Effect of *Epichloë* symbiosis on arbuscular mycorrhizal fungi community. A metagenomic approach

M. Victoria Vignale[1,2], Lucía Ortiz Rocca[3], Marcelo Soria[3], Leopoldo Iannone[1,2], M. Victoria Novas[1,2]

[1]Institute de Micología y Botánica (INMIBO), Buenos Aires (Argentina)
[2]Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (Argentina)
[3]Cátedra de Microbiología, Facultad de Agronomia, Universidad de Buenos Aires, INBA-CONICET (Argentina)
mvnovas@gmail.com

Studies in native grasses have shown that endophyte-infected (E+) plants present higher colonization of AMF than endophyte-free (E-) plants, suggesting a positive association between arbuscular mycorrhizal fungi (AMF) and endophytic *Epichloë* species.

The objective of this work was to study if *Epichloë* species in two different *Bromus auleticus* ecotypes, modified the AMF species present in the rhizosphere by comparing E+ to E- plants.

E+ and E- seeds of *Bromus auleticus*, a native grass in Argentina, symbiotic with *Epichloë* sp. (EP ecotype) or with *Epichloë pampeana* (LP ecotype) were used in the study. Plots were established in the agricultural experimental station EEA-INTA, Concepción del Uruguay, Entre Ríos Province, Argentina. Five years later, three independent soils samples from the rhizosphere of EP E+, EP E-, LP E+ and LP E- plants were collected, total DNA was isolated, a fragment of the gene coding for the 18S rRNA was amplified with primers that showed higher specificity for Glomeromycotina and sequenced with a Roche 454 pyrosequencer.

When replicates yielded very few reads, they were discarded from the analysis, such that every treatment had duplicate samples except EP E+, which had triplicates. The reads were subjected to a filtering process. Sequences not aligned to the expected region or assigned to taxa different from known AMF were removed. Bioinformatic and data analyses were carried out with mothur (https://mothur.org/) and R (https://www.r-project.org/).

The number of reads per sample ranged from 4719 to 16101 and to facilitate the analysis the reads were randomly normalized to 4700 for all replicates. A distance matrix between reads was built and they were clustered into Operational Taxonomic Units (OTUs) at a genetic distance cutoff of 0.03. In total, 1093 OTUs were obtained and each received a consensus taxonomic designation. The abundance of OTUs per treatment was used to calculate a Bray-Curtis distance matrix among treatments.

There were differences in the fungal microbiome composition between ecotypes, but not between endophytic status.

The presence of the endophyte did not significantly alter metrics of alpha diversity such as observed richness, Chao's richness estimator or inverse-Simpson measure of diversity.

Although the global analysis did not reveal differences between E+ and E- plants we observed several OTUs with large differences associated with the endophyte presence.

Most of the OTUs were unclassified genera or members of the *Septoglomus* genus, which was one of the most frequent genera found. Species of this genus are found in different environments, especially in soils under extreme conditions. The soil in Concepcion del Uruguay is a vertisol with high concentration of clay that forms deep cracks in dry seasons.

In conclusion, these results suggest that the higher mycorrhizal colonization observed in E+ plants are due to the facilitation of the establishment of the symbioses and not necessarily to the diversity of AMF species found in the rhizosphere of E+ plants.