ISSN 1064-2293, Eurasian Soil Science, 2022, Vol. 55, No. 2, pp. 221–234. © Pleiades Publishing, Ltd., 2022.
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SOIL BIOLOGY

Microbiological Indicators of Soil Ecological Functions: A Review

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Received June 11, 2021; revised July 16, 2021; accepted July 31, 2021

Abstract—The review summarizes the most relevant microbiological characteristics that can serve as indicators of soil ecological functions: bioresource, phytosanitary, as well as the functions of carbon transformation and plant nutrition. The indices of diversity and taxonomic structure of microbial communities as well as abundance of certain groups of microorganisms are estimated to characterize the bioresource function of soils. The main microbiological indicators of carbon transformation are microbial biomass carbon, the ratio of bacterial to fungal biomasses, soil respiration, enzyme activities, and the rate of soil organic matter decomposition. The appropriate microbiological indicators of the plant nutrition function of soil are the enzyme activities associated with the nitrogen and phosphorus cycles, as well as molecular species-specific markers of arbuscular mycorrhiza. Gene abundances of phytopathogens and their antagonists can serve as indicators of soil phytosanitary function. Since a high variation of some characteristics and certain difficulties in their interpretation are currently the relevant problems in microbiological indication of soil functions, the key goal is a careful selection of the parameters with the best applicability as indicators of soil ecological functions.

Keywords: soil biodiversity, carbon transformation in soil, plant root nutrition, soil suppressiveness

DOI: 10.1134/S1064229322020090

INTRODUCTION

Soil is the basis for existence of terrestrial ecosystems and a valuable nonrenewable resource in terms of food security [134]. Soil fulfils numerous key ecological, social, and economic functions [58]. An increase in the anthropogenic impact causes soil degradation [73], which demonstrates the need for indicators allowing the soil state to be assessed and the first signs suggesting the disturbed performance of soil ecological functions to be detected [86]. Soil microorganisms are the key player in biogeochemical cycles of nutrient elements and rapidly respond to the changes in the environment [7]. Microorganisms are directly or indirectly involved in a wide range of soil ecological functions and, thus, can serve as efficient highly sensitive indicators of these functions [71, 120].

When considering the use of microbiological indicators, it is first necessary to define the classification of soil functions. Dobrovol'skii and Nikitin [8, 10] proposed one of the first systems for soil functions in the biosphere and ecosystems; they distinguished the biogeocenosis-level and global biosphere-level soil functions. The biogeocenosis-level functions comprise physical, physicochemical, informational, and integral functions as well as soil fertility as a separate function [8, 10]. Soil functions represent part of the ecological functions of landscape [16]. In the relevant international literature, soil functions and their grouping are regarded as an intermediate stage that connects the traditionally measured soil properties with the ecosystem services; consequently, the soil functions are defined in accordance with the corresponding ecosystem services [47, 69, 77, 115]. Since any consensus on the soil functions is yet absent, we try in this review to define soil functions in ecosystems in the way most clearly reflecting the essence of the corresponding processes in soil that provide the function of other ecosystem components. Any microbiological indicators are absent for several soil ecological functions (physical support, hydrological, and production functions). However, microbiological characteristics are applicable to assess the bioresource, plant nutrition, and phytosanitary functions of soil as well as the function of carbon transformation (Fig. 1). The review discusses the role of microorganisms in maintaining soil functions and summarizes the most relevant microbiological characteristics applicable as the indicators of these functions.

BIORESOURCE FUNCTION OF SOIL

Soils are the largest biodiversity reservoir comprising at least a quarter of the living species of the planet [7, 14, 137]. That is why, the bioresource function is

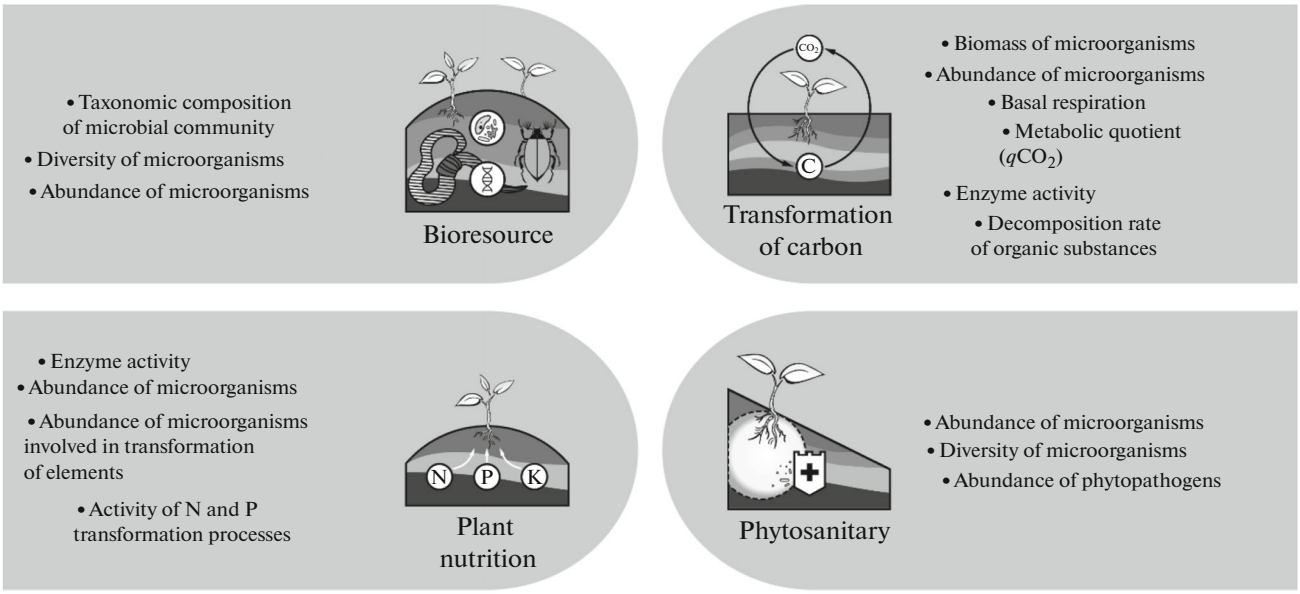

Fig. 1. Main microbiological characteristics of soil ecological functions.

one of the most important functions performed by soil; this function consists in maintenance of biodiversity and abundance of the communities of soil organisms or the organisms associated with soil [56]. The key role of this function is to provide the mechanisms underlying the ecosystem resistance and self-regulation; correspondingly, the related issues have been included into the list of sustainable development goals [55] and the GLOBIO3 (a framework to investigate options for reducing global terrestrial biodiversity loss after 2020) [48]. Soil biological diversity plays an important role in mineralization of organic compounds, carbon sequestration, and maintenance of the cycles of biophilic elements, as well as plant nutrition and health [82]. As a consequence, a decrease in biodiversity and abundance of organisms (i.e., soil bioresource function) slows down the intensity of biogenic processes of soil formation and decomposition of organic substances and decreases the total soil fertility, soil buffer capacity, and its ability to self-restore after degradation [137]. The biodiversity has not been quantified for most areas of the world since this is a difficult task [84]. However, prognostic maps and atlases of biodiversity of soil bacteria [68], fungi [136], nematodes [144], and earthworms [109] are already available for individual regions.

Several types of biodiversity are distinguished. α-Diversity characterizes the taxa within a community, their richness (number of taxa in the community), and the evenness (relative abundance) [22, 44, 45]. β-Diversity refers to the similarity/dissimilarity of different communities, demonstrating the degree of differentiation in species distribution or the rate of changes in the species composition. Mere counting of the number of taxa is poorly informative for basic and applied ecology because the species differ in their abundances. Correspondingly, α-diversity is usually studied taking into account four theoretical models: geometric, logarithmic, lognormal, and MacArthur broken stick model [146]. Note that the applicability of particular methods for assessing biodiversity of communities in classical ecology is well known; however, this issue is rather vague for microorganisms [44]. Soil microbiome has not only a specific structure because of the absence of dominants, but also an extraordinary taxonomic diversity, making some classical biodiversity indices insufficient for its analysis [146].

The Margalef and Menhinick indices (Table 1) are among the most widespread characteristics for biodiversity; they do not take into account the relative abundances of taxa and require for calculation only the number of the observed taxa and the total number of individuals [44]. The Shannon–Weaver index is also a popular tool; its specific feature is the focus on rare species [26]. The Pielou evenness index is also widely used; this index normalizes the Shannon index to the range between 0 and 1. In addition, the Simpson index is used; it describes the probability of two individuals randomly selected from an indefinitely large community to belong to the same species. The larger the value of this index, the lower is the species diversity. The diversity decreases with increasing Simpson's index [44]; it is most sensitive to the presence of most abundant species in the sample but weakly depends on the species richness [26].

Manifold natural (temperature, moisture content, acidity, quality and quantity of organic matter, character and composition of plant cover, etc.) and anthro-

Table 1. Biodiversity indices

Index	Equation	Legend
Margalef's	$DMg = (S-1)/\ln N$	S, number of observed taxa and
Menhinick's	$DMn = S/(N)^{1/2}$	N, total number of individuals
Shannon–Weaver	$H = -\sum_{i=1}^{n} p_i \cdot \log_2(p_i)$	$p_i = \frac{x_i}{\sum_{i=1}^{n} x_i}$ and x_i is number of species in a community
Pielou's	$E = \frac{H}{\log N}$	H, Shannon's index and N, number of species in a community
Simpson's	$D = \frac{\sum n_i \cdot (n_i - 1)}{N \cdot (N-1)}$	N, number of species and n, number of individuals of the ith species
Williams polydominance index	$\frac{1}{D}$	D, Simpson's index
Chao1	$\text{Chao1} = S_{obs} + \frac{a^2}{2b}$	S_{obs} is the observed number of taxa; a, number of taxa containing one sequence; and b, number of taxa containing two sequences

pogenic (pollution, abundance and quality of fertilizers, type of land use and agricultural soil management) factors influence the diversity of soil microbiome [66, 153]. Either an extreme change in environmental conditions (desertification, drastic loss of organic matter, salinization, strong acidification/alkalinization, etc.) or a decrease in the number of ecological niches (for example, because of long-term soil plowing) usually causes a decrease in the biodiversity of microorganisms in both natural and agricultural soils [117]. For example, the bioresource function of soils is disturbed by a long-term application of mineral fertilizers [35, 153], plowing [23, 25], or pollution [21, 25] since all these factors reduce the biodiversity of microorganisms.

Detection of soil microbial diversity for a long time relied on the cultivation of microorganisms in nutrient media [57], allowing the abundance of their ecological and functional groups (nitrogen fixers, cellulolytic species, amylolytic species, etc.) to be assessed [19, 24]. However, 90–99% of all bacteria, archaea, and microscopic fungi are currently uncultivable [132]. The advent of molecular gene methods allows the total microbiological diversity to be estimated using the total genetic material directly extracted from the soil [35, 146].

Quantitative polymerase chain reaction (real-time qPCR) is used to assess the abundances of different groups of soil microorganisms; qPCR enables to determine the abundances of ribosomal or functional genes per unit soil weight. The diversity of microorganisms is estimated using 16S rRNA gene for prokaryotes, and 18S rRNA gene or ITS region for fungi [35]. This method determines the copy number of the genes coding for the key enzymes (for example, nitrogenase, nitrate reductase, etc.), thereby characterizing the potential activity of different ecological and functional microbial groups (see below).

Denaturing gradient gel electrophoresis (DGGE) [101] and temperature gradient gel electrophoresis (TGGE) [78] were among the first methods used for profiling the genetic diversity of microorganisms. However, the resolution of DGGE and TGGE is rather poor as compared with the real microbial diversity in soil [103]. Currently, the genetic diversity of soil microbiome is assessed using next generation sequencing (NGS) technique, which is able to read hundreds of thousands and even millions of DNA regions. The NGS technology has enhanced the advance in the methods for assessing the structure and diversity of soil microbiomes, in particular, metabarcoding, i.e., the study of genetic diversity by analyzing the amplicon libraries of marker genes (16S rRNA, 18S rRNA, ITSs, and functional genes) [35, 100].

High-throughput sequencing using the shotgun technology gives the possibility to analyze not only marker genes, but also the entire soil metagenome [29, 114]. Although the productivity of NGS techniques is high, analysis of that large amount of information as a whole soil metagenome remains an extremely laborious process [54, 140]. The high-throughput sequencing methods still have a number of limitations in analyzing soil biodiversity, first and foremost, a high cost of such analysis and incompleteness of the resulting databases. Nonetheless, the sequencing costs have significantly decreased over the last 10 years, and this trend is retained [35]. In turn, further development of NGS technologies and updating of the databases contribute to the efficiency of these methods in the analysis of soil bioresource function [100].

FUNCTION OF CARBON TRANSFORMATION

The regulation of carbon cycle is one of the most important soil functions in the biosphere [131]. A wide range of compounds in soils contains organic carbon; collectively, these compounds are referred to as soil organic matter (**SOM**). Most of the soil properties depend on the quantitative and qualitative SOM compositions, thereby determining the key role of SOM in functioning of ecosystems [31]. The current intensification of agriculture has led to a ubiquitous active SOM mineralization, decreasing the productivity of arable lands [16]. The conversion of plant residues to SOM and the processes of SOM mineralization, stabilization, and destabilization are of a paramount importance in the soil carbon dynamics available to microbiological indication [33, 122, 130]. Soil saprophytic microorganisms are the main agents in SOM transformation and their biomass is a dynamic source of SOM renewal and carbon sink [63]. The main microbiological indicators of the processes underlying the carbon cycle are the carbon of microbial biomass, soil respiration, rates of SOM decomposition and mineralization, and enzyme activity.

Microbial biomass carbon (C_{mic}) is the most active and dynamic part of SOM, typically accounting for 3–5% of the soil organic matter content [80, 135]. Microbial biomass carbon can be used as an indicator for assessing the productivity of ecosystems as well as an early indicator of the changes in SOM [135]. The C_{mic} to C_{org} ratio is an indicator of the availability of soil organic carbon [1, 2].

The C_{mic} content in soil can be determined using direct, physiological, biochemical (biocide), and molecular (biomarker) methods. The *direct methods* include luminescence microscopy with a set of fluorescent dyes allowing the microbial cells to be counted [27, 57] and *physiological,* the technique of substrate-induced respiration. The latter consists in measuring the emission of CO_2 produced by soil microorganisms during 3–5 h after addition of a known amount of readily available substrate (usually, glucose) to soil. An example of *molecular methods* is quantitative assessment of biomarkers, such as DNA and RNA [93, 123], phospholipid fatty acids (PLFAs) [150], and ATP in cells [3]. In general, the main problem in all methods for assessing soil microbial biomass is the variation in correction factors for a measured characteristic (respiration, fumigated carbon, DNA, and PLFAs) when calculating the microbial carbon content [11, 123].

Soil respiration (SR) is the CO_2 emission from soil during SOM mineralization and respiration of soil biota [13, 18]. The SR consists of heterotrophic (microbial and zoogenic) and autotrophic (root) respirations. SR correlates well with the SOM content and microbial biomass and is, thus, one of the classical methods for assessing soil biological activity [2, 18, 57]. SR is determined by measuring the evolved CO_2 or the O_2 consumption [149]. The CO_2 assessment is more sensitive since its concentration in the atmosphere is approximately 0.04% versus about 20% of oxygen. SR is determined using either the methods based on CO_2 absorption by alkali followed by titration or gas chromatography and infrared spectroscopy. Since 10 to 90% of the CO_2 emission from soil is provided by microorganisms [107], the intensity of CO_2 emitted during the SOM decomposition in the sites free from roots and plant residues is usually assessed; this is referred to as basal respiration (**BR**) [1, 36, 149]. BR values are important for modeling and estimation of the carbon budget in terrestrial ecosystems [12], it is helpful in determining the dependence of SOM mineralization rate on various climatic factors [5] or agrogenic impacts [122, 142]. A decrease in the content of microbial biomass causes a decrease in the soil carbon stock and slows down the carbon cycle in ecosystems in general [3]. The microbial biomass characteristics are believed to be more sensitive for assessing quantitative and qualitative changes in SOM [31].

Metabolic or *respiratory quotient* (qCO_2) is one of the indicators of the microbial contribution to the carbon cycle; its value is determined according to the ratio of BR to substrate-induced respiration [49]. This quotient reflects the efficiency of C_{org} transformation into microbial biomass by heterotrophic microorganisms and, correspondingly, is applicable as an indicator of the changes in soil quality [70]. The metabolic quotient and C_{org} mineralization rate in soil are decisive for the carbon cycle in terrestrial ecosystems [52]. In addition, qCO_2 describes the state of the community: the larger its value, the less favorable are the conditions in ecosystem. A low qCO_2 suggests a decrease in the intensity of biogeochemical carbon cycle in the soil [52].

Rate of organic matter decomposition. The decomposition of SOM components is usually assessed according to a decrease in the weight of material in net bags, change in the litter amount, and $C–CO_2$ formation determined by closed chamber technique [120]. The use of litter decomposition as a functional indicator has some problems associated with standardization because the differences in litter quality have a pronounced effect on the rate of its degradation [89]. This shortcoming is surmountable by using a standard cellulose substrate as a substitute for organic residues [138], for example, linen/hempen or cotton strips [81] or bags with black and green tea. A rapid decomposition of cellulose strips suggests a rapid destruction of the litter [81, 138]. Biokinetic method, utilizing the $C–CO_2$ emission from an incubated soil sample, makes it possible to concurrently assess the availability of organic carbon for decomposition and the ability of a soil community to decompose organic matter [30, 32]. In addition, these methods determine the amount of decomposed SOM or plant residues as well as the decomposition rate constant [33, 133]. Community-

level physiological profiling (CLPP), assessing the utilization activity of several organic substrates [4, 92], also gives vast information about the trophic status of soil microbial community.

Enzyme activities (EAs). Soil enzymes contribute to the SOM decomposition and transformation, release available nutrients for biota, and are involved in the cycles of biogenic elements [59, 121]. The intracellular enzymes exist only within the organisms providing their vital activities and break down after the organisms die, having almost no effect on soil. As for the extracellular enzymes, organisms release them into environment, where they oxidize/hydrolyze polymeric organic compounds and thereby control the balance between the transformation of stable and labile SOM species [59, 95, 129].

EAs change considerably faster as compared with most physical and chemical characteristics of soil, which makes it possible to detect SOM transformation at early stages [88]. Typically, amylase, glycosidase, cellulase, chitinase, phosphatase, protease, and urease activities are detectable in the soil [126]. The main methods used for measuring EAs utilize spectrophotometry, fluorescence, and radioactively labeled isotopes. The measurements of potential soil EA are based on a zero-order kinetics, which allows the reaction rate proportional to enzyme concentration to be rapidly reached [59]. Any commonly recognized procedures for assessing the soil EAs are still absent [53, 126]. An original method for determining cellulase activity by cellophane membrane method consists in recording of the decrease in the membrane rupture strength after its incubation in the soil [20]. A leading role of EA characteristics in evaluating the effect of pollutants (pesticides, heavy metals, oil products, etc.) on the ecological state of soils has been demonstrated [6, 15]. Simplicity of the method and its high sensitivity enhance the use of EAs as an indicator of the soil ecological function, which reflects the transformation of carbon.

FUNCTION OF NUTRIENT SUPPLY TO PLANTS

One of the soil functions is supplying plants with nutrients; microbiome plays a considerable role in this function by transforming nutrients (first and foremost, nitrogen and phosphorus compounds) into the form available for plants [82]. Microorganisms transform nitrogen-containing substances by nitrogen fixation, ammonification, nitrification, denitrification, etc. [13, 29, 43, 118]. For example, intensive cultivation of farmlands can activate denitrification processes and, as a consequence, decreases the total nitrogen content in the soil [119]. Traditionally, the processes of nitrogen transformation in soil are studied by chromatography [41, 46]. Nitrogenase activity is mainly determined using the classical gas chromatography C_2H_2 reduction method [41, 46]. Denitrification intensity is also measured by adding C_2H_2, which inhibits N_2O reductase, catalyzing the last stage in the denitrification pathway. A promising method for measuring the activities of microbiological processes (for example, denitrification) utilizes the substrates enriched with ^{15}N [60].

Less dynamic characteristics associated with microbial counts and abundances of their functional genes and transcripts are also applicable as indicators of the potential activity of the nitrogen cycle. For example, the *nifH* gene codes for one of the nitrogenase subunits and is most frequently used as a molecular marker for nitrogen fixation [111]. The genes *narG* and *napA* as well as *norB, qnorB, nirS, nirK,* and *nosZ* are used as molecular markers for different stages of denitrification (Table 2). The *amoA* (bacterial and archaeal), *NxrA,* and *NxrB* genes are molecular markers for nitrification intensity [74]. On the other hand, it is most important to keep in mind that the abundance of a certain gene does not always correlate with the intensity of the corresponding process in soil [35]. Meta-analysis has shown that the abundance of functional genes associated with nitrification and denitrification processes considerably increases after application of nitrogen fertilizers in different forms [105], suggesting that the functional genes of nitrogen cycle are applicable as indicators at least for agroecosystems. A long-term field experiment has shown that the intensity of denitrification and nitrification processes correlates with the abundance of genes rather than with the diversity of nitrogen-cycling communities [75]. Many studies report the correlation between the abundance of nitrifier genes and nitrification activity [147].

Microorganisms are involved in mineralization and immobilization of organophosphorus compounds as well as in mobilization of insoluble inorganic phosphorus compounds [82, 152]. Mycorrhizal fungi and rhizospheric microorganisms are the most active players in phosphorus mobilization [79]. The ability of soil microbial community to mineralize organophosphorus compounds is assessed by phosphatase activities [102] and the abundance of the genes coding for phosphatases, *phoC* and *phoD* [72].

The majority (to 98%) of higher plants are in symbiotic associations with mycorrhizal fungi. The mutualistic relationship between these organisms consists in that mineral nutrients, vitamins, and growth stimulators are supplied to plants via hyphae and the plant photosynthetic products are supplied to mycobionts. Arbuscular, endotrophic, exotrophic, and ericoid mycorrhizae are distinguished [37]. Mycorrhiza can stimulate the growth and development of host plants and improve their nutrition and tolerance to drought, salinization, and heavy metals [51] as well as trigger the mechanisms underlying resistance to phytopathogens [112]. The mycorrhizal symbiosis allows plants to develop in low fertility soils, where the nutrients near the roots soon become deficient [64]. Characteristic

Table 2. Functional genes involved in the nitrogen [108, 131] and phosphorus [65] cycles

Process	Functional gene
Nitrogen fixation	*nifH*
Ammonification	*amoA*
Nitrification, stage 1 $NH_2OH \rightarrow NO_2^-$	*amoA* (bacterial and archaeal)
Nitrification, stage 2 $NO_2^- \rightarrow NO_3^-$	*Nxr*
Denitrification, stage 1 $NO_3^- \rightarrow NO_2^-$	*narG* and *napA*
Denitrification, stage 2 $NO_2^- \rightarrow NO$	*nirS* and *nirK*
Denitrification, stage 3 $NO \rightarrow N_2O$	*norB* and *qnorB*
Denitrification, stage 4 $N_2O \rightarrow N_2$	*nosZ*
Hydrolysis of organophosphorus compounds	*phoD*
Process	Functional gene
Nitrogen fixation	*nifH*
Nitrification, stage 1 $NH_2OH \rightarrow NO_2^-$	*amoA* (bacterial and archaeal)
Nitrification, stage 2 $NO_2^- \rightarrow NO_3^-$	*NxrA* and *NxrB*
Denitrification, stage 1 $NO_3^- \rightarrow NO_2^-$	*narG* and *napA*
Denitrification, stage 2 $NO_2^- \rightarrow NO$	*nirS* and *nirK*
Denitrification, stage 3 $NO \rightarrow N_2O$	*norB* and *qnorB*
Denitrification, stage 4 $N_2O \rightarrow N_2$	*nosZ*
Hydrolysis of organophosphorus compounds	*phoD*

of most agricultural species is the arbuscular mycorrhiza [85]. The use of mycorrhization enhances an increase in crop yields and improves their quality [61]. The mycelium of arbuscular mycorrhiza considerably increases the absorption capacity of plant roots, including phosphates and phytates, poorly available phosphorus compounds [61, 110]. An increase in the abundance of mycorrhiza-forming fungal cultures in soil is directly associated with an increase in the efficiency of plant nitrogen nutrition [145].

Counting of the spores of the mycobiont, phospholipid fatty acid (PLFA) content, and genetic markers are used as indicators of the quality and quantity of arbuscular mycorrhiza [148]. The signature PLFAs characteristic of the fungi that form arbuscular mycorrhiza are listed in the review by Olsson [104]. The 18S and LSU rRNA sequences are the genetic markers for the Glomeromycota fungi, forming the arbuscular mycorrhiza typical of 80% of the vascular plants [37]; their abundance is assessed by quantitative PCR using group-specific and species-specific primers [116].

SOIL PHYTOSANITARY FUNCTION

Soil phytosanitary function consists in prevention of the growth and development of the microorganisms pathogenic to plants [38]. Under certain conditions, phytopathogenic viruses, bacteria, and microscopic fungi are able to attack plants and inhibit their vital activities [94, 143]. The plant infectious diseases originating from soil are among the most important problems in agriculture [42, 67, 94]. According to the FAO (Food and Agriculture Organization) data, diseases and pests are responsible for up to 30% losses in the potential crop yields [40].

One of the main components of soil phytosanitary function is the suppressive (biocontrol) activity [28, 42, 143]. Soil suppressiveness (**SS**) is the parameter that characterizes the ability of soil to inhibit and/or eliminate individual pathogenic species from the living soil system, which is determined by the integral impact of biological, physicochemical, and agrochemical properties [38]. Characteristic of the highly suppressive soils is a very low level of disease development even in the presence of a virulent pathogen and a susceptible plant [62, 97]. Primarily, SS depends on the activities of soil microorganisms and their metabolites [87, 127] as well as on soil physicochemical properties and environmental factors. The mechanisms underlying SS are associated with several factors, including microbiostasis and fungistasis; competition between phytopathogens for the host plant; synthesis of antibiotics, lytic enzymes, and nonvolatile antifungal compounds; activation of plant disease

Table 3. Characteristics of soil ecological functions

Characteristic	Unit	Method	Cost	Soil function
Diversity and abundance (colony forming units) of cultivated microorganisms	Number of taxa and CFU/g soil	Microbiological cultivation	Medium	Bioresource and phytosanitary
Total taxonomic diversity of microorganisms	Number of taxa/g soil	NGS	High	Bioresource and phytosanitary
α-Diversity indices of a microbiological community	Dimensionless	Microbiological cultivation and NGS	Low/high	Bioresource
Abundance of microbial genes	Copy number of genes/g soil	Real-time PCR	Medium	Bioresource
Colony number of cultivable phytopathogens	CFU/g soil	Microbiological cultivation	Medium	Phytosanitary
Gene abundance of phytopathogens	Copy number of genes/g soil	Quantitative PCR	Medium	Phytosanitary
Microbial biomass carbon	μg C/g soil	Gas chromatography; fumigation–extraction; determination of PLFA, DNA, or ATP content; and luminescence microscopy	Low/medium	Carbon transformation
Counts of living and dead microbial cells	Number of cells/g soil or %	Luminescence microscopy	Medium	Carbon transformation
Fungi-to-bacteria ratio	Dimensionless	Measurement of PLFA content and luminescence microscopy	Medium	Carbon transformation
Basal respiration	μg CO_2–C/(g soil h)	Gas chromatography	Medium	Carbon transformation
Metabolic quotient (qCO_2)	μg CO_2–C/(mg C_{mic} h)	Gas chromatography	Low	Carbon transformation
Substrate decomposition rate in soil	Days or weeks	Weighing	Low	Carbon transformation
Community-level physiological profiling (CLPP)	Absorption units	Measurement of absorption	Medium	Carbon transformation
Potential enzyme activity	mg enzyme/(100 g soil h)	Measurement of absorption/fluorescence	Medium	Carbon transformation and plant nutrition
PLFA content of the fungi forming arbuscular mycorrhiza	nmol/g soil	Gas chromatography methods	Medium	Plant nutrition
Gene abundance of the fungi forming arbuscular mycorrhiza	Copy number of genes/g soil	Quantitative PCR	Medium	Plant nutrition
Spore abundance of the fungi forming arbuscular mycorrhiza	Spore counts/g soil	Direct light microscopy	Low	Plant nutrition
Abundance of functional genes associated with particular process of N and P cycles	Copy number of genes/g soil	Quantitative PCR	Medium	Plant nutrition
Potential activity of processes of N and P cycles	mg of N or P/(kg soil h)	Gas chromatography methods	Medium	Plant nutrition

resistance genes; and improvement of nutrition and disease resistance of the whole plant [67, 87, 92]. A microbiome with high biodiversity and high abundance of microbial antagonists considerably limits the development of phytopathogens [87, 113, 121].

Development of objective methods for assessing SS is of a paramount importance for phytopathology and plant protection [42]. The total SS can be assessed using plate counting [83] and dilution technique. The degree of plant infection in this method is characterized according to the infection of plant underground organs with the help of microbial plating and calculation of disease development indices. Toropova et al. [42] propose a new index to characterize SS, namely, the coefficient of parasitic activity of a pathogen, which is determined by the ratio of disease development index to the population density of pathogen's infectious structures. Thus, the coefficient of parasitic activity shows the degree of plant affection induced by one infectious unit of a phytopathogen [42].

Currently, microbiological indicators of SS are actively searched for; most of the indicators are associated with the estimation of the abundance of either microbial phytopathogens or their antagonists [96, 97, 141, 142]. The abundance of such microorganisms is assessed either by cultivation of the target phytopathogen groups on nutrient media [28, 39, 42] or with the help of molecular gene approaches (real-time PCR, metabarcoding, etc.) [50, 94]. Since the counting of phytopathogenic microorganisms on selective nutrient media is a time- and labor-consuming procedure [42, 139] and many phytopathogens are poorly cultivable or uncultivable at all [50, 128], more reliable indicators able to adequately estimate SS are demanded. One of the promising approaches to assessment of soil phytosanitary state is determination of the copy number of ribosome genes of the microorganisms that according to the existing databases [76, 98, 106] belong to the most abundant phytopathogens, such as *Ralstonia* and *Erwinia* bacteria*; Fusarium, Alternaria, Rhizoctonia, Phoma,* and *Verticillium* microscopic fungi*;* oomycetes *Phytophthora* and *Pythium* [50]. For example, an increase in the abundance of *Ralstonia* species in soil causes an increase in plant infection rate; however, the application of organic fertilizers decreases the abundance of phytopathogens and restores soil phytosanitary function [99, 151].

CONCLUSIONS

The considered microbiological parameters can be used to assess the directions of the processes associated with the ecological functions performed by soil, namely bioresource, carbon transformation, plant root nutrition, and phytosanitary. The main microbiological indicators of soil ecological functions are listed in Table 3. The indicators have their own advantages and shortcomings; some of them report only a single function and others can record two functions at once; the principles underlying these methods and their cost vary.

Diversity indices, taxonomic richness/profile of microbiome, and abundances of certain microbial groups are best applicable to characterize the soil bioresource function. The main microbiological indicators of the carbon transformation function are microbial biomass carbon, soil respiration, enzyme activity, and SOM decomposition rate. The optimal microbiological indicators for assessing the plant nutrition is the enzyme activities responsible for a particular stage in biogeochemical cycles of macroelements, the abundance of genes coding for the corresponding enzymes, and species-specific molecular markers of arbuscular mycorrhiza. Determination of the copy numbers of the markers genes of the most abundant phytopathogens—bacteria *Ralstonia* and *Erwinia;* microscopic fungi *Fusarium, Alternaria, Rhizoctonia, Phoma,* and *Verticillium;* and oomycetes *Phytophthora* and *Pythium*—is proposed as the method to assess soil phytosanitary function.

Since a high variation of some characteristics and certain difficulties in their interpretation are currently the relevant problems in microbiological indication of soil, the key problem is a careful choice of the parameters most pertinent to the indication of soil ecological functions.

Most of the considered classical methods in microbiology are applicable to assessment of only a small part (sometimes rather far from the existing diversity in soil) of the taxonomic and functional diversities of microorganisms. Molecular biological methods have a considerably higher resolution and, correspondingly, are absolutely necessary to resolve the key problems in profiling the soil microbiome. For example, cultivation of microorganisms is inapplicable to the assessment of taxonomic diversity of uncultivable species and luminescence microscopy gives considerable errors in determination of prokaryotic and fungal biomasses. Molecular methods—quantitative PCR, metabarcoding, metagenomics, abundance of functional genes, genetic markers for arbuscular mycobionts and phytopathogens, and the content of PLFAs and adenosine triphosphates—are the most appropriate for this purpose.

When using microbiological indicators of soil functions, it is important to understand that there is no "optimal" soil or a universal set of soil characteristics. They should mainly serve for a comparative characterization of the soils similar in their other characteristics. Although some microbiological characteristics are highly variable and poorly interpretable with respect to microbiological indication of soil, they have a high potential as integrated sensitive indicators of soil functions. Taking into account the progress in molecular biological methods, the number of various soil microbiological characteristics will undoubtedly increase. Thus, a key problem is to carefully select the

parameters with the best applicability as indicators of soil ecological functions.

FUNDING

The study was supported by the Ministry of Education and Science of the Russian Federation (agreement no. 075-15-2020-805 of October 02, 2020).

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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Translated by G. Chirikova