Final Report

Project Title: A multiple option demonstration, based on life cycle analysis, to transition from a coal mining past to a CO_2 sequestering future through diverse prairie establishment in reclaimed mine land for “beginning,” “limited resource” and marginal farmers/ranchers and landowners.

Project Manager: Corine Peugh

Timeframe Covered: September 15, 2010 to September 14, 2013

Grant Number: 69-3A-75-10-132

Date of Submission of Report: November 18, 2013

Deliverables Identified on the Grant Agreement:

- Establish four 15-acre sites in reclaimed coal land to demonstrate based on life cycle analysis of carbon balance and energy use how to grow high diversity prairie and how to support grazing for meat production.

- Conduct a series of workshops for farmers and ranchers to learn about the project options.

- Conduct outreach activities to disseminate the demonstration option to students, teachers and other partners.

- Document the workshops and outreach efforts with written narrative and pictures.

- Attend at least one NRCS CIG Showcase or comparable NRCS event during the period of the project agreement.

- Submit semi-annual performance progress report and a final report documenting project results.

- Develop a factsheet describing the new technology or approach

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Executive Summary

The Wilds, with the assistance of the Ohio State University Carbon Management and Sequestration Center and Applied Ecological Services, Inc, was able to successfully demonstrate the establishment of 60 acres of native, warm-season prairie on marginal land for use in an agricultural setting including showing the establishment process, management techniques, monitoring practices, costs, benefits and uses.

This demonstration project showed the multiple uses of prairie including use in grazing, hay and biomass production, carbon sequestration, soil improvement and wildlife habitat. These uses can provide multiple sources of revenue for landowners who incorporate this type of land conversion into their existing operation. Each use was assessed through monitoring of associated benefits, along with completing a life-cycle analysis comparing the land preparation and management techniques employed to help determine which method is most conducive to sequestering carbon. Results of these monitoring events were then compiled, along with establishment methods, costs and benefits into outreach events such as day-long workshops held at *the Wilds* each year as well as at several other events both at *the Wilds* and at outside events. These workshops allowed for an open discussion on the possibilities that come with prairie establishment, questions and concerns from landowners and a chance to help educate the public about cost-share options that would allow them to implement these practices.

Introduction

Based in the heart of economically disadvantaged Appalachian Ohio, *the Wilds* established a 60-acre demonstration project with four distinct replicable options of land preparation and land use management practices to initiate growing high diversity prairie on reclaimed coal mined land or other marginal lands, which are typically severely degraded and have poor productivity. The demonstration is based on established methodology that has already been tested and adapted at *the Wilds*. It enables ranchers and land-holders to chose options based on Life Cycle Analysis (LCA), to regenerate the land and support biodiversity while generating income through biomass production as feedstock for bioenergy or through high-quality grass-fed meat production, payments for participation in conservation reserves and through carbon-credit payoffs in the future for sequestering carbon through prairie roots and soils. Though based in Muskingum County, the demonstration is relevant for areas with similar land use history or risk of drought in neighboring states and beyond. Moreover, *the Wilds*, through its visitor operation (serving over 115,000 visitors each year from around the country and abroad,) outreach and education programs and the Conservation Science Training Program, has disseminated the model well beyond its boundaries to various stakeholders.

Background

Located on nearly 10,000 acres of reclaimed coal-mined land in the heart of Appalachian Ohio, *the Wilds* is a unique center practicing conservation through innovation, education and personal experience. In Ohio alone, there are over 700,000 acres of previously mined land (pers comm. 2005 T. Jackson, ODNR). Research to date has shown that high diversity prairie production is CO_2-negitive. Moreover, in a decade, high diversity prairie on marginal land can produce 238% more bioenergy than monocultures (Tilman et al., 2006) while avoiding pressure on food-growing land and thus avoiding the food vs. fuel controversy. Another estimate shows that the average sequestration potential for Ohio minesoils is about 3.50 Tg CO_2 (1Tg= 1 million metric tons) (Ussiri and Lal, 2005). Improving management practices including introducing high diversity perennial mixtures and nitrogen fixing plants may further increase the potential for soil organic carbon (Conant et al., 2001.)

In 2008, *the Wilds* with sponsorship from Rentech, Inc. and in partnership with the Ohio State University launched an experiment on 20 acres to establish high diversity prairie on reclaimed coal mine land. Through the interim results of the various treatment options, *the Wilds* has gained insights about the land preparation, land management and species combination necessary for a high-diversity prairie establishment (Sengupta et al., 2010).

Situated in the economically disadvantaged and previously coal mined area, *the Wilds* is well suited to offer practical and replicable options in the form of four demonstrated models for farmers, ranchers and other land owners to regenerate the land while earning economic benefits. This is a critical first step to transitioning to a CO_2 sequestering economy of the future. There are four primary uses that were demonstrated including soil improvement and carbon sequestration, facilitated by deep prairie roots. Secondly, prairie shoots can serve as a feedstock for bioenergy, grazed for high quality prairie-fed meat production or can be pyrolyzed for bio-char production, which as a soil additive will increase CO_2 sequestration. Third, prairie can provide drought-tolerant forage during the hot summer months when incorporated into a rotational grazing system. Lastly, prairie reserves may be worth subsidies from government agencies. Each of the four options will provide cost-benefit choices and practical steps to regenerate the land, support biodiversity and address climate change adaptation by enhanced CO_2 sequestration, while earning income.

Review of Methods

Based on many years experience of establishing high diversity prairie in reclaimed mine soils, *the Wilds* employed three different land preparation methods for the 60 acre demonstration: (a) sub-soil and till, (b) till only, (c) two parcels of no-till, one with bison grazing which began in 2013 (Figure 1).

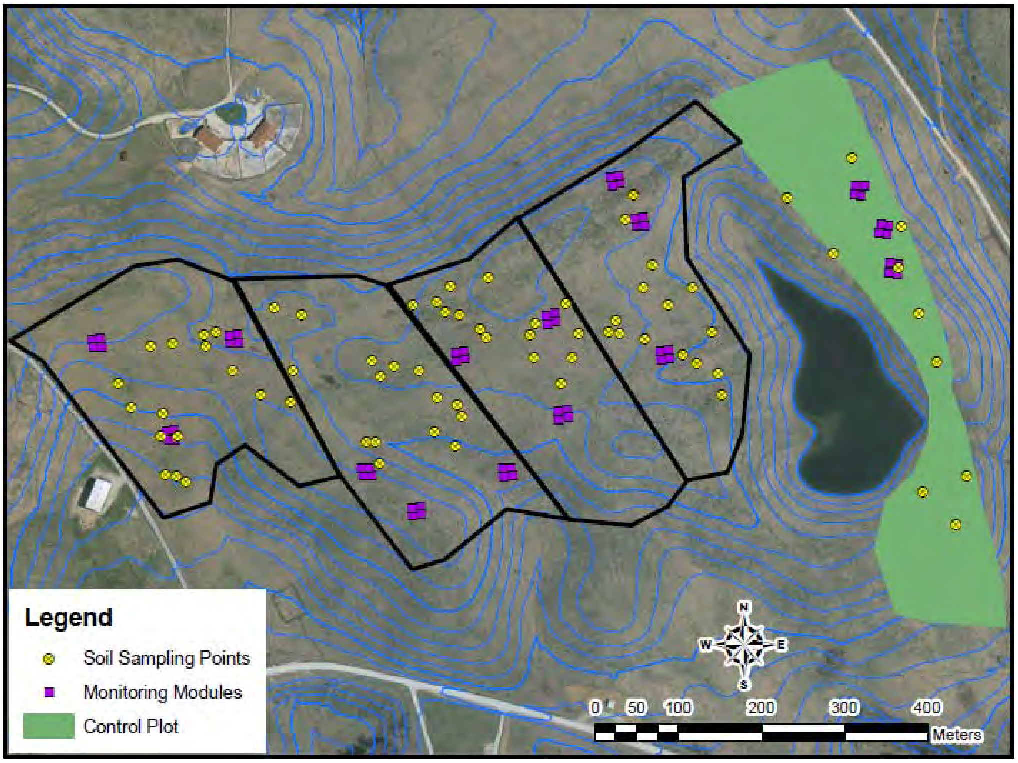


Figure 1. *Map of the 60-acre demonstration site on the Wilds' property with location of sampling modules used for monitoring. The four 15-acre plots from left to right are: no-till with grazing in 2013, no-till, till only, and till with subsoiling.*

Site Preparation

All four of the plots within the 60 acre demonstration site were originally dominated by cool season grasses used to establish cover during post-mining reclamation. In order to prepare the site and ensure good stand establishment, autumn olive (*Elaeagnus umbellata*) shrubs were treated and removed and a prescribed burn was conducted across the entire site. Following green-up, cool-season grasses were treated with an herbicide application and the land preparation treatments were applied (tilling and tilling with subsoiling) prior to seeding. All four plots were planted with the same seed mix using a no-till drill in June 2011. The species and ratios used in the seed mix are shown in Table 1. Regular maintenance to date has included mowing for weed control and maintaining the firebreaks surrounding the plots.

Table 1*. Seed mix planted in 2011 for all 4 treatment plots.*

Quantity	UOM	Species	Unit Price	Ext. Price
0.12400	lb	Big Bluestem, ‘Niagra’ (*Andropogon gerardii)*	$10.00	$1.24
0.11790	lb	Switchgrass, ‘Shawnee’ (*Panicum virgatum)*	$7.00	$0.83
0.12730	lb	Eastern Gamagrass (Tripsacum dactyloides)	$10.00	$1.27
0.10790	lb	Indiangrass, PA Ecotype (*Sorghastrum nutans)*	$16.00	$1.73
0.06440	lb	Canada Wild Rye (*Elymus canadensis)*	$7.00	$0.45
0.06370	lb	Little Bluestem, ‘Camper’ (*Schizachyrium scoparium)*	$13.00	$0.83
0.02740	lb	Round Head Lespedeza (*Lespedeza capitata)*	$140.00	$3.84
0.04000	lb	Showy Tick Trefoil, PA Ecotype (*Desmodium canadense)*	$140.00	$5.60
0.05700	lb	Wild Senna, WV & VA Ecotype (*Senna hebecarpa)*	$32.00	$1.82
0.03730	lb	Purple Prairie Clover (*Dalea purpurea)*	$36.00	$1.34
0.08060	lb	Purple Coneflower (*Echinacea purpurea)*	$28.00	$2.26
0.04500	lb	Maximillian’s Sunflower (*Helianthus maximiliani)*	$40.00	$1.80
0.03380	lb	Ox Eye Sunflower, PA Ecotype (*Heliopsis helianthoides)*	$48.00	$1.62
0.01560	lb	Wild Bergamot (*Monarda fistulosa)*	$196.00	$3.06
0.00810	lb	Grey Headed Coneflower, OH Ecotype (*Ratibida pinnata)*	$48.00	$0.39
0.00810	lb	Sweet Black Eyed Susan (*Rudbeckia subtomentosa)*	$96.00	$0.78
0.00940	lb	Tall Coreopsis, OH Ecotype (*Coreopsis tripteris)*	$300.00	$2.82
0.01500	lb	Cup Plant (*Silphium perfoliatum)*	$40.00	$0.60
0.01440	lb	New England Aster, PA Ecotype (*Symphyotrichum novae-angliae)*	$248.00	$3.57
0.00310	lb	Slender Mountain Mint (*Pycnanthemum tenuifolium)*	$360.00	$1.12
1.00000	**lb**	**Total**	**$36.97**	**$36.97**

Monitoring Methodology

In order to assess the performance of prairie for the multiple uses being demonstrated, the four demonstration plots as well as two reference sites. These reference sites serve to provide an idea of what results might be expected in landscapes not planted into prairie. The first reference site is a 15-acre control plot adjacent to the 60-acre demonstration on the same previously surface mined and reclaimed grassland. The second reference site is located on a partner farm in nearby Chandlersville, OH, where a calf cow operation has been run on unmined land for several generations. All monitoring activity can be seen in Figure 2. Methods include vegetation surveys for composition, small mammal diversity through trapping and arthropod diversity via sweep netting. The potential of the landscape to sequester carbon is being estimated through annual soil samples which are analyzed for carbon content as well as bulk density. In the third year a Life Cycle Analysis (LCA) was conducted for each of the treatments to compare the net carbon balance of each of the treatment plots. Forage quality, biomass yield and weight gain by bison were also assessed in the third year along with an ethogram study to determine differences in grazing behavior of bison on warm versus cool-season forage.

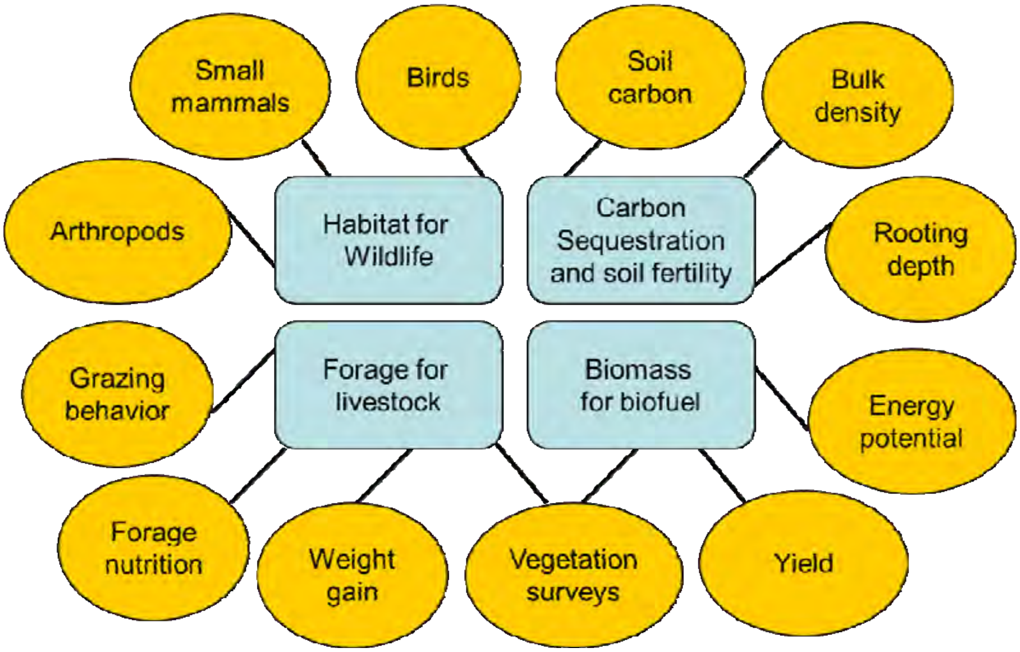


***Figure 2**. Monitoring efforts indicated by yellow ovals, and the associated prairie crop that they are being used to assess are indicated by the blue squares.*

<u>Soil Analysis</u>

Soil sampling was conducted by Applied Ecological Services (AES). Sections from the final report provided by AES are included below and the full document can be found in Appendix A. Using GIS tools, a boundary area of the plots was randomly generated which intersects the four management zones, and randomly generated 14-16 sample points distributed within each zone (Fig. 3a). The area is mapped as Morristown silty-clay loam soil, though the entirety of the site is reclaimed coal mined land. As shown in the sections below, the soils vary tremendously in depth throughout the site. The site includes mostly flat topography, with some slopes less than 25%. Baseline sampling of the study area before management practices began was compared with measurements made during and at the conclusion of the 3-year study to determine if any measureable change occurred in the soil carbon levels of the different strata.

Figure 3a: *Soil Sampling – the Wilds Demonstration Area, image by AES.*

At *the Wilds'* partner farm in Muskingum County, Ohio, owned by Cathie Kreager, nine samples were collected during each year to serve as a reference site (Fig. 3b). A 7.3-acre zone was chosen that encompasses portions of three pastures used for grazing beef cattle on a rotational basis. During the baseline year (2011), the samples were collected from a small area in the lower field only. In 2012, field staff from AES and the Wilds decided that the nine samples should be more widely distributed among the three primary pastures. Three samples from the lower field were retained, while the remaining six were distributed amongst the two upper pastures. At the Kreager farm, the soils are mapped as Guernsey-Upshur silty clay loam soils (6 to 15 percent slopes, eroded).

Figure 3b: *Soil Sampling – Partner Farm off-site reference, image by AES.*

Pre-sampling

On March 3, 2011, Steven Apfelbaum and Carl Christopher of AES visited *the Wilds’* demonstration site and the partner-farm (Cathie Kreager’s farm) to perform preliminary soil sampling. Soil core samples were collected from ten plots at the Wilds and three additional plots at the Kreager farm. For each sample, the soil core was divided into fourths to a depth of 1 meter (40 inches). A sub-sample was drawn from each of these depth segments, which were then mixed to form a single homogenized sample (for a given plot). Four of *the Wilds’* samples and one of the partner farm samples were shipped to the University of Missouri’s Soil Characterization Laboratory (UM Soils Lab), for analysis of % Total Carbon, % Organic Carbon, % Total Nitrogen, pH Water, and pH Salt (Table 2).

The % Total Carbon values for the four *Wilds*’ plots were analyzed to get a general idea of the soil carbon content and to estimate how many samples would need to be collected for baseline sampling. For the margin of error to be within +/- 10% of the sample mean with

95% confidence, we calculated that 32 samples should be collected at *the Wilds* if the % Total Carbon is represented by a normal distribution. Since it is unlikely that the actual carbon distribution is normal, it was decided that 61 samples would be taken from randomly distributed locations across the entire study area; at least 15 from within each zone (Figure 3a).

Table 2: *Soil Pre-sampling Lab Results (2011)table provided by AES.*

	Sample	**% Total Nitrogen**	**% Organic Carbon**	**% Inorganic Carbon**	**% Total Carbon**	**pH water**	**pH salt**
The Wilds	Plot 1	0.08	0.63	0.61	1.24	7.78	7.39
	Plot 2	0.10	1.61	0.60	2.21	7.20	7.08
	Plot 3	0.10	0.76	1.04	1.80	7.88	7.49
	Plot 4	0.07	0.59	0.68	1.27	7.90	7.47
	Average:		0.90		1.63		
	SD:		0.48		0.46		
Partner Farm	KRE-1	0.18	1.57	0.00	1.57	6.71	6.34

Sampling Methods

During 2011, soil samples were collected using a pneumatically powered JMC Environmentalist's Sub-soil Probe (ESP) sampler. A plastic liner was inserted into the hollow, stainless steel, tubular body of the probe, which was then pneumatically hammered into the ground to a depth of 1 meter, or until resistance impeded further insertion of the probe. All soil carbon analyses were conducted with soil samples from the plastic liners. In addition, at three of the sample locations within each zone at *the Wilds* and three of the sample locations at the partner farm, at least two bulk density samples were obtained from pits dug at the plots. A visual determination of approximate layer boundaries was made in the field and, from within the top layer, soil from the pit was scooped to fill a 2-oz metal canister. This was repeated for layers discovered down to a depth of approximately 30 cm. A total of 35 individual samples were collected from the different layers at the different sample plots, and shipped to the UM Soils Lab for drying, final weight measurement and bulk density determination.

During 2012 and 2013, soil samples were collected using a Giddings hydraulic soil probe mounted on a John Deere Gator ATV. At the sample site, a 2" diameter plastic liner was inserted into the hollow, stainless steel tubular body of the probe, which was then pneumatically pushed into the ground to a depth of 1 meter, or until resistance impeded further insertion of the probe. All soil carbon and bulk density analyses were conducted with soil samples from the plastic liners.

Laboratory Analysis Methods

To determine the amount of carbon in the soil at a site requires the measurement of three primary variables in the laboratory:

- Volume of soil for which calculations are being done: the amount of soil mass per unit volume (for a given soil sample, considered a point location, the measured dimension is depth; individual layer depths determined by analysis by a soil pedologist in the lab).
- Amount of carbon in the soil as a percentage of the mass of the soil (% Carbon – from laboratory analysis of the soil sample of a homogenous layer); and
- Density of the soil: the amount of soil mass per unit volume (bulk density – calculated by comparing the mass of a known volume of a freshly collected sample with the mass of the sample after it is baked to dryness, thereby removing all water from the soil).

Soil Core Descriptions:

When the soil cores were sent to the lab for analysis, the Lab was instructed to split the soil core based on genetic horizons or observed layers, with a maximum of three to four "samples" per core,. The question then would be, "What sampling increments would be representative of rooting depths and pedogenic carbon (carbon from plant roots and other plant detritus) across the diversity of materials in this reclaimed landscape?”

A cursory investigation of the organic carbon depth distribution data analyzed in the laboratory quickly confirms that the root-rich surface horizons were the major soil pool for carbon sequestration on a percentage basis. One needs only to look at the variability in the thicknesses of the surface horizons to realize that a uniform sampling depth increment would have homogenized the results and patterns of the most important active carbon pool in this system. In addition, it would be difficult to compare the soil organic carbon (SOC) in reclaimed soils to the reference soils if a "horizon-based" approach had not been followed. If a study is being conducted to assess carbon sequestration or SOC pools, it is important to sample in such a way that does not mask the native variability of the landscape being measured.

A “horizon” was generally distinguished by material color, texture, structure and presence of biological activity, including worms and roots. Most of the observed surface horizons appeared to have some component of a pedogenic A-horizon, which had probably been scraped from somewhere in the landscape, then replaced on the surface of the reclaimed land. A horizon structure generally was fine to medium granular, and the transition to the underlying replaced materials was usually abrupt and smooth, from granular structure to massive or platey structure, with obvious color differences and abrupt decreases in root abundance.

Underlying materials were distinguished by the same criteria. Rooting depth and color were the primary discriminators for determining the "horizons" of the reclaimed materials. Most of the layering in the reclaimed materials was clearly from different sources. Multiple colors often were described because the overburden materials used in the reclamation process were usually mottled with distinct patterns and colors.

The University of Missouri’s Soil Characterization Lab Director was actively engaged in mine soil reclamation research in Missouri for 12 years, and always used morphological features to determine sampling increments. He stated that he never used uniform sampling increments that ignored both the reclamation process and subsequent plant rooting and pedogenic processes. The project was a multi-state collaboration that included Illinois, Kentucky, Indiana and North Dakota, and all five states used the same sampling protocol.

Bulk Density Analysis:

Soil bulk density (Db) is a measure of the mass of soil per unit volume (solids + pore space) and is usually reported on an oven-dry basis.

$$D_b = \frac{mass\ of\ dry\ soil}{volume\ of\ solids\ \&\ pore\ spaces}$$

Unlike the measurement of particle density, the bulk density measurement accounts for the spaces between the soil particles (pore space) as well as the soil solids. Soils with a high proportion of pore space have lower mass per unit volume, and therefore have low bulk density. Typical mineral soils have bulk densities that range from 1.0 to 1.6 g/cm^3. A bulk density greater than 1.6 g/m^3 may indicate soil compaction, which means these soils have a low proportion of pore space and, therefore, low porosity. Alternatively, soils with a high proportion of organic matter tend to have bulk densities that are less than 1.0 g/cm^3.

The bulk density of different soils varies based largely on soil texture and the degree of soil compaction. Sandy soils with low organic matter tend to have higher bulk density than clayey or loamy soils. Soil bulk density is usually higher in subsurface soils than in surface horizons, in part due to compaction by the weight of the surface soil.

The bulk densities reported in *the Wilds*' data were reported on a rock-free basis. If the rock fragment mass is included, the bulk densities would increase accordingly.

Soil Carbon Analysis:

SOC is reported based on the percent of soil mass "lost on ignition." In other words, the accurate measure of percentage of SOC is on a rock-free basis. The sample preparation procedures were the same during all three years. All fragments larger than 2mm were separated in the sample preparation by the standard screening process. Visible coal fragments were removed by hand. Darker shale fragments were high in organic matter, and the shale decomposed readily and could not be removed. Some of the soils contained coal dust too fine to be separated. This is one of the realities of dealing with reclaimed mine soils.

One of the reasons to sample by horizons rather than by depth increments is that the surface horizon SOC is very precisely quantified, thus enhancing the ability to measure whole profile SOC.

The University of Missouri's Soil Characterization Lab Director indicated that they never acid wash samples to remove calcium carbonate because their 28 years of experience with the Leco "loss on ignition" method shows that carbonate is released at different temperatures than SOC. As a result, they "burn off" the carbonate rather than go through the time consuming, more costly, messy and imprecise acid-washing procedure. The National Soil Survey Laboratory in Lincoln, Nebraska, has verified that the "two-step loss on ignition" process precisely distinguishes between SOC and carbonate C.

Data Analysis Methods

Combining this information for each soil layer of a sample core, we can calculate the carbon content at that plot location in units of soil carbon per unit area. A generalized version of the calculation is:

$$SC_y = \sum_{l=1}^{L} sd_l \cdot sdens_l \cdot \%sc_l \qquad (1)$$

Where:

SC_y = Total measured soil carbon per unit area at plot y

L = The number of soil layers measured within the calculated depth

l = Individual soil layer

sd_l = The average depth (thickness) of soil layer l within the plot

$sdens_l$ = The average bulk density of soil layer l within the plot

$\%sc_l$ = Percentage carbon (The average mass of soil carbon in layer l , as a percentage of the total mass of the sample, as measured in the laboratory)

To address the variability in soil horizons and soil cores by year, an additional step was completed in 2013. After the carbon levels were converted to kg/m^3 by horizon (from % carbon), the carbon levels for each soil core were normalized to a consistent depth of 100 cm for comparison purposes. Standard adjustments to account for differences in soil mass, or soil core lengths, are common for comparison purposes. However, it should be noted that this always results in estimates that may be inflated or deflated. By normalizing all data across zones and years for *the Wilds’* 2011-2013 soil carbon data, it ensures that the soil cores can be compared across all years and appropriate statistical analyses (ANOVA) can be completed.

Vegetation

Three to four sampling modules were established within each of the four plots at the 60 acre demonstration, as well as in the control plot and at the Partner Farm. Modules were setup by random sampling using a Mobile Mapper GPS. From the random GPS point, a module was created by measuring a 10x10m area in the northward direction. The southeast corner of the module was marked with a flag and the northeast corner was marked with a stake which also held the module number. A modified version of the Carolina Vegetation Survey Protocol (CVS, Peet et. al., 1998) was used to collect data on the presence and relative abundance of the species in each treatment plot. Rather than the 20x50m configuration of 10x10m modules used in the CVS-EEP protocol, individual 10x10m modules were surveyed. Each of the standard depth assignments for each module was used (Figure 4.)

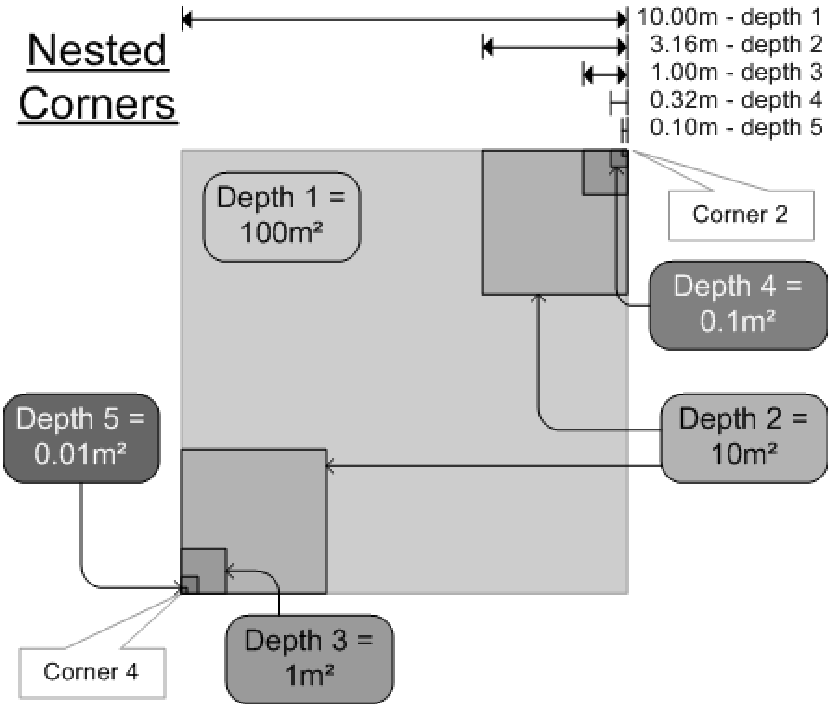
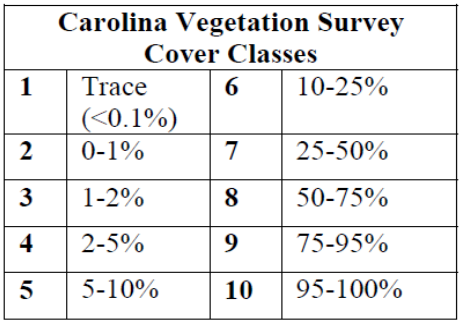


Figure 4. *Module configuration as used in the Carolina Vegetation Survey Protocol (Figure from CVS-EEP Protocol for Recording Vegetation, Lee et al, 2008)*

Each module was intensively surveyed starting in corner 2, at the depth of 5. Each species was recorded as it was encountered and given an initial score according to the depth it was encountered in and a secondary score according to the relative percent cover occupied within the entire 100m^2 module based on a scale from 1 to 10 (Table 3.) This data was used to establish which species were most abundant, as well as to determine if the percentage of non-native, invasive vegetation was higher or lower than that of the planted species.



Carolina Vegetation Survey Cover Classes			
1	Trace (<0.1%)	**6**	10-25%
2	0-1%	**7**	25-50%
3	1-2%	**8**	50-75%
4	2-5%	**9**	75-95%
5	5-10%	**10**	95-100%

Table 3. *Cover class scores as described in the CVS-EEP protocol (table from CVS-EEP Protocol for Recording Vegetation, Lee et al, 2008)*

Arthropods

Arthropod surveys were conducted by using the same randomly selected sampling modules used in vegetation surveys (Figure 1). Invertebrates were sampled in each of the 4 plots, the control and partner farm using a sweep netting technique. For sweep net samples, invertebrates were collected by making full sweeps throughout the module for a sampling period of two minutes. Samples were collected during late-morning or early-afternoon on days where there was little to no rain. Once a sample was taken the invertebrates were placed into plastic bags and labeled with the module number. The invertebrates were taken to the lab and were then identified to family classification.

Small Mammals

Trapping helps determine the quality of habitat provided by each of the practices. Trapping has been conducted under the guidance of Dr. Jim Dooley in 2011, 2012 and 2013. In 2011, Sherman live traps were used to sample the four plots for small mammals. A total of 28 traps were set for each trap night, two in each sampling module, for a total of at least 6 per plot, with peanut butter as bait and quilt batting for warmth (Figure 5). Traps were set for 2 non-consecutive trap nights, between November 15th and 23rd. Traps were set at 4pm and checked at 8am each trap night. Traps were not set if it rained or if the chance of rain was greater than 50%. Traps were collected each morning and a note was made if the trap was sprung or not. Following capture, each animal was identified, sexed, weighed and then marked for recapture. The trap location and temperature were recorded as well as whether the animal was a recapture or not. Once the data was recorded the animal was released in the same location it was captured.

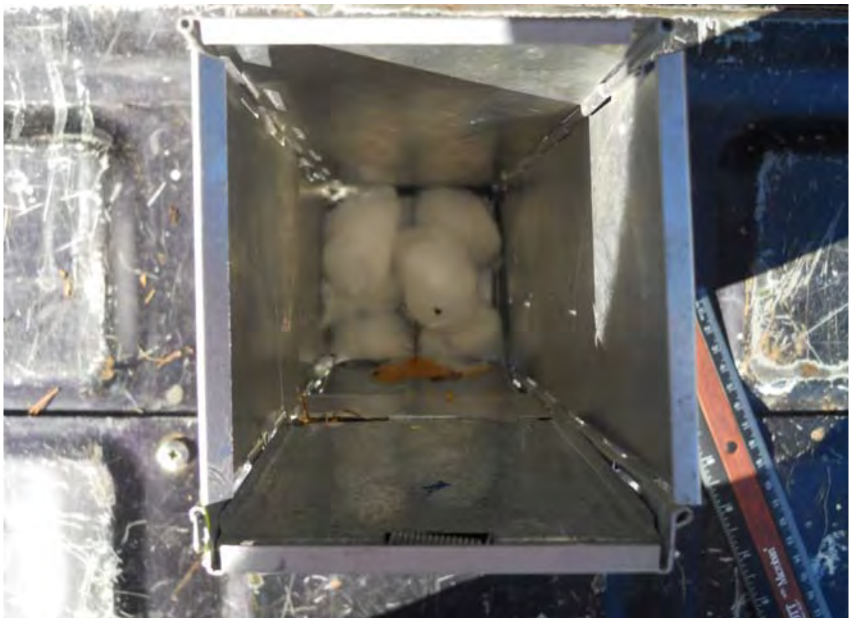


Figure 5. *Sherman traps used for small mammal trapping that were set with cotton material and peanut butter as bait.*

Trapping in 2012 was conducted the week of September 24th-28th. Methods in 2012 were modified slightly from those the year before in order to improve capture rate. This was done by increasing the number of traps in each plot by using a transect method. Traps were set at 6pm and checked at 7am each morning (these times were altered slightly to allow for an equal amount of daylight trap time as the daylight hours decreased). In each of the four 15 acre demonstration plots a total of 500 meters of transects were established running parallel to each other from north to south. Traps were set every 10 meters for a total of 50 Sherman traps in each plot. Traps were not set in the rain or if the chance of rain was above 50%. Both 7 and 9 inch Sherman traps were set with peanut butter bait and cotton. As temperatures dropped, extra cotton was added to the traps for added insulation for captured mammals. Low lying microhabitat areas were avoided for trap setting in case of heavy rain and flooding. Traps were collected each morning and noted whether the door was sprung or not. Captures were identified, sexed, weighed and then checked or marked for recapture. Similar methods were used in 2013, but with 10 additional traps in each 15 acre plot to further increase capture rates.

Life Cycle Analysis (LCA)

The LCA was conducted in the fall of 2013 by Dr. Jose Guzman from the Ohio State University Carbon Management and Sequestration Center. The full report can be found in Appendix B with sections included below.

Goal and scope

There are many environmental impacts which can be assessed including greenhouse gas (GHG) emissions, water quantity and quality, resource depletion, primary energy, waste, and toxicity. The purpose of this LCA is to determine whether prairie ecosystems can be an effective tool in sequestering C as well as which management technique is the best. Many studies in agriculture and reclaimed mine land have shown that the establishment of prairies are a significant atmospheric C sink, resulting in lower global warming potential (GWP, CO_2-C, CH_4-C, and N_2O-C) and improved soil quality compared to practices that are a source of C (Shrestha and Lal, 2006; Shrestha et al., 2009). The LCA consists of the preparation, establishment and harvest of prairie as a crop. It does not include processing or transport off property, nor does it include monitoring activities related to the demonstration that would not typically be carried out by farmers. The temporal boundary is the 3 year timeframe required for establishment, and the spatial boundary is limited to the 24 ha demonstration site. The scope of this LCA covers the ‘cradle-to-gate’ stage of the life cycle, from extraction of raw materials through land use change (LUC), agricultural activities, and production to the point where the prairie is harvested for bioenergy or feed (Figure 6).

Inventory analysis

In end of August of 2013, aboveground biomass was measured to quantify potential C outputs from plant biomass. Three plant samples were collected within a 50 cm^2 frame in each treatment plot. Plant biomass was dried at 60°C for 7 days, and weighed to determine dry matter weight (kg ha^{-1}). A total C concentration value of 43% from plant biomass (Guzman and Al-Kaisi, 2010) was used and multiplied by the plant biomass (dry matter m^{-2}) to determine the aboveground potential C outputs in kg ha^{-1}. Root biomass was estimated using a root:shoot ratio of 1.1 for tall-grass species and 0.8 for legumes and root turnover ratio of 0.5 for both which were derived from studies done in switchgrass (*Panicum virgatum*) and legume mixture plots (Bolinder et al., 2002).

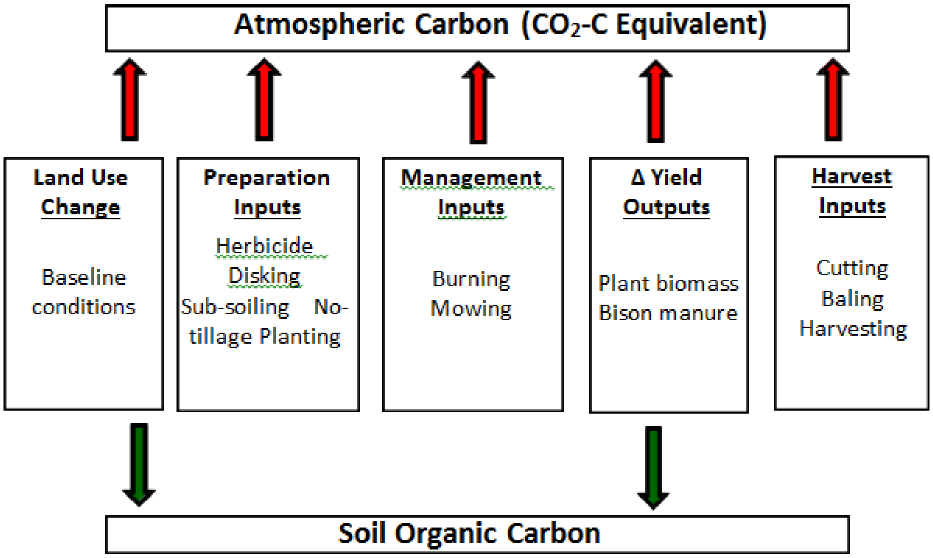


Figure 6. *'Cradle-to-gate' stage of the life cycle, from extraction of raw materials through land use change, management activities, and production to the point where the prairie is harvested for bioenergy or feed.*

The impact of CO_2 emissions from management inputs such as tillage, seed bed preparation, planting, herbicide applications, mowing, and burning due to combustion of fuel are also evaluated as seen in Figure 6. Since units between these inputs vary (i.e. L, oz, MJ), conversion factors were needed to express CO_2 emissions into kg of C equivalent (CE) for comparisons between inputs and outputs (Lal, 2004). Management CE inputs were calculated using fuel usage during operations. One kg of diesel and gasoline had a CE of 0.94 and 0.85 kg, respectively, and one kg of 2, 4-D herbicide had a CE of 1.7 kg (Lal, 2004). Additionally, CO_2 emitted from burning of prairies was accounted by assuming that 50% of the aboveground biomass was consumed and 63% of burned biomass was emitted as CO_2 (notes from *the Wilds*). Records of fuel usage, herbicide, fertilizers, and burning for each treatment plot has been kept since the project began in 2011 by *the Wilds* team.

Calculation of changes in soil quality and CO_2 emissions requires estimates of the effects of land preparation and management practices on soil organic carbon (SOC). Changes in net soil C were calculated using data collected from Applied Ecological Services, Inc. Net changes in SOC concentrations (%) that were measured in 2011 and 2013 were multiplied by mean bulk density (Mg m^{-3}) values and soil depth thickness to convert SOC concentrations to mass per area basis (kg ha^{-1}) for all treatment plots by soil horizon. An average A soil horizon depth of 6 cm, and B horizon depth of 34 cm was used across treatments. It is assumed that all C sequestered as SOC, comes from atmospheric CO_2 through photosynthesis and bison manure (recycled C from biomass consumption), and that all SOC losses are emitted as CO_2 to the atmosphere. Additionally, C losses from soil erosion and leaching were assumed to be negligible.

Impact assessment

To evaluate the C balance and energy use of each of the land preparations being demonstrated, an index of sustainability (I_s) was used to assess temporal changes in the output/input ratio of C using a holistic approach (Lal, 2004):

$$I_s - \left[\frac{C_0}{C_I}\right] t$$

where C_O is the sum of all outputs expressed in CE, C_I is the sum of all inputs expressed in CE, and *t* is the time in years. The reference unit C equivalent (CE), i.e. the reference measure for which the environmental burden (CO_2) from farming operations is expressed as kg CE ha^{-1} yr^{-1}. In order for a management practice to be considered sustainable, the I_s ratio as expressed by net output of C, must be >1 and be increasing with time.

Grazing Observations and Forage Analysis

The purpose of this aspect of the study is to demonstrate the suitability of native prairie for use as pasture on marginal land compared to traditional cool-season pastures, also on marginal land. Two sites were used, both on *the Wilds'* property. The first is a 15-acre warm-season grass prairie in the 60-acre demonstration area at *the Wilds*. This prairie pasture was reclaimed in the 1980's then left as grassland until prairie establishment in 2011. The second pasture, of the same acreage, was also reclaimed to grassland and has been used as animal pasture. The cool-season pasture grasses established during reclamation were renovated to include endophyte free fescue over ten years ago.

Observations began in 2013 once the prairie had a chance to establish and the bison were introduced. The bison used in the study were from *the Wilds*' existing herd. Bison of similar lineage, health, and age (one-year old heifers) were chosen. Prior to the study the bison had been raised on a cool-season grass pasture at *the Wilds*. Ten bison were divided into two groups of five each with approximately the same group weight. Body condition scores were also recorded for each bison. During the first week of July 2013 the groups were introduced to the previously described pastures. Body condition scores were assigned again on August 1st and bison were reweighed 83 days after the first weighing.

The grazing behavior study design and definitions of behavior were developed in collaboration with Dr. Jane Packard, of Texas A&M University's Department of Wildlife & Fisheries Sciences, who has experience conducting animal studies at Fossil Rim Wildlife Center (FRWC) and *the Wilds*. Both groups of bison were observed for 30 minutes in the early morning between 7:00 and 8:30am then again in the afternoon between 1:30 and 3:00pm when the calves were most likely to be grazing. Each session consisted of 30 minutes of continuous observation during which a focal animal was the subject. Focal animals were chosen from the groups at random each day and identified with binoculars by their ear tags. During continuous observation the start and stop times of grazing behavior as well as the bite counts were recorded for the focal animal only. In addition, instantaneous observations were recorded on the entire group every 60 seconds to determine how many were grazing.

Forage samples were collected from each pasture on July 17th, August 12th and August 30th. The method used for collecting samples was to clip a handful of forage about five inches above the ground from a corner of each of the vegetation survey modules. These subsamples were then chopped into finer pieces, put in a bucket and mixed together with the other subsamples from the same plot. A portion of this mix was then bagged and sent to Holmes Laboratory for determination of metabolizable energy (ME), crude protein, fiber and trace mineral content. Results from the lab were then shared with Dr. Stephen Boyles of Ohio State University's Department of Animal Science, who provided a comparison of nutrition required for bison/cattle and the nutrition available in each pasture.

Biomass Potential and Yield

Samples of aboveground biomass were collected from the four plots of the 60-acre demonstration site on two separate occasions. The first sampling was conducted on August 29th, 2013 by Dr. Jose Guzman as part of the LCA with three random samples collected per plot using a 50cm quadrat. The second sampling was conducted on October 17th in order to determine the potential energy gained from prairie grown as a biomass crop. These second set of samples were collected later in the year in order to ensure a greater fiber content and lower nutrient content which results in fewer contaminants and greater potential energy during conversion to biofuel. Each 15-acre plot was categorized by percent slope (0-8%, 9-15% and 16-20% slope) then 5 samples were collected from within each of the three categories in each plot for a total of 15 samples per plot. These were collected using a 25cm quadrat and clipping 8cm above the ground.

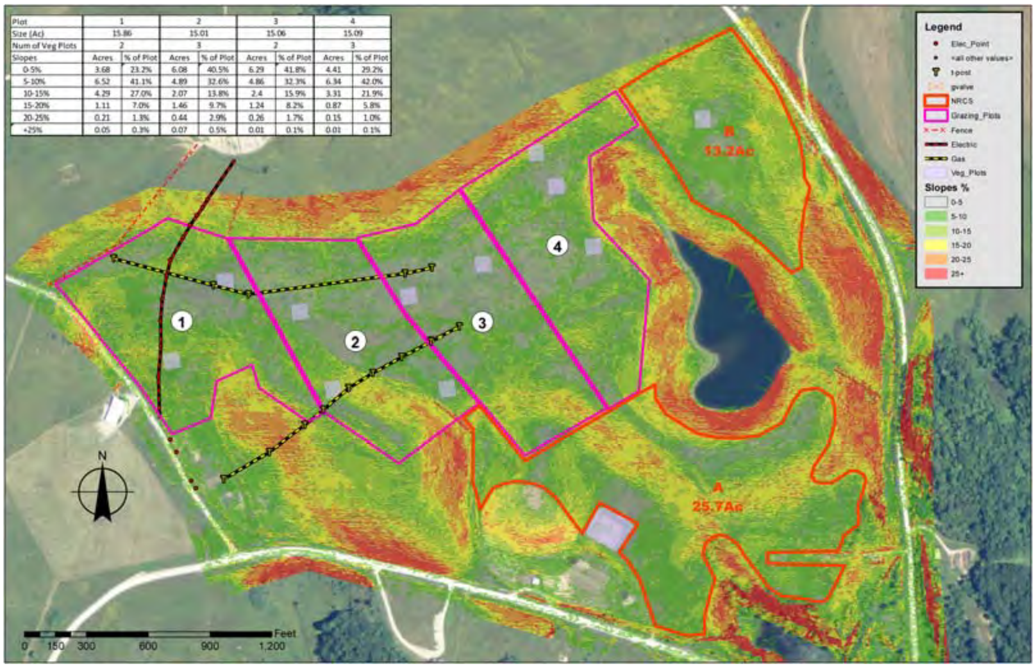
Samples were then dried at 60°C for 7 days and then weighed to determine dry matter weight (kg ha^{-1}). Average weights were then compared between plots. The weights were also used to estimate biofuel product from conversion to cellulosic ethanol at the rate of 0.255 L kg^{-1} of dry biomass (Tilman et al., 2006, Sheehan et al., 2004).

Discussion of Quality Assurance

Project site description: characteristics of the site, sample locations, rationale for locations, map.

This project was conducted on 60 acres of previously surface-mined land at *the Wilds* conservation center in Cumberland, Ohio and was chosen to meet the objectives of assessing the potential for utilizing warm-season grasses and forbs as a crop on marginal lands. The project area was reclaimed to cool-season grasses in the 1980’s following completion of mining activities and was left as such until the start of the project. The site was divided into 4, 15 acre plots. Plot delineation was completed by the Ohio Wetlands Foundation (OWF) and done in such a manner as to ensure as much homogeneity between plots as possible with regard to slope, aspect, vegetation, etc (Figure 7). Sampling locations within each 15 acre plot (including vegetation, soil and small mammals) were chosen at random and marked as permanent plots for each subsequent sampling event.

Figure 7. *The 60-acre demonstration site delineated by OWF showing percent slope. Image provided by OWF.*



Plot	1		2		3		4	
Size (Ac)	15.86		15.01		15.06		15.09	
Num of Veg Plots	2		3		2		3	
Slopes	Acres	% of Plot	Acres	% of Plot	Acres	% of Plot	Acres	% of Plot
0-5%	3.68	23.2%	6.08	40.5%	6.29	41.8%	4.41	29.2%
5-10%	6.52	41.1%	4.89	32.6%	4.86	32.3%	6.34	42.0%
10-15%	4.29	27.0%	2.07	13.8%	2.4	15.9%	3.31	21.9%
15-20%	1.11	7.0%	1.46	9.7%	1.24	8.2%	0.87	5.8%
20-25%	0.21	1.3%	0.44	2.9%	0.26	1.7%	0.15	1.0%
+25%	0.05	0.3%	0.07	0.5%	0.01	0.1%	0.01	0.1%

<u>Sampling design. Include the precision level of measurements, completeness (will data be sufficient), how samples and measurements truly represent what is occurring, and comparability (can the project situation be compared to real-life situations).</u>

A mid-diversity prairie seed mix was chosen for the site based on both cost, suitability for marginal land and overall value toward the four end uses. Each plot was designated a land preparation technique (no-till, tilling, as well as tilling with subsoiling) which was implemented using full-scale farm equipment to demonstrate feasibility with existing equipment. All plots were planted using the same no-till native seed drill, which is available both commercially and as a rental unit at most Soil and Water Conservation District offices. Data collected on biodiversity parameters was done in conjunction with partnering universities and demonstrate changes in biodiversity as prairie becomes established. Other data collected on soil carbon, forage quality and biomass production were done using accredited laboratories and may be used by landowners to evaluate their stand for the end-use(s) being targeted. For details on the precision and methods used please see the details in previous methods section.

Sampling procedures: Describe collection methods, collection frequency, equipment used, volume or amounts sampled, and how samples are handled, stored, and transported.

Each plot was sampled on an annual basis for changes in biodiversity, including vegetation, arthropods and small mammals; and soil characteristics, including bulk density and organic carbon. In the final year of the project, sampling was done for biomass production, forage quality, as well as bison weights and body condition scores (BCS) several times throughout the year. Data collection methods and equipment used were outlined previously in ‘Methods Review’ section in greater detail.

Custody procedures: Describe chain-of-custody procedures for samples and data.

Soil samples were taken by Applied Ecological Services, Inc. and delivered by the technicians to the University of Missouri’s soils lab for analysis. Forage analyses were done through Holmes Laboratory and were collected by Wilds’ staff and mailed to the lab immediately following collection. Biomass yield determination and biodiversity collections involved no sample transport; this data was recorded in the field by project personnel.

Calibration: What, if any, field equipment will require calibration & how will it be done.

Herbicide application and seeding for the entire project was done using spray equipment and a no-till native seed drill, operated by FDC Enterprises Inc. Prior to herbicide application and seeding, the equipment was calibrated by the operators. Herbicide sprayers and seed drills were calibrated prior to use according to the manufactures instructions as they are different for each make and model.

Sample analysis, quality control: Cite analytical procedures to be used in the field or laboratory, sub-sampling or sample preparation, units of measure to be used. Describe limits of detection.

Soil and forage samples were processed according to laboratory procedures for each lab used (as above.) Biomass samples were collected from random points throughout each plot, weighed and then dried. Dry weights were then used to estimate the yield for each plot.

Discuss data reduction, analysis, review, and reporting: How raw data is converted and presented, who reviewed it, and how the final presentation was derived.

All data were recorded in Excel spreadsheet files. All data recording, reduction, analysis and review for the soils were conducted by Applied Ecological Services. Data from the LCA and biomass yields were recorded by project personnel and analyzed by Dr. Rattan Lal and Dr. Jose Guzman at The Ohio State University Carbon Management and Sequestration Center. All other data was collected and analyzed by project staff using the statistical program R and then reviewed by advisors. Please see results section for details on tests used for each dataset.

Findings

Soil Carbon

Baseline core depths ranged from 9 to 78 cm across all sites with depths at the Partner farm being 16cm greater on average. Prior to prairie establishment average rooting depths were 22 to 28cm at the 60-acre demonstration with an average depth of 52cm at the Partner Farm. There was slightly deeper rooting depth in the no-till plot prior to prairie establishment which may indicate differences in soil properties between plots since similar results were found after prairie establishment as well. Bulk density results were slightly lower than expected but proportional between sites with less density in soil from the Partner Farm (Table 4). These lower bulk density values may be due to rock removal prior to calculation.

Table 4. *Baseline (2011) Average A, B and C Horizon Bulk Density Values. Summary table provided by AES.*

$sdens_t$	**Horizon**	**n**	**Mean g cm^{-3}**	**SD g cm^{-3}**	**SEM g cm^{-3}**
The Wilds	A	11	**0.92**	0.08	0.02
	B	12	**1.31**	0.09	0.03
	C	6	**1.32**	0.06	0.02
Kreager Farm	A	3	**0.87**	0.05	0.03
	B	3	**0.96**	0.19	0.03

To summarize all sample points across the four plots, a baseline estimate of 20.8 to 24.2 kg/m2 was determined for total carbon and 8.8

to 11.0 kg/m2 for organic carbon with the Partner Farm having lower soil total carbon (Table 5). However, when looking at organic carbon the Partner farm had slightly higher levels than the plots at the 60-acre demonstration site (Table 6). This is likely due to the fact that the farm was not previously mined and is actively grazed. Additionally, inorganic carbon is found in soil samples from the 60-acre demonstration in the form of calcium carbonate as well as coal fragments.

Table 5: *Baseline (2011) Soil Total Carbon, 95% Confidence Intervals. Summary table provided by AES.*

Total Carbon	Sample plots	Carbon			Zone Area	Zone CO_2e			Relative Error
	n	kg/m^2			acre	MT			+/- %
Zone 1	16	21.7	*to*	30.3	13.5	4,340	*to*	6,070	16.6%
Zone 2	15	16.9	*to*	23.3	15.8	3,960	*to*	5,470	16.0%
Zone 3	15	20.2	*to*	26.6	14.4	4,330	*to*	5,670	13.5%
Zone 4	15	16.9	*to*	86.8	15.0	3,770	*to*	5,270	16.6%
Zones 1-4	61	20.8	*to*	24.2	58.7	18,100	*to*	21,000	7.4%
Kreager	9	10.4	*to*	15.4	7.3	1,120	*to*	1,670	19.7%

Table 6: *Baseline (2011) Soil Organic Carbon, 95% Confidence Intervals. Summary table provided by AES.*

Organic Carbon	Sample plots	Carbon			Zone Area	Zone CO_2e			Relative Error
	n	kg/m^2			acre	MT			+/- %
Zone 1	16	6.2	*to*	13.2	13.5	1,250	*to*	2,640	35.7%
Zone 2	15	7.2	*to*	11.0	15.8	1,690	*to*	2,570	20.7%
Zone 3	15	8.7	*to*	11.5	14.4	1,850	*to*	2,470	14.3%
Zone 4	15	8.8	*to*	12.4	15.0	1,950	*to*	2,770	17.2%
Zones 1-4	61	8.8	*to*	11.0	58.7	7,660	*to*	9,580	11.1%
Kreager	9	9.0	*to*	15.2	7.3	977	*to*	1,640	25.4%

Soil samples collected in the second and third years showed some change from the baseline results. In the third year, average rooting depth in the established prairie across all plots of the 60-acre demonstration was approximately 45cm as seen in Table 7, compared to the average 25cm rooting depth of the cool-season grass pasture prior to prairie establishment. Similar to the baseline rooting depths, the no-till plot (2) had the greatest average rooting depth again with approximately 53cm mean length, yet there were little differences in soil total and organic carbon between plots. Total carbon (Table 8) and organic carbon (Table 9) seem to have decreased on average since establishment and had not yet recovered to previous levels by the third year. These results emphasize the importance of long-term monitoring as well as the role that planting and management methods can have in soil carbon changes.

Table 7: *Year Three Soil Sample Rooting Depths (2013), table from AES.*

	Cores n	Mean Length cm	Std Dev cm	Median cm	Minimum cm	Maximum cm
Zone 1	16	40.94	15.70	37.5	22	66
Zone 2	15	52.60	22.15	49	26	91
Zone 3	15	39.00	10.28	36	27	60
Zone 4	15	46.87	18.80	39	24	94
Zones 1-4	61	44.79	17.66	39	22	94
Zone 5 – Control	10	43.30	14.80	40.50	24	71
Kreager Farm	9	90.11	16.95	97	48	100

Table 8: *Year Three Soil Total Carbon, 95% Confidence Intervals (2013). Summary table provided by AES.*

Total Carbon	Sample plots	Carbon			Zone Area	Zone CO_2e			Relative Error
	n	kg/m^2			acre	MT			+/- %
Zone 1	16	14.6	*to*	21.2	13.5	2,930	*to*	4,240	18.2%
Zone 2	15	10.3	*to*	15.9	15.8	2,420	*to*	3,720	21.1%
Zone 3	15	14.8	*to*	22.2	14.4	3,170	*to*	4,730	19.8%
Zone 4	15	14.1	*to*	73.7	15.0	3,140	*to*	4,470	17.5%
Zones 1-4	61	15.2	*to*	18.2	58.7	13,300	*to*	15,800	8.9%
Zone 5 –	10	13.4	*to*	21.8	15.4	3,060	*to*	4,990	24.0%
Kreager Farm	9	11.5	*to*	22.9	7.3	1,250	*to*	2,480	33.1%

Table 9: *Year Three Soil Organic Carbon, 95% Confidence Intervals (2013). Summary table provided by AES.*

Organic Carbon	Sample plots	Carbon			Zone Area	Zone CO_2e			Relative Error
	n	kg/m^2			acre	MT			+/- %
Zone 1	16	6.5	*to*	9.3	13.5	1,300	*to*	1,860	17.5%
Zone 2	15	4.9	*to*	7.5	15.8	1,140	*to*	1,770	21.4%
Zone 3	15	6.3	*to*	9.5	14.4	1,340	*to*	2,030	20.3%
Zone 4	15	6.7	*to*	8.9	15.0	1,490	*to*	1,980	14.2%
Zones 1-4	61	6.8	*to*	8.0	58.7	5,890	*to*	7,000	8.7%
Zone 5 –	10	7.2	*to*	10.2	15.4	1,640	*to*	2,330	17.3%
Kreager Farm	9	6.7	*to*	19.9	7.3	724	*to*	2,160	49.7%

The achievable soil carbon levels will be somewhat determined by soil nitrogen availability due to the nature of the reclaimed substrate. With adequate soil macronutrient management, soil carbon levels at the 60-acre demonstration site should be able to attain levels similar to those at the unmined partner farm with annual increases between 0.0804kgC m^{-2} and 0.1166kgC m^{-2} (Appendix A).

Vegetation

Results from 2011 vegetation surveys were used to calculate diversity among treatments, which was then compared using ANOVA, finding no significant differences ($P = 0.22$). Although there were no significant differences indicated by the test, there are similarities in the community composition of the no-till with grazing and no-till plots (1 & 2) indicated by an NMDS ordination. The till-only and till with sub-soiling plots (3 & 4) also showed similar community composition, indicating slight but not significant differences in prairie establishment between plots. Once established in the third year, vegetation survey results indicated similar findings with no significant difference between the four prairie plots.

Table 10. *Results of 2013 vegetation surveys*

Plot/ Treatment	**Average SR/Plot**	**Average Diversity /Plot**	**Average Planted Species/Plot**	**Average Invasive Species/Plot**	**Average Grass/ Legume/Forb**
No till/Grazing	24	2.00	7.6	1.6	6 / 2 / 17
No till	22	2.20	6.3	2.6	4 / 2 / 17
Till	28	2.08	8.6	1.6	6 / 3 / 15
Till & Subsoil	28	2.23	8	2	6 / 4 / 17
Control	10	1.17	N/A	1.6	4 / 2 / 5
Partner Farm	18	1.33	N/A	2.6	4 / 3 / 13

Arthropods

Sampling in 2011 resulted in a total of 34 different families of arthropods found within the plots. Although family richness by plot did not vary much, as seen in Table 11, an ANOVA was used to determine if there was a significant difference in Arthropod family richness between the 4 plots. It was found that there was no significant statistical difference (p= 0.14). When plant species richness increased within the module, arthropod family diversity increased (p=0.03). This indicates there is a positive relationship between plant species richness and arthropod family richness within the prairie plots.

Sampling in 2013 was completed using the same methods in late July and early August. Results show greater family richness from the first year, when the prairie was establishing (Table 12). No significant difference was found between the arthropod abundance or richness of the prairie plots. However, sampling was also conducted at the Partner Farm in 2013 for comparison to a typical farm in the area. The area sampled at the partner farm was cool season grass pastures grazed by cattle and horses. There was significantly greater family richness (p = 0.02) and abundance of arthropods (p = 0.035) at the partner farm using a two-way t-test with unequal variances. The difference seen in Figure 8 may likely be due to the presence of manure from livestock but requires further investigation.

***Table 11**. Family richness of arthropods by treatment type 2011*

Sampling Module	Treatment (plot number)	Family Richness
1	No-till with grazing beginning 2013 (1)	11
2	No-till with grazing beginning 2013 (1)	10
3	No-till with grazing beginning 2013 (1)	8
4	No-till only (2)	14
5	No-till only (2)	11
6	No-till only (2)	10
8	No-till only (2)	11
7	Tilling (3)	12
9	Tilling (3)	12
10	Tilling and Subsoiling (4)	11
11	Tilling and Subsoiling (4)	15
12	Tilling and Subsoiling (4)	9
13	Tilling and Subsoiling (4)	8
14	Control (5)	8

***Table 12**. Family richness of arthropods by Treatment type 2013*

Sampling Module	Treatment (plot number)	Richness
1	No-till with grazing beginning 2013 (1)	13
2	No-till with grazing beginning 2013 (1)	21
3	No-till with grazing beginning 2013 (1)	23
4	No-till only (2)	13
5	No-till only (2)	18
6	No-till only (2)	22
7	No-till only (2)	20
8	Tilling (3)	26
9	Tilling (3)	27
10	Tilling and Subsoiling (4)	25
11	Tilling and Subsoiling (4)	23
12	Tilling and Subsoiling (4)	24
13	Tilling and Subsoiling (4)	27
14	Control (5)	12
15	Control (5)	24

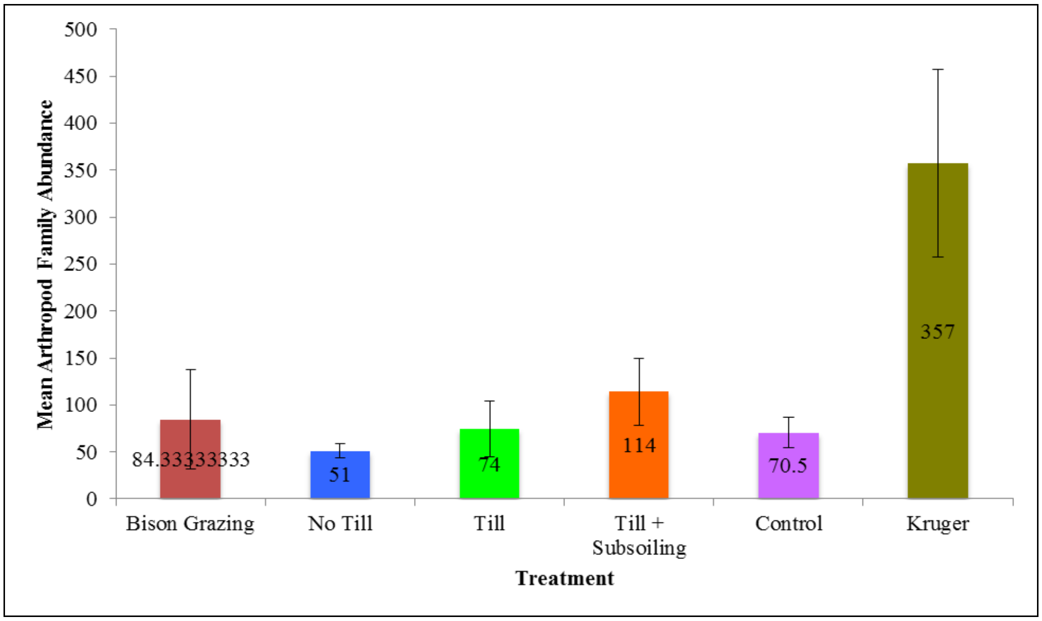


***Figure 8.** Mean arthropod family abundance between plots and Partner Farm in 2013.*

<u>Small Mammal</u>

In 2011 a total of eight small mammals were captured during the trapping period. Seven of the mammals were *Peromyscus maniculatus* (deer mouse) and one was *Sylvilagus floridanus* (Eastern cottontail.) The number of captures were found to be greatest in the no-till only treatment plot. Where vegetation diversity increased the number of small mammal captures also increased. The weights of the small mammals were higher in the no-till and no-till with grazing plots compared to the tilled and the tilled and sub-soiled plots.

In 2012 there were a total of 183 captures with the most abundant being *Microtus pennsylvanicus* (meadow vole), followed by *Peromyscus laucopus* (white-footed field mouse), *Mus musculus* (house mouse) and one *Mustela nivalis* (least weasel). The average abundance or capture success of small mammals by plot were similar, as was the species richness as seen in Table 13.

Trapping in 2013 revealed a total of 7 different species total compared to a total of 4 species in the prairie plots in 2012. These species included *Microtus pennsylvanicus* (meadow vole) as the most dominant, as well as several species with similar abundance, *Peromyscus maniculatus* (deer mouse), *Peromyscus laucopus* (white-footed field mouse), *Blarina brevicauda* (Northern short-tailed shrew), as well as one instance of *Neovison vison* (mink) and *Napaeozapus insignis* (woodland jumping mouse). This range of species indicates a quality habitat for small mammals and their predators.

Table 13. *Number of species and capture success of small mammal survey 2012.*

Plot/Treatment	Species	Captures
No till/Grazing	3	19%
No till	3	23.5%
Till	3	24%
Till & Subsoil	4	18.5%
Partner Farm	1	1%

Life Cycle Analysis

Components considered in the LCA were converted to carbon equivalents (CE) and categorized as either inputs or outputs to the system. Outputs, or produced carbon, divided by the inputs, or used carbon, gives us a ratio indicating sustainability termed “Index of Sustainability” (Is). Results that are greater than 1 indicate that the process is sustainable because less carbon is being used than stored. The till only plot emits the least carbon equivalents for the carbon outputs of that plot with an I_s of 8.53 (Table 14a). However, the expectation is that over time the till and till with sub-soiling plots may prove to be less sustainable since disturbed soils tend to be drier leading to potentially less biomass production. Burning as a management tool also decreases sustainability by emitting carbon.

Table 14a. *LCA results as Index of Sustainability by plot*.

Sustainability index	**I_s (Outputs/Inputs)**				
		No-till, grazing	**No-till**	**Till only**	**Till & Sub-soil**
I_s	(Outputs/Inputs) non-burning year	1.94	2.50	8.53	1.44
I_s	(Outputs/Inputs) 50% removal	-	1.65	5.46	0.93
I_s	(Outputs/Inputs) 100% removal	-	0.81	2.68	0.43
I_s	(Outputs/Inputs) burning year	0.86	1.09	2.01	0.71

In addition to this carbon LCA, energy use was tracked in order to compare the land management and preparation treatments. This comparison included the no-till, till only and till with subsoil plots but excluded the grazing plot which would not be used to produce biomass crops. The scope was limited to fuel used during establishment and management and did not include energy used in the production or transport of seed, herbicide or biofuel. For the purposes of comparing the treatments it is reasonable to assume all other inputs would be the same for each treatment. If expanded into a complete energy LCA these results could then be compared to other biofuel crops and would likely be much lower than the current results in Table 14b. One factor that could increase the energy output is inclusion of energy from combustion of lignin during ethanol production resulting in cogeneration of approximately 0.54 MJ L^{-1} of electricity (Hill et al., 2009), although this would be a relatively minor addition. All inputs and outputs were converted to MJ/ha at the rates of 32.4 MJ L^{-1} for gasoline, 35.9 MJ L^{-1} for diesel (Pradhan et al., 2011) and 21.2 MJ L^{-1} for cellulosic ethanol (Schmer et al., 2008). Results were similar to those found in the carbon LCA in that the no-till plot has the highest ratio of outputs to inputs. However, in this case the difference is due to lower energy inputs during establishment, rather than carbon loss during conversion. Again, these comparisons only represent the first three years and should be conducted again in several years to capture potential changes in biomass yield between the plots.

***Table 14b**. Comparison of plots by energy ratio*

Inputs	Practice	No-till (MJ/ha)	Till only (MJ/ha)	Till & Subsoil (MJ/ha)
Fuel usage	burn torches (2:1 diesel:gasoline)	9.63	9.63	9.63
	burn-vehicles (gasoline)	48.40	48.40	48.40
	mowing (diesel)	559.76	559.76	559.76
	spraying (diesel)	26.81	26.81	26.81
	subsoil (diesel)	0	0	556.41
	tillage (diesel)	0	388.81	388.81
	planting (diesel)	26.81	26.81	26.81
Total Inputs		**671.42**	**1060.23**	**1616.64**
Outputs				
Biofuel	Low heating value	39471	17939	23435
Total Outputs		**39471**	**17939**	**23435**
Net Energy Ratio (NER)	Outputs/Inputs*	**59**	**17**	**14**

Grazing Observations and Forage Analysis

Observations of a different focal bison for 44 mornings show a difference in how many times the focal bison was grazing in each pasture. The focal chosen from the warm season herd was seen grazing 20 times, and the focal chosen from the cool season herd was seen grazing 33 times (Table 15). A chi-square test indicated that the grazing is not independent of the pasture being grazed. This was also the case when looking at both morning and afternoon observations as seen in Table 16. However, when you look at how many of the herd are grazing during the 30 minute observations there doesn't seem to be a difference between the two herds.

***Table15**. Times focal bison were observed grazing on each pasture in the morning.*

AM only	***Grazing***	***Not Grazing***
Warm-season pasture	***20***	***24***
Cool-season pasture	***33***	***11***

***Table16**. Times focal bison were observed grazing on each pasture morning & evening.*

AM & PM	***Grazing***	***Not Grazing***
Warm-season pasture	***26***	***40***
Cool-season pasture	***39***	***27***

No difference was seen between pastures from instantaneous observations of the each herd. The number of bison grazing out of 5 at 60 second intervals was similar between pastures as seen in Table 17. These findings indicate that the bison may be grazing more frequently on the cool-season pasture but a longer term study would be needed since little difference was found between herd grazing behaviors. Additionally, the forage results indicate similar nutritional quality between the pastures, with both meeting the requirements of a pregnant cow or bison when available with free-choice mineral as seen in Tables 18 and 19.

Table 17. *Average number of bison grazing during instantaneous scans of each herd*

	Average number of grazers out of 5
Cool season pasture	*1.24*
Warm season pasture	*1.34*

Table 18. *Requirements of a pregnant cow or bison compared to the nutrition available from prairie at the Wilds.*

Nutrient	**Requirement**	**Forage (100% DM)**	**Diet**
As Fed Intake, lbs			*68*
Dry Matter Intake, lbs			*19.4*
TDN, %	*53*	*53*	*Cow should be able to be maintained on pasture and free-choice mineral.*
Crude Protein, %	*8*	*14*	
Calcium, %	*.2*	*.63*	
Phosphorus, %	*.17*	*.26*	
Magnesium , %	*.1*	*.21*	
Potassium, %	*.65*	*2.0*	
Sodium, %	*.08*	*.08*	
Copper, ppm	*> 8*	*5*	
Iron, ppm	*50*	*20*	
Zinc, ppm	*30*	*47*	

Table 19. *Requirements of a pregnant cow or bison compared to the nutrition available from cool-season pasture at the Wilds.*

Nutrient	Requirement	Forage (100% DM)	Diet
As Fed Intake, lbs			63
Dry Matter Intake, lbs			19.4
TDN, %	53	54	Cow should be able to be maintained on pasture and free-choice mineral.
Crude Protein, %	8	13.8	
Calcium, %	.2	.74	
Phosphorus, %	.17	.24	
Magnesium , %	.1	.15	
Potassium, %	.65	1.52	
Sodium, %	.08	.03	
Copper, ppm	> 8	4	
Iron, ppm	50	39	
Zinc, ppm	30	24	

Biomass Potential and Yield

Biomass sampling for the LCA in August revealed the greatest yield in the no-till plot (Table 20) and similar results were found when sampling biomass again in October of 2013 (Table 21). The grazing plot was not sampled because harvest for biofuel would typically not occur on grazed pasture. Estimates of potential cellulosic ethanol yield from dry biomass range from 342 to 753 liters of ethanol per acre.

Table 20. *Aboveground biomass collected in August by Dr. Jose Guzman*

Land preparation and management practice	Aboveground biomass (kg ha^{-1})
Control, pre-existing vegetation	5,435 ± 551[†]
No-till plus grazing	8,971 ± 1148
No-till	11,130 ± 1905
Tilling	9,881 ± 625
Tilling plus sub-soiling	6,660 ± 625

[†] Standard deviation

Table 21. *Biomass yields by plot in October 2013*

Land Preparation and Management Practice	Average (kg ha^{-1})	Standard deviation	Liters of ethanol/ha	Liters of ethanol/ac
No-till	7301.3	±6163.3	1861.84	753.7813765
Till only	3318.4	±3334.7	846.192	342.5878543
Till with Subsoiling	4334.9	±3846.2	1105.408	447.5336032

Conclusions and Recommendations

1) Our large-scale project indicates that native tallgrass prairie is a viable crop for landowners with marginal lands, such as surface mined land, which offers many benefits including the ability to grow under poor soil conditions.

2) Future work should include the development of individual mixes tailored to the desired use(s) to maximize productivity for outcomes such as biofuel production.

3) Further research is needed regarding the productivity of soils at former mine sites reclaimed to native warm-season grasses and forbs with regard to the end use(s).

4) More time and monitoring are needed in order to be able to draw strong conclusions from the data collected, as three years is not sufficient time to be able to detect trends in data, especially with regard to soil composition and carbon sequestration. Therefore, continuation funding is needed.

5) In terms of stand establishment, it appears that the no-till establishment method is potentially the best method in terms of growing the highest yields for biomass production on mined lands.

6) There is an extremely limited availability of bio-refineries, especially in the Appalachian region, therefore in order to truly make biofuel production a viable option for landowners, additional facilities need to be built in this region and a supply chain needs to be developed to create a system for harvest, transport, conversion to fuel and sale of biomass products.

7) In addition, viable carbon markets need to be fully developed and implemented in order to allow for the sale and trade of carbon credits in the U.S.

In conclusion, we feel this project was successful in demonstrating the potential of native, warm-season grasses and forbs for use on marginal land in an agricultural setting. Education and outreach events were well received by the agricultural and ranching communities, and hundreds of landowners, educators, researchers and students were able to learn about and experience this project first-hand. Informational materials were developed and distributed to help educate landowners on the establishment, management and uses of prairie on marginal lands, creating an opportunity for income on otherwise fallow lands that benefit both people and the environment. *The Wilds* plans to seek out additional funding sources in order to continue monitoring and research efforts related to this project as well as expand on the original idea to incorporate other aspects of prairie habitats and develop partnerships with companies working to develop the infrastructure necessary to make all options demonstrated here available to landowners.

Appendices

Appendix A - AES Final Soil Report

Appendix B - OSU LCA Report

Appendix C - Forage Analysis - Nutrition Results

Appendix D - Publications

Appendix E - Maps

Appendix F - References

The Wilds CIG Final Report
Appendix A
AES Final Soil Report

The Wilds
Soil Carbon Sampling
Final Report for 2011–2013

Cumberland, Ohio

August 2013

Applied Ecological Services, Inc.™

The Wilds
Soil Carbon Sampling
Final Report for 2011–2013

Cumberland, Ohio

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Contents

Introduction

In 2010, staff of The Wilds received a Conservation Innovation Grant (CIG) award from the US Department of Agriculture's Natural Resources Conservation Service to test land-use change benefits to soil carbon levels on the reclaimed coal mine landforms present at the Wilds. The Wilds contracted with Applied Ecological Services (AES) to assist in some tasks associated with the CIG award, specifically those focused on field sampling and analysis of soil carbon levels, and contributions to life cycle analysis.

One of the CIG award focused areas of inquiry was to understand how mined lands might be improved, and how such improvements in soil carbon levels might benefit local farmers who also own and operate farming and livestock grazing operations on similar reclaimed former coal mined lands.

Specific to the Wilds was a desire to test how grazing of bison might benefit soil carbon levels, and how different tillage management strategies of the substrates might benefit carbon levels. These tests are explained below. This report summarizes the AES contribution over the program life, to helping understand answers to these questions.

Background – Task 1: Soil Testing, Analysis, and Reporting

From 2011-2013, AES conducted the following activities to fulfill its contract responsibilities to the Wilds: three visits to collect soil samples; laboratory analysis by an AES-contracted soil characterization laboratory; and an assessment of current soil carbon levels (both total and organic) in the four land management zones at the Wilds' demonstration site (during 2013, sampling was also conducted in a "control plot") and on the nearby Kreager farm reference site. A summary report was prepared for the Wilds following each of the first two sampling years. This report constitutes the final report prepared for the Wilds for the three-year study.

Stratification

At the Wilds demonstration site, four adjacent areas were chosen for different management practices, which constitute the individual strata, and an additional area has been designated a "control plot" for vegetation monitoring throughout all years and soil sampling in 2013 only. After the various treatments were completed in zones 1-4, a mix of native prairie grasses and forbs was planted at the Wilds site. No treatments occurred in Zone 5; cool season grasses typical on the larger site dominated the area. The management activities utilized in the four adjacent areas at the Wilds include:

- Zone 1 (13.5 acres) – No-till and Prairie-based Bison Grazing (though not yet introduced when Year 3 sampling was complete in June 2013);
- Zone 2 (15.8 acres) – No-till Only;
- Zone 3 (14.4 acres) – Till Only;
- Zone 4 (15.0 acres) – Sub-soil and Till; and
- Zone 5 (15.4 acres) – Control Plot

Using GIS tools, a boundary area of the test plots was randomly generated which intersects the four management zones, and randomly generated sample points distributed within each zone (Fig. A1). The area is mapped as Morristown silty-clay loam soil, though the entirety of the site is reclaimed coal mined land. As shown in the sections below, the soils vary tremendously in depth throughout the site. The site includes mostly flat topography, with some slopes less than 25%. Baseline sampling of the study area before management practices began is compared with measurements made during and at the conclusion of the 3-year study to determine if any measureable change has occurred in the soil carbon levels of the different strata.

At the Wilds' partner farmer in Muskingum County, Ohio, owned by Cathie Kreager, nine samples were collected during each year to serve as a reference site (Fig. A2). A 7.3-acre zone was chosen that encompasses portions of three pastures used for grazing beef cattle on a rotational basis. During the baseline year (2011), the samples were collected from a small area in the lower field only. In 2012, field staff from AES and the Wilds decided that the nine samples should be more widely distributed among the three primary pastures. Three samples from the lower field were retained, while the remaining six were distributed amongst the two upper pastures. At the Kreager farm, the soils are mapped as Guernsey-Upshur silty clay loam soils (6 to 15 percent slopes, eroded).

Sampling Methods

During 2011, soil samples were collected using a pneumatically powered JMC Environmentalist's Sub-soil Probe (ESP) sampler. A plastic liner was inserted into the hollow, stainless steel, tubular body of the probe, which was then pneumatically hammered into the ground to a depth of 1 meter, or until resistance impeded further insertion of the probe. All soil carbon analyses were conducted with soil samples from the plastic liners. In addition, at three of the sample locations within each zone at the Wilds and three of the sample locations at the Kreager farm, at least two bulk density samples were obtained from pits dug at the plots. A visual determination of approximate layer boundaries was made in the field and, from within the top layer, soil from the pit was scooped to fill a 2-oz metal canister. This was repeated for layers discovered down to a depth of approximately 30 cm.

During 2012 and 2013, soil samples were collected using a Giddings hydraulic soil probe mounted on a John Deere Gator ATV. At the sample site, a 2" diameter plastic liner was inserted into the hollow, stainless steel tubular body of the probe, which was then pneumatically pushed into the ground to a depth of 1 meter, or until resistance impeded further insertion of the probe. All soil carbon and bulk density analyses were conducted with soil samples from the plastic liners.

Laboratory Analysis Methods

To determine the amount of carbon in the soil at a site requires the measurement of three primary variables in the laboratory:

- Volume of soil for which calculations are being done: the amount of soil mass per unit volume (for a given soil sample, considered a point location, the measured dimension is depth; individual layer depths determined by analysis by a soil pedologist in the lab).
- Amount of carbon in the soil as a percentage of the mass of the soil (% Carbon – from laboratory analysis of the soil sample of a homogenous layer); and
- Density of the soil: the amount of soil mass per unit volume (bulk density – calculated by comparing the mass of a known volume of a freshly collected sample with the mass of the sample after it is baked to dryness, thereby removing all water from the soil).

Soil Core Descriptions:

When the soil cores were sent to the lab for analysis, the Lab was instructed to split the soil core based on genetic horizons or observed layers, with a maximum of three to four "samples" per core, due to budget limitations. The question then would be, "What sampling increments would be representative of rooting depths and pedogenic carbon (carbon from plant roots and other plant detritus) across the diversity of materials in this reclaimed landscape?"

A cursory investigation of the organic carbon depth distribution data analyzed in the laboratory quickly confirms that the root-rich surface horizons were the major soil pool for carbon sequestration on a percentage basis. One need only to look at the variability in the thicknesses of the surface horizons to realize that a uniform sampling depth increment would have homogenized the results and patterns of the most important active carbon pool in this system. In addition, it would be difficult to compare the SOC in reclaimed soils to the reference soils if a "horizon-based" approach had not been followed. If a study is being conducted to assess carbon sequestration or SOC pools, it is important to sample in such a way that does not mask the native variability of the landscape being measured.

A “horizon” was generally distinguished by material color, texture, structure and presence of biological activity, including worms and roots. Most of the observed surface horizons appeared to have some component of a pedogenic A-horizon, which had probably been scraped from somewhere in the landscape, then replaced on the surface of the reclaimed land. A horizon structure generally was fine to medium granular, and the transition to the underlying replaced materials was usually abrupt and smooth, from granular structure to massive or platey structure, with obvious color differences and abrupt decreases in root abundance.

Underlying materials were distinguished by the same criteria. Rooting depth and color were the primary discriminators for determining the "horizons" of the reclaimed materials. Most of the layering in the reclaimed materials was clearly from different sources. Multiple colors often were described because the overburden materials used in the reclamation process were usually mottled with distinct patterns and colors.

The University of Missouri’s Soil Characterization Lab Director was actively engaged in mine soil reclamation research in Missouri for 12 years, and always used morphological features to determine sampling increments. He stated that he never used uniform sampling increments that ignored both the reclamation process and subsequent plant rooting and pedogenic processes. The project was a multi-state collaboration that included Illinois, Kentucky, Indiana and North Dakota, and all five states used the same sampling protocol.

Bulk Density Analysis:

Soil bulk density (Db) is a measure of the mass of soil per unit volume (solids + pore space) and is usually reported on an oven-dry basis.

$$D_b = \frac{mass\ of\ dry\ soil}{volume\ of\ solids\ \&\ pore\ spaces}$$

Unlike the measurement of particle density, the bulk density measurement accounts for the spaces between the soil particles (pore space) as well as the soil solids. Soils with a high proportion of pore space have lower mass per unit volume, and therefore have low bulk density. Typical mineral soils have bulk densities that range from 1.0 to 1.6 g/cm^3. A bulk density greater than 1.6 g/m^3 may indicate soil compaction, which means these soils have a low proportion of pore space and, therefore, low porosity. Alternatively, soils with a high proportion of organic matter tend to have bulk densities that are less than 1.0 g/cm^3.

The bulk density of different soils varies based largely on soil texture and the degree of soil compaction. Sandy soils with low organic matter tend to have higher bulk density than clayey or loamy soils. Soil bulk density is usually higher in subsurface soils than in surface horizons, in part due to compaction by the weight of the surface soil.

The bulk densities reported in the Wilds data were reported on a rock-free basis. If the rock fragment mass is included, the bulk densities would increase accordingly.

Soil Carbon Analysis:

SOC is reported based on the percent of soil mass "lost on ignition." In other words, the accurate measure of percentage of SOC is on a rock-free basis. The sample preparation procedures were the same during all three years. All fragments larger than 2mm were separated in the sample preparation by the standard screening process. Visible coal fragments were removed by hand. Darker shale fragments were high in organic matter, and the shale decomposed readily and could not be removed. Some of the soils contained coal dust too fine to be separated. This is one of the realities of dealing with reclaimed mine soils.

One of the reasons to sample by horizons rather than by depth increments is that the surface horizon SOC is very precisely quantified, thus enhancing the ability to measure whole profile SOC.

The University of Missouri's Soil Characterization Lab Director indicated that they never acid wash samples to remove calcium carbonate because their 28 years of experience with the Leco "loss on ignition" method shows that carbonate is released at different temperatures than SOC. As a result, they "burn off" the carbonate rather than go through the time consuming, more costly, messy and imprecise acid-washing procedure. The National Soil Survey Laboratory in Lincoln, Nebraska, has verified that the "two-step loss on ignition" process precisely distinguishes between SOC and carbonate C.

Data Analysis Methods

Combining this information for each soil layer of a sample core, we can calculate the carbon content at that plot location in units of soil carbon per unit area. A generalized version of the calculation is:

$$SC_y = \sum_{l=1}^{L} sd_l \cdot sdens_l \cdot \%sc_l \qquad (1)$$

Where:

SC_y = Total measured soil carbon per unit area at plot y

L = The number of soil layers measured within the calculated depth

l = Individual soil layer

sd_l = The average depth (thickness) of soil layer l within the plot

$sdens_l$ = The average bulk density of soil layer l within the plot

$\%sc_l$ = Percentage carbon (The average mass of soil carbon in layer l , as a percentage of the total mass of the sample, as measured in the laboratory)

To address the variability in soil horizons and soil cores by year, an additional step was completed in 2013. After the carbon levels were converted from to kg/m^2 by horizon (from % carbon), the carbon levels for each soil core were normalized to a consistent depth of 100 cm for comparison purposes. Standard adjustments to account for differences in soil mass, or soil core lengths, are common for comparison purposes. However, it should be noted that this always results in estimates that may be inflated or deflated. By normalizing all data across zones and years for the Wilds 2011-2013 soil carbon data, it ensures that the soil cores can be compared across all years and appropriate statistical analyses (ANOVA) can be completed.

Pre-sampling (March 2011)

On March 3, 2011, Steven Apfelbaum and Carl Christopher of AES visited the Wilds' demonstration site and partner-farmer Cathie Kreager's farm to perform preliminary soil sampling. Soil core samples were collected from ten plots at the Wilds and three additional plots at the Kreager farm. For each sample, the soil core was divided into fourths to a depth of 1 meter (40 inches). A sub-sample was drawn from each of these depth segments, which were then mixed to form a single homogenized sample (for a given plot). Four of the Wilds samples and one of the Kreager samples were shipped to the University of Missouri's Soil Characterization Laboratory (UM Soils Lab), for analysis of % Total Carbon, % Organic Carbon, % Total Nitrogen, pH Water, and pH Salt (Table 1).

The % Total Carbon values for the four Wilds plots were analyzed to get a general idea of the soil carbon content and to estimate how many samples would need to be collected for baseline sampling. For the margin of error to be within +/- 10% of the sample mean with 95% confidence, we calculated that 32 samples should be collected at the Wilds if the % Total Carbon is represented by a normal distribution. Since it is unlikely that the actual carbon distribution is normal, we decided to collect samples at 61 plots randomly distributed across the entire study area; at least 15 from within each zone (Figure A1).

Table 1: Soil Pre-sampling Lab Results (2011)

	Sample	% Total Nitrogen	% Organic Carbon	% Inorganic Carbon	% Total Carbon	pH water	pH salt
The Wilds	CIG-1	0.08	0.63	0.61	1.24	7.78	7.39
	CIG-5	0.10	1.61	0.60	2.21	7.20	7.08
	CIG-7	0.10	0.76	1.04	1.80	7.88	7.49
	CIG-10	0.07	0.59	0.68	1.27	7.90	7.47
	Average:		0.90		1.63		
	SD:		0.48		0.46		
Kreager Farm	KRE-1	0.18	1.57	0.00	1.57	6.71	6.34

Baseline (Year One) Sampling (April 2011)

Steven Apfelbaum and Carl Christopher returned to the Wilds and Kreager Farm on April 17 and 18, 2011, to collect the baseline samples. Casey Brooks of the Wilds assisted them. Sample cores were collected from 61 plots at the Wilds and 9 plots at the Kreager Farm. During 2011, soil samples were collected using the soil sampling methods described in the Background section above.

Core Depth and Rooting Depth

The core sampling depths achievable until resistance was experienced showed wide variation, ranging from 9 cm to 78 cm, although the sample cores obtained at the Kreager Farm were on average about 16 cm deeper than the Wilds samples (Table 2). In all cases, the sample cores collected were shallower than the ideal, pre-chosen sampling depth for the experiment of 1 meter[1]. The plastic sleeves containing the freshly removed cores were capped and shipped directly to the UM Soils Lab. At the lab, a soil pedologist described them, including determination of layer thicknesses based on Munsell colors. Bulk density and chemical analyses were completed.

Table 2: Baseline (Year One) Soil Sample Total Core Depths (2011)

	Cores n	Mean Length cm	Std Dev cm	Median cm	Minimum cm	Maximum cm
Zone 1	16	54.44	16.42	58	22	78
Zone 2	15	48.07	14.84	49	25	71
Zone 3	15	36.73	15.23	36	16	73
Zone 4	15	36.80	13.82	38	9	60
Zones 1-4	61	44.18	16.64	42	9	78
Kreager Farm	9	60.22	10.17	62	39	70

During the laboratory analysis, the pedologist carefully noted the rooting depth within each soil core. In 2011, the rooting depths at the Wilds ranged from 5 cm to 50 cm, with an average of 25 cm; while the rooting depths observed at the Kreager farm were on average about 27 cm deeper than the Wilds samples (Table 3). On average, the rooting depth was 57% of the depth of the soil core. Zones 2 had a slightly deeper rooting depth than Zones 1, 3 and 4, and Zone 4 had the shortest maximum rooting depth.

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[1] This sampling impediment is not unusual; it is often experienced in former mined lands due to the presence of rock fragments within the respreads substrates that are now serving as rooting medium and topsoils.

Table 3: Baseline (Year One) Soil Sample Rooting Depths (2011)

	Cores n	Mean Length cm	Std Dev cm	Median cm	Minimum cm	Maximum cm
Zone 1	16	22.44	11.31	18	5	48
Zone 2	15	28.20	12.03	25	8	50
Zone 3	15	24.00	10.20	25	8	48
Zone 4	15	24.73	8.97	27	8	39
Zones 1-4	61	24.80	10.65	23	5	50
Kreager Farm	9	52.00	20.06	58	4	70

Bulk Density

At three of the sample locations within each zone at the Wilds, and three of the sample locations at the Kreager farm, at least two bulk density samples were obtained from pits dug at the plots. A visual determination of approximate layer boundaries was made in the field and, from within the top layer, soil from the pit was scooped to fill a standard bulk density 2-oz metal canister. This was repeated for layers to a depth of approximately 30 cm.

The topmost layer, referred to as the "A-horizon," had fairly consistent thicknesses of approximately 6 cm for the Wilds sample locations. The A-horizon was slightly thicker, averaging 8.5 cm (but results were more variable), at the Kreager farm. The next lowest layer below the A-horizon that was sampled for bulk density was taken from between the bottom of the A-horizon and the approximate 30 cm maximum depth to which the pit was dug, or until a distinct change in color was detected, and this was identified as the B-horizon bulk density sample. At several of the pits, there was a distinct third layer below the B-horizon. This layer, labeled as the C-horizon, was sampled for bulk density in a similar manner. A total of 35 individual samples[2] were collected from the different layers at the different sample plots, and shipped to the UM Soils Lab for drying, final weight measurement and bulk density determination.

Table 4: Baseline (Year One) Average A, B and C Horizon Bulk Density Values (2011)

$sdens_l$	Horizon	n	Mean g cm^{-3}	SD g cm^{-3}	SEM g cm^{-3}
The Wilds	A	11	**0.92**	0.08	0.02
	B	12	**1.31**	0.09	0.03
	C	6	**1.32**	0.06	0.02
Kreager Farm	A	3	**0.87**	0.05	0.03
	B	3	**0.96**	0.19	0.03

Soil Carbon

Analysis of Total Carbon and Organic Carbon content for each of the individual core samples was completed. Grouping the sample data points based on their strata boundaries (Figs. A1, A2), the 95%

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[2] Of the 35 individual specimens: 7 were collected from Zone1, 9 were collected from Zone 2, 6 were collected from Zone 3, 7 were collected from Zone 4, and the remaining 6 were collected from the Kreager farm.

confidence intervals for the carbon density (kg/m^2) and the total carbon content (kg CO_2 equivalents) are reported for each management zone (Table 5, Table 6). For each of the strata the uncertainty in both the total and organic carbon calculations was highly variable.

A summary of all sample points across Zones 1-4 yields a baseline estimate of 20.8 to 24.2 kg/m^2 for total carbon and 8.8 to 11.0 kg/m^2 for organic carbon. To achieve a similar level of accounted-for variance (to explain the statistic variance in the individual strata) for post-management comparison, 15-16 additional samples would need to be collected in each zone. If a similar mean and standard deviation were obtained among 30 soil cores, the relative error would be reduced by an additional 4-5% in each zone and 2% in the grouped (Zones 1-4) zone for total carbon—and even greater amounts for organic carbon (e.g. 5-12%).

An alternative to collecting more samples would be to reduce the maximum calculated depth. Choosing only to compare soil carbon content down to a certain depth (e.g. 30cm) would reduce much of the uncertainty associated with combining carbon content calculations at different points having widely varying calculating depths, but would include the A-horizons where most of the organic carbon exists, as seen in the raw data tables. Additionally, this could help to ensure that coal fragments or coal dust, typically found in the lower horizons, does not artificially inflate the carbon values.

Table 5: Baseline (Year One) Soil Total Carbon, 95% Confidence Intervals (2011)

Total Carbon	Sample plots	Carbon			Zone Area	Zone CO_2e			Relative Error
	n	kg/m^2			acre	MT			+/- %
Zone 1	16	21.7	*to*	30.3	13.5	4,340	*to*	6,070	16.6%
Zone 2	15	16.9	*to*	23.3	15.8	3,960	*to*	5,470	16.0%
Zone 3	15	20.2	*to*	26.6	14.4	4,330	*to*	5,670	13.5%
Zone 4	15	16.9	*to*	86.8	15.0	3,770	*to*	5,270	16.6%
Zones 1-4	61	20.8	*to*	24.2	58.7	18,100	*to*	21,000	7.4%
Kreager Farm	9	10.4	*to*	15.4	7.3	1,120	*to*	1,670	19.7%

Table 6: Baseline (Year One) Soil Organic Carbon, 95% Confidence Intervals (2011)

Organic Carbon	Sample plots	Carbon			Zone Area	Zone CO_2e			Relative Error
	n	kg/m^2			acre	MT			+/- %
Zone 1	16	6.2	*to*	13.2	13.5	1,250	*to*	2,640	35.7%
Zone 2	15	7.2	*to*	11.0	15.8	1,690	*to*	2,570	20.7%
Zone 3	15	8.7	*to*	11.5	14.4	1,850	*to*	2,470	14.3%
Zone 4	15	8.8	*to*	12.4	15.0	1,950	*to*	2,770	17.2%
Zones 1-4	61	8.8	*to*	11.0	58.7	7,660	*to*	9,580	11.1%
Kreager Farm	9	9.0	*to*	15.2	7.3	977	*to*	1,640	25.4%

Year Two Sampling (November 2012)

Two AES Ecologists, Ry Thompson and Jarrett Cellini, returned to the Wilds and Kreager Farm on November 19, 2012, to collect the year two samples, assisted by Jessica Spencer of the Wilds. Sample cores were collected from 61 plots at the Wilds (Fig. A1) and 9 plots at the Kreager Farm (Fig. A2). During 2012, soil samples were collected using the soil sampling methods described in the Background section above.

Core Depth and Rooting Depth

The core sampling depths achievable until resistance was experienced showed less variation than in 2011. This was likely due to the collection method changing from the JMC soil sampler to the Giddings hydraulic soil sampler. In 2012, the core depths at the Wilds ranged from 23 cm to 100 cm, with an average of 62 cm, while the sample cores obtained at the Kreager farm were on average about 11 cm deeper than the Wilds samples (Table 7). In most cases, the sample cores collected were shallower than the ideal, pre-chosen sampling depth for the experiment of 1 meter, but they were 17 cm deeper on average than in 2011. At most sampling locations, the hydraulic soil sampler reached a restrictive layer and met resistance at a depth shallower than 1 meter. This often occurred at a distinct shale layer on the site. The plastic sleeves containing the freshly removed cores were capped and shipped directly to the University of Missouri Soil Characterization lab for core description and splitting for chemical analysis based on the observed horizons.

Table 7: Year Two Soil Sample Total Core Depths (2012)

	Cores n	**Mean Length cm**	**Std Dev cm**	**Median cm**	**Minimum cm**	**Maximum cm**
Zone 1	16	62.81	24.67	63	23	100
Zone 2	15	62.80	23.70	67	27	100
Zone 3	15	56.07	18.88	53	30	90
Zone 4	15	64.73	23.14	70	24	98
Zones 1-4	61	61.62	22.41	63	23	100
Kreager Farm	9	72.67	12.42	68	61	100

During the laboratory analysis, a soil pedologist described and split the soil cores into genetic horizons as described above. In addition, the pedologist carefully noted the rooting depth within each soil core, following the standard methods described in *Field book for describing and sampling soils, Version 3.0* (Shoeneberger et. al). In 2012, the rooting depths at the Wilds ranged from 6 cm to 77 cm, with an average of 36 cm; while the rooting depths observed at the Kreager farm were on average about 34 cm deeper than the Wilds samples (Table 8). On average, the rooting depth was 62% of depth of the soil core, and ranged from 18% - 100% of the core depth. Zones 2 and 4 had a slightly deeper average rooting depth than Zones 1 and 3, though Zone 4 had the shortest maximum rooting depth.

Table 8: Year Two Soil Sample Rooting Depths (2012)

	Cores n	Mean Length cm	Std Dev cm	Median cm	Minimum cm	Maximum cm
Zone 1	16	33.31	11.89	29	20	63
Zone 2	15	36.13	10.61	36	22	57
Zone 3	15	34.73	16.49	32	14	78
Zone 4	15	38.07	16.41	36	6	77
Zones 1-4	61	35.52	13.82	34	6	78
Kreager Farm	9	69.44	9.76	67	58	86

Bulk Density

At all of the sample locations within each zone at the Wilds, and at all of the sample locations at the Kreager farm, bulk density samples were obtained from the soil cores and run at the lab. A bulk density sample was collected and run from every described soil horizon within every soil core. The topmost layer is referred to collectively as the "A-horizon." The A-horizon refers to any horizon beginning with "A" (A1, A2, and AB). Below the A-horizon, the next lowest layer sampled for bulk density was identified as the "B-horizon," taken from where a distinct change in color was detected. The B-horizon includes B1, B2, B1c, B2c, and Bc. At a majority of the soil core locations, below the B-horizon there was a distinct third layer (labeled as the "C-horizon"), which was sampled for bulk density in a similar manner. If encountered, a restrictive (R) layer was also documented. Average bulk density values were re-measured in 2012 (Table 9). The standard deviation and standard error of the mean (SEM) were computed and are included.

Table 9: Year Two Average A, B and C Horizon Bulk Density Values (2012)

$sdens_l$	Horizon	n	Mean g cm^{-3}	SD g cm^{-3}	SEM g cm^{-3}
The Wilds	A	61	**0.98**	0.14	0.02
	B	52	**1.26**	0.16	0.02
	C	66	**1.28**	0.23	0.03
Kreager Farm	A	12	**1.14**	0.17	0.05
	B	16	**1.34**	0.18	0.05
	C	4	**1.33**	0.26	0.13

Soil Carbon

Analysis of Total Carbon and Organic Carbon content for each of the individual core samples was completed. Grouping the sample data points based on their strata boundaries (Figs. A1, A2), the 95% confidence intervals for the carbon density (kg/m^2) and the total carbon content (kg CO_2 equivalents) are reported for each management zone (Table 10, Table 11). For each of the strata the uncertainty in both the total and organic carbon calculations is high, but it is less than the 2011 uncertainty.

A summary of all sample points across Zones 1-4 yields a baseline estimate of 19.0 to 22.8 kg/m^2 for total carbon and 7.8 to 9.0 kg/m^2 for organic carbon. To achieve a similar level of accounted-for variance (to explain the statistical variance in the individual strata) for post-management comparison, 15-16 additional samples would need to be collected in each zone. If a similar mean and standard deviation were obtained among 30 soil cores, the relative error would be reduced by an additional 3-8% in each zone and 2.5% in the grouped (zone 1-4) zone for total carbon and similar amounts for organic carbon.

An alternative to collecting more samples would be to reduce the maximum calculated depth, as stated above. Choosing only to compare soil carbon content down to a certain depth (e.g. 30 cm) would reduce some of the uncertainty associated with combining carbon content calculations at different points having varying calculating depths, but would include the A-horizons of soil where most of the organic carbon exists, as seen in the raw data tables. Additionally, this could help to ensure that coal fragments or coal dust, typically found in the lower horizons, does not artificially inflate the carbon values.

Table 10: Year Two Soil Total Carbon, 95% Confidence Intervals (2012)

Total Carbon	**Sample plots**	**Carbon**			**Zone Area**	**Zone CO_2e**			**Relative Error**
	n	kg/m^2			acre	MT			+/- %
Zone 1	16	23.1	*to*	33.1	13.5	4,640	*to*	6,620	17.6%
Zone 2	15	15.0	*to*	18.6	15.8	3,520	*to*	4,350	10.5%
Zone 3	15	16.3	*to*	23.3	14.4	3,470	*to*	4,990	17.9%
Zone 4	15	13.7	*to*	85.3	15.0	3,060	*to*	5,180	25.7%
Zones 1-4	61	19.0	*to*	22.8	58.7	16,600	*to*	19,800	8.9%
Kreager Farm	9	13.0	*to*	23.6	7.3	1,410	*to*	2,560	29.0%

Table 11: Year Two Soil Organic Carbon, 95% Confidence Intervals (2012)

Organic Carbon	**Sample plots**	**Carbon**			**Zone Area**	**Zone CO_2e**			**Relative Error**
	n	kg/m^2			acre	MT			+/- %
Zone 1	16	8.9	*to*	12.3	13.5	1,790	*to*	2,450	15.6%
Zone 2	15	6.5	*to*	8.7	15.8	1,540	*to*	2,030	13.8%
Zone 3	15	6.3	*to*	8.7	14.4	1,340	*to*	1,860	16.2%
Zone 4	15	6.3	*to*	9.1	15.0	1,410	*to*	2,020	18.0%
Zones 1-4	61	7.8	*to*	9.0	58.7	6,760	*to*	7,870	7.6%
Kreager Farm	9	9.0	*to*	20.8	7.3	972	*to*	2,250	39.7%

Year Three Sampling (June 2013)

Two AES Ecologists, Ry Thompson and Jarrett Cellini, returned to the Wilds and Kreager farm on June 18, 2013, to collect Year Three samples. Jessica Spencer and two summer interns from the Wilds assisted. Sample cores were collected from 71 plots at the Wilds (Fig. A1) and 9 plots at the Kreager Farm (Fig. A2). During 2013, soil samples were collected using the soil sampling methods described in the Background section above.

Core Depth and Rooting Depth

The core depths showed a similar variation to those in 2012, though they averaged slightly less in 2013. In 2012, the core depths at the Wilds ranged from 23 cm to 99 cm, with an average of 57 cm, while the sample cores obtained at the Kreager farm were on average about 41 cm deeper than the Wilds samples (Table 12). In most cases, the sample cores collected from the Wilds were shallower than the ideal, pre-chosen sampling depth for the experiment of 1 meter. At most sampling locations, the hydraulic soil sampler reached a restrictive layer and met resistance at a depth shallower than 1 meter. The plastic sleeves containing the freshly removed cores were capped and shipped directly to the Soil Characterization lab for description of soil horizons by Munsell colors and splitting for chemical analysis based on these horizons.

Table 12: Year Three Soil Sample Total Core Depths (2013)

	Cores n	Mean cm	Std Dev cm	Median cm	Minimum cm	Maximum cm
Zone 1	16	53.94	28.30	48.5	23	98
Zone 2	15	71.47	21.44	73	32	98
Zone 3	15	53.13	26.41	41	27	99
Zone 4	15	48.67	18.67	49	24	94
Zones 1-4	61	56.75	25.05	52	23	99
Zone 5 – Control	10	47.70	23.04	40.5	24	99
Kreager Farm	9	97.33	2.45	98	94	100

During the laboratory analysis, a soil pedologist described and split the soil cores into genetic horizons as described above. In addition, the pedologist carefully noted the rooting depth within each soil core. In 2013, the rooting depths at the Wilds ranged from 22 cm to 94 cm, with an average of 45 cm; while the rooting depths observed at the Kreager farm were on average about 45 cm deeper than the Wilds samples (Table 13). On average, the rooting depth in zones 1-4 was 86% of the depth of the soil core, and ranged from 22% - 100% of the core depth. Zones 2 and 4 had a slightly deeper rooting depth than Zones 1 and 3. Zone 5 had a slightly shorter average rooting depth than zones 1-4.

Table 13: Year Three Soil Sample Rooting Depths (2013)

	Cores n	Mean Length cm	Std Dev cm	Median cm	Minimum cm	Maximum cm
Zone 1	16	40.94	15.70	37.5	22	66
Zone 2	15	52.60	22.15	49	26	91
Zone 3	15	39.00	10.28	36	27	60
Zone 4	15	46.87	18.80	39	24	94
Zones 1-4	61	44.79	17.66	39	22	94
Zone 5 – Control	10	43.30	14.80	40.50	24	71
Kreager Farm	9	90.11	16.95	97	48	100

Bulk Density

At all of the sample locations within each zone at the Wilds, and at all of the sample locations at the Kreager Farm, bulk density samples were obtained from the soil cores and run at the lab. A bulk density sample was collected and run from every described soil horizon within every soil core. The topmost layer, referred to collectively as the "A-horizon," refers to any horizons beginning with A (A1, A2, and AB). The next lowest layer below the A-horizon that was sampled for bulk density was taken from where a distinct change in color was detected, and this was identified as the B-horizon and includes B1, B2, B1c, B2c, and Bc. At a majority of the soil core locations, there was a distinct third layer below the B-horizon, which was sampled for bulk density in a similar manner, and labeled as the C-horizon. If encountered, a restrictive (R) layer was also documented. Average bulk density values re-measured in 2013 (Table 14) below, and averaged slightly lower than those observed in 2012.

Table 14: Year Three Average A, B and C Horizon Bulk Density Values (2013)

$sdens_l$	**Horizon**	**n**	**Mean g cm^{-3}**	**SD g cm^{-3}**	**SEM g cm^{-3}**
The Wilds	A	56	**0.92**	0.17	0.02
	B	70	**1.06**	0.22	0.03
	C	44	**1.11**	0.22	0.03
Kreager Farm	A	21	**1.03**	0.18	0.04
	B	20	**1.23**	0.13	0.03

Soil Carbon

Analysis of Total Carbon and Organic Carbon content for each of the individual core samples was completed. Grouping the sample data points based on their strata boundaries (Figs. A1, A2), the 95% confidence intervals for the carbon density (kg/m^2) and the total carbon content (kg CO_2 equivalents) are reported for each management zone (Table 15, Table 16). For each of the strata the uncertainty in both the total and organic carbon calculations is high, but it is less than the 2011 uncertainty and similar to the 2012 uncertainty.

A summary of all sample points across Zones 1-4 yields a baseline estimate of 15.2 to 18.2 kg/m^2 for total carbon and 6.5 to 9.3 kg/m^2 for organic carbon. To achieve a similar level of accounted-for variance (to explain the statistical variance in the individual strata) for post-management comparison, 15-16 additional samples would need to be collected in each zone. If a similar mean and standard deviation were obtained among 30 soil cores, the relative error would be reduced by an additional 6-8% in each zone and 2.5% in the grouped (zone 1-4) zone for total carbon and similar amounts for organic carbon.

An alternative to collecting more samples would be to reduce the maximum calculated depth, as stated above. Choosing only to compare soil carbon content down to a certain depth (e.g. 30 cm) would reduce some of the uncertainty associated with combining carbon content calculations at different points having varying calculating depths, but would include the A-horizons of soil where most of the organic carbon exists, as seen in the raw data tables. Additionally, this could help to ensure that coal fragments or coal dust, typically found in the lower horizons, does not artificially inflate the carbon values.

Table 15: Year Three Soil Total Carbon, 95% Confidence Intervals (2013)

Total Carbon	**Sample plots**	**Carbon**			**Zone Area**	**Zone CO_2e**			**Relative Error**
	n	kg/m^2			acre	MT			+/- %
Zone 1	16	14.6	*to*	21.2	13.5	2,930	*to*	4,240	18.2%
Zone 2	15	10.3	*to*	15.9	15.8	2,420	*to*	3,720	21.1%
Zone 3	15	14.8	*to*	22.2	14.4	3,170	*to*	4,730	19.8%
Zone 4	15	14.1	*to*	73.7	15.0	3,140	*to*	4,470	17.5%
Zones 1-4	61	15.2	*to*	18.2	58.7	13,300	*to*	15,800	8.9%
Zone 5 – Control	10	13.4	*to*	21.8	15.4	3,060	*to*	4,990	24.0%
Kreager Farm	9	11.5	*to*	22.9	7.3	1,250	*to*	2,480	33.1%

Table 16: Year Three Soil Organic Carbon, 95% Confidence Intervals (2013)

Organic Carbon	**Sample plots**	**Carbon**			**Zone Area**	**Zone CO_2e**			**Relative Error**
	n	kg/m^2			acre	MT			+/- %
Zone 1	16	6.5	*to*	9.3	13.5	1,300	*to*	1,860	17.5%
Zone 2	15	4.9	*to*	7.5	15.8	1,140	*to*	1,770	21.4%
Zone 3	15	6.3	*to*	9.5	14.4	1,340	*to*	2,030	20.3%
Zone 4	15	6.7	*to*	8.9	15.0	1,490	*to*	1,980	14.2%
Zones 1-4	61	6.8	*to*	8.0	58.7	5,890	*to*	7,000	8.7%
Zone 5 – Control	10	7.2	*to*	10.2	15.4	1,640	*to*	2,330	17.3%
Kreager Farm	9	6.7	*to*	19.9	7.3	724	*to*	2,160	49.7%

Results and Discussion

In this section, an analysis of variance (ANOVA) is presented. This is followed by a discussion of existing soil carbon levels and hypotheses associated with these levels. The section closes with a discussion of future soil carbon accrual potential in the experimental management zones at the Wilds.

Statistical Data Analysis Summary

Analysis of variance (ANOVA) is a collection of statistical models used to analyze the differences between group means and their associated procedures (such as "variation" among and between groups). In its simplest form, ANOVA provides a statistical test of whether or not the means of several groups are equal, and therefore generalizes t-test to more than two groups. ANOVAs are useful in comparing (testing) three or more means (groups or variables) for statistical significance.

For the Wilds data, ANOVA was conducted with pooled data from the three years of investigations of organic C as dependent variables for the four zones and the reference site (Kreager Farm). The organic soil C was transformed by natural logarithm function to meet the normality assumption for ANOVA analysis (Figure 1 & Table 17). The assumption of homogeneity for the variance analysis was also tested by the standard approach of Levene's test with an option of the squared residuals, which showed that our analysis did not violate the assumption (F=2.16 and p=0.0744).

Figure 1: Normality test for the soil organic carbon

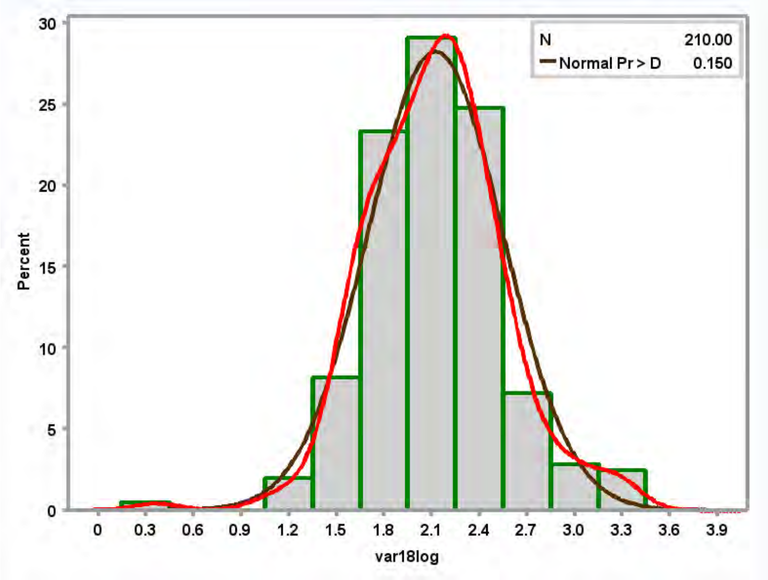
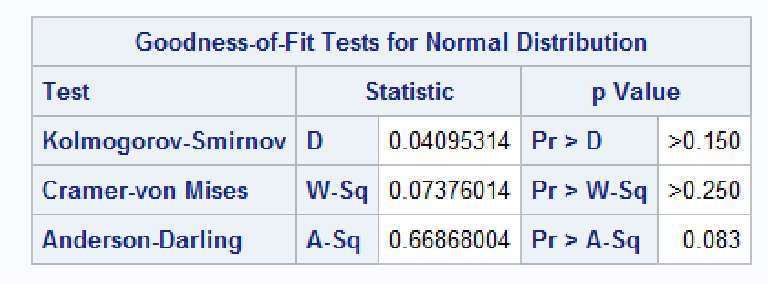


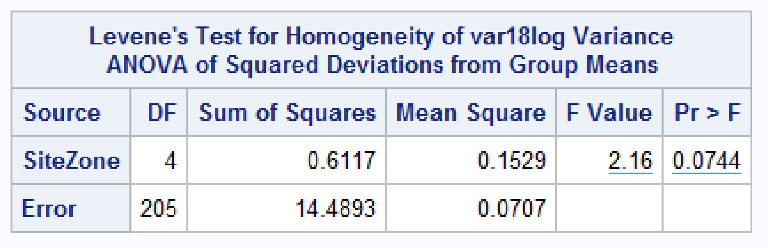
Table 17: Normality test for the soil organic carbon



Goodness-of-Fit Tests for Normal Distribution

Test	Statistic		p Value	
Kolmogorov-Smirnov	D	0.04095314	Pr > D	>0.150
Cramer-von Mises	W-Sq	0.07376014	Pr > W-Sq	>0.250
Anderson-Darling	A-Sq	0.66868004	Pr > A-Sq	0.083

Table 18: Homogeneity test

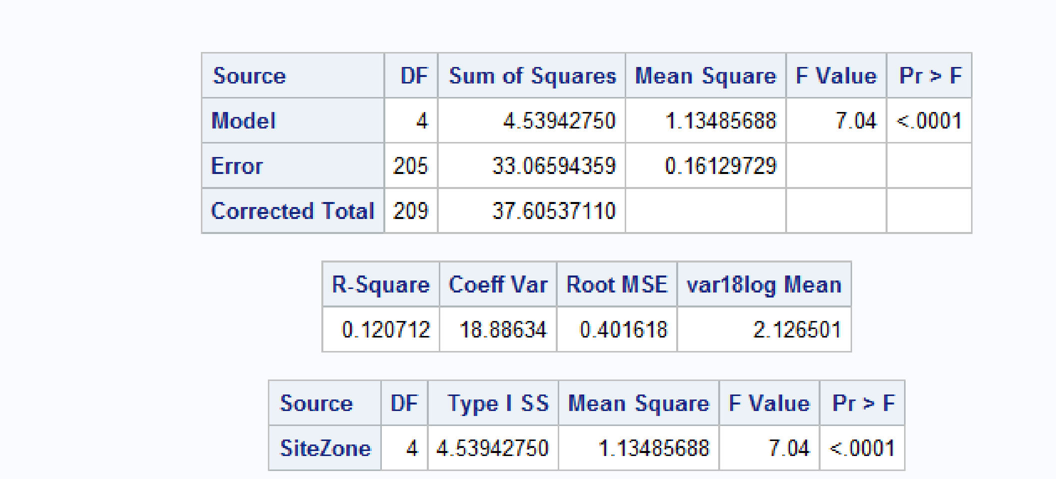


Levene's Test for Homogeneity of var18log Variance
ANOVA of Squared Deviations from Group Means

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
SiteZone	4	0.6117	0.1529	2.16	0.0744
Error	205	14.4893	0.0707		

When we were sure that the major assumptions of the analysis were not violated, we conducted the final analysis with SAS PROC GLM procedure. The analysis showed that derived F=7.04 exceeds the tabled critical values of F=2.41 at p=0.05 with df1=4 and df2=205. Therefore, H0 is rejected, and it was concluded that at least one mean was significantly different from one other mean for the zones. To determine the pattern of mean differences, a Tukey post hoc test was conducted, and it showed that the organic soil C at the reference site (Kreager farm) was significantly different from the carbon at each of the four zones. In contrast, among the four zones, the means of the carbon were not significantly different from each other (Figure 2 and Table 20).

Table 19: Variance analysis



Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	4.53942750	1.13485688	7.04	<.0001
Error	205	33.06594359	0.16129729		
Corrected Total	209	37.60537110			

R-Square	Coeff Var	Root MSE	var18log Mean
0.120712	18.88634	0.401618	2.126501

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SiteZone	4	4.53942750	1.13485688	7.04	<.0001

Figure 2: Post-hoc test of means of the soil organic C in the five sites (a significant difference of the mean at the level of 0.05 indicated by the difference of the letters at the top of the error bar).

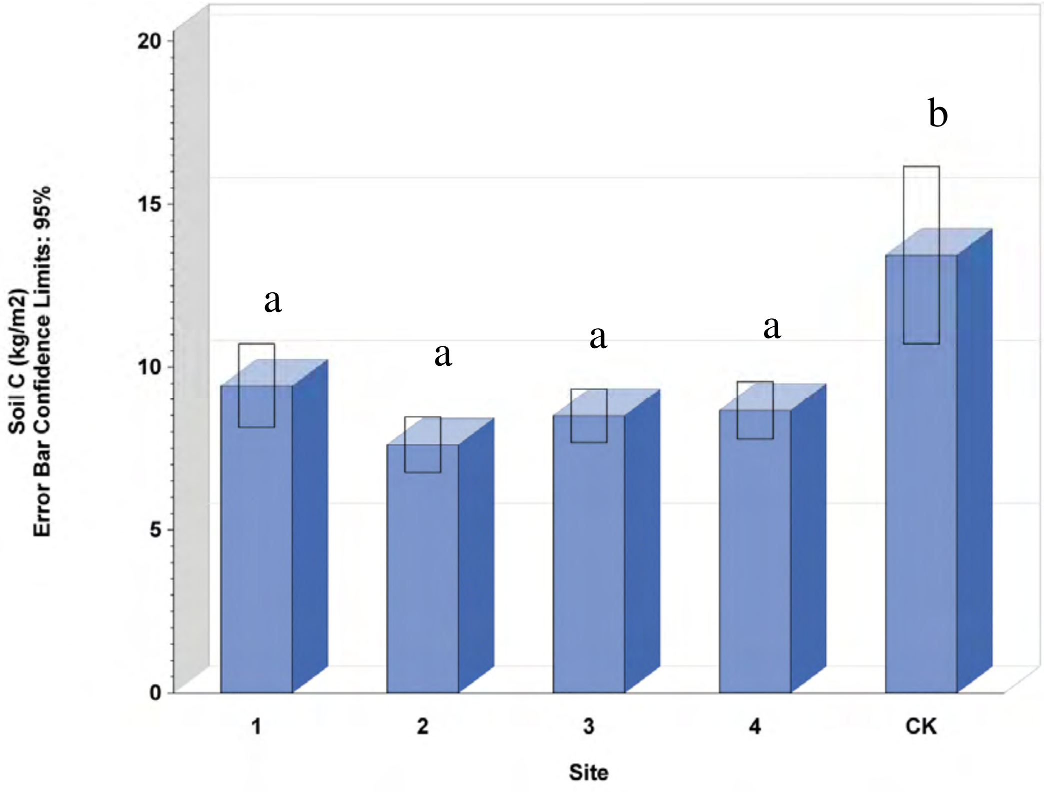
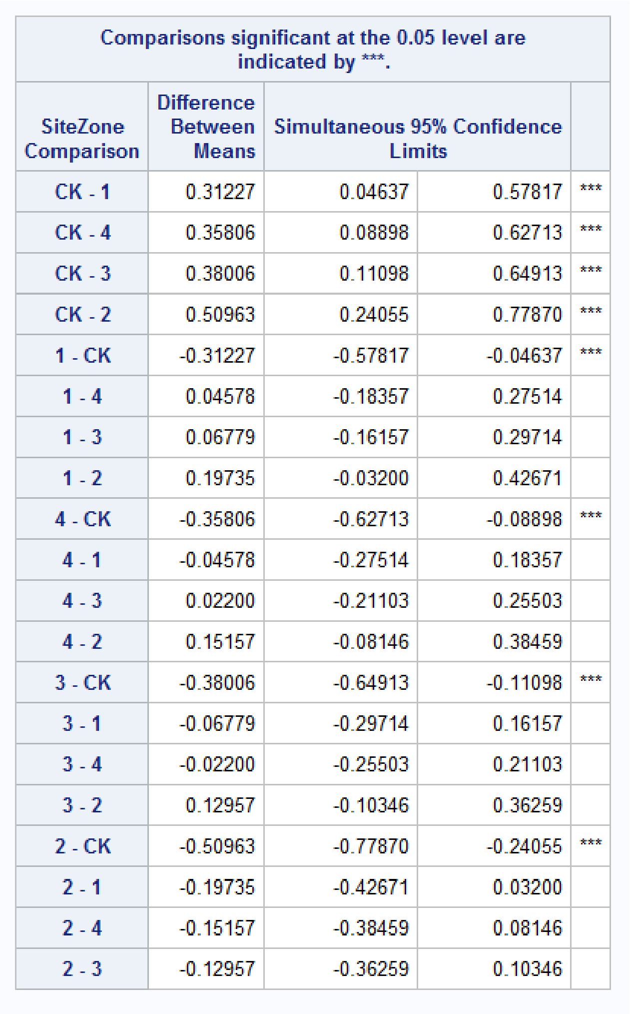


Table 20: Post-hoc test of means of the soil organic C in the five sites.



Comparisons significant at the 0.05 level are indicated by ***.

SiteZone Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
CK - 1	0.31227	0.04637	0.57817	***
CK - 4	0.35806	0.08898	0.62713	***
CK - 3	0.38006	0.11098	0.64913	***
CK - 2	0.50963	0.24055	0.77870	***
1 - CK	-0.31227	-0.57817	-0.04637	***
1 - 4	0.04578	-0.18357	0.27514	
1 - 3	0.06779	-0.16157	0.29714	
1 - 2	0.19735	-0.03200	0.42671	
4 - CK	-0.35806	-0.62713	-0.08898	***
4 - 1	-0.04578	-0.27514	0.18357	
4 - 3	0.02200	-0.21103	0.25503	
4 - 2	0.15157	-0.08146	0.38459	
3 - CK	-0.38006	-0.64913	-0.11098	***
3 - 1	-0.06779	-0.29714	0.16157	
3 - 4	-0.02200	-0.25503	0.21103	
3 - 2	0.12957	-0.10346	0.36259	
2 - CK	-0.50963	-0.77870	-0.24055	***
2 - 1	-0.19735	-0.42671	0.03200	
2 - 4	-0.15157	-0.38459	0.08146	
2 - 3	-0.12957	-0.36259	0.10346	

Existing Soil Carbon Levels

The quantity of carbon contained in soils is directly related to the diversity and health of soil biota. Since virtually all organic carbon sequestered in soils is extracted from the atmosphere by photosynthetic organisms and converted to complex molecules by bacteria and fungi, in synergy with insects and animals, it has been proposed that restoring degraded grasslands worldwide is an effective and sustainable method for increasing soil organic carbon. Existing organic and total soil carbon levels were documented within the four management zones (covering the approximately 60-acre research site) at the Wilds between 2011 and 2013.

The Wilds implemented four distinct replicable options of land preparation and land use/management practices to initiate growing high diversity prairie on reclaimed coal mined land. This land is typically severely degraded and has poor productivity. Non-native, cool season grasses dominated the study area. The Wilds employed three different land preparation methods for the four 15-acre management zones, including a) sub-soil and till; b) only till; and c) two parcels with no-till, one of which will later support prairie-based bison grazing.

As described above and shown in Figures 3-6, the sampling data shows reasonably consistent year-to-year levels of soil carbon at the Wilds. Because of the dominance of cool season grasses found growing homogenously over most of the study area, it is unlikely that the variable timing of the annual sampling events has contributed to major deviations in soil carbon levels.

It will likely take longer than the 3-year duration of the study for soil carbon stocks to reach their new equilibrium levels based on new management practices. Because of this, proportionate scaling to project soil carbon levels may be effected more by the scaling changes than real on the ground carbon levels. As mentioned above under data analysis, the soil carbon levels presented in this report have been adjusted, or normalized, to represent 100cm core lengths for comparison purposes. As shown in Tables 2, 7, and 12 above, the actual measured average soil core lengths were considerably shorter than 100cm so the soil carbon estimates provided are inflated accordingly.

Figures 3-6 provide a summary of the soil carbon data from all zones between 2011 and 2013.

Figure 3: Total Soil Carbon Levels, Grouped by Year

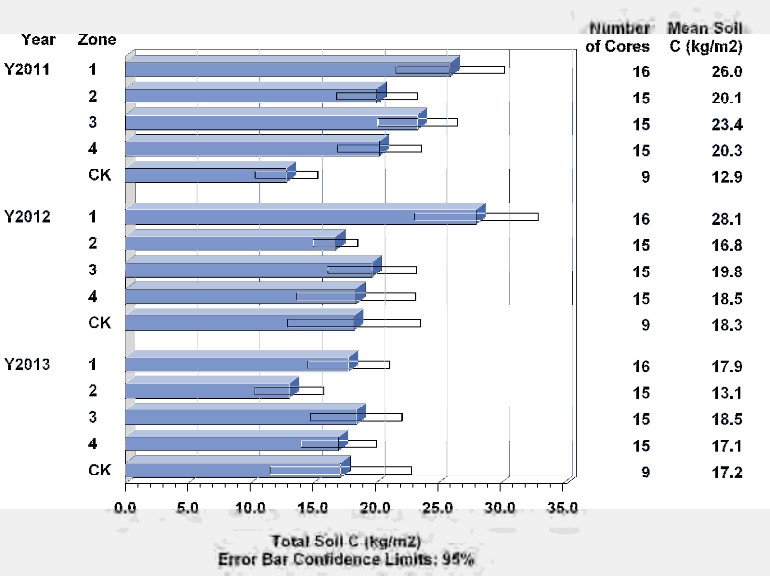


Figure 4: Organic Carbon Levels, Grouped by Year

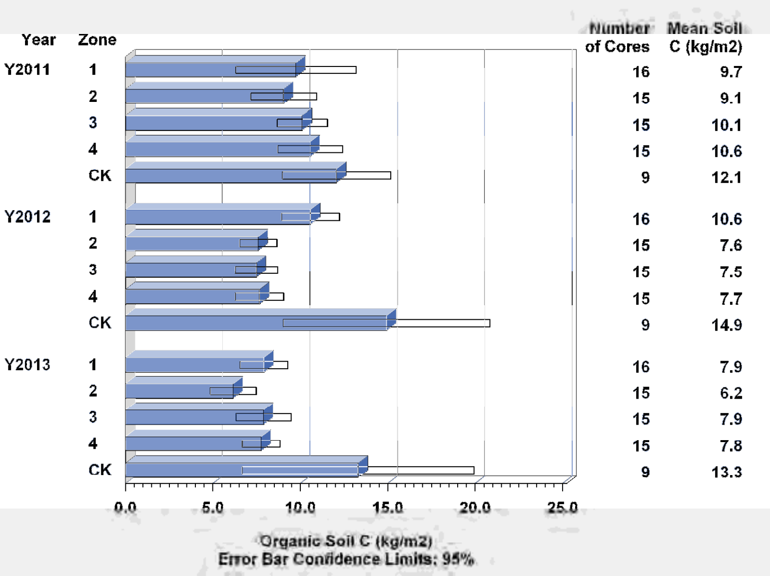


Figure 5: Total Soil Carbon Levels, Grouped by Zone

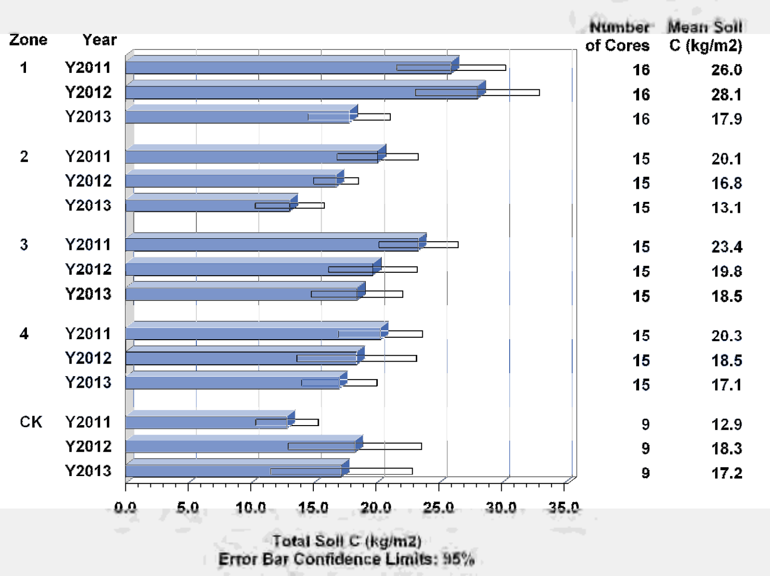
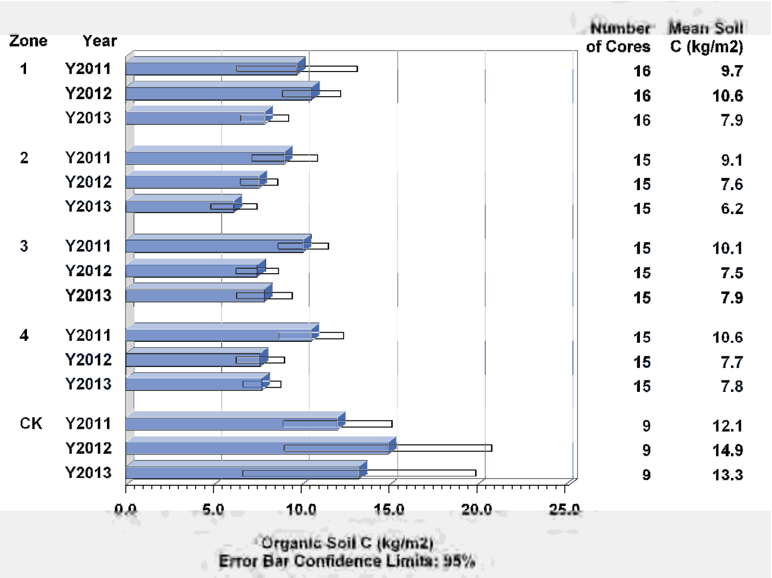


Figure 6: Organic Carbon Levels, Grouped by Zone



Zone	Year	Number of Cores	Mean Soil C (kg/m2)
1	Y2011	16	9.7
	Y2012	16	10.6
	Y2013	16	7.9
2	Y2011	15	9.1
	Y2012	15	7.6
	Y2013	15	6.2
3	Y2011	15	10.1
	Y2012	15	7.5
	Y2013	15	7.9
4	Y2011	15	10.6
	Y2012	15	7.7
	Y2013	15	7.8
CK	Y2011	9	12.1
	Y2012	9	14.9
	Y2013	9	13.3

Future Carbon Accrual Potential

The Kreager farm site serves as an appropriate reference site for the Wilds, as it relates to future organic soil carbon accrual potential. The organic soil carbon levels observed at the Kreager farm appear to be a reasonable target for what might be achievable over time with improvements in substrate conditions on the Wilds. The ANOVA analysis, as described above, showed that the organic soil C at the Kreager farm reference site was significantly different from the carbon at each of the four zones. In contrast, among the four zones, the means of the carbon were not significantly different from each other.

Because of the nature of the respreads substrates used as rooting medium as a part of the reclamation of the Wilds from the former coal mining days, an estimate of the achievable carbon levels in the soils, over time, will closely relate to the availability of soil nitrogen. Based on the sampling and laboratory and statistical analysis completed for the project, we see no reason why, with adequate soil macronutrient management (nitrogen, phosphorus and carbon balances), levels of soil carbon as measured in the Kreager farm site would not be achievable.

The following figures document the annual soil carbon accruals expected in each management zone to achieve the Kreager farm organic carbon levels in 50 years. With appropriate management of the soils, as described above, it may be possible to exceed the projected levels of annual soil carbon increase and achieve reference levels in less than time.

Figure 7: Existing and Reference Org C Levels, with Expected Annual Accrual Rate (Zone 1)

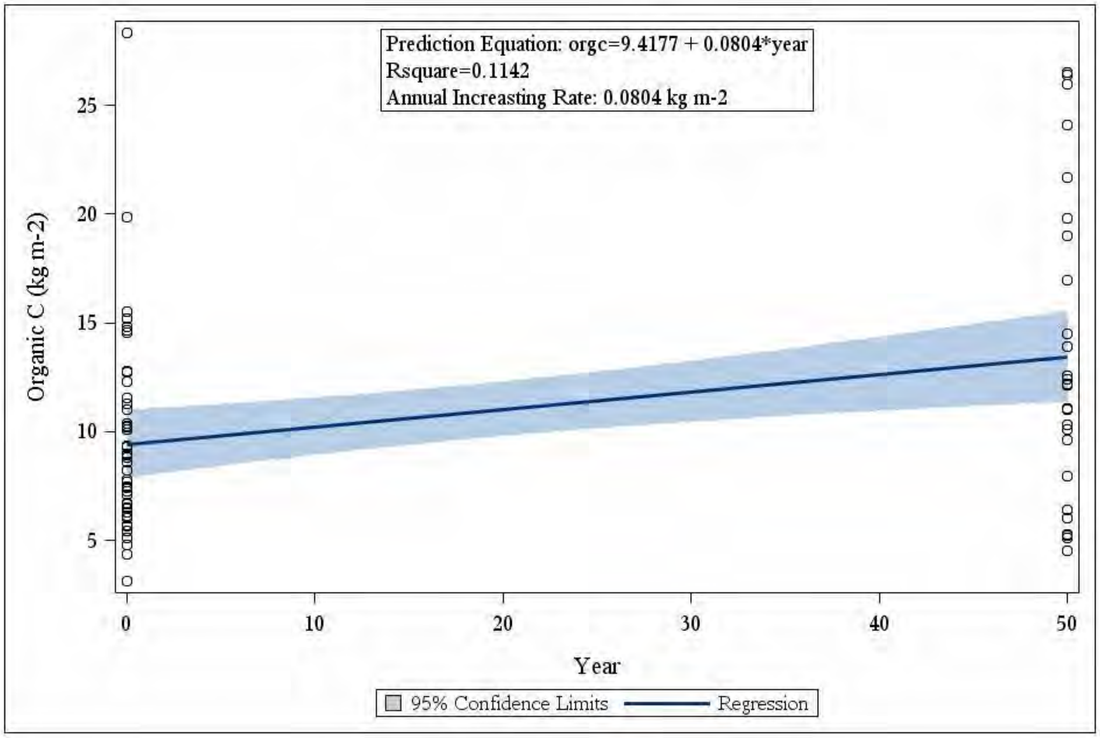


Figure 8: Existing and Reference Org C Levels, with Expected Annual Accrual Rate (Zone 2)

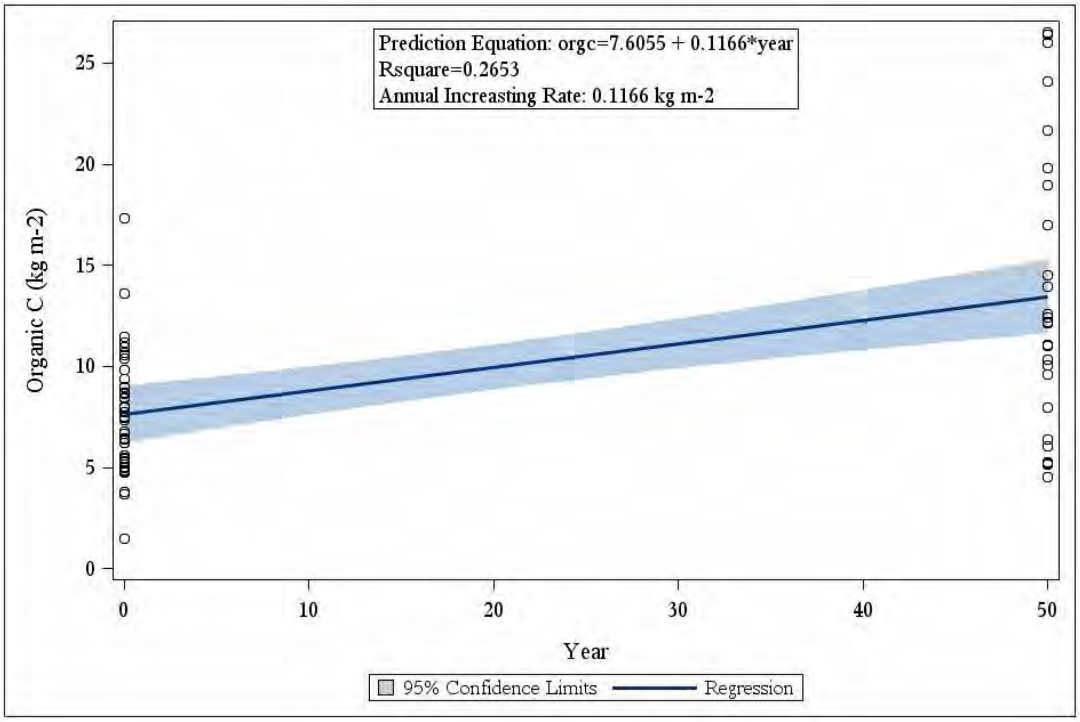


Figure 9: Existing and Reference Org C Levels, with Expected Annual Accrual Rate (Zone 3)

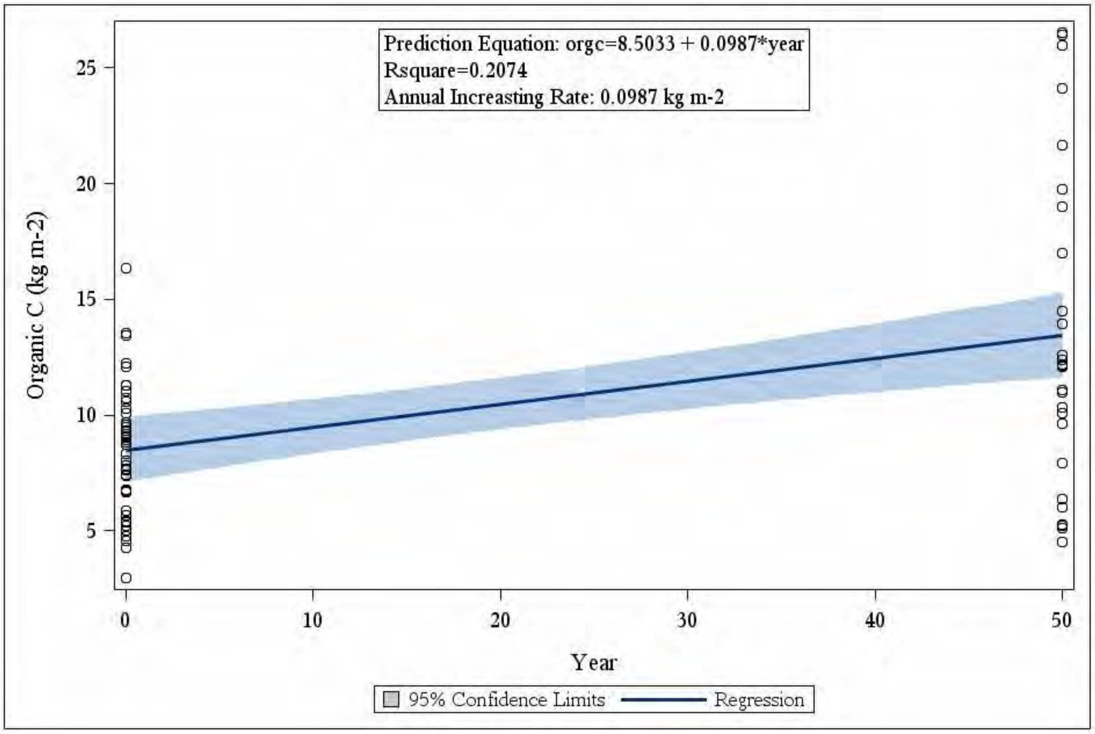
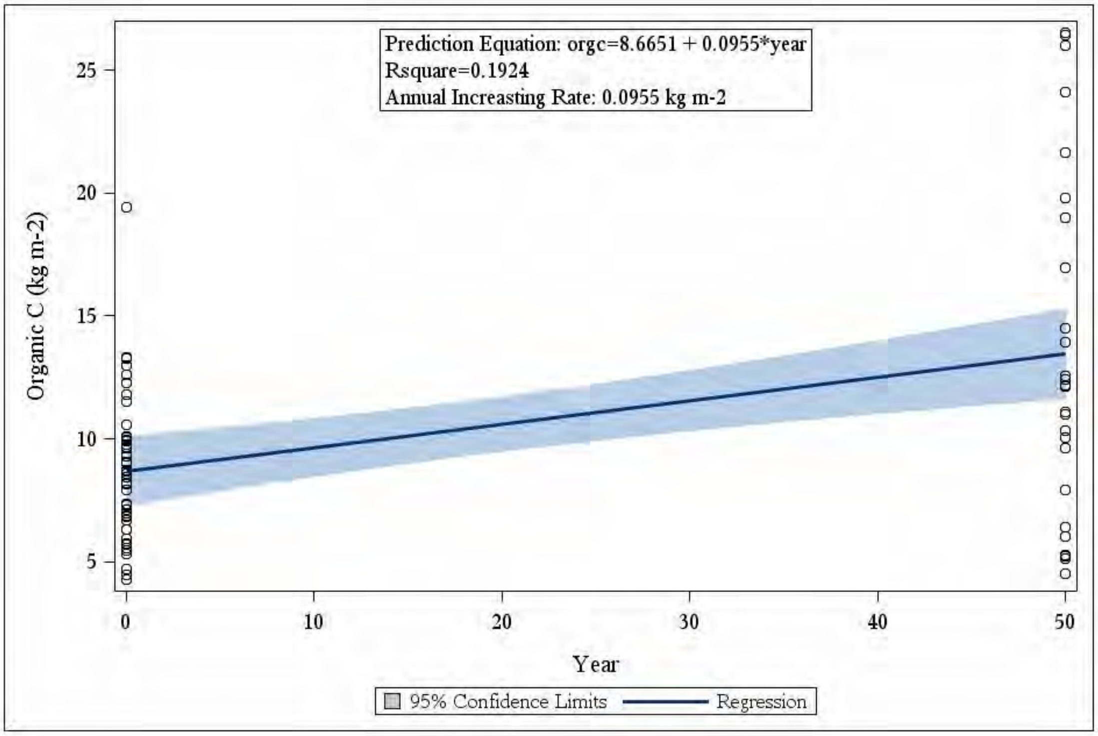


Fig. 10: Existing and Reference Org C Levels, with Expected Annual Accrual Rate (Zone 4)



References

Schoeneberger, P.J., D.A. Wysocki, E.C. Benham and Soil Survey Staff. 2012. *Field book for describing and sampling soils, Version 3.0*. Natural Resources Conservation Service, National Soil Survey Center, Lincoln, NE.

Appendix A: Site Maps

(Full-resolution versions of Figures A1, A2 included with report as separate attachments)

Figure A1: Soil Sampling – the Wilds Demonstration Area

Figure A2: Soil Sampling – Kreager Farm Reference Area

Appendix B: Data Tables of Soil Carbon Data

Table B1: Year Three (2013) Soil Carbon Sampling Statistical Summary, 95% Confidence Intervals

	Measurement	*Layer*	*Mean*	*SD*	*N*	*SEM*	*CL*	*α*	*p**	*DF*	*t-score*	*ME*	*95% CI*			*% Error*
Zones 1-4	**Total C kg/m^2 layer**	**Full Core**	16.70	5.80	61	0.743	95%	0.05	0.975	60	2.00	1.49	15.21	*to*	18.19	8.9%
	Organic C kg/m^2-layer	**Full Core**	7.40	2.50	61	0.320	95%	0.05	0.975	60	2.00	0.64	6.76	*to*	8.04	8.7%
	Horizon Thickness cm	**Full Core**	56.75	25.05	61	3.207	95%	0.05	0.975	60	2.00	6.42	50.33	*to*	63.17	11.3%
Zone 1	*Measurement*	*Layer*	*Mean*	*SD*	*N*	*SEM*	*CL*	*α*	*p**	*DF*	*t-score*	*ME*	*95% CI*			*% Error*
	Total C kg/m^2 layer	**Full Core**	17.90	6.10	16	1.525	95%	0.05	0.975	15	2.13	3.25	14.65	*to*	21.15	18.2%
	Organic C kg/m^2-layer	**Full Core**	7.90	2.60	16	0.650	95%	0.05	0.975	15	2.13	1.39	6.51	*to*	9.29	17.5%
	Horizon Thickness cm	**Full Core**	53.94	28.30	16	7.075	95%	0.05	0.975	15	2.13	15.08	38.86	*to*	69.02	28.0%
Zone 2	*Measurement*	*Layer*	*Mean*	*SD*	*N*	*SEM*	*CL*	*α*	*p**	*DF*	*t-score*	*ME*	*95% CI*			*% Error*
	Total C kg/m^2 layer	**Full Core**	13.10	5.00	15	1.291	95%	0.05	0.975	14	2.14	2.77	10.33	*to*	15.87	21.1%
	Organic C kg/m^2-layer	**Full Core**	6.20	2.40	15	0.620	95%	0.05	0.975	14	2.14	1.33	4.87	*to*	7.53	21.4%
	Horizon Thickness cm	**Full Core**	71.47	21.44	15	5.536	95%	0.05	0.975	14	2.14	11.87	59.60	*to*	83.34	16.6%
Zone 3	*Measurement*	*Layer*	*Mean*	*SD*	*N*	*SEM*	*CL*	*α*	*p**	*DF*	*t-score*	*ME*	*95% CI*			*% Error*
	Total C kg/m^2 layer	**Full Core**	18.50	6.60	15	1.704	95%	0.05	0.975	14	2.14	3.65	14.85	*to*	22.15	19.8%
	Organic C kg/m^2-layer	**Full Core**	7.90	2.90	15	0.749	95%	0.05	0.975	14	2.14	1.61	6.29	*to*	9.51	20.3%
	Horizon Thickness cm	**Full Core**	53.13	26.41	15	6.819	95%	0.05	0.975	14	2.14	14.63	38.50	*to*	67.76	27.5%
Zone 4	*Measurement*	*Layer*	*Mean*	*SD*	*N*	*SEM*	*CL*	*α*	*p**	*DF*	*t-score*	*ME*	*95% CI*			*% Error*
	Total C kg/m^2 layer	**Full Core**	17.10	5.40	15	1.394	95%	0.05	0.98	14	2.14	2.99	14.11	*to*	20.09	17.5%
	Organic C kg/m^2-layer	**Full Core**	7.80	2.00	15	0.516	95%	0.05	0.98	14	2.14	1.11	6.69	*to*	8.91	14.2%
	Horizon Thickness cm	**Full Core**	48.67	18.67	15	4.821	95%	0.05	0.98	14	2.14	10.34	38.33	*to*	59.01	21.2%
Zone 5 (Control)	*Measurement*	*Layer*	*Mean*	*SD*	*N*	*SEM*	*CL*	*α*	*p**	*DF*	*t-score*	*ME*	*95% CI*			*% Error*
	Total C kg/m^2 layer	**Full Core**	17.60	5.90	10	1.866	95%	0.05	0.98	9	2.26	4.22	13.38	*to*	21.82	24.0%
	Organic C kg/m^2-layer	**Full Core**	8.70	2.10	10	0.664	95%	0.05	0.98	9	2.26	1.50	7.20	*to*	10.20	17.3%
	Horizon Thickness cm	**Full Core**	47.70	23.04	10	7.286	95%	0.05	0.98	9	2.26	16.48	31.22	*to*	64.18	34.6%
Kreager Farm	*Measurement*	*Layer*	*Mean*	*SD*	*N*	*SEM*	*CL*	*α*	*p**	*DF*	*t-score*	*ME*	*95% CI*			*% Error*
	Total C kg/m^2 layer	**Full Core**	17.20	7.40	9	2.467	95%	0.05	0.975	8	2.31	5.69	11.51	*to*	22.89	33.1%
	Organic C kg/m^2-layer	**Full Core**	13.30	8.60	9	2.867	95%	0.05	0.975	8	2.31	6.61	6.69	*to*	19.91	49.7%
	Horizon Thickness cm	**Full Core**	97.33	2.45	9	0.817	95%	0.05	0.975	8	2.31	1.88	95.45	*to*	99.21	1.9%

Table B2: Year Two (2012) Soil Carbon Sampling Statistical Summary, 95% Confidence Intervals

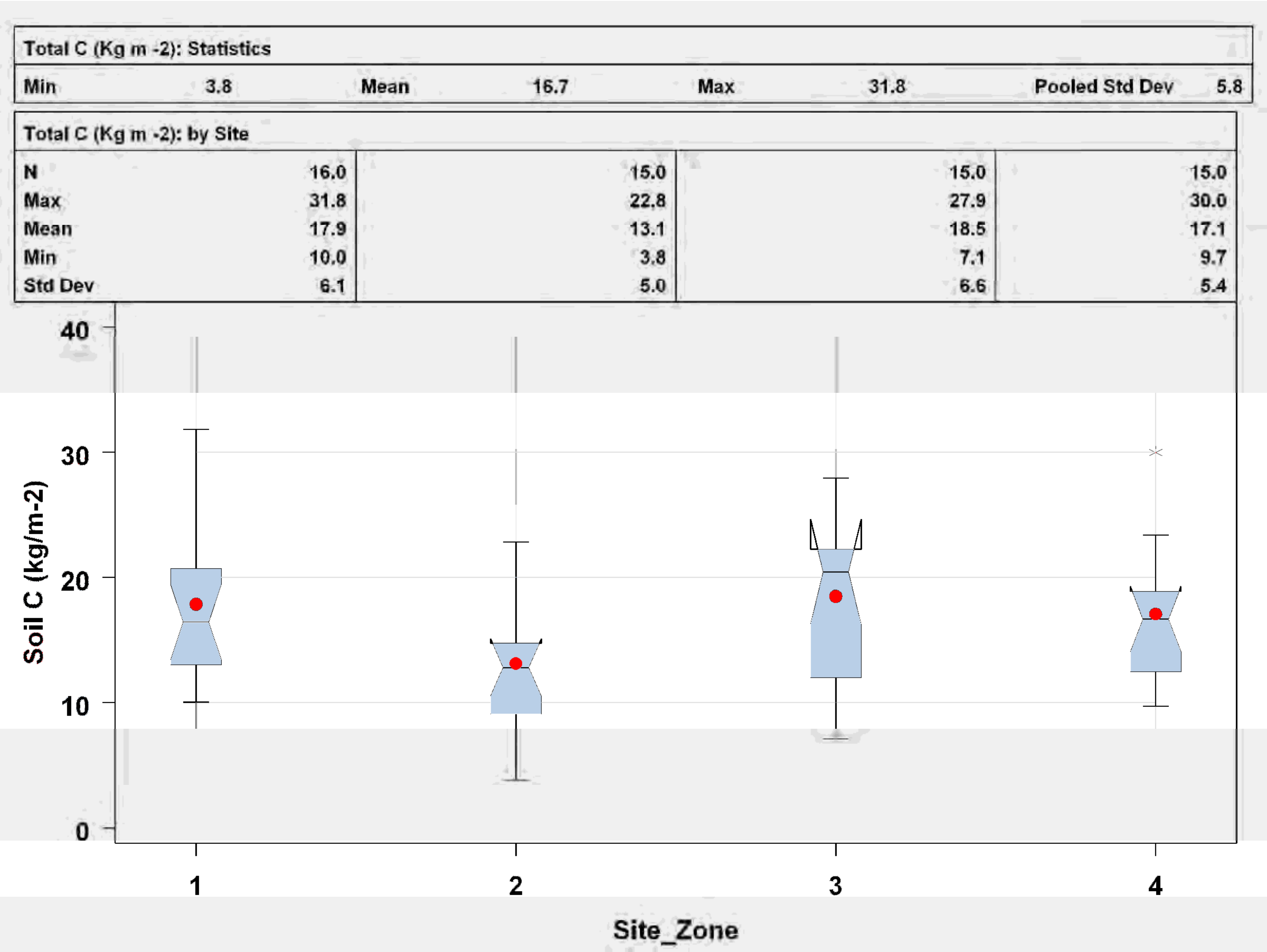
	Measurement	*Layer*	*Mean*	*SD*	*N*	*SEM*	*CL*	*α*	*p**	*DF*	*t-score*	*ME*	*95% CI*			*% Error*
Zones 1-4	Total C kg/m^2 layer	Full Core	20.90	7.30	61	0.935	95%	0.05	0.975	60	2.00	1.87	19.03	to	22.77	8.9%
	Organic C kg/m^2-layer	Full Core	8.40	2.50	61	0.320	95%	0.05	0.975	60	2.00	0.64	7.76	to	9.04	7.6%
	Horizon Thickness cm	Full Core	61.62	22.41	61	2.869	95%	0.05	0.975	60	2.00	5.74	55.88	to	67.36	9.3%
Zone 1	*Measurement*	*Layer*	*Mean*	*SD*	*N*	*SEM*	*CL*	*α*	*p**	*DF*	*t-score*	*ME*	*95% CI*			*% Error*
	Total C kg/m^2 layer	Full Core	28.10	9.30	16	2.325	95%	0.05	0.975	15	2.13	4.96	23.14	to	33.06	17.6%
	Organic C kg/m^2-layer	Full Core	10.60	3.10	16	0.775	95%	0.05	0.975	15	2.13	1.65	8.95	to	12.25	15.6%
	Horizon Thickness cm	Full Core	62.81	24.67	16	6.168	95%	0.05	0.975	15	2.13	13.15	49.66	to	75.96	20.9%
Zone 2	*Measurement*	*Layer*	*Mean*	*SD*	*N*	*SEM*	*CL*	*α*	*p**	*DF*	*t-score*	*ME*	*95% CI*			*% Error*
	Total C kg/m^2 layer	Full Core	16.80	3.20	15	0.826	95%	0.05	0.975	14	2.14	1.77	15.03	to	18.57	10.5%
	Organic C kg/m^2-layer	Full Core	7.60	1.90	15	0.491	95%	0.05	0.975	14	2.14	1.05	6.55	to	8.65	13.8%
	Horizon Thickness cm	Full Core	62.80	23.70	15	6.119	95%	0.05	0.975	14	2.14	13.12	49.68	to	75.92	20.9%
Zone 3	*Measurement*	*Layer*	*Mean*	*SD*	*N*	*SEM*	*CL*	*α*	*p**	*DF*	*t-score*	*ME*	*95% CI*			*% Error*
	Total C kg/m^2 layer	Full Core	19.80	6.40	15	1.652	95%	0.05	0.975	14	2.14	3.54	16.26	to	23.34	17.9%
	Organic C kg/m^2-layer	Full Core	7.50	2.20	15	0.568	95%	0.05	0.975	14	2.14	1.22	6.28	to	8.72	16.2%
	Horizon Thickness cm	Full Core	56.07	18.88	15	4.875	95%	0.05	0.975	14	2.14	10.46	45.61	to	66.53	18.6%
Zone 4	*Measurement*	*Layer*	*Mean*	*SD*	*N*	*SEM*	*CL*	*α*	*p**	*DF*	*t-score*	*ME*	*95% CI*			*% Error*
	Total C kg/m^2 layer	Full Core	18.50	8.60	15	2.221	95%	0.05	0.98	14	2.14	4.76	13.74	to	23.26	25.7%
	Organic C kg/m^2-layer	Full Core	7.70	2.50	15	0.645	95%	0.05	0.98	14	2.14	1.38	6.32	to	9.08	18.0%
	Horizon Thickness cm	Full Core	64.73	23.14	15	5.975	95%	0.05	0.98	14	2.14	12.81	51.92	to	77.54	19.8%
Kreager Farm	*Measurement*	*Layer*	*Mean*	*SD*	*N*	*SEM*	*CL*	*α*	*p**	*DF*	*t-score*	*ME*	*95% CI*			*% Error*
	Total C kg/m^2 layer	Full Core	18.30	6.90	9	2.300	95%	0.05	0.975	8	2.31	5.30	13.00	to	23.60	29.0%
	Organic C kg/m^2-layer	Full Core	14.90	7.70	9	2.567	95%	0.05	0.975	8	2.31	5.92	8.98	to	20.82	39.7%
	Horizon Thickness cm	Full Core	72.67	12.42	9	4.140	95%	0.05	0.975	8	2.31	9.55	63.12	to	82.22	13.1%

Table B3: Baseline (2011) Soil Carbon Sampling Statistical Summary, 95% Confidence Intervals

	Measurement	*Layer*	*Mean*	*SD*	*N*	*SEM*	*CL*	*α*	*p**	*DF*	*t-score*	*ME*	*95% CI*			*% Error*
Zones 1-4	**Total C kg/m^2 layer**	**Full Core**	22.50	6.50	61	0.832	95%	0.05	0.975	60	2.00	**1.66**	**20.84**	*to*	**24.16**	7.4%
	Organic C kg/m^2-layer	**Full Core**	9.90	4.30	61	0.551	95%	0.05	0.975	60	2.00	**1.10**	**8.80**	*to*	**11.00**	11.1%
	Horizon Thickness cm	**Full Core**	44.18	16.64	61	2.131	95%	0.05	0.975	60	2.00	**4.26**	**39.92**	*to*	**48.44**	9.6%
	Measurement	*Layer*	*Mean*	*SD*	*N*	*SEM*	*CL*	*α*	*p**	*DF*	*t-score*	*ME*	*95% CI*			*% Error*
Zone 1	**Total C kg/m^2 layer**	**Full Core**	26.00	8.10	16	2.025	95%	0.05	0.975	15	2.13	**4.32**	**21.68**	*to*	**30.32**	16.6%
	Organic C kg/m^2-layer	**Full Core**	9.70	6.50	16	1.625	95%	0.05	0.975	15	2.13	**3.46**	**6.24**	*to*	**13.16**	35.7%
	Horizon Thickness cm	**Full Core**	54.44	16.42	16	4.105	95%	0.05	0.975	15	2.13	**8.75**	**45.69**	*to*	**63.19**	16.1%
	Measurement	*Layer*	*Mean*	*SD*	*N*	*SEM*	*CL*	*α*	*p**	*DF*	*t-score*	*ME*	*95% CI*			*% Error*
Zone 2	**Total C kg/m^2 layer**	**Full Core**	20.10	5.80	15	1.498	95%	0.05	0.975	14	2.14	**3.21**	**16.89**	*to*	**23.31**	16.0%
	Organic C kg/m^2-layer	**Full Core**	9.10	3.40	15	0.878	95%	0.05	0.975	14	2.14	**1.88**	**7.22**	*to*	**10.98**	20.7%
	Horizon Thickness cm	**Full Core**	48.07	14.84	15	3.832	95%	0.05	0.975	14	2.14	**8.22**	**39.85**	*to*	**56.29**	17.1%
	Measurement	*Layer*	*Mean*	*SD*	*N*	*SEM*	*CL*	*α*	*p**	*DF*	*t-score*	*ME*	*95% CI*			*% Error*
Zone 3	**Total C kg/m^2 layer**	**Full Core**	23.40	5.70	15	1.472	95%	0.05	0.975	14	2.14	**3.16**	**20.24**	*to*	**26.56**	13.5%
	Organic C kg/m^2-layer	**Full Core**	10.10	2.60	15	0.671	95%	0.05	0.975	14	2.14	**1.44**	**8.66**	*to*	**11.54**	14.3%
	Horizon Thickness cm	**Full Core**	36.73	15.23	15	3.932	95%	0.05	0.975	14	2.14	**8.43**	**28.30**	*to*	**45.16**	23.0%
	Measurement	*Layer*	*Mean*	*SD*	*N*	*SEM*	*CL*	*α*	*p**	*DF*	*t-score*	*ME*	*95% CI*			*% Error*
Zone 4	**Total C kg/m^2 layer**	**Full Core**	20.30	6.10	15	1.575	95%	0.05	0.98	14	2.14	**3.38**	**16.92**	*to*	**23.68**	16.6%
	Organic C kg/m^2-layer	**Full Core**	10.60	3.30	15	0.852	95%	0.05	0.98	14	2.14	**1.83**	**8.77**	*to*	**12.43**	17.2%
	Horizon Thickness cm	**Full Core**	36.80	13.82	15	3.568	95%	0.05	0.98	14	2.14	**7.65**	**29.15**	*to*	**44.45**	20.8%
	Measurement	*Layer*	*Mean*	*SD*	*N*	*SEM*	*CL*	*α*	*p**	*DF*	*t-score*	*ME*	*95% CI*			*% Error*
Kreager Farm	**Total C kg/m^2 layer**	**Full Core**	12.90	3.30	9	1.100	95%	0.05	0.975	8	2.31	**2.54**	**10.36**	*to*	**15.44**	19.7%
	Organic C kg/m^2-layer	**Full Core**	12.10	4.00	9	1.333	95%	0.05	0.975	8	2.31	**3.07**	**9.03**	*to*	**15.17**	25.4%
	Horizon Thickness cm	**Full Core**	60.22	10.17	9	3.390	95%	0.05	0.975	8	2.31	**7.82**	**52.40**	*to*	**68.04**	13.0%

Appendix C: Data Figures of 2013 Soil Carbon Data

Figure C1: Total Soil Carbon – Full Core – the Wilds – Zone 1-4 (2013)



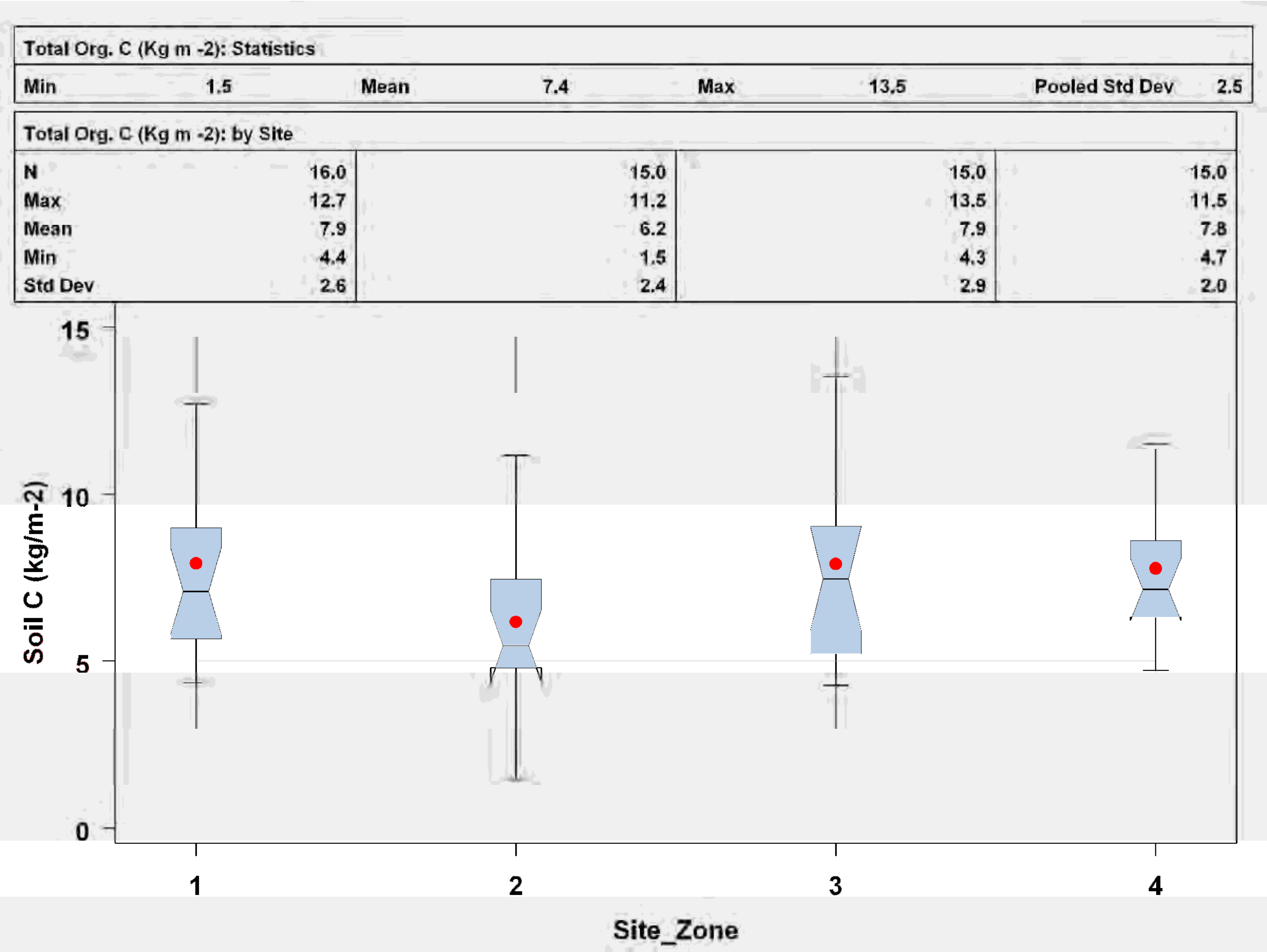
Total C (Kg m -2): Statistics

Min	3.8	Mean	16.7	Max	31.8	Pooled Std Dev	5.8

Total C (Kg m -2): by Site

N	16.0	15.0	15.0	15.0
Max	31.8	22.8	27.9	30.0
Mean	17.9	13.1	18.5	17.1
Min	10.0	3.8	7.1	9.7
Std Dev	6.1	5.0	6.6	5.4

Figure C2: Organic Soil Carbon – Full Core – the Wilds – Zone 1-4 (2013)



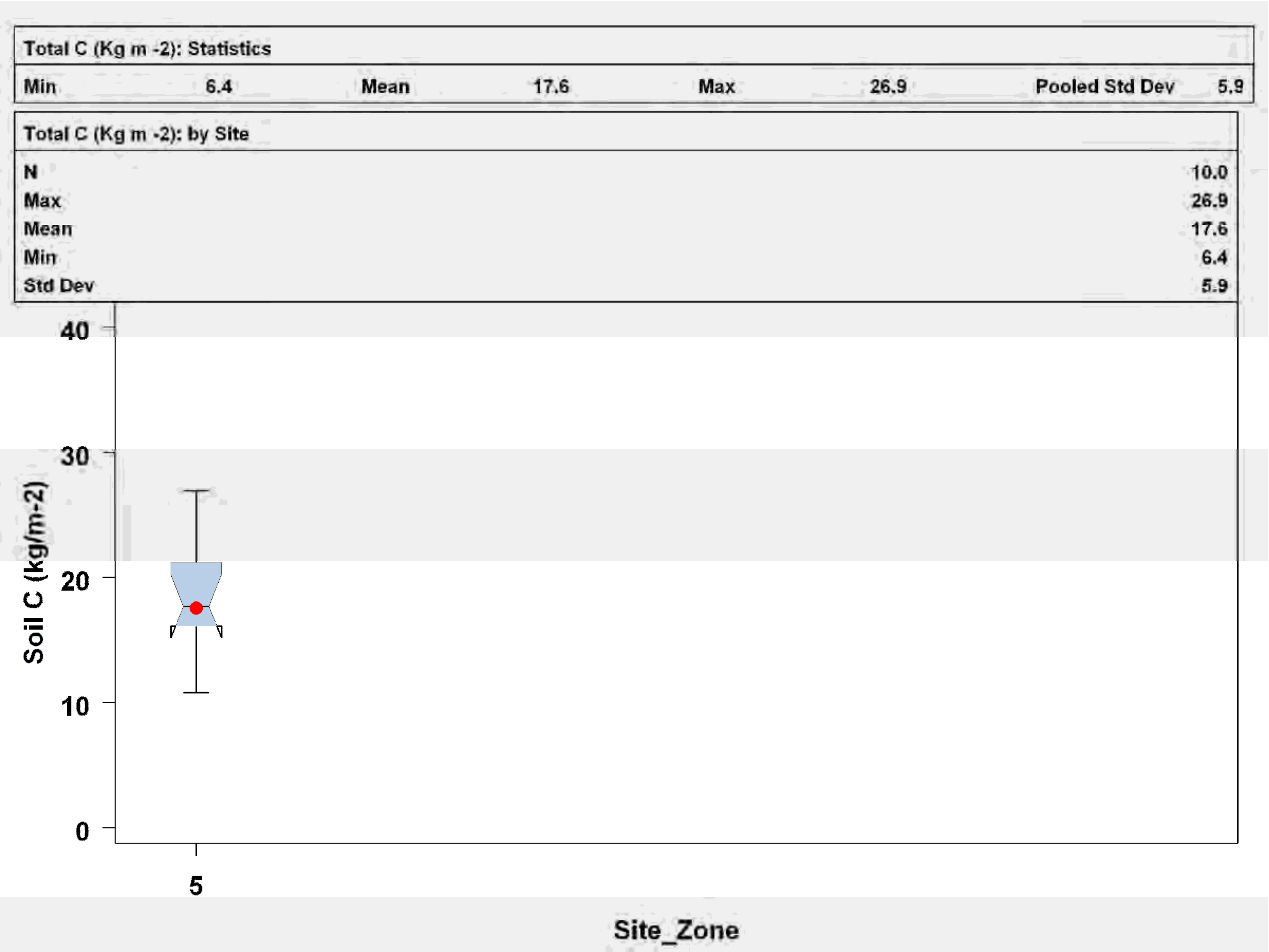
Total Org. C (Kg m -2): Statistics

Min	1.5	Mean	7.4	Max	13.5	Pooled Std Dev	2.5

Total Org. C (Kg m -2): by Site

N	16.0	15.0	15.0	15.0
Max	12.7	11.2	13.5	11.5
Mean	7.9	6.2	7.9	7.8
Min	4.4	1.5	4.3	4.7
Std Dev	2.6	2.4	2.9	2.0

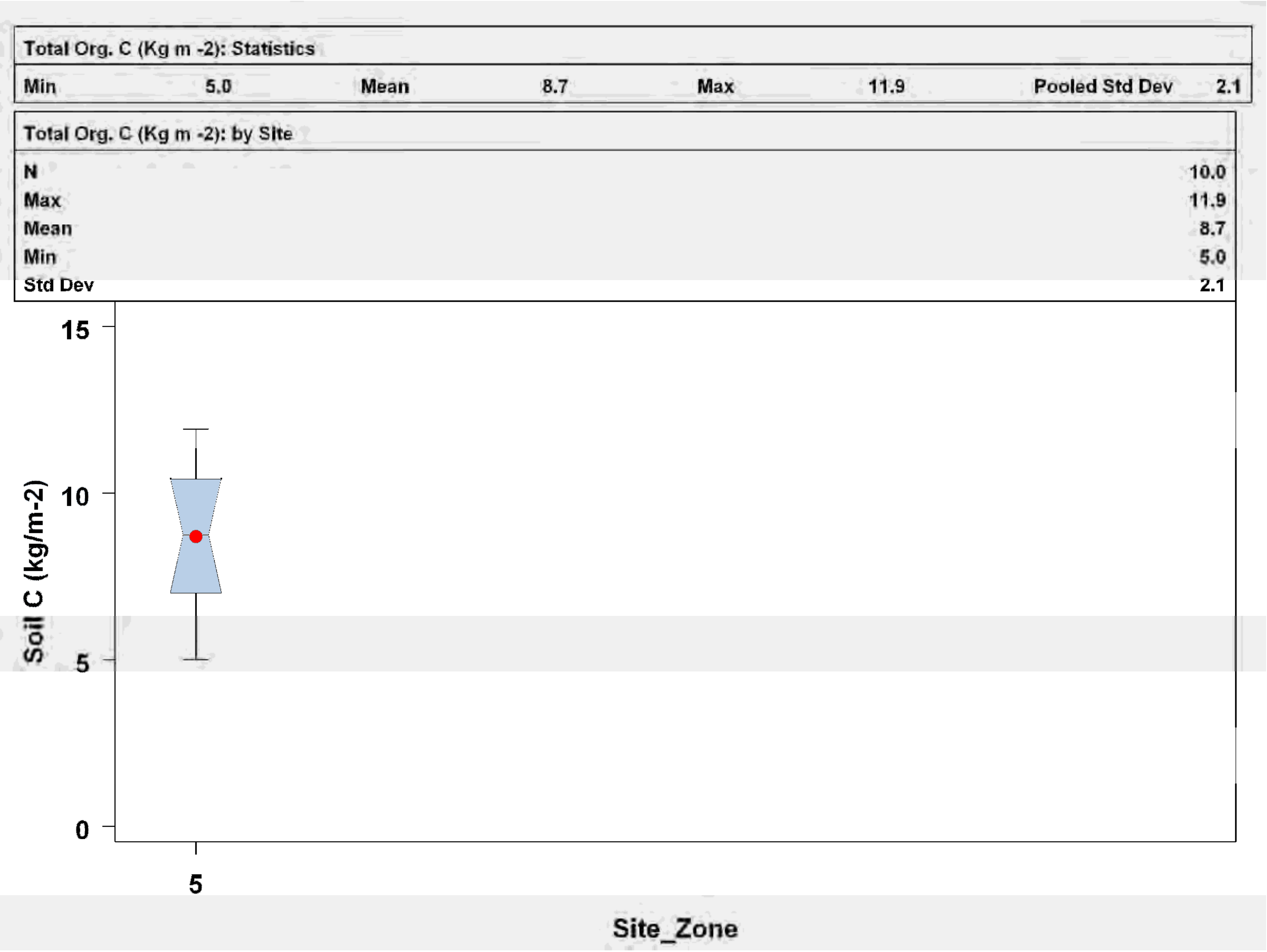
Figure C3: Total Soil Carbon – Full Core – the Wilds – Zone 5 Control Plot (2013)



Total C (Kg m -2): Statistics							
Min	6.4	Mean	17.6	Max	26.9	Pooled Std Dev	5.9

Total C (Kg m -2): by Site	
N	10.0
Max	26.9
Mean	17.6
Min	6.4
Std Dev	5.9

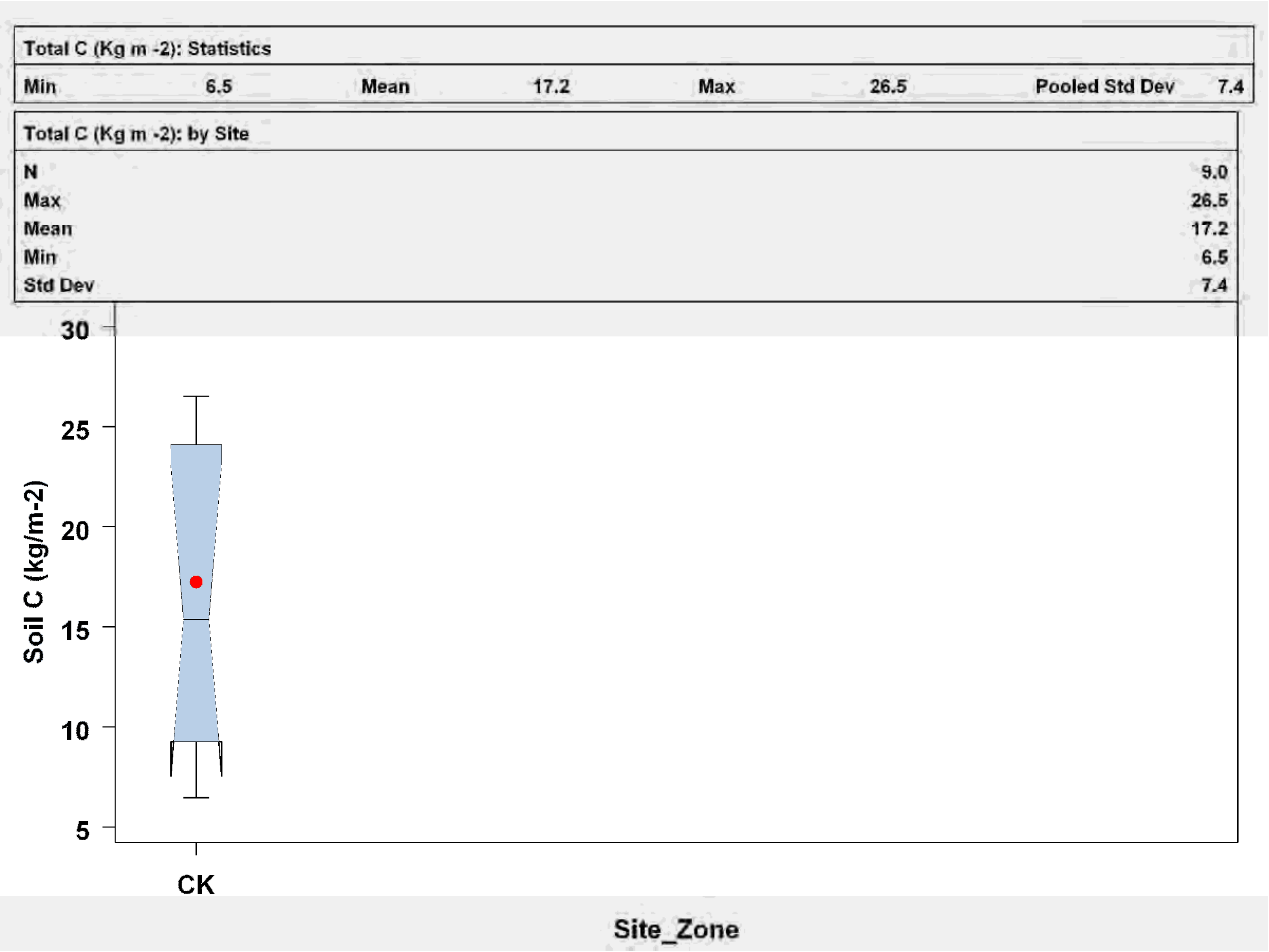
Figure C4: Organic Soil Carbon – Full Core – the Wilds – Zone 5 Control Plot (2013)



Total Org. C (Kg m -2): Statistics							
Min	5.0	Mean	8.7	Max	11.9	Pooled Std Dev	2.1

Total Org. C (Kg m -2): by Site	
N	10.0
Max	11.9
Mean	8.7
Min	5.0
Std Dev	2.1

Figure C5: Total Soil Carbon – Full Core – Kreager Farm (2013)



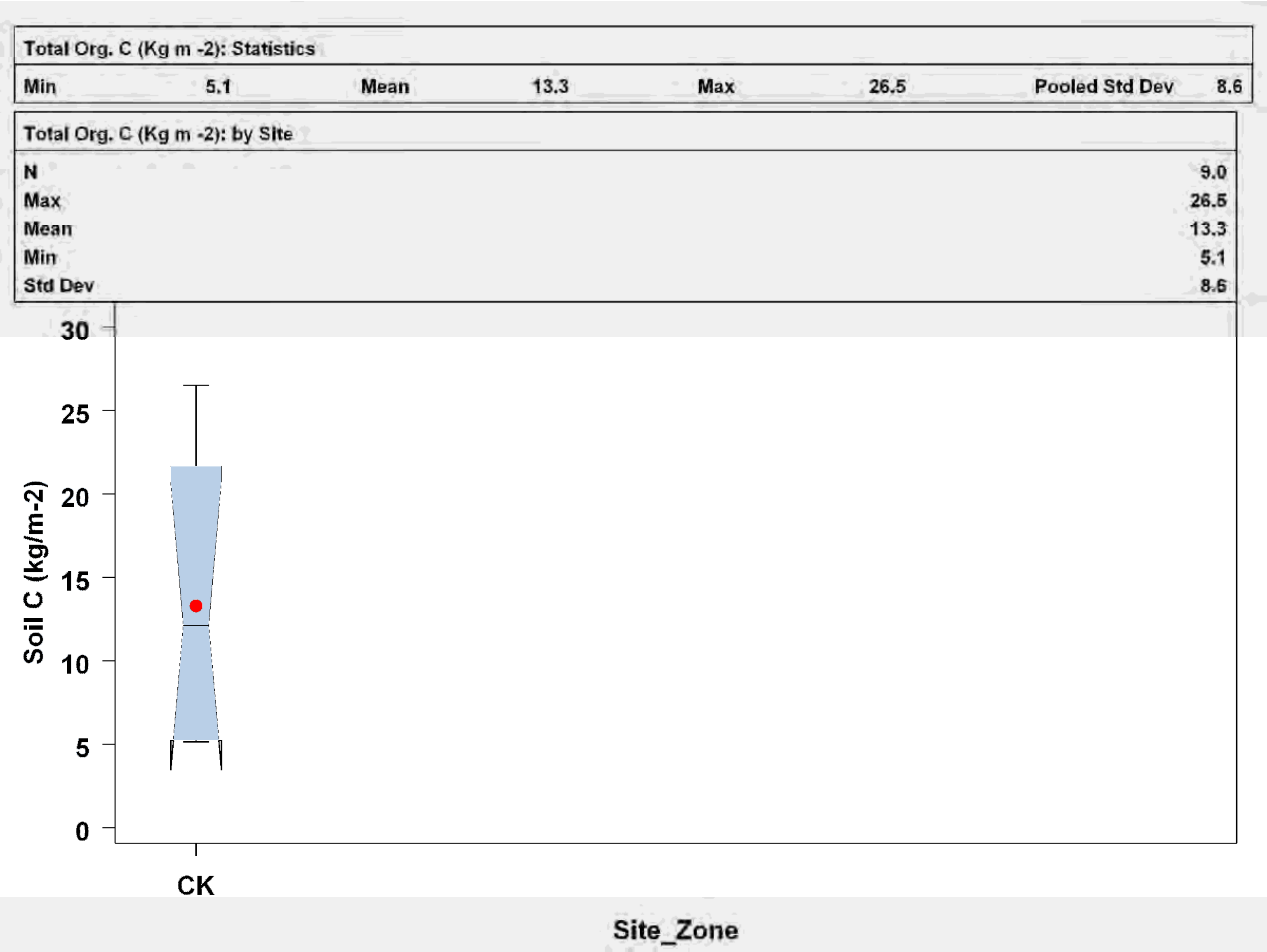
Total C (Kg m -2): Statistics

Min	6.5	Mean	17.2	Max	26.5	Pooled Std Dev	7.4

Total C (Kg m -2): by Site

N	9.0
Max	26.5
Mean	17.2
Min	6.5
Std Dev	7.4

Figure C6: Organic Soil Carbon – Full Core – Kreager Farm (2013)



Total Org. C (Kg m -2): Statistics							
Min	5.1	Mean	13.3	Max	26.5	Pooled Std Dev	8.6

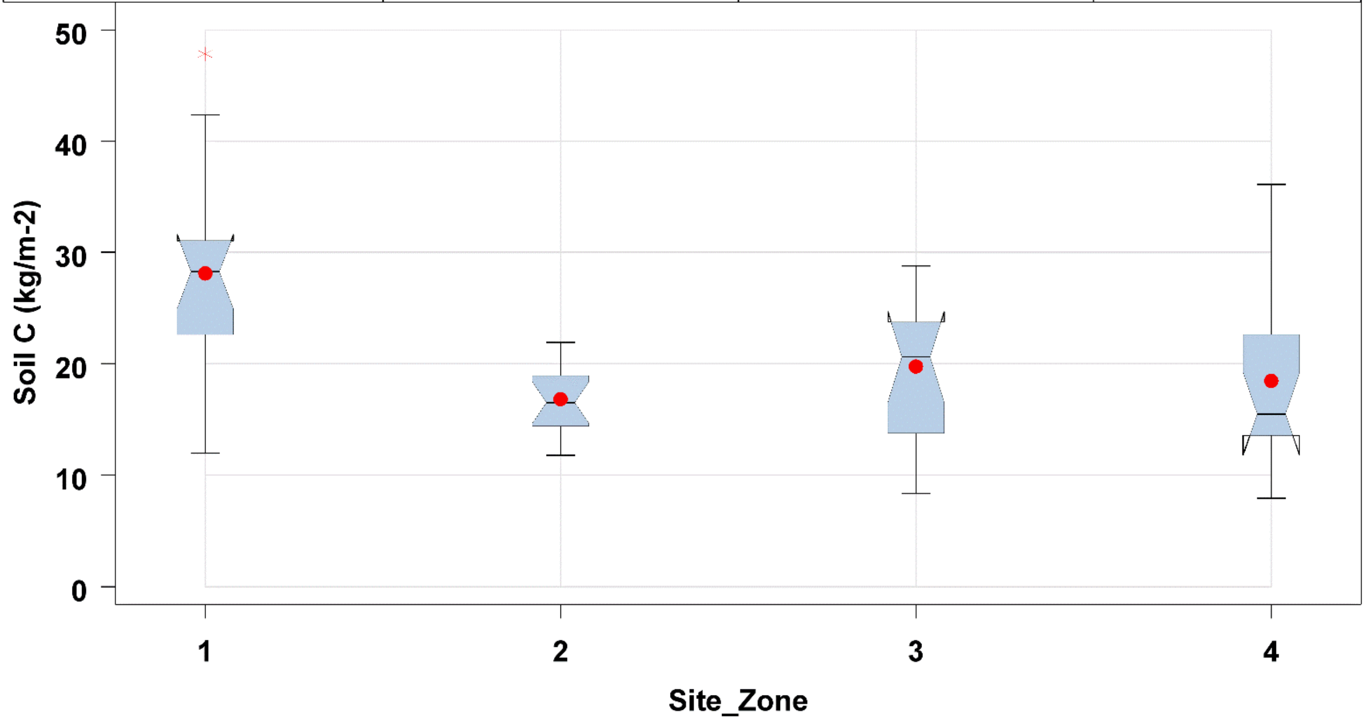
Total Org. C (Kg m -2): by Site	
N	9.0
Max	26.5
Mean	13.3
Min	5.1
Std Dev	8.6

Appendix D: Data Figures of 2012 Soil Carbon Data

Figure D1: Total Soil Carbon – Full Core – the Wilds (2012)

Total C (Kg m -2): Statistics							
Min	7.9	Mean	20.9	Max	47.8	Pooled Std Dev	7.3

Total C (Kg m -2): by Site				
N	16.0	15.0	15.0	15.0
Max	47.8	21.9	28.8	36.1
Mean	28.1	16.8	19.8	18.5
Min	12.0	11.8	8.4	7.9
Std Dev	9.3	3.2	6.4	8.6



Soil Carbon – Full Core – the Wilds (2012)

tistics					
Mean	8.4	Max	15.5	Pooled Std Dev	2.5

Site			
16.0	15.0	15.0	15.0
15.5	10.9	10.6	13.3
10.6	7.6	7.5	7.7
6.5	5.3	3.0	4.3
3.1	1.9	2.2	2.5

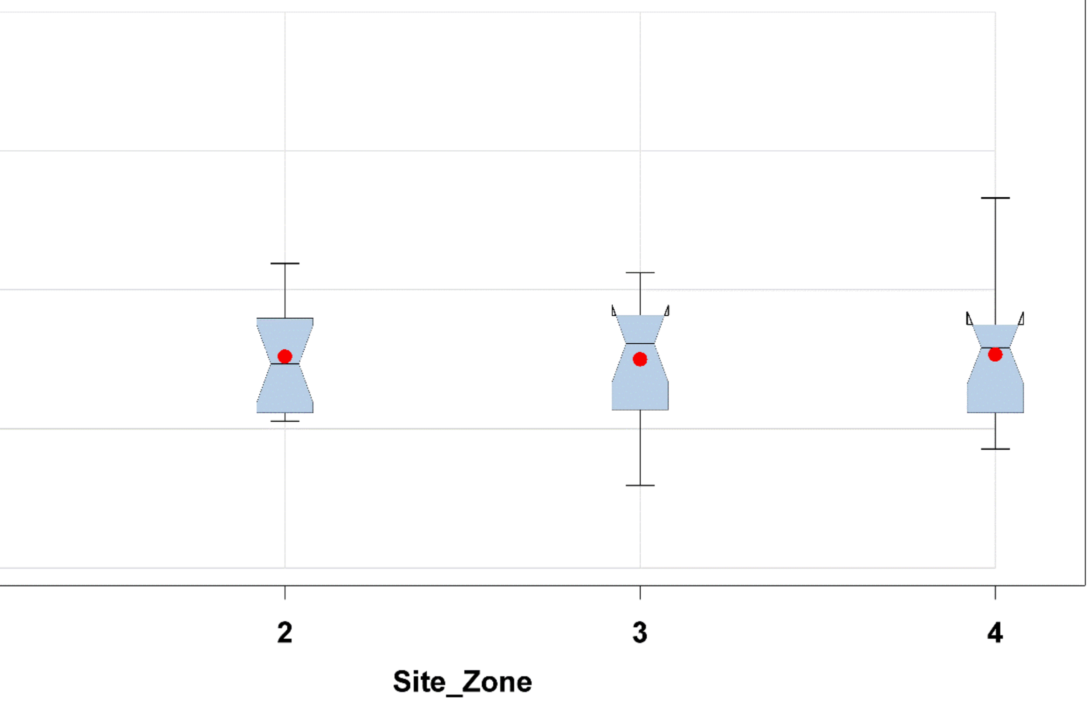


Figure D3: Total Soil Carbon – Full Core – Kreager Farm (2012)

Total C (Kg m -2): Statistics							
Min	10.3	Mean	18.3	Max	26.4	Pooled Std Dev	6.9

Total C (Kg m -2): by Site	
N	9.0
Max	26.4
Mean	18.3
Min	10.3
Std Dev	6.9

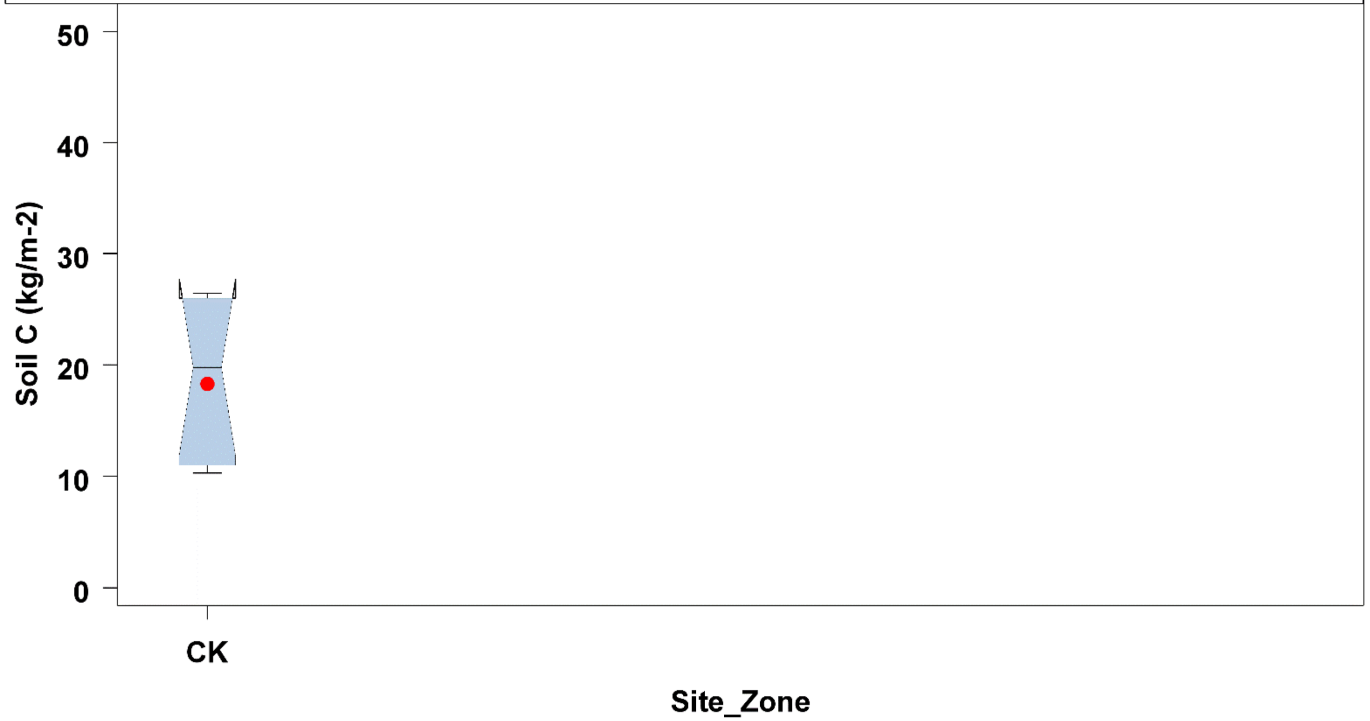
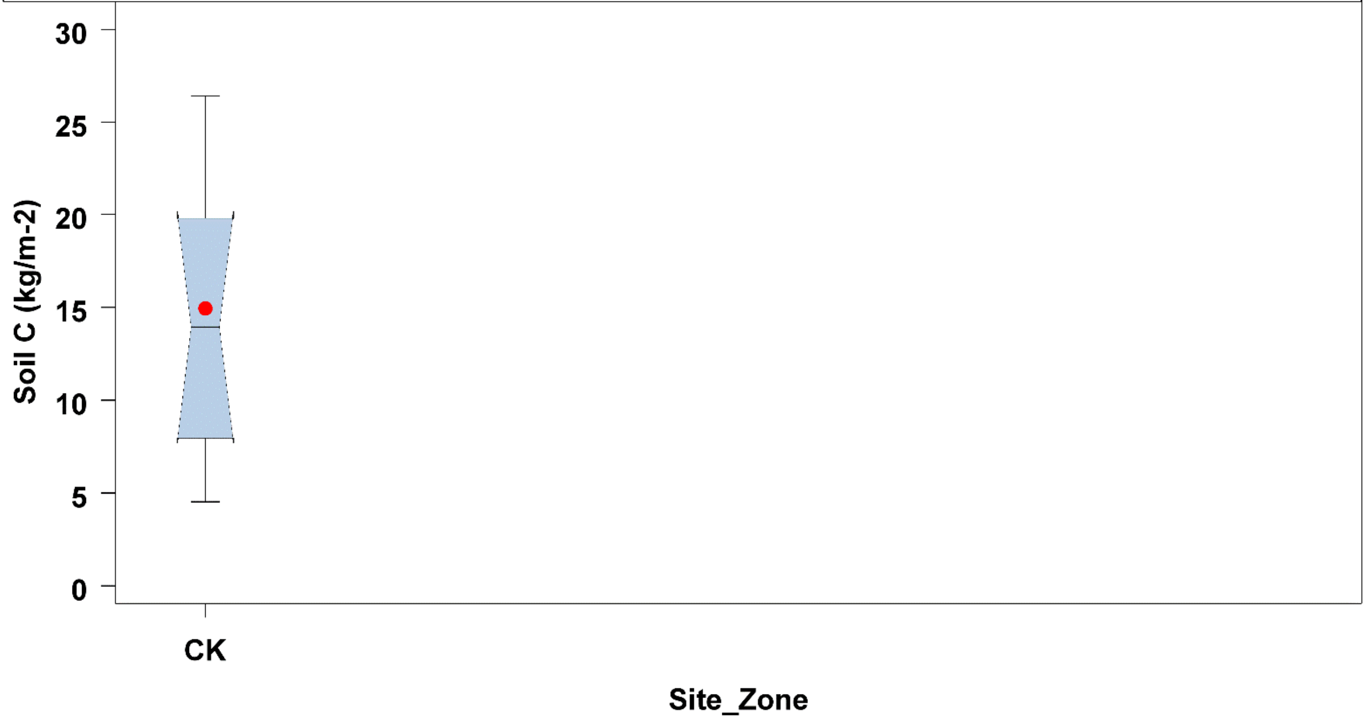


Figure D4: Organic Soil Carbon – Full Core – Kreager Farm (2012)

Total Org. C (Kg m -2): Statistics							
Min	4.5	Mean	14.9	Max	26.4	Pooled Std Dev	7.7

Total Org. C (Kg m -2): by Site	
N	9.0
Max	26.4
Mean	14.9
Min	4.5
Std Dev	7.7



Appendix E: Data Figures of 2011 Soil Carbon Data

Figure E1: Total Soil Carbon – Full Core – the Wilds (2011)

Total C (Kg m -2): Statistics

Min	**9.0**	**Mean**	**22.5**	**Max**	**38.2**	**Pooled Std Dev**	**6.5**

Total C (Kg m -2): by Site

N	**16.0**	**15.0**	**15.0**	**15.0**
Max	**38.2**	**33.6**	**32.6**	**31.0**
Mean	**26.0**	**20.1**	**23.4**	**20.3**
Min	**9.3**	**9.0**	**11.3**	**11.2**
Std Dev	**8.1**	**5.8**	**5.7**	**6.1**

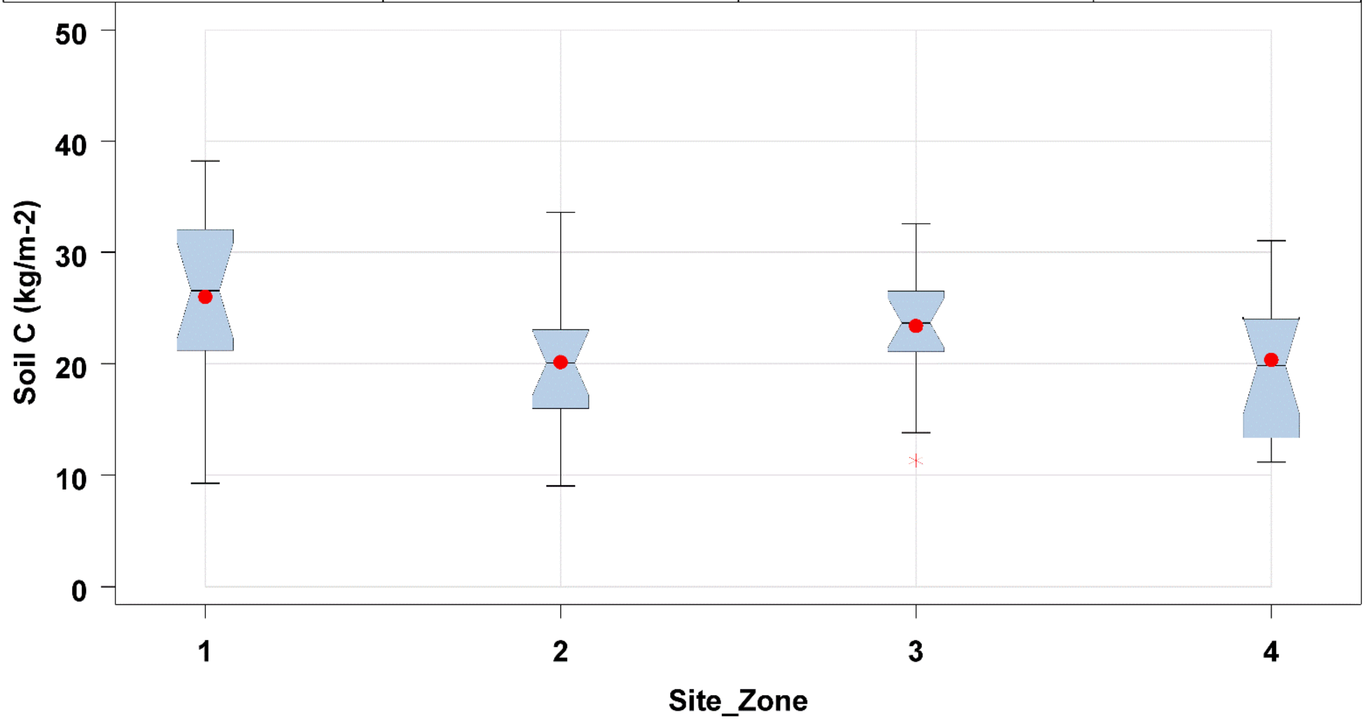


Figure E2: Organic Soil Carbon – Full Core – the Wilds (2011)

Total Org. C (Kg m -2): Statistics							
Min	3.1	Mean	9.9	Max	28.3	Pooled Std Dev	4.3

Total Org. C (Kg m -2): by Site				
N	16.0	15.0	15.0	15.0
Max	28.3	17.3	16.3	19.4
Mean	9.7	9.1	10.1	10.6
Min	3.1	3.8	6.7	5.7
Std Dev	6.5	3.4	2.6	3.3

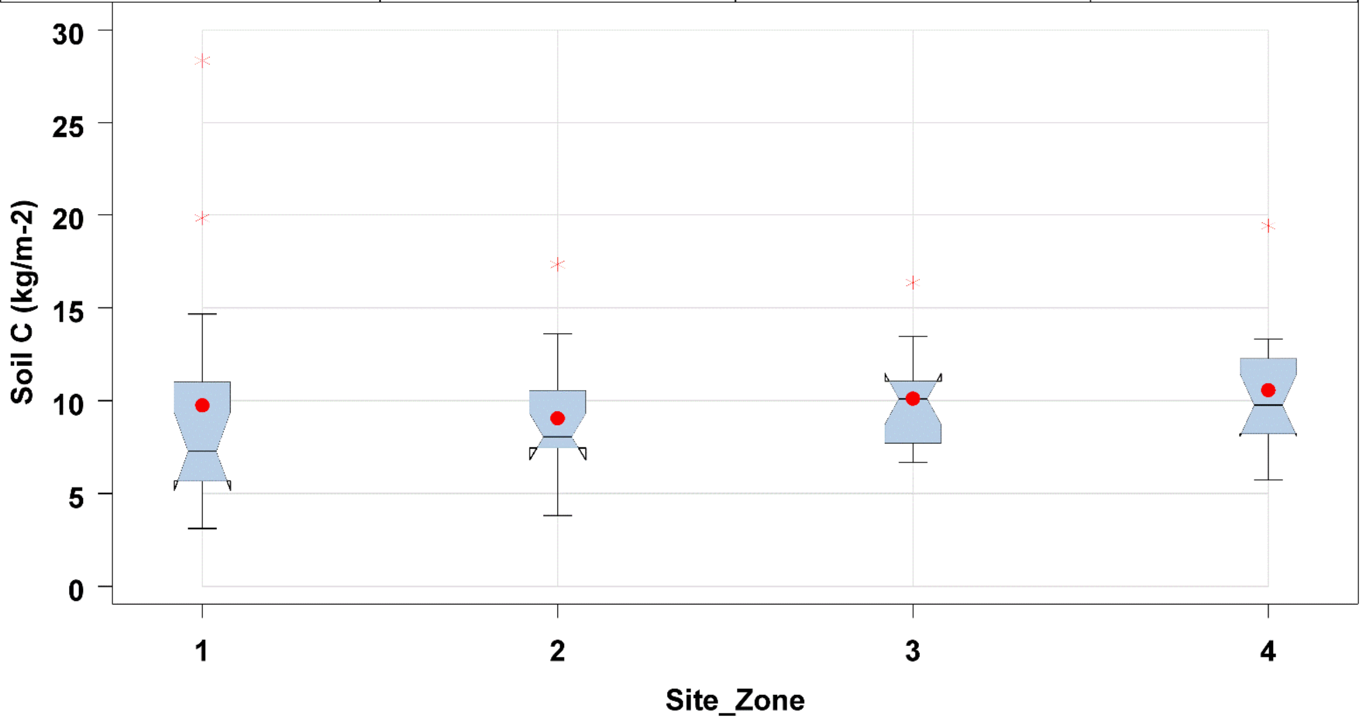


Figure E3: Total Soil Carbon – Full Core – Kreager Farm (2011)

Total C (Kg m -2): Statistics							
Min	9.6	Mean	12.9	Max	19.0	Pooled Std Dev	3.3

Total C (Kg m -2): by Site	
N	9.0
Max	19.0
Mean	12.9
Min	9.6
Std Dev	3.3

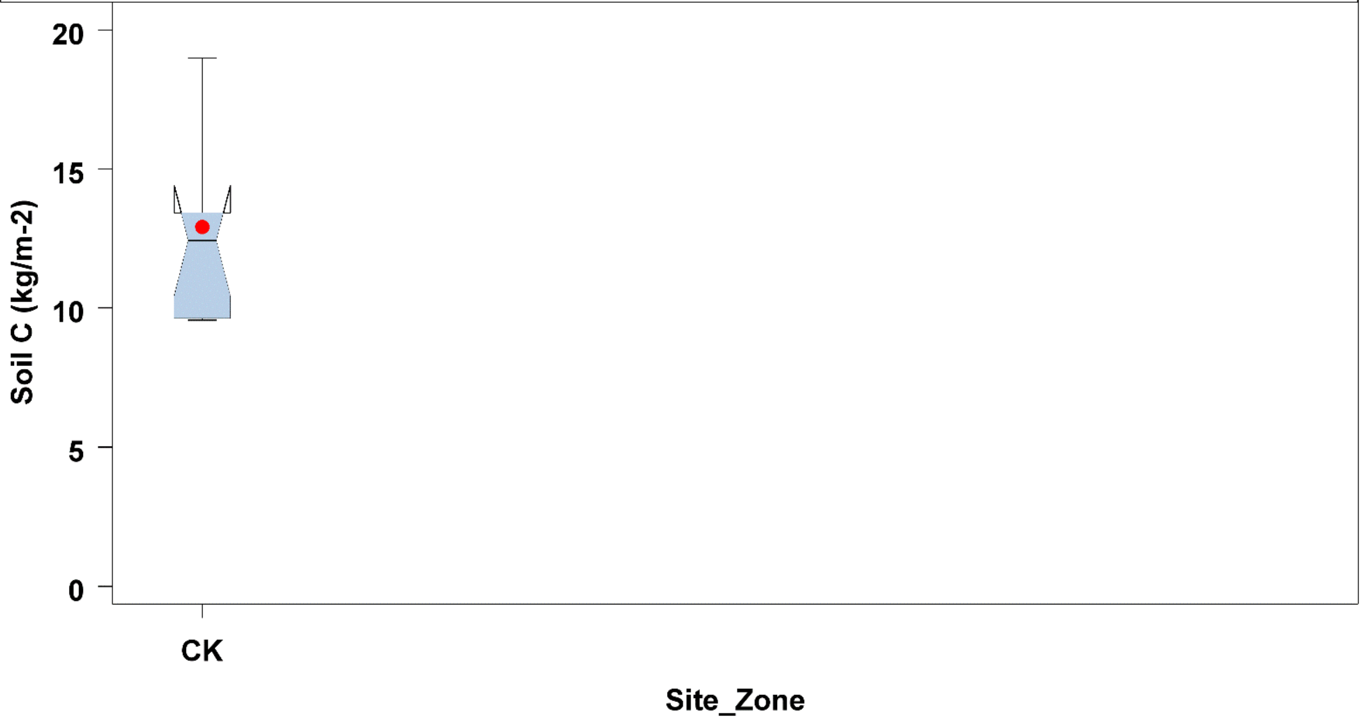
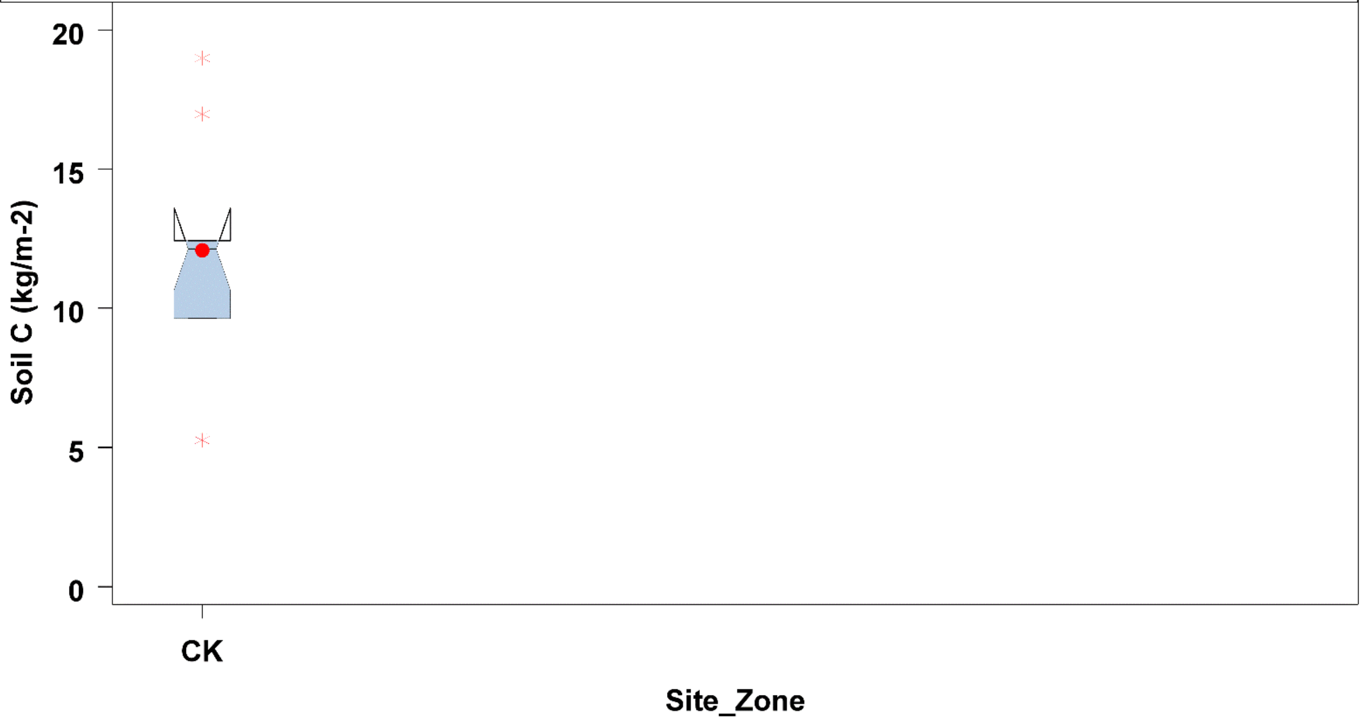


Figure E4: Organic Soil Carbon – Full Core – Kreager Farm (2011)

Total Org. C (Kg m -2): Statistics							
Min	5.3	Mean	12.1	Max	19.0	Pooled Std Dev	4.0

Total Org. C (Kg m -2): by Site	
N	9.0
Max	19.0
Mean	12.1
Min	5.3
Std Dev	4.0



The Wilds CIG Final Report
Appendix B
OSU LCA Report

Carbon Life Cycle Assessment for Prairie as a Crop in Reclaimed Mine Land

Main Authors:

Jose German Guzman
Rattan Lal

FINAL REPORT

for The Wilds

Report Issued: August 15, 2013

Period of Performance: April 1, 2011 to September 15, 2013

Name and Address of Submitting Organization:

The Ohio State University
Carbon Management and Sequestration Center
422B Kottman HallColumbus
OH 43210-1063

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LIST OF ABBREVIATIONS

C - Carbon

CE - Carbon equivalents

cm - Centimeter

CO_2 - Carbon dioxide

DK - Disking only

DK-S - Disking plus sub-soiling

GHG - Greenhouse gas

GWP - Global warming potential

ha - Hectare

kg - Kilo gram

L - Liter

LUC -Land use change

LCA - Life cycle assessment

Mg - Mega gram

NPP - Net primary productivity

NT - No-tillage

NT-G - No-tillage with grazing

SOC - Soil organic carbon

yr - Year

Executive Summary

In 2011, the Wilds began a 3 year study to demonstrate the additional income opportunities for farmers on marginal land through the use of native prairie. One of the objectives was to determine the best balance of productivity and reduced environmental damage under different land preparation and management practices for establishing prairies using a Life Cycle Assessment (LCA) with carbon (C) as the reference unit. The scope of the LCA covered the 'cradle-to-gate' stage of the life cycle, from extraction of raw materials through land use change (LUC), agricultural activities, and production to the point where the prairie is harvested for bioenergy or feed. Land preparation and management practices included disking with sub-soiling (DK-S), disking only (DK), no-tillage (NT), and no-tillage with grazing (NT-G, bison were introduced in the spring of 2013). To evaluate the C balance and energy use of each of the land preparations being demonstrated, an Index of Sustainability ($I_s = C_O/C_I$, where C_O is the sum of all outputs, and C_I is the sum of all inputs) was used to assess temporal changes in C. In order for a management practice to be considered sustainable, the I_s ratio as expressed by net output of C, must be >1 and be increasing with time.

Results from the LCA show that changes in soil organic carbon (SOC) dominate the C emissions from establishment of prairies, followed by burning. Of the four land preparation and management practices, the DK treatment had the highest I_s at 8.53. This was due to having the least degradation of SOC during LUC (-730 kg ha^{-1} yr^{-1}), and second highest aboveground biomass production (9,881 kg ha^{-1}). The highest aboveground biomass production occurred with NT practice (11,130 kg ha^{-1}), although C emissions from SOC losses were similar to DK-S practice, which on average was 2,899 kg ha^{-1} yr^{-1}. The I_s values for NT and DK-S were 2.50 and 1.44, respectively. Grazing from bison reduced the aboveground biomass to 8,971 kg ha^{-1} compared to NT with no grazing,

although stocking density was low enough that I_s was still 1.94. When considering harvesting 50% of the aboveground biomass, NT and DK practices still had values >1. This was not the case for DK-S which resulted in a I_s value of 0.93. Additionally, when 100% of aboveground biomass was considered for harvesting, DK (I_s of 2.68) was the lone practice with an I_s value >1.

Background on LCA

Land use changes (LUC) can have harmful and beneficial effects to the environment. In this study, to balance the benefits of land preparation practices of establishing prairies as a crop for marginal land with reduced environmental damage requires a holistic approach. One tool that can manage these complexities is a Life Cycle Assessment (LCA), which involves the "compilation and evaluation of the inputs, outputs and the potential environmental impacts of a product system throughout its life cycle within a given set of boundary conditions" (ISO, 1997). In principle, a LCA can be used to lessen the environmental impacts of LUC by guiding in the decision making process. There are four major steps in a LCA (ISO, 2006):

1) **Goal and scope –** Determines the framework of the study and specifies the functions or performance of the product or system which is to be assessed.
2) **Inventory analysis –** Lists and quantifies the inputs and outputs of processes at each stage in the life cycle.
3) **Impact assessment –** Provides a quantitative analysis of the system with all its inputs and outputs from an environmental point of view.
4) **Interpretation –** Provides summary and derived recommendations from inventory and impact assessment.

Goal and scope

There are many environmental impacts which can be assessed including greenhouse gas (GHG) emissions, water quantity and quality, resource depletion, primary energy, waste, and toxicity. The purpose of this LCA is to determine whether prairie ecosystems can be an effective tool in sequestering C as well as which management technique is the best. Many studies in agriculture and reclaimed mine land have shown that the establishment of prairies are a significant atmospheric C sink, resulting in lower global warming potential (GWP, CO_2-C, CH_4-C, and N_2O-C) and improved soil quality compared to practices that are a source of C (Shrestha and Lal, 2006; Shrestha et al., 2009). The intended audience of this study is farmers and ranchers on marginal land who might be able to use prairie as a source of multiple incomes. The LCA consists of the preparation, establishment and harvest of prairie as a crop. It does not include processing or transport off property, nor does it include monitoring activities related to the demonstration that would not typically be carried out by farmers. The temporal boundary is the 3 year timeframe required for establishment, and the spatial boundary is limited to the 24 ha demonstration site. The scope of this LCA covers the ‘cradle-to-gate’ stage of the life cycle, from extraction of raw materials through LUC, agricultural activities, and production to the point where the prairie is harvested for bioenergy or feed (Fig. 1).

Inventory analysis

In end of August of 2013, aboveground biomass was measured to quantify potential C outputs from plant biomass. Three plant samples were collected within a 50 cm^2 frame in each treatment plot. Plant biomass was dried at 60°C for 7 days, and weighed to determine dry matter weight (kg ha^{-1}). A total C concentration value of 43% from plant biomass (Guzman and Al-Kaisi, 2010) was used and multiplied by the plant biomass (dry matter m^{-2}) to determine the aboveground potential

C outputs in kg ha^{-1}. Root biomass was estimated using a root:shoot ratio of 1.1 for tall-grass species and 0.8 for legumes and root turnover ratio of 0.5 for both which were derived from studies done in switchgrass (*Panicum virgatum*) and legume mixture plots (Bolinder et al., 2002).

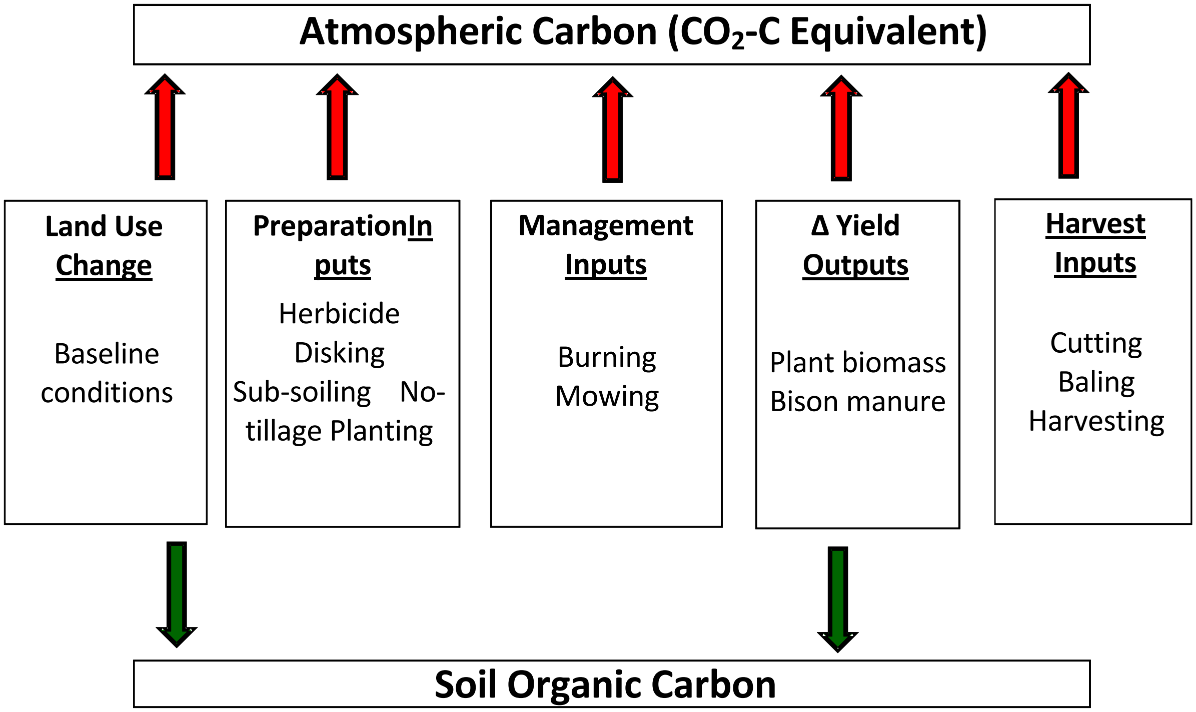

Figure 1. 'Cradle-to-gate' stage of the life cycle, from extraction of raw materials through land use change, management activities, and production to the point where the prairie is harvested for bioenergy or feed.

The impact of CO_2 emissions from management inputs such as tillage, seed bed preparation, planting, herbicide applications, mowing, and burning due to combustion of fuel are also evaluated (Fig. 1). Since units between these inputs vary (i.e. L, oz, MJ), conversion factors were needed to express CO_2 emissions into kg of C equivalent (CE) for comparisons between inputs and outputs (Lal, 2004). Management CE inputs were calculated using fuel usage during operations. One kg of diesel and gasoline had a CE of 0.94 and 0.85 kg, respectively, and one kg of 2, 4-D herbicide had a CE of 1.7 kg (Lal, 2004). Additionally, CO_2 emitted from burning of prairies was accounted by assuming that 50% of the aboveground biomass was consumed and 63% of burned biomass was emitted as CO_2 (notes from the Wilds). Records of fuel usage, herbicide, fertilizers, and burning for each treatment plot has been kept since the project began in 2011 by the Wilds team.

Calculation of changes in soil quality and CO_2 emissions requires estimates of the effects of land preparation and management practices on soil organic carbon (SOC). Changes in net soil C were calculated using data collected from Applied Ecological Services, Inc. Net changes in SOCconcentrations (%) that were measured in 2011 and 2013 were multiplied by mean bulk density (Mg m^{-3}) values and soil depth thickness to convert SOC concentrations to mass per area basis (kg ha^{-1}) for all treatment plots by soil horizon. An average A soil horizon depth of 6 cm, and B horizon depth of 34 cm was used across treatments. It is assumed that all C sequestered as SOC, comes from atmospheric CO_2 through photosynthesis and bison manure (recycled C from biomass consumption), and that all SOC losses are emitted as CO_2 to the atmosphere. Additionally, C losses from soil erosion and leaching were assumed to be negligible.

Impact assessment

To evaluate the C balance and energy use of each of the land preparations being demonstrated, an index of sustainability (I_s) was used to assess temporal changes in the output/input ratio of C using a holistic approach (Lal, 2004):

$$I_s = \left[\frac{C_O}{C_I}\right] t$$

where C_O is the sum of all outputs expressed in CE, C_I is the sum of all inputs expressed in CE, and t is the time in years. The reference unit C equivalent (CE), i.e. the reference measure for which the environmental burden (CO_2) from farming operations is expressed as kg CE ha^{-1} yr^{-1}. In order for a management practice to be considered sustainable, the I_s ratio as expressed by net output of C, must be >1 and be increasing with time.

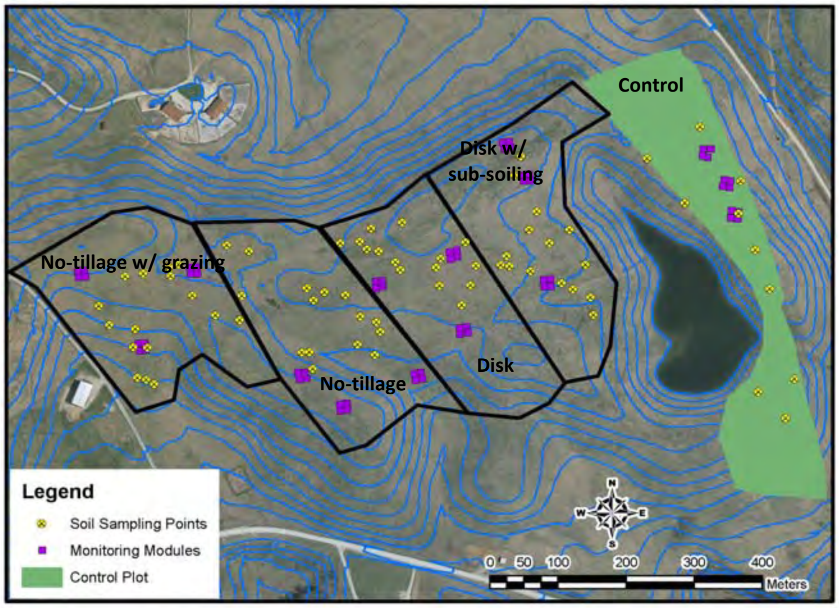

Figure 2. Land preparation treatments on 24 ha demonstration site at the Wilds. Image provided by the Wilds.

Objectives and Hypotheses

In order to highlight the multiple benefits of prairie ecosystems, the Wilds began a 3 year project in 2011 through funding from the United States Department of Agriculture's Conservation Innovation Grant Program. The objective was to demonstrate additional income opportunities for farmers on marginal land through the use of native prairie. The benefits include:

1) Sustainable forage for livestock to be incorporated into rotational grazing
2) Wildlife habitat, especially pollinators, small mammals and grassland birds
3) Biomass production for hay or biofuel
4) C sequestration in soils, which in turn improves soil quality

Land preparation and management practices are being compared to determine which methods are best for each benefit. There were four treatments, each on 6 ha for a total of 24 ha. All plots were planted with the same seed mix utilizing no-tillage drill equipment. Soil preparation treatments included disking with sub-soiling (DK-S), disking only (DK), no-tillage (NT), and no-tillage with grazing (NT-G, bison were introduced in the spring of 2013) (Fig. 2).

Site Description

The study site is located on the Wilds land that had been mined in the early 1980's, and was once part of the Muskingum Mine. All overburden was removed above the coal seams during mining and was piled in previously mined areas. As required by law, the mine spoil was then contoured to approximate the original rolling hill landscape and then covered with stockpiled topsoil approximately 20 cm deep. A mix of forage grasses and legumes were seeded to establish vegetation ground cover. The reclaimed soil in this region is classified as the Morristown series (loamy-skeletal, mixed, active, calcareous, mesic Typic Udorthents), which consists of a 20 cm surface layer of dark brown silty clay loam above light gray silty clay loam overburden that is alkaline and contains 10 to 40 percent coarse fragments (Soil Survey Staff, 1996). Selected baseline soil conditions for control and treatment plots and present vegetation ground cover are listed in Table 1.

Results

Soil quality (soil organic carbon and bulk density)

Many studies have shown that SOC can be used as an indicator for soil quality within LCA of ecosystems (Karlen et al., 1997; Bendfeldt et al., 2001). An increase in SOC due to the soil management practices implies a benefit, whereas any decrease in SOC is considered as harmful to the system. The effect of the different land preparation and management practices on soil quality by quantifying changes in SOC after 3 years since tall-grass prairie establishment is shown in Table 2. Average SOC pool across treatments during baseline year was 39.68 Mg ha^{-1}, at a soil depth of 40 cm (Table 1). The SOC pool was predominantly in the A horizon (0 to 6 cm), ranging from 39 to 49% of total SOC pool. This is likely due to root growth and decay that occurred since the land was reclaimed and seeded with forage grass and legume species, resulting in higher SOC pool and increased pH (due to weathering of over burden soil high in carbonates) compared to the original undisturbed forest soils. For comparison, an undisturbed forest soil 50 km east of the Wilds, SOC pool in the top 40 cm soil depth was on average 31.74 Mg ha^{-1} and a pH of 5.4 (Lal et al., 2012).

Consequently, it was no surprise that SOC sequestration rates were negative, on average -2.93 Mg ha^{-1} yr^{-1}, except to a lesser extent in the DK land preparation which on average was -0.73 Mg ha^{-1} yr^{-1}. This could be attributed to reduced potential C inputs from above- and belowground biomass and higher rates of decomposition during the first 2 years of tall-grass prairie establishment compared to pre-existing conditions. Lower rates of SOC losses in DK practice could be due to the creation of a more porous soil media, resulting in increased biomass production (above- and belowground) and greater potential for C additions into the soil when compared to NT practice when high soil moisture conditions and cold temperatures are limiting factors (Staricka et al., 1991; Al-Kaisi and Yin, 2005). However, C additions from aboveground tall-grass prairie treatments after 3 years since establishment were on average 40% greater than the control (Table 3). This implies that current negative (input of CE emissions) from net soil C changes due to LUC,

Table 1. Establishment year, plant species ground cover and baseline conditions for selected soil properties for each treatment plot.

Characteristic of ecosystem	Land preparation and management practice				
	Control	No-tillage plus grazing	No-tillage	Disking	Disking plus sub-soiling
Establishment	1980s	2011	2011	2011	2011
Plant species ground cover based on 2013 sampling	*Bromus-inermis* (32%) *Poa-pratensis* (23%) *Lotus-corniculatus* (45%)	*Panicum-virgatum* (30%) *Monarda-fistulosa* (8%) *Poa pratensis* (3%) *Sorghastrum-nutans* (2%) *Echinacea-purpurea* (22%) *Asclepias-L.* (3%) *Lotus-corniculatus* (32%)	*Panicum-virgatum* (60%) *Heliopsis-helianthoides* (15%) *Monarda-fistulosa* (12%) *Echinacea-purpurea* (3%) *Lotus corniculatus* (10%)	*Panicum-virgatum* (32%) *Monarda fistulosa* (7%) *Poa-pratensis* (2%) *Sorghastrum-nutans* (8%) *Andropogon- gerardii* (13%) *Echinacea-purpurea* (3%) *Lotus-corniculatus* (35%)	*Panicum-virgatum* (17%) *Heliopsis-helianthoides* (15%) *Monarda-fistulosa* (15%) *Festuca* (3%) *Poa-pratensis* (5%) *Lotus-corniculatus* (45%)
Soil organic carbon (Mg ha^{-1}) (0-6 cm soil depth)†	–	12.56 ± 5.51	14.68 ± 3.80	17.17 ± 2.82	17.99 ± 6.48
Soil organic carbon (Mg ha^{-1}) (6-40 cm soil depth)‡	–	30.94 ± 3.97	22.87 ± 1.55	17.43 ± 6.80	25.59 ± 2.13
Bulk density (g cm^{-3}) (0-6 cm soil depth)†	–	0.91 ± 0.05	0.93 ± 0.04	0.98 ± 0.14	0.94 ± 0.08
Bulk density (g cm^{-3}) (6-40 cm soil depth)‡	–	1.30 ± 0.05	1.14 ± 0.17	1.25 ± 0.04	1.25 ± 0.05
pH (in water) (0-40 cm soil depth)	–	7.78	7.20	7.88	7.90

† Soil horizon A

‡Soil horizon B

will eventually trend positive and be a sink for CE emissions (Table 4). Additionally, bulk density at the 0 to 6 cm A layer on average was 0.94 Mg m^{-3} and 1.04 for soil depth at 6 to 40 cm. These values are much lower compared to other studies done in reclaimed mine land in Southeast Ohio, ranging from 1.05 to 1.40 for the top A horizon and 1.40 to 1.80 at lower depths due to the nature and compaction of overburden soil during contouring of landscape (Lal et al., 2012; Ussiri et al., 2006).

Net primary production (aboveground biomass)

Net primary productivity (NPP) is the basis for ecosystem function and is critical in restoring core processes to disturbed landscapes. Plant species varied by treatment (Table 1), and consequently resulted in differences in aboveground biomass production (Table 3). The lowest aboveground biomass (5,435 kg ha^{-1}) occurred in the pre-existing treatment (control), where smooth brome (*Bromus inermis*) and Kentucky bluegrass (*Poa pratensis*)were the prevailing grass species. These plant species have been shown to produce less biomass compared to tall-grass prairie species such as switchgrass (*Panicum virgatum*), Indian grass (*Sorghastrum nutans*), and big bluestem (*Andropogon gerardii*)which ranges from 4,000 to 12,000 kg ha^{-1}in reclaimed mine land (Skousen et al., 2008).

The highest aboveground biomass was observed in NT (11,130 kg ha^{-1}), followed by DK (9,881 kg ha^{-1}), and the least in the DK-S (6,660 kg ha^{-1}). Aboveground biomass was reduced due to grazing in treatment with bison (8,971 kg ha^{-1}), although bison density in the plot was low enough that aboveground biomass was still significantly greater than the control and DK-S treatment. These data clearly demonstrate that the aboveground biomass was affected by land preparation and management practices (e.g. tillage, grazing) and emphasizes the need to account for LUC and management effects on NPP in LCA and C budgets.

Table 2. Effect of land preparation and management practices on soil organic carbon, bulk density, and soil organic carbon sequestration rate 3 years after establishment.

Land preparation and management practice	Soil horizon	SOC pool (Mg ha^{-1})	Bulk density (Mg m^{-3})	SOC sequestration rate (Mg ha^{-1} yr^{-1})
No-tillage plus grazing	A, 0 - 6 cm	16.70 ± 4.49[†]	0.95 ± 0.14	-3.00 ± 1.09
	B, 6 - 40 cm	17.80 ± 9.56	0.97 ± 0.16	
No-tillage	A, 0 - 6 cm	11.20 ± 2.52	0.86 ± 0.08	-2.95 ± 1.44
	B, 6 - 40 cm	17.50 ± 6.93	0.97 ± 0.05	
Disking	A, 0 - 6 cm	13.25 ± 3.66	0.94 ± 0.10	-0.73 ± 1.19
	B, 6 - 40 cm	19.16 ± 9.77	1.15 ± 0.13	
Disking plus sub-soiling	A, 0 - 6 cm	17.07 ± 14.49	1.02 ± 0.08	-2.83 ± 1.95
	B, 6 - 40 cm	17.58 ± 15.65	1.10 ± 0.05	

[†] Standard deviation

Table 3. Abovegroundbiomass 3 years after prairie establishment.

Land preparation and management practice	Aboveground biomass (kg ha^{-1})
Control, pre-existing vegetation	5,435 ± 551[†]
No-tillage plus grazing	8,971 ± 1148
No-tillage	11,130 ± 1905
Disking	9,881 ± 625
Disking plus sub-soiling	6,660 ± 625

[†] Standard deviation

Carbon emissions

Until recently, LCA studies did not consider LUC and generally concluded that biofuel crop production led to a reduction of GHG emissions compared to fossil fuels (Farrell et al. 2006). Recent studies have found that the LUC component of GHG emissions from conversion of annual grain based biofuels and grassland systems are often large enough to offset this benefit (Brandao et al., 2011). When converting from forage grass vegetated reclaimed mine land (over 10 years) to tall-grass prairie, the largest source of C emissions was from LUC, ranging from 730 to 3,000 CE kg ha^{-1} (Table 4). It was assumed that all losses in SOC were emitted to the atmosphere as CO$_2$ for reasons explained above in the soil quality section. The next largest contributor to C emissions was burning of the aboveground biomass, which ranged from 902 to 1,561 CE kg ha^{-1}. Total CE emissions from management inputs including the manufacturing and combustion of fuel, and herbicide applications were relativity small compared to LUC and burning, ranging from 6.88 to 36.26 CE kg ha^{-1}. In prairie ecosystems, management inputs are typically low due to the nutrient recycling of perennial grasses and nitrogen fixation from legumes. In comparison, annual grain crop systems such as corn have high inputs of fertilizer, resulting in C emissions that can exceed 200 CE kg ha^{-1} (Clemens et al., 1995; Lal, 2004).

Sustainability of different land preparations and management practices

An Index of Sustainability (I_s) ratio was used to evaluate C use efficiency; values >1 indicate sustainable practices (Table 4). Carbon emissions and sinks resulting from the different land preparation and management practices are also shown in Table 4. These C emissions are clearly dominated by changes in SOC and whether aboveground biomass was burned or harvested. It is expected that SOC losses observed in this study will eventually turn into gains, due to higher C additions compared to original vegetation establishment, resulting in higher I_s values over time. In the scenario when aboveground biomass was neither burned nor harvested, all the land preparation and management practices had I_s values >1. This was due to NPP (above and belowground biomass) more than compensated for C emissions from SOC losses and management inputs. Comparing across land preparation practices, DK had the highest I_s (8.53), due to having the lowest SOC losses. The DK-S had the lowest I_s (1.44) due to having the lowest NPP, yet having similar SOC losses compared to NT practices. When considering harvesting 50% of the aboveground biomass, NT and DK practices still had I_s values >1. This was not the case for DK-S which resulted in a I_s value of 0.93. Additionally, when 100% of aboveground biomass was considered for harvesting, DK (I_s of 2.68) was the lone practice with an I_s value >1.

Conclusions

The focus of this study had been on C balance, and it is clear that LUC from reclaimed mind land to tall-grass prairie had a positive impact, mainly due to atmospheric C fixation from the increase in aboveground biomass. All land preparation and management practices had sustainability index (I_s) values >1 when neither burning nor aboveground biomass harvesting was done. When harvesting of 100% of the aboveground biomass was considered, I_s values were reduced to <1, except for DK practice. Changes in SOC dominated the C emissions from establishment of prairies, followed by burning. Of the four land preparation and management practices studied, the DK practice had the best impact on soil quality and C emissions. This was due to having the lowest degradation of SOC during the LUC, and also the second highest aboveground biomass production. Although, it should be noted that LUC are multi-faceted (e.g. including effects on biodiversity and water quantity/quality), and SOC does not indicate all possible impacts on soil quality and sustainability of a system. Alternative or complementary indicators such as soil erosion and eutrophication potential should be evaluated. Additionally, 3 years is too short of a time to definitively determine which land preparation and management practices are the most productive and sustainable, since I_s values due to LUC should increase with time to be considered sustainable and could vary by management practice.

Table 4. Carbon balance and sustainability index under different land preparation and management practices.

		Carbon emissions (kg CE/ha)			
Inputs	**Description**	**No-till & grazing**	**No-till**	**Disking only**	**Disking & sub-soiling**
Fuel usage	Disking (L diesel/ha)	0	0	1.43	1.43
	Mowing (L diesel/ha)	2.06	2.06	2.06	2.06
	Spraying (L diesel/ha) 19.6 L total	0.10	0.10	0.10	0.10
	Seed bed prep (subsoil) L diesel/ha	0	0	0	2.05
	Burning- Torch 7.6 L total	0.04	0.04	0.04	0.04
	Burning- vehicles (gasoline/ha) 37.9 L total	0.20	0.20	0.20	0.20
	Planting (no-till for all, L diesel/ha) 5.1 total	0.10	0.10	0.10	0.10
	Windrower (L diesel/ha), from Lal, 2004	4.8	4.8	4.8	4.8
	Rake (L diesel/ha), from Lal, 2004	1.7	1.7	1.7	1.7
	Baler ,large round (L diesel/ha), from Lal, 2004	5.8	5.8	5.8	5.8
	Forage harvesting (L diesel/ha), from Lal, 2004	13.6	13.6	13.6	13.6
Herbicide	2 applications of 2,4-D (oz/ha) 87.1 L total	4.39	4.39	4.39	4.39
Burning	C loss (kg/ha)	1,215	1,561	1,338	902
Fertilizers	None	0	0	0	0
Net soil C[†]	SOC kg/ha/yr	3,000	2,950	730	2,830
Total Inputs	Non-burning year	3,006	2,956	738	2,840
Total Inputs	Forage harvest (50 and 100% removal)	3,032	2,982	764	2,866
Total Inputs	Burning year	4,221	4,518	2,076	3,742
Outputs		**Carbon sinks (kg CE/ha)**			
ΔYield	Aboveground biomass (kg/ha) non-burning year	3,857	4,957	4,248	2,863
ΔYield	Belowground biomass (kg/ha)	1,728	2,429	2,050	1,240
ΔYield	Aboveground biomass (kg/ha) 50% removal	-	2,479	2,124	1,432
ΔYield	Aboveground biomass (kg/ha) 100% removal	-	0	0	0
ΔYield	Ash and aboveground biomass (kg/ha) burning year	-	2,479	2,124	1,432
Manure	Bison manure (kg/ha) at density of 1bison/ha	241[‡]	0	0	0
Sustainability index		**I_s (Outputs/Inputs)**			
I_s	(Outputs/Inputs) non-burning year	1.94	2.50	8.53	1.44
I_s	(Outputs/Inputs) 50% removal	-	1.65	5.46	0.93
I_s	(Outputs/Inputs) 100% removal	-	0.81	2.68	0.43
I_s	(Outputs/Inputs) burning year	-	1.09	2.01	0.71

[†] These data assumes negligible losses by erosion and leaching. [‡] Recycled C from aboveground biomass consumption. Gain in animal weight was not accounted for.

Future work and recommendations

This study has shown that tall-grass prairie ecosystems are a significant net sink for atmospheric CO_2 three years afterestablishment, due to high biomass yields compensating for SOC losses from LUC. Continuation of monitoring changes in SOC and aboveground biomass production is necessary to confirm that these results are indicative of a positive trend in sustainability, and not just seasonal related short term effects. Future collaborations between the Wilds and the Carbon Management and Sequestration Center (CMASC) would entail additional physical (water holding capacity, soil penetration resistance, aggregate stability, aggregate strength), chemical (NO_3^- , NH_4^+, P, K), and biological (microbial biomass C) soil properties measurements to better evaluate soil quality. In addition to C emissions from SOC losses and management inputs, GHG emissions (CO_2, N_2O and CH_4) should also me measured to have a better understating of these prairie ecosystems affect on GWP. Complimentary to the C emissions and soil quality LCA done in this study, water quantity and quality LCA can also be used to evaluate the sustainability of theses prairie ecosystems. The CMASC is currently evaluating this aspect at the Wilds in the NT tall-grass prairie and control plots. This study can be expanded to cover the rest of land preparation and management practices plots and be included in future grant proposals such as the Conservation Innovation Grant.

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Appendix C

Forage Analysis - Nutrition Results

The Wilds
Prairie Agriculture Demonstration Project
Forage Analysis Results

HOLMES LABORATORY INC.
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FORAGE/FEEDSTUFF ANALYSIS REPORT

[A Vital Key to Todays Agriculture]
www.holmeslab.com
Test Performed: AG

Customer: The Wilds-Control

The Wilds
14000 Internatinoal Rd.
Cumberland, OH 43732

Date Reported: 07/30/2013
Lab Number: 13-2680
SAMPLE I.D.: Grass Pasture
Cool Season

Item		Units	As Sampled Basis	Dry Matter Basis
Moisture		%	66.93	
Dry Matter		%	33.07	
Crude Protein		%	3.70	11.19
Available Protein		%		
Adjusted Crude Protein		%		
A.D.F. Protein		%		
N.D.F. Protein		%		
Soluble Protein		%		
Protein Solubility		%		
Lignin		%		
Acid Detergent Fiber		%	12.57	38.00
Neutral Detergent Fiber		%	17.15	51.86
NFC (Non-Fiber Carbohydrate)		%	8.98	27.15
Sugar		%		
Starch		%		
NSC = Starch + Sugar		%		
Crude Fat		%		
TDN		%	16.95	51.25
NEl		Mcal/lb.	.170	.515
NEm		Mcal/lb.	.152	.459
NEg		Mcal/lb.	.069	.209
Ash		%		
Lignin Insoluble Ash		%		
Calcium	(Ca)	%	.21	.62
Phosphorus	(P)	%	.07	.20
Magnesium	(Mg)	%	.04	.13
Potassium	(K)	%	.41	1.24
Sulfur	(S)	%		
Sodium	(Na)	%	.019	.056
Chloride	(Cl)	%		
Copper	(Cu)	ppm	1	3
Manganese	(Mn)	ppm	11	34
Zinc	(Zn)	ppm	4	13
Iron	(Fe)	ppm	7	21
Molybdenum	(Mo)	ppm		
Aluminum	(Al)	ppm		
Nitrate	(NO3)	%		
pH				
RFV (Relative Feed Value)				106
Horse	DE	Mcal/lb.		
Horse	TDN	%		
Crude Fiber		%		
DCAD		meq./100g DM		
DCAD		meq./1 lb.DM		

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Test Performed: AG

Customer: The Wilds-K. Farm

The Wilds
14000 Internatinoal Rd.
Cumberland, OH 43732

Date Reported: 07/30/2013
Lab Number: 13-2679
SAMPLE I.D.: Grass Pasture
Cool Season and Forb

Item		Units	As Sampled Basis	Dry Matter Basis
Moisture		%	79.54	
Dry Matter		%	20.46	
Crude Protein		%	2.88	14.06
Available Protein		%		
Adjusted Crude Protein		%		
A.D.F. Protein		%		
N.D.F. Protein		%		
Soluble Protein		%		
Protein Solubility		%		
Lignin		%		
Acid Detergent Fiber		%	7.08	34.58
Neutral Detergent Fiber		%	12.67	61.91
NFC (Non-Fiber Carbohydrate)		%	2.91	14.23
Sugar		%		
Starch		%		
NSC = Starch + Sugar		%		
Crude Fat		%		
TDN		%	11.42	55.84
NEl		Mcal/lb.	.116	.566
NEm		Mcal/lb.	.108	.530
NEg		Mcal/lb.	.056	.276
Ash		%		
Lignin Insoluble Ash		%		
Calcium	(Ca)	%	.13	.63
Phosphorus	(P)	%	.05	.26
Magnesium	(Mg)	%	.04	.21
Potassium	(K)	%	.41	2.00
Sulfur	(S)	%		
Sodium	(Na)	%	.016	.077
Chloride	(Cl)	%		
Copper	(Cu)	ppm	1	5
Manganese	(Mn)	ppm	10	47
Zinc	(Zn)	ppm	4	20
Iron	(Fe)	ppm	16	76
Molybdenum	(Mo)	ppm		
Aluminum	(Al)	ppm		
Nitrate	(NO3)	%		
pH				
RFV (Relative Feed Value)				93
Horse	DE	Mcal/lb.		
Horse	TDN	%		
Crude Fiber		%		
DCAD		meq./100g DM		
DCAD		meq./1 lb.DM		

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Customer: The Wilds

The Wilds
14000 Internatinoal Rd.
Cumberland, OH 43732

Date Reported: 07/25/2013
Lab Number: 13-2625
SAMPLE I.D.: Mixed Pasture
Warm Ssn.Grs.& Forbs

Item		Units	As Sampled Basis	Dry Matter Basis
Moisture		%	68.93	
Dry Matter		%	31.07	
Crude Protein		%	2.26	7.27
Available Protein		%		
Adjusted Crude Protein		%		
A.D.F. Protein		%		
N.D.F. Protein		%		
Soluble Protein		%		
Protein Solubility		%		
Lignin		%		
Acid Detergent Fiber		%	12.84	41.32
Neutral Detergent Fiber		%	18.36	59.09
NFC (Non-Fiber Carbohydrate)		%	6.97	22.44
Sugar		%		
Starch		%		
NSC = Starch + Sugar		%		
Crude Fat		%		
TDN		%	15.59	50.17
NEl		Mcal/lb.	.156	.503
NEm		Mcal/lb.	.137	.442
NEg		Mcal/lb.	.060	.193
Ash		%		
Lignin Insoluble Ash		%		
Calcium	(Ca)	%	.39	1.26
Phosphorus	(P)	%	.06	.19
Magnesium	(Mg)	%	.06	.20
Potassium	(K)	%	.48	1.54
Sulfur	(S)	%		
Sodium	(Na)	%	.010	.031
Chloride	(Cl)	%		
Copper	(Cu)	ppm	2	7
Manganese	(Mn)	ppm	19	61
Zinc	(Zn)	ppm	11	34
Iron	(Fe)	ppm	15	48
Molybdenum	(Mo)	ppm		
Aluminum	(Al)	ppm		
Nitrate	(NO3)	%		
pH				
RFV (Relative Feed Value)				89
Horse	DE	Mcal/lb.		
Horse	TDN	%		
Crude Fiber		%		
DCAD		meq./100g DM		
DCAD		meq./1 lb.DM		

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Customer: The Wilds

The Wilds
14000 Internatinoal Rd.
Cumberland, OH 43732

Date Reported: 08/19/2013
Lab Number: 13-2888
SAMPLE I.D.: Grass Pasture
Cool Season-Con.1

Item		Units	As Sampled Basis	Dry Matter Basis
Moisture		%	68.15	
Dry Matter		%	31.85	
Crude Protein		%	4.39	13.79
Available Protein		%		
Adjusted Crude Protein		%		
A.D.F. Protein		%		
N.D.F. Protein		%		
Soluble Protein		%		
Protein Solubility		%		
Lignin		%		
Acid Detergent Fiber		%	11.16	35.05
Neutral Detergent Fiber		%	16.35	51.34
NFC (Non-Fiber Carbohydrate)		%	7.98	25.07
Sugar		%		
Starch		%		
NSC = Starch + Sugar		%		
Crude Fat		%		
TDN		%	17.58	55.21
NEl		Mcal/lb.	.178	.559
NEm		Mcal/lb.	.166	.521
NEg		Mcal/lb.	.085	.267
Ash		%		
Lignin Insoluble Ash		%		
Calcium	(Ca)	%	.24	.76
Phosphorus	(P)	%	.09	.27
Magnesium	(Mg)	%	.05	.15
Potassium	(K)	%	.51	1.60
Sulfur	(S)	%		
Sodium	(Na)	%	.003	.010
Chloride	(Cl)	%		
Copper	(Cu)	ppm	1	4
Manganese	(Mn)	ppm	13	41
Zinc	(Zn)	ppm	10	31
Iron	(Fe)	ppm	18	56
Molybdenum	(Mo)	ppm		
Aluminum	(Al)	ppm		
Nitrate	(NO3)	%		
pH				
RFV (Relative Feed Value)				112
Horse	DE	Mcal/lb.		
Horse	TDN	%		
Crude Fiber		%		
DCAD		meq./100g DM		
DCAD		meq./1 lb.DM		

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Customer: The Wilds

The Wilds
14000 Internatinoal Rd.
Cumberland, OH 43732

Date Reported: 08/19/2013
Lab Number: 13-2889
SAMPLE I.D.: Grass Pasture
Cool Season-Con.2

Item		Units	As Sampled Basis	Dry Matter Basis
Moisture		%	72.39	
Dry Matter		%	27.61	
Crude Protein		%	4.55	16.48
Available Protein		%		
Adjusted Crude Protein		%		
A.D.F. Protein		%		
N.D.F. Protein		%		
Soluble Protein		%		
Protein Solubility		%		
Lignin		%		
Acid Detergent Fiber		%	9.41	34.07
Neutral Detergent Fiber		%	12.14	43.98
NFC (Non-Fiber Carbohydrate)		%	8.21	29.74
Sugar		%		
Starch		%		
NSC = Starch + Sugar		%		
Crude Fat		%		
TDN		%	15.62	56.56
NEl		Mcal/lb.	.158	.574
NEm		Mcal/lb.	.150	.542
NEg		Mcal/lb.	.079	.286
Ash		%		
Lignin Insoluble Ash		%		
Calcium	(Ca)	%	.23	.85
Phosphorus	(P)	%	.07	.25
Magnesium	(Mg)	%	.05	.17
Potassium	(K)	%	.47	1.72
Sulfur	(S)	%		
Sodium	(Na)	%	.006	.020
Chloride	(Cl)	%		
Copper	(Cu)	ppm	1	4
Manganese	(Mn)	ppm	11	40
Zinc	(Zn)	ppm	8	28
Iron	(Fe)	ppm	11	39
Molybdenum	(Mo)	ppm		
Aluminum	(Al)	ppm		
Nitrate	(NO3)	%		
pH				
RFV (Relative Feed Value)				132
Horse	DE	Mcal/lb.		
Horse	TDN	%		
Crude Fiber		%		
DCAD		meq./100g DM		
DCAD		meq./1 lb.DM		

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The Wilds
14000 Internatinoal Rd.
Cumberland, OH 43732

Date Reported: 08/19/2013
Lab Number: 13-2890
SAMPLE I.D.: Mixed Pasture
Warm Seasn.Prairie 1

Item		Units	As Sampled Basis	Dry Matter Basis
Moisture		%	73.89	
Dry Matter		%	26.11	
Crude Protein		%	2.93	11.23
Available Protein		%		
Adjusted Crude Protein		%		
A.D.F. Protein		%		
N.D.F. Protein		%		
Soluble Protein		%		
Protein Solubility		%		
Lignin		%		
Acid Detergent Fiber		%	8.78	33.63
Neutral Detergent Fiber		%	12.68	48.58
NFC (Non-Fiber Carbohydrate)		%	7.57	28.99
Sugar		%		
Starch		%		
NSC = Starch + Sugar		%		
Crude Fat		%		
TDN		%	15.45	59.17
NEl		Mcal/lb.	.157	.603
NEm		Mcal/lb.	.152	.581
NEg		Mcal/lb.	.084	.322
Ash		%		
Lignin Insoluble Ash		%		
Calcium	(Ca)	%	.32	1.23
Phosphorus	(P)	%	.08	.30
Magnesium	(Mg)	%	.06	.23
Potassium	(K)	%	.53	2.04
Sulfur	(S)	%		
Sodium	(Na)	%	.005	.021
Chloride	(Cl)	%		
Copper	(Cu)	ppm	2	7
Manganese	(Mn)	ppm	11	41
Zinc	(Zn)	ppm	8	30
Iron	(Fe)	ppm	7	25
Molybdenum	(Mo)	ppm		
Aluminum	(Al)	ppm		
Nitrate	(NO3)	%		
pH				
RFV (Relative Feed Value)				120
Horse	DE	Mcal/lb.		
Horse	TDN	%		
Crude Fiber		%		
DCAD		meq./100g DM		
DCAD		meq./1 lb.DM		

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Customer: The Wilds

The Wilds
14000 Internatinoal Rd.
Cumberland, OH 43732

Date Reported: 08/19/2013
Lab Number: 13-2891
SAMPLE I.D.: Mixed Pasture
Warm Seasn.Prairie 2

Item	Units	As Sampled Basis	Dry Matter Basis
Moisture	%	72.02	
Dry Matter	%	27.98	
Crude Protein	%	3.39	12.11
Available Protein	%		
Adjusted Crude Protein	%		
A.D.F. Protein	%		
N.D.F. Protein	%		
Soluble Protein	%		
Protein Solubility	%		
Lignin	%		
Acid Detergent Fiber	%	9.08	32.44
Neutral Detergent Fiber	%	14.20	50.76
NFC (Non-Fiber Carbohydrate)	%	7.26	25.93
Sugar	%		
Starch	%		
NSC = Starch + Sugar	%		
Crude Fat	%		
TDN	%	16.96	60.61
NEl	Mcal/lb.	.173	.619
NEm	Mcal/lb.	.169	.603
NEg	Mcal/lb.	.096	.342
Ash	%		
Lignin Insoluble Ash	%		
Calcium (Ca)	%	.29	1.04
Phosphorus (P)	%	.07	.26
Magnesium (Mg)	%	.08	.28
Potassium (K)	%	.47	1.68
Sulfur (S)	%		
Sodium (Na)	%	.006	.020
Chloride (Cl)	%		
Copper (Cu)	ppm	1	5
Manganese (Mn)	ppm	14	50
Zinc (Zn)	ppm	9	33
Iron (Fe)	ppm	7	24
Molybdenum (Mo)	ppm		
Aluminum (Al)	ppm		
Nitrate (NO3)	%		
pH			
RFV (Relative Feed Value)			117
Horse DE	Mcal/lb.		
Horse TDN	%		
Crude Fiber	%		
DCAD	meq./100g DM		
DCAD	meq./1 lb.DM		

The Wilds CIG Final Report

Appendix D

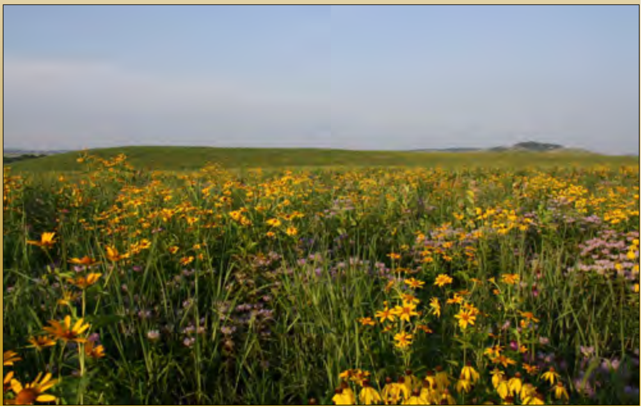
Publications

PRAIRIE IN AGRICULTURE

Utilizing the multiple benefits of native warm season grasses and forbs

What is a Prairie?

Prairies are open areas with few or no trees, dominated by native warm-season grasses such as Big Bluestem *(Andropogon gerardii)*, Indian Grass *(Sorghastrum nutans)*, and Switchgrass *(Panicum virgatum)* and forbs such as Purple Coneflower *(Echinachea purpurea)* and Black-eyed Susan *(Rudbeckia hirta)*. There are three types of prairie: shortgrass, tallgrass and mixed-grass. Tallgrass prairies exist in areas that receive enough rainfall to allow these grasses to grow up to 9ft tall.



Threats to Our Grassland Prairies

More than 142 million acres of tallgrass prairie once existed throughout the U.S.; today only about 1% of that remains. The biggest threats to grassland habitats come from:

- Agricultural development
- Urban sprawl
- Climate change
- Pesticides and herbicides

Once the steel plow was invented in 1837, much of the Tallgrass prairie landscape was converted to agriculture. The rich soils created by the deep roots and frequent fires were perfect for growing crops and those with drier soils were converted to grazing land for livestock. Climate change is also affecting these unique ecosystems. As weather patterns continue to change, grassland prairies are not getting the amount of annual rainfall necessary to grow to their full potential.

Why do Grassland Prairies Matter?

Grasslands ecosystems have many types of environmental, economic and intrinsic benefits including:

- Erosion control
- Improving water quality
- Sequestering carbon.

Their extensive root systems, are excellent at rebuilding depleted soils, stabilizing soil and filtering storm water. Prairie also recharges groundwater sources, absorbing up to 9 inches of rainfall per hour before runoff occurs. Many species of plants, insects, birds and mammals rely on grassland prairies; therefore, they are very important wildlife habitat areas.

Prairie as a Crop

When most people think of prairie, an agricultural crop isn't usually the first thing that comes to mind. But at *the Wilds*, an innovative research and conservation center in southeastern Ohio, that is exactly what is being demonstrated on a 60 acre section of the property planted in warm-season grasses and forbs in 2011. The project was funded by the USDA-NRCS Conservation Innovation Grant as a three year demonstration, using native warm season grasses to show the multiple uses of prairie for farmers and ranchers on marginal land.

The four beneficial uses being demonstrated are wildlife habitat, forage for livestock, biomass production for biofuels and carbon sequestration as part of soil improvement. Providing habitat for wildlife is a potential source of income for landowners who qualify for the Farm Service Agency's Conservation Reserve Program (CRP). When incorporated into a rotational grazing system to provide summer forage, prairie can provide savings in hay cost. Biomass from prairie can be sold as hay, pellets to burn for heat and electricity, or as a feedstock for biofuel production. Lastly carbon sequestration improves soil quality which increases crop yields and may eventually provide income as carbon credits.

Prairie is a versatile crop that can be established just about anywhere, and establishes much faster than forest .

Uses

Prairie ecosystems provide:

- **Forage for livestock** in summer months and during drought
- **Habitat for wildlife** including pollinating insects, small mammals and birds
- **Biomass crop** for biofuel production
- **Carbon sequestration and soil improvement**

Seed Selection:

Prairie seed can be very costly, so it is important to chose species that compliment the region, site conditions and intended purpose of your stand.

- Choose **local ecotypes**, these are best suited to the conditions in your area.
- Include **grasses, forbs and legumes** into your mix, ratios should be determined by the main end use (i.e. for grazing 75-80% of your mix should consist of grasses and legumes.)
- Include an annual **cover-crop**, such as Plains Coreopsis, to help suppress weeds and hold soils together while the prairie is establishing.

Establishing Prairie On Your Farm

Good stand establishment requires appropriate site preparations.

- Choose a site that is conducive to management regimes such as mowing or grazing.
- Eliminate existing vegetation using two herbicide applications. This is best done after mowing, spray the new growth in the fall and then again in the spring.
- Seeds should be planted using a no-till drill for best establishment (a broadcast seeder can also be used, but the site should be tilled following herbicide application to allow for good seed-to-soil contact.)

The stand will be weedy during the first year, **this is expected**, manage for weeds by mowing or using a selective herbicide if necessary. Weed pressure should decrease during the second and third years.

Costs & Benefits:

The cost of seed will vary depending on region, quantity and species selection, ranging from $10/lb to over $140/lb, but should average around $40/lb for a mid-diversity mix.

Cost of establishment can be inexpensive if labor can be done in-house with existing equipment. Cost of herbicide averages between $20 and $40 per acre. Management costs are about $47/acre each year after establishment (for mowing)

Estimates for income potential include:

- $650-$1100 - Grazing (@1-2lb/day for 150 days, @ $0.70-1.00/lb)
- $45-$315 - Biomass (Producing 3-7 tons/acre @$15-45/ton)

Resources:

- To purchase native seed (in the Eastern US): www.ernstseed.com
- Establishment help and Equipment rental (Ohio): http://ohiodnr.com/tabid/9093/Default.aspx
- Conservation reserve program: http://www.nrcs.usda.gov/programs/crp/

Equipment List:

- Native no-till seed drill (check your local SWCD)
- Herbicide spraying equipment
- Rotary-type mower adjustable to 10" cut height

A broadcast seeder can be used in place of a no-till drill, but will require tilling.

Prairie Management

The best method for managing your prairie is with prescribed burning. This removes dead debris, suppresses woody vegetation and weeds, and promotes healthy stands. It is best to burn in between February and April, prior to new growth in the spring. If burning is not an option, mowing is a suitable alternative. Mowing should be done prior to March 1st or after July 15th to avoid wildlife reproductive periods.

Calculating Pure Live Seed (PLS):

Pure live seed (PLS) is a measure used by the seed industry to describe the percentage of a quantity of seed that will germinate.

$$\text{PLS} = \frac{\%\text{Purity x } (\%\text{Germination} + \%\text{Dormant/Hard})}{100}$$

To determine how much bulk seed is needed to equal 1 PLS lb, 1 is then divided by the %PLS (i.e. 1 / .768 = 1.3, therefore 1.3 bulk lb of seed is equal to 1 PLS)

Innovative Approaches to Land Management: The Agricultural Benefits of Prairie Crops

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Introduction

The Wilds, a conservation center on previously surface-mined land, began a 3 year project in 2011 demonstrating the multiple benefits of prairie ecosystems. Funded by the United States Department of Agriculture's Conservation Innovation Grant Program, the project's objective is to demonstrate the use of native prairie species on marginal land to generate the following income opportunities for farmers:

- Sustainable forage for livestock to be incorporated into rotational grazing (savings)
- Wildlife habitat, especially for pollinators, small mammals and grassland birds(CRP)
- Biomass production for hay or biofuel (income)
- Carbon credits sequestration in soils, which in turn improves soil quality (income)

Land preparation and management techniques are being compared to determine which methods are best for each benefit. There are four treatments, each on 15 acres, totaling 60 acres. All four plots were planted with a single mix of native, warm-season grasses and forbs using a no-till drill in June 2011. Soil preparation treatments included: i) tilling only, ii) tilling & subsoiling, iii) no-till planting, and iv) no-till planting with grazing (bison introduced Spring of 2013). Additionally, a local farm which grazes cattle on cool season grasses is incorporated as a second site with conditions similar to that of local farmland.

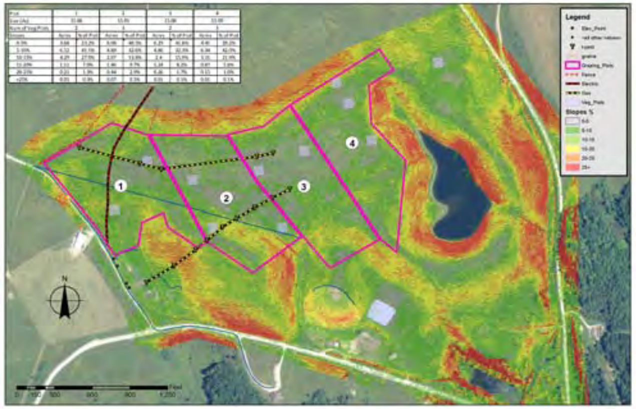


Figure 1. Map of the 60-acre demonstration site on the Wilds' property with location of sampling modules used for monitoring vegetation, arthropods and 2011 small mammals.

Site Preparation

All four plots within the 60 acre site were originally dominated by cool season grasses, used to establish cover during post-mining reclamation. In order to prepare the site and ensure good stand establishment, autumn olive (*Elaeagnus umbellata*) shrubs were removed. In addition, a prescribe burn was conducted across the entire site. Following green-up, cool-season grasses were treated with a glyphosate (2%) herbicide application and the soil preparation treatments were applied prior to seeding.

Figure 2. Soil preparation treatments in Plot 4 included subsoiling followed by tilling.

Figure 3. Following site preparations, all four plots were seeded using a no-till drill.

Monitoring

Surveys are conducted annually on the 60 acre site as well as on the partner farm to assess changes in diversity of vegetation, arthropods, and small mammals. The potential of the landscape to sequester carbon is being estimated through annual soil samples which are analyzed for carbon content as well as bulk density. In the third year, a Life Cycle Analysis (LCA) will be conducted for each of the treatments to compare the net carbon balance and energy use in each of the treatment plots. Forage quality, biomass yield and weight gain by bison will also be assessed in the third year. An ethogram study will determine the intensity with which bison are grazing on warm season versus cool season grasses.

Figure 4 (top). A modified version of the North Carolina Vegetation Survey Protocol (NCVS, Peet et al., 1998) is used to collect data each year on the presence and relative abundance of the species at several points in each treatment plot.

Figure 5 (middle). Small mammal surveys are conducted on an annual basis to detect changes in populations over time as well as differences between treatments and the partner farm to determine the quality of habitat provided by warm-season grasses.

Figure 6 (bottom). Soil samples are collected each year using a pneumatically-powered JMC Environmentalist's Sub-soil Probe (ESP) to determine the bulk density, % total carbon, % organic carbon, % total nitrogen. Measurements are compared to baseline samples and those of the partner farm to determine the effects of the seed mix and treatments used on soil health.

Methods

Vegetation surveys – In order to gauge the planting success, vegetation surveys have been conducted each year. Randomly distributed 100 m2 sampling modules were established within all four treatment plots as well as a control plot just outside of the study area. (represented by the squares on the map in Figure 1.) A modified version of the North Carolina Vegetation Survey Protocol (NCVS-EEP, Peet et. al., 1998) was used to collect data on the presence and relative abundance of the species in each treatment plot, as well as to determine the percentage of non-native, invasive vegetation that persists.

Soil Analysis **–** A total of 60 soil samples are collected each year from the four treatment plots at *the Wilds* and 9 from the partner farm. To characterize the total carbon content of the soil, stratification is used to determine the boundary of an area where individual soil carbon measurements will be combined, considering factors such as the existing soil depth, texture and parent material, geomorphic position, land use and management history, or any other factors that may have influenced the deposition or removal of carbon. The uniformity of the measurements within a stratum is analyzed statistically to determine the expected overall carbon content (mass) in terms of a confidence interval at a chosen confidence level. Samples were then analyzed for bulk density, % total carbon, % organic carbon, % total nitrogen.

Life Cycle Analysis – One aspect of the project is to provide farmers with options for establishing prairie based on Life Cycle Analysis (LCA). Our goal is to apply energy and carbon LCA to the 4 land preparation and management techniques being demonstrated for establishing native tall grass prairie as a crop. The purpose is to determine whether prairie can be an effective tool in sequestering carbon (carbon LCA) and producing biomass for biofuel (energy LCA) as well as which management technique is the best for achieving each. Records of fuel usage and project management have been kept since the project began in 2011. The LCA will be conducted in the fall of 2013.

Grazing Study and Bison Ethogram **–** The purpose of this aspect of the study is to compare the health of bison grazed on a cool-season pasture with that of a warm-season pasture. Two 15 acre pastures will be used on *the Wilds*' property. The first is located in Plot 1 within the 60 acre demonstration area. The second site is a cool-season pasture which was renovated to include endophyte free fescue. Five, 1 year-old bison calves, of similar weight and age will be introduced to the sites in April 2013. Weight and body condition scores (BCS) will be obtained for each animal prior to introducing them into pasture and at the conclusion of the study. Vegetation surveys will be conducted prior to, during and after grazing and exclosures will be installed in both pastures to determine what species are being favored. Forage samples will be taken from both sites in April, June and August and analyzed for metabolizable energy (ME). An ethogram study will be conducted daily through August, 2013. Behavior of both herds will be observed including time spent foraging, resting and locomotion, as well as bite counts and size. These results will provide an indication of how much is being consumed to meet the animals' needs and which type of forage results in the highest overall weight gain and animal health.

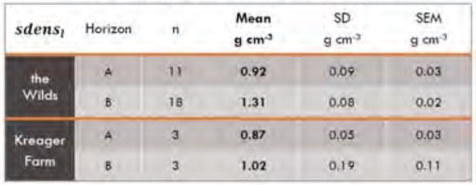
Discussion

Vegetation- Preliminary results from 2012 vegetation surveys show no significant difference in species richness or diversity between treatments. One exception, the partner farm, had significantly lower plant diversity than the tilled (p=0.007) and tilled plus sub-soiled (p=0.009) treatments. The number of invasive and planted species did not vary between treatments as determined by a Chi-squared test (p-value = 0.9778).

Insects- Results from 2011 surveys of arthropods showed a positive correlation between arthropod family diversity and plant species richness (p=0.03) across treatments.

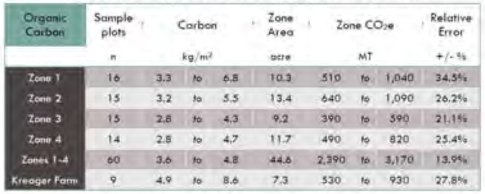
Soils- Samples taken in 2012 shortly after planting showed organic carbon density across the four treatments to be 3.6 – 4.8 kg/m^2 with a 95% confidence interval and little difference between the treatments. Samples collected from the partner farm at the same time indicate 4.9 – 8.6 kg/m^2 organic carbon density Average bulk densities determined from the same samples were similar at both sites.

This study demonstrates the feasibility of utilizing prairie crops in a farm setting, as well as any differences in the outcome of each treatment. Based on these results, as well as those from bison grazing and LCA (to be completed this year), farmers establishing warm-season grasses on marginal lands will be able to make informed decisions about which of the proposed uses best suit their needs, and which land preparation and management techniques are most appropriate. This demonstration is intended to serve as a model and provide guidance for farmers who would like to incorporate prairie into their current grazing practices.



$sdens_l$	Horizon	n	Mean g cm^{-3}	SD g cm^{-3}	SEM g cm^{-3}
the Wilds	A	11	**0.92**	0.09	0.03
	B	18	**1.31**	0.08	0.02
Kreager Farm	A	3	**0.87**	0.05	0.03
	B	3	**1.02**	0.19	0.11

Table 2. Baseline average A and B-horizon bulk density values of the Wilds' 60 ac. demonstration site and the partner farm's traditional cool-season pasture.



Organic Carbon	Sample plots	Carbon			Zone Area	Zone CO$_2$e			Relative Error
	n	kg/m^2			acre	MT			+/- %
Zone 1	16	3.3	to	6.8	10.3	510	to	1,040	34.5%
Zone 2	15	3.2	to	5.5	13.4	640	to	1,090	26.2%
Zone 3	15	2.8	to	4.3	9.2	390	to	590	21.1%
Zone 4	14	2.8	to	4.7	11.7	490	to	820	25.4%
Zones 1-4	60	3.6	to	4.8	44.6	2,390	to	3,170	13.9%
Kreager Farm	9	4.9	to	8.6	7.3	530	to	930	27.8%

Table 1. Baseline soil organic carbon density with 95% confidence intervals, for the Wilds' 60 ac. demonstration site and the partner farm's traditional cool-season pasture.

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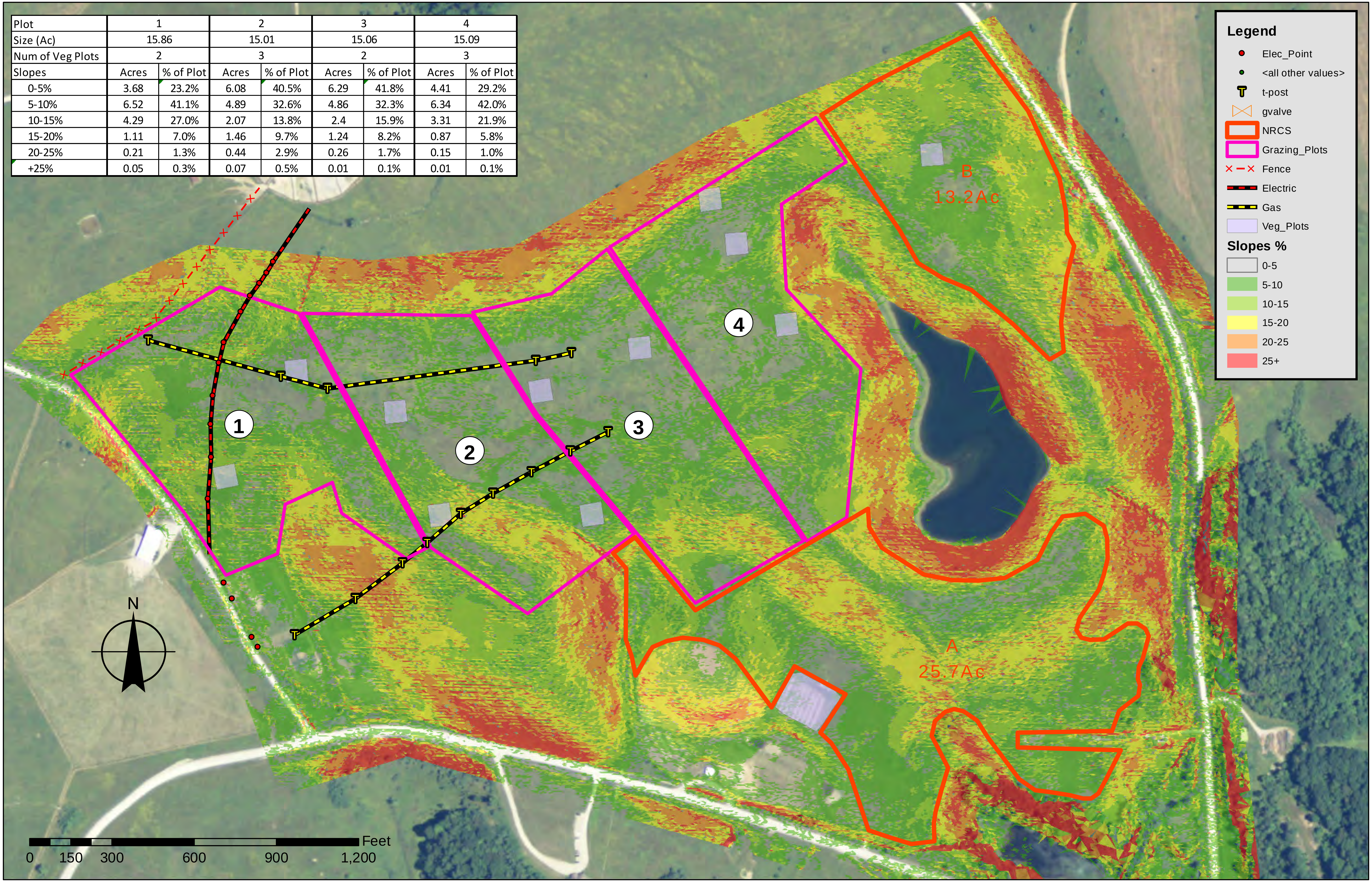
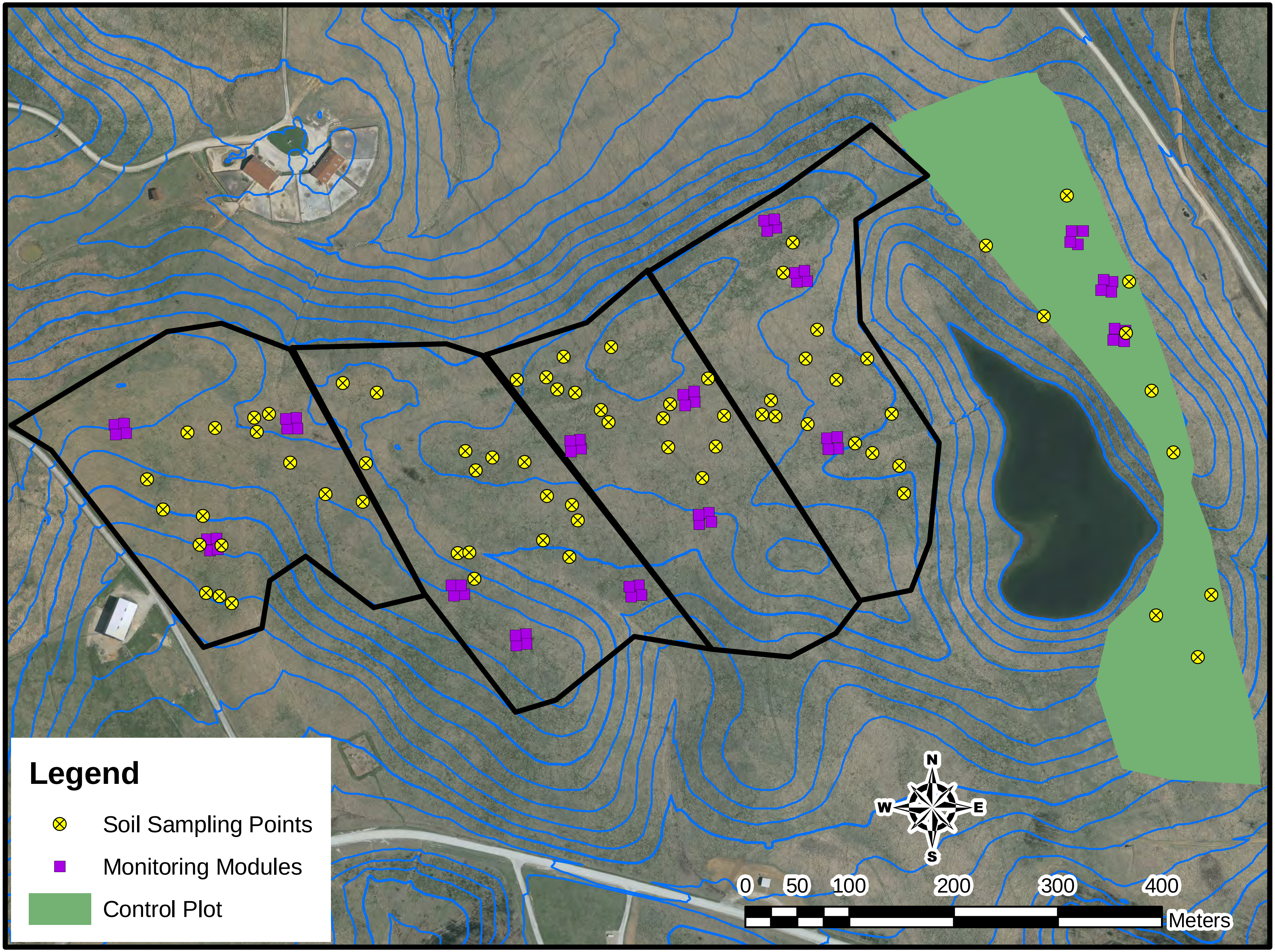
Appendix E
Maps

1..............................Prairie Demonstration Site Map with % Slope Provided by the Ohio Wetland Foundation

2..............................Prairie Demonstration Site Map with Sampling Point Locations for Soils and Biodiversity

Plot	1		2		3		4	
Size (Ac)	15.86		15.01		15.06		15.09	
Num of Veg Plots	2		3		2		3	
Slopes	Acres	% of Plot	Acres	% of Plot	Acres	% of Plot	Acres	% of Plot
0-5%	3.68	23.2%	6.08	40.5%	6.29	41.8%	4.41	29.2%
5-10%	6.52	41.1%	4.89	32.6%	4.86	32.3%	6.34	42.0%
10-15%	4.29	27.0%	2.07	13.8%	2.4	15.9%	3.31	21.9%
15-20%	1.11	7.0%	1.46	9.7%	1.24	8.2%	0.87	5.8%
20-25%	0.21	1.3%	0.44	2.9%	0.26	1.7%	0.15	1.0%
+25%	0.05	0.3%	0.07	0.5%	0.01	0.1%	0.01	0.1%




Legend
Soil Sampling Points
Monitoring Modules
Control Plot
N
W
E
S
0
50
100
200
300
400
Meters

The Wilds CIG Final Report

Appendix F

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