Nonpoint Source Nitrogen Pollution

Challenges, Solutions, and Sustainable Approaches

Edited by
Tonni Agustiono Kurniawan
Abdelkader Anouzla

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Elsevier
Radarweg 29, PO Box 211, 1000 AE Amsterdam, Netherlands
125 London Wall, London EC2Y 5AS, United Kingdom
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States

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ISBN: 978-0-443-29070-1

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Publisher: Peter Llewellyn
Acquisitions Editor: Pamela Sadhukhan
Editorial Project Manager: Vasowati Shome
Production Project Manager: Prem Kumar Kaliamoorthi
Cover Designer: Mark Rogers

Typeset by MPS Limited, Chennai, India

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Contents

Section I Emission sources of nitrogen pollution

Section II How does nitrogen pollution decrease soil quality?

Section III Toward a sustainable nitrogen management

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Anouzla conducts research in the areas of water and waste treatment, wastewater treatment plant operation, leachate discharge treatment, solid waste sorting, technical landfill management, composting of solid waste and sludge from wastewater treatment plants, algae as a natural solution for water-food-energy nexus and microplastic pollution in the environment, digitalization in the water sector, saving water in business and irrigation, and nitrogen pollution.

Preface

Nitrogen, a vital nutrient for plant and microbial growth, plays an essential role in supporting agricultural productivity, ecosystem function, and the global food supply. Yet, its unchecked mobilization in the form of reactive nitrogen species (NO_3^-, NH_4^+, NOx, and organic nitrogen) has created one of the most pressing environmental challenges of our time: nonpoint source (NPS) nitrogen pollution. Unlike emissions from identifiable and discrete origins (point sources), NPS pollution arises diffusely from myriad land surfaces and water runoff, making it far more complex to quantify, trace, regulate, and mitigate.

This book addresses the science, policy, and management frameworks necessary to confront the growing threat of diffuse nitrogen pollution. It offers a multidisciplinary, evidence-based, and forward-looking perspective—bringing together insights from environmental chemistry, agronomy, hydrology, ecology, remote sensing, and socio-economics. This volume is aimed at a diverse audience: academic researchers, environmental engineers, policy practitioners, planners, students, and anyone engaged in water resource management and sustainable land-use planning.

The idea for this book was born from the mounting realization that achieving clean water, healthy ecosystems, and climate-resilient agriculture will remain elusive unless the challenge of NPS nitrogen pollution is addressed head-on. Globally, the release of nitrogen from fertilizers, animal husbandry, urban runoff, and atmospheric deposition continues to increase. As a result, eutrophication of freshwater and marine environments, groundwater contamination, and emissions of nitrous oxide—a potent greenhouse gas—are intensifying.

The insidious nature of NPS pollution lies in its invisibility. It does not originate from a single pipe or factory outlet, but rather from thousands of hectares of fertilized farmland, stormwater drains, leaky septic systems, and atmospheric deposition—often acting synergistically. This diffuseness makes detection difficult and regulatory enforcement even more so. At the same time, the complexity of nitrogen pathways—spanning surface runoff, subsurface leaching, plant uptake, microbial transformation, and atmospheric transport—necessitates integrated scientific tools and holistic management responses.

The urgency of addressing NPS nitrogen pollution cannot be overstated. According to UNEP's 2022 Global Nitrogen Assessment, over 80% of nitrogen fertilizers applied globally are not taken up by plants and instead escape into air, water, and soil. NPS nitrogen is a key driver of hypoxic dead zones, including in the Gulf of Mexico, Baltic Sea, and East China Sea. In rural and peri-urban areas, nitrate-contaminated drinking water is associated with elevated risks of methemoglobinemia and potential carcinogenic effects. In developing countries, poor regulatory oversight combined with rapidly intensifying agriculture and urbanization exacerbates these risks, especially where wastewater infrastructure is inadequate or nonexistent.

This book was written in recognition of a paradox: while the sources and consequences of NPS nitrogen pollution are well-documented, the solutions remain underimplemented, underfunded, and often undervalued in policy agendas. One of the key contributions of this volume is its attempt to bridge the science-policy-practice divide, showing how advanced knowledge and modeling can translate into scalable, context-sensitive action.

As authors and editors, our collective hope is that this book will serve as both a scientific reference and a catalyst for real-world change. It seeks to empower professionals and students alike with the conceptual clarity and technical tools needed to confront NPS nitrogen pollution as a climate, agriculture, public health, and water security issue.

We acknowledge the contributions of numerous colleagues, institutions, and datasets that have informed this work. In particular, we express gratitude to the research teams advancing integrative watershed science, to field practitioners testing solutions in real-world environments, and to the global scientific networks that have fostered interdisciplinary collaboration on nitrogen issues. We are also deeply grateful to Elsevier for supporting the publication of this book and for their commitment to advancing sustainable environmental science.

Let this book be a contribution to a broader movement: toward a nitrogen-smart planet—where food systems, ecosystems, and human health thrive together, guided by the principles of science, stewardship, and sustainability.

Tonni Agustiono Kurniawan
Abdelkader Anouzla

SECTION I

Emission sources of nitrogen pollution

CHAPTER 9

Nitrogen cycling in the ecosystem and its role in air, water, and soil pollution

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Nitrogen cycle is an important biogeochemical process for ecosystem balance. It converts nitrogen into various chemicals needed by living organisms. However, human activities have disrupted the balance of the nitrogen cycle, causing air, water, and soil pollution. Air pollution occurs through nitrogen oxide gas emissions from fossil fuel combustion and industry, contributing to the formation of acid rain and smog. Water pollution is caused by nitrogen-rich agricultural runoff, leading to eutrophication and water quality degradation. Soil pollution results from the overuse of nitrogen fertilizers, which disrupts soil structure and fertility. This chapter emphasizes the importance of understanding the nitrogen cycle and the impacts pose by how human activities on ways to develop pollution mitigation strategies and maintain ecosystem integrity. It starts from identifying sources of nitrogen pollution, designing targeted solutions, and encouraging technological innovation to reduce pollution and improve nitrogen use efficiency, which becomes the basis for more sustainable policies in the industry and agriculture sectors.

The nitrogen cycle is a complex biogeochemical process that involves the transformation of nitrogen from one chemical to another. Nitrogen is an essential building block of amino acids, proteins, and nucleic acids (DNA and RNA), vital components for all living things (Kou-Giesbrecht et al., 2023). In the ecosystem, it moves from producers (plants) to consumers (herbivores and carnivores). An understanding of the nitrogen cycle is essential for maintaining ecosystem balance, ensuring nutrient availability for plants, and addressing the challenges of climate change.

Nitrogen in gaseous form (N_2) is the most abundant form in the atmosphere, but plants cannot use it directly (Gong et al., 2024). They need nitrogen in absorbable forms such as ammonia (NH_3), nitrate (NO_3^-), or ammonium ions (NH_4^+). The nitrogen cycle converts atmospheric nitrogen into a form usable by plants through the process of nitrogen fixation by bacteria and other microorganisms.

Nonpoint Source Nitrogen Pollution
DOI: https://doi.org/10.1016/B978-0-443-29070-1.00013-6

A balanced nitrogen cycle is essential for ecosystem health. If this cycle is disrupted, for example, due to excess nitrogen fertilizer, it can lead to eutrophication (excessive algae growth) in waters, which can damage aquatic ecosystems and reduce biodiversity (Heisler et al., 2008). Processes in the nitrogen cycle, such as denitrification, produce greenhouse gases like nitrous oxide (N_2O), which is much more potent than carbon dioxide at trapping heat in the atmosphere (Wallman et al., 2022).

In agriculture an understanding of the nitrogen cycle is important to optimize the use of nitrogen fertilizers. Overuse of fertilizers can lead to water and soil pollution, while nitrogen deficiency can limit crop growth (Rodríguez-Espinosa et al., 2023). By understanding the nitrogen cycle, we can implement more sustainable agricultural practices, such as crop rotation and the use of organic fertilizers (Liandy et al., 2016; Noor & Soewondo, 2016).

9.1 Nitrogen cycle

Nitrogen in the atmosphere can be converted into various chemical forms that can be used by living organisms (Bebber, 2021). Fig. 9.1 illustrates a schematic representation

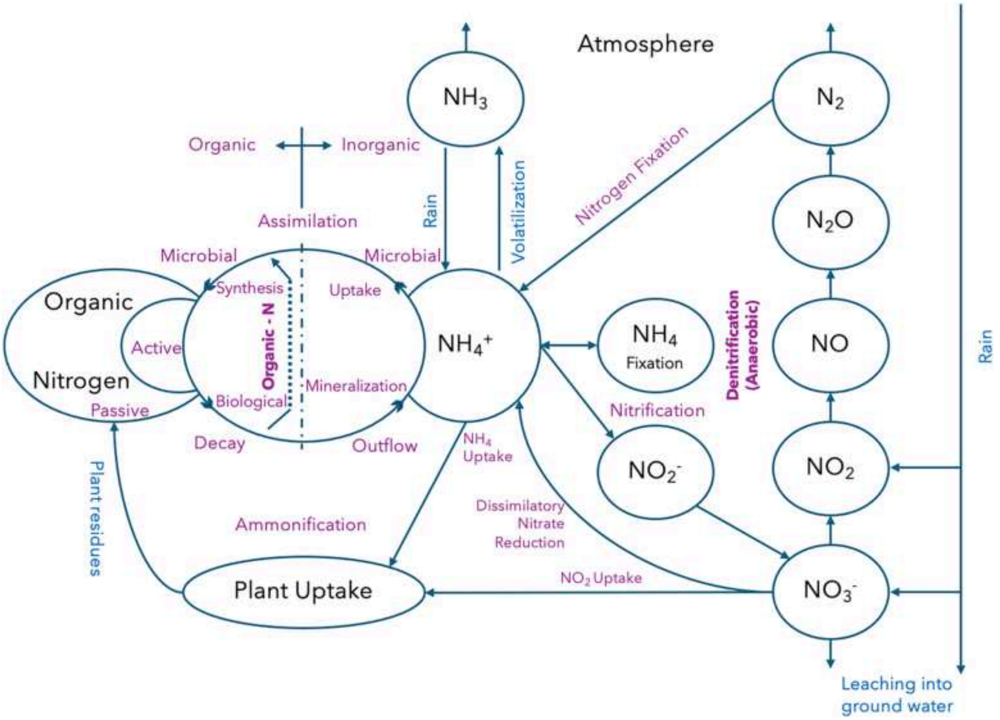

Figure 9.1 Nitrogen cycle in nature.

of the nitrogen cycle in nature, which consists of both biological and nonbiological processes. The biological processes include ammonification/mineralization, nitrification, denitrification, nitrogen fixation, assimilatory nitrogen reduction, microbial synthesis of ammonium and organic nitrogen into microbial cells, plant uptake, and the transformation of ammonium and nitrate nitrogen into plant proteins (Ward & Jensen, 2014). Meanwhile, the nonbiological processes involve ammonia volatilization, leaching of nitrite and nitrate nitrogen into groundwater, ammonium fixation within soil clay minerals, and the precipitation of nitrate and ammonium nitrogen.

9.1.1 First stage

The first stage is fixation, which converts nitrogen gas (N_2) in the atmosphere into ammonia (NH_3) by bacteria. This process is quite complex and requires significant energy (Zahran, 1999). Here is a more detailed explanation of nitrogen fixation, both biologically and nonbiologically.

9.1.1.1 Biological nitrogen fixation

The most common method of nitrogen fixation is biological, which is carried out by prokaryotic microorganisms, namely bacteria. There are two types of biological nitrogen fixation (Lindström & Mousavi, 2020).

9.1.1.2 Symbiotic nitrogen fixation

Symbiotic nitrogen fixation occurs when certain bacteria, like *Rhizobium,* form symbiotic relationships with legumes (beans, soybeans, alfalfa). These bacteria live in the plant's root nodules and provide ammonia to the host, while the plant provides carbohydrates and other organic compounds to the bacteria. This mechanism occurs as the bacteria's nitrogenase enzyme catalyzes the conversion of N_2 to NH_3. The enzyme is very sensitive to oxygen, so bacteria have a special mechanism to protect nitrogenase from oxygen (Yang et al., 2022). In symbiotic bacteria, root nodules provide a microaerobic (low oxygen) environment through the oxygen-binding protein leghemoglobin. The nitrogen fixation reaction can be written as follows:

$$N_2 + 8H + +8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi$$

This reaction requires a large amount of energy in the form of adenosine triphosphate (ATP). This symbiotic nitrogen fixation process is highly efficient and contributes significantly to nitrogen fixation in terrestrial ecosystems.

Bacteroids lack the necessary enzymes for ammonia assimilation. As a result the NH_3 produced from N_2 reduction is released into the root cell, where it undergoes assimilation through the GS-GOGAT pathway (Fig. 9.2). This process leads to the formation of glutamine, glutamate, and subsequently other nitrogen-containing

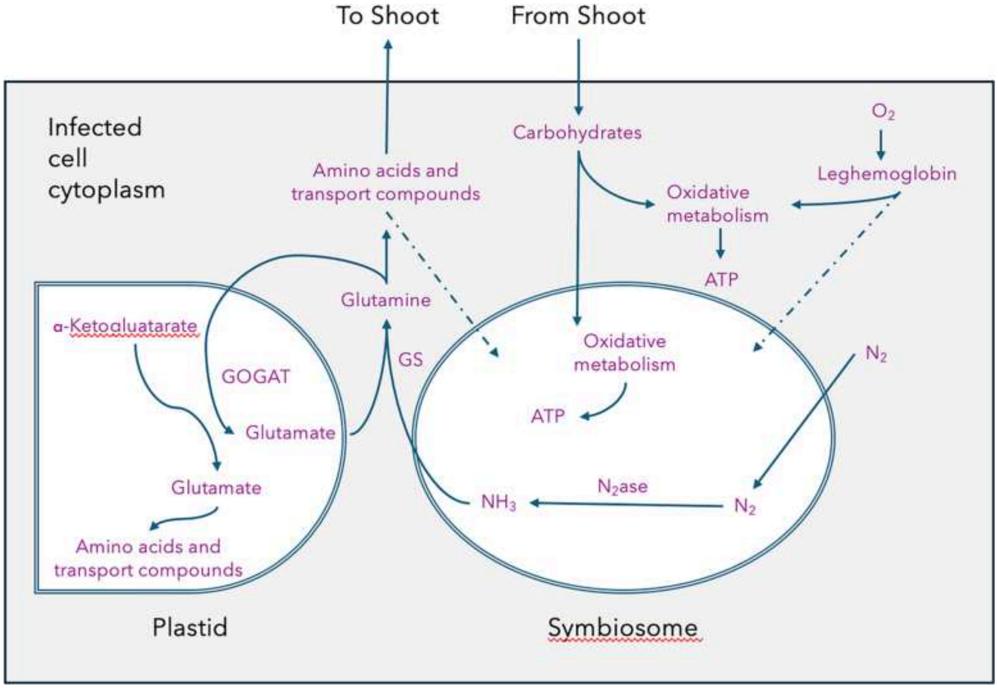

Figure 9.2 Nitrogen fixation and ammonia assimilation within an infected cell of a legume root nodule.

transport compounds. While some of these organic compounds are returned to the bacteroids, the majority are transported to the plant shoot via the xylem. To sustain N_2 fixation the host plant must provide bacteroids with a carbon source, which is delivered to the root nodule through the phloem in the form of sucrose. However, this sugar is metabolized within the host cell and converted into C_4 dicarboxylates, primarily malate. These dicarboxylates are then transported across the peribacteroid membrane, serving as the main carbon source for N_2-fixing organisms.

9.1.1.2.1 Nonsymbiotic nitrogen fixation

Free-living bacteria in soil or water, such as Azotobacter, Cyanobacteria (blue-green algae), and some anaerobic bacteria, can also fix nitrogen. Although nonsymbiotic nitrogen fixation is less efficient than symbiotic fixation, it still plays an important role, especially in aquatic environments. Among free-living diazotrophs, cyanobacteria have garnered significant interest due to their widespread and abundant presence across terrestrial and aquatic ecosystems, as well as their unique photosynthetic metabolism, which makes nitrogen fixation seemingly paradoxical. When nitrogen levels are depleted, heterocysts emerge at regular intervals along the cyanobacterial filament, serving as specialized anaerobic sites for N_2 fixation despite external aerobic conditions

(Kumar et al., 2010). The ability to fix nitrogen results from structural and metabolic changes in vegetative cells as they differentiate into heterocysts (Fig. 9.3).

During this transformation, heterocysts develop a thick cell wall with an inner laminated glycolipid layer that acts as an oxygen permeability barrier, preventing nitrogenase inactivation. These specialized cells maintain connections with adjacent vegetative cells through thin cytoplasmic channels (microplasmodesmata), which traverse the septum separating them and extend into the polar body (plag) filling the nearby region. Additionally, the photosynthetic system undergoes extensive reorganization during heterocyst differentiation: phycobilisomes disappear, and the oxygen-producing photosystem II is completely dismantled, while photosystem I, responsible for ATP production via cyclic photophosphorylation, remains functional in the thylakoid membranes.

In heterocysts the ATP required for nitrogenase activity is generated through cyclic photophosphorylation, whereas the reducing power necessary for N_2 fixation is supplied by neighboring photosynthetic cells in the form of maltose. The breakdown of sugar and the oxidation of glucose via the pentose phosphate pathway produce NADPH, which is then used for ferredoxin reduction, facilitating nitrogen fixation.

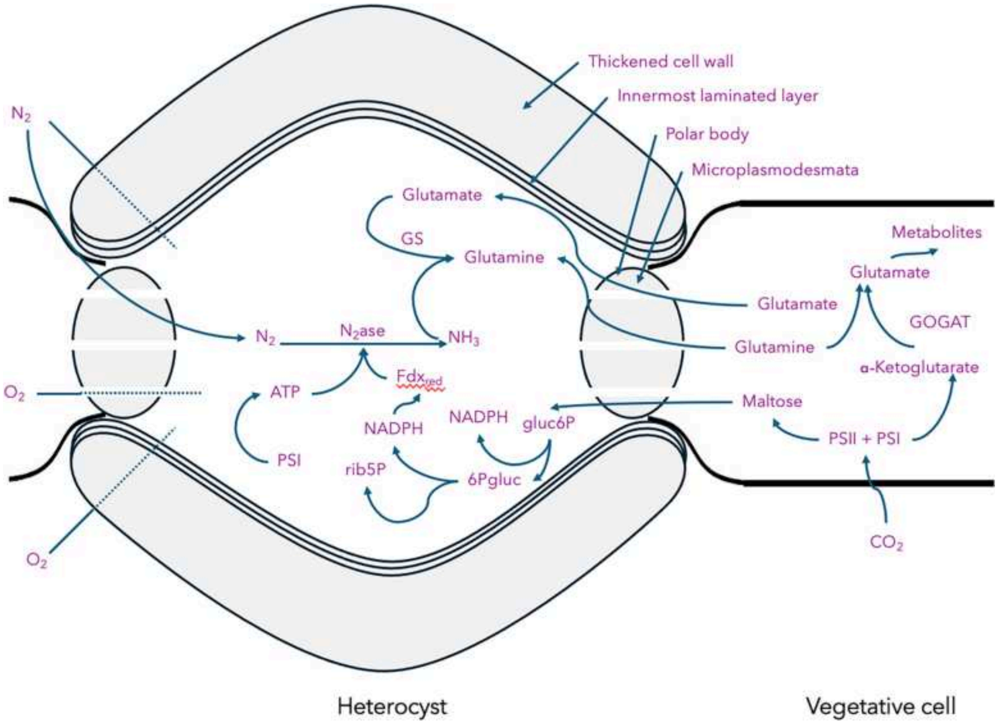

Figure 9.3 Cyanobacterial heterocyst depicting nitrogen fixation and metabolite transfer.

9.1.1.3 Nonbiological nitrogen fixation

Besides biological fixation, there is also nonbiological nitrogen fixation, which occurs through the following processes (Verma et al., 2021).

1. Atmospheric Fixation: Lightning can provide enough energy to break down N_2 molecules and allow reaction with oxygen to form nitrogen oxides (NOx). NOx then dissolves in rainwater and enters the soil as nitrate.
2. Industrial Fixation: The Haber-Bosch process is used industrially to produce ammonia from nitrogen and hydrogen at high temperature and pressure with the help of a catalyst. The ammonia produced is used for the manufacture of nitrogen fertilizers.

9.1.2 Second stage

The second stage is nitrification, which is the process of biological oxidation of ammonia (NH_3) to nitrite ($NO^{(2-)}$) and then to nitrate ($NO^{(3-)}$) by autotrophic nitrifying bacteria (Kuypers et al., 2018). This is an important stage in the nitrogen cycle because it converts ammonia, which can be toxic to, into easily absorbed nitrate. Nitrification requires oxygen to occur (Sahrawat, 2008). Nitrification happens in two stages—nitrification and nitration, as follows:

9.1.2.1 Ammonia oxidation to nitrite (nitrification)

The first stage of nitrification is carried out by ammonia-oxidizing bacteria (AOB) (Ramdewor et al., 2013). These bacteria oxidize ammonia into nitrite using oxygen as an electron acceptor. The reaction that occurs in general can be simplified as follows:

$$2NH_3 + 3O^2 \rightarrow 2NO^{2-} + 2H + +2H_2O$$

Some common AOB bacterial genera involved in this process include the following:

- *Nitrosomonas*, is one of the most common AOBs found in soil and aquatic environments.
- *Nitrosospira*, a bacterium that plays an important role in nitritization, especially in acidic soils.
- *Nitrosococcus*, a bacterium commonly found in marine environments.

9.1.2.1.1 Oxidation of nitrite to nitrate (nitratation)

The second stage of nitrification is carried out by nitrite-oxidizing bacteria (NOB) (Zhang et al., 2021). These bacteria oxidize nitrite into nitrate, also using oxygen as an electron acceptor. The reaction that occurs in general can be simplified as follows:

$$2NO^{2-} + O_2 \rightarrow 2NO^{3-}$$

Some common NOB bacterial genera involved in this process include the following:

- *Nitrobacter* is one of the most common NOBs found in soil and aquatic environments.
- Nitrospira, a bacterium that plays an important role in nitratation, and recent studies have shown that in some environments *Nitrospira* is found to be more abundant than.
- *Nitrococcus* is a bacterium more commonly found in marine environments.

The rate of nitrification in the nitrogen cycle is influenced by several environmental factors, namely (Ibrahim, 2018):

1. Oxygen availability because nitrification is an aerobic process, so oxygen availability is very important. Anaerobic conditions (without oxygen) will inhibit nitrification.
2. pH or the acidity of the environment, because pH that is too acidic or too alkaline can inhibit bacterial activity. Nitrifying bacteria grow optimally at neutral to slightly alkaline pH (pH 7–9).
3. Temperature, as too low or too high temperatures, can slow down or stop the nitrification process. The optimal temperature for nitrification ranges from 25°C to 30°C.
4. Substrate availability (ammonia and nitrite): The concentration of ammonia and nitrite in the environment will affect the rate of nitrification.
5. Presence of inhibitors. Some chemical compounds, such as heavy metals and certain pesticides, can inhibit the activity of nitrifying bacteria.

9.1.3 Third stage

The third stage is assimilation, which is the process of reducing inorganic nitrogen, such as ammonia (NH_3), nitrate (NO_3^-), and nitrite (NO_2^-) into organic compounds that can be used by living organisms themselves (Zayed et al., 2023). This process is an important step in introducing nitrogen into the food chain. The important things to pay attention to in this assimilation stage are as follows:

9.1.3.1 Nitrogen uptake by plants

Nitrogen uptake by plants, as plants are the main players in assimilation. Most plant species take up and utilize nitrate (NO_3^-), ammonium (NH_4^+), urea, and amino acids as nitrogen sources; however, the preference for these sources varies among different plant species (Fig. 9.4). For tomato roots the ideal nitrate-to-ammonium ratio is 3:1, and an increase in ammonium concentration can inhibit growth. In contrast, white spruce thrives with higher ammonium levels in the soil, while certain Arctic sedges show a preference for amino acids as their primary nitrogen source (Ali, 2020).

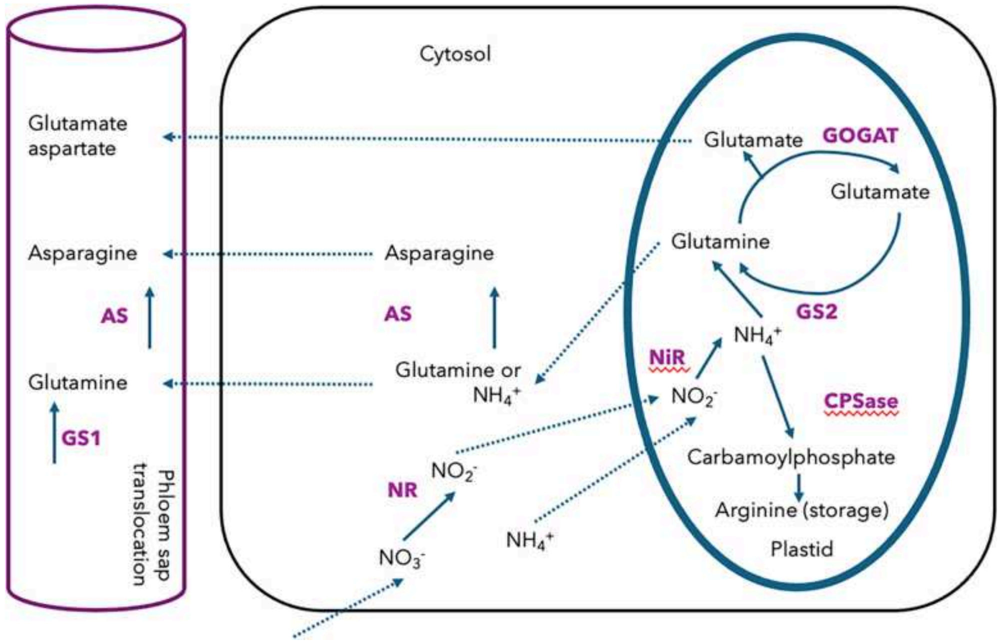

Figure 9.4 Uptake of nitrogen in plants.

Plants absorb inorganic nitrogen, mainly nitrate (NO_3^-) and ammonium (NH_4^+), from the soil through their roots (Ramond et al., 2022).

Nitrate (NO_3^-) is the most common form of nitrogen absorbed by plants from the soil. Nitrate is highly water-soluble and mobile in the soil, making it easily accessible to plant roots. Plants absorb nitrate from the soil and transfer it to leaf or root cells, in which nitrate is reduced to nitrite (NO_2^-) and then to ammonium (NH_4^+). This process requires energy and is catalyzed by the enzyme nitrate reductase (NR) by using the coenzyme nicotinamide adenine dinucleotide reduced (NADH) or nicotinamide adenine dinucleotide phosphate reduced (NADPH) as an electron donor. The reaction equation is as follows:

$$NO_3^- + NADH/NADPH + H^+ \rightarrow NO_2^- + NAD/NADP^{++} + H_2O$$

NR is a cytosolic enzyme (located in the cytosol of the cell) and is a highly regulated enzyme, the activity of which is affected by nitrate availability and environmental conditions such as light and temperature.

Furthermore, there is a reduction of nitrite to ammonium (NH_4^+) by nitrite reductase (NiR). Nitrite (NO_2^-) produced in the previous stage is toxic to plant cells if allowed to accumulate. Therefore nitrite must be further reduced to ammonium (NH_4^+). The reduction process is catalyzed by NiR and occurs in chloroplasts (in leaf cells) or in plastids (in root cells). The reaction equation is as follows:

$$NO_2^- + 6\text{Ferredoxin}(\text{red}) + 8H^+ \rightarrow NH_4^+ + 6\text{Ferredoxin}(\text{ox}) + 2H_2O$$

The NiR enzyme is more efficient and performs the reduction of nitrite to ammonium in one reaction step.

Ammonium (NH_4^+) is absorbed by plants although in smaller amounts than nitrate. Ammonium is usually available where organic matter decomposition occurs or in areas with high microbial activity. The ammonium (NH_4^+) will be converted into amino acids. Ammonium (NH_4^+) produced at this stage will be assimilated into amino acids. This ammonium assimilation process involves two main enzymes, glutamine synthetase (GS) and glutamate synthase (GOGAT).

- GS will catalyze the reaction between ammonium (NH_4^+) and glutamate to form glutamine;
- GOGAT will catalyze the transfer of amide group from glutamine to α-ketoglutarate to form two glutamate molecules.

The resulting glutamate is then used as a precursor for the synthesis of other amino acids and various organic nitrogen compounds. Although nitrite (NO_2^-) is also a form of inorganic nitrogen, it is usually not absorbed directly by plants in large quantities. Nitrite is usually an intermediate form in the nitrification process and will be converted quickly to nitrate.

9.1.3.2 Microorganism role

Some microorganisms, especially that live in the soil, play an important role in three assimilating mechanisms, that is, direct assimilation, symbiotic mutualism with Leguminosae plants (legumes), and increasing nitrogen availability (Ramoneda et al., 2021).

- Direct assimilation by soil bacteria and fungi, that is. inorganic nitrogen is directly assimilated into the cell biomass of the soil bacteria and fungi. These microorganisms convert inorganic nitrogen into nitrogenous organic compounds that become part of their body cells. This process is important because these microorganisms are a source of organic nitrogen for other organisms. Microorganisms living in aquatic ecosystems, that is, phytoplanktons, also assimilate, by absorbing inorganic nitrogen from the water for their growth. Phytoplankton and seaweed are the main producers in aquatic ecosystems and form the basis of the food chain.
- Some nitrogen-fixing bacteria, especially those belonging to the genus *Rhizobium*, have a mutualistic symbiosis with Leguminosae (legume) plants. These bacteria fix atmospheric nitrogen (N_2) and convert it into ammonium (NH_4^+) that can be used by plants. The legume provides carbohydrates for the bacteria, while the bacteria provide nitrogen in a usable form. In addition, mycorrhizal fungi are also capable of forming symbiotic relationships with plant roots. These fungi expand the root network and increase the uptake of nutrients, including nitrogen, from the soil. These fungi can also assist plants in assimilating nitrogen.

- Increasing nitrogen availability is done through the process of decomposition and mineralization of organic matter (plant and animal residues). Nitrogenous organic compounds are converted into ammonium (NH_4^+) through the ammonification process. This ammonium is then assimilated by other microorganisms or plants. Increased nitrogen availability also occurs due to the process of nitrification by microorganisms. Although nitrification itself is not assimilation, this process is important because nitrifying microorganisms (such as Nitrosomonas and Nitrobacter) are able to convert ammonium (NH_4^+) into nitrate (NO_3^-), which is most easily assimilated by plants (Ladha et al., 2022; Zulfarina et al., 2017).
- Nitrogenous organic compounds produced through assimilation include amino acids, proteins, nucleic acids (DNA and RNA), and various other essential organic nitrogen compounds. These compounds become part of plant tissues and are passed on to animals when they consume plants. After inorganic nitrogen (nitrate and ammonium) is absorbed by plants and microorganisms, it is converted into various nitrogen-containing organic compounds (Ladha et al., 2022). These compounds are immobilized in the cells of plants and microorganisms, for growth, development, and cellular functions. Several types of nitrogenous organic compounds are produced during assimilation:
  - Amino acids, the basic building blocks of proteins. Plants and microorganisms combine ammonium (NH_4^+) with carbon skeletons (derived from photosynthesis or metabolism) to form various types of amino acids. There are 20 standard amino acids commonly used in protein synthesis. Amino acids are not only used to make proteins but also act as precursors for the synthesis of other compounds, such as hormones, vitamins, and pigments.
  - Proteins and complex macromolecules made up of chains of amino acids bound through peptide bonds. Proteins have a variety of important functions in cells, including as enzymes (biological catalysts), structures (e.g., collagen), transport proteins (e.g., hemoglobin), and regulatory proteins (e.g., hormones).
  - Nucleic acids, namely DNA (deoxyribonucleic acid) and RNA (ribonucleic acid), are molecules that carry genetic information. Nucleic acids are composed of nucleotides; each contains a nitrogenous base (adenine, guanine, cytosine, thymine in DNA, and uracil in RNA), a pentose sugar, and a phosphate group. DNA stores genetic information, and RNA plays a role in protein synthesis.
  - Chlorophyll is a green pigment found in the chloroplasts of plants and algae. Chlorophyll plays an important role in photosynthesis, that is, converting light into chemical energy. Chlorophyll contains nitrogen atoms in its porphyrin ring structure. Without chlorophyll, plants cannot photosynthesize and produce food.
  - Other organic nitrogen compounds, such as alkaloids (e.g., caffeine and nicotine), amines, and amides. These compounds play various functions in physiological processes in organisms. Some vitamins and hormones also contain nitrogen and are made of assimilated nitrogen.

9.1.3.3 Nitrogen transfer

When an organism dies, the organic nitrogen compounds in its biomass are broken down by decomposers (bacteria and fungi), producing ammonium (NH_4^+). This ammonium can then undergo nitrification again, or be absorbed directly by plants and microorganisms, continuing the nitrogen cycle (Gong et al., 2024). It is therefore apparent that assimilation is a key stage as it converts inorganic nitrogen into a form that can be used by living things, allowing nitrogen to enter into the food chain and nutrient cycle. The nitrate reduction process in plants involves changing nitrate (NO_3^-) into ammonium (NH_4^+) through a series of steps catalyzed by enzymes, mainly NR and NiR. Factors that affect the assimilation stage, especially the nitrate reduction process in plants, can be grouped into:

9.1.3.4 Environmental factors

1. Nitrate (NO_3^-) availability, as nitrate is the main substrate for this process. The higher the nitrate concentration in the soil, the greater the potential rate of nitrate reduction (up to the limit of enzyme saturation). Effective nitrate transport from the soil into plant cells is essential. Factors such as soil conditions (aeration, pH, moisture) and the presence of nitrate transporters in the roots affect the availability of nitrate in the cell.
2. Light, required for photosynthesis, which produces carbohydrates and reductants (NADH or NADPH) needed by NR. Light can induce NR gene expression and increase enzyme synthesis. Light is also essential to produce reduced ferredoxin, which is used by NiR to reduce nitrite to ammonium.
3. Temperature affects the activity of NR and NiR enzymes. Each enzyme has an optimum temperature; temperatures that are too low or too high can inhibit its activity. Extreme temperatures can also damage the structure of the enzyme and decrease its effectiveness.
4. The pH of the environment affects enzyme activity. Changes in pH within the cell or the surrounding environment can affect the speed of enzymatic reactions. Soil pH also affects the availability of other nutrients, which in turn can affect nitrate reduction.
5. Water availability is crucial as lack of water causes stomata to close, reducing CO_2 uptake for photosynthesis and the production of reductants necessary for nitrate reduction. Drought can cause stress in plants, inhibiting growth and metabolic activities, including nitrate reduction. Water availability is also important for nitrate transport from the roots to other parts of the plant.
6. The availability of other nutrients, such as Molybdenum (Mo), is an important cofactor for NR enzymes. Mo deficiency can inhibit NR activity. Iron (Fe) is required for the synthesis of ferredoxin, which is utilized by NiR. Other nutrients, such as phosphorus and potassium, also play a role in overall plant metabolism, including the process of nitrate reduction.

9.1.3.5 Internal plant/microorganism factor

1. Carbohydrates (sugars) produced from photosynthesis provide energy for the nitrate reduction process. Carbohydrates provide the carbon skeleton for amino acid synthesis after ammonium is produced. Carbohydrate levels can affect NR gene expression and enzyme activity.
2. Ammonium is the end product of nitrate reduction and can act as a feedback inhibitor for NR. High ammonium concentrations can decrease NR activity. Ammonium can affect NR gene expression, by decreasing enzyme production when ammonium levels are high.
3. Stage of plant development, such as nitrate reduction activity, tends to be higher in leaves that are actively photosynthesizing. Nitrate reduction activity can also occur in the roots if nitrate is available in high amounts.
4. Each species has genetic characteristics that affect the efficiency and regulation of nitrate reduction. Plants living in environments with different nitrate availability may have developed different adaptation mechanisms.
5. Some plant hormones, such as cytokinin and auxin, may affect nitrate reduction activity, although the mechanism is not fully understood. These hormones may interact with environmental signals to regulate the nitrate reduction process.

9.1.4 Fourth stage

The fourth stage is ammonification, which occurs when bacteria and fungi decompose and convert complex organic compounds into ammonium (NH_4^+) (Chen et al., 2024). These complex organic compounds come from organic matter, such as plant and animal remains, and waste organisms that are converted into ammonia through the process of hydrolysis and release of nitrogen from organic compounds. Decomposer microorganisms (bacteria and fungi) are found living in soil or aquatic environments. Ammonification aims to recycle organic nitrogen into an inorganic form (ammonium), to be reused by other organisms in the nitrogen cycle (Zeng et al., 2014). Ammonification can occur in soil, sediment, or aquatic environments where decomposition takes place.

Although the third (assimilation stage) and the fourth stage (ammonification) of the nitrogen cycle both produce ammonium (NH_4^+), assimilation and ammonification are two different processes, particularly in the initial nitrogen source, the reaction mechanism, and the microorganisms involved (Table 9.1) (Ding, 2023).

It therefore appears that nitrate reduction is part of nitrogen assimilation, that is plants and some microorganisms convert nitrate into organic nitrogen form (NH_4^+) for their growth. Meanwhile, ammonification is part of decomposition and mineralization,

Table 9.1 Key differences between nitrate assimilation and ammonification

Feature	Nitrate reduction to ammonium (nitrate assimilation)	Ammonification
Nitrogen source	Inorganic nitrate (NO_3^-)	Organic nitrogen compounds
Organisms	Plants, algae, some microorganisms	Decomposer microorganisms (bacteria, fungi)
Mechanism	Stepwise enzymatic reduction	Decomposition and hydrolysis of organic compounds
Purpose	Assimilating nitrogen for growth	Recycling organic nitrogen into inorganic form
End Product	Ammonium (NH_4^+) for organic compound synthesis	Ammonium (NH_4^+) for recycling

whereby microorganisms convert organic nitrogen compounds (NH_4^+) back into ammonium to be reused in the nitrogen cycle.

9.1.5 Fifth stage

The fifth stage is denitrification, where some bacteria convert nitrate back into nitrogen gas (N_2), releasing it into the atmosphere to maintain the global nitrogen balance. Under anaerobic conditions, some bacteria can use nitrate (NO_3^-) as an electron acceptor in the denitrification process. It converts nitrate into nitrogen gas (N_2), which is released into the atmosphere. Some form of nitrogen intermediate (N_2O) can also be produced. If the ratio of carbon to nitrogen (C:N) in the decomposed organic matter is high, then microorganisms will store or assimilate more ammonium from their environment to meet their nitrogen needs for growth (van der Sloot et al., 2022). In addition, there is a temporary storage called immobilization, where ammonium is "stored" in the microbial biomass when it is not available to plants or other processes. Under alkaline soil pH conditions, ammonium can be converted to gaseous ammonia (NH_3). Ammonia volatilization causes nitrogen losses from the soil system and reduces the availability of nitrogen for plant growth (van der Sloot et al., 2022). Identification and quantification of denitrifying bacteria requires a combination of microbiological, biochemical, and molecular techniques. Here are some commonly used methods (Stief, 2013):

9.1.5.1 Classical microbiology methods

1. Soil sampling was conducted with a soil corer or sterile shovel from various depths and representative locations. Water sampling is done with sterile bottles from various points and depths. Samples should be processed immediately or stored at low temperatures to prevent changes in microbial composition.

2. Samples are inoculated on selective media containing nitrate as a nitrogen source and a specific carbon source. These media are designed to promote the growth of denitrifying bacteria while inhibiting the growth of others. Incubation is conducted under anaerobic conditions by using anaerobic containers or by replacing air with an inert gas (e.g., nitrogen). After incubation, bacterial colonies grown on selective media are transferred to pure agar media to obtain pure cultures.
3. The biochemical test consists of a nitrate reduction test to see the ability of bacteria to reduce nitrate to nitrite or nitrogen gas. Griess reagent can be used to detect the presence of nitrite. The nitrogen gas production test is carried out to detect the production of nitrogen gas (N_2) or oxides of nitrogen (N_2O) as a denitrification product. The use of Durham tubes in liquid media can help detect gas production. Catalase and oxidase tests are performed to distinguish denitrifying bacteria from other bacteria based on catalase and oxidase enzyme activities. Identification based on morphology and Gram staining is used to distinguish Gram-positive and Gram-negative bacteria. Microscopic observations can also help in initial identification.

9.1.5.2 Molecular methods

1. Total DNA is extracted from soil or water samples using appropriate DNA extraction kits. This DNA will be used as a template for polymerase chain reaction (PCR) analysis and sequencing.
2. PCR is used to amplify specific genes associated with denitrification, such as nirS, nirK, nosZ, and narG genes. The primers used should be specific for the genes of denitrifying bacteria. PCR products are separated by agar gel electrophoresis to confirm the presence of the target genes.
3. Quantitative PCR (qPCR) is used to quantify the amount of denitrification genes in a sample. qPCR requires a curve standard with a known concentration of DNA to quantify the amount of the target gene in the sample. qPCR can also be used to measure the expression of denitrification genes, which indicates metabolic activity.
4. DNA sequencing (Metagenomics and 16S rRNA) allows identifying all genes and organisms present in a sample, including denitrifying bacteria. Metagenomic data provides information on the diversity and functional potential of microbial communities. The 16S rRNA gene sequencing can identify the types of denitrifying bacteria present in the sample. 16S rRNA sequencing provides information on the diversity of bacteria in the sample.
5. Fluorescence in situ hybridization uses fluorescently labeled DNA probes that are specific to the gene sequences of denitrifying bacteria. The probe will bind to the target bacteria and can be observed with a fluorescent microscope.

9.1.5.3 Biochemical and physiological methods in the field

1. Measurement of denitrification rate using the acetylene block method. This method uses acetylene to inhibit the reduction of N_2O to N_2 so that N_2O can be measured as an indicator of denitrification rate. Direct measurement of N_2 gas production can also be done using gas chromatography techniques. Denitrification rates can also be estimated by measuring the decrease in nitrate concentration over time.
2. Redox potential measurements can provide information on environmental conditions that favor or inhibit denitrification. The use of redox electrodes to measure the reductive conditions (without oxygen) required for denitrification.
3. Measurement of total organic carbon availability in samples can provide information on the availability of carbon sources for denitrifying bacteria.
4. Measurements of other nutrient availability, such as concentrations of phosphorus, potassium, and other nutrients, can provide information on nutrient availability for denitrifying bacteria.
5. Stable Isotope Method. The use of nitrate enriched with 15N isotopes can trace the denitrification pathway and measure the denitrification rate. Measuring the 15N/14N isotope ratio in N_2 gas can provide information on denitrification rates and pathways.

The field implementation strategy begins with sampling planning to determine representative sampling locations. Samples are taken from various depths and at different times. Sampling is done sterilely, and samples are stored properly. A combination of microbiological, molecular, and biochemical methods is usually used to obtain a comprehensive picture of the denitrifying bacterial community. The qPCR method was implemented for denitrification gene quantification and denitrification rate measurement to confirm functional activity. Furthermore, statistical data analysis was conducted to obtain accurate conclusions. Data were obtained to develop effective nitrate pollution control strategies.

9.2 Human disturbance and nitrogen pollution in ecosystems

Human activities have significantly increased the amount of reactive nitrogen in the environment, causing various pollution problems in air, water, and soil (Du et al., 2024).

9.2.1 Nitrogen pollution in the air

Airborne nitrogen pollution, especially by nitrogen oxides (NOx), is a serious environmental problem with significant impacts on human health, the environment, and climate. NOx contributes to the formation of acid rain, smog, and respiratory problems (Badr & Probert, 1993).

Nitrogen pollution in the air is mainly caused by nitrogen oxide (NOx) compounds, which are a group of gases consisting of nitrogen monoxide (NO) and nitrogen dioxide (NO_2). These compounds are highly reactive and have a significant impact on air quality and human health.

Nitrogen naturally occurs in the environment and undergoes continuous transformation between organic and inorganic forms. The production of fertilizers further contributes to natural nitrogen sources. Both naturally occurring and human-induced nitrogen production constitute the modern nitrogen cycle. Human activities are now the dominant source of nitrogen and play a significant role in altering the nitrogen cycle. These changes can impact other biogeochemical cycles, such as those of carbon, sulfur, and oxygen. Globally, agricultural activities and large-scale biomass burning are the primary sources of nitrous oxide (N_2O) emissions. The rise in agricultural emissions is primarily driven by fertilizer application to farmlands, livestock grazing, and the spreading of animal manure. Additionally, the annual variability in agricultural N_2O emissions is influenced by seasonal savanna burning (Liandy et al., 2016). In total, human activities contribute approximately 140 Tg N per year to terrestrial ecosystems. Fig. 9.5 illustrates the various sources and pathways of nitrogen that lead to both direct and indirect N_2O emissions from soil and water (Ghaly & Ramakrishnan, 2015).

The main sources of NOx pollution are as follows:

1. Fossil fuel combustion (Rinanti et al., 2024).
    a. Motor vehicles, such as cars, trucks, and motorcycles, are major contributors to NOx emissions through the combustion of fossil fuels (gasoline and diesel).
    b. Coal and natural gas power plants also release NOx into the atmosphere through fuel combustion.
    c. Industrial processes that involve burning fossil fuels, such as cement, steel, and chemical plants, are also significant sources of NOx emissions.
2. Other industrial processes (Dasti et al., 2021).
    a. Industries that produce nitric acid, which is used in the manufacture of fertilizers and other chemicals, also release NOx as a by-product.
    b. Biomass burning, such as garbage burning and forest burning, can also produce NOx.
3. Although most of the nitrogen contained in fertilizers enters and contaminates the soil, a small amount of nitrogen can evaporate into the air and form ammonia (NH_3), which can then react with other compounds in the atmosphere to form NOx.

The sources of NOx pollution mentioned above have the following negative impacts (Dangal et al., 2019):

1. Acid rain forms because NOx reacts with water, oxygen, and other compounds in the atmosphere to form nitric acid (HNO_3) and nitric acid (HNO_2). Acid rain can

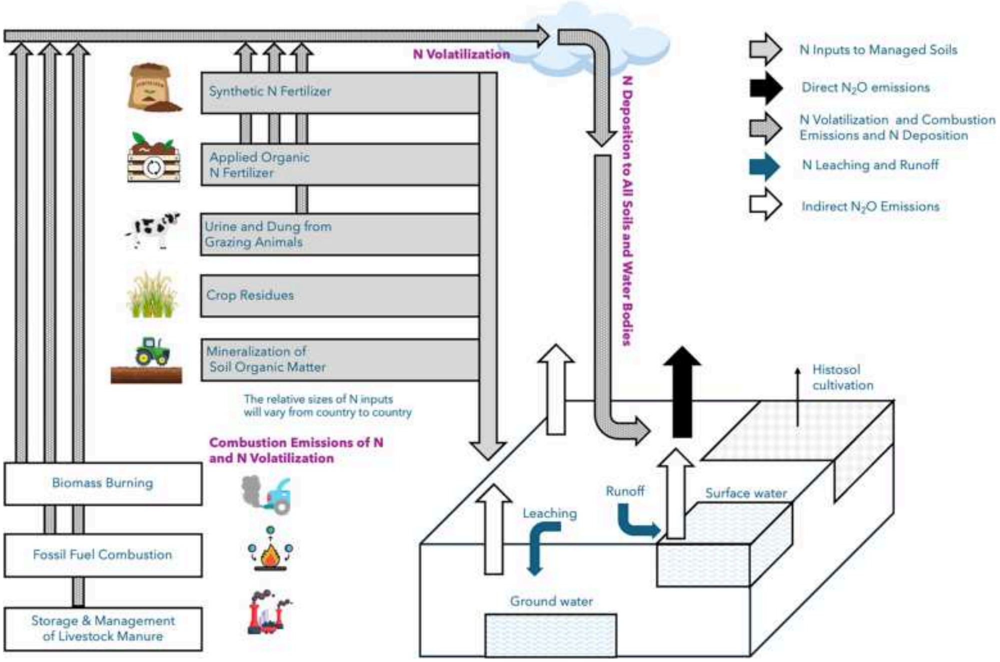

Figure 9.5 Sources of nitrogen that results N_2O emissions. *Modified from Ghaly, A. E., & Ramakrishnan, V. V. (2015). Nitrogen sources and cycling in the ecosystem and its role in air, water and soil pollution: A critical review.* Journal of Pollution Effects & Control, 3*(2).* *https://doi.org/10.4172/2375-4397.1000136*.

damage aquatic ecosystems, soil, forests, and buildings. Acid rain can also leach heavy metals from the soil and contaminate water sources.

2. Formation of Smog. NOx reacts with volatile organic compounds (VOCs) in sunlight to form tropospheric ozone (O_3) and other compounds that are the main components of smog. Photochemical smog can cause eye, nose, and throat irritation, as well as respiratory problems. Smog can also reduce visibility and damage crops.
3. Exposure to NOx can cause irritation to the respiratory tract, such as coughing, shortness of breath, and aggravate existing respiratory conditions (e.g., asthma). Long-term exposure to NOx may increase the risk of chronic respiratory diseases, such as bronchitis and chronic obstructive pulmonary disease (COPD).
4. The greenhouse effect arises because one form of NOx, dinitrogen monoxide (N_2O), is a powerful greenhouse gas that contributes to global warming.
5. NOx falling to the earth's surface through dry or wet deposition can contribute to nutrient enrichment (eutrophication) in terrestrial and aquatic ecosystems.

Some of the strategies that can be implemented to control NOx pollution mentioned above are (Tian et al., 2024).

1. Develop and utilize more efficient and low NOx emission vehicle technologies, such as electric vehicles and hybrid vehicles.
2. Switch to cleaner alternative fuels, such as compressed natural gas (CNG), biofuels, and hydrogen.
3. Implement emission control technologies in industry, such as catalytic converters, to reduce the release of NOx into the atmosphere.
4. Improve energy efficiency in various sectors to reduce the need to burn fossil fuels.
5. Switch to renewable energy sources, such as solar, wind, and hydro power, to reduce dependence on fossil fuels.
6. Implement strict policies and regulations to control NOx emissions from various sources.
7. Conduct regular air quality monitoring to identify pollution sources and evaluate the effectiveness of control efforts.

9.2.2 Nitrogen pollution in water

Nitrogen pollution in water occurs when there is an excess of nitrogen compounds, mainly nitrate (NO_3^-) and ammonia (NH_3), in water bodies such as rivers, lakes, seas, and groundwater (Hakiki et al., 2019). This excess nitrogen can disrupt the balance of aquatic ecosystems and negatively affect human health. Sources of nitrogen pollution in waters are as follows:

1. Agricultural runoff. Overuse of nitrogen fertilizers on farms is a major source of nitrogen pollution in water. Rain can wash fertilizers that are not absorbed by plants into water bodies (Huang et al., 2025).
2. Livestock waste. Livestock manure contains ammonia and other nitrogen compounds. If livestock waste is not managed properly, these nitrogen compounds can contaminate surface water and groundwater.
3. Domestic sewage. Domestic wastewater contains urea and other nitrogen compounds from human urine and feces. If wastewater is not treated properly before discharge, it can cause nitrogen pollution in water.
4. Industrial waste. Some industries, such as the fertilizer and textile industries, may also produce waste containing nitrogen compounds.
5. Fossil fuel combustion. It produces nitrogen oxides (NOx) that can be deposited into waters through acid rain.

Nitrogen pollution in waters causes negative impacts that are outlined below:

1. Excess nitrogen in waters leads to eutrophication, which is the process of nutrient overenrichment in an aquatic ecosystem, often triggered by elevated levels of nitrogen and phosphorus (Selman et al., 2002). How excess nitrogen can cause eutrophication can be explained as follows:

a. Eutrophication begins with the availability of a source of nitrogen in the water, which comes from:
  i. Nitrogen fertilizers used in agriculture are carried by runoff into rivers, lakes, and other waters.
  ii. Domestic and industrial waste often contains nitrogen in the form of ammonia and nitrate (Minarti et al., 2024).
  iii. Atmospheric deposition, by oxides of nitrogen (NOx) emissions from fossil fuel combustion and ammonia (NH_3) from agriculture, falls into waters through rain and dry deposition (Hirel et al., 2011).
  iv. Feed and manure residues from aquaculture can also be a source of nitrogen in the water.

b. Nitrogen is an essential nutrient for the growth of algae and other aquatic plants. When nitrogen levels in the water increase excessively, it triggers rapid algal growth (*algal bloom*) (Tazkiaturrizki et al., 2024).

c. Algae (including phytoplankton and cyanobacteria) utilize excess nitrogen to grow very quickly, leading to a drastic increase and growth of algal biomass (*Algal Bloom*). Algal blooms often cause water discoloration to green, brown, or red, depending on the dominant type of algae (Ayuwaningsih et al., 2018).

d. When algal blooms die, they sink to the bottom of the water and are decomposed by bacteria. This decomposition process depletes dissolved oxygen in the water. If decomposition occurs massively, dissolved oxygen levels in the water can decrease dramatically (hypoxia) or even run out completely (anoxia). Hypoxia/anoxia conditions can kill fish and other aquatic organisms that need oxygen to breathe.

e. Algal blooms and hypoxia/anoxia conditions can alter the structure of aquatic ecosystems, leading to the loss of sensitive species and the dominance of species tolerant of low oxygen conditions. This means that there is a decrease in biodiversity that causes ecosystem imbalance. Some types of algae, such as cyanobacteria, can produce toxins that are harmful to animals and humans. Furthermore, eutrophication can alter the food chain in waters, affecting organisms from producer to consumer levels. Excessive algae growth can cover the water surface, reduce sunlight penetration to the bottom, and destroy the habitat of other aquatic plants (Abascal et al., 2022).

f. Eutrophication has negative impacts on the environment and humans.
  i. Economic loss, because:
    i. Eutrophication can lead to decreased fish catches and damage to fisheries.
    ii. Algal blooms and polluted water can reduce tourist attractions and harm the tourism industry.
    iii. Eutrophication can increase the cost of treating clean water for human consumption.

  - **ii.** Negative effects on human health:
    - **i.** Consumption of water contaminated with algal toxins can cause poisoning in humans and animals (Bijay-Singh & Craswell, 2021).
    - **ii.** Algal blooms can release toxic compounds into the air that can cause respiratory problems.
  - **iii.** Ecosystem damage, due to:
    - **i.** Eutrophication can lead to loss of biodiversity and extinction of sensitive species. In addition, there are changes in the species composition of aquatic ecosystems, due to the loss of some pollution-sensitive species and the dominance of pollution-tolerant species.
    - **ii.** Eutrophication can alter aquatic ecosystem functions and disrupt biogeochemical cycles.

2. High concentrations of nitrate in drinking water can be harmful to human health, especially infants. Nitrates can be converted to nitrites in the body, which can impair the blood's ability to transport oxygen (methemoglobinemia or "blue baby syndrome").
3. Ammonia is toxic to aquatic organisms, especially fish. Ammonia can interfere with breathing and other organ functions in fish.
4. Nitrogen pollution can negatively impact the fisheries and tourism sectors due to reduced water quality and loss of biodiversity (Basu et al., 2022).

Table 9.2 presents nitrogen inputs into major rivers worldwide and the corresponding nitrogen exports to coastal waters. Notably, agriculture accounts for 17%–92% of the total annual nitrogen input into these rivers. Similarly, its contribution to nitrogen exports in coastal waters ranges from 2% to 83%. The relatively low agricultural contribution to nitrogen flow in rivers such as the Amazon and Zaire reflects the limited agricultural development within their watersheds. In contrast the high nitrogen inputs observed in the Ganges and Chinese rivers indicate intensified agricultural activity, likely driven by the increased use of fertilizers over the past few decades.

9.2.3 Nitrogen pollution in soil

Soil nitrogen pollution occurs when there is an excess of nitrogen in the soil, either in the form of inorganic nitrogen compounds (such as nitrate and ammonium) or organic nitrogen compounds, causing negative impacts on the environment and human health. Excessive use of nitrogen fertilizers can compromise soil structure and fertility. Excess nitrogen can acidify the soil, reduce biodiversity, and pollute groundwater (Zhang et al., 2021).

The pathway of fertilizer nitrogen (N) in agroecosystems is illustrated schematically in Fig. 9.6. Nitrogen is either absorbed by plant roots or lost through leaching and

Table 9.2 The role of agriculture in nitrogen input to major rivers worldwide and its subsequent discharge into coastal waters (Bijay-Singh & Craswell, 2021).

River (country)	N input into rivers (kg N km^{-2} per year)	Contribution of agriculture (%)	N export to coastal waters (kg N km^{-2} per year)	Contribution of agriculture (%)
Mississippi (USA)	7489	89	597	63
Amazon (Brazil)	3034	17	692	6
Nile (Egypt)	3601	67	268	37
Zaire (Zaire)	3427	18	632	9
Zambezi (Zambia, Zimbabwe, Mozambique)	3175	47	330	2
Rhine (Germany)	13,941	77	2795	49
Po (Italy)	9060	81	1841	56
Ganges (India)	9366	81	1269	55
Changjiang (China)	11,823	92	2237	83
Huanghe (China)	5159	88	214	24

gaseous emissions from the small mineral-N pool, which consists of nitrate and ammonium ions. Although the applied fertilizer N directly contributes to this pool, it is continuously replenished through the mineralization of organic N stored in the larger soil N pool. Only a fraction of the applied fertilizer N is utilized by the crops, while the remaining portion is lost through various processes, including nitrate leaching from the soil–plant system. A significant amount, however, integrates into the large pool of organically bound nitrogen in the soil.

Nitrogen fertilizers are essential for plant growth as nitrogen is a major component of chlorophyll, amino acids, proteins, and nucleic acids. Nitrogen *fertilizers* are available in various forms and formulas, namely inorganic nitrogen, organic nitrogen, and *slow-release* nitrogen fertilizers (Hu et al., 2014).

1. Inorganic (synthetic) nitrogen fertilizer (Zhang et al., 2021).
   - **a.** Urea ($CO(NH_2)_2$) fertilizer is the most commonly used solid nitrogen fertilizer worldwide, containing about 46% nitrogen, making it the fertilizer with the highest nitrogen content. This fertilizer is available in granular or prill form. Urea is easily soluble in water, and in the soil, it will be converted to ammonium through hydrolysis by the enzyme urease. Subsequently, ammonium is converted into nitrate through the process of nitrification. It has high nitrogen content, easy to apply, and relatively cheap. However, it has the limitation of being susceptible to nitrogen loss through ammonia volatilization, especially in alkaline soil conditions.
   - **b.** Ammonium sulfate ($(NH_4)_2SO_4$) fertilizer, containing about 21% nitrogen and 24% sulfur, is available in crystalline or granular form. Ammonium sulfate is soluble in water and releases ammonium ions which are then converted to nitrate through nitrification. This fertilizer provides nitrogen and sulfur needed by

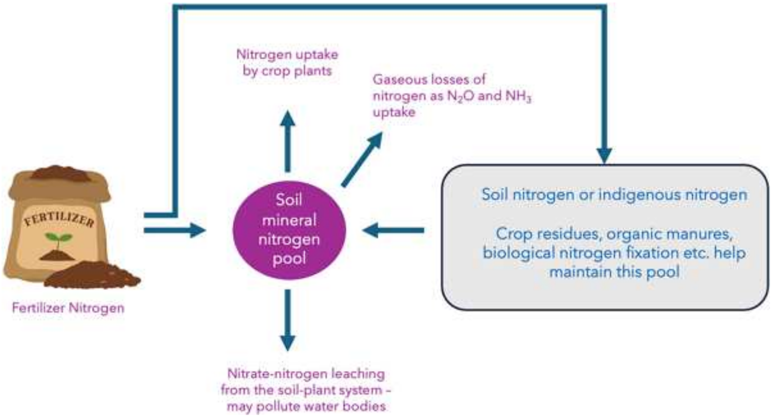

Figure 9.6 Fate of fertilizer N applied to agricultural.

plants, is relatively stable and nonvolatile, but has a lower nitrogen content than urea and can cause a decrease in soil pH (acidification) if used excessively.

   - **c.** Ammonium nitrate fertilizer (NH_4NO_3), containing about 33%–35% nitrogen, half in ammonium form and half in nitrate form, is available in granular form. Ammonium nitrate dissolves easily in water and immediately releases ammonium and nitrate that can be directly absorbed by plants. The advantage of this fertilizer is that nitrogen is available in the form of ammonium and nitrate which are quickly absorbed by plants. This fertilizer has limitations that are hygroscopic (easily absorb water), easily explode under certain conditions, and can cause soil acidification.
   - **d.** Calcium nitrate fertilizer ($Ca(NO_3)_2$), containing about 15.5% nitrogen and 19% calcium, is available in granular form. Calcium nitrate dissolves easily in water and releases nitrate and calcium ions that can be directly absorbed by plants. This fertilizer provides nitrogen in the form of rapidly available nitrate and also calcium which is important for plant growth but has a relatively low nitrogen content and tends to be expensive.
   - **e.** Liquid nitrogen fertilizer, available in various formulas, including urea-ammonium nitrate (UAN) solution and ammonium nitrate solution. Liquid nitrogen fertilizers can be applied through foliar application or through irrigation systems (fertigation). Liquid nitrogen fertilizers are easy to apply, can be absorbed faster, and can be combined with other fertilizers or pesticides. However, liquid nitrogen fertilizers require special equipment for application and are more expensive than solid fertilizers.

2. Organic nitrogen fertilizer
   - **a.** Manure, derived from animal manure (cows, chickens, goats) that has decomposed, is solid. Nitrogen in manure is released slowly through the mineralization process by soil microorganisms. Manure provides nitrogen and other nutrients, improves soil structure, and increases the activity of soil microorganisms, but it has a relatively low nitrogen content, and nitrogen is not available in a form that is easily absorbed by plants.
   - **b.** Compost, derived from decomposed organic matter (crop residues, kitchen waste), is a solid. Nitrogen in compost is released slowly through mineralization. Compost fertilizer provides nitrogen and other nutrients, improves soil structure, and increases soil water holding capacity. However, the nitrogen content in compost is relatively low, and nitrogen is not available in a form that is easily absorbed by plants (Gu et al., 2021).
   - **c.** Green fertilizer comes from plants grown specifically for fertilization purposes and then buried in the soil. Green manure can be either fresh or dried organic matter. Nitrogen from green manure plants is released through decomposition. Some green manure plants can also fix nitrogen from the air (e.g., legume

plants). The advantage of green manure is that it can increase the nitrogen and organic matter content of the soil and increase the availability of other nutrients, but green manure application requires time and special planning.

   d. Bone meal fertilizer is derived from ground animal bones. This fertilizer is in powder form, containing phosphorus and nitrogen, which will be released slowly after decomposition. Thus the advantage of this fertilizer is that it can provide phosphorus and nitrogen slowly, and help improve soil structure. However, the nitrogen content is relatively low, and nitrogen is not easily absorbed by plants.

3. *Slow-release* nitrogen fertilizers.

These fertilizers are formulated to release nitrogen slowly over a period of time and are available in various forms, including coated granules or organic fertilizers. Nitrogen is released gradually through decomposition, hydrolysis, or layering processes. Thus the use of *slow-release fertilizers* can reduce the risk of nitrogen loss due to leaching or volatilization, provide nitrogen sustainably, and reduce the need for repeated fertilizer applications. These fertilizers are also more expensive than conventional nitrogen fertilizers.

Forest growth generally continues to increase at nitrogen input levels where negative effects on plant species diversity and soil quality are already evident (Fig. 9.7). However, when nitrogen deposition exceeds 10–15 kg N ha^{-1} yr^{-1}, nitrogen leaching begins to rise as the forest nears nitrogen saturation, leading to soil acidification and increased leaching of essential base cations. Prolonged exposure to high nitrogen deposition can cause nutrient imbalances in roots and leaves, along with the release of aluminum (Al) due to soil acidification, which has toxic effects on fine roots and mycorrhiza. Additionally, excessive nitrogen accumulation in foliage may reduce frost tolerance, escalate the severity and frequency of insect and pathogenic infestations, and heighten the risk of drought stress due to increased canopy size, a higher shoot-to-root ratio, and the decline of mycorrhizal associations. As a consequence of these factors, forest growth diminishes at high nitrogen input levels, typically exceeding 20–30 kg N ha^{-1} yr^{-1} (Vries, 2021).

To determine excess nitrogen, it is necessary to measure nitrogen levels in the soil. Nitrogen in soil can be found in various forms, both organic and inorganic. Thus the methods of measurement also vary depending on the form of nitrogen to be analyzed (Qiao et al., 2022).

1. Methods for measuring total nitrogen in soil

   a. The Kjeldahl method is the classic and most commonly used method for measuring total nitrogen in soil. The principle is to convert all forms of nitrogen in the soil sample to ammonium (NH_4^+) through a deconstruction process using concentrated sulfuric acid and a catalyst. The resulting ammonium is then distilled and titrated to determine its concentration. Destruction begins by heating the soil sample with concentrated sulfuric acid and a catalyst to convert all nitrogen to ammonium sulfate. Next, the solution containing ammonium sulfate is distilled with the addition of a base (e.g., NaOH) to liberate ammonia (NH_3). In the final stage the distilled ammonia is collected in a boric acid solution and then titrated with a standard acid solution (e.g., HCl) to determine the amount of ammonia equivalent to the total nitrogen in the sample.

  b. The Dumas method (combustion method) involves burning the soil sample under excess oxygen conditions at high temperatures (around 900°C–1000°C) to convert all the nitrogen into nitrogen gas (N_2). The N_2 gas is then measured using a thermal conductivity detector. The soil sample is first burned in a furnace at high temperature. The resulting N_2 gas is measured using a thermal conductivity detector. It is relatively more expensive compared to the Kjeldahl method.

2. Methods for measuring inorganic nitrogen in soil.

  a. To measure nitrate (NO_3^-), it can be done by:

    i. Spectrophotometric method. Nitrate in the soil extract is reacted with certain reagents to form colored compounds that can be measured by a

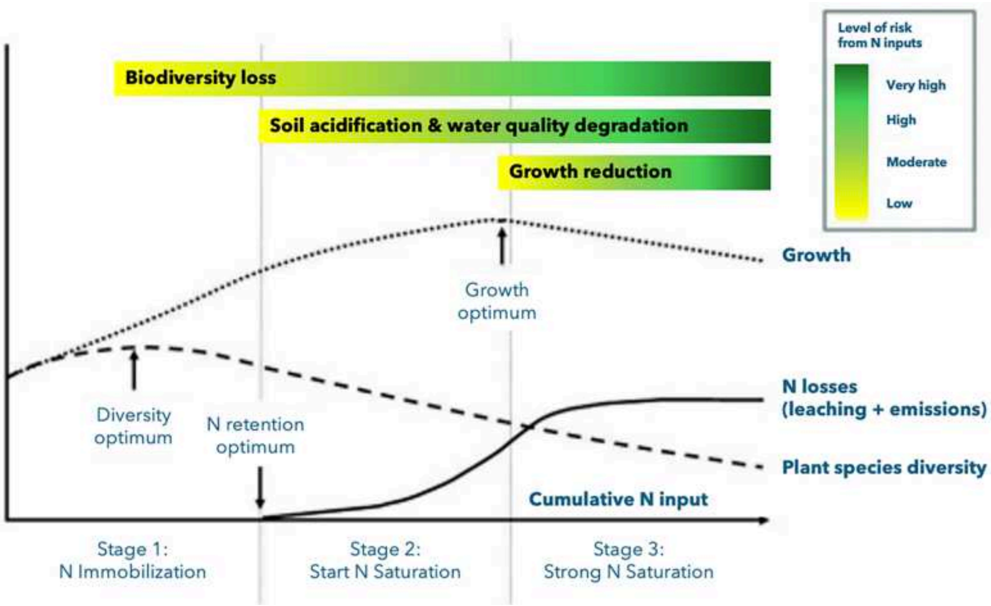

Figure 9.7 The conceptual link between nitrogen saturation stages and their impacts on terrestrial ecosystems, particularly concerning soil processes, vegetation dynamics, and growth. *Modified from Vries. (2021). Impacts of nitrogen emissions on ecosystems and human health: A mini review.* Current Opinion in Environmental Science & Health, *21*. *https://doi.org/10.1016/J.COESH.2021.100249*.

    spectrophotometer at a specific wavelength. Soil extracts are prepared, reacted with reagents, and measured with a spectrophotometer. This method is simple, fast, and inexpensive, but not necessarily accurate.
    - **ii.** Ion chromatography method. Nitrate ions were separated from other ions in the soil extract by ion chromatography and then measured with a conductivity detector. The soil extract is injected onto an ion chromatography column and analyzed. This method is specific and accurate but requires more expensive equipment.
    - **iii.** Ion-selective electrode (ISE). Electrodes sensitive to nitrate ions are used to measure nitrate concentrations directly in soil samples or extracts. The electrode is dipped into the sample or extract, and the electrical potential is measured. The method is fast, easy, and flexible to use, but less accurate.
  - **b.** As with measuring nitrate (NO_3^-), to measure ammonium (NH_4^+), it can be done by:
    - **i.** Spectrophotometric method, with the same process as for nitrate.
    - **ii.** ISE. Electrodes sensitive to ammonium ions are used to measure ammonium concentrations directly in soil samples or extracts.

3. Methods of measuring organic nitrogen in soil. Organic nitrogen content can be estimated by subtracting the inorganic nitrogen content (nitrate + ammonium) from the total nitrogen measured by the Kjeldahl or Dumas method. Further analysis can be performed by high-performance liquid chromatography (HPLC) or spectroscopic methods.

Nitrogen pollution is often the result of human activities and can upset the balance of the ecosystem. The causes of nitrogen pollution in the soil can be explained as follows:

1. The overuse of synthetic nitrogen fertilizers in intensive agriculture with the aim of increasing crop yields. This excess nitrogen is not fully absorbed by plants and can accumulate in the soil. In addition, waste from livestock farming is also rich in nitrogen and can be a source of soil pollution.
2. Some industries produce nitrogen-containing waste, such as the food processing, textile, and chemical industries. If this waste is not treated properly, nitrogen from the waste can contaminate the soil. Similarly, liquid waste from households (wastewater) and septic tanks also contains nitrogen in the form of urea and ammonium. It has the potential to contaminate the soil.
3. Atmospheric deposition, due to emissions of oxides of nitrogen (NOx) from fossil fuel combustion (motor vehicles, power plants) that react in the atmosphere and form nitric acid, can then fall to the soil through acid rain. In addition, ammonia (NH_3) emissions from agriculture (especially livestock) and industry can also be deposited onto the soil.

4. Overcultivated farmland or intensive tillage can increase the rate of mineralization of organic nitrogen in the soil, converting it to inorganic forms (ammonium and nitrate) which are more soluble and can pollute the soil if not absorbed by plants.

Nitrogen pollution in the soil, as described above, causes the following negative impacts:

1. Groundwater and surface water pollution. Water-soluble leachate nitrate (NO_3^-) can seep into the soil and contaminate groundwater. Nitrate can also be carried by surface runoff, causing eutrophication, which triggers excessive algae growth (*algal bloom*), reduces dissolved oxygen, and harms aquatic life.
2. Excess nitrogen in the form of nitrate can cause soil acidification. This acidification can affect the activity of soil microorganisms, rendering nutrient imbalances and interfering with the uptake of other nutrients.
3. Excess nitrogen can alter biodiversity by changing plant compositions in natural ecosystems, reducing species diversity, damaging the forest, and increasing plant susceptibility to diseases and pests.
4. Some processes in the nitrogen cycle, such as denitrification, can produce nitrous oxide (N_2O) which is a powerful greenhouse gas.
5. Consumption of nitrate-contaminated drinking water has a negative impact on human health. Excess nitrate causes methemoglobinemia (*blue baby syndrome*), which impairs the blood's ability to carry oxygen.

To address the problem of nitrogen pollution in soils, a multifaceted approach is required, including:

1. Proper understanding of the specific nitrogen pollution sources in an area to design effective solutions.
2. Design targeted solutions need to be tailored to specific pollution sources.
3. Encourage technological innovation to help reduce nitrogen emissions from industry and improve nitrogen use efficiency in agriculture.
4. Sustainable policies related to the regulation of fertilizer use, emission standards for industry, and incentives for sustainable practices can drive systemic change (Sitawati et al., 2022).

Sustainable agriculture aims to produce enough food without harming the environment, including reducing the negative impacts of nitrogen pollution. Here are some practices that can be implemented (Mahmud et al., 2021):

1. Efficient management of nitrogen fertilizer

a. Fertilizer use based on crop needs with:
   i. regular soil analysis to determine the content of nitrogen and other nutrients, so as to apply fertilizer efficiently.

        - **ii.** leaf analysis to monitor the nutritional status of the crop and adjust fertilizer application if necessary.
        - **iii.** timely fertilizer application in accordance with crop's specific phase, thus maximizing nitrogen uptake and reducing loss risk.
    - **b.** Use of *slow-release fertilizers*. Slow-release fertilizers release nitrogen gradually, reducing the risk of nitrogen loss. It can also reduce the frequency of fertilizer application, thus saving resources.
    - **c.** Use of organic fertilizers (Li et al., 2023), namely manure and compost that are rich in organic matter and nitrogen. Nitrogen in organic fertilizers is released slowly, reducing loss and improving soil health. In addition, planting cover crops like legumes can fix nitrogen and increase soil nitrogen content.
    - **d.** Proper fertilizer application techniques, that is, applying fertilizer near the crop row instead of across the field, to minimize nitrogen losses. In addition, fertilizer can be incorporated into the soil immediately to prevent ammonia volatilization. Fertigation through a drip irrigation system can also increase the efficiency of plant's nitrogen uptake.

- **2.** Sustainable land management
    - **a.** Soil conservation (Roesch-Mcnally et al., 2018), in:
        - **i.** No-Till Farming to reduce tillage, preventing erosion and excessive mineralization of organic nitrogen.
        - **ii.** Building terraces on sloping land to reduce surface flow and erosion.
        - **iii.** Plant crops according to the contour lines of the land to reduce erosion.
        - **iv.** Cover cropping between main periods to protect the soil from erosion, increase soil organic matter, and absorb residual nitrogen in the soil.
    - **b.** Crop rotation with different species, including legumes, to improve soil fertility, reduce dependence on fertilizers, and break the cycle of pests and diseases.
    - **c.** Use of organic mulches to cover the soil surface, reduce evaporation, increase soil organic matter, and suppress weed growth.
- **3.** Efficient water management
    - **a.** Appropriate Irrigation uses a drip irrigation system to deliver water precisely to plant roots, reducing water and fertilizer loss through percolation and surface runoff (Wang et al., 2020).
    - **b.** Scheduled Irrigation based on crop needs and weather conditions to avoid overwatering.
    - **c.** Drainage management, by establishing a good drainage system to prevent waterlogging that can cause denitrification and nitrogen loss in the form of gas (Singh et al., 2020).

4. Agricultural waste management
    a. Processing organic waste, such as animal manure and crop residues, into compost that can be used as organic fertilizer (Eska et al., 2024).
    b. Construct an effluent treatment system to remove nitrogen and other polluting compounds before discharge into the environment (Omar et al., 2024).
5. Biodiversity enhancement
    a. Agroforestry combines agriculture with tree planting to increase biodiversity, reduce erosion, and increase carbon sequestration.
    b. Conserve natural habitats around farmland to support pollinating insects, natural predators of pests, and beneficial soil microorganisms.
6. Technology and innovation
    a. Using sensor technology, GPS, and geographic information systems (GIS) to apply fertilizer, water, and other agricultural inputs precisely according to crop needs (Ayaz et al., 2019).
    b. With biotechnology approaches, plant varieties that are more efficient in nitrogen uptake and resistant to pests and diseases, as well as soil microorganisms that increase nitrogen availability to plants, are developed (Hirel et al., 2011).

Based on the description above the benefits of sustainable agricultural practices are (Murray et al., 2017):

1. Reduce nitrogen loss to the environment through leaching, volatilization, and denitrification.
2. Increase soil organic matter content, soil structure, and soil microorganism activity.
3. Minimize the use of synthetic nitrogen fertilizers and switch to more sustainable nitrogen sources.
4. Reduce water wastage through efficient irrigation practices.
5. Increase crop yields in the long term by maintaining soil fertility and plant health.
6. Supporting biodiversity on the farm and beyond.
7. Reduce greenhouse gas emissions associated with the use of nitrogen fertilizers and conventional agricultural practices.

AI disclosure

During the preparation of this work the author(s) used Kakak.ai. In the preparation of this manuscript, AI was used solely as a tool to expand perspectives and assist in structuring the framework or outline. The final content was carefully refined, rewritten based on the authors' critical thinking, and supported by credible and validated references to ensure accuracy and reliability. After using this tool/service the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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