Do Mycorrhiza Play a Role in the BNI Performance of Brachiaria humidicola?

Julian Ruggaber[1], Konrad Egenolf[1], Philipp Schad[1], Nikola Teutscherová[2], Eduardo Vazquez[2], Ashly Arevalo[3], Jacobo Arango[3], Frank Rasche[1]

[1]University of Hohenheim, Inst. of Agric. Sci. in the Tropics (Hans-Ruthenberg-Institute), Germany
[2]Technical University of Madrid, Dept. of Agricultural Production, Spain
[3]International Center for Tropical Agriculture (CIAT), Colombia

Contact:
Julian.ruggaber@uni-hohenheim.de

Context and research objectives

Biological Nitrification Inhibition (BNI) has been demonstrated in a wide variety of plants, but an *in situ* effect was only shown for the pasture grass *Brachiaria humidicola (Bh)*. *Bh* is commonly used as a model to demonstrate the BNI effect as a possibility to tighten the nitrogen cycle in tropical grasslands. BNI was thought to be a purely allelochemical reaction with brachialactone as the compound mainly responsible for the inhibition of nitrifying prokaryotes (Subbarao et al. 2009). However, microbial competition for N and N uptake by Arbuscular Mycorrhizal Fungi (AMF) has not been considered so far. AMF colonization may be one factor that influences BNI performance of crops *in situ*.

Fig 1: Picture of the pot experiment in a greenhouse in CIAT, Palmira, Colombia

Objectives of this study were to evaluate the BNI capacity and mycorrhization of three different *Bh* genotypes under different soil conditions. We aimed to answer the following research questions:

(i) Should AMF be considered in future studies on BNI in other crops?
(ii) Are there differences in the community structure of AMF among the different treatments?

3 Genotypes:
- 679 (high BNI)
- 16888 (high BNI)
- 26146 (mid-low BNI)

X

2 Ferralsoils:
- Porvenir (36% clay)
- Taluma (16% clay)

Fig 2: Two factorial setup of the pot experiment

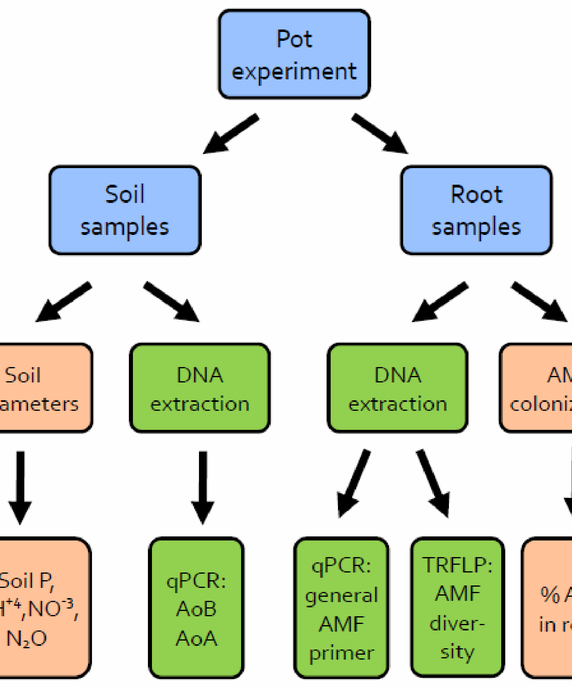

Fig 3: Scheme of the experimental setup. Green boxes indicate a molecular method. Orange boxes indicate a non-molecular method.

Methodology

- Soil and root samples were taken at the end of a two year pot trail with 3 soils and 3 Bh genotypes (Fig 1& 2.)
- Fertilization with N-source and follow up monitoring of $NO3^-$ and $NH4^+$ in soil solution with suction cups.
- Root biomass determination after removal of soil
- AMF colonization: Quantification after root staining with ink and vinegar method (Vierheilig et al. 1998) (Fig 5)
- Net nitrification was measured in a two week fertilization experiment as soil nitrate (NO_3^-) evolution using micro-suction cups and validated by classical N_{min} analysis
- qPCR for AMF primer and TRFLP for community structure analysis will be performed (ongoing)

Preliminary results and discussion

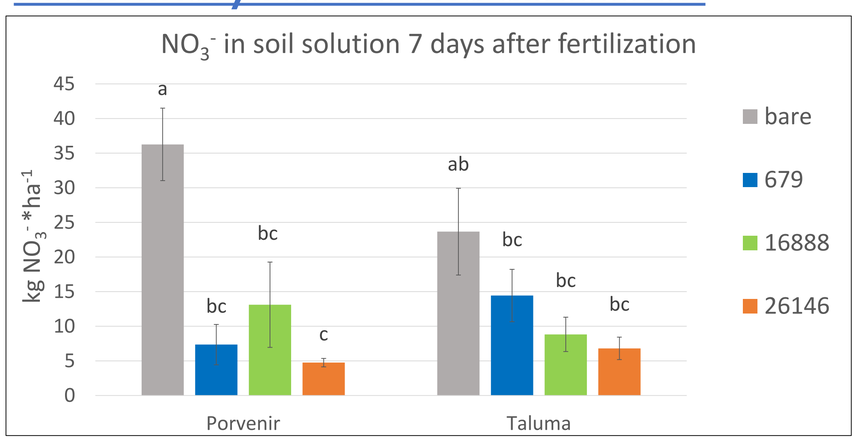

Fig 5: Results of the nitrate levels I n the lysimeter trial after 7 days. The bars indicate standard error, letters indicate grouping with the HSD-test with p=0.05.

NO_3^- levels in soil solution (Lysimeter trail):

- No significant differences in NO3 between genotypes and soils, 7 days after fertilization
  -> no difference in BNI effect ?
  -> Other forms of N immobilization?

Root biomass:

- Linear correlation in limed Porvenir soil treatment: higher biomass means lower NO3- concentration in soils (not shown)
- 26146 (low BNI) has highest root biomass.
  -> Are high BNI roots more effective in N uptake?

AMF colonization:

- No significant difference in AMF colonization between genotype and soil.
- Root staining is a low cost method to reveal mycorrhization and function of AMF (Fig. 5)
- Tendency: Soil texture is determinant for plant AMF colonization (p=0,09)

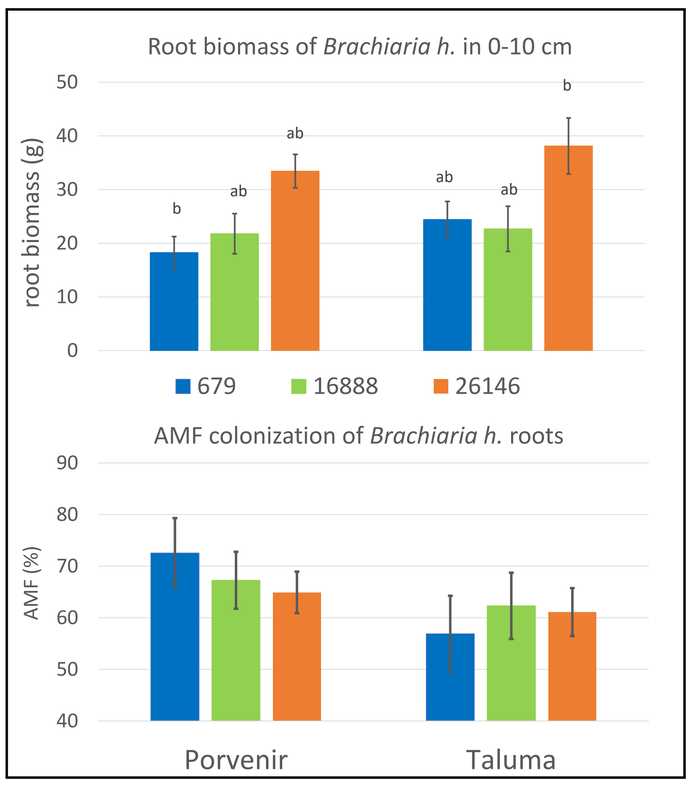

Fig 4: Results of the root biomass determination (top) and AMF colonization analysis (bottom). The bars indicate standard error, letters indicate grouping with the HSD-test with p=0.05.

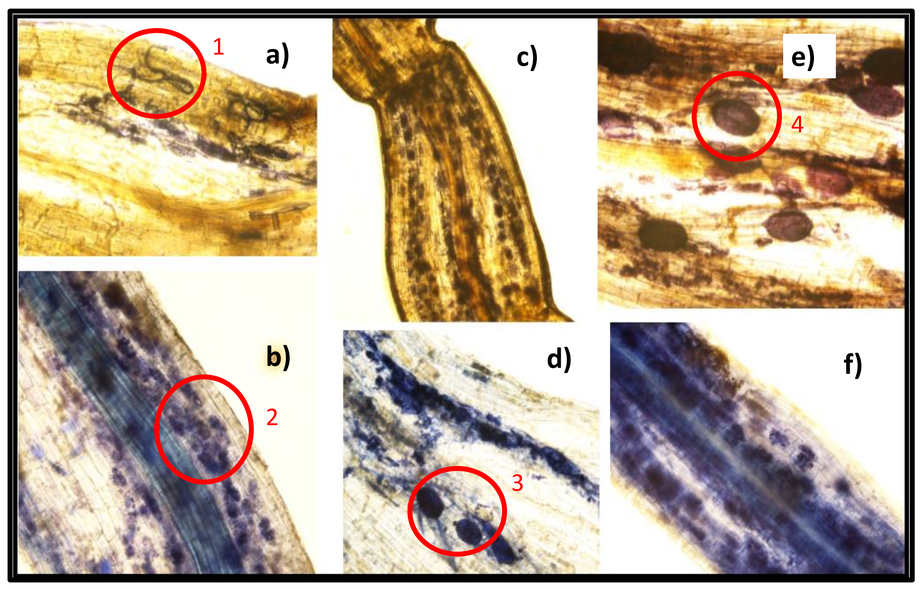

Fig 5: a) and b) genotype 26146; c) and d) genotype 679; e) and f) genotype 16888.
Red circle 1: Hyphae in root; Red circle 2: Popped vesicles; Red circle 3: Vesicle; Red circle 4: Vesicle

Further experiments

- qPCR of extracted root DNA: Will there be significant differences in AMF DNA between genotypes or soil type?
- TRFLP for community fingerprint
- Is data bank TRFLP a viable option to quickly analyze mycorrhizal community structure?

Outlook

- Experiment in soil without mycorrhizal spores can be conducted to demonstrate BNI effects without AMF.
- AMF mediating factor between plants and microbes?
- Remediation strategy: High BNI Bh with AMF spores to restore degraded soils?
- In healthy soils AMF spore numbers are usually sufficiently high to allow sufficient colonization.

References:
Subbarao, G. V., Nakahara, K., Hurtado, M. D. P., Ono, H., Moreta, D. E., Salcedo, A. F., ... & Yoshida, M. (2009). Evidence for biological nitrification inhibition in Brachiaria pastures. Proceedings of the National Academy of Sciences, pnas-0903694106.
Vierheilig, H., Coughlan, A. P., Wyss, U. R. S., & Piché, Y. (1998). Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Applied and environmental microbiology*, *64*(12), 5004-5007.

This study is financed by
Anton & Petra Ehrmann-Stiftung Research Training Group
„Water – People – Agriculture“ at the University of Hohenheim.

This study is supported by
„Foundation fiat panis“

This study was conducted within the framework of the LivestockPlus project funded by the CGIAR Research Program (CRP) on Climate Change, Agriculture and Food Security (CCAFS), which is a strategic alliance between the CGIAR and Future Earth. In addition, this work was also carried out as part of the Livestock CRP. We thank all the donors who support the work of the program globally through their contributions to the CGIAR system.