



Ungulates alter plant cover without consistent effect on soil ecosystem functioning

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ABSTRACT

Shifts in animal husbandry and landscape use have significantly changed ungulate grazing effects on ecosystem functioning. These changes are now the subject of extensive research with respect to plant and soil communities, but the results of these studies are highly varied and context dependent. The objective of this study is to address contextual variation by holding all sampling methods and analytical approaches constant and analyse the effect of the feral goat (*Capra hircus*) population of Mallorca Island, Spain, on soil physical, chemical and biological characteristics across five controlled sites. Specifically, vegetation cover and soil properties in fenced plots excluded from ungulates were compared with adjacent grazed plots in five independent mountain areas of Mallorca. Soil microbial activity measured as Community-Level Physiological Profiles (CLPP) using EcoPlate™ increased when ungulates were excluded. However, all other physical and chemical measures of the soils did not vary significantly when we considered ungulate exclusion across all plots, and this may be caused by a soil community that is simply robust to the effects of the herbivores. Or, it may be due to the high heterogeneity that was detected among pair plots comparisons within each of the five sites. Indeed, we find more variability within a site than among our independent sites leading us to hypothesize that grazing does influence biogeochemical cycles, but it does it by increasing variability of the system in general. Our well-controlled multilevel meta-analysis confirms the notion that ungulate effects are highly context dependent, and soil heterogeneity makes resolving clear patterns very challenging. Apparently, context persistently drives the soil response more than the grazing itself, and this is seen even at very small scales.

1. Introduction

In recent decades, human land use has changed, and this includes animal husbandry and grazing practices. The changes in grazing practice are causing profound transformation of plant productivity and community composition at landscape level scales (Capó et al., 2021; Peco et al., 2006; Sebastià et al., 2008; Sjödin et al., 2007). Therefore, populations of ungulates, especially domestic livestock but also wild species, have been extensively studied to determine their effects on the composition and functioning of plant (Jia et al., 2018) and soil communities (for recent meta-analysis and synthesis see: Andriuzzi and Wall, 2018;

Forbes et al., 2019). However, all of this extensive research seems to reveal more questions than answers. Specifically, we know that herbivory directly affects plant growth and plant community dynamics. Therefore, we would expect to find subsequent effects below ground in the soils. This is because soils are directly connected to the plant community via the rhizosphere and bidirectional plant and soil feedbacks that occur therein (van der Putten et al., 2016). As such, they provide important ecosystem services like decomposition and nutrient cycling that sustains soil productivity and future plant growth (Coleman et al., 2004; Crowther et al., 2019; Roger-Estrade et al., 2010) or even plant resistance to abiotic disturbances and stress (Brussaard et al., 2007).

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However, a clear connection between ungulate effects above-ground on the plant community and that of the soils below-ground has not been realized. This disconnect may be because soils are highly heterogeneous, and their functioning is subject to multiple and varied environmental drivers (Wall et al., 2012). Therefore, we question the role of environmental heterogeneity to moderate when, or under what conditions ungulates can affect soil functioning.

The influence of diverse animal grazers on the landscape and the soils has been highly mixed and is context dependent. The presence of livestock has been shown to significantly degrade soil in some cases (Gizicki et al., 2018), but it can have positive consequences in other cases (Pulido et al., 2018). Grazing by ungulates in high densities is responsible for a reduction in the organic carbon in the soil by reducing the amounts of necromass that is returned to the soil. However, other studies report significant increases in organic matter due to return via feces (Peco et al., 2006; Pulido et al., 2018). The variability in organic matter return to the soil may be caused by differences in ungulate feeding activity associated with plant quality and ungulate efficiency (*sensu* Krumins et al., 2015). Logically, shifts in carbon allocation to the soil will directly influence the functioning of microbial communities there. However, the responses of microbes to ungulate grazing, as in other responses, can be quite mixed. Microbial biomass can be reduced by high pressure from herbivores and the destruction of structures such as mycelia or bacterial biofilms (Damacena De Souza et al., 2006), although in other studies it has been observed that it can increase at high grazing intensities (Mohr et al., 2005) or differing conditions (Stark et al., 2002). Effects on the microbial community will cascade to ecosystem services like decomposition. Yet again, this is context dependent. For instance, when moose were excluded from a plot, decomposition was not affected (Ellis and Leroux, 2017; Kolstad et al., 2018). However, in a different environmental context, decomposition was reduced when moose and hares were excluded (Kielland et al., 1997).

All of these varied responses to herbivory may be a product of the abiotic context of the parent soil and the landscape in which it sits. The return of organic carbon to the soil may be directly impacted when grazed soils have greater bulk density. This can be associated with excessive trampling (Alegre and Lara, 1991) and to the reduction in some areas of the amount of organic matter which is capable of creating clay aggregates that would maintain more porous soils. The pH can also be affected by modifying the microbial and root activity (Jeddi and Chaieb, 2010), although this effect varies according to the environmental conditions and the type of soil (Bardgett et al., 2001; Noe and Abril, 2013). As is likely the case with respect to this work, trampling and therefore its effects on bulk density may be high when water is limiting and soil organic matter is otherwise low (Reichert et al., 2018).

We do know that ungulates are responsible for regulating biogeochemical cycles, the most notable being the acceleration in the nitrogen and carbon cycle (Fleurbaey et al., 2011; Patra et al., 2005). It has been shown that ungulates are able to inhibit ecosystem functions like nitrification or ammonification due to the reduced return of nitrogen to the soil and/or the compaction of the soil which consequent decrease in soil moisture (Pan et al., 2018; Xu et al., 2008). Simultaneously, grazing may lead to increased nitrogen losses by leaching or volatilization by removing the vegetation cover and making the soil more susceptible to erosion (Núñez et al., 2010). This can be overcome when the presence of ungulates increases nitrogen mineralization due to the contributions of organic nitrogen with feces and urine (Frank and Groffman, 1998; Furusawa et al., 2016), but this effect will be spatially dispersed as the animals move through the landscape and will be moderated by local environmental conditions. For instance, xeric soils can be more oxygenated, moderating microbially mediated enzyme activities and nitrogen transformations (Ghiloufi et al., 2018), likely interacting with other physical effects of ungulates to the soil.

As a means to address this conundrum in soil ecology, we exploit an existing ungulate exclusion experiment in which anti-herbivory plots

were established from 5 to 20 years ago. The objective of this study is to analyse the differences in physical, chemical and biological characteristics of the soil between areas without ungulates, feral goats (*Capra hircus*) and free-ranging flocks of sheep (*Ovis aries*), compared to areas under continuous ungulate grazing in five distinct mountainous localities with Mediterranean vegetation in Mallorca, Spain. Specifically, we wanted to know if the variability in grazing upon the soil was greater within each site or among the five individual sites. One strength of this study is that we measured response to the same ungulate across well-known (Table 1) and climatically and geographically similar sites while maintaining experimenter identity constant. Given that each site is independent, we expected to see differences among them. However, we wanted to know if the differences all followed the same trend. Our results are relevant locally and globally. This information will help in the analysis of the impacts derived from variation of ungulate populations that many rural areas suffer. However, they are also globally relevant because they help to inform what environmental conditions determine the impact of the ungulates on the soil more broadly.

2. Materials and methods

2.1. Study sites

We address the role of ungulate grazers on soil factors across five independent sites in the mountainous region on the Island of Mallorca, Spain (Fig. 1). Steep slopes predominate the sites, and the soil is generally shallow with limestone outcropping (See Table 1). The climate is Mediterranean and ranges from humid to semiarid subtypes along the topographical gradients. The study sites were named according to the toponym where they are located (Fig. 1).

All five study sites are located in natural areas with pre-established herbivore exclusion plots. There are two types of habitats between the study sites, holm oak forest and Mediterranean scrubland. Holm oak forest was formed by *Quercus ilex* as the only tree species at Son Moragues, with some pines (*Pinus halepensis*) at Binifaldo or some Strawberry trees (*Arbutus unedo*) and pines at Sobreamunt. In the scrubland areas, La Victoria is dominated by the large tussock grass *Ampelodesmos mauritanica* meanwhile at Na Burguesa predominate the perennial grass *Brachypodium retusum* with *Cistus* sp. scrubs. Within each of the five areas there is at least one fenced ungulate enclosure. Each site allows for two treatments, ungulate herbivore presence (Ungulate) or exclusion (Ungulate-free). Each area has a different size and age of exclusions (Table 1), depending on the history of the site, some are designed as a large exclusion that include several study plots within, while in other sites there were multiple small exclusions, including only one plot within each. Outside of the enclosures, in all sites, feral goats (*Capra aegagrus hircus*) graze freely, and in Son Moragues and Binifaldo, domestic sheep herds occasionally graze with the goats. To assess herbivory effects at each site, we established Ungulate/Ungulate-free paired sampling plots for a binary comparison (Table 1).

2.2. Sampling protocol

In each study site, we established three 10 × 10 m plots for each treatment, except in Binifaldó, where only two plots per treatment could be established (28 plots in total). Each of these plots within the ungulate excluded zone was compared with a plot in the open zone that was as close as possible (but always >1 m from the fence). We conducted a vegetation survey in all plots using the point intercept technique (Elzinga et al., 1998), in which we measured vegetation and plant species (excluding trees higher than 1.5 m) at 25 m intervals. We measure the percent vegetation cover as the relative number of points that are vegetated or bare ground. Due to the complexity of the vegetation structure, sometimes multiple individuals of different species occurred at the same point intersection. Therefore, we measure vegetation complexity as the relative number of points that support multiple

Table 1

Principal characteristics of the five study sites of Majorca Mountains: type of habitat, soil texture classification, mean elevation, annual mean temperature, Net Primary Production (NPP). The Time of exclusion indicates the approximate years of ungulates exclusion establishment until the data collection, and Design indicates the number of fenced areas with respect to the number of plots inside each fence area.

Site	Habitat	Textural Soil Class	Elevation (m asl)	T ^a	NPP kgC/m ² /yr ^b	Time of exclusion	Design
Binifaldó	Forest	Clay	626	13.3 °C	1.398	>15	1/2
Sobremunt	Forest	Loam	663	13.9 °C	1.303	>20	1/3
Son Moragues	Forest	Loam	522	13.8 °C	1.401	>15	3/1
La Victoria	Scrubland	Silty clay	97	17.2 °C	1.232	5	1/3
Na Burguesa	Scrubland	Silt loam	479	15.5 °C	1.298	>20	3/1

^a Climatological data are from Climatologies at High Resolution for the Earth Land Surface Areas, project CHELSA (Karger et al., 2017).

^b Net Primary Production Gap-Filled Yearly L4 Global 500 m SIN Grid (MOD17A3HGF v006) (Running and Zhao, 2019).

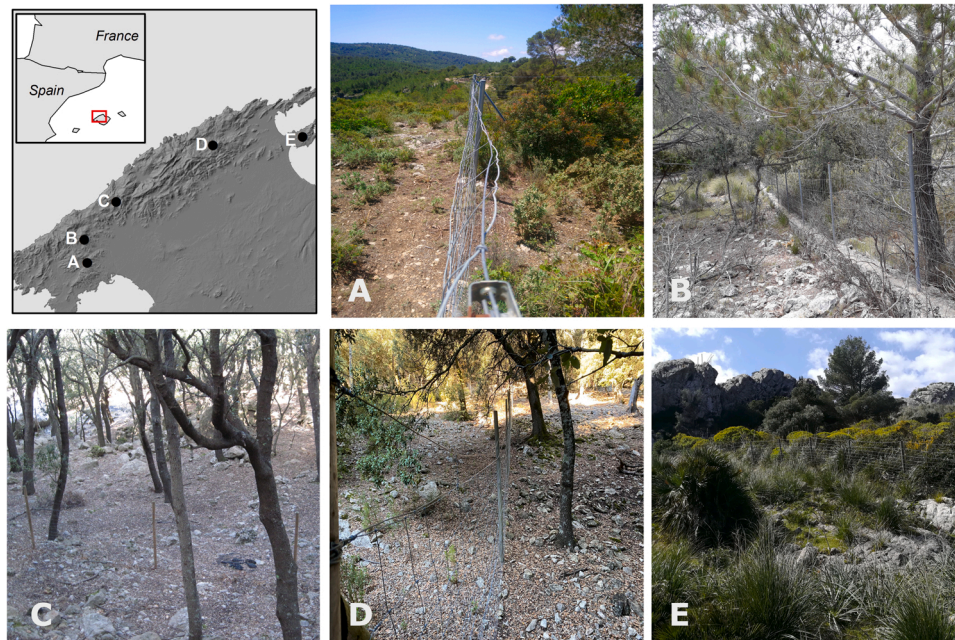


Fig. 1. The first slot indicates the geographical location of the study sites. The rest of the slots show photographic details of the habitats for the fenced study sites. A (Na Burguesa) and E (La Victoria) represent scrubland. The forest areas are related to the letters B (Sobremunt), C (Son Moragues) and D (Binifaldó).

different species (vertically through the point). We exclude trees higher than 1.5 m because they are not likely relevant to ungulate herbivory in this system.

Within each plot, we randomly established three 0.01 m² sampling frames (84 samples in total) to collect the litter layer and two bulk soil samples. In the laboratory, the litter was oven dried (24 h at 105 °C) and weighed, and the bulk soil samples were stored at 4 °C before further analysis. We used 200 gr of dry, 2 mm sieved soil for chemical analysis. All analyses were performed by Eurofins Laboratories Ltd., Lleida, Spain for the following parameters: pH, electrical conductivity, C (elemental carbon), organic matter content, N (elemental nitrogen) and five soil textural classes (fine sand, coarse sand, fine silt, coarse silt and clay). The C:N ratio was also calculated.

We used the excavation method described in (Blake and Hartge, 1986) to measure the bulk soil density due to the stony nature of the sites (Capó-Bauçà et al., 2019). This procedure included digging a 10 cm³ hole by using the same square frame and a gardening shovel. The volume of the excavation was determined by lining the hole with plastic film and filling it completely with a measured volume of water. Coarse fragments (diameter > 2 mm) were sieved out and bulk density was calculated as the mass of dry, coarse fragment-free soil per volume of the excavated soil, where volume was also calculated on a coarse fragment-free basis. We were not able to assess soil bulk density at Sobremunt since the soil nature is too rocky and there is insufficient depth to extract samples.

2.3. Soil microbial community profiling

We measured the functional diversity of the microbial community in soil with the community-level physiological profiles method (CLPP) (Garland and Mills, 1991) using Biolog EcoPlates™ (Biolog Inc., Hayward, California, USA). EcoPlates contain 31 distinct carbon sources in triplicate as well a colour dye that turns purple if a given carbon source is metabolized by the microbial community present in the well. Following the colour development over time allows one to estimate the rate of carbon source utilization in addition to which carbon sources were used.

To prepare a microbial inoculum from each soil, we diluted 5gr. sub-samples of fine soil in saline (45 ml, 0.9% NaCl) then centrifuged for 10 min at 2,400rpm. The microbial suspension was left to stand for 30 min at 4 °C then the sub-samples were again diluted 1:100 in saline before inoculation in to the EcoPlates at the rate of with 100 µL of sample in each well. We incubated the EcoPlates in the dark at a constant 25 °C and measured optical density (OD) at 590 nm with a plate reader every 12 h for up to 96 h plus an additional reading at 160 h. We scored a carbon source as positive when two out of three wells reached an OD of at least 0.2 after subtraction of the median blank from all wells. We modelled the colour development in each well that we scored as positive with a modified Gompertz equation (Zwietering et al., 1990).

We used the R script and nlsLM function of the package “minpack.lm” 1.2–1 version (Elzhov et al., 2016) to fit the Gompertz function

(Roger et al., 2016). The function is a sigmoidal curve describing microbial community growth and is determined by 3 parameters, lag phase (l), maximum uptake rate (r) and maximum population growth (k) (Kahm et al., 2010). Functional diversity was calculated considering each carbon source as community trait, and the maximum uptake rate (r) of the carbon source was taken as trait value. We weighed all carbon sources by their uptake rate and calculated diversity of the community according to the Shannon Index using the diversity function in the vegan package (Oksanen et al., 2019).

The average well colour development (AWCD) parameter represents the metabolic capacity of the soil microorganisms grown in the EcoPlates. AWCD was derived from the mean difference among OD values of the wells following (Garland and Mills, 1991) formula: $AWCD = \sum (C_i - R) / 31$ where C_i is the optical density of the substrate i , R is the optical density of the control wells and 31 is the number of different carbon sources of each well (in triplicate). An AWCD value is generated for each reading increment, and we then select the time increment in which maximum AWCD value occurs to assess soil metabolic capacity.

2.4. Organic matter decomposition

We assessed ungulate effects on soil biological activity by calculating the organic matter degradation rate on standard plant material following the Tea Bag Index (TBI) method of (Keuskamp et al., 2013). The tea bags were labelled with a waterproof marker, oven dried at 70°C for 48 h and weighed (+/- 0.01 g). Subsequently, three replicates of each tea type were buried in each plot in 8-cm deep separate holes of recorded location (a total of 84 sample units). Sample burial was carried out between March 26th to March 29th, 2018, and they were retrieved 90 days later. Post recovery, bags were cleaned of roots and debris, dried and weighed. According to the weight loss, we calculated the TBI parameters: S (stabilization factor) and k (decomposition rate constant). Green tea has a very labile fraction that has a rapid initial decomposition rate. After 90 days we were able to determine how much of the labile fraction of the material is decomposed (k) and how much is stabilized (S). Rooibos tea decomposes much more slowly. After 90 days, it is still in the first phase of decomposition. Thereby, the weight loss of rooibos tea is a proxy of the initial decomposition rate (k). TBI parameters were calculated using the spreadsheet template provided by TBI research team in the website <http://www.teatime4science.org>.

2.5. Statistical analysis

Data from the vegetation transects were analysed using mixed regression models considering the treatment (Herbivory Yes or No) as a fixed factor and plot nested in site as a random intercept term. The data fulfilled the assumptions of normality and homoscedasticity. The mixed effect models were conducted in the nlme package 3.1–152 (Pinheiro et al., 2021) in R (R Core Team, 2020).

We detected very high heterogeneity in soil characteristic data including many outliers and significant differences among plots within the same study area. Furthermore, when the sample size is small or the intensity of an effect is low (as was the case in our study), an alternative to control for Type II statistical error is the meta-analysis (Arnquist and Wooster 1995). Following the criteria established by Gómez-Aparicio et al. (2004), the effect of ungulate exclusion (ungulate versus ungulate-free) on soil physicochemical and biological characteristics was analyzed using a meta-analysis. We performed a three level meta-analysis to address variation across paired plots while also accounting for the dependency of the ungulate exclusion effects within study sites. Each paired plot was considered as an individual study and weighed according to its robustness to assess the overall effect of herbivory exclusion on soil properties.

In order to structure the multilevel meta-analysis workflow, we compute the effect size and variance for each set of paired plots within each individual study in the context of response ratios described in

(Hedges et al., 1999). Summary statistics (mean (μ), standard deviation (sd) and sample size (n)) for each response variable were reported to determine the weighted effect size for each plot. The effect size of ungulate exclusion on the response variables was calculated as $\ln(\mu_{\text{ungulate-free}} / \mu_{\text{ungulate}})$, hereafter referred to as the log response ratio, $\ln(RR)$ (Hedges et al., 1999). The $\ln(RR)$ quantifies the proportional change that results from herbivore exclusion and is appropriate given that the absolute value in the response variables varied widely among and within sites (Goldberg et al., 1999; Hedges et al., 1999). Negative values of $\ln(RR)$ indicate that the exclusion of ungulates decreases the response variable, so ungulates presence increases it. The overall $\ln(RR)$ was back-transformed and converted to a percentage of change for ease of interpretation.

The response ratios were weighted with the inverse sample variance to ensure a greater contribution of the most robust studies (Rosenberg et al., 2000), therefore, we estimate the variances associated with the response ratio $V_{\ln(RR)}$ (Hedges et al., 1999) as:

$$V_{\ln(RR)} = \frac{SD_{\text{ungrazed}}^2}{n_{\text{ungrazed}}(X_{\text{ungrazed}})^2} + \frac{SD_{\text{grazed}}^2}{n_{\text{grazed}}(X_{\text{grazed}})^2}$$

We assessed the consistency of the $\ln(RR)$ across studies for each response variable by fitting a three-level meta-analytic model. For this purpose, we use the `rma.mv` function of the metafor (Viechtbauer, 2010) package in R, by running the syntax:

```
rma.mv(data, yi, V, random = ~1|site/plot)
```

Data is the dataset containing the summary statistics of each variable; y_i is the effect size Ungulate-free, and V is the sampling variance. Random argument specifies the random-effect structure of the model. In three level meta-analysis, the heterogeneity is distributed between the sampling variance (I_{Level1}^2), the variance within plots (I_{Level2}^2) and between site (I_{Level3}^2). We used the `var.comp` function in the `dmatar` package (Harrer et al., 2019) in R to calculate the multilevel variance I^2 . The heterogeneity variation between plots and/or sites can be regarded as substantial if less than 75% of the total amount of variance is attributed to sampling variance (I_{Level1}^2) (Hunter et al., 1991). When that occurred, we performed a test of moderators by introducing in the model the covariates: elevation, Net Primary Production (NPP), temperature, soil textural classes (summarized with the first component of a Principal Component Analysis with the five texture components) and the categorical covariate of habitat, which may explain the observed heterogeneity. We derived the mean effect of each moderator with the 95%CI and the results of the omnibus test under the test of moderators (Table S1). If the p-value associated with the test of moderators Q_M is larger than the significance level of 0.05 we concluded that the overall effect is not moderated by the covariates included (Viechtbauer, 2010).

With respect to the CLPP, we also used a multivariate approach to summarize the data. We first calculated normalized Biolog absorbance of each substrate after 96 h of incubation by dividing the mean value of absorbance of the three wells per substrate by the AWCD value of each plate. With these values we conducted a PCA with 'prcomp' function in base R, comparing the carbon source utilization patterns of soil microbial communities with respect to the presence or absence of ungulates (Capó-Bauçà et al., 2019).

3. Results

3.1. Vegetation response to ungulate exclusion

The exclusion of ungulate herbivores generates significant changes in vegetation cover. In the absence of ungulates, vegetation cover and complexity (% coverage with several layers of vegetation) increased significantly (Table 2). Likewise, the percent cover of bare soil decreased with the absence of ungulates (Table 2).

Table 2

The ungulate exclusion effects on the percentage of bare soil, total vegetation cover and percentage of cover with multiple different species (complexity) as analyzed by a mixed general lineal model GLMM.

Plant Cover	Ungulates (mean±SE)	Ungulates-free (mean±SE)	GLMM
Bare soil	46.12 ± 10.11	26.63 ± 9.58	$F_{(1,3)} = 16.14$; $P=0.0015$
Cover	45.52 ± 11.07	62.58 ± 19.95	$F_{(1,3)} = 10.73$; $P=0.0060$
Complexity	17.75 ± 6.29	32.80 ± 8.92	$F_{(1,3)} = 5.22$; $P=0.0398$

3.2. Soil response to ungulate exclusion

For 11 of the 12 variables examined, the CI of the mean effect size overlapped zero (Table 3, Fig. 2). Soil microbes were more active in ungulates excluded plots, since the activity of microbial communities measured by CLPP with EcoPlates, changed significantly (Table 3) with higher activity (AWCD) in the absence of ungulates (Fig. 2; Table 3). All

Table 3

Statistical results of the overall effect of the exclusion over the response variables analysed by multi-level meta-analytic models. In the first column a global increase in the studied variable was indicated by ↑ whereas a decrease was indicated by ↓. The P-value of the fitted model is outlined in bold when the effect is statistically significant. The percentage of response was calculated as the inverse transformation of logarithm of the effect size $\ln(RR)$. N° indicates the number of comparisons corresponds to the number of pair of plots analysed. The variance is distributed across the sampling variance (I^2_{Level1}), variance within plots (I^2_{Level2}), and between site (I^2_{Level3}).

Variable	P- value	% Response	N°	I^2_{Level1}	I^2_{Level2}	I^2_{Level3}
Bulk density↓	0.093	-10.0	11	14.47	85.52	0
Litter↑	0.213	0.22(-0.14,0.58) 24.6	14	7.16	92.84	0
Electrical conductivity↑	0.194	0.11(-0.07,0.29) 12.1	14	52.97	47.03	0
Elemental Carbon↑	0.278	0.12(-0.111,0.35) 13.0	14	39.17	60.83	0
Organic Matter↑	0.304	0.13(-0.14,0.40) 14.3	14	15.62	69.17	15.21
Elemental Nitrogen↑	0.152	0.16(-0.07,0.38) 16.9	14	31.57	54.88	13.55
pH↑	0.580	0.01(-0.02,0.03) 0.5	14	100	0	0
C/N↓	0.349	0.03(-0.09,0.03) -2.7	14	31.61	68.39	0
AWCD ↑	0.008	0.25(0.08,0.43) 28.9	14	10.03	89.97	0
Shannon (H') ↑	0.099	0.13(-0.03,0.28) 13.4	14	10.13	89.87	0
Stabilization factor (S) ↑	0.820	0.01(-0.08,0.10) 1.0	13	34.42	65.58	0
Decomposed rate (k) ↑	0.892	0.02(-0.25,0.28) 1.6	8	10.84	84.81	4.35

physicochemical parameters measured (BD, Electric conductivity, elemental carbon, soil organic matter, elemental nitrogen, C/N ratio and pH), microbial functional diversity (H) and OM decomposition measured by TBI did not significantly change with ungulates exclusion (Table 3). For these soil characteristics, effect size varied from negative to positive across paired plot comparisons inside the same studied area (Fig. 2, Fig. 1S). Forest diagrams representing the estimate of the variance between studies show no consistent effect inside the same area (Fig. 2, Fig. 2S).

In fact, for all the variables, less than 75% of the total amount of variance is attributed to sampling variance (I^2_{Level1}), and the percentage of variance within plots (I^2_{Level2}) is the highest in all cases. (Table 3). However, this could not be explained by geographic factors or even environmental characteristics because meta-regressions showed no significant influence of elevation, NPP, temperature, soil textural classes on the effect sizes within each site (Table S1). Only the habitat covariate had a marginal significant effect on the effect size of elemental nitrogen and stabilization factor (S) variables (Table S1).

With respect to the carbon source utilization patterns of soil microbial communities, the PCA of the absorbance of all the available substrates in the EcoPlates™ read at 96 h shows no clear variation between ungulate and ungulate-free treatments (Fig. 2S).

4. Discussion

Apparently, local environmental heterogeneity may be a more critical driver of soil ecosystem properties than the effects of ungulate herbivory and subsequent shifts in plant community composition and productivity. These are the findings in a mountainous Mediterranean ecosystem (Mallorca, Spain), but they also inform the broader literature that often finds context dependent and idiosyncratic responses of soils to herbivory (Andriuzzi and Wall, 2018; Forbes et al., 2019; Vermeire et al., 2021). In this experiment, we compared the effects of ungulate exclusions on the above ground vegetation but also on the physical, chemical and bacteriological characteristics of soils. As expected, excluding the ungulates, significantly affected understory vegetation (cover and complexity). However, this was not realized belowground where there were very few meaningful changes to the soil structure, chemistry and biology. We suspect that our results are due to two possible explanations. First, the abiotic and microbiological properties of the soil but not aboveground vegetation were robust to the effects of grazing. This would be in agreement with previous works such as that of Greenwood et al. (1997) where they conclude that soil physical properties appear to be relatively insensitive to stocking rate in the long term. In fact, as Vermeire et al. (2021) suggested, we can expect relatively small changes in microbial communities due to natural disturbance when soil microbial communities have evolved with disturbance. In our case, microorganisms in the Mediterranean basin have adapted to soils with little availability of water (Yuste et al., 2014) making them resistant to change. And second, we assume that the environmental heterogeneity of the different plots clouds our ability to see consistent effects of ungulate grazing exclusion. In fact, herbivores may even increase this heterogeneity by trampling some areas, defecating in other and consuming vegetation in patches (Eldridge et al., 2019; Zhang et al., 2020). Moreover, it is likely both, as these two hypotheses are not mutually exclusive.

Here, we attempt to tackle heterogeneity and variability of environmental factors across different experimental sites with a three-level meta-analysis in which each of the comparisons among plots was treated as a study dealing with the dependency of the effect sizes within sites. Across our sites, ungulate exclusion had no effect on belowground soil processes and ecosystem functioning such as: decomposition, mineralization, C/N ratio or nutrient stock. Therefore, we have assumed that other factors controlling the biochemical activity of soil are likely more impactful than ungulate grazers. In reality however, our meta-

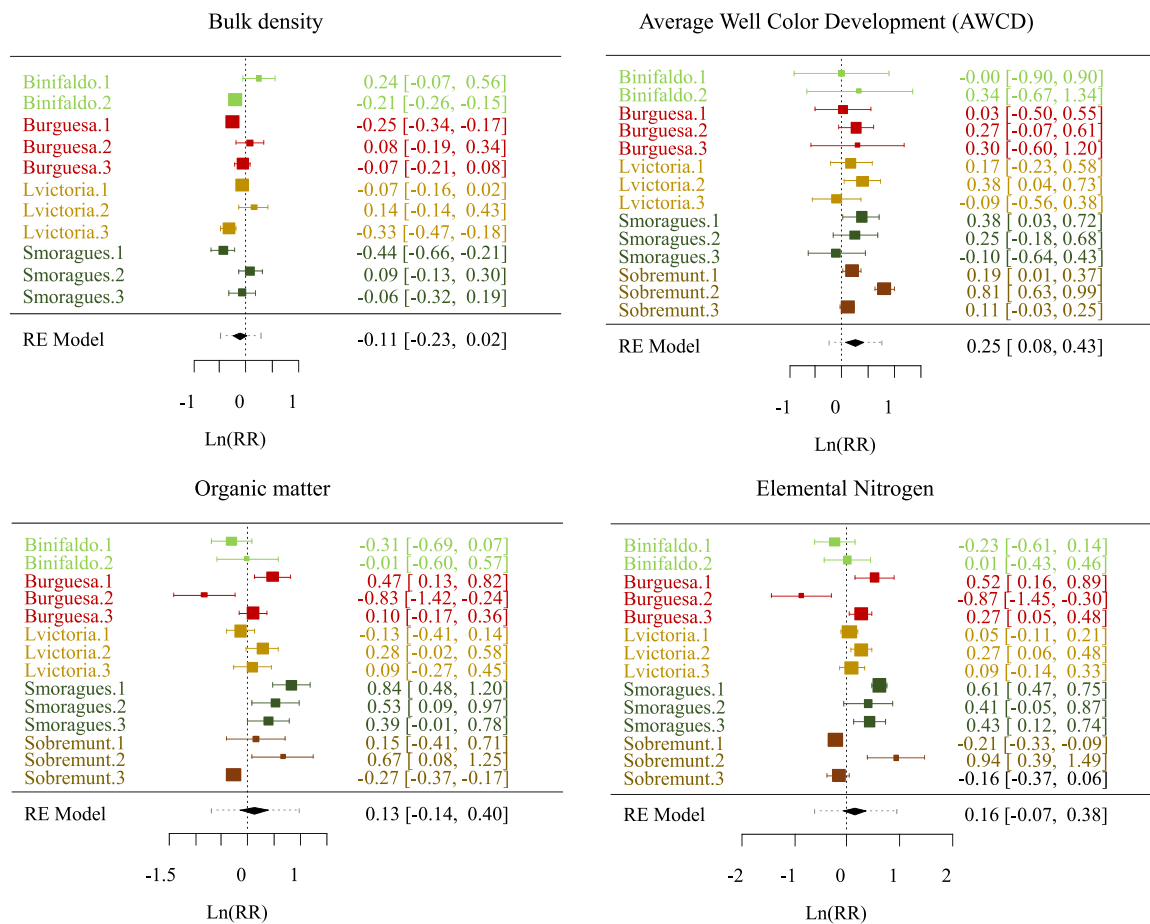


Fig. 2. Forest plot of the effect of the herbivory on four key response variables (Bulk density, AWCD, organic matter and soil organic nitrogen content). In each plot, the names on the left identify the individual plots in each of the five study areas. The boxes represent the $\ln(RR)$ of the individual studies, and the horizontal lines their 95% confidence intervals. The size of the boxes expresses the weight (see methods) of each study in the total effect, which is represented by a diamond. Response rates less than zero (vertical dotted line) indicate negative ungulate exclusion effect, while values greater than zero indicate a positive effect. If the diamond does not cross the zero line, the overall effect is significant. For a full list of comparisons see Table 3 or Table S1.

analysis reveals no consistent driver of soil characteristics in this system but high heterogeneity between plots. Many of the environmental parameters of the soil were highly variable and did not obviously respond to the impacts of ungulates in spite of changes to the vegetation. For instance, the comparison of one pair of plots can result in an important increase of soil organic matter, nitrogen and pH when ungulates are excluded while other pair plot comparisons showed the contrary effect (Fig. 2, Fig. 1S).

Our results here, and those of Ellis and Leroux (2017), found that decomposition rate k of standardized litter was not affected by ungulate exclusion. However, this is in contrast to Kielland et al. (1997) who found a decrease in decomposition rate of standardized litter (cellulose) inside herbivore exclusion fences in the Alaskan Tiaga. But also, Cárdenas et al. (2012) demonstrated that herbivory accelerates the decomposition of organic matter in a neotropical cloud forest. Similarly, we found higher nitrogen concentrations in eight of the twelve ungulate exclusion comparisons (Fig. 2d). That is, in our study, ungulates reduced soil nitrogen content. This was expected since ungulates have a preference for plants rich in N, and it is documented that their increase is related to a decrease in soil N (Pastor and Naiman, 1992). Similar responses have been observed with *Cervus elaphus* (Bardgett et al., 2001; Donkor et al., 2002; Gass and Binkley, 2011; Kumbasli et al., 2010). However, the relationship of both N and C concentration to ungulate exclusion is mixed but often neutral among our sampling sites with effect size very close to 0 in most of the cases (Fig. 1S). No change in the soil C/N ratio is frequently reported in herbivore exclusion studies (Gass

and Binkley, 2011; Harrison and Bardgett, 2004; Stark et al., 2010; Wardle et al., 2001).

Although there were few consistent effects of excluding ungulates upon soil properties, the capacity of the microbial communities to metabolize diverse carbon sources increased. The AWCD of the EcoPlates was significantly higher in soils that were excluded from the ungulates. This did not coincide with an increase on soil bacterial functional diversity, but it did coincide with a general decrease in the bulk density of the soil. This result follows the findings of other works with domestic goats by Holdaway (2014), with feral goat and deer by Kardol et al. (2014) and with moose by Gass and Binkley (2011) reporting that ungulate trampling produces soil compaction and may limit microbial activity.

In summary, when all of our five study sites were analysed, we find significant effects of ungulate grazing on vegetation cover but few significant effects on soil properties. Indeed, our lack of statistical significance is due to within plot variability that is greater than the variability between treatments or among sites. We propose the hypothesis that grazing does influence biogeochemical cycles, but it does it by increasing variability of the system in general. This is a testable hypothesis, and we propose that a study of ecosystem stability as opposed to static measures of ecosystem functions may be a more useful metric to study the effects of herbivory and its changing patterns with respect to the landscape. For instance, repeated measures of soil respiration, with respect to variable grazing intensity, would reveal the stability of soil functioning under herbivores. Regardless, increased sampling will

always be needed, and even a cross-study meta-analysis has revealed similar clouded results with respect to soil (Andriuzzi and Wall, 2018). Apparently, the spatial variation in the soil biotic and abiotic conditions was so great that our sampling effort was insufficient. This is supported by the high heterogeneity of results among comparison plots inside the same site (Fig. 2) indicating that most of the variation is at an even smaller spatial scale than site (that is between plots). A primary goal of this project was to control for methodological differences such that we could resolve the role of ecosystem context (Forbes et al., 2019). In practice, we achieved methodological control. However, apparently variability of an ecosystem is more important than the direct effects of ungulate grazers on the soil.

5. Conclusions

The last century has seen a profound transition in ungulate land use and herd management globally. The outcome of this is major shifts in plant community dynamics and the distribution of grassland and forested ecosystems. The implications of this span conservation, ecosystem stability and carbon sequestration. The results of our three-level meta-analysis studying 14 ungulate enclosure experiments reveals that although plant communities are directly affected by ungulate activity, apparently, the effects are not consistently realized below-ground in the soil. Therefore, we assume that, soil environmental heterogeneity dilutes grazing effects, and this is realized even at very small scales (<10 m). Variability in the soil response to ungulates was greater within each of our experimental plots than among the five sites. This finding is important because soil community processes drive plant community dynamics and ultimately, ecosystem functioning (van der Putten et al., 2016). Our research findings inform a deeper understanding of the role of ungulates in terrestrial ecosystems. However, the spatial and temporal scale at which ungulates affect soil may be difficult to capture in experiments and a challenge for herders and land managers. On the other hand, the impact of ungulate on soil integrity might be different in more homogenous biomes with a lower micro-landscape complexity and more homogeneous vegetation cover.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2021.107796.

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