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Genetic mitigation strategies to tackle agricultural GHG emissions: The case for biological nitrification inhibition technology

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Citation:

Subbarao, Guntur; Arango, Jacobo; Masahiro, Kishii; Hooper, A.M.; Yoshihashi, Tadashi; Ando, Y.; Nakahara, Kazuhiko; Deshpande, Santosh; Ortiz-Monasterio, Ivan; Ishitani, Manabu; Peters, Michael; Chirinda, Ngonidzashe; Wollenberg, Eva; Lata, J.C; Gerard, Bruno; Tobita, Satoshi; Rao, Idupulapati; Braun, Hans J.; Kommerell, Victor; Tohme, Joe; Iwanaga, Masa. 2017. Genetic mitigation strategies to tackle agricultural GHG emissions: The case for biological nitrification inhibition technology. *Plant Science*. 1-4 p.

Publisher's DOI:

<http://dx.doi.org/10.1016/j.plantsci.2017.05.004>

Access through CIAT Research Online:

<http://hdl.handle.net/10568/81137>

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Manuscript Details

Manuscript number	PSL_2017_82
Title	Genetic mitigation strategies to tackle agricultural GHG emissions: The case for biological nitrification inhibition technology
Article type	Review article

Abstract

ABSTRACT Accelerated soil-nitrifier activity and rapid nitrification are the cause of declining nitrogen-use efficiency (NUE) and enhanced nitrous oxide (N₂O) emissions from farming. Biological nitrification inhibition (BNI) is the ability of certain plant roots to suppress soil-nitrifier activity, through production and release of nitrification inhibitors. The power of phytochemicals with BNI-function needs to be harnessed to control soil-nitrifier activity and improve nitrogen-cycling in agricultural systems. Transformative biological technologies designed for genetic mitigation are needed, so that BNI-enabled crop-livestock and cropping systems can rein in soil-nitrifier activity, to help reduce greenhouse gas (GHG) emissions and globally make farming nitrogen efficient and less harmful to environment. This will reinforce the adaptation or mitigation impact of other climate-smart agriculture technologies.

Keywords global warming; climate change; nitrous oxide emissions; genetic mitigation; soil nitrifier activity, nitrification control; biological nitrification inhibition, greenhouse gas emissions; agro-pastoral systems; pastures; field crops, BNI traits, BNI technology

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Suggested reviewers William Schlesinger, Dave Chadwick, Laurent Philippot, Achim Doberman

Submission Files Included in this PDF

File Name [File Type]

BNI-PS-opinion-CL-submit-2017.docx [Cover Letter]

PlantScience-BNI-Opinion-2017-submitted.docx [Manuscript File]

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Dated 6th February, 2017

Prof. Thomas W Okita

Review Editor - Plant Science

Institute of Biological Chemistry

Washington State University, Pullman, WA 99164-6340; Email: okita@wsu.edu

Sub: Submitting the Commissioned critical 'Opinion' article for publication in 'Plant Science' journal

Dear Prof. Okita:

Please refer our earlier correspondence (your email dated 29th November 2016) suggesting commissioning of an "Opinion" article on BNI (a copy of the email is attached with this cover letter).

Enclosed, please find the files of the manuscript titled 'Genetic mitigation strategies to tackle agricultural GHG emissions: The case for biological nitrification inhibition technology', authored by GV Subbarao, J. Arango, K. Masahiro, A.M. Hooper, T. Yoshihashi, Y. Ando, K. Nakahara, S. Deshpande, I. Ortiz-Monasterio, M. Ishitani, M. Peters, N. Chirinda, L. Wollenberg, J.C. Lata, B. Gerard, S. Tobita, I.M. Rao, H.J. Braun, V. Kommerell, J. Tohme and M. Iwanaga.

I would like to suggest the following subject specialists as potential reviewers for this article.

- Prof. William H Schlesinger, Director, Cary Institute of Ecosystem Studies, Millbrook, NY, USA; Email: schlesingerw@caryinstitute.org
- Prof. Achim Doberman, Director, Rothamsted Research, UK; Email: achim.dobermann@rothamsted.ac.uk
- Dr. Laurent Philippot, Director, INRA, UMR 1229, F-21000 Dijon, France (Email: Laurent.Philippot@dijon.inra.fr)
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- Prof. Cristina Cruz, Dept. Biologia Vegetal, Faculdade de Ciencias de Lisboa, 1749-016 Lisboa, Portugal (Email: cristinacruzoughton@gmail.com)

All the co-authors of this manuscript are members of the recently formed BNI consortium. The discussions during the 2nd International BNI consortium meeting (held at JIRCAS, Tsukuba,

Japan, during September 14-15 2016) led to the development of this critical 'opinion' manuscript draft.

Please keep us informed on the processing of this manuscript.

With kind regards,

Sincerely,

GV Subbarao, Senior Scientist & Group Leader

BNI Research Team, Crop, Livestock & Environment Division, JIRCAS, Japan

cc: all co-authors of this manuscript

From: *Okita, Thomas W* <okita@wsu.edu <mailto:okita@wsu.edu>>

> Date: Tue, Nov 29, 2016 at 8:11 PM
> Subject: RE: Commission for Review Article - Plant Science 'Opinion'
> category article on BNI
> To: Subbarao guntur <subbagv2000@gmail.com
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> **PLEASE INCLUDE A COPY OF THIS EMAIL WITH YOUR SUBMISSION**

>
> Dear Dr. Subbarao, ____

>
> Thank you for your proposal as the scope is just what the journal is
> looking for. Please accept a commission to turn it into a critical
> opinion paper on need to employ genetic strategies
> to mitigate GHG emissions. ____

>
> Please make sure to minimize "cataloging" to the necessary and
> maximize critical reviewing. If there is reason for cataloging -
> try to do it in Tables, and then discuss the principles

>
> in the text, and not each individual case. Please update the
> literature since the publication of your 2015 Plant Science Review
> article. ____

>
> As I mentioned in my earlier email, we would like to have plenty of
> extrapolation, synthesis and speculation in reviews. Go beyond the
> facts to the implications, and describe missing

>
> experiments, etc. needed to advance the field, but clearly delineate
> where facts end and speculation begins. ____

OPINION article

**Genetic mitigation strategies to tackle agricultural GHG emissions:
The case for biological nitrification inhibition technology**

***G.V. Subbarao^a, J. Arango^b, K. Masahiro^c, A.M. Hooper^d, T. Yoshihashi^a, Y. Ando^a, K. Nakahara^a, S. Deshpande^e, I. Ortiz-Monasterio^c, M. Ishitani^b, M. Peters^b, N. Chirinda^b, L. Wollenberg^f, J.C. Lata^g, B. Gerard^c, S. Tobita^a, I.M. Rao^b, H.J. Braun^c, V. Kommerell^c, J. Tohme^b and M. Iwanaga^a**

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ABSTRACT

Accelerated soil-nitrifier activity and rapid nitrification are the cause of declining nitrogen-use efficiency (NUE) and enhanced nitrous oxide (N₂O) emissions from farming. Biological nitrification inhibition (BNI) is the ability of certain plant roots to suppress soil-nitrifier activity, through production and release of nitrification inhibitors. The power of phytochemicals with BNI-function needs to be harnessed to control soil-nitrifier activity and improve nitrogen-cycling in agricultural systems. Transformative biological technologies designed for genetic mitigation are needed, so that BNI-enabled crop-livestock and cropping systems can rein in soil-nitrifier activity, to help reduce greenhouse gas (GHG) emissions and globally make farming nitrogen efficient and less harmful to environment. This will reinforce the adaptation or mitigation impact of other climate-smart agriculture technologies.

1. Introduction

Agriculture has become the largest source of man-made greenhouse gases (GHGs) on the planet [1]. It generates 14,000 Tg CO₂.eq.yr⁻¹, about 24% of total GHG emissions [1]. To put this in perspective, CO₂ emissions from automobiles contribute to 14% of global GHG emissions [1-2]. A major portion of agricultural GHG emissions is associated with the production and use of nitrogen (N-fertilizers, based on life-cycle analysis), which is energy and carbon intensive [2]. It is ironic that nearly 70% of N-fertilizers applied to agricultural soils is lost and returned to atmosphere as oxides of N and N₂ (through microbial nitrification and denitrification processes), before the crops can absorb and assimilate it into plant protein with no net benefits to humans [3]. Nearly 80% of global emissions of nitrous oxide (N₂O), a GHG 300 times more potent than CO₂, comes from the production and utilization of N-fertilizers in agriculture [4]. Providing farmers with new nitrogen-use efficiency options requires a major research and development effort, in combination with effective extension approaches.

1.1. The Paris Climate Agreement

With global food demand projected to double by 2050, agricultural emissions will grow further, unless agriculture becomes climate-smart [1]. Annual N-fertilizer use is expected to reach 300 Tg by 2050; global N₂O emissions will double compared with present levels and reach 7.5 Tg N₂O-N in such a 'business as usual' scenario [4,5,6]. The Paris Agreement (PA) signed in 2015, set the goal to reduce GHG emissions by 80% from 2005 levels by 2050 to limit global temperature rise to <2°C [7-8]. Reducing GHG emissions from agriculture is thus critical to meeting PA emission targets [7].

1.2. Global cropping intensification to maximize yield resulted in weakened soil-health

Development of fertilizer-responsive crops (e.g. semi-dwarf wheat, -rice, and maize) has transformed global cereal production, but inadvertently unleashed a cascading effect of N-pollution in the environment [8,9]. Farmers in many intensive production systems are being forced to apply more N-fertilizer to sustain higher yields. Selection and breeding under high N-input environments and crop intensification have resulted in the development of nitrate (NO_3^-)-responsive cultivars and high-nitrifying soil environments, leading to a decline in NUE (<30% at present) in crop production [3,10-11]. Nitrate leaching and N_2O emissions are an indication of weakening soil health (due to declining soil-carbon levels and shifts in soil microbial ecology conducive for accelerated nitrifier-activity) [10-11]. We need a course correction now to increase food production, whilst improving soil health and minimizing GHG emissions.

1.3. The need for genetic mitigation to tackle N_2O emissions

Genetically enhanced mitigation technologies that are easily deployable and scalable, to reduce nitrification and N_2O emissions, would make agricultural systems more N-efficient and reduce emissions. Biological nitrification inhibition (BNI) is the ability of certain plant roots to suppress soil-nitrifier activity, through production and release of biological nitrification inhibitors (BNIs) [3]. BNI is a natural plant behavior, found in certain climax ecosystems where plants and microbes compete fiercely for limited mineralized soil-N [12-13]. We should learn from nature and introduce these biological mechanisms to manage N-cycling in agricultural systems. Plant roots produce BNIs to suppress nitrifier activity (which converts immobile soil-ammonium (NH_4^+) to mobile

soil-nitrate (NO_3^-) and retain soil-N in NH_4^+ form to facilitate plant absorption and transfer into immobile microbial/organic-N (Fig. 1) [3,10]. Soil- NO_3^- , once formed, is highly prone to leaching, and is also a substrate for soil denitrifying microbes that convert it into N_2O , NO (nitric oxide) and ultimately N_2 gas [3] (Fig. 1) - a net loss for plant production. N_2O is primarily produced during both nitrification and denitrification processes [3] and BNI function suppresses N_2O emissions by reducing nitrification and limiting NO_3^- availability to denitrifiers (Fig. 1) [3,10]. The challenge is to redesign agricultural systems with crops and pastures that produce sufficient BNIs from root systems to suppress wasteful nitrification processes, increase N-flow to the plant and retention in soils, thus significantly improving nitrogen-use efficiency [3,14]. The power of BNI-enabled phytochemical secretions/additions from crop/pasture root systems should be unleashed to limit GHG emissions while sustaining future growth in food production.

2. BNI technology to benefit agriculture and the environment

BNI technology exploits the understanding of BNI chemistry, and its impact on the soil microbiome, to develop genetic components that include BNI-enabled genetic stocks and genetic tools. These would facilitate introduction of BNI traits into major food and forage crops in the near future [3,10,14-18]. Production and release of BNIs from plant roots require the presence of NH_4^+ in the rhizosphere and soil-microsites where NH_4^+ is present, which are also the hot-spots for nitrifier populations [3,10,14,19]. As the BNIs release from roots is localized (i.e. BNI release is confined to parts of the root system exposed to NH_4^+)[14], the delivery of BNIs is thus essentially targeted to where there is a high probability of nitrifier-activity. In addition, sustained release of BNIs from root

systems is functionally linked with the uptake and assimilation of NH_4^+ , which acts as a switch mechanism for BNI function. This results in a more effective delivery of BNIs to soil-nitrifier sites in the field [20-21]. In addition, the diverse chemical structures of BNI molecules and their multi-mode of inhibitory action on *Nitrosomonas*, could provide a lasting-control over nitrifier activity in agricultural soils compared to synthetic nitrification inhibitors [3,22]. The inhibitory effect from synthetic nitrification inhibitors does not last more than a few weeks at the most (often less than a week) and their delivery in the field is fraught with many challenges. They are expensive to apply and are often ineffective in the field, which may explain the lack of their wide-spread adoption by farmers [23]. BNI technology is suitable for integrated crop-livestock and cropping systems.

2.1 Crop-Livestock systems

Brachiaria grasses are the most widely planted forage crops in the tropics with as many as 100 million hectares planted as pastures in Brazil alone [24]. Among forage crops tested, *Brachiaria humidicola* has the highest BNI-capacity and produces brachialactone (a powerful nitrification inhibitor) in its deep-root systems [14]. Each year, from root turnover alone, well-managed *Brachiaria* pastures could add 14 kg brachialactone ha^{-1} and enrich the soil-C by up to 5 t ha^{-1} [25]. In addition, nearly 2.6 to 7.5 million units of BNI-activity $\text{ha}^{-1} \text{d}^{-1}$ (depending on the genetic stock) is released from roots, equivalent to annual additions of 6.2-18kg of nitrapyrin ha^{-1} (a synthetic nitrification inhibitor) [10,14]. Field studies with *Brachiaria* grasses showed that while they suppressed nitrification and N_2O emissions [14], the reduced nitrifier activity has improved ^{15}N -retention in soils, ^{15}N -recovery and NUE of maize in an integrated maize-

Brachiaria (crop-livestock) system for several years [26-27].

2.2. Cropping Systems

Sorghum, a climate-smart cereal, releases sorgoleone from its roots, which mediates BNI-activity [15,28]. Genetic improvement for enhanced levels of sorgoleone release is one route to develop BNI-enabled cereal production [3,10]. Wheat, the most important food crop (grown on 240 million ha globally), uses about 20% of all fertilizer applied globally [16-17]. However, modern wheat cultivars do not have strong BNI-activity in their root systems [16-17]. Development of BNI-enabled wheat varieties using wild relatives or progenitors as sources of effective BNI-traits can be achieved using chromosome engineering [16-17]. Wheat yield potential can be doubled from present levels to reach 20 t ha⁻¹, but requires substantial improvements in NUE to make this economically attractive. The potential for improving BNI-capacity in wheat, sorghum and *Brachiaria* pastures has been illustrated [3,16-18].

2.3. Deploying BNI technology

Mitigation strategies/technologies to reduce agricultural GHG emissions must be cost-effective and politically feasible to implement if they are to be adopted widely to reduce costs and deliver benefits to society. For example, mitigation technologies such as alternate wetting and drying in paddy fields can be challenging to implement for social and political reasons [29]. Similarly, the patchy distribution of urine-N (a major N source) in grazed grasslands makes it difficult to control N-losses using synthetic nitrification inhibitors [6]. With 220 million cattle in Brazil alone [30], N-inputs from urine are estimated at 12.8 Tg N y⁻¹ (based on the assumption that the average cow

excretes 160 g N in its urine per day) and nearly 90% of this N is lost due to rapid nitrification and denitrification [3,6]. BNI-enabled pastures can effectively suppress these nitrification associated N-losses [6,14]. When bovine urine was applied to high-BNI *B. humidicola* (CIAT 679) pastures in the field, N₂O emissions were 60% less compared to low-BNI *Brachiaria* (*Brachiaria* hybrid 'Mulato') pastures [31]. In Brazil, the potential impact on N-losses and N₂O emissions from bovine urine N-inputs that may result from replacing low-BNI and/or degraded *Brachiaria* pastures with high-BNI *Brachiaria* pastures could be high. BNI-technology, could become an important piece in the puzzle to render agriculture more nutrient and resource-efficient, while protecting the environment. Breeding BNI-enabled food crops and forages and integrating these BNI-enabled components into crop-livestock systems could be the key genetic mitigation option to reduce N₂O emissions. This genetic mitigation technology can be deployed without additional cost to the farmers, and is easy to adopt and scalable, as it does not require specialized or additional farm equipment or changes in water management.

2.4 The case for policy change

The PA came into force in November 2016; COP22 (Conference of Parties; organized in Marrakech, Morocco) initiated deliberations to assess technological options (i.e. those available or that can be developed in the near future) and develop the required policy framework to advance implementation of the PA agenda. Breeding crop varieties with BNI-traits and development of BNI-enabled production systems may take up to 30 years (that includes delivery, time for adoption and for deployment) and requires a major change in the direction of agricultural research. It could be funded from part of

the earmarked funds (i.e. about 150 billion US\$ per annum) to implement the PA agenda. A policy decision at this stage is thus necessary to identify suitable potential technologies that can transform the agricultural sector by improving NUE and facilitate tightening of N-cycling in agricultural systems to reduce GHG emissions; BNI-technology could be considered as one of the key biological options.

3. Outlook

Current agricultural practices need transformative changes. Other sectors, e.g. industry, energy production and transport are making major progress in increasing efficiency (thereby reducing GHG emissions), due to technological advances. New biological technologies must be developed for the agriculture sector to improve soil-N residence time and reduce N-losses to improve N-efficiency, which requires a tight control over soil-nitrifier activity. In addition, a closer coupling of crop and animal husbandry is needed to facilitate the recycling of organic-N through agricultural soils and reduce annual increases in N-fertilizer use. Nearly 175 Tg of fixed-N (biologically fixed-N from legumes + industrially fixed-N as N-fertilizer) enters into agricultural systems annually, but <1% of this Nr (reactive-N) is retained in human bodies. The remainder is returned to the atmosphere through nitrification and denitrification processes (as NO_x and N₂ gas, strongly impacting human health, ecosystem functions, and contributing to climate change), which in turn drives year-on-year increases in N-fertilizer application to sustain food production [3,10]. The economic value of this wasted Nr alone from agricultural systems is estimated at US\$ 81 billion per year [9]. For example, the European Union, which consumes only 11 Tg Nr (N-fertilizer) annually, faces major challenges from N pollution on human health and ecosystems in economic-terms that

1 reaches US\$ 102-320 billion y^{-1} [32]. When considering agricultural production in low
2 and middle-income countries with high population growth rates, global damage to
3 ecosystems and human health from Nr pollution could therefore be enormous. We
4 should not treat agriculture as merely a commodity-producing industry with profit as the
5 *sole motto*, but manage agriculture as part of a larger ecosystem that provides life-
6 support and services to human society. We need to ask ourselves why is 99% of the Nr
7 that enters into farming systems each year allowed to return to the atmosphere [33],
8 without being productively rerouted through agricultural soils and cycled back into
9 sustainable agri-food systems.

10
11 A fundamental shift is needed in the way Nr is managed in agricultural systems to
12 curtail the increasingly insatiable 'soil-hunger' for N fertilizers. This requires the
13 introduction of novel BNI-traits into main-stream breeding, coupled with changes in
14 crop management and integrated crop-livestock systems to limit soil-nitrifier activity.
15 Suppressing soil-nitrifier activity can have a cascading effect on soil-N retention, soil
16 organic matter buildup and shifts in microbial ecology that, over time, can help improve
17 soil health [3,10]. The second Green Revolution must integrate plant traits that improve
18 soil health, in addition to traits that enhance yield potential and stability. While the
19 scientific goals of using BNI for a better NUE are inextricably linked to the
20 amelioration of the worst predictions of GHG production and potential changes in
21 climate, few farmers will change their practices for the altruistic goals of reducing their
22 C-footprint and N_2O generation. However, the bottom line of protecting biologically
23 fixed or synthetic-N supplies through BNI-technologies means that less N-fertilizer is
24 required for the same yield, and the gross excesses of some practices can be reined in by

demonstrating that increased application is an unnecessary cost. Money talks in the end.

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Figure Captions

Fig. 1. Biological nitrification inhibition where plant root systems produce nitrification inhibitors to suppress nitrifier activity in soils to reduce NO_3^- formation, facilitate NH_4^+ immobilization, plant uptake of NH_4^+ and reduction of N_2O emissions.

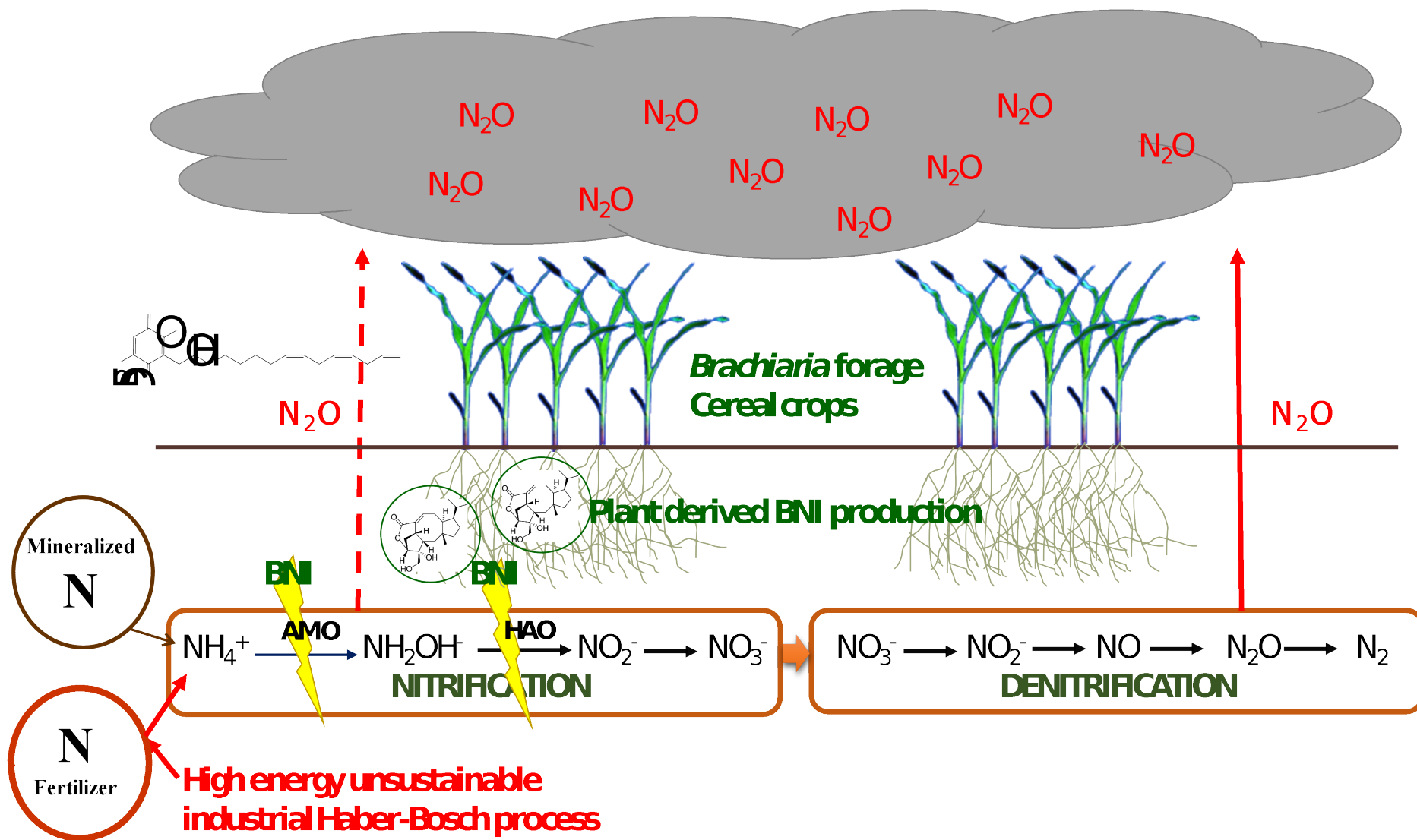


Fig. 1.

1
2 **Title**

3 **Genetic mitigation strategies to tackle agricultural GHG emissions:**
4 **The case for biological nitrification inhibition technology**
5

6 **Highlights**

7 Accelerated soil-nitrifier activity and rapid nitrification are the cause of declining NUE and
8 enhanced N₂O emissions from farming. Transformative biological technologies designed for
9 genetic mitigation are needed where BNI-enabled crop-livestock and cropping systems can rein
10 in nitrifier activity, reduce GHG emissions and make farming N-efficient.
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