The gut microbiome, which is the collection of bacteria, fungi, viruses and protozoa inhabiting the gastrointestinal tract, is intricately involved in many aspects of host physiology throughout life. The diet plays a central role in shaping the composition, function and diversity of gut microbiome. The interplay between diet and gut microbiome composition is important for health and nutrition of mammals.

Little is known about how seasonal changes in diet composition and quality determine gut microbial communities, which is important in the digestive process and performance of wild herbivores including parasite resistance. Studies on diet and microbiome linkages in domestic and captive animals do not fully reflect the relationships observed in natural populations. Therefore, undertaking research on the gut microbiome in wild large herbivores is important for better understanding of the relationship between foraging strategies and microbiome diversity and its function.

We plan to study the gut microbiome of the European bison (*Bison bonasus*), the largest European herbivore, rescued from extinction and characterised by low genetic variability. The high sexual dimorphism and the diversity of occupied habitats and foraging conditions (including supplementary feeding) of different bison herds create a natural field experiment and make the species an ideal model to study the influence of environmental and biological factors on microbiome diversity and host resistance to parasites.

We hypothesisee that_microbiome diversity will be higher when bison forage on a wider variety of plants and higher quality forage. It will also be higher in males. Supplementary feeding and the use of agricultural crops will strongly modify the bison microbiome. We also expect that diet, by altering the microbiome, will influence parasite resistance in bison.

We intend to test the above aims and assumptions by analysing the composition of the gut microbiome and the quality and composition of the diet using fresh faecal samples collected in different seasons and from European bison herds.

The microbiome diversity will be analysed using advanced NGS sequencing methods (16S rRNA metabarcoding sequencing and shotgun metagenome sequencing). The metabarcoding of the DNA fragments encoding the P6 loop of the trnL chloroplast intron and 28S rRNA will be used to study diet composition of bison and identify nematodes in faecal sample. We will also use various modern bioinformatic tools and sequence databases to identify groups/taxa of microorganisms, plants and nematodes. Finally, we will use advanced statistical modelling to test the effects of diet composition and quality (including supplementary feeding), body size and environmental conditions on the diversity of the gut microbiome and the presence of bacteria inhibiting parasitic nematodes.

The project represents an innovative approach as it combines non-invasive collection of research material and next-generation DNA sequencing, DNA-based dietary analysis, near-infrared diet quality analysis and statistical modelling. This is the first time such research will be carried out on the European bison, the largest land mammal in Europe and one of the last remnants of the legendary megafauna. This species has been recognised as a refugee species, adapted to open habitats and restricted to sub-optimal forest habitats. A highly innovative aspect of the study is the investigation of the influence of host diet and gut microbiome on parasite resistance. As parasites are a major threat to the conservation of European bison and other wildlife, the results of this project may help to identify mechanisms responsible for parasite prevalence and dynamics, and provide tools for adaptive management of large mammals.