UNDERSTANDING AND MEASURING ORGANIC MATTER IN SOIL

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WASHINGTON STATE UNIVERSITY EXTENSION

Key Points

- Soil organic matter provides numerous functions in soil. Understanding its composition and how it interacts with clay, plant nutrients, other compounds, and the soil microbial community is key to improving soil management.
- Soil organic matter has long been studied using an alkaline extraction which produces humic substances: humic and fulvic acids. This tool has allowed researchers to separate organic matter from soil minerals to study the structure of the organic molecules present in soil.
- The term humus has been used for more than 200 years to describe organic materials produced through decomposition of crop residues and other organic inputs to soil. A portion of soil humus was thought to be composed of material that was stable and resistant to further decomposition because of large size or lack of digestibility to microbes.
- New tools have allowed soil organic matter to be studied in conditions closer to the natural environment and have also shown that the soil environment, not inherent properties of organic molecules themselves, greatly affects decomposition of organic molecules. Terms, such as humus, humification, humic and fulvic acids, which are tied to the alkaline extraction procedure, have changed through time, and are not descriptive of soil organic matter functions or stability. The concept of large, stable, older organic molecules resistant to decomposition is no longer valid.
- Smaller organic molecules that are products of decomposition may behave like oil in water to form larger conglomerates (known as supramolecules) with a hydrophobic interior and a hydrophilic exterior.
- The location of organic matter affects its reactivity in the soil. Organic matter can adhere to mineral particles, be protected in aggregates, be dissolved in the soil water, or remain as organic particles. Organic matter that is associated with mineral particles is thought to be more stable in the soil, but interactions with root exudates can mobilize mineral-associated organic matter and associated nutrients.
- Measuring soil organic matter has also advanced. Although there are many soil health indicators related to soil organic matter, research has started to narrow the number of tests that are both useful for management and commercially available. Two such tests are active carbon and carbon mineralization representing both the accumulation of soil organic matter and its use (loss) as an energy source by soil microbes.

Soil organic matter (SOM) content is often linked to soil health. Producers are encouraged to increase SOM to improve soil functions, such as water-holding capacity, improvement of soil structure and infiltration, nutrient exchange, nutrient supply from decomposition, and carbon sequestration. While it is commonly included in basic soil tests, increased attention to soil health and biology has encouraged interest in more advanced understanding of SOM. Past concepts of SOM structure, composition, location in the soil, and interaction with microbes, minerals, and chemical compounds have recently been challenged. Likewise, laboratory methods to study SOM have evolved. As new producer-oriented soil tests emerge, those who rely on these tests should understand their potential and limitations. This publication will describe current conceptual models for SOM and emerging tools to monitor changes in SOM structure, composition, and function.

The Living Component of Soil

Organic materials are formed from living organisms, such as plants and animals. They are largely composed of carbon and provide energy and nutrients to soil microorganisms. As plant and animal materials enter the soil, they are broken into smaller pieces by soil arthropods. New surface area is exposed and colonized by bacteria and fungi that secrete enzymes capable of breaking carbon bonds and releasing energy and nutrients stored in plant and animal tissues. New microbial biomass and microbial products (e.g., enzymes and polysaccharides) are formed and also recycled as microbes are eaten by other microbes. Most carbon introduced into the soil is converted to carbon dioxide and released to the atmosphere in a matter of days or years, but some decomposed materials and plant inputs persist as soil organic matter for hundreds of years. The nature and physical location of this more stable organic matter is particularly important for its potential to store carbon removed from the atmosphere through photosynthesis and keep it in the soil as a means of mitigating agriculture's contribution to climate change.

Soil **organic carbon** (SOC) and soil organic matter are not synonymous but are frequently used interchangeably. Carbon atoms account for about 50–55% of soil organic matter, but SOM also includes other elements bound to carbon, such as hydrogen, oxygen, phosphorous, nitrogen, sulfur, potassium, and calcium. Organic carbon is carbon associated with living or dead organisms, but not all carbon in the soil is organic. Minerals such as limestone or gypsum are carbon-based and can be found in many soils.

Soil Humus Past and Present

The behavior and persistence of organic materials in soils has interested chemists for centuries. **Soil organic matter** (SOM) includes both recognizable organic debris from living and dead organisms and **biomolecules** that are the direct and indirect result of decomposition of the original organic inputs.

The term **humus** has historically referred to SOM that has been transformed and has lost the visible features of the organic residue from which it was formed (McBride 1994). It was also considered to be a portion of the total SOM more resistant to decomposition. In this context, humus is formed by **humification** of organic inputs. Two important ideas that underpin this concept of humification are: (1) new **organic molecules** are preserved or created through soil processes and (2) these new organic molecules (i.e., humus) are resistant to degradation by microorganisms. Humification is thought to involve both biotic and abiotic processes acting on organic inputs and the byproducts of decomposition. **Lignin** was considered a major precursor to soil humus because it was considered to be difficult to decompose and therefore selectively preserved (Kögel-Knabner and Rumpel 2018). The humification concept was developed in association with a specific technique for studying soil organic matter called alkaline extraction.

Operation Separation: Extracting Organic Matter from Soil for a Closer Look

The study of soil organic matter is confounded by the fact that soils are composed primarily, about 98% by weight, of mineral particles—sand, silt, and clay—which are non-organic materials. Organic molecules form strong bonds with mineral particles, especially the smaller-sized silt and clay (see Figure 1, steps c–e) making the removal of organic matter from mineral particles for further study difficult.

Historically, soil chemists have separated the organic and mineral fractions with an **alkaline extraction**. Reacting soil with a highly basic solution (high pH) causes organic compounds to be more soluble in water and allows for isolation and study of more of the individual molecules than extraction with water alone. The resulting soluble portion is referred to as **humic substances** and the insoluble portion as **humin**. The humic substances were further separated by reaction with an acid solution to separate **humic acids** (soluble at high pH, but insoluble at low pH) and **fulvic acids** (soluble at both high and low pH). These procedures have generated an entire vocabulary that was helpful in describing lab methods, but potentially confusing in terms of actual soil processes. What has become apparent over time is that these elements isolated through alkaline extraction are actually "operational" components since the fractions are the result of a laboratory operation that creates conditions not present in soil.

Alkaline extraction has been criticized because the high pH used in the process changes the molecules that are being studied. It may also form new molecules that were not in the original sample. Furthermore, because the method was not developed from a theory of organic matter composition and behavior, some argue that scientists have been tempted to formulate explanations for soil processes and carbon cycling that conform to observations from alkaline extraction (Lehmann and Kleber 2015; Kögel-Knabner and Rumpel 2018).

For example, several models for the chemical structure of humic and fulvic acids have been proposed by examining materials following alkaline extraction and sequential degradation of the extracted products (Burdon 2001). Large molecular substances observed in alkaline extracts were historically thought to be a novel class of structures formed in the soil and resistant to degradation (Kelleher and Simpson 2006). However, the lack of similarly obtainable products in plants or microorganisms and analysis with new methods suggests that these *are* artifacts of the extraction process and not products synthesized in the soil, at least not in significant amounts (Burdon 2001; Kelleher and Simpson 2006; Lehmann and Kleber 2015).

The historical idea of humus as relatively large, old, and stable organic molecules is now viewed as outdated and widely discredited (Sutton and Sposito 2005; Lehmann et al. 2008). Some researchers suggest that terms associated with the old concept such as "humification," "humus," "humic acid," and "fulvic acid" should be used sparingly, if at all. "Humic substances" may be appropriate terminology within the context of alkaline extraction or commercial products made from alkaline extraction, though preferred terminology for these substances could be "organic alkaline extracts."

However, the matter is not completely settled. Controversy continues over whether organic matter is stabilized in soil through *creation* of unique organic molecules (i.e., through humification) that are resistant to microbial degradation. Though stability of organic matter is now thought to be controlled more by local conditions and microenvironments than by the inherent chemical properties of organic molecules (Kleber 2010), others defend the idea that humification creates new biomolecules through chemical processes. Defenders argue that it is short-sighted and simplistic to dismiss the existence and importance of novel substances that are created through the microbial degradation of organic materials, are stable in soil, and interact dynamically with mineral components of soils, plant roots, and soil organisms. They propose creation of new and larger biomolecules through the accumulation of **phenols** in the soil as microbial byproducts or remnants of incomplete lignin decomposition. Phenols then react with free **amino acids** and **aliphatic** carbon compounds to form larger molecular structures (Horwath 2015; Gerke 2018).

Alkaline extraction also remains an important tool. Using it to extract and purify organic molecules from soil is still a primary way to describe organic molecule structure and potential reactions in the soil. Alkaline extraction is also valuable because it is difficult to isolate sufficient quantity of organic molecules for study from typical soils with water or weaker extracts. Nonetheless, when alkaline extraction is used, it is difficult to be certain that the process of extracting the molecules did not change the molecular structure or that a representative fraction was isolated. Proponents of alkaline extraction assert that alkaline extracts do correspond to molecules existing in soil (Piccolo 2016). While this may be true, it is also true that alkaline extraction does not discriminate between plant **root exudates** (e.g., melanins or tannins), microbial products (e.g., antibiotics), and any novel molecules formed from chemical (abiotic) processes—that are the specific target (i.e., humic

Size Conversions

Soil organisms, aggregates, mineral particles, and individual molecules span a wide range of sizes. Millimeters, microns, and nanometers are all units used to describe size and distance in soil. Rock fragments (gravel) are larger than 2 mm. Larger organisms, like earthworms and soil arthropods, can be one to several mm. Sand particles are 0.050 mm to 2 mm and are similar in size to large aggregates (macroaggregates) which are 0.250 mm or larger. Silt particles (0.002–0.050) are smaller than sand and larger than clay (< 0.002 mm or 2 µm). Bacteria are around 1 µm while an individual clay particle is about 10–100 nm thick.

1 millimeter (mm) = 1,000 microns (µm) = 1,000,000 nanometers (nm)

1 nanometer (nm) = 0.001 microns (µm) = 0.000001 millimeters (mm)

1 inch = 25.4 mm

substances) (Kleber and Lehmann 2019). A comprehensive analysis of alkaline extracts from a surface soil and from a humic substance standard showed that nearly all organic molecules from both sources could be identified as microbial and plant biopolymers and their degradation products, not a distinct category of novel substances (Kelleher and Simpson 2006).

The controversy over how to classify soil organic matter molecules in various stages of decomposition will certainly continue. Both methods that require extracted organic matter and methods that allow characterization of soil organic matter in a more natural state will be valuable to increase our understanding of soil organic matter and its behavior in soil (Kögel-Knabner and Rumpel 2018).

Stable, Assembled, and Dry on the Inside: A New Understanding of Soil Organic Materials

An emergent model for the structure and behavior of soluble organic materials views associations of smaller molecules as **supramolecules**. This model proposes that smaller organic molecules from microbial degradation of plant inputs self-assemble in weakly bound clusters (Piccolo 2002, 2016; Sutton and Sposito 2005). These clusters are stabilized and partially protected from further microbial attack by the formation of hydrophobic regions, where microbial degradation is hindered. Much like oil droplets in water, or **micelles**, the organic molecules collect in progressively larger clusters or supramolecules (Figure 1, step f). Though **humic substances**, by definition, have been historically linked to alkaline extraction, Wells (2019) proposes that organic supramolecules share properties traditionally associated with these materials. Specifically, they form through abiotic processes (i.e., hydrogen bonding and other intermolecular forces that are not physical or chemical reactions and not biologically-mediated processes) and are resistant to microbial attack *because of their chemical form*. Hydrogen bonds, which hold together smaller organic molecules, can be quite strong. In addition to hydrophobic and oxygen-limited areas inside the organic supramolecule, these molecules are also protected from microbial enzymes because of their random structure (many enzymes are secreted by microbes to degrade specific molecules and organic supramolecules may not be a suitable enzyme target) and ability to bind to and inhibit enzymes (Allison 2006; Wells 2019).

As discussed in the section “Icing on a Multilayered Cake: Organic Matter on Clay Surfaces,” organic molecules interface with soil surfaces in a layered fashion that can also be driven by micelle-type dynamics (Figure 1, step e). The hydrophobic core of an organic supramolecule can adhere to a clay particle with the hydrophilic outer layer exposed to the soil water. Because soil water is integral to supporting supramolecules and also influences attraction to clay surfaces, if soils dry out, the association as a supramolecule or with clay can weaken and not necessarily reform in rewetted soils. Thus soils with frequent wet/dry cycles do not support the persistence of supramolecules or clay-associated organic matter as well as soils that are more consistently moist (Kleber et al. 2007; Horwath 2015).

Organic molecules may move between clay particles and the soil solution when plant root exudates or microbes change local chemical conditions (Keiluweit et al. 2015; Piccolo 2016). Plant roots release sugars that serve as a food source for the microbial community. This can increase overall enzyme activity and increase decomposition of unprotected organic matter (Kuzyakov et al. 2000). Additionally, root exudates of **organic acids** can also release protected organic molecules from clay surfaces and increase their solubility and availability to decomposition.

Organic Matter Location, Protection, and Activity

Many of the benefits of soil organic matter to agriculture come from its decomposition and not necessarily its storage. As dead plant and animal residues enter the soil and are decomposed by microorganisms, some of the nutrients held in the residue are made available to plants. For soil microbes, the location of food sources and the local environment determines whether that potential meal can be consumed.

For soil microbes, otherwise available food sources may be effectively locked in **aggregates** or strongly bound to clay surfaces as **mineral-associated organic matter**. Larger litter particles, called **particulate organic matter** (POM), can be freely available or isolated in aggregates. Extremely small organic particles can be dissolved in the soil water (**dissolved organic matter** [defined as < 0.45 µm]). Soil particles can also be separated based on their density; **heavy fraction organic**

matter is assumed to be associated with mineral particles, while **light fraction organic matter** is roughly correlated with POM.

Along with chemical extraction methods, analyzing soil carbon in different physical locations and examining the activity (i.e., how available is carbon for decomposition) of soil organic matter have improved our understanding of SOM dynamics and storage mechanisms. These approaches hold promise for helping to link management practices to soil organic matter data.

Tiny Houses: Aggregate Organic Matter

Soil aggregates are stable clusters of mineral particles (e.g., sand, silt, and clay) and organic matter (Figure 1, steps a and b). They affect soil structure and create pore spaces in soil that allow for water storage, drainage, and air exchange. Organic molecules released from microbes or plants, such as polysaccharides and **proteins**, act as glue and cement to hold together microaggregates. These organic molecules, along with fungal hyphae and plant roots, connect microaggregates (< 0.250 mm) together into macroaggregates (> 0.250 mm) (Totsche et al. 2018).

As much as 90% of SOM can be associated with aggregates. The size distribution of aggregates and how they intertwine determines the size and architecture of soil pores, creating a multitude of microenvironments that affect exposure of aggregate-contained SOM to air and microbes. Channels and caverns of various sizes weave through the spaces between and within aggregates. Some connect to larger pores, creating opportunity for gas exchange and water movement, while others are closed off within the aggregate structure. Small organisms, such as bacteria (< 0.001 mm diameter), can grow in tiny pores (< 0.01 mm) where they are protected from larger predatory organisms, such as protozoa (0.005 to 0.5 mm diameter) and nematodes (0.005–0.100 mm diameter). However, if the organic matter is sealed in pores with no gas exchange then the environment is not conducive for decomposition and organic matter can persist. Pores with the largest bacterial populations are smaller but also connected to larger pores (Totsche et al. 2018).

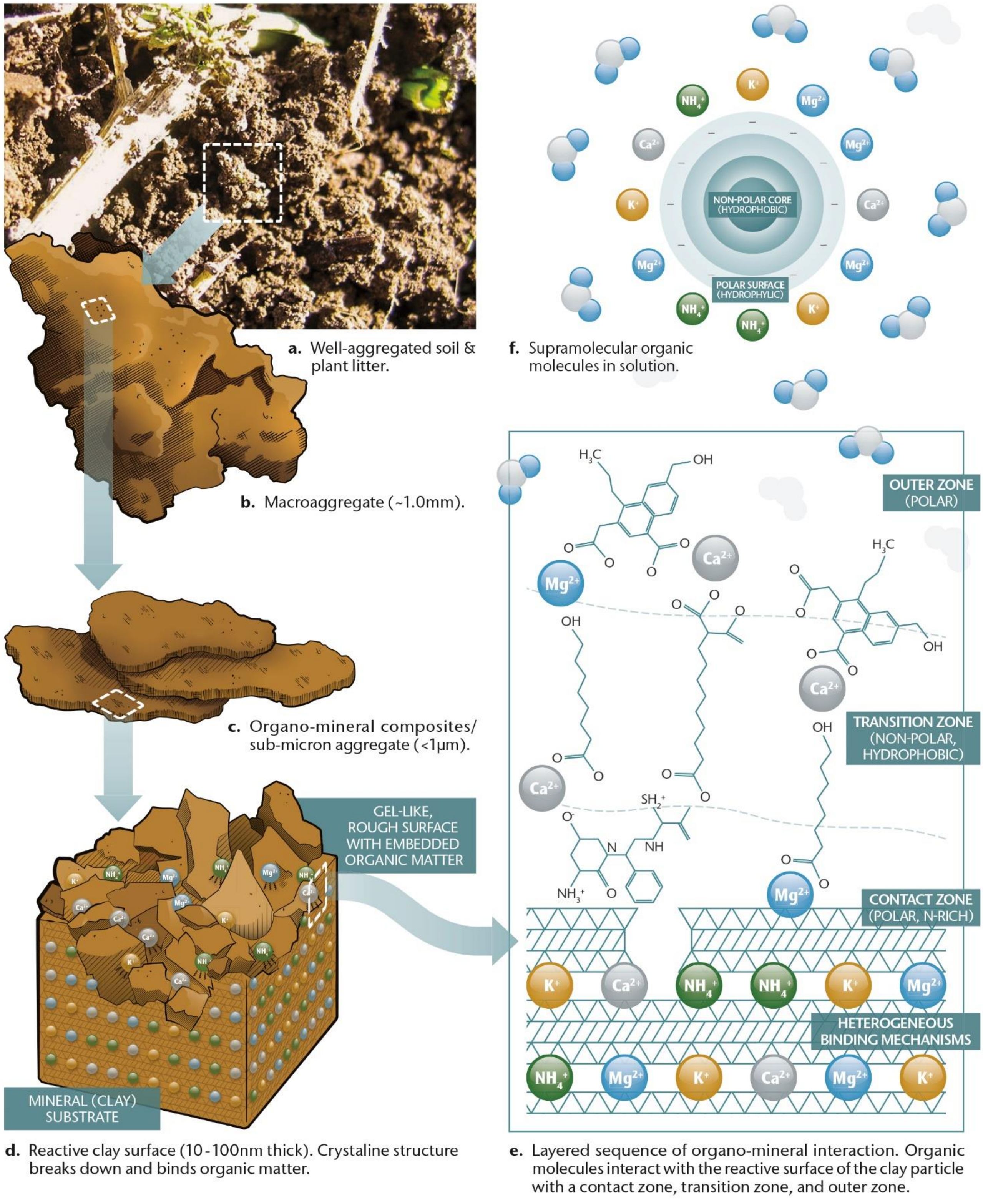
Carbon inside large and small aggregates (known as “occluded POM”) is unavailable for decomposition as long as the aggregate remains intact. Tillage disrupts larger aggregates and frees up organic matter trapped inside (Six et al. 1998). Quantifying aggregate SOM is time-consuming and the method is not likely to be used outside of research in the near future.

Frosting a Multilayered Cake: Organic Matter on Clay Surfaces

The surface area of soil particles is where most of the action occurs in terms of nutrient exchange. The vast majority of surface area is on clay particles (< 0.002 mm or < 2 µm). Individual clay particles are solid minerals primarily composed of oxygen, silica, and aluminum atoms with smaller quantities of magnesium, iron, potassium, and other atoms. Clay minerals have an ordered, flat, sheet-like structure (Figure 1, step e). Organic matter, especially pieces of cell envelopes of dead microbes, such as proteins and fatty acids, can coat these surfaces of clay and smaller silt particles. The bonding of SOM to soil particles occurs by electrostatic connection (i.e., positively charged parts of organic compounds are attracted to negative charges on soil mineral particles), sharing of electrons with atoms in soil particles (**ligand exchange**), and cation bridges (cations with two positive charges, such as Ca^{2+} or Mg^{2+}, “bridge” the negative charge on a clay particle and the negative charge on an organic molecule) (Figure 1, step e). Mineral-associated organic matter is considered to be chemically protected from decomposition, and the amount in soil is related to the soil texture with greater quantities usually in finer-textured soils (Six et al. 2002). Soil scientists can separate mineral-associated organic matter so that it can be quantified.

The protection afforded SOM by its bonding to clay particles has led to the idea of **carbon saturation** in soils. Soils with more clay and small-sized silt can accommodate higher amounts of chemically stable, mineral-associated organic matter. When all the available area on a soil’s clay and silt particles is filled with SOM, that soil is said to be saturated. Soils that are further from their carbon saturation point will more readily build mineral-associated organic matter when organic amendments or residues are added (Stewart et al. 2008). The amount of carbon that can associate with mineral surfaces appears to plateau (Figure 2), but continued application of litters will increase the unprotected particulate and dissolved organic matter pools, keeping the soil engine that supports soil life and provides nutrients.

Even though mineral-associated organic matter is considered to be relatively stable, plants can alter the local environment and disrupt the association. Root activity can counteract the bonds by exuding compounds—root exudates—such as oxalic acid that can remove SOM from the mineral particles (Keiluweit et al. 2015).



a. Well-aggregated soil & plant litter.

b. Macroaggregate (~1.0mm).

c. Organo-mineral composites/ sub-micron aggregate (<1μm).

d. Reactive clay surface (10 - 100nm thick). Crystaline structure breaks down and binds organic matter.

e. Layered sequence of organo-mineral interaction. Organic molecules interact with the reactive surface of the clay particle with a contact zone, transition zone, and outer zone.

f. Supramolecular organic molecules in solution.

Figure 1. Soil organic matter interactions with mineral surfaces. Organic matter in aggregates (steps a–b) is associated with clay particles in organo-mineral composites, also called sub-micron aggregates. Organic matter accumulates in patches (step c) and extends below the surface of the weathering clay mineral (step d). Organic matter interacts with external and internal mineral surfaces in multiple layers (step e). In the contact layer, organic molecules attach, or adsorb, to the negatively charged clay surface through positive charges on proteins (shown on left side of step e), cation bridging (show on right side of step e), hydrophobic interactions, ligand exchange, and weak bonding forces. Cations bridge from the contact zone to a hydrophobic transition layer and then to an outer layer where polar organic molecules interact with soil water. Organic supramolecules form a similar zonal sequence with a hydrophobic inner core and polar surface that interacts with soil water (step f). (Adapted from Miltner et al., 2012 [step c] ; Basile-Doelsch et al., 2015 [step d]; Kleber et al., 2007 [step e]; and Fischer, 2017 [step f]).

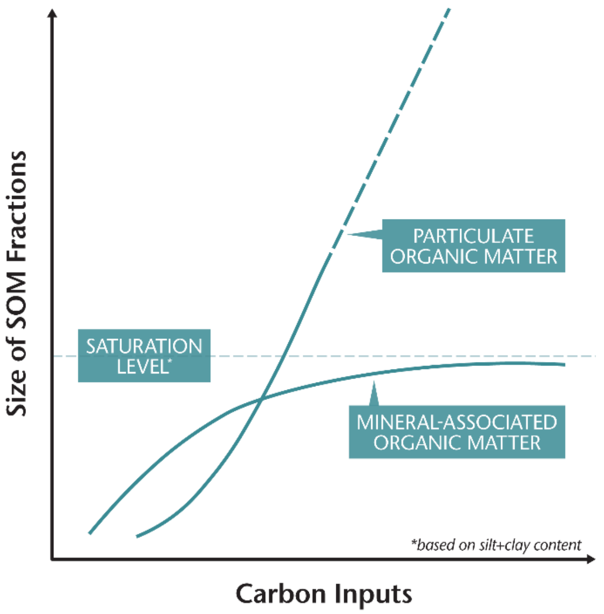


Figure 2. Amount of soil organic matter associated with minerals or as free particulate organic matter with increasing carbon inputs (Adapted from Castellano et al. 2015).

While the ultimate source for SOM is primarily plant material, the material coating soil particles is likely to have been digested, consumed, and recycled by soil microbes. Soil microbes digest plant residues by excreting enzymes that break the chemical bonds holding the material together. They then reabsorb the smaller resulting molecules to build new cell parts as well as divide to form new cells. Throughout the decomposition process, much of the litter carbon is released into the atmosphere as carbon dioxide. Depending on how efficiently microbes are able to utilize the food source, this portion of carbon returned to the atmosphere can be high for complex litters (i.e., 70 to 90%), such as lignin, or low for simple sugars (i.e., 25%), such as glucose (Cotrufo et al. 2013).

As individual bacteria die and break apart, their cell membranes, which include proteins and lipids, enter the soil matrix. Proteins can directly interact with negatively charged mineral surfaces in the contact zone and lipids form the hydrophobic transition zone (Figure 1, step e). Also, microbial cell membranes consist of fragments and macromolecules, which persist longer in soils than single molecules (for example, peptides and short chains of amino acids are not degraded as easily as free amino acids). Thus, these microbially-assimilated residues are more likely to coat clay particles than the original plant residues (Miltner et al. 2012).

Because of the importance of microbes in stabilizing organic matter on mineral surfaces, their food sources are important. The type of organic material added to the soil affects the degree to which carbon can be stabilized in the mineral fraction. “High quality” residues, having low carbon to nitrogen ratios (C:N) and low phenol and lignin concentrations are more likely to be converted to stable SOM. Nitrogen-rich materials are easily digestible by microbes while phenols and lignins are more resistant to degradation. Degradation of these residues will thus produce more microbial-derived organic matter (i.e., dead cells) and lead to more carbon stabilized in association with silt and clay particles than residues with low N or higher levels of phenol or lignin. Cotrufo et al. (2013) dubbed the relationship between organic material quality and stabilization the **Microbial Efficiency-Matrix Stabilization** (**MEMS**) **framework**.

It is noteworthy that this concept is a departure from previous concepts of stabilization of organic matter where lignin was considered an important precursor to “stable” humic materials. It follows that management practices aimed at stabilizing organic matter in soils should be adjusted to include more high quality inputs (as suggested by the MEMS framework), though application of N-rich litters beyond agronomic goals should still be avoided. Also, if soils are far from carbon saturation, it is hypothesized that quality of residue inputs will have less of an effect on rates of stabilization than soils that are closer to saturation (Castellano et al. 2015).

The Debris Field: Particulate Organic Matter

Particulate organic matter (POM) is defined as sand-sized pieces of organic matter (0.053 mm–2.000 mm or 53–2000 µm), which is much larger than mineral associated SOM (clay minerals are < 0.002 mm or 2 µm and silt particles are between clay and sand in size), POM is composed of partially decomposed litter from plant shoots or roots, fungal hyphae, spores, seeds, fecal pellets, or bodies of soil-dwelling animals. POM can be found in aggregates where it is more protected from microbes (see “Tiny Houses: Aggregate Organic Matter” section above). POM is generally a lower quality food source for microbes than mineral-associated organic matter because it has a higher C:N and consists of larger polymers that are not readily decomposed (Lavallee et al. 2019). However, most POM is thought to be loose in the soil and therefore available for decomposition (i.e., labile) (Marriott and Wander 2006; Chenu et al. 2015). Because of its higher availability to microbes, the amount of POM in a soil is linked to nutrient cycling in soils, total microbial biomass, soil respiration (release of CO_2), and soilborne disease suppression (Stone et al. 2001).

Compared to mineral-associated organic matter, POM is more available, but it may not be of good quality to microbes. Mineral-associated SOM is a good food source but must first be mobilized from the mineral surface. There are several methods employed by researchers for separating POM from mineral-associated organic matter. Because of their different pathways for use by microorganisms and residence times in soil (mineral-associated organic matter may exist for decades to centuries while POM is generally decomposed in less than ten years), a standard and widely available method to test POM and mineral-associated organic matter is warranted (Lavallee et al. 2019).

Carbon on the Move: Dissolved Organic Matter

Dissolved organic matter is a mixture of extremely small molecules that may aggregate together into clusters (i.e., a supramolecular structure, see Figure 1, step f) (Sutton and Sposito 2005). Small SOM-mineral complexes can also be dissolved. Dissolved organic matter generally represents less than one percent of organic carbon in agricultural soils, and a smaller amount in forest soils, but can be an important source of carbon for soils below the root zone. Even though it can be dissolved in water, these molecules are not necessarily all easily degraded by soil microbes (Kleber 2010; Chenu et al. 2015).

Par Baked Carbon: Biochar or Pyrolized Organic Matter

Biochar is organic material that has been combusted without addition of oxygen (known as pyrolysis) with the intention of using it as a soil amendment. Carbon in the soil that was previously burned, which can occur naturally in a forest or prairie fire, is known as black carbon. Adding biochar to soils increases the amount of black carbon, and this may have positive effects on important soil properties. Biochar is porous and has a large surface area, which can decrease soil compaction and improve fertility and aggregation. Any organic material can be used to make biochar, though some high moisture organic waste products are better suited for composting. Production of biochar soil amendment that can store carbon in the soil and also improve soil quality may be a good use for woody trimmings from tree fruit production or waste from paper and lumber industries, which may otherwise be completely burned or incinerated. Like many organic amendments, moving biochar from areas of high biomass production to areas with less biomass is likely cost prohibitive.

When organic materials are heated, the molecular structure of the original materials are changed. At lower temperature and heating duration, carbon structures are more amorphous and may still contain volatile compounds while, at more intensive temperatures, graphite-like crystalline structures are formed and volatile chemicals are nearly completely lost (Kleber 2010). In other words, extensive heating of organic material creates a material similar to an inorganic or mineral form of carbon (graphite). In addition to its mineral-like structure, another explanation for the observed stability of biochar in soils could be the lack of a decomposer community capable of using the material as a food source (Kleber 2010).

The relative stability of biochar means it is not a soil input that will directly build the microbial community (i.e., as a food source), though changes to soil structure may alter the soil environment and create new habitat. Amendments that are richer in degradable carbon move energy through the soil microbial community and support important soil functions (see the Interpretation of Soil Organic Carbon Measurements section below).

Can I Eat That?: Organic Matter Activity

In addition to the structure and location of SOM, researchers have studied the rate and degree of decomposition of SOM by microbes. The results generally indicate that some SOM decomposes quickly (a fast pool) while the remaining portion decomposes at a slower rate (slow pool). Mineral-associated and aggregate-protected organic matter usually correspond to a slow pool, but changing conditions, such as through tillage, can expose even these protected food sources to microbial decomposition. POM may contain both slow and fast pools depending on the amount and type of recent additions of organic matter. By better understanding the physical location of these pools and how management affects their size, producers can improve their ability to manage soils for multiple, sometimes competing, objectives.

For example, the fertility contribution of soil organic matter requires decomposition of the material (building the active or fast pool) while storing carbon for decades to centuries requires some degree of protection of soil organic matter (building the slow pool). High quality (low C:N) litters are valuable for both increasing fertility and also for providing microbial byproducts likely to associate with clay particles.

Measuring Soil Organic Matter Accumulation and Loss

As discussed above, soil organic matter is not a uniform substance but instead a complex mixture of organic molecules. The location of these molecules is also complex. They can be stuck to mineral particles and be simultaneously folded into aggregates. They cluster in supramolecular structures dissolved in soil water or adhering to surfaces, or exist as free or occluded particulate matter. However, the measurements of total soil organic matter are fortunately not nearly as complex.

The Whole Organic Pie

Soil organic matter is mostly carbon atoms with other elements bound to the carbon. Measurements target either organic carbon or actual soil organic matter. Organic carbon is measured by dry combustion. The soil is heated to 1350°C and the CO_2 loss is measured, giving the soil organic carbon (SOC). This is an accurate method, but not widely used outside of research. Instead, commercial soil labs use the less expensive and loss-on-ignition method to estimate soil organic matter. Temperature, duration of heating, and sample size vary and can affect results, but a common methodology involves heating soils at 360°C for two hours. Organic matter (carbon and associated atoms) are lost from the sample and the difference in weight is calculated as soil organic matter. Loss-on-ignition data is often modified by a correction factor for local soils to approximate the dry combustion method.

In soils with significant inorganic carbon (carbon in the form of calcium or magnesium carbonates), the dry combustion and loss-on-ignition methods described above must be preceded by a pretreatment to remove this inorganic carbon. A third method, called Walkley-Black wet oxidation, gives different values than dry combustion (generally higher), but the two tests are highly correlated in soils with pH less than 7.2. However organic matter is measured, it is important to know which method is being used when comparing data from different labs or regions. Sullivan et al. (2019) discuss general principles for accurate soil sampling methods as well as variability in different laboratory tests for SOM.

The measured SOC, given as percent by weight of soil, is converted to percent soil organic matter by a conversion factor based on the carbon in soil organic matter. The standard factor to use has been 1.74, with one percent SOC equal to 1.74 percent SOM, but a factor of 2.0 has been offered as a more accurate factor for many soils (Pribyl 2010).

Taking Slices of Total Soil Organic Matter

Because of the various ways in which soil organic matter is protected in the soil and because the amount of SOM in soils is huge relative to annual plant biomass inputs, the total soil organic matter changes slowly in response to management. It is often three to five years before an increase in total SOM can be seen following a change in practice. However, soil scientists have worked to find a measurement of SOM that responds more quickly to management changes. This would allow farmers to quickly evaluate practices and adjust accordingly. “Active” is the term used to describe some of these methods which, in this case, means they are more easily removed from the soil than other components of SOM, but they may still be protected on mineral surfaces or in aggregates. Some methods for measuring active pools of soil carbon, such as the various fractionation methods (e.g., aggregate-carbon, mineral-associated-carbon, dissolved carbon), are useful for research but are too slow or expensive for commercial use. Other quick or inexpensive tests have proven to be difficult to interpret or too variable. There are, however, two methods: permanganate-oxidizable carbon (POXC) and mineralizable carbon (MinC), that are relatively quick and inexpensive (Hurisso et al. 2016). These two methods give contrasting views of how SOM is changing in the soil. POXC shows the accumulation of soil carbon while MinC shows the loss of soil carbon from both SOM and from decomposition of plant materials. These two processes are the source of many of the benefits of SOM and so are important to measure.

POXC

POXC is thought to represent the more recent storage of carbon inputs in the soil (Hurisso et al. 2016). Increases in POXC values are likely related to the practices that increase biomolecules in the soil solution and in association with mineral particles: plant biomass decomposition by microbes and direct contribution by microbes through their products and necromass (dead biomass). Practices that lead to SOC stabilization and accumulation include reduced tillage and soil amendments, such as compost.

POXC is sensitive to tillage-driven changes in the soil (Awale et al. 2017) and to different soil amendment practices (Bongiorno et al. 2019). In a Europe-wide study, POXC was the indicator most related to other fertility, physical, and biological measurements (Bongiorno et al. 2019). It was also related to total soil carbon, microbial biomass carbon, and particulate organic carbon but showed a greater sensitivity to changes in management or rotation than those other indicators across 12 studies (Culman et al. 2012).

In addition to being sensitive to management, relative to other measurements it is quick, cheap, and easy to run. Commercial soil labs have begun to offer POXC testing.

Mineralizable Carbon

Mineralization is the use of soil organic carbon as an energy source (food) by soil microbial populations. This organic carbon comes mostly from plants but also from microbes and other soil organisms. In extracting energy, much of the carbon in the biomass leaves the soil as CO_2. This loss of CO_2 is a good indicator of potential soil biological activity. As with POXC, MinC has been found to be better than most other soil measurements in predicting agronomic performance in soils (Culman et al. 2013). Both POXC and MinC were able to distinguish differences in both rotation and in soil management.

In research, MinC, also called soil respiration, is usually measured over long time periods, several days to weeks. The most robust measurements of carbon mineralization take several weeks. Although this more complete mineralization test is recognized as an important index of soil health, there are no standardized methods and the tests are neither quick nor cheap to conduct (Wade et al. 2018).

However, research has found that the flush of CO_2 released from soil when it is rewetted from a dry condition is related to carbon mineralization and has the benefit of being quick, and easy to conduct (Franzluebbers 2018). There are standards for 4-day, 3-day, and 24-hr incubation periods. The latter has been commercialized (e.g., Solvita) and is available in some commercial soil labs. Research has found that the variability between labs in these carbon mineralization tests is 2–20 times greater than other commercial soil tests, so data from different labs should be compared with caution.

The most valuable attribute of POXC and MinC is that they have been shown to be more related to crop yield (Hurisso et al. 2016) than other tested measurements.

Soil Protein

Another slice of soil organic matter that has potential in evaluating soils is organic nitrogen. Nitrogen is a critical nutrient for crops and for soil microbial communities. As soil organic carbon is mineralized, so is organic nitrogen; the carbon and nitrogen cycles are linked. One promising measure of the organic-nitrogen component is soil protein. Soil protein represents the largest N source in soil organic matter that is available to microbes and, through them, to crops (Hurisso et al. 2018). Although once called glomalin, after the fungal group *Glomales*, it is now recognized that these proteins are from a variety of sources. Soil protein can be measured by a method called autoclaved-citrate extraction (ACE-protein), which has been found to be sensitive to management changes and differences in soil health (Hurisso et al. 2018). The method extracts protein from a variety of organic sources and so can be viewed as an indicator of soil organic nitrogen released (mineralized) to the soil. The nitrogen-supplying ability of a soil's organic matter has been linked closely with positive yield effects, much more so than the other benefits of soil organic matter (Schjønning et al. 2018). ACE protein is offered by an increasing number of commercial soil testing services.

Interpretation of Soil Organic Carbon Measurements

Measurements must be interpreted. With soil organic matter and its related indexes, it is often thought that more is always better. Although there have been several attempts, a minimum threshold of SOM for crop production has not been found (Loveland and Webb 2003). This is probably because management, including tillage (in the short term) and fertilizer inputs, can often substitute for many of the benefits of higher levels of SOM. Interpretation of SOM calls for a more nuanced view.

Research is finding that the flow of carbon through a soil may be as or more important than the accumulation of carbon stored in the soil as SOM (Schjønning et al. 2018; Kästner and Miltner 2018). The effects of SOM on crop production has been likened to a hydroelectric plant (Janzen 2006). The height of the water behind the dam (see Figure 3) is what determines the potential amount of energy that can be produced. The flow through the turbines is this potential being used to produce power. If too much water is let through the turbines, the water level drops. In the soil, the amount of organic matter is the potential for biological activity, estimated by POXC. The release of organic carbon, MinC, is the actual flow through the soil's microbial community. If MinC is too high, SOM will drop unless carbon is added through amendments or plant biomass. In contrast, just building SOM levels with low MinC levels will limit benefits, such as N release to crops. It is best to keep in mind both accumulation and flow.

This is why tests of SOM storage, like POXC, and of SOM flow, as measured by mineralization, are valuable for management. However, there are still factors to consider when interpreting these measurements.

To evaluate management changes, POXC and MinC should be measured regularly, every year or two, at the same time of the year. The direction of change should then be determined for each measurement. POXC and MinC, are then best interpreted together (Table 1), and with consideration of recent field practices. Although target levels of these measurements have been established for some regions, this work is ongoing for the West and Washington State.

Field practices affect both POXC and MinC. Cover crops can affect both indicators depending on their carbon to nitrogen ratios (C:N) and tillage. Legumes with lower C:N can lead to increased rates of carbon mineralization compared to non-legumes because of the added nitrogen. However, more of the legume residue is likely to end up as SOM. A no-till cereal rye cover crop will affect POXC more than MinC because of reduced soil disturbance relative to conventional tillage (Hurisso et al. 2016). Mineralization rates are increased by tillage and application of low C:N soil amendments, such as chicken manure. They are decreased by lack of carbon inputs, water logging (reduced oxygen levels), and by application of high C:N amendments, such as wheat straw.

Depth Considerations with SOM

Because building SOM can be slow and costly, it is best to concentrate SOM where it can do the most good, at the soil surface (Franzluebbers 2002). While the total amount of soil organic matter in the root zone is important to storage of nutrients and water, it is the surface organic matter that is important for many other soil functions. A functioning soil surface, especially if also covered with crop residue, prevents wind and water erosion, supports free exchange of air, enhances biological activity, allows seedlings to emerge unhindered and plant roots to proliferate, and promotes quick water infiltration. When the soil surface does not function, the direct and indirect consequences on the profits, productivity, and environmental impact of agriculture are large.

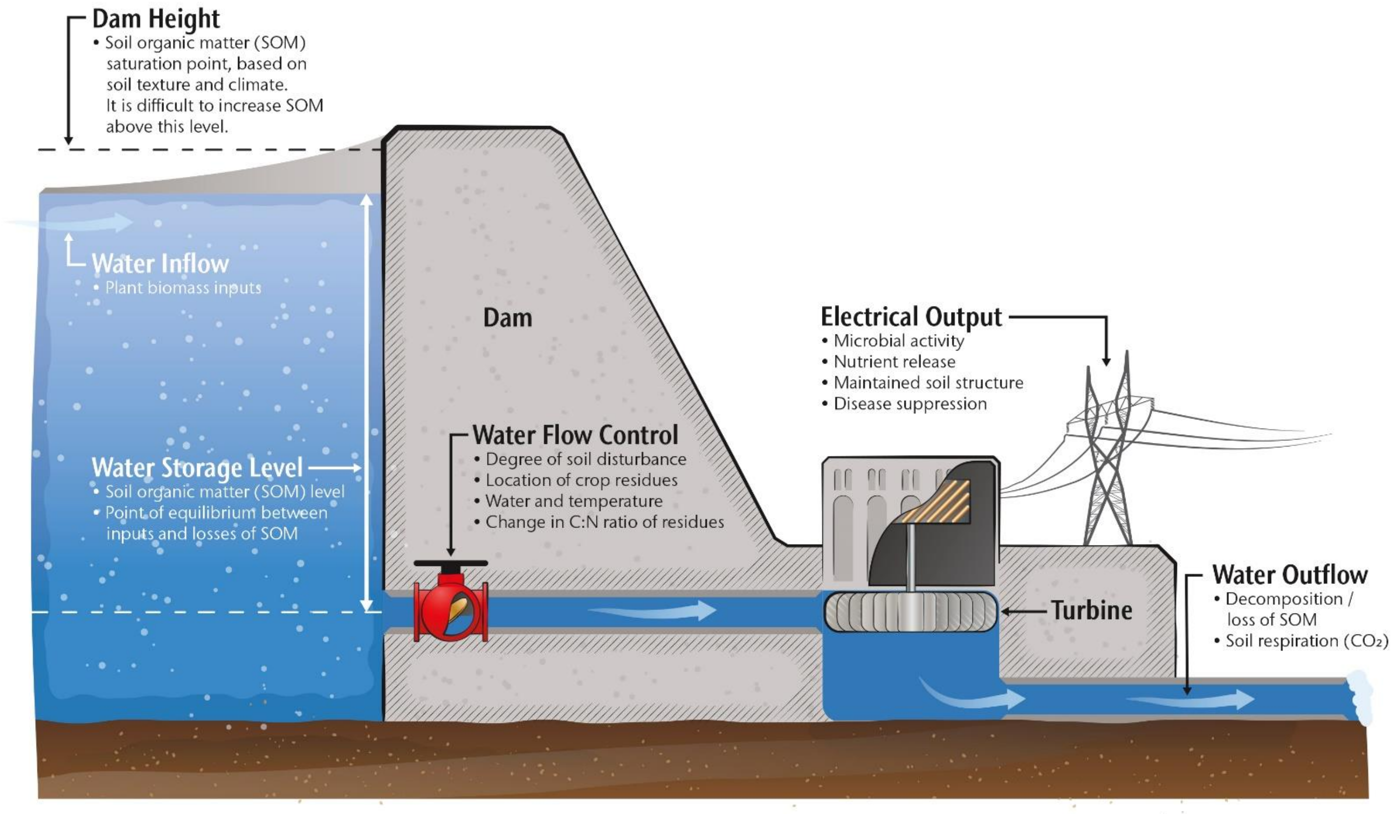


Figure 3. Soil organic matter storage and flux can be thought of as water behind a dam (storage) and conversion to energy (flux). (Adapted from Janzen, 2006.)

Table 1. Considerations when interpreting POXC and MinC values together. This table only considers soil management practices that affect inputs of plant biomass or amendments and losses of soil organic matter. Other factors, such as pH, salinity, compaction, etc. should also be kept in mind.

		MinC (Loss of SOM)		
		Decreasing	Steady	Increasing
POXC (Gain of SOM)	Decreasing (Inputs < Losses)	Increase plant inputs.	Increase plant inputs, Decrease soil disturbance.	Did recent practices affect MinC, such as tillage? Increase plant or organic amendment inputs, Decrease soil disturbance.
	Steady (Inputs = Losses)	Increase decomposition of SOM?[1]	Are you getting the benefits you want from your SOM?	Decrease decomposition?[2]
	Increasing (Inputs > Losses)	Increase decomposition of SOM?	Are you getting the benefits you want from your SOM?	Decrease decomposition?

[1] Practices that increase decomposition: tillage; keeping soil moisture high, but not saturated; increasing plant inputs while maintaining other factors at current levels, incorporating plant residues.

[2] Practices that decrease decomposition: reducing tillage, that is, not incorporating crop residues; keeping soil dry; reducing crop residues while keeping other factors at current levels.

Managing the soil so that organic matter is concentrated in the top two to three inches of the soil is supported by research in several countries (Franzluebbers 2013). The same amount of carbon concentrated at the surface rather than distributed uniformly in the top foot can yield greater benefits. In one test, increased total organic matter improved water infiltration by 27%, but concentrating organic matter at the surface improved water infiltration by nearly 300%. This only applies when surface SOM levels are below saturation levels and silt and clay levels are conducive to storing carbon at the surface.

This change in mindset from total SOM to depth of SOM also requires a change in measurements. To measure the effects of surface-concentrated organic matter, a surface concentration ratio (SCR) is used:

SCR = (organic matter in the top 2–4 in.) / (organic matter in the 6–12 in. zone). (There are not yet standard depths for this indicator.)

The surface concentration ratio (also called a stratification ratio by researchers) (Franzluebbers 2010) can be a useful indicator of overall soil health. The SCR is not limited to SOM, but can be used with POXC, MinC, and other such indicators. The downside of this measurement is that each SCR value requires two analyses, one for each depth, doubling testing costs. Also, the relationship between SCR and overall soil health breaks down if there are compaction layers below the surface.

A Simple Suite of Soil Health Measurements

Research on soil health indicators is ongoing. However, the synthesis of many studies is pointing to a few useful soil health measurements to consider (Fine et al. 2017; van Es 2019):

- SOM or SOC
- POXC
- MinC
- ACE-N (soil protein)
- Aggregate stability
- Soil penetration resistance (highly affected by soil moisture levels)

Aggregate stability and soil penetration resistance indicate a soil's physical condition. Although they are related to soil organic matter, they involve other factors, such as soil texture, disturbance, and moisture levels. Using all or several of these proven measurements can help you evaluate your soil building efforts over time.

Conclusion

Organic matter has long been considered a critical parameter in evaluating soil health. However, describing, measuring, and managing organic matter is complicated. The combination of changing soil conditions, the intimate relationships formed between microbes, biomolecules, soil minerals, and aggregates, and the practical needs of crop production make SOM management a daunting task. This publication has presented the latest in research on SOM and its measurement in hopes of making this task a little easier for farmers, gardeners, and anyone else who manages soils.

Analytical Variability of Various Soil Measurements Compared

(Hurisso et al. 2018)

Highest to lowest: MinC > SOC = POXC > Protein >Mehlich P > pH

Variability in time: MinC > SOC = POXC = Mehlich P > Protein > pH

MinC and POXC have analytical variability similar to SOC by loss on ignition

Protein is similar to Mehlich-P testing

Variability over time and space similar to other routine soil nutrient tests

Glossary

aggregate: A clump of sand, silt, clay, and organic matter that is relatively stable in the presence of water or mild force. There is a hierarchy of aggregation in soils: submicron aggregates or organo-mineral composites (< 0.001 mm or < 1 µm) may link together to form microaggregates (< 0.250 mm or 250 µm), which link together to form macroaggregates (> 0.250 mm or 250 µm). Descriptions of aggregation for a particular soil are sensitive to the specific method chosen and specifically to the energy intensity imparted to the soil sample.

alkaline extraction (also called "progressive extraction" and "soil organic matter extraction," see Figure 4): Bulk soil organic matter has historically been fractioned into different components through a progressive extraction that begins with reaction of soil in a strongly alkaline solution (pH 13), producing an insoluble material (humin) and a soluble portion (humic substances). The high pH solution increases solubility of organic molecules by decreasing bonding with divalent or trivalent cations (such as Ca^{2+} or NH^{3+}) and reducing or eliminating hydrogen bonding and hydrophic interactions that adhere organic molecules to each other and to soil particles. At high pH, the organic molecules act as salts and become soluble by bonding with the monovalent cations Na^{+} or K^{+}. The alkali-soluble extract (humic substances) is then reacted in acid solution (pH 1 or 2) to produce an insoluble precipitate (humic acid) and an acid soluble portion (fulvic acid). This can be described as an "operational" definition of soil organic matter since the fractions are observed following a laboratory operation that creates conditions not present in soil. Alkaline extraction has been criticized for creation of artifacts during the procedure.

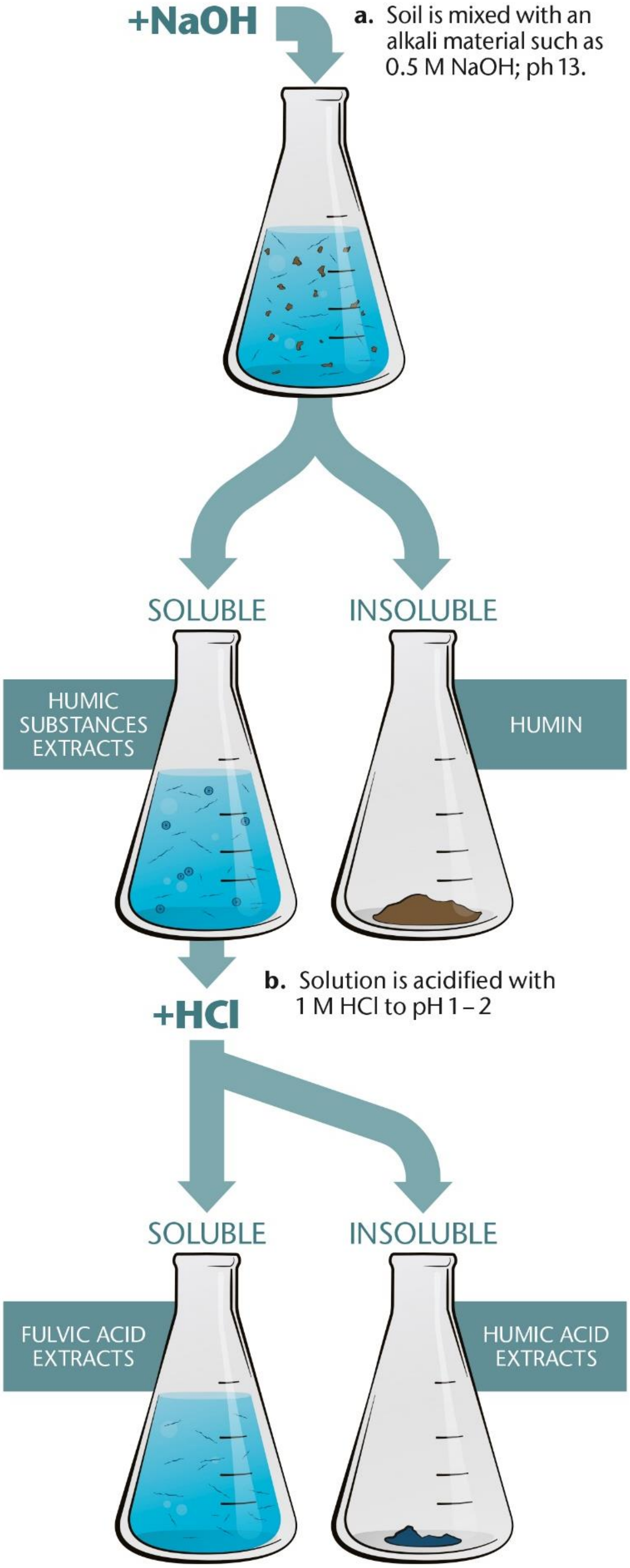


Figure 4. Progressive extraction of organic matter with alkaline and acid solutions.

aliphatic (compounds): Hydrocarbons that are open chains or form rings. Unlike aromatic compounds, they do not contain a stable ring. Aliphatic compounds are hydrophobic and non-polar.

amino acids: Building blocks of protein that contain both an amine group (NH_2) and a carboxylic acid (COOH) attached to a carbon backbone. The specific characteristics of the carbon backbone affect the chemical behavior of the amino acid (polar, non-polar, neutral, positive, or negative). See also: **polypeptides** and **proteins**.

amphiphilic: Characteristic of molecules with a polar (both positive and negative charges) water-soluble group attached to a water-insoluble hydrocarbon chain.

aromatic compounds: Hydrocarbons that are cyclic and planar with a stable ring. Aromatic compounds are hydrophobic and non-polar.

biomolecules: Molecules that are produced by living organisms, including carbohydrates, polypeptides, amino acids, **nucleic acids**, organic acids, and aliphatic and aromatic compounds.

carbohydrates: Six or five membered carbon rings containing an oxygen with –OH groups as constituents. Polymers are called oligosaccharides and polysaccharides. Historically considered important precursors to humic acids.

carbon saturation: The concept that soils can stabilize a limited amount of carbon on the surface of mineral particles. Soils with greater clay content have the ability to stabilize more carbon on the surfaces. See also: **mineral-associated organic matter**.

chemically-protected organic matter: Organic matter that is protected from microorganism degradation due to chemical bonds that adhere molecules to mineral surfaces (see **mineral-associated organic matter**).

dissolved organic matter: Soil organic matter smaller than 0.45 µm after extraction with solutions ranging from cold water to saline solutions.

fulvic acid: Operationally defined as organic material that is soluble in alkaline extraction and soluble in a subsequent acid extraction.

heavy fraction organic matter: Roughly corresponds to **mineral-associated organic matter** but could also include dense particulate organic matter. The association with clay minerals makes the material more dense (> 1.6–1.85 g[cm^{-3}]).

humification: A proposed process of biochemical and physiochemical transformation of organic molecules that leads to unique structures in soil not inherited directly from organisms. The humification process is reported to lead to the progressive accumulation of non-polar (i.e., hydrophobic) compounds in domains excluded from water and microbial activity. Humification is perceived to involve both biotic and abiotic processes acting on litter inputs and the byproducts of decomposition. Critics of this terminology (humification, humus, humic acid) argue that it is tied to alkaline extraction and not to an actual soil process. A proposed replacement is *decaying organic material*.

humic acid: Operationally defined as organic material that is soluble in alkaline extraction and insoluble in acid extraction.

humic substances: Operationally defined as material that is soluble in alkaline extraction.

humin: Operationally defined as material that is insoluble in alkaline extraction. Thought to be organo-mineral complexes of organic matter.

humus: Frequently used synonymously with soil organic matter. Sometimes considered to be a narrower portion of the total soil organic matter that does not include undecomposed litter (i.e., recognizable organic debris from animals and vegetation) or **biomolecules**. The term humads has been used to describe "active" humus in contrast to "passive" humus. Organic matter (humus) has been historically subdivided via **alkaline extraction/progressive chemical extraction**. See also: **humic substances**, **humic acid**, **fulvic acid**, and **humin**.

hydrolysable and nonhydrolyzable organic matter: Hydrolysable molecules are broken apart (decomposed) by water or acid. Acid hydrolysable organic matter, for example, is quantified with 6 M HCl acid and the nonhydrolyzable organic matter (identified as mineral-associated molecules and some long-chain aliphatic and aromatic compounds) resists acid.

ligand exchange: Weathering soil clay particles are characterized by Al or Si atoms bound to a hydroxyl group (OH^-) that can carry a negative charge at high pH. The charged (polar) heads of **amphiphilic** molecules can share electrons with these hydroxylated metal ions in a ligand exchange.

light fraction organic matter: Roughly corresponds to **particulate organic matter** but is separated based on density not size. Separation is primarily done with sodium polytungstate and the light fraction is considered to be < 1.6–1.85 g/cm^3).

lignin: Dense, hydrophobic, hydrocarbon molecules that are resistant to breakdown by enzymes. Some fungi are capable of decomposing lignin. Lignin compounds are found in cell walls of terrestrial plants.

micelles: A supramolecular assembly of surfactant molecules dispersed in a liquid colloid. In aqueous solution, hydrophilic regions face outward and protect an inner hydrophobic region.

nucleic acids: Include DNA and RNA. They are composed of monomers, which are nucleotides made of three components: a five-carbon sugar, a phosphate group, and a nitrogenous base.

organic acids: Organic compounds with acidic properties. The most common are carboxylic acids. They are generally weak acids and do not dissociate completely in water. Examples include acetic acid, oxalic acid, lactic acid, and citric acid.

organic carbon: Carbon associated with other elements to form molecules that do not exist as a gas (e.g., carbon dioxide or methane) or form mineral carbon compounds such as carbonate-carbon (carbon in the form of calcium or magnesium carbonates). Carbonates can occur in arid soils, so it can be important to clarify that one is referring to "soil organic carbon" and not "total carbon."

organic molecules: A molecule containing the elements hydrogen and carbon.

particulate organic matter (POM): Organic matter that is greater than 0.052 mm (52 µm) in size and not closely associated with mineral surfaces. POM is a heterogeneous mixture (i.e., the same fraction could include fresh litter as well as pieces of biochar) but is generally less dense and less nitrogen rich than **mineral-associated organic matter**. Occluded POM is POM protected from microbial attack in aggregates.

phenols: Cyclic, planar (aromatic) organic compounds. Polyphenolic molecules, such as tannins, are a significant component of some terrestrial plants.

polypeptides: Short chains of amino acid monomers linked by peptide (amide) bonds. Proteins are polypeptides that have more well defined secondary, tertiary, and quaternary structure.

proteins: Complex molecules made from linking amino acids.

Microbial Efficiency-Matrix Stabilization (MEMS) framework: A hypothesis that connects decomposition of plant litters with stabilization of organic matter in soil, particularly on clay surfaces in organo-mineral complexes. High quality litters, with low carbon to nitrogen ratios and low concentrations of phenol and lignin, are more easily converted to microbial biomass. Microbial biomass is more likely to associate with mineral surfaces where it may be protected from further decomposition.

mineral-associated organic matter: Carbon-containing compounds that are bound to soil particles, especially clay-sized particles that account for most of the surface area in soils. Carbon molecules bind to clay through electrostatic connection (i.e., positive charged parts of organic compounds are attracted to negative charges on soil particles), by sharing of electrons with atoms in soil particles (ligand exchange), and cation bridges (cations with two positive charges, such as Ca^{2+} or Mg^{2+}, "bridge" the negative charge on a clay particle and the negative charge on an organic molecule). See Figure 1 (step e).

root exudates: Ions, amino acids, sugars, mucilage, organic acids, and other small molecular weight compounds either leaked from root cells or actively secreted into the area surrounding roots. Organic acids, such as acetic and oxalic acid, can increase nutrient solubility and mobilize adsorbed organic molecules from mineral surfaces. Amino acids and sugars provide significant energy and nutrients to soil microbes. Root exudates are a part of the total root deposition, which also includes root turnover and sloughing off of cells. Between 5% and 21% of the total photosynthetically-fixed carbon can be deposited in soil (Balestrini et al. 2015)

soil organic matter: Includes recognizable organic debris from living and dead organisms and biomolecules that are the result of decomposition of the original organic inputs. Biomolecules include carbohydrates, polypeptides, amino acids, nucleic acids, organic acids, and aliphatic and aromatic compounds. It contributes to soil fertility by providing N, P, S, and other nutrients through decomposition. Soil organic matter is typically 50% to 58% carbon. **Humic substances** are also considered to be part of the soil organic matter.

supramolecules: Assemblages of two or more smaller molecules. Smaller organic molecules derived from microbial degradation of plant inputs are thought to form supramolecules in soils. The smaller molecules self-assemble in weakly bound clusters that are stabilized and partially protected from further microbial attack by the formation of hydrophobic regions, where microbial degradation is hindered.

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