

UNCOVERING MICROBIAL DRIVERS OF GROUNDWATER POLLUTANT BIODEGRADATION VIA TOTAL RNA

Ivan Perez Samos , Erkin Gözdereliler, Lucas Horstmann, Laurine Leray, Lea Ellegaard-Jensen,
Anders Priemé, Carsten Suhr Jacobsen

Acknowledgements

Supervision

Carsten Suhr Jacobsen
Erkin Gözdereliler

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Aarhus University

Department of Environmental Science

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Thank you!

DNA and RNA in microbial communities



DNA:

- Potential community
- Potential functions



RNA:

- Active community
- Expressed functions



DNA and RNA in microbial communities



DNA:

- Potential community
- Potential functions

Complementary

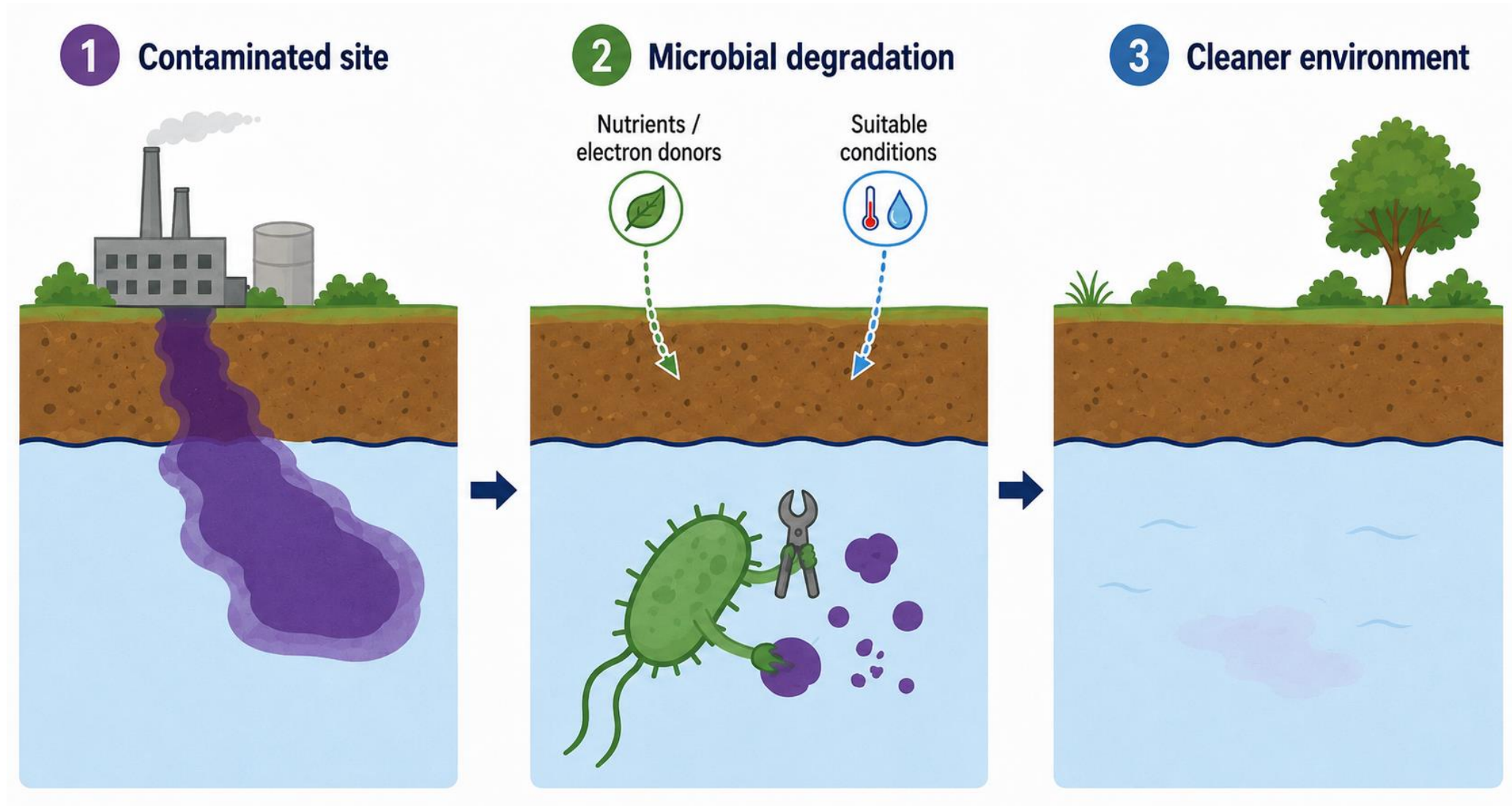


RNA:

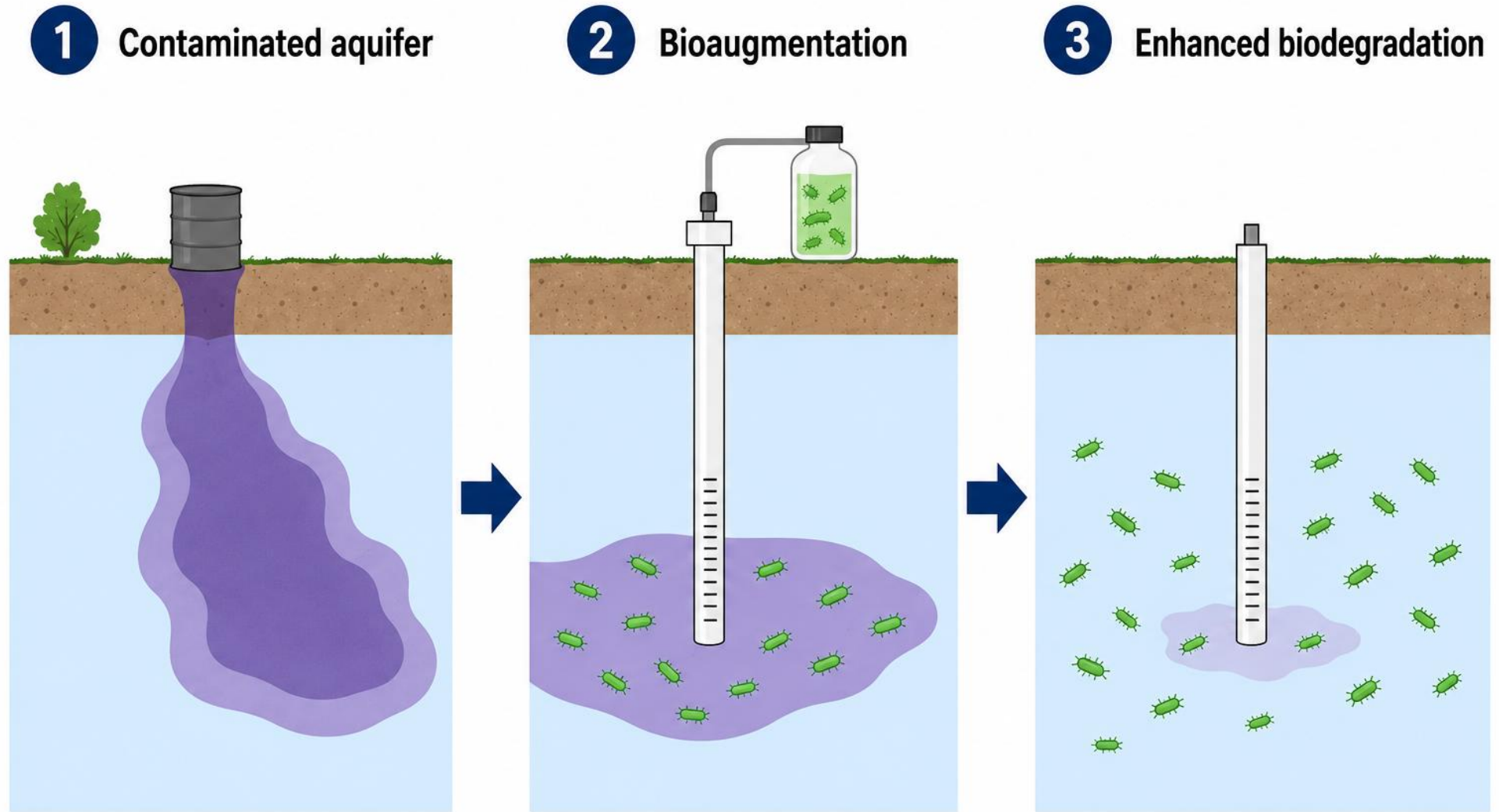
- Active community
- Expressed functions



Bioremediation: process in which biological systems are used to remove environmental pollutants.



Bioaugmentation: Addition of microorganisms that can biodegrade contaminants



Let's fix all the contaminated aquifers!

Contamination



Measure
contaminant
REALLY HIGH 😞



Bioaugmentation



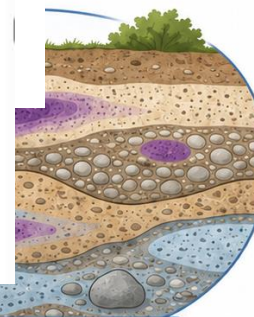
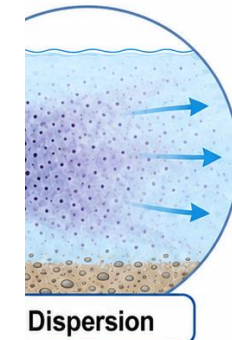
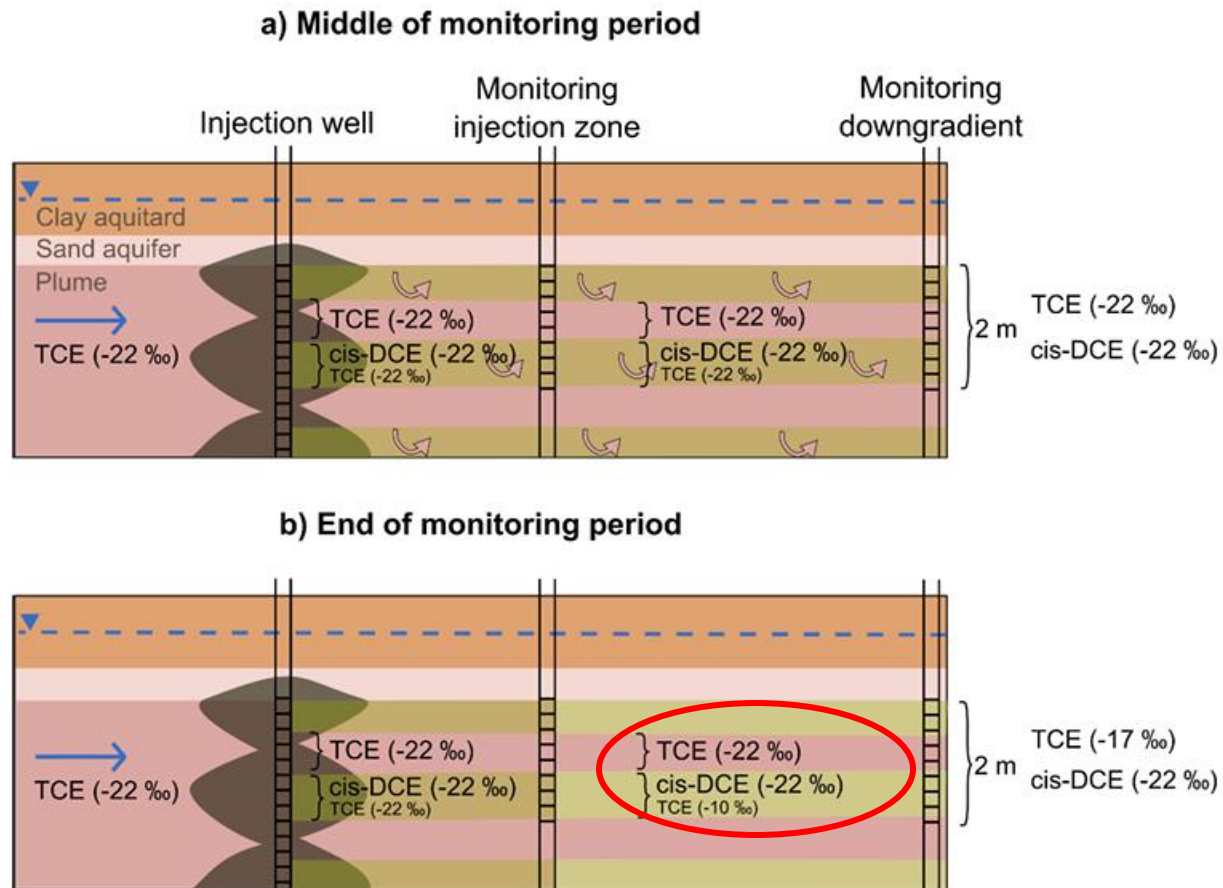
Measure
contaminant
IT DROPS 😊



SUCCESS



Chemical monitoring is not enough



Ottosen, C. B., Bjerg, P. L., Hunkeler, D., Zimmermann, J., Tuxen, N., Harrekilde, D., Bennedsen, L., Leonard, G., Brabæk, L., Kristensen, I. L., & Broholm, M. M. (2021). Assessment of chlorinated ethenes degradation after field scale injection of activated carbon and bioamendments: Application of isotopic and microbial analyses. *Journal of Contaminant Hydrology*, 240, 103794. <https://doi.org/10.1016/j.jconhyd.2021.103794>

Complement chemical monitoring with microbial activity



Chemical monitoring is **crucial** and **necessary**, but **not enough** in its own



DNA provides information of **functional potential** and **presence**



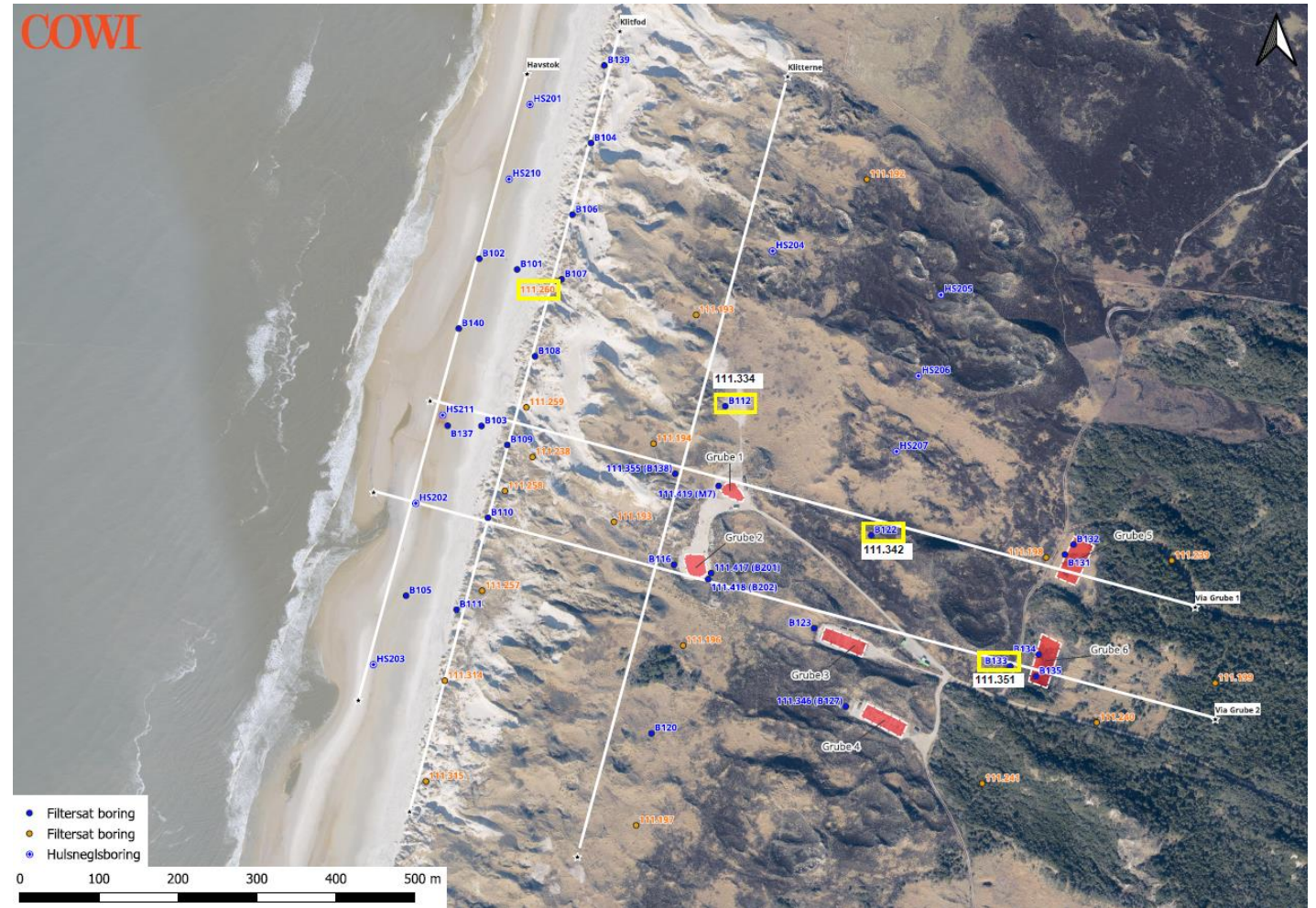
RNA provides information about **active biological response**.

Experimental design: Kægård Klitplantage

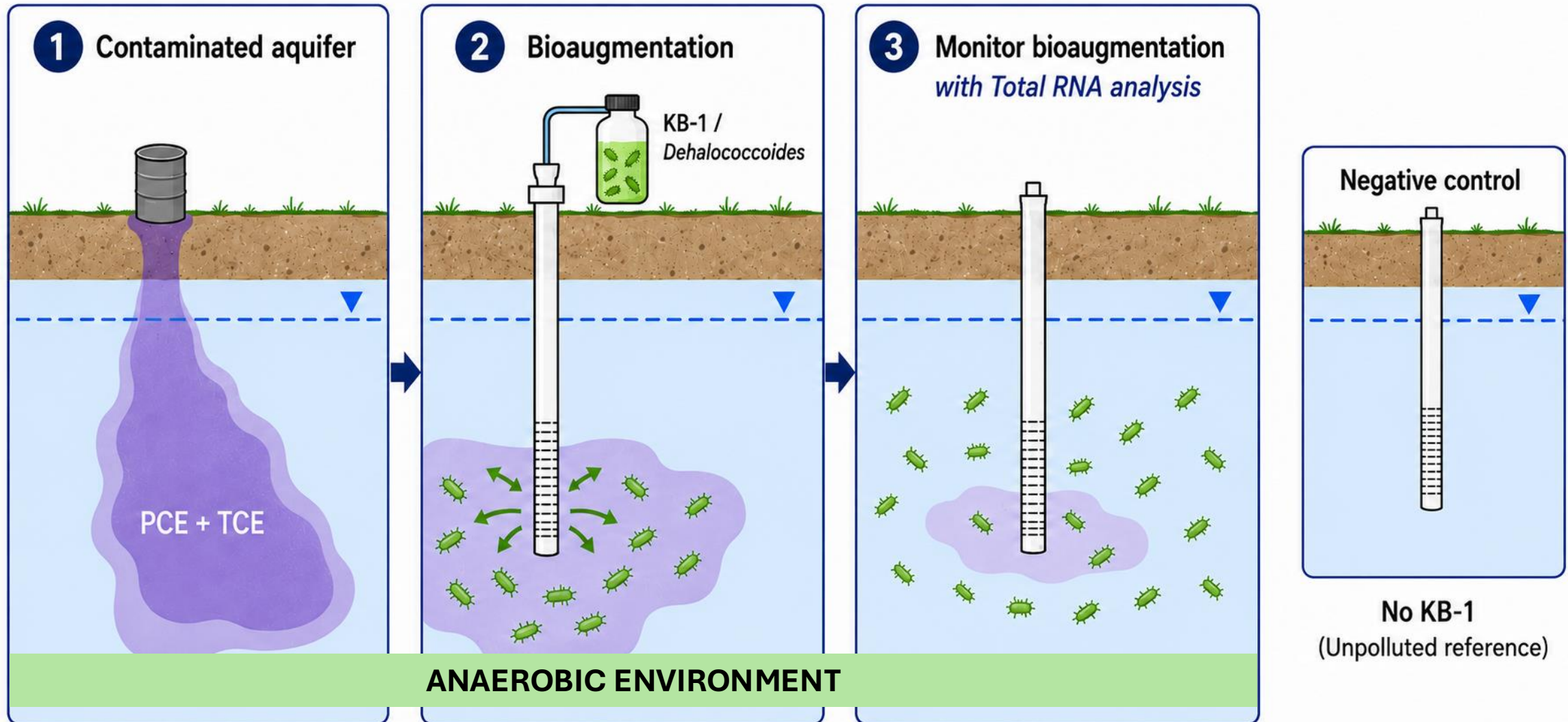
Location: Western coast of Denmark, near the North Sea.

Main point history:

- **280,000 metric tons of pharmaceutical and industrial waste disposed between 1956-1973.**
- Contaminants include pharmaceutical waste, **chlorinated solvents (PCE, TCE), ...**



Experimental design: Situation

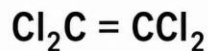
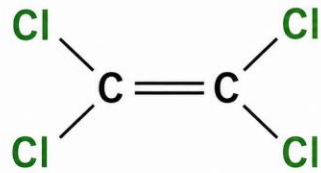


Experimental design: Target compounds and bacteria

Compounds to remove:

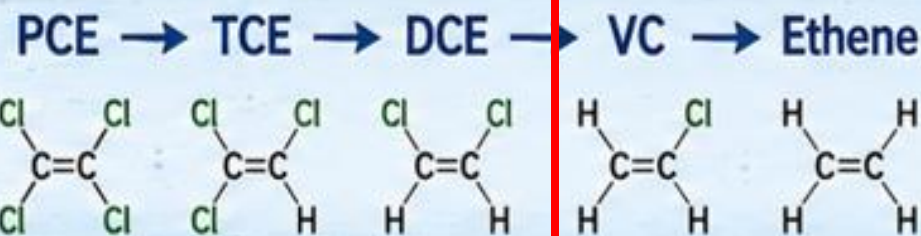
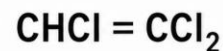
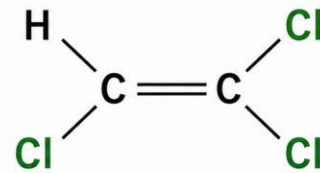
PCE

Tetrachloroethene
(Perchloroethylene)



TCE

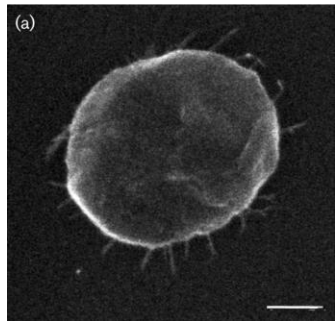
Trichloroethene
(Trichloroethylene)



Target organism:

Dehalococcoides mccartyi:

- Key organism in **KB-1[®]** culture
- Specialized in **organohalide respiration** in **anaerobic conditions**



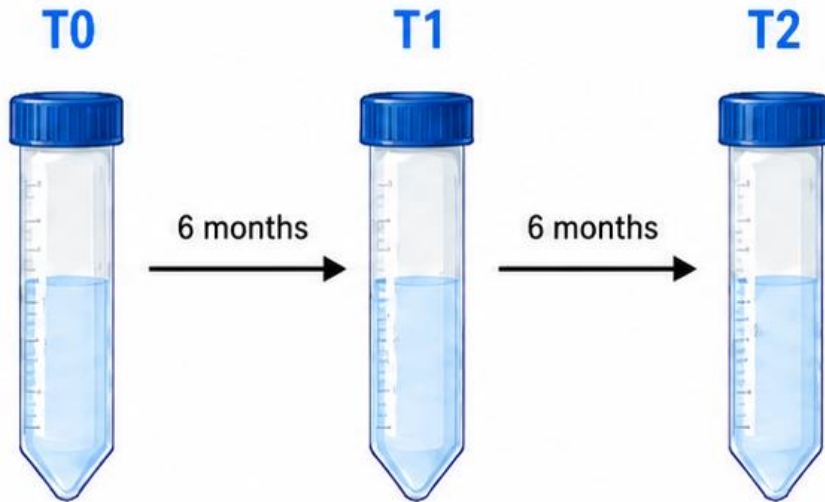
Source: Löffler et al. (2013)

Harbor **reductive dehalogenase** genes: *vcrA* and *bvcA*

Experimental design: Samples

NON-POLLUTED AREA

Untreated (Reference site)



POLLUTED AREA

Untreated
(Native conditions)

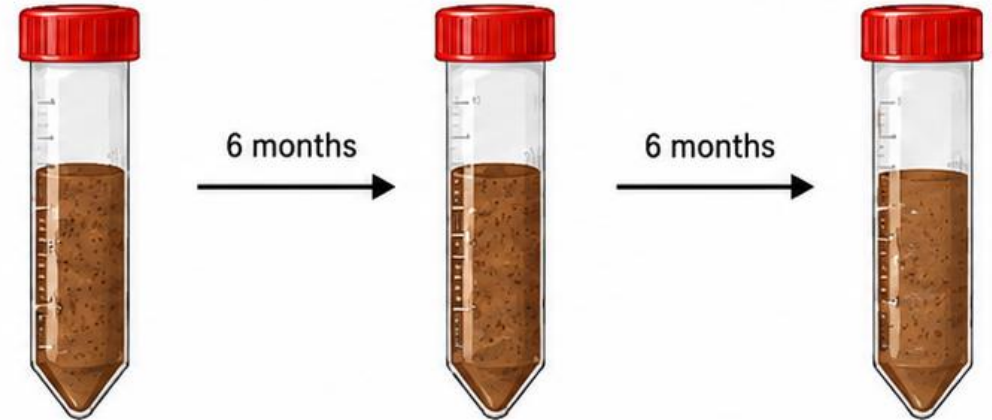
T0

T1

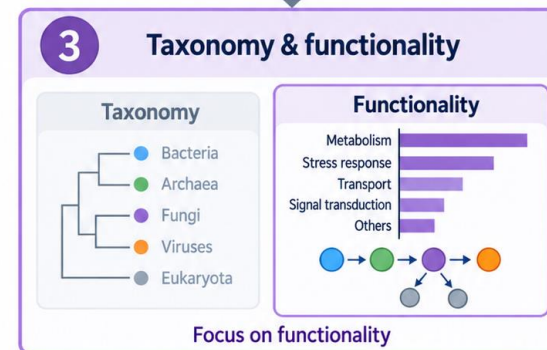
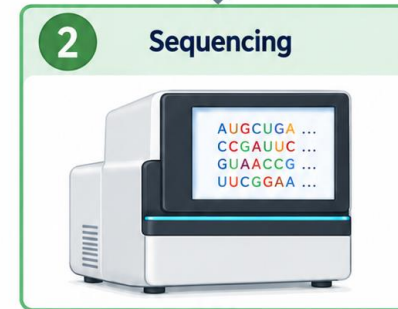
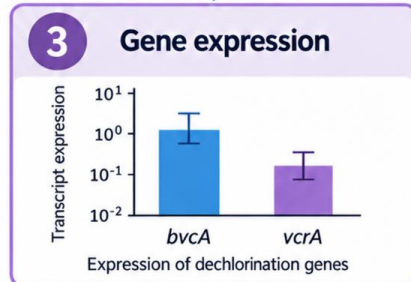
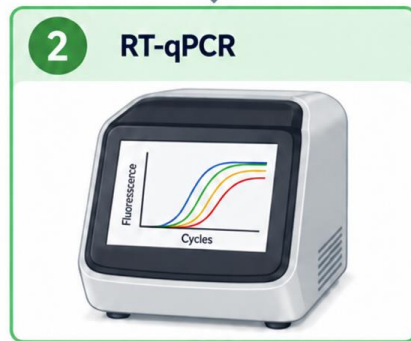
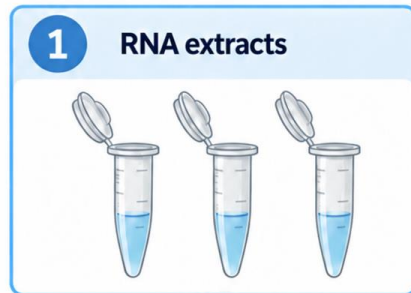
T2

Treated

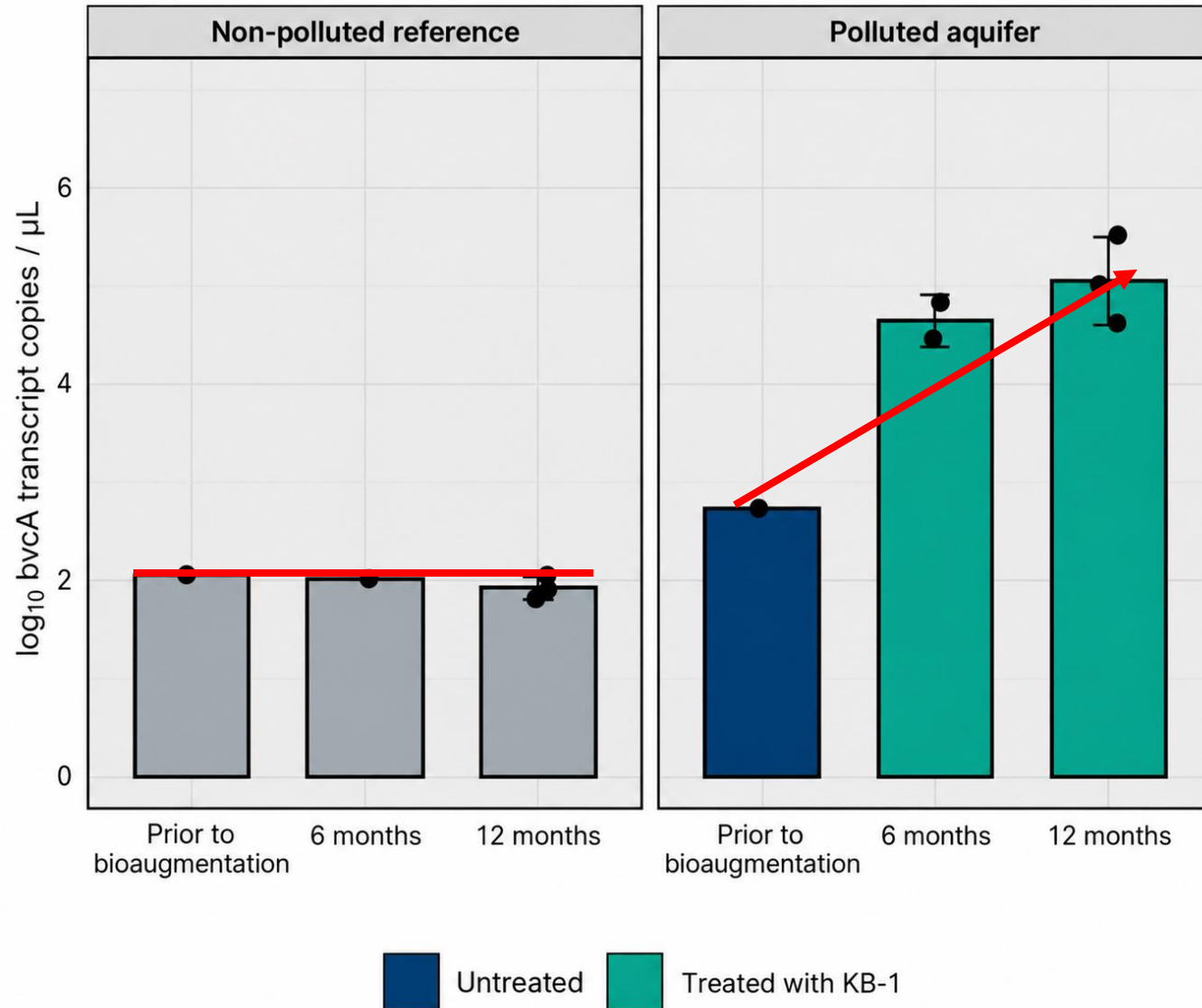
Treated



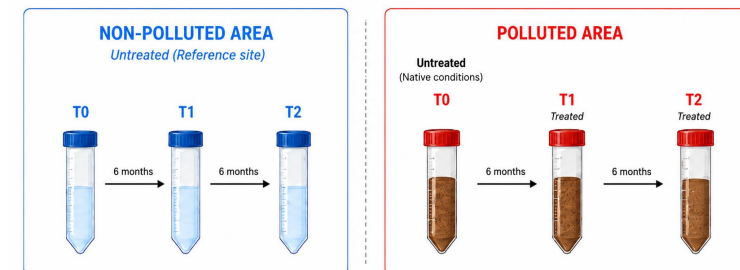
Experimental design: Techniques



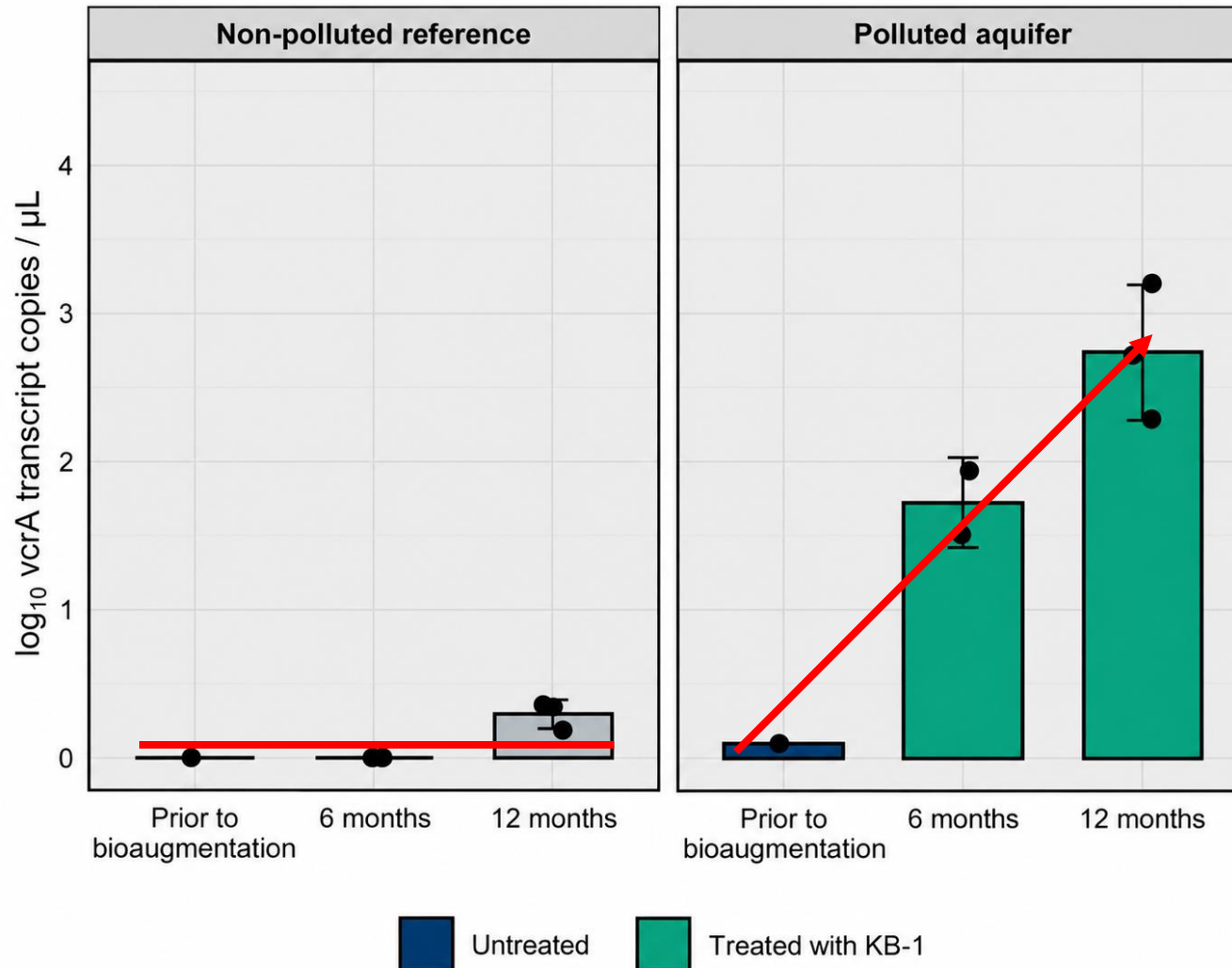
Dehalogenase genes progression: *bvcA*



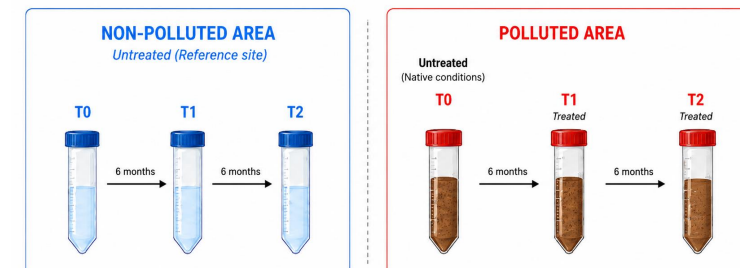
- **Stable expression** of the gene in the **reference**
- **Increasing *bvcA* expression** indicates **active reductive dechlorination**.



Dehalogenase genes progression: *vcrA*

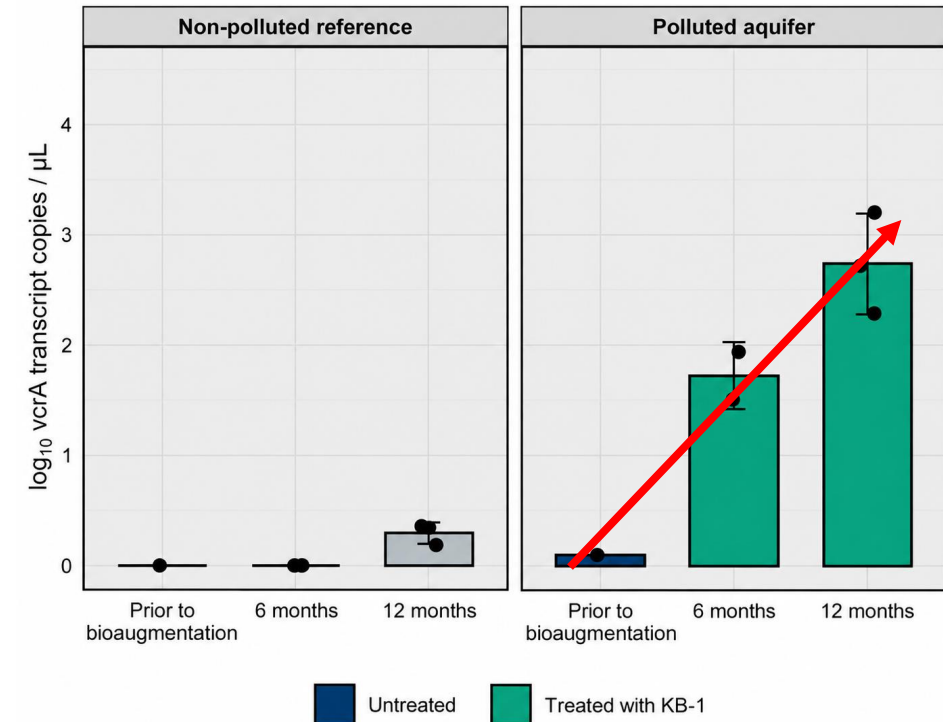
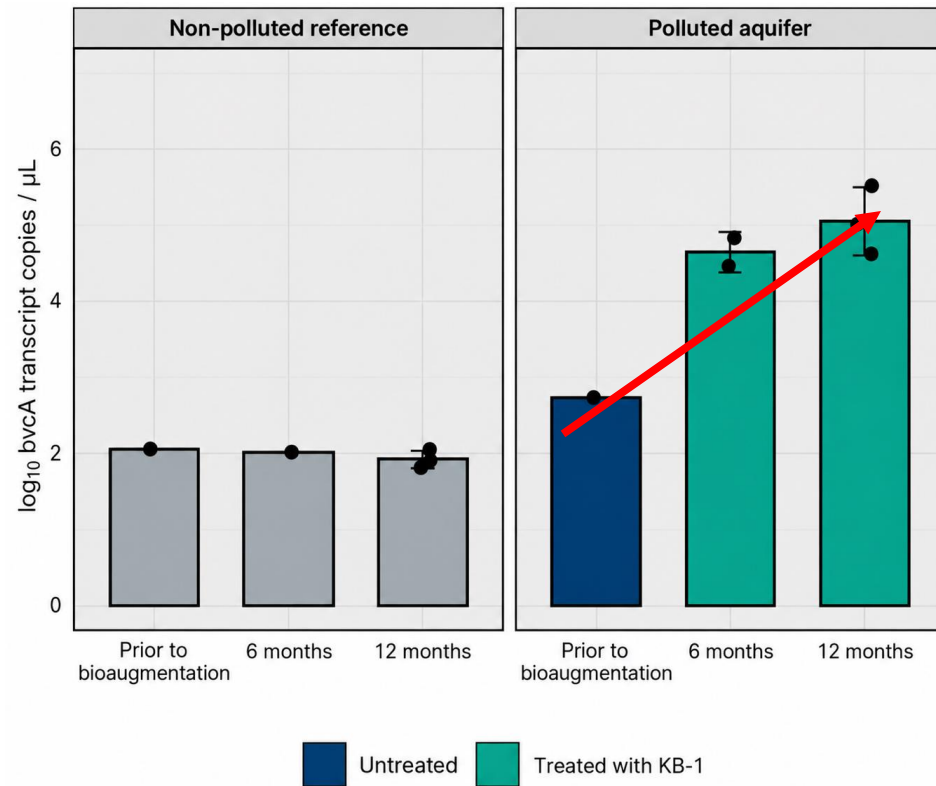


- **Stable expression** of the gene in the **reference**
- **Increasing *vcrA* expression** indicates **active reductive dechlorination**.

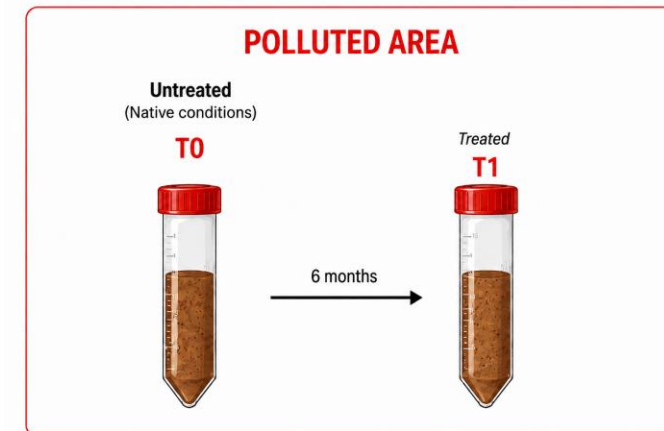
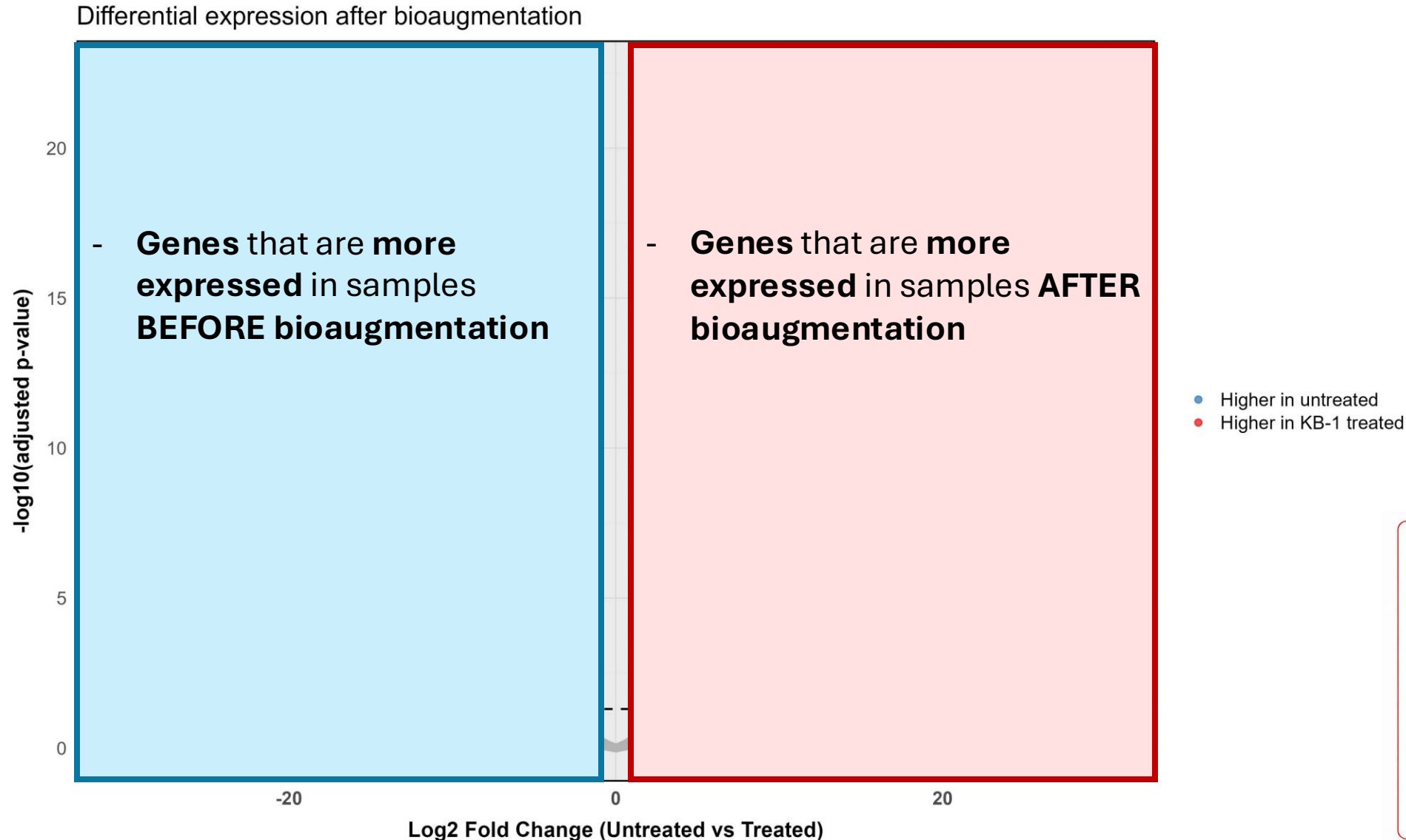


Dehalogenase genes progression: Important message

 ***Dehalococcoides* is performing more dechlorination after KB-1[®], it increases and it can be detected.**

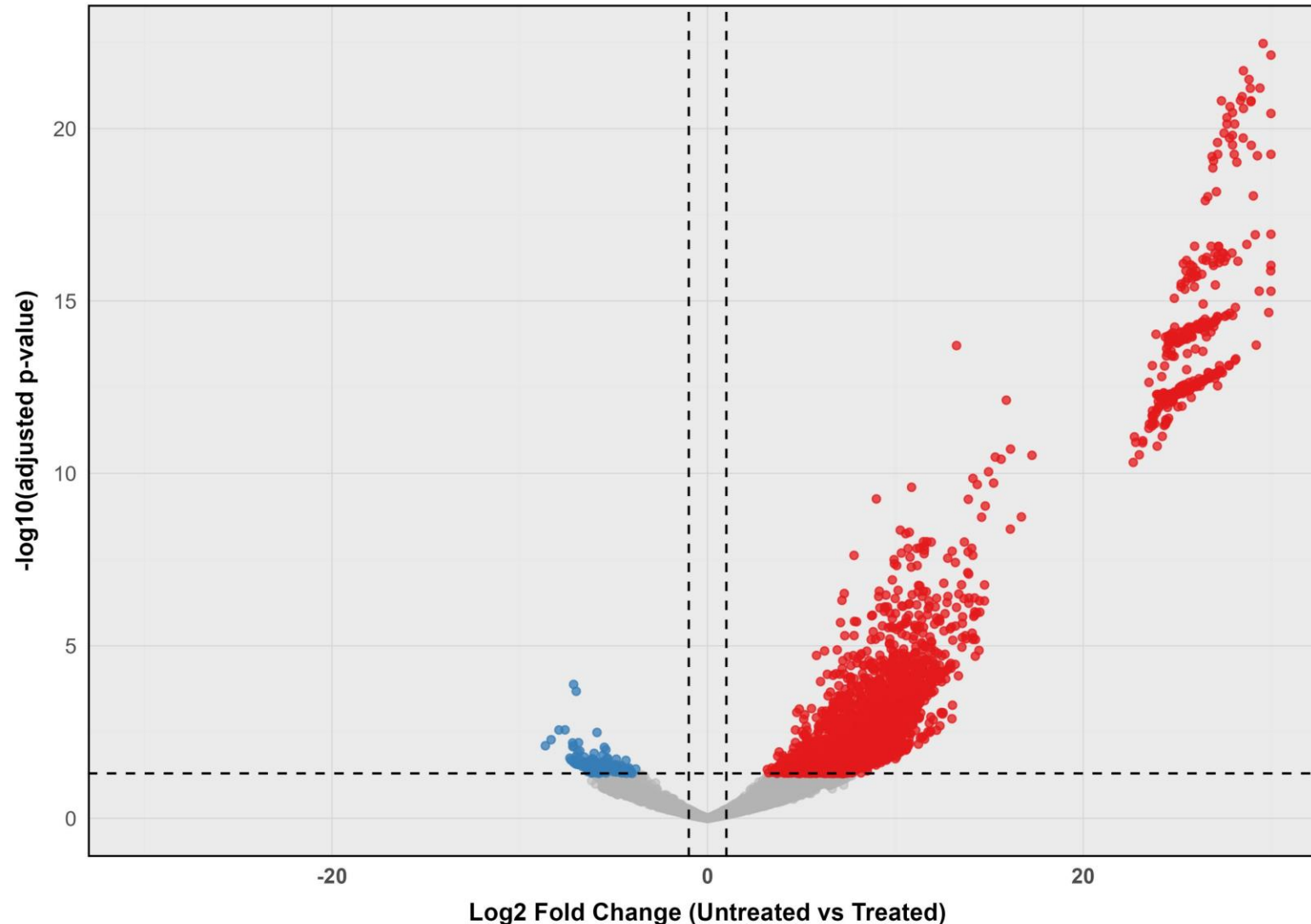


Community response – Untreated vs Treated



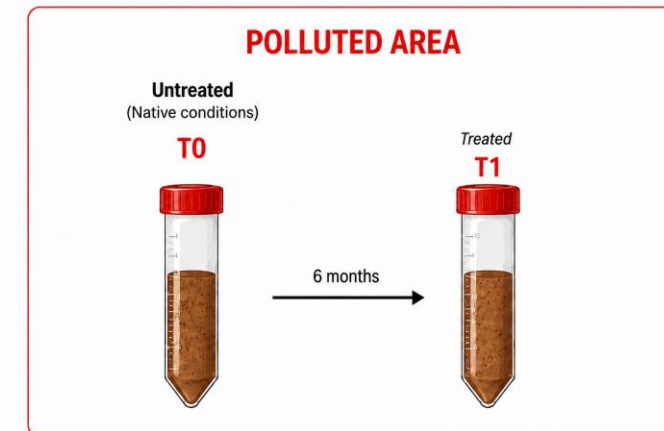
Community response – Untreated vs Treated

Differential expression after bioaugmentation



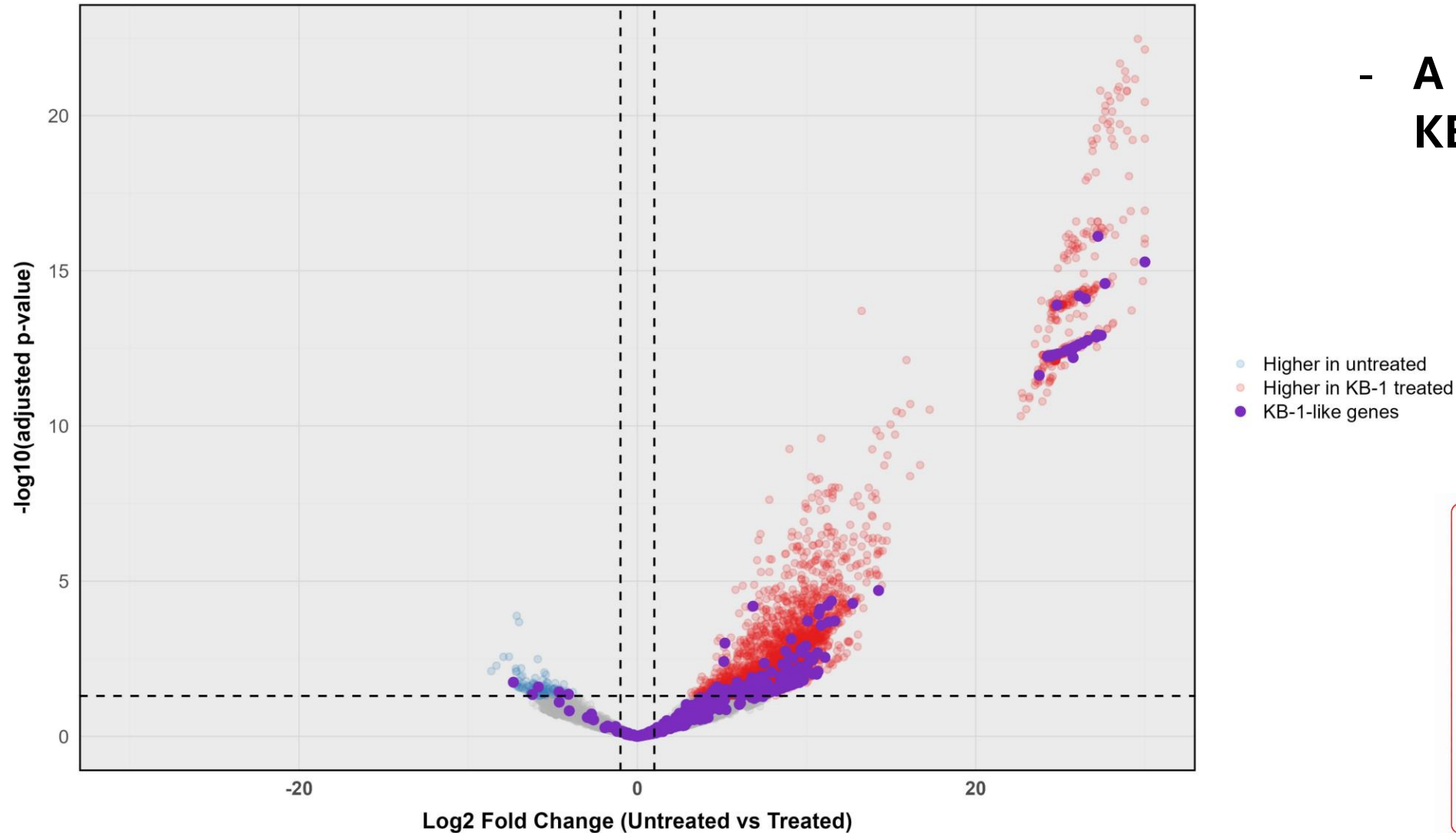
- **Many up-regulated genes in KB-1[®] treated**

• Higher in untreated
• Higher in KB-1 treated

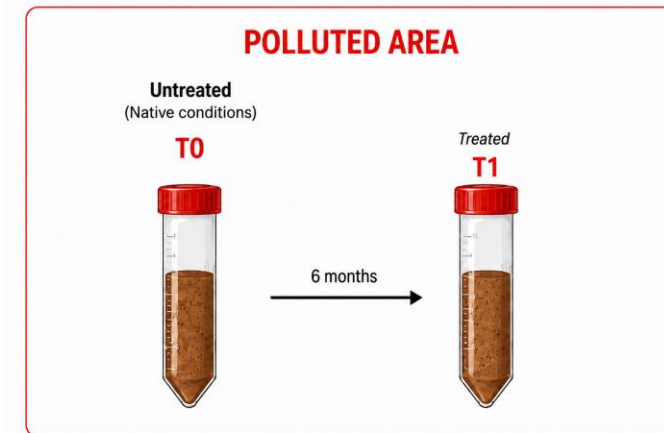


Community response – Untreated vs Treated

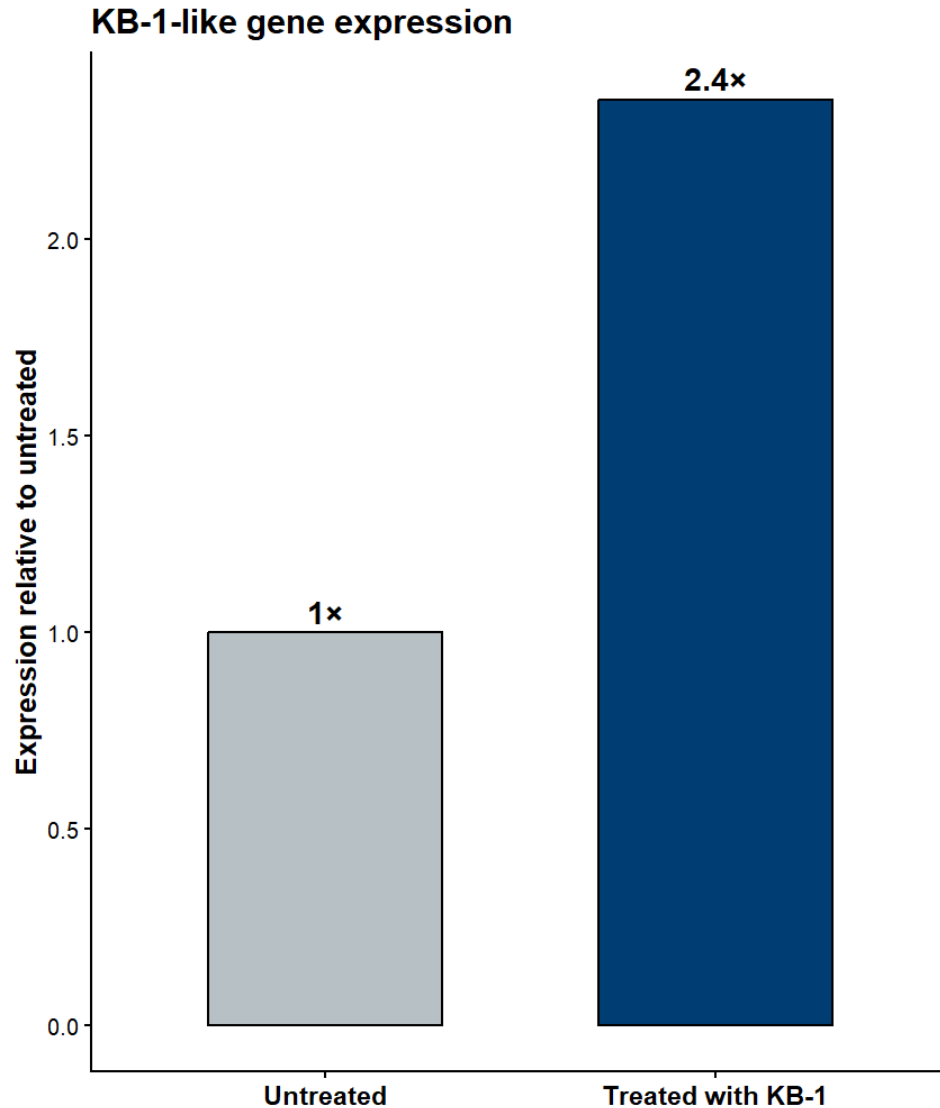
KB-1-like transcriptional response after bioaugmentation



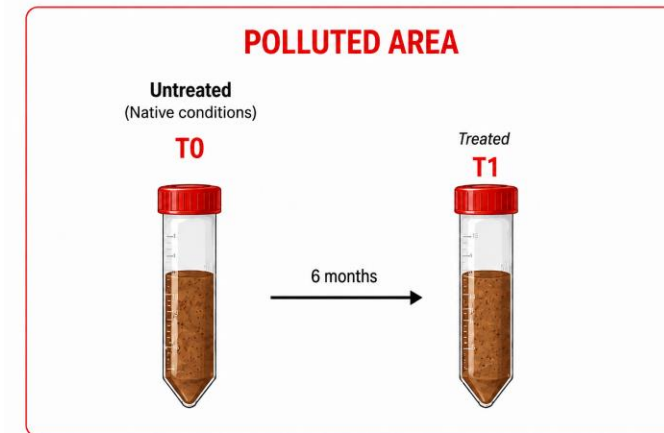
- **A lot of them are KB-1[®]-like genes**



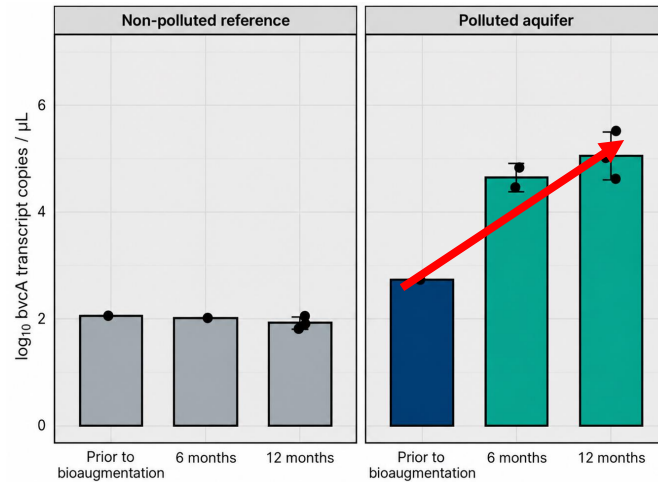
Community response - Untreated vs Treated



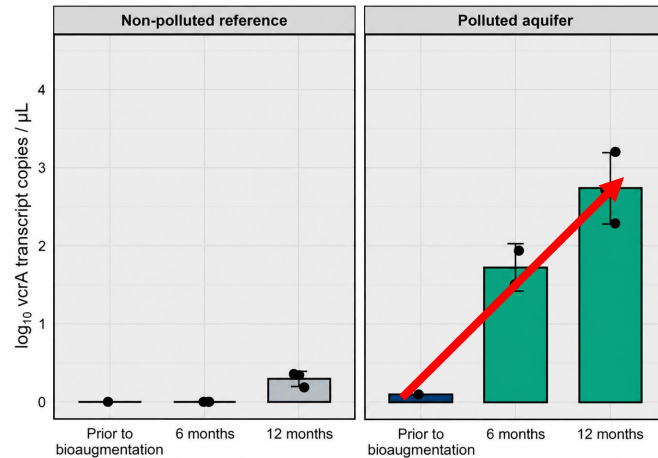
- KB-1[®]-like genes are 2.4 times more expressed in samples after KB-1 addition



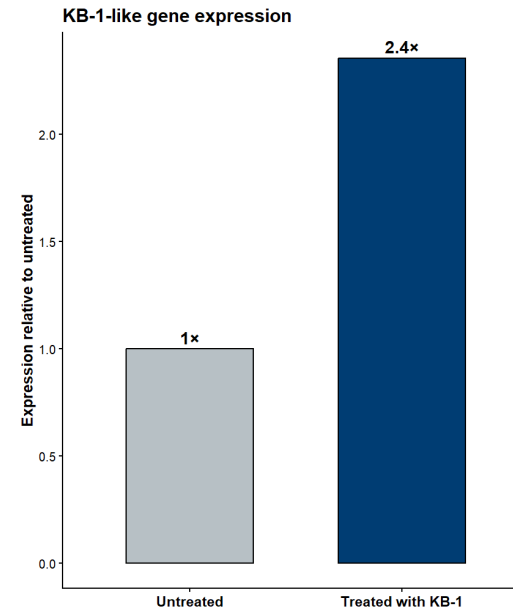
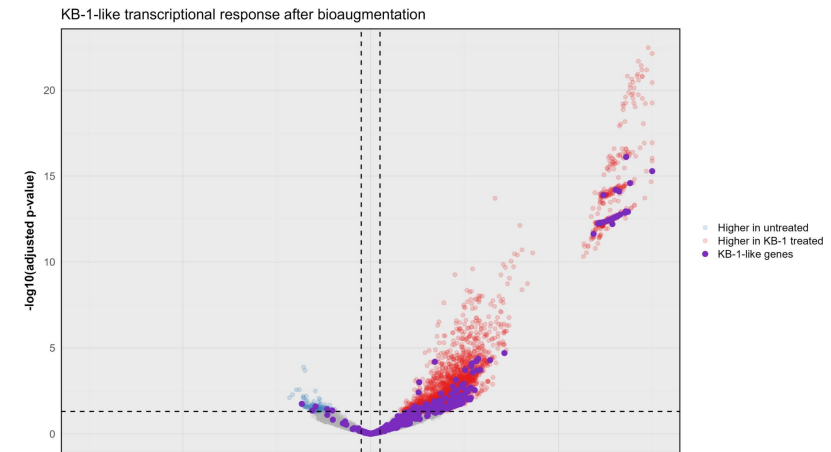
Combining biology-based results



■ Untreated ■ Treated with KB-1



■ Untreated ■ Treated with KB-1



Conclusion

Through RT-qPCR:

- Detected **specific gene expression** successfully.

TotalRNA pipeline analysis:

- **Response** of the whole **community**
- **Assess** the **effect** of specific **bioaugmentation** treatments

Providing a more complete bioaugmentation monitoring

Take-home message

RNA monitoring complements chemistry and is less affected by flow and hydrogeochemical variability.

Contaminant concentrations can change for many physical reasons; RNA provides direct biological evidence that the dechlorination machinery is active.

These results demonstrate the **potential of RNA-based monitoring** for assessing **KB-1[®] bioaugmentation** at field scale, although further validation is required.

THANK YOU VERY MUCH FOR LISTENING!

