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REPORT ON EXPEDITION / PROJECT

Expedition/Project Title: Puszta 2023: impacts of grazing on the composition and traits of grasses in the Kiskunság Hungarian Steppe

Travel Dates: 06/05/2023-28/05/2023

Location: Kiskunság National Park, Hungary

Group Members: Susan Eshelman (Group Leaders), Lorna MacKinnon (Research Assistant)

Aims: The final aims of this project were to quantify the impacts of grazing on the species diversity and structure of alkali and loess grassland and to increase the body of information for temperate ecosystems in the discussion of grazing ecosystems globally.

Photography consent form attached: Yes
(*please refer to your award letter*) No

Outcome (a minimum of 500 words):-

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Davis Expedition Report

Project Title: Puszta 2023: impacts of grazing on the composition and traits of grasses in the Kiskunság Hungarian Steppe



May 6-27, 2023

Prepared by Susan Eshelman, PhD Student, The University of Edinburgh, Royal Botanic Garden Edinburgh, and Royal Botanic Gardens Kew

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Photo Provided by Susan Eshelman

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Introduction:

Grassland ecosystems cover approximately 40% of the Earth's land surface and are key to many industries including livestock, agriculture, and ecotourism (White et al., 2000). Grasslands provide vital ecosystem services such as carbon storage, improve water availability, and are biodiversity hotspots for many specialised animals and plants (Egoh et al., 2009; Lemaire et al., 2011). However, globally grasslands are under threat due to climate change and anthropogenic activities, and conservation efforts are required to protect and restore these ecosystems (Ceballos et al., 2010).

Hungarian grasslands, situated within the Pannonian biogeographical region (also referred to as the Puszta or Pannonian steppe), are of great conservation importance, with higher biodiversity than most other European grasslands (Fourth National Report to the Convention on Biological Diversity- Hungary, 2009). These steppe grasslands, including alkali or loess grassland, (Figure 1) have been present since the last ice age (100-25 k years ago) and formerly were home to many extinct megafauna (Molnár & Borhidi, 2003). The grasslands have experienced grazing for millennia, resulting in the evolution of a grazing-adapted flora that needs grazing for its continued existence and richness (Gordon & Prins, 2008; Stanová et al., 2008).



Figure 1. Boundary between open alkali and loess steppe grasslands within Kiskunság National Park (Eshelman, 2023)

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Since 50-7k years ago, European ecosystems have lost most of their native megafauna, with livestock replacing native fauna (such as deer) in many regions (Malhi et al., 2016; Owen-Smith, 1988). This has led to extensive ecosystem changes, altering the mosaic of open and closed habitats once found across Europe (Svenning, 2002). Currently, intensive livestock management is a major threat to Hungarian grasslands as grazing intensities are too high, resulting in overgrazing, woody encroachment, and homogenising of the landscape (Stanová et al., 2008). In recent years, extensive (low/medium intensity) grazing by livestock or native grazers has become a more sustainable farming practice and an important conservation tool for maintaining many European natural and semi-natural grasslands (Metera et al., 2010; Tälle et al., 2016). A growing number of restoration and rewilding projects within European grasslands aim to reinitiate or restore ecological processes through the reintroduction or management of herbivore grazing, trampling, and browsing (Griffiths et al., 2013; Lundgren et al., 2020). When done properly, low-intensity grazing regimes help to maintain an open ground canopy while also increasing the grassland biodiversity (Metera et al., 2010; Török et al., 2016). Within Hungary, traditional low-intensity grazing by livestock has been a major part of the country's cultural and landscape history for centuries (Gordon & Prins, 2008). This has led to unique and highly diverse ecosystems of grazing-adapted flora. Hence, the Hungarian Steppe presents an excellent opportunity to study how extensive grazing influences the composition and functional traits of a European grassland. This will contribute to the understanding of grazing systems worldwide, and aid in developing effective regional management, restoration, and rewilding strategies.

Selective grazing by herbivores can create and maintain patches of quality forage, called grazing lawns (Hempson et al., 2015; McNaughton, 1984) (See Figure 2 for examples). Grazing lawns are taxonomically defined by the dominance of short grasses that are often prostrate with short stolons or rhizomes and small seeds (Cromsigt & Olf, 2008; Hempson et al., 2015; McNaughton, 1984). Functionally, they are characterised by high densities of grazing herbivores that, through grazing and trampling, consistently modify the vegetation structure over time (Arnold et al., 2014; McNaughton, 1984). Grazing lawns provide herbivores with the high-quality forage they need to meet daily nutritional requirements and can be important resources of nutrition in seasonal and nutrient-poor environments (Verweij et al., 2006; Waldram et al., 2008). Grazing lawns are traditionally seen as tropical grassland ecosystems with large mammal herbivores, but an increased understanding of the processes shaping grassland systems has led to increased application of the term to other ecosystems (Person et al., 2003; Prins et al., 1980; Roberts et al., 2011). Little work has been done, however, to understand the structure and dynamics of grazing lawns within temperate ecosystems. Therefore, this project aims to improve understanding of the structure and function of European grazing lawns within the Hungarian Steppe.

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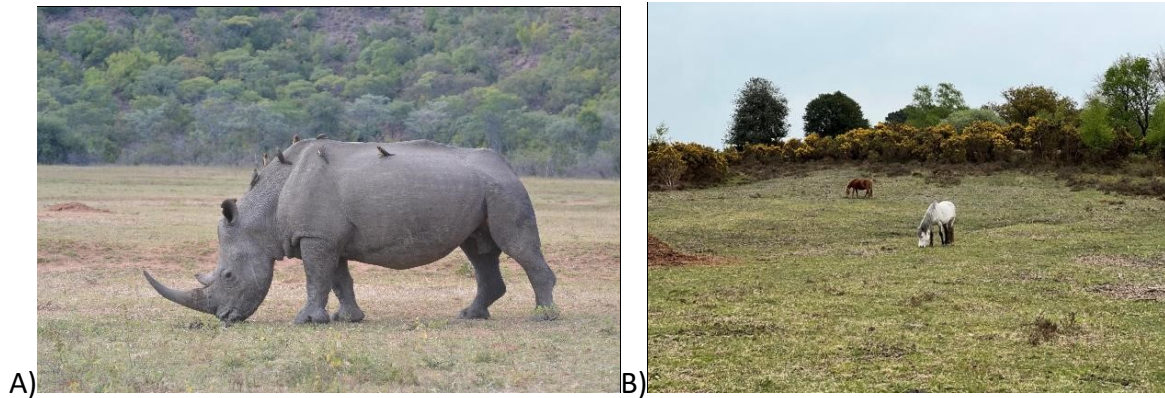


Figure 2- (A) Example of a tropical grazing lawn at Welgevonden Private Game Reserve, Vaalwater, South Africa. This grazing lawn consists of highly palatable, laterally growing, mat-forming C4 grass. This lawn is maintained by a variety of large herbivores, including white rhino (*Ceratotherium simum*), blue wildebeest (*Connochaetes taurinus*), common warthog (*Phacochoerus africanus*), Impala (*Aepyceros melampus*), and plains zebra (*Equus quagga*). (B) Example of temperate grazing lawn in New Forest National Park, England, United Kingdom. Grazing lawn consists of a mixture of laterally growing, mat-forming C3 grasses and sedges. New Forest Ponies (*Equus caballus*) regularly graze this lawn.

This project explores how grazing influences the composition and structure of grasses in alkaline and loess steppe grasslands (Figure 1). Currently, most grazing research focuses on tropical ecosystems. Temperate and tropical grazing ecosystems differ in climatic space and support specialised floras and faunas adapted to these contrasting environmental conditions. Tropical ecosystems are significantly more productive than temperate ecosystems, with high rates of species turnover and primary productivity (Frank et al., 1998). Tropical grazing are shown to significantly alter the composition of grasslands influencing the dynamics of these ecosystems (Mcivor et al., 2005; Stock et al., 2010). This, however, is less understood in temperate ecosystems, particularly European temperate. European grazing ecosystems are largely understudied outside of an agricultural perspective and it is unclear if grazing influences the structure and assembly of temperate (and particularly European) grazing ecosystems in a functionally equivalent manner to its role in tropical ecosystems. Therefore, this project investigates whether grazing alters the composition of Puszta grasslands and whether this is associated with changes in the functional traits of grazing-resistant grasses. Our findings will contribute to a broader body of information on grass traits associated with resistance to (and thriving under) grazing by large herbivores. Because of their high conservation importance and unique biodiversity, the alkali and loess grasslands of central Hungary were an ideal ecosystem to investigate the impact of grazing on grassy vegetation within Europe. The results of this project will also help to update the concept of grazing lawns across both tropical and temperate ecosystems and how their assembly and dynamics are impacted by mega faunal extinction and replacement.

Project Aims and Objectives:

We conducted a series of vegetation plots along a grazing gradient in Kiskunság National Park, Hungary to quantify the impacts of grazing on the composition and structure of grassy vegetation within the Puszta ecosystem, increasing the body of information for temperate ecosystems in the discussion of grazing ecosystems globally. We addressed the following main objectives:

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1. Quantified the impacts of grazing on the diversity and species composition of alkali and loess grasslands.
2. Assessed differences in composition and soil for adjacent loess and alkaline grasslands
3. Quantify the impacts of grazing on grass functional traits related to growth and resource acquisition within alkaline grasslands.

Methods:

Study Area

We conducted all fieldwork within Kiskunság National Park (570 km²) in the Carpathian Basin of central Hungary (Ladányi et al., 2015) (Figure 3). Established in 1975, Kiskunság National Park is a UNESCO biosphere reserve and Natura 2000 site. All sites surveyed were in Milka-puszta (approximately 65 km²) in the southern portion of the park. This area includes alkali, loess steppe, open alkali grassland, and alkali meadow with chernosem or solonetz soils. For this project we focused on open alkali and loess steppe habitats as there were the most commonly grazed by livestock according to land managers. Managed grazing throughout the park occurs yearly from April to October, primarily by sheep and cattle at densities of 0.3-1 livestock unit per ha (Molnár et al., 2020). Our field project took place during May, which is at the start of the main flowering season for Hungarian grasses and is the prime period for identification.

Field Surveys

We surveyed 17 plots along a grazing gradient for two habitat types, loess and alkaline grasslands ($N_{\text{loess}}=8$, $N_{\text{alkaline}}=9$). Sites were located a minimum of 300 m away from each other. At the request of the park rangers, sampling was restricted to the north and south areas of the park.

We utilized the Global Grassy Group's protocol (Lehmann et al., 2022) to characterize the ground layer vegetation's taxonomic and functional composition at each site. This protocol was designed to allow for uniform data collection across a variety of environments.

Compositional surveys

Sites were placed in an area of homogenous vegetation cover that was representative of the environment being sampled. Each individual site covered an area of 2500 m² (0.25 ha) and consisted of a 50 m x 50 m central cross, laid out using a measuring tape (Figure 3). Along each 50 m transect, 1 m circular plots were placed at a 5m distance, as well as a 1 m plot placed at the centre point where the transect intersected ($N=21$ plots). This arrangement allowed for a better picture of the compositional variation than sampling a single larger circular plot with the same total area. Counts in the 21 plots allow for the estimation of site-level species richness and the species rank frequency distribution. Grass species presence/absence surveys and plant specimen collection (see Functional Trait

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Measurements for more) were conducted within each of the 21 x 1 m diameter circular plots arranged along the cross. For loess grasslands, presence/absence was recorded for species from all functional groups (mat-forming grass, tussock-forming grass, sedge, forb, legume, geophyte, spiny, succulent, moss, woody).

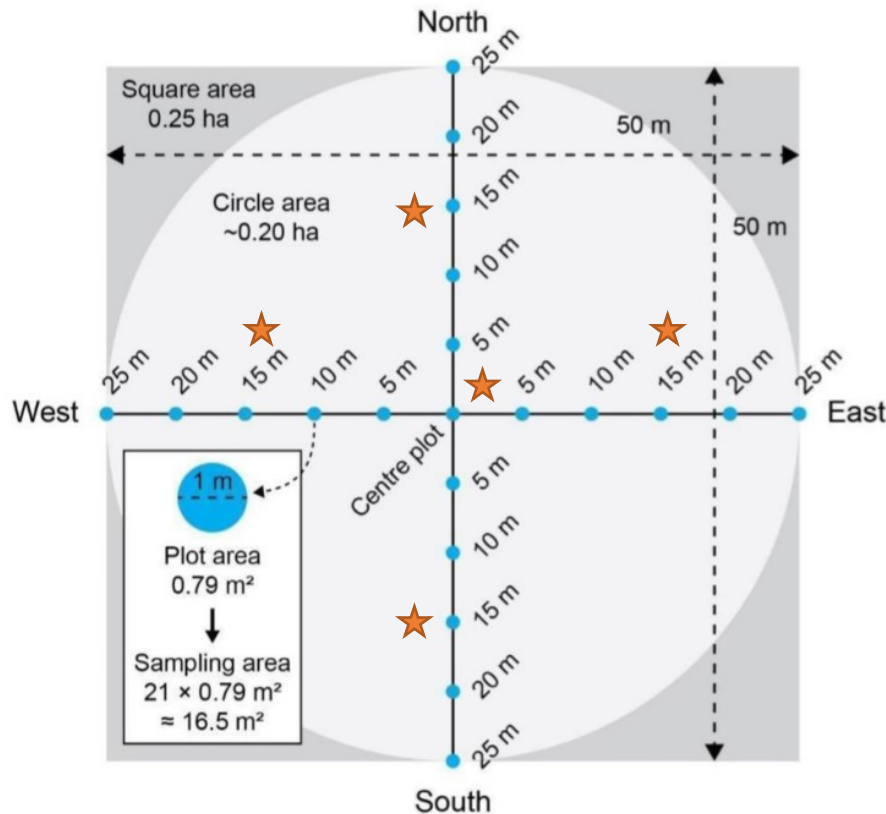


Figure 3- Diagram showing an example of 50 m x 50 m layout for a site taken from Lehmann et al., 2022. The diagram is not drawn to scale. At the centre of the site is a 1 m diameter plot from which four 25 m transects branch off, each perpendicular to the other. The resulting area sampled is 16.5 m² over a 2500 m² (0.25 ha) area. Orange stars represent approximately, where soil samples were extracted.

Within each plot, bare, plant, and litter ground cover was estimated from 100%. Plant ground cover was further subdivided into percent cover for each functional group (listed above). Animal dung counts and evidence of grazing were recorded within each circular plot to estimate herbivore densities and grazing intensity within sites. Animal dung counts were used to calculate the percent animal dung occurrence by dividing the number of plot in which dung was recorded by the total number of plots at each site (N=21). This percentage of animal dung was used to create the grazing gradient utilized in data exploration and analysis.

Functional Trait Measurements

A functional trait is a defined, measurable property of an individual organism that strongly influences its performance (growth and survival) or fitness (McGill et al., 2006). Functional traits of plants consist of morphological, physiological, and phenological

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attributes that influence individual species' performance (Violle et al., 2007). We measured functional traits of grass species recorded in the 21 compositional plots used after the survey were conducted, following standard protocols (Wigley et al., 2020). At each site, we measured the following traits related to growth and disturbance tolerance in three individuals per species: culm diameter, bulk density, stem length, leaf thickness, and plant height. Traits to be collected in the lan include specific leaf area, specific leaf length, and leaf nitrogen content.

Specimens, leaf samples, and soil samples were exported to the UK for chemical analysis. Exported material will be processed within the lab at the University of Edinburgh for foliar nitrogen, carbon, and phosphorous content, specific leaf area (SLA), and specific stem length (SSL). Chemical analysis and SLA both of require destructive sampling. All specimens collected have been sent to the Royal Botanic Gardens Edinburgh to be added to the herbarium collections.

Soil Sampling

Soil texture, type, colour, and density were collected during composition surveys. For each plot, five soil cores (at 5-10cm depth) were combined as a composite soil sample per plot (Frank 2008; Rahmanian et al, 2019). Cores were extracted adjacent to the centre plot and the 15m point along each transect to ensure an accurate representation of the area. Samples were dried in a convection oven at 120°C for 1 hour before being mixed and subsampled. All soil subsamples were exported back to Edinburgh for analysis. In the upcoming months, soil samples will be dried in oven and then ground to a fine powder in preparation for analysis. Proposed soil analysis will include carbon, nitrogen and phosphorous content as well as soil texture analysis. The James Hutton Institute can provide a broad chemical and soil property analysis.

Preliminary Results

During the 3-week expedition, we conducted 17 plots across two habitat types (9 open alkaline and 8 loess) (Figure 4). Preliminary outcomes of the expedition data are outlined below:

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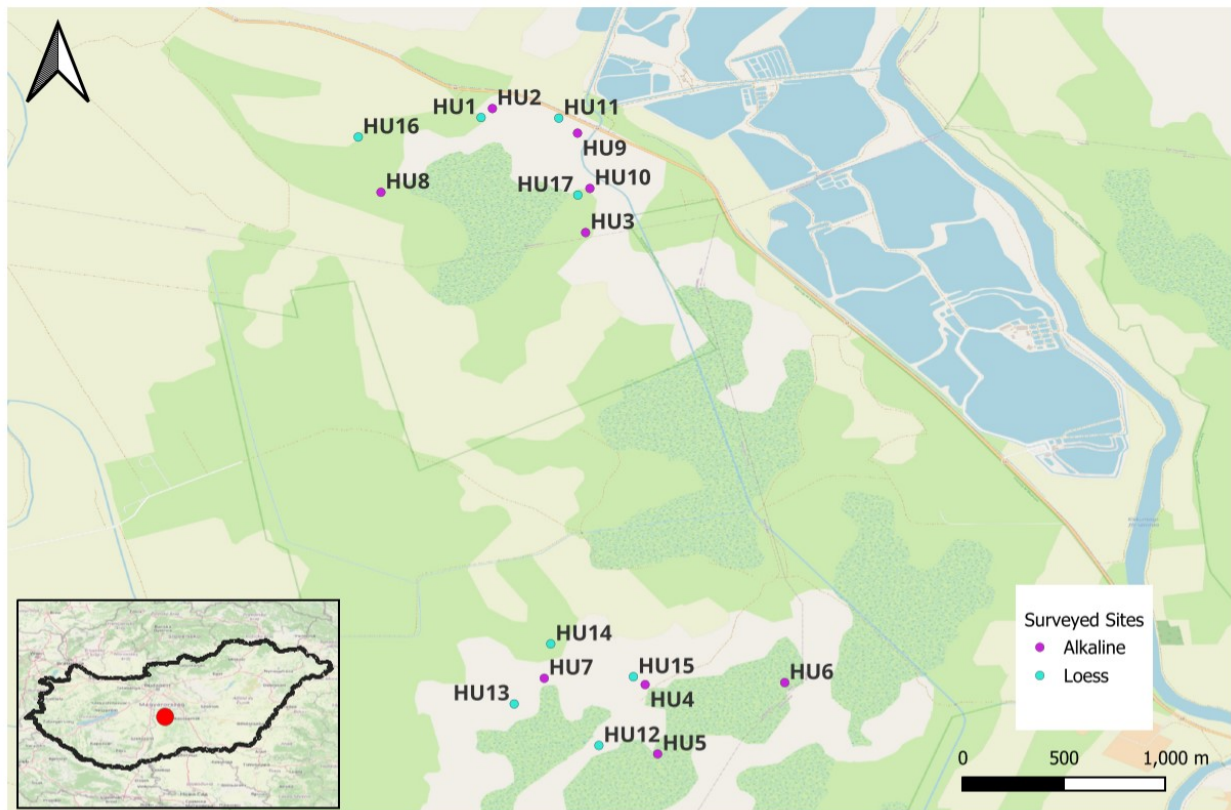


Figure 4- Map showing survey site location within Kiskunság National Park, Hungary. Sites were categorized as open alkaline or loess grassland habitat. A total of 17 sites were surveyed across the area.

Vegetation height and Grass Species Richness

As expected, grass height decreased as grazing increased in both the alkaline and loess habitats (Figure 5A). Repeated grazing concentrates grass biomass at the soil surface, reducing canopy height (McNaughton, 1976, 1983, 1984; Verweij et al., 2006). Litter depth decreased with grazing in the loess habitat however remained consistently low in the alkaline habitat (Figure 5B). This is likely because the open alkaline habitats flood frequently, preventing litter biomass build-up (Figure 6A). Grass species richness was consistent across all alkaline sites, ranging between one to three species. Across loess sites we saw a wider range of species richness that increased as grazing increased (Figure 6B). We expected loess grasslands to have higher diversity than the alkaline as loess grasslands are one of Hungary's most species-rich habitat types (Csontos *et al.*, 2022).

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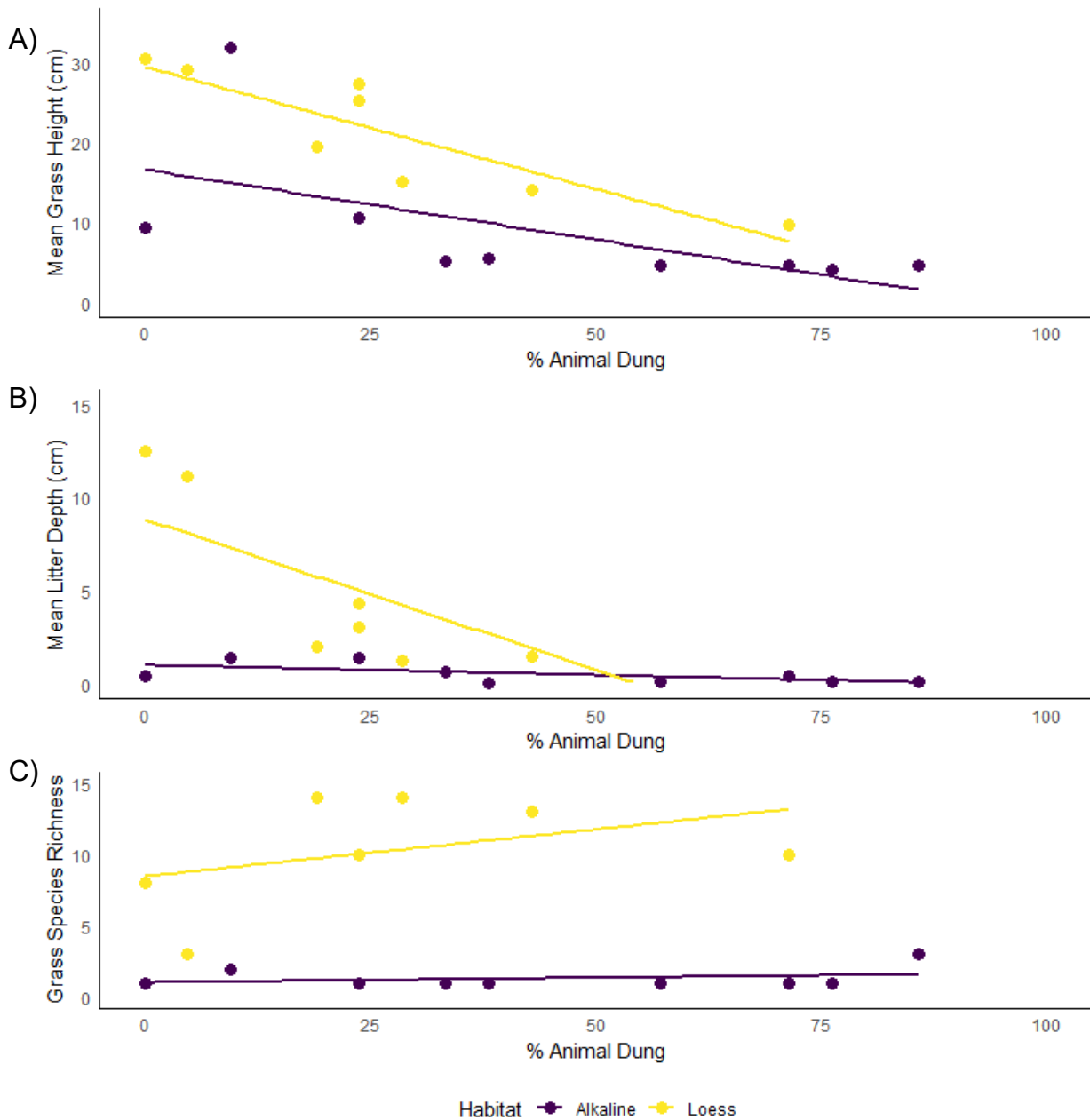


Figure 5- Relationship between grazing intensity and three ecological metrics: (A) Mean grass height (cm), identified at the 80th percentile of vegetation height, (B) Mean litter depth (cm), measured as the height of grass and leaf litter above bare ground, (C) Grass species richness. Measurements were collected from alkaline and loess sites across a grazing gradient, represented as the percentage of circular plots containing animal dung.

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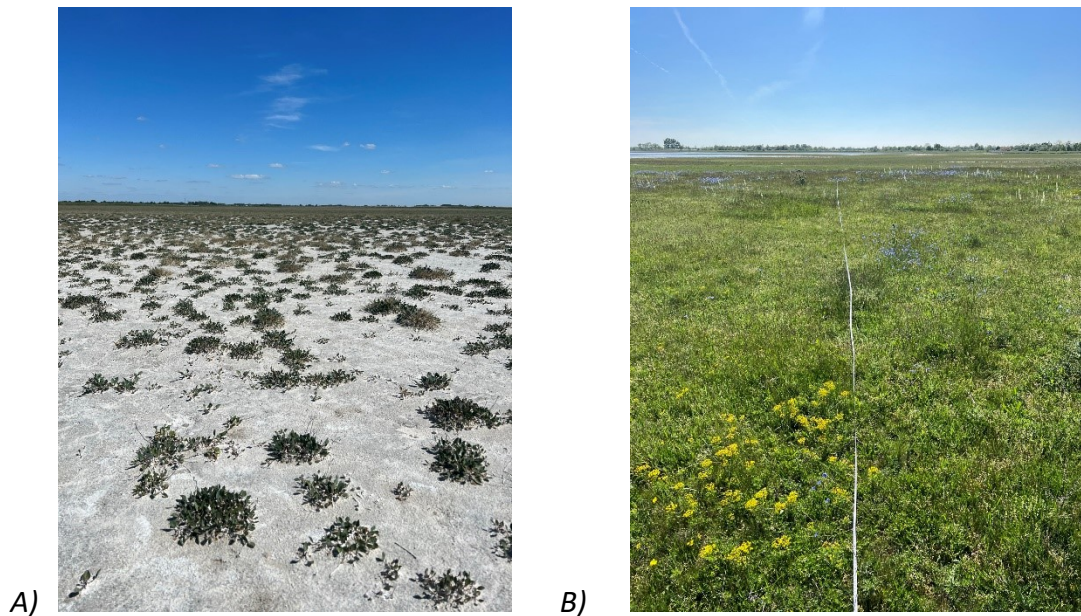


Figure 6- Photos of (A) alkaline and (B) loess habitats surveyed within Kiskunság National Park, Hungary in May 2023. These photos show the significant differences between the two habitat types.

Percent Ground Cover

Within alkaline habitats, low bare ground and high plant cover were observed at both high and low grazing intensities (Figure 7 A, B, E, F). In both habitats, low grazing intensities allows plant biomass to build as biomass is not removed, resulting in higher ground canopy and plant cover. At high grazing intensities, bottom-up compensatory growth enables plants to rapidly replace damaged tissue quickly while keeping biomass close to ground level (McNaughton, 1979, 1983). As mentioned before, these alkaline grasslands flooded frequently which is a contributing factor to the high bare ground and low litter cover in comparison to loess grasslands, which had relatively low bare ground cover, and high plant and litter cover (Figure 7 C, D).

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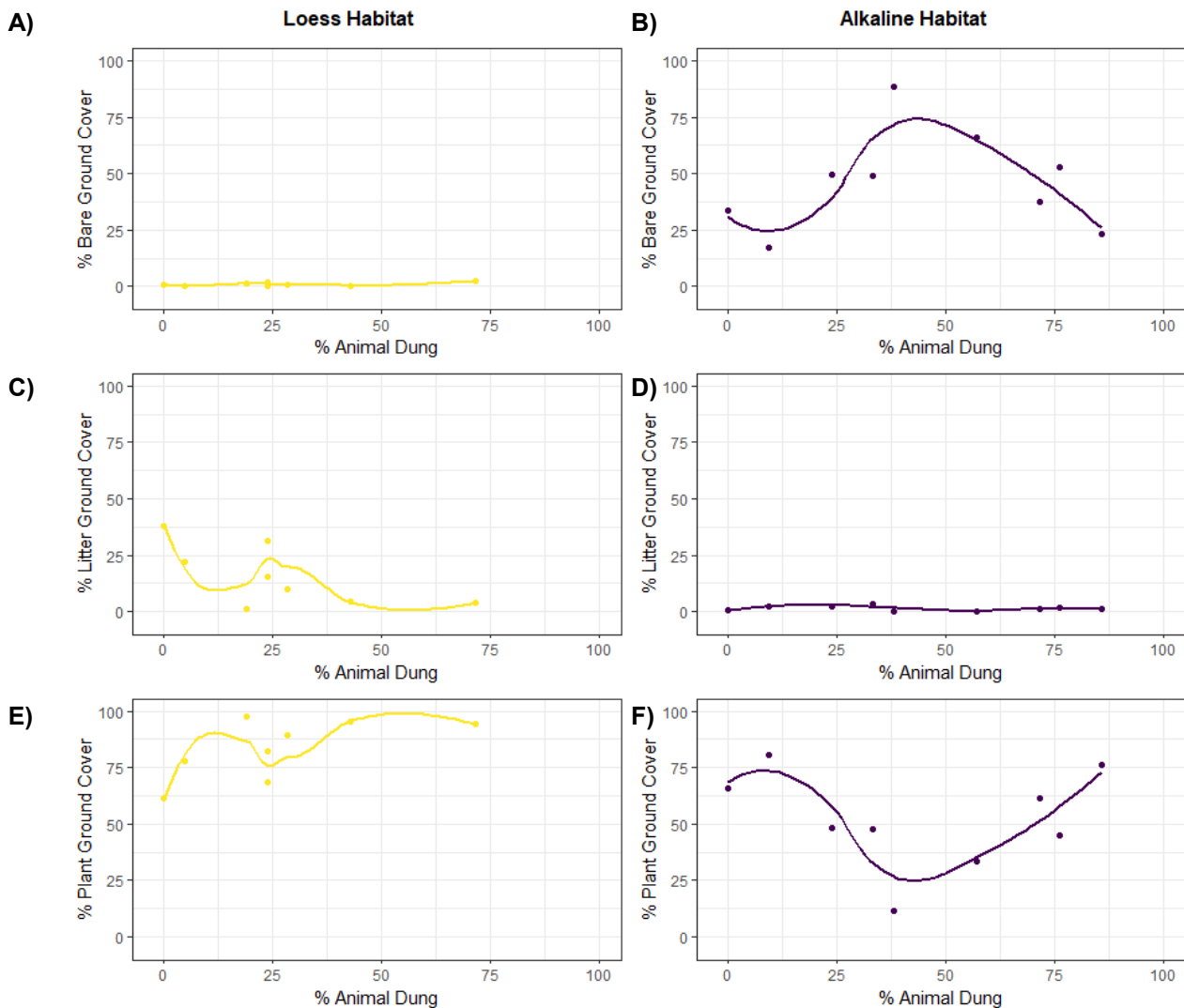


Figure 7- Relationship between grazing intensity and ground cover: Ground cover was recorded as the percentage (out of 100) of bare, litter, and plant cover within each of the 21 circular plots at each site. Measurements were collected from alkaline and loess sites across a grazing gradient, represented as the percentage of circular plots containing animal dung.

Percentage Plant Cover

For alkaline grasslands, we observed few changes in plant cover as grazing increased (Figure 8 B, D, F, H, J). At medium grazing intensities, tussock grasses reduced in cover however, this is due to an increase in bare ground cover (Figure 8 H, Figure 7 B). Alkaline habitats were dominated by tussock forming grasses that can persist through repeated flooding. In contrast, loess habitats contained a mixture of functional groups (Figure 8 A, C, E, G, I). At low grazing intensities, loess sites' plant cover was dominated by tussock-forming grasses with little forb or legume forb cover. As grazing intensity increases, overall diversity increases with more forb, legume forb, mat-forming grasses occurring. It appears that diversity in the loess and alkaline habitats peak at medium grazing intensities (25-60%) which is in agreement with Török et al, (2016).

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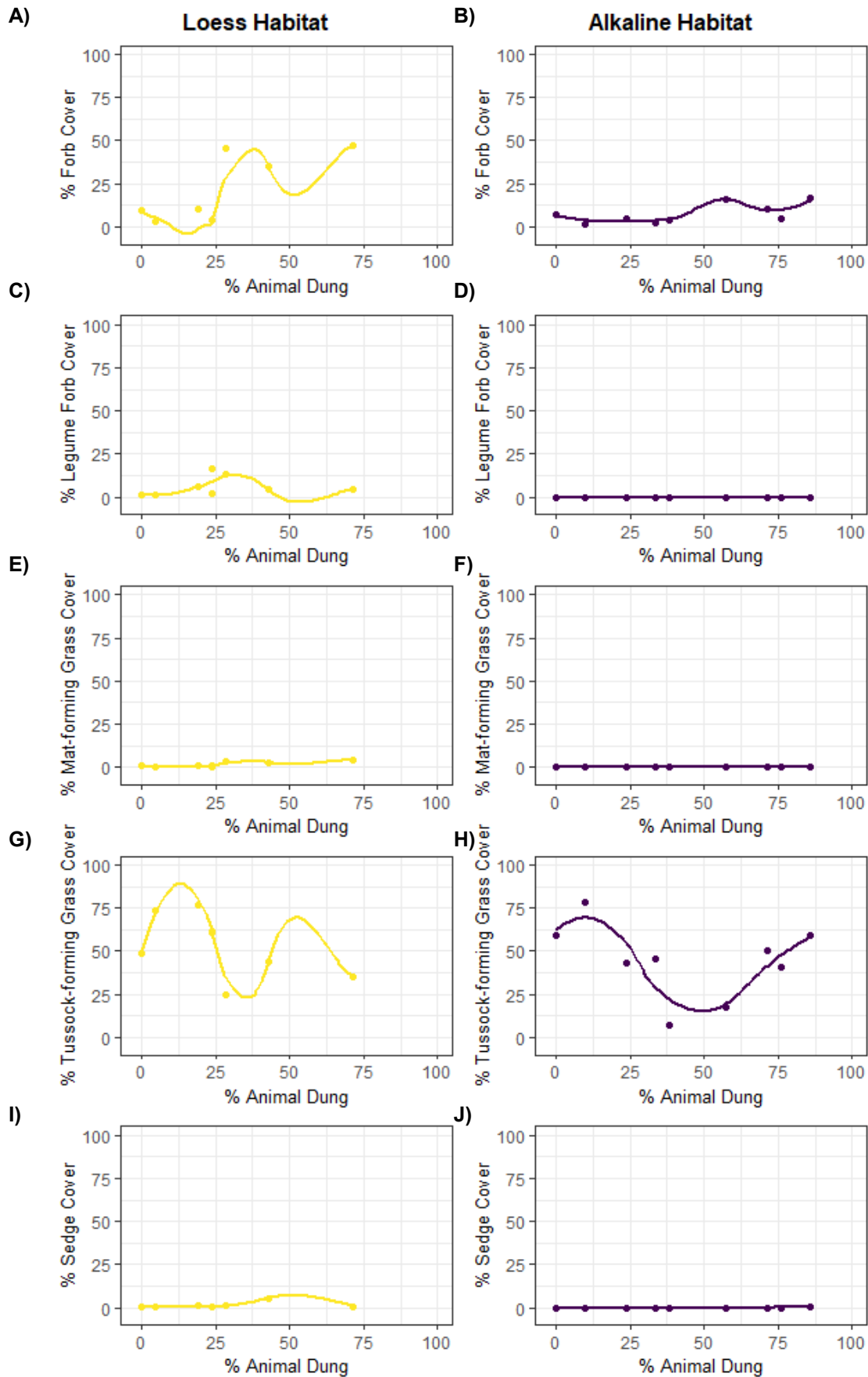


Figure 8- Relationship between grazing intensity and plant cover analysed for five plant functional groups: Data was collected within each of the 21 circular plots at each site with the percentage of plant cover divided into ten

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distinct plant functional group, five of which are shown here (Forb, Legume Forb, Mat-forming grass, Tussock-forming grass, and Sedge). The plant functional group cover within a plot sums up to the total percentage of plant cover. Measurements were collected from alkaline and loess sites across a grazing gradient, represented as the percentage of circular plots containing animal dung.

Functional Trait and Chemical Analysis

Functional trait and chemical analysis have yet to be conducted as data is still being collected and processed. Chemical analysis will be conducted on leaf and soils samples from all sites. Leaves will be ground to a fine powder and analysed for leaf nitrogen, carbon, and phosphorus content. Leaf nitrogen content is positively correlated with maximum photosynthetic rates and increases with the palatability of grasses making it essential to understanding the impacts of grazing on plants traits (Field et al., 1983). As mentioned before, chemical analysis will also be conducted on soils collected at each site. Soil analysis will allow us to disentangle changes in plant composition and traits caused by grazing from those resulting from environmental differences.

Expedition Expenditure

Item	Total Cost
International Flights +Luggage + Airport Transfers	£1425
Accommodation	£955
In country Transportation (Car Rental + Petrol + Trains)	£759
Food (Groceries + Restaurant)	£620
Field Kit	£314
Misc. Costs	£96
Plant/Soil Chemical Analysis, Consumables, and Equipment (Pending)	£2300.00
Total Expedition Cost	£6469.00

The remaining post-expedition expenses include the essential plant and soil chemical analysis of sampled material. Costs of this analysis, equipment, and consumables are estimated to be £2300. This soil and plant analysis will be completed in the next 6-8 months pending lab availability.

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Project Participants

Group Members:

Group Leader: Susan Eshelman, Ph.D. Student at the University of Edinburgh, Royal Botanic Garden Edinburgh, and Royal Botanic Gardens Kew

Susan's Ph.D. research focuses on understanding grazing as a process shaping grassy ecosystems by studying compositional and functional trait shifts in grassy floras. She investigates grazed ecosystems globally by evaluating the processes that shape community assembly within grazing lawns. She is a trained botanist with over 5 years of field experience in a variety of ecosystems. This project will form a part of her Ph.D. thesis and allow her to explore a different ecosystem type to her previous work and will build her leadership, fieldwork, and data handling skills. Susan will lead planning for the expedition, development of field survey techniques, and liaison with project collaborators in Hungary. Susan will also lead data handling and analysis with the student team members after the return of the expedition.

Research Assistant: Lorna MacKinnon, University of Edinburgh alumna

Lorna is an experienced botanist and plant taxonomist. She completed her MSc Biodiversity & Taxonomy of Plants from the University of Edinburgh & Royal Botanic Garden Edinburgh in 2007. Since graduating, Lorna has worked in the Middle East and tropical Africa. She continues to work closely with Royal Botanic Garden Edinburgh through various projects.

Hungarian Scientific Collaborators: Lendület Seed Ecology Research Group, Institute of Ecology and Botany, Centre for Ecological Research, Vácrátót, Hungary

This project will collaborate with the Lendület Seed Ecology Research Group, led by Dr. Orsolya Valkó and Dr. Balázs Deak. This research group focuses on the restoration and conservation of grassland ecosystems through seed and landscape ecology approaches and has conducted research in Kiskunság National Park for almost five years. Dr. Valkó and Dr. Deak have local expertise in grass identification and have also been involved in the identification of appropriate and safe field sites and will advise on accommodation, site access, and permit requirements. One of the team members will work with the field project in the first week of work to facilitate local orientation.

Local Collaborators:

Dr. Balázs Deak and Dr. Orsolya Valkó (*Lendület Seed Ecology Research Group, Centre for Ecological Research, Vácrátót, Hungary*)

Project Advisors:

Dr. Graham Stone (*University of Edinburgh, Institute of Ecology and Evolution*)

Dr. Caroline Lehmann (*University of Edinburgh, School of Geoscience and Royal Botanic Garden Edinburgh*)

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Dr. Maria Vorontsova (*Royal Botanic Gardens, Kew*)

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