Microbial complex adaptation in soils of different cultivation degrees

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Abstract. Throughout years-long observation, the properties of cultured soils in European Russia were studied (including the geographical location, physical, chemical, and biological properties of soils, the composition and structure of bacterial communities in them, etc.). Groups of microorganisms with various reactions to anthropogenic impacts were analyzed. Biological and fermentation power indicators manifest the ongoing soil-forming processes. The increase in the numbers of microorganisms of their biological activity (denitrification) is observed along the North-South line with the maximum values in the steppe zone. For acidic soils, the following indicators and susceptible soil microorganisms were determined: Actinomadura roseola, Methylococcus sp., Nocardia carnea, Sphingomonas capsulata, as well as for alkaline soils: Rhodococcus equi, Streptomyces sp. The following dominating species were found in the microbial consortium of the taiga zone soils: – Rhodococcus rhodochrous, and the dominant species for the wooded steppe, steppe, and dry steppe zones is the association of anaerobic Ruminococcus sp. with vesicular-arbuscular micromycetes. The data obtained are crucial for environmental monitoring and the development of nature protection actions to support soil fertility.

1. Introduction
Soil microorganisms are notable for their high susceptibility to environmental impacts in the short term, and they can support the biodiversity and the fungistasis of the soil for a significantly long time. The structural changes to the functioning of ecosystems in various biomes are determined by the participation of numerous microorganism groups in biochemical processes, which is vital in regulating the efficient soil fertility and plant growth conditions (nutrition) in the agricultural industry. For example, after the harvesting of the vicia-oats mix on the soddy-podzolic loamy sand soils, the anaerobic genus Clostridium was dominating, after the barley it was the aerobic bacterium species Arthrobacter globiformis, and after maize, it was the aerobic actinomycetes Rhodococcus sp. [1] This helps characterize the microbiological processes in the soil as anaerobic or aerobic, as well as its productiveness and the resilience of the agricultural ecosystem components under intensive farming conditions, during prolonged non-cultivating periods and in the soils outside the crop rotation (fallows). It is noticed that in soddy-podzolic soils, the disintegration of plant residues is largely
The biological activity of the soil under the influence of cultivation processes (technogenesis) in various soil and climate conditions.

2. Materials and Methods
The years-long monitoring to study the biological activity and soil fertility of farmlands was conducted in European Russia in various biomes, such as taiga, wooded steppe, steppe, and dry steppe (semidesert). A large set of artificial ecosystem parameters were studied for agricultural ecosystems and agrocoenoses that form a sequence running from North to South. The properties of soils (podzol; soddy-podzolic; chernozem; chestnut soils, etc) were assessed using the classic methods of plant ecology, soil science, and microbiology. The sampling locations were grouped according to the differences in the cultivated soil formation: a) fallows (outside crop rotation for 5 or more years), b) black fallsows, c) tilled land (soils under intensive farming – grain crop rotation), d) wildland and native areas. The species composition (the consortium) of the microbial community was determined using the gas chromatography and mass spectrometry (GCMS) method based on the identification of molecular structures or specific chemical markers of microbial cell walls (fatty acids, aldehydes, oxyacids) at the genera and/or species level: bacteria, actinomycetes, fungi and viruses [3, 4].

The long-term data were analyzed using the methods of mathematical statistics in MS Excel (2013) and Statistica (2010) software packages. To interpret the results, correlation and regression analysis was performed. The significance of differences between the compared properties was evaluated at $P \leq 0.05$. The following characteristic parameters of biological activity of soil were calculated: $X_0$ - average number of microorganisms, cells/g; dispersion - $\pm \sigma$; $N$ - number of species; $K_v$ – coefficient of variation, $\%$; $r^2$ - coefficient of determination; $IR$ - incidence rate, $\%$; $SRI$ - overall soil respiration index, equal to the ratio between the total emission of carbon dioxide and methane and the total number of microorganisms; $DNI$ - denitrification index, equal to the ratio between the total emission of nitrogen oxide and the organic carbon content in soils from various habitats.

3. The study of the microbial complex in soils of different cultivation
In natural phytocoenoses, the plant community is in a dynamic equilibrium adapted to the natural conditions and soils. In farming, soil fertility recovery relies on a combination of natural and anthropogenic factors influencing the soils and transforming natural soils into cultivated ones where properties and conditions comply with the requirements of the cultivated plants [5]. The ranges for the main environmental properties of cultivated lands in European Russia are as follows: organic substance content in carbon equivalent – 2.7 to 4.5 $\%$; pH$_{water}$ – 6.3 to 7.9; cation exchange capacity – 3.5 to 18 mg-eq/100 g; native humidity during vegetation – 5 to 22 $\%$ of the TMC; total number of microorganisms – $45\pm17\cdot10^7$ cells/g; total number of fungi – $12\pm9\cdot10^7$ cells/g; carbon dioxide emission – $44\pm9$ mg CO$_2$/(10 g per day). In agricultural ecosystems of various cultivation levels in terms of spatial and temporal incidence rate, the following genera were found: dominating (>60 $\%$) – Acetobacter, Rhodococcus, Streptomyces; frequent (30–60 $\%$) – Agrobacterium, Arthrobacter, Butyrivibrio, Rhodococcus, Staphylococcus, Xanthomonas; rare (10–30 $\%$) – Acetobacterium, Actinomadura, Actinomycetes, Bacillus, Bifidobacterium, Clostridium, Eubacterium, Methylococcus, Micromonaspora, Propionibacterium, Pseudomonas, Ruminoococcus; accidental (<10 $\%$) – Acinetobacter, Aeromonas, Caulobacter, Chlamydia, Clostridium, Micrococcus, Nitrobacter, Nocardia, Pseudonocardia, Rhodococcus, Sphingobacterium. Among the phytopathogenic bacteria, the majority is represented by the gram-negative specimen of the Pseudomonas and Bacillus genera [6].

With the increase of the degree of soil cultivation, the biodiversity (the number of species) reduced and the rank distribution shifted towards the increase of the specific species in the consortium. The species diversity of the microbial coenosis in the wild, fallow, and lea lands was two times higher due to the anaerobic clostridia specimens which were not present in the tilled soils (Fig. 1). In the
microbial community of the tilled lands, the incidence of the following species exceeded 20%: *Eubacterium lentum, Ruminococcus sp.*, *Rhodococcus rhodochrous*; in the fallow land soil: *Acetobacter diazotrophicus* (*present in this variant only), *Rhodococcus rhodochrous, Arthrobacter globiformis*; in the lea land soil: *Bifidobacterium sp.*, *Clostridium pasteurianum, Eubacterium lentum,* *Nocardi a asteroids, Ruminococcus sp.*, *Rhodococcus rhodochrous*; in the lea land soil: *Actinomadura roseola, Methylococcus sp.*, *Arthrobacter globiformis, Bifidobacterium sp., Clostridium pasteurianum, Eubacterium lentum, Rhodococcus rhodochrous, Ruminococcus sp.*, *Streptomyces sp.*

No significant difference was observed in the numbers of microorganisms belonging to genera *Actinomycetes, Acetobacter, Bifidobacterium, Clostridium,* and *Ruminococcus* in the soils of various habitats (tilled land, fallow, lea land, wildland). The functioning of aerobic/anaerobic consortium, in which the microorganisms are in a trophic relationship, is vital for the formation of humic substances in the soils of various habitats. A significant positive correlation was found between the number of the obligate aerobic bacterium *Clostridium putrificum*, the humus content ($r^2=0.58$), and the aerobic species of the actinomycete line of *Nocardia asteroides* ($r^2=0.59$) that can decompose complex polymer carbon compounds in the soil.

The maximum number of *Actinomadura roseola* and *Arthrobacter globiformi* (5.8–86.5·$10^6$ cells/g) is determined in natural (wild) soils, that of *Nocardia carnea* (30.9–88.5·$10^6$ cells/g) – in the fallows, and *Aspergillus sp.* (1.3–4.2·$10^6$ cells/g) in the soils from actively farmed lands (Fig. 2). This calls for the assumption that the soil is resilient to anthropogenic impacts, there is an abundance of *Actinomadura roseola, Methylococcus sp.*, *Arthrobacter globiformis*.

![Figure 1. Rank distribution of species in the soils with various degrees of cultivation: a – fallow land, b – lea land, c – tilled land, d – wild land](image1)

![Figure 2. The reaction of susceptible microorganisms to the degree of soil cultivation. I group – podzol, soddy-podzolic, meadow brown, and meadow gley degraded soils; II group– grey forest, chernozem and chestnut soil](image2)
content in cultivated lands: it is a direct dependence for the group I \((r^2 \leq 1.0)\) and an inverse dependence for group II \((r^2 \geq 0.8)\). The data array was grouped the following way: I – soil with \(\text{pH} < 6.9\) (podzol, soddy-podzolic, meadow brown, and meadow gley degraded soils); II – soil with \(\text{pH} > 7.0\) (grey forest soil, chernozem and chestnut soil). We conditionally determined some of the microorganism species as geographic (zoning) soil formation disturbance indicators: for the acidic soils of taiga, they include *Arthrobacter globiformis*, *Aspergillus* sp.; for neutral and alkaline soils of wooded steppe and steppe, they are *Actinomadura roseola*, *Methylococcus* sp., *Nocardia carnea*.

Irrespective of the research area, the number of species in the main taxonomic microorganism groups was uneven: Gram-negative bacteria amounted to 14.3 % of the total number of species; Gram-positive bacteria – 42.9 %; actinomycetes – 21.4 %; micromycetes – 14.3 %, and other – 7.1 %. In this context, the Menchinnik index was used to assess the species abundance, as it is recommended for the comparison of collections (selections) of various sizes. The results call for a conclusion that a high species abundance was observed under intensive farming conditions (lea and tilled land) due to the abundance of actinomycetes and Gram-positive bacteria irrespective of the zoning conditions (Table 1). From the phytosanitary prospect, the best environmental conditions for plants corresponded with the requirements of soil phytopathogens that quickly adapted and populated both the soils and he cultivated crops causing root rots and other diseases.

### Table 1. Microorganism species diversity in soil with various cultivation levels

| Taxonomic group          | Soil collection | fallow (a) | lea land (b) | tilled land (c) | wildland (d) |
|--------------------------|-----------------|------------|--------------|-----------------|--------------|
| actinomycetes            | I               | 0.48       | 0.87         | 0.68            | 0.59         |
| gram-negative bacteria   | I               | 0.31       | 0.48         | 0.41            | 0.39         |
| gram-positive bacteria   | I               | 0.52       | 0.68         | 0.68            | 0.30         |
| micromycetes             | I               | 0.44       | 0.64         | 0.40            | 0.30         |
| other                    | I               | 0.22       | 0.10         | 0.21            | 0.13         |
|                          | \(X_a \pm \sigma\) | 0.39±0.11 | 0.53±0.29    | 0.48±0.18       | 0.34±0.15   |
|                          | \(K_V\), %      | 28         | 55           | 38              | 44           |
| actinomycetes            | II              | 0.26       | 1.36         | 0.65            | 0.34         |
| gram-negative bacteria   | II              | 0.37       | 0.45         | 0.38            | 0.23         |
| gram-positive bacteria   | II              | 0.70       | 0.36         | 0.97            | 0.40         |
| micromycetes             | II              | 0.31       | 0.31         | 0.51            | 0.31         |
| other                    | II              | 0.13       | 0.10         | 0.21            | 0.23         |
|                          | \(X_a \pm \sigma\) | 0.35±0.19 | 0.49±0.46    | 0.54±0.26       | 0.30±0.07   |
|                          | \(K_V\), %      | 54         | 92           | 47              | 22           |

Note: see conventions in the text.

The main nutritious substrate for soil microorganisms is plant residues that determine the productivity of an agricultural ecosystem. The increase in the biological activity and abundance of microorganisms is observed along the North-South line from \(26.1 \cdot 10^7\) to \(64.3 \cdot 10^7\) cells/g, which corresponds with the distribution of DNI from 1.79 to 2.49 arbitrary units (the maximum was observed in the steppe zone).

The SRI index varied between 0.09 and 1.53 \((K_V < 18\ %)\), and reached its maximum in the taiga forest zone due to the active participation of micromycetes \((X=20.9 \times 10^7\ \text{cells/g})\) in the biological cycle under the limiting temperature and percolative water conditions, as well as the chemical properties of the soil material.

In the steppe zone, the amounts of fungi did not exceed \(2.79 \times 10^7\ \text{cells/g}^\), and their position in the micromycete community was taken over by bacteria and actinomycetes. These conclusions confirm the significance of the Menchinick index. Its value for group II soil fallow fields is 0.31, which is almost two times lower as compared with I group (0.64).
4. Conclusion
Agricultural ecosystems are not sustainable, as they do not have mechanisms of self-regulation and recovery. Therefore, they transform into natural bioceonoses with a high abundance of microbial communities typical of wildlands as compared with tilled lands – \((1.5-22.1) \times 10^7\) cells/g and \((0.2-5.0) \times 10^7\) cells/g respectively – if left without the human’s support for a prolonged period. Structural changes in agricultural ecosystems and natural bioceonoses (wildlands) of European Russia were determined by the participation of taxonomic groups of microorganisms in the soil-forming processes. Dominant species of the microbial consortium were determined, and for the northern soils of taiga, they include *Rhodococcus rhodochrous*; while for the southern soils of the wooded steppe, steppe, and dry steppe, it is the association of anaerobic *Ruminococcus* sp. with vesicular-arbuscular micromycetes. The role of aerobic and anaerobic microorganism species in the formation soil fertility is vital: in the wild and fallow lands are characterized by active anaerobic free-living fixer *Clostridium* sp.; the lea lands – by actinomycetes and actinobacteria of the genera *Actinomyces* sp., *Nocardia asteroids*, *Rhodococcus* sp., *Rhodococcus rhodochrous*; and the intensive farming lands are dominated by *Clostridium pasterianum*, *Nocardia asteroids*, *Nocardia* sp., *Rhodococcus* sp., *Rhodococcus rhodochrous*.

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References
[1] Pashkevich Ye B, Verkhovtseva N V, Osipov G A, Fless N A and Mineyev V G 2002 The changes in the composition of the microbial community of soddy-podzolic loamy sand soil dressed with cover crop and straw as organic fertilizers Reports of the Russian Academy of Agricultural Sciences 525–8
[2] Orlova O V, Andronov E E, Vorobyov N I et al 2015 Composition and functioning of microbial communities in the decomposition of straw cereals in Soddy-podzolicic soil Agricult. Biol. 50(3) 305–14
[3] Dworzanski J P and Snyder A P 2005 Classification and identification of bacteria using mass spectrometry-based proteomics Expert Rev. Proteomics 2(6) 863–78
[4] Popov D A, Ovseyenko S T, Osipov G A and Vostrikova T Y 2013 The express mode of identification of agents of bacteriemias using the technique of gas chromatography-mass spectrometry Klin. Lab. Diagn. 5 54–8
[5] Lipiec J and Usowicz B 2018 Spatial relationships among cereal yields and selected soil physical and chemical properties The Sci. of the total environment 633 1579–90 DOI:10.1016/j.scitotenv.2018.03.277 Corpus ID: 46892433
[6] Brenner D J, Krieg N R, Staley J T and Carrity G M 2005 Bergey’s Manual of Systematic Bacteriology 2rd ed, vol 2, parts A B and C (New York: Springer-Verlag) 1136 p