# **2021 GLBRC Standard Sampling Report**

# **Biofuel Cropping System Experiment (BCSE)**

#### Soil

Soil was sampled from all the BCSE plots once in 2021. Some treatments were sampled more frequently alongside gas sampling (see BCSE Gas section below). Soil samples were taken December 2-6 in all blocks or replicates (R) 1-5 of all treatments (G) 1-10. Soil was sampled using <sup>3</sup>/<sub>4</sub>" diameter push corers. Three cores (0-25cm) were taken from each of the three sampling stations and all 9 cores composited for each plot. On December 8, soil was sampled from the BCSE subplots. Nine cores (0-25cm) were composited from each subplot. Soil was also sampled from rainout sheltered areas of G5R2-5 (see Rainfall Manipulation Experiment) on December 8. Four cores (0-25cm) were taken from each of those areas.

Inorganic nitrogen extractions and soil moisture determinations were done on subsamples from all soil sampled. Analysis of the extracts for nitrate and ammonium should be completed by Cathy McMinn during winter 2021-2022. All samples were air-dried, archived, and subsamples sent to the MSU Soil and Plant Nutrient Lab for standard analysis which includes pH, lime requirement, P, K, Ca, Mg, and estimated cation exchange capacity.

#### Gas

Greenhouse gases were measured from round static chambers installed in replicates 1-4 of G1-5 beginning May 6. Analysis of nitrous oxide, carbon dioxide and methane was completed instantaneously in the field using the Mobile Integrated Cavity Offset Spectroscopy (MICOS) system twelve times between April 23 and July 23. The MICOS was not working after July 23. Beginning August 6, gas samples were put into Labco exetainers and transported back to the lab for analysis. They were analyzed for nitrous oxide, carbon dioxide and methane by Kevin Kahmark and Cathy McMinn using the Agilent/Gerstel automated GC in Academic 330. This was done five more times between August and November. Soil samples (0-25cm depth) were collected from each plot as well. The soil was sampled simultaneously with most gas sampling and taken from 1-2m away from each static chamber. Soil was sampled sixteen times in 2021. Inorganic nitrogen extractions and soil moisture determinations were done on subsamples from all soil sampled. Analysis of the extracts for nitrate and ammonium should be completed by Cathy McMinn during winter 2021-2022.

The automated gas sampling trailer now measures  $N_2O$ ,  $CO_2$ , and NO from six chambers on G5R1 and six chambers on G1R1. Sampling started in April and stopped in December due to a power module issue on the ICOS unit. Incubations were continuous throughout the day and night. All data is now collected via web application. In early 2022, a methane gas analyzer will be added.

#### Water

From May through December, 188 water leachate samples in total were collected from the BCSE and Switchgrass Nitrogen Rate Experiment lysimeter sites. BCSE samples were analyzed for ions (F, Cl, Br, NO3-N, SO4, Na, K, Mg, Ca, and NH4-N). Other collection attempts were made where the lysimeters yielded no water due to dry weather conditions. Sampling and analysis was completed by Dave Weed, Hamilton lab.

## Plants

Plant biomass samples were taken from the BCSE plots at their peak to measure above-ground net primary production (ANPP) or just prior to agronomic operations. No species separations were completed on samples taken in 2021. Plots were sampled as follows: G3 cover crop on May 18 ahead of harvest; G8 understory on July 20-21 ahead of mowing; G4, 5, 7, 9, 10 ANPP on August 31-September 7; G2 and G3 ANPP on October 5-8; G1 ANPP on October 13 and G6 ANPP on October 18-20. Species composition and plant heights were measured with three transects per plot following the line-point intercept protocol. Transects were completed around the same time that each treatment was sampled for ANPP, except for G1 where no transect data was collected. On November 23, the residue remaining following machine corn stover collection was sampled from the ground in G1 to evaluate collection efficiency. Two ground traps were deployed in each G8 plot on July 26 to collect leaf litter as leaves fell from the trees. Litter from the traps was collected every two weeks until the trees were bare, and traps were pulled from the field on November 22. On December 15, to track poplar growth, ten trees were randomly chosen from each G8 plot and the main stem diameter measured at 15cm above the ground. All plant material from all sampling was dried at 60°C for at least 48 hours. The dried biomass will be weighed, ground and archived. Combustion analysis for carbon and nitrogen determination will be completed by Stacey VanderWulp.

Switchgrass stand frequency was determined in May with stand counts done in all replicates of G5 main plot only, no microplots.

Plant phenological development was tracked using cameras positioned on each plot in block 1. These cameras were programmed to take a picture about every 30 minutes between 5am and 9pm. A subset of this data is included in our database while the entire collection is saved with the PhenoCam Network (https://phenocam.sr.unh.edu/webcam/).

Cheyenne Lei, graduate student with Jiquan Chen, has albedo towers installed in G1, 2, 5, 6, 7, 9 and 10 of block 1 of BCSE. These towers have been functioning continuously since May 2018. Instruments on these towers measured solar radiation, near infrared radiation, albedo, air temperature, precipitation, soil moisture, wind speed, wind direction, barometric pressure, relative humidity and recorded images of vegetative growth. Personnel from the Chen lab also measured leaf chlorophyll concentrations periodically using a SPAD meter and instantaneous measurements of solar radiation for the calculation of albedo using a survey pole at BCSE blocks 1, 2 and 4 (as well as LTER MCSE). These measurements were completed biweekly. No instantaneous measurements were completed at GLBRC Scale-Up or MLE.

Around twelve drone flights were completed over the BCSE from early April to December 2021. Flights collected albedo data from each plot. All flights concluded with a 30 second to one-minute flight over a white tarp as reference. All flying was done by Kevin Kahmark. Data collection is on a raspberry pi computer developed by Sven Bohm.

Yield data was collected from all plots and subplots during mechanical harvest. A sub-sample from the machine harvest of these plots was saved. This biomass was dried and will be ground, archived, and analyzed for carbon and nitrogen.

## **BCSE Subplots and Microplot**

**Stover non-removal subplot (G1 R1-5):** This is a 15-foot-wide strip along the west edge of each G1 plot where corn stover is not collected. The corn stover in the rest of the plot is collected after harvest. Besides the soil samples collected in December (see BCSE soil), there was no other sampling done here. Yield data was collected from mechanical harvest.

**Unfertilized subplot (G2, 3, 5-7, 9 R1-5):** This is a 15-foot-wide strip along either the east or west edge of each G2, G3, G5, G6, G7 and G9 plot where no nitrogen fertilizer is applied while the rest of the plot received fertilizer. Besides the soil samples collected in December (see BCSE soil), there was no other sampling done here. Yield data was collected from mechanical harvest.

**Fertilized subplot (G10 R1-5):** This is a 15-foot-wide strip along either the east or west edge of each G10 plot where nitrogen fertilizer is applied while the remainder of the plot receives none. Besides the soil samples collected in December (see BCSE soil), there was no other sampling done here. Yield data was collected from mechanical harvest.

**Rainfall Manipulation Experiment (G5 R2-5):** Beginning in 2018, rainout shelters were installed in G5R2-5 during the growing season. The shelters are 12' long x 12' wide. Initially the shelters excluded about 60% of rainfall. In April 2019, the exclusion was increased to nearly 100% with full roof panels installed. In 2021, shelters were put in place on June 11 and removed for the season on September 14. The area under the rainout shelter and a comparable unsheltered area nearby were harvested by hand just prior to machine harvest in these plots. Soil samples were collected from the sheltered area in December (see BCSE soil).

# Scale-up Experiment

#### Soil

Soil was sampled on November 18 in all the GLBRC scale-up fields at Lux Arbor and Marshall Farms by Thelen and Robertson lab personnel. Soil was sampled using <sup>3</sup>/<sub>4</sub>" diameter push corers. Ten cores were taken to a depth of 25 cm at each of the 10 stations in each plot. Inorganic nitrogen extractions and soil moisture determinations were done on subsamples from all soil sampled. Analysis of the extracts for nitrate and ammonium will be completed by Cathy McMinn during winter 2021-2022.

These soil samples were air-dried and archived. Subsamples from all were sent to the MSU Soil and Plant Nutrient Lab for standard analysis.

# Gas

Gas was not sampled in the Scale-up plots in 2021. Each of the seven fields has a  $CO_2$  flux tower that measures the flux of carbon dioxide into and out of the canopy/soil and allows for development of a complete carbon budget based on rates of carbon sequestration and loss from these different systems.

# Plants

Plant biomass samples for ANPP were not taken from any scale-up plots in 2021. Transects through the switchgrass, prairie and reference fields were used, following the same line-point intercept

protocol as on the BCSE, to collect data on species composition and plant height. This data was collected September 21-24.

Plant phenological development was tracked using a camera positioned in the Marshall corn field (M1). As on the BCSE, this camera is part of the PhenoCam Network.

The Basso Lab used a DJI M100 and DJI M600 Pro to fly the GLBRC Scale-Up fields from May to November 2021. Approximately every 2 weeks the corn, native prairie and switchgrass fields were flown (L1, L2, L3, M1, M2, M3) as well as the GLBRC BCSE and Marginal Land Experiment at Lux Arbor. The UAVs flew at 122m (400ft) and were equipped with a visual camera and either Micasense RedEdge 3 or Micasense Altum. Over the course of the season at least 6 NDRE, NDVI, RGB and Thermal images per field were collected.

At the time of this report (January 2022), yield data had been collected from all harvested sites (L1, L3, M1, M2) when mechanically harvested. Switchgrass sites (L2, M3) have not been harvested.

# Marginal Land Experiment (MLE)

## Soil

Soil samples were taken from all MLE sites in the fall of 2021. They were sampled in this order between October 5 and November 1: WC (Hancock), MC (Lake City), MS (Lux Arbor), MN (Escanaba) and WN (Rhinelander). Each half of the split plots (fertilized and unfertilized) was sampled separately with six 0-25cm cores/split plot composited. Beginning in 2018, as on the BCSE, rainout shelters were installed in each G5 plot at all the MLE sites during the growing season. The shelters are 12' long x 12' wide, exclude nearly 100% of rainfall and are installed on the fertilized split plot. Shelter were put in place in mid-June and removed for the season in late August or early September. Soil was sampled in the sheltered areas on the same day as the split plots. Four cores (0-25cm) were taken from each sheltered area. Inorganic nitrogen extractions and soil moisture determinations were done on subsamples from all soil sampled. Analysis of the extracts for nitrate and ammonium should be completed by Cathy McMinn during winter 2021-2022. All samples were air-dried, archived, and subsamples sent to the MSU Soil and Plant Nutrient Lab for standard analysis. This analysis is done every three years.

# Plants

Plant biomass samples were taken from all MLE sites for ANPP in late August and September. Samples were not sorted to species. Species composition and plant height data was collected in all plots, at all sites in September using a line-point intercept protocol. Species were identified and height measured along two transects in both the fertilized and unfertilized split plots.

Yield data was collected from all of the mechanically harvested plots (G5-7, 9, 10) separately in the fertilized and unfertilized split plots. G11 was not harvested, so hand samples were taken there to look at production. Additionally, the area under the rainout shelter and a comparable unsheltered area nearby were harvested by hand. Subsamples of all harvested materials (with the exception of the G5 unsheltered comparison area) were saved and will be ground, cataloged and stored on MSU main campus. The majority of this work was done by personnel from Kurt Thelen's lab, led by Todd Martin. Species composition transects were completed by a four-person crew from the Robertson lab. No drone flights were performed in 2021.

## Weather

A weather station is installed at each MLE site. Each weather station collects air temperature, precipitation, solar radiation, relative humidity, soil moisture and temperature at 10cm and 25cm depths, and wind speed. Two replicates of G5 at each site have loggers to measure soil moisture and temperature at 10cm and 25cm depth within the shelter footprint and in a nearby ambient subplot. Water profilers installed in switchgrass, poplar, and native prairie plots allow researchers to study the dynamics of water and water use efficiency in MLE soils.

# Switchgrass Nitrogen Rate Experiment (formerly Nitrogen/Harvest)

## Soil

Soil was not sampled in 2021. Soil is sampled every 3 years and is due next in 2022.

## Water

Soil water samplers were used to collect water from the soil in blocks 2-4. Collections were made in May-December. Leachate samples were analyzed for the same ions as on the BCSE, as well as for non-purgeable organic carbon (NPOC) and total dissolved nitrogen (TDN). They may be analyzed for total dissolved phosphorus (TDP). All sampling and analysis was completed by Dave Weed.

## Plants

Switchgrass stand frequency was determined in May with stand counts done in all plots.

Yield data was collected from all plots during mechanical harvest. A sub-sample from the machine harvest of these plots was saved. This biomass is dried and will be ground, archived, and analyzed for carbon and nitrogen.

# **Switchgrass Variety Experiment**

**Project 1:** Determination of differences in associative nitrogen fixation rates between four highyielding switchgrass varieties, Cave-in-Rock, Southlow (upland ecotype), Alamo, and Kanlow (lowland ecotype).

Biomass and soil samples collected in 2020 were analyzed at the Ostrom Lab for <sup>15</sup>N isotopic analysis. Gas samples were analyzed for  $CO_2$  and  $SF_6$  at the Robertson Lab, and later at the Ostrom Lab for <sup>15</sup>N isotopic analysis. Soil inorganic N were analyzed at the Robertson Lab.

Complete metagenome analysis of switchgrass soil and roots was done at the JGI, as a second alternative. Our samples had a very low <sup>15</sup>N signature, and JGI could not use them for a <sup>15</sup>N-DNA SIP determination.

**Project 2:** Study of phenological and chemical composition differences among all switchgrass varieties

Root biomass samples collected by Samantha Mosier only from the fertilized subplots during late fall 2020 were washed and processed in May by Zahraa Al-Tameemi. The total number of deep root cores taken was 36, only from the interstitial portion (i.e., 10cm from the crown). Each core was split into four depths (0-10, 10-25, 25-50, and 50-100cm), and soil and roots were separated. Soil

samples were analyzed for carbon and nitrogen content at the Robertson Lab and isotopic analysis at the Ostrom Lab. Roots were washed using an elutriator on MSU main campus, pulverized, and packed for carbon and nitrogen content at the Robertson Lab during late summer 2021. A soil subsample from each soil depth was archived for future use.

Written by Stacey VanderWulp with contributions from Sven Bohm, Carolina Cordova, Kevin Kahmark, Cheyenne Lei, Ruben Ulbrich and Dave Weed

Experiment	sample type	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
GLBRC BCSE	plants														
GLBRC BCSE	surface soil														
GLBRC BCSE	deep core soil														
GLBRC Scale-up	plants														
GLBRC Scale-up	surface soil														
GLBRC Scale-up	microplot surface soil														
GLBRC Scale-up	deep core soil														
GLBRC Scale-up	microplot deep core soil														
GLBRC MLE	plants											campus	campus	campus	campus
GLBRC MLE	surface soil														
GLBRC MLE	deep core soil														
GLBRC Switchgrass Gradient	plants														
GLBRC Switchgrass Gradient	deep core soil														

## **Archived Material**

## **Agronomic Soil Analysis**

Experiment	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
LTER MCSE														
LTER Resource Gradient														
GLBRC BCSE main												G1-3	G1-3	
GLBRC BCSE micro												G1-3	G1-3	
GLBRC BCSE deep core														
GLBRC Scale-up												L1, M1	L1, M1	
GLBRC MLE														
GLBRC Switchgrass Gradient														