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Microscopic Counting of the Total Number of Bacteria and Metabolically Active Bacteria in Soil Samples: Their Relationship and Oscillation Dynamics of Number

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Abstract—Experimental results of daily counting under a microscope for 30 days of prokaryotic cells in preparations from soil samples when stained with different specific dyes and published results on daily counting of bacteria in soil using different methods are presented. The FITC dye, which stains the entire set of bacterial cells, revealed a wavelike dynamics of cell numbers with different numbers of oscillations in the form of peaks in all experiments. Using the SFDA dye, which detects only living, metabolically active cells, wavelike dynamics were also revealed, but their oscillating number was significantly less. The reliability of oscillations and differences in cell numbers when using different dyes were confirmed statistically by harmonic analysis. The wavelike dynamics of living, metabolically active cells is a consequence of the cycles of growth and death of bacterial cells and short-term trophic succession in the microbial community. External disturbing influences did not affect the manifestation of wavelike population dynamics, either in the population of living cells or in the total number of cells. The phenomenon of wavelike dynamics of nonliving bacterial cells and their numerical superiority is explained by the fact that cells, losing viability, lyse and disintegrate not immediately after dying, but with some delay in time. This leads to the accumulation and permanent superiority of the pool of dead cells when microscopically counting the total number of bacteria in the soil and explains the discrepancy in bacterial numbers between different counting methods. The presented experimental and published material will serve as a substantiation for microbiologists and biotechnologists of the need to control the dynamics of the numbers of introduced populations and communities of microorganisms into the natural environment, as well as a source of knowledge for the successful management of natural microbial communities.

Keywords: bacteria, numbers, soil, fluorescent dyes, living cells, dead cells, dynamics, oscillations, fluctuations

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INTRODUCTION

Since the time of S.N. Winogradsky, who proposed counting bacterial cells in soil samples under a microscope on preparations stained with erythrosine, a strong opinion was formed that nature, especially soil, contains a huge amount, up to 2×10^9 and more, bacterial cells (Vinogradsky, 1952). These discoveries of S.N. Winogradsky and his proposals to use selective media and conditions were aimed at the most complete isolation of microorganisms from natural sources and the identification of microorganisms with previously unknown metabolism and microorganisms with specific properties and functions, which stimulated further search for media and cultivation conditions. However, a significant numerical excess of bacterial cells found in soil samples when counted under bright-field and then fluorescent and electron microscopes over the number when counted by plating remained for many reasons, unexplained or not convincing (Somova et al., 2020; Trolldenier, 1972; Stevens, 1995; van Bruggen and Semenov, 2001).

The method of identifying and recording viable forms of microorganisms by plating on appropriate media is considered the most reliable among microbiologists, but there are references to the publication of Staley, one of the editors of the most complete identification of bacteria by Bergey, which states that no more than 1% of the number of bacterial cells grows on traditionally used media (Staley and Kanopka, 1985; Holt et al., 1994).

An attempt to solve the problem of the inconsistency of the available results on the number and biodiversity of bacteria in natural substrates and uncertainty about their state (all living or part) and the desire to

find correlations of the number and activity of microorganisms in natural environments stimulated specialists to use different approaches. A search was carried out for a universal method, a marker that would allow the detection and identification of all microdiversity (Blagodatskaya and Kuzyakov, 2013). The genetic material of microorganisms was chosen as such a marker, and molecular biological methods (MBM) were proposed for determining the biodiversity, taxonomy, and even activity of microorganisms. At the same time, advances in understanding the biochemistry of the bacterial cell and advances in the chemical synthesis of substances created the possibility of such an influence on the bacterial cell, which made it possible to determine the activity of bacterial cells without isolating them from their habitat—soil, water, etc. Methods have been developed for differential staining of bacterial cells to determine the viability of the entire set of prokaryotes with minimal impact on them. Such methods, unlike MBM, do not require the destruction of microbial cells and are based on the use of their entire organismal structure (Yurshenas and Kashirskaya, 2022; Haugland, 1996).

There are a few very important remarks to make here. First, as taught by the outstanding theorist and successful "microbe hunter" Academician G.A. Zavarzin—only in that case the process is significant and noticeable if there are many microorganisms carrying it out and they are active (Semenov and Đukić, 2019, 2020). Second, as noted earlier, for identifying and recording viable forms of microorganisms, the most reliable method is plating on appropriate media. Third, all technological processes known at the end of the first quarter of the 21st century in which microorganisms are used are based on their cultivated forms. Fourth, all known dangerous (for humans and animals) pathogenic microorganisms are cultivated and maintained in collections. Fifth, in natural substrates, samples of which are used to identify microorganisms and determine their abundance and diversity, populations are in the late stationary growth phase (Semenov et al., 2022). In this phase, cells of most populations exist in a state of starvation and survival (El-Registan et al., 2022). The presence and, especially, the detection of a population of bacterial cells in the exponential stage in the natural environment is a very short-term and rare phenomenon, and therefore difficult to record (Zvyagintsev, 1987). When a substrate appears, cells can return from the "starvation stage" to an active state or not return and, moving into the "dead kingdom," replenish the organic matter of the soil and soil ecosystem (Semenov and Kogut, 2015). Thus, the multifaceted scientific and practical problem of determining the number of bacteria in natural environments and the ratio of active, living, and dead cells found in natural substrates by microscopic account in comparison with the number of bacterial cells growing in laboratory media, as well as the discrepancy between their functional and taxonomic biodiversity data obtained by predictive methods, that is, molecular biological, continues to need a solution.

The task of identifying and counting microbial cells with the difference between living cells and dead ones in the natural environment is especially in situ not idle (Blagodatskaya and Kuzyakov, 2013). The identification of living, metabolically active cells (MACs) is important for the successful application of biologics in ecosystems for a variety of socially beneficial purposes. It is important to know how many viable MACs have been introduced into the ecosystem and how many living and metabolically active cells of the native microbiota are already contained in the soil that will compete with the introduced population (Limar et al., 1984). Knowledge about the amount of MACs is also important for determining the risks of the spread of pathogenic microorganisms that form part of the microbial community (MC) in the ecosystem cycle of microorganisms (Kupriyanov et al., 2010).

However, determining the number of bacterial cells in the soil ecosystem is complicated by the fact that their quantitative dynamics significantly oscillate not only in space (from sample to sample), but most importantly in each sample in time (Semenov et al., 1999, 2013, 2022; Zelenev et al., 2005b; van Bruggen et al., 2017). A one-time (single) determination of the number of bacterial cells in a soil sample leads to colossal (millions or more) errors in population estimates and, as a consequence, to the adoption of erroneous decisions. Consequently, it is necessary to have an idea of the dynamic changes in the number of not so much the total number of cells, but rather the number of living cells in the soil ecosystem.

For this purpose, differentiating dyes have been developed that do not stain all cells, as was the case with S.N. Winogradsky, but only metabolically active ones, considered alive and detectable by fluorescence microscopy (Haugland, 1996). A fairly modern and widespread dye that allows one to identify and take into account the total number of cells is the FITC (fluorescein-5-isothiocyanate) dye. The method of counting bacteria using FITC, described in 1970, is still used almost unchanged (Babiuk and Paul, 1970). Further improvement and development of fluorescent dyes and deepening of knowledge about the structure of the bacterial cell and its membranes and energy have led to the fact that modern dyes make it possible to differentiate and stain either all cells, as is achieved with FITC, or only metabolically active, living ones with fluorescent dye FDA (fluorescein diacetate) and SFDA (5-(and 6-)sulfofluorescein diacetate) (Lundgren, 1981; Tsuji et al., 1995).

The next step in improving methods for staining microorganisms, which makes it possible to differentiate living and dead cells in one preparation, is the complex vital dye "Live/Dead kit" (Life Technologies, USA) (Haugland, 1996). This method, on one hand, helps speed up the counting procedure, but on

the other hand, owing to the rather rapid discoloration of the dyes in the preparation, and/or if the "operator" has problems distinguishing colors, as the authors' experience has shown, when using this method, tangible problems arise (Semenov and Shatalov, 2003; Ushakova et al., 2012).

The purpose of this work is to show, using our own results and published data, that the wavelike dynamics of changes in the number of bacteria in the soil is not a consequence of external influences, but an object phenomenon that occurs in accordance with the third law of ecology of the existence of microbial populations; to an explanation for the fact of wavelike changes in the soil of not only the number living bacteria but also dead ones, numerically significantly exceeding living ones; and to demonstrate that the number of cells detected during a total microscopic count is significantly higher than the number counted in the form of CFU—a consequence of the fact that the majority of bacterial cells during a total microscopic count are simply not viable.

MATERIALS AND METHODS

Setting up and Conducting Experiments

The soil was collected in the Botanical Garden of Moscow State University near a sea buckthorn plant (*Hippophae rhamnoides* L.). A sample of the humus-accumulative soil horizon was taken from a depth of 0–10 cm. Roots, pebbles, and soil animals were removed and sifted through a sieve with a pore size of 2 mm. The soil for the first experiment was collected in June. Its moisture content immediately after sampling was about 20%. For the second and third experiments, the soil was collected in September of the same year at the same location. The moisture content of the sampled soil was 22%. The first and second experiments with exposure to soil and subsequent recording of bacteria in the samples were carried out immediately after soil sampling. The third experiment was carried out in January of the following year, that is, after several months of soil storage. The soil was stored in a plastic bag at 4°C. After storage, the soil moisture was 21%. Immediately before the count, the soil was divided into two parts of 0.5 kg each and the initial moisture content was determined. To determine the moisture content, soil samples were dried at 105°C for 5 h. The moisture content was calculated using the formula $m = (a - b)/(a - c)$, where a is the weight of wet soil and container, b is the weight of dried soil and container, and c is the weight of container. Next, one part of the soil was used as a control, and the second part was dried. In the first experiment, the soil was dried at 45°C for a day. In the second and third experiments, the soil was dried for the same time, but at 70°C. The purpose of drying is twofold—the destruction of possible protozoa and nematodes in order to exclude their possible impact on the number of soil microorganisms by consuming the latter and testing the assumption that external disturbing influences are not the cause of the oscillating dynamics of the number and functional activity of microorganisms in natural substrates, but the drying that occurs when warming up and subsequent moistening, and there is a natural effect. For drying, the soil was spread in a thin layer of ~0.5–1.0 cm in a drying oven. After drying the soil, its moisture content was determined and the moisture content was restored to its original state with distilled water sterilized by filtration. After moistening, the soil of both variants was quickly packaged into 90 tubes for each variant, 5 g/tube, and each tube was covered with wrap to prevent drying. The tubes were incubated at 25°C during the time of the experiment.

Preparation, Staining of Preparations, and Counting of Cells

The dyes used were FITC (fluorescein-5-isothiocyanate) (Babiuk and Paul, 1970) and SFDA (5-(and 6-)sulfofluorescein diacetate) (Tsuji et al., 1995). The operating principle of the dyes used is as follows. FITC dye reacts with cell proteins, both surface and intracellular. The SFDA dye penetrates bacterial cells by passive diffusion. Nonfluorescent SFDA, under the action of intracellular esterases, is converted into a fluorescent product—FSA (fluorescein-5-(6-)sulfonic acid), which accumulates in the cytoplasm. There is no accumulation of dye in dead cells, even if esterases are active and continue to work, since membrane degradation occurs in dead cells and the fluorescent dye is easily released (washed out) from the cell. When using SFDA, more than 98% of living cells are stained, which makes it possible to distinguish between dead and living microorganisms with great accuracy. Moreover, the degree of fluorescence of SFDA is 3 times higher than that of FDA (Tsuji et al., 1995).

Every day, three test tubes were selected from the control and experimental variants using a random sampling method. The moisture content in the soil from each test tube was determined. Preparations were prepared for fluorescence microscopy. For this purpose, soil suspensions were subjected to ultrasonic treatment according to Zvyagintsev (1987). The soil suspension was prepared using tap water and sterilized by filtration, using a dilution of up to 10^{-4} for total cell counting when stained with fluorescent dye FITC and up to 10^{-3} for counting living cells using the fluorescent dye SFDA. The total number of bacterial cells was taken into account after staining the soil suspension with FITC (Babiuk and Paul, 1970) and the number of living MACs was taken into account after staining with SFDA (Tsuji et al., 1995).

Bacteria were counted using a fluorescence microscope Mikmed 2 Lumam RPO –11 (JSC LOMO, St. Petersburg, Russia) with an excitation light wavelength of 480 nm, ×100 objective, ×10 eyepiece. The

total number of bacterial cells in soil samples was counted in 20 fields of view. The number of cells (M) in the soil was calculated using the formula

$$M = (((S/s \times N/n) \times D)/V)/(1 - m/100),$$

where M is the number of cells per 1 g of dry soil; S is the filter area, μm^2; s is the microscope field of view area, μm^2 (S/s is the entire number of fields of view for a given filter); N is the number of cells on a given filter; n is the number of fields of view (N/n is the average number of cells in one field of view); D is the dilution used for cell counting; V is the volume of the filtered sample, mL (if a volume not equal to 1 mL was used); and m is the soil moisture in percent.

Determination of Water-Soluble Organic Matter in Soil

In the third experiment in the soil, in addition to recording the total number and number of living cells, daily determination of dissolved organic matter (DOM) was carried out. The DOM concentration was determined using the bichromate method (Semenov et al., 2013).

Statistical Processing of Results

During the third experiment, bacteria were counted on 1080 filters and 21 600 fields of view were calculated. Statistical processing of the results was carried out in the same way as was done in the publication with the participation of one of the authors (Zelenev et al., 2005b). The graphs show the average value with standard deviation calculated in Excel.

To confirm the reliability of oscillations in the obtained experimental data, statistical harmonic Fourier analysis was used (Smirnov, 1974; Gorbenko and Kryshev, 1985). Such an analysis is used to identify periodicity in dynamic processes, and in particular, it allows one to identify periodicity in the dynamics of the growth of microbial populations. The essence of such an analysis is described in more detail in the previous publication of the authors (Semenov et al., 2022).

RESULTS AND DISCUSSION

Several consecutive experiments were carried out with daily recording of the number of bacterial cells in preparations prepared from soil samples taken from the same locus, but in different climatic seasons. This was done deliberately to exclude possible explanations of the results obtained as a consequence of the influence of such natural factors as temperature, humidity, and solar radiation (Semenov, 2005; Semenov et al., 2013; van Bruggen, et al., 2006).

Accounting for the Total Number of Bacteria in Soil Samples under a Fluorescence Microscope, Stained with FITC Dye

It was found that the number of bacteria during daily counting is characterized by significant variation. Moreover, both the increase in numbers and the decrease in the overwhelming majority of observations lasted a day or more, thereby indicating a regularity of the phenomenon, and not randomness (Fig. 1). D.G. Zvyagintsev and G.N. Zaitseva (1979) in their observations proposed to consider as the most significant peaks formed by at least three values (points) on the ascending or descending sides. This approach appears to have been proposed owing to the inability to perform appropriate statistical evaluations that are appropriate for processing long data series. However, with the approach proposed by these authors, part of the experimental data may not be taken into account and may even be forced to be ignored; namely, peaks formed by rapidly growing populations and consisting of only three points will not be taken into account. In this case, only peaks formed by four or more experimental points that are slowly growing in the population will be taken into account on the graphs. As a result of this approach, the biological essence of the processes occurring in the microcosm of the soil ecosystem is formalized. The authors of this publication solved this problem using harmonic analysis, the results of which are shown in Figs. 4–7.

In the first experiment, which was carried out in June–July with freshly selected soil, and in the control (without heating the soil) on the first day of the experiment, a drop in the total number of cells was observed from 5.60×10^9 to 2.06×10^9 on the third day, and from the third to the fifth day, there was an increase in the number of cells to 4.59×10^9 (Fig. 1a). Over the entire observation period, peaks of maxima were identified in the control soil on days 5, 9, 15, 20–21, 24, and possibly after 29 days, and minima were observed on days 3, 7, 12, 16, 22, and 27. In the soil of the test sample, dried at 45°C, from the first day of the experiment to the third day, just as in the control, the number of cells decreased to the level of 2.07×10^9, and the first peak of the maximum was recorded on the fifth day and amounted to 4.18×10^9 cells per gram of absolutely dry (GADS) soil. Subsequently, in the dynamics of the experimental sample, maxima in the number of bacteria were found on days 5, 8, 11, 14, 20, 24, and 28, and minima corresponded to days 3–4, 6, 9, 13, 18, 22, and 27. The minimum number of cells during the population count observations in the control soil sample was 2.06×10^9, the maximum was 7.71×10^9, and in the experimental sample, it was 1.29×10^9 and 7.06×10^9, respectively.

In the second experiment, cell counting was carried out in September–October, that is, immediately after sampling. When counting bacteria daily, fluctuations in the total number of cells were also found, both in

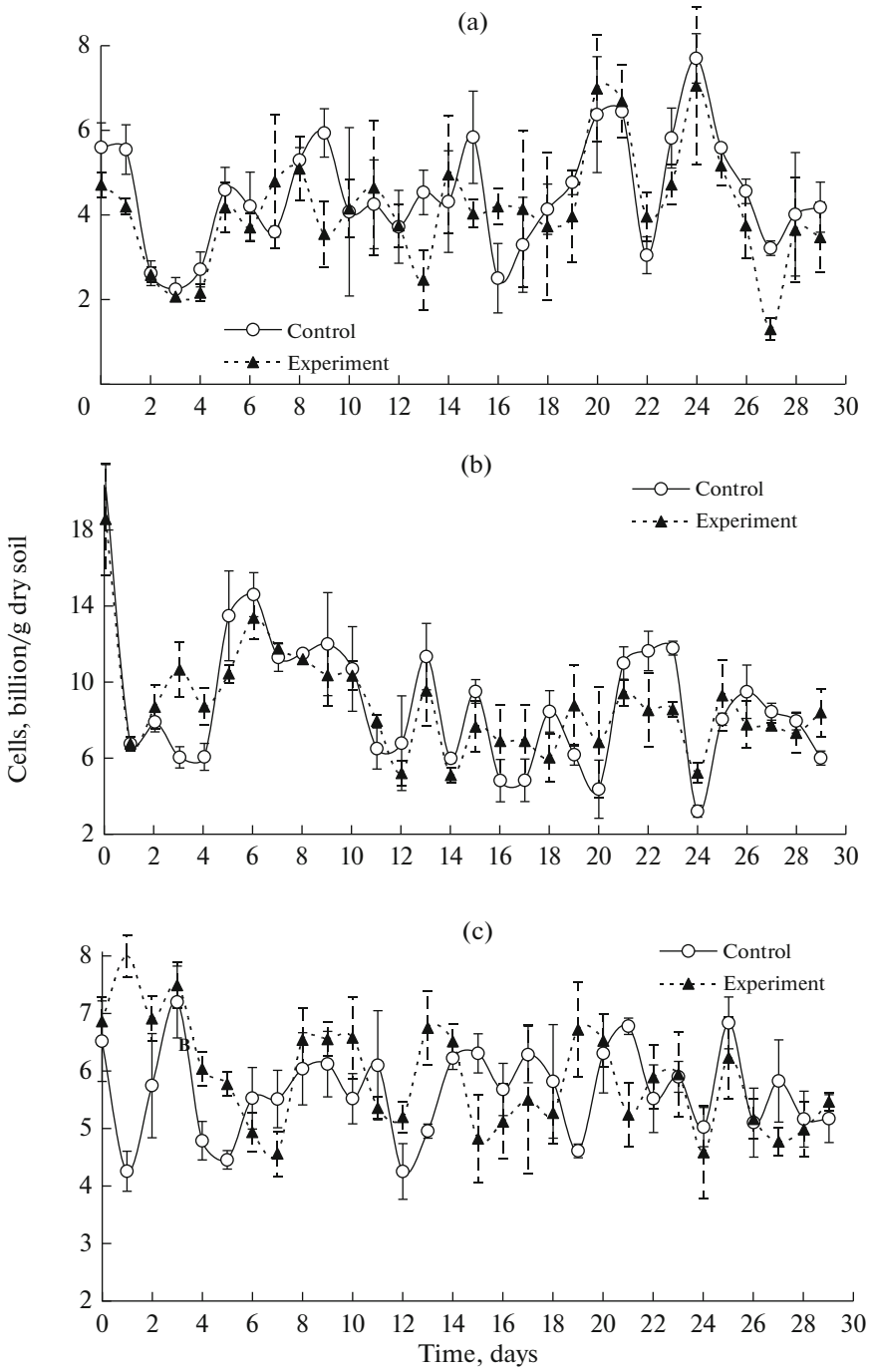

Fig. 1. Accounting for the total number of bacterial cells in soil using FITC dye in experimental and control variants in three experiments ((a, b) and (c), respectively). The values indicated by dots in Figs. 1–3 are represented as arithmetic averages; the values of deviations on the graphs are presented as results calculated in the standard Excel program.

the experiment and in the control. From the first day of the experiment, in the control sample, there was a decrease in the number of cells and then an increase (Fig. 1b). Maximum cell numbers in the control fall on days 6, 9, 13, 15, 18, 23, and 26, and the days of minima in the control correspond to days 3–4, 7, 11, 14, 16, 20, 24, and 29. In the experimental sample, the maximum number of cells occurs on days 3, 6, 10, 13, 15, 19, 21, 25, and 29 of the experiment and the minimum values in the soil of the experiment correspond to days 1, 4, 9, 12, 14, 18, 20, 24, and 28. The minimum number of cells during observations in the con-

trol soil sample was 3.14×10^9, and the maximum was 1.45×10^{10}, and in the experimental sample, it was 5.04×10^9 and 1.33×10^{10}, respectively.

In the third experiment, in the control soil sample, the number of bacteria decreased from the first day of the experiment to 4.26×10^9 and then began to increase to 7.20×10^9 on the third day of the experiment (Fig. 1c). During the experiment, peaks of maximum cell numbers were noted on days 4, 7, 9, 11, 15, 17, 21, 25, and 27, and minimum values were on days 1, 5, 10, 12, 16, 19, 24, 26, and 28 days. In the experimental soil sample, from the beginning of the experiment, there was an increase in the number of microorganisms from 6.87×10^9 to 8.00×10^9 on the first day of the experiment. In the experimental version, the peaks of maxima corresponded to days 1, 3, 8–10, 13, 17, 19, 23, 25, and 29, and the peaks of minima corresponded to days 2, 7, 12, 15, 18, 21, 24, and 27 of the experiment. The minimum number of cells in the third experiment during the observation period in the control soil sample was 4.27×10^9, and the maximum was 7.20×10^9. The minimum number of cells during the observation period in the soil of the experiment was 4.57×10^9, and the maximum was 8.00×10^9.

So, a visual analysis of the daily dynamics of the total number of cells in soil samples shows that there is a pronounced wavelike dynamic in all three experiments, regardless of the time of soil sampling, the time of storage of samples, and the time of counting bacteria. All this confirms the objectivity of the phenomenon of wavelike oscillations in the number of bacteria in the soil. During a comparative visual analysis, the following patterns can be noted in the presented results: an increase in the number of peaks from the first experiment to the third. At the peaks of the maxima, a noticeable increase in the number of cells can be noted only in the second (autumn) experiment. This coincides with the known fact of seasonal variability in the number of microorganisms in natural substrates (Samtsevich, 1955; Chernov and Zhelezova, 2020). The number of cells, both in control samples and in experimental ones, in all experiments differs little at the points of minimums. Regardless of the factors noted above, the timing of sampling, the storage time of samples, and the experimental disturbances carried out in the form of heating the soil do not lead to catastrophic changes in the total number of bacterial cells in soil samples, as shown by a total count of cells.

Accounting for the Number of Living, MAC of Bacteria in Preparations of Soil Samples Stained with SFDA Dye

The dynamics of the number of living, MAC of bacteria during daily counting, just like the dynamics of total counting, is characterized by a significant variation in number. In the monthly dynamics of counting numbers, the identified increases or decreases in cells lasted several days, thereby indicating a pattern of processes of wavelike oscillations (Fig. 2). The approach to taking into account significant peaks in the dynamics of the number of living cells was used the same as when counting the total number of cells.

In the first experiment, in the control sample in the population dynamics, a decrease in the number of living cells was observed from the first to the fourth day from 5.71×10^8 to 1.06×10^8 (Fig. 2a). Subsequently, minima in the number of cells were found on days 7, 11, 14, 16, 20, 22, 25, and 28. Maxima were detected on days 5, 10, 13, 15, 18, 21, 23, and 26. In the experimental sample, a drop in the number of cells was also recorded in the first days of the experiment from 4.23×10^8 to 5.45×10^7 on the fourth day. Subsequently, minimum cell numbers were discovered corresponding to the following days: 8–10, 14, 16, 20, 25, 27–29. Peaks of maxima occurred on days 5, 13, 15, 17, 22, and 26. The minimum number of living cells during observations in the control sample was 1.06×10^8; the maximum was 6.36×10^8. In the experimental sample, these indicators were 5.45×10^7 and 4.97×10^8, respectively.

In the second experiment, in the control sample on the first day, there was a slight drop in the number of cells from 7.28×10^8 to 6.72×10^8 and then an increase in number to 1.43×10^9 on the fourth day (Fig. 2b). The then observed peaks of maxima corresponded to days 11, 15, 21–22, and 25, and the peaks of minima corresponded to days 8, 12, 19, 23–24, and 27. In the test sample, just as in the control sample, there was a decrease in the number of cells from the beginning of the experiment from 1.19×10^9 to 6.66×10^8 on the second day. Then the number of cells increased to 1.43×10^9 on the fourth day of the experiment. Subsequently, peaks of minima are observed on days 5, 8, 13, 20–21, 24, and 28, and peaks of maxima are observed on days 4, 6, 12, 15, 23, 25, and 29. The minimum number of living cells during the observation period in the control soil sample was 1.09×10^8; the maximum quantity in the control was 1.43×10^9. In the experimental sample, these figures values were 1.15×10^8 and 1.43×10^9, respectively.

In the third experiment, in the control variant, growth of living cells was observed on the first day of the experiment from 3.05×10^8 to 4.35×10^8 cells, and then there was a slow decline, and on the third day of the experiment, the number was 3.65×10^8 (Fig. 2c). Subsequently, fluctuations in the number of living cells were observed with maxima on days 4, 7, 11, 13– 14, 18, 24, 26, and 28 and minima on days 6, 8, 12, 15, 20, 22, 25, and 27 of the experiment. While in the control the number of living cells increased on the first day, in the soil of the experimental sample, the number of living cells fell from 3.05×10^8 to 1.08×10^8. Subsequently, the number of cells had maxima on days 6, 10, 13, 15, 19, 21, 24–25, 28, and 29 and minima on days

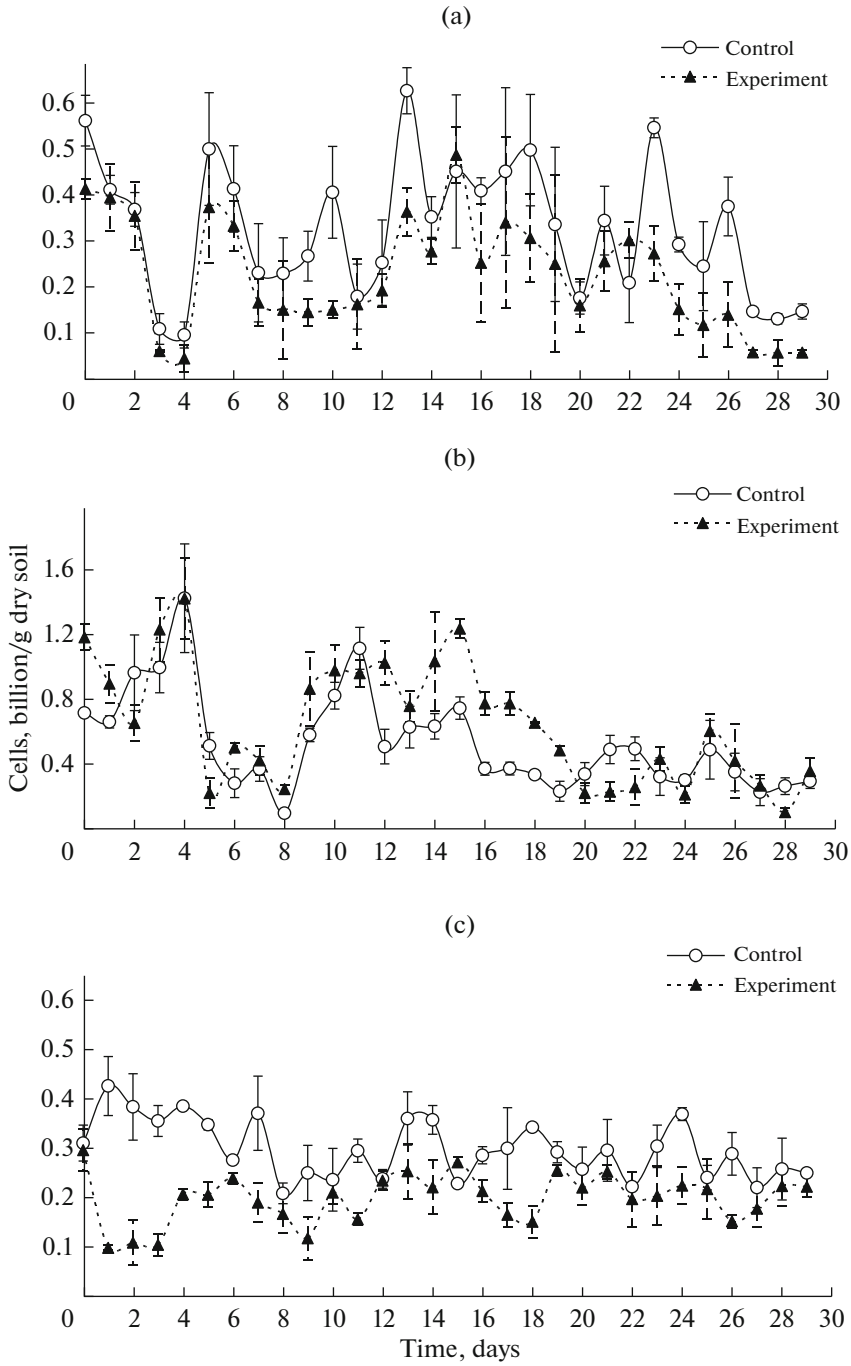

Fig. 2. Accounting for the number of physiologically active bacterial cells in the soil using the SFDA dye in the experimental and control variants in three experiments ((a, b) and (c), respectively).

1–3, 9, 11, 14, 18, 20, 22, and 26. The minimum number of living cells during the observation period in the control soil sample was 2.19×10^8; the maximum quantity in the control was 4.35×10^8. In the experimental sample, these values were 1.08×10^8 and 3.06×10^8, respectively.

Visual analysis of the daily dynamics of the number of MACs shows that, just as when taking into account the total number of cells, there is a pronounced wave-like dynamic in all three experiments, regardless of the disturbing influence. Drying the soil samples did not affect the waveform dynamics and the number of living bacteria in the soil. A comparative analysis of the values amplitude of fluctuations in the number of living cells in these experiments, as well as in the experiments of counting all cells, shows that the deviations of

the amplitude values from the average are reduced, that is, the fluctuations are "evened out."

On the Ratio of the Number of Living and Dead Cells in Soil Samples

A comparison of the dynamics of cell numbers during total counting (Fig. 1) and MAC (Fig. 2) clearly shows that the total number of bacteria in the soil is significantly, at least an order of magnitude, greater than the number of living bacteria. Subtracting the number of living cells from the total number of cells gives the number of dead cells, which also greatly exceeds the number of living bacteria. The dynamics of the number of living cells, the number of which is an order of magnitude less than the total number, also has a wavelike appearance, which does not repeat the dynamics of the total number of cells, but is not in antiphase with it. In this case, fluctuations and peaks of maxima and minima of dead cells repeat the dynamics of fluctuations in the total number of cells (figure not shown). The significant quantitative superiority of dead cells over MAC can be explained by the assumption that dead cells do not immediately destruct, losing the integrity of their cell walls and the electrochemical function of their membranes, and therefore can be stained with FITC and other general dyes for some time. As is well known, the FITC dye reacts with cell proteins, and taking into account the physicochemical conditions in the soil, it is quite objectively possible to believe that a cell, even if it has lost its electrochemical membrane potential or dies, will remain intact for some time (Babiuk and Paul, 1970).

Thus, in microbiological practice, researchers, when counting bacteria in the soil, using traditional fluorescent dyes common in microscopy, identify a huge number of bacteria, but most of them are dead cells. Taking into account the remark of G.A. Zavarzin that, only that the process is significant and noticeable, if that the process carry it out many microorganisms and they are active, it turns out that biosphere processes are carried out in only 10% of the total number found in the general count of bacteria, which is emphasized by other researchers with other methods (Blagodatskaya and Kuzyakov, 2013).

When counting bacteria by inoculating suspensions on various agar media, researchers obtain their number in the form of CFU, which, as a rule, is an order of magnitude or even lower than the total number determined by microscopic counting. The obvious conclusion is that the results of estimating the number of bacteria in the soil are influenced by the accounting method used. Staining preparations with general dyes gives overestimated results, and method of plating on the media gives significantly underestimated results. At the same time, by conducting a single, one-time count, and not in dynamics, researchers can "get" either to the peak of maximum or minimum numbers, which significantly increases the error. Only the use of specific dyes, the action of which occurs through the functional activity of cells, can gives the most objective idea of the number of living ones that are significant for assessment of biosphere processes, which is emphasized by other researchers (Blagodatskaya and Kuzyakov, 2013).

Determination of the Concentration of Dissolved Organic Matter and Soil Moisture during Daily Recording of the Number of Cells in the Sample of the Third Experiment

The main mechanism for the wavelike dynamics of cell numbers is periodic cycles of the death of some cells and the resumption of growth of living cells, in particular, due to the dissolved organic matter (DOM) of dead cells. It was decided to study the daily dynamics of DOM concentration in the soil of the analyzed sample. The authors recognized that the analytical method used to determine DOM concentrations and the expected values of DOM concentrations in the analyzed samples could create significant difficulties in interpreting the results if the results of the daily dynamics of DOM concentrations contradict the wavelike dynamics of cell numbers. Determination of DOM concentration over time was carried out only in samples of the third experiment (Fig. 3). Both in the control soil sample and in the soil after drying (experiment), impressive fluctuations in the concentration of DOM were found. By analogy with the analysis of the results of counting the number of cells in soil samples, we note the values of the minimum and maximum concentrations of DOM in the control and in the experiment of the analyzed sample. The minimum concentration of DOM in the control sample was found on the fifth day and was equal to 315.73 μg/g.d.s., the maximum concentration was found on the 14th day and was equal to 1899.11 μg/g.d.s. In the test sample, the minimum concentration of DOM was found on day 23 and was equal to 356.71 μg/g.d.s.; the maximum concentration of DOM was found on day 19 and was equal to 1869.12 μg/g.d.s. To confirm the role of the bacterial mortmass as a substrate for the growth of living bacteria, the concentrations of organic carbon in the mass of dead cells were calculated for the control and test samples at the points of minimum and maximum DOM. Calculations showed that, on the fifth day of the dynamics, the carbon concentration was 411 μg/g.d.s., and on the 14th day (maximum), it was 585 μg/g.d.s. In the experimental sample the similar indicators are equal to 436 μg/g.d.s. (minimum biomass) and 789 μg/g.d.s. (maximum biomass). Thus, experimental and calculated data not only do not contradict, but show the presence of significant fluctuations in carbon concentrations that determine the wavelike dynamics of microbial populations.

In all analyzed samples, soil moisture was determined daily before counting the number of cells, since a significant, uncontrolled change in soil moisture can

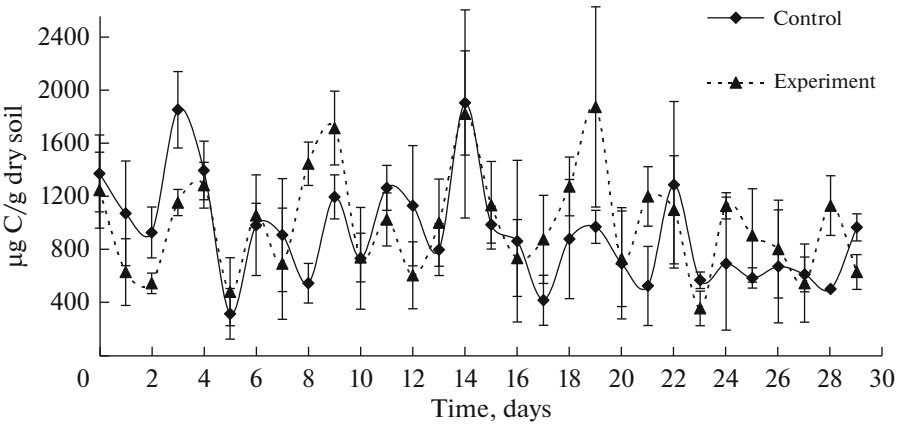

Fig. 3. Dynamics of DOM in the soil in the control and experiment in the third experiment.

create serious difficulties with the interpretation of the results of cell numbers (figures not shown). The importance of soil in the samples used to count bacteria ranged from 17.5 to 20.5%. With these data, a correlation comparison was made of the dynamics of the number of living cells. The correlation coefficients varied from 0.04 to 0.22 in the control and from 0.12 to 0.16 in the experiment, with a significance level in all experiments of 0.95. Thus, a change in the moisture content within the specified limits cannot be considered as one of the factors of disturbing influences causing oscillatory dynamics of the number of bacteria.

Harmonic Analysis of Experimental Data on the Number of Bacteria in Preparations Stained with FITC

For statistical processing of the results, harmonic analysis was used, which confirmed the reliability of fluctuations in the number of microorganisms in soil samples. Important characteristics of harmonic analysis are such parameters as the number of harmonics, the amplitude, phase, and period of oscillations, and the F-criterion (Smirnov, 1974; Gorbenko and Kryshev, 1985). Harmonic analysis of the results of the overall count of cells in the control of the first experiment revealed three harmonics with orders 1, 2, and 6 and with similar amplitudes, but significantly different periods and phases of oscillation (Fig. 4a; Table 1). Since harmonic analysis "rejects" (hides) all those harmonics that do not correspond to a given level of significance criterion, the number of harmonics on the graph after harmonic analysis may not coincide with the number of peaks on the curves of dynamics before harmonic analysis. Harmonic analysis of results of the number of cells in the experimental sample of the first experiment revealed only two harmonics of the first and second orders (Fig. 5a). In this case, the first peak of the harmonic is formed by two points corresponding to 7–8 days, and the second peak is formed by three points and is revealed by harmonic analysis on days 20–24 (Table 1). Thus, harmonic analysis revealed that the disturbing effect in the form of drying led to a decrease in amplitudes and to a slight extension of the period of oscillations in cell numbers. From the point of view of microbial ecology, this indicates succession in the MC soil with a "change" of dominant populations, although visually, without harmonic analysis, the differences in the dynamics of cell numbers in the control and experimental samples are not impressive (Fig. 1).

In the results of the control soil sample of the second experiment, harmonic analysis revealed three significant harmonics of the first, fourth, and seventh orders (Fig. 4b; Table 1). Note that harmonic analysis assumes the "absorption" of harmonics of lower orders by harmonics of higher orders, and therefore harmonics of lower orders are not reflected in the figures. This "absorption" is also reflected in the periods of oscillations, where the period of the first harmonic is 30 days, the period of the fourth harmonic is 7.5 days, and the period of the seventh harmonic is 4.29 days, although such indicators as coefficients of variation and the Fisher coefficient (F_{expert}) differ only for the seventh harmonic. Harmonic analysis of the results of the experimental soil sample of the second experiment showed only one first-order harmonic with a period of 30 days and a significant coefficient of variation (Fig. 5b; Table 1). Note that, in the dynamics of cell numbers, when analyzing the second experiment, both with general accounting and with taking into account the MAC, first-order harmonics were revealed.

Harmonic analysis of the results of the number of cells of the control sample of the third experiment (Fig. 4c; Table 1) discovered two harmonics of the fourth and fifth orders with periods of 7.5 and 6.0 days. At the same time, visual analysis shows that the amplitude of the oscillations noticeably decreases from the first to the thirtieth day of observation. Harmonic analysis of the results of the experimental soil sample of the third experiment reveals only one third-order harmonic with a period of 10 days (Fig. 5c; Table 1).

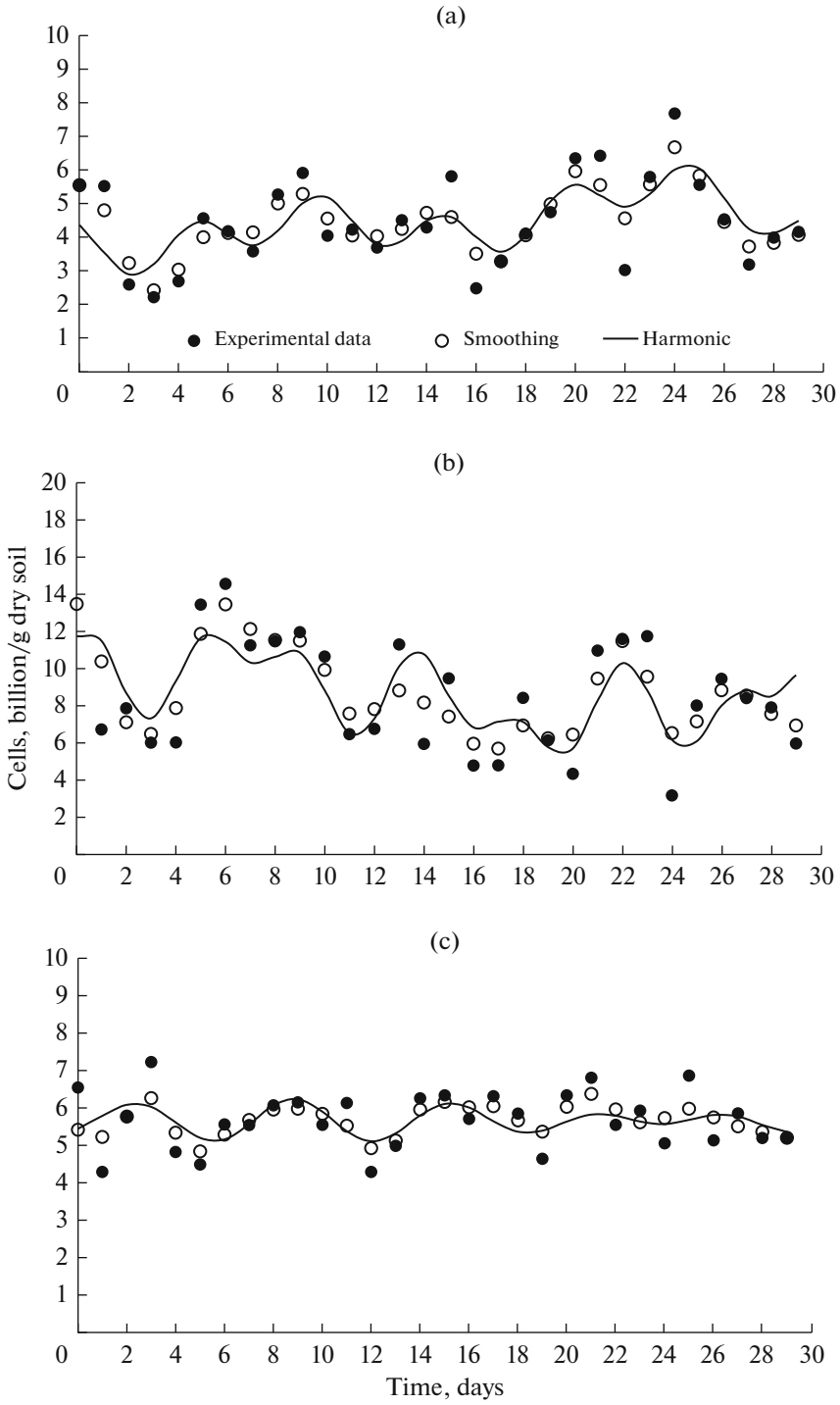

Fig. 4. Harmonic analysis of the results of accounting for the total number of bacterial cells in the soil in the control variant in three experiments ((a, b) and (c), respectively).

Thus, the effect of drying the soil MC by drying the samples leads to a slight decrease in the number of oscillations (harmonics), a decrease in the amplitudes of the oscillations and an extension of the periods of oscillations, but at the same time, this does not lead to a significant decrease in the number of bacterial cells in these samples. From the point of view of the manifestation of the functional activity of MC, expressed in the growth rate and number of bacteria in the soil, the revealed facts obtained as a result of harmonic analysis indicate a change in the dominant populations in the bacterial community, a change in the availability of nutritional resources, and possibly other biotic and abiotic factors.

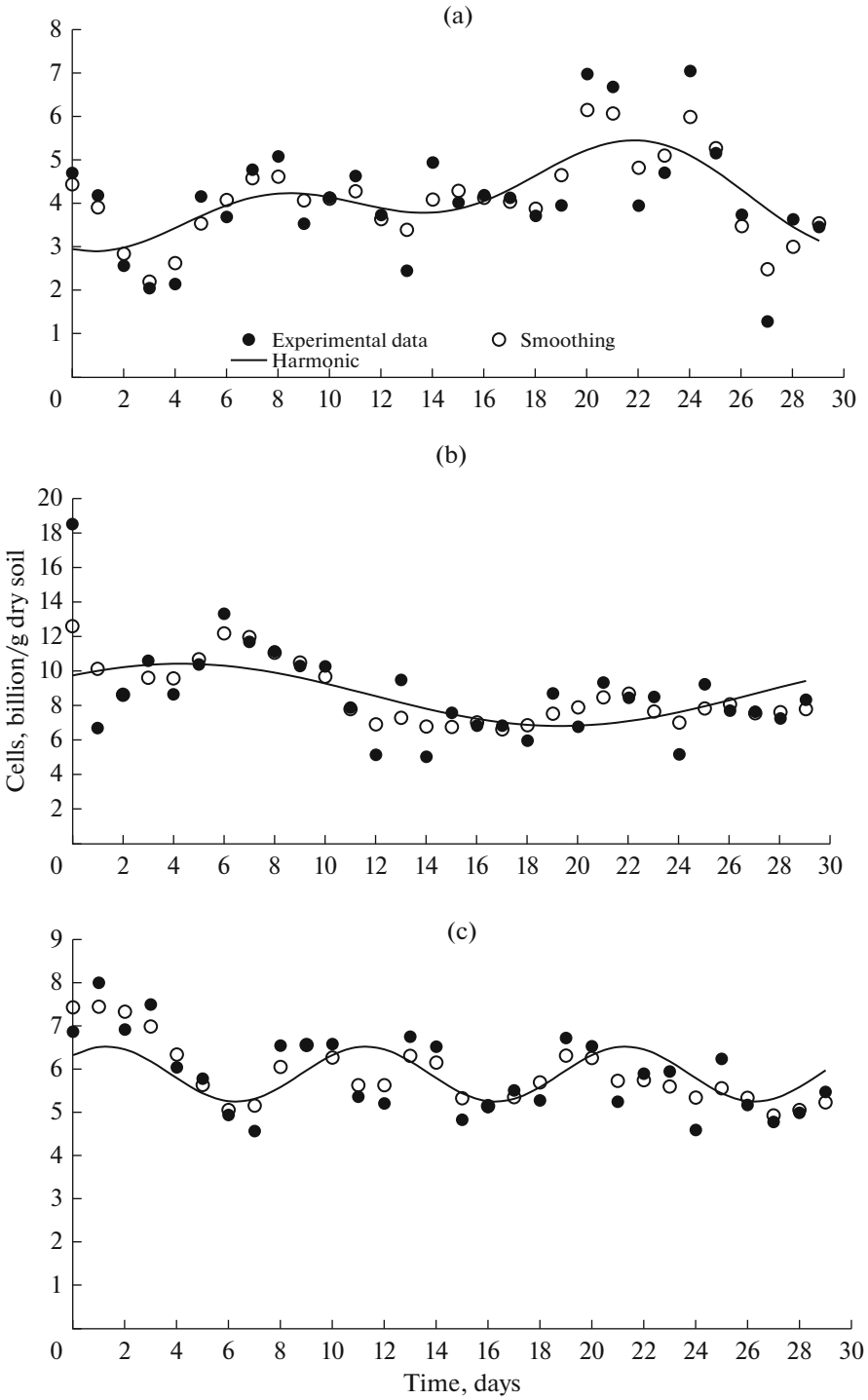

Fig. 5. Harmonic analysis of the results of recording the total number of bacterial cells in the soil in the experimental variant in three experiments ((a, b) and (c), respectively).

Harmonic Analysis of Experimental Data on the Number of Bacteria in Preparations Stained with SFDA

Harmonic analysis of the results of counting the number of living bacterial cells in the control soil sample of the first experiment (Fig. 6a; Table 1) identified three harmonics of first, fifth, and seventh orders with periods of 30.0, 6.0, and 4.29 days. The parameters of the MAC analysis basically coincide with the parameters of the harmonic analysis of the general count of bacteria in the soil of the first experiment, with the exception of the magnitudes of the vibration amplitudes. The values of the number amplitudes with a

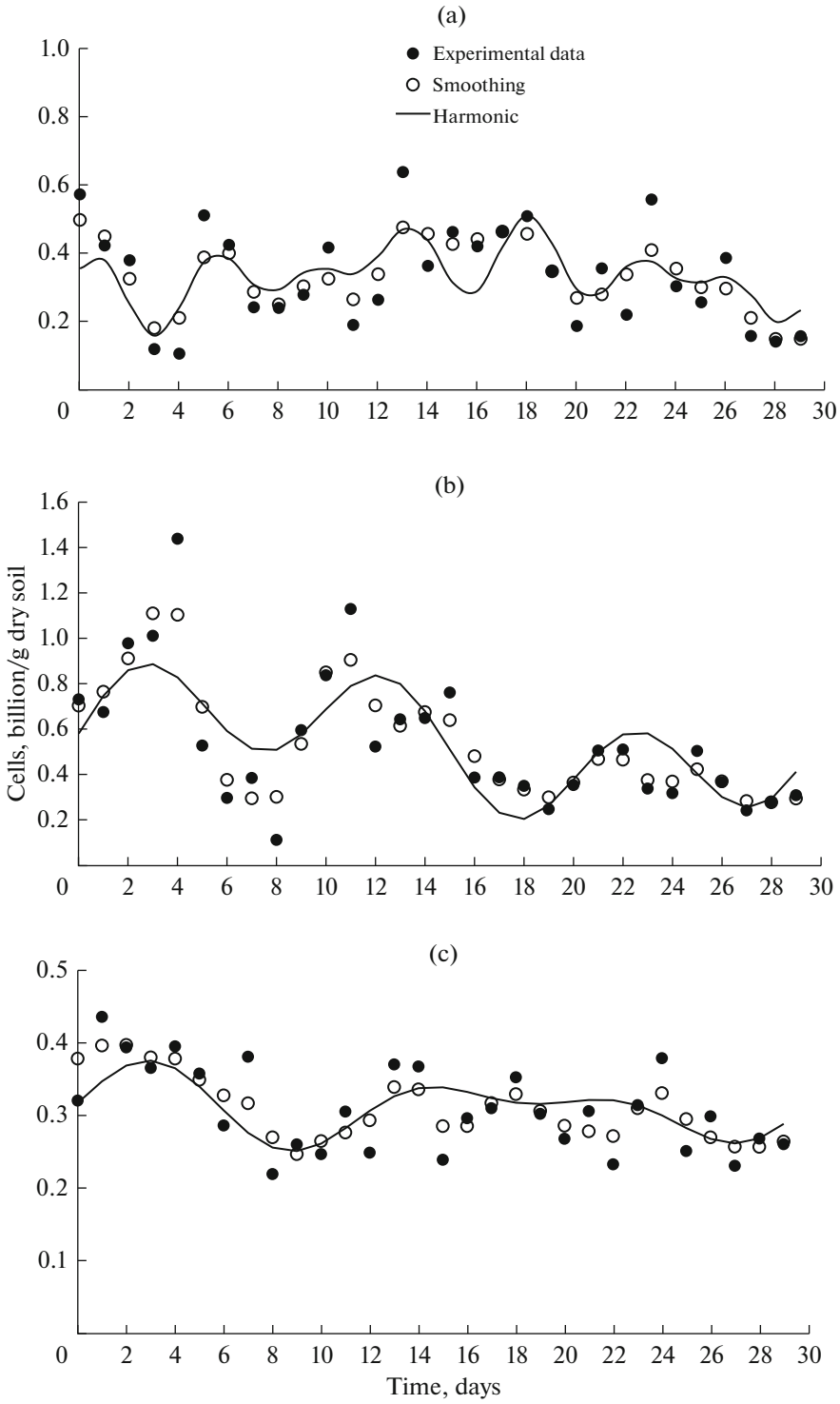

Fig. 6. Harmonic analysis of the results of taking into account the number of physiologically active bacterial cells in the soil in the control variant in three experiments ((a, b) and (c), respectively).

general count of cells range from 5.76 to 6.54 billion, and when counting living cells, the amplitude values are an order of magnitude smaller, from 0.62 to 0.63 billion, which is statistical confirmation of a lower number of living cells. By harmonic analysis of the results of recording the number of cells of living bacteria in the experimental soil sample of the first experiment (Fig. 7a; Table 1), two harmonics were detected—the second and fourth orders with periods of 15.0 and 7.5 days. The values of the amplitudes of

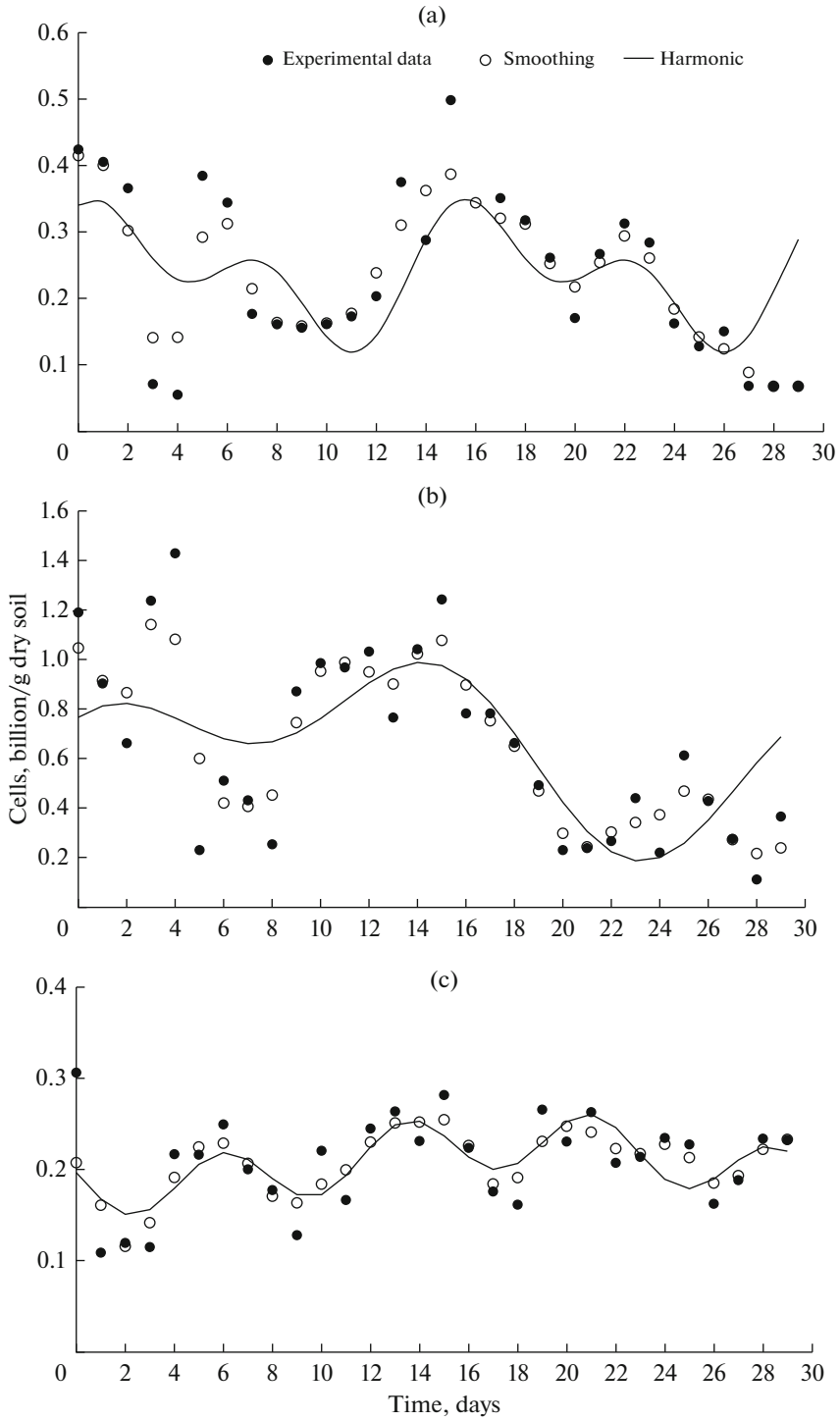

Fig. 7. Harmonic analysis of the results of taking into account the number of physiologically active bacterial cells in the soil in the experimental variant in three experiments ((a, b) and (c), respectively).

the number of living cells were 0.60 and 0.71 billion, while in the test sample with a general count, the values were 6.97 and 7.68 billion; that is, this represents statistical confirmation of a lower number of living cells by an order of magnitude.

When performing a harmonic analysis of the results of the number of living bacteria in the control soil sample of the second experiment, two harmonics were found—first and third orders with periods of 30 and 10 days, respectively (Fig. 6b; Table 1). The ampli-

Table 1. Harmonic characteristics of the total number and number of physiologically active cells in the soil in three experiments obtained using Fourier analysis at significance levels of 0.1 and 0.05

Experim. number	Characterized object	Harmonic no.	Amplitude bln	Phase	Period days	Frequency	Variation coeff., %	F exp	F tab 0.1 sign.lev	Quantity of analysis points	F tab 0.05 sign.lev
1	Cells_contr_total	1	5.76	21.50	30.00	0.03	18.32	2.66	2.50	30	3.30
	Cells_contr_total	**2**	**6.54**	**8.82**	**15.00**	**0.07**	**23.65**	**3.43**	**2.50**	**30**	**3.30**
	Cells_contr_total	6	6.21	–0.30	5.00	0.20	21.34	3.09	2.50	30	3.30
	Cells_exper_total	**1**	**7.68**	**19.40**	**30.00**	**0.03**	**29.96**	**4.34**	**2.50**	**30**	**3.30**
	Cells_exper_total	**2**	**6.97**	**7.44**	**15.00**	**0.07**	**24.69**	**3.58**	**2.50**	**30**	**3.30**
	Cells_ contr _alive	1	0.62	15.45	30.00	0.03	19.60	2.84	2.50	30	3.30
	Cells_ contr _alive	5	0.62	0.29	6.00	0.17	19.18	2.78	2.50	30	3.30
	Cells_ contr _alive	7	0.63	0.81	4.29	0.23	20.33	2.95	2.50	30	3.30
	Cells_exper_alive	**2**	**0.71**	**2.17**	**15.00**	**0.07**	**25.81**	**3.74**	**2.50**	**30**	**3.30**
	Cells_exper_alive	4	0.60	0.14	7.50	0.13	18.36	2.66	2.50	30	3.30
2	**Cells_contr_total**	**1**	**15.69**	**4.87**	**30.00**	**0.03**	**23.75**	**3.44**	**2.50**	**30**	**3.30**
	Cells_contr_total	**4**	**15.49**	**–0.57**	**7.50**	**0.13**	**23.14**	**3.35**	**2.50**	**30**	**3.30**
	Cells_contr_total	7	14.09	0.67	4.29	0.23	19.17	2.78	2.50	30	3.30
	Cells_exper_total	**1**	**17.98**	**4.29**	**30.00**	**0.03**	**52.60**	**7.62**	**2.50**	**30**	**3.30**
	Cells_ contr _alive	**1**	**1.81**	**6.65**	**30.00**	**0.03**	**26.73**	**3.88**	**2.50**	**30**	**3.30**
	Cells_ contr _alive	**3**	**2.22**	**2.48**	**10.00**	**0.10**	**40.30**	**5.84**	**2.50**	**30**	**3.30**
	Cells_exper_alive	**1**	**2.53**	**9.53**	**30.00**	**0.03**	**33.10**	**4.80**	**2.50**	**30**	**3.30**
	Cells_exper_alive	**2**	**2.24**	**0.38**	**15.00**	**0.07**	**25.77**	**3.74**	**2.50**	**30**	**3.30**
3	Cells_contr_total	4	2.44	1.29	7.50	0.13	19.17	2.78	2.50	30	3.30
	Cells_contr_total	**5**	**3.37**	**2.82**	**6.00**	**0.17**	**36.60**	**5.30**	**2.50**	**30**	**3.30**
	Cells_exper_total	**3**	**6.37**	**1.27**	**10.00**	**0.10**	**39.20**	**5.69**	**2.50**	**30**	**3.30**
	Cells_ contr _alive	**2**	**0.38**	**2.54**	**15.00**	**0.07**	**36.40**	**5.28**	**2.50**	**30**	**3.30**
	Cells_ contr _alive	3	0.29	3.10	10.00	0.10	21.79	3.16	2.50	30	3.30
	Cells_exper_alive	**1**	**0.25**	**18.12**	**30.00**	**0.03**	**28.13**	**4.08**	**2.50**	**30**	**3.30**
	Cells_exper_alive	**4**	**0.32**	**–1.52**	**7.50**	**0.13**	**45.50**	**6.60**	**2.50**	**30**	**3.30**

tudes of the harmonic of living cells in the control sample of the second experiment are 1.81 and 2.22 billion versus 14.09 and 15.69 billion when were account the total number. Thus, the amplitudes of the harmonics of the number of living cells are less than those of the total number, not by an order of magnitude, but by 7–8 times. Harmonic analysis of the results of recording the number of living bacterial cells in the experimental sample of the second experiment (Fig. 7b; Table 1) revealed two harmonics of the first and second orders with periods of 30 and 15 days. The amplitudes of living cells were 2.24 and 2.53 billion; the amplitude of the single harmonic of the total number of cells was 17.98 billion, that is, 7–8 times higher than the amplitudes of the harmonics of the number of living cells, as in the control of this experiment.

By harmonic analysis of the results of the number of living bacteria in the control sample of the third experiment (Fig. 7c; Table 1), two harmonics of the second and third orders with periods of 15 and 10 days were detected. The amplitudes of living cells were equal to 0.29 and 0.38 billion, while the amplitudes of the harmonics of the total number of cells were 2.44 and 3.37 billion, that is, almost an order of magnitude greater, which was also observed in the first experiment. Harmonic analysis of the results of counting living cells in the experimental soil sample of the third experiment revealed two harmonics of the first and fourth orders with periods of 30.0 and 7.5 days (Fig. 7c; Table 1), with amplitudes of 0.25 and 0.32 billion. The harmonic amplitude of the total number of cells was 6.37 billion. In this experiment, the amplitudes of the harmonics of the number of living cells are 20–25 times less than the amplitudes of the harmonics of the total number of cells. Thus, taking into account the total number of bacterial cells revealed a significant excess of their number—an order of magnitude or more than the number of living bacteria. Harmonic analysis confirmed the pattern of wavelike dynamics of changes in the number of bacterial cells in the soil as a consequence of the death of part of the microbial community and subsequent growth.

ANALYSIS AND DISCUSSION OF RESULTS FROM PUBLICATIONS OF THE AUTHORS OF THE ARTICLE AND OTHER LITERATURE SOURCES

To emphasize the significance and importance of the results presented in this work, the authors consider it necessary to briefly mention and discuss some of their experimental results obtained and published earlier (Nizovtseva et al., 1995; Lavrentieva et al., 2009; Semenov et al., 1999, 2013, 2022; Emer et al., 2014; Semenov and Semenova, 2018; van Bruggen et al., 2000, 2006, 2008, 2017; Zelenev et al., 2005a, 2005b; van Diepeningen et al., 2005; He et al., 2017).

For objectivity, the authors of this work present a certain minimum of experimental results of other authors who conducted similar experiments many years ago primarily under the leadership of T.V. Aristovskaya and published in a collection edited by her (Aristovskaya, 1974). This team carried out extensive studies of the number of bacteria in the soil and rhizosphere by long-term daily counting of the total number of cells under a microscope, and some of the authors also counted bacteria in the form of CFU. These studies convincingly demonstrate the oscillatory dynamics of bacterial numbers in communities of different soils. The peculiarity of these results is that in some publications the authors present not only the results in the form of graphs, which clearly show the oscillatory dynamics of the number of bacterial cells in soil samples, but also digital data in the form of tables, which allows them to be subjected to additional statistical processing (Egorova, 1974; Efremova, 1974; Zykina, 1974; Shchapova, 1974).

Previously, one of the authors of this publication and a team of researchers studied the relationship between a complex natural disturbance effect on the soil microbial community and the dynamics of the MC response upon daily recording of the number of bacteria and some MC metabolites in this soil (Zelenev, et al., 2005b). A natural disturbance was a one-time application of a freshly harvested clover-grass mixture to the soil as a source of organic carbon and nitrogen. The response of the bacterial community was recorded by daily recording of the total number of bacteria under a microscope using different luminescent dyes and the number of metabolically active bacteria (live), with daily recording of CFU, and also the daily dynamics of concentrations of NO_3 and NH_4, pH, and Rh in the soil was monitored. The daily dynamics of DOM concentration was not determined in these experiments, because so much organic carbon was added to the soil with the freshly harvested clover-grass mixture that it was pointless to detect fluctuations in DOM because of the death of some bacteria using available methods. In these studies, all methods of counting the number of bacteria revealed reliable wavelike dynamics of bacteria, confirmed by harmonic analysis. Since CFU counts were carried out from soil samples immediately after adding a larger amount of fresh clover-grass mixture to this soil, the number of CFU in the first half of the month-long experiment was even greater than the number counted under a fluorescence microscope with FDA staining. The number of bacteria counted under a fluorescence microscope using FITC staining was an order of magnitude greater than the number of CFU, and in the first half of the month-long experiment, it was even two orders of magnitude greater than the number using FDA staining. At the same time, daily determination of concentrations of NO_3 and NH_4 and the values of pH and Rh did not reveal their wavelike dynamics.

Thus, the given excerpts of results from publications confirm and complement the experimental

results presented in this work. The first very significant addition and confirmation of the results is that the majority of bacteria taken into account when calculating their total number with nonspecific dyes are represented by dead cells. The next, no less significant confirmation of the fact that the number of bacteria identified and counted in the soil by plating a soil suspension on a medium and in conditions that most closely correspond to the medium in composition and cultivation conditions from which the bacteria are sown is determined primarily by the state of the dominant bacterial populations and their viability and activity. The composition of the media used and cultivation conditions are only conditions for the implementation of the organism's activity strategy. As a continuation of the above, it is appropriate to conclude that, when counting bacteria in the soil by sown suspensions on appropriate media, it is possible to count most or all living cells or obtain a number comparable to the number obtained by microscopic counting with the appropriate fluorescent "vital" dyes. These results also confirm that only living organisms and the direct products of their metabolism, which directly depend on the vital activity of organisms, exhibit wavelike dynamics (Semenov et al., 2019).

Confirming and complementary conclusions were obtained in other publications of the authors (Semenov et al., 2013). The authors carried out a daily count of CFU of bacteria for a month in soil suspensions prepared from soil samples from plots that were treated using biological or intensive farming technology, determined daily CO_2 emissions and daily dynamics of the composition of amplicons obtained after PCR and DGGE analysis of DNA samples isolated from soil samples (Semenov et al., 2013). CFU counting was carried out using differentiating media for copiotrophs (salt medium with 2.5 g/L glucose and 0.2 enzymatic hydrolyzate of casein) and oligotrophs (salt medium contained 100 times less glucose and enzymatic hydrolyzate of casein, and high-purity Noble Agar was used). CFU of copiotrophs were counted after 60 h of incubation, and oligotrophs, after 14 days. The number of CFU of bacteria and dynamics of CO_2 emission was wavy regardless of the trophic affiliation of the bacteria and regardless of the soil treatment technology. The reliability of the wavelike dynamics of CFU of copiotrophic and oligotrophic bacteria was confirmed by harmonic analysis. In this count, the number of CFU of copiotrophs in some seasonal periods of the experiment varied from 1.44×10^7 (CFU/g dry soil) to 1.19×10^8, and the number of CFU of oligotrophs varied from 5.53×10^7 to 2.81×10^8 (CFU/g dry soil). Analysis of the daily dynamics of amplicons obtained after PCR and DGGE analysis of DNA samples revealed wavelike changes in the structure of the MC (Semenov et al., 2013). At the same time, the analytical parameters of the amplicons changed in the same way within each peak when comparing the dynamics of amplicons with the dynamics of the CFU numbers of both copiotrophs and oligotrophs. Changes in the dynamics of amplicons within each peak, and not from peak to peak, indicate the cyclical nature of changes not only in the structure of the MC but also in the numbers of populations. Note that the same phenomenon was shown when studying the dynamics of MC amplicons along the root of wheat plants (van Diepeningen et al., 2005). Oscillatory changes in gene activity and abundance over time have been observed in microbial communities of other ecosystems (Gómez-Brandón et al., 2020). Thus, by inoculating suspensions from samples of natural media on to selected laboratory media, it is possible to identify and count the number of bacterial cells comparable to the number detected by microscopic counting with fluorescent dyes, such as FDA and SFDA. We emphasize that researchers who reviewed methods for determining and identifying active microorganisms in soil concluded that of the three most significant methods for identifying active microorganisms in soil, the first method is microscopic counting with specific fluorescent dyes, and the third is counting microorganisms on media (Blagodatskaya and Kuzyakov, 2013). Therefore, the critical point when counting bacterial cells in soil samples by any method is the viability of the microorganisms. If a large number of bacterial cells in natural environments at the time of counting turn out to be dead, but not yet disintegrated, then they provide the majority that is revealed when counting bacteria under a microscope with nonspecific dyes.

Additional confirmation that, when counting cells under a microscope, already dead cells can be taken into account, is our data presented in the article by Semenov et al. (2022). This publication provides data on long-term (60 days) daily counting of pure culture cells of *Pseudomonas fluorescens* 32 *gfp*, labeled with the ability to synthesize green fluorescent protein (GFP). It was shown that, when analyzing the same culture sample taken from the medium, the number of cells counted under the microscope was much greater than the number of cells with GFP growing on agar medium in Petri dishes in the form of CFU. Consequently, cells that are not capable of growth on the medium can rightfully be called dead, and the possibility of identifying them under a microscope simply confirms that they are not yet disintegrated!

CONCLUSIONS

Topics and questions about the number of bacterial cells and prokaryotic cells in nature and primarily in the soil ecosystem, their viability, the number of truly living, but not reproducing (not growing, surviving) bacterial cells, and the ratio of living, surviving, and dead cells and about methods of counting bacterial cells in natural samples and methods for recognizing living, surviving, and dead cells are long-standing and

far from closed topics for research in microbiology (Blagodatskaya and Kuzyakov, 2013). The importance of these topics is obvious and does not require justification or belief in their relevance. With the results presented, the authors, of course, are not able to cover all the topics and answer all the questions listed above, but they believe that the results presented convincingly explain some of them.

First, in the soil ecosystem with microscopic recording with nonspecific dyes, the high number of bacteria detected is represented mainly by nonliving cells. Our results and published data demonstrate that the number of metabolically active, living cells is at least an order of magnitude less than the total number of bacterial cells when counted under a microscope. This phenomenon is explained by the fact that when bacterial cells die, they are not instantly lysed and disintegrated, but the dyes used are "held" for some time by the polymer structures of the cell, which is what ensures their abundance. S.N. Winogradsky, a hundred years ago, while counting bacterial cells in soil samples, could not have known that the colossal number of bacteria he discovered was represented mainly by nonliving cells, but his discovery gave a powerful impetus to the knowledge of the number and diversity of microorganisms in nature and the development of methods for understanding the microworld.

Note that phenomena known in microbiology, such as VBNC (viable but not cultured cells), persister cells, and the persistence phenomenon, take place. However, to extend these phenomena, discovered in pure cultures in laboratory conditions, to all natural populations of prokaryotes, and even more so to all microbial communities, and to apply these phenomena to explain the significant excess in the number of cells in a total count of bacteria in soil with nonspecific dyes and, thereby, essentially "translate" all cells counted in this way into the category of viable ones is neither logical nor justified for the current time period. If the phenomena of uncultivated nature and persistence are extended to all results obtained using specific dyes, such results will have to be invalidated, and the absence of methods that would somehow help to distinguish between dead bacterial cells in natural substrates will have to be recognized. The extension of the above-mentioned phenomena to the assessment of the entire set of bacteria in nature leads to the recognition of this entire set as viable and, thereby, disavows ecological concepts about the pool of organisms and the size of the econiche, etc., as well as the recognition of the nonexistent wavelike dynamics of microorganisms, which is based on the cyclic death and growth of remaining living cells at the expense of the biomass of primarily dead cells, etc. (Gendugov et al., 2011; El Registan et al., 2022; Blazewicz et al., 2020).

The next rather important topic discussed in this work is the effect of a smaller number of bacteria detected when inoculating soil suspensions on agar media and counted as CFU, compared to the number of bacteria detected when counting bacteria from the same samples when staining suspensions with common dyes. This is explained by the fact that most bacterial cells observed under a microscope in preparations from soil suspensions are not viable, but are simply dead. The work provides examples and links to results about a comparable number of bacterial cells detected by microscopic counting using specific dyes and in the form of CFU, when plating was carried out on media enriched in various substrates, or when plating was carried out on media that, in composition and conditions, corresponded to the physiological state of the bacterial community. The work contains not only links to publications but also excerpts from the results of publications. The review publication of Staley and Kanopka (1985), often cited by experts, initiated the development of methods for determining abundance and microdiversity. However, it should be noted that the authors' assumption that only 1% of the total diversity of microorganisms was detected on media was made on the basis of observations and data obtained using traditional "copiotrophic" laboratory media in composition, and the doctrine of oligotrophs and a significant excess of the number of oligotrophs over copiotrophs at the time of writing and publication of this review had not yet been formed (Poindexter, 1981; Semenov, 1991; Zelenev et al., 2005a).

Let us note once again the central theme of this work—the objectivity of the phenomenon of wavelike dynamics of bacteria in populations and communities of microorganisms. Wavelike dynamics also occur when microscopically counting all microorganisms or only living ones when counting cells in the form of CFU in soil samples and pure cultures, and when determining some physiological functions of microorganisms: N_2 fixation, cellulolytic activity, and, most importantly, respiration in communities of microorganisms, which made it possible to justify, develop and propose for practical use a method for determining the health of the soil and soil ecosystem (Semenov and Semenova, 2018).

Our experimental results with heating of soil samples convince us that external disturbances on microbial communities are not the cause of oscillations in the abundance and functional activity of MPs and MCs. Our experimental results and many results from the literature clearly confirm the wavelike dynamics of microorganisms as a consequence of cycles of growth and death of bacterial populations and short-term trophic succession in microbial communities (Gendugov et al., 2011). This both confirms and corroborates the concepts of D.G. Zvyagintsev about the excess of the number of bacteria over the amount of substrate available to bacteria in a specific microniche and, as a consequence, starvation of some populations and communities. It is the manifestation of trophic succession in the MC that explains the "variation in height" and duration of the peaks that arise in the MC. Different

"speed" of growth of peaks and height of peaks (i.e., the number of cells) is a consequence of the local, temporary dominance of microorganisms of different trophic (ecological) strategies, and primarily copiotrophs and oligotrophs in the MC (Semenov, 1991; Zelenev et al., 2005a), which is determined by the availability of concentration and the appropriate quality of substrates for the growth of microorganisms, as once again confirmed by researchers (Blagodatskaya and Kuzyakov, 2013; Stone et al., 2023). The objectivity of the phenomenon of wavelike dynamics of bacteria is shown not only by the number of MC cells and their activity but also by the oscillations of amplicons obtained after PCR and DGGE analysis of DNA isolated from soil samples.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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