



Adaptation to salt in *Archaea* vs. *Bacteria*: a new “osmoregulatory” point of view



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INTRODUCTION

EXTREME ENVIRONMENTS → Exhibit particularly high or low levels of one or more physicochemical factors, such as pH, solar radiation, temperature, pressure, nutrients, or salinity.

HYPERSALINE ENVIRONMENTS = SALTERNS



These environments, characterized by high salt levels and nutrient scarcity, have been previously considered to harbour a low diversity of *Archaea* and *Bacteria*. Rising salinity levels not only decrease diversity but reshape the prokaryotic community, tipping the scales in favor of the *Archaea*.

Microbial capacity to survive in such extreme conditions is driven by different strategies, including specialized osmoregulation processes and dedicated metabolic pathways. However, our knowledge on these adaptation strategies is derived largely from studies on isolated microbes or on select hypersaline environments, with a focus on extreme salt concentrations. As such, we know little about salt adaptation strategies employed by environmental microbial populations and how they shape the dynamics of populations across a range of salt concentrations.

Marine salterns are a unique form of hypersaline environment that can serve as a natural laboratory to study such questions, as they comprise a series of ponds that range in salinity from seawater to salt saturation (Fig. 1 and 2).



Fig. 1. Isla Cristina salterns (Huelva).



Fig. 2. Sample collection in July 2021 in Isla Cristina salterns (Huelva).

1. Explore microbial taxonomic and functional diversity across a salinity gradient in marine salterns.
2. Determine whether microbial communities exhibit a tipping point along the salinity gradient, at which diversity is significantly reduced and *Archaea* become dominant.
3. Elucidate the mechanisms of osmoregulation and metabolic adaptations of microbial populations that shape the community at different salinities.

METHODOLOGY

A total of 34 metagenomes from the Isla Cristina and Isla Bacuta salterns (Huelva, southwest Spain), with salinities between 13‰ and 44‰ (w/v), were sequenced by Illumina NovaSeq (Fig. 3 and 4).

We taxonomically profiled the metagenomes into species-level units using the MOTUs database and assessed how diversity and community turnover shifted across the salinity gradient.

From these samples 1,478 MAGs were recovered using the cross-mapping binning technique and their metabolic capabilities were compared with MAGs from nine seawater metagenomes in the surrounding area of the studied salterns, together with over 800 reference genomes of cultured halophilic taxa. Bioinformatic tools like metaSPAdes, SqueezeMeta, GTDB-Tk, HMMER or eggNOG, among others, were used to perform this study.

This analysis enabled an exploration of the osmoregulatory mechanisms and their distribution across phyla, as well as the metabolic interactions within the community.

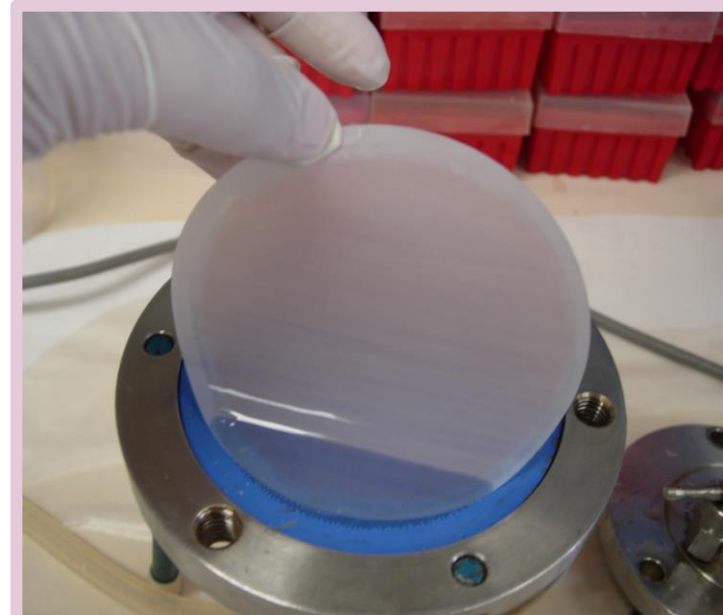


Fig. 3. Filter used in the first stage of metagenomic DNA extraction.



Fig. 4. Extraction of metagenomic DNA using the phenol:chloroform:isoamyl alcohol protocol.

RESULTS & DISCUSSION

DIVERSITY & COMMUNITY DYNAMICS

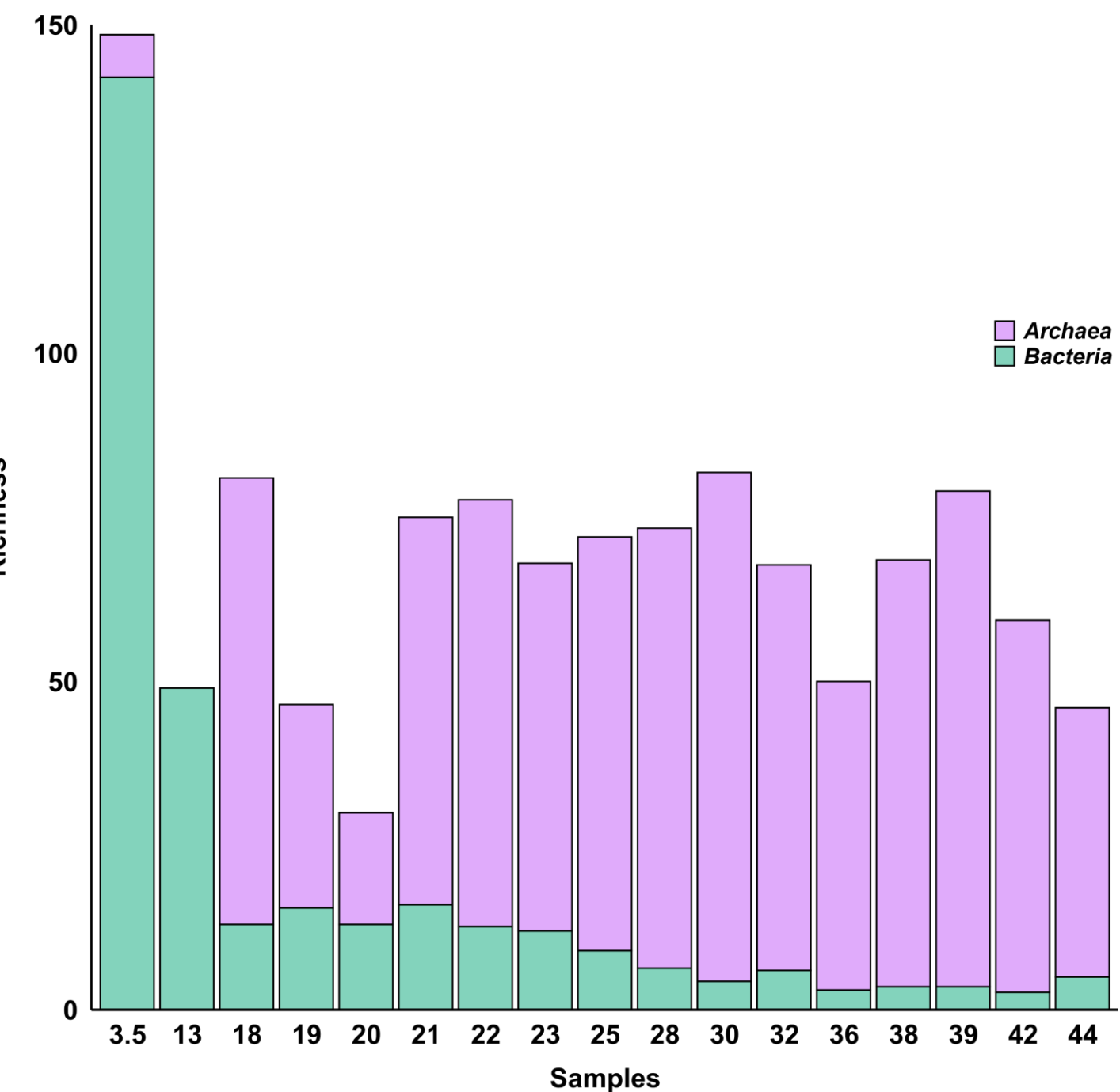


Fig. 5. Alpha diversity (Richness) calculated for the whole set of metagenomes.

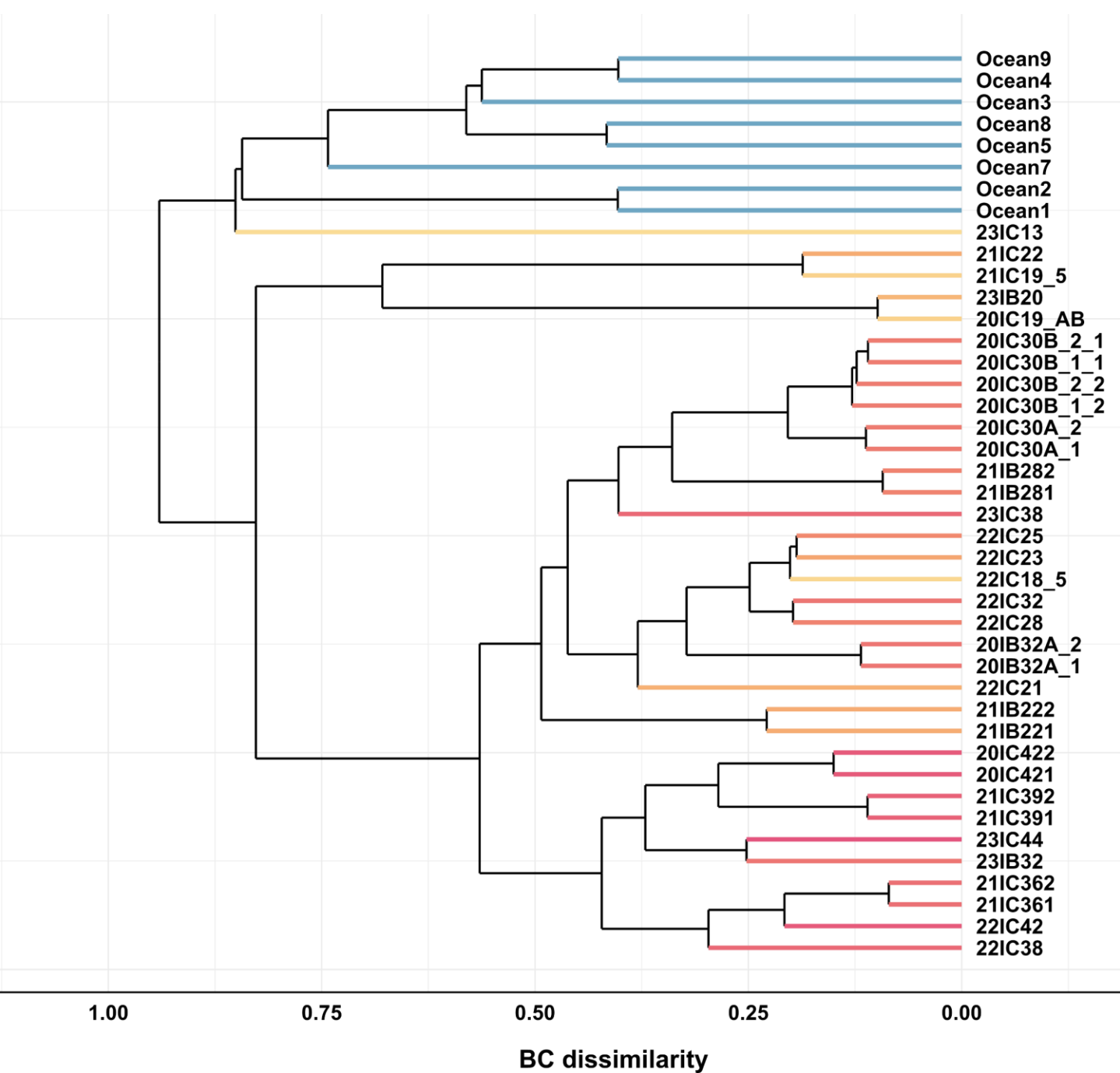


Fig. 6. Bray Curtis dissimilarity between samples.

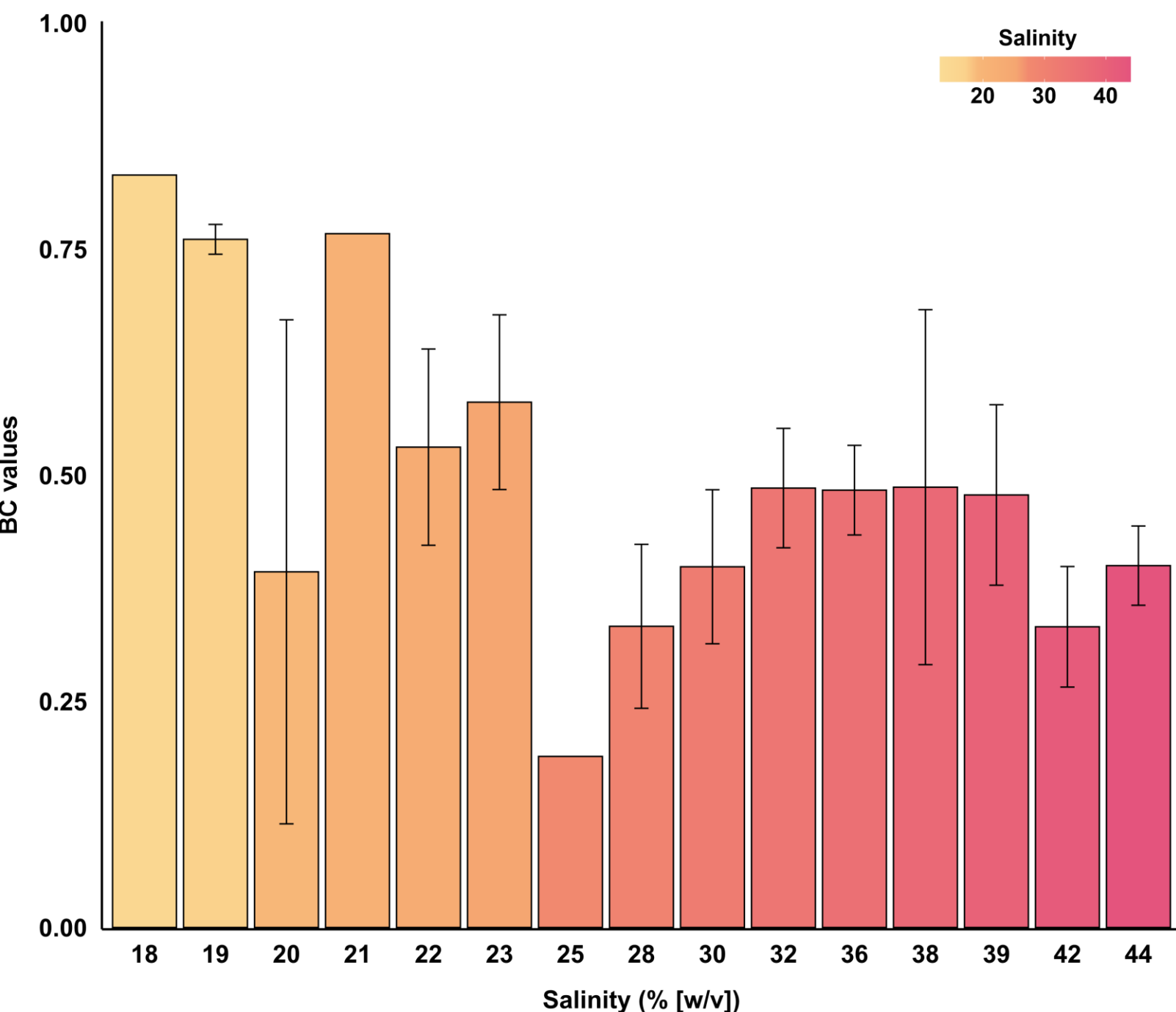


Fig. 7. Turnover rates between consecutive samples.

Fig. 5 shows that diversity does not decrease drastically with increasing salinity, as initially expected. However, *Archaea* do maintain their dominance within the community across the entire salinity range and even increase in abundance at higher salinities.

On the other hand, at intermediate salinities, we observe relatively stable diversity. Bray Curtis dissimilarities, based on the relative abundances of MOTUs, were calculated, and the samples were clustered accordingly in a dendrogram (Fig. 6). As shown, samples from low and high salinities group into a single cluster, respectively. However, the samples from intermediate salinities display a clear disorganization, which gradually converges toward the high-salinity samples.

Subsequently, turnover was assessed by comparing the abundances between samples from immediately successive salinity levels (Fig. 7). As observed, turnover values are high across the community, with a marked increase at intermediate salinities.

This suggests that the community undergoes multiple and continuous changes at these salinity levels.

The various osmoregulatory mechanisms known to occur in such environments (salt-in and salt-out strategies) were investigated across all the MAGs recovered and reference genomes.

These MAGs were analyzed by grouping them taxonomically according to the GTDB classification into three groups: *Bacteria*, *Archaea*, and a group referred to as “Nano”. The “Nano group” includes taxa formally classified within the *Archaea* domain (*Nanobdellota* and “Ca. Nanohaloarchaeota”) but which, a priori, display distinctive features—such as smaller, simpler genomes and a tendency toward symbiosis with other archaea.

As shown in the Venn diagram (Fig. 8), and contrary to expectations, there are very few differences in osmoregulatory mechanisms between *Archaea* and *Bacteria*, with only seven genes found to be exclusive to the *Bacteria* domain. A closer examination of the “Nano group” reveals that, surprisingly, it shares similar osmoregulatory traits with both *Bacteria* and *Archaea*, despite belonging to the latter.

Finally, a phylum-level analysis of osmoregulation (Fig. 9) reveals that the relevant genes are distributed in a nearly universal manner, blurring the phylogenetic boundaries in salt adaptation strategies and showing that osmoregulatory mechanisms are not domain-specific as was previously assumed.

CONCLUSIONS

- Diversity is not strictly inversely proportional to salinity, as expected.
- The community dynamics involve an initial drop in diversity, followed by a recovery and stabilization. However, the rate of change is very high and eventually decreases as saline saturation is reached.
- Osmoregulatory mechanisms are not domain-specific, blurring the phylogenetic boundaries in salt adaptation strategies.

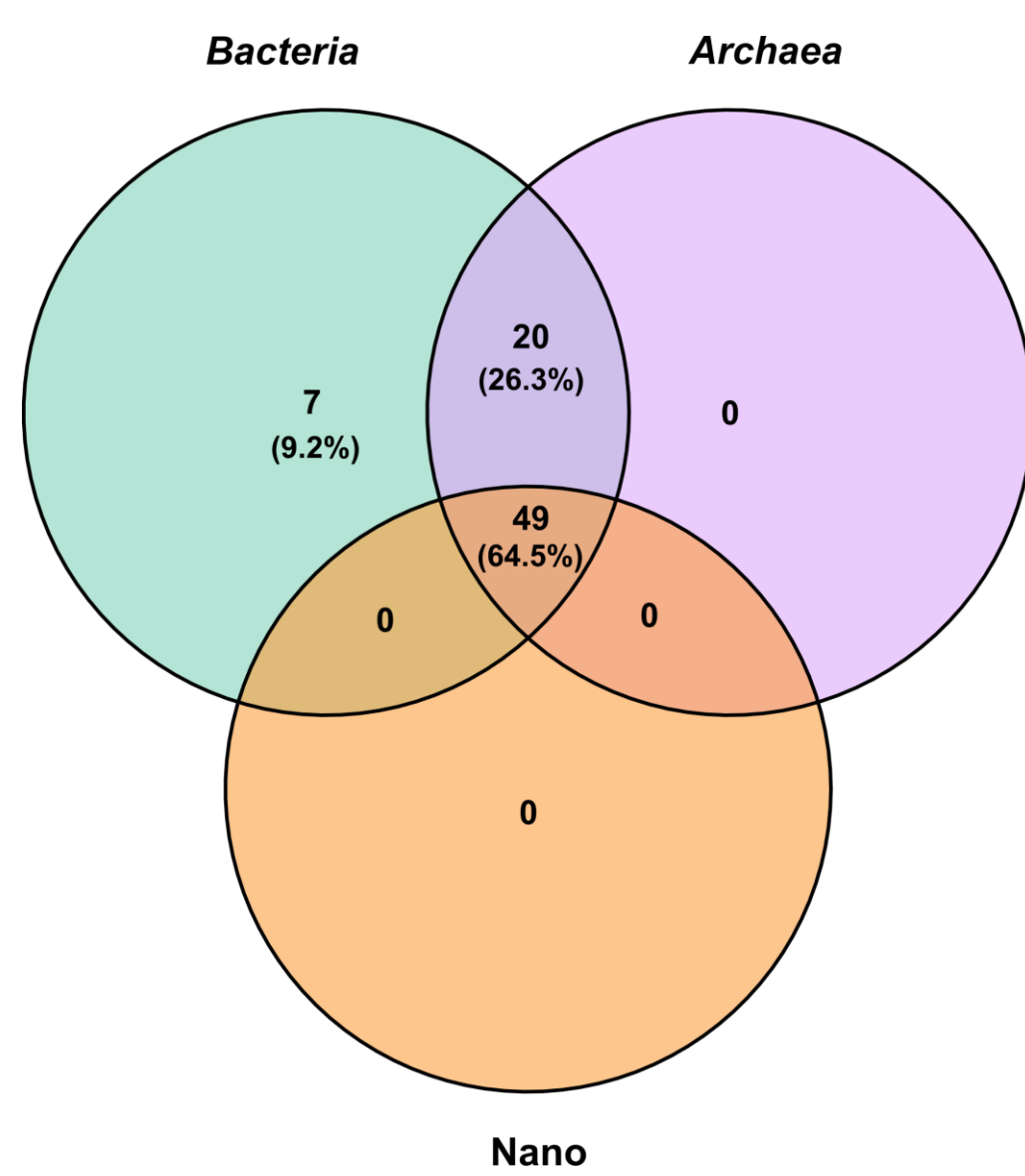


Fig. 8. Distribution of genes related to osmoregulation in the domains Archaea, Bacteria and “Nano group”.

OSMOREGULATORY MECHANISMS

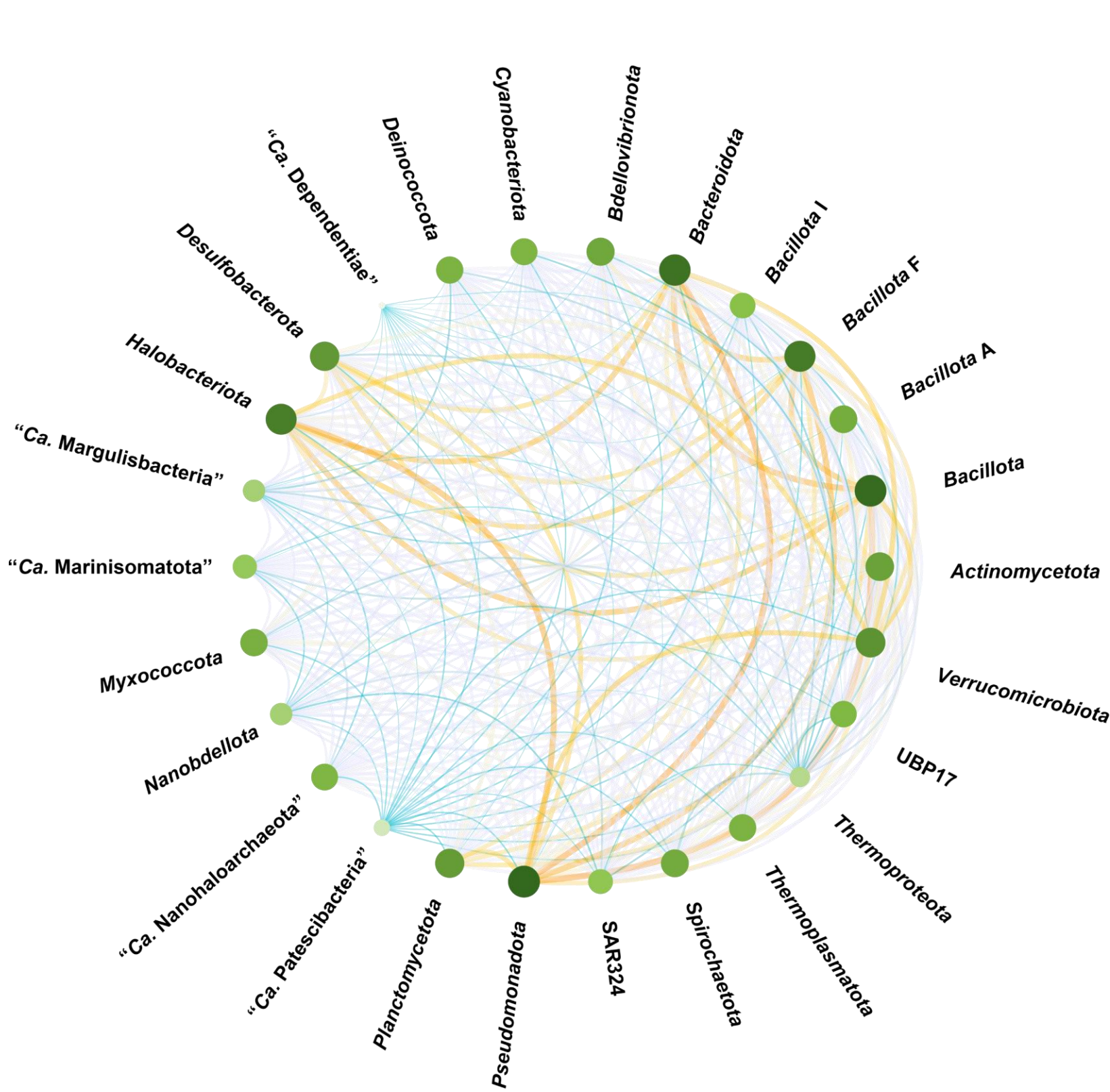


Fig. 9. Distribution of genes related to osmoregulation across all taxa associated with halophiles, as well as the degree of gene sharing exhibited by each phylum.

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