

Review

Nitrogen Losses and Potential Mitigation Strategies for a Sustainable Agroecosystem

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Abstract: Nitrogen (N) in the agricultural production system influences many aspects of agroecosystems and several critical ecosystem services widely depend on the N availability in the soil. Cumulative changes in regional ecosystem services may lead to global environmental changes. Thus, the soil N status in agriculture is of critical importance to strategize its most efficient use. Nitrogen is also one of the most susceptible macronutrients to environmental loss, such as ammonia volatilization (NH₃), nitrous oxide (N₂O) emissions, nitrate leaching (NO₃), etc. Any form of N losses from agricultural systems can be major limitations for crop production, soil sustainability, and environmental safeguard. There is a need to focus on mitigation strategies to minimize global N pollution and implement agricultural management practices that encourage regenerative and sustainable agriculture. In this review, we identified the avenues of N loss into the environment caused by current agronomic practices and discussed the potential practices that can be adapted to prevent this N loss in production agriculture. This review also explored the N status in agriculture during the COVID-19 pandemic and the existing knowledge gaps and questions that need to be addressed.

Keywords: nitrogen; nitrate leaching; nitrous oxide; soil resilience; soil microbiome; regenerative agriculture; ecological ditch



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1. Introduction

The current global population of 7.8 billion is projected to reach over 9 billion by 2050 [1]. This projected boom in population would mandate an approximately 70% increase in global agricultural production to ensure food security in the developed and nearly 100% in the developing countries [2]. To keep up with this demand, global agriculture will continue to consume more amendments in both inorganic and organic forms that can support agricultural production and simultaneously battle the food waste crisis where one-third of the annual produced food goes to waste (1.3 billion tons) [3]. Nitrogen (N) is a critical element for all living organisms and assimilation of N by both terrestrial and aquatic plants is limited by its forms in the ecosystem [4].

Over the last five decades, the global N cycle has changed significantly due to the incessant input of nitrogen fertilizers. Nitrogen cycling involves five major steps; biological N fixation, ammonification, nitrification, N assimilation into microbial biomass pool, and finally denitrification [5]. However, both chemical N fertilizers and organic manure are often applied to soil in exceeding amounts for crop growth requirements lead to N loss. Of the applied N for crop growth, only 45–50% is being incorporated into the agricultural products [6] and the remainder is subjected to substantial loss [7]. A significant

amount of N in soil and may be lost to the environment as NO_3 , NH_3 , or N_2O [8,9]. NO_3 may also continue to undergo recycling in the soil–water–air system and convert to N_2O and N_2 through the denitrification process and released back to the atmosphere [10]. Particularly, N_2O emission is substantial from production agriculture at the beginning stage of N fertilizer application during the cropping season [11]. Furthermore, the potential warming effect of N_2O and the short-term cooling effect of NH_3 and NO_x both has major consequences on global human and environmental health [12,13]. Additional complications in estimating N_2O arises from the nonlinear nature of N_2O emission in response to N fertilizer application along with other soil and environmental controlling factors [14]. Intergovernmental Panel on Climate Change (IPCC) linear model, one of the approaches to estimating N loss in terms of N_2O , which may overestimate N loss at lower N application rates while underestimating higher rates [15–17] (Figure 1).

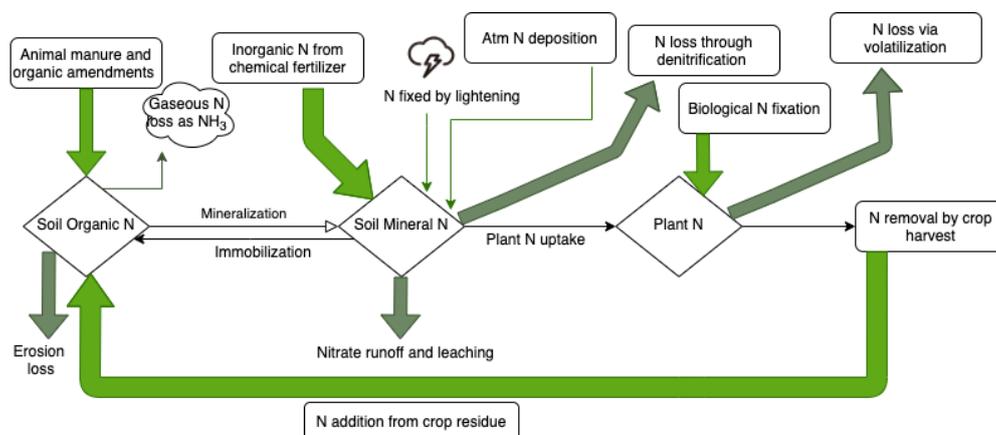


Figure 1. The nitrogen cycle in the soil.

Agricultural contribution to global N pollution requires constant monitoring and adoption of necessary efforts at the farm, regional, and national levels to mitigate and regulate environmental degradation. Nitrogen footprint, under agricultural context, is the amount of N released from resource use in agricultural production at every step of the production line both upstream or downstream [18]. Nitrogen footprint can serve as an indicator of the usage and losses of N in the production and consumption of food and energy, thereby, an ecosystem service. The objective of this review was to explore the agricultural status of N in the current agronomic production system and possible mitigation strategies to address and minimize N loss for sustainable agriculture.

2. Nitrogen Footprint in the World

Nitrogen plays such a cardinal role in ecosystem productions and the Earth's energy balance that any changes in the N cycle will bring about profound impacts on the global ecosystem and human health [19–21]. It is necessary to track the gains and losses in the N cycle and there are several tools to do just that. For instance, a quantifying tool that serves as an indicator of N losses to the environment from different stages of the N cycle ranging from production to consumption levels is called N footprint [22]. Globally, agriculture dominates N footprints [23]. Furthermore, global trades connecting different countries in importing and exporting agricultural commodities also leave a major mark on the N footprint. Nitrogen footprint can be a useful tool to help resolve the critical dilemma between the optimized N use (nitrogen use efficiency; NUE) and minimize negative impacts associated with N use. The food N footprint is the dominant component of the per capita N footprint [24,25]. On average, the per capita N footprint of ten countries from different regions of the world ranges from 15 to 47 kg N per capita per year and the principal reason behind this is the difference in the protein consumption rates and N losses during agronomic production [22]. In Asia, China has a big per capita food N

footprint and it has increased almost 50% over 30 years (i.e., 1980 to 2008), however, it was still close or lower than that of countries in North America or Europe [24,26]. Of this per capita food N consumption, almost 40% is from protein, which is much lower than in other countries [26,27]. In the case of fossil fuel consumption, China leaves a lower N footprint than the USA [18] but since it has limited to no regulatory measures in place to reduce NO_x emission [28], hence, China has a higher energy N footprint compared to some of the European countries like UK and Germany [24–26]. Australia, a country in the Asia–Pacific region, due to a high protein diet and affordable food prices has a high food N footprint and a high energy N footprint due to higher N emission from electricity generation [22]. In North America, the USA has the largest N footprint for food production where the N footprint per capita is 39 kg N. This is followed by transportation where a relatively higher N footprint is observed compared to other countries [18]. In Europe, agricultural innovation was pioneered by the industrial revolution primarily at the turn of the 18th century, and again in the early 20th century with the arrival of the Haber–Bosch process. This nitrogen-based synthetic fertilizer production dramatically increased the productivity in agricultural sector, thus, countries like UK, Netherlands, Austria, and Germany have a higher food N footprint than many parts of Asia, typically ranging from 24 to 29 kg N capita⁻¹ year⁻¹ [18,29]. Table 1 details per-capita nitrogen footprints from different countries adopted from Oita et al. (2016) of nations [30].

Table 1. Ranked per-capita nitrogen footprints of nations (NFP = nitrogen footprint).

| Country (High-Ranked in NFP) | NFP (kg N cap ⁻¹ yr ⁻¹) | Region | Country (Low Ranked in NFP) | NFP (kg N cap ⁻¹ yr ⁻¹) | Region |
|---------------------------------|---|----------------|--------------------------------|---|----------------------|
| Hong Kong | 225 | Southeast Asia | Liberia | 2 | West Africa |
| Luxemburg | 145 | Western Europe | Moldova | 3 | Eastern Europe |
| Kuwait | 102 | Western Asia | Côte d’Ivoire | 5 | West Africa |
| Singapore | 98 | Southeast Asia | Papua New Guinea | 7 | Oceania |
| Uruguay | 90 | South America | Tajikistan | 9 | Central Asia |
| Australia | 88 | Oceania | Malawi | 10 | South-Eastern Africa |
| UAE | 70 | Western Asia | Sri Lanka | 11 | South Asia |
| Paraguay | 67 | South America | North Korea | 12 | East Asia |
| Canada | 65 | North America | Mozambique | 15 | Southern Africa |
| USA | 62 | North America | Burundi | 17 | East Africa |

3. Nitrogen Losses in the Environment

Nitrogen contains readily converted reactive chemical (reactive nitrogen; Nr) [24] species causing a cascading effect on the environment and impacting the global ecosystems [31]. These reactive species are N₂O, NO₃⁻, nitrite (NO₂⁻), NH₃, and ammonium (NH₄⁺) and mostly of anthropogenic origins such as fossil fuel combustion and agriculture (both legume cultivation and use of industrial fertilizers) (Figure 2).

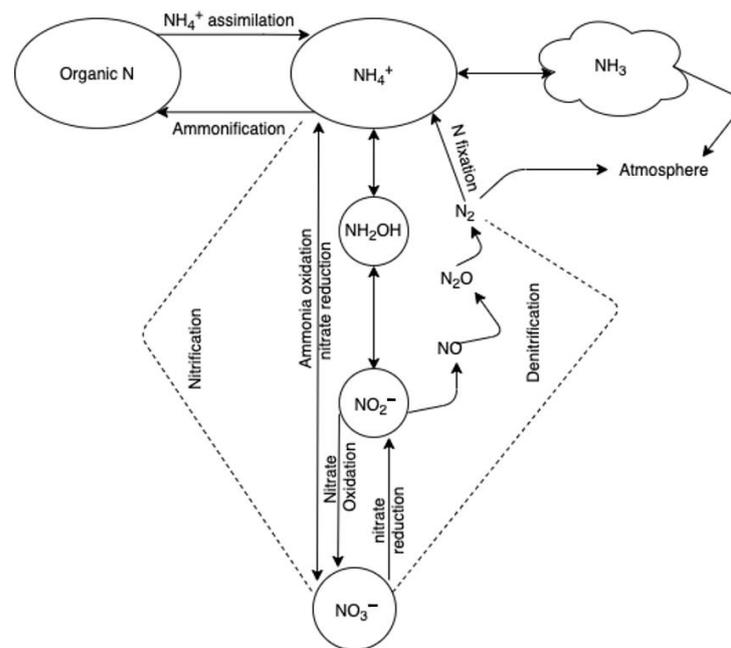


Figure 2. The major processes of the nitrogen cycle in soil.

3.1. Ammonia Volatilization

Ammonia volatilization is one of the major sources of N loss from arable farms worldwide. Different soil conditions affect the volatilization rate of NH₃ from the soil. Soils with high pH are generally prone to lose significant amounts of NH₃, however, neutral or acid soil may also lose NH₃, especially after inorganic fertilizer application like urea or organic amendments such as urine [32,33]. Moreover, soil and atmospheric temperature greatly affect the urea hydrolysis, thereby, the NH₃ (aqueous) transfer rate from the soil solution to atmospheric NH₃ (gas) [34]. Furthermore, low soil moisture tends to stimulate high soil solution concentration, thus, higher loss of NH₃ [35]. When NH₄ containing fertilizers such as ammonium nitrate (NH₄NO₃) or ammonium sulfate (NH₄SO₄) or urea (CH₄N₂O) are applied to soil during different stages of crop growth [36], they are typically subjected to immediate NH₃ volatilization. For instance, upon application, urea undergoes hydrolysis ensuing higher soil pH in the microsites of soil causing transformation of NH₄⁺ to NH₃ [37,38]. The greatest amount of NH₃ is released through anthropogenic activities [39], which releases around 7.6 million kg ha⁻¹ of NH₃ emission is from crop and animal husbandry accounting for more than 90% of the total emission [40]. Ammonia volatilization is a major problem to human health and the environment because it can react with acidic components of the environment such as sulfate (SO₄²⁻) or NO₃⁻ and form a secondary inorganic aerosol. NH₃ emissions, moreover, significantly contribute to the formation of acid rain in the atmosphere and can be an indirect source of N₂O emissions, and promote eutrophication of surface water bodies [35,41]. The economic implications of NH₃ emission are particularly threatening to developing countries, for instance, India, as an earlier simulation study showed that almost one-third of the applied N fertilizers or manure is lost to the atmosphere as NH₃ [42,43].

3.2. Nitrous Oxide and Oxides of Nitrogen (NO_x) Emissions

The simultaneous process of nitrification (aerobic condition) and denitrification (anaerobic condition) produces N₂O as the common byproduct in soil [44]. During nitrification, NH₄-N is microbially converted to an intermediate product, hydroxylamine (NH₂OH) followed by the production of NOH, and finally NO₂. During nitrification, N₂O is produced both at NH₂OH and NO steps, while, on the contrary, denitrification, transforms NO₃ or NO to N₂ or N₂O [22]. Nitrous oxide is considered one of the most critical greenhouse

gasses due to its prolonged atmospheric lifetime (120 years) and more potent in trapping heat than CO₂. Moreover, N₂O is responsible for ozone depletion by reacting with stratospheric O₂ and forming nitric acid [45]. Agricultural activities are the largest anthropogenic sources of atmospheric N₂O emissions [46]. An increase in fertilizer application rates, N sources, are the dominant factors of N₂O production in an agroecosystem [47]. Livestock, especially, traditionally grazed ruminants on poor quality fodder in pastures also plays a significant role in N₂O and methane (CH₄) emissions [48].

NO₂ is released to the stratosphere mostly through fossil fuel combustion from vehicles and industrial plants in addition to natural processes of nitrogen in the soil. It has been highlighted by the WHO as a potential health risk and that exposure to this pollutant should be minimized [49]. Several pulmonary and cardiovascular diseases have been linked to long-term exposure to elevated levels of NO₂. Some of the most documented cases include lung function growth deficit in children and compromise lung function in adults [50,51].

3.3. Nitrate Leaching

Apart from NH₄⁺, NO₃ produced during nitrification in the aerobic condition is a preferable form of N for plant uptake. However, it is highly mobile in soil due to its anionic nature and in well-drained soil, NO₃ is the first nutrient to be washed off from the soil profile. The amount and distribution of rain and irrigation affect the NO₃ loss below the root zone in the soil profile [52]. Studies done in the early 90s' suggested that depending on the soil type, almost 80% of the applied N can be lost as NO₃ runoff [53]. Colder temperature contributes considerably to nitrate leaching due to greater precipitation and slower plant uptake of nitrate [54]. Earlier studies on nitrate loss in soil suggested that more than 100 kg N ha⁻¹ year⁻¹ of nitrate was lost from grazeland after tillage through the soil profile over the next winter [55]. Soil structure and texture may also affect nitrate movement through the soil profile. Nitrate leaching is greatest in sandy soil with poor structure and slowest in clay soil [35]. Additionally, soil macrofauna movement and plant root growth often allow rapid nitrate movement in soil [56]. Nitrate leaching and runoff lead to severely deteriorated both ground and surface water quality levels and resulting in eutrophication and algal bloom. Severe cases of NO₃ contamination in human is evident by Blue-Baby Syndrome [57] where NO₃ molecules in drinking water combine with blood hemoglobin and hinder blood oxygen transportation [58]. Table 2 shows the major sources, amounts, and pathways of global reactive nitrogen (Nr) emission [30].

Table 2. Major sources, amounts, and pathways of global reactive nitrogen (Nr) emission.

| Reactive Nitrogen (Nr) | Amount (Tg) | Source | Pathway |
|---|-------------|-----------------------------|-----------------------------|
| Nitrate (NO ₃ ⁻) | 161 | Industries and Agriculture | Leaching and surface runoff |
| Ammonia (NH ₃) | 45 | Agriculture | Volatilized |
| Nitrous Oxide (N ₂ O) | 6.2 | Agriculture | Gaseous emission |
| NO _x | 35 | Transportation and emission | Gaseous emission |
| Nitrogen emissions potentials to water mainly as NO ₃ ⁻ | 28 | Consumer | Mainly Sewage |

4. Mitigation Strategies

4.1. Farming System Design

To establish an effective and productive resource partitioning system, a collaborative organization of the agricultural production systems, which involves the adoption and eventual adaptation of a combination of a less expansive, minimal resource extraction crop-livestock production of high-value commodities, is required. The integrated pro-

duction system (IPS) is not essentially a location restrained system, rather a synergistic approach towards agricultural resource utilization with minimal external input compared to intensive systems, and IPS helps materialize the concept of integrating several on-site farming components such as crop residues and animal waste [59,60]. A recent case study from Nigeria where herdsman and farmers exchange resources, for instance, manure (collected by farmers) and crop stubble (used by herdsman as cattle feed) exchange helped ease societal tension and offering a limited but promising solution to an on-site N loss solution [61]. Another example of IPS has been demonstrated in the Benin Republic, West Africa where a local farm (Songhai Farm) adopted all the IPS components and successfully collaborated between the different stages of agriculture such as production, selective breeding, harvesting, product processing, product placement in the local market, and finally on-site waste recycling to reuse nutrients [62]. Thus, IPS allows all participating parties alike to place a value on the on-site waste and help in sharing spatially separated resources.

4.2. On-Farm Best Available Techniques (BATs)

Preventing the loss of ammonia can be achieved by a combination of BATs such as placing N-fertilizers subsurface or injecting, applying urea fertilizer before rainfall events and after application irrigate the crops, applying N-fertilizers with acidifying agents such as elemental sulfur (not gypsum) or urease inhibitor, etc. [35,63]. Reducing nitrate loss through the soil profile, however, requires the adoption of a wide range of management strategies, for instance, split application of N-fertilizers according to crop nitrogen requirements and if applying animal slurries as N source then according to the crop demand, spraying nitrification inhibitor, maintain a constant vegetative cover, especially over the drainage period, cultivating soil in spring rather than in fall, etc. [64–71]. Many of the mitigation strategies adopted for N₂O emission overlap with the strategies for mitigating NH₃ volatilization and nitrate loss with a few exceptions, for instance, using lime to increase soil pH, avoiding anaerobic pockets in soil by maintaining optimum irrigation rate, and reduce animal traffic to avoid soil compaction [35,72].

4.3. Improved Nitrogen Use Efficiency (NUE)

Achieving nitrogen use efficiency (NUE) in crop and livestock production will involve implementing the best management practices (BMPs) leading to increase recycling within the system [73,74]. Some BMPs of NUE are discussed hereunder.

4.3.1. Enhanced Efficiency of Fertilizer Material

The use of enhanced efficiency fertilizers composed of coatings of low permeable materials attached to an inhibitor (nitrification or urease inhibitor) as an additive may be used to regulate processes such as nitrification or urea hydrolysis to simultaneously reduce the N loss and increase N uptake by plant and soil microbial population [75–77] under both laboratory and field setup. Such examples of inhibitors are *N*-(*n*-butyl) thiophosphoric triamide (NBPT), phenylphosphorodiamidate, dicyandiamide (DCD), and 3,4-dimethyl pyrazole phosphate (DMPP). For instance, the use of NBPT coated urea compared to uncoated-urea reduced NH₃ emission by 42% for sunflower in Spain [78]; from 9.5% to 1.0% of applied N for winter wheat in Australia [79], and finally in the UK, for multiple grassland and winter cereal species [80]. In New Zealand, the volatilization of NH₃ in grazing pastures was reduced through the application of NBPT (18%–28%) [81]. Moreover, the application of nitrification inhibitors such as DCD and DMPP in conjunction with urea significantly reduced N₂O emission from agricultural soils of Louisiana, USA by more than 76% and 67%, respectively [82].

4.3.2. Site-Specific Nutrient Management (SSNM) and Real-Time Nitrogen Management (RTNM)

Crops typically respond to applied N in varying degrees and the fate of that applied N depends vastly on the soil conditions. The traditional broad-spectrum blanket recommendation or “one size fits all” often fails to address crop N requirements and

cannot surveil N availability from all possible sources leading to severe economic and environmental drawbacks. A possible solution could be the site-specific nutrient management strategy (SSNM). The SSNM considers several factors in production agriculture, for instance, yield potential of the crop, plant nutrition, inherent soil capability to supply N, calibrated N dosage, and subsequent N recovery calculations [22]. The SSNM addresses the crop-specific nutritional needs, utilizes maximum available resources to obtain N, calculates the gap of N required to fill the nutrient deficit gap, and finally recommends optimum N application recommendation [83]. Contrary to the blanket application of N, split applications according to the crop requirements, based on the growth stage, could prove to be an important strategy to enhance N recycling in soil, minimize loss of N as NH_3 and N_2O . Without the destructive collection of samples, sensor-based tools have the potential to identify and correct N stress, which has already occurred during the growing season for plant production [84]. Normalized difference vegetative index (NDVI) data of the greenness of plant leaf and previous crop yield and N application data can be used to implement the splits and may lead to an increase in nitrogen use efficiency and N recovery by the plant [22]. Studies on wheat (*Triticum aestivum* L.) produced primarily on the Central Great Plains of the United States showed that sensor data converted to NDVI was used to formulate a response index and showed a 15% greater NUE compared to whole-field techniques [85]. An emerging technology for site-specific N application for maize is real-time fluoro-sensing for variable-rate nutrient management, especially at earlier growth stage (V2) of the plants [86]. Moreover, proximal sensor-based variable rate N management strategy was reported to achieved greater grain yield and improved NUE for maize in Colorado, USA [87]. Similarly, in the UK, applications based on real-time sensor data saved 15 kg N ha⁻¹ that increased the NUE without any reduction in wheat grain yield [88]. In a five-year study on N recovery, approximately 25–50 greater N was recovered with RTNM in maize cropping over-scheduled N application in Punjab, India [89]. In Guangxi Zhuang, China, NUE in rice (*Oryza sativa*) was 75% higher in densely planted reduced and delayed N applied rice plants compared to conventional farmers practice (broadcast) [90].

4.3.3. Deep Placement of Urea Super Granules

If there is an anoxic layer overlying a reduced zone in the soil, which is very typical of low-lying flooded rice-production systems, N loss can be encouraged by nitrification and denitrification, simultaneously [22]. Deep placement of nitrogenous fertilizers, for instance, large urea granules in rice-fields has successfully prevented the conversion of NH_4 to NO_3 and subsequent losses. Early studies in the 90s' showed that deep placement of urea reduced N loss by 65% and resulted in a greater rice yield by 50% in grain yield compared to split application of granulated urea [91]. Recent studies also showed that NH_3 and N_2O loss were significantly suppressed by 94% with the deep placement of large urea granules (≥ 0.7 g) in rice-growing areas [92,93]. Furthermore, policymakers across Europe have indicated a relatively new method termed as "Closed-Slot Injection Method" to reduce NH_3 emissions with inorganic fertilizers or organic inputs and this technique has proven promising due to the wide-spread availability among European farmers. For instance, NH_3 loss was reduced in maize by 75% for mineral fertilizer and 96% for organic amendments compared to surface broadcast [94].

4.4. Pasture and Livestock Management

Reducing N_2O emissions while keeping the ruminant population at the higher end of the production spectrum if not decreasing, requires either a top-quality ruminant diet or improved yield, thus, making this a livestock nutrition issue. Ruminant excretion quality is largely dependent upon ingested feed quality and depending on soil factors such as soil moisture and temperature, the N-component of the excretion may be subject to significant losses, thus, causing environmental hazards, such as increased nitrous oxide emission and greater nitrate loss [95,96]. Apart from reducing P loss to runoff [97], to facilitate enhanced nitrogen mineralization and to reduce the loss of NO_3 in the soil,

strategic cattle grazing in pastures along with overseeding of susceptible areas with annual grasses may offer a sustainable solution in livestock management as opposed to traditional grazing [72]. Additional approaches, such as supplementation of animal feed and anaerobic digestion may help increasing nutrient use efficiency by recycling nutrients on-site [98]. These management strategies are particularly important for developing countries like China, India, and Brazil that are major players in the beef and dairy industries [99] where ruminant feed is mostly associated with inferior quality of feed leading to a low-efficiency animal diet [100]. On the other hand, adopting alternative approaches, such as rearing mono-gastric non-ruminants, for instance, poultry, rabbit, swine, and horses (which are typically on a better and balanced diet than beef and dairy cattle) may potentially lead to efficient nutrient use and lower nitrogen loss [98]. Additionally, supplementing animal feed with amino acids such as lysine has the benefits of reduced N loss from swine and poultry by 30% compared to traditional feeding routine [101]. In managing pasture soil health, alternative ways of adding N have been investigated. In addition to adding organic fertilizers such as poultry [102] in managed pastures, using N-fixing legumes to supply soil N is an ecologically safe practice [103]. For instance, in long term pastures in Australia and New Zealand, and organic farming in the UK, white clover (*Trifolium repens*) is grown with other grass species and the fixed N is released slowly to the grass once released into the soil through root exudates and dead legume tissue [104–106]. In more recent studies on drought-tolerant forages, inter-seeding alfalfa into established Old World bluestem (OWB) grass helped restore soil health and enhance soil microbial community complexities [107].

4.5. Managing Livestock Wastewater

On-site livestock wastewater may be managed with the use of microalgae in the wastewater where livestock feces and urine (high N content). Due to its high concentration of N content, this animal waste tends to produce pollutants such as NH_3 and N_2O , which are essential for microalgal growth [108]. In addition to inorganic P, microalgae are known to assimilate inorganic N species and transform them into organic nitrogenous compounds [109]. Nitrogen oxide emissions from the wastewater, for instance, were reduced by 80% with *Chlorella* sp. [110] and entirely with *Gracilaria birdiae* (red seaweed) [111]. Seaweed can reduce N_2O emissions by assimilating and storing N in high concentration [112] and decreasing available NO_x in the wastewater system [113].

4.6. Carbon-Rich Sources

In terms of regulating N loss or impacting the N cycle, manure or litter broadcast leads to enhanced N_2O emission [114], whereas, the sole application of biochar or a combination of lime and biochar (livestock slurry) showed a significant reduction in NH_3 emissions [115] and cumulative N_2O loss [116]. In production agriculture, especially, where litter or manure is used, ammonia is the precursor of nitrous oxide emission and volatilized ammonia can travel up to 5 km from its source of origin and a minor portion (1% proportion) has the potential to be re-emitted as nitrous oxide upon redeposition [117]. The application of biochar has been shown to reduce the emission of nitrous oxide from redeposited ammonia by 69% and this was attributed to the increased aeration caused by the inherent porosity of the biochar [118]. Similar high C sources, obtained as an agro-industrial byproduct, known as char (contains 30% of total C) have shown a reduction in NH_3 volatilization up to 37% under fertilized soil compared to control [119]. In current agronomic practices, sewage sludge is commonly used, however, its use is associated with a greater risk of toxic substance accumulation, for instance, heavy metals, polycyclic aromatic hydrocarbons, or polychlorinated biphenyls, phenanthrene, and pyrene [120,121]. Furthermore, upon application, sewage sludge may phyto-accumulate and can be trophic transferred in agroecosystem food webs [122]. However, in recent years, the use of sewage sludge in conjunction with carbon adsorbents like activated carbon or biochar is gaining popularity both in terms of environmental safety and soil health [123–125]. In a recent study, conjunctive use of sewage sludge and biochar as soil amendments showed a dramatic

decrease in sludge toxicity shortly after application due to nutrient immobilization and a significant reduction in nitrate loss from the soil profile [126]. Therefore, in addition to the phyto-stabilization of heavy metals [127], the use of high C sources in regulating N loss is a potentially promising avenue for further research.

4.7. Engineering Cereal Crops for Nitrogen Fixation

For the last five decades, scientists and agronomists have been studying the prospects of N fixation in cereal crops and evidently, there has been tremendous progress in areas like the expression of nitrogenase gene in eukaryotes and the nodule formation workflow in plants [128]. These efforts have led to investigate the scope of engineering cereal crops to perform symbiotic or autonomous N fixation, however, several factors, namely, population growth and a high rate of N application in production agriculture can outwit the constant efforts of developing N fixing transgenic cereals [129]. The primary tenet behind expressing nitrogenase in cereals is to re-envisage the legume-rhizobia symbiosis leading to nodule formation. The presence of building blocks for nodule formation in cereals is indicated by the presence of several common plant hormones [130,131].

Furthermore, *Nod* factors (legume-rhizobia symbiosis) and *Myc* factors (cereals and arbuscular mycorrhizal symbiosis) are structurally similar, indicating the possibilities, although abstract but promising, to engineer cereal crops to express *Nod* factors and initiate the first step toward nodulation [130,132,133]. On the contrary, considering the technical challenges of engineering cereals crops for nitrogen fixation, a sustainable and potential alternative approach such as root-associated diazotrophs in fixing and supplying N to cereals may offer a solution in N management with a short turnover time [134,135]. Although less sensitive than the legume-rhizobia symbiosis, nevertheless, the association between cereal crops and the rhizosphere shares a sophisticated signal route between microbes and plant host [136–139]. A recent ground-breaking study from the N depleted areas of Oaxaca, Mexico, showed mucilage associated with the aerial roots of Sierra Mixe maize can colonize free-living diazotrophic bacteria and the estimated up to 82% of the nitrogen content of maize [140]. The known association between plant and diazotrophs may improve the growth and yield of cereals in low N soils but the performance of these microbial strains is often not reproducible in the field [141,142].

4.8. Plant Growth Promoting Microbial Consortia

Soil microbes are key players in organic matter decomposition, macro, and micronutrient cycling, and facilitating nutrient availability for plant uptake [143,144]. Microbial communities associated with plants are also capable of abating environmental pollution [145]. Rhizosphere dwelling microbes are also known as plant growth-promoting microorganisms (PGPMs) [146] because they encourage plant growth and foster soil health by N fixation, P solubilization, mineralization of macro (calcium, magnesium, and potassium)/micronutrients (zinc, iodine, and nickel) and secreting phytohormones, and finally suppressing pathogens [147,148]. Additionally, animal wastes like poultry litter or composted poultry litter is a major source of organic N in production agriculture but poultry manure can cause a high loss of nitrogen via ammonia volatilization [149]. Therefore, farmers, industries, and researchers are giving considerable attention to the formulation of these microbial communities on-farm or off-site and using them to fortify the resilience of the agroecosystems. However, the function and propagation of these concocted exogenous microbes may be limited to their ability to survive under a highly diverse, competitive, and constantly changing medium such as soil [150,151]. In Southeastern USA, recent research on the production and application of locally derived exogenous microorganisms in managing nutrient availability from animal waste and utilizing them in nutrient cycling, especially in N-cycling, has shown higher potential nitrogen mineralization in soil 0–5 and 0–15 cm depth indicating a more robust and complex microbial community composition [152–154]. Additionally, microbially mediated decomposition of nitrogenous

compounds in soil may enhance the N availability and consequently be intercepted by plant roots and taken up [155].

4.9. Phytogetic Approach and Fungal Utilization

As discussed above, nutrients excreted by livestock reflect the diet consumed and are an indicator of N, P, and CH₄ release into the environment. Tannins and saponin-rich plants such as *Acacia mearnsii*, *Delonix regia*, *Enterolobium cyclocarpum*, and *Musa paradisiac* may be used as animal feed supplementation to enhance N retention [156–159]. For instance, alfalfa silage has a higher crude protein and nitrate content, which upon feeding may lead to higher N₂O emission as opposed to feeding a combination of corn silage and grass hay [160]. In some countries in Asia, for instance, China and India, rice and wheat straws are burned, thus, causing environmental havoc like, smog. However, inoculating the straws with fungal species like *Aspergillus terreus* may reduce the lignocellulose content and enhance the decomposition process by soil microbes, which in turn enhance the nitrogen mineralization process [98].

4.10. Organic Agriculture as a Tool in Nitrogen Pollution Remediation

The history of organic agriculture is somewhat contentious and at the turn of the 21st century, some critics portrayed organic farming as an ideologically driven inefficient food production system [161–163]. Nonetheless, globally, the organic farming community experienced a continuous increase in the number of organic farms, acreage of land, the consumer market for organic foods, and organic agriculture-focused research funding [164]. As of now, more than 160 countries practice organic farming and more countries continue to join the community [164]. Worldwide, nearly 2.3 million organic farmers are growing organic produce in 0.99% of the total cultivable land [165] (Table 3).

Table 3. Regional distribution of land area (Mha and percent) under organic agricultural land.

| Region | Area (Mha) | Percent (%) of Total Organic Agriculture Land |
|---------------|------------|---|
| Oceania | 17.3 | 40 |
| Europe | 11.6 | 27 |
| Latin America | 6.8 | 15 |
| North America | 3.7 | 8 |
| Asia | 3.6 | 7 |
| Africa | 1.3 | 3 |

There are some intrinsic weaknesses in organic farming practices, especially, in the timely release of nutrients that coincide with plant N demand from typical organic amendments such as compost [166,167] and consequent potential higher nutrient loss mainly as nitrate [168]. Nitrate leaching losses have been reported to be similar or minutely lower in conventional agriculture compared to organic practices in several studies [169]. Nevertheless, organically managed agricultural systems can potentially contribute to the mitigation of climate change through efficient nutrient management techniques leading to reduced emission of nitrogenous gases from the production system and sequester carbon in the soil. Probably the most powerful aspect of organic agriculture, especially in developing countries, is its capacity to compete and often attain equal or higher yields as compared to traditional farming practices [170]. Organic farming practices have been shown to safeguard water quality in rivers as well. In a simulation study, a researcher has shown that a combined effect of higher precipitation and ethanol production from common biofuel crops could cause the river N level to rise by 24%, whereas, simple practices that are common to organic farming such as cover cropping, use of legumes in crop rotations, etc., could decrease the river N level by 7% [171]. Organic cropping systems to succeed, a few factors need to be considered, for instance, the synchronicity between crop N demand and N delivery from animal waste, greater flexibility in designing crop rotations, pretreating

the compost with microbial inoculum to facilitate greater and rapid N availability from compost for plant uptake, etc. [154,169].

4.10.1. Limited External Input

In organic agriculture, inputs such as synthetic fertilizers, chemical pesticides, and herbicides are strictly restricted, hence, external energy for the chemical synthesis of nitrogen or phosphorus fertilizer is not required. In a conventional wheat-growing system, typically, 56% of the energy burden falls on chemical fertilizers and 11% on pesticides [172], thereby, increased the chances of nutrient loss. Although, organic agriculture avoids this requirement of energy but often highly dependent on the use of fossil fuels, especially in mechanical weed management. A study in the UK compared crops grown on seven conventional and organic farms and realized that although there is a higher energy demand of machinery to produce foods, the energy balance still tilted toward energy savings in organic farms (indicated by a 15% lower energy demand) gained by waiving the use of synthetic fertilizers and pesticides [173].

4.10.2. Crop Diversification

Cropping of diverse assemblages of local plant varieties fosters resilience in agroecosystems to counter sudden environmental stresses such as droughts and economic volatility such as price variations [174]. Additionally, cropping diversity encourages the efficient use of soil nutrients and optimum yield [175].

4.11. Ecological Ditch

In addition to N₂O emission and NH₃ volatilization from agricultural fields, a major source of nitrogen pollution is agricultural drainage [176,177]. Typically, the compositional nature of agricultural runoff is complex due to the sheer number and types of nutrients, for instance, runoff often contains nitrate, ammonium, inorganic phosphorous, organic pollutants, and heavy metals [178]. These N and phosphorus (P) nutrients play a crucial role in the growth of aquatic plants, which upon a lack of regulation can lead to eutrophication in the downstream receiving aquatic systems [179]. Heavy nutrient loads from agricultural lands can potentially cause eutrophication, hypoxia, and ecological damages in nearby water bodies [180].

The “ecological ditch” (eco-ditch) is an effective component in alleviating non-point agricultural pollution. Eco-ditches are examples of best management practices and a stark contrast to the traditional agricultural drainage ditches. Eco-ditches create an exclusive ecosystem where the participating parties are aquatic plants and associated microbial communities fueled by the constant nutritional substrates [181]. Eco-ditches are designed to absorb nutrients that are otherwise lost through surface runoff and make those nutrients available for root uptake or be incorporated into microbial metabolites [182,183]. Eco-ditches designed with *Leersia oryzoides* and *Typha latifolia* reduced the load of inorganic N from 2.5% to 1.5%, accounting for more than 50% of the total reduction over 2 years in Northern Mississippi [184]. However, in designing eco-ditches, plant population diversity needs greater attention and highly efficient ditch plants should be selected. One possible constraint of these ditches could be the variability in nutrient removal capacity by plants, which is strictly dependent upon the growth stages of plants. For instance, during the growing period, plants tend to uptake more nutrients as opposed to the senesce period [185]. Plant harvesting continuously in the eco-ditches may offer a possible solution [186].

4.12. Genetic Improvement

4.12.1. Identifying Candidate Gene in Plants for Improved NUE

Nitrogen use efficiency of plants is genetically regulated; thus, nutrient use varies vastly among plant species. These variations lead to differences in several aspects in different plants including N assimilation, uptake, and remobilization capability, hence, alludes to the need to screen for potential genetic traits across these genotypes.

- a. Differentially expressed genes (DFEs) to validate their roles in NUE of different genotypes of crop species can be profiled globally for different genotypes under different N treatments. DFEs have four components and these are:
 - i. Hybridization based transcriptome analysis to identify differentially expressed traits with low abundance [187];
 - ii. Analyzing short sequence tags of individual mRNA and then linked to form long sequences and finally cloning them [188];
 - iii. Probe-targeted hybridization of immobilized cDNA molecules to generate a large amount of data and analysis of the whole genome [189];
 - iv. RNA sequencing involves the sequencing of every RNA molecule and subsequently profiling a particular gene expression [190].
- b. Functional validation of genes by mutation and transgenic studies.

Both the mutant population and natural variants can be studied to identify genes of interest in crop NUE. The steps involve propagation of the mutated population and screening for mutated phenotypes, finally followed by gene recovery through map-based cloning strategies.

4.12.2. Discovery of Genes by Mapping Studies

Biomarker-based mapping studies by biparental linkage analysis and association studies in naturally existing genotypes are two possible strategies in identifying the position of the NUE genes in crops. In low and optimum N systems, a meta-analysis of the quantitative trait loci (QTLs) for yield was mapped to discover linked markers with the gene that controls the specific trait, which in this case was N use and the study revealed a total of 22 meta-QTLs under low N [191]. Additionally, another association study in 196 accessions of wheat for yield components expressed 23 N-responsive regions, which can be exploited by breeders to develop highly N responsive varieties of wheat [192].

In the coming decades, one of the greatest challenges humanity faces is climate change, and agriculture is both a key contributor to crisis and will be immensely impacted by this problem [193]. Thus, minimizing the loss and emission of reactive N is crucial in slowing down the rate of climate change [194,195] (Table 4).

Table 4. Mitigation strategies to prevent potential N loss.

| Mitigation Strategies | Approach |
|--|-------------------------|
| Farming System Design | Agronomic |
| On-Farm Best Available Techniques (BATs) | Agronomic |
| Improved Nitrogen Use Efficiency (NUE) | Agronomic |
| Pasture and Livestock Management | Agronomic and landscape |
| Managing Livestock Wastewater | Hydrologic |
| Carbon-rich sources | Agronomic |
| Engineering Cereal Crops for Nitrogen Fixation | Molecular |
| Plant Growth Promoting Microbial Consortia | Agronomic and molecular |
| Phytogenic Approach and Fungal Utilization | Agronomic and molecular |
| Organic Agriculture | Agronomic |
| Ecological Ditch | Landscape |
| Genetic Improvement | Molecular |

Adapting to a combination of these mitigation strategies will enable individual growers and the farming community at large. For instance, agronomic approaches such as formulating microbial inoculum from a local source (discussed in Section 4.8) are affordable and particularly is very important for agriculture in developing countries, while molecular techniques such as N-fixing cereal crops, NUE gene identification, and mapping (discussed in Sections 4.7 and 4.12) are time-consuming and requires a long-term research investment. Nonetheless, policy-driven and ecosystem service-oriented mitigation strategies will help combat future N losses from agriculture and offer ecological safeguard.

5. Nitrogen Status in Agriculture during the COVID-19 Pandemic

On 11 March 2020, the World Health Organization (WHO) declared that the world faces a pandemic by the novel coronavirus (COVID-19) [196,197]. The tremendous disruptions across the globe caused by the COVID-19 pandemic are affecting the entire realm of human activities. A recent study investigating the relationship between long-term exposure to NO₂ and coronavirus mortality through the analysis of tropospheric NO₂ mapping and distribution data generated by the Sentinel-5P satellite showed that 78% of death cases were in five regions located in Northern Italy and Central Spain that displayed the highest NO₂ concentration levels and low circulation of air to disperse the pollution. These findings suggest that long-term exposure to this pollutant may be a major contributor to COVID-19 death in these regions and possibly in other parts of the world [198]. However, the COVID-19 outbreak and consequent social distancing activities led to an extensive decline in traffic and allowed comparisons of air quality during and before the decline to document the impacts of COVID-19 on NO₂ concentration in Florida Counties through March 2020. The results indicated a 54.07% decrease in NO₂ in the atmosphere [199]. In the context of agricultural safeguard, According to World Economic Outlook, the emerging and developing nations will face extreme severity of negative growth [200], and according to the Food and Agriculture Organization (FAO) and World Food Program (WFP), there will be food insecurity at an unprecedented level [201]. In this time of global crisis, now more than ever, soil plays a pivotal role as production agriculture and bedrock of resilience in food security [202]. The impact of this global pandemic on agriculture will likely be learned in waves in the coming years. Assessing the current soil N, carbon (C), and P content as impacted by the pandemic for devising future strategies to recover from the pandemic and establish long-term sustainable goals to maintain soil health for future needs is very crucial. Likely lower livestock production and with lower fertilizer application due to the COVID-19 restrictions, global agriculture may see improved farm management scenarios resulting in reduced GHGs, like, N₂O, lower NH₃ emissions, and low nutrient loss to surface water. For instance, there have been limited agricultural activities in livestock production that are being reflected in the reduced greenhouse gas emission such as NO_x in countries such as China and Italy; however, the same study also concluded that agricultural pollution via NH₃ emission has not changed significantly compared to the pre-pandemic era [203]. This may consequently result in improved ecosystem services and higher food quality. A closer look at the examination of macronutrients such as N and P is crucial, where N limits crop growth and P fertilization plays a crucial role in crop yield. However, during this pandemic, due to lockdown, food supply chains have been massively disrupted on a regional and global scale, thus, raising the most imminent threat from the huge addition of organic waste as mass disposal from the dairy and vegetable industry. Additionally, reduced meat consumption in the USA during the earlier months of 2020 has led to the massive burial of swine and poultry in many parts the country [202]. Such an influx of surplus organic matter to the soil, especially, P in the organic matter, may result in an imbalanced soil nutrient status. Therefore, for future crop fertilization strategies, application based on N requirements of the plant may result in over-application of P, which in turn may be fixed in the soil–mineral complex, making the soil depleted in plant-available P. In this context, the long-term consequences of the massive burial of organic waste may introduce additional complication is land use, surface, and groundwater quality, soil macro and microfauna and flora diversity, critical ecosystems services, and human well-being.

6. Knowledge Gaps and Questions

The agricultural system is complex and often the feedbacks and processes are non-linear. Nitrogen loss mitigation strategies require sustainable, resilient, and redundant ways of producing and consuming that can also be adopted in climate change strategies in a broader sense [204]. Immediate attention is required to explore and interpret data from low-income countries in this regard. Although, the greatest emission of GHGs such as

N₂O is estimated to be higher in the low to middle-income parts of the world from food waste there exists close to no empirical studies on how to tackle these challenges [205,206]. The problem is further complicated as the mean income in these countries increases, the dietary habits change. High protein and carbohydrate diets lead to intense livestock and cereal production systems ensuing in greater GHGs emissions [207]. However, climate implication is yet to be explored in those countries as compared to developed parts (high-income) of the globe [208]. Another aspect of identifying and abating N loss is investing resources into research on post-harvest management of crops and crop residues [209]. Inclusive public policies and equitable funding mechanisms that are equitable and resilient are the two major catalysts that will lead to lower agricultural system N footprints. A major shortfall in global climate change strategies is the lack of financial allocation for tackling emission problems, especially in developing countries, and uncertainties shrouded by the lack of research to indicate whether the allocated money is being used for climate-smart agriculture. For instance, in 2015, out of \$391(US) billion only \$8 (US) billion was issued to address and adopt strategies to mitigate climate strategies like nitrogen use efficiency, soil health, and GHGs emission mitigation [210]. Furthermore, assigning sustainability indexes to agricultural commodities and assimilating sustainability benchmarks in dietary intake guidelines may lead to change in dietary regimens fostering healthy and low N footprint foods and low emission diets [211,212]. Countries such as Brazil, Germany, Qatar, and Sweden have taken steps towards this goal already, and the USA has thus far failed to adopt [213]. Government policies now must realize that voluntary measures are not adequate in lowering N footprints at farm level production, rather strict regulation, financial incentives, and subsidies for regenerative agriculture are obligated [214]. Now, the world is faced with an entirely new challenge of the COVID-19 pandemic, and the existing soil and agricultural management strategies must include the complex task of including the COVID-19 pandemic as a variable along with the existing ones, for instance, climate change, food insecurity, freshwater crisis, and continuously endangered and fading biodiversity. A radical shift from the dependencies on industrialized mono-agriculture to more diverse agroecology may be required in the coming days as the global population traverse through this pandemic.

7. Conclusions and Future Directions

The nitrogen challenge at its core requires societal recognition and indicates a potential opportunity to steer away from a fragmented policy approach, rather than toward rapid solutions [215]. Addressing the loss of N in agricultural production and proposing possible mitigation strategies should aim to be inclusive, realize the current shortcomings, and most importantly should be realistic to attain. From simple measures such as, the inclusion of nitrogen-fixing legumes in production agriculture as cover crops [216] to more complicated efforts that aim to materialize artificial symbioses or associative nitrogen fixation in non-legume plants, especially in cereals [128] are some of the possible ventures that may be undertaken. Moreover, undertaking efficient N management measures, for instance, controlled release of N or drip N fertilization in rice and maize, respectively, can direct the cost-benefit balance to lean toward profit [217,218]. Additionally, 4R (Right Source, Right Rate, Right Time, and Right Placement) N guidelines for corn are extremely profitable (40% increase) while decreasing the N application rate (21% reduction) [219]. Thus, connecting socioeconomic requirements with landscape potentials should be the central aspect in future N loss-related plans and policies [220]. Another key element to be explored is soil resilience and should be implemented wherever possible. Resilience will enable degraded or depleted soil to recover and possibly stop the soil from being a sink but a source. In the coming days, the soil should not be discussed as an isolated component rather it should be thought of as an essential tool of regenerative agriculture where possible answers to pollution questions can be sought after. Including soil as a resource in strategizing grass-root policies may reduce the risk of market vulnerabilities and associated risks. Finally, to summarize, while policy and funding apparatuses have been proposed amply but few have

been implemented to address and mitigate the global N pollution status for regenerative and sustainable agriculture.

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