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Considerations regarding some factors that influence the soil microbiome diversity in agroecosystems

Laurentia ARTIOMOV[1], **Nina FRUNZE**[1]

[1] Institute of Microbiology and Biotechnology, (Republic of Moldova), lara_09@rambler.ru

Abstract

At the system level, the microbiome plays an integral role in almost all soil processes, so that abundance, composition and microbial activity largely determine the sustainable productivity of agricultural land. Metagenomic methods are an effective tool for rapid analysis of microbial heterogeneity. Ribosomal RNA sequencing allows the identification of most soil microorganisms. Soil microbiome can be influenced by soil management or by natural or anthropogenic disturbances leading to taxonomic and functional changes. Agricultural practices, such as intercalation of forest strips, reduced tillage, organic fertilization; crop rotation, etc. have a positive impact on the abundance and richness of specific groups of soil organisms and on soil microbial diversity. Ecological (organic) agriculture contributes to the increase of the of species richness, heterogeneity and to the modification of the soil microbiocenosis structure compared to the soil for which conventional agricultural practices. This effect is largely ensured by the use of organic fertilizers, which stimulate the development of existing microorganisms, but also bring new species of microorganisms. Fertilized cultivated soils have a higher nutrient mobility and in their microbiome the microorganisms with copiotrophic nutritional strategy are favoured. In uncultivated soils the content of labile, easily accessible nutrients is lower and the development of oligotrophic microorganisms is promoted. Crop rotation, as a management practice for agroecosystems, is beneficial for soil microbial diversity. In conclusion, we mention that the anthropogenic load of the soil, the agricultural management system influences the formation of prokaryotic communities in the soils. Thus, by adapting agricultural management practices, it is possible to encourage the recruitment of specific groups of soil organisms and increase microbial diversity.

Keywords: soil microbiome diversity, metagenomics, agroecosystems, agricultural practices

1. Introduction

Soil micro-organisms are a key component of both natural and agro-ecosystems. They produce and consume atmospheric gases, influence soil acidity, regulate carbon dynamics, mediate nutrient cycling in the soil profile, modify soil water availability. Soil-borne plant and animal pathogens can influence vegetation dynamics and animal populations, with far-reaching effects on the functioning of terrestrial ecosystems. Soil microorganisms contribute to plant growth, soil fertility and pathogen biocontrol. The results of the research in recent decades indicate that conventional and ecological agriculture systems have a major influence on the composition of the microbial communities of the soil, and the microbiome modification influences the soil health and agroecosystems productivity. Soil microorganisms are also indicators of soil quality, given their participation in many biochemical processes essential for the environment and also for soil ecological functions.

Most soil-dwelling prokaryotes (90-99% of the total community composition) cannot be cultured in the laboratory, so their functions cannot be studied by classical microbiological methods. Recent advances in the development of metagenomic methods have greatly expanded the ability to investigate the soil microbiome and identify factors that shape soil microbial communities over space and time.

Methodology

The research combines bibliographic research on the influence of agro-technical factors on soil microorganisms with metagenomic study of the soil microbiome at the *Biotron Experimental Base*. The bibliographic study addressing the topic of factors influencing the diversity of the soil microbiome of agroecosystems was carried out through a systematic search on this topic in Google Scholar databases. For the search we used appropriate combinations of the keywords: soil microbiome, metagenomic methods, agroecosystems, and agricultural practices. We selected and analyzed articles addressing the topic of the influence of agricultural practices on soil microbiome diversity, mainly published in the last 15 years. Metagenomic research of the soil microbiome was carried out in the GENOMIC TECHNOLOGIES section of the Center for the Collective Use of Scientific Equipment "Genomic Technologies, Proteomics and Cell Biology" of the All-Russian Research Institute of Agricultural Microbiology, St. Petersburg, Russia.

3. Results and discussion

3.1 The influence of the agricultural management system on the soil microbiome

The soil is extremely important for the biodiversity of the ecosystem and plays a role in the productivity of the ecosystem. One of the most important challenges in agriculture is to determine the effectiveness and environmental impact of certain farming practices. Excessive chemical fertilizers use in conventional agriculture can cause serious environmental problems. Most pesticides adversely affect the functioning of soil microorganisms, their diversity, composition and biochemical processes. Pesticides cause an imbalance in soil fertility, which directly affects crop yields. Organic agriculture is considered more friendly for the environment and has less harmful effects on the ecosystem, including soil. Agricultural practices affect soil microorganisms, which are the key players of many ecosystem processes. The complexity of soil microbiocenosis makes it difficult to try to propose universally valid mechanisms regarding the influence of agricultural management systems on different functional groups of soil microorganisms. Organic agriculture increases richness, reduces dispersal and changes the structure of the soil microbiota [Hartmann *et al*., 2018]. Li R. and colleagues [Li *et al*., 2012] demonstrated experimentally that bacteria that promote the growth of plants belonging to the genera *Burkholeria*, *Stenotrophomonas* and *Pseudomonas* were more abundant in a system of organic farming. Other papers mention that organic fertilized soil is caracterized by more abundant speccific taxa of Bacteria (Rhizobiales, Thiotrichaceae, Micromonosporaceae, Desulfurellaceae and Myxococcales), which contribute to the nutrient cycle (C, N, S and PThe application of conventional agricultural practices has led to an increase in the relative abundance of acid-tolerant and alkali-tolerant bacteria. *(Acidobacteriaceae* and *Sporolactobacillaceae*) [Wang *et al*., 2016]. These results indicate that organic farming leads to the formation of a more stable microflora of the bacterial community. A higher abundance of acid and alkaline resistant microorganisms (*Acidiphilium*, *Acidobacteriaceae*, *Sporolactobacillaceae*, *Alicyclobacillus* and *Alkaliphilus*) in the soil of the conventional agricultural management system, suggests that conventional soil cultivation creates stressful conditions for soil microorganisms and soil it is an ecological model that encourages sustainable development.

Organic farming is considered an important factor in the suppression of phytopathogens from the soil occupied by various crops [Li *et al*., 2012]. Disease-suppressing bacteria play a key role in disease suppression. The abundance of *Firmicutes* and *Actinobacteria* bacteria increases in the soil under the conditions of ecological management. These bacteria have been usually associated with plant disease control. Other bacteria of the genera *Pseudomonas*, *Bacillus*, *Burkholderia*, and *Actinomycetes*, frequently have been found as numerous populations in disease suppressive soils [van Bniggen *et al*., 2003]. Zarraonaindia I. and co-workers note that bacteria of the order *Myxococcales* (*Cystobacteraceae* and *Haliangiaceae*), which are more abundant in the soil of organic farms, have the ability to synthesize bioactive compounds *Althiomycin* and *Myxovirescin* with important role in the biocontrol of bacterial and fungal diseases [Zarraonaindia *et al*., 2020]. *Mixobacteria* are thought to have the potential to suppress plant diseases and play a key role in organic farming.

It is necessary to understand the consequences of land use change on soil the ability of the to support the functions of a multitude of living beings and to interact through the cycle of matter and energy with the components of the abiotic environment.

When deciphering these aspects, it is very important to understand how the soil microbiome changes, under the influence of which factors and how agricultural productivity is affected. Conventional farming practices (use of synthetic chemical fertilizers, pesticides, herbicides and other continual inputs, genetically modified organisms, tillage etc.) alter the physico-chemical properties of the soil. Trivedi P. et al. [2016] carried out a bibliographic analysis of more than 100 publications to understand how agricultural practices influence the structure and diversity of the soil microbiocenosis identified by modern sequencing techniques (Next Generation NGS sequencing). The authors identified higher relative abundances of some bacterial phyla in agricultural systems (e.g. *Actinobacteriota*, *Firmicutes*, *Verrumicrobiota* and *Chloroflexi*) and of other phyla in natural ecosystems (*Acidobacteria*, *Proteobacteria*, *Cyanobacteria*, *Planctomycetota*). These trends correlated with some soil properties (soil C, soil N, soil pH) .

Agroecosystems are more dynamic, more heterogeneous, have a more variable physical structure and chemical composition compared to natural systems, providing microorganisms with various ecological niches for development. The authors note that in arid and temperate zones there is a greater diversity of microorganisms in the soil of agroecosystems than in the soil of natural ecosystems. This phenomenon can be explained by the insufficiency of nutrient resources in the arid soil in particular. [Trivedi *et al*.,2016]. Conversion of uncultivated, natural soils to agricultural soils increases the taxonomic diversity of soil prokaryotes, but communities become more homogeneous as some rare, endemic soil taxa are lost. The diversity of soil microorganisms supports several soil ecosystem functions (ensuring productivity, control of phytopathogenic agents, bioremediation of polluted soil, etc.), and the reduction of the diversity of microorganisms during the conversion of natural lands could have a negative impact on the health and productivity of agricultural ecosystems. [Delgado-Baquerizo, 2016]. Within the project 20.80009.5107 "Efficiency of the use of soil resources and microbial diversity by applying the elements of biological (organic) agriculture", the structural taxa of prokaryotes, identified by nucleotide sequencing of the amplified 16S rRNA gene (Illumina 3.rar), were analyzed [Artiomov et al., 2021]. The aim of the research was to elucidate the influence of crop rotation and the type of soil fertilization on the structure and diversity of the microbiome. About 19 phyla, 38 classes, 97 orders, 146 families and 305 genera of prokaryotes have been identified. About 19 phyla, 38 classes, 97 orders, 146 families and 305 genera of prokaryotes have been identified. The phyla with the highest abundance in the *Bacteria* domain: *Proteobacteria* (14.3-22.5%), *Actinobacteriota* (16.1- 22%), *Firmicutes* (0.78- 5.9%), *Acidobacteriota* (3.0- 7,5%). In the *Archaea* domain the most abundant was the phylum *Thaumarchaeota* (2.3-7.9%). The phyla of rare bacteria, with low abundance: *Deinococcota* (0.008%), *Abditibacteriota* (0.009%) - were detected only in the control variant of the rotation without alfalfa. The *Halobacterota* phylum (0.02%) of the *Archaea* domain was found only in the variants with organic fertilization. The phyla with higher abundances in the soil of the forest strip adjacent to the experimental lot were *Acidobacteriota* and *Verrumicrobiota.* Most representatives of these phyla have an oligotrophic nutrition strategy and metabolize recalcitrant carbon sources of virgin soil in the forest strip more easily than copiotrophic microorganisms. The highest Shannon diversity index was recorded for the Proteobacteria phylum of the Bacteria domain in the intercropping without alfalfa, control variants (3.42) and mineral fertilization (3.35).

3.2 Influence of soil fertilization system

Fertilization and amendments traditionally used in agriculture have an important impact on soil and plants. Most nutrients (N, P, S) are resulting from the degradation of organic matter and must be mineralized by bacteria or fungi to be available for plants. Under natural conditions, microbial mineralization is the determining factor in plant growth. The exudates of the plants shape the microbiome, improve the mineralization of organic residues by soil microorganisms and the bioavailability of nutrients for plants. Chemical fertilization can unbalance the relationship between plants and soil microorganisms. The administration of nitrogen fertilizers can have an impact on the development of diseases.

High nitrogen concentrations are often positively correlated with an increase in the susceptibility of plants to diseases. Fierer et al. [2012] demonstrated that nitrogen fertilization increases the rate of copiotrophic bacteria, manifested by increased DNA, RNA and protein metabolism. This change has been confirmed by metagenomic analyzes. The decrease in urea decomposition has also been observed, which suggests a diminished use of organic nitrogen forms. Nitrogen fertilization, by limiting competition for resources, increases the incidence of plant diseases [Delitte *et al*., 2021]. When nitrogen is not limiting, pathogens can easily acquire nitrogen and cause more diseases to plants than on the land where nitrogen is limiting. However, in vitro experiments, it has been shown that the activity of several bacterial and fungal genes, expected to be involved in pathogenicity, is stimulated during growth under limiting conditions of nutrients [Snoeijers *et al*., 2000]. Wolny-Koładka K. et al. [2022] established that high-dose nitrogen fertilizers alter the qualitative composition of microbiocenosis; there is a decrease in the abundance of *Arthrobacter, Azotobacter* and *Streptomyces* bacteria, the dominant genera until fertilizer administration. The dominance of microbiocenoses is replaced by other microorganisms, mainly *Deuteromycetes* fungi. Soil fertilization with phosphorus decreases the ability of soil microorganisms to solubilize phosphorus [Pantigoso et al., 2018].

Organic fertilisation with compost or manure usually has the most significant effects on the microbial community in agricultural soils. Organic amendments shape the soil microbiome, primarily through nutrient availability, but also through pH or modulation of other soil parameters. Although chemical fertilisation appears to make the soil microbiome 'dependent' on these nutrients, favouring copiotrophic bacteria and decreasing the solubilising and mineralising abilities of N and P cycling bacteria, organic amendments offer more possibilities. In this regard, Ling et al [2016] found that long-term organic amendments support stronger functional potential and more interactions within soil communities than chemical fertilization, most likely due to better soil stability and buffering capacity. Liu S. et al [2021] found that the administration of soil fertilizers contributed to a decrease in the number of plant phytopathogenic fungi (Rhizopus, Penicillium and Fusarium) in the soil. Organic fertilizers in complex with the application of a mixture of effective microorganisms ensured the increase of the diversity of bacteria. The use of compost together with mixtures of microorganisms - phytopathogen control agents, is an effective method of ecological agriculture.

Soil treatment with cattle manure and lime resulted in increased soil pH and as a result led to changes in the soil microbiome [Chen *et al*., 2022]. *Actinobacteriota*, and *Proteobacteriota* phyla became more abundant in the rhizosphere upon application of organic materials with lime.

Within the *Actinobacteriota* phylum, combined application of organic materials (manure or biochar) and lime significantly increased the relative abundance of the *Nocardioides* and *Phycicoccus* genera. In the *Proteobacteria* phylum, all tested amelioration measures increased the relative abundance of the genera *Sphingomonas* and *Microvirga*. Chen, D., et al [2022] demonstrated that the application of soil amendments in acidified cropland in southern China increased soil microbial diversity in the plant rhizosphere, which limited pathogenic fungal spore proliferation and hyphal growth. In addition, reprogramming the rhizosphere microbial community by increasing the abundance of *Streptomyces*, *Streptacidiphilus* and *Sinomonas* rhizobacteria enhanced the physiological activity of plant roots and increased the disease resistance of seedlings. Therefore, these findings highlighted that building the rhizosphere microbiome by applying soil amendments in acidified croplands in southern China may be a key approach for suppressing soil-borne diseases.

Wang, Y. F. et al. [2022] studied the contribution of biodiversity of soil organisms and community structures (clusters) to soil ecosystem multifunctionality in a 25-year organic fertilization experiment. The researchers' experiment demonstrated that organic fertilization resulted in the formation of a unique community of soil organisms compared to other treatments. Organic fertilization essentially enriched the genera *Cellvibrio*, *Thermomonas*, *Adhaeribacter*, *Stenotrophomonas*, *Streptomyces*, *Actinocorallia*, *Clostridium sensustricto1*, while decreasing the abundance of *Nocardioides*, *Solirubrobacter*, *Agromyces* and *Xylophilus* compared to the no-fertilization variant. Significant positive relationships between soil organism biodiversity and ecosystem multifunctionality were not demonstrated. This may be due to the functional redundancy of soil organisms in agroecosystems, which means that different species can perform the same ecosystem function [Li *et al*., 2021]. Other researchers argue that soil functions may be directed by specialized microorganisms rather than the whole community [Bastida *et al*., 2016]. It is assumed that specialized microorganisms are gathered in ecological clusters to maintain multiple soil functions [Delgado-Baquerizo *et al*. 2018].

Soil salinisation is a major factor negatively influencing agricultural development worldwide. Highly efficient and environmentally beneficial management measures are needed to mitigate soil salinization. Mao X. and co-workers [2022] applied bovine manure, biochar and a bioorganic fertilizer to soil with mild salinity to investigate the remedial effect of organic materials in saline soils cultivated with melon (*Cucumis melo* L). Genome sequencing of soil prokaryotes demonstrated that organic fertilization significantly improved the structure of the soil microbiome, contributing to an increase in the relative abundance of the phyla *Acidobacteriota* and *Firmicute*s and of the halotolerant bacterial genera *Flavobacterium*, *Bacillus* and *Arthrobacter.* The three organic materials mitigated soil salinization, improved the soil microbiome, and promoted the growth of melon plants, especially the biochar treatment. Organic-mineral fertilizers produced from sewage sludge are also used in agricultural practices. The most commonly used fertilizers are mineral or organic fertilizers, but increasing attention is being paid to organo-mineral fertilizers produced from sewage sludge. Hawrot-Paw M. et. al [2022] investigated the effect of fertilization with a product obtained from sewage sludge on the qualitative and quantitative indicators of soil microbiocenosis and on the morphological and physiological characteristics of corn. This product positively influenced the soil microbiome throughout the maize growing season. The activity of microorganisms in the soil fertilized with the organo-mineral product was higher compared to the control in the range of 8 to 43%. Microbiological investigations showed that the number of bacteria of the genus *Bacillus* was higher in the soil fertilized with organo-mineral fertilizer produced from sewage sludge compared to the control. Bacteria of the genus *Bacillus* (phylum *Firmicutes*), which exist in the rhizosphere, are biological control agents, inhibit the growth of many phytopathogenic fungi and play an important role in stimulating plant growth [Akinrinlola *et al.*, 2018].

3.3 Influence of crop rotation

The practice of monocultures in agriculture disrupts the natural balance of soils, drastically diminishes above-ground and underground biodiversity, decreases soil fertility and leads to the spread of plant diseases. Alternatives to monoculture are crop rotation and intercropping to improve quality.

Crop rotation has a significant impact on soil microbial diversity. Venter Z.S. and co-authors [2016] found that increasing crop diversity in rotation has a positive impact on soil microorganism abundance (+ 15.11%) and diversity (+ 3.36%)]. Crop rotation induces diversity of microbial community composition and increased metabolic capacity. These changes could be related to the variety of crop root exudates and the chemical composition of crop residues. No-till tillage combined with crop rotation could be a potential agricultural management system to improve soil stability and biological activity. The relationship between biodiversity and agro-ecosystem functioning is complicated, and the link between aboveground plant and belowground micro-organism diversity needs further research.

The application of intercropping has a positive effect on soil characteristics and results in increased abundance and diversity of soil bacteria at the taxonomic genus level compared to maize monoculture [Volinska *et al*., 2022]. The genera *Massilia* and *Haliangium* have been proposed as bacterial indicators of susceptibility to monoculture, while *Sphingomonas* has been recommended to be an indicator of long-term maize monoculture resistance. Overall, the researchers' results showed that the use of a intercropping system can be a sustainable farming practice.

Our metagenomic sequencing research of the soil microbiome at the Multiannual Station of the "Biotron" Experimental Base (Chisinau) demonstrated that agricultural practices influence some beneficial microorganisms involved in atmospheric nitrogen fixation. The genus *Ensifer*, which includes bacteria capable of inducing the formation of root nodules in legumes, was detected only in alfalfa soil with the mineral background variant (N45-90P30-60K60-90). Bacteria of the genus *Streptomyces*, which have potential as biocontrol agents, had the highest relative abundance (1.3%) in the organic background variant of alfalfa rotation [Artiomov et al., 2021]. The analysis of the Jaccard coefficient at the phylum level demonstrates the lowest similarity when comparing the variants: control II/ organic fertilization -0.84, mineral fertilization I / Forest strip - 0.85, mineral fertilization I / organic fertilization - 0.85. The highest degree of similarity was observed when comparing the variants organic fertilization/ forest strip in both crop rotations – 0.95.

4. Conclusions

The ability of soil microorganisms to support a variety of ecosystem services is of increasing interest, but understanding of the link between farmland ecosystem services and the soil microbiome in different agricultural management systems is still limited. Unfortunately, many beneficial functions of the soil microbiome are currently under threat due to changing climate and precipitation patterns, soil degradation, and poor land management practices.

Soil microorganisms contribute to plant growth, soil fertility and pathogen biocontrol. Intensive farming practices alter the physico-chemical properties of soil and as a result the diversity and composition of soil microbial communities is affected. Organic farming leads to a significant increase in the abundance of some nutrition-related bacteria, while reducing some of the abundance of acid and alkaline resistant bacteria.

Long-term organic amendments support stronger functional potential and more interactions within soil communities than chemical fertilisation. Chemical fertilisation can unbalance the relationship between plants and soil micro-organisms. Nitrogen fertilizer administration can impact disease development. Nitrogen fertilisation, by limiting competition for resources, increases the incidence of plant diseases.

Monocultures cause the spread of pests and diseases, which must be controlled using even more chemicals. Crop rotation and intercropping have a positive effect on soil characteristics and microbiome structure. Anthropogenic activities lead to the erosion of the soil microbiome diversity of microorganisms through the loss of rare, endemic taxa. Thus, by adapting agricultural management practices it is possible to stimulate the development of specific groups of soil organisms and increase microbial diversity. Currently, the complexity of the diversity of the soil microbiome makes it difficult to draw firm conclusions about the differences between the soil *Bacteria* and *Archaea* communities of different agricultural systems.

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