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REVIEW ARTICLE

Nitrification inhibition in tropical soil under no-tillage system

Inibição da nitrificação em solos tropicais sob plantio direto

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ABSTRACT: In no-tillage systems (NTS), biological activity and nitrification process in the soil are higher due to higher organic matter and moisture contents, resulting in higher levels of nitrate compared with areas under conventional tillage systems. However, nitrogen in the form of nitrate is more easily leached, helping to reduce nitrogen use efficiency (NUE) by plants, besides contaminating water resources. Therefore, the use of alternatives that promote the persistence of ammonium in the soil contribute to increased nitrogen use efficiency in agricultural systems, as well as to their sustainability. The purpose of this review was to discuss the process of nitrification inhibition. We observed that it is possible to increase the content of ammonium in the soil, contributing to the reduction of N loss through leaching and increasing the efficiency of nitrogen use by crops, thus providing sustainability to agricultural systems. To this end, the use of cover crops, such as Brachiaria species, and synthetic nitrification inhibitors incorporated to nitrogen fertilizers are among the alternatives. Nevertheless, studies should be developed in order to clarify the conditions under which these cover crops can be introduced in agricultural systems for this purpose and also which factors interfere on the efficiency of synthetic nitrification inhibitors.

RESUMO: *No sistema plantio direto (SPD), em razão dos maiores teores de matéria orgânica e umidade, a atividade biológica é maior, bem como o processo de nitrificação, resultando em teores mais elevados de nitrato em relação às áreas sob sistema convencional de manejo do solo. No entanto, o nitrogênio na forma nítrica é mais facilmente lixiviado, contribuindo para a redução da eficiência do aproveitamento do N pelas plantas, além de poder contaminar os recursos hídricos. Assim, o uso de alternativas que favoreçam a permanência do amônio no solo contribuirá para o aumento da eficiência de utilização do N nos sistemas agrícolas, bem como para a sustentabilidade dos mesmos. O objetivo desta revisão foi discutir o processo relacionado à inibição da nitrificação. Observou-se que se podem elevar os teores de amônio no solo, contribuindo para a redução das perdas de N por lixiviação e aumentando a eficiência de utilização do nitrogênio pelas culturas, proporcionando maior sustentabilidade aos sistemas agrícolas. Entre as alternativas, há o uso de plantas de cobertura, como as do gênero Brachiaria, além do uso de inibidores sintéticos da nitrificação incorporados aos fertilizantes nitrogenados. Entretanto, estudos devem ser desenvolvidos no sentido de esclarecer em que condições essas plantas de cobertura podem ser introduzidas nos sistemas agrícolas para esse fim e também quais os fatores que interferem na maior ou na menor eficiência dos inibidores sintéticos de nitrificação.*

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1 Introduction

Nitrogen (N) is one of the most dynamic nutrients in the soil (NASCENTE et al., 2011). Most of it is contained in its organic fraction (over 90%), a large reservoir of forms more readily available, such as nitrate and ammonium, and these mineral forms, despite responding for a small portion of the total N, are of utmost importance from the nutritional viewpoint because they are absorbed by plants and microorganisms (MARSCHNER, 1995).

Most plants absorb nitrate and ammonium indistinctly (MALAVOLTA, 1980). However, there are plants, such as rice, that prefer to absorb nitrogen in the form of ammonium early in their development (MALAVOLTA, 1980; NASCENTE; CRUSCIOL; COBUCCI, 2012). The combined use of the two forms of N can lead to better performance of the plant (TISDALE; NELSON; BEATON, 1985). Nevertheless, the low efficiency of the agronomic use of this nutrient, observed in most agricultural systems, is the result of N losses associated with nitrification, that is, N losses through leaching and denitrification of N-NO_3^- (MALAVOLTA, 1980; NASCENTE; CRUSCIOL; COBUCCI, 2012). In this sense, in aerated soils, N is mineralized to ammonium and is quickly transformed into nitrite and then nitrate, with the latter being the predominant form (BREDEMEIER; MUNDSTOCK, 2000).

However, larger amounts of nitrate lead to higher losses of N, especially in environments with high rainfall (CRUSCIOL et al., 2011). This nutrient is the element most easily lost through leaching, following the downward movement of the water that percolates into the soil profile (MALAVOLTA, 1980). This leaching process not only results in considerable losses of nitrogen, but also represents a risk of pollution to surface and ground water (ALCÂNTARA; CAMARGO, 2010). Nevertheless, in tropical regions such as Brazil, Latosols present low organic matter content and are rich in variable charges, and for this reason, they may have net positive charge, favoring the retention of nitrate, which is an anion with predominantly electrostatic adsorption (ALCÂNTARA; CAMARGO, 2010). In addition, the absorption of nitrogen as ammonium is more beneficial to plants, because N assimilation by plants is a highly demanding process in terms of energy, requiring the transfer of two electrons per NO_3^- converted to NO_2^-, six electrons per NO_2^- converted to NH_4^+, and two electrons and one adenosine triphosphate (ATP) per NH_4^+ molecule converted to glutamate (MALAVOLTA, 1980). Therefore, the assimilation of NO_3^- demands more energy compared with the assimilation of NH_4^+ (BREDEMEIER; MUNDSTOCK, 2000).

Nitrogen use efficiency and loss processes in the soil-plant system present economic and environmental consequences, especially when N-oxides are emitted into the atmosphere. Nitrous oxide (N_2O) has received increased attention, mainly because it contributes to the greenhouse effect and the destruction of the ozone layer (CERRI; CERRI, 2007, CHIEN; PROCHNOW; CANTARELLA, 2009).

Therefore, the development of technologies that provide increased nitrogen use efficiency could contribute to the reduction of losses and contamination of water resources (nitrate) and atmosphere (nitrous oxide). Thus, knowledge about the processes involving the transformations of N in the soil is crucial to the search of viable alternatives to reduce losses and increase nitrogen use efficiency in agricultural systems. The objective of this review was to discuss the processes of N loss and a form to improve the utilization of this element in agricultural systems.

2 Development

2.1 Nitrogen dynamics in soil

Nitrogen is present in the soil in three main forms: organic compounds in plant residues (i), organisms and humic substances (ii), and inorganic forms (iii). In this latter form, nitrogen is represented by NH_4^+ fixed to clay minerals by NH_4^+, NO_3^- and NO_2^- present in the soil solution, and also in the gas fraction, which may contain N in the forms of NH_3, N_2, NO and N_2O. Figure 1 shows the average proportion of occurrence of each form (McLAREN; CAMERON, 1996).

Nitrogen occurs in the soil mainly in its inorganic form (Figure 1) and it is available to plants through biological processes. In agricultural systems, the physicochemical processes of volatilization, fixation and leaching usually occur associated with nitrogen releases after microbiological transformations. Nitrogen availability in the soil depends, in part, on the balance between the processes of mineralization and immobilization. The balance between these processes may vary according to time, formation of organic residue decomposition, soil microbial activity (MARSCHNER, 1995), and the agricultural system used. Gonçalves, Ceretta and Basso (2000) observed that after six years of soil management under NTS, only 4% of the nitrogen was found in the mineral form (NO_3^-, NO_2^- and NH_4^+).

The mineralization process is influenced by several factors, chiefly temperature, moisture, aeration, quantity and composition of the organic material. These factors regulate the velocity of the transformation of organic N in mineral N (MALAVOLTA, 1980; MARSCHNER, 1995).

2.2 Action of biological processes

The accumulation of organic residues on soil surface promotes increased biological activity and, as a consequence,

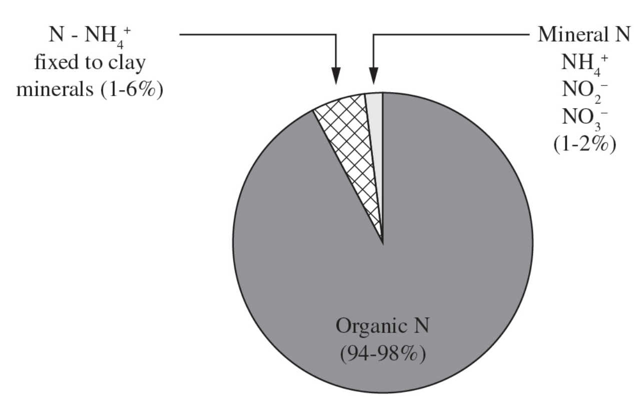

Figure 1. Distribution of forms of nitrogen in the soil (McLAREN; CAMERON, 1996).

transformations of organic material. Regarding N, the main transformations of biological origin are mineralization (ammonification and nitrification), denitrification, and immobilization.

- Mineralization

The transformation of organic nitrogen into inorganic forms is called mineralization. In the mineralization process of organic matter, nitrogen goes through the following phases: organic N, ammoniacal N, and nitric N (nitrite N and nitrate N). The ammonium and nitrate produced are a consequence of two different microbiological processes: ammonification and nitrification (MARSCHNER, 1995).

- Ammonification

The process of ammonification is the deamination of complex organic nitrogen compounds such as proteins, amino acids and nucleic acids. When decomposing microorganisms (saprophyte bacteria and fungi) act on the nitrogenous organic matter, they release various residues to the environment, including ammonia (NH_3). Combined with ground water, ammonia forms ammonium hydroxide, which ionizes and produces NH_4^+ (ammonium ion) and OH^- (hydroxyl) (MARSCHNER, 1995).

- Nitrification

The nitrification process involves conversion of soil NH_4^+ to NO_3^-, by oxidation reactions (NASCENTE; CRUSCIOL; COBUCCI, 2012). This reaction is ruled by the activity of autotrophic bacteria and occurs in two stages. The first stage, called nitritation, involves conversion of NH_4^+ to NO_2^- by *Nitrosomonas* and *Nitrosospira*, (MARSCHNER, 1995) according to Equation 1.

$$2NH_4^+ + 3O_2 \text{ ---}\textit{Nitrosomonas}\text{---> } 2NO_2^- + 2H_2O + 4H^+ \quad (1)$$

This nitrification stage can be subdivided into two others according to the activity of the enzymes ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO), present in the bacteria of genus *Nitrosomonas* (SUBBARAO et al., 2007a, b). These enzymes catalyze the essential reactions in the oxidation of ammonia (SUBBARAO et al., 2008). AMO acts in the conversion of ammonia to hydroxylamine (Equation 2) and HAO acts in the conversion of nitrite to hydroxylamine (Equation 3).

$$NH_3 + O_2 + 2H^+ \text{ ----AMO----> } NH_2OH + H_2O \quad (2)$$

$$NH_2OH + H_2O \text{ -----HAO-----> } NO_2^- + 5H^+ \quad (3)$$

The second stage of the nitrification process is called nitration, that is, the oxidation of NO_2^- to NO_3^-. It occurs in a single phase by action of the nitrite oxidoreductase (NOR) enzyme present in bacteria of genus *Nitrobacter* (Equation 4). This conversion occurs rapidly and, therefore, nitrite rarely accumulates in the soil (MARSCHNER, 1995).

$$2NO_2^- + O_2 \text{ ---NOR---> } 2NO_3^- \quad (4)$$

Operations with machines accelerate the process of nitrification in agricultural systems (MALAVOLTA, 1980). These operations, such as soil correction, are required for some cases, as in acidic soil, resulting in greater aeration. But there are others that could be overcome, such as lack of crop rotation and excessive use of nitrogenous fertilizers (SUBBARAO, 2009a).

Thus, the main form of N found in aerated soils is NO_3^-, regardless of the source of N applied. Moreover, roots can aerate the soil (in the rhizosphere region) favoring nitrification even more (MALAVOLTA, 1980).

- Denitrification

Many bacteria are able to utilize NO_3^- instead of oxygen as electron acceptors in their terminal respiratory chain and thus reduce NO_3^- to (NO_2^-) and hipo-nitrite (HON_2O_2), and then to elemental nitrogen (N_2), nitrous oxide (N_2O) and nitric oxide (NO), whose destination is the atmosphere. This process is known as denitrification (CANTARELLA, 2007).

Agriculture is considered one of the activities that most contribute to emissions of N_2O. Due to strong the Brazilian agricultural vocation, the percentage of N_2O emissions attributable to agriculture can reach 94% (CERRI; CERRI, 2007). It should also be considered that there are two, sometimes three, crops per year with the use of irrigation in the Brazil. In addition to the intensive use of the land throughout the year, the N doses applied to the soil are higher owing to the low content of organic matter found in tropical regions.

The significant contribution of agriculture to emissions of N_2O indicates that it is necessary to develop strategies and techniques to reduce greenhouse gas emissions. In order to achieve positive results, it is necessary to identify the environmental factors and the forms of soil management that contribute to increase or reduce emissions of N_2O (CERRI; CERRI, 2007). Studies show that the increase in the pore space occupied by water results in higher emissions of N_2O (CHIEN; PROCHNOW; CANTARELLA, 2009); this is an expected result, considering that denitrification is a biological process that occurs under anaerobic conditions. Therefore, agricultural practices that improve water infiltration in the soil are essential to prevent the formation of environments that favor N losses by denitrification. However, according to Subbarao et al. (2006b), denitrification should not just be seen as a process of N loss of agricultural systems and emission of gases into the atmosphere. This biological process is part of the N cycle, and it is one of the ways to reduce pollution in systems with excess nitrate.

A solution to prevent loss of nutrients would be the use of no-tillage systems (NTS). Nevertheless, adjustments are needed for the success of this system in avoiding nutrient losses. Adjustments and improvement of the NTS occur when integrated production systems are used, an example of these systems is the crops-livestock integration (CLI). CLI fits perfectly to NTS, besides allowing for adjustment of the triple concept that underpins and support the system. In the traditional concept, the basic NTS tripod comprises a) no soil disturbance, b) crop rotation, and c) permanent ground cover. To this concept, CLI adds an important ingredient to the success of production systems in tropical regions, especially for sandy soils subject to nutrient losses through leaching: the maintenance of a photosynthetically active species and a consortium of species during the longest period possible within a year. Note that the connotation of year has lost its importance, since the system will be planned in the long term. Thus, the basis of the NTS would be formed by a broader and more powerful concept, and would have its tripod supported by the following factors: a) no soil disturbance, b) crop rotation with intercropping of species, and c) maintenance of vegetated soil. Clearly, this vegetation will leave residues that will form permanent ground cover and will yield a continuous cycling and release of nutrients to the system.

- Immobilization/remineralization

Nitrogen immobilization is a process that occurs simultaneously with mineralization, but in the reverse direction. Immobilization is defined as the conversion of inorganic N into organic N. This process is mediated by microorganisms that incorporate the inorganic nitrogen available in the soil to their cells. When these microorganisms die, the N assimilated can be remineralized or incorporated into the cells of other microorganisms (MARSCHNER, 1995).

Nitrogen availability in the soil is, therefore, controlled by microbial processes of mineralization and immobilization, which basically depend on the C/N ratio and on the biochemical composition of decomposing crop residues (CRUSCIOL et al., 2011).

Leguminous species present low C/N ratio in comparison with other plant families. This characteristic, coupled with the presence of soluble compounds, favors the mineralization of crop residues. On the other hand, non-leguminous species generally present high C/N ratio, which favors the temporary immobilization of nitrogen in the microbial biomass (MALAVOLTA, 1980).

2.3 Action of physicochemical processes

Concurrently with the biological transformations of nitrogen compounds there are the physicochemical processes, which are usually associated with N releases in agricultural systems. The main physicochemical processes are volatilization of $N\text{-}NH_3$, fixation of $N\text{-}NH_4^+$, leaching of $N\text{-}NO_3^-$, and emission of N_2O and N_2. Most of the nitrogen is released during the early decomposition, which may coincide with a low nitrogen demand by the growing crop. If mineral N is available precociously, nutrient losses may occur by volatilization of ammonia and leaching of $N\text{-}NO_3^-$ (NASCENTE; CRUSCIOL; COBUCCI, 2012).

The highest loss of nitrate through leaching in NTS occurs due to lower evaporation and better structuring along the profile, which favors water infiltration into the soil. Ground cover can also promote the downward movement of NO_3^- via macropores owing to lower water evaporation (CRUSCIOL et al., 2011).

The nitrate ion accompanies this flow to deeper layers. Nitrate is easily lost through leaching (NASCENTE; CRUSCIOL; COBUCCI, 2012), following the downward movement of water that percolates into the soil profile. This occurs because of the predominance of negative charges in topsoil and the low chemical interaction of the anion with soil minerals (CRUSCIOL et al., 2011). However, tropical soils, which typically have low organic matter content and are rich in variable charges, may present net positive charge and provide a reduction in nitrate losses, mainly in subsurface (ALCÂNTARA; CAMARGO, 2010).

2.4 Nitrification inhibition

Nitrification inhibition may be the most important process that determines the efficiency of the N cycle, that is, the proportion of N that remains in the ecosystem over a full cycle. Efficiency in nitrogen utilization (dry matter produced per unit of N applied) is an intrinsic physiological function; therefore difficult to be genetically manipulated (SUBBARAO et al., 2009a). Improvements in the efficiency of nitrogen utilization result from the way this element is absorbed. Reduction of the nitrification rate of plants enables better opportunity to absorb N, while it still remains in the region of the roots. For instance, in the case rice cultivation, regulation of nitrification is important to balance the proportion between NH_4^+ and NO_3^- in the soil, condition wherein the crop develops best (MALAVOLTA, 1980).

In soils under aerated conditions and high temperatures, ammoniacal N is oxidized to NO_3^- in a relatively short period of time, between 15 and 30 days. Thus, in the short term, even soils fertilized with ammoniacal N tend to present N predominantly in the form of NO_3^-. Therefore, in many cases, the advantage with respect to the leaching of fertilizers with ammoniacal N compared with those that have N in the form of nitrate can be very small and transient (CANTARELLA, 2007). One of the ways to regulate or reduce the nitrification rate in well-managed and aerated soils would be the use of nitrification inhibitors.

Synthetic nitrification inhibitors provide nitrogen fertilizers with the characteristics of slow nitrogen release and prolonged stay in the soil, in the form of ammonium, making it less susceptible to leaching. Therefore, the use of nitrogen sources requires optimized application techniques, so that producers can obtain maximum economic benefit. The use of synthetic inhibitors is a strategy to adjust nitrogen balance in the soil, but it is not the only one; to adjust the dynamics of N in production systems, all tools available for this purpose should be used.

- Acidity affecting nitrification

Ammonifying microorganisms are little affected by soil pH, but nitrification activity is highly dependent on pH. In acidic soils, the population of nitrifying groups (*Nitrossomonas* and *Nitrobacter*) is extremely small. Nitrification rate decreases below pH 6.0 in water and it is negligible below pH 4.5 (CRUSCIOL et al., 2011). According to Crusciol et al. (2011), nitrification is limited in soil layers with a pH ($CaCl_2$, 0.1 M) of approximately 4.0. On these grounds, the predominant form of mineral N in soils under native vegetation and pasture (usually acidic, pH <6.0), practically along the entire profile, is the ammoniacal form.

- Biological nitrification inhibitors

In an attempt to avoid the prevalence of nitrate in the soil, any practice which favors the maintenance of N in its ammoniacal form would be desirable to prevent losses through leaching, and also to create a more favorable environment to the development of some crops such as rice. One of these practices would be the use of cover crops. According to Scivittaro; et al. (2003), the combination of green manure and mineral fertilizers as a source of N for crop is a promising management practice, which rationalizes the use of mineral sources, without dispensing high yields. Malavolta (1980) reported that the presence of N in the form of NH_4^+ is favored by substances secreted by the roots of grasses, which inhibit nitrification, and the existence of lower pH values, which generally occur under such conditions.

According to Subbarao et al. (2006b), to minimize N losses associated with nitrification in agricultural production systems, it is necessary to keep N in the soil in its ammoniacal form as long as possible. This strategy will allows synchrony between N supply and crop demand. Regulation of nitrification could be the key to improving N recovery and agronomic efficiency of N use in situations where N loss by nitrification is significant (SUBBARAO et al., 2007c), particularly for species that require balance between ammonium and nitrite in the soil, such as rice (NASCENTE; CRUSCIOL; COBUCCI, 2012).

Several researchers observed low nitrification rate in soils under tropical forage grasses and forest soils, which led to the hypothesis that compounds released by plant roots may influence nitrification (SUBBARAO et al., 2006a, b).

Recently, it was demonstrated that nitrification can be stimulated or suppressed depending on the type of ecosystem (SUBBARAO et al., 2006a). In most cases, the active chemical constituents were not identified or there was no release of active ingredients in amounts sufficient to maintain inhibition. Subbarao et al. (2007c) tested several plant species with respect to the production capacity of nitrification inhibitors. Forage, grain and legume species were tested. Genus *Brachiaria* were among the forage species that excelled in the inhibition of biological nitrification. In soils where *Brachiaria humidicola* was cultivated, nitrification suppression was higher than 90%. Nitrification inhibition occurred through the release of root exudates, but only when the source of nitrogen was NH_4^+.

The natural ability of a plant to inhibit nitrification by means of root exudates is called biological nitrification inhibition - BNI (SUBBARAO et al., 2006b). Such mechanism was observed in *Brachiaria humidicola* (SUBBARAO et al., 2006a, 2007a, c). The bioassay-guided fractionation of exudates of *Brachiaria humidicola* roots allowed the isolation of a cyclic diterpene, which was designated “brachialactone”. Nitrification inhibition *in vitro* system in experiment with *Nitrosomonas* (N.) *europaea* was linearly related to the concentration of brachialactone. Brachialactone can be considered a potent biological nitrification inhibitor (BNI) compared to nitrapyrin or dicyandiamide, which are two of the most commonly used synthetic nitrification inhibitors. The contribution of brachialactone to the total inhibitory activity of exudates ranged from 60% to 96% (SUBBARAO et al., 2009b).

The BNIs of *Brachiaria humidicola* roots seem to block the way of the enzymes ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO) present in the bacteria *Nitrosomonas* (N.) *europaea* (SUBBARAO et al., 2007a, b). AMO and HAO are enzyme systems that catalyze the essential reactions of the oxidation of ammonia to NO_3^- (SUBBARAO et al., 2008).

A bioluminescent experiment using a recombinant strain of *Nitrosomonas europaea* was adopted to detect and quantify the presence of nitrification inhibitors released in plant roots (SUBBARAO et al., 2006a). The functional relationship between the emission of bioluminescence and the production of nitrite in the experiment proved to be linear. The inhibition of bioluminescence caused by 0.22 mM of AT (synthetic inhibitor - allylthiourea) was 80% (SUBBARAO et al., 2006a).

Using a concentration gradient of AT (standard curve, dose-response), the inhibitory effect of samples (for example, root exudates or plants extracts) can be expressed and compared. With these methodological tools, it is possible to determine and compare the BNI capacity of crops or pastures. The inhibitory effect of 0.22 mM AT in an experiment containing 18.9 mM of NH_4^+ is defined as an activity unit of AT (SUBBARAO et al., 2006a).

Studies of tropical forage, grain and legume species indicated that there is ample BNI capacity among plant species. Eighteen species were tested with respect to BNI production (ATU activity^{-1} by root dry weight), and the results varied from 0 (that is, no detectable activity) to 18.3 AT units (SUBBARAO et al., 2007c).

The highest BNI capacity was found in species of the genus *Brachiaria*. *B. humidicola* and *B. decumbens* pastures, which are highly adapted to environments with low levels of N (SUBBARAO et al., 2007d). However, there are variations between different cultivars of *B. humidicola* regarding BNI production. Furthermore, regardless of the cultivar, the production of inhibitors was identified only with the supply of N-NH_4^+ (SUBBARAO et al., 2007a).

BNI release is related to plant N status (SUBBARAO et al., 2006a). Particularly, the N form applied (NH_4^+ or NO_3^-) has great influence on the synthesis and release of BNIs in the roots of *Brachiaria humidicola* (SUBBARAO et al., 2007a, b).

Plants cultivated with NO_3^- as the source of nitrogen did not stimulate the release of BNIs in the roots (SUBBARAO et al., 2007a). Release of BNIs in the roots was observed in plants cultivated with N-NH_4^+ as N source (SUBBARAO et al., 2007a, b, 2009a, b). Moreover, even for plants cultivated with N-NH_4^+, the presence of NH_4^+ in the rhizosphere is essential for the synthesis and release of BNIs (SUBBARAO et al., 2007a, b). The number of BNIs released was three times higher when plants were cultivated with N-NH_4^+ instead of N-NO_3^- (SUBBARAO et al., 2006a).

The continuous presence of NH_4^+ and, perhaps, the side effect in the reduction of pH in the rhizosphere due to its absorption, are essential for sustaining the development and exudation of BNI compounds. Low pH protects BNIs from inactivation and keeps them functionally active after exudation. The combination of low pH and the presence of NH_4^+ in the rhizosphere has a synergistic effect on the exudation of BNIs in the roots (SUBBARAO et al., 2007a).

The regulatory role the NH_4^+ and pH play in the synthesis and release of BNIs suggests that this is an adaptive mechanism to protect NH_4^+ from nitrifiers in natural systems with N limitation (SUBBARAO et al. 2006b). Thus, stress due to lack of N could be the stimulus for the evolution of plants in producing nitrification inhibitors (MALAVOLTA, 1980). This theory is relevant, since NO_3^- absorption is favored in acidic medium (low pH), with a value of approximately 4.0. In contrast, NH_4^+ absorption is favored in alkaline medium (MARSCHNER, 1995).

A split root system was used to investigate the dependence of the BNI synthesis mechanism on the presence of NH_4^+ in the rhizosphere. Initially, the plants were cultivated only with $(NH_4)_2SO_4$ as the source of nitrogen. Thereafter, half of the root system was exposed to NH_4^+ and the other half to NO_3^-, in separate vases. Exudation of the BNI (brachialactone) was activated only in the part of the root system exposed to NH_4^+, and not in the whole root system (SUBBARAO et al., 2009b).

In leguminous plants, it is likely that the production of BNIs would have little or no adaptive value, since they present ability to fix nitrogen by symbiosis. This way, the maintenance of N in the ammoniacal form would not bring advantages to the legume species, in addition to favoring concurrent species that do not perform symbiosis (SUBBARAO et al., 2009a). Preliminary laboratory studies indicate that exudates of soybean roots stimulate nitrification (SUBBARAO et al., 2007d).

Through the use of cover crops in agricultural systems, it is possible to add amounts of crop residues that can affect the soil microbial biomass and, consequently, alter the mineralization process of nitrogen and other elements. However, differential effects on microbial biomass have been observed depending on the type of residue added to the soil (MARSCHNER, 1995).

There is an estimate that each year 30% of the root mass of pastures of *Brachiaria humidicola* remains in the soil, which is equivalent to one ton of dry matter of roots per hectare. This amount of roots can contain significant amounts of BNIs, and may be a major reason for the low nitrification rates observed in areas cultivated with this species (SUBBARAO et al., 2006a).

- Synthetic nitrification inhibitors

Synthetic nitrification inhibitors are compounds that delay the oxidation of NH_4^+ to NO_3^-. Nitrification inhibition occurs by blocking the enzyme AMO (SUBBARAO et al., 2007a, b) present in the bacteria *Nitrosomonas* (Figure 2).

AMO (critical enzyme involved in the oxidation of ammonia) presents a wide range of substrates for catalytic oxidation, and the inhibitory effects of many compounds occur because of the competition of the active site of this enzyme (SUBBARAO et al., 2006b). This fundamental weakness in the functioning of the enzyme AMO enables a wide range of molecules with diverse chemical structures to act as inhibitors

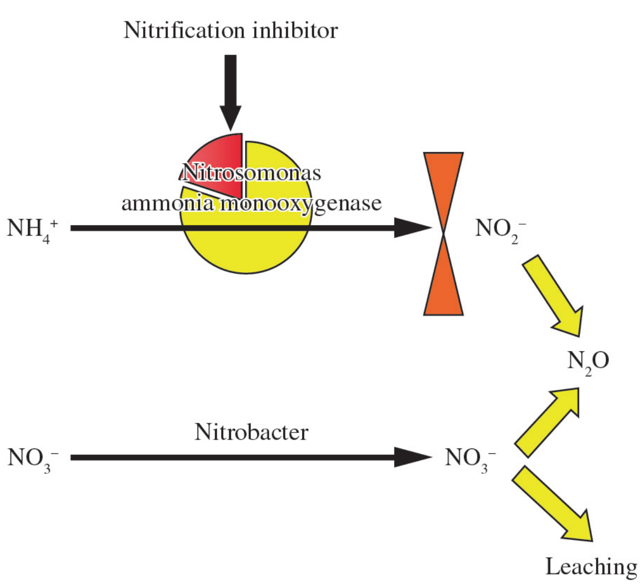

Figure 2. Action of the synthetic nitrification inhibitor (adapted from DI; CAMERON, 2002a).

of nitrification. This has been exploited for the development of chemical nitrification inhibitors (SUBBARAO et al., 2006b).

The maintenance of N in the form of NH_4^+ would bring advantages to improve the utilization of N in agricultural systems. However, in order to obtain these advantages, besides the use of cover plants capable of producing BNI, the use of synthetic nitrification inhibitors will often be required (SUBBARAO et al., 2006b), especially when nitrification is favored, because under these conditions the plants would not be stimulated to produce BNI.

There are numerous compounds registered as nitrification inhibitors (SUBBARAO et al., 2006b), but only a few have been extensively studied and tested under field conditions. The main compounds are nitrapyrin, DCD (dicyandiamide), and DMPP (3,4-dimethylpyrazole phosphate) (DI; CAMERON, 2002a; SUBBARAO et al., 2006b).

DCD nitrification inhibitor retards the first nitrification stage, deactivating the enzymes of the bacteria that convert ammoniacal N to nitric N, resulting in significant reduction in the leaching of NO_3^- (DI; CAMERON, 2002a). In addition to the benefits resulting from the reduction in the leaching of NO_3^-, the inhibitors can also be used as strategies for reducing emissions of N_2O. The effects of DCD and DMPP in N losses by N_2O emission and through leaching of NO_3^- depend on the dose applied, the time, and methods of nitrification inhibitors (DI; CAMERON, 2002b).

The advantage in the use of DCD lies on the lower financial cost compared with other inhibitors such as nitrapyrin and on its high solubility in water, allowing it to be applied in liquid form (SOARES; CANTARELLA; MENEGALE, 2012). It is also less volatile than nitrapyrin and it decomposes into NH_4^+ and CO_2 in the soil, being classified as a non-toxic substance (DI; CAMERON, 2002b). Marcelino (2009) reported reduction of 76% in the oxidation of ammonium to nitrate in urea treated with DCD 15 days after incubation of the fertilizer to the soil. Soares, Cantarella and Menegale (2012) observed that the use of DCD was more effective in reducing N losses than the urease inhibitor, and its effect last up to 23 days after application. The authors also found that, after assessment of nitrate losses, the treatment with application of DCD provided higher concentrations of ammonium and lower concentrations of nitrate compared to treatments without inhibitors and to those with urease inhibitor.

Another product developed as a nitrification inhibitor, highly specific and with low concentration applied per area, is 3,4-dimethylpyrazole phosphate (DMPP). This inhibitor can be incorporated into solid and liquid fertilizers; its main features are specific effect on *Nitrosomonas* bacteria, temporary bacteriostatic and non-bactericidal action, harmless to aquatic and terrestrial organisms, and biologically degraded in soil with no residual effect (BAÑULS et al., 2000).

According to Zerulla et al. (2001), DMPP added to fertilizers sulfonitrate ammonium, urea and ammonium sulfate resulted in yield gains in wheat, irrigated rice and vegetables, in addition to providing regular productivity, even when subjected to high amounts of rainfall and/or irrigation. Bañuls et al. (2000) reported efficiency of DMPP incorporated to fertilizer NPK (14-08-20), containing 75% of its N content in ammonium, in tomato crop in soil of clay loam texture, with increased yield of 4% compared with the control (without DMPP). Soratto and Souza (2006) also obtained higher plant height and insertion of the first corn cob when they performed fertilization with ammonium sulfonitrate + DMPP in comparison to the use of urea. Lana et al. (2008) obtained a significant increase in corn yield when they used urea in conjunction with DMPP.

Another synthetic inhibitor is nitrapyrin [2-chloro-6-(trichloromethyl) pyridine], which has a highly selective effect on *Nitrosomonas*; it is used as a nitrification inhibitor. NO^{-}_{3} concentration in the soil reduced significantly when ammonium sulfate and urea were associated with nitrapyrin. According to Cantarella (2007), this inhibitor is recommended for use in mixtures with ammoniacal nitrogen fertilizers, such as anhydrous ammonia, ammonium sulfate, furan, ammonium nitrate, and animal manures. The author adds that nitrapyrin caused nitrification reduction when added to urea or ammonium nitrate and applied on the soil surface under corn in NTS.

Under conditions of intense rainfall, significant amounts of nitrate can be lost in sandy soils. Inhibition of oxidation of ammonia to nitrite and then to nitrate (nitrification) preserves N in its ammoniacal form, which is less mobile in the soil and less susceptible to losses through leaching, which increases the efficiency of nitrogen use. Nitrification inhibitors restrain the oxidation of ammonia to nitrite by *nitrossonas* spp. and to nitrate by *nitrobacter* and *nitrosolobus* spp. (CHIEN; PROCHNOW; CANTARELLA., 2009).

Despite the positive results obtained with synthetic inhibitors, these products have not yet been widely adopted as a technological tool, and there are doubts about the cost-benefit relation. These doubts arise from lack of consistent results in different agricultural environments and types of soil (SUBBARAO et al., 2006b).

3 Final remarks

The concentration of ammonium in the soil can be high, contributing to the reduction of nitrate losses through leaching and increasing the efficiency of nitrogen use by crops, increasing the sustainability of agricultural systems. The use of cover crops, such as Brachiaria species, and synthetic nitrification inhibitors are among the alternatives. However, studies should be developed in order to clarify the conditions under which these cover crops can be introduced in agricultural systems for this purpose and also which factors interfere on the efficiency of synthetic nitrification inhibitors.

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