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

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Introduction

Up to 70% of the nitrogen (N) fertilizers applied to agricultural systems is lost due to nitrification and denitrification. Nitrification is a microbiological process that generates nitrate (NO_3^-) and promotes the loss of N fertilizers by leaching and denitrification. Nitrification and denitrification are the only known biological processes that generate nitrous oxide (N_2O), a powerful greenhouse gas contributing to global warming. There is an urgent need to suppress nitrification processes 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_2O 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 3 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 a subsequent maize crop.

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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). A soybean crop and bare soil, which lack such BNI capacity, were used as controls. Ammonium sulfate was applied to each plot. Copy numbers of *amoA* genes of ammonia-oxidizing bacteria (AOB) and archaea (AOA) were determined through Real-Time PCR to quantify the impact of inhibitory effects from *B. humidicola* 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 a subsequent maize crop*

A 1-ha field was selected from each of 3 contrasting land uses: a 15-year-old pasture of *B. humidicola* CIAT 679 (cv. Tully) 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. Hybrid maize (Pioneer 30K73) was sown on 17 July 2012 at all 3 field sites. Nitrogen fertilizer was applied at 3 rates (60, 120 and 240 kg N/ha) at each 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* accession 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 than the controls (soybean and bare soil) and the other tropical grasses (Figure 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 contrasting BNI capacities (Figure 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 rates 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 (Figure 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 with soybean and bare soil plots. The wide variation of nitrification rates observed in a set of apomictic *B. humidicola* hybrids contributed to the identification of hybrids with contrasting BNI capacity. Accumulation of BNIs in soil of a long-term *B. humidicola* pasture improved grain yield and agronomic NUE of the subsequent maize crop.

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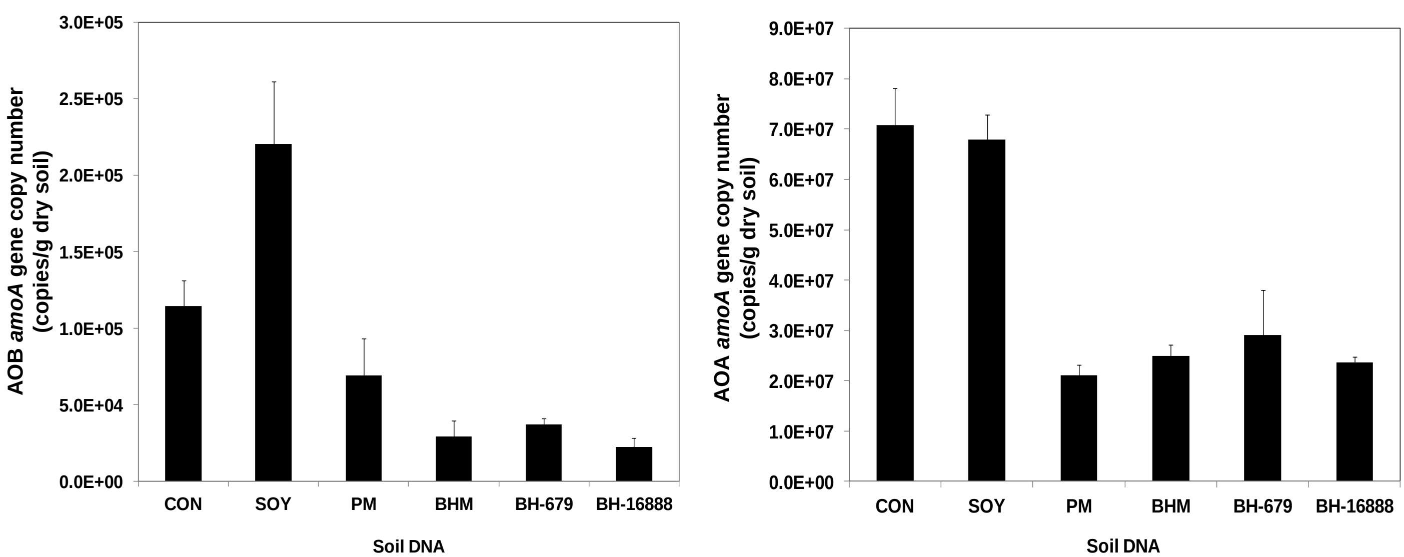

Figure 1. Gene copy numbers of ammonia-oxidizing bacteria (AOB) *amoA* gene (left), and ammonia-oxidizing archaea (AOA) *amoA* gene (right) at 1 day after ammonium sulfate application. CON = control (bare soil); SOY = soybean; PM = *Panicum maximum*; BHM = *Brachiaria* hybrid cv. Mulato; BH-679 = *B. humidicola* CIAT 679 (standard cv. Tully); 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 ± s.e. from 3 replications.

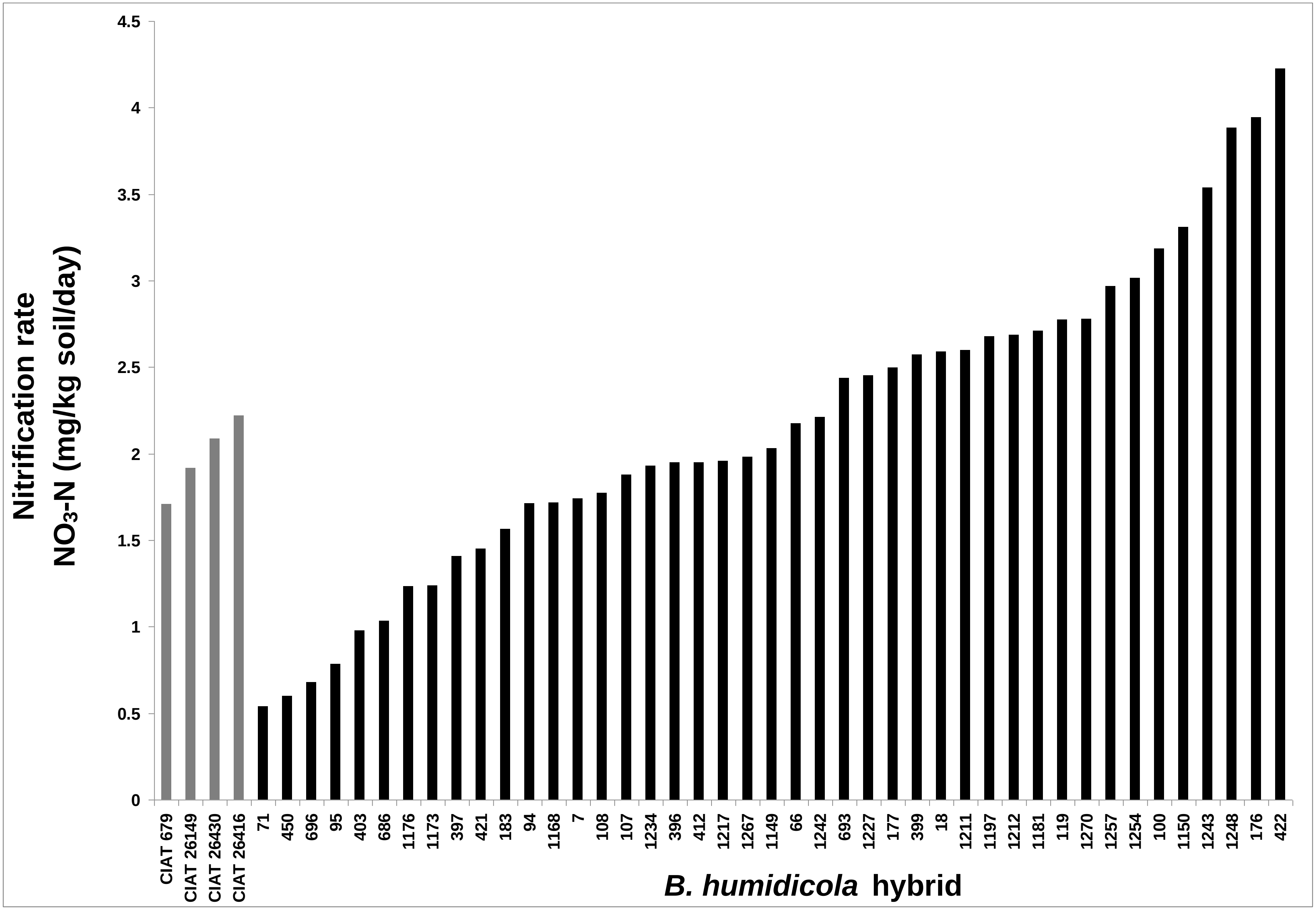

Figure 2. Genotypic differences in nitrification rates – expressed as NO_3-N (mg/kg soil/d) in field plots of *B. humidicola* hybrids. Gray bars represent *B. humidicola* CIAT accessions used as controls.

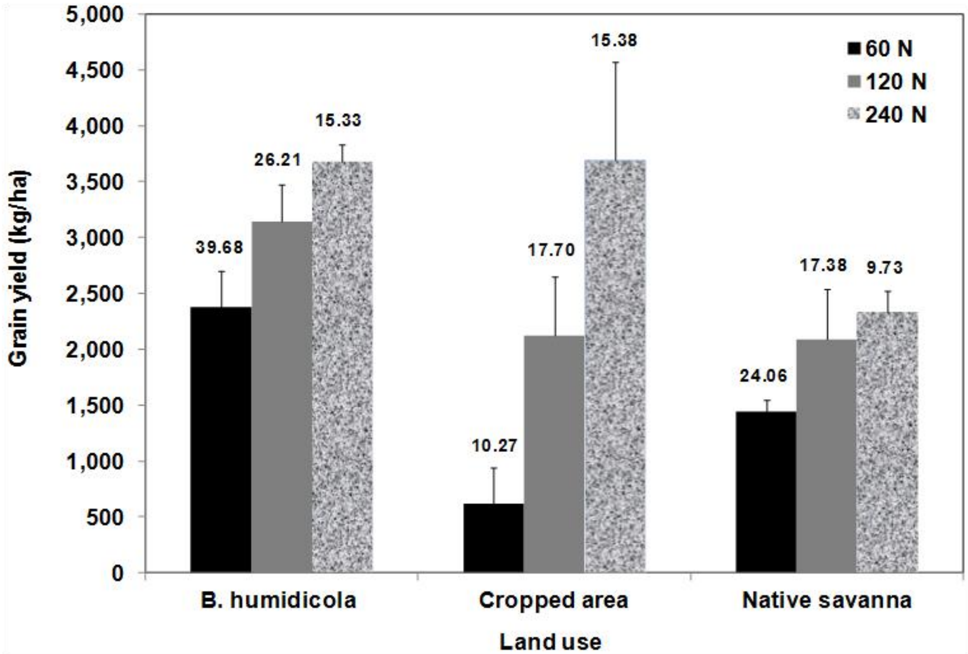

Figure 3. Grain yield (kg/ha) from maize plots fertilized with 60, 120 and 240 kg N/ha, on areas with different previous land uses (15-yr-old *B. humidicola* pasture; 4 years maize-soybean rotations; and native savanna). Agronomic nitrogen use efficiency (kg grain yield/kg N applied) values are shown above the s.e. bars. Values are means ± s.e. from 3 replications.

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