AQUIFER COMMUNITIES – CAN THEY PREDICT CHANGES IN AOTEAROA'S SOURCE (GROUND)WATER

Louise Weaver, Judith Webber, Philip Abraham, Bronwyn Humphries, Erin McGill, Pierre-Yves Dupont, Murray Close (Institute of Environmental Science and Research)

ABSTRACT

Groundwater is an extremely valuable resource for municipal, industrial, farm water and domestic supplies. In New Zealand, 40 per cent of drinking water comes from groundwater aquifers (LAWA, 2022). Unfortunately, due to land use activities, human (anthropogenic) influences can contaminate the aquifer system. While the effects of anthropogenic contaminants are well documented on surface water, little is known about their impact on groundwater resources, particularly how they impact bacterial communities. Molecular methods provide an opportunity to examine the bacterial taxa in aquifers and how they respond to contaminants.

In this study, we used Miseq sequencing to assess the bacterial taxa in the aquifer systems across four geographic regions (Southland, Canterbury, Nelson, and Hawkes Bay) of New Zealand. Physicochemical parameters were also measured, and the correlations between physiochemical parameters and bacterial taxa were evaluated.

There were significant differences in bacterial diversity between regions (Adonis, p-value = 0.001). Bacterial assemblages in samples from Nelson and Hawkes Bay were similar to and thus clustered among those from Canterbury, while samples from Southland clustered to the left of the ordination and separated into two smaller clusters along the Y axis.

The presence of Nitrososphaeria across all regions indicates its potential importance in the nitrogen cycle of groundwater ecosystems. The detection of Methanobacteria in the Canterbury and Hawkes Bay regions and its absence in the Southland and Nelson regions points to regional differences in methane-producing bacterial communities.

Significant correlations (Spearman Rank Correlation) between bacterial phylum and physicochemical parameters indicated environmental factors' influence on the bacterial community composition. There was a strong correlation between Thaumarchaeota abundance with total dissolved nitrogen (rho 0.533, p<0.0001) and nitrate (r=0.539, p<0.0001), suggesting Thaumarchaeota's potential role in nitrogen cycling processes in groundwater.

KEYWORDS

Groundwater, aquifers, contaminants, bacteria, DNA, sequencing

PRESENTER PROFILE

Judith is a groundwater scientist at ESR in Christchurch. Her research focuses on how human activities affect aquifers, especially the health of the microbial communities within these ecosystems. Judith's research currently focuses on sequencing and bioinformatics to identify correlations between microbes and groundwater contaminants. Her research aims to produce an early warning tool to help predict changes in aquifer communities to mitigate harmful contaminants in drinking water supplies.

INTRODUCTION

Globally 2.5 billion people depend solely on groundwater to satisfy their daily drinking water needs (Grönwall and Danert, 2020). As the global population continues to increase, there is not only a greater demand for freshwater resources but also the risk that groundwater aquifers will be affected by human activities that release contaminants into the environment, potentially impacting and altering the natural aquifer communities (Kroeze et al., 2016).

While the effects of anthropogenic contaminants are well documented on surface water, little is known about their impact on groundwater resources. Of particular concern is the lack of information on how the increasing concentration of contaminants will influence the bacterial communities within the aquifer (Korbel et al., 2017). These bacterial communities are highly diverse and functionally complex and play an essential role in driving the dynamics of aquatic ecosystems (Ushio, 2019).

Bacteria that live within aquifer communities utilise contaminants as energy (carbon) sources and remove harmful pollutants from groundwater sources. A better understanding of the biodiversity of bacterial aquifer communities and their interactions is vital and urgently required to enable the future protection of these ecosystems and protect global drinking water supplies.

While studies in subterranean aquifers are complex due to the lack of accessibility to the subsurface, environmental DNA (eDNA) provides an opportunity to examine what taxa are present and relate these to the aquifer's water quality and environmental conditions.

This study identifies bacterial communities in shallow groundwater aquifer systems across four geographic regions (Southland, Canterbury, Nelson, and Hawkes Bay) of New Zealand. Physicochemical parameters, bacterial diversity and abundance were evaluated and assessed for significant correlations.

MATERIALS AND METHODS

WELLS AND REGIONS

Forty-eight existing wells located across the North and South Islands of New Zealand were chosen for this study. The wells were located in four geographic regions, Southland (5 wells), Canterbury (29 wells), Nelson (5 wells), and Hawkes Bay (9 wells) (Figure 1).



Figure 1: New Zealand well locations and regions

CLIMATE CONDITIONS

In the South Island of New Zealand, the Southland region generally has mild summer conditions, dominated by cool coastal breezes. High temperatures occasionally occur during summer due to the hot northwesterly winds (Macara, 2013). The Canterbury region has warm summers typically moderated by a cool northeasterly sea breeze. High summer temperatures can occur when the hot, dry northwesterly winds blow over the Alps and plains. The annual mean rainfall in Canterbury is low, with long dry spells (Macara, 2016a). Nelson is the sunniest region of New Zealand, with warm, dry and settled weather predominating through summer. Nelson has mild winters, with light frosts and winds (Macara, 2016b). In the North Island of New Zealand, the Hawkes Bay region experiences less wind than other regions and has warm, dry and settled weather during summer, with cool frosty winters (Chappell, 2013).

SAMPLING AND ANALYSIS

Groundwater samples were collected from the wells and analysed for dissolved organic carbon, NO_3 -N (nitrate), and total dissolved nitrogen. Temperature and pH were measured in the field using a YSI combined meter.

DNA from groundwater samples was extracted using the Qiagen DNeasy Power Water[®] extraction kit (Qiagen, Hilden, Germany) and sent to New Zealand Genomes Ltd (Auckland, New Zealand) for Illumina MiSeq sequencing. The V3-V4 region of the 16S rRNA gene of bacteria was amplified with the 515F/806RB primer pair (Parada et al., 2016). Returned sequences were quality checked, trimmed to remove barcodes and primers and processed using the DADA2 pipeline. Sequences were analysed statistically using R.

RESULTS AND DISCUSSION

There was a significant difference in pH for the regions studied (Kruskal-Wallis p-value = 0.003). The Southland wells had the lowest median pH of 5.46, with the other regions having median pH levels of 6.63 to 7.01. Southland aquifers are typically shallow and unconfined and are recharged by rainfall infiltration. Low pH often occurs in these systems where pH values less than six are not uncommon (Southland, 2010).

There was a significant difference in well temperatures between regions (Kruskal-Wallis p-value = 0.005). The Southland median temperature of 10.4 °C was significantly lower than Nelson's 14.1 °C (Dunn's p-value = 0.002). The Southland region has a cooler climate and fewer sunshine hours than Nelson. Nelson has the highest number of sunshine hours across New Zealand. The wells in Canterbury had the most significant variation in groundwater temperature ranging from 6.9 °C in well SR2 to 16.6 °C in well M35/7542. This variability might be attributed to differences in aquifer properties, depth to the water table, and local climatic conditions.

Dissolved organic carbon was lowest in the Southland wells (0.6 mg/L (median)), indicating this region's relatively low organic carbon content. The Canterbury and Nelson regions had higher median organic carbon levels, but there was variation within these regional measurements. This variation in organic carbon may indicate localised sources of organic matter input, such as decaying vegetation or anthropogenic activities. The lack of a significant difference in dissolved organic carbon concentrations between regions (Kruskal-Wallis p-value = 0.056) suggests that the variations in organic carbon content might not be strongly influenced by regional factors but rather by local sources and hydrogeological conditions.

The wells in the Southland region had the highest median nitrate concentration of 5.5 Southland's groundwater resource is susceptible to mq/L. nitrate contamination due to the region's shallow aguifers and recharge by percolation through the soil from areas with intensive land use. Areas of Southland have ongoing land use intensification, which has accelerated in many places over the last two decades (Southland, 2010). In contrast, the Hawkes Bay region's lower agricultural impacts on groundwater guality meant it had the lowest median nitrate concentration of 1.1 mg/L. The Canterbury and Nelson aquifers are often much deeper than Southland (up to 100 meters) (Southland, 2010) and these regions had median nitrate concentrations of 2.0 mg/L and 2.5 mg/L, respectively. Localised pollution sources can result in higher nitrate levels infiltrating aguifer ecosystems. K38/1081 in the Canterbury region had the highest nitrate concentration of 11 mg/L, which could signify a localised pollution source. Furthermore, the lack of a significant difference in nitrate concentrations between regions (Kruskal-Wallis p-value = 0.206) indicates that nitrate pollution is a widespread issue across the study areas and calls for effective management strategies to control nitrate levels in groundwater.

The highest median total dissolved nitrogen was found in Southland, at 5.7 mg/L. Higher total dissolved nitrogen may be attributed to agricultural activities in this region and nitrogen inputs from fertilisers and animal waste. The Nelson and Canterbury regions had median total dissolved nitrogen concentrations of 2.6 mg/L and 2.2 mg/L, respectively, while the Hawkes Bay region had the lowest median concentration of 1.1 mg/L. The most significant variation in total dissolved nitrogen was observed in the Canterbury wells, with concentrations ranging from 0 to 10.9 mg/L, potentially due to varying land use practices and nitrogen sources in this region. The lack of a significant difference in total dissolved nitrogen pollution might be a common issue throughout these regions.

There were significant differences in bacterial diversity between regions (Adonis, p-value = 0.001). Bacterial taxa clustered by regions according to the 95 % confidence ellipses on the PCoA plots (Figure 2). Bacterial assemblages in well samples from Nelson and Hawkes Bay were similar to and thus clustered among those from Canterbury, while samples from Southland clustered to the left of the ordination and separated into two smaller clusters along the Y axis. Two Canterbury wells (K37/3306 and M35/2677) were outliers from the 95 % ellipse for the Canterbury region, and both were similar in bacterial composition to Southland groundwater samples.



Figure 2: Principal Coordinates ordination of bacterial assemblages in groundwater samples from wells in four regions of New Zealand based on sequencing of the 16S rRNA gene

Significant variations in the relative abundance of bacterial taxa at the Class level were observed. The presence of

Nitrososphaeria was present in the majority of wells across all regions. The relative abundance of Nitrososphaeria was relatively high in the Southland, Canterbury, and Nelson regions, possibly indicating its potential importance in the nitrogen cycle of these groundwater ecosystems (Figure 3). The detection of Methanobacteria in the Canterbury and Hawkes Bay regions and its absence in the Southland and Nelson regions points to regional differences in methane-producing bacterial communities (Figure 3).



Figure 3: Relative abundance of bacterial classes in groundwater samples from wells in four regions of New Zealand

Significant correlations between bacterial phylum and physicochemical parameters indicated environmental factors' influence on the bacterial community composition. The strong correlation between Thaumarchaeota abundance, total dissolved nitrogen (Spearman Rank Correlation, (rho 0.533, p<0.0001) and nitrate (r=0.539, p<0.0001) suggest their potential role in nitrogen cycling processes in groundwater.

CONCLUSIONS

This research sheds light on the complex and diverse aquifer communities in groundwater ecosystems in different regions of New Zealand. The results highlight the role of environmental factors in shaping bacterial communities' composition and emphasise the importance of understanding regional variations in diversity for groundwater management and conservation efforts. Further research is needed to explore the functional roles of specific bacterial groups and their potential implications for groundwater quality and ecosystem functioning.

ACKNOWLEDGEMENTS

The authors want to acknowledge ESR's Strategic Science Investment Funding for funding this research.

REFERENCES

- CHAPPELL, P. R. 2013. The climate and weather of Hawke's Bay. *NIWA Science and Technology Series.* 3rd ed.
- GRÖNWALL, J. & DANERT, K. 2020. Regarding groundwater and drinking water access through a human rights lens: Self-supply as a norm. *Water*, 12, 419.
- KORBEL, K., CHARITON, A., STEPHENSON, S., GREENFIELD, P. & HOSE, G. C. 2017. Wells provide a distorted view of life in the aquifer: implications for sampling, monitoring and assessment of groundwater ecosystems. *Scientific reports,* 7, 1-13.
- KROEZE, C., GABBERT, S., HOFSTRA, N., KOELMANS, A. A., LI, A., LÖHR, A., LUDWIG, F., STROKAL, M., VERBURG, C. & VERMEULEN, L. 2016. Global modelling of surface water quality: a multipollutant approach. *Current opinion in environmental sustainability*, 23, 35-45.
- LAWA. 2022. *Q&A: LAWA Groundwater Quality* [Online]. Land Air Water Aotearoa. Available: https://www.lawa.org.nz/get-involved/news-and-stories/national-news/2022/march/qalawa-groundwater-

quality/#:~:text=Groundwater%20is%20a%20vital%20resource,groundwater%20for%20their %20drinking%20water. [Accessed].

- MACARA, G. R. 2013. The climate and weather of Southland. *NIWA Science and Technology Series*. 2nd ed.
- MACARA, G. R. 2016a. The climate and weather of Canterbury. *NIWA Science and Technology Series.* 2nd ed.
- MACARA, G. R. 2016b. The climate and weather of Nelson and Tasman. *NIWA Science and Technology Series.* 2nd ed.
- PARADA, A. E., NEEDHAM, D. M. & FUHRMAN, J. A. 2016. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environmental microbiology*, 18, 1403-1414.
- SOUTHLAND, E. 2010. State of the Environment: Groundwater Quality Technical Report.
- USHIO, M. 2019. Use of a filter cartridge combined with intra-cartridge bead-beating improves detection of microbial DNA from water samples. *Methods in Ecology and Evolution*, 10, 1142-1156.