

***In the search for Soil Aerobic Methane Emission as a result of Microbial metabolism of Organophosphonates (SAMEMO)***

Methane, next to carbon dioxide, is the most important greenhouse gas, responsible for about 20% of global warming. Over the past 10 years, atmospheric methane concentrations have increased by 5.9%. According to long-standing scientific theory, the most important biological source of methane is the activity of methanogenic microorganisms inhabiting anaerobic environments, including rice fields and rumen in livestock. However, global methane budget is not balanced, indicating additional unidentified biogeochemical sources. Recent studies have revealed that methane can be formed under aerobic conditions as a result of the organophosphonate metabolism performed by the enzymatic carbon-phosphorus lyase pathway. Organophosphonates are natural and synthetic phosphonic acid derivatives whose unique feature is the presence of a direct bond between carbon and phosphorus atoms. The PhnJ protein, a key component of the carbon-phosphorus lyase pathway, is responsible for breaking the bond between carbon and phosphorus, which allows further bioconversion of the compounds. In the marine environment, the activity of this enzymatic pathway has been shown to be a major source of methane emission from oxygen-rich zones. These observations have clarified the research problem referred to as the “*marine methane paradox*”. One of the prevalent natural organophosphonate is methylphosphonic acid, the transformation of which is the source of the aforementioned methane emissions from the marine environment. In contrast, the most widely used synthetic organophosphonate is the herbicide glyphosate, whose global annual consumption is projected at  $9.2 \times 10^8$  kg in 2025. Also prominent among synthetic organophosphonates is aminotris(methylenephosphonic acid), which is commonly used to soften water in industrial and household cooling systems due to its chelating properties. These compounds directly or indirectly end up in the environment, including the soil, where they can contribute to methane emissions.

The main objective of the project is to investigate whether soil microorganisms metabolising organophosphonate compounds under aerobic conditions contribute to methane emissions. For this purpose, microcosm and *in vitro* experiments will be performed. Methane emissions will be measured using gas chromatography, and DNA will be isolated from organophosphonate-treated samples and sequencing of 16S rRNA gene fragments will be performed to determine the composition of the microbial community. The sequencing results will be enriched with a quantitative aspect through the use of digital polymerase chain reaction. This method will also be used to analyse the effect of organophosphonates on the expression of the *phnJ* gene transcripts encoding the aforementioned PhnJ protein.

This project is the first attempt to investigate the process of biological methane emission from soil under aerobic conditions. The results obtained will contribute to a more complete understanding and fill knowledge gaps on the global methane budget. The topic is highly relevant in the face of ongoing climate change and may be of interest to a wide audience.