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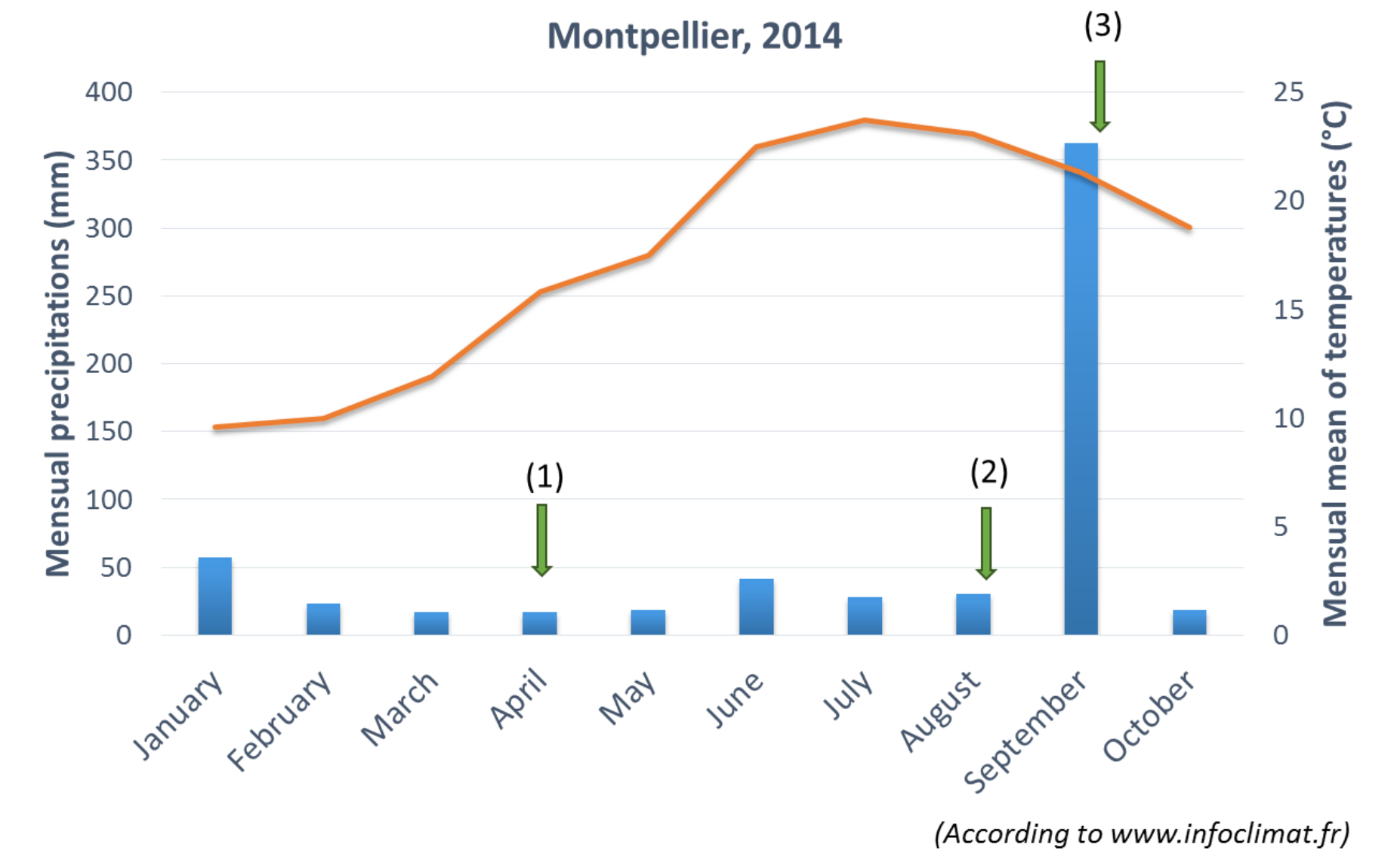
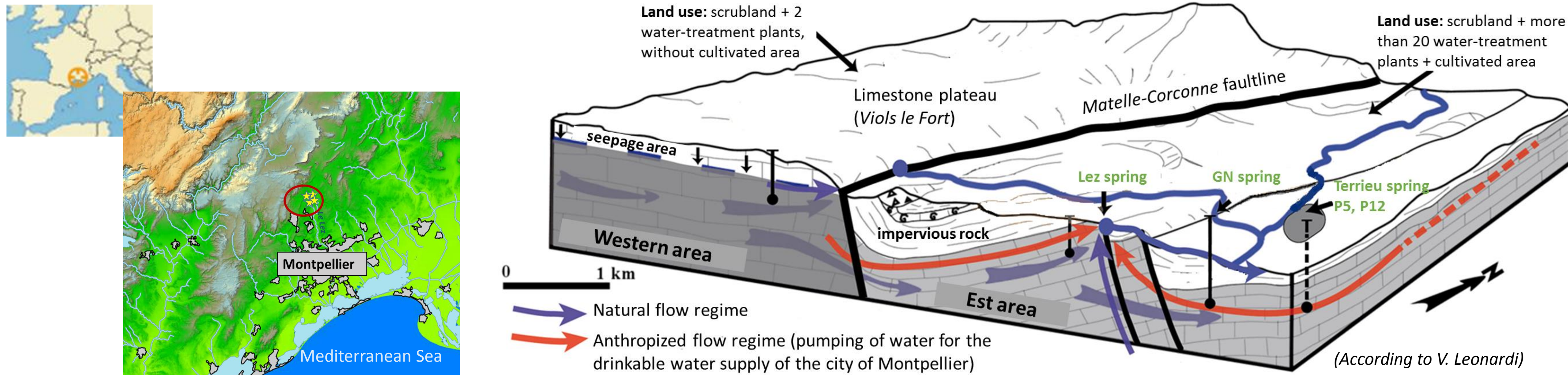
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Waters play a crucial role in interconnecting different ecosystems like humans, animals, soils and hydrosystems, contributing to the circulation of antibiotic-resistant bacteria. Karstic hydrosystems are commonly known to be vulnerable to anthropogenic contaminations due to direct connections between surface water and groundwater. Karst is also heterogeneous due to the contrast between waters stored in some compartments with long residence time and waters with fast transit. Considered as integrator ecosystems, natural waters are of great interest to survey the antibiotic (ATB) resistance in the environment, which is relevant to human health. Among natural hydrosystems, karstic aquifers are poorly investigated for emergence and dissemination of antibio-resistance. The question of their role as reservoir for antimicrobial resistance according their hydrogeological characteristics remains open.

Study area, water sampling and methods

The Lez karst hydrosystem, in southern France is studied through 4 sampling sites corresponding to contrasted hydrogeological conditions and anthropization:

- Lez spring, which supplies drinking water to the metropolitan region of Montpellier city
- Gour Noir (GN) spring, not particularly exposed to anthropic pollution
- Terrieu spring, exposed to anthropic activities, with rapid flow (P12 well) and slow flow (P5 well)

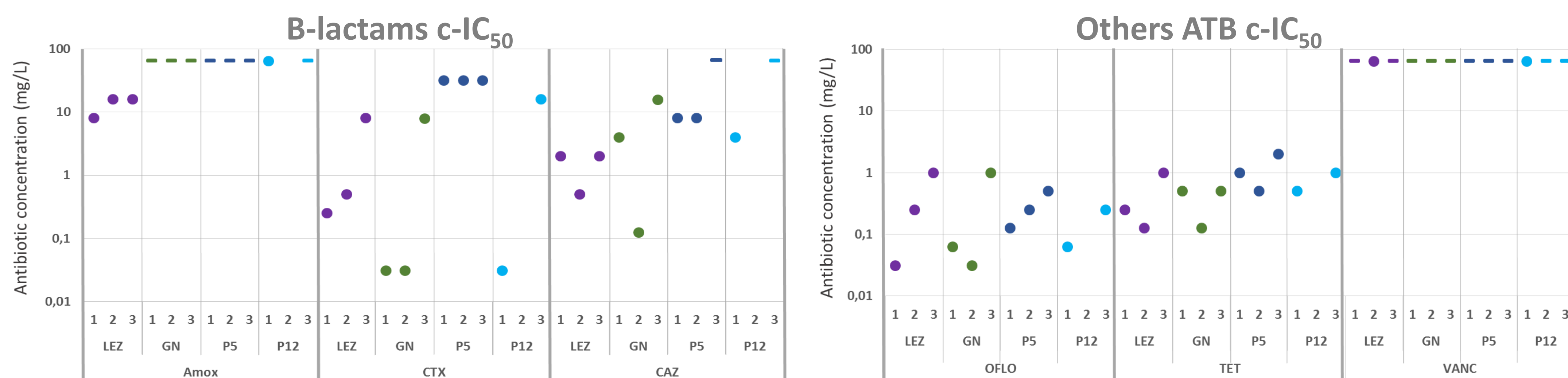


Water samples are collected:

- during the spring (1),
- during the dry summer conditions (2),
- and after local autumnal heavy rains (3).

To evaluate the resistance level and its dynamics, and the biodiversity of total and resistant bacterial communities, we develop an original mixed method associating i) selective culture conditions, using culture media containing tetracycline (TET), vancomycin (VANC), ofloxacin (OFLO), amoxicillin (Amox), cefotaxime (CTX) or ceftazidime (CAZ) at increasing concentrations (from 0.0312 mg/L to 64 or 128 mg/L), and ii) molecular approaches, using 16S rRNA gene PCR-Temporal Temperature Gradient Gel Electrophoresis (TTGE) and sequencing.

Determination of the concentration of antibiotic inhibiting 50% of the cultivable community



Here we present the antibiotics c-IC₅₀ concentrations inhibiting 50% of the whole culturable community for the sampling periods 1 to 3.

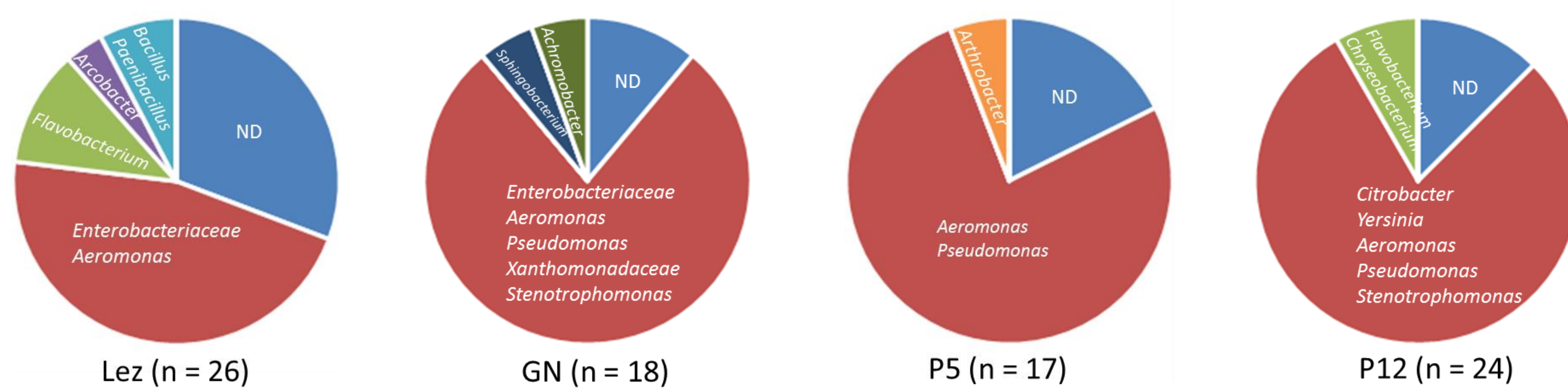
Resistance to beta-lactams, particularly to 3rd generation cephalosporins (CAZ and CTX) varied according both the geological site and the period of sampling. Variation of resistance observed with the others antibiotics were not so marked.

Dashes indicate that for some samples, the c-IC₅₀ cannot be determined, because it is over 64 mg/L, showing that some culturable bacterial communities are majoritary composed with resistant bacteria. **These results show that conditions such as the season and the speed of water circulation in the karst aquifer influence the level of resistance in the cultivable bacterial communities. The impact of the level of anthropization on the community resistance is lower.**

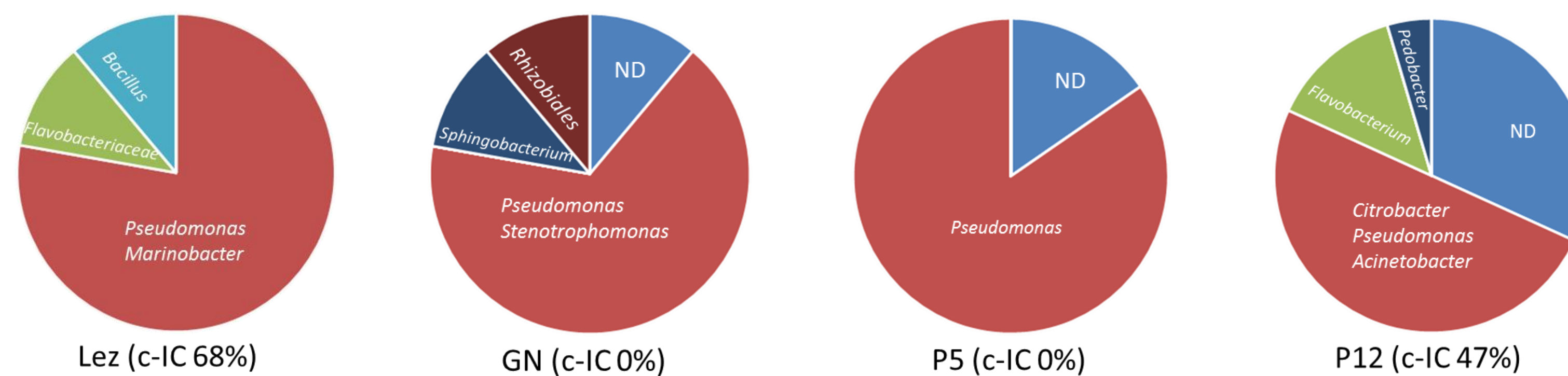
Taxonomic Diversity in total and antibiotic-tolerant bacterial communities

Results presented here are obtained with waters sampled during the spring period. TTGE migration produces a banding pattern; *n*, the number of TTGE bands by pattern represents the overall cultivable bacterial diversity. The bacterial diversity is presented to the genus level. The genera are gathered according their high-level taxa affiliation.

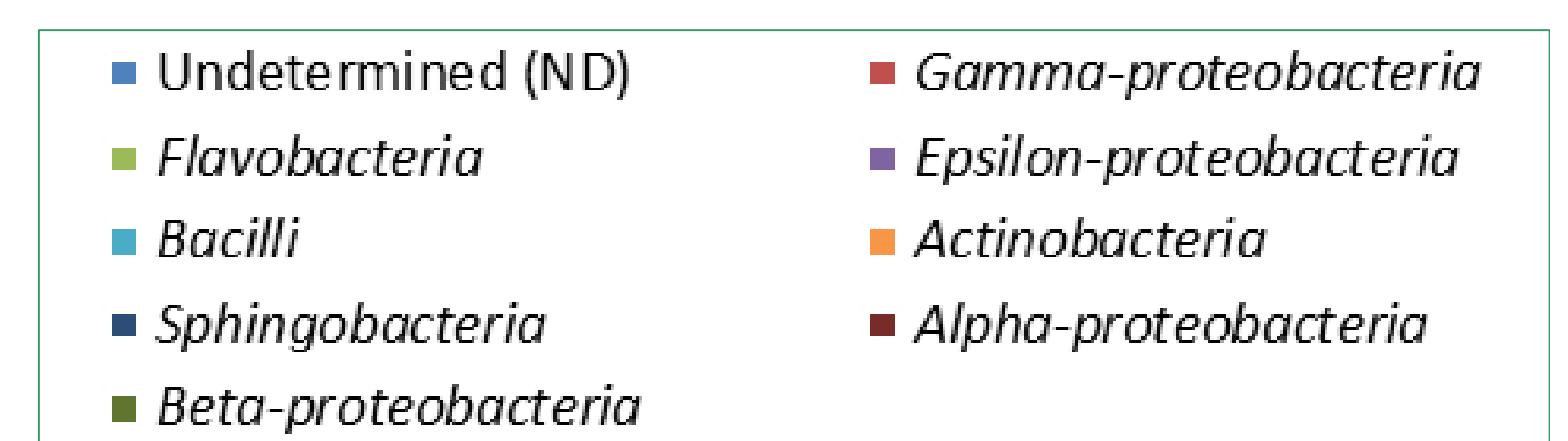
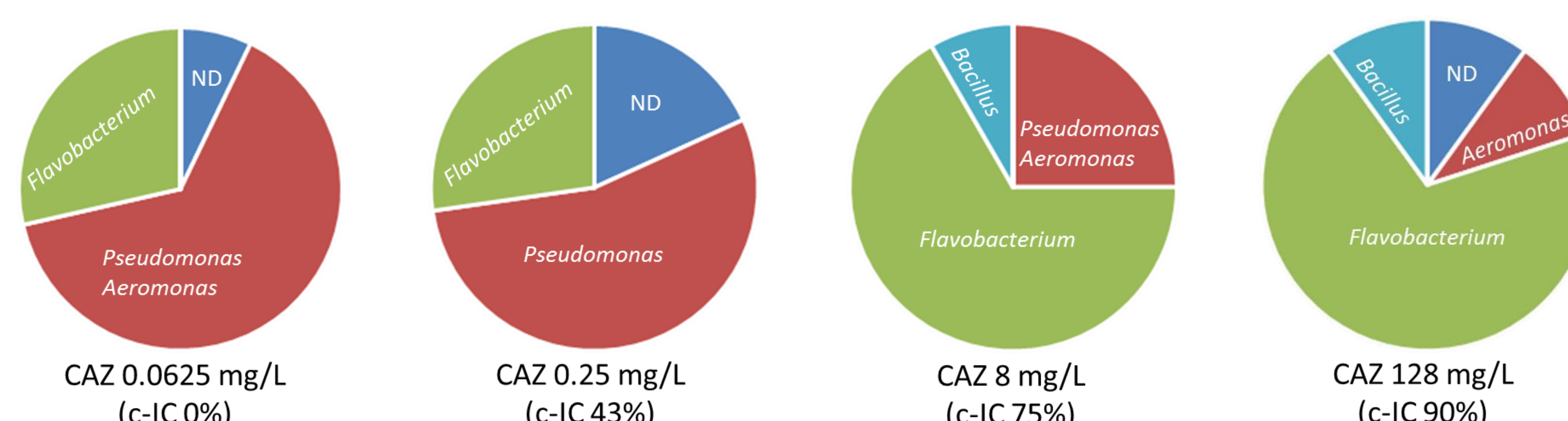
Taxonomic diversity of waters from different karstic compartments:



In vitro effect of low ATB concentrations on community composition: example of ofloxacin 0.0312 mg/L



In vitro effect of increasing concentrations of ATB on water community: example of CAZ on P12 water



Gamma-proteobacteria are mostly represented by potentially human pathogens. Their relative proportion and diversity vary according the site of sampling but appear not clearly linked to the level of anthropization.

We observed a clear impact of low ofloxacin concentration on the relative proportion of genera and high-level taxa as well as on the genus diversity within each high-level taxon.

The increase of CAZ concentration changes the community composition. Flavobacteria are clearly selected by high concentrations while *Pseudomonas* population decreases. For the potentially human pathogen *Aeromonas*, the dynamics observed suggests the co-existence of two populations with different behavior against ATB.

Conclusion and perspectives: The original approach presented herein allows to assess the resistant bacterial community in natural waters. A single experiment allows c-IC determination and raw community barcoding. It appears efficient to follow-up the dynamics of resistant communities according sites, seasons and ATB concentrations. Based on culture, the method avoid the non-cultivable community but resistance to ATB is a phenotypic trait generally assessed by culture in medicine. Therefore, the approach should provide data able to be compared with resistance epidemiology in human populations.