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Title: Two-Dimensional Visualization and Quantification of Labile, Inorganic Plant Nutrients and Contaminants in Soil

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **Y**

Videographer: All screen capture files provided, do not film

3. Interview statements: Considering the covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

☒ Interviewees wear masks until the videographer steps away (≥6 ft/2 m) and begins filming. The interviewee then removes the mask for line delivery only. When the shot is acquired, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **44**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Stefan Wagner**: The biogeochemical cycling of elements in soil plays a crucial role in environmental systems. With this protocol, the distribution of plant-available element fractions can be imaged in 2D using DGT-mass-spectrometry-imaging **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **Jakob Santner**: This method is unique in its ability to visualize and quantify ultratrace-levels of multiple inorganic solute species at the soil-root interface, substantially exceeding the spatial resolution of alternative methods **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

OPTIONAL:

1.3. **Jakob Santner**: In addition to the investigation of labile element fluxes in soils and sediments, this method can be applied to investigate how plant roots take up nutrient and contaminant elements **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Videographer: Can cut for time*

Protocol

2. Diffusive Gradients in Thin Films (DGT) Gel Fabrication and Rhizotron Preparation

2.1. For DGT (D-G-T) gel fabrication, first coat a thin film of polyurethane-based mixed anion and cation binding gel suspension onto a glass plate **[1]** and place the plate into an oven to initiate gel formation by solvent evaporation **[2]**.

2.1.1. WIDE: Talent adding suspension to plate *Videographer: Important step*
2.1.2. Talent placing glass plate into oven *Videographer: Important step*

2.2. After repeating the application and evaporation three times, hydrate the resulting triple-coated glass plate in a water bath **[1]** to obtain a 0.1-millimeter-thin, tear-proof mixed anion and cation binding gel **[2]**.

2.2.1. Talent placing glass plate into water bath
2.2.2. Shot of final gel in storage vial

2.3. To assemble the rhizotron, use two clamps to attach one small acrylic plate at the bottom of the rhizotron with the pressure of the clamps directed onto the rhizotron frame so that the plate does not bend inwards **[1-TXT]**.

2.3.1. Talent applying clamp(s)/attaching plate **TEXT: Weigh all materials before assembly**

2.4. Incline the rhizotron slightly toward the small plastic plate **[1]** and fill the rhizotron with pre-moistened soil up to an approximate height of 4 centimeters **[2]**.

2.4.1. Rhizotron being inclined
2.4.2. Rhizotron being filled

2.5. Agitate the rhizotron slightly to evenly distribute the soil **[1]** and use a compaction tool to gently compress the soil by a few millimeters **[2]**.

2.5.1. Rhizotron being agitated
2.5.2. Soil being compressed

2.6. Repeat the filling and compression until the rhizotron is filled with soil, leaving a 3-centimeter gap at the top **[1]**.

2.6.1. Shot of 3-cm gap

2.7. Use tape to carefully fix a 13 x 22-centimeter piece of PTFE (P-T-F-E) foil to the rhizotron frame one corner at a time **[1-TXT]**, applying tension to ensure a flat foil surface **[2-TXT]**.

2.7.1. Talent taping foil **TEXT: PTFE: polytetrafluoroethylene**
2.7.2. Shot of corner being taped with tension **TEXT: Open and re-fix individual corners until folds removed as necessary**

2.8. When the PTFE foil is flat and contiguous with the soil surface, attach a second piece of foil on the lower end of the rhizotron, overlapping the upper PTFE foil piece by 1 centimeter, in the same manner **[1]**.

2.8.1. Foil being taped

2.9. When the second piece has been secured, apply a protective plastic foil cover **[1]**, place a front plate onto the soil-filled and foil-covered rhizotron **[2]**, and place one rail around each side of the rhizotron **[3]**.

2.9.1. Talent placing cover
2.9.2. Talent placing plate onto rhizotron
2.9.3. Talent placing rail around at least one side

2.10. Then tighten the screws by hand to fix the rails and front plate to the rhizotron with the screws positioned toward the closed side of the rhizotron **[1]**.

2.10.1. Screw(s) being tightened

2.11. To water the soil, push pipette tips into the watering holes **[1]** and let the water flow into the soil by gravity **[2]**.

2.11.1. Talent pressing pipette into watering hole(s)
2.11.2. Water flowing into soil

3. Plant Cultivation

3.1. To grow plants, plant up to two seedlings into the rhizotron **[1-TXT]** and add 5 milliliters of water directly to the seedlings to support their growth **[2]**.

3.1.1. WIDE: Talent planting seedling(s) *Videographer: Important step* **TEXT: Alternative: Cultivate plant cuttings**
3.1.2. Talent adding water to seedling(s) *Videographer: Important step*

3.2. Cover the top opening of the rhizotron for the first two days after planting with a transparent, moisture-retaining film **[1]** and wrap the rhizotron in aluminum foil to prevent microphytic growth **[2]**.

3.2.1. Film being placed
3.2.2. Talent wrapping rhizotron with foil

3.3. Then place the planted rhizotron into a growth room with the environmental conditions set to the specific plant requirements **[1]** and incline the rhizotron 25-35 degrees to ensure root development along the front plate via gravitropism **[2]**.

3.3.1. Talent placing rhizotron into growth room
3.3.2. Rhizotron being inclined

4. Solute Distribution Sampling

4.1. To apply the fabricated DGT gel, cut a 10-micron-thick polycarbonate membrane with a 0.2-micron pore size to an at least greater than 1-centimeter width and length of each side of the gel **[1]** and place the membrane onto the gel **[2]**.

4.1.1. WIDE: Talent cutting membrane
4.1.2. Talent placing membrane onto gel

4.2. Apply water to remove air bubbles from the stack **[1]** and use vinyl electrical tape to fix the membrane to the plate along all four edges of the gel **[2-TXT]**.

~~4.2.1. Water being applied *Videographer: Important/difficult step*~~ NOTE: This shot is included in shot 4.1.2.
4.2.2. Membrane being taped *Videographer: Important/difficult step* **TEXT: Use tweezers to remove bubbles between gel and membrane as necessary**

4.3. After removing the front plate and protective foils, align the view finder of a digital single-lens reflex camera equipped with a macro lens to the center of the region of interest in the gel **[1]** and, including a scale bar in the image, acquire an orthogonal photo of the region of interest **[2]**.

4.3.1. Talent adjusting camera focus
4.3.2. LAB MEDIA: 4.3.2_ROI with ruler

4.4. Then align one edge of the plate equipped with the gel-membrane stack with an edge of the open rhizotron **[1]**, gently bend the plate toward the soil **[2]**, and use the rails and screws to attach the plate to the rhizotron **[3]**.

4.4.1. Plate being aligned *Videographer: Important/difficult step*
4.4.2. Plate being bent *Videographer: Important/difficult step*
4.4.3. Plate being attached to rhizotron *Videographer: Important/difficult step*

5. Gel Retrieval and Chemical Analysis Preparation

5.1. After the solute sampling period, transfer the front plate from the rhizotron to a laminar flow hood with the gel-membrane stack side facing up **[1]** and carefully remove the tape and the polycarbonate membrane covering the gel **[2]**.

5.1.1. WIDE: Talent placing plate in hood
5.1.2. Tape and membrane being removed

5.2. Apply water to help the gel to float freely on a thin film of water on the plate with the soil-contact side facing up **[1]** and transfer the gel onto a polyethersulfone membrane with a 0.45-micron pore size **[2]** and blotting paper support **[3-TXT]**.

5.2.1. Water being applied
5.2.2. Gel being transferred onto membrane **TEXT: Maintain gel orientation**
5.2.3. Added shot: Gel on membrane being transferred onto blotting paper support

5.3. After covering the gel stack with a protective foil, place the stack in a vacuum gel drier **[1]**.

5.3.1. Gel stack being placed into gel drier

5.4. When the gel has fully dried, use double-sided adhesive tape to fix the dried gel together with other gel samples on a glass plate **[1]**.

5.4.1. Gel being fixed to glass plate

6. Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) Line Scan Analysis

6.1. To perform a laser ablation inductively coupled plasma mass spectrometry line scan analysis of the dried DGT gels, first fix the sample, blanks, and standards onto the laser ablation sample stage **[1]** and lock the laser stage into the ablation cell of the laser ablation system **[2]**.

6.1.1. WIDE: Talent fixing plate to stage
6.1.2. Stage being locked

6.2. In the laser ablation software, move to the region of interest on the gel surface **[1]** and

draw a single, approximately 1-millimeter-long line across the surface of a gel standard **[2]**.

6.2.1. ~~Talent selecting line tool~~ Talent moving to region of interest in LA software, with monitor visible in frame
6.2.2. SCREEN: screenshot_1: 00:00-00:05

6.3. Right click the line in the **Scan Patterns** window to verify that the laser ablation parameters have been set and adopted and use the **Duplicate Scans** tool to duplicate this line four times with a interline distance larger than the spot diameter **[1]**.

6.3.1. SCREEN: screenshot_2: 00:00-00:15 *Video Editor: can speed up*

6.4. After repeating this line drawing for each gel standard, calibration blank, and method blank **[1]**, draw a single line along the top edge of the rectangular area of the gel sample to be analyzed and duplicate the line to create parallel lines for the entire sample area as demonstrated, using an interline distance of 300-400 micrometers **[2]**.

6.4.1. SCREEN: screenshot_3.1: 00:17-00:28
6.4.2. SCREEN: screenshot_4: 00:02-00:30 *Video Editor: please speed up*

6.5. Verify that each start and end point of each line is properly focused on the gel surface **[1]** and click **Analyze Batch** to initiate the sample sequence on the inductively coupled plasma mass spectrometer **[1-TXT]**.

6.5.1. SCREEN: screenshot_5.1: 00:00-00:13 *Video Editor: please speed up* **TEXT: Acquisition initiated upon first laser pulse**
6.5.2. SCREEN: screenshot_5.2: 00:02-00:13 *Video Editor: please speed up*

6.6. Click **Emission** in the **Laser Energy** window to recharge the laser head, click **Run** to open the **Run Experiment** window, and select **Selected Patterns Only [1]**.

6.6.1. SCREEN: screenshot_6: 00:00-00:14

6.7. Set the **Washout Delay** to 20-30 seconds, select the **Enable Laser During Scans** box, and set the **Laser Warmup Time** to 10 seconds **[1]**.

6.7.1. SCREEN: screenshot_7: 00:00-00:11

6.8. Then click **Run** and **OK** to start the line-scan analysis **[1]** and monitor the raw signal intensity in counts per second for each isotope on the inductively coupled plasma mass spectrometer in real time. Each line should start and end with a gas blank **[2]**.

6.8.1. SCREEN: screenshot_8.1: 00:00-00:36 *Video Editor: please speed up*
6.8.2. SCREEN: screenshot_8.2: 00:05-00:54

7. Data Processing and Calibration

7.1. After the analysis, import the raw data file for each ablated line into a spreadsheet **[1]**.

7.1.1. WIDE: Talent importing data, with monitor visible in frame

7.2. The raw data table shows the inductively coupled plasma mass spectrometry readings for each isotope in counts per second and the corresponding time points in seconds. List all the lines next to each other in different columns **[1]**.

7.2.1. SCREEN: screenshot_9: 00:06-00:13

7.3. Calculate an average gas blank for each isotope from all of the gas blank values recorded before the line ablations and subtract the average gas blank from the corresponding raw intensities for each isotope to correct for the background signal **[1]**.

7.3.1. SCREEN: screenshot_10: 00:00-00:39 *Video Editor: please speed up*

7.4. To apply internal normalization, divide the gas blank-corrected signal intensity of each isotope by the gas blank-corrected signal intensity of the internal standard carbon-thirteen for each datapoint to correct for variations in the amount of material ablated and instrumental drift **[1]**.

7.4.1. SCREEN: screenshot_11: 00:00-00:13

7.5. Crop the data before the start and after the end of each ablated line to remove the gas blank background signal and transpose the data table to obtain a grid matrix in which each row corresponds to an ablated line and each column corresponds to a normalized isotope intensity value **[1]**.

7.5.1. SCREEN: screenshot_12: 00:00-00:31 *Video Editor: please speed up*

7.6. Then apply the calibration function obtained from the analysis of the gel standards and save the calibrated data matrix as a text file **[1]**.

7.6.1. SCREEN: screenshot_13: 00:00-00:25 *Video Editor: please speed up*

8. Image Generation

8.1. To generate an image, import the calibrated sample data matrix into the image analysis

software as a text image **[1]** and apply the aspect ratio correction factor and a **Look Up Table** to visualize the chemical gradients in the solute image **[2]**.

8.1.1. WIDE: Talent importing data matrix *Videographer: Important step*
8.1.2. SCREEN: screenshot_14: 00:00-00:20 *Video Editor: please speed up*

8.2. Adjust the image color balance to control the lower and upper limits of the display range, add a **Calibration bar**, and save the solute image as tiff-file **[1]**.

8.2.1. SCREEN: screenshot_15: 00:00-00:28 *Video Editor: please speed up*

8.3. Use the **Copy to System** command to copy the solute image and paste the image into desktop publishing software **[1]**.

8.3.1. SCREEN: screenshot_16: 00:00-00:08

8.4. Then scale-match, align, and compose the solute image with the photo of the region of interest and the other solute images **[1]**.

8.4.1. SCREEN: screenshot_17: 00:00-00:29 *Video Editor: please zoom in onto solute images after scale-matching as possible*

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
2.1., 3.1., 4.2., 4.4., 8.1.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
4.2., 4.4. The most difficult aspect of this procedure refers to the gel application on the rhizotron front plate and subsequently on the region of interest in the rhizotron. This is critical because a close and geometrically-stable contact between the gel and the soil surface is required for accurate sampling of the solute distribution at the soil-root interface. Successful gel application on the front plate can be ensured by visual inspection of the gel-membrane stack for trapped air bubbles between the layers. If air bubbles are present, the gel-membrane stack needs to be reassembled. Successful gel application on the region of interest can be ensured by 'bending' the gel-equipped front plate onto the open rhizotron.

Results

9. Results: Representative Sub-mm 2D Solute Flux Distribution of Multiple Nutrients and Contaminants Across Different Soil-Root Interfaces

9.1. Alignment of the solute images with a photographic image of the region of interest **[1]** reveals that the sub-millimeter, 2D solute flux distribution of different elements is highly variable according to the soil structure and root morphology **[2]**.

9.1.1. LAB MEDIA: Figure 7 *Video Editor: please emphasize ROI images*
9.1.2. LAB MEDIA: Figure 7 *Video Editor: please emphasize Mg-Fe images*

9.2. For example, in this analysis of a young buckwheat root grown in carbonate-free soil fertilized with ammonium nitrate **[1]**, the sub-millimeter solute distribution showed zones of decreased aluminum **[2]**, phosphorus **[3]**, and iron fluxes alongside older root sections due to root uptake **[4]**, and highly increased magnesium **[5]**, aluminum **[6]**, phosphorus **[7]**, manganese **[8]**, and iron fluxes at the root apex due to localized nutrient mobilization processes **[9]**.

9.2.1. LAB MEDIA: Figure 7A
9.2.2. LAB MEDIA: Figure 7A *Video Editor: please emphasize Al image near top of image*
9.2.3. LAB MEDIA: Figure 7A *Video Editor: please emphasize P image near top of image*
9.2.4. LAB MEDIA: Figure 7A *Video Editor: please emphasize Fe image near top of image*
9.2.5. LAB MEDIA: Figure 7A *Video Editor: please emphasize Mg image near root tip*
9.2.6. LAB MEDIA: Figure 7A *Video Editor: please emphasize Al image near root tip*
9.2.7. LAB MEDIA: Figure 7A *Video Editor: please emphasize P image near root tip*
9.2.8. LAB MEDIA: Figure 7A *Video Editor: please emphasize Mn image near root tip*
9.2.9. LAB MEDIA: Figure 7A *Video Editor: please emphasize Fe image near root tip*

9.3. In this analysis **[1]**, a distinct depletion of zinc **[2]**, cadmium **[3]**, and lead can be observed at the immediate root position **[4]**, illustrating that roots of the metal-tolerant willow species *Salix smithiana* act as a localized sink for labile trace metals in contaminated soil **[5]**.

9.3.1. LAB MEDIA: Figure 7B
9.3.2. LAB MEDIA: Figure 7B *Video Editor: please emphasize Zn image*
9.3.3. LAB MEDIA: Figure 7B *Video Editor: please emphasize Cd image*
9.3.4. LAB MEDIA: Figure 7B *Video Editor: please emphasize Pb image*

9.3.5. LAB MEDIA: Figure 7B

9.4. In this analysis, the distribution of labile trace metals alongside roots of *Salix smithiana* was co-localized with the distribution of pH using a combined, single-layer planar optode-DGT cation binding gel **[1]**.

9.4.1. LAB MEDIA: Figure 7C

9.5. This method combination revealed that increased solute fluxes of manganese **[1]**, iron **[2]**, cobalt **[3]**, nickel **[4]**, copper **[5]** and lead **[6]** were associated with a pH decrease by approximately one unit **[7]**, suggesting pH-induced metal solubilization **[8]**.

9.5.1. LAB MEDIA: Figure 7C *Video Editor: please emphasize Mn image*
9.5.2. LAB MEDIA: Figure 7C *Video Editor: please emphasize Fe image*
9.5.3. LAB MEDIA: Figure 7C *Video Editor: please emphasize Co image*
9.5.4. LAB MEDIA: Figure 7C *Video Editor: please emphasize Ni image*
9.5.5. LAB MEDIA: Figure 7C *Video Editor: please emphasize Cu image*
9.5.6. LAB MEDIA: Figure 7C *Video Editor: please emphasize Pb image*
9.5.7. LAB MEDIA: Figure 7C *Video Editor: please emphasize pH image*
9.5.8. LAB MEDIA: Figure 7C

Conclusion

10. Conclusion Interview Statements

10.1. **Stefan Wagner**: It is critical to ensure a close and stable contact between the DGT gel and the soil surface to avoid analytical artefacts. If in doubt, repeat the gel application procedure **[1]**.

10.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (4.2., 4.4.)

10.2. **Jakob Santner**: This method can be combined with other diffusion-based imaging techniques, such as planar optodes, to simultaneously assess a range of parameters involved in plant element uptake **[1]**.

10.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera