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The Importance of Reducing Nitrate Concentration in Soil

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ABSTRACT

Excessive nitrogen fertilization leads to a high nitrate concentration in plants (vegetables). Nitrates enter the human body via food and convert to nitrites which have a toxic effect. With the aim of preserving biodiversity, plant health, human health and a series of other ecological factors, microbial processes; denitrifications, Dissimilatory Nitrate Reductions to Ammonium (DNRA) and the anaerobic ammonium oxidation (annamox) will be presented in this paper. These are the three key processes for reducing nitrates in the soil with the goal of maintaining balance.

Keywords: *Nitrates; Health; Denitrification; DNRA; Anammox*

Introduction

Nitrogen is an integral part of many compounds in the plant cell; it promotes root and above-ground part growth, which is why nitrogen fertilizers are often used in agricultural soils. The lack of nitrogen in the plant causes slow growth of the plant and the phenomenon of chlorosis. However, it is important to be careful when applying nitrogen fertilizer because it affects the amount of nitrates in vegetables. In the human body, nitrates ingested *via* food and water convert to nitrites, which can endanger human health by oxidizing hemoglobin to methemoglobin. Excess nitrogen in the soil results in an intense growth of vegetative organs and a darker, cyan leaf color with more negative (agronomic, economic and environmental) consequences. Therefore, it is necessary to comprehend the way that nitrates get into the soil and vegetables, the way that affects human health, and how to reduce nitrate concentrations in the soil. In addition, due to the correlation of nitrates and plants in the soil, it is necessary to understand the way of reducing the nitrate concentration in fruitage (vegetables), thus ensuring prevention from their possible toxic effects on the human body [1]. The aim of this paper is to highlight the importance of reducing the excess nitrate in the soil via microbial processes of denitrification, Dissimilatory Nitrate Reduction to Ammonium (DNRA) and anaerobic ammonium oxidation, in order to avoid the negative effect of excess nitrogen in the soil. More precisely, in order to avoid a number of environmental and ecological health issues, of which water eutrophication, biodiversity loss, illicit quantities of certain elements in food and drinking water, global warming and ozone depletion should be emphasized [2].

Literature Review

Nitrogen Circulation

Ammonification is the process by which microorganisms break down protein molecules to amino acids and then to ammonia. Ammonia, unused by plants and microorganisms, is further subjected to the nitrification process. In the first stage of nitrification, ammonia is oxidized to nitrite, and this phase is called nitration. In the second stage, nitration occurs, which is the reaction of oxidation to nitrate [3]. When nitrates are found under anaerobic conditions, their reduction (i.e. nitrification) occurs, where the nitrates are reduced to the appearance of gaseous nitrogen, which leaves the atmosphere and becomes inaccessible to plants. However, there is a special group of microorganisms that binds elemental nitrogen from the air and transforms it into forms that are readily available to plants. This process is called nitrogen fixation and is divided into abiotic nitrogen fixation, which takes place without the presence of microorganisms, whether it is naturally or artificially initiated, and the biological fixation of atmospheric nitrogen, which takes place in the presence of a special group of microorganisms [4]. Moreso, biotic fixation can be divided into free, associative and symbiotic, and it is important to note that nodule bacteria (Rhizobium, Bradyrhizobium) and leguminous plants are involved in symbiotic nitrogen fixation. The nitrogenase enzyme, which allows the reduction of elemental nitrogen to ammonia, is crucial throughout the process, and the respiratory pigment leghemoglobin is responsible for the red color of the nodules when they are cut, it maintains a low oxygen concentration in bacterial cells, and thus activaties the enzyme nitrogenase (Figure 1).

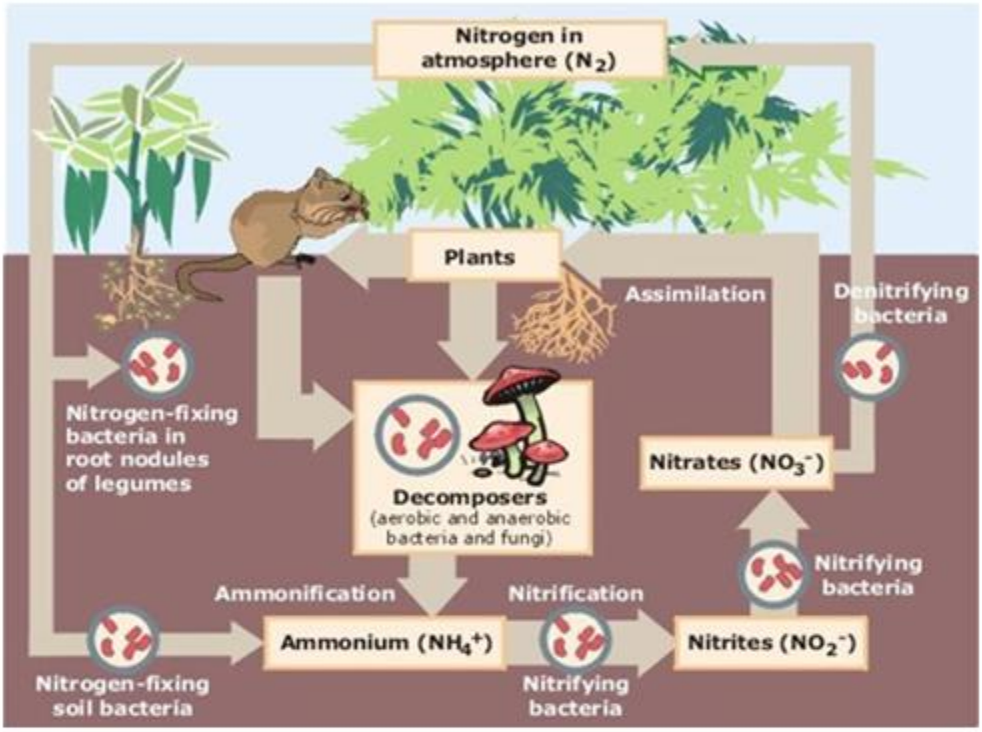

Figure 1: U.S. Environmental protection agency-nitrogen cycle.

Nitrates in the Soil and Plants

Nitrogen is an important element in plant nutrition because it is considered to be a major limiting factor for plant growth and development, and for achieving optimal yields [5,6]. Nitrogen is an integral part of many compounds in the plant cell, e.g. nucleic acids, amino acids, proteins, amides, amines, photosynthetic pigments and other compounds that form the basis of life. For this reason, the chemistry and metabolism of this element are justifiably considered the most important part of plant nutrition. Because of this, nitrogen fertilizers are often used in agricultural soils. The lack of nitrogen in the plant causes slower growth of the plant and the appearance of chlorosis. However, the applying of nitrogen fertilizers should be performed with caution. Nitrates are an important metabolite of nitrogen circulation in nature. They are produced by nitrite (NO^{2-}) oxidation caused by the action of nitrogen bacteria (*Nitrobacter*). Nitrate ions, as anions, do not have the ability to bind to the soil adsorption complex and are therefore susceptible to leaching into deeper soil layers and groundwater. Food processing, fertilizer use and growth conditions (especially soil temperature and daylight) affect the amount of nitrate in vegetables [7-9]. Crops such as beetroot, lettuce, radish and spinach often contain nitrates at concentrations higher than 2500 mg/kg, especially when grown in greenhouses.

Nitrates are most commonly found in cellular vacuoles from which they are transported to the xylem. Xylems transfer water and nutrients from the roots to the leaves, while the phloem transports photosynthesis products from the leaves to the plant's growth points. The said process further affects the distribution of nitrates between leaves and storage organs such as seeds or fruits. The system of this transfer results in young leaves having a smaller nitrate concentration than older ones [10].

Nitrates in the Human Body

In the human body, nitrates themselves are not really toxic but nitrites are. They are produced in the body by the reduction of nitrates or they come with food or water that we consume (nitrate reduction in nature). The reduction of nitrate to nitrite starts in the oral cavity by the action of commensal bacteria located on the tongue. Nitrites are a strong oxidizing agent, hence their toxicity. Nitrites can endanger human health by oxidizing hemoglobin to methemoglobin [11]. Methemoglobin is hemoglobin that is produced by the oxidation of iron atoms in heme molecules, which makes it unable to bind and transport oxygen, so the effect is similar to blood loss or carbon monoxide poisoning. However, it is unlikely that such nitrite/nitrate quantities introduced into the body at one time would be life- threatening, but sub-toxic in the long term can weaken the body. The oxidation of nucleic acid bases as a result of nitrate or nitrite consumption is another type of cellular damage, latent, but much more dangerous because DNA alterations can lead to cancer. It is precisely the replacement of uracil (in the RNA) with thymine (in the DNA) that is a way of protecting the cell. Oxidized thymine can be recognized, but oxidation of uracil produces cytosine, a second DNA base, so the repair is uncertain. International estimates of nitrate intake from food in Europe are 31-185 mg/day and in the United States ≈40100 mg/day. Nitrate intake from food other than vegetables, drinking water and dried meat is estimated to be around 35-44 mg/person/day for a person weighing 60 kg [12]. In 2002, the Scientific Committee on Food (SCF) established an Acceptable Daily Nitrate Intake (ADI) of 0-3.7 mg/kg, ie. weight/day, which is equivalent to an intake of 222 mg of nitrate for an adult person weighing 60 kg. However, increased levels of acid in the stomach destroy most of the nitrate-reducing bacteria and reduce the risk of developing methemoglobinemia. Herbal compounds polyphenols have a strong antioxidant activity, and together with vitamin C reduce the formation of nitrate in the stomach, so it is recommended that people who eat larger amounts of vegetables to intake higher amounts of vitamin C and polyphenols.

How to Reduce Nitrate Quantities in Vegetables

Vegetables should be conserved and processed because that way, they prevent contamination caused by bacteria. The removal of stems results in a 30%-40% reduction in nitrate content in lettuce and spinach [13]. Peeling potatoes and beets reduces the nitrate content by 20%-62%. When washing vegetables, nitrate levels are reduced by 10%-15%. In thermal treatment, this nitrate loss is further increased. By boiling peas, cabbage, beans, carrots, potatoes, spinach and other vegetables in water, the nitrate level is reduced by 16%-79% [14].

Reducing the Concentration of Nitrates in the Soil

High levels of nitrates in the soil are correlated with their higher content in the leaves of green plants, so it is necessary to reduce the concentration of nitrates in the soil in order not to exceed the Maximum Permitted Levels (MPL) of nitrates in vegetables, which are prescribed by Commission Regulations (EC) No 1881/2006, at the European Union level. Microbial processes of denitrification, Dissimilatory Reduction of Nitrate to Ammonia (DNRA) and anaerobic oxidation of ammonia (anammox) are the three key processes for reducing nitrate in the soil [15].

Denitrification: Denitrification is a chemical process in the soil in which nitrate and nitrite nitrogen, due to the influence of denitrifying bacteria (e.g. *Bacterium denitrificans, Bacterium fluorescens, Bacterium pyiocyaneum, Thiobacillus denitrificans*), transforms into free nitrogen or nitrogen oxides. Denitrification occurs when oxygen levels are reduced and nitrates become the main source of oxygen for microorganisms. The process is performed under anaerobic conditions, when the dissolved oxygen concentration is less than 0.5 mg/L, ideally less than 0.2 [16]. When bacteria separate nitrate (NO^{3-}) to produce oxygen (O_2), the nitrate is reduced to nitrous oxide (N_2O) and then to nitrogen gas (N_2). Because nitrogen gas has low solubility in water, it escapes into the atmosphere in the form of gas bubbles. Free nitrogen is a major component of air, so its release does not raise environmental concerns (The Water Planet Company). Shortening the denitrification process is a major factor that affects N_2O emissions in soil [17]. In addition, the present microbial community affects both the rate of production and the gaseous products obtained by denitrification, which are primarily controlled through the presence/absence, abundance, and activation of responsible genes. Several environmental factors that control the rate of denitrification are known, including O_2 and soil water content, NO_3, carbon, pH and temperature. However, soil texture and biological activity also play a crutial role in O_2

availability, producing O_2 gradients as a result of diffusion and aerobic respiration. The importance of water for denitrification has been demonstrated and found that increasing pore water (WFPS) increases denitrification rates, but also induces higher N_2 production. Plant roots have a variable effect on denitrification. The root releases significant amounts of soluble carbon. Oxygen in the soil is consumed during root breathing. In contrast, plant roots dry the soil while drawing in moisture for evapotranspiration and depleting nitrate from the soil as the plant grows. Variations in soil texture and water retention capacity in the landscape will also affect denitrification losses. A fundamental strategy to control denitrification is to maintain the minimum nitrate concentration required for plant nutrition. Nitrogen oxide emissions are generally low if high N yield is achieved, so excessive fertilizer or manure should always be avoided. Nutrient management practices that optimize crop N recovery will also minimize N_2O loss and nitrate leaching [18]. The concept of reduced yield N_2O emissions focuses on obtaining the highest possible crop yields while minimizing undesirable emissions (International Plant Nutrition Institute (IPNI)) [19].

Dissimilatory Nitrate Reduction to Ammonium (DNRA): Another, less characterized nitrate reduction process is DNRA, or nitrate ammonification, in which NO_3 is reduced to NO_2 and NH^{4+}, with N_2O being produced at the NO_2 reduction stage as a by-product. The DNRA process can be divided into two steps. The first step is the reduction of nitrate to nitrite, such as denitrification, which is catalyzed in the periplasm or the membrane-bound Nitrate Reductases (NARs). napA and narG are genes that encode these NARs. After nitrate reduction, nitrite is reduced to ammonia by Nitrite Reductase (NIR), encoded by the nrfA gene [20]. In comparison, nirS and nirK are related nitrite reductase genes involved in denitrification, while nosZ encodes nitric oxide reduction. Quantifying these functional genes results in a proportion of DNRA bacteria in nitrate depletion. Moreso, DNRA bacteria are diverse. In addition to fermentative bacteria, some sulfur and anammox bacteria can disguise themselves into DNRA bacteria. However, DNRA converts nitrate to ammonium *via* nitrite and stores nitrogen in estuaries. The estuary is a submerged river mouth in which, due to the reduced energy and the speed of the river flow, some of the sediment brought in is deposited. In recent years, it has been reported that nitrogen can be eliminated due to anammox along with the dissimilatory reduction of nitrate to ammonia [21,22]. DNRA exists in various aquatic systems, including estuaries, which play an important role in sediments. The distribution of DNRA bacteria in estuaries is an important factor affecting water pollution. Currently, a lot of research has been conducted on estuaries at the DNRA in Europe and America, while in the case of China, the DNRA has been mainly studied in the Pearl River estuary. The effect of carbon sources and the rate of nitrate reduction were examined in these studies. The contributions of anammox, denitrification and DNRA to nitrate reduction in the Yangtze estuary. However, community abundance and composition have not been investigated based on nrfA gene analysis. DNRA volumes varied statistically between July and January. The absence of significant seasonal differences in DNRA indicates that temperature was not an important factor controlling DNRA activity in the Yangtze estuary. Previous studies have reported that the production of ammonia by DNRA is associated with organic carbon content.

Anaerobic ammonium oxidation (Anammox): Anammox is an anaerobic oxidation of ammonia by Planctomycetes bacteria. In a typical Anammox process, nitrate (NO^{3-}) formation is bypassed by nitrification because nitrite (NO^{2-}) and ammonium (NH^{4+}) are directly converted to nitrogen gas (N_2). Currently, five genera of anammox have been discovered: Brocadia, Kuenenia, Anammoxoglobus, Jettenia (all freshwater species) and Scalindua (marine species). Anammox bacteria are characterized by several striking properties. Primarily by the fact that they all possess a single anammoxosome, a membrane-bound compartment within the cytoplasm that is the site of anammox catabolism. Furthermore, the membranes of these bacteria are mainly composed of laderan lipids. The conversion to hydrazine (commonly used as a high-energy rocket fuel and toxic to most living organisms), as a mediator, is particularly interesting. The final striking feature of the organism is its extremely slow growth rate. The doubling time ranges from 7 to 22 days. Anammox bacteria are aimed toward converting their substrates at very low concentrations, in other words, they have a very high affinity for the ammonia and nitrite substrates (submichromolar range). Anammox cells are loaded with cytochrome c type proteins (≈30% protein complement), including enzymes that carry out key catabolic reactions in the anammox process, making the cells distinctly red. The anammox process was originally thought to happen only from 20°C to 43°C, but more recently anammox has been noticed at temperatures of 36°C to 52°C in hot springs and 60°C to 85°C at hydrothermal vents along the Mid-Atlantic ridge. Today, one of the most important applications of the Anammox system is the process of purifying wastewaters. Generally, because it can shorten the conventional nitrogen cycle, the Anammox process has received a great deal of attention among other biological treatment methods. For wastewater containing large amounts of ammonia (NH^{4+}) and nitrite (NO^{2-}), denitrification plays an important role as it can primarily determine the success of the treatment plant by transforming NH^{4+} to N_2 and finally releasing N_2 into the environment. Consequently, the cost of wastewater treatment is reduced by about 90% using the Anammox wastewater treatment process. Recently, using new Anammox bacteria, Brocadia sinica, with sulfate-dependent anammox bacteria, Anammoxoglobus sulfate and Bacillus benzoevorans, the highest nitrogen removal efficiency, in laboratory UASB reactors in the world, can be achieved (74.3-76.7 kg-N/m^3/d). Furthermore, one study of the Anammox process involves fertilizer removal in a rice field in southern China. Due to the use of fertilizers, a large amount of ammonia has been accumulated in rice fields, with values of 6.2-178.8 mg/kg soil. This research shows that the application of the Anammox process is not limited to the treatment of wastewater, but the Anammox process can also be applied to the treatment of solid waste (Figure 2).

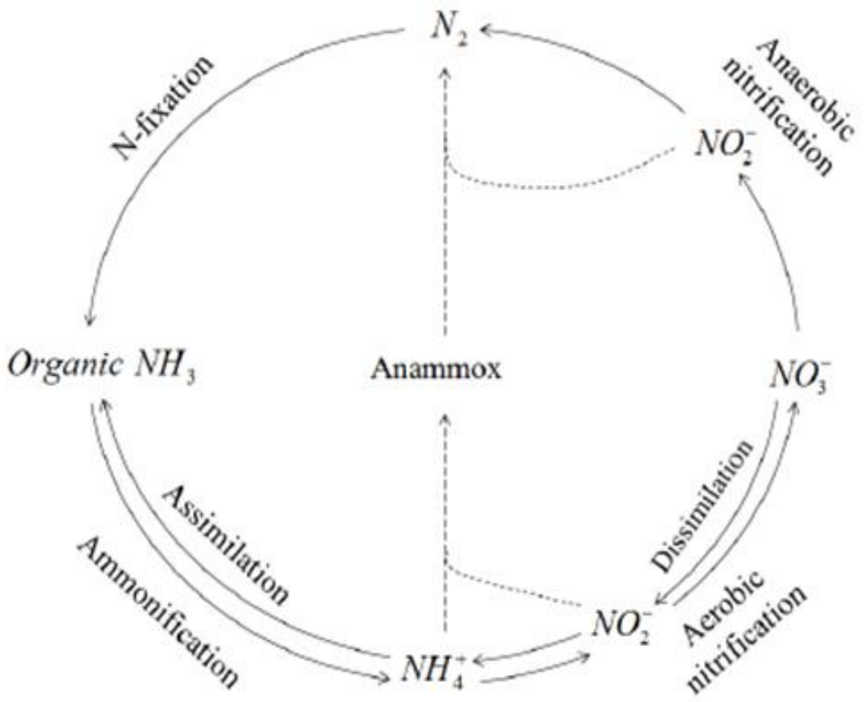

Figure 2: Nitrogen cycle in nature and in Anammox pathway.

Discussion

Fertilizer application directly affects the amount of nitrogen available in the soil and plant nutrition. It also contributes to suitable conditions for achieving high and persistent yields of adequate quality, while preserving soil fertility. The main objective of agricultural production is the economically viable production of quality food in an environmentally friendly way. Agro-technical fertilization and the soil repair measures are implemented when an inadequate supply of soil with nutrients may limit the production or even effect the soil degradation. In intensive crop production, nitrogen is almost regularly a limiting factor, and the application of nitrogen fertilizers leads to a significant increase in yields and also a potential environmental burden if inadequate fertilization results in leaching of nitric nitrogen into watercourses or underground water. Nitrates in underground water directly affect human health.

Conclusion

The World Health Organization allows a concentration of 45 mg/l nitrites which are harmful to humans because in a gastrointestinal tract nitrates to nitrites reduction can occur, as well as a reaction with amines which produces carcinogenic compounds nitrosamines. Thereby, nitrate water pollution endangers human health and pollutes the environment. Because of that, with the aim of preservation the biodiversity, plant health, human health and many other environmental factors, it is concluded that the knowledge of the decrease of nitrite concentration in the soil is obligatory. In other words, it is concluded that the education about nitrogen fertilization is equivalent to the education on the process of reducing nitrate over-concentration in soil.

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