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## Responses of microbial communities exposed to combined stressors in hyporheic zone

Laura Kergoat, Aymeric Dabrin, Thibault Datry, Abdelkader Azougui, Bernadette Volat, Bernard Motte, Christophe Rosy, Chloé Bonnineau

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# ContaSed 2021

June 9-11 2021

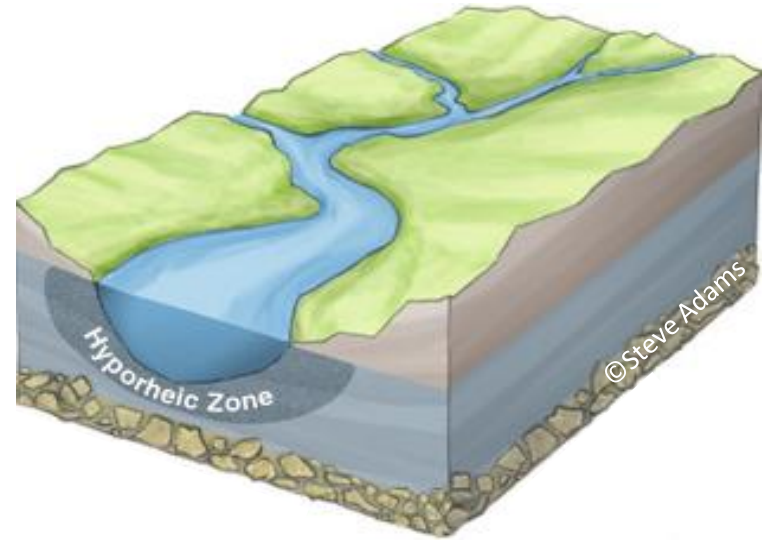
## Responses of microbial communities exposed to combined stressors in hyporheic zone

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Laura Kergoat<sup>1</sup>, Aymeric Dabrin<sup>1</sup>, Thibault Datry<sup>1</sup>,  
Abdelkader Azougui<sup>1</sup>, Bernadette Volat<sup>1</sup>, Bernard  
Motte<sup>1</sup>, Christophe Rosy<sup>1</sup>, Chloé Bonnineau<sup>1</sup>

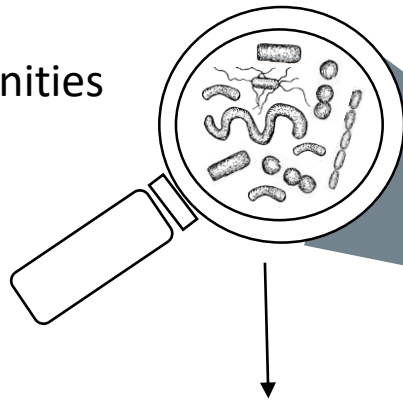
<sup>1</sup> INRAE, UR Riverly, Lyon-Villeurbanne, France

**Hyporheic zone** : “the saturated interstitial areas beneath the stream bed and into the stream banks that contain some proportion of channel water” <sup>1</sup>



<sup>1</sup> White, D. S. (1993). Journal of the North American Benthological Society 12, 61–69.

Microbial communities



- Mineralization of organic matter<sup>2</sup>
- Retention and degradation of pollutants<sup>3,4</sup>

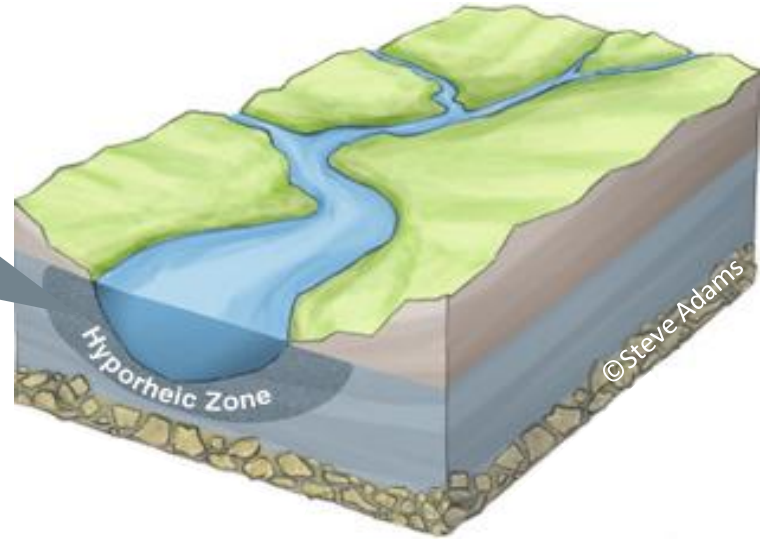
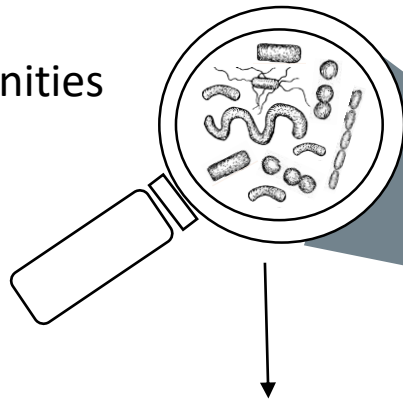
Filter for the river !

<sup>2</sup> Piscart, C. (2011). Science of The Total Environment 409, 4373–4380.

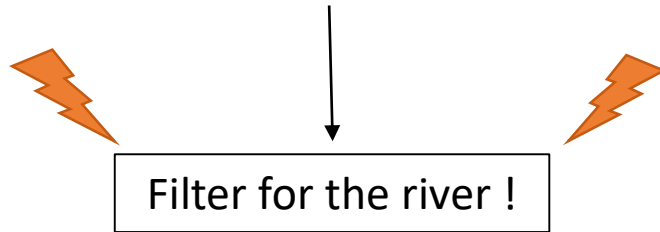
<sup>3</sup> Gandy, C. J. (2007). Science of The Total Environment 373, 435–446.

<sup>4</sup> Peralta-Maraver, I. (2018). Science of The Total Environment 610–611, 267–275.

Microbial communities



- Mineralization of organic matter<sup>2</sup>
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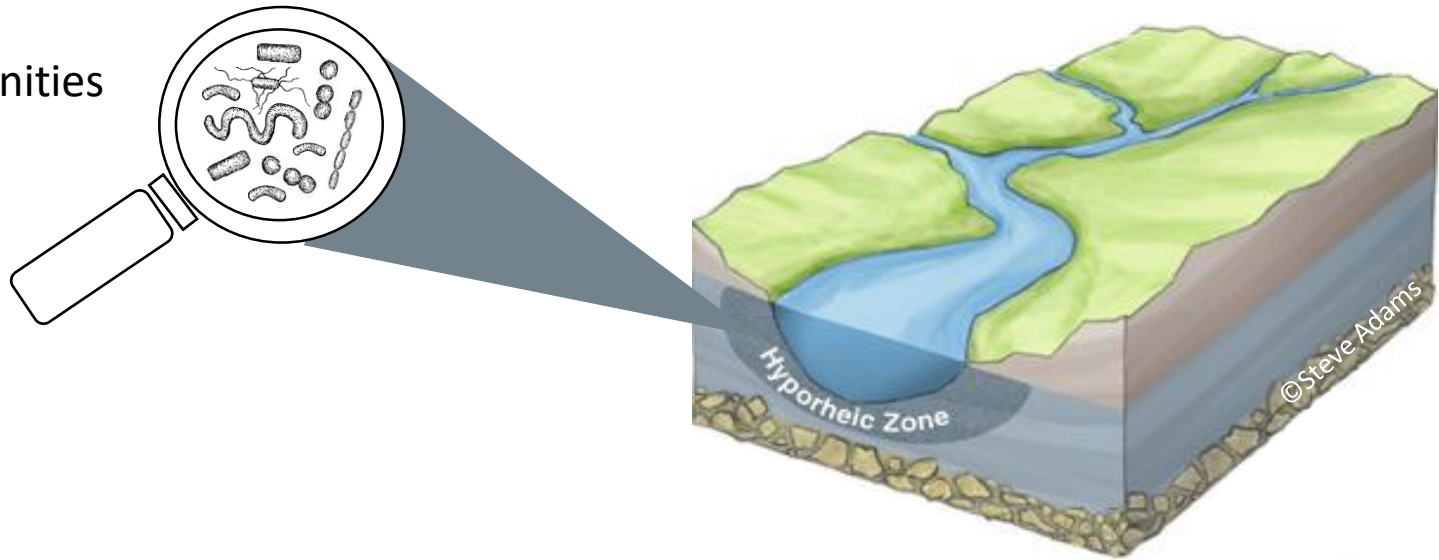
<sup>2</sup> Piscart, C. (2011). *Science of The Total Environment* 409, 4373–4380.

<sup>3</sup> Gandy, C. J. (2007). *Science of The Total Environment* 373, 435–446.

<sup>4</sup> Peralta-Maraver, I. (2018). *Science of The Total Environment* 610–611, 267–275.



Microbial communities



**Clogging**

**Clogging** = fine sediment deposition onto the riverbed and their gradual infiltration into the hyporheic zone <sup>5</sup>



- Input of fine sediment and organic matter -> **stimulation of microbial activities** <sup>6,7</sup>
- Reduction of oxygen supply -> **anaerobic microbial communities** development <sup>8</sup>

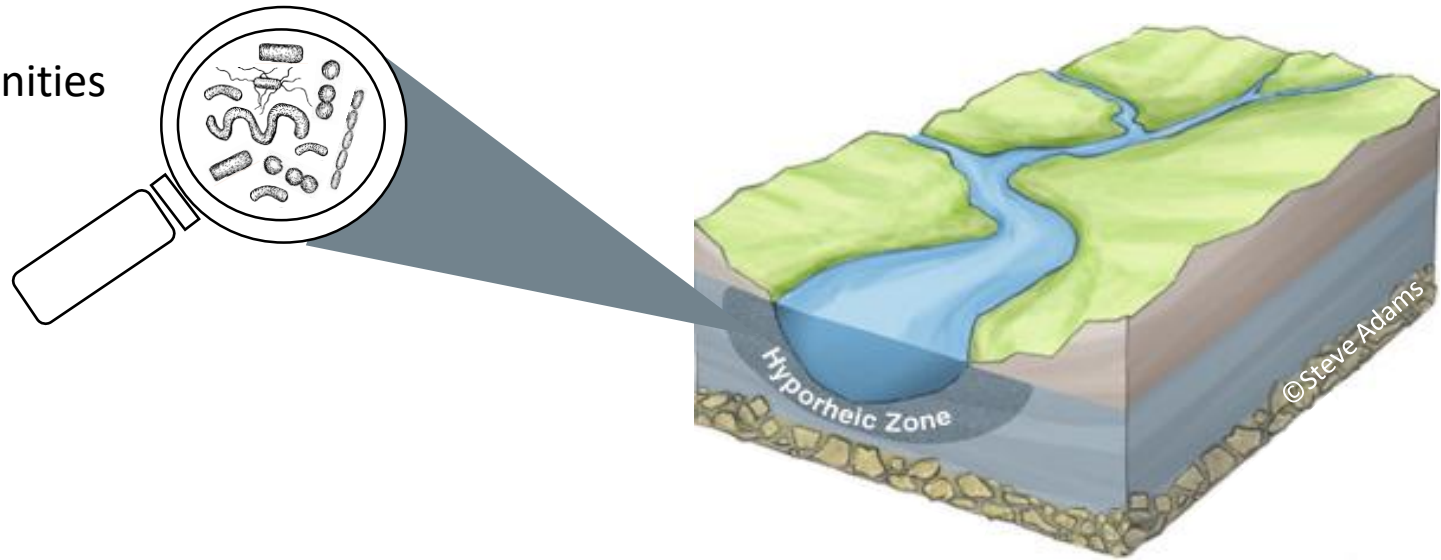
<sup>5</sup> Wood, P. J. (1997). *Environmental Management* 21, 203–217.

<sup>6</sup> Hartwig, M. (2015) *Ecohydrol.* 8, 961–975.

<sup>7</sup> Nogaro, G. (2007). *Science of The Total Environment* 377, 334–348.

<sup>8</sup> Navel, S. (2011). *Microb Ecol* 61, 968–979.

## Microbial communities



## Copper

- Used as a **fungicide** in agriculture
- Transferred to aquatic systems by **leaching**
- Frequently found in **sediment**

[Cu] = 21,7 mg/kg (median concentration in France) <sup>9</sup>

- **Ecotoxicological effects**

-> microbial functions (respiration, denitrification, mineralization of organic matter) <sup>10, 11</sup>

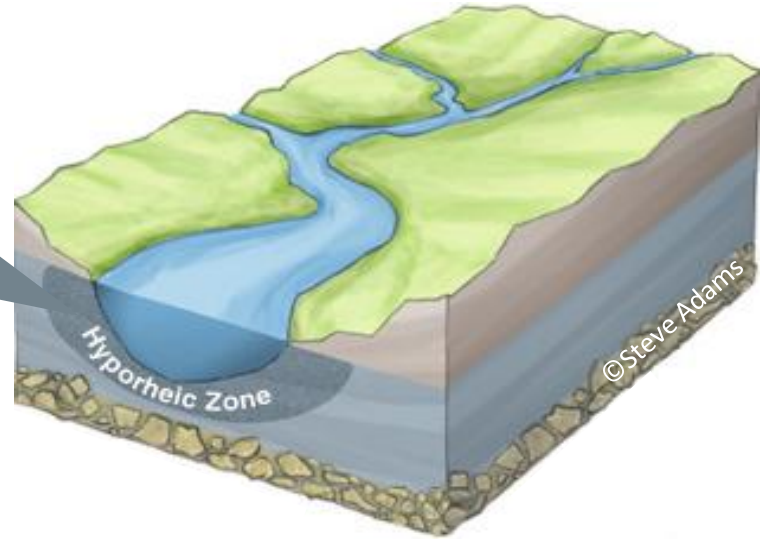
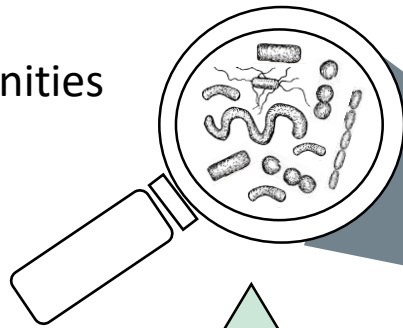
-> microbial structure <sup>10, 11</sup>

<sup>9</sup> INERIS 2010, Rapport d'étude

<sup>10</sup> Mahamoud Ahmed, A. (2018). *Front. Microbiol.* 9, 1852.

<sup>11</sup> Sutcliffe, B.(2019). *Environmental Pollution* 247, 1028–1038.

Microbial communities



Combined effects ?



**Clogging**

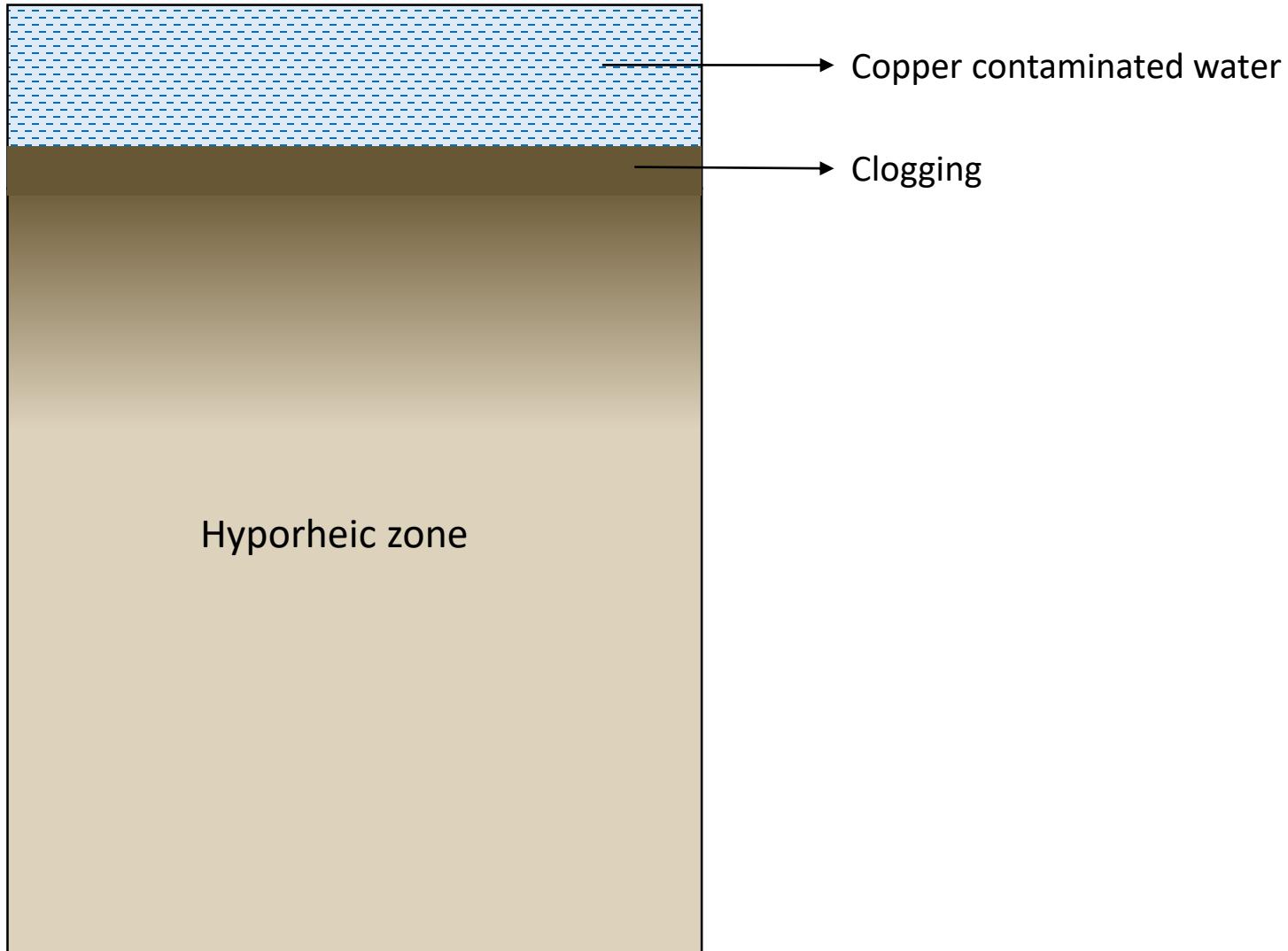


**Copper**

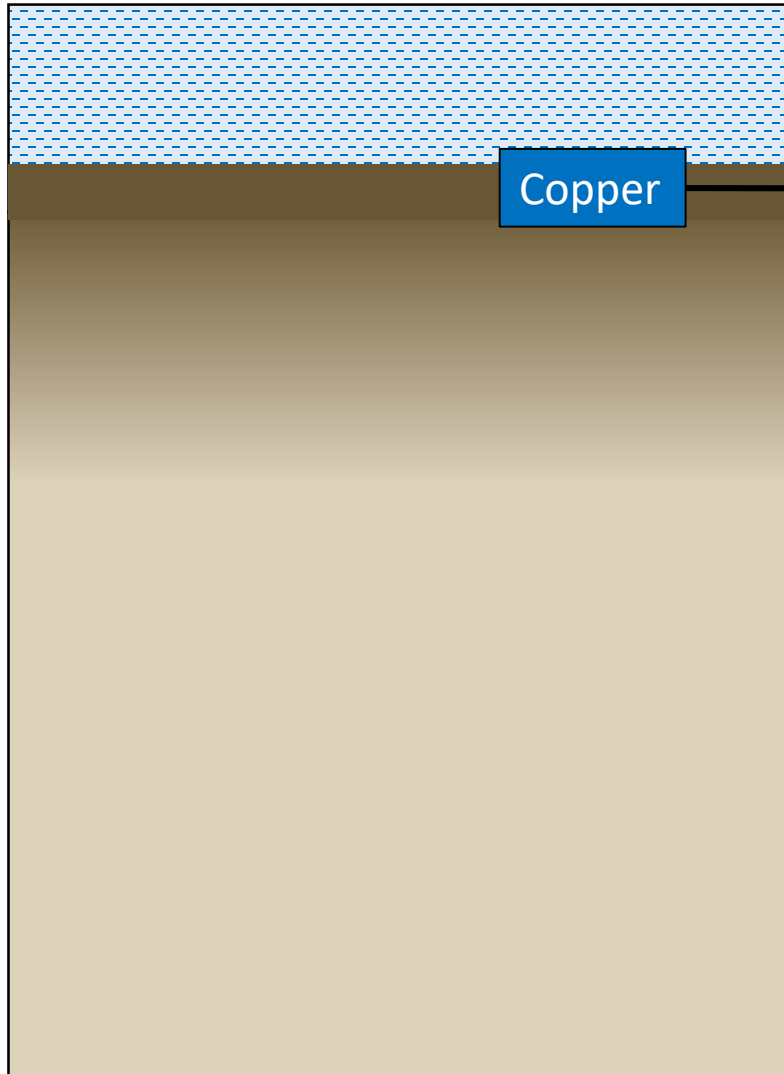




What are the combined effects of copper contamination and clogging on microbial communities in the hyporheic zone?



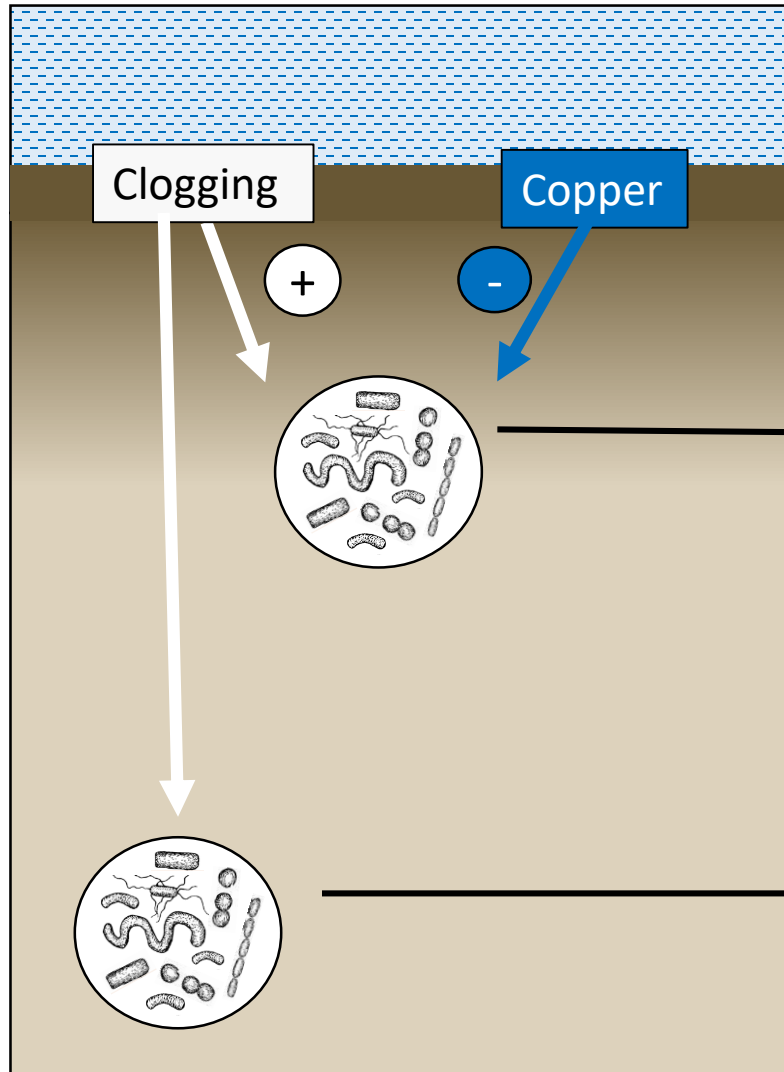
What are the combined effects of copper contamination and clogging on microbial communities in the hyporheic zone?



## Hypothesis 1

Copper fixation in the first centimeters

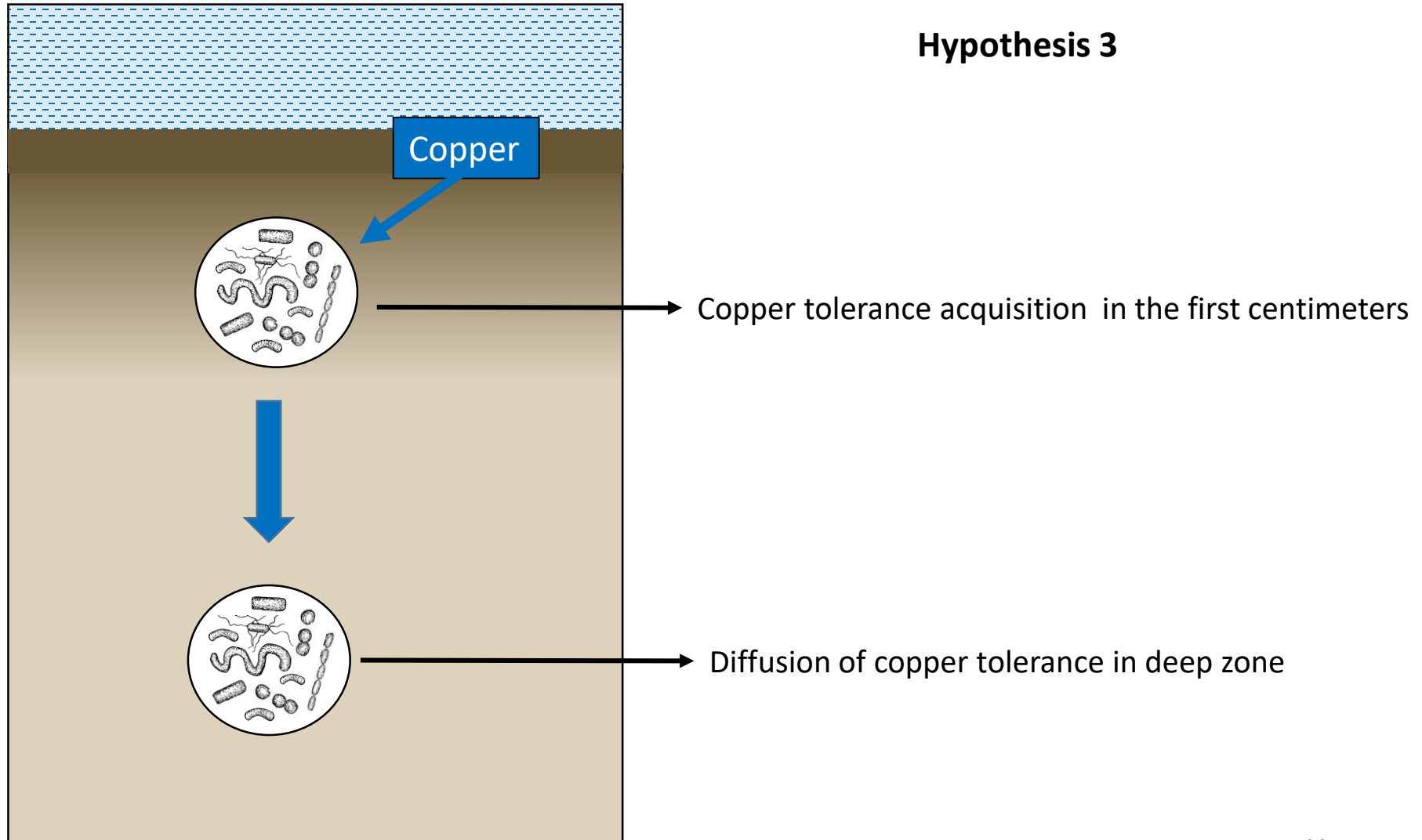
What are the combined effects of copper contamination and clogging on microbial communities in the hyporheic zone?

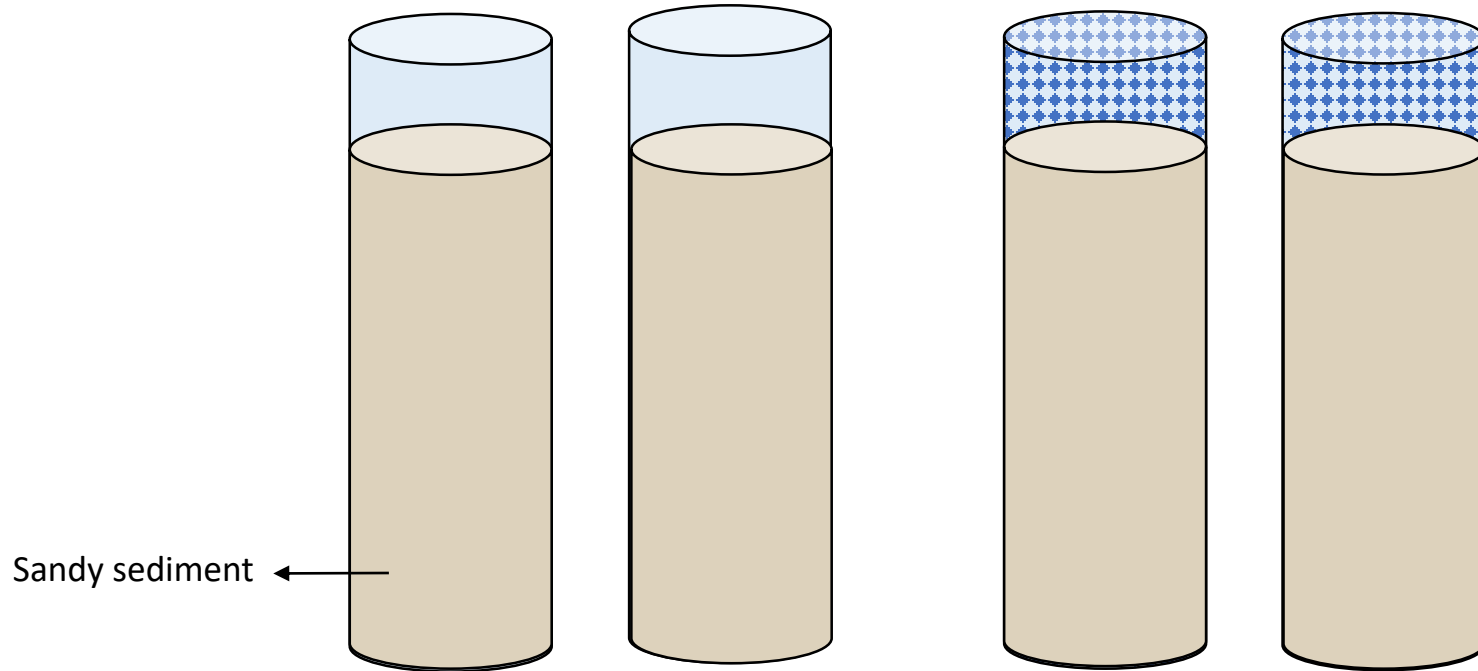


## Hypothesis 2

- Antagonistic effects of copper and clogging on microbial functions
- Modification of microbial structure
- Only the effects of clogging on microbial functions and structure (development of anaerobic microbial communities)

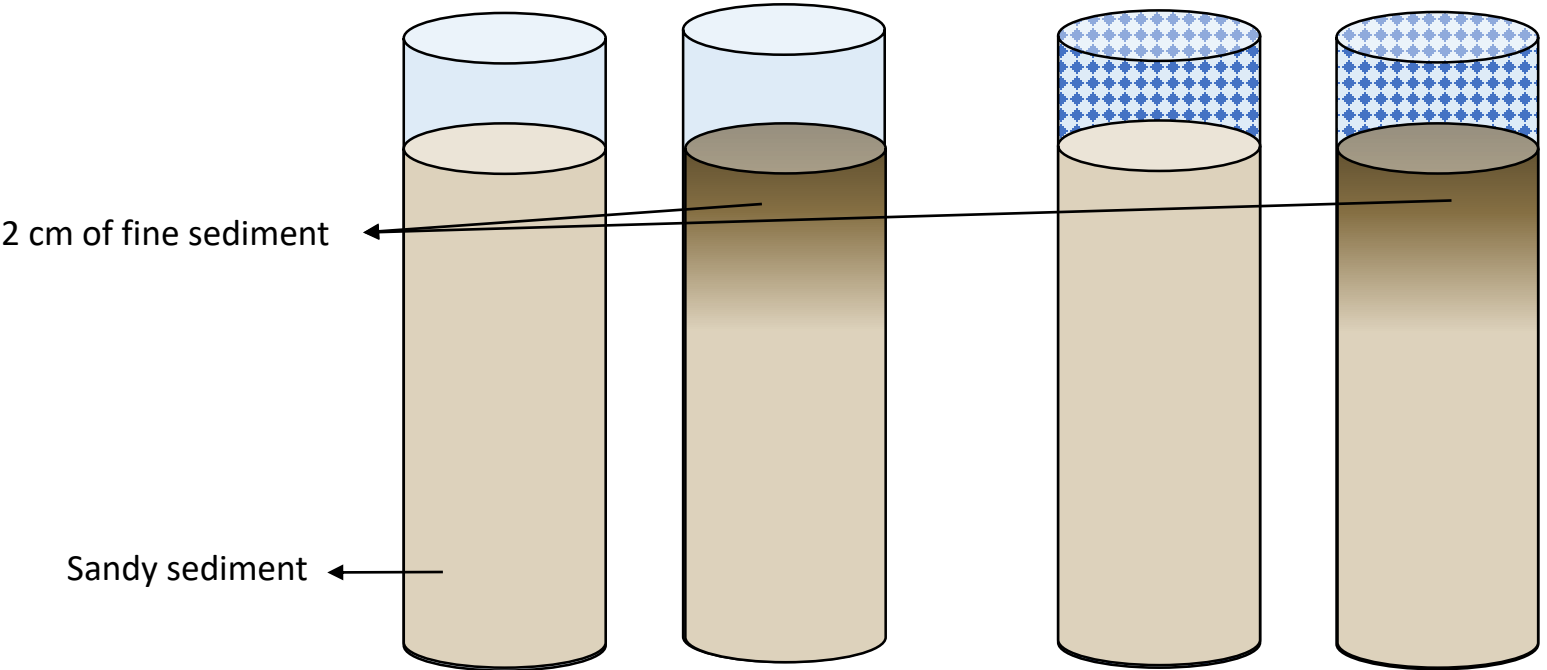
What are the combined effects of copper contamination and clogging on microbial communities in the hyporheic zone?



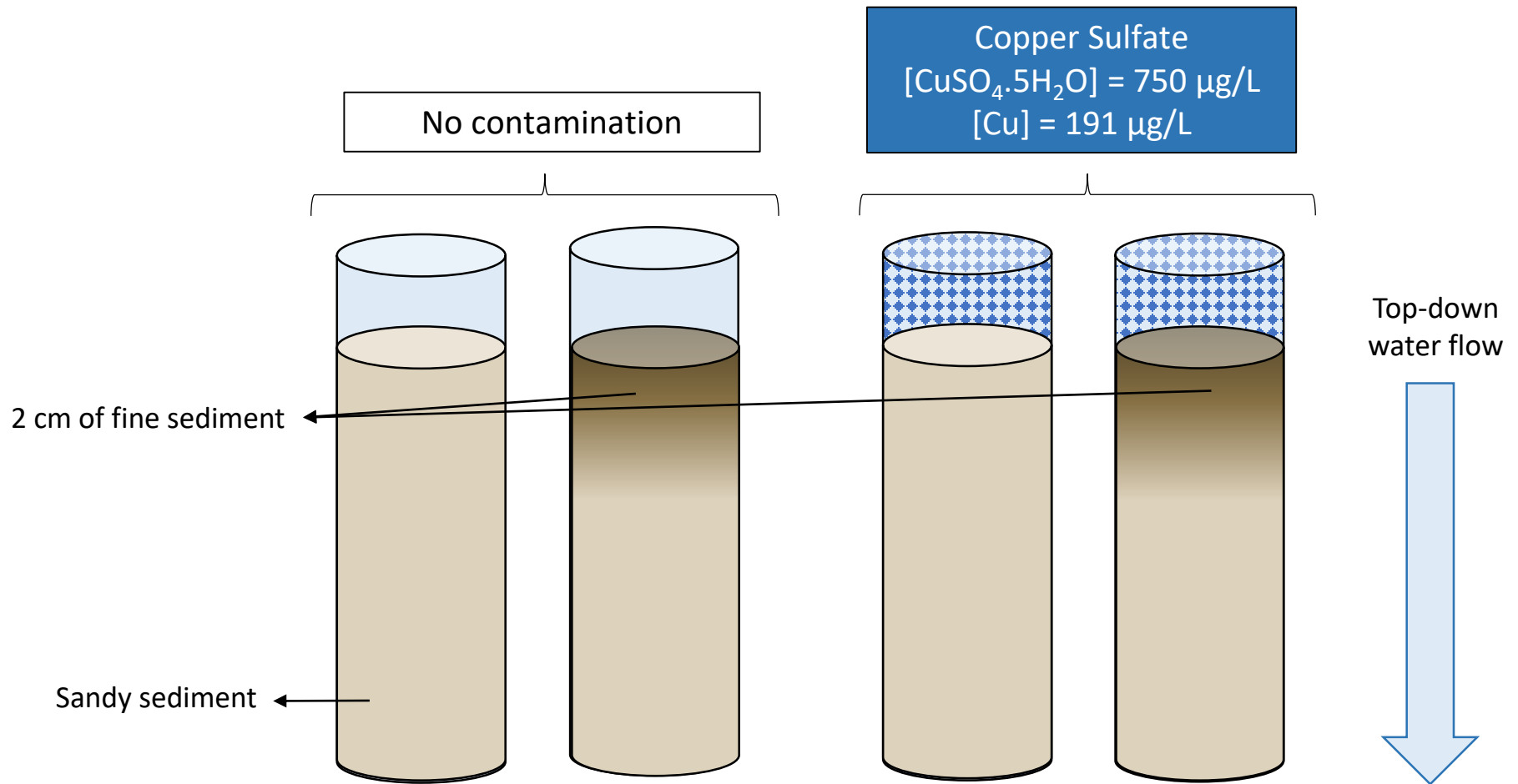




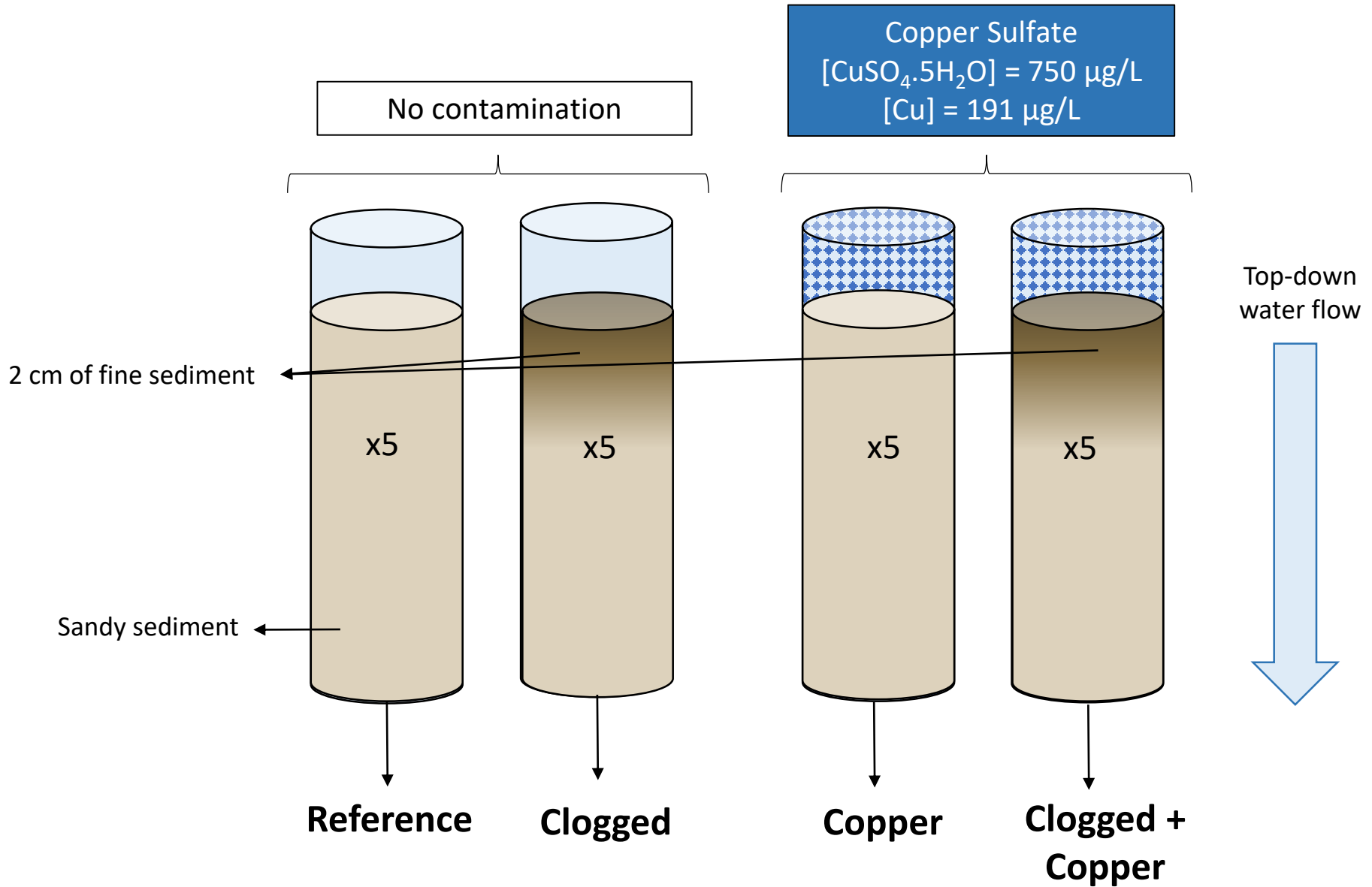
# Experimental design



# Experimental design



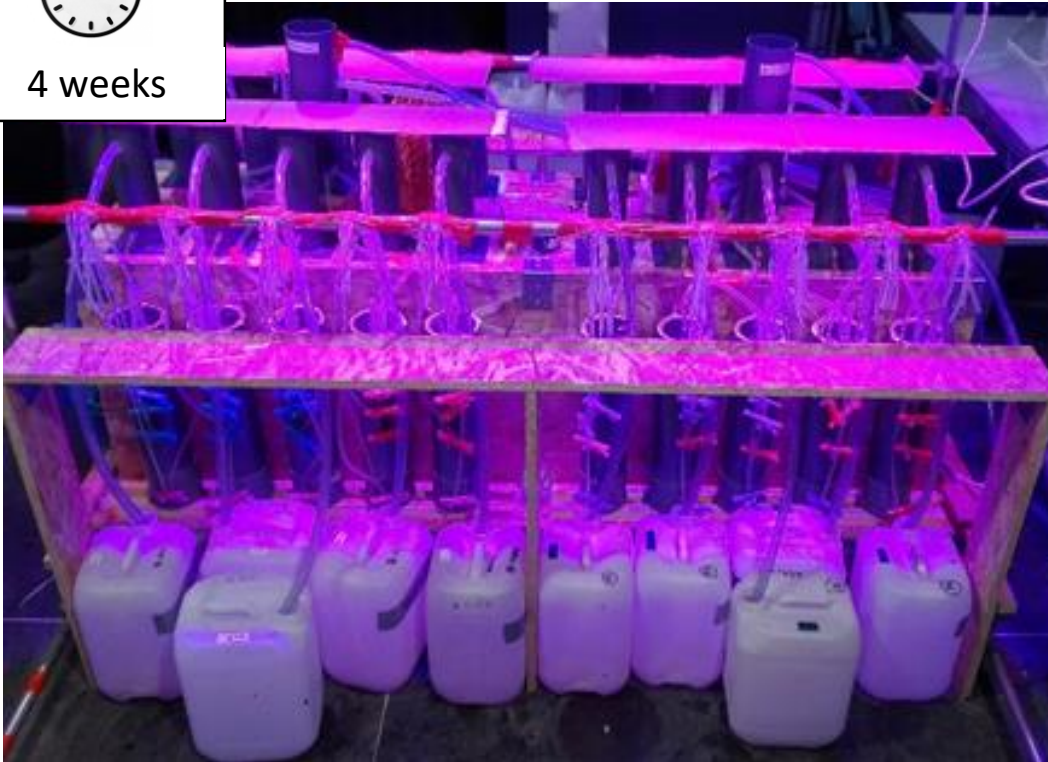
# Experimental design



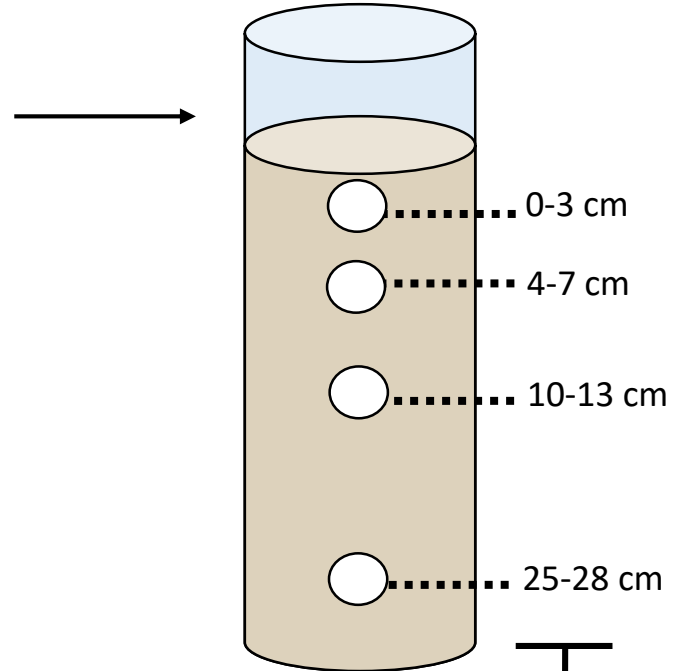
# Experimental design



4 weeks



## Sediment sampling



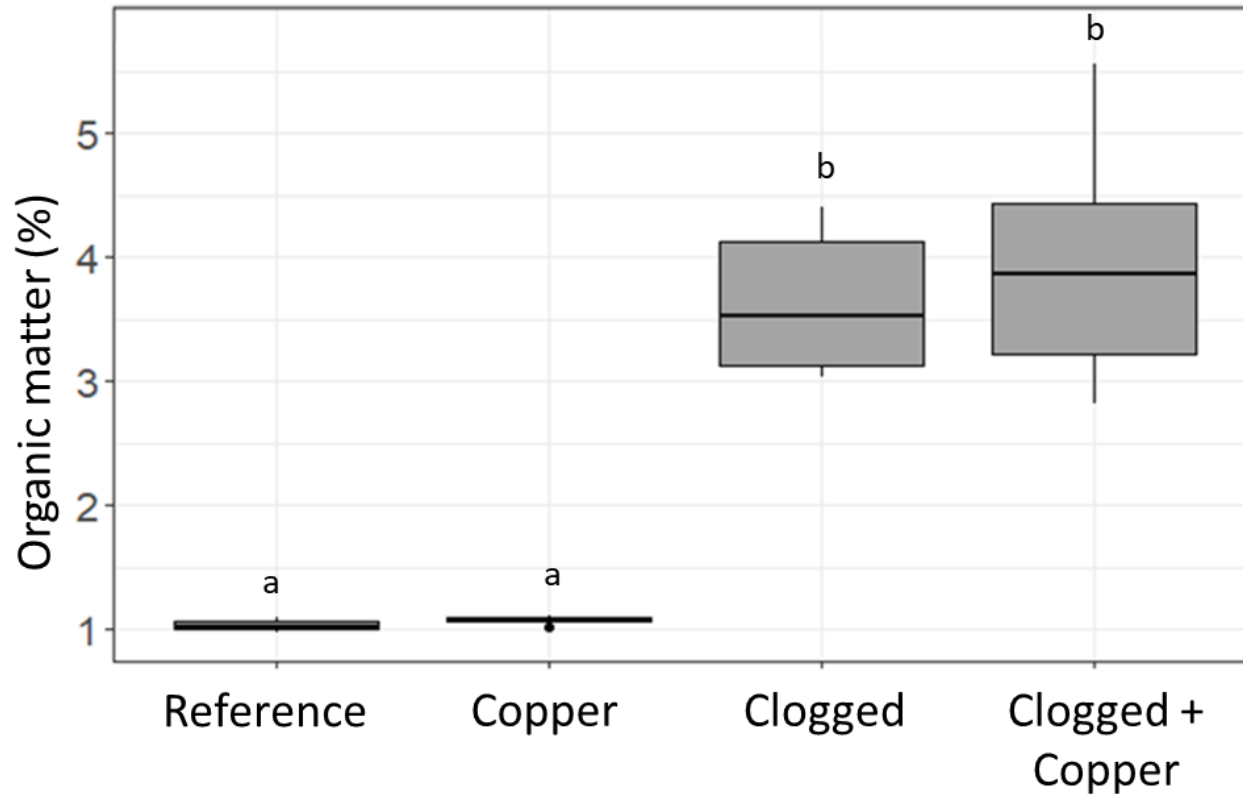
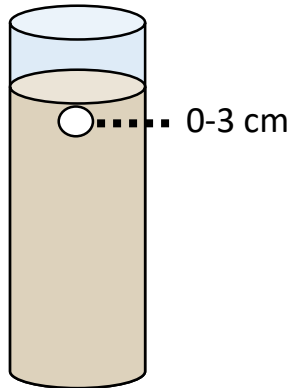
Physical and chemical parameters



Function/structure of microbial communities



## ❖ Organic matter in sediment

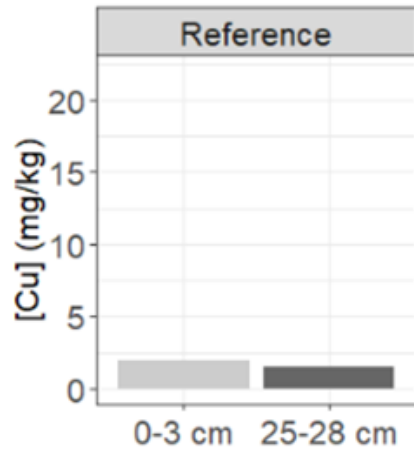
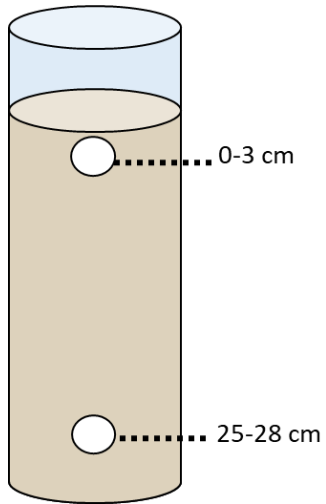


The first centimeters of the clogged columns are rich in organic matter



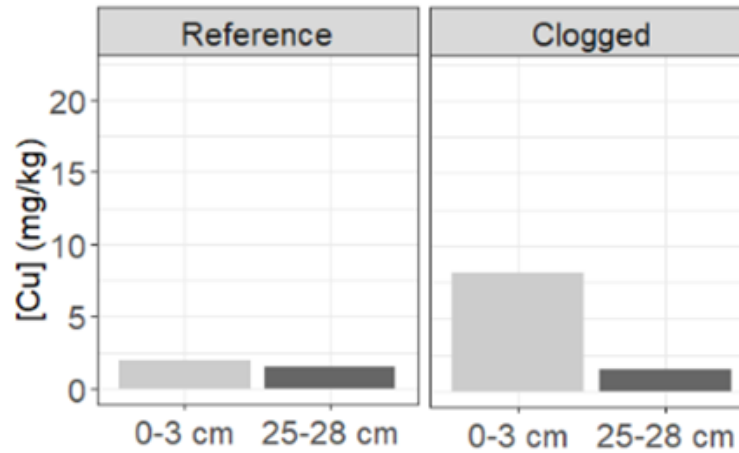
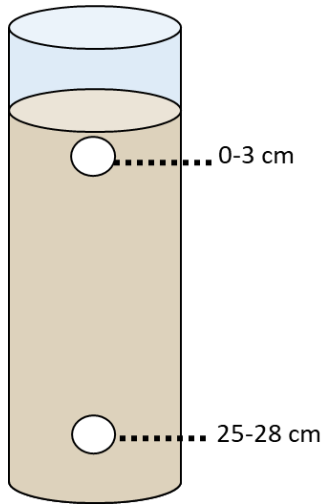


## ❖ Copper distribution in sediment



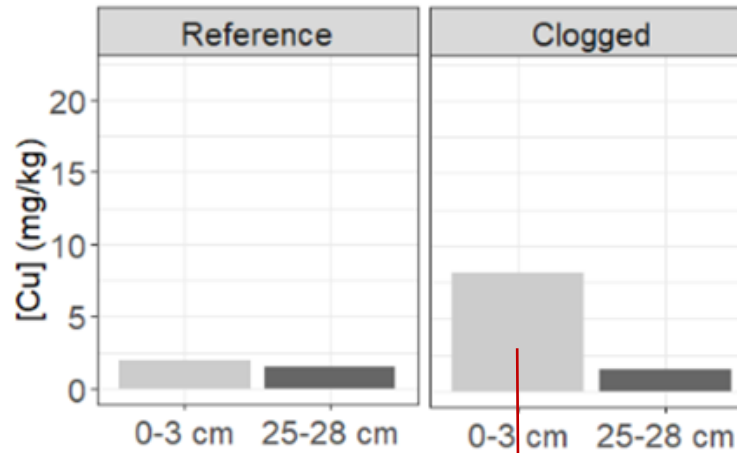
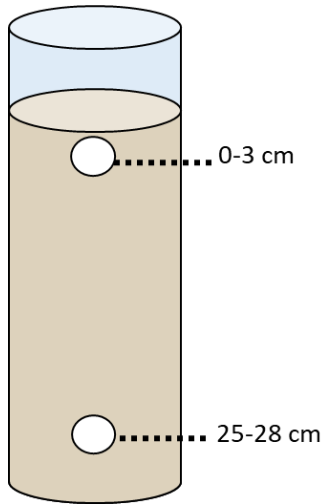


## ❖ Copper distribution in sediment





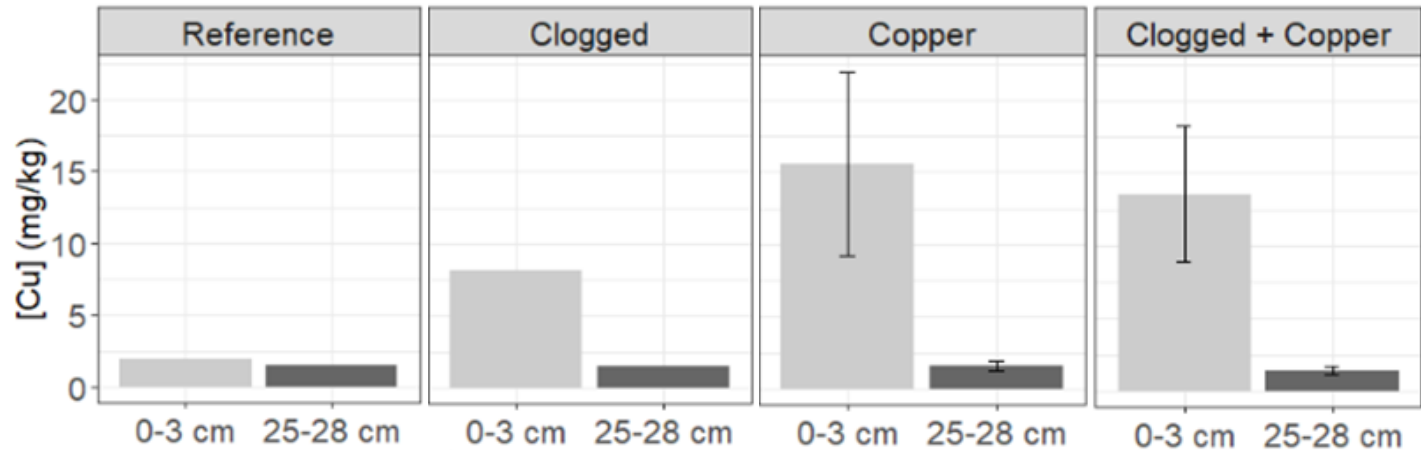
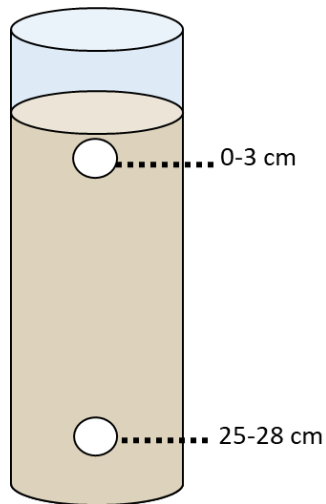
## ❖ Copper distribution in sediment



Sediment used for clogging was  
copper contaminated  
(30.19 mg Cu/kg in dry sediment)

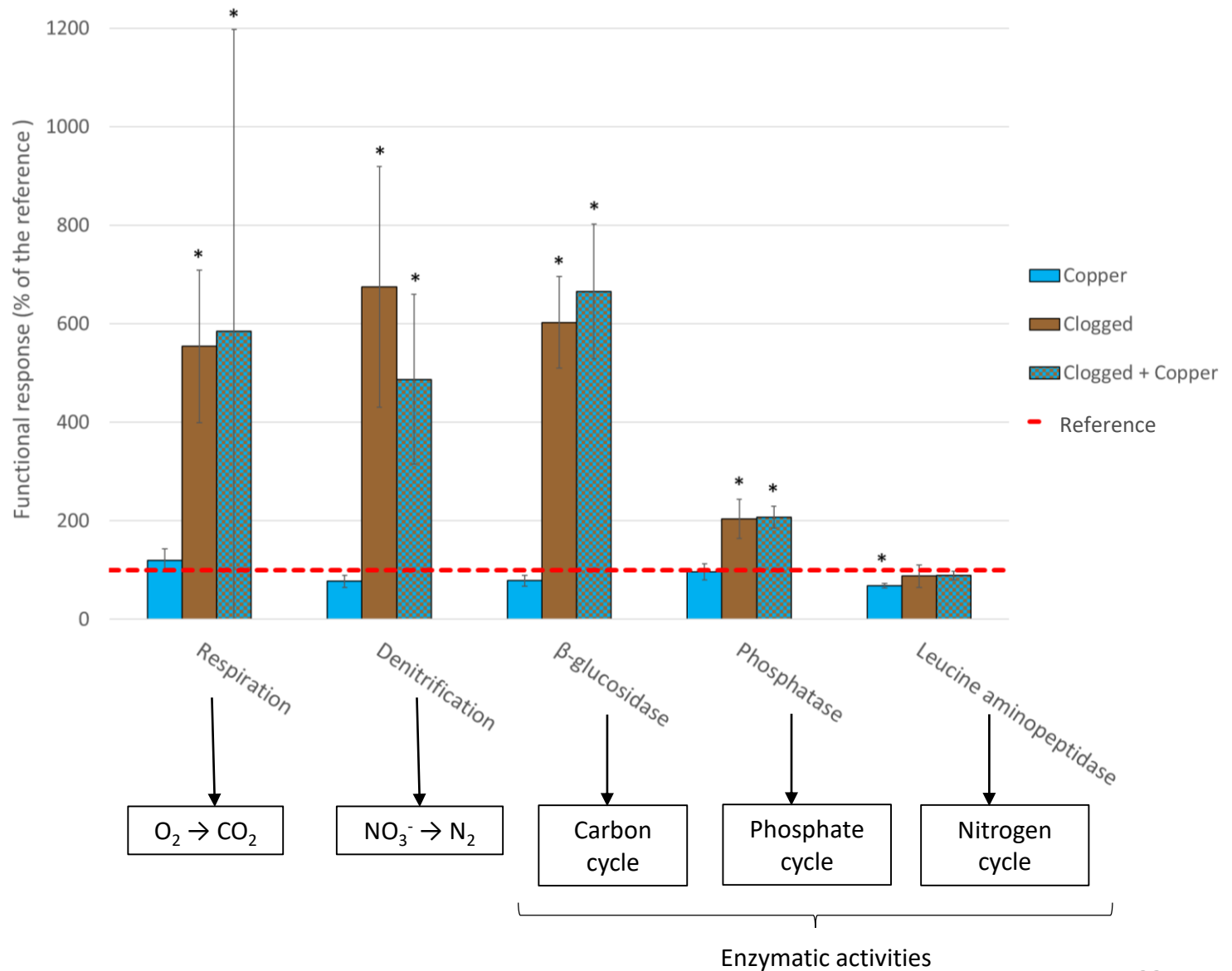
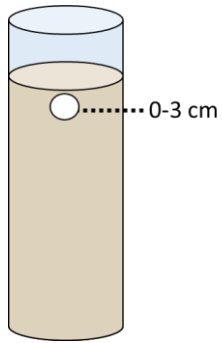


## ❖ Copper distribution in sediment

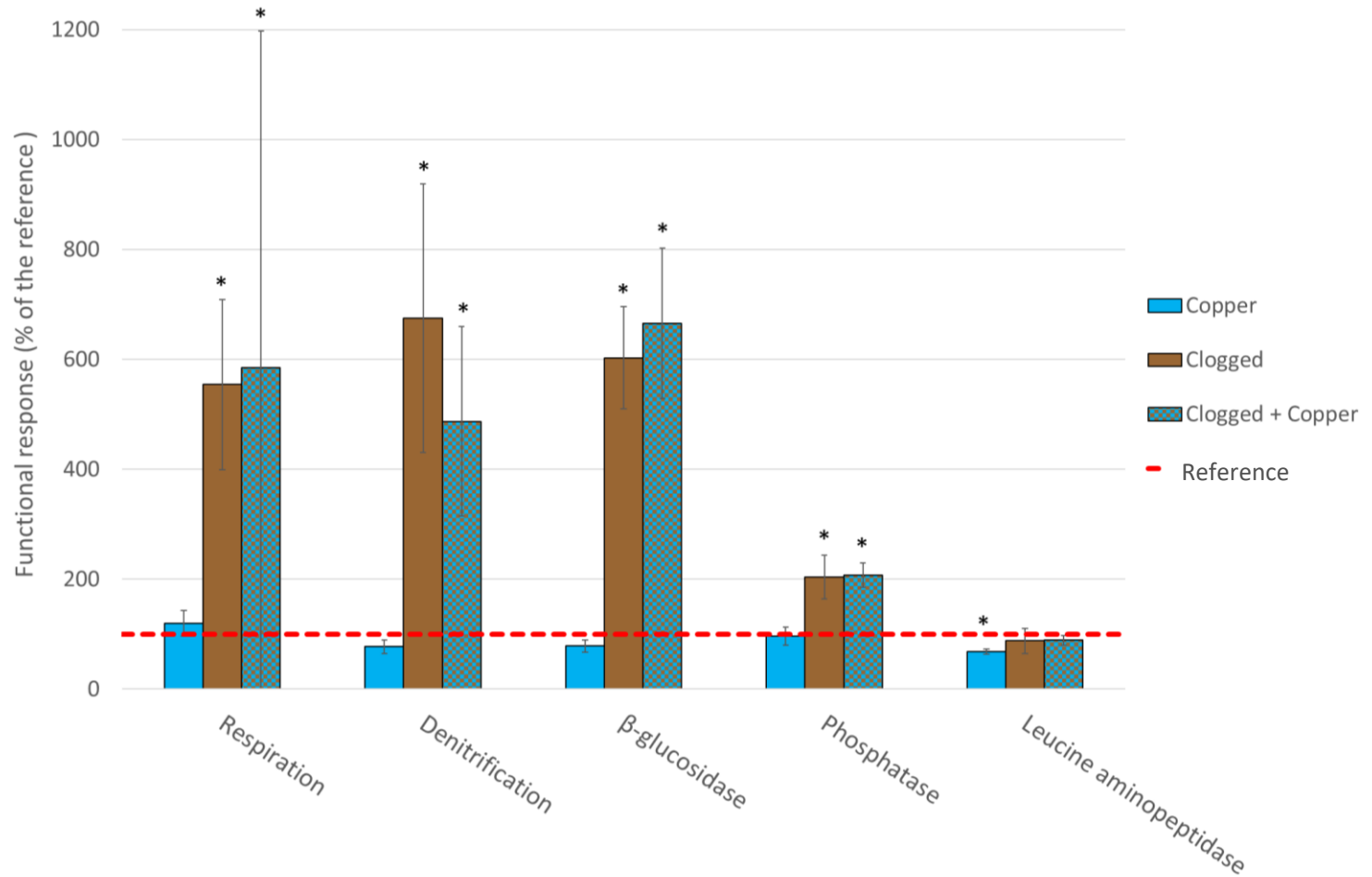
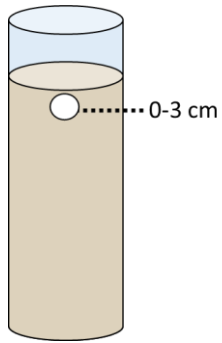


similar copper distribution profiles

- Clogging did not change the distribution of copper in the column
- No diffusion of copper in depth in the presence or not of clogging sediment

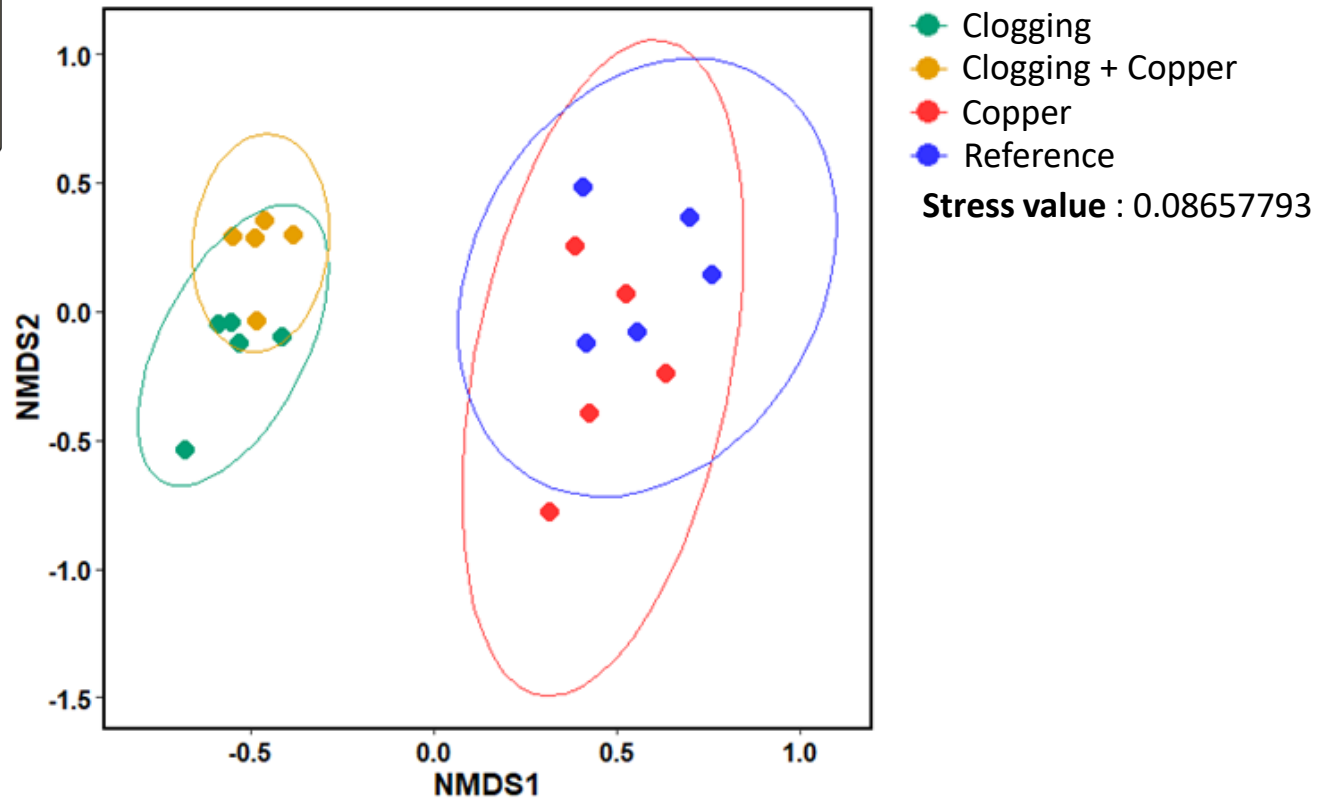
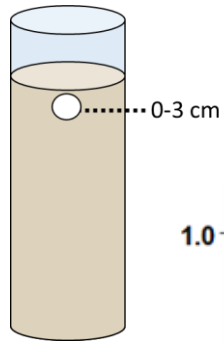




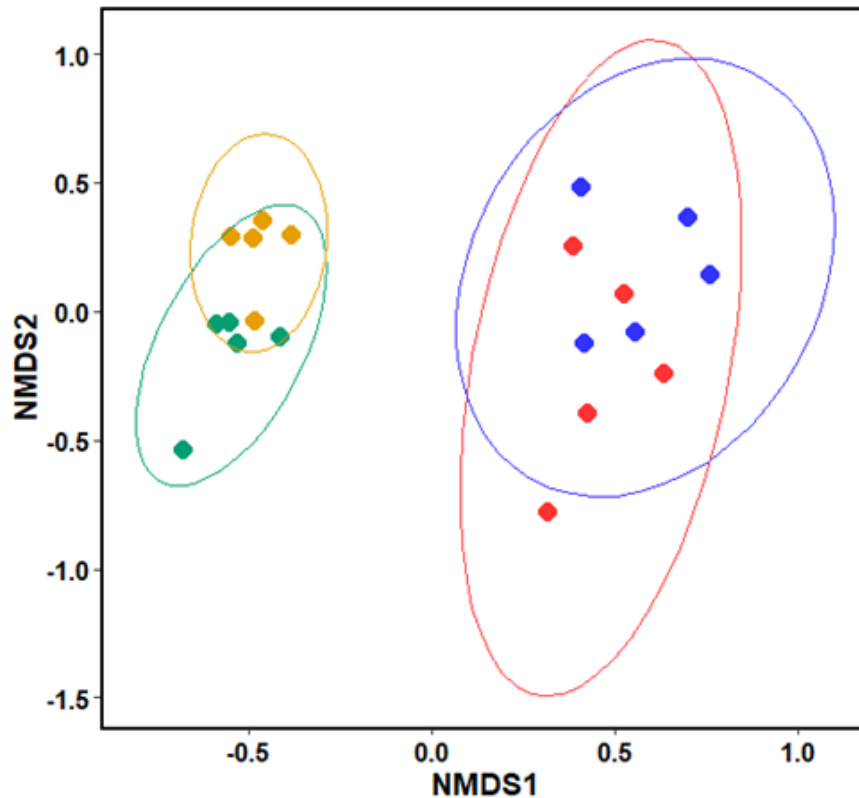
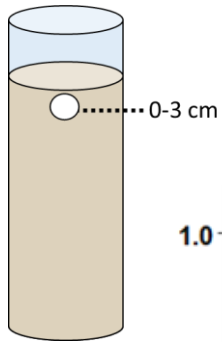


No effect of copper except for leucine aminopeptidase

Stimulation of microbial activities in the presence of clogging except for leucine aminopeptidase



Separation in two groups : exposed or not exposed to clogging  
 Small effect of copper at the tested concentration



- Clogging
- Clogging + Copper
- Copper
- Reference

**Stress value** : 0.08657793

Adonis2 test results (999 permutations)

Factor	Pr (>F)
Clogging	0,001 ***
Copper	0,040 *
Copper*Clogging	0,018 *

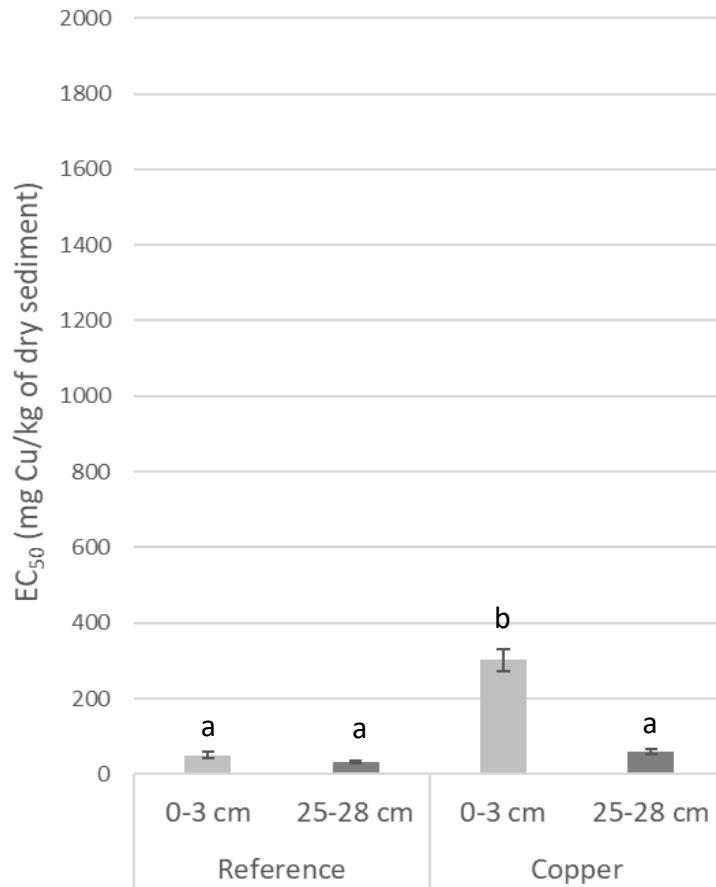
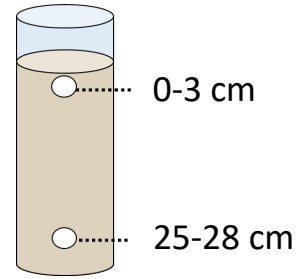
Separation in two groups : exposed or not exposed to clogging  
 Small effect of copper at the tested concentration



**EC50** = Concentration causing 50% reduction in microbial activity measured in an acute toxicity test (PICT method)

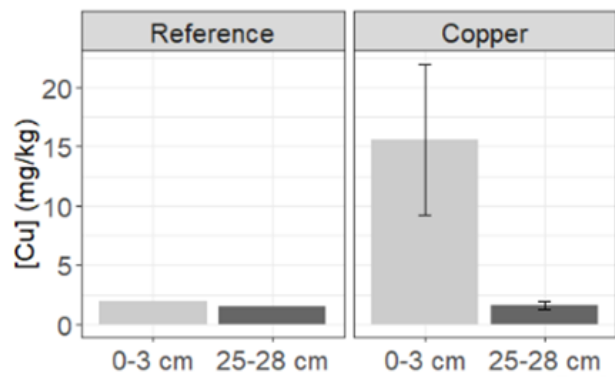
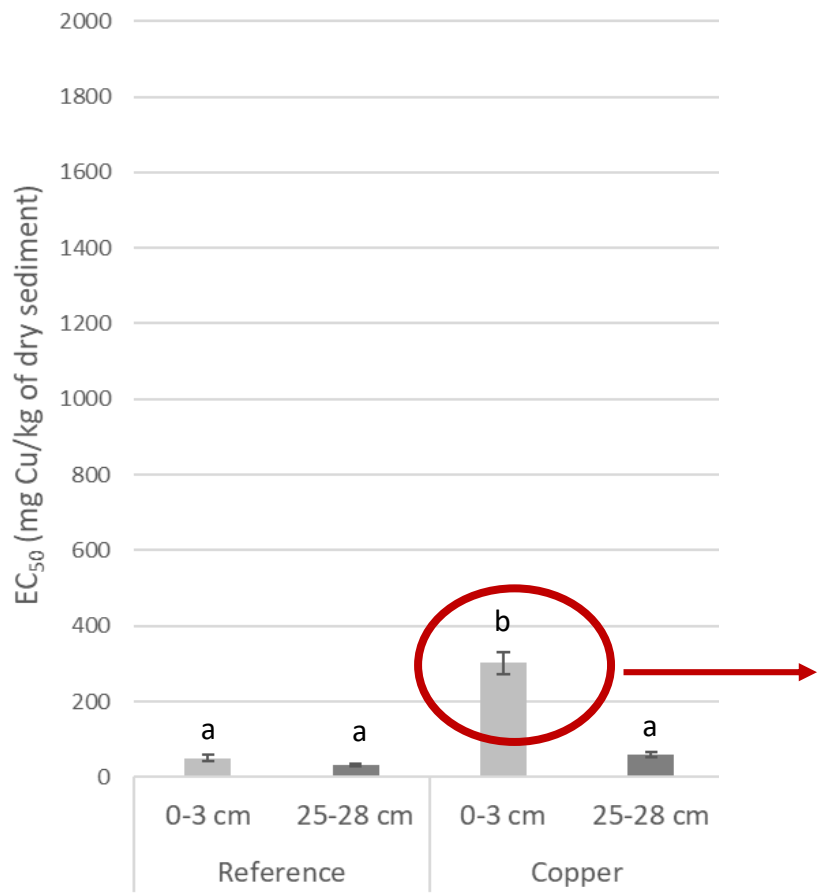
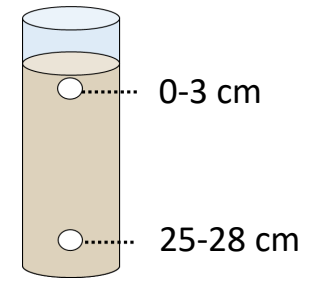


**EC<sub>50</sub>** = Concentration causing 50% reduction in microbial activity measured in an acute toxicity test (PICT method)

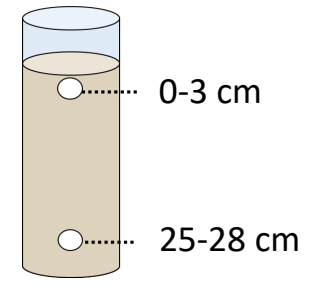
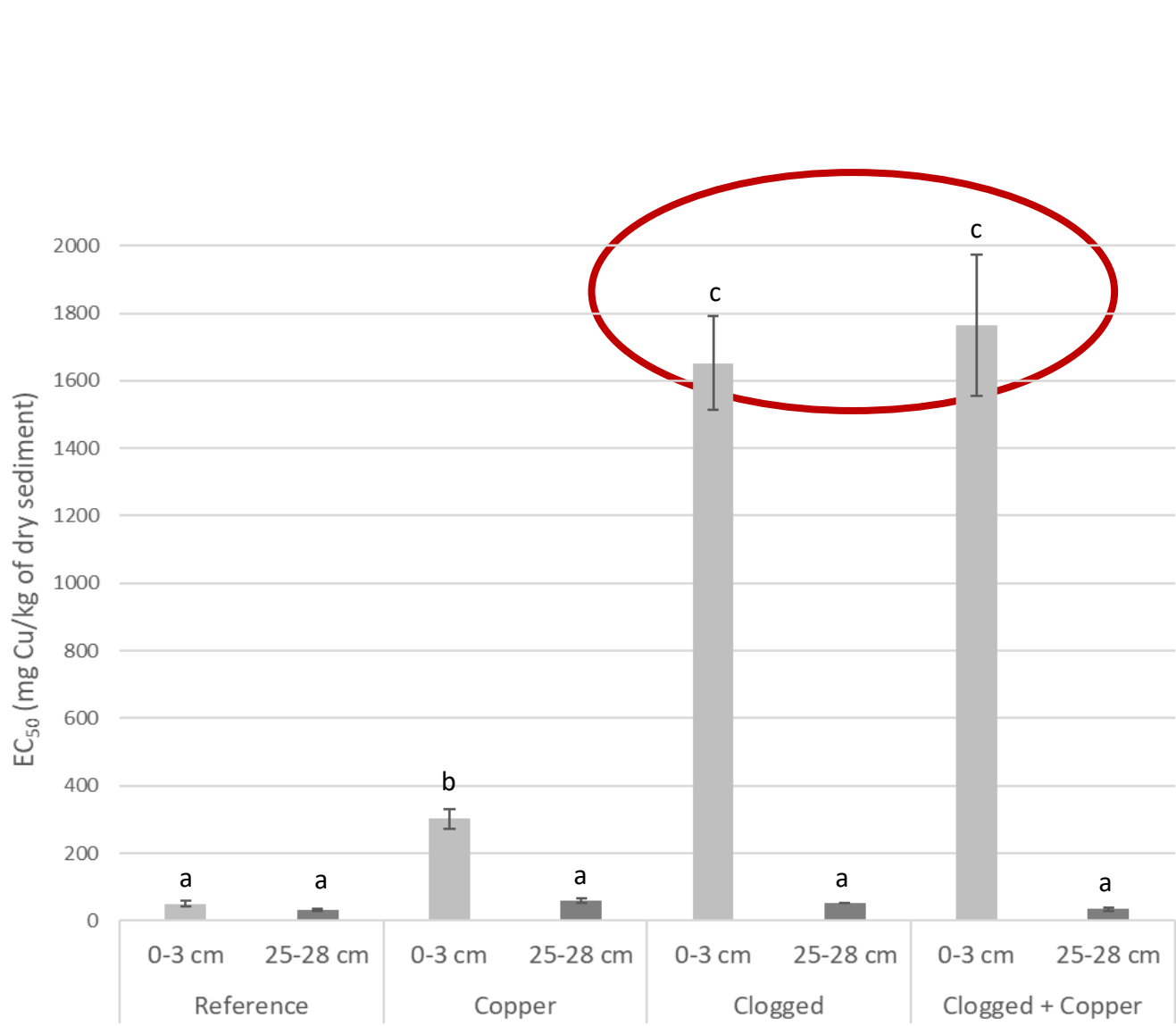


(Different letters indicate significant differences between two treatments)

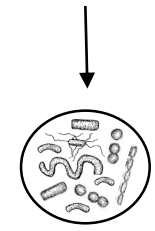
# Results - Acquisition of copper tolerance in microbial communities



(Different letters indicate significant differences between two treatments)



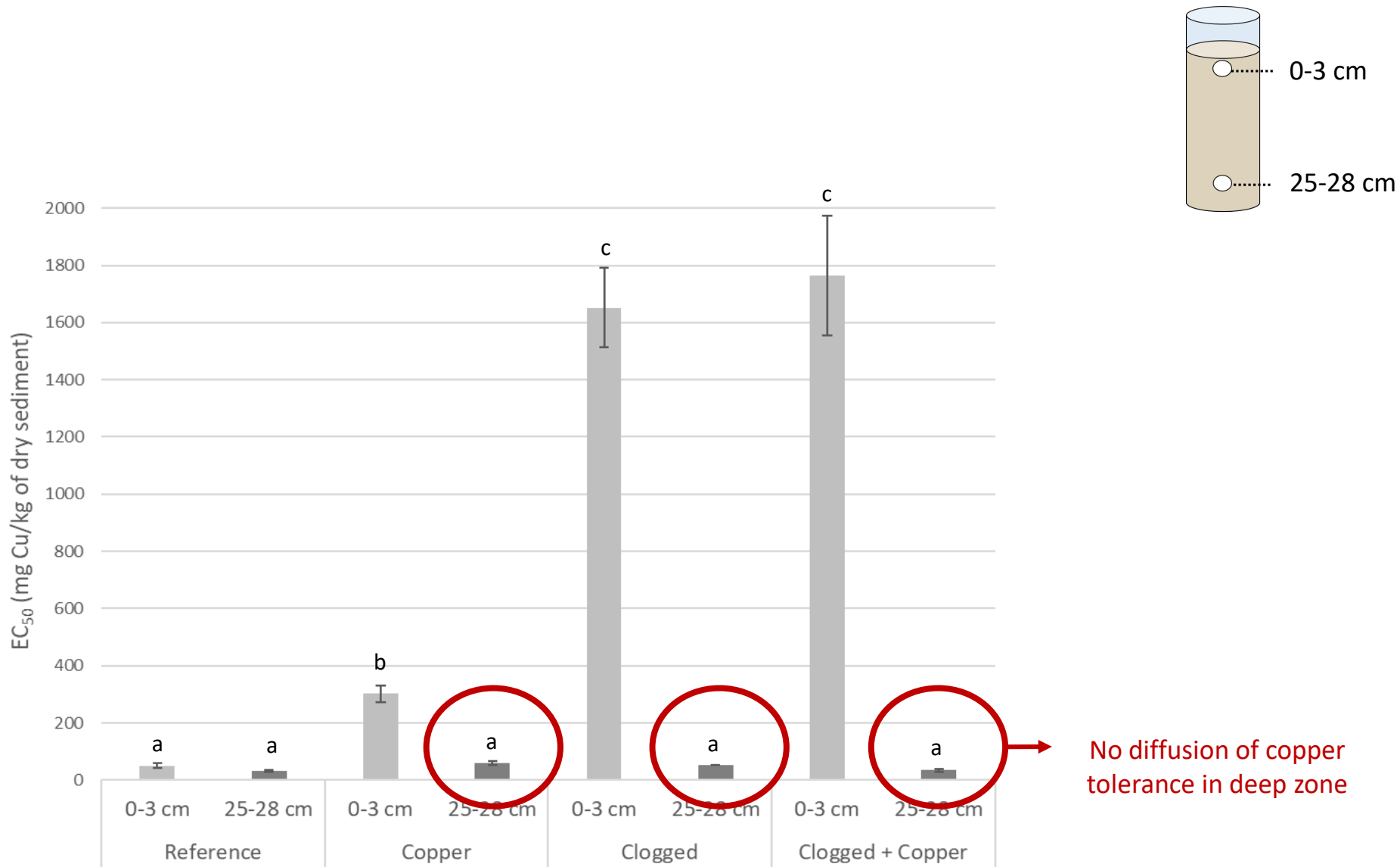
Sediment used for clogging was copper contaminated (30.19 mg Cu/kg in dry sediment)



Already copper tolerant?

(Different letters indicate significant differences between two treatments)

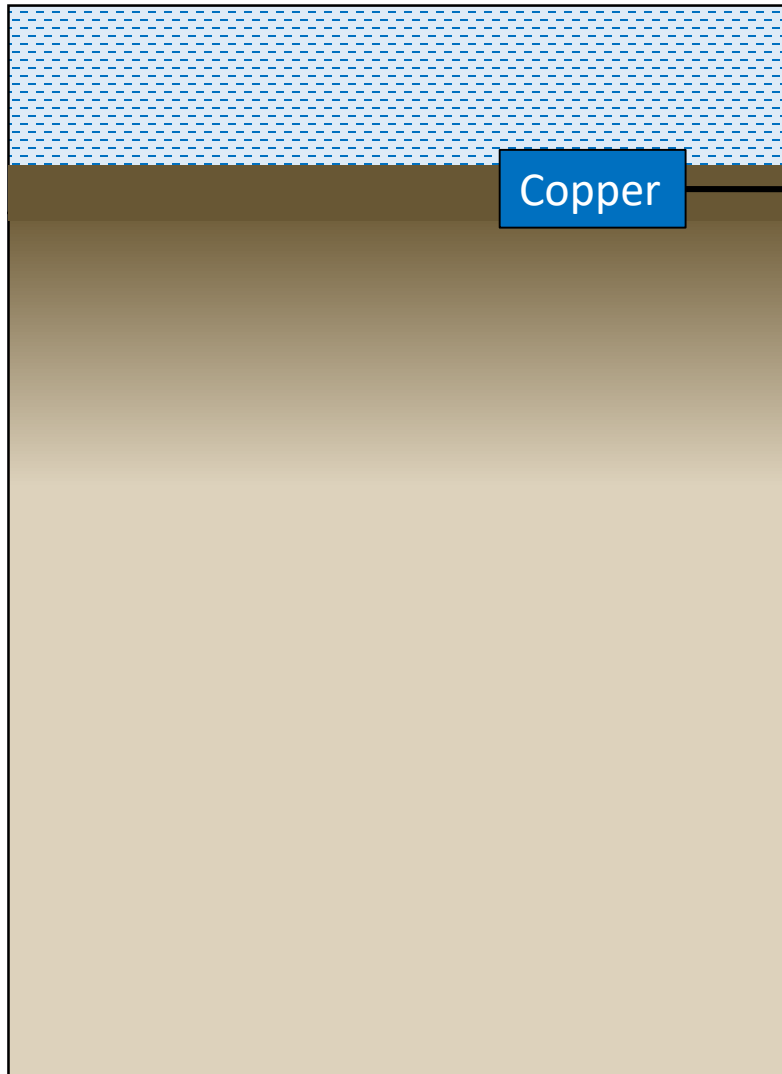
# Results - Acquisition of copper tolerance in microbial communities



(Different letters indicate significant differences between two treatments)



What are the combined effects of copper contamination and clogging on microbial communities in the hyporheic zone?



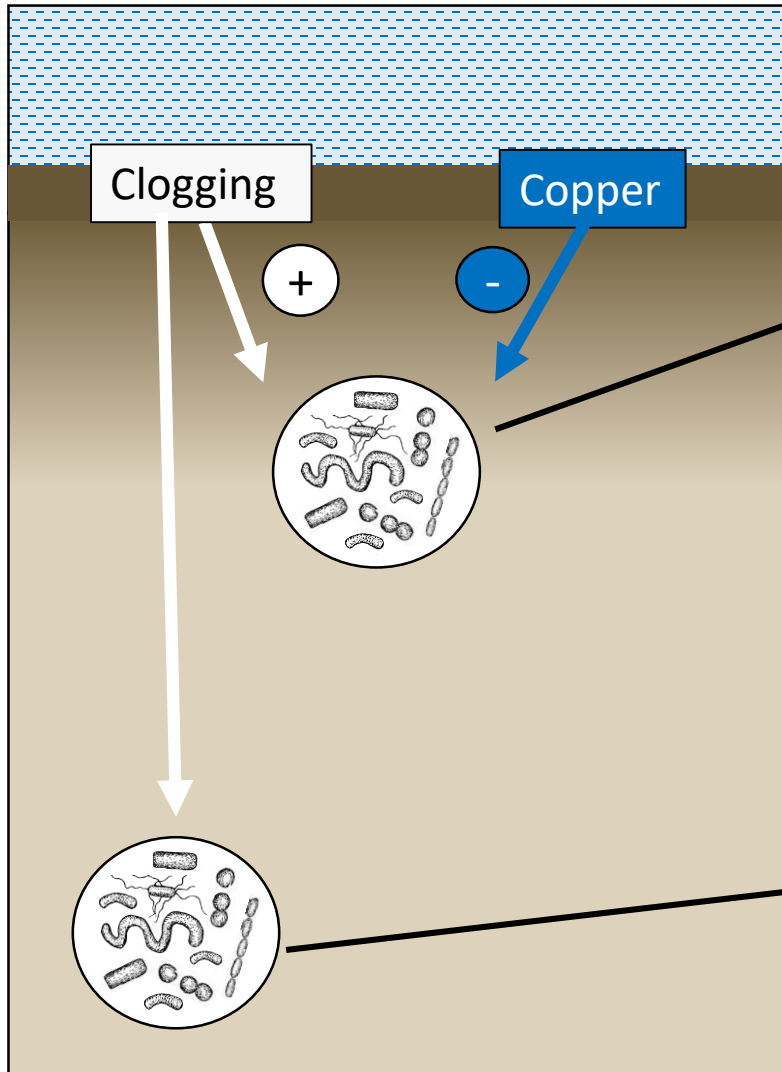
## Hypothesis 1

Copper fixation in the first centimeters



Copper Fixation in the first centimeters with or without clogging

What are the combined effects of copper contamination and clogging on microbial communities in the hyporheic zone?



## Hypothesis 2

- Antagonistic effects of copper and clogging on microbial functions
- Modification of microbial structure



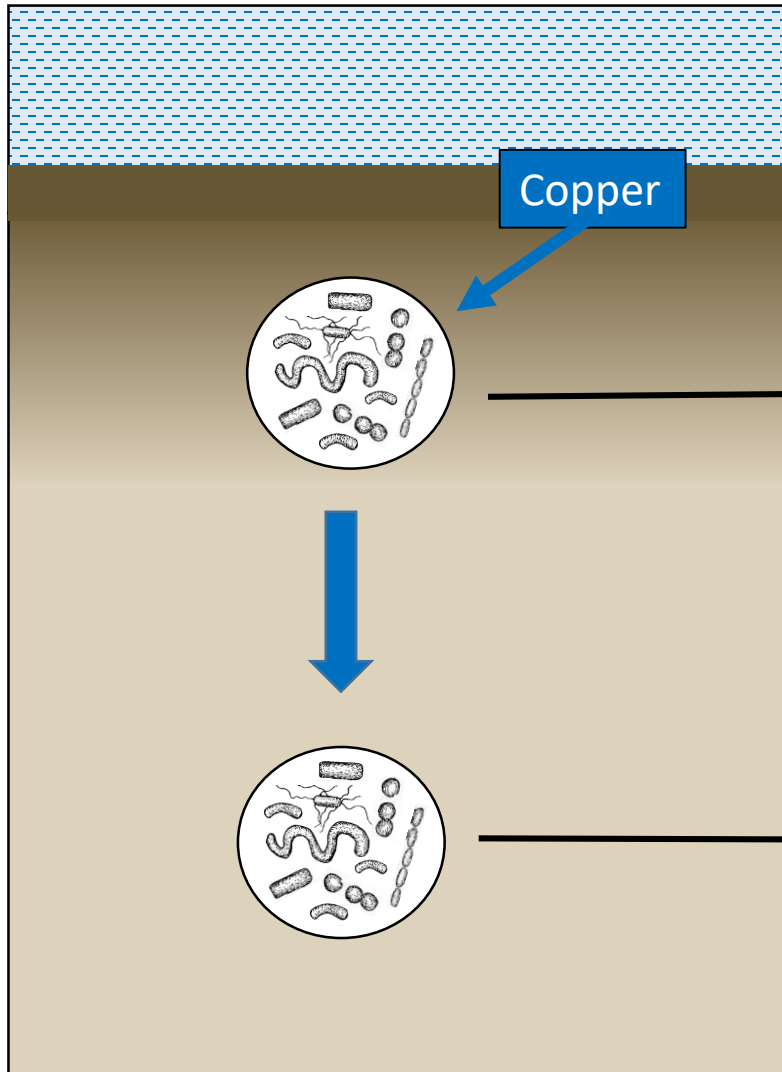
- ✓ Clogging strongly affects microbial functions (stimulation) and microbial structure
- ✓ Clogging reduces the copper effects on leucine aminopeptidase

- Only the effects of clogging on microbial functions and structure (development of anaerobic communities)



- ✓ No difference in microbial function and structure between treatments

What are the combined effects of copper contamination and clogging on microbial communities in the hyporheic zone?



## Hypothesis 3

Copper tolerance in the first cm

- ✓ Copper tolerance in the first cm of sediment exposed to “copper” treatment without clogging
- ✓ Microbial communities of clogging sediment already copper tolerant ?

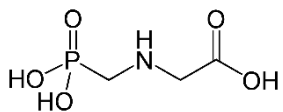
Diffusion of copper tolerance in deep zone

- ✓ No diffusion of copper tolerance
- ✓ Copper tolerance linked to copper distribution at a micro habitat level

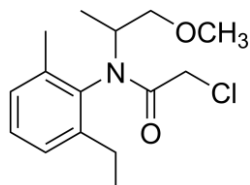
Copper filtered in the first centimeters of the hyporheic sediments



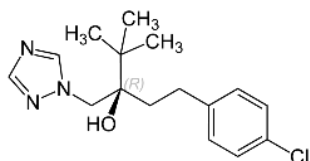
How clogging can modify the filter role of a more mobile contaminant ?



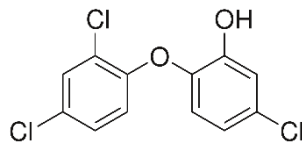
**Glyphosate**  
(Herbicide)



**Metolachlore**  
(Herbicide)



**Tébuconazole**  
(Fongicide)



**Triclosan**  
(Biocide)

- Organic contaminants
- Frequently found in streams
- Biodegradation by microorganisms

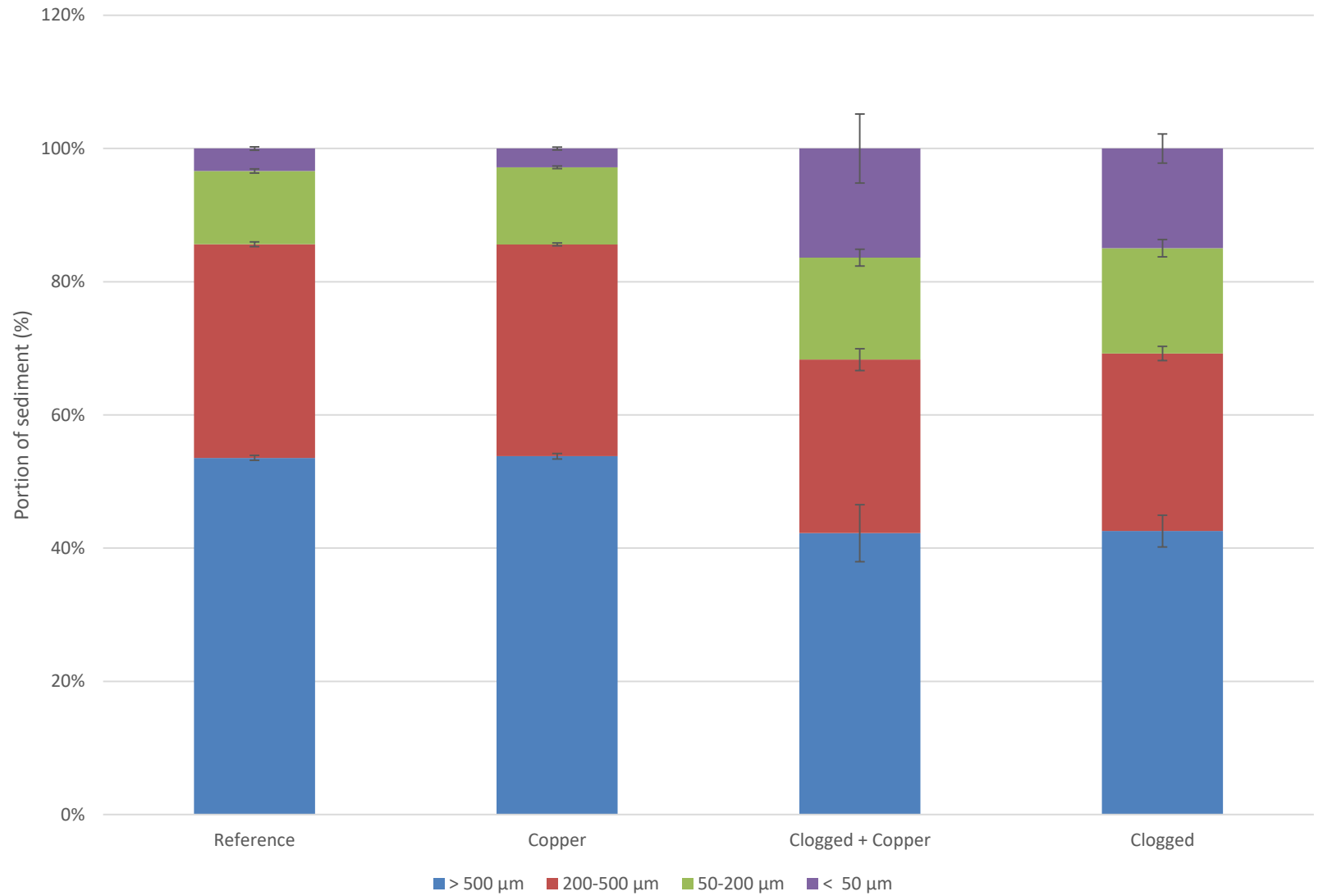


[laura.kergoat@inrae.fr](mailto:laura.kergoat@inrae.fr)

> Thank you for your attention !







# PICT method

