Longer perturbations are thought to cause permanent elimination of native microbial species and allow incursion of new species which may come to dominate the microbial community (Herzyk et al. 2017).

Since landfills leach a complex mixture of pollutants, a combination of technologies is required to achieve better treatment results (Remmas et al. 2017; Ye et al. 2019). However, the versatile metabolic capabilities of microorganisms make them suitable for remediation of a wide range of contamination cases (Majone et al. 2015), and are frequently applied in monitored natural attenuation of landfill-impacted environments (Mouser et al. 2005). Microbially-catalysed reactions dominate the processes that drive natural attenuation of both organic and inorganic contaminants in the environment (Smets and Pritchard 2003). Monitored natural attenuation is by far the cheapest, but also an efficient and eco-friendly remediation strategy compared to other remediation techniques such as the conventional pump and treat remedial option (Majone et al. 2015; Logeshwaran et al. 2018).

Providing unequivocal evidence of intrinsic bioremediation involves use of metabolic functional analysis, stable isotope probing, reactive transport modelling, recording decrease in contaminant concentrations and identifying resident microbes to unravel their ecological characteristics such as pollutant transformation capabilities, abundance and distribution (Smets and Pritchard 2003; Mouser et al. 2005; Scow and Hicks 2005; Majone et al. 2015; Zhang et al. 2016; Lueders 2017). Notwithstanding, data from landfill-impacted groundwater microbial ecology utilising the latest available molecular techniques are scarce. Despite contaminations from landfills being numerous globally, attention has mostly been given to characterising the groundwater geochemistry or the leachate chemistry, although microbiological studies on leachate microbiology using the latest molecular techniques are now gaining momentum. Several previous studies have investigated the microbiology of landfill-impacted aquifers (Albrechtsen, Heron and Christensen 1995; Ludvigsen et al. 1999; Mouser et al. 2005; Lin et al. 2007; Chen et al. 2017; Taş et al. 2018). However, the spatiotemporal and seasonal microbial community dynamics in general remains understudied (Smith et al. 2018a), which highlights an important knowledge gap. Focusing not only on the abundance of degrading microbes but also on the diversity and dynamics provides a better understanding of contaminated groundwater ecosystems (Pilloni et al. 2019). The present study gives an insight into the microbiome of groundwater contaminated by a municipal landfill, by examining the diversity, abundance and changes in microbial community composition, as a function of the groundwater chemistry, distance, time and season over a period of 2 years.

MATERIALS AND METHODS

Study area

The study site is in a complex of quaternary deposits consisting of moraines, till, subglacial glaciofluvial deposit and glaciofluvial delta deposits (Klempe 2004). The presence of a few kettle holes in the delta deposits attracted dumping of waste and in the period 1958–1974, one of the largest kettle holes was directly filled up with MSW. Due to the isolation of the site from the town centre and the demand for a landfill for Bø and Sauherad Municipalities (now merged to form Mid-Telemark Municipality), four cells were opened to establish the Revdalen Landfill, which was operational from 1974 to 1996 (Abiriga, Vestgarden and Klempe 2020). Due to lack of liners and leachate collection system (less stringent regulation at the time), the leachate from the 1974 to 1996 cells have migrated and contaminated the confined aquifer underneath which is inside a submoraine glaciofluvial deposit (Klempe 2004). Additional information on the study site is available elsewhere (Klempe 2004, 2015; Abiriga, Vestgarden and Klempe 2020, 2021b).

Experimental procedure

Groundwater and aquifer sediment sampling

Groundwater samples were collected from four wells: R0, located in a nearby uncontaminated aquifer; R1, R2 and R4, located downgradient of the landfill in the contaminated aquifer (Fig. 1). R1 has five levels (R101–R105) at 126, 125, 124, 123 and 122 m above sea level; R2 has four levels (R201–R204) at 122, 121, 119 and 118 m above sea level; R4 has three levels (R401–R403) at 118, 117 and 114 m above sea level. R1, R2 and R4 were placed along the groundwater flow direction at the proximal, intermediate

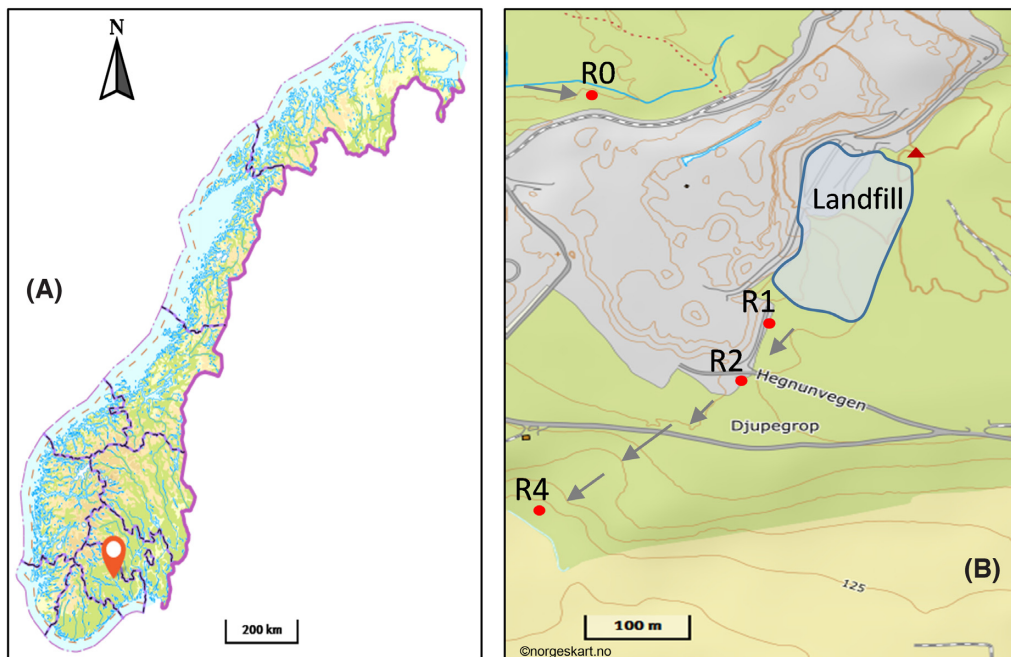


Figure 1. Location of Revdalen Landfill on a map of Norway (A) and a detailed map showing the location of the landfill and the sampling wells R0, R1, R2 and R4 (B). R0 is the background well located in a nearby uncontaminated aquifer, while R1, R2 and R4 are located in the contaminated aquifer, placed at the proximal, intermediate and distal positions from the landfill. The arrows indicate the groundwater flow direction, and the red triangle depicts the location of a seasonal rivulet which recharges the confined aquifer. For clarity of readability, the location for the aquifer sediment samples is not shown; it is only ~30 cm downgradient of R4. Green shading indicates woodland, yellow indicates farmland and grey indicates industrial land, including the landfill and an adjacent active gravel pit/quarry. [Mapping source: Norwegian Mapping Authority, www.norgeskart.no, with permission].

and distal positions, respectively, and are hereafter referred to as such. Groundwater samples were collected twice a year in spring and autumn in 2018 and 2019 from all the levels in the four wells. Groundwater sampling procedures have been described elsewhere (Abiriga, Vestgarden and Klempe 2020), as well as groundwater chemical analysis (Abiriga et al. 2021a). Samples for analysis of the diversity of the 16S rRNA genes were collected in sterile 350 mL PETE bottles. A total of 300 mL of the water was filtered through 0.2 μm polycarbonate membrane filters within 48 h and the filters were stored at -70°C prior to DNA extraction. A total of 52 samples were collected from the four monitoring wells. In addition, one sample was taken from a rivulet which feeds the aquifer at the outcrop area. Further, three aquifer sediment samples were obtained at depths of 6–7, 8–9 and 9–10 m using the piston method. 25 g saturated sediment subsamples were taken and stored at -70°C prior to DNA extraction.

DNA extraction, sequencing and bioinformatics

The procedure for the extraction of DNA from the filters has been described previously (Abiriga et al. 2021a). DNA extraction from the aquifer sediment was done using DNeasy PowerSoil Kit following the manufacturer's instructions. The amount of genomic DNA in the samples was quantified using Qubit Fluorometer 3.0 (Life Technologies, Malaysia) and the quality evaluated by gel electrophoresis on 2% agarose. All the samples were subjected to 16S rRNA gene metabarcoding of the V3–V4 hypervariable region using the universal primer set 319F (5'-ACTCCTACGGGAGGCAG CAG-3') and 805R (5'-GGACTACNVGGGTWTCTAAT-3'). Samples were sequenced using Illumina MiSeq (600 cycles) by applying the 300 bp paired-end protocol. PCR amplifications and library preparation, as well as the bioinformatics pipeline implemented in sequence data analysis are described in Abiriga et al. (2021a).

The library statistics for the samples are provided in the supplementary material (see supplementary data).

Statistical data analysis

All statistical analyses were conducted in R version 4.0.2 (R Core Team 2020). Water chemistry data was subjected to principal component analysis. Due to the dimensionally heterogeneous physicochemical data, the data was standardised (centred and normalised) prior to principal component analysis to ensure all parameters are given equal weight. Difference in groundwater chemistry across sampling wells and between spring and autumn was tested using the nonparametric Kruskal–Wallis and Mann–Whitney tests respectively, as the data showed nonnormal distribution. Because the levels of solutes in the background well were different from the other wells (assessed during data exploration), data from the background well was excluded in the Kruskal–Wallis analysis to minimise type-I error. In all the above analyses, iron has been excluded because it was below the limit of detection in all the wells except R4, while temperature was excluded due to many missing observations.

Nonmetric Multidimensional Scaling (NMDS) was used to visualise spatial group clustering based on Bray–Curtis dissimilarity distance. The operational taxonomic unit (OTU) data was transformed using fourth-root transformation (except for intermediate well which was subjected to square root transformation as fourth-root transformation was too strong for it) and standardised using Wisconsin double standardisation. Differences in the microbial community composition among sampling wells, between 2018 and 2019, and between autumn and spring were tested using permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) by applying the function *adonis* from package *vegan* (Oksanen et al. 2019) on 999 permutations.

The analysis was performed on Bray–Curtis dissimilarity measure calculated from fourth/square root-transformed and Wisconsin double-standardised community data. The assumption of homogeneity of group dispersion was checked using the function *betadisper* (Anderson 2006).

Canonical ordination i.e. redundancy analysis (RDA) was conducted on fourth-root-transformed and Hellinger standardised (Legendre and Gallagher 2001) OTU abundance data and standardised water chemistry data. Water chemistry variables that were significantly associated with variation in microbial species composition were selected using forward selection based on 999 permutations at 0.05 significance level (Akaike Information Criterion if there are ties) by implementing the function *ordstep* from package *vegan* (Oksanen et al. 2019). To quantify the contributions of water chemistry, season, year and distance (well) to the variation in microbial composition, variation partitioning (Borcard, Legendre and Drapeau 1992) was conducted and significance was tested by permutation (999 permutations). In total, two parameters: total nitrogen and temperature were removed from the data during the constrained ordination as they contain numerous missing values.

RESULTS

Groundwater chemistry

The groundwater chemistry was characteristically different across the four wells (Figures S1 and S2, Supporting Information). Overall, the wells in the contaminated aquifer were more related to each other than to the background well. Within the contaminated aquifer, similarity in water characteristics was greatest between the proximal and intermediate wells (Figure S2, Supporting Information). Nonparametric Kruskal–Wallis test showed significant differences across the wells in the contaminated aquifer for most of the groundwater parameters, except nitrate, total nitrogen and manganese (Table S1, Supporting Information). None of the parameters showed statistically significant differences across the depth profiles in the proximal, intermediate or distal wells (not shown). Seasonal variation was strongest in the proximal well, followed by the intermediate well (Table S2, Supporting Information). A total of eight groundwater variables showed significant differences between spring and autumn, however, none of the 15 variables showed significant seasonal changes in the distal and background wells. Similarly, interannual change in the groundwater chemistry was greatest in the proximal well (nine variables), followed by the intermediate well (six variables; Table S2, Supporting Information). The distal and background wells had only moderate (four variables) and marginal (one variable) significant interannual changes.

Groundwater and aquifer sediment microbiology

Microbial composition and abundance

There were 1763 OTUs detected from the contaminated groundwater samples, which were classified to 62 phyla, constituting 98.7% bacteria, 1.24% archaea and 0.06% unclassified. The background groundwater samples had 485 OTUs classified to 39 phyla, consisting of 97.73% bacteria, 2.06% archaea and 0.21% unclassified. The eight most abundant phyla from both the background and contaminated aquifer were *Proteobacteria*, *Patescibacteria*, *Actinobacteria*, *Acidobacteria*, *Verrucomicrobia*, *Bacteroidetes*, *Chloroflexi* and *Nitrospirota* (Fig. 2). The aquifer recharge sample had 599 OTUs, all from domain bacteria. These were classified to 29 phyla, of which, *Proteobacteria*, *Bacteroidetes*, *Chloroflexi*,

Verrucomicrobia, *Acidobacteria*, *Actinobacteria*, *Patescibacteria* and *Cyanobacteria* were the top eight phyla. From the aquifer sediments, 652 OTUs were detected, comprising 39 phyla. Of these, *Acidobacteria*, *Proteobacteria*, *Chloroflexi*, *Actinobacteria*, *Firmicutes*, *Methylomirabilota*, *Planctomycetota* and WPS.2 (candidate phylum) formed the top eight phyla (Fig. 2).

In order to simplify comparison, the top ten most abundant OTUs in each sample were identified. In the background well, these included genus *Duganella*, which displayed a higher abundance in spring. Other OTUs were *Collimonas*, *Pseudomonas*, *Polaromonas*, *Alkanindiges* and *Rhodoferax*; with *Duganella*, *Alkanindiges* and *Rhodoferax* as the most abundant OTUs in the spring samples of the first sampling campaign (Figure S5, Supporting Information). The proximal well showed that *Aquabacterium*, *Janthinobacterium*, *Oxalobacteraceae* and *Pedobacter* were highly abundant in the uppermost level of the well, while *Sulfurifustis* and *Sulfuritalea* were more abundant in the deeper levels (Figure S6, Supporting Information). Taxa *Methylobacter* and *Polaromonas* were ubiquitous throughout the levels in this well. Other taxa include *Gallionella*, *Polynucleobacter*, *Pseudomonas*, *Rhodanobacter* and *Rhodoferax*. A medically relevant genus among the top 10 taxa in this well was *Enterococcus*. In the intermediate well, the top 10 taxa showed modest abundances, except *Oxalobacteraceae*, *Saccharimonadales*, *Brevundimonas*, *Rhodoferax*, *Pseudomonas*, *Pedobacter*, *Caulobacter*, LWQ8 (uncultured family of *Saccharimonadales*) and *Undibacterium* (Figure S7, Supporting Information). Taxa of medical relevance in the top 10 OTUs were *Legionella* and *Stenotrophomonas*. Genera *Caulobacter* and *Methylobacter* were ubiquitous in the intermediate well. The most abundant taxa in the distal well were *Oxalobacteraceae*, *Rhodoferax*, *Polynucleobacter* and *Undibacterium* (Figure S8, Supporting Information), but *Saccharimonadales* was also moderately abundant. Genus *Duganella* appeared in the second year of the sampling campaign, but only occurred in high abundance in spring. A similar seasonal trend was observed for *Polynucleobacter* and GK598 (uncultured *Alcaligenaceae*).

Spatial and seasonal variation in microbial community composition

The microbial community compositions of the water samples collected from the four sampling wells clustered separately from each other, although the proximal and intermediate wells showed some slight overlap (Fig. 3). The aquifer sediment samples also clustered separately and well away from the nearby distal well, and the recharge sample was also well isolated from the other groups. PERMANOVA results from a global comparison ($F = 7.14$, $df = 4$ and $P = 0.001$) and pairwise comparisons (Table 1) revealed significant differences in microbial community composition across and between the wells, respectively. The overall quantitative contribution of distance to the microbial community composition is provided under RDA (Fig. 7).

In the proximal well, an overall analysis of variance (PERMANOVA) showed a statistically significant difference among the levels ($F = 1.47$, $df = 4$ and $P = 0.002$). However, performing pairwise comparisons between the levels did not give significant differences after Bonferroni correction (Table S3, Supporting Information). In moving from top to bottom within the proximal well, a higher dispersion was observed among the samples from the topmost level (Fig. 4 and Figure S4, Supporting Information). No significant overall difference was observed among the depth profiles in the intermediate or distal wells ($F = 0.89$, $df = 3$ and $P = 0.651$ and $F = 1.08$, $df = 2$ and $P = 0.322$, respectively). Similarly, no significant differences in the pairwise comparisons between the levels in each well were observed (Table S3, Supporting Information). As in the proximal well, samples from the

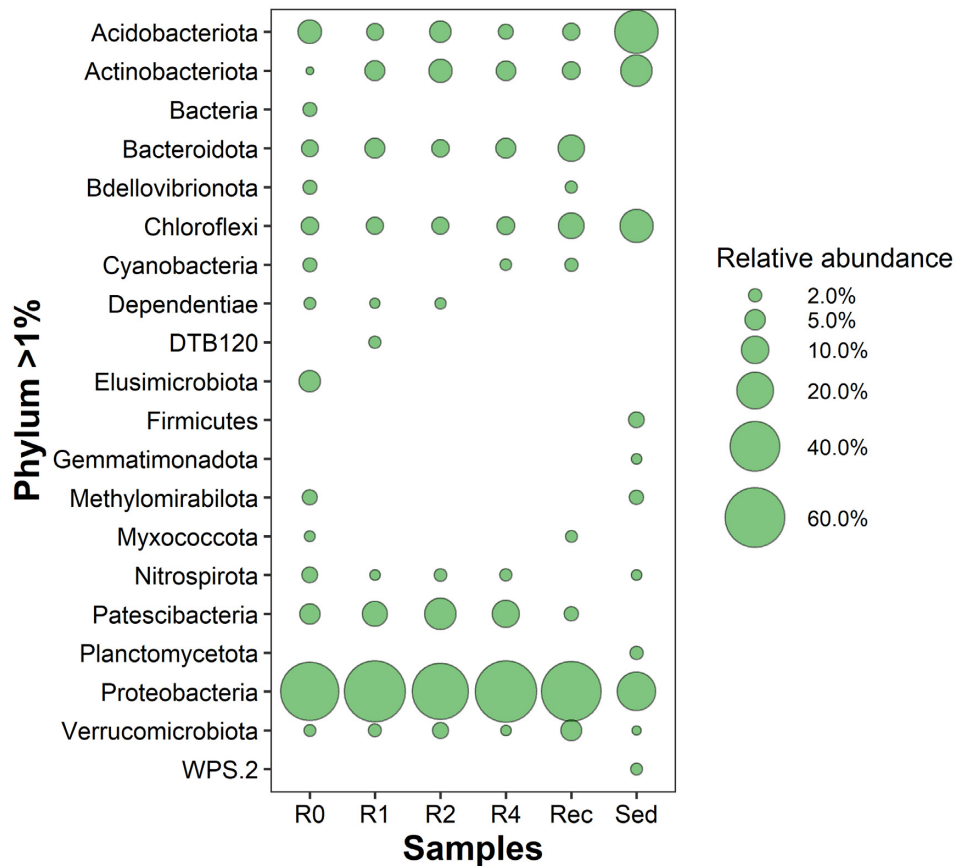


Figure 2. Abundant microbial phyla in the nearby uncontaminated aquifer (R0), in the contaminated aquifer (R1, R2 and R4), recharge water (Rec) and aquifer sediment (Sed) near R4. R1, R2 and R4 were placed along the groundwater flow direction at the proximal, intermediate and distal position from the landfill, respectively.

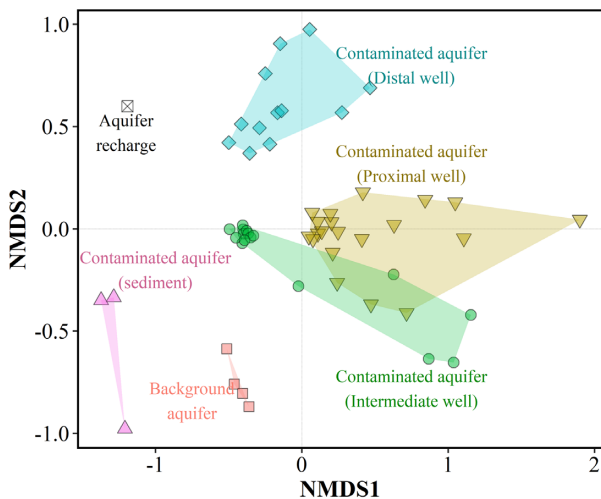


Figure 3. Nonmetric multidimensional scaling (NMDS) plot showing the beta diversity (Bray-Curtis) among the samples: the background aquifer (R0), the contaminated aquifer (proximal R1, intermediate R2, distal R4 and aquifer sediment), and the aquifer recharge.

topmost level in the intermediate well (R201) showed the highest dispersion (Fig. 4 and Figure S4, Supporting Information). In the distal well, the dispersion among samples was comparable, although the dispersion seemed higher in samples of the middle level (R402; Fig. 4 and Figure S4, Supporting Information). Among

Table 1. PERMANOVA result of pairwise comparisons of the microbial community composition between the samples. R0 is the background well located in a nearby uncontaminated aquifer, while R1, R2 and R4 are the wells located in the contaminated aquifer at the proximal, intermediate and distal positions from the landfill. Sed is the aquifer sediment sample.

Pairs	F. Model	R ²	P. adjusted ^a
R0 vs. R1	6.621	0.231	0.01
R0 vs. R2	7.022	0.281	0.01
R0 vs. R4	7.791	0.358	0.02
R0 vs. Sed	3.655	0.422	0.23
R1 vs. R2	7.379	0.178	0.01
R1 vs. R4	8.373	0.218	0.01
R1 vs. Sed	4.525	0.177	0.02
R2 vs. R4	8.191	0.240	0.01
R2 vs. Sed	3.680	0.178	0.01
R4 vs. Sed	3.822	0.227	0.03

^aP-value adjusted using Bonferroni correction.

The recharge sample was removed during the permutation testing as it has only one observation.

the levels in the proximal and intermediate wells, a lower microbial diversity was observed in the uppermost levels, while no clear trend was observed among the levels in the distal well (Figure S3, Supporting Information).

PERMANOVA conducted on seasonality showed significant differences in the microbial community composition between spring and autumn only in the proximal and intermediate wells

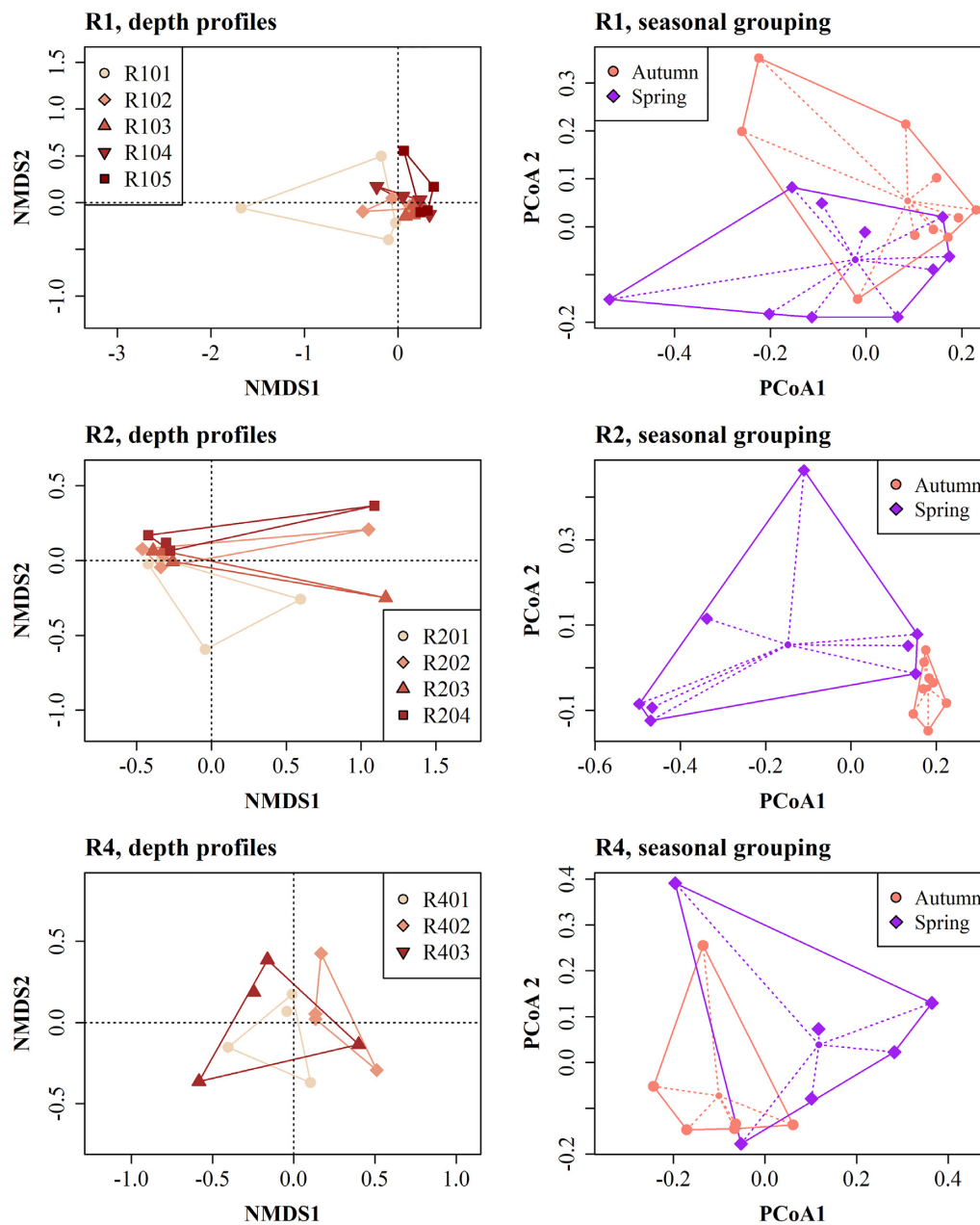


Figure 4. Nonmetric Multidimensional Scaling (NMDS) plots of the depth profiles and Principal Coordinate Analysis plots of seasonal grouping (the centres to which the samples are connected are the centroids) from the depth profiles. Colour schemes of light to dark indicate increasing depth. R1, R2 and R4 are the wells located in the contaminated aquifer at the proximal, intermediate and distal positions from the landfill.

(Table 2); this was accompanied by a large increase in dispersion in the intermediate well (Fig. 4 right hand panel). The overall seasonal effect on the microbial community is presented under RDA (Fig. 7).

Interannual variation (2018 and 2019)

Beta dispersion (Fig. 5) suggests difference in microbial community composition between 2018 and 2019. However, this was significant only for the intermediate well ($F = 2.29$, $df = 1$ and $P = 0.024$). No statistically significant change in the microbial community between 2018 and 2019 was observed for the proximal ($F = 1.34$, $df = 1$ and $P = 0.104$), distal ($F = 1.09$, $df = 1$ and $P = 0.355$) or background ($F = 1.22$, $df = 1$ and $P = 0.148$) wells.

Redundancy analysis

The first two axes of the RDA (Fig. 6) account for 12.1% of the total constrained variance (31.4%). Note that the inferred variance (explained proportion) is the adjusted R^2 . A total of five canonical axes were statistically significant (RDA1-RDA3, $P = 0.001$; RDA4, $P = 0.016$ and RDA5, $P = 0.024$; after 999 permutations). Groundwater chemical parameters that significantly influenced the microbial composition were pH, sodium, calcium, magnesium, manganese, alkalinity, nitrate and total organic carbon (TOC). These are the variables which have also demonstrated stronger gradients in the studied ecosystem (Figure S1, Supporting Information). Notwithstanding the output from the statistical model selection, all the variables in the global model were retained in

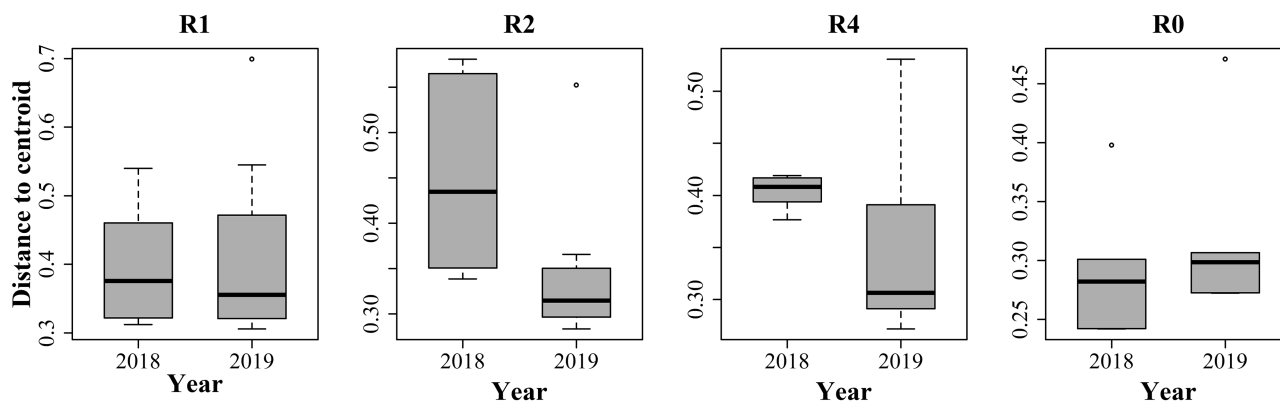


Figure 5. Beta dispersion between 2018 and 2019 for R1, R2, R4 and R0. R0 is the background well located in a nearby uncontaminated aquifer, while R1, R2 and R4 are the wells located in the contaminated aquifer at the proximal, intermediate and distal positions from the landfill.

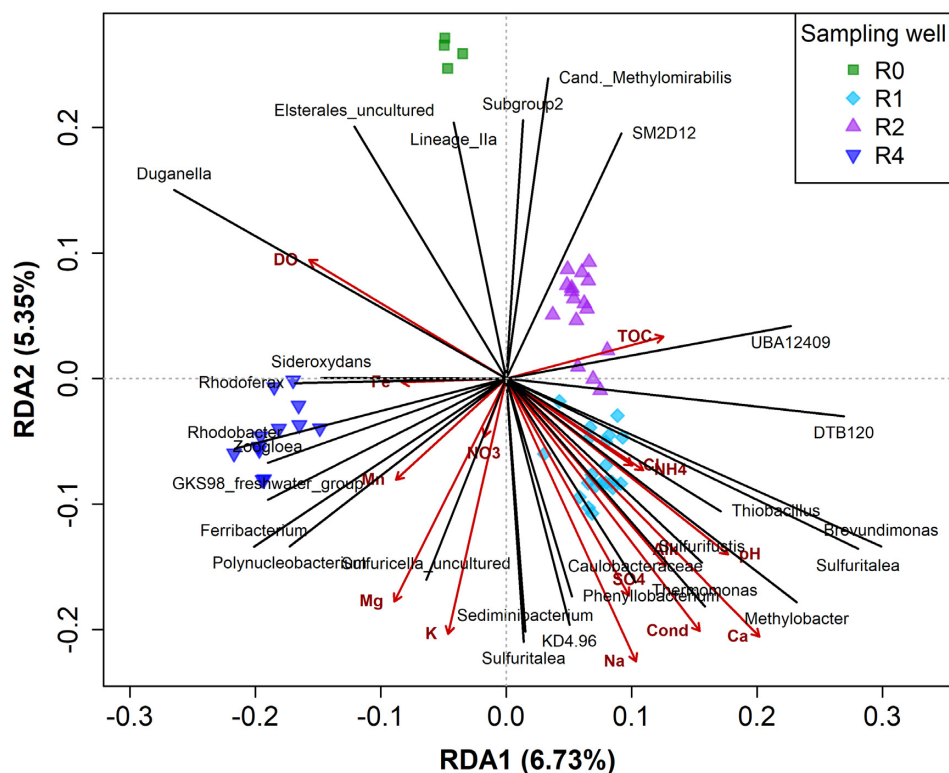


Figure 6. Redundancy analysis (RDA) performed on fourth-root transformed and Hellinger-standardised OTU abundance data. To distinguish water chemistry variables from OTUs, OTU scores are plotted without arrows. Only the prominent species vectors are shown. R0 is the background well located in a nearby uncontaminated aquifer, while R1, R2 and R4 are the wells located in the contaminated aquifer at the proximal, intermediate and distal positions from the landfill.

the final model to explore potential biogeochemical processes in the contaminated aquifer, moreover such automatic variable selection strategies would sometimes choose ecologically irrelevant models (Ramette 2007). In the RDA triplot (Fig. 6), dissolved oxygen showed a strong but opposite gradient to pH, ammonium and chloride along RDA1. Conductivity, sodium, potassium, calcium, magnesium and sulphate, on the other hand, showed stronger gradients along RDA2. The geochemical and microbial data have structured the sampling sites first along RDA1, separating the background and the distal wells which correlated negatively with the axis, from the proximal and intermediate wells which correlated positively with the axis. The second axis (RDA2) separated mainly the uncontaminated groundwater

from the contaminated groundwater. The proximal and intermediate wells clustered close to each other.

Output from variation partitioning (Fig. 7) shows that all the four explanatory variables (water chemistry, year, season and well) collectively accounted for 33.2% of the total variance, while 66.8% variance remained unexplained. Much of the explained variance was shared by water chemistry and well (18.5%), while only 0.2% of the variation in microbial composition was jointly explained by all the four variables. The result also showed that the unshared variation in microbial composition (excluding the effects of covariables) explained individually by the variables was higher for water chemistry (7%) and well (5.9%), but lower for season (0.8%) and year (<0%). The overall contribution with-

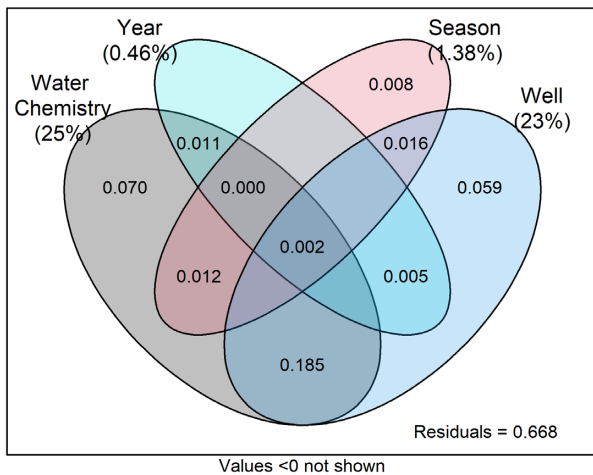


Figure 7. Variation partitioning of proportions of variation in microbial community composition explained by water chemistry, year, season and distance (well). Values in parenthesis indicate variations explained by the variables without removing the effects of covariables.

Table 2. PERMANOVA results of seasonal microbial community variation in the sampling wells R0, R1, R2 and R4. R0 is the background well located in a nearby uncontaminated aquifer, while R1, R2 and R4 are the wells located in the contaminated aquifer at the proximal, intermediate and distal positions from the landfill.

Well	Number of sample	Number of OTU	F. Model	R ²	P-value
R1	20	927	1.71	0.087	0.007
R2 ^a	16	1342	3.54	0.202	0.001
R4	12	1027	1.36	0.12	0.086
R0	12	616 ^b	1.43	0.125	0.064

^a OTU data was square-root transformed, as fourth-root transformation was too strong in this case.

^b OTUs based on 12 samples, but only 4 samples were used in all the other analysis.

out removing the effects of covariables was 25% for water chemistry, 0.46% for year, 1.3% for season and 23% for well. Testing for these proportions based on 999 permutations yielded significant results for water chemistry ($F = 1.83$ and $P = 0.001$), well ($F = 2.00$ and $P = 0.001$), and season ($F = 1.37$ and $P = 0.035$), but not for year ($F = 0.97$ and $P = 0.539$). Thus, the variables can be ordered based on their importance in explaining the variation in the microbial composition as water chemistry > distance > season > year.

DISCUSSION

Spatial variation

Microbial community composition showed a significant spatial variation. The background well, which is an unperturbed environment, was most dissimilar to the other wells. This is a clear manifestation of the impact of the landfill leachate on the aquifer ecosystem. However, as the background and contaminated aquifers are not connected, their original communities may have been different. In the contaminated aquifer, there was clear evidence of variation of the microbial community along the line of flow, with the two wells nearest the landfill and each other being more similar. Similar findings have been reported

for a landfill leachate plume in Banisveld landfill using denaturing gradient gel electrophoresis community profiling (Brad et al. 2013). Such spatial differentiation of microbial communities has been suggested to contribute to better degradation of contaminants (Brad et al. 2013) and, in addition, provides useful information, as it gives insight into differential adaptation of microbes to leachate (Mouser et al. 2005). The ecological gradient in Revdalen is short, where samples primarily differ in species abundances (Ramette 2007). The water chemistry demonstrated two maximum directions (Fig. 6): towards the proximal well (for eight variables) and towards the distal well (for six variables). On a similar pattern of structuring, the abundant OTUs in the proximal and distal wells were mostly culturable, while those in the intermediate and background wells were predominantly uncultured taxa.

Depth-resolved variation in microbial composition indicated a significant difference across the levels in the proximal well, which could be attributed to the disproportionately high abundance of certain OTUs, e.g. *Aquabacterium*, *Janthinobacterium*, *Oxalobacteraceae* and *Pedobacter* in the uppermost level, and *Sulfurifustis* and *Sulfuritalea* in the deeper levels (Figure S6, Supporting Information). In addition, beta diversity analysis (Fig. 4) indicates that samples from the uppermost level have larger dispersion. Both the intermediate and distal wells showed non-significant differences across the discrete depth profiles and in pairwise comparisons, which suggest that the microbial floras of the intermediate and distal wells were compositionally similar across the depths. This agrees with the observation that only three most abundant OTUs in the intermediate well (*Brevundimonas*, *Pseudomonas* and *Saccharimonadales*) and one in the distal well (*Oxalobacteraceae*) showed disproportionately higher abundance in one level (Figures S7 and S8, Supporting Information). Similar lack of vertical variation within sampling wells has been reported in a crude-oil contaminant plume (Fahrenfeld et al. 2014). However, a study on microbial communities from wells at depths 10–17 m but having different hydraulic conductivities recorded significant vertical variation (Lin et al. 2012). In Revdalen, the aquifer matrix comprises sand and gravel (~5 m thick), which can be considered to have relatively less varied hydraulic conductivities compared to matrices made up of completely different geological layering materials that can influence microbial and nutrient distribution (Smith et al. 2018a). In addition, Revdalen has a different hydrological regime than the unconfined aquifer studied by Lin et al. (2012). Depth-resolved microbial community variation in various types of geological strata remains poorly understood (Smith et al. 2018a), which calls for more elucidation.

Variation partitioning showed that both water chemistry and distance (wells) were intercorrelated and they jointly accounted for most of the variation in the microbial composition. This implies that the microbial composition and water chemistry characteristics have a similar spatial structuring, presumably caused by the landfill leachate plume. A gradient of an increase in concentrations of dissolved oxygen, magnesium, potassium, manganese and sulphate, and a decrease in pH, conductivity, calcium and alkalinity were observed along the proximal-intermediate-distal path (Figures S1 and S2, Supporting Information). This highlights the importance of distance on attenuation of pollutants which has been described previously (Abiriga, Vestgarden and Klempe 2020; 2021b). This likely influenced the microbial composition and structure. Thus, homogenisation force from the leachate plume should be strongest at the proximal and intermediate wells, which makes the microbial composition and structure more similar in these wells,

although the close proximity would also imply microbial propagation from proximal to intermediate well. At the distal well, such homogenisation force is diminished, and other processes become increasingly important in driving the microbial composition and structure.

Seasonal and Interannual variations

By the virtue of their formation, aquifers are viewed as being environmentally stable (Zhou, Kellermann and Griebler 2012; Pilloni et al. 2019). In Snowbelt countries such as Norway, there can be considerable aquifer recharge after snowmelt, accounting for 60–80% of the yearly recharge in some regions (Kløve et al. 2017). This is likely to cause shifts in the geochemical processes within an aquifer and is particularly important in unconfined aquifers, where seasonal groundwater table fluctuations can lead to redistribution of contaminants (Fretwell et al. 2005). This causes shifts in plume movement, which can exert control over microbial community assemblages (Pilloni et al. 2019). In Revdalen, a significant difference in water chemistry between spring and autumn was observed, but only in the proximal and intermediate wells. The variables with significant changes were sulphate, TOC, sodium, potassium and calcium in the proximal well, and conductivity, ammonium and TOC in the intermediate well (Table S2, Supporting Information).

Considering aquifers as microbial habitats, their intrinsic features such as changes in geochemical and hydraulic regimes are now acknowledged as key influencers of activity and population of microorganisms (Griebler and Lueders 2009; Brad et al. 2013; Lueders 2017). Results suggest that microbial composition in Revdalen is influenced by season, but it seems that although statistically significant, season has only a marginal effect, as it accounted for only 1.3% of the variation. Previous studies have reported seasonal variation in composition and diversity of microbes in pristine aquifers (Farnleitner et al. 2005; Lin et al. 2012; Zhou, Kellermann and Griebler 2012) and in industry-impacted urban aquifers (Smith et al. 2018b). Beta diversity analysis indicated significant seasonal variations in the proximal and intermediate wells. Given the locations of these wells relative to the landfill, these observations suggest that the microbial communities in them are more susceptible to seasonal changes. This agrees with the observation that more geochemical parameters have shown significant seasonal fluctuations in the proximal and intermediate wells than in the distal and background wells (Table S2, Supporting Information). However, seasonal variation was also evidenced in the distal and background well, where taxa *Duganella*, *Polynucleobacter* and GKS98 (uncultured *Alcaligenaceae*), showed higher abundances in spring. It is unclear why these taxa showed higher abundances only in spring. It can be speculated that the recharge water brings along nutrients that specifically favoured these microorganisms. *Duganella* for instance, might be responding to dissolved oxygen, as dissolved oxygen was found to be replenished in spring (Abiriga, Vestgarden and Klempe 2020) and the genus showed a positive correlation with dissolved oxygen (Fig. 6). In a batch experiment (Griebler et al. 2016), growth of *Duganella* was strongly stimulated when nutrient source (R2A) was amended in a reactor. Our study is based on field observation and unambiguous interpretation of such dynamics is difficult (Pilloni et al. 2019). More study is required to understand this dynamism, but the results suggest that the microbial communities were less variable in autumn than in spring, probably due to calmer groundwater flow conditions in autumn. The higher recharge

occurring in spring (Kløve et al. 2017) may be followed by mixing and this causes instability in the subsurface (Smith et al. 2018a).

Results from beta diversity analysis and variation partitioning suggest that the microbial communities were less variable over the study period. A similar observation of less community variability over a one-year period have been made from pristine aquifers (Farnleitner et al. 2005). However, a closer look into the data indicated that the microbial community in the intermediate well was more variable, as the beta diversity analysis gave a significant difference between 2018 and 2019 ($P = 0.024$). Even so, the temporal variation was related to season, especially with samples collected in spring 2018 (not shown). The meteorological conditions in 2018 were exceptional, with unusually high winter snowfall, leading to a higher aquifer recharge, followed by very dry weather in the summer. This likely lowered the water table, creating zones of unsaturation around the proximal and intermediate wells that favoured oxidation reactions. Consequently, concentrations of sulphate, dissolved oxygen and nitrate were highest in autumn (Figure S2, Supporting Information). How long such implied community stability will prevail in aquifers is a matter of future studies as apparently no data exists for field observation >2 years.

CONCLUSION

Literature on landfill-leachate-impacted aquifers is scarce. Here, we examined key influencers of the microbial community in an aquifer contaminated by a municipal landfill leachate in south-east Norway to understand the interplay between microbial community composition and environmental factors: groundwater geochemistry, distance, season and time. The explanatory variables explained 33.2% of the variation in microbial composition, thus a bigger proportion (66.8%) of the variation remained unexplained. The unexplained proportion likely represents both deterministic but unmeasured variables (that the present data was unable to capture) and stochastic processes. The explained variation was largely jointly accounted for by the groundwater chemistry and distance, which were intercorrelated. Season had only a marginal effect on the microbial communities, as it explained only 1.3% of the variation. Interannual variation was negligible, which suggests that the microbial communities were less variable over the study timeframe. The findings of the study are important in understanding how environmental factors influence microbial composition of anthropogenically impacted aquifers, which is very useful in ensuring proper management of remediation sites.

DATA AVAILABILITY

The raw sequence data supporting the study have been deposited in Sequence Read Archive under BioProjects PRJNA677875 (groundwater; biosamples SAMN16775936–SAMN16775995 and recharge SAMN16776020) and PRJNA677889 (aquifer sediment). The groundwater biosamples consisted of samples collected in 2018 and 2019.

ACKNOWLEDGEMENT

The authors thank Frode Bergan and Tom Aage Aarnes for participating in fieldwork. We also extend our sincere appreciation to Karin Brekke Li for technical assistance in chemical analysis of groundwater samples. The sequencing service was provided by the Norwegian Sequencing Centre (<https://www.sequ>

encing.uio.no/), a national technology platform hosted by the University of Oslo and supported by the Functional Genomics and Infrastructure programs of the Research Council of Norway and the Southeastern Regional Health Authorities. We thank the two anonymous reviewers and the editor for their constructive comments to the manuscript.

SUPPLEMENTARY DATA

Supplementary data are available at [FEMSEC](#) online.

Conflict of interest. None declared.

REFERENCES

- Abiriga D, Jenkins A, Alfsnes K et al. Characterisation of the bacterial microbiota of a landfill-contaminated confined aquifer undergoing intrinsic remediation. *Sci Total Environ* 2021a;785:147349.
- Abiriga D, Vestgarden LS, Klempe H Groundwater contamination from a municipal landfill: effect of age, landfill closure, and season on groundwater chemistry. *Sci Total Environ* 2020;737:140307.
- Abiriga D, Vestgarden LS, Klempe H Long-term redox conditions in a landfill-leachate-contaminated groundwater. *Sci Total Environ* 2021b;755:143725.
- Albrechtsen H-J, Heron G, Christensen TH. Limiting factors for microbial Fe (III)-reduction in a landfill leachate polluted aquifer (Vejen, Denmark). *FEMS Microbiol Ecol* 1995;16:233–47.
- Anderson MJ. A new method for non-parametric multivariate analysis of variance. *Austral Ecol* 2001;26:32–46.
- Anderson MJ. Distance-based tests for homogeneity of multivariate dispersions. *Biometrics* 2006;62:245–53.
- Basberg L, Banks D, Sæther OM. Redox processes in groundwater impacted by landfill leachate. *Aquat Geochem* 1998;4:253–72.
- Bjerg PL, Tuxen N, Reitzel LA et al. Natural attenuation processes in landfill leachate plumes at three Danish sites. *Ground Water* 2011;49:688–705.
- Borcard D, Legendre P, Drapeau P. Partialling out the spatial component of ecological variation. *Ecology* 1992;73:1045–55.
- Brad T, Oberfell C, van Breukelen B et al. Spatiotemporal variations in microbial communities in a landfill leachate plume. *Groundwater Monitor Remed* 2013;33:69–78.
- Chapman DV. *Water Quality Assessments: A Guide to the use of Biota, Sediments and Water in Environmental Monitoring*. 1996, CRC Press.
- Chen Q-L, Li H, Zhou X-Y et al. An underappreciated hotspot of antibiotic resistance: the groundwater near the municipal solid waste landfill. *Sci Total Environ* 2017;609:966–73.
- Christensen TH, Bjerg PL, Kjeldsen P. Natural attenuation: a feasible approach to remediation of ground water pollution at landfills? *Groundwater Monitor Remed* 2000;20:69–77.
- Eggen T, Moeder M, Arukwe A. Municipal landfill leachates: a significant source for new and emerging pollutants. *Sci Total Environ* 2010;408:5147–57.
- Fahrenfeld N, Cozzarelli IM, Bailey Z et al. Insights into biodegradation through depth-resolved microbial community functional and structural profiling of a crude-oil contaminant plume. *Microb Ecol* 2014;68:453–62.
- Farnleitner AH, Willhartitz I, Ryzinska G et al. Bacterial dynamics in spring water of alpine karst aquifers indicates the presence of stable autochthonous microbial endokarst communities. *Environ Microbiol* 2005;7:1248–59.
- Fretwell B, Burgess W, Barker J et al. Redistribution of contaminants by a fluctuating water table in a micro-porous, double-porosity aquifer: field observations and model simulations. *J Contam Hydrol* 2005;78:27–52.
- Griebler C, Briellmann H, Haberer CM et al. Potential impacts of geothermal energy use and storage of heat on groundwater quality, biodiversity, and ecosystem processes. *Environ Earth Sci* 2016;75:1391.
- Griebler C, Lueders T. Microbial biodiversity in groundwater ecosystems. *Freshwater Biol* 2009;54:649–77.
- Griebler C, Malard F, Lefebvre T. Current developments in groundwater ecology—from biodiversity to ecosystem function and services. *Curr Opin Biotechnol* 2014;27:159–67.
- Haarstad K, Ludvigsen G. Ten years of pesticide monitoring in Norwegian ground water. *Groundwater Monitor Remed* 2007;27:75–89.
- Haarstad K, Mæhlum T. Pesticides in Norwegian landfill leachates. *Open Environ Biol Monitor J* 2008;1:8–15.
- Herzyk A, Fillinger L, Larentis M et al. Response and recovery of a pristine groundwater ecosystem impacted by toluene contamination—a meso-scale indoor aquifer experiment. *J Contam Hydrol* 2017;207:17–30.
- Kjeldsen P, Barlaz MA, Rooker AP et al. Present and long-term composition of MSW landfill leachate: a review. *Crit Rev Environ Sci Technol* 2002;32:297–336.
- Klempe H. Identification of quaternary subsurface glacial deposits using 3D databases and GIS. *Norsk Geografisk Tidsskrift-Norwegian Journal of Geography* 2004;58:90–95.
- Klempe H. The hydrogeological and cultural background for two sacred springs, Bø, Telemark County, Norway. *Quat Int* 2015;368:31–42.
- Kløve B, Kvitsand HML, Pitkänen T. et al. Overview of groundwater sources and water-supply systems, and associated microbial pollution, in Finland, Norway and Iceland. *Hydrogeol J* 2017;25:1033–44.
- Kvæerner J, Eklo OM, Solbakken E et al. An integrated approach for assessing influence of agricultural activities on pesticides in a shallow aquifer in south-eastern Norway. *Sci Total Environ* 2014;499:520–32.
- Lapworth D, Baran N, Stuart M et al. Emerging organic contaminants in groundwater: a review of sources, fate and occurrence. *Environ Pollut* 2012;163:287–303.
- Legendre P, Gallagher ED. Ecologically meaningful transformations for ordination of species data. *Oecologia* 2001;129:271–80.
- Lin B, Braster M, Röling WF et al. Iron-reducing microorganisms in a landfill leachate-polluted aquifer: complementing culture-independent information with enrichments and isolations. *Geomicrobiol J* 2007;24:283–94.
- Lin X, McKinley J, Resch CT et al. Spatial and temporal dynamics of the microbial community in the Hanford unconfined aquifer. *ISME J* 2012;6:1665–76.
- Logeshwaran P, Megharaj M, Chadalavada S et al. Petroleum hydrocarbons (PH) in groundwater aquifers: an overview of environmental fate, toxicity, microbial degradation and risk-based remediation approaches. *Environ Technol Innovat* 2018;10:175–93.
- Ludvigsen L, Albrechtsen H-J, Ringelberg D et al. Distribution and composition of microbial populations in a landfill leachate contaminated aquifer (Grindsted, Denmark). *Microb Ecol* 1999;37:197–207.
- Lueders T. The ecology of anaerobic degraders of BTEX hydrocarbons in aquifers. *FEMS Microbiol Ecol* 2017;93:fiw220.

- Majone M, Verdini R, Aulenta F et al. In situ groundwater and sediment bioremediation: barriers and perspectives at European contaminated sites. *New Biotechnol* 2015;**32**:133–46.
- Mouser PJ, Rizzo DM, Röling WF et al. A multivariate statistical approach to spatial representation of groundwater contamination using hydrochemistry and microbial community profiles. *Environ Sci Technol* 2005;**39**:7551–9.
- Oksanen J, Blanchet FG, Friendly M et al. *vegan: Community Ecology Package*. 2019, <https://CRAN.R-project.org/package=vegan>.
- Pilloni G, Bayer A, Ruth-Anneser B et al. Dynamics of hydrology and anaerobic hydrocarbon degrader communities in a tar-oil contaminated aquifer. *Microorganisms* 2019;**7**:46.
- R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing, 2020, <https://www.R-project.org/>.
- Ramette A. Multivariate analyses in microbial ecology. *FEMS Microbiol Ecol* 2007;**62**:142–60.
- Remmas N, Melidis P, Zerva I et al. Dominance of candidate Saccharibacteria in a membrane bioreactor treating medium age landfill leachate: effects of organic load on microbial communities, hydrolytic potential and extracellular polymeric substances. *Bioresour Technol* 2017;**238**:48–56.
- Scow KM, Hicks KA. Natural attenuation and enhanced bioremediation of organic contaminants in groundwater. *Curr Opin Biotechnol* 2005;**16**:246–53.
- Smets BF, Pritchard P. Elucidating the microbial component of natural attenuation. *Curr Opin Biotechnol* 2003;**14**:283–8.
- Smith HJ, Zelaya AJ, De León KB et al. Impact of hydrologic boundaries on microbial planktonic and biofilm communities in shallow terrestrial subsurface environments. *FEMS Microbiol Ecol* 2018a;**94**:fy191.
- Smith RJ, Paterson JS, Wallis I et al. Southern South Australian groundwater microbe diversity. *FEMS Microbiol Ecol* 2018b;**94**:fy158.
- Taş N, Brandt BW, Braster M. et al. Subsurface landfill leachate contamination affects microbial metabolic potential and gene expression in the Banisveld aquifer. *FEMS Microbiol Ecol* 2018;**94**:fy156.
- Ye J, Chen X, Chen C et al. Emerging sustainable technologies for remediation of soils and groundwater in a municipal solid waste landfill site—A review. *Chemosphere* 2019;**227**:681–702.
- Zaporozec A, Miller JC *Groundwater Pollution*. 7, place de Fontenoy, 75700, Paris: United Nations Educational, Scientific and Cultural Organisation. 2000
- Zhang D, Vahala R, Wang Y et al. Microbes in biological processes for municipal landfill leachate treatment: community, function and interaction. *Int Biodeterior Biodegrad* 2016;**113**:88–96.
- Zhou Y, Kellermann C, Griebler C. Spatio-temporal patterns of microbial communities in a hydrologically dynamic pristine aquifer. *FEMS Microbiol Ecol* 2012;**81**:230–42.