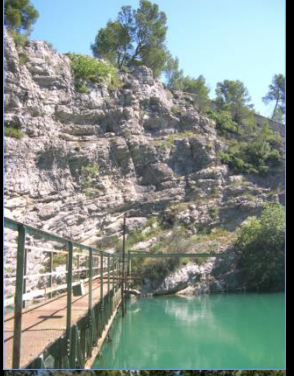

Bacterial community structure and antibioresistance in a Mediterranean Karst aquifer

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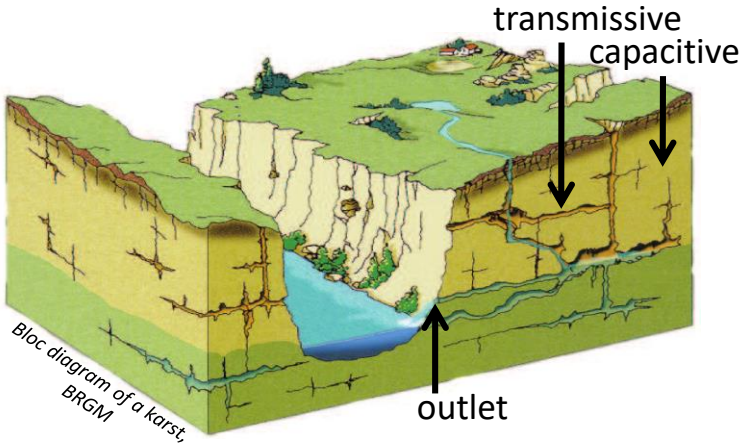


Bloc diagram of a karst, BRGM

Mediterranean karst aquifer:

✓Heterogeneous (capacitive/transmissive elements)
✓Role in interconnecting surface and subsurface waters
✓Major water ressource
✓ **Living ecosystem** vulnerable to anthropogenic contaminations

Karst microbial communities:

✓Karstic springs: diverse and stable autochtonous communities
➢ Other parts of the aquifer = « black box »

Antibiotic resistant populations

➢Emergence and dissemination of antibioresistance?

Mediterranean karst aquifer

➢ **Investigate the spatial and temporal dynamics of the karst bacterial communities**

➢ **Total bacterial communities**

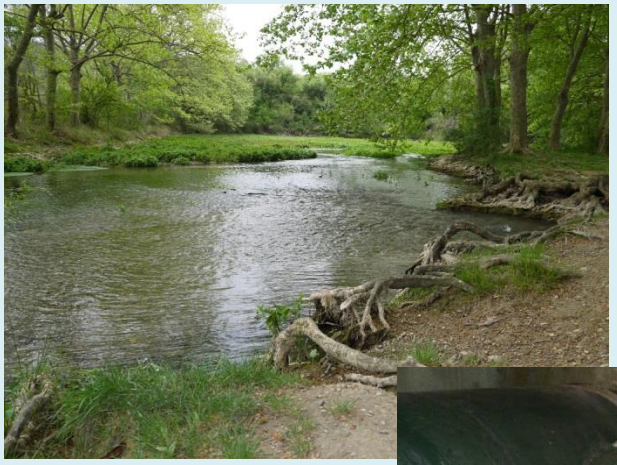
➢ **Antibiotic resistant populations**

Is Mediterranean Karst a reservoir for antibacterial resistance?

Karst microbial communities

➢ **Assess the relevance of bacterial community monitoring to investigate karst functioning (in combination with geochemistry and hydrodynamic)**

e Lez aquifer

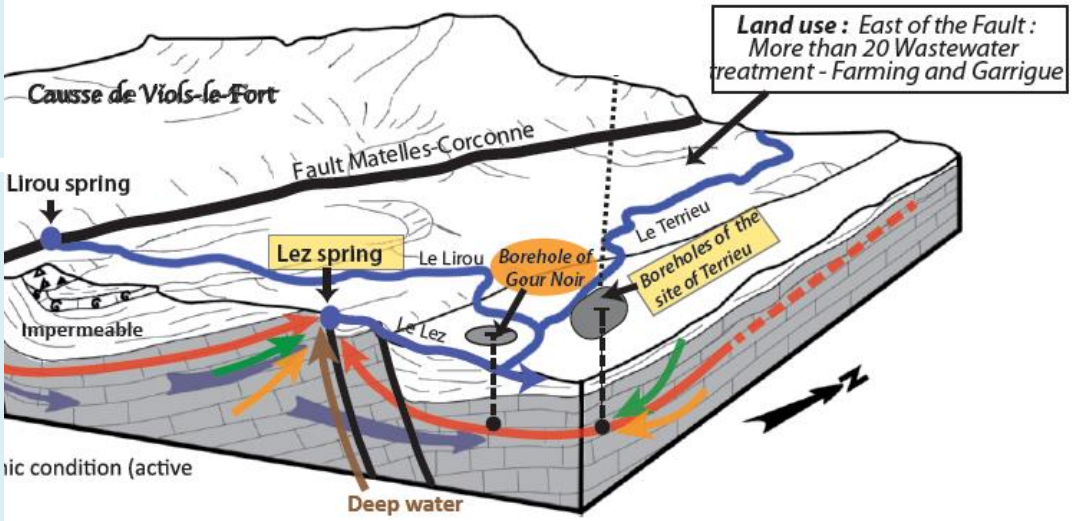


Lez spring

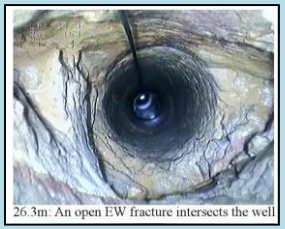
- main outlet of the aquifer
- drinking water supply for Montpellier
- mixing of waters of different origins

Gour Noir

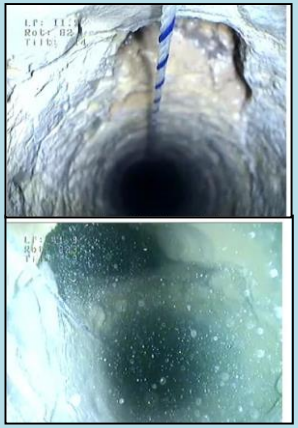
- captive aquifer



Adapted from Leonardi 2015



P5

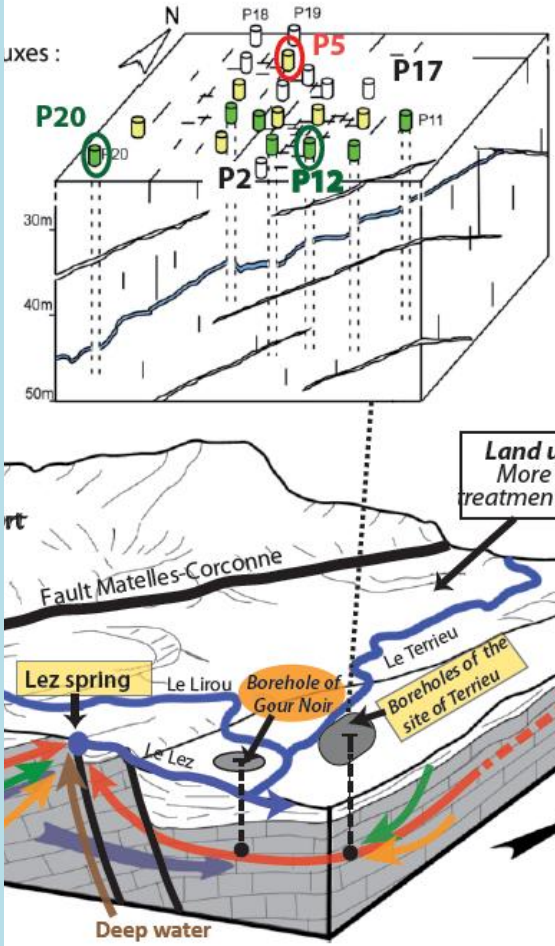


P12/P20

Terrieu experimental site

- Limestone outcrop
- **P5** : capacitive element (matrix/fracture)
- **P12 and P20 :** transmissive element (drain)

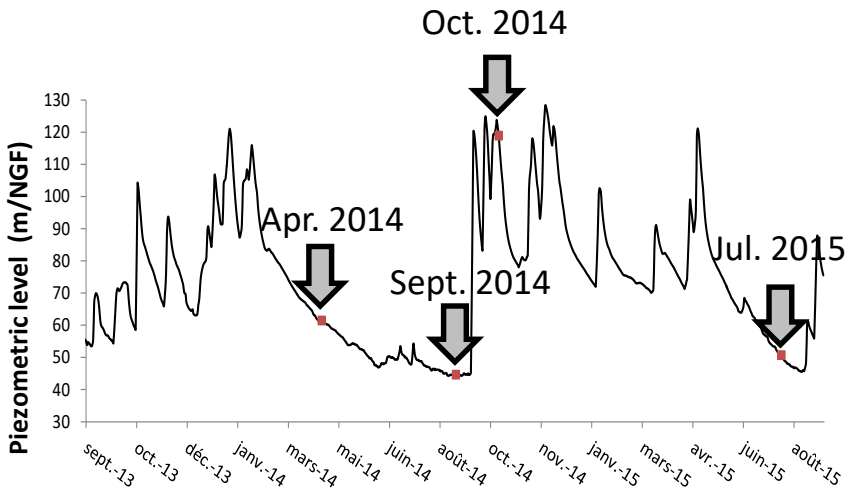
uifer



Adapted from Leonardi 2015

4 sampling sites:
- Lez spring
- Gour Noir
- P5
- P12 or P20

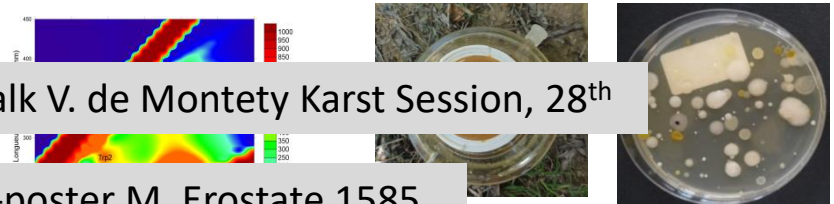
4 sampling campaigns:
- Apr. 2014: middle flow
- Sep. 2014: very low flow
- Oct. 2014: very high flow
- July 2015: low flow



***in situ* measurements:**
- ✓temperature
- ✓pH
- ✓conductivity
- ✓O_2

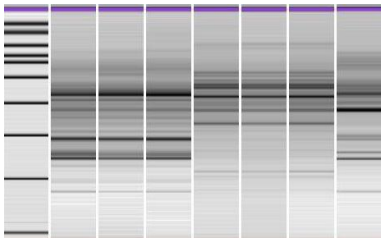
Chemical analyses:
- ✓Dissolved OM
- ✓Major, trace elements
- ✓Dissolved gases (noble gases, CFC, SF6)

Microbial analyses:
- ✓Total bacterial community structure: Automated Ribosomal Intergenic Spacer Analysis (ARISA)
- ✓ATB resistant populations : Culture on media with ATB -> c-MIC50



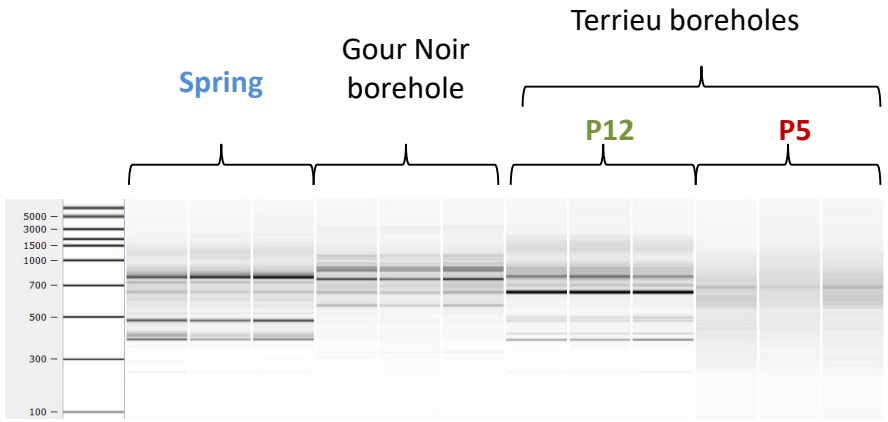
Talk V. de Montety Karst Session, 28th

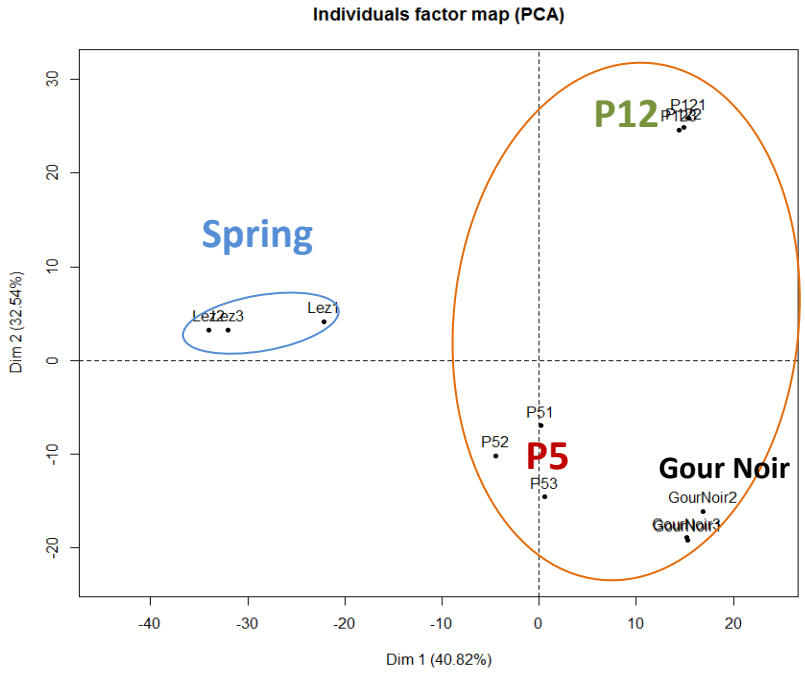
E-poster M. Erostate 1585



Total bacterial community structure
Spatial dynamic

- April 2014 (middle flow)





❖ Discrimination spring *vs* boreholes (associated with distinct geochemical characteristics)

❖ Discrimination **P5** *vs* **P12** (waters with very similar geochemical characteristics)

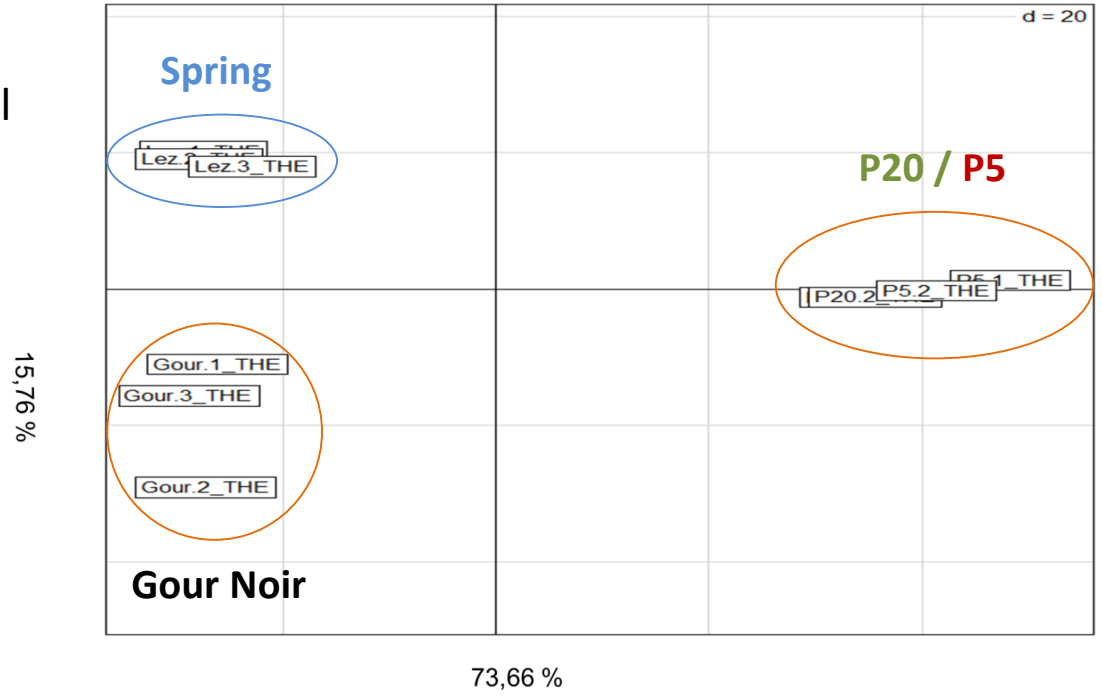
➔ **Microbiology more sensitive to discriminate between capacitive and transmissive flow**

Total bacterial community structure
Spatial dynamic

- October 2014 (flood event)

❖ **P5** and **P20**: very similar bacterial communities

➔ contribution of surface water?

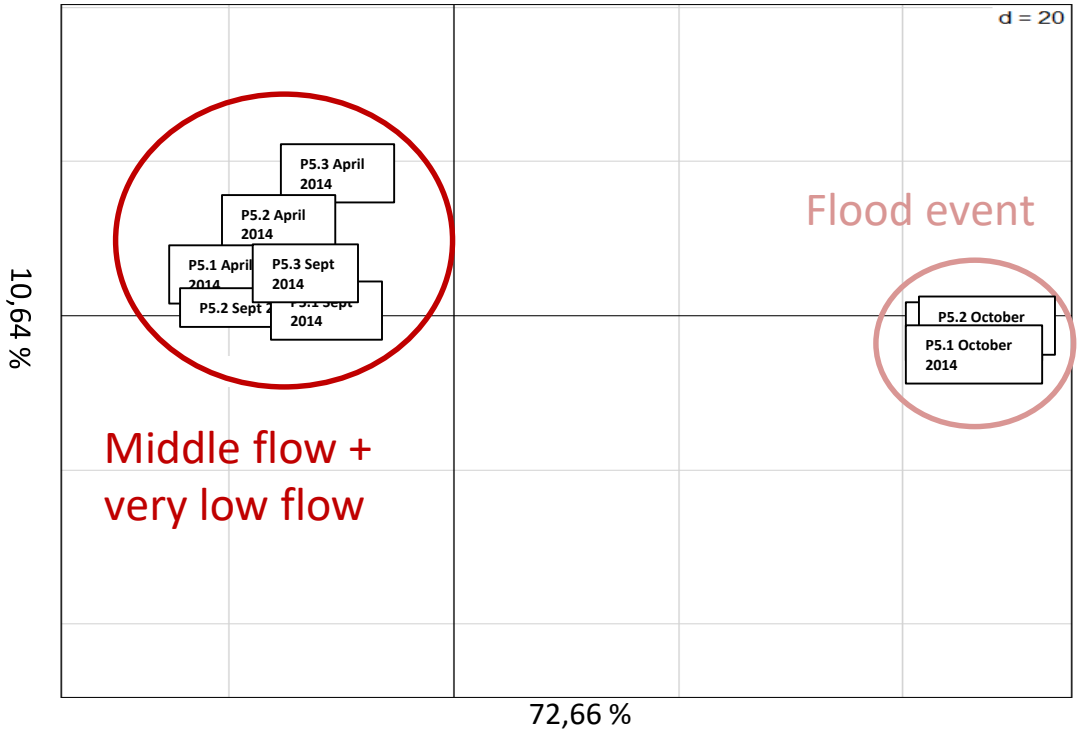


Total bacterial community structure
Temporal dynamic

- P5 borehole

➔ Stable **autochtonous** communities under middle or low flow

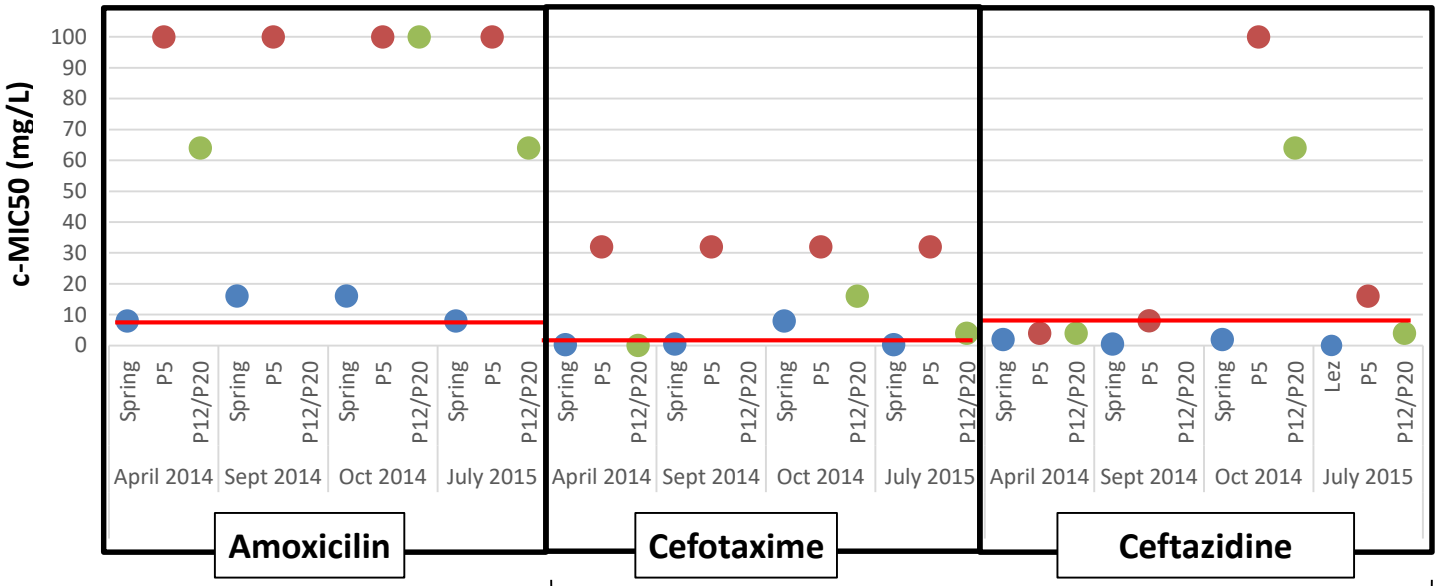
➔ Flood event : contribution of **allochtonous** populations originating from the surface?



Antibiotic resistant populations
Spatio-Temporal dynamic

- P5 (capacitive)
- P12/P20 (transmissive)
- Spring
- clinical breakpoints

c-MIC50: Minimum concentration necessary to inhibit 50% of the cultivable community



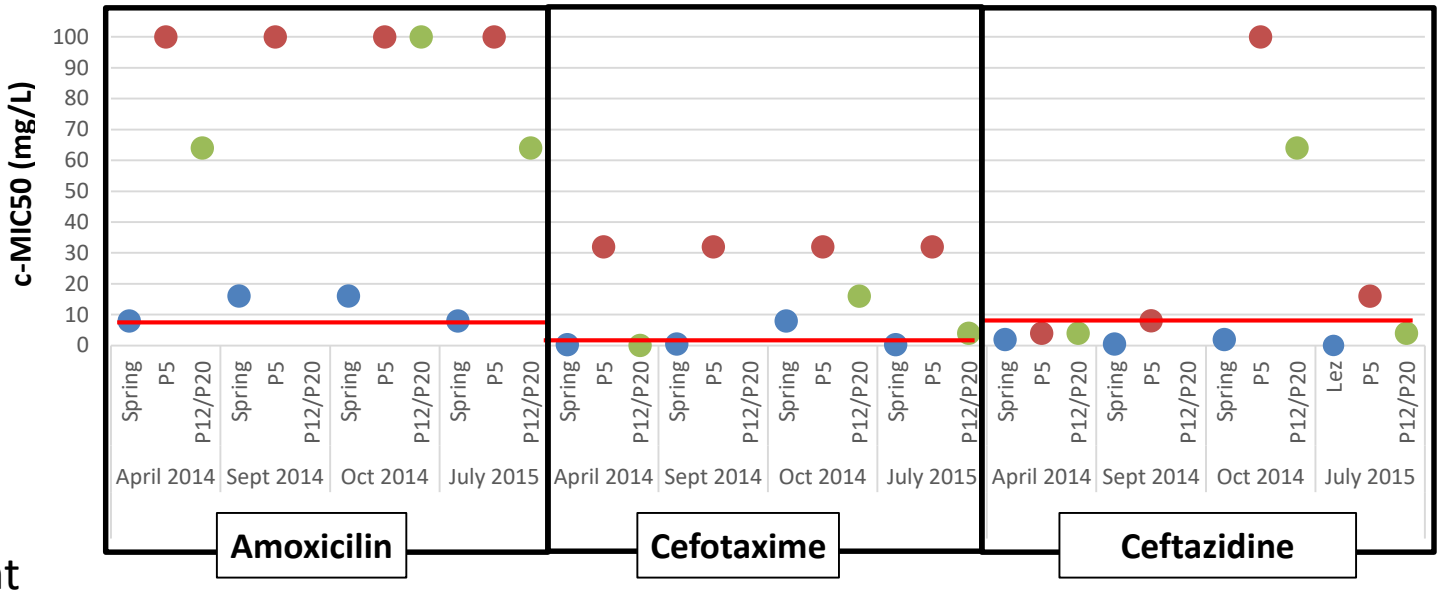
- 1st generation
- Broad spectrum
- Massive use

- 3rd génération (more extended-spectrum)
- resistance is of great concern for human therapy

- Level of ATB resistance **P5 (capacitive)** > **P12/20 (transmissive)** > **Spring**
- Higher level of resistance during flood event
- Resistance often higher than the clinical breakpoint

Antibiotic resistant populations Spatio-Temporal dynamic

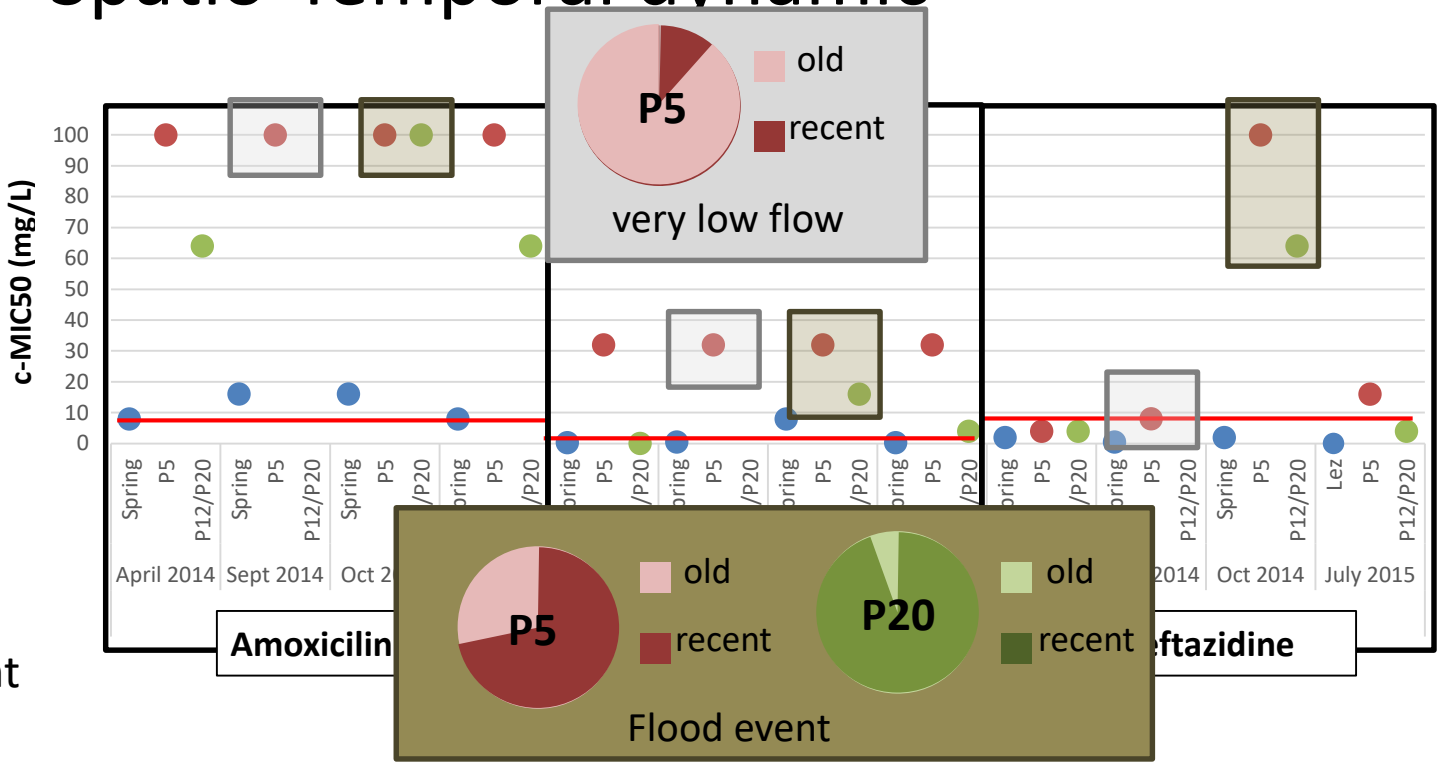
- P5 (capacitive)
- P12/P20 (transmissive)
- Spring
- clinical breakpoints



- **Dissolved gas SF6 :** proxy of mean residence time -> estimation of the proportion of recent and older water

Antibiotic resistant populations
Spatio-Temporal dynamic

- P5 (capacitive)
- P12/P20 (transmissive)
- Spring
- clinical breakpoints



❖ **Dissolved gas SF6 :** proxy of mean residence time -> estimation of the proportion of recent and older water

➢ Low flow -> « old » water in **P5** associated with high level of ATB resistance

➢ Flood event: high proportion of « recent » water associated with an increase of the resistance (**P20**)

- Spring :
  - Distinct total bacterial communities compared to boreholes (associated with distinct chemistry due to mixing of waters of diverse origins)
  - Lower level of ATB resistance than **P5** or **P12/20** (but sometimes higher than clinical breakpoints)

- Capacitive part of the aquifer (water circulation slow)
  - **stable autochtonous** community (distinct from those in transmissive element)
  - higher ATB resistance than in transmissive element
  - **stable autochtonous antibioresistant populations**

- Flood event: influence of surface water (anthropogenic contamination)
  - Increase of resistance level particularly in transmissive elements
  - Unlikely to persist

Conclusions

- Hydrogeological and hydrological conditions impact bacterial community structure and level of ATB resistance

- **Bacterial communities** relevant as **complementary tracers**
  - To better understand Karst functioning
  - For the monitoring of water quality

- Origin of ATB resistance in the Lez karstic aquifer:
  - **Anthropogenic** : contamination during flood events
  - **Environmental** : autochtonous resistant populations

Perspectives

- **Mediterranean Karst as a potential reservoir for antimicrobial resistance?**
  - "Resistome": intrinsic resistance *vs* genetically acquired resistance

  → Potential risk in term of resistance transferability and relevance in clinical epidemiology?

Thanks…

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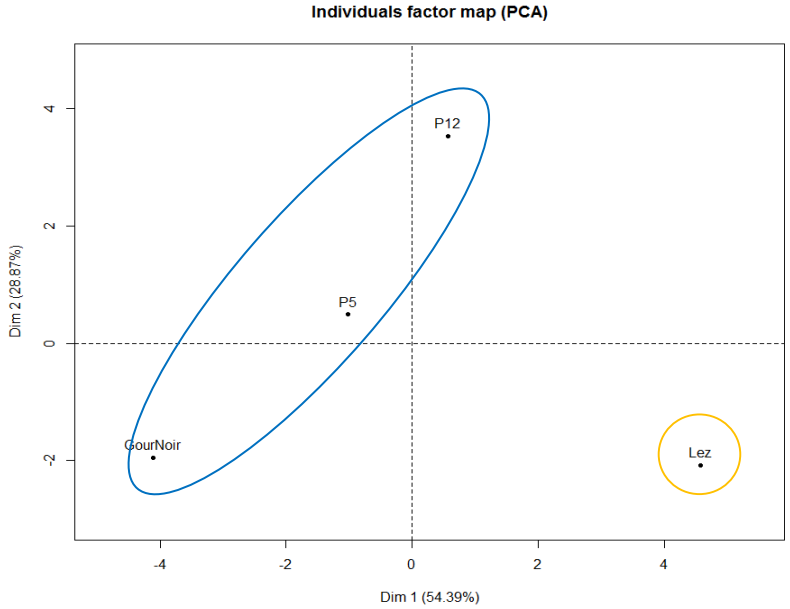
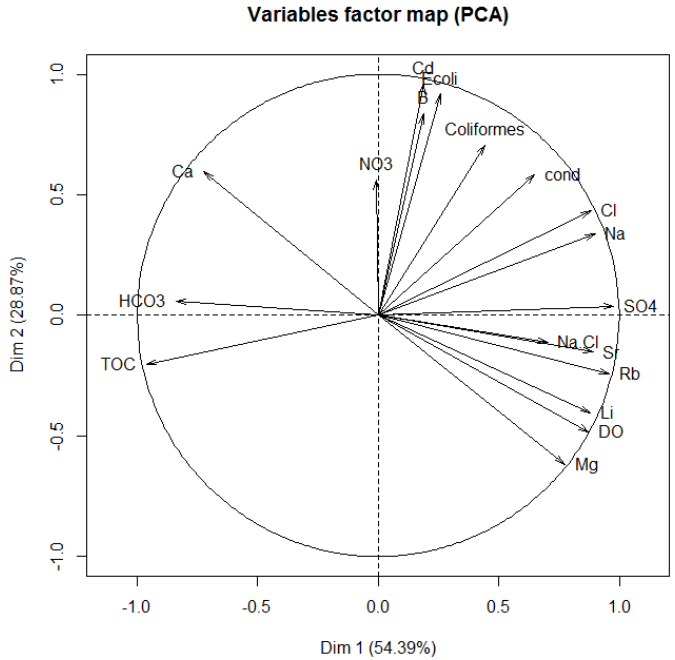
Students:
- Mickael Hardy
- Celia Roure
- Elia Laroche

Field team:
- Remy Muller
- Fred Hernandez
- Pierre Marchand

Geochemical characterization

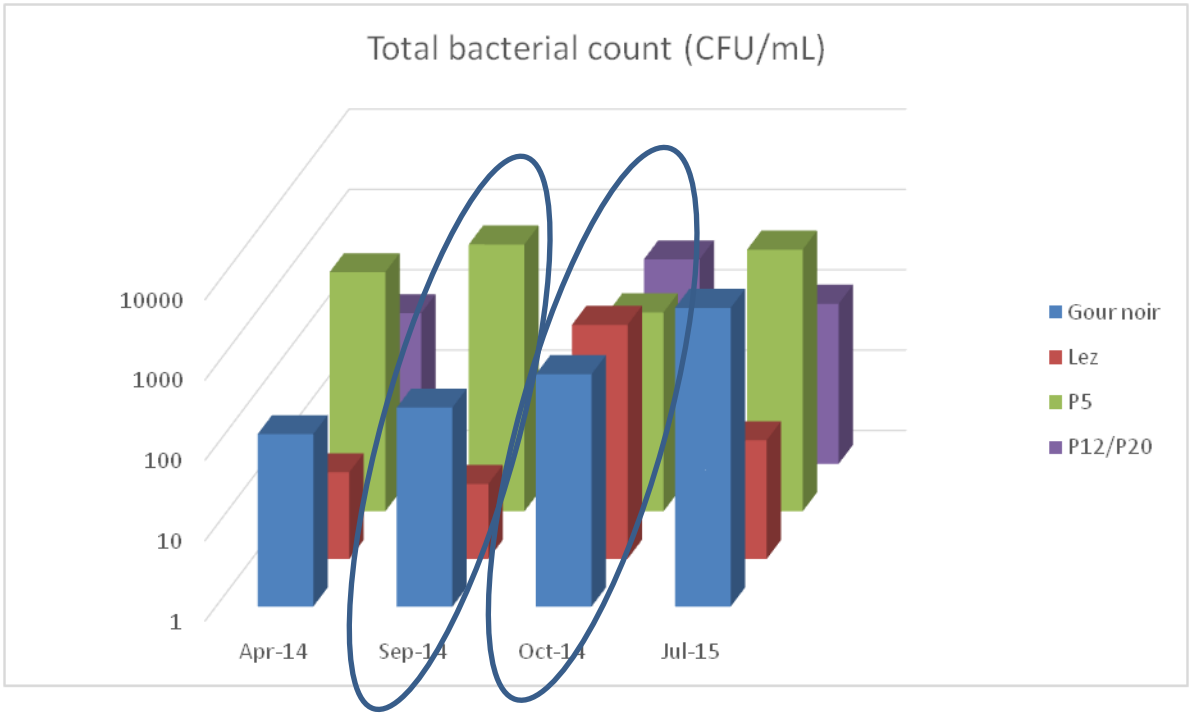
- April 2014 campaign (middle flow)





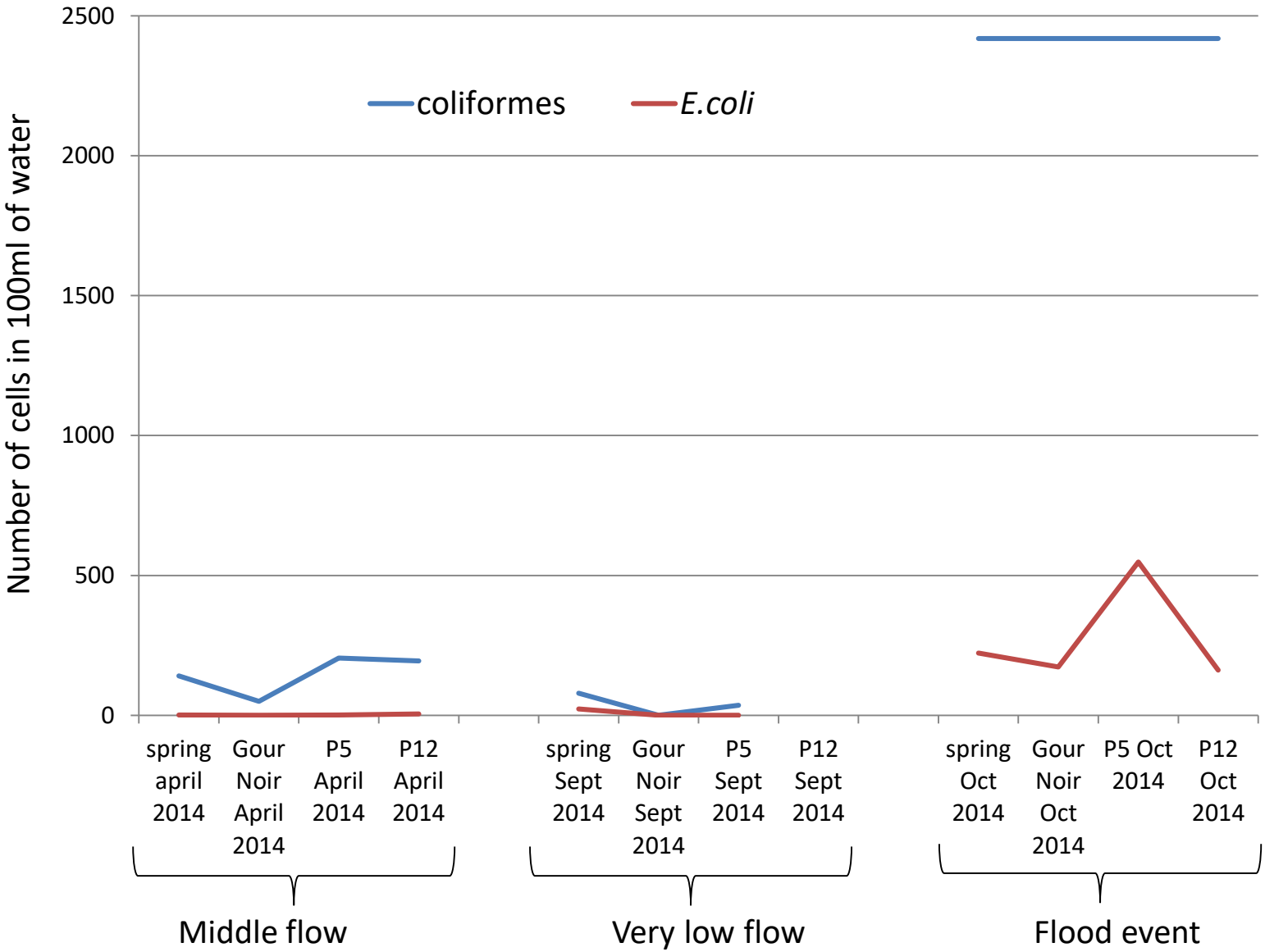
- Discrimination spring *vs* boreholes
- Spring ➔higher level of mineralization (higher conductivity) reflects the contribution of water from a deep compartment (Caetano Bicalho et al., 2012)
- Boreholes:
  - ➔ geochemical feature typical of karstic systems (high [carbonate] and [Ca])
  - ➔ anthropogenic contamination P12 > P5 (fecal indicator bacteria)

Cultivable bacterial communities

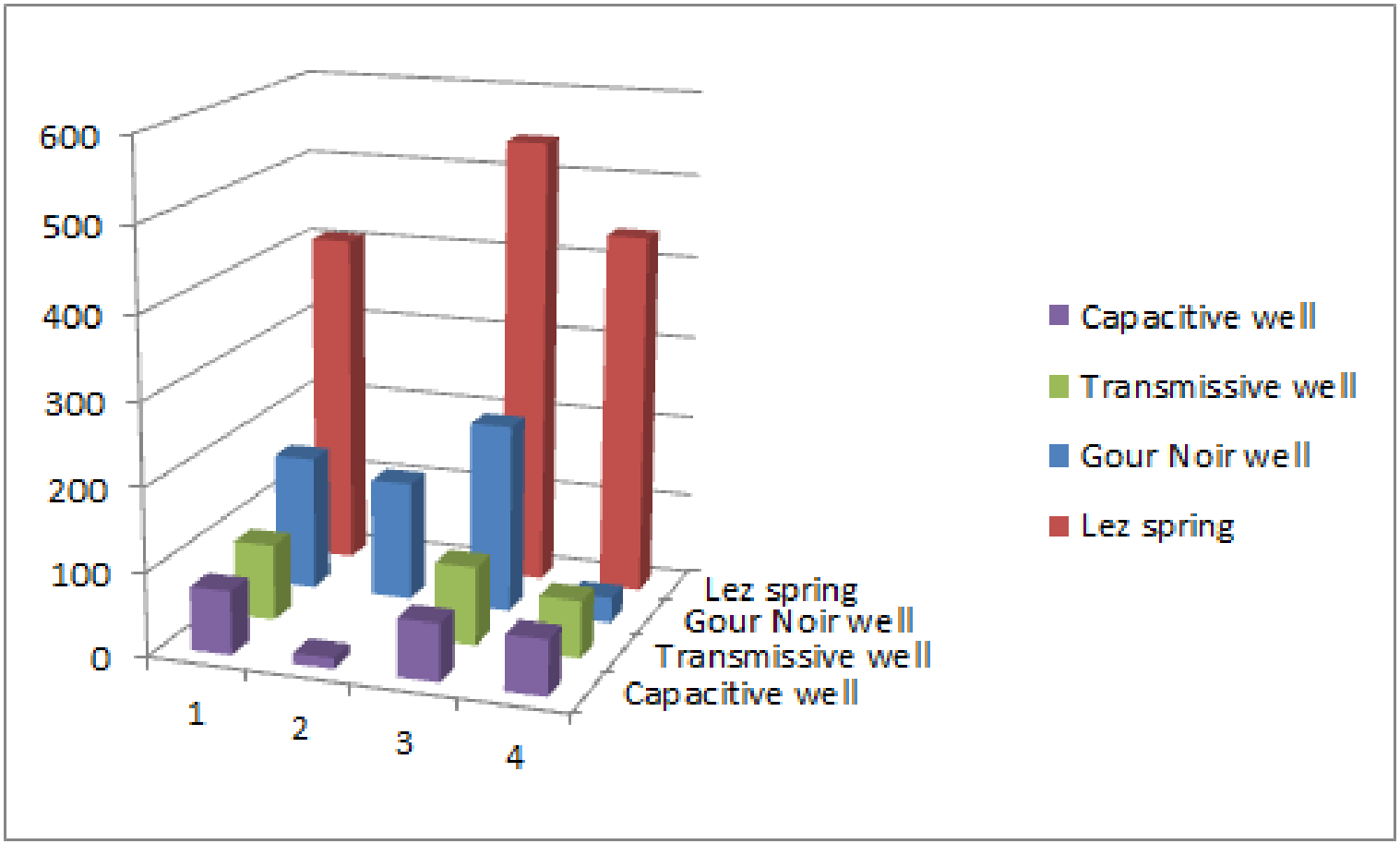


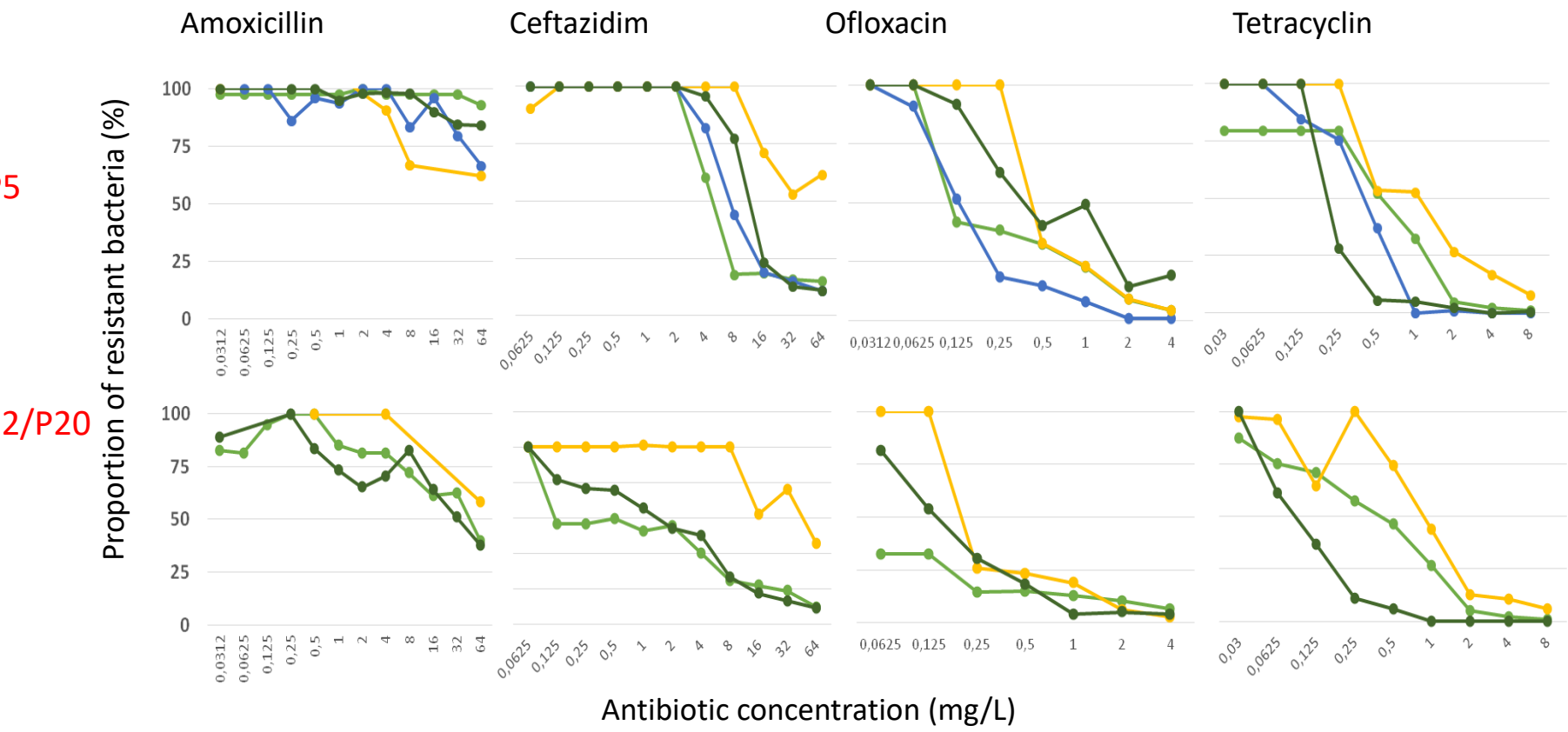
April 2014: middle flow
Sept 2014: very low flow
Oct 2014: very high flow
July 2015: low flow

Lez biomass maximum under very high flow conditions
Biomass fluctuations during hydrological cycling differ according to the aquifer compartment.
Bacterial dynamics in P5 ≠ P12/P20


Number of cells in 100ml of water
2500
2000
1500
1000
500
0
coliformes
E.coli
spring april 2014
Gour Noir April 2014
P5 April 2014
P12 April 2014
spring Sept 2014
Gour Noir Sept 2014
P5 Sept 2014
P12 Sept 2014
spring Oct 2014
Gour Noir Oct 2014
P5 Oct 2014
P12 Oct 2014
Middle flow
Very low flow
Flood event

1 : april 14,
2 : sept 14,
3 : oct 14,
4 : jul 15.

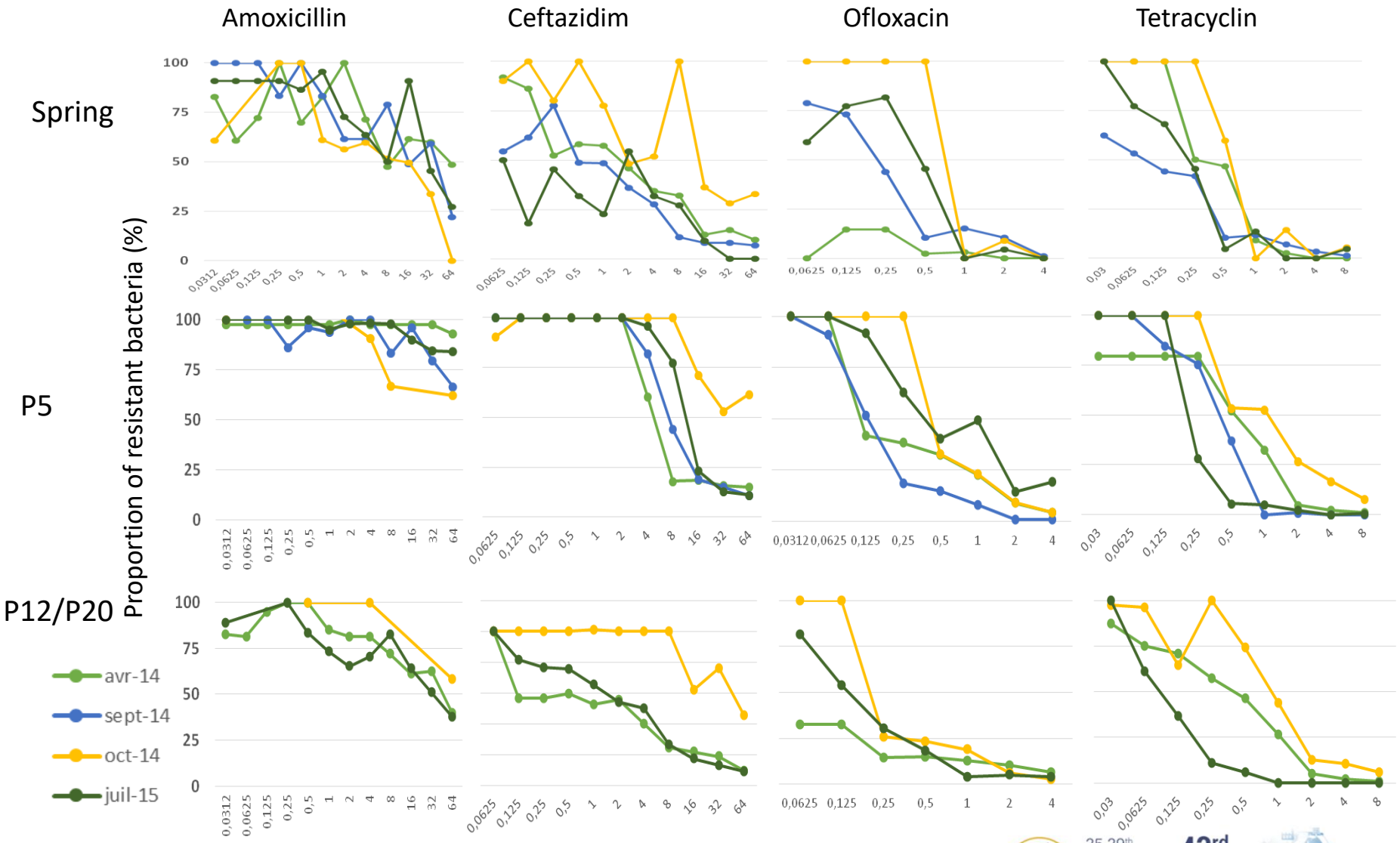


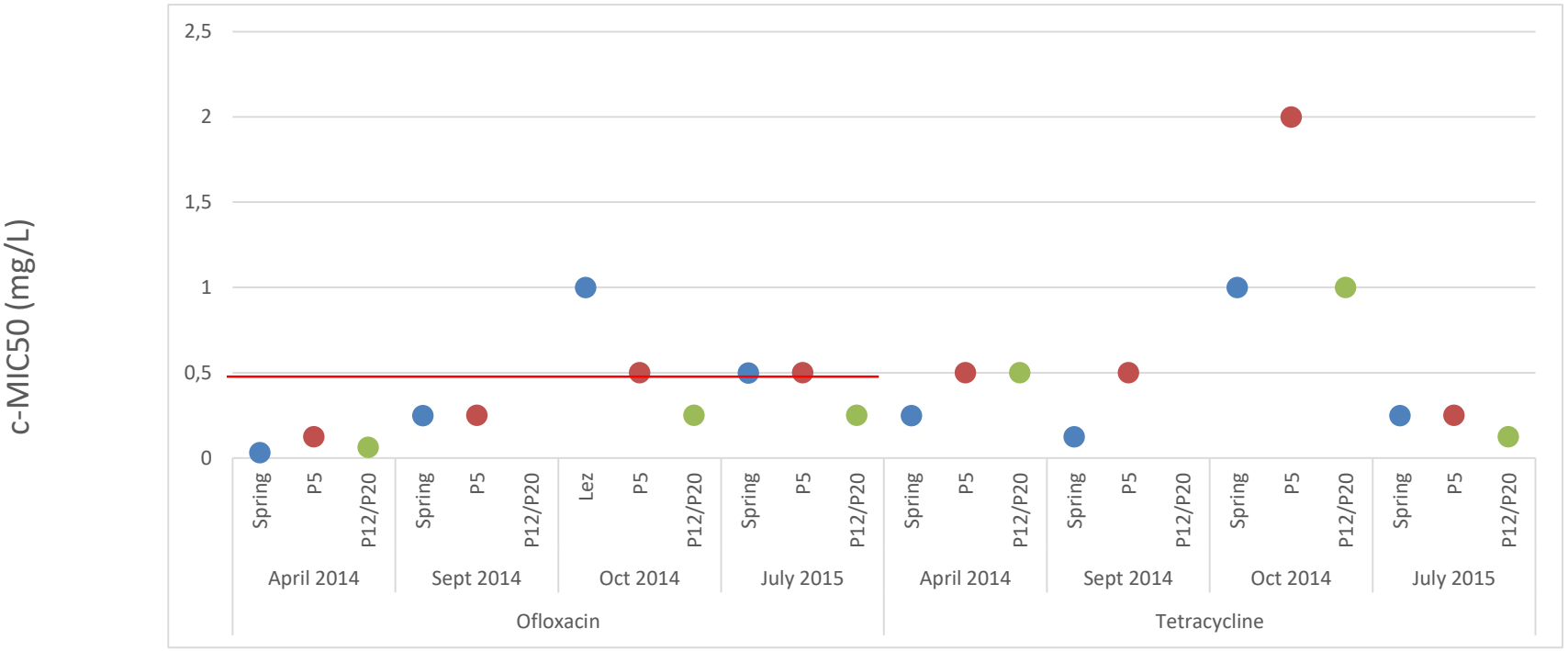


- P5: high resistance to amoxicillin whatever the hydrological conditions
- Very high flow : higher level of resistance to Ofloxacine and Tetracyclin

- P12/P20: lower levels of resistance than P5
- Higher resistance level during very high flow

- Spatio temporal dynamics of ATB resistance




c-MIC50 (mg/L)
2,5
2
1,5
1
0,5
0
Spring
P5
P12/P20
Lez
April 2014
Sept 2014
Oct 2014
July 2015
Ofloxacin
Tetracycline