Humic Substances Extracted by Alkali Are Invalid Proxies for the Dynamics and Functions of Organic Matter in Terrestrial and Aquatic Ecosystems

Markus Kleber* and Johannes Lehmann

Abstract

The conceptual rigor of using organic materials extracted from soil by alkali, called *humic substances*, as proxies for soil organic matter has been questioned for almost 180 years. However, the humic substances paradigm, i.e., the understanding that alkali-extracted humic substances are newly synthesized materials with unique properties that are distinct from non-humic organic matter, continues to be propagated throughout the literature. Here, we revisit the mechanistic background of the extraction process to show that alkaline extraction is unable to discriminate for the chemical history of a compound. For this reason, alkaline extraction cannot distinguish between (i) materials that have undergone secondary synthesis or humification ("humic" substances), and (ii) materials that are decorated with ionizable functional groups for other reasons, such as oxidative depolymerization ("non-humic" substances). While this mechanistic consideration alone renders invalid a distinction between humic substances and non-humic substances based on alkaline extraction, we further show that the evidence available to date does not support the assumption that processes of secondary synthesis create quantitatively significant proportions of "chemically reactive, yet recalcitrant" materials in natural environments. Any definition of humic substances that invokes both alkaline extraction and secondary synthesis is thus flawed on at least two accounts: (i) alkaline extraction is unable to achieve its purpose of separating humic from non-humic substances, and (ii) the assertion that the extracted materials have unique molecular properties as a consequence of secondary synthesis cannot be proven because alkaline extraction cannot separate materials created by secondary synthesis from other, ionizable organic compounds. Finally, we point out that since the definition of humic substances is tied to the alkaline-extraction procedure, neither the existence of operationally defined humic substances in the environment nor their chemical integrity during the course of alkaline extraction can be independently verified. We conclude that organic materials extracted by alkali require appropriate nomenclature and rigorous definition to merit consideration in teaching, research, and application.

Core Ideas

- Alkaline extraction cannot distinguish between humic substances and non-humic substances.
- Alkaline extraction cannot discriminate for products of secondary synthesis.
- The humic paradigm needs to be set aside.

*Corresponding author (Markus.Kleber@oregonstate.edu).

SOIL ORGANIC MATTER is an important ecosystem property that influences plant growth, water quality, and atmospheric greenhouse gas concentrations, among many other soil functions (Weil and Brady, 2016). The science and management of soil rely on an understanding of the composition of soil organic matter to evaluate and predict moisture retention, nutrient cycling, and the dynamics of gas exchange. Throughout the history of soil organic matter science, the concepts of humus, humification, and humic substances have played an important role in shaping this understanding (Stevenson, 1994). Here, we review the historic development of this concept, outline its fundamental challenges with respect to conceptual rigor and analytical methodology, and conclude that both the nomenclature and the underlying concepts are best set aside.

The Humic Substances Paradigm— Definitions

The term *soil humus* was defined by Stevenson (1994) as the total of the organic compounds in soil exclusive of (i) undecayed plant and animal tissues, (ii) their "partial" decomposition products, and (iii) the soil biomass. Closely following Wershaw (2000), we call the resulting understanding that soil humus is composed of the end products of synthetic reactions that alter the structure of plant degradation products in a way that provides these newly synthesized materials with unique properties that are distinct from non-humified organic matter the *humic substances paradigm*. The merits of the humic substances paradigm have been debated throughout the history of soil organic matter research (Waksman, 1936). The fundamental contention in this debate is the question of whether there is indeed a naturally occurring process of secondary synthesis in the biosphere that operates independent of and in addition to standard processes of decay and, in doing so, reassembles plant degradation products into new, molecularly and functionally distinct compounds at a quantitatively relevant scale. This potential process is widely called *humification*, and the resulting materials are called *humic substances*. Following Kononova's (1958, 1966) postulate that humic substances are macromolecules with resulting properties and features, the humic substances paradigm saw an increase in popularity during the second half of the 20th century. The significance of secondary synthesis processes is seen in the fact

M. Kleber, Dep. of Crop and Soil Science, Oregon State Univ., 2750 SW Campus Way, Corvallis, OR 97331; J. Lehmann, Soil and Crop Science, School of Integrative Plant Science, 909 Bradfield Hall, and Atkinson Center for a Sustainable Future, Rice Hall, Cornell Univ., Ithaca, NY 14853, and Institute for Advanced Studies, Technical Univ. Munich, Lichtenbergstrasse 2a. 85748 Garching, Germany.

that these processes will render the newly synthesized humic substances "highly chemically reactive yet recalcitrant with respect to biodegradation" (IHSS, 2019). Kononova's (1958, 1966) suggestion that humic substances are macromolecules generated via polymerization processes is reflected in the definition of humic substances originally published by Stevenson (1994) and currently phrased in Soil Science Society of America's *Glossary of Soil Science Terms* as "A series of relatively high-molecular weight, yellow to black colored substances formed by secondary synthesis reactions in soils" (SSSA, 2019).

Is the Humic Substances Paradigm Still Relevant?

By the beginning of the 21st century, researchers involved in soil organic carbon cycling began to abandon the terminology associated with the humic substances paradigm (Lützow et al., 2006) or to redefine *humic substances* without implication of specific functionality as the fraction of organic matter that remains molecularly uncharacterized (Baldock and Broos, 2011; Hatcher, 2004; Hedges et al., 2000). They did so on the basis of three main evolving insights:

1. Alkaline extraction is unable to separate humic substances from non-humic substances (i.e., from functional biomolecules, their partial decomposition products, and from microbial residues).
2. There was an apparent lack of relationship between biological functioning of soil organic matter and its alkaline extractability.
3. Alkali-extracted materials did not possess the properties they were supposed to have acquired during the proposed process of secondary synthesis, or "humification."

The Mechanism of Alkaline Extraction

The study of soil organic matter is complicated by the fact that depending on soil type, depth in the soil profile, and other factors, a relatively small amount of organic matter is diluted throughout a much larger pool of colloidal-sized mineral matter, with which it can form associations of variable structural complexity and bonding strength. An investigation into the chemical properties of soil organic matter thus requires either (i) a preparatory step to separate the mineral and the organic phases for subsequent independent observation or (ii) a technique that allows for the direct investigation of organic matter chemistry in situ, while still associated with the mineral phase.

The latter option was not available to the scientific community until the advent of solid state nuclear magnetic resonance spectrometry (Wilson et al., 1981) and the subsequent development of synchrotron-based soft X-ray spectromicroscopy (Meyer-Ilse et al., 1995; Schmahl, 1993), and it must be conceded that even these sophisticated methods of direct, in situ observation have limitations. During the centuries of organic matter research preceding these developments, the scientific community was restricted to physical (sieving, handpicking, flotation) or chemical attempts to separate the organic from the mineral phase. The alkaline extraction procedure, first performed by Achard (1786), became the most widely used separation method and is still used

today, likely because it extracts more organic matter than most alternatives (Hayes, 2006; Schnitzer and Monreal, 2011).

The question then arises, Why were the extracted materials called *acids*? When Achard (1786) subjected several varieties of peat to a large catalog of extraction procedures, he made the following key observations:

- Peat did not dissolve in water: $H_3C\text{-}R\text{-}COOH + H_2O = H_3C\text{-}R\text{-}COOH + H_2O$
- Peat did not dissolve in water when H^+ (acid) was added to the system: $H_3C\text{-}R\text{-}COOH + H^+ + H_2O + = H_3C\text{-}R\text{-}COOH + H^+ + H_2O$
- Peat became partially soluble in water only when base (OH^-) was added to the system: $H_3C\text{-}R\text{-}COOH + OH^- + H_2O + = H_3C\text{-}R\text{-}COO^- + 2\,H_2O$

Hence, Achard (1786) discovered that a certain, variable fraction of organic matter is decorated with functional groups that can be ionized by the addition of OH^- ions (a base). To react with a base, these functional groups must have the ability to dissociate a proton (H^+), and this ability is known as *acidity*. The conversion of a carboxyl group into its dissociated carboxylate form has momentous consequences for the solubility of the respective compound, as it dramatically (by one order of magnitude, Table 1) increases the heat of solution that can be released by hydrating the carboxylate ($R\text{-}COO^-$) form, compared with the intact carboxyl ($R\text{-}COOH$). In other words, alkaline extraction solubilizes all such organic compounds that are decorated with ionizable functional groups, but it is most efficient when a given organic compound is decorated with many carboxylic groups.
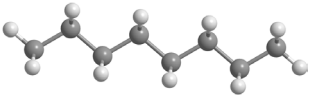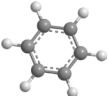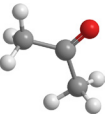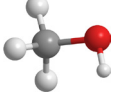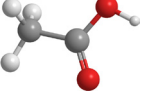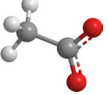
Next to common physical factors, such as temperature and pressure, the efficiency of solubilization through alkalinization depends on:

1. the kind of functional group (alcoholic, phenolic, carbonyl, carboxyl, etc.) present;
2. the propensity of a given functional group to dissociate its proton, quantified by the associated acidity constant (pK_a);
3. the size and makeup of the organic residue to which the functional group is attached;
4. the number and distribution of functional groups per molecule (e.g., is the molecule an amphiphile with a polar head and a nonpolar tail?); and
5. the concentration of OH^- offered, i.e., the pH of the extractant.

It follows that for an alkaline extraction to be as exhaustive as possible, the pH of the extractant needs to be chosen such that it is higher than the pK_a values of the functional groups targeted by the extraction. Table 2 gives an overview of the ionization state of functional groups associated with common biomolecules, illustrating that a treatment at pH 13 (equivalent to 0.1 M NaOH) will ionize many groups that would not be ionized at normal soil pH (such as a phenolic OH). This opens up unpredictable avenues of potential reactions between the newly ionized groups and the myriad of mineral and organic constituents of soil, reactions that would never be able to occur under the pH conditions prevailing in natural soil systems.

These considerations illustrate that alkaline extraction will be most successful when the organic matter to be extracted is highly decorated with abundant phenolic and carboxylic groups. In

Table 1. Heat of solution of simple organic compounds in water. Positive number indicates energy input is required for solvation, negative values indicate energy is released on solvation. Data from Anslyn and Dougherty (2006).

Compound			Functionality	Hydration energy
Octane		$CH_3(CH_2)_6CH_3$	nonpolar	12
Benzene		C_6H_6	quadrupole	−4
Acetone		$(CH_3)_2CO$	polar/ carbonyl	−16
Methanol		CH_3OH	polar/ alcoholic	−21
Acetic acid		CH_3COOH	polar/ carboxyl	−28
Acetate		CH_3COO^-	ionic/ carboxylate	−335

Table 2. Variations in the ionization state of functional groups as a function of proton concentration. The pH range from 4 to 7 is commonly encountered in soil; pH 13 and higher is used by alkaline extraction procedures.

Compound		Group	pK_a†	Fraction (%) in ionized form at a pH of			
				4	5.5	7	13
Lipid (ethane)		CH	50	0	0	0	0
Alcohol (ethanol)		OH	16	0	0	0	0.1
Carbohydrate (glucose)		OH	12.3	0	0	0	84.0
Fatty acid (octanoic acid)		COOH	4.89	11.4	80.3	99.2	100
Carboxylic acid (oxalic acid)		COOH (I)	1.25	99.8	100	100	100
		COOH (II)	4.14	42.0	95.8	99.9	100
Phenol		OH	9.95	0	0	0.1	99.9
Para-hydroxybenzoic acid		COOH	4.48	24.9	91.3	99.7	100
		OH	9.32	0	0	0.5	100

† From Haynes (2012).

soil organic matter, acquisition of oxygen-containing functional groups is tied to the process of oxidative decomposition (Fig. 1).

Thus, the more decomposed the organic matter, the greater the abundance of oxygen-containing functional groups and the better the resulting alkaline extractability. Since the compounds listed in Table 2 all qualify as known, chemically identifiable compounds, they would fall under the category of non-humic substances. However, with the exception of ethane and ethanol, all of these non-humic substances would be ionized by the alkaline extraction procedure. This emphasizes the fact that alkaline extraction does not have the discriminatory power necessary to allow for a separation of humic substances (materials that have been transformed by humification) from non-humic substances (materials that have not undergone secondary synthesis, or humification).

The Alkaline Extraction and the Humic Substances Paradigm Are Intertwined

Four decades after the procedure of alkaline extraction was first performed and published (Achard, 1786), the scientific community began to converge on a terminology to categorize the extracted organic materials: the term *humic acids* was first used by Döbereiner (1822) and Sprengel (1826). The importance of this timeline cannot be overstated: the extraction procedure preceded efforts to develop a matching nomenclature, and the development of the nomenclature for its resulting products was not informed by an a priori theoretical or conceptual assumption about the nature and functionality of the extracted materials.

The concept that alkali-extractable compounds might be the result of secondary synthesis also evolved long after the development of the extraction procedure and has its roots in laboratory experiments aiming to re-create the hypothetical synthesis process. Attempts to synthesize humic substances began in the early 19th century, when Braconnot (1819) submitted starch and sucrose to the action of strong acids and obtained a black precipitate that was soluble in alkali—and suggested this process might emulate the formation of the humic substances that Achard (1786) had also obtained through extraction of soil with alkali. Further attempts at re-creating humic substances in the laboratory can be divided into three strategies: (i) treatment of organic precursor materials with acid or alkali (Braconnot, 1819; Maillard, 1916, 1917), (ii) enzymatic synthesis reactions (Haider and Martin, 1975; Naidja et al., 1998), and (iii) synthesis reactions catalyzed
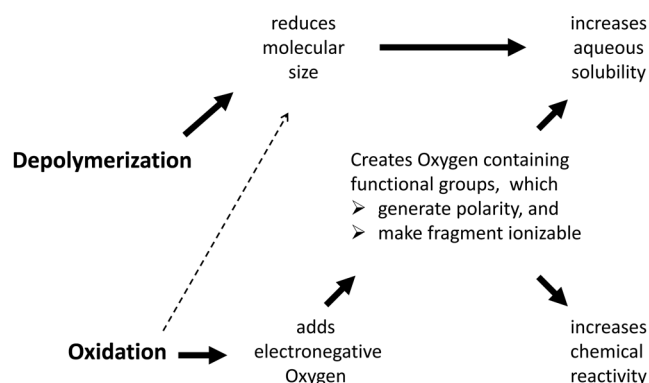
by mineral surfaces (Huang and Hardie, 2009). In these experiments, the suitability of eventual reaction products as models for humic substances was always asserted by confirming their ability to dissolve in alkali. Hence, solubility in alkali became established as the defining characteristic of humic substances. To date, solubility in alkali is still the overriding criterion for the definition of humic substances, as reflected in the definitions posted by the International Humic Substances Society (IHSS, 2019) and the Soil Science Society of America (SSSA, 2019).

Why Should the Humic Substances Paradigm Be Set Aside?

Absence of a Rigorous Definition: "You Cannot Prove a Vague Theory Wrong"

Two decades ago, Wershaw (2000) stated that "there are no universally accepted definitions of the terms humic substances and humus." It is easy to show that this assessment is as true today as it was two decades ago. The fact that the definitions of humus and humic substances have never been rigorously constrained has been openly acknowledged in the past (Huang and Hardie, 2009; MacCarthy et al., 1990) and is illustrated in Fig. 2 and Table 3. But to enable constructive debate, it needs to be clear
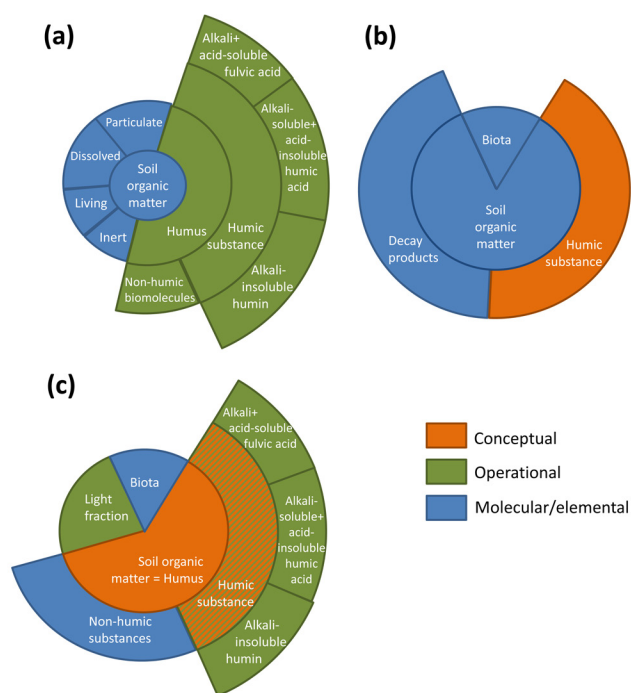

Fig. 2. Traditional views of soil organic matter composed of "humic substances" or "humus." Multilevel pie charts (in which the inner circles depict higher hierarchies) of common classifications based on the concept that humic substances are either (a) defined by their extractability in an alkaline solution but may not necessarily be molecularly characterized (operational definition) (Baldock and Broos, 2011); (b) actually synthesized in soil and have distinct properties (process definition) (Horwath, 2015); or (c) a combination of (a) and (b), both synthesized in soil and can be isolated by alkali (Kononova, 1966; Huang and Hardie, 2009; Schnitzer and Monreal, 2011; Tan, 2014,) which is the most common definition currently found in textbooks; definitions invariably include some fractions that are identifiable by their molecular structure, size, or solubility (Molecular definition), such as microorganisms, dissolved or particulate organic matter (amounts in fractions vary between soils and are illustrative).

Fig. 1. Decomposition creates functionalized organic fragments through depolymerization and oxidation.

Table 3. Attributes used to define the molecular characteristics of humic substances and their use by different authors. An X indicates the attribute was mentioned as a defining element by the source heading the column.

Attribute	Kononova (1966)	Schnitzer (1978)	Aiken et al. (1985)	Stevenson (1994)	Essington (2015)	SSSA (2019)	Publications that refute or challenge the attribute
Naturally occurring			X	X	X†	X	Kelleher and Simpson, 2006; Lehmann et al., 2008, Masoom et al., 2016
High molecular weight	X	X‡	X	X		X	Myneni et al., 1999; Piccolo, 2001; Sutton and Sposito, 2005; Wershaw, 1986, 1993
Predominantly aromatic	X	X					Baldock et al., 1992
Refractory			X		X		Gramss et al., 1999a, 1999b, 1999c; Kononova, 1966; Mathur and Paul, 1967; Tatzber et al., 2009
Formed by secondary synthesis	X			X		X	Burdon, 2001; Lehmann and Kleber, 2015
Based on solubility characteristics	X	X		X		X	This attribute forms the basis of the extraction procedure: solubilization with an ionizing liquid

† Implied.

‡ "a few 100 to several thousand Da."

what is being debated. That this debate is challenging can be illustrated by the following quote: "Unfortunately the terms humic and fulvic acids, originally chosen to describe fractions isolated from soils, have been purloined by those working with freshwater and marine organic substances, which may be very different both in origin and in chemical composition" (Parsons, 1988). In water science, other isolates from water such as those obtained by XAD resins (Filella, 2009; Thurman and Malcolm, 1981), are also called "humic substances." In comparison, total natural organic matter is typically obtained from water by reverse osmosis (Serkiz and Perdue, 1990) and is mostly seen as separate from humic substances (Maurice et al., 2002).

Broken Chain of Arguments

A point of agreement among the majority of definitions of humic substances is the requirement that they be soluble in alkali, an argument that Wershaw (2000) addressed in a manner that deserves to be quoted verbatim:

> The second shortcoming of humic substance research arises when one tries to isolate a material that fits whatever definition one chooses for a humic substance. Any attempt to exclude a group of natural organic matter components from the definition of humic substances or humus requires that methods exist that allow one to remove the nonhumic components from the crude humic isolates normally obtained. If one cannot reliably separate humic substances from nonhumic substances, then whatever analytical technique one uses will not uniquely characterize the humic substance of interest. If one accepts Stevenson's definitions of humic substances and humus then the first step in any study of humic substances is to separate those substances formed by secondary synthesis from all other NOM [natural organic matter] components. If one cannot accomplish this task then, of necessity, one cannot claim to have analyzed or characterized a humic substance. (p. 2)
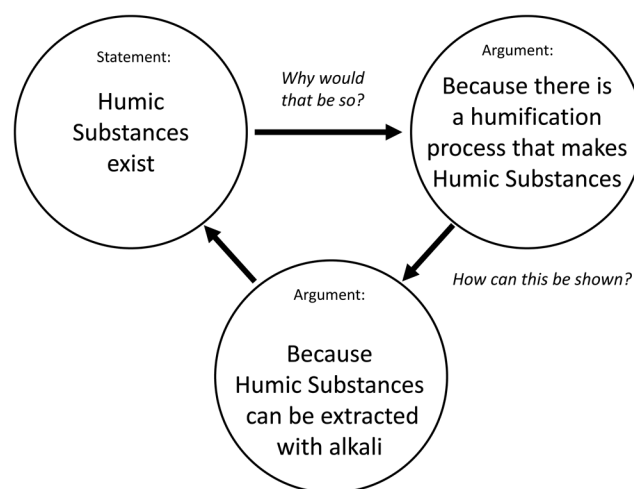
Figure 3 illustrates the importance of Wershaw's (2000) conclusion. The logic underlying a definition based on solubility in alkali, besides being circular, breaks down at two points: (i) the alkaline extraction procedure is unable to discriminate between humic and non-humic substances, that is, it is unable to extract

humic substances; and (ii) there is no publication to date that has convincingly demonstrated the quantitative relevance of secondary synthesis reactions outside of the laboratory and in the natural soil environment.

The Biological Functioning of Soil Organic Matter Is Not Reflected in Its Alkaline Extractability

When it became apparent in the 1970s and 1980s that atmospheric CO_2 levels were on the rise, the focus of soil organic matter research shifted from agricultural concerns to more geochemically motivated questions of soil carbon cycling. Researchers were in need of parameters for the study of decomposition processes and evaluated numerous proxies and indicators, including chemical extractions, particle-size separates, and density fractions. One of the first comprehensive studies to compare the usefulness of physical fractions with that of chemical extracts was offered by Oades and Ladd (1977). They saw the ability of alkaline extracts to support inference about organic carbon cycling as very limited, for a number of reasons:

1. "The use of E4:E6 ratios and k values on (chemical) extracts

Fig. 3. The reasoning underlying the classic humic substances paradigm. Wershaw (2000) argues that this reasoning, besides being circular, is invalid because alkaline extraction cannot distinguish between humic and non-humic substances.

from the different particle sizes has failed to produce data from which generalizations can be drawn. . . . Interpretation of spectrophotometric work in terms of condensation should be viewed with caution. Condensation has also been confused with 'humification' " (p. 137).

2. "It is abundantly clear that the classical chemical fractionation scheme fractionates microbial tissue and that the presence of soil alters this fractionation" (p. 143).

3. "Fungal material is less soluble than bacterial components, and is concentrated in humin fractions . . . [A] number of soils, including acidic soils and soils of arid regions, have high humin components due in part to resistant, insoluble fungal materials" (p. 143).

4. "Neither plant nor microbial tissues can be considered as being humified (sensu secondary synthesis) just because they appear in humic and humin fractions" (p 145).

5. "A further disadvantage in using extractants such as alkali is that the extracted material would be derived in part from several sources, e.g., part decomposed plant fragments, the microbial biomass and clay organic complexes. Thus there is no way of deciding whether, for example, the hydrogen-bonded protein isolated from a humic acid fraction may not have been derived from material which was not humified in the soil" (p. 151).

The arguments offered by Oades and Ladd (1977) remain valid today and, together with the emerging recognition of the importance of physical protection for the cycling of soil organic matter, have prompted the scientific community to increasingly explore physical soil fractions for process research (Six et al., 2004).

Alkali-Extracted Materials Do Not Possess the Properties They Were Supposed to Have Acquired during the Proposed Process of Secondary Synthesis ("Humification")

Following the humic substances paradigm, the process of humification would alter the structure of plant degradation products in a way that confers to the newly synthesized materials unique properties that are distinct from non-humified organic matter; such a newly created chemical uniqueness is the main justification for the scientific interest in humic substances. Since the postulated features are quite specific, it must be possible to identify chemical compounds that possess such properties as would result from humification in the environment, particularly so if humification is a quantitatively relevant phenomenon in natural ecosystems. Also, if these materials do exist, such identification must be possible independent of the method of analysis; that is, it must be possible to find products of secondary synthesis that are macromolecular, highly aromatic, refractory, and so on, without having to use an operational extraction to isolate them. However, the postulated properties could not be rigorously demonstrated in alkaline extracts: recalcitrance, also known as "inherent resistance to decomposition," was already refuted by Kononova (1966, p. 153), who found that "humus substances can be utilized to a greater or lesser extent by microorganisms." The "predominantly" aromatic character of humic substances was challenged by Baldock et al. (1992). Macromolecularity

of alkali-extracted materials was refuted by Piccolo (2001) and Sutton and Sposito (2005); the existence of a thermodynamic rationale for secondary synthesis was rejected by Burdon (2001); and the postulate of "natural occurrence" is invalid since it is impossible to demonstrate the natural existence of humic substances as long as their definition is solely tied to the alkaline extraction procedure. Table 3 summarizes some of the pertinent literature and also demonstrates the inability to converge on a generally accepted definition for these materials.

Special Case: Secondary Synthesis as an Explanation of *Recalcitrance*

The concept of recalcitrant humic substances is at the heart of several of the definitions that can be found in textbooks (i.e., Essington, 2015) and is also emphasized in the definition offered by IHSS (2019). A brief review of the history of this view is helpful to illustrate how assumptions of the past have been surpassed by present-day evidence with respect to humic substances as defined by alkaline extraction. The first critical consideration of the concept of organic matter stability in a popular textbook was offered by Waksman (1936). Waksman's book is about humus, which he initially defined as that part of plant and animal residues that is "more or less resistant to decomposition; is of dark brown to black color and possesses certain physical and chemical properties" (p. ix), before recommending that the term *humus* "should be used to designate the organic matter of the soil as a whole" (p. 62). Based on observations of carbon loss following fallowing and cultivation of native prairie, Waksman (1936, pp. 253–255) made the following inferences:

1. "It is the nature of the humus in the soil, rather than its total concentration, that is primarily concerned with soil productivity";

2. "Humus does not decompose in the soil as a whole, certain constituents of the humus decompose more readily than others";

3. "One may be tempted to consider the existence of unstable and stable forms of humus."

Table 4 emphasizes that to Waksman, the classification of humus as unstable is conditional: the postulated instability only sets in when the material is placed "under favorable conditions" for decomposition. In other words, once the peat bog is drained, the purported "inherent" stability of the organic matter preserved therein is lost. Current science in the 1930s therefore provided a great deal of nuance to the concept of stable humus.

However, the observation that certain constituents of the humus decompose more readily than others and the perception of a chemical difference between stable and unstable humus motivated researchers in the following decades to look for a mechanistic connection between chemistry and stability. Kononova (1958) synthesized earlier work to arrive at the suggestion that humic substances are high-molecular weight compounds and that humic substances of different soils are systems of polymers.

During 1960s and 1970s, several studies provided supporting arguments, including observations of high molecular weight of organic materials extracted with alkali (30–50 kDa; Flaig, 1958) and an influential paper by Swaby and Ladd (1962, p. 201)

arguing that during the production of synthetic humic acids in laboratory experiments, "polymerization and polycondensation were more rapid in the presence of amino acids than in their absence." Swaby and Ladd (1962; p. 201) proceeded to infer that "the resistance of humic acid to microbial and to chemical decomposition could be explained if it were a large spherical molecule, consisting of many heterogeneous units, irregularly crosslinked by different covalent bonds, so that innumerable extra-cellular enzymes from many different microorganisms would be needed to dismember it piece by piece from the outer surface. This would be a lengthy process unless a greater variety of microorganisms were encouraged by the addition of readily decomposable debris."

Further support for the "recalcitrance" idea came from radiocarbon analysis, a technique that allows estimation of the age of carbon atoms in organic compounds. Campbell et al. (1967) were able to show that nonhydrolyzable humic acid extracts from two soils had an age of 1230 and 1400 yr, whereas the respective hydrolysates were only 465 and 25 yr old, and that the "humus" of Podzolic soils was less stable (i.e., inferred from being younger) than that of Chernozems (equivalent to Mollisols). Campbell et al. (1967) also observed an inverse relationship between ^{14}C-derived mean residence times and the E465/E650 ratios (ratio of the light extinction of a humic substance suspended in 0.02 M $NaHCO_3$ at wavelengths of 465 and 650 nm, respectively) of alkaline extracts, indicating that older humic substances may possess more condensed aromatic phases than younger ones. Note that stability is here expressed on a time scale; that is, the longer carbon persists, the more stable it is considered to be. But persistence in the environment can have many other causes than intrinsic recalcitrance, such as mineral protection and microaggregation (Kleber et al., 2005; Lehmann et al., 2007).

The assumption that molecular complexity and degree of polymerization might be causal to slow decomposition received strong support in the following decade of the 1970s (Haider et al., 1975; Haider and Trojanowski, 1975; Martin and Haider, 1971; Martin et al., 1982). These authors introduced ^{14}C into the molecular structure of phenolic acids and used the labeled monomers to prepare phenolic polymers subsequently in the laboratory. This allowed them to identify the molecular origin of the carbon in the $^{14}CO_2$ that evolved during decomposition of these molecules. In the laboratory, Haider and Martin (1975) showed how linkage into polymeric structures was able to retard the decomposition of organic carbon during a 12-wk incubation period. However, Haider and Martin (1975) did not demonstrate that linkage of phenolic polymers into polymeric macromolecules occurs in a quantitatively relevant fashion in natural soil environments.

Therefore, the chain of arguments underlying the paradigm of "secondary synthesis leads to chemical stabilization", as postulated by Swaby and Ladd (1962), can be summarized as:

1. polymerization leads to "inherent" resistance to decomposition →
2. "inherent" resistance to decomposition leads to long residence time →
3. organic matter persists because it is polymeric and macromolecular = chemically stable.

This chain of arguments falls apart when there is no secondary synthesis of polymeric macromolecules. Multiple independent publications (Myneni et al., 1999; Piccolo, 2001; Sutton and Sposito, 2005; Wershaw, 1986, 1993) have provided overwhelming evidence that the materials extracted by alkali are not macromolecular.

The Humic Nomenclature as a Matter of Convenience

From the very beginning of systematic soil organic matter research, the scientific community has been divided about the usefulness of operationally defined *humic acids* as a research tool. Waksman (1936, p. 28 ff) described the struggle of the scientific community to find terminology (crenic, apocrenic, ulmic, humic acids, and many more) for the materials extracted by numerous researchers following Achard's exploratory efforts. But as early as 1840, we find evidence for the realization that the type and quality of the materials obtained was always a function of the extraction procedure, represented in Liebig's (1840) statement that "there is not the slightest reason to believe that one or another of these [alkali soluble] products should have the shape or the properties we assign to the humus existing in nature" (see Supplemental Table S1 for the complete original quote). Others who openly criticized the practice of considering humus as being composed of alkali-extractable humic substances included Van Bemmelen (quoted in Waksman 1936), who stated that "the

Table 4. Properties of stable and unstable humus as identified by Waksman (1936).

	Definition	Properties	Found where
Stable humus	"That type of humus which has undergone extensive decomposition, similar to what has taken place in well decomposed composts or cultivated mineral soils." (p. 254)	Almost complete disappearance of cellulose and hemicelluloses; considerable increase in the percentage of lignin-like complexes and protein; a narrowing of the C/N ratio to about 10:1 or less.	Mineral soils below the surface horizon; low-moor peats; well-drained peat soils; the A2 and B horizons of forest soils
Unstable humus	"Those forms of humus which still undergo rapid† decomposition when placed under favorable conditions, as by correcting the reaction by proper aeration (drainage), by a more favorable temperature, or by the addition of mineral nutrients or available nitrogen essential for the activities of microorganisms." (p. 254)	The C/N ratio in this form of humus is much wider, varying from 20:1 to 30:1 in composts and in the forest floor, to as high as 80:1 in high-moor peats.	Decomposing composts; undrained peat bogs; forest floor

† Note how Waksman here gets into conflict with his general definition of humus as being "more or less resistant to decomposition" by acknowledging that this supposed "resistance" can be overcome through adjustment of external controls.

determination of that part of the soil humus, which is soluble in dilute alkalis [...] can teach us very little. Such simple procedures are entirely unsatisfactory to judge the value of the humus."

The matter seemed to have been settled when Waksman (1936, p. 62), after careful examination of over 1300 research papers (a tremendous achievement at a time without electronic access to publications), came to the conclusion that "one may feel justified in abandoning the whole nomenclature of humic substances. These labels designate, not definite chemical compounds but merely certain preparations." It is enlightening to review how the author of the most widely used definition of humic substances (Stevenson 1994) reacted to this verdict. He argued that "abandonment of these terms would cause even greater confusion than their continued use" (Stevenson 1994, p. 31). In other words, Stevenson recommended maintaining the nomenclature not because of some specific scientific merit but for sake of convenience. A similar argument has been made in favor of the isolation method, the alkaline extraction procedure (Schnitzer and Monreal 2011): "dilute aqueous alkalis, especially 0.1–0.5 NaOH solutions, have been, by a wide margin, the most efficient extractants until today. Over 95% of all SOM [soil organic matter] researchers have used and are still using dilute NaOH solutions for this purpose, and their work has produced several thousand scientific peer-reviewed papers. Are we now going to throw rigorous scientific published articles into the waste paper basket and disregard this huge scientific literature?"

Recommendations

Several different steps are possible in a way forward that we recommend for consideration:

1. Separate the materials obtained via alkaline extraction from the products of a tentative humification process. We propose calling the extraction procedure *alkaline extraction*, without an implicit or explicit inference to an ecological pool. We advise against separating the terms *humus*, *humic substances* (and its subcategories), and *humification* from each other and redefining them individually. This has been attempted earlier (Waksman, 1936), without success.

2. Define and clarify what alkaline extraction is. We propose defining alkaline extractions as an operational procedure to extract a large proportion of organic matter from soil. The user may need to recognize that the extraction is never fully efficient, with important caveats regarding the production of artifacts. Alkaline extracts have demonstrable practical uses (Rose et al., 2014), opening an interesting and potentially important avenue in understanding certain, well-constrained aspects of soil biogeochemistry and plant–soil interactions, when these are investigated on a mechanistic level.

3. Adopt a terminology that separates a process occurring in soil from its properties, which allows more flexibility to adapt to new evidence in the future. Definitions of *humus* come with many qualifications and in so many variations (see Supplemental Table S1) that there is no expectation of agreement in the foreseeable future. Besides, there is no clear chemical rationale why such a subsection of organic matter should be distinguished. It is much more straightforward to utilize the term *soil organic matter*, including live, dead and decaying materials. Soil organic matter undergoes continuous breakdown, creating a continuum of more or less decomposed materials, as described by the Soil Continuum Model (Lehmann and Kleber, 2015). Neither the terms *stabilization* or *humification* nor related terms and concepts allow unbiased evaluation of the process of organic matter transformation in soil. A forward-looking terminology needs to be agnostic to the type of organic matter soil contains or the process by which it is transformed or retained in one form or another.

Concluding Remarks

The humic substances paradigm, as it still stands, is based on an argument that is invalid (alkali is unable to separate humic substances from non-humic substances) and on a supposition that is unproven (there is no proof for a quantitatively significant production of synthesis products that have no physiological purpose but are biogeochemically "active," nor is there a compelling thermodynamic rationale why such a process would happen). This suggests that humic substances may be best set aside as organic matter categories.

The debate surrounding secondary synthesis must be treated as an independent issue with the character of a contest between a (i) synthesis pathway: "many scientists ....considered...the substances formed from reactions between simple organic chemicals set free in the soil environment" (Hayes and Swift, 1990, p. 251); and a (ii) degradative pathway: "Organic matter is a thermodynamic anomaly atop a free energy precipice that drops off on all sides to dispersed, stable ingredients such as carbon dioxide, water, nitrate and phosphate" (Hedges et al., 2000). The existence of a "degradative pathway" is established beyond doubt: soil respiration would not occur if this process did not exist. The degradative pathway would also be a necessary condition for the postulated "synthesis pathway"; without its action, there would be nothing to synthesize. This means we can be sure of one thing: organic matter is progressively decomposed to ever smaller molecular units, thereby forming a compositional continuum spanning from large biomacromolecules to tiny carboxylic acids and, eventually, carbon dioxide or bicarbonate.

Modern-day analytical tools should make it possible to design experiments that can unequivocally accept or refute the question whether a hypothetical pathway of secondary synthesis, or "humification," has quantitative relevance in natural environments. Isotopic labels combined with spectrometric techniques may allow tracking of decomposition products as well as eventual synthesis products along their journey through the soil, revealing any propensity to become resynthesized. However, as Oades and Ladd (1977) pointed out more than 40 years ago, neither plant nor microbial tissues are "humified" just because they appear in operationally defined humic, fulvic, and humin fractions. Ultimate proof (or refutation) of secondary synthesis is hence possible only through the application of observational techniques other than alkaline extraction. These secondary synthesis products must be chemically and functionally different (Wershaw, 2000) from non-humic substances that constitute identifiable biomolecules such as polysaccharides, proteins, and lipids (Stevenson, 1994). If products of secondary synthesis do exist in nature in meaningful quantities, it must be possible (i) to

observe them independent of operational extraction procedures, (ii) to offer a thermodynamic rationale for their formation, and (iii) to define them without resorting to alkaline extraction. As long as these conditions are not met, the relevance of such materials for the dynamics and functions of organic matter in terrestrial and aquatic ecosystems cannot be postulated.

Supplemental Material

Supplemental Table S1 provides an overview over the variety of definitions used over the past 200 years. Definitions that deviate to some extent from the classic humic substances paradigm are highlighted in blue.

References

Achard, F.K. 1786. Chemische Untersuchung des Torfs. Crell's Chemical Annals 2:391–403.

Aiken, G.R., D.M. McKnight, R.L. Wershaw, and P. MacCarthy. 1985. An introduction to humic substances in soil, sediment, and water. In: G.R. Aiken, editor, Humic substances in soil, sediment, and water: Geochemistry, isolation and characterization. Wiley-Interscience, New York. p. 1–9.

Anslyn, E.V., and D.A. Dougherty. 2006. Modern physical organic chemistry. University Science Books, Sausalito, CA.

Baldock, J.A., and K. Broos. 2011. Soil organic matter. In: Handbook of soil sciences. CRC Press, Boca Raton, FL. p. 11-11–11-52.

Baldock, J.A., J.M. Oades, A.G. Waters, X. Peng, A.M. Vassallo, and M.A. Wilson. 1992. Aspects of the chemical structure of soil organic materials as revealed by solid-state 13C NMR spectroscopy. Biogeochemistry 16:1–42. doi:10.1007/BF02402261

Braconnot, H. 1819. Sur la conversion du corps ligneaux en gomme, en sucre et en un acide d'une nature particuliere, par le moyen de l'acide sulphurique; conversion de la meme substance ligneuse en ulmine par la potasse. Annales des Chimie et des Physique 12:172–195.

Burdon, J. 2001. Are the traditional concepts of the structures of humic substances realistic? Soil Sci. 166:752–769. doi:10.1097/00010694-200111000-00004

Campbell, C.A., E.A. Paul, D.A. Rennie, and K.J. McCallum. 1967. Applicability of the carbon-dating method of analysis to soil humus studies. Soil Sci. 104:217–224. doi:10.1097/00010694-196709000-00010

Döbereiner, J.W. 1822. Zur pneumatischen Chemie. III. Zur pneumatischen Phytochemie. Jena, Germany. p. 64–74.

Essington, M.E. 2015. Soil and water chemistry: An integrative approach. CRC Press, Boca Raton, FL.

Filella, M. 2009. Freshwaters: Which NOM matters? Environ. Chem. Lett. 7:21–35. doi:10.1007/s10311-008-0158-x

Flaig, W. 1958. Die Chemie organischer Stoffe im Boden und deren physiologische Wirkung. In: VII. Congress of the International Soil Science Society. International Soil Science Society, Hamburg, Germany. p. 11–45.

Gramss, G., K.D. Voigt, and B. Kirsche. 1999a. Degradation of polycyclic aromatic hydrocarbons with three to seven aromatic rings by higher fungi in sterile and unsterile soils. Biodegradation 10:51–62. doi:10.1023/A:1008368923383

Gramss, G., K.D. Voigt, and B. Kirsche. 1999b. Oxidoreductase enzymes liberated by plant roots and their effects on soil humic material. Chemosphere 38:1481–1494. doi:10.1016/S0045-6535(98)00369-5

Gramss, G., D. Ziegenhagen, and S. Sorge. 1999c. Degradation of soil humic extract by wood- and soil-associated fungi, bacteria, and commercial enzymes. Microb. Ecol. 37:140–151. doi:10.1007/s002489900138

Haider, K., and J.P. Martin. 1975. Decomposition of specifically C14 labeled benzoic and cinnamic acid derivatives in soil. Soil Sci. Soc. Am. J. 39:657–662. doi:10.2136/sssaj1975.03615995003900040025x

Haider, K., J.P. Martin, and Z. Filip. 1975. Humus biochemistry. In: E.A. Paul and A.D. McLaren, editors, Soil biochemistry. Marcel Dekker, New York. p. 195–244.

Haider, K., and J. Trojanowski. 1975. Decomposition of specifically C-14-labeled phenols and dehydropolymers of coniferyl alcohol as models for lignin degradation by soft and white rot fungi. Arch. Microbiol. 105:33–41. doi:10.1007/BF00447109

Hatcher, P.G. 2004. The CHNs of organic geochemistry: Characterization of molecularly uncharacterized non-living organic matter. Mar. Chem. 92:5–8. doi:10.1016/j.marchem.2004.06.014

Hayes, M.H.B. 2006. Solvent systems for the isolation of organic components from soils. Soil Sci. Soc. Am. J. 70:986–994. doi:10.2136/sssaj2005.0107

Hayes, M.H.B., and R.S. Swift. 1990. Genesis, isolation, composition, and structures of soil humic substances. In: M.F. De Boodt, M.H.B. Hayes, and A. Herbillon, editors, Soil colloids and their associations in aggregates. Plenum Press, New York. p. 245–305. doi:10.1007/978-1-4899-2611-1_10

Haynes, W.M. 2012. CRC handbook of chemistry and physics. CRC Press, Boca Raton, FL.

Hedges, J.I., G. Eglinton, P.G. Hatcher, D.L. Kirchman, C. Arnosti, S. Derenne, et al. 2000. The molecularly-uncharacterized component of nonliving organic matter in natural environments. Org. Geochem. 31:945–958. doi:10.1016/S0146-6380(00)00096-6

Horwath, W. 2015. Carbon cycling: The dynamics and formation of organic matter. In: E.A. Paul, editor, Soil microbiology, ecology and biochemistry. Elsevier, London. p. 339–382. doi:10.1016/B978-0-12-415955-6.00012-8

Huang, P.M., and A.G. Hardie. 2009. Formation mechanisms of humic substances in the environment. In: N. Senesi, B. Xing, and P.M. Huang, editors, Biophysico-chemical processes involving natural nonliving organic matter in environmental systems. John Wiley & Sons, Hoboken, NJ. p. 41–109. doi:10.1002/9780470494950.ch2

IHSS. 2019. What are humic substances? International Humic Substances Society. http://humic-substances.org/ (accessed January 2019).

Kelleher, B.P., and A.J. Simpson. 2006. Humic substances in soils: Are they really chemically distinct? Environ. Sci. Technol. 40:4605–4611. doi:10.1021/es0608085

Kleber, M., R. Mikutta, M.S. Torn, and R. Jahn. 2005. Poorly crystalline mineral phases protect organic matter in acid subsoil horizons. Eur. J. Soil Sci. 56:717–725.

Kononova, M.M. 1958. Die Humusstoffe des Bodens. Deutscher Verlag der Wissenschaften, Berlin

Kononova, M.M. 1966. Soil organic matter. Pergamon Press, Oxford, UK.

Lehmann, J., J. Kinyangi, and D. Solomon. 2007. Organic matter stabilization in soil microaggregates: Implications from spatial heterogeneity of organic carbon contents and carbon forms. Biogeochemistry 85:45–57. doi:10.1007/s10533-007-9105-3

Lehmann, J., and M. Kleber. 2015. The contentious nature of soil organic matter. Nature 528:60–68.

Lehmann, J., D. Solomon, J. Kinyangi, L. Dathe, S. Wirick, and C. Jacobsen. 2008. Spatial complexity of soil organic matter forms at nanometre scales. Nat. Geosci. 1:238–242. doi:10.1038/ngeo155

Liebig, J.v. 1840. Die organische Chemie in ihrer Anwendung auf Agricultur und Physiologie. Friedrich Vieweg und Sohn, Braunschweig, Germany.

Lützow, M., I. Kögel-Knabner, K. Ekschmitt, E. Matzner, G. Guggenberger, B. Marschner, et al. 2006. Stabilization of organic matter in temperate soils: Mechanisms and their relevance under different soil conditions- a review. Eur. J. Soil Sci. 57:426–445. doi:10.1111/j.1365-2389.2006.00809.x

MacCarthy, P., R.L. Malcolm, C.E. Clapp, and P.R. Bloom. 1990. An introduction to soil humic substances. In: P. MacCarthy, R.L. Malcolm, C.E. Clapp, and P.R. Bloom, editors, Humic substances in crop and soil sciences: Selected readings. SSSA, Madison, WI. p. 1–12.

Maillard, L.C. 1916. Synthèse des matières humiques par action des acides aminés sur les sucres réducteurs. Ann. Chim. 5:258–317.

Maillard, L.C. 1917. Identité des matières humiques de synthèse avec les matières humiques naturelles. Ann. Chim. 7:113–152.

Martin, J.P., and K. Haider. 1971. Microbial activity in relation to soil humus formation. Soil Sci. 111:54–63. doi:10.1097/00010694-197101000-00007

Martin, J.P., H. Zunino, P. Peirano, M. Caiozzi, and K. Haider. 1982. Decomposition of ^{14}C-labeled lignins, model humic acid polymers, and fungal melanins in allophanic soils. Soil Biol. Biochem. 14:289–293. doi:10.1016/0038-0717(82)90039-6

Masoom, H., D. Courtier-Murias, H. Farooq, R. Soong, B.P. Kelleher, C. Zhang, et al. 2016. Soil organic matter in its native state: Unravelling the most complex biomaterial on Earth. Environ. Sci. Technol. 50:1670–1680. doi:10.1021/acs.est.5b03410

Mathur, S.P., and E.A. Paul. 1967. Microbial utilization of soil humic acids. Can. J. Microbiol. 13:573–580. doi:10.1139/m67-074

Maurice, P.A., M.J. Pullin, S.E. Cabaniss, Q. Zhou, K. Namjesnik-Dejanovic, and G.R. Aiken. 2002. A comparison of surface water natural organic matter in raw filtered water samples, XAD, and reverse osmosis isolates. Water Res. 36:2357–2371. doi:10.1016/S0043-1354(01)00442-0

Meyer-Ilse, W., H. Medecki, L. Jochum, E. Anderson, D. Attwood, C. Magowan et al. 1995. New high-resolution zone-plate microscope at beamline 6.1 of the ALS. Synchrotron Radiat. News 8:29–33. doi:10.1080/08940889508602811

Myneni, S.C.B., J.T. Brown, G.A. Martinez, and W. Meyer-Ilse. 1999. Imaging of humic substance macromolecular structures in water and soils. Science 286:1335–1337. doi:10.1126/science.286.5443.1335

Naidja, A., P.M. Huang, and J.M. Bollag. 1998. Comparison of reaction products from the transformation of catechol catalyzed by birnessite or tyrosinase. Soil Sci. Soc. Am. J. 62:188–195. doi:10.2136/sssaj1998.03615995006200010025x

Oades, J.M., and J.N. Ladd. 1977. Biochemical properties: Carbon and nitrogen metabolism. In: J.S. Russell and E.L. Greacen, editors, Soil factors in crop production in a semi-arid environment. University of Queensland Press, St. Lucia, QLD, Australia. p. 127–160.

Parsons, J.W. 1988. Isolation of humic substances from soils and sediments. In: F.H. Frimmel and R.F. Christman, editors, Humic substances and their role in the environment. John Wiley & Sons, Chichester, UK. p. 3–14.

Piccolo, A. 2001. The supramolecular structure of humic substances. Soil Sci. 166:810–832. doi:10.1097/00010694-200111000-00007

Rose, M.T., A.F. Patti, K.R. Little, A.L. Brown, W.R. Jackson, and T.R. Cavagnaro. 2014. A meta-analysis and review of plant-growth response to humic substances: Practical implications for agriculture. Adv. Agron. 124:37–89.

Schmahl, G. 1993. X-ray microscopy studies. Optik (Stuttg.) 93:95–102.

Schnitzer, M. 1978. Humic substances: Chemistry and reactions. In: M. Schnitzer and S.U. Khan, editors, Soil organic matter. Elsevier, Amsterdam. p. 1–64. doi:10.1016/S0166-2481(08)70016-3

Schnitzer, M., and C.M. Monreal. 2011. Chapter three–Quo vadis soil organic matter research? A biological link to the chemistry of humification. Adv. Agron. 113:143–217.

Serkiz, S.M., and E.M. Perdue. 1990. Isolation of dissolved organic matter from the Suwannee River using reverse osmosis. Water Res. 24:911–916. doi:10.1016/0043-1354(90)90142-S

Six, J., H. Bossuyt, S. Degryze, and K. Denef. 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil Tillage Res. 79:7–31. doi:10.1016/j.still.2004.03.008

Sprengel, C. 1826. Über Pflanzenhumus, Humussäure und humussaure Salze [About plant humus, humic acids and salts of humic acids]. Arch. Gesammte Naturl. 8:145–220.

SSSA. 2019. Glossary of soil science terms. Soil Science Society of America. https://www.soils.org/publications/soils-glossary (accessed January 2019).

Stevenson, F.J. 1994. Humus chemistry: Genesis, composition, reactions. 2nd ed. John Wiley & Sons, New York.

Sutton, R., and G. Sposito. 2005. Molecular structure in soil humic substances: The new view. Environ. Sci. Technol. 39:9009–9015. doi:10.1021/es050778q

Swaby, R.J., and J.N. Ladd. 1962. Chemical nature, microbial resistance, and origin of soil humus. In: Transactions of Joint Meeting Comms. IV & V. International Society of Soil Science, Palmerston North, New Zealand. p. 197–202.

Tan, K.H., 2014. Humic matter in soil and the environment: principles and controversies. CRC Press. Boca Raton, FL. doi.org/10.1201/b17037

Tatzber, M., M. Stemmer, H. Spiegel, C. Katzlberger, F. Zehetner, G. Haberhauer, et al. 2009. Decomposition of carbon-14-labeled organic amendments and humic acids in a long-term field experiment. Soil Sci. Soc. Am. J. 73:744–750. doi:10.2136/sssaj2008.0235

Thurman, E.M., and R.L. Malcolm. 1981. Preparative isolation of aquatic humic substances. Environ. Sci. Technol. 15:463–466. doi:10.1021/es00086a012

Waksman, S.A. 1936. Humus: Origin, chemical composition and importance in nature. Williams and Wilkins, Baltimore, MD. doi:10.1097/00010694-193605000-00010

Weil, R., and N.C. Brady. 2016. The nature and properties of soils. 15th ed. Pearson Education, Harlow, UK.

Wershaw, R.L. 1986. A new model for humic materials and their interactions with hydrophobic organic chemicals in soil–water or sediment–water systems. J. Contam. Hydrol. 1:29–45. doi:10.1016/0169-7722(86)90005-7

Wershaw, R.L. 1993. Model for humus in soils and sediments. Environ. Sci. Technol. 27:814–816. doi:10.1021/es00042a603

Wershaw, R.L. 2000. The study of humic substances: In search of a paradigm. In: E.A. Ghabbour and G. Davies, editors, Humic substances: Versatile components of plants, soil, and water. Royal Society of Chemistry, Cambridge, UK. p. 1–7. doi:10.1016/B978-1-85573-807-2.50005-9

Wilson, M.A., R.J. Pugmire, K.W. Zilm, K.M. Goh, S. Heng, and D.M. Grant. 1981. Cross-polarization C13-NMR spectroscopy with magic angle spinning characterizes organic matter in whole soils. Nature 294:648–650. doi:10.1038/294648a0