## Bacterial and fungal diversity of Alpine pastures

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Alpine pastures are a complex, multidimensional terrestrial habitat in which animal and plant species interact with each other and with large numbers of bacteria and fungi (*microbiota*), forming an ecological network that extends both above ground and within the soil. Although soil-, plant-, and animal-associated microbiota are known to provide fundamental services for their hosts, microbial diversity of ecosystems in general, and Alpine pastures in particular, is still largely unexplored. In addition, the extent to which climate changes in Europe and especially in the Alpine space will impact such micro-biodiversity is completely unknown. Here, we investigated changes in bacterial and fungal diversity associated with more than 900 samples of soil, rhizosphere (*Carex* spp. and *Festuca* spp.), invertebrates (nematodes, collembolans, earthworms, beetles) and vertebrates (using faecal eDNA of hares, wild ungulates and livestock) along an elevational gradient spanning 1,500 metres (a proxy for climate change). Characterization of bacterial and fungal communities of each sample was performed by targeting the bacterial 16S rRNA gene V3-V4 region and the fungal ITS2 loci as barcodes for amplicon sequencing-based meta-taxonomics. Diversity metrics of ASVs (amplicon sequence variants) and phylogenetic distances (Bray-Curtis, Unifrac) were used in redundancy analyses (db-RDA) to compare microbiota composition across sample types and elevation gradient. We found that soil, plant, invertebrate, and vertebrate microbiota were characterized by only partially overlapping bacterial and fungal communities, displaying specific associations with host, elevation, temperature, nutrient availability, and plant community composition. Faecal microbiota of wild and farmed animals revealed different patterns across bacterial and fungal microbiota, with bacterial communities being markedly shaped by host-interaction dynamics, and fungal communities showing closer associations with habitat and elevation. Analyses of fungal and bacterial taxa shared between sample types established greater overlaps between soil, rhizosphere and soil-dwelling invertebrates, compared to other invertebrates and vertebrates, highlighting above/belowground and host/habitat-specific associations in the Alpine meta-community.

*Keywords:* microbiota, 16S rRNA gene, ITS2, amplicon sequencing, meta-taxonomy

### Connection to biodiversity conservation, connectivity, and restoration

The micro-biodiversity of Alpine pastures is almost completely unknown, yet pivotal to the definition of biodiversity conservation strategies in this characteristic agro-ecosystem in light of climate warming. In addition, evaluating the level of connectivity between bacterial and fungal communities along an altitudinal gradient hosted by Alpine soil, plants, soil-dwelling invertebrates and above ground fauna is one of the main goals of this research project.

### Policy implications

By studying microbiota along an altitudinal gradient, our results provide additional insights regarding how future climate change may affect the bacterial and fungal diversity of soil and dominant vertebrate and invertebrate animal species in one of the most characteristic, species-rich, and economically and culturally important Alpine habitats. Thus, our results will have implications for management of mountain agriculture, biodiversity conservation, landscape preservation, and climate change mitigation strategies.