**INTRODUCTION**: Critical Zone Observatories (CZOs) fill an important role in characterizing the complex interactions that occur in the interval from the top of the vegetation canopy to the base of drinking water aquifers. The existing CZOs, while differing in their climatic and topographic settings, share a common trait in that they are all situated in moderate to high relief landscapes. The physical and biogeochemical processes within the current CZOs are thus closely coupled to fluvial processes. To date *there exists no CZO that resides in a low relief environment despite the immense ecological importance of these settings.* We propose to create a new CZO in the prairie pothole region (PPR) of the Northern Great Plains that will depart from this paradigm. We have assembled a team with expertise in hydrology (Rosenberry), ecology (Euliss, Mushet), environmental and geochemistry (Arnold, Chin, Toner), geology (Goldhaber, Ito), microbiology (Lower), soil science (Nater), biometeorology (Griffis), and outreach (Hamilton) to fully characterize critical zone evolution and its links to ecology within a representative portion of the PPR. The PPR CZO will be located at the Woodworth Field Station (WFS) in North Dakota and will leverage past (US Geological Survey; USGS and US Fish and Wildlife Service; USFWS) and future (USGS, National Ecological Obervatory Network; NEON) scientific efforts and investments. The theme of the CZO is the exploration of the co-evolution of geology, hydrology, geochemistry, and biota in an ecologically and economically significant post-glacial landscape. Within this framework, we will conduct research on 1) the influence of post-glacial geologic, hydrologic, geochemical and biotic evolution of the PPR on its present ecology; 2) the drivers behind the biogeochemical cycling of redox sensitive elements and pollutants, and 3) the processes that drive greenhouse gas fluxes. Underlying the proposed studies is the issue of scale. We expect that processes characterized at the WFS are generalizable to the broader PPR.

***Why the Prairie Pothole Region?***

The PPR, which encompasses approximately 780,000 km$^2$ of the north-central United States and south-central Canada (Figure 1), contains a high density of small, shallow, depressional wetlands, ponds, and lakes (henceforth called prairie pothole wetlands or PPWs) embedded in a complex landscape of moraines, glacial meltwater channels, outwash plains, and lacustrine plains. By one estimate, the total number of PPWs prior to European settlement was 12.6 million.[1] These topographically closed wetlands are hydrologically linked through groundwater and occasional ephemeral overland flow only. Collectively, the PPWs and their associated watersheds constitute one of the largest inland wetland systems on Earth[2] and are of unparalleled importance to a diverse community of waterfowl, amphibians, invertebrates, and plants. The PPR represents one of the major waterfowl breeding habitats and waterfowl migration corridors in North America. It is a critical breeding habitat for ~22 million ducks of 12 species and produces from 50% to 75% of the continent's duck population.[3] Primary productivity of the PPR is similar in magnitude to tropical rain forests.[4] Moreover, PPWs provide important ecosystem services including biomass production, groundwater recharge, floodwater storage, carbon sequestration, and pollutant degradation.[5]

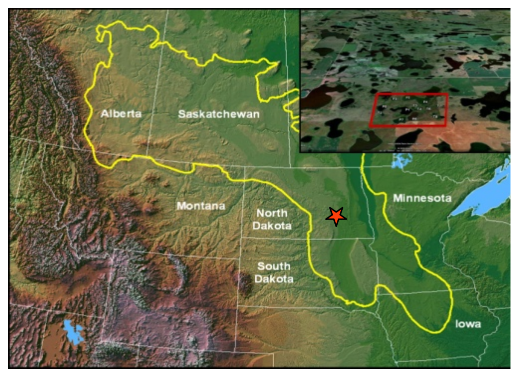


**Figure 1**: The Prairie Pothole Region. The proposed PPR CZO site is marked. The inset shows the density of PPWs.

The proposed PPR CZO fills a major gap in the existing CZO network because its geological and hydrological structure is not of orogenic origin. Rather, it is nested within the Northern Great Plains, one of the Nation's major crop growing regions. The landscape was established beginning 16,000 years ago upon the retreat of late Pleistocene glaciers and has been affected by weathering processes linked to strong seasonality. The PPR is located in a tectonically stable low relief part of North America. The surface geology is dominated by glacial till that is tens of meters thick (much thicker than in current CZOs). We equate till with regolith because it consists of disaggregated rock generated by weathering and glacial transport that covers the underlying bedrock. The critical zone in the PPR comprises the till, overlying soil (dominantly Mollisols, that generally correlate with grassland vegetation), and the embedded wetlands and biota.

The PPR critical zone is dominated by hydrological and biogeochemical cycling of fluids, solids, and gases, rather than by erosional and depositional sediment transport. Today these interconnected processes are under substantial anthropogenic influence. During the last 150 years, the landscape has been altered to facilitate crop and livestock production. This often included the draining of PPWs as agriculture expanded through the region resulting in hydrologic changes to the system. In North Dakota, approximately 50% of the wetland area has been converted to croplands,[6] and land use in the PPR is still evolving. Crops being grown have changed due to market pressures from wheat to corn and soybeans driven by increasing needs for biofuel and feed-stocks. More recently, energy extraction activity in the western reaches of the PPR has increased significantly and the environmental impacts of this expanding activity are becoming apparent.[7]

The hydrology of PPWs is dominated by atmospheric exchange in the form of spring snowmelt and summer convective storms and evapotranspiration that greatly exceeds annual precipitation.[8] As a result, these wetlands are hydrologically dynamic in response to to changes in climate. Many shallow wetlands dry up during the summer and many others disappear during periods of drought. The hydrology of PPWs also reflects the interaction of nested topographically closed basins. Within individual subbasins, upland wetlands act as groundwater recharge zones that are linked to intermediate elevation flow-through wetlands and local terminal low elevation discharge wetlands (Figure 2). These individual small sub basins may be linked to larger regional groundwater flow systems.[9,10]

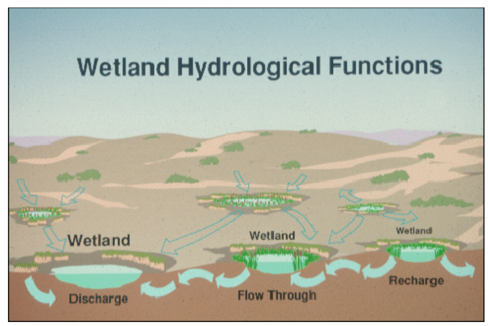

**Figure 2:** Hydrologic linkages of PPWs.

The interaction of geology and hydrology within the critical zone of the PPR has resulted in unique surface and groundwater composition that varies greatly over time and space. Recharge wetlands have surface water composition similar to rainwater, while flow though and discharge wetland waters have higher ionic strength and may range from slightly brackish to hypersaline. A striking aspect of PPWs is that this dramatic change in salinity often occurs over distances as small as tens of meters. Reactive minerals within the till are the source of the very high concentration of oxidized and reduced sulfur species in these systems as well as the dominant cations. As a result, the macro- and microbial ecosystems that have evolved since the last glacial maximum are diverse and intimately linked to the complex hydrology and hydrochemistry of the region.[10]

The PPR is a region of effective moisture deficit reflecting the interplay of three air masses: warm-dry Pacific air, cold-dry Arctic air, and warm-moist Gulf air.[11-13] The result is an ecosystem complex that repeatedly experienced major episodes of drought and deluge and is particularly susceptible to climate change.[14-17] The PPR is predicted to become hotter and wetter under current emission scenarios.[18] Climate change will alter the hydrologic, biogeochemical, and ecological balances of the system and could lead to even greater forced changes in land use. In a system that is vulnerable to change due to alterations in land use, climate, and energy production, it is essential to understand how critical zone processes operate and how they are altered by anthropogenic forcings. Characterizing a system of this complexity involves reconstruction of past conditions, measurement and modeling of current conditions, and prediction of future trends. *Establishing a low-relief grassland dominated PPR CZO would represent a major step towards understanding the system as it exists today and assessing its vulnerability to human driven change.*

**SCIENTIFIC JUSTIFICATION:** The major research activities of the PPR CZO will address three central hypotheses listed below. We will use the CZO Common Measurement Protocols and additional measurements as described below and test these hypotheses. The data collected will inform our conceptual model (Figure 3).Wetlands drive the biological communities of the PPR, and the wetlands are in turn driven by the climate and their hydrologic setting. The wetland continuum model proposed by

Euliss et al.[10] organizes wetland biological data based on their hydrologic relation to groundwater (recharge, flow through, or discharge) and climate conditions/atmospheric water (drought to deluge). We will seek to extend this model to determine how the hydrologic drivers and underlying geology of the system influence the hydrochemistry, sediment chemistry, microbiology, and greenhouse gas (GHG) emissions. The outcomes of the creation of a PPR CZO will be an integrated understanding of critical zone processes in this important region, elucidation of the interconnected hydrologic, biogeochemical, ecological, and climatic drivers of the system, and the ability to predict how anthropogenic influences will alter these processes and drivers.

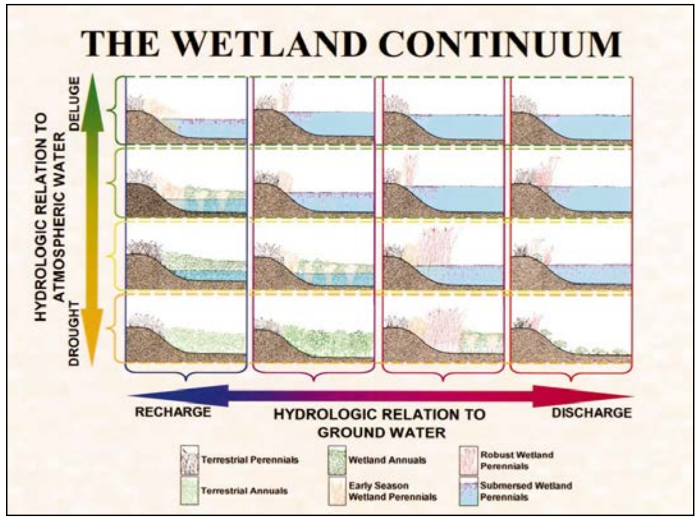


**Figure 3**: In the wetland continuum framework, the ecology, chemistry, and microbiology of the PPWs are driven by their relation to atmospheric water and groundwater.

*Hypothesis 1*. Influenced by the highly variable climate in the temperate midcontinent since the last glacial maximum, the interaction between till weathering and groundwater is the major long-term control on evolution of the critical zone in PPR ecosystems. This hypothesis will be tested through detailed paleoecological, lithologic, geochemical, mineralogical, biogeochemical and geophysical studies of the till and wetland sediments via cores as well as elemental (major, minor and trace) and isotopic (H, C, N, O, and Sr) characterization of groundwater. This hypothesis addresses two of the fundamental CZO questions (http://criticalzone.org/national/research/): How does the critical zone form and How does it evolve?

*Hypothesis 2:* Biogeochemical and ecological processes are coupled to the unique hydrology of the PPR (i.e, the existence of recharge, flow through and discharge wetlands that are hydrologically linked by groundwater). This has created chemical and redox gradients ranging from near rainwater composition for recharge wetlands to highly saline discharge wetlands, that in turn, drive the ecology and biology of the system (i.e., the wetland continuum model, Figure 3). Aquatic plant communities are both affected by and affect geochemical processes in wetlands. Thus, plant communities provide a direct link between geochemical processes, climate, and biotic components of PPWs. These wetlands also attenuate pollutants ranging from nutrients to synthetic organic compounds. We will also investigate the "assimilative capacity" of this system to accommodate anthropogenic contaminants. Hypothesis 2 will be tested by conducting hydrologic and biogeochemical measurements (e.g.,specific elements and compounds and their isotopic signatures), and investigating micro- and macro-biological communities (e.g. detailed characterization of upland and wetland plant communities) in catchments along the continuum from recharge to discharge wetlands. Hypothesis 2 addresses the fundamental CZO questions: How does the critical zone operate? and How may it function in the future?

*Hypothesis 3*: The strong spatial and temporal gradients in hydrology and hydrochemistry within the PPR set the stage for a very dynamic response of GHG fluxes to climate and anthropogenic perturbations such as drought/deluge, draining wetlands to establish agriculture, and the invasion of exotic species. Using the wetland continuum model (Figure 3) as a framework, we hypothesize that there are unique response functions for each GHG ($CO_2$, $CH_4$, and $N_2O$) that are generally dependent on hydrologic regime, the hydrochemistry, and the associated plant communities. We will also measure trace gas emissions of various wetland plant species (both native and introduced) and relate observed differences in plant physiological processes to their effects on geochemical processes and climate drivers. Using the continuum model as a framework with high resolution LiDAR and remote sensing products provides a capacity to scale up our greenhouse gas budgets to the PPR. Given the geographic extent of the

PPR, the response of GHG fluxes to climate and anthropogenic perturbations are likely to be significant at the continental scale. These hypotheses will be tested by measuring elemental fluxes in the groundwater, wetlands, atmosphere, and soils and by linking microbiology, geochemistry, and vegetation gradients to GHG fluxes. Hypothesis 3 also addresses how the critical zone functions and is important to understand in the context of future change.

**Current Status of Knowledge**

Much of the current knowledge about the PPR is derived from 40 years of hydrological and ecological measurements by Canadian scientists at the St. Denis Saskatchewan site[18b] and the USGS at the Cottonwood Lakes Study Area (CLSA). The PPR CZO will use what has been learned at CLSA, and expand this knowledge into a CZO at WFS which will allow a partnership with NEON. The CLSA cannot be the primary CZO site, because the infrastructure required would disrupt the ongoing, long-term research of biotic communities. By identifying how wetlands at WFS fit into the recharge to discharge continuum, we will be able to use information derived from previous detailed studies at the CLSA to further our understanding about how biotic communities will respond to the geochemical processes that will be studied at WFS.

*Basic geologic and geochemical development of the PPR since the last glacial maximum:* The southwestern border of the PPR follows the maximum extent of late Pleistocene glaciation that shaped the surficial landscape and provided the geologic material that defines the hydrologic and geochemical characteristics of the region.[19] Melting of the ice sheet left behind a blanket of heterogeneous and unsorted glacial sediment (i.e. glacial till) resulting in a hummocky topography. The low permeability of the till is what causes the ponding of water on the surface, forming wetlands. The till-covered area is divided geographically into the glaciated plains on the northeast and the Missouri Coteau to the southwest. The Missouri Escarpment separates these two regions. The proposed CZO occurs within the Coteau and overlies an area of stagnation.

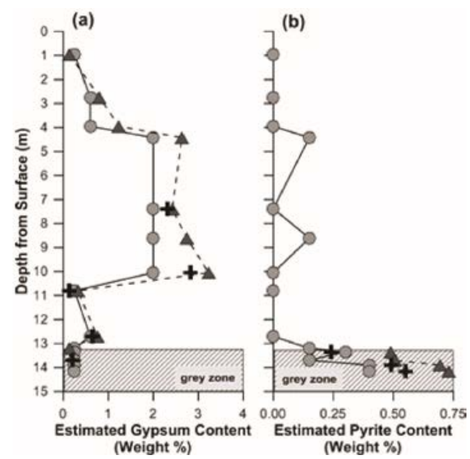

**Figure 4.** Depth profiles of estimated (a) gypsum and pyrite (b) content of select glacial till samples from a CLSA core.

The till contains minerals including quartz and feldspar derived from the Canadian Shield, dolomite and calcite from southern Canadian Paleozoic carbonates, and pyrite-bearing Pierre Shale derived from late Cretaceous organic rich marine units that directly underlie portions of the PPR.[20] Weathering of reactive sulfide and carbonate minerals along groundwater flow paths in the till is an important control on the chemical composition of water in PPWs.[21-27] The negative sulfur isotope signature of the shale-derived pyrite[27b] provides a tracer for sulfur in this system. Dissolution of carbonate minerals is a major source of $Ca^{2+}$, $Mg^{2+}$ and $HCO_3^-$ ions and pyrite oxidation is the source of $SO_4^{2-}$ to PPR groundwater and wetland water. Figure 4 illustrates the weathering (oxidation) of till from a core in the CLSA. Presence of an oxidized, nearly pyrite-free upper brown zone (upper 13.2m) and an underlying pyrite-bearing gray zone is apparent. The upper oxidized zone contains gypsum, an oxidation product of pyrite. The same color boundary has been observed widely in the PPR.[22,24,28] An analysis of nearly 1800 drill hole logs in a two county area that contains the WFS and CLSA documented that the upper oxidized zone is nearly ubiquitous and extends from 1.2 to 28.2 meters in depth.[29] A coupled 1D fluid-flow and chemical reaction model of the formation of this zone by oxidation of pyrite in the till under water-saturated conditions is limited by the slow diffusion of oxygen in water[30] implying that oxidation occurred in the vadose zone. The modern water table in the uplands portion of the CLSA is roughly 1 to 4 meters below the land surface, far shallower than the oxidation interface. We hypothesize that an oxidation event occurred in the past when conditions were drier and the water table deeper than today, allowing oxygen in a thick vadose zone. Testing this hypothesis will require detailed study of the till coupled with investigation of the regional paleoclimatology to determine when a deeper vadose zone may have been present.

*Paleoecology of the PPR through the sediment record.* The climatic history of the PPR is a key constraint on the long-term evolution of its critical zone. Most of this historical record is derived from coring in lakes (rather than small wetlands). The discontinuous sedimentary record of many small wetlands has discouraged serious attempts to reconstruct detailed Holocene history of interplay between climate and wetland function. The very existence, however, of the Central Flyway for North American bird migration suggests that even during the severest prolonged drought, not all PPR water bodies went dry. Studies of deeper PPR lakes that did not dry during the Holocene indicate that the Early Holocene landscape included spruce trees but was taken over by grasses and prairie forbs ~10,000 years ago.[31,32a] Diatom and ostracodes species assemblages indicate initially dilute water that became more saline between 8,000-9,000 years ago.[16,32a,b] The Mid-Holocene arid period is characterized by ragweed-type and goosefoot-family pollen whose abundances fluctuate widely.[31] Ragweed is indicative of warm season precipitation and suggests variable summer precipitation.[31] The Late Holocene, since ~3,000 years ago, has been wetter than the Mid- Holocene.[16,32a,33] Decadal to semi-decadal resolution studies of diatom and ostracode species assemblages and ostracode shell chemistry for the last 2,000 years record multi-decadal arid periods occurring at approximately 400 year intervals within the generally wet Late Holocene.[17]

*Hydrology, Hydrochemistry, Biogeochemistry, and Pollutant Processing:* The hydrology of the PPR is devoid of stream networks and is dominated by temporary and perennial open-water wetlands that grow and shrink in response to variability and timing of precipitation. These wetlands play different roles in the regional hydrogeologic setting depending upon topographic location (Figure 2). Water enters these wetlands as precipitation, overland runoff, or groundwater discharge and is lost through evapotranspiration (ET) and to the local aquifer for recharge and flow-through wetlands. Thus, this system is sensitive to climate variability. While water entering these wetlands through precipitation and to a lesser degree overland flow is quantifiable, inputs from groundwater (for flow through and discharge wetlands) and loss processes such as ET and advection from pore fluids to the aquifer are difficult to assess. Indeed, during dry periods ET from wetland plants can reverse groundwater advection to discharge wetlands[34,35] resulting in accelerated shrinking of wetland surface areas, as detected by remote sensing. These events alter the wetland hydrochemistry, which ultimately impacts the microbial and macro-ecology. There are large temporal variations in the water balance due to seasonal, inter-annual, and decadal changes in water balance. Seasonal variability is controlled by a yearly cycle of concentration by evaporation in summer, further concentration by freezing in winter, and dilution during thaw and melting of accumulated snowpack in spring. Longer-term climate shifts lead to episodes of major evaporation or dilution. Thus, we will need to make hydrologic measurements on relatively high-resolution time scales and use various techniques to determine the degree of evaporation/dilution and residence times.

The chemistry of PPW waters is highly variable in both salinity and solute composition along flow-paths from recharge to flow-through to discharge PPWs.[36,37] Wetland waters generally evolve with increasing salinity towards Ca-depletion and PPW solute evolution never reaches gypsum saturation, although gypsum may be present in the till. Instead, sulfate evaporite minerals (mirabilite, thenardite and epsomite) ultimately precipitate during periods of drought around wetland margins.[38] Microbially mediated sulfate-reduction produces carbonate alkalinity furthering the Ca-depletion. As a result of the complex wetland groundwater geochemistry, oxidation-reduction (redox) gradients also develop in these systems and play critical roles in biogeochemical reactions such as denitrification, mercury methylation, and the attenuation of synthetic organic contaminants. The wetland sediment and aquifer redox profiles differ depending upon their position in the hydrologic gradient. Our past studies[39,40] at CLSA showed that wetlands with high concentrations of sulfate in the water column possess abundant levels of reduced sulfur species in sediment pore waters that include sulfite, thiosulfate, sulfide (mM levels), and polysulfides. The sulfides are capable of degrading a variety of pesticides via nucleophilic pathways or reductive reactions.[39,40] The general water chemistry, redox zonation, and synthetic organic contaminant attenuation potential has not been adequately surveyed either spatially or temporally in the PPR.

Organic matter (OM) in the form of soil/sediment organic carbon (SOC) or dissolved organic carbon (DOC) plays a critical role in the biogeochemical cycling of elements, as a substrate for microbial respiration, the release of greenhouse gases, the availability of micronutrients, and the attenuation of synthetic organic compounds such as pesticides. SOC and DOC in the PPR are unique in both composition (grassland precursors) and quantity (unusually high levels of DOC that range from 20-200 mg/L).[39,41-43] Work conducted by our team shows that OM is involved in the formation of reactive oxygen species in sunlit PPW waters and that it can participate in both redox and nucleophilic substitution reactions in sediments.[40,44] What is less known is the role that OM plays in other important biogeochemical reactions (e.g., denitrification, methanogenesis, mercury methylation, etc), and this is an important goal for the proposed research.

Nitrogen inputs into the prairie potholes include biological, geologic, and anthropogenic sources and its fate is linked to that of OM. Nitrogen fixation by cyanobacteria and plants are expected to be a dominant source, while the Pierre Shale is anticipated to be a secondary source.[45,46] Anthropogenic nitrogen is introduced to PPWs from croplands, while N in deeper groundwater is expected to originate from geologic sources. Previous work at CLSA determined that groundwater had a broad range of inorganic nitrogen concentrations (<3 to ~1000 µM N), with $NH_4^+$ as the dominant species.[46] Further $^{15}N$-$NH_4^+$ data (3.6 to 14.7‰; n=11) suggest different cycling mechanisms, but additional data is necessary to discriminate between Pierre Shale, wetland OM, and anthropogenic sources, as well as more carefully defining the relationship between hydrologic flowpaths and N speciation.

Iron is an important biogeochemical element, and is linked with the cycling of elements such as carbon, sulfur, and mercury. Iron is a required nutrient and can act as an electron donor or acceptor for microbes depending on speciation. As a result it can serve as a redox buffer in PPW sediments. Further, iron reducers are thought to out-compete methanogens for substrates such as acetate,[47] and may enhance or inhibit mercury methylation.[48,49] In addition to the dynamic links to other elemental cycles, iron is an essential piece of the PPR CZO because iron-bearing minerals such as pyrite are the source of sulfur to water in oxidizing conditions. In the wetlands with less sulfur, mixed valence iron minerals such as magnetite can participate in various contaminant attenuation reactions.

Sulfur species (oxidized and reduced) gradients are intimately tied to the iron biogeochemistry in PPWs. In recharge wetlands where sulfur compounds are in low abundance, we anticipate iron to be the dominant electron/donor acceptor (see previous), but in water bodies with mM level quantities of sulfur, sulfate becomes the terminal electron acceptor and reduced sulfur quickly reacts with iron to form iron sulfides. We observed no soluble iron species in sulfur-dominated wetland pore fluids in the CLSA,[43] but sediments contain high levels of total iron (ca. 20 g/kg) and total sulfur (ca. 13 g/kg).[50] Using X-ray absorption near edge structure (XANES) spectroscopy, we have been able to show that the sulfur pools - pyritic S, reduced organic S (mono- and disulfide), and oxidized S (inorganic sulfate, ester sulfate, and sulfonate) - in the sediments are seasonally dynamic, with reduced organic sulfur cycling to oxidized sulfur and back. Thus, we suspect that the magnitude of specific important biogeochemical reactions in the PPR (denitrification, methylation of Hg, attenuation of pesticides, methanogenesis) is largely dependent upon the most available terminal electron acceptor present.

_Ecology:_ The ecology of PPR wetlands has been the primary focus of study at the CLSA since research was initiated at the site in 1967.[9] The study of modern ecological processes and paleoecological reconstruction at WFS will be informed by information gleaned from this long-term ecological monitoring. Study results from CLSA will be calibrated against instrumental climate records and thus provide a test bed for some of the approaches and tools used by the CZO and NEON at WFS. Over the history of the CLSA, relationships among climate, hydrology, geology, and chemistry and their combined effects on biotic communities have been explored. This work has led to the development of the wetland continuum concept that serves as the PPR CZO conceptual model.

While the amount of water a PPW receives from groundwater is typically small compared to atmospheric water inputs, the dissolved salts that either enter/leave a wetland through groundwater discharge/recharge profoundly influence the plant and animal communities.[10,51-53] The plant communities of PPR discharge wetlands consist of species that tolerate high salinity. By contrast, recharge wetlands are vegetated by species that thrive under freshwater conditions. Aquatic invertebrate communities also vary by wetland type.[52] Ultimately, the plant and invertebrate communities provide both structural and food components of wetland habitats used by the region's diverse suite of vertebrates, including waterfowl, other wetland birds, small mammals, and amphibians. Because a major influence on the ecology of PPW is the delivery of atmospheric water into wetland basins,[10] drought and deluge conditions influence PPW chemical composition and contribute to great inter-annual changes in both floral and faunal communities. Anthropogenic factors that have altered historic relationships between infiltration and runoff are conversions of native vegetation in upland areas to agricultural crops, cultivation practices (e.g., conventional vs. conservation tillage[54]), and the introduction of nonnative invasive species. Even conservation practices significantly alter groundwater recharge when non-native grasses that have higher water demands than native species such as smooth brome (*Bromus inermis*) are used to revegetate upland catchments.[55]

*Microorganisms:* We are unaware of any comprehensive study that examines microbial metabolism in the PPR, but microorganisms are expected to be major contributors to elemental cycles because of the diverse range of electron donors and acceptors in the soils and sediments of this area. Indeed, this notion is supported by recent metagenomic analysis performed on a single sample of agricultural soil from Waseca County, MN, which is in the PPR.[56,57] This analysis was not conducted to determine the microbial community structure in the PPR. Rather, the authors were simply testing their new shotgun sequencing method to determine taxonomic compositions in a complex, heterogeneous environmental sample. Using their new metagenomic analysis, Tringe et al.[56] discovered enormous diversity represented by more than 3000 bacterial phylotypes (or ribotypes) in the single soil sample. Based on the geochemistry of the PPR , nitrate-reducers, sulfate-reducers, and methanogens are likely to be prominent. Iron and/or manganese-reducers[58] may also play a role in redox reactions within the PPR. Nitrate reducers are an important potential source of the greenhouse gas $N_2O$.[59,60] Sulfate-reducers produce various forms of reduced sulfur and, as described above, they are important in the processing of xenobiotics. Additionally, sulfate reducers may suppress methanogens in wetland environments[61,62] by outcompeting methanogens for available resources. Lastly, the role of methanogens is also important in GHG budgets, because they produce ~85% of the global methane.[63,64]

*Greenhouse Gases in the PPR*: Although PPR wetlands cover an extensive area and store a significant amount of carbon, their contemporary carbon budget and GHG budget is poorly quantified or understood. For example, the magnitude, direction, and spatial variability of $CO_2$ net ecosystem exchange (NEE) is largely unknown. There is evidence that PPWs sequester organic carbon in their sediments[65,66] and that some years experience a net gain of atmospheric $CO_2$ (negative NEE)[67] indicating their potential to act as a carbon sink. In other years these same PPWs act as net sources of $CO_2$ (positive NEE) indicating that heterotrophic processes dominate over the autotrophic processes. A recent study[67] suggests that carbonate precipitation plays an important role in $CO_2$ efflux from bicarbonate-rich wetlands, and this may make them a more important source of $CO_2$ emissions than previously thought.[68] Microorganisms are key to the mineralization of organic matter and can also be important autotrophic members of wetland/lake ecosystems. In addition, both heterotrophic (e.g., sulfate-reducers) and autotrophic (e.g., cyanobacteria) microorganisms promote carbonate precipitation and promote $CO_2$ production. Aquatic plant species also alter microorganism communities through oxidation of their rhizospheres. Thus, knowledge of microbial biogeochemistry and ecology of PPR wetlands and lakes is required to adequately characterize their role in the carbon cycle and to interpret GHG fluxes.

A recent analysis estimated that North American mineral wetlands emit 70% more $CH_4$ than peatlands.[69] This assessment highlighted the paucity of $CH_4$ emissions data from the PPR. Initial GHG flux estimates for wetland catchments within the PPR established the potential of this region to play a significant role in the continental $CH_4$ budget.[66,70,71] Given the high levels of sulfate in some wetlands, however, sulfate reducing bacteria may also play an important role by inhibiting methanogenesis.[66,71] Thus, these dynamics need further study. To date the majority of flux information comes from short-term chamber-based observations that do not adequately capture the episodic nature of methane emissions or the large spatial variability.[66,70-72] This severely limits our ability to estimate the $CH_4$ budget of PPR wetlands and to validate $CH_4$ emission models. In the proposed work, we will make use of near-continuous eddy flux measurements by NEON and CZO towers of $CO_2$ and $CH_4$ combined with carbon isotope measurements on these two species, to give high spatial and temporal resolution measurement to allow estimation of annual budgets and to improve our understanding of the biogeochemical and environmental controls. Further, we will complement these measurements with additional flux chamber observations within specific plant communities.

Nitrous oxide ($N_2O$) emissions from the PPR are also poorly quantified. Although $N_2O$ emissions are hypothesized to be relatively low from ponded PPR wetlands compared to agricultural ecosystems,[66,71,73] the expansion of row crop agriculture into the region[74] and associated large synthetic nitrogen inputs[74] are expected to have an increasing impact on nitrate concentration in surface and groundwater flows. Thus there is a need to gather baseline information on $N_2O$ emissions from wetlands not impacted by agricultural runoff. Knowledge of the effects of the historic drought/deluge cycles, current geochemical gradients, and different land uses on microbial biogeochemical processes will lead to an understanding of the degree and direction of feedbacks on future GHG emissions.

## IMPLEMENTATION PLAN

*Site description*: The main activities of the PPR CZO will take place at the WFS in east central North Dakota (Figures 1 and 5). This site has five major advantages: 1) it will be co-located with the NEON core site for the Northern Plains Domain (see Letter of Support), and the CZO will be able to leverage measurements taken at this site. Importantly, the vision and mission of the CZO and NEON are distinct; 2) There is a long history of research at the WFS and the nearby (11 km southeast) CLSA by USGS and USFWS researchers; We will be able to use the CLSA as a companion/comparison site; 3) Having NEON and the USGS within the PPR CZO will allow interaction and collaboration with ecologists, *which is an explicit goal of this round of CZO funding*, 4) The WFS includes a diversity of wetland hydrologic and geochemical types and geologic substrates spanning much of the range found in the broader PPR; and 5) there are several outstanding opportunities for education and outreach activities.

The WFS (Figure 5) is a 1200 hectare Waterfowl Production Area managed by the USFWS. The station contains approximately 550 wetland basins and is dedicated to research activities. Climate is cool temperate subhumid, with long-term average annual precipitation of 44 cm. Ecological studies at the site are extensive.[76] Native grasslands consist of xeric mixed-grass prairie vegetation. Agricultural fields are present adjacent to the WFS. Most of the WFS is underlain by a thick layer of glacial till typical of much of the PPR. The eastern edge and southeast corner of the WFS, however, intersect coarser-grained glaciofluvial sediments that were deposited in an ice-walled gravel train (Figure 5). Thus, the site provides the opportunity to study wetlands located in different hydrologic and hydraulic environments. The hummocky stagnation moraine portion of WFS contains up to 40 wetlands per $km^2$. Soils in the study area are Mollisols, and include calciaquolls, calciupdolls, endoaquolls, hapludolls, and natroaquolls.[77]

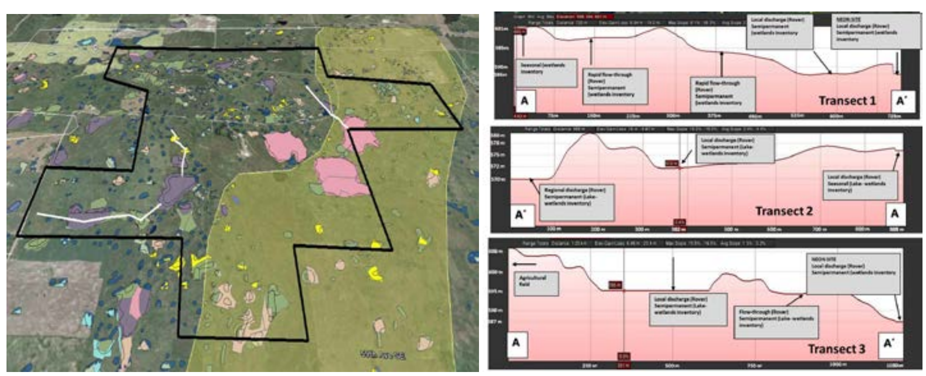


**Figure 5**: *Left*: Google Earth image of the Woodworth Study Area (outlined in black). Tentative hydrologic functions are coded by color from an analysis of Landsat (1973-2011). The lighter, eastern region traversing the WSA is cross bedded sand. The rest of WFS is underlain by permeable glacial till. The proposed initial transects are shown as is the NEON core aquatic site. *Right*: Elevation plots along the three transects.

The entire range of PPWs described by Stewart and Kantrud[78] is found at WFS: ephemeral, temporary, seasonal, semi-permanent, and permanent[79] (Figure 5). The three largest wetlands within the WFS lie within or near the glaciofluvial sediments. Two of these were sampled in 1972 and 2012. The overall range of conductivities was 1160 to 1366 $\mu S\ cm^{-1}$. The 2012 analysis of these two permanent wetlands show them to be brackish with sulfate and bicarbonate as the dominant anions. In contrast, 16 temporary recharge wetlands sampled in 1993 and 1994 by Detenbeck et al.[80] had mean conductivities of ~300 μS $cm^{-1}$. Chemical analyses of these wetlands confirm that they are relatively fresh with bicarbonate as the dominant anion. Substantially higher hydrologic conductivity of the course-grained sediments likely results in the largest wetlands being more dependent on groundwater input than other wetlands in the WFS.

*Preliminary Sampling Sites*: The majority of CZO infrastructure and sampling will occur on three nested transects (Figure 5) that have been initially located based on topography and wetland function. Two of the three transects (1 and 3) terminate at a wetland that is the focus of the NEON aquatic science team, so that the data collected by NEON can be leveraged in the CZO. The upland portion of transect 3 terminates in a large agricultural field. Transect 2 terminates in one of the permanent wetlands described above. An on the ground assessment of the vegetation (which varies based on water salinity) will be performed before finalizing the transects. The first round of sampling will focus on water level, water chemistry, and water age. The initial data will allow us to make adjustments if it is determined that the hydrologic connections are different than our initial evaluation. Concentrating data collection along these transects will provide samples along hydrological, geochemical, and biotic gradients and allow for data sharing across hypotheses.

*CZO Infrastucture and Measurements*: The PPR CZO is committed to the infrastructure necessary to collect the data regarding critical zone architecture/evolution and fluxes described in the "Common Critical Zone Observatory (CZO) Infrastructure and Measurements". A summary is provided here, and specific details are incorporated into the research tasks described below. **Land-Atmosphere exchange** measurements will leverage the micrometeorological towers (i.e. turbulence, eddy $CO_2$/$H_2O$/heat/ momentum fluxes, radiation balance, wet/dry deposition samplers, phenological cameras, and other supporting meteorological observations) in the NEON fundamental instrumentation unit (FIU) at the Woodworth site. We will also be able to take advantage of the annual peak-vegetation LiDAR dataset that will be collected each year beginning in 2014, and for minimal incremental cost, will extend this area covered to CLSA. We have also budgeted for an additional LiDAR pass each year. Further, we will use (and augment if necessary) NEON's key measurements of **vegetation, soil, and microbiota** variables (i.e. phenology, biodiversity, abundance, invasive species, net ecosystem productivity, water balance, soil temperature and water content, etc). Microbiological measurements in the soils, regolith, and wetlands are a key component of the PPR CZO and will inform our other biogeochemical studies. **Soil** and **regolith/saprolite** and key **biogeochemical indicators** such as elemental composition, mineralogy, carbon and nutrient stocks, and paleo/architectural traits will be quantified. A strong emphasis will also be placed on measurements that will characterize the **hydrology** (regolith/ground water), **wetland sediments**, and **water chemistry**. The diversity in surface water chemistry in the PPR CZO is unlike that at any other current CZO. Critical measurements will include detailed time series for surface and groundwater chemistry, water level in the wetlands and associated groundwater (to be measured by pressure transducers), and PPL sediment/porewater chemistry (coring). All of the above supporting measurements will be critical for the overall data interpretation, hypothesis testing, and the parameterization for model development. We will follow the proposed NEON data quality control and assessment procedures outlined by the FIU group to establish high quality and consistent data for our group, other CZOs, and the broader scientific community.

*PPR CZO and NEON Synergy:* Given the diversity in hydrological and ecological setting of the PPR there is an important need for multiple research sites and micrometeorological towers to help capture the key endpoints and transitions within the study domain. The collaboration between the proposed CZO and NEON Woodworth Field Stations (WFS, Ecological Domain 9) provide an excellent opportunity to better represent the PPR and should provide a number of synergistic opportunities within the science and local communities.

The NEON core site with the FIU, including a flux tower is within the WFS. In addition, two additional roving NEON tower units are to be located within about 10 km of the core site. The NEON core aquatic site in WFS is to be situated within a local discharge wetland, and basic water chemistry and sediment chemistry measurements will be made. Together these sites will represent one end point within our study area. The proposed CZO sites will expand the coverage by targeting recharge, flow-through, and discharge wetlands along multiple transects, and with data from the CLSA. A suite of common measurements (i.e. energy, $CO_2$, and water vapor fluxes) will be measured at each of these stations providing an opportunity to assess the relative importance of spatial variability, hydrometeorological, and ecological sensitivity. The CZO measurements of water flows, chemistry, and microbiology described below cover a wider range of parameters and provide greater spatial and temporal resolution that those of NEON. Thus, the broad range of measurements throughout the study area will be important for expanding the potential variables for model development and evaluation and scaling to regional interpretations. As noted on the NEON website – an important feature of their work is to use NEON data to help drive and evaluate models such as the National Center for Atmospheric Research Community Land Model (CLM), which includes hydrology, ecology, and climate parameters, as well as physical chemical, and biological processes. The proposed NSF CZO will make an important contribution toward such efforts, and we will seek to work with NEON on using the CLM for the data collected at WFS. Our group and collaborators use a broad range of Earth System Models including CLM.[81] It should also be highlighted here that the CZO-NEON synergy goes beyond spatial representation and measurement overlap. A broad array of measurements proposed for the CZO will not be possible in the early phases of NEON, but could be added over time. For example, we will attempt to measure methane and nitrous oxide emissions, organic matter composition, and regolith structure and composition across the study domain and have plans for very detailed hydrological and geochemical monitoring that should prove invaluable for expanding the explanatory power of the NEON observatory.

*Hypothesis 1. Influenced by the highly variable climate in the mid-latitude midcontinent since the last glacial maximum, the interaction between till weathering and groundwater is the major long-term control on evolution of the critical zone in PPR ecosystems.*

*Paleoreconstruction*. Using sediment cores in wetlands and soil/sediment cores from the uplands, the goal is to reconstruct till evolution, paleoecology (e.g., diatoms, ostracodes, pollen), hydrologic function change, and geochemistry. The goal is to reconstruct the pre-European settlement history with a focus on climate-induced (past climate) changes. These include wetland hydrogeochemical function over time and the combined influence on paleoecology with emphasis on ostracode species assemblage and possibly other proxy records (diatoms, pollen, and biomarker molecules). Isotopic studies of C and O of the ostracode calcite shells along with species assemblage data will be used to determine whether the specific wetland-and adjacent uplands served recharge, flow-through or discharge roles as a function of time. Contemporary measurements at the WFS will allow a comparison of the current structure/function of the site (see Hypotheses 2 and 3) in a relatively undisturbed prairie ecosystem to the historic, pristine conditions as determined from the cores. Wetland cores will also be taken at the CLSA to allow comparison of the two systems.

*Geochemistry/till structure/dynamics.* A series of approximately 8-15m long cores will be collected using a Geoprobe direct push tool along all 3 transects. The initial goal is for a total of 9 cores in year one, three along each transect. These will be augmented with additional cores in subsequent years. Geochemistry and mineralogy as a function of depth will be determined for all cores, and the data will be used to describe the redox status of the system (e.g. solid and dissolved electron donors and acceptors). The specific sampling intervals will depend on visual inspection of the cores. Major and trace elements will be determined for all intervals via sample digestion and ICP-MS/AES. Given the importance of sulfur in the system, sulfate (gypsum), pyrite content, and organic sulfur will be determined via a combination of wet chemical and X-ray absorption spectroscopy (XAS) approaches.[50] Mineralogy of crystalline phases will be characterized by powder X-ray diffraction (XRD), while poorly crystalline (oxyhydr)oxides of iron and manganese will be described by XAS.[82,83] Sulfur isotopes will also be determined on solid phases to determine S fractionation patterns resulting from repeated reduction and oxidation processes. The chemical, isotopic and hydrologic data will be combined to calculate the thickness of the brown zone[30] and estimate sulfur mass balances for the system. Regolith production (i.e.

till alteration) rates will be calculated using uranium-series isotopes by Lin Ma at UTEP (see attached letter). This method has been used to effectively characterize regolith development at the Shale Hills CZO[84] and has been shown to be applicable at the CLSA.[85] At CLSA, preliminary modeling of data indicates that the development of the brown zone shown in figure 4 required 9.3 kyr. These isotopic measurements will be conducted in year 2 after the initial suite of core samples has been analyzed.

The *physical structure* of the glacial till aquifer includes features such as sand lenses and high salinity zones that can be detected using geophysical techniques, including electrical resistivity. Two and three dimensional maps of subsurface electrical resistivity can be generated using well-established geophysical methods. Based on an initial resistivity mapping study at the CLSA, we expect that subsurface resistivity will provide two key pieces of information: relative hydraulic conductivity of subsurface material[86] and relative pore water salinity.[27,87] Both of these are important for understanding the control that the regolith imparts on wetland hydrochemistry. The USGS Crustal Geophysics and Geochemistry Science Center in Denver has the tools and expertise to generate electrical resistivity maps at the WFS. Electromagnetic (Geophex GEM-2) and direct current resistivity (AGI SuperSting, Geometrics OhmMapper TR5) tools will be used to map both upland terrain and wetland sediment. Of particular interest will be delineation of the subsurface interface between the glacial till and outwash sand that runs through the WFS. Subsurface electrical resistivity maps will be calibrated with borehole geophysical measurements made on all wells drilled and with solid phase and groundwater chemistry.

*Summary:* These studies will address how the critical zone forms and evolves. The information collected will allow detailed characterization of the subsurface including an assessment of the information on the Holocene climatic record and critical zone evolution incorporated in both upland and wetland portions of the topography.

*Hypothesis 2: Biogeochemical and ecological processes are coupled to the unique hydrology of the PPR, i.e., the existence of recharge, flow through and discharge wetlands that are hydrologically linked by groundwater.*

*Hydrology:* Water flux measurements are central to all CZO's. The proposed CZO will be instrumented with wells along the topographic gradients shown in Figure 5 equipped with pressure transducers sensitive enough to detect small changes in potentiometric head as well as stage gauges deployed in the wetland to monitor changes in water flow between wetlands and the adjacent aquifer. The well network will be supplemented by lysimeter nests at the wetland margin to determine rapid transpiration-driven hydrologic and geochemical transformations.[35,88] During times of drought and excessive precipitation flow reversals can occur on a continuous basis. For un-instrumented wetlands portable hydraulic potentiomanometers[89] will be used to measure head and sample pore fluids for biogeochemical analyses described elsewhere. This will allow us to assess how hydrologic alterations can affect the chemical composition of wetland surface and pore waters and groundwater. Deeper wells may be deployed in subsequent years based on year 1 data (time and funds permitting) to study the hydrology and hydrogeochemistry of aquifers disconnected from the shallow groundwater system by aquitards.

We will instrument the site with rain gauges and meteorological (met) sensors to quantify atmospheric exchanges, which are needed for water and chemical budgets. The NEON Fundamental Instrument Unit (FIU) tower deployed nearby will be used to determine ET from eddy covariance measurements. ET measurements beyond the FIU tower will be estimated using site-specific met sensors and well-established equations; e.g., Priestly-Taylor, Penman. We have extensive experience with the implementation of these approaches.[90] We will test the robustness of our site-specific estimates via comparisons with measurements made from the NEON FIU tower.

We will measure the hydraulic conductivity (K) for shallow and deep groundwater over time and space using standard single-well slug tests or the steady-state drawdown low flow method[91] if the water table drops to within the screened interval of a well. Values for K using the low flow approach will be compared to those determined by slug tests.

The contribution of meteoric and evaporated water to the composition of the PPW sediment pore fluids and groundwater as a function of time and space will be determined by measuring water isotopes.

$\delta^{18}O$ and $\delta^{2}H$ of water will be determined by cavity ringdown spectroscopy (Picarro 2130i). Our Picarro cavity ring down spectrometer makes these measurements inexpensive and routine. Finally, we will conduct the full spectrum of standard water quality analyses common to all CZO e.g., major anions and cations, pH, conductivity, etc. on both pore and surface waters.

The kinetics of many of the biogeochemical and environmental processes investigated at the proposed CZO will vary greatly, and residence time measurements of the various hydrologic compartments will need to be made. Chlorofluorocarbons (CFC) will be used to assess hydrologic residence times. Water samples will be collected using USGS recommended protocols and analyzed by purge and trap gas chromatography with electron-capture detection.

On a broader scale, we will investigate hydrology via a combination of aerial information (National Agricultural Imagery Program (NAIP) and NEON/CZO LiDAR information) and satellite data (Landsat and high-resolution optical satellite imagery from commercial sensors the Polar Geospatial Center or PGC at the University of Minnesota). The PGC works with The National Geospatial-Intelligence Agency (NGA) to task and license commercial mono sub-meter imagery, is available free to federally funded researchers (see Letter of Support). This will allow a determination of stage-area-volume relations for all wetlands across the study areas, which will lead to much improved understanding of short-term hydrological responses to climate as quantified by various indicies, such as the standard precipitation index and Palmer drought severity index[92,93] Wetland density and water body surface area can be modeled using power laws[92,94] and we will use this tool to assess surface water and to a lesser degree groundwater storage as a function of time. Wetland responses to these changing atmospheric processes will be compared to detailed measurements of evaporation and/or evapo-transpiration over the adjacent landscape from the NEON FIU towers. The hydrologic data is critical to putting the chemical and microbiological measurements below into the wetland continuum context. These same areal and satellite data will be employed to scale our results to broader portions of the PPR.

*Hydrochemistry:* The hydrochemistry of pond/lake water, groundwater, and pore water will be investigated at several spatial and temporal scales. Wetland, soil pore water, and groundwater chemistry will be monitored on a weekly basis for water chemistry, including major, minor and some trace ions, DIC and /DOC. Samples will be collected on a or more frequent basis to evaluate effects of overland flow from snowmelt and storms, and to determine diel cycles of redox species. Near continuous measurements of temperature, dissolved oxygen, pH, turbidity, and specific conductance will be taken for selected periods using Hydrolab DX-5 sondes available from UMN. Analyses will be performed at UMN and USGS Denver. Major and trace cations will be analyzed by inductively coupled plasma (ICP)-mass spectrometry and ICP-atomic emission spectroscopy. Alkalinity will be measured by Gran-titration. Anions and ammonium will be determined by ion chromatography. Concentrations of $NO_3^-$ below the IC detection limit and total N will be analyzed by chemiluminesence. $NH_4^+$ will be analyzed by colorimetry when Na interferences impede measurement by IC. Total phosphorus will be determined by ICP-MS and orthophosphate by colorimetry (phosphomolybdenum blue method). Dissolved gasses ($CH_4$, $CO_2$, $N_2O$) will be extracted by shaking water samples with ultra-high purity nitrogen[95] and analyzing the gas phase by gas chromatography at the NPWRC.

Stable isotopes of C, S, and N of selected chemical species will be determined for select samples. Sulfate will be precipitated as $BaSO_4$ and the $\delta^{34}S$ value of sulfate determined by elemental analysis-isotope ratio mass spectrometry (IRMS) at USGS Denver laboratories. The $\delta^{13}C$ value of $CO_2$ and $CH_4$ will be simultaneously determined by cavity ringdown spectroscopy (Picarro 2201i). Water for $\delta^{13}C$ and concentration of DIC and DOC will be filtered inline to avoid exposure to atmosphere and stored over ice in dark until analysis. Analysis will be performed on OI Analytical TIC/TOC analyzer coupled to Thermo-FisheDelta V Plus IRMS. Sources and cycling of N will be evaluated using $\delta^{15}N$ of water measured at the USGS Reston Stable Isotope Lab. Given sufficient concentrations, $NH_3$ will be extracted by diffusion and reacted with Cu to form $N_2$, which will be analyzed for $\delta^{15}N$-$NH_4^+$ by IRMS[98b]. A microbial culture of *Pseudomonas aureofaciens* will be used to react $NO_3^-$ to $N_2O$, which will be analyzed for $\delta^{15}N$-$NO_3^-$ by IRMS.[96] Soil and sediment N concentrations and $\delta^{15}N$ will be analyzed by pyrolysis and IRMS at the USGS Denver Stable Isotope Lab.

*Organic Matter Analysis:* SOC will be extracted from sediment and soils using standard base extraction methods. DOC will be used unaltered or isolated by solid phase extraction.[97] Light absorbance (specific UV absorbance, spectral slope) and fluorescence spectroscopy (excitation emission matrix or EEM and fluorescence indices) will be used to characterize changes in organic matter composition.[98-101] EEMs can be analyzed quantitatively using parallel factor analysis (PARAFAC),[100] and we will create a model specific to PPR organic matter. Seasonally, we will conduct more robust OM analyses by high-pressure size exclusion chromatography (HPSEC) to determine seasonal changes in the molecular weight distribution and by Electrospray Ionization Fourier Transform Ion Cyclotron Mass Spectrometry (ESI-FT-ICRMS) to determine OM structure[102,103] and multi-dimensional aqueous phase 1H and 13C-NMR using water suppression techniques[104] as well as solid state NMR for SOC. We will be able to perform these measurements at Old Dominion University (see letter of support). These analyses will allow us to catalog the diversity of OM composition in the PPR over time and space. Finally, we will conduct OM bacterial productivity studies to assess OM bioavailability over time and space using the leucine uptake method. Incubations will be done with either DOC or SOC using membrane filtered inoculum (from wetland water) for two weeks and subsamples taken and spiked with tritiated leucine to assess incorporation into bacterial biomass.[105,106]

*Redox cycling of major elements (Mn, Fe, S)*: We will collect/probe pore-water using various methods including squeezing core material, lysimeters, and peepers. Anoxic field and laboratory collection techniques will be used as necessary. Pore water chemistry will be measured as described above for wetland and groundwater. Reduced sulfur (bisulfide, polysulfides), Fe(II), Fe(III), and Mn(II) will be measured in-situ by voltammetry. These measurements can be made continuously using mercury-gold amalgam electrodes that are made at Ohio State and connected to a DLK-70 voltammeter.[107] The electrodes are robust and will be inserted into the sediments using an aluminum drivepoint or simply placed down a well. The voltammeter will be housed in a secure box and can be powered by a car battery that can be exchanged as needed. Cross-checks and calibrations of electrodes will be made with colorimetric determinations of redox species concentrations on select samples using standard colorimetic methods (e.g., methylene blue method for bisulfide and ferrozine for Fe(II)).

X-ray absorption spectroscopy (XAS) will be used to assess the speciation of solid phase iron, manganese, and sulfur-bearing phases in the soils, sediments, and suspended particles using published methods.[50,108,109] Co-P.I. Toner has led continuous synchrotron instrument proposals at the Advanced Light Source, Lawrence Berkeley National Laboratory, since 2005. Stable sulfur isotope measurements of sulfate and reduced sulfur will be made on select samples using the methods previously described. In addition to the major redox active elements (O, N, Mn, Fe, S, C) certain trace metal/metalloids of concern will also be characterized. For example, the content of Se, As, and Mo is enriched in the till (hosted by a component of black shale) and will be measured in solid and liquid samples during ICP-MS analyses. Mercury deposition and methylation in region are also of interest, because past works have shown mercury methylation is higher in prairie wetlands than other aquatic systems.[110] Of particular interest to this study is methylation along sulfate gradients. Co-PIs Nater and Toner have extensive experience with ultraclean Hg sample collection and handling protocols and quantification via cold vapor atomic fluoresence spectroscropy.

*Ecology/macrobiology:* Plant communities of each study wetland bisected by the CZO primary transects (Figure 5) will be quantified and compared to earlier studies.[76] Aerial photography (e.g., from NAIP) will be used to delineate the extent of each plant community within study wetlands. A list of plant species in each delineated community will be developed and cover (%) of each species will be estimated within ¼ $m^2$ plots. Vegetation height will also be measured in each plot. The number and location of plots will reflect the size and shape of delineated communities. Plant identifications will be verified at the NPWRC's herbarium. We will also quantify upland plant communities through the placement of randomly located sampling plots located along secondary transects that will radiate from a wetlands edge to the topographic high dividing adjacent watersheds. Detailed measurements of plant communities will be augmented with data collected by NEON and LandSat/MODIS derived plant metrics, e.g., biomass, normalized difference vegetation index (NDVI). We will also explore the use of data generated from

LiDAR flights to estimate canopy height and other plant community metrics. We will relate plant community data to hydrogeochemical data using a variety of statistical techniques, including ordinations from non-metric multidimensional scalings.

*Microbiological community structure and function:* We will determine the microbial community structure and functions of the both upland and wetland soil/sediment at different spatial and temporal scales. Microbial community structure will be assessed through pyrosequencing of 16s rDNA and microbial membrane phospholipid fatty acid (PLFA) analysis. Pyrosequencing provides very detailed information about the specific microorganisms present and PLFAs provide a better quantification of changes in microbial populations and structure over short time periods.[111] In addition, stable carbon isotope measurements of PLFAs provide information on microbial carbon sources/cycling and are especially useful for quantifying the importance of $CH_4$ as an ecosystem carbon source.[112-116] Microbial function will be assessed through functional gene analysis (e.g. mca genes for methanogenesis, dsr genes for sulfate reduction) as well as pore water geochemical and stable isotope measurements, solid phase S and Fe speciation measurements by X-ray absorption spectrometry, and in-situ redox measurements.

Microbial genomic DNA will be extracted from these samples using established protocols.[117-119] Briefly, microorganisms will be isolated and the cells lysed. DNA will be pelletized and its quality, purity, and concentration of DNA will be determined by agarose gel electrophoresis and UV spectrophotometric measurements. The purified microbial genomic DNA will be amplified by multiple displacement amplification. Approximately 10 μg of the purified genomic DNA will be sent to the Plant-Microbe Genomics Facility at The Ohio State University for massively-parallel DNA pyrosequencing. Microbial 16S rRNA genes will also be amplified and sequenced. for-fee basis by staff working in the Plant-Microbe Genomics Facility at The Ohio State University. The staff at the Plant-Microbe Genomics Facility will assist the PIs with sample preparation, as well as perform library construction, sequencing, and data analysis to identify the operational taxonomic units and their concentrations associated with different temporal or spatial attributes of the Prairie Potholes.

Total lipids will be recovered from splits of samples collected for rDNA using a modified Bligh-Dyer extraction (USGS, Denver).[120] Phospholipids will be isolated using silica gel solid phase extraction cartridges and then methylated.[121] The resulting fatty acid methyl esters will be quantified by gas chromatography-flame ionization detection and mass spectrometry will be used for compound identification. Stable carbon isotopes of select PLFAs will be measured by gas chromatography-combustion-IRMS.[114,115]

*Contaminant processing:* PPWs can serve as potential sinks for anthropogenic pollutants. Both contaminant classes have the potential to disrupt ecosystem functions. We will assess the contaminant "assimilative capacity" across the PPW continuum. This work will emphasize transect 3 which is downslope from a large agricultural field. In the water column we will study direct and indirect (photochemically produced reactive intermediates e.g., ROS, triplet state organic matter) photolysis of pesticides. Using molecular probes and quenchers specific to ROS and triplet DOM we can quantify steady state concentrations of these species in laboratory experiments across the wetland continuum. Data from the CLSA[44] show that these photoreactive intermediates are important for pesticide attenuation, but the photochemistry of other PPR wetlands is not well known.

Our past work[39,40] has shown that reduced sulfur species and minerals in the sediments are able to degrade pesticides by nucleophilic and reductive reactions. While the geochemical characterization of wetland sediments will identify and quantify these reactive species, we will also investigate attenuation by sorption and reduction. Sorption is likely important for even moderately hydrophobic compounds due to the high solids-to-ratio found in sediments. We will collect PPW sediments for sorption experiments on yet to be determined pesticides and treat it (autoclaving, exposure to oxygen, etc.) to eliminate analyte losses due to degradation. Sorption isotherms will be conducted in batch systems and the data fitted to an appropriate isotherm equation. We will also conduct transformation experiments with untreated PPW sediment. The reactions will be performed in a deoxygenated buffer solution to delineate sediment-mediated transformation of pesticides from pore water processes. Experiments will be executed in anaerobic conditions and analytes assayed by liquid chromatography. Reduction reaction will be quantified as additional pesticide loss beyond that expected by sorption. We will assess the "assimilative capacity" of the sediment by repeating the experiments using the same sediments multiple times.

*Summary:* The results of these interrelated hydrologic, chemical, microbial and ecological measurements will reveal how hydrology and geology dictate biogeochemical and ecological proceses along the continuum from recharge to discharge wetlands in water and sediments. Understanding these gradients will allow prediction of important biogeochemical processes and pollutant processing. Additionally, the studies will assess the complex relationship between microbial metabolism and geochemical processes within the wetland continuum framework and will inform CLM modeling.

*Hypothesis 3*: *Using the wetland continuum model (Figure 5) as a framework, we hypothesize that there are unique response functions for each greenhouse gas ($CO_2$, $CH_4$, and $N_2O$), that are dependent on hydrologic regime and associated plant communities. The variations will depend on the seasonal precipitation, water table, vegetation, and $O_2$ concentrations in the near-surface soil profile.*

The contemporary GHG budget of the recharge, flow through, and discharge wetlands will be evaluated using flux chambers and micrometeorological (i.e. Eddy Covariance, EC, and Flux-Gradient, FG) techniques. The specific site selection will be informed by the data acquisition and analyses related to Hypothesis 2. The goal is to select representative wetlands along the continuum model domain that have adequate aerodynamic fetch for micrometeorological applications. The measurements will then determine how location within this framework affects GHG budgets. Using supporting data such as temperature, high resolution LiDAR, satellite estimates of NDVI, geospatial analyses, and site specific models, these findings will be generalized/extrapolated across the PPR.

*GHG Chamber Measurements:* We will measure trace gas emissions of various wetland plant species (both native and introduced) and relate observed differences in plant physiological processes to their affects on geochemical processes and climate drivers. We will collect weekly GHG samples from spring thaw through approximately October using the static chamber method.[122] Samples will be collected from within stands of a minimum of four dominant wetland plant species. One species will be reed canary grass (*Phalaris arundinacea)*, a species known to alter oxic conditions in its rhyzosphere. At each site, we will insert a chamber base (polyvinyl chloride ring) into the soil. To collect gases, a chamber made of polyvinyl chloride equipped with a pressure vent and a septum for syringe sampling will be sealed to the chamber base. Headspace gas samples will be withdrawn from each chamber at the end of a 30-minute accumulation period (sufficient to detect and quantify emissions of $CO_2$, $N_2O$, and $CH_4$) using a syringe. Gas emission sampling will be conducted from 10:00–14:00 h during each sampling event. Selected additional measurements will be made in conjuction with the micrometeorlogical measurements described below. All samples will be transferred to, and stored over-pressurized in, pre-evacuated serum bottles fitted with gas-impermeable septa. Samples will be analyzed within one week via gas chromatography, but tests have shown that $N_2O$, $CH_4$, and $CO_2$ concentrations remain stable for four weeks.

*Micrometeorological Measurements of Energy, Water and Greenhouse Gases:*Simultaneous micrometeorological flux measurements of $CO_2$, $CH_4$, and $N_2O$ provide a unique opportunity to quantify the GHG budget and the associated radiative forcing of representative sites within the PPR. Eddy covariance (EC) measurements of evapotranspiration will also be made to help constrain the water balance of these wetlands and the PPR. The detailed hydrologic, hydrochemical, biogeochemical, microbial, and ecological data and analyses, described in the previous sections, will be used to help interpret the temporal, seasonal, and inter-annual variability in the GHG fluxes. Together, these data and analyses will be used to compute site-specific annual GHG budgets and will be used to help improve the description of the biophysical processes represented in process models. These models will be used to help scale up to the larger PPR and assess the sensitivity of PPR GHG budgets to perturbations in climate, hydrology, land use, or other anthropogenic factors.

The GHG budgets will be assessed using a roving EC ($CO_2$ and $CH_{4;}$ measured using the latest open-path and closed-path gas analyzer technologies (LI-7700 and LI-7200, respectively Licor Inc), and flux-gradient (FG) ($N_2O$; using a novel gas filter correlation technique (Teledyne Instruments, API, M3120EU2 analyzer)[123] system and chamber-based approaches. A set of common simultaneous measurements (i.e. $CO_2$ and $H_2O$) will also be made at the NEON tower site. Given equipment limitations, the micrometeorological equipment will be rotated among the transects every 3-4 weeks with the NEON site serving as an anchor point for comparisons. Instrument operation, data processing and handling of data gaps will follow the standard eddy-covariance protocols. We will follow AmeriFlux and NEON guidelines regarding best measurement practices and quality control of the data. Our EC system will be co-located with the NEON tower at the beginning of the study to evaluate the measurement quality and our flux processing procedures.

The relationships observed among species composition, LAI/NDVI, nutrient status, and hydrologic regime will be used to interpret the NEE observations and to help scale-up NEE estimates for the PPR. Methane emissions will also be measured simultaneously using the same roving EC system and are expected be a function of soil/water column temperature, water table height, and NEE.[124] The very high-frequency (20 Hz) measurements of $CH_4$ using an open-path sensor will provide an excellent opportunity to examine the role of ebullition events and their potential influence on the methane budget.-In addition, we will use chamber-based approaches to capture these events and examine their correlation with the EC-based detection approach. The total carbon budget of these systems will be determined from the EC fluxes of $CH_4$ and $CO_2$, but will also need to consider the flux of DIC/DOC in the form of runoff, drainage, and ground water flow. Because we are using a roving tower technique (i.e. there are limited resources to make simultaneous measurement at each of the key sites) the annual budget information will require interpolation through time using process-based functions. The multiple years of measurements will provide an opportunity to evaluate these response functions by testing them against independent data from different years or boot-strapping approaches using all data.[124]

$N_2O$ emissions are expected to be highly episodic and dependent on precipitation events and fluctuations in water table position with peak emissions correlating with sites that have high nutrient input and high NEE. We hypothesize that wetlands with ground water input that has an agricultural fingerprint will be associated with the highest $N_2O$ emissions due to N inputs in the form of synthetic fertilizer and manure. Consideration of the total GHG budget will show that carbon sequestration in the most productive wetlands (highest NEE) will largely be offset by $N_2O$ and $CH_4$ emissions. When considering the global warming potential of each gas on a 100-year time horizon, even the most productive wetlands will likely have a positive radiative forcing on climate, the exception might be the upland/recharge zones where NEE is expected to fully compensate $N_2O$ and $CH_4$ emissions. An important goal of our work is to use these contemporary fluxes and environmental relations, to understand how these systems might have changed historically, and how future environmental conditions are likely to impact GHG budgets and radiative forcing effects.

The "net climate" impact will be assessed by considering the annual exchange of $CO_2$, $N_2O$, and $CH_4$ as well as net annual energy exchange. The latter encompasses latent and sensible heat exchange between surface and atmosphere, ground heat storage and release, absorption of solar radiation, and net exchange of thermal infrared (longwave) radiation. All of these transfers of mass or energy will potentially be variable along the wetland continuum and will be sensitive to seasonal and interannual variations in precipitation, temperature etc. Our GHG fluxes will be expressed as the $CO_2$-equivalent global warming potential (GWP). Shortwave radiation changes will be expressed as global radiative forcing by scaling these changes with the total surface area of the Earth and adjusting for atmospheric absorption.[125-127]

**Timeline that includes which PIs/SPs are responsible for guidance of specific activities.**

**ENGAGEMENT PLAN:** The infrastructure put in place at WFS and the co-location with a NEON core site will provide unique opportunities for the scientific community to conduct research at the PPR CZO. We will use multiple avenues to ensure that scientists not directly affiliated with this proposal are able to conduct relevant research at the site. 1) To inform nonparticipant scientists about the details of the PPR CZO, we will hold public forums in which the science plan and instrumentation of the PPR CZO will be described. Additionally, question and answer sessions will be held at national meetings of the American Geophysical Union and the American Society of Limnology and Oceanography. 2) In years 2 and 3 of the project, we will host on-site 2-day workshops for up to ten scientists interested in conducting work at the site, so the ongoing activities and infrastructure can be viewed first hand. The workshops will be advertised via various email listservs, and applicants for the workshops will have to put forth a research idea to be considered for acceptance. We anticipate providing funds for ten participants ($10,000 budgeted in Y2 and Y3) per offering, but additional participants will be welcome if they are able to fund their own travel. 3) The PPR CZO will also fund seed grants (ranging from $2,500- to $10,000) to allow non-CZO participants to conduct research at the PPR CZO. A requirement of seed projects will be that they seek to conduct research that does not duplicate ongoing efforts. Funds for these seed grants ($90,000 total) are included in the project budget. 4) The PPR CZO will make collected data available on the web as specified via the CZO Network. Providing data to the community at large will allow researchers not directly affiliated with the CZO to provide insight, develop new models and analyses, and suggest new measurements that would not be possible if data were not available. 5) As described in more detail below, we will hire Julie Zickefoose (a writer, naturalist, and National Public Radio commentator) to serve as the PPR CZO blogger. By using a blog to highlight CZO activities at regular intervals, the scientific community and public will be able to follow our activities more closely. We will ensure that the blog is disseminated widely by using social media (e.g., Twitter and Facebook) to allow “followers” to know when new content is available.

**DISSEMINATION PLAN:**The dissemination plan will follow a multi-pronged approach to ensure that the scientific community, stakeholders, and the public learn about the scientific activities and findings of the PPR CZO. Collected samples will be registered with the System for Earth Sample Registration and given an IGSN number so that samples can be tracked within the CZO and requested by other researchers. Data, models, and other tools will be shared via the CZO National website and conform to any protocols developed by the CZO National Office. Funds for a data manager have been included in each budget year to ensure that there is an individual responsible for handling the associated server and web pages.

Scientific products will also be disseminated via presentations at national conferences, peer reviewed publications, and technical reports. The PPR CZO will work closely with NEON to organize sessions at relevant national conferences that will attract scientists from both the geoscience and ecology communities e.g., American Society of Limnology and Oceanography, American Geophysical Union, the Ecological Society of America, etc. We will also work with the public relations offices at our respective institutions to develop press releases highlighting major research findings and brochures/flyers/magazine articles that highlight the PPR CZO research.

In each summer of the project, we will recruit two high school teachers (one from Minneapolis/St. Paul, MN and one from Columbus, OH) to work as a field and lab assistant at the PPR CZO for a two week period. This will be modeled after the Teachers and Researchers Experiencing and Collaborating (TREC) program through the Arctic Research Consortium of the U.S. Co-PI Chin has hosted TREC teachers on NSF sponsored projects and has served on the participant selection panel. Funds for this activity (dubbed Prairie-TREC) are included as participant support costs. Each teacher will be selected from a pool of applicants.The Science Museum of Minnesota (SMM) will aid in the annual selection of a Twin Cities teacher for PrairieTREC by informing teachers of this opportunity through its *Science House* and *Nexus*. *Science House* is SMM’s onsite resource center where educators can check out classroom sets of hands-on materials for their students and engage in formal and informal consultation and professional development. *Nexus* is an SMM initiative for district-based leaders, curriculum coordinators and others with responsibility for K–12 STEM education to address racial, class, and gender-based achievement gaps. The teachers will keep an electronic journal of their experiences and be active participants in sampling, on site data collection and analyses, and research team meetings. The individuals will be expected to transfer knowledge into the classroom and integrate CZO research topics into their lessons. We will work closely with the teachers to help them develop teaching modules for their classrooms and to share the classroom implications of their PrairieTREC experiences with other teachers.

The PPR CZO research will be disseminated to the public via interactive activities at the SMM. **ONCE AND FUTURE EARTH** is the SMM's strategic initiative to help a wide range of audiences appreciate that humans now rival natural processes in creating global change and that a diverse array of innovations is needed for people to thrive on this planet. Patrick Hamilton, SMM's Director of Global Change Initiatives, will be responsible for integrating the work of the Prairie Pothole Region CZO into the work of the museum. In particular, the PPR CZO will take advantage of two existing SMM assets, namely **FUTURE EARTH and EARTH BUZZ.** SMM's Future Earth exhibit (DRL 0741760) opened to the public in April 2012 and explores how humans are now the dominant agents of planetary change. SMM will incorporate the PPR CZO into this exhibit. In particular, SMM will contract with Twin Cities Public Television to produce a short two-three minute video with one or more PPR CZO researchers for inclusion into the menu of choices available in the Future Earth theater. SMM created Science Buzz (ESI 0337389) in 2002 to design and test digital and exhibit templates that permit museums to rapidly add current science content to their exhibit floors. It has since grown into a dynamic, popular online destination with a global audience and a physical presence on the exhibit floors of 15 U.S. museums. EARTH BUZZ is a subset of Science Buzz that focuses on environmental issues. Once a month, SMM, in collaboration with the PPR CZO steering committee, will select a PPR CZO topic to highlight and then will use the various EARTH BUZZ templates – Scientist-on-the-Spot, Buzz Blog, Science in the News – to facilitate online discussions of this topic. SMM will work to encourage graduate students involved with the PPR CZO to use Earth Buzz to develop writing styles that are interesting and accessible to Earth Buzz's online audiences and to visitors who explore the kiosks installed in museums across the U.S.

Julie Zickefoose has agreed to participate in the proposed CZO as its official nature blogger. Julie is best known for her nature commentaries on NPR's All Things Considered, her art, and her books, *The Blue Bird Effect* and *Letters from Eden*. Julie is also one of the guest guides at the annual Prairies and Potholes Birding Festival held each June. Her blog (http://juliezickefoose.blogspot.com/) has a large following and she will create a separate blog linked through her own blog and the CZO website that will explain the science being conducted there. Julie will post her PPR CZO stories both on her blog and also on SMM's Earth Buzz. She has a huge following among birders and given the importance of the PPR as a stopover on the North American Migration Flyway, we believe that Julie is an excellent outreach conduit to the birding community and public at large. We have budgeted travel expenses (twice annually) for Julie to come out into the field with us as well compensation ($8,000 to $9,000 annually) for writing roughly twenty blog articles annually.

**Broader Impacts:** The PPR CZO will *advance discovery promoting teaching, training and learning* via 1) professional development of teachers (Prairie-TREC); 2) student participating in professional meetings; 3) postdoctoral training in teaching; 4) holding a PPR CZO video conference seminar for all investigators twice per month; 4) development of joint hydrology/biogeochemistry "May-mester" field camp for both UMN and OSU students. Ito will work with her contacts at Sitting Bull College to *establish summer internships for Native American students* involving summer fieldwork at WFS. Ito currently directs an NSF OEDG (Opportunities for Enhancing Diversity in Geosciences) program with collaborators at Fond du Lac Reservation and through her affiliation with Geoscience Alliance (see support letter from Diana Dalbotten) has the necessary connections for this effort.

We will *build research infrastructure* through the collaboration among two major universities, government scientists, and NEON. The PPR CZO will serve as a new multi-user field facility, and leverage investments made by NEON and the USGS. Our team will *enhance scientific understanding* via the SMM and blogging efforts describe above. Additionally, the seed grants, conference presentations, scientific publications, and data availability will inform the broader scientific community. Lastly, the *benefits to society* of the PPR CZO are that improved understanding of critical zone function and GHG emissions in a unique North American ecosystem will lead to improved resource protection, land management/development, and agricultural practices. The partnership with federal scientists will lead to integration of the work into USGS programs of national interest.

**Results from Prior NSF Support:** All published papers resulting from prior NSF support are listed in the References list. **Euliss, Goldhaber, Mushet, Nater,** and **Rosenberry** have not had NSF support in the past five years.

**Arnold and Chin:** a) EAR-0910692 and EAR-0911296; $152,784, July 2009-June 2011 b) *Collaborative Research: Redox Processes in the Sedimentary Pore waters of Prairie Pothole Lakes: Implications for the Attenuation of Pesticides; Chin and Arnold.* c) *Intellectual Merit:* We showed that the pore water constituents are highly reactive to pesticides, and the results greatly enhance our understanding of how these PPWs function to attenuate agrochemicals used in the region. Additionally, the sulfur speciation/dynamics in the sediment were quantified via XANES spectroscopy. *Broader Impact:* It will be possible to better plan pesticide application and tillage practices based upon the known geochemistry of the neighboring lakes. Pesticide attenuation also provides a reason to maintain and restore prairie lakes/wetlands. We initiated a research collaboration with USGS scientists, funded the education of two graduate students, and worked with 8-11$^{th}$ grade students through on-campus summer camps. Results were broadly disseminated via national conferences. d) Five papers have been published. e) The major research products are the publications and the extensive data sets in the associated electronic supporting information files. Although this pilot study demonstrated the potential for the millions of PPWs to mitigate the harmful effects of pesticides, more work is needed to understand the links between the hydrologic and biogeochemical processes in PPWs, and this is one of the motivating factors for the CZO proposal. **Chin, Arnold,** and **Goldhaber** have a pending proposal to study how ET and local hydrology affect sulfur dynamics, DOC levels, and pesticide attenuation in porewaters at CLSA. The proposal would complement CZO efforts, but has minor overlap due to more focused efforts/goals.

**Griffis**: a) ATM-0546476; $600,904; 5/2006-4/2012. b) *CAREER: Measurement and Modeling of Land-Atmosphere Isotopic $CO_2$ Exchange in the Upper Midwest, United States"* c) *Intellectual Merit:* Results demonstrate that we can partition the relative contributions of C3 and C4 species to the regional atmospheric carbon budget. Our research also demonstrated the importance of short-term variations in the carbon isotope composition of respiration and its influence on partitioning net ecosystem $CO_2$ exchange into the component fluxes. Through this grant we also worked through the major details of measuring isotopic water vapor fluxes and the canopy scale kinetic fractionation effect for isotopes of $CO_2$ and water vapor and the validation of the SiLSM model. *Broader Impacts:* This work will lead to improved prediction of greenhouse gas fluxes in agricultural landscapes. Two undergraduate and three graduate students and one post doctoral scientist were supported. Results have been presented at several national meetings. d) A total of ten papers have been published. e) The major research products are the publications and the extensive data sets in the associated electronic supporting information files. This work forms the basis for the study of GHG budgets propose herein.

**Hamilton:**a) DRL-0741760, $ 2,999,377, 08/31/2008 to 09/01/2013; b) *Future Earth Initiative*; c) *Intellectual Merit:* The Future Earth Initiative brought the SMM together with the University of Minnesota's Institute on the Environment and six NSF Science and Technology Centers (STCs) to develop educational deliverables exploring the concept of humans as the dominant agents of global environmental change. Deliverables include a 2,000 square-foot exhibit at SMM, web based Earth Buzz kiosks, and workshops with decision makers on the local and regional implications of climate change. The Future Earth Initiative catalyzed SMM to make a long-term commitment to global change education. *Broader Impacts:* SMM worked with George Mason University's Center for Climate Change Communication to conduct an audience segmentation analysis of the museum's audience. The results of this work were widely shared with the informal science education community and resulted in several other institutions conducting analyses of their audiences and the sharing of these results. Spawned by the Future Earth Initiative, a monograph (Future Earth: Advancing Civic Understanding of the Anthropocene) is being prepared for publication in 2013 by the American Geophysical Union. This book will be an anthology of research-based papers from those conducting innovative research into public attitudes toward environmental issues to those engaging with a wide range of public and policymaker audiences to advance civic appreciation of the implications of the Anthropocene. d) Future Earth: Advancing Civic Understanding of the Anthropocene. AGU Geopress (October 2013); e) SMM's reports on its global warming audience segmentation analyses are available at http://www.smm.org/researchandeval/samples.

**Ito** a) EAR -519255, $48,501; August 2005-August 2009 b) *Collaborative Research: Testing Potential of Paired Lakes on the NE Tibetan Plateau for Studying Interaction Between Hydrochemical Evolution and Environmental Change; Yu and Ito.* c) *Intellectual Merit:* Holocene millennial-scale climate variations in arid northeastern Qaidam Basin on the northeastern Tibetan Plateau were investigated using two cores from Hurleg Lake. The core lithology and ostracode species assemblage indicate that lake level has changed by many meters in the last 10,000 years, suggesting today's large groundwater inflow and perennial stream inflow may not have been reliable source of waters. Climate reconstructions highlighted the complexities involved in the spatial and temporal patterns of regional climate changes in regions of complex topography under two dominant climate drivers. *Broader Impacts*: One graduate student at Lehigh University and 5 graduate students at Lanzhou University were trained. One Lanzhou student came to U of MN-Duluth and completed her PhD. Technicians and graduate students at Lanzhou University were trained how to collect sediment cores. d) and e) Five papers have been published and are the major products of the work.

**Lower:** a) EAR-0525297, EAR-0525340, and EAR- 0745808; $940,983; 10/05- 3/14 b) *CAREER: The protein-mineral bond; Unraveling the bacterium-mineral interface - Nanoscale structures and forces.* c) *Intellectual Merit*: Molecular investigation of biogenic macromolecules and their fundamental reactions with mineral or material surfaces. Results from experimental and theoretical approaches provide deeper appreciation of phenomena at the interface between biological molecules and minerals/material surfaces. *Broader Impact:* We know much more about how bacteria form elementary bonds with inorganic surfaces. These grants supported 1 undergraduate Honors student, 7 graduate students (3 M.S. and 4 Ph.D.), and 3 post-docs. Collaborations were established with researchers from 6 U.S. and 3 international universities. PI created and teaches an annual, 1-week science field camp for 400 middle school students and 40 high school counselors (2009-2013). d) Over a dozen peer-reviewed research articles published in journals including *Science* and the *Proceeding of the National Academy of Sciences USA*. e) The major research products are the publications and conference presentations.

**Toner:** a) OCE-1038055; $198,147, 9/10 –10/13. b) *Collaborative Research: Integrating geochemistry, microbiology, and hydrodynamics: A model for trace element transport and fate in hydrothermal plumes.* c) *Intellectual Merit:* There is abundant energy available to microorganisms in hydrothermal plumes, but the net effect of microbial activity on hydrothermal fluxes of elements such as iron, sulfur and carbon to the ocean is not known for any site. The over-arching goal is to integrate geochemical and microbiological observations in a physical-geochemical model. *Broader Impact:* The project supports one Ph.D. student. The project supported the training of one undergraduate in analysis of X-ray diffraction data and travel to present at an international conference. Toner presented research results to public audiences, including 8th – 12th grade girls of color, as a 2011 Ridge 2000 Distinguished Lecturer. d) Two articles have been published. e) The major research products are conference abstracts, two publications, and mineralogical databases for hydrothermal plumes.