Vindication of humic substances as a key component of organic matter in soil and water

Michael H.B. Hayes[a], Roger S. Swift[b],*

[a]Department of Chemical Sciences, University of Limerick, Limerick, Ireland
[b]Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Gatton, QLD, Australia
*Corresponding author: e-mail address: r.swift@uq.edu.au

Contents

Abstract

Soil organic matter is a complex mixture partly of recognizable, largely unaltered plant components plus a group of highly modified materials that bear no morphological resemblance to the original components. Their formation results from transformation and decomposition processes collectively known as "humification." Humic substances, a family of closely related compounds, are considered to be a major product of this process.

In recent years, several articles have questioned the role and even existence of soil humic substances as a distinct entity in soil organic matter. They regard soil organic matter as a continuum of degradation reactions by unspecified processes from the original biomass inputs to carboxylic acids, and eventually to carbon dioxide without the

formation of new classes of compounds along the way. We disagree fundamentally with these views and highlight the errors, misconceptions, misinterpretations, inbuilt bias, lack of scientific rigor, and failure to consider works and evidence that run counter to their preconceived views.

In response, we present an evidence-based rebuttal, detailing the decomposition processes of plant components and their transformation to a range of products, some of which, including humic substances, have a degree of resistance to microbial degradation. We provide an appraisal of information about the genesis and compositions of humic substances, and the importance of compositional knowledge to devise treatments to modify their rate of decomposition.

We note that all reputable turnover models that accurately predict soil organic matter behavior include one or more persistent pools of carbon which is not compatible with the concepts in the soil continuum model.

1. Introduction

In an opinion article, Lehmann and Kleber (2015) challenged a number of well-established views and concepts about soil organic matter (SOM), including the formation and even the existence of humic substances (HSs). In a more recent paper, the same authors (Kleber and Lehmann, 2019) have stated "*Humic substances extracted by alkali are invalid proxies for the dynamics and functions of organic matter in terrestrial and aquatic ecosystems.*" In this paper, we examine their various claims and show that their major premises are based on misinterpretations of existing data and/or on conjecture with little or no experimental justification.

A central theme of their earlier paper (Lehmann and Kleber, 2015) is the proposal of a "Soil Continuum Model" (SCM) to explain the overall behavior of organic matter (OM) in the soil. This model focuses on the capacity of decomposer organisms to access SOM and on its protection from decomposition provided by soil mineral components. They view SOM "*as a continuum spanning the full range from intact plant material to highly oxidized carbon in carboxylic acids.*" In this, so called "continuum," they suggest that organic fragments are "*continuously processed by a decomposer community from large plant and animal residues towards smaller molecular size. At the same time, greater oxidation of the organic materials increases solubility in water as well as affording the opportunity for protection against further decomposition through association with mineral surfaces and incorporation into aggregates,*" and they add that "*it represents robust* (sic) *science and will facilitate the way we communicate between disciplines and with the public.*" This notion of a "continuum" does not take account of products of the transformation process that have been shown

to have a degree of innate persistence resulting from their composition, and not just that provided by protection through interaction with soil minerals. We agree that all naturally occurring organic materials entering the soil will decompose eventually and, as Jenkinson (1981) has stated, "*if this were not so, any completely resistant fraction would by now cover the surface of the earth,*" but there certainly are some materials, and classes of materials that have greater resistance to degradation than others.

We acknowledge that at any given time in a mature, well vegetated soil, all stages of the decomposition process will be represented, ranging from fresh, unaltered plant debris through a multitude of transformed products, and finally to CO_2. However, given that SOM is a heterogeneous mixture consisting of a wide range of ***discrete*** materials with different compositions (as will be outlined below); then it is both incorrect and inappropriate to refer to it as a "continuum" or expect it to behave like one since it does not satisfy the basic definition of a continuum.

We respect the right of all scientists to question and challenge concepts, both old and new, which help to establish a firmer base on which to move forward. While the L and K paper raises some interesting questions, we are concerned about erroneous assumptions and misinterpretations, the inbuilt bias, the failure to cite work that runs counter to their views, as well as the general lack of chemical and scientific rigor in the arguments presented.

Our intention here is to give a considered, balanced and evidence-based response to their papers. In order to do so we find it necessary to elaborate on data and concepts that differ from those presented in articles involving Lehmann and Kleber (2015) and Kleber and Lehmann (2019). We will present a definition that divides SOM into a number of subcomponents, including HSs (Section 2) and show that some of these (including HSs) have a degree of resistance to decomposition that is not dependent on associations with soil mineral colloids. We will outline concepts of the origins of HSs (Section 3); describe modern methods for the isolation and fractionation of the different components of OM in soils and water (Section 4); outline the nature of the transformations of organic residues in soils (Section 5); and point out differences between components of SOM in terms of molecular sizes and chemical composition based on data from modern spectroscopic procedures (Section 6). We will show that the SCM model suggested by Lehmann and Kleber (2015) is unlikely to be of any value for describing decomposition rates for SOM as a whole or predicting the effects of either global warming or of soil management systems on SOM levels (Sections 7 and 8).

2. Definitions of soil organic matter and humic substances

In order to advance this discussion it is necessary to define the terms humus, humification, and humic substances (HSs). The terms humus and humification are used in general parlance, but without scientific rigor. Humification refers to the breakdown of organic materials in soils and composts leading to the formation of humus. Humus is a general term used to describe the transformed products formed from a wide variety of organic substrates without any intent to assign chemical composition or structure. In contrast, the term humic substances (HSs) is used scientifically to describe specific components formed during the humification process which can be isolated and fractionated in a variety of ways. Based on an approach of Kononova (1966, 1975), Hayes and Swift (1978) proposed that SOM is a heterogeneous mixture of all organic components found in soil which can be subdivided into two groups with different morphological and chemical characteristics:

(1) Unaltered materials that include fresh organic debris and nontransformed components of older debris; and.

(2) Transformed products, or *humus*, that bear no morphological resemblances to the structures from which they were derived. These transformed components are referred to as *humified* products. They consist of both humic and nonhumic substances and can therefore be subdivided as follows:

(2a) Amorphous, brown-colored humic components differentiated on the basis of solubility properties into humic acids (HAs), fulvic acids (FAs), and humins.

(2b) Other components belonging to recognizable classes, such as polysaccharides, polypeptides, altered lignins, etc. These can be synthesized by microorganisms or can arise from modifications of similar compounds in the original debris. Such materials, though components of humus, would not be regarded as HSs.

In the classical definitions soil humic acids (HAs) are materials precipitated when the aqueous base extracts are adjusted to pH values between 2 and 1, those remaining in solution in the acidified media are called fulvic acids (FAs), and the insoluble materials released from parent plant or microbial substrates, or formed in secondary synthesis reactions are called humins. Contrary to statements made by Kleber and Lehmann (2019), it should

be noted that these definitions do not involve considerations of mode of synthesis, chemical composition or molecular structure and size of the HSs. We will show in Section 4 how applications of modern separation procedures can give more specific compositional information of what are commonly described as the HA, FA, and humin fractions. These definitions are, of course operational, and are not intended to indicate materials that are compositionally homogeneous. They provide a basis for sensible discussion based on shared understandings, and allow us to develop an awareness of "*families* of molecules" that share very similar properties which can be described in broad compositional terms. It is worth noting that, whereas these commonly used definitions were published 40 years ago, Lehmann and Kleber (2015) were either not aware of them or chose to ignore them.

Much negative comment is made about HSs being operationally defined rather than chemically or structurally defined. No self-respecting scientist would willingly accept such a definition unless there was no suitable alternative. The reason why there is currently no obvious alternative is relatively simple. The synthesis of common biological macromolecules such as proteins and carbohydrates is genetically controlled and the amino acids or monosaccharide units are linked by repeating peptidyl or glycosidic linkages which can be readily hydrolyzed. In contrast, HSs are formed from a multitude of aromatic and aliphatic component units through a range of transformation processes that include biologically mediated degradation and re-synthesis reactions. In addition, the many different "monomer" components are linked by strong, nonhydrolyzable C–C and C-O-C bonds. As a consequence, a rapidly increasing number of different, nonrepeating molecular structures are formed and many of these have very similar, but not identical, compositions and structures. Consequently, they constitute a *family* of closely related compounds which display similar properties and behavior and are referred to as HSs.

It is also worth noting that similar operational definitions are used to describe other complex, naturally-occurring materials, for example "kerogen." This term is commonly used by geologists and petroleum scientists to describe an organic component in mineral deposits and is defined in terms of its solubility (or lack of it) in particular solvents.

An example of a term commonly to describe another *family* of closely related compounds in soil is "soil polysaccharides." This broad term includes a wide variety of polysaccharides of plant and microbial origin which differ greatly in their detailed molecular composition but have considerable commonality in the nature of their "monomer" units and the linkages

between them and exhibit similar gross chemical properties such as solubility, polarity and reactivity.

As we proceed to consider the composition and reactivity of HSs, it is important to take account of their origins and definitions and also the complex nature of the processes leading to their formation as outlined above.

3. The formation and persistence of humic substances

At any given time, depending on the type of vegetation and the season, the SOM will contain various amounts of fresh, largely unaltered plant materials. It is safe to assume that rapidly decomposing plant components, such as simple sugars, oligosaccharides, amino acids, peptides, proteins, and starch will have a transient presence due to their rapid utilization by microorganisms and will usually be found in the soil in small, if any, quantities. Some of the more resistant plant components, such as complex hemicelluloses, structural cellulose and lignin, plus cuticular and suberized materials, together with charred residues, all of which can remain intact for longer periods and collectively make up a significant component of the total SOM. It is only when the complexity of the processes and the resulting products are understood that the magnitude and the nature of the problems surrounding the composition of SOM can be realized, and the necessary experimental approaches and techniques applied. Consequently, in order to study this complex mixture, it is necessary to simplify the system by isolating particular components wherever possible. This inevitably involves isolation, followed by fractionation and characterization.

We have referred in Section 1 to the comment by Lehmann and Kleber (2015) that a degree of protection of SOM is afforded by "*interactions with soil mineral surfaces and incorporation into aggregates.*" In order to interact with soil minerals the organic biomass substrates will need to have, or to develop functional groups or properties that will enable such interactions to take place, and that is recognized by Lehmann and Kleber. Cellulose, hemicellulose, and lignin, major components of ligno-cellulosic plants, do not interact per se with soil minerals, and transformations brought about by soil microorganisms are needed for the interactions to take place.

Contrary to the erroneous claims made by Lehman and Kleber (2015), there is ample evidence to support the concept that the transformed components of SOM are more resistant to decomposition than the original plant material from which they are formed. A good example of such evidence is that obtained from long-term bare fallow experiments in which

experimental plots are kept completely free from vegetation for extended periods of time so that no plant residues entered the soil after the commencement of the experiments. In one such study, Barré et al. (2010) examined the results from trials carried out in Denmark, France, Russia, Sweden and the United Kingdom which had run for periods of time ranging from ~30 to 80 years. Some of the sites were originally arable land and some grassland, and the soils varied in texture from site to site, ranging from coarse sandy to silty clay loam. As would be expected, the grassland soils initially lost OM at a faster rate than the arable soils for several years, but thereafter declined at a similar rate as the equivalent arable soil. The amount of organic matter remaining across all sites ranged from 79% to 35%, with mean values of 68% for the 30–49 years duration sites and 38% for the 50–80 years duration sites. The remaining, more stable carbon pool is made up of resistant, slowly-decomposing, humified SOM and varying amounts of pyrogenic carbon depending mainly on previous practices such as crop residue or forest burning. Most importantly, in terms of the current discussion, is the fact that the SOM in the coarse sandy soil behaved the same as in those soils with considerable clay content, indicating that the persistence of SOM is an *inherent property* and occurs even without the presence of clay for protection of the OM.

Taking a different approach Skjemstad et al. (2008) used carbon isotopic ratios to study SOM persistence in continuous production systems, thereby avoiding the use of long-term bare fallow experiments. They compared the organic matter formed in the surface 15 cm of a C4 forested soil to that in the similar depth of a neighboring soil from which the forest had been cleared 90 years prior to introduction of C3 grasses. They used $\delta^{13}C/^{12}C$ ratios, and photo-oxidation to remove OM not contained in micro-aggregates, which showed the extent that OM originating in the forest was preserved in the aggregates from the grassland soil. The use of this isotopic ratio technique avoids the contamination problems associated with ^{14}C dating and shows unequivocally that SOM fractions persist in the soil for more than 100 years. This persistence was shown to be the result of a combination of the chemical composition of the OM as well as protection by association with fine minerals, or occlusion within aggregates.

The changes that take place when plant residues decompose arise from what is referred to as the *humification process*, and humic substances (HSs) are among the products formed in this process. The degree of resistance to degradation of these substances in soil does not necessarily involve protection by soil minerals. For example, HSs are major components of peats,

brown coals and lignites even where soil minerals are absent. The long term existence of these substances is evidence that the SCM is incorrect. In that model such organic substances would not persist without mineral or other forms of protection. These substances and the extracts from them are clearly not the same as the original biomass from which they were formed, and there is a vast literature of spectroscopic evidence to show this (e.g. Del Rio et al., 1994; Hatcher et al., 1981, 1989; Sun et al., 2015). There are other examples where protection of OM does not occur through mineral associations, such as in sandy soils in which the dynamics of SOM are similar to those for soils which contain clay minerals. These examples of organic matter behavior in sandy and organic soils (Loveland and Webb, 2003) clearly show that resistance to decomposition of components of SOM are the result of inherent properties of the organic components and are not dependent on the presence of clay minerals.

Lehmann and Kleber (2015) have also considered three concepts for the transformations of organic matter in the soil and they regard these as the "*humification*" model, the "*Selective Preservation*" concept, and the "*Progressive Decomposition*" model. Our comments on these concepts follow.

3.1 The humification model

It is accepted that biological transformations of biomass have a major role in the genesis of HSs, but there is controversy about the nature and composition of the transformation products. In their considerations of the "*humification*" model, Lehmann and Kleber (2015) refer to assumptions of "*further transformation or synthesis of the initial decomposition products into large dark-coloured compounds.*" That statement especially applies to what is regarded as secondary synthesis, or the classical concept of the formation of macromolecular HSs from "building blocks" from the metabolic activities of organisms that breakdown and transform the OM. Lehmann and Kleber state that "*a consolidated assessment of published evidence reveals that secondary synthesis of 'humic substances' facilitated by minerals or enzymes has not been shown to be relevant in natural systems.*" We will present evidence to refute that statement.

In addition they state "*on these grounds we find it inadvisable to support the classic 'humification' model.*" They quote literature suggesting that "*upon cell death, materials that are synthesized in the course of microbial anabolism are released into the soil, where they are subject to further degradation*" and suggest that, based

on a review by Hedges et al. (2000) "*these materials released into the soil from microbial anabolism are subjected to further degradation, and throughout the process the materials remain on an energetic downhill trajectory as opposed to giving rise to the hypothetical 'humic substances' whose 'secondary synthesis' would require energy investments.*" In expressing their viewpoint Hedges et al. stated that "*nonliving organic molecules persist in all natural environments, where on average they greatly outweigh biochemicals in living organisms from which they derive.*" In supporting their statement Lehmann and Kleber refer to Burdon (2001) who considered that an organism "*making a substance for which it has no use would lead to its extinction because of competition from organisms that did not waste energy and resources in this way.*" However, within a particular ecosystem, it is not unusual for organisms to invest energy in the production of substances that provide future benefits for them and their progeny (e.g. in building its web a spider uses a lot of energy as an investment to secure a future supply of food). In soils the conversion of readily decomposable materials into more resistant, but still slowly decomposable forms could well be a collective mechanism or strategy adopted or evolved by micro-organisms to assist their survival during a period when there is little or no source of fresh plant material, as occurs in natural and managed systems.

Contrary to the statements of Lehmann and Kleber, quoted above, there is convincing evidence for the biological synthesis of HSs from phenolic precursors. The work of Martin, Haider and their colleagues (Bondietti et al., 1971; Haider and Martin, 1967, 1975; Haider et al., 1977; Martin et al., 1967, 1972, 1974, 1975; Martin and Haider, 1969, 1971, 1976a,b), which, once again, was not referenced in the Lehmann and Kleber (2015) contribution, provides strong evidence to support the potential for biological synthesis. Their work showed how phenolic compounds could be transformed to macromolecular substances with characteristics of HAs. In the work involving Martin and Haider (reviewed by Hayes and Swift, 1978) a number of fungal species have been listed that are considered to be important for the biological synthesis of HAs from phenolic precursors. It is important to note that phenols and humic-like substances can be generated by some of these fungi when no aromatic materials are included in the starting substrates (Martin and Haider, 1971). Inclusion of clays accelerated macromolecule synthesis (Bondietti et al., 1971). The catalytic effects of the clays were considered to result from a concentration of enzymes and of substrates at the clay surface, and the removal of metabolic wastes by adsorption. The omission by Lehmann and

Kleber (2015) of the relevant work by Haider, Martin and their colleagues is surprising because some of that work is referenced by Kleber (2010) and Kleber and Lehmann (2019).

In addition, there are a number of soil enzymes, including phenoloxidase, which give rise to radicals of phenols which polymerize and have been shown to play an important role in the formation of new compounds (Martin et al., 1975). Laccase is widely distributed among fungi, and is especially abundant in white rot fungi. It also produces phenoxy radicals that initiate a polymerization process (Hollman and Arends, 2012; Jong-Rok et al., 2012; Richter et al., 2015). Chefetz et al. (1998) isolated and purified laccase from a compost and showed that macromolecules were formed when guaiacol (2-methoxyphenol) was added and incubated. Most of the phenols found in fungal culture media were also found as products of sodium amalgam degradation of HAs from peat and soil (Martin et al., 1974).

Given the abundance in soil of laccase and of other enzymes capable of degradation and synthesis of macromolecular substances (Hollman and Arends, 2012; Jong-Rok et al., 2012; Richter et al., 2015), it is surprising that more focussed attention has not being given to the role of fungal microorganisms in the genesis of HSs. In a similar vein the abundance of phenoloxidases in soils is such that they can catalyze the extracellular formation of macromolecular substances. It is worth noting in passing that the production extracellular enzymes is another example of organisms investing energy and resources in a process from which other organisms can benefit (Burns et al., 2013). This highlights again the complex web of interactions that are involved in the decomposition of SOM and the formation of new substances.

Work involving Haider and Martin (1975), Haider et al. (1977), and Martin et al. (1975) showed that phenolic compounds with unsaturated aliphatic side chains had a relatively high degree of resistance to microbial decomposition, and could be further protected from biodegradation by incorporation into humic macromolecules.

The macromolecular substances formed by fungal metabolism/synthesis have a dark color similar to HSs. This would provide a much better explanation for the origins of color in SOM than the experimentally unsubstantiated, nonsensical explanation of Lehmann and Kleber (2015) (viz: "*the dark colour of 'humic' extracts generated in laboratory experiments can be satisfactorily explained by a combination of two processes: the degradation of natural pigments and the accumulation of molecules containing random conjugated bonds (which appear dark in the mixture).*" It should also be noted that the

formation of conjugated C═C double bonds (which cannot be random if they are conjugated) has frequently been suggested to occur as part of the humification process but this is much more likely to result from the degradation of a major plant component such as lignin rather than minor amounts of readily degraded pigments.

In their recent publication Kleber and Lehmann (2019) have referred to the works of Martin and Haider (1971), Haider and Martin (1975) and Haider, Martin, and Reitz (1977) that involved microbially synthesized polymeric substances from ^{14}C-labeled benzoic and cinnamic acid and from coniferyl alcohol. They stated that linkages into polymeric structures were able to retard the decomposition of organic carbon during a 12-week incubation period, and also stated, *without any supporting experimental evidence*, that they did not consider "*linkage of phenolic polymers into polymeric macromolecules occurs in a quantitatively relevant fashion in natural soil environments.*"

In considering the possibility of abiotic synthesis of HSs, Lehmann and Kleber (2015) agree with Burdon (2001) who stated that the Maillard "Browning Reaction" (Maillard, 1916, 1917) is unlikely to be important because of the low concentration of the reactants (reducing sugars and amino acids) in the soil solution. However, these reactions are more likely to take place within the decomposing organic matrix where greater concentrations of the reactants are localized. The related work of Enders (1943a,b), Enders and Fries (1936), Enders and Sigurdsson (1948), Enders et al. (1948) focuses on abiotic synthesis involving reactions of methylglyoxal (2-oxoproponal) with glycine and amino compounds to give structures having properties similar to HSs. Schuffelen and Bolt (1950) and Hayes (1960, 2009) have shown that reactions of methyl glyoxal (2-oxopropanal) with glycine can give macromolecular products with many properties similar to those of soil humic acids.

Based on Burdon's (2001) mechanistic comments, Lehmann and Kleber (2015) chose to disregard the extensive work of Flaig (1960) and of Flaig et al. (1975) on the role of phenols (from transformations of lignin) in the genesis of HSs. However, that view is contrary to the reaction pathways proposed by Flaig et al. (1975) and also by Ziechmann (1980) for the polymerization of phenolic units arising from the degradation of lignin. More recent work by Wallis et al. (2006) demonstrated carbon to carbon bond formation using simple phenols and iron (III)–clay complexes in mild conditions in water, and this concept could be relevant to abiotic synthesis in soil.

Given the range of possible reactions and pathways addressed above, the following assessment may be helpful: After considering all of the available

evidence, our opinion is that microbially mediated processes are much more likely to be the dominant pathways in both decomposition and secondary synthesis processes taking place in soil, but it is possible that under certain circumstances (e.g. high, localized concentrations of OM) abiotic reactions could make some contribution to these transformation processes.

3.2 The selective preservation concept

Based on the "selective preservation" (or "preferential decomposition") concept (Lützow et al., 2006), L&K noted "*that major difficulties in the understanding and prediction of SOM dynamics originate from the simultaneous operation of several mechanisms,*" and that "*organic inputs are composed of both labile and relatively recalcitrant compounds, the latter being used by microorganisms only when the former are exhausted.*" They (Lehmann and Kleber, 2015) consider that now there is "*robust evidence that, under suitable conditions, appropriately adapted decomposer organisms have the ability to decompose even presumably persistent materials*" and "*decomposition of presumably recalcitrant lignin is fastest at the early stages of decomposition, as long as it is easily accessible and small organic molecules* (Klotzbucher et al., 2011) *are available as a source of energy to help mineralize the lignin.*"

In contrast to views expressed about the decomposition of lignin, we consider that there is strong evidence for the role of lignin in the genesis of HSs. We have referred to contributions by Flaig (1960) and colleagues (Flaig et al., 1975), by Ziechmann (1980), and by Wallis et al. (2006). The Hatcher group (DiDonato et al., 2016; Waggoner et al., 2015) applied electrospray ionization coupled to Fourier Transform ion cyclotron resonance mass spectrometry (ESI–FTICR–MS), and nuclear magnetic resonance spectroscopy (NMR) in their studies of the role of lignin in the genesis of HSs. Their data for samples from various ecosystems, climatic regions and soil textural classes indicated that the HAs "*are distinguished by the presence of three predominant molecular components: lignin-like molecules, carboxyl-containing aliphatic molecules, and condensed aromatic molecules* "*that bear similarity to black carbon.*" This work provided evidence for lignin as the primary source of major components of SOM, and "*particularly condensed aromatic organic molecules often categorized as black carbon.*" That would suggest an alternative and/or complimentary nonpyrogenic source, to forest (Knicker, 2007; Knicker et al., 2008) and vegetation fires for the presence of fused aromatic structures and black carbon in soils. They also suggest that much of the carboxyl–containing aliphatic components are sourced from lignin.

The data of DiDonato et al., and of Waggoner et al. for HSs from several soils with different degrees of humification showed that molecular formulae of well humified soils fell into two predominant regions of van Krevelen plots consisting of condensed aromatic molecules, as well as high H/C, low O/C carboxyl-containing aliphatic molecules (CCAM). The spectral data for HAs from a poorly humified spodosol soil had relatively more molecular formula plotting in the lignin-like region and relatively fewer condensed aromatic molecules. Thus the less humified samples contained relatively more components indicative of lignin origins, and the more humified samples were composed of relatively more molecular formulas in the CCAM and condensed aromatic regions. These more humified samples also contained relatively more carboxylated molecular functionalities than the less humified samples. To explain the molecular formulae observed, they proposed a humification process that involves photo- or microbially generated reactive oxygen species in soils. These would transform the fresh organic matter, mainly lignin, to the molecules observed in the ESI–FTICR–MS data. Waggoner et al. (2015) showed that black carbon-like and alicyclic aliphatic compounds could be generated by hydroxyl radical-initiated degradation of lignin, further indicating that lignin transformations are important contributors to the humification process.

These detailed, high-quality studies, show that lignin and its components are major contributors to the formation of HSs and impart a degree of recalcitrance to the new structures, which are not readily decomposed in soil.

3.3 The progressive decomposition model

This model is described by Lehmann and Kleber (2015) as soil organic matter consisting of "*a range of organic fragments and microbial products of all sizes at various stages of decomposition.*" We agree with this statement which is implicit in the definitions proposed by Hayes and Swift (1978) and referred to in this article. They refer to "*mixtures of identifiable compounds such as fragments or microorganisms that are distributed in different locations of micro-aggregates, showing similarity to the humic extract, and having small size.*" It is not surprising that identifiable fragments of plants and of microorganisms could be occluded in micro-aggregates and, when aggregates are dispersed, some could be coextracted in base-soluble humic extracts. The more hydrophobic humin components among these are likely to sorb strongly to soil mineral colloids and thereby could be afforded further protection (Hayes et al., 2017).

A comparison of humin materials isolated from different soil types in three different continents indicated that their compositions were essentially the same and that caused Hayes and Swift (2016) to ask "is humin the same everywhere"? As already stated, we agree that all of the organic components will transform, and eventually decay. The transformation rates will be vastly different for: fresh plant materials and microbial substrates; humified material, and for the humin materials, whether they are free in soil or sorbed on soil inorganic colloids.

4. Isolation and fractionation of the organic components in soil and water

The great advances made in understanding the composition, structure, and reactivity of biological molecules have evolved from the isolation and fractionation of component structures. Lehmann and Kleber state that "*the traditional 'humification' concepts limit observations of soil organic matter to its solubility in alkaline extracts, unlike the emergent view of organic matter based on solubility in water and its accessibility to microorganisms.*" They proffer the view that HSs "*extracted by alkali from soil environments are artefacts of the extraction process, with humic acids and fulvic acids having an exaggerated chemical reactivity relative to* "*true isolates.*" It is clear that their viewpoint has not been altered in the 3 year interim since their original publication and their more recent paper (Kleber and Lehmann, 2019).They use the term "true isolate" to represent the whole SOM and not just the alkali–extractable fractions. We find it difficult to recognize their concept of "true isolates" since neither they, nor in so far as we know, anyone else, has managed, or even attempted to obtain what might be considered to be a "true isolate." Experimental evaluation of this claim requires not only isolated FA and HA samples but also isolates more representative of the whole SOM. They state that "*extraction is always incomplete leaving 50–70% of the organic carbon unextracted which is then defined as the insoluble humin fraction*" (in passing, it is worth noting that partial extraction is, in itself, indicative of the existence of different, discrete entities in SOM). In any event, it is clear to us that they were not aware of the detailed studies that have been carried out with different extraction systems (including nonalkaline extractants) used for the isolation of OM components from soil and water (e.g. Aiken, 1985; Clapp et al., 2005; Green et al., 2015; Hayes, 1985; Hayes et al., 2008, 2012;

Malcolm and MacCarthy, 1992, Mylotte et al., 2015, 2016; Ritchie and Perdue, 2003; Serkiz and Perdue, 1990; Song et al., 2008, 2011; Swift, 1996).

In the absence of an effective means of obtaining this "so-called" "true isolate" from soil, a direct experimental test of these claims is not possible, but alternative approaches are suggested below. In their considerations of extractions of SOM, Lehmann and Kleber (2015) cited extractions with alkali as the most efficient "*although incomplete*" *(with 50–70% of the SOM unextracted),* "*selective, and prone to creating artefacts*" They agreed with Waksman (1936) (and still do, Kleber and Lehmann, 2019) who stated that "humic" "*nomenclature should be dropped because the term relates only to material obtained by a specific procedure.*" It should be noted that (much like Lehmann and Kleber) Waksman *presented no experimental evidence whatsoever to support his views.*

Following a consideration of the relevance of the pH of the medium and pK_a values of the dissociable functional groups, Kleber and Lehmann (2019) state that "*alkaline extraction will be most successful when the organic matter to be extracted is highly decorated with abundant phenolic and carboxylic groups.*" They outline how "*acquisition of oxygen-containing functional groups is tied to the process of decomposition.*"

We agree that a dilute sodium hydroxide solution could, over an extended period and under the right conditions, cause some hydrolysis of weak ester bonds and facilitate minor oxidation. It is clear, however, that the authors were not familiar with (or chose to ignore) the work of Swift and Posner (1972) almost two generations ago. Their data confirmed that some oxidation of a HA extract did take place when the extract was stored in 1 M NaOH for 30 days **under an atmosphere of oxygen**. No oxidation was observed when the HAs were stored under the same conditions in an atmosphere of dinitrogen gas. Oxidative effects can be minimized by the exclusion of oxygen, minimizing extraction time and the rapid neutral-ization of the extracts. However, anyone who accepts the artifact sugges-tion should consider how the OM in soil could, when subjected to dilute base for short periods of time at ambient temperatures, give rise to such a sizeable component (∼15–35%) of materials that resemble the NMR spectra of HSs (Fig. 1). Dilute NaOH at room temperature would not lead to the creation of aromatic or aliphatic entities or to the formation of C–C linking bonds. We would welcome proofs of mechanisms that would enable

realistic precursors to undergo reactions that would give rise to such large quantities of materials in such a short space of time under such mild conditions. In the absence of such evidence, the suggestion that HSs are artifacts created during extraction by weak alkali is farcical and should be ignored.

4.1 Extraction of humic substances from soil

The possibility of oxidation of organic matter in alkaline media caused Bremner (1949) and Bremner and Lees (1949) to carry out extractions with neutral salt reagents. They used 0.1 M sodium pyrophosphate (neutralized to pH 7) for their best results. The pyrophosphate complexes and removes strongly held divalent and polyvalent cations that occupy charge sites in the HSs rendering them insoluble, and replaces these cations with readily dissociated sodium ions. This results in the solubilization of a significant fraction of the SOM. Yields in pyrophosphate are less than those obtained in dilute alkali because of the lower degree of ionization of carboxylic acid functionalities at pH 7 (as compared to pH 13). Based on the observations of Swift and Posner (1972) and others, recommendations have been developed for the extraction from soil at neutral and alkaline pH values, at room temperature (up to 25 °C), excluding air or using an atmosphere of nitrogen to minimize the possibility of oxidation of the extract. The procedure involves: minimizing the concentration of the alkali; minimizing the extraction time, excluding light; and neutralizing or acidifying the isolate as soon as possible after extraction. Thus the FA and HA fractions from soil are not likely to differ dramatically from an isolate that is truly representative of those components of SOM. These considerations were taken into account when 0.1 M NaOH was selected as the extractant of choice for the isolation of the soil HSs standards of the International Humic Substances Society (IHSS) (Swift, 1996).

Olk et al. (2019a,b) found no evidence to indicate that extractions in base under the recommended conditions give rise to the formation of artifacts or alter the reactivities of the organic matter. It is worth noting that their publications agree with our rebuttal of several of the claims made by Lehmann and Kleber (2015).

Hayes (1985) and Clapp et al. (2005) have compared the properties of organic solvents that may be considered for the extraction of HSs from soils, Swift (1996) has described the IHSS procedure for isolations from soil, and resin (Aiken, 1985; Malcolm and MacCarthy, 1992) and reverse osmosis (Green et al., 2015; Serkiz and Perdue, 1990) techniques are used to

isolate HSs and natural organic matter from waters. Hayes et al. (2008, 2012) used an exhaustive sequential extraction procedure at increasing pH values, to isolate humic and other components from soil, then they then introduced 0.1 M alkali in 6 M urea as a sequel to the extraction with 0.1 M NaOH to extract all of the humic and some other components from soil (Song et al., 2008, 2011), and finally they used acidified (H_2SO_4) dimethyl sulfoxide (DMSO) to isolate the remaining humin (Hayes et al., 2017; Mylotte et al., 2015, 2016; Song et al., 2008, 2011). They have carried out extensive analyses of the fractions isolated by the IHSS procedure and by a resin in tandem procedure in which highly diluted and acidified extracts were passed on to XAD-8 ((poly)methyl methacrylate) and XAD-4 (styrenedivinylbenzene) resins in tandem. The more hydrophobic, humic and fulvic acids, are retained by the XAD-8 resin, and the more hydrophilic (mainly nonhumic components) coextracted with the FAs and HAs are retained by the XAD-4 resin. Using these procedures up to 95% of the SOM was isolated and recovered. This approach provides a complete cross section of the major components of SOM without the formation of artifacts.

The suggestion of Lehmann and Kleber (2015) for a single "true isolate" of SOM is not achievable. Consequently, the approach outlined above is as close as is currently possible to obtain a complete extraction of SOM components, the study of which will provide a better understanding of this complex mixture.

Importantly, *it is only through studies such as these where extractable yields are greatly increased and components with very different compositions and properties are isolated and characterized, that we can get a true indication of the composition of SOM*. The increased yields as outlined above counter the criticism of low yields of extracts leveled by Lehmann and Kleber (2015), and provide a greater assurance that, collectively, the materials isolated and recovered represent the majority of the components of SOM. Using this approach at least three chemically distinct component groups, namely; carbohydrate–rich carboxylic acids, the HSs and humin have been identified. This outcome could not have been achieved if the proposed SCM was accepted as describing the reality of SOM transformations in soil.

Fig. 1 shows the CPMAS ^{13}C NMR spectra of three SOM fractions and clearly illustrate the substantial compositional differences between humic acids, the carbohydrate-rich carboxylic acids, and the humin components isolated from soil. The humic acid spectrum shows strong peaks for aliphatic
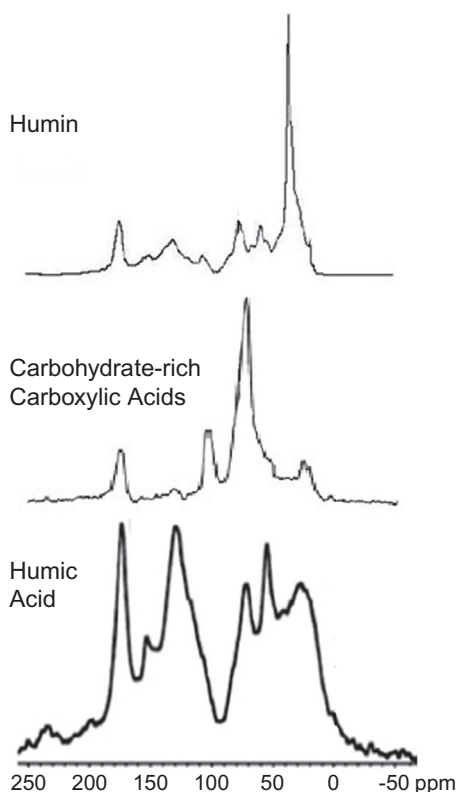
Fig. 1 CPMAS ^{13}C NMR spectra showing substantial compositional differences between the following soil isolates: *Humic acid*—exhibits very strong aromatic C and carboxyl C contents, strong alkyl and moderate carbohydrate (or C–O) C contents; *Carbohydrate-rich carboxylic acids*—exhibit very strong carbohydrate C content plus small carboxyl C and very low alkyl C contents; *Humin*—exhibits very strong dominant alkyl C content with small amounts of; carbohydrate C, aromatic C and carboxyl C; (Note: Approximate ranges of signal assignments are aliphatic hydrocarbon, 0–50 ppm; carbohydrate C (including anomeric C) and other C–O, ~60–105 ppm; aromatic hydrocarbon (including 0-aromatic), ~125–150 ppm, carboxylic C, ~160–185 ppm).

hydrocarbon (~0–50 ppm), aromatic hydrocarbon (123–150 ppm) and carboxyl components (~160–185 ppm) and a moderate amount of carbohydrate content (~50–90 ppm) which is typical of this fraction of SOM. The carbohydrate-rich fraction differs greatly from the HA spectrum and is naturally dominated by the carbohydrate peak (50–90 ppm) as well as the distinct anomeric-C peak (105 ppm), there is a moderate carboxylic acid peak and a modest amount of aliphatic hydrocarbon, but there is only a very small aromatic carbon in this fraction. In stark contrast to both of the

other two spectra, the humin spectrum is dominated by a very large aliphatic hydrocarbon peak with small amounts of aromatic hydrocarbon, carbohydrate and carboxylic acid groups.

Quite clearly, these three spectra are very different and are very different each from the others and this is indicative of the existence of discrete fractions of SOM with different compositions, properties and behavioral characteristics; including different levels of resistance to further decomposition,

4.2 Isolation of humic substances from water

Lehmann and Kleber (2015) state that "*to date 'humic substances' as extracted by alkali constitute the organic workhorse that is investigated by the community of aquatic chemists.*" They argue that "*the persistence and movement of terrestrial-derived organic carbon compounds entering aquatic ecosystems will rely on their protection by minerals, solubility in water and microbial degradation rather than primarily their chemical properties.*"

Before addressing these statements in detail it is necessary to consider some basic principles with respect to dissolved OM in soil and water environments. In aquatic systems, OM arises from the surrounding environment in the forms of dissolved or particulate OM, or OM in association with transported soil mineral particles. These organic materials arising from *outside* the water body are identified as allochthonous, whereas those that arise from biological processes *within* water bodies are referred to as autochthonous. It is important to recognize that the focus of interest of water scientists is on dissolved organic matter (DOM) in filtered samples and was initiated by the USGS (as described by Averett et al. (1989) for samples from the Suwannee River). Aiken (1985) has described the use of XAD–8 resin for the isolation of HAs and FAs, from waters, and subsequently XAD–4 resin was used in tandem with XAD–8 to recover an additional fraction (Malcolm and MacCarthy, 1992). In addition to using this resin procedure, reverse osmosis (RO) technology (Serkiz and Perdue, 1990; Green et al., 2015) has been used to process large volumes of water from riverine sources. Since isolations from the Suwannee River in south eastern Georgia (USA) began nearly 40 years ago, numerous samples of HAs and FAs isolated by the IHSS resin procedure, and natural organic matter (NOM), isolated by the reverse osmosis (RO) process have been distributed by the IHSS to the worldwide community of scientists. These samples have been used in more than 800 published papers between 1980 and 2018, resulting in 42,000 citations (Web of Science, March 2019).

Based on this information we reject the suggestion that alkali extraction is the "workhorse of the aquatic chemists," since alkali is not involved in the extraction process. In addition when untreated and alkali-treated RO extracts from the Suwanee River were made alkaline and subjected to capillary electrophoresis (Schmitt-Kopplin and Junkers, 2003), the results of the treated and untreated samples were identical. These observations demolish the speculative claim by Lehmann and Kleber (2015), not substantiated by experimental data, that contact with strong base leads to an exaggerated chemical reactivity relative to "true isolates."

Work by the authors (Hayes et al., 2008, 2012) has shown that the HSs isolated from soils at pH 7 most closely resembled that isolated from the drainage waters from the soils. There were compositional differences in the isolates at the higher pH values directly related to solvation of the conjugate base functionalities from acid dissociations under these conditions. Such observations are ignored by Lehmann and Kleber.

Lehmann and Kleber (2015) cited references in regard to electron shuttling relevant to HSs. They state that "*extracts of 'humic substances' typically used for investigations of electron shuttling phenomena may have developed this capacity not as a result of 'humification', but because alkaline solutions extract quinones that are present in soil as a result of known microbial metabolism* (Newman and Kolter, 2001) *or in carbon thermally altered by fire* (Solomon et al., 2007) *which has been shown to be electrochemically active* (Kappler et al., 2014; Klupfel et al., 2014)" There is abundant evidence for quinones in soil HSs. derived from the biological oxidation, during humification, of phenolic components of lignin (Hayes and Swift, 1978). It is therefore inevitable that quinone functionalities will be present in aquatic HSs with origins in soils.

5. Products of transformation of organic residues in soils

There is an extensive body of literature dealing with the decomposition in soil of residues of all origins. In general, the decomposition processes have been well characterized, but there still is disagreement about the nature and composition of the products. There is no disagreement that the readily decomposable components of plant residues (e.g. simple sugars and starch, peptides, proteins, nucleic acids, etc.) are broken down and transformed rapidly, whereas the more resistant plant components (e.g. cellulose, lignin, tannins, cutins, etc.) are transformed more slowly. The main disagreements arise over the nature and composition of the breakdown and decomposition

products of all of these components as well as the products of microbial activity produced during the decomposition processes.

In addressing this issue Lehmann and Kleber (2015) wrote "*The vast portfolio of options for variations in carbon turnover dynamics in the SCM provides a full explanation of organic matter properties as observed by contemporary, in situ spectromicroscopic techniques without invoking 'humification' processes or 'humic substances'.*" We disagree fundamentally with this statement and question the evidence they have used to support it, based on papers by Gillespie et al. (2011), Kelleher and Simpson (2006), Lehmann et al. (2008) and Mylotte et al. (2015).

In contradiction to the comments of Lehmann and Kleber (2015) based on their work, Kelleher and Simpson (2006) actually state "that the vast majority of operationally defined humic material in soils is a very complex mixture of microbial and plant biopolymers and their degradation products," and went on to state that "it is important to note this work in no way rules out the existence of a distinct category of humic macromolecules, either at low abundance in the soluble fraction from young soils, in diagenetically evolved samples (for example lignites, etc.), or in the nonextractable humin fraction."

The paper by Mylotte et al. (2015) deals with the alkaline extracts, and in a subsequent paper (Mylotte et al., 2016) with the acidified DMSO extracted materials (humin) from a sediment core. The HSQC spectrum of the humin isolated by Hayes et al. (2017) and by Mylotte et al. (2016), and the TOCSY spectrum for the Mollisol clay humin (Hayes et al., 2017; Song et al., 2008) were dominated by resonances that could be attributed to aliphatic hydrocarbon and long chain aliphatic hydrocarbon acid/ester functionalities, and minor resonances attributable to carbohydrate, peptide, and even lignin-derived functionalities. It is important to stress that this work dealt with the humin fraction, and what was regarded as the humic components had been removed by an exhaustive extraction process (Hayes et al., 2012; Song et al., 2008). Contrary to the statement above made by Lehmann and Kleber, the data in these three papers do not undermine the concept of humification or the presence of humic substances.

Applications of near-edge X-ray fine structure (NEXAFS) spectroscopy indicated to Lehmann et al. (2008) that organic C forms in widely different soils were similar. In contrast they observed highly variable carbon functional groups within different spatially distinct regions, of scales of nano- and micrometers. That is as would be expected. However, it does not provide information about the organic functional groups in the soil as a

whole. For example, Clapp and Hayes (1999) using several analytical pro-
cedures (elemental and δ^{13}C analyses, ^{13}C NMR, sugar, amino acid and
titration analyses) showed distinct, assignable differences in the organic mat-
ter compositions, and were able to show that the HAs from the clay-sized
fractions were more oxidized than those from the microaggregates in the
form of silt-sized aggregates.

It is not sufficient to compare spectra of selected materials with HSs
isolates unless the HSs have been carefully fractionated as referenced in
Section 4, and analyzed to gain a better knowledge the extents to which car-
bohydrate, peptide and other materials are components of HA, FA, and
humin structures. The citations quoted by Lehmann and Kleber (2015)
do not prove that HSs are composed of mixtures of plant and of microbial
compounds but it is clear that derivatives of plant and microbial compounds
can be found in associations with or in proximity to HSs and other trans-
formed products.

6. Chemical and physical evidence for humic substances

Lehmann and Kleber (2015) state that "*among the thousands of publica-
tions on 'humic substances' not one independently confirms- for example by direct
spectroscopic observation- that the 'humic substances' extracted by alkali are compo-
nents of organic matter that exist separately in soil environments.*" Since SOM is a
complex intimate mixture there would be little point in applying spec-
troscopic procedures for direct identification of a multitude of functional
groups within a multitude of components. *Applying spectroscopy to such a mix-
ture would be like trying to identify the voice of one person in a crowded stadium if
all were to shout their names in unison.* In Section 4 we have addressed the
question of the postulated effect of alkali and shown that in the concentra-
tions, and under the conditions used, it does not form new compounds or
alter the compositions of existing compounds. In addition, there is ample
evidence to show that the materials extracted in base are unaltered com-
ponents of the SOM (Section 4).

The ^{13}C NMR and the wet chemistry data referred to in Section 4
have revealed distinct differences between the humic fractions exhaustively
extracted at the different pH values, and between these and the humin. Each
of the base-extracted fractions in Section 4 satisfies criteria for defining HSs
and nonhumic substances. The most prominent humic components (HAs
and FAs) were compositionally and spectroscopically distinct from the
hydrophilic (nonhumic) fractions isolated as described. NMR spectroscopy

and wet chemistry analytical procedures showed that the hydrophilic carbohydrate-rich fractions are largely derived from microbial transformation processes and are not bound to the humic structures. The humic components isolated by the resin-in-sequence procedure had significantly lesser amounts of sugars and amino acids than those isolated using the IHSS procedure.

6.1 Chemical compositions of humic substances

A first step in understanding the composition and structure of any organic substance is to gain an understanding of its component molecules and a second step is to understand the sequences of the component molecules and how they are joined together. Where readily hydrolyzable, highly labile repeating linkages are involved, it is possible to gain an understanding of molecular composition and structure, such as those found in common biological systems.

Such an approach does not apply for HSs in which the linking bonds are more difficult to cleave, and the structural units are more diverse and do not occur in a regular sequence. Some molecules identified in the digests can be related to structural units in the humic molecules, but given the strength of the reaction conditions required, additional artifacts are formed that render the molecular reconstruction process extremely difficult. It is, however, clear that there is a need to provide a rudimentary explanatory guide to the composition of the building blocks and the bonds that link them.

Schnitzer and his colleagues (see Schnitzer and Khan (1972, 1978), and others, extensively referenced by Hayes and Swift (1978), made significant contributions by identifying digest products in the oxidative and reductive degradations of HSs, but Hayes and Swift (1978), and Clapp et al. (2005), by applying degradation mechanisms, showed that the digest products were often derivatives of the structural units that make up the humic structures. The humeomics approach of the Piccolo Group (Nebbioso and Piccolo, 2011, 2012; Nebbioso et al., 2014) uses "a stepwise separation of molecules from humic suprastructures by progressively breaking intermolecular bonds and characterizing their structure by advanced analytical instrumentation." The approach is logical but the high energy inputs applied at the later stages of the sequence could give rise to intractable artifacts. The use of a sequence of enzymes could provide an attractive alternative approach.

Based on the information available from many degradation studies, together with that from ^{13}C and proton NMR spectra, we are now getting better indications of the types of components and linkages in HSs and humin structures. ^{13}C NMR data indicate that there are aliphatic components containing methyl, methylene, and methine functionalities, aromatic, and O-aromatic structures that can include methoxyl (characteristic of lignin), carboxyl/ester/amide structures that can include carboxyls of aliphatic and aromatic groups, the amides of peptides, and ester structures, and with evidence for lesser amounts of carbohydrate, peptide and tannin structures.

6.2 Physico-chemical properties of humic substances

Considerations of the molecular sizes of HSs are matters of considerable debate and some controversy. The traditional view (Hayes and Swift, 1978) has been that HSs are polydisperse, macromolecular structures formed from random condensation reactions between the products of soil microbial metabolism. To accommodate their hydrophobic and hydrophilic components, Wershaw (1999) proposed a "membrane-micelle" model for humus in soils and sediments, and Engebretson and von Wandruszka (1998) deduced from observations of the quenching of fluorescence by Br$^-$ that the humic molecules could spontaneously aggregate through hydrophobic bonding and that pyrene associated with the hydrophobic molecules within the aggregated structures would be protected from the Br$^-$ ions. Kenworthy and Hayes (1997) showed that pyrene was more protected from Br$^-$ quenching in HAs and FAs extracted at the higher pH values.

Piccolo (2002) and colleagues have advocated the concept of supramolecular associations of smaller molecules using High Pressure Size Exclusion Chromatography (HPSEC) of HAs following amendments, such as low molecular weight organic acids (a concept introduced in their earlier studies) to rupture the intermolecular associations which give assemblages that mimic large molecules. More recently Wells and Stretz (2019), based on a "Comprehensive Hydrogen Bond Theory" (Gilli and Gilli, 2013) have presented a conceptual model for supramolecular aggregation of OM based on hydrogen bonding forces of varying strengths, some of which have strong interactions.

The concept of supramolecular associations has adherents, and has acceptance from Lehmann and Kleber (2015). Plausible explanations can be put forward for mechanisms by which associations of small and large molecules can occur. Indeed it is certain that in the solid state HSs

and other components of SOM are in associations which help them to remain in the soil.

Given that macromolecules are major components of plants, it is understandable that some colleagues have difficulties in reconciling the supramolecular association concept with earlier results obtained by many workers using gel chromatography (Cameron et al., 1972; deNobili, 1999; Swift, 1985), ultracentrifugation, together with other techniques as reviewed by Swift (1985). For example, using gel chromatography, Swift et al. (1992) separated a soil HA into four distinct size fractions. These were repeatedly refined and recovered until they were stable with respect to molecular size and elution volume. ^{13}C-CPMAS NMR spectra of all fractions were different. The contributions of aromaticity, carboxyl, and lignin increased with decreasing molecular size whilst carbohydrate content decreased. These differences in composition would not have occurred if the molecules in the fractions had reassembled in accordance with the concept of supramolecular associations (Swift, 1999).
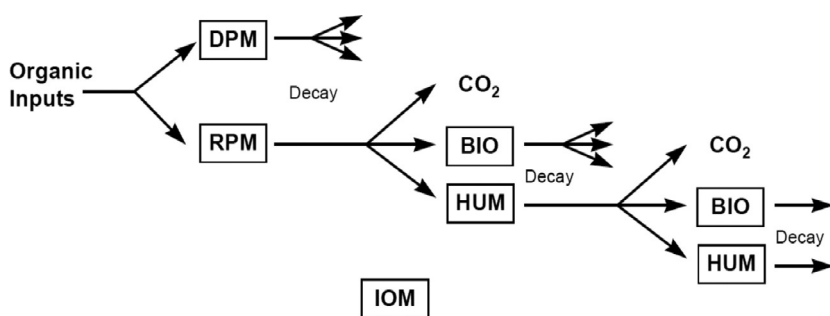
7. Modeling the turnover of soil organic matter

From the inception of studies of SOM, it has been important to understand both the processes involved in the turnover and the amounts or rates of turnover that occur under different conditions. There is an increasing interest in the amounts and the behavior of soil carbon arising from issues related to climate change caused by increasing atmospheric levels of CO_2. Because of the strong link between SOM and atmospheric CO_2 it has become increasingly important to model the behavior of soil carbon in order to predict future changes in SOM levels, or to back-calculate historical levels which may not have been measured previously. A number of models have been developed which address these issues (Smith et al., 1997). The most common are the RothC (Coleman and Jenkinson, 2014; Jenkinson, 1990), CENTURY (Parton, 1996) and APSIM (McCowan et al., 1996). In the CENTURY and APSIM models, the SOM is a component of a larger crop production model, whereas RothC focusses on the turnover of SOM. Detailed comparative studies have been carried to test the reliability, accuracy, and applicability of these and other SOM turnover models (McCowan et al., 1996; Ranatunga et al., 2001; Skjemstad et al., 2004). Each of the three models performed very well in these comparisons.

In stark contrast to the SCM model proposed by Lehman and Kleber (2015), all of the above models have similar constructs in that they utilize a combination of discrete pools of SOM. As a result of soil microbial activity, each of these pools turns over at a different rate following first order kinetics. The rate of turnover for each pool can be modified to some extent by other factors such as temperature, water content, clay content, and vegetative cover. Typically, these discrete pools will cover a wide span of turnover times ranging from; (a) rapid (weeks to months), (b) moderate (years to decades), and (c) slow (centuries), plus, in some cases, an inert fraction (millennia).

For example, in the case of the RothC model (Fig. 2) the pools used are: Decomposable Plant Material (DPM); Resistant Plant Material (RPM); Microbial Biomass (BIO); Humified Organic Matter (HUM); and Inert Organic Matter (IOM). Values for the decomposition rate constants for each of these organic matter pools, together with their derived radiocarbon age were obtained from a long-term, rigorously-managed experimental site at Rothamsted (Jenkinson, 1990) (Table 1).

Subsequently, Skjemstad et al. (2004) proposed a procedure through which the organic matter pools, inferred or calculated in the Roth C

RPM : Resistant Plant Material
DPM : Decomposable Plant Material   **HUM : Humified OM**
BIO : Microbial Biomass   **IOM : Inert Organic Matter**

Fig. 2 Schematic structure of the RothC Model (Coleman and Jenkinson, 2014) showing the different organic matter pools each of which is allocated a different decomposition rate constant (see Table 1). *Source: Adapted from Coleman, K., Jenkinson, D.S., 2014. RothC—a Model for the Turnover of Carbon in Soil: Model Description and User Guide;* www.rothamsted.ac.uk/sites/default/files/RothC_guide_WIN.pdf.

Table 1 RothC decomposition rate constants used and the resultant radiocarbon ages of the SOM Pools in a long-term experiment at Rothamsted Experimental Station (Coleman and Jenkinson, 2014; Jenkinson, 1990).

RothC SOM pool	Decomposition rate constant (year^{-1})	Equivalent radiocarbon age (year)
DPM	10.0	0.2
BIO	0.66	21.8
HUM	0.02	116.9
IOM[a]	∼0	50,000[a]

[a]The age of the IOM fraction is arbitrarily set to 50×10^3 years implying that it is geological rather than pedological in origin. However, this may not be the case in soils where the vegetation is subject to regular burning events and the carbon could be of more recent origin. Source: Adapted from Coleman, K., Jenkinson, D.S., 2014. RothC—a Model for the Turnover of Carbon in Soil: Model Description and User Guide; www.rothamsted.ac. uk/sites/default/files/RothC_guide_WIN.pdf.

modeling process, can be replaced by using actual measurable SOM pools. The application of this technique opens up new opportunities in the experimental examination of SOM, of the carbon pools, and their rates of turnover.

The most important point in the context of the current discussion is that it has been possible to describe the actual observed changes in SOM levels in long-term experiments only by using models with several organic matter pools turning over at different rates. It has proved to be impossible to fit the observed data from many sites using a single pool with a single value for the rate of decomposition, with or without a modifier, and first order kinetics. Yet these are essentially the conditions which would appear to apply using the SCM concept. It is difficult to conceive that the massive differences in decomposition rates required to match the observed data could be attributable solely to the level of access to SOM of microorganisms, as proposed by Lehmann and Kleber (2015). Importantly, it is worth noting that the dynamics of SOM decomposition in sandy soils (Joivet et al. 2003, Sanderman et al. 2013) follow a similar pattern even though access by microorganisms and their enzymes is not inhibited by the presence of clay or aggregates.

In addition, the established models referred to fit more closely with real-world experience based on many hundreds of observations of the decomposition of plant materials in soils of widely different compositions. All of these observations support the fact that the rates of decomposition in soil of different plant components vary greatly, and the same is true for the

new components formed in the soil through the transformation by microorganisms of these plant materials.

Such observations provide powerful evidence that SOM is made up of components that differ widely in terms of composition and rates of decomposability. This runs counter to the assumptions made in the SCM and on this basis alone that model lacks credibility.

8. Overview and future directions

Lehmann and Kleber (2015) view SOM (see Section 1) "*as a continuum spanning the full range from intact plant material to highly oxidized carbon in carboxylic acids.*" Within this "continuum" they state that the organic fragments are "*continuously processed by a decomposer community from large plant and animal residues towards smaller molecular size. At the same time, greater oxidation of the organic materials increases solubility in water as well as affording the opportunity for protection against further decomposition through association with mineral surfaces and incorporation into aggregates.*"

In their more recent publication, 3 years after the first, Kleber and Lehmann (2019) have not changed their perspective. They conclude that "*the humic substances paradigm, as it still stands is based on an argument that is invalid (alkali is unable to separate humic substances from nonhumic 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 compelling thermodynamic rationale why such a process would happen). This suggests that humic substances may be best set aside as organic matter categories.*"

In our rebuttal of these highly controversial and somewhat confusing statements, we question the evidence that the authors provide to support their concepts. We have provided definitions for SOM that have long been accepted as well as for the humic and nonhumic components of SOM. On the basis of emerging information we recognize that some minor adjustments to these definitions (Hayes and Swift, 1978) would be appropriate. For example, recent evidence indicates that slowly-degradable, largely aliphatic, plant cuticular materials (Hayes et al., 2017; Song et al., 2008, 2011) are major components of humin. Thus, humin, though a major component of SOM, may not now be regarded as a component of the humic fraction because it is largely composed of recognizable components of plants and of other biological molecules, as outlined in (2b), Section 2. In mineral soils, humin materials can associate strongly with soil inorganic

colloids, with hydrophobic bonding as the major adsorption mechanism (Hayes et al., 2017). We have also provided convincing evidence showing that the humic substances, carboxylated carbohydrates and the humin components resulting from the humification process are distinct categories of materials with different degrees of resistance to microbial degradation, and have compositional properties that are different and are identifiable using a range of modern spectroscopic procedures.

In their considerations of genesis and secondary synthesis, Kleber and Lehmann (2019) cite us (Hayes and Swift, 1990) in terms of "*the substances formed from reactions between simple organic chemicals set free in the soil environment*" In that reference and in our earlier Chapter (Hayes and Swift, 1978) we have indeed indicated that microbial synthesis could take place using molecules released from (microbial) degradation of biomass, and we have indicated in Section 3 of this chapter how abiotic synthesis could occur in the decaying biomass matrix of the SOM. We agree with Kleber and Lehmann (2019) that degradative pathways are involved in the transformations of OM eventually to CO_2, but we convincingly defend the thesis of the formation in the degradation process of humic materials with very different compositions from the starting materials and with a higher degree of recalcitrance that slows their degradation.

We agree that resistance to microbial decomposition is enhanced by physical protection resulting from occlusions in microaggregates and sorption onto clay and other mineral surfaces, but we can find no evidence to indicate that the decomposition process mainly involves water soluble organic components (from Lehmann and Kleber, 2015). We have found in our studies of the OM in soils and in their drainage waters (Hayes et al., 2008, 2012) that the amounts of OM in solution were low, and the major components could be classified as HSs which had resisted degradation. It is well established that a wide range of soil microorganisms produce a multitude of extracellular enzymes which are responsible for decomposing both simple and complex plant macromolecules (e.g. lignocellulose) (Burns et al., 2013). This is recognized as a key mechanism in humification; a complex, intertwined web of microbiological, chemical and physical processes.

Our analysis of modeling procedures that successfully represent the turnover of SOM use a combination of discrete pools of SOM, each of which decays and turns over at a different rate following first order kinetics. Importantly, it has been impossible to fit the data from many sites using a single pool with a single value for the rate of decomposition and first order

kinetics, as would appear to be implied for the SCM concept with adsorption onto clay as the only decomposition rate modifier.

Lal (2004, 2014) and many other authors, have stressed, even from antiquity, the importance of SOM to soil fertility and to soil conservation. For example, in the works of Columella (04–70 AD, assembled by Ash, 1941) there is a description of how organic wastes needed to be processed (essentially composted in modern terms) before being used for soil amendment. Lehmann and Kleber (2015) duly cite contributions of SOM to soil fertility. However, they do not agree with the emphasis placed on a "stable humus pool," building or augmenting the return of carbon lost through agricultural activities by "*drawing on the outdated concept of humification.*" In contrast, taking a countervailing viewpoint Swift (2001) has described how building up the persistent components of SOM (Hayes et al., 2017) could be used to sequester carbon into the soil, particularly in soils where SOM levels have been depleted by long-term cropping practices. This would also reduce CO_2 levels in the atmosphere and, thereby, represent a win-win outcome by simultaneously increasing soil fertility and decreasing the rate of global warming.

In our discussion of humification (Section 3), we have taken account of the transformations of biomass giving rise to a distinct category of materials that constitute the "family" of HSs, and also presented clear evidence for biotic synthesis of HSs, as well as possibilities for abiotic synthesis. As noted, we know that plant cuticular materials have a high resistance to biodegradation (Hayes et al., 2017) and these and other recalcitrant bio-(macro) molecules (Lorenz et al., 2007) are major components of the humin. Such relatively hydrophobic molecules strongly associate with the soil mineral colloids, and they can also associate with and protect the HSs (Hayes et al., 2017; Lorenz et al., 2007).

We view humification as playing a vital role in agriculture and we recognize its important role in providing a degree of stability within SOM and a reliable source of energy for the microbial processes that are essential in a productive soil. The formation of recognizable humic substances with a degree of resistance to degradation is highly important for "soil health" and fertility. Gerke (2010, 2018) has also outlined many of the compositional and stability aspects of HSs and has emphasized the importance of humic-trace metal complexes in plant nutrition.

A possible way forward for the maintenance of SOM levels and soil fertility (Hayes et al., 2017) would be to breed crops which, without diminishing the yield of the main commercial product, would have greater

abundances of components with a high degree of resistance to decomposition when they are returned to the soil. Such crops could also be rotated with legumes to maintain or enhance soil fertility in addition to rebuilding SOM content.

The world population at the present time is 7.7 bn, and estimated to rise to 9.7 bn by 2050, and 11.2 bn by 2100. The preindustrial revolution level of CO_2 in the atmosphere was 278 ppm (in 1750) and now it is at 415 ppm, and is increasing by ca 2 ppm p.a. The depletion of SOM resulting from agricultural practices is estimated to contribute about 50% to the total emissions of greenhouse gases (Lal, 2004, 2014). However, it is the CO_2 arising from the longer-term loss of indigenous SOM, and not that arising from the annual transforming amendments, that is a major contributor to increased levels of atmospheric CO_2.

A high proportion of the world's most fertile soils are under long-term cultivation and that has led to a very significant depletion of the SOM in these soils. Inevitably that will lead to soil degradation and loss of fertility unless the depletion is arrested. A recent communication by Piccolo et al. (2018) has outlined how a water soluble iron porphyrin structure arrested the loss of SOM from conventional tillage when added at a rate of $10 \, kg \, ha^{-1}$ for 3 years. Concepts such as these and others will need to be developed and further studied as we seek to find ways to not only retain but increase SOM levels, particularly in highly productive soils.

References

Aiken, G.R., 1985. Isolation and concentration techniques for aquatic humic substances. In: Aiken, G.R., McKnight, D.M., Wershaw, R.L., MacCarthy, P. (Eds.), Humic Substances in Soil, Sediment, and Water. Wiley, New York, pp. 363–385.

Ash, H.B., 1941. Translation of on Agriculture Vol 1: Books 1–4 by Columella. Harvard University Press.

Averett, R.C., Leenheer, J.A., McKnight, D.M., Thorn, K.A., 1989. Humic Substances in the Suwannee River, Georgia: Interactions, Properties, and Proposed Structures. U. S. Geological Survey, Open-File Report, pp. 87–557.

Barré, P., Eglin, T., Christensen, B.T., Ciais, P., Houot, S., Kätterer, T., van Oort, F., Peylin, P., Poulton, P.R., Romanenkov, V., Chenu, C., 2010. Quantifying and isolating stable soil organic carbon using long-term bare fallow experiments. Biogeosciences 7, 3839–3850.

Bondietti, E., Martin, J.P., Haider, K., 1971. Influence of nitrogen source and clay on growth and phenolic polymer production by *Stachybotrys* species, *Hendersonula torulidea* and *Aspergillus sydowi*. Soil Sci. Soc. Am. Proc. 35, 917–922.

Bremner, J.M., 1949. Some observations on the oxidation of soil organic matter in the presence of alkali. J. Soil Sci. 1, 198–204.

Bremner, J.M., Lees, H., 1949. Studies on soil organic matter. II. The extraction of organic matter from soil by neutral reagents. J. Agric. Sci. 39, 274–279.

Burdon, J., 2001. Are the traditional concepts of the structures of humic substances realistic? Soil Sci. 166, 752–769.

Burns, R.G., DeForest, J.I., Marxsen, J., Sinsabaugh, R.I., Stromberger, M.E., Wallenstein, M.D., Weintraub, M.N., Zoppini, A., 2013. Soil enzymes in a changing environment: current knowledge and future directions. Soil Biol. Biochem. 58, 216–234.

Cameron, R.S., Thornton, B.K., Swift, R.S., Posner, A.M., 1972. Molecular weight and shape of humic acid from sedimentation and diffusion measurements on fractionated extracts. J. Soil Sci. 23, 394–408.

Chefetz, B., Chen, Y., Hadar, Y., 1998. Purification and characterization of laccase from *Chaetomium thermophilium* and its role in humification. Appl. Environ. Microbiol. 64, 3175–3179.

Clapp, C.E., Hayes, M.H.B., 1999. Characterisation of humic substances isolated from clay- and silt-sized fractions of a corn residue amended agricultural soil. Soil Sci. 164, 899–913.

Clapp, C.E., Hayes, M.H.B., Simpson, A.J., Kingery, W.L., 2005. The chemistry of soil organic matter. In: Tabatabai, A., Sparks, D.L. (Eds.), Chemical Processes in Soils. Book Series No. 8, Soil Science Society of America, Madison, WI, pp. 1–150.

Coleman, K., Jenkinson, D.S., 2014. RothC—a Model for the Turnover of Carbon in Soil: Model Description and User Guide. www.rothamsted.ac.uk/sites/default/files/RothC_guide_WIN.pdf.

Del Rio, J.C., Gonzales-Vila, F.J., Martin, F., Verdejo, T., 1994. Characterization of humic acids from low-rank coals by ^{13}C-NMR and pyrolysis-methylation. Org. Geochem. 22, 885–891.

deNobili, M., 1999. Size exclusion chromatography of humic substances: limits, perspectives and prospectives. Soil Sci. 164, 825–833.

DiDonato, N., Chen, H., Waggoner, D., Hatcher, P.G., 2016. Potential origin and formation for molecular components of humic acids in soils. Geochim. Cosmochim. Acta 178, 210–222.

Enders, C., 1943a. Wie ensteht der Humus in der Natur. Die Chemie 56, 281–292.

Enders, C., 1943b. Uber den Chemismus den Huminsaurbildung unter physiologischen Bedungen. IV. Mitt. Die Role der Mikroorganismen bei den Humifizierunsvorgangen. Biochem. Z. 315, 259–292.

Enders, C., Fries, G., 1936. Zur Analogie von Melanoiden und Huminsauren. Kolloid Z. 76, 289–291.

Enders, C., Sigurdsson, S., 1948. Uber den Chemismus der bildung unter physiologischen Bindungen. VII Mitt. Biochem. Z. 318, 44–46.

Enders, C., Tschapek, M., Glane, R., 1948. Vergleichende Untersuchungen einer kolloider Eigenschaften von naturlichen Huminsauren und Synthetischen Melanoidenen. Kolloid Z. 110, 240–244.

Engebretson, R.R., von Wandruszka, R., 1998. Kinetic aspects of cation enhanced aggregation in aqueous humic acids. Environ. Sci. Technol. 32, 488–493.

Flaig, W., 1960. Comparative chemical investigation of soil humic compounds and model substances. Sci. Proc. R. Dublin Soc. 4, 49–62.

Flaig, W., Butelspacher, H., Reitz, E., 1975. Chemical composition and physical properties of humic substances. In: Gieseking, J.E. (Ed.), Soil Components, vol. 1. Springer, Berlin, pp. 1–211.

Gerke, J., 2010. Humic organic matter-Al(Fe)-phosphate complexes: an underestimated phosphate form in soils and source of plant available phosphate. Soil Sci. 175, 417–425.

Gerke, J., 2018. Concepts and misconceptions of humic substances as the stable part of soil organic matter: a review. Agronomy 8, 1–16.

Gillespie, A.W., Walley, F.L., Ross, A.S., Leinweber, P., Eckhardt, K.-U., Regier, T.Z., Blyth, R.I.R., 2011. Glomalin-related soil protein contains non-mycorrhizal-related heat-stable proteins, lipids and humic materials. Soil Biol. Biochem. 43, 766–777.

Gilli, G., Gilli, P., 2013. The nature of the hydrogen bond. In: Outline of a Comprehensive Bond Theory. Oxford University Press.

Green, N.W., McInnis, D., Hertkorn, N., Maurice, P.A., Perdue, E.M., 2015. Suwannee River natural organic matter: isolation of the 2R101N reference sample by reverse osmosis. Environ. Eng. Sci. 32, 38–44.

Haider, K., Martin, J.P., 1967. Synthesis and transformation of phenolic compounds by *Epicoccum nigrum* in relation to humic acid formation. Soil Sci. Soc. Am. Proc. 31, 766–772.

Haider, K., Martin, J.P., 1975. Decomposition of specifically carbon–14–labeled benzoic acid and cinnamic acid derivatives in soil. Soil Sci. Soc. Am. J. 39, 657–662.

Haider, K., Martin, J.P., Reitz, E., 1977. Decomposition in soil of ^{14}C-labeled coumaryl alcohols: free and linked into dehydropolymer and plant lignins and model humic acids. Soil Sci. Soc. Am. J. 41, 556–562.

Hatcher, P.G., Breger, I.A., Earl, W.L., 1981. Nuclear magnetic resonance studies of ancient buried wood. I. Observations on the origin of coal to the brown coal stage. Org. Geochem. 3, 49–55.

Hatcher, P.G., Lerch, H.E., Bates, A.K., Verheyen, T.V., 1989. Solid-state ^{13}C nuclear magnetic resonance studies of coalified gymnosperm xylem tissue from Australian brown coals. Org. Geochem. 14, 145–155.

Hayes, M.H.B., 1960. Subsidence and Humification in Peats. Ph.D Dissertation, The Ohio State University.

Hayes, M.H.B., 1985. Extraction of humic substances from soil. In: Aiken, G.R., McKnight, D.M., Wershaw, R.L., MacCarthy, P. (Eds.), Humic Substances in Soil, Sediment and Water. Wiley, New York, pp. 329–362.

Hayes, M.H.B., 2009. Evolution of concepts of environmental natural non-living organic matter. In: Senesi, N., Xing, B., Huang, P.M. (Eds.), IUPAC Series on Biophysico-Chemical Process in Environmental Systems. Vol 2 Biophysico-chemical Process involving Natural Nonliving Organic Matter in Environmental Systems. Wiley, New York, pp. 1–39.

Hayes, M.H.B., Swift, R.S., 1978. The chemistry of soil organic colloids. In: Greenland, D.J., Hayes, M.H.B. (Eds.), The Chemistry of Soil Constituents. Wiley, Chichester, pp. 175–320.

Hayes, M.H.B., Swift, R.S., 1990. Genesis, isolation, composition and structures of soil humic substances. In: DeBoodt, M.F., Hayes, M.H.B., Herbillon, A. (Eds.), Soil Colloids and Their Associations in Aggregates. Plenum, New York and London, pp. 245–305.

Hayes, M.H.B., Swift, R.S., 2016. Is Humin the Same Everywhere? 01–3 Presentation, Proceedings of the 18th International IHSS Conference. Kanazawa, Japan.

Hayes, T.M., Hayes, M.H.B., Skjemstad, J.O., Swift, R.S., 2008. Compositional relationships between organic matter in a grassland soil and its drainage waters. Eur. J. Soil Sci. 59, 603–616.

Hayes, T.M., Hayes, M.H.B., Swift, R.S., 2012. Detailed investigation of organic matter components in extracts and drainage waters from a soil under long term cultivation. Org. Geochem. 52, 13–21.

Hayes, M.H.B., Mylotte, R., Swift, R.S., 2017. Humin: its composition and importance in soil organic matter. Adv. Agron. 143, 47–138.

Hedges, J.I., Eglinton, G., Hatcher, P.G., Kirchman, D.L., Arnosti, C., Derenne, S., Evershed, R.P., Kögel-Knabner, I., de Leeuw, J.W., Littke, R., Michaelis, W., Rullkötter, J., 2000. The molecularly-uncharacterized component of nonliving organic matter in natural environments. Org. Geochem. 31, 945–958.

Hollman, F., Arends, I.W.C., 2012. Enzyme initiated radical polymerisations. Polymers 4, 759–793.

Jenkinson, D.S., 1981. The fate of plant and animal residues in soil. In: Greenland, D.J., Hayes, M.H.B. (Eds.), The Chemistry of Soil Processes. Wiley, Chichester, pp. 505–561.

Jenkinson, D.S., 1990. The turnover of organic carbon and nitrogen in soil. Philos. Trans. R. Soc. B 329, 361–368.

Joivet, C., Arrouays, D., Leveque, J., Andreux, F., Chenu, C., 2003. Organic carbon dynamics in soil particle size separates of sandy spodosols when forest is cleared for maize cropping. Eur. J. Soil Sci. 54, 257–268.

Jong-Rok, J., Baldrianm, P., Murugesan, K., Chang, Y.-S., 2012. Laccase-catalysed oxidations of naturally occurring phenols: from *in vivo* biosynthetic pathways to green synthetic applications. J. Microbial. Biotechnol. 5, 318–332.

Kappler, A., Wuestner, M.L., Ruecker, A., Harter, J., Halama, M., Behrens, S., 2014. Biochar as an electron shuttle between bacteria and Fe(III) minerals. Environ. Sci. Technol. Lett. 1, 339–344.

Kelleher, B.P., Simpson, A.J., 2006. Humic substances in soils: are they really distinct? Environ. Sci. Technol. 40, 4605–4611.

Kenworthy, I.P., Hayes, M.H.B., 1997. Investigations of some structural properties of humic substances by fluorescence quenching. In: Hayes, M.H.B., Wilson, W.S. (Eds.), Humic Substances, Peats, and Sludges. Health and Environmental Aspects. The Royal Society of Chemistry, Cambridge, pp. 39–45.

Kleber, M., 2010. What is recalcitrant soil organic matter? Environ. Chem. 7, 320–332.

Kleber, M., Lehmann, J., 2019. Humic substances extracted by alkali are invalid proxies for the dynamics and functions of organic matter in terrestrial and aquatic ecosystems. J. Environ. Qual. 48, 207–216.

Klotzbucher, T., Kaiser, K., Guggenberger, G., Gatzek, C., Kalbitz, K., 2011. A new conceptual model for the fate of lignin in decomposing plant litter. Ecology 92, 1052–1062.

Klupfel, L., Keiluweit, M., Kleber, M., Sander, M., 2014. Redox properties of plant-biomass derived black carbon (biochar). Environ. Sci. Technol. 48, 5601–5611.

Knicker, H., 2007. How does fire affect the nature and stability of soil organic nitrogen and carbon? A review. Biogeochemistry 85, 91–118.

Knicker, H., Hischer, A., Gonzales-Vila, F.J., Almendros, G.A., 2008. A new conceptual model for the structural properties of char produced during vegetation fires. Org. Geochem. 39, 935–939.

Kononova, M.M., 1966. Soil organic matter. In: Its Nature, its Role in Soil Formation and in Soil. Pergamon Press, Oxford.

Kononova, M.M., 1975. Humus of virgin and cultivated soils. In: Gieseking, J.E. (Ed.), Soil Components, vol. 1. Springer Verlag, Berlin, pp. 475–526.

Lal, R., 2004. Soil carbon sequestration to mitigate climate change. Geoderma 123, 1–22.

Lal, R., 2014. Soil carbon sequestration impacts on global climate change and food security. Science 304, 1623–1627.

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

Lehmann, J., Solomon, D., Kinyangi, J., Dathe, L., Wirick, S., Jacobsen, C., 2008. Spatial complexity of soil organic matter forms at nanometer scales. Nat. Geosci. 1, 238–242.

Lorenz, K., Lal, R., Preston, C.M., Nierop, K.G., 2007. Strengthening the soil organic carbon by increasing contributions from recalcitrant aliphatic bio(macro)molecules. Geoderma 142, 1–10.

Loveland, P., Webb, J., 2003. Is there a critical level of organic matter in the agricultural soils of temperate regions?: a review. Soil Tillage Res. 70, 1–18.

Lützow, M.V., Kogel Knabner, I., Ekshmitt, K., Matzner, E., Guggenberger, G., Marschner, B., Flessa, H.B., 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.

Maillard, L.C., 1916. Synthes des matieres humique par action des acides amines sur les sucres reductours. Ann. Chim. 5, 258–317.

Maillard, L.C., 1917. Identite des matieres humiques de synthese avec les matieres humiques naturelles. Ann. Chim. 7, 113–152.

Malcolm, R.L., MacCarthy, P., 1992. Quantitative evaluation of the XAD-8 and XAD-4 resins used in tandem for removing organic solutes from water. Environ. Int. 18, 597–607.

Martin, J.P., Haider, K., 1969. Phenolic polymers of *Stachybotrys atra, Stachybotrys chartarum*, and *Epicoccum nigrum* in relation to humic acid formation. Soil Sci. 107, 260–270.

Martin, J.P., Haider, K., 1971. Microbial activity in relation to soil humus formation. Soil Sci. 111, 54–63.

Martin, J.P., Haider, K., 1976a. Decomposition of specifically carbon-14-labeled ferulic acid: free and linked into model humic-acid type polymers. Soil Sci. Soc. Am. J. 40, 377–380.

Martin, J.P., Haider, K., 1976b. Decomposition of specifically ^{14}C labelled DHP and corn stalk lignins, model humic acid–type polymers, and coniferyl alcohols. In: Proc. of the IAEA–FAO Agrochem. Symp. (Braunschweig) 2, pp. 23–32.

Martin, J.P., Richards, S.J., Haider, K., 1967. Properties and decomposition and binding action in soil of 'humic acid' synthesized by *Epicoccum nigrum*. Soil Sci. Soc. Am. Proc. 31, 657–662.

Martin, J.P., Haider, K., Wolf, D., 1972. Synthesis of phenols and phenolic polymers by *Hendersonula toruloideain* relation to humic acid formation. Soil Sci. Soc. Am. Proc. 36, 311–315.

Martin, J.P., Haider, K., Saiz-Jimenez, C., 1974. Sodium amalgam reductive degradation of fungal and model phenolic polymers, soil humic acids and simple phenolic compounds. Soil Sci. Soc. Am. Proc. 38, 760–765.

Martin, J.P., Haider, K., Bondietti, E., 1975. Properties of model humic acids synthesized by phenoloxidase and autoxidation of phenols and other components formed by soil fungi. In: Proceedings of the International Meeting Humic Substances (Wageningen, the Netherlands), pp. 171–186.

McCown, R.L., Hammer, G.L., Hargreaves, J.N.G., Holzworth, D.P., Freebairn, D.H., 1996. APSIM a novel software system for model development, model testing and simulation in agricultural systems research. Agr. Syst. 74, 255–271.

Mylotte, R., Verheyen, T.V., Reynolds, A., Dalton, C., Patti, A.F., Chang, R.R., Burdon, J., Hayes, M.H.B., 2015. Isolation and characterisation of recalcitrant organic components from an estuarine sediment core. J. Soil. Sediment 15, 211–224.

Mylotte, R., Sutrisno, A., Farooq, H., Masoom, H., Soong, R., Hayes, M.H.B., Simpson, A.J., 2016. Insights into the composition of recalcitrant organic matter from four estuarine sediments using NMR spectroscopy. Org. Geochem. 98, 155–165.

Nebbioso, A., Piccolo, A., 2011. Basis of a humeomics science: chemical fractionation and molecular characterization of humic biosuprastructures. Biomacromolecules 12, 1187–1199.

Nebbioso, A., Piccolo, A., 2012. Advances in humeomics: enhanced structural identification of humic molecules after size fractionation of a soil humic acid. Anal. Chim. Acta 720, 77–90.

Nebbioso, A., Piccolo, A., Lambschift, M., Spiteller, M., 2014. Molecular characterization of an end-residue of humeomics applied to a soil humic acid. RSC Adv. 4, 23658–23665.

Newman, D.K., Kolter, R., 2001. A role for extracted quinones in electrocellular electron transfer. Nature 405, 94–97.

Olk, D.C., Bloom, P.R., de Nobili, M., Chen, Y., McKnight, D.M., Wells, M.J.M., Weber, J., 2019a. Using humic fractions to understand natural organic matter processes in soil and water: selected studies and applications. J. Environ. Qual. 48, 1633–1643.

Olk, D.C., Bloom, P.R., Perdue, E.M., McKnight, D.M., Chen, Y., Farenhorst, A., Senesi, N., Chin, Y.-P., Schmitt-Koplin, P., Hertkorn, N., Harir, M., 2019b. Environmental and agricultural relevance of humic fractions extracted by alkali from soils and natural waters. J. Environ. Qual. 48, 217–232 and 1126 (for correction).

Parton, W.J., Powlson, D.S., Smith, P., Smith, J.U., 1996. The CENTURY model. In: Evaluation of Soil Organic Matter Models. NATO ASI Series (Series I: Global Environmental Change), vol. 38. Springer, Berlin.

Piccolo, A., 2002. The supramolecular structure of humic substances. A novel understanding of humus chemistry and implications in soil science. Adv. Agron. 75, 57–134.

Piccolo, A., Spaccini, R., Cozzolino, V., Nuzzo, A., Drosos, M., Zavattaro, L., Grignani, C., Puglisi, E., Trevisan, M., 2018. Effective carbon sequestration in Italian agricultural soils by *in situ* polymerization of soil organic matter under biomimetic photocatalysis. Land Degrad. Dev. 29, 485–494.

Ranatunga, K., Hill, M.J., Probert, M.E., 2001. Comparative application of APSIM, RothC and century to predict soil carbon dynamics. In: MODSIM, vol: 2. Natural Systems (Canberra, Australia 2001), pp. 33–738.

Richter, M., Schulenburg, C., Jankowska, D., Heck, T., Faccio, G., 2015. Novel materials through nature's catalysts. Mater. Today 18, 459–467.

Ritchie, J.D., Perdue, E.M., 2003. Proton-binding study of standard and reference fulvic acids, humic acids, and natural organic matter. Geochim. Cosmochim. Acta 67, 85–96.

Sanderman, J., Fillery, I.R.P., Jongepier, R., Massalsky, A., Roper, M.M., Macdonald, L., Maddern, T., Murphy, D.V., Baldock, J.A., 2013. Carbon sequestration under subtropical perennial pastures II: carbon dynamics. Soil Res. 51, 771–780.

Schmitt-Kopplin, P., Junkers, J., 2003. Capillary zone electrophoresis of natural organic matter. J. Chromatogr. A 998, 1–20.

Schnitzer, M., Khan, S.U., 1972. Humic Substances in the Environment. Marcel Dekker, New York.

Schnitzer, M., Khan, S.U., 1978. Soil Organic Matter. Elsevier, New York.

Schuffelen, A.C., Bolt, G.H., 1950. Some notes on the synthesis of humic compounds. In: Landbouwk. Tijdschr. 62, No. 4/5.

Serkiz, S.M., Perdue, E.M., 1990. Isolation of dissolved organic matter from the Suwannee River using reverse osmosis. Water Res. 24, 911–916.

Skjemstad, J.O., Spouncer, L.R., Cowie, B., Swift, R.S., 2004. Calibration of the Rothamsted organic carbon turnover model (RothC version 26.3), using measurable soil organic carbon pools. Aust. J. Soil Res. 42, 79–88.

Skjemstad, J.O., Krul, E.S., Swift, R.S., Szarvas, S., 2008. Mechanisms of protection of soil organic matter under pasture following clearing of rainforest in an oxisol. Geoderma 143, 231–242.

Smith, P., Smith, J.U., Powlson, D.S., McGill, W.B., Arah, J.R.M., Chertov, O.G., Coleman, K., Franko, U., Frolking, S., Jenkinson, D.S., Jensen, L.S., Kelly, R.H., Klein-Gunnewiek, H., Komarov, A.S., Li, C., Molina, J.A.E., Mueller, T., Parton, W.J., Thornley, J.H.M., Whitmore, A.P., 1997. A comparison of the performance of nine soil organic matter models using datasets from seven long-term experiments. Geoderma 81, 153–225.

Solomon, D., Lehmann, J., Thies, J., Schafer, T., Liang, B., Kinyangi, J., Neves, E., Petersen, J., Luizao, F., Skjemstad, J., 2007. Molecular signature and sources of biological recalcitrance of organic C in Amazonian Dark Earths. Geochim. Cosmochim. Acta 71, 2285–2298.

Song, G., Novotny, E.H., Clapp, C.E., Hayes, M.H.B., 2008. Sequential exhaustive extractions and characterisations, using solid and solution state NMR, of the humic, including humin, components in a Mollisol soil. Eur. J. Soil Sci. 59, 505–516.

Song, G., Hayes, M.H.B., Novotny, E.H., Simpson, A.J., 2011. Isolation and fractionation of soil humin materials using alkaline urea and DMSO plus sulphuric acid. Naturwissenschaften 98, 5–13.

Sun, Q., Weimin, D., Yuncong, L., Guodong, L., Jiuai, S., Qishuo, D., 2015. Characterization of humic acids derived from leonardite using a solid-state NMR spectroscopy and effects of humic acids on growth and nutrient uptake of snap bean. Chem. Spec. Bioavailab. 27, 156–161.

Swift, R.S., 1985. Fractionation of soil humic substances. In: Aiken, G.R., McKnight, D.M., Wershaw, R.L., MacCarthy, P. (Eds.), Humic Substances in Soil, Sediment and Water. Wiley, New York, pp. 387–429.

Swift, R.S., 1996. Organic matter characterization. In: Sparks, D.L. (Ed.), Methods of Soil Analysis, Part 3. Chemical Methods, Book Series No. 5, Soil Science Society of America, Madison, WI, pp. 1011–1069.

Swift, R.S., 1999. Macromolecular properties of soil humic substances: fact, fiction and opinion. Soil Sci. 164, 790–802.

Swift, R.S., 2001. Sequestration of carbon by soil. Soil Sci. 166, 858–871.

Swift, R.S., Posner, A.M., 1972. Autoxidation of humic acid under alkaline conditions. J. Soil Sci. 23, 381–393.

Swift, R.S., Leonard, R.L., Newman, R.H., Theng, B.K.G., 1992. Changes in humic acid composition with molecular weight as detected by ^{13}C-nuclear magnetic resonance spectroscopy. Sci. Total Environ. 117, 53–61.

Waggoner, D.C., Chen, H., Willoughby, A.S., Hatcher, P.G., 2015. Formation of black carbon-like and alicyclic aliphatic compounds by hydroxyl radical initiated degradation of lignin. Org. Geochem. 82, 69–76.

Waksman, S.A., 1936. Humus: Origin, Chemical Composition, and Importance in Nature. Williams & Wilkins, Baltimore.

Wallis, P., Booth, K., Patti, A.F., Scott, J.L., 2006. Oxidative recoupling revisited. Solvent free, heterogeneous and in water. Green Chem. 8, 333–337.

Wells, M.J.M., Stretz, H.A., 2019. Supramolecular architectures of natural organic matter. Sci. Total Environ. 671, 115–1133.

Wershaw, R.L., 1999. Molecular aggregation of humic substances. Soil Sci. 64, 803–813.

Ziechmann, W., 1980. Huminstoffe. Verlag Chemie, Weinheime, Germany.