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Plant diets in the Italian Alps: Refining species-level resolution from fecal shotgun sequencing

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Accurate diet characterization is fundamental to understanding the ecology and resilience of wildlife in sensitive alpine ecosystems. While DNA metabarcoding has become a standard tool, it often lacks the taxonomic resolution to distinguish closely related plant species for precise ecological monitoring. We evaluate a high-precision framework based on shotgun sequencing to achieve species-level resolution in plant diet analysis, providing a robust alternative for monitoring mountain wildlife. Our approach is being validated using the Alpine rock ptarmigan (*Lagopus muta helvetica*), a boreal, cold-adapted glacial relict inhabiting high-altitude environments in the Italian Alps, serving as an ideal model for testing dietary resolution across alpine flora. We evaluate shotgun sequencing with the Kraken2 classifier across diverse reference databases, including full genomes, chloroplast markers, and ITS sequences. A key component involves enhancing reference databases by integrating the latest sequences from NCBI-GenBank in a quality-controlled and curated manner, ensuring the highest possible taxonomic coverage for alpine-specific plants. Results highlight the critical role of database selection and demonstrate that shotgun metagenomics allows for parallel identification of plant content and microbial communities within the same fecal samples. Our findings suggest functional links between diet and the gut microbiome; for instance, medicinal plants correlate with reduced pathogenic bacteria, suggesting a role in modulating animal health. Furthermore, detecting soil-health indicators like *Pseudomonas putida* and plant pathogens like *Rhizobium tumorigenes* demonstrates that single fecal samples simultaneously inform on host health, diet, and habitat-level integrity. This scalable approach is highly transferable to the conservation of other alpine species and their habitats, such as those of the Alpine chamois (*Rupicapra rupicapra rupicapra*) or mountain hare (*Lepus timidus*).