# **2020 GLBRC Standard Sampling Report**

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

#### Soil

Soil was sampled from all the BCSE plots once in 2020. Some treatments were sampled more frequently alongside gas sampling (see BCSE Gas section below). Samples were taken December 1 in all blocks or replicates (R) 1-5 of all treatments (G) 1-10. Soil was sampled using  $\frac{3}{4}$ " 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 2, 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 2. Four cores (0-25cm) were taken from each 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 2020-2021. All samples will be air-dried and archived. Subsamples from the annual treatments only (G1-3) will be sent to the MSU Soil and Plant Nutrient Lab for standard analysis which includes pH, lime requirement, P, K, Ca, and Mg in January 2021.

#### Gas

Greenhouse gases were analyzed in the field from replicates 1-4 of G1-3 and 5 using the Mobile Integrated Cavity Offset Spectroscopy (MICOS) system. Analysis took place twelve times between April 28 and December 8, 2020. Soil samples (0-25cm depth) were collected from each plot as well. The soil was sampled simultaneously with gas sampling and 1-2m from each static chamber. 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 2020-2021.

The automated gas sampling trailer was moved to the alleyway just east of G5R1. The trailer has electric and fiber which was run from the old position near G10R1. After troubleshooting in the summer months,  $N_2O$  and  $CO_2$  data was collected from six chambers located in G5R1 and five chambers located in G1R1. Incubations were continuous throughout the day and night. A minimum detection limit study run from October thru early December utilized the same chambers and trailer system. All data is now collected via web application. In early December, the trailer greenhouse gas system was shutdown. Work will be done toward adding NOx measurements over the winter.

#### Water

From May through November 2020, 109 water leachate samples were collected from the BCSE and Switchgrass Nitrogen Rate Experiment lysimeter sites from soil water samplers buried in the plots. These were analyzed for anions, cations, and NH<sub>4</sub>-N via ion chromatography. Several collection attempts were made where the lysimeters simply yielded no water due to dry weather conditions over the summer. Sampling and analysis was completed by Dave Weed, Hamilton lab.

GIS points for all BCSE lysimeters were also added and/or verified in the spring with field work by Hsun-Yi Hsieh.

#### 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 2020. Plots were sampled as follows: G3 cover crop on June 1 ahead of harvest; G8 on June 1 and July 24 ahead of mowing each time; G9 ANPP on August 12; G5, 7, 10 ANPP on August 19-27; G2 and G3 ANPP on September 16-18; G1 ANPP on September 24 and G6 ANPP on October 12-14. 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 20, 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 August 10 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 23. Since the trees were small and herbaceous growth around the trees extensive, ground traps may not have accurately estimated the litter fall. Poplar leaf litter was collected from the ground near each station in each plot on November 23 to compare to the litter trap biomass. On December 3, 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/).

In addition, 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. Instruments on these towers measured radiation, temperature, precipitation, soil moisture 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 radiation for the calculation of albedo using a survey pole at BCSE blocks 1, 2 and 4.

Around twelve drone flights were completed over the BCSE from early April to December 4, 2020. Flights collected albedo data from each plot. All flights included a 30 second to one-minute flight over a white tarp as reference. All flying was done by Kevin Kahmark.

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. 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. 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 19 in all the GLBRC scale-up fields at Lux Arbor and Marshall Farms by Thelen 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 by the Robertson lab. Analysis of the extracts for nitrate and ammonium will be completed by Cathy McMinn during winter 2020-2021.

These soil samples will be air-dried and archived. Subsamples from the annual treatments only (L1, M1) will be sent to the MSU Soil and Plant Nutrient Lab for standard analysis which includes pH, lime requirement, P, K, Ca, and Mg in January 2021.

#### Gas

Gas was not sampled in the Scale-up plots in 2020. Each of the seven fields has a  $CO_2$  flux tower that measures the flux of  $CO_2$  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 2020. 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 2-3. The scale-up prairie fields were not fertilized in 2020, so no microplot plant samples were taken.

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 2020. 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 the Marginal Land Experiment. 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.

Yield data was collected from all harvested sites (L1-3, M1-3) when mechanically harvested.

#### Marginal Land Experiment (MLE)

#### Soil

Soil samples were taken from all MLE sites in the fall of 2020. They were sampled in this order between October 6 and October 29: WN (Rhinelander), WC (Hancock), MN (Escanaba), MC (Lake City) and MS (Lux Arbor). 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. The shelters are 12' long x 12' wide, exclude nearly 100% of rainfall and are installed on the fertilized split plot. 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 will be completed by Cathy McMinn during winter 2020-2021. All samples will be air-dried and archived.

## 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 G5 and G8 plots, at all sites in early September using a line-point intercept protocol. Species were identified and height measured along two transects in both the fertilized and unfertilized split plots. Data could not be collected in all treatments due to personnel availability related to the COVID-19 pandemic\*.

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 two-person crew from the Robertson lab.

Approval was received from all site managers to fly drones as necessary. Due to COVID-19, flights were only occasionally completed for visual imagery. All flying was done by Kevin Kahmark

#### 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 10 and 25cm depths, and wind speed. Two replicates of G5 at each site have loggers to measure soil moisture and temperature at 10 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 2020. Soil is sampled in the fall of odd-numbered years only.

## Water

Soil water samplers were used to collect water from the soil in blocks 2-4. Collections were made in May-October 2020. Like the BCSE water samples, these were analyzed for anions, cations, and NH4-N via ion chromatography. In addition, these were also processed and analyzed for non-purgeable organic carbon (NPOC) and total dissolved nitrogen (TDN), and will 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).

Two whole-plants of each variety from plots in blocks 1-3 were dug up (0-30 cm deep), potted, and transported to the greenhouse. Isotopic methods ( $^{15}N_2$  tracer gas) were used to determine the amount of associative nitrogen fixation of these plants. Biomass and soil samples were sent to the Ostrom Lab for isotopic analysis, gas samples were analyzed for CO<sub>2</sub> and SF<sub>6</sub> at the Robertson Lab and later at the Ostrom Lab for <sup>15</sup>N isotopic analysis. Soil inorganic N analysis will be done at the Robertson Lab.

DNA was extracted for <sup>15</sup>N-DNA SIP analysis at the JGI with the help of Evans lab personnel and should be submitted by early January 2021.

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

From May 31 through October 13, aboveground switchgrass biomass samples were collected from the unfertilized subplot only from 9 varieties (except EG1101, EG1102, EG2101). Sampling was done approximately every two weeks or more, for a total of eight harvests. These samples were sorted according to their phenological stage, oven-dried, and analyzed carbon and nitrogen content at the Robertson Lab. Additionally, LAI measurements were taken. LAI measurements were taken from the same plots manually (same week as biomass harvest) with a ceptometer. The Basso lab flew a drone over the experiment about every two weeks starting at peak until harvest, collecting RGB, NDVI, and NDRE imagery. Additional root biomass samples were collected by Samantha Mosier with a Geoprobe from the fertilized portion of each plot right after harvest (early November). 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 4 depths (0-10, 10-25, 25-50, and 50-100 cm), and soil and roots were separated. Soil will be analyzed for carbon and nitrogen content at the Robertson Lab and for isotopic analysis at the Ostrom Lab. Roots will be washed using an elutriator on MSU main campus in spring 2021. A soil subsample from each soil depth will be archived for future use.

Thelen lab personnel recorded switchgrass plant density and harvest yields from the fertilized subplots in the Variety Experiment.

\*The COVID-19 pandemic greatly altered the way most sampling and sample processing could be completed in 2020. The start of the sampling season was delayed from early April to late April/early May 2020 due to pandemic-related restrictions. However, most standard sampling was able to be completed.

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

## **Archived Material**

Experiment	sample type	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
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
GLBRC MLE	surface soil													
GLBRC MLE	deep core soil													
GLBRC Switchgrass Gradient	plants													
GLBRC Switchgrass Gradient	deep core soil													

## Agronomic Soil Analysis

Experiment	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
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													