2022. January 10. NE-1938 Multistate Meeting Agenda (DRAFT) 9 am eastern time

1) Review of what we have said we would do in the proposal.

   At the end of the agenda is the methods cut from the proposal. Please review and comment on anything that needs to be changed or included.

2) Discussion of timing of each method including time of year, length of deployment, how many deployments, ….

3) Update from each participant… Do you have a site picked out? Is it instrumented to measure hydrology at each transect point? What sampling and analysis have you completed or need to do?….

   Marty
   Mark
   Jim
   John
   Patrick
   Bruce
   Karen
   Colby
   Judy

4) Methodology to share?

5) Tea and litter bag results.

6) Discussion on potential publications.

7) Additional discussion.

Field and Laboratory Methods Multistate Project NE-1938

Plot layout

Within each site, **nine research plots** have been identified **along three transects** as illustrated in Figure 3. Each of the transects extends radially outwards from the center of the vernal pool (zone 1) through zone 2 and into the upland. Along each transect, a single plot was centrally located within each of the hydrological zones. Locations of the transects were randomized based upon compass orientation.

Elevations along each transect will be measured using appropriate tools such as a level or total station. Microtopographic differences will be documented by recording elevations at 1 meter intervals along the transects.

Hydrology

The depth of ponded water or the depth to the water table (below the surface) will continue to be recorded at each site. Depth of ponded water is measured using a staff gauge. Monitoring ports consisting of a well screen installed to a depth of 100 cm **have been placed at each plot and water tables will continue to be measured periodically** (Figure 3).

Along a single transect at each site, water table recording devices have been installed and programmed to record water table levels daily. The detailed (daily) data set from the recording devices will be extended to the other transects based on the periodic observations in the monitoring ports.

Soil Morphology

In the vicinity o**f each plot**, a soil profile description has been made to a depth of 1 to 2 m according to standard protocols (Schoeneberger et al., 2012). Samples collected from each horizon have been stored for laboratory analysis. Morphological descriptions will be compared with approved field indicators of hydric soils to determine whether there is any need for additional hydric soil indicators for use in depressional wetlands (USDA-NRCS, 2017).

Vegetation Analysis

Plant communities **in each of the three zones** will be assessed by methods outlined in the 1987 USACOE Wetland Delineation Manual (Environmental Laboratory, 1987) and the appropriate regional supplement (USACOE, 2010, USACOE, 2012a, USACOE, 2012b).

Weather and Climate Data

In order to generalize and extend hydrological observations from the period of this study to the broader context, weather data will be obtained **from the nearest weather station** that maintains a long term (30+ years) record of daily precipitation and air temperatures. Daily records of

precipitation and of minimum and maximum temperatures will be collected for the period of this study and will also be obtained for a minimum of the previous 30 years.

NOTHING ABOUT SOIL TEMPERATURES

Quantification of Carbon and Nitrogen Stocks

Carbon and nitrogen stocks will be determined at plots **along each transect** (Vasilas et al., 2013). A soil core will be collected from the upper 50 cm in a way that permits simultaneous calculation of horizon thickness and soil bulk density. While most approaches to calculating carbon stocks generate independent errors associated with determining bulk density and measuring horizon thickness, this approach decreases sampling error by combining these two components. Within each plot, a section of aluminum tubing (sharpened on the leading edge) (60 cm long and 5 cm diameter) will be driven 50 cm into the soil. The tube will then be excavated and capped. Upon return to the lab, cores will be frozen to assist in extrusion (alternatively, cores will be opened with sheet metal shears). Once opened, the cores will be divided into vertical sections based on observed soil horizons, and the thickness of each horizon will be carefully measured. All soil material from each horizon will then be homogenized and weighed. The bulk density of each horizon will then be calculated as the weight of the horizon divided by the horizon volume (calculated from the thickness of horizon multiplied by the cross-sectional area of the tube). The soil organic C percentage will be determined using a homogenized subsample of each horizon**. Total carbon will be determined in duplicate by dry combustion** (Nelson and Sommers, 1996) using a high temperature CNH Analyzer with an IR detector. These data will be used in conjunction with measurements of horizon thickness and bulk density to calculate the total C stocks in the soil to a depth of 50 cm.

Soil Inorganic Nitrogen

Soil nitrate and ammonium will be measured on samples **collected from each plot i**n the middle to end of the aerobic phase (August -September**). Four to six replicate cores will be collected** using a 30 cm push probe, and will be aggregated into a single composite homogenized sample for analysis. Samples will be analyzed using the HACH 8171 method, similar to that used by Spokas et al. (2010). These data will be used to provide insight into OM decomposition data.

Soil Redox Assessment

IRIS (indication of reduction in soil) films will be used to assess the reducing soil conditions **within each plot (**Rabenhorst, 2008, 2018; Rabenhorst and Burch, 2006; Rabenhorst et al., 2008; Vasilas et al., 2013). Both traditional Fe-coated and newly developed Mn-coated devices will be utilized (Rabenhorst and Persing, 2017; Rabenhorst and Post, 2018). Five replicate IRIS films of each type (Fe and Mn) will be deployed at each plot to a depth of 50 cm. IRIS films will be deployed for one month periods in the Spring when water tables are expected to be high. Deployment dates at the various sites will be scheduled to follow local weather conditions and will target the beginning of the growing season as determined by US Army Corps of Engineers guidance (USACOE, 2010; USACOE, 2012; USACOE, 2012). The extent of reduction on IRIS films will be assessed using digital image analysis (Rabenhorst, 2012). Mn- coated IRIS devices

may also be deployed prior to the normal growing season in an attempt to document biogeochemical conditions during colder, but saturated, periods.

Carbon Inputs

Replicate measurements of litterfall will be **made within each plot along the central transect at each site**. Leaf litter deposition will be measured between the months of December to August, and September to November using plastic devices to collect litter. These selected sampling periods were chosen to align with the period of major leaf fall in the forested wetlands of the eastern United States (September to November) (Ricker et al., 2014). **Three randomly placed C inputs as deadfall will be determined in each plot.** Deadfall will be considered as any woody debris greater than 1 cm in diameter. Existing deadfall and leaf litter will be cleared from the forest floor upon delineation of each plot. Flags, placed at the corners of each plot, will be left in place throughout the study. Each year deadfall that has accumulated in the plots will be collected. Leaves and deadfall will be dried to a constant weight at 60o C, in order to determine carbon contributions at the various hydrologic zones throughout the sites. Carbon inputs will be estimated assuming a concentration of 0.50 g C g-1 of leaf litter (Davis et al., 2010).

Organic Matter Decomposition

During the previous study northern white birch (*Betula papyrifera*) sticks (9.5 mm dowels, 30 cm long) were inserted into the soil and then extracting following one year of burial in order to assess the relative rates of organic matter decomposition. This approach was based upon other studies showing that wooden sticks can be used to indicate organic matter decomposition rates in several different types of settings (Baker et al., 2001; Gulis et al., 2004; Ostertag et al., 2008). To complement these data already collected, metrics of leaf litter and woody deadfall decomposition will be examined at each study plot**. Five replicate nylon mesh leaf-litter bags** will be filled with dried, pre-weighed leaves of species native **to each site** (such as White Oak (*Quercus alba),* Black Oak (*Quercus velutina),* or Red Maple (*Acer rubrum)*) and secured at the soil surface in each zone. After retrieval, the bags will be rinsed and dried to a constant weight (60oC) and mass loss will be calculated by comparing with initial weights. **Two sets of five replicate pre-weighed northern white** birch (*Betula papyrifera*) dowels (15 cm in length and either 1 cm or 2 cm in diameter) will be secured at the soil surface **at each research plot** at the same time as the leaf litter bags. **The bags and dowel rods will be left on the soil surface for a year (May to May),** dried in the oven, and the difference in weight before and after will be calculated as a measure of degree of decomposition. **We will repeat this measure of decomposition for three years** to document and understand temporal variability. We will use the number of growing degree days for each year, and among the study sites, to identify any difference in energy in the soil system between the sites and years and relate those differences to decomposition rates. Growing degree days are an index of solar energy a given site receives each day and is based on air temperature. It is strongly correlated with soil heat which in turn is an index related to soil microbial activity (Douglas and Rickman, 1992). We will compare the decomposition rates to organic inputs from leaf and woody deadfall studies to understand net carbon fluxes from the primary sources of SOC to each system and how temperature, inundation, and soil surface saturation control carbon fluxes in wetlands.

Greenhouse Gas Flux

Flux rates of major greenhouse gasses **will be measured at each research plot on each of the three transects** at each site, using a closed chamber approach, thus providing data for each of the three hydrologic zones. Two cylindrical plastic chambers (16 cm in height, 20 cm in diameter) will be placed at each site and pushed approximately 2.5 cm into the soil. Using a 20 ml gas-tight syringe, an initial gas sample will be collected after securing the chamber's lid, which contained a rubber septum to allow for sampling, followed by samples taken 15 and 30 minutes after the initial sample. The headspace of the chamber will be mixed prior to sampling. After sample collection, syringe contents are immediately transferred into a 15 ml evacuated tube (Amador and Azivinis, 2013). In each sample, CO2, CH4, and N2O will be determined.

Sampling date will be based on GDD in the spring, summer, and fall. In the field, internal chamber temperatures are measured when each gas sample is collected and averaged in order to obtain the average chamber temperature during the sampling period. Soil temperature and moisture content at a depth of 10 cm, and specific chamber volume (m3) will be recorded at each sample period (Ricker et al., 2014; Waggoner, 2016).

Gas concentrations (CO2, CH4, and N2O) will be measured with a Shimadzu gas chromatograph and recorded in units of ppm (Altor and Mitsch, 2008). Concentrations are plotted against time and fitted with a linear regression in order to calculate the CO2 flux rates. The mass of each gas present in the sampling chamber, or n (mol), is calculated using the Ideal Gas Law, n=PV/RT, where *n*=mol CO2 per mol air, R=universal gas constant (0.0821 L atm/mol K), T= chamber internal temperature (K), P=atmospheric pressure (atm), and V= chamber volume (L). The rate of GHG production per unit area is calculated using the slope of the best- fit line, cross-sectional area of the chamber, and volume of air in the chamber (Waggoner, 2016).