

# Nitrification Inhibitors-Role in Mitigating Nitrous Oxide Emissions from Soil

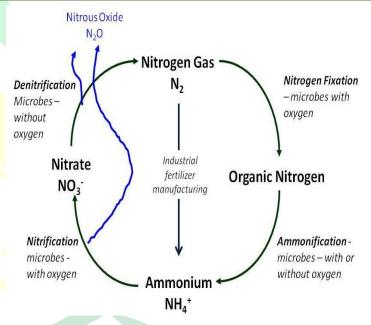
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# Introduction

The nitrogen cycle is a process which involves several key stages. The atmospheric nitrogen (N<sub>2</sub>) is transformed into ammonia (NH<sub>3</sub>) by specialized bacteria and archaea known as diazotrophs, utilizing the nitrogenase enzyme to break the N<sub>2</sub> triple bond. Within soil, NH<sub>3</sub> can convert into ammonium ions (NH<sub>4</sub><sup>+</sup>), which further undergo oxidation to form



nitrate ions (NO<sub>3</sub><sup>-</sup>) through a process called nitrification, occurring in three steps. Nitrification generates nitrite (NO<sub>2</sub><sup>-</sup>) and NO<sub>3</sub><sup>-</sup> ions, and these compounds can undergo reduction through denitrification, a sequential process involving four enzymes that ultimately convert NO<sub>3</sub><sup>-</sup> back to N<sub>2</sub>, with intermediate products such as NO<sub>2</sub><sup>-</sup>, NO, and N<sub>2</sub>O. Additionally, during the ammonification of NO<sub>3</sub><sup>-</sup> to NH<sub>4</sub><sup>+</sup> via NO<sub>2</sub><sup>-</sup>, there is a possibility of NO<sub>3</sub><sup>-</sup> reduction, resulting in the production of N<sub>2</sub>O (Thomson et al., 2012).

Nitrous oxide ( $N_2O$ ), now the primary ozone-depleting gas from human activities and a top concern among greenhouse gases (GHGs), demands attention in agricultural practices amidst climate change discussions. Agricultural activities contribute significantly to anthropogenic nitrous oxide (N2O) emissions, accounting for approximately 70%. N<sub>2</sub>O is a



potent greenhouse gas with a global warming potential nearly 300 times that of  $CO_2$  and is a major source of environmental nitrogen (N) pollution. Though its atmospheric presence is low compared to carbon dioxide (CO<sub>2</sub>), at about 0.32 ppm versus CO<sub>2</sub>'s 380 ppm, its potency—310 times stronger than CO<sub>2</sub>—and atmospheric longevity of 120 years (USEPA, 2012) are concerning.

In the U.S., soil management in agriculture contributes to 69 percent of N<sub>2</sub>O emissions, constituting 3 percent of total GHG emissions (USEPA, 2012). These emissions stem from natural soil processes influenced by various factors like moisture, temperature, microbial activity, aeration, and organic matter. Primarily, N<sub>2</sub>O results from denitrification, converting nitrate (NO<sub>3</sub><sup>-</sup>) to N<sub>2</sub> gas when oxygen is limited. Additionally, N<sub>2</sub>O can occur during nitrification, converting ammonium  $(NH_4^+)$  to  $NO_3^-$  under suitable aerobic conditions. Denitrification represents a significant pathway for N<sub>2</sub>O emissions and is the main pathway of N<sub>2</sub>O emissions. Denitrification occurs under anaerobic conditions and is carried out by heterotrophic bacteria that utilize carbon (C) as an energy source and thereby reduce NO<sub>3</sub><sup>-</sup> to nitrogen gas (N<sub>2</sub>). During denitrification, NO<sub>3</sub><sup>-</sup> acts as an electron acceptor and is reduced to nitrite (NO<sub>2</sub><sup>-</sup>) followed by nitric oxide (NO). Nitric oxide is toxic to bacterial cells and is quickly transformed into  $N_2O$ , which is eventually converted back to atmospheric  $N_2$ . An additional pathway for agricultural N<sub>2</sub>O emissions is nitrification. During nitrification, NH4<sup>+</sup> ions and NH3 are oxidized and converted to NO2<sup>-</sup> and NO3<sup>-</sup> by nitrifying bacteria. When NH<sub>4</sub><sup>+</sup> oxidizes NO<sub>2</sub><sup>-</sup>, N<sub>2</sub>O is produced as an intermediate product and is lost to the atmosphere. Other potential negative impacts of excess N include aquatic acidification, loss of biodiversity, and negative human health impacts such as methemoglobinemia in children (blue baby syndrome) following consumption of water with  $NO_3^-$  concentrations exceeding 10 mg L<sup>-1</sup>

Increased inorganic nitrogen concentrations from fertilizers or organic sources enhance  $N_2O$  emissions, albeit not as significantly as nitrogen loss via leaching or volatilization. Managing  $N_2O$  emissions is gaining importance due to environmental concerns, despite its limited impact on crop growth.  $N_2O$  produced by nitrification can be decreased when nitrification inhibitors are used. Autotrophic aerobic nitrification involving ammonia-oxidizing and nitrite-oxidizing bacteria, alongside anaerobic denitrification mediated by specific bacteria, are key microbial pathways in the soil's nitrogen cycle. Additional microorganisms play roles in these processes: anammox bacteria under anaerobic conditions convert  $NH_4^+$  and



 $NO_2^-$  into  $N_2$ , while certain fungi engage in denitrification and codenitrification, producing  $N_2$ and  $N_2O$ . Archaea also contribute, mediating nitrification in marine ecosystems and facilitating denitrification in soils (Hayatsu et al., 2008). A small proportion of  $N_2O$  arises from nonbiological pathways, such as the chemical breakdown of nitrite (chemidenitrification) and the oxidation of hydroxylamine (NH<sub>2</sub>OH) (Bremner, 1997). Chemidenitrification occurs in neutral and acidic soils, leading to the volatilization and fixation of  $NO_2^-$  within the soil organic matter (Bremner et al., 1980; Bremner, 1997). However, the  $N_2O$  produced through this process is nearly negligible (Bremner et al., 1980; Bremner, 1997). Hydroxylamine, an intermediate compound during NH<sub>4</sub><sup>+</sup> to  $NO_3^-$  oxidation, has the potential to generate considerably more  $N_2O$ compared to chemidenitrification (Bremner, 1997). In neutral and acidic soils, NH<sub>2</sub>OH oxidation results in  $N_2O$  as the primary product due to its reaction with Mn and Fe. Conversely, in calcareous soils with pH ranging from 7.8 to 8.2, NH<sub>2</sub>OH reacts with CaCO<sub>3</sub>, with the main product being  $N_2$  (Bremner et al., 1980).

# Soil as a Sink of N<sub>2</sub>O

In spite of the reports on production and emission of N<sub>2</sub>O, soils can sink N<sub>2</sub>O from the atmosphere. This phenomenon is still poorly understood, but it can be extremely important in agricultural systems. Factors influencing the consumption of N<sub>2</sub>O by soils are still unclear, but negative fluxes have been reported in a wide range of conditions frequently (but not always) associated to low availability of N and O<sub>2</sub> in soils, i.e., favorable conditions to reduce N<sub>2</sub>O to N<sub>2</sub>. The consumption of N<sub>2</sub>O also depends on soil properties, water content, soil temperature, pH and availability of labile organic C and N. Such variety of conditions suggests that a great number of processes should be related to this phenomenon. The longer the N<sub>2</sub>O remains in the soil, whether because it was produced in deeper layers or due to slow diffusion to the atmosphere, the greater amounts of N<sub>2</sub>O will be used as electron acceptor and greater will be the emissions of N<sub>2</sub> (Chapuis-Lardy et al. 2007).

# **Impact of Nitrification on Environment**

Nitrification has direct implications for water quality, as the end result, nitrates (NO<sup>-</sup>), is a highly mobile nitrogen form that can easily seep into groundwater, particularly during heavy rainfall. The leaching of nitrates into groundwater is contingent on factors like soil composition, bedrock conditions, and groundwater depth. Shallow groundwater combined with sandy soil increases the risk of nitrate leaching, while deeper groundwater with heavy clay soil



reduces or eliminates this risk. Once nitrates enter groundwater, infants and pregnant animals face heightened health concerns, as boiling water does not eliminate nitrates and can, in fact, exacerbate their concentration. Elevated nitrate levels can be toxic to newborns, leading to conditions such as "blue-baby syndrome," characterized by bluish pigmentation around the eyes and mouth. Aside from nitrate leaching, another significant environmental challenge associated with nitrification is the emission of potent greenhouse gases, namely N<sub>2</sub>O and NO. Approximately 50% of available nitrogen is lost through leaching or the production of gaseous N<sub>2</sub>O, a greenhouse gas with a warming intensity 300 times greater than CO<sub>2</sub>. Increased concentrations of NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup> in the soil contribute to NH<sub>3</sub> volatilization, leading to environmental pollution and posing threats to human health. Soil acidification is another concern linked to nitrification, as NH<sub>4</sub><sup>+</sup> fertilizer addition converts to leachable NO<sub>3</sub><sup>-</sup>, leaving behind H<sup>+</sup> and acidifying the soil. Soil acidity is detrimental to plant growth and associated microflora, leading to soil degradation.

The application of Nitrification Inhibitors (NIs) addresses these issues by reducing nitrate leaching, enhancing nitrogen uptake efficiency, and mitigating environmental pollution. Plants heavily rely on soil for nitrogen uptake, and the leaching and volatilization of nitrogen forms can reduce available nitrogen concentrations, negatively impacting plant productivity. NIs help counteract these effects and contribute to environmental conservation and climate change mitigation by targeting the essential nitrification process crucial for nitrogen assimilation by plants. The judicious use of NIs holds promise in alleviating environmental pollution and mitigating the effects of climate change.

# **Nitrification Inhibitors**

Nitrification inhibitors are chemicals that impede the nitrification process by targeting nitrifying bacteria responsible for producing specific enzymes: ammonia monooxygenase (AMO), hydroxylamine oxidoreductase (HAO), and nitric oxide reductase (NOR). Nitrification comprises two stages: firstly, ammonia (NH<sub>4</sub><sup>+</sup>) oxidation to hydroxylamine (NH<sub>2</sub>OH) through the AMO enzyme, followed by NH<sub>2</sub>OH conversion to nitrite (NO<sub>2</sub><sup>-</sup>) via HAO. In the subsequent phase, NO<sub>2</sub><sup>-</sup> transforms to nitrate (NO<sub>3</sub><sup>-</sup>) using the NOR enzyme. NO<sub>3</sub><sup>-</sup> generated during nitrification can lead to nitrogen losses through leaching or denitrification. Most nitrification inhibitors function by targeting and inhibiting the AMO enzyme during the initial stage of nitrification (Ruser *et al.*, 2015). Nitrification inhibitors can



deactivate ammonia monooxygenase, an enzyme catalyzing the first and rate-limiting step of nitrification through at least one of mechanistic actions of binding the active site of the enzyme (competitive inhibition), changing the conformation of the enzyme (non-competitive inhibition), scavenging co-factors (e.g., Cu) of the enzyme, and damaging the enzyme and other membrane-bound enzymes as a result of oxidation (suicide inhibition)

Nitrification inhibitors have gained interest as a possible mitigation strategy for lowering N<sub>2</sub>O emissions as well as reducing water pollution associated with use of fertilizers. Some examples of nitrification inhibitors include DCD and DMPP, thiourea, carbon sulfide (CS2), thioethers, ethylene: C2-H2, 2-ethynylpyridine, 3-amino-1,2,4-triazole, N-2,5-dichlorophenyl succinamic acid (DCS), 2-amino-4-chloro-6-methyl pyrimidine (AM), nitrapyrine (NP) and ammonium thiosulphate (ATS), and N-[3(5)-methyl-1H-pyrazol-1-yl) methyl] acetamide (MPA).

#### Mode of action:

Nitrification inhibitors (NIs) defer the initial and rate-limiting step of nitrification by impeding the activity of ammonia mono-oxygenase enzymes, causing the conversion of NH<sub>4</sub><sup>+</sup> to NH<sub>2</sub>OH. Consequently, the accumulation of NH<sub>4</sub><sup>+</sup> can be adsorbed onto sites of soil cation exchange, reducing the likelihood of leaching loss, as observed with NO<sub>3</sub>. Moreover, the inhibition of NH<sub>3</sub> oxidation from NIs may indirectly diminish denitrification losses by restraining the availability of the substrate for denitrification (NO<sub>3</sub><sup>-</sup>). Notably, nitrification inhibitors have demonstrated a substantial reduction in N<sub>2</sub>O and NO emissions in both chemical and organic fertilizers. The presence of NH<sub>4</sub><sup>+</sup> can be advantageous for plant utilization due to the NI effect, potentially enhancing the quality and productivity of nitrogen use in crops. However, the postponed nitrification allows the plant to optimize NO<sub>3</sub> production and utilization once nitrification occurs, thereby improving overall productivity.

DCD stands out as a widely used nitrification inhibitor, effectively curbing N<sub>2</sub>O emissions. It has demonstrated substantial reductions in gross nitrification rates—up to 57.9% in neutral silt loam and 62.4% in alkaline clay soils—resulting in significant drops of N<sub>2</sub>O emissions by 93.2% and 46.8%, respectively. Additionally, it lowered NO<sub>3</sub>– accumulation by roughly 20% across both soil types. In drier sandy-loam soils, N<sub>2</sub>O emission rates decreased by as much as 40%. Combining DCD with urea slashed N<sub>2</sub>O emissions by 58–78% in grassland and barley fields. However, its effectiveness varies between arable and grassland soils,



displaying superior inhibition in arable soils, with reductions of 81% in contrast to 58% in grassland, likely due to differing nitrogen concentrations. Despite its popularity, DCD faces challenges due to its high cost and the need for relatively large doses (15–30 kg DCD ha–1) considering its comparatively weaker inhibitory effect. Additionally, concerns about phytotoxicity have been raised, causing leaf tip scorching and compromising the visual appeal of leafy crops, affecting their marketability.

# Dicyandiamide (DCD)

DCD, an inorganic solid, undergoes biodegradation in soil, breaking down into urea,  $CO_2$ , and  $NH_4^+$ . Its physicochemical properties, including high water solubility and polar nature (reflected in its log Kow value of -1.0), render it susceptible to leaching from the intended root zones where it aims to inhibit nitrification. DCD inhibits the essential enzyme AMO in nitrifying bacteria by chelating copper in its active site, slowing the conversion of  $NH_3$  to hydroxylamine. This inhibition delays the formation of  $NO_2^-$  and  $NO_3^-$ , reducing  $N_2O$  emissions and preventing N loss through leaching and atmospheric release. DCD's attraction lies in its ability to mitigate air and water pollution by retaining  $NH_4^+$  in soil, reducing leaching risks. In laboratory cultures, DCD exhibits inhibition at 10 to 50 mg kg<sup>-1</sup> in soil, lasting 4–8 weeks based on environmental conditions. Toxicology tests conducted by ECHA classify DCD as virtually non-toxic, and it is registered under REACH regulations.

#### Nitrapyrin

Nitrapyrin, introduced in the USA in 1974, has been extensively used in North America as a tool to control ammonium conversion to nitrate during nitrification, primarily in corn but also in wheat and sorghum cultivation. This selective inhibitor acts as a bactericidal agent, targeting nitramonas bacteria metabolism, effectively inhibiting nitrification at concentrations of 1.0 mg kg<sup>-1</sup> in pure cultures and 10 to 40 mg kg<sup>-1</sup> in soil. While stable in cool soils, it degrades within 30 days in warmer conditions, making it preferable for winter and autumn nitrogen applications. Nitrapyrin is noted for effectively curbing  $NO_3^-$  leaching from waterlogged soils. However, it's categorized as an organic chlorine compound, raising environmental fate concerns. Nitrapyrin shows low acute toxicity but is considered a Category II eye irritant. Toxicology studies conducted by ECHA indicate no genotoxicity but highlight eye irritation potential.

3, 4-Dimethylpyrazole Phosphate (DMPP)



The nitrification inhibitor 3, 4-dimethylpyrazole phosphate (DMPP) is recognized as a copper-selective metal chelator, acting on the AMO enzyme crucial for nitrification. It's compatible with solid, liquid fertilizers, and slurry applications, offering stronger inhibition compared to DCD at a lower application rate of 0.5–1.5 kg ha–1. DMPP exhibits reduced mobility in soil due to sorption, providing advantages over DCD. Its nitrification inhibitory effects typically last 4–10 weeks, contingent on environmental factors. Extensive testing has been conducted to assess ecological and toxicological impacts, leading to its approved use. As per the Globally Harmonized System of Classification (GHS), DMPP is classified as causing severe eye damage (category 1) and harmful if swallowed.

# 3, 4-Dimethylpyrazole Succinic (DMPSA)

DMPSA stands as a relatively novel form of nitrification inhibitor, displaying enhanced stability in alkaline conditions compared to DMPP, attributed to the presence of a succinic group. Compatible with CAN (calcium ammonium nitrate) and diammonium phosphate (DAP), DMPSA is used as a nitrification inhibitor. Research by Pacholski *et al.*, highlighted the efficacy of DMPSA in combination with urea and CAN fertilizers, showcasing a substantial 60–90% reduction in N<sub>2</sub>O emissions compared to untreated standard fertilizer. Additionally, the study noted significantly lower levels of NO3– with DMPSA application, as the inhibitor facilitated sustained higher ammonium levels.

Nitrification inhibitor	Mode of action
2-chloro 6-(trichloromethyl) pyridine	Decrease nitrate formation and
[Nitropyrin]	N2O emissions
3-4 dimethylpyrazole phosphate	Stabilize fertilizers and soil
(DMPP)	ammonium concentration
Dicyandiamide	Inhibit Nitrosomonaseuropea
Glyphosate	Inhibit 5-enolpyruvylshkimitae
Gryphosate	3-phosphate synthase (EPSS)
Ethylene and acteylene	Inhibit ammonia nitrifiers

#### Synthetic nitrification inhibitors

#### Effect of NI's on denitrification and other gaseous emissions

Nitrification not only acts as a source of  $N_2O$  in soils but also supplies  $NO_3$  as a substrate for denitrification. By reducing the  $NO_3$  content in soils after the application of  $NH_4^+$ 



or urea fertilization, nitrification inhibitors (NIs) play a role in lowering the risk of  $N_2O$  production by denitrification results in an increased  $N_2/N_2O$  ratio as  $NO_3$  concentrations decrease. This indirect inhibitory effect on denitrification is considered a crucial mechanism for reducing  $N_2O$  emissions through the use of NIs. Studies have shown that the application of NIs can coincide with lower  $NO_3$  concentrations in the topsoil, contributing to reduced  $N_2O$  flux rates. However, contrasting results in some experiments suggest additional mechanisms influencing N2O production during denitrification. In addition to the indirect effect, some authors have reported direct effects of NIs on  $N_2O$  production. For instance, certain NIs, like 3-mercapto-1,2,4-triazole, have been found to significantly decrease  $NO_3$  losses and enrich  $NO_2$ , resulting in the lowest  $N_2/N_2O$  ratio among treatments.

While some studies demonstrate a reduction in  $N_2O$  emissions with NIs, others show no such parallelism, indicating complex interactions. The effectiveness of NIs varies, with different NIs showing different effects on denitrification and  $N_2O$  production. The impact of NIs on NH<sub>3</sub> emissions also needs consideration, as non-incorporation of N fertilizers into the soil may lead to an increase in NH<sub>3</sub> emission. Furthermore, besides  $N_2O$  and NO, the use of NIs can influence the emission of greenhouse gases such as  $CO_2$  and  $CH_4$ .

#### **Role of Nitrification Inhibitors**

The incorporation of nitrification inhibitors (NIs) with nitrogen fertilizer during the autumn typically results in enhanced crop yields and nitrogen uptakes compared to the application of fertilizer alone. Although spring application of fertilizer alone may yield higher crop productivity and nitrogen uptakes, it is often impractical. In certain experiments, the application of inhibitors during the autumn has shown no advantages over fertilizer application alone. Several reasons could elucidate their inefficacy in such experiments. Firstly, negligible leaching and denitrification losses occurred between the inhibitor application date and the onset of nitrogen assimilation by the crop, making nitrification inhibition unnecessary. Secondly, the soils often contained excessive amounts of nitrogen, either ammonified from soil organic matter or due to excessive nitrogen fertilizer application. Even with significant nitrogen losses through leaching and denitrification, there would still be sufficient nitrogen available for the crop, regardless of whether the inhibitor had reduced nitrate losses. Thirdly, in some trials, either ineffective inhibitors or insufficient amounts of effective inhibitors were applied. Many



inhibitors require soil incorporation after application to prevent the volatilization of the active ingredient.

In certain experiments, inhibitors such as nitrapyrin were not incorporated after application. Numerous inhibitors assessed in laboratories have proven ineffective in field conditions due to various factors. Additionally, many of these compounds are impractical for commercial use due to their cost, toxicity, and lack of specificity. Most studies report a benefit from inhibitor use if an effective inhibitor is correctly applied under conditions conducive to leaching and denitrification, and excessive nitrogen is not applied.

Several factors may reduce an inhibitor's efficacy. If an inhibitor is applied with ammoniumproducing fertilizers to the soil surface without subsequent incorporation, ammonia volatilization losses may increase. Inhibition of nitrification retains a pool of ammonium susceptible to volatilization. A high ammonium:nitrate ratio may be unsuitable for plants because ammonium is phytotoxic unless rapidly converted to amino acids after assimilation by a plant. Conversely, high nitrate levels can accumulate in plant tissues without detrimental effects. The preference of a crop for ammonium or nitrate depends on various factors, and this preference may change during the life of a plant. An indirect effect of ammonium assimilation, but not nitrate, may be a reduction in the uptake of cations, particularly Ca<sup>2+</sup> and Mg<sup>2+</sup>. Ruminants fed fodder grown on soil fertilized with ammonium and treated with a nitrification inhibitor may experience grass tetany and frothy bloat. Some inhibitors, at high concentrations, may be directly phytotoxic to certain crops.

In addition to reducing leaching/denitrification losses, inhibitor use may offer other benefits. Inhibitors can reduce nitrate levels in various horticultural crops and in grass. The predominant form of inorganic nitrogen in soil may affect the severity of some fungal and bacterial crop diseases. However, there is a large grey area where equivocal results indicate that inhibitor use is more of an insurance policy than a certain method of increasing crop recovery of fertilizer nitrogen. Experiments with injected urea on grass in the U.K. demonstrated that injection with an inhibitor between October and December usually resulted in increased nitrogen uptake by the sward compared to urea alone. The advantage of injection in autumn or winter was that high yields of grass could be obtained from the first cut in early spring, a time when forage is often in demand. Factors affecting the efficacy of inhibitors include soil type, organic matter content, and the rooting depth of the crop. Inhibitors are more



effective on light soils with little organic matter, and their necessity may vary based on the rooting depth of a particular crop. The timing and amount of nitrogen fertilizer applied are crucial, as inhibitor application may not be cost-effective if the inhibitor's price exceeds that of applying extra or remedial nitrogen to compensate for leaching/denitrification losses.

#### **Plant Extracts as Nitrification Inhibitors**

Primarily derived from vegetable sources, products in India have been tested and utilized to enhance the efficiency of nitrogen fertilizers. These materials, which contain modest amounts of nitrogen, were historically employed as fertilizers before their nitrifying capabilities were recognized. Notably, there is a particular emphasis on utilizing Neem, Karanjin, and Mahua as nitrification inhibitors. Neem (and Mahua) cakes were previously used as manure, but their efficiency was found to be inferior to organic fertilizers. This reduced effectiveness was attributed to the presence of bitter and odorous compounds, particularly nimbinan acrid alkaloids. To improve nitrification efficiency, Neem cake can be blended with inorganic fertilizers, predominantly urea. Alternatively, integrating extracts of oil cake with acetone, petroleum ether, or alcohol has shown potential in enhancing nitrification efficiency.

The optimal ammonium nitrification inhibition was observed after a 30-day incubation period with 12% Neem oil (containing 12% nitrogen). Among the fractions, the bitter compounds were identified as the primary contributors to the inhibitory effects. Urea treated with acetone extracts from Neem crush resulted in higher rice output and demonstrated greater effectiveness compared to 100 kg/hectare slow-release urea or untreated urea.

Nitrification inhibitor		Source	Mode of action
Cinnamic acid ar	nd	Root exudates of	Inhibit
flavanones		plants	Nitrosomonaseuropea
Methyl furylate and		Grass root	
Methyl	4-		Inhibit nitrification enzymes
hydroxycinnamate		Brachariahumidicola	
Caffeic acid an	nd	Needles and bark of	Inhibit nitrification and ymag
chlorogenic acid		pine Pinus ponderosa	Inhibit nitrification enzymes

#### Natural nitrification inhibitors



Flavanones- Sakuranetin and Gallocatechin	Root exudates	Inhibit ammonia monooxygenase (AMO) and hydroxylamine oxideoreductase (HAO) pathways
Karajin	Seeds of Karanja tree	Inhibit nitrification enzymes
Brachialactone	Brachariahumidicola	Inhibit Nitrosomonaseuropea
Sorgeolactone	Root exudates of sorghum	Inhibit H+-ATPase in root microsomal membrane and herbicidal property on photosystem II
1,9-Decandiol	Root exudates of rice	Inhibit AMO pathway
Linoleic acid and Linolenic acid	Shoots of Brachariahumidicola grass	Inhibit AMO and HAO pathways
Mint	Menthaspicata oil	Decrease urease activity and nitrifiers inhibition, increase nitrogen use efficiency
Neem	Tissues of Azadirachta indica	Increase nitrogen use efficiency

# Factors Influencing Inhibitor Activity

The effectiveness of nitrification inhibitors (NIs) in the field is determined by various physical, chemical, and biological factors. Compounds proposed as nitrification inhibitors must possess both persistence and bioactivity in the field for successful application. Nitrification inhibitors exhibit variability in water solubility and flexibility, influencing their stability, durability, and efficacy. Ideally, an inhibitor should have similar soil mobility to NH4<sup>+</sup>-N, but many nitrification inhibitors deviate from this criterion. Nitrapyrin has lower mobility than NH4<sup>+</sup>-N, while DCD exhibits significantly higher mobility, limiting its performance.

In general, soil organic matter strongly adsorbs organic inhibitors, reducing their mobility, bioactivity, and efficacy while prolonging their persistence through adsorption. Soil pH is a critical factor influencing nitrifiers and overall heterotrophic biological activity. Chemical instability of inhibitors at soil pH  $\leq$  4 renders them unsuitable for acidic soils. Nitrapyrin remains stable at pH ranges of 2.7 to 11.9 but degrades at pH levels exceeding 6.5,



especially in soils with higher organic matter content. Soil structure and organic matter content impact the efficacy and durability of nitrification inhibitors. Soil organic matter adsorption diminishes the mobility, volatility, bioactivity, and, consequently, the effectiveness of inhibitors. In light-weight soils with low organic matter (1%), nitrification inhibitors exhibit less biosorption, remaining bioreactive and efficient. Conversely, in highly textured soils with high organic matter levels, inhibitors like nitrapyrin are more effective.

Genetic variability within Nitrosomonas strains and the sensitivity of nitrification inhibitors could influence their efficacy. Different strains of Nitrosomonas europaea exhibit variations in nitrapyrin sensitivity, with certain strains oxidizing nitrapyrin to 6-chloropicolinic acid, rendering the inhibitor less effective. Soil temperature significantly impacts the continuity of many nitrification inhibitors. The nitrification rate increases linearly with temperature until reaching a limit of about 30°C. Nitrapyrin, DCD, AM, and DMPP are highly effective at temperatures  $\leq 5^{\circ}$ C, with their inhibitory effects lasting up to six months at low temperatures, making them suitable for winter and autumn applications. However, as temperatures rise above 10°C, the efficacy of most nitrification inhibitors linearly decreases. At temperatures around 25°C, the inhibitory effect typically lasts for only two to three weeks, reflecting the inverse relationship between soil temperature and inhibitor efficacy due to reduced effectiveness and increased organic activity of nitrifiers at higher temperatures.

# **Conclusion and Future Perspectives**

In summary, employing nitrification inhibitors emerges as a compelling approach to counteracting nitrous oxide emissions from soil, addressing a significant contributor to greenhouse gas levels. These inhibitors play a pivotal role by slowing down the conversion of ammonium to nitrate, effectively reducing the production of nitrous oxide, a potent greenhouse gas that significantly impacts climate change. The effectiveness of nitrification inhibitors has been substantiated through research and field studies, particularly in agricultural systems where nitrogen fertilizers are extensively used. By optimizing nitrogen utilization and minimizing losses through denitrification, these inhibitors not only contribute to the sustainability of the environment but also improve the economic viability of agricultural practices.

Nevertheless, it is crucial to recognize that the efficacy of nitrification inhibitors is subject to various factors such as soil type, climate conditions, and specific crop management



practices. A comprehensive understanding of these factors is essential for the successful application of nitrification inhibitors across diverse agricultural settings.

In addressing the challenges posed by climate change and the imperative to reduce greenhouse gas emissions, the integration of nitrification inhibitors into sustainable agricultural practices shows great potential. The ongoing commitment to research, innovation, and the widespread adoption of these inhibitors, combined with a holistic approach to soil and nutrient management, can make significant contributions to achieving environmental and agricultural objectives. Collaboration among scientists, policymakers, and farmers is imperative to promote the widespread adoption of nitrification inhibitors as part of a comprehensive strategy aimed at mitigating nitrous oxide emissions and fostering a more sustainable future.

#### References

- Bremner, J. M. (1997). Sources of nitrous oxide in soils. *Nutrient cycling in* Agroecosystems, 49, 7-16.
- Bremner, J. M., Blackmer, A. M., & Waring, S. A. (1980). Formation of nitrous oxide and dinitrogen by chemical decomposition of hydroxylamine in soils. *Soil Biology and Biochemistry*, 12(3), 263-269.
- Byrne, M. P., Tobin, J. T., Forrestal, P. J., Danaher, M., Nkwonta, C. G., Richards, K., ... & O'Callaghan, T. F. (2020). Urease and nitrification inhibitors—As mitigation tools for greenhouse gas emissions in sustainable dairy systems: A review. *Sustainability*, *12*(15), 6018.
- Chapuis-Lardy, L. Y. D. I. E., Wrage, N., Metay, A., CHOTTE, J. L., & Bernoux, M. (2007). Soils, a sink for N2O? A review. *Global Change Biology*, *13*(1), 1-17.
- Hayatsu, M., Tago, K., & Saito, M. (2008). Various players in the nitrogen cycle: diversity and functions of the microorganisms involved in nitrification and denitrification. *Soil Science and Plant Nutrition*, 54(1), 33-45.
- Kumawat, K. & Nagpal, Sharon & Singh, Ummed & Sharma, Shivani. (2021). Nitrification inhibitors: Precursor for higher nitrogen use efficiency and soil health. *Nutrient use efficiency through next generation fertilizers*, 12, 213-249
- Ruser, R., & Schulz, R. (2015). The effect of nitrification inhibitors on the nitrous oxide (N2O) release from agricultural soils—a review. *Journal of Plant Nutrition and Soil Science*, 178(2), 171-188.



- Ruser, R., & Schulz, R. (2015). The effect of nitrification inhibitors on the nitrous oxide (N2O) release from agricultural soils—a review. *Journal of Plant Nutrition and Soil Science*, 178(2), 171-188.
- Thomson, A. J., Giannopoulos, G., Pretty, J., Baggs, E. M., & Richardson, D. J. (2012). Biological sources and sinks of nitrous oxide and strategies to mitigate emissions. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 367(1593), 1157-1168.



