



MICROBIAL COMMUNITIES TRAITS AND HYDROGEOCHEMISTRY IN A COASTAL KARST AQUIFER SUBJECT TO SALINIZATION: A CLOSER LOOK AT INTERACTIONS

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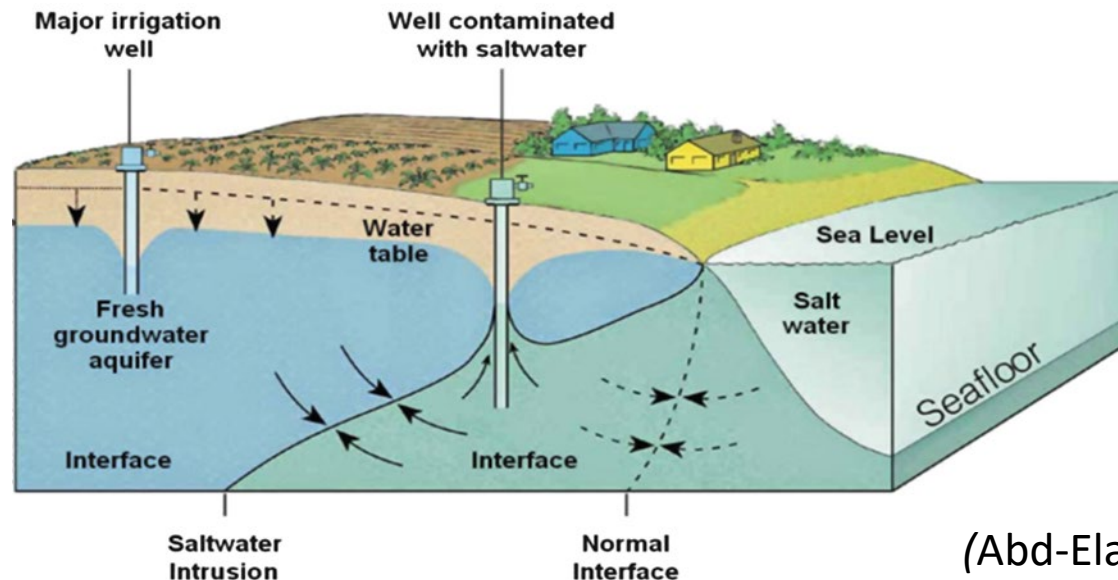


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Introduction

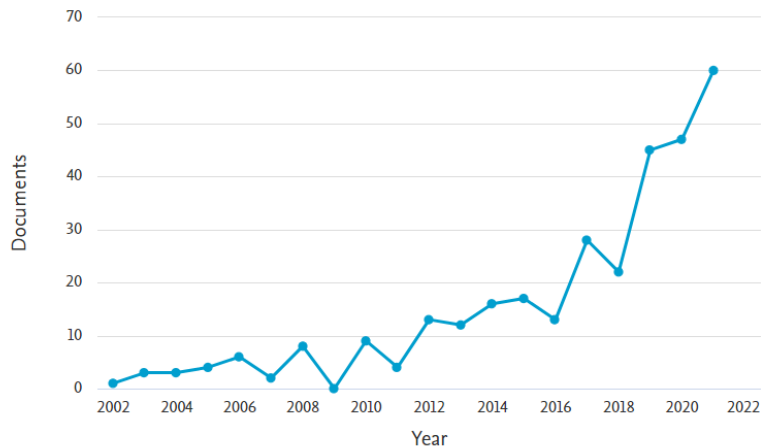
- ✓ 11% of the total groundwater is hosted in karst aquifers all over the world and supply potable water to nearly 25% of the world's population (WHO Report , 2011).
- ✓ Karst Coastal aquifer are prone to the salinization by owing to:
 - high circulation rate of groundwater determined by the lithological features
 - space-time variations in the extension of the recharge events of the aquifers
 - over-pumping in highly anthropized coastlines



(Abd-Elaty et al. 2018).

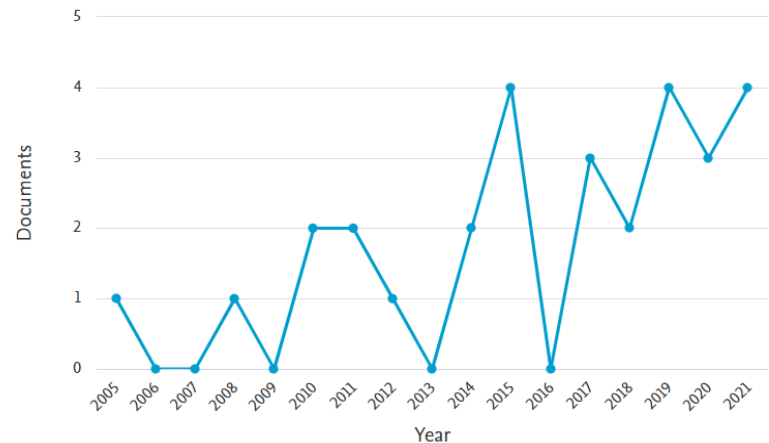
The issue of freshwater salinization effects on the microbial community in literature

Documents by year



Number of documents on **salinization** and **microbial communities** over the past 20 years (source: SCOPUS).

Documents by year



Number of documents on **groundwater** and **seawater intrusion** and **microbial community** over the past 20 years (source: SCOPUS).

Study aims

The VIOLA Project , co-funded by the European Regional Development Fund (**ERDF**) is aimed at identifying the natural background levels for groundwater bodies subject to saline intrusion.

In this frame, a scientific effort was dedicated to deepen the knowledge on the groundwater microbial ecology.

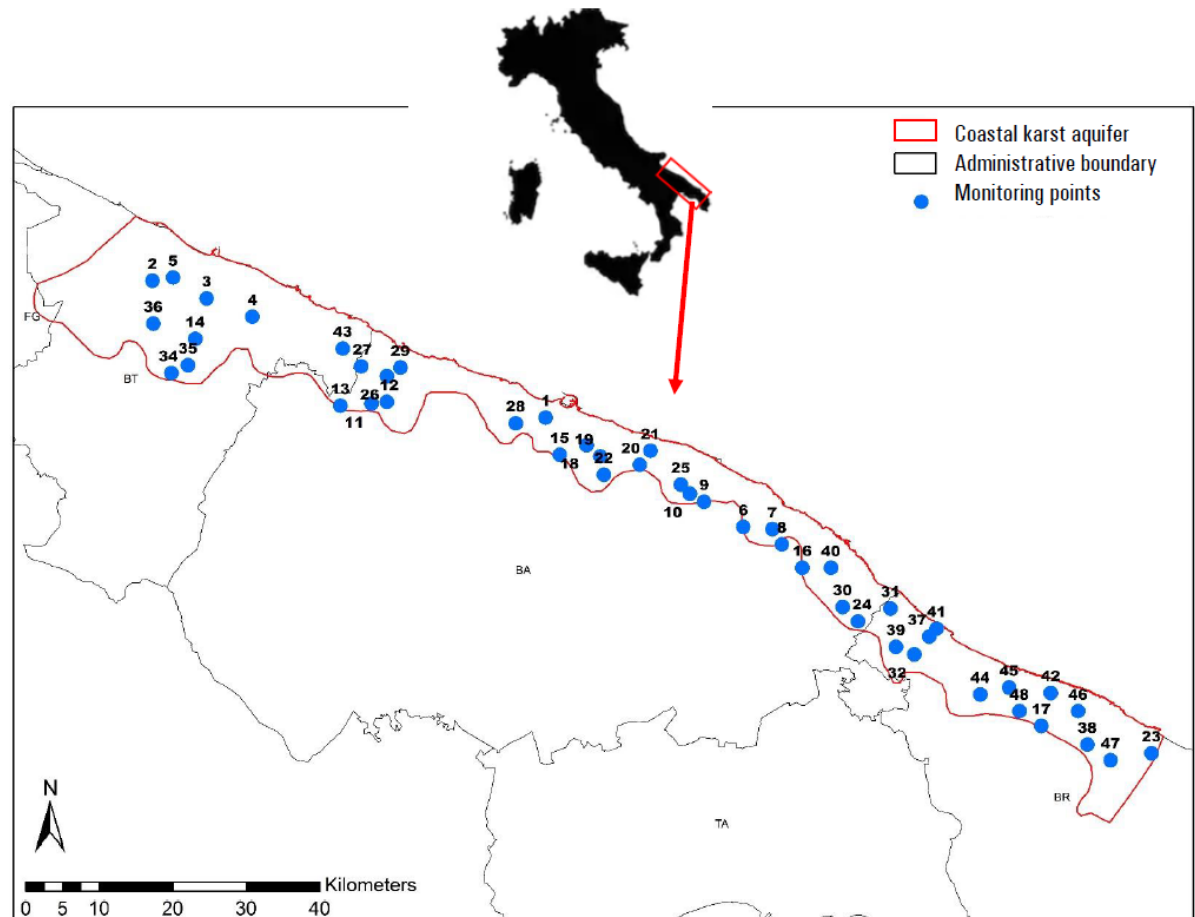
The main goals were:

- ✓ To explore the possible effects of the imbalance between freshwater and seawater in a coastal aquifer on the microbial functional traits and community structure across a salinity gradient
- ✓ To test a set of rapid and reliable assays to determine the sensitivity of microbial metabolic responses to environmental changes in a large-scale space-time study

Study area

The study has been conducted in the coastal sector of the Murgia karst aquifer located in a semi-arid climatic region in southern Italy (Apulia region) with an elevated agricultural vocation and consequent withdrawals of water for irrigation.

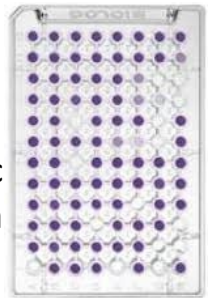
- ✓ An investigated area of 1,227 Km²
- ✓ 48 public/private wells
- ✓ Four sampling campaigns
- ✓ Total samples = 188



Study area and sampling sites.

Materials and Methods

- ✓ **Microbial cell abundance** : The total prokaryotic cell counts were measured through flow-cytometry analysis. This technique allows to discriminate cells with two different nucleic acid content (cells with low NA content - LNA; cells with high NA content - HNA)
- ✓ **Microbial metabolic potential and functional diversity**: These parameters were measured through the Biolog EcoPlates assay. The test detects the microbial degradative activity performed on 31 organic carbon sources after 24h of incubation.
- ✓ **Microbial respiration**: The intrinsic microbial ability to mineralize the dissolved organic carbon present in the samples was measured by the Biolog MT2 assay by detecting a colorimetric reaction after 24h of incubation.



- ✓ **Microbial extracellular enzyme activity**: The expression of 19 enzymatic activities belonging to five enzyme macroclasses was detected by a colorimetric reaction of the API ZYM assay after 72h of incubation.

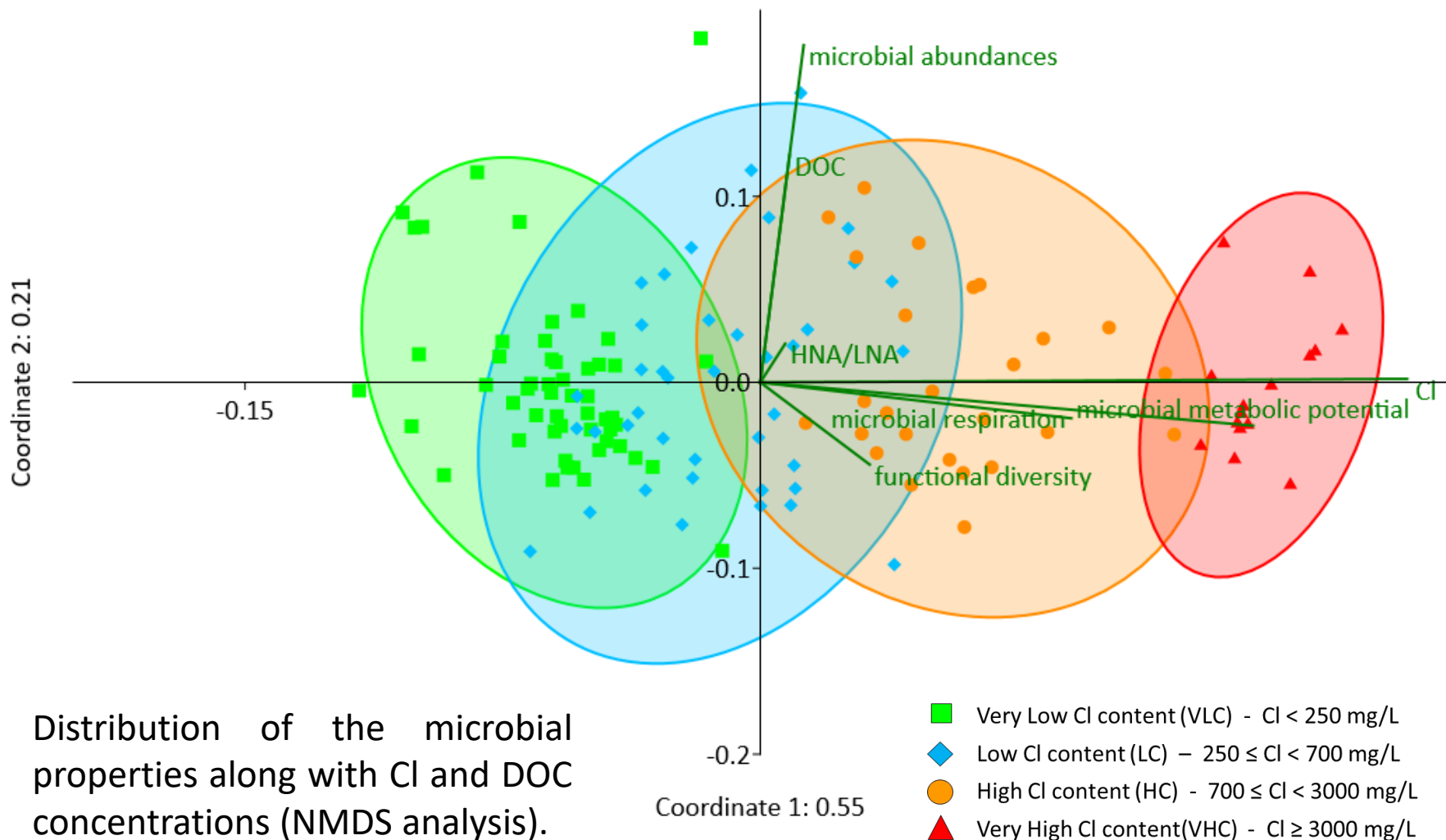
- ✓ **Faecal indicators**: The presence of total coliforms and E. coli in the samples was detected through the colorimetric reaction provided by the Colilert-18 assay after 24h of incubation.



- ✓ **Microbial biodiversity**: The phylogenetic composition of the microbial community was assessed by 16S rRNA gene amplicon sequencing, to identify the dominant taxa retrieved in the samples

Results: microbial properties across the salinization gradient

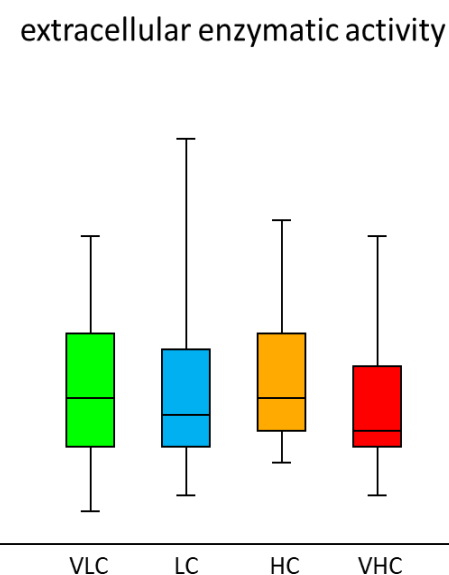
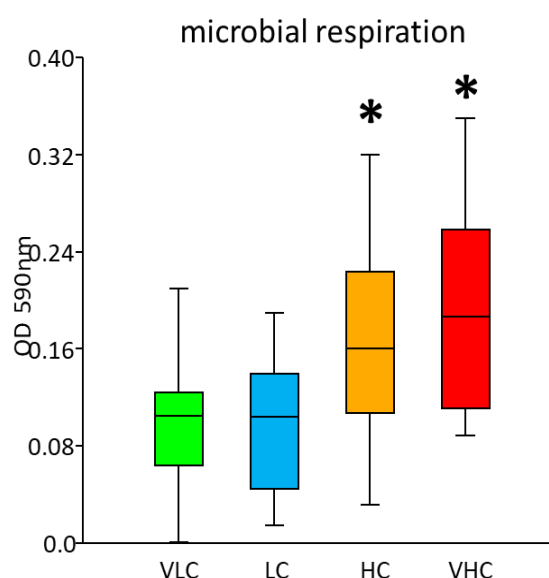
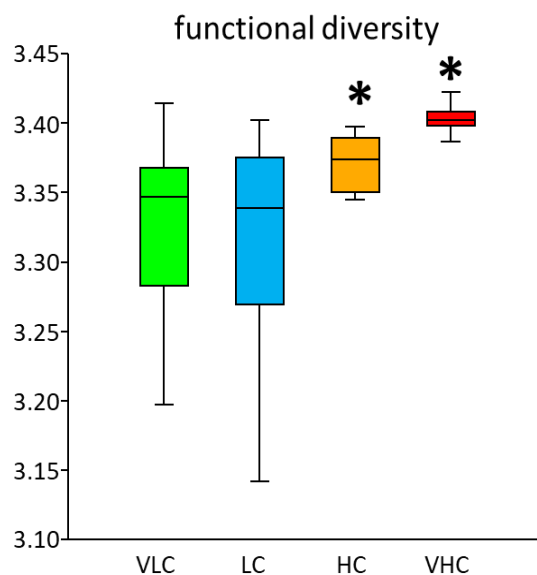
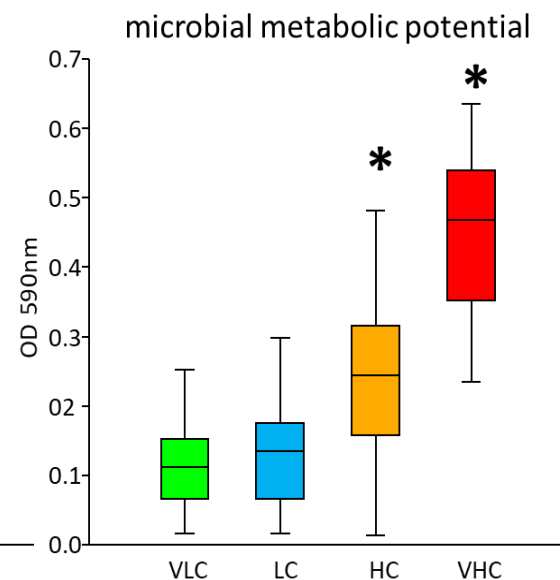
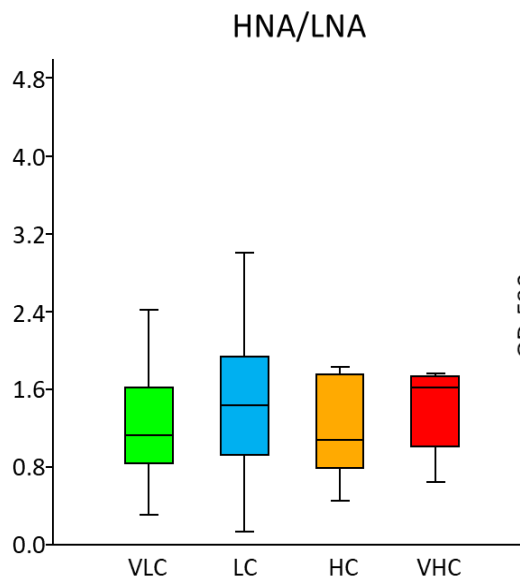
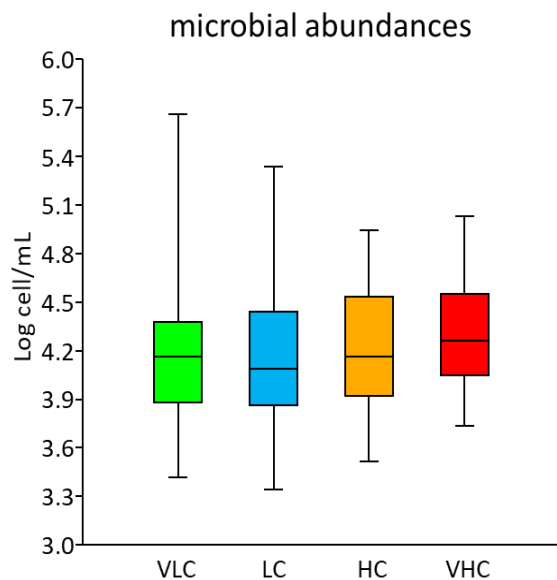
Four statistical different groups of groundwater samples were identified by the chlorides concentration, (PERMANOVA, $p < 0.05$).



Seawater Cl content: approx. 19000 mg/L

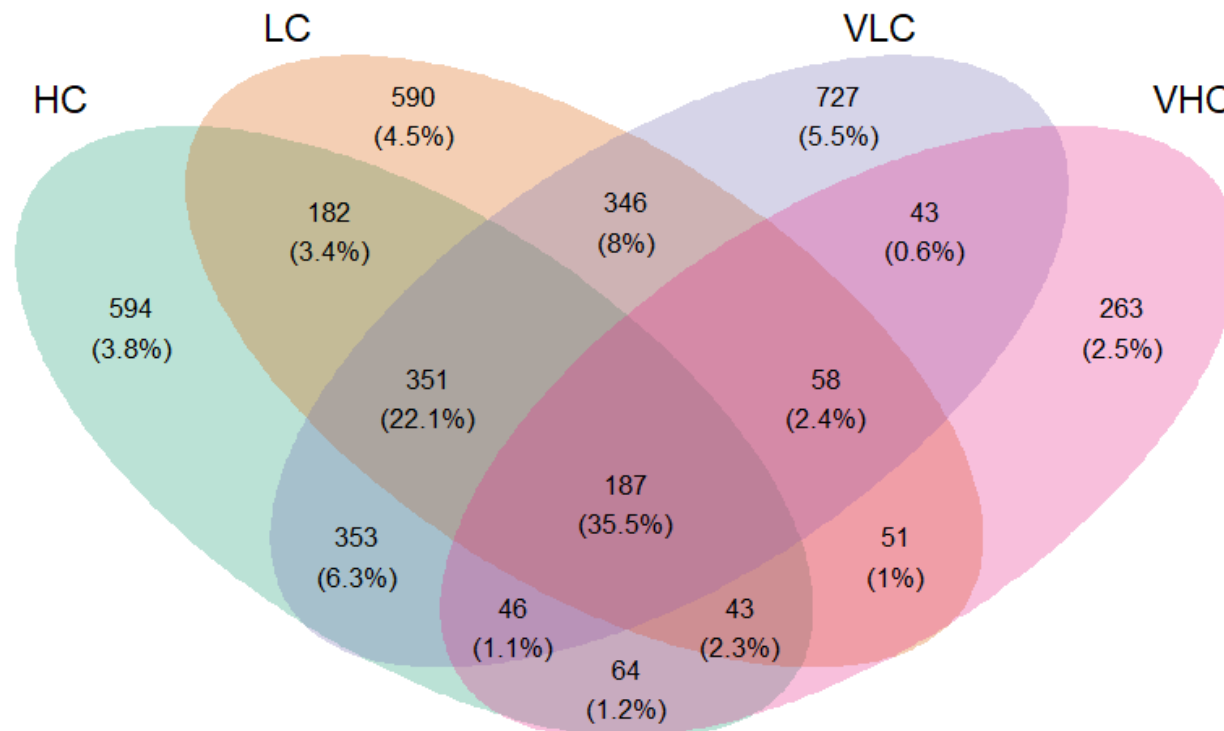
Results: microbial properties

- Very Low Cl content (VLC) - $Cl < 250$ mg/L
- Low Cl content (LC) - $250 \leq Cl < 700$ mg/L
- High Cl content (HC) - $700 \leq Cl < 3000$ mg/L
- Very High Cl content (VHC) - $Cl \geq 3000$ mg/L



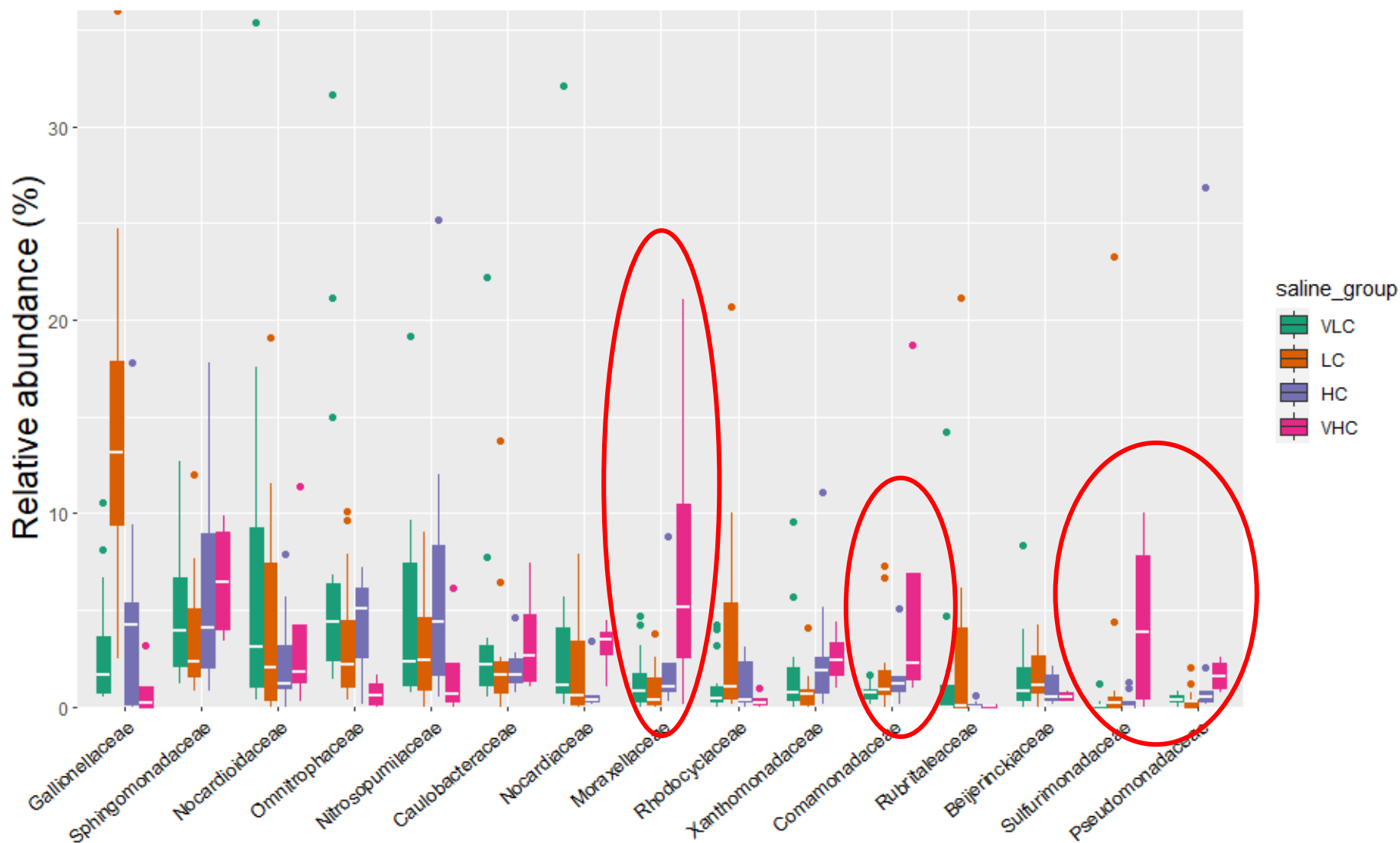
Results: phylogenetic analyses

- ✓ A total of 3898 Amplicon Sequence Variants (ASVs) belonging to 482 different microbial families were found.
- ✓ The Venn diagram shows the number of shared and unique ASVs among the bacterial communities characterizing the four groundwater groups.
- ✓ A decreasing percentage of unique ASVs was observed along the seawater intrusion gradient, while a common taxa core (>35% ASVs) is shared among all samples.



Preliminary results – phylogenetic analyses

15 dominant families were retrieved in the four groundwater sample groups



Conclusions

- ✓ Salinization affected >60% of sampling sites in the target region
- ✓ The microbial functional assays were proved as fast and reliable early-warning tools to discriminate the response of the resident microbiota to the saline gradient.
- ✓ The increase in microbial metabolism, associated to high salinity levels, may suggest the adoption of energy-consuming adaptation strategies to face environmental stressors.
- ✓ The salinity increase did not affected the total cell abundance, but induced a biodiversity loss with a shifts in the relative occurrence of selected taxa.
- ✓ A significant increase of sulfate-reducing and nitrogen-fixing microorganisms was likely to foster differences in the biogeochemical cycling across the salinity gradient.



..Thanks for your attention!

