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# Microbial Nitrification Paradox: A Paradigm Shift on Nitrogen Uptake by Rice

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Nitrogen fertilization is an integral agronomic practice to increasing productivity and profitability in agriculture. But the poor nitrogen use efficiency (NUE, about < 40%) causes many economic and environmental challenges. The microbial oxidative process of converting ammonia to nitrite to nitrate (nitrification) is the rate-limiting step in the N loss. The century-old, conventional theory of nitrification with the involvement of two functionally different bacterial groups as ammonia-oxidizing bacteria and nitrite-oxidizing bacteria has been upturned by the recent discoveries of archaeal members involved in ammonia oxidation (Ammonia-oxidizing archaea, AOA), anaerobic ammonia-oxidizing bacteria (Anammox bacteria) and complete ammonia oxidizing bacteria (Comammox bacteria) in the last two decades, largely due to the advances in molecular and metagenomic methods introduced to study the microbial ecology. What is interesting to know is that nitrate-transporters of host plants, as in rice, are involved in the assembly of the microbiome associated with roots. Besides, rice plants produce the biological nitrification inhibiting (BNI) compounds released through root exudates. Involvement of diverse microbiome members and the plant genome through nitrate transporters on the rice rhizosphere microbiome assembly necessitates the reappraisal of the nitrogen fertilization management options. This paper also highlights the need for gathering new knowledge on the plant-microbe interactions, from the genome to metabolite levels, and conserving these resources for sustainable rice cultivation.

## Introduction

Prokaryotic microorganisms can react with molecular dinitrogen ($N_2$) to convert it to ammonium, the first step in the biological nitrogen cycle. An alternative to this step is the application of chemical nitrogen fertilizers, which is practiced mainly in intensive agriculture. The nitrogen input in the form of ammonium ($NH_4^+$), nitrate ($NO_3^-$), or urea provides the substrates for microbial processes, such as nitrification and denitrification. The discovery of nitrifying microorganisms by Winogradsky (1891) led to an understanding that the oxidation of ammonia to nitrite to nitrate involves two bacterial functional groups: (i) autotrophic ammonia-oxidizing bacteria (AOB) and (ii) autotrophic nitrite-oxidizing bacteria (NOB). These autotrophic bacteria, along with heterotrophic (bacterial- as well as fungal) nitrifiers, have adverse consequences, depending on the type and amount of N fertilizers applied, such as (i) poor N-fertilizer use efficiency (NUE), (ii) losses of different N forms, (iii) nitrate-contamination of freshwater resources, and (iv) increased nitrous oxide emissions. The century-old, conventional theory about nitrification has been challenged in the last two decades, by the reports on the presence of anaerobic ammonia-oxidizing bacteria (Anammox bacteria, discovered in 1999), ammonia-oxidizing archaea (AOA in 2005), the coupled ferric-iron reducing- and ammonia-oxidizing bacteria (Feammox in 2006), and complete ammonia-oxidizing bacteria (Comammox in 2015) in soils (Hayatsu *et al*., 2021). These discoveries are primarily due to the advances in molecular detection and metagenomic analyses of nitrifying microorganisms. A better understanding of nitrification processes is necessary to create opportunities for improving the NUE and minimizing the adverse environmental impact of intensive cultivation.

Traditionally, rice is cultivated under lowland (waterlogged) conditions where ammonium is considered the major form of available N to plants. The predominantly anaerobic soil conditions in the rice-paddy ecosystem impede nitrate accumulation or retention. However, some ammonium gets oxidized to nitrate in the rhizosphere by the molecular oxygen, which is transported through specialized aerenchyma; nitrate subsequently gets converted to $N_2$ or other gaseous forms. In modern, intensive rice cultivation, urea or ammoniacal fertilizers are applied to increase productivity. About 50-70% of applied N is lost either via volatilization or nitrate leaching, contributing to the poor nitrogen use efficiency (NUE) in rice. The oxidative process of ammonium to nitrate via nitrite can occur in

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**Author for Correspondence: Email-b.ramakrishnan@icar.gov.in ; ramakrishnanbala@yahoo.com* the presence of AOB and AOA, and nitrite to nitrate by the NOB, or ammonium to $N_2$ by the anammox bacteria (i.e., *Candidatus* Brocadia, *Candidatus* Kuenenia, *Candidatus* Anammoxglobus, and *Candidatus* Jettenia) and comammox bacteria (i.e., *Nitrospira* sublineage II). Until the discoveries of these novel microbial entities in the last two decades, nitrification has been considered the foremost and rate-limiting step in the N-loss. The nitrification process has been the key target for mitigating nitrogen loss from agricultural fields. Therefore, synthetic nitrification inhibitors (NIs such as 2-amino-4-chloro-6-methyl pyrimidine, dicyandiamide (DCD), 3, 4-Dimethylpyrazole phosphate (DMPP) and nitrapyrin) are employed to inhibit the activities of nitrifiers from decreasing the accumulation of nitrate in soils and nitrous oxide emissions from soils, including from the rice-paddy fields. Nevertheless, the application of these synthetic NIs has several drawbacks, such as high cost, poor efficiency, increased ammonia volatilization, non-target effects, and the risk potential related to trophic transfer, food safety, and the environment (Subbarao *et al.,* 2006).

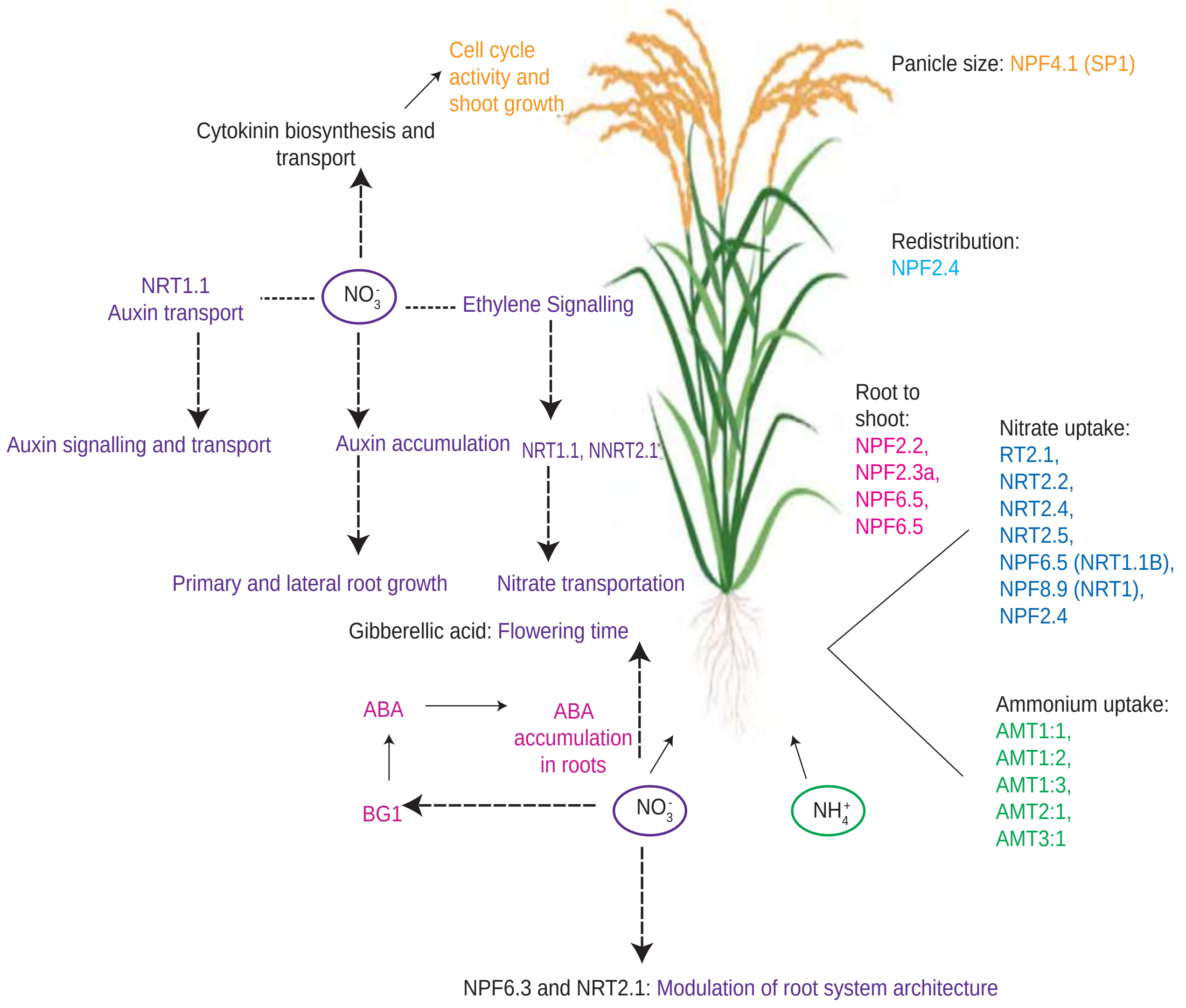
### Nitrification: A Conundrum in the Rice-Paddy Fields

While nitrification inhibition appears promising to prevent oxidative N losses via nitrate, the paradox is that nitrate is another available form of N for plant uptake. Interestingly, nitrate has recently been recognized as an equally important N source as ammonium for rice cultivation (Fig. 1). Even the lowland rice plants can take up about 15–40% of total N in the form of nitrate. Additionally, the plant-microbe interactions related to nitrification have never received enough attention. The rice plant genome has several nitrate transporters, such as nitrate and peptide transporter family (NPF) members (about 93), nitrate transporters (NRT2, 4), and high-affinity nitrate transport-activating protein members (NAR2, 2), suggesting their significant involvement in the N uptake and distribution of N within the plant (Cai *et al*., 2009; Li *et al*., 2017). These nitrate transporters are critical for improving nitrogen use efficiency (NUE) in rice. Many *Indica* rice cultivars exhibit superior growth on nitrate than ammonium, and their combined presence [i.e., $NH_4^+$:$NO_3^-$ at 75:25] acts synergistically. Thus, nitrate availability is equally essential as ammonium for plant uptake. The predominantly anaerobic flooded rice fields are neither conducive to nitrate formation nor nitrate retention. Even if a small amount of nitrate is produced, subsequent denitrification processes prevent nitrate accumulation. Likewise, only a small amount of N is lost via nitrate leaching.

On the contrary, ammonium toxicity is of utmost concern in rice-paddy fields. Increased ammonium levels can suppress the uptake of other cations (e.g. $K^+$, $Ca^{2+}$, and $Mg^{2+}$) and even inhibit plant growth. What is more interesting is that nitrate transporters of rice are a significant factor in the rhizosphere microbiome assembly (Zhang *et al.,* 2019). The rhizosphere microbiome, especially the diversity and composition and functions such as niche creation, resource competition, antagonism, and cooperation, influences the plant growth and development, resistance to pathogens, and stress

**Non-target effects of synthetic nitrification inhibitors (SNIs)**

Synthetic nitrification inhibitors (SNIs) have numerous modulating effects on the non-target organisms, including the loss of diversity and functions of different microbial guilds. For example, higher concentrations of Chloro-methyl-pyrazole phosphate (ClMPP), Dicyandiamide (DCD), and 4-dimethylpyrazolephosphate (DMPP) inhibited the growth of non-target microorganisms. SNIs suppress not only the abundance of ammonia-oxidizing archaea, but also the total population of archaea. Nitrapyrin has substantial effects on the relative abundance of non-target archaea (*Thaumarchaeota*), bacteria (*Proteobacteria* and *Nitrospirae*) and fungi (*Basidiomycota*) in the wheat rhizosphere. Specifically, the abundances of bacteria (*Gaiella, Nitrososphaera* and *Rhodoplanes)* and fungi *(Gliomastix,* and *Ganoderma*) are more susceptible to the nitrapyrin addition (Schmidt *et al.*, 2022). The soil treatment with nitrapyrin leads to the selective enrichment of copiotrophs (bacteria- Alphaproteo bacteria and Betaproteo bacteria; *Ascomycota* fungi) with the suppression of oligotrophs (bacteria – *Acidobacteria* and *Planctomycetes* and *Basidiomycota* fungi). These findings suggest that SNIs can disturb the plant-microbe interactions and natural soil functioning processes that contravene the desired effect of nitrification inhibition.



Nitrate modulates auxin-signalling, biosynthesis, and transport (blue), affecting both primary and lateral root growth. Nitrate induces ABA build up (pink) in the root tips by the upregulation of the gene BG1 that codified the enzyme B-glucosidase 1. Nitrate also controls ethylene signalling (purple) that regulates the expression of essential nitrate transporters, NRT1.1, and NRT2.1. In shoot apical meristem, cytokinin (red) play a role in regulating cell cycle activity which depends on nitrate availability. Similarly, localization of nitrate transporters is highlighted. NRT2.1, NRT2.2, NRT2.4, NPF6.5 (NRT1.1B), NPF8.9 (NRT1), and NPF2.4 are involved in nitrate uptake (blue). NPF2.2, NRT2.3a, NPF6.5, and NPF2.4 participate in root to shoot nitrate translocation (pink). NPF4.1 (SP1) is expressed in the panicle and controls panicle size (orange). AMT1, AMT2, and AMT3 are involved in ammonium uptake (green). Different coloured tagged characters or arrows indicate different transporters and nitrate roles in corresponding physiological processes.

**Fig. 1. Physiological functions of nitrate and localization of nitrate transporters in rice**

tolerance. Hence, excessive ammoniacal N fertilizers or NIs can deny the synergy of plant-microbe interactions in the rhizosphere. Future research should also include the plant-microbe interactions at the phyllosphere to know their influences on the NUE since recent attempts have been to provide nano-N fertilizers through foliar application.

## Chemical Dialogues: Biological Nitrification Inhibitors in the Rhizosphere

In the rhizosphere, plants' roots are closely associated with microorganisms, colonized on the surface and inside the roots as endophytes. These microbial associations are collectively referred to as the microbiome. Since the microbial interactions can be neutral, beneficial, or harmful, the host plant determines the community structure by attracting the desired microorganisms using specific chemical compounds through root exudation. The rhizosphere-associated microorganisms provide additional genetic and functional diversity to the host plants; the rhizosphere microbiome is considered the secondary genome of plants. Interestingly, the root exudates of rice contain a nitrification inhibitor (i.e., 1,9-decanediol) which can inhibit the ammonia monooxygenase (AMO) and there is a strong correlation between the amounts of 1,9-decanediol in the root exudates and the biological nitrification inhibition (BNI) abilities (Sun *et al*., 2016). The regulation of the N cycle in the rhizosphere depends primarily on the plant-microbe interactions. The mechanistic understanding of the microbiome assembly processes, factors that drive the assembly, and niches the specific microbial members can occupy are essential to benefit from these plant-microbe interactions, improve the NUE, and protect the environment sustainably.

## Future Perspectives

1. The chemical dialogues between the rhizosphere microbiome and the host plants require intensive studies. The biological nitrification inhibitors can be superior to the synthetic nitrification inhibitors. It will be better to breed, design, or engineer rice cultivars with higher capacities to modulate nitrification processes for better plant N uptake.
2. The chemical dialogues in the rhizosphere or phyllosphere as the driver of the microbiome assembly also need scrutiny in all the crops to identify the crop-specific stimulators or inhibitors.
3. The beneficial characteristics of BNIs necessitate breeding rice cultivars with desired traits associated with the plant-microbe interactions. The relevant fundamental researches on the identification of genetic loci, specificity, compatibility with other useful traits, and biosynthetic pathways associated with the plant-microbe interactions are critical for making a positive impact in agriculture.
4. Due to their critical roles in the plant-microbe interactions, the microbial genomic resources need conservation and utilization. Future strategies need to include the creation of the genomic database of beneficial microorganisms, besides the culture collections for utilizing them as bio-stimulants or as the sources of genes for use in agriculture.

## References

Cai H, Y Zhou, J Xiao, X Li, Q Zhang and X Lian (2009) Overexpressed glutamine synthetase gene modifies nitrogen metabolism and abiotic stress responses in rice. *Plant Cell Rep.* **28**: 527-537.

Dong D, W Yang, H Sun, S Kong and H Xu (2021) Nitrous oxide emissions in response to long-term application of the nitrification inhibitor DMPP in an acidic luvisol. *Appl. Soil Ecol.* **159**: 103861.

Hayatsu M, C Katsuyama and K Tago (2021) Overview of recent researches on nitrifying microorganisms in soil *Soil Sci. Plant Nutr.* **67:** 619-632.

Li H, B Hu and C Chu (2017) Nitrogen use efficiency in crops: lessons from *Arabidopsis* and rice. *J. Exp. Bot.* **68**: 2477-2488.

Schmidt R, XB Wang, P Garbeva and É Yergeau (2022) The nitrification inhibitor nitrapyrin has non-target effects on the soil microbial community structure, composition, and functions. *Appl. Soil Ecol.* **171**: 104350.

Subbarao GV, O Ito, KL Sahrawat, WL Berry, K Nakahara, T Ishikawa, T Watanabe, K Suenaga, M Rondon and IM Rao (2006) Scope and strategies for regulation of nitrification in agricultural systems—challenges and opportunities. *Crit. Rev. Plant Sci.* **25**: 303-335.

Sun L, Y Lu, F Yu, HJ Kronzucker and W Shi (2016) Biological nitrification inhibition by rice root exudates and its relationship with nitrogen-use efficiency. *New Phytol.* **212**: 646-656.

Winogradsky S (1891) The formation of nitrates in the process of nitrification. In: *Annales de l'Institut Pasteur* (Vol. 5, pp. 577-616).

Zhang J, YX Liu, N Zhang, B Hu, T Jin, H Xu, Y Qin, P Yan, X Zhang, X Guo and J Hui (2019) NRT1.1B is associated with root microbiota composition and nitrogen use in field-grown rice. *Nature Biotech.* **37**: 676-684.