

Multi-disciplinary approach for assessing the impact of a flood event in a shallow karst cavity (Pindal Cave, Spain)

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1. Introduction

Pindal Cave (Asturias, Spain) and its Paleolithic art have been part of the UNESCO World Heritage List since 2008. Since 2017, a multidisciplinary team have been carrying out research focused on deciphering the relationships between environmental conditions and microbial activity to the design of conservation strategies. On October 23, 2019, the cave was flooded. After the flood, a specific study was performed to evaluate the environmental changes caused in the underground ecosystem. This study revealed a massive entry of material from a cattle farm located vertically in the cavity. Thereafter, in 2021, the livestock activity in the vicinity of the cave was stopped.



Fig. 1. Paleolithic art and Pindal Cave location.

3. Methods

One week after the flood, the sediments were sampled at three points for their subsequent biogeochemical and microbiological analysis [2,3]. The results were compared with those obtained before and one year after the flooding. For microbiological analysis, DNA was extracted from moonmilk, cave and exogenous sediments. Then, 16S rRNA gene amplicon sequencing was performed on Illumina, and reads were analyzed using QIIME 2 and the SILVA 132 database [3].

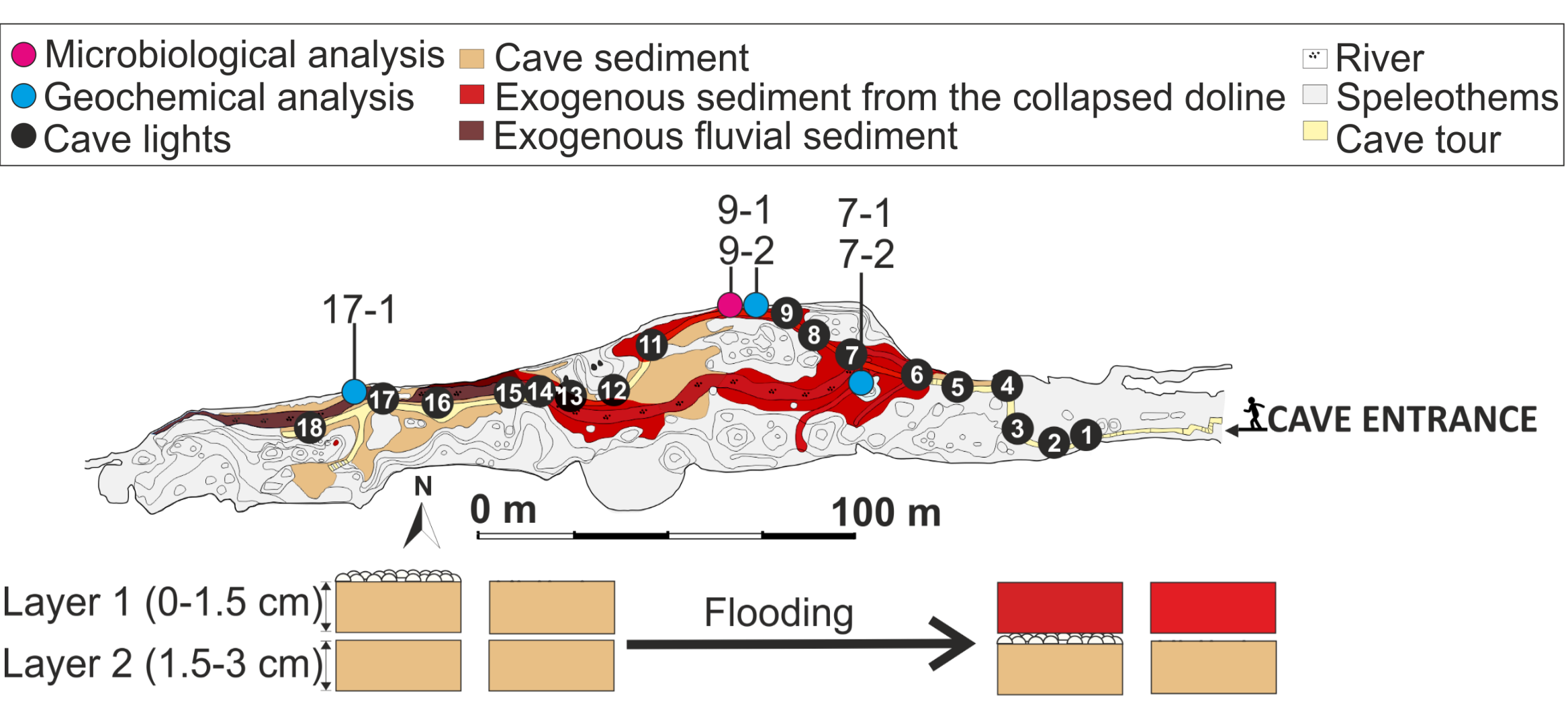


Fig. 4. Flood affected areas and sampling strategy.

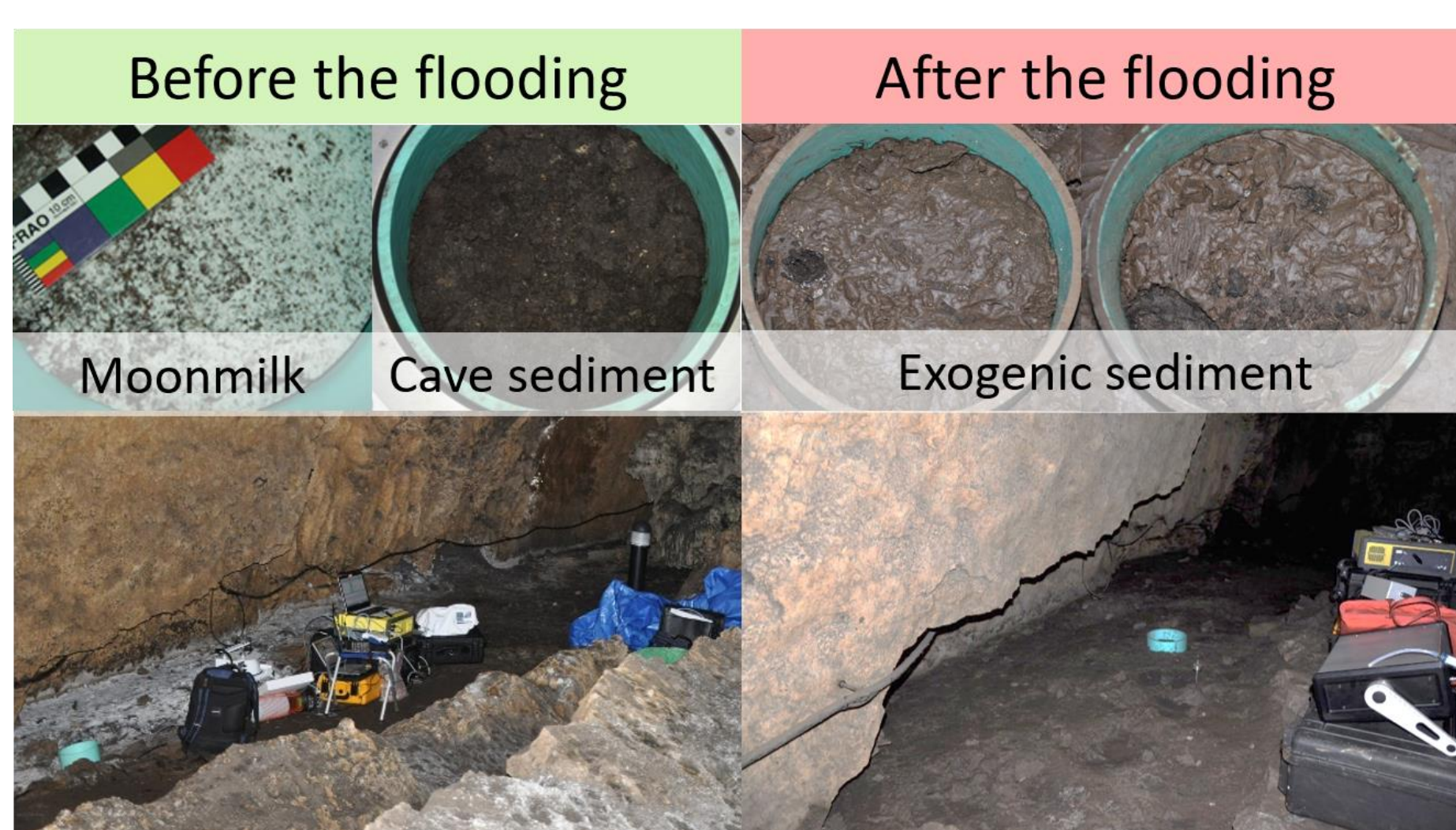


Fig. 5. Microbiological sampling area (site 9).

2. Why did the flood occur?

The Pindal karstic system develops in a calcareous massif (Carboniferous) modeled in the form of an erosional marine terrace (rasa) by coastal morphogenetic processes. This marine terrace level is located at an elevation of 30-68 meters above current sea level and constitutes the preferred catchment area for runoff water from another higher level (140-170 m) developed on quartzite layers with very low permeability (Ordovician). The cave is the main endokarstic feature of the system. On the surface of the 30-68 m rasa there are numerous exokarstic structures of sinkhole and polje type. On one of the sinkholes, located almost vertically to the cave, a cattle farm was installed in 1995 (Fig. 2).

Between October 19 and 23, 2019, an extreme episode of rainfall occurred in the area with a cumulative total of 209 l/m². This event caused a strong accumulation of water in the sinkhole that finally collapsed, flooding the cave for several days (Fig. 3).

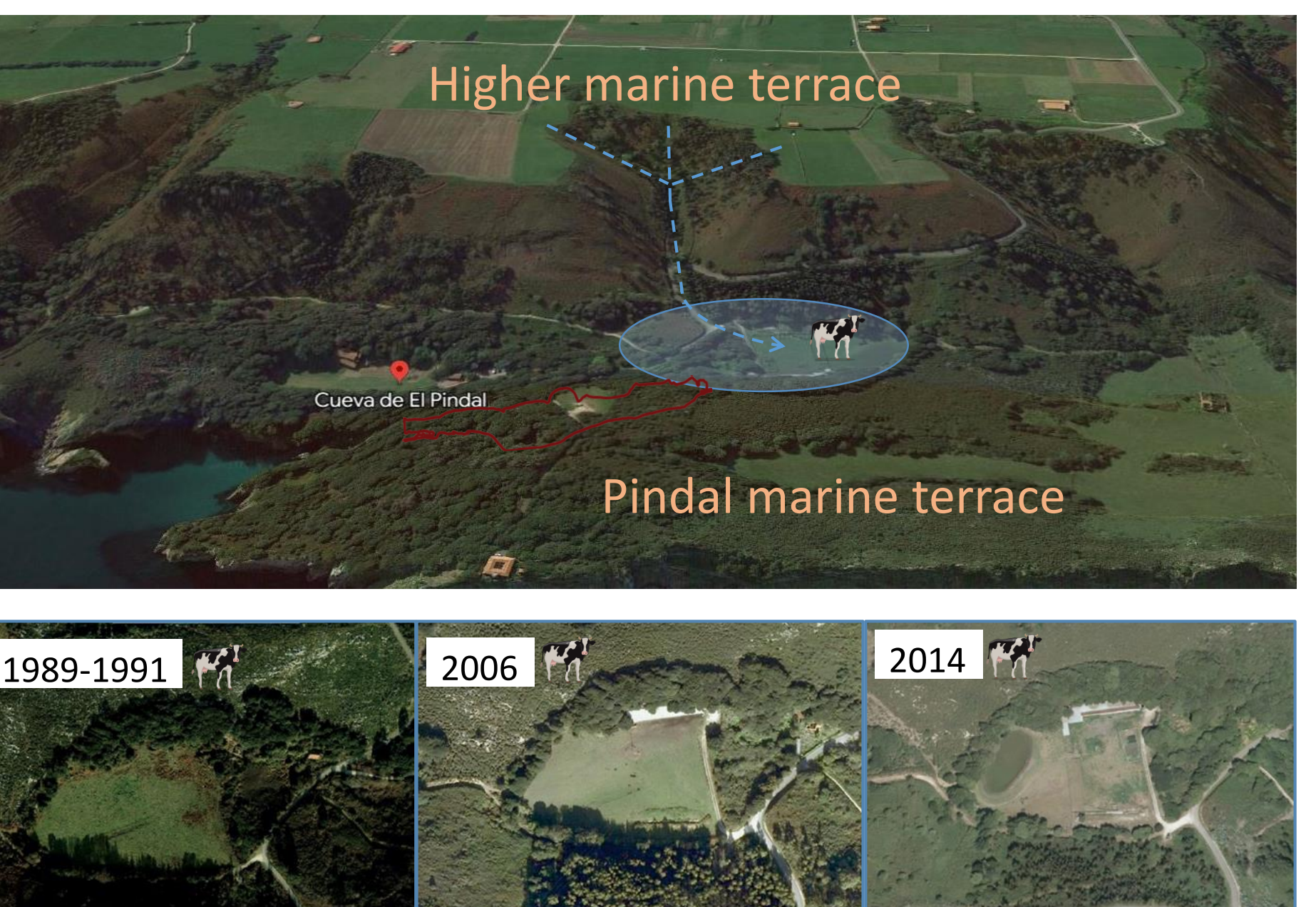


Fig. 2. Aerial view of the rasas (top) and evolution of the farm (bottom). Blue indicate the discharge area.



Fig. 3. Collapsed sinkhole (top) and flooded cave (bottom).

4. Geochemical results

The cave sediments and exogenous sediments of sites 7 and 9 showed high values of organic matter, nitrogen phosphorus and metals. These values were higher than those of the innermost sites (17) that were not directly affected by the collapse of the sinkhole and the entry of water contaminated with slurry.

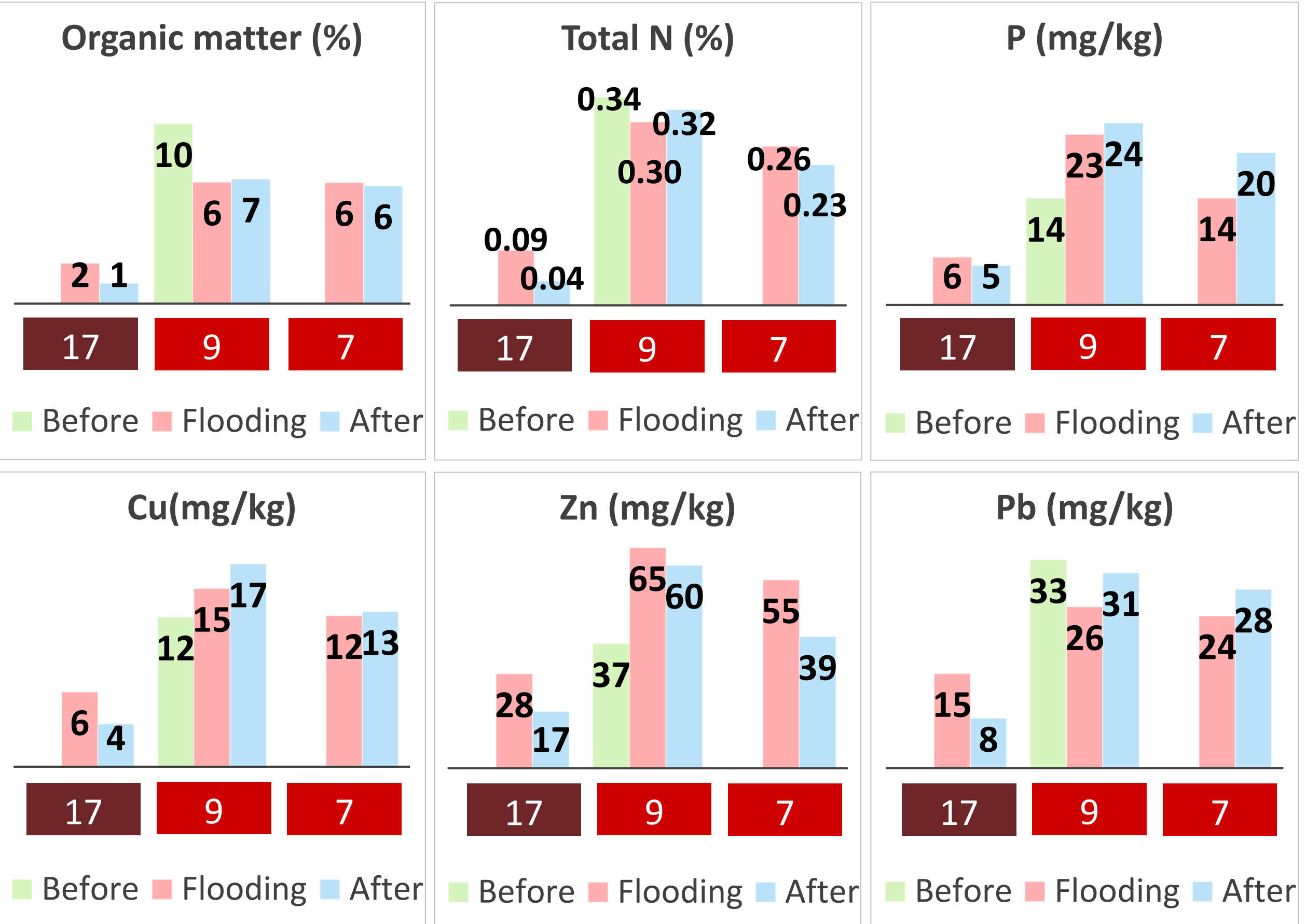


Fig. 6. Results of the geochemical analysis. Before: before the flooding, Flooding: one week after the flooding, After: one year after the flooding. Red: sediments contaminated with slurry (sites 9 and 7). Maroon: uncontaminated sediments (site 17).

5. Microbiological results

One week after the flooding the exogenous sediments (In Fig. 8: 9-1 in red) showed high relative abundances of *Thauera*, *Corynebacteriaceae*, *Bacillota*, *Bacteroidota* and methanogenic archaea characteristic of the gastrointestinal tract and contaminated environments.

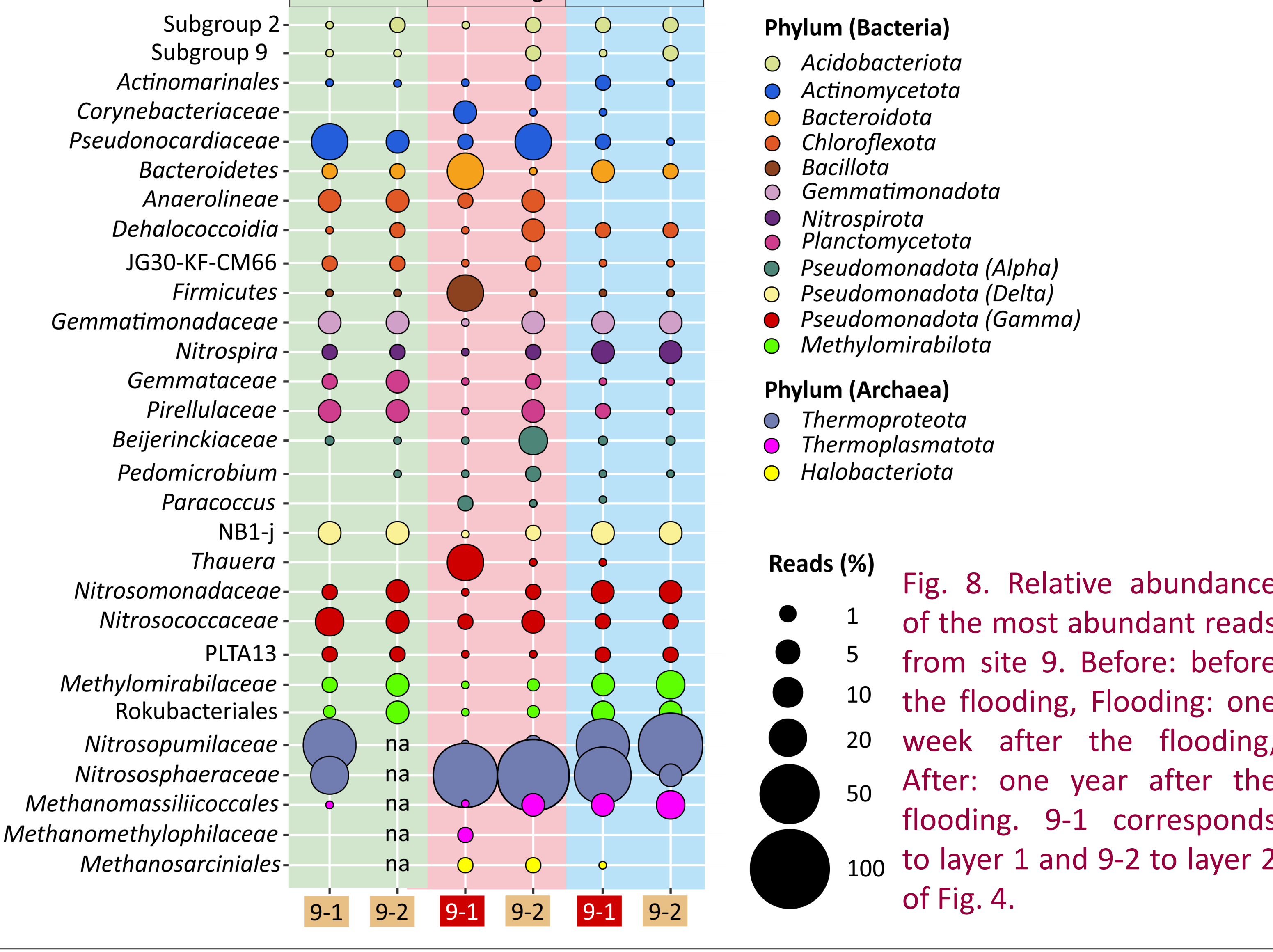


Fig. 8. Relative abundance of the most abundant reads from site 9. Before: before the flooding, Flooding: one week after the flooding, After: one year after the flooding. 9-1 corresponds to layer 1 and 9-2 to layer 2 of Fig. 4.

References

[1] Jennings, J.N., 1985, Karst Geomorphology: Oxford, Basil Blackwell, 293 p.
 [2] Martin-Pozas, T., Sánchez-Moral, S., Fernández-Cortés, A., et al. (2020). Biologically mediated release of endogenous N₂O and NO₂ gases in a hydrothermal, hypoxic subterranean environment. *Science of the Total Environment*, 747, 141218. <https://doi.org/10.1016/j.scitotenv.2020.141218>.
 [3] Martin-Pozas, T., Cuezva, S., Fernandez-Cortés, A., et al. (2022). Role of subterranean microbiota in the carbon cycle and greenhouse gas dynamics. *Science of the Total Environment*, 831, 154931. <https://doi.org/10.1016/j.scitotenv.2022.154921>.

6. Conclusions

- The entry of contaminated water was located at site 13 and affected the areas inside the cavity located between site 6 and site 16 (red zone in Fig. 4).
- The influence of livestock activity in the cave is not recent, it has taken place over a prolonged time and results in high organic matter content that favors the development of microbial populations in the cave.
- The flooding modified the microbial communities of the affected areas, introducing genetic material from anaerobic bacteria and methanogenic archaea characteristic of the intestinal tract of mammals.