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SPECIFICITIES OF NITROGEN TRANSFORMATION IN THE BIOSPHERE

**Leka Mandić^{1,*}, Ivana Bošković², Marina Stojanova³, Vesna Đurović¹,
Monika Sojanova⁴, Dragutin Đukić¹**

¹University of Kragujevac, Faculty of Agriculture, Čačak, Serbia

²University of East Sarajevo, Faculty of Agriculture, Sarajevo, Bosnia and Herzegovina

³University of Ss. Cyril and Methodius, Faculty of Agricultural Sciences and Food,
Skopje, North Macedonia

⁴Association for Scientific-Research, Educational and Cultural Activities "Open Science",
Ohrid, North Macedonia

*Corresponding author: lekamg@kg.ac.rs

ABSTRACT

This review paper directs us towards a better understanding of the fundamental meaning of the biological nitrogen cycle, the role of diazotrophic bacteria in this process, the negative consequences of intensive use of nitrogen mineral fertilizers, the application of diazotrophic bacteria in modern agriculture, as well as the symbiosis between these bacteria and higher eukaryotic organisms (plants, animals).

Keywords: Nitrogen fixation, bacteria, nitrogen cycle, symbiosis.

THE MEANING OF THE BIOLOGICAL NITROGEN CYCLE

Advancements in studying the specifics of nitrogen transformation in soil over the past two decades have not led to a change in existing understandings of the role of terrestrial microorganisms in the nitrogen cycle at the biosphere level.

In return, the lack of such knowledge manifests not only in the inferiority of the overall biological understanding of everything but also prevents addressing the most crucial practical problems of modern civilization, among which the sustainability of biosphere development and food security of the planet's population hold a particularly significant place.

The role of nitrogen as one of the basic chemical carriers of the properties of living matter is well known. Also known are the general direction of nitrogen cycling in nature and the specificities of its components – nitrogen fixation, nitrification, ammonification, and denitrification. The biological significance of such nitrogen cycling lies in the fact that during cyclic transformations of nitrogen in the biosphere, various compounds with different durations are continuously formed, which enable all living organisms to obtain nitrogen in a form suitable for them.

The transformation of nitrogen in nature is associated with crucial issues in contemporary biology, such as the preservation of biological diversity and the sustainable development of Earth's biosphere.

In an even closer relationship with the nitrogen cycle is the problem of providing the planet's population with dietary protein (nitrogen-containing), whose scarcity has existed throughout all epochs of civilization and remains relevant today.

According to existing estimates, approximately 110-120 million tons of nitrogen are removed from the soil worldwide each year through yields, while around 80 million tons are applied to fields in the form of nitrogen mineral fertilizers, and 30 million tons are incorporated as part of organic fertilizers. Taking into account the nitrogen uptake coefficient by plants (up to 50% for mineral fertilizers and 30% for organic fertilizers) from these sources, the average global yield includes approximately 40-45 million tons of nitrogen, or about one-third of its removal.

Modern nitrogen fertilizer production meets at most one-third of the total needs of plant production worldwide in that element, and the primary quantity of nitrogen for agricultural plants is obtained from soil nitrogen reserves, which are formed and maintained thanks to the activity of nitrogen-fixing microorganisms.

THE ROLE OF DIAZOTROPHIC BACTERIA

Nitrogen-fixing bacteria (diazotrophs) are the only inhabitants of the planet that assimilate atmospheric nitrogen (N_2) and provide not only for themselves but also for other organisms with this element, making the process of atmospheric nitrogen fixation play a leading role in nitrogen balance both in nature and in fields.

Nitrogen fixation is the primary source of accessible nitrogen for all living organisms in the biosphere in all periods of its development, although only a negligible part (around 0.0007%) of its total reserves on Earth simultaneously represents "biological" nitrogen (Reed et al., 2011; Đukić et al., 2007).

It is presumed that the phenomenon of nitrogen fixation originated as early as the early precambrian and has existed without significant changes for several billion years (Mišustin, Šiljnikova, 1968; Jemcev, Đukić, 2000). Initially, nitrogen fixation appeared in anaerobic prokaryotes, which use the energy of fermentation in the "primitive broth," and then the ability of nitrogen fixation spread among all groups of bacteria and archaea.

A probable evidence of the "ancientness" of nitrogen fixation can be considered the low substrate specificity of the nitrogenase enzyme, which carries out this process, i.e., the ability to reduce not only N_2 but also a range of other compounds with a triple bond in their molecules (acetylene, cyanides, etc.), which distinguishes it from other enzymes known for their extremely high substrate specificity (Kretovič, 1995).

Contrary to the previously prevalent understanding of diazotrophy being present only in a narrow group of highly specialized bacteria, there is now a growing belief in the ability of nitrogen fixation among all prokaryotes (bacteria and archaea), belonging to various physiological and taxonomic groups – lithotrophs, phototrophs, and heterotrophs, aerobic, microaerophilic, and anaerobic, filamentous, budding, and mycelial, gram-positive and gram-negative (Raymond et al., 2004).

As an indirect confirmation of the "relatedness" of all bacteria in terms of this property, it can be noted that nitrogen-fixing enzymes – nitrogenases from different bacteria containing molybdenum – are not only similar in structure but also capable of forming active "hybrid" nitrogenases from components extracted by different organisms (Eady, 1996).

The specificity of nitrogenase lies in its slow activity, with a very low turnover rate (about 1.5 s at 23 °C), requiring a large amount of energy (28 M ATP for reducing 1 M N_2 , or 12 g of glucose for fixing 1 g of nitrogen), and nitrogen fixation is the most intensive process in a living cell (Kretovič, 1995).

In natural ecosystems, nitrogen as a biofilming element does not limit their productivity due to the high balance of all components of its biogeochemical cycle, where

the nitrogen income fully covers all expenditure items, and a part of nitrogen becomes part of the soil organic matter. Contrary to that, in agroecosystem soils, nitrogen is a fundamental element that determines their productivity because the nitrogen cycle in these soils is not only highly disrupted but also disturbed due to frequent soil tillage and continuous crop rotation, which disrupt microbial communities responsible for nitrogen fixation. Along with the regular removal of nitrogen with the yield, all of this leads to its deficit in arable soils and necessitates the regular application of nitrogen fertilizers. Exporting products from agroecosystems increases the imbalance between providing agricultural plants with accessible nitrogen and its availability through plant residues and waste from yield processing. Moreover, this imbalance worsens because the main consumption of nitrogen-containing products is geographically separated from their production. Nitrogen mineral fertilizers serve as a palliative measure for this issue.

NEGATIVE CONSEQUENCES OF INTENSIVE USE OF NITROGEN FERTILIZERS

The intensive use of nitrogen mineral fertilizers (in tenfold quantities) and the implementation of plant varieties that require high doses of nitrogen led to the success of the "green revolution" in crop production in the 1950s and 1960s. However, it did not completely solve the world's food problem. In contemporary society, such technologies provide balanced protein nutrition for the population of industrially developed countries, while developing countries still face a chronic protein deficit in their diets.

The widespread use of nitrogen fertilizers in developed countries has become the cause of well-known negative effects in nature: the influx of nitrates into groundwater, accelerated mineralization of soil organic matter (dehumification), and the consequent increase in greenhouse gas emissions (N_2O , CO_2 , CH_4) from the soil. This not only leads to local deterioration of the natural environment but also results in an imbalance of the nitrogen cycle at the global level, which is already affecting the stability of Earth's climate and ozone layer, manifesting in various disruptions of fundamental biosphere functions. It is also known that the synthesis, transport, storage, and application of nitrogen fertilizers require significant amount of energy. Every year, approximately 35% of the total energy consumed in agriculture worldwide is used for the production of 80 million tons of fertilizer (Šumnij, 1991). According to calculations, increasing yields from 1 to 2 tons per hectare, which is necessary to combat the existing food deficit worldwide, increases energy losses by 10 times (Žučenko, 1996). The rising cost of energy sources increases the price of nitrogen fertilizers, whose high cost is the main reason for their limited use in developing countries.

APPLICATION OF DIAZOTROPHIC BACTERIA IN MODERN AGRICULTURE

Bacteria reduce nitrogen, mostly using solar energy, and their application enables significant reductions in energy losses in the production of nitrogen fertilizers. Additionally, microbiological fixation of atmospheric nitrogen is the only environmentally clean way to supply plants with accessible nitrogen, practically eliminating pollution of soil, water, and air. This is due to the tight coupling between nitrogen fixation and photosynthesis in most nitrogen-fixing systems, where all fixed nitrogen is immediately used for the synthesis of nitrogen compounds in plant cells, after their death, it binds to soil organic matter, improving its quality and reducing nitrogen leaching and loss in the form of gases.

It's not a coincidence that in the system of so-called biological (biological, sustainable, alternative) farming, which is becoming increasingly recognizable and widespread, there is a recognized necessity, on the one hand, to limit the use of mineral nitrogen fertilizers as much as possible, and on the other hand, to increase the share of environmentally friendly and cost-effective "biological" nitrogen.

The prevailing opinion is that the stability of global agriculture and the growth of plant productivity are impossible without strengthening the activity of diazotrophic bacteria in the soil (Canfield et al., 2010).

Predicting the situation regarding food (protein) security in modern civilization, it can be argued that the role and share of "biological" nitrogen in agriculture and plant production will continue to grow steadily throughout the 21st century.

As a result, in the mid-1970s, the issue of "biological nitrogen" gained priority status in science, and the global scientific community began actively studying its specifics. Many achievements in this field of science have general biological significance, confirming the leading role of microbial nitrogen fixation in the biosphere.

Until recently, nitrogen fixation was believed to be inherent to a narrow range of microorganisms, including representatives of the genera *Azotobacter*, *Clostridium*, *Rhizobium*, and cyanobacteria – Mišustin, Šiljnikova (1968).

As noted, this capability has been identified in representatives of most physiological taxonomic groups of bacteria and archaea, which has led to the postulation of its presence in all prokaryotes.

Nitrogen-fixing organisms finally exclude eukaryotes such as fungi, algae, plants, and animals, and nitrogen fixation is included in the list of fundamental differences that separate prokaryotes from eukaryotes.

A new and unexpected discovery was the ability of many nitrogen-fixing bacteria to switch to the opposite process - denitrification in the presence of mineral nitrogen compounds (nitrates) in the environment. This duality of behavior has been observed in representatives of genera such as *Agrobacterium*, *Alcaligenes*, *Aquaspirillum*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Desulfovibrio*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Klebsiella*, *Methanobacterium*, *Pseudomonas*, *Rhizobium*, *Spirillum*, *Thiobacillus*, *Vibrio* and this list continues to expand (Umarov, 2001; Roesch et al., 2007).

SYMBIOSIS BETWEEN DIAZOTROPHIC BACTERIA AND HIGHER EUKARYOTIC ORGANISMS

To understand the role of nitrogen-fixing bacteria in maintaining the productivity and stability of the biosphere, it is important to discover, in terms of composition, the various symbioses with eukaryotic organisms, not only with plants but also with animals.

According to modern understanding, symbiosis is not just the ability of organisms of different species to live together, but also a special form of life in which the combination of different components transforms into an integrated system that has its own unique morphology, anatomy, physiology, and ecology (Golovlev, 2000).

It is also assumed that the continuous existence of life on Earth is a result of organisms' persistent ability to form symbioses, which are more of a rule than an exception for various biocenoses.

Nitrogen-fixing symbioses vary greatly in the composition of organisms that make them up, but they share one common characteristic - a tight linkage of nitrogen and carbon biogeochemical cycles (Tihonovič et al., 2004). Such integration of nitrogen and carbon metabolism is most characteristic of symbioses between bacteria and plants (Table 1).

In nature, important roles are played by cyanosymbioses - symbioses of cyanobacteria with protists, animals, fungi, and plants. Being the oldest symbiotic relationships on Earth, characterized by pronounced structural-functional plasticity and combining the ability of photoautotrophy and diazotrophy, they are the most prevalent in the biosphere (Skripnikov, 2006).

Table 1. Nitrogen-fixing symbioses between plants and bacteria

Plants (families)	Bacteria (genera)	N ₂ fixation (kg/ha/season)
<i>Fabaceae</i>	<i>Rhizobium, Azorhizobium, Bradyrhizobium, Mesorhizobium, Sinorhizobium</i>	350
<i>Ulmaceae</i>	<i>Bradyrhizobium</i>	70
<i>Betulaceae</i>	<i>Frankia</i>	300
<i>Casuariaceae</i>	<i>Frankia</i>	50
<i>Coriariaceae</i>	<i>Frankia</i>	No data available
<i>Datisacaceae</i>	<i>Frankia</i>	No data available
<i>Elaeagnaceae</i>	<i>Frankia</i>	No data available.
<i>Myricaceae</i>	<i>Frankia</i>	No data available.
<i>Rhamnaceae</i>	<i>Frankia</i>	No data available.
<i>Rosaceae</i>	<i>Frankia</i>	No data available.
<i>Azolla</i>	<i>Anabaena (Nostoc)</i>	120
<i>Ceratozamia</i>	<i>Anabaena (Nostoc)</i>	60

Cyanobacteria form symbioses with representatives of four major phylogenetic groups of higher plants - bryophytes, ferns (azolla), gymnosperms, and angiosperms. In addition, the localization sites of cyanobacteria (cyanobiont) in the host plant's body can significantly differ in various symbioses. In Anthocerotophyta, the cyanobiont is localized in specialized cavities on the lower side of the thallus, where special outgrowths are found, significantly increasing the surface area of the mutual connection and facilitating the transport of sucrose from the plant and bound nitrogen in the opposite direction. The efficiency of such a system is very high - less than 1% of fixed nitrogen is released externally.

In symbiosis with cycads, cyanobacteria localize in specialized coralloid roots (coralloid nodules), where the cyanobiont occupies a distinct zone. In symbioses with angiosperms, cyanobacteria inhabit specialized glands at the base of leaflets.

The most well-known and prevalent cyanosymbiosis is that of the water fern *Azolla* with the nitrogen-fixing bacterium *Anabaena (Nostoc)*, according to contemporary data), which has been used in tropical agriculture for centuries to improve nitrogen nutrition in crops. Symbioses between marine cyanobacteria and diatomaceous algae play an important role in the global biological nitrogen fixation system.

One of the most important characteristics of all cyanosymbioses is the production of polysaccharide mucilage, which can be produced by either the host plant, the cyanobiont, or both partners simultaneously. The abundant production of mucilage is also characteristic of the rhizoplane of higher plants, which is inhabited by heterotrophic diazotrophic bacteria. It can be assumed that the mucilage prevents the diffusion of oxygen and thereby protects nitrogenase from its inhibitory effects.

However, despite the high efficiency of nitrogen fixation in symbioses, their contribution to the overall balance of "biological" nitrogen in the biosphere is relatively

small, this is due to the limited spread of such communities - even in agroecosystems, the share of leguminous crops does not exceed 10% of the total cultivated area, and in natural phytocenoses, leguminous plants are present only in the early stages of plant succession and are practically absent in climax ecosystems.

In nature, nitrogen is primarily fixed through associative nitrogen fixation, through the interaction of bacteria and plants that do not create specialized organs (nodules) on their roots and stems (Döbereiner, Pedrosa, 1987; Peix et al., 2015).

The type of nitrogen fixation is widely prevalent on the planet and plays a leading role in maintaining the nitrogen balance of the biosphere. According to current estimates, thanks to associative nitrogen fixation in temperate climate zones, at least 30-50 kg N₂/ha of nitrogen enters the soil annually, while in tropical zones, it can be as high as 100 kg/ha.

In the animal world, nitrogen fixation was first discovered in insects - termites, cockroaches, aphids, and locusts, which mainly feed on cellulose, pectin, and other carbohydrate compounds that do not contain nitrogen. These compounds are broken down by cellulolytic microorganisms in the animals' gastrointestinal tract into monomers, which are then utilized by diazotrophic bacteria that live symbiotically with them. The amount of nitrogen that enters the host organism can be judged, for example, based on termites, where the contribution of "biological" nitrogen reaches 60% of their total nitrogen content in their bodies (Tayasu, 1998).

About twenty years ago, microbial nitrogen fixation was discovered in vertebrates, with the most active being in herbivorous mammals (voles, beavers, etc.), which primarily consume green parts of plants that are low in nitrogen (Naumova et al., 2000).

Based on the data obtained, bacterial nitrogen fixation can be considered to have significant physiological importance for phytophagous mammals. It is very likely that other ecological niches with high levels of microbial nitrogen fixation will be found. According to preliminary data, nitrogen fixation actively occurs in the rumen of ruminants, and "biological" nitrogen plays a prominent role in their nitrogen nutrition.

The data about the significant role of microbial nitrogen fixation in nature becomes even more impressive when considering that the total amount of nitrogenase simultaneously present in the biosphere is estimated to be only a few kilograms (Vens, 2002).

In the biosphere, four types of bacterial nitrogenases are known, which are complementary to each other: the "classic" molybdenum-dependent nitrogenase and three "alternative" nitrogenases - vanadium-dependent, iron-dependent, and superoxide-dependent. Undoubtedly, such duplication allows not only diazotrophic bacteria but also all organisms to avoid a deficit of necessary or bound nitrogen in the absence of molybdenum in the soil. Molybdenum is a relatively rare element in Earth's crust (its concentration is around 1 g/t) and it is unevenly distributed, consequently, its deficiency in many areas could lead to the blocking not only of nitrogen fixation but also of other nitrogen cycle processes, since the enzyme systems that carry them out contain molybdenum.

NATURAL ASYMBIOTIC NITROGEN FIXATION

The binding of molecular nitrogen can also occur in non-biological high-temperature processes - during electrical discharges in the atmosphere, volcanic eruptions, fires, etc. However, their overall contribution to the planet is not greater than 10% of microbial nitrogen fixation.

Even a brief overview of information about "biological" nitrogen in bacteria allows us to conclude that nitrogen fixation is one of the main functions in the biosphere and the primary way to supply it with accessible nitrogen.

In terms of significance for living nature, nitrogen fixation and assimilation of N_2 can be compared only to another global process - photosynthesis.

The specificity of nitrogen, which distinguishes it from other biophilic elements like (carbon, phosphorus, and sulfur), lies in the fact that it practically does not accumulate in a living cell (with the exception found in some cyanobacteria in the form of a reserve protein - cyanophycin). Moreover, as first noted by Libig, the more nitrogen enters the organism with food, the more it is excreted in the form of nitrogen metabolism products.

Another specificity of nitrogen lies in the high solubility and mobility of all its compounds, which leads to the absence of nitrogen minerals and ores in nature, with primary minerals containing "traces" of nitrogen.

The only exception is soils that are capable of retaining and accumulating nitrogen in organic matter, becoming a unique natural reservoir of accessible forms of this element. It is thanks to this ability that soil maintains important properties such as high biological diversity and productivity (fertility), which at the biosphere level manifest in its stability and sustainable development.

As observed, soils are characterized by the closed biogeochemical cycles of most chemical elements. However, the nitrogen cycle in soil is open because its important links (nitrification, denitrification) lead to the formation of gaseous compounds (N_2 , N_2O , NO , etc.), which enter the atmosphere and only gradually return to the soil.

About 70 years ago, the global nitrogen balance, primarily determined by the amount of nitrate (NO_3^-) in soil and natural waters, and the content of nitrous oxide (N_2O) in the atmosphere, was close to neutral and did not raise any questions. Since then, there has been a continuous increase in the concentration of NO_3^- and N_2O , which has already attracted the attention of not only geochemists but also many other specialists. The main concern is the nitrogen biogenic gases in the greenhouse effect and global climate change.

As is known, the amplification of this effect is primarily associated with the increase in the concentration of so-called greenhouse gases (CO_2 , CH_4 , and N_2O) in Earth's atmosphere due to disrupted global carbon and nitrogen cycles (Bouwman, 1990).

In addition to contributing to the greenhouse effect, nitrous oxide is the primary cause of planetary ozone layer degradation, leading to an increase in the dose of strong ultraviolet radiation from the Sun on Earth's surface, directly impacting the biological diversity of the biosphere and its sustainability.

CONCLUSION

This review paper directs us towards a better understanding of the fundamental concept of the nitrogen cycle, the role of diazotrophic bacteria in this process, the negative consequences of intensive use of nitrogen mineral fertilizers, the application of diazotrophic bacteria in modern agriculture, as well as the symbiosis between these bacteria and higher eukaryotic organisms (plants, animals).

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