COMPARING LONG-TERM SOIL CARBON CONTENT AND THERMAL STABILITY
AMONG DIFFERENT AGRICULTURAL SYSTEMS

Hang Zhao
Master of Environmental Studies

Spring 2024

Alain F. Plante, Ph.D. Department of Earth and Environmental Science
Jon Hawkings, Ph.D. Department of Earth and Environmental Science

ABSTRACT

COMPARING LONG-TERM SOIL CARBON CONTENT AND THERMAL STABILITY AMONG DIFFERENT AGRICULTURAL SYSTEMS

Hang Zhao

Soil carbon stability is vital for maintaining the global carbon cycle balance, biodiversity conservation, and sustainable agriculture. While the earth's climate is experiencing unprecedented changes in recent years and will be more unpredictable in the future, it is urgent to determine the factors influencing soil organic matter content and its composition and stability, in order to ensure the soil health and food security. Agricultural management and treatments including organic treatments, tillage, crop rotation, and nutrients management are critical components that are closely linked with soil carbon retention, composition, and stability. Rodale Institute, located in Kutztown, Pennsylvania, has established a program that compares the organic and conventional cropping systems in a field trial since 1981, called Farming System Trial (FST). Total of 72 soil samples collected from FST in 1981, 2007, and 2020 will be analyzed in this study using the simultaneous thermal analysis (STA) of Thermogravimetry (TG) and Differential Scanning Calorimetry (DSC) coupled with the evolved CO_2 gas analysis, to investigate and compare the long-term changes in soil organic matter and SOC thermal stability between conventional and organic agricultural systems. Statistical methods including Analysis of Variance (ANOVA) and Principal Component Analysis (PCA) were performed in R to analyze the data. Results have revealed that the soil carbon differs the most in 2020, since it is 12 years after the till and no-till separation, large variance in mean and is significantly distinct among three different treatments. Analysis of several indicators across various soil groups indicates that there is no significant difference attributable to the treatment with regard to carbon quality. Long-term observations should be conducted with consistently controlled treatment groups for tillage and systems to detect more pronounced patterns and differences.

Introduction

Soil is the most substantial carbon pool in the terrestrial ecosystem, storing more carbon than the biomass and atmosphere combined (Fontaine et al., 2007; Schmidt et al., 2011). It plays a significant role in regulating global carbon cycle and is essential for living organisms. While most of the carbon present in soil as the soil organic matter (SOM), which is composed of different materials such as microbes, bacteria, and decompositions of once living organisms, contributing massively to soil water and nutrient retention, soil structure, and overall soil quality for agricultural uses, the depletion of soil organic matter due to recent anthropogenic activities including land use changes, deforestation, and wildfires, has largely impacted the soil health and productivity, which further reduced the crop yields, releasing more carbon into the atmosphere, and thus exacerbating climate change (Ontl & Schulte, 2012; Wang et al., 2023). Soils in arctic biomes have accumulated substantial amount of organic matter during the Quaternary Period, making the soils under permafrost the huge reservoir of organic matter, where their 3-meter soil layer containing 30-40% of the global soil carbon pool (Alekseev & Abakumov, 2022). In the United States, soil organic carbon is broadly distributed throughout the states, with the highest concentrations of soil organic carbon storage found in the Northeast, Mid-North, Mid-South, West, and Southeast regions, as illustrated in **Figure 1**. Carbon is often lost from soil by decomposition of soil organic matter releasing CO_2 into the atmosphere, though it could also be loss through long-term erosion and leaching (Lal, 2019; Trumbore, 1997). Soil carbon stability is defined as the resistance to change or loss due to microbial degradation, and is characterized by physical, chemical, biological, and thermal indices (Li et al., 2022). As SOM is one of the most key components of healthy soil for agronomic activities, investigating the factors that affect soil organic matter composition and stability which largely determine the resistance of SOM decomposition, is crucial for mitigating the soil organic carbon (SOC) loss and thus enhancing crop productivity.
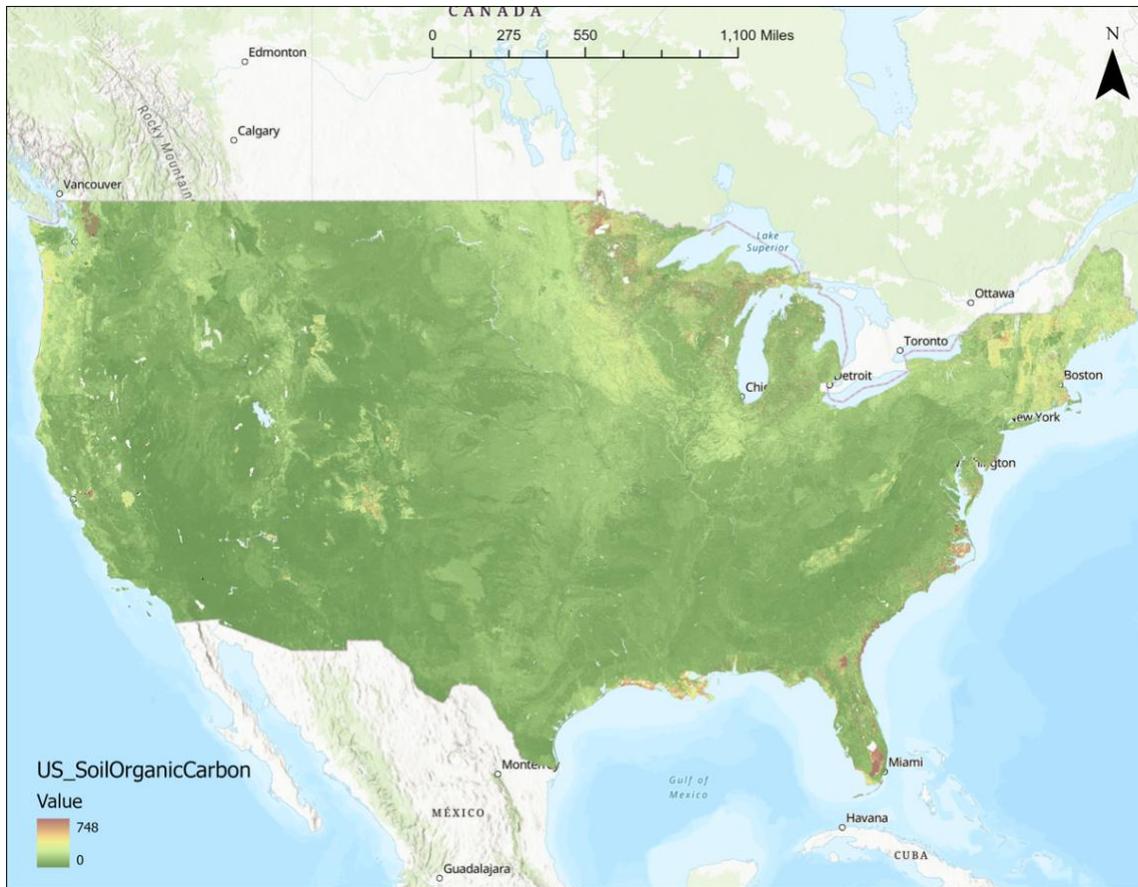
Figure 1. Soil Organic Carbon Content in national scale. **Source**: Global Soil Organic Carbon Map v1.5 (GSOC), Food and Agriculture Organization of the United States (FAO).

Rodale Institute launched a farming trial system (FST) beginning in 1981, has been comparing the conventional chemically based system with organic cropping systems in Kutztown, Pennsylvania for about 40 years, which was aimed for evaluating the differences between cropping systems, mainly organic manure, organic legume, and conventional, alongside with common practices including tillage, crop rotation and nutrient management, to better understand their impact on yields, health, and soil carbon retention (Rodale Institute, 2024; Seidel et al., 2017). To be more specific, the organic manure system operates with organic dairy or beef, based on a long rotation of annual grain crops and perennial forage crops, applying manure or composted manure and legume cover and forage crops rotation for fertility; Organic legume system does not involve livestock, based on mid-length rotation of annual grain crops and cover crops, using leguminous plants extracting nitrogen from the air for fertility; Neither of the organic approaches use herbicides or pesticides; Conventional system, which represents a typical

U.S. grain operation, uses synthetic nitrogen (N) as fertilizer and herbicides for weed control (Seidel et al., 2017, Rodale Institute, 2023). Till and no-till were officially divided for these three systems since 2008, resulting in six groups of farming systems for FST. Prior to this tillage measurement, all systems were treated in full conventional tillage. However, for analytical purposes, different sections of the same plots were designated as 'till' and 'no-till' samples to evaluate the variance between the plots for 1981 and 2007 samples. **Figure 2.** demonstrates an example system and tillage configuration of side-by-side FST plots at the Rodale Institute Farmland.

Figure 2. A top view of the Rodale Farming Systems Trial with plot map and soil survey map. System & Tillage labeled are hypothetical arrangement, which may not reflect actual scenarios. **Source:** Soil Survey Map. Created via ArcGIS Pro.

As it has been suggested that improving agronomic management practices can enhance the quantity and resiliency of the soil organic matter, this study compares soil carbon content and thermal stability between the soil samples collected at the FST in three significant years: 1981, 2007, and 2020, and in conventional and organic agricultural systems respectively.

Literature Review

2.1 Definitions

Increasingly, thermal analysis techniques such as thermogravimetry (TG), differential thermal analysis (DTA) and differential scanning calorimetry (DSC) have become the predominant methods for studying soil organic matter, which were a conventional technique having a long history being used in material science and geosciences (Plante et al., 2009). Thermogravimetry is a method that displays the weight variations of a sample during a heat cycle, or with temperature change (Gao et al., 2015). Sample composed of multiple materials will exhibit distinct peaks of weight loss at varying temperatures, hence the quantities of these materials which have different thermal stabilities, can be determined from the thermogravimetry curve, reflecting the sample's thermal stability and composition (Gao et al., 2015; Plante et al., 2009). Differential thermal analysis (DTA) is very similar to heat flux differential scanning calorimetry, which are techniques to measure temperature difference, or to observe the degree of temperature movements between sample and inert reference. DTA is generally being replaced by DSC in current studies as more sensitive DSC instruments are available (McElhaney, 1982). The integral of the exothermic differential scanning calorimetry (DSC) curves, acquired under a dry air flux and referenced against the curve obtained under a nitrogen flux, yields the combustion heat of the soil, expressed in kilojoules per gram (Barros et al., 2011). In general DSC measures the energy required to proceed the decomposition of organic materials and minerals which could be either endothermic or exothermic reactions. Evolved gas analysis (EGA) is a method used to identify and quantify the volatile substances produced when materials decompose under heat, and it was proposed to simply use H_2O and CO_2 as the principal gases (Fernández et al., 2012).

2.2 Thermal Analysis in SOC Research

Though have been received poor attention for decades, thermal analysis, especially differential thermogravimetry (DTG) has been used for studying SOM and soil classification already during 1960s (Schnitzer & Hoffman, 1966). Even earlier than that, in 1964 the comparably new approach for thermal analysis – differential scanning calorimetry was being advised which

7

measures the energy required to keep temperature of the sample and reference the same (Watson et al., 1964), or the heat flow rate difference (Höhne et al., 2003). Further advancement of DSC enables precise quantification of SOM and sample heat capacity, making it more advantageous than DTA which only measures temperature difference (Barros et al., 2007; Plante et al., 2009). The advancement of thermal analysis has been marked by the innovation of coupling techniques that allow the products of thermal reactions to be analyzed by additional detectors (Plante et al., 2009). After proving the viability of applying thermal analysis in characterizing SOM quality under certain experimental conditions such as sample quantity and heat rate by Fernández et al. (2011), the coupling TG-DSC with CO_2/H_2O evolved gas analysis (EGA) method demonstrated high validity and accuracy for determining SOM compositions (Fernández et al., 2012), which also established the foundation methodology used in this study.

2.3 Agricultural Treatments

Soil organic carbon quantity and quality can be affected by several factors mainly agricultural management. Tillage is used to mix and oxygenate the soil, as well as for integrating cover crops, crop residues, manure, fertilizers, and pesticides into the root layer of the soil. It can facilitate soil respiration, increase the availability of substrates, the potential for oxidation-reduction reactions, and boost the diversity and population of microbial life (Liu et al., 2006). However, numerous research have revealed that no-till is the practice that preserve soil moisture and increases the retention period of soil organic carbon, whereas conventional tillage could result in more rapid residue decay, increase exposure of protected SOM to microbial decomposition, and depletion of water content, especially in 0-15 cm soil depth (Awale et al., 2017). Long-term effects study conducted by Shrestha *et al.* (2015) found that in no-tillage (NT) systems, soil organic carbon (SOC) and soil organic nitrogen concentrations, along with SOC stock, were approximately 50% greater at a soil depth of 0–10 cm compared to the conventional tillage (CT) system. Conservation tillage is a technique that maintains crop residues to cover at least 30% of the soil surface after tillage practices, which could in long term enhance soil quality and soil organic matter composition, minimize the impact of water and wind erosion, and overall improve crop productivity and sustainability (Bergtold & Sailus, 2020; Man et al., 2021). Rodale Institute

employed this type of conservative reduced tillage method for their organic no-till management (**Table 1**).

Rotation is another common practice as a key factor of keeping healthy amount of organic carbon stock and soil productivity. For example, it was suggested that soybean and corn rotation increase the yields by 0.3 mgHa^{-1} than monoculture of soybeans during a three-year observation from 2012-2015, and there was a significant difference on N_2O and CO_2 emission among various rotation types (Behnke et al., 2018). The size of soil microbial community is larger under crop rotation, related to the nutrients such as C (carbon) and N (nitrogen) availability and influence the soil organic carbon content in all soil depths (Liu et al., 2006). Rodale Institute applies more diverse crop rotation on organic practices with up to seven crops in eight years than conventional system with two crops per three years (**Table 1**).

Table 1. Detailed description of crops type, cover crops, and tillage methods for each system.

System Input	Conventional Synthetic	Organic Legume	Organic Manure
Cash Crop Type & Rotation	Corn - Corn - Soy Three-year Rotation	Corn - Oats - Soy - Wheat Four-year Rotation	Corn - Oats - Soy - Wheat - 3 years - Hay_Corn - silage_Wheat Nine-year Rotation
Cover Crop Use of herbicides	No Cover Crop Herbicides & Pesticides	Rye, Clover, Hairy Vetch No Herbicides & Pesticides	Rye, Clover, Hairy Vetch No Herbicides & Pesticides
Tillage After 2008	Full Tillage (Chisel Plow) Continuous No-Till	Full Tillage (Moldboard Plow) Rotational No-Till (Reduced Tillage)	Full Tillage (Moldboard Plow) Rotational No-Till (Reduced Tillage)

Several research have focused on the soil characteristics difference under conventional and organic management using the soil samples from Rodale FST. Wander et al. (1994) investigated the impact of organic practices on soil fertility and active soil organic matter in a 10-year scale, suggesting that the changes in overall SOM contents in all three management were negligible, while the variation in biological active SOM can be attributed to the organic treatment and the SOM quality and quantity were higher under animal-based rotation and cover-cropped treatment

respectively. Two years later, Wander et al. (1996) researched on the soil carbon turnover rate, where they found that the differences in the microbial activities, carbon cycling characteristics, and respiration rate of the organic manure-amended and organic cover-cropped soils had mostly diminished, while both these organic systems ended up demonstrating more effective carbon cycling characteristics than the conventionally managed soil. In more recent findings, it was suggested that about 16-132% increase in SOC was observed in organic practices, within those the organic manure treatment had a substantially greater growth than organic-legume operation (Littrell et al., 2021). Reducing tillage in long term was suggested to be beneficial for SOC accumulation due to its contribution to the size and stability of soil aggregates. Organic manure system is also more effective than organic legume system in enhancing the amount of SOC aggregates measuring 1-2 mm, attributed to the combination effects of using composted manure, deep-root systems of perennial forage crops, and minimal disturbance via rotations (Littrell et al., 2021). Effects on soil hydraulic properties have also been investigated, as it is increasingly important to assess the ability of retaining and draining water in the soil under the escalating effects of climate change and extreme weather. Despite these soil hydraulic properties did not exhibit significant difference among the three management practices, the soil organic carbon content was again proved to be higher in organic-manure treatment than organic-legume treatment and conventional management (Alfahham et al., 2021). Our hypothesis is that the soil samples from organic agricultural treatments exhibit higher organic carbon content and thermal stability compared to soils under conventional practices.

Methodology

3.1 Soil Geography

The 6.1 ha farming system trial (FST) at Rodale Institute is the longest-running, side-by-side comparison of organic and conventional agricultural systems in North America, running from 1981 to present. It is located in Kutztown, Berks County, within the state of Pennsylvania, which has a humid continental climate with warm summers and cold winters. Mean annual precipitation is about 42 inches and mean annual temperature is 52 degree F (approximately 11.1 degree C). According to the USDA Web Soil Survey (2024), the main soil type of FST should be: BkB (Berks-Weilert complex, 3-8% slopes), CmA (Clarksburg silt loam, 0-3% slopes), and CmB (Clarksburg silt loam, 3-8% slopes).
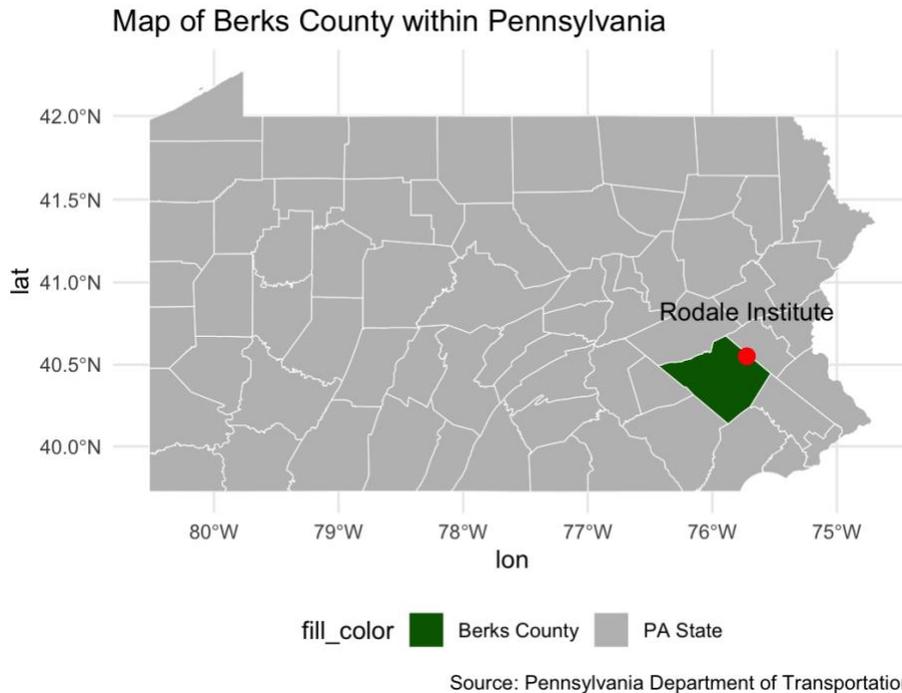

Figure 3. Location of Rodale Institute Headquarter & Farming System Trial (FST).

In total, 72 soil samples were collected from the FST across three distinct years: 1981, 2007, and 2020. Each year, an equal number of samples were obtained, yielding 24 samples per annum. Within the set of 24 samples from each year, three different agricultural treatments were

distributed evenly, which were further divided into two categories based on the farming practice employed: tilled and no-till. Soil carbon percentage was also included in the datasheet. Before transferring them to thermal analysis, the samples were carefully weighed into crucibles, with weights ranging from 40 to 60 mg.

3.2 Lab Experiment

Thermal analysis was conducted by the Netzsch STA449 F5 Jupiter with an automatic sample carrier with a capacity of 18 samples. The instrument is coupled to a LICOR LI-840a infrared gas analyzer (IRGA) for measuring CO_2 and H_2O in the evolved gas during ramped combustion. Soil samples were heated from 120 degree Celsius to 800 degrees Celsius. Thermogravimetry (TG), Differential Scanning Calorimetry (DSC), and Evolved Gas Analysis (EGA) are all happening simultaneously so that the whole process could be more efficient and consistent.

3.3 Statistical Analysis

All statistical analysis were performed in RStudio Version 2023.06.2, and by default the statistical significance level is p<0.05.

3.3.1 Analysis of Variance (ANOVA)

Analysis of Variance (ANOVA) is a statistical method used to analyze the differences among group means in a sample or dataset. Different from Student's t test (t test) which is used for comparisons between two groups, ANOVA can compare means among three or more groups. ANOVA helps in testing the null hypothesis that all group means are equal against the alternative hypothesis that at least one group mean is different, where the hypothesis for k population means could be expressed as follows:

$$Null\ Hypothesis\ H_0: \mu_1 = \mu_2 = \mu_3 = \cdots \mu_k$$
$$Alternative\ Hypothesis\ H_1: Not\ all\ the\ means\ are\ equal$$

If P value is less than the normal significance level of 0.05, then it could be statistically significant to reject the null hypothesis so that there is difference in means among the groups.

The test statistics for ANOVA is expressed as F, the ratio of Mean Square Between and Mean Square Within. To elaborate it further, F statistics is closely related to the sum of squares for treatment or the between group sum of square which is often expressed as SST, and the sum of squares for error or the within group sum of square which is often expressed as SSE (Penn State, 2024), where:

$$SST = \sum_{i=1}^{k} n_i(\bar{y}_i - \bar{y})^2$$

$$SSE = \sum_{i=1}^{k} \sum_{j=1}^{n_i} (y_{ij} - \bar{y}_i)^2$$

In these equations, i is the ith population, j is j^{th} observation from the ith population, k is the total number of groups, n_i is the sample size from the ith population. While F is the ratio of mean square between (MSB) and mean square within (MSW), these two expressions are defined as follows:

$$MSB = \frac{SST}{k-1}$$

$$MSW = \frac{SSE}{N-k}$$

$$F = \frac{MSB}{MSW}$$

Where N is the total number of observations across all groups.

Both one-way and two-way ANOVA were used in this study, where the three dependent variables are: Soil carbon percentage, CO2t50_degC (temperature at which 50% of carbon is escaped), and CO2max_degC (temperature at which maximum carbon is escaped), and independent variables are years, systems, and tillage. One-way ANOVA will be used for detecting the difference among three distinct years, two-way ANOVA will be applied on combination effects of system and tillage on those dependent variables.

The problem with ANOVA is that it only tells the information that there are differences among group means but does not indicate where specifically the differences are. Therefore, a post-hoc analysis called Tukey Honestly Significant Difference (HSD) Post Hoc test for pairwise comparisons were also applied in cases where significant differences among the various groups were observed, which could identify the specific groups that are significantly different.

3.3.2 Principal Component Analysis (PCA)

Multivariate analysis is often used when dealing with large dataset. Among those statistical methods, principal component analysis (PCA) is one of the most commonly used. It is to reduce the dimensionality of a dataset, while preserving as much statistical information (such as variance) as possible. To elaborate further, reduce dimensions can be achieved by identifying new variables that are linear functions of the original dataset's variables, optimize variance and remain uncorrelated with one another (Jolliffe & Cadima, 2016). The first principal component (PC1) always contains the most variance, whereas the second principal component (PC2) will be orthogonal to the PC1 while preserving the most variance on that direction. Values of these new variables are called factor scores, which can be seen as the projections of the observations onto principal components (Abdi & Williams, 2010).

Result

4.1 Total Carbon Percentage

Total carbon percentage represents the proportion of all carbon forms contained in the soil
organic matter, which include organic carbon, inorganic carbon (carbonates/bicarbonates), and
elemental (insignificant in most soil). First, boxplots of total carbon percentage in each group of
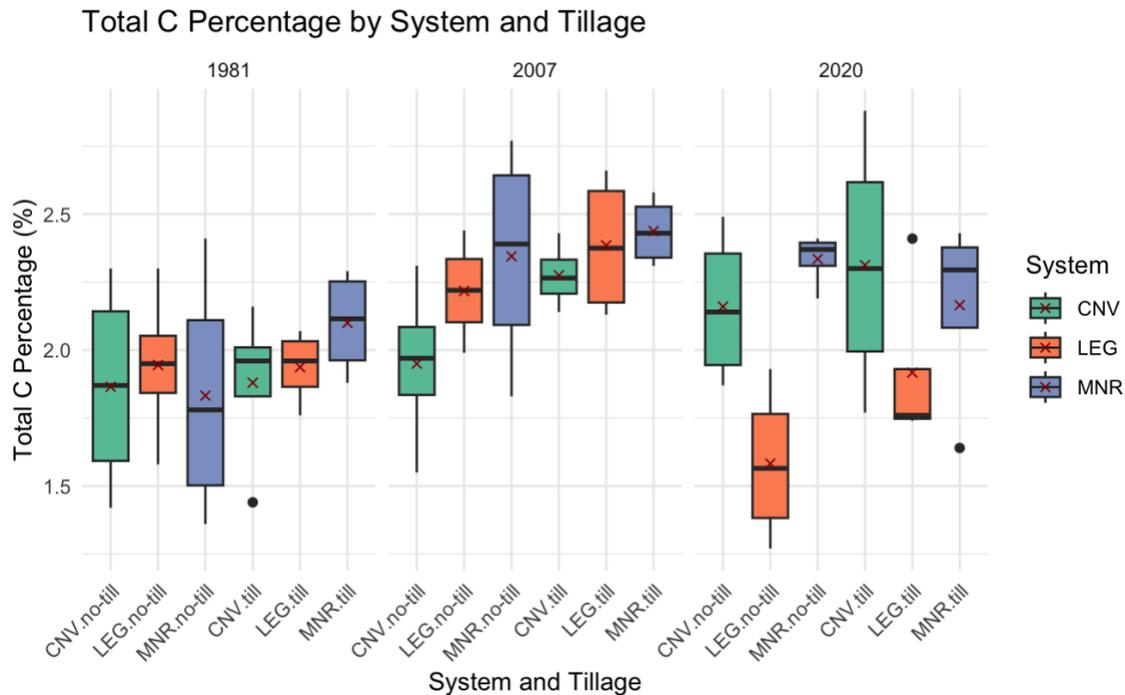soil and year were plotted (**Figure 4**).

Figure 4. Boxplot for showing total carbon percentage in each combination.

The boxplot presented the distribution of total carbon percentage across different agricultural
systems and tillage practices over three years: 1981, 2007, and 2020. The agricultural systems
are categorized into Conventional (CNV), Legume (LEG), and Organic Manure (MNR), each
with no-till and till practices. Note that tillage was not divided for 1981 and 2007 samples, so no-
till and till simply indicate two soil samples collected from one same FST plot. Boxplot can
demonstrate the maximum, minimum, upper and lower quantile, and median value of the dataset
effectively. From this plot, medians of the total carbon percentage can be seen to fluctuate over
time within each system-tillage combination, which is shown as the splitting line in the middle.

Outliers are depicted as individual points, indicating values that fall outside the expected range based on the interquartile range. Red cross indicates mean value of the dataset. Significant variations in the carbon percentage are observed in the year 2020, which presents a pattern different from the preceding years. Noteworthy is the fact that 2020 marks the first year in this dataset where tillage practices are categorized into full-tillage and reduced-tillage, while tillage system was uniformly conducted as full tillage prior to 2008. Therefore, it could be deduced that the large variance of total carbon percentage among 2020 samples may be attributed to the differentiation in tillage practices.

Comparing across the years, one might infer that certain practices show a trend towards increasing or decreasing soil carbon percentages. Based on the one-way ANOVA result which test the carbon percentage as a function of the three distinct years (**Table 2**), It seems that the year is only the significant factor in both till and no-till legume systems, showing p-value lower than 0.05 level, which is not surprising considering the substantial variance observed in carbon percentage under the legume system in 2020, strikingly lower compared to the preceding two years.

Table 2. One-way ANOVA output for carbon percentage in response to three years under different combination of agricultural practices.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	System_Tillage
Year	2	0.184466666666666	0.0922333333333332	0.806470416788108	0.476245021389406	CNV - no-till
Residuals	9	1.0293	0.114366666666666	NA	NA	CNV - no-till
Year	2	0.459316666666666	0.229658333333333	1.96443082186898	0.195916041268459	CNV - till
Residuals	9	1.052175	0.116908333333333	NA	NA	CNV - till
Year	2	0.81185	0.405925	5.75100354191263	0.0246041058020896	LEG - no-till
Residuals	9	0.63525	0.0705833333333334	NA	NA	LEG - no-till
Year	2	0.558949999999999	0.279475	4.25992886781268	0.0499118541600981	LEG - till
Residuals	9	0.59045	0.0656055555555555	NA	NA	LEG - till
Year	2	0.687016666666664	0.343508333333332	2.50112250470237	0.136836439647818	MNR - no-till
Residuals	9	1.236075	0.137341666666667	NA	NA	MNR - no-till
Year	2	0.256516666666668	0.128258333333334	2.08446571260892	0.180349058616655	MNR - till
Residuals	9	0.553775000000001	0.0615305555555557	NA	NA	MNR - till

Moving on to two-way ANOVA, System is statistically significant in 2020 dataset (**Table 3**). Based on previous observation, this difference is attributed to the abnormal low SOC in legume system.

Table 3. Two-way ANOVA output for carbon percentage in response to combination of agricultural practices (Tillage*System) in three years.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Year
1981.Tillage	1	0.0504167	0.0504167	0.4851514	0.4949954	1981
1981.System	2	0.0377083	0.0188542	0.1814306	0.8355826	1981
1981.Tillage:System	2	0.0932583	0.0466292	0.4487049	0.6454045	1981
1981.Residuals	18	1.8705500	0.1039194	NA	NA	1981
2007.Tillage	1	0.2281500	0.2281500	3.2868062	0.0865505	2007
2007.System	2	0.3238083	0.1619042	2.3324463	0.1256810	2007
2007.Tillage:System	2	0.0563250	0.0281625	0.4057185	0.6724419	2007
2007.Residuals	18	1.2494500	0.0694139	NA	NA	2007
2020.Tillage	1	0.0672042	0.0672042	0.6118663	0.4442549	2020
2020.System	2	1.2976750	0.6488375	5.9073987	0.0106553	2020
2020.Tillage:System	2	0.2615583	0.1307792	1.1906906	0.3268512	2020
2020.Residuals	18	1.9770250	0.1098347	NA	NA	2020

Post-hoc test specifically on 2020 dataset was performed to see the detailed differences of group means. From the Tukey's HSD plot (**Figure 5**), it is evident that there is significant difference in the mean SOC values between the no-till manure and no-till legume systems. The confidence interval, though very close, does not cross zero, indicating this significance. Correspondingly, the boxplot (**Figure 4**) clearly shows that the no-till organic manure system has a substantially higher carbon content compared to the no-till organic legume system. Moreover, no-till legume and till conventional appear to have nearly significant difference (p = 0.056) as the confidence interval is slightly passing over zero line, meaning that no-till legume has nearly significant lower value of carbon content than till conventional system, while there is higher possibility that this difference is due to random chance.
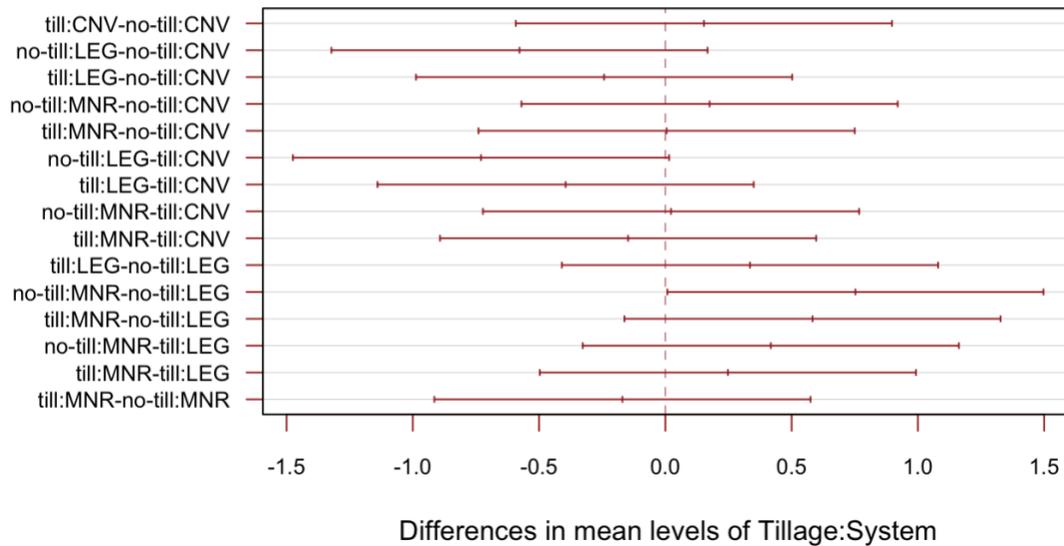
95% family-wise confidence level

Figure 5. Tukey's HSD post-hoc test for 2020 dataset on system*tillage.

4.2 CO$_2$t50_degC

CO$_2$t50_degC demonstrates the temperature at which half of the carbon dioxide gas is evolved, which could further indicate the thermal stability of SOC as carbon dioxide can only be produced by burning organic matter in the soil. Similar to DSCt50_degC which represents the temperature at which half of the energy is released in differential scanning calorimetry, a higher CO$_2$t50_degC value suggests greater thermal stability, more diverse structure, higher level of formation of humic substances, and more resilience to microbial decomposition (Gao et al., 2015; Peltre et al., 2013). Boxplot (**Figure 6**) is showing the CO$_2$t50_degC by system and tillage across three years. The temperature overall ranges from about 363 – 377 degrees Celsius. Overall, the manure-till system consistently exhibits least variance across the groups and years. It is noteworthy that the year 2020 shows a marked increase in range variability and in general lower temperature across all six system-tillage combinations compared to the preceding years. Comparing mean and median temperatures, it reveals that the conventional-till system recorded a higher average and median temperature in both 1981 and 2007. Conversely, in 2020, the legume

18

no-till and manure-till systems exhibit the highest average temperatures, with the manure-till system also presenting the highest median temperature.
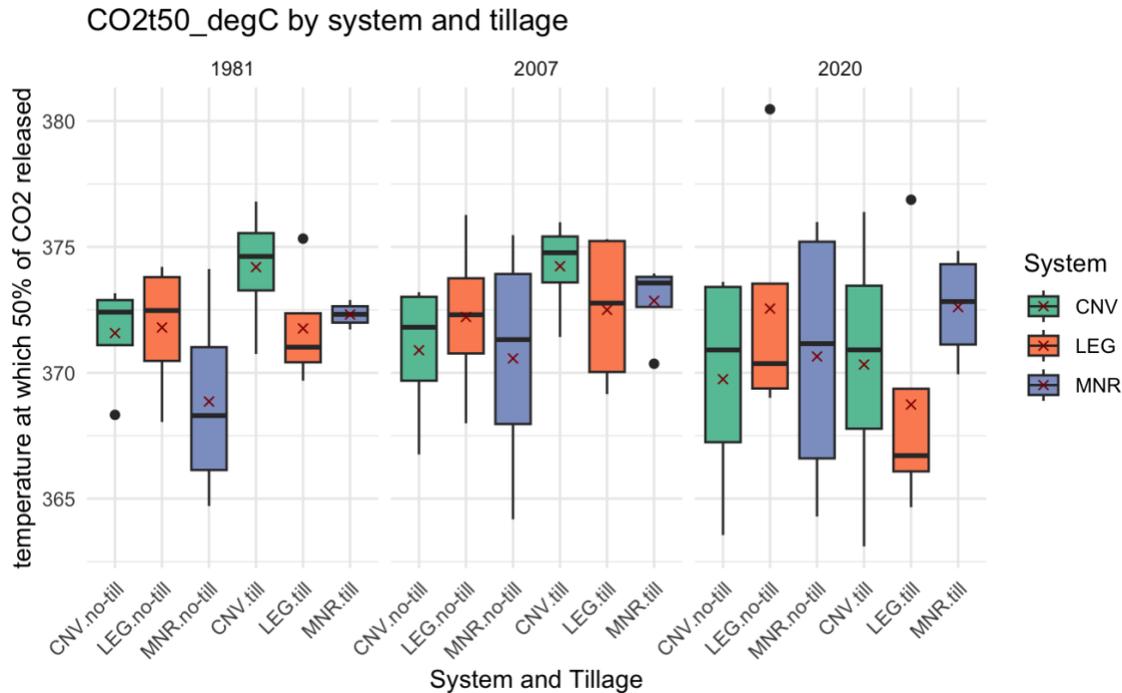

Figure 6. Boxplot for showing co2t50_degC for combinations of system and tillage.

Both one-way and two-way ANOVA for CO_2t50_degC against years, systems, and tillage are not showing any significant difference among groups.

4.3 CO_2max_degC

CO_2max_degC suggests the temperature at which the maximum or the peak of the CO_2 is released. Similar to CO_2t50_degC, this is also an indicator to assess soil thermal stability. Temperature ranges from 332 – 344 Celsius Degrees. Boxplot (**Figure 7.**) is showing the CO_2max_degC by system and tillage across three years. Broadly, this graph exhibits quite similar patterns with those observed in the CO_2t50_degC data, though it may display greater variability in peak temperature ranges. Manure till and no-till systems in 2020 are clearly showing higher

temperatures than all other systems, while this trend may not be obvious for 1981 and 2007 samples.
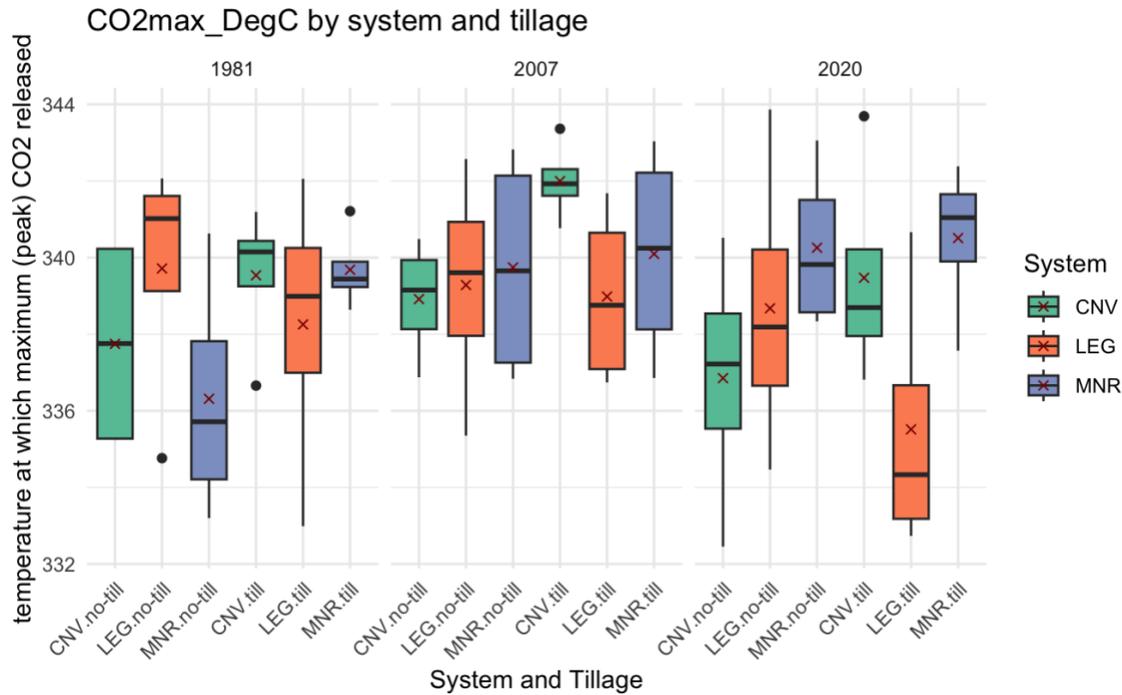

Figure 7. Boxplot for showing co2max_degC for combinations of system and tillage.

Both one-way and two-way ANOVA for CO_2max_degC against years, systems, and tillage are not showing any significant difference among groups.

4.4 Principal Component Analysis

Principal component analysis (PCA) was performed for dataset containing proportion of CO_2 released through the combustion of soil organic matter at each temperature level from 120 to 750 Celsius degree measured during the EGA analysis. It is commonly used to determine similar chemical compositions and characteristics between groups (Plante et al., 2023). The dataset was initially visualized using a dot plot, with the y-axis representing the proportion of CO_2-C released to total CO_2-C, and the x-axis indicating the temperature in degrees Celsius (**Figure 8**). Each data point is depicted as a dot on the graph. It displays that all FST samples are having consistent trends and patterns, where the temperatures at which peak CO_2 emissions occur are closely gathered indicated by the blue vertical line at 340 degrees Celsius.
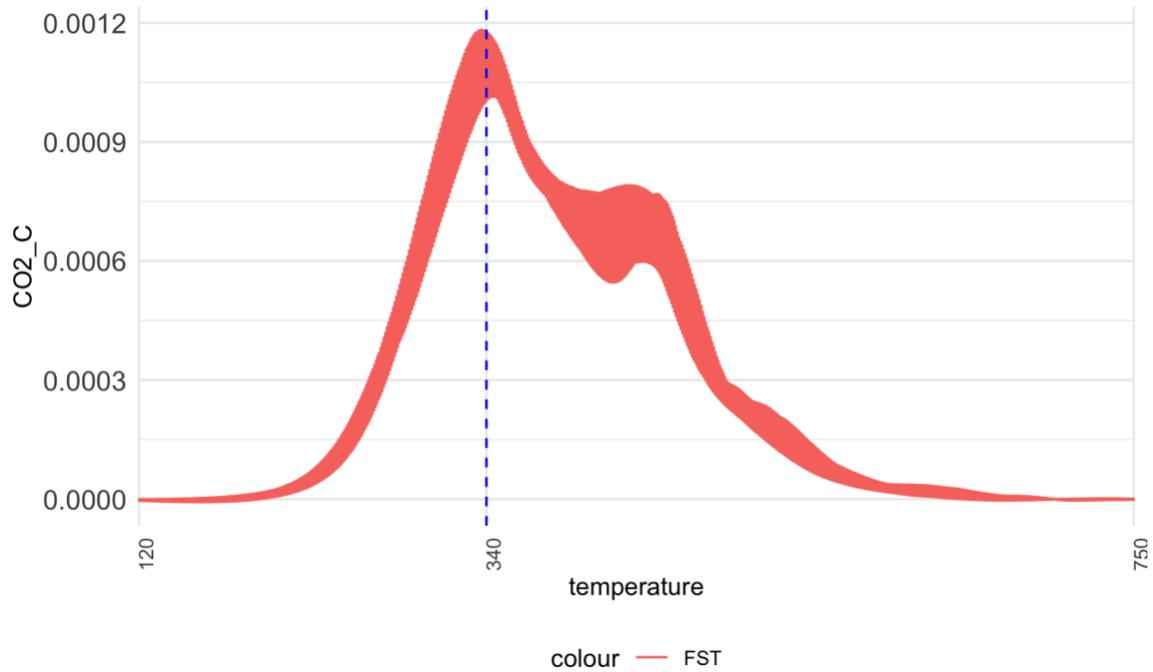
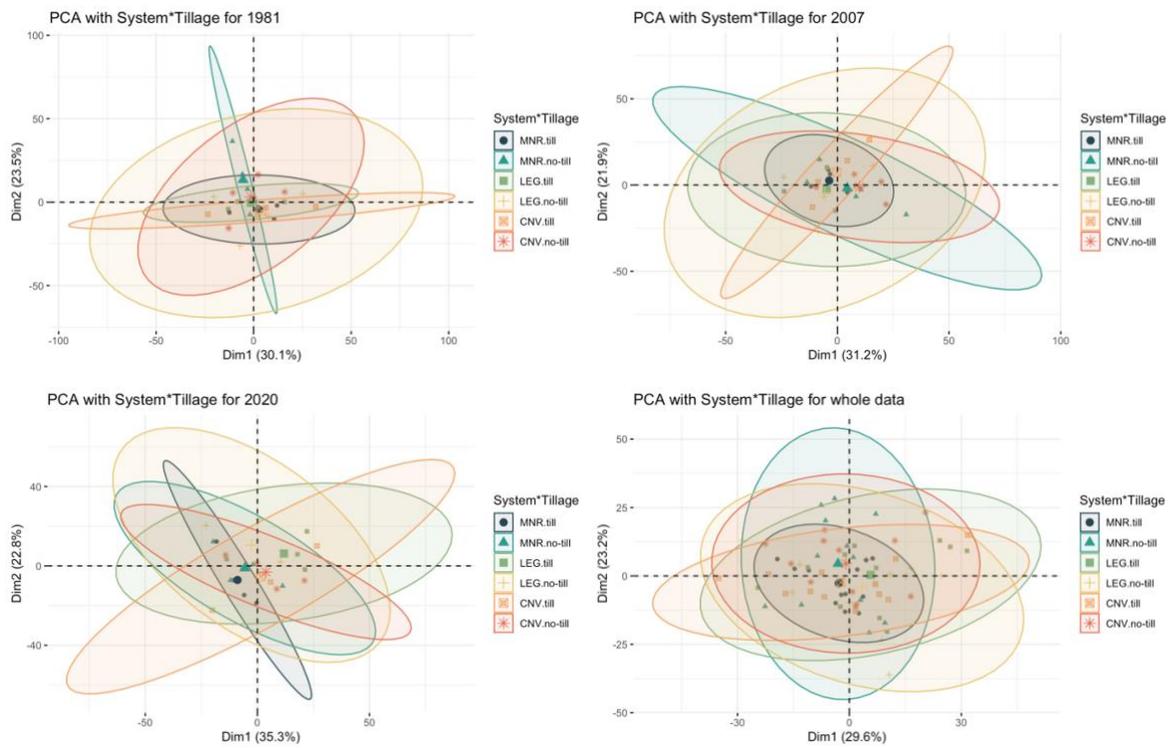**Figure 8**. EGA dot plot for all 72 FST soil samples.

Figure 9. PCA score plot (confidence ellipses) of 1981, 2007, 2020, and the whole dataset.

No difference can be observed from the PCA score plot with confidence ellipses (**Figure 9.**) for all three years analysis, as the ellipses are overlapping with each other so no distinct pattern could be seen. Therefore, we could conclude that no cluster or difference among six different combinations of system and tillage could be determined, and they are all behaving similarly.

Discussion

The boxplot (**Figure 4.**) comparing total soil carbon percentages reveals that in 2007, both the till and no-till organic manure systems had the highest median soil carbon levels, marginally surpassing the organic legume till system and significantly outperforming conventional till systems. In addition, the no-till organic manure system displayed a broad range of carbon percentages, including a peak value indicative of the beneficial impact of organic manure application combined with minimal soil disturbance on the overall SOC content. From 1981 to 2007, there was a pronounced upward trend in carbon percentages for treatments involving organic legumes and manure, while this also applied to conventional till system. Despite this, in 2020, while the median carbon contents in both the no-till and tilled organic manure systems remained the highest, with the no-till organic manure system in particular standing out, the conventional till system showed an unexpected spike, exhibiting the highest maximum soil carbon content among all treatment groups. Meanwhile, SOC in organic legume systems dropped dramatically in 2020, whereas the median carbon level in conventional systems have trend of incline from 2007 level. This could be attributed to the application of no-till (reduced tillage), and addition of genetically modified (GM) corn and soybean in both systems since 2008 (Seidel et al., 2017). Though some studies have revealed that no-till or conservation tillage management could increase the SOC content (Cooper et al., 2021), in general the advantageous effects will be observed after a long-term treatment for example, more than 10 years while also merely observed in top soil layer (Haddaway, 2017), whereas in this case only 12 years after the change in tillage treatment, it may not achieve the anticipated result. Studies also found that there are considerable uncertainties to observe evident increase in SOC stock under no-till treatment, and it is largely dependent on soil type and climate conditions (Ogle et al., 2019). In addition, as tillage was not separated and GM crops were not included before 2008, comparing data from 1981 and 2007 with the 2020 data seems inconsistent and scientifically inaccurate. Therefore, further observations from future soil samples under longer treatment of systems and tillage would provide more robust basis for comparisons.

It should also be noted that the relationship between biogeochemical sense of SOM stability and measure of system energy by thermal analysis is not straightforward or inherently obvious, given

that the stability of SOM is not solely dependent on its quality, instead it also includes several other processes such as physio-chemical interactions with clay surface, accessibility to microbes and aggregation, and accumulations of biomacromolecules (Plante et al., 2011). Therefore, though no significant difference were shown in temperature at which maximum or half of CO_2 released across the six treatment groups, the benefits and advantages of organic systems, particularly in combination with no-till or reduced tillage, may be reflected in other aspects of soil properties.

Conclusion

To investigate whether the organic agricultural management would allow higher soil organic matter and carbon stability compared to conventional approach, 72 soil samples collected from the Rodale Institute FST in 1981, 2007, and 2020 were evaluated using simultaneous thermal analysis. The results obtained from analysis of Variance (ANOVA) and multivariate statistical methods – Principal Component Analysis (PCA) determined few statistical significance of sample SOC differences among the six groups, which are categorized as manure, legume, and conventional systems, each further subdivided into till and no-till. Moreover, the primary SOC difference in 2020 is likely due to the separation of tillage practices and the addition of genetically modified crops after 2008. For the samples from 1981 and 2007, the 'till' and 'no-till' labels represent two samples taken from the same plot, rather than distinct tillage treatments. No significant difference was observed for CO_2t50_degC and CO_2max_degC, from both ANOVA and PCA results. Overall, prolonged period of observations after the new applications in 2008 and consistency in sample variables are recommended for more valuable comparisons between the effects of no-till and till management, organic and conventional systems on soil carbon content and thermal stability.

Reference

Abdi, H., & Williams, L. J. (2010). Principal component analysis. *WIREs Computational Statistics*, *2*(4), 433–459. https://doi.org/10.1002/wics.101

Alekseev, I., & Abakumov, E. (2022). Soil organic carbon stocks and stability of organic matter in permafrost-affected soils of Yamal region, Russian Arctic. *Geoderma Regional*, *28*, e00454. https://doi.org/10.1016/j.geodrs.2021.e00454

Alfahham, A., Amato, M. T., Omondi, E., Giménez, D., & Plante, A. F. (2021). Assessing the impact of organic versus conventional agricultural management on soil hydraulic properties in a long-term experiment. *Soil Science Society of America Journal*, *85*(6), 2135–2148. https://doi.org/10.1002/saj2.20314

Awale, R., Emeson, M. A., & Machado, S. (2017). Soil Organic Carbon Pools as Early Indicators for Soil Organic Matter Stock Changes under Different Tillage Practices in Inland Pacific Northwest. *Frontiers in Ecology and Evolution*, *5*. https://doi.org/10.3389/fevo.2017.00096

Barros, N., Salgado, J., & Feijóo, S. (2007). Calorimetry and soil. *XIVth ISBC Proceedings Special Issue*, *458*(1), 11–17. https://doi.org/10.1016/j.tca.2007.01.010

Barros, N., Salgado, J., Villanueva, M., Rodriquez-Añón, J., Proupin, J., Feijóo, S., & Martín-Pastor, M. (2011). Application of DSC–TG and NMR to study the soil organic matter. *Journal of Thermal Analysis and Calorimetry*, *104*(1), 53–60. https://doi.org/10.1007/s10973-010-1163-4

Behnke, G. D., Zuber, S. M., Pittelkow, C. M., Nafziger, E. D., & Villamil, M. B. (2018). Long-term crop rotation and tillage effects on soil greenhouse gas emissions and crop production in Illinois, USA. *Agriculture, Ecosystems & Environment*, *261*, 62–70. https://doi.org/10.1016/j.agee.2018.03.007

Bergtold, J. & Sailus, M. (Eds). (2020). Conservation Tillage Systems in the Southeast: Production, Profitability and Stewardship. https://www.sare.org/wp-content/uploads/Conservation-Tillage-Systems-in-the-Southeast_compressed.pdf

Cooper, H. V., Sjögersten, S., Lark, R. M., Girkin, N. T., Vane, C. H., Calonego, J. C., Rosolem, C., & Mooney, S. J. (2021). Long-term zero-tillage enhances the protection of soil carbon

in tropical agriculture. *European Journal of Soil Science*, *72*(6), 2477–2492. https://doi.org/10.1111/ejss.13111

Fernández, J. M., Peltre, C., Craine, J. M., & Plante, A. F. (2012). Improved Characterization of Soil Organic Matter by Thermal Analysis Using CO2/H2O Evolved Gas Analysis. *Environmental Science & Technology*, *46*(16), 8921–8927. https://doi.org/10.1021/es301375d

Fernández, J. M., Plante, A. F., Leifeld, J., & Rasmussen, C. (2011). Methodological considerations for using thermal analysis in the characterization of soil organic matter. *Journal of Thermal Analysis and Calorimetry*, *104*(1), 389–398. https://doi.org/10.1007/s10973-010-1145-6

Fontaine, S., Barot, S., Barré, P., Bdioui, N., Mary, B., & Rumpel, C. (2007). Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature*, *450*(7167), 277–280. https://doi.org/10.1038/nature06275

Food and Agriculture Organization of the United States (FAO). (2019). Global Soil Organic Carbon Map v1.5 (GSOC). https://data.apps.fao.org/catalog/iso/7730e747-eb73-49c9-bfe6-84ebae718743

Gao, W., Zhou, T., & Ren, T. (2015). Conversion from Conventional to No Tillage Alters Thermal Stability of Organic Matter in Soil Aggregates. *Soil Science Society of America Journal*, *79*(2), 585–594. https://doi.org/10.2136/sssaj2014.08.0334

Haddaway, N.R., Hedlund, K., Jackson, L.E. et al. (2017). How does tillage intensity affect soil organic carbon? A systematic review. Environmental Evidence, 6, 30. https://doi.org/10.1186/s13750-017-0108-9

Höhne, G. W. H., Hemminger, W. F., & Flammersheim, H.-J. (2003). Introduction. In *Differential Scanning Calorimetry* (pp. 1–7). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-662-06710-9_1

Jolliffe, I. T., & Cadima, J. (2016). Principal component analysis: A review and recent developments. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, *374*(2065), 20150202. https://doi.org/10.1098/rsta.2015.0202

Lal, R. (2019). Accelerated Soil erosion as a source of atmospheric CO2. *Soil Carbon and Climate Change: The 4 per Mille Initiative*, *188*, 35–40. https://doi.org/10.1016/j.still.2018.02.001

Li, Z., Xiao, L., Deng, C., Yuan, Z., Liang, C., Xiong, Q., Li, Z., & Nie, X. (2022). Thermal stability of soil organic carbon subjected to water erosion as a function of edaphic factors. *International Journal of Sediment Research*, *37*(1), 26–36. https://doi.org/10.1016/j.ijsrc.2021.06.002

Littrell, J., Xu, S., Omondi, E., Saha, D., Lee, J., & Jagadamma, S. (2021). Long-term organic management combined with conservation tillage enhanced soil organic carbon accumulation and aggregation. *Soil Science Society of America Journal*, *85*(5), 1741–1754. https://doi.org/10.1002/saj2.20259

Liu, X., Herbert, S. J., Hashemi, A. M., Zhang, X., & Ding, G. (2006). Effects of agricultural management on soil organic matter and carbon transformation—A review. *Plant, Soil and Environment*, *52*(12), 531–543.

Man, M., Wagner-Riddle, C., Dunfield, K. E., Deen, B., & Simpson, M. J. (2021). Long-term crop rotation and different tillage practices alter soil organic matter composition and degradation. *Soil and Tillage Research*, *209*, 104960. https://doi.org/10.1016/j.still.2021.104960

McElhaney, R. N. (1982). The use of differential scanning calorimetry and differential thermal analysis in studies of model and biological membranes. *Chemistry and Physics of Lipids*, *30*(2), 229–259. https://doi.org/10.1016/0009-3084(82)90053-6

Ogle, S.M., Alsaker, C., Baldock, J. et al. (2019). Climate and Soil Characteristics Determine Where No-Till Management Can Store Carbon in Soils and Mitigate Greenhouse Gas Emissions. *Scientific Reports*, 9, 11665. https://doi.org/10.1038/s41598-019-47861-7

Ontl, T. A. & Schulte, L. A. (2012). Soil Carbon Storage. *Nature Education Knowledge* 3(10):35

Peltre, C., Fernández, J. M., Craine, J. M., & Plante, A. F. (2013). Relationships between Biological and Thermal Indices of Soil Organic Matter Stability Differ with Soil Organic Carbon Level. *Soil Science Society of America Journal*, *77*(6), 2020–2028. https://doi.org/10.2136/sssaj2013.02.0081

Pennsylvania State University. (2023). STAT 500 Lesson 10: Introduction to ANOVA. Eberly College of Science. https://online.stat.psu.edu/stat500/lesson/10

Plante, A. F., Fernández, J. M., Haddix, M. L., Steinweg, J. M., & Conant, R. T. (2011). Biological, chemical and thermal indices of soil organic matter stability in four grassland soils. *Soil Biology and Biochemistry*, *43*(5), 1051–1058. https://doi.org/10.1016/j.soilbio.2011.01.024

Plante, A. F., Fernández, J. M., & Leifeld, J. (2009). Application of thermal analysis techniques in soil science. *Geoderma*, *153*(1), 1–10. https://doi.org/10.1016/j.geoderma.2009.08.016

Plante, A. F., Sanderman, J., Asanopoulos, C. H., Bell, S., & Baldock, J. (2023). Interpreting ramped combustion thermograms using 13C NMR spectroscopy to characterize soil organic matter composition. *Geoderma*, *432*, 116415. https://doi.org/10.1016/j.geoderma.2023.116415

Rodale Institute. (2024). Farming System Trial 40-Year Report. https://rodaleinstitute.org/wp-content/uploads/FST_40YearReport_RodaleInstitute-1.pdf

Schmidt, M. W. I., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D. A. C., Nannipieri, P., Rasse, D. P., Weiner, S., & Trumbore, S. E. (2011). Persistence of soil organic matter as an ecosystem property. *Nature*, *478*(7367), 49–56. https://doi.org/10.1038/nature10386

Schnitzer, M., & Hoffman, I. (1966). A Thermogravimetric Approach to the Classification of Organic Soils. *Soil Science Society of America Journal*, *30*(1), 63–66. https://doi.org/10.2136/sssaj1966.03615995003000010024x

Seidel, R., Moyer, J., Nichols, K., & Bhosekar, V. (2017). Studies on long-term performance of organic and conventional cropping systems in Pennsylvania. *Organic Agriculture*, *7*(1), 53–61. https://doi.org/10.1007/s13165-015-0145-z

Shrestha, B.M., Singh, B.R., Forte, C. and Certini, G. (2015). Long-term effects of tillage, nutrient application and crop rotation on soil organic matter quality assessed by NMR spectroscopy. *Soil Use and Management*, 31: 358-366. https://doi.org/10.1111/sum.12198

Trumbore, S. E. (1997). Potential responses of soil organic carbon to global environmental change. *Proceedings of the National Academy of Sciences*, *94*(16), 8284–8291. https://doi.org/10.1073/pnas.94.16.8284

United States Department of Agriculture (USDA). (2024). Web Soil Survey. Natural Resources Conservation Service. https://websoilsurvey.nrcs.usda.gov/app/

Wander, M.M., Dudley, R.B., Traina, S.J., Kaufman, D., Stinner, B.R. and Sims, G.K. (1996). Acetate Fate in Organic and Conventionally Managed Soils. *Soil Science Society of America Journal*, 60, 1110-1116. https://doi.org/10.2136/sssaj1996.03615995006000040021x

Wander, M. M., Traina, S. J., Stinner, B. R., & Peters, S. E. (1994). Organic and Conventional Management Effects on Biologically Active Soil Organic Matter Pools. *Soil Science Society of America Journal*, *58*(4), 1130–1139. https://doi.org/10.2136/sssaj1994.03615995005800040018x

Wang, C., Cheng, T., Tang, S., Li, M., Zhang, D., & Pan, X. (2023). Protective-mechanism-exclusive thermal stability modes of soil organic matter: Novel implication for wildfire effect on soil organic carbon. *Science of The Total Environment*, *861*, 160734. https://doi.org/10.1016/j.scitotenv.2022.160734

Watson, E. S., O'Neill, M. J., Justin, Joshua., & Brenner, Nathaniel. (1964). A Differential Scanning Calorimeter for Quantitative Differential Thermal Analysis. *Analytical Chemistry*, *36*(7), 1233–1238. https://doi.org/10.1021/ac60213a019