Research Progress Related to Sorghum Biological Nitrification Inhibitors

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Abstract: To meet the growing population’s demand for food, humans have introduced large amounts of nitrogen fertilizers into agricultural systems, resulting in highly nitrified environments in most farmland soils. In highly nitrified environments, the application of nitrogen fertilizer easily leads to the formation of nitrate (NO$_3^-$) and subsequent leaching, resulting in very low utilization rates. Moreover, nitrogen loss can cause harm to both the environment and human health, making it necessary to inhibit the nitrification process. Nitrification inhibitors can suppress nitrification, and inhibitors derived biologically from plant roots are gaining attention due to their low cost and environmental friendliness. Sorghum, as a crop capable of growing in arid environments, holds economic value and also possesses the ability to secrete biological nitrification inhibitors. This article utilizes sorghum as a case study to review different types of BNIs (MHPP, sorgoleone, and sakuranetin), their mechanisms of inhibition, and influencing factors. This article summarizes the contributions of these inhibitors in reducing N$_2$O emissions and increasing food production, while also providing insight into future research directions for sorghum’s biological nitrification inhibitors in terms of agricultural production efficiency. BNIs are expected to play an important role in improving agricultural production and reducing environmental pollution.

Keywords: biological nitrification inhibitors; nitrification; agricultural production; environmental friendliness; nitrogen fertilizers

1. Introduction

Since the Industrial Revolution, humanity has introduced large amounts of reactive nitrogen into the biosphere, thereby altering the traditional natural nitrogen cycle [1,2]. In order to sustain the growing population and enhance food production, nitrogen fertilizers are extensively employed in agricultural systems [3–5]. Presently, annual nitrogen fertilizers usage has reached 150 million tons, with projections expecting it to double by 2050 [6–8]. An estimated 70% of nitrogen fertilizers applied to soil annually are lost through nitrification and cause a loss of USD 30 billion [9]. Nitrification, a crucial aspect of the nitrogen cycle, holds significant implications for agriculture and the environment [10–12]. Nitrification widely occurs in most agricultural soils, proceeds rapidly, and is challenging to control, resulting in significant losses to agricultural production [13–15]. Thus, finding strategies to inhibit the nitrification process is essential to mitigate pollution caused by nitrogen leakage. Inhibiting nitrification is essential for the development of agricultural systems with low N$_2$O emissions [16,17].

To inhibit the nitrification process and enhance nitrogen use efficiency, thereby reducing the environmental impact of nitrogen loss, the application of nitrification inhibitors...
(NIs) has become a widely adopted method for improving nitrogen utilization [18,19]. Synthetic nitrification inhibitors have been used in places such as the United States and Europe to prolong the retention time of ammonium nitrogen in the soil. This practice effectively mitigates losses due to nitrate nitrogen leaching and has demonstrated success, offering practical instances for continued research on nitrification inhibitors [20,21]. Nitrapyrin, dicyandiamide (DCD), and 3,4-dimethylpyrazole phosphate (DMPP) represent frequently employed synthetic nitrification inhibitors [22–25]. These NIs are typically mixed with fertilizers to delay the nitrification process, thereby meeting the nutritional requirements of crops at various growth stages [26]. This method prolongs the retention time of ammonium nitrogen in the soil by suppressing microbial activity during nitrification, thereby notably enhancing efficacy, significantly improving the utilization efficiency of nitrogen fertilizers. However, as the understanding of nitrification inhibitors (NIs) deepens, it has been discovered that the use of synthetic NIs may lead to additional issues. For instance, nitrapyrin, a commonly used synthetic inhibitor, has inherent toxicity that, when applied to soil, could potentially impair plant growth [27]. DCD has the potential to leach groundwater, and its application is relatively costly [28–30]. Furthermore, the inhibitory effects of synthetic nitrification inhibitors in soil typically persist for no more than a few weeks, often less than one week [13]. Consequently, considering the cost and environmental side effects of synthetic nitrification inhibitors, scientists are compelled to seek more economical and environmentally friendly alternatives to mitigate nitrification.

Nitrogen is an indispensable nutrient in natural ecosystems, and various mechanisms have evolved in nature to reduce nitrogen loss and maintain microbial nutritional balance. In 1966, researchers discovered a phenomenon of reduced nitrification in forest and grassland soils, which subsequently led to the introduction of the concept of biological nitrification inhibition (BNI). BNI originates from plants and is readily degraded and utilized by microbes, rendering it an environmentally friendly inhibitor of nitrification [31,32]. The inhibitory effect of 0.22 \( \mu \text{M} \) AT on 18.9 mM \( \text{NH}_4^+ \) is defined as one ATU. Research has demonstrated that adding 20 ATU of BNIs per gram of soil, derived from the root exudates of Brachiaria humidicola, can sustain its activity for approximately fifty days [33]. In comparison to synthetic nitrification inhibitors, this BNI is characterized by its prolonged duration of action and minimal adverse effects on crops, enabling precise transport to nitrification sites upon root release [34,35]. Consequently, harnessing BNIs secreted by plants to inhibit the nitrification process emerges as a crucial strategy for improving nitrogen fertilizer efficiency and augmenting food production [34].

Plants confirmed to have the ability to secrete BNIs include sorghum (Sorghum bicolor) [36], rice (Oryza sativa) [37], pasture grass (Brachiaria humidicola) [31], pine resin [38], Hibiscus splendens, Solanum echinatum [39], spruce (Picea abies), and Nordmann fir (Abies nordmanniana) [40]. Many plants capable of secreting BNIs are believed to remain undiscovered in nature. Recent research on BNIs has revealed that these plants predominantly inhabit environments with low nitrogen content, guiding scientists in their search for additional sources of biological nitrification inhibitors [10,41].

Sorghum, a vital grain and animal feed crop, is a primary agricultural plant in many arid and semi-arid regions worldwide due to its drought resistance and broad adaptability [42–44]. In response to the concurrent challenges of nitrogen fertilizer efficiency and environmental impact, there has been a surge in the discovery and application of biological nitrification inhibitors (BNIs) in recent years, presenting effective strategies to bolster nitrogen utilization and mitigate environmental nitrogen losses. Despite the relatively late initiation of research on sorghum BNIs, it has already demonstrated considerable potential for both research and practical application [17,45]. This article, using sorghum as an example, reviews several BNI compounds and their functions, and discusses in detail the types of BNIs secreted by sorghum, influencing factors, and release mechanisms. Moreover, this article explores the practical applications of BNIs in agricultural production, including their potential contributions to reducing greenhouse gas emissions and increasing food production. We hope this review can provide a theoretical basis for enhancing nitrogen
efficiency in agriculture and offer new insights and practical approaches for agricultural environmental management.

2. Biological Nitrification Inhibitors and Their Functions That Have Been Identified in Sorghum

To date, several BNIs have been identified to be released primarily from the roots of sorghum (*Sorghum bicolor*), including sorgoleone, methyl 3-(4-hydroxyphenyl)propionate (MHPP), and sakuranetin [36,46–48]. Studies have shown that sakuranetin, sorgoleone, and MHPP all possess significant nitrification inhibitory effects. Based on the effective doses of these three BNIs to inhibit 80% of *Nitrosomonas*, sakuranetin showed the strongest inhibitory effect on *Nitrosomonas*, followed by sorgoleone and MHPP. Among them, sorgoleone showed a linear inhibitory effect on *Nitrosomonas* in the concentration range of 0 to 13 µM [31,49]. Additionally, sakuranetin can also suppress fungal diseases in rice [50,51]. Meanwhile, MHPP can inhibit primary root growth and promote lateral root development in sorghum roots, optimizing root structure. This optimization increases the total root volume and enhances the plant’s efficiency in absorbing mineral nutrients from the soil [52].

Sorgoleone not only plays a key role in inhibiting nitrification in the soil but is also considered a potential natural herbicide [53–61]. The release of this unique compound is influenced by various factors, such as the form of nutrients, the action of plant hormones, and exposure to light. Further research has revealed that the state of root hair development may also indirectly affect the synthesis and release of sorgoleone [53,56,62–64]. Moreover, in soil, sorgoleone significantly inhibits the oxidation of ammonium ions (NH$_4^+$), demonstrating its dose-dependent effects [46,47,65,66]. Among various sorghum varieties, sorgoleone has been identified as the only hydrophobic compound associated with BNI functionality to date. Importantly, these varieties exhibit genetic differences in sorgoleone and BNI release, suggesting the potential utilization of these genetic variations through breeding programs to develop improved sorghum varieties. This aims to establish a more sustainable and environmentally friendly production system compared to those currently cultivated in most regions worldwide [17,47,56,58,59,67,68]. The compounds discovered in sorghum root exudates have shown nitrification inhibitory functions, which are crucial for revealing the nature of BNIs. It should be noted that the above compounds are currently isolated under hydroponic conditions, and there are still some difficulties in studying their mechanisms of action under soil conditions.

3. The Inhibition Mechanism of Biological Nitrification Inhibitors

BNIs primarily slow down the nitrification process by targeting two key biochemical pathways: the ammonia monooxygenase (AMO) pathway and the hydroxylamine oxidoreductase (HAO) pathway [69]. Ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) can independently exert their functions through the AMO pathway. Conversely, the HAO pathway relies solely on AOB, which oxidizes hydroxylamine into nitrite during nitrification. AMO of AOB is a trimeric membrane-bound protein consisting of three subunits: *amoA*, *amoB*, and *amoC*, with *amoA* serving as the active site [70]. The gene composition of AOA’s AMO differs from that of AOB, possibly containing only *amoA*, which may lead to different responses to BNIs due to this molecular difference [71,72]. In the nitrification process, the ammonia-oxidation step is carried out jointly by AOA and AOB, and it is the rate-limiting step in the entire process [73]. Consequently, research on the inhibitory mechanisms of BNIs primarily focuses on AOA and AOB.

Studies show that when BNIs affect the AMO pathway, the activity of both AOA and AOB decreases, as evidenced by the reduced abundance of both in the rhizosphere soil of signal grass (*Brachiaria humidicola*) [74,75]. However, BNIs targeting the HAO pathway primarily affect AOB. This is evidenced by the reduction in AOB genes in the rhizosphere soil of tall ryegrass (*Leymus racemosus*), indicating that the impact of the HAO pathway on AOA remains unclear [17]. Research also shows that BNIs can inhibit AMO, while compounds like arm lactose, sorgoleone, and sakuranetin can inhibit HAO
(Figure 1). The initial step of nitrification, where ammonia is converted by AMO into hydroxylamine, represents the slowest phase of the process; subsequently, hydroxylamine is further converted by HAO into nitrite. While these inhibitory effects have been validated under hydroponic conditions, their confirmation in soil conditions remains limited. Studies that transferred sorghum root exudates from hydroponic setups to soil environments demonstrated significant inhibition of AOB [76].

While AOA and AOB are known to regulate and inhibit the nitrification process, nitrifying bacteria of the *Nitrobacter* genus also hold significance in this process. NOB are categorized into four genera, with *Nitrobacter* and *Nitrospira* being the most extensively studied [77,78]. Moreover, studies have shown that the litter of fir trees in forests can inhibit the nitrification process by suppressing the activity of the *Nitrobacter* genus [40,79]. Exploring how NOB respond to BNIs is a critical area for future research in nitrification inhibition and for future exploration of nitrification inhibition technologies. Understanding this process can not only shows the effects of BNIs on specific NOB types such as *Nitrobacter* and *Nitrospira* but may also help scientists develop more effective strategies to control the nitrogen cycle in agricultural and environmental systems.

### 4. Environmental Factors Affecting the Secretion of BNIs in Sorghum

The effectiveness of BNIs may be impacted by a range of soil and environmental factors [13,49,80–82] including soil type, chemical properties, pH levels, organic matter content, and microbial activity, among others [80–85]. Understanding and considering these factors is crucial for determining applicability, considering how BNIs can fluctuate depending on soil type, with instances where they might prove ineffective in alkaline soils. Moreover, the organic matter content within the soil can impact decomposition and release dynamics of these inhibitors, consequently affecting their longevity. Typically, soils with higher organic matter content may harbor more bacteria, potentially accelerating the degradation of BNIs [86]. Additionally, under high temperatures, the degradation of NIs accelerates, leading to speculation that BNIs may also be affected by high temperatures [80,81,87]. Soil moisture conditions are also an important factor [88,89]. Soils with lower water content may affect the mobility of soluble BNIs, causing their accumulation in the roots, which could potentially hinder plant growth. Studies suggest that higher soil moisture levels lead to reduced secretion of hydrophobic BNIs, such as sorgoleone, by sorghum, underscoring the significance of soil moisture in BNI release. Therefore, emphasizing soil moisture management is essential to promote optimal plant growth and facilitate nitrogen cycling [65].

The form of nitrogen significantly affects the synthesis and secretion of sorghum BNIs, and the plant’s response mechanism to different forms of nitrogen is a key factor in adapting to various soil environments. NH$_4^+$ and NO$_3^-$ are the main nitrogen sources for plants, and their abundance and form in the soil directly influence plant growth and development [90–92].
BNIs are commonly released around the root systems of sorghum plants; however, their impacts can extend further through soil microbial-mediated processes. The abundance of NH$_4^+$ in the rhizosphere can significantly stimulate the synthesis and secretion of BNIs in plant roots, thus regulating the activity of nitrifying bacteria in the soil and affecting the nitrogen transformation process. Studies have shown that the richer the NH$_4^+$ content in the soil, the more BNIs (sorgoleone) sorghum secretes [65]. An environment enriched with NO$_3^-$ may reduce the production of BNIs in plant roots [36,93,94]. The presence of NH$_4^+$ in soil, whether through mineralization of soil organic nitrogen or through the application of nitrogen fertilizers such as urea or ammonium sulfate, can enhance the activity of nitrifying bacteria [95,96]. Generally, sorghum facilitates the secretion of BNIs in low-nitrogen environments. However, compared to soils where sorghum is not planted, sorghum can also secrete BNIs under high-nitrogen conditions [76]. To minimize nitrogen runoff, natural ecosystems employ diverse strategies to regulate nitrogen flow, including inhibiting nitrification and utilizing various forms of nitrogen, both organic and inorganic, as nitrogen sources. This limits the flow of nitrogen through the nitrification pathway [97,98].

The quantity of BNIs released by sorghum varies at different growth stages. Studies have shown that the release amount of BNIs from “Hybridsorgo” sorghum roots reaches its peak on the 50th day of plant growth, and the activity of BNIs within the root tissue is generally higher than that released from the roots [17]. Research shows that the release of BNIs from sorghum roots reaches its peak on the 50th day of plant growth, with the activity of BNIs within the root tissue typically surpassing that of the BNIs released from the roots themselves. Sorghum roots secrete two types of BNIs: hydrophobic and hydrophilic (Figure 2) [17]. Hydrophobic BNIs, owing to their low solubility in water, predominantly impact the region near the rhizosphere, effectively inhibiting nitrification in that vicinity [57]. At this stage, root activity is mainly focused on the establishment of a healthy root structure and rapidly absorbing nutrients from the surrounding soil. As sorghum enters a more mature growth stage, the secretion of hydrophilic BNIs begins to increase, balancing the contribution of hydrophobic BNIs [17,66]. Hydrophilic BNIs, owing to their enhanced water solubility, can disperse through water flow to more distant soil regions, thereby inhibiting nitrification across a broader expanse. This helps stabilize the nitrogen cycle over a larger area, especially when the root system is well developed and needs to impact the soil environment more broadly. The complementary actions of these two types of BNIs in distinct soil environments effectively inhibit the nitrification process.

The pH value of plant roots serves a regulatory role in the release of sorghum BNIs. BNIs exhibit stable functionality within soil where pH ranges from 3.0 to 9.0. Research suggests that the secretion activity of sorghum BNI peaks when the rhizosphere soil pH falls between 5 and 6 [66]. A comprehensive understanding of the regulatory mechanisms governing soil pH’s impact on BNI release can facilitate the optimization of soil management strategies, bolster nitrogen use efficiency, and offer substantial backing for sustainable agricultural development. However, many aspects of the relationship between BNI release and soil pH still require further research to reveal their underlying mechanisms and enhance their application in agricultural production. These findings have facilitated in-depth research into the release process and physiological mechanisms of BNIs [99]. Various sorghum varieties have varying abilities to secrete BNIs, and we hope to reveal the key genes and genetic mechanisms that affect this trait [100]. This will provide breeders with valuable information, enabling them to selectively improve sorghum varieties to enhance the activity of their BNIs, thereby yielding greater environmental and economic benefits in agricultural production.
5. The Contribution of Biological Nitrification Inhibitors in Reducing Greenhouse Gas Emissions and Improving Crop Yields

BNIs have a significant impact on greenhouse gas emissions in agricultural production. N₂O, a greenhouse gas closely associated with nitrogen fertilizer application and nitrification, possesses a global warming potential 298 times greater than that of CO₂ [101]. BNIs play a crucial role in mitigating the release of N₂O from soil, consequently diminishing the impact of agricultural practices on greenhouse gas emissions. The ability of plants to suppress N₂O emissions is linked to their capacity to secrete BNIs; the stronger the BNI capability, the greater the suppression of N₂O emissions [93]. In a long-term field experiment with B. humidicola, researchers found that soils planted with this BNI-secreting grass exhibited a significant reduction in N₂O emissions, achieving a 90% decrease compared to soils planted with soybeans [34,46,100,102]. Additionally, in an intercropping study featuring sorghum and maize, a marked reduction in N₂O emissions was observed [103]. The 1,9-Decanediol secreted by rice has also been demonstrated to reduce N₂O emissions [104]. In a paddy field experiment, researchers used two types of BNI (Nimin and Karanjin) and one NI (DCD), finding that the two BNIs were significantly more effective at suppressing N₂O emissions in rice soil than DCD. Specifically, N₂O emissions were significantly inhibited in the Nimin-applied plots during both the wet and dry seasons, with reductions of 69% and 85% over the control, respectively [105].

BNIs reduce nitrogen loss by inhibiting nitrification in the soil, thereby enhancing plant nitrogen uptake and utilization, which positively affects crop yield. Studies have shown that crop yields significantly increase after BNI application. Through extensive long-term experiments, researchers have found that adding neem cake and karanja cake to fertilizers significantly increases rice and wheat yields [102,106]. Furthermore, Zhang and colleagues observed that vegetable yields were significantly higher in plots treated with BNIs compared to those treated with urea. Datta and Roy, along with other researchers, also noted similar phenomena, where the application of BNIs (such as Nimin) and fisetin notably increased rice yields [105,107]. Additionally, besides directly adding BNIs to the soil, field planting of grasses with BNI functionality can also increase the yield of subsequently planted maize [108].

Therefore, we can consider introducing plants that release high levels of BNIs into agricultural systems to increase food production and mitigate N₂O emissions.
using BNIs, multiple factors such as application methods, dosage, and soil type should be considered to develop rational application strategies that maximize the reduction of greenhouse gas emissions and enhance the efficiency of nitrogen fertilizer use.

6. Perspectives

The future prospects for the development of sorghum BNIs are promising, particularly concerning the improvement of nitrogen fertilizer efficiency and environmental protection. With the growing demand for sustainable agricultural practices, effective nitrogen source management has become crucial to increase crop productivity and mitigating environmental impacts. Future studies need to explore in depth the biochemical mechanisms of BNIs and their inhibitory effects in different soil environments. At the same time, it is important to explore the economic feasibility of BNI implementation in large-scale agriculture and further optimize the extraction and application of BNIs from various crops. Additionally, the integration of transgenic technology with sorghum is crucial for sustainable agriculture and is one of the important directions for future research. Educational outreach and policy support will also be crucial in promoting the widespread application of BNIs. Through these measures, the development of sorghum BNI technology can enhance agricultural efficiency and also help build a more environmentally friendly and sustainable agricultural system.

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Agronomy 2024, 57.


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