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$\delta^{15}N$ leaf signature in *Brachiaria humidicola* reflects the potential Biological Nitrification Inhibition (BNI)

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Objectives and Hypothesis

Investigate if high and low BNI *Brachiaria humidicola (Bh)* CIAT accessions show contrasting $\delta^{15}N$ signatures in leaves and link this to nitrification inhibition indicators

- ✓ High BNI *Bh* genotypes (CIAT 16888 & CIAT 679) feed on NH_4^+ with a lower $\delta^{15}N$ signature due to nitrification inhibition, whereas low BNI CIAT 26146 (high nitrification) should feed on a respective $\delta^{15}N$ enriched NH_4^+ pool. This should be reflected in the $\delta^{15}N$ signature of leaves
- ✓ Long-term cultivation of *Bh*s with high BNI potential should results in less leaching losses of ^{15}N depleted NO_3^- and therefore in relative lower $\delta^{15}N$ leaf signatures compared to low BNI *Bh* genotypes, where higher $\delta^{15}N$ leaf signature are expected

Background

- ✓ The tropical forage grass *Brachiaria humidicola* (*Bh*) reduces soil microbial nitrification through release of nitrification inhibitors (NIs) and consequently reduces formation of nitrate (NO_3^-) in soils
- ✓ NO_3^- leaching and nitrous oxide (N_2O) emission might therefore be reduced by biological nitrification inhibition (BNI)
- ✓ Intraspecific contrasting BNI potentials in *Bh* have been observed but screening methods for field plot application need to be further developed to identify promising *Bh* candidates with high BNI potential
- ✓ Nitrification discriminates strongly against the stable isotope ^{15}N and consequently leads to a ^{15}N enriched ammonium (NH_4^+) and a ^{15}N depleted NO_3^- pool
- ✓ Long-term losses of ^{15}N depleted NO_3^- via leaching enrich the remaining soil N relatively with ^{15}N

Results

- ✓Sig. correlation among NO_3^- in soil and $\delta^{15}N$ in leaves at 8 days after N fertilization
- ✓High BNI *Bh*s showed respective lower $\delta^{15}N$ signatures and less NO_3^- in topsoil compared to *Bh* with low BNI potential
- ✓Soil incubation indicated lower nitrification for high BNI genotypes compared to low BNI genotype
- ✓Equal $\delta^{15}N$ signatures in Oct 2013 for the 3 genotypes. Two years later high BNI accessions had sig. lower $\delta^{15}N$ values in leaves compared to low BNI *Bh*

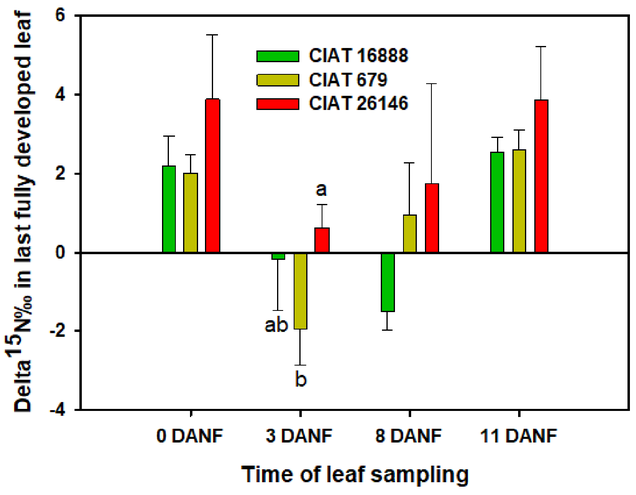


Fig. 2
$\delta^{15}N$ signature of the last fully developed leaf. Sampling from 0-11 days after N fertilization (DANF). CIAT 16888 & CIAT 679 = high BNI, CIAT 26146 = low BNI. Different letters indicate sig. different means (α = 0.05)

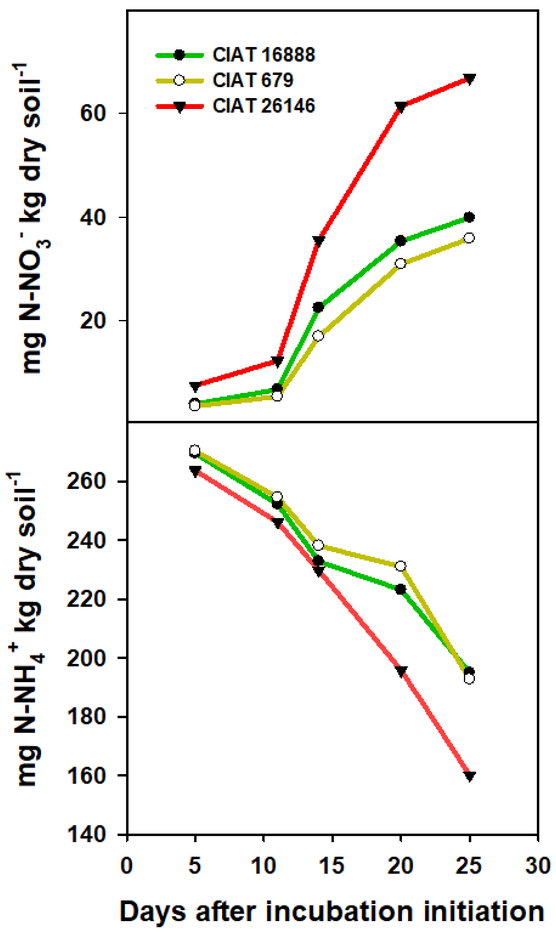


Fig. 3
Soil incubation mineralization results for CIAT 16888 & CIAT 679 = high BNI, CIAT 26146 = low BNI. Soil samples were taken from –N plots and incubated with NH_4^+ solution

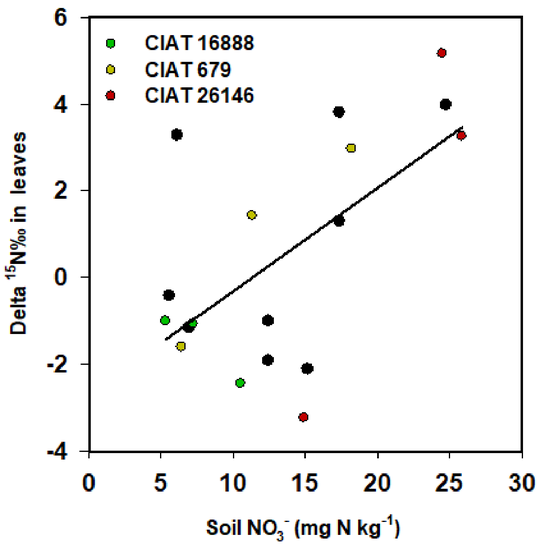


Fig.1 Correlation among NO_3^- in soil and $\delta^{15}N$ in leaves at 8 days after N fertilization (p=0.006, R^2=0.38)

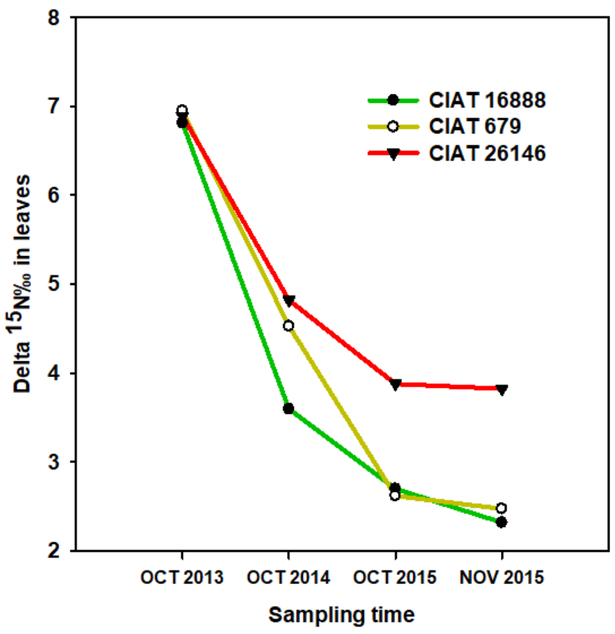
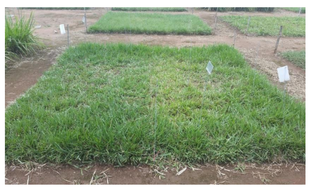


Fig. 4 $\delta^{15}N$ signature of leaves sampled after the rainy season from 2013-2015. CIAT 16888 & CIAT 679 = high BNI, CIAT 26146 = low BNI

Material & Methods

- ✓ Study site: CORPOICA La Libertad Research station, Llanos of Colombia
- ✓ Contrasting BNI *Bh* genotypes were grown since 2012 in a fully randomized block design. In October 2015 plots were split and either fertilized (+N) or not (-N) with 70 kg N ha^{-1}. Soil was collected from -N plots and incubated for potential nitrification determination. Leaves were collected from both split plots of two high BNI (CIAT 679 and CIAT 16888) and one low BNI (CIAT 26146) *Bh* hybrids at 0, 3, 8 and 11 days after fertilization (DAF) and ^{15}N was measured with an IRMS
- ✓ ^{15}N natural abundance of the sample relative to the standard (atmospheric N) was expressed as: $\delta^{15}N‰ = [(R_{sample} / R_{standard}) -1 \times 1000$ (‰) where R represents the isotope ratio ($^{15}N/^{14}N$) and $R_{standard}$ is $^{15}N/^{14}N$ of atmospheric N_2 i.e. 0.3663 ± 0.0004 atom ^{15}N‰



Conclusions

- ✓Expected high BNI potential of CIAT 16888 & CIAT 679 was confirmed by lower soil nitrification
- ✓$\delta^{15}N$ leaf signature was linked to soil nitrification
- ✓Decreasing $\delta^{15}N$ values in leaves over time indicated reduced losses of (^{15}N depleted) NO_3^- due to expected reduction of soil nitrification
- ✓The $\delta^{15}N$ method has the potential to detect:
  - ✓differences of contrasting *Bh* accessions in terms of BNI (preferentially low/no uptake of ^{15}N depleted NO_3^-)
  - ✓differences of long-term N losses in the field

Further work of the authors:

- Karwat H, Moreta D, Arango J, Núñez J, Rao I, Rincón A, Rasche F, Cadisch G (2017) **Residual effect of BNI by *Brachiaria humidicola* pasture on nitrogen recovery and grain yield of subsequent maize**. Plant and Soil. Article in press. DOI: 10.1007/s11104-017-3381-z
- Karwat H, Sparke MA, Arango J, Núñez J, Moreta D, Rao I, Rasche F, Cadisch G (201X) **Nitrate reductase activity in leaves as an indicator of biological nitrification inhibition ability of *Brachiaria humidicola.*** Submitted to Plant and Soil, Article under review
- Núñez J, Arevalo A, Karwat H, Egenolf K, Rao I, Cadisch G, Rasche F, Subbarao G, Miles J, Arango J (201X**) Biological nitrification inhibition activity in a soil-grown biparental population of the forage grass *Brachiaria humidicola.*** Submitted to Plant and Soil, Article under review

We would like to thank for financial support of our research:

Federal Ministry for Economic Cooperation and Development

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