Examining the impacts of regenerative agriculture on soil carbon: A community-based participatory study.

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Abstract

Innovative farmers in the southeastern Cumberland Plateau region are stewarding their lands using regenerative practices, such as improved cover cropping and rotational grazing. These practices have been shown to increase and sequester soil carbon, offering an important tool for mitigating climate change caused by rising atmospheric CO_2 concentrations. In partnership with the Southeast Tennessee Young Farmers (SeTNYF) Coalition, we initiated a pilot project to examine the impact of rotational grazing and cover cropping on soil carbon on seven local small farms. We employed a community-based participatory approach to design the research around the needs of our community and to ensure that farmers were consulted at every step. We compared soil carbon at two depths (0-15 and 15-30 cm) between parcels managed with regenerative practices (rotational grazing or cover cropping) and adjacent non-regenerative plots. Using a sampling protocol employed by the Soil Inventory Project, we sampled at a minimum density of 3 samples per acre, although on some farms we sampled more, for a total of 406 samples. We hypothesized that soils under rotational grazing or cover crops would have higher organic carbon than adjacent non-regeneratively managed land. We found no significant difference in soil carbon between rotational grazing and adjacent plots. On one farm we found significantly higher carbon under soils that had been cover cropped. Interviews with farmers, informed by carbon maps developed from the data, suggested that the sampling was not directed enough, nor was the density high enough to detect changes in carbon resulting from highly localized regenerative practices, especially given the inherent heterogeneity of soil, compounded by differing land use histories. More research on other factors influencing soil is crucial for future carbon studies to better understand the environmental benefits of regenerative agriculture.

Our data provide a baseline for continued research on small farms which has been enhanced through our use of the community-based participatory approach.

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Introduction

Today, industrial farming is a leading cause of climate change and soil depletion. The agricultural sector makes up 10 to 20% of the United States' total greenhouse gas emissions and 75% of the N_2O emissions [1]. The majority of CO_2 emissions result from deforestation and the use of fossil fuels in operating machinery [2]. These emissions have increased by 7% since 1990 [1]. Not only is this system contributing to the climate crisis, but fertilizer use and tilling practices also threaten soil health and increase erosion [3]. Every decade, we are losing over two centimeters of topsoil which equates to a pickup truck full of dirt for every family in the country [4]. Erosion leads to organic soil carbon loss which is essential to supporting the soil food web, threatening soil function, structure and productivity. This can result in reduced water retention which exacerbates fertilizer runoff and water pollution [5]. A loss of soil productivity contributes to crop failure and food insecurity. As such, the industrial agriculture sector has serious environmental, economic, and health consequences. This underscores the importance of finding ways to sustainably improve our agricultural system to reduce greenhouse gas emissions and restore soil health.

Regenerative agriculture (RA) offers to both offset climate change and regenerate soil [6]. The scientific definition of regenerative agriculture varies with such individualized practices, but the management primarily aims to rebuild soil organic matter based on five principles: (1) minimizing soil disturbance, (2) keeping the soil covered year-round, (3) keeping live plants and roots in the soil for as long as possible, (4) incorporating biodiversity, and (5) integrating animals [7]. These practices are not just sustainable, but regenerative because they help protect and sequester soil carbon [8]. In doing so, atmospheric CO_2 is also removed from the atmosphere and sequestered in the soil, thus helping to mitigate greenhouse gas emissions.

Keeping the soil covered year-round through cover cropping and the integration of animals through rotational grazing are two regenerative practices that are relatively well-known and more widely applied by small farmers. Rotational grazing is the frequent rotation of enclosed livestock throughout a pasture. Conventional grazing typically results in overgrazing and soil degradation because livestock are allowed to roam freely and graze in one preferred area or on a preferred forage [9]. During rotational grazing, livestock are moved frequently to small paddocks, ensuring that no amount of soil is overgrazed or under grazed. Rotational grazing applies the disturbance ecology theory, where periodic disturbance through grazing keeps the ecosystem healthy and increases species diversity [5, 10]. Grazing stimulates greater root growth and encourages nutrient cycling in grass biomass and manure. Rotational grazing maintains photosynthetic activity and hydrological function, while also giving the pasture time to rest. Because no one forage species is overgrazed, it helps maintain a more biodiverse ecosystem [11, 12].

Cover cropping is another practice that improves soil health, as crops are grown in-between plantings to keep the soil "covered" at all times. They prevent erosion by providing a protective cover to the soil and reducing raindrop impact and runoff [13]. Along with erosion prevention, the additional above and below-ground carbon inputs improve soil health and regenerate carbon [13]. Cover crops generally contain legume species that fix atmospheric nitrogen and/or grasses that can improve water absorption, raise microbial diversity and suppress undesirable weeds [14]. Both rotational grazing and cover cropping have been shown to maximize soil carbon through increased photosynthetic production of organic matter that both protect and sequester carbon [9, 15, 16] . Soil carbon is essential for supporting healthy soil food webs that maintain nutrient cycling, soil fertility, and overall soil health.

Along with these environmental benefits, RA practices also have socioeconomic benefits. Increased soil organic matter and soil water retention increases productivity and crop yields [17]. RA also requires less costly fertilizer input, and it creates an ecological soil environment that can sustain itself over time by supporting healthy soil food webs [17]. Through maximized yield and self-sustaining soil, RA has the ability to improve financial stability for farmers, particularly small rural farmers [17]. RA can provide additional sources of diversified revenue when multispecies animals and crops are integrated, improving financial resilience too [18]. More consistent, diversified yields also ensure stabilized food security for local consumers.

While RA offers promising potential environmental and climate solutions, its application and efficacy can vary greatly in different contexts. Because it affects complex ecosystem processes, the impacts on soil carbon sequestration and other ecosystem services can be difficult to quantify [6]. This is particularly true on small farms, where the implementation of RA practices tends to be localized and individualized to the farm context, which includes farmers' management objectives and access to resources. More research is needed to understand the impact of the application of RA in the context of small farms, especially on soil carbon sequestration.

RA is gaining traction in farming sectors and governments, but it is still understudied. In particular, small farmers who employ regenerative techniques need experimental data to more effectively implement regenerative practices . In this study, we aimed to quantify the impacts of RA rotational grazing and cover cropping on soil organic carbon on seven small farms on and around the Cumberland Plateau in Southeastern Tennessee. An important objective of the study was to support local farmers by carrying out research that directly benefited them. Working with the Southeast Tennessee Young Farmers' (SeTNYF) Coalition, we employed a community-based

participatory approach that allowed farmers to inform and lead research based on their needs and interests. Our participatory approach helped us fully consider the unique context, land use history and practices of each farm. We carried out this pilot study with the intention of using our findings to guide future studies in small communities. We studied farms employing either cover cropping or rotational grazing. We hypothesized that these regenerative practices would increase soil carbon compared to adjacent non-regeneratively managed lands.

Methods and Materials

Study sites.

The study took place on seven smallholder farms in southeastern Tennessee and northern Georgia (Figure 1; Table 1). The farms were located both on top of the southeast Cumberland Plateau (upland) and in the valleys at the base of the plateau (cove) (Figure 2). The upland and cove locations on the Cumberland Plateau differ in soil parent material, moisture and light availability due to their different geological strata and topographic positions. Derived from Pennsylvanian and Warren Point sandstone, upland soils are acidic, well drained and naturally support a mix of drought resistant oak-hickory-pine forests. The finer grained soils in mesic coves are weathered from limestone and have a higher pH and base cation status [19, 20]. In this study, four farms were located on upland sites, and the other three were in the cove (Table 1).

Community-based participatory research approach.

We used a community-based participatory research design (CBPR), a model traditionally used in agroecology [21] and public health research [22] (Figure 3). CBPR models create a collaborative bridge between academic scientists and community participants in research that prioritizes the needs of the community [23]. The initial research objective was developed in response to farmers' interest in knowing how their regenerative practices affected soil carbon. We established a partnership with local farmers through the Southeast Tennessee Young Farmers' (SeTNYF) coalition, a regional organization that supports innovative and sustainable farming and small family farms. SeTNYF facilitates communication among local farmers and community organizations, and supports the generation and dissemination of sustainable food system-related education. The coalition president identified seven farmers using regenerative practices that were

interested in volunteering for the study. We consulted with farmers at each step of the research process to ensure that their interests and concerns were integrated into the study (Figure 3).

We collaborated with researchers from Skidmore College's The Soil Inventory Project (TSIP) and Caney Fork Farms in Carthage, Tennessee. TSIP's mission is to "connect people to soil carbon data" by building a continental scale inventory that provides actionable data for farmers and other agricultural innovators [24]. We used TSIP's sampling protocol so that the data from the farms in this study could be added to the database. Caney Fork Farms researches and applies carbon farming practices to produce affordable food, demonstrating the benefits that regenerative organic farming practices offer Tennessee farmers [25]. Both Caney Fork Farms and TSIP scientists assisted with the sampling design and methods. SeTNYF facilitated communication between the research team and farmers throughout the project.

Initial farm characterization.

To better inform our soil sampling strategy in the context of each farm, we developed a survey that asked open-ended questions about years of management, land use history, and current regenerative management practices (Table 1). To integrate the needs of farmers in the study design, the survey also asked farmers what they hoped to gain from participating. The survey was sent to farmers using the platform Google Forms [26] prior to visiting their farm. To visualize the impact of past and current practices, we met with farmers and walked their land with them prior to sampling. This allowed farmers to share information about their practices and identify specific locations of management.

Farm site selection and sampling protocol.

Farm sites were identified based on the availability of volunteers. Small farms in the southern Tennessee and northern Georgia region vary greatly in size, with the smallest farm we

sampled being less than 0.5 hectares and the largest being 140 hectares. Farmers in this study employed two main regenerative practices: cover cropping in growing fields or rotational grazing in pasture with livestock such as cattle, alpacas, goats, and sheep. Chickens were also often moved throughout the pastures.

Sample points on each farm were located both on regeneratively managed land (cover cropping or rotational grazing) and unmanaged or conventionally-managed parcels that were located in close proximity to the areas under regenerative management (adjacent unmanaged). The sampling locations were chosen remotely by TSIP on regenerative and adjacent unmanaged sites at a density of approximately 3 points per acre using random Global Stratified Sampling software. This software uses an algorithm considering both vegetative productivity and topography/slope/aspect data for sampling [27]. The point coordinates were loaded into ArcGIS (ArcGIS Desktop: Version 3.1.2) and Field Maps (ArcGIS Enterprise 10.8.1).

In the field, we navigated to each sampling point using the ArcGIS Field Maps Application. We also used this application to record information about the site and the regenerative practices used at each sampling location. We took photographs of the point location for reference.

To collect soil samples at depths of 0-15 and 15-30 cm, we used a soil drill developed by TSIP. This method uses an electric drill to power a bit (2-cm diameter, 15-cm length) into the ground to collect the loosened soil in a metal box attached to the bit. The number of samples collected per farm varied according to farm size. Across all farms, we collected soil at both depths at 203 points, for a total of 406 samples.

The samples collected using the drill were air dried in paper bags and sent to Ward Lab (Kearney NE, USA) analyzed for total organic carbon (%) using Loss on Ignition (LOR). To

calculate soil carbon content (mg C/kg soil) we measured bulk density (0-15 cm) at each sampling point. Variation in soil bulk density alters the mass of carbon in soil. Soil carbon content (Mg C/ha), calculated as the product of % carbon x bulk density (g soil/cm^3) at 0-15 cm depth, provides a more accurate measurement of soil carbon mass value across different soil types. To calculate bulk density, samples were extracted using an AMS (American Falls, ID, USA) 2 x 6" split core sampler placed 15 cm into the ground adjacent to the drill samples. These samples were collected at two or three geospatially-located points per acre on each farm. Bulk density samples were collected and transported to the lab in labeled brown paper bags. The soil bulk density samples were dried in a forced air oven at 60 degrees C until they reached a constant mass. After recording the final weight for bulk density calculation, these soils were sieved through a 2-mm mesh, and mailed to the University of Georgia's Agriculture and Environmental Services (AES) Laboratory (Athens, GA, USA) for routine soil fertility analysis, including pH, cation exchange capacity, Melich III extractable P, Ca, Mg and K. At the UGA lab, C and N were also analyzed using Carlo Erba mass spectroscopy to measure total soil C (mg/kg soil) and nitrogen (N mg/kg).

Statistical analyses

The rotational grazing data were analyzed statistically using a General Linear Model (GLM) analysis of variance (ANOVA) in R (R core team 2021) to determine the effects of treatment (rotational grazing vs adjacent unmanaged), depth, farm, topographic location (upland vs cove) and their interactions on soil organic carbon percentage [28]. We removed organic carbon percentages above 15 due to likely historical landscape influences and transformed the percent carbon (arcsine(sqrt(%carbon/100)) to normalize the residuals. We also removed non-significant interactions, and plotted the data using R.

The cover cropping data were analyzed statistically using a General Linear Model (GLM) three-way analysis of variance (ANOVA) to determine the effects of treatment cover cropping and adjacent unmanaged), depth, farm, topographic location (upland and cove) and their interactions on soil organic carbon percentage. We used a paired t-test to compare carbon content (Mg C/ha) on control plots and cover cropped plots, since depth was not a variable factor.

GIS mapping.

Responding to the farmers' desire to see the results visually across their fields, the soil carbon data were used to create maps that demonstrated the % carbon at each sampling point. Interpolation methods (IDW) were used to depict an estimation spread of %C across the sampling areas on each farm. Equal Area Intervals were used to keep data breaks consistent and focus on carbon hot spots [29]. We scheduled Zoom meetings with each farmer to share and discuss the maps, and solicited their insights, predictions, and questions about the initial data.

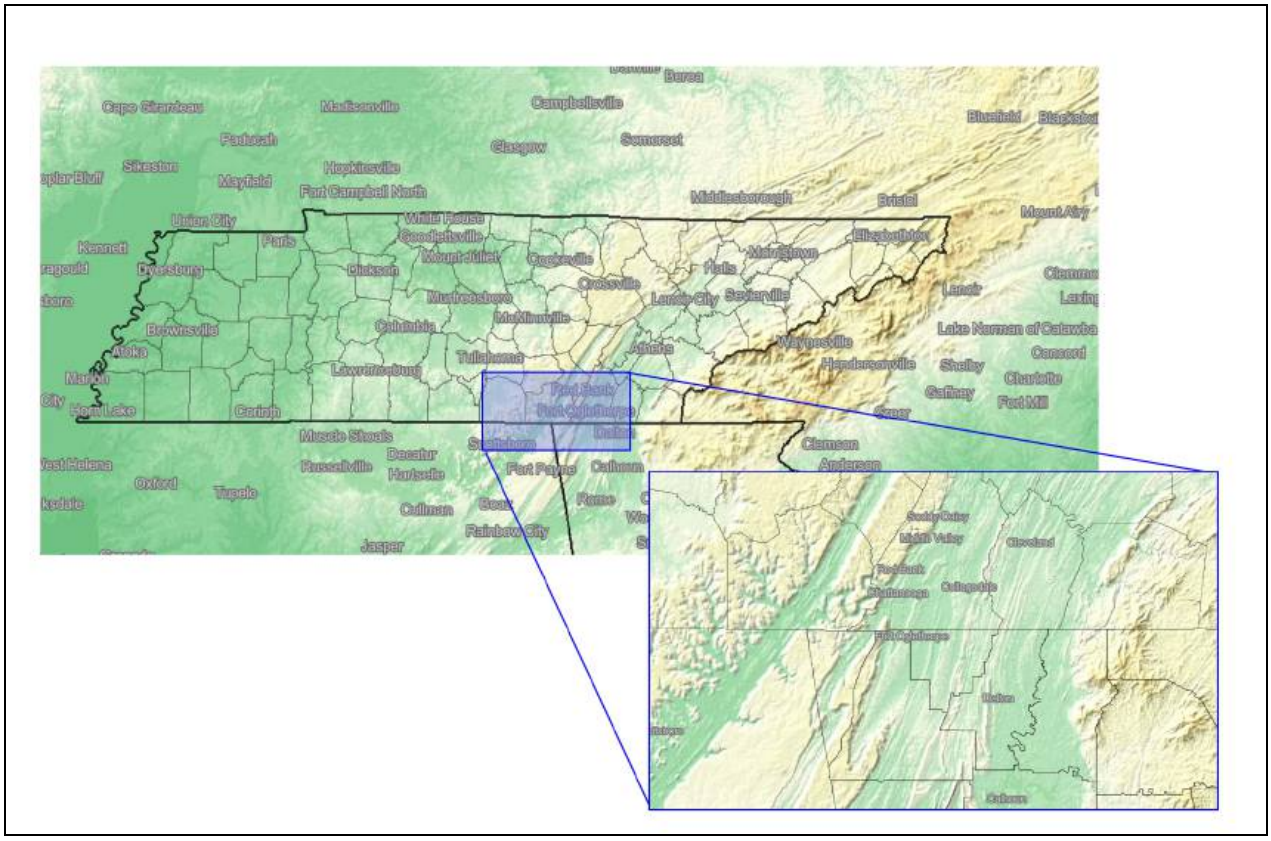

Figure 1. The seven study sites were located around the Cumberland Plateau in southeastern Tennessee and northern Georgia, U.S.A.

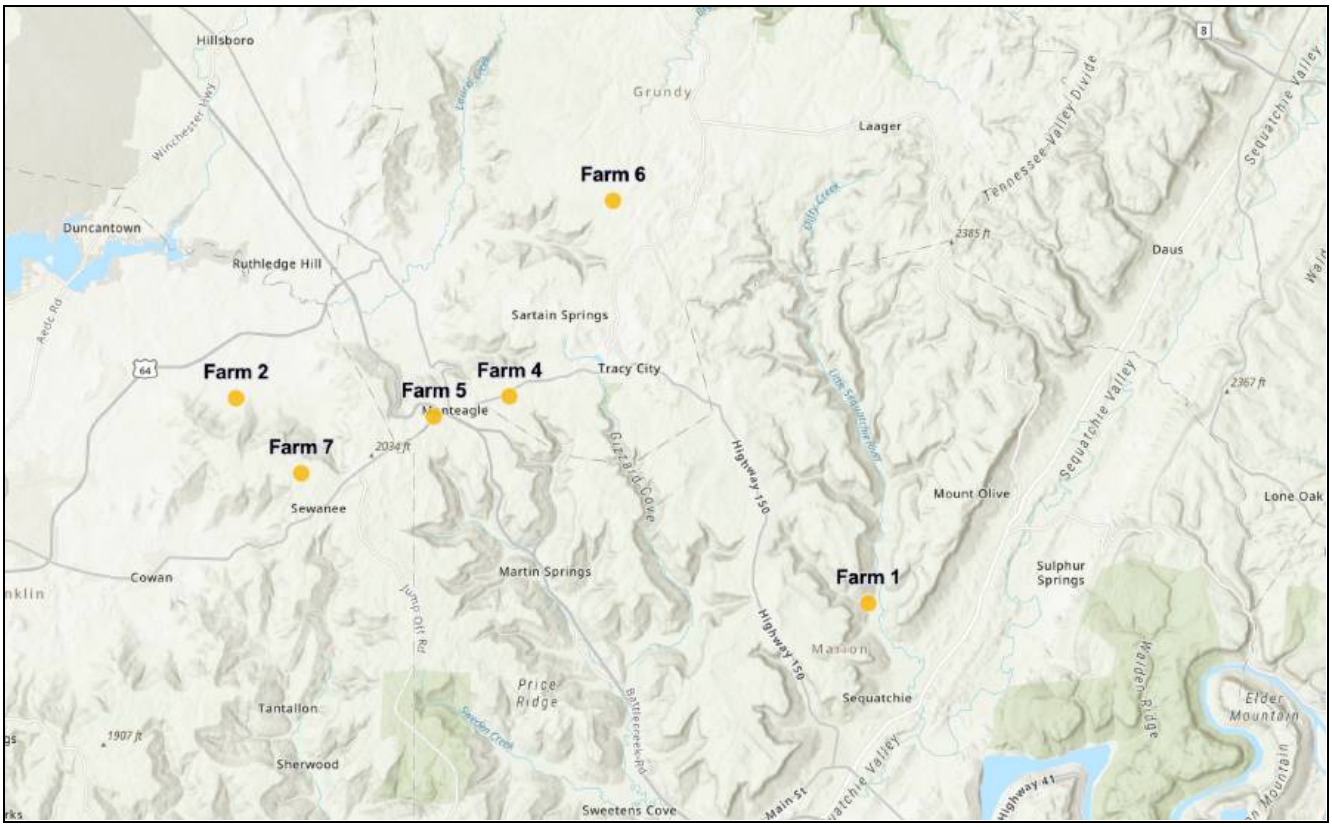

Figure 2. Zoomed in map of six farm sites located throughout the southeastern Cumberland Plateau in Southeastern Tennessee, U.S.A. Farm 3 in Northern Georgia is not visible on this map.

Table 1. The 7 farms participating in the pilot study were located on and around the Southeastern Cumberland Plateau in Tennessee and in northern Georgia on both cove and upland sites. The soil was sampled at two depths (0-15 and 15-30 cm) and at a minimum density of 7 samples per hectare, although smaller farms were sampled at higher densities. Number of sample replicates indicates samples taken at one depth. A total of 406 samples were collected. Farm sites were volunteered by farmers in the Southeastern TN Young Farmers (SeTNYF) Coalition who use regenerative practices.

Cove Farms				
Farm No	Location	Practice	Years under practice	Managed sample replicates (n) / Unmanaged replicates(n)
1	Sequatchie, TN	Rotational grazing	25	29/21
2	Alto, TN	Rotational grazing	12	17/9
3	Rock Spring, GA	Cover Cropping and Rotational Grazing	< 1 year	11/11

Upland Farms				
Farm No	Location	Practice	Years under practice	Managed sample replicates (n) / Unmanaged replicates (n)
4	Monteagle, TN	Rotational grazing	10	10/10
5	Monteagle, TN	Rotational grazing	4	25/17
6	Coalmont, TN	Rotational grazing	8	20/9
7	Sewanee, TN	Cover cropping	3	10/5

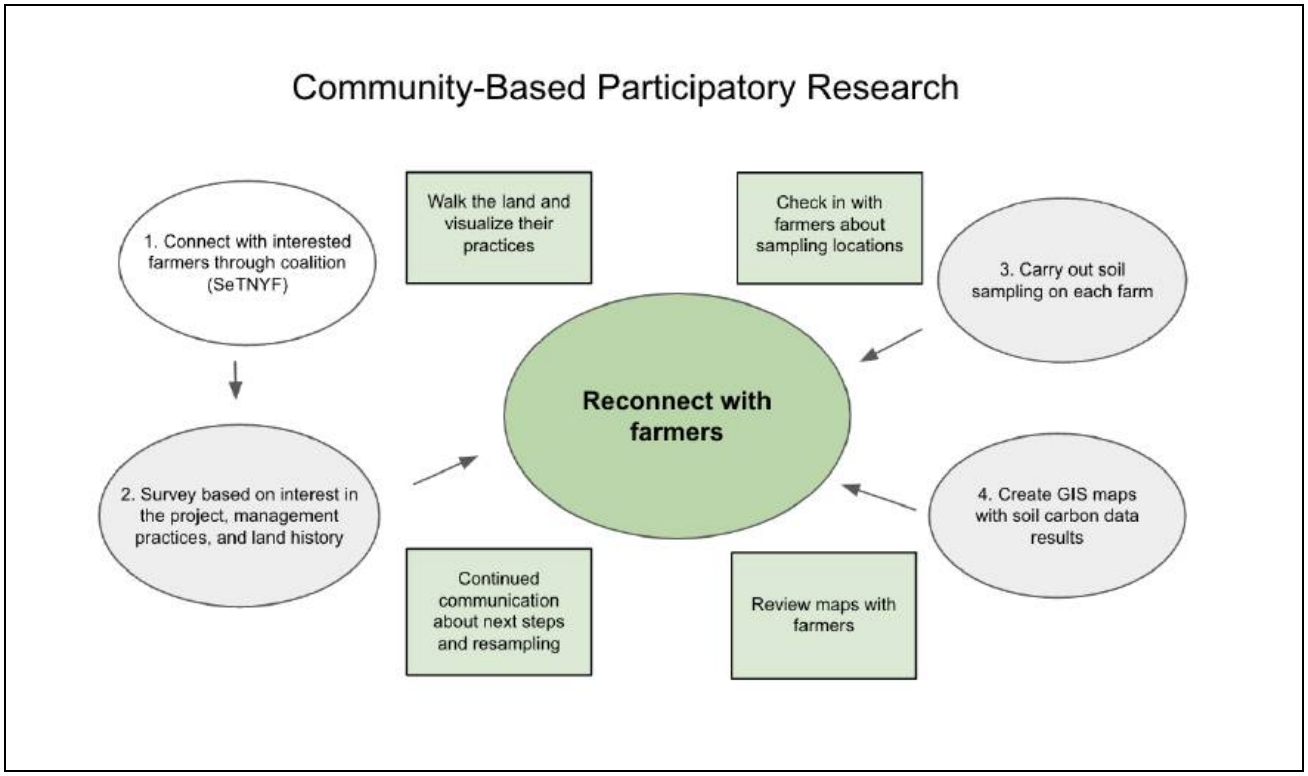

Figure 3. Community-based participatory research model that shows the four initial steps (gray/white) in the participatory process, each of which provided points of contact for consultation with farmers (green).

Results

Soil organic carbon percentage

A total of 406 soil samples were statistically analyzed for organic soil carbon percentage (%) at depths of 0-15 cm and 15-30 cm (Figure 4; Figure 5).The average organic soil carbon percentage varied across different rotational grazing farms (Table 2). There were no statistically significant differences between rotational grazing treatments and non-regenerative adjacent land ($p > 0.05$). Our analysis of variance found significantly less carbon at lower depths of 15-30 cm and in the cove environment for rotationally grazed farms (Table 5).

The two farms employing cover cropping showed differences in organic soil carbon percentage (%) that depended on depth and farm (Figure 5). On the cove farm, cover cropping decreased soil carbon and on the upland farm cover cropping increased soil carbon, but only at depths of shallower depths of 0-15 cm (Table 3).

Soil carbon content

204 soil samples were statistically analyzed for soil carbon content at 0-15 cm (MG/Ha) (Figure 6; Figure 7). The average soil carbon content varied across different farms (Table 4; Table 5).There was no statistically significant difference between rotational grazing treatments and non-regenerative adjacent land ($p > 0.05$).

Mean soil carbon content values for cover crop managed land ranged from 28.74 (± 1.99) to 91.25 (± 9.05) across two farms (Table 5). Mean soil carbon content values for unmanaged land on cover cropped farms ranged from 40.12 (± 3.82) to 63.42 (± 1.66) across two farms (Table 5). Only two farms, both employing cover crops, showed significant differences in managed and unmanaged land soil carbon content (Figure 7). The cove farm showed significantly higher organic carbon on unmanaged land ($p = 0.02$; Table 5). The upland farm showed significantly higher organic carbon on managed cover cropped land ($p = 0.01$; Table 5).

Sharing results using carbon maps

Carbon layer maps allowed farmers to visualize the distribution of carbon among sampling sites (Appendix 1-7). We held meetings with the farmers where conversations focused on areas of high or low soil carbon on the maps, and farmers were able to make hypotheses about specific management practices, such as frequent animal congregation, hay feeding, or changes in land elevation. These conversations were critical in our collaborative research process and significantly informed the next steps of the project.

Table 2. A comparison of mean (±one std err) percent soil carbon (%) at two depths (0-15 and 15-30 cm) between land that is rotationally grazed and adjacent unmanaged parcels on farms located on top (upland) or in the coves of the Southeastern Cumberland Plateau, USA.

Depth (cm)	Upland (n = 3 farms*)		Depth (cm)	Cove (n = 3 farms*)	
	Rotational grazing	Adjacent unmanaged		Rotational grazing	Adjacent unmanaged
0-15	3.10 ± 0.16	3.80 ± 0.32	0-15	2.23 ± 0.09	2.28 ± 0.102
15-30	1.69 ± 0.20	2.29 ± 0.32	15-30	0.996 ± 0.06	1.10 ± 0.06

*Multiple soil sample replicates were collected from each farm, the number of which was based on the size of the farm. Therefore the total number of samples per farm varies. An equal number of soil replicates were collected from both 0-15 and 15-30 cm depths.

Table 3. A three-way analysis of variance (ANOVA) summary comparing effects of cover cropping, depth, and farm at two depths (0-15 cm and 15-30 cm) and two locations (cove and upland) on percent soil carbon (%).

	Sum Sq.	Df	F Value	ANOVA p-value
Treatment	0.002069	1	2.0639	0.1561965
Depth	0.007478	1	7.4608	0.0083406
Farm	0.001071	1	1.0689	0.3054906
Treatment:Depth	0.000980	1	0.9775	0.3269264
Treatment:Farm	0.012481	1	12.4516	0.0008247
Depth:Farm	0.000028	1	0.0277	0.8684783
Treatment:Depth:Farm	0.006598		6.5827	0.0129046
Residuals	0.058137	58		

*There was only one farm per Plateau location for cover cropping. The total number of samples per farm varies, due to differing farm size. An equal number of soil replicates were collected from both 0-15 and 15-30 cm depths.

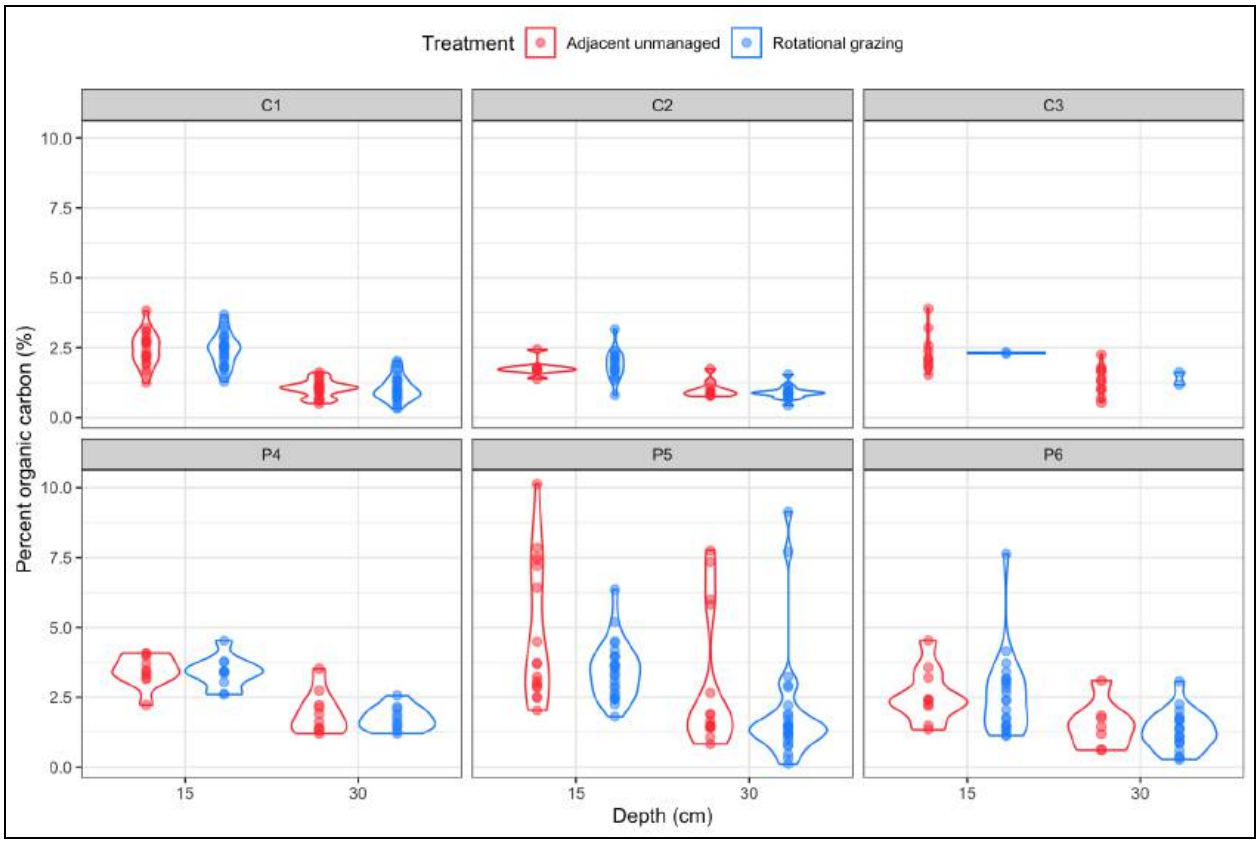

Figure 4. Violin plot of differences in percent soil carbon (%) across six farms at different locations (Cove=C, Upland=P) and corresponding treatments rotational grazing (blue) and adjacent unmanaged land (red) at depths of 0-15 cm and 15-30 cm.

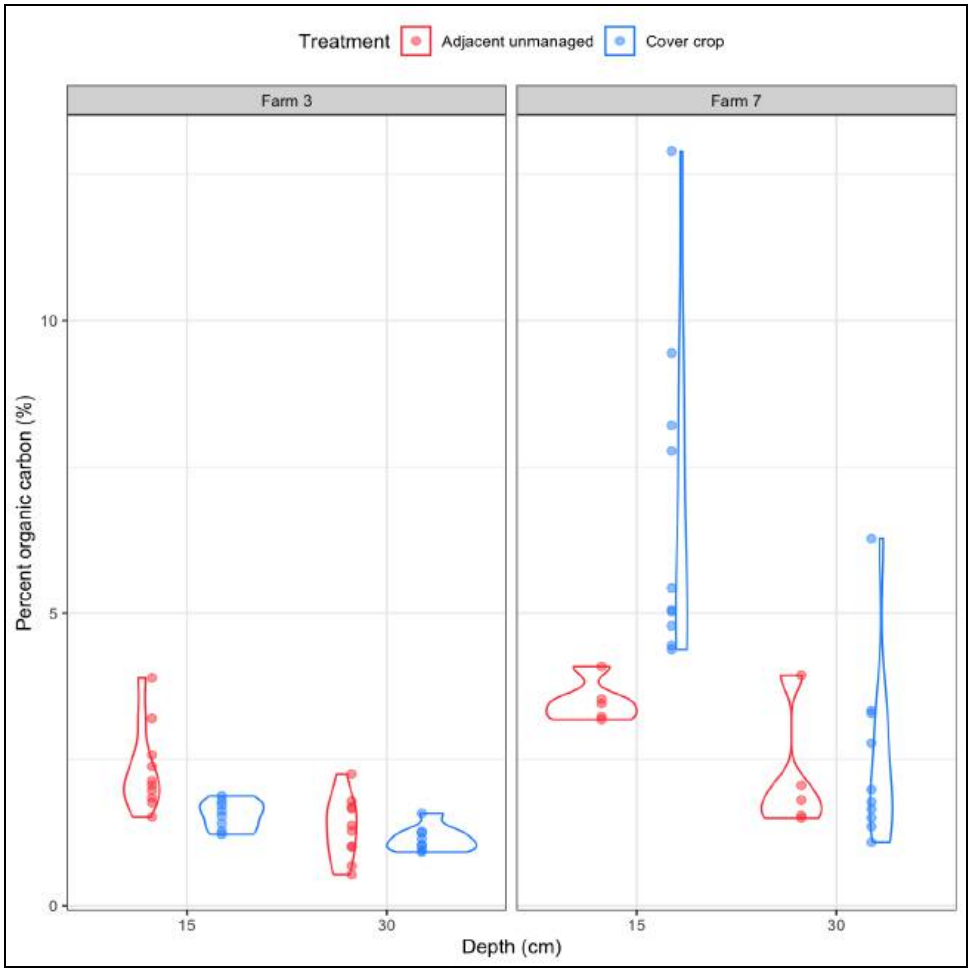

Figure 5. Violin plot of differences in percent soil carbon (%) across two at different locations and corresponding treatments cover cropping (blue) and adjacent unmanaged land (red) at depths of 0-15 cm and 15-30 cm.

Table 4 . A comparison of mean (± se) soil carbon content (Mg/Ha) at 0-15 cm between land that is rotationally grazed and adjacent unmanaged on farms located on top (upland) or in the coves of the Southeastern Cumberland Plateau.

Upland (n = 3 farms)*		Cove (n = 3 farms)	
Rotationally grazed	Adjacent unmanaged	Rotationally grazed	Adjacent unmanaged
57.9 ± 2.90	67.9 ± 5.08	41.3 ± 1.55	41.8 ± 1.82

*Multiple soil sample replicates were collected from each farm, the number of which was based upon the size of the farm. Therefore the total number of samples per farm varies.

Table 5. A comparison of mean (± se) soil carbon content (Mg/Ha) at 0-15 cm between land that is cover cropped on two farms, one located on top (upland) and one in the cove of the Southeastern Cumberland Plateau.

Upland (n = 15 soil replicates)*			Cove (n = 20 replicates)		
Cover cropped	Adjacent unmanaged	t-test p-value	Cover cropped	Adjacent unmanaged	t-test p-value
91.25±9.05	63.42±1.66	0.01	28.74±1.99	40.12±3.82	0.02

*There was only one farm per Plateau location for cover cropping. The total number of samples per farm varies, due to differing farm size.

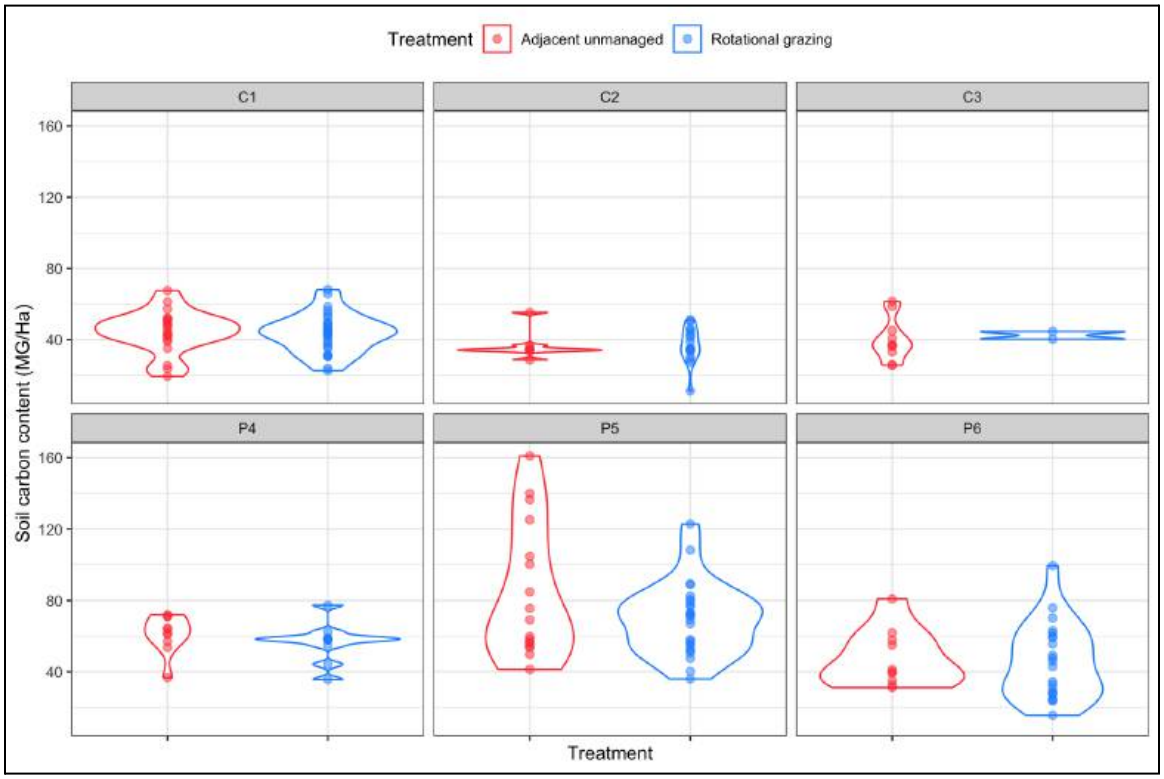

Figure 6. Violin plot demonstrating the distribution of soil carbon content (Mg/Ha) across six farms at different locations (Cove=C, Upland=P) and corresponding treatments rotational grazing (blue) and adjacent unmanaged land (red) at depths of 0-15 cm.

Table 5. An analysis of variance (ANOVA) summary comparing effects of rotational grazing treatment, depth, and location at two depths (0-15 cm and 15-30 cm), two locations (cove and upland) on percent soil carbon (%).

	Sum Sq.	Df	F Value	ANOVA p-value
Treatment	0.00029	2	0.2501	0.61731
Depth	0.21778	1	189.1642	2.2e-16
Location	0.06163	1	53.5331	1.735e-12
Treatment:Location	0.00594	1	5.1551	0.02378
Residuals	0.40526	352		

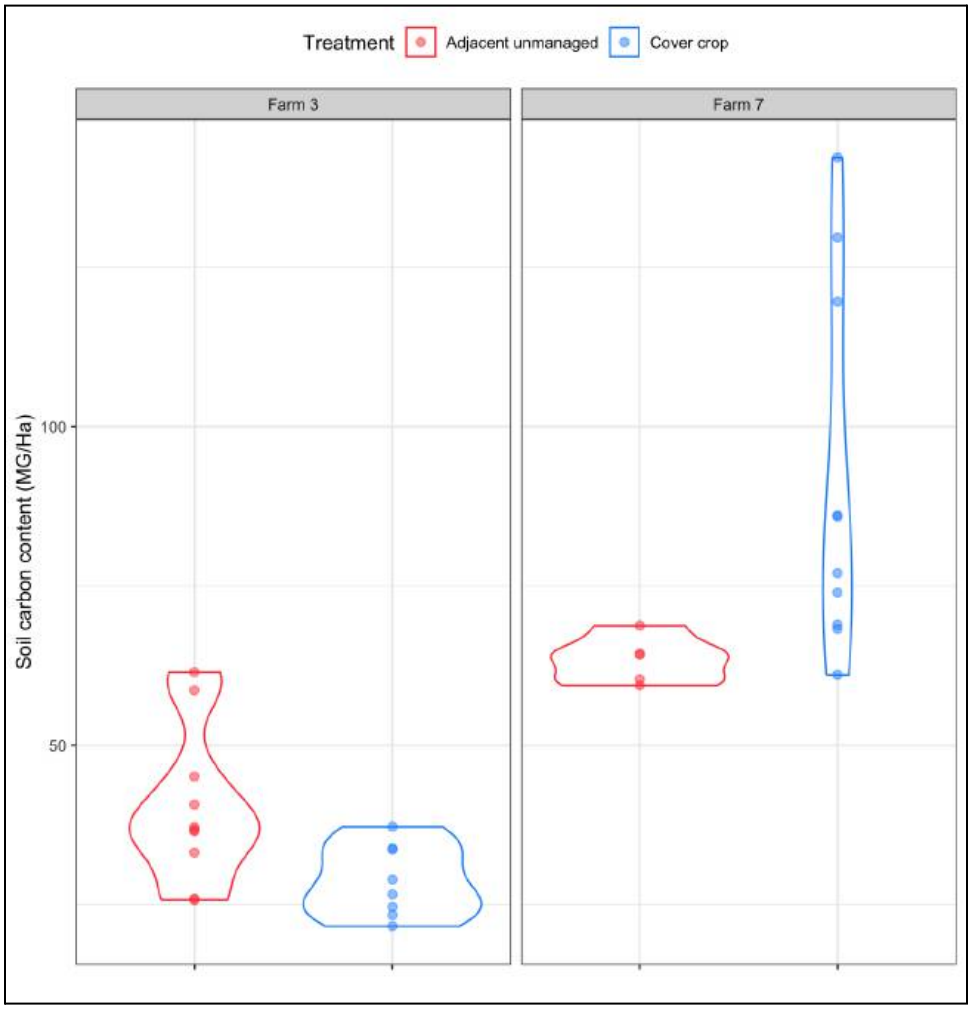

Figure 7. Violin plot demonstrating the distribution of soil carbon content (Mg/Ha) across two farms and corresponding treatments cover cropping (blue) and adjacent unmanaged land (red) at depths of 0-15 cm.

Discussion

Effects of management on soil carbon.

In this study, we hypothesized that soil carbon would be higher on parcels where the regenerative practices of rotational grazing and cover cropping were employed compared to adjacent lands where no regenerative management occurred. Previous studies have demonstrated this effect [9, 15, 16]. Adjacent "unmanaged" land varied farm-to-farm (Table 6), but this "control" comparison mostly included soil covered by grasses, forbes, and shrubs that were not rotationally grazed or cover cropped. Some adjacent land samples on Farms 3, 5, and 7 included soil sampled from lawns beneath grass that was mowed. Other adjacent areas on Farms 1 and 2 were conventionally grazed, that is, cows were allowed to remain in the same large parcel over extended periods of time. Overall, our analysis showed no significant difference in percent organic soil carbon (%) or soil carbon content (MG/Ha) between land under rotational grazing and adjacent land. We did find significant differences in percent organic carbon and soil carbon content between cover cropping and adjacent unmanaged land at 0-15 cm depth, but not at 15-30 cm depth, and this effect depends on both treatment, farm, and depth. Lower carbon concentrations at 15-30 cm are expected due to reduced biological activity and root growth, and more time is needed at this lower depth to see a change from regenerative management [30]. Our averages for percent organic soil carbon (%) range from 0.996 ± 0.06 to 3.80 ± 0.32, and the West Australian government cites similar numbers for agricultural dryland (0.7-4%) and even higher for intensive dairy soils (14%) [31].

Although our study was a pilot with seven farm sites, we still expected to see an impact of regenerative management on the soil, because most of the farmers had been practicing rotational grazing for at least five years [9, 16]. One farm in particular had practiced "mob

grazing" for nearly a decade in which a herd of animals of different species and ages (cows, sheep and alpacas) were moved through small parcels every day. This labor intensive practice forces the animals to consume all species of forage, not just those that are preferred, which increases biodiversity by reducing the dominance of one plant. The grazing animals are then moved to another paddock, giving the grazed paddocks months to recover to minimize over-and-selective grazing. Another benefit of confining animals to small parcels for short periods of time is that the manure is more evenly distributed across each parcel. Other farms moved their cattle frequently and also ran mobile chicken coops over the paddocks to further enrich the manure of chickens, who consumed insects on these sites. As such, these regenerative rotational grazing practices would be expected to maximize grass net primary productivity (NPP) because each parcel has time to regrow after being grazed and fertilized with manure. Grazing stimulates root growth, and higher above-and below-ground NPP can lead to greater organic matter accumulation below ground, leading to higher soil carbon as demonstrated in other studies[9, 16]. However, results vary greatly among studies because regenerative agriculture practices tend to be unique to the farmer and location, presenting a challenge to quantify carbon impact on a large scale. In this study, we suspect three other factors may have had a more pronounced influence on baseline soil carbon: the soil-forming factors of parent material, time, and organisms (i.e. vegetation, animals, and microorganisms) which is controlled by land management (Figure 8) [32]. Many other studies with variable results cite the effects of (i) parent material [11, 33, 34, 35], (ii) climate [33, 35, 36], (iii) topography [37, 38], (iv) time [5, 11, 39], and (v) organisms [10, 40, 41, 42, 43, 44, 45]. We briefly explore the influence of these factors in consideration of our community-based participatory approach and choice of sampling density.

Parent material and plateau location.

The geologic parent material from which soil is generated determines its chemical and physical properties [46]. It takes time for factors such as vegetation cover to override the fundamental geochemical characteristics of soil determined by parent material. Our farms were located on the upland and valley/cove regions of the Cumberland Plateau. The parent material differs between these two Plateau locations, with upland soils derived from sandstone strata and cove soils weathered primarily from limestone. These differences in geology affect soil pH, particle size and moisture, which may, in turn, have an impact on NPP and soil organic matter production and carbon accumulation. We found significant effects of plateau location on soil carbon with the two highest concentrations of organic carbon found on the upland sites of Farm 5 and Farm 7. This was unexpected because some studies found that sandy loam soils show less change in carbon [11, 34]. Bonin et al. (2014) also stated that clay soils are expected to store more carbon. In general, limestone (calcium carbonate) derived cove soils have a higher percentage of clay and inorganic carbon, with the latter being a large but under-appreciated source of soil carbon that is vulnerable to loss through soil acidification [33, 49]. Yost and Hartemink (2019) found that organic carbon was high in forest and grassland in sandy environments similar to our upland regions, but low under shrub or agriculture [35]. We found that carbon was higher in sandy upland soils, regardless of management practices in adjacent areas.

Climate can also influence baseline soil carbon. Temperature and precipitation affect soil moisture and determine vegetation types that influence carbon inputs [36]. In general, the upland and cove environments produce different microclimates, with the cove and valley soils retaining greater moisture from the run-off they receive from the sandy upland sites. However, with one

seasonal sampling, we cannot infer the impact of microclimate on net primary productivity and carbon sequestration. It is also challenging to separate the effects of climate from topography, as topography can create microclimates. Still, it was unexpected to find more carbon at the higher elevation upland locations, and it's unlikely that topography is not the direct cause of these findings.

Time.

Many regenerative agriculture studies sample and compare soil carbon repeatedly over time. It takes time for the signal of soil carbon accumulation to be distinguishable from the heterogeneity caused by other factors, such as previous land use [5]. Although the amount of time varies, it can take up to 8 years of repeated sampling, or even longer in drier environments [5, 11] to detect the influence of management practice on soil carbon. In this pilot study, we researched farms that had been managed for various amounts of time, from as little as one year to eight years. We gathered management duration data through surveys and interviews with farmers, as a part of the community-based participatory approach. Our comparison between managed and unmanaged land represents a single snapshot during one season, instead of a comparison of samples collected in the same locations over time. It is possible that some of the farms had not been regeneratively managed long enough to see an increase in soil carbon, and this was particularly notable at lower depths of 15-30 cm where we did not see change. There are also potential interactions between the landscape and time. Sanderman et al. [39] found that mesic rangelands under rotational grazing management in Australia responded more quickly, producing changes in soil carbon, potentially due to shorter recovery times for vegetation. This was in contrast to our findings of higher carbon on the upland sites. Overall, our study

established baseline soil carbon for the seven farms that will be monitored over time through repeated sampling in the future.

Land management (manipulation of organisms).

Soil is directly affected by the organisms that exist within it and live off of it. This can occur at microbiological scale, moving up the food web to invertebrates to fungi, plants, arthropods, and grazing ruminants. The manipulation of organisms can influence the fundamental quality of soil already determined [10, 40, 41, 42, 43, 44, 45]. The seven farms we sampled varied in how they manipulate biota (Table 6). Rotationally grazed farms employed animals such as chickens, heritage breed cattle, sheep, goats, and alpacas, with some farms using multi-species mob grazing. Grazing animals stimulate root growth by clipping above ground plants, and they deposit manure that adds nutrients to the soil. Greater root growth supports bacteria [40] and invertebrate diversity [41] in the soil food web with the addition of organic matter and the ecological disturbance effect [10]. There is little research on the impacts of multispecies grazing, but research suggests that including animals with biodiverse forage preferences may further improve rotational grazing outcomes [42].

Regenerative farmers also control organisms through improved and diverse cover crops that provide ecosystem services, such as nitrogen fixation, pollinator services, improved forage and/or weed and pest suppression. Cover cropping has also been shown to support biodiversity with an increased species richness of bacteria [43], invertebrates [44], and a compositional change in fungal communities [45]. Farm 7 employed cover crops with the addition of organic compost, which we hypothesize may have contributed to the significantly higher organic carbon percentage, particularly at shallower soil depths. As each farmer employed a different use of biota, with individualized grazing practices, it is still difficult to consistently define a specific

regenerative treatment. Previous land history and management may also have had an impact on carbon levels. We collected information on land history and previous management through surveys and interviews with farmers. Farm 5 in particular had a unique history as a conventional dairy farm for over 100 years and then as a municipal waste treatment field prior to regenerative management in pasture. Adjacent unmanaged land points were selected randomly using TSIP's protocol, and some of these points were located near an old railroad track. These samples appeared darker and more gritty in texture, suggesting the presence of coal, a complex mixture of inorganic and organic carbon, transported by a historical railroad when coal was still actively mined in the region. This may explain the overall high carbon content found on Farm 5 in both regenerative lands due to the municipal waste and in the adjacent lands due to the railroad.

Participatory method.

Our use of a community-based participatory approach directed us to consult farmers at every step of the research process. CBPR models excite interest, encourage participation, enable mutual learning, and strengthen community bonding [47]. We began our study by reaching out to a local farmer and president of SeTNYF to develop a survey of interest. This provided important information about land history and management prior to sampling. We also interviewed farmers in person while walking on their land. In the future, we will use farmer input to inform our sampling locations due to the highly localized nature of their implementation of regenerative practices. After data collection, at the request of the farmers, we shared results through maps created using GIS which they could view on an online, interactive website. For privacy protection, this website has stayed restricted to farmer access. This collaborative analysis of results improved our dissemination of findings, helped us solicit farmer insight to interpret and form a better understanding of the landscape history and provided key insights on how to

improve future sampling protocols. Sharing data in an open, accessible and transparent manner built trust that strengthened our partnership and ensured our continued collaboration with the farming community, so that the study can continue into the future.

Sampling density.

Sampling density and location vary across regenerative agriculture studies. Working on small farms and private property can present challenges for sampling controlled applications of treatments. There is a wide range of variability within small, individualized regenerative farms, and it can be difficult to capture this. We used the Global Stratified Sampling protocol that was developed for and by researchers at the Soil Inventory Project (TSIP). The Global Stratified Sampling protocol generates random sampling point locations using software that accounts for variation in slope and aspect. While this randomization followed the TSIP protocol, we believe that sampling at a minimum density of 7 samples per hectare was not high enough in the heterogeneous soil landscape to detect a soil carbon signal from the highly localized regenerative management practices implemented by farmers. This may have diminished our ability to quantify the effects of their management on soil. Our conversations about the location of carbon hotspots in relation to their knowledge of where they implemented regenerative practices suggest that we missed locations in which they had applied regenerative management most intensively. In future samplings, we will consult closely with farmers to sample in a more more directed and intensive manner in parcels where they have most intentionally implemented regenerative practices.

The choice of adjacent land may have had an impact on our results, particularly in unmanaged samples. We consistently selected sampling points for adjacent land within walkable distance of the managed pastures in an attempt to sample the same soil series. Available

unmanaged land varied from farm to farm. For example, on Farms 3, 5, and 6 adjacent land was predominantly covered in dense overgrowth and brush. This overgrowth may have sequestered similar amounts of carbon to regenerative management, potentially due to its longstanding, undisturbed presence on the soil. On Farm 7, some adjacent land resembled a mowed lawn, and Farm 4 had a couple points in a lawn where clippings had been mulched. Byrnes et al. [48] found that rotational grazing can capture more carbon than traditional grazing, but may not show a difference when compared to unmanaged land because of site-specific environmental differences.

Conclusion.

While our rotational grazing soils showed no difference in mean percent carbon (%) compared to adjacent unmanaged land, the cover-cropped Farm 7 had significantly higher soil organic carbon in the top 15 cm. It is interesting to note that this farm had been cover cropped for three years, suggesting that cover cropping has the potential to raise soil carbon quickly. Our conversations with farmers as part of the participatory process suggests that "rotational grazing" practices differ considerably by farm. Indeed, regenerative agriculture is not a monolithic practice, but a matrix of individualized practices that have at the basis certain foundational ideas. As we saw, what farmers call regenerative and rotational grazing, varies farm by farm. Therefore, the research on regenerative agriculture may need to look beyond the simple cause-and-effect model of management impact on soil carbon. There are many nuanced complexities of the local landscape and the personalized practice a farmer chooses to employ, and these complexities must be considered before sampling occurs. In particular, other factors affecting soil may mean that it takes a long time before the signal of regenerative farming on soil carbon becomes detectable. In this inherently heterogeneous environment, the sampling density

must be higher, especially in areas where parent material and previous land history may have strong influences.

Table 6. Description of specific regenerative management, adjacent land, and land use history for each farm.

	Regenerative Management	Adjacent Land	Land Use History
Farm 1	Rotational grazing of cattle, chickens, and sheep	Conventional hay farm	Second generation farm
Farm 2	Flexible rotational grazing of cattle	Conventional cattle farm	N/A
Farm 3	Cover cropping and rotational grazing of sheep; clover cover and mycorrhizal inoculation	Mowed grass and adjacent woodland	Previous conventional hay farm
Farm 4	Rotational grazing of sheep	Mowed grass and adjacent woodland	N/A
Farm 5	Rotational grazing of cattle	Adjacent forage and brush	Previous conventional dairy farm, municipal waste water treatment, and historic railroad
Farm 6	Mob rotational grazing of cattle, alpaca, and goats	Adjacent forage and brush	Previous strip mine
Farm 7	Cover cropping, compost application	Mowed grass	N/A

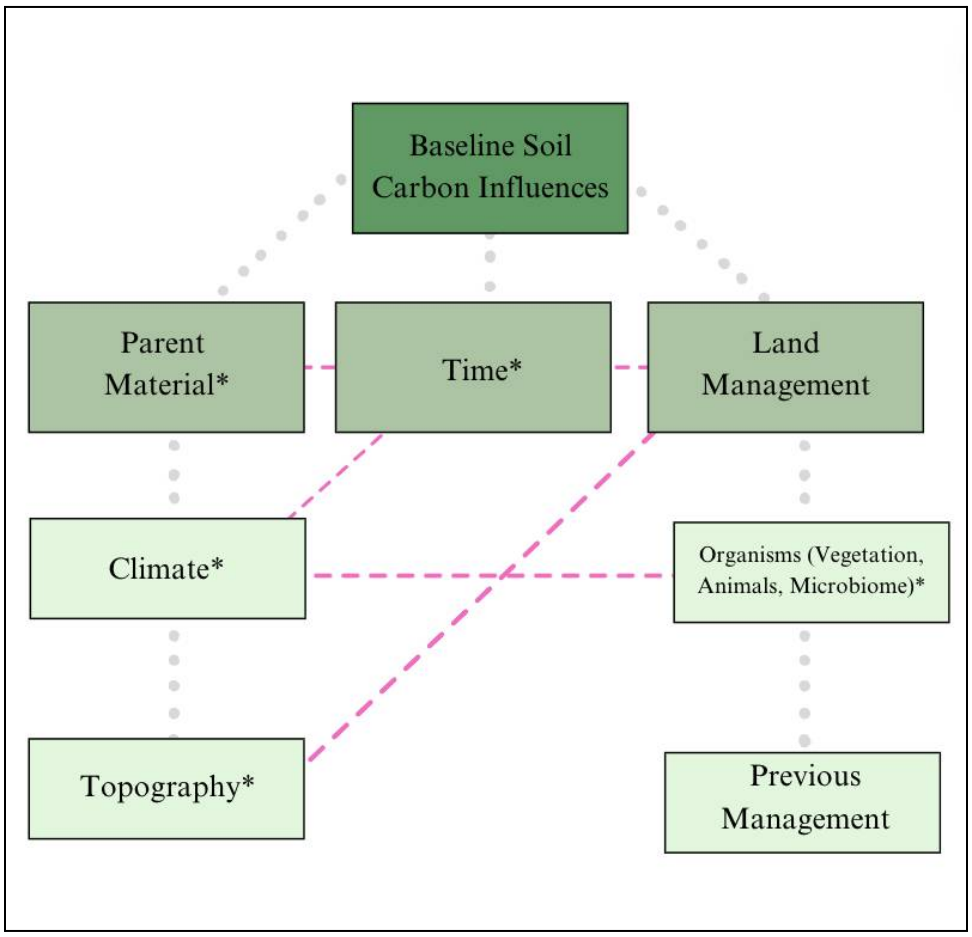

Figure 8. Baseline soil carbon influences and their relation to the five soil-forming factors.* Gray lines represent their relationship between factors in our discussion and pink lines represent biological connections.

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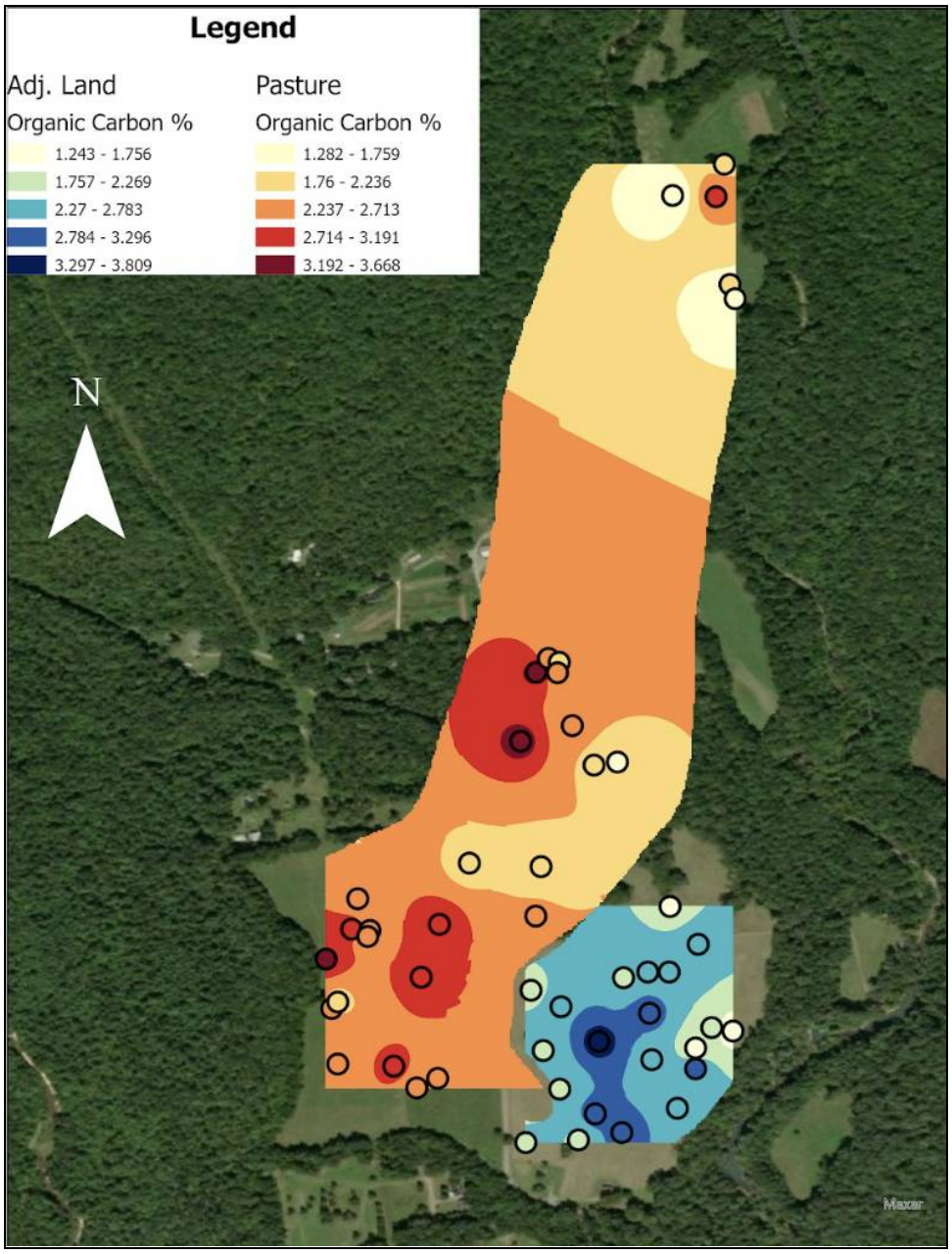
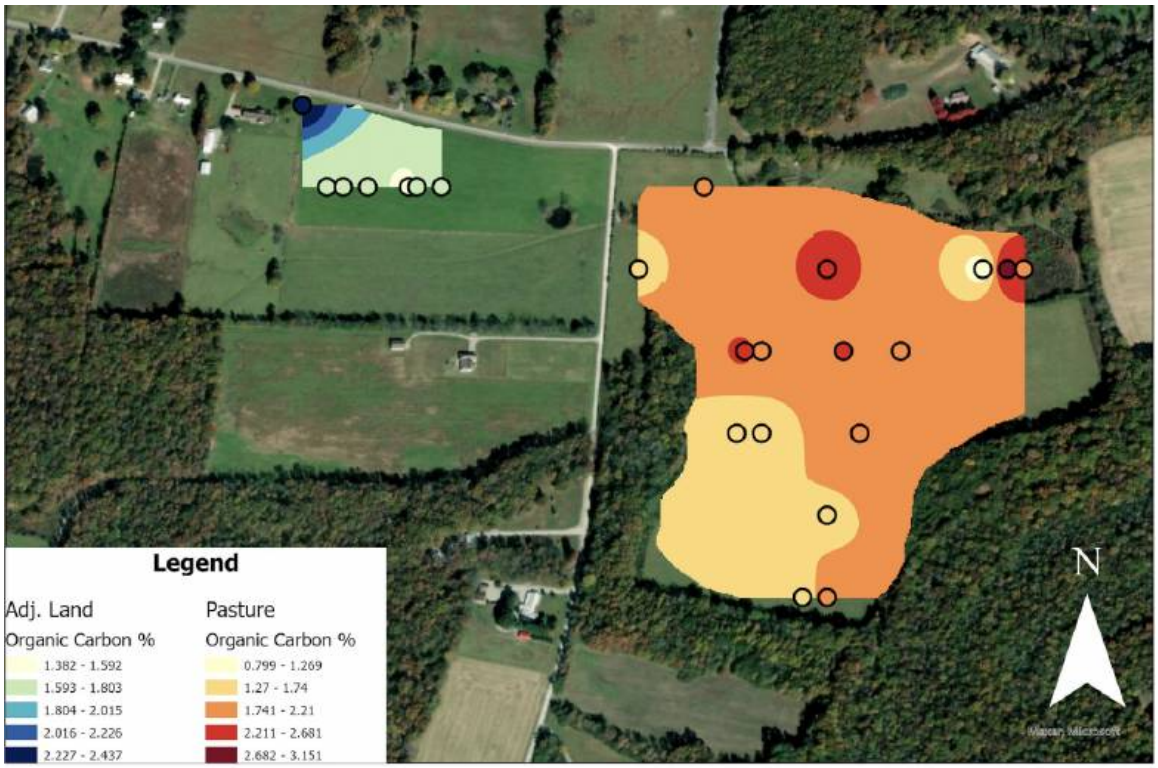
Appendices

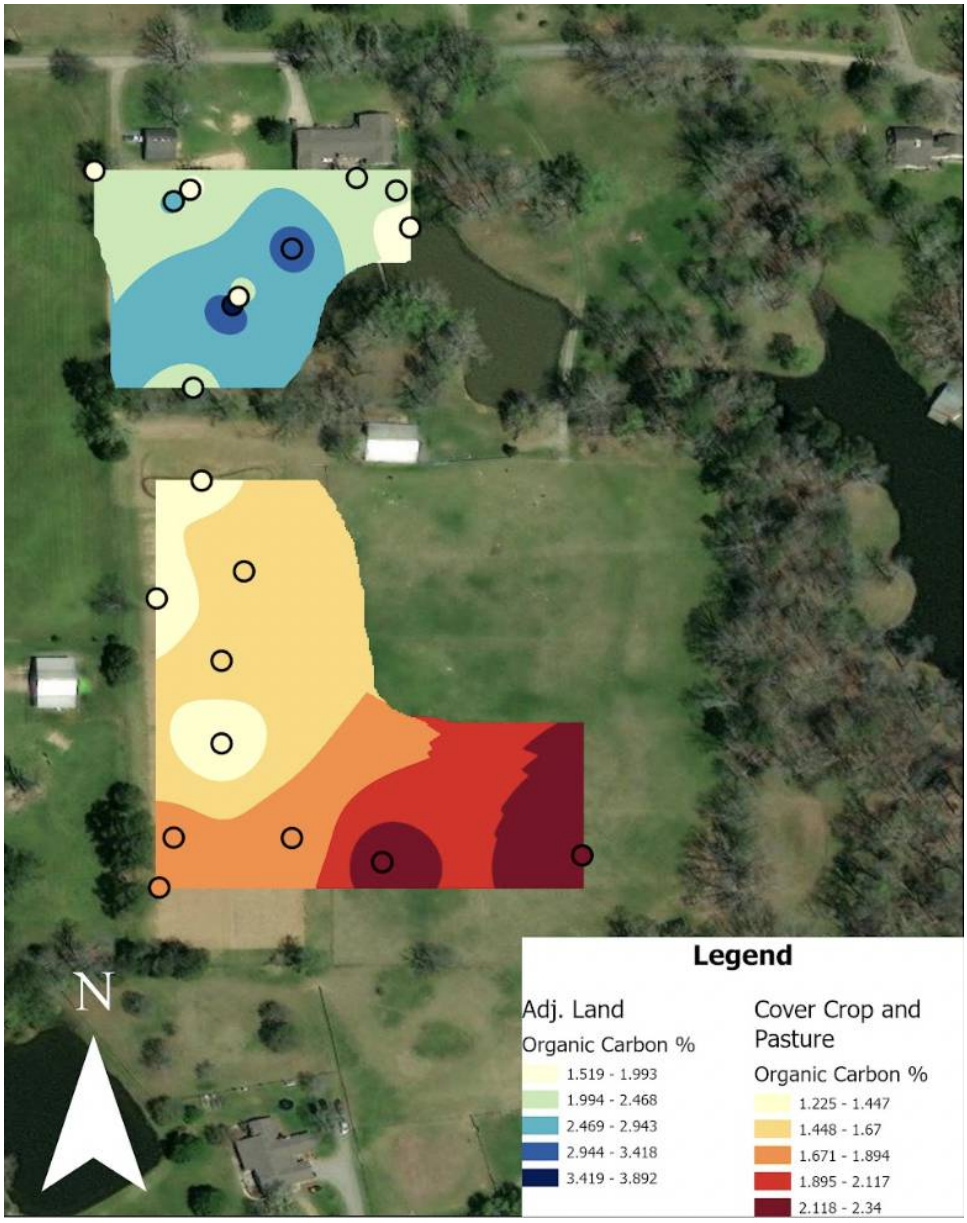
These are the seven carbon maps used as the basis for discussions with farmers regarding results and potential hotspots. Maps were an important tool in the community-based participatory process as we shared insights about findings. Comments in the appendix legend refer to feedback provided by farmers about specific practices.



Appendix 1. Example of the GIS map of percent soil carbon (%) across Farm 1 at 0-15 cm. Hot spots in the pasture coincide with more frequent rotation of chickens and feeding of cows.

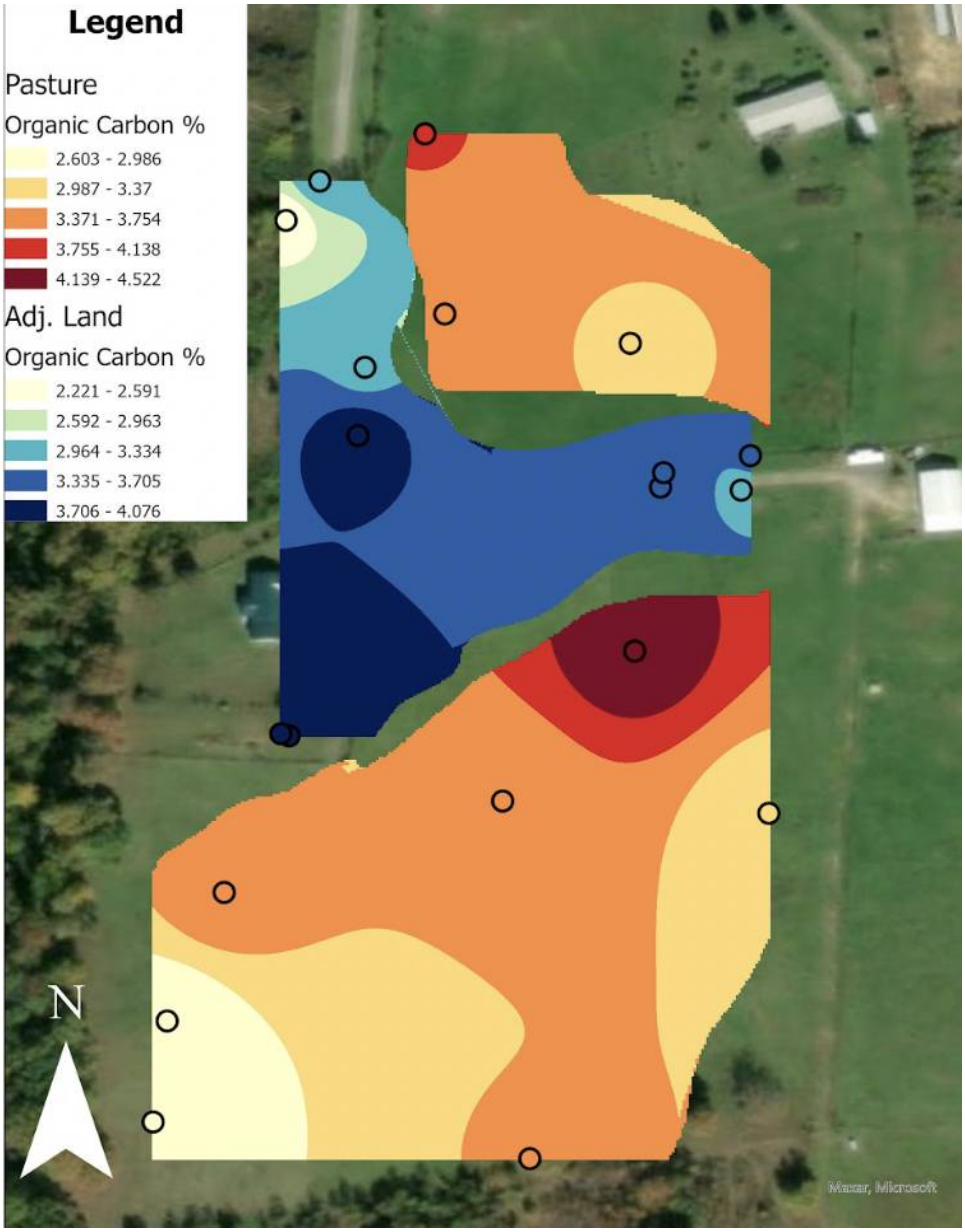


Appendix 2. Example of the GIS map of percent soil carbon (%) across Farm 2 at 0-15 cm. Hot spots in the pasture coincide with feeding areas of cattle.

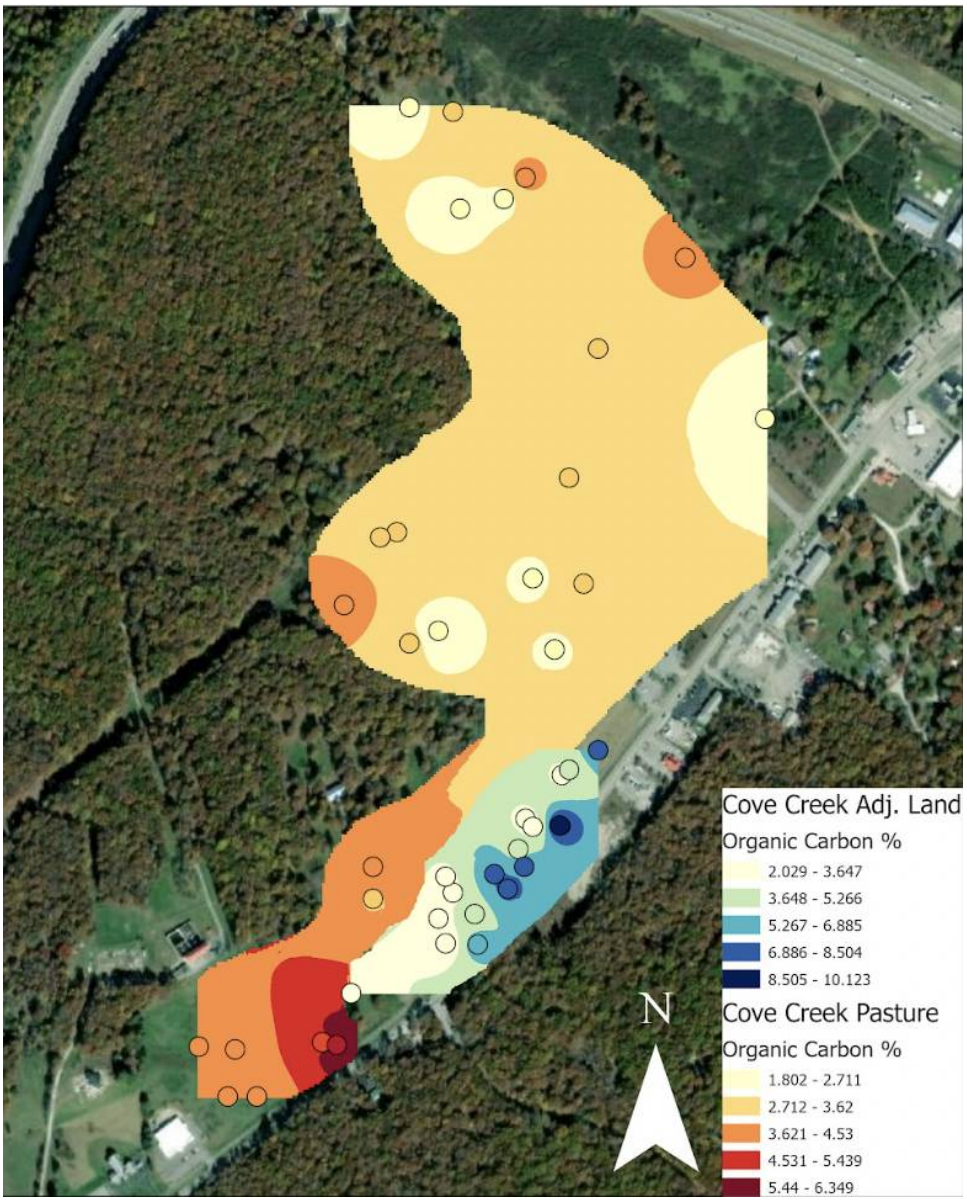


Appendix 3. Example of the GIS map of percent soil carbon (%) across Farm 3 at 0-15 cm.

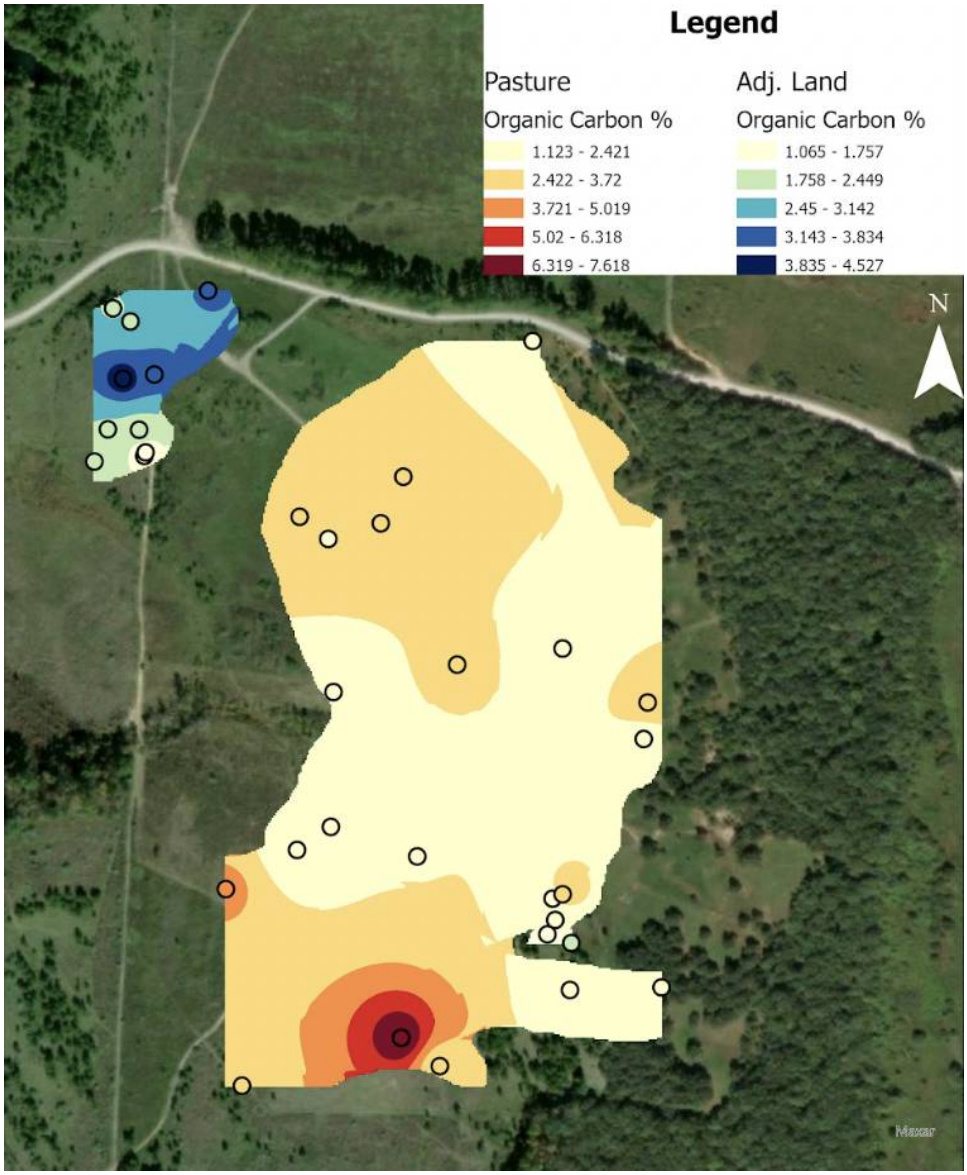
Hot spots coincide with pasture sheep rotational grazing.



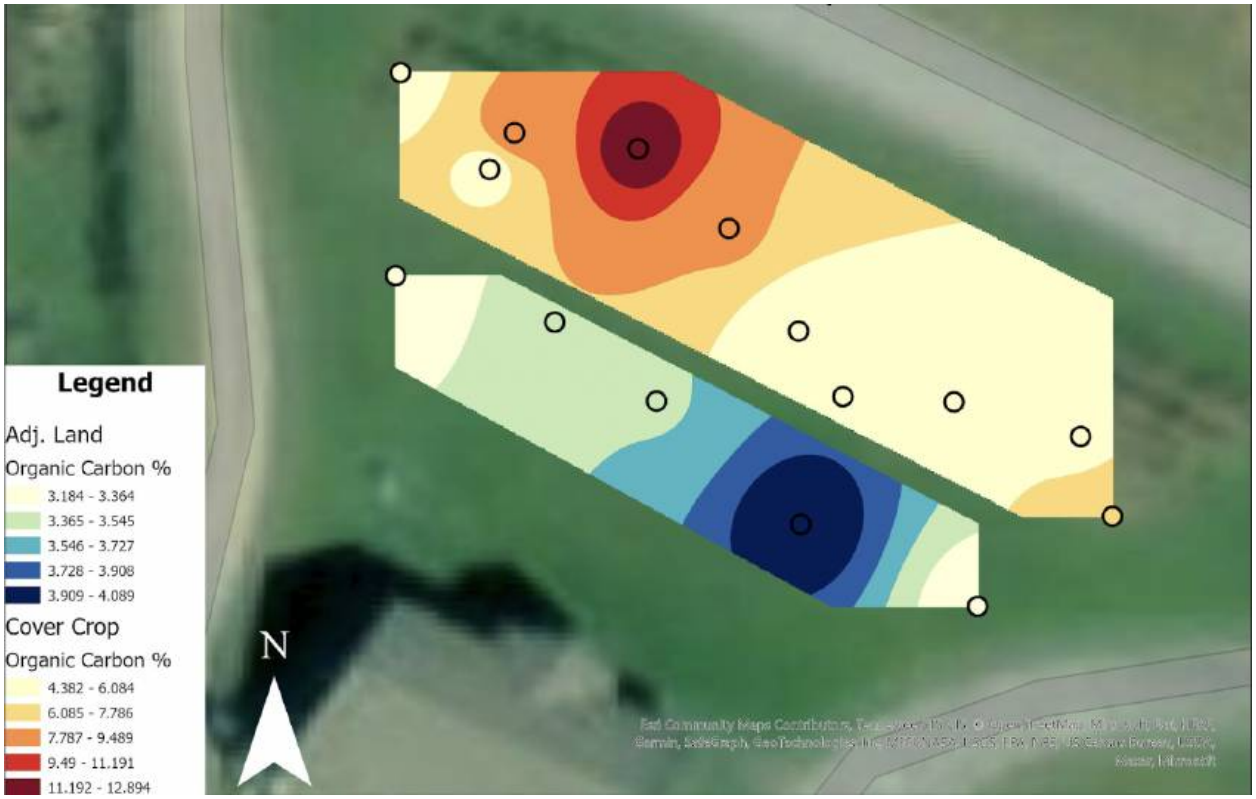
Appendix 4. Example of the GIS map of percent soil carbon (%) across Farm 4 at 0-15 cm. Hot spots in the pasture coincide with seasonal management differences and concentrated hay feeding areas.



Appendix 5. Example of the GIS map of percent soil carbon (%) across Farm 5 at 0-15 cm. Hot spots in the pasture coincide with land history of municipal waste management.



Appendix 6. Example of the GIS map of percent soil carbon (%) across Farm 6 at 0-15 cm. Hot spots in the southern pasture coincide with residue from trampling.



Appendix 7. Example of the GIS map of percent soil carbon (%) across Farm 7 at 0-15 cm. Hot spots coincide with addition of organic matter compost.