

# **Prokaryotic diversity and ecosystem functioning in hypersaline environments: a metagenomic study of the Cagliari salterns**

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Solar salterns are extreme environments of high scientific interest, hosting specialized microbial communities shaped by steep physicochemical gradients. Here we present the first comparative metagenomic analysis of two solar salterns in southern Sardinia: Conti Vecchi (SCV, active) and Molentargius (SM, inactive), located ~15 km apart and exposed to similar climatic conditions. This setting provides a unique opportunity to assess the impact of saltwork management on microbial diversity along a salinity gradient.

Water and sediment samples were collected from ponds with varying salinities in winter and summer. A total of 2786 metagenome-assembled genomes (MAGs) were reconstructed, 1537 from SCV and 1249 from SM, including 1131 MAGs from sediments and 1655 from water. Physicochemical analyses identified seasonal variation as the primary driver of environmental differences ( $p$ -value < 0.001), explaining 61% of total variance, although site-specific differences in thermal range and pond structure were also observed.

Benthic trophic conditions varied markedly across ponds. The medium-low salinity pond SCV-1 (57 psu) was eutrophic, with organic carbon exceeding microbial processing capacity, while the high-salinity pond SCV-17 (275 psu) showed near-complete depletion of biopolymeric carbon. Metagenomic results supported these patterns, with *Pseudomonadota* and *Bacteroidota* dominating at low salinities, and *Halobacteriota* prevailing in high-salinity ponds.

In SM, the high salinity pond SM-3 (260 psu) exhibited a striking seasonal shift, with high degradation rates and rapid turnover in winter, and reduced activity in summer, suggesting substantial changes in microbial community. Taxonomic analysis confirmed this frame, revealing an unexpected dominance of Bacteria despite the high salinity. In addition, a large fraction of MAGs could not be assigned to known taxa, suggesting unexplored novelty spanning from species to higher taxonomic levels. Overall, these findings position southern Sardinia salterns as unexplored reservoirs of prokaryotic novelty and underscore the importance of expanding metagenomic investigations to other under-characterized Mediterranean extreme environments.