Experimental Consideration, Treatments, and Methods in Determining Soil Organic Carbon Sequestration Rates

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In agricultural land areas, no-tillage (NT) farming systems have been practiced to replace intensive tillage practices such as, moldboard plow (MP), chisel plow (CP), and other systems to improve many soil health indicators, and specifically to increase soil organic carbon (SOC) sequestration and reduce soil erosion. Numerous approaches to estimate the amounts and rates of SOC sequestration as a result of a switch to NT systems have been published, but there is a concern regarding protocol for assessing SOC especially for different tillage systems. Therefore, the objectives of this paper are to: (i) define and understand concepts of SOC sequestration, (ii) quantify SOC distribution and the methodology of measurements, (iii) address soil spatial variability at field- or landscape-scale for potential SOC sequestration, and (iv) consider proper field experimental design, including pretreatments baseline for SOC sequestration determination. For SOC sequestration to occur, as a result of a treatment applied to a land unit, all of the SOC sequestered must originate from the atmospheric CO_2 pool and be transferred into the soil humus through land unit plants, plant residues, and other organic solids. The SOC stock present in soil humus at end of a study must be greater than the pretreatment SOC stock levels in the same land unit. However, one should recognize that a continuity equation showing drawdown in atmospheric concentration of CO_2 may be difficult, if not impossible, to quantify. Therefore, SOC sequestration results of paired comparisons of NT to other conventional tillage systems with no pretreatments SOC baseline, and if the conventional system is not at a steady state, will likely be inaccurate where the potential for SOC loss exists in both systems. To unequivocally demonstrate that the SOC sequestration has occurred at a specific site, a temporal increase must be documented relative to pretreatment SOC content and linked attendant changes in soil properties and ecosystem services and functions with proper consideration given to soil spatial variability. Also, a standardized methodology that includes proper experimental design, pretreatment baseline, root zone soil depth consideration, and consistent method of SOC analysis must be used when determining SOC sequestration.

Abbreviations: BMPs, best management practices; CP, chisel plow; GHG, greenhouse gas; IPCC, Intergovernmental Panel on Climate Change; MP, moldboard plow; MRT, mean residence time; NT, no tillage; PT, plow tillage; SIC, soil inorganic C; SOC, soil organic carbon; SOM, soil organic matter; TSC, total soil carbon; WL, woodlot.

DEFINITION OF SOIL ORGANIC CARBON SEQUESTRATION

Terrestrial carbon (C) sequestration can be defined as the capture and secure storage of atmospheric C into biotic and pedologic C pools that would otherwise be emitted to or remain in the atmosphere (Lal, 2007). The idea of C storage is: (i) to prevent C emission caused by human activities from reaching the atmosphere by capturing and diverting it to secure storage, or (ii) to remove it from the atmosphere by various means and to enhance its mean residence time (MRT) in the

soil. Soil organic carbon (SOC) sequestration is also defined by Olson (2013a; p. 203) as

> *process of transferring CO$_2$ from the atmosphere into the soil of a land unit through unit plants, plant residues and other organic solids, which are stored or retained in the unit as part of the soil organic matter (humus). Retention time of sequestered carbon in the soil (terrestrial pool) can range from short-term (not immediately released back to atmosphere) to long-term (millennia) storage. The sequestered SOC process should increase the net SOC storage during and at the end of a study to above the previous pre-treatment baseline.*

Carbon sequestration by agricultural land has generated international interest because of its potential impact on and benefits for both agriculture and climate change adaptation and mitigation. Where proper soil and residue management techniques are implemented, agriculture can be one of many potential amelioration practices to alleviating the problem of greenhouse gas (GHG) emissions. Additionally, agriculture conservation practices (e.g., the use of different cropping systems and plant residue management, as well as organic management farming) can enhance soil C storage. Farmers, as well as the soil and environment, receive benefits from C sequestration. Agricultural ecosystems represent an estimated 11% of the earth's land surface (USDA–NRCS, 1996, 1998) and include some of the most productive and C-rich soils. As a result, they play a significant role in the storage and release of C within the terrestrial C cycle (Lal et al., 1995). The negative and positive considerations of the soil C balance and emission of GHGs from the soil are: (i) potential increase of CO$_2$ emissions from soil contributing to the increase of radiative force, (ii) the potential increase in emissions of other GHGs (e.g., N$_2$O and CH$_4$) from soil as a consequence of land management practices and fertilizer use, and (iii) the potential for increasing C (as CO$_2$) storage into soils, which equals 1.3 to 2.4 Pg or 10^{15} g C yr^{-1} (Tans et al., 1990), and to help reduce future increases of CO$_2$ in the atmosphere.

The objectives of this review paper are to: (i) define and understand concepts of SOC sequestration, (ii) quantify SOC distribution and the methodology of measurements, (iii) address soil spatial variability at field- or landscape-scale for potential SOC sequestration, and (iv) consider field experimental design including pretreatments baseline for determination of SOC sequestration.

LITERATURE REVIEW

Because of burning fossil fuels, cultivation and draining of grasslands and wetlands, deforestation, and land-use conversions, there has been increased interest in developing methods to sequester atmospheric CO$_2$ (Sundermeier et al., 2005; Baker et al., 2007; Lal, 2009). In agricultural land areas, NT systems have been proposed as an alternative to replace intensive tillage systems such as MP and CP as a way to sequester SOC (Ogle et al., 2012; Luo et al., 2001). Many researchers (Johnson et al., 2005; Liebig et al., 2005; Franzluebbers, 2005; Franzluebbers and Follett, 2005; Lal et al.,1998; Kumar et al., 2012) have suggested that converting from MP or CP systems to NT has a large potential for SOC sequestration, but NT does affect the distribution of SOC within the soil profile (Baker et al., 2007; Luo et al., 2010; VandenBygaart et al., 2003; Sundermeier et al., 2005; Ogle et al., 2012). When NT, CP, and MP are under a non-steady state (Olson, 2010) all three tillage systems may fail to sequester a significant amount of SOC.

Conservation tillage and NT systems along with crop rotation have been implemented to maximize soil C storage or SOC sequestration. Lal et al. (1998) suggested that conversion of a conventional system to NT could result in a 0.50 Mg or 10^6 g C ha^{-1} yr^{-1} sequestration rate, and West and Post (2002) found from global analysis of long-term agricultural management experiments that conversion of plow tillage (PT) to NT can sequester 0.57 Mg C ha^{-1} yr^{-1} over 15 to 22 yr. Results from another study suggested that a NT system sequestered SOC in the top 0- to 20-cm layer at 0.19 Mg C ha^{-1} yr^{-1} for Hoytville soil and 0.17 Mg C ha^{-1} yr^{-1} for Wooster soil in Ohio, USA (Kumar et al., 2012).

While Johnson et al. (2005), Liebig et al. (2005), and Franzluebbers (2005) reviewed and synthesized the results for the central, northwestern, and southeastern regions of the USA, there are questions as to the steady state of the soils reported (Table 1). These specific regional SOC sequestration rates were apparently determined based on many comparison studies within each region (Christopher et al., 2009) with the SOC concentration measured at the end of the tillage study and it was reported that SOC stock was greater in soil under NT than those under MP. These findings of SOC sequestration were based on results of paired comparisons of NT to other conventional tillage systems with no pretreatments baseline SOC concentration measurement and assuming that conventional system baseline was at steady state. Changes in SOC concentration with changes in management can either be related to enhanced tillage disturbance or changes in C inputs. Carbon inputs from outside a

Table 1. Published Worldwide and Regional USA soil organic C (SOC) retention rates or rates of net SOC storage change for a switch from conventional till (CT) to no-till (NT).

Region, USA Worldwide	SOC Retention Rate (Mg C ha^{-1} yr^{-1})	Source reference
North Central	0.48 ± 0.59	Johnson et al. (2005)
Northwest	0.27 ± 0.19	Liebig et al. (2005)
South East	0.45 ± 0.04	Franzluebbers (2010)
North East	-0.07 ± 0.27	Gregorich et al. (2005)
South West	0.30 ± 0.21	Franzluebbers and Follett (2005)
USA	0.50	Lal et al. (1998)
Worldwide	0.48 ± 0.13	West and Post (2002)
Humid climates	0.22	Six et al. (2004)
Arid climates	0.10	Six et al. (2004)

land unit, such as animal waste or other C sources, are considered to be a redistribution of C already in storage and from external sources and not sequestered SOC. These C rich amendments need to be accounted for when they are applied to the land unit. The impact of tillage disturbance applied to a land unit on SOC concentration has been documented. Land unit crop yield, biomass, and residue returned to the soil are usually not significantly different as a result of the tillage treatment (Olson et al., 2013). If the residue returned to the land unit soil is significantly different between tillage treatments and resulted in humus formation increase it could be as a result of SOC sequestration. When the impacts of tillage treatments on SOC sequestration are measured, the C inputs are often statistically similar for all tillage treatments. Therefore, only the effects of tillage disturbance which does have a significant effect on SOC loss were considered.

Balesdent et al. (1990) compared PT with NT and observed that PT practice enhances SOC and N mineralization by incorporating crop residues, disrupting soil aggregates and increasing aeration causing a reduction in SOC and N stocks. Plowing was the primary cause of SOC oxidation and emission of CO_2 to the atmosphere (Al-Kaisi and Yin, 2005a). Drainage of poorly drained, nearly level soils (Olson et al., 2002) and well drained, sloping and eroded soils (Olson, 2010) often resulted in the accelerated rates of SOC oxidation and emission of CO_2. Kern (1994) estimated historical soil C losses in the top 30 cm after cultivation for major field crops in the contiguous USA to be 16% primarily as a result of plowing which caused oxidation of SOC and emissions of CO_2 to the atmosphere. Franzluebbers and Follett (2005) reported that the SOC stock of timberland and prairie soils declined with cultivation in North America. The SOC rate of decline as a result of cultivation was 22 ± 10% for the northeast, 34 ± 24% for the northwest, no value was reported for the central, 25 ± 33% for southwest, and 36 ± 29% for southeast.

Many researchers (Clark et al., 1985; Lal, 2003) have reported that accelerated soil erosion exacerbates C emissions. Lal (1995) suggested that 20% of the C translocated by erosion may be released eventually as CO_2 into the atmosphere, 10% to transport as dissolved C in water bodies, and 70% remains on the landscape often on a lower landscape position. Van Oost et al. (2007a) found that the eroded C gets buried at the bottom of the slope where decomposition rates are slower. According to Baker et al. (2007), this difference in SOC distribution can be attributed to different properties of PT vs. NT soils. In general, NT soils are more compacted with greater bulk density, particularly at the soil surface, than recently plowed soils (Kitur et al., 1993). Furthermore, crop residues left on the soil in NT causes a decrease in soil temperature early in the growing season, which limits root growth (Baker et al., 2007).

West and Post (2002) reviewed 137 paired studies, but in the studies considered, SOC concentration was only measured in the top 15 or 30 cm of soil (Baker et al., 2007). Another review by VandenBygaart et al. (2003), however, included studies where SOC concentration was measured to a depth > 30 cm.

The only difference found was the location of the C accumulation within the soil profile. In NT plots, SOC stocks were concentrated in the top 30 cm, but were dispersed to greater depths in tilled plots (Baker et al., 2007; Blanco-Canqui and Lal, 2008; VandenBygaart et al., 2003).

The many land-use and agricultural system changes over time have had an impact on the SOC concentrations and trends (gains, steady-state, or loss) in the USA. Each change affected the SOC concentrations and trends. Some management practices increased SOC stocks and some reduced them, but SOC concentration seldom reached a true "steady state" since many of these land use and practice changes were adopted at various times and had an impact over different periods (Fig. 1). It usually takes a significant number of years to reach a steady state, especially if the soils are sloping and eroding and SOC-rich sediment is being transported from the land unit (Guzman and Al-Kaisi, 2010).

The real issue that needs to be clarified here is the concept of SOC sequestration as related to the system characteristics, such as NT (Schlesinger, 1999). There is some validity about NT superiority to intensive tillage in terms of SOC retention. However, annual losses of C from SOC storage as a result of oxidation and emissions of CO_2 to the atmosphere at specific sites could be greater than the additional amount of annually stored SOC (in humus) by any agricultural system (Olson, 2010). Thus, the SOC sequestration concept needs to be applied to a specific land unit by establishing boundaries such as pretreatment baseline to ensure rigorous measurements and accurate interpretation of findings in evaluating the effectiveness of management practices in removing CO_2 from the atmosphere and positively increasing the SOC stock above the baseline (Fig. 2) or with reference to the SOC stock before the establishment of treatments. While the NT 0- to 5-cm layer sequestered SOC, the 0- to 75-cm root zone layer lost SOC (above a root restricting fragipan with only trace amounts of SOC) during the 20 yr. In addition to the management and land-use effects on SOC retention, the soil sampling protocol must account for spatial variability.
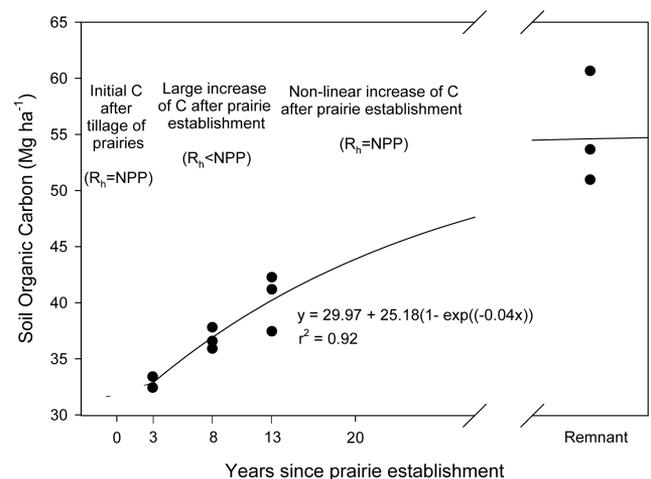

Fig. 1. Soil organic carbon content over a 2- to 14-yr period of reconstructed prairies and potential future increase (Guzman and Al-Kaisi, 2010: J. Environ. Quality 39:136–146).

SOIL CARBON DISTRIBUTION WITH DEPTH

When determining SOC sequestration, storage, or retention it is important to include the entire root zone, which is commonly to a depth of between 1 and 2 m unless there is a root restrictive layer present, such as a very dense horizon, fragipan, or bedrock. Tillage systems can influence SOC distribution and storage or retention in the surface and subsurface layers. Deep tillage, such as MP or chisel, can significantly alter SOC distribution in the soil profile. Soil inversion can completely translocate surface soil SOC to lower depths (Fig. 3). In a long-term tillage study in Illinois (Fig. 2), the NT system showed SOC increase or sequestration in the upper 0- to 5-cm layer, but there was a SOC loss within the 5- to 75-cm layer. The SOC stocks need to be accounted for in the entire root zone to assess tillage system effect and plant contributions to SOC change. Because of the expense and effort involved, most soil sampling techniques focus on SOC measurements within the soil surface layer as a primary metric for evaluating SOC change.

The soil surface however is a reliable marker only for measurement of C concentration characteristics as directly related to a C concentration characteristic below the soil surface at the time of sampling (Wuest, 2009). Deeper than surface soil sampling will not completely overcome a bias caused by bulk density variations and various other soil variables including change in soil surface elevation except when SOC concentration is universally absent (appr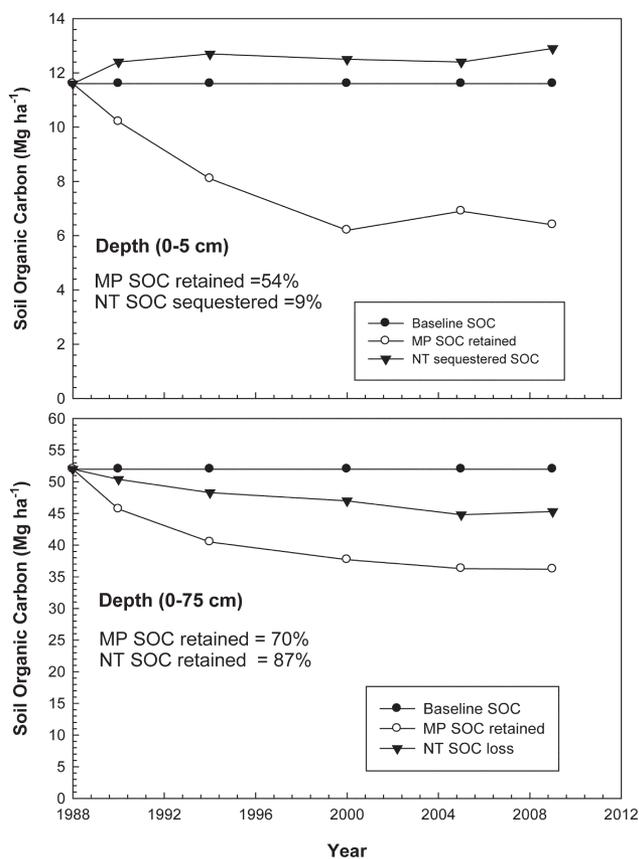oaching zero) at lower depths. Sampling soils to a depth that the SOC concentration is universally absent is recommended (Lee et al., 2009; Wuest, 2009). In most soils, SOC concentration is approaching zero but trace amounts are still present which does not significantly change the root zone total SOC stock in the soil profile at a depth ranging from 0.75 to 1.0 m (Soil Survey Staff, 1968). Equivalent soil mass (mass-depth) instead of linear depth can be used to correct for tillage treatment differences in soil bulk density allowing more precise and accurate quantitative comparison of SOC stocks (Lee et al., 2009; Wuest, 2009; Ellert and Beltany, 1995). Doetterl et al. (2012) proposed an alternative method to measure soil variability with depth. The SOC data should be expressed on equal soil mass per unit area to appropriate depth where SOC concentration is absent. An example of such units would be Mg C ha^{-1} to a 1-m depth.

METHODS TO SEPARATE SOIL ORGANIC CARBON FROM SOIL INORGANIC CARBON AND TOTAL SOIL CARBON

Methods used to quantify SOC concentration can be used to distinguish soil inorganic C (SIC) from SOC. The latter can be measured by dry combustion methods (Soil Survey Staff, 2004) if HCl pretreatment is used to eliminate the SIC before ignition and measurement where the total soil carbon (TSC) value becomes the SOC value (Harris et al., 2001). Soil pH greater than 7.1 has been used by researchers (David et al., 2011) to identify samples with SIC and selected for pretreatment with HCl. The SOC can be measured by wet chemistry method as well as modified acid-dichromate organic C procedure (Soil Survey Staff, 2004). The accuracy of this method is less than CN analyzers since oxidation of organic C is incomplete and requires the use of a correction factor which varies with conditions (Lettens et al., 2007; Meersmans et al., 2009). Another method for determining SIC if the soil pH was > 7.1 is by using a modified pressure calcimeter method (Sherrod et al., 2002; Al-Kaisi

Fig. 2. Soil organic carbon levels in 0- to 5-cm and 0- to 75-cm layers in Grantsburg soils during a 20-yr tillage experiment.
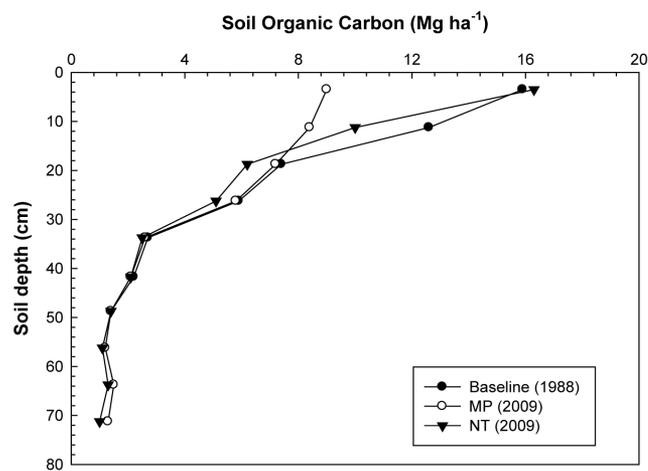

Fig. 3. Soil organic carbon changes with depth in Grantsburg soils after 20-yr tillage treatments. The baseline for the no tillage and moldboard plots were statistically similar and mean was used as baseline for both treatments.

and Grote, 2007) and subtract from the TSC concentration values determined initially by the dry combustion.

An alternative approach, when carbonates are high would be to measure SIC or carbonate and subtract this from TSC using dry combustion to determine the SOC by difference (Soil Survey Staff, 2004). When SOC is measured multiple times in the course of a long-term study, the same laboratory method should be used (Mulvaney et al., 2010). Researchers should not assume soil samples do not have SIC sources, and should take under consideration past liming or naturally formed carbonates even if the soils samples have a pH below 7.0 and then report their total C findings (dry combustion as SOC when no pretreatment with HCl was used [Mulvaney et al., 2010]). This is especially critical consideration if the intention is to determine soil C sequestration and to be in compliance with the definition, any additional carbons (i.e., organic manure, inorganic natural occurrence such $CaCO_3$, or as an amendment as liming) must be assessed to determine SOC change during the study.

SOIL SPATIAL VARIABILITY CONSIDERATION

Natural soil spatial variability occurs at both large and small scales including on almost all plot areas regardless of size and it should be addressed in the experimental design with sufficient replication (Olson and Kitur, 1993). Natural variability in soils will be reflected in different soil parameters and the outcome can vary significantly, if sampling is insufficient to account for such variability (Olson et al., 1985). Different soil texture and type of clay mineralogy can have significant effects on SOC retention. Increase in clay content has been shown to increase SOC concentration (Bationo et al., 2007) and type of clay whether it is 1:1 or 2:1 can have an effect on SOC retention or storage.

Careful evaluation of soil before establishing studies dealing with evaluating SOC sequestration with different tillage and cropping systems is essential. Sequestration of SOC as it was previously defined depends on the interaction between soil and plant. Therefore, soil differences can be reflected in plant performance, productivity, and biomass production as main contributors to SOC input in the soil system. It is helpful to document and demonstrate spatial variability utilizing tools such as three-dimensional (3-D) map of the soil to understand the interaction effects of land management practices and soil type on SOC distribution (Grunwald et al. 2001a, 2001b, 2001c; Arriaga and Lowery, 2005). The use of a soil sampling grid that accounts for surface variability, but also accounts for below soil surface variability within the root zone will provide more accurate accounting for SOC stocks changes or retention.

MICROBIAL ROLE IN SOIL ORGANIC CARBON SEQUESTRATION OR NET STORAGE

To understand SOC sequestration it is imperative to shed light on the role of soil microbial activities and how they influenced by management practices such as cropping and tillage systems and subsequent effects on SOC sequestration (Lupwayi

et al., 1999; West and Post, 2002). Soil microbial activity and biodiversity are essential components for any agro-ecosystem to sufficiently sequester SOC. Soil microbial contribution to soil organic matter (SOM) is influenced by the microbial community size, dynamics, and process of formation and decomposition that influence their stability (Six et al., 2006). However, changes in tillage and cropping systems are some of agricultural practices can influence the dynamic microbial activities, which ultimately affect SOC dynamic and stability (Scow, 1997; Paustian et al., 1997). Soil organic C change is essentially determined as a balance between organic C input from above and below ground plant sources and organic C losses as result of decomposition, erosion, and leaching, which influenced by soil topography, drainage class, and management systems (i.e., tillage and crop rotations). As soil microbial activity influenced by tillage and cropping systems, SOC change will eventually be affected as demonstrated by root and basal respiration rate, where significant differences have been observed between different tillage and cropping systems (Wardle, 1995; Guzman and Al-Kaisi, 2010). During the respiration and decomposition process, which will be accelerated by intensity of tillage system, the C in plant residue and some in various other soils' C pools are released to atmosphere as CO_2. This release rate of CO_2 can be accelerated over time as more intensive tillage takes place, which can influence SOC stability (i.e., steady state of SOC; Cole et al., 1997). Intensive tillage increases soil aeration resulting in increased residue decomposition due to increase in biological activities.

Since soil microbial communities play a significant role in regulating SOC dynamics, any shift in such activities in response to different tillage and cropping systems (Fig. 1) play critical role in determining the outcome and rate of SOC gain or loss as CO_2. Determining the quantity of atmospheric CO_2 sequestered in the soil depends on the stability of the agriculture system (no disturbance), metabolism of the ecosystem, where atmospheric CO_2 is fixed by plants, and converted into organic C compounds through photosynthesis as a mechanism for C sequestration. During SOC decomposition, CO_2 is released through heterotrophic respiration, which is strongly correlated with soil temperature and moisture regimes (Linn and Doran, 1984; Davidson et al., 1998; Luo et al., 2001), residue lignin/nitrogen ratio (Geng et al., 1993; Melillo et al., 1989), and the role of different microbial types in decomposition process. Portions of root and litter that resist decomposition develop into a stable form of SOM that can last for hundreds and thousands of years before it is broken down by microbes (Luo and Zhou, 2006). However, only about 10% of soil surface CO_2 efflux is derived from decomposition of older, more recalcitrant C compounds (Gaudinski et al., 2000). When coupled with potential C input from above- and belowground biomass, C loss from soil surface as CO_2 efflux can be used to determine annual C sequestered as shown by researchers using a C budgeting approach (Dugas et al., 1999; Frank and Dugas, 2001; Flanagan et al., 2002; Yazaki et al., 2004; Kucharik et al., 2006).

During a long-term experiment (20–50 yr), most of the SOC sequestered in early years could be released in later years and before the end of the experiment. Therefore, the SOC sequestered during the long-term experiment is not in permanent storage. This loss will accelerate each year with some of the previous net SOC storage being released in subsequent years as a result of tillage disturbance during planting or synthetic N application leading to change in soil environment and subsequent change in soil microbial activities and increase in mineralization process, which would effectively reduce SOC sequestration rate over time (Fig. 4). The interaction between management practices and soil microbial community is complex, but it plays a significant role in influencing the pathways of SOC retention and loss.

IDENTIFYING APPROPRIATE EXPERIMENTAL DESIGN

Identifying proper experimental design and treatments are critical considerations in addressing the question of soil C sequestration in any tillage and cropping systems experiments. Chappell et al. (2013) found that measuring an increase in SOC stocks over a C estimation area over time is a prerequisite to demonstrating that SOC has been stored in the land unit. Given the well-known spatial variability and dynamic nature of soil C, the inclusion of a pretreatment baseline particularly in tillage studies is essential to monitor over time the different tillage and cropping systems effects on the rate of SOC sequestration. Many current studies have been established with omission of such consideration particularly in the case of evaluating NT as compared with intensive tillage systems for SOC sequestration. However, it is possible to somewhat overcome this deficiency if time-sequence samples are taken. It is suggested that these be taken on a temporal scale of a minimum of yearly sampling.

The underlying assumption in paired comparison studies is that intensive tillage is at a steady state, which may or may not be the case. Significant body of published research used paired comparison between various tillage treatments with one treatment, such as MP, used as baseline or control is in steady state
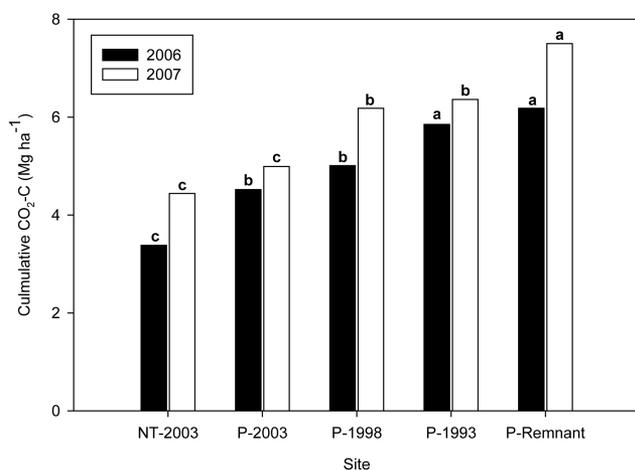

Fig. 4. Cumulative soil CO_2–C effluxes for each site for 2006 and 2007 growing seasons (Guzman and Al-Kaisi, 2010: J. Environ. Quality 39:136-146).

may be in error (Franzluebbers, 2010; Johnson et al., 2005; Liebig et al., 2005; Franzluebbers, 2005; Franzluebbers and Follett, 2005; Lal et al., 1998). Moreover, these comparisons are often sampled only once during or at the end of a long-term study to determine the amount and rate of SOC sequestration. If the assumption of steady state is true then any increase in SOC of the comparison treatment (NT) with above baseline treatment (MP) at the end of study would represent the amount of SOC sequestered. Without a pretreatment baseline SOC values (MP), the SOC sequestration magnitude and rate findings cannot be verified (Olson, 2010; Fynn et al., 2009; Sanderman and Baldock, 2010a, 2010b). It has been shown that the SOC sequestration rate based on a NT comparison with SOC stocks of the baseline (MP) treatment in the final study year (West and Post, 2002) might not be correct if baseline (MP) treatment is not at a steady state. It is possible that all treatments including baseline treatment (MP) lost SOC over time (Olson, 2010) and if so the differences between treatments at the end of the study means that one treatment (NT) lost less SOC or retained more SOC in storage and it is losing SOC at a lower rate. Therefore, any loss in pretreatment SOC stocks for baseline treatment (MP) during the long-term experiment must be subtracted from the comparison treatment (NT) SOC stocks gain since the MP baseline is not at a steady state.

STANDARDIZED PROCEDURE FOR MEASURING SOIL ORGANIC CARBON STOCKS

There is a need for a standardized approach to measuring SOC concentration with respect to SOC sequestration, and pre-experimental sampling is suggested. In this regard a paired comparison design should be approached carefully. Paired comparison can work for many agronomic and soil determinations, but may not be ideal for SOC sequestration. Paired comparisons are a valid approach if the objective is to determine differences between treatments in a soil property after a long-term study and the difference can be determined in the last year if replications are sufficient. It is often reasonable to assume each treatment, such as different tillage treatments in a randomized design, had the same level of a property, such as SOC concentration, at the beginning of the study even if that concentration is not known. The pair comparison method has been used successfully in many agronomic studies where the change in soil property by treatment or yield response difference between treatments is important (Olson et al., 2013). However, this is not the case for SOC sequestration since the difference between treatments only highlights gain or loss in SOC stocks over certain time.

Losses in SOC stocks can be associated with water erosion, conventional tillage soil disturbance, and disturbance during NT planting, intensification of mono-cropping systems, disturbance during N injection, leaching of soluble SOC, aeration, and SOM mineralization. This can and often does offset the gains in SOC stocks from the aboveground biomass, root system, and crop residue C inputs to the humus or SOM.

Previously eroded soils with low SOC concentration were identified as having significant potential to sequester SOC (Lal et al., 1998; Lal, 1999). In a 20-yr study of previously eroded soils on 6% slopes with low SOC concentration, the amount and rates of SOC storage and retention as a result of a conversion to NT or CP tillage systems using the comparison method with MP tillage SOC stocks as a baseline have also been quantified (Olson, 2010). The findings of this study showed no SOC sequestration occurred in the NT, CP, and MP plots since the SOC stocks of the plot area was higher at the start of the experiment than at the end of the study (Table 2). The NT plots did retain or store more SOC stocks after 20-yr than MP. However, there was no increase in sequestered SOC in NT system as compared with pretreatment baseline SOC stocks of the same treatment plots.

Any loading of external SOC rich sediment or materials (not directly from the atmosphere through unit plants to the soil humus) such as sediment deposition must be accounted for in the analysis and should be deducted from the amount claimed as SOC sequestration since it does not satisfy the definition or concept of SOC sequestration. Organic amendments represent OC source that was fixed in a different location and transported and applied to the land unit and incorporated into existing SOC (Schlesinger, 1999). Such practice does not involve any transfer or storage of atmospheric CO_2. On the contrary it can be source for CO_2 emission due to OC decomposition. It is the stored SOC that is transported and applied to the plots from outside the land unit that can be considered retained C. The addition of C amendments to a land unit is not likely a very stable C pool and would most likely increase the amount of CO_2 emission from the land unit during decomposition to the atmosphere.

DYNAMICS AND CONTRIBUTION OF AGRICULTURAL MANAGEMENT PRACTICES TO SOIL ORGANIC CARBON STORAGE OVER TIME

There is a growing body of scientific literature related to the contribution of agronomic best management practices (BMPs) to SOC sequestration (Huggins et al., 2007; Luo et al., 2010, Sanford et al., 2012; Kumar et al., 2012, Van Doren et al., 1976; Pierce and Fortin, 1997; Lyons et al., 1997; Hendrix,

1997; Olson, 2010). These researchers found that SOC stocks declined from pretreatment levels even with long-term NT studies. Sanford et al. (2012), evaluated the influence of six cropping systems on SOC stocks at the Wisconsin Integrated Cropping System Trial over a 20-yr period. Analysis of SOC on either a concentration or mass per volume of soil basis indicated a significant decline (lost 5.5 Mg C ha^{-1} or 0.28 Mg ha^{-1} yr^{-1}) across all of the systems. Results from this study demonstrate the importance of: (i) comparing current and initial soil samples when evaluating SOC sequestration and (ii) evaluating changes in SOC stocks throughout the entire soil profile and not limited to the top depth. The losses of SOC stocks at depths below the tillage zone are either a result of loss of SOC inputs from roots, translocation or due to oxidative loss at these depths or both.

In Ohio, SOC data were collected from Wooster and Hoytville plot areas before establishments of the tillage treatments (Van Doren et al., 1976). It was reported that before the establishment of the Wooster tillage plots in 1962 the SOC concentration was 14 g kg^{-1} for the 0 to 15 cm (Ap horizon). The Hoytville plots had a 22 g kg^{-1} SOC concentration for the 0 to 15 cm (Ap horizon). In 2011, Kumar et al. (2012) reported SOC data for the same sites for PT treatments for the 0- to 40-cm layer in 10-cm intervals. One can adjust these values for comparison between 1962 and 2011 by proportionally weighting the 2011 SOC values by combining the 0- to 10-cm top layer with half of the 10- to 20-cm layer. The adjusted 2011 SOC concentrations for the adjusted 0- to 15-cm layer are estimated to be 12.5 g kg^{-1} in Wooster PT plots and 14.3 g kg^{-1} in the Hoytville plots (Olson, 2013b).

Based on the measured SOC concentrations from Van Doren et al. (1976) in 1962 and 1964, and the 2011 data from Kumar et al. (2012), one can estimate a loss in the Wooster plots of 1.5 g kg^{-1} (11%) and in the Hoytville plots of 7.7 g kg^{-1} (35%). These data suggest that the Wooster and Hoytville PT plots were not at a steady state and were losing SOC at a significant rate, 11 and 35%, respectively. This finding does not support the use of PT as the baseline for NT when determining SOC sequestration. The exact amount of net SOC sequestration can only be documented with pretreatment SOC concentration and bulk density measurements which were not reported. There are en-

Table 2. Twenty-year effects of tillage treatments (six replications) on soil organic C (SOC; Mg C ha^{-1} layer^{-1}) of the Grantsburg soil. Paired comparison with 2009 MP baseline and pretreatment 1988 baseline methods (Olson, 2010).

Tillage treatment[†]	Depth	September 1988 (pretreatment baseline)	June 2009	Pretreatment 1988 baseline method 20-yr SOC loss (below pretreatment 1988 baseline)	Paired comparison method with MP as baseline NT vs. MP 20-yr SOC retention rate difference (NT above 2009 MP baseline)
	cm	——Mg C ha^{-1} layer^{-1}——		————Mg C ha^{-1} layer^{-1}————	
NT	0–15	28.5a**	25.2a**	-0.17a	+0.40
	15–75	23.6a	20.1a	-0.18a	+0.06
	0–75 (all)	52.1a	45.3a	-0.35a	+0.46
MP	0–15	28.3a	17.3b	-0.55b	
	15–75	23.1a	18.9a	-0.21a	
	0–75	51.4a	36.2b	-0.76b	

** Mean of six replications with the same letter and in the same year and depth with a different tillage treatment are not significantly different at $P = 0.05$.
† NT, no-till; MP, moldboard plow tillage.

vironmental conditions and production input variables that could result in continuous variations in the rate of SOC concentration or stock gains and losses over time, but these were not reported. Furthermore, in 2011 the Wooster PT plots were still showing a 37% SOC loss when compared with a woodlot (WL) and Hoytville PT plots are still showing a 60% SOC loss when compared with WL (Olson, 2013b). If the PT was truly at steady state then all this SOC loss had to occur in the 25 to 30 yr (between 1930s and 1962 or 1964) before the tillage treatments were applied. After 47 and 49 yr of NT treatments, the losses were only 32 and 55%, respectively or a reduction of 5%. This comparison suggests that SOC sequestration is less than reported by Kumar et al. (2012). This example highlights the need to use pre-treatment SOC concentrations or stocks for C sequestration studies. Support for treatment of C sequestration should be a net increase in SOC storage during and at the end of a study above the pretreatment baseline SOC stocks (Olson, 2013b).

Huggins et al. (2007), in a study of tillage and crop rotations effects on SOC in southern Minnesota, reported losses of 3.7 and 1.6 Mg SOC ha^{-1} for low productivity or aggressively tilled soils and high productivity or minimally tilled systems, respectively. These findings were supported by Luo et al. (2010) in a meta-analysis of 69 paired experiments, where they found that conversion from conventionally tilled to NT farming practices affected the SOC stocks location, but not the amount of SOC (<40-cm soil depth) under similar crop rotations. Other researchers (Hendrix, 1997; Lyons et al., 1997; Pierce and Fortin, 1997; Olson, 2010) findings also documented a decline in SOC stock under NT as compared with pretreatment SOC stocks over time. All these findings warrant the consideration and careful use of pretreatment baseline as we deal with very dynamic systems regardless of the type of tillage or cropping system for proper interpretation and documentation of those systems effects on SOC sequestration. The value of conservation systems and NT in particular is the potential reduction in soil CO_2 emission as relative to tillage disturbance where SOC oxidation and mineralization can lead to significant SOC loss. Therefore, NT systems may not be fully adequate for SOC sequestration, but comparatively to CT systems can be very effective in minimizing soil C emission, thus increasing soil C retention and its value for improving soil health.

METHODS FOR DETERMINING SOIL ORGANIC CARBON SEQUESTRATION

As it was discussed previously, different experimental designs and methods have been used in determining SOC sequestration, thus this section presents a case study designed to compare common methods used to determining SOC sequestration using paired comparison and pretreatment baseline methods. The paired comparison method evaluates the relative SOC dynamics between treatments whereas the pretreatment baseline method evaluates absolute changes in SOC concentrations or stocks that can be used to determine SOC sequestration rates. A study site was selected at Dixon Springs, IL to

determine the SOC sequestration rate for a 20-yr time period (Fig. 2 and 3). The plots are on 6% slopes with a moderately eroded phase of Grantsburg silt loam (fine-silty, mixed, mesic Oxyaquic Fragiudalfs). The pair comparison method used by many researchers with MP tillage system as baseline suggested that 0.455 Mg C ha^{-1} yr^{-1} of SOC sequestration occurred during the 20-yr experiment with NT (Table 2). However, the use of pretreatment baseline SOC of the experiment before establishment in 1988 approach did not validate the SOC sequestration rate value. At the same site, the sloping and eroding NT plot using a pretreatment baseline approach indicated that a reduction of 0.34 Mg C ha^{-1} yr^{-1} occurred during the 20-yr study (Olson, 2010). The fact that the same plot area was used and the findings were so different (0.455 Mg C ha^{-1} yr^{-1} of SOC sequestration vs. 0.34 Mg C ha^{-1} yr^{-1} loss) suggests that the paired comparison was not valid to determine SOC sequestration since the MP treatment was not at steady state and continuously losing SOC (lost 30% of the SOC [15.2 Mg C ha^{-1} layer^{-1}]) to the lower slope, waterway, or into the atmosphere during the 20-yr experiment. The initial 17% gain (9.1 Mg C ha^{-1} layer^{-1}) in SOC stocks (NT vs. MP) would have to be reduced by the magnitude of the decline in MP- SOC stocks over the 20-yr experiment. Therefore, NT would have lost 13% (6.8 Mg C ha^{-1} layer^{-1}) of the net SOC stored in those plots before the 20-yr experiment and that no actual SOC sequestration occurred (Table 2).

Research findings of several studies (Huggins et al., 2007; Luo et al., 2010; Sanford et al., 2012; Kumar et al., 2012, Van Doren et al., 1976; Pierce and Fortin, 1997; Lyons et al., 1997; Hendrix, 1997; Olson, 2010) suggest that a pretreatment for SOC baseline is essential in all tillage comparison studies to determine the amount and rate of SOC sequestration, storage, retention, or loss.

If the researcher (Olson, 2010) had decided to use NT on the entire plot area instead of including MP or any intensive tillage treatments, the NT plots would have resulted in retention of more SOC storage after 20-yr or lost significantly less per year (0.455 Mg C ha^{-1} yr^{-1}) from the areas converted to NT (Table 2). Consequently, the reduction in GHG emissions as a result of using only NT in the plot area would have resulted in more net SOC storage or retention after 20-yr and would have resulted in less GHG emissions from the pretreatment SOC stocks stored or retained in the soil. In this study, NT increased SOC retention on sloping and eroding sites and reduced the GHG emissions from soil when compared with MP or any of the intensive tillage treatment, but this does not mean NT is sequestrating SOC; it is often just retaining more or losing less.

The effect of plot size relative to landscape position is also important. If the MP plot being measured is confined to a convex shoulder or steep back slope, significant amounts of SOC will be lost due to translocation from the plot area, but not necessarily from the field or landscape. A significant amount of SOC may be found in depositional parts of the landscape. In this case the magnitude of the negative SOC sequestrations rates reported for the MP plots would not apply to the entire hillslope or field.

Additionally, some of the SOC stocks could be buried in the depositional areas and less subject to microbial decomposition. This issue was discussed by Liu et al. (2003) and by Van Oost et al. (2007b). Liu et al. (2003) found that failing to account for the impact of soil erosion and deposition may potentially contribute to an overestimation of the total historical C released from soils. Using Cs^{137} and C inventory measurement from a large-scale survey, Van Oost et al. (2007b) observed consistent evidence for an erosion-induced sink of atmospheric C equivalent to approximately 26% of the C transported by erosion. Overall, soil erosion and deposition reduced CO_2 emissions from the soil into the atmosphere by exposing low carbon bearing soil at eroding sites and by burying SOC stocks at depositional sites. Van Oost et al. (2007b, 2012) suggested agricultural erosion does not represent an important source or sink for atmospheric CO_2.

DIFFERENTIATION BETWEEN SOIL CARBON LOADING, TRANSLOCATION, AND CARBON SEQUESTRATION

One source of the conflicting research findings relate to the general nature of the definition of SOC sequestration (Olson, 2013a; Lal, 2007), that does not account for the loading of SOC from rich sediment (e.g., eroding and transported sediment C from outside the land unit) and any applied organic amendments (e.g., manure or waste materials) and considered as SOC sequestration. These external inputs can include natural input processes, such as, soil erosion of a sloping soil and sediment-rich C deposition on a soil located on a lower landscape position or in a waterway. The land unit could be a plot, plot area, parcel, tract, field, farm, landscape position, landscape, wetland, forest, or prairie with defined and identified boundaries. Atmospheric C is cycled to the plant by photosynthesis, the plant cycles the organic C to the soil and it becomes part of the SOC pools. The SOC sequestration definition needs meaningful boundaries to be used by researchers who want to measure actual changes in a specific part of a terrestrial (soil) C pool.

Loading of SOC-rich amendments from an external source onto a land unit creates a number of issues. There is a perception by some researchers that the application of manure or other source of C from external or outside the land unit sources can be considered as C sequestration (Nafziger and Dunker, 2011). This assumption is not valid (Schlesinger, 1999) since it violates the definition of SOC sequestration stated earlier in this paper. The same argument holds for application of biochar (Lehmann and Joseph, 2009) and other C-rich amendments. No atmospheric CO_2 is converted and stored as a result of amendment transfer and application and it does not help reduce global warming that is the essence of C sequestration. These amendment cases illustrate the necessity of specifying the land unit with borders and identification of the origin of the SOC sources to accurately measure the change in SOC content derived from atmospheric CO_2 by a specific land unit. This specification prevents the loading of SOC rich sediment or materials from outside the land unit boundaries to be claimed as SOC sequestration. The addition of land unit boundaries in the definition of SOC sequestration prevents overestimation (David et al., 2011) or underestimation of the SOC sequestered. However, the use of organic amendments, such as animal manure and biochar is a valuable source for improving SOM and nutrients pool that are essential for sustaining soil productivity and health.

EXTRAPOLATING FIELD SCALE FINDINGS OF SOIL ORGANIC CARBON SEQUESTRATION TO REGIONAL AND GLOBAL SCALES

Credible information on SOC stocks and fluxes is needed at different temporal and spatial scales to make these data suitable for policy imperatives and for promoting adoption of BMPs (Wang et al., 2010). Estimates of SOC stocks and fluxes can vary widely over geographical space and through time (Zhong and Xu, 2011). Yet, quantification of SOC stocks at high spatial and temporal resolutions in response to land use and management is needed as a decision-support tool, such as meeting the commitments of the Kyoto treaty and other subsequent ones for trading C credits (Wise et al., 2009). However, there are no standardized methods of scaling up data from a point source (pedon) to regional, national, or global levels, and there are no standardized procedures to quantify uncertainties associated with such estimates. Thus, regional estimates for SOC stocks and fluxes can differ according to the scaling procedure (Krogh et al., 2003) and the specific variable(s) used. For example, Wang et al. (2010) observed that soil texture had a greater effect on SOC density than some climate factors at the city and the county scales. Further estimates of SOC stocks at the national level can differ by a factor of 2 or more (e.g., estimates of SOC stocks in soils of UK by Howard et al. (1995) vs. Smith et al., 2000).

Two requirements of scaling up the data are choice of key soil parameters and the scaling methods. Choice of a simple group of parameters is often as good as or better than more complex variables (Bridgham et al., 1998). The parameters(s) chosen must facilitate scaling efforts in landscape analysis. Schindlbacher et al. (2008) concluded that even a single parameter can be sufficient to scale up for example the temperature-sensitivity of CO_2 flux. Thus, selection of site variables is critical to the scaling (Tan et al., 2004, 2006; Kumar and Lal, 2011).

Among the scaling methods, geostatistical techniques have been widely used in assessing SOC stocks (Stutter et al., 2009). These techniques are able to characterize the spatial continuity of regionalized variables. The Universal Kriging technique (Simon et al., 2013) has been revised and updated for site-specific situations (Tan et al., 2006; Mishra et al., 2009, 2010). Kriging techniques can be used to map spatial distribution of SOC stocks over a state or a region (Kumar and Lal, 2011). Orihuela-Belmonte et al. (2013) proposed a nested approach in which point-scale data can be used to map landscape and regional estimates of SOC stocks.

Uncertainties in scaling up are also exacerbated by hydrological flow paths which can influence the biogeochemical processes (over a watershed scale). There is a strong sensitivity of

watershed level processes to spatial scale, and a large variation in elemental fluxes can occur in the first 10 to 20 ha of the drainage area (Johnson et al., 2000). Thus, understanding the fate of SOC transported by erosional processes (Lal et al., 1998; 2003; Van Oost et al., 2007a) is also affected by spatial scale within the watershed. Similarly, scaling up the data on SOC depletion by land use conversion may be affected by the north-south climate gradient across the watershed or a region (Elberling et al., 2003). On the other extreme of the spatial scale, protection of SOC in microaggregates (<200 mm) within large macroaggregates can strongly influence the long-term stabilization of SOC stocks (Bossuyt et al., 2005).

Despite the great need for credible data, there is lack of standardized procedures of scaling up the SOC stocks data and rate of change (Suuster et al., 2012). Thus, scaling procedures need to be developed and standardized from a molecular to watershed levels, and nanosecond to decadal and millennial scales. The need for scaling up is simply to transfer field and laboratory studies findings to regional and global application in a standardized approach to address the concerns of C sequestration in meeting the increasing concerns with global warming.

A modeling approach has been used by many scientists to quantify or extrapolate based on changes in SOC stocks which take place as a result of treatments that have been implemented in the field such as, tillage management, crop rotation, conservation practices, integration of prairie system, etc. The extrapolation of field findings can be accurate to a certain degree if the assumptions and the clustering of soil physical and chemical properties are accurate. It is well documented that soil physical properties such as texture, drainage class, soil erodibility, topography, type of cropping system or vegetation, and regional climate are critical factors in determining the potential of C sequestration.

One of the challenges in determining global C sequestration and the movement of the C between the atmosphere, pedosphere, lithosphere, and biosphere is measuring the change between each sphere which is impossible. The current state of the science makes it impossible for most researchers, with the possible exception of modelers, to quantify changes in both the terrestrial and atmospheric pools. The alternative to making global measurement is to establish a unit field, plot, forest, prairie, farm, or tract of landscape with boundaries and then account for any SOC transported, redistributed, or loaded on to that land unit. Once the SOC sequestration amount and rate is determined one can use pretreatment and after treatment measurement to determine if the SOC has been sequestered, stored, retained, or lost. The measured land unit findings can then be scaled up to a global scale.

Some of the challenges in developing a standardized approach for global SOC sequestration is the lack of uniform experimental design or approach that will include essential parameters such as the determination of SOC concentration using the same method (e.g., dry combustion), standardized soil depths that will extend below the top 30 cm, actual measurements of bulk density for each depth and for different tillage systems, lack of pretreatment baseline, and similar cropping systems to name

few. When confronted with a lack of actual bulk density measurements several scientists have suggested or practiced the use of estimation approach such as regression functions to determine bulk density based on clay and sand particle fractions and percentage of SOM (West and Post, 2002; Chen et al., 1998). There are several problems with such approach which may lead to over or underestimation of SOC sequestration. One, the bulk density is not only affected by clay and sand contents, but also by field moisture conditions when bulk density is collected and potential compaction during sampling (Lee et al., 2009), two, type of tillage system has great influence on soil bulk density where well established NT tends to have much different bulk density values than conventional tillage, especially in the tillage zone of 30 cm or more (Al-Kaisi et al., 2005c). Another pitfall in determining regional and global SOC sequestration tends to be focusing only on the top 30 cm or less (West and Post, 2002). It is well documented that SOC allocation with depth is highly influenced by tillage system and the degree of soil inversion, especially with conventional tillage systems, such as MP (Al-Kaisi et al., 2005c).

The Intergovernmental Panel on Climate Change (IPCC) developed guidelines for SOC accounting including C sinks in forest and agricultural ecosystems (Houghton et al., 1997). The recommendation by the IPCC was to use a multiplication factor of 1.1 for converting from CT to NT (Houghton et al., 1997), which means an increase in SOC by 10%. This assumption view is also not valid based on the following facts: (i) there is an inherent assumption that CT is at steady state, which is not correct in all cases; and (ii) there is no pretreatment baseline for SOC stock, which is essential for determining or validating the SOC sequestration. These concerns need to be addressed in attempting or developing regional or global account for SOC sequestration. To extrapolate from field scale to regional or global scale, experiments must be standardized to meet the criteria outlined previously to avoid any confounding effects when extrapolating from small scale to a large scale in accounting for SOC sequestration.

One of the hazards of IPCC approach is implicit and explicit discontinuities that occur from the scaling process. Empirical studies by definition set boundaries to their measurements. Scaling issues arise when transfers occur across boundaries with feed forward and feed backward loops between bounded units resulting in nonlinearities that are difficult to predict. Another modeling approach that is not addressed in this review is process based models that are less dependent on extrapolation of empirical results in the same way as a statistical model. Process models are based on empirical studies for elucidation of universal relationships, but are less dependent on unaccounted quirks associated with bounded empirical studies.

CONCLUSIONS

Previous soil science research has built an essential foundation to assess the capacity of soil to store and retain SOC. However, there are inconsistencies in the understanding and application of SOC sequestration concepts and experimental designs and methods used to determine SOC sequestration. Most critical is the

inability to accurately verify C drawn from the atmosphere and sequestered in the land unit soil. It is important to determine SOC sequestration rates for various agricultural land treatments and to establish a protocol to validate the rate of SOC sequestration.

In this paper, it has been proposed that field experimental methods and procedures should be selected carefully to measure, monitor and assess internal and external C inputs. The amount of SOC lost from the soil storage during the time of treatments application in an experiment needs to be accounted for before and during the determination of SOC sequestration amount to determine the change in net SOC storage in a highly dynamic and variable system. The proposed protocols that have been discussed are necessary to move the science forward and to attempt to address future predicted climate trends. The amount of SOC sequestered as a result of alternative agricultural systems such as NT and its effects on the net change in SOC stocks storage in terrestrial C pool and SOC released to the water and atmospheric pools need to be measured or calculated. If losses from the terrestrial C pool are greater than gains in SOC stocks during the time of the experiment then no net SOC sequestration would have occurred and the release of CO_2 gas would have increased rather than being depleted in the atmosphere. Additional investments in SOC research is needed to better understand the agricultural management practices that are most likely to sequester SOC or at least retain more net SOC stocks.

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