Microbial diversity of the Heiðmörk groundwater system and potential sources of drinking water contamination

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Report summary

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Ágrip á íslensku:	Neysluvatnið í Reykjavík er almennt talið mjög hreint og laust við bæði náttúruleg aðskotaefni og mengun af mannavöldum og er þar af leiðandi ómeðhöndlað fyrir neyslu. Hins vegar getur örverumengun átt sér stað í grunnvatninu í miklum úrkomu- og hlákuatburðum. Markmiðið með þessu verkefni var að rannsaka örveruflóruna í borholuvatninu á Heiðmerkursvæðinu, sem sér Reykjavík fyrir köldu neysluvatni og að meta mögulegan uppruna örverumengunar í grunnvatni. Þessi skýrsla lýsir niðurstöðum mælinga á örveruflóru í neysluvatninu yfir árs tímabil. Við venjulegar aðstæður var örverufjöldinn í borholuvatninu mjög lár en á sama tíma var fjölbreytileiki örvera mjög mikill. Samanburðarrannsóknir á borholuvatni og jarðvegssýnum í leysingum og í manngerðri eftirlíkingu af úrkomuburði sýndu fram á að fjölbreytileiki örvera í neysluvatninu eykst í flóðaatburðum sem og hlutfallslegt magn jarðvegsbaktería. Þessar niðurstöður sýna að jarðvegsbakteríur geta borist í grunnvatnskerfið með yfirborðsvatni og þar með haft áhrif á gæði neysluvatnsins. Þessi rannsókn hefur lagt grunn að framtíðar rannsóknum á fjölbreytileika örvera í neysluvatninu á höfuðborgarsvæðinu og mögulegum uppruna örverumengunar.		
Lykilorð á íslensku:	*Örveruflóra, grunnvatn, Heiðmörk, neysluvatn, mengun*		

Summary in English:	Drinking water in Reykjavík is generally considered free of anthropogenic or natural contaminants and therefore does not undergo any regular water treatment measures. However, microbial contamination can occur during periods of high melt and rain water runoff. The objective of this report was to analyse the microbial community in well water from the Heiðmörk groundwater area, supplying the capital area with drinking water, and assessing potential sources of groundwater contamination. This report describes the drinking water microbial community structure over the period of one year. During normal well operation the cell count in the drinking water was very low. At the same time an extremely high diversity of microbial taxa was detected in the water samples. Comparative analysis of well water and soil samples during periods of meltwater runoff and during the simulation of a high precipitation event showed changes in the well water microbiome along with a higher relative abundance of soil-associated bacteria. This demonstrates that surface bacteria can enter the groundwater system potentially posing a risk to drinking water safety under such extreme weather conditions. This study lays the baseline for future exploratory studies on the dynamics of the drinking water microbiome in the capital area and potential sources of contamination.
English keywords:	*Microbiome, groundwater, Heiðmörk, drinking water, contamination*

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1. Background

Drinking water in Iceland is generally considered free of anthropogenic or natural contamination and is not treated unless there are dangers of surface water intrusion (Gunnarsdottir et al., 2016). Most of the drinking water sources in Iceland are from groundwater flow located in porous bedrock which has originated from meteoric water percolated through soil and rock (Gunnarsdóttir and Gissurarson, 2008; Kløve et al., 2017). The city of Reykjavík draws is drinking water from boreholes located in the Heiðmörk nature reserve, which receives groundwater flow from the Húsafellsbruni and Bláfjöll mountains. The different areas in Heiðmörk in which wells are maintained are *Jaðar*, *Gvendarbrunnar* and *Myllulækur* which receive water from the Elliðaárstraumur groundwater flow, and *Vatnsendakriki* which receives water from the Straumsvíkurstraumur groundwater flow (Cypaité, 2015).

Drinking water quality is monitored according to the Icelandic Drinking Water Regulation (IDWR) and through implementation of Hazard Analysis and Critical Control Points (HACCP) (Gunnarsdottir et al., 2015a; Gunnarsdóttir and Gissurarson, 2008). In addition, high water quality is maintained by closing shallow wells during periods of intensive rain and snow melt (Gunnarsdóttir and Gissurarson, 2008). As part of the IDWR, microbiological indicators are regularly monitored to ensure safe drinking water. This includes enumeration of the Heterotrophic Plate Counts (HPC) at 22 °C (according to ISO 6222:1999 mod.) and of coliforms and *Escherichia coli* (according to ISO 9308-1:1990 and ISO 9308-1:2000) as potential indicators for drinking water contamination.

In January 2018, during a period of unusually high precipitation and melt water runoff in Heiðmörk, *E. coli* was detected in well water and counts for HPC exceeded the maximum recommended value outlined in the IDWR leading to the announcement of a public warning in the capital area and the closing of several drinking water wells.

This raised the questions as to how and from which sources surface water contamination can enter the groundwater system in Heiðmörk under extreme weather events as experienced in January 2018. Despite regular sample analysis of well water which displays the abundance of cultivable microorganisms, these usually represent less than 0.1 % of the entire microbial diversity found in a given environment. The entire microbial community, often referred to as the microbiome, of drinking water is globally understudied (Hull et al., 2019). However, using novel methods such as High-Throughput Sequencing (Caporaso et al., 2010) it is now possible to analyse whole microbial assemblages and track their dynamics across time and between different environments. This offers an opportunity to characterise the Heiðmörk groundwater microbiome and assess the risks of drinking water contamination.

2. Objective

The microbial community composition of the Heiðmörk groundwater system providing drinking water to the capital area in Iceland, as well as its dynamics during surface water intrusion have previously been unstudied. In 2018, Matís ohf. was commissioned by Veitur ohf., the utilities operator of the drinking water distribution in Reykjavík, to analyse the well water microbiome in the Heiðmörk groundwater system and study potential sources of well water contamination. This included analysing the soil and vegetation microbiology from various locations in the area to assess if certain locations are more at risk of introducing surface microorganisms into the well water. Further, the project was aimed at creating the first set of baseline data describing the microbial community in the Heiðmörk groundwater system over the period of one year, which acts as a reference point for further studies on the drinking water microbiology from this area and potential sources of drinking water contamination.

The main objectives of the project were:

1. **To analyse the microbial community composition in well water at the sites Gvendarbrunnar, Myllulækur, Jaðar and Vatnsendakriki**
    a. during normal weather conditions.
    b. during a natural melt water runoff event.
    c. during the simulation of a high precipitation event.

2. **Compare the well water microbial communities to those found in soil and vegetation in the same areas.**

3. **Assess potential sources of groundwater contamination.**

3. Results and Discussion

3.1 Microbial diversity of well water from the Heiðmörk water catchment area

Summary: Although the total number of cells was low in the tested well water samples during normal operation, the diversity of different bacterial species was strikingly high. Many of these bacteria belong to taxonomic groups often found in freshwater and other environmental habitats and are likely benign to humans and do not pose a health risk. Because the community composition of bacteria was not similar between wells, we suggest that many bacteria detected in well water live in association with the subsurface rock layer (e.g. on biofilms) surrounding each well and are not freely distributed throughout the groundwater layer in the Heiðmörk water catchment area.

Sampling location and abiotic parameters

Between November 2018 and October 2019, 72 well water samples and 33 soil and vegetation samples from the Heiðmörk water catchment area were collected and analysed (see map in Figure 1 for location of sampling sites). Well water samples were collected from 12 wells and one distribution system located in the areas Jaðar, Myllulækur, Gvendarbrunnar and Vatnsendakriki (Table 1). Depths of the boreholes ranged from 12.3 m to 136 m with varying estimated intact casing depths.

Table 1: List of sampled wells, year of construction, registered depth and estimated intact casing depth.

Well name	Site	Year	Registered depth [m]	Est. intact casing depth [m]
V-01	Jaðar	1974	32	Dotted liner
V-03	Jaðar	1973	63.7	7.7
V-04	Jaðar	1973	15.5	7.2
V-05	Jaðar	1974	15.1	7.2
V-10	Jaðar	1978	15.3	Dotted liner
V-11	Jaðar	1978	13.1	12
V-12	Myllulækur	1975	103.6	23.4
V-13	Myllulækur	1975	50.5	20.1
V-14	Myllulækur	1976	54	38.5
V-22	Gvendarbrunnar	1985	12.3	4.8
VK-01	Vatnsendakriki	1990	96.8	71
VK-05	Vatnsendakriki	1993	136	60.7
T-2	Distribution system Vatnsendakriki	-	NA	NA

All well water samples were collected from taps attached to the main outlet pipe of the pumps located in the respective pump houses. In order to prevent microbial contamination of samples, either from the environment or from the taps, each tap was disinfected on the outside with 70% ethanol, flamed briefly with a Bunsen burner and opened for 5 minutes to remove stagnant water and reduce the detection of resident microbes in the pipe (Figure 2). Pumps that had not been in operation prior to sampling were started at least 30 minutes before sampling of the well occurred.

The temperature of well water remained at an average of 3.8 ± 0.3 °C throughout the sampling period. The average pH value for well water samples collected in December 2018 was 8.9 ± 0.2.

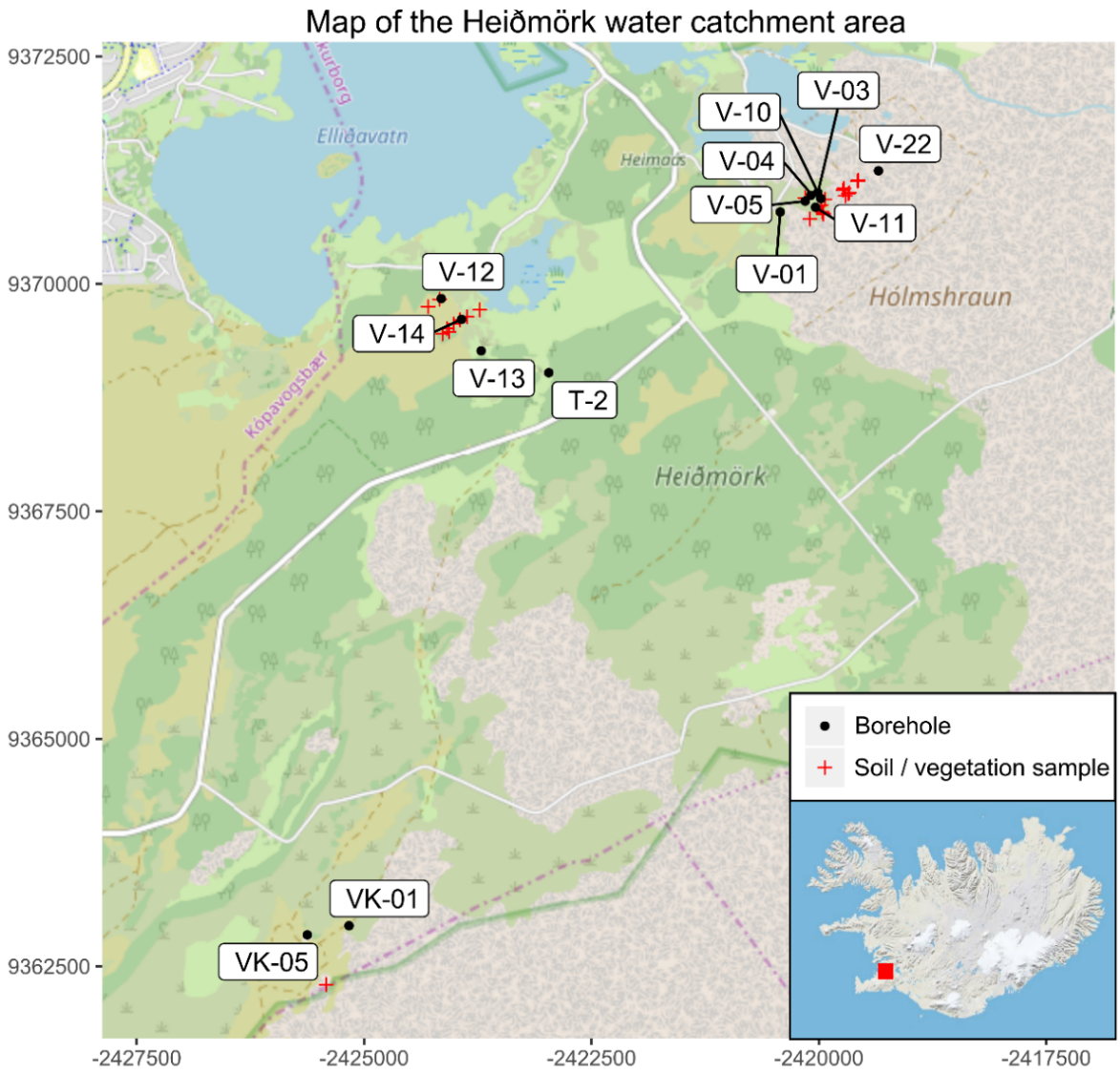

Figure 1: Map of the Heiðmörk water catchment area providing drinking water for the capital area in South-West Iceland. Selected boreholes used for well water sampling are marked with labels. Red crosses indicate sites of soil and vegetation sample collection. Coordinates are given as EPSG:3857 projection coordinates. Source: OpenStreetMap.

Other general biochemical factors of aquifers in Iceland can be found in the publications by Gunnarsdottir et al. (2016, 2015b). Values specifically for drinking water in Reykjavik are published in the environmental report from Orkuveita Reykjavíkur (2016), the parent company of Veitur ohf. Values for well water from the Heiðmörk groundwater system were generally below maximum recommended values.

Figure 2: Images from well water sampling. Taps located after the well pumps were disinfected with 70% ethanol, flamed and rinsed by opening the tap for 5 minutes before collecting water samples for microbiome analysis (top left). Water samples for microbiome analysis were collected in sterile plastic containers directly from a pump outlet (top right). Water samples for cell counts were collected in sterile 200 ml plastic containers (bottom left). Water samples for microbiome analysis were filtered on 0.2 µm membrane filters and stored at -80°C before DNA extraction (bottom right).

Total cell counts and viable cell counts

The number of cells in drinking water varies and is highly dependent on the use of water treatment methods (Hammes et al., 2008), the source of the drinking water (Gillespie et al., 2014), temperature (Liu et al., 2013) and other environmental factors (Van Nevel et al., 2017). Studies enumerating Total Cell Counts (TCC) in drinking water, measured using flowcytometric methods, report between 1,000 – 500,000 cells per ml during normal operation (Van Nevel et al., 2017).

In January 2019, Veitur connected an automated flow cytometry instrument, BactoSense, to well V-05 in the Jaðar area. This well was chosen as it is known to be sensitive to surface water contamination. The instrument was in operation during this whole study and can thus be used for comparison. It measures total cell counts (TCC). The average TCC during normal operation at well V-05 (excluding values collected during technical malfunctions, instrument calibration or the simulated high precipitation experiment where the instrument was moved to well V-14) between January 2019 and January 2020 was 7,575 ± 4,345 cells per ml (Figure 3), thus being on the lower side of previously reported values of drinking water.

Whereas TCC is a recommended method for measuring total cells in water samples it includes counts of dead cells, the proportion of which in TCC has not yet been estimated conclusively (Van Nevel et al., 2017). Therefore drinking water safety standards require the measurement of the number of heterotrophic plate counts (HPC) in drinking water, which gives an indication of the living microorganisms in a sample and their subsequent health risk (Allen et al., 2004; Gensberger et al., 2015). However, it must be noted that this measure does not reflect the total number of living cells in a sample nor their diversity, as it is estimated that less than 0.1 % of all bacteria are readily cultivable in a laboratory (Hammes et al., 2008; Staley and Konopka, 1985).

Table 2: List of sample collection, sample type and type of analysis.

Sampling date	Sample number	Sample type	Analysis	Comment
November 2018	33	Soil, vegetation, bird droppings	Plate counts, 16S rRNA seq.	Samples collected surrounding well houses
December 2018	12	Well water	Plate counts, 16S rRNA seq.	Normal operation
February 2019	9	Well water	Plate counts, 16S rRNA seq.	Normal operation with melt water runoff
May 2019	11	Well water	Plate counts, 16S rRNA seq.	Normal operation
July 2019	11	Well water	Plate counts, 16S rRNA seq.	Normal operation
September 2019	11	Well water	Plate counts, 16S rRNA seq.	Normal operation
Sep.-Oct. 2019	18	Well water and surface water	Plate counts, 16S rRNA seq.	Simulation of high precipitation (“Watering experiment”)

Average heterotrophic plate counts (HPC) grown on nutrient agar at 22 °C were 15 ± 42 cells per ml (Figure 3). This included a high count of 320 viable cells per ml in well V-12 in September 2019 during normal well operation. A count of 2500 cells per ml in well V-12 in December 2018 was removed from the dataset as an outlier since a sample collected from the same well one month later in January 2019 indicated an HPC of 4 cells per ml (data not shown). However, these high HPC counts, which lie above official recommendations for drinking water (*Icelandic Drinking Water Regulation IDWR 536/2001*, 2001), could indicate the presence of high HPC during rare events that are not connected to high precipitation or other extreme weather conditions and warrant further investigation. It should be mentioned that TCC and HPC in drinking water have so far only been weakly correlated or not at all (Siebel et al., 2008; Van Nevel et al., 2017).

No bacterial cells characterised as Coliforms or *E. coli* were detected in the well water during the sampling period.

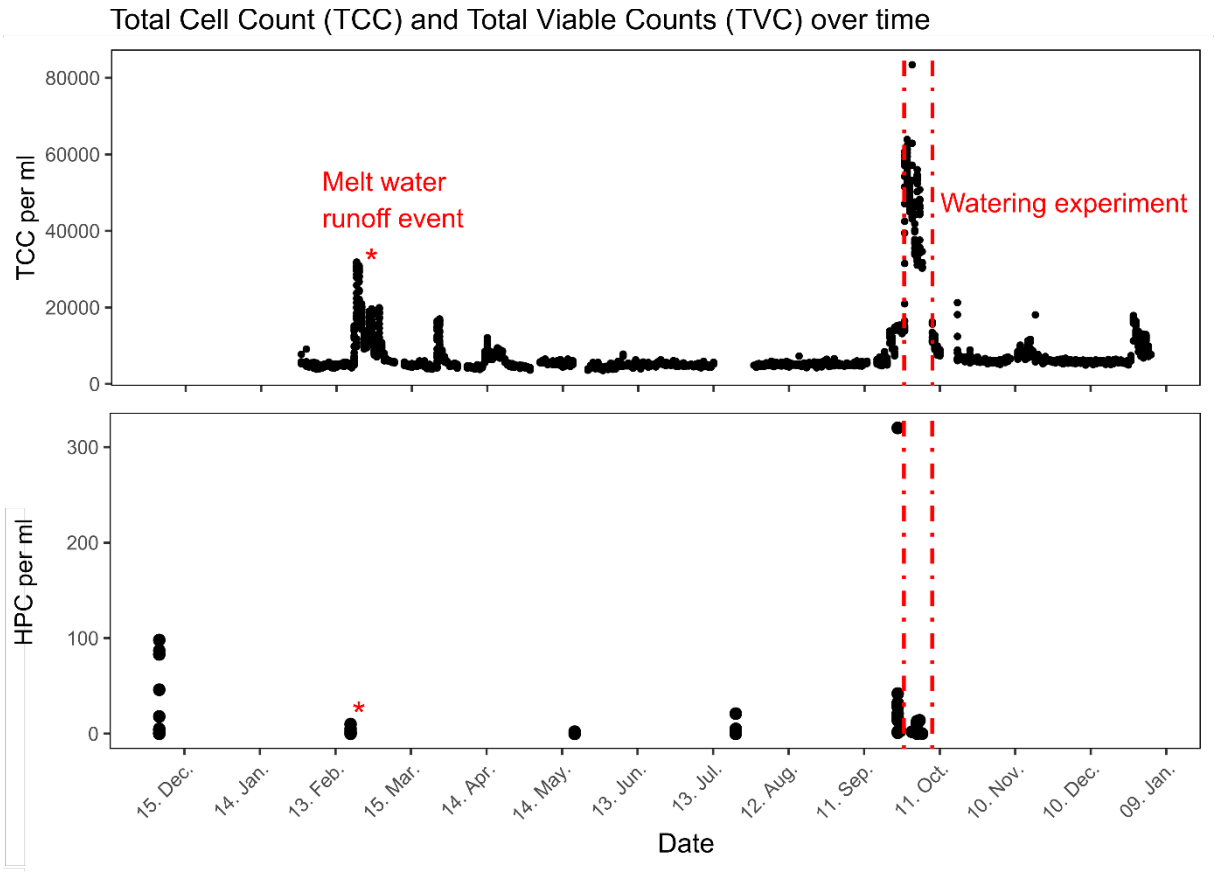

Figure 3: TCC based on flowcytometric cell counts in water from well V-05 and well V14 during the "Watering experiment" (top) and HPC counts based on plate counts (bottom) from December 2018 to January 2020. A melt water runoff event occurring in February 2019 is marked with a red asterisk. The simulated precipitation event ("Watering experiment") conducted at well V-14 from September 23 to October 4 is marked with red dashes. One HPC (2500 TCV per ml) measured in December 2018 was marked as an outlier and removed from the dataset. Peaks due to technical malfunctions were removed from the TCC dataset.

Microbial diversity

The prokaryotic (i.e. taxa belonging to the kingdoms *Bacteria* and *Archaea*) diversity of well water collected during normal operation varied between samples, ranging from 240 to 3069 Amplicon Sequence Variants (ASVs, computational equivalent to a microbial strain) or 226 to 2637 Operational Taxonomic Units at 97% sequence similarity (OTUs, computational equivalent to microbial species) (Figure 4). The total number of distinct ASVs and OTUs across all samples was 27,101 and 15,437, respectively. This represents a higher diversity compared to previous studies on well or drinking water microbiomes using similar sequencing technology, which report up to 9,000 distinct taxa in the entire drinking water microbiome (Van Nevel et al., 2017). Similarly, the average number of unique taxa per sample is higher compared to previous studies (Bautista-de los Santos et al., 2016). However, it must be mentioned that most previous studies focus on chlorinated or chloraminated drinking water and thus potentially record a reduced number of detectable diversities.

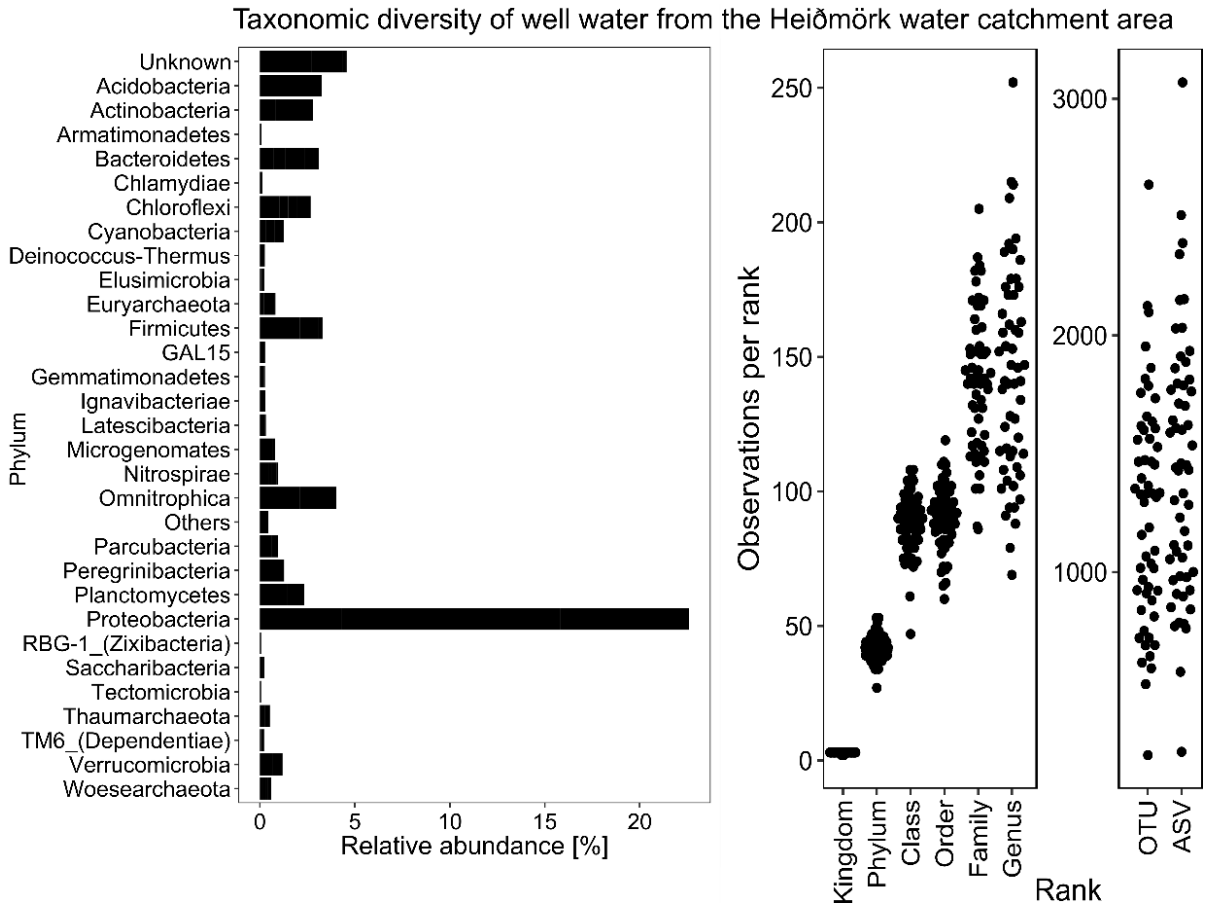

Figure 4: Taxonomic diversity as relative abundance (left) and number of observations per taxonomic tank. Data from all wells was normalised and summarised. Sequence variants with less than two representations are omitted from the dataset.

Microbial community structure

The prokaryotic taxa were further assigned to an average of 143 genera, 141 families, 90 orders, 87 classes and 41 phyla (Figure 4). Most taxa were assigned to the bacterial phyla *Proteobacteria*, *Omnitrophica*, *Firmicutes*, *Acidobacteria*, *Actinobacteria*, *Bacteroidetes* and *Chloroflexi*. The archaeal phyla *Euryarchaeota*, *Thaumarchaeota*, *Woesearchaeota* represented 3.2 % of the relative prokaryotic abundance.

Previous studies on drinking water microbiomes report a similar microbial community structure at the phylum level, with *Proteobacteria* being the dominant taxa and a high relative abundance of *Bacteroidetes*, *Acidobacteria*, *Actinobacteria*, *Planctomycetes* and *Chloroflexi* (Bautista-de los Santos et al., 2016; Proctor and Hammes, 2015; Smith et al., 2012).

A large proportion of the relative abundance was not assigned to a known phylum (see bar labelled "Unknown" in Figure 4) indicating the presence of yet uncharacterised bacterial clades in the groundwater system of the Heiðmörk water catchment area. In addition, the 20 most abundant bacterial OTUs included five OTUs with less than 86.0% sequence identity to previously cultivated and characterised bacteria (Table 3). Three of these OTUs (OTU4, OTU7 and OTU19) were previously detected in freshwater sources in Iceland using molecular methods, showing highest similarity to GenBank sequences LR595445.1, LR595684.1 and LR595226.1, respectively. The drinking water microbiome has previously been described as an understudied environment compared to the microbiome of other aquatic or terrestrial habitats (Hull et al., 2019). The detection of abundant and previously uncharacterised bacterial taxa in the well water from the Heiðmörk water catchment area highlights this discrepancy and warrants further research into the yet uncharacterised microbial taxa of the drinking water microbiome from this area.

Other highly abundant OTUs included ubiquitous environmental bacteria related to *Bacillus* spp., bacteria belonging to the diverse group of *Escherichia* and *Shigella*, *Pseudomonas* spp., *Rhodococcus* spp. and *Brevundimonas* spp. In addition, several highly abundant OTUs were related to bacteria previously detected in freshwater or groundwater systems including OTUs related to *Aeromonas* spp., *Polaromonas eurypsychrophila*, *Aquabacterium commune*, *Sideroxydans lithotrophicus*, *Cavicella subterranean*, *Sulfuriflexus mobilis*, *Sediminibacterium goheungense*. Other OTUs were related to bacteria from soil or plant root habitats such as *Bradyrhizobium* spp. and *Vicinamibacter silvestris*.

Table 3: List of the 20 most abundant OTUs in well water from the Heiðmörk water catchment area during normal operation. OTUs with an undetermined phylum rank are marked with an asterisk and represent yet unknown bacterial clades.

Taxa ID	Relative abundance [%]	Detection in % of samples	Phylum	Closest cultivated relative	Sequence identity [%]	Description
OTU1	4.3	49	Firmicutes	Bacillus spp.	100	Ubiquitous environmental bacteria
OTU2	3.3	18	Proteobacteria	Aeromonas spp.	100	Ubiquitous fresh water bacteria
OTU3†	2.5	100	Proteobacteria	Escherichia / Shigella spp.	100	Diverse group of animal-associated bacteria / potential lab contaminant
OTU4*	**1.9**	100	**Undetermined**	**Clostridium tepidum**	**84.35**	**Uncultured bacterium from spring source water**
OTU5	1.9	100	Proteobacteria	Polaromonas eurypsychrophila	100	Previously isolated from glacial ice core
OTU6	1.6	95	Proteobacteria	Aquabacterium commune	99.24	Previously isolated from drinking water systems
OTU7*	**1.6**	82	**Undetermined**	**Limisphaera ngatamarikiensis**	**85.88**	**Uncultured bacterium from spring source water**
OTU8	1.1	93	Proteobacteria	Sideroxydans lithotrophicus	98.47	Previously isolated from freshwater source
OTU9	1.0	96	Proteobacteria	Pseudomonas spp.	100	Ubiquitous environmental bacteria
OTU10	1.0	33	Actinobacteria	Rhodococcus spp.	100	Ubiquitous environmental bacteria
OTU11*	**0.9**	96	**Undetermined**	**Moorella humiferrea**	**84.73**	**Uncultured bacterium from subsurface microbial community**
OTU12	0.8	53	Proteobacteria	Cavicella subterranea	98.47	Previously isolated from deep mineral-water aquifer
OTU13	0.8	81	Proteobacteria	Sulfuriflexus mobilis	92.75	Uncultured bacterium from drinking water distribution system
OTU14	0.7	96	Proteobacteria	Brevundimonas spp.	100	Ubiquitous environmental bacteria
OTU15*	**0.6**	96	**Undetermined**	**Desulfonatronum parangueonense**	**83.21**	**Uncultured bacterium from groundwater habitat**
OTU16	0.6	89	Bacteroidetes	Sediminibacterium goheungense	100	Previously isolated from a freshwater reservoir
OTU17	0.6	89	Proteobacteria	Bradyrhizobium spp.	100	Plant root associated bacteria
OTU18	0.6	96	Acidobacteria	Vicinamibacter silvestris	95.42	Uncultured bacterium from soil habitats
OTU19*	**0.5**	44	**Undetermined**	**Syntrophorhabdus aromaticivorans**	**85.88**	**Uncultured bacterium from spring source water**
OTU20*	**0.5**	93	**Undetermined**	**Moorella humiferrea**	**85.88**	**Uncultured bacterium from groundwater habitat**

Despite their relatively high abundance, some OTUs were not present in all samples. OTU1 and OTU2, for example, were found in only 49 and 18 % of the well water samples during normal operation, respectively. In addition, OTU2 was only detected in wells sampled in February 2019 at a high relative abundance, indicating that an increase of this bacterium could be due to the melt water runoff during the time of sampling or other seasonal variations, such as nutrient flux in the groundwater systems. On the other hand, OTU3, OTU4 and OTU5 were detected in all well water samples, suggesting that these bacteria belong to the stable microbial community of the groundwater ecosystem.

Differences in the presence and absence of OTUs could also be explained through the presence of localised microbial communities that occur only in specific sites or wells of the catchment area. This was demonstrated by the relatively low core microbial community (i.e. the number of taxa detected in all well water samples during all seasons) and consisted of only two ASVs or seven OTUs in total (data not shown).

This high variability was also observed between samples from the same site with less than 12 % of the microbial community (based on the relative abundance of shared OTUs) being shared between all wells at the site Jaðar (Figure 5). The average shared relative abundance between samples of the same site across seasons, as well as between all samples from the same site on each sampling date was relatively low with less than 50 %, apart for samples collected in Jaðar in February 2019 (Figure 5). This shows that the well water microbiome is not uniform between wells or seasons and is therefore likely to represent not only the microbiome of the groundwater, but also the microbiomes inhabiting the porous bedrock surrounding each well.

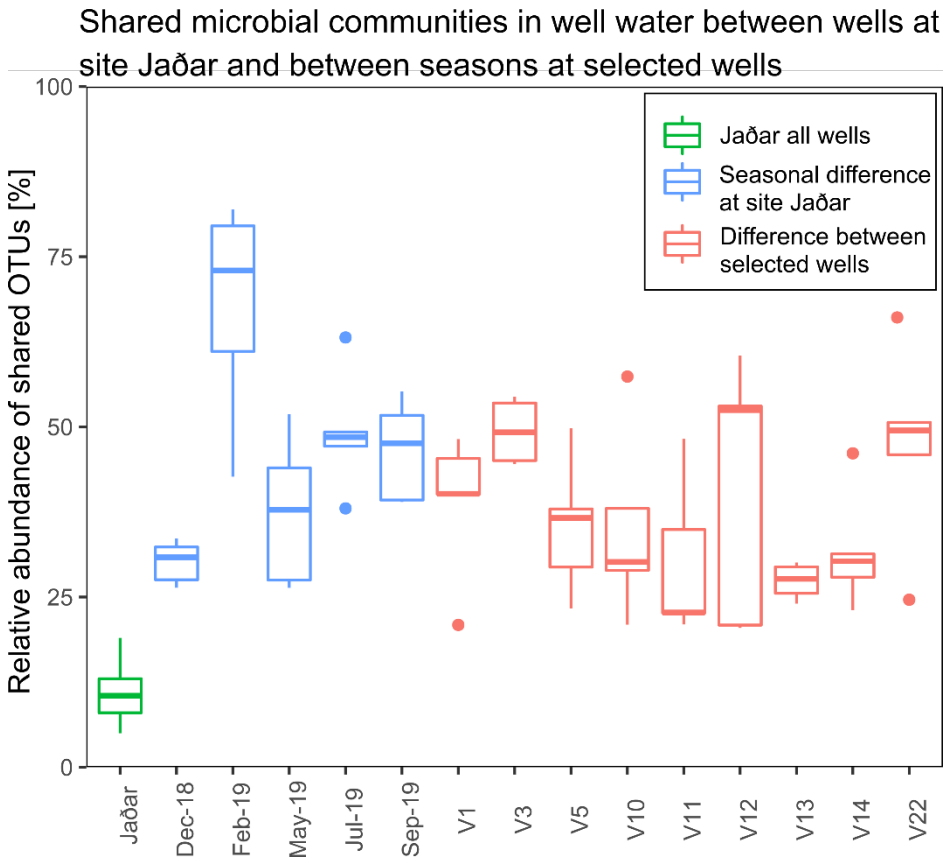

Figure 5: Box plot of the relative abundance of shared OTUs between all well water samples from Jaðar (green), between all well water samples at Jaðar at each sampling date (blue) and between well water samples across sampling dates from selected wells (red).

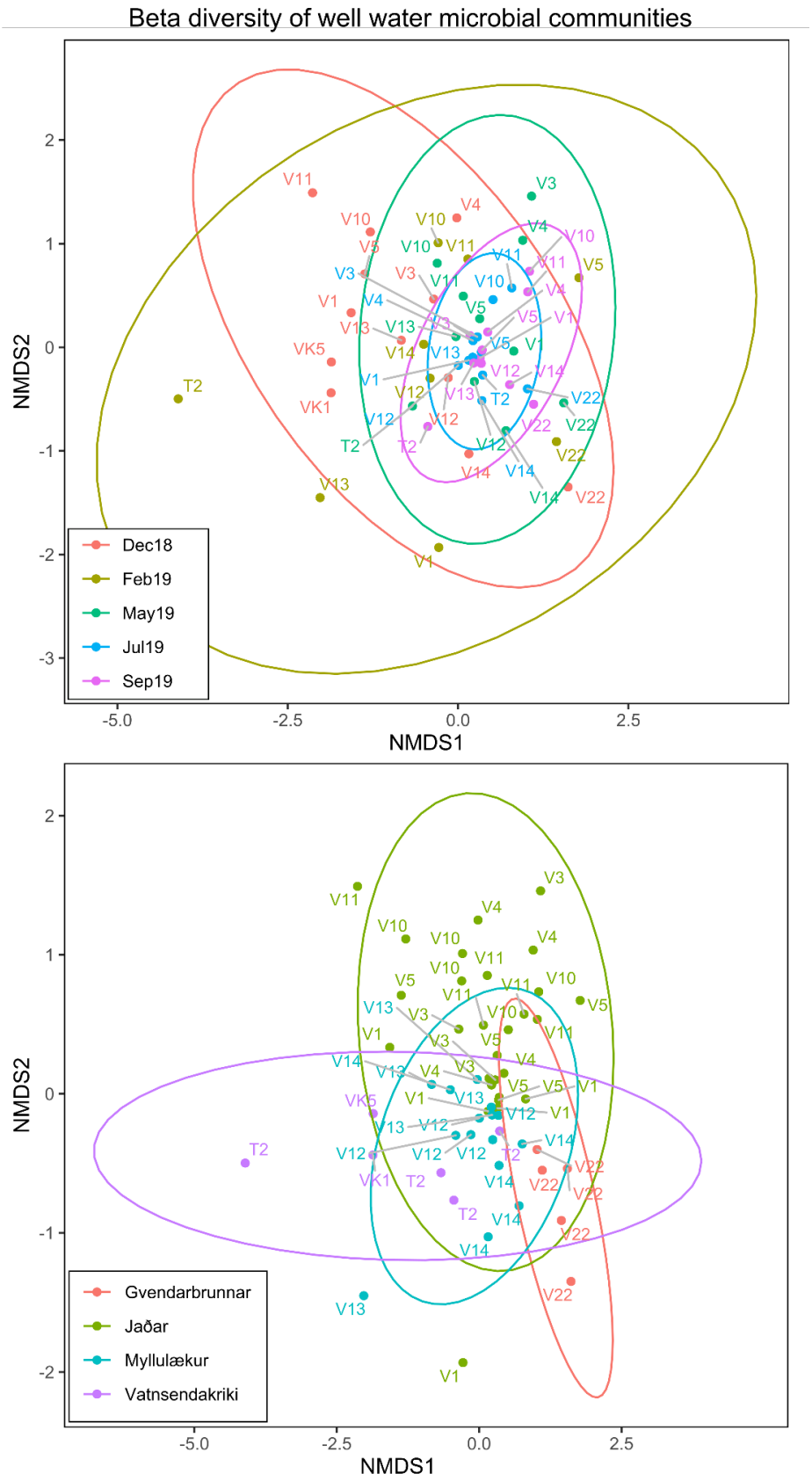

Figure 6: NMDS ordination of well water microbial beta diversity based on Jaccard indices and presence/absence transformation, marked according to season (top) or sampling sites (bottom). Stress value: 0.22.

Comparing the microbial communities using ordination of distance matrices showed a large variation between samples from the same sampling occasion without a clear differentiation between dates (Figure 6), suggesting that the seasonal influence on the microbial community structure is lower than the inter-sample difference between wells. Comparing the microbial community structure between the four sampling sites, however, showed a stronger differentiation (Figure 6). This was supported by PERMANOVA statistical testing which rejected the null hypothesis that the centroids of the groups are equivalent ($p < 0.01$). However, a higher number of samples from the sites Gvendarbrunnar and Vatnsendakriki would be necessary to validate this finding.

3.2 Comparison of soil and well water microbial communities

Summary: Bacteria detected in the soil and vegetation samples were also found in the well water samples collected during normal well operation. However, they only represented a small number of bacterial species in the overall well water bacterial community. This shows that there is either a transfer or overlapping habitat of bacteria living in surface soil layers and the well water. It was not possible to correlate if certain locations or soil types had an increased bacterial presence in the well water samples. Detection of E. coli *in one soil sample close to a well housing indicates the presence of potential pathogens in the environment that could enter the groundwater system. Further analysis is needed to detect the origin of this* E. coli *strain.*

Sample collection and cell counts

In order to describe the surface microbial community of the Heiðmörk water catchment area and compare them to well water, 33 samples were collected in November 2018. All samples were collected in close proximity to the well houses sampled in the subsequent months (see map in Figure 1). These consisted of soil samples from various depths and soil types, vegetation (e.g. moss, birch, lupin, ling) and bird droppings (Figure 7). All samples were homogenised prior to processing for sequencing in order to capture the majority of microbial taxa present in the samples.

HPC from soil and vegetation samples ranged from 12,000 cells per g in a moss and soil sample from Vatnsendakriki to 9,400,000 cells per g from a reworked area with gravel less than 5 m from the well house V-14. Interestingly the latter sample was also the only sample in which the presence of *E. coli* was detected (4 cells per g of soil). Samples from bird droppings contained between 7,500,000 and 10,000,000 viable cells per g. However, none of these samples contained *E. coli* or coliform counts.

Microbial community composition

The microbial communities of soil and vegetation samples at the class level were largely similar. This was likely due to an overlap between sample types (i.e. soil samples containing vegetation and vice versa) and shared microbial communities previously reported for plants and soil. The major classes associated with these habitat types were *Alphaproteobacteria*, *Betaproteobacteria* and *Planctomycetacia* (Figure 8). Within these classes, dominant bacteria were assigned to the *Escherichia*/*Shigella* group, *Bradyrhizobiaceae*, the DA101_soil_group and the group KD4-96 (phylum Chloroflexi), all of which contain potentially soil associated bacteria (Gołębiewski et al., 2014; Ishii et al., 2006; Lanzén et al., 2015; Marcondes de Souza et al., 2014; Nautiyal et al., 2010).

Figure 7: Images of soil sample collection in the Heiðmörk water catchment area in November 2018. (Top left) Soil sample collection in reworked area above well V-22. (Top right) Section of moss and soil. (Bottom left) Bird droppings in collected at Vatnsendakriki. (Bottom right) Hole dug for soil sample collection. Photographs courtesy of Veitur ohf.

The samples of bird droppings (n = 3, grouped and labelled as “Animal” in Figure 8) was dominated by *Gammaproteobacteria*, *Alphaproteobacteria* and *Sphingobacteriia*. Within these classes highly abundant bacteria were associated with the groups *Pseudomonas*, *Sphingomonas* and *Faecalitalea*, *Pedobacter*, which contain species that are either ubiquitous in the environment (Chatterjee et al.,

2017) or associated with animals and soil (De Maesschalck et al., 2014; Glaeser and Kämpfer, 2014; Steyn et al., 1998).

Compared to soil and vegetation samples, the well water microbial communities (grouped and labelled as “Water” in Figure 8), had a lower abundance of *Acidimicrobiia*, *Acidobacteria*, the KD4-96 group, *Spartobacteria* and *Thermoleophilia*, all of which are largely associated with soil environments. The 20 most abundant OTUs detected in the well water samples were underrepresented in the soil and vegetation communities, representing less than 1.5 % of the total relative abundance after excluding the soil-associated OTUs OTU3 and OTU17 from the dataset (see Table 3).

Comparing the microbial communities from each environmental habitat type based on distance matrices showed a clear differentiation between well water samples and the other habitats (Figure 9), indicating different community structures. This does not, however, exclude the presence of taxa that can be found across different habitats. When comparing the number of shared OTUs between habitats, well water shared 2005 OTUs, or 7.9 % of the total detected OTUs, with the other habitats (Figure 10). This shows that certain taxa are either suitable to survive in all habitats, or that there is a mode of transfer between the different environments. Such overlap in taxa between soil and groundwater layers have previously been reported (Meier et al., 2017).

Based on a soil sample site above well V-14 where samples were collected at successive depths of 0, 10 and 50 cm it was shown that soil at 50 cm depth was more dissimilar to other surface soil samples and, indeed, showed less overlap of shared abundance to the well water microbial community (data not shown). This could indicate that transfer between soil microbes and well water occurs mainly from surface soil and water layers, possibly through fissures in the ground. Though more data on the soil microbial community at different depth would be needed to confirm this hypothesis.

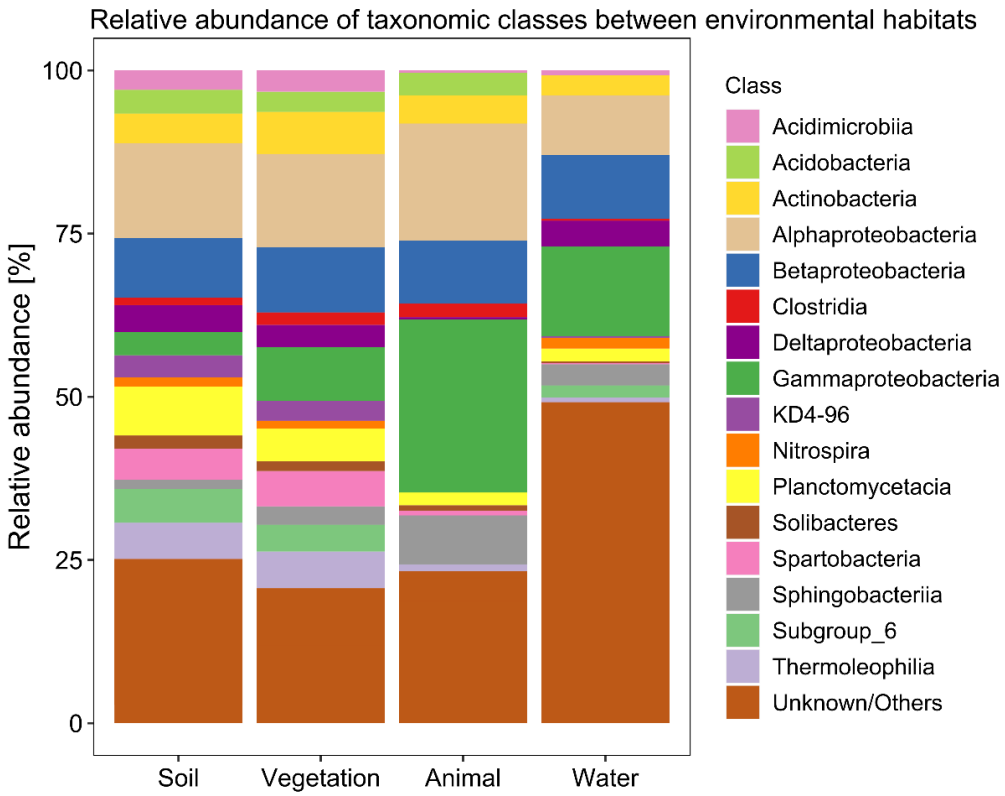

Figure 8: Relative abundance of taxonomic classes between different environmental habitats. Low abundant taxa and taxa with an undetermined class are summarised under the group "Unknown/Others".

Comparing well depth and shared soil microbes in well water, showed that well VK-01 with the deepest intact casing had a higher number of shared abundance to the soil sample taken in its close vicinity compared to V-22, which had the shallowest intact casing depth. However, the number of shared soil OTUs was lower in the deeper cased well VK-01 compared to V-22. The well water samples VK-01-1, for instance, shared 36 OTUs with its corresponding soil samples VKS1, which accounted for 6.1 % of its OTUs and 13.2 % of its relative abundance. The well water sample V-22-1, on the other hand, shared 81 OTUs with the soil sample GVS1 which accounted for only 3.8 % of the OTUs found in the well water and 7.4 % of the relative abundance. Similar values were found between V-22-1 and other soil samples collected in close proximity to the well (GVS2 – GVS9, data not shown). Only one sample was available close to VK-01 for comparison.

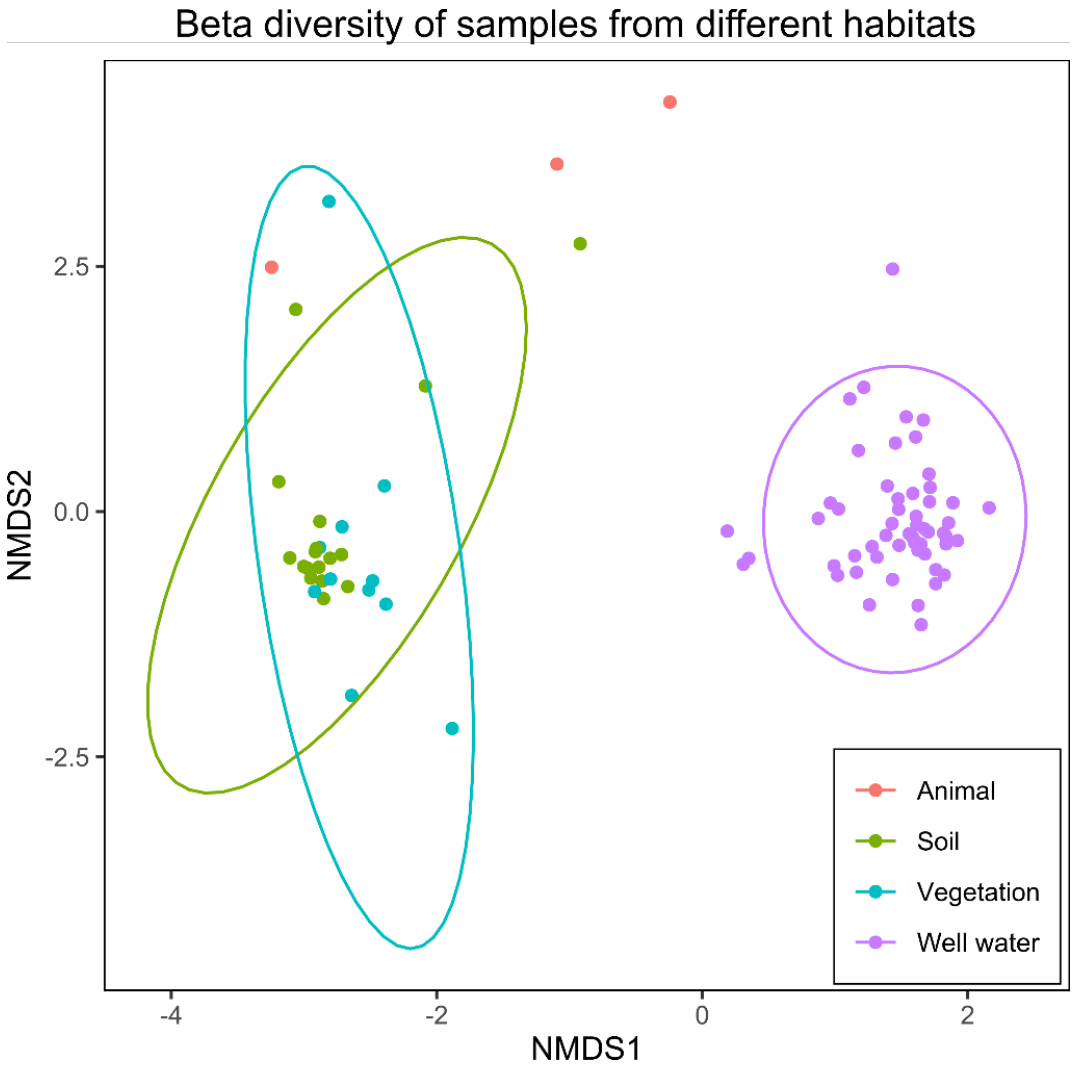

Figure 9: NMDS ordination of microbial beta diversity based on Jaccard indices and presence/absence transformation. Soil and vegetation samples cluster further away from well water samples indicating distinct differences in their microbial communities. "Animal" refers to collected bird droppings. Stress value: 0.22.

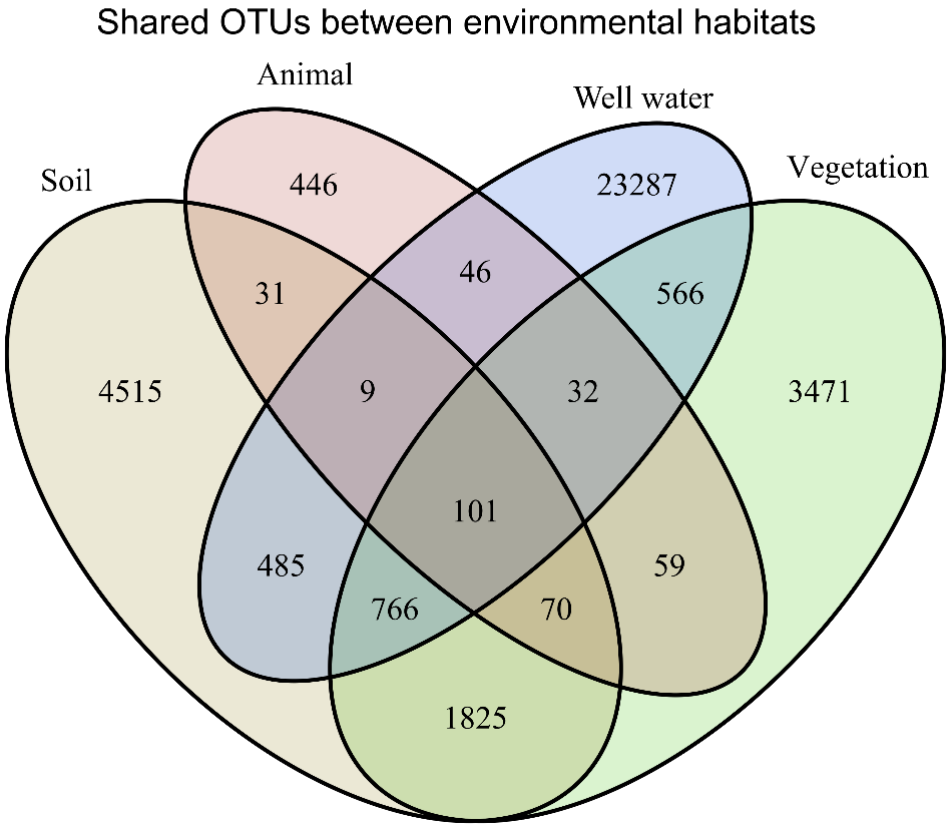

Figure 10: Number of shared OTUs between environmental habitats. "Animal" refers to collected bird droppings.

Only 46 OTUs were found in the animal related samples and also in the well water samples. This accounted for less than 0.3 % of the relative abundance of the well water. The microbiomes from each habitat contained more OTUs that were not shared with another habitat. This was especially apparent for the well water samples which did not share 92.1 % of its OTUs compared to 58.1, 50.4 and 56.2 % for the Soil, Vegetation and Animal samples, respectively. This indicates that the transfer of microbes is directed from surface layers to subsurface layers, rather than vice versa.

3.3 Microbial community of well water during a natural melt water runoff event

Summary: A natural melt water runoff and precipitation event in February 2019 appeared to have an effect on the bacterial community in the well water. This included a reduction of the overall bacterial diversity and an increase in the abundance of certain bacterial species including an Aeromonas *and* Bacillus *species. Differences were more pronounced in some wells compared with others. Whereas the magnitude of the melt water runoff was likely smaller than recorded in January 2018, this gives an indication as to the susceptibility of well water during such weather*

Water catchment areas in the Nordic countries retrieve significant water replenishment during snow melting period and during events of high precipitation (Kløve et al., 2017). One objective of this study was to measure differences in the groundwater microbial community during events of significant water discharge to the groundwater systems. In February 2019, a melt water runoff event was recorded coinciding with previous snow cover, temperatures above 0°C, strong winds and rainfall

The number of observed taxa were similar between well water samples collected at different times of the year, however, the diversity, measured through the Shannon diversity index, was significantly lower ($p < 0.05$, ANOVA) for samples collected in February 2019 during the meltwater runoff event (Figure 11). The Shannon diversity index accounts for both the abundance and evenness of observed taxa in a sample. Since the abundance did not change significantly between sampling dates the lower diversity score for the samples collected in February are likely caused be a reduction of the evenness. Looking at the community structure of samples collected in February this could be validated, since a few taxa accounted for larger shares of the relative abundance than in the samples collected at the other dates. This could indicate the intrusion of certain bacteria into the well water during the high melt water runoff and precipitation event which then accounted for an unusually high relative abundance in the microbial community.

The distribution of the major microbial taxa in well water was largely similar between December, May, July and September, however, in accordance with the diversity data, samples collected during the high melt water and precipitation events in February had a higher relative abundance of *Bacilli* and *Gammaproteobacteria* (Figure 12), corresponding largely to OTU1, OTU2 and OTU3 listed in Table 3, or *Bacillus* spp., *Aeromonas* spp. and *Escherichia*/*Shigella* spp., respectively. This could give a first indication as to which bacterial taxa are responsible to changes in the microbial community during surface water intrusion into the groundwater during extreme weather conditions.

Whereas OTU3 was distributed relatively equally between well water samples taken in February 2019, OTU1 was predominantly found in wells V01, V10, V12 and V22 and OTU2 in wells V01, V05, V10, V11 and V12 and V14. Interestingly, OTU1 was also detected as the dominant bacterium in a sample collected in a pump sump visually covered with layers of biofilm in the well house of V11 (data not shown), possibly indicating the association of this bacterium with biofilms. Further sampling during high snowmelt and rain events and a focus on why these boreholes are affected by the presence of certain dominant taxa whereas others are not could give further indications as to the potential source of contamination.

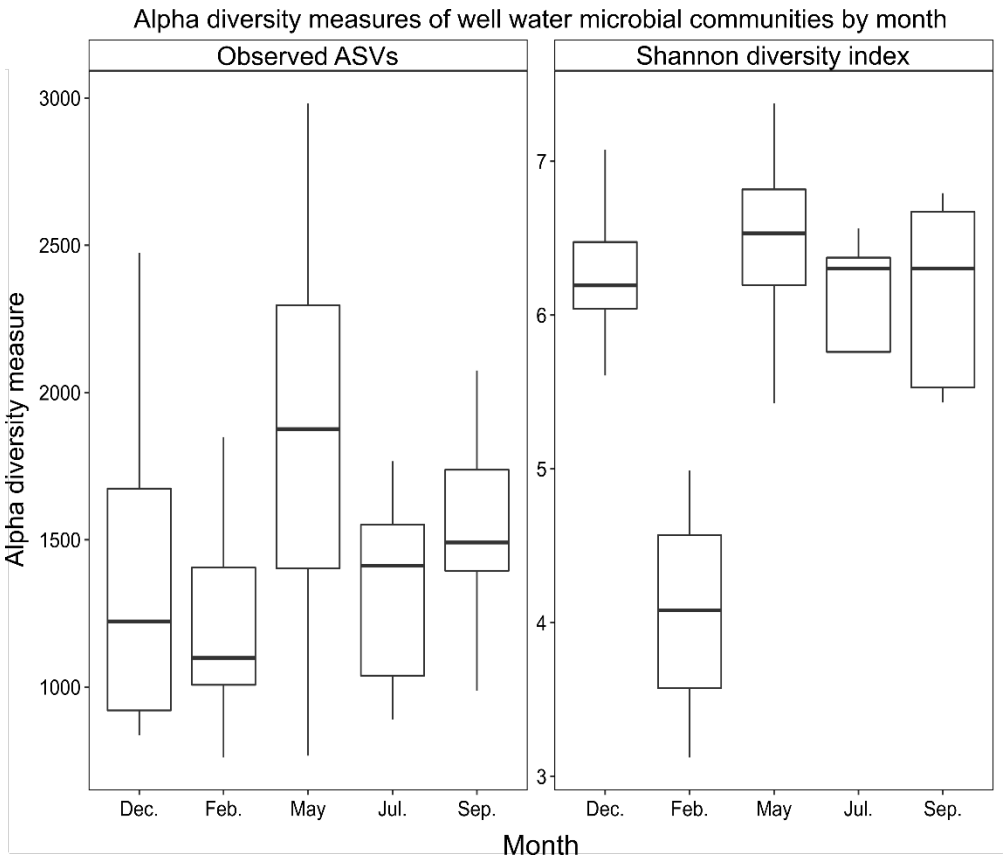

Figure 11: Box plot of the number of observed ASVs and Shannon diversity index of well water samples grouped by sampling date.

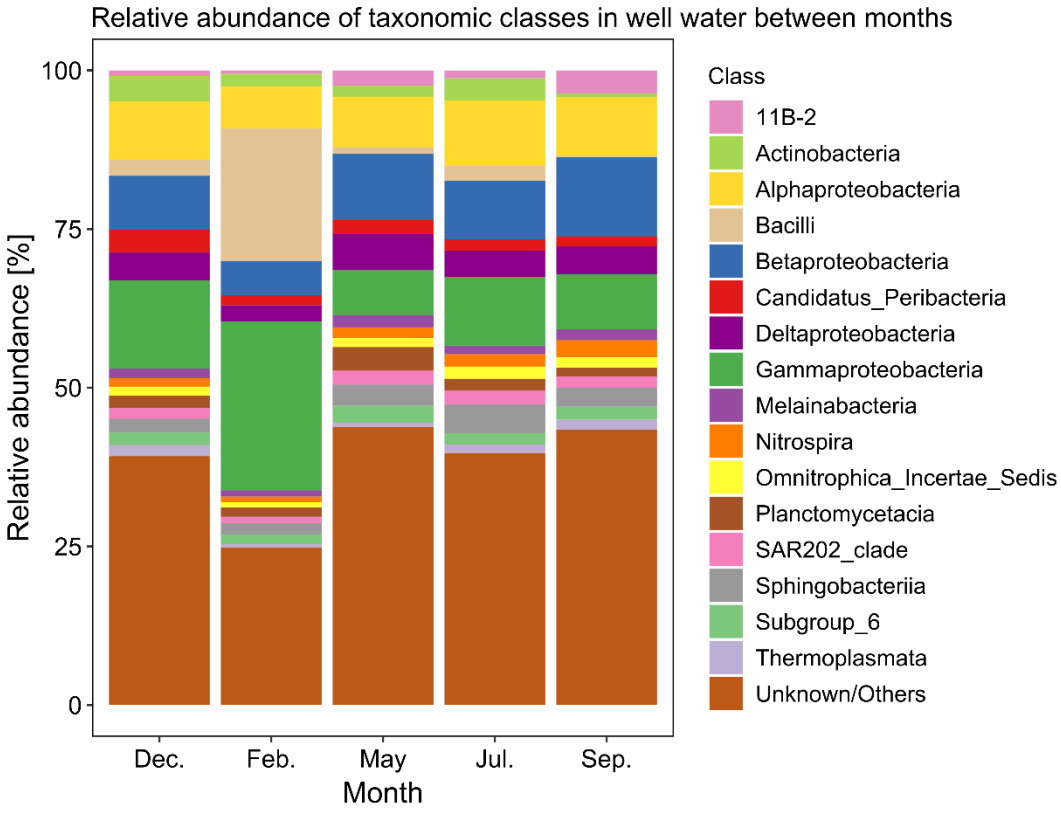

Figure 12: Relative abundance of taxonomic classes in well water collected during different months of the year.

3.4 Microbial community of well water during a simulated high precipitation event

Summary: Simulation of a high precipitation event at well V-14 showed that cell counts in the well water increased simultaneously with the presence of high amounts of surface water. Microbial analysis further showed that the surface water introduced bacteria associated with surface soil layers into the well water. Despite this microbial intrusion, soil bacteria represented only a small fraction of the increased cell counts in the well water, indicating that most surface soil bacteria are filtered before entering the well.

An experiment was set up at well V-14 to introduce a high volume of water into the close vicinity of the well house in order to simulate a high precipitation event under controlled conditions. For this, well water from well V-14 was first flushed 20 m away from the well house, then 70 m away and finally next to the V-14 well house (Figure 13). The experiment was carried out over the course of six days. One well water sample was collected prior to the experiment and subsequently samples were collected during the watering of the surface surrounding the well house.

Figure 13: Distribution of well water around the pump house of well V14 during the simulated high precipitation event („Watering experiment"). Photograph courtesy of Veitur ohf.

During the watering of the surrounding environment, TCC increased from an average of 14,423 to 48,416 cells per ml (Figure 14). The distance from the well house at which the water was introduced (distinguishable through the three peaks in Figure 14) did not seem to have a large impact on the cell counts.

NMDS ordination showed a clear differentiation between well water samples taken during normal operation and during the watering experiment (Figure 15) demonstrating that high amounts of precipitation can have an impact on the well water microbiome. Although the microbial community structure at higher taxonomical levels was similar between both groups of well water samples, the

samples collected during the watering experiment, for example, had a higher abundance of OTU26 (Phylum: *Proteobacteria*, 93 % sequence similarity to *Thiobacter subterraneus*), OTU248 (Phylum: *Acidobacteria*, 90 % to *Luteitalea pratensis*), OTU543 (Phylum: *Actinobacteria*, 93 % to *Aciditerrimonas ferrireducens*), OTU838 (Phylum: *Proteobacteria*, 93 % to *Racemicystis persica*) compared to samples collected during normal operation (excluding sample V14 from February 2019). These had a highest sequence similarity to GenBank sequences HM187255.1, MH524131.1, KY892080.1, MH526105.1, which were isolated from subsurface sediment or soil habitats. Whereas this list of differentially abundant taxa is not conclusive, this give a first insight into the associated habitat of bacteria entering the wells during events of high precipitation.

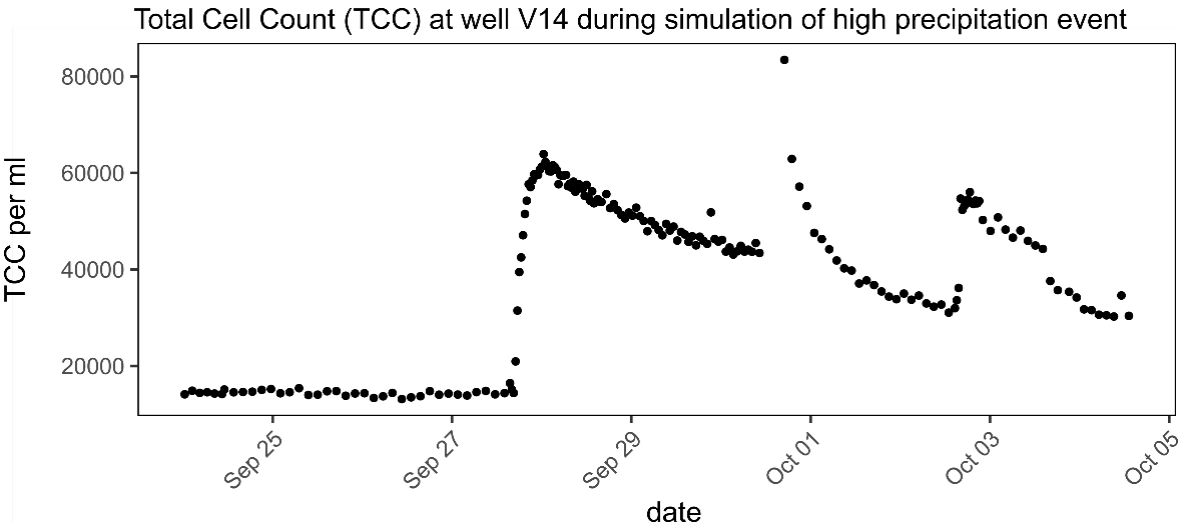

Figure 14: TCC increases after starting the watering experiment on the 27th of September 2019. The three peaks refer to the area of water distribution: First, 20 m away from the pump house. Subsequently, 70 m from the pump house. Lastly, in close proximity to the pump house (see Figure 13).

Comparing the relative abundance of shared OTUs between selected well water samples collected at well V-14 before and during the watering experiment, it could be shown that during the watering experiment well water samples shared a higher abundance with each other compared to before the watering experiment started (Figure 16). In addition, well water collected during the watering experiment shared a higher number of OTUs and relative abundance with soil samples from the environment surrounding V-14 compared to well water taken before the experiment at well V-14.

Along with the increase of TCC during the experiment, this shows that bacteria from soil and surface layers are capable of entering the groundwater system at Heiðmörk by percolating through surface layers or by directly entering the well water through gaps in the well casing. Previous studies on well water contamination in the Nordic countries have shown that a variety of factors can influence microbiological contamination of groundwater, including improper well design, wellhead completion, type and thickness of superficial deposits, land use and distance from contamination sources (Gaut,

2005; Kløve et al., 2017; Pitkänen et al., 2011). Therefore, further analysis of the well structures at Heiðmörk could provide additional information as to the route through which surface microbes can enter the wells and potentially pose risks to drinking water safety.

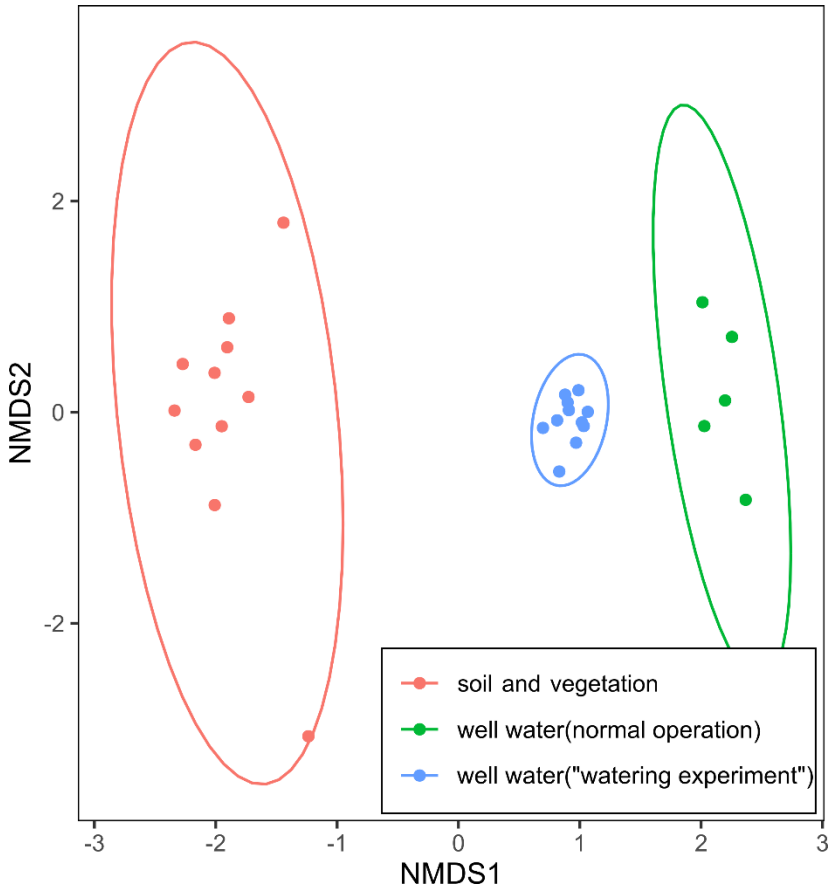

Figure 15: NMDS ordination of microbial beta diversity based on Jaccard indices and presence/absence transformation. Well water samples collected during the "watering experiment" cluster away from well water samples taken during normal operation indicating distinct differences in their microbial communities. Stress value: 0.05

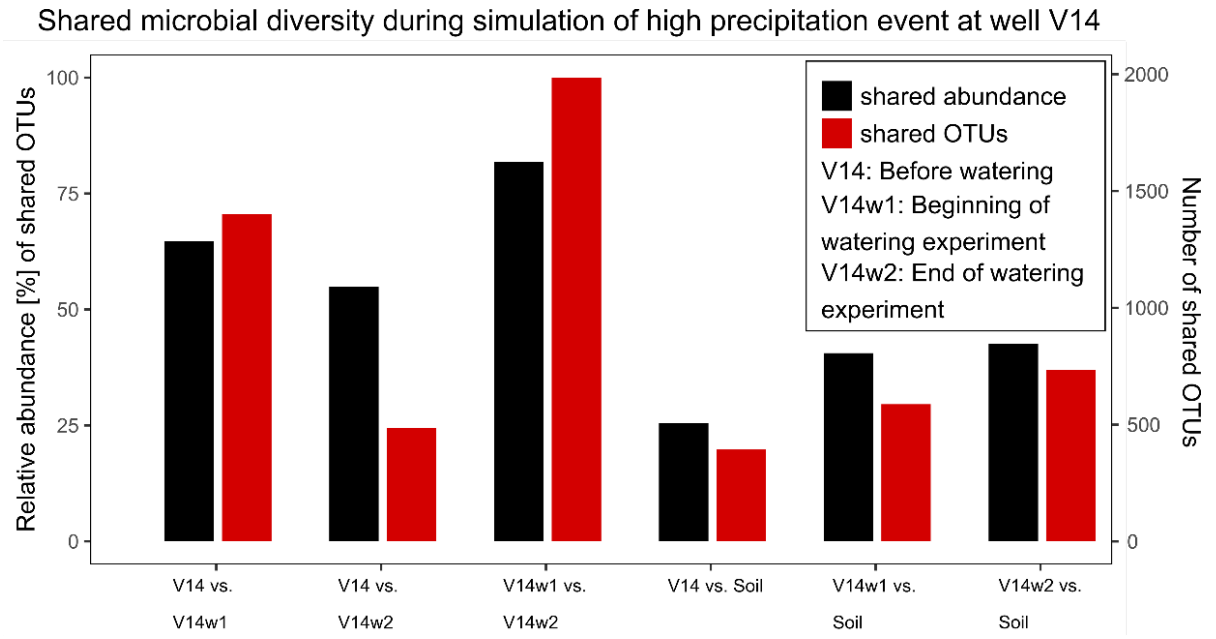

Figure 16: Well water samples collected during the „watering experiment" share a higher relative abundance and number of shared OTUs than samples taken before and immediately after beginning the watering experiment. Well water samples taken during the watering experiment share a higher relative abundance and number of shared OTUs with soil samples taken in close proximity to the pump house than well water collected before the "watering experiment".

4. Conclusion

The current study analysed the microbial diversity in well water from the Heiðmörk groundwater system in Iceland using culture-dependent and -independent methods in order to characterise the microbial communities in the drinking water across seasons and analyse potential sources of drinking water contamination. It could be shown that well water from the Heiðmörk groundwater system carries a very low microbial abundance, but with an extremely high microbial diversity, the latter surpassing previous estimates on the microbial diversity in drinking water. Whereas these bacteria are likely benign, further research into the yet uncharacterised portion of this microbiome is warranted.

Comparing the microbial community structure in the well water across seasons and at higher taxonomic levels showed a stable presence and relative abundance of taxa, indicating similar functional attributes of the groundwater microbiome. However, at lower taxonomic levels (i.e. species and strain level) few taxa were shared between wells and across seasons, showing that the detected microbial communities are specific to each well rather than uniform across the entire water catchment area. This also implies that the shared microbiome of the groundwater in Heiðmörk contributes less to the drinking water microbiome than the microorganisms associated with the subsurface environment surrounding each well.

Well water collected during a period of melt water runoff in February 2019 had undergone changes compared to samples collected during normal operation, including an increased abundance of bacteria assigned to the phyla *Bacilli* and *Gammaproteobacteria*, implicating the intrusion of non-native bacteria into groundwater during water runoff events. Differences in the abundance of these taxa in different wells, could offer further clues as to the risks of contamination for each well in the area.

During the simulation of a high precipitation event in September 2019 it could be shown that microbial counts in the well water increased more than threefold upon introducing water to the surface surrounding the well house. In addition, a shift in the microbial community structure was detected with a higher abundance of bacteria previously associated with the surrounding soil environment. This demonstrates that surface bacteria are likely able to enter the groundwater during extreme events of high precipitation or melt water runoff, presenting risks when animal or human-related microbial contaminants are present.

The results from this study describe the microbial community in the drinking water supplied to the Reykjavík area in Iceland and contribute to an understanding of the potential sources and dynamics of the drinking water microbiome. Based on these results, future studies using metagenomic tools to trace strain level taxa between habitats and locations will enable further analysis of the dynamics in the drinking water microbiome, as well as help track potential sources of contaminants.

5. Material and Methods

5.1 Sample collection

5.1.1 Environmental samples surrounding well houses in Heiðmörk

Soil, vegetation and bird dropping samples were collected in November 2018 surrounding drinking water wells in the Heiðmörk area (see Figure 1). Care was taken to collect samples from different types of locations, including a forested area, a reworked gravel area, moss layers, between a fault line and in a marsh. Soil samples were collected using a metal tube soil sampler which was cleaned with sterile water and 70 % ethanol between samples. Vegetation was removed using sterile gloves. All samples were kept in sterile plastic bags at below 4°C until being transported to the laboratory where they were stored at -80°C until DNA extraction.

5.1.2 Well water samples during normal operation

Water samples during normal well operation were collected on five occasions (Table 2). Wells that had not been in operation on the day of sampling were restarted at least 30 min before the samples were collected. In order to prevent contamination or introduce stagnant water from the pipes leading away from the pumps, all samples were collected in the same way: The outside of the pipe outlet tap was disinfected with 70 % ethanol and flamed briefly, followed by opening the outlet for 5 minutes to remove stagnant water from the pipes. Then at least 2 l of the well water was collected in sterile plastic containers for microbiome analysis and additional 200 ml of water was collected in sterile containers for microbial plate counts. All samples were kept cool until being transported to the laboratory where they were immediately filtered on 0.2 µm membrane filters (Whatman). Filters with residue were stored at -80°C until DNA extraction. Two negative extraction controls were processed to exclude the introduction of contamination during these steps. The first control consisted of a filter attached to the filtering apparatus and filtering air for 30 sec and the second control consisted of a filter which filtered 1l of sterile deionised water. These samples were processed along with the well water samples in the subsequent steps.

5.1.3 Simulated high precipitation event

An experiment was conducted in late September to simulate high precipitation around well V-14 and track the entry of surface microbes into the well water. On the 27th of September water from well V-14 was discharged 22 m away from the well house which led to an increase of cell counts in the well water from V-14. On the 30th of September the discharge pipe was lengthened to 70 m which still resulted in an increase of cell counts. On the 2nd of October a part of the discharged well water was distributed onto the road surrounding the well house. Well water samples were collected throughout

the experiment as described in section 5.1.2. All samples were kept cool, brought to the laboratory within two days and processed as described above.

5.2 Cell counts and cultivation

Total cell counts (TCC) were measured using a BactoSense automatic flow cytometer (Sigrist). The device was set up by Veitur and took measurements at well V-05 from January 2019 to 2020, apart from during the simulated high precipitation event when it was set up at well V-14. Data points correlating to instrument malfunction, calibration or other non-biological incidents were removed from the dataset.

Heterotrophic plate counts were conducted according to ISO 6222:1999mod at food safety laboratory at Matís. In short, water samples were serially diluted, plated on nutrient agar and incubated at 22°C. Counts were given as viable cells per ml water. Counts of coliforms and *Escherichia coli* were also conducted at the food safety laboratory at Matís according to ISO 9308-1:1990 and ISO 9308-1:2000.

5.3 16S rRNA gene amplicon sequencing

5.3.1 DNA extraction

Soil and vegetation samples were defrosted, weighed and approximately 150g transferred to a sterile laboratory blender (Waring) with 150 ml of sterile laboratory grade water. Each sample was blended for 60 seconds at low speed. 200 µl of the homogenised samples was then transferred to the bead tube of the PureLink Microbiome DNA purification Kit (Invitrogen) and DNA was extracted according to the manufacturer's instructions.

For the well water samples, filters with residue were defrosted and the cell residue scraped from the filtered using a sterile knife and 150 µl of sterile water. The cell suspension was then transferred to a new tube and DNA was extracted using the MasterPure Compete DNA and RNA Purification Kit (Epicentre) according to the manufacturer's instructions for total DNA purification.

DNA quality and quantity were analysed for all samples on a Nanodrop 1000 spectrophotometer (Thermofisher).

5.3.2 PCR and sequencing

PCR amplification of the partial 16S rRNA gene was conducted using the universal prokaryotic primer pair 515F (5'-GTGYCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACNVGGGTWTCTAAT-3') (Apprill et al., 2015; Parada et al., 2016). The PCR reaction was prepared with 10 ng of template DNA, 12.5 µM of each primer, and 0.25 µl of Q5 high fidelity polymerase (NEB). The thermocycler conditions were set to an initial denaturation step of 98°C for 30 sec, followed by 35 cycles of 98°C for 10 sec, 52°C for 30

sec and 72°C for 30 sec, and a final elongation step at 72°C for 2 min. PCR products were checked on a 1 % agarose gel. All negative extractions controls as well as the negative PCR control were not visible.

The PCR products were subsequently purified and sequencing libraries were constructed using the Nextera XT barcoding kit (Illumina) and following the "16S Metagenomic Sequencing Library Preparation guide" from Illumina. After normalisation and quantification, 9 pM of the final pooled library was loaded on a MiSeq Desktop sequencer (Illumina) and sequenced with V3 chemistry and 2 x 300 cycles across two sequencing runs.

The total output from sequencing was 28 million paired end reads each with 300 base pair length.

5.4 Bioinformatic analysis and statistics

Bioinformatic analysis was conducted in RStudio running R version 3.6.0 (R Core Team, 2019; RStudio Team, 2016). Sequence variants were inferred using the R Package DADA2 version 1.4 (Callahan et al., 2016) with the filterAndTrim variables set to truncLen = c(260,240), maxEE = 3, trimLeft = 15, truncQ = 2 and the learnError command performed on a subset of 108 reads. Sequence variants smaller than 200 bp and larger than 300 bp were removed and chimeric sequences were removed with the command removeBimeraDenovo. Taxonomic assignment was performed against a training set of the Silva v132 database (Quast et al., 2013). For comparison of similarities between samples, reads were clustered together into OTUs at 97% sequence similarity using Usearch (Edgar, 2010) and the -cluster_smallmem command sorted by coverage. All statistics were conducted in R using the packages phyloseq (McMurdie and Holmes, 2013) and vegan (Oksanen et al., 2013). Plots were created in R using packages ggplot2 (Wickham, 2009) and phyloseq.

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Annex

List of partial 16S rRNA sequences from the 20 most abundant OTUs in well water from the Heiðmörk water catchment area.

>OTU1
GTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGA
AAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGT
GTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGG
AGCGAAAGCGTGGGGAGCGAACAGGATTAG

>OTU2
GTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTGGATAAGTTAGATGTG
AAAGCCCCGGGCTCAACCTGGGAATTGCATTTAAAACTGTCCAGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGT
GTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAG
GTGCGAAAGCGTGGGGAGCAAACAGGATTAG

>OTU3
GTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTG
AAATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGT
GTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAG
GTGCGAAAGCGTGGGGAGCAAACAGGATTAG

>OTU4
GTAAGACAGAGGTGGCGAGCGTTGTTCGGATTTACTGGGCGTAAAGAGTTCGTAGGCGGTTTTACATGTCTGTTGTGA
AATCCCGAGGCTTAACCTCGGAACTGCATCAGAAACGGTATCACTAGAGGACAGGAGGGGGAAGTGGAATTCCAGGT
GTAGCGGTGAAATGCGTAGATATCTGGAAGAACACCGGTGGCGAAGGCGGCTTCCTGGACTGTCCCTGACGCTGAGG
AACGAAAGCGTGGGTAGCAAACAGGATTAG

>OTU5
GTAATACGTAGGGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTCTATAAGACAGATGTG
AAATCCCCGGGCTCAACCTGGGAATGGCATTTGTGACTGTAGAGCTAGAGTACGGTAGAGGGGGATGGAATTCCGCG
TGTAGCAGTGAAATGCGTAGATATGCGGAGGAACACCGATGGCGAAGGCAATCCCCTGGACCTGTACTGACGCTCATG
CACGAAAGCGTGGGGAGCAAACAGGATTAG

>OTU6
GTAATACGTAGGGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGCTTTGCAAGACAGATGTG
AAATCCCCGGGCTTAACCTGGGAACTGCATTTGTGACTGCAAGGCTAGAGTACGGTAGAGGGGGATGGAATTCCGCGT
GTAGCAGTGAAATGCGTAGATATGCGGAGGAACACCGATGGCGAAGGCAATCCCCTGGACCTGTACTGACGCTCATGC
ACGAAAGCGTGGGGAGCAAACAGGATTAG

>OTU7
GTAAGACAGAGGTGGCGAGCGTTGTTCGGATTTACTGGGCGTAAAGCGTGCGTAGGCGGTTGTGTGTGTCCGATGTG
AAATCCCGGGGCTTAACCCCGGAGCTGCATCGGAAACTGCGCGACTTGAGGATAGGAGGGGGAAGCGGAATTCCCGG
TGTAGCGGTGAAATGCGTAGATATCGGGAAGAACACCGGTGGCGAAGGCGGCTTCCTGGCCTATTTCTGACGCTGAG
GTACGAAAGCGTGGGGAGCAAACAGGATTAG

>OTU8
GTAATACGTAGGGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTTTGTAAGACAGATGTG
AAATCCCCGGGCTTAACCTGGGAACTGCATTTGTGACTGCAAGGCTAGAGTACGGCAGAGGGGGGTAGAATTCCACGT
GTAGCAGTGAAATGCGTAGATATGTGGAGGAATACCGATGGCGAAGGCAGCCCCCTGGGTCGATACTGACGCTCATG
CACGAAAGCGTGGGGAGCAAACAGGATTAG

>OTU9
GTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTTGTTAAGTTGGATGTGA
AATCCCCGGGCTCAACCTGGGAACTGCATTCAAAACTGACTGACTAGAGTATGGTAGAGGGTGGTGGAATTTCCTGTG
TAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTAATACTGACACTGAGGT
GCGAAAGCGTGGGGAGCAAACAGGATTAG

>OTU10
GTAATACGTAGGGTGCAAGCGTTGTCCGGAATTACTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCGTCTGTGA
AAACCAGCAGCTCAACTGTTGGCTTGCAGGCGATACGGGCAGACTTGAGTATTTCAGGGGAGACTGGAATTCCTGGTG

TAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGTCTCTGGGAAATAACTGACGCTGAGG
AGCGAAAGCGTGGGTAGCGAACAGGATTAG

>OTU11
GTAATACGTAGGGGGCAAGCGTTACCCGGAATTACTGGGCGTAAAGTGTGCGTAGGCGGCAGGGTAAGTCTTCTGTG
AAAGCTCCCGGCTTAACTGGGAGAGGTCAGGAGATACTACCCAGCTTGAGGGCAGTAGAGGAAGGCGGAATTCCCGG
TGTAGCGGTGAAATGCGTAGATATCGGGAGGAACACCAGTGGCGAAGGCGGCCTTCTGGGCTGTGCCTGACGCTGAG
GCACGAGAGCGTGGGGAGCAAACAGGATTAG

>OTU12
GTAATACAGAGGGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGCGGTTATGTAAGTTGGATGTG
AAATCCCCGGGCTTAACCTGGGCACTGCATTCAAAACTGCATAGCTAGAGTATGGGAGAGGAAGGTAGAATTCCAGGT
GTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGATGGCGAAGGCAGCCTTCTGGCCTAATACTGACGCTGAGG
TGCGAAAGCATGGGGAGCAAACAGGATTAG

>OTU13
GTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGGGCGCGTAGGTGGTTTGGTAAGTTGGATGTG
AAATCCCCGGGCTCAACCTGGGAATTGCATTCAATACTGCTTGACTAGAGTATGGTAGAGGGAAGCGGAATTCCACAT
GTAGCGGTGAAATGCGTAGATATGTGGAGGAACATCAATGGCGAAGGCAGCTTCCTGGACCAATACTGACACTGAGG
CGCGAAAGCGTGGGTAGCAAACAGGATTAG

>OTU14
GTAATACGAAGGGGGCTAGCGTTGCTCGGAATTACTGGGCGTAAAGGGAGCGTAGGCGGACATTTAAGTCAGAGGTG
AAATCCCGGAGCTTAACTTCGGAACTGCCTTTGATACTGGGTGTCTTGAGTGTGAGAGAGGTATGTGGAACTCCGAGT
GTAGAGGTGAAATTCGTAGATATTCGGAAGAACACCAGTGGCGAAGGCGACATACTGGCTCATTACTGACGCTGAGGC
TCGAAAGCGTGGGGAGCAAACAGGATTAG

>OTU15
GTAAGACAGAGGTGGCAAGCGTTGTTCGGATTCATTGGGTGTAAAGGGCAGGTAGGTGGTTATGTAAGTCTGGTGTG
AAATCCCACGGCTCAACCGTGGAACTGCATTGGAAACTACATGACTTGAGTACCGGAGAGGTGAGGGGAATTCTCGGT
GTAAGGGTGAAATCTGTAGATATCGAGAGGAACACCAGTGGCGAAGGCGCCTCACTGGCCGGTTACTGACACTGAGC
TGCGAAAGCAAGGGGAGCAAACAGGATTAG

>OTU16
GTAATACGGAGGGTGCAAGCGTTATCCGGATTCACTGGGTTTAAAGGGTGCGTAGGCGGGCAGGTAAGTCAGTGGTG
AAATCCTGGAGCTTAACTCCAGAACTGCCATTGATACTATCTGTCTTGAATATTGTGGAGGTAAGCGGAATATGTCATG
TAGCGGTGAAATGCTTAGATATGACATAGAACACCTATTGCGAAGGCAGCTTACTACGCATATATTGACGCTGAGGCAC
GAAAGCGTGGGGATCAAACAGGATTAG

>OTU17
GTAATACGAAGGGGGCTAGCGTTGCTCGGAATCACTGGGCGTAAAGGGTGCGTAGGCGGGTCTTTAAGTCAGGGGTG
AAATCCTGGAGCTCAACTCCAGAACTGCCTTTGATACTGAGGATCTTGAGTTCGGGAGAGGTGAGTGGAACTGCGAGT
GTAGAGGTGAAATTCGTAGATATTCGCAAGAACACCAGTGGCGAAGGCGGCTCACTGGCCCGATACTGACGCTGAGG
CACGAAAGCGTGGGGAGCAAACAGGATTAG

>OTU18
GTAATACAGAGGGGGCAAGCGTTGTTCGGAATTACTGGGCGTAAAGGGCGCGTAGGCGGCCTTCTAAGTCGAACGTG
AAATCCCTGGGCTCAACCCAGGAACTGCGTCCGAGACTGGAAGGCTCGAATCCGGGAGAGGGATGTGGAATTCCAGG
TGTAGCGGTGAAATGCGTAGATATCTGGAGGAACATCGGTGGCGAAGGCGGCATCCTGGACCGGTATTGACGCTGAG
GCGCGAAAGCCAGGGGAGCAAACGGGATTAG

>OTU19
GTAATACAGAGGTGGCGAGCGTTGTTCGGATTTACTGGGCGTAAAGCGTACGTAGGCGGTTGTGTGTGTCCGATGTGA
AATCCCGGGGCTTAACCCCGGGACTGCATCGGAAACTGCATGGCTTGAGGACAGGAGGGGGAAGCGGAATTCCCGGT
GTAGCGGTGAAATGCGTAGATATCGGGAAGAACACCGGTGGCGAAGGCGGCTTCCTGGCCTGTTTCTGACGCTGAGG
TACGAAAGCGTGGGGAGCAAACAGGATTAG

>OTU20
GTAATACGTAGGGAGCGAGCGTTACCCGGAATCACTGGGCGTAAAGGGCGCGTAGGCGGCCTGGCAAGTCTCTTGTG
AAAGCTCCCGGCTTAACTGGGAGAGGTCAAGGGATACTACCAGGCTCGAGGGCAGTAGAGGAAGGCGGAATTCCCGG
TGTAGCGGTGAAATGCGTAGATATCGGGAGGAACACCAGTGGCGAAAGCGGCCTTCTGGGCTGTGCCTGACGCTGAG
GCGCGAGAGCGTGGGTAGCAAACAGGATTAG

Complete list of samples and metadata used in this study

Sample ID / well no.	Site	Sample type	Sample description	Date	Sequencing	Plate counts	Comments
GVS1	Gvendarbrunnar	Soil /Vegetation	Reworked area, grassy, some lupin, on top of gravel	27/11/2018	yes	yes	
GVS2	Gvendarbrunnar	Soil /Vegetation	Reworked area, grassy, some lupin, on top of gravel	27/11/2018	yes	no	
GVS3	Gvendarbrunnar	Soil /Vegetation	Reworked area, grassy, some lupin, on top of gravel	27/11/2018	yes	no	
GVS4	Gvendarbrunnar	Soil /Vegetation	Moss layer from surface area in 50ml tube	27/11/2018	yes	no	
GVS5	Gvendarbrunnar	Soil /Vegetation	Birch, moss on top of soil on lava	27/11/2018	yes	yes	
GVS6	Gvendarbrunnar	Soil /Vegetation	Only soil layer of GSV5	27/11/2018	yes	no	
GVS7	Gvendarbrunnar	Soil /Vegetation	Moss and soil on lava	27/11/2018	yes	yes	
GVS8	Gvendarbrunnar	Soil /Vegetation	Vegetation between birch trees in 50 ml tube	27/11/2018	yes	no	
GVS9	Gvendarbrunnar	Soil /Vegetation	Soil sample between birch trees	27/11/2018	yes	yes	
JAS1	Jaðar	Soil /Vegetation	Grassy, by the road next to V5	27/11/2018	yes	yes	
JAS2	Jaðar	Soil /Vegetation	Birch, moss, vegetation, just above V3, similar to GVS9	27/11/2018	yes	yes	
JAS3	Jaðar	Soil /Vegetation	Frozen moss on soil similar to GVS7	27/11/2018	yes	yes	
JAS4	Jaðar	Berries	Berries either from bird feces or vomit lyin on moss	27/11/2018	yes	no	Not used for analysis
JAS5	Jaðar	Bird droppings	Bird droppings on moss stone	27/11/2018	yes	yes	
JAS6	Jaðar	Berries	Berries either from bird feces or vomit lyin on moss	27/11/2018	yes	yes	Not used for analysis
JAS7	Jaðar	Soil /Vegetation	mixed vegetation in a lava field depression above V5	27/11/2018	yes	no	
JAS8	Jaðar	Soil /Vegetation	From same hole as JAS7 but at a depth of 15cm	27/11/2018	yes	no	
JAS9	Jaðar	Soil /Vegetation	From same hole as JAS7 but at a depth of 50cm	27/11/2018	yes	no	
JAS10	Jaðar	Soil /Vegetation	In the same pine tree patch	27/11/2018	yes	no	
MYS1	Myllulækur	Soil /Vegetation	Gras, lupine, moss, worked area with gravel just above V14	27/11/2018	yes	yes	
MYS2	Myllulækur	Soil /Vegetation	Lupine, moss, grass, above V14	27/11/2018	yes	yes	

MYS3	Myllulækur	Soil /Vegetation	Moss, soil, ash alyer below V14	27/11/2018	yes	no	
MYS4	Myllulækur	Soil /Vegetation	Moss, grass, lyng, near water below V14	27/11/2018	yes	yes	
MYS5	Myllulækur	Soil /Vegetation	Lupine, grass, sandy soil above V12	27/11/2018	yes	no	
MYS6	Myllulækur	Soil /Vegetation	Grass, moss, different types of willow, taken in the fault	27/11/2018	yes	no	
MYS7	Myllulækur	Soil /Vegetation	Inside the pine forest	27/11/2018	yes	yes	
MYS8	Myllulækur	Soil /Vegetation	soil sample in MYS7 hole 10 cm from surface	27/11/2018	yes	no	
MYS9	Myllulækur	Soil /Vegetation	soil sample in MYS7 hole 50 cm from surface	27/11/2018	yes	no	
MYS10	Myllulækur	Soil /Vegetation	Inside the pine forest	27/11/2018	yes	no	
MYS11	Myllulækur	Soil /Vegetation	Inside the pine forest	27/11/2018	yes	no	
VKS1	Vatnsendakriki	Bird droppings	on stone in 50 ml tube	27/11/2018	yes	yes	
VKS2	Vatnsendakriki	Soil /Vegetation	Moss and soil on lava	27/11/2018	yes	yes	
VKS3	Vatnsendakriki	Bird droppings	on stone in 50 ml tube	27/11/2018	yes	no	
V-22	Gvendarbrunnar	Water	Bore hole water	05/12/2018	yes	yes	
V-04	Jaðar	Water	Bore hole water	05/12/2018	yes	yes	
V-03	Jaðar	Water	Bore hole water	05/12/2018	yes	yes	
V-10	Jaðar	Water	Bore hole water	05/12/2018	yes	yes	
V-11	Jaðar	Water	Bore hole water	05/12/2018	yes	yes	
V-11 pump sump	Jaðar	Water	Water from the pump sump in the pump house, lots of biofilms	05/12/2018	yes	no	Not used for analysis
V-05	Jaðar	Water	Bore hole water	05/12/2018	yes	yes	
V-01	Jaðar	Water	Bore hole water	05/12/2018	yes	yes	
V-12	Myllulækur	Water	Bore hole water	05/12/2018	yes	yes	
V-14	Myllulækur	Water	Bore hole water	05/12/2018	yes	yes	
V-13	Myllulækur	Water	Bore hole water	05/12/2018	yes	yes	
VK-01	Vatnsendakriki	Water	Bore hole water	05/12/2018	yes	yes	
VK-05	Vatnsendakriki	Water	Bore hole water	05/12/2018	yes	yes	
GvG1	Gvendarbrunnar	Water	Water from spring in lake next to V-22 house (Gjá við Gv.)	06/12/2018	yes	yes	Not used for analysis
V-12_b	Myllulækur	Water	Bore hole water	15/01/2018	yes	yes	

V-22_2	Gvendarbrunnar	Water	Bore hole water	22/02/2018	yes	yes	
V-10_2	Jaðar	Water	Bore hole water	22/02/2018	yes	yes	
V-11_2	Jaðar	Water	Bore hole water	22/02/2018	yes	yes	
V-05_2	Jaðar	Water	Bore hole water	22/02/2018	yes	yes	
V-01_2	Jaðar	Water	Bore hole water	22/02/2018	yes	yes	
T-2_2	Vatnsendakriki	Water	Water distr. system from Vatnsendakriki	22/02/2018	yes	yes	
V-12_2	Myllulækur	Water	Bore hole water	22/02/2018	yes	yes	
V-14_2 before UV	Myllulækur	Water	Bore hole water	22/02/2018	yes	yes	Not used for analysis
V-13_2	Myllulækur	Water	Bore hole water	22/02/2018	yes	yes	
V-14_2 after UV	Myllulækur	Water	Bore hole water	22/02/2018	no	yes	
Hraunbrún_2	Hraunbrún	Water	Distribution system	22/02/2018	no	yes	Not used for analysis
V-22_3	Gvendarbrunnar	Water	Bore hole water	02/05/2019	yes	yes	
V-10_3	Jaðar	Water	Bore hole water	02/05/2019	yes	yes	
V-11_3	Jaðar	Water	Bore hole water	02/05/2019	yes	yes	
V-04_3	Jaðar	Water	Bore hole water	02/05/2019	yes	yes	
V-03_3	Jaðar	Water	Bore hole water	02/05/2019	yes	yes	
V-05_3	Jaðar	Water	Bore hole water	02/05/2019	yes	yes	
V-01_3	Jaðar	Water	Bore hole water	02/05/2019	yes	yes	
T-2_3	Vatnsendakriki	Water	Water distr. system from Vatnsendakriki	02/05/2019	yes	yes	
V-12_3	Myllulækur	Water	Bore hole water	02/05/2019	yes	yes	
V-14_3	Myllulækur	Water	Bore hole water	02/05/2019	yes	yes	
V-13_3	Myllulækur	Water	Bore hole water	02/05/2019	yes	yes	
V-22_4	Gvendarbrunnar	Water	Bore hole water	22/07/2019	yes	yes	
V-10_4	Jaðar	Water	Bore hole water	22/07/2019	yes	yes	
V-11_4	Jaðar	Water	Bore hole water	22/07/2019	yes	yes	
V-04_4	Jaðar	Water	Bore hole water	22/07/2019	yes	yes	
V-03_4	Jaðar	Water	Bore hole water	22/07/2019	yes	yes	
V-05_4	Jaðar	Water	Bore hole water	22/07/2019	yes	yes	

V-01_4	Jaðar	Water	Bore hole water	22/07/2019	yes	yes	
T-2_4	Vatnsendakriki	Water	Water distr. system from Vatnsendakriki	22/07/2019	yes	yes	
V-12_4	Myllulækur	Water	Bore hole water	22/07/2019	yes	yes	
V-14_4	Myllulækur	Water	Bore hole water	22/07/2019	yes	yes	
V-13_4	Myllulækur	Water	Bore hole water	22/07/2019	yes	yes	
V-22_5	Gvendarbrunnar	Water	Bore hole water	27/09/2019	yes	yes	
V-10_5	Jaðar	Water	Bore hole water	27/09/2019	yes	yes	
V-11_5	Jaðar	Water	Bore hole water	27/09/2019	yes	yes	
V-04_5	Jaðar	Water	Bore hole water	27/09/2019	yes	yes	
V-03_5	Jaðar	Water	Bore hole water	27/09/2019	yes	yes	
V-05_5	Jaðar	Water	Bore hole water	27/09/2019	yes	yes	
V-01_5	Jaðar	Water	Bore hole water	27/09/2019	yes	yes	
T-2_5	Vatnsendakriki	Water	Water distr. system from Vatnsendakriki	27/09/2019	yes	yes	
V-12_5	Myllulækur	Water	Bore hole water	27/09/2019	yes	yes	
V-14_5	Myllulækur	Water	Bore hole water	27/09/2019	yes	yes	
V-13_5	Myllulækur	Water	Bore hole water	27/09/2019	yes	yes	
V14_6_1	Myllulækur	Water	Bore hole water while flushing 22m	28/09/2019	yes	yes	
V14_6_2	Myllulækur	Water	Bore hole water while flushing 22m	30/09/2019	yes	yes	
V14_6_3	Myllulækur	Water	Bore hole water while flushing 70m	01/10/2019	yes	yes	
V14_6_4	Myllulækur	Water	Bore hole water while flushing 70m	02/10/2019	no	yes	
V14_6_5	Myllulækur	Water	Bore hole water while flushing 70m	02/10/2019	yes	yes	
V14_6_6	Myllulækur	Water	Bore hole water while watering the road around V14	02/10/2019	yes	yes	
V14_6_7	Myllulækur	Water	Bore hole water while watering the road around V14	02/10/2019	yes	yes	
V14_6_8	Myllulækur	Water	Bore hole water while watering the road around V14	02/10/2019	no	yes	
V14_6_9	Myllulækur	Water	Bore hole water while watering the road around V14	02/10/2019	yes	yes	
V14_6_10	Myllulækur	Water	Bore hole water while watering the road around V14	02/10/2019	no	yes	

V14_6_11	Myllulækur	Water	Bore hole water while watering the road around V14	02/10/2019	no	yes	
V14_6_12	Myllulækur	Water	Bore hole water while watering the road around V14	02/10/2019	yes	yes	
V14_6_13	Myllulækur	Water	Bore hole water while watering the road around V14	02/10/2019	no	yes	
V14_6_14	Myllulækur	Water	Bore hole water while watering the road around V14	02/10/2019	no	yes	
V14_6_15	Myllulækur	Water	Bore hole water while watering the road around V14	02/10/2019	yes	yes	
V14_6_16	Myllulækur	Water	Bore hole water while watering the road around V14	03/10/2019	yes	yes	
V14_vegpollur	Myllulækur	Water	Water from surface water induced through flushing	02/10/2019	yes	yes	Not used for analysis
V14_6_17	Myllulækur	Water	Bore hole water after watering the road around V14	04/10/2019	yes	yes	