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Faecal nutrient deposition of domestic and wild herbivores in an alpine grassland

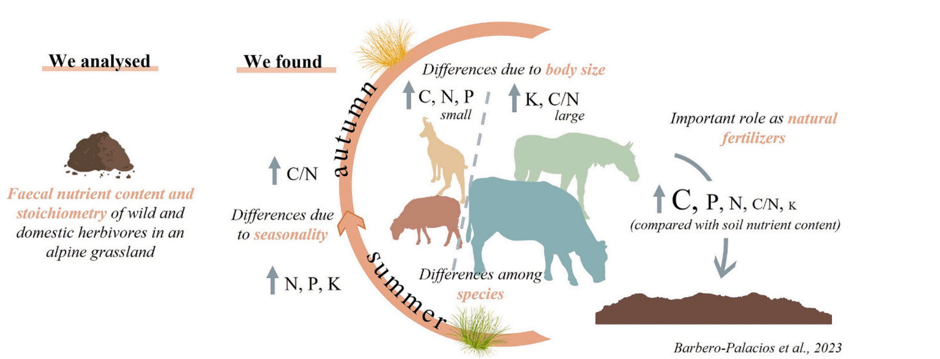
Laura Barbero-Palacios[a,*], Kristy M. Ferraro[b], Isabel C. Barrio[a], Jennifer A. Krumins[c], Jordi Bartolomé[d], Elena Albanell[d], Laia Jarque-Bascuñana[e], Santiago Lavín[e], Juan A. Calleja[f,g], José A. Carreira[h], Emmanuel Serrano[e]

[a] Faculty of Environmental and Forest Sciences, Agricultural University of Iceland, Árleyni 22, Keldnaholt, IS-112 Reykjavík, Iceland
[b] Yale University School of the Environment, 370 Prospect Street, New Haven, CT 06511, USA
[c] Department of Biology, Montclair State University, Montclair, NY, USA
[d] Grup de Recerca en Remugants, Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona (UAB), Bellaterra, Spain
[e] Wildlife Ecology & Health Group (WE&H) and Servei d'Ecopatologia de Fauna Salvatge (SEFaS), Departament de Medicina i Cirurgia Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona (UAB), 08193 Bellaterra, Barcelona, Spain
[f] Departamento de Biología (Botánica), Centro de Investigación en Biodiversidad y Cambio Global (CIBC-UAM), Universidad Autónoma de Madrid, Spain
[g] CREAF, Cerdanyola del Vallès, Spain
[h] Departamento de Biología Animal, Vegetal y Ecología, Universidad de Jaén, 23071 Jaén, Spain

HIGHLIGHTS

- Herbivore populations impact ecosystem biogeochemistry.
- Faecal nutrient content differed among species, body sizes, and seasonality.
- Herbivores, especially cattle, had an important role in C, N, and P soil fertilization.
- This is the first quantification of faecal nutrient content in an alpine ecosystem.
- Management should consider contributions of different herbivores to nutrient fluxes.

GRAPHICAL ABSTRACT



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ABSTRACT

The contribution of herbivores to ecosystem nutrient fluxes through dung deposition has the potential to, directly and indirectly, influence ecosystem functioning. This process can be particularly important in nutrient-limited ecosystems such as alpine systems. However, herbivore dung content (carbon, C; nitrogen, N; phosphorus, P; potassium, K) and stoichiometry (C/N) may differ among species due to differences in diet, seasonality, body type, feeding strategy, and/or digestive system with consequences for soil biogeochemistry. Here we explore how species, body size, and seasonality may result in differences in dung stoichiometry for four alpine herbivores (chamois, sheep, horse, and cattle). We found that herbivore dung nutrient content often varies among species as

* Corresponding author.
E-mail addresses: laura@lbhi.is (L. Barbero-Palacios), isabel@lbhi.is (I.C. Barrio), kruminsj@montclair.edu (J.A. Krumins), jordi.bartolome@uab.cat (J. Bartolomé), elena.albanell@uab.cat (E. Albanell), santiago.lavin@uab.cat (S. Lavín), juan.calleja@uam.es (J.A. Calleja).

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Phosphorus
Potassium
Nitrogen

well as with body size, with the dung of small herbivores having larger C, N, and P faecal content. Seasonality also showed marked effects on faecal nutrient content, with a general pattern of decreasing levels of faecal P, N and an increase of C/N as the summer progresses following the loss of nutrient value of the vegetation. Moreover, we showed how herbivores play an important role as natural fertilizers of C, N, and P in our study area, especially cattle. Our study highlights the importance of considering the relative contribution of different herbivores to ecosystem nutrient fluxes in management practices, especially with ongoing changes in wild and domestic herbivore populations in alpine ecosystems.

1. Introduction

In recent years, there has been a growing interest in and recognition of the link between terrestrial animals and biogeochemical cycles (termed zoogeochemistry), and how animals may mediate nutrient cycling and ecosystem functioning (Ellis-Soto et al., 2021; Enquist et al., 2020; Forbes et al., 2019; Malhi et al., 2016). Large terrestrial herbivores have an important role in soil nutrient fluxes, with dung deposition acting as one of the main mechanisms of input and nutrient cycle acceleration (Sitters et al., 2017). Herbivore waste has relatively low C/N ratios compared to other inputs (i.e. leaf litter, standing dead wood) allowing for nutrients within the waste to be rapidly released into the soil (Frank and Groffman, 1998; Hobbs, 1996; Pastor et al., 1993). Additionally, from daily activities and seasonal migrations, herbivores actively transport these nutrients across landscapes and create spatial heterogeneity and dynamism in the distribution of nutrients (Bauer and Hoye, 2014; Ferraro et al., 2022). However, it has been estimated that wild animal-mediated nutrient transport has declined by >90 % since the late Pleistocene due to declining herbivore population densities, species loss, and constraints on animal movement (Doughty et al., 2016). Large herbivore communities are facing rapid changes at global and local scales – changes that will affect animal-vectorized nutrient subsidies (Ripple et al., 2015).

Faecal nutrient content varies both among herbivore species and through time, thus impacting the spatial patterning and primary production of the terrestrial plant communities that benefit from these nutrient inputs (Sitters and Olde Venterink, 2018, 2021b; Valdés-Correcher et al., 2019). The temporal variation in dung quality has been well studied; nutrient concentration and stoichiometry vary across seasons, reflecting changes in plant phenology and herbivore's diet quality and composition (Villamuelas et al., 2016; Verheyden et al., 2011). In contrast, the drivers of the interspecific variation in dung quality are less well understood. Theory suggests that body size may be an important factor; increasing body size correlates with higher total metabolic requirements, requiring animals to expand their diet to lower-quality forage which translates into higher dung C/N ratios (Demment and Van Soest, 1985). Small herbivores, on the other hand, have higher metabolic rates and select N-richer food which decreases faecal C/N ratios (Jarman, 1974; Sitters and Olde Venterink, 2021a). Similarly, the P requirement for skeletal investment also increases with body size, and consequently, larger herbivores will excrete proportionally lower P (Le Roux et al., 2020; Sitters et al., 2017). However, this body-size dependent theory for differences in dung stoichiometry has been recently questioned (Sitters and Olde Venterink, 2021a) as other factors might affect faecal stoichiometry and overall nutrient content, such as feeding strategies (i.e., grazer, browser, mixed-feeder; Grant et al., 1995) or digestive strategies (i.e., ruminant and hindgut fermenters; de Iongh et al., 2011).

We explore these questions within the context of Pyrenean grasslands. These systems have been traditionally used for extensive livestock grazing during summer (García-González et al., 2005). Pastoralism, however, is decreasing in such ecosystems, and livestock is gradually being replaced by an increasing population of wild herbivores (Espunyes et al., 2019a, 2019b; Muñoz-Ulecia et al., 2021). In the alpine ecosystem of the Spanish Pyrenees, a large population of a wild herbivore, the Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*), is present the whole year, and from June to October they cohabit with sheep (*Ovis aries*), horses (Cavall Pirinenc Català horses *Equus ferus caballus*), and free-roaming cattle (Bruna dels Pirineus cattle, *Bos taurus*). How pastoralism abandonment and consequently changes in the abundance and richness of the herbivores present in the Pyrenees will affect soil nutrient cycling and therefore ecosystem functioning is uncertain. With the aim of understanding the potential contribution of dung to soil nutrient fluxes in the Pyrenees, we assessed the faecal nutrient content (carbon, C; nitrogen, N; phosphorus, P; potassium, K) and stoichiometry (C/N) of the four herbivores (chamois, sheep, horse, and cattle) present in the system. We collected samples from June to October when all species coexist in the system. Our objectives were to 1) explore if herbivore faecal nutrient content and stoichiometry differed between species and body size groups, 2) determine if seasonal differences in herbivore faecal nutrient content and stoichiometry persisted in our study system, 3) compare soil nutrient content of the study area with the faecal nutrient content of the four herbivores, and 4) quantify the amount of nutrients released annually by each herbivore.

2. Methodology

2.1. Study area

The study was conducted in the Freser-Setcases National Game Reserve (FSNGR) in the eastern Pyrenees, Catalonia, Spain (42° 22′ N, 2° 09′ E; Fig. 1a). FSNGR is a 20.200 ha mountainous area with an average altitude of 2000 m a.s.l. (1200–2910 m a.s.l. at Puigmal Peak) dominated by metamorphic rocks (Vigo i Bonada, 2008). Soils in the study area are young with little or no profile development (Entisol order according to IUSS Working Group WRB, 2022). In particular, soils have young erosional surfaces (Orthents suborder), on slopes where the depth to bedrock is very shallow (Cryorthent and Udorthent groups).

Sampling was carried out in two areas separated by 20 km of rough terrain (Fig. 1b): Costabona, a 410 ha area located in the north-eastern part of the FSNGR (42° 24′ N, 2° 20′ E, ranging from 1093 to 2429 m a.s.l.), and Fontalba, a 717 ha area in the western part (42° 22′ N, 2° 08′ E, ranging from 1660 to 2248 m a.s.l.). Both areas are characterised by similar vegetation composition and structure, typical of the sub-humid subalpine and alpine bioclimatic belts of the southern slopes of the Pyrenees with a noticeable climatic Mediterranean influence (Vigo i Bonada, 2008). Above 2000 m a.s.l., the vegetation consists of alpine grasslands dominated by graminoids (e.g., *Festuca* and *Carex* genus). At lower elevations (1200 to 2000 m a.s.l.), the vegetation is dominated by *Pinus uncinata* forest patches and dispersed patches of woody understory shrubs, including *Arctostaphylos uva-ursi*, *Calluna vulgaris*, *Cytisus scoparius*, and *Juniperus communis*. During the study years (2011 and 2012), the annual mean temperature was 6 °C (monthly min = 1.7 °C, monthly max = 10.8 °C) and the mean yearly accumulated rainfall was 955.2 mm. During the study period, June to October, the mean temperature was 10.4 °C (min = 6.5 °C, max = 16.3 °C) and the mean accumulated rainfall was 355.15 mm (Catalan Meteorological Office, 2022).

2.2. Studied species

Pyrenean chamois are present all year long in FSNGR, but change habitats seasonally. During autumn and winter, chamois are mostly present below the treeline, and in spring and summer, they are usually observed in open grasslands above the tree line, where they share habitat with livestock species. According to Gálvez Cerón (2015) in Costabona, a population of 80 chamois (0.04 livestock units/ha, 7.6 % of total herbivore population) cooccurs with herds of 300 sheep (0.15 LU/ha, 28.6 % of the total herbivore population), 70 horses (0.12 LU/ha, 6.7 % of the total herbivore population), and 600 cattle (1.46 LU/ha, 57.1 % of the total herbivore population) from June to October. In Fontalba, 150 chamois (0.04 LU/ha, 33.3 % of the total herbivore population) share habitat with 50 horses (0.05 LU/ha, 11.1 % of the total herbivore population) and 250 cattle (0.35 LU/ha, 55.6 % of the total herbivore population); sheep are not present in this study area (Table 1). Other large herbivores such as roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), and mouflon (*Ovis aries musimon*) reside in FSNGR at very low densities and were not considered in this study; no large predators are present within the park.

Of the four study species, chamois has the smallest body mass, followed by sheep, horses, and finally, cattle (Table 1). The diet of the domestic species largely overlaps and is dominated by grasses and forbs. Although chamois is generally considered a mixed feeder in the summer months, in our study area it acts as a browser with a clear preference for shrubs like *Calluna vulgaris* and *Cytisus scoparius* (Espunyes et al., 2019a). It is hypothesized that this behaviour is due to niche partitioning, as they are displaced to more rocky or forested areas to avoid competition with livestock (Espunyes et al., 2019a).

2.3. Faecal sampling and nutrient content assessment

Faecal samples were collected following the sampling procedure of Gálvez Cerón (2015). Fresh faecal samples of the studied herbivores were collected monthly from June to October for two years (2011 and 2012) in both study areas (Fontalba and Costabona). At least two observers visited each study area twice a month and walked two transects (one per location) of about 5 km. These transects were placed between 1900 and 2400 m a.s.l. and encompassed the main vegetation communities and the altitudinal movement of chamois throughout the months within each study area. Groups of chamois and livestock herds were

Table 1
General information of the herbivore species present in the study area. Data on daily defecations events was averaged across literature sources, except for chamois where sheep defecation rates were used. Dry dung weight was measured from 5 faeces collected in the field in the present study.

	Chamois	Sheep	Horse	Cattle
Average population size*	230	300	120	850
Body size	Small	Small	Large	Large
Feeding strategy	Browser[a]	Browser[a]	Grazer[b]	Grazer[b]
Digestive system	Ruminant	Ruminant	Non ruminant	Ruminant
Average body weight (kg)	30[c]	60[d]	700[e]	800[d]
Defecation events per day	16[f,g,h]	16[f,g,h]	12[i,j]	15[k,l]
Dry dung weight (g)	25.66	25.66	251.55	282.6
Average nr. of days in the field	365	153	153	153

* Average population represents the average numbers of animals in 2011 and 2012 summed across sites.
[a] Gálvez Cerón, 2015.
[b] Hofmann, 1989.
[c] Smith et al., 2003.
[d] Generalitat de Catalunya, 2010.
[e] González, 2011.
[f] Welch, 1982.
[g] Longhurst, 1954.
[h] de Bie, 1976.
[i] Westendorf, 2006.
[j] Lamoot et al., 2004.
[k] Villettaz Robichaud et al., 2011.
[l] Aland et al., 2002.

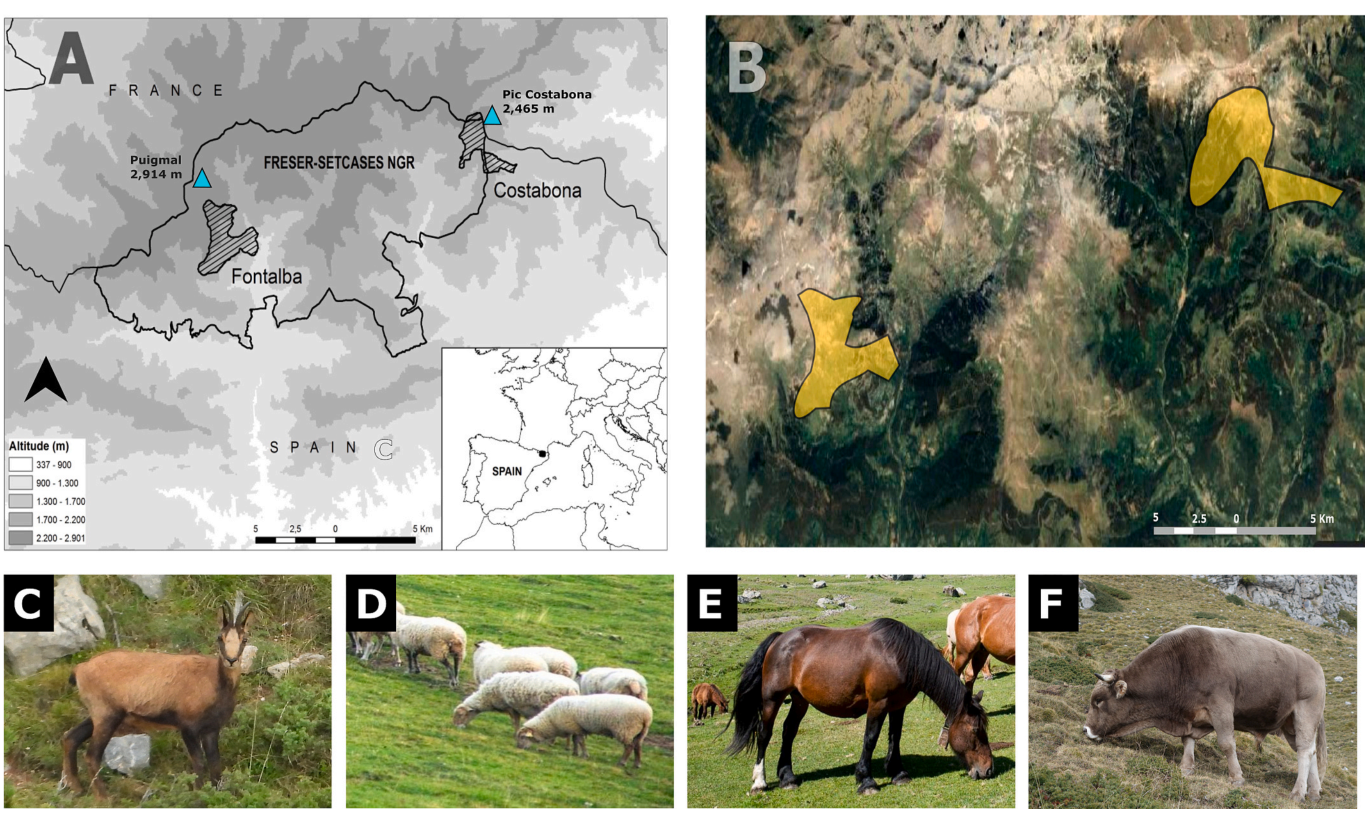

Fig. 1. Location of both study areas (Fontalba and Costabona) in Freser-Setcases National Game Reserve, Spain (A, B) where chamois (C), sheep (D), horse (E), and cattle (F) are present.

located with binoculars and spotting scopes. Observers collected fresh droppings of all herbivore species at the exact location where animals were sighted and in their surroundings. At the site of collection, group size, composition, and precise location of the animals were recorded. Faecal samples were collected in separate labelled plastic bags. Based on colour, texture, and the presence of mucus, we were able to determine that all samples were collected within a maximum of 5 h after defecation (Hibert et al., 2011). To avoid double-sampling of the same individual, groups of animals observed twice were only sampled once. A group of droppings collected in one bag was considered a single faecal sample, even if the droppings could have belonged to different individuals. Samples were transported to the laboratory and stored frozen at −20 °C. Before processing, samples were thawed for 24 h and then dried in an oven at 80 °C for 48 h.

To assess the nutrient content of chamois, horse, and cattle faeces, we analysed two faecal samples per species for each site, month, and year. For sheep, we analysed three samples for each month and year, as sheep were only present in Costabona. Some months had a lower number of samples because no fresh faeces were found. A total of 121 samples (33 for chamois, 23 for sheep, 31 for horse, and 34 for cattle) were sent to the University of Jaen for chemical analysis. Faecal samples were ground and homogenized using an MM400 Retsch mixer ball mill. Duplicated or triplicated analyses were carried out in aliquots of two-thirds of the samples to account for analytical variability. C and N concentrations were measured by gas chromatography using a LECO TruSpec Micro CNHS autoanalyzer, and P and K concentrations were measured by Inductively Coupled-Plasma (ICP) spectrometry (Agilent model 7900), after H_2SO_4-H_2O_2 acid wet digestion of faecal samples (Parkinson and Allen, 1975) using a BD40 block digestion system (Seal Analytical). Analytical precision (i.e., the mean and standard deviation of the coefficients of variation of the different replicated analyses) was 2.04 ± 2.07 % for total C, 3.15 ± 2.43 % for total N, 2.7 ± 2.2 % for total P and 4.8 ± 4.3 % for total K. Nutrient contents are expressed as % of a particular element of the total dry weight of dung; means and standard errors are presented.

2.4. Data analyses

We used linear mixed-effect models (LMMs) to analyse the relationship between faecal nutrient concentrations (C, N, P, K) and stoichiometry (C/N) and herbivore species (chamois, sheep, horse, and cattle), body size (small or large herbivores), and seasonality (from June to October). Before analysis, we excluded one faecal nitrogen data point for chamois, which was a clear outlier 4 standard deviations away from the mean.

We first built models for each of the nutrients to assess if the average concentration for each nutrient (C, K, N, P, C/N) was different among herbivores. We included a nested random effect of the sampling site and a variable for each independent month across the two years. To compare each species, we performed a posthoc Tukey test on these models using the package 'multcomp' (Hothorn et al., 2016). To assess if average nutrient values were different among body sizes, we then divided the herbivore species into two groups: small herbivores (chamois and sheep) and large herbivores (horse and cattle). We classified herbivores per body size and not per feeding strategy (i.e., grazer, browser, and mixed feeder) due to our low sample size of each guild (see Table 1). We then built a set of repeated measures ANOVAs to explore if seasonal differences existed for each species for each of the nutrients (C, N, P, K C/N). Repeated measures ANOVAs were chosen as they are robust to non-normality (Blanca et al., 2023), and while most of our models contained normally distributed data, or data that met the assumptions of normality after log-transformation, two models did not. Both models, however, met the assumption of sphericity, indicating that the results of the repeated measures ANOVA are robust and had non-significant results (Blanca et al., 2023). For the models with significant seasonal differences (i.e., $p < 0.05$), we performed a posthoc Tukey test to evaluate if any seasonal patterns persisted across nutrients. All statistical analyses were conducted using the 'lme4' (Bates et al., 2023) and 'lmerTest' (Kuznetsova et al., 2017) packages in the R Statistical Software 3.6.2 version (R Core Team, 2021).

To understand the faecal content of each nutrient in the context of alpine ecosystems, we compared the average faecal composition (%) of each nutrient for each herbivore with the background soil content (%). Since soil biogeochemistry information from our study area was not available, we extracted soil nutrient content values from the literature from sites within the Pyrenean mountain range that were comparable to ours. Concentrations of soil organic C data were extracted from Garcia-Pausas et al. (2007), the concentration of total soil N and C/N ratio from Rodríguez et al. (2020), and soil P and K concentration from Badía et al. (2008).

Finally, to assess the total nutrient contribution of each herbivore in alpine ecosystems yearly, we compiled information on daily defecation rates for each herbivore from the literature. Sheep defecation events are reported as 17 (Welch, 1982), 13 to 16 (Longhurst, 1954) and 18 (de Bie, 1976) events per day; in this study, we used the average (16 defecation events per day). Sheep defecation rates were used also for chamois since literature about the topic was not found. Horse defecation rates are reported from 4 to 13 events per day (Westendorf, 2006), and 20 (Lamoot et al., 2004); in this study, we used an average (12 defecation events per day). Finally, cattle defecation rates are reported as 3 to 18, 9 to 23 (Villettaz Robichaud et al., 2011) and 8 to 29 (Aland et al., 2002) events per day; we use the average (15 defecation events per day). To estimate the total nutrient contribution of each species, defecation rates were multiplied by the average number of days each species was present at the sites, the average dry weight of a defecation event (total defecation dry weight), and the density. The average dry weight of a defecation event for each herbivore was calculated based on five randomly chosen faecal samples per species. We collected and weighed the whole dung defecation from five cows and horses as well as the droppings from five sheep. We subsampled 20 g of the fresh sample to dry at 65 °C for 24 h. The dry material was used to calculate the specific dry weights per defecation event in our animal models. The population density of each herbivore (individuals per hectare) was estimated using the average population of both study years and areas retrieved from Gálvez Cerón (2015) and dividing it by the area of each study site (410 ha for Costabona and 717 ha for Fontalba).

3. Results

3.1. Faecal nutrient content variation between herbivore species, body sizes and seasonality

Differences in faecal nutrient concentration among herbivore species were found for all nutrients (Fig. 2; Appendix A: Table A1). Sheep had on average the largest concentrations of C, N, and P within their faecal matter, while horses had the largest C/N ratios and K concentrations (Table A2). Sheep faecal concentration differed from cattle and horse for every nutrient, while chamois differed from cattle and horse in N and K concentrations as well as C/N ratios (Table A3). Chamois and sheep had no differences in any faecal nutrient content except for P and generally, cattle and horses had similar faecal nutrient content except for K and C/N ratios (Table A3). Aggregating by body size resulted in similar findings, with large-bodied and small-bodied herbivores having significantly different faecal nutrient contents; large-bodied herbivores had relatively lower C, N, and P content yet higher K content and C/N ratios (Table A4).

While in a vast majority of cases, faecal nutrient concentration varied through time, there were several notable exceptions. Chamois was the only species to exhibit seasonal differences in faecal C concentrations (Fig. 3; Table 2), a result specifically driven by significantly lower values in July. On the other hand, horses exhibited no monthly differences in faecal N, and cattle only had a weakly significant seasonal variation in

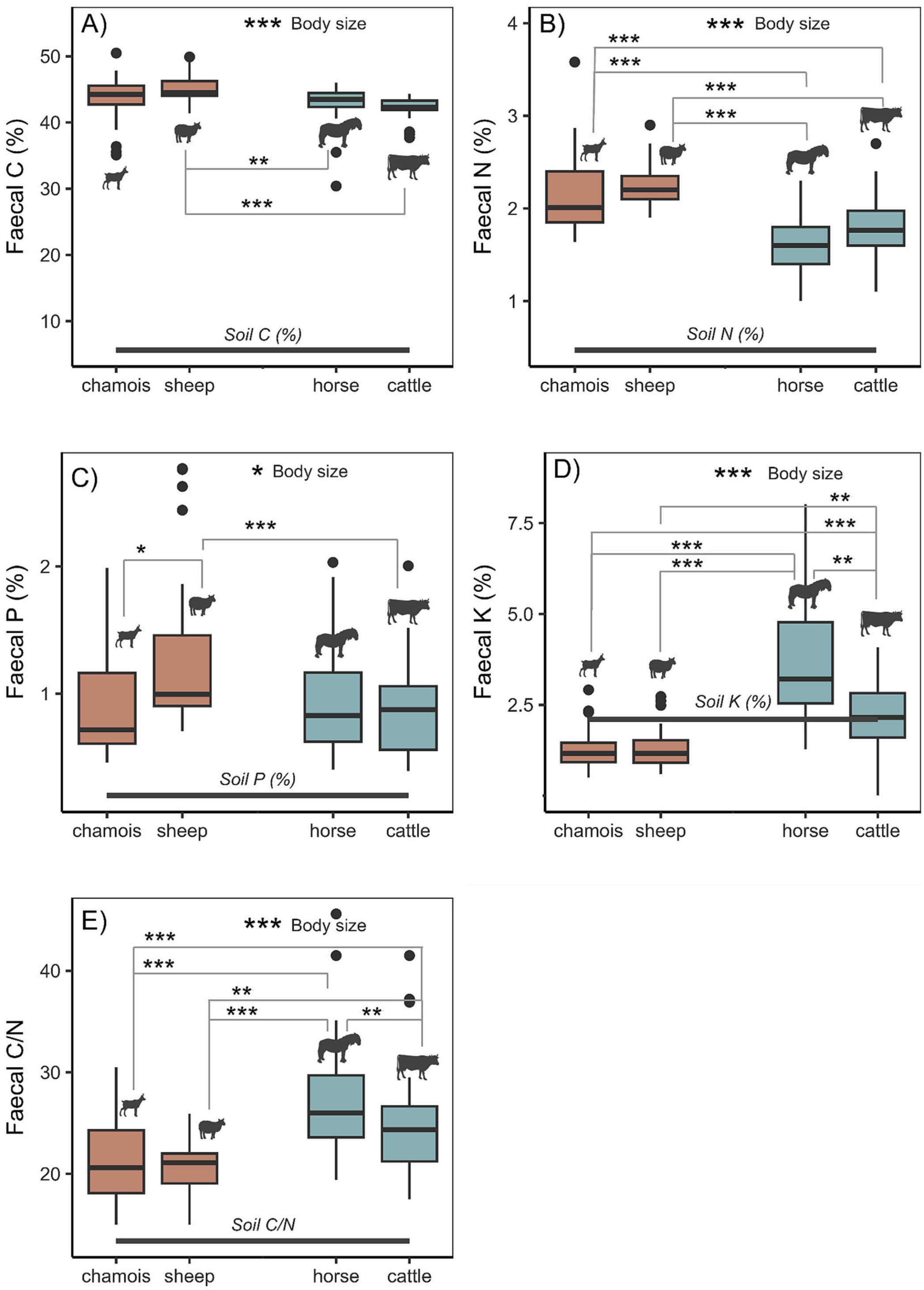

Fig. 2. Average faecal content (%; mean and SE) of carbon (A), nitrogen (B), phosphorus (C), potassium (D), and carbon to nitrogen ratio (E) of the four herbivores studied. Post-hoc pairwise Tukey test for each herbivore species is represented with grey lines and asterisks (see Table A3). Average soil percentage of each nutrient is shown with a solid line (see Table A2). Small herbivores (chamois and sheep) are presented in orange and large herbivores (horse and cattle) are presented in blue; significant differences between body sizes (small vs. large) are indicated at the top of the graph. Each species' n can be found in Table A1 (*** = $p < 0.001$; ** = $p < 0.01$; * = $p < 0.05$).

faecal P. There were also several seasonal patterns among herbivores; most species had increased faecal N in June compared to other months (Table A5). Small herbivores also had generally enriched *P* values in June compared to other months. Finally, faecal C/N ratios remained relatively consistent in the fall months for all domestic herbivores (August–October).

3.2. Faecal nutrient deposition

Comparing the average nutrient content (%) in the faeces of herbivores to the average nutrient content in the soil of the Pyrenees reveals that the herbivores' faeces contain approximately 8 times more C, 4 times more N, 5 times more P, and 2 times higher carbon-to-nitrogen ratio (C/N). Concentrations of faecal K were comparable to those from soil (Fig. 2; Table A2).

In terms of annual nutrient input into the ecosystem, cattle had by far the largest inputs of all nutrients among the studied herbivores, a result of their higher population density (Fig. 4). Among the four species, the wild ungulate (i.e., chamois) deposited the smallest amount of nutrients into the ecosystem. In fact, cattle deposited 28 times more C, 14 times more K, 23 times more N, and 24 times more P than chamois. Sheep deposited the second largest quantity of K and P, while horses deposited

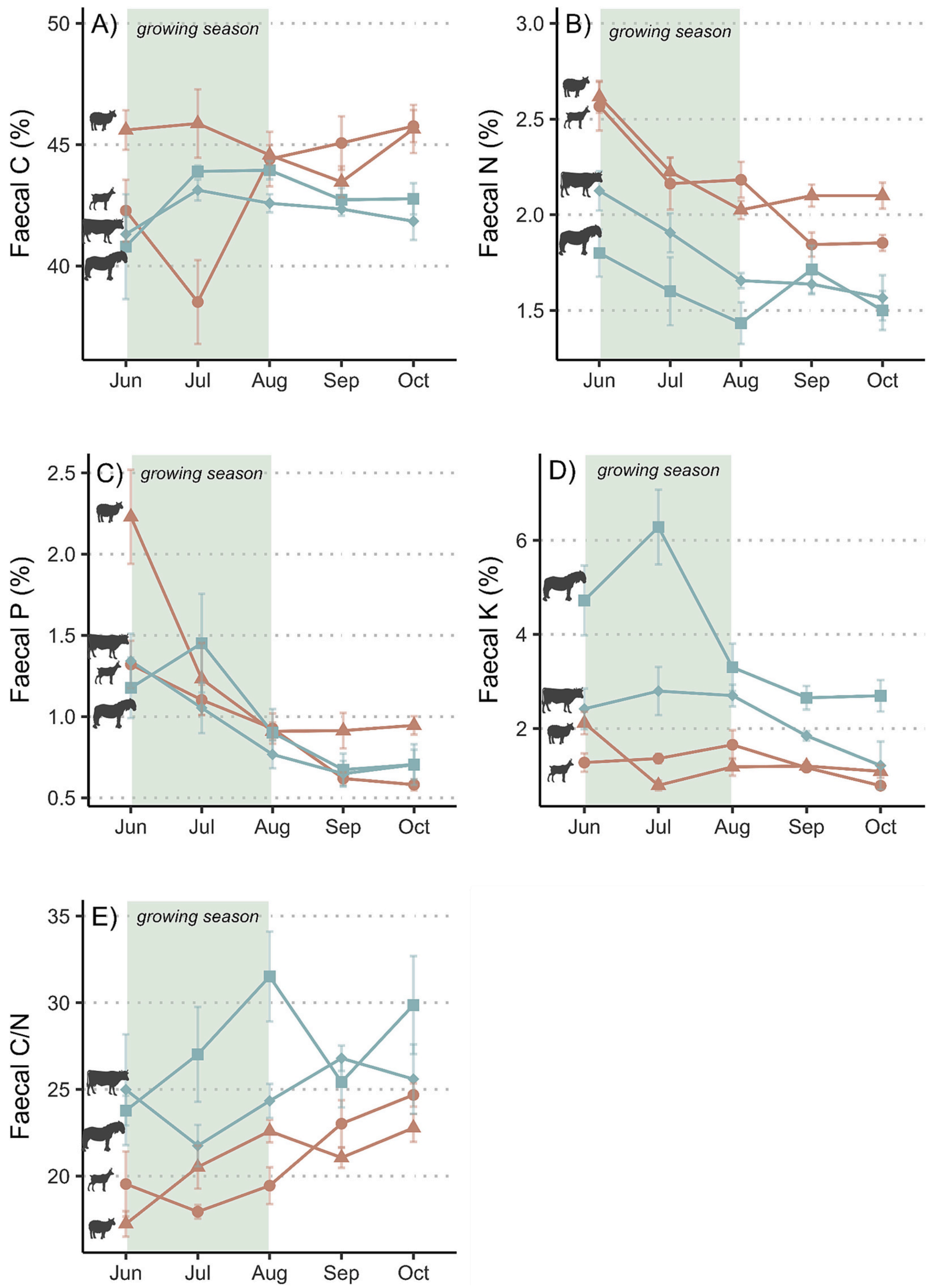

Fig. 3. Monthly faecal nutrient content variation (%; mean and SE) is shown for carbon (A), nitrogen (B), phosphorus (C), potassium (D), and carbon to nitrogen ratio (E). Small herbivores (chamois and sheep) are presented in orange and large herbivores (horse and cattle) are presented in blue. Growing season is presented with a green section. Monthly *n* can be found in Table A1.

the second largest quantity of C and N.

4. Discussion

Our analyses revealed considerable variation in faecal nutrient content among four alpine herbivores, largely depending on their body size. Nutrient contributions by the different herbivores also differed along the growing season, highlighting the importance of considering the whole herbivore assemblage and seasonality when studying nutrient dynamics in alpine environments. Our results demonstrate the important role of herbivores as natural fertilizers in alpine ecosystems, as they contribute material with higher nutrient concentrations than those typically found in the soils.

Unsurprisingly, there was significant variation between species in terms of faecal nutrient content. The fact that the horse had the lowest levels of faecal N is consistent with its non-ruminant digestion. Non-ruminants have greater tolerance of structural plant fiber in their diet and low losses of bacterial protein (de Iongh et al., 2011; Edwards, 1991;

Table 2
Summary of the repeated measures ANOVA analysing the monthly variation of faecal nutrient content (%) of each herbivore species (chamois, sheep, horse, and cattle). C = carbon, K = potassium, N = nitrogen, P = phosphorus, C/N = carbon to nitrogen ratio.

	Sum sq	Mean sq	F value
C			
Chamois	168	42	5.013 **
Sheep	14.76	3.689	0.85
Horse	38	9.501	0.967
Cattle	4.88	1.22	0.895
N			
Chamois	2.466	0.617	11.11 ***
Sheep	1.295	0.324	12.45 ***
Horse	0.645	0.161	1.812
Cattle	0.910	0.227	3.946 *
P			
Chamois	2.855	0.714	11.25 ***
Sheep	7.011	1.753	9.553 ***
Horse	2.336	0.584	3.833 *
Cattle	1.898	0.474	2.438
K			
Chamois	2.758	0.689	2.923 *
Sheep	5.334	1.333	8.442 ***
Horse	49.63	12.41	7.088 ***
Cattle	11.56	2.89	2.877 *
C/N			
Chamois	367.9	91.97	22.85 ***
Sheep	77.46	19.365	5.452 **
Horse	307.3	76.84	2.859 *
Cattle	211.7	52.93	3.991 *

Significance levels: *$p < 0.1$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.*

Macandza et al., 2014). On the other hand, small ruminants have higher fermentation rates and a large surface-to-volume ratio in the alimentary tract compared to large ruminants (Arman et al., 1975), and this could explain why chamois and sheep had the highest levels of faecal N. Different feeding strategies could also correspond to species differences in dung stoichiometry. Our results show a higher N concentration in the dung of the browsers (chamois and sheep) compared to the grazers (horse and cattle), as seen in other studies (Codron et al., 2007b; Grant et al., 1995; Le Roux et al., 2020; Sitters et al., 2014; Sitters and Olde Venterink, 2021a; Zhu et al., 2020). This might be a consequence of the ability of browse plants to survive and retain most of their nutritional value throughout the seasons, contrary to grass species (Ravhuhali et al., 2022). However, despite its widespread acceptance, there is a big disagreement in the distinction of feeding guilds (i.e., grazer, browser, mixed-feeder) since this classification is insufficient to explain the full extent of dietary diversity among herbivores (reviewed in Ditchkoff, 2000), and comparisons between feeding strategies should be made with caution (Codron et al., 2007a, 2007b).

Our results also showed that body size had a significant effect on dung stoichiometry, with small herbivores having larger C, N, and P faecal concentrations and smaller K concentrations and C/N ratios than large herbivores. This supports the theory that small body-sized herbivores select N-richer food (Jarman, 1974), and contrasts the prediction that large mammal dung is richer in N and poorer in P due to metabolic processes and skeletal investment (Le Roux et al., 2020; Sitters et al., 2017). Higher levels of faecal C, N, and P content in small herbivores have been reported before (Codron et al., 2007b; Edwards, 1991; Zhu et al., 2020) however other studies have found contrasting results, where higher levels were reported for large herbivores (de Iongh et al., 2011; Pérez-Barberia et al., 2004). Moreover, higher levels of K (Esse et al., 2001) and C/N ratio (Zhu et al., 2020) in large herbivores has also been previously reported. Of note, our comparisons are based on a few herbivore species. Data from a larger range of herbivores will be needed to reach robust conclusions about the influence of body size, digestive system and/or feeding strategy on the faecal nutrient content and stoichiometry. In addition, previous literature about herbivore dung stoichiometry mainly focuses on faecal N and/or diet quality and is mostly focused on savannah ecosystems (e.g. Codron et al., 2007b; Sitters et al., 2014), which makes it difficult to compare our results with other studies with the same characteristics.

As a general pattern, we found decreasing levels of faecal N and P, and an increase of C/N as the summer progressed; a result of the loss of the nutrient value of the most abundant grasses and forb species in the study area (Marinas et al., 2003). These results are in line with previous studies linking faecal concentration with seasonality (e.g., Arnuti et al., 2020; Espunyes et al., 2022; Gálvez-Cerón et al., 2013; Leslie and Starkey, 1985; Valdés-Correcher et al., 2019). However, seasonal changes were inconsistent across nutrients and species. For example, chamois' faecal C increased throughout the summer months, while horses and cattle had consistent faecal C concentrations. This may reflect diet shifts, where chamois shift to a more ligneous diet composition from summer to autumn, whereas horses and cattle maintain similar feeding habits (Espunyes et al., 2019a). Interestingly, sheep followed the same diet pattern as chamois but did not exhibit the same changes in faecal C among seasons. Further, while the general pattern of nutrient concentration across the seasons may mirror overall plant nutrient patterns (Jarque-Bascuñana et al., 2022), we did not find a link between diet and herbivores with the rest of the nutrients, since similar diet choices among domestic herbivores (i.e., sheep, horse, and cattle), did not result in similar concentrations of faecal nutrient content.

Herbivore faeces represented an important local source of soil fertilization in our study area, especially for C, N, and P. N and P are particularly important inputs, as they are limiting nutrients for plant productivity in terrestrial ecosystems (Penning de Vries et al., 1980; Vitousek and Howarth, 1991). Greater levels of herbivore's faecal C, N, and P content compared with soil have also been found in savannah (Augustine et al., 2003) and boreal forest ecosystems (Pastor et al., 1993), but the difference between faecal and soil nutrient content in these systems are not as marked as in our study. Additionally, in the Pyrenees, a positive effect of livestock on nutrient content has been seen before for organic soil C (Badía et al., 2008; Rodríguez et al., 2020), which our study further supports.

5. Implications for management

Herbivore contributions to nutrient fluxes through waste products have the potential to influence ecosystem nutrient cycling (Barbero-Palacios et al., 2020) and functioning (Le Roux et al., 2018; Schmitz et al., 2018; Ferraro et al., 2022). Alpine ecosystems have relatively nutrient-poor soils, and therefore herbivore dung can provide a disproportionate contribution to nutrient dynamics in those systems. In the Spanish Pyrenees, as in other alpine ecosystems across Europe, there has been a dramatic decrease in pastoralism activities (Espunyes et al., 2019b). Abandonment of traditional grazing practices has a negative impact on grassland production (Jarque-Bascuñana et al., 2022), allowing bush encroachment. Consequently, this leads to an increase in soil carbon-to-nitrogen ratio and the reduction of N, P or K content (Grau et al., 2019), impoverishing ecosystem services. Nutrient deposition by herbivores in alpine areas might enhance nutrient cycling in soils and ultimately in the resilience of alpine grasslands to global changes (Kinugasa et al., 2012).

6. Conclusions

This is the first quantification of the nutrient content of chamois, sheep, horse, and cattle faeces in an alpine ecosystem. Moreover, this is the first seasonal examination of excreted nutrient content in these

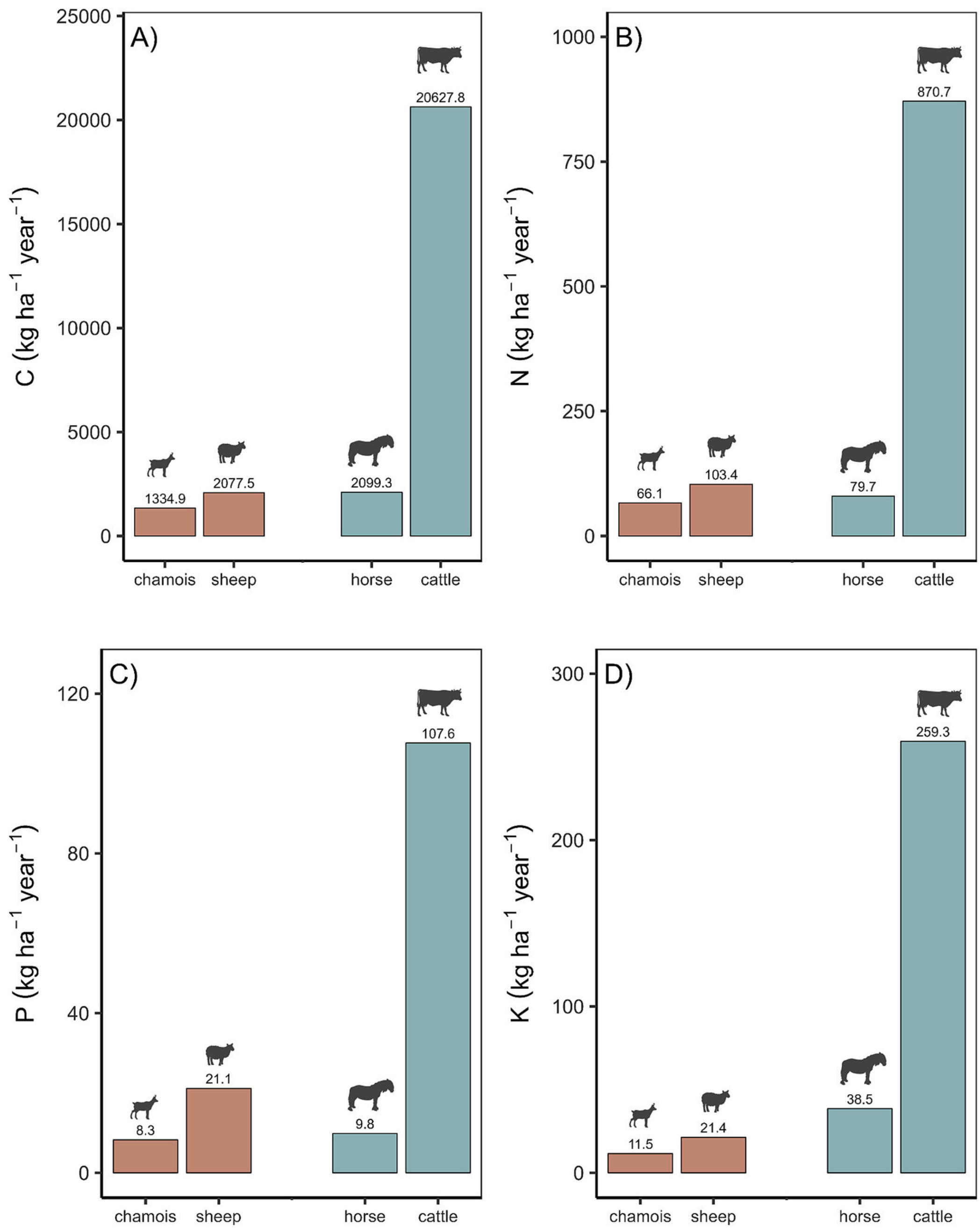

Fig. 4. Estimates of the nutrient contribution of each herbivore per hectare and year, based on their relative abundance, average defecation rates and time spend in the field (see Methodology and Table 1 for details on calculations). Small herbivores (chamois and sheep) are presented in orange and large herbivores (horse and cattle) are presented in blue.

ecosystems. This field study showed that the faecal nutrient content of four alpine herbivores varies considerably among species, body size, and seasons, and highlights the important local fertilization effect they can have. Our study suggests that changes in species density and/or composition of the herbivore community in a specific location might change soil biogeochemistry. Therefore, special attention should be paid when planning management practices, given the current trend of changes in wild and domestic herbivore populations. Finally, we want to make a call for more data about nutrient stoichiometry in the dung of herbivores which vary in body size, feeding strategy, and digestive system, and a synthesis of available data.

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

Raw data and statistical analysis are available on Mendeley data https://doi.org/10.17632/gcg645gwhk.1.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.166616.

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