# Bromide tracer study to investigate depth of water uptake by switchgrass underneath vs. outside rainout shelters

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## INTRODUCTION

In 2021 we conducted a bromide tracer experiment at the GLBRC Bioenergy Land Experiment (formerly known as the Marginal Land Experiment) at Lux Arbor Reserve to investigate whether switchgrass taps into deeper soil water under rainout shelters as the surface soil water dries out. Tapping deeper water could explain how switchgrass survives weeks without rainfall under the shelters and during natural dry periods.

Bromide is useful as a conservative solute tracer because it is present at very low concentrations in soil solutions, is generally unreactive and remains in solution in soils, and can be readily measured using ion chromatography. Previous studies at KBS employing bromide as a tracer of soil water movement include Jin et al. (2008), Hess et al. (2018), and Hamilton (2020).

Various studies in the literature have shown that bromide is taken up by plants, but this is mostly described in relation to how plant uptake may interfere with its use as a hydrologic tracer in wetlands. A prior study of bromide as a tracer by Bowman et al. (1997) documented plant uptake and considered toxicity, but not in switchgrass.

Prior to the experiment with switchgrass under rainout shelters, we conducted a test for bromide uptake and toxicity in switchgrass to ensure that bromide was taken up into foliage in proportion to concentrations in the soil, and that the growth of the grass was not affected by high bromide in soil solutions. That study is summarized below.

## TEST FOR BROMIDE UPTAKE AND TOXICITY

Katherine Egeler, a GLBRC Undergraduate Research Assistant (URA) at KBS in 2021, conducted this work in reserve plot G607 in the Biofuel Cropping System Experiment at KBS, which was planted in switchgrass (variety Cave-in-Rock). One of two strips harvested the previous year was selected because grass growth there was not inhibited by a thatch layer. Four 28.5cm wide x 23 cm tall, open stainless-steel cylinders were hammered 4 cm into the ground across the site to create individual plots. Four tracer solution levels were made using sodium bromide and deionized (DI) water: 0, 100, 500, and 1000 mg/L Br<sup>-</sup>.

Plots were watered with 500 mL of their assigned solution triweekly on M/W/F between 14 June and 9 July. Plant heights were measured weekly on Mondays. On 12 July, switchgrass leaves were collected from each plot. Leaves outside plots were also collected as a reference. The experiment began during a dry period, then several heavy rains fell during the bromide addition.



The collected leaves were dried in an oven at 175°C for four days, then ground using a coffee grinder. Extracts were created by boiling 2.5 grams of dried and ground leaves from a single plot in 50 mL of DI water, in triplicate for each plot. After cooling and filtration, Br<sup>-</sup> in the extracts was analyzed using membrane suppression ion chromatography.

Results showed that Br- appeared in the foliage in approximately linear proportion to the concentrations in the added water, supporting its use as a tracer of soil water uptake (Fig. 1).



Fig. 1. Bromide concentrations in switchgrass foliage in relation to bromide concentrations in added water. Six samples were averaged for the control (reference) point (2 sets of triplicates) while nine samples (3 sets of triplicates) were averaged for all other data points.

Switchgrass heights did not diminish with increasing bromide concentrations, and significant growth was observed during the bromide addition period in all plots (Table 1). No visual evidence of toxicity was apparent when comparing the high bromide plots to the reference plot and to plants outside of the plots; all of them developed some yellowing over the course of the experiment.

Sample	Initial Height of Plot (6/14)	Final Height of Plot (7/28)	Final Height Near Plot (7/28)	Inside vs. Near Plot (7/28)	Growth
Reference	76 cm	136 cm	N/A	N/A	60 cm
DI Water	75.9 cm	138 cm	143 cm	-5 cm	62 cm
100 mg/L Br <sup>-</sup>	76.8 cm	123 cm	129 cm	-6 cm	46 cm
500 mg/L Br <sup>-</sup>	77.6 cm	134 cm	137 cm	-3 cm	56 cm
1000 mg/L Br <sup>-</sup>	74.4 cm	131 cm	134 cm	-3 cm	57 cm

Table 1. Mean plant heights at the start and end of the addition period.



Based on results of this study, we chose to add 100 mg/L Br<sup>-</sup> in the main experiment described below.

#### TEST FOR DEPTH OF SOIL WATER UPTAKE BY SWITCHGRASS

We used bromide as a tracer to determine if there were differences in depth of water uptake by switchgrass underneath vs. outside rainout shelters, testing the hypothesis that plants under rainout shelters will take up more bromide (and thus water) from deeper soil layers.

Bromide solutions (100 mg Br<sup>-</sup>L<sup>-1</sup> as NaBr) were injected over ~4 hrs by gravity drainage from PVC pipes between 20–22 July 2021. The pipes were sealed at the bottom and drilled with 1/8 inch holes to allow drainage from near the bottom. Three injection pipes were arranged in a triangle with sides of about 0.75–1 m. The deepest pipes were installed with the GeoProbe.

In four replicate switchgrass plots at the Lux Arbor Marginal Lands Experiment, the pipes were set to drain into either 0.1–0.2 m or 0.75–0.85 m depth ranges. Solutions were injected into these two depths both underneath and outside of the rainout shelters in each plot (Fig. 2). Each injection pipe received ~750 mL of the bromide solution. We verified that solutions had drained out of the pipes on the day after injection began.



Fig. 2. Schematic depiction of the experimental design.

Switchgrass foliage was sampled soon after (7–10 leaves per sampling), and then weekly for four weeks, from the area inside each triangle defined by the injection pipes.



Fig. 3. Rainfall in the KBS area during the deployment of rainout sheltersand the bromide addition experiment in 2021 compared to longterm data compiled by Grant Falvo.



Rainfall before and during the experiment in comparison to long-term means is shown in Fig. 3. The

growing season was unusual in that it began under very dry conditions, then quickly caught up to normal rainfall during the period of shelter deployment.

Soil water availability at 10- and 25-cm underneath and outside the rainout shelters is shown in Fig. 4. At 10 cm depth, soil water was evidently drawn down to the lower limit of plant extraction. Despite loss of sensor data at 25 cm underneath the shelter, soil water availability would certainly have been much lower than outside the shelter.

*Fig. 4 (right). Volumetric water contact measured by soil water sensors underneath and outside the shelters in 2021.* 

Analysis of foliage samples showed that bromide was taken up and remained in the leaves throughout the sampling period (Fig. 5). When the data are viewed separately by plot, it is apparent that there were no differences in bromide concentrations from switchgrass underneath compared to outside the rainout shelters (Fig. 6). Expressing the data as means for the four sampling dates leads to the same conclusion, which is also supported by ANOVA (Fig. 7).





Fig. 5. All measurements of bromide concentrations in switchgrass leaves.





Fig. 6. Bromide concentrations in switchgrass leaves over time, divided by depth and location either underneath the shelter ("In") or outside the shelter ("Out").



Fig. 7. Means and standard deviations for bromide concentrations in switchgrass leaves over the second through fourth sampling dates.

Although the rainfall that occurred during the experiment may have flushed some of the bromide from the root zone outside the rainout shelters (see below), the concentrations in foliage were not greatly different overall between outside versus underneath the rainout shelters (Fig. 7).



The amount of bromide in the foliage can be compared to the amount injected into the root zone. We added 225 mg Br to each triangular area (750 mL of 100 mg L<sup>-1</sup> times three pipes). If we assume that bromide spread over 1 m<sup>2</sup> and that the aboveground biomass of switchgrass was ~700 g m<sup>-2</sup>, the observed tissue concentrations of about 100-150  $\mu$ g g<sup>-1</sup> equate to 70–105 mg Br, which is 31–47% of the injected bromide.

We also took soil cores for bromide analysis to look at the vertical distribution of bromide and to verify that the deeper injections did not spread upward. The cores were taken on 24 Aug with the GeoProbe from injection sites outside the shelter, as well as in a nearby alley where no bromide had been added to check background concentrations. As in an earlier study of percolation (Hamilton 2020), the soil cores were subdivided and leached in tap water with low Br<sup>-</sup> concentrations (DI water was in short supply at the time). The leachate was filtered and analyzed for Br<sup>-</sup> by ion chromatography.

The soil core samples showed almost no bromide above background, with very low concentrations overall, close to the lower limit of measurement (Fig. 8). This method of leaching core segments in water should have worked because we readily measured bromide this way previously (Hamilton 2020). Possible explanations for the low bromide in the cores include: 1) the plume beneath each injection pipe was not wide and thus could have been missed by the coring; 2) most of the added bromide (and water) was taken up by the plants; 3) percolation of new rainfall had flushed remaining bromide downward; and/or 4) the new rainfall had diluted the added bromide to close to background concentrations.



*Fig. 8. Leachable Br<sup>-</sup> in soil core segments taken outside the rainout shelters at the end of the experiment (24 August 2021). The tap water used for leaching is shown as well.* 



#### CONCLUSIONS

The bromide addition was successful in tracing soil water uptake by switchgrass, and there was no evidence of toxicity or growth inhibition. The hypothesis that switchgrass underneath the rainout shelters would draw more water from the deeper depth was not supported, as there were no consistent differences in bromide concentrations between switchgrass underneath compared to outside the rainout shelters.

If this kind of experiment is to be conducted again in the future, suggestions include:

- 1) Conduct the addition earlier in the growing season;
- 2) Include deeper injections (1.5 or 2 m?) and perhaps use a higher Br concentration (500 mg L<sup>-1</sup>);
- 3) Consider a second injection if heavy rainfall occurs;
- 4) Measure growth rates over time.

Bromide tracing could also be designed to detect soil water uptake by plants underneath the shelters from outside of the shelter footprint by adding bromide near the edges and seeing how far into the shelter footprint bromide appears in the foliage.

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