D409 | EGU2020-7630

VIOLA
natural background levels
in Apulian groundwater bodies

The VIOLA Project: Functional responses of groundwater microbial community across the salinity gradient in a coastal karst aquifer

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EGU General Assembly 2020
Online | 4–8 May 2020
HS8.2.9 - Groundwater flow and geofluids system understanding with regard to environmental problems and resource management

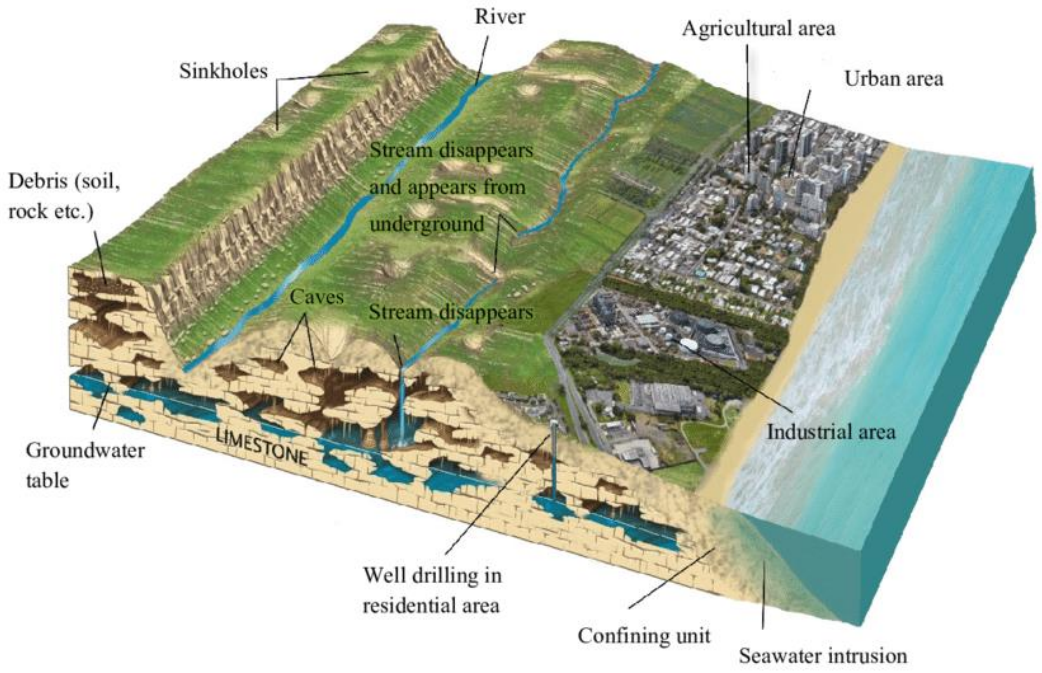
IRSA CNR

Introduction

Karst aquifers are among the most important ecosystems to supply potable water to nearly 25% of the world's population (WHO Report 2011). In particular, coastal karst aquifers are vulnerable systems, sensitive to several impact factors, including the intrusion of saline water.

The seawater intrusion into coastal karst systems can be influenced by geologic and lithologic heterogeneity, localized surface recharge, natural and anthropogenic activities (Xu et al., 2016). Furthermore, the over-pumping near coastal areas can exacerbate seawater intrusion by reducing the hydraulic pressure of the freshwater aquifer.

Microbial communities play an important role in the groundwater ecosystem as are posed at the base of the food web. Increasing salinity can affect microbial community structure and functioning, leading to significant changes in geogenic cycles and organic matter turnover (Grieblier & Abramov 2015).



Structural characteristics of a coastal karst aquifer

Objectives

- The main goal of the VIOLA project was to investigate innovative approaches to optimize the evaluation of natural background levels in coastal karst aquifers. The groundwater chemical quality (e.g., salinization) was investigated along with the resident microbial community properties responsible for biogeochemical cycles.

- The acquisition of data related to the properties of microbial communities performed by traditional approaches, is time consuming and unsuitable to be applied on regional scale surveys. In this investigation, we aimed to apply rapid and multiparametric tests in order to obtain an immediate response on changes occurring to microbial communities living under different environmental conditions.

- To address these objectives, the properties of the resident microbial communities were described by total microbial cells counts, nucleic acid content (flow cytometry), microbial metabolic potential and functional diversity (BIOLOG™ EcoPlates), microbial respiration (BIOLOG™ MT2) and extracellular enzyme activities (APYZYM®). Moreover, the groundwater quality was analysed for presence of total coliforms and *Escherichia coli* (Colilert-18® test).

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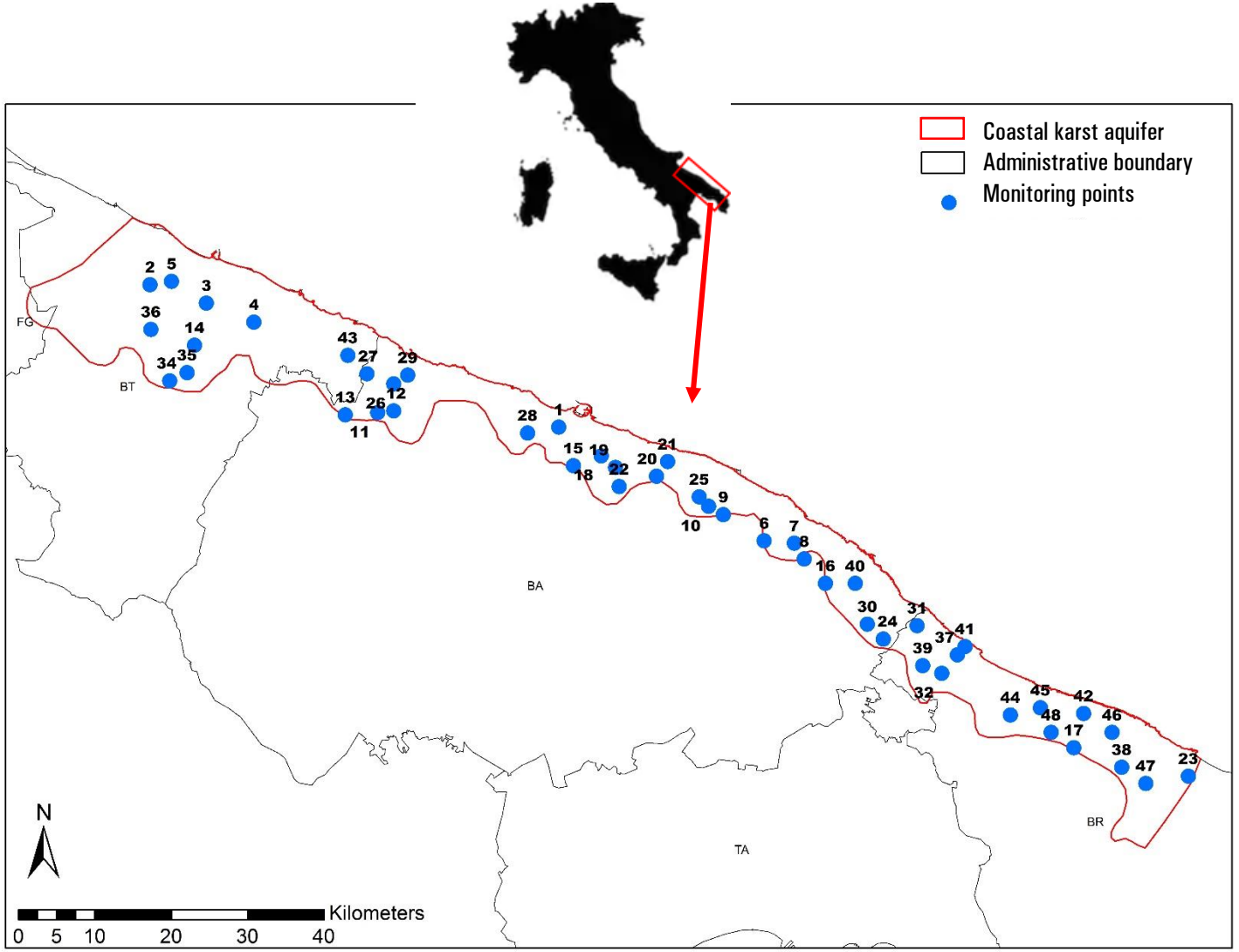
Study area

The study was conducted in a coastal karst aquifer located in a semi-arid climatic Region (Apulia) in Southern Italy. The area is characterised by an elevated agricultural vocation and affected by significant water withdrawals for irrigation.

The VIOLA project relies on four sampling campaigns scheduled along two years (2019-2020) .

The result reported herein are related to the sampling campaign carried out in September - October 2019 across a network of 47 monitoring points spread on an area of 1227 Km^2.



Map of study area

Materials & Methods

Groundwater quality was characterised by a multidisciplinary approach including hydrological, geochemical and microbiological analyses

- **Physical-chemical analyses performed *in situ:***
  pH, oxidation-reduction potential, dissolved oxygen, electric conducibility and temperature were measured by a flow cell equipped with AQUAREAD probes. *In situ* UV-VIS determinations were performed for ammonia, nitrites and cyanides.

- **Chemical analyses performed in laboratory**
  Major anions were analysed through Ionic Chromatography (Dionex DX-120) within 48/72 h. Bicarbonates were determined by automatic titration. major cations were measured with inductively Coupled Plasma – Optical Emission Spectrometry (Perkin Elmer P400). Trace elements were measured trough ICP-Mass Spectrometry (Agilent technologies 7500c) within one month.

  (see: Parrone et al., EGU2020-7561 for details).

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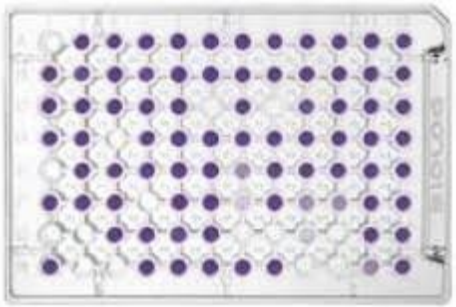
Materials & Methods

- **Microbial analyses**

✓ The **total prokaryotic cell counts** (TCC) were performed by **flow cytometry** by staining 2mL samples with SYBR Green I. The intensity of green fluorescence emitted by SYBR positive cells allows the discrimination among cell groups exhibiting low (LNA) and high (HNA) nucleic acid content, indicating different cell status (Amalfitano et al., 2014)

✓ The **microbial metabolic potential** (MMP) was measured by the **Biolog™ EcoPlates assay**. This test allows to analyse the resident microbial community ability in degrading organic carbon substrates belonging to different class of chemical compounds. The sample is inoculated in 96 wells containing 31 substrates, in triplicate, spiked with tetrazolium dye. The development of formazane, due to the microbial metabolic activity, is detected after 24 h incubation (20°C), through the colour development at the absorbance at 590 nm. The MMP is estimated by the mean degradative activity on all substrates as the average of adjusted absorbance of the whole microplate (Melita et al., 2019). The metabolic profiles obtained can be used to estimate functional microbial diversity by the Equitability index : (E =H/lnS).



✓ The **microbial respiration (MR)** was assayed to describe the organic matter mineralization process. The **Biolog™MT2 assay**, based on the transformation of the respiration-sensitive tetrazolium dye in formazane, was utilised. After sample inoculation in 96 well microplates, the development of the color after 24h incubation (20°C) is detected through the measurement of the absorbance at 590 nm (Dos Santos et al., 2002).

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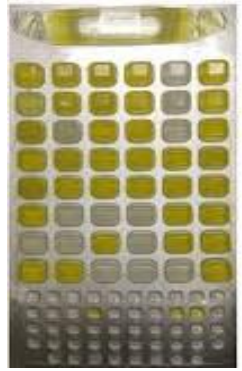
Materials & Methods

- **Microbial analyses**

✓ The extracellular enzymes are utilised by the microbial cells to feed on organic polymers of large size (>600Da) and their measurements give important information on the source of food on which their metabolism relies. The extracellular enzyme activities were measured by **API ZYM®** assay (bioMérieux) that consists in microwells containing different spiked susbstrata relative to the 19 enzymatic activities: three phosphatases, three esterases, three aminopeptidases, two proteases, and eight glycosyl-hydrolases. After sample inoculation and 24 h incubation at 20°C, manufacturer reactives are added and the enzymatic activity is detected by the colour development according to the intensity scale provided by the manufacturer (Tiquia, 2011).

✓ The abundance of total coliforms and *Escherichia coli* was estimated by **Colilert-18®/Quanti-Tray system** (IDEXX). This assay relies on β-d-galactosidase activity, expressed by both total coliforms and *Escherichia coli (E. coli).* This enzyme can hydrolyse ONPG, releasing the yellow-coloured product o-nitrophenol. β-glucuronidase, expressed by the majority of *E. coli*, can hydrolyse MUG, forming the fluorescent product 4-methylumbelliferone. The Colilert-18 reagent was dissolved in the water sample and added to a Quanti-Tray 2000™, sealed and incubated. After 24 hours incubation (35°C), the development of the color allows to estimate the cell abundances (MPN/100ml) of these microorganisms.



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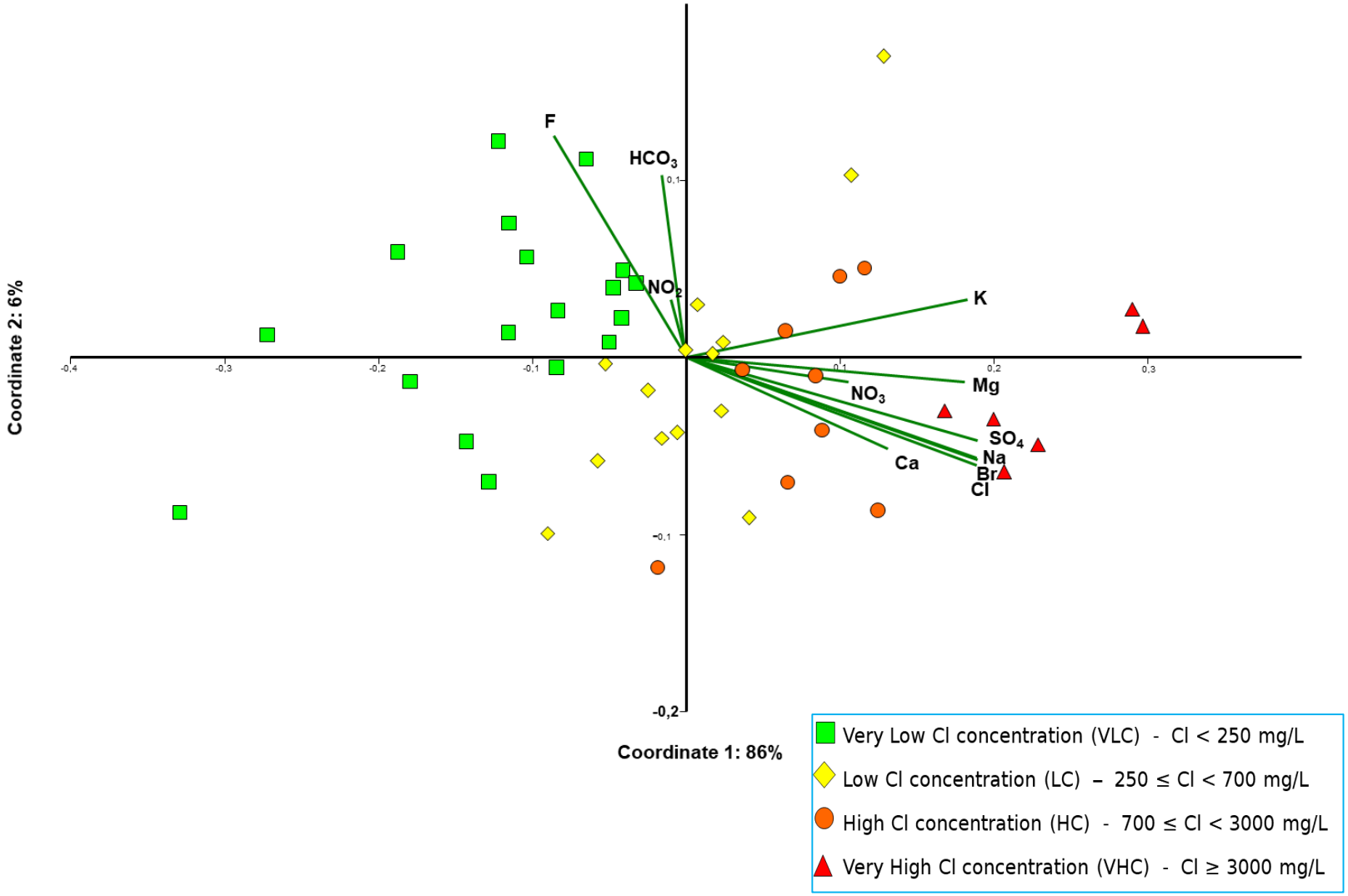
Results

The groundwater geochemical characterization has highlighted at least two main processes driving the water chemistry patterns: the water-rock interaction inside the carbonate stratum and the mixing with salt water caused by saline intrusion (see Parrone et al., EGU2020-7561 for details).
The concentration of chlorides was used as a chemical indicator of salinization.

The nMDS ordination plot, based on Bray-Curtis distance matrix of log-transformed data, was used to represent the groundwater hydro-geochemical characteristics (major compounds and trace elements). Following Cl concentration and electrical conductivity at different salinization levels, four clusters of samples were identified (ANOSIM; $p < 0.05$).

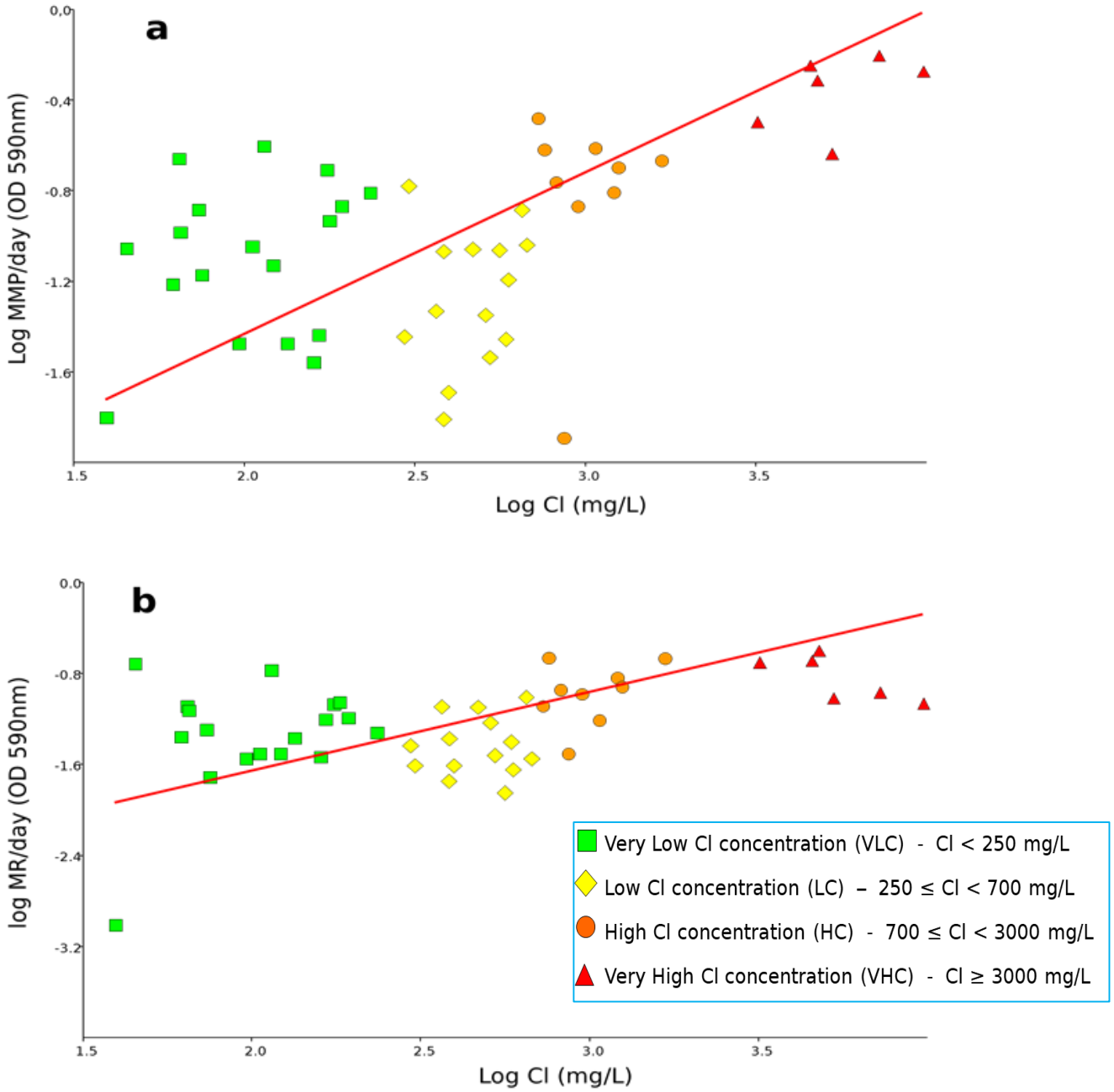


Results

Positive and significant correlations (Spearman's Ranks correlation) were observed between Cl concentration and microbial metabolic potential (MMP) (a) and microbial respiration rates (MR) (b). The clusters of samples are indicated by different symbols as displayed in the legend.

The slopes of the linear correlations, the values of the intercepts, Spearman's correlation coefficients (r), and p-values are reported in the table below.

Variable	Slope	Intercept	r	p
MMP	0.38	-19.84	0.53	0.0001
MR	0.32	-20.59	0.46	0.0011



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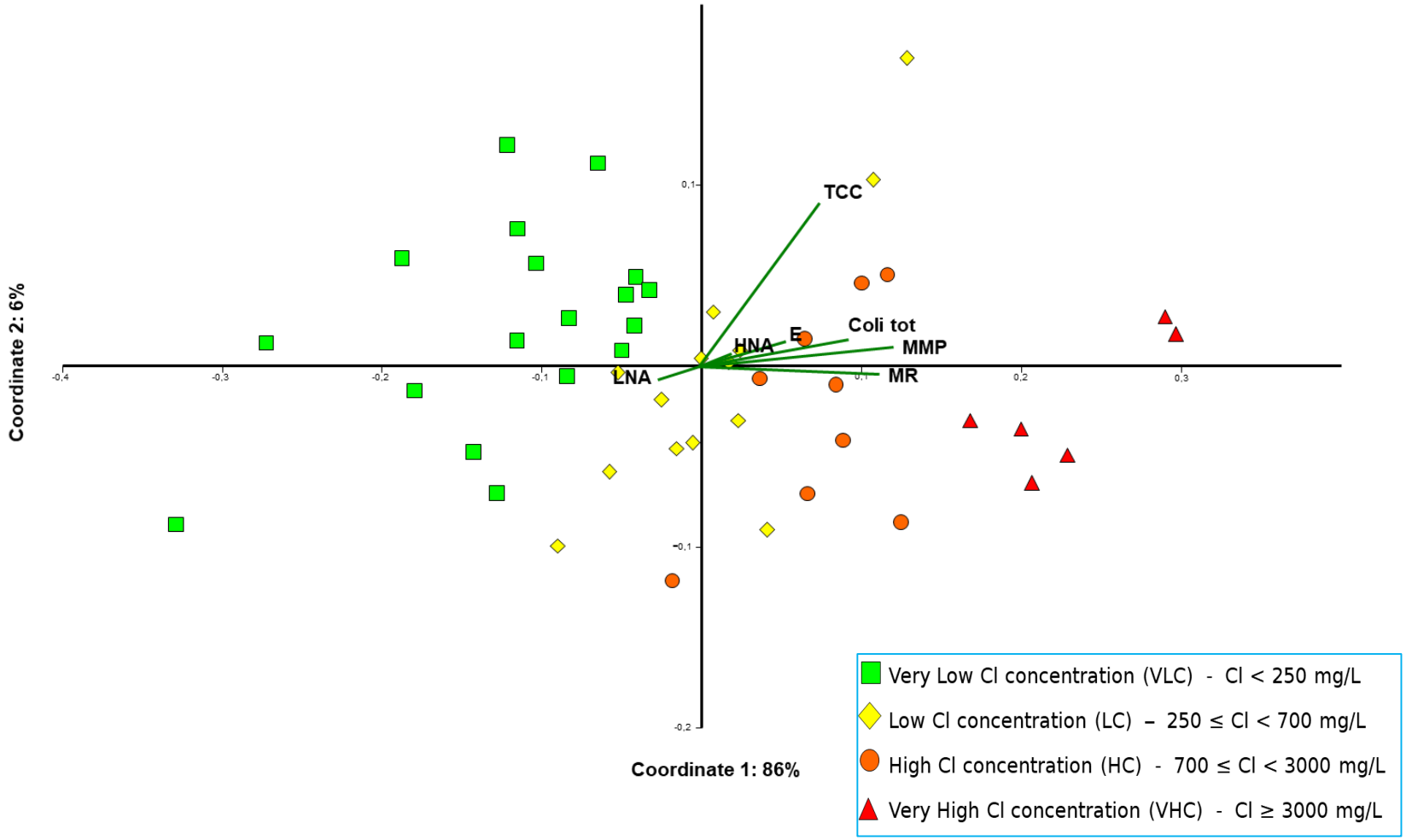
Results

The hydro-geochemical and microbiological data were integrated by nMDS analysis. The position of each sampling point is given by hydrochemical properties (major and trace elements). Vectors represent the microbial community properties that concurred to the characterization of four groundwater clusters.

The tendency to low nucleic acid content cells (LNA), was observed in samples characterized by low and very low Cl content. Total cell counts (TCC), metabolic activities (MMP and MR) and functional diversity (E) showed relatively high values at high and very high Cl concentration as displayed in the legend.



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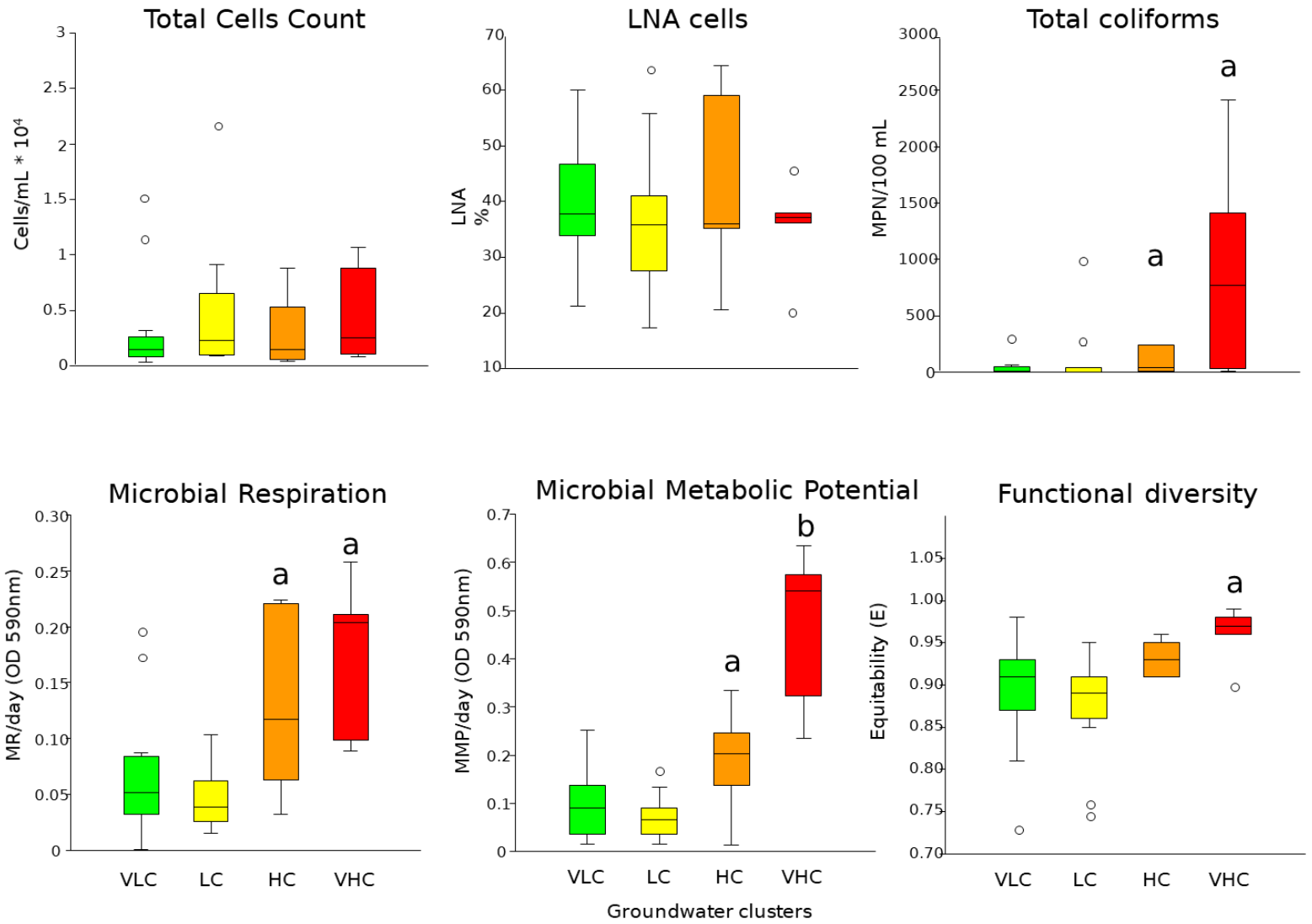
Results

In this box ordination plot, microbial properties in four groundwater clusters are displayed. The median values at 25° and 75° percentile along with outliers are shown. Statistical differences (Kruskall-Wallis, $p < 0.05$) among groundwater clusters are indicated by different letters.
Microbial abundance (TCC) and nucleic acid content (LNA) were characterized by heterogeneous distribution. Total coliforms resulted significantly higher at high range of Cl concentration (HC and VHC) along with microbial respiration and microbial metabolic potential rates. Interestingly, VHC conditions differed from the rest of groundwater clusters for high values of functional diversity. Faecal pollutants, traced by *E. coli*, were not detected in any samples.

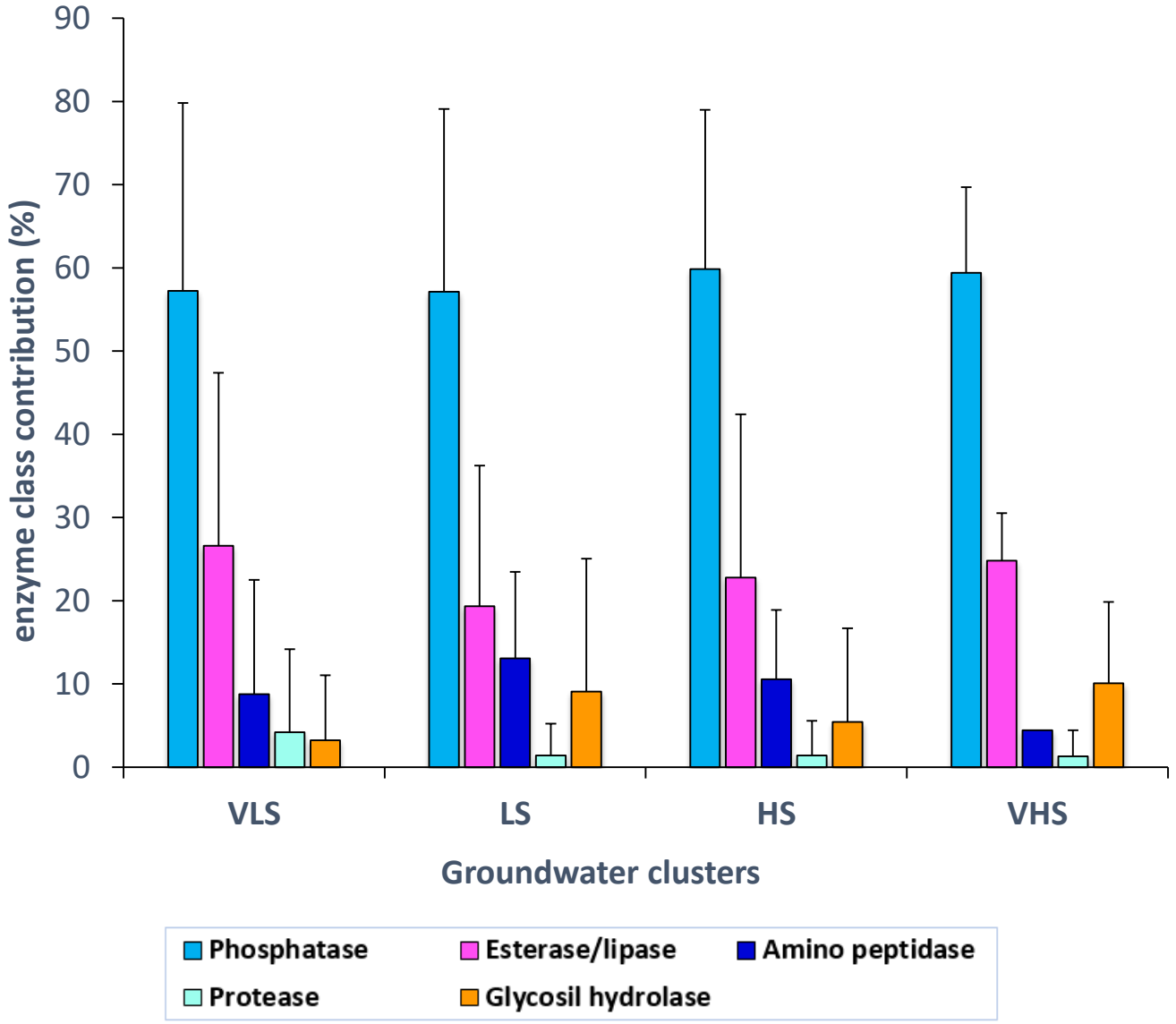


Results

In this graph are reported the percentage contributions of five classes of extracellular enzymes, as displayed in the legend, to the total enzyme activity relatively to four groundwater clusters (VLS, LS, HS, VHS).

The multivariate statistical approach showed no significant differences in the enzyme profiles among the groundwater clusters (ANOVA, $p > 0.05$).

Anyway, the class of enzymes belonging to phosphatase played the main role in the mineralization of the organic matter in any groundwater cluster, contributing on average for 57% to the total, followed by the enzymes belonging to esterase/lipase class (23%) and aminopeptidase class (13%). The contribution of the rest of the enzyme classes was below 10%.



Discussion and preliminary conclusions

- Groundwater chemical analyses highlighted qualitative heterogeneity of the samples, mainly due to salinization traced by chloride concentration.
- The results show as the salinization gradient can shape resident microbial communities metabolic properties, with the potential to affect biogeochemical cycles. High levels of salinization imply organic matter utilization at rates up to four (MR) and eight (MMP) folds higher than those observed at low salinity levels. Although no groundwater cluster was affected by faecal pollution (*E. coli*), at high salinization conditions were observed significantly high total coliform abundances (allochthonous microorganisms for the groundwater environment), along with the tendency to microbial cells with high nucleic acid content (HNA). Furthermore, significantly high values of the equitability index observed in the high salinity clusters highlighted a wider heterogeneity in the substrate degradation ability (MMP) that might imply a wider functional diversity. Differences in the metabolic profiles observed among groundwater clusters might be associated to different microbial taxa whose specific properties allow a better response to the environmental changes.
- The analysis of extracellular enzyme activity highlighted the common feature of the predominance of the phosphatase enzyme class, used for the acquisition of phosphorus from organic matter. This result indicates a diffuse condition of phosphorus limitation (Arnosti et al., 2014). The enzyme profile was also characterized by the glycosyl-hydrolase enzyme class used by microbes for the acquisition of carbon (Patel et al. 2018). Interestingly, the aminopeptidase enzyme class, one of the key enzymes for the acquisition of carbon and nitrogen, assumed relatively low values, very likely indicating a low nitrogen limitation in these environments (Zoppini et al., 2014)
- In conclusion, the microbial analyses provide insights on the changes occurred within the aquifer with the potential to affect the biogeochemical cycles. The fast microbial test utilized were reliably to characterize the environmental changes. The ongoing analysis (e.g. concentration of organic matter, stable isotopes and microbial diversity) will further help to define the genesis of these alterations (natural vs anthropogenic) and the effect on the microbial component.

Linked displays

Other displays on the VIOLA project

EGU2020-7716

The VIOLA Project: Natural background levels for the groundwater bodies of Apulia Region (Southern Italy)

Masciale R., Amalfitano S., Frollini E., Ghergo S., Melita M., Parrone D., Preziosi E., Vurro M., Zoppini A., and Passarella G.

EGU2020-7561

The VIOLA project: Geochemical characterization and natural background levels in a coastal groundwater body of the Apulia Region (Southern Italy)

Parrone D., Frollini E., Amalfitano S., Ghergo S., Masciale R., Melita M., Passarella G., Vurro M., Zoppini A., Preziosi E.

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Acknowledgements

The VIOLA project has been funded by **Apulia Region** within the frame of the P.O. F.E.S.R. Puglia 2014-2020

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