

Towards soil micro-zymography: comparison of staining and impregnation strategies

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1 OBJECTIVES

- ❖ Developing the **micro-zymography** approach to visualize enzymes activity at the microscopic level combining the microinjections of fluorogenic substrates with epifluorescence microscopy.
- ❖ Visualization of the distribution of enzyme activity at the surface of soil **micro-aggregates**.
- ❖ Comparing different **fixation** approaches.

2 METHODS

- ❖ Visualization of samples were performed under the fluorescence microscope by both **normal** and **UV light**.
- ❖ Soil aggregates were selected from **maize rhizosphere** as we expected higher microbial and enzymatic activity in the rhizosphere compared with bulk soil.
- ❖ Soil samples were mounted on the glass slide with different methods; **soil suspension**, **2% agarose gel**, and **silicon**.
- ❖ Visualization of enzyme activities was carried out by 10 mM **phosphatase-substrate**.

3 RESULTS

No.	Approach	Fluorescent	Bright field
1	Soil suspension mounted on the glass slide		
2	Soil particles fixed on glass slide with 2% agarose gel		
3	Soil particles fixed on glass slide with silicon		

4 CONCLUSIONS

- ❖ The liquid environment of soil suspension makes the particles mobile
- ❖ Agarose gel demonstrates high auto-fluorescence which interferes the signals of enzyme activity
- ❖ Silicon is hydrophobic so substrate should be added before fixation.
- ❖ Comparing the pros and cons of each approach, **silicon** is introduced as the best fixative in normal microscopic methods.