More than meets the eye: unravelling anthropic land use impacts on skin microbiota of an opportunistic amphibian species

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As amphibians register population declines worldwide as a result of human-driven habitat modifications and emerging diseases, their skin microbiota has attracted major interest as a means of adaptation to the changing environment and a barrier against pathogens. A substantial body of research has been published on the characterization of the bacterial taxa found on amphibian skin, or their variation between species and/or life stages, as well on the interaction between amphibian skin microbiota and potential pathogens, especially the fungus *Batrachochytrium dendrobatidis*, showing how bacterial skin communities can influence the resistance of amphibian wild populations to this pathogen. However, the diversity of amphibian skin mycobiota and its potential interaction with the bacterial component of microbiota is still largely unexplored. This study aimed to characterize the diversity patterns of both bacterial and fungal communities living on the skin of *Bombina variegata* (Linneaus 1758), and to highlight the associations between these patterns with biotic and abiotic parameters of their habitats, including the microbiota of the wetland itself. Skin swabs of about ten individuals were collected from populations of four habitat categories, with varying degrees of human impact. Alpha and beta diversity indices were calculated for both communities from the 16S (bacteria) and ITS1 (fungi) amplicon variants (ASVs) retrieved. Differences across habitats were statistically tested, as well as the influence of abiotic factors and that of one community on another. We found a clear association between skin and water microbial communities in terms of relative abundance of bacterial and fungal ASVs shared between the two microbial communities. Both alpha and beta diversity indices calculated from *B. variegata* skin swabs were found to be statistically different across habitats, although different patterns were found for bacterial and fungal diversity. Beta diversity indices also showed close clustering of water and skin communities for each habitat and sampling site. Water pH, temperature and dissolved oxygen proved to be statistically significant factors affecting both bacterial and fungal diversity, but, again, with different patterns between alpha and beta diversity.

Keywords: amphibians, skin microbiota, land use, anthropic impact, pathogens

Connection to biodiversity conservation, connectivity, and restoration

This study contributes to biodiversity conservation, as amphibian skin microbiota has an important role in both adaptation to the changing environment and as barrier against pathogens. This research is one of the first focusing on both bacterial and fungal diversity, providing relevant insights into this relatively unexplored component of wild population biodiversity. In fact, we highlighted complex, and sometimes opposing, trends, which will require more studies to be fully understood. These first promising results also support the hypothesis that human activities contribute to shaping amphibian skin microbiota. We also demonstrated the existence of a strong association between skin and environmental microbial communities. Overall, we argue that conservation strategies for B. variegata, and amphibians more in general, should be based on a better understanding of the aquatic/environmental microbiota and the impact of anthropogenic activities in these environments.

Policy implications

This study highlighted that some habitats, such as farm ponds or agricultural water tanks, can be important breeding sites for the target species. Therefore these sites could benefit from small interventions or ad-hoc management practices like constructing wooden fences that do not allow cattle to enter the farm pond and disturb the water.