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RICERCA IN IDROLOGIA MEDICA
E MEDICINA TERMALE

TERME DI COMANO

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DI TRENTO E ROVERETO

A longitudinal characterization of microbiome of alpine water springs used for human consumption

Olivier Jousson

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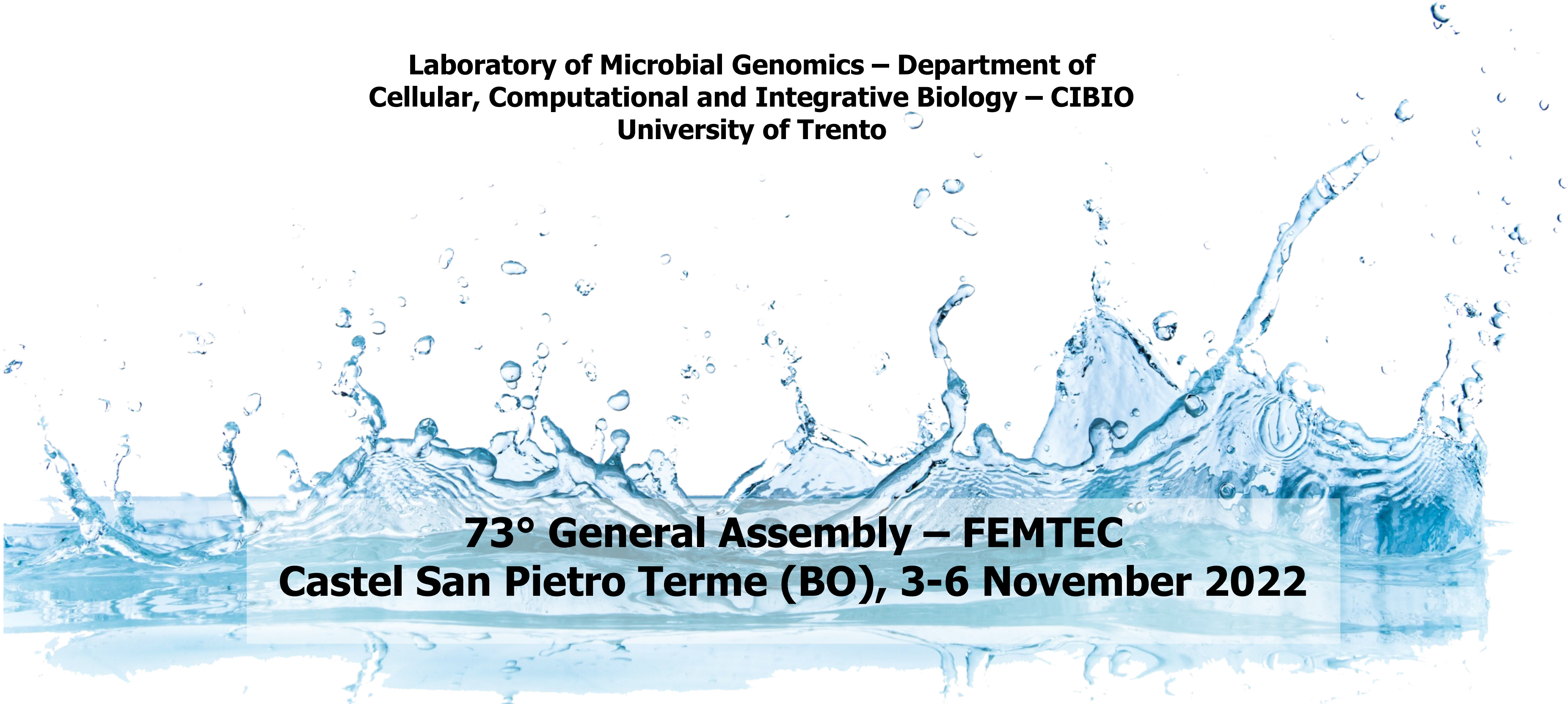
Cellular, Computational and Integrative Biology – CIBIO
University of Trento**



**73° General Assembly – FEMTEC
Castel San Pietro Terme (BO), 3-6 November 2022**

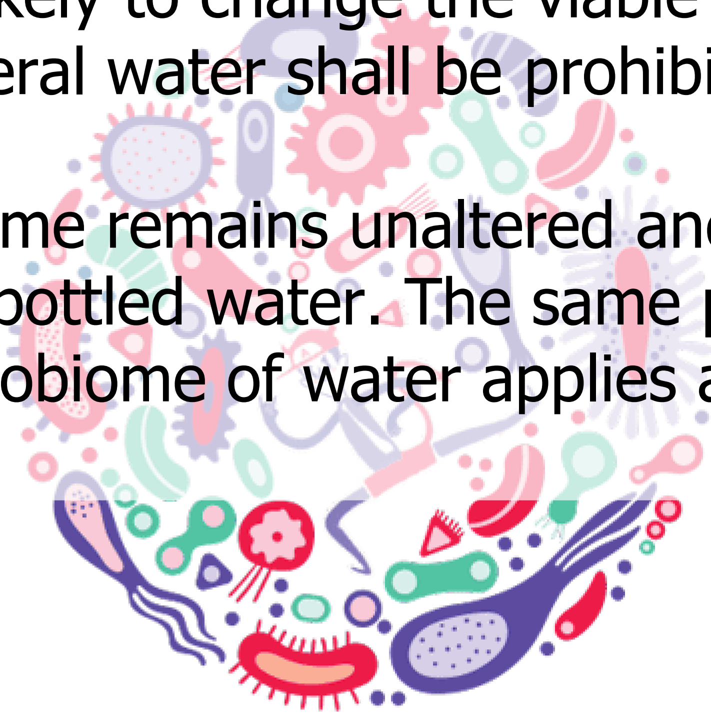
The groundwater microbiome

- Microbiome of **water springs** has been deeply explored by a large number of studies, but the majority of them focused on **extreme environments**
- The microbiome of **drinking water** has been explored mainly to describe spatial and temporal taxonomic compositional patterns, or to investigate the effects of distribution and purification plants on water quality and safety
- Springs that provide water destined for **human consumption** have been instead scarcely investigated
- The presence of bacteria in water sources used for human consumption is a **major concern for health authorities** and the management of microorganisms for water safety is strictly regulated, but it is focused only on total microbial load and on seeking for the presence of pathogenic species that represent a minor fraction of the microbiome diversity

The groundwater microbiome

- European Council Directive 80/777/EEC on the approximation of the laws of the Member States relating to the exploitation and marketing of natural mineral waters. The directive states that: "Any disinfection treatment … and … the addition of bacteriostatic elements or any other treatment likely to change the viable colony count of the natural mineral water shall be prohibited."

- Thus the spring microbiome remains unaltered and is consequently present in bottled water. The same principle of leaving intact the microbiome of water applies also to Spa and Medical Spa.



Our previous work

TERME DI COMANO

G ITAL DERMATOL VENEREOL 2000;135

Valutazione dell'efficacia dell'acqua della fonte termale

Comano's (Trentino) thermal water interferes with tumour necrosis factor-α expression and interleukin-8 production and secretion by cultured human psoriati
anti-pso

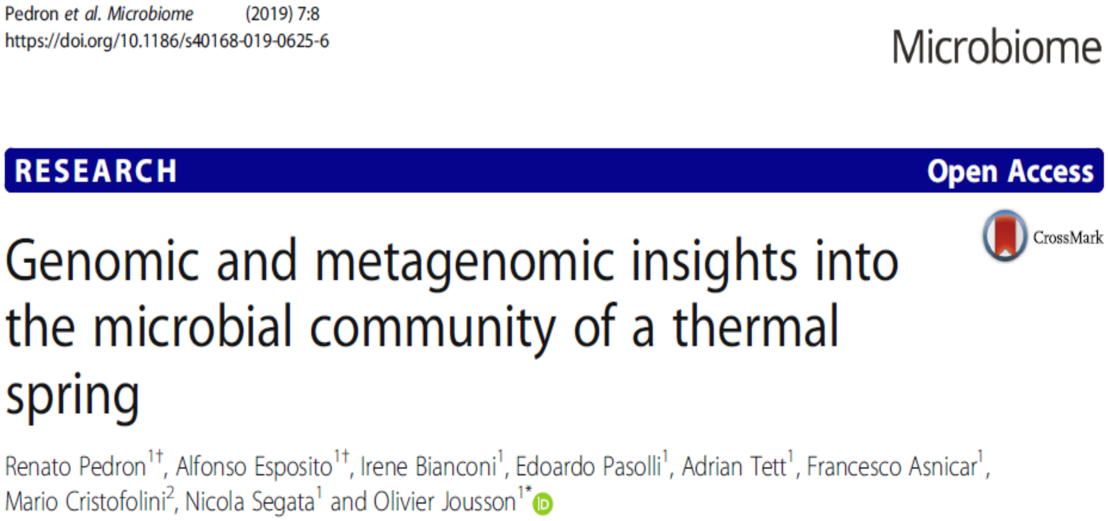
Authors: Ilari
Zanoni, Ubald

Research article

Balnotherapy for atopic dermatitis in children at Comano spa in Trentino, Italy

Stefania Farina, **Paolo Gisondi** ✉, Mauro Zanoni, Manuela Pace, Laura Rizzoli, Ermanno Baldo & Giampiero Girolomoni

Page 366-371 | Received 22 Apr 2010, Accepted 08 Jul 2010, Published online: 22 Jan 2011



Pedron *et al. Microbiome* (2019) 7:8
https://doi.org/10.1186/s40168-019-0625-6

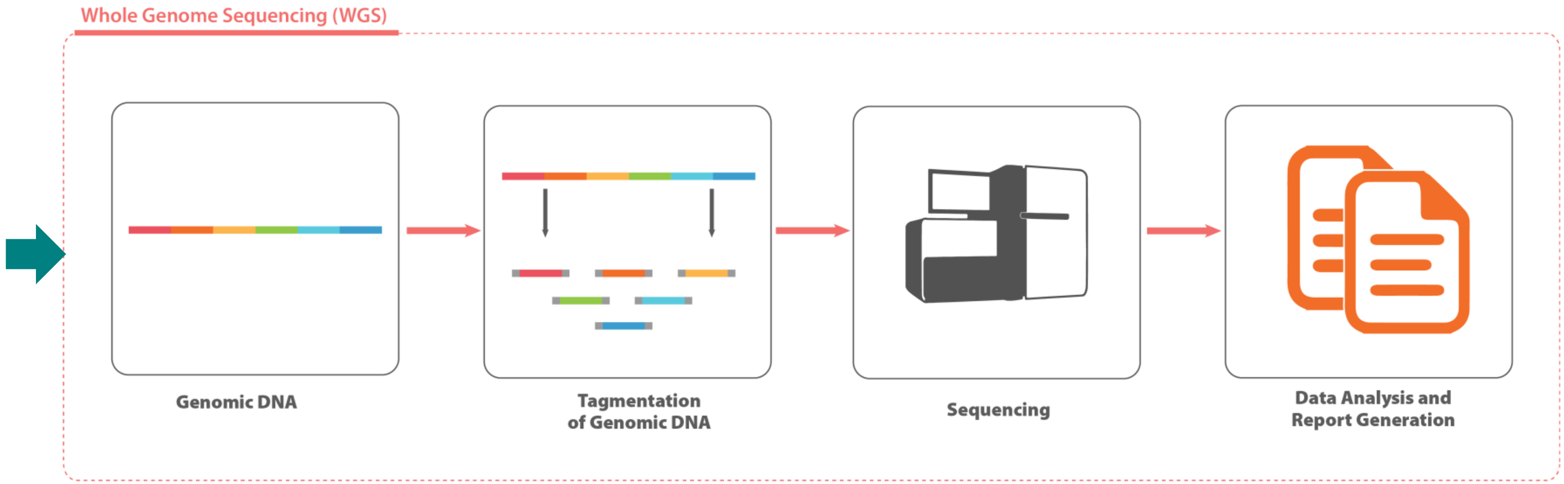
Microbiome

RESEARCH **Open Access**

Genomic and metagenomic insights into the microbial community of a thermal spring

Renato Pedron[1†], Alfonso Esposito[1†], Irene Bianconi[1], Edoardo Pasolli[1], Adrian Tett[1], Francesco Asnicar[1], Mario Cristofolini[2], Nicola Segata[1] and Olivier Jousson[1*]

Next Generation Sequencing Workflow



Whole Genome Sequencing (WGS)

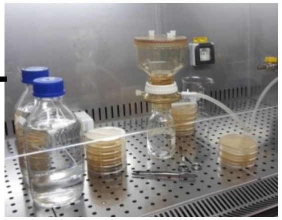
Genomic DNA

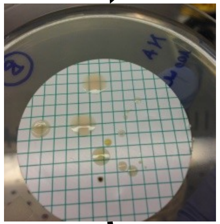
Tagmentation of Genomic DNA

Sequencing

Data Analysis and Report Generation

Strain isolation workflow





Set terreni di coltura in Deepwell 96

Semina 10 µL di Acqua di Comano

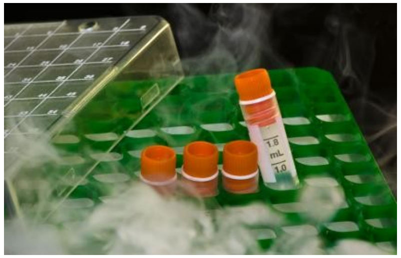
Incubazione a diverse condizioni

Preparazione di stock di glicerolo conservati a -80°C

16S rRNA sequencing

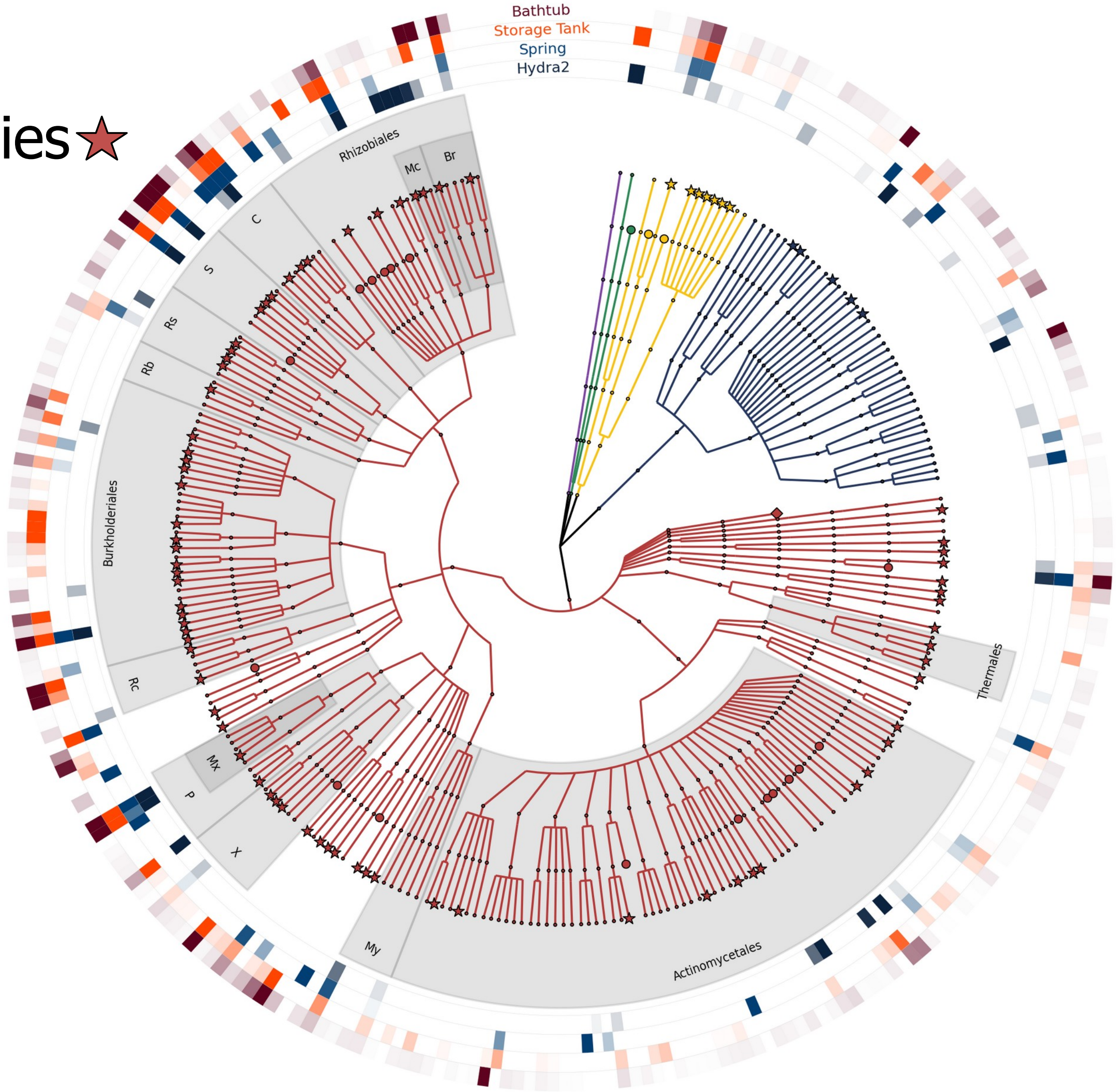
Identificazione

A collection of 181 bacterial isolates

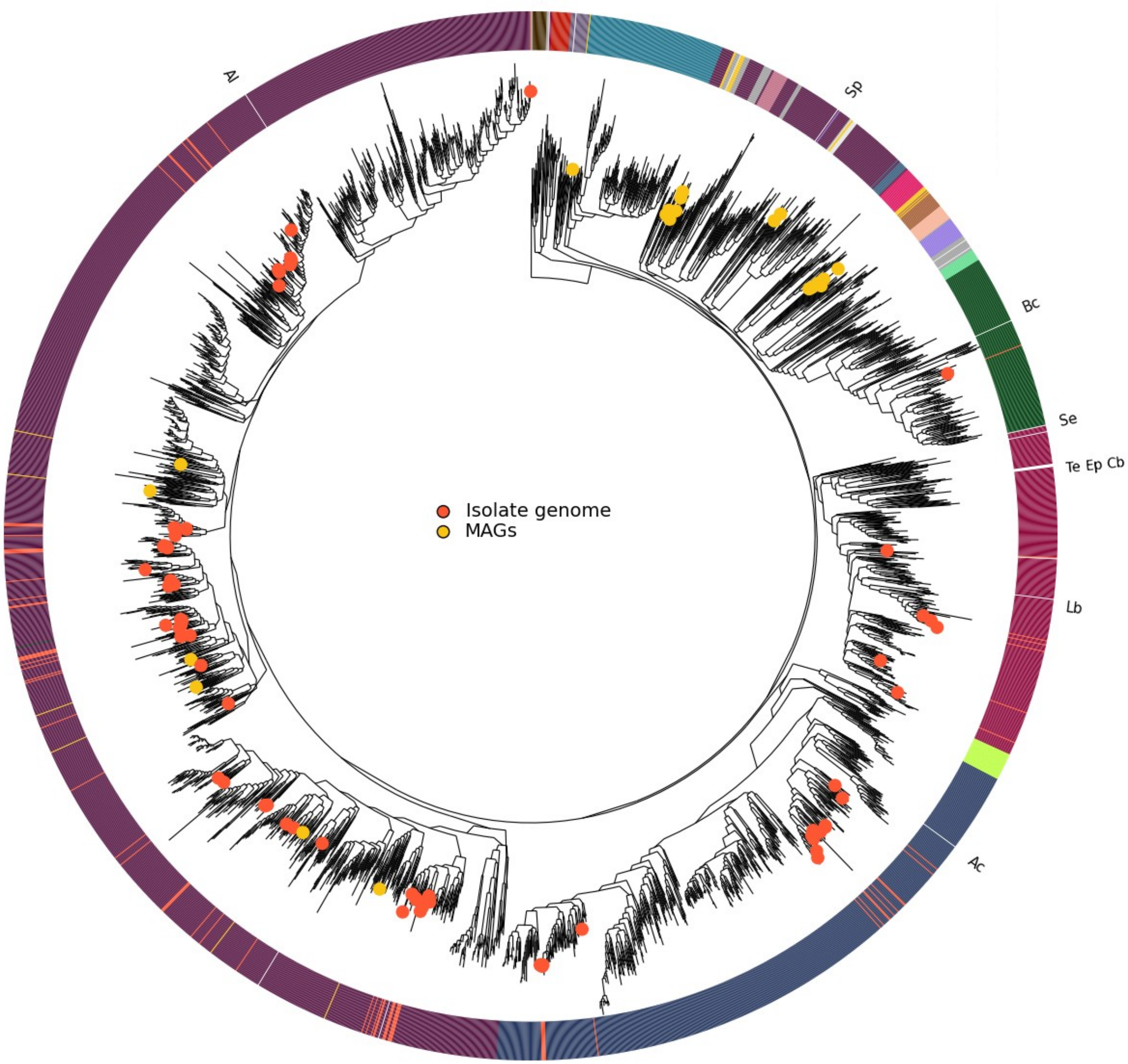


Comano Terme Microbiome

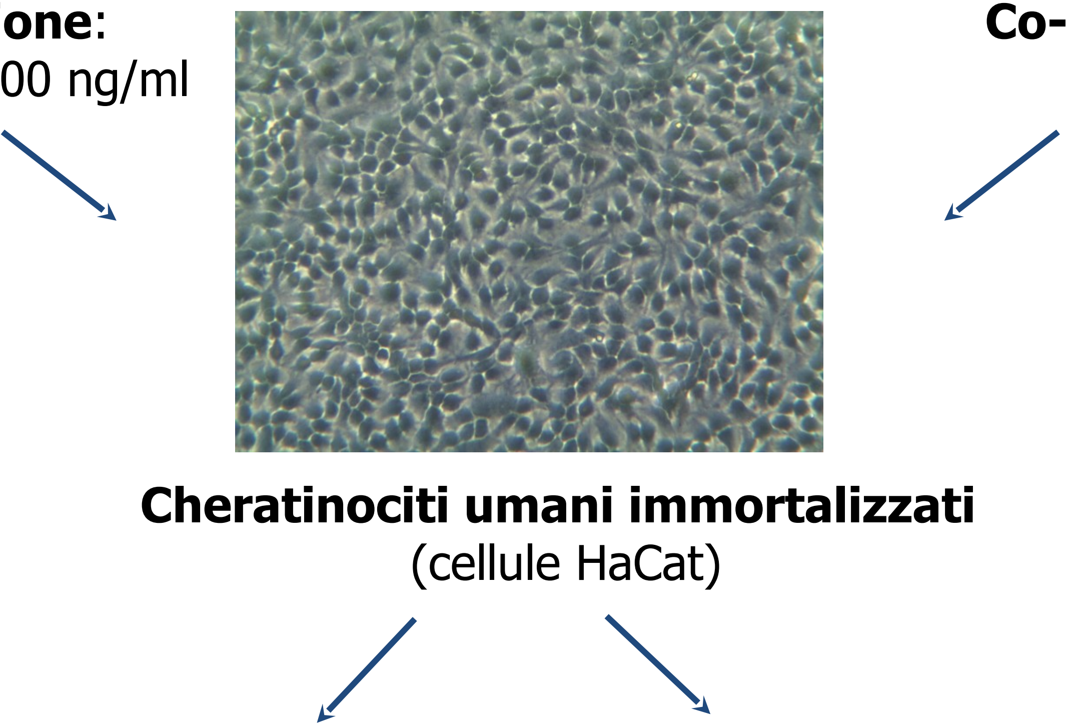
About 40% of unknown species★



Isolate Genomes vs Metagenome-Assembled Genomes



Characterization of the strain collection: seeking for immunomodulatory properties



Infiammazione:
IL-17A e IFN-γ 100 ng/ml

Co-trattamento:
5mg lisato
batterico
sterile

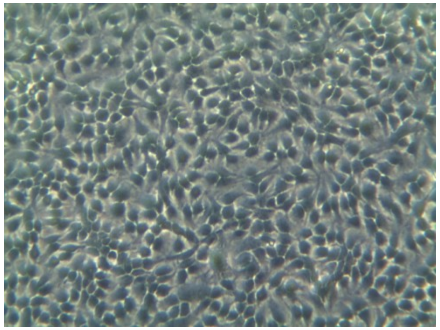
Cheratinociti umani immortalizzati
(cellule HaCat)

Valutazione visiva:
crescita e morfologia

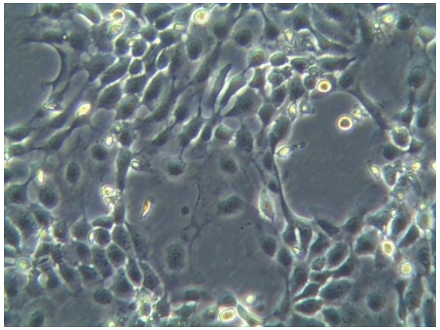
Analisi trascrizionale:
livello mRNA IL-6

Effect of bacterial lysates on Hacat cells

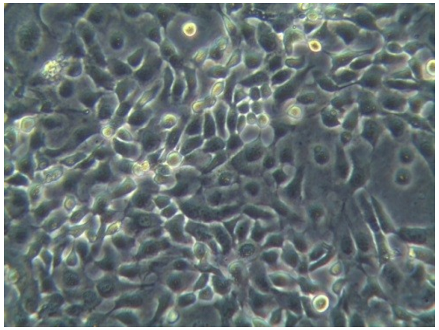
Cellule non trattate



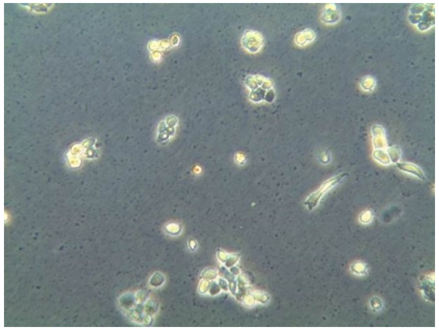
Cellule infiammate:
IL-17A + IFN-γ



IL-17A + IFN-γ + Lisato batterico

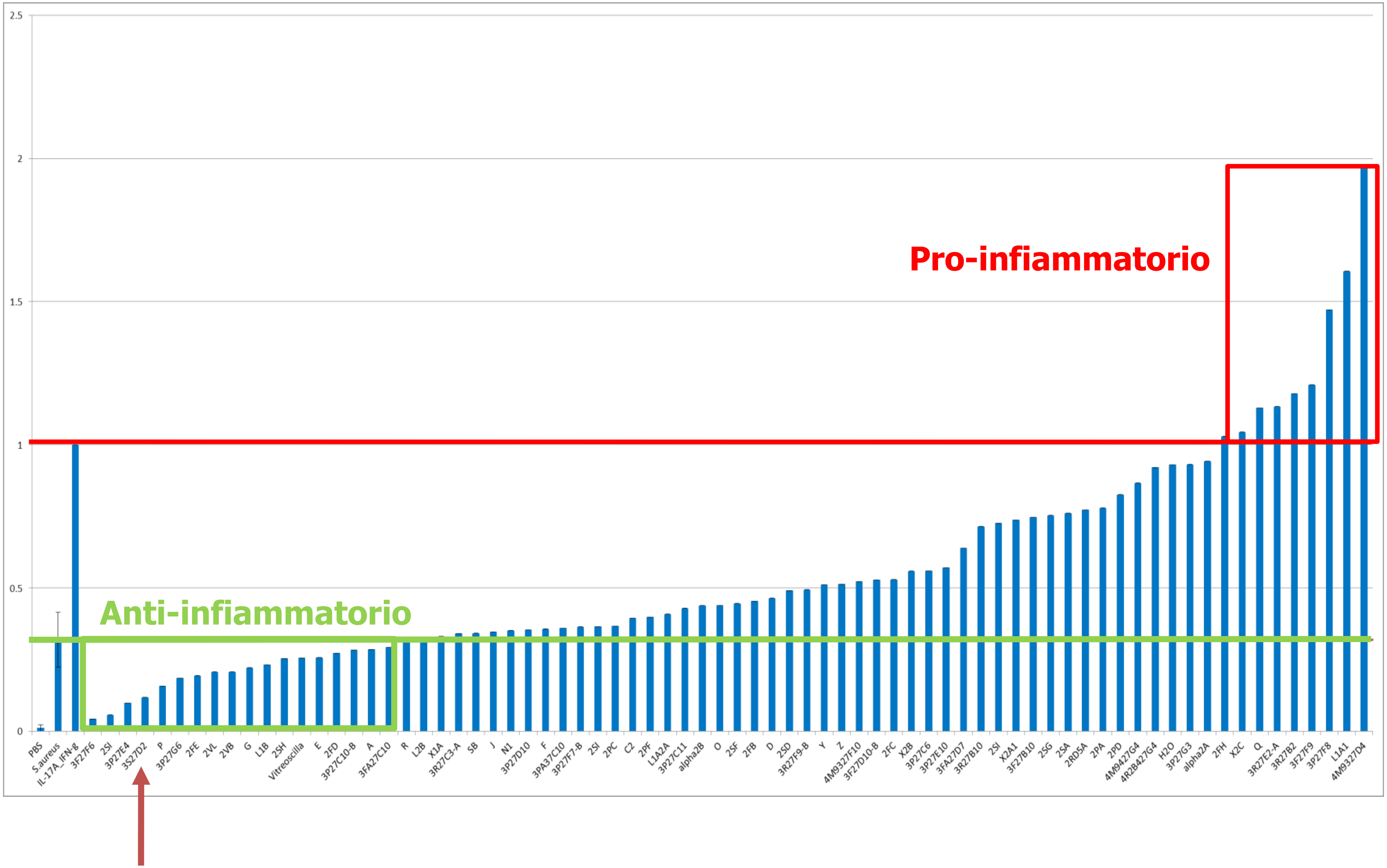


Effetto positivo:
incremento crescita



Effetto negativo:
morte cellulare

Screening output



Pro-infiammatorio

Anti-infiammatorio

Mesorhizobium comanense sp. nov.

INTERNATIONAL
JOURNAL OF **SYSTEMATIC**
AND EVOLUTIONARY
MICROBIOLOGY

TAXONOMIC DESCRIPTION
Pedron *et al*., *Int. J. Syst. Evol. Microbiol.* 2021;71:005131
DOI 10.1099/ijsem.0.005131

MICROBIOLOGY SOCIETY

Mesorhizobium comanense sp. nov., isolated from groundwater

Renato Pedron[1], Elena Luchi[1], Marta Acin Albiac[2], Raffaella Di Cagno[2], Daniele Catorci[1,3]†, Alfonso Esposito[1], Irene Bianconi[1], Davide Losa[1], Mario Cristofolini[4], Graziano Guella[3] and Olivier Jousson[1,*]

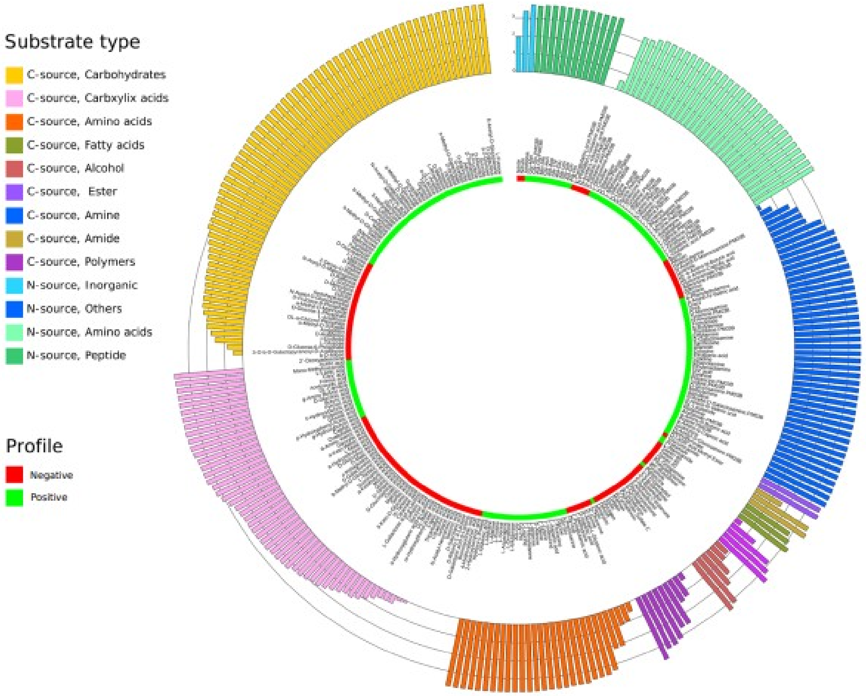
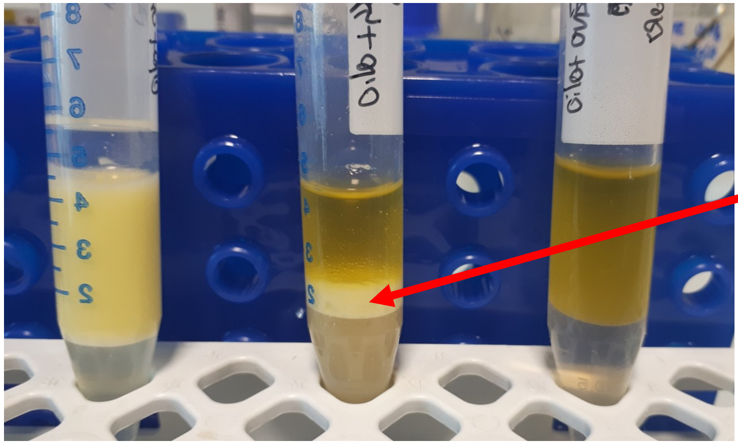
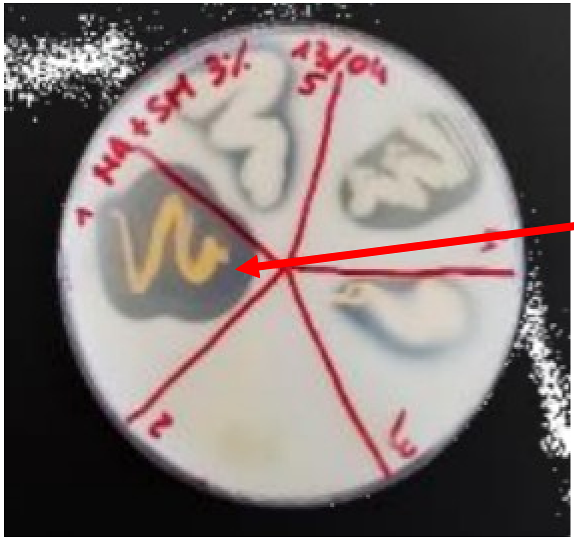


Fig. 3. Phenotypic profile of strain 3P27G6^T. The tested compounds are grouped by substrate family and displayed as bar charts in the outer ring of the figure. The represented value is the log10 normalized mean of three biological replicates of the area under the curve calculated using non-parametrical free spline methods. The inner ring marks the compounds for which the strain tested positive (green) or negative (red).

Other bioactivities of potential medical and industrial interest

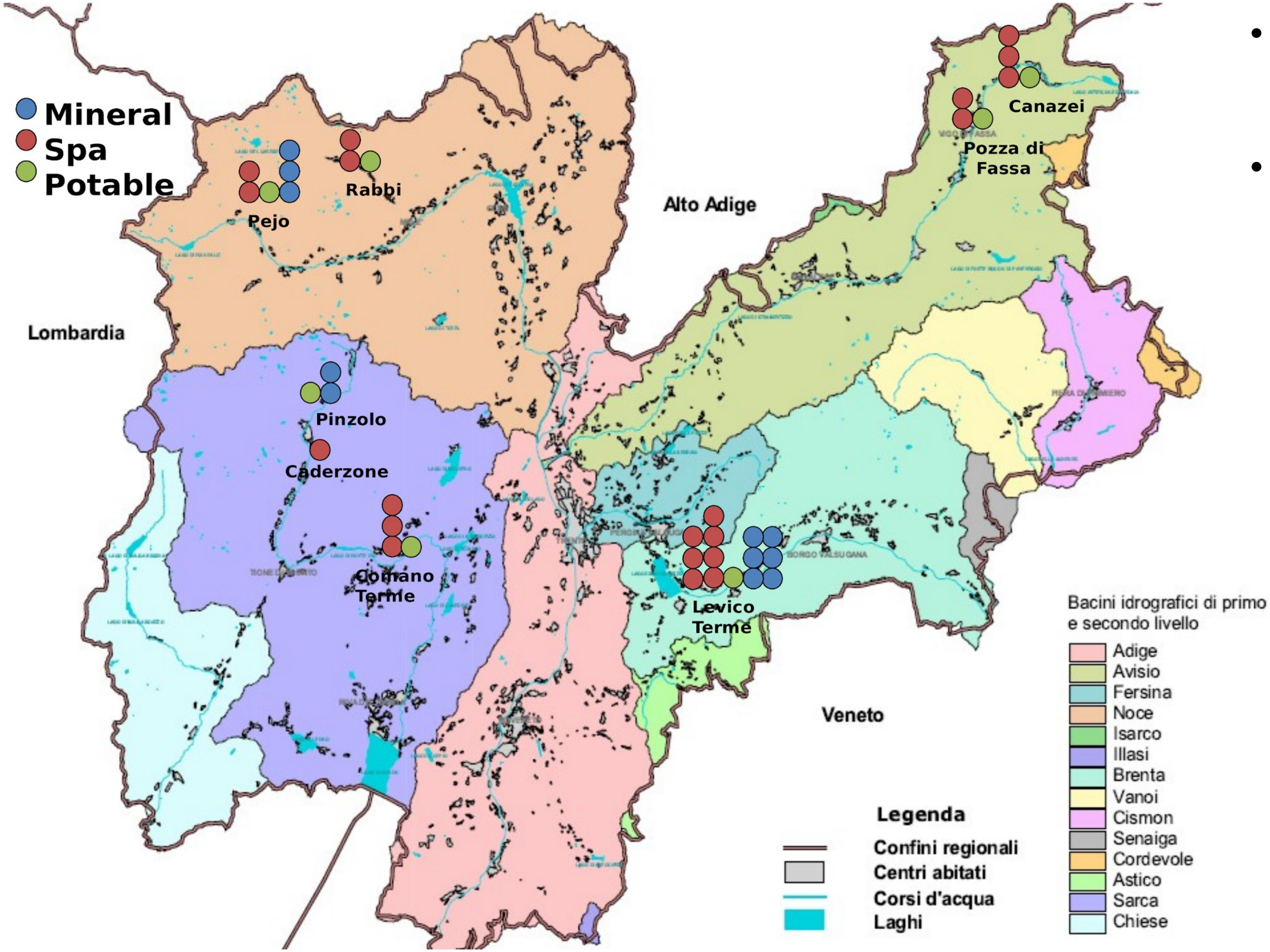


Biosurfattanti



Biodegradazione (di proteine, lipidi, zuccheri)

The Microbiome of Trentino springs



- 38 springs in Trentino
- 8 time points over a two-year period

frontiers | Frontiers in Microbiology

Microbiome characterization of alpine water springs for human consumption reveals site- and usage-specific microbial signatures

Renato Pedron[1†], Alfonso Esposito[2†], William Cozza[1], Massimo Paolazzi[3], Mario Cristofolini[4], Nicola Segata[1] and Olivier Jousson[1*]

[1]Department of Cellular, Computational and Integrative Biology – CIBIO, University of Trento, Trento, Italy, [2]International Centre for Genetic Engineering and Biotechnology – ICGEB, Trieste, Italy, [3]Agenzia provinciale per la protezione dell'ambiente – APPA, Trento, Italy, [4]Istituto G.B. Mattei, Stenico, Italy

Integration of microbial community composition with physico-chemical metadata

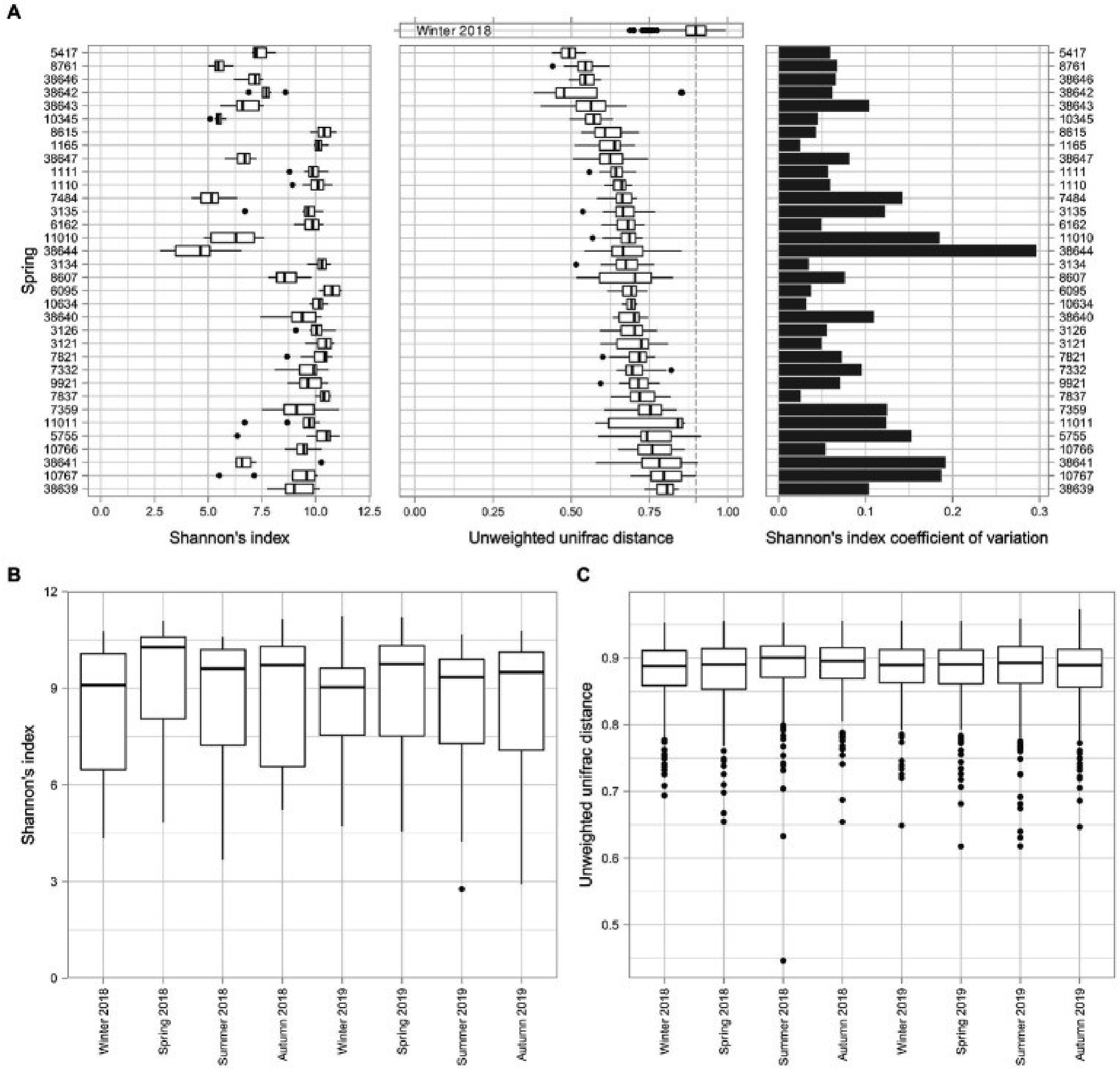
Sample collection

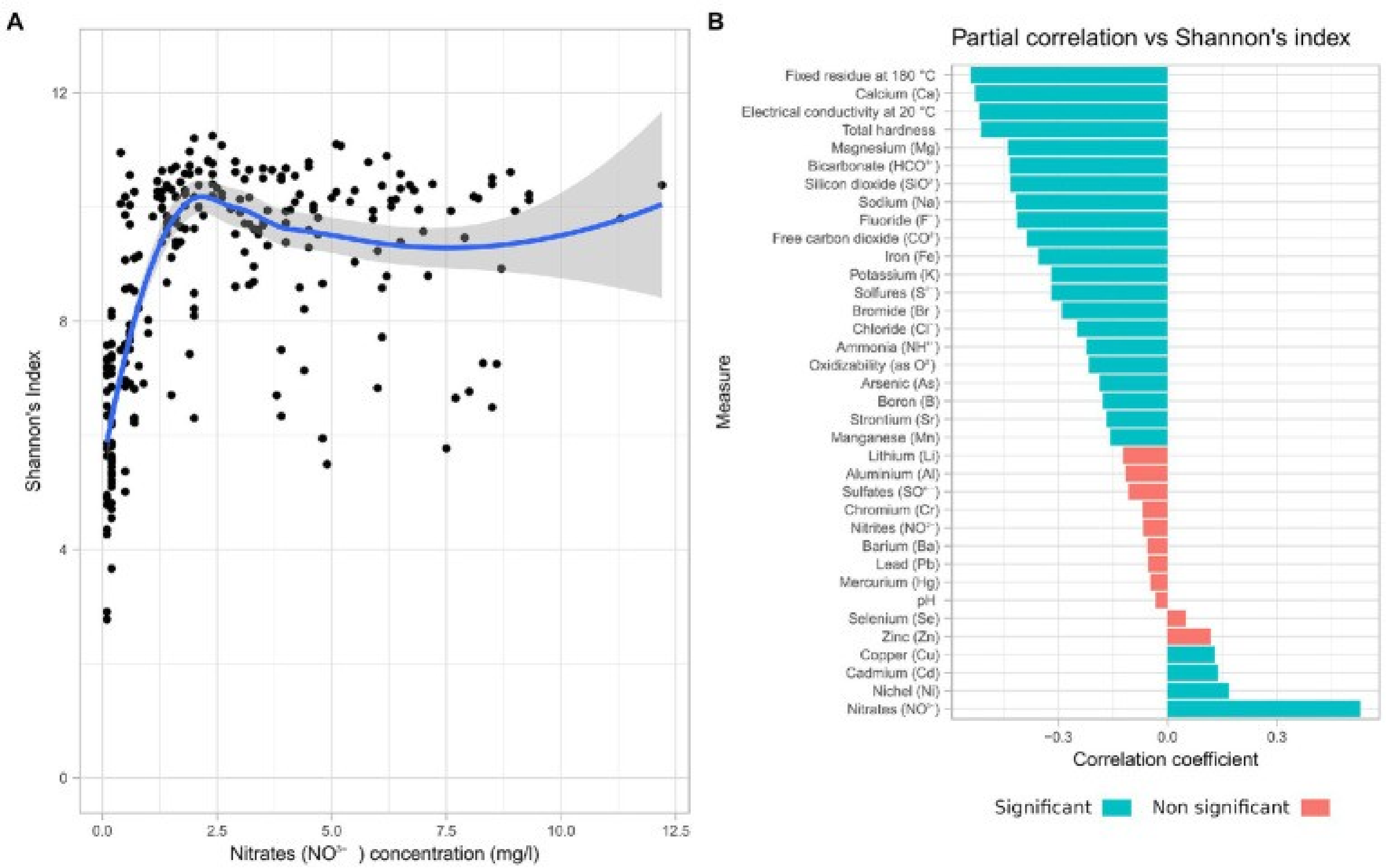
Physico-chemical analysis

Microbiome characterization

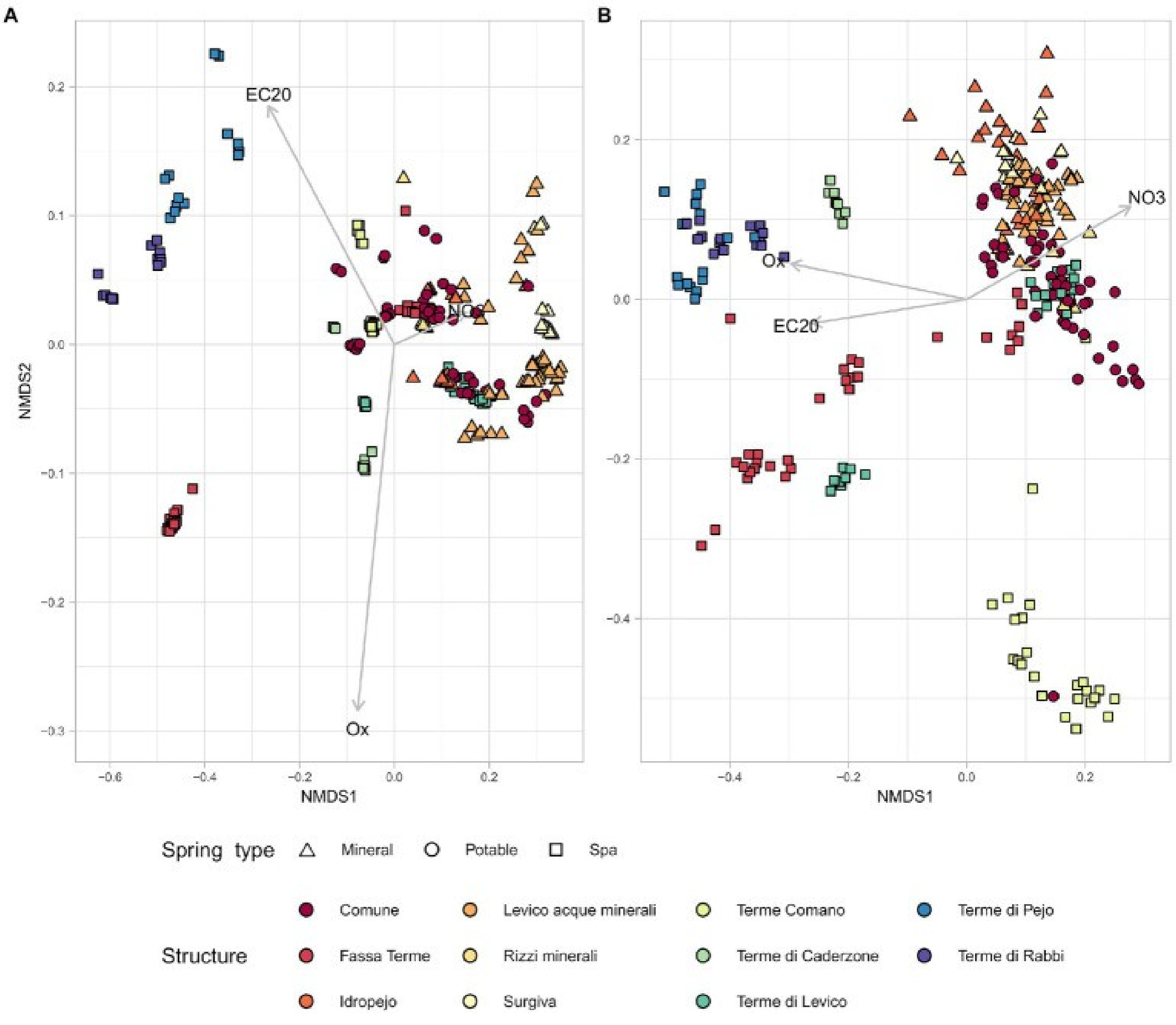
Evalutation of the microbiome stability of the springs

Microbiome diversity is affected by seasonal cycle

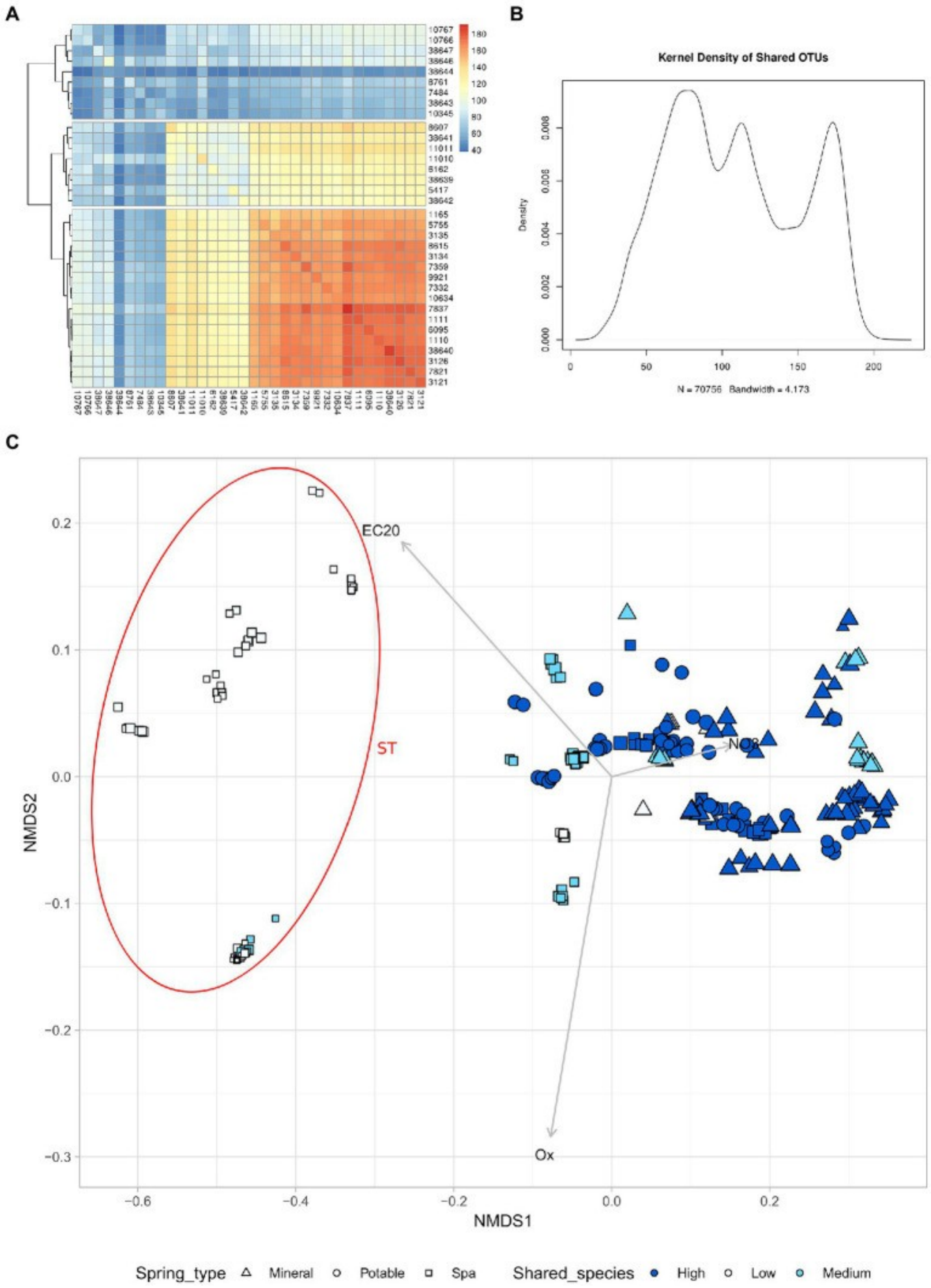




Nitrate concentration influences the microbiome diversity. Correlation of chemical data with alpha diversity. **(A)** Scatterplot of Shannon alpha diversity index of all samples in the study vs. Nitrate concentration. **(B)** Spearman partial correlations, given Nitrates, of all measured chemical parameters.



The physico-chemical parameters and the sequencing data present similar behavior



High nitrates concentration leads to a higher alpha diversity and **more shared species.**

(A) Heatmap of the number of shared species between different springs (mean of all timepoints)

(B) Kernel density of shared ASV

(C) NMDS of all samples based on chemical parameters

The samples can be been divided in 3 groups based on the number of shared species with other springs. **High number of shared species (High), Medium number of shared species (Medium), low number of shared species (Low)**.

Conclusions and future perspectives

- Diversity of spring microbiome is affected by seasonal cycle
- Environmental parameters are partially correlated with microbial community composition
- The analysis of the number of shared species reveals three distinct groups of springs
- Our results sum up to the recent literature in support of the use of microbial communities as bioindicators for groundwater quality assessment
- The present study constitutes an example for standard water spring monitoring integrated with microbial community composition on a regional scale, and provides information which could be useful in the design and application of future water management policies

Acknowledgements

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PROVINCIA
AUTONOMA DI TRENTO

- Mario Cristofolini (Istituto G.B. Mattei)
- Renato Pedron, Alfonso Esposito, Mattia Benedet, Gregory Vincenti (CIBIO)
- Nicola Segata (CIBIO)
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- Provincia Autonoma di Trento