

## **Application of fluorescent microscopy and cascade filtration methods for analysis of soil microbial community**

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Methods establishment of soil microbial cells size estimation called from the importance of current needs of research in microbial ecology. Some of the methods need to be improved for more detailed view of changes happen in microbiome of terrestrial ecosystems. The combination of traditional microscopy methods, fluorescence and filtration in addition to cutting-edge DNA analysis gives a wide range of the approaches for soil microbial ecologists in their research questions.

In the most of the cases the bacterial cells size is limited of the natural conditions such as lack of nutrients or stress factors due to heterogeneity of soil system. In the samples of soils, lakes and rivers sediments, snow and rain water the bacterial cells were detected minimally of 0.2 microns. We established the combination of the cascade filtration and fluorescent microscopy for complex analysis of different terrestrial ecosystems and various soil types. Our modification based on the use of successively filtered soil suspension for collection of microbes by the membrane pores decrease. Combination with fluorescence microscopy and DNA analysis via FISH method gave the presentation of microbial interactions and review of ecological strategies of soil microorganisms.

Humus horizons of primitive arctic soil were the most favorable for bacterial growth. Quantified biomass of soil bacteria depends on the dominance of cells with specific dimensions caused of stress factors. The average bacterial size of different soil varied from 0.23 to 0.38 microns, however in humus horizons of arctic soil we detected the contrast dominance of the bigger bacterial cells sized of 1.85 microns. Fungi in this case contributed to increase the availability of organic matter for bacteria because the fungal mycelium forms the appreciable part of microbial biomass of primitive arctic soil. The dominant content of bigger bacterial cells in forest and fallow soil as well as the opposite situation in arable soils caused by the availability of nutrients (glucose) and the degree of agricultural anthropogenic stress. Various combinations of factors such as stressful conditions (anaerobiosis, acidity and temperature) influenced on bacterial size. The decrease of these stress factors resulted in return to the original bacterial cell size in soil.

Furthermore the modification of gram-negative bacteria quantification was performed and combined with FISH method and DNA extraction. We established the methodological comparison of gram-negative bacteria groups in aerobic and anaerobic conditions. Due to absence of significant difference between the most frequent soil gram-negative bacteria groups we concluded the important ecological role of gram-negative bacteria as common group of microorganisms in natural polymer degradation. Depending on nutrient (glucose, cellulose, chitin) gram-negative bacteria competed with actinomycetes for available nutrients at the different time, what explained by the ecological flexibility of this soil bacteria group. The experiments showed expressed faster chitinolytic activity of soil gram-negative bacteria compare to actinomycetes.

Thus our approaches to use the combination both traditional and cutting-edge methods, forms the unique basement for various research and mostly open the wide doors to design new scientific experiments in ecology of terrestrial ecosystems and especially in soil microbial ecology.