Biological Nitrification Inhibition (BNI) in *Brachiaria* Pastures: A Novel Strategy to Improve Eco-Efficiency of Crop-Livestock Systems and to Mitigate Climate Change

Danilo E. Moreta  
*Centro Internacional de Agricultura Tropical, Colombia*

Jacobo Arango  
*Centro Internacional de Agricultura Tropical, Colombia*

Mauricio Sotelo  
*Centro Internacional de Agricultura Tropical, Colombia*

Daniel Vergara  
*Centro Internacional de Agricultura Tropical, Colombia*

Alvaro Rincón  
*Corporación Colombiana de Investigación Agropecuaria, Colombia*

See next page for additional authors

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Presenter Information
Danilo E. Moreta, Jacobo Arango, Mauricio Sotelo, Daniel Vergara, Alvaro Rincón, Manabu Ishitani, Aracely Castro, John Miles, Michael Peters, Joe Tohme, Guntur V. Subbarao, and Idupulapati M. Rao

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Biological nitrification inhibition (BNI) in *Brachiaria* pastures: A novel strategy to improve eco-efficiency of crop-livestock systems and to mitigate climate change


A International Center for Tropical Agriculture (CIAT), A.A. 6713, Cali, Colombia  
B Corporación Colombiana de Investigación Agropecuaria (Corpoica), E. E. La Libertad, km 17 vía Puerto López, Meta, Colombia  
C Japan International Research Center for Agricultural Sciences (JIRCAS), Ibaraki 305-8686, Japan  
Contact email: i.rao@cgiar.org

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**Introduction**

Up to 70% of the nitrogen (N) fertilizers applied to agricultural systems are lost due to nitrification and denitrification. Nitrification is a microbiological process that generates nitrate (NO$_3^-$) and promotes the losses of N fertilizers by leaching and denitrification. Nitrification and denitrification are the only known biological processes that generate nitrous oxide (N$_2$O), a powerful greenhouse gas contributing to global warming. There is an urgent need to suppress nitrification process in soil to improve N-recovery and N use efficiency (NUE) of agricultural systems and to mitigate climate change (Subbarao et al. 2012). Certain *Brachiaria* grasses (*B. humidicola*) can suppress soil-nitrification by releasing biological nitrification inhibitors (BNIs) from roots, thereby reducing N$_2$O emissions. This phenomenon, termed biological nitrification inhibition (BNI), has been the subject of recent research to characterize and validate the concept under field conditions (Subbarao et al. 2009). Advances on three aspects of BNI research are reported here: (1) gene quantification of soil nitrifying microorganisms to determine BNI activity in *B. humidicola*; (2) screening of *B. humidicola* breeding materials to identify hybrids with contrasting levels of BNI; and (3) quantification of the BNI-residual effect from *B. humidicola* on N-recovery and agronomic-NUE of the subsequent maize crop.

**Methods**

**Gene quantification of soil nitrifying microorganisms to determine BNI activity in *B. humidicola***

A proof of concept work was designed to monitor the dynamics of nitrification in soils as influenced by *Brachiaria* spp. with differential BNI capacities (Subbarao et al. 2009). Soybean crop and bare soil, which lack such BNI capacity, were used as controls. Ammonium-sulfate was applied to each plot. Copy number of *amoA* genes of ammonia-oxidizing bacteria (AOB) and archaea (AOA) were determined through Real-Time PCR to quantify the impact of inhibitory effect from *Brachiaria* sp. under field conditions at 1 day after the ammonium-sulfate application.

**Screening of *B. humidicola* breeding materials to identify hybrids with contrasting levels of BNI**

A set of apomictic *B. humidicola* hybrids were screened by determining nitrification rates in soil samples taken from unreplicated field plots established for seed production. Four CIAT accessions were used as controls for BNI activity.

**Quantification of the BNI-residual effect from *B. humidicola* on N-recovery and NUE of the subsequent maize crop**

One-hectare of field area was selected from each of the three contrasting land uses that included a long-term *B. humidicola* CIAT 679 pasture (15-year-old) with accumulated inhibitory effect in soil (i.e., high BNIs in soil), a nearby agricultural field (in which a crop rotation of maize and soybean was practiced for 4 years) with low BNIs in soil, and a native savanna field with moderate level of BNIs in soil. These three sites were considered as land use treatment sites. Maize hybrid (Pioneer 30K73) was sown on 17 July 2012 in all three field sites. Nitrogen fertilizer was applied at three rates (60, 120 and 240 kg/ha) at each field site. Grain yield and agronomic NUE were determined to assess the BNI residual effect on subsequent maize cultivation.

**Results**

Molecular data confirmed that *B. humidicola* CIAT 16888 has the capacity to inhibit soil nitrification (BNI activity). Rhizosphere soil from *B. humidicola* CIAT 16888 plots exhibited a lower gene copy number of AOB and AOA *amoA* genes compared to the controls (soybean and bare soil) and the other tropical grasses (Fig. 1). Different values of nitrification rates observed in field plots of *B. humidicola* breeding materials suggested genetic variation for BNI and contributed to identification of hybrids with
Figure 1. Gene copy number of ammonia-oxidizing bacteria (AOB) amoA gene (left), and ammonia-oxidizing archaea (AOA) amoA gene (right) at 1 day after ammonium-sulfate application. CON = control (bare soil); SOY = soybean; PM = Panicum maximum; BHM = Brachiaria hybrid cv. Mulato; BH-679 = B. humidicola CIAT 679 (standard cultivar); BH-16888 = B. humidicola CIAT 16888 (a high-BNI capacity germplasm accession). Gene copy number was expressed as copy number per g of dry soil. Values are mean ± SE from three replications.

Figure 2. Genotypic differences in nitrification rates – expressed as NO3-N (mg/kg soil/day) in field plots of B. humidicola hybrids. Gray bars represent B. humidicola CIAT accessions used as controls contrasting BNI capacities (Fig 2). The higher grain yields of maize observed from B. humidicola pasture land use were associated with greater values of agronomic NUE, particularly at lower rate of N applied (60 kg/ha). This observation indicates the importance of accumulated BNIs from this pasture over time in improving the agronomic NUE of maize crop (Fig. 3).

Conclusion

BNI activity in Brachiaria humidicola plots was confirmed by observing a lower copy number of amoA genes from bacterial and archaeal populations compared to soybean and bare soil plots. The wide variation of nitrification rates observed in a set of apomictic B. humidicola hybrids contributed to identification of hybrids with contrasting BNI capacities. Accumulation of BNIs in soil of long-term B. humidicola pasture improved grain yield and agronomic NUE of the subsequent maize crop.

References
