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Herbicides Effect on Nitrogen Cycling in Agroecosystems

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1. Introduction

With the widespread use of post-emergent herbicides and their increased use in areas under conservation management systems (no-till and minimum tillage), concerns about environmental and public health problems due to herbicidal molecules has been growing. The use of herbicides has been justified by the resultant reduction in production costs because expenses regarding application and product price are lower with these treatments compared with existing alternatives. However, studies in the literature have demonstrated that there can be an increasing need for greater doses of nitrogen (N) fertilizers and pesticides after herbicides have been applied (Cakmak, 2007; Damin et al., 2008, 2009).

The use of nitrogen fertilizers in doses that are enough to supply the demand of crops is one of the main practices associated with high productivity. However, these fertilizers are expensive, and their indiscriminate use can cause the emission of green-house gases (Sherlock et al., 1989), the contamination of superficial waters with nitrate and the destruction of ozone in the stratosphere, with N₂O as an intermediary (Groffman, 2000). In addition, fossil fuels, which are non-renewable resource, are consumed in the manufacture of N fertilizers.

Recent research has shown that about 15 to 20% of the N introduced by fertilization can be lost after plants have been desiccated by herbicides. This losses can be even greater when the N that was already in the system is considered. Moreover, some laboratory studies have shown an increase in the emission of N₂O, a gas that has a global heating potential 298 times greater than that of CO₂, in areas using desiccants.

Despite the importance of awareness about the effects of herbicides in the nitrogen cycle, this subject has been seldom discussed and studied in the literature. Within this context, this chapter will discuss the effects of herbicides in the nitrogen cycle processes that determine its availability to plants in agricultural systems.

2. Nitrogen in the agroecosystems

In an attempt to minimize the production costs and environmental impacts that occur because of the use of N fertilizers, the adoption of management strategies that minimize the

N losses in agroecosystems has been proposed. Such strategies include the efficient application of N at the moment the crop needs it most. N presents a great difficulty, however, because it is a highly reactive element that is subject to various changes in the soil and in plants (Moreira & Siqueira, 2006). Most of the changes of N in soil are intermediated by the microbial community, which fluctuates in both time and space. Therefore, besides properties such as pH, organic matter and CTC content, N dynamic is influenced by factors that affect the microbiota. These factors include aeration, water availability and the presence of toxic or stimulants substances to microorganisms.

The effects of herbicides on soil microbiota depend on the mechanism and other features of the product; type of metabolism of the microorganism (e.g., photolithotrophic, photoorganotrophic, chemolithotrophic, chemoorganotrophic), which determines if the metabolic routes that are affected by the herbicide are present in the microorganism; and also edaphic and climatic factors. Some herbicides, such as the paraquat, are toxic to biota in general. Others, such as glyphosate and glufosinate ammonium show low toxicity to non-target organisms. In fact, an increase in microbial biomass after the application of these products has been observed in some situations. This result has been attributed to the products serving as a source of nutrients. It is worth stressing, however, that some studies have shown that glyphosate is toxic to some organisms, such as certain strains of nitrogen-fixing bacteria (NFB).

Besides affecting processes of the N cycle that are intermediated by the microbiota, the herbicides can change the metabolism of N inside the plant, depending on the mechanism of the product. The herbicide glufosinate ammonium, for example, inhibits the incorporation of NH_4^+ in organic compounds. This effect can cause a build-up of NH_4^+ in vegetal tissue. Because its ions are toxic to plants, part of the accumulated NH_4^+ can be exuded through the roots and/or converted into NH_3 and lost in the atmosphere, reducing the N availability in the system. The exit of N from the plant can increase the C/N relation of the formed stubble, thus reducing its mineralization rate.

Some of the processes of the N cycle and the effect desiccant herbicides have over them are presented below.

2.1 Nitrogen losses

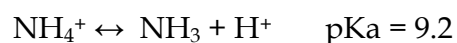
Among the main forms of nitrogen loss in agroecosystems are removal by crop, the volatilization of soil ammonium, the gas loss of nitrogen oxides (NO_2 , N_2O , NO) and elemental nitrogen (N_2), the lixiviation of soil nitrate leaving the root system's target range and NH_3 loss by the aerial parts of plants.

Sutton et al. (1993) reported that losses of nitrogen by the aerial parts of vegetables can increase ammonium levels in the atmosphere, noting emission values ranging from 5-15 $\text{kg ha}^{-1} \text{ year}^{-1}$ for N- NH_3 . This nitrogen output may be responsible for 15-20% of the total NH_3 gas emissions (Asman et al., 1998).

The magnitude of nitrogen losses through the aerial parts of crops varies according to each species. Wetselaar & Farquhar (1980) estimated from research available in the literature that the total N losses through the aerial parts of wheat, rice, grassy sorghum, cotton and rye crops were within a range of 16 to 40, 48, 48, 21 and 42 $\text{kg ha}^{-1} \text{ N}$, respectively. For barley crops, Schjoerring et al. (1993) found losses of 1-2 $\text{kg ha}^{-1} \text{ year}^{-1}$ of N- NH_3 under three fertilization regimes with N. For corn, Francis et al. (1993) found losses of 45 to 81 $\text{kg ha}^{-1} \text{ N}$, with application rates ranging from 50 to 300 $\text{kg ha}^{-1} \text{ N}$. NH_3 losses through the aerial parts of sugar cane were indirectly estimated by Trivelin (2000) at around 50 $\text{kg ha}^{-1} \text{ year}^{-1}$. This number represents half of the dose of N fertilizer applied to the crop sprouts.

Aside from its effects on various species, N losses can be linked to the developmental stages of plants, N availability in the soil, stomatal conductance, leaf temperature, NH_3 concentrations in the atmosphere, ammonium concentrations in the plant, the activity of the glutamine synthetase enzyme and environmental stresses (Schjoerring et al., 1998; Parton et al., 1988). Another important factor that affects not only the intensity but also the way in which ammonium gas is exchanged among leaves and the atmosphere is the ammonium compensation point, which was defined by Farquhar et al. (1980). When NH_3 concentrations in the atmosphere are below the ammonium compensation point of the plant, emission will occur through the leaves; for concentrations above, it is absorbed (Farquhar et al., 1980; Holtan-Hartwig & Bockman, 1994).

Senescence is the developmental stage that shows the highest NH_3 emission potential. During this stage, there is an increase in protein degradation, and the liberated N is transferred to the glutamate. Afterward, the glutamate N is converted to NH_4^+ by the glutamate dehydrogenase (GDH) enzyme. GDH is an enzyme of N metabolism that usually reaches a higher degree of activity during senescence (Ragster & Chrispeels, 1981; Laurière & Daussand, 1983). NH_4^+ is available for transformation in the composts of N transport, such as glutamine and asparagine (Ghosh et al., 1995; Nakasathien et al., 2000), and some of the ions are converted into NH_3 according to the following balanced reaction:



When the pH is 9.2, 50% of the molecules are dissociated (NH_4^+), and 50% are in the molecular form (NH_3). In the cytoplasm, the pH is usually around 7.2. At this pH value, only 1% of the molecules are in the NH_3 form. However, it is important to stress that the NH_4^+ build-up in the cytoplasm can promote H^+ exit to the apoplast, transiently increasing the interior pH of the cell and, consequently, the NH_3 concentration (Britto & Kronzucker, 2002). There is evidence that NH_3 transport is easier through membranes (Kleiner, 1981), and that it can be stomatally lost.

2.1.1 Effect of herbicides

Herbicides are used in agricultural areas to induce the senescence of plants. The duration of the senescence phase deriving from herbicide application is very short, and the intervention may occur at any developmental stage. Therefore, it is possible that the behavioral standards of N are different from those observed in natural senescence conditions. Besides inducing senescence, glyphosate and glufosinate ammonium herbicides also affect the plant's N metabolism.

The herbicidal action of glyphosate is attributed to its inhibition of the EPSPs (enolpyruvylshikimate phosphate synthase) enzyme that is responsible for one of the synthesis phases of the tryptophan amino acids phenylalanine and tyrosine. However, as a secondary effect, there is an inhibition in the synthesis of phenolic compounds deriving from those amino acids, which increases the activity of the PAL (phenylalanine ammonia lyase) enzyme due to a response effect (Duke & Hoagland, 1985). The PAL acts in the lyase of phenylalanine and tyrosine amino acids, resulting in the formation of phenolic acids and NH_4^+ (Hoagland et al., 1979).

The glufosinate ammonium herbicide inhibits the action of the glutamine synthetase, which is responsible for the glutamate synthesis from ammonium and glutamine. This metabolic route is considered the main form of ammonium assimilation in organic composts (Schjoerring et al., 1998). Consequently, the application of glufosinate ammonium results in endogenous NH_4^+ in the plant (Manderscheid et al., 2005).

Therefore, the increase of NH_4^+ in plants desiccated with glyphosate and glufosinate ammonium should not be connected to the induction senescence alone. Damini et al. (2010b) quantified N losses by *Brachiaria decumbens* after desiccation with glyphosate, glufosinate ammonium and paraquat, all of which promoted senescence. However, only the first two increased the losses of N in the system.

Manderscheid et al. (2005) assessed the ammonium concentration in five weed species after applying glufosinate ammonium. They observed an NH_4^+ increase in studied species. Damini et al. (2008; 2010a) observed losses of around 10 to 20% of applied N fertilizer after the application of glyphosate and glufosinate ammonium in *Brachiaria decumbens* and *Pennisetum glaucum* (Table 1). In those same studies, a reduction of around 40% of the total-N in the plant was observed after the application of herbicides.

From a practical point of view, the effect of herbicides on the total N of plants used for soil covering is important because it can affect the mineralization rate due to a raise in the C:N ratio, and it can effect the immobilization of N by soil microorganisms, a process that reduces the nutrient's availability to plants.

3. N mineralization/immobilization

When organic residue is added to the soil, extra-cellular enzymes are liberated by the biota, which degrade the residue into substances with smaller molecular weights (monomers). These monomers are absorbed and metabolized by microbial cells, which transform them into inorganic forms, a process called mineralization. Mineralization occurs at the same time as immobilization, which refers to the incorporation of mineral nutrients to the microbial cells to meet the nutritional demand of the soil microbiota.

| Treatments | Above ground part | | Roots | | Entire plant | | Soil | | Losses on soil-plant system | |
|-------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|-----------------------------|--------------------|
| | P. g. ¹ | B. d. ² | P. g. ¹ | B. d. ² | P. g. ¹ | B. d. ² | P. g. ¹ | B. d. ² | P. g. ¹ | B. d. ² |
| | g m ⁻² | | | | | | | | | |
| Control | 13.8 a | 11.5 | 3.4 a | 6.1 a | 17.2 a | 17.6 a | 2.4 b | 2.1 b | 5.4 b | 9.9 b |
| Glyphosate | 10.8 b | 8.1 | 2.7 b | 2.9 b | 13.6 b | 11.1 b | 3.4 a | 3.4 a | 8.0 a | 15.1 a |
| Glufosinate | 11.0 b | 8.1 | 2.0 b | 2.4 b | 12.9 b | 10.5 b | 4.0 a | 3.1 a | 8.0 a | 16.0 a |
| LDS (0,05) | 2.1 | 3.8 | 0.5 | 1.8 | 3.0 | 4.9 | 0.7 | 0.8 | 2.3 | 4.9 |
| P | ** | ns | * | ** | ** | ** | * | ** | * | * |
| C.V. (%) | 19.2 | 26.3 | 31.7 | 32.3 | 8.9 | 25.1 | 28.3 | 18.9 | 14.9 | 24.2 |

¹Measurement of ten replicates. ²Measurement of six replicates. *Means followed by different letters in columns differ from each other by Tukey's Test ($\alpha = 0.05$). **Means followed by different letters in columns differ from each other by Tukey's Test ($\alpha = 0.01$). The total dose of N was 25 g m⁻² in pearl millet and 15 g m⁻² in signal grass.

Table 1. Data of N recovered and lost from ¹⁵N-labeled fertilizer (Ndff) in the soil-plant system after herbicide application in *Brachiaria decumbens* (B.d.) and *Pennisetum glaucum* (P.g.). Retrieved from Damini et al. (2010a).

From the incorporation of vegetable residues in soil to their mineralization, several living organisms act in this process. In the initial phase, the vegetable residues are fragmented into smaller litter by the soil's macro-fauna. Subsequently, a diverse microbial community acts to liberate extra-cellular enzymes, which turn the substances that are easily decomposed into monomers that can then be absorbed by microbial cells. Substances that are difficult to degrade, such as cellulose and lignin, are degraded by a more specialized group of microorganisms, which compose the secondary and tertiary mineralizers.

Residues that are rich in lignin and polyphenols have slower mineralization rates in the soil due to the resistance these substances have to degradation (Moreira & Siqueira, 2006; Oliveira et al., 2002). The C/N ratio also has a major influence on the mineralization rate of vegetable residues, as it determines the N availability in the microbiota. Crop residues with a C/N ratio around 15:1 to 20:1 (e.g., soybeans, beans, lupine, forage turnips) tend to degrade faster than those with a C/N ratio higher than 30:1 (e.g., black oat, pearl millet, corn, sorghum). Heinzmann (1985) studied the N dynamics after winter green fertilization with black oat, forage turnip, hairy vetch, lupine and wheat and their effects on soy, corn and bean crops. He observed that the N liberation from legume and forage turnip residues occurred in the first weeks, whereas the black oat liberated more N in the blooming and graining stages.

3.1 Effect of herbicides

In addition to chemical composition, the mineralization of crop residues added to the soil depends on factors that affect the composition and activity of the decomposing microbial community. These factors include temperature, humidity, aeration and the presence of toxic or nurturing substances. Concerning the effect of herbicides on mineralization rates, some observations should be made: 1) Herbicides can increase vegetable residue decomposition rates due to a greater detachment of leaves and roots caused by senescence. Thus, they may have a beneficial physical effect on stubble mineralization (Snapp & Borner, 2005). 2) However, the increased C/N ratio in the stubble caused by the application of these products may decrease the mineralization rate. 3) Furthermore, these herbicidal molecules may alter the soil's microbial community and cause an increase or a decrease in mineralization, depending on the affected microorganism populations.

Argenta et al. (2001) observed an increase in the C:N ratio on the aerial parts of black oats 16 days after the application of glyphosate, glufosinate and paraquat. Although the paraquat did not decrease the N content of the plant during senescence, there was a remobilization of the nutrient to the reproductive structures and to the root system (Marschner, 1995). With the increase in the C/N ratio, the stubble mineralization rate can decrease. In fact, Damin et al. (2009; 2010c) assessed the N decomposition rate present in pearl millet and black oat stubble after the application of glyphosate and glufosinate ammonium. They observed an increase in the stubble dry matter remaining on the soil surface and a decrease in N liquid mineralization (Tables 2 and 3). In their studies, the absorption of N in stubble (enriched with ^{15}N) by corn plants was also assessed, and the herbicide-treated stubble was observed to be less effective in supplying N to plants.

The mineralization of organic substances is mainly mediated by chemoorganotrophic microorganisms, which should not be damaged by the application of glyphosate and glufosinate ammonium because these herbicides act on metabolic routes that are typical of lithotrophic organisms (chemolithotrophic or photolithotrophic). Therefore, the reduction in mineralization observed by Damin et al. (2009; 2010c) was attributed to the increase in the stubble C/N ratio.

In fact, Acinelli et al. (2002) observed that the application of the recommended doses of glyphosate and glufosinate ammonium did not interfere in the activity or in the soil microbial carbon biomass. The application of higher doses than recommended resulted in greater microbial activity, which can be connected to the death of lithotrophic micro-organisms, ensuring a competitive advantage to heterotrophs. Similar results were found for glyphosate by Haney et al. (2000), Ratcliffe et al. (2006) and Zabaloy & Gómez (2008).

Some studies have shown that the N mineralization that is already in the soil is increased by the application of glyphosate. Grossbard (1985) assessed the effect of glyphosate on soil N mineralization and observed an increase in mineralization when the herbicide was used. Haney et al. (2002) evaluated under laboratory conditions the effect of applying atrazine a mix of atrazine and glyphosate on the mineralization of total-C and total-N present in the soil. These authors observed a greater C and N mineralization in the soil treated with the mixture. The results showed that the glyphosate favored the chemoorganotrophic microbiota, causing an increase in the soil edaphic organic matter mineralization.

In tillage systems in Brazil's Cerrado, a reduction in deposited stubble mineralization is desirable because it increases the permanence time of residue over the soil. However, the lower effectiveness of the residue in supplying N to plants and microorganisms can reduce productivity when the N supply through fertilization is not appropriate because the addition of low N content organic residues to the soil results in the immobilization of the nutrient by the microbiota, reducing its availability to plants. Therefore, the use of herbicides for covering crop desiccation in tillage system should be considered in the decision making processes of N fertilization management.

| Treatments | DM | Carbon | Nitrogen | C:N | Δ DM | Δ C | Δ N |
|-------------|---------------|--------|------------|--------|-------------|------------|------------|
| | — g per pot — | | mg per pot | | — % — | | |
| Control | 6.5 b | 2.6 b | 117.2 b | 22.6 b | 49.9 a | 49.4 a | 69.6 a |
| Glyphosate | 8.5 a | 3.7 a | 159.9 a | 24.3 b | 34.1 b | 22.1 b | 51.8 b |
| Glufosinate | 9.3 a | 3.8 a | 160.2 a | 28.6 a | 28.3 b | 23.2 b | 49.7 b |
| F-test | ** | ** | ** | * | ** | ** | ** |
| CV (%) | 15.5 | 13.6 | 16.2 | 12.4 | 25.6 | 30.3 | 12.3 |

DM = Dry mass; C = carbon; N = nitrogen; C:N = Carbon to Nitrogen ratio; Δ = (Non-recovered fraction/ total amount added)*100. Means followed by the same letter in a given column indicates nonsignificant differences at the 5% level by Tukey test. **, * = Significant at 1% and 5% levels, respectively.

Table 2. Dry mass, carbon, nitrogen, and C:N ratio in above-ground parts of black oat treated with herbicides at 101-days-old grown in soil.

4. N exuding through the roots

The rhizosphere may be defined as the soil zone that is influenced by the roots. The deposition of exuded matter and root fragments in this region favors the growth and activity of the soil microbial community, which has important functions in cycling and in the supply of nutrients to plants, especially N. About 15% of the total CO₂ set by plants is deposited in the rhizosphere in a cereal crop cycle (Nguyen, 2003). Sugars, amino acids,

organic acids, phenols, exoenzymes and ions such as NH_4^+ are the main compounds exuded by the roots (Krafczyk et al., 1984; Marschner, 1995; Dakora & Phillips, 2002; Nguyen, 2003; Paterson, 2003).

Exudation may be an important way for N to exit the plant. Klein et al. (1988) estimated that the C:N ratio of exuded compounds for the *Poaceae* family species ranged from 2 to 2.7. However, other studies have demonstrated that the exuding of compounds containing N is very low, and the exuded compounds are rich in sugars (Deubel et al., 2000; Paterson, 2003; Merbach et al., 2003). The main factors affecting the amount and composition of material exuded from the roots are vegetable species, plant development stage, environmental stress, nutritional state, injuries, application of chemical products and activity and composition of the soil microbial community (Marschner, 1995; Holland et al., 1996).

| Treatments | Net N mineralization | Non-recovered | Soil - Plant |
|-------------|------------------------|---------------|--------------|
| | ----- mg per pot ----- | | |
| Control | 201.2 a | 52.9 | 418.0 a |
| Glyphosate | 150.0 b | 43.9 | 383.9 ab |
| Glufosinate | 148.5 b | 74.6 | 368.3 b |
| F-test | ** | NS | * |
| CV (%) | 8.8 | 54.6 | 8.9 |
| | ----- % ----- | | |
| Control | 42.3 a | 11.2 | 88.8 |
| Glyphosate | 35.3 b | 10.3 | 90.2 |
| Glufosinate | 33.5 b | 16.8 | 83.1 |
| F-test | ** | NS | NS |
| CV (%) | 8.7 | 55.4 | 8.8 |

Means followed by the same letter in a given column indicates nonsignificant differences at the 5% level by Tukey's test; **, * = Significant at 1% and 5% levels, respectively; NS = nonsignificant difference.

Table 3. Net N mineralization, non-recovered fraction and soil-plant recovery of the nitrogen arising from black oat residue with or without herbicide previous application.

4.1 The effect of herbicides in exudated N

Herbicides may affect some of the mentioned factors and, consequently, root exudation. Kremer et al. (2005) evaluated amino acid and carbohydrate exudation after glyphosate was applied to conventional crops and crops genetically modified for herbicide tolerance. They concluded that the exudation was stronger in both crops after the product was applied. Similar results were obtained in bean plants that were desiccated with glyphosate (Liu et al., 1997). These authors found aspartic acid, glutamic acid, serine, glycine, amino butyric acid, valine, isoleucine, leucine, tyrosine, phenylalanine and proline in the material exuded from the roots. They observed that the valine, isoleucine and glycine concentrations were higher in the plants desiccated with the product.

The herbicides that were used as a replacement for the glyphosate also increased root exudation. Damin et al. (2010b) evaluated the compound exudation containing N and the root detachment in *Brachiaria decumbens* plants after the application of glyphosate, glufosinate ammonium and paraquat. They observed an increase in exudation (Figure 1) and the detachment of roots after the application of three products. Paraquat, unlike

glyphosate and glufosinate ammonium, does not have its action connected to the N metabolism of the plant. As all herbicides increase N exudation, this process is probably not connected exclusively to the product's active mechanism. Physiologic events characteristic of senescence could justify the results obtained in this research. However, Kremer et al. (2005) observed that the exudation of sugars and amino acids was higher in soybeans genetically modified for tolerance to glyphosate than in the conventional soybeans. In this case, the increase in exudation was not connected to the development of senescence after the application of the herbicide.

It is worth stressing that the losses from exudation and root detachment together represented less than 4% of the N applied to the entire plant and, therefore, they should not affect in a relevant way the N content of the same plant (Damin et al., 2010b). The compound N exudation, notwithstanding, may modify the composition and activity of the soil microbial community due to the supply of carbon sources that are highly soluble and readily available.

There is a great diversity of microorganisms in the soil, which, according to the kind of nutrition, may be grouped in photolithotrophic, photoorganotrophic, chemolithotrophic and chemoorganotrophic. Phototrophic microorganisms (photolithotrophic and photoorganotrophic) are rare, and chemotrophic microorganisms are abundant in the soil. All kinds of fungi are chemoorganotrophic, i.e., they use organic molecules as a source of carbon and energy, whereas bacteria and actinomycetes may come from a variety of groups. Processes such as nitrification and N biological fixation are mainly mediated by chemolithotrophic organisms, which use CO₂ as a carbon source and obtain energy from inorganic compound oxidation (Moreira & Siqueira, 2006).

Due to the nutritional type, the supply of easily decomposable organic carbon sources caused by exudation offers a competitive advantage to chemoorganotrophic microorganisms, modifying the proportion of microorganism populations in the soil microbial community. In addition, chemoorganotrophs may be favored by the use of herbicides such as glyphosate and glufosinate, which act in metabolic routes that are present only in lithotrophic organisms (photolithotrophic and chemolithotrophic).

Among the chemoorganotrophic microorganisms that are important for agriculture are the decomposers of organic residues and most of the plants' pathogens. Kremer et al. (2005) observed an increase in fungi biomass of the kind *Fusarium* sp. after the application of glyphosate to soybeans. Liu et al. (1997) observed an increase in the number of colonies and a higher development of the *Pythium* sp. fungi after addition to the environment through the bean roots cultivated with glyphosate desiccated or exuded herbicide. As the glyphosate can also be exuded by the roots (Kremer et al., 2005; Tuffi Santos et al., 2008), it is possible that the population increase of pathogenic microorganisms after the application of the herbicide is connected to the N compound exudation as well as to the presence of the herbicide.

Aside from those processes, a higher colonization of roots by pathogenic microorganisms may be associated with the effects the herbicide has on the plant's defense system. Liu et al. (1997) also observed that bean plantlets growing in the middle of *Pythium* sp. showed a higher lignin content when glyphosate was not added to the environment. Studies in sterile environments have shown that the increase in *Pythium* and *Fusarium* sp. is connected to the effectiveness of the glyphosate herbicide (Johal & Rahe, 1984; Levesque & Rahe, 1992; Levesque et al., 1992; Levesque et al., 1993; Descalzo et al., 1996).

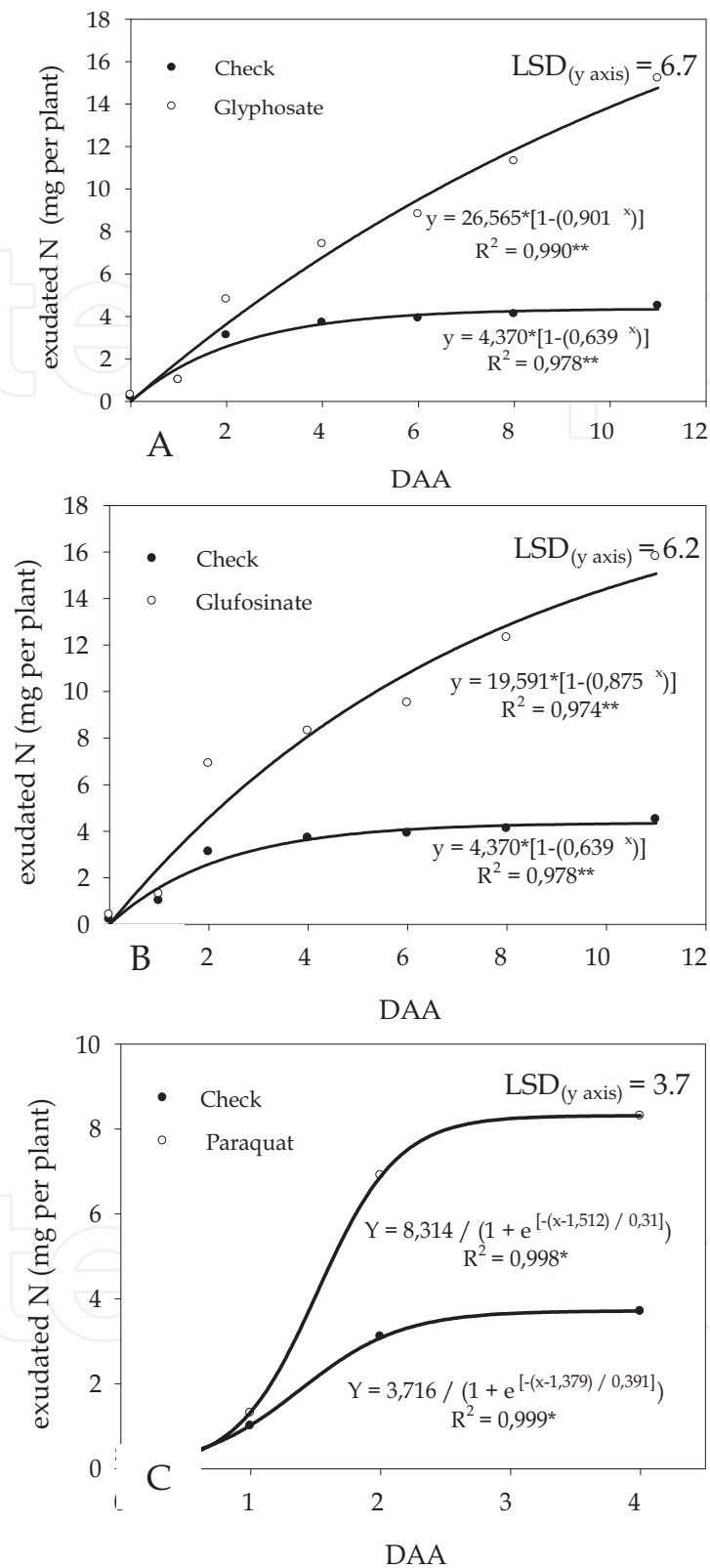


Fig. 1. Time accumulated exudation of nitrogen after herbicide application on *Brachiaria decumbens* Stapf. A - Glyphosate ($F_{\text{herb}}=2.8^*/F_{\text{DAA}}=13.5^{**}/F_{\text{Herb} \times \text{DAA}}=4.3^{**}$); B - Glufosinate-ammonium ($F_{\text{herb}}=4.9^*/F_{\text{DAA}}=15.4^{**}/F_{\text{Herb} \times \text{DAA}}=4.9^{**}$); C - Paraquat ($F_{\text{herb}}=6.7^*/F_{\text{DAA}}=15.2^{**}/F_{\text{Herb} \times \text{DAA}}=3.1^*$). DAA = days after herbicide application

It is likely that other chemoorganotrophic microorganisms, such as denitrificants, are favored by the use of glyphosate and glufosinate, as the highest exudation results in increased C and N availability in both the soil and these herbicidal molecules (unlike paraquat) do not affect the soil's chemoorganotrophic microbiota.

5. Nitrification/denitrification

In addition to reducing the agronomic effectiveness of N fertilization, N losses because of the denitrification-nitrification processes in soil may have a negative impact on the environment because considerable amounts of N_2O are generated in these processes. This gas is considered the fourth-greatest contributor to the greenhouse effect, although it is present in the atmosphere and in the stratosphere in small amounts. Moreover, N_2O is an intermediary in the ozone layer destruction process (Tabatabai et al., 1981; Griffith, 2005). It is estimated that approximately 20% of the global gas emissions of nitrogen oxides and elemental N are connected to agricultural activity. However, in countries such as Australia, agriculture may be responsible for 80% of these emissions (Australian Greenhouse Office, 2001).

The main processes that result in the gas loss of N oxides and elemental N are nitrification and denitrification. During nitrification (in the oxidation of NH_4 into NO_2), there may be slight production of nitrous oxide by a chemical dismutation of nitroxyl (NOH) or by the action of nitrite reductase (Schmidt, 1982; Bremner, 1997). In this case, the formation of N_2O may occur under aerobic conditions, during the nitrification. Although the N_2O production by nitrification might, in some situations, have an emission potential similar to that of the denitrification process (Granli & Bockman, 1994; Wang et al., 1997), denitrification seems to be the main N_2O source in the soil (Tiedje, 1994).

Biological denitrification is defined as an anaerobic breathing process mediated by microorganisms that are able to use NO_3 or NO_2 as final electron acceptors. The main products of denitrification are N_2 and N_2O , and the proportion of production of each gas varies according to the environmental conditions. There are more than 125 bacterial species that are capable of performing denitrification, including phototrophs, lithotrophs and organotrophs (the most important group).

The main environmental factors that interfere in denitrification rates are O_2 supply, water content, temperature, organic matter, the presence of organic substances and ammonium (NH_4^+) and nitrate (NO_3^-) concentration (Firestone & Davidson, 1989). A number of studies have demonstrated that an increase in carbon content results in higher N_2 and N_2O emissions. Furthermore, pH may interfere in N_2O and N_2 emissions, as it affects nitrification, when it is reduced below 3.5 (Focht & Verstraete, 1977).

5.1 The effect of herbicides in nitrification/denitrification

As discussed in section 4.1, chemoorganotrophic microorganisms may be favored by the application of some herbicides, and such is the case for glyphosate and glufosinate ammonium. Laboratory studies have demonstrated the increase of N_2O emission in soils treated with glyphosate (Bollag & Henninger, 1976; Yeomans & Bremner, 1985; Carlisle & Trevors, 1986), which might be attributable to the herbicide serving as a nutrient source to organisms and offering a competitive advantage to denitrificants through the killing of chemolithotrophic microorganisms. However, higher N_2O emissions were also observed for soils with plants desiccated by these molecules (Robertson et al., 1987; Tenuta & Beauchamp, 1996). This observation may be attributed to the indirect effects of the herbicide

in the rizosphere due to the killing of the plants, such as the increase in promptly available carbon content and a reduction in the environment's NO_3 absorption, leaving it more susceptible to microbial attack.

Most nitrifying microorganisms are chemolithotrophic and, therefore, they can show the metabolic routes present in the plants, including those that were affected by glyphosate or glufosinate ammonium. Damin et al. (2009) evaluated the NO_3^- content of two soils (haplustox and quartzsament) 90 days after the permanence of millet stubble desiccated with these herbicides. The N- NO_3^- content in the glyphosate and glufosinate treatments was reduced in the quartzsament, but not in the haplustox. The haplustox soil had a higher sorting capacity than the quartzsament, as it could be noticed in the contents of the clay, the CTC and the organic matter of soils (Table 4). In fact, the NH_4^+ availability to nitrifying microorganisms was lower in the haplustox than in the quartzsament due to the higher ion absorption to the colloidal fraction of the soil. Moreover, the glyphosate showed a great affinity toward the iron and aluminum oxides and the hydroxides (Prata et al., 2000) common in the oxisoils. The strong connection between the herbicide and these fractions reduced the bioavailability of the product to the microorganisms.

Some authors have observed a rise in NO_3^- content in the soil after the plants are killed. This result can be attributed to a higher availability of NH_4^+ in the soil as it stops being absorbed by the plant. Although the decrease in nitrification indicates a damaging effect to the microbiota, it is important to highlight that the conversion of NH_4^+ into NO_3^- in the soil may promote a higher risk of superficial water contamination by nitrate and a lower availability of the nutrient to future crops.

| Treatments | NH_4^+ | | NO_3^- | |
|---------------|-----------------|-----------|-----------------|-----------|
| | Quartzsament | Haplustox | Quartzsament | Haplustox |
| | mg per pot | | | |
| Witness+ | 229.7 | 297.7 | 117.2baA | 31.5 aB |
| Glyphosate | 197.7 | 331.6 | 59.3 bA | 34.2 aA |
| Glufosinate | 212.6 | 314.5 | 82.6 bA | 21.4 aB |
| Average | 213.3 b | 314.6 a | 86.4 | 29.1 |
| Herbicide F | 0.0 ns | | 3.8* | |
| Soil F | 24.3** | | 43.7** | |
| Interaction F | 0.9 ns | | 4.1* | |

+Witness (stubble with no application of herbicides); Means followed by the same letter in a given column indicates nonsignificant differences at the 5% level by Tukey's test. **, * = Significant at 1% and 5% levels, respectively

Table 4. NH_4^+ and NO_3^- content in two soils 90 days after the permanence on the soil of *Pennisetum glaucum* stubble desiccated with herbicides (taken from Damin et al., 2009)

6. Biological N fixation

Biological N_2 fixation (BNF) is an important entrance path for N in agroecosystems, and it is the main source of the nutrient in leguminous farming. In these plants, BNF is performed through the symbiosis between N fixing bacteria (NFB), which break the N_2 triple bond through the action of the nitrogenase enzyme, reducing it to NH_3 , while the plants supply photosynthates to the bacteria.

Only a small percentage of prokaryotes are able to accomplish BNF. Among these prokaryotes, however, there is high morphological, physiological and phylogenetic diversity. In addition, N fixing micro-organisms are found among the heterotrophs, the anoxygenic phototrophs, the archaeobacteria and the cyanobacteria. The main kinds of fixing bacteria, which are economically important, are *Rhizobium* and *Bradyrhizobium*, which are predominantly chemoorganotrophic metabolic proteobacteria, although there are some chemolithotrophic strains of *Bradyrhizobium japonicum*.

6.1 Effect of herbicides

Due to the diverse kinds of metabolism of N fixing bacteria, it is difficult to establish which conditions are favorable or unfavorable to NFB, as the effects of a determined factor will depend on the bacteria community present in the soil. This difficulty may be one of the reasons for the contradictory results in the literature concerning the effect of herbicides on nodulation.

The growth, survival and nitrogenase activity results vary according to the herbicide doses applied, the species/strains studied and the experimental conditions (Faizah et al., 1980; Mallik & Tesfai, 1983; Eberbach & Douglas, 1989; Martensson, 1992; Moorman et al., 1992; Mallik & Tesfai, 1985; Moorman, 1986). Recently, Santos et al. (2004) observed differentiated responses in the growth of strains of native Brazilian *Bradyrhizobium* spp. because of the application of different commercial formulations of glyphosate. This differentiation reveals the importance of considering the adjuvant components of those herbicides in impact evaluations. With soybeans that are tolerant to glyphosate (Roundup Ready, RR), King et al. (2001) reported that the herbicide increased the number and decreased the weight of nodes. Jaworski (1972) observed a reduction of BNF after the application of glyphosate.

Some authors have attributed the BNF reduction after glyphosate application to the inhibition of the EPSPs enzymes in microorganisms (Malkores, 2000). Moorman (1986) evaluated the effect of glyphosate on the development of *Bradyrhizobium* spp. in the environment with and without the addition of amino acids. It was observed that the herbicide reduced the *Bradyrhizobium* spp. population only when no amino acids were added. It is worth stressing, however, that only chemolithotrophic microorganisms, such as some strains of *Bradyrhizobium* spp., should show this metabolic route.

Some authors have attributed the BNF reduction after glyphosate application to the physiological changes caused to the plants. It is common for nutritional disorders to happen in transgenic soybeans after glyphosate application, and two of them are particularly important: manganese (Mn) and iron (Fe). The lack of manganese in the tissue may increase the ureide concentration in the aerial parts, signaling to the BNF to stop fixation (Gordon, 2007). Moreover, the application of the herbicide reduces the production of flavonoids, substances that stimulate the genetic expression of nodulation.

7. Final consideration

The effect of herbicides on a soil-plant system's N loss and on the mineralization rates of vegetable residues should be considered when predicting the N availability to plants, in nutrient-caused environmental impact studies, in production cost estimations and in decision making concerning the appropriate time to apply N doses in areas under a tillage system. This kind of information is important for creating managing strategies that increase

the efficiency of N fertilization and reduce the environmental impacts caused by the use of fertilizers and herbicides.

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Herbicides are much more than just weed killers. They may exhibit beneficial or adverse effects on other organisms. Given their toxicological, environmental but also agricultural relevance, herbicides are an interesting field of activity not only for scientists working in the field of agriculture. It seems that the investigation of herbicide-induced effects on weeds, crop plants, ecosystems, microorganisms, and higher organism requires a multidisciplinary approach. Some important aspects regarding the multisided impacts of herbicides on the living world are highlighted in this book. I am sure that the readers will find a lot of helpful information, even if they are only slightly interested in the topic.

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