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Impact of grazing abandonment and phosphoric fertilization on *Nardus* grasslands floristic diversity in Gorbeia Natural Park (Bizkaia, Northern Spain)

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Abstract. Among the different grazing habitats of Gorbeia Natural Park, *Nardus stricta* species-rich grasslands (habitat of community interest 6230*, Council directive 92/43/EEC) have special importance due to its large coverage, ecological value, landscape relevance, and its social-economic importance as the territorial base of livestock farms. This study focuses on this particular habitat and aims to assess the impact of grazing exclusion (which simulates abandonment) and the use of phosphoric fertilization on the floristic diversity of these grasslands in Gorbeia. Experimental treatments (4 replicates per treatment) included "non-grazing", "phosphoric fertilization" and the control ("grazing and non-fertilization"). In each replicate (experimental plot of 10 × 10 m), randomly selected sampling squares (50 × 50 cm) were used to study species presence and cover (in 2012, 10 square per replicate; in 2013, 5). Differences between treatments and years in both richness and the equivalent number of species (i.e., the number of equally-common species, a proper measure of species diversity) were studied through curves of accumulated richness and diversity. The results showed different patterns according to the year. In 2012, the lowest richness and species diversity were found in the fertilization treatment. No differences were detected between the control and exclusion treatments. In 2013, the highest richness and species diversity were observed in the control; the species richness was intermediate in the fertilization treatment and lowest in the exclusion treatment. Our results will contribute to the knowledge and implementation of management guidelines aiming to the conservation of biodiversity of mountain habitats.

Keywords. Diversity – Species richness – *Nardus stricta* – Natura 2000 network – Floristic composition – Community interest habitat 6230 *

Impact de l'abandon du pâturage et de la fertilisation phosphorique sur la diversité floristique des prairies de* Nardus *dans le Parc Naturel Gorbea (Bizkaia, Nord de l'Espagne)

Résumé. *Parmi les différentes formes de végétation liées au pâturage dans le Parc Naturel de Gorbea, les prairies de nard raide (*Nardus stricta*) (habitat d'intérêt communautaire 6230*, Directive 92/43/CEE) présentent une importance toute particulière en raison de leurs grandes surfaces, leurs valeurs écologique et paysager ainsi que d'un point de vu socio-économique du à leurs rôles comme base territoriale des exploitations d'élevage. L'objectif de cette étude était d'évaluer les effets de l'abandon du pâturage et de la fertilisation phosphorée sur la diversité botanique des prairies de* N. stricta *dans le massif de Gorbeia. Les traitements expérimentaux (4 répétitions par traitement) étaient constitués par le "non-pâturage" et "fertilisation phosphorée" ainsi qu'un traitement témoin "pâturage et non-fertilisation". Dans chaque répétition (parcelle expérimentale de 10 × 10 m) un inventaire botanique et le % de couverture végétale ont été réalisés dans la surface d'un carré (0,5 × 0,5 m) lancé au hasard. En 2012, dix lancements par répétition ont été réalisés et cinq en 2013. Les résultats ont montré des tendances différentes selon l'année. En 2012, le traitement "fertilisation phosphorée" a montré les valeurs les plus petites de richesse et de diversité botanique. Aucune différence n'a été décelée entre les traitements témoin et d'exclusion. En 2013, les valeurs de richesse et de diversité les plus élevées ont été observées dans le traitement "fertilisation phosphorée"; la valeur de la richesse de la flore était intermédiaire pour le témoin et la plus faible dans le traitement de l'exclusion. Les résultats de cette étude contribueront à la connaissance et la mise en œuvre des directives de gestion qui visent à la conservation de la biodiversité de ces habitats de montagne.*

Mots-clés. *Diversité – Richesse en espèces* – Nardus stricta – *Réseau Natura 2000 – Composition floristique – Communauté d'intérêt habitat 6230 **

I – Introduction

Pastoral mountain landscape in the Basque Country is dominated by community interest priority habitat 6230*. Currently, it remains well-conserved despite threats such as the abandonment of its use caused by the lack of generational handover and some management practices (like fertilization, clearing and herbicide application) that could be deemed as inadequate (Rigueiro *et al*., 2009). Grazing abandonment is a medium term threat that can lead to a secondary succession with the invasion of pastures by tall grasses, shrubs and trees (Galvanek and Janák, 2008; Mandaluniz *et al*., 2007). In fact, the technical report 2008/14/24 of the European Commission Management of "Natura 2000 habitats * Species-rich *Nardus* grasslands 6230" recommends grazing as one of the main practices for this habitat management. On the other hand, several guidelines in the context of agricultural practices have been defined on the use of mineral fertilizers in order to avoid serious and irreversible biodiversity loss (Rodriguez *et al*., 2001). The *Nardus stricta* priority species present in this habitat 6230* requires the prohibition of actions that modify soil conditions where it develops (Rigueiro *et al*., 2009).

In this context, the aim of this research was to evaluate the impact of grazing exclusion and phosphoric fertilization on the structure of herbaceous communities by using biodiversity indexes, such as species richness and the equivalent number of species (i.e., a proper measure of the 'true species diversity'; see Jost, 2006; Jost and González-Oreja, 2012).

II – Materials and methods

1. Study area and design

The study was carried out on Oderiaga grazing area, located in the Gorbeia Natural Park (Bizkaia; Northern Spain), with a surface of 1555 ha of which 147 ha correspond to the habitat 6230* (dominant species: *Festuca gr. rubra, Agrostis curtisii, A. capillaris, Gallium saxatile, Potentilla erecta*). The area lays on siliceous parent material and predominantly faces the south. It is the most important area within the natural park in terms of grazing livestock, with 24.5% of the total stocking rate (Albizu *et al*., 2010).

Experimental treatments (4 replicates per treatment) included "non-grazing", "phosphoric fertilization" and the control ("grazing and non-fertilization"). Applied ecologic phosphoric fertilization was FERTIGAFSA TDVIDA TD 1-32, in a dose of 192 kg ha^{-1}. In each replicate (experimental plot of 10 × 10 m), randomly selected sampling squares (50 × 50 cm) were used to register species presence and cover (in 2012, 10 square per replicate; in 2013, only 5). Differences in richness (observed and extrapolated number of species) and the "true diversity" (exponential of Shannon diversity index) of plant species between treatments and years were studied through curves of accumulated richness and diversity.

Conceptually, species richness is the most intuitive and direct parameter for measuring biodiversity (Gotelli and Colwell, 2001). However, its determination is not simple due to the increase in the number of observed species along with the sampling effort. As a result, it is necessary to undertake a complete, laborious, time-consuming and thorough sampling in order to obtain a comprehensive measure of the true richness of a plant community. EstimateS version 9.10 (Colwell 2013) was used for calculating diversity indexes, and also for obtaining curves of accumulated richness and diversity, as well as extrapolation of richness for 120 and 60 squares for 2012 and 2013, respectively. At comparable levels of sampling effort (as measured by the number of sampled squares), mean values and corresponding 95% confidence intervals (CI) for species accumulation and diversity curves were computed following Colwell *et al*. (2004); differences were judged as statistically significant ($P < 0.05$) if CI did not overlap. For more details on CI, please see analytical formulas as presented in Colwell (2013). The considered effects were "grazing, non-fertilization", grazing with "phosphoric fertilization" and "non -grazing".

III – Results and discussion

In 2012, the curves of accumulated richness of "grazing, non-fertilization" and "non-grazing" treatments were parallel to each other and had only minor differences (both in terms of specific richness and extrapolated until 120 squares) (Fig. 1). The curve for "phosphoric fertilization" always remained lower, which means that the fertilization treatments were linked to less species richness in studied grasslands. However, in 2013 the observed pattern was different: the richness was higher in fertilized grasslands, while the number of extrapolated species in control ("grazing, non-fertilization") was intermediate and lowest in non-grazing areas.

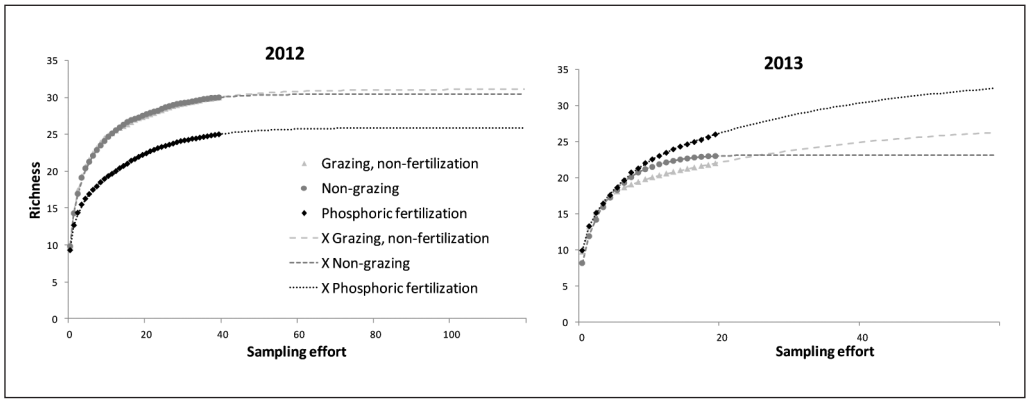

Fig. 1. Observed and extrapolated (X) richness for grazing, non-fertilization, non-grazing and phosphoric fertilization treatments during the years of 2012 and 2013, for a sampling effort of 40 (2012) and 20 (2013) quadrants treatment^{-1}. Sampling effort measures the number of sampled quadrats.

As for the diversity index, the number of equivalent species was similar to that observed in 2012: similar curves between "non-grazing" and "grazing, non-fertilization" treatments while in phosphoric fertilization, species diversity was the lowest. During 2013, diversity was the maximum in the control treatment ("grazing, non-fertilization") for any sampling effort, followed by the "phosphoric fertilization" and finally, "non-grazing" treatments. There was no overlapping between the confidence intervals corresponding to 95 % (dashed lines, Fig. 2). Thus, we can accept that the differences between treatments were statistically significant. In 2013, when the number of observations was greater than 10, differences were significant among treatments. Differences were higher with increasing sampling efforts. That is, diversity in 2013 was highest in "grazing, non-fertilization" treatment, followed by "grazing, non-fertilization" and finally for "non-grazing".

The results are in line with many other studies that have shown the importance of grazing in the conservation of plant biodiversity and the decline of it with abandonment (see, for instance, Mariotte *et al*., 2013). In this regard, it is necessary to highlight the importance of the type of grazing for conservation. In trials under the project FARMING LIFE REGEN carried out for testing free versus guided grazing, higher biodiversity values were detected for directed grazing (Mandaluniz *et al*., 2016, accepted for EGF congress). At the same time, timescale effect is important and thus, it is necessary to analyze grazing abandonment effect on biodiversity in a longer term. As for the effect of phosphoric fertilization, our results are in line with studies in which it was observed that pastures with phosphoric input have lower species richness and diversity, and showed a decrease of diversity with a progressive increase in phosphoric fertilization doses (Puerto *et al*., 1990) or with studies that related a higher floristic richness in soils with low or intermediate nutrients levels (Rodriguez *et al*., 2001).

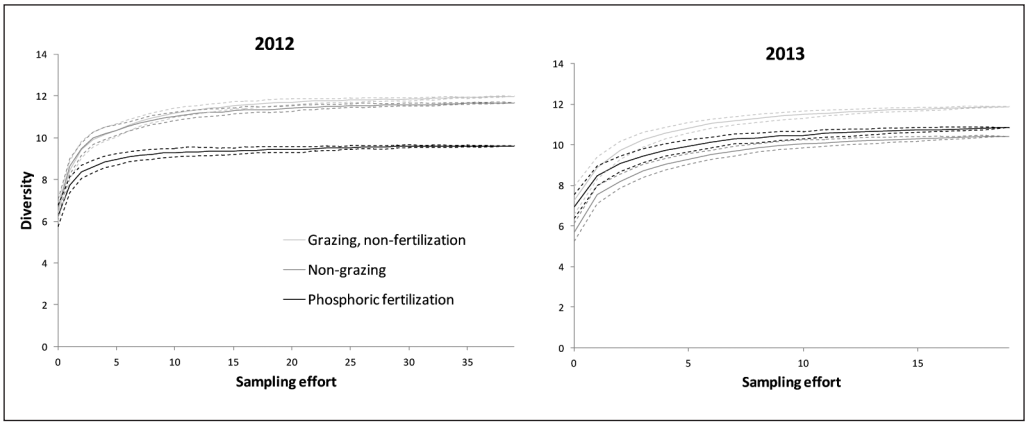

Fig. 2. Shannon exponential diversity values (and 95% confidence intervals) for grazing, non-fertilization, non-grazing and phosphoric fertilization treatments during the years of 2012 and 2013, for a sampling effort of 40 (2012) and 20 (2013) quadrants treatment^{-1}. Sampling effort measures the number of sampled quadrats.

IV – Conclusions

The effect of abandonment and phosphorus fertilization treatments differ depending on the time lag between the application and observations. In this sense, the fertilization has a negative impact on the richness and diversity of species when it is applied. However, the effect of the exclusion is increasing so that, although at first no effect is detected, after one year from the applications, the decline of diversity values is significant. All of the tested experimental treatments involve a loss of species diversity.

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