Chapter

Advancement of Nitrogen Fertilization on Tropical Environmental

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Abstract

The nitrogen (N) fertilization synthetic or biological is primordial for food production worldwide. The consumption of N fertilizers in agricultural systems increased in exponential scale, mainly in developing countries. However, some negative points are associated to industrial N consumption; consequently the industry promoted ways to minimize N losses in production systems of tropical agriculture. Biological nitrogen fixation is a very important natural and sustainable process for the growth of leguminous plants, in which many micronutrients are involved, mainly as enzyme activators or prosthetic group. However, other mechanisms in the rhizosphere and molecular region still need to be clarified. Therefore, the aim of this chapter is to compile information about the historical and current affairs about the advances in N fertilization in tropical environments through a history from N fertilization worldwide, N balance in the main agricultural systems, introduction of alternative ways to avoid N losses, advances between BNF and micronutrients, as well as the effects of N absence in plant metabolisms. Biological nitrogen fixation is a very important natural process for the growth of leguminous plants, in addition many metallic nutrients, micronutrients, are involved in BNF metabolism, mainly as enzyme activators or prosthetic group. But other mechanisms in the rhizosphere and molecular region still need to be clarified.

Keywords: ammonia synthesis, biological N fixation, humic substances, N balance, volatilization

1. Introduction

Hellriegel and Wilfarth showed definitive evidence for N\(_2\) fixation by microbes in legumes in 1886, but the industrial process to fertilizer production known as the Haber-Bosch was established just in 1906, which uses a catalytic agent at high pressure and high temperature [1].

Actually, the world population has now been increasingly relying on nitrogen (N) fertilizers in order to keep up with the demands of food and economic growth rates; on the other hand, less than 30% of synthetic fertilizers would actually be
utilized; the unused chemicals sprayed on crops would be lost in the field and could subsequently cause serious environmental problems.

Urea is a popular N source in developing countries due to its advantages of a high N content, safety, and easy transportation [2]. However, the increase of pH and surface soil NH$_4^+$ concentrations resulting from urea hydrolysis can exacerbate NH$_3$ emission.

This causes low N use efficiency, especially in alkaline soils or soils with low sorption capacity, which limits the use of urea fertilizer in Europe [3]. In tropical areas, increasing the adoption of no-till systems also induces to high N losses from urea fertilization, in tropical soils, due to high temperatures and moisture; NH$_3$ losses exceeding 40% of the surface-applied urea N have been reported, especially under no-till or perennial crops where plant residues are kept on the soil surface [4].

Nitrogen losses by NH$_3$ emission not only brings economic loss to farmers, but also detrimental effects to ecosystems and human health, while the biological nitrogen fixation (BNF) has the advantage of being environmentally friendly and therefore would be ideal for sustainable agriculture.

Enormous progress in almost all aspects of BNF has been made in the past century, especially in the recent two decades, in genetics and biochemistry, culminating in the determination of the crystallographic structures of both nitrogenase components and micronutrients metabolism.

These information collaborated to elucidate N assimilation routes in plants clarifying further its essentiality and allowing to infer that plants can be affected negatively in molecular even genetic level in N absence.

Therefore, the aim of this chapter is to compile information about the historical and current concerns about the advances in N fertilization in tropical environments through a history from N fertilization worldwide, N balance in the main agricultural systems, introduction of alternatives ways to avoid N losses, advances between BNF and micronutrients, as well as the effects of N absence in plant metabolism.

2. History of nitrogen fertilization on tropical environmental

Nitrogen is an essential element to all organisms, because it is part of protein, acids, and other organic compounds [5]. The importance of this nutrient for plants is already known since the 1660s; however, only at 1804 De Saussure received credits for N essentiality after observations of nitrate uptake from soil solution. In this same period, other researchers, as Liebig at 1840, fortified the idea of plants absorb N from atmosphere [6, 7].

Around 78% of the atmosphere gas is compound for N however in gaseous form chemically unavailable. In front of the increased demand by food production and need of N restitution after crop harvests, Fritz Haber at 1909 synthetizes the gaseous element to ammonia (NH$_3$) through a reaction with hydrogen and iron on high pressure and temperatures, which posteriorly was industrially developed by Carl Bosch in 1912–1913, resulting at the known Haber-Bosch process [8].

The N sources used on agricultural activities, even at the end of the eighteenth century, were from crop residues and animal manure modified or not through composting. The production and management of N fertilizers to increase crop yield, as well as corn [9, 10] and wheat [11] around the world [12] have begun at the Green Revolution of the nineteenth century, followed by ammonia synthesis in the beginning of the twentieth century and the increased need of high yield on agricultural areas [13].
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DOI: http://dx.doi.org/10.5772/intechopen.90699

| World                | 2015    | 2016    | 2017    | 2018    | 2019    | 2020    | Reference |
|----------------------|---------|---------|---------|---------|---------|---------|-----------|
| Total capacity NH₃  | 174.781 | 181.228 | 185.222 | 186.804 | 186.920 | 188.310 | [11]      |

| Africa               |         |         |         |         |         |         |           |
|----------------------|---------|---------|---------|---------|---------|---------|-----------|
| Total capacity NH₃  | 8.310   | 9.545   | 10.739  | 10.700  | 10.700  | 11.000  |           |

| Americas             |         |         |         |         |         |         |           |
|----------------------|---------|---------|---------|---------|---------|---------|-----------|
| Total capacity NH₃  | 24.301  | 27.618  | 28.688  | 29.304  | 29.320  | 29.346  |           |

| Asia                 |         |         |         |         |         |         |           |
|----------------------|---------|---------|---------|---------|---------|---------|-----------|
| Total capacity NH₃  | 99.959  | 101.188 | 101.703 | 101.734 | 101.734 | 102.799 |           |

| Europe               |         |         |         |         |         |         |           |
|----------------------|---------|---------|---------|---------|---------|---------|-----------|
| Total capacity NH₃  | 40.378  | 41.044  | 42.338  | 43.211  | 43.311  | 43.311  |           |

| Oceania              |         |         |         |         |         |         |           |
|----------------------|---------|---------|---------|---------|---------|---------|-----------|
| Total capacity NH₃  | 1.833   | 1.833   | 1.854   | 1.854   | 1.854   | 1.854   |           |

**Table 1.**
Estimative of supply capacity of N (NH₃) in continents (in thousand tons) of 2015–2020 (adapted of FAO [12]).

| World                | 1960    | 1980    | 2000    | Reference |
|----------------------|---------|---------|---------|-----------|
| Animal manure applied in soil | 22%     | 16%     | 14%     | [14]      |
| Animal feces         | 56%     | 40%     | 40%     |           |
| Synthetic fertilizers | 22%     | 44%     | 46%     |           |

| Africa               |         |         |         |           |
|----------------------|---------|---------|---------|-----------|
| Animal manure applied in soil | 4%      | 4%      | 4%      |           |
| Animal manure left in pasture | 91%     | 84%     | 84%     |           |
| Synthetic fertilizers | 5%      | 12%     | 12%     |           |

| Americas             |         |         |         |           |
|----------------------|---------|---------|---------|-----------|
| Animal manure applied in soil | 16%     | 13%     | 13%     |           |
| Animal manure left in pasture | 60%     | 50%     | 47%     |           |
| Synthetic fertilizers | 24%     | 37%     | 40%     |           |

| Asia                 |         |         |         |           |
|----------------------|---------|---------|---------|-----------|
| Animal manure applied in soil | 20%     | 13%     | 12%     |           |
| Animal manure left in pasture | 61%     | 34%     | 30%     |           |
| Synthetic fertilizers | 19%     | 53%     | 58%     |           |

| Europe               |         |         |         |           |
|----------------------|---------|---------|---------|-----------|
| Animal manure applied in soil | 40%     | 28%     | 30%     |           |
| Animal manure left in pasture | 27%     | 17%     | 17%     |           |
| Synthetic fertilizers | 33%     | 55%     | 53%     |           |

| Oceania              |         |         |         |           |
|----------------------|---------|---------|---------|-----------|
| Animal manure applied in soil | 2%      | 3%      | 4%      |           |
| Animal manure left in pasture | 96%     | 91%     | 77%     |           |
| Synthetic fertilizers | 2%      | 6%      | 19%     |           |

The percentual represents averages from the 1960s, 1980s, and 2000s (adapted of FAO [12]).

**Table 2.**
Global cumulative of N fertilization from animal manure and fertilizers between 1961 and 2014.
Data from the FAO [14] estimated that the global capacity of N ammonia offer increases annually of 1.5% in average, with production of 174,781–188,310 thousands of tons of 2015–2020 (Table 1).

In addition, during this period, Africa, Oceania, Europe, and the Americas increased the capacity to 32.4, 1.1, 7.3, and 20.8%, respectively, however, stands out to Asia continent with the highest productive capacity estimated to 102,799 thousands of tons of N to 2020 (Table 1).

Estimates in global scale from FAOSTAT [15] show N inputs from animal manure increased from 66 to 113 million from 1961 to 2014, while N fertilizers applied in soils increased from 18 to 28 million of tons of N, respectively.

The use of N fertilizer at Europe continent increased 33% (about 5 million of tons of N), as a similar tendency observed in others regions (Table 2).

Brazil is one of the biggest fertilizer consumers in the world. The significant increase in fertilizer consumption occurred between 1988 and 2010 [16] as a consequence of public policy implementation and Brazilian agriculture modernization.

Nitrogen had a higher growth consumption among the nutrients from NPK in the analyzed period, around 250%, from 814,952 to 2,854,189 tons; however, N fertilizers consumption was 12,211,855 tons from 2010 to 2013 and to around 15,469,549 tons from 2014 to 2017 [17, 18].

3. Nitrogen balance in the tropical agricultural systems

Nitrogen balance in the systems becomes a concern for tropical agriculture as a result of the high scale of N fertilizer production. Nutrient balance is a parameter that analyzes the relation between quantity of vegetable biomass produced and nutrient applied. Besides, nutrient balance is a tool with easy application and able to guide the management to efficient fertilization [19].

Nitrogen balance as a management technique accounts the nutrient exportation by crops, residual in soil and the N losses [20]; thus it is essential to a balanced fertilization strategy aiming to maximize the economic return and ensure the environmental quality.

The calculations of nutrient balance evaluation must account for the input and output of N because this nutrient can be distributed by soil, plant, and animal (Table 3). Between 95 and 100% of the total N input into soil is from the surface through rainfall or dust and aerosols, irrigation, runoff and groundwater, biological fixation by phototrophic and heterotrophic organisms, organic and inorganic fertilization, and seed reserves. Besides the plants exports, the N output occurs by erosion, leaching and drainage, ammonia volatilization, denitrification, and senescence plants [21, 22].

Brazilian crop exports 50% of N in harvested product mainly by the largest exportations of soybean (70%), corn (15%), sugarcane (8%), rice (2%), and wheat (2%) [17]. However, these N quantities have contribution from the N biologic fixation (NBF), mainly from soybean with 82% of the total N input in crops production.

Soybean occupied the largest area of agriculture in Brazil between 2013 and 2016 and also was responsible for the largest nutrient exportation, although N is not applied in this crop, it comprised 70% of the total N exported by all crops, while phosphorus and potassium reached 57.5 and 56.8%, respectively [23]. Analyze nutrient exportation nutrient exportation for area unity in this period was found out the largest nutrient exporters were soybean (181 kg ha\(^{-1}\)), tomato (159 kg ha\(^{-1}\)), and cotton (129 kg ha\(^{-1}\)).
4. Ways to avoid N losses from agricultural systems

In agricultural systems there are losses in general; however, N losses are considered highly relevant [24, 25]. Nitrogen losses are a potential contaminant and can impact production cost. Nitrogen is a dynamic element in soil and can be lost to the atmosphere by denitrification and ammonia volatilization [24, 25].

Ammonia volatilization is a concerning problem because it represents high N losses in soil–plant system besides to be a threat for global environmental [26], while the N losses by denitrification in tropical areas are less significant in consequence of its restriction in the use of nitrate as fertilizer due its explosive potential [25–28].

Global agricultural production is responsible for 50% of N losses by ammonia volatilization meaning 37 tons of N for year; however, the losses can be higher according to the N source, application way, soil management, climate, soil temperature, and humidity [29–34].
Urea is the most N source used in the world; however, also it has high susceptibility to be lost in agricultural systems [24, 25]. The high presence of urease enzyme in soil causes a rapid hydrolysis of urea and, consequently, ammonia losses to the atmosphere [35].

Variable quantities of ammonia lost to the atmosphere were related by urea use in agriculture [35–37] according to the exemplified in Table 4.

Urease is an extracellular enzyme naturally presents in soil, plants, and microorganisms acting as a catalyst of urea in the hydrolysis process [30–32]. This chemical process induces excess of protons (H⁺); consequently it rises pH in soil around the fertilizer granules of 6.5–8.8 or until 9.0 causing unbalance between ammonium (NH₄⁺) and ammonia (NH₃) [33, 34].

During hydrolysis ammonium carbonate is formed, which is dissociated to produce ammonia ions and hydroxide; however, the relative concentration of ammonia and ammonium is determined by the pH in soil solution, and ammonia is favored under high pH condition according to equations [28].

\[
\text{NH}_4^+ + \text{OH}^- \leftrightarrow \text{NH}_3 + \text{H}_2\text{O} \quad (1)
\]

\[
(\text{NH}_2) \text{CO} + 2\text{H}_2\text{O} \rightarrow (\text{NH}_4) \text{CO}_3 \rightarrow \text{NH}_4^+ + \text{NH}_3^+ + \text{CO}_2 + \text{OH}^- \quad (2)
\]

Researches about urease inhibition in soil have begun over than 70 years ago, resulting in many compounds evaluated and patented as urease inhibitors [38]. Urease has a great effect on the soil-plant system through plant N efficiency, as well as being a versatile enzyme, presenting technological, biotechnological and transgenic applications [39].

Nitrogen losses can be avoided or reduced through organic or inorganic chemical compounds included in urea as an able technology to increase the efficiency of N fertilization at low cost [40–42]. Urea with urease inhibitor can cost around 30% higher than conventional urea [43].

The phosphorotriamides, hydroquinone, catechol, copper, boron, and zinc are the most evaluated additives as urease inhibitor [44]. There are more than

| Rate of applied (kg N ha⁻¹) | Mean % N volatilized | Location       | Reference |
|-----------------------------|----------------------|----------------|-----------|
| Grassland soils             |                      |                |           |
| 180                         | 22.8                 | Argentina      | [16–22]   |
| 15–200                      | 17.6                 | New Zealand    |           |
| 50                          | 36.0                 | USA            |           |
| 30–150                      | 26.7                 | UK             |           |
| 25                          | 7.5                  | New Zealand    | [11, 17, 23–29] |
| Arable soils                |                      |                |           |
| 50                          | 55                   | Brazil         |           |
| 150                         | 30                   | Brazil         |           |
| 120                         | 77                   | Brazil         |           |
| 90                          | 128                  | Denmark        |           |
| 200                         | 30                   | India          |           |
| 60                          | 79                   | Argentina      |           |
| 46                          | 23                   | Australia      |           |

Table 4. Examples of ammonia volatilization due to urea application in different soils, modified from [20].

40 phosphorotriamides synthetized considered the most effective compound to urease inhibition because its composition comprises a functional group containing P=O or P=S bonded for at least one free amide (NH$_2$) to react with urease active sites and they are considered [45].

Urease inhibitor known as NBpt (N-(n-butyl) thiophosphoric triamide) has been the most used additives in Brazil, in which urea is the most used N source.

This additive is dissolved in a nonaqueous solvent to adding characteristics as (i) larger stability to NBpt molecule under temperature, humidity and transportation variances, and (ii) higher solubility; (iii) improves adherence of mix solvent + NBpt to urea granule, (iv) low toxicity, and inflammable potential; and (v) acts as buffer agent to keep alkaline pH similar to hydrolysis environment of urea in soil providing NBpt stability [43].

The largest of compounds used along with urea are low efficient when applied in soil [43]. NBpt aim is to retard the ammonia volatilization peak [46]. Generally, chemical compounds with similar structure as urea can be more efficient to retard the volatilization; thus, the bond sites and length of amide of phosphoryl triaside are similar to urea; however, there are no substrates for urease [45].

Recently, lab researches reported beneficial and/or synergic effects of the humic substances use with urea [47–49]; however, the action mechanism is still unknown [49]; also depending of humic substances, the results can be contradictory [50, 51], but there are hypotheses that urease enzymes reduce with the association of humic acid and urea [48]; besides it minimizes N losses, it can improve buffer effect in soil pH [52].

Urease inhibitor and humic substances with urea at adjusted pH (pH = 7) provided reduction of 50% from total N volatilization on a Latossolo Vermelho on sugar cane [53].

5. Interaction between biological N fixation (BNF) and micronutrients to higher plants

Biological N fixation (BNF) is an important process to global agricultural systems. This phenomenon was discovered in the mid of the nineteenth century by the German chemist Hermann Hellriegel (1831–1895); however, factors on root nodules were unknown, until the Dutch microbiologist and botanic Martinus Beijerinck (1851–1931) identifies microorganisms on root nodules able to realize chemical process to transform atmospherically N to ammonia allowing fixation and absorption by plants, proving the symbiosis between legumes and bacterial [54].

Fixation biological of N$_2$ (BNF) through the bacteria from genus *Bradyrhizobium* can supply N quantity necessary in legume crops as soybean, besides it is currently observed for many researchers as a clean technology contributing to replace mineral N fertilizers in legume crops [55].

Nitrogen fixation by bacteria already is well described [56]; however, currently studies are focused in nutrients involved in this metabolism, especially micronutrients [57, 58]. Among the micronutrients able to influence the BNF are boron, copper, zinc, cobalt, iron, nickel, manganese, and molybdenum, essential as structural components and enzyme activators in plants [56–59].

Iron is necessary to the production of cofactor FeMo that acts along with nitrogenase enzymes, which can affect significantly the BNF [60]. Excess or default of zinc and nickel can affect the established bacteria inside of the nodules and its symbiosis with plants [57].

There was an increase in BNF and N uptake as a result of the growth of nodules in number and mass with boron foliar application, and these results were attributed
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to the role of boron in the induction of nitrate assimilation by increasing protein synthesis by plant [58].

Manganese has direct role on many enzymatic processes on the BNF, including amide hydrolase enzyme which is directly dependent of Mn$^{2+}$, and it is responsible for ureide degradation being able to control the BNF under hydric deficiency [61].

Low copper affects the nodule formation and reduces the quantity of fixation bacteria; this element is essential for both bacteria and plants; however, its direct role on BNF is still unclear [59].

Molybdenum is an essential nutrient to BNF taking part on nitrate reductase with the reduction of nitrate (NO$_3^-$) to nitrite (NO$_2^-$) and on the nitrogenase process in conversion of dinitrogen (N$_2$) to ammonia (NH$_3$) by fixation bacteria. The low quantity demand of molybdenum allows its application on soil and foliar or even by seeds treatments, which is a form of quality aggregation to the seeds by affecting positively on germination [62].

Cobalt is a component of cobalamin and leghemoglobin synthesis, which is controlling their levels on nodules and avoiding nitrogenase enzyme inactivation; thus this element can be considered essential to N$_2$ fixation [63].

Nickel can affect directly the presence and quantity of fixation microorganisms because it is a hydrogenase component (Ni-Fe), which can recycle H$_2$ that is

![Figure 1. Root nodules from legume. A1, longitudinal section; A2, approximated image on nodules developed with no Ni; B1, longitudinal section; B2, approximated image on nodules developed with 0.5 g dm$^{-3}$ of Ni; C1, longitudinal section; C2, approximated image on nodules developed with 10 g dm$^{-3}$ of Ni [64].](image-url)
generated from N reduction and could affect positively or negatively the legume metabolism [64]. Nickel balance on BNF can be seen on fixation nodules where in its absence causes large formation of internal cells according to Figure 1.

6. Recent reports about N absence on plants metabolism

Even though the essentiality had been established for N at higher plants, there are still remained doubts about how the N absence can affect the metabolism. Recently, by modern techniques and sensible equipment, it was possible to determine clearly as N absence affects plant metabolism and production.

The N deficiency exposure of *Olea europaea* plants was described as a significant decrease on chlorophyll a and net photosynthetic rate (Figure 2). Photosynthesis is a process that involves light absorption by the photosynthetic pigments present in

![Figure 2.](image)

*Chlorophyll a (Chl a), nitrogen content, and net photosynthetic rate (Amax) in Olea europaea plants exposed to nitrogen deficiency* [65].
light-harvesting complexes, being crucial for plant development and largely dependent on the leaf N content, because N composes the chlorophyll molecules [65].

The effects of N deficiency in the leaves of *Oryza sativa* seedlings were verified that the fluorescence parameters were negatively modulated in N-deficient plants [66]. While Figure 2 presents few modifications until the fifth day in N-deficient plants, when compared with control plants, however as nitrogen deficiency continued, chlorophyll fluorescence of N-deficient plants was significantly impacted, in comparison with control plants.

The decrease in the ratio Fv/Fm of plants under water deficit indicates reduction in the photochemical activity, leading to the inhibition of the photosynthetic rate and the generation of reactive oxygen radicals in the chloroplast, causing damages to PSII components. Additionally, the decline in ETR values of plants under water deficit is due to the deficiency of plastoquinone (PQ) used in oxidation-reduction reactions.

7. Concluding remarks

Nitrogen fertilizer consumption follows the increasing demand by food, fiber, and energy production. The quantification of nitrogen inputs and outputs on agricultural system has been a useful and efficient tool to the evaluation of management, mainly to the tropical agricultural.

Biological fixation is an important nitrogen input to productive systems comprising benefits in economic and environmental concerns, mainly for tropical agriculture; however, the narrow relation among this process and micronutrients and its metabolic routes still needs to be clarified.

Advances of the N fertilization on tropical environment reported at this chapter are focused mainly in an attempt to reduce ammonia volatilization from urea in consequence of its largest use as N source.

Among urease inhibitors used in tropical agriculture, NBPt has been highlighted; however, humic substances have been shown as a future alternative to reduce ammonia volatilization that still requires knowledge about its origin, molecular composition, and environmental questions.

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