Chapter

Organic Nitrogen in Agricultural Systems

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Abstract

This work summarizes information about organic nitrogen (N) in the agricultural system. The organic N forms in soils have been studied by identifying and quantifying the released organic compounds when soils are acid treated at high temperature, in which the following organic N fractions are obtained: hydrolyzable total N, subdivided into hydrolyzable NH_4^+-N, amino sugars-N, amino acids-N, and unidentified-N and acid insoluble N, a fraction that remains associated with soil minerals after acid hydrolysis. Nitrogen mineralization and immobilization are biochemical processes in nature. This chapter summarizes how these processes occur in the agricultural system. Then, soluble organic nitrogen (SON), volatilization and denitrification processes, and biological nitrogen fixation (BNF) as a key component of the nitrogen cycle and how it makes N available to plants are also discussed. Finally, we discuss the use of organic fertilizers as N source to satisfy the worldwide demand for organic foods produced without synthetic inputs.

Keywords: biological N fixation, immobilization, mineralization, organic fertilization

1. Introduction

Nitrogen (N) is the fourth most abundant element in cellular biomass and comprises most of the Earth's atmosphere. In the surface layer of most soils, over 90% of N occurs in organic forms. Soil organic N can be divided into two categories: (1) N from organic residues and (2) N from soil organic matter or humus [1]. All these materials are important in maintaining or improving soil fertility and plant nutrition through direct and indirect effects on microbial activity and nutrient availability [2]. Analysis of organic fractions has been highlighted due to the increasing application of organic fertilizers and their direct and indirect effects on crop growth and yield and soil attributes. Thus, we will discuss about organic N forms, N mineralization and immobilization, volatilization and denitrification, soluble organic N, biological N fixation, and organic fertilization with emphasis on N.

2. Organic nitrogen

Nitrogen is an essential element for plants, being constituent of important biomolecules such as adenosine triphosphate (ATP), reduced nicotinamide adenine dinucleotide (NADH), nicotinamide adenine dinucleotide phosphate (NADPH), chlorophylls, amino acids and proteins (glyco- and lipoproteins), nitrogenous bases and nucleic acids, and various enzymes [3, 4]. Soil organic N consisting of proteins, chitins, amino acids, and nucleic acids represents about 90–98% of total soil N [1, 5]. Mineralized N forms are transient in the soil so that the existing amount depends on numerous processes such as mineralization, immobilization, nitrification, denitrification, leaching, and plant uptake. Therefore, the study of mineral N may not represent the N availability during the crop growing. On the other hand, the study of organic N fractions and their transformations over time can help in predicting the N availability for crops, in estimating the N supply to the soil, and in evaluating the potential release of mineral N by organic fertilizers.

Many compounds account the soil organic N, being approximately 40% protein material (proteins, peptides, and amino acids), 5–6% amino sugars, 35% heterocyclic nitrogen compounds (including purines and pyrimidines), and 19% NH_3, with ¼ fixed as NH_4^+. Thus, protein materials and heterocyclic compounds predominate in the total soil N, and organic N fractionation may inform about the mineralization susceptibility of compounds [6]. The organic N forms in soil have been studied by identifying and quantifying the released organic compounds when soils are acid treated at high temperature. The organic N fractions obtained by acid hydrolysis are hydrolyzable total N, subdivided into hydrolyzable NH_4^+-N, amino sugars-N, amino acids-N, and unidentified-N and acid insoluble N, a fraction that remains associated with soil minerals after acid hydrolysis [7].

The fractionation allows separating the labile N forms from the soil, such as amide-N and amino-N (acid hydrolyzable), which can be rapidly synthesized in the mineralization process, releasing inorganic N (NH_4^+ and NO_3^-) to the soil solution. However, most of the organic N can compose more stable fractions in the soil, such as non-hydrolyzable-N and unidentified-N. Variation in the non-hydrolyzable-N may be related to soil management, because the higher the hydrolysis intensity of organic N fractions in the soil, the higher the presence of finer particles that form clay-metal-humus complexes that constitute the non-hydrolyzed N. In Brazil, studies are reported in soils from Amazônia [8], São Paulo [9–12], and Espirito Santo [13].

In Latosols and Argisols from Amazônia, determination of the organic N forms indicated that the immobilization was mainly from microbial origin and the ^{15}N immobilized in the soil was found as acid-soluble N and undistilled-N [8]. In São Paulo, in sugarcane-cultivated soil, amino acid-N fractions predominated, and, after 12 weeks incubation, the total hydrolyzable-N did not vary, but the hydrolyzable NH^{4+}-N decreased [9]. In soil samples under different cover plants [10], the amino acid-N fraction predominated, with the following distribution: 14–38% hydrolyzable NH_4^+-N, 36–52% adenosine triphosphate as NH_4^+-N + amino sugars, 10–32% amino sugar-N, 26–46% amino acid-N, and 3–28% unidentified-N.

Moreover, in São Paulo, in a soil under maize cultivation, it was observed that topdressing N fertilization decreased the N content of the most labile fractions (hydrolyzable NH_4^+-N and amino sugars-N) in the surface layer of the soil, and the amino acid-N and amino sugar-N fractions were considered the organic N reservoirs that control the soil N availability [11]. In contrast, fertilization with cattle manure [12] increased the most easily mineralized (up to 100 days) organic N fractions and subsequently increased the more stable organic N fractions, mainly in clay soil. In Espirito Santo, in soil under eucalyptus, [13] observed that the amino-N was predominant (39%), followed by unidentified-N (27%), amide-N (18%), and hexosamine-N (15%).

Several theories have been developed to explain the resistance of some N compounds to microbial attack. It is mentioned that N compounds are probably protein constituents (amino acids, peptides, and proteins) that are stabilized by reactions with lignins, tannins, quinones, and reducing sugars. Moreover, N compounds would adsorb to the clay fraction of soil and thereby would be protected against the action of protease enzymes. Also, the formation of organic N complexes and polyvalent cations (iron and aluminum) is another biologically stable form of protection [14]. Accumulation and/or decrease of organic C and N is more dynamic in sandy soils than in clayey ones, probably due to the highest oxygenation capacity and lower residue input of sandy soils due to its low productive potential, which gives it less resilience.

3. Nitrogen mineralization and immobilization in the agricultural systems

Nitrogen mineralization and immobilization are biochemical processes widely discussed in the literature. We will focus on how these processes occur in the agricultural system. N mineralization occurs through hydrolysis and biodegradation of soil organic matter when N content in the substrate exceeds the metabolic N requirement by microbial cells. The process is mediated by heterotrophic soil microorganisms [15] that use nitrogenous organic substances as a source of C, N, and energy, releasing NH_4^+ ions as a residue (ammonification). In its turn, immobilization is defined as the transformation of inorganic N (NH_4^+, NH_3, NO_3^-, NO_2^-) to microbial forms. Microbiota assimilates inorganic forms of N by incorporating them into the amino acids, which will participate in protein synthesis during soil biomass formation [14].

N mineralization and immobilization occur simultaneously and oppositely in the soil. The net balance between these processes is controlled by several factors: (a) environmental, such as soil temperature, aeration, and moisture; (b) soil physical, such as texture, structure, and size of aggregates [16]; (c) soil chemical, such as pH; (d) agricultural management system adopted [17]; and (e) quality parameters of the decomposing waste (such as C/N, C/P, and C/S ratios), content of easily decomposable and recalcitrant fractions, type of associated decomposers, size and activity of microbial biomass, and inorganic N availability [18]. Carbon/nitrogen (C/N) ratio less than 25 in organic waste favors N mineralization and fast decomposition, while greater than 30 strongly favors N immobilization and fast decomposition [19]. The crop developmental stage also influences waste C/N ratio. For instance, wastes from millet plants cut at the flowering or milky grain stages present high C/N ratio which delays mineralization. On the other hand, wastes from millet cut at the flag leaf stage, even though phytomass is lower, present less C/N ratio which favors N mineralization for the next crop [20].

In residue plant, considering ^{13}C-CPMAS NMR spectral regions [21], observed that the carbonyl C and N-alkyl and methoxyl C regions had the most significant positive correlation with N mineralization, while the di-O-alkyl C and O-alkyl C were strongly associated with N immobilization. This study demonstrates that the biochemical quality of organic C defined by 13C-CPMAS NMR is capable of predicting N dynamic pattern better than C/N ratio. Abbasi et al. [22] observed positively correlated with the initial residue N contents and negatively correlated with lignin content C/N ratio, lignin/N ratio, polyphenol/N ratio, and (lignin +

polyphenol)/N ratio indicating a significant role of residue chemical composition and quality in regulating N transformations and cycling in soil.

In the N compartments, N from the most labile fractions is released in the early mineralization process, and its mineralization estimate can be used to adjust the nitrogen fertilization recommendations. In fact, it was observed that the mineralization potential and the respective mineralization rate can be used to predict the N availability for plants in the agricultural system. Camargo et al. [23] found that the potentially mineralizable nitrogen values in 10 soils from Rio Grande do Sul ranged from 108.6 to 210.8 mg kg^{-1}.

In respect to the management system adopted, time is essential for N mineralization, mainly in the no-tillage (NT) system. Siqueira et al. [24] found that in soil under NT system for 12 and 22 years, the averages for N mineralization were 0.19 and 0.26 g m^{-2} day^{-1}, respectively. For organic compounds such as sludge, the N mineralization rate is generally below 50%, 5–38% [25], 14–43% [26], 7–16% [27], and 24–31% [28]. Among the species used in straw production, Fabaceae plants stand out for fixing atmospheric N_2 and presenting low C/N ratio tissues, in addition to the high soluble compound content and low lignin and polyphenol contents. This fact favors the fast decomposition and mineralization, with significant N input to the soil–plant system, but with reduced soil cover, which is essential for NT system [29]. On the other hand, Poaceae plants present relatively high dry matter content and high C/N ratio (> 30), which increase the persistence of soil cover although increase N immobilization [30, 31].

4. Nitrogen volatilization and denitrification

Volatilization is the main cause of N loss where ammonia gas (NH_3) is produced according to the simplified equation: $NH_4^+ + OH^- \leftrightarrow NH_{3(g)} + H_2O$. NH_3 loss increases with increasing soil pH. Ammonium ion (NH_4^+) can be adsorbed by soil colloids (clays in humus); thus the largest losses are found in sandy soils and poor in soil organic matter (SOM). Denitrification is another factor that favors N loss, which is mainly controlled by organic matter content, pH, and soil temperature. This process is performed by anaerobic bacteria such as *Pseudomonas*, *Bacillus*, *Micrococcus*, and *Achromobacter*, which are heterotrophic and get energy from carbon, through oxidation of organic compounds. Some autotrophic species also participate in the process such as *Thiobacillus denitrificans* and *T. thioparus* [32].

NH_3 losses by volatilization in agriculture occur due to many factors: ambient temperature, soil moisture at fertilization time, urease enzyme activity, soil pH, cation exchange capacity, soil cover, rainfall after fertilization, and SOM content [33, 34]. Tasca et al. [34] reported 4.6-fold less NH_3 volatilization when topdressing urea was performed at 18°C temperature, compared to 35° C, which demonstrates that N losses increase with increasing temperature. Low volatilization rates are also reported under higher soil moisture values, around 20%, because fertilizer hydrolysis facilitates the NH_4^+ diffusion, making it less susceptible to volatilization, even considering the increased soil biological activity in that moisture. In contrast, higher N losses occur under around 10% humidity values, because the NH_4^+ incorporation is inefficient, resulting in higher N-NH_3 emissions [34]. Moreover, NH_3 losses by volatilization are higher during the driest periods of the year. Soil moisture at fertilization time directly interferes with urea hydrolysis and consequently with NH_3 volatilization losses. Thus, soil wetting soon after urea application is more important than the soil moisture at the application time [35]. According to Ros et al. [36], water applied after urea fertilization or the occurrence of rainfall may decrease NH_3 volatilization if it is sufficient to dilute the hydroxyl (OH^-)

concentration around the urea granules produced during the hydrolysis, besides providing the incorporation of urea in the soil.

Plant cover also influences N-NH_3 volatilization. Pinheiro [37] found the removal of sugarcane straw from the soil decreased NH_3 volatilization rates. The analysis of topsoil and straw indicated higher urea and NH_4^+ retention in the largest amounts of straw on the soil, besides effective urea hydrolysis occurring directly in the straw. These results demonstrated a direct contribution of the straw mulches on NH_3 volatilization. However, despite NH_3 volatilization decreases with straw removal, the choice of straw amount to be removed cannot be based only on NH_3 volatilization of N fertilizer. Analyzing fertilizer mixtures in laboratory, Vitti et al. [38] found that mixing urea (330 mg) with ammonium sulfate (300 mg) significantly reduced N-NH_3 losses (97.47 mg) relative to urea (121.52 mg), without affecting the physicochemical quality attributes of the mixture for technical and agronomic efficiency purpose. In Brazil, urea is the most used mineral N fertilizer, but it has volatilization losses due to the enzymatic hydrolysis that consumes H^+ and increases soil pH. For that reason, even in acidic soils, urea is subject to N losses by volatilization [39]. In agricultural systems, the largest N losses by volatilization occur 3–5 days after fertilizer application [40]. Santos [41] observed that from total N-NH_3 loss by volatilization, 92.5% occurred until the fifth day after fertilization, negatively affecting the corn grain yield.

Fertilizer type may also influence N-NH_3 volatilization. The application of polymer and organic compound-coated urea promoted the lowest ammonia losses by volatilization [42, 43]. In soil under pasture (*Brachiaria decumbens*), Lana et al. [44] observed NH_3 losses 2 days after urea application (2765 mg) and that the use of an inhibitor (NBPT) reduced the volatilization peak by up 4 days. The use of urea plus Uremax NBPT 500® decreased volatilization by approximately 75% after 11 days. Also, adding acid fertilizers may reduce NH_3 losses by 29% [45]. According to Gurgel et al. [46], mineral fertilizers mixed with urea and humic acid (5 and 10%) and urea and zeolite (10%) reduced N-NH_3 losses up to 38%. Results were even more effective in sandy soils.

The use of liquid and solid organic biofertilizers such as poultry and swine residues are also alternative means to reduce N losses, since N is present in biofertilizers as organic form, thereby requiring more time to be mineralized by microorganisms for plant uptake. Niraula et al. [47] reported that cattle manure applied in corn had 11% lower cumulative NH_3 emission than urea, without affecting grain yield, despite having higher CO_2 and CH_4 emissions. Thus, after comparing the ammonia volatilization levels reported in 92 studies, Bouwman et al. [48] concluded that the average NH_3 emissions from the synthetic urea fertilizer and manure slurry were 21.0 and 21.2% from applied N fertilizer, respectively. Moreover, acidification has been a resource used to minimize urea volatilization with liquid waste. Park et al. [49] observed the application of acidified slurry reduced NH_3 emissions by 78.1%, N_2O emissions by 78.9%, and NO_3^- leaching by 17.81% compared to control (non-pH-controlled pig slurry), over the course of the experiment.

Quantifying ammonia volatilization from various organic N sources (castor bean cake, bokashi, legume fertilizers, cattle manure), Rocha et al. [50] observed (i) the N loss rate by NH_3 volatilization varies from 3 to 25% in winter/spring and 2 to 38% in summer/autumn among the studied organic fertilizers; (ii) when incorporating organic fertilizers into the soil, volatilization was significantly lower than when they are maintained on the soil surface, with a volatilization reduction by 80% for castor cake, 78% for bokashi, and 67% for legume fertilizer, while for cattle manure there was no difference; and (iii) when on surface, potential NH_3 volatilization from the total N applied in winter/spring and summer/autumn seasons, respectively, was 25.5 and 38.1% for castor cake, 16.6 and 13.7% for bokashi, 8.2 and 8.8% for legume fertilizer, and 3.4 and 2.4% for cattle manure.

In Planosol under irrigated rice, the addition of cover plants on the soil and water management by intermittent irrigation were practices that mitigated N_2O emissions. Zschornack et al. [51] observed an increase in N_2O emissions by more than 200% in a drained area than continuous water blade area. Thus, soil drainage during rice cultivation increases N_2O emissions by stimulating nitrification and denitrification processes. In addition, N_2O emissions depend on the input waste quality and increase significantly when legumes are inserted into cover plants. Moreover, analyzing biochar in rice, He et al. [52] suggested that the combination of biochar and HQ (urease inhibitor-hydroquinone) or the combined application of urease and nitrification inhibitors to soil enriched with biochar at least 1 year previously could be an effective practice for reducing NH_3 emissions and increasing rice yields.

Finally, microorganism respiration may also contribute to retaining N into the soil. By dissimilatory nitrate reduction to ammonium (DNRA), a respiratory process antagonistic to denitrification, nitrate is used by microorganisms, mainly *Bradyrhizobium* and *Mesorhizobium* bacteria, as electron acceptors. This process results in N retention and production of the less mobile ammonium cation (NH_4^+), thereby reducing the contribution to the total N_2O pool [53]. In addition to N fixation, the potential N retention by microorganisms through DNRA becomes a relevant feature in the reduction of N losses by denitrification [54]. This suggests DNRA may act as a mechanism for conserving N in agricultural systems.

5. Soluble nitrogen

Soluble organic nitrogen is a labile source of N for microorganisms and is an important soluble N reservoir in agricultural soils. Plant species (associated or not with mycorrhizae) can directly uptake simple organic N present in the SON pool [55]. The SON pool is composed of high (protein oligomers), medium (small peptides) [56], and low molecular weight compounds (monomers such as amino acids) [57]. As plants uptake organic and inorganic N, the relative proportion of these different N sources in soils is a determinant of N management.

SON is suggested as a transitional phase during N transformation between soil organic matter and inorganic N (NH_4^+-N) and considered an intermediate step in microbial mineralization of organic N [58]. The SON pool can regulate the N transformation rate in the soil, i.e., the ammonification and nitrification rates, affecting the substrate associated with different plant species. Thus, soil organic N fractions and SON pools are important indicators of soil fertility and plant nutrition requirements [59], inferring the potential supply of N mainly in low N mineralization soils [60].

Besides an important component of soil total soluble N, SON plays a key role in N cycling and therefore in determining soil N availability in agricultural systems [61]. The amount of SON represents a relatively high proportion of the total soluble nitrogen (TSN) pool. It has been reported that SON constitutes 17–90% and 32–50% of TSN in pasture and agricultural soils, respectively [46, 47, 62, 63]. Like in mineral N, SON dynamics are affected by mineralization, immobilization, leaching, and plant uptake, but its pool size is more constant than mineral N [64]. Although remains unclearly understood, SON is an important pool in N transformations and plant uptake.

Biotic and abiotic processes are involved in the SON generation in soil [58]. By biotic processes, SON can be produced directly from microbial turnover and indirectly through the microbial excretion of extracellular enzymes [61]. However, as plants and microorganisms can compete for soil organic N, it is also possible that SON reservoirs vary spatially due to the variation in activity and density of microbial population between different types of agricultural management. Zhang et al. [65] reported that SON fractions were significantly and positively correlated with the no-tillage system practices and that this agricultural system is beneficial and effective for increasing soil N turnover.

Proteins are the most abundant nitrogen compounds in SON. Depolymerization of these organic macromolecules in monomeric SON (amino acids) can be considered rate-limiting for the total N cycling in soils [66]. Soil amino acids can contribute, in relative and absolute terms, to the SON pool in agricultural soils, which was observed in soil under fertilized sugarcane [55]. Also, plants can use proteins as N source without the help of other organisms [67]. Although the relative contribution of amino acids to N supply for crops remains unclear, all studied plants have shown the ability to uptake and metabolize amino acids as well as soils containing amino acids [68].

Organic agriculture practices can increase the content of SON, protein, and free amino acids in the soil as a result of frequent and long-term inputs of organic matter. In addition, agricultural production quantity may also influence the SON pool abundance. However, the effect of organic cultivation on specific free amino acids and protein pools remains unclear [66].

Soil organic matter, pH, total C, total N, and C/N ratio are the main factors affecting soil SON abundance. SON dynamics can be significantly affected by mineralization and immobilization during microbial growth and decomposition of organic matter. Besides that, agricultural practices such as irrigation management, fertilization, plowing, harrowing, harvesting, and the plant growth stage can also play an important role in SON dynamics [59, 63]. Furthermore, high temperatures may increase the SON content by stimulating decomposition of organic matter [69]. Knowing the temporal dynamics of organic N pools in the soil may help to understand how these pools are affected by soil properties, climate and crop management, and whether SON can contribute to N supply of crops.

6. Biological nitrogen fixation

Nitrogen in the gaseous form (N_2) represents 78% of the atmospheric gases but is inert and unavailable to plants. Only nitrogen-fixing microorganisms, including bacteria, cyanobacteria, and fungi, are able to break the triple bond between the atoms ($N \equiv N$) of the atmospheric nitrogen, thus transforming it into ammonia (NH_3) through the nitrogenase enzyme ($N_2 + 8H^+ + 6e^- \rightarrow 2NH_3 + H_2$) [70]. Biological nitrogen fixation is a key component of the nitrogen cycle and responsible for most of the nitrogen available to plants.

BNF is performed by symbiotic, endophytic, or free-living microorganisms [71, 72]. Symbiotic bacteria associate with plants forming root nodules (rhizobia), where they fix nitrogen while benefiting from plant photoassimilates. It has been observed that this symbiosis occurs not only in plants from the Leguminosae family [71] but also in cereals such as rice, maize, and wheat from the Poaceae family [73]. BNF also occurs in nonsymbiotic associations. Endophytic bacteria colonize plant tissues and fix N while benefiting from plant photoassimilates, although the amount of N fixed is lower than in symbiosis [73, 74]. Also, free-living microorganisms inhabiting rhizosphere, soil region around plant roots, fix nitrogen while feeding on root exudates (amino acids, peptides, proteins, enzymes, vitamins, and hormones), which stimulate growth of diazotrophic bacteria from genera *Acetobacter*, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Herbaspirillum*, *Klebsiella*, *Paenibacillus*, and *Pseudomonas* [71].

Nitrogen-fixing microorganisms occur naturally in soil [71] and in water [72] or colonize seeds [74]. However, in the agricultural environment, conventional practices such as plowing, harrowing, chemical fertilization, and pesticide application reduce the soil microorganism populations, which make these areas depending on the application of nitrogen fertilizers [75, 76]. Chemical fertilizers require a great amount of energy to be produced, energy that is derived from fossil fuels. Moreover, they are potential soil and water contaminants and expensive and scarce for many developing country farmers [77]. Therefore, strategies have been studied to increase BNF by plants and thus reduce dependence on chemical fertilization.

Conservation practices such as minimum tillage, no tillage, and cover crops stimulate BNF as they increase the population and activity of soil microorganisms (bacteria, actinomycetes, and mycorrhizae) [78, 79]. In addition to capturing soil N, reducing N loss by leaching, and becoming an N source for succeeding crops, mixing cover crops (legumes and grasses) provide additional N through BNF [79, 80].

Another alternative for increasing BNF is to inoculate nitrogen-fixing microorganisms in crops. Inoculated into the seeds, roots, or leaves, these microorganisms may increase the formation of root nodules, stimulate root growth, improve nutrient uptake, stimulate antioxidant defense system, increase tolerance to biotic (pest and pathogen) and abiotic (drought and salinity) stresses, and thereby increase crop productivity. Inoculation of nodulating as well as endophytic fungi or bacteria stimulates growth in both legumes and grasses and represents a viable and sustainable alternative (**Table 1**). Among the most used microorganisms are *Rhizobium* and *Bradyrhizobium* genera bacteria inoculated in legumes and *Azospirillum* and *Enterobacter* genera in grasses (**Table 1**).

Studies also focus on the application of nitrogen-fixing microorganisms through irrigation water, on the genetic improvement for BNF by legume crops [96], on becoming plants able to self-fertilize by stimulating root fungal associations in grasses, and on providing cereals with the nitrogen-fixing enzyme (nitrogenase) [77]. Estimations indicate these practices can reduce fertilizer application costs by billions of dollars annually.

Crop	Scientific name	Inoculated microorganism	Reference
Rice	*Oryza sativa*	*Bacillus amyloliquefaciens*, *Enterobacter cloacae*, *Klebsiella variicola*	[81, 82]
Sugarcane	*Saccharum officinarum*	*Gluconacetobacter diazotrophicus*, *Herbaspirillum seropedicae*, *H. rubrisubalbicans*, *Burkholderia tropica* e *Azospirillum amazonense*	[83]
Cowpea	*Vigna unguiculata*	*Actinomadura*, *Bradyrhizobium elkanii*, *B. pachyrhizi*, *B. yuanmingense*, *Paenibacillus graminis*, *Rhizophagus irregularis*	[84–88]
Common bean	*Phaseolus vulgaris*	*Rhizobium leguminosarum* bv. phaseoli, *R. tropici*	[89]
Maize	*Zea mays*	*Azospirillum brasilense*, *Herbaspirillum seropedicae*	[90, 91]
Soybean	*Glycine max*	*Bradyrhizobium japonicum*, *Bacillus megaterium*, *Methylobacterium oryzae*,	[92, 93]
Wheat	*Triticum aestivum*	*Azospirillum brasilense*, *A. insolitus*, *Enterobacter* sp., *Microbacterium arborescens*, *Serratia marcescens*, *Zoogloea ramigera*	[94, 95]

Table 1.
Legume and cereal crops and nitrogen-fixing microorganisms used for inoculation.

7. Nitrogen and organic fertilization

The worldwide demand for organic foods, produced without the use of synthetic inputs, has driven the use of conservation practices, especially fertilization using organic wastes. The application of organic wastes to the soil improves soil fertility by increasing the organic matter (OM) and nutrient contents, such as N and phosphorus (P), and soil microbiota population, as well as improving the cation exchange capacity (CEC) [97].

Organic fertilization improves yield and quality of vegetables such as lettuce (*Lactuca sativa* L.) [98], tomato (*Solanum lycopersicum* Mill.) [99], and carrot (*Daucus* carota L.) [100]; fruits such as papaya (*Carica papaya* L.) [101], citrus (*Citrus* spp.) [102], and raspberry (*Rubus* idaeus L.) [103]; and annual crops such as maize (*Zea mays* L.) [104] and cowpea (*Vigna unguiculata* (L.) Walp) [105]. Most organic fertilizers used as N source are derived from (a) agricultural wastes (cattle, swine and poultry manure), slaughterhouses (bone and blood meal), composting, and vermicomposting; (b) agro-industrial wastes (oilseed pies, sugarcane bagasse, and vinasse) and biochar; and (c) household wastes and sewage sludge composting (**Table 2**).

N input by organic fertilizers occurs predominantly through mineralization of organic N, although some mineral N fractions may be released [107, 119]. The organic N mineralization rate is regulated by N fractions and C/N ratio of the decomposing waste, as well as by environmental temperature and humidity [120, 121]. Under favorable conditions, high N content organic fertilizers mineralize quickly similarly to synthetic fertilizers, while those with low N content and high C/N ratio mineralize slowly [122]. Thus, knowing the mineralization rate allows choosing the best organic fertilizer to be used in agriculture (**Table 2**).

Manures are the main used organic fertilizers worldwide, especially as N source, though the amount and quality of N in manure may vary according to animal species, age, and feed. Forage-based diets increase the residue production, although reduce the quality that is provided by a concentrate-based diet [97, 119]. Cattle, equine, sheep, goat, and swine manures present similar N content, ranging from 0.77 to 3.90%. In its turn, poultry litter may have 2.80–4.60% N content, due to concentrate-based feed supplied to poultries, being a fast mineralizing fertilizer [106, 107]. Thus, manure fertilization has been efficient for many crops, such as sweet pepper (*Capsicum annuum* L.) [123] and radish (*Raphanus sativus* L.) [124].

Residues from the castor bean (*Ricinus communis* L.; *Euphorbiaceae*) chain stand out due to the high N content which is found in the pie (7.54% N), in the oil extraction residue (12.82% N), and in the pulp from direct oil transesterification for biodiesel production [106, 125–127]. Castor pie mineralization rate is more intense than in other composts and thus quickly releases N and other readily available nutrients to plants. As reported by [126], evaluating microbial respiration, who obtained mineralization rates 6 times faster than those obtained in cattle manure and 14 times faster than in sugarcane bagasse, other pies, such as peanut (*Arachis* spp.) and cotton (*Gossypium* spp.), may also have high N (4.0–7.0%) content and similar mineralization characteristics [128, 129].

The product obtained from the composting of organic wastes is rich in stable organic matter. Wastes are transformed through biological decomposition, and the process is affected by environmental conditions and N content. As nitrogen compounds are food for microbiota, N deficiency in waste may retard the maturation process, and the excess may increase the N volatilization as ammonia (NH_3), consequently affecting N stabilization processes in composting [130]. Also, humus from vermicomposting (usually by using *Eisenia foetida* species) is highly stable and presents high contents of N and humic acids, which indicate a better relationship between the mineralization and humification processes of OM, with decreasing C/N ratio [115, 131].

Source	N content (%)	C/N ratio	Reference
Cattle manure	0.8–3.2	16.0–21.0	[97, 106]
Equine manure	1.4–3.9	21.9–25.0	[97, 107]
Sheep manure	1.2–1.8	9.0–29.0	[108, 109]
Swine manure	1.9–2.8	10.0–12.0	[97, 107]
Poultry litter	2.8–4.6	4.2–22.0	[97, 106, 107]
Blood meal[1]	11.8–12.9	—	[110, 111]
Bone meal	4.1–4.2	4.0–7.0	[97, 112]
Meat and bone meal	5.5–6.6	6.0	[106, 112, 113]
Castor pulp[1]	12.8	—	
Castor pie	5.2–7.5	6.0–9.0	[97, 106, 112]
Cotton pie[1]	4.5	—	[106]
Filter pie	1.5–1.8	21.0–24.0	[97, 112]
Sugarcane bagasse	0.9–1.5	85.0	[106, 111, 114]
Vinasse	0.3–1.2	4.0–17.0	[97, 112]
Compost	0.7–2.6	11.3–64.0	[107, 115]
Humus	1.3–2.6	11.0–34.0	[115, 116]
Millicompost	2.0–2.2	15.0–19.0	[98, 117]
Biochar	0.1–5.0	7.0–400.0	[118]
Sewage sludge	0.8–3.5	9.0–50.0	[97, 112]
Household waste	0.9–2.6	7.0–27.0	[97, 107, 112]

[1]*C/N ratio not found.*

Table 2.
Nitrogen content and carbon/nitrogen ratio (C/N) in organic fertilizers.

In addition to the earthworms, arthropods that constitute the edaphic macrofauna [87, 132, 133] are also of great interest. Millipedes (Myriapoda: Diplopoda) fragment and feed on organic wastes and excrete low C/N ratio feces (2.2% N) producing the millicompost [134–136]. Studies suggest that millicompost is similar to vermicompost and commercial substrates in relation to N supply and other macro- and micronutrients for seedling production, such as in lettuce (*Lactuca sativa*) [98] and pitaya (*Hylocereus* spp.) (Cactaceae) [137].

In relation to slow-release organic fertilizers, biochar is an alternative. A by-product from carbonization (pyrolysis) of biomass under low-oxygen atmosphere, biochar is fine-grained carbonaceous material with decomposition resistance [118]. N content in biochar depends on the source material (biomass) as well as on the pyrolysis temperature. Biochars from wood have high C/N ratio and low N content (0.1%), while those from manures have low C/N ratio and high N content (5.0%). For instance, biochar from eucalyptus wood (*Eucalyptus urophylla* S. T. Blake and *Corymbia citriodora* (Hook.) K.D. Hill and L.A.S. Johnson) contains 0.66 and 0.48% N, respectively, while from coffee husks (*Coffea* spp.) contains 2.74% N [138]. Besides slowly releasing nutrients, the use of biochars increases N uptake via ion exchange and NH_3 removal by adsorption, stimulates immobilization (reducing NO_3^- losses), and reduces N_2O emissions [139–142]. Moreover, biochar improves mycorrhizal associations and nitrogen biological fixation [118].

Urban wastes have also been used in agriculture. Sewage sludge showed to be an excellent N source (0.80 and 3.47% N) besides slowly mineralizing. N mineralization rates from 20 to 38% were found after 105 days [143], which depends on source material characteristics and treatment processes as well as on heavy metal content that accelerate or limit mineralization [107, 144]. Slaughterhouse residues, such as bone and blood meal, present high N rates, but they are not yet used in agriculture because studies on its adoption and behavior as organic fertilizer are scarce [97, 110, 112].

8. Concluding remarks

In the surface layer of most soils, the soil organic N can be divided into two categories: N from organic residues and N from soil organic matter or humus. N mineralization and immobilization processes occur simultaneously and oppositely in the soil. The net balance between these processes is controlled by several factors such as environmental conditions, soil physicochemical factors, agricultural management adopted, quality of the decomposing residues, and content of easily decomposable and recalcitrant fractions. As organic agriculture increases soluble organic nitrogen content, this fraction has been extensively studied. Also, being biological nitrogen fixation a key component of the nitrogen cycle and responsible for most of the nitrogen available to plants, it was also discussed in this chapter.

Finally, we discussed nitrogen and organic fertilization, since the worldwide demand for organic foods produced without the use of synthetic inputs has driven the use of conservation practices, especially fertilization using organic wastes. Most organic fertilizers used as N source is derived from agricultural and agro-industrial wastes, slaughterhouse wastes, composting and vermicomposting, biochars, household wastes, and sewage sludge composting.

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