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Fluorescent microscopy approaches of quantitative soil microbial analysis

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Classical fluorescent microscopy method was used during the last decades in various microbiological studies of terrestrial ecosystems. The method provides representative results and simple application which is allow to use it both as routine part of amplitudinous research and in small-scaled laboratories. Furthermore, depending on research targets a lot of modifications of fluorescent microscopy method were established. Combination and comparison of several approaches is an opportunity of quantitative estimation of microbial community in soil.

The first analytical part of the study was dedicated to soil bacterial density estimation by fluorescent microscopy in dynamic of several 30-days experiments. The purpose of research was estimation of changes in soil bacterial community on the different soil horizons under aerobic and anaerobic conditions with adding nutrients in two experimental sets: cellulose and chitin. Was modified the nalidixic acid method for inhibition of DNA division of gram-negative bacteria, and the method provides the quantification of this bacterial group by fluorescent microscopy. Established approach allowed to estimate 3-4 times more cells of gram-negative bacteria in soil.

The functions of actinomyces in soil polymer destruction are traditionally considered as dominant in comparison to gram-negative bacterial group. However, quantification of gram-negative bacteria in chernozem and peatland provides underestimation of classical notion for this bacterial group. Chitin introduction had no positive effect to gram-negative bacterial population density changes in chernozem but concurrently this nutrient provided the fast growing dynamics at the first 3 days of experiment both under aerobic and anaerobic conditions. This is confirming chitinolytic activity of gram-negative bacteria in soil organic matter decomposition.

At the next part of research modified method for soil gram-negative bacteria quantification was compared to fluorescent in situ hybridization method (FISH). This approach was used for evaluation of contribution of each gram-negative bactera group. No significant difference between the main soil gram-negative bacterial groups (phylum Proteobacteria and Bacteroidetes) was found both under anaerobic and anaerobic conditions in chernozem in the topsoil. Thus soil gram-negative bacteria play an important ecological role in natural polymer degradation as common group of microorganisms.

Another approach with using cascade filtration technique for bacterial population density estimation in chernozem was compared to classical method of fluorescent microscopy. Quantification of soil bacteria with cascade filtration provided by filters with different diameters and filtering of soil suspension in fixed amount. In comparison to the classical fluorescent microscopy method the modification with filtration of soil suspension provided to quantify more bacterial cells. Thus biomass calculation results of soil bacteria by using classical fluorescent microscopy could be underestimated and combination with cascade filtration technique allow to avoid potential experimental error.

Thereby, combination and comparison of several fluorescent microscopy methods modifications established during the research provided miscellaneous approaches in soil bacteria quantification and analysis of ecological roles of soil microorganisms.