



## Pyric herbivory increases soil microbial diversity but has a site-dependent effect on soil mesofauna in the mid-term

Leticia San Emeterio <sup>a,\*</sup>, Enrique Baquero <sup>b,d</sup>, Rodrigo Antón <sup>c,f</sup>, Rafael Jordana <sup>d</sup>, Leire Múgica <sup>a</sup>, José L. Sáez <sup>e</sup>, Iñigo Virto <sup>c</sup>, Rosa M. Canals <sup>a</sup>

<sup>a</sup> Institute for Innovation and Sustainable Development in Food Chain (IS-FOOD), Department of Agricultural Engineering, Biotechnology and Food, Public University of Navarre (UPNA), Campus de Arrosadia, 31006 Pamplona, Spain

<sup>b</sup> University of Navarra, Biodiversity and Environment Institute BIOMA, Irunlarrea 1, 31080 Pamplona, Spain

<sup>c</sup> Institute for Innovation and Sustainable Development in Food Chain (IS-FOOD), Department of Science, Public University of Navarre (UPNA), Campus de Arrosadia, 31006 Pamplona, Spain

<sup>d</sup> University of Navarra, Faculty of Sciences, Department of Environmental Biology, University Campus, 31008 Pamplona, Spain

<sup>e</sup> Instituto Navarro de Tecnologías e Infraestructuras Agroalimentarias-INTIA, Edificio de Peritos, 31610 Villava, Spain

<sup>f</sup> INRAE, Info&Sols, Avenue de la Pomme de Pin, 45075, Orléans, France

### ARTICLE INFO

#### Keywords:

Prescribed burnings  
Horse targeted grazing  
DNA metabarcoding  
Temperate mountain grasslands  
Soil compaction

### ABSTRACT

Woody plant encroachment threatens grassland biomes at a global scale. Pyric herbivory combines prescribed burnings and targeted grazing to restore open habitats and has proved to be successful in promoting landscape and plant community diversity. However, less is known on the effects of pyric herbivory practices on below-ground biodiversity. We evaluated the midterm effect on mesofauna, bacteria and fungi of prescribed burns and targeted horse grazing regimes implemented to restore a grassland encroached by gorse (*Ulex gallii* Planch.). We hypothesized that 1) low-intensity shrub-to shrub burnings had no effect or had a transient effect of low magnitude on soil microbial diversity, and that 2) targeted horse grazing after burning increased soil mesofauna and microbial diversity in the midterm. We established an experiment in two shrub-encroached grasslands in western Pyrenees with three treatments (no burning and no grazing as control, burning but no grazing, and burning and grazing). We measured soil properties and soil diversity of fungi and bacteria (DNA-metabarcoding) just after fire, and vegetation structure, soil properties and soil diversity of fungi, bacteria and mesofauna after two periods of targeted grazing (a year and a half after the burning). The response to pyric herbivory differed among soil organisms. Fungi were more sensitive to burning than bacteria, but both recovered a year and a half after burning –fungi only recovered in the presence of grazing–. Grazing increased soil fungi and bacteria diversity indexes (~20 % and ~5 % increase, respectively) at the two sites. A year and a half after burning, burned and ungrazed areas had a 30 % more mesofauna diversity than control areas whereas grazing of the burned areas decreased mesofauna diversity by 30 % at one of the sites compared to the control. Since the responses to pyric herbivory vary among soil organisms, a wide range of management intensities across space and time are recommended for maximizing soil biodiversity.

### 1. Introduction

Woody plant encroachment threatens grassland biomes at a global scale (Archer et al., 2017; Sala and Maestre, 2014) with woody-cover increases per decade around 10–20 % in North America, 11 % in South America, 1.1 % in Australia, 2.5 % in Africa and 1.3 % in Southern Europe (Barger et al., 2011; Fuchs et al., 2013; Stevens et al., 2017;

Venter et al., 2018). Despite different reasons are behind this global process (Archer et al., 2017; García et al., 2020; Venter et al., 2018), the maintenance of a disturbance regime of fire and grazing (natural or anthropogenic) that prevents biomass accumulation is known to be crucial for many open biomes (Pausas and Bond, 2019).

In western Pyrenees, land abandonment and the decline in pastoral activities in the last decades has favored woody plant encroachment and

\* Correspondence to: Institute for Innovation and Sustainable Development in Food Chain (IS-FOOD), Department of Science, Public University of Navarre (UPNA), Campus de Arrosadia, 31006 Pamplona, Spain.

E-mail address: [leticia.sanemeterio@unavarra.es](mailto:leticia.sanemeterio@unavarra.es) (L. San Emeterio).

<https://doi.org/10.1016/j.agee.2023.108632>

Received 27 January 2023; Received in revised form 1 June 2023; Accepted 8 June 2023

Available online 16 June 2023

0167-8809/© 2023 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

mountain grasslands have experienced profound changes (Canals, 2019). In central Pyrenees, 24 % of sparse grasslands (vegetation cover <50 %) and 19 % of dense grasslands (grass cover >50 %) became shrublands between the mid-1980 s and mid-2000 s (Gartzia et al., 2014). Preserving these grasslands and the ecosystem services that supply requires the adoption of sustainable ecosystem practices that allow the maintenance and restoration of open habitats and mosaic landscapes.

Pyric herbivory –grazing driven by fire– combines prescribed burnings and targeted grazing to maintain open habitats and increase landscape heterogeneity (Fuhlendorf et al., 2009). This management, focused on enhancing resources heterogeneity, relies on the coupling of fire and grazing and emulates an historical disturbance regime. Pyric herbivory creates a dynamic mosaic of patches (new burned, burned with regrowth and unburned) that are grazed at different intensities due to selective preference of livestock for recently re-growth vegetation (Wilcox et al., 2022). Pyric herbivory has been proven to be a suitable restoration management practice, capable of creating resilient landscapes that promote biodiversity and decrease the risk of extreme wildfire events while maintaining an economic activity around the livestock farming (Bowman et al., 2016; Fuhlendorf et al., 2017; Wilcox et al., 2022). Since heterogeneity prompts biodiversity due to the differences in habitat selection of different species –from recently disturbed to less disturbed habitats (Christensen, 1997; Fuhlendorf et al., 2006)–, pyric herbivory increases biodiversity of vegetation (Mcgranahan et al., 2012; Múgica et al., 2021), small mammals (Fuhlendorf et al., 2010; Ricketts and Sandercock, 2016), birds (Hovick et al., 2015) and aboveground invertebrates (Doxon et al., 2011). However, much less it is known on the effects of pyric herbivory on soil belowground biodiversity.

Fire and grazing affect soil biodiversity by multiple direct (i.e., thermal shock, soil compaction) and indirect pathways (i.e., changes in composition and structure of vegetation and changes in soil spatial heterogeneity). Direct effects depend on fire severity (Barreiro and Díaz-Raviña, 2021; Dooley and Treseder, 2012), grazing intensity (Wang and Tang, 2019) and grazer species (Wang et al., 2019) and indirect effects may be modulated by the prevailing environment (Adkins et al., 2020). Both disturbances eliminate plant biomass (by combustion or consumption) and add nutrients into the soil through ashes, excreta or grazing-induced root exudates and C through the last two (Hamilton et al., 2008). Soil physical properties can also be affected since fire can increase or decrease soil water repellence depending on the temperature reached during fire (DeBano, 2000) and grazing increases soil bulk density, and decreases porosity and water infiltration by trampling and treading (Drewry et al., 2008).

Fire and grazing effects on soil biodiversity differ among taxa. In low-intensity fires, only the topsoil is affected by high temperatures, thus organisms' resistance will depend on its ability to avoid the thermal shock by moving deeper into the soil or through a fire-resistant spore stage. Thermal shock has been proved to have more significant negative effects on soil microbiota (<100  $\mu\text{m}$ ) than in meso (80  $\mu\text{m}$ –2 mm, e.g. collembola, acari) and macro-biota (500  $\mu\text{m}$ –50 mm, e.g. earthworms, termites) (Alcañiz et al., 2018; Swift et al., 1979), and on fungi more than on bacteria, being the latter more resistant to fire and recovering faster after it (Ammitzboll et al., 2022; Pressler et al., 2019). Regarding soil physical changes, compaction hinders the movement of soil organisms (non-burrowing would be more affected than burrowing species, (Larsen et al., 2004)) and diminishes the availability of oxygen through changes in the distribution of soil pores, affecting differently aerobic/anaerobic microbial populations.

The magnitude and final response of soil biodiversity after fire and grazing are difficult to predict because they may trigger different processes with opposite directions, which may affect differently to different taxa. In essence, the combination of both low-intensity fires and grazing can increase soil biodiversity by promoting new microhabitats that offer a variety of niche compartments for species characterized by its low

mobility (Ettema and Wardle, 2002; Sulkava and Huhta, 1998). Fire can increase bacterial diversity through increasing microhabitat heterogeneity of burned soils with distinct patches of pyrogenic organic matter (Zhang et al., 2021). Grazing increases the spatial heterogeneity of bulk density and water content through trampling (Stavi et al., 2008), creates hotspots of nutrient accumulation and soil  $\text{N}_2\text{O}$  fluxes via deposition of faeces and urine (Cowan et al., 2015) and changes in the spatial heterogeneity of litter cover and soil pH through selective defoliation of plant species (Eldridge et al., 2020).

Although fire and grazing are intimately linked, their effects have been usually studied separately so, our objective was to evaluate the effect on diversity of soil mesofauna, bacteria and fungi of a prescribed burn and a targeted horse grazing regime two years after burning. The pyric herbivory practice was implemented to restore an open dominant-grassland community in an area becoming encroached by the gorse (*Ulex gallii* Planch.). Our hypotheses were: 1) low-intensity shrub-to shrub burnings have no effect or have a transient effect of low magnitude on soil microbial diversity because of the short increase of temperatures reached during fire, and 2) targeted horse grazing after burning increases soil mesofauna and microbial diversity because of the priming effect of C and N inputs and the increase in heterogeneity of resources and micro-habitats.

## 2. Material and methods

### 2.1. Study site

The study site is located in Roncesvalles (Fig. 1), at the western side of the Special Area of Conservation (SAC) Roncesvalles-Selva de Irati (Natura 2000 network site code ES0000126), in the SW Pyrenees (43°1'N 1°19'W). The climate is humid temperate (Cfb) according to Köppen climate classification, with snowy winters and cold and misty summers. The mean annual temperature and precipitation are 9.3 °C and 1638 mm, respectively, according to data collected during 1972–2021 at the nearest climatic station, Auritzberri-Espinal (872 m asl), located ca. 5 km from the sampling plots (Gobierno de Navarra, 2020). Soils, developed from shales, are loamy, organic (~20 % of soil organic matter), acidic (~4) and with high cation exchange capacity (~23  $\text{cmol}(+) \text{kg}^{-1}$ ). According to the USDA, soils are classified as Humic Dystrudept and Typic Humudept. The vegetation is a mosaic of beech forests, shrubland communities dominated by gorse (*Ulex gallii* Planch.) and accompanied by heath species such as *Calluna vulgaris* (L.) Hull. and *Erica vagans* L., and grassland communities dominated by perennial grasses such as *Festuca rubra* gr., *Agrostis capillaris* L., *Agrostis curtisii* Kerguelen., forbs such as *Galium saxatile* L. and *Potentilla erecta* (L.) Ræusch and a small proportion of legumes such as *Trifolium repens* L.

In the last decades, extensive stock farming has sharply decline in the area. Livestock grazing abandonment has favored shrub encroachment and gorse expansion in particular. Gorse is a legume-shrub that develops tall and dense covers that are very flammable and with a high calorific power (Marino et al., 2011). Regional government promotes and financially supports prescribed burns of these shrublands with two objectives: 1) restoration purposes by promoting plant diversity and the entry of new species and 2) fuel control to minimize the risk of wildfires that can expand to nearby beech forests. Dense gorse lands are usually burned by professional firefighters according to a burning plan (Múgica et al., 2018), whereas areas with a lower gorse cover are burned by shepherds and rangers using the traditional practice of shrub-to-shrub, in which single shrubs are burned while the nearby herbaceous vegetation remains unburned (San Emeterio et al., 2016).

### 2.2. Experimental design

In order to evaluate the effect of pyric herbivory we used the demonstrative pilot experience set up in the Open2preserve project (SOE2/P5/E0804, <https://open2preserve.eu/>). In 2018 we established

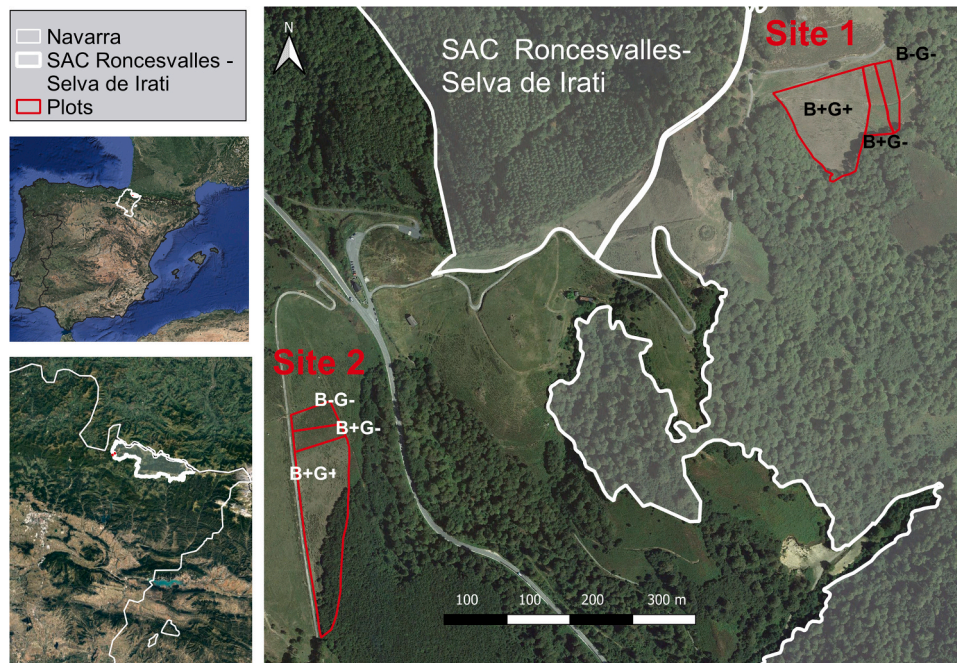


Fig. 1. Study area location map. B- no burning; B+ burning; G- no grazing; G+ grazing. Site 1: Erdiko bizkar, Site 2: Girizu.

the experiment in two sites in which we implemented three treatments: no burning and no grazing as control (B-G-), burning and no grazing (B+G-) and burning and grazing (B+G+) (Fig. 1). The characteristics of each site are shown in Table 1. Both sites have similar altitudes and soils but differ in aspect, slope, initial vegetation and previous management. Site 1 has a southern aspect with slopes over 40 % and in the last decades it has been practically abandoned being burned in 2014 and lightly grazed only in 2018 by a small herd of mares. Site 2 has an eastern aspect with slopes below 40 % and in the last decades shrub encroachment has been reduced through a prescribed burning in 1998 and a mechanical clearing in 2006, combined with an extensive horse and sheep grazing.

Fine ecological monitoring of restoration measures is often logistically complicated, particularly in the cases of grazing and fire in which large areas are needed and which implementation is highly expensive in terms of financial and human effort. For this reason, fire and grazing were not replicated within each site in this experience and the resulting data were analysed according to the suggestions of Davies and Gray (2015) and Oksanen (2001) 1) which are: 1) minimize potential confounding effects (see below), 2) when possible, examine the magnitude of differences between the treatment and the control areas before and after the experiment, 3) use inferential statistics and let the reader judge the reliability of the descriptive statistics and 4) clearly state the statistical inferences that can be drawn from data sets and do not overinterpret. In detail, we followed these approaches:

- 1) To avoid pre-existent spatial differences before the treatment application (burning and/or grazing), treatment plots within each site had similar soil and treatments were applied parallel to the slope line in order to avoid slope effects (upper vs bottom).
- 2) Due to the lack of data, we cannot compare soil diversity of mesofauna, fungi and bacteria before and after burning, but we can compare soil fungi and bacteria diversity before and after grazing.
- 3) and 4) To make inference of both effects (immediate burning and grazing after burning) we considered sampling point within plots as replicates for burning and grazing for two main reasons. First, soil biota shows patchy distributions at the scale of centimetres to metres within topographically and textural uniform soil surfaces. These patches are structured depending on plant size, type of organism and the scale of the study. At plot scale, these patches can range, from 1 to

3 in forests to 5 cm in arable soil (Ettema and Wardle, 2002). Therefore, the minimum distance between sampling points (> 5 m) suggested that the samples were probably independent. Second, we expect that burning and grazing impacts on soil diversity occur mainly at small spatial scales (Loucougaray et al., 2004). However, to avoid overinterpretation, when we do not have data before and after treatment (before and after burning for soil microbial and mesofauna diversity and before and after grazing for soil mesofauna diversity) we compare the responses of soil diversity between treatments, and we discuss how likely the observed differences in soil diversity are caused by the treatment (burning and/or grazing). When we have data before and after the treatment (microbial diversity before and after grazing) we assume that the differences are caused by the treatment.

### 2.3. Prescribed burnings

Regional legislation requires a burning permission that depends on the risk of the controlled burn. Burnings at the two sites were classified at the lowest risk level (1 over a 3-risk category) which meant that the burnings could be performed by local farmers and forest rangers according to a previous established burning plan. However, professional firefighters helped in the burning because of their experimental and demonstrative goal. At each site, a 2 m-strip of land was cleared of vegetation to avoid the unwanted burning of the control plot. Burnings at the two sites were performed on March 28th, 2019. At each site, three sets of four k thermocouples were set up to monitor the temperature profile of the burnings: flame (35–45 cm above soil level), soil surface, 1 cm-soil depth and 5 cm-soil depth. Temperatures were recorded by three data loggers (4 Channel Thermocouple Data Logger HH-521BT, Thermosense) from minutes before the burning until at least one hour after the passing of the flames. Soils were between 9 and 10 °C at the beginning. The burning at site 2 reached higher temperatures and spread faster than the burning at site 1 (Table 2). At the two sites the result of the burning was a mosaic of burned patches (shrubs) and practically unburned grass, but at site 1 the burned patches were bigger due to the greater shrub cover than site 2. At the two sites, the soil temperature at 1 cm and 5 cm-depth did not considerably increase since the maximum temperature reached was 19.2 °C.

**Table 1**

Site characteristics, soils, initial vegetation and previous management. Soil parameters are represented by the mean  $\pm$  standard error (n = 3).

	Site 1	Site 2
Name place	Erdiko Bizkar	Girizu
Area (ha)	2.55	3.86
Altitude (m asl)	1100	1070
Aspect	S	E
Slope (%)	43–50	34
<b>Soils (0–10 cm)</b>		
Texture (%)		
Coarse sand (2–0.2 mm)	15.15 $\pm$ 1.47	15.62 $\pm$ 1.91
Medium sand (0.2–0.1 mm)	3.96 $\pm$ 0.21	4.32 $\pm$ 0.48
Fine sand (0.1–0.05 mm)	8.17 $\pm$ 0.32	7.44 $\pm$ 0.43
Coarse silt (0.05–0.02 mm)	15.23 $\pm$ 1.91	10.17 $\pm$ 1.17
Fine silt (0.02–0.002 mm)	32.12 $\pm$ 1.61	38.50 $\pm$ 2.14
Clay (< 0.002 mm)	25.37 $\pm$ 0.40	27.92 $\pm$ 1.25
pH	4.16 $\pm$ 0.12	4.11 $\pm$ 0.09
CEC (mmol <sup>(+)</sup> kg <sup>-1</sup> )	23 $\pm$ 2	23 $\pm$ 2
SOM (%)	18 $\pm$ 2	23 $\pm$ 1
C/N	15 $\pm$ 1	14 $\pm$ 0.3
Total N (%)	0.72 $\pm$ 0.07	0.94 $\pm$ 0.04
Available P (P <sub>2</sub> O <sub>5</sub> ) (mg·kg <sup>-1</sup> )	14 $\pm$ 3	18 $\pm$ 1
Available K (K <sub>2</sub> O) (mg·kg <sup>-1</sup> )	181 $\pm$ 30	192 $\pm$ 28
USDA classification	Humic dystrodept	Typic humidept
<b>Vegetation</b>		
Initial cover (%)		
Shrubs	43	27
Grasses	39	47
Forbs	18	25
Species richness	25	32
<b>Previous management</b>		
Shrub clearing	Prescribed burning in 2014 (Múgica et al., 2018)	Prescribed burning in 1998 Mechanical clearing 2006
Grazing Previous year (2018)		
Grazer	Mares	Mares and sheep
Grazing period	3 months (starting late August)	7.5 months (starting April)
Peak stocking rate 2000–2017	16.8 livestock units Small herd of horses, sporadically	64.3 livestock units Summer grazing by a sheep herd and, since 2014, a small herd of horses

CEC, cation exchange capacity; SOM, soil organic matter.

**Table 2**

Burning characteristics at the two sites.

	Site 1	Site 2
<b>Burning</b>		
*Temperatures (°C)		
Flame	709–200	784–393
Soil surface	246–65	693–124
1 cm depth	19.2–10.9	5.6–6.2
**Residence time (min)	2–9	1
Area (Ha)	1.95	2.5
Duration (h)	1.5	1

\*Range of maximum reached temperatures at three points, \*\*Total time above 50 °C on soil surface.

## 2.4. Targeted horse grazing

We chose mares of the autochthonous breed Burguete for the targeted experimental grazing. Horse grazing has been successfully used as a management tool for reducing shrub encroachment in Atlantic regions (González-Hernández et al., 2020). In the two sites, the grazing area was fenced and mobile drinking troughs were installed. In 2019, the grazing period started 65 days after burning and lasted 139 days, from May 31st till October 17th. In 2020, the grazing period lasted 121 days, from May 4th till September 2nd. During the grazing period, five mares rotated between the two sites depending on mare nutritional state (evaluated through body condition), grass availability and shrub regrowth. Over the two years as a whole, site 1 was grazed more days than site 2 (Table 3). The mares firstly grazed the herbaceous vegetation and then shift to consume shrubs when the availability of the grassy component decreased. The mares were moved to the other site when the forage offer was too low or when the body condition score was below 2.5 (over 5). This management was complemented with specific external forage inputs (food baits) in the form of lucerne pellets, placed in strategic sites of the pasture to promote a more homogeneous use of the area.

## 2.5. Establishment of permanent plots and vegetation monitoring

To evaluate the effect of the targeted grazing on the control of shrub regrowth, 32 permanent plots (1 m<sup>2</sup>) were distributed across each site, 8 plots in the B-G- treatment in the two sites, 4 plots in the B+G- treatment in site 2, 8 plots in the B+G- treatment in site 1, 20 plots in the B+G+ treatment in site 2 and 16 plots in B+G+ in site 1. Cover of shrubs, grasses and forbs and bare ground was monitored in the 1 m<sup>2</sup> plots at two times 1) in November 2019 after the first period of grazing, nine months after burning and 2) in November 2020 after the second period of grazing, one year and nine months after burning. The percentage cover (vertical projection) for each plant group was visually estimated by two experimented observers. Here, we present the results of the last monitoring.

## 2.6. Soil sampling and analysis

### 2.6.1. Soil parameters

To evaluate the effects of targeted grazing after burning on soil parameters, 32 soil samples were collected next to the vegetation permanent plots. Samples were taken with an intact soil core sampler and the sample was divided in two depths 0–3 and 3–10 cm. Subsamples of ~5 g were collected from thoroughly mixed 0–3 cm samples, and immediately frozen at –80 °C for microbial diversity analysis. Soil samples were collected three times, in March 2019 just after burning, in October 2019 after one period of grazing and 9 months after burning, and in November 2020 after the second period of grazing and 21 months after burning. Here, we present the results of the first and the last sampling. The soil parameters were analysed as described by San Emeterio et al. (2021). Briefly, the main chemical parameters were determined in air-dried samples by standard methods in a certified laboratory (Nasertic, Pamplona, Spain): SOM by oxidation with chromate in the presence of sulfuric acid and total N by the Dumas method. In fresh samples we measured soil water content by gravimetry, pH in 1:2.5 water extracts, Olsen available P and ammonium and nitrate contents in KCl 2 M extracts with an AA3 segmented flow analyser (Braun+Luebbe, Norderstedt, Germany). Microbial biomass C and N (MBC and MBN, respectively) contents were determined by chloroform fumigation-direct extraction (Davidson et al., 1989), assuming a fumigation efficiency of 0.54 (K<sub>N</sub> and K<sub>C</sub>) (Joergensen et al., 2011). Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) contents were measured and calculated as described by San Emeterio et al. (2014). Total amino acid contents were determined following the spectrofluorometric method presented by Jones et al. (2002) and modified by Darrouzet-Nardi et al. (2013). Soil penetration resistance measurements were

**Table 3**  
Grazing rotation between sites and days of grazing.

	First rotation			Second rotation			Third rotation			Total grazing days
	Starting date	Final date	Days	Starting date	Final date	Days	Starting date	Final date	Days	
<b>Site 1</b>										
2019	June 16th	July 18th	32	August 17th	September 10th	24	October 7th	October 31st	24	80
2020	May 4th	May 21st	17	June 9th	July 4th	25	July 21st	August 11th	21	63
<b>Site 2</b>										
2019	May 31st	June 16th	16	July 18th	August 17th	30	September 10th	October 7th	27	73
2020	May 21st	June 9th	19	July 4th	July 22nd	18	August 11th	August 27th	16	53

conducted in the field to characterize soil compaction in each site. Measurements were carried out with a RIMIK CP401 digital cone penetrometer (Rimik Pty., Ltd., Toowoomba, Australia) along a sampling grid defined for this purpose of 13 × 13 m for site 1 and 10 × 10 m for site 2. The maximum depth studied was 60 cm and the penetrometer was set to register the resistance to penetration (MPa) at 1.5 cm intervals.

### 2.6.2. Soil mesofauna

To study the soil mesofauna diversity, we followed the method proposed by the LIFE-SOIL MONTANA project (Martín et al., 2013). For each site, and in November 2020 coinciding with the soil sampling, we took 6 soil samples in the no burning no grazing plot (B-G-), 6 samples in the burning no grazing plot (B+G-) and 18 samples in the burning and grazing plot (B+G+). At each sampling point, we collected a cylindrical soil sample of 10 cm diameter and 5 cm deep and split it into two depths 0–2 and 2–4 cm. We extracted the animals using a Berlese-Tullgreen method consisting of a 2 mm metal mesh on a funnel to support the soil cylinder, a 50 W incandescent lamp at 20 cm to emit light and heat and drive out the organisms, and a small container containing ethyl alcohol (70 %) under the funnel to collect them. We observed the organisms under a stereomicroscope and classified them into mesofauna groups (Protura, Diplura, Collembola, Microcoryphia, Zygentoma, Dermaptera, Orthoptera, Embioptera, Blattaria, Psocoptera, Hemiptera, Thysanoptera, Coleoptera, Hymenoptera, Diptera, Holometabolous, Acari, Araneae, Opiliones, Palpigradi, Pseudoscorpiones, Isopoda, Chilopoda, Diplopoda, Pauropoda, Symphyla). We counted the individuals of each group. This extraction method may have a bias since some mesofauna groups are less efficiently extracted. For this reason, we focused on the most abundant groups and better indicators such as Collembola and Acari.

### 2.6.3. Microbial diversity (fungi and bacteria)

To evaluate the immediate effects of burning on microbial diversity (fungi and bacteria) we used soil subsamples (0–3 cm) from the first soil monitoring (immediately after burning), five from the burned area of each site and another five from the unburned area (n = 20). To evaluate the effects of targeted horse grazing after burning we used soil subsamples from the last soil monitoring (after two periods of grazing, 21 months after burning), four from each treatment of each site (n = 24). Soil samples were kept at – 80 °C until further analyses.

Soil DNA was isolated using the DNeasy PowerSoil Pro DNA isolation kit (QIAGEN) from 280 mg of soil eluted in 50 µL. qPCR analyses and DNA metabarcoding were carried out by AllGenetics & Biology SL (www.allgenetics.eu). Fungal and bacterial DNA detection and quantification were performed using a quantitative PCR (qPCR) assay. For fungi, we targeted an ITS genomic region of around 300 bp using primers ITS86F (5' GTG AAT CAT CGA ATC TTT GAA 3') and ITS4 (5' TCC TCC GCT TAT TGA TAT GC 3'), described by Turenne et al. (1999) and White et al. (1990), respectively. For bacteria, we targeted a 16 S genomic region with a size of 290 pb using primers 515 F (5' GTG YCA GCM GCC GCG GTA A 3') (Parada et al., 2016) and 806 R (5' GGA CTA CNV GGG TWT CTA AT 3') (Apprill et al., 2015). Details on the qPCR protocol can be found in Box1, Supplementary material.

For fungi and bacteria library preparation we amplified the same genomic regions using the same primers as describe for qPCR. These primers included the Illumina sequencing primer sequences attached to their 5' ends. The taxonomy was assigned using a pre-trained classifier of the UNITE reference database for fungi (Abarenkov et al., 2020) (updated on May 2021) and a pre-trained classifier of the SILVA reference database for bacteria (Quast et al., 2013) release 138.1 August 2020). Details on the full metabarcoding pipeline (DNA extraction, amplification, sequencing and bioinformatic processing) are described in Box2, Supplementary material.

### 2.6.4. Statistical analyses

All statistical analyses and data visualizations were performed using R (R Core Team, 2022).

Effects on soil properties were analysed using Canonical Correspondence analysis (CCA). We used soil properties as the response variables and site, sampling depth and burning as the explanatory variables for evaluating the immediate effects of burning (B-, B+) and using management (B-G-, B+G-, B+G+) instead of burning for evaluating the effects of grazing after burning. The significance of each explanatory variable was evaluated using Monte Carlo permutation tests with the permutations restricted within sites (n = 999). We used bootstrapping to estimate confidence intervals (at 97.5 %) of the variance of each soil parameter.

Grazing effects after burning on soil compaction were evaluated using general least square models (GLS) with resistance to penetration as the response variable and site, depth, management and their interactions as factors. A spatial correlation term was included when the model with a spatial correlation term was more parsimonious (smaller AIC). Semi-variograms were adjusted to the most used models (nugget, spherical and exponential). We estimated values of the variables at non-sampled points by 'ordinary kriging' interpolation using the semi-variograms (Cressie, 1993). We subjected the models to leave-one-out cross-validation and used the root mean square error (RMSE) as a measure of precision. We selected the models with the lowest RMSE and we generated map contours with them. We used the nlme package (Pinheiro et al., 2022) for adjusting the GLS models and the gstat R package (Gräler et al., 2016) for calculating and adjusting the semi-variograms, the kriging, and contour maps.

Grazing effects after burning on vegetation structure were evaluated using GLS models with shrubs, herbaceous (grasses, graminoids and forbs) and bare ground cover (in percentage), as response variables and site, management (B-G-, B+G-, B+G+) and their interaction as factors. A spatial correlation term was included when needed. We used bootstrapping to estimate confidence intervals (95 %) of the variance.

Effects on OTUs richness of soil fungi and bacteria, diversity (Shannon and Simpson indexes) and number of ITS and 16 S copies (fungi and bacteria qPCR), were analysed using two-way ANOVA with site, burning and their interaction as factors for the immediate effects of burning, and using management instead of burning for the effects of grazing after burning. For evaluating the effects of grazing we used the difference between the second sampling (after grazing) and the first sampling (before grazing). For burned soils, we subtracted the mean value of burned soils in the first sampling to the values of the second sampling

and for unburned soils we subtracted the mean value of unburned soils in the first sampling to the values in the second sampling.

Effects on soil fungi and bacteria composition were evaluated using distance based (Bray Curtis) Redundancy Analysis (db-RDA). We used OTUs reads as the response variables and site, burning and soil properties (pH, SWC, total C, total N, DOC, DON, DTN, nitrate, ammonium, available P and total amino acids) as the explanatory variables for analysing immediate burning effects and site, management, soil properties and vegetation structure (shrubs, herbaceous and bare soil cover) as explanatory variables for analysing grazing effects after burning. We used the ordiR2step function of the vegan package (Oksanen et al., 2022) to select the explanatory variables to be included the final model. Variables were included if the new variable was significant and the adjusted  $R^2$  of the model with the new variable did not exceed the adjusted  $R^2$  of the global model. We evaluated the significance of each explanatory variable included in the final model using Monte Carlo permutation tests with the permutations restricted within sites ( $n = 999$ ). We evaluated the importance of the drivers of fungi and bacteria composition (site, management, soil and vegetation) using variation partitioning analysis (varpart function of the vegan package).

Effects on soil mesofauna diversity (Shannon and Simpson indexes) were evaluated using three-way ANOVA with site, sampling depth, management and their interactions as factors, and type III sum of squares for unbalanced designs. Effects on soil mesofauna composition were analysed using transformed based Redundancy Analysis (tb-RDA). We used Hellinger-transformed data of the composition of soil mesofauna as the response variables and site, sampling depth and management as the explanatory variables. We selected the explanatory variables and tested their significance following the procedure previously described.

### 3. Results

#### 3.1. Immediate response of soil properties to prescribed burnings

The first axis of the CCA explained  $\sim 14\%$  of the variance and separated deep soils on the left from topsoils on the right (Fig. 2). The second axis explained  $\sim 7\%$  of the variance and separated soils from site 1 on top and site 2 at the bottom. Neither first CCA axis nor second CCA axis separated burned from unburned soils.

The variance of mineral N, DON and DOC was greater in burned than unburned soils, whereas the variance of SWC, pH, P, total amino acids, and MBN was greater in unburned than burned soils (Fig. S1,

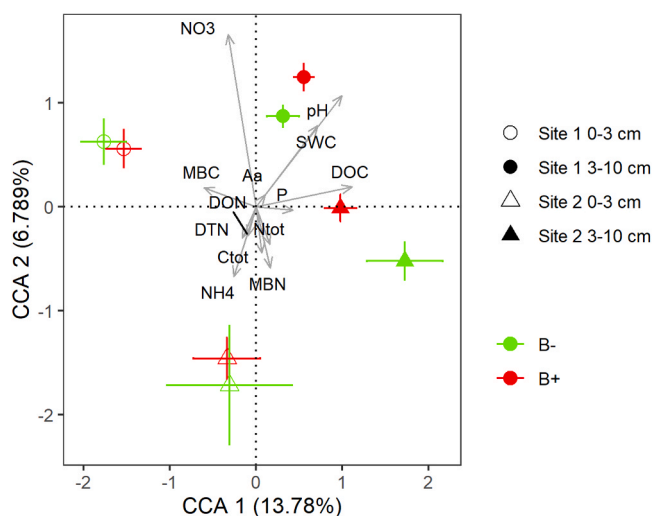


Fig. 2. Canonical correspondence analysis (CCA) of soil properties of burned and unburned soils. Symbols represent mean  $\pm$  SEM of 'sites' loadings (soil samples) grouped by site and sampling depth.

Supplementary material).

#### 3.2. Immediate response of abundance, diversity and composition of soil microbial populations (fungi and bacteria) to prescribed burnings

Soil fungal richness was similar in burned and unburned plots in both sites ( $F = 2.0$ ,  $p = 0.180$ ) but fungal diversity showed different responses to prescribed burns among sites (Fig. 3). In site 1 the burned plot presented less Shannon ( $-18\%$ ) and Simpson ( $-7\%$ ) diversity indexes than the unburned control (Site  $\times$  Burning  $F = 8.5$ ,  $p = 0.010$  and  $F = 8.9$ ,  $p = 0.009$ , respectively) but in site 2 Shannon and Simpson diversity indexes were similar between treatments. More fungal abundance (number of ITS copies) was detected in site 2 compared to site 1 ( $F = 5.9$ ,  $p = 0.027$ ). Fungi richness was similar in burned and unburned plots in both sites ( $F = 2.0$ ,  $p = 0.180$ ). On the contrary, soil bacteria diversity showed similar responses to prescribed burns at the two sites (Fig. 3). Burned plots showed less abundance ( $-1\%$ ,  $F = 3.1$ ,  $p = 0.071$ ), less richness ( $-6\%$ ,  $F = 4.3$ ,  $p = 0.030$ ) and lower Shannon diversity indexes ( $-2\%$ ,  $F = 3.5$ ,  $p = 0.053$ ) than their respective unburned controls.

The final db-RDA model for soil fungi and bacteria composition after burning included site ( $F = 2.2$ ,  $p < 0.004$ ,  $F = 2.6$ ,  $p = 0.002$ , respectively) and MBN ( $F = 1.3$ ,  $p = 0.134$ ,  $F = 0.9$ ,  $p = 0.479$ , respectively) as constrained variables (Fig. 4). For both, fungi and bacteria, the first db-RDA axis explained  $\sim 12\%$  of the variance and separated soil microbial composition by sites with site 1 on the left side (more abundance of Archaeorhizomycetes) and site 2 on the right (more abundance of Claviceptaceae and more MBN) (Fig. 4).

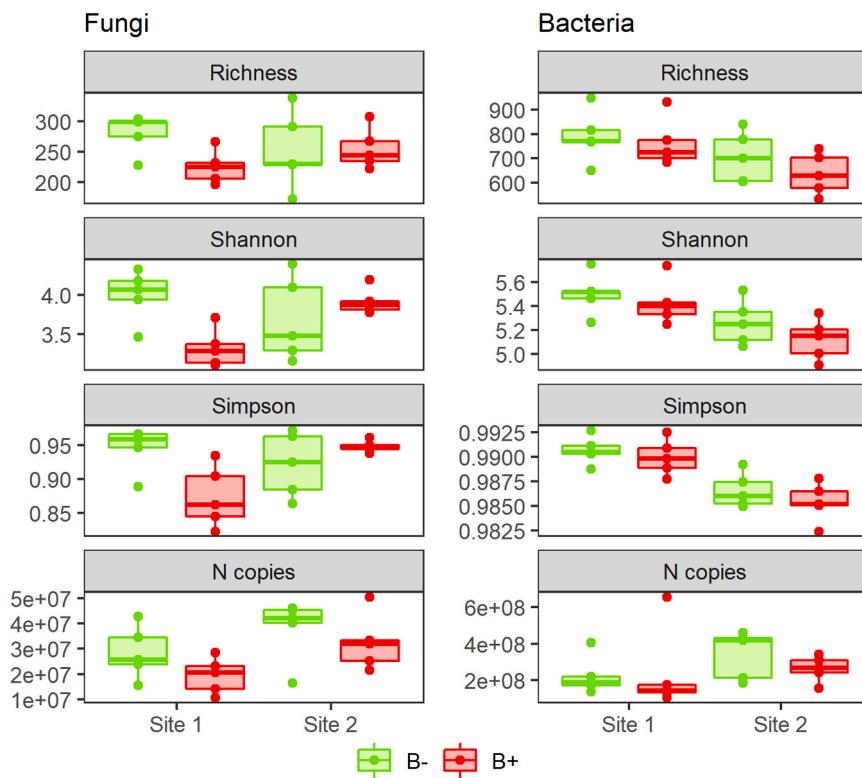
#### 3.3. Response of soil properties and vegetation structure to the pyric herbivory

The first axis of the CCA explained  $\sim 28\%$  of the variance and separated grazed soils with higher cover of bare ground and nitrate content, on the right, from ungrazed soils with higher cover of shrubs, on the left (Fig. 5). The second axis explained  $\sim 8\%$  of the variance and separated deep soil samples (3–10 cm) from topsoil samples (0–3 cm) at the bottom.

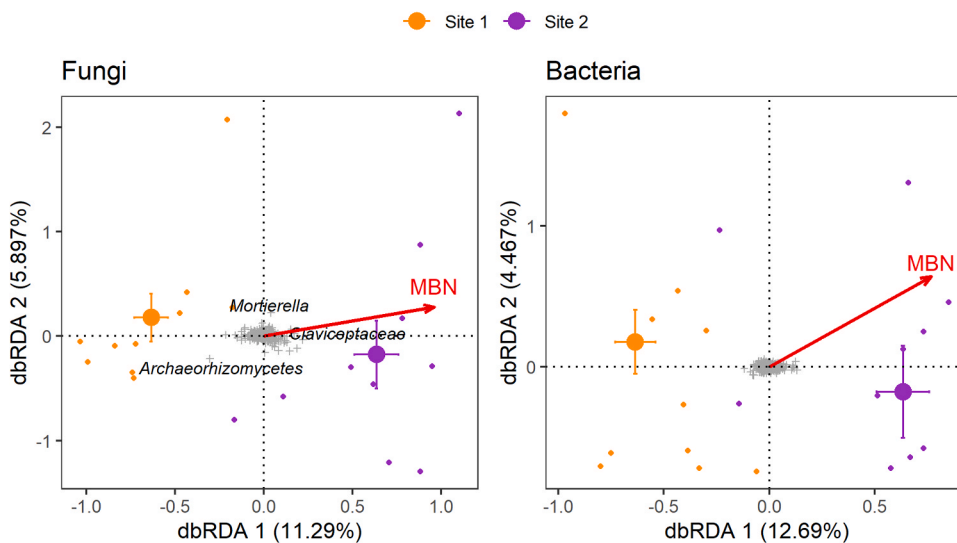
Soil compaction was similar between grazed and ungrazed plots ( $F = 0.12$ ,  $p = 0.884$ ), but it was different between sites ( $F = 94.24$ ,  $p < 0.001$ ) and depths ( $F = 79.94$ ,  $p < 0.001$ ) (Fig. 6). Soils from site 2 were more compacted (at 6 cm depth,  $1338 \pm 37$  kPa  $\text{cm}^{-1}$ ; at 30 cm depth,  $1600 \pm 28$ ) than soils from site 1 (at 6 cm depth,  $1074 \pm 25$  (mean  $\pm$  SEM); at 30 cm depth,  $1317 \pm 23$ ). At the scale of the study, semi-variograms showed a moderate spatial structure in site 1 and no spatial structure in site 2 (Fig. S2, Supplementary material) and contour maps reflected the differences in spatial heterogeneity of soil compaction between sites (Fig. S2, Supplementary material).

Cover of shrubs was higher in ungrazed than grazed plots at both sites ( $F = 19.9$ ,  $p < 0.001$ ) and did not differ between sites ( $F = 1.8$ ,  $p = 0.183$ , Fig. 6). Cover of grasses and forbs did not differ between management treatments ( $F = 0.8$ ,  $p = 0.432$ ) and was higher at site 2 than at site 1 ( $F = 5.8$ ,  $p = 0.019$ , Fig. 6). The differences between management treatments on cover of bare ground was site dependent ( $F_{\text{treatment} \times \text{site}} = 5.6$ ,  $p = 0.006$ ). At both sites, cover of bare ground was higher at grazed than ungrazed plots but on site 1 increased from  $\sim 5\%$  to  $\sim 35\%$  and at site 2 increased from  $\sim 0\%$  to  $\sim 13\%$ .

Regarding the effect of pyric herbivory on the variance of soil properties and vegetation structure, grazed plots presented high variance in pH, soil mineral N content, and bare ground cover whereas unmanaged plots presented high variance in soil microbial abundance (Fig. S3, Supplementary material).



**Fig. 3.** Responses of richness, diversity and abundance of soil microbial populations (fungi and bacteria) to prescribed burnings. N copy (ITS for fungi, 16 S for bacteria), B- no burning, B+ burning.



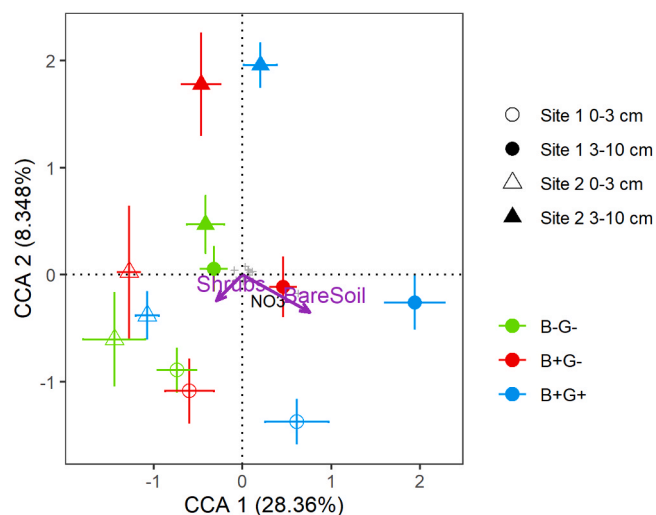
**Fig. 4.** Distance based (Bray-Curtis) Redundancy Analysis (db-RDA) of soil fungi (left) and bacteria (right) composition immediately after burning constrained by site and soil variables (MBN, Microbial biomass nitrogen). Small symbols represent soil samples and big symbols represent mean  $\pm$  SEM of soil samples grouped by sites. Red arrows represent vectors of soil constraining variables. Gray crosses represent OTUs scores. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**3.4. Response of abundance, diversity and composition of soil mesofauna and soil microbial populations (fungi and bacteria) to the pyric herbivory**

In Fig. 7, we disentangle potential differences on richness, diversity and abundance of soil fungi and bacteria due to different sampling seasons from differences due to management. Seasonal differences –difference between the two sample dates in control soils (no burned and no grazed) – depended on site and type of microorganism. At site 1, richness and diversity of fungi in control soils was similar at both dates but richness and diversity of bacteria was lower in fall than in the previous early spring. Fungi abundance in control soils was greater in fall than in the previous early spring and bacteria abundance was similar at

both dates (Fig. 7). At site 2, fungi richness, diversity and abundance in control soils were lower in fall than in the previous early spring whereas bacteria richness and diversity remained similar, and abundance was lower in fall than in the previous early spring (Fig. 7).

Pyric herbivory showed significant effects on the richness and diversity of fungi and bacteria (for fungi,  $F = 22.3$ ,  $p < 0.001$ ,  $F = 14.3$ ,  $p < 0.001$ ; for bacteria,  $F = 6.6$ ,  $p = 0.007$ ;  $F = 6.5$ ,  $p = 0.007$ ,  $F = 5.1$ ,  $p = 0.017$ , for richness, and Shannon and Simpson diversity, respectively) but no effects on fungi and bacteria abundance (for fungi  $F = 0.1$ ,  $p = 0.892$ ; for bacteria  $F = 1.2$ ,  $p = 0.335$ ; Table S1, Supplementary material). At both sites the differences in richness and diversity of fungi and bacteria were always bigger in grazed than

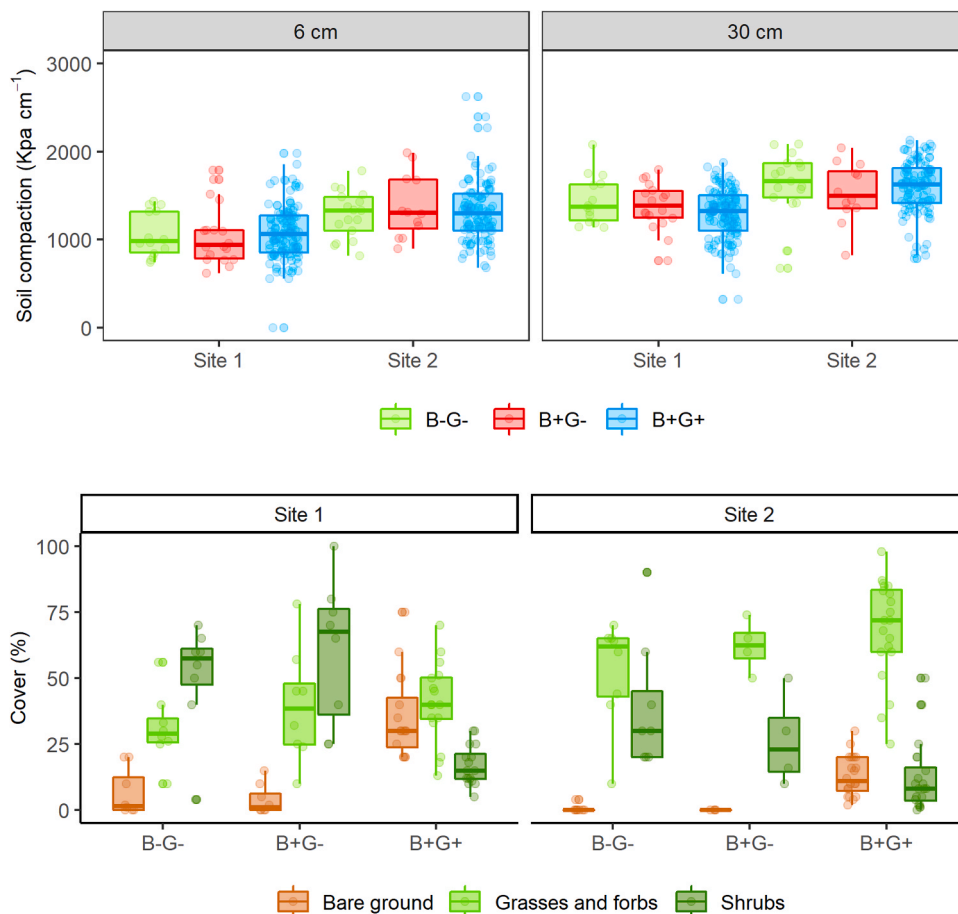


**Fig. 5.** Canonical correspondence analysis of soil properties constrained by site, sampling depth, management, and shrubs and bare ground cover. Symbols represent mean  $\pm$  SEM of soil sample loadings grouped by site, sampling depth and management. Purple arrows represent vectors of vegetation constraining variables. Gray crosses represent ‘species’ scores (soil properties). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

ungrazed soils. Therefore, grazing clearly increased richness and diversity of bacteria and fungi (Fig. 7). When there were no differences between sampling dates in control soils, the differences after grazing were positive, and when the difference between sampling dates in control soils were negative, there were no differences after grazing or the differences were positive. For example, at site 1 fungi richness remained similar in control soils but increased in  $\sim 80$  OTUs after grazing whereas bacteria richness decreased in  $\sim 100$  OTUs in control soils but increased in  $\sim 20$  OTUs after grazing (Fig. 7). All this implied that grazed soils showed greater richness and diversity indexes of bacteria and fungi than ungrazed soils (Fig. S4, Supplementary material).

The final db-RDA model for soil fungi composition after targeted horse grazing included site ( $F = 2.8, p = 0.005$ ), management ( $F = 2.4, p = 0.003$ ), vegetation (bare soil  $F = 1.1, p = 0.272$ ) and soil (pH  $F = 1.2, p = 0.206$ ) as constrained variables (Fig. 8, left). The first db-RDA axis explained  $\sim 17\%$  of the variance and separated soil fungi composition by management. On the left, burned and grazed soils were characterized by less acidic pH, more bare soil cover and more abundance of Auriculariales. On the right, burned and ungrazed soils were characterized by more acidic pH, less bare soil cover and more abundance of Archaeorhizomycetes. The second axis explained around 8% of variation and separated soil fungi composition by site, with site 2 on top and site 1 at bottom.

The final db-RDA model for soil bacteria composition after targeted horse grazing included site ( $F = 3.6, p = 0.001$ ), management ( $F = 1.6, p = 0.018$ ), vegetation (bare ground  $F = 0.9, p = 0.544$ ) and soil (DOC:  $F = 1.7, p = 0.054$ ; C:  $F = 1.7, p = 0.046$  and P:  $F = 1.1, p = 0.340$ ) as constrained variables (Fig. 8, right). The first db-RDA axis explained  $\sim 15\%$  of the variance and separated soil fungi composition by site, with



**Fig. 6.** Responses of soil compaction ( $\text{kpa cm}^{-1}$ ) at two depths (6 and 30 cm) –top panel– and of vegetation structure –bottom panel– to management. B-G-, no burning and no grazing; B+G-, burning and no grazing; B+G+, burning and grazing.



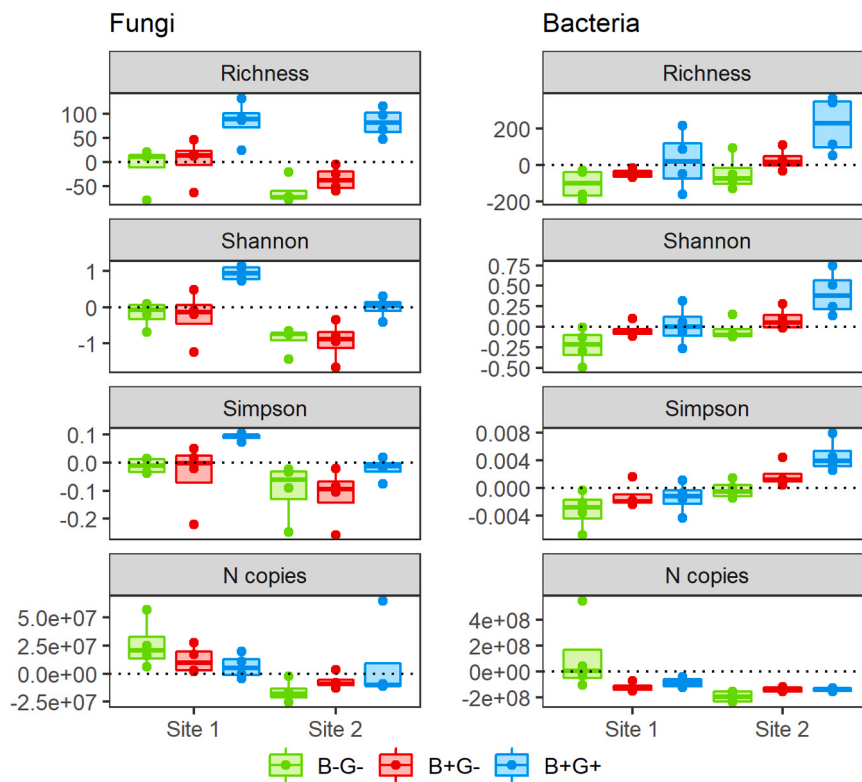


Fig. 7. Differences (November 2020-March 2019) in richness, diversity and abundance of soil microbial populations (Fungi, left; Bacteria, right) in soils with different management. Differences > 0 mean greater values in fall after applying the grazing treatment than in the previous early spring. B-G- no burning and no grazing; B+G- burning and no grazing, B+G+ burning and grazing.

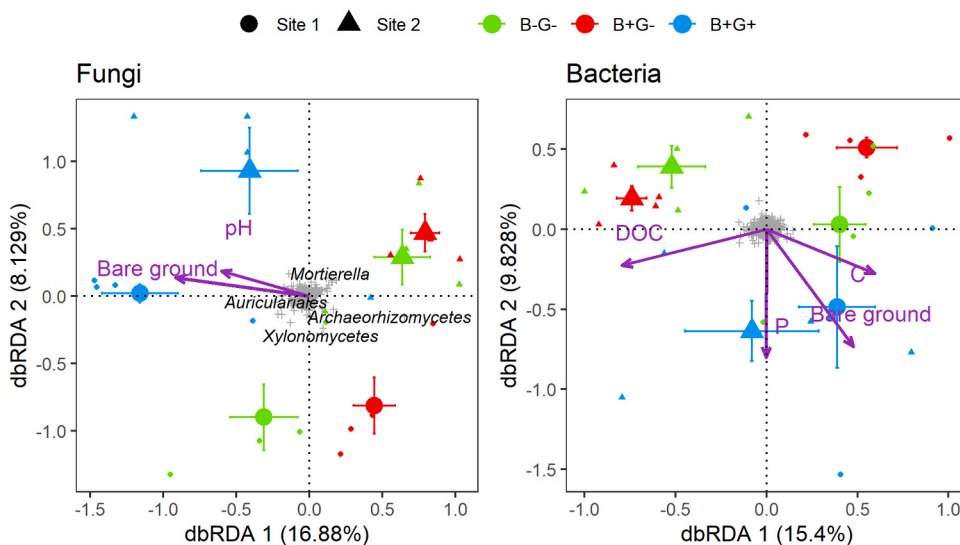


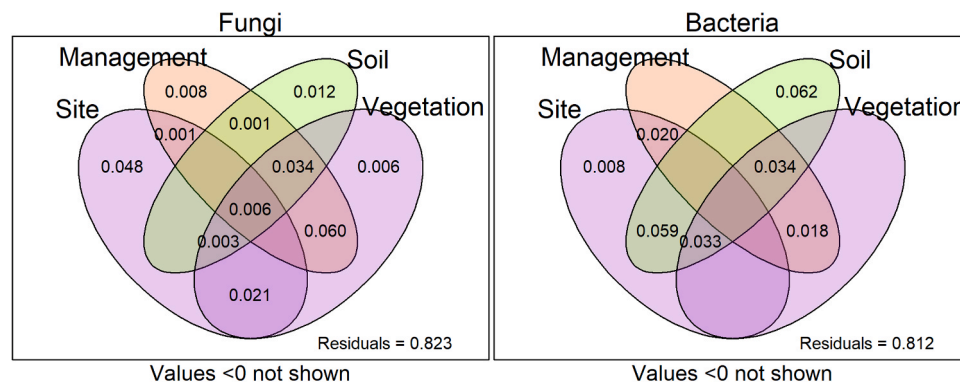
Fig. 8. Distance based (Bray-Curtis) Redundancy Analysis (db-RDA) of fungi (left) and bacteria (right) composition from soils after two periods targeted grazing constrained by site, management, soil (DOC, dissolved organic carbon) and vegetation structure variables. Small symbols represent soil sample loadings and big symbols represent mean  $\pm$  SEM of soil sample loadings grouped by site and management. Purple arrows represent vectors of soil and vegetation constraining variables. Gray crosses represent species scores.

the site 2 on the left with more soil DOC and site 1 on the right with more C content. The second axis explained around 10 % of variation and separated soil bacteria composition by management, at the bottom burned and grazed soils were characterized for having high P and bare ground cover.

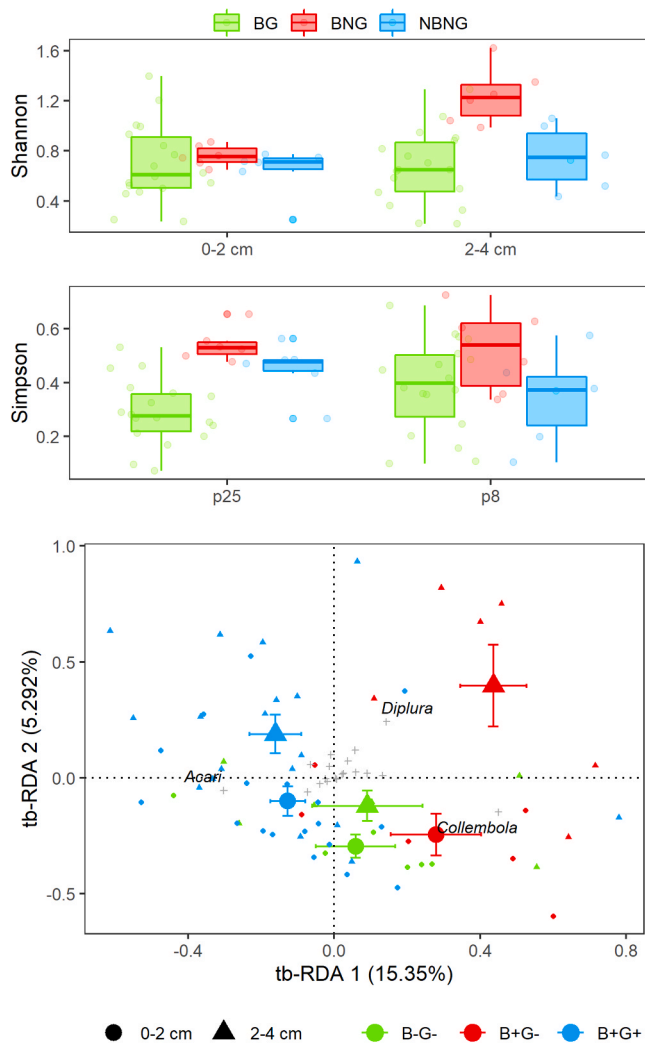
Vegetation explained the largest percentage of variation in fungi composition (13 %). However, most of the variation was shared with management alone (6 %) or with management plus soil (3.4 %) (Fig. 9, left). Soil explained the largest percentage of variation in bacteria composition (18.8 %) but most of the variation was shared with site alone (5.9 %), with site plus vegetation (3.3 %) and with vegetation plus

management (3.4 %) (Fig. 9, right).

Abundances of mesofauna for the two sites can be found in Table S2. The response of Shannon diversity of soil mesofauna to management depended on soil depth ( $F_{\text{Management:Depth}} = 4.3, p = 0.019$ , Table S3). Topsoils with different managements had similar Shannon diversity indexes whereas, in deeper soils (2–4 cm), mesofauna from burned but not grazed plots (B+G-) showed a greater Shannon diversity index than no managed (B-G-) and burned and grazed (B+G+) soils. (Fig. 10). The response of Simpson diversity of soil mesofauna to management depended on site ( $F_{\text{Management:Site}} = 2.7, p = 0.077$ ). Burned and grazed soils showed lower Simpson diversity than ungrazed soils (burned and



**Fig. 9.** Venn diagram of variation partitioning of fungi and bacteria composition. Each area represents the proportion of variation in fungi or bacteria composition by Site, Management, Vegetation and Soil. Vegetation: Bare ground; Soil: pH for fungi and DOC, C and P for bacteria.



**Fig. 10.** Response of soil mesofauna diversity (Shannon and Simpson indexes) to management (top panels). Transformed based Redundancy Analysis (tb-RDA) of mesofauna composition from soils after two periods of targeted horse grazing constrained by management and depth (bottom panel). Small symbols represent soil sample loadings and big symbols represent mean  $\pm$  SEM of soil sample loadings grouped by depth and management. Gray crosses represent species scores. B-G- no burning and no grazing; B+G- burning and no grazing, B+G+ burning and grazing.

unburned) in site 2 (Fig. 10).

The final tb-RDA model for soil mesofauna composition included management ( $F = 6.1, p < 0.001$ ) and depth ( $F = 2.8, p = 0.030$ ). The first tb-RDA axis explained 15.35 % of the variance and separated soil mesofauna composition by management. Mesofauna from burned and grazed soils (on the left) seemed to have different composition (more relative abundance of Acari) than ungrazed soils (on the right) characterized by more relative abundance of Collembola (Fig. 10). The second tb-RDA axis explained 5.3 % of the variance and separated mesofauna by depth with topsoils (0–2 cm) below deeper soils (2–4 cm) (Fig. 10).

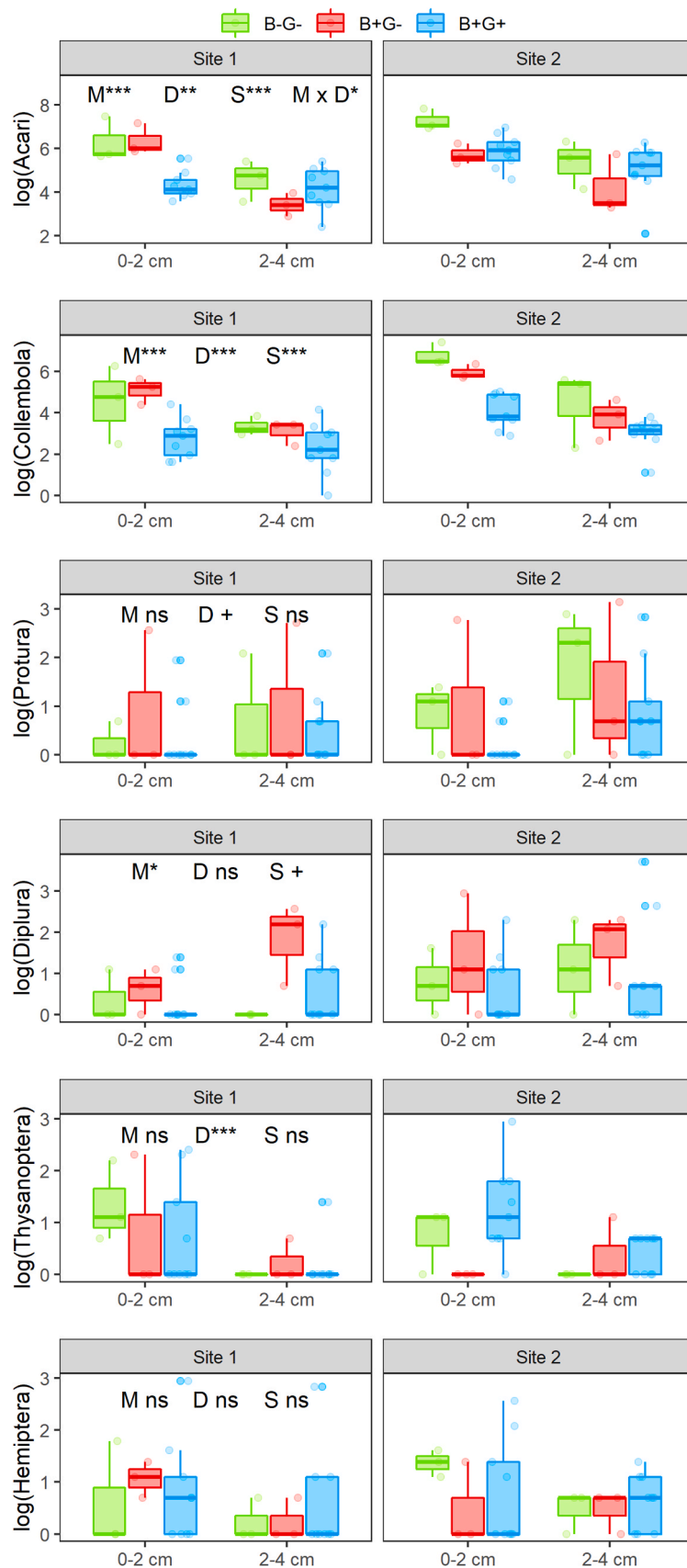
The abundance of different soil mesofauna groups responded differently to management (Fig. 11). The abundance of Collembola ( $F = 15.8, p < 0.001$ ), Diplura ( $F = 3.4, p = 0.041$ ) and Acari (only on topsoil,  $F_{\text{Management} \times \text{Depth}} = 4.9, p = 0.011$ ) was higher in ungrazed (burned or unburned) soils than in grazed soils. The abundance of Protura ( $F = 1.7, p = 0.198$ ), Thysanoptera ( $F = 0.8, p = 0.470$ ) and Hemiptera ( $F = 0.1, p = 0.869$ ) was similar across soils with different managements.

Soil mesofauna abundance along the soil depth profile also depended on the mesofauna group (Fig. 11). Collembola ( $F = 20.7, p < 0.001$ ), Acari ( $F = 11.3, p < 0.001$ ) and Thysanoptera ( $F = 12.8, p < 0.001$ ) were more abundant on the topsoil whereas Protura tended to be more abundant on the deep soil ( $F = 3.3, p = 0.076$ ). Diplura ( $F = 2.7, p = 0.110$ ) and Hemiptera ( $F = 2.6, p = 0.110$ ) were similarly abundant along the soil profile.

Some mesofauna groups were more abundant on site 2 than on site 1 (Acari  $F = 14.2, p < 0.001$ ; Collembola  $F = 15.9, p < 0.001$ ; Diplura  $F = 3.8, p = 0.058$ ) whereas some of them showed similar abundances in both sites (Protura  $F = 1.4, p = 0.246$ ; Thysanoptera  $F = 0.2, p = 0.626$ ; Hemiptera  $F = 0.2, p = 0.665$ ).

#### 4. Discussion

Andriuzzi and Wall (2017) proposed a conceptual framework for predicting soil biological responses to grazing based on the balance between grazing disturbance (trampling, soil compaction, plant removal) and resource inputs (excreta, compensatory plant growth and increase of root exudation). In our case, two factors complicate this conceptual framework: 1) the existence of a previous burning with its potential negative (immediate thermal shock) and positive effects (increase in heterogeneity and resource inputs once the thermal shock is overcome), and 2) the ecological restoration goal of this pyric herbivory practice. We, indeed, look for a change in the vegetation structure reducing the shrub cover and increasing the grassy component to restore an open habitat. We followed the approach in Fig. 12 to discuss the final effect of the pyric herbivory on the diversity of soil fungi, bacteria and mesofauna linking it to changes in soil chemistry and vegetation structure.



**Fig. 11.** Response of absolute abundances (logarithmic scale of counts) of soil mesofauna groups to management at two soil depths and two sites. B-G- no burning and no grazing; B+G- burning and no grazing, B+G+ burning and grazing. M, management; D, depth, S, Site. \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$ , \*  $p < 0.05$ , +  $p < 0.010$ , ns no significant  $p \geq 0.010$ .

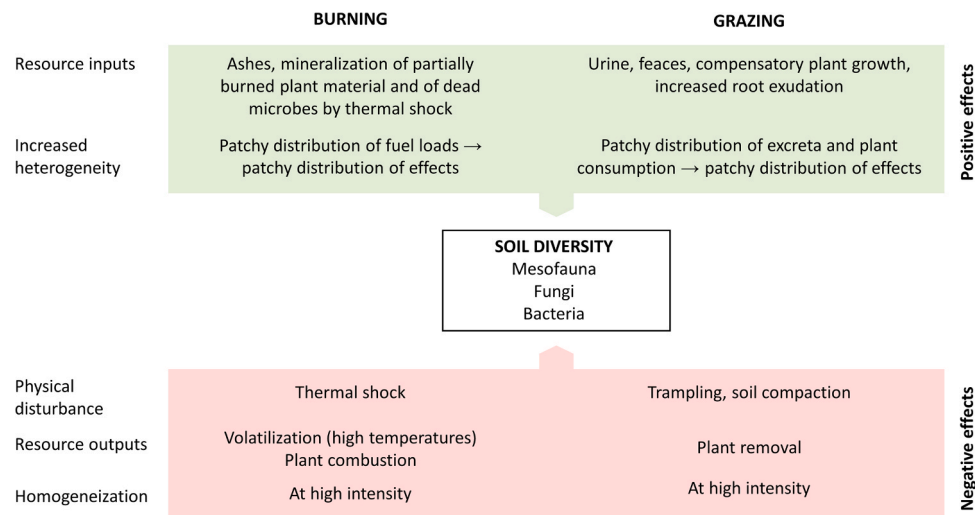


Fig. 12. Conceptual framework of pyric herbivory effects on soil diversity.

Pyric herbivory with targeted horse grazing changed the chemistry of the soil and the structure of the vegetation. Prescribed burnings did not immediately change the availability of soil nutrients but increased the variance of mineral N, DON and DOC in burned soils (Fig. S1, Supplementary material). 18 months after burning and in the absence of grazing, the shrub cover was recovered (Fig. 6). After two periods of targeted horse grazing, grazed plots had more nitrate in soil, less shrub cover and more bare ground cover than ungrazed plots (Fig. 5). Grazing also increased the variance of soil pH and mineral N content and bare ground cover (Fig. S3, Supplementary material).

#### 4.1. Immediate response of biodiversity of soil fungi and bacteria to the prescribed burning

We observed strong differences in fungi diversity (~20 % less Shannon diversity index in burned than in unburned soils) but only slightly differences in bacteria diversity (~2 % less Shannon diversity index in burned than in unburned soils). The most plausible explanation for the differences observed is the thermal shock of burning. Fungi have been reported to be more sensitive than bacteria to the thermal shock (Ammitzbohl et al., 2022; Pressler et al., 2019), and the highest difference was observed at site 1 which endured the greatest flame residence time (9 min vs 1 min), indicating that the topsoil experienced temperatures above 50 °C during more time. Eventually, we did not find differences in fungi or bacterial community composition between burned and unburned soils (Fig. 5), which suggested that differences in fungi and bacteria diversity were more likely explained by the effect of prescribed burnings than by potential pre-existent spatial differences.

#### 4.2. Two-year effect of pyric herbivory on soil compaction

After two periods of horse targeted grazing, shrub encroachment is reduced without significantly affecting soil compaction (Fig. 6). Horses rotate between sites, therefore the grazing pressure in the same area is not constant permitting resting periods in which herbaceous vegetation can recover. However, site 2 has more compacted soils than site 1 (Fig. 6) probably due to legacy effects of a more intensive grazing by sheep and cattle in the last two decades (Drewry, 2006).

#### 4.3. Two-year effect of pyric herbivory on biodiversity and composition of soil fungi and bacteria

A year and a half after the burnings, bacteria richness and diversity was similar in ungrazed burned and unburned soils and fungi richness

and diversity remained lower in ungrazed burned soils at the site with the most intense burning compared to unburned soils (Fig. S2, Supplementary material). However, after two years of grazing, richness and diversity of fungi and bacteria increased (Fig. 7), equalling or exceeding the diversity and richness of ungrazed soils (Fig. S2, Supplementary material). After the thermal shock, several factors can favor a fast recovery of microbial diversity. Fire-resistant microbial populations may enhance their activity thanks to new available resources released from dead fire-sensitive soil microbes and mineralization of burned and partially-burned plant material (Barreiro and Díaz-Raviña, 2021), and a greater heterogeneity in the availability of resources may promote diversity of microhabitats and thus, microbial diversity (Ettema and Wardle, 2002). Burning increased the variance of resources for fungi and bacteria such as mineral N, DON and DOC (Fig. S1, Supplementary material) and grazing increased the variance of resources (mineral N) and drivers such as pH and the percentage of bare ground cover (Fig. S3, Supplementary material). As we hypothesized, pyric herbivory increased fungi and bacteria diversity coinciding with the enhanced heterogeneity of resources and niches.

Bacterial and fungal composition were different in grazed and ungrazed soils (Fig. 10) and the factors that explained most of the variation in OTUs composition varied in each case. Changes in vegetation structure due to the management explained most of the variation in fungal composition (6 %, Fig. 9) whereas soil properties in the different sites explained most of the variation in bacterial composition (5.9 %, Fig. 9). Seaton et al. (2022) in a study of 57 temperate pastures, Nielsen et al. (2010) in moorlands and birch-forests, and Vermeire et al. (2021) in African savannas also found that bacteria composition is better explained by soil chemistry whereas fungal composition is better explained by management and vegetation structure and composition. Urbanová et al. (2015) found similar results in forests. Tree identity explained most of the variation in soil fungal composition whereas the tree effect on bacterial composition was partially mediated by pH. Furthermore, fungal taxa (~35 %) were more tree-specific than bacterial taxa (~3 %). Proteins and polysaccharides exuded by roots and fungi bond mineral particles together, can limit microbe dispersion and can create specific chemical niches explaining the presence or absence of determined microbial taxa (Ljadó et al., 2018). The proportion of explained variation in our study was low compared with other studies. Huhe et al. (2017) studied the effect of a strong gradient of grazing intensities (from 0 to 8.67 sheep ha<sup>-1</sup>) on fungi and bacteria composition and founded an unexplained variance of ~50 % for fungi and bacteria. Yu et al. (2021) studied a gradient of degradation in temperate grasslands (from ~90–30 % of plant cover) and founded an unexplained

variance of 30 % for fungi and 15 % for bacteria. Firstly, other unaccounted factors in our study, such as microclimatic or microtopographic conditions, can influence soil diversity. And secondly, the previously cited studies with higher proportion of explained variance evaluated gradients with stronger contrasts than our study.

#### 4.4. Two-year effect of pyric herbivory on biodiversity and composition of soil mesofauna

Differences on soil mesofauna diversity among management treatments were site and depth dependent. In ungrazed soils at 2–4 cm depth, soil mesofauna diversity was consistently greater in burned soils, compared to unburned (Fig. 10). This increase of diversity at 2–4 cm can be explained by indirect effects of burning. The burning produces distinct patches of diverse pyrogenic organic matter (Zhang et al., 2021) which release nutrients that percolate deeper into the soil. Sulkava and Huhta (1998) showed that increasing the spatial heterogeneity of different resources increased soil microarthropod diversity. Regarding grazing, the effect on the diversity of soil mesofauna was inconsistent among sites and burned and grazed areas displayed a similar (site 1) or a lower diversity than ungrazed and unburned soils (site 2).

Grazing may negatively affect soil mesofauna through trampling, soil compaction and plant removal (Andriuzzi and Wall, 2017). In our case, soil compaction did not seem to play a determinant role in the decrease of soil mesofauna diversity. We did not find differences in soil compaction among grazed and ungrazed areas (Fig. 6) and although site 2 showed more compacted soils than site 1, both sites had similar soil mesofauna diversity indexes (Fig. 10). The soil compaction values that we observed were relatively low compared to values from other studies in Atlantic mountain grasslands (Aldezabal et al., 2015). At 10 cm depth, we found soil compaction mean values of  $1.1 \pm 0.3$  Mpa in site 1 and  $1.4 \pm 0.3$  Mpa in site 2 whereas Aldezabal et al. (2015) in grazed plots found values 3 times greater than our values ( $3.31 \pm 0.86$  Mpa) and in plots with 5 years of grazing exclusion found similar values ( $1.28 \pm 0.21$  Mpa).

Besides potential effects on soil compaction, trampling of grazers may increase the bare ground cover, and this effect has been observed in the grazed plots (Fig. 6). Disappearance of the vegetation cover, even in very small areas, may cause the loss of the topsoil organic horizon and the destruction of the microhabitat for soil mesofauna. At the topsoil of site 2, the grazed plot had 23 % less Shannon and 38 % less Simpson mesofauna diversity than the ungrazed plot and differences decreased in depth (grazed plot 18 % less Shannon and 30 % less Simpson mesofauna diversity; Fig. 10). At site 1 we did not find differences in soil mesofauna diversity between grazed and control plots (Fig. 10). The different response between sites may be explained by legacy effects and the different structure of the vegetation at the beginning of the experiment. Despite its current misuse, site 2 had a longer history of optimal grazing than site 1 (Table 1) and it had developed a more continuous grassy cover than site 1, without bare ground spots. Therefore, soil mesofauna community from site 2 could not have responded as well as mesofauna from site 1 to a new mosaic of shrubs, grasses and bare ground patches characterizing site 2.

Two years of pyric herbivory changed the composition of soil mesofauna and affected differently to different soil mesofauna groups. Grazed plots had greater relative abundance of Acari and less relative abundance of Collembola than ungrazed plots (Fig. 10), although both groups had less absolute abundance in grazed than ungrazed soils, particularly on the topsoil (Fig. 11). Acari and Collembola are the most abundant groups of mesofauna (George et al., 2017). Acari have a wide range of diet preferences (Oribatids are consumers of organic matter and fungi and Mesotigmatids are mobile predators of other mesofauna) (Gulvik, 2007) and prefer moorlands and heathlands with highly organic and moist soils (Black et al., 2003) whereas Collembola consume fungi, bacteria, actinomycetes, algae or plant detritus (Rusek, 1998) and prefer grasslands and deciduous woodlands (Black et al., 2003). Again,

the differences in Acari and Collembola abundance seems to be more linked to changes in microhabitat –increase in bare ground cover after grazing– than to changes in food resources since grazing did not affect the fungi abundance (Fig. 7) or the soil organic matter on the topsoil ( $F = 1.6$ ,  $p = 0.201$ , data not shown). Nielsen et al. (2010) also found that Oribatids respond more to changes in vegetation than to soil properties. Despite the results, the differences in Collembola and Acari abundances between grazed and ungrazed plots are likely to be transient since site 2 with a longer legacy history of grazing had more abundance of Collembola and Acari than site 1. Furthermore, targeted horse grazing is usually planned for the first 2–3 years after burning to decrease the shrub cover and then, a mixed grazing with sheep, cattle and horses is advised to consolidate the open grassland.

#### 4.5. Final remarks and management recommendations for maximizing soil biodiversity

Different soil organisms responded differently to burning and grazing. Fungi were more sensitive to burning than bacteria, but both recovered a year and a half after it –fungi enhanced by the presence of grazers–. Grazing had a positive effect on soil fungi and bacteria diversity. Burning was positive to mesofauna diversity in the midterm and grazing displayed effects that were site dependent, negative or null, which we related to the occurrence of bare soil and the legacy history of the site.

According to these results, we propose some management recommendations for maximizing soil biodiversity in the restoration of temperate mountain grasslands by means of pyric herbivory practices. It is convenient to plan a gradient across space and time of different management intensities to ensure the existence of a wide range of soil organisms that according to their ecology respond differently to the different intensities of burning and grazing. For planning the management gradient consider 1) that mesofauna and fungi diversity are more sensitive to changes in vegetation and microhabitat and bacteria diversity is more sensitive to changes in soil chemistry, 2) that potential legacy effects from previous management can lead to site dependent responses, and 3) that fire and grazing provide heterogeneity of resource availability on space and time. Finally, our results indicate the importance of monitoring key variables to avoid irrecoverable disturbance effects, such as soil compaction and bare ground cover for grazing and flame residence time and soil temperature profile for burning.

#### Funding

This work was supported by the European Regional Development Fund through the Interreg Sudoe Programme [grant number SOE2/P5/E0804] and the Spanish Ministry of Science [grant number PID2020-116786RB-C31]. Founding sources were not involved in the conduct of the research or the preparation of the article.

#### CRedit authorship contribution statement

**Leticia San Emeterio:** Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing – original draft. **Enrique Baquero:** Methodology, Investigation, Data curation, Writing – review & editing. **Rodrigo Antón:** Methodology, Investigation, Data curation, Writing – review & editing. **Rafael Jordana:** Methodology, Investigation, Data curation, Writing – review & editing. **Leire Múgica:** Methodology, Investigation, Data curation, Writing – review & editing. **Jose Luis Sáez:** Methodology, Investigation, Resources, Writing – review & editing. **Íñigo Virto:** Methodology, Investigation, Data curation, Writing – review & editing. **Rosa Maria Canals:** Conceptualization, Investigation, Writing – review & editing, Supervising, Project administration, Funding acquisition.

## 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.

## Data availability

Data will be made available on request.

## Acknowledgements

The authors want to thank La Real Colegiata de Navarra for allowing the establishment of the experiment in their land; to Jone Lizarza, Luis Echeverría, Iñaki and Beñat at INTIA for his help with the establishment of the experiment, to Amaia Ibarrola and María Durán at UPNA for his help with sampling and laboratory analyses; to Lorena, Mikel, Ainara, Paula, Cusi and Martín for his help during field work; to Asier Ganboa and Telmo Rebolé for his help as Interreg Volunteers; to Javier Peralta at UPNA for his help identifying plant specimens; and finally, to Mikel and Marta soil scientists from the Government of Navarre for his help classifying the soils. Open access funding provided by Universidad Pública de Navarra.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.agee.2023.108632](https://doi.org/10.1016/j.agee.2023.108632).

## References

- Adkins, J., Docherty, K.M., Gutknecht, J.L.M., Miesel, J.R., 2020. How do soil microbial communities respond to fire in the intermediate term? Investigating direct and indirect effects associated with fire occurrence and burn severity. *Sci. Total Environ.* 745, 140957 <https://doi.org/10.1016/j.scitotenv.2020.140957>.
- Alcañiz, M., Outeiro, L., Francos, M., Úbeda, X., 2018. Effects of prescribed fires on soil properties: a review. *Sci. Total Environ.* 613–614, 944–957. <https://doi.org/10.1016/j.scitotenv.2017.09.144>.
- Aldezabal, A., Moragues, L., Odrizola, I., Mijangos, I., 2015. Impact of grazing abandonment on plant and soil microbial communities in an Atlantic mountain grassland. *Appl. Soil Ecol.* 96, 251–260. <https://doi.org/10.1016/j.apsoil.2015.08.013>.
- Ammitzboll, H., Jordan, G.J., Baker, S.C., Freeman, J., Bissett, A., 2022. Contrasting successional responses of soil bacteria and fungi to post-logging burn severity. *Ecol. Manag.* 508, 120059 <https://doi.org/10.1016/j.foreco.2022.120059>.
- Andriuzzi, W.S., Wall, D.H., 2017. Responses of belowground communities to large aboveground herbivores: meta-analysis reveals biome-dependent patterns and critical research gaps. *Glob. Chang. Biol.* 23, 3857–3868. <https://doi.org/10.1111/gcb.13675>.
- Apprill, A., McNally, S., Parsons, R., Weber, L., 2015. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat. Microb. Ecol.* 75, 129–137. <https://doi.org/10.3354/ame01753>.
- Archer, S.R., Andersen, E.M., Predick, K.L., Schwinning, S., Steidl, R.J., Woods, S.R., 2017. Woody plant encroachment: causes and consequences. In: Briske, D.D. (Ed.), *Rangeland Systems: Processes, Management and Challenges*. Springer International Publishing, Cham, pp. 25–84. [https://doi.org/10.1007/978-3-319-46709-2\\_2](https://doi.org/10.1007/978-3-319-46709-2_2).
- Barger, N.N., Archer, S.R., Campbell, J.L., Huang, C., Morton, J.A., Knapp, A.K., 2011. Woody plant proliferation in North American drylands: a synthesis of impacts on ecosystem carbon balance. *J. Geophys. Res. Biogeosci.* 116, G00K07. <https://doi.org/10.1029/2010JG001506>.
- Barreiro, A., Díaz-Raviña, M., 2021. Fire impacts on soil microorganisms: mass, activity, and diversity. *Curr. Opin. Environ. Sci. Health* 22, 100264. <https://doi.org/10.1016/j.coesh.2021.100264>.
- Black, H.I.J., Parekh, N.R., Chaplow, J.S., Monson, F., Watkins, J., Creamer, R., Potter, E. D., Poskitt, J.M., Rowland, P., Ainsworth, G., Hornung, M., 2003. Assessing soil biodiversity across Great Britain: national trends in the occurrence of heterotrophic bacteria and invertebrates in soil. *J. Environ. Manag.* 67, 255–266. [https://doi.org/10.1016/S0301-4797\(02\)00178-0](https://doi.org/10.1016/S0301-4797(02)00178-0).
- Bowman, D.M.J.S., Perry, G.L.W., Higgins, S.I., Johnson, C.N., Fuhlendorf, S.D., Murphy, B.P., 2016. Pyrodiversity is the coupling of biodiversity and fire regimes in food webs. *Philos. Trans. R. Soc. B Biol. Sci.* <https://doi.org/10.1098/rstb.2015.0169>.
- Canals, R.M., 2019. Landscape in motion: revisiting the role of key disturbances in the preservation of mountain ecosystems. *Geogr. Res. Lett.* 45, 515–531. <https://doi.org/10.18172/cig.3634>.
- Christensen, N.L., 1997. Managing for heterogeneity and complexity on dynamic landscapes. In: Pickett, S.T.A., Ostfeld, R.S., Shachak, M., Likens, G.E. (Eds.), *The Ecological Basis of Conservation: Heterogeneity, Ecosystems, and Biodiversity*. Springer, US, Boston, MA, pp. 167–186. [https://doi.org/10.1007/978-1-4615-6003-6\\_17](https://doi.org/10.1007/978-1-4615-6003-6_17).
- Cowan, N.J., Norman, P., Famulari, D., Levy, P.E., Reay, D.S., Skiba, U.M., 2015. Spatial variability and hotspots of soil N<sub>2</sub>O fluxes from intensively grazed grassland. *Biogeosciences* 12, 1585–1596. <https://doi.org/10.5194/bg-12-1585-2015>.
- Cressie, N.A.C., 1993. *Statistics for Spatial Data*. John Wiley & Sons, New York.
- Darrouzet-Nardi, A., Ladd, M.P., Weintraub, M.N., 2013. Fluorescent microplate analysis of amino acids and other primary amines in soils. *Soil Biol. Biochem.* 57, 78–82. <https://doi.org/10.1016/j.soilbio.2012.07.017>.
- Davidson, E.A., Eckert, R.W., Hart, S.C., Firestone, M.K., 1989. Direct extraction of microbial biomass nitrogen from forest and grassland soils of California. *Soil Biol. Biochem.* 21, 773–778. [https://doi.org/10.1016/0038-0717\(89\)90169-7](https://doi.org/10.1016/0038-0717(89)90169-7).
- Davies, G.M., Gray, A., 2015. Don't let spurious accusations of pseudoreplication limit our ability to learn from natural experiments (and other messy kinds of ecological monitoring). *Ecol. Evol.* 5, 5295–5304. <https://doi.org/10.1002/ece3.1782>.
- DeBano, L.F., 2000. The role of fire and soil heating on water repellency in wildland environments: a review. *J. Hydrol.* 231–232, 195–206. [https://doi.org/http://dx.doi.org/10.1016/S0022-1694\(00\)00194-3](https://doi.org/http://dx.doi.org/10.1016/S0022-1694(00)00194-3).
- Dooley, S.R., Treseder, K.K., 2012. The effect of fire on microbial biomass: a meta-analysis of field studies. *Biogeochemistry* 109, 49–61. <https://doi.org/10.1007/s10533-011-9633-8>.
- Doxon, E.D., Davis, C.A., Fuhlendorf, S.D., Winter, S.L., 2011. Aboveground macroinvertebrate diversity and abundance in sand sagebrush prairie managed with the use of pyric herbivory. *Rangel. Ecol. Manag.* 64, 394–403. <https://doi.org/10.2111/REM-D-10-00169.1>.
- Drewry, J.J., 2006. Natural recovery of soil physical properties from treading damage of pastoral soils in New Zealand and Australia: a review. *Agric. Ecosyst. Environ.* 114, 159–169. <https://doi.org/10.1016/j.agee.2005.11.028>.
- Drewry, J.J., Cameron, K.C., Buchan, G.D., 2008. Pasture yield and soil physical property responses to soil compaction from treading and grazing - a review. *Aust. J. Soil Res.* 46, 237–256. <https://doi.org/10.1071/SR07125>.
- Eldridge, D.J., Travers, S.K., Val, J., Wang, J.T., Liu, H., Singh, B.K., Delgado-Baquerizo, M., 2020. Grazing regulates the spatial heterogeneity of soil microbial communities within ecological networks. *Ecosystems* 23, 932–942. <https://doi.org/10.1007/s10021-019-00448-9>.
- Ettema, C.H., Wardle, D.A., 2002. Spatial soil ecology. *Trends Ecol. Evol.* 17, 177–183. [https://doi.org/10.1016/S0169-5347\(02\)02496-5](https://doi.org/10.1016/S0169-5347(02)02496-5).
- Fuchs, R., Herold, M., Verburg, P.H., Clevers, J.G.P.W., 2013. A high-resolution and harmonized model approach for reconstructing and analysing historic land changes in Europe. *Biogeosciences* 10, 1543–1559. <https://doi.org/10.5194/bg-10-1543-2013>.
- Fuhlendorf, S.D., Engle, D.M., Kerby, J., Hamilton, R., 2009. Pyric herbivory: rewinding landscapes through the recoupling of fire and grazing. *Conserv. Biol.* 23, 588–598. <https://doi.org/10.1111/j.1523-1739.2008.01139.x>.
- Fuhlendorf, S.D., Fynn, R.W.S., McGranahan, D.A., Twidwell, D., 2017. Heterogeneity as the basis for rangeland management. In: Briske, D.D. (Ed.), *Rangeland Systems: Processes, Management and Challenges*. Springer International Publishing, Cham, pp. 169–196. [https://doi.org/10.1007/978-3-319-46709-2\\_5](https://doi.org/10.1007/978-3-319-46709-2_5).
- Fuhlendorf, S.D., Harrell, W.C., Engle, D.M., Hamilton, R.G., Davis, C.A., Leslie, D.M., 2006. Should heterogeneity be the basis for conservation? Grassland bird response to fire and grazing. *Ecol. Appl.* 16, 1706–1716. [https://doi.org/10.1890/1051-0761\(2006\)016\[1706:SHBTBF\]2.0.CO;2](https://doi.org/10.1890/1051-0761(2006)016[1706:SHBTBF]2.0.CO;2).
- Fuhlendorf, S.D., Townsend, D.E., Elmore, R.D., Engle, D.M., 2010. Pyric-herbivory to promote rangeland heterogeneity: evidence from small mammal communities. *Rangel. Ecol. Manag.* 63, 670–678. <https://doi.org/10.2111/REM-D-10-00044.1>.
- García, M., Isla, C., Lehmann, C.E.R., Stevens, N., Bjorkman, A.D., 2020. Woody plant encroachment intensifies under climate change across Tundra and Savanna biomes. *Glob. Ecol. Biogeogr.* 29, 925–943. <https://doi.org/10.1111/gcb.13072>.
- Gartzia, M., Alados, C.L., Pérez-Cabello, F., 2014. Assessment of the effects of biophysical and anthropogenic factors on woody plant encroachment in dense and sparse mountain grasslands based on remote sensing data. *Prog. Phys. Geogr.* 38, 201–217. <https://doi.org/10.1177/0309133314524429>.
- George, P.B.L., Keith, A.M., Creer, S., Barret, G.L., Lebron, I., Emmett, B.A., Robinson, D. A., Jones, D.L., 2017. Evaluation of mesofauna communities as soil quality indicators in a national-level monitoring programme. *Soil Biol. Biochem.* 115, 537–546. <https://doi.org/10.1016/j.soilbio.2017.09.022>.
- González-Hernández, M.P., Mouronte, V., Romero, R., Rigueiro-Rodríguez, A., Mosquera-Losada, M.R., 2020. Plant diversity and botanical composition in an Atlantic heather-gorse dominated understorey after horse grazing suspension: comparison of a continuous and rotational management. *Glob. Ecol. Conserv.* 23. <https://doi.org/10.1016/j.gecco.2020.e01134>.
- Gräler, B., Pebesma, E.J., Heuvelink, G., 2016. Spatio-temporal interpolation using gstat. *R. J.* 8, 204–218.
- Gulvik, M.E., 2007. Mites (Acari) as indicators of soil biodiversity and land use monitoring: a review. *Pol. J. Ecol.* 55, 415–440.
- Hamilton, E.W., Frank, D.A., Hinchey, P.M., Murray, T.R., 2008. Defoliation induces root exudation and triggers positive rhizospheric feedbacks in a temperate grassland. *Soil Biol. Biochem.* 40, 2865–2873. <https://doi.org/10.1016/j.soilbio.2008.08.007>.
- Hovick, T.J., Elmore, R.D., Fuhlendorf, S.D., Engle, D.M., Hamilton, R.G., 2015. Spatial heterogeneity increases diversity and stability in grassland bird communities. *Ecol. Appl.* 25, 662–672. <https://doi.org/10.1890/14-1067.1.sm>.
- Huhe, Chen, X., Hou, F., Wu, Y., Cheng, Y., 2017. Bacterial and fungal community structures in Loess Plateau grasslands with different grazing intensities. *Front. Microbiol.* 8, 1–10. <https://doi.org/10.3389/fmicb.2017.00606>.

- Joergensen, R.G., Wu, J., Brookes, P.C., 2011. Measuring soil microbial biomass using an automated procedure. *Soil Biol. Biochem.* 43, 873–876. <https://doi.org/10.1016/j.soilbio.2010.09.024>.
- Jones, D.L., Owen, A.G., Farrar, J.F., 2002. Simple method to enable the high resolution determination of total free amino acids in soil solutions and soil extracts. *Soil Biol. Biochem.* 34, 1893–1902. [https://doi.org/10.1016/S0038-0717\(02\)00203-1](https://doi.org/10.1016/S0038-0717(02)00203-1).
- Larsen, T., Schjønning, P., Axelsen, J., 2004. The impact of soil compaction on euedaphic Collembola. *Appl. Soil Ecol.* 26, 273–281. <https://doi.org/10.1016/j.apsoil.2003.12.006>.
- Lladó, S., López-Mondéjar, R., Baldrian, P., 2018. Drivers of microbial community structure in forest soils. *Appl. Microbiol. Biotechnol.* 102, 4331–4338. <https://doi.org/10.1007/s00253-018-8950-4>.
- Loucougaray, G., Bonis, A., Bouzillé, J.B., 2004. Effects of grazing by horses and/or cattle on the diversity of coastal grasslands in western France. *Biol. Conserv.* 116, 59–71. [https://doi.org/10.1016/S0006-3207\(03\)00177-0](https://doi.org/10.1016/S0006-3207(03)00177-0).
- Marino, E., Guijarro, M., Hernandez, C., Madrigal, J., Díez, C., 2011. Fire hazard after prescribed burning in a gorse shrubland: implications for fuel management. *J. Environ. Manag.* 92, 1003–1011. <https://doi.org/10.1016/j.jenvman.2010.11.006>.
- Gobierno de Navarra, 2020. Ficha climática Irabia. <http://meteo.navarra.es/climatologia/selfichaclima.cfm?IDEstacion=32&tipo=AUTO>. (Accessed 3 June 2020).
- Martín, I., Anza, M., Mijangos, I., Garbisu, C., 2013. Soil health evaluation based on microarthropod communities in pastures of Gorbeia Natural Park. In: Lombardi, G., Mosimann, E., Gorlier, A., Iussig, G., Lonati, M., Pittarello, M., Probo, M. (Eds.), *Proceedings of the Meeting of the FAO-CIHEAM Mountain Pasture Network - Pastoralism and Ecosystem Conservation*. 5–7 June 2013, Trivero, Italy, 196–198.
- Mcgranahan, D.A., Engle, D.M., Fuhlendorf, S.D., Winter, S.J., Miller, J.R., Debinski, D. M., 2012. Spatial heterogeneity across five rangelands managed with pyric herbivory. *J. Appl. Ecol.* 49, 903–910. <https://doi.org/10.1111/j.1365-2664.2012.02168.x>.
- Múgica, L., Canals, R.M., San Emeterio, L., 2018. Changes in soil nitrogen dynamics caused by prescribed fires in dense gorse lands in SW Pyrenees. *Sci. Total Environ.* 639, 175–185. <https://doi.org/10.1016/j.scitotenv.2018.05.139>.
- Múgica, L., Canals, R.M., San Emeterio, L., Peralta, J., 2021. Decoupling of traditional burnings and grazing regimes alters plant diversity and dominant species competition in high-mountain grasslands. *Sci. Total Environ.* 790, 147917 <https://doi.org/10.1016/j.scitotenv.2021.147917>.
- Nielsen, U.N., Osler, G.H.R., Campbell, C.D., Burslem, D.F.R.P., van der Wal, R., 2010. The influence of vegetation type, soil properties and precipitation on the composition of soil mite and microbial communities at the landscape scale. *J. Biogeogr.* 37, 1317–1328. <https://doi.org/10.1111/j.1365-2699.2010.02281.x>.
- Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2022. *vegan: Community Ecology Package*.
- Oksanen, L., 2001. Logic of experiments in ecology: is pseudoreplication a pseudoissue? *Oikos* 94, 27–38. <https://doi.org/10.1034/j.1600-0706.2001.11311.x>.
- Parada, A.E., Needham, D.M., Fuhrman, J.A., 2016. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environ. Microbiol.* 18, 1403–1414. <https://doi.org/10.1111/1462-2920.13023>.
- Pausas, J.G., Bond, W.J., 2019. Alternative biome states in terrestrial ecosystems. *Trends Plant Sci.* 1–14. <https://doi.org/10.1016/j.tplants.2019.11.003>.
- Pinheiro, J., Bates, D., Team, T.R. core, 2022. *nlme: Linear and nonlinear mixed effects models*. R Package Version 3.1–157. <https://doi.org/https://CRAN.R-project.org/package=nlme>.
- Pressler, Y., Moore, J.C., Cotrufo, M.F., 2019. Belowground community responses to fire: meta-analysis reveals contrasting responses of soil microorganisms and mesofauna. *Oikos* 128, 309–327. <https://doi.org/10.1111/oik.05738>.
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 41, D590–D596. <https://doi.org/10.1093/nar/gks1219>.
- R Core Team, 2022. *R: a Language and Environment for Statistical Computing*.
- Ricketts, A.M., Sandercock, B.K., 2016. Patch-burn grazing increases habitat heterogeneity and biodiversity of small mammals in managed rangelands. *Ecosphere* 7, 1–16. <https://doi.org/10.1002/ecs2.1431>.
- Rusek, J., 1998. Biodiversity of Collembola and their functional role in the ecosystem. *Biodivers. Conserv.* 7, 1207–1219. <https://doi.org/10.1023/A:1008887817883>.
- Sala, O.E., Maestre, F.T., 2014. Grass-woodland transitions: determinants and consequences for ecosystem functioning and provisioning of services. *J. Ecol.* 102, 1357–1362. <https://doi.org/10.1111/1365-2745.12326>.
- San Emeterio, L., Canals, R.M., Herman, D.J., 2014. Combined effects of labile and recalcitrant carbon on short-term availability of nitrogen in intensified arable soil. *Eur. J. Soil Sci.* 65, 377–385. <https://doi.org/10.1111/ejss.12133>.
- San Emeterio, L., Durán, M., Múgica, L., Jiménez, J.J., Canals, R.M., 2021. Relating the spatial distribution of a tall-grass to fertility islands in a temperate mountain grassland. *Soil Biol. Biochem.* 163, 108455 <https://doi.org/10.1016/j.soilbio.2021.108455>.
- San Emeterio, L., Múgica, L., Ugarte, M.D., Goicoa, T., Canals, R.M., 2016. Sustainability of traditional pastoral fires in highlands under global change: effects on soil function and nutrient cycling. *Agric. Ecosyst. Environ.* 235, 155–163. <https://doi.org/10.1016/j.agee.2016.10.009>.
- Seaton, F.M., Griffiths, R.I., Goodall, T., Lebron, I., Norton, L.R., 2022. Pasture age impacts soil fungal composition while bacteria respond to soil chemistry. *Agric. Ecosyst. Environ.* 330, 107900 <https://doi.org/10.1016/j.agee.2022.107900>.
- Stavi, I., Ungar, E.D., Lavee, H., Sarah, P., 2008. Grazing-induced spatial variability of soil bulk density and content of moisture, organic carbon and calcium carbonate in a semi-arid rangeland. *Catena* 75, 288–296. <https://doi.org/10.1016/j.catena.2008.07.007>.
- Stevens, N., Lehmann, C.E.R., Murphy, B.P., Durigan, G., 2017. Savanna woody encroachment is widespread across three continents. *Glob. Chang. Biol.* 23, 235–244. <https://doi.org/10.1111/gcb.13409>.
- Sulkava, P., Huhta, V., 1998. Habitat patchiness affects decomposition and faunal diversity: a microcosm experiment on forest floor. *Oecologia* 116, 390–396. <https://doi.org/10.1007/s004420050602>.
- Swift, M.J., Heal, O.W., Anderson, J.M., Anderson, J.M., 1979. *Decomposition in terrestrial ecosystems*. Univ. Calif. Press 5.
- Turenne, C.Y., Sanche, S.E., Hoban, D.J., Karlowsky, J.A., Kabani, A.M., 1999. Rapid identification of fungi by sequencing the ITS1 and ITS2 regions using an automated capillary electrophoresis system. *J. Clin. Microbiol.* 37, 1846–1851. <https://doi.org/10.1128/JCM.37.6.1846-1851.1999>.
- Urbanová, M., Šnajdr, J., Baldrian, P., 2015. Composition of fungal and bacterial communities in forest litter and soil is largely determined by dominant trees. *Soil Biol. Biochem.* 84, 53–64. <https://doi.org/10.1016/j.soilbio.2015.02.011>.
- Venter, Z.S., Cramer, M.D., Hawkins, H.J., 2018. Drivers of woody plant encroachment over Africa. *Nat. Commun.* 9, 1–7. <https://doi.org/10.1038/s41467-018-04616-8>.
- Vermeire, M.L., Thoresen, J., Lennard, K., Vikram, S., Kirkman, K., Swemmer, A.M., Te Beest, M., Siebert, F., Gordijn, P., Venter, Z., Brunel, C., Wolfaard, G., Krumins, J.A., Cramer, M.D., Hawkins, H.J., 2021. Fire and herbivory drive fungal and bacterial communities through distinct above- and belowground mechanisms. *Sci. Total Environ.* 785, 147189 <https://doi.org/10.1016/j.scitotenv.2021.147189>.
- Wang, C., Tang, Y., 2019. A global meta-analysis of the response of multi-taxa diversity to grazing intensity in grasslands. *Environ. Res. Lett.* 14, 114003 <https://doi.org/10.1088/1748-9326/ab4932>.
- Wang, L., Delgado-Baquerizo, M., Wang, D., Isbell, F., Liu, Jun, Feng, C., Liu, Jushan, Zhong, Z., Zhu, H., Yuan, X., Chang, Q., Liu, C., 2019. Diversifying livestock promotes multidiversity and multifunctionality in managed grasslands. *Proc. Natl. Acad. Sci. USA* 116, 6187–6192. <https://doi.org/10.1073/pnas.1807354116>.
- White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, Thomas, J. (Eds.), *PCR Protocols*. Academic Press, San Diego, pp. 315–322. <https://doi.org/10.1016/B978-0-12-372180-8.50042-1>.
- Wilcox, B.P., Fuhlendorf, S.D., Walker, J.W., Twidwell, D., Wu, X., Ben, Goodman, L.E., Treadwell, M., Birt, A., 2022. Saving imperiled grassland biomes by recoupling fire and grazing: a case study from the Great Plains. *Front. Ecol. Environ.* 20, 179–186. <https://doi.org/10.1002/fee.2448>.
- Yu, Y., Zheng, L., Zhou, Y., Sang, W., Zhao, J., Liu, L., Li, C., Xiao, C., 2021. Changes in soil microbial community structure and function following degradation in a temperate grassland. *J. Plant Ecol.* 14, 384–397. <https://doi.org/10.1093/jpe/rtaa102>.
- Zhang, L., Ma, B., Tang, C., Yu, H., Lv, X., Mazza Rodrigues, J.L., Dahlgren, R.A., Xu, J., 2021. Habitat heterogeneity induced by pyrogenic organic matter in wildfire-perturbed soils mediates bacterial community assembly processes. *ISME J.* 15, 1943–1955. <https://doi.org/10.1038/s41396-021-00896-z>.