



Nitrogen biotransformation: its ecological control and risk assessment in soil

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ARTICLE INFO	ABSTRACT
Keywords: Pesticides Nitrification inhibitors Side effects NO effect level Risk assessment methods Article history Submitted: 2025-03-09 Revised: 2025-06-13 Accepted: 2025-08-08 Available online: 2025-10-20 Published regularly: December 2025 * Corresponding Author Email address: ferisman.tindaon@uhn.ac.id	The Haber-Bosch (H-B) process, which enables the industrial production of ammonia from nitrogen and hydrogen, fundamentally changed food production. This process is crucial for synthesizing nitrogen-based fertilizers, which are essential for boosting crop yields and supporting the world's growing population. Monoculture farming, particularly when combined with high nitrogen input, poses significant environmental risks. It leads to soil degradation, increased vulnerability to pests and diseases, and water pollution. Reliance on synthetic fertilizers to offset nutrient depletion further worsens these problems. The question explores whether current analytical methods adequately identify and evaluate the side effects of urease (UI), nitrification (NI), and denitrification (DI) inhibitors used in nitrogen management strategies for high-yield monoculture farming. While inhibitors are designed to improve nitrogen use efficiency and reduce losses, their effectiveness must be weighed against their unintended consequences, necessitating the development of more comprehensive and holistic analytical approaches that better balance productivity and environmental protection. This research examines the impact of various nitrogen fertilizer strategies, combined with pesticide use, on non-target organisms in ecosystems. It specifically examines the impacts of urea, nitrate manipulation, and stabilized nitrogen fertilizers, such as urease inhibitors (UI), nitrification inhibitors (NI), and dual inhibitors (DI), on ecological balance. The study also examines the broader environmental implications of these practices, including nitrogen loss and greenhouse gas emissions. It highlights how these agrochemicals can affect wild plants, pollinators, and other non-target species, potentially disrupting ecosystem functions.
How to Cite: Tindaon, F. & Benckiser, G. (2025). Nitrogen Biotransformation: its ecological control and risk assessment in soil. <i>Sains Tanah Journal of Soil Science and Agroclimatology</i>, 22(2), 282-299. https://doi.org/10.20961/stjssa.v22i2.100306	

1. INTRODUCTION

Agricultural practices can have a negative impact on soil health, leading to degradation and reduced productivity over time. These practices can disrupt the delicate balance of soil ecosystems, leading to issues such as erosion, loss of organic matter, compaction, and pollution. Disturbances from cultivation, improper pest control, and the use of synthetic nitrogen fertilizers, particularly those derived from the Haber-Bosch process, are major contributors. These practices can lead to erosion, compaction, nutrient imbalances, and pollution of water resources (Bardgett & van der Putten, 2014; Benckiser, 2017; Benckiser et al., 2016; Geisseler et al., 2017; Joergensen & Wichern, 2018). Strategies to mitigate these negative impacts involve optimizing nitrogen use efficiency, minimizing runoff, and exploring alternative fertilization and water management methods. Farmers and administrations must adopt strategies that minimize nitrogen

runoff and optimize nitrogen use efficiency, while simultaneously exploring alternative methods for crop fertilization and water management (Albornoz, 2016; Andr n et al., 2008; Anshori et al., 2018; Chen et al., 2018; Coskun et al., 2017; Pfromm, 2017; Singh, 2018). The observation that plants control the activity of ammonium-oxidizing bacteria (AOB) and ammonium-oxidizing archaea (AOA) inspired industries to curtail the activity of nitrifying and denitrifying AOB, AOA, and fungi by using urease inhibitors (UI), nitrification inhibitors (NI), stabilized N fertilizers (Chinnadurai et al., 2014; Leithold et al., 2015; Qiao et al., 2015; Subbarao et al., 2017; Yahya et al., 2017). Soil properties and the activity of urease, nitrification, and denitrification all play a role in nitrogen cycling, and these processes can be influenced by the presence of compounds that inhibit or promote them. These processes compete with

soil organisms and plants for nitrogen, with urease converting urea to ammonium, nitrification oxidizing ammonium to nitrate, and denitrification converting nitrate to gaseous forms of nitrogen (N_2O and N_2) (Benckiser et al., 2015; Chen et al., 2016; Herbold et al., 2017; Lehtovirta-Morley et al., 2013; Maeda et al., 2015; Marco, 2014; Stempfhuber et al., 2017; Stempfhuber et al., 2016; Subbarao et al., 2015). Nitrogen dioxide (NO_2) can negatively impact plant growth and yield, particularly at higher concentrations. While NO_2 can be a source of nitrogen for plants, excessive amounts can lead to phytotoxicity, meaning it can directly damage plant tissues and reduce overall productivity. While NO_2 rarely accumulates in significant amounts in soil naturally, excessive nitrogen fertilization can lead to its build-up and contribute to soil acidification, which can be detrimental to plant life. In the nitrogen cycle, nitrogen dioxide (NO_2) is indeed an intermediate compound and is quickly converted to nitrite (NO_2^-) and then nitrate (NO_3^-) by microorganisms. This conversion is part of the nitrification process, where ammonia (NH_3) is first converted to nitrite and then to nitrate (Ghaly & Ramakrishnan, 2015). In well-aerated soils, the application of urea, ammonium, and nitrate fertilizers can significantly alter soil carbon storage by influencing microbial communities and their metabolic processes. These nitrogen (N) sources affect microbial activity, potentially leading to changes in carbon decomposition rates, soil organic matter formation, and overall soil health. Nitrogen fertilizers, in both their ammonium and nitrate forms, can alter the rates of organic matter decomposition, nutrient cycling, and overall soil health. The specific impacts depend on factors like the type of fertilizer, application rate, soil pH, and the presence of other soil amendments. The specific effects depend on factors like soil type, water content, and the type of nitrogen fertilizer used. Urea, upon hydrolysis in soil, releases ammonium, which can impact soil pH and affect microbial activity. Nitrate, being more mobile, can leach from the topsoil, potentially affecting carbon storage in deeper layers. Nitrogen fertilization, particularly from fertilizers, can significantly alter the composition and activity of soil microbial communities, especially those involved in nitrogen cycling. Increased nitrogen availability, often from fertilizer application, promotes the abundance, growth, and metabolic activity of nitrifying microbes, specifically ammonia-oxidizing bacteria (AOB) and archaea (AOA). This increase in nitrogen boosts nitrification, the process by which ammonia is converted to nitrite and then to nitrate. AOB and AOA play key roles in this process, with their relative abundance and activity influenced by nitrogen levels (Ai et al., 2013; Ameloot et al., 2014; Gao et al., 2018; Ouyang et al., 2017; Papp et al., 2019; Wang et al., 2015; Xue et al., 2016). Ammonia-oxidizing bacteria (AOB) and archaea (AOA) utilize CO_2 , electrons derived from ammonia oxidation, protons, nitrogen, and other necessary elements to produce biomass. However, the sensitivity of this process to water stress is not a defining characteristic of AOB, as both AOB and AOA are affected by water availability, but AOA have been observed to be more sensitive to drought in some studies (Bello et al., 2019). At prevailing anaerobic conditions, nitrifying bacteria and archaea can switch to nitrate respiration (Benckiser et al.,

2016). Other agriculturists prefer precision farming and employ UI, NI, DI stabilized urea, NH_4 , NO_3 fertilizers to control electron (e) donor- NH_4 / NO_3 -acceptor ratio balancing in soils (Benckiser, 2017; Benckiser et al., 2016; Leithold et al., 2015; Wu et al., 2017; Yang et al., 2016). Urease, nitrification, and denitrification inhibitors (UI, NI, DI) need to reach the water-retention zones within the soil to effectively inhibit nitrogen transformations. Increased nitrogen availability, often from fertilizer application, promotes the abundance, growth, and metabolic activity of nitrifying microbes, specifically ammonia-oxidizing bacteria (AOB) and archaea (AOA). This increase in nitrogen enhances nitrification, the process by which ammonia is converted into nitrite and subsequently into nitrate. AOB and AOA play key roles in this process, with their relative abundance and activity influenced by nitrogen levels. If the inhibitors don't reach the soil pores where these processes primarily occur, they won't be able to exert their intended effect (Bore et al., 2017; Martin et al., 2012; Trapp et al., 2016). Organic and precision farmers, seeking both environmental friendliness and high productivity, can benefit significantly from agricultural decision support systems (AgriDSS). These systems can help optimize resource allocation, manage inputs, and make informed decisions for both organic and precision farming practices (Habibullah et al., 2018; Lindblom et al., 2017; Lundström & Lindblom, 2016; Vestergaard et al., 2017). The methods listed, including x-ray analysis, enzyme analytics, gas-liquid chromatography, light and fluorescent contrast microscopy, and confocal Raman imaging, are all powerful tools used in various scientific disciplines for detailed analysis and imaging of materials, including biological samples. Several techniques allow scientists to investigate the structure, composition, and behavior of molecules, cells, and tissues at different scales. These include microscopy (light and electron), spectroscopy, and various imaging techniques. (Domeignoz-Horta et al., 2016; Kniggendorf et al., 2016; Subbarao et al., 2017; Supriyadi et al., 2021). Pesticides, although effective in controlling target pests, can disrupt soil microbial communities, which are essential for nutrient cycling and overall ecosystem health. These disruptions can lead to shifts in microbial community composition and function, potentially impacting soil fertility and ecosystem resilience. These changes can have far-reaching consequences, including biochemical and tissue-level damage in non-target organisms, and potential links to various human diseases (Bardon et al., 2016; Benckiser et al., 2016; Ghosh et al., 2017; Kafarski & Talma, 2018; Kurniawati et al., 2023; Rodrigues et al., 2018; Subbarao et al., 2017). There is a strong interest in developing cost-effective, sensitive, and easy-to-use bioassays to detect side effects of pollutants across different trophic levels in ecosystems. This is driven by the need to assess potential impacts on farmers, scientists, administrators, and the general public, with a focus on practical, field-deployable methods (Benckiser, 2017; Grenni et al., 2018; Pronk et al., 2017). This review analyzes how do different types of nitrogen fertilizers interact with various soil types and microbial communities to affect overall soil health. The goal is to assess the benefits and risks of these inhibitors, including their effects on non-target organisms, exposure

pathways, and potential health effects, while also identifying knowledge gaps.

2. Urease, ammonia mono-oxygenase, and denitrification-inhibiting compounds

Agricultural production highly depends on water and soil factors, which must be utilized efficiently. Precision agriculture technology has emerged as a transformative force in modern agriculture, revolutionizing how farmers manage their crops and livestock. This innovative approach leverages advanced technologies, data analytics, and automation to optimize farming practices, increase crop yields, and minimize environmental impact. To achieve highly productive and sustainable organic farming, a precision approach to nitrogen fertilization is crucial. This involves using CropS, a pre-calculating program that adapts nitrogen application to the plant's specific nitrogen demand, aiming for an eco-friendly food and feed production system. Despite the availability of precision farming technologies like CropSAT, some farmers are not adopting site-specific, low-emission practices due to factors such as high initial costs, complex technology, and a lack of perceived benefits or knowledge, despite the potential for increased efficiency and reduced environmental impact. Instead, they were largely relying on traditional chemical inputs, such as herbicide-resistant crops, microbial inoculants, pesticides, and stabilized nitrogen fertilizers. This indicates a preference for established methods over the potential benefits of precision farming, such as reduced resource consumption and environmental impact. This preference for established chemical solutions over more sustainable, technology-driven approaches highlights a gap in the adoption of precision farming techniques. This observation highlights a gap between the available technology for more sustainable farming practices, and the choices farmers are making. While precision agriculture (PA) offers potential for optimized resource use and environmental benefits, farmers may opt for alternative strategies due to factors such as perceived yield gains from conventional methods, ease of implementation, and familiarity with established practices. The adoption of PA is influenced by a complex interplay of factors, including economic considerations, access to technology, and individual farmer characteristics. This is reflected in the adoption rates of precision agriculture technologies, which are often lower than expected despite the potential benefits (Table 1; Fig. 1) (Leithold et al., 2015; Lindblom et al., 2017; Lundström & Lindblom, 2016; Tindaon & Benckiser, 2019; Tindaon et al., 2012). Agriculturally applied urease and nitrification inhibitors, designed to enhance nitrogen use efficiency and reduce emissions, can indeed be found in non-target environments. While these inhibitors are primarily used to manage nitrogen in agricultural soils, they can be transported to other areas through various pathways, potentially impacting ecosystems and processes beyond their intended application. A significant portion of soil microbial diversity remains uncultured in labs, making it difficult to fully grasp soil health and its impact. While precision agriculture (PA) offers significant benefits, such as optimized resource use and environmental protection, some farmers may opt for

conventional methods due to perceptions of higher yields, ease of use, and familiarity with established practices. To improve their understanding of microbial communities, researchers are developing methods to assess both microbial diversity and function at the species level. It refers to the combined approach of improving the ability to grow (cultivate) microorganisms in the lab, alongside the use of metagenomics to study microbial communities without needing to culture them. Metagenomics uses molecular techniques to analyze the genetic material directly from environmental samples, providing a more complete picture of microbial diversity and function than traditional culture-based methods alone (Bardon et al., 2016; Du & Liu, 2012; Hirayama et al., 2015; Jung et al., 2014; Marmann et al., 2014; Nai & Meyer, 2018; Prakash et al., 2013; Schütte et al., 2017). An urease inhibitor, such as N-(n-butyl) thiophosphoric triamide (NBPT; Fig. 1), can be used to reduce nitrogen loss from urea-based fertilizers. Specifically, it inhibits the conversion of urea to ammonia (NH_3), carbon dioxide (CO_2), and water, which is catalyzed by the urease enzyme. The addition of NBPT to urea fertilizer, potentially in combination with nitrification inhibitors like DCD, can further enhance nitrogen retention in the soil by reducing ammonia volatilization and other nitrogen losses. A marketed product is Agrotain (NBPT, 6.5%, DCD, 81.2%) or the urease and nitrification inhibiting ammonium thiosulfate $(\text{NH}_4)_2\text{S}_2\text{O}_3$, field applied at rates of 1 or 10 $\mu\text{g g}^{-1}$ soil (Table 2), meanwhile detected in non-target aquatic environments (Margon et al., 2015; Scheurer et al., 2016).

Nitrification, a crucial part of the nitrogen cycle, is a two-step microbial process in which ammonium (NH_4^+) is converted to nitrite (NO_2^-) and subsequently to nitrate (NO_3^-). This process is essential for converting nitrogen into forms that plants can use. It's carried out by specialized bacteria and archaea under aerobic conditions. This process is carried out by specific microorganisms: ammonia-oxidizing bacteria (AOB) and archaea (AOA). AOB are more common in alkaline soils ($\text{pH} \geq 8$), while AOA tend to dominate in acidic conditions. While bacteria are well-known for their role in nitrification (the conversion of ammonia to nitrite and then to nitrate), other microorganisms like archaea and even some fungi can also participate in these processes under anaerobic conditions with available carbon (Robertson & Groffman, 2007). The enzyme ammonium monooxygenase (AMO) plays a crucial role in the nitrification process, specifically in the initial step of converting ammonia (NH_3) to nitrite (NO_2^-). This initial oxidation is crucial for the overall conversion of ammonia to nitrate (NO_3^-). However, AMO activity can be inhibited in various environments, including monoculture and fertilized soils, the guts of ruminants, and aquatic systems, potentially due to factors like nitrite toxicity or the presence of inhibitors like phenylacetylene, listed in Table 1. Among the in Table 1 listed NI mostly applied are nitrapyrin, DCD, and DMPP (chemical structures Fig. 1), which are similarly effective in inhibiting the AOB nitroso group at recommended application rates but less the AOA soil fraction, which in abundance and activity seem to be more affected by the wet-up of dry soils (Barrena et al., 2017; Fisk et al., 2015). Then the spread NI, nitrapyrin, DCD, DMPP (Fig. 1).

Table 1. Agriculturally applied urease and nitrification inhibitors, partly found in non-target Environments

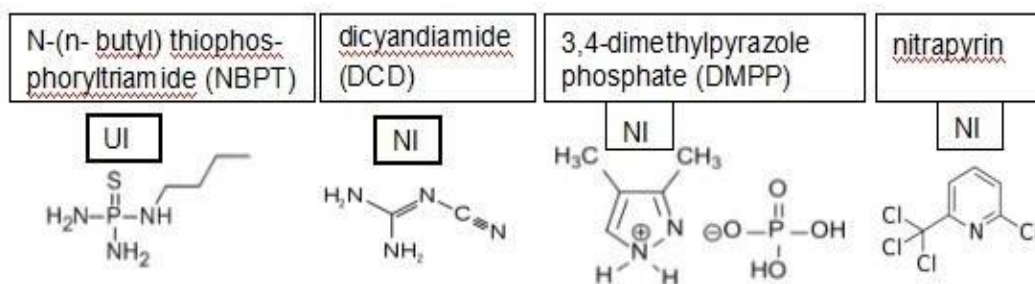
Urease inhibitors	References
phosphoroamides (N-phenylphosphorictriamide)	Li et al. (2023)
Ar-(i-butyl)thiophosphorictriamide (TPTA)	Byrne et al. (2020)
N-(n-butyl)thiophosphoryltriamide (NBPT)	Cruchaga et al. (2011); Zanin et al. (2016); Mazzei et al. (2017)
N-(2-Nitrophenyl)phosphorictriamide(2-NPT)	Adhikari et al. (2018); Adhikari et al. (2021)
Phenylphosphorodiamidate	Nugrahaeningtyas et al. (2022)
N-(diaminophosphinyl)benzeneacetamide	Chakrabarti et al. (2024)
W-(diaminophosphinyl)benzamide ; 4-fluoro-N-(diaminophosphinyl)benzamide ; 2,5-dichloro-1,4-benzoquinone ; 2,5-dimethyl-1,4-benzoquinone ; 2,6-dichloro-1,4-benzoquinone ; sodium-4-chloromercuribenzoate	Yeomans (1986)
chiral 3-substituted-4-amino-5-thioxo-1H,4H-1,2,4-triazoles	Kolovou et al. (2023)
Catechol	Dimkpa (2014),
Hydroquinone, 1,4-benzoquinone	Valenzuela-Hormazabal et al. (2024)
Nitrification inhibitors	
2-amino-4-chloro-6-methylpyrimidine	Yeomans (1986)
nitrapyrin (N-Serve, 24, 24E), potassium azide	Adhikari et al. (2021); Adhikari et al. (2018)
Wood- pronitridine	Ward et al. (2018); Habibullah et al. (2018)
2-mercaptobenzothiazole, sulfathiazole	Arora and Srivastava (2013)
4-amino-1,2,4-triazole, 3-mercapto-1,2,4-triazole ;2,4-diamine-6-trichloromethyl-s-triazine , potassiumamylxanthate, potassiummethylxanthate sodiummethylxanthate, sodiumisopropylxanthate thiourea, 2-chloroacetamide, 2-fluoroacetamide ; 2-4-nitrobenzotrithloride, 4-mesylbenzotrithloride, caffeic acid, chlorogenic acid, p-coumaric acid, ferulic acid, gallic acid, sodium thiocarbonate, sodium diethyldithiocarbamate, phenylmercuracetate	Kumar et al. (2015)
3-methoxy furano-2', 3', 7, 8-flavone (C ₁₈ H ₁₂ O ₄)	
dicyandiamide (DCD)	Di and Cameron (2011)
3-methylpyr3,4-dimethylpyrazole phosphate (DMPP) azole-carboxamide	Shi et al. (2017); Yang et al. (2016)
(NH ₄) ₂ S ₂ O ₃	Abbasi et al. (2011); Margon et al. (2015)
DMPSA, 2-(2,3-dimethyl-1 H-pyrazole-1) 2-(3,4-dimethyl-1H-pyrazole-1)	Huérano et al. (2016);
succinic acid mixture	Rodrigues et al. (2018)
Ensin, DCD, 1,2,4 triazole mixture	Šima et al. (2013)
Piadin, 1H-1,2,4 triazole-3-methylpyrazole mixture	Wu et al. (2017)

The enzyme AMO acts not very specifically by oxidizing not only NH₃ but also methane (CH₄), ethylene (C₂H₄), propylene (C₃H₆), phenol (C₈H₆O), or cyclohexane. Nitrification inhibitors, such as DCD, DMPP, and nitrapyrin, are beneficial for farmers because they improve nitrogen use efficiency by slowing down the conversion of ammonium to nitrite and nitrate in the soil. This results in reduced nitrogen loss through leaching and denitrification, ultimately leading to improved plant growth and yield. These compounds help retain nitrogen in the ammonium form, a form plants can readily utilize, while minimizing the formation of harmful nitrogen oxides (N₂O) (Benckiser et al., 2015; Fisk et al., 2015).

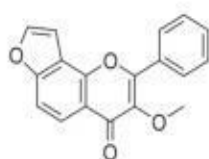
3. Evaluation of nitrogen use efficiency improvement and UI, NI, DI risk assessment

3.1. Nitrogen use efficiency

Nitrogen use efficiency (NUE) is the fraction of applied N that is absorbed and used by the crop. Under ideal conditions, all applied fertilizer N would go to the crop or be stored in the soil for later crops, but this is unrealistic in field settings and particularly when N is applied as inorganic forms because the loss pathways described above (NO₃⁻ leaching, N₂O emissions, and NH₃ volatilization) remove N from the system. Nitrification, performed by microorganisms, converts ammonium (NH₄⁺) to nitrite (NO₂⁻) and then to nitrate (NO₃⁻).

A Industrially designed and marketed urease (UI) and nitrification inhibitors (NI)**B Plant seed extracted compound with nitrification inhibitory properties**

Karanja, a 3-methoxyfurano-2', 3', 7, 8-flavone

**C Inhibitor of *Pseudomonas brassicacearum* NFM 421 denitrification**

Procyanidin, a proanthocyanidin flavonoid

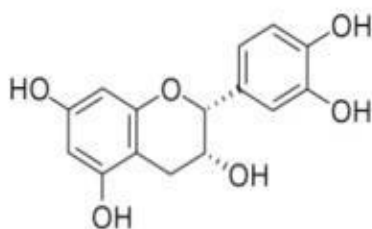


Figure 1. Chemical structures of the urease inhibitor N-(n-butyl) thiophosphoryl triamide (NBPT; Mazzei et al. (2017)) (A 1), the nitrification inhibitors dicyandiamide DCD, DMPP, and nitrapyrin (Yang et al. (2016); Habibullah et al. (2018); A 3-4), the Karanja plant seeds nitrification inhibitor Karanja, a 3-methoxy furano-2', 3', 7, 8-flavone (Majumdar, 2008); B), and procyanidin, a denitrification inhibitor (Bardon et al. (2016); C)

Subsequently, denitrification returns nitrite and nitrate to the atmosphere, often as nitrogen gas (N_2). In soil, ammonium (NH_4^+), which originates from biological nitrogen fixation (BNF) and total nitrogen fixation (TNF), undergoes transformations mediated by microbial nitrate reductases. These enzymes, including Nap, Nar, and Nas, convert ammonium into nitrite (NO_2) and nitrate (NO_3). Nitrifying bacteria and archaea convert ammonia to nitrite and then to nitrate. Denitrifying bacteria, fungi, and archaea then utilize these compounds, converting them back into gaseous nitrogen (N_2) and releasing them into the atmosphere (Benckiser et al., 2016; IPCC, 2014; Maeda et al., 2015; Nacry et al., 2013; Regan et al., 2017; Robertson & Groffman, 2007; USEPA, 2016). While Nap, Nar, and Nas nitrate reductases share the ability to reduce nitrate to nitrite, they differ significantly in their cellular location, operon organization,

and active site structures. Nap (periplasmic) and Nar (respiratory) have distinct catalytic subunit structures and electron transfer pathways, while Nas (assimilatory) is often found in the cytoplasm. Nap (periplasmic nitrate reductase) is found in the periplasm, Nar (respiratory nitrate reductase) is membrane-bound, and Nas (assimilatory nitrate reductase) is located in the cytoplasm. These differences reflect their distinct roles in nitrogen metabolism: Nap and Nar are involved in dissimilatory nitrate reduction (denitrification). While Nas is used for assimilatory nitrate reduction (incorporating nitrogen into biomass) (Sparacino-Watkins et al., 2014). Nitrifying and denitrifying bacteria, archaea, and fungi play a crucial role in the global nitrogen cycle, influencing plant nutrition, climate, and human health. Proteobacteria, particularly those expressing NapABC and NarGHI, are key players in aerobic respiratory electron

transport and redox-balancing within this cycle (Martikainen, 2022). In *Paracoccus pantotrophus*, nitrate respiration involves two distinct systems: NapABC and NarGHI. NapABC dissipates reducing equivalents derived from ubiquinol, while NarGHI harnesses them to create a proton gradient. Interestingly, a *Fallopia* spp. An extract containing procyanidin, when applied to *Pseudomonas brassicacearum* NFM 421, leads to the overexpression of the narG gene, specifically inhibiting the NO reductase (Bardon et al., 2016). Procyanidins, a type of plant-derived flavonoid, can disrupt the cell membrane of *Pseudomonas brassicacearum* NFM 421, affecting the structure of membrane-bound enzymes like nitrate reductase. This interaction can lead to membrane disturbances and potentially inhibit nitrate reduction. This interaction can inhibit denitrification, a process where nitrate is converted to gaseous nitrogen. Similar to procyanidins and/or NI, the herbicides acetochlor, bensulfuron-methyl, or fenoxaprop-p-ethyl + tribenuron-methyl applied to rice can interact with nitrifying and denitrifying bacteria, archaea, and fungi. It can be hypothesized from the up to 30% observed N₂O emission decrease of in the presence of such compounds and other NUE, pesticide risk assessment of UI and NI studies (Abalos et al., 2014; Bahram et al., 2018; Florio et al., 2014; Jiang et al., 2015; Tindaon et al., 2011; Tindaon et al., 2012; Yang et al., 2016). To improve Nitrogen Use Efficiency (NUE), applied Urease Inhibitors (UI), Nitrification Inhibitors (NI), and Denitrification Inhibitors (DI) should effectively target specific soil microbes (nitrifying, denitrifying bacteria, archaea, and fungi) within water-retaining soil pores. These inhibitors should not be overly persistent, adsorbing strongly to soil particles or degrading rapidly, and they should not negatively impact non-target organisms. Essentially, these inhibitors should act specifically and efficiently to improve NUE without becoming a detriment to other soil processes, similar to how long-term pesticide applications can have unintended consequences (Lam et al., 2018; Singh et al., 2013). In a study using soil columns filled with New Zealand grassland soil, decomposing organic matter released ammonium (NH₄⁺), which was rapidly transformed into nitrite (NO₂⁻) and nitrous oxide (N₂O) in the absence of DMPP. When DMPP, a nitrification inhibitor, was applied to the top of the soil columns, it suppressed the transcription of ammonia-oxidizing bacteria (AOB) but did not inhibit the amoA genes of ammonia-oxidizing archaea (AOA). This suggests DMPP's inhibitory effect on nitrification is selective, targeting AOB more strongly than AOA. (Duan et al., 2017). Thus despite DMPP NH₄⁺ is transformed and very likely also in poorly drained New Zealand dairy farm grassland with restricted grazing and to the surface applied DCD that accumulated to 9% in the top centimeters and to <10% moved below the soil depth of 10 cm by causing a measurable NO₃⁻ leaching reduction, which majorly must be attributed to a DCD manipulated nitrifying, denitrifying activity in the upper soil centimeters (Kim et al., 2012; Romera et al., 2017). In a meta-analysis across maize farm field sites, DCD inhibited equally efficiently as DMPP the transformation of N, but the monetary revenues differed (Yang et al., 2016). In maize farming, Dicyandiamide (DCD) treated fields, when fertilized with stabilized nitrogen granules, generated significantly

higher revenue compared to fields treated with DMPP. Specifically, DCD treated fields yielded an extra \$109.49 per hectare per year, while DMPP treated fields resulted in only \$15.67 in additional revenue. This difference is primarily attributed to DCD's more substantial impact on crop yield and its ability to increase revenue in maize farms. However, a southeastern Australian study on Nitrogen Urease Efficiency, involving various fertilization strategies with and without nitrification and urease inhibitors (DMPP, NBPT), concluded that these inhibitors have limited scope in reducing N₂O emissions. Furthermore, the effectiveness of nitrification inhibitors like nitrapyrin, DCD, and DMPP is highly dependent on soil type, temperature, organic matter, and water availability (Barrena et al., 2017; Doran et al., 2018; Marsden et al., 2015; Wallace et al., 2018; Woodward et al., 2016; Yang et al., 2016). In a study of nitrous oxide (N₂O) emissions from agricultural practices, Wallace et al. (2018) found that in-season rainfall and fertilization at sowing, rather than nitrification inhibitors (NIs), were the primary drivers of low N₂O emissions in the studied variants (UI and NI). This finding, along with a European Commission (EC) cost/benefit analysis, led to hesitancy in recommending widespread adoption of NI-stabilized N-fertilizers. The EC argumentation is: (a) monoculture cereal and maize crop yield improvements are not satisfyingly documented, (b) N- saving effectiveness is not sufficiently tested, (c) NI treated post-harvest soils emit increasingly NH₃ (Scheer et al., 2017), and (d) UI, NI impacts on N, C cycling and non-target organisms are not clearly defined and evaluated (Folina et al., 2021).

Nitrification inhibitors nitrapyrin and DCD are transformed in a first degradation step to 6-chloropicolinic acid and guanyleurea/guanidine, respectively. DMPP, another nitrification inhibitor, oxidizes in the top 0.5 cm of clay loam soil with a half-life of 5 days, and in the 2.5 cm profile with a half-life of 21-28 days, regardless of application rate, to diacetyl, methylglyoxal, acetic acid, and formic acid. The more complete DMPP degradation may be an explanation for the lower monetary revenues of DMPP compared with DCD (Yang et al., 2016). The effectiveness of urease (UI), nitrification (NI), and denitrification (DI) inhibitors is influenced by the specific soil microbial community composition, particularly the ratio of Nitrosotalea devanattera to ammonia-oxidizing bacteria (AOB). These inhibitors, designed to manage nitrogen cycling in soil, exhibit variable efficacy depending on the microbial landscape they encounter (Benckiser, 2017; Gong et al., 2013; Lehtovirta-Morley et al., 2013; Li et al., 2018; Stempfhuber et al., 2017; Stempfhuber et al., 2016). While nitrification inhibitors like DCD and DMPP are used to reduce nitrogen loss from soil, their environmental safety is complex and not fully understood. While some studies show DCD and DMPP effectively reduce nitrogen loss and N₂O emissions, other research highlights variations in their efficacy and potential impacts on soil microorganisms and nitrogen cycling. Factors like diffusion, degradation rates, and effects on nitrogenase and dehydrogenase activity complicate the assessment of their overall environmental impact (Benckiser, 2012, 2017; Tindaon & Benckiser, 2019; Tindaon et al., 2012).

3.2. UI, NI, DI risk assessment

Both ammonia-oxidizing archaea (AOA) and bacteria (AOB) contribute to nitrous oxide (N_2O) emissions, and their relative contributions can be influenced by factors like soil pH and the presence of nitrification inhibitors (NIs). AOA and AOB both perform the initial step of nitrification, the oxidation of ammonia, which produces N_2O as a byproduct. AOA are often more dominant in acidic soils, while AOB are more prevalent in alkaline soils. Nitrification inhibitors can also affect the balance, potentially favoring one group over the other. In alkaline soils, AOB tend to dominate N_2O production from nitrification, while in acidic soils, AOA and AOB contribute more equally. AOA and AOB are differentially affected by NIs, with AOB often being more susceptible. Nitrification inhibitors (NIs) like DMPP and DCD are designed to reduce nitrous oxide (N_2O) emissions by specifically targeting and slowing down the nitrification process in the soil. Nitrification is the biological conversion of ammonia (NH_4^+) to nitrite (NO_2^-) and then to nitrate (NO_3^-), and it's a key source of N_2O emissions from agricultural soils. By inhibiting this process, NIs help reduce the amount of nitrate formed, which is a substrate for denitrification, another process that can produce N_2O . This reduction in nitrification helps to decrease the amount of nitrate available for denitrification, a process that can produce N_2O as a byproduct. However, their effectiveness can fluctuate due to variations in soil conditions and the specific populations of ammonia-oxidizing archaea (AOA) and bacteria (AOB) present. Understanding the interplay between soil properties, NI effectiveness, and the activity of AOA and AOB is crucial for effective N_2O emission mitigation strategies. DCD (dicyandiamide) and DMPP (3,4-dimethylpyrazole phosphate) are commonly used NIs that aim to reduce N_2O emissions by inhibiting nitrification, the process by which ammonia is converted to nitrite and then nitrate. NIs can have varying effects on AOA and AOB. While they are often more effective at inhibiting AOB, some studies suggest that AOA can still contribute to N_2O production, especially in acidic conditions or at higher NI application rates (Fisk et al., 2015; Gong et al., 2013; Kong et al., 2016; McGeough et al., 2012). Field measurements of N_2O emissions often show lower increases with NI application compared to laboratory experiments, where higher N_2O yields might be observed with NI application. The different effects of NIs on AOA and AOB are linked to their different ammonia monooxygenase (AMO) enzymes. Understanding the extent of AMO inhibition by NIs on both AOA and AOB is crucial for accurately predicting N_2O emissions. Soil pH, organic matter content, and nitrogen availability are key factors that influence the effectiveness of nitrification inhibitors (NIs) and the relative contributions of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) to nitrous oxide (N_2O) emissions (Duan et al., 2017; Shi et al., 2017; Waldrip et al., 2016; Waseem et al., 2017). The application of pesticides and inhibitors to soil surfaces can lead to significant environmental problems, including water contamination and harm to both aquatic and terrestrial ecosystems. These substances can leach into nearby water bodies or run off into them, impacting aquatic

life and potentially disrupting the natural balance of the ecosystem. Furthermore, plants can absorb these substances from the soil, potentially leading to reduced crop yields, contamination of food sources, and further health risks. These include urease inhibitors (1H-1,2,4-triazole), nitrification inhibitors (nitrapyrin and DCD), herbicides (glyphosate, nonanoic acid, dichlorprop-P), insecticides (potassium oil, malathion, pyrethrins), and fruit-packaging industry products (ortho-phenylphenol, diphenylamine, ethoxyquin). The insecticide pyrethrins have a No Observed Effect Concentration (NOEC) of $3 \mu g L^{-1}$. Imazalil has a half-life range (DT50) of 47.0 to 150.8 days, and ortho-phenylphenol and diphenylamine have DT50s of 0.6 and 1.3 days, respectively (Bahram et al., 2018; Ghosh et al., 2017; Guo et al., 2016; Jiang et al., 2015; Papadopoulou et al., 2016; Scheurer et al., 2016; Woodward et al., 2016). Ethoxyquin, a food preservative, undergoes rapid transformation in the body and feed materials, yielding a short-lived imine (a major metabolite) and a more persistent compound called 2,4-dimethyl-6-ethoxyquinoline. This transformation is relevant to its use as a nitrification inhibitor and its impact on soil microbes. Urease and nitrification inhibitors, including those containing chloropicrin, can potentially affect rumen microbial consortia when ingested by ruminants, leading to altered nitrogen cycling and metabolic processes. While these inhibitors are primarily used in agriculture to reduce nitrogen losses from soils, their presence in feed can impact the complex microbial ecosystem within the rumen. Chloropicrin (CCl_3NO_2), a soil fumigant, has antimicrobial, fungicidal, herbicidal, insecticidal, and nematocidal properties, and shares structural similarities with nitrapyrin. In silty loam soil, the conversion of ammonia to nitrite, a step in the nitrogen cycle, is slower compared to sandy loam soils. This delay can impact nitrogen availability for plants and potentially lead to increased nitrogen loss from the soil (Yan et al., 2017).

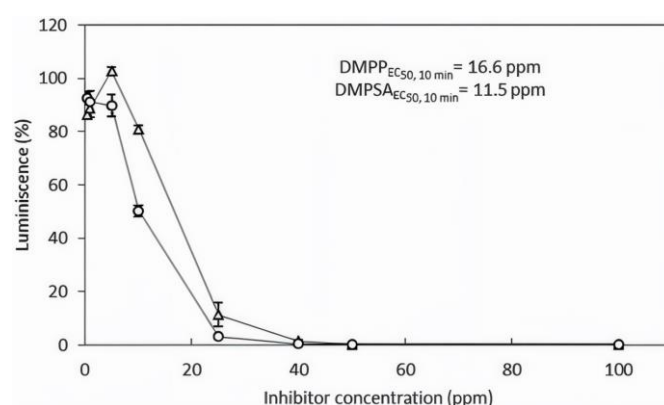


Figure 2. Decreasing luminescence of the aquatic, gram-negative bacterium *Vibrio fischeri* in presence of increasing DMPP (Δ) and DMPSA ($\circ$) concentrations. The luminescence (%) started to decrease in presence of DMPP and DMPSA at around 7 ppm until to around 27 ppm then *Vibrio fischeri* was obviously completely inhibited by both NI. The EC 50 values for the negative control with sucrose and the positive control with zinc sulfate were 0 and 5.6mg/L, respectively (graphic Rodrigues et al. (2018)).

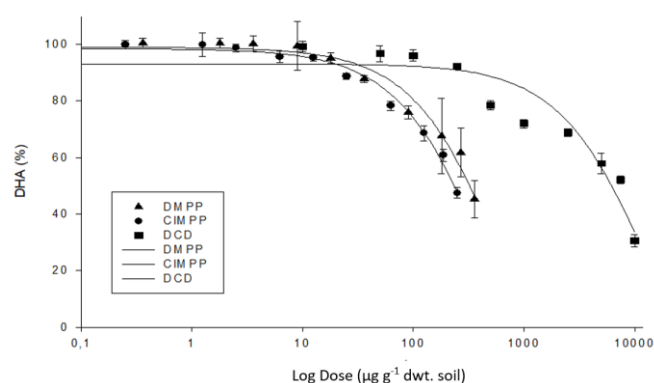


Figure 3. DMPP, CIMPP and DCD concentration dependent side effects on DHA for their visualisation the recommended field dosage of 0.36 μg DMPP; 0.25 μg CIMPP; 10 μg DCD per gram dry soil, corresponding to 90 kg N applied as ammonium sulphate ha⁻¹ must be significantly surpassed (Tindaon & Benckiser, 2019).

The CCl_3NO_2 example invites to hypothesize that UI, NI, DI may inhibit not only target organisms but also side-affect non-target organisms, perhaps more severe as traced from measured N_2O emissions, NH_4 , NO_3 , and NI concentration changes, fauna population abundance estimates, NA, and DHA dose–response relationships, soil mega genome analysis, or ecotoxicity tests with cultivable bacteria as the aquatic, gram-negative bacterium *Vibrio fischeri* (Fig. 2 and 3) (Kong et al., 2016; Kumar et al., 2015; Qin et al., 2017; Tindaon & Benckiser, 2019; Tindaon et al., 2012). Bremner and Yeomans (1986) studied nitrous oxide (N_2O) release from soil using 30 different nitrification inhibitors (NIs). The study started with an application rate of 10 μg NI per gram of soil. This research focuses on how nitrification inhibitors (NIs) affect nitrous oxide (N_2O) emissions from soil, likely by examining their ability to reduce or inhibit the nitrification process, a key step in the nitrogen cycle that can lead to N_2O release. Nitrification inhibitors (NIs) are compounds used in agriculture to slow down the nitrification process, which is the conversion of ammonium to nitrate in the soil. Nitrification inhibitors (NIs) help improve nitrogen use efficiency by reducing nitrogen loss through leaching and denitrification, and potentially reducing greenhouse gas emissions like nitrous oxide (N_2O) by inhibiting the process of nitrification. Nitrification is the microbial process that converts ammonium (NH_4^+) to nitrate (NO_3^-), a form of nitrogen more susceptible to leaching and denitrification. At a higher NI concentration (50 μg NI g^{-1} soil), 2,4-diamino-6-trichloromethyl-s-triazine also became effective. In field applications on New Zealand dairy pastures, dicyandiamide (DCD) with bovine urine showed no impact on earthworm or springtail populations. But bovine urine did influence bacterial composition (Bhaduri et al., 2022). Climate, soil properties, and agricultural practices significantly influence the effectiveness of urease, nitrification, and denitrification inhibitors on soil organisms. These factors affect the soil's environment, which in turn impact. These factors affect how these inhibitors function and their impact on nitrogen cycling processes like ammonia volatilization, nitrification, and denitrification.

Table 2. Dehydrogenase- and dimethylsulfoxide reduction activity in soil samples from the control soil, not treated with the used nitrification inhibitors

Soil type	Dehydrogenase activity (μg INF g^{-1} dry soil h^{-1})	Dimethylsulfoxide reduction activity (ng DMS g^{-1} dry soil h^{-1})
Silty clay	431.6 $\pm$ 3.4	369.8 $\pm$ 2.5
Silt	274.2 $\pm$ 4.3	321.1 $\pm$ 4.6
Loamy sand	121.0 $\pm$ 0.9	96.5 $\pm$ 1.2

Remarks: ¹ = Average of 5 replicates, Tindaon et al. (2012)

A bacterial consortium, enriched with a nitrifying medium after inoculation with soil from a DMPP-treated field experiment, showed morphological changes under a transmission electron microscope at 10 times the DMPP concentration than the field-recommended rate of 0.25 μg DMPP g^{-1} dry soil. Specifically, the study by Norton and Ouyang (2019) and the research by Benckiser et al. (2013) both observed these changes, indicating a higher sensitivity to DMPP at the higher concentration. Bacteria as *Rhodobacter sphaeroides*, *Rhodopseudomonas palustris*, *Azospirillum brasilense*, and *Rhizobium leguminosarum* bv. *trifolii*, *Sinorhizobium meliloti*, *Bradyrhizobium* sp., or *Anabaena doliolum* showed in the presence of DMPP, the herbicides terbutryn, simazine, methabenzthiazuron, prometryn 2,4-D, quinalphos, monocrotophos, the fungicides captan, carbendazim, imazetapir, thiram, and the insecticide carbofuran, and studied by counting nodule numbers, measuring CO_2 -production, N_2 fixation (NA), and calculating dose–response relationships (Fig. 2), adverse reactions (Das & De, 2018; Purwanto et al., 2014; Tindaon et al., 2011; Tindaon et al., 2012). The dehydrogenase activity (DHA) DCD, CIMPP (4-chloromethylpyrazole), DMPP concentration dependent dose–response relationships (field recommended application rate: 10, DCD, 0.36, CIMPP, 0.25 μg DMPP g^{-1} dry soil and 5, 10, 25, 50, 100, 250, 500, 750, up to 1,000 times higher application rates; for details see Tindaon et al. (2012). In the clayey soil that the DMPP, CIMPP must surpass the recommended field application rate approximately by 50 times, DCD by about 250 times, before the photosynthesis, N_2 fixation dependent DHA started showing a side effect (Fig. 2). In the loamy and sandy soil, the DHA reacted earlier than in the clayey soil. Dehydrogenase- and dimethylsulfoxide reduction activity in soil samples from the control soil, not treated with the used nitrification inhibitor, were measured as a basis for the dose–response relationship (Table 2) (Tindaon et al., 2012).

To assess the impact of urease and nitrification inhibitors, as well as other nitrogen-stabilized fertilizers and pesticides, farmers and administrators often rely on measurements of DHA, NH_4 , NO_3 , and N_2O , alongside organism-based laboratory tests (*Vibrio fischeri* example, Fig. 3). To better assess the impact of products on soil health and biodiversity, farmers and administrators could utilize readily available, sensitive, and cost-effective bioassays that provide detailed species-level information. These bioassays offer a more practical approach compared to complex multi-omic or environmental monitoring methods, especially for understanding the effects of products on soil biological diversity (Bahram et al., 2018; DeLong, 2012; Du & Liu, 2012;

Li et al., 2018; Nguyen et al., 2017; Pal et al., 2016; Prakash et al., 2013; Rodrigues et al., 2018; Schütte et al., 2017; Subbarao et al., 2017; Vestergaard et al., 2017; Wallace et al., 2018; Waseem et al., 2017; Zhang et al., 2017). Integrating sensitive and cost-effective bioassays with existing methods will provide a more holistic view of the impact of nitrogen management practices on soil health and biodiversity, leading to more informed decision-making for farmers and administrators.

4. DISCUSSION

The use of urease and nitrification inhibitors in N-stabilized fertilizers presents a complex situation where the inhibitors, designed to improve nitrogen use efficiency, can interact with soil components and potentially impact adjacent water and plant life. Understanding these interactions, including the diffusion of inhibitors and their effects on soil microbes, is crucial for assessing potential risks at recommended application rates. Urease and nitrification inhibitors are valuable tools in agriculture for reducing nitrogen loss from soil. Urease inhibitors, such as 1H-1,2,4-triazole, slow down the conversion of urea to ammonia, while nitrification inhibitors, including nitrapyrin and dicyandiamide (DCD), inhibit the conversion of ammonium to nitrate. Both processes can lead to nitrogen loss, which impacts crop yields and contributes to environmental issues such as greenhouse gas emissions. This helps to reduce nitrogen losses through ammonia volatilization and leaching, ultimately improving nitrogen use efficiency for plants. By inhibiting these processes, these compounds help keep nitrogen in the soil for longer, making it more available for plant uptake and reducing losses through volatilization and leaching. However, their diffusion from fertilizer granules within the soil pore hierarchy (clay, silt, sand, humus) is affected by various factors. In agriculture, inhibitors are used to reduce ammonia and nitrous oxide emissions, potentially improving water and air quality. While nitrogen inhibitors in agriculture can reduce fertilizer needs and potentially increase yields, they also pose risks like toxicity to aquatic life and potential harm to human health. Several factors significantly influence the effectiveness of nitrogen (N) loss inhibitors, impacting crop yield and environmental outcomes. These factors include soil properties, such as texture and pH, as well as environmental conditions like temperature and rainfall, and management practices, including fertilizer application timing and method. Further research is needed to understand their effectiveness across different conditions and to mitigate these negative impacts. Studies have shown that the use of inhibitors can lead to increased revenue for farmers, offsetting the cost of the inhibitors. While inhibitors in agriculture can reduce emissions, their use presents several potential downsides. These include concerns about toxicity to humans and the environment, variable efficacy depending on conditions, knowledge gaps regarding long-term impacts, and the cost of implementation. Inhibitors, such as those used to reduce nitrogen emissions, may contain substances that are toxic to aquatic organisms or pose health risks to humans. Environmental impacts can include unintended consequences for non-target organisms and the global

nitrogen cycle. The effectiveness of inhibitors, such as nitrification inhibitors (NIs) or urease inhibitors (UIs), is indeed influenced by various soil and management factors. These factors can impact the inhibitor's ability to reduce nitrogen loss, ultimately affecting crop yield and environmental sustainability. Further research is needed to understand their behavior and long-term effects in different agricultural systems. The cost of inhibitors and their application can also be a consideration. Plant ingesting animals, sustaining in the rumen 10¹³ to 10¹⁴ microbes, the food chain is thus reachable by UI, NI, DI (Cruchaga et al., 2011; Doran et al., 2018; Ishaq et al., 2017; Legay et al., 2016; Pronk et al., 2017; Raul et al., 2016; Rodrigues et al., 2018; Scheurer et al., 2016; Soliveres et al., 2016; Subbarao et al., 2017; Tindaon et al., 2012; Vestergaard et al., 2017; Woodward et al., 2016). The microbial communities in the soil and root environments of *Dactylis glomerata* (orchard grass) and *Zea mays* (corn) are significantly influenced by land use intensity, nitrogen availability, and plant characteristics. Different land use practices, nitrogen levels, and plant traits significantly influence the composition and structure of microbial communities in both the rhizosphere and bulk soil. The rhizosphere, being the soil zone immediately surrounding plant roots, is particularly susceptible to these factors due to the direct interactions between plants and microbes. These factors interact in complex ways, leading to distinct microbial profiles in these two soil zones. Understanding these relationships is crucial for optimizing agricultural practices and promoting sustainable soil health. The research investigates the role of *Pseudomonadaceae*, *Enterobacteriaceae*, and *Comamonadaceae* (all Proteobacteria) in these environments, particularly concerning ammonia-oxidizing archaea (AOA) and bacteria (AOB), Nitrospira-like bacteria (NS), and other nitrogen-cycling bacteria. This research examines the impact of land use, nitrogen levels, and root exudates on soil microbial communities. It acknowledges the limitations of using 16S rRNA gene sequencing and N₂O measurements in identifying specific microbial catalysts beyond ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), and nitrous oxide reducers (NS). The study aims to understand how these factors affect microbial composition and function within the soil ecosystem. The study aims to understand how these factors influence the composition and function of soil microbial communities. The study will focus on how these factors affect the overall microbial community structure and function, even with the inability to pinpoint every single microbial player involved (Bakker et al., 2015; Estendorfer et al., 2017; Schulz et al., 2017; Stempfhuber et al., 2017; Stempfhuber et al., 2016; Subbarao et al., 2017). In addition to O₂, NH₄ /NO₂ /NO₃, organic substrate soil organism interconnectivities and farmers' soil tillage and pesticide applications, other than UI, NI, the low cultivation success at the microorganism species level and a continuous discovering of novel enzymes and bio-surfactants complicate to improve NUE, to find with the presently available methodology coherent explanations for composition structure shifts and to identify and formulate UI, NI risk assessments (Cai et al., 2016; Domeignoz-Horta et al., 2016; Duan et al., 2017;

Gottschalk, 2015; Marco, 2014; Stempfhuber et al., 2016; Thies et al., 2016; Yan et al., 2017). The existing methodologies can significantly expand our understanding of cell functioning, environmental nitrogen cycling, and soil genetic diversity. While the nitrogen cycle is complex and involves numerous processes, advancements in molecular biology and analytical techniques offer powerful tools for investigation. We understand, meanwhile, reasonably how and when bacteria and fungi make C, N, C, P, Fe, and trace elements palatable for their own, plant and animal use, and that not only the nitrifying, denitrifying microflora suffers under an overdosed spreading of nano-sized, with soil particles and cell surfaces interact UI, NI, DI (Bardgett & van der Putten, 2014; Benckiser et al., 2013; Nacry et al., 2013; Vestergaard et al., 2017; Vogel et al., 2009). While the precise mechanisms are still being researched, urease inhibitors (UI), nitrification inhibitors (NI), and denitrification inhibitors (DI) are known to influence soil microbial diversity at the species level by impacting specific microbial groups and overall diversity. These inhibitors, used to enhance nitrogen use efficiency in agriculture, can alter the composition and activity of soil microbial communities. UIs and NIs primarily affect the abundance and activity of ureolytic and nitrifying bacteria, respectively, while DIs can influence the diversity and activity of denitrifiers. Nitrapyrin added to an undisturbed semi-arid steppe soil shifted the NH₄⁺ + NO₃⁻ ratio and organic matter (SOM) decomposition (Austin et al., 2006). In calcareous Uzbekistani soil under cotton, the application of urea and ammophos fertilizer along with the nitrification inhibitor potassium oxalate (PO) has a complex impact on soil microorganisms. While PO can suppress ammonifying and nitrifying bacteria, it may also promote the growth of oligonitrophilic bacteria like *Corynebacterium* and *Nocardia*, which are involved in cellulose breakdown. This suggests a shift in the microbial community composition, potentially favoring organisms that can utilize less readily available nitrogen sources. In contrast, urea fertilizer in a Minnesota corn system can lead to lower nitrous oxide (N₂O) emissions compared to anhydrous ammonia. Additionally, studies on grassland soils treated with bovine urine and dicyandiamide (DCD) show that the soil's microbial composition is more significantly altered by the urine component than the DCD (Nguyen et al., 2017; O'Callaghan et al., 2010; Venterea et al., 2010). Several studies using molecular biological techniques, including TEM, have investigated the effects of nitrification inhibitors (NIs) like DMPP (3,4-dimethylpyrazole phosphate) and DCD (dicyandiamide) on soil nitrogen dynamics and nitrous oxide (N₂O) emissions. These studies reveal that: 1) DMPP can cause side effects when applied at rates significantly higher than recommended, and that 2) both DCD and DMPP do not completely inhibit all microorganisms responsible for N₂O production, such as ammonia-oxidizing bacteria (AOB), ammonia-oxidizing archaea (AOA), and certain fungi. Additionally, 3) in-situ measurements in agricultural fields show a stronger correlation between N₂O and N₂ emissions with NO₂⁻, the intermediate product of nitrification, rather than with the initial substrate, NH₄⁺, or the final product, NO₃⁻ (Cai et al., 2016; Gong et al., 2013; McGeough et al.,

2012; Xue et al., 2016; Yang et al., 2016; Zebarth et al., 2012; Zhu et al., 2013). The use of UI, NI, and DI to reduce increasing N leaching, nitrous oxide (N₂O), ammonia (NH₃) emissions, eutrophication, and the pollution of adjacent non-target aquatic environments after H-B invention (Chen et al., 2018) enabled the provision of plant-available nitrogen (N) in synchrony with crop requirements. A 62 NI treated sites comprising field study evidenced a NH₃ emission increase of 20% due to UI, NI application (mean, 95% confidential interval: 33–67%), a dissolved inorganic 48% N leaching (–56% to –38%), 24% NO emission (–38% to –8%) and 44% N₂O reduction (–48% to –39%), a 58% plant N recovery increase (34–93%), a 16.5% net N reduction, an in total, up to 20% crop yield increase (grain, straw, vegetable, pasture hay productivity by 9% (6–13%), 15% (12–18%), 5% (0–10%) and 14% (8–20%), respectively), and \$ 163 ha⁻¹ yr⁻¹ maize farm revenues (equivalent to a 8.95%) (Qiao et al., 2015). Despite such UI, NI advantages a EC cost/benefit analysis hesitates to recommend a large-scale application of NI stabilized N-fertilizers, inter alia because the hand in hand working of the NH₄⁺ to NO₃⁻ - converting nitroso-, the NO₂⁻ to NO₃⁻ - converting nitro-groups, the soil properties, microbial, plant N uptake, and soil urease activity dependent soil NH₄⁺ /NO₃⁻ ratio changes, UI, NI, DI non-target organism side effects are not understood in a way that monoculture organic, precision farming can successfully adapt to plant demand and a high productivity (Leithold et al., 2015; Zhang & Kovacs, 2012).

Inhibitors can be effective in reducing emissions, but their impact varies and requires further research to fully understand their effects on the environment and human health. Some inhibitors can be toxic to aquatic organisms, and there's a need to evaluate the potential for unintended consequences on non-target organisms and the nitrogen cycle. Agricultural decision support systems (DSS) like CropSAT, which utilize technologies like GPS and GNSS, are instrumental in enhancing precision farming practices, including in monoculture and organic farming, by optimizing nitrogen fertilization. These systems enable farmers to make more informed decisions regarding input management, leading to improved resource utilization and potentially increased yields. Nitrogen management systems aim to optimize fertilizer use by considering plant needs and soil characteristics, enhancing efficiency and potentially increasing yields. CropSAT, a decision support system, utilizes data from drones and other technologies to guide precision farming practice (Lindblom et al., 2017). Precision agriculture, enabled by GPS and GNSS-connected equipment, allows for accurate field positioning and detailed measurement of various soil and crop parameters. The data mentioned can be leveraged to create vegetation index maps for risk assessment, which in turn helps optimize resource allocation, minimize negative impacts, and stabilize revenue. This is achieved by using the maps to identify areas vulnerable to environmental changes, allowing for proactive measures to be taken to mitigate potential risks and maximize resource utilization (Benckiser, 2017; Lindblom et al., 2017; Lundström & Lindblom, 2016). The research questions focus on understanding how environmental conditions and agricultural practices influence inhibitor performance, their degradation, and potential uptake by crops. Specifically, the questions

explore how soil temperature, pH, moisture, fertilizer rate, placement, and timing affect inhibitor performance. What environmental factors contribute to the degradation and persistence of applied inhibitors in the soil? Some inhibitors are designed to be systemic, meaning they are absorbed by the plant and transported throughout its tissues. Degradation products can also be taken up, potentially leading to different effects than the parent compound. The extent of uptake depends on the specific inhibitor, the crop, environmental conditions, and the chemical properties of the degradation products. By addressing these questions, researchers can gain a deeper understanding of how inhibitors interact with the soil environment, how they degrade over time, and how they might impact crop health and safety.

5. CONCLUSIONS

The convergence of advanced single-atom detection techniques has increased computational power, and interdisciplinary research is enhancing our understanding of soil and its interactions with various systems. This progress is likely to lead to more environmentally sustainable agricultural practices, including targeted pesticide applications and nitrogen fertilizer use that minimizes environmental impact. Omics technologies offer a robust approach for studying complex biological systems by integrating data from various levels, including genomics, transcriptomics, proteomics, and metabolomics. However, the current methodologies for assessing side effects, including those beyond the existing nitrogen index (NI) evaluation systems, face challenges, particularly in cultivating diverse organisms. Evaluating the specific impairments caused by nitrogen-stabilized urea and NH_4^+ fertilizer remains partially unidentifiable; however, this evaluation provides a more comprehensive picture. Farmers are challenged to align natural nutrient cycling and productivity within nitrogen-deficient ecosystems while engaging in soil cultivation, fertilization, and pesticide applications. Further research is essential to optimize nitrogen fertilizer inhibitors, seeking a balance between their effectiveness in increasing crop yields and reducing their adverse environmental impacts. This includes developing strategies to enhance nitrogen use efficiency (NUE) while addressing issues like nitrate pollution, air quality degradation, and greenhouse gas emissions. A thorough understanding of how inhibitors behave and their effects on agronomic and ecological systems will be vital for creating sustainable agricultural practices that minimize environmental impact while maximizing crop yields.

Declaration of Competing Interest

The authors declare that no competing financial or personal interests may appear to influence the work reported in this paper.

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