Effect of grazing abandonment on arbuscular mycorrhizal diversity in Gorbeia Natural Park

Sarrionandia E., Muguerza E., Anza M., Lanzén A., Garbisu C., Epelde L.

in

Casasús I. (ed.), Lombardi G. (ed.).
Mountain pastures and livestock farming facing uncertainty: environmental, technical and socio-economic challenges

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 116

2016
pages 271-274

To cite this article / Pour citer cet article

Sarrionandia E., Muguerza E., Anza M., Lanzén A., Garbisu C., Epelde L. **Effect of grazing abandonment on arbuscular mycorrhizal diversity in Gorbeia Natural Park.** In : Casasús I. (ed.), Lombardi G. (ed.). *Mountain pastures and livestock farming facing uncertainty: environmental, technical and socio-economic challenges.* Zaragoza : CIHEAM, 2016. p. 271-274 (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 116)

Effect of grazing abandonment on arbuscular mycorrhizal diversity in Gorbeia Natural Park

E. Sarrionandia[1], E. Mugerza[2], M. Anza[2], A. Lanzén[2], C. Garbisu[2] and L. Epelde[1]

[1]Department of Plant Biology and Ecology, University of the Basque Country
E-01006 Vitoria-Gasteiz (Spain)
[2]Soil Microbial Ecology Group, NEIKER-Tecnalia, E-48160 Derio (Spain)

Abstract. Mountain pastures in the Basque Country (northern Spain) are the result of extensive grazing dating from the Neolithic. However, current management practices are resulting in the progressive abandonment of grazing in these mountain areas. Soil microbial communities are vital for many soil processes and the delivery of essential ecosystem services; in particular, arbuscular mycorrhizal fungi play a key role in plant nutrient acquisition. The influence of grazing on the diversity of arbuscular mycorrhizal communities remains poorly explored. Then, our main objective was to evaluate the effect of the abandonment of grazing on arbuscular mycorrhizal diversity through the study of twelve grazed versus non-grazed (simulated by 100 m^2 exclusions) areas in Atlantic mountain grasslands. We determined the diversity of arbuscular mycorrhizal fungi using both morphological (spore morphotyping under the microscope) and genetic (Illumina sequencing of ITS amplicons) approaches. After two years, only slight differences on arbuscular mycorrhiza were observed between grazed and non-grazed areas. In fact, the type of vegetation under which the exclusions were established accounted for the greatest amount of data variability. On the other hand, both approaches (morphological and molecular) gave similar results, highlighting the complementarity of these approaches for the study of the biodiversity of arbuscular mycorrhiza.

Keywords. Glomeromycota – Morphotypes – Metabarcoding.

Effet de l’abandon du pâturage sur la diversité mycorhizienne dans le Parc Naturel Gorbea

Résumé. *Les pâturages de montagne du Pays Basque (nord de l’Espagne) sont le résultat d’une pratique de pâturage extensif datant du néolithique. Toutefois, les pratiques de gestion actuelles se traduisent par l’abandon progressif du pâturage dans ces zones de montagne. Les communautés microbiennes du sol sont essentielles pour de nombreux processus du sol et la fourniture de services écosystémiques essentiels; en particulier, les mycorhizes arbusculaires jouent un rôle clé dans l’acquisition des éléments nutritifs des plantes. L’influence du pâturage sur la diversité des communautés mycorhiziennes arbusculaires reste mal exploré. Notre objectif principal était d’évaluer l’effet de l’abandon du pâturage sur la diversité mycorhizienne à travers l’étude de douze zones pâturées par rapport aux zones non pâturées (simulé par des exclusions de 10 m^2) dans les prairies de montagne de l’Atlantique. Nous avons déterminé la diversité des champignons mycorhiziens arbusculaires en utilisant à la fois des approches morphologique (spores morphotype sous le microscope) et génétique (séquençage Illumina des amplicons ITS). Après deux ans, seules des légères différences sur les communautés mycorhiziennes ont été observées entre les zones pâturées ou non. En fait, le type de végétation dans lesquelles les exclusions ont été établies ont expliqué la plus grande part de la variabilité observée. D’autre part, les deux approches (morphologiques et moléculaires) ont donné des résultats similaires, mettant en évidence la complémentarité de ces approches pour l’étude de la biodiversité des mycorhizes arbusculaires.*

Mots-clés. *Glomeromycota – Morphotypes – Metabarcoding.*

I – Introduction

Soil microbial communities are vital for many soil processes and the delivery of essential ecosystem services. Arbuscular mycorrhizal fungi (AMF), in particular, are considered natural biofertilizers, since they provide the host with water and nutrients, as well as pathogen protection, in exchange for photosynthetic products (Berruti *et al*., 2016). However, plant species respond differently to different fungal species; AMF biodiversity might affect plant community (van der Heijden *et al*., 1998) and, consequently, overall ecosystem services.

In mountain pastures of the Basque Country (Spain) grazing has been the most important economic activity since the Neolithic (Barandiaran and Manterola, 2000). These semi-natural grasslands deliver many valuable ecosystem services (Bullock *et al*., 2011). If the management trends observed continue, a progressive medium- or long-term grazing abandonment could be expected. The question of whether diversity of AMF may be modified by livestock grazing remains poorly explored, and we hypothesize that thousands of years of herding activity has shape a distinct AMF diversity. In this respect, the main objective of our study was to evaluate the effect of the abandonment of grazing on AMF diversity through the study of twelve grazed versus non-grazed areas in Atlantic mountain grasslands.

II – Materials and methods

1. Study area and experimental design

The current study was carried out in the Gorbeia Natural Park (43ºN 2.5ºW), in the Atlantic region of the Basque Country (northern Spain). Within the Natural Park, four locations with distinct type of grassland vegetation were chosen; 3 in the mountain area at 630-720 m.a.s.l. (mountain 6230a, mountain 6230c and mountain 6170), and one in the valley at 240-410 m.a.s.l. (Valley). In spring 2012, three permanent exclusions of 10 x 10 m were placed in these locations, twelve in total.

2. Determination of arbuscular mycorrhizal diversity

A total of 24 soil samples (12 inside and 12 outside) were collected two years after the establishment of the exclusions. For the morphological characterization of mycorrhiza, spores were isolated from 50 g dry weigh of soil from each sample by wet sieving, decanting and centrifugation in sucrose medium. After manually selecting all the spores present under a microscope at 40x magnifications, they were mounted on slides in either polyvinyl alcohol –lactic acid– glycerol (PVLG) or a mixture of PVLG-Melzer reagent. Spore characteristics were observed under a microscope at 200x and 400x magnifications for the establishment and quantification of morphotypes. For the genetic characterization, DNA extraction was carried out from aliquots corresponding to 0.25 g of dry-weight soil from all samples using PowerSoil DNA Isolation kits. Fungal ITS amplicon library preparation, Illumina MiSeq sequencing, sequence data processing and taxonomic classification were performed as described in Lanzén *et al*. (2015). The effect of grazing on arbuscular mycorrhizal diversity was assessed through univariate (ANOVA analysis of variance followed by post-hoc Tukey test using Microsoft StatView software) and multivariate (canonical correspondence analyses using Canoco 5 to study the influence of experimental factors on mycorrhizal composition) statistical tests.

III – Results and discussion

A total of 18 spore morphotypes and 832 OTUs belonging to Glomeromycota division were distinguished. Regarding alpha diversity, the indexes calculated showed no significant differences between grazed and ungrazed areas nor with the morphological neither with the genetic approach (Table 1). However, the type of vegetation accounted for significant differences in both richness and Shannon´s diversity index with the genetic approach, being the greatest in the valley. From this we can interpret that the edaphoclimatic conditions in the mountain may not favour the establishment of mycorrhiza (Lugo *et al*. 2012), but other factors, such as grazing pressure, could be involved since arbuscular mycorrizal species diversity has been shown to increase with the altitude (Bonfim *et al*. 2016).

Table 1. Diversity indexes (average ± standard deviation, n = 3, different letters denote significant differences according to Tukey test)

	Morphological approach		Genetic approach	
	Shannon	Richness	Shannon	Richness
Mountain 6230a NON GRAZED	1.75 ± 0.19^{a}	14.0 ± 1.0^{a}	2.03 ± 0.33^{a}	18.9 ± 1.1^{a}
Mountain 6230a GRAZED	1.79 ± 0.11^{a}	13.7 ± 1.2^{a}	2.01 ± 0.35^{a}	19.8 ± 5.1^{a}
Mountain 6170 NON GRAZED	1.62 ± 0.06^{a}	14.3 ± 1.2^{a}	2.79 ± 0.29^{ab}	40.2 ± 3.4^{a}
Mountain 6170 GRAZED	1.67 ± 0.14^{a}	10.0 ± 1.7^{a}	2.64 ± 0.50^{ab}	35.1 ± 14.9^{ab}
Mountain 6230b NON GRAZED	1.83 ± 0.13^{a}	13.0 ± 1.0^{a}	2.52 ± 0.31^{a}	26.5 ± 4.4^{a}
Mountain 6230b GRAZED	1.72 ± 0.19^{a}	13.0 ± 2.0^{a}	2.36 ± 0.10^{a}	23.3 ± 2.8^{a}
Valley NON GRAZED	1.32 ± 0.32^{a}	14.3 ± 2.3^{a}	3.54 ± 0.36^{b}	61.6 ± 12.8^{b}
Valley GRAZED	1.42 ± 0.29^{a}	14.3 ± 3.2^{a}	3.49 ± 0.23^{b}	60.4 ± 3.8^{b}

The results are similar in respect to the composition of the AMF community (Fig. 1). With both approaches, the variable that accounts for the greatest amount of data variability is the type of vegetation and not the grazing treatment. In the canonical correspondence analyses performed, valley samples separate from the rest along CCA-1, while the habitat 6170 separates from habitats 6230 along CCA-2. Having a closer look at the individual taxonomic level, there are four morphotypes in the 6170 habitat and some OTUs classified as "uncultured Glomerales" in the 6230c habitat that increase significantly ($P<0.05$; data not shown) inside the exclusions. Some studies have previously reported changes in soil microbial parameters (e.g., enzyme activities, microbial biomass, CO_2 emissions) as a consequence of grazing abandonment (Aldezabal *et al*., 2015), which have been strongly related with the occurrence of certain fungal species (Bonfim *et al*. 2016).

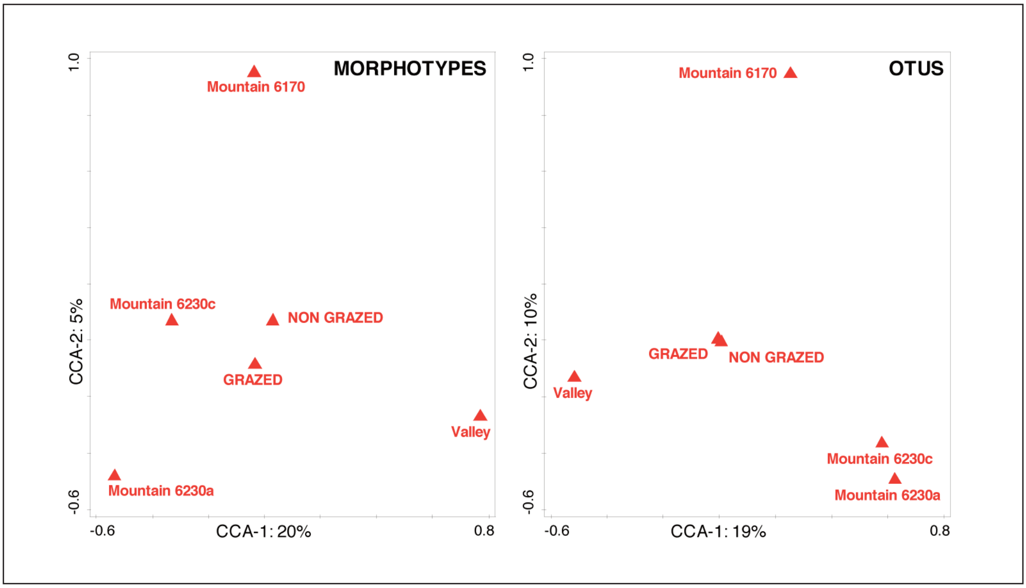

Fig. 1. Scatterplots of the canonical correspondence analyses performed using the treatments and Habitats as explanatory variables and the spore morphotypes (left; pseudo-F = 1.9, P = 0.014) or OTUs of the Glomeromycota division (right; pseudo-F = 2.8, P = 0.002) as response variables.

The reason for the few differences found in our case might be the short term character of the study (the exclusions were established just two years before the sampling) or the need to perform a more exhaustive sampling to overcome the huge spatial heterogeneity of soil microbial communities. Finally, the genetic approach allowed the detection of more OTUs than morphotypes found with the morphological approach. Therefore, it seems that several OTUs were considered to belong to the same morphotype under the microscope. Interestingly, both approaches gave similar results, strengthening the reliability of the data obtained.

IV – Conclusions

A longer period of time under exclusion and a more exhaustive sampling might be needed to observe consistent differences on AMF diversity as a consequence of grazing abandonment. Apart from that, a higher alpha diversity of AMF was found in valley areas compared to mountain areas. This study highlights the complementarity of the morphological and genetic approaches for the study of the biodiversity of AMF.

Acknowledgments

This work has been financially supported by the Spanish Ministry of Economy and Competitiveness through the BIOPASTO project (AGL2013-48361-C2-2-R).

References

Aldezabal A., Moragues L., Odriozola I. and Mijangos I., 2015. Impact of grazing abandonment on plant and soil microbial communities in an Atlantic mountain grassland. *Applied Soil Ecology,* 96, 251-260.

Barandiaran J.M. and Manterola A., 2000. *Ganaderia y pastoreo en Vasconia*. Etniker Euskalerria-Eusko Jaurlaritza, Bilbao.

Berruti A., Lumini E., Balestrini R. and Bianciotto V., 2016. Arbuscular mycorrhizal fungi as natural biofertilizers: let´s benefit from past successes. *Frontiers in Microbiology,* 6, 1559.

Bonfim J.A., Figueiredo Vasconcellos R.L., Gumiere T., Colombo Mescolotti, D.L., Oehl F. and Cardoso E.J.B., 2016. Diversity of arbuscular mycorrhizal fungi in a Brazilian atlantic forest toposequence. *Microbial Ecology,* 71, 164-177.

Bullock J.M., Jefferson R.G., Blackstock T.H., Pakeman R.J., Emmett B.A., Pywell R.J., Grime J. and Silverton J., 2011. Chapter 6: Semi-natural grasslands. In: *The UK National Ecosystem Assessment Technical Report,* UK National Ecosystem Assessment. UNEP-WCMC, Cambridge, UK.

Lanzén A., Epelde L., Garbisu C., Anza M., Martín-Sánchez I., Blanco F. and Mijangos I., 2015. The community structures of prokaryotes and fungi in mountain pasture soils are highly correlated and primarily influenced by pH. *Frontiers in Microbiology,* 6, 1321.

Lugo M.A., Negritto M.A., Jofre M., Anton A. and Galetto L., 2012. Colonization of native Andean grasses by arbuscular mycorrhizal fungi in Puna: a matter of altitude, host photosynthetic pathway and host life cycles. *FEMS Microbiology Ecology,* 81, 455-466.

van der Heijden M.G.A., Klironomos J.N., Ursic M., Moutoglis P., Streitwolf-Engel R., Boller T., Wiemken A. and Sanders I.R., 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature,* 396, 69-72.