Going underground

Dr Inés C Wilhartitz has dedicated much of her career to understanding the bacterial communities found in karst aquifers. In this interview, she explains why groundwater microbiology is so important, and introduces her current research interests

By way of an introduction, can you outline the core objectives of your research?

Microbiological research in certain environments, like the sea or lakes, has a longstanding tradition. However, people have recently come to realise that the enormous volume of the saturated, terrestrial underground forms one of the largest environments for microorganisms on Earth. It is estimated that up to 40 per cent of prokaryotic biomass is hidden within this terrestrial subsurface. Although they may be involved in many geochemical processes, we have little information on natural bacterial communities inhabiting these systems. My main concern is to contribute to understanding these important habitats.

What led you to develop an interest in aquatic research and groundwater microbiology?

I have always been fascinated by water. From a chemist's point of view, it is unique; for a biologist, it is vital for all known forms of life. More than 70 per cent of the drinking water demand in Switzerland, Austria, Germany, Hungary and Italy is covered by groundwater resources, mainly from the Alps. In Vienna, where I started my research, it is more than 95 per cent. These resources are coming under increased pressure and are in great need of thorough investigation and sustainable management. All these reasons make this a thrilling and extremely important research topic.

Most of the research in such areas has so far focused on organisms relevant for its evaluation as drinking water. In contrast, what is the role of natural aquifer communities?

Given that surface water has enough time to seep slowly through an aquifer, the rock matrix can act as a huge filter – providing high quality water at the spring outlet. Biofilms on the rock surface can add to this self-purification process by, for example, enhancing the capability to adsorb organic material and by assisting the degradation of various substances. The fewer organic substances left in the abstracted water, the smaller the regrowth potential of undesired bacteria in the pipes during water transport. Furthermore, autochthonous microbial endokarst communities might be closely linked to geology by affecting the driving force of karst formation, namely the balance between lime and carbonic acid.

The production of carbon dioxide and acid compounds would inevitably affect the surrounding bedrock. However, one has to understand that these resident microorganisms are different from health relevant organisms, that don't come from the aquifer itself but mostly from surface influence.

Groundwater aquifers have a different structure than lakes or rivers. How are the bacterial communities organised there?

Karst aquifers are generally very oligotrophic. They have low nutrient levels, which results in very low cell numbers in the spring water. This is actually one reason why they are good drinking water resources under base flow conditions. We could demonstrate that most of the planktonic cells (about 90 per cent) are in a so-called 'dormant state' and only have low or no metabolic activity; it is generally concluded that attached microorganisms dominate subsurface environments in biomass and that planktonic cells are subsets of the attached organism. We have found that heterotrophic prokaryotic production is approximately 10^6 times higher in karst aquifer biofilms than in the respective water column. This means that metabolic processes and all kinds of reactions linked to bacteria will mainly take place in the biofilm compartment.

Are you collaborating with other organisations in the course of your current investigations? Has a multidisciplinary approach proved important to the success of the project?

A multidisciplinary approach is crucial for our current project. Although new sequencing methods might be powerful, thorough interpretation of the data is only possible when one can link the results to other environmental parameters. We are collecting a wealth of lithological, chemical, physical and hydrological background data. In that respect, I am working together with water authorities, National Parks and the University and Geological Survey in Slovenia. Furthermore, we also have a collaborative relationship with my former working group in Vienna, whose focus is more on risk assessment and the usage of these resources as safe drinking water.

Examining underground **microbial communities**

Despite increasing interest in the science of karst aquifers in recent years, understanding of the bacterial communities in this unique environment is still limited. However, a team from **Eawag** is helping to give this important topic the attention it requires

'KARST' IS A specific geological formation that is caused by the dissolving action of water on carbonate bedrock, over a period of thousands of years. As a result of this distinctive geological process, karst landscapes develop idiosyncratic landforms, including complex networks of surface and underground springs, drainage systems, sinkholes, caves and streams. Characterised by a series of caves that provide a huge water storage capacity, karstic aquifers are a geological phenomenon widespread in alpine regions. The groundwater that accumulates as part of such subterranean drainage systems is an increasingly important resource. With the exception of glaciers and ice caps, groundwater accounts for a staggering 97 per cent of all freshwaters available on Earth. Combined with the vast distribution of karst, this results in 20-25 per cent of the global population being partly or completely supplied by groundwater from karstic areas.

Problematically, although karstic aquifers are significant freshwater resources, they can be difficult to exploit and are also incredibly vulnerable to contamination. More than ever, these aquifers require protection and sustainable management. However, this is a feat far easier said than done; karstic aquifers exhibit unique characteristics in terms of hydrogeology, biogeochemistry and vulnerability, and in-depth information is still scarce. The most elusive information in this area is related to microbial communities living in alpine and mountainous karst aquifers, and their importance for ecosystem functioning. As there is limited accessibility to information on the topic, a team from the Swiss Federal Institute of Aquatic Science and Technology (Eawag) is working to bridge this significant knowledge gap. Led by Dr Inés Wilhartitz, the research group has dedicated many years to studying the bacterial communities in alpine aquifers in Central Europe. Continuing to compile their results along with hydrological, chemical and geological data from the same locations, the team consistently provides greater insight and understanding on the functioning and structure of this unique ecosystem.

INVESTIGATING AMECS

Wilhartitz's research interest has been a long time in the making. The project, in its current incarnation, stems back to previous work in which Wilhartitz (who at that time was working in a team at the Technical University of Vienna) provided the first evidence of an autochthonous microbial endokarst community (AMEC) in alpine karst aquifers. Here, two contrasting springs which had nearby catchment areas, but different hydrogeological conditions – such as contrasting water storage capacity – were the subjects of investigation. The evidence showed that, depending on the environmental characteristics, distinct natural AMECs inhabited the two different aquifer types. The results of this work were well received and have since gained much recognition.

Ongoing work will revisit the research on AMECs. Using new high-resolution methods, Wilhartitz et al are re-evaluating their original concept, expanding it to the Central European region, including 40 different aquifers. The work will try to establish whether there are indeed any fundamental differences between microbial communities living therein. If this proves to be the case, the team will then analyse which environmental factors might cause such differences.

TO CAPTURE THE COMPLEXITY OF MICROBIAL COMMUNITIES

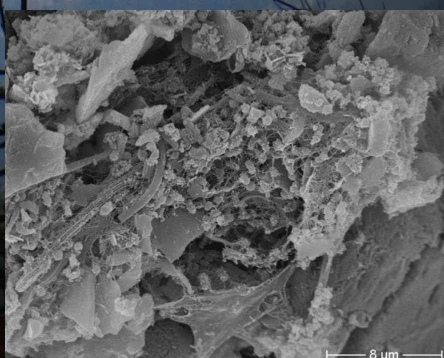
The description of community composition is essential for understanding how they react to transient perturbations, such as flooding, falling water tables and starvation, or chronic environmental changes, like potential alterations in nutrient concentrations due to anthropogenic influence. Indeed, AMECs are frequently subjected to a great deal of environmental stress, which they deal with by changing their protein expression. However, this alteration is not matched by immediate genomic rearrangements. Therefore, the mere study of individual genes is not sufficient to fully understand microbial adaptation strategies. In order to move away from the descriptive approach to ecosystem functioning, Wilhartitz uses a combination of metagenomics with metaproteomics, which involves mapping all proteins in a sample at a certain time point, to provide better insight.

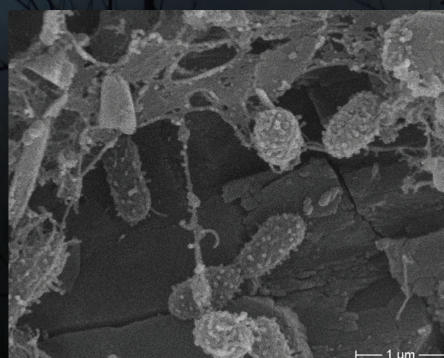
In the longer term, global warming will have a profound influence on alpine regions and their hydrological cycle, also changing environmental conditions within aquifers. This can ultimately lead to changes in the community composition itself. Knowledge of the status quo is essential in order to monitor and judge the consequences of this process.

HIGH-TECH APPROACHES

Given the complexity of AMEC research, it is unsuprising that it relies heavily on the recent, rapid developments in technology that have taken place over recent years. For instance, the development of

Scanning electron microscope pictures of a bacterial colony, inhabiting the rock surface in a karst aquifer, and a close up of the same colony.





novel throughput parallel sequencing approaches and innovative tools, such as total community divergence measures, has been vital to the progress in microbial research.

By using total community-based divergence measures, for example, it is now possible to calculate the fundamental α-diversity for individual communities, as well as the β-diversity between microbial communities, as it has been customary in the field of macroecology for decades. "The application of these new analytical tools has led to new fundamental insights in the composition and control of microbial communities on a global scale. But most importantly, target-orientated hypothesis testing on genetic microbial community structures and their controlling mechanisms has become possible," Wilhartitz explains. It is the latter feature that is a particularly exciting prospect for Wilhartitz and her team. Such technology could help their endeavours to discover the underlying patterns which might help explain why communities in different aquifers contrast.

CHALLENGING WORK

Sophisticated technology notwithstanding, examining microbial communities in such a specific environment is not without it challenges. In fact, even the first step of such research – selecting appropriate aquifers – is fraught with difficulty. Unlike other bodies of water, such as lakes or rivers, where samples can be taken from one particular place, alpine aquifers are more problematic as they can store water up to hundreds of metres inside a mountain. Moreover, when sampling at a spring outlet, there is no guarantee that it is a source of the genuine groundwater that needs to be studied, as there are numerous factors that can influence the composition of the water within the last few metres of its journey. Another difficulty is the extremely low cell numbers within samples, which makes collecting sufficient quantities of genetic material extremely difficult.

Yet, in spite of the challenges posed by this field of research, improvements and new discoveries are constantly being made. One recent, exciting development is the proposed construction of a base for a robust eco-phylogentic framework in the karstic subsurface. This is set to be the first scientific foundation to systematically understand the distribution and origin of autochthonous microbial aquifer communities, with an unprecedented level of resolution. "The possible scientific and applied impacts go far beyond the level of microbial ecology," states Wilhartitz; indeed, it could be of potential importance for geobiology, ecotoxicology, evolutionary science and water resource management. In particular, this platform will provide important data, assisting the international effort to develop a reference-system for groundwater quality. Not only could this have significant impacts on aspects such as risk assessment in groundwater, it could also provide invaluable data to future projects, like Wilhartitz's, on the subject of karstic aquifers and their ecosystems.

INTELLIGENCE

MICROBIAL COMMUNITIES INHERENT TO KARSTIC AQUIFERS – ECOLOGICAL DRIVING FORCES IN THE DEPTH

OBJECTIVES

Karst aquifers are unique groundwater ecosystems and important drinking water resources. Therefore, the project aims to establish a biogeographically based framework describing occurrence and distribution of microbial communities in representatively chosen karstic areas of Central Europe and to link the phylogenetic information to basic subsurface ecosystem processes in alpine karstic aquifers.

KEY COLLABORATORS

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