

The nitrogen cycle plays a crucial role in cycling nutrients through ecosystems. In this cycle, nitrification is an important step, in which ammonium is oxidized to nitrites and then nitrates. For many years, it was believed that nitrification was performed by only two cooperating groups of microorganisms. In the first step, bacteria (AOB - ammonia oxidizing bacteria) and archaea (AOA - ammonia oxidizing archaea) oxidize ammonia. The product of their metabolism is nitrite, which in the second step, are oxidized to nitrates by nitrite oxidizing bacteria (NOB). However, in 2015, a group of bacteria capable of carrying out both of these steps on their own was discovered. These bacteria belonged to the genus *Nitrospira* and were named "comammox" (COMplete AMMonium OXidation). This groundbreaking discovery initiated ongoing work to determine the role of comammox bacteria, factors influencing their occurrence, ecological niches that they occupy, and their relationship with canonical nitrifiers. One area that requires further investigation is the ecological significance of the comammox process and the extent to which comammox *Nitrospira* are involved in nitrification. Therefore, the objective of the proposed project is to assess the prevalence of comammox bacteria in bacterial communities in various environments and to analyze their co-occurrence network with other groups of bacteria, in particular with bacteria involved in nitrogen transformations.

In traditional microbiology, microorganisms are cultured and studied in laboratories, far away from their natural environment. However, the vast majority of bacteria, including comammox, cannot be grown under laboratory conditions. Therefore, the study of these microorganisms requires the use of molecular methods based on the analysis of nucleic acids and next-generation sequencing. In the proposed project, two modern methods will be used: metagenomics and metatranscriptomics. Metagenomics is the study of the total DNA obtained directly from the environment (the metagenome). It allows the entire population of microorganisms that form a microbiocenosis to be studied. Metatranscriptomics, in contrast, is the study of the total mRNA obtained directly from the environment (the metatranscriptome) and thus provides information about all the genes that are expressed in the entire complex of microorganisms. Using these methods allows for insight into the genetic structure of the microbial community without the need to culture the microorganisms in a laboratory.

The data obtained from the methods described above will be used to create a co-occurrence network of the bacteria forming the studied communities, including comammox *Nitrospira*. Microorganisms form complex communities, the shape and functioning of which are influenced not only by environmental factors but also by interspecies interactions, both antagonistic and non-antagonistic. One of the methods of studying the interactions between microorganisms is to create a network that represents the studied community and allows it to be comprehensively analyzed. By visualizing and analyzing such networks, key taxa in communities can be determined, as well as taxa that are potentially interdependent or that potentially compete with each other. Mapping the ecological networks of microbial communities can help predict the effects of changes and disturbances in an ecosystem, and allow complex microbial communities to be engineered.

The results that will be obtained will provide information about the prevalence of comammox bacteria in various environments, correlations between the abundance of comammox bacteria and that of other bacteria, the ratio of comammox bacteria to canonical nitrifiers, and differences in the structure of genes determining ammonia oxidation between comammox bacteria that inhabit different environments.