Review Comments	Response(s) to Comments
Reviewer 1 Comments	
More work could be done on identifying/verifying the source of new black carbon sites and tying them to specific anthropogenic activities.	Our current goal is to quantify black carbon. We hope to elucidate the source of black carbon in follow-up studies. We will be exploring site history where historic aerial photos are available. A new methods section titled "Site History" was added to the project outline.
Make sure all your members actually sign up for the project, so far only Dr Turk and Dr Rabenhorst are listed. Which is likely below the minimum threshold.	We are working on getting everyone signed up.
Reviewer 2 Comments	
In the project overview/abstract is says "Our goal is to study depressional wetlands across 11 different states with varying climates from Northeast Region across the Midwest and into the Mountain West in order to assess the impacts of temperature, hydrology, and soil properties on soil carbon storage." This statement really limits the project in that later on under the organization/governance section it is stated "Additional participants with expertise in pedology, mineralogy, soil ecology, hydrology, soil-environmental science, and other related disciplines will be invited to join the project." Perhaps amend the initial statement to indicate the study will include the Northeast Region as well as additional participants from across the United States. One major missing area is the southern USA, which has many famous examples of depressional wetlands (cypress domes, Carolina bays, etc.) that are in the thermic/hyperthermic soil temperature regimes. As stated in the proposal, it seems like this project will be held in focus to the initial 11 states.	We have reframed the project to emphasize the goal of determining the range of soil C storage and C fluxes across the 11 study sites, and de-emphasized quantifying temperature as the main factor under investigation. This is primarily to address comments from Reviewer 6. It also leaves the door open to additional participants from other regions, which would expand the potential ranges of characteristics that we will document. Unfortunately, multistate projects can be a bit limited in what types of sites (in this case, type of depressional wetland) based on what individuals sign on to the project from different states. We would warmly welcome additional participants - especially from the famous examples mentioned by Reviewer 2.
Under the section of Organic Matter Decomposition: The methods as outlined are generally	We will adopt the ashing method for the sites that are still conducting or are repeating litter bag studies. Our methods have been revised

acceptable, however the participants will need to determine mean ash weight of litter samples initially and compare to each collection due to seasonal ponding/flooding that can introduce sediment weight to the sample bags. In methods it mentions rinsing bags upon collection, this can lead to loss of litter from bags. Careful hand sorting and ash weights (mean before versus after field incubation) are typically used to correct for possible mineral/organic additions to the litter bags. Handling loss can also occur during transport (unless samples are fully sealed in containers), triplicate mesh bags can be weighed before and after transport to field locations to estimate this minor source of loss (Baker et al., 2001 cited in the references outlines this procedure). In areas with large destructive fauna (bears, hogs, bison) exclosures may need to be constructed to obtain viable data from the decay bags. Five sets of bags can easily be destroyed by large animals before 1 year, if not protected.	accordingly.
Reviewer 3 Comments	
My concerns with the proposed work include (1) the addition of the four sites in the Midwest (1 site), Plains (2) and western mountains (1) are not sufficient to really develop a climate gradient, (2) Methods to measure decomposition and greenhouse greenhouse gases are inadequate to characterize fluxes. For example, red maple and oak will be used to measure decomposition across all sites? Seasonal (3 seasons) measurements of greenhouse gases? It is not clear whether these will be done every year for 5 years or not. (3) Understanding the recent and long-term history of the sites is essential for C storage and fluxes. The proposal does not address this at all. I would be more positive about this proposal if it included some products (publications, etc) from the 5 year study in the NE	Regarding concerns about the climate gradient, please see our response to comments from reviewer 6. Greenhouse gas sampling will be performed for at least one field season with "normal rainfall". We now note this in the project outline. We will also add the ashing method in response to comments from Reviewer 2. The recent history of most of these sites is known. Long-term history will be summarized to the extent possible by the time of publication based on aerial imagery. We will also identify the ESD state based on current vegetation to understand previous land use, assuming ESDs are available for each wetland in the respective 11 MLRAs. Products resulting from previous funding cycles are now noted in the project outline. See the new section titled "Summary of Outputs and Impacts from Previous Funding Cycles"

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Overall, it seems that the proposal is an easy extension of the work done in the NE over the past 5 years. While the proposed black carbon measurements are new, I do not expect black C to account for a significant amount of C stored. It might help though with fire history (see comments below). It would make more sense to add more climatically diverse sites, focus on C storage (and drop the decomposition and greenhouse gas work) and factors (hydrology, climate variables - temperature and precipitation, plant productivity) that affect it, and a thorough understanding of the history (agricultural, grazing, forestry, fire and associated drainage activities and ditches) of the sites.	We will try to include site history regarding fire, coal mining, etc. in the final report and subsequent publication(s). Unfortunately, site selection for multistate projects is limited by the recruitment of additional collaborators from states not currently represented on the project. Our current data reveals a significant amount of black carbon in the West Virginia and Pennsylvania sites. We also expect significant amounts of black carbon in the Nebraska and Kansas sites due to prairie fires, and in the case of the Kansas site documented prescribed fires approximately every 2 to 3 years.
<u>Reviewer 4 Comments:</u>	
This is an exciting project that will have considerable impacts for understanding of wetland soil carbon that can support environmental sustainability. For the soil organic carbon measurements consider using the equivalent soil mass calculation (Ellert & Bettany, 1995) to appropriately compare between wetlands were bulk density is likely to differ. Consider reported soil carbon to 0-30 cm as well to align with historical studies of other depressional wetlands in North America, although the 0-50 cm sampling depth is preferrable and important. Consider methods for how the soil organic carbon or nitrogen at sampling points within the wetland will be used to calculate soil carbon stocks for the entire wetlands (ie. measure the area for each of the three zones within the wetland or alternative methods?). For the greenhouse gas measurements the frequency of the sampling is not indicated (ie. every X days or weeks). It is important to consider temporal and spatial hotspots of greenhouse gas emissions, especially for N2O.	The Ellert and Bettany (1995) manuscript was added to our method. We are analyzing carbon by genetic horizon to a depth of at least 50 cm. Distinct horizons will be described and characterized discreetly. Other researchers are welcome to use our data to quantify C down to 30 cm, if desired. Calculating areas represented by the three zones is a good idea. We will estimate areas represented within each of our sites based on Goldman et al. (2020). This was added to the outline. Gas sampling will be done on at least a quarterly basis, weather and site access permitting. Sampling will be performed while the soil in zone two is at or near saturation during the wet season and sampled in the week following a rain event during the dry season. This was clarified in the outline.
<u>Reviewer 5 Comments:</u>	
Sites will vary in hydrology as temperature and rainfall amounts gradually decrease from east to west. This may make comparisons among sites more difficult.	Following a reframing of the project to address comments from Reviewer 6, the project now focuses on capturing the range of properties exhibited across the sampled sites.

	Because these are all depressional, closed-basin wetlands the hydrology should be similar across all sites. The biggest differences in hydrology will occur between zones within a given site. The rainfall amounts do decrease from east to west. However, this has more of an impact on the soil moisture of upland areas which would transition from Udic to Ustic soil moisture regimes across this gradient. However, the center and edge zones of our experimental design should exhibit an Aquic soil moisture regime, and this should be consistent across all sites.
Suggest rainfall be measured on site to compare with nearest available weather data.	This is not practical for many of the study sites, especially the more remote ones like Wyoming and Virginia, and is problematic for forested sites since a clearing is required.
Redox measurements from IRIS tubes may not be able to say much about potential formation of methane. Methane production may also be retarded by sulfates.	Sulfates are expected to be minimal based on these being inland, freshwater, depressional wetlands. Budget constraints limit other methods of documenting reduction (eg. redox electrodes with data loggers).
Bulk density may be useful for expressing C levels on a volume basis.	We are measuring bulk density. See "Quantification of Carbon and Nitrogen Stocks" in our methods.
<u>Reviewer 6 Comments:</u>	
The main question presented is "how such an increase in temperature will affect carbon stocks in wetlands?" With the suggested route to answering the question being "find wetland with similar soils, hydrologies, and geomorphic settings but in a range of temperatures." The idea, then, it seems is to utilize sites that behave similarly with respect to all the soil forming factors other than the temperature component of climate. Intuitively, this makes sense – hold everything but temperature constant and try to see what the variation in carbon dynamics is. To this end, the researchers have identified 11 different sites to instrument and measure in closed-drainage systems.	The project outline was revised to address these very useful critiques from Reviewer 6. We reframed the project by changing research objectives 1 and 2. This refocuses the project on documenting the range of characteristics of depressional wetlands across the 11 study sites. Edits were made throughout the outline to reflect this reframing.

 These include, based on Figure 1: Wyoming, 8000' elevation and either granitic residuum or Pinedale-aged till of mixed mineralogy, and a conifer-dominant ecosystem Nebraska, 1000' elevation, in Peorian loess, and likely a native prairie ecosystem that has been modified by landuse Kansas, ~1000', in pre-Illinoisian glacial landscape, likely native prairie with major modifications Michigan, ~800', in Wisconsinan-aged till, likely native hardwood forest Pennsylvania, ~1000', in ridge and valley province, likely hardwood forest as native community, with at least 2 clear cut histories, if not fully converted to agriculture Delaware and Maryland, <500' on either the piedmont or coastal plain, hardwood forest with modifications WV and VA, ~200-2500', in ridge and valley province, native hardwoods with strong potential for modifications RI, >500', in coastal plain or glacial outwash? With a hardwood native vegetation that has seen modification And MA, ~500-1000', in glaciated landscape, also native hardwood, with unclear landuse history 	
The researchers intent seems to be to say that these spatially spread sites have similar soil forming conditions and therefore can be compared against each other to use temperature as a controlling factor of carbon dynamics.	
To this reviewer, the locations, site histories, and variables of CLORT are not held constant to the exception of temperature. There is strong variation in pedogenic processes and controls. And while there may be a distinct variation in soil temperature, the differences in local climate do not make these sites a temperature-sequence.	
Hydrology: the researchers are targeting hydric to non-hydric hillslopes. Yet, all hydric	

soils are not alike – if they were we would not have and be constantly updating and revising the hydric soils indicators - we would have one indicator. We have regionality to the indicators as well as texture-dependent indicators. The researchers will be monitoring with wells (Figure 4 mentions piezometers and wells, but piezometers are not mentioned in the methods), which can help elucidate water flow patterns - which is important. Surface and subsurface hydrology can have significant impacts on distribution of dissolved and particulate organic carbon. I am not convinced (based on my experiences observing landscape hydrology at similar systems around the US and elsewhere) that the hydrologies of these systems are likely to be similar enough to discount hydrology as a modifier of OC distribution in the landscape meaning that temperature is not the primary driver of the carbon dynamics.

Parent Materials: there are at least three different parent material types I would expect to see for these various sites based on their approximate geographic locations – glacial till, residuum, and outwash. Without full knowledge of the sites, I might also include colluvium and alluvium. Additionally, the geochemistry of the parent materials represented by the sites looks to have high potential variation. That chemistry, and the resultant soil chemistry (e.g. pH, carbonates, ...) can have a strong influence on the carbon decomposition dynamics.

Organisms: The variations in precipitation, temperature, and evapotranspiration mechanics at these sites have resulted in distinct vegetative communities. The conifer and prairie systems are different than the hardwood systems. The chemical makeup of the vegetative litter is different and, combined with the chemistries, hydrologies (e.g. precipitation (amounts and seasonalities), vapor pressure deficits), and fine-earth differences make me think that decomposition will not be controlled by temperature alone. The described methods of measuring decomposition and carbon inputs may not be appropriate for the given geographies. Herbaceous plant senescence in the western sites begins much earlier than the eastern sites. And this is markedly so in a western vernal pool system in an ustic or xeric region. Focusing on fall litter collection at 8000' is a bit late. No mention of adjusting sampling protocol to fit regional plant cycling is mentioned.

Black Carbon: The authors are intrigued by the pyrogenic carbon that has been observed at some of these sites. This carbon is a definite long-term storage type for carbon in soils as it is slow to react and decompose. In western forests pyrogenic carbon has been shown to have a strong influence on nutrient cycling. What I find interesting is the lack of discussion as to the genesis of this carbon. In fire-adapted landscapes, this carbon can be added repetitively over 100's to 1000's of years. But only if the systems are burning. In the sites west of the Mississippi, the pyrogenic carbon addition is still a strong possibility unless landuse has eliminated the vegetation that carries fire. In the eastern sites, what is the source of the carbon? Is it from periodic burning of the wetlands? Or is was it contributed by slopewash in the past after sites were logged and burned. Or cyclic wildfire. I would have liked to have been provided some more site-specific details to evaluate whether black carbon is something to pursue in this study. Impact of historic landuse: Each of these sites has been managed differently and exposed to different degrees of erosion, accumulation, fire, native vegetation conversion, altered hydrology, etc. A full accounting of those potential impacts are required to assess how they can be compared and how historic landuse may have impacted current carbon levels. In an undisturbed system, the carbon balance is likely a 100-1000 year dynamic that reflects long-term trends in hydrology, vegetation, and climate. In the last 100 years, in almost all the proposed systems, we have set the cycle out of balance in drastic ways. The current carbon cycling may not reflect the actual influence of climate any more as the system

may still be finding a new equilibrium post disturbance. We could compare this to the idea of isostatic rebound – the glaciers have been gone for ~10,000 years and the land masses are still adjusting. Carbon in a wetland-upland system could be in the same scenario, adjusting to the violent plowing or timber harvests that occurred and drastically upset the balance. No indication of that was provided to help assess the historic landuse changes and potential influence on the carbon cycle	
In summary, with respect to the idea that these sites can help isolate temperature as a control on carbon dynamics (a main thesis from the introduction) and then be used to model carbon storage with climate change, there is not enough information provided to support that the project can achieve this. And based on my knowledge/experience, there will be too much environmental noise to achieve that expected outcome. That said, much like the wetland soils project of the 1990's, a longitudinal study across these ecosystems to help elucidate the dynamics of carbon in wetland-upland systems across these highly varied systems will provide valuable scientific knowledge to aid in our management on a regional basis. It just won't do what is being proposed as the main thesis.	
 The researchers propose the below objectives. I give an impression with each one. To better understand the hydrological, biogeochemical and pedological properties and processes that affect SOM decomposition, CO2 and CH4 greenhouse gas fluxes, and C sequestration in depressional wetland ecosystems, as expressed across geographical and climatic gradients. Certainly, can be done for each site and compared between sites. This objective hints that differences other than control by temperature are expected. That doesn't jive 	We removed "as expressed across geographical and climatic gradients" from this objective.
with that thesis that differences will be related	

to temperature alone, and therefore using these sites as a way to develop a standardized model how SOC stocks will change as temperature increases due to climate change. And that was suggested as the premise for the research.	
 To determine the relationship between soil and air temperature and accumulated soil C stocks and fluxes in depressional wetland systems. About the same response as to the previous objective 	We revised this objective to state: "To document the range in accumulated soil C stocks and fluxes across these 11 depressional wetland systems." as part of our reframing of the project to address this reviewer's comments above.
• To determine the relationship between soil and air temperature and accumulated soil C stocks and fluxes in depressional wetland systems.	
About the same response as to the previous objective	
• To determine the relationship between hydroperiod (i.e. duration of saturation and inundation) and accumulated soil C stocks and fluxes in depressional wetlands.	
Good for developing regional concepts, but not for applying as a blanket across all the ecoregions represented in the study	
 To seek to develop morphological indices of the hydroperiod within depressional wetlands in order to estimate or predict C stocks. 	We removed this objective.
This will be a challenge. If we take indicator A12, thick dark surface – that is a morphological property. Can we estimate carbon from that? Not likely. We can infer that SOC is high (relative to the surrounding soils) but the SOC content can be highly variable across different regions in soils that have the	

A12 Indicator applied. That would be a fun NASIS exercise. if only indicators were consistently included in NASIS data population in pedons that got lab analyses. • To quantify black carbon in depressional wetland systems. With this limited selection of sites, are the researchers proposing to assign relative black carbon content to depressional wetlands? The black carbon to the depressional wetlands? The black carbon is a reflection of local fire history and may or may not be applicable across all systems. Andto what end? How does specifically identifying black carbon heresting information, but limited in scope and applicability. 3. Appropriate scope of activity to accomplish objectives: The activities suggested for this project can get at the proposed thesis of how temperature increases due to climate change will impact carbon stocks in wetlands. 4. Potential for significant outputs(products) and outcomes and/or impacts: This research can provide further wetland dynamic information that is needed as these systems are managed and threatened. Wetlands are storage systems for carbon and understanding the dynamics across different ecotones adds to the database already built by previous projects. Information gained in this project can help further quantify the role of wetlands in providing ecosystem services and can be used to refine landuse policy at state, regional, and national evels. Given the recert judicial decisions that decreased the scope of what wetlands fall under CWA jurisdiction, adding to the quantification of wetland types, ecosystem processes and services is highly valuable.		
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	5. Overall technical merit:	

As a general scientific knowledge pursuit, this proposed project has strong technical merit. With respect to answering the question about how future temperature changes will impact carbon storage, the project proposal falls short.	
If there is a desire to better quantify wetland soil carbon dynamics, this project should be funded. If the desire is to answer whether changing temperature due to climate change will impact carbon stocks, then this project does seem to meet that desire and should not be funded.	