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Review

Suppression of soil nitrification by plants

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ABSTRACT

Nitrification, the biological oxidation of ammonium to nitrate, weakens the soil's ability to retain N and facilitates N-losses from production agriculture through nitrate-leaching and denitrification. This process has a profound influence on what form of mineral-N is absorbed, used by plants, and retained in the soil, or lost to the environment, which in turn affects N-cycling, N-use efficiency (NUE) and ecosystem health and services. As reactive-N is often the most limiting in natural ecosystems, plants have acquired a range of mechanisms that suppress soil-nitrifier activity to limit N-losses *via* N-leaching and denitrification. Plants' ability to produce and release nitrification inhibitors from roots and suppress soil-nitrifier activity is termed 'biological nitrification inhibition' (BNI). With recent developments in methodology for *in-situ* measurement of nitrification inhibition, it is now possible to characterize BNI function in plants. This review assesses the current status of our understanding of the production and release of biological nitrification inhibitors (BNIs) and their potential in improving NUE in agriculture. A suite of genetic, soil and environmental factors regulate BNI activity in plants. BNI-function can be genetically exploited to improve the BNI-capacity of major food- and feed-crops to develop next-generation production systems with reduced nitrification and N_2O emission rates to benefit both agriculture and the environment. The feasibility of such an approach is discussed based on the progresses made.

Contents

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1. Introduction

Nitrification, a critical aerobic process that evolved about 2.5 billion years ago, was considered a relatively minor component of the N-cycle until about 50 years ago, when synthetic fertilizer applications in agriculture became widespread [1]. Two groups of soil microorganisms, ammonia-oxidizing bacteria (mainly *Nitrosomonas* spp. and *Nitrosospira* spp.) and ammonia-oxidizing archaea, are largely responsible for the biological oxidation of NH_4^+ to NO_3^- [2,3]. Cationic ammonium is electrostatically held by negatively charged clay surfaces and functional groups of soil organic matter (SOM), and often remains bound to the soil. In contrast, anionic NO_3^- does not bind to the soil and is prone to leaching from the root zone. Several heterotrophic soil bacteria denitrify NO_3^- under anaerobic or partially anaerobic conditions and produce nitrous oxide (N_2O), a colorless gas known as 'laughing gas'. However, N_2O emissions from agricultural systems is no laughing matter as N_2O is a powerful greenhouse gas with global warming potential 300 times greater than that of CO_2, and is the third most important contributor to global warming [4–6]. Nearly 70% of global N_2O emissions come from agricultural ecosystems, where nitrification and denitrification are the major biological processes responsible for its production [7–9]. N_2O levels in the atmosphere are increasing at an alarming rate and are expected to quadruple by 2050 [10–13], unless measures are taken to reduce such emissions.

1.1. Nitrification: A biological process of critical importance for the sustainability of agricultural systems with implications for climate change

Nitrogen fixation, SOM mineralization, immobilization, ammonification, nitrification, and denitrification are the major processes/pathways of the N-cycle in soils (Fig. 1). Nitrification has a relatively minor role in undisturbed ecosystems, whether temperate or tropical as they retain large amounts of N and minimize N-leakages from these systems. Nitrification in some natural systems seems severely restricted, but the underlying mechanism(s) governing N-flow is still poorly understood [14,15]. For example, polyphenols released from leaf litter in certain pine forests can form complexes with dissolved organic N [16]. These organic-N-polyphenol complexes resist soil mineralization, but are absorbed by ecto-mycorrhizae colonizing pine root systems where they are mineralized and supplied to the pine host, thereby tightly regulating N-flow within such ecosystems [17,18]. A range of N conserving mechanisms have evolved in natural ecosystems including direct uptake of organic N by plants (by short-circuiting mineralization) and suppression of nitrification. These mechanisms essentially close the N cycle and facilitate soil-N buildup [18–23].

Unlike most undisturbed ecosystems, modern intensified agricultural systems typically have open N cycles, and have become extremely leaky and inherently inefficient [22,24–28]. While less than 10% of total N undergoes nitrification in undisturbed ecosystems [29], over 95% of total N flows through the nitrification–denitrification pathway in modern production systems [30]. High-nitrifying soil environments in modern production systems are largely responsible for low-N recovery and low-NUE [30,31]. The intensification of agricultural production systems and the decoupling of crop production from livestock operations have disrupted nutrient cycling, depleted SOM stocks, altered soil physical and chemical properties, and driven major shifts in soil microbial activity, resulting in the creation of present high-nitrifying soil environments where NO_3^- accounts for >95% of crop N uptake [10,30–33]. In addition, soil microbial biomass and its nutrient-cycle regulation power has been severely weakened in modern agricultural systems, leading to de-synchrony between soil-N mineralization and plant N demand [34].

Soil nitrification rates have indeed increased several-fold in modern production systems compared to traditional agricultural systems [31,35–37]. Our studies with Alfisols managed under traditional farming practices, i.e. rainfed cropping [Alfisol-rainfed – only single crop is grown during rainy season with limited fertilizer inputs and rotating periodically with legumes] or under irrigated conditions [Alfisol-irrigated – Full irrigation with liberal fertilizer regimes to raise two to three crops per year] over 30 years showed a 5-fold increase in soil nitrification rates in Alfisol-irrigated fields compared to Alfisol-rainfed fields (Subbarao and Sahrawat, Unpublished research, 2013), reinforcing that intensification of agricultural practices resulting in hyper soil-nitrifier activity and accelerated nitrification rates. Despite all the advances in agronomic management of N applications in production agriculture, nearly 70% of N-fertilizer applied to production systems is consequently lost through NO_3^- leaching and gaseous N-emissions (N_2O, NO and N_2) [28,38,39]. The NUE (weight of cereal grain produced per weight of N fertilizer applied) in cereal production systems has accordingly declined from about 80 in 1960s to 20 at present [32], suggesting diminishing returns from N-fertilizer applications. Synthetic nitrification inhibitors were developed in the 1960's, but they have not been widely adopted due to inconsistent performance and lack of economic viability for their use in production agriculture [40]. Urea is the most commonly used nitrogen fertilizer in production agriculture, hydrolyzes (within 24 h from application to the soil by enzyme 'urease' produced by soil bacteria) and releases ammonia, and the nitrogen becomes available to the plant. Urease inhibitors such as NBPT [N-(n-butyl) thiophosphoric triamide, also known as 'agrotarin'] is available and extensively tested in production systems, but has not been adopted due to reasons similar for nitrification inhibitors [30,31,40]. Fertilizer-N use is projected to double and is expected to reach 300 Tg N y^{-1} by 2050 [11,39]. Nitrogen lost from NO_3^- leaching is likely to reach 61.5 Tg N y^{-1} [11], while N_2O emissions are projected to reach 17 Tg N y^{-1} [10,11,41] unless measures are taken to reduce these emissions. These projections suggest that N pollution is reaching a tipping point and that urgent action is needed to improve NUE in production agriculture and minimize N leakages [42].

2. Biological nitrification inhibition (BNI)

2.1. The BNI concept

The ability of certain plant roots to produce and release nitrification inhibitors to suppress soil-nitrifier activity is termed 'biological nitrification inhibition' (BNI). As nitrification is the most important process determining N-cycling efficiency (i.e. proportion of N retained in the ecosystem during a complete cycling loop), restricting nitrification will minimize N-leakage and facilitate N-flow through NH_4^+ assimilation pathways [30]. Most plants and microbes have the ability to utilize NH_4^+ or NO_3^- as mineral-N source [43]; yet, few studies have integrated plant utilization of these N-forms on ecosystem functioning [44]. Suppressing soil-nitrifier activity thus, should not limit the availability of inorganic-N for plant growth or soil microbial activity. Moreover,

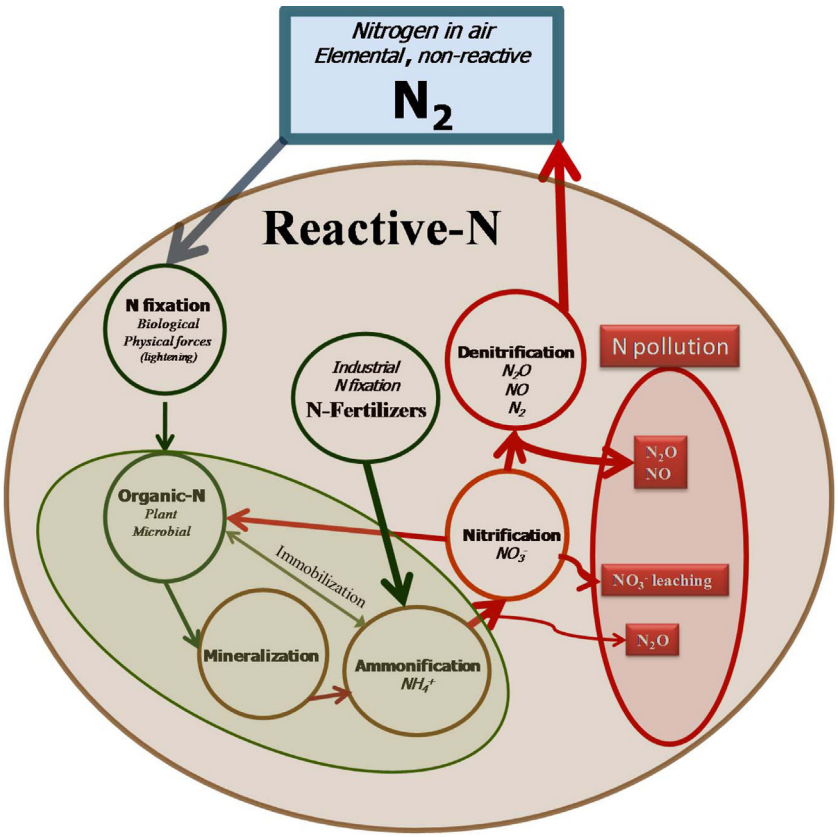

Fig. 1. Major processes of nitrogen cycle in soils.

H^+ is released by plant roots when NH_4^+ is absorbed and assimilated, leading to the acidification of the rhizosphere, which improves P availability to growing plants [45].

Agronomic NUE ($NUE_{agronomic}$ = grain yield per unit of applied N) is a function of both intrinsic-NUE ($NUE_{intrinsic}$ = dry matter produced per unit N absorbed), HI (harvest index) and N uptake [46]. $NUE_{intrinsic}$ is physiologically conserved [47]; thus, improvement in $NUE_{agronomic}$ can only come from improvements in crop-N uptake, which is largely a function of recovering applied fertilizer-N [48]. BNI function in plants impacts $NUE_{agronomic}$ by improving N uptake, facilitate N-retention and reduce N-losses associated with nitrification–denitrification processes [30,49], a conclusion further supported by both field and modeling studies [44,50–53].

Recent methodological developments have facilitated the detection and quantification of nitrification inhibitors from plant roots using a recombinant luminescent *Nitrosomonas* construct [54,55]. The recombinant strain of *N. europaea* carries an expression vector for the *Vibrio harveyi luxAB* genes (Fig. 2) and produces a distinct two-peak luminescence pattern during a 30-s analysis period [55]. The functional relationship between bioluminescence emission and nitrite production in the assay has been shown to be linear using a synthetic nitrification inhibitor, allylthiourea (AT) [55]. The inhibition caused by 0.22 μM AT in assay (about 80% inhibition in bioluminescence and NO_2^- production) is defined as one allylthiourea unit (ATU) [55]. Using the response to a concentration gradient of AT (i.e., standard dose-response curve), the inhibitory effects of root exudates, soil or plant extracts are determined and expressed in ATU [55]. These research methodologies have facilitated the evaluation and characterization of BNI-function in plants [55]. Soil-based assays to determine changes in nitrification potential in the rhizosphere [56] and analysis of nitrifier populations can further complement these efforts to characterize BNI function [49,57].

2.2. Evidence for BNI-function

Most plants release chemical compounds from root systems that either stimulate or suppress nitrifier activity. The assay system based on recombinant luminescent *Nitrosomonas* can be used to detect and quantify nitrification inhibitors (i.e. BNI-activity) or stimulators (i.e. negative BNI-activity) released from roots [54,55]. The root exudates of most legumes (*Glycine max*, *Vigna unguiculata* and *Phaseolus vulgaris*) did not have detectable inhibitory activity in the assay, whereas most cereals evaluated have varying levels of inhibitory activity in the assay [58]. Isoflavones such as genistein and daidzein found in soybean root exudates have stimulatory effect on *Nitrosomonas* when tested in the assay (GV Subbarao, unpublished results). Tropical pasture grasses that are adapted to low-N environments, in particular *Brachiaria* spp. have the highest BNI-activity in root systems [58]. In contrast, *Panicum* spp. adapted to high-N (in comparison to *Brachiaria* spp.) environments have relatively weak BNI-activity in their root systems [58,59]. Among field crops, sorghum (*Sorghum bicolor*) adapted to low-N input environments appears to have stronger BNI-capacity than crops adapted to high-N input environments such as wheat (*Triticum aestivum*) and maize (*Zea mays*) [58]. Nitrification inhibition is likely an adaptation mechanism to retain and use N efficiently in N limiting natural systems [30,52,59,60]. It is not surprising that N-fixing legumes have low BNI-capacity in root systems as BNI-function may have no adaptive value to them; indeed BNI may favor the attraction of non-legume competitors in N-limiting environments [58]. However, the intensity of N-fixation itself depends on N availability [61], and therefore should be modified by the BNI-capacity of other

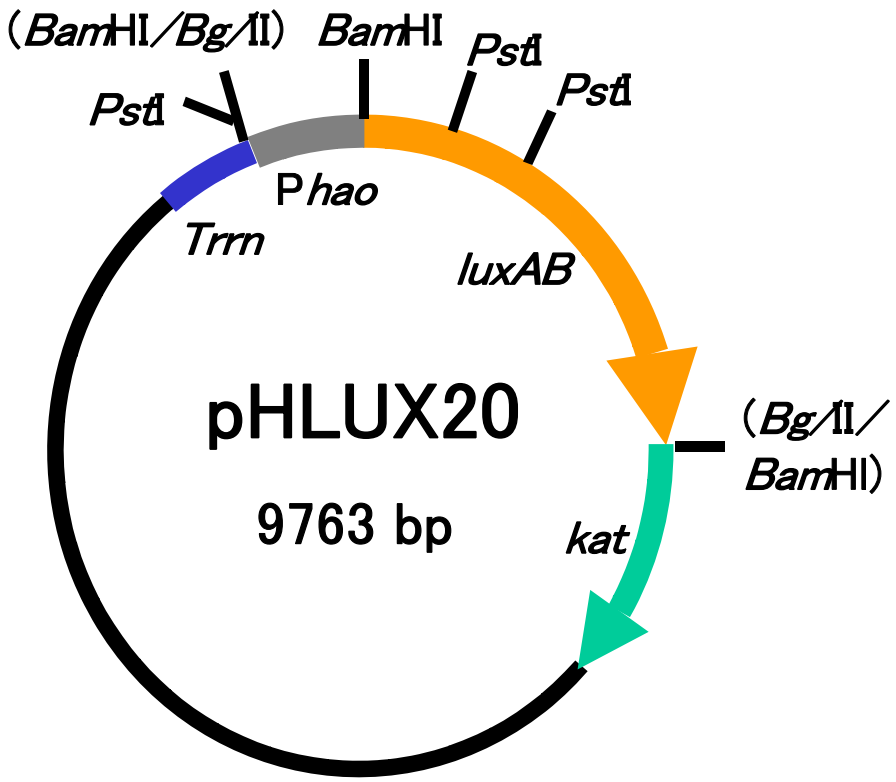

Fig. 2. Map of recombinant luminous *Nitrosomonas europaea* (pHLUX20) developed to detect and quantify nitrification inhibitors in the plant–soil system [54].

plants, which raises questions about the evolution and plasticity of this process in complex plant communities.

2.3. Chemical identity of BNIs and their mode of action

Several BNIs have been isolated from root exudates and plant tissues (root and shoot) of *B. humidicola* and sorghum [49,55,62–64] (Table 1). These BNIs have chemical structures belonging to diverse functional groups – fatty acids [linoleic acid, and linolenic acid], phenylpropanoids [Methyl 3-(4-hydroxyphenyl)propionate (MHPP), methyl-*p*-coumarate, and methyl ferulate], flavonoids [sakuranetin and karanjin, quinones [sorgoleone], diterpenoids [brachialactone] [30] and isothiocyanates [2-propenyl-glucosinolate, methyl-isothiocyanate, 2-propenyl-isothiocyanate, butyl-isothiocyanate, phenyl-isothiocyanate, benzyl-isothiocyanate, butyl-isothiocyanate, phenyl-isothiocyanate, benzyl-isothiocyanate and phenethyl-isothiocyanate] [68–70].

Dominant compounds such as hydrophilic-brachialactone released from *B. humidicola* roots or hydrophobic-sorgoleone released from sorghum roots account for a major portion (>80%) of BNI-activity in those species [30,49,65]. The sorgoleone biosynthetic pathway is known and its genetic control is well-understood [71,72]. In contrast, the biosynthesis pathway for brachialactone is still unknown. Brachialactone has a dicyclopenta[a,d]cyclooctane skeleton (5–8–5 ring system) with a γ-lactone ring bridging one of the five-membered rings and the eight membered ring [49,73]. Certain fungi and plants have the ability to synthesize 5–8–5 tricyclic terpenoids such as ophiobolanes and fusicoccanes [73–75], but the lactone ring of brachialactone is a novel cyclic diterpenoid. Fusicoccin type cyclic diterpenes are biologically synthesized from geranylgeranyl diphosphate by a two-step cyclization catalyzed by terpene cyclases [75]. In higher plants, terpenoid biosynthesis is through either HMG-CoA reductase pathway (mevalonic acid pathway, located in cytoplasm) or 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate pathway (MEP/DOXP pathway, located in plastids) [76,77]. Operon-like gene clusters control the biosynthesis pathways of certain diterpenoids such as 'momilactone' in rice [76–80], or certain phytoalexins such as sakuranetin [81]. If such an operon cluster for brachialactone biosynthesis is identified, metabolic engineering of brachialactone biosynthesis and introduction of BNI-capacity into root systems of major food crops using transgenic approaches may be possible.

Crude BNI-activity extracted from root exudates or plant tissues is likely composed of a cocktail of nitrification inhibitors, each with a single mode or in some cases multi-mode of inhibitory effects on enzymatic pathways of *Nitrosomonas* [30]. BNIs such as linoleic acid, linolenic acid, sorgoleone and brachialactone inhibit *Nitrosomonas* through blocking of both ammonia mono-oxygenase and hydroxylamine oxidoreductase enzymatic pathways involved in ammonia oxidation in *Nitrosomonas* [49,62,65]. Also, some BNIs (e.g. sorgoleone) could disrupt the crucial electron transfer pathway from hydroxylamine oxidoreductase to ubiquinone and cytochrome. This pathway needs to be maintained to generate reducing power (i.e. NADPH), which is crucial to the metabolic functions of *Nitrosomonas* [82–84]. Most synthetic nitrification inhibitors [(e.g., nitrapyrin, dicyandiamide, and 3,4-dimethylpyrazole phosphate (DMPP)] inhibit *Nitrosomonas* activity by suppressing the ammonia monooxygenase enzymatic pathway [60,85], but they have no effect on the hydroxylamine oxidoreductase enzymatic pathway. BNIs such as hydrophilic-MHPP and certain monoterpenes (e.g. limonene, produced by *Pinus ponderosa*) also inhibit *Nitrosomonas* in a similar way, i.e. by blocking only the ammonia monooxygenase pathway [22,63,67].

2.4. Characterization of BNI-function

Two categories of BNIs are released from plant root systems: hydrophilic-BNIs and hydrophobic-BNIs (Fig. 3) [65]. Their

Table 1
Relative effectiveness of various BNIs, and their mode of action on *Nitrosomonas* in *in vitro* bioassay (AMO, ammonia monooxygenase; HAO, hydroxyl aminooxidoreductase).

Serial No.	BNI compound	Isolated from	Inhibit AMO or HAO enzymatic pathway	ED_{80} [(μM) *in vitro* bioassay]	Ref.
1	Brachialactone	*B. humidicola* root exudate	AMO and HAO	10.6	[49]
2	Methyl *p*-coumarate	*B. humidicola* root tissue	NA	>40.0	[64]
3	Methyl ferulate	*B. humidicola* root tissue	NA	>20.0	[64]
4	Linoleic acid (LA)	*B. humidicola* shoot tissue	AMO and HAO	16.0	[62]
5	Linolenic acid (LN)	*B. humidicola* shoot tissue	AMO and HAO	16.0	[62]
6	Sorgoleone	Sorghum root exudate	AMO and HAO	12.0	[65,66]
7	MHPP	Sorghum root exudate	AMO	>120.0	[63]
8	Sakuranetin	Sorghum root exudate	AMO and HAO	0.6	[65]
9	Limonene	*Pinus ponderosa* leaf	AMO	NA	[67]
	Synthetic nitrification inhibitors				
10	©Allylthiourea		AMO	0.22	[62]
11	©Nitrapyrin		AMO	17.32	[62]
12	©Dicyandiamide		AMO	2200.00	[62]

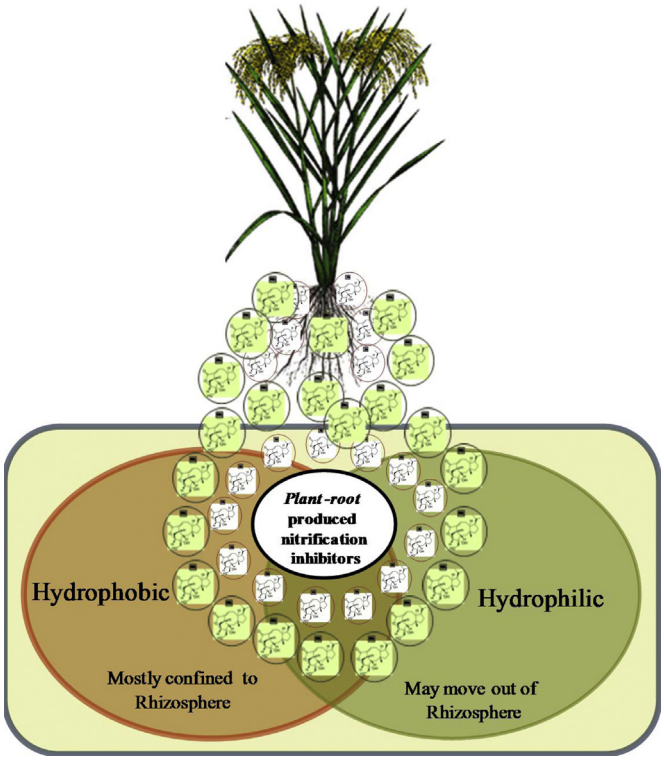

Fig. 3. Hydrophobic- and hydrophilic-nitrification inhibitors (BNIs) released from plant roots and their significance to BNI function.

relative contribution to BNI-capacity varies among plant species and even across growth stages. For example, in sorghum hydrophobic-BNI activity is the major contributor during early growth stages [up to 14 days after planting], but hydrophobic- and hydrophilic-BNIs contribute equally at later growth stages (30 days and after) [30,65]. Hydrophobic- and hydrophilic-BNIs differ in their mobility in soil due to differential solubility and/or affinity to water. The hydrophobic-BNIs may remain close to the root systems as they are strongly sorbed to soil mineral or organic particles, which may further increase their persistence. Their movement in soil is primarily *via* diffusion across concentration gradients and is likely confined to the rhizosphere [65,82]. In contrast, water soluble hydrophilic-BNIs are more likely to move out of the rhizosphere which may enhance their capacity to suppress nitrification in bulk soil [65]. Thus the distribution of hydrophobic- and hydrophilic-BNIs in the rhizosphere may have complementary functional roles [65].

Based on the observations of BNI-activity in sorghum in several greenhouse studies, it is estimated that the total amount of BNIs (hydrophilic- plus hydrophobic-) released during 130-days growing period (coinciding with the physiological maturity of this crop) can suppress nitrification up to 50% in about 500 g soil per plant [65]. Field and greenhouse studies with *Brachiaria humidicola* provide evidence for strong nitrification inhibition potential. Assuming 1.5 Mg ha^{-1} average live root biomass from a long-term grass pasture [86], and BNI-capacity of 17–70 ATU g^{-1} root dry wt. d^{-1} [59], it is estimated that *B. humidicola* can potentially release 2.6×10^6 to 7.5×10^6 ATU ha^{-1} d^{-1} of hydrophilic-BNIs 49,59; no published results are available on the estimates of hydrophobic-BNI activity from *Brachiaria* spp.). This estimate amounts to an inhibitory potential equivalent to that by the application of 6.2–18 kg of nitrapyrin ha^{-1} y^{-1} (based on 1 ATU being equivalent to 0.6 μg of nitrapyrin, a synthetic nitrification inhibitor), which is sufficient to have a significant influence on nitrifier activity and nitrification rates in the soil [49]. Field studies indicate a 90% decline in soil ammonium oxidation rates and N_2O emissions within three years of establishment of *B. humidicola* pastures [49]. These reduced emissions are attributed to the extremely small nitrifier populations present in the established pastures. In the same study, field plots planted to soybean (*Glycine max*, a plant species with no significant BNI-capacity) did not inhibit soil ammonium oxidation rates or N_2O emissions [49] (Fig. 4A and B). Based on the monitoring of N_2O emissions over a 3-year period from fields planted with tropical grasses with a wide range of BNI-capacity, a negative relationship was observed between the BNI-capacity of a species and N_2O emissions [30].

2.5. BNI release mechanisms

BNI synthesis and release are highly regulated plant attributes, which are stimulated by the presence of NH_4^+ in the rhizosphere [59]. The N-form (NH_4^+ *vs.* NO_3^-) in the soil has a major influence on the synthesis and release of BNIs by *B. humidicola*, sorghum, and *Leymus racemosus*, a wild relative of wheat [49,59,63,65,87,88]. Plants grown with NO_3^- as their N-source did not release BNIs, whereas plants grown with NH_4^+ as the N-source did release BNIs. Despite high levels of BNIs detected in the root tissues of NH_4^+ grown plants, BNIs were only released when plant roots were directly exposed to NH_4^+ during the collection of root exudates [49,59,65]. In addition, BNIs release from roots is a localized phenomenon confined to the part of the root system exposed to NH_4^+ and was not extended to the remaining parts of the root system (Fig. 5) [49,88]. A localized release of BNIs ensures relatively high concentrations of BNIs in the soil micro-sites where nitrifiers are active and NH_4^+ is present [49]. The regulatory role of NH_4^+ in the synthesis and release of BNIs suggests a possible adaptive role in protecting NH_4^+ from nitrifiers [49].

The activation and operation of proton pumping activity of root plasma membranes has been hypothesized as a functional

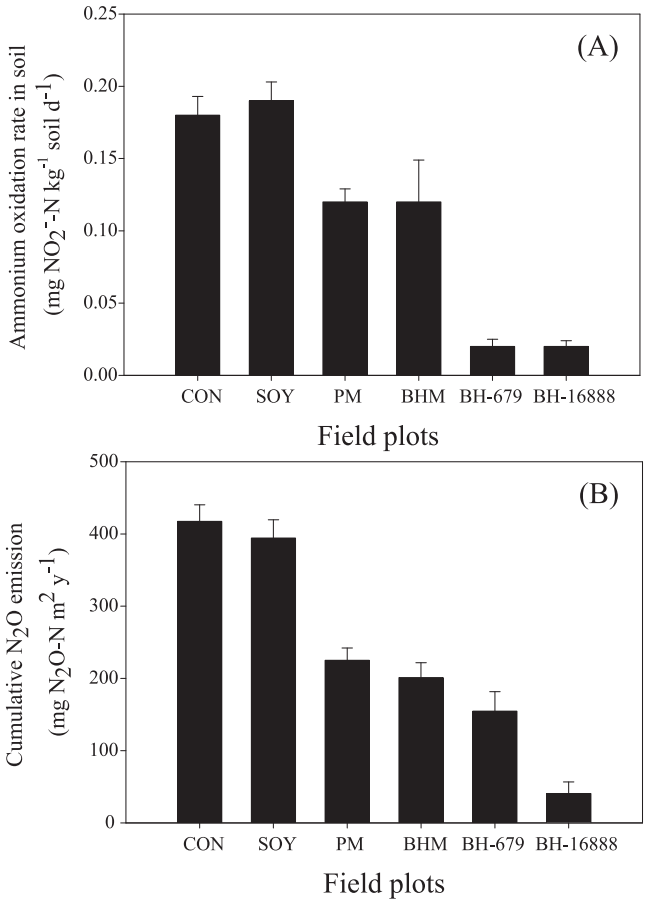

Fig. 4. (A) Soil ammonium oxidation rates (mg of NO_2^- N kg^{-1} soil d^{-1}) in field plots planted with tropical pasture grasses (differing in BNI capacity) and soybean (lacking BNI capacity in roots) [over 3 years from establishment of pastures (September 2004 to November 2007); for soybean, two planting seasons every year and after six seasons of cultivation]. CON, control (plant-free) plots; SOY, soybean; PM, *P. maximum*; BHM, *Brachiaria* hybrid cv. Mulato; BH-679, *B. humidicola* CIAT 679 (standard cultivar); BH-16888, *B. humidicola* accession CIAT 16888 (a germplasm accession). Values are means ± SE from three replications [49]. (B) Cumulative N_2O emissions (mg of N_2O N m^{-2} per year) from field plots of tropical pasture grasses (monitored monthly over a 3-year period, from September 2004 to November 2007). Plots are identified in Fig. 3 legend. Values of means ± SE from three replications [49].

link between BNI release (presumably organic anions) and NH_4^+ uptake and assimilation. If BNIs are transported through voltage-dependent anion channels, their release will be closely related to the regulation of proton pump-ATPase. We speculate that the transport of BNIs, driven by proton pump-ATPase, is associated with NH_4^+ uptake and assimilation in sorghum (Fig. 6) [88]. The rhizosphere pH also influences the release of BNIs from roots. Recent results indicate that sorghum plants do not release BNIs from their roots in the presence of NH_4^+ when the rhizosphere pH is 7 or higher; the optimum BNI release was observed at a rhizosphere pH of 5.0–6.0, which stimulates the functioning of the proton pumps [63,65]. These results imply that the suppression of nitrification by BNI is likely to be restricted to sorghum grown on acid soils. Light-textured soils with low buffering capacity and moderate acidity (pH <6.0), which is the case for most tropical grasslands or savannas, might be better suited for the expression and exploitation of BNI function in sorghum [65,89]. The results of recent studies suggest that unlike hydrophilic-BNI release in sorghum, hydrophobic-BNI release is not sensitive to pH changes in the rhizosphere as its release is not associated with proton pumping activity (Tingjun and Subbarao, unpublished results). Moreover, nitrifier activity and nitrification are suppressed by *B. humidicola* pasture in heavy black

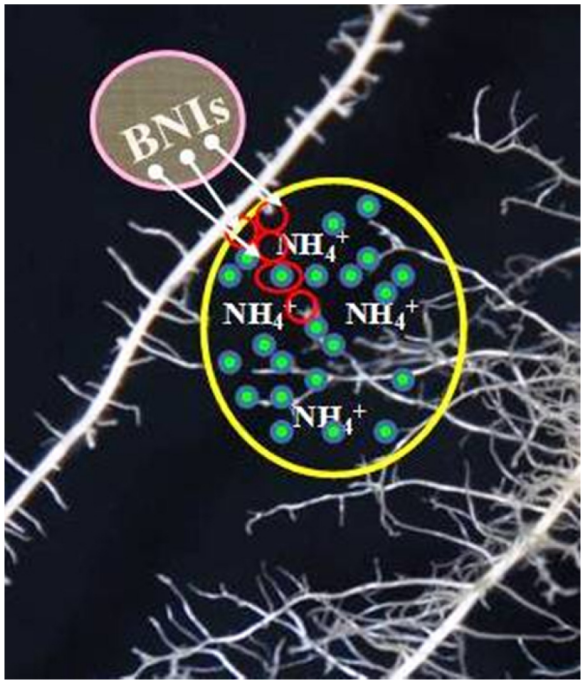

Fig. 5. A hypothesis proposed for localized release of BNIs from roots when NH_4^+ is sensed in the rhizosphere [49].

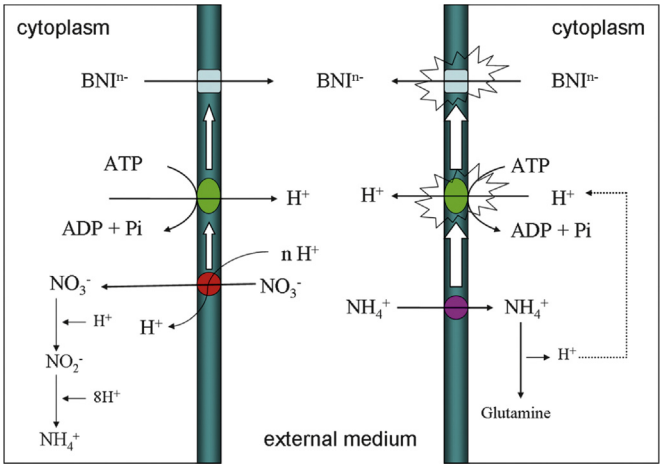

Fig. 6. A hypothesis on the transport of BNIs, driven by PM H^+-ATPase, associated with NH_4^+ uptake and assimilation in source [88].

soils (Vertisols) with pH of 7.2 following two years after its establishment [49].

2.6. Stability of BNIs in soil systems

Although quantification of BNI-activity using an *in vitro* assay during 30 min exposure to pure cultures of *Nitrosomonas* spp. is a useful initial screening tool for determining BNI-capacity, we should expect that not all BNIs released from root system of plants are effective in suppressing soil nitrifier activity in the field. For BNIs to be effective in soil-based systems, these compounds must persist and be effective in the soil. BNI-compounds isolated from root exudates of *B. humidicola* were effective when added to the soil at 10–20 ATU g^{-1} soil and incubated for 55 days at 20 °C; soil nitrification was suppressed by 50–90% [55,90]. Certain BNIs such as linoleic acid and linolenic acid (BNI-compounds isolated from leaf tissues of *B. humidicola*) partially lost their effectiveness in soil after

80 days and the activity was completely lost after 100 days [62]. However, the BNI effect from *Hyparrhenia* sp. on tropical savanna soils remained functional even after air drying and storing soil in the dark [50,51]. Sorgoleone (the most important component of hydrophobic-BNI activity in sorghum) and MHPP (the compound isolated from hydrophilic-BNI-activity in sorghum) are effective in suppressing soil nitrification [65,66], whereas sakuranetin, a BNI-compound isolated from hydrophilic-BNI-activity of sorghum, has no inhibitory effect on soil-nitrification. These findings indicate and confirm that not all BNIs released from root systems are actually effective in suppressing nitrification in the field soil [65].

2.7. Potential for genetic improvement of BNI-capacity

The existence of genetic variation in available germplasm is a prerequisite for the improvement of any plant trait through conventional and/or molecular breeding approaches. Significant genetic variability exists for BNI-capacity in *B. humidicola* germplasm (7–46 ATU g^{-1} root dry wt. d^{-1}), suggesting that there is a strong potential to breed *Brachiaria* for enhanced BNI-capacity by selection and recombination [58,84]. Substantial genetic variability for sorgoleone release has also been found in sorghum germplasm [65,66]. Efforts are currently underway to map the quantitative trait loci (QTL) associated with the sorgoleone-trait in sorghum as well as brachialactone release in a bi-parental population of *B. humidicola.* The identification of markers linked to the genetic regions affecting BNI may be particularly useful considering the expense and difficulty of phenotyping large numbers of breeding materials for BNI-activity and brachialactone release. BNI-activity in *B. humidicola* appears to be normally distributed, indicating a quantitative mode of inheritance and control by a number of QTLs with moderate to minor effects (Ishitani and Selvaraj, unpublished data). The probable role of multiple small effects from QTLs and the difficulty of backcrossing self-intolerant *B. humidicola* may complicate marker assisted selection for brachialactone release.

Wild relatives of crops, which have not been selected in high-N input environments, may hold the best hope to introgress BNI-capacity into major food crops [84]. A wild relative of wheat, *Leymus racemosus*, has high BNI-capacity. Genetic control of BNI-capacity in *L. racemosus* has been located to chromosome *Lr#n*, and successfully introduced and expressed in a cultivated wheat background [87,91]. Efforts are currently underway to identify the specific sub-chromosomal region controlling the BNI-capacity in *L. racemosus* and transfer high-BNI-capacity to root systems of elite wheat cultivars (Kishii and Subbarao, unpublished results).

Cultivated wheat lacks BNI-capacity in its root systems, perhaps a consequence of decades of selection pressure under intensively managed high-nitrifying environments [84,87]. One novel idea is to use synthetic hexaploid wheats (i.e. artificially re-synthesized wheat lines) [92,93] as a source for BNI-capacity, if they have substantial BNI-capacity in their root systems. Synthetic hexaploid wheats are created by crossing tetraploid durum wheat (*Triticum turgidum*) and diploid wild goat-grass (*Aegilops tauchii*). This cross produces an unstable triploid F1, which is treated with colchicine to double the chromosome number to produce stable synthetic hexaploid wheat. These 'new-born' wheats have not been subjected to any kind of selection pressure in high nitrifying environments and provide opportunities to capture traits linked to BNI-capacity in their root systems (a kind of "reset" button to erase the entire selection history of breeding efforts from the last century). BNI-capacity can be introduced into elite cultivated wheat by crossing modern cultivars with synthetic hexaploid wheats. There are also a number of amphiploids between wheat and related species available in gene-banks, which can provide additional potential sources for enhanced BNI-capacity. Finally, transgenic approaches may enable the development of genetically modified maize and wheat with the ability to produce and release powerful BNIs similar to brachialactone. However, a clear understanding of the genes involved in the regulation, biosynthesis and release of BNI compounds will be necessary to introduce new BNI genes from foreign sources to important food crop species.

2.8. Deploying BNI function in production agriculture

Our understanding of BNI-function in plants is new and still developing. Substantial resources and efforts will be required to develop further insights in the chemical nature and identity of BNIs released from major food and feed crops, release mechanisms, stability and effectiveness of these biologically-produced nitrification inhibitors in various soil types and environmental conditions. As described in the earlier sections, a range of mechanisms have evolved that suppress nitrifier activity and limit N-flow towards nitrification pathway to reduce leakage and facilitate closure of N-cycle in some natural undisturbed ecosystems. Controlling soil nitrifier activity is thus central to restore soil-N retention ability and facilitate N-flow towards immobilization, the first step towards buildup of SOM and long-term buffering capacity and contribute to agro-ecosystem productivity and sustainability.

The ideal and most efficient way to control nitrifier activity and reduce nitrification in agricultural systems is to exploit the BNI-capacity in plants to deliver biologically-produced nitrification inhibitors directly to nitrifier sites in the soil through their root systems. This strategy has many advantages over the use of synthetic nitrification inhibitors, where delivery to nitrifier sites is not effective and their use is uneconomical [30,60]. The ability to secrete/exude a vast array of compounds into the rhizosphere is one of the most remarkable metabolic features of plant roots; nearly 25% (30% in some cases) of all photosynthetically fixed carbon is transferred to the rhizosphere through root exudation [45,94]. The development of conventional, marker assisted, and transgenic breeding strategies to enhance the production and release of BNIs are all urgently needed to suppress nitrifier activity and lower nitrification rates in production agriculture. In addition, agro-pastoral systems based on the rotation of annual crops and perennial forage grasses such as *Brachiaria* spp. will likely exploit the high BNI-capacity of these pasture grasses and reduce soil nitrifier activity, enhance soil-N retention, and increase fertilizer-N recovery and yield of food crops with low BNI-capacity [30,40,95]. Incorporating nitrification inhibiting plant tissues [68–70] (for example, isothiocyanates found in some *Brassicaceae* family members show inhibitory effect on soil nitrification) into soil systems (similar to green manure application) could be one of the ideas need to be tested to control nitrification as part of cropping systems approach.

3. Perspectives

Modern production systems are human-centric ecosystems, driven largely by massive infusion of industrially fixed-N, have become high-nitrifying due to augmented soil nitrifier activity [32,38,96,6,97,98]. Nearly 95% of the reactive-N that enters these agricultural systems goes through rapid nitrification and nitrate has become the dominant, if not the sole inorganic-N source for crop uptake and assimilation. This has resulted in the low NUE of modern agricultural systems and enhanced N pollution [30,33,99–103]. Of the 175 Tg N (industrially fixed-N) annually applied to global-agricultural systems, less than 1 Tg N is retained by human body [104]; the rest (>99%) is lost [(*via* nitrification and denitrification) and returns to the atmosphere as elemental non-reactive N_2], necessitating continued application of N-fertilizer to maintain food production [30,104]. Ideally, most reactive-N entering into human-centric ecosystems should be cycled/re-cycled and

nitrification/denitrification in soils should be tightly controlled and regulated) to reduce the need for continued application of industrially fixed-N to support food production.

Accelerated nitrification rates in agricultural soils have resulted in a decline in NUE since the advent of Green Revolution, and led to diminishing returns on N-fertilization [32,98,104]. Nearly 70% of applied N-fertilizer is lost (*via* NO_3^- leaching and gaseous-N emissions) from production systems, before the crop has a chance to absorb and assimilate it into plant-protein [46,105,106]. Annual economic loss from lost N-fertilizer is estimated at 90 US$ billion [30,99]. If this trend continues, annual N-fertilizer application is expected to reach 300 Tg N by 2050; and the global N_2O emissions will reach 19 Tg y^{-1} by 2100 (from 10 Tg in 1990) [11,39,42,107]. There is an urgency to develop next-generation technologies to reduce N_2O emissions from agricultural systems as the IPCC set a target to cut global greenhouse gas emissions by 80% by 2100 [13]; EU, USA and China have committed to cut emissions by 30–40% (at the 1990 levels) by 2025.

Controlling soil nitrification is critical to reverse the present trend in declining NUE, and to improve N-retention and reduce N_2O emissions from agricultural systems. A paradigm shift is needed to move away from an inherently inefficient NO_3^--centric nutrition and towards NH_4^+-centric crop nutrition. Synthetic nitrification inhibitors are neither cost-effective nor functionally stable [30,60,89]. BNI-function, where plant-root systems deliver powerful BNIs at nitrifier-sites, should be genetically exploited as a plant trait for developing low-nitrifying, low-N_2O emitting next-generation N-efficient production systems.

Though, early observations of nitrification inhibition were mostly made on tropical grassland systems, BNI function seems not confined to plants either from humid- or sub-humid tropics as certain temperate forest ecosystems (such as pine forests) also suppress nitrification; selected temperate grasses likely also have BNI capacity in their root systems (recent unpublished reports and personal communications); also *Brasicaceae* members that are adapted to temperate climate have BNIs (isothiocyanates) in their root and shoot tissues and probably release these BNIs into the soil as well. In addition, some of the wheat wild relatives have high BNI-capacity, further suggesting that BNI function is widespread in both tropical and temperate plants.

High BNI-capacity root systems can be developed in both tropical- and temperate- crops and pastures using classical and modern breeding tools and approaches. Nitrification inhibitor producing plants such as *Brasicaceae* members can be incorporated in the soils (similar to green manures) and cropping and rotations can be developed with the primary objective of controlling soil nitrifier activity to improve NUE of production systems. High-BNI capacity *Brachiaria* pastures can be integrated with low-BNI capacity crops such as maize or upland rice in agro-pastoral systems which could fit well with the current move towards ecological-intensified agriculture. Low-nitrifying soil environment is an essential requirement for reducing N_2O emissions from agricultural systems and to limit nitrogen leakage into the larger environment. However, low-nitrifying soil environments must be complemented with restoration of key microbial communities to facilitate synchronization between SOM mineralization and crop-N demand [34]. Genetic and agronomic exploitation of BNI function in crops and pastures can facilitate moving towards low-nitrifying and low-N_2O emitting agricultural systems which can be an integral part of second Green Revolution.

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