

Capítulo 6

Los herbívoros y el banco de semillas de una comunidad de plantas anuales: productividad, identidad del herbívoro y endozoocoria

Este capítulo reproduce íntegramente el texto del siguiente manuscrito:

Lucía Gálvez Bravo, Matra Rueda & Salvador Rebollo (*In preparation*). Herbivores and the soil seed bank of an annual plant community: productivity, herbivore identity and endozoochory

Resumen

Los efectos de los herbívoros sobre el banco de semillas de la comunidad herbácea pueden ser positivos, negativos o neutros, y a través de varios mecanismos. Factores tales como la identidad de los herbívoros y la productividad local tendrán una influencia sobre los efectos netos de los herbívoros sobre la comunidad de plantas anuales, y la endozoocoria es uno de los mecanismos más importantes a través de los cuales los herbívoros pueden ejercer su influencia. El objetivo principal de este estudio fue investigar los efectos de herbívoros de distinto tamaño sobre el banco de semillas de los pastizales anuales mediterráneos, haciendo especial énfasis en el papel que juega la productividad de las plantas y la endozoocoria. Se consideraron pastizales con diferente productividad (alta y baja), y dos herbívoros de diferente tamaño, el conejo Europeo (*Oryctolagus cuniculus*) y la oveja (*Ovis aries*). Cinco bloques con tres tratamientos de pastoreo cada uno fueron colocados en zonas de baja (LPS) y alta productividad (HPS). Cada bloque incluyó tres parcelas con los siguientes tratamientos de pastoreo: 1) pastado libremente por los dos herbívoros; 2) exclusión de ovejas, pastado únicamente por conejos; 3) exclusión total. Dentro de cada parcela pastada se recogieron muestras del banco de semillas de 3 subparcelas en las que se retiraban los excrementos de los herbívoros mensualmente (subparcelas sin excrementos), y 3 subparcelas que no se alteraban (subparcelas con excrementos). Se encontraron grandes diferencias en los efectos del pastoreo sobre el banco de semillas de lugares con distinta productividad. En HPS, la presencia de herbívoros tuvo un efecto positivo sobre la abundancia y diversidad del banco de semillas. En LPS, a pesar de encontrar efectos específicos para ciertos grupos y especies concretas, no se encontró una influencia clara sobre la densidad y diversidad total del banco de semillas. La endozoocoria fue relevante para el banco de semillas de ciertas especies y grupos de plantas, pero la contribución final de los excrementos de los herbívoros sobre el banco de semillas germinable no fue fácil de determinar. En LPS, los conejos tuvieron una mayor influencia que las ovejas sobre los parámetros del banco de semillas, mientras que en zonas de alta productividad los herbívoros grandes (ovejas) parecían intensificar los efectos de los pequeños herbívoros. Nuestros resultados sugieren que la interacción entre la identidad de los herbívoros y la productividad local es crucial para comprender los efectos de los herbívoros sobre el banco de semillas de las comunidades de pastizales mediterráneos.

Herbivores and the soil seed bank of an annual plant community: productivity, herbivore identity and endozoochory

Gálvez Bravo, L. & Rueda, M., Rebollo, S.

Departamento de Ecología, Edificio de Ciencias, Universidad de Alcalá, E-28871 Alcalá de Henares, Madrid. Spain. Phone: +34 918856406; Fax: +34 918854929, E-mail: marta.rueda@uah.es

Abstract

Herbivores can exert either positive, negative or neutral effects on the soil seed bank of plant assemblages, through various mechanisms. Factors such as herbivore identity and site productivity will influence the net effects of herbivores on annual plant communities, and endozoochory is one of the most important mechanisms through which they can exert such effects. The main objective of this study was to investigate the effects of different-sized herbivores on the soil seed bank of annual Mediterranean pastures, with special emphasis on the role of plant productivity and potential effects through endozoochory. We considered pastures with different productivity (high and low), and two herbivores of contrasting size, European rabbits (*Oryctolagus cuniculus*); and free-ranging sheep (*Ovis aries*). Five replicate blocks with three grazing treatments were set up at both low productivity (LPS) and high productivity sites (HPS). Each block had three plots with the following grazing treatments: 1) free herbivore grazing; 2) sheep exclusion, only grazed by rabbits; and 3) full exclusions. Inside each grazed plot, soil seed bank samples were collected from 3 subplots where dung was removed monthly (subplots without dung), and 3 untouched subplots (subplots with dung). There were marked differences in influence of grazing on the soil seed bank of sites with different productivity. In HPS herbivore presence had a positive effect on both total seed abundance and diversity. In LPS, in spite of specific effects found for certain guilds and individual species, no clear influence on total seed density and diversity was observed. Endozoochory influenced the soil seed bank for certain species and plant guilds, but the final contribution of herbivore faeces to the readily germinable soil seed bank was not easy to assess. In LPS, rabbits had greater effects than sheep on seed bank parameters, whilst under high productivity the large herbivore (sheep) seemed to intensify small herbivore effects. Our results suggest that the interaction between herbivore identity and site productivity is crucial to understand herbivore effects on the seed bank of annual Mediterranean pastures.

Keywords: dehesa ecosystems, *Oryctolagus cuniculus*, sheep, herbivore exclusions

1. Introduction

The soil seed bank contributes significantly to the community dynamics of plant assemblages (e.g. Simpson *et al.* 1989). Seed banks act as a seed reserve, reducing local extinction risk; become a source of propagules that contribute to community regeneration after disturbances; and increase community diversity, since species coexist with established vegetation as seeds (Díaz-Villa *et al.* 2003). In fact, the quantification of floristic diversity in a vegetation patch should take into account species present in the soil seed bank (Major & Pyott 1966).

Herbivores can exert either positive (e.g. Russi *et al.* 1992a), negative (e.g. McDonald *et al.* 1996), or neutral (e.g. Ortega *et al.* 1997; Meissner & Facelli 1999) effects on the soil seed bank, through antagonistic mechanisms. On the one hand, they can reduce seed production through defoliation and/or flowerhead consumption (e.g. Williams 1984; Edwards & Crawley 1999) although the strength of effects depends on factors such as time of year (i.e. whether flowerheads are eaten before or after setting seeds). On the other hand, they can increase seed availability. For example, they can disperse large quantities of seeds (e.g. Malo & Suarez 1995; Cosyns *et al.* 2005b; Pakeman *et al.* 2002); gut passage can improve germination success (Cosyns *et al.* 2005b); and they can enhance their chances of germination and establishment in the vegetation through gap creation (Bullock *et al.* 1994). Herbivore density at a given location may shift the balance towards either positive or negative effects of herbivory. If densities are too high, herbivores may have negative effects on populations of certain/less competitive species (Vellend *et al.* 2006). The side towards which the balance will finally shift may depend on other factors such as herbivore identity (Demmet & van Soest 1985; Cosyns *et al.* 2005b; Mouissie *et al.* 2005), and site productivity (Osem *et al.* 2006, Bakker *et al.* 2006).

Herbivore identity can determine the net effects of grazers on plant communities, because different herbivores use habitats in different ways, and they also differ in selectivity and digestive systems. Large herbivores can have profound effects on the vegetation through defoliation and flowerhead consumption (Crawley 1997), but they also consume vast amounts of seeds whilst grazing, becoming potentially relevant dispersers (Janzen 1984). In many cases, seeds survive gut passage and may benefit from dispersal and the nutrient package that being deposited in dung provides, which can be especially relevant for the dispersal of subordinate species (Bakker & Olff 2003). For example, sheep are important legume dispersers (Russi *et al.* 1992a), and cattle transport vast amounts of seeds in dung which benefit from both nutrients and gap creation when dung is deposited (Cosyns *et al.* 2005b; Malo & Suarez 1995a; Mouissie *et al.* 2005). Small mammals, on the contrary, are often more selective and may have a more dramatic effect through the consumption of certain species or plant parts (Edwards & Crawley 1999; Gibbens *et al.* 1993). They may also crush seeds that would pass untouched through large herbivores, but seeds undergo less chemical damage inside the gut (Bakker & Olff 2003). In fact, species composition of seeds dispersed will often vary with the type of herbivore (e.g. Malo & Suarez 1995b; Cosyns *et al.* 2005a). There will also be differences in the dispersal ability of different-sized herbivores, for large mammals have a longer digestive tract and larger home ranges, and thus are likely to disperse seeds further (Bakker & Olff 2003). However, small grazers have been shown to deposit large amounts of dung and disperse more seeds relative to larger herbivores (e.g. rabbits vs. fallow deer, Malo *et al.* 2000). If herbivores consume functional seeds, the final outcome will depend on the passage through the herbivore's intestinal tract. In some cases, a greater proportion of seeds are able to germinate after passage through the

gut of a hindgut fermenter than through a ruminant (e.g. rabbits vs. sheep, Cosyns *et al.* 2005b).

With respect to resource availability and site productivity, Osem *et al.* (2006) observed that herbivores had a more dramatic effect on the soil seed bank of sites with higher productivity, whilst their effects were negligible in less productive areas within a semi-arid annual community. They found that changes in seed bank density and composition following herbivore exclusion were determined by species characteristics (plant traits) interacting with site conditions. The importance of plant traits shifts from plant size to seed size with decreasing availability of soil resources, which also reflects differences in species composition between areas of different productivity (i.e. less productive areas are likely to have more annual species, which produce small, abundant seeds).

The main objective of this study was to investigate the effects of different-sized herbivores on the soil seed bank of annual Mediterranean pastures, with special emphasis on the role of plant productivity and potential effects through endozoocory. These pastures are characterised by a high spatial heterogeneity in resources, mainly due to topography, which has a large influence on the soil seed bank (Ortega *et al.* 1997), and promotes different plant communities (Bernáldez & Pineda 1980). We considered pastures with different productivity (high and low) under the same climatic and management regime, and two herbivores of contrasting size and digestive strategy, European wild rabbits (*Oryctolagus cuniculus*, a hindgut fermenter); and free-ranging sheep (*Ovis aries*, ruminants). We attempted to answer the following questions: 1) what are the net effects of herbivory on the soil seed bank and how do they depend on site productivity? 2) do herbivores of different size have a different influence on the soil seed bank? 3) what is the contribution of seeds inside herbivore dung to the soil seed bank, and is this contribution dependent on herbivore size?

2. Material and Methods

Study area

The present study was carried out in central Spain on a 300 ha dehesa situated in Chapinería, south-west of Madrid (40° 23' N, 4° 12' W). Mean altitude is 670 m a.s.l., and climate is continental Mediterranean, with 12°C and 432.6 mm of mean annual temperature and precipitation, respectively. The substrate is sandy to sandy-loamed, and lays upon a fractured bedrock of granite. The vegetation is dominated by *Quercus ilex* L. subsp. *rotundifolia* (Lam.). Herbaceous vegetation can be divided into xerophytic (on ridges, slopes and predominantly dry areas), and mesic pastures (on lowlands and water accumulation areas), with marked differences in productivity (115 g/m² and 530 g/m², respectively, Rueda 2006). Both communities are dominated by annual plants that germinate after the first heavy autumn rains, flower during spring and die at the beginning of summer after setting seed. Individuals withstand the summer drought as dormant seeds in the soil. More productive areas include some perennial species (6 species, 7.4%) which may become locally abundant (e.g. *Agrostis castellana*, *Gaudinia fragilis*, *Cynodon dactylon*, up to 50% cover). Land management is based on traditional practices, and includes grazing by a free-ranging trashumant herd of about 600 sheep. The areas is also specifically managed for small game hunting, mainly rabbits (*Oryctolagus cuniculus*), with a high warren density (8.3 warrens/ha).

Experimental design

In August 2001, five replicate blocks with three grazing treatments were set up at both low productivity sites (LPS) and high productivity sites (HPS), which were at least 900 m apart. The three grazing treatments were free herbivore grazing (rabbits+sheep plots) (which represents the natural situation or 'control'); sheep exclusion, only grazed by rabbits (rabbit plots), and non-grazed (full enclosure) (see **Figure 1**).

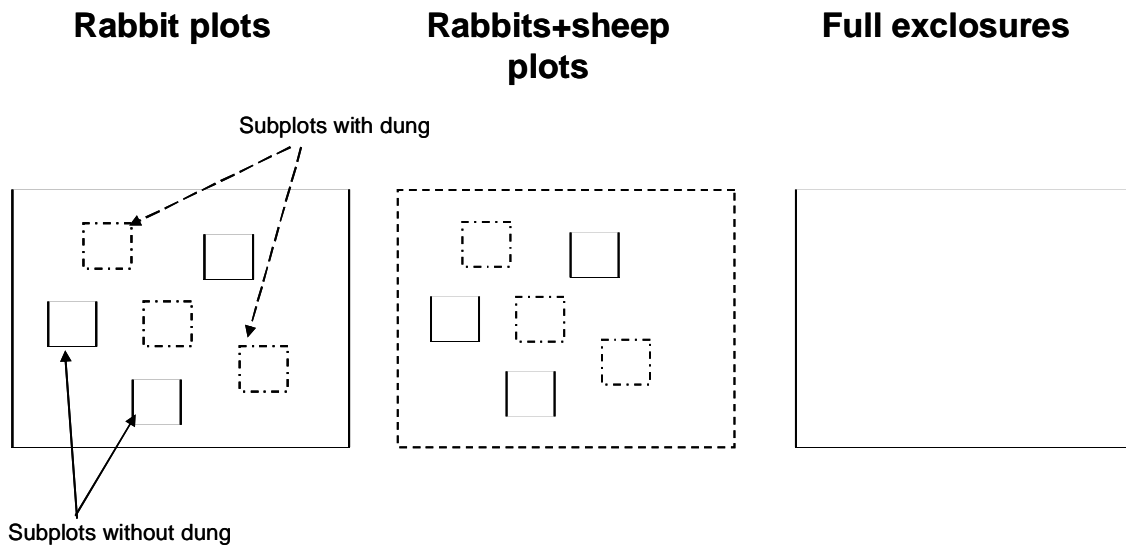


Figure 1. Experimental setup. Each replicate block (10 in total, 5 at each productivity level) had three plots with the following grazing treatments: free herbivore grazing (rabbits+sheep plots); sheep exclusion, only grazed by rabbits (rabbit plots); and full enclosures. Inside each grazed plot, samples were collected from 3 subplots where dung was removed monthly (subplots without dung), and 3 untouched subplots (subplots with dung).

Treatments were applied to 36 m² fenced enclosure plots with a 1 m high chicken mesh (width 2.5 cm). Rabbits+sheep plots were not fenced to allow access to both herbivores. The mesh in rabbit plots was lifted 20 cm above ground level to allow rabbit access but exclude sheep. Full enclosures were completely fenced and the mesh was buried 30 cm into the soil forming a "L" shape to avoid rabbits burrowing underneath, thus preventing access of both herbivores.

Additionally, three 1 m x 1 m subplots were established inside the grazed treatments (rabbit and rabbits+sheep plots). From January 2002 onwards, these subplots were visited monthly and cleared of all herbivore faecal pellets, therefore constituting areas without endozoochorous input. Collected pellets were also used to estimate relative herbivore abundance inside each grazed plot. Pellet counts are appropriate as indicators of relative abundance for both sheep (Bailey & Putman 1981) and rabbits (Wood 1988). Pellet degradation times and

therefore persistence may vary between habitats and seasons (Taylor & Williams 1956). Previous studies of faecal pellet decay rates have revealed that monthly collections are appropriate for the study area (M. Rueda, unpublished data).

Collection and estimation of the readily germinable soil seed bank

In September 2003, just before the first heavy autumn rains, ten 4 cm diameter and 3 cm deep soil cores were collected from random locations within each plot and subplot, in order to determine the density and composition of the germinable soil seed bank (*sensu* Thompson & Grime 1979). Ten cores were collected for each subplot (10 with and 10 without dung at each grazed plot, and 10 at full enclosures, 50 in total) and in each replicate block (5 replicates in LPS and 5 in HPS, total: 50 x 10 = 500 cores).

The contents of each core were spread out in 6 cm x 6 cm x 7 cm deep pots after breaking up

any soil aggregates and discarding vegetation remnants (we specifically removed stolon and rhizome fragments in the soil to avoid counting vegetative growth as seed abundance) and litter. This allowed the sample to be spread inside the pot forming a uniform layer which was never deeper than 1.5 cm. The pots also contained a 5.5 cm deep layer of vermiculite, separated from the soil by thin gauze. This prevented any mixing, as well as loss of seeds and soil, while being permeable to water and roots. Pots were watered when necessary.

The samples were left to germinate under controlled greenhouse conditions for over 11 months (October 2003 to September 2004), until no more seedlings emerged. Seedlings were identified up to species level where possible, and daily counted and removed from each pot in order to avoid competitive interactions and flowering. At the same time, the soil was stirred to stimulate further seed germination. If identification required further growth, seedlings were transplanted into separate pots. Four pots with sterile soil were placed among the soil seed bank samples in order to account for potential seed rain in the greenhouse. Pots were periodically shifted around the greenhouse to standardise growing conditions.

Seed bank parameters

Seedlings emerged were used to estimate seed density (seeds/m²) for total vegetation, individual species, and floristic guilds (Gramineae, Non-grass monocots, Forbs, Compositae and Legumes). Several parameters were calculated from data of seedlings emerged from soil samples: species richness (S); Shannon-Weaver diversity index (Shannon & Weaver 1949), ($H' = - \sum p_i \log_2 p_i$) where p_i is the proportion of species i ; and Pielou's (1966) evenness index ($J' = H'/H'_{\max}$) where H' is the Shannon-Wiener diversity index. We also created a synthetic variable representing floristic composition by applying a Non-Metric Multidimensional Scaling ordination (NMDS) (Clarke 1993) to individual species data.

The scores of the one-dimensional solution obtained were used as values representing the composition of each sample in further analyses. The distance matrix was calculated using the Sørensen (Bray-Curtis) index, more sensitive in heterogeneous data sets such as the one considered, and giving less weight to outliers than Euclidean distances (Roberts 1986). Additionally, sixteen of the most abundant species were chosen for individual comparisons (see **Appendix**).

Data analyses

Data were analysed by pairwise comparisons using randomization tests with 2000 permutations (α level of $p < 0.001$, Manly 2006). The difference between the two means was chosen as the "Y" parameter, and the observed value of Y was compared with the distribution of Y that was obtained by randomly reordering the data. If the null hypothesis is true, the observed Y value should appear as a typical value of the randomization distribution of Y. If this is not the case, the null hypothesis can be rejected and we can consider that alternative hypothesis of a significant difference between the two means considered is valid. The p level is calculated as the percentage of values that are as extreme as, or more extreme than the observed value in the randomization distribution. If Y falls within the most extreme 5% of values, then $p < 0.05$, if within 1%, then $p < 0.01$, etc. This non-parametric approach was considered the most appropriate given the nested, unbalanced experimental design (**Figure 1**), and the fact that we only had 5 replicates at each productivity category, increasing the risk of type II errors.

Pairwise comparisons and p estimation was carried out in three steps. First, data from rabbits+sheep plots (the 'control' situation) at LPS and HPS were compared. These tests confirmed that there were significant differences in soil seed bank parameters under different productivity, so we considered data from LPS and HPS separately thereafter. Secondly, we tested herbivore effects on soil seed bank parameters,

comparing data from the different treatments (rabbit plots, rabbits+sheep plots and full exclosures), at both LPS and HPS. The data from grazed plots used for these comparisons corresponded to subplots with dung. Third, in order to study the contribution of herbivore dung to the soil seed bank, only grazed plots were considered at both LPS and HPS. In each case, subplots with and without dung were compared.

3. Results

Influence of productivity on the soil seed bank

A total of 32477 seedlings emerged from the collected soil seed bank samples. Mean seed density was 48383.9 seeds/m². Only 2527 seedlings (7.8 %) could not be identified up to species level. In order to test for significant differences in soil seed bank parameters between LPS and HPS, comparisons were carried out using rabbits+sheep plots only (which represent the natural situation in the study area). Seed density was much higher in the more productive sites, which also harboured more species (**Table 1a**). The floristic composition of the soil seed bank was very different at sites with different productivity (Axis II, $p < 0.01$) (**Figure 2**). With respect to species guilds, productive sites had more Legumes, Gramineae, and Non-grass monocots. Low Productivity sites (LPS) were richer in Forb seeds (**Table 1b**).

In LPS, most of the soil seed bank (89%) was composed of 13 species, of which 3 were the most abundant (*Crassula tillaea*: 31%; *Sagina apetala*: 13% and *Aphanes microcarpa*: 12%; Total: 56%). Sixteen species contributed to 78% of the soil seed bank in HPS, and the three most abundant species were (*Juncus bufonius*: 17%; *Vulpia bromoides*: 13.5%, and *Bromus squarrosus*: 10.8%; Total: 41.3%). Juncaceae (17.3%) were the most abundant family, followed by Legumes (8.3%) (**Appendix 1**). Given these differences, the effects of different-sized herbivores on the soil seed bank will be considered separately for LPS and HPS hereafter.

Herbivore dung inputs

More dung was deposited by herbivores in LPS than at HPS, reflecting a more intense overall use of low productivity areas, which occupy a greater proportion of the study site (**Figure 3**). The contribution of rabbits to dung collected in rabbits+sheep plots was about 76%, whilst sheep contributed about 23%, both for HPS and LPS. In LPS, the mean weight of dung deposited by rabbits each month was highest in rabbit plots than in rabbits+sheep plots (means: 3.06 g ± 0.44 s.e. and 0.89 g ± 0.12 s.e, respectively, $p < 0.001$), but there were no significant differences in monthly dung deposition by rabbits in HPS (rabbit plots: 1.34 g ± 0.35 s.e., rabbits+sheep plots: 1.30 g ± 0.48 s.e.,

Table 1. Results of the comparison of soil seed bank parameters in rabbits+sheep plots of low and high productivity sites (LPS and HPS respectively). a) Means ± s.e seed density (seeds/m²) and diversity parameters (S = species richness; E = Pielou's Evenness index; H' = Shannon's diversity index) in low and high productivity sites (LPS and HPS, respectively); b) means ± s.e. seed density (seeds/m²) of each plant guild at low and high productivity sites.

| | LPS | HPS | p |
|---------------------------|-------------------------|-------------------------|--------|
| a) | | | |
| Total seed density | 35350.3 ± 2321.5 | 74028.7 ± 4520.0 | <0.001 |
| S | 8.4 ± 0.3 | 14.26 ± 0.54 | <0.01 |
| E | 0.94 ± 0.003 | 0.78 ± 0.01 | NS |
| H' | 2.02 ± 0.04 | 2.04 ± 0.05 | NS |
| b) | | | |
| Gramineae | 4856.7 ± 1160.1 | 34235.7 ± 9763.4 | <0.01 |
| Compositae | 2054.1 ± 299.4 | 1162.4 ± 344.8 | NS |
| Legumes | 191.1 ± 54.0 | 7977.7 ± 3554.3 | <0.01 |
| Forbs | 30031.8 ± 1954.7 | 21624.2 ± 3348.5 | <0.01 |
| Non-grass monocots | 414.0 ± 283.1 | 16783.4 ± 5582.6 | <0.01 |

$p > 0.05$). Monthly dung deposition by sheep was significantly higher in HPS than in LPS (means: $0.41 \text{ g} \pm 0.25 \text{ s.e.}$ and $0.26 \text{ g} \pm 0.10 \text{ s.e.}$, respectively, $p < 0.01$).

With respect to temporal patterns of dung deposition, they varied between the different treatments in LPS, where rabbit dung was more abundant in rabbit plots than in rabbits+sheep plots (**Figure 4a,b**). In rabbit plots, the greatest amount of dung was deposited in LPS sites during the spring months (March-May), dropped during summer, and increased again in autumn (from October). In rabbits+sheep plots, both rabbit and sheep dung followed similar trends, with no significant variations except for a small peak in May-June. In HPS, however, rabbit dung in both rabbit plots and rabbits+sheep plots followed a similar temporal pattern. They highest rabbit dung densities were recorded in summer (May-August), and during March and June for sheep.

Effects of herbivory at low productivity sites (LPS)

In low productivity sites, herbivory treatments did not have a significant effect on total seed density, or on the diversity parameters of the soil seed bank (**Table 2a**). Floristic composition only differed between rabbit plots and full exclusures (Axis II of NMS ordination, $p < 0.05$). Rabbit plots had significantly fewer Compositae

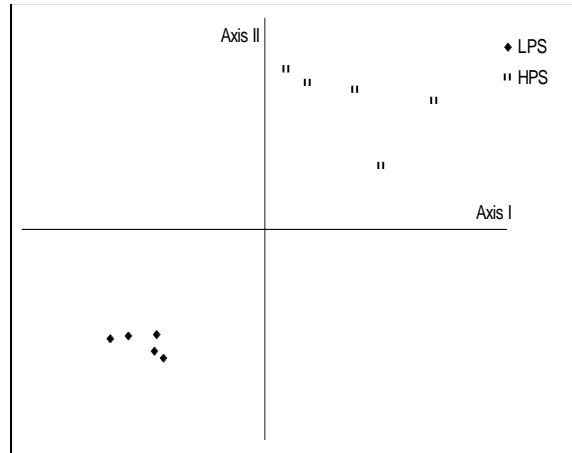


Figure 2. Results of the NMS ordination representing the floristic composition of the soil seed bank of the rabbits+sheep plots in low and high productivity sites (LPS and HPS, respectively).

seeds than sheep+rabbit plots and full exclusures ($p < 0.01$ and $p < 0.05$ respectively) (**Table 2b**). Three species out of 16 showed significant differences between treatments in LPS. *Andryala integrifolia* seed density was significantly higher in full exclusures when compared with grazed plots ($p < 0.05$). For *Crassula tillaea*, rabbits+sheep plots had higher seed densities when compared with full exclusures ($p < 0.01$), while *Polycarpon tetraphyllum* seeds were more abundant in rabbit plots than in rabbits+sheep plots ($p < 0.01$) (**Appendix 2**).

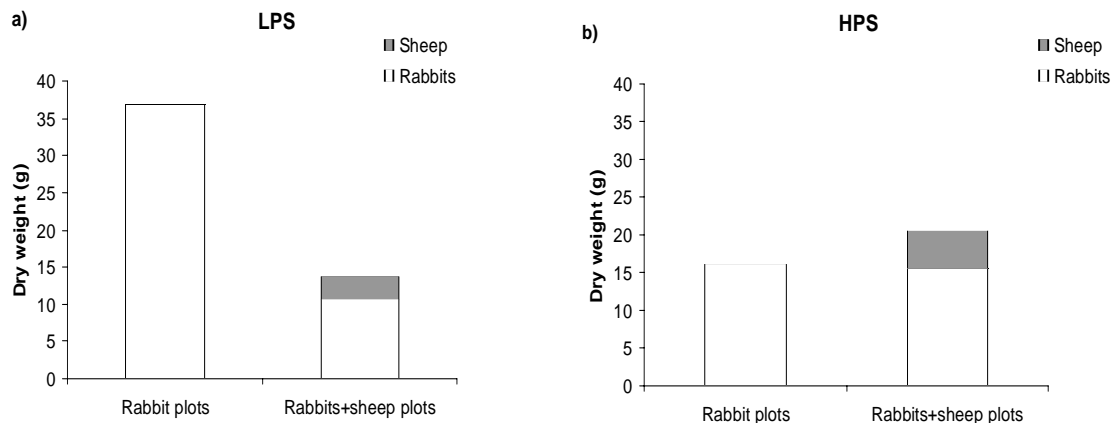


Figure 3. Total dung collected for a) rabbits and b) sheep in both low productivity (LPS) and high productivity sites (HPS), and in both grazing treatments.

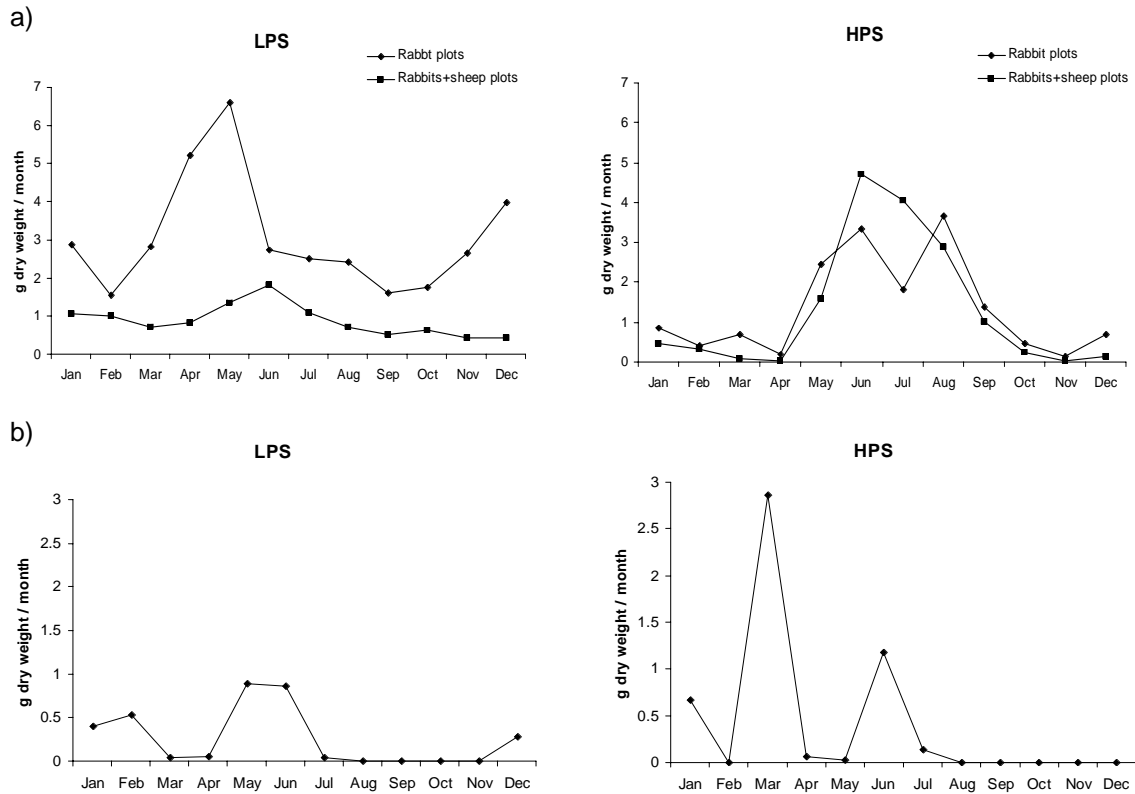


Figure 4. Annual trends (mean monthly pellet production) of a) rabbit and b) sheep pellet deposition in grazed plots in both low and high productivity sites (LPS and HPS, respectively). Note that there is an absence of sheep dung during the summer months because they are driven away from the system (trashumance).

Effects of herbivory on the seed bank at high productivity sites (HPS)

Differences between treatments at high productivity sites were more evident than in less productive areas. Full enclosures had significantly lower seed densities than grazed plots ($p < 0.001$) and there were no significant differences between grazed treatments (**Table 3**). Species richness and diversity were significantly greater in rabbits+sheep plots than in full enclosures ($p < 0.01$) (**Table 3a**). Floristic composition between treatments in highly productive areas did not differ significantly (Axis I and II of NMS ordination, $p > 0.05$).

Gramineae were more abundant in the grazed plots than in full enclosures ($p < 0.001$), and Legume abundance was significantly greatest in plots grazed by both rabbits and sheep

($p < 0.001$). These plots have also a greater abundance of Forbs and Compositae when compared with full enclosures ($p < 0.01$ and < 0.05 , respectively) (**Table 3b**).

Four species out of 16 showed significant effects of treatments in HPS. Rabbit plots had significantly higher *Cerastium glutinosum* seeds than full enclosures ($p < 0.05$), and the same trend was found for *Poa annua* ($p < 0.05$). In the case of *Trifolium campestre*, plots grazed by both herbivores had higher seed densities than both rabbit plots and full enclosures ($p < 0.001$ in both cases). In the case of *Vulpia ciliata*, both grazed treatments had higher densities than full enclosures ($p < 0.001$ in both cases), but did not differ between them (**Appendix 2**).

Contribution of endozoochory to the soil seed bank

In both LPS and HPS, total seed density, diversity parameters and floristic composition showed no significant effects of the removal of either rabbit or sheep dung (all $p > 0.05$, **Table 4a and 5a**). For plant guilds, in the rabbit plots of LPS Gramineae were significantly more abundant in subplots with dung ($p < 0.01$, **Table 4b**). A similar result was observed in HPS rabbit plots for non-grass monocots, they were more abundant in subplots with dung ($p < 0.001$, **Table 5b**). With respect to individual species, in HPS, both *Cerastium glutinosum* and *Juncus bufonis* seeds were more abundant in rabbit plots if dung was present ($p = 0.001$ and $p = 0.05$, respectively). In HPS the effects of dung removal were strongest for *Vulpia ciliata*, which had much lower seed densities in subplots without dung, both within rabbit plots ($p < 0.05$) and rabbits+sheep plots ($p < 0.01$) (**Appendix 2**). Surprisingly, in LPS *Sagina apetala* seed density was greatest in rabbit plots without dung ($p < 0.01$), and a similar result was observed for *Trifolium arvense* in rabbits+sheep plots ($p < 0.05$). In HPS rabbits+sheep plots, Gramineae were significantly more abundant in subplots without dung ($p < 0.01$).

4. Discussion

Effects of herbivory on sites of different productivity

The large differences in seed density and seed bank species composition found between sites of different productivity are similar to results of other studies carried out in Mediterranean pastures (e.g. Ortega *et al.* 1997, Osem *et al.* 2006). In our experiment, productivity played an important role in the relationship between herbivory and the soil seed bank. Herbivores had a stronger effect on soil seed bank parameters in high productivity sites (HPS) than in low productivity sites (LPS), mainly due to the marked differences in species composition and the main limiting factors acting at each site

(Osem *et al.* 2006; Ortega *et al.* 1997).

In LPS, the soil seed bank inside full enclosures did not show significant changes in total seed density or diversity with respect to the grazed treatments, which is similar to results obtained by Ortega *et al.* (1997). However, we did observe a significant influence of heavier rabbit grazing in rabbit plots on floristic composition (**Figure 3**), reflected in the results obtained for certain plant guilds and species. Note that rabbit dung collected in rabbit plots was three times greater than dung collected in rabbit+sheep plots. Compositae seed density was lower in rabbit plots than in full enclosures. This is probably explained by the fact that rabbits prefer to consume Compositae in the absence of other herbivores (Soriguer 1983), and these preferences have probably caused a decrease in the seed abundance of this guild. This is reinforced by the clear effect of herbivore exclusion on *Andryala integrifolia* (a Composite), which was more abundant in the soil seed bank in full enclosures. The flowerheads of this species are readily eaten in grazed plots (personal observation). This trend in the soil seed bank is in contrast with results obtained for the cover of this species in the established vegetation, which is higher in rabbit plots than full enclosures (Rueda 2006). This suggests that rabbits may be reducing seed production, but may be encouraging prostrate and rosette growth of this species, and therefore a higher overall cover (McIntyre *et al.* 1999).

There were some species which had higher seed densities under grazing, either by both herbivores (*Crassula tillaea*) or by rabbits alone (*Polycarpon tetraphyllum*). These trends were also observed in the standing vegetation of grazed plots (Rueda 2006). These species are clearly benefited by herbivory, probably because their small size implies adaptations to low resource availability in LPS and better avoidance of grazing than taller plants (Osem *et al.* 2004). Previous research in similar Mediterranean communities has frequently shown that plant size is negatively related to

Table 2. Seed parameters of the soil seed bank in both grazed treatments (data from subplots with dung) and full closures in low productivity sites (LPS). a) Mean \pm s.e. total seed density (seeds/m²) and diversity parameters (S = species richness; E = Pielou's Evenness index; H' = Shannon's diversity index); b) mean \pm s.e. seed density (seeds/m²) of the different plant guilds. Different letters indicate significant differences ($p < 0.05$), no letters indicate no significant differences.

| | Rabbit plots | Rabbits + Sheep plots | Full closures |
|---------------------------|---------------------------------------|---------------------------------------|---------------------------------------|
| a) | | | |
| Total density | 33901.3 \pm 1902.6 | 35350.3 \pm 2321.5 | 36672.0 \pm 2872.3 |
| S | 9.3 \pm 0.4 | 8.4 \pm 0.3 | 10.1 \pm 0.4 |
| E | 0.9447 \pm 0.00292 | 0.9368 \pm 0.00309 | 0.9478 \pm 0.00264 |
| H' | 2.10 \pm 0.04 | 2.02 \pm 0.04 | 2.16 \pm 0.04 |
| b) | | | |
| Gramineae | 5175.2 \pm 836.2 | 4856.7 \pm 1160.1 | 5047.8 \pm 2146. |
| Compositae | 1226.1 \pm 267.2a | 2054.1 \pm 299.4b | 2197.5 \pm 595.1b |
| Legumes | 191.1 \pm 81.97 | 191.1 \pm 53.99 | 414.0 \pm 185.69 |
| Forbs | 26544.6 \pm 1589.7 | 30031.8 \pm 1954.679 | 28423.6 \pm 5828.7 |
| Non-grass monocots | 302.6 \pm 178.7 | 414.0 \pm 283.1 | 796.2 \pm 395.7 |

Table 3. Seed bank parameters of the soil seed bank in both grazed treatments (data from subplots with dung) and full closures in high productivity sites (HPS). a) Mean \pm s.e seed density (seeds/m²) and diversity parameters (S = species richness; E = Pielou's Evenness index; H' = Shannon's diversity index); b) mean \pm s.e seed density (seeds/m²) of the different plant guilds. Different letters indicate significant differences ($p < 0.05$), no letters indicate no significant differences.

| | Rabbit plots | Rabbits + Sheep plots | Full closures |
|---------------------------|---|--|---|
| a) | | | |
| Total density | 68885.4\pm5440.2 a | 74028.7\pm4520.0 a | 47659.2\pm3661.9 b |
| S | 12.9\pm0.64 ab | 14.3\pm0.54 a | 11.7\pm0.60 b |
| E | 0.79 \pm 0.01 | 0.78 \pm 0.01 | 0.77 \pm 0.02 |
| H' | 1.93\pm0.04 ab | 2.04\pm0.05 a | 1.84\pm0.06 b |
| b) | | | |
| Gramineae | 30684.7\pm8055.9 a | 34235.7\pm9763.4 a | 14777.1\pm2861.3 b |
| Compositae | 716.56\pm291.4 ab | 1162.42\pm344.8 a | 382.17\pm160.0 b |
| Legumes | 3742.04\pm1705.9 a | 7977.71\pm3554.3 b | 3105.10\pm1031.7 a |
| Forbs | 16799.4 \pm 4103.3 ab | 21624.2 \pm 3348.6 a | 13773.9 \pm 2507.5b |
| Non-grass monocots | 20636.9 \pm 7074.3 | 16783.4 \pm 5582.6 | 17465 \pm 5207.3 |

Table 4. Seed bank parameters in subplots with and without dung of both grazed treatments in low productivity sites (LPS). a) Mean \pm s.e. seed density (seeds/m²) and diversity parameters (S = species richness; E = Pielou's Evenness index; H' = Shannon's diversity index); b) mean \pm s.e. seed density (seeds/m²) of the different plant guilds. Different letters indicate significant differences ($p < 0.05$), no letters indicate no significant differences.

| a) | Rabbit plots | | Rabbits + Sheep plots | |
|---------------------------|---------------------------------------|--|------------------------|----------------------|
| | With dung | Without dung | With dung | Without dung |
| Total Density | 33901.3 \pm 1902.6 | 37611.5 \pm 2281.2 | 35350.3 \pm 2321.5 | 34538.2 \pm 1910.3 |
| S | 9.3 \pm 0.4 | 8.9 \pm 0.3 | 8.4 \pm 0.3 | 9.1 \pm 0.4 |
| E | 0.94 \pm 0.002 | 0.94 \pm 0.003 | 0.94 \pm 0.003 | 0.94 \pm 0.003 |
| H' | 2.1 \pm 0.04 | 2.02 \pm 0.04 | 2.02 \pm 0.04 | 2.03 \pm 0.05 |
| b) | | | | |
| Gramineae | 5175.2 \pm 836.2a | 2515.9 \pm 347.6 b | 4856.7 \pm 1160.1 | 4840.8 \pm 1388.7 |
| Compositae | 1226.1 \pm 267.2 | 1480.9 \pm 345.8 | 2054.1 \pm 299.4 | 2181.5 \pm 617.0 |
| Legumes | 191.1 \pm 82.0 | 111.5 \pm 59.6 | 191.1 \pm 54.0 | 318.5 \pm 168.9 |
| Forbs | 26544.6 \pm 1589.7 | 29952.2 \pm 2771.3 | 30031.8 \pm 1954.679 | 26496.8 \pm 1764.8 |
| Non-grass monocots | 302.6 \pm 178.7 | 589.2 \pm 411.1 | 414.0 \pm 283.1 | 525.5 \pm 284.4 |

Table 5. Seed bank parameters in subplots with and without dung of both grazed treatments in high productivity sites (HPS). a) Mean \pm s.e. seed density (seeds/m²) and diversity parameters (S = species richness; E = Pielou's Evenness index; H' = Shannon's diversity index); b) mean \pm s.e. seed density (seeds/m²) of the different plant guilds. Different letters indicate significant differences ($p < 0.05$), no letters indicate no significant differences.

| a) | Rabbit plots | | Rabbits + Sheep plots | |
|---------------------------|--|--|--|--|
| | With dung | Without dung | With dung | Without dung |
| Total density | 68885.4 \pm 5440.2 | 67452.2 \pm 4653.0 | 74028.7 \pm 4520.0 | 80891.7 \pm 4677.0 |
| S | 12.9 \pm 0.64 | 12.4 \pm 0.58 | 14.3 \pm 0.54 | 14.0 \pm 0.55 |
| E | 0.79 \pm 0.01 | 0.80 \pm 0.01 | 0.78 \pm 0.01 | 0.78 \pm 0.01 |
| H' | 1.93 \pm 0.04 | 1.97 \pm 0.04 | 2.04 \pm 0.05 | 2.03 \pm 0.05 |
| b) | | | | |
| Gramineae | 30684.7 \pm 8055.9 | 36035.0 \pm 3493.1 | 34235.7\pm9763.4 a | 45939.5\pm4001.5 b |
| Compositae | 716.56 \pm 291.4 | 700.64 \pm 257.3 | 1162.42 \pm 344.8 | 875.80 \pm 506.1 |
| Legumes | 3742.04 \pm 1705.9 | 3789.81 \pm 601.2 | 7977.71 \pm 3554.3 | 9729.30 \pm 1350.3 |
| Forbs | 16799.4 \pm 4103.3 | 15748.4 \pm 3847.6 | 21624.2 \pm 3348.6 | 23391.7 \pm 4873.6 |
| Non-grass monocots | 20636.9\pm7074.3 a | 14665.6\pm4727.7 b | 16783.4 \pm 5582.6 | 9888.5 \pm 3857.8 |

grazing, and short plants are also more frequent in less productive environments (Noy-Meir *et al.* 1989; Hadar *et al.* 1999; Lavorel *et al.* 1999; Osem *et al.* 2004).

Summarising, in LPS we found a detrimental effect of rabbit grazing on the soil seed bank of Compositae, probably due to flowerhead consumption; and a positive effect of rabbits and sheep on the seed bank of small plants due to an increase of these species in the established vegetation. These effects did not translate into significant effects of grazing on total seed density or diversity under low productivity.

In HPS the effects of grazing treatments were stronger, with a clear detrimental effect of herbivore exclusion on the total density, species richness and diversity of soil seed bank. The soil seed bank of full enclosures was less diverse, and seed density was lowest for almost all plant guilds and several individual species. This trend is opposite to results shown in Osem *et al.* (2006), but concurs with results from Russi *et al.* (1992b) and Sternberg *et al.* (2003). Our results are also supported by data from the established vegetation in full enclosures, which have the lowest vegetation cover (Rueda 2006). This means that herbivory is a significant element for the preservation of diversity in the seed bank of HPS. This is reflected in the higher abundance of Gramineae and Legume seeds in grazed plots, especially those grazed by both rabbits and sheep. Furthermore, Rabbits seemed to favour the seeds of certain species in HPS, for example *Cerastium glutinosum* and *Poa annua*. This is probably also due to specific adaptations to resist herbivory such as waxy hairs (*C. glutinosum*); or a preference for more nitrophilous and disturbed environments induced by rabbit activities (*P. annua*).

It is important to highlight that the established plant community at HPS had a high vegetation cover (95%) and a relatively high proportion of perennials (about 30% cover) (Rueda 2006). The positive effects of herbivory on species richness of the HPS seed bank suggest that in

these areas herbivores may exert their most relevant effects through gap creation (Bakker & Olf 2003). In HPS, herbivory encourages subordinate annual species that produce large amounts of seeds (Malo & Suarez 1995a), releasing them from light competition in the face of the dense sward of tall plants that are dominant under highly productive conditions (e.g. Sternberg *et al.* 2003; Osem *et al.* 2004). In unproductive habitats there is a greater proportion of bare ground (about 55%, Rueda 2006), so space availability for germination does not seem to be a limiting factor (Zobel *et al.* 2000).

Overall, there were marked differences in influence of grazing on the soil seed bank of sites with different productivity. In HPS herbivore presence has a positive effect on both seed abundance and seed bank diversity. In LPS, in spite of specific effects found for certain guilds and individual species, no clear influence on total seed density and diversity was observed, perhaps because herbivore effects on different species counteract each other. At LPS, observed effects seemed greatly conditioned by herbivore density and abiotic factors. In these less productive areas, nutrient and water availability play an important role in determining plant abundance, species composition and diversity, and this will be reflected in the soil seed bank (Ortega *et al.* 1997). However, evidence from rabbit plots suggests that defoliation and/or flowerhead consumption may have a strong influence when herbivore densities are high.

It is also important to consider the possible influence of the different seed bank types found in these pastures on our results. Summer transient seed banks, with a small fraction of persistent seeds, are more frequent in the upper, less productive zones of Mediterranean pastures (Ortega *et al.* 1997). A persistent seed bank is more frequent in lowland productive areas, where there is a higher proportion of perennials and higher plant cover. These are believed to be strategies that compensate for the lack of available gaps for establishment in the case of persistent banks, and to ensure therophyte

persistence in sites of greater abiotic unpredictability in the case of summer transient banks. The dissection of effects seen in the present study would greatly benefit from data from several consecutive years in order to include the possible influence of these and other seed bank strategies and their interactions with grazing.

What is the contribution of endozoochory to the soil seed bank?

In this study, the contribution of endozoochorous seeds to the soil seed bank was assessed using a pellet removal experiment. We compared subplots with and without dung inside each grazed treatment (rabbit plots and rabbits+sheep plots). We expected to find greater seed densities in subplots with dung for those guilds and species for which dispersal through herbivore dung could be a significant mechanism. We found that rabbits played a clear dispersal role for Gramineae in LPS and Non-grass monocots in HPS. Furthermore, the seed density of *Cerastium glutinosum* (a forb) in HPS was significantly higher in rabbit plots with dung, and *Vulpia ciliata* (a grass) seeds were most abundant if dung was present for both grazed treatments, which concurs with the relatively high abundance of *Vulpia* spp. found in herbivore dung by Malo & Suarez (1995).

The endozoochorous potential of herbivores, including rabbits and sheep, has been widely studied (e.g. Malo *et al.* 2000; Malo & Suarez 1995a, 1995b; Russi *et al.* 1992b; Janzen 1984). Using data from Malo & Suarez (1995) for rabbits (6.5 seeds /g dung), and Russi *et al.* (1992b) for sheep (up to 28 seeds/g dung), we calculated the overall dispersal potential for rabbits and sheep in the study area. In grazed plots, rabbit endozoochory would have contributed with 38.4% of seeds, and sheep with 16.8%. Results from the dung removal experiment clearly show that this is an overestimation, because if it were true we would have found greater significant effects of dung removal. In fact, the greatest difference found between plots with

and without rabbit dung was about 8% decrease in Gramineae in LPS rabbit plots when dung was removed (**Appendix 1**).

This reveals that although dispersal potential may be high, the final contribution of herbivore faeces to the readily germinable soil seed bank is not so easily assessed, as observed by Pakeman *et al.* (1999). Malo *et al.* (1995), however, argued that the role of rabbits was very important, but they studied the contribution of rabbit endozoochory to seed bank build-up in disturbed gaps, whilst we looked at undisturbed areas. This discrepancy supports the idea that the main short-term influence of herbivores on the germinable seed bank is mediated through gap creation, as suggested by Cosyns *et al.* (2006), and not through direct seed input.

There are several other reasons why seeds found in dung may not end up contributing to the soil seed bank of these pastures. 1) Longer-term experiments may be necessary in order to detect the real relevance of seed inputs through dung, especially in heterogeneous habitats with very different local pellet degradation rates, which are important for seed release from the highly hydrophobic dung pellets (Verdu & Galante 2004; Van der Wal *et al.* 2004). 2) Other authors have argued that seed production is so high in certain habitats, that slight variations may have no relevant effects (Pakeman *et al.* 1999). This may be coupled, in the case of sheep, with the low yearly densities, considering they spend 4 months away from the system. 3) Another mechanism is increased seed mortality inside rabbit pellets since they are more likely to suffer attacks by microbes and fungi if they are trapped in a nutrient-rich package such as a dung pellet (Van der Wal *et al.* 2004). 4) Finally, differential post-dispersal seed predation and seed loss. Up to 72% of seeds present at the beginning of summer can be lost from the seed bank through various mechanisms by autumn (Traba *et al.* 2006).

Influence of herbivore identity

At LPS, we did not find significant effects of the large herbivore (sheep) on the soil seed bank. Rabbits (the smaller herbivore), did have a significant effect in plots where sheep were excluded. This was mainly because rabbit densities were very high in these plots. Rabbits promoted differences in floristic composition of the soil seed bank, and certain species were encouraged under rabbit grazing in rabbit plots. Additionally, they seemed to benefit Gramineae through endozoochorous dispersal.

In contrast, at HPS the combination of both sheep and rabbit grazing produced significant effects with respect to full enclosures. Rabbit grazing in rabbit plots induced higher total and Gramineae seed density. Rabbits also increased the density of non-grass monocots, *Cerastium glutinosum* and *Poa annua* seeds through endozoochory (higher densities in subplots with dung vs. subplots without dung). The presence of sheep in rabbits+sheep plots reinforced the trends found for total seed density and Gramineae, and they had a significant effect on parameters such as species richness and diversity. Sheep presence also increased seed density of Forbs, Compositae and Legumes. This result is probably due to the fact that large herbivores such as sheep have a greater potential for gap creation. Sheep also seemed to increase the abundance of *Trifolium campestre* with respect to the other grazing treatments, which may be a confirmation of the beneficial influence of sheep for Legumes (Russi *et al.* 1992b). Additionally, sheep dung seemed to be a relevant source of *Juncus buffonius* and *Vulpia ciliata* seeds in these more productive sites, both species also significantly influenced by rabbits.

Our results on the effects of herbivore identity concur with those of Bakker *et al.* (2006), who found a clear effect of the interaction between large herbivore grazing and productivity on species richness, with greater effects in sites of higher productivity. However, they were not able

to find clear trends when small herbivores were excluded.

In our case, effects found at LPS were mainly mediated by the very high densities of the small herbivore (rabbits), which suggests that the interaction between local conditions and small herbivore density is the key to explain grazing effects in low productivity areas. HPS are by nature more sensitive to grazing because herbivory modifies the conditions under which plants compete (they are released from competition for light and must withstand defoliation by herbivores). Additionally, in unproductive habitats most of the plant biomass tends to be located belowground (root:shoot ratio seems higher in LPS, Rueda 2006), whilst with increasing productivity more biomass is allocated aboveground, increasing the potential for herbivores to affect species composition (Milchunas & Lauenroth 1993; Osem *et al.* 2002). Our results suggest that under high productivity large herbivores intensify small herbivore effects through an increase in gaps for recruitment because their potential for disturbance is greater.

Conclusions

We can conclude that productivity and herbivore density and identity are important factors that condition the effects that herbivores may have on the soil seed bank of annual Mediterranean pastures. It is also clear that the interaction of plant consumption and endozoochorous dispersal by herbivores with other mechanisms such as gap creation may be important to shift the final balance of herbivore effects on grasslands. The alleviation of herbivore pressure seems to have little or no effects on total seed density or floristic composition of the soil seed bank of the resource-limited sites upslope, and causes a decline in seed density and diversity in lowland, productive areas. Since the soil seed bank is only the reservoir state of the plant community, it would be interesting to study whether herbivores affect the relationship between the seed bank and the established vegetation, and

analyse their role in the regeneration/resilience of these communities.

Acknowledgements

We would like to thank the management of the Dehesa Santo Ángel de la Guarda, Chapinería, for allowing us to work in the study site. Gonzalo García for help with the soil seed bank collection and in the greenhouse. We are also grateful to the staff and management of Jardín Botánico Juan Carlos I (Alcalá de Henares), especially Beatriz Díaz, Gema Lucas and Paloma Millán. We would also like to thank Luis M^a Carrascal for statistical advice. This work is part of the projects REN2000-0783/GLO and REN2003-05553/GLO, Ministerio de Ciencia y Tecnología (MCyT). Marta Rueda was supported by a FPI grant from the MCyT and Lucía Gálvez Bravo by a FPI grant from the Comunidad Autónoma de Madrid.

References

- Bailey, R.E. & Putman, R.J. (1981) Estimation of fallow deer (*Dama dama*) populations from faecal accumulation. *Journal of Applied Ecology* 14: 287-291
- Bakker, E.S. & Olf, H. (2003) Impact of different-sized herbivores on recruitment opportunities for subordinate herbs in grasslands. *Journal of Vegetation Science* 14: 465-474.
- Bakker, E.S., Ritchie, M.E., Olf, H., Milchunas, D.G., & Knops, J.M.H. (2006) Herbivore impact on grassland plant diversity depends on habitat productivity and herbivore size. *Ecology Letters* 9: 780-788.
- Bernaldez, F.G. & Pineda, F.D. (1980) Bases para la tipificación integrada de los pastizales de dehesa. *Pastos* 10: 20-43.
- Bullock, J.M., Clear Hill, B., Dale, M.P., & Silvertown, J. (1994) An experimental study of the effects of sheep grazing on vegetation change in a species-poor grassland and the role of seedling recruitment into gaps. *Journal of Applied Ecology* 31: 439-507.
- Clarke, K.R. (1993) Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* 18: 117-143.
- Cosyns, E., Claerhout, S., Lamoot, I., & Hoffmann, M. (2005a) Endozoochorous seed dispersal by cattle and horse in a spatially heterogeneous landscape. *Plant Ecology* 178: 149-162.
- Cosyns, E., Delporte, A., Lens, L., & Hoffmann, M. (2005b) Germination success of temperate grassland species after passage through ungulate and rabbit guts. *Journal of Ecology* 93: 353-361.
- Cosyns, E., Bossuyt, B., Hoffmann, M., Vervaeke, H., & Lens, L. (2006) Seedling establishment after endozoochory in disturbed and undisturbed grasslands. *Basic and Applied Ecology* 7: 360-369.
- Crawley, M.J. (1997). Plant-herbivore dynamics. In *Plant Ecology* (ed M.J. Crawley), pp. 401-474. Blackwell Science, Oxford.
- Demmet, M.W. & van Soest, P.J. (1985) A nutritional explanation for body-size patterns of ruminant and non-ruminant herbivores. *American Naturalist* 125: 641-672.
- Díaz-Villa, M.D., Marañón, T., Arroyo, J., & Garrido, B. (2003) Soil seed bank and floristic diversity in a forest-grassland mosaic in southern Spain. *Journal of Vegetation Science* 14: 701-709.
- Edwards, G.R. & Crawley, M.J. (1999) Herbivores, seed banks and seedling recruitment in mesic grassland. *Journal of Ecology* 87: 423-435.
- Gibbens, R.P., Havstad, K.M., Billheimer, D.D., & Herbel, C.H. (1993) Creosotebush vegetation after 50 years of lagomorph exclusion. *Oecologia* 94: 210-217.
- Hadar, L., Noy-Meir, I., & Perevolotsky, A. (1999) The effect of shrub clearing and grazing on the composition of a Mediterranean plant community: functional groups versus species. *Journal of Vegetation Science* 10: 673-682.
- Janzen, D.H. (1984) Dispersal of small seeds by big herbivores: Foliage is the fruit. *American Naturalist* 123: 338-353.
- Lavorel, S., McIntyre, S., & Grigulis, K. (1999) Plant response to disturbance in a Mediterranean grassland: How many functional groups? *Journal of Vegetation Science* 10: 661-672.
- Major, J. & Pyott, W.T. (1966) Buried, viable seeds in two California bunchgrass sites and their bearing on the definition of a flora *Plant Ecology* 13: 253-282.
- Malo, J.E., Jiménez, B., & Suarez, F. (1995) Seed bank build-up in small disturbances in a Mediterranean pasture: the contribution of endozoochorous dispersal by rabbits. *Ecography* 18: 73-82.
- Malo, J.E., Jiménez, B., & Suarez, F. (2000) Herbivore dunging and endozoochorous seed deposition in a Mediterranean dehesa. *Journal of Range Management* 53: 322-328.
- Malo, J.E. & Suarez, F. (1995a) Establishment of pasture species on cattle dung: the role of endozoochorous seeds. *Journal of Vegetation Science* 6: 169-174.

- Malo, J.E. & Suarez, F. (1995b) Herbivorous mammals as seed dispersers in a Mediterranean dehesa. *Oecologia* 104: 246-255.
- Manly, B.F.J. (2006) *Randomization, Bootstrap And Monte Carlo Methods in Biology*, 3rd edn. Chapman & Hall/CRC, New York.
- McDonald, A.W., Bakker, J.P., & Vegelin, K. (1996) Seed bank classification and its importance for the restoration of species-rich flooded-meadows. *Journal of Vegetation Science* 7: 156-164.
- McIntyre, S., Lavorel, S., Landsberg, J., & Forbes, T.D.A. (1999) Disturbance response in vegetation - towards a global perspective on functional traits. *Journal of Vegetation Science* 10: 621-630.
- Meissner, R.A. & Facelli, J.M. (1999) Effects of sheep exclusion on the soil seed bank and annual vegetation in chenopod shrublands of South Australia. *Journal of Arid Environments* 42: 117-128.
- Milchunas, D.G. & Lauenroth, W.K. (1993) Quantitative effects of grazing on vegetation and soils over a global range of environments. *Ecological Monographs* 63: 327-366.
- Mouissie, A.M., Vos, P., Verhagen, H.M.C., & Bakker, J.P. (2005) Endozoochory by free-ranging, large herbivores: Ecological correlates and perspectives for restoration *Basic and Applied Ecology* 6: 547-558.
- Noy-Meir, I., Gutman, M., & Kaplan, Y. (1989) Response of Mediterranean grassland plants to grazing and protection. *Journal of Ecology* 77: 290-310.
- Ortega, M., Levassor, C., & Peco, B. (1997) Seasonal dynamics of Mediterranean pasture seed banks along environmental gradients. *Journal of Biogeography* 24: 177-195.
- Osem, Y., Perevolotsky, A., & Kigel, J. (2002) Grazing effect on diversity of annual plant communities in a semi-arid rangeland: interactions with small-scale spatial and temporal variation in primary productivity. *Journal of Ecology* 90: 936-946.
- Osem, Y., Perevolotsky, A., & Kigel, J. (2004) Site productivity and plant size explain the response of annual species to grazing in a Mediterranean semi-arid rangeland. *Journal of Ecology* 92: 297-309.
- Osem, Y., Perevolotsky, A., & Kigel, J. (2006) Similarity between seed bank and vegetation in a semi-arid annual plant community: The role of productivity and grazing. *Journal of Vegetation Science* 17: 29-36.
- Pakeman, R.J., Engelen, J. & Attwood, J.P. (1999) Rabbit endozoochory and seedbank build-up in an acidic grassland. *Plant Ecology* 145: 83-90.
- Pakeman, R.J., Digneffe, G., & Small, J.L. (2002) Ecological correlates of endozoochory by herbivores. *Functional Ecology* 16: 296-304.
- Pielou, E.C. (1966) The measurement of diversity in different types of biological collections. *Journal of Theoretical Biology* 13: 131-144.
- Roberts, D.W. (1986) Ordination and the basis of fuzzy set theory. *Vegetatio* 66: 123-131.
- Rueda, M. (2006) Selección de hábitat por herbívoros de diferente tamaño y sus efectos sobre la vegetación. El papel del conejo (*Oryctolagus cuniculus*) en ecosistemas de dehesa. Tesis Doctoral. Universidad de Alcalá, Alcalá de Henares.
- Russi, L., Cocks, P.S., & Roberts, E.H. (1992a) The fate of legume seeds eaten by sheep from a Mediterranean grassland. *Journal of Applied Ecology* 29: 772-778.
- Russi, L., Cocks, P.S., & Roberts, E.H. (1992b) Seed bank dynamics in a Mediterranean grassland. *Journal of Applied Ecology* 29: 763-771.
- Shannon, C.E. & Weaver, W. (1949) *The Mathematical Theory of Communication* University of Illinois Press, Urbana, Illinois.
- Simpson, R.L., Leck, M.A., & Parker, V.T. (1989). Seed banks: general concepts and methodological issues. In *Ecology of Soil Seed Banks* (eds M.A. Leck, V.T. Parker & R.L. Simpson), pp. 3-8. Academic Press Inc., San Diego.
- Soriguer, R.C. (1983) Consideraciones sobre el efecto de los conejos y los grandes herbívoros en los pastizales de la Vera de Doñana. *Doñana Acta Vertebrata* 10: 155-168.
- Sternberg, M., Gutman, M., Perevolotsky, A., & Kigel, J. (2003) Effects of grazing on soil seed bank dynamics: An approach with functional groups. *Journal of Vegetation Science* 14: 375-386.
- Taylor, R.H. & Williams, R.M. (1956) The use of pellet counts for establishing the density of populations of wild rabbit *Oryctolagus cuniculus* (L.). *New Zealand Journal of Science and Technology* 38: 239-256.
- Thompson, K. & Grime, J.P. (1979) Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. *Journal of Ecology* 67: 893-921.
- Traba, J., Azcárate, F., & Peco, B. (2006) The fate of seeds in Mediterranean soil seed banks in relation to their traits. *Journal of Vegetation Science* 17: 5-10.
- Van der Wal, R., Bardgett, R.D., Harrison, K.A., & Stien, A. (2004) Vertebrate herbivores and ecosystem control: cascading effects of faeces on tundra ecosystems. *Ecography* 27: 242-252.
- Vellend, M., Knight, T.M., & Drake, J.M. (2006) Antagonistic effects of seed dispersal and herbivory on plant migration. *Ecology Letters* 9: 319-326.
- Wood, D.H. (1988) Estimating Rabbit Density by Counting Dung Pellets. *Australian Wildlife Research* 15: 665-671.

- Verdu, J.R. & Galante, E. (2004) Behavioural and morphological adaptations for a low-quality resource in semi-arid environments: dung beetles (Coleoptera, Scarabaeoidea) associated with the European rabbit (*Oryctolagus cuniculus* L.). *Journal of Natural History* 38: 705-715.
- Williams, E.D. (1984) Changes during 3 years in the size and composition of the seed bank beneath a long-term pasture as influenced by defoliation and fertilizer regime. *Journal of Applied Ecology* 21: 603-615.
- Zobel, M., Otsus, M., Liira, J., Moora, M., & Mols, T. (2000) Is small-scale species richness limited by seed availability or microsite availability? *Ecology* 81: 3274-3282.

Appendix 2 - Means \pm s.e seed density (seeds/m²) for 16 of the most abundant species, in both high and low productivity sites, and under the different grazing treatments. All species are annuals. (B) = Boraginaceae; (C) = Compositae; (Ca) = Caryophyllaceae; (Cra) = Crassulaceae; (G) = Gramineae; (L) = Leguminosae; (J) = Juncaceae; (R) = Rosaceae; (S) = Scrophulariaceae

| | Low Productivity Sites | | | | | | High Productivity Sites | | | | | |
|-------------------------------------|------------------------|-------------------------|-------------------------|-------------------------|-------------------------|--------------------------|-------------------------|-------------------------|-----------------------|------------------------|-------------------------|--------------|
| | Rabbit plots | | Rabbits+sheep plots | | Full enclosures | | Rabbit plots | | Rabbits+sheep plots | | Full enclosures | |
| | With dung | Without dung | With dung | Without dung | With dung | Without dung | With dung | Without dung | With dung | Without dung | With dung | Without dung |
| <i>Agrostis pourreii</i> (G) | 127.4 \pm 73.8 | 31.8 \pm 31.8 | 0 | 63.7 \pm 29.8 | 31.8 \pm 19.5 | 7993.6 \pm 5422.5 | 1226.1 \pm 64 1.7 | 1608.3 \pm 1141.5 | 1958.6 \pm 1490. | 2038.2 \pm 1687.7 | 1162.4 \pm 557.1 | |
| <i>Andryala integrifolia</i> (C) | 828.0 \pm 309.0 | 111.5 \pm 73.8 | 127.4 \pm 40.6 | 47.8 \pm 19.5 | 366.2 \pm 194.4 | 31.8 \pm 31.8 | 0 | 0 | 0 | 0 | 31.8 \pm 31.8 | |
| <i>Aphanes microcarpa</i> (R) | 2245.2 2 \pm 489 | 4315.3 \pm 11 50.9 | 3996.8 \pm 732.5 | 4172.0 \pm 1132.6 | 5477.7 \pm 2810.6 | 388.1 \pm 125.9 | 95.5 \pm 15.9 | 175.2 \pm 11 9.2 | 31.8 \pm 3 1.8 | 47.8 \pm 47.8 | 47.8 \pm 47.8 | |
| <i>Cerastium glutinosum</i> (Ca) | 1958.6 \pm 448.0 | 589.2 \pm 164.3 | 716.6 \pm 253.0 | 111.5 \pm 19.5 | 429.9 \pm 203.9 | 7197.5 \pm 1541.5 | 2070.1 \pm 95 9.4 | 557.3 \pm 266.5 | 1831.2 \pm 609.5 | 1735.7 \pm 53 8.5 | 1003.2 \pm 577.8 | |
| <i>Crassula tillaea</i> (Cra) | 56162. 4 \pm 769 | 11608.3 \pm 1190.4 | 10700.6 \pm 1384.4 | 11273.9 \pm 1160.8 | 8901.3 \pm 30111.9 | 191.1 \pm 54.0 | 15.9 \pm 15.9 | 31.8 \pm 19. 5 | 79.6 \pm 3 5.6 | 15.9 \pm 15.9 | 47.8 \pm 31.8 | |
| <i>Crepis capillaris</i> (C) | 2245.2 \pm 864.7 | 183.3 \pm 82.0 | 183.3 \pm 82.0 | 720.1 \pm 322.0 | 552.8 \pm 247.2 | 493.6 \pm 194.0 | 127.4 \pm 73.8 | 159.2 \pm 83 .5 | 95.5 \pm 5 8.5 | 31.8 \pm 31.8 | 79.6 \pm 61.7 | |
| <i>Juncus buffonius</i> (J) | 2547.8 \pm 1389. | 286.6 \pm 177.3 | 286.6 \pm 177.3 | 493.6 \pm 259.7 | 796.2 \pm 395.7 | 57531.8 \pm 14639.4 | 14187.9 \pm 4018.7 | 11035.0 \pm 3397.6 | 12277. 1 \pm | 7547.8 \pm 2804.2 | 12484.1 \pm 4048.7 | |
| <i>Myosotis discolor</i> (B) | 1640.1 \pm 895.0 | 143.3 \pm 63.7 | 143.3 \pm 63.7 | 334.4 \pm 156.0 | 382.2 \pm 190.8 | 8519.1 \pm 2880.5 | 1656.1 \pm 15.9 | 2133.8 \pm 1 144.8 | 1815.3 \pm 713.2 | 1210.2 \pm 397.6 | 1703.8 \pm 558.2 | |
| <i>Poa annua</i> (G) | 47.8 \pm 3 1.8 | 31.8 \pm 31.8 | 0 | 0 | 0 | 1480.9 \pm 785.2 | 414.0 \pm 297.3 | 382.2 \pm 285.3 | 286.6 \pm 92.8 | 238.9 \pm 120.7 | 159.2 \pm 87.2 | |
| <i>Polycarpon tetraphyllum</i> (Ca) | 302.5 \pm 92.2 | 63.7 \pm 39.0 | 15.9 \pm 15.9 | 47.8 \pm 31.8 | 95.5 \pm 39.0 | 63.7 \pm 46.4 | 0 | 0 | 31.8 \pm 31.8 | 0 | 31.8 \pm 19.5 | |
| <i>Sagina apetala</i> (Ca) | 24729. 3 \pm 777 | 3646.5 \pm 1637.4 | 3646.5 \pm 1637.4 | 4904.5 \pm 1863.4 | 4697.5 \pm 878.3 | 1719.8 \pm 611.9 | 111.5 \pm 92.8 | 222.9 \pm 95 .5 | 270.7 \pm 82.0 | 207.0 \pm 96.2 | 907.6 \pm 563.9 | |
| <i>Spergularia rubra</i> (Ca) | 1751.6 \pm 339.3 | 1385.4 \pm 424.0 | 1385.4 \pm 424.0 | 1210.2 \pm 398.4 | 1544.6 \pm 814.5 | - | - | - | - | - | - | |
| <i>Trifolium arvense</i> (L) | 525.5 \pm 252.5 | 47.8 \pm 15.9 | 47.8 \pm 15.9 | 175.2 \pm 81.2 | 127.4 \pm 78.0 | 238.9 \pm 94.2 | 47.8 \pm 47.8 | 15.9 \pm 15. 9 | 31.8 \pm 31.8 | 79.6 \pm 15.9 | 63.7 \pm 39.0 | |
| <i>Trifolium campestre</i> (L) | 63.7 \pm 4 6.4 | 15.9 \pm 15.9 | 15.9 \pm 15.9 | 15.9 \pm 15.9 | 0 | 9522.3 \pm 2894.0 | 1273.9 \pm 498.5 | 1433.1 \pm 455.3 | 3200.6 \pm | 2738.9 \pm 587.0 | 875.8 \pm 334.0 | |
| <i>Veronica arvense</i> (S) | 47.8 \pm 3 1.8 | 15.9 \pm 15.9 | 15.9 \pm 15.9 | 0 | 15.9 \pm 15.9 | 1098.7 \pm 607.1 | 286.6 \pm 15.6 | 286.6 \pm 21 1.5 | 222.9 \pm 131.8 | 222.9 \pm 145.5 | 79.6 \pm 43.6 | |
| <i>Vulpia ciliata</i> (G) | 7866.2 \pm 1660. | 2070.1 \pm 638.4 | 1666.1 \pm 481.9 | 1369.4 \pm 521.7 | 1656.1 \pm 743.2 | 8566.9 \pm 4445.0 | 2579.8 \pm 1771.1 | 1321.7 \pm 572.8 | 3359.9 \pm | 1210.2 \pm 520.5 | 95.5 \pm 46.4 | |
| | | | | | | 2065.4 | | | | | | |