

Nitrogen management in grasslands and forage-based production systems – Role of biological nitrification inhibition (BNI)

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Abstract

Nitrogen (N), the most critical and essential nutrient for plant growth, largely determines the productivity in both extensive and intensive grassland systems. Nitrification and denitrification processes in the soil are the primary drivers of generating reactive N (NO_3^- , N_2O and NO), largely responsible for N loss and degradation of grasslands. Suppressing nitrification can thus facilitate retention of soil N to sustain long-term productivity of grasslands and forage-based production systems. Certain plants can suppress soil nitrification by releasing inhibitors from roots, a phenomenon termed ‘biological nitrification inhibition’ (BNI). Recent methodological developments [e.g. bioluminescence assay to detect biological nitrification inhibitors (BNIs) from plant-root systems] led to significant advances in our ability to quantify and characterize BNI function in pasture grasses. Among grass pastures, BNI capacity is strongest in low-N environment grasses such as *Brachiaria humidicola* and weakest in high-N environment grasses such as Italian ryegrass (*Lolium perenne*) and *B. brizantha*. The chemical identity of some of the BNIs produced in plant tissues and released from roots has now been established and their mode of inhibitory action determined on nitrifying *Nitrosomonas* bacteria. Synthesis and release of BNIs is a highly regulated and localized process, triggered by the presence of NH_4^+ in the rhizosphere, which facilitates release of BNIs close to soil-nitrifier sites. Substantial genotypic variation is found for BNI capacity in *B. humidicola*, which opens the way for its genetic manipulation. Field studies suggest that *Brachiaria* grasses suppress nitrification and N_2O emissions from soil. The potential for exploiting BNI function (from a genetic improvement and a system perspective) to develop production systems, that are low-nitrifying, low N_2O -emitting, economically efficient and ecologically sustainable, is discussed.

Resumen

El nitrógeno (N), el nutriente más crítico y esencial para el crecimiento de las plantas, es determinante para la productividad de las pasturas, tanto de tipo extensivo como intensivo. Los procesos de nitrificación y desnitrificación en el suelo son los principales responsables de la generación de formas de N reactivo (NO_3^- , N_2O y NO) y, como consecuencia, de la pérdida de N y la degradación de las pasturas. Por tanto, la supresión de la nitrificación puede facilitar la retención de N en el suelo necesario para mantener, a largo plazo, la productividad de pastizales y sistemas de producción basados en forrajes. Algunas plantas pueden suprimir la nitrificación en el suelo mediante la liberación de sustancias inhibitoras desde sus raíces, un fenómeno llamado ‘inhibición biológica de la nitrificación’ (BNI, por su sigla en

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inglés). Metodologías recientemente desarrolladas, por ej., pruebas de bioluminiscencia para detectar inhibidores biológicos de la nitrificación (BNIs) en el sistema radicular de plantas, han permitido mejorar las posibilidades de cuantificar y caracterizar la función de BNI en gramíneas forrajeras. Dentro de las gramíneas, la más alta capacidad de BNI se ha encontrado en especies de ambientes bajos en N como *Brachiaria humidicola*, y la más baja en especies de ambientes altos en N como *Lolium perenne* y *B. brizantha*. Actualmente se conoce la identidad química de algunos BNIs producidos en tejidos de plantas y liberados en las raíces, igualmente su modo de acción inhibitoria sobre la nitrificación de las bacterias *Nitrosomonas*. La síntesis y liberación de los BNIs es un proceso altamente regulado y localizado, estimulado por la presencia de NH_4 en la rizósfera, lo que facilita la liberación de los BNIs cerca de los sitios de nitrificación en el suelo. En *B. humidicola* se ha encontrado una amplia variación genotípica en la capacidad de BNI, lo que abre un camino para su manipulación genética. Estudios a nivel de campo sugieren que las gramíneas del género *Brachiaria* reducen la nitrificación y la emisión de N_2O del suelo. Se discute el potencial de explotar la función de BNI, desde la perspectiva de mejoramiento genético y de sistema, para desarrollar sistemas de producción con baja nitrificación y baja emisión de N_2O , y que sean económicamente eficientes y ecológicamente sostenibles.

Introduction

Grass pastures are the largest land user, occupying 3.2 billion ha of the 4.9 billion ha of available agricultural land worldwide (Steinfeld et al. 2006). In addition, a significant portion of the cultivated land (0.5 billion ha) is used for growing forage grasses and feed-grain crops (e.g. sorghum, barley, maize and soybean) to support intensive livestock production (Steinfeld and Wassenaar 2007; Herrero et al. 2010, 2011). Mineralization of soil organic matter (SOM) is the major N source in extensive grassland systems. For intensive grass pastures, fertilizer N inputs can reach from 200 to 600 kg N/ha/yr, with only 30% recovered by plant protein and entering into the animal system, while the remaining 70% is lost to the environment in reactive N forms (i.e. NO_3^- , N_2O , NO) (Galloway et al. 2009). Nitrogen-use efficiency (NUE) in grassland systems (meat or milk protein produced/kg plant protein N intake) ranges from 5 to 10%, depending on whether milk or meat is the output (van der Hoek 1998). Grazing animals typically retain about 5% of the N intake (from the grass consumed) in their bodies and excrete the rest through urine (about 90% of the total N intake) and dung, which becomes an N source for the grass pasture (Worthington and Danks 1992). Much of this N, however, is lost through NO_3^- leaching and gaseous N emissions (N_2O , NO and N_2), causing ecological damage and economic loss (Tilman et al. 2002; Steinfeld and Wassenaar 2007; Herrero et al. 2011; Subbarao et al. 2013b).

N losses from agricultural systems impact the global environment and contribute significantly to global warming

Due to the development of high-nitrifying soil environments (where NO_3^- accounts for >95% of the plant N

uptake), intensive pasture and feed-grain production systems have become extremely “leaky” and inherently inefficient (Subbarao et al. 2012); nearly 70% of the 150 Mt N fertilizer applied annually to global agricultural systems is lost through NO_3^- leaching and N_2O and NO emissions; annual economic loss from the lost N is estimated to be US\$ 90 billion (Subbarao et al. 2013b). Fertilizer N use is projected to reach 300 Mt/yr by 2050 (Tilman et al. 2001) and N lost through NO_3^- leaching from agricultural systems could reach close to 61.5 Mt N/yr (Schlesinger 2009). Currently 17 Mt N is emitted as N_2O and this is expected to quadruple by 2100, due largely to an increase in the use of N fertilizers (Galloway et al. 2008).

Nitrification opens several pathways for N loss and weakens the soil N retention capacity in grassland systems

Nitrification, the biological oxidation of NH_4^+ to NO_3^- , opens several pathways for production of N_2O and NO, generated through nitrifier-denitrification or heterotrophic denitrification processes (Davidson and Verchot 2000; Zhu et al. 2013). Nitrification and denitrification are the major drivers for global emissions of N_2O , the most aggressive and powerful greenhouse gas, directly affected by human activity, with a global warming potential 300 times greater than that of CO_2 (Hahn and Crutzen 1982). As a cation, NH_4^+ is held by the negatively charged surfaces of clay minerals and SOM, that reduce the NH_4^+ loss by leaching. In contrast, the negatively charged NO_3^- does not readily bond to the soil, and is sufficiently labile to be leached out of the root zone. Nitrogen enters grass pastures primarily as N fertilizers (in intensive systems) or is derived from SOM-mineralization (in extensive systems) or hydrolysis of

urea N from urine excreted from grazing animals, where NH_4^+ is produced either through SOM-mineralization-ammonification or urea hydrolysis, as the first product of inorganic N. Heterotrophic soil microorganisms convert NH_4^+ into microbial N, i.e. immobilization, and pasture roots and nitrifying bacteria compete for this NH_4^+ as an N source (Figure 1). Nitrogen flow into microbial immobilization or plant uptake is desirable. However, N flows into nitrification pathways generate reactive N forms (NO_3^- , N_2O and NO), that are not retained by the soil, and are lost to the environment, leading to the degradation of grassland systems.

Restricting the N flow to the nitrification pathway by inhibiting soil nitrifier activity facilitates NH_4^+ uptake by plants; this also allows N flow into the microbial pool (Hodge et al. 2000). The immobilization and mineralization loop of the N cycle dominates to keep soil N cycling within the system, and creates a slow-release N pool to sustain grassland productivity in such systems (Figure 1). Most plants have the ability to use NH_4^+ or NO_3^- as

their N source (Haynes and Goh 1978; Boudsocq et al. 2012). Reducing nitrification rates in agricultural systems does not alter the intrinsic ability of plants to absorb N, but does increase retention time of N in the root zone as NH_4^+ , which is less mobile and less energetically costly for uptake and assimilation than NO_3^- , providing additional time for plants to absorb N. Many of the advantages, associated with inhibiting nitrification to improve productivity and NUE of intensive grassland systems and feed-grain production systems, have been demonstrated using chemical nitrification inhibitors (Subbarao et al. 2006a; Dennis et al. 2012).

Biological nitrification inhibition (BNI)

The BNI concept

The ability to produce and release nitrification inhibitors from plant roots to suppress soil nitrifier activity is termed 'biological nitrification inhibition' (Figure 1).

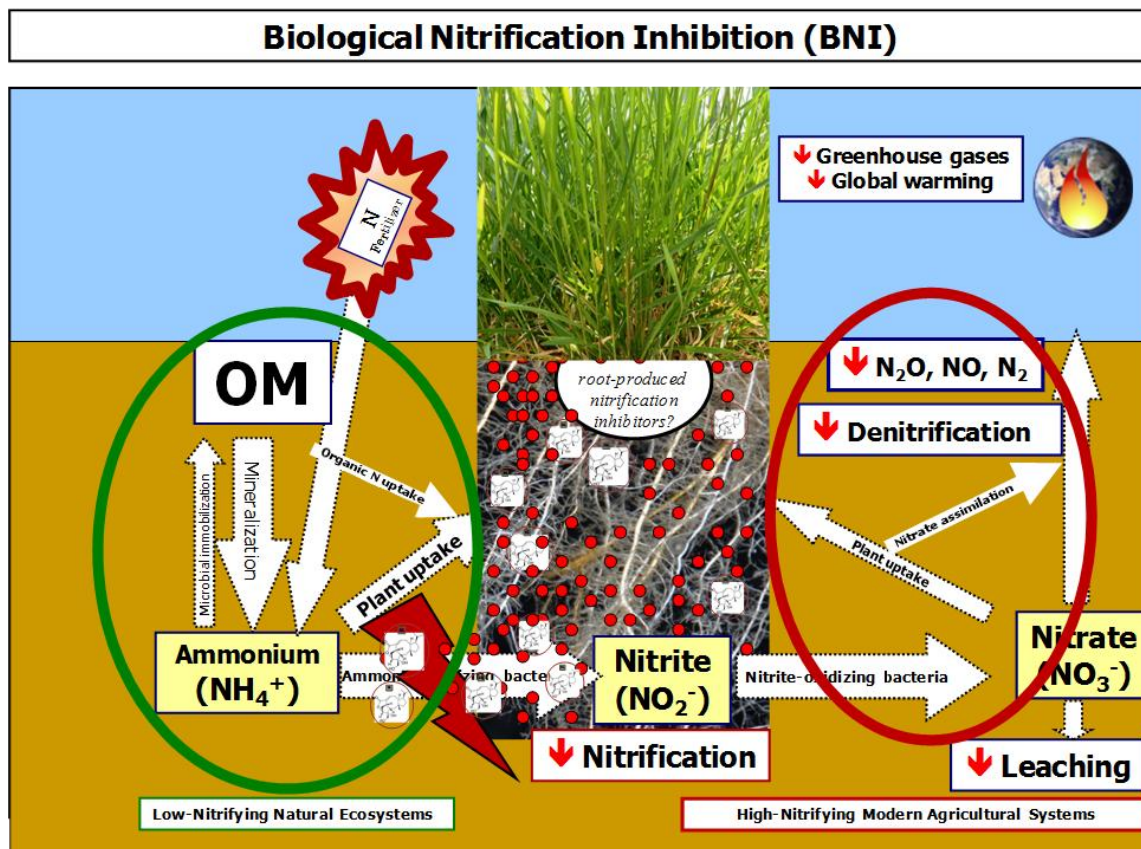


Figure 1. Schematic representation of the biological nitrification inhibition (BNI) interfaces with the N cycle. The BNI exuded by roots inhibits nitrification that converts NH_4^+ to NO_2^- . In ecosystems with large amounts of BNI (e.g. brachialactone), such as in *Brachiaria* grasses, the flow of N from NH_4^+ to NO_3^- , via NO_2^- , is restricted, and it is NH_4^+ and microbial N rather than NO_3^- that accumulates in the soil. In systems with little or no BNI, such as modern agricultural systems, nitrification occurs rapidly, leaving little time for plant roots to absorb NO_3^- ; thus NO_3^- is lost from the system through denitrification and leaching; (adapted from Subbarao et al. 2012).

Nitrification largely determines the N-cycling efficiency (i.e. proportion of N that stays in the ecosystem during a complete N-cycling loop); the BNI function has the potential to improve agronomic NUE (Subbarao et al. 2012; 2013b). Recent modeling studies coupled with in-situ measures suggest that tropical grasses, which inhibit nitrification, exhibit a 2-fold greater productivity than those that lack such ability (Lata 1999; Boudsocq et al. 2012).

BNI characterization in pasture grasses

Recent methodological advances have facilitated the detection and quantification of nitrification inhibitors from intact plant roots using a recombinant *Nitrosomonas* construct (Subbarao et al. 2006b). Nitrification inhibitors released from roots measured as ‘BNI activity’, are expressed in ATU (allylthiourea unit) and this ability is termed BNI capacity (Subbarao et al. 2007b). Root systems of tropical pasture grasses showed a wide range in BNI capacity. *Brachiaria humidicola*, a grass adapted to low-N production environments of South American savannas, showed the greatest BNI capacity (range from 15 to 50 ATU/g root dry wt/d) (Subbarao et al. 2007b). By contrast, *Lolium perenne*, *B. brizantha* and *Panicum maximum*, that are adapted to high-N environments, showed the least BNI capacity (2–5 ATU/g root dry wt/d) (Figure 2). Sorghum is the only field crop that showed a significant BNI capacity (5–10 ATU/g root dry wt/d) among the cereal and legume crops evaluated (Subbarao et al. 2007b; 2013b).

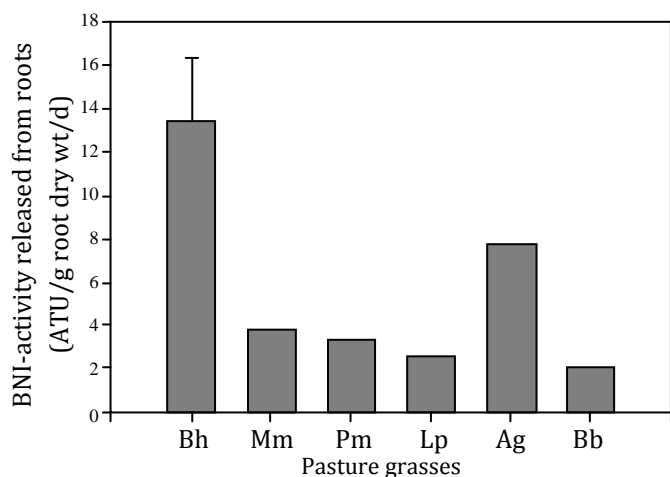


Figure 2. BNI activity released from intact roots of various pasture grasses grown in sand-vermiculite (3:1 v/v) culture for 60 days; Bh – *Brachiaria humidicola*, Mm – *Melinis minutiflora*, Pm – *Panicum maximum*, Lp – *Lolium perenne*, Ag – *Andropogon gayanus*, Bb – *B. brizantha*. Vertical bar represents LSD (0.05); (based on Subbarao et al. 2007b).

The BNI capacity of root systems arises from their ability to release 2 categories of BNIs: (a) hydrophobic BNIs; and (b) hydrophilic BNIs. These BNI fractions differ in their mobility in the soil and their solubility in water; the hydrophobic BNIs may remain close to the root as they could be strongly adsorbed on the soil particles, increasing their persistence. The mobility of the hydrophobic BNIs is via diffusion across a concentration gradient; thus this form is likely to be confined to the rhizosphere (Raynaud 2010; Subbarao et al. 2013a). In contrast, the hydrophilic BNIs may move further from the point of release due to their solubility in water, and this may improve their capacity to control nitrification beyond the rhizosphere (Subbarao et al. 2013a). The relative contributions of hydrophobic BNIs and hydrophilic BNIs to the BNI capacity may differ among plant species. For *Brachiaria* grasses, both fractions make equal contributions to the BNI capacity; for sorghum, the hydrophobic BNIs play a dominant role in determining the BNI capacity, whereas in wheat, hydrophilic BNIs determine the root system’s inhibitory capacity (G.V. Subbarao and T. Tsehaye, unpublished data).

For *Brachiaria* spp., the amount of inhibitors released from root systems could be substantial. Based on the BNI activity release rates observed (17–50 ATU/g root dry wt/d) and assuming the average live root biomass from a long-term grass pasture at 1.5 t/ha (Rao 1998), it was estimated that BNI activity of 2.6×10^6 – 7.5×10^6 ATU/ha/d is potentially released (Subbarao et al. 2009a). This amounts to an inhibitory potential equivalent to that achieved by the application of 6.2–18.0 kg of nitrpyrin/ha/yr, which is large enough to have a significant influence on the functioning of the nitrifier population and nitrification rates in the soil. Field studies indicate a 90% decline in soil ammonium oxidation rates due to extremely small populations of nitrifiers (ammonia-oxidizing bacteria, AOB, and archaea, AOA, determined as *amoA* genes) within 3 years of establishment of *B. humidicola* (Figure 3). Nitrous oxide emissions were suppressed by >90% in field plots of *B. humidicola* compared with soybean, which lacks BNI capacity in its root systems (Subbarao et al. 2009a).

Chemical identities of BNIs and their mode of inhibitory action

The major nitrification inhibitor released from the roots of *B. humidicola* is a cyclic diterpene, named ‘brachialactone’ (Subbarao et al. 2009a). This compound has a dicyclopenta (a,d) cyclooctane skeleton (5-8-5 ring system) with a γ -lactone ring bridging one of the 5-membered rings and the 8-membered ring (Figure 4)

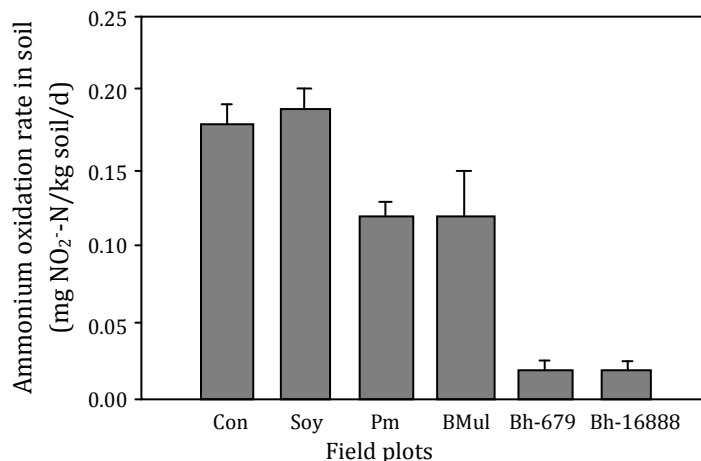


Figure 3. Soil ammonium oxidation rates in field plots planted to tropical pasture grasses (differing in BNI capacity) and soybean (lacking BNI capacity in roots); grasses: covering 3 years from establishment (September 2004–November 2007), soybean: 6 seasons of cultivation over 3 years. Con – control plots (plant free); Soy – soybean; Pm – *Panicum maximum*; BMul – *Brachiaria* hybrid cv. Mulato (apomictic hybrid that contains germplasm from *B. ruziziensis*, *B. decumbens* and *B. brizantha*, but NOT from *B. humidicola*); Bh-679 – *B. humidicola* CIAT 679 (standard cultivar Tully); Bh-16888 – *B. humidicola* accession CIAT 16888. Values are means \pm s.e. of 3 replications; (adapted from Subbarao et al. 2009a).

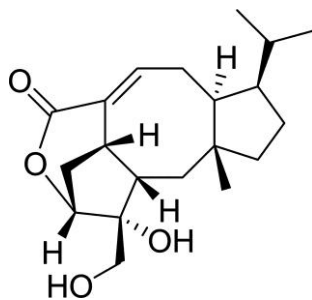


Figure 4. Chemical structure of brachialactone, the major nitrification inhibitor isolated from root exudates of *Brachiaria humidicola*; (from Subbarao et al. 2009a).

(Subbarao et al. 2009a). Brachialactone, with an IC_{80} of 10.6 μ m, is considered one of the most potent nitrification inhibitors as compared with nitrapyrin or dicyandiamide (DCD), 2 of the synthetic nitrification inhibitors most commonly used in production agriculture (IC_{80} , concentration for 80% inhibition in the bioassay, of 5.8 μ m for nitrapyrin and 2200 μ m for DCD). Brachialactone inhibits *Nitrosomonas* sp. by blocking both ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO) enzymatic functions, but appears to have a relatively stronger effect on the AMO than on

the HAO enzymatic pathway. About 60–90% of the inhibitory activity released from the roots of *B. humidicola* is due to brachialactone. Release of brachialactone is a regulated plant function, triggered and sustained by the availability of NH_4^+ in the root environment (Subbarao et al. 2007a; 2009a). Brachialactone release is restricted to those roots that are directly exposed to NH_4^+ , and not the entire root system, suggesting a localized release response (Subbarao et al. 2009a).

Genetic improvement of BNI capacity of pasture grasses

Significant genetic variability (ranging from 7.1 to 46.3 ATU/g root dry wt/d) exists for BNI capacity in *B. humidicola*, indicating a significant potential for genetic manipulation of BNI capacity by conventional plant breeding (Subbarao et al. 2007b; 2009b). Recent findings suggest substantial genetic variability for brachialactone release among *B. humidicola* germplasm accessions, nearly 10-fold differences, suggesting the potential for breeding *Brachiaria* genotypes with high brachialactone capacity. Efforts are underway to develop molecular markers for brachialactone release capacity in *Brachiaria* spp.

Perspectives

Sustainable intensification of grasslands and feed-crop production systems is needed to meet the global demands for meat and milk, particularly in developing countries. As the demand for meat and milk is expected to double by 2050 (Herrero et al. 2009), there will be further efforts to intensify grasslands and feed-crop-based systems. Most increases in productivity are, however, achieved through massive inputs of industrially produced N fertilizer. Nearly 70% of the 150 Mt N applied to global agricultural systems is lost, largely due to the high nitrifying nature of soil environments (Tilman et al. 2001; Subbarao et al. 2013b). As nitrification and denitrification are the primary biological drivers of NO_3^- , N_2O and NO production (i.e. reactive N forms largely responsible for environmental pollution), suppressing nitrification is critical to reduce N losses and to retain soil N for longer periods in the grassland systems. The BNI function in forage grasses and feed-crops such as sorghum can be exploited using genetic and crop- and/or production system-based management to design low-nitrifying agronomic environments to improve NUE. In addition, the high BNI capacity in *Brachiaria* spp. can be utilized for the benefit of feed-crop systems such as maize, that receive most of the N fertilization but do not have inherent BNI capacity in their root systems. This

could be achieved by integrating *Brachiaria* pastures with high BNI capacity and maize production using agro-pastoral systems (Subbarao et al. 2013b). In grazed grassland systems, most of the plant protein N is excreted by livestock (through urine) and thus returned to the soil. Grassland systems that retain N excreted by livestock are likely to maintain/sustain productivity over time. The BNI function could be most effective in controlling nitrification in grassland systems if genetically manipulated, either by conventional plant breeding or by genetic engineering. Most grasses develop extensive root systems and are perennial (Rao et al. 2011); if this is combined with high BNI capacity, these grassland systems can potentially suppress soil nitrifier activity to retain and use N more efficiently than at present. As grazing animals usually deposit urine and dung in a random, patchy manner, soil N is redistributed. The patchy distribution makes it difficult to control nitrification using synthetic nitrification inhibitors. The BNI function in forage grasses could be more effective in controlling nitrification to sustain system productivity and to protect these systems from degradation.

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