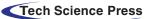


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REVIEW





Plant Nitrogen Metabolism: Balancing Resilience to Nutritional Stress and Abiotic Challenges

Muhammad Farhan^{1,#}, Manda Sathish², Rafia Kiran¹, Aroosa Mushtaq³, Alaa Baazeem⁴, Ammarah Hasnain⁵, Fahad Hakim¹, Syed Atif Hasan Naqvi^{1,#,*}, Mustansar Mubeen⁶, Yasir Iftikhar^{6,*}, Aqleem Abbas⁷, Muhammad Zeeshan Hassan¹ and Mahmoud Moustafa⁸

¹Department of Plant Pathology, Faculty of Agricultural Sciences & Technology, Bahauddin Zakariya University, Multan, 60800, Pakistan

²Centro de Investigación de Estudios Avanzados del Maule (CIEAM), Vicerrectoría de Investigación y Postgrado, Universidad Católica del Maule, Talca, 3460000, Chile

³College of Food Science and Nutritional Engineering, China Agricultural University, Beijing, China

⁴Department of Biology, College of Science, Taif University, P.O. Box 11099, Taif, 21944, Saudi Arabia

⁵Department of Biological Sciences, Lahore University of Biological and Applied Sciences, Lahore, Pakistan

⁶Department of Plant Pathology, College of Agriculture, University of Sargodha, Sargodha, 40100, Pakistan

⁷Department of Agriculture and Food Technology, Karakorum International University, Gilgit, Pakistan

⁸Department of Biology, Faculty of Science, King Khalid University, Abha, Saudi Arabia

*Corresponding Authors: Syed Atif Hasan Naqvi. Email: atifnaqvi@bzu.edu.pk; Yasir Iftikhar. Email: yasir.iftikhar@uos.edu.pk

[#]These authors have contributed equally to this work

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ABSTRACT

Plant growth and resilience to abiotic stresses, such as soil salinity and drought, depend intricately on nitrogen metabolism. This review explores nitrogen's regulatory role in plant responses to these challenges, unveiling a dynamic interplay between nitrogen availability and abiotic stress. In the context of soil salinity, a nuanced relationship emerges, featuring both antagonistic and synergistic interactions between salinity and nitrogen levels. Salinity-induced chlorophyll depletion in plants can be alleviated by optimal nitrogen supplementation; however, excessive nitrogen can exacerbate salinity stress. We delve into the complexities of this interaction and its agricultural implications. Nitrogen, a vital element within essential plant structures like chloroplasts, elicits diverse responses based on its availability. This review comprehensively examines manifestations of nitrogen deficiency and toxicity across various crop types, including cereals, vegetables, legumes, and fruits. Furthermore, we explore the broader consequences of nitrogen products, such as N₂O, NO₂, and ammonia, on human health. Understanding the intricate relationship between nitrogen and salinity, especially chloride accumulation in nitrate-fed plants and sodium buildup in ammonium-fed plants, is pivotal for optimizing crop nitrogen management. However, prudent nitrogen use is essential, as overapplication can exacerbate nitrogen-related issues. Nitrogen Use Efficiency (NUE) is of paramount importance in addressing salinity challenges and enhancing sustainable crop productivity. Achieving this goal requires advancements in crop varieties with efficient nitrogen utilization, precise timing and placement of nitrogen fertilizer application, and thoughtful nitrogen source selection to mitigate



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losses, particularly urea-based fertilizer volatilization. This review article delves into the multifaceted world of plant nitrogen metabolism and its pivotal role in enabling plant resilience to nutritional stress and abiotic challenges. It offers insights into future directions for sustainable agriculture.

KEYWORDS

Synthetic nitrogen; nitrogen signaling; sustainable agriculture; eutrophication; ammonium; nitrate

1 Introduction

Nitrogen, represented by the chemical symbol N, plays a vital role in sustaining life on Earth. It is indispensable for plant health as it enables the production of amino acids, the building blocks of proteins. Proteins, in turn, are pivotal for promoting robust plant growth. Additionally, nitrogen is a fundamental component of DNA, RNA, and nucleic acids, essential for all living organisms. When nitrogen is deficient in the soil, plants suffer from stunted growth due to their inability to synthesize essential proteins and maintain critical nucleic acid structures [1,2]. Nitrogen plays a crucial role in plant growth and is a cornerstone of global food production. Research has indicated that nitrogen is the primary factor influencing genome size, with phosphorus being of lesser significance in this context [3]. The efficient use of nitrogen is responsible for an increase in root length, diameter, branching, and biomass content in the roots. As the root network spreads to a larger area, water absorption is also improved [4]. The concentration of nitrogen in the atmosphere is about 78%, but unfortunately, plants cannot use this nitrogen directly. First, this nitrogen is fixed through fertilizer manufacturing or by microorganisms. Nitrogen is also present in the soil, but 98% of it is organic nitrogen that plants cannot uptake [5]. The cycle of interconversion between inert Nitrogen (N₂) and reactive nitrogen (that plants can uptake and is responsible for cellular life) is known as the Nitrogen cycle. Before 1909, nitrogen was naturally fixed; however, after that, the Haber-Bosch process was invented for artificial nitrogen fixation on an industrial scale [6]. Nitrogen for plants is available in various forms, such as nitrate and ammonium (in inorganic form), urea, free amino acids, and short peptides (in organic forms); however, urea must undergo hydrolysis to transform into ammonium nitrogen before becoming accessible for crop utilization [7]. Nitrogen is responsible for chlorophyll production, which increases the rate of photosynthesis, gives plants greening, and increases crop quality and yield. Additionally, it enhances plant resistance to abiotic stresses by promoting protein synthesis, enzyme activation for stress response, and osmotic regulation [8]. Salinity is one of the major abiotic stresses that can be mitigated by applying appropriate amounts of nitrogen, which helps regulate ion balance, alleviate osmotic stress, and reduce competition for water. For example, 12 g/kg of soil proved to be effective in enhancing salinity tolerance in sorghum crops [9]. China, India, the USA, Pakistan, Iran, Egypt, Thailand, Australia, Argentina, and South Africa are among the countries where salinity poses a significant problem for crop productivity. Additionally, salinity is a concern in other countries such as Chile, Turkey, Syria, Algeria, Tunisia, Sudan, and the Gulf states. In the case of Iran, the decline in wheat yield occurred first, followed by a decline in barley yield due to salinization [10]. The influence of salinity on nitrogen availability in soils significantly affects the crop, leading to symptoms of chlorosis, stunted growth, discoloration, and necrosis of older leaves in severe cases. Many crops also exhibit curled small leaves and grains with low protein content [11,12]. Two of the processes including leaching (loss of Nitrogen with the flowing water) and denitrification (reduction of nitrogen from nitrite to gaseous nitrogen due to microbial activity) are losing nitrogen from soil [5]. Over the past three decades, the use of Nitrogen has increased as it is an essential nutrient for plant growth, development, and yield [13].

The use of Nitrogenous fertilizers has increased by 48% since 2008 increasing food production [2]. More use of nitrogenous fertilizers has increased the crop yield but is also polluting the environment [14]. Excessive application of Nitrogen also causes yield losses due to soil acidification. It also decreases the available content of potassium and phosphorus [13]. Agricultural practices (mainly fertilizer applications) have enhanced plant growth by increasing reactive nitrogen compounds (ammonia, nitrate, and nitrite), but this enhancement is harmful to the ecosystem. Additionally, climate changes alter the transport, availability, and timing of nitrogen-related processes, including nitrogen fixation, nitrification, and denitrification [15]. Excessive nitrate due to synthetic fertilizers is the second chemical threat to the surface and groundwater [16,17]. In water, nitrogen exists in four forms nitrate, ammonia, ammonium, and nitrite, but nitrate is the most toxic of all. Increasing levels of nitrate in water is becoming fatal for aquatic life and human beings because it causes cancer and blue-baby syndrome in adults and infants respectively due to the reaction with protein [17,18]. The excessive use of nitrogen is the primary cause of salinity and the aforementioned problems, making it a major issue for discussion. The harmonization of agronomic, genetic, and biotechnological tools will enhance nitrogen assimilation efficiency in crops, aligning agricultural systems with global needs to preserve environmental functions and resources [19]. A small amount of copper is also required for good utilization of Nitrogen by the plants from the soil [20]. The preservation of a high GSH/GSSG ratio, which is essential for DHAR (dehydroascorbate reductase) and other GSH-dependent enzymes engaged in antioxidant defense mechanisms, was also aided by increases in NO-mediated GR (glutathione reductase) activity during salinity stress [21]. Applications of nanoparticles (Nano-fertilizers) have demonstrated significant potential in enhancing plants' ability to withstand environmental challenges, such as drought, high and low temperatures, and salinity, by modulating the physiological, biochemical, and molecular processes of plants [22]. Therefore, this review summarizes the literature on problems created by the excessive use of N, its relation with salinity, and different approaches for improving NUE.

2 Salinity as Abiotic Stress

Water evaporates from desert playas, which are flat, arid regions located at the base of undrained desert basins. These areas are covered with water in spring, leading to an increase in salt concentrations from 7000 to 14000 mM NaCl [23]. This salinity is an important abiotic stress factor in the arid and semi-arid areas of the world due to its effect on the productivity of different crops by suppressing photosynthesis. In this case, the concentration of sodium ions is increased while that of potassium ions is decreased [24]. Currently, 20% of the global land area is affected by salinity. It is believed that 30% of agricultural land could be lost within the next 25 years due to salt stress, with a total loss that could reach 50% by 2050. According to the geography and environmental conditions, different types of soluble salts are found in different fields including sodium sulfate and sodium chloride [9]. Depending on the nitrogen source, salinity has a variable impact on the intake of several nutrients like plants becoming more sensitive when ammonia is used as a source of fertilizer. Main cereal crops including rice, wheat, maize, and sorghum, and similarly important cash crops like sugarcane and cotton are greatly affected by salinity. Molecular studies showed that there are three ways by which salts suppress the growth of plants. First is the osmotic stress (water is drawn out from the cell due to higher salts conc.) that increases the concentrations of plant toxins (ion toxicity due to accumulation of Na⁺ and Cl⁻ ions), second includes ionic stresses and the third one is the oxidative stress carried out by Reactive Oxygen Species (ROS). Photosynthetic activity is disturbed by the imbalance of ions, imbalance of nutrients, and hormonal imbalance while the most important is the disturbance in water uptake caused by salinity [25]. Salinity stress results in severe oxidative damage, MG toxicity, cell death, and nuclear damage, which significantly decreases the growth of the seedlings [26]. Strongly salinized soils have a lower capacity to absorb CH_4 than moderately salinized soils do. According to one of the studies, CH₄ uptake was limited in severely salinized soil. This was probably due to the poor activity of *Methylocella* spp.; the most prevalent methanotrophs, and

the fact that few of them were even able to oxidase methane in these soils. The inorganic nitrogen content of soil decreases with increasing salinity [27]. Nitrogen fertilization promotes N_2O emission from saline soils because of large quantities of nitrogen present as a substrate for soil nitrification and denitrification, thereby facilitating N_2O emission [28]. The relation between salinity and different doses of Nitrogen was checked in an experiment on *Crocus sativus* L. According to that the negative impact of salinity was mitigated by 50 kg N/ha while 100 kg of N/ha enhanced the negative impact of the crop. The negative impact of salinity can be assessed by the number of leaves, length of leaves, weight of dry shoots, number of corms, and weight of corms [29].

3 Importance of Nitrogen

3.1 In Plants

The Sun is the sole source of energy for life on Earth. Sunlight, specifically blue and red light, is captured by chlorophyll in plants, algae, and Cyanobacteria. Through photosynthesis, these organisms convert sunlight, carbon dioxide, and water into chemical energy, producing glucose as the final product. Chlorophyll is found in the green parts of plants within the thylakoid membrane of the chloroplast. In the structure of chlorophyll, four nitrogen atoms surround the magnesium ion, forming a complex structure. This is often referred to as a porphyrin ring or tetrapyrrole ring [30]. The chlorophyll content is dependent on the chloroplast structure, which is influenced by the availability of nitrogen for the plants. When the effect of nitrogen was examined on maize crops using a low level of nitrogen fertilizer, it resulted in a reduction in the photosynthetic area and the chlorophyll content [31]. The main element to control the dynamics of various ecosystems on Earth is Nitrogen [32]. Wheat uses Nitrogen in greater quantity as compared to the other nutrients and due to its deficiency, the surface area of the leaf and the photosynthetic area is decreased, and the plant's green color is reduced [33]. When a proper amount of fertilizer was applied (138 kg/ha) it increased the pigment PS II and photosynthetic area of the leaf [2]. In the case of sugar beet, the highest content of chlorophyll and highest productivity was obtained when the application of Nitrogen was 120 kg/ha [34]. High nitrogen concentration increases potassium, phosphorus, iron, calcium, magnesium, and copper uptake in plants by promoting root growth, altering PH, stimulating microbial activity, and increasing the solubility of these fertilizers. Since 1980, due to the use of Nitrogenous fertilizers in China 70% increase in grain has been recorded [35]. Nitrogen is also responsible for the structure and composition of cell walls [36]. In the case of Oats Nitrogen is also responsible for reproduction and vegetative growth [37]. In saline soils, the application of nitrogen increased the concentration of chlorophyll, thereby enhancing photosynthesis and yield in oat crops. The augmentation in grain concentration (amount or density of grains in crops like cereals) was attributed to the alleviating effect on salinity through the use of nitrogen [38]. According to Jiang et al. [39] soil salinity decreases by increasing the Nitrogen concentration up to a limit while excessive (more the recommended dose, which is different for different crops) application of Nitrogen promotes Salinity [39]. The use of nitrogen fertilizers appeared to have a beneficial effect on reducing alfalfa's salt stress and preserving the production of shoot biomass, but N fertilization may restrict the plant's capacity to fix atmospheric N [40]. N fertilizer was successful in alleviating the adverse impacts of NaCl and Na₂SO₄ in Sorghum when the applied concentration of Nitrogen was 12 g N kg^{-1} of soil [9].

3.2 Nitrogen's Dual Nature: Essential Nutrient and Industrial Marvel

Humans are incapable of directly utilizing elemental nitrogen and must absorb alternative forms such as NO_2^- and NO_3^- , with NO_3^- being obtainable from green plants as well as inadvertent ingestion of contaminated water [41]. The protein structure encompasses 16% nitrogen, while certain neurotransmitters and peptide hormones are composed of amino acids (building blocks of protein) [42]. Carbon, Phosphorus, and Nitrogen are the building blocks of DNA and RNA. Five nitrogen's are present in pyrimidine and three nitrogen's in purine are the main nucleotides [43]. Children need higher protein

(35%) for growth, while adults require 15%. Understanding metabolism, intake, and health effects is crucial because of increasing Nitrogen demand [41]. In adults, the body maintains a nitrogen balance, with protein neither stored nor accumulated. However, during growth, reproduction, illness, or injury, the body's nitrogen demand increases, resulting in a positive balance. Conversely, if protein utilization exceeds intake or catabolism outpaces consumption, a Negative Nitrogen Balance occurs. Nitric oxide acts as a crucial messenger molecule, facilitating physiological functions like blood vessel dilation, platelet activation, and immune modulation. Proteins provide 2%–5% of the body's energy, equivalent to 4 calories per gram, akin to carbohydrates [41]. During the first month, human milk contains 1.3 grams of proteins per 100 ml and this quantity goes to 1.1 grams per 100 ml after the first month [42]. Sometimes NO₃⁻ is converted into NO2⁻ that can cross the placental barrier and can cause the death of the fetus [41]. In Earth's troposphere, N_2O serves as the primary source of O_3 while nitrogen and argon were initially used in lamps to prevent blackening, however, nitrogen lamps offer color to light, enhance filament brilliance, and maintain consistent characteristics [44,45]. There are many other uses of Nitrogen as it is used in the pharmaceutical industries for the preservation of Blood, eggs, and sperm. Similarly, Nitrous oxide can be used as an anesthetic. In the process of metal production, Nitrogen is an important component that prevents oxidation. It can also be used as a blanketing agent. It also has applications in the food industry as it is used for freezing and cooling food due to its property of low temperature. It also reduces the damage to food and helps the food to transfer over long distances. Nitrogen is also used to remove unstable organic chemicals from the reaction [32] (Fig. 1).

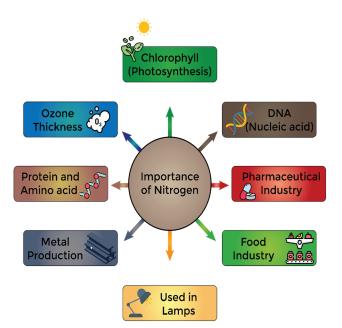


Figure 1: Some common uses of nitrogen in different fields

4 Nitrogen Cycle and Fixation (Biological, Industrial, Abiotic and Lightening)

The number of major pools of Nitrogen on this earth are two which first one being the atmosphere where Nitrogen exists in molecular form (N_2) while the second one is the reactive nitrogen pool that exists in biological molecules. The interconversion of Nitrogen between these two pools is known as the Nitrogen cycle [46]. Five major steps are involved in the Nitrogen Cycle that includes Nitrogen Fixation, Mineralization, Immobilization, Nitrification, and Denitrification [47]. The process of conversion of molecular Nitrogen into Biological Nitrogen is known as Nitrogen Fixation [48,49]. Nitrogen fixation is

carried out by symbiotic and free-living bacteria; it can also be fixed artificially through lightning [50]. Biological nitrogen fixation is carried out by prokaryotes that convert atmospheric nitrogen molecules into ammonia with the help of the nitrogenase enzyme. Beijerinck discovered this process in 1901. Among these prokaryotes, some include free-living bacteria like Azotobacter, which inhabits soil, Cyanobacteria found in water, as well as Azospirillum and Rhizobium, which form symbiotic associations with legumes [51]. Nitrogenase enzyme contains molybdenum, iron, and vanadium [52]. These bacteria that are involved in nitrogen fixation are named diazotrophs [53]. The nitrogen molecule is composed of two nitrogen atoms joined by a triple covalent bond, Nitrogenase catalyzes the breaking of this bond and the addition of three hydrogen atoms to each nitrogen atom. Since the Nitrogenase enzyme can only function in an anaerobic environment, Leg Hb is a pigment found in leguminous plants. It serves as a scavenger of oxygen. For Nitrogenase to function, it exhausts all the oxygen and generates an anaerobic environment. Nitrogen is fixed using hydrogen donors. Numerous substances, including pyruvic acid, glucose, sucrose, etc.; are hydrogen donors. Energy is obtained by the utilization of ATP. The molecules of generated ammonia must be bound by another carbon-containing chemical [54]. In the center of each mature nodule, there are billions of bacteria present that fix nitrogen [55]. The white or grey color of nodules represents that nodules are young and do not fix the nitrogen. If the color of nodules is pink or reddish it means they are fixing the Nitrogen. Old nodules that are not fixing the nitrogen their color become green [56]. Cyanobacteria may form a relation to the roots of plants intracellularly or intercellularly [57]. Heterotrophic and Cyanobacterial nitrogen fixation depends upon the quality of carbon and the availability of micronutrients respectively and the rate of nitrogen fixation on land is decreased due to a low supply of phosphorus [58]. In non-symbiotic associations, bacteria release nitrogen through the breakdown of bacterial cells, utilizing carbon and energy from the environment for nitrogen fixation. Energy from plant roots, in the form of carbohydrates, fuels this process. Factors influencing biological nitrogen fixation include tillage, acidic soils, intercropping, fungicides, pesticides, insecticides, nitrogen fertilization, temperature, and moisture. Among free-living heterotrophs, aerobic organisms like Methanosarcina and Clostridium exist, while heterotrophic microaerophiles include Frankia, Azospirillum, and Bradyrhizobium, and aerobic heterotrophic free-living organisms include Azobacter and Derxia. Root-associated species comprise microaerophilic Herbaspirillium and endophytic Acetobacter, with Frankia and Bradyrhizobium in symbiotic association. Free-living autotrophs include microaerophilic organisms like Rhodospirillum and Bradyrhizobium, aerobic free-living autotrophs like Cyanobacteria, and symbiotic autotrophs such as Anabaena azalea and cyanobacteria [59,60]. Atmospheric nitrogen can be fixed through lightning, where nitrogen and oxygen combine to form NO, which then reacts with rainwater to produce nitrous acid or nitric acid, subsequently converted into nitrates for plant uptake [61,62] (Fig. 2). Many chemical reactions were conducted to combine Nitrogen and Hydrogen after the detection of ammonia in the chemical lab of the Margulies brothers. Haber decided to synthesize ammonia by using heat and iron as a catalyst [63]. In 1909 he forcefully combined the nitrogen with hydrogen at a high temperature of 500°C and high pressure of about 150-200 atm [64]. Haber thought that this reaction would be reversible and then he detected the movement of ammonia, H, and N to opposite sides when he experimented with normal atmospheric pressure [63]. For the production of ammonia, 2% of the world's natural gas is being used as a source of hydrogen and it uses 1%-2% of the world's energy sources. Annually 140 million tons of Nitrogen are being fixed with the help of Haber Bosch process [63]. The Chemical equation for synthetic Nitrogen fixation is given below:

 $N_2 + 3H_2 \rightarrow 2NH_3 + 46.1 \text{ KJmol}^{-1}$ [64]

In the case of abiotic nitrogen fixation in soil, 10 T g of Nitrogen can be fixed per year in the desert through photochemical fixation. In photochemical Nitrogen Fixation, organic nitrogen is used to generate ammonium and sometimes it supplies more than half of the bioavailable nitrogen in the ecosystem.

Photochemical Nitrogen fixation is important because sometimes microorganisms are unable to decompose organic Nitrogen into ammonium but it can be fixed by sunlight [65]. The second step of the Nitrogen cycle in which ammonium is oxidized into nitrite is known as Nitrification. Nitrite ions are further oxidized to nitrate ions by other nitrifying bacteria like Nitrobacter. Both nitrite and nitrate are forms of nitrogen that plants can readily absorb [66,67]. The last step of the Nitrogen cycle includes the conversion of nitrate into molecular Nitrogen and the activity is carried out by microorganisms [68]. Among different communities of plants, the measurements of denitrification differ [69] (Fig. 2).

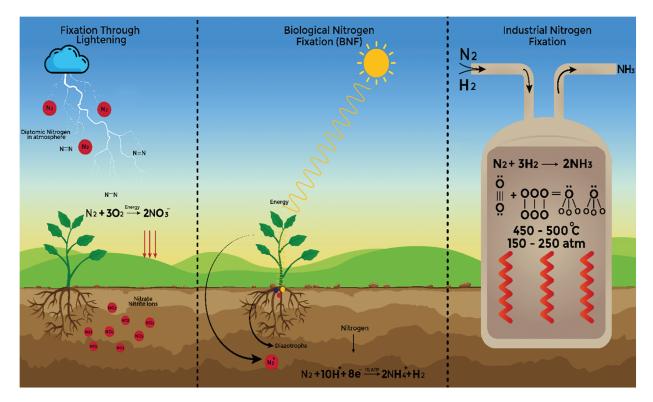


Figure 2: Comparison between BNF, artificial nitrogen fixation and nitrogen fixation through lightening

5 What is Nitrogen Use Efficiency, Importance and Improvements

As nitrogen is a crucial factor in crop production, its excessive application not only boosts plant growth and yield but also escalates production costs and contributes to environmental degradation, underscoring the necessity for users to grasp nitrogen use efficiency and cultivate varieties with improved nitrogen use efficiency. Nitrogen use efficiency is commonly employed to estimate the conversion of nitrogen inputs into agricultural products and to gauge the risk of nitrogen losses to the broader environment, determined by factors such as nitrogen uptake, mobilization, and assimilation, with only 30%–50% of applied nitrogen being utilized by plants, while the remainder is wasted [5]. Agronomists and genetic researchers propose two different definitions of NUE. According to agronomists, NUE is the relation between available Nitrogen for the plant or Nitrogen uptake by plants and the generation of grains per Unit of Nitrogen. According to genetic researchers, NUE relates to the number of genes like glutamine synthetize, to are important for better Nitrogen Use Efficiency [70]. When the roots of many plants take up nitrate, some of it is assimilated into the roots, but the majority is transported to the shoot, where it is first reduced to nitrite by nitrate reductase in the cytoplasm. Then further converted to ammonium by nitrite reductase in the plastids and glutamine synthetase (GS) in the plastids and cytoplasm.

makes glutamine important for enhancing NUE [71]. By altering the expression of genes for nitrate transport, the Indica subspecies of rice was genetically improved for better nitrogen use efficiency (NUE) through increased absorption of nitrates. In China, the NUE of the rice cultivar Wuyunjing 7 was enhanced through the same genetic changes [72,73]. It can also be defined as determining the amount of Nitrogen stored by plants or released into the atmosphere in the form of nitrous oxide. NUE of leguminous crops is better because they store maximum Nitrogen inside them, while it is not completely known whether they do not release Nitrogen into the atmosphere or release it in very small amounts. Overall, Nitrogen use efficiency is the amount of Nitrogen available for plants how much of that is used by plants, and how much Nitrogen they retain at the time of harvest [74]. Nitrogen use efficiency is the product of utilizing the efficiency of Nitrogen. Nitrogen uptake efficiency depends upon the amount of ammonium and nitrate present in soil and the ability of plant cells to store them [75].

In the case of cereal crops, the worldwide nitrogen use efficiency remained at 30%. Improvements in NUE can control the use of high amounts of Nitrogen. According to an estimate about England fields, through better crop management NUE can extend but will remain below 75%. The was a difference in Nitrogen use efficiency when both ammonium and nitrates were present as compared to when only nitrate was present. In the case of High ammonium NUE was found to be lower [70]. The Nitrogen Use efficiency in China is lower as compared to the developed countries that is 10%–20% [76]. It has been found that microbes show high NUE even when there is less Nitrogen [77]. During the last years, 47 Mt of Nitrogen was applied to cereal crops out of a total of 83 Mt, Southeast Asia, and North East Asia used 71% and 32% of that Nitrogen, respectively [78]. Recovery of Nitrogen depends upon the crop type, fertilizer type, cultural practices, and environmental factors [79]. NUE is lower when farmers use conventional methods to grow their crops. It is also lowered when there is poor coordination among plant requirements and the time of application (mostly applied earlier that is lost due to the temperature or any other abiotic stress) [74]. NUE is also decreased due to the balance in the nutrient's applications [80].

NUE can be improved using fertilizers with a slow-release process [81]. NUE has been reported to improve in Pakistan using potassium fertilizer [80]. Improvements in cultural practices can improve NUE [82]. Nitrogen Use Efficiency depends upon translocation of Nitrogen in sensing leaves, and it can be improved by Mycorrhizal fungi [83]. NUE can also be improved by using genetically improved verities [78] Urea is the most abundantly used fertilizer worldwide and for its better use efficiency, it is coated with Sulphur. Nitrogen use efficiency can also be improved by using a Urea-Triazone solution as an alternative to granular urea as it dissolves or releases nitrogen slowly [84]. Application of water and nutrients directly to the rhizosphere of plants improves the NUE [81]. Broad and healthy rhizosphere also have higher NUE as experimented in rice. We can prevent the environment by using varieties with high NUE by studying their genetic makeup [76]. Different tillage techniques have varying degrees of soil disturbance, which impacts soil N dynamics and plant N availability. The conservation tillage (less or no tillage) approach frequently results in higher NUE overall, however, the link between the two varies throughout the studies [85]. Cotton yields were higher in conservation tillage plots than in conventional tillage plots with an optimal application of nitrogen, according to a lengthy (10-year) study done in the southern United States of America [86]. Slow-release nitrogen fertilizers may reduce nitrogen leaching and denitrification losses and enhance the timing of nitrogen release and uptake in response to crop needs. Similarly, slow-release synthetic urea-based fertilizers like isobutylidene diurea (IBDU) and crotobylidene diurea (CDU), which are coated N sources, have also enhanced the NUE. When compared to broadcasting, N fertilizer injection, band application, and placement under the seeds during planting all resulted in higher NUE and lower NH₃ volatilization in winter wheat. Above-discussed factors to enhance NUE play a vital role in sustainable agriculture by reducing pollution and enhancing crop productivity. For NUE, the plant's intrinsic capability must be enhanced and chosen to promote effective uptake,

utilization of N, and production of higher yield under conditions of moderate or minimal N availability [19]. In an experiment conducted in India nanotechnology was used for the efficient application of Nitrogen and Zinc along with the practice of organic forming [87]. The loading of Urease enzymes upon the fertilizers limits the release of Nitrogen from urea via hydrolysis. These enzyme-loaded fertilizers (nano fertilizers) are termed nanoparticles and the technology used to make these particles is called nanotechnology [88]. In nanotechnology, fertilizers are used in small amounts, and during the experiments, a great increase in yield was recorded (Table 1).

| Sr. No. | Crop name | Nanofertilizers used | Production increased (% age) | References |
|---------|--------------|---------------------------|------------------------------|------------|
| 1 | Wheat | N and Zn Nanofertilizers | 5.35% | [87] |
| 2 | Sesame | N and Zn Nanofertilizers | 24.24% | [89] |
| 3 | Pearl millet | Zn Nanofertilizers | 37.7% | [90] |
| 4 | Mustard | N and Zn Nanofertilizers | 8.4% | [87] |
| 5 | Cotton | N and MgO Nanofertilizers | 22% | [89] |
| | | N and ZnO Nanofertilizers | 33% | |
| | | N and CuO Nanofertilizers | 18% | |
| 6 | Black Pae | Fe Nanofertilizers | 23.3% | [91] |

Table 1: Effect of nanofertilizers on crop yield improvement: a comparative analysis

6 Symptoms of Nitrogen Deficiency and Excess

6.1 Nitrogen Deficiency

The content of chlorophyll is reduced, and degradation of enzymes occurs due to the reduced level of nitrogen in plants. As degradation of protein leads to a change in the enzyme activity, which causes the leaf senescence. The level of Rubisco activase, endase, ATP synthesis, glutamine synthesis, etc. also reduced due to the reduced level of nitrogen. At the initial stage of senescence, the level of mRNA increases, and it becomes very low when leaves become completely senescent [92]. The degradation of chlorophyll, leading to leaf yellowing, contributes to the decline in crop plant growth, primarily due to low nitrogen levels, affecting overall crop yield [93]. This nitrogen deficiency reduces cell division, initially manifesting as lower leaf yellowing, potentially leading to leaf drop in severe cases, and decreases seed protein content. Furthermore, nitrogen deficiency induces early maturity, further reducing yield [93,94]. During vegetative growth, the surface area of the leaf is reduced [95]. Low fertilization rates may result in seed yields that fall short of expectations [96]. Reduction in yield of seed yield was measured up to 30%. In the case of photosynthetic machinery both chlorophyll inflorescence and chlorophyll concentration were found to be reduced due to a deficiency of Nitrogen [97] (Fig. 1). The primary metabolism of plants is reduced due to the deficiency of Nitrogen in the soil. Due to less availability of Nitrogen, the rate of transpiration and photosynthesis is reduced [98]. Due to this plant growth is affected [82]. When there was a low level of Nitrogen in Arabidopsis the flowering was promoted in it [98]. At the heading and seedling stage, the deficiency of Nitrogen does not have much effect on yield while yield losses are maximum when the plant is deficient with Nitrogen at the tillering stage [99].

6.2 Nitrogen Excess

A higher concentration of nitrogen than required causes problems in plants and disturbs our environment. It affects the growth, development, and composition of plants, and seed quality is affected.

Using higher amounts of nitrogen limits the content of phenolic and flavonoids, reducing antioxidant power [100]. The excessive use of nitrate increases plant biomass, but the excessive application of ammonium shows a negative impact on plant growth [101]. Nitrogen toxicity causes leaves to turn dark green, extending the period of vegetative growth. The height of the plant increases while the stem remains thin, eventually leading to lodging. The water use efficiency of the plant decreases due to nitrogen toxicity, resulting in the burning of leaves and stunted growth. Lesions form on leaves, stems, and roots, and the leaves also exhibit downward rolling [12]. The breakdown of particles and formation of harmful chemicals named carcinogens and allergens are due to Nitrogen toxicity. Disease attacks become more by the use of higher amounts of Nitrogen (Fig. 3) [101].

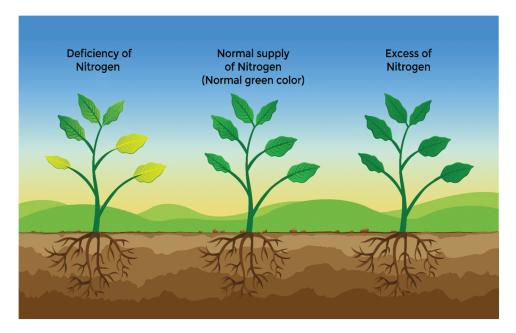


Figure 3: Response of plants at different concentrations of applied Nitrogen in the ecosystem

7 Nitrogen and Cereal Crops

7.1 Nitrogen Use Efficiency of Cereals

The maximum cultivable land in the world is under the cultivation of cereal crops. Additionally, approximately 90% of cereal production comes from wheat, rice, and maize. The yearly application of nitrogen to cereal crops is 94 million tons, of which 60% is wasted, posing a threat to the environment. Nitrogen Use Efficiency (NUE) in cereal crops depends on two things: how the plant uptakes nitrogen and how much of it can be used. NUE can also be calculated at the time of harvest. Moreover, the higher application of nitrogen decreases NUE. It can be enhanced by the availability of other micro and macronutrients and by crop rotation. The use of nano-fertilizers can also increase NUE [79]. Agronomic practices like time of application, place, and proper amount of fertilizer enhance the NUE [102].

7.2 Wheat, Symptoms of Nitrogen Deficiency and Excess

Wheat provides humans with a lot of carbohydrates and proteins (10%-15%) that are useful for human health, so it is one of the vital staple crops. Due to the plantation of new industries and the movement of people toward the cities, the demand for this staple food is being increased. Dietary fibers are also present in wheat along with Vitamin B [103]. Older leaves first show the stress of nitrogen as their color changes

from green to light green and finally, it turns yellow due to the shortage of chlorophyll. The whole field gives a yellowish appearance due to nitrogen stress [104]. Due to the deficiency of nitrogen in wheat the synthesis of chloroplast, in which the photosynthesizing pigment chlorophyll is present, is reduced which directly affects the process of photosynthesis. The physiology of the plant is disturbed as plant growth is stunted, the size of the leaf is reduced which decreases the area of photosynthesis, and the yield of a crop is reduced. Grain quality is also disturbed as the content of protein and amino acids is reduced [105]. When nitrogen supply increases the demand for nitrogen for wheat crops, the number of spikes and spikelet's is reduced. weight of grain is also reduced. NUE of the crop is reduced and reproductive growth becomes poor which results in yield loss [106]. The toxicity of nitrogen breaks the resistance of the wheat plants and the plant show excessive vegetative growth. Weed problems are more common in such types of fields [107].

7.3 Rice, Symptoms of Nitrogen Deficiency and Excess

Rice is one of the three important food crops. In terms of food, about half of the world's population depends on rice. Regarding cultivation area, rice ranks second. Ninety percent of the world's rice is produced in Asia. If we include the cultivation in Latin America and Africa along with Asia, these three regions together account for 98% of the world's rice production [108]. The deficiency of nitrogen in rice occurs when demands for nitrogen increase like at the tillering stage. In addition, older leaves start changing their color from the tip resulting in yellowing of the whole leaf. Moreover, under severe conditions of N deficiency leaves may be died. The size of leaves is also affected as they become smaller with short length and width disturbing the area of photosynthesis and color becoming yellowish [109]. Under nitrogen toxicity, the greenery of rice plants is increased which attracts the insects and increases the disease attack. At the stage of maturity lodging of the plant occurs, and leaves become folded along with patches on them [110]. Plants show stunted growth due to the lack of photosynthesis which is dependent on the content of chlorophyll. Chlorophyll is present in the chloroplast, whose structure is destroyed in rice plants due to the toxicity of nitrogen [111].

8 Maize, Symptoms of Nitrogen Deficiency and Excess

Worldwide cultivation of maize comes at number three after Rice and Wheat [112]. The Oueen of cereals needs a short span for its growth. It can be used as food for humans and to feed the animals as a feeder crop [113]. It can be used in different ways by making its flour that is then used to make bakery products, it can be eaten after roasting, and its fructose level is high and provides a lot of amino acids [112]. Maize provides us with many nutrients as described below. Symptoms appear by the yellowing of older leaves showing necrosis at the tip and the color of other nitrogen-deficient leaves becomes pale green [114]. Nitrogen is the component of cell protoplast, which plays an important role in plant growth, so nitrogen stress retarded the growth of plants resulting in yield losses. Number of barren plants also increased [115]. Due to the nitrogen deficiency, the shoot biomass of the plant is also affected [116]. In an experiment of spectral and true color response, the maize leaves were tested in which nitrogen-deficient leaves showed high spectral reflection and it was found that it was due to the lack of chlorophyll [117]. Nutritional deficiency limits the growth of scarlet eggplant by altering the absorption and use efficiency of macronutrients, leading to reduced plant height, stem diameter, leaf number, and leaf area, as well as decreased dry matter production in both above and below ground biomass. Nitrogen deficiency hinders cell division, affects cell elongation in later leaf development, and reduces the production of photosynthetic pigments. This, in turn, impacts the absorption of light and the metabolism of magnesium, essential for chlorophyll molecules. Additionally, nitrogen deficiency decreases the rate of transpiration and the efficiency of absorbing and using other nutrients. Moreover, excessive nitrogen use lowers the soil pH, which can impede crop growth and reduce yield [118–120].

9 Barley, Symptoms of Nitrogen Deficiency and Toxicity in Barley

As nitrogen is important in the production of barley, the plant is disturbed due to the deficiency of nitrogen. Symptoms of nitrogen stress first appear on older leaves, which start changing their color from the tip. The color of the lower leaves becomes light green. Under continuous stress, the color of other leaves starts diverting from green, while the pale green color of older leaves changes to white. Finally, it results in a reduction in protein levels and also in yield [121]. Due to nitrogen stress, a 50% reduction in photosynthesis has been observed in barley. It also reduces the shoot growth. Although the growth rate of plants becomes slow the level of starch proteins and amino acids is increased due to nitrogen stress. The size of leaves has been observed smaller as compared to the nitrogen-sufficient plants [122]. The toxicity of nitrogen in plants shows effects on the growth of plants and the pathogenic and non-pathogenic stresses on plants are increased [123]. Due to excessive use of ammonia yellowing of leaves occurs and in highly affected verities yield may decrease up to 60%. Sometimes it can be fatal for the plants. Roots and shoots may also lower [124].

10 Sorghum, Symptoms of Nitrogen Deficiency and Toxicity in Barley

Sorghum, the fifth most important crop after the previously discussed four, provides sustenance for approximately 750 million people. It thrives in hot regions, requiring a temperature of 25°C for optimal yield. During water scarcity, it conserves water by rolling its leaves. With a protein content of 17%, sweet sorghum is cultivated in about 100 countries across 44 million hectares worldwide. Its potential for ethanol production, coupled with low production costs, makes it an economically viable option [125,126]. Sorghum plants facing the nitrogen stress show chlorosis of leaves, reduction in growth and sometimes it can be fatal. It decreases the number of leaves and the length of roots. The appearance of the purple color is also a symptom of nitrogen deficiency in sorghum [127]. Chlorosis occurs due to a decrease in leaf chlorophyll contents while leaf reflectance is increased [128]. The amount of nucleic acid, proteins, chlorophyll, and lipids in plants is decreased due to the production of relative oxygen species that are the result of nitrogen toxicity. Its toxicity produces some poisonous compounds that affect the germination of seeds and reduce plant growth. It also causes toxic problems in men [129].

11 Importance of Legumes (Nutritional and Nitrogen Fixation)

Among the flowering plants, the Leguminosae family ranks as the third largest family, comprising 800 genera. Within this family, some members are classified as grain crops, known as pulses, while others are considered weeds. Legumes are of great economic importance due to their high nutritional value, containing two to three times more protein than cereal crops. In addition to protein, they are rich in vitamins, folic acid, minerals, fatty acids, dietary fibers, and carbohydrates. They play a vital role in providing sustenance for millions of people. Soybean and pulses are cultivated over 121.5 million hectares and 82.4 million hectares of the world's cultivable area [130,131]. The popularity of grain legumes is because of their higher content of proteins or oils [132]. The third most important crop is chickpea with 15% of total production among the pulses [133]. It acts as a vital source of protein for small farmers and is also used to feed animals [134]. Cowpeas have high nutritive value and improve soil fertility [135]. The protein content of soybean is 40%-42% while oil contents are 20%-22%. Among vegetable proteins, soybean contributes about 60% [136]. Lentil is cultivated worldwide as it provides maximum levels of essential amino acids, minerals, and Vitamin B1. Its major producers are Canada, Turkey, and India [137]. Legumes fix atmospheric nitrogen into ammonia by making symbiotic associations with bacteria known as rhizobia [138]. The association between the roots of legumes and soil bacteria results in the formation of nodules that release Nitrogenase enzymes and fix the atmospheric molecular nitrogen. This is a symbiotic type of association because both partners are providing benefits to each other. Bacteria get carbohydrates from plants and use them as a source of energy and to facilitate the

reaction of Nitrogen fixation and nodule formation that provide reduced nitrogen to the plants. Biological Nitrogen fixation is important as it reduces the cost of production and prevents the environment from pollution that is caused by excessive use of industrial nitrogen. About 50–200 Mt of nitrogen is being fixed biologically per year [131]. Although legumes fix atmospheric nitrogen the amount of nitrogen fixed biologically is not too much to get a high yield. Therefore, we have to provide them with nitrogen by artificial means [132]. For the growth and germination of chickpeas there should be a small amount of nitrogen up to the establishment of symbiotic association and in nitrogen are 15–20 kg per hectare [134], while in an experiment 40 kg of nitrogen proved to be optimum for cowpea yield and sometimes there is no effect of N on its yield [135]. Similarly, in peanuts, the same amount of nitrogen application rates were from 30–80 kg per hectare [136].

11.1 Symptoms of Nitrogen Deficiency and Excess in Legumes: Chickpeas, Cowpeas, Lentils, Beans

Symptoms of nitrogen deficiency first appear on older leaves showing yellowing and pigments of pink color on the upper surface of older leaves. These pigments also appear on the stem [140]. Pink pigmentation on leaves is caused by severe deficiency of nitrogen and first leaves become red then pink. The whole plant gives a yellowish appearance. Older leaves finally become white and drop [134]. Due to nitrogen deficiency, plant growth is retarded, leading to a change in the color of mature leaves, which become light yellow-green. Since nitrogen is a key component of chlorophyll, photosynthesis is also reduced in the presence of nitrogen deficiency. The overall growth of the plant is stunted, exhibiting a pale yellow color due to the lack of nitrogen. Cupping of upper-middle leaves occurs, and old leaves display a yellow color, sometimes leading to their death. Nitrogen deficiency in the late season results in reduced biomass and ultimately decreases the yield of lentil crops [135–137]. The maximum Nitrogen Use Efficiency of lentils is determined to be 110.1%. Nitrogen deficiency in the early season is observed during nodule development, and in the late season, it may be attributed to various biotic or abiotic factors. Initial symptoms manifest on older leaves, while younger leaves remain unaffected. Under mild conditions, a light green color predominates in the plant. Yellowing is evident in the upper three leaves, while the lower three leaves may become necrotic [138–140].

In case of excessive use of nitrogen in fava beans, the number of nodules and the dry weight is decreased. Moreover, in underground parts, the secretion of flavonoids are reduced due to the toxicity of nitrogen fertilizer. In an experiment, the reduction in flavonoid was observed at 13.2% in the case of a nitrogen-rich crop compared to the nitrogen-deficit crop [141]. In the case of soybean, excessive nitrogen affects the growth of nodules and the Nitrogenase activity. The food moving towards the nodules is decreased due to N toxicity. Finally, the Nitrogen Use Efficiency and the Nitrogen fixation is reduced [142].

12 Vegetables and Nitrogen

Nitrogen is essential for the primary process of photosynthesis, and any disruption in its concentration can disturb this vital process. Despite its importance for plant growth, the application of Nitrogen fertilizer can lead to emissions of nitrogen oxide and dinitrogen oxide, subsequently decreasing the Nitrogen Use Efficiency of crops. The Nitrogen Use Efficiency varies among vegetables, ranging from 5% to 67.3% across different crops, with an increase observed when industrial nitrogen is combined with manure [143]. Leafy vegetables like spinach and lettuce necessitate nitrogen during their vegetative growth phase, as they are harvested before reaching the reproductive stage, requiring rapid leaf size development. Failure to apply nitrogen during the initial growth stage of spinach may result in smaller leaves. Conversely, tuber vegetables require nitrogen equally during both their vegetative and tuber phases [144]. Cucurbits require nitrogen levels ranging from over 110 kg to under 220 kg per hectare for optimal

growth and development. Onions achieve optimal growth and yield with 100 kg of nitrogen per hectare, while potatoes exhibit maximum production with nitrogen application rates between 100 and 150 kg per hectare [145–147]. Optimal growth for radishes is achieved with 2.15 kg of nitrogen per 1000 kg of fleshy leaves, while tomatoes benefit from 240 kg of nitrogen for better growth, development, and yield. Vegetables are essential for human body growth as they supply dietary fibers, vitamins, plant chemicals, and minerals, enhancing vision, reducing heart diseases, certain cancers, and diabetes. Crucifers contain 40% non-starch parts and 50% dietary fibers in dry weight, while Alliaceae members contain 5% sulfur in dry weight, with onions being the highest fiber provider in the family. Tomatoes serve as a potassium source in First World countries and aid in skin protection, while potatoes offer ample carbohydrates, containing 7% starch when cooked and 13% when cooled, as well as essential amino acids [148–150].

12.1 Symptoms of Nitrogen Deficiency and Toxicity in Vegetable Crops: Cucurbits, Onion, Potato, Radish, Tomato

Optimal growth for radishes is achieved with 2.15 kg of nitrogen per 1000 kg of fleshy leaves, while tomatoes benefit from 240 kg of nitrogen for better growth, development, and yield. Vegetables are essential for human body growth as they supply dietary fibers, vitamins, plant chemicals, and minerals, enhancing vision, reducing heart diseases, certain cancers, and diabetes. Crucifers contain 40% non-starch parts and 50% dietary fibers in dry weight, while Alliaceae members contain 5% sulfur in dry weight, with onions being the highest fiber provider in the family. Tomatoes serve as a potassium source in First World countries and aid in skin protection, while potatoes offer ample carbohydrates, containing 7% starch when cooked and 13% when cooled, as well as essential amino acids [151]. The symptoms of nitrogen deficiency include chlorosis in the leaves. The size of the bulb is also reduced due to the deficiency of nitrogen [152]. Other symptoms include yellowing of younger leaves due to decreased chloroplast production, with symptoms evenly distributed across the entire leaf [153]. This yellowing signifies a reduction in chlorophyll content and impacts overall photosynthetic efficiency. Concurrently, plant growth is stunted as cell division decreases, while protein levels also decline, affecting the plant's metabolic processes. Additionally, reduced nitrogen levels result in smaller leaves, altering the mass-tovolume ratio and diminishing light absorption, ultimately hindering photosynthesis. Notably, nitrogen deficiency can prove fatal for cotyledons and radishes demonstrate heightened anthocyanin levels under nitrogen stress, indicative of physiological responses to nutrient deficiencies. Similarly, in tomatoes, nitrogen stress leads to a decline in leaf nitrogen content, thereby reducing nitrogen levels in tissues and resulting in a subsequent decrease in plant biomass production [153-157]. The color of leaves deviates from green to less green [158]. Nitrogen stress disturbs the metabolic activities of tomato plants [159]. The toxicity of ammonium causes the change in PH between the cells, disturbs the balance of osmosis, and causes the unavailability of minerals to the plant. It decreases the calcium and magnesium in the plant. The toxicity of ammonium decreases the synthesis of chlorophyll which is the main factor in the process of photosynthesis. So, the process of photosynthesis is decreased, and less food is produced which causes the stunted growth of plants. The symptoms of yellowing and necrosis also appear on the leaves and the underground parts of the plant [160]. The middle and lower leaves are curved. The size of fruits is also reduced [151]. Nitrogen toxicity reduces the immunity of onion and it becomes affected by biotic or abiotic stress. The maturity of onion is delayed with two centers at the bulb due to the excessive use of nitrogen [161]. The toxicity of nitrogen causes a decrease in yield [162]. The yield of potatoes is decreased due to the toxicity of nitrogen because amino acids are not converted into protein. The plant focuses on the growth of stems and leaves [163]. Initially, by the excessive use of nitrogen, the growth of plants is increased and then goes on decreasing. And Nitrogen Use Efficiency is also decreased [164]. The toxicity of ammonium in radish causes a decrease in the processes of photosynthesis. It also decreases the rate of transpiration. In the case of below-ground parts, the accumulation of dry matter is reduced. While ammonium level is increased in the chloroplasts that block the passage of materials and

affect the enzymatic activity of plants [165]. The toxicity of nitrogen causes to decrease in the immunity of the plant against biotic stress [166]. It affects the quality of fruits, has an effect on secondary metabolites of plants and disturbs the level of vitamins in fruits. The fruits with an excessive supply of nitrogen become soft and are destroyed earlier. The yield of tomatoes is decreased because the plant focuses on vegetative growth when there is excessive application of nitrogen. And it results in a decrease in yield [167]. It also causes a delay in the ripening of fruits and their size becomes smaller [168].

13 Nitrogen and Fruits

Nitrogen is important in the growth and development of fruits as it is part of the cell wall and is also vital for the metabolism of plants [169]. In biology fruit means the part of the plant that can be eaten [170]. Fruits are important for human beings as they contain dietary fibers, which help lower the level of cholesterol. They provide a high amount of minerals, plant chemicals, and vitamins, therefore they are considered protective [171]. Different fruits contain 61%–89.1% water, 0.5%–1.1% protein, Fats up to 4.4%, 4.4%–34.8% sugars, 2%–14.8% dietary fibers, and starch from trace amounts to 3% [172]. Citrus contains 0.17% protein, Banana contains 0.18% and Apples contain 0.15%. In the case of carbohydrates, Apples contain 1.29% carbohydrates, Banana contains 1.27% and citrus contains 0.72% carbohydrates [170].

13.1 Symptoms of Nitrogen Deficiency and Toxicity in Fruits: Apple, Banana, Citrus, Grapes, Mango

The color of the leaves changes uniformly without patches or streaking due to nitrogen deficiency in apples, resulting in smaller-sized leaves. Additionally, symptoms initially manifest on older leaves before progressing toward the upper leaves, potentially leading to necrosis in severe conditions. Terminal shoots emerge but remain shorter. In terms of fruit development, apples under nitrogen deficiency bear fewer fruits, which ripen early and are smaller in size. Furthermore, plants experiencing nitrogen deficiency produce fruits of lower quality and nutritional value [173]. Due to nitrogen deficiency, the level of chlorophyll is decreased in leaves due to which it loses its green color. Initially, symptoms appear on lower leaves then in severe conditions upper leaves show a pink color [174]. The stem becomes thin which is pseudostem. The size of petioles becomes smaller and the life of leaves decreases. The growth of plants is also decreased [175]. First lower leaves are affected and then deficiency symptoms progress toward upper leaves with a light green to vellow color. Fresh leaves are smaller and slenderer. In addition, the older leaves before shedding change their color to yellow, and green streaks appear on the leaves. It also affects the color of fruits that become pale. The plants facing nitrogen deficiency may remain smaller due to a reduction in growth [176]. Sometimes the color of the veins becomes yellow but another part of the leaf remains green [177]. The color of leaves changes from green to light green and finally yellow while other parts of the plant show red or pink color. Reduction in growth of shoots appears. In fruits the available Nitrogen for yeast become decreased [178]. This occurs in leaves due to the low protein and break up of plastids, so the level of chlorophyll is decreased which is responsible for chlorosis. The vegetative growth of plants is decreased [179]. As in other fruits deficiency symptoms start appearing from older leaves and then move toward younger leaves, similar is the case here. Moreover, in severe conditions the whole plant becomes yellow. Chlorophyll content is reduced which reduces vegetative growth and finally, plant growth is reduced. Finally, the production of mango is decreased [180].

Fruits are produced with low calcium levels due to excessive nitrogen usage, and bananas exhibit the development of slender pseudostems under nitrogen toxicity, causing the slender stems to bend when bearing fruits. This delay in reproductive growth onset later in the season is accompanied by decreased Nitrogen Use Efficiency and elevated soil pH. In citrus, nitrogen toxicity impairs root conduction of nitrates towards the leaves, resulting in reduced enzyme activity, lower fruit quality, and compromised cell membranes. Moreover, heightened vegetative growth from nitrogen toxicity shortens the reproductive growth period, ultimately leading to decreased yield [181]. In grapes, poor stem growth results in delayed and low-quality fruit maturation, accompanied by darkening and cupping of the leaves, indicative of a

vegetative growth dominance over reproductive growth. Excessive nitrogen application in mangoes renders the fruits more vulnerable to insect and pathogenic attacks, impeding proper color development upon ripening and leading to lower calcium levels. This disturbance in the vegetative growth phase ultimately diminishes yield [182].

14 Nitrogen and Climate: Role in Climate Change

Humans play a crucial role in altering the nitrogen cycle, and various nitrogen forms contribute to environmental degradation by influencing the climate. Certain nitrogen forms yield cooling effects, whereas others result in warming. Di nitrogen, a significant nitrogen form, plays a key role in cooling the environment. Similar to ozone, which captures sunlight rays from the sun, N_2 gas enhances the ozone layer, trapping more sunlight and generating cooling effects. In contrast, nitrous oxide is a gas that depletes the ozone layer, leading to a warming effect as more rays reach the Earth. Both dinitrogen and nitrous oxide are classified as greenhouse gases [183]. Excessive nitrogen can cause air pollution, and water pollution by producing algal blooms, disturbing aquatic life, and destroying our soil. Therefore, the gasses emitted due to the toxicity of nitrogen and are responsible for pollution; include ammonia, Nitrous oxide, Nitrogen dioxide, and Nitric oxide.

14.1 Atmospheric Effects: Emission of Ammonia and Nitrous Oxide

Ammonia is a gas with a bad smell and it is harmful to both humans and the environment as it causes a disturbance for the eyes and skin and if it is present in high amounts then it disturbs our air passageways and lungs. Moreover, it leads to death, while in the case of eves, it causes permanent damage to the eves. Ammonia also causes diseases of the heart and sometimes heart attacks may occur due to this gas [184]. Oxides of Nitrogen (NO) inhibit the synthesis of different enzymes including aconitase, NADPH-oxidase, Ribonucleotide reductase, and other oxidoreductases [185]. High NO₂ levels can prolong and exacerbate common viral infections and gravely damage the lungs [186]. According to recent estimates, NO2 pollution is responsible for 4.0 (1.8-5.2) million new cases of pediatric asthma each year, or 13% (range, 6%-16%) of the world's asthma incidence [187]. In the case of the environment, it is deposited in the lakes, in the air, and soil. It increases the acidity of lake water which can be fatal for the aquatic life. Then ammonia can be deposited into the land and create problems. As the ratio of carbon to nitrogen in the soil is disturbed it causes the emission of nitrogen from the soil in any form. Ammonia can be deposited into the soil in two ways including dry deposition and wet deposition. In the case of dry deposition, ammonia combines with the dust particles present in the atmosphere and the force of gravity brings it to land. Ammonia combines with precipitation in wet deposition, where the agriculture sector contributes up to 70% of total anthropogenic emissions, primarily from livestock waste or excreta and the excessive use of fertilizers, mainly nitrogen [184].

Out of total anthropogenic gasses agricultural sector contributes two-thirds of it and the main contributor among the agricultural sector is the application of Fertilizers. Moreover, it contributes in the form of BNF, ammonia, and other nitrogen oxides. Two processes, denitrification and nitrification emit these gases. In addition, out of these gases, the emission of nitrous oxide is exceeding the predicted limits. As total emission of this gas in 1860 was 5.3 ± 0.4 Tg N₂O-N a–1 exceeded up to 13.9 ± 1.4 Tg N₂O-N a–1 up to the year 2020. It has been estimated that in previous years 15%–70% of the applied nitrogen is used by the plants while 5%–25% is being wasted in the soil, water, and environment [188]. The important factors responsible for the change in the climate are ozone depletion and global warming. Moreover, the main factor for ozone depletion and warming is the emission of greenhouse gases. China's temperature rose by 0.91°C in the last century, exceeding the global increase of 0.85°C. Northern China experienced a greater temperature rise than the south. Nitrous oxide, a potent greenhouse gas from nitrogenous fertilizers, has a high warming potential, 25 times that of methane and 298 times that of carbon dioxide. Decreasing nitrogen fertilizer use can help control these harmful gas emissions [189]. Initially, inorganic fertilizers were promoted, but now Organic Nitrogen is recommended due to its favorable yield results and fewer harmful effects [190]. Similarly, Nitrogen dioxide is also responsible for respiratory diseases and for the diseases of Heart [191] (Fig. 4).

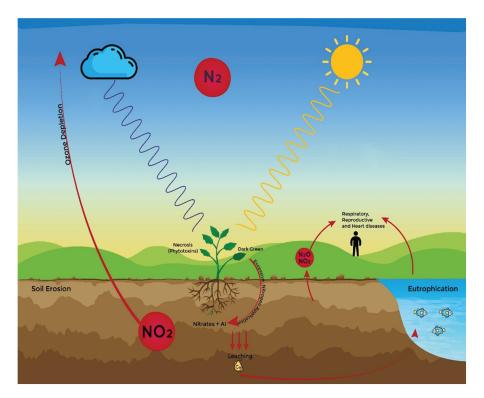


Figure 4: Effects of excessive nitrogen on different factors of the environment including aquatic life, soils, atmosphere, humans, and plants. Please increase the size of the very small letters. As they are, they are unreadable

14.2 Effect of Nitrogen on the Aquatic Environment

Ammonium, nitrate, nitrite, and ammonia are water-soluble; they can leach into groundwater, lakes, and rivers. After humans consume this water, nitrate can have various harmful effects on human health, while ammonium is generally considered non-toxic. Drinking water contaminated with nitrate can cause chest cancer, birth defects, and many problems in a child of above three months inside the mother. While the rest of the contaminated water is added to the lakes and becomes a threat to aquatic life. As that water contains more nitrogen in the form of nitrate the production and growth of aquatic plants and algae is increased. In addition, the competition for food and other requirements is increased in the aquatic ecosystem. When the growth of algae is increased so much that it covers the surface of the water this condition is known as algal bloom. Moreover, due to this algal bloom light cannot enter the water as it acts as a translucent object and also consumes much of the minerals and available food in the aquatic ecosystem. Besides these common blooms, some toxic blooms produce toxic compounds. These toxins are not only harmful to aquatic life but they have toxic effects on the terrestrial ecosystem. As humans consume fish these toxic compounds with the help of fish enter the human body and cause different problems in humans and sometimes, they can be fatal. They also affect other animals like dogs and birds etc. by drinking contaminated water or consuming the fishes affected with that algal bloom. By the death

of algal bloom, the concentration of carbon dioxide in the water is increased and the available oxygen is reduced which proved to be fatal for the aquatic animals. Not only do algal blooms affect aquatic life but the direct addition of different nitrogen forms also affects aquatic animals. That has many impacts on the lifestyle of the organisms, decreasing available food for them, and calcium carbonate becomes more acidic which is used for making the skeleton of some organisms. Therefore, the skeleton made with acidic calcium carbonate is weak. Water also becomes more acidic that have negative impacts. It converts ammonia into ammonium which affects the central nervous system and can be fatal [184].

14.3 Effect on Carbon Cycle and Biodiversity

The excessive use of nitrogen is a key factor disrupting the carbon cycle. Nitrogen plays a crucial role in aiding microbes during the decomposition process, releasing carbon dioxide. Nitrogen-loving plants thrive, while those intolerant to high nitrogen levels perish. The imbalance also negatively impacts various species, rendering some more susceptible to drought and exhibiting poor growth under less drought stress [192]. When excessive nitrogen is applied to the plants it is not taken up by plants and cannot leach down with the water. So, it is added up into the soil and reacts with soil in different ways that cause soil acidification by decreasing soil PH. Except for nitric acid other forms of Nitrogenous fertilizers do not contribute directly to changing the PH but they aid in different ways to change it. In addition, one of the major problems caused by acidic soil is that it disturbs the growth of roots. Roots become weaker and cannot absorb nutrients properly which will result in stunted growth of the plants. Another problem caused by the low PH of soil is the addition of a toxic element. Aluminum is present in the clay and cannot dissolve in high PH, but due to higher concentrations of Nitrogenous fertilizers, the soil PH is decreased supporting the aluminum to dissolve and become available to the plants. When plants uptake that aluminum they face many problems as it is toxic. It affects the leaves, roots, and shoots of plants, and internally causes disturbance in cell division, metabolism, respiration, and many other processes of the plant body. It also disturbs the uptake of nutrients like magnesium, phosphorous, etc. Nitrogen toxicity causes soil salinity, which affects plant growth by changing the morphology, physiology, and molecular structures of plants. It also changes the morphology of soil.

Excessive fertilizers that are added to the soil decrease the length and strength of roots. Similarly, they are toxic to soil microorganisms and even can be fatal. Both of these things support soil erosion. Soil is compact and is held together by soil microorganisms and roots of plants and through many different processes. So, water, wind, snow, and animals can remove the topsoil that is rich in nutrients, that flows with the water into rivers and oceans. This is known as soil erosion. So the soil behind is not fertile for the growth of plants and the plants produced show weak health status and the symptoms of different nutrient deficiencies. Similarly, when there are short roots and no microorganisms to hold the compact layers of land together. Then topsoil will easily flow and the remaining weak soil will not be favorable for plant growth, as it does not contain minerals. Another problem related to excessive nitrogen is the leaching of other minerals. Nitrate combines with other minerals like calcium and when nitrate leaches down it brings those minerals with itself. It also helps in the addition of toxic metals to plants. As PH is lowered, it changes the forms of toxic metals like lead, cadmium, etc. Moreover, these forms become available for the plants and can be fatal for them. Moreover, it reduces the organic carbon in the soil [184]. Due to the emission of nitrogen into the atmosphere, rainwater becomes acidic. In addition, when it rains the acidity of plants increases causing many problems like loss of minerals by the plant body and it can lead to the death of the plant. The effect of acid rain is not limited to the plants but the microbial communities are also affected due to the acid rain. The balance of the ecosystems is [184]. Denitrification is a process in which bacteria convert Nitrogen into Nitrous oxide and Nitrogen gas.

As we are using excessive Nitrogenous fertilizers the nitrates increase the process of denitrification [192] (Fig. 4).

14.4 Impact of Climate Change on the Nitrogen Cycle

The nitrogen cycle is being disturbed due to human activities like excessive use of Nitrogenous fertilizers, deforestation, construction sites, burning of fossil fuels, pharmaceutical industries, and growing leguminous crops [193]. It has been found that burning fossil fuels produces nitrous oxide gas and contributes to the addition of nitrogen to the environment. Therefore, this Nitrous oxide gas causes acid rain which is a problem for plant health. The nitrogen Cycle has been disturbed in the last 300 years. Burning of fossil fuels is one of the major factors in disturbing this nitrogen cycle by adding reactive Nitrogen into the Atmosphere [194]. Excessive nitrogen poses numerous harmful effects on plant health, contributing to the loss of nitrogen into lakes, rivers, and through leaching down. Consequently, the natural system of Biological Nitrogen Fixation is disrupted. Additionally, deforestation leads to soil erosion, carrying away topsoil, minerals, and nitrogen [193]. Chemicals that are released from pharmaceutical industries are considered pollutants that disturb the nitrogen cycle in soil and water environments. Drugs that are being used by humans and other animals are also being added to the environment and becoming one of the factors for pollution. The waste of these drugs is being added to the water bodies and nitrifying bacteria are present there. These added chemicals disturb the nitrifying bacteria that inhabit the process of nitrification. It has been reported that harmful chemical compounds are removed during the process of nitrification. Nitrification involves chemicals that are more sensitive to harm than ammonification because a wide variety of microbes are involved in the process of ammonification. Different types of pharmaceutical chemicals disturb ammonification while some of them do not affect the formation of ammonia. When the complex of nystatin and pharmacies are present in the water in amounts of hundred mili grams per kilogram then they affect the formation of NH₃⁻ Similarly, when the quantity of erythromycin is 20 mg per kilogram of water then it reduces the synthesis of NH_3^{-1} However, the presence of benzylpenicillin does not affect too much. Denitrification is less affected by the chemicals from the pharmaceutical industry, but certain types of chemicals can completely halt the denitrification process. For example, when Acetaminophen is present in the water in amounts of 250 mg per litter of water then it completely stops the process of denitrification. Anammox helps in nitrogen fixation. These bacteria have five different types but they are sensitive to abiotic stress and are also affected by the pollutants like that of pharmaceuticals [195]. Nitrogen assimilation is also disturbed by Cd toxicity so the foliar application of Alpha lipoic acid (Tomato plant) limits the cadmium uptake and enhances the nitrogen assimilation [196].

15 Conclusions

Nitrogen is necessary not only for plants but for all forms of life on the planet Earth. Similarly, its deficiency leads to a lack of chlorophyll giving the plants yellow color and a decrease in photosynthesis so the food produced is not enough for the consumers. It also pollutes the atmosphere and causes the problem of global warming by depleting the ozone layer. It also disturbs human health as it affects the respiratory system, Reproductive system, and heart. The rhizosphere is also being disturbed due to its excessive applications. Because we are utilizing excessive amounts to increase output, all of it is doing is making things worse for us rather than helping our crops produce more. The nitrogen use efficiency of different plants is different so we should apply nitrogen according to the requirements of the plant and in split form. Therefore, to overcome the problems of excess, deficiency, and pollution caused by Nitrogen we should analyze the soil before applying the Nitrogen which will decrease the chances of these

problems. In addition, we should decrease the application of Nitrogenous fertilizer by improving the Nitrogen Use efficiency of plants which will help us to control pollution caused by Nitrogen. To lower production costs and boost productivity, we need to transition to nano-fertilizers.

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