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Measurement of soil carbon stocks

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Soil versus non-soil

Since the dawn of agricultural science, humans have been interested in measuring soil carbon. This typically involved collecting a soil sample from the field and analyzing the carbon content in the laboratory. The lateral and vertical variability of soils requires that the sampling locations, sampling depths, and even the sampling time are taken into consideration. To preserve the soil organic carbon at the time of sampling, soils typically are air dried to curtail biological activity. Even within the soil sample, the heterogeneous distribution of soil requires some form of processing before analyses can be repeated with confidence. The standard method of processing soil samples is to pass them through a sieve with 2 mm openings. The material that passes through the sieve is regarded as soil, while that which does not often is regarded as non-soil. The non-soil consists of stones or solid mineral fragments larger than 2 mm, but it invariably contains roots as well. Too often the non-soil has been merely discarded as something irrelevant to the study of soil science, but as will become apparent, the non-soil components often need to be taken into account when estimating the ecosystem quantities of C and other nutrients.

Below-ground plant carbon

Once the soil sample is removed to the laboratory, careful consideration must be taken not only on the physical dimensions of the sample but also on the nature of the materials (both soil and "non-soil") contained within it. Seldom discussed is the extent to which plant roots should be included in the soil sample. Many regard roots that fail to pass the 2 mm cut-off as non-soil and exclude the larger materials, some attempt to exclude most root and surface plant residues from soil samples, while some of us cut and shred the larger pieces of organic matter so that it might pass the 2 mm sieve and thus included with the soil. Root carbon and above-ground plant residues on the soil surface are the most problematic pools to quantify in terrestrial carbon cycles. The size of the soil organic C (SOC) pool in terrestrial ecosystems usually is expressed as the SOC mass per unit area to a specified depth or mass of soil. The balance between SOC inputs (ultimately from plants) and outputs (decomposition of SOC back to CO_2 emitted from the soil) determines SOC stock or pool size. Measuring plant C inputs from the above-ground is straight-forward relative to those from below-ground (mainly roots, but also rhizomes and other plant structures formed below the soil surface). Below-ground plant C inputs and some portion of above-ground residues on the soil surface invariably are collected with soil samples. Often the larger and most obvious portion of these constituents are simply discarded, other times these constituents are carefully removed from and excluded from the SOC pool, and some argue that these

should be included with the soil sample. While such constituents typically account for less than 20% of the SOC pool, they usually are the fraction that is most sensitive to land use and management. We suggest, therefore, that these components should either be quantified as a separate pool or included in the >2 mm soil sample. The below-ground plant C inputs and soil surface residues (only present in soil samples from the upper-most soil layer) must not be discarded in a haphazard fashion because they are a critical and dynamic part of the terrestrial C stock.

Once soil samples have been removed from the field, air-dried and crushed to pass 2mm, they are fundamentally distinct from the soils in their original natural state. The liquid phase has been largely eliminated, the gas phase has been homogenized and largely dissociated from biological activity, and the structural arrangement of the soil particles has been extensively disrupted. What is regarded as soil, especially the array of organic constituents included as soil, is operationally-defined by the sample processing procedures. Methods that seek to separate macro-organic matter from the soil must be uniformly and quantitatively impose across all samples, recognizing that below-ground plant C inputs may encompass coarse roots as well as fine rootlets and root exudates (now dried onto the soil particles) and even mycorrhizal hyphae. Again the distinction rarely is clear-cut, and so fractions such as macro-organic matter, are defined by the procedures used to isolate them from the soil. Often it may be preferable to include macro-organic matter with the <2 mm soil sample, but in many settings, it is inappropriate to simply discard the material. Before soil samples may be analyzed to determine chemical composition, they must be processed (drying and sieving, at minimum). Since processed soil samples often bear little resemblance to the natural soil profile or pedon from which they were obtained, it is critical to account for changes during processing soil that analytical data may be placed back into the context of the pedon.

Soil organic carbon versus organic matter (SOC vs SOM)

Soil organic matter refers to the non-mineral portion of the soil, whereas soil organic C refers to carbon atoms that are present within organic molecules. Organic matter consists of a diversity of molecules, ranging from simple organic anions like acetate through to large hetero-polymers known as humic substances. Confusion persists regarding the distinction between SOM and SOC, and many farmers and extension personnel still seem to refer to SOM, even though analyses of it are archaic. Conceptually, there is nothing fundamentally erroneous about SOM and discussing how it might be managed and how it might influence ecosystem function. Analytically, however, soil organic matter is difficult to assess reliably, and most assays are defined by the method used rather than the substance sought to be assessed. SOM typically is determined via wet oxidation whereby soil is mixed with an acidic solution of potassium dichromate ($K_2Cr_2O_7$). Over the years a diversity of oxidation conditions (>2 mm or >0.25 mm soil particles; with or without heating) and assays have been used to estimate the mass fraction of SOM in the aliquot of soil taken for the assay. Often the amount of unreacted dichromate was determined by redox titration, such that the amount of oxidant remaining was inversely proportional SOM content. Correction factors to account for incomplete oxidation of SOM usually were required. Not only is this analytical approach empirical, with oxidant consumption equated to SOM (despite the common occurrence of other soil constituents that consume the oxidant), but it also generates chromium-laden wastes (including hexavalent chromium) that are known to be toxic. Although deficiencies in wet

oxidation methods for SOM were noted as early as 1930 by English researchers (Walkley and Black 1934), and have been acknowledged repeatedly (e.g. Chatterjee et al. 2009), such methods continue to be regarded as simple and inexpensive to perform.

The continued use of wet oxidation methods by soil testing labs likely contribute to the persistence reference to SOM. Producers and extension agents often are more familiar with SOM contents than with corresponding SOC contents. More careful considerations of how management might influence SOM soon raise questions about the differences in the chemical nature and molecular structure of SOM relative to the plant materials entering the soil. Compared to recently deposited plant residues (roots, stems, leaves, chaff, flowers, seed coats, etc.) a majority of SOM has been modified by soil biological processes and chemical interactions with soil minerals. To construct carbon budgets to compare soil inputs and outputs with the amount present, the carbon atom is conveniently adopted as the common denominator. Because the SOC concentration and stock provide useful insights to C cycling and interpretations of management effects, SOC often is estimated as SOM ÷ 1.724. The latter factor often is referred to as the von Bemmelen factor after a German chemist who in 1890 asserted that SOM contained 58% C. Of course, there can be no single conversion factor to enable a straight-forward interconversion between SOC and SOM because SOM is variable and heterogeneous. Relative to the first half of the 20th century, now there is a much greater appreciation for the association and even organo-mineral complexation between mineral and organic soil particles. Isolating SOM from mineral soils typically involves some chemical alteration of it, so data on the elemental composition of SOM isolated from mineral soil are rare. An even simpler but related problem is the elemental composition of plant roots because elemental analyses of root samples from mineral soils indicate that the root organic matter has been diluted by mineral particles

Soil carbon analysis

Contemporary methods of soil C analysis involve requiring the use of automated combustion analyzers that convert carbon to CO_2 which subsequently is analyzed. Many of these analyzers also determine total soil N as well via automation of the Dumas method, named for Jean Baptiste Andre Dumas, a French Chemist working in the mid-1800s. The high temperatures required for complete combustion or organic matter and thermal decomposition of carbonates also convert soil N to gaseous oxides, which subsequently are reduced to N_2 by passing through reduced copper grains in a heat tube. The ability to reliably determine both C and N contents in a single analysis is very useful because the resulting C/N ratio provides valuable information of the chemical nature of these crucial elements, as well as important checks on analytical performance. Most of these instruments can be configured to determine C and N at much greater concentrations than those encountered in mineral soils, and so they may also be used for analyzing plant and animal tissues, and other organic materials. Most contemporary CN analyzers are equipped with an automated sampler which permits unattended analyses of sample sets that may vary in size from 30 to 300 individual samples. Most of the analyzers use pure gases, such as He, O_2, N_2 or Ar, typically supplied from compressed gas cylinders or gas generators. The pure gases are essential to flush away ambient CO_2 and especially N_2 in the background atmosphere, and O_2 often is required to ensure complete combustion of organic materials to CO_2. When operating properly, the analyzer should convert all soil C to CO_2, regardless whether it is present as organic matter, char or

inorganic carbonate. In 2016 the costs of such analyzers typically lie in the range of $50,000 to $150,000 USD, depending on configuration. The analyzers vary considerably in the sample size typically required for a single analysis (5 to 5000 mg mineral soil), in combustion process (tube orientation, reagents/catalysts, temperatures, ash removal, etc.), in the approaches used to separate the combustion gases, and finally in the analytical principle used to quantify the amount of CO_2 or N_2 in the combustion gases.

Automated soil CN analyzers measure total carbon and total nitrogen concentration originally present in the soil sample and leave behind non-combustible ash in the combustion tube or sample crucible/boat. Many soils contain appreciable amounts of carbonate, sometimes at the surface, but often in the sub-surface layers. Soil carbonate or inorganic C is fundamentally distinct from SOC, as it is subject to formation and dissolution by abiotic processes, albeit with considerable influence of biological processes. Usually the time-scale associated with changes in soil inorganic C are considerably longer (100s to 1000s of years or more), than with changes in SOC (10s to 100s of years). Of course, soil inorganic C may change rapidly in response to application of agricultural lime to manage soil acidity, or to other carbonate-rich amendments, including carbonate-rich irrigation water. On geological time scales, precipitation and dissolution processes dominate the circulation of carbon among the lithosphere, hydrosphere, and atmosphere. On the time scale typically associated with anthropogenic land management, the primary focus tends to be on SOC rather than inorganic C. Consequently, it is important to analytically distinguish soil inorganic carbon from SOC or total soil C. Wet oxidation techniques are unreliable, as they rarely recover all of the SOC. Only slightly better are dual combustion temperature approaches that attempt to exploit the fact that many carbonate minerals remain stable at temperatures above those at which SOC combusts to CO_2. Dual combustion techniques cannot be implemented on analyzers that employ dynamic flash combustion because high temperatures (1000 °C and above) and exothermic reactions typically exceed the thermal stability of carbonates. The fundamental problem with the dual temperature approaches is that soil chemistry is messy, and rather than being present as some well-defined crystal structure, soil carbonate is co-precipitated with an array of other elements, and the structure ranges from crystalline to amorphous. Consequently, soil carbonate may decompose at lower temperatures than well-ordered minerals. Conversely, SOC may be occluded or protected from combustion by close association with mineral surfaces, such that greater temperatures are required for combustion that typically expected for non-complexed SOC.

Soil inorganic C may be eliminated from soil samples by acidification before direct automated combustion analysis of SOC persisting after acidification, or inorganic C may be determined directly as CO_2 produced upon acidification of the soil, and SOC then may be determined as total minus inorganic C. Fully automated instrumentation specifically designed for determining soil inorganic C is uncommon (with the exception of those sold by UIC Inc. in Joliet IL USA). Many reliable techniques (including manual apparatus) for determining soil inorganic C are available, and detection of evolved CO_2 often has been automated through the use of non-dispersive infrared gas analyzers or gas chromatographs, and related modes of detection. Various acids (H_2SO_3, H_3PO_4, HCl) and assorted acidification procedures have been used to eliminate soil inorganic C before using an automated CN analyzer to determine total C which is equivalent to SOC, provided inorganic C has been eliminated successfully. Potentially

confounding effects of improper acidification procedures is the solubilisation and subsequent loss of organic C (e.g. caused by washing or rinsing the sample with aqueous acid) and by weight changes associated with acidification reactions. Stoichiometrically, for example, the reaction between calcium carbonate and HCl will liberate CO_2, the resulting $CaCl_2$ will have a greater mass than the carbonate it replaced. To get around these issues, instead of acidifying a bulk soil sample, many analysts perform a small-scale acidification of only the aliquot of soil to be introduced to the automated CN analyzer. In this way, the mass of the original non-acidified sample is used in the calculation of SOC concentration, and any mass changes caused by acidification are immaterial because these occur after the original mass is recorded. Many analysts use HCl, as after acidification any excess can be eliminated by gentle heating in a vacuum oven, and solubilisation is a non-issue because the sample is dried before analysis without rinsing or washing that might remove solubilized SOC.

Soil sample collection

Soil sample collection is the most critical aspect of measuring soil carbon stock. The most sophisticated instrument producing the most accurate and precise determinations of soil carbon concentration in the laboratory is likely to provide questionable and even misleading assessments of SOC stock if the soils have not be sampled properly. Assorted approaches to evaluate SOC *in situ*, without collecting samples and analyzing them in a laboratory are being investigated, but the standard reference approach, for all but the most complex setting, involves collecting soil samples in the field and returning them to the laboratory for processing, preparation, and analysis. Soils are highly variable in space, and the size or more specifically, the area captured in the soil sample often is small relative to the extent of the pedon or soil management unit. Inevitably and justifiably, spatial variability combined with the small area captured by soil sampling leads to concerns about how well the sample represents the area of interest. When the primary goal is to measure the temporal change in soil carbon storage, contributions from spatial variability are even more concerning. A strategy of simplification may be adopted to lessen the influence of spatial variability on temporal changes in soil carbon stocks. In this instance, the scope is narrowed to focus on temporal changes at a microsite (e.g. an area of 10 to 15 m^2). Sufficient microsites must be sampled to provide estimates of the dispersion in measured SOC stocks about the mean. Within an individual microsite, however, it is assumed that spatial variability is less important than temporal changes.

It is crucial that the investigator is fully aware of what is being measured. The soil sampling approach discussed here focusses on the effects of biological processes, namely the balance between plant C inputs and heterotrophic decomposition. In some landscapes, SOC stocks also are influenced by geomorphological processes or the balance between soil deposition and soil removal by erosion. The approach presented here is the simplest case where erosion and deposition may be negligible. Quantifying changes in SOC stocks in landscapes with appreciable deposition or erosion requires more involved techniques applied at the landscape or catchments scale, and are beyond the scope of this discussion. Failure to recognize the complexity of geomorphological processes influencing SOC stocks in erosion and depositional landscapes has contributed much confusion to assessments of land-atmosphere C exchange. The biggest errors tend to be associated with inadequate consideration of the fate of SOC in eroded soil. Usually it is assumed to be rapidly decomposed back to atmospheric CO_2,

whereas more careful assessments often suggest a sizable fraction of SOC becomes stabilized in deposited soil and/or sediment, and that the rate of SOC accumulation at recently eroded sites may exceed the pre-erosional rate of accumulation. The microsite approach discussed here will measure temporal changes in SOC at sites where erosion and deposition are negligible. The approach is also applicable to rare instances where the rate of soil deposition or erosion at the site is precisely known.

Soil samples must be collected in such a manner that the analytical concentrations determined for a highly processed sub-sample combusted in automated analyzers may be placed back into the context of the field. To do this, soil samples must be collected from a well-defined area and a carefully measured depth increment. The area and depth increment define the volume sampled, and the soil weight per unit volume typically is called soil bulk density. The SOC stock is calculated as the product of soil bulk density, layer thickness, and SOC concentration. For example, if the SOC concentration is 2% or 20 kg SOC Mg^{-1} soil for a 15 cm thick soil layer with a bulk density of 1.4 Mg m^{-3}, the SOC stock may be calculated as:

$$\frac{20\ kg\ SOC}{1\ Mg\ soil} \times 0.15\ m \times \frac{1.2\ Mg\ soil}{1\ m^3} = \frac{3.6\ kg\ SOC}{m^2}$$

Investigators usually recognize the relevance of soil thickness to estimating SOC stocks, but may not fully appreciate how difficult it may be to accurately measure thickness in the field, or that simple estimate of soil thickness rarely provide quantitative insight to soil erosion. Simple calculations like that given above also have alerted investigators to the importance of measuring soil bulk density to estimate SOC stocks. Unfortunately, this awareness sometimes elicits complicated and independent methods to measure *in situ* soil bulk density that might be relevant to characterize soil physical processes like water infiltration or gas diffusion. For the purpose of estimating SOC stocks, bulk density measurements do not have to be complicated, but the measurements should be determined for the very same samples collected to determine SOC concentrations.

In practice, mechanically-driven soil coring equipment provides the most efficient means of collecting samples for determining SOC stocks in agroecosystems. The cross sectional area of the inside diameter of the soil core tube bit defines the area of soil being collected. Depending on the setting, a core diameter of 5 to 10 cm provides for an adequate visual assessment of soil characteristics, and manageable amount of soil for processing in the laboratory. In the field, the core tube containing the soil is removed from the hydraulic apparatus used to insert the tube and withdraw the soil core, the tube is placed in an horizontal orientation, and the soil core is carefully pushed from the tube and cut into appropriate depth increments. As it is pushed from the sampling tube, the soil core is inspected for breakage, excessive compaction, adhesion to the core tube walls, channels carved by stones or woody plant materials, etc. The end of the core tube may be used as a cutting guide to improve the accuracy of thickness measurements. The core may crumble as it is transferred into trays or bags for subsequent processing, but the original volume must be well-defined and precisely known. The dry mass of soil and any stones (>2 mm) will be determined in the laboratory, and the stone-free dry mass per unit volume (bulk density) will be used to estimate SOC stocks. The recommended depth of soil sampling should be adjusted to comply with study objectives and environmental setting, but typically will be between 40 and 120 cm, with increments of 15 cm or smaller. Ideally, all increments for a particular sampling point

will be drawn from the same core or cores. Sometimes two or three cores may be combined for a single sampling point, thereby increasing the area sampled and lessening the potential effects of spatial variability. The important aspect is that bulk density is determined for the cores actually collected and that the entire layer sampled is collected in a contiguous fashion (i.e. layers sectioned carefully so that no increment is excluded).

The configuration of soil sampling points within a microsite may vary depending on the desired number of cores sampled from within each microsite, and the number of times the microcosm will be sampled. For example, with a 4 x 7 m rectangular area six initial samples might be collected along two rows at 2 m intervals. At some subsequent sampling time (say 5 to 10 years later) six additional cores are collected, but these will be offset by 1 m but interspersed with the cores collected initially. To precisely mark the locations where the initial cores were collected, electromagnetic markers, such as those used by urban utility companies may be buried well below the depth of tillage. To minimize microsite disturbance, and especially burial of topsoil from within the microsite, the holes left by the initial sampling should be refilled with cores collected from some distance (perhaps 10 m) outside of the microcosm. The six cores may be combined so the mean SOC stock might be estimated for the initial sampling times at each microcosm, and so that analytical resources may be devoted to sampling a greater number of microcosms within the field or soil management unit. In practice, careful sampling is expensive, and investigators may analyze SOC stocks for each core within the microsite.

Soil sample processing and preservation

Soil coring equipment usually works best in slightly moist, but not wet or very dry conditions. On the Canadian Prairies, our preferred time of soil sampling is in the fall after soil moisture has been depleted and the crop has been harvested. The soil samples are in a field moist condition, in most years ambient humidity is conducive to air-drying as a means to slow microbial transformations and preserve the soil sample. Soon after the soil samples are transferred from the field to the drying room, the total wet weight is recorded and the cores are crumbled to obtain representative sub-samples to determine field moisture content (by drying a sub-sample at 105 °C; subsequently discarded) and to retain a sample at field moisture content if required for microbial analyses (preserved by refrigeration or freezing). Typically, bulk samples are dried in Al foil trays (re-usable, within limits), and samples are stirred on a daily basis to accelerate drying at ambient temperature (20 °C).

Soils typically are crushed to pass a sieve with 2 mm apertures to separate soil from the stones. As discussed above, procedures to handle coarse fragments of plant tissues, especially in soils from the surface layer must be defined at the outset. We recommend against arbitrary distinctions between soil and non-soil C, and instead, prefer to retain all materials collected in the soil core at sampling. Some labs continue to use perforated (2 mm round holes) drum mills similar to the Rukuhia grinder described by Waters and Sweetman (195?). The mineral soil aggregates and well as a majority of the plant material is crushed to particles smaller than 2mm.

Since most automated CN analyzers typically combust a 5 to 500 mg aliquot of mineral soil to represent an entire sample that may weight on the order of 1 kg (even more if samples are composited), fine-

grinding is essential. Fine grinding must reduce particle size to ensure the SOC and total N is homogenously distributed, and that the small aliquot introduced to the analyzer represents the entire sample. A general guideline is that a representative subsample is collected from the 2 mm material, and crushed to pass a No. 60 or100 mesh sieve with openings of 250 or 150 um. Representative sub-sampling and careful fine-grinding is essential to obtain reliable results, and some testing of sub-sampling variability to probes sources and amounts of sampling variability may be instructive. The size of the sub-sample and consequently the amount ground may vary with available grinding procedures and equipment. For example, for high volume projects we often use a roller mill in which a 7 g sub-sample is left to tumble for 18 hours in metal canisters containing metal bars to effect pulverization. This mill can grind more than 100 samples simultaneously, but the 7 g sub-sample must represent the entire sample. For some projects we use a vibratory 'dish ring and puck mill', similar to those used to crush ore samples for metal analyses. Depending on dish size, sub-samples of 70 g or more are pulverized to a fine powdery consistency, so sub-sampling tends to be less problematic. Sample throughput, however, is reduced and labor requirements are increased, because samples must be ground one at a time. In many cases the 2 mm soil in the catch tray for the perforated drum mill may be thoroughly mixed and random scoops taken to collect a representative sub-sample. In other cases the cone and quartering technique or manual riffle dividers may be used to obtain representative sub-samples. Since the sample may have to go through such a process multiple times to obtain a manageable sub-sample, such procedures may become costly, dusty and error-prone. In these instances some sort of mechanical sample divider, such as a rotating tube sub-sampler, may be used to create a flowing stream of dry soil so that representative and unbiased subsamples may be obtained from it.

Soil analysis

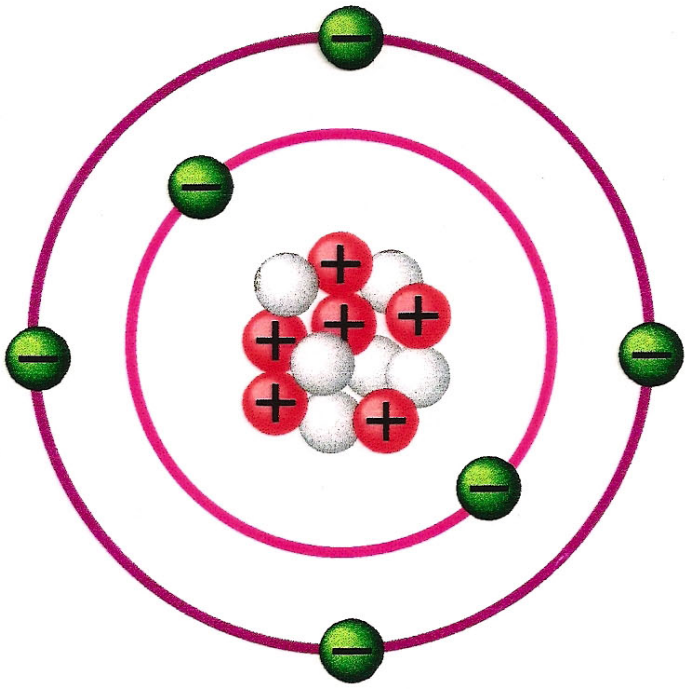
After fine grinding, soils are retained in sample vials or even high-grade paper coin envelopes. Despite a small risk of contributions from the paper fiber, such envelopes are inexpensive, easy to label, expose a large surface area to drying or equilibration to ambient humidity, and most important are less prone to the build-up of static charge which otherwise may thwart the accuracy of micro-analytical weighing. Analyses of total C and N are calibrated using pure organic chemicals (e.g. acetanilide, glutamine, EDTA), matrix relevant reference materials (e.g. soils, sediments, ores) developed in-house or purchased from commercial suppliers, or reference materials with concentrations assigned by recognized government agencies or certification bodies (e.g. agencies responsible for chemical metrology).

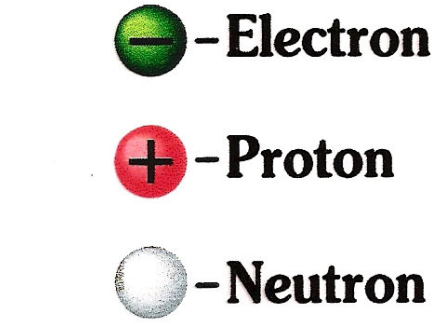
Calculation of temporal change in SOC stocks

Assuming that sampling, processing and analysis of the soil samples has been done correctly, and further assuming the stone-free soil bulk densities at the initial and subsequent sample time are identical, SOC stocks for successive soil thicknesses or volumes may be calculated simply as the product of SOC concentration, layer thickness and bulk density, as described previously. In practice, however, bulk densities vary among microcosms, and especially between soil sampling times. Often soil management of environmental conditions will be dissimilar at initial and subsequent sampling times. The analytical concentrations of C and N determined for the soil must be placed back into the field

context to assess whether appreciable changes in SOC stocks have occurred. Many investigators fail to recognize the interdependence of soil mass and SOC mass, but greater bulk densities for the same soil thickness means a greater soil mass, and this will tend to inflate SOC stock. In some settings, there may be good reasons (e.g. based on soil profile morphology) to vary the thicknesses of the soil layers sampled, but this will further accentuate differences in the masses of soil being compared.

To avoid errors associated with comparing unequal soil masses in settings where soil redistribution is negligible, the thicknesses of soil being compared are adjusted to attain an equivalent soil mass. The approach does not have to be complicated, but some investigators initially find it unsettling to compare SOC stocks in unequal soil volumes or thicknesses. The equivalent soil mass approach simply adheres to the premise of mass conservation, and adjusts soil thickness so that soil mass is equivalent. Some assumptions are inherent in this approach, because the thicknesses of the soil layers being compared may not exactly coincide with those sampled, but in practice the required interpolations usually are palatable, and the uncertainties about SOC concentrations diminish as deeper layers are considered where variations with depth tend to be small.

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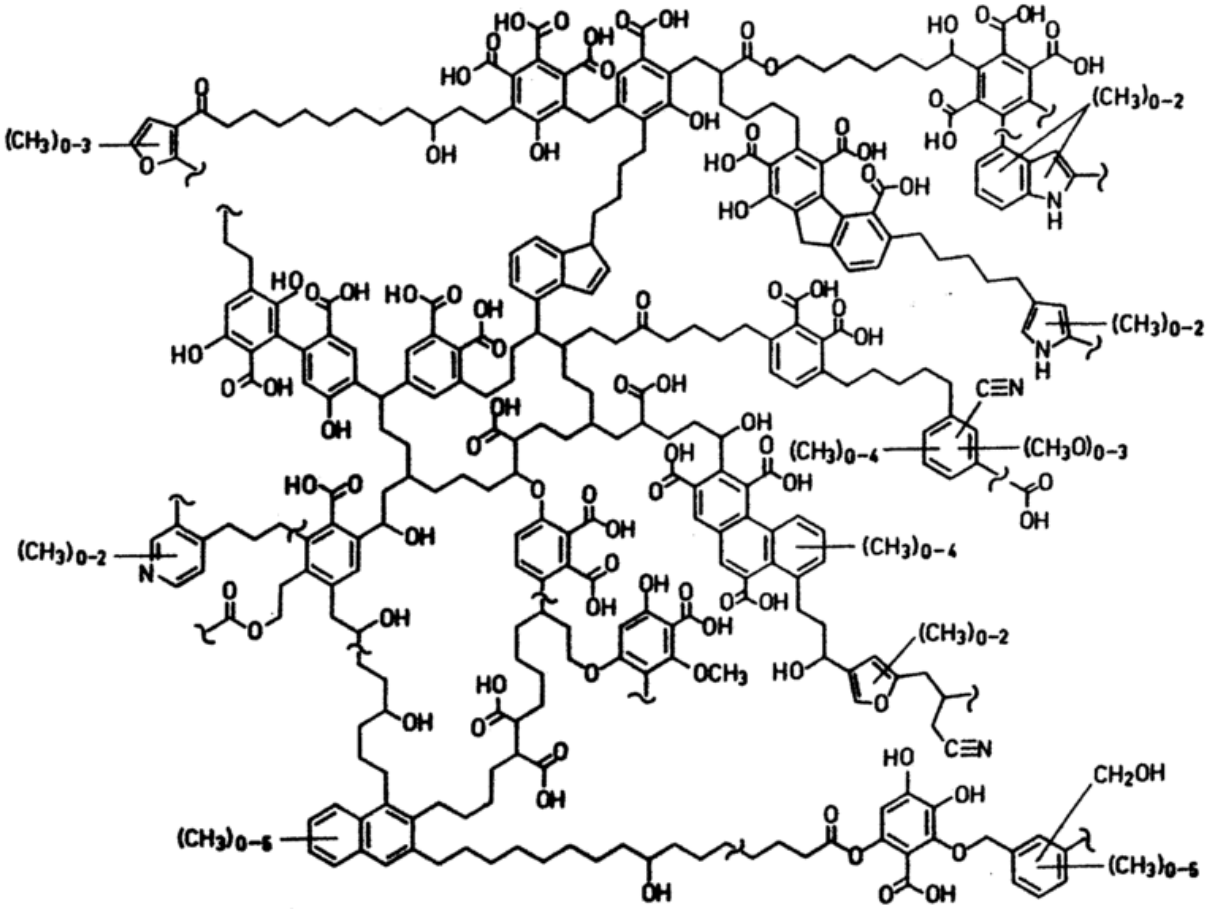
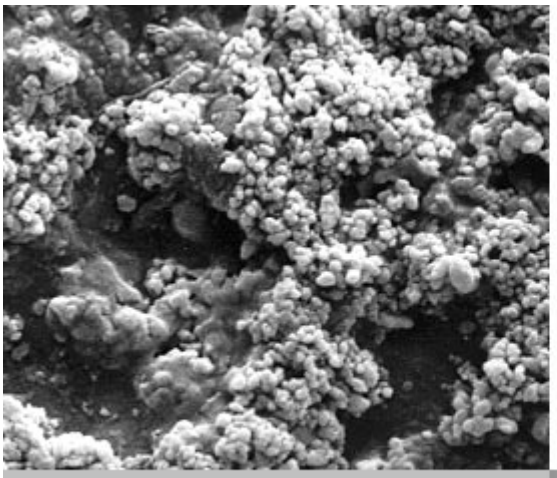
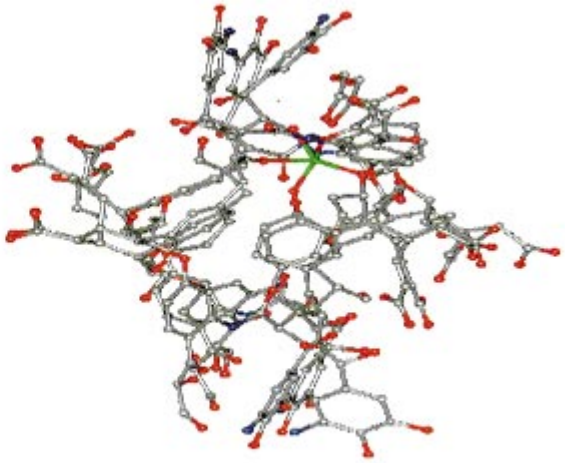
-Proton
-Neutron

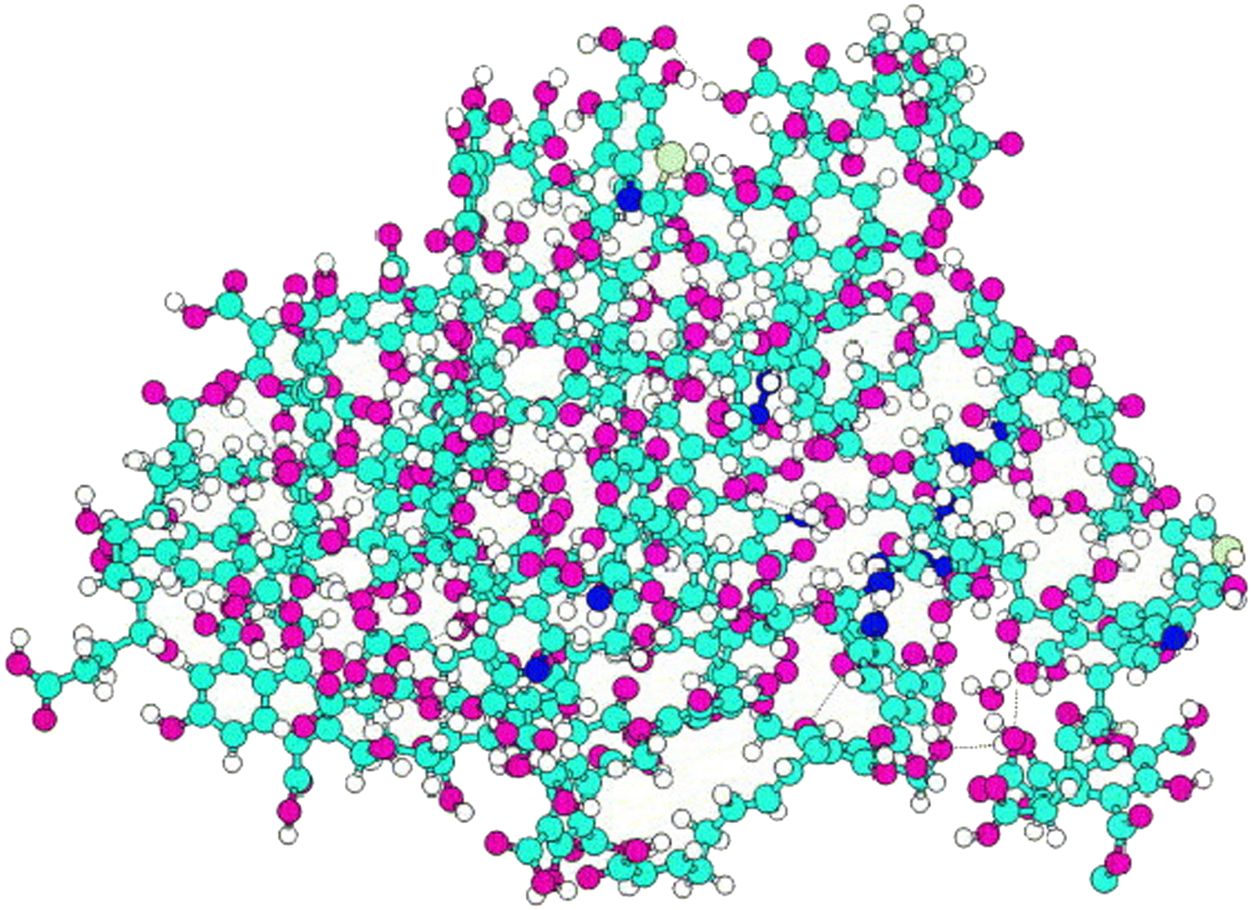




SEM image (approx 2000 times enlarged) of a solid humic acid (www.hagroup.neu.edu)



Proposed humic acid building block with a hollow interior for water retention (Davies et al., 1997)



Model of a dissolved organic matter molecule or humic substance. Color codes for atom types are as follows: carbon (cyan), hydrogen (white), oxygen (pink), nitrogen (blue), and sulfur (yellow). from: Schulten H-R (1999) Analytical pyrolysis and computational chemistry of aquatic humic substances and dissolved organic matter. J. Anal. Appl. Pyrolysis 49(1):385–415.

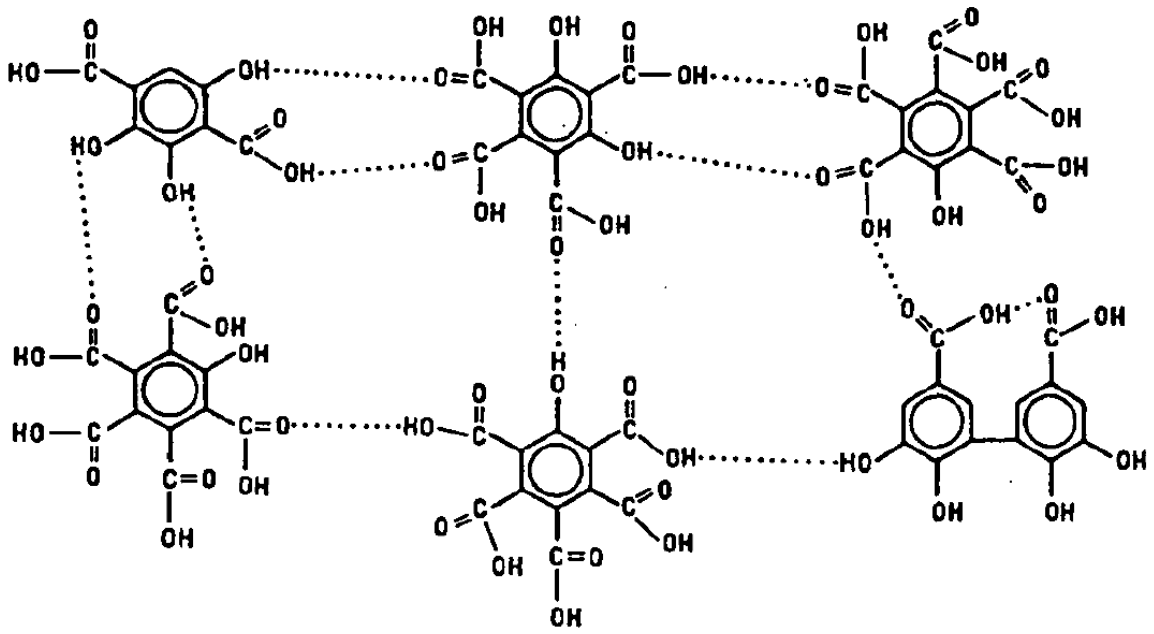


Figure 6. Structure of a fulvic acid, after Schnitzer (1978)

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