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Soil organic matter black box

Examining alkaline extraction and humic substances research

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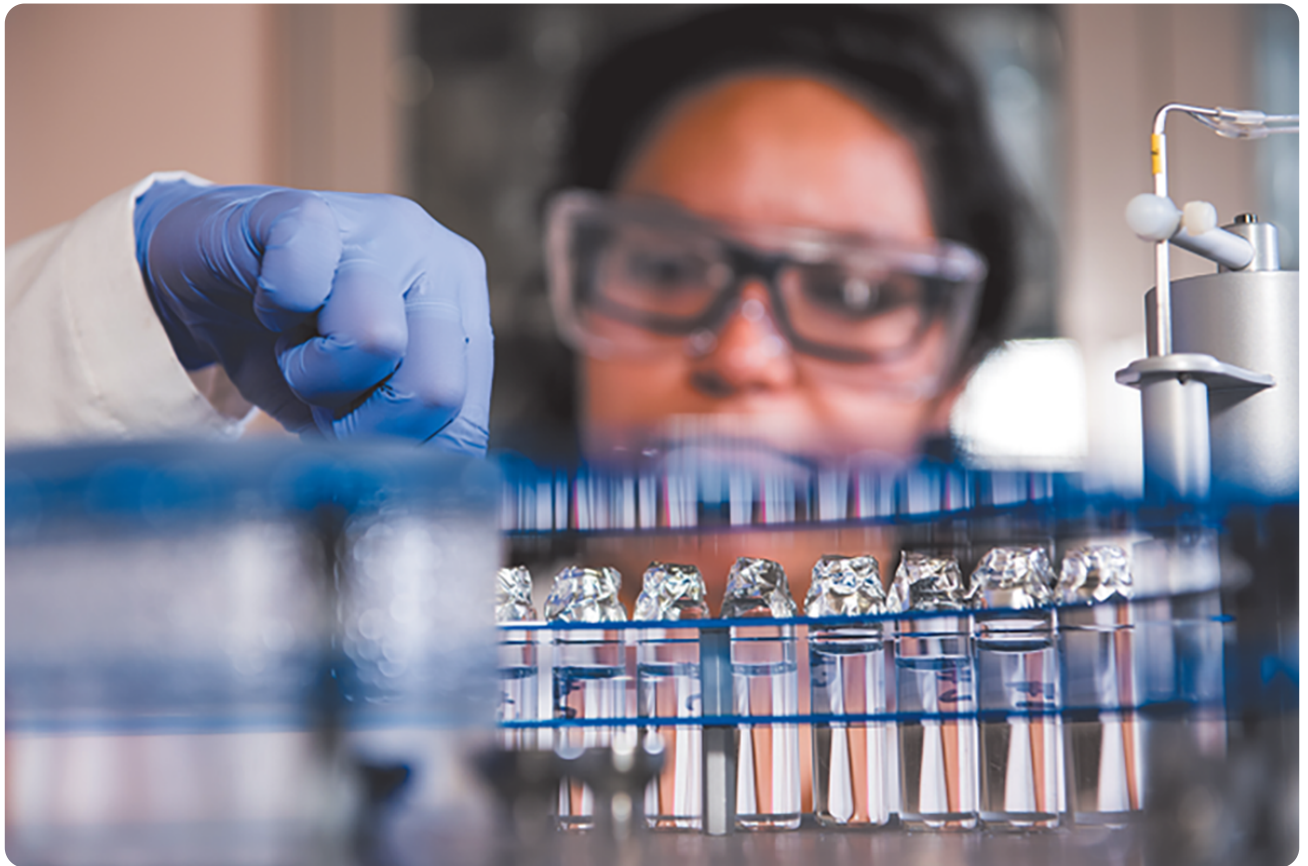


Photo courtesy of Andrea Starr, Pacific Northwest National Laboratory.

- A recent special section in the *Journal of Environmental Quality* collected more than 10 papers to examine the state of research on alkaline extraction and humic substances.
 - Both proponents and opponents of the method published papers discussing the viability and reliability of alkaline extraction.
 - Alkaline extraction represents a tool in soil scientists' toolkit to better understand the effect of management and environmental practices on soil organic matter.
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The famed "black box," present in every aircraft, records critical flight data and conversations in the cockpit at the time of a disaster. When the black box is rescued from the rubble and cracked open, it can tell investigators what went awry. In the soil, organic matter acts like that black box, holding the history of carbon and nutrient cycling over time and illuminating the intricate relationships between those resources and plant life.

This information in soil organic matter (SOM) may be a means of understanding what goes wrong when crops fail or yields fall. But these secrets have not been forthcoming. The role of SOM has shown itself slippery to quantify, at best.

Understanding exactly what is going on in the soil has implications for both management practices and environmental applications. Soil amendments, crop

rotations, cover crops, tillage, grazing, and environmental perturbations (among many other factors) affect the makeup of organic matter in the soil. Knowing how these variables interact and affect SOM will help researchers develop the best management practices for the highest yields and healthiest fields.

“Since soil organic matter is such a big pool of carbon—it holds roughly twice as much carbon as CO₂ does in the air—the more you know about it, the more you're probably able to design management systems that can sequester more carbon in the soil,” says Tsutomu Ohno, a professor of soil chemistry at the University of Maine and technical editor of a recent special section in the *Journal of Environmental Quality* focused on humic substances.



Photo courtesy of NCDA&CS.

This special section sought to examine one distinct method for characterizing SOM: alkaline extraction. Titled “Using Alkaline Extraction to Understand Environmental Processes,” the section brought together more than 10 papers discussing the controversy surrounding the method.

To get you up to speed: researchers using alkaline extraction seek to quantify components of soil humus, getting at the makeup of the organic component of soil. Humic substances are made up of plant and microbial remains which undergo “humification” as they decay and decompose.

Using a strong base (often sodium hydroxide), researchers mix soil in solution to separate out SOM fractions based on their acid–base solubility. The highly basic solution is then neutralized to prevent excess oxidation of the dissolved substances.

The inquisitive researcher gathers three fractions: humic acid, fulvic acid, and humin.

These three substances are not classified based on their chemical makeup or biological function, per se, but solely on their solubility under high- and low-pH conditions. At high pH, both humic and fulvic acids remain in solution, turning it a dark brown. When the extraction is acidified, the humic acid precipitates from solution and is thought to be made up of compounds with a higher molecular weight than fulvic acid. Fulvic acid remains in solution, leaving it a clear yellow color. Humin, then, is whatever solid organic matter is left behind, not extracted by alkali. It is thought to be the most stable component of SOM, and thus the least accessible to breakdown by chemical means.



The vials show the fulvic acid fraction, which remains in solution after humic acid is precipitated out. Fulvic acid gives the solution its characteristic yellow color, ranging from light to deep red-yellow. Photo courtesy of Lucretia Sherrod.

It follows that some researchers using the method equate fulvic and humic acid with fast- and intermediate-cycling carbon pools, respectively. Fast-cycling carbon pools are thought to be most quickly affected by management practices and environmental perturbations and offer the greatest amount of usable organic matter to plant life, while medium-/slow-cycling pools are less sensitive and take longer to register changes in the environment or management.

Recalcitrant or passive pools are most resistant to change. These pools are considered “mineral bound,” or tightly tied to mineral content in the soil and thus largely unavailable for metabolism.

As Lucretia Sherrod, an SSSA member and soil scientist with the USDA–ARS puts it, “The idea is to try to rake out the whole organic matter story into pieces that are fast–cycling, slow–cycling, intermediate–cycling...to try and look at different systems, different conditions. We want to have another tool in our toolbox to say that we're really degrading this specific pool with this system, and how do we change that?”

With the fundamentals of the method in mind, the debate over humic substances research is best summarized by a query in an article (<https://doi.org/10.2134/jeq2019.01.0001c>) on the topic published earlier in the year by Henry Janzen, Agriculture and Agri–Food Canada: “Can using alkaline extraction accurately represent the character of the intact humus?”

Reviewing the State of Humic Substances Research

Alkaline extraction has been used to test SOM for more than 200 years, long before modern analytical techniques and experimental methods were developed. Though humin, fulvic acid, and humic acid have been measured in the lab for years as a proxy for understanding the state of soil organic matter in situ, researchers do not have a strong grasp on the isolates’ molecular structure or composition.

In conjunction with Tsutomu Ohno, Nancy J. Hess and Nikolla P. Qafoku, both of the Pacific Northwest National Laboratory, served as guest editors. The team invited two groups of researchers to write advance review articles for the section with one group in favor of humic substances and the other opposed. As Ohno, Hess, and Qafoku mentioned in their introduction to the section (<https://doi.org/10.2134/jeq2019.08.0292>), they asked the authors: “Does the ‘humic substances’ perspective, as defined using alkaline extraction of soils and sediments, still promise meaningful advances in our understanding of organic matter in terrestrial and aquatic ecosystems?”

Markus Kleber and Johannes Lehmann (2019) wrote a review critiquing humic substances research (<https://doi.org/10.2134/jeq2019.01.0036>). They argued that (i) alkaline extraction is unable to differentiate between humic and non-humic substances, (ii) the biological function of SOM does not relate to its alkaline extractability, and (iii) researchers cannot definitively demonstrate that alkali-extracted materials have undergone humification.

That is, the primary argument against alkaline extraction is that researchers cannot be sure they are measuring SOM as it is found in actual soil. The extraction process may be modifying organic matter, creating laboratory artifacts not found in nature.

Kleber and Lehmann argue that the method also creates a highly basic environment, which “opens up unpredictable avenues of potential reactions...that would never be able to occur under the pH conditions prevailing in natural soil systems.”

They also argued that oxygen-containing functional groups are best isolated via alkaline extraction. During the process of decomposition, these oxygen-containing functional groups increasingly concentrate in the soil as organic molecules are broken down, thus creating more readily oxidizable substances. These substances *can* be differentiated from soil that has undergone humification, lending credence to the idea that alkaline extraction “does not have the discriminatory power necessary to allow for a separation of humic substances...from non-humic substances.”

Proponents of the method argue that it is not necessary to understand exactly *what* humic substances are on a chemical level if their measurement can help solve problems in the field. In the invited paper in favor of humic substance research, Dan Olk and his co-authors (2019) presented a review of studies in which alkaline extraction was used to examine agronomic and environmental problems in both soil

and natural waters (<https://doi.org/10.2134/jeq2019.03.0100>).

Olk, the lead author on the paper and a soil biochemist at the USDA-ARS, has worked with alkaline extraction in a variety of environments, from cotton fields in California to rice paddies in the Philippines.

“For me, it works very well for studying nutrient cycling,” he says. “It seems to work very well for studying the absorption of things that are bound up by the more aromatic parts of soil organic matter, like heavy metals, pesticides, and persistent organic pollutants.”

The group's review paper also discusses the major issues raised by critics of the technique. In particular, they rebutted the idea that using alkaline extraction requires a belief in a single macromolecular structure of humic substances. Rather, Olk expressed the idea that understanding exactly *what* humic substances are made of is less important than using them to effectively solve problems in the field.

“We're trying to study a black box that we cannot recover from the soil, and we don't have the chemical tools available to get the whole structure,” Olk says. “Some critics claim that proponents of alkaline extraction exclusively believe that humic acid is a defined molecule, it has a certain structure...and that fulvic acid is another molecule, with another structure. I personally don't agree: I don't know if there is a humic acid molecule, but there's a humic acid fraction you can extract from the soil and study.”

Olk and his co-authors also argue that humic substances are not laboratory artifacts, but a viable means of understanding whole soil organic matter, particularly when the problem is suited to fulvic or humic acid characterization. As Olk puts it, “Think function: pick the method that best fits your objectives.”

For example, Olk mentioned his work in the Philippines on rice paddy management and his research team's use of heavy, isotopically labeled nitrogen fertilizer to examine nitrogen cycling under different management strategies (Olk et al., 2006). The team found a demonstrable difference in nitrogen content under aerobic and anaerobic tilling strategies, demonstrating questions of management and nitrogen cycling as solid applications for the humic substance research paradigm.

In short, Olk and his co-authors presented an alternative to the criticisms set out by Kleber and Lehmann (2019): that alkaline extraction, when used as a tool in the proper context, can be a valuable means of quantifying changes in SOM, even if the chemical makeup of humic substances is not well understood.

Comparing Humic Substances and Other Techniques for Quantifying SOM

Another article published in the special section sought to compare different methods of quantifying SOM. Lucretia Sherrod headed a research team that tested traditional alkaline extraction against several common, less aggressive methods of studying organic carbon (<https://doi.org/10.2134/jeq2019.03.0104>).

The team measured organic carbon with a slew of methodologies, including the following: whole soil C, water-soluble C (WSOC), permanganate-oxidizable C (POX-C), soil microbial biomass C (SMBC), particulate organic matter C (POM-C), and passive mineral-associated organic C (PMAOC).

Though the team was hoping to find positive correlations between fulvic acid and quantifiers measuring fast-cycling organic carbon (i.e., WSOC, SMBC, or POM-C), and humic acid with slower-cycling quantifiers (i.e., POM-C and PMAOC), this was not the case. Instead, both fulvic acid and humic acid were found to be significantly correlated with multiple forms of measurement.

“There's all this overlap,” Sherrod says. “What we were hoping to get was two different things—it would be only fulvic acid strongly correlated to POX or POM or water-soluble organic C, but instead this pool, along with humic and humin, are significant for everything, pretty much, but not any one big winner. The exception was that the POM-C pool was not significant at the 5% level for any of the humic pools, which was unexpected.”

Overlap between these methods of measurement is just one more indication that the black box of soil organic matter research has yet to be understood. With the current methodologies, clarifying how fast-, intermediate-, and slow-cycling carbon pools are affected by management may mean that researchers are not necessarily discussing the same carbon pools, depending on the tool they use. It's this reality that drives further research into the best techniques for measuring SOM, pushing today and tomorrow's scientists to find out how, exactly, we can manage our soils in the best possible way for crop growth and environmental health.

There is room at the table for more than one method of understanding soil organic matter—perhaps, for now, using the method that best fits the research question at hand is a good start. As Olk succinctly puts it, “We should be looking for the relative strengths of all these approaches and build them together into an integrated approach, taking the best of these different methods.”

With this integrated approach, using multiple tools from our toolkit to examine soil organic matter, we can crack open the black box found in the rubble and truly understand the secrets it holds.

DIG DEEPER

Check out the special section, "Using Alkaline Extraction To Understand Environmental Processes," in the November–December 2019 issue of the *Journal of Environmental Quality* at

<https://dl.sciencesocieties.org/publications/jeq/tocs/48/6>

Reference

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