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## **THE SPECIFITIES OF MICROORGANISMS LIFE IN SOIL**

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### **ABSTRACT**

*Despite the importance, scale, and intensity of the nitrogen cycle processes in Earth's biosphere, they are poorly understood, and the role of soil in the creation and absorption of nitrogen gases remains unclear, even though nitrogen largely determines the soil's ability to sustain productivity in terrestrial ecosystems. The state of terrestrial ecosystems and, of course, the stability of the biosphere directly depend on the content and rate of nitrogen transformation in soils, however, the investigation of nitrogen behavior in terrestrial ecosystems is only done in a fragmented manner. Therefore, the task of this review is to further clarify these phenomena and to focus the attention of younger generations of researchers in this field.*

**Keywords:** Nitrogen fixation, denitrification, microorganisms, nitrification, soil.

### **INTRODUCTION**

In terms of the number of microorganisms, terrestrial ecosystems (soils) significantly surpass all other geospheres (water, sludge, air). From this, it follows that they also significantly influence the intensity and extent of microbial transformation processes of various chemical elements, resulting in alterations of the properties of terrestrial ecosystems (soils) to the same extent reflecting on the state of the entire biosphere. However, the contribution of the terrestrial microbial community (soils) to the global cycling of chemical elements has so far been relatively well-defined only for carbon, while their role in the transformation of other biogenic elements, especially nitrogen, is still insignificant.

### **THE ROLE OF MICROORGANISMS IN THE TRANSFORMATION OF VARIOUS ELEMENTS**

As known, unlike higher organisms (eukaryotes), microorganisms (mostly prokaryotes) actively transform most other chemical elements that are not utilized in animal cell metabolism. Among them are mercury (Hg), arsenic (As), selenium (Se), antimony (Sb), cadmium (Cd), chromium (Cr), lead (Pb), tin (Sn), tellurium (Te), palladium (Pa), bismuth (Bi), aluminum (Al), silver (Ag), gold (Au), lithium (Li), uranium (U), technetium (Tc) and others. According to existing data, microorganisms are capable of transforming a total of 65–68 chemical elements. Due to the action of microorganisms, the migratory capability of these elements typically increases significantly, leading to their

dispersion over large territories, causing what Kovda figuratively described as the growing 'metalization of the biosphere'. Early research by Vernadsky and Polinov (1992) and their followers convincingly testifies to the primary role of biological factors in the fragmentation of (mountain) rocks and primary pedogenesis. According to existing data, microbiological processes, in terms of the scale and depth of reactions, occupy a leading position in the transformation of elements in the Earth's crust during the degradation of minerals and rocks. It has been proven that under natural conditions, all rocks and minerals (not only on the surface, but also at significant depths— 2 to 5 km deep) are abundantly inhabited by various types of microorganisms. Under favorable conditions, they become active and degrade them, extracting (leaching) various chemical elements. Degradation of rocks and minerals can occur under the direct or indirect influence of microorganisms and their life activity products. Several mechanisms of such action are known: oxidation and reduction of elements with variable valence, action of biogenic acids and bases (acidolysis and alkalolysis), complexolysis (chelation), and biosorption.

Elements of variable valence (S, N, Fe, Mn, Sb, Mo, Cu, As, etc.), when present in rocks and minerals in a reduced state, are capable of serving as an energy source for autotrophic bacteria, transitioning into their oxidized form. Bacteria most easily oxidize divalent sulfur in sulfide minerals (antimonite, bismuthinite, galena, molybdenite, pyrite, sphalerite, etc.). In this process, sulfur from sulfide converts into hexavalent sulfate, leading to the formation of various secondary sulfate minerals such as gypsum, alunite, and jarosite which are characteristic of many soils (Watkinson and Blair, 1993). Nitrification is another widely spread process in soils. It involves the bacterial oxidation of trivalent nitrogen (in the form of ammonia), resulting in the formation of pentavalent nitrogen nitrate. Apart from sulfur and nitrogen, other chemical elements such as divalent iron, manganese, antimony, monovalent copper, molybdenum, uranium, and a range of other elements can serve as an energy source for autotrophic bacteria. Under the influence of microorganisms, minerals containing these elements are degraded, transforming into other minerals that are usually more soluble and mobile.

The opposite process—microbial reduction of elements with variable valence is also widely spread in soil and plays an important role in the biogeochemical cycles of modern biogeocenoses. The most characteristic is the microbial reduction of Fe (III), Mn (IV), Co (III), Cr (VI), Mo (VI), Hg (II), and several other metals, which actively occurs during changes in soil moisture (aerobic conditions) and is associated with the cycles of nitrogen, oxygen, and sulfur, significantly impacting the state of the natural environment. The process is mainly carried out by heterotrophic microorganisms, which use these elements as electron acceptors during the oxidation of organic matter in the absence of free oxygen during anaerobic respiration. Examples of processes that occur during this include gel formation (reduction of trivalent iron), denitrification (reduction of pentavalent nitrogen), sulfate reduction (reduction of hexavalent sulfur) methanogenesis, and others (Li et al., 2012).

### **ACIDOPHILICITY AND ALKALOGENICITY OF MICROORGANISMS**

Acidity (alkalinity), influenced by organic and mineral acids and bases of microbiological origin, is another important natural factor in mineral formation and primary pedogenesis. Microorganisms, without exception, possess the ability to produce acids and bases, but autotrophic bacteria and microfungi (microscopic fungi) are the most active in this regard. Nitrifying bacteria produce nitric and nitrous acid, sulfur bacteria produce sulfuric and sulfurous acid and many sulfate-reducing bacteria release hydrogen

sulfide. Various microorganisms carry out ammonification and produce ammonia - a biogenic alkali, which also acts as an active geochemical agent. Microscopic fungi are known as producers of strong organic acids - acetic, oxalic, succinic, citric acids, among others, under whose influence any rocks and minerals gradually decompose.

### **MICROBIAL DEGRADATION OF COMPLEXES**

Complexolysis (helactogenesis) is a widely prevalent process of biological weathering of rocks and minerals. The formation of organometallic complexes can occur simultaneously with the action of biogenic acids, alkalis, and other microbial metabolites. According to modern understanding, helactogenesis is the fundamental mechanism of rock and mineral degradation during the development of heterotrophic microorganisms. Therefore, one of the main characteristics of chelates is their good solubility. Chelation significantly increases the mobility of chemical elements. Conversely, during the degradation of chelates, which is also carried out by microorganisms utilizing the organic portion of their molecule, there is a local concentration of these elements.

### **MICROORGANISMS' INVOLVEMENT IN BIOLOGICAL ELEMENT SORPTION**

Biosorption is another way microorganisms influence chemical elements in natural environments, as pointed out by Vernadsky (1992). The basis of biosorption lies in processes where chemical elements interact with the surface structures of microbial cells, their metabolites, and exopolymers. Many bacteria and microscopic fungi accumulate significant amounts of various elements in their biomass, leading to their concentration in areas of massive growth or aggregation of these organisms.

Microbiological biosorption of certain metals (gold, uranium, copper, cadmium) from low-concentration natural solutions is widely applied in modern biotechnology (Abbas et al., 2014; Đukić and Jemcević, 2003; Đukić et al., 2013; Đukić et al., 2015; Đukić and Mandić, 2016; Đukić et al., 2018; Đukić et al., 2020).

### **MICROBIAL DEGRADATION OF MINERALS**

It has been established that in the surface layers (2–5 km) of the Earth's crust (in the zone of hypergenesis), microorganisms are the main factor in the degradation of all major groups of minerals - sulfides, arsenates, silicates, oxides, and hydroxides. The oxidation of sulfide minerals is the most widespread example of microbial mineral degradation in soil. Bacteria oxidize copper sulfides (pyrite and marcasite, pyrrhotite, covellite, chalcopyrite, bornite, etc.), nickel (millerite), cobalt (cobaltite), zinc (sphalerite, marmatite), molybdenum (molybdenite), lead (galena, geokronite). The oxidation of arsenic compounds - arsenite (realgar, orpiment, etc.) is also widespread. These processes are most active during increased soil aeration - during drying, plowing, and tilling. Various microorganisms degrade silicates by breaking down siloxane and alumino-silicate bonds, which form the basis of the crystal structure of these minerals. Among other minerals that undergoing microbial transformation in soil, compounds of manganese and iron are highly prevalent (Lamers et al., 2012). There are over 100 minerals known to contain manganese, including oxides, carbonates, and silicates. The most important oxides are psilomelane, birnessite, pyrolusite, and manganite; among carbonates, rhodochrosite is notable, and



among silicates, rhodonite. All these minerals are secondary and result from microbial degradation of primary minerals of volcanic origin during primary pedogenesis. The role of nitrogen in the biological productivity of soil and water. As it is known, the activity of terrestrial microorganisms has led to the rapid complexity of microzones and the emergence of numerous microsites in the soil profile where the physicochemical conditions significantly differ. This has ultimately contributed to the prevailing belief, among which microbial nitrogen fixation is the most important, providing not only for the immediate needs of organisms but also for the accumulation (reserving) of nitrogen in the form of various nitrogen compounds. Nitrogen fixation, which appeared in the earliest living organisms (bacteria, archaea), practically simultaneously with the origin of life on Earth, has gained global significance with the planet's expansion, and the nitrogen bound during this process has begun to play a crucial role in the biosphere. Microbial nitrogen fixation actively occurs in various loci (microcosms) with a wide range of physical and chemical conditions (different types of soil, river and marine water, mud, plant rhizosphere and phyllosphere, animal gastrointestinal tract, etc.), ultimately contributing to maintaining the dynamic balance of nitrogen compound concentrations in nature. Soil microorganisms play a particularly significant role in forming and maintaining nitrogen biogeochemical cycles, primarily through biological fixation (James, 2000).

During evolution, the products of former biospheres in the form of various organic residues-caustobiolites and kerogens (coal, oil, sapropel, peat, humus)-gradually became diverse, and their total quantity increased. Simultaneously, the amount of reserve nitrogen in them increased, mainly as a result of the activity of nitrogen-fixing bacteria, transforming into various nitrogen compounds that differ in composition and duration in the natural environment. Among biogenic elements, nitrogen stands out due to the high mobility of all natural compounds and the rapid rate of metabolism, which explains the lesser occurrence of their accumulations in nature (in the form of minerals and agronomic ores) and in the composition of reserve substances in living cells. The longest-lasting nitrogen in soil organic matter, mainly humus, is the primary reservoir of 'biological' nitrogen in the biosphere. Only soils have the ability to accumulate (immobilize) nitrogen bound during nitrogen fixation and serve as the only long-term depot of this element in the biosphere. However, this nitrogen is resistant to mineralization and cannot serve as an easily accessible source for most organisms, leading them to rely on other methods to supplement it, among which symbioses and associations with diazotrophic bacteria are the most widespread.

### **MICROBIAL NITRIFICATION**

Another important microbiological component in the global nitrogen cycle is nitrification. Nitrification is carried out by two fundamentally different groups of microorganisms. The first group consists of taxonomically and physiologically homogeneous bacteria that are highly specialized in oxidized substrates and perform autotrophic nitrification. The second group includes taxonomically and physiologically diverse bacteria and fungi that perform heterotrophic nitrification. For a long time, it was believed that autotrophic nitrifiers played the leading role in the global process of oxidizing ammonia to nitrite and then to nitrate, while the activity of heterotrophic nitrifiers was not even considered. In recent years, thanks to new methods of studying nitrification in the natural environment, these assumptions are being reconsidered. According to the obtained data, heterotrophic nitrification plays an important, often leading role in the oxidation of reduced nitrogen compounds in many soils, and therefore, in their global cycling (Beeckman et al.,

2018). It is a widely held belief that nitrates are the end product of nitrification, easily leached from the soil and carried away by water, leading to the majority of bound nitrogen being transported from land to the ocean, remaining unchanged in the process. However, there is an increasing amount of data indicating that significant nitrogen losses from soil occur not only due to nitrate leaching but also due to the release of its gaseous forms. During nitrification, the primary gas produced is nitrous oxide ( $N_2O$ ).

### **MICROBIAL DENITRIFICATION**

Microbial denitrification is the final step in the microbiologically ‘controlled’ biogeochemical nitrogen cycle, where bound nitrogen is transformed back into atmospheric  $N_2$ . In recent years, it has been clarified that during microbial denitrification, along with molecular nitrogen ( $N_2$ ), a significant amount of nitrous oxide ( $N_2O$ ) is also produced, which is often the primary end product of the process. Despite its relative chemical inertness, nitrous oxide participates in the greenhouse effect, leading to an increasing content of this gas in Earth's atmosphere, thereby altering the planet's heat balance. Additionally, its interaction with ozone ( $O_3$ ) in the upper layers of Earth's troposphere is one of the main causes of the planet's ozone layer degradation, which protects living organisms from solar ultraviolet radiation. There is increasing evidence that under the influence of this effect, there is a slow but continuous decline in biological diversity on Earth.

Based on data obtained in the last three decades regarding the role of soil microbial communities in global nitrogen (and carbon) cycles, a new approach has been enabled to explain the causes of climate change on Earth. It is assumed that this change may be due to a highly pronounced deterioration of soil properties, accompanied by rapid loss of organic matter stocks, leading to disruption of the existing dynamic balance in the global carbon and nitrogen cycle - reducing nitrogen stocks in soil and increasing them (primarily in the form of nitrous oxide) in the atmosphere (Đukić and Jemcević, 2000; Đukić et al., 2007).

### **DEHUMIFICATION OF SOIL**

The long-maintained dynamic equilibrium in the content of different forms of nitrogen in the biosphere, mainly determined based on the amounts of nitrates in soil and water and the concentration of nitrous oxide in the atmosphere, began to change about 60 years ago. The increase in nitrate and  $N_2O$  content is conditioned by a complex of causes, encompassed by the common term ‘soil degradation’ which leads to accelerated mineralization of soil organic matter-dehumidification. Monitoring the dynamic organic matter of the soil, which has been ongoing for over 200 years (coordinated by the international program Global Change and Terrestrial Ecosystems), leaves no doubt about the acceleration of this process. Soil organic matter and its most important component - soil humus-have accumulated over a long period (from several hundred to a thousand years) and reflect the history of pedogenesis on Earth. The content of humus and other organic compounds rapidly decreases under various influences on the soil: fertilizer application, liming, soil pollution by acid rain, heavy metals, pesticides, and many other substances. Humus reacts quickly to soil drying and wetting, plowing and cultivation, whereby its content generally decreases.

‘Biological nitrogen’ which remains in the humus composition for a long time, transitions into other compounds during dehumidification, ultimately transforming into nitrous oxide and molecular nitrogen. The relationship between these gases is also

influenced by a complex set of environmental factors. Thus, changes in environmental conditions quickly impact the biogeochemical nitrogen cycle in the biosphere. Conversely, changes (increases) in the rate of nitrogen and carbon cycling affect the Earth's climate - the higher the average atmospheric temperature, the faster soils dry out and lose organic matter. The interdependence and mutual conditioning of soil properties and Earth's climate are the most important issues in contemporary fundamental pedology. Despite the importance, scale, and intensity of the nitrogen cycle processes in Earth's biosphere, they are poorly understood, and the role of soil in the creation and absorption of nitrogen gases remains unclear, even though nitrogen largely determines the soil's ability to sustain productivity in terrestrial ecosystems (Robertson and Groffman, 2015). The state of terrestrial ecosystems and, of course, the stability of the biosphere directly depend on the content and rate of nitrogen transformation in soils, yet the investigation of nitrogen behavior in terrestrial ecosystems is only done in a fragmented manner. Therefore, the task of this review is to further clarify these phenomena and to focus the attention of younger generations of researchers in this field.

## CONCLUSION

'Biological nitrogen' which remains in the humus composition for a long time, transitions into other compounds during dehumification, ultimately transforming into nitrous oxide and molecular nitrogen, the relationship between these gases is also influenced by a complex set of environmental factors. Thus, changes in environmental conditions quickly impact the biogeochemical nitrogen cycle in the biosphere. Conversely, changes (increases) in the rate of nitrogen and carbon cycling affect the Earth's climate - the higher the average atmospheric temperature, the faster soils dry out and lose organic matter. The interdependence and mutual conditioning of soil properties and Earth's climate are the most important issues in contemporary fundamental pedology. Despite the fact that the state of terrestrial ecosystems and, of course, the stability of the biosphere directly depend on the content and rate of nitrogen transformation in soils, the investigation of nitrogen behavior in terrestrial ecosystems is only done in a fragmented manner.

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