Advances in measuring soil organic carbon stocks and dynamics at the profile scale

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1 Introduction

Quantification of soil organic carbon (SOC) stocks and dynamics is required for and applied to different frameworks, situations and scales, ranging from an experimental plot (for evaluating treatment effects) to whole fields (e.g. in SOC-accounting or crediting systems) and inventories of whole countries or continents (Orgiazzi et al., 2018, Saby et al., 2008, Bolinder et al., 2010, Viscarra Rossel and Brus, 2018). Accurate estimates of belowground stocks of carbon are necessary for understanding ecosystem responses to changes in land use, management, climate and other environmental stresses. Monitoring of SOC stock responses to management has gained interest in recent years due to the call to raise ambitions for countries to include SOC storage in climate change negotiations (Wiese et al., 2019). At the same time, the opposite is also the case: understanding the impact of these drivers and underlying processes is key for designing appropriate frameworks for monitoring, reporting and verification (MRV) of SOC stock changes (Smith et al., 2020).

The estimation of SOC stocks is challenging at any scale because it requires measurement of several key parameters including SOC content, bulk density and rock fragments, some of which are highly variable in space and time, and are prone to sampling biases and other pitfalls. It is therefore even more

challenging to measure and verify changes in SOC stock, which are usually small compared to the total SOC stock and its spatial heterogeneity (Smith, 2004). Of all the parameters mentioned, the one with the largest spatial heterogeneity (both vertically and horizontally) is usually SOC content (Goidts et al., 2009). An appropriate sampling design, which accounts for the scale-dependent *in situ* variability, is thus crucial for representative sampling and quantification of SOC changes (VandenBygaart and Angers, 2006, VandenBygaart, 2006). Detecting change in SOC stock usually requires a large number of samples to account for spatial variability, that are collected at sufficiently long intervals to allow for changes to be detectable (Saby et al., 2008, Schrumpf et al., 2011, Heikkinen et al., 2020, de Gruijter et al., 2018, Schöning et al., 2006, Viscarra Rossel et al., 2016). Our focus in this chapter will be to describe the methods, techniques and approaches used to measure SOC stocks and dynamics in the soil profile. The spatial variability of SOC stocks and their associated parameters and the sampling designs to best tackle this issue will not be discussed in detail. Instead, our objective here is to provide a general introduction to traditional, state-of-the-art and novel techniques and frameworks used to quantify SOC stocks and their dynamics.

Characterizing SOC dynamics refers not only to quantitative changes in bulk SOC over time, but also to the partitioning of SOC in fractions with distinct turnover kinetics, their residence times and stabilization pathways. The concept of fractionating SOC to derive these functional pools is introduced, as well as the use of C isotopes to accurately trace inputs in the soil profile. We also discuss techniques used to characterize and predict changes in SOC stocks for evaluating environmental or management changes.

2 Accurate estimation of parameters needed to calculate soil organic carbon stocks

2.1 Sampling and sensing methods

Three parameters are required to estimate SOC stocks at a given soil depth: (1) SOC content, which is usually measured in the fine soil fraction < 2 mm; (2) bulk density (mass of soil per volume); and (3) the fraction of rock fragments > 2 mm, which are usually considered to be free of SOC. An appropriate sampling design for the determination of these parameters for a particular spatial unit depends primarily on the size and properties of that unit (Goidts et al., 2009). Collecting a sample that contains a representative amount of SOC for the spatial unit of interest is not only the major goal, but also a major challenge of soil sampling. Here, we focus on the smallest spatial unit of field sampling – the soil profile – to introduce some basic techniques and pitfalls for sampling soils for subsequent measurements of the required parameters.

The nature of the 'soil profile' and adequate sampling depth are important considerations when measuring and evaluating SOC stocks. Globally, the thickness of the layer that is usually defined as soil, that is, loose material above the bedrock that underwent (initial) pedogenesis and is biologically active, mostly exceeds 1 m (https://www.isric.org/explore/wise-databases). Only in regions characterized by strong erosion processes, such a mountain areas or deserts, as well as regions of initial soil development, can the soil thickness be much lower. Accordingly, the upper 30 cm of soil, which is the standard sampling depth in many experiments and inventories, stores only half of the total SOC stock on a global scale (Batjes, 2014), which implies that SOC storage and storage potential are strongly underestimated when only topsoils are considered. Furthermore, neglecting SOC in deeper layers may also lead to false conclusions about SOC dynamics (Luo et al., 2010, Hicks Pries et al., 2017).

The ability to detect either temporal or spatial changes in SOC stocks depends on the depth of sampling because deeper sampling usually increases the variability of the measurement (Ellert et al., 2008). However, increasing the depth of sampling increases the cost and effort to do so. Thus, a primary consideration with regard to the appropriate sampling depth relates to the soil management or process under investigation. Factors such as land use, cropping system, tillage management, rooting depth, landscape position, drainage class and other soil-forming factors affect the quantity and distribution of SOC with depth, and will therefore influence decisions on appropriate sampling depth. For example, in studies comparing contrasting tillage systems, the depth of sampling required to determine difference in SOC stocks may be just below the depth of plowing (e.g. 30-40 cm). In contrast, an assessment of SOC in systems with deep-rooted crops or those that receive additions of large amounts of organic amendments may require sampling below this depth (Tautges et al., 2019).

The sampling techniques and methods used to determine the SOC content can be used for any other variable to be measured in 'disturbed' soil samples (as opposed to intact soil cores). However, consideration of the depth gradients within the soil profile is of particular importance for elements (e.g., C, N and P) contained in soil organic matter. In the vast majority of soils, the distribution of SOC along the soil profile shows a clear depth dependency, with decreasing SOC at increasing soil depth. This is primarily related to the OC inputs to the soil, which are highest close to the surface and exponentially decline with depth (Jackson et al., 1996). In agricultural soils, particularly in tilled arable soils, the depth distribution of SOC differs from that of soils under natural vegetation, due to the homogenizing effect of tillage. The depth gradient within the plow layer can thus be expected to be relatively small, which, however, causes a sharp decline of SOC at the border between plow layer and the underlying subsoil (Fig. 1a). In any case, the depth gradient of
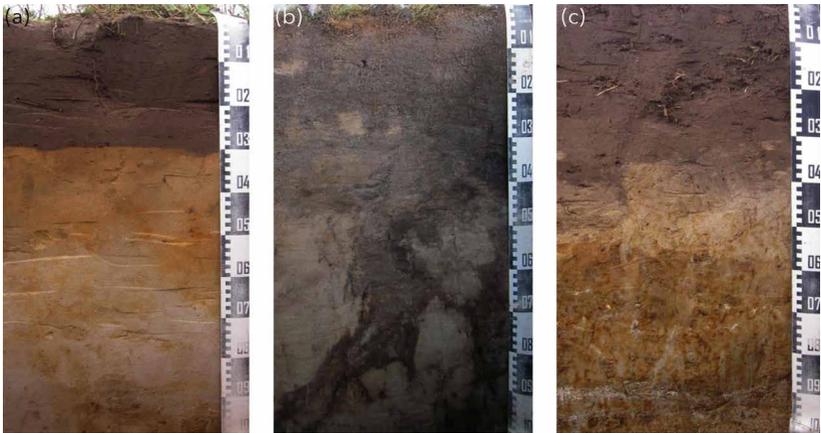
Figure 1 Example profile pictures of (a) a plowed arable soil with a sharp decline in soil organic carbon (SOC) below the plow layer; (b) a gleysol with a more complex depth distribution of SOC; and (c) a Stagnosol with hydromorphic properties. Photos belong to the Thünen Institute of Climate-Smart Agriculture, Germany.

SOC needs to be considered during soil sampling to obtain reliable estimates of SOC content in a given volume of soil. When sampling a particular vertical distribution, it is necessary that the whole depth of the increment is covered in approximately equal shares. This might be the most basic, but is also the most important rule of soil sampling; disregarding this rule can cause large biases in estimates of SOC. There are exceptions to the typical sharp gradients in SOC with depth (e.g. soils with large amounts of allochthonous SOC, such as those observed in fluvisols or those characterized by pedogenesis that involves SOC redistribution with depth, such as podsols (Schneider et al., 2020)). For these soils, it is even more important to ensure that each layer or soil horizon is well represented in the sample used to estimate SOC stocks (Fig. 1b).

The simplest and cheapest technique to sample a soil is to use a spade to extract a slice of topsoil material from above ground (Fernández-Ugalde et al., 2020b). However, this method can be particularly prone to a sampling bias, since the spade does not ensure collection of a homogeneously distributed sample for a given depth increment. Moreover, the sampling depth is not easily controllable and reproducible with a spade, since sampling usually occurs in a V-shape (http://kvkcachar.nic.in/soil.html). Finally, sampling multiple depth increments becomes almost impossible, unless a full soil pit is excavated first. The latter will be discussed as a separate technique in the following section. Spade sampling has the advantage of being cheap, quick and applicable to a wide range of situations. Therefore it has also been used in continental scale soil inventories such as the European LUCAS Soil Survey (Orgiazzi et al., 2018). However, it is not a volumetric sampling method, which precludes deriving the

other two parameters (i.e., bulk density and rock fragment fraction) needed for SOC stock estimation from the same sample. Therefore, this technique is used mainly when only the SOC content of a single layer of topsoil is of interest, not the SOC stock of a whole profile.

A hand-operated gouge auger or a core sampler of any kind is often used for soil sampling because it allows for controlled depth sampling at multiple depths, and to obtain a representative sample of each depth increment. Depending on the diameter of the core, an undisturbed core of soil can be obtained with relatively low effort. For these reasons, a gouge auger or a core sampler are the most common tools used for soil sampling. A machine-driven hydraulic system is usually preferred if the diameter of the core exceeds 4–5 cm and the desired sampling depth exceeds ~30 cm. The disadvantages of the auger or core sampler include deformations of the core (compression and stretching) as well as a partial mixing of topsoil and subsoil (Walter et al., 2016). Wet, coarse-textured soil is specifically prone to compaction. Also, a high rock fragment fraction, a dense network of coarse roots in forest topsoils, frozen or extremely dry soil can aggravate or impede auger or core sampling. In specific situations, e.g. in permafrost soils, a drilling auger is indispensable to extract cores of sufficient depth and quality. The fact that the extracted cores derived from auger sampling or soil coring resemble relatively undisturbed soil samples, allows for simultaneous determination of physical (e.g. bulk density) and chemical soil properties in the same sample, which helps to reduce the uncertainty of SOC stock estimation. Also, the fraction of rock fragments, at least those not exceeding the diameter of the auger/core sampler, can be estimated. Thus, if the soil core has a sufficient diameter (e.g. 4–10 cm) for volumetric and relatively undisturbed soil sampling, all parameters for estimating SOC stocks can be derived from one sample.

Another commonly used method to sample and describe soils is the soil pit method. This is usually the most labor-intensive method, but it is also a low-tech method which enables the most unbiased description of the soil profile and thus provides information related to pedogenesis (Vadeboncoeur et al., 2012). For example, hydromorphic properties, such as concretions of pedogenic oxides, are an important indicator for the presence of partial oxygen limitation which is directly coupled to SOC cycling (Poeplau et al., 2020). These properties are often only visible at the profile scale (see Fig. 1c). In addition, the abundance of macropores, the proportion of larger rock fragments, root distribution, as well as exact borders of pedogenic horizons can be best determined by a direct view of the entire soil profile. The length and depth of a soil pit can range from a few decimeters to several meters and are adjusted to the purpose of sampling and the site properties. Representative soil sampling of a profile pit for SOC content determination can best be achieved by (i) taking a thin slice of the whole depth increment or horizon, or (ii) using small metal cylinders to extract a

known volume of soil. The latter has the advantage that bulk density can also be measured in the same sample. Due to the strong negative relationship between bulk density and SOC content (Kaur et al., 2002), the sampling distance for both parameters should generally be minimized.

The preference of sampling by horizon or depth increment is context-specific. Usually, when SOC stocks are targeted, depth-specific values are of interest. However, the profile pit sampling provides the opportunity of a mixed approach, that is, a general sampling by fixed depth increments, with additional increments if horizon borders vary strongly from depth increment borders (Jacobs et al., 2018). Finally, the profile pit method is also of advantage in more stony soils, or soils with a high share of larger stones that will not be included in auger sampling or soil coring. The volumetric proportion of the rock fragment fraction can be visually estimated on the profile wall. If many spatial replicates are required, the profile pit sampling is less suitable than taking soil cores. It should be noted that two different sampling techniques will not yield identical results. In the German Agricultural Soil Inventory, profile pit sampling has been accompanied by eight core samples around the pit. Figure 2 shows that depth-specific sampling biases were detected for bulk density, SOC content and stocks. In the topsoil (0–30 cm), all parameters tended to be higher in the soil pit, while in the subsoils (50–100 cm), the opposite was true. A change in sampling method between sampling dates should be avoided.

Representative sampling of larger spatial units, for example, the field or landscape scale, requires greater sampling effort and ideally some knowledge about the spatial distribution of SOC (Viscarra Rossel and Brus, 2018, Heikkinen et al., 2020, VandenBygaart, 2006). In recent years, cost-effective indirect measures such as proximal sensing or even mobile phone-based image analysis tools have been developed for non-destructive 'sampling' of soils to estimate SOC and bulk density (Aitkenhead et al., 2016, Viscarra Rossel et al., 2016, Wills et al., 2007). Mobile phone applications,
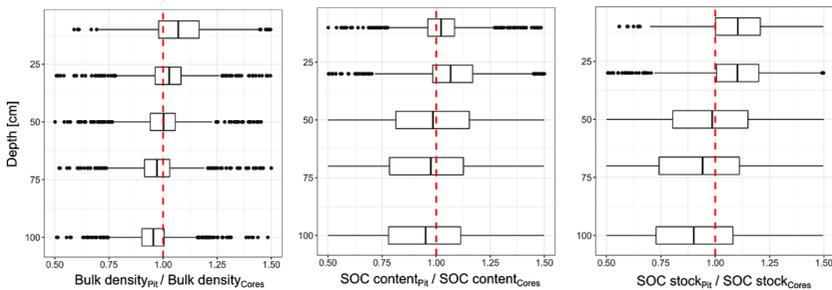

Figure 2 Ratios of bulk density, SOC content and stock, determined via profile pit (n = 1 per site) or core sampler (n = 8 per site), for a total of 1236 sampling sites of the German Agricultural Soil Inventory. Dashed red line indicates no change.

which are usually based on soil color sensing and a statistical model in the background (machine learning), might have the advantage that citizen science projects can easily be designed and big datasets can be gathered. However, among currently available proximal sensing techniques, visible near-infrared spectroscopy for SOC content estimation and active gamma-ray attenuation for bulk density have been found to be the most accurate measures (England and Viscarra Rossel, 2018). Claims have been made that these indirect methods might be as accurate, or even more accurate than composite sampling (Viscarra Rossel and Brus, 2018), and could be used for SOC stock estimation and even SOC stock change detection. For this reason, mobile proximal-sensing techniques are already used in official carbon accounting schemes such as the Emissions Reduction Fund of the Australian Government (https://www.industry.gov.au/regulations-and-standards/methods-for-the-emissions-reduction-fund/measurement-of-soil-carbon-sequestration-in-agricultural-systems-method). The accuracy of aerial means of proximal sensing might be higher than simple random composite sampling for determining aerial means of SOC in certain situations, since the spatial coverage is higher and more information about SOC heterogeneity can be obtained. Therefore, it can be a way forward for soil mapping, deriving field–landscape-scale baseline SOC stocks and the development of sampling designs (see Chapter 11 of this book). However, it is doubtful that proximal sensing, or even remote sensing, will be accurate enough to verify changes in SOC stocks. Even with a large spectral library, Jaconi et al. (2017) used stationary (not mobile) near-infrared spectroscopy to determine SOC contents in dried and homogenized soil, and the uncertainty was 3.2 g C kg^{-1}, which exceeds the expected changes – as for example, induced by agricultural management – in reasonable timescales. The analytical error of, for example, elemental analyses, is one order-of-magnitude lower (Saby et al., 2008). Mobile field sensors are even less accurate (England and Viscarra Rossel, 2018). The cost efficiency of sensor-based estimation of soil properties is often highlighted (Viscarra Rossel and Brus, 2018). However, this applies only to mobile sensors that are used in the field (with high uncertainty), while for stationary laboratory spectrometers, the costs of sampling and sample preparation for spectroscopy are equal to those of actual measurements. Furthermore, sensors for determining the rock fragment fraction are in an early stage of development (Jiang et al., 2021). Therefore, estimates of SOC contents or stocks should not rely on proximal sensing or image analysis methods alone. However, the major advantage and the great potential of these techniques are that the easily and rapidly obtained spectra contain information on a wide range of chemical, physical and even biological soil properties (Cécillon et al., 2009, Jaconi et al., 2019, Vohland et al., 2014, Hermansen et al., 2017). The development of global comprehensive spectral

libraries is a pre-condition for the routine use of infrared spectroscopy, for example, in soil monitoring (Cécillon et al., 2009).

As indicated above, sampling soil cores is more rapid and less labor-intensive than excavation of soil pits. Research involving spectroscopic analysis of soil cores has shown that this approach allows for rapid and data-rich investigation of the vertical distribution of soil properties, including SOC, at the scale of centimeters (Doetterl et al., 2013). Research using hyperspectral imaging of soil cores in the laboratory illustrates that even more detailed information can be obtained at even finer microscales (Steffens and Buddenbaum, 2013). The very fine resolution of this type of imaging can provide data about the hot- and cold-spots of SOC vertically through the profile (Hobley et al., 2018), potentially allowing for detailed assessments of subsoil changes in SOC.

In summary, all of these sampling techniques have their strengths and weaknesses and should be selected depending on the scientific question (including, for example, the desired accuracy and spatial extent of the area of interest), labor and resources available for sampling, expected soil properties, sampling depth and of course, cost–benefit considerations. However, especially in repeated samplings, there should not be a change in sampling methodology, since this can severely bias all three parameters of SOC stock estimation (Walter et al., 2016) and thus impede the interpretation of temporal trends or at least add significant noise to the data.

2.2 Calculation of soil organic carbon stocks and the equivalent-soil-mass concept

Once the required parameters are obtained, the calculation of SOC stocks based on the amount of fine soil present and its SOC content should be straightforward. However, careful consideration must be given to all of the parameters.

2.2.1 Case study 1

It has been shown recently that up to 87 out of 100 randomly picked studies overestimated SOC stocks due to misuse of the parameters 'bulk density' and 'rock fragment fraction' in various ways (Poeplau et al., 2017). In brief, the following three biases (M1–M3) were found (summarized in Fig. 3):

  M1: Bulk density of the whole soil (including stones with a bulk density of
      ~2.6 g cm^{-3}) was determined, and the volume occupied by fine soil was
      not corrected for the volume of stones. This method, which basically
      neglects the presence of stones, is correct when the soil has no rock
      fragments at all (e.g., eolian deposits). In all other cases, such as those with
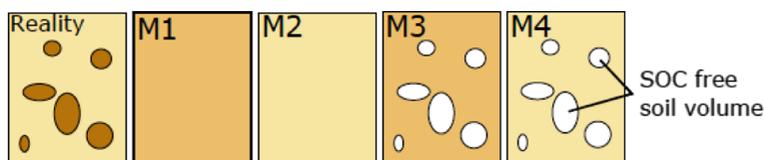
Figure 3 Schematic figure of four methods to calculate SOC stocks (Poeplau et al., 2017) in samples with rock fragments. The different shades of brown indicate the density of the material. The highest bulk density is found in stones (oval) and the lowest in fine soil.

> missing data on the rock fragments fraction, the method will overestimate the SOC stock (i.e. fine soil bulk density is overestimated and stones are considered to be filled with fine soil). Sampling with a spade, a narrow auger or by using proximal sensing all lead to this type of error, since no reliable data on the rock fragment fraction can be obtained and it is not possible to estimate this parameter using other soil properties (e.g. by using pedotransfer functions).

> M2: The rock fragment fraction was estimated and used to calculate the bulk density of the fine soil, but was not used to correct the total volume of soil. Therefore, the amount of fine soil was overestimated, as was the SOC stock.

> M3: The opposite happened in this method: the bulk density of the whole soil was used (which is an overestimation) while the volume of the soil was corrected for the rock fragment fraction.

Only when the rock fragment fraction was used to determine the correct fine soil mass and the correct soil volume (M4), unbiased SOC stock estimates were obtained. It is particularly relevant to apply M4 when investigating stony soils. More details and equations for all methods, as well as a simplified version of M4 can be found in (Poeplau et al., 2017). This method was applied to the dataset of the German agricultural soil inventory, which contained relatively few soil profiles with a high rock fragment fraction; the average bias caused by methods M1–M3 ranged from 2.1% to 10.1%, depending on depth increment and method.

For unbiased comparison of SOC stocks in time series or the valuation of treatment effects, the SOC stock estimates must be calculated for equal masses of soil (von Haden et al., 2020). The equivalent-soil-mass approach has been proposed under various names for a long time (Ellert and Bettany, 1995, Sposito et al., 1976), but is often overlooked or solved in different ways. Due to the fact that SOC content and bulk density are negatively correlated, the effect can be quite severe and usually leads to underestimation of SOC stock differences when fixed depth increments are compared. When a soil accumulates SOC in a specific depth increment (e.g. 0–30 cm), its bulk density decreases. In the case

of resampling after SOC accumulation to the same depth of the initial sampling, several centimeters of soil will be missed and the mass of soil will be lower than at initial sampling. Since it is almost impossible to account for this change in soil mass per depth increment during sampling, this is only done in rare cases (Don et al., 2019). The best option in the majority of cases is to correct the soil mass arithmetically. In the above-mentioned example with a single-layer sampling, no data is available for the depth increment below 30 cm. Therefore, the only possible correction is to 'cut' the heavier soil to a depth that equals the mass of the lighter soil in 0–30 cm. In single-layer sampling, this is straightforward, especially when this layer does not cross a pedogenic horizon border in which there is a strong shift in SOC content and bulk density. However, when multiple layers are sampled, the problem of equivalent soil mass to a given depth becomes more complex and can be solved (approximately) using post hoc non-linear models which are fitted to cumulative SOC stocks, to derive specific SOC stocks for any desired soil mass (Wendt and Hauser, 2013, Rovira et al., 2015). As discussed by Rovira et al. (2015), such a correction has limitations in stony soils, in which differences in fine soil mass can be driven by random differences in the rock fragment fraction.

For comparison of cumulative SOC stocks, such approximations are a reduction of the fixed depth bias, and in most cases, reasonably accurate. However, a serious problem related to the equivalent soil mass remains unsolved: if a depth increment includes the border between two pedogenic horizons (A and B horizons), a change in bulk density within one horizon (e.g. the Ap layer in tilled arable soils), will lead to a shift in the proportions of A and B horizon material, characterized by distinct SOC contents (Fig. 1a). Such 'dilution effects' can mask actual treatment effects and increase the noise in SOC stock data. This is another argument for exceeding the typical sampling depth of 30 cm and estimating the SOC stock in deeper layers.

In summary, even if all parameters for SOC stock quantification are estimated with high accuracy, major biases can still be introduced when calculating SOC stocks.

3 Understanding soil organic carbon dynamics

3.1 Long-term experiments, repeated inventories and space-for-time substitution

Changes in SOC that occur in response to alterations in land use, management or environmental factors are slow (often on the timescale of decades) and occur at relatively low rates compared to the total SOC stock and its variability (Smith, 2004). Therefore, detecting significant changes in SOC can take many years, depending on the effects of a given treatment and

the background variability in SOC. The effects of agricultural management, such as tillage, fertilization, residue management or crop rotation on the SOC stock are relatively small, usually ranging from <0.1–0.5 Mg C ha^{-1} yr^{-1} (Freibauer et al., 2004, Bolinder et al., 2020). The management effects are therefore mostly studied in long-term field experiments (Debreczeni and Körschens, 2003), in which treatment effects are compared to a reference or control treatment and spatial variability is ideally tackled by random, block-wise replication. Having a control treatment is crucial to distinguish between treatment effects and changes in storage that represent the absolute transfer of C between soil and atmosphere (Fig. 4). In this case, the average initial SOC stocks in the plots of treatment A and B were equal. However, in reality, this is rarely the case because many of the existing older experiments were not designed for rigorous statistical analysis. For example, many old, long-term experiments have only two replicates per treatment. When plots are laid out in a field having a gradient in SOC, it is likely that two treatments may differ in initial SOC stocks, which would be a major pitfall in quantifying treatment effects on SOC dynamics. For unbiased quantification of treatment effects, this difference should be accounted for if there is information on the initial SOC contents available. In ten Swedish long-term experiments on mineral fertilization with a total of 1116 treatment pairs, we found that initial absolute SOC content differences between plots were on average 1.3 g C kg^{-1}, while the final differences after about 50 years were 1.5 g C kg^{-1}, with 47% of the
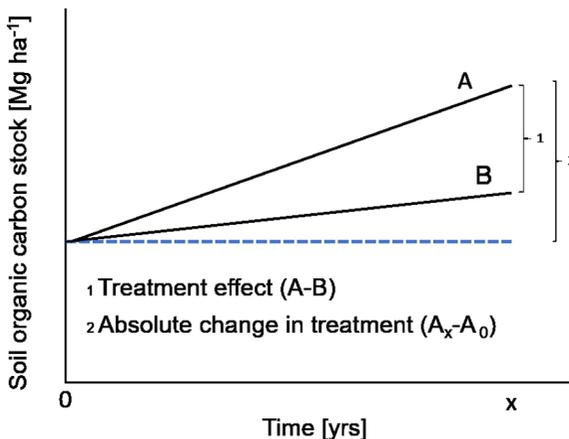

Figure 4 An example of SOC changes between two treatments, A and B. For treatment A, the absolute change is the difference in SOC at time x, compared to that at time 0. The net change is the difference between SOC in treatment A and that in treatment B, at time x, assuming that SOC was the same in both treatments at time 0. The latter approach is often used to measure the effect on SOC of a proposed treatment (e.g. no-till) compared to a standard 'control' (e.g. conventional tillage). Figure content adapted from (Ellert et al., 2001).

treatments having larger initial differences than final differences (Poeplau et al., 2016). We also found that the initial and final differences were significantly correlated, indicating a long-lasting legacy effect of different starting conditions across plots. Ideally, treatment effects at time x should thus be calculated as $(A_x − A_0) − (B_x − B_0)$, rather than $A_x − B_x$.

On larger spatial scales, repeated soil inventories are useful to evaluate SOC stock changes over large areas and long periods of time (Bellamy et al., 2005). It has been suggested that a period of ten years is necessary for detecting changes in average SOC stocks on a national scale (Schrumpf et al., 2011), while the continental-scale soil monitoring network, LUCAS Soil, was able to detect significant SOC losses (decline in SOC contents) in European croplands between 2009 and 2015 (Fernández-Ugalde et al., 2020a). For a correct interpretation of the regional trend in SOC stocks, careful records of land use and management practices are important. For example, Bellamy et al. (2005) suggested that SOC losses in Great Britain were mainly climate change-driven, while Smith et al. (2007) later showed that the reported losses were most likely overestimated and mainly attributable to changes in agricultural management. Other difficulties arise if the types of soil are not fully characterized across the sampling area or if the sampling is not conducted uniformly in space and time. If SOC inventories are carried out over long periods of time, factors that significantly affect the soil profile depth (e.g., erosion and deposition) or bulk density (e.g., compaction) should also be quantified repeatedly. A closer look at those inventories that have repeatedly sampled soils also reveals that even if the exact same sampling plots are resampled, the reproducibility of site-specific SOC stocks can be limited (Heikkinen et al., 2013). Figure 5 depicts the distribution of observed SOC changes in European topsoils (0–20 cm) between 2009 and 2015. Only 25% of the 16145 resampled soils had a relative deviation in SOC content between +/-10%, a range that could be related to changes caused by management or even climate change within six years. The other 75% of soils were out of this range, indicating that resampling caused a strong random error, masking potential management effects. It is not known what causes such large deviations in repeated sampling, but the reasons could be manifold, including slight shifts in sampling position, sampling depth, sample preparation or the above-mentioned problem on changes in bulk density and a related 'dilution' of depth increments. Although this effect is not only observed in the LUCAS dataset (Heikkinen et al., 2013), the relatively large deviations in this case could be related to the low reproducibility of the spade sampling method, as described above. Soil monitoring networks are currently set up in many parts of the world. The issue of the random resampling error should be further evaluated.
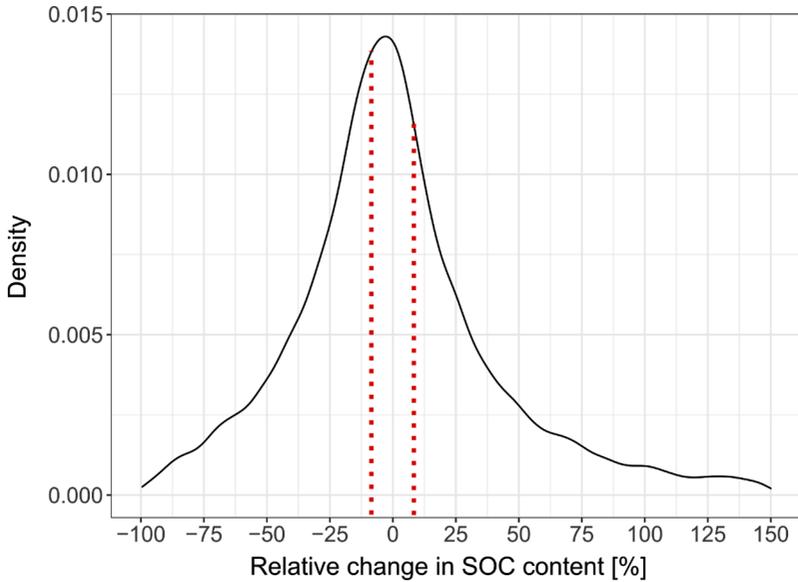
Figure 5 Distribution of relative deviations in SOC content between the LUCAS Soil sampling campaigns in 2009 and 2015. A total of 16 145 sampling locations could be compared, while only 15 406 are displayed here, due to even more extreme deviations of 739 sampling locations. The area between the red dotted lines indicates the range of deviations that could potentially be attributed to actual changes in SOC over time (+/-10%).

The space-for-time substitution approach is a common and powerful way to overcome the problem that SOC dynamics occur relatively slowly, while research questions often need to be answered in the short term (Pickett, 1989). This approach is based on the extrapolation of a temporal trend from a series of differently aged sites, also known as a chronosequence. For example, forest sites of different ages (i.e. time since afforestation) are compared to each other and to a long-term cropland to estimate the temporal dynamic of SOC since afforestation (Vesterdal et al., 2002, Quezada et al., 2019). Of course, the major assumption is that all sites had the same initial SOC stock at the time of conversion. This may rarely occur (see above), but site selection should account for this by minimizing pedological or topographical differences as well as different management across sites. Complementary to chronosequences, other naturally occurring gradients can be useful to study SOC dynamics. One example is thermosequences – a series of plots in close proximity to a geothermal source that are used to study soil-warming effects on SOC dynamics (O'Gorman et al., 2014, Sigurdsson et al., 2016). In addition, translocation experiments, along altitudinal gradients for example,

can be used to mimic climate change effects on SOC dynamics (Luan et al., 2014).

In summary, SOC dynamics are assessed in a wide range of scenarios, frameworks and experimental approaches, all of which require a high degree of standardization and reproducibility.

3.2 Soil organic carbon fractionation

SOC is the main constituent element of soil organic matter, a highly complex material consisting of countless compounds of various chemical properties and biogeochemical stability. For a better understanding, predicting and modeling of its dynamics and turnover in the soil, SOC is often conceptualized into different functional pools with distinct turnover kinetics (von Lützow et al., 2007). SOC models distinguish roughly between 2 and 7 partly interrelated pools, and experimental attempts have been made to quantify these pools by various fractionation schemes. Those approaches developed over time, following the prevailing paradigms of SOC stabilization in soils or vice versa (Dynarski et al., 2020). Starting from pure and harsh chemical extractions (Waksman, 1925), SOC fractionation has become more related to physical separation methods, such as size or density fractionation (Carter et al., 2003). A problem with SOC fractionation is that a myriad of different fractionation methods and method variations have been used, which usually preclude cross-study comparisons. A total of 20 different fractionation methods were evaluated by Poeplau et al. (2018) to investigate their efficiency to isolate pools with different turnover times. It turned out that methods separating clay or clay and silt-associated SOC from coarse and free particulate SOC were among the most effective. These methods were only outperformed by methods that additionally included a step to obtain an oxidation-resistant SOC fraction. How meaningful such chemical fractionation methods are with respect to *in situ* biogeochemical stability (Poeplau et al., 2019, Lutfalla et al., 2014) or regarding their representativeness of model pools has often been debated (Elliott et al., 1996, Luo et al., 2014). It has recently been proposed that the mere separation of particulate organic matter (POM) and mineral-associated organic matter (MAOM), two pools that have been shown to be substantially different in their mean residence time and response to changes, should be a possible way forward toward an improved understanding and prediction of SOC dynamics (Lavallee et al., 2020). This suggestion will be examined by many researchers around the globe, since this efficient simplification strongly reduces the workload compared to other, more complex methods and increases the comparability across studies at the same time. However, the choice of a method should always suit the purpose, and a reduction to the mentioned two fractions might not always be the optimal solution.

3.3 Use of carbon isotopes to quantify soil organic carbon turnover

3.3.1 ^{13}C natural abundance (discrimination with age)

Well-drained soils under stable vegetation (i.e. forest or grassland) are characterized by regular inputs of litter and root material which are gradually decomposed and mixed as they move down through the soil profile. Thus, a gradient is established in these profiles as the undecomposed organic material at the surface is transformed by decomposition into smaller particles/compounds that become more mobile and are transported deeper in the profile. The soil profile under forest or grassland contains an undisturbed, long-term record of decomposition and mixing processes useful for evaluating the rate of SOC turnover and determining the factors that regulate it. This evaluation can be conducted by coupling measurements of the stable C isotope composition ($δ^{13}$C) and SOC at different depths through the soil profile.

The observation that the natural abundance of $δ^{13}$C increases as SOC concentration decreases from the surface litter to deep layers in forest and grassland soils has been widely reported in different ecosystems and climates (Powers and Schlesinger, 2002, Wang et al., 2018, Garten Jr. et al., 2000). These observations show that $δ^{13}$C increases vertically in the profile, until it reaches a maximum steady-state value (Acton et al., 2013, Kohl et al., 2015). This increase in $δ^{13}$C with depth is attributed to several factors, including the mixing of two isotopically different sources of C (Garten et al., 2000). The change in $δ^{13}$C values with depth may reflect the depletion of atmospheric $δ^{13}$C-CO_2 due to combustion of $δ^{13}$C-depleted fossil fuels (a decrease of about 2‰ since the pre-industrial era) (Keeling et al., 2017). The change in isotopic composition with depth may also be attributed to the mixing of above- and below-ground vegetative sources of C because of the isotopic differences in, for example, root inputs compared to leaf litter inputs. Soil C may also be enriched with ^{13}C due to isotopic discrimination against the heavier isotope in biological processes.

Soils formed under similar conditions exhibit similar trends in $δ^{13}$C with depth, and the generally observed opposing trends of $δ^{13}$C increases and SOC concentration decreases with depth result in a negative linear relationship between $\log_{10}$-transformed SOC and $δ^{13}$C. The slope of the gradient, β, has been proposed as a proxy for SOC turnover (Garten Jr. et al., 2000). More recently, litter quality and mean annual temperature (MAT) were observed to be related to β (Campbell et al., 2009), as are soil texture and drainage (Powers and Schlesinger, 2002). It should be noted that these observations hold true only for well-drained forest and grassland soils, because it has been observed that the gradient in $δ^{13}$C with depth was reversed in soils with very high clay content

(Krull and Skjemstad, 2003) and in water-logged soils with poor drainage due to the production of ^{13}C-depleted methane (Krull and Retallack, 2000).

Recently a meta-analysis was conducted with data from studies that measured the vertical gradients of stable C isotopes and SOC in 176 forest and grassland soil profiles to evaluate the controls of climate, soil properties and nutrients on SOC turnover in different biomes worldwide (Wang et al., 2018). A significant relationship between β and modeled SOC turnover rates was observed. Also, relationships observed between β and MAT, MAP, soil N concentration and clay content suggest that these data could be used at a regional level to estimate SOC turnover. This would help to clarify the role of SOC in the climate system and could reduce modeling uncertainty.

3.3.2 C_3-C_4 vegetation change

The natural ^{13}C abundance of SOC is also useful as a quantitative tracer in systems when two plant species sources differ in their isotopic composition. For example, introduction of C4 plants to soil previously developed under C3 vegetation (or vice versa) results in the SOC containing two isotopically different sources of C. The δ^{13}C signature of the soil organic matter integrates the relative contribution of the fresh C4 plant-derived carbon and the C3-derived SOC. In this situation, a two-end-member mixing model (Chalk et al., 2020) is used to partition the SOC to origin, thereby allowing for an *in situ* estimate of SOC turnover:

$$\delta M = f_A \delta_A + f_B \delta_B$$

where f_A and f_B are the fractions or proportions of A (e.g. C4-C) and B (e.g. C3-C) in M, and δ_M, δ_A and δ_B represent the mean ^{13}C signatures for the mixture M and sources A and B, respectively. The estimate of the proportion of C4-C derived from this model best characterizes turnover for the period of time following the vegetation change. The turnover rate of bulk soil and physical fractions of soil can also be determined using this mixing model (Derrien and Amelung, 2011).

The natural δ^{13}C abundance method has been widely used to estimate SOC turnover in soils and soil fractions under different climates and types of vegetation (Balesdent, 1987, Gregorich et al., 1995). The strength of the method is evident in its use as a soil profile indicator of both known and presumed changes in vegetation and/or climate, in timescales ranging from years to centuries (depending on the approach). In a comprehensive review, Chalk et al. (2020) summarized studies using δ^{13}C measurements at different depths/horizons in the soil profile to indicate shifts in ecotone boundaries and changes in vegetation (e.g. induced by fire, grazing or climate change) within ecosystems.

3.3.3 Case study 2

In this case study, several of the approaches discussed above were applied to study SOC dynamics at the soil profile scale. These include: the space-for-time substitution (change from annual crops to the perennial bioenergy crop *Miscanthus*), SOC fractionation and the natural $\delta^{13}C$ abundance method, to study fraction and depth-specific SOC turnover (Poeplau and Don, 2014). *Miscanthus*, a perennial C4 grass from Eastern Asia, is considered a bioenergy crop in European agro-ecosystems, due to its high biomass production under low input conditions (Clifton-Brown et al., 2015). It was hypothesized that SOC sequestration could be an additional, climate-smart benefit of *Miscanthus* cultivation on soil that has been used as long-term cropland earlier. Soils of six *Miscanthus* plantations and their adjacent croplands across central Europe (Germany, the Netherlands, Switzerland and Denmark) were sampled to a depth of 80 cm to assess total SOC stock changes as well as C4-carbon accumulation and C3 depletion along the soil profile. For the topsoil (0–10, 10–20 and 20–30 cm increments), an SOC fractionation was conducted and the fraction of C4 carbon was determined for each of the five following fractions (Zimmermann et al., 2007): POM, dissolved organic C (DOC), sand and stable-aggregates-associated-C (S+A), silt and clay associated C that was (rSOC) or was not resistant to a chemical oxidation treatment (S+C-rSOC). Conceptually, the turnover rate of the different fractions follows the order POM > DOC > S+A > S+C−rSOC > rSOC. After 16 years of *Miscanthus* cultivation, SOC stocks increased at an annual rate of 0.4±0.2 Mg C ha^{-1} yr^{-1}. As expected, sequestration of *Miscanthus*-derived SOC was highest in the topsoil and decreased exponentially with depth (Fig. 6a). However, even in the deepest subsoil increment (50–80 cm), a clear *Miscanthus* signal was detected at all sites. The fractionation revealed that the POM fraction was most enriched in *Miscanthus*-derived SOC, while the lowest enrichment was found in the oxidation-resistant carbon of the silt and clay fraction (rSOC) (Fig. 6b). This case study represents a proof of concept for the applied fractionation method.

3.4 Other isotope approaches and alternatives to quantify soil organic carbon turnover

The use of C isotopes is not restricted to the natural abundance and stable isotopes ^{12}C and ^{13}C. The much lower natural abundance of the radioactive isotope ^{14}C is also used for studying SOC age and turnover, in the so-called radiocarbon dating method (Balesdent, 1987, Trumbore et al., 1989). This powerful method, which is widely used in age determination in many subject areas, is based on the radioactive decay of ^{14}C. It has fundamentally contributed to our understanding of SOC ages, which range from years to millennia (Paul
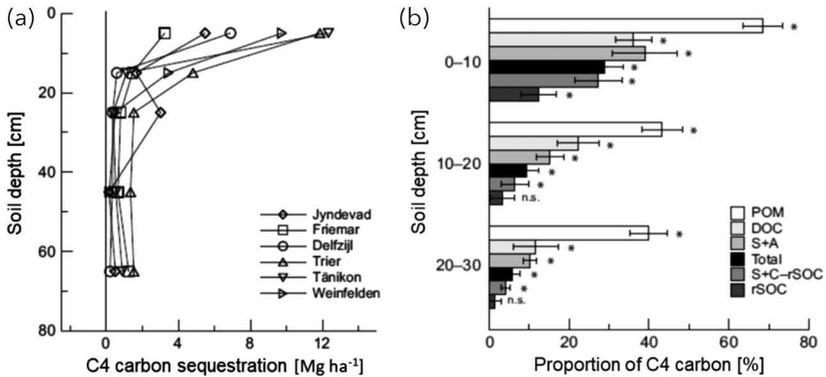
Figure 6 (a) *Miscanthus*-derived carbon sequestration as a function of depth at six central European sites; and (b) average proportion of *Miscanthus*-derived carbon in bulk SOC (total) and five different SOC fractions (POM = particulate organic matter, DOC = dissolved organic carbon, S + A = sand and stable aggregate-associated organic carbon, S + C − rSOC = silt and clay-associated organic carbon that is not resistant to oxidation with NaOCl, rSOC = NaOCL-resistant SOC). Adapted from Poeplau and Don (2014).

et al., 1997). Whole-profile SOC dynamics were extensively studied using radiocarbon dating, leading to improved understanding of soil–atmosphere carbon transfers (Balesdent et al., 2018). Paul et al. (2001) measured ^{14}C natural abundance in the profiles of agricultural soils in mid-west USA and determined the mean residence time (MRT) of SOC at different depths. The MRTs of the SOC of surface horizons ranged from modern to 1100 years (mean: 560 years). The MRT increased to an average of 1700 years in the 25–50-cm depth increment and 2757 years at 50–100 cm. One important feature of radiocarbon dating is the use of the so-called 'bomb peak,' which relates to the testing of nuclear weapons in the 1950s and 1960s, which increased the atmospheric ^{14}C content by as much as 70%. This peak helps to distinguish modern (post-bomb peak) from older (pre-bomb peak) carbon and can thus be used to estimate several parameters of SOC dynamics, such as annual input and turnover time (Goh, 1990). The steep depletion of atmospheric ^{14}C after the bomb peak can further be used as a calibration curve for exact dating of entirely modern (post-bomb peak) organic matter (Wild et al., 2000). Apart from natural abundance, ^{13}C and ^{14}C are also used as tracers in labeling studies, mainly to study shorter-term (days to years) SOC dynamics and related processes (Gregorich et al., 2015, Ziegler et al., 2005). When applied as pulses to living plants, carbon allocation within the plant and to the soil (microbes) can be directly quantified (Johnson et al., 2002, Epron et al., 2012, Swinnen et al., 1994).

Apart from the coupled use of isotopes and SOC fractionation, other methods, such as thermal analysis techniques, have been used (Plante et al., 2009). The main idea is to link SOC thermal stability to its biogeochemical

stability, and in recent years, thermogravimetry, differential scanning calorimetry and temperature-controlled thermal analysis (e.g. Rock Eval pyrolysis) have been found to be promising high-throughput approaches (Peltre et al., 2013, Barré et al., 2016, Disnar et al., 2003). Several indices from temperature-controlled thermal analysis have been developed to successfully describe *in situ* biogeochemical stability (Cécillon et al., 2018, Poeplau et al., 2019). In combination with a machine-learning model, Rock Eval pyrolysis was found to be potentially applicable to estimate the proportion of centennially stable SOC in large sample sets, such as from soil inventories (Cécillon et al., 2018).

The ratio of SOC stocks and inputs or output (i.e. heterotrophic respiration) is often used as a measure of the MRT of SOC (Chen et al., 2013, Carvalhais et al., 2014). Due to several critical assumptions associated with using this approach, the absolute numbers should be interpreted with care, and the correct terminology is also important, as pointed out by Sierra et al. (2017). Moreover, it has recently been pointed out that the MRT of SOC is strongly underestimated by the ratio of total SOC divided by total annual C input or efflux, since a major part ~80% of fresh C inputs to the soil is decomposed rapidly and never appears in the pool that is usually defined as SOC (that is, organic carbon quantified in the < 2 mm soil fraction) (Luo et al., submitted, Poeplau et al., 2021). The stock/input or stock/output ratio might therefore define the MRT of organic carbon that freshly enters the soil rather than that of stabilized SOC, which should also explain the discrepancies usually detected when comparing estimates of MRT or SOC turnover based on δ^{13}C approaches (Sanderman et al., 2003).

4 Conclusion

This chapter gives a broad overview on advances in measuring SOC stocks and dynamics. Owing to its high relevance for scientists, land owners and policy makers, soil organic matter research has a long history in many parts of the globe. The focus of this research in the past has been on surface soils. However, in the past 2–3 decades, more attention has been given to SOC in the subsoil. Thus, there has been more effort to characterize SOC stocks and dynamics within the soil profile. The complexity and diversity of soils has led to the development of a wide range of different methods and approaches to sample soils, to design experiments and monitoring schemes, calculate SOC stocks, fractionate SOC and investigate its dynamics through the soil profile. This diversity can be problematic because one method, technique or concept may not be universally applicable or comparable to other methods. However, even in situations where no change in method and geographical position occurs, it remains a challenge to 'reproduce' SOC stock estimates, hampering the verification of SOC stock changes. Soil-sensing methods have become

more widely used in recent years and more attention is currently devoted to developing proximal and remote-sensing-based methods for estimating SOC stocks. However, in the short term, it seems unlikely that soil-sensing methods will fully replace traditional soil sampling and analysis. It is therefore necessary to continue to work toward improving our techniques for measuring SOC stocks and dynamics at the profile scale.

5 Where to look for further information

A text book with a good overview on soil sampling and analysis methods

- Carter, M. R. and Gregorich, E. G. (Eds.). (2007). *Soil Sampling and Methods of Analysis*. CRC Press.

Further material on sensing methods, including mobile phone applications:

- http://blog.our-sci.net/2019/01/15/an-open-strategy-to-build-soil-carbon -part-2/.
- https://www.quickcarbon.org/.
- https://www.hutton.ac.uk/research/departments/information-and -computational-sciences/esmart.

A webpage on SOC fractionation methods, based on an international method comparison (Poeplau et al., 2018):

- https://www.somfractionation.org/.

Comprehensive guidelines for measuring and modeling SOC stocks and changes:

- http://www.fao.org/3/CA2934EN/ca2934en.pdf.

Project page of the European soil monitoring system LUCAS Soil, with links to data repositories:

- https://esdac.jrc.ec.europa.eu/projects/lucas.

Tea time! – An inspiring global citizen science project related to organic matter (litter) decomposition:

- http://www.teatime4science.org/.

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