# Exudate Dynamics of the Rhizosphere Visualized Using MALDI-Imaging

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## Introduction

Carbon and nutrient soil cycling is regulated by a plant's rhizosphere, with the local microbial environment relying on the exudates of the rhizosphere's root structure. Currently, the dynamics of root exudates remains understudied. Recent studies utilizing mass spectrometry imaging have begun to provide some insights. Challenges remain due to difficulties in extracting information from the roots and soil while preserving the spatial distribution of the exudate molecules. Here, we implement an extraction method that extracts exudants to an anodized aluminum oxide (AAO) membrane to preserve spatial information, then conduct MSI to examine the spatial distribution of the exudate relative to the rhizosphere. This implementation is used to inspect the differences in exudate between root samples exposed to watered and droughted conditions.

## Methods

*Zea Mays* were grown in rhizoboxes in a regulated growth chamber, with the only difference being in the watering of the samples. Exudate was transferred to the AAO membrane by placing the membrane on the roots and patting the membrane. The membrane was fixed to a glass plate using double-sided conductive copper tape and sprayed with NEDC using an HTX M3+ sprayer. The default method for NEDC that is provided with the M3+ sprayer was used, as it was found to provide the best sensitivity for small molecules. The AAO filter was imaged using a timsTOF fleX MALDI-2 system (TIMS off, MALDI, negative mode) at a spatial resolution of 50 or 20 µm. Tentative annotations were performed in MetaboScape® 2023b.


timsTOF fleX

SCiLS

Met

## Results

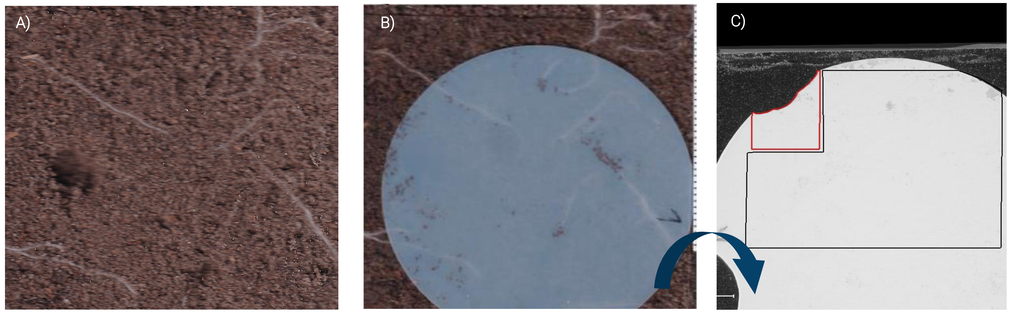


Figure 1. A) Optical image of the soil and root system of the droughted rhizobox. B) Optical image of the AAO filter laid over the root system. Water was used to wet the filter paper and it was blotted to promote extraction of metabolites into the AAO filter. C) The filter was flipped and then applied to a piece of copper tape before being mounted onto a MALDI Big Slide. The red outlined region was acquired at a spatial resolution of 20 µm, and the black outlined region was acquired with a spatial resolution of 50 µm.

- Ion images of metabolites show localization to and around the root structure root structure.
- The delocalization of the metabolic exudates is greater in the samples subjected to drought-like conditions relative to the samples that were watered
- Increased exudates observed in droughted species could arise for several reasons.
- Plant stress may lead to an increase in exudation in order to improve nutrient uptake and improve overall plant fitness

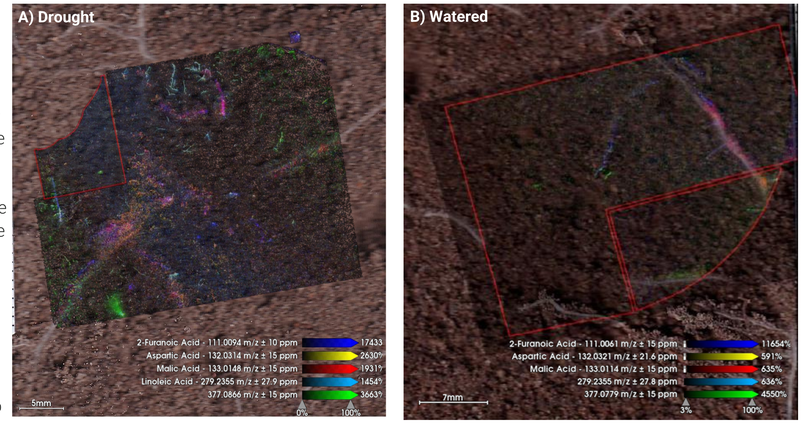


Figure 2. A) Overlaid photograph of sample acquisition region with soil showing where the root structure exudates were extracted from. B) Equipment used in this study, including the timsTOF fleX MALDI-2 system

## Select Metabolite Ion Images

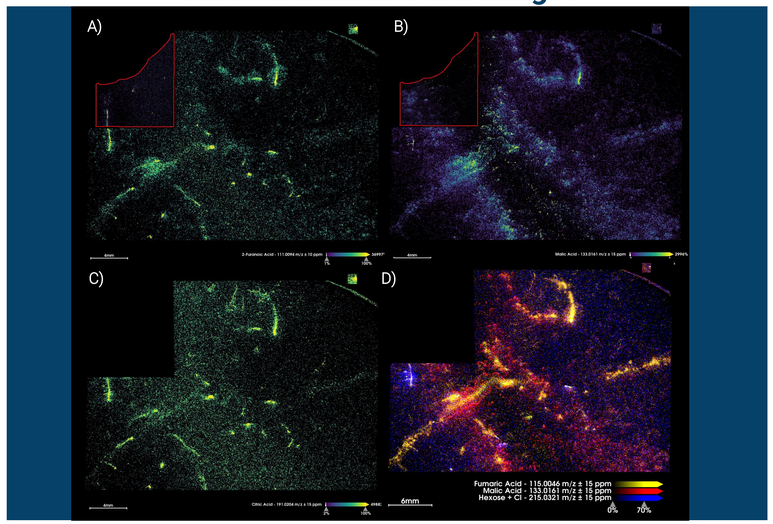


Figure 3. Select ion images of known plant metabolites. A) Ion image of 2-furanoic acid. B) Malic acid. C) Citric acid. D) False-color plot of common metabolites found in the citric acid cycle. Note all assignments are tentative based only on exact mass

## Summary

AAO membranes were used to extract root exudates directly from soil contained in a rhizobox. MALDI Imaging was used to detect various metabolites throughout the soil. Metabolites were detected in higher abundance around the roots in the droughted sample relative to the watered sample. This increase in metabolites is potentially attributed to an increased abundance of microbial activity around the roots.

## Conclusion

- A novel method utilizing AAO membranes was used to extract root exudates directly from soil contained in a rhizobox.
- Due to the high relative abundance of metabolites found surrounding the roots of the droughted sample, it is possible that exudates have increased to improve nutrient uptake.

Imaging: Spatially-Resolved Omics