RESEARCH LETTER

Camera illustration of Indicator of Reduction in Soils (IRIS) reduction dynamics

Olivia Victoria LeFevre | Thorsten Knappenberger | Joey Nathan Shaw | Yaniv Olshansky

Dep. of Crop, Soil & Environmental Sciences, Auburn Univ., 201 Funchess Hall, Auburn, AL 36849, USA

Correspondence
Thorsten Knappenberger, Dep. of Crop, Soil & Environmental Sciences, Auburn Univ., 201 Funchess Hall, Auburn, AL 36849, USA. Email: knappi@auburn.edu

Abstract
Indicator of Reduction in Soils (IRIS) tubes or films are used with coatings of iron or manganese oxide to observe depth or occurrence of reducing conditions, with coating removal often assessed weekly. We evaluated the use of a rhizosphere camera to capture iron and manganese reduction (coating removal) at high temporal resolution. A rhizosphere tube was coated with iron and manganese oxide (two sections of each oxide) and inserted into a saturated column filled with a surface horizon from a wet soil (Fluvaquent). Images were taken hourly over 28 d and compared with Eh and pH data. Reducing conditions were observed for manganese and iron after 1 and 4 d, respectively. This technology builds upon an existing approach and could be used to evaluate real-time reducing soil conditions with IRIS as well as to improve oxide coating composition and tube/film development (e.g., coating thickness).

1 INTRODUCTION

Redox potentials can be measured in the field by determining voltage differences between platinum (Pt) probes and a reference electrode (Rabenhorst et al., 2009). An alternative for evaluating reduced soil conditions is the Indicator of Reduction in Soils (IRIS). IRIS tubes made out of polyvinylchloride (PVC) pipes or plastic films have been used with coatings of iron (Castenson & Rabenhorst, 2006; Jenkinson & Franzmeier, 2006; Liggett et al., 2020; Rabenhorst & Burch, 2006) or manganese oxide(s) (Dorau & Mansfeldt, 2015; Dorau et al., 2016; Rabenhorst, 2018; Rabenhorst & Persing, 2017) to observe reducing conditions in the field. Recently, Scott et al. (2021) found coating removal from clear tubes to be similar to PVC.

The oxide coating removal depends on soil saturation, anaerobic-microbial activity, and a sufficient source of nutrients to develop biochemically reducing conditions (Castenson & Rabenhorst, 2006). The IRIS method is destructive, and removed tubes are not reinserted after evaluation. IRIS tubes are typically field deployed in nests of five tubes for 28 d. For temporal analyses, a set of IRIS tubes or films are removed after 7 d and compared with a different set removed after 14 d, and so on (Dorau & Mansfeldt, 2015; Rabenhorst & Persing, 2017). Given the spatial variability of microbial communities, labile carbon, and nutrients, a comparison of different tubes/films even from close proximity can potentially result in coating removal variability not caused by the difference in IRIS tube deployment time. Weekly measurements also do not allow observation of reduction dynamics at higher temporal resolution.

Developing automated methods to measure coating removal from the tube or film will improve the efficacy of the IRIS approach. Scott et al. (2021) recently used a borehole...

Abbreviations: IRIS, Indicator of Reduction in Soils; PVC, polyvinylchloride.
camera to capture images in clear IRIS tubes, which required manual camera movement through the tube. Expanding on their findings, we present a fully automated method of hourly image acquisition and image processing for finer temporal resolution of IRIS. In addition, we compare these results with measurements of reduction (Eh-pH data). Therefore, the objective of this study was to evaluate the use of a rhizotron camera to capture iron and manganese oxide coating removal at high temporal resolution.

2 MATERIALS AND METHODS

Iron (Fe) oxide coating was manufactured according to the Quick (7 d) IRIS Tube Paint Recipe (Rabenhorst, 2016) and stored in a refrigerator. This recipe produces about 60–70% ferrihydrite and 30–40% goethite (Rabenhorst & Burch, 2006). The manganese (Mn) oxide coating was made followed the procedure by Händel et al. (2013) with modifications by Rabenhorst and Persing (2017).

Rhizosphere tubes (referred to as tubes) sourced from the manufacturer of the rhizosphere camera (CID Bio-Science) were cut to 47 cm in length (6.3 cm i.d.) and placed in a column. The outside of the tube was sanded, and each coating was applied in two coatings on two-quarter sections of the tube (Figure 1). A hot air gun was used to dry the coating to increase durability (Dorau & Mansfeldt, 2015). A target with six shades of gray and 12 colors was added to the top of the tube for image color correction (Buchaillot et al., 2019). A sandy loam textured soil sampled from a surface horizon from a coarse-loamy, mixed, semiactive, thermic Fluvaquent (54% sand, 27% silt, 19% clay, soil organic carbon 1.7%) was used to pack the column. The soil was air-dried, passed through a 2-mm sieve, and homogenized. Peat moss (0.05 g g⁻¹) was added as a carbon source to stimulate microbial activity.

One tube with a rhizosphere camera (CI-600, CID Bio-Science) was centered in a 20-L plastic bucket (column), and a 20-cm layer of soil was placed over 8 cm of washed sand for drainage. The soil was added in 2-cm increments and was gently tapped to reduce air pockets. A well (PVC, 3.8 cm i.d., bottom 25-cm slotted, covered with nylon membrane) was placed in the column. Two reference Ag-AgCl saturated KCl probes, and one Quad redox probe (Paleo Terra) were placed in the column, and redox potential was measured at 3-, 8-, 13-, and 18-cm depths. Four type K thermocouples (TC Direct) were added to the column at 10-cm depth. Data from thermocouples and redox probes were acquired with a CR-1000X data logger (Campbell). Water was added to the column through the well, and a water level of 3 cm below the surface was maintained throughout the study. Water level and pH were monitored regularly throughout the 28-d experiment.

The rhizosphere camera software was controlled by a Python (Python Software Foundation, 2020) script using the PyAutoGUI package (Sweigart, 2020), and hourly images were automatically recorded. The images were converted from RGB (red, green, blue) to the CIE L*a*b* color space (Russ, 2002). The L*, a*, and b* values were extracted from the 18 colors of the color target and regressed against the known color target L*a*b* values provided by the manufacturer. All pixels were corrected based on regression parameters for each channel (Buchaillot et al., 2019). The corrected images were then transferred back to the RGB color space and saved as uncompressed TIFF files. The corrected images were filtered with a median filter (5-by-5 pixels) and reduced in size to 500-by-561 pixels. Finally, the four areas representing the iron and manganese coatings were cropped from the reduced images and saved as individual files.

In comparison to IRIS tubes that use PVC and therefore have a white background or films that can be placed in front of any background color, the rhizosphere tubes are clear, and the background is the surrounding soil. The reduction is detected when a sufficient change in color parameters, including hue, saturation, or brightness, occurs. The Fe reduction was detected by converting the images to the CIE L*a*b* color space. Images were inspected to determine where Fe reduction had occurred. Oxidized sections had L*a*b* values of 18.5, 23, 12 based on the color of the coating, whereas reduced sections had values of 23, 13, 20 representing the color of the soil. The Euclidian distances of each pixel to both of these colors were calculated, and a pixel was considered to represent coating removal when the Euclidian distance was shorter to the reduced color than to the oxidized color. Mostly due to solubility differences, ferrihydrite was reduced more readily than the goethite component. Thus, image analysis reflects mostly the reduction of ferrihydrite. Manganese images were converted to the HSV (hue, saturation, value) color space (Russ, 2002), and reduced conditions were found when the saturation channel of the image was below 20%. For each window, a binary image was saved, and the reduction was assessed in 0.5-cm increments. Reducing conditions were present when a minimum of 30% reduction was measured for

Core Ideas

• Rhizosphere cameras can be used to observe Mn and Fe oxide coating removal.
• Mn and Fe oxide coating removal was assessed at a high temporal resolution.
• This technology can be used to improve real-time in-situ redox measurements.


RESULTS AND DISCUSSION

The average column temperature was 20.9 °C (+/−1.3 °C). The pH at the start of the experiment was 7.29 and quickly decreased to an average value of 6.40 (+/−0.13). Redox potential data indicate Mn reducing conditions were present after 1.2, 0.4, 0.4, and 0.3 d at 3-, 8-, 13-, and 18-cm depths, respectively (Figure 2a). Iron reducing conditions were not present in the first two depths during the 28-d experiment and after 4.8 and 4.1 d at 13- and 18-cm depths, respectively. The assumed concentrations of Mn²⁺ and Fe²⁺ for reduction half-reactions were 10⁻⁶ M (Essington, 2015).

Image analyses indicated 30% reduction and removal was reached after 4.9, 9.0, 9.5, and 13.4 d in the MnA, FeA, MnB, and FeB windows, respectively (Figure 2b). The maximum reduction rates (maximum slopes in Figure 2b) were 20, 16, 13, and 8% d⁻¹ for the MnA, MnB, FeA, and FeB windows, respectively. The Mn coating in window MnA was removed entirely, whereas the percentages of coating removed in windows FeA, FeB, and MnB were 85, 70, and 67%, respectively. An upper depletion depth, defined as the depth below which there is a significant change in coating removal (Jenkinson & Franzmeier, 2006), is shown after 28 d in Figure 1.

Based on thermodynamic reduction half-reaction equations and the redox potentials, Mn reduction occurs before Fe. However, FeA showed coating removal before MnA and MnB, although MnA surpassed FeA after about 4 d (Figure 2b). We attribute this early coating removal of FeA—before redox potential data indicated reducing conditions—to ferric iron in the coating that was detached shortly after saturation. It is of
note that this detachment was not detectable in FeB, which could possibly be attributed to slightly uneven coating application resulting in a thicker Fe-oxide coating on FeB. The pore water was stagnant during the experiment, and as Mn and Fe coating was reduced, Mn\(^{2+}\) and Fe\(^{2+}\) concentrations in solution increased, and likely altered dissolution processes as the experiment went on.

### 4 CONCLUSIONS

Our results and the findings of Scott et al. (2021) demonstrate that it is possible to observe redox conditions in situ using cameras and clear tubes. We used a rhizosphere camera to observe iron and manganese coating removal as an indicator of reducing conditions in a simulated wetland soil. The advantage of using a rhizosphere camera to monitor reducing conditions is the high temporal resolution and continuous data collection on the same tube. The high temporal resolution reduction curves can be used to evaluate complex saturation and temperature regimes, and therefore this technology could be used to improve protocols further for evaluating reducing soil conditions in the field based on IRIS tubes or films.
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