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Accelerating the development of biological nitrification inhibition as a viable nitrous oxide mitigation strategy in grazed livestock systems

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Abstract

This position paper summarizes the current understanding of biological nitrification inhibition (BNI) to identify research needs for accelerating the development of BNI as a N_2O mitigation strategy for grazed livestock systems. We propose that the initial research focus should be on the systematic screening of agronomically desirable plants for their BNI

potency and N_2O reduction potential. This requires the development of *in-situ* screening methods that can be combined with reliable N_2O emission measurements and microbial and metabolomic analyses to confirm the selective inhibition of nitrification. As BNI-induced reductions in N_2O emissions can occur by directly inhibiting nitrification, or via indirect effects on other N transformations, it is also important to measure gross N transformation rates to disentangle these direct and indirect effects. However, an equally important challenge will be to discern the apparent influence of soil N fertility status on the release of BNIs, particularly for more intensively managed grazing systems.

Introduction

Nitrous oxide (N_2O) is a powerful greenhouse gas (GHG) with a global warming potential close to 300 times that of carbon dioxide. Globally, agriculture contributes around 52% of anthropogenic N_2O emissions, with animal urine patches the largest N_2O source in grazed livestock systems (Tian et al. 2020). The inhibition of soil nitrification, which is the conversion of ammonium to nitrate, has been shown to reduce N_2O emissions, with much of the existing understanding of the abatement potential based on studies using synthetic nitrification inhibitors (SNIs; de Klein et al. 2011; Di and Cameron 2016; Minet et al. 2016a; Chadwick et al. 2018). However, there is increasing evidence that plant-induced biological nitrification inhibition (BNI), defined here as attenuation of the nitrification process resulting from the introduction of plant secondary metabolites into the soil through root exudation or turnover of plant tissue, can also reduce N_2O emissions (Subbarao et al. 2013; Byrnes et al. 2017; Villegas et al. 2020). Although SNIs provide the flexibility to target applications at specific times, locations and doses to maximize N_2O reduction, BNIs offer other advantages such as i) a root delivery network reaching into nitrifying sites in the soil, ii) affecting both ammonia mono-oxygenase (AMO) and hydroxylamine oxidoreductase (HAO), two enzymes involved in nitrification (compared to SNI which only acts on the AMO pathway), iii) not requiring synthetic production and mechanical application and therefore potentially lowering costs, iv) potential for continuous formation in growing plants, and v) being more natural, thus offering the potential for greater public acceptance. On the other hand, the effectiveness of BNI relies on soil incorporation of plant tissue containing BNI compounds, or the rhizodeposition of BNI active compounds into the soil. The latter is modulated by many plant-soil interactions and as yet poorly understood (Nardi et al. 2020). We acknowledge that SNI and BNI could be complementary as N_2O mitigation options for grazed livestock systems, but we focus here specifically on the potential of BNIs and their development as a recognized N_2O mitigation strategy for livestock systems. The following sections summarize our current understanding and identify key research needs for accelerating this development along key stages of the innovation pipeline (Figure 1):

(1) Identifying candidate forage species with the genetic capacity to synthesize BNI compounds (discovery)
(2) Maximizing the BNI capacity of these compounds in soils with agronomically viable species (proof of concept)
(3) Managing species within systems to maintain BNI effect and productivity (proof of function)
(4) Implementing systems to incentivize farmers to adopt BNI as a N_2O mitigation strategy (recognized mitigation option)

Discovery: Which source-plants have the genetic capacity to regulate BNI?

Much of the work to date has focused on (sub)tropical systems and common agricultural plants that have been shown to exhibit the BNI trait naturally including *Brachiaria humidicola* (syn. *Urochloa*), wheat, sorghum, maize, rice, (Subbarao and Searchinger 2021), and Elymus grass (Li et al. 2022). There is some evidence that the temperate forb species plantain (*Plantago lanceolata*) may also exhibit BNI effects (Judson et al. 2019). For all these species BNI-active root exudates have been identified and many of these plants have genetic variation in BNI capacity among wild populations and modern cultivars (Navarrete et al. 2016; Nardi et al. 2020; Subbarao et al. 2021). Recent research has also demonstrated that the BNI trait from the wild grass *Leymus racemosus* can be successfully transferred via inter-specific hybridization into elite wheat cultivars without disrupting agronomic features or using regulated gene technologies (Subbarao et al. 2021). Therefore, key elements of success at the 'discovery' stage are that high potency source-plants containing the BNI trait are identified and that interventions to transfer the trait from potentially raw germplasm sources into elite forage cultivars can occur within agronomic constraints. These efforts will benefit from deciphering the fundamental genetic control of BNI traits in source plants, knowledge of the candidate genes influencing BNI trait expression, and highly efficient means of screening for BNI expression in candidate source populations and large-scale breeding populations.

Proof of concept: What is the N_2O reduction potential and how can the BNI effect be maximized?

The reduction potential of N_2O emissions through BNI depends on the microbial community composition, abundance and activity of nitrifiers. The common understanding is that N_2O originating from nitrification is largely produced by ammonia oxidizing bacteria (AOB) and much less so by ammonia oxidizing archaea (AOA) (Prosser et al. 2020). However, a recent study with pure cultures of the AOA *Nitrosopumilus maritimus* showed that this AOA can also produce N_2O from nitrification (Kraft et al. 2022). These authors showed that under the anaerobic conditions of the study, the AOA was capable of generating and re-using oxygen

(O_2) to support their metabolic activity. This suggests that AOA can perform nitrification and produce N_2O under anaerobic conditions. However, the implications of this finding for managed livestock systems requires further investigation, as increased N availability in these systems is likely to favor AOB over AOA (Egenolf et al. 2022). In addition, the relative contribution of AOA vs AOB to nitrification in different ecosystems is not fully understood yet.

N_2O reductions due to BNI have been measured for some key tropical and subtropical grass species, including *Brachiaria humidicola* and Guinea grass (*Megathyrsus maximus*) (Subbarao et al. 2013; Byrnes et al. 2017; Villegas et al. 2020). Byrnes et al. (2017) showed that soils containing a *Brachiaria* cultivar with high BNI capacity emitted 60% less N_2O from urine patches than soils with low BNI capacity cultivars, and Villegas et al. (2020) identified varieties of Guinea grass with high N_2O reduction potentials. Both studies found a direct link between N_2O reduction and BNI, i.e., reduced nitrifier bacteria abundance and nitrification rates. In temperate climate systems, plantain (*Plantago lanceolata*) has been suggested as a species with BNI activity (de Klein et al. 2020), but comprehensive investigation into a direct link between N_2O reduction and BNI activity is lacking. Furthermore, experimental results on the effect of plantain on N_2O emissions from livestock urine are inconsistent, with both reductions and increases in N_2O observed (Luo et al. 2018; Simon et al. 2019; Pijlman et al. 2020; Bracken et al. 2021). It is commonly accepted that BNI is an adaptive mechanism that plants use to conserve mineral nitrogen (N) in soils where the competition between plants and microbes for limited N is high. So, one hypothesis is that the inconsistency in the results from intensively managed systems could be attributable to variation in soil N fertility status, with high soil N fertility possibly downregulating the expression of the BNI trait, and thus the N_2O reduction potential. A recent study indeed suggested that, while BNI seems to determine net nitrification rates in extensive pasture systems with *B. humidicola*, inter- and intracompetition for N between microbes and plants appeared to be the main determinant in intensive systems (Egenolf et al. 2022). However, the impact of soil N fertility status on BNI-trait expression is yet to be systematically investigated; more studies into this effect are needed. This should include experiments under controlled conditions in greenhouses that enable a focus on specific controlling factors, as well as field trials to investigate the impact under grazing conditions. There are also other potential factors that regulate the release of BNI compounds, including soil pH, soil moisture content, soil aeration and nematode activity (Wurst et al. 2010; Zhang et al. 2022), that warrant systematic testing in field studies. As it is difficult to separate BNI effects from other plant effects on soil N transformations and microbial community, such studies should measure gross N transformation rates to disentangle the direct and indirect effects of root exudates on soil nitrification (Nardi et al. 2020; Ma et al. 2021). In addition, studies should combine N_2O measurements with metabolomics and microbial analysis to confirm both the release of BNI compounds as well as nitrifier inhibition of nitrification, thus directly linking any reduction in N_2O emissions with BNI under field conditions.

Proof of function: How can the BNI trait for N_2O reduction be optimized in grazed systems?

Once our understanding of the links between BNI-induced N_2O reduction and soil N status or other regulators is improved, the question is how this can be optimized in grazed livestock systems? This may be especially relevant in legume-containing pasture systems, where there is a strong interaction between soil N fertility status and legume content of the sward, or in grazed systems, where urine deposition results in localized rapid increases in soil N and soil pH. Furthermore, to meet improved productivity as well as environmental outcomes, enhancement of the BNI trait should not compromise the viability of the system through unintended consequences on agronomic characteristics of the species such as, productivity, palatability, nutritional value, persistence, winter hardiness, and drought resilience. To date, there is no evidence to suggest that the BNI-trait has a yield penalty either in pastures or in grain crops when comparing BNI-capable varieties with non-BNI capable varieties of the same species (Subbarao and Searchinger 2021). In addition, a BNI-induced increase in farm N use efficiency (NUE) provides the opportunity to reduce farm N inputs and any associated N_2O emissions. A recent LCA modelling study suggested that the impacts from BNI-wheat with 40% nitrification inhibition by 2050 could reduce both N fertilizer requirements and GHG emissions by about 15%, and improve NUE at the farm scale by almost 17% (Leon et al. 2021). However, there is limited research on the effects of BNI species on soil, rumen and farm level N cycling in grazed systems, which severely limits our ability to assess the full impact of BNI species on farm scale GHG emissions. More specifically, due to the apparent inverse relationship between soil N fertility and BNI, a key question is whether there is a 'sweet spot' of N fertility in managed grazed livestock systems: one that supplies N sufficient to promote exudation of BNI compounds and thus conserve N, yet not too low that plant production is significantly compromised? Another key question is if, and how, the release of BNI compounds is affected by transient changes in soil N and pH in urine patches in grazed systems. In addition, the effect of grazing intensity on soil aeration and root exudation (Sun et al. 2017), and their subsequent impacts on microbial community composition and function also warrant further investigation. Finally, for optimizing BNIs within grazed systems there may be advantages in synthesizing 'BNI active' plant compounds that are delivered to the soil via surface application or in animal feeds (Minet et al. 2016b). Although this would eliminate some advantages of BNIs over SNIs, as discussed above, it could provide a solution in the shorter-term, whilst longer-term plant screening and breeding programmes are developed and root-delivery of BNI compounds is maximized.

Recognized mitigation option: How can farmers be recognized for BNI-induced N_2O reduction in grazing systems?

For farmers to be recognized for achieving N_2O reductions, the effect of the intervention on total N_2O emissions needs to be accounted for in GHG inventory methodologies and on-farm accounting tools. To the best of our knowledge, BNI is not (yet) recognized as a N_2O mitigation technology in national GHG inventories nor in on-farm accounting tools. This not only requires robust evidence of the efficacy of BNI and the ability to predict N_2O reductions under a range of temporal and spatially variable conditions, but it also requires the ability to accurately estimate and record the BNI 'activity' of plants. For BNI-active plants in grazed systems this means being able to demonstrate and verify the effects of their presence in the swards and the conditions that influence their efficacy in N_2O reduction.

Conclusions

For BNI to be successfully exploited as a N_2O mitigation option in grazed livestock systems, we identified key questions along key stages of the innovation pipeline and possible approaches to address these (Table 1). We propose that the initial research focus should be prioritized on the 'discovery' and 'proof of concept' stages. Firstly, the systematic screening of agronomically desirable plants and cultivars to identify their ability to synthesize and exude BNI compounds (i.e., do these plants have the genetic blueprint for BNI?) requires the development of *in-situ* screening methods that can be combined with reliable N_2O emission measurements as well as measurements of gross N transformation rates. To ensure that any N_2O reduction can be assigned to BNI, these measurements should also be accompanied by microbial and metabolomic analyses to confirm the selective inhibition of nitrification. Secondly, whilst understanding the genetic regulation of BNI is a key first step, an equally important challenge will be to discern the apparent influence of soil N fertility status or other soil and climatic factors on the release of the BNIs, particularly for more intensively managed grazing systems. The expansion of an existing BNI consortium (Subbarao and Searchinger 2021) to develop a coordinated global programme to address the research gaps we identified here may be a key step towards accelerating the development of BNI as a N_2O mitigation option in both (sub)tropical and temperate livestock systems.

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Statements and Declarations

Conflict of Interest: The authors declare that they have no conflict of interest

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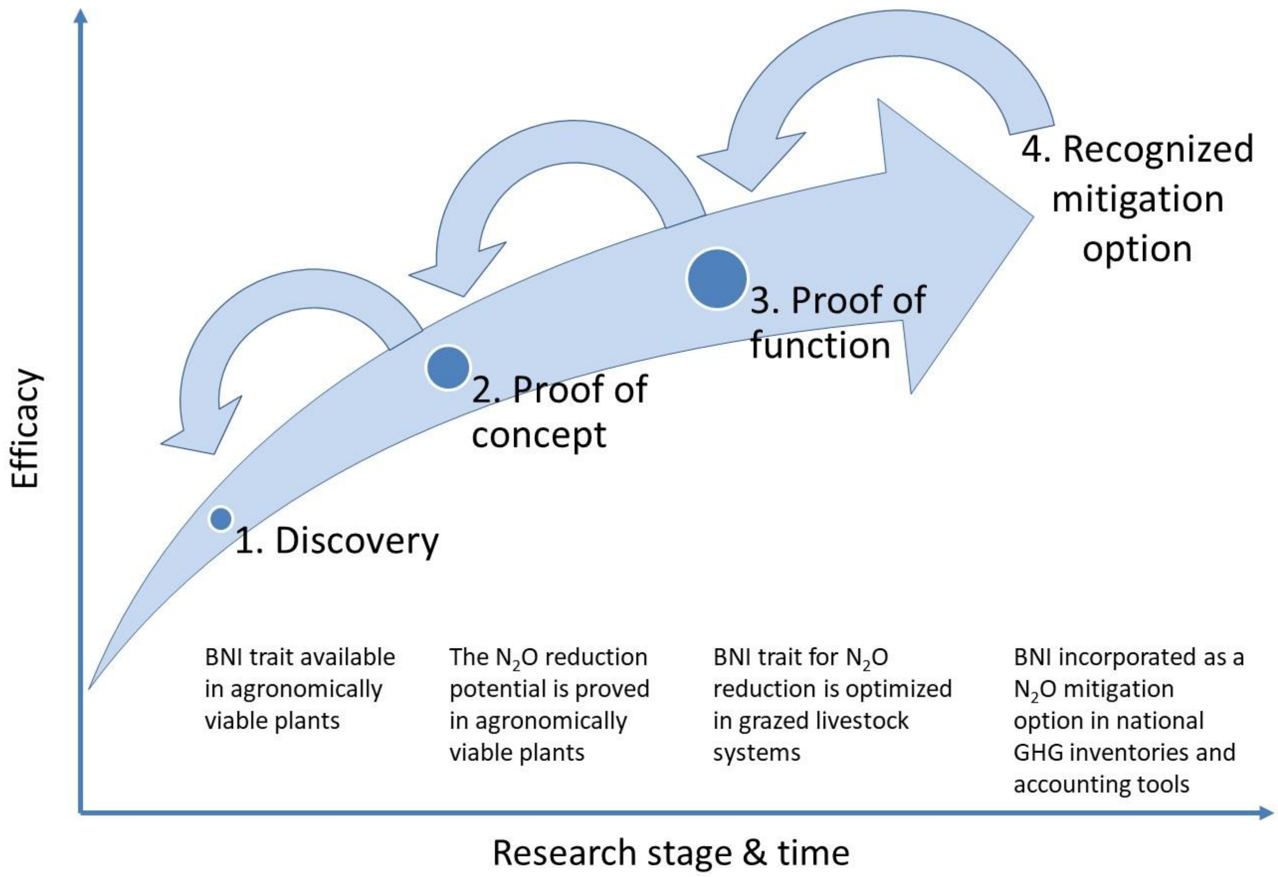

Figure 1: Stages of development pipeline for BNI as a N_2O mitigation option and desired outcome of each stage.

Table 1: Biological nitrification inhibition – key research questions and recommendations for future work for developing BNI into a viable strategy for reduction N_2O emissions from grazed livestock systems.

	1. Stage of development Discovery	2. Proof of concept	3. Proof of function	4. Recognized mitigation option
Desired outcome at each stage	**Existence of BNI trait** Trait is available in agronomically viable plants	**Expression of BNI trait for N_2O reduction** Factors that maximize the expression of the BNI trait in plants are understood. The N_2O reduction potential is proved in BNI capable plants that are agronomically viable.	**BNI trait optimised in systems** BNI-induced N_2O reduction potential optimized in agronomically viable systems	**BNI recognised in calculators** BNI-induced N_2O reduction potential incorporated into national GHG inventories and on-farm GHG accounting tools
Summary of knowledge or capability gaps	Lack of knowledge of high potency sources. Lack of predictive tools to identify candidate sources. Lack of genetic knowledge and rapid screening methods.	Lack of understanding of the drivers of the expression of the BNI trait in grazed systems.	Lack of understanding of the impact of BNI-capable species or mixed swards on other important outcomes. Lack of understanding of impacts of treading-induced changes in aeration and exudation on BNI.	Lack of consistent and robust evidence on BNI technology for mitigating N_2O emissions.
Selected key research questions	What high potency BNI sources are available for pasture species? Are those sources amenable to plant breeding?	What conditions maximize the expression of the BNI trait in agronomically viable temperate species? How does the BNI trait expression change in urine patches, and in legume-based pastures?	How can the use of BNI-capable plants be optimized in livestock systems to optimize agro-economic and environmental benefits? How to balance N fertility to promote BNI expression without compromising yield.	What is the effect of incorporating BNI-capable plants in livestock systems on the annual N_2O emission factors for urine and other N sources?
Approach to fill knowledge gaps	Develop and apply consistent rapid screening methods to survey within and among agronomically viable species for BNI potency and N_2O reduction potential.	Screen BNI-capable plants under different conditions to elucidate relationships between key variables and BNI trait expression to maximize efficacy. Measure N_2O and gross N transformation rates and combine with microbial and metabolomic analysis.	Field studies measuring the effect of the management of BNI-capable plants on a range of agro-economic and environmental metrics. Grazing studies to measure the effect of grazing pressure on soil aeration and root exudation, and subsequently N_2O emissions.	Comprehensive validation under range of conditions quantifying the effect of BNI on N_2O emission factors for urine and other N sources.