

Environmental and Agricultural Relevance of Humic Fractions Extracted by Alkali from Soils and Natural Waters

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Abstract

To study the structure and function of soil organic matter, soil scientists have performed alkali extractions for soil humic acid (HA) and fulvic acid (FA) fractions for more than 200 years. Over the last few decades aquatic scientists have used similar fractions of dissolved organic matter, extracted by resin adsorption followed by alkali desorption. Critics have claimed that alkali-extractable fractions are laboratory artifacts, hence unsuitable for studying natural organic matter structure and function in field conditions. In response, this review first addresses specific conceptual concerns about humic fractions. Then we discuss several case studies in which HA and FA were extracted from soils, waters, and organic materials to address meaningful problems across diverse research settings. Specifically, one case study demonstrated the importance of humic substances for understanding transport and bioavailability of persistent organic pollutants. An understanding of metal binding sites in FA and HA proved essential to accurately model metal ion behavior in soil and water. In landscape-based studies, pesticides were preferentially bound to HA, reducing their mobility. Compost maturity and acceptability of other organic waste for land application were well evaluated by properties of HA extracted from these materials. A young humic fraction helped understand N cycling in paddy rice (*Oryza sativa* L.) soils, leading to improved rice management. The HA and FA fractions accurately represent natural organic matter across multiple environments, source materials, and research objectives. Studying them can help resolve important scientific and practical issues.

Core Ideas

- Humic substances have long been extracted from soils, waters, and organic materials.
- Their chemical composition has well represented that of natural organic matter.
- Compost maturation and composition are well represented by their properties.
- Soil humic studies elucidated metal and organic xenobiotic binding and nutrient cycling.
- Their quantities and composition in soil respond to field treatments.

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FOR MORE THAN 200 years, natural organic matter (NOM) has been extracted from soil through alkali extraction to obtain fractions for quantitative and qualitative analyses. Scientists most commonly use sodium hydroxide (NaOH) for alkali extraction but also use potassium hydroxide (KOH) and sodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7$). Alkali extraction can remove as much as 80% of soil organic matter (SOM) from a mineral soil (Stevenson, 1982). Orlov (1985) summarized the humic acid (HA) and fulvic acid (FA) contents of 200 samples of sub-Podzolic soils by stating that alkali extractions recovered $57 \pm 15\%$ of total SOM. Alkali extractions have been used to address several research objectives. Perhaps the best known has been the elucidation of the chemical structure of NOM, often using wet chemical and instrumental methods that are incompatible with the inorganic soil matrix, thus requiring separation of the organic component of NOM.

Scientists who work with alkali extracts commonly divide SOM into three major fractions: HA, which is solubilized by alkali but remains insoluble in subsequent acidification; FA, which has been historically defined as the material soluble in both alkali and acid; and humin, which is the fraction that is not extracted by alkali. Both the HA and FA fractions can be considered as *humic substances* (HS). More recently the International Humic Substances Society has defined FA as the material that is soluble in both alkali and acid and that will adsorb to a nonionic resin, distinguishing it from a NOM fraction that is very hydrophilic even at low pH and does not

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Abbreviations: DOM, dissolved organic matter; FA, fulvic acid; HA, humic acid; HPSEC, high-performance size exclusion chromatography; HS, humic substances; MHA, mobile humic acid; NMR, nuclear magnetic resonance; NOM, natural organic matter; POP, persistent organic pollutant; RO, reverse osmosis; SEC, size exclusion chromatography; SHM, Stockholm humic model; SOM, soil organic matter.

adsorb on the resin (Swift, 1996; IHSS, 2018b). The resin sorption step to purify FA was adopted from the procedure for isolating HA and FA from natural waters, discussed below. Note that HA and FA fractions are defined operationally, not by chemical composition.

Aquatic scientists extract NOM from natural waters to provide concentrated samples for the study of the structure and behavior of aquatic NOM. Scientists often need well-characterized, freeze-dried samples that can be easily stored, transported, and used to prepare concentrated solutions that are required for some types of analysis, such as nuclear magnetic resonance (NMR) spectroscopy. The average quantity of NOM in river waters is only about 20 mg L⁻¹ of NOM (9.6 mg C L⁻¹) (Perdue and Ritchie, 2014). Concentration by rotary evaporation or reverse osmosis (RO) can be useful, but this also concentrates salts, which can interfere with chemical analyses and is especially problematic in hard water systems. As an alternative to concentration, various sorbents such as silica gel, alumina, and activated carbon had been used for HA and FA extraction from natural waters before it was discovered that macroporous resins with low polarity are effective sorbents. The method currently used by most scientists involves the use of XAD-8 resin (Thurman and Malcolm, 1981; Aiken, 1985), which is also used to separate FA from the hydrophilic fraction in NaOH soil extracts (Swift, 1996). Though used extensively in the past, this moderately polar macroporous poly(methylmethacrylate) resin is no longer commercially available. Supelite DAX-8 (Sigma-Aldrich), which has the same chemistry and similar porosity, can be substituted for XAD-8 (Peuravuori et al., 2002; Chow, 2006).

In the XAD-8/DAX-8 method, filtered water is acidified to pH 2 with HCl and passed through a column of resin, to which SOM components are adsorbed. The HA and FA are subsequently desorbed from the column with 0.1 M NaOH, followed by separation of FA from HA after acidification to pH 1, then by desalting and freeze-drying (Swift 1996). The XAD-8/DAX-8 method is used by the International Humic Substances Society to prepare standard and reference samples of aquatic HA and FA (IHSS, 2018a).

Over the many years that strong alkali has been used in HS studies, critics have periodically expressed concern that alkali-extractable fractions are laboratory artifacts and are not suitable to accurately study NOM processes in field conditions (Schnitzer and Khan, 1972; Orlov, 1985; Lehmann and Kleber, 2015). As part of renewed debate, this report addresses the question: Does the study of humic substances extracted by alkali advance our understanding of the dynamics and function of organic matter in terrestrial and aquatic ecosystems? In other words, does humic-based research enable accurate identification of processes for resolving important scientific issues and practical problems in water and soil systems? In response, this review first responds to conceptual criticisms of alkaline extractions and then provides selected examples of published humic studies that have generated useful knowledge. These examples address environmental issues in both water and soil systems, as well as agricultural issues in soil. They are a representative selection, not a comprehensive review, of published studies involving alkali extractions and humic fractions.

General Justification for Alkali Extractions

Before presenting specific applications of humic fraction-based research, we first respond to three general criticisms of alkali extractions that have led some scientists to presume that humic fractions cannot represent in situ NOM.

The first criticism is the potential for chemical alteration during alkali extraction. This appears a valid concern, but has thorough evidence for substantial alterations been presented? A point of debate should be the extent of its alteration—although the extent has seldom been quantified, much less has its specific impact on the results and their interpretations been discussed. Here, we begin to address the extent of alteration. An important condition and widespread practice for minimizing chemical alteration is for alkali extractions to be performed under inert atmospheres, such as nitrogen gas (N₂), or otherwise protected from all but brief contact with air.

Summarizing earlier work, Schnitzer and Khan (1972) noted that (i) the optical properties of HA extracted from soils with 0.5 M NaOH and 1 M NaF were the same, (ii) carboxyl contents and bulk elemental compositions of HA extracted from soils with 0.4 M NaOH and 1 M sodium fluoride (NaF) were essentially the same, (iii) HA extracted from soil with Na₄P₂O₇ and NaOH were very similar in all respects, and (iv) FA extracted from soil using water or NaOH had identical properties. Schnitzer and Skinner (1968) reported that FA extracted from a Podzolic soil with 0.5 M NaOH (under N₂) or with 0.1 M hydrogen chloride (HCl) had very similar (i) bulk elemental compositions, (ii) contents of oxygen-containing functional groups, (iii) infrared spectra, and (iv) molecular weight distribution by gel permeation chromatography. They concluded that claims of alteration during extraction with NaOH were exaggerated. Citing earlier work, Orlov (1985) summarized that soil FA recovered from water without alkali extraction are very similar in their properties to FA extracted by alkali from soil, that FA extracted from organic materials by water and by NaOH are similar, and that the visible reflectance spectra of humus-rich soils almost completely reproduced the shape and intensity of the reflectance spectra of soil HA.

In more recent studies, HA extracted from six uncultivated soils had similar chemical characteristics (as measured by ¹³C NMR analysis) as did the <1.6 Mg m⁻³ (occluded) density fraction of aggregates (Golchin et al., 1994). The HA fraction, the <1.6 Mg m⁻³ density fraction, and four other density fractions all reflected the general chemical nature (e.g., degree of aromaticity vs. aliphaticity) for each of the six soils. Pyrolysis-field ionization mass spectroscopy identified the same 10 classes of biochemical compounds in a soil that were also found in its HA, FA, and humin fractions (Schnitzer and Monreal, 2011). Relative abundances of each class were identical across all three fractions and SOM for 8 of the 10 classes, although abundances differed for specific compounds. Olk et al. (2002) also used pyrolysis-mass spectrometry to demonstrate that an increasing number of annual paddy rice (*Oryza sativa* L.) crops affected classes of biochemical compounds similarly in two HA fractions as in SOM, including trends in lignin phenol residues, fatty acids, and heterocyclic N. An accumulation of lignin phenols with increasing paddy rice cropping was shown for both HA fractions and

SOM by ^{13}C NMR and cupric oxide (CuO) oxidation, respectively (Olk et al., 1996). The ^{13}C NMR spectra of these two HA fractions, in coordination with spectra of the light fraction and particulate organic matter, approximated the spectrum for SOM in a corn (*Zea mays* L.)–soybean [*Glycine max.* (L.) Merr.] soil, lacking only the aliphatic C peak of SOM that can be attributed to the unextracted humin (Cao et al., 2011). Finally, Peuravuori et al. (2006) reported relatively minor differences between an HA fraction and its source lignite ore, as measured by ultraviolet-visible, ^1H NMR, ^{13}C NMR, and Fourier-transform infrared spectroscopies.

In contrast to the widespread use of inert atmospheres for alkali extractions, modification of humic substances by exposure to NaOH under oxygenated atmospheres is well known, and one example is given here. Swift and Posner (1972) found that exposure of a peat HA to oxygen gas (O_2) in a 1 M NaOH solution for 30 d resulted in the conversion of almost half of the HA to FA and other low-molecular-weight compounds and the release of a small amount of carbon dioxide (CO_2). Also, amino acid N was lost from the HA. Under a N_2 atmosphere over 30 d, however, very little C was oxidized to CO_2 , the conversion to FA and other low molecular weight compounds was only about 15% of the original HA, and much less amino N was lost from the HA than under O_2 . Using a shorter extraction time (24 h) that is more representative of soil HS studies, Swift and Posner (1972) found that HA was not significantly altered by exposure to 1 M NaOH under N_2 . The more typical conditions for extraction of HA and FA from soils are yet milder, with 0.1 M NaOH being the most widely used extractant.

Strongly alkali solutions can also hydrolyze esters in NOM, increasing the concentration of carboxylic acid groups. Ritchie and Perdue (2008) studied titration hysteresis with four standard FAs and two standard HAs from the International Humic Substances Society. Hysteresis is evident when titration curves (plots of organic charge density vs. pH) for forward and reverse titrations are not superimposable. These acid samples were titrated from approximately pH 3 to 10.5 with NaOH under N_2 , and after waiting either 30 min or 24 h, they were back-titrated with HCl. In all cases, the organic charge density at pH 8 was used to estimate carboxyl content. The concentrations of carboxyl groups in the samples increased by 13 to 18% during the 24-h period between the two reverse titrations. These observations are generally consistent with those of other studies of hysteresis (Bowles et al., 1989; Antweiler, 1991; Leenheer et al., 1995; Sierra et al., 2004).

More recent data for samples taken from the Suwannee River (Georgia, USA) further illustrate that exposure to NaOH does not greatly alter NOM (Perdue et al., unpublished data). The samples were collected from the Suwannee River, a blackwater stream with very high concentrations of dissolved organic C, from 30 to 80 mg dissolved organic C L^{-1} , that is highly acidic, with $\text{pH} < 4$. A variety of related samples was collected in 2003, including (i) the original Suwannee River water (unprocessed); (ii) RO-NOM (concentrated 21-fold by reverse osmosis, RO, in 2003; (iii) HA and FA (collected using the XAD-8 method, with completion of laboratory processing in 2004); and (iv) hydrophilic fraction (dissolved organic matter [DOM] that failed to adsorb to XAD-8 resin at pH 2).

These samples were obtained at the site where the Suwannee River drains from the Okefenokee Swamp, where the International Humic Substances Society isolates standard and reference samples of HA, FA, and RO-NOM for distribution. The DOM in the Suwannee River is largely derived from local soils by leaching with meteoric waters at non-alkaline pH. The RO fraction was isolated directly using RO and contains almost all the NOM species that occur in the water. This fraction was isolated with no involvement of NaOH or HCl and can be compared with its corresponding HA and FA fractions that were isolated by adsorption–desorption using columns of XAD-8/DAX-8 resins, which involves NaOH and HCl.

Exact molecular masses of many thousands of compounds in each of the above samples were determined using electrospray ionization Fourier-transform ion cyclotron resonance mass spectrometry, and CHOFIT software was used to assign molecular formulas to exact masses (Green and Perdue, 2015). Only molecular formulas containing C, H, O, and heteroatoms N (0–6 atoms) and S (0–2 atoms) were included in the calculation of formulas. Data for the number of formulas calculated from mass spectra illustrate the great diversity in the composition of HS (Table 1).

For comparison of the dominant peaks in mass spectra of different samples, the “Gold Standard” was defined as the subset of molecular formulas to which both the ^{12}C and ^{13}C isotopologues were assigned independently (Table 1). The Gold Standard requirement tends to identify the molecular formulas of relatively intense peaks in a mass spectrum because of the low natural abundance of ^{13}C . Thus, Gold Standard molecular formulas were assigned to $14.3 \pm 5.9\%$ of the fitted peaks, while they accounted for $26.0 \pm 13.9\%$ of fitted peak intensity.

Using only Gold Standard molecular formulas, pairwise comparisons were made between two samples by computing the ratio of the number of identical molecular formulas common to both samples to the number of molecular formulas for the sample with the lesser number of molecular formulas. When two samples have different numbers of molecular formulas, the lower number is the upper limit for the number of shared formulas. The comparison ratio is thus constrained to be in the range of 0 to 1. For example, for the 2480 molecular formulas in the original river water and the 1760 molecular formulas in the RO concentrate (RO-NOM), 1566 molecular formulas are found in both samples (0.89). It follows that the RO process is largely conservative.

Table 1. Formulas identified by Fourier-transform ion cyclotron resonance.

Sample	Total fit formulas†	Fit intensity‡	Gold CHOS§	Gold NS¶
River water	13,308	6.27E+10	2460	20
RO-NOM	11,278	4.15E+10	1726	34
2004 fulvic acid	16,340	1.15E+11	3400	24
2004 humic acid	9,794	2.29E+10	724	22
Hydrophilic fraction	10,967	3.73E+10	902	68

† Total fit formulas = the number of formulas assigned by CHOFIT.

‡ Fit intensity = sum of intensity of all fit formulas, arbitrary units.

§ Gold CHO = Gold Standard formulas composed only of elements C, H, and O.

¶ Gold NS = Gold Standard formulas that also contain elements N and S.

Table 2. Similarity of Fourier-transform ion cyclotron resonance peaks for samples from the Suwannee River. Pairwise comparisons were made between two samples by computing the ratio of the number of identical Gold Standard formulas common to both samples to the number of Gold Standard formulas for the sample with the lesser number of Gold Standard formulas.

	River water	Reverse osmosis natural organic matter	Humic acid	Fulvic acid	Hydrophilic fraction
River water	x				
Reverse osmosis natural organic matter	0.89	x			
2004 fulvic acid	0.80	0.66	x		
2004 humic acid	0.85	0.92	0.88	x	
Hydrophilic fraction	0.84	0.84	0.32	0.78	x

Table 2 contains comparison ratios for all pairwise combinations of the five samples. Comparison ratios for river water with RO-NOM, HA, FA, and hydrophilic fraction ranged from 0.80 to 0.89—a high degree of similarity that argues strongly against significant modification of NOM by NaOH and/or HCl when HA, FA, and hydrophilic NOM are obtained via the XAD-8/DAX-8 method. The RO-NOM has the greatest similarity to river water (comparison ratio = 0.89). As expected, the hydrophilic fraction was rather different from HA, with a comparison ratio of 0.32, while it was more similar to the FA, with a comparison ratio of 0.78. Somewhat unexpectedly, the comparison ratio for HA and RO-NOM was only 0.66.

Overall, the analysis of Fourier-transform ion cyclotron resonance mass spectroscopic results demonstrates that very minor chemical modification of NOM is caused by RO or by isolation of HA and FA using XAD-8 resin, NaOH, and HCl.

Given these findings, it may be assumed that the molecular formulas in detected HA, FA, and the hydrophilic fraction are also in the original water. This possibility is demonstrated graphically using two van Krevelen plots of H/C versus O/C atomic ratios. Figure 1 shows all Gold Standard molecular formulas that are found in the original river water and also in the HA, FA, and/or the hydrophilic fraction. The black symbols represent molecular formulas containing only C, H, and O, including ^{13}C . The red symbols represent molecular formulas that also contain N and/or S. Figure 2 shows all Gold Standard molecular formulas that are found in the original river water but not in HA, FA, or hydrophilic fraction.

Clearly, nearly all Gold Standard molecular formulas contain only C, H, and O. It is also evident that 89.2% ($100 \times [(2208 + 4)/(2208 + 4 + 252 + 16)]$) of the molecular formulas in river water are also found in at least one of the three fractions (HA, FA, hydrophilic NOM). This value is quite similar to the corresponding comparison ratios for this subset of samples. These plots demonstrate visually that most of the molecular formulas in river water appear in at least one of the fractions.

The fact that humic fractions approximated trends in NOM composition in the numerous studies cited above indicates that for soil and aquatic HS fractions extracted using currently recommended procedures, the recovered materials can reasonably depict NOM. We conclude that some, probably minor, modifications of HA and FA are likely caused by exposure to alkali

under a N_2 gas atmosphere, but by themselves, such minor modifications are not grounds for dismissing humic fractions as irrelevant for studies of NOM behavior in field conditions.

A second—and confusing—criticism by Kleber and Johnson (2010) and Lehmann and Kleber (2015) presumes that use of alkali extractions is necessarily bound to belief in a humic macromolecular structure. This structure has been popular among humic chemists, but already several decades ago practitioners of alkali extractions debated multiple other models for NOM

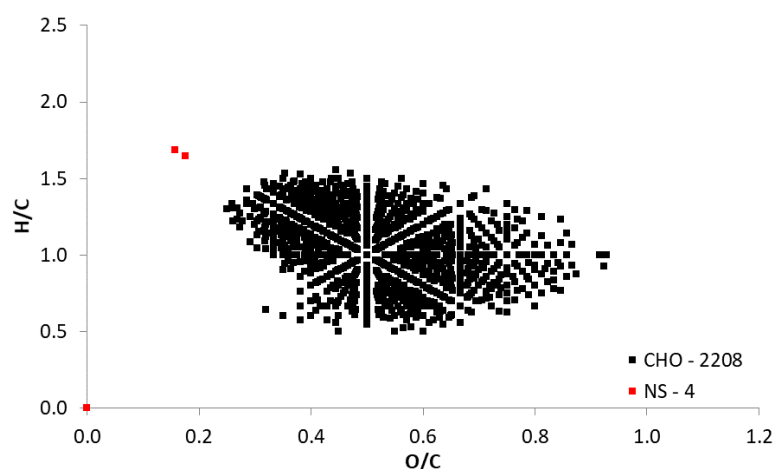


Fig. 1. Van Krevelen diagram showing Gold Standard molecular formulas found in river water and in 2004 humic acid, 2004 fulvic acid, and/or 2014 hydrophilic compounds. CHO: molecular formulae containing only C, H, and O. NS: molecular formulae also containing N and/or S.

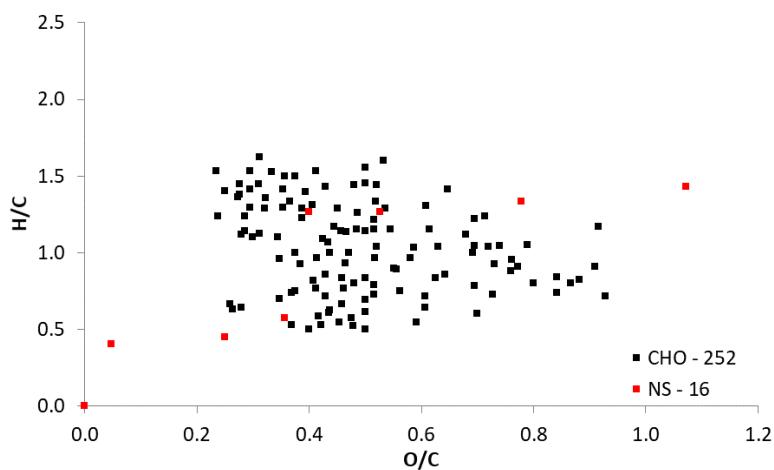


Fig. 2. Van Krevelen diagram showing Gold Standard molecular formulas found in river water but not in the 2004 humic acid, 2004 fulvic acid, or 2014 hydrophilic compounds. CHO: molecular formulae containing only C, H, and O. NS: molecular formulae also containing N and/or S.

structure. These have included the classical model of a humic aromatic core (Haworth, 1971; Stevenson, 1982) but also more loosely arranged networks of phenols and acids (Schnitzer and Khan, 1972) or alkyl aromatics (Schulten and Schnitzer, 1997), ordered aggregates of amphiphilic molecules composed mainly of relatively unaltered plant polymer segments (Wershaw, 1993), self-assembling supramolecular units bound together by hydrogen and hydrophobic bonds (Piccolo, 2001; Sutton and Sposito, 2005), mixtures of condensed domains and expanded domains (Xing and Chen, 1999), and no regular structure at all—that is, various components are randomly bound together into super mixtures (MacCarthy, 2001). Such compositional complexity is compounded by the multitude of chemical mechanisms known to stabilize SOM components through bonding with other organic components, to soil mineral surfaces, or to multivalent cations. They include H bonding, van der Waals forces, ionic attraction, and coordination complexes (Stevenson, 1994).

Molecular weight data are useful for describing the complexity of the composition of humic substances. Both number-average (M_n) and weight-average (M_w) molecular weights are often reported in the literature. Only M_n values may be determined using colligative methods (e.g., cryoscopy and vapor pressure osmometry). Noncolligative methods (e.g., size exclusion chromatography [SEC], flow field-flow fractionation, ultraviolet scanning ultracentrifugation, and molar absorptivity–ultraviolet spectrophotometry) provide more insight into distributions of molecular weight, from which both M_n and M_w are generally calculated (Stevenson, 1994). If there is any dispersity in molecular weights of a sample, M_w values will exceed M_n . Perdue and Ritchie (2014) summarized data for 130 samples of aquatic HA, FA, and RO-NOM (concentrated by reverse osmosis). The average M_n values of HA, FA, and RO-NOM were 1700, 834, and 1100 Da, and the average M_w values of HA, FA, and NOM were 3310, 1805, and 1590 Da. Recently, McAdams et al. (2018) reported M_n and M_w values that were determined using the most recent methods in high-performance size exclusion chromatography (HPSEC) for eight samples of aquatic FA. The average M_n and M_w values were 627 ± 181 Da and 1394 ± 468 Da, respectively. It is clear that aquatic HS contain little, if any, large macromolecular components, speaking against a large macromolecular structure.

Between 1988 and 2012, Perdue (unpublished data, 2018) isolated one sample of HA, three samples of FA, and six samples of RO-NOM from the Suwannee River, Georgia, for which M_n values were determined using vapor pressure osmometry. The average M_n values were 697, 717 ± 82 , and 727 ± 77 Da for the HA, FA, and RO-NOM, respectively. The similarity of M_n values confirms that average molecular weight was not modified by exposure to strong base and acid in the XAD-8/DAX-8 extraction procedure used for the HA and FA.

In comparison to aquatic HA, determination of the intrinsic molecular weight of soil HA has been much more difficult. Reported molecular weights for soil HA have varied from a few thousand to a million daltons (Stevenson, 1982, 1994). However, more recent studies suggest the older studies overestimated molecular weights of HS components. An HPSEC study of 33 soil HAs yielded M_n of 1347 ± 485 and M_w of $13,200 \pm 12,000$ Da. (Fujitake et al., 2012). The high apparent M_w values and high ratio of M_w to M_n , a measure of polydispersity, suggests

that soil HA is more polydisperse than are aquatic HA and FA, with a significant content of higher molecular weight components. Asakawa et al. (2011) also reported HPSEC data indicating M_n and M_w values for three soil HA that ranged from 2790 to 3610 and 5350 to 28,200, respectively, with polydispersity values ranging from 3.1 to 14.9. Fractionation of one soil HA using a preparatory SEC column produced 10 humic subfractions with a range of molecular weights but each with low polydispersity. When these separated subfractions were recombined, the subsequent HPSEC analysis of their combined material yielded chromatograms similar to that of the original sample. These HPSEC studies further demonstrate the wide range of molecular weights of HS, illustrating their chemical complexity and arguing against one unifying structure.

Sutton and Sposito (2005) reviewed evidence for the idea that soil HS are “collections of diverse, relatively low molecular mass components forming dynamic associations stabilized by hydrophobic interactions and hydrogen bonds.” Piccolo (2001), for example, found that the addition of acetic acid (CH_3COOH) can disrupt H bonds in a lignite HA and result in HPSEC chromatograms that show a higher content of smaller molecular weights than without acetic acid addition. However, the data also indicated a significant proportion of components with molecular weights of >100 kDa, even after treatment with acetic acid. Sutton and Sposito (2005) attributed this large fraction to identifiable biomolecules. We agree that plant and microbial biomolecules are important components of HS, adding to their chemical diversity.

In their discussion of the implications of the supramolecular model, Sutton and Sposito (2005) noted that soil humic substances typically possess average ^{14}C ages ranging from hundreds to thousands of years, while many biomolecule components are normally readily decomposed by microbes. They suggested that binding to humic fractions can result in protection from microbial degradation.

Lehmann et al. (2008) used carbon-synchrotron near edge X-ray fine structure spectrometry to study the distribution of four different classes of soil organic C in natural soil aggregates, and they found spatial segregation at the nanoscale. They were easily able to identify black carbon nanoparticles as areas very dense in aromatic C but low in the three other classes of C: aliphatic, carboxylic, and phenolic/pyrimidine or imidazole. They also identified a wide distribution of clusters containing different ratios of the four classes of C, including some areas with little or no aromatic C. This variability argues against SOM being predominantly large polymers of somewhat similar composition (Schmidt et al., 2011), but it is compatible with the argument that HS (and SOM) are composed of a variety of biomolecules and products of abiotic reactions.

The complexity of HS is further illustrated by Hertkorn et al. (2008), who compared molecular formulas found by electrospray ionization Fourier-transform ion cyclotron resonance mass spectrometry in FA from the Suwannee River with the theoretically possible formulas within a range of molecular weights, H/C, and O/C. Estimating from their figure 7, approximately 80% of all possible CHO formulas having molecular weight of 250 to 650 Da, H/C of 0.42 to 1.38, and O/C of 0.16 to 0.64 were found in the FA. This is an incredible level of complexity for a natural sample. These results confirm the futility of trying

to account for all observed properties of HS in a single average molecular “structure,” because those properties might in reality be distributed among thousands of compounds.

The molecular-structure debates will continue among practitioners of all NOM extraction procedures, while practical applications of humic fractions will proceed independently. Many researchers use alkali extractions to obtain representative NOM fractions to study NOM chemical structure, elemental cycling involving NOM, or its interactions with nutrients, metals, or pesticides. Successful application of their results is not contingent on the existence of a theoretical humic macromolecular structure, as is demonstrated in the case studies we present below.

Finally, a third criticism, related to the conjectured humic macromolecular structure, claims there is no evidence for the occurrence of “humification,” which would bring about the unique structures in soil not inherited directly from organisms, as advocated by Haworth (1971), Stevenson (1982, 1994), and others. Humification has been generally defined as an accumulation over time of lipid-derived alkyl compounds, aromatic compounds with carboxyl and phenolic substituents, along with depletion of carbohydrates and amino acids (Zech et al., 1997). Critics instead attributed the abundance of aromatic compounds in humic fractions to unwanted chemical alterations that occur during alkali extraction (Kleber and Johnson, 2010). Schmidt et al. (2011) and Lehmann and Kleber (2015) also argued that lignin, often presumed to be the long-term source of aromatic compounds, degrades as quickly in soil as do all other classes of biochemical compounds and therefore its degradation products cannot evolve into a long-lasting aromatic core of humic structures. Lehmann and Kleber (2015) proposed what they call the soil continuum model for the evolution of all soil C to CO₂, a model that does not involve preferential preservation of lignin-derived components or any abiotic reactions between NOM components.

In response to these criticisms, we point out that evidence for humification has been demonstrated in studies that do not involve alkali extractions. Zech et al. (1997) described its occurrence with increasing depth in litter-rich surface horizons of forest soils. During incubations of *Eucalyptus* litter for as short as 7 d, Skene et al. (1997) observed the formation of heterocyclic N; they hypothesized this occurrence was due to abiotic reactions between specific nitrogenous compounds with lignin and other polyphenolic compounds.

Bernal et al. (2009) reviewed the significance of humification in the assessment of compost maturation and noted the numerous studies that demonstrated humification in maturing composts. In particular, Chen (2003) reported several studies of ¹³C NMR and pyrolysis–gas chromatography–mass spectrometry that showed preferential degradation of carbohydrates during composting, resulting in relative enrichment of modified lignin residues. These trends have been reproduced in humic extractions of compost-amended soils (Plaza and Senesi, 2009). Bernal et al. (2009) also equated a humified nature of compost with its stability against microbial decomposition, often measured as diminished aerobic respiration. The authors considered the degree of humification as the most important measure of compost maturity for assessing the agricultural value of compost and underscored the widespread recognition of humification within the compost industry.

Independent lines of evidence from field and laboratory settings demonstrate the relative longevity of lignin. In perhaps the most comprehensive global review of litter decomposition studies, Zhang et al. (2008) associated lignin content with increased longevity of plant material for natural ecosystems. Contradictory results exist in other studies, perhaps due to differences in specific plant materials, land use, or local climatic and soil conditions, and the possibility that subfractions of lignin might differ in their labilities (Thevenot et al., 2010). Wickings et al. (2012) found selective preservation of lignins in corn and grass litters during three growing seasons, and the selective preservation became more pronounced over that time. Waggoner et al. (2015) and Waggoner and Hatcher (2017) showed that partially oxidized lignin in soil and surface waters can be transformed by reactions involving OH[−] radicals into long-chain molecules containing both alicyclic rings rich in carboxyl groups and black-carbon-like condensed structures similarly rich in carboxyl groups. The proposed pathways to alicyclic molecules are a series of reactions known to occur during fungal degradation of lignin (DiDonato et al., 2016). DiDonato and Hatcher (2017) identified alicyclic molecules in soil HA. These types of functional groups have been commonly reported elsewhere in studies on the chemical nature of HA components. This type of reaction pathway would result in the inability of current phenol analyses to detect lignin-derived phenolic groups, when the lignin residues persist at stages short of complete oxidation.

Across longer time periods, carbon-14 dating of multiple humic fractions extracted from the same soil has determined older ¹⁴C age (older mean residence time or smaller fraction of modern values) for those fractions that show larger proportions of aromatic and carboxyl C or other chemical signals of more humified NOM (Campbell et al., 1967; Olk et al., 1995, 1996; Arai et al., 1996; Legorreta-Padilla, 2005). A gradual trend over geologic time toward a dominant accumulation of aromatic compounds paired with depletion of carbohydrates and amino acids is well known for the temporal sequence from composts through peats onto lignite and then hard coals. The dominance of aromatic C by the lignite stage was attributed by González-Vila et al. (1996), Peuravuori et al. (2006), and Wang et al. (2017) to transformation of lignin residues that were apparent at earlier stages. Reviewing the chemical structure of coal, Haenel (1992) considered lignin, in terms of quantity, as possibly the single most important source material of coal, even though lignin residues are not prevalent in coal. Carbohydrate depletion and aromatic compound enrichment over geologic time were also demonstrated by ¹³C NMR analysis of HA fractions extracted from peat, leonardite, and coal by Piccolo et al. (1996) and Lu et al. (2000).

We postulate that humification is a valid set of processes that occur over time and that humic fraction studies of humification reasonably depict changes over time in NOM. It has always been clear that humification has both biological and abiotic components (Stevenson, 1994; Gerke, 2018). Microbial processes likely provide the energy needed to drive chemical rearrangements, but it is still disputed whether the altered chemical nature is due entirely to microbially driven production of new materials or whether it also includes abiotically driven reconfigurations of transient-state molecules that form during degradation. Multiple explanations have been proposed for the exact processes that

result in more humified materials, including progressive microbial oxidation and abiotic alterations of biomolecules (Zech et al., 1997), alteration of lignin initiated by hydroxyl radicals (Waggoner et al., 2015), or deposition of complex microbial polyketides (alkylaromatic, polyaromatic, and polyphenolic compounds) that can undergo further abiotic transformations (Schnitzer and Monreal, 2011). Reactive oxygen species might drive photochemical alterations of terrestrial NOM during transport from rivers to marine bodies (Waggoner et al., 2017). Polymerization of phenolic residues from lignin degradation can be catalyzed by exoenzymes (Gerke, 2018), and HA components can increase in molecular weight due to covalent linkages catalyzed by peroxidase (Cozzolino and Piccolo, 2002). Enzymatic coupling reactions have also been shown for xenobiotic phenolic compounds, which form covalent bonds to HS in the presence of phenoloxidase enzymes (Dec and Bollag, 2000). Similar enzymatic-catalyzed coupling to humic constituents can also occur for xenobiotic anilines (Dec and Bollag, 2000). Distinguishing all specific roles of each mode of alteration has not yet been achieved (Schnitzer and Monreal, 2011). This area deserves further investigation.

It can thus be concluded that some, probably minor, chemical modifications of HA and FA are likely caused by exposure to NaOH. Nevertheless, HS closely represent persistent NOM components and are therefore useful tools for studying the structure and functions of NOM in soil and water. We also conclude that humification is a real process in natural settings, regardless of whether NOM consists of specified macromolecular aromatic structures or not.

The following section presents case studies that address how humic fractions can be used in environmental research to gain an understanding of important processes involving NOM in soil and water.

Case Studies

Reactions between Persistent Organic Pollutants and Humic Fractions in Dissolved Organic Matter

Dissolved organic matter and the humic fraction in particular are known to bind persistent organic pollutants (POPs) through hydrophobic interactions (Uhle et al., 1999; Wei-Haas et al. (2014) and references therein). While this binding may result in natural water concentrations higher than would be expected for these sparingly soluble organic compounds, it is the speciation of POPs by humic DOM that ultimately affects their aquatic fate. For example, humic binding makes POPs less bioavailable to organisms (Akkanen and Kukkonen, 2003), yet binding could mediate various reactions that affect attenuation, such as

sorption and photochemical degradation reactions (Remucal, 2014). The degree to which this binding occurs is dependent on both the POP and humic DOM properties, such as octanol/water partition coefficients (K_{ow}) and other determinants of polarity. Because humic DOM is significantly more polar than octanol, humic DOM partition coefficients (K_{DOM}) are typically 1 to 10% of the analytes' K_{ow} values (Chiou et al., 1986; Chin et al., 1997; Neale et al., 2011, 2012; Wei-Haas et al., 2014). Persistent organic pollutants that have a K_{ow} of 10^4 L kg⁻¹ or greater will readily partition into humic DOM with K_{DOM} values of $>10^2$ to 10^3 L kg⁻¹, depending on the humic DOM properties.

The importance of humic DOM properties in determining the strength of this partitioning was demonstrated by Uhle et al. (1999). In solubility-enhancement experiments conducted using FAs isolated by XAD-8 chromatography from the Great Dismal Swamp (a temperate wetland in Virginia, USA) and from Pony Lake (a hypereutrophic saline pond in Antarctica), the plant-soil-derived FA from the Great Dismal Swamp had much higher binding constants for three substituted polychlorinated biphenyl congeners compared with the Pony Lake FA (Table 3). These results indicate that the greater aromaticity and polarizability of the Great Dismal Swamp FA enable stronger interactions with the polychlorinated biphenyls and explain why the humic DOM fraction, given its greater aromaticity, is more important than other fractions of DOM in controlling the speciation of POPs in aquatic ecosystems.

The partitioning of hydrophobic POPs into humic DOM can affect its photochemical fate. For example, nonpolar analytes that are susceptible to oxidation by singlet oxygen (¹O₂) are more rapidly degraded if they can partition into the humic DOM phase, which is a potent ¹O₂ photosensitizer (Latch and McNeill, 2006). Thus, humic DOM can participate in the photoreduction of halogenated hydrophobic POPs such as hexachlorobenzene, whereby the analyte partitions into the excited state of humic DOM, which in turn acts as both an electron and hydrogen donor (Uhle et al., 1999; Grannas et al., 2012; Remucal, 2014; Porras et al., 2014). Thus, humic DOM plays a critical role in the photochemical fate of POPs by sensitizing the reaction that renders many compounds susceptible to degradation through indirect pathways.

These photolytic pathways are expected to be particularly important in the Arctic, where POPs are concentrated due to global-scale distillation processes (Hageman et al., 2015), resulting in bioconcentration or biomagnification of POPs in food webs of significance for subsistence-hunting communities. During periods of near-continuous sunlight, surface waters are ice-free, and concentrations of humic DOM are high due to the flushing of the tundra by snowmelt. Grannas et al. (2012) studied the photosensitization process with humic DOM isolated

Table 3. Octanol/water partition coefficient (K_{ow}) values for selected polychlorinated biphenyls (PCBs) and dissolved organic C partition coefficients (K_{doc}) values for PCBs for partitioning into fulvic acid (FA).

PCB	Pony Lake FA K_{doc} (L/kg DOC†) (95% CI)	Great Dismal Swamp FA K_{doc} (L/kg of DOC)	aqueous solubility (mol/L)	PCP log K_{ow}
2,2',5	4,620 ± 720‡	14,400 ± 3,700	1.98×10^{-6}	4.73 ± 0.72; <i>n</i> = 9
2,2',5,6'	4,270 ± 400	14,300 ± 2,200	1.63×10^{-7}	5.46; <i>n</i> = 1
3,3',4,4'	38,000 ± 5,800	52,300 ± 7,500	1.87×10^{-9}	6.23 ± 0.44; <i>n</i> = 5

† DOC, dissolved organic carbon.

‡ Confidence interval.

from different Arctic surface waters and found that humic DOM stimulated the photodegradation of hexachlorobenzene (Fig. 3) but not of lindane (hexachlorocyclohexane), which does not have an affinity for humic DOM (data not shown).

In summary, the aromatic nature of humic substances makes them especially effective binders of some POPs. Their binding was shown in these examples to alter the ecological persistence of POPs in compound-specific manners. Hence, the chemical interactions between POPs and HS have clear significance for explaining solubility and longevity of POPs in aquatic ecosystems.

Modeling of Metal Ion Binding in Soils

The retention of plant-available metal ions in soils is largely due to association with carboxyl and hydroxyl weak acid groups in humic substances. This includes divalent exchangeable cations, such as Ca^{2+} and Mg^{2+} , trivalent Al^{3+} , which is abundant in acid soils, and environmentally important metal cations such as Cd^{2+} , Fe^{2+} , Fe^{3+} , Cr^{3+} , Co^{2+} , Cu^{2+} , Ni^{2+} , and Pb^{2+} . The weak acid groups also provide most of the pH-dependent charge and pH buffering in noncalcareous surface soils (Bloom et al., 2005; Bloom and Sklyberg, 2012). At low soil pH, the weak acid groups are bound to H^+ and Al^{3+} , while at higher pH the charge-neutralizing ions are mostly Ca^{2+} and Mg^{2+} .

Several investigators have developed computer-based models for calculating H^+ and metal ion binding to humic substances, using binding constants developed from data for HA and FA.

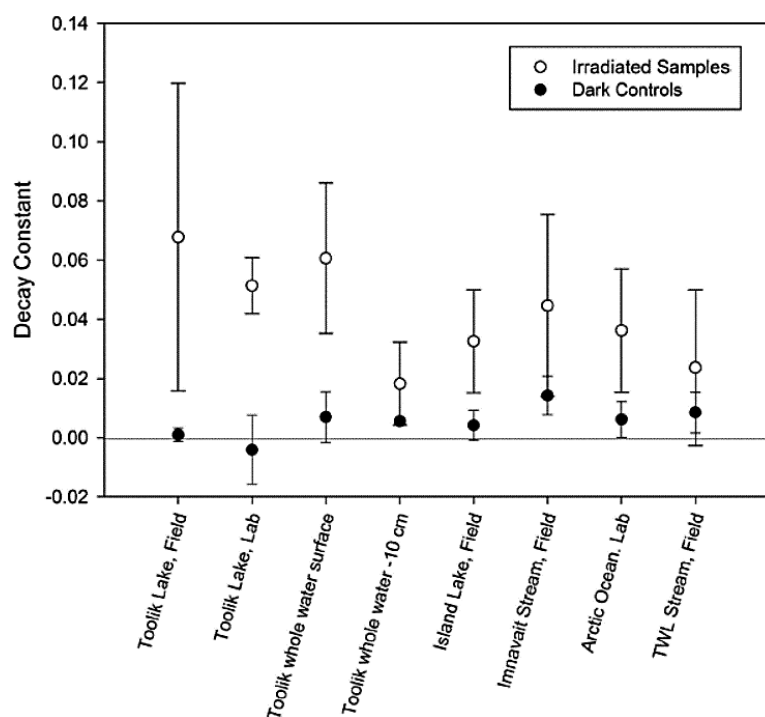


Fig. 3. Effect of humic dissolved organic matter (DOM) on photodegradation of hexachlorobenzene (HCB) in different Arctic surface waters. Plots show differences in degradation rates with and without irradiation in water samples with DOM. First-order decay constants (calculated from the slopes of the linearized data for initial time points of decay curves) for HCB degradation in the presence of humic DOM were determined from different surface waters and one whole water sample from Toolik Lake, Alaska. The experiments were conducted under different conditions in both laboratory and field settings. Error bars represent the 90% confidence interval, calculated using analysis of variance, of linearized concentration data from replicate experiments. Source: Grannas et al. (2012).

These include the NICA–Donnan model (Kinniburgh et al., 1996), Model VII (Tipping et al., 2011), and the Stockholm humic model (SHM, Gustafsson, 2001). The NICA–Donnan model is a continuous distribution model, whereas Model VII and the SHM are discrete site models. In this case study, we illustrate how these models can be used to understand the behavior of metals and metal ion–HS interactions in soils by discussing some applications of the SHM.

In the development of the SHM, Gustafsson (2001) assumed two classes of sites defined by central pKa values that each define four binding sites, $\pm(\Delta\text{pKa} + 2\Delta\text{pKa})$. This results in eight distinct pKa values (Gustafsson, 2001) and is similar to the approach taken by Tipping et al. (2011) in Model VII. The SHM is implemented in Visual MINTEQ, a freely available equilibrium speciation model (Gustafsson, 2019). Gustafsson (2001) reported central pKa values for the stronger acid sites of 3 to 4.45 (carboxyl) and 8.4 to 9.2 (mostly phenolic) for the weaker acid sites in HA and FA. In addition, the SHM includes a term to account for electrostatic effects. In the case of divalent ions, both monodentate and bidentate complexation are allowed. For bidentate binding, SHM allows for metal association with two of the stronger acid carboxyl sites or with one carboxyl group and one OH. The model also allows for nonspecific electrostatic binding of weakly bound ions.

Gustafsson (2001) conducted an evaluation of SHM for H^+ binding and the binding of Ca^{2+} , Cd^{2+} , Pb^{2+} , Cu^{2+} , and Al^{3+} by a peat HA over a pH range of 4 to 10 at ionic strengths of 0.01 and 0.1 M. This study also included evaluation of Ca–Cd, Ca–Al, and Pb–Al competition. The results showed the model did well in describing H^+ binding and H^+ /metal-ion interactions with the HA over a wide range of solution concentrations, and it adequately described Cd competition with Ca, but it did less well for Ca–Al and Pb–Al competition. The model also did not describe well the effects of ionic strength on metal ion binding (Gustafsson, 2001). In a subsequent batch study of two mor layer forest soils, Gustafsson and van Schaik (2003) reparameterized the model and generated generic constants for use in soil metal–humic modeling. This gave better results but underpredicted soluble Cu and Pb, two very strongly bound ions, due to the failure to account for the Cu and Pb associated with soluble FA.

When binding to soluble FA was included in the SHM modeling of data from a column study of heavy metal leaching of two moderately contaminated urban soils (34 and 17 g organic C kg^{-1} soil), the modeling successfully predicted the solution concentrations of Cu^{2+} , Cr^{3+} , and Pb^{2+} . In solution, these ions are mostly associated with soluble FA. The model was also able to predict that solution concentrations of Cd, Ni, and Zn would increase with additions of sodium chloride (NaCl) to the leaching solution and that salt had little effect on the strongly bound ions, Cu^{2+} , Cr^{3+} , and Pb^{2+} .

In a batch study of eight Spodosols, Tibergh et al. (2018) investigated the sorption of Pb and Cd in Bs horizons that contained poorly crystalline oxyhydroxides of Al and Fe. The authors included in the SHM model a high affinity adsorption site for Pb in addition

to the weaker sites that had been included in their generic parameters. This site was needed to account for the underprediction of soluble Pb at low additions (Gustafsson et al., 2011). They also had to reparameterize the active humic fraction of SOM from the generic value of 0.55 to about 0.10, and it varied with the soil. This was necessary because of the adsorption of HS to, and inclusion into, Fe and Al oxyhydroxides. Also, the point of zero charge of the Fe oxyhydroxide was increased to account for the sorption of HS. When the changes were made, Visual MINTEQ was able to predict solution Cd and Pb over a wide pH range. Both Cd and Pb were found to be bound mostly to SOM, with a small fraction of the Pb associated with ferrihydrite.

These modeling studies of metal ion binding to solid phase HA and FA in SOM and soluble FA illustrate how mechanistic modeling based on an understanding of humic substances not only can provide tools for prediction but also can be used to help gain an understanding of the role of SOM in the retention and mobility of metal ions in soils.

Pesticide Sorption in Soils

Based on an estimate using Google Scholar (<https://scholar.google.com/>), about 10% of the peer-reviewed articles discussing HS refer to their interactions with pesticides. Humic substances are of interest because they are generally more chemically reactive with pesticides than are nonhumified materials (Farenhorst, 2006). Humic fractions are relatively unique in that they are, by definition, heterogeneous in molecular weight and structure as influenced by their environment and time (Niederer et al., 2007; Amir et al., 2010). Despite this heterogeneity, generalized statements can be made about the extent to which various types of HS interact with pesticides, indicating reproducible behavior of HS in pesticide studies. For example, during incubations under controlled conditions, pesticides tend to have a greater affinity for HA than FA, and a greater affinity for HS richer in aromatic C than in aliphatic C (Chiou et al., 1986; Piccolo et al., 1992; Kulikova and Perminova, 2002; Farenhorst et al., 2010; Chowdhury et al., 2014). Also, a number of studies reported that pesticides have a greater affinity for HS derived in terrestrial than in aquatic environments (Chiou et al., 1986; Niederer et al., 2007), and HS with larger molecular sizes sorb more strongly to pesticides than do those with smaller molecular sizes (Piccolo et al., 1992, 1996).

A range of techniques has been used to aid in the characterization of HS, particularly Fourier-transform infrared spectroscopy, ^{13}C NMR spectroscopy, and, increasingly, synchrotron-based Fourier-transform infrared–attenuated total reflectance spectroscopy and carbon near edge X-ray fine structure spectroscopy (Davis et al., 1999; Kulikova and Perminova, 2002; Solomon et al., 2005; Christl and Kretzschmar, 2007; Amir et al., 2010; Prietzel et al., 2018). The application of these techniques has contributed significantly to our knowledge of the mechanisms responsible for pesticide sorption to HS (Piccolo et al., 1996; Wang et al., 2011; Dutta et al., 2015). Pesticides can be categorized as cationic, ionizable (i.e., weakly acidic, weakly basic, or zwitterionic), or nonionic (nonpolar) molecules. Charge transfer and ionic bonding are mechanisms that specifically apply to the sorption of cationic pesticides by HS (Senesi and Chen, 1989; Senesi, 1992). For nonionic pesticides, hydrophobic bonding (partitioning) is most common, but other possible mechanisms

for sorption by HS include charge-transfer complexes, covalent bonding, hydrogen bonding, and Van der Waals attractions (Senesi and Chen, 1989; Senesi, 1992). Bonding mechanisms commonly observed for ionizable pesticides and HS include charge-transfer (electron donor–acceptor) complexes, hydrogen bonding, ligand exchange, and Van der Waals attractions (Fusi et al., 1988; Senesi and Chen, 1989; Hermosin and Cornejo, 1993; Piccolo and Celano, 1994; Senesi et al., 1995, 1997; Ferreira et al., 2002; Sheals et al., 2002).

It is generally accepted that for most pesticides, the pesticide sorption capacity of a soil is determined by characteristics of its organic components. Yet, for soil samples collected along a catenary sequence, the sorption of a pesticide per unit organic C (K_{OC}) was not significantly correlated with SOC (Farenhorst et al., 2010). However, K_{OC} was significantly correlated with the amount of HA that was extracted by NaOH from these soils, as well as with parameters indicative of greater HA and SOC aromaticity. Similarly, SOM aromaticity was the only factor to explain the observed variations in pesticide K_{OC} values across soils collected from a wide range of ecosystems in Australia and Pakistan (Ahmad et al., 2001). Thus, the aromatic content of soil may be the decisive factor for sorption of some pesticides, and this parameter is better estimated by HA content than by SOC.

Pesticide sorption parameters are among the most sensitive input parameters in pesticide fate models (Dubus et al., 2003; Fan et al., 2008; Farenhorst et al., 2009; Nolan et al., 2015). These models are essential tools in regulatory exposure assessments of currently used pesticides and in the registration process of new pesticide products. Knowledge of the spatial variability of pesticide sorption parameters in soil landscapes has been found to be more important to the refinement of risk assessments than the choice of the pesticide fate model itself (Dann et al., 2006). Since HS affect the value of a pesticide sorption parameter in soil, advancing knowledge of the spatial distribution of HS and their characteristics in soil landscapes could play an important role in the refinement of regulatory exposure assessments that rely on pesticide fate modeling.

Humic Acid as an Indicator of Compost Maturity and the Capacity of Compost to Enhance Plant Growth

Composting is a management tool for treating organic wastes that results in rapid humification and formation of products that are much more stable than raw wastes. Compost can be used as a soil amendment in agricultural or horticultural production and as a high-quality peat substitute for container-grown plants (Chen et al., 1985, 1988; Chen and Hadar, 1986; Inbar et al., 1989). During composting, total alkali-extractable HS increases with time and the newly formed HS resemble those of “young” HS formed in soils (Niemeyer et al., 1992).

Composting of municipal solid waste has been studied extensively since industrialized countries began to emphasize the recycling of solid wastes, especially municipal solid waste, due to its vast and rapidly growing quantities and a severe decrease in availability of landfill space. Composting of municipal solid waste has the capacity to reduce the volume and mass by approximately 50%, resulting in a product that can be beneficial to agriculture (He et al., 1992). In a study of municipal solid waste composting over 132 d, the HA content of the compost

increased to a maximum plateau at Day 112 and the compost at this time produced the highest plant dry weight in a cucumber bioassay of compost maturity (Chefetz et al., 1996). The authors concluded that their plant-growth bioassay, HA content, and diffuse reflectance infrared Fourier transform spectra of the composting materials provided reliable and useful parameters for determining compost maturity.

Senesi and coworkers studied the products of an abiotic treatment of olive oil mill wastewater that was designed to eliminate problems associated with the anaerobic soil conditions caused by direct land application (Senesi and Brunetti, 1996; Brunetti et al., 2008). Abiotic catalysis with manganese (IV) oxide plus bubbling with air for 30 and 60 d tripled the extractable HA content of the wastewater compared with open air treatment in a lagoon for the same time period. The HA produced after 60 d with abiotic catalysis was similar chemically to soil HA, which encouraged adoption of the abiotic catalysis (Brunetti et al., 2008). This treatment has been successfully adopted industrially to induce humification in wastewaters before their application as liquid amendments to agricultural soils, especially in semiarid regions.

Soil Humic Fractions as Indicators of Soil Organic Matter Responses to Organic Amendments

Plaza and Senesi (2009) reviewed nearly 30 studies in which HA fractions were extracted for chemical characterization from soils that had received organic amendments of various natures, including animal manures, composts, sewage sludge, and olive oil mill wastewaters. The authors compared the chemical properties of the extracted humic fractions with those from corresponding unamended control soils. All compositional analyses detected some level of alteration in humic properties following organic amendments, suggesting that the organic amendments had been partially incorporated into the humic fraction, but the degree of change varied by analysis. For example, the humic fractions from amended soils showed elemental compositions intermediate between each amendment and the native humic fractions from the unamended control soils. Intermediate values for the humic fractions of amended soils were also observed for the ratio of light absorption at 465 and 665 μm , (E4/E6 ratio), fluorescence spectra, Fourier-transform infrared spectra, ^{13}C NMR spectra, organic free radical concentrations, and types and strengths of metal bonding. In some cases, the HA responses strengthened with increasing rate of amendment incorporation, while a limited number of time series studies showed a gradual diminishing of the alteration with increasing time after application. These results indicate that the HS fractions were responsive to recent land management, in this case organic amendments.

Soil Humic Fractions as Indicators of Young Soil Organic Matter for Nutrient Cycling in Agriculture

Soil organic matter pools can cycle over timescales ranging from weeks or months to millennia. Determining the contributions of SOM in production agriculture requires the study of SOM fractions that cycle over relatively short timescales so that they match the generally recent histories of agricultural practices.

Extraction procedures for young SOM fractions are often based on disrupting soil agents that stabilize SOM against short-term cycling, including binding to clay mineral surfaces (Christensen, 1992), aggregation (Tisdall and Oades, 1982; Six et al., 2004; Sutton and Sposito, 2005), and polyvalent cations (Baldock and Skjemstad, 2000). One of the most common soil cations, Ca^{2+} , can stabilize organic ligands against microbial decomposition in controlled conditions, and its soil abundance has been positively linked to SOM levels in the field (Baldock and Skjemstad, 2000).

To explore the capacity of Ca binding to distinguish more recalcitrant SOM from labile materials, Olk and colleagues followed previous studies by Tyrurin (Kononova, 1966) and Campbell et al. (1967) in modifying alkali extraction to recover an HA fraction not bound to Ca (Olk, 2006). This unbound mobile humic acid (MHA) fraction was extracted by NaOH before the routine soil decalcification step of repeated HCl washes (Swift, 1996), while the Ca-bound calcium humate (CaHA) fraction was extracted by NaOH immediately after soil decalcification.

In multiple studies, MHA has shown the characteristics of young SOM—modern ^{14}C age (Campbell et al., 1967; Olk et al., 1995, 1996), relatively high N and H concentrations, and the significant presence of multiple C and organic P forms, including labile compounds (Mahieu et al., 2002). By contrast, the chemical composition of the CaHA was dominated by compounds associated with more advanced humification—carboxyl-C, unsubstituted aromatic C, organic free radicals, heterocyclic N, and monoester P (Mahieu et al., 2002), together with greater ^{14}C age (Campbell et al., 1967; Olk et al., 1995, 1996). Characterization studies of CaHA and MHA have been performed on soils cropped to cotton (*Gossypium* spp.), rice, and wheat (*Triticum* spp.). Corn–soybean soils have given similar results with or without animal manure amendment (Mao et al., 2008; Cao et al., 2011; He et al., 2011; Wingeyer et al., 2012).

Characterization of both fractions has enabled a meaningful understanding of soil nutrient cycling and soil-C forms. Seeking to explain the kinetic parameters in a two-pool first- and zero-order model for net N mineralization in paddy rice soils, Ve et al. (2004) predicted only 27 and 36% of the variability in the rate constants of both pools through stepwise regression against soil properties. Inclusion of extracted HA mass and chemical nature parameters for the MHA and CaHA into the regressions improved their predictions to 64 and 75%, respectively. The biochemical natures of the MHA and CaHA varied reproducibly in rice field trials in response to fertilizer treatments and the number of annual paddy rice crops, establishing highly significant correlations of humic visible light absorption, organic free radical levels, and H concentrations with the proportions of functional groups measured by ^{13}C , ^{31}P , and ^{15}N NMR (Mahieu et al., 2002). These correlations underscore the reliability of extracting humic fractions for nutrient-cycling studies.

Following is a case study in which chemical characterization of the MHA and CaHA fractions proved useful for elucidating soil C and nutrient cycling in paddy rice soils. The HA results guided the development of improved rice management practices.

Soil Organic Matter and Soil N Cycling for the Sustainability of Monoculture Paddy Rice

Long-term field trials of double- and triple-cropped monoculture paddy rice in the Philippines have shown a striking long-term yield decline across multiple decades (Cassman et al., 1995). Agronomic evidence suggests that the decline is due primarily to a late-season decrease in crop uptake of N mineralized from SOM, despite maintained or increased quantities of soil N in these SOM-rich soils and continued plant uptake of fertilizer N. In a series of publications, Olk and colleagues found that the MHA and CaHA fractions from these long-term field trials responded in quantity and chemical nature to the degree of rice cropping intensity, especially the MHA (Olk, 2006). Analysis of these humic fractions found that under monoculture paddy rice, phenolic lignin residues accumulated in the HA fractions and soil N cycling was inhibited during the flooded conditions that prevail during a paddy-rice growing season. Schmidt-Rohr et al. (2004) used advanced NMR spectroscopy to identify an anilide structure in the MHA fraction of a triple-cropped rice soil and concluded this aromatic N structure formed through the presence of phenolic lignin residues. Together, this body of work provides a plausible mechanism for explaining the long-term yield declines by postulating the binding of soil organic N forms with phenolic lignin residues during the flooded soil conditions of the rice-growing season.

This hypothesis was tested in Arkansas, where introduction of a continuous rice monoculture (one annual crop) led within a few years to a 19% grain yield loss compared with the dominant rice–soybean rotation (Anders et al., 2004, 2007). Rice leaf N concentrations suggested a late-season N deficiency, similar to the Philippines' yield decline. Nitrogen fertilizer was applied only once early in the season, compelling late-season crop N uptake to become dependent on soil N mineralization. Olk et al. (2009) applied the concepts gained from HS research in the Philippines to measure whole soil properties of the low-C Arkansas soils and reported an accumulation of soil phenols under continuous rice monoculture compared with rice–soybean rotation. Similar to the Philippines study, ^{15}N -fertilizer measurements demonstrated that late-season crop uptake of soil N was inhibited under continuous rice cropping compared with rice following soybean, while plant uptake of fertilizer N was relatively unaffected. These measurements on whole soil N and crop tissue N in Arkansas agreed with the hypothesis developed through HA extractions from the considerably richer in C Philippines soils. Therefore, the humic-based studies in the Philippines paddy soils were reliable proxies for SOM processes in the Arkansas paddy soils.

Based on the association of long-term flooded soil conditions with soil phenol accumulation and inhibition of soil N cycling in both the Philippines and Arkansas, together with the desire to conserve water, new crop management treatments were designed that used alternate wetting and drying cycles to increase soil aeration during the cropping period, in part to degrade phenolic lignin residues that may bind soil N forms. When coordinated with timely N fertilizer applications, these treatments were subsequently shown to reduce irrigation water needs by >25%, greenhouse gas emissions by nearly 90%, fungal disease incidence, and grain contents of arsenic and mercury (Massey et al., 2014; Linquist et al., 2015; LaHue et al., 2016).

Based on these multiple benefits, the in-season aeration treatments are now recommended and supported by the USDA-NRCS for sustainable rice production and are currently used on about 45,000 ha in US rice fields, with further increases planned to more than 200,000 ha. They are also now recommended internationally through the Sustainable Rice Platform (<http://www.sustainable-rice.org/>) as a means of achieving sustainable rice production. Their development was partially based on concepts gained through HA extraction and characterization, demonstrating that HA fractions can represent SOM dynamics under crop management treatments and that the generated information can be successfully applied to resolve issues of soil nutrient cycling and agronomic sustainability. Overall, the studies cited herein demonstrate the capacity of soil humic fractions to respond reproducibly in quantity and chemical nature to environmental conditions.

Discussion and Conclusion

Humic and fulvic acids are composed of a vast array of components. They contain both biological molecules and the products of humification reactions, and the majority of their components have molecular weights much lower than thought several decades ago. Based on reviews of the soil and aquatic literatures presented here, any modifications of HA and FA that are caused by exposure to NaOH appear to be minor. The constructive applications of HS presented in this review demonstrate that a minor degree of modification does not diminish the validity of humic fractions in representing NOM. In short, HS accurately reproduced the chemical nature of their source materials in soils, waters, and organic sediments. They are not laboratory artifacts that arise from reactions caused by NaOH. Instead, they are suitable for the study of NOM processes in field conditions, thereby refuting Lehmann and Kleber (2015).

Evidence is presented here for the occurrence of humification in studies that did not use alkali extraction. These included increasing ages of composts and geologic organic deposits, increasing depth in forest soils, and decomposition of plant litter material. There is convincing evidence that coupling reactions involving phenolic compounds with other phenolic compounds and with HA contribute to humification. In addition, there is evidence that reactions of OH^- radicals with partially oxidized lignin produce polyaromatic and alicyclic products. This is in contradiction to the argument of Lehmann and Kleber (2015) that the formation of SOM involves only biological reactions.

The alkali extraction procedures described in this review are practical for extracting both soil and aquatic organic fractions, and they provide fractions whose properties help explain NOM composition, behavior, and chemical cycling in these media. Specifically, an understanding of the properties and chemical makeup of HS was key to understanding environmental pollution due to POPs, toxic metals, and pesticides, as well as the effects on soil performance of land management practices, including organic amendments. A modified alkali extraction procedure obtained two soil HA fractions that differed consistently in their chemical composition and degree of humification. The younger HA fraction extracted without prior removal of Ca, which can stabilize HA in soil, was shown to be useful in gaining an understanding of N cycling in intensively cropped paddy rice

soils, leading to improved crop management options for reversing long-term grain yield declines.

The studies presented here demonstrate the use of alkali-extracted humic fractions to resolve practical and scientifically important issues without invoking any preconception of humic molecular structure. Indeed, the best evidence presented here shows that HA and FA are composed of a vast array of components, and it is not reasonable to propose a single structure. Efforts by Lehmann and Kleber (2015) and others to link use of alkali extractions to a belief in a defined aromatic macromolecular structure of HS may hinder a constructive dialogue among practitioners of different fractionation procedures. The existence of humification is not predicated on a defined humic molecular structure. Gradual alteration in the chemical structures of NOM components does not require them to comprise a specific macromolecular structure.

This report has illustrated scientific advances and practical solutions to issues that were realized through the study of HS, and in cases that would not have been clearly identified merely through study of total NOM or perhaps alternative fractionation procedures. We reject the argument by Lehmann and Kleber (2015) that all research involving humic fractions is “hampering scientific progress.” The terms *humic fraction* and *fulvic fraction* are concise descriptions of substances that are indeed more humified than other NOM components. We believe scientific progress would be enhanced if opposing researchers would (i) stop insisting that alkali extractions are necessarily bound to belief in a macromolecular humic structure and (ii) acknowledge the mass of evidence that alkali extractions do produce fractions that reasonably represent NOM, instead of presuming that the potential for chemical alteration is by itself sufficient proof for unacceptable alteration (Semenov et al., 2013; Lehmann and Kleber, 2015).

Lehmann and Kleber (2015) proposed the soil continuum model as a framework for understanding NOM, in which materials amended to soils degrade solely into successively smaller molecules. In light of evidence presented in this report, the soil continuum model appears simplistic. It presumes that the chemical nature of amended material has no effect on its subsequent degradation rate, whereas literature reviewed above indicates the relative longevity of lignin residues, sometimes in forms that currently popular chemical analyses do not recognize as lignin residues. Preferential degradation of carbohydrates and proteinaceous compounds and preferential accumulation of lipid-derived alkyl compounds with increasing age were found in a number of studies, as reviewed above. The soil continuum model also fails to account for substantial chemical alterations of materials following incorporation into soil. Yet the occurrence of humification has been demonstrated in studies described herein, including those that did not involve alkali extractions or that associated increasing humification nature with increasing ^{14}C age of HS. Study of humic fractions enables greater focus on more aromatic components of NOM, which can play specific roles in NOM cycling and behavior.

We maintain that alkali extractions are a suitable approach for studying the chemical nature and activity of NOM in terrestrial and aquatic processes. Declaring humic fractionation to be an unacceptable approach to NOM would first require refutation of all accomplishments described in this report. How can a

fatally flawed procedure generate the quality of results described here, in both water and soil systems, and for both environmental and agricultural purposes? A fatally flawed procedure is incapable of such constructive results. Therefore, humic fractions are a suitable approach to NOM.

Yet we agree that humic fractionation is neither a perfect nor a complete approach to studying SOM. For example, aggregate-based fractions are much more suitable for study of SOM positioning within the three-dimensional architecture of soil. Particulate organic matter has been used impressively to demonstrate long-term C losses with tillage of native soils (Cambardella and Elliott, 1992). But humic extractions allow the study of other, more chemically based stabilization modes, such as varying stages of humification or intrinsic chemical recalcitrance, binding with soil minerals, and Ca and Al binding.

More broadly, we maintain that SOM can be stabilized through multiple modes, which will vary in their relative significance by soil properties and land management practices (e.g., no-tillage vs. tillage, puddling of rice soils). Stabilization of SOM components creates pools of varying cycling rates, which generates the need to distinguish them based on research objectives. Yet every procedure for extracting NOM fractions becomes imperfect at some point; none will fully distinguish all labile NOM from all recalcitrant NOM for all research objectives and for water, soil, and organic substances in all possible settings. Fractionations of all types share the undesirable potential for overlap of NOM materials among fractions during extraction. There will be situations and research objectives that are better suited to one fractionation approach than to others. This concept should be the guide for selection of a fractionation procedure, not the insistence that one imperfect procedure is more flawed than all other imperfect procedures.

As an example, aquatic scientists use methods other than XAD-8/DAX-8 and XAD-4 to obtain DOM fractions for different types of studies. They include extraction with other types of resins (e.g., Li and Minor, 2015) and RO for waters with very low ionic strength and high DOM, or RO coupled with electrodialysis to remove salts from more typical surface waters (Vetter et al., 2007; Koprivnjak et al., 2009).

There have been multiple calls for integrated use of chemical and physical fractionations to better approximate the reality that SOM is a complex mixture of many components and can be bound in any soil through multiple stabilization agents (Trumbore and Zheng, 1996; Olk and Gregorich, 2006; von Lützow et al., 2007). A 2004 symposium summarized by Olk and Gregorich (2006) was organized to encourage constructive interaction among practitioners of these imperfect approaches. Unfortunately, the tenor of discussion among practitioners has too often degraded to attacks based on selective literature reviews. Once again, we call on soil and aquatic researchers of NOM to recognize all honest data sources and acknowledge strengths and shortcomings of each fractionation procedure when interpreting results. Each fractionation procedure in current use for studying NOM, including alkali extraction for humic fractions, has limitations. Yet each procedure can and has provided useful results—within the bounds of its capabilities—that would not be possible by studying only total NOM.

Conflict of Interest

The authors declare that they have no conflict of interest. D. Olk currently serves as volunteer scientific advisor for the Humic Products Trade Association and receives no financial support from HPTA.

References

- Ahmad, R., R. Kookana, A. Alston, and J.O. Skjemstad. 2001. The nature of soil organic matter affects sorption of pesticides: 1. Relationships with carbon chemistry as determined by ^{13}C CPMAS NMR spectroscopy. *Environ. Sci. Technol.* 35:878–884.
- Aiken, G.R. 1985. Isolation and concentration techniques for aquatic humic substances. In: G.R. Aiken, et al., editors, *Humic substances in soil, sediment, and water: Geochemistry and isolation*. John Wiley & Sons, New York. p. 363–385.
- Akkanen, J., and J.V. Kukkonen. 2003. Measuring the bioavailability of two hydrophobic organic compounds in the presence of dissolved organic matter. *Environ. Toxicol. Chem.* 22:518–524. doi:10.1002/etc.5620220308
- Amir, S., A. Jouraiphy, A. Meddich, M. El Gharous, P. Winterton, and M. Hafidi. 2010. Structural study of humic acids during composting of activated sludge-green waste: Elemental analysis, FTIR and ^{13}C NMR. *J. Hazard. Mater.* 177:524–529.
- Anders, M.M., D.C. Olk, T. Harper, T. Daniel, and J. Holzhauser. 2004. The effect of rotation, tillage, and fertility on rice grain yields and nutrient flows. *Tech. Bull.* 321. [CD-ROM]. North Carolina Agric. Res. Serv., Raleigh, NC.
- Anders, M.M., K.B. Watkins, K.A.K. Moldenhauer, J.W. Gibbons, and R.W. McNew. 2007. The effect of rotation, tillage, fertility, and variety on rice grain yield. In: R.J. Norman, et al., editors, *B.R. Wells rice research studies series 2006*. Univ. Ark. Res. Ser. 550. Ark. Agric. Exp. Stn, Fayetteville. p. 251–258.
- Antweiler, R.C. 1991. The hydrolysis of Suwannee River fulvic acid. In: R.A. Baker, editor, *Organic substances and sediments in water*. Vol. 1: Humics and soils. Lewis, Chelsea, MI. p. 163–177.
- Arai, S., T. Hatta, U. Tanaka, K. Hayamizu, K. Kigoshi, and O. Ito. 1996. Characterization of the organic components of an Alfisol and a Vertisol in adjacent locations in Indian semi-arid tropics using optical spectroscopy, ^{13}C NMR spectroscopy, and ^{14}C dating. *Geoderma* 69:59–70.
- Asakawa, D., Y. Iimura, T. Kiyota, Y. Yanagi, and N. Fujitake. 2011. Molecular size fractionation of soil humic acids using preparative high performance size-exclusion chromatography. *J. Chromatogr. A* 1218:6448–6453. doi:10.1016/j.chroma.2011.07.030
- Baldock, J.A., and J.O. Skjemstad. 2000. Role of the soil matrix and minerals in protecting natural organic materials against biological attack. *Org. Geochem.* 31:697–710. doi:10.1016/S0146-6380(00)00049-8
- Bernal, M.P., J.A. Albuquerque, and R. Moral. 2009. Composting of animal manures and chemical criteria for compost maturity assessment. A review. *Bioresour. Technol.* 100:5444–5453. doi:10.1016/j.biortech.2008.11.027
- Bloom, P.R., and U.L. Skjellberg. 2012. Soil pH and pH buffering. In: P.M. Huang, Y. Li, and M.E. Sumner, editors, *Handbook of soil science*. 2nd ed. CRC Press, Boca Raton, FL. p. 19-1–19-14.
- Bloom, P.R., U.L. Skjellberg, and M.E. Sumner. 2005. Soil acidity. In: A. Tabatabai and D. Sparks, editors, *Chemical processes in soils*. SSSA Book Series 8. SSSA, Madison, WI. p. 411–460.
- Bowles, E.C., R.C. Antweiler, and P. MacCarthy. 1989. Acid-base titrations and hydrolysis of fulvic acid from the Suwannee River. In: R.C. Averett et al., editors, *Humic substances in the Suwannee River, GA: Interaction, properties, and proposed structures*. USGS Open File Rep. 87-557. USGS, Reston, VA. p. 209–229.
- Brunetti, G., N. Senesi, and C. Plaza. 2008. Organic matter humification in olive oil mill wastewater by abiotic catalysis with manganese(IV) oxide. *Bioresour. Technol.* 99:8528–8531. doi:10.1016/j.biortech.2008.02.047
- Cambardella, C.A., and E.T. Elliott. 1992. Particulate soil organic-matter changes across a grassland cultivation sequence. *Soil Sci. Soc. Am. J.* 56:777–782. doi:10.2136/sssaj1992.03615995005600030017x
- Campbell, C.A., E.A. Paul, D.A. Rennie, and K.J. McCallum. 1967. Applicability of the carbon-dating method of analysis to soil humus studies. *Soil Sci.* 104:217–224. doi:10.1097/00010694-196709000-00010
- Cao, X., D.C. Olk, M. Chappell, C.A. Cambardella, L.F. Miller, and J. Mao. 2011. Solid-state NMR analysis of soil organic matter fractions from integrated physical-chemical extraction. *Soil Sci. Soc. Am. J.* 75:1374–1384. doi:10.2136/sssaj2010.0382
- Cassman, K.G., S.K. De Datta, D.C. Olk, J. Alcantara, M. Samson, J. Descalsota, and M. Dizon. 1995. Yield decline and the nitrogen economy of long-term experiments on continuous, irrigated rice systems in the tropics. In: R. Lal and B.A. Stewart, editors, *Soil management: Experimental basis for sustainability and environmental quality*. CRC Press, Boca Raton, FL. p. 181–222.
- Chefetz, B., P.G. Hatcher, Y. Hadar, and Y. Chen. 1996. Chemical and biological characterization of organic matter during composting of municipal solid waste. *J. Environ. Qual.* 25:776–785. doi:10.2134/jeq1996.00472425002500040018x
- Chen, Y. 2003. Nuclear magnetic resonance, infra-red and pyrolysis: Application of spectroscopic methodologies to maturity determination of composts. *Compost Sci. Util.* 11:152–168.
- Chen, Y., and Y. Hadar. 1986. Composting and use of agricultural wastes in container media. In: M. De Bertoldi, M.P. Feranti, P. L'Hermite, and F. Zucconi, editors, *Compost production, quality and use*. Elsevier, New York. p. 71–77.
- Chen, Y., Y. Hadar, and Y. Inbar. 1985. The use of composted waste as peat substitute in horticulture. In: P. L'Hermite, editor, *Processing and use of organic sludge and liquid agricultural wastes*. Reidel, Boston, MA. p. 388–392.
- Chen, Y., Y. Inbar, and Y. Hadar. 1988. Composted agricultural wastes as potting media for ornamental plants. *Soil Sci.* 145:298–303. doi:10.1097/00010694-198804000-00009
- Chin, Y.P., G.R. Aiken, and K.M. Danielsen. 1997. Binding of pyrene to aquatic and commercial humic substances: The role of molecular weight and aromaticity. *Environ. Sci. Technol.* 31:1630–1635. doi:10.1021/es960404k
- Chiou, C.T., R.L. Malcolm, T.I. Brinton, and D.E. Kile. 1986. Water solubility enhancement of some organic pollutants and pesticides by dissolved humic and fulvic acids. *Environ. Sci. Technol.* 20:502–508. doi:10.1021/es00147a010
- Chow, A.T. 2006. Comparison of DAX-8 and XAD-8 resins for isolating disinfection byproduct precursors. *J. Water Supply Res. Technol. Aqua* 55:45–55. doi:10.2166/aqua.2005.063
- Chowdhury, R., J.W. Atwater, and K.J. Hall. 2014. Role of soil humic and fulvic acid in the sorption of endosulfan (alpha and beta). *Chem. Ecol.* 30:755–764. doi:10.1080/02757540.2014.917171
- Christensen, B. 1992. Physical fractionation of soil and organic matter in primary particle size and density separates. *Adv. Soil Sci.* 20:1–90. doi:10.1007/978-1-4612-2930-8_1
- Christl, I., and R. Kretzschmar. 2007. C-1s NEXAFS Spectroscopy reveals chemical fractionation of humic acid by cation-induced coagulation. *Environ. Sci. Technol.* 41:1915–1920. doi:10.1021/es062141s
- Cozzolino, A., and A. Piccolo. 2002. Polymerization of dissolved humic substances catalyzed by peroxidase. Effects of pH and humic composition. *Org. Geochem.* 33:281–294. doi:10.1016/S0146-6380(01)00160-7
- Dann, R.L., M.E. Close, R. Lee, and L. Pang. 2006. Impact of data quality and model complexity on prediction of pesticide leaching. *J. Environ. Qual.* 35:628–640. doi:10.2134/jeq2005.0257
- Davis, W.M., C.L. Erickson, C.T. Johnston, J.J. Delfino, and J.E. Porter. 1999. Quantitative Fourier transform infrared spectroscopic investigation of humic substance functional group composition. *Chemosphere* 38:2913–2928. doi:10.1016/S0045-6535(98)00486-X
- Dec, J., and J.-M. Bollag. 2000. Phenoloxidase-mediated interactions of phenols and anilines with humic materials. *J. Environ. Qual.* 29:665–676. doi:10.2134/jeq2000.00472425002900030001x
- DiDonato, N., H.M. Chen, D. Waggoner, and P.G. Hatcher. 2016. Potential origin and formation for molecular components of humic acids in soils. *Geochim. Cosmochim. Acta* 178:210–222. doi:10.1016/j.gca.2016.01.013
- DiDonato, N., and P.G. Hatcher. 2017. Alicyclic carboxylic acids in soil humic acid as detected with ultrahigh resolution mass spectrometry and multi-dimensional NMR. *Org. Geochem.* 112:33–46. doi:10.1016/j.orggeochem.2017.06.010
- Dubus, I.G., C.D. Brown, and S. Beulke. 2003. Sources of uncertainty in pesticide fate modelling. *Sci. Total Environ.* 317:53–72. doi:10.1016/S0048-9697(03)00362-0
- Dutta, A., A. Mandal, S. Manna, S.B. Singh, A.E. Berns, and N. Singh. 2015. Effect of organic carbon chemistry on sorption of atrazine and metsulfuron-methyl as determined by ^{13}C -NMR and IR spectroscopy. *Environ. Monit. Assess.* 187:620. doi:10.1007/s10661-015-4837-3
- Fan, Z., F. Casey, H. Hakk, and G.L. Larsen. 2008. Modeling coupled degradation, sorption, and transport of 17β -estradiol in undisturbed soil. *Water Resour. Res.* 44:W08424. doi:10.1029/2007WR006407
- Farenhorst, A. 2006. Importance of soil organic matter fractions in soil-landscape and regional assessments of pesticide sorption and leaching in soil. *Soil Sci. Soc. Am. J.* 70:1005–1012. doi:10.2136/sssaj2005.0158
- Farenhorst, A., D.A.R. McQueen, I. Saiyed, C. Hilderbrand, S. Li, D.A. Lobb, P. Messing, T.E. Schumacher, S.K. Papiernik, and M.J. Lindstrom. 2009. Variations in soil properties and herbicide sorption coefficients with depth in relation to PRZM (pesticide root zone model) calculations. *Geoderma* 150:267–277. doi:10.1016/j.geoderma.2009.02.002
- Farenhorst, A., I. Saiyed, T.B. Goh, and P. McQueen. 2010. The important characteristics of soil organic matter affecting the sorption of 2,4-dichlorophenoxyacetic acid sorption along a catenary sequence. *J. Environ. Sci. Health Part B* 45:204–213. doi:10.1080/03601231003613542

- Feireira, J.A., L. Martin-Neto, C.M.P. Vaz, and J.B. Regitano. 2002. Sorption interactions between imazaquin and a humic acid extracted from a typical Brazilian Oxisol. *J. Environ. Qual.* 31:1665–1670. doi:10.2134/jeq2002.1665
- Fujitake, N., D. Asakawa, and Y. Yanagi. 2012. Characterization of soil humic acids by C-13 NMR spectroscopy and high performance size exclusion chromatography. *Bunseki Kagaku* 61:287–298. doi:10.2116/bunsekikagaku.61.287
- Fusi, P., M. Franci, and M. Bosetto. 1988. Interaction of fluazifop-butyl and fluazifop with smectites. *Appl. Clay Sci.* 3:63–73. doi:10.1016/0169-1317(88)90006-3
- Gerke, J. 2018. Concepts and misconceptions of humic substances as the stable part of soil organic matter: A review. *Agronomy* 8(5):76. doi:10.3390/agronomy8050076
- Golchin, A., J.M. Oades, J.O. Skjemstad, and P. Clarke. 1994. Soil structure and carbon cycling. *Aust. J. Soil Res.* 32:1043–1068. doi:10.1071/SR9941043
- González-Vila, F.J., J.C. Del Rio, F. Martin, and T. Verdejo. 1996. Pyrolytic alkylation–gas chromatography–mass spectrometry of model polymers: Further insights into the mechanism and scope of the technique. *J. Chromatogr. A* 750:155–160. doi:10.1016/0021-9673(96)00539-0
- Grannas, A.M., R.M. Cory, P.L. Miller, Y.P. Chin, and D.M. McKnight. 2012. The role of dissolved organic matter in arctic surface waters in the photolysis of hexachlorobenzene and lindane. *J. Geophys. Res. Biogeosci.* 117(G1):G01003. doi:10.1029/2010JG001518
- Green, N.W., and E.M. Perdue. 2015. Fast graphically-inspired algorithm for chemical formulae assignment in ultrahigh resolution mass spectrometry. *Anal. Chem.* 87:5086–5094. doi:10.1021/ac504166t
- Gustafsson, J.P. 2001. Modeling the acid-base properties and metal complexation of humic substances with the Stockholm humic model. *J. Colloid Interface Sci.* 244:102–112. doi:10.1006/jcis.2001.7871
- Gustafsson, J.P. 2019. Visual MINTEQ 3.1. <https://vminteq.lwr.kth.se/download/>.
- Gustafsson, J.P., C. Tiberg, A. Edkymish, and D.B. Kleja. 2011. Modelling lead(II) sorption to ferrihydrite and soil organic matter. *Environ. Chem.* 8:485–492. doi:10.1071/EN11025
- Gustafsson, J.P., and J.W.J. van Schaik. 2003. Cation binding in a mor layer: Batch experiments and modelling. *Eur. J. Soil Sci.* 54:295–310. doi:10.1046/j.1365-2389.2003.00526.x
- Haenel, M. 1992. Recent progress in coal structure research. *Fuel* 71:1211–1223. doi:10.1016/0016-2361(92)90046-Q
- Hageman, K.J., C. Bogdal, and M. Scheringer. 2015. Long-range and regional atmospheric transport of POPs and implications for global cycling. *Compr. Anal. Chem.* 67:363–387. doi:10.1016/B978-0-444-63299-9.00011-9
- Haworth, R.D. 1971. The chemical nature of humic acid. *Soil Sci.* 111:71–79. doi:10.1097/00010694-197101000-00009
- He, X.T., S.T. Traina, and T.J. Logan. 1992. Chemical properties of municipal solid waste composts. *J. Environ. Qual.* 21:318–329. doi:10.2134/jeq1992.00472425002100030003x
- He, Z., D.C. Olk, and B.J. Cade-Menun. 2011. Forms and lability of phosphorus in humic acid fractions of Hord silt loam soil. *Soil Sci. Soc. Am. J.* 75:1712–1722. doi:10.2136/sssaj2010.0355
- Hermosin, M.C., and J. Cornejo. 1993. Binding mechanism of 2,4-dichlorophenoxyacetic acid by organo-clays. *J. Environ. Qual.* 22:325–331. doi:10.2134/jeq1993.00472425002200020013x
- Hertkorn, N., M. Frommberger, M. Witt, B.P. Koch, P. Schmitt-Kopplin, and E.M. Perdue. 2008. Natural organic matter and the event horizon of mass spectrometry. *Anal. Chem.* 80:8908–8919. doi:10.1021/ac800464g
- Inbar, Y., Y. Chen, and Y. Hadar. 1989. Solid-state carbon-13 nuclear magnetic resonance and infrared spectroscopy of composted organic matter. *Soil Sci. Soc. Am. J.* 53:1695–1701. doi:10.2136/sssaj1989.03615995005300060014x
- International Humic Substances Society. 2018a. Isolation of IHSS aquatic humic and fulvic acids. <http://humic-substances.org/isolation-of-ihss-aquatic-humic-and-fulvic-acids>.
- International Humic Substances Society. 2018b. What are humic substances? IHSS. <http://humic-substances.org/what-are-humic-substances-2/>.
- Kinniburgh, D.G., C.J. Milne, M.F. Benedetti, J.P. Pinheiro, J. Filius, L.K. Koopal, and W.H. Van Riemsdijk. 1996. Metal ion binding by humic acid: Application of the NICA–Donnan model. *Environ. Sci. Technol.* 30:1687–1698. doi:10.1021/es950695h
- Kleber, M., and M.G. Johnson. 2010. Advances in understanding the molecular structure of soil organic matter: Implications for interactions in the environment. *Adv. Agron.* 106:77–142. doi:10.1016/S0065-2113(10)06003-7
- Kononova, M.M. 1966. Soil organic matter: Its nature, its role in soil formation and in soil fertility. 2nd ed. Pergamon Press, Oxford, UK.
- Koprivnjak, J.-F., P.H. Pfromm, E. Ingall, T.A. Vetter, P. Schmitt-Kopplin, N. Hertkorn, M. Frommberger, H. Knicker, and E.M. Perdue. 2009. Chemical and spectroscopic characterization of marine dissolved organic matter isolated using coupled reverse osmosis–electrodialysis. *Geochim. Cosmochim. Acta* 73:4215–4231. doi:10.1016/j.gca.2009.04.010
- Kulikova, N., and I. Perminova. 2002. Binding of atrazine to humic substances from soil, peat, and coal related to their structure. *Environ. Sci. Technol.* 36:3720–3724. doi:10.1021/es015778e
- LaHue, G.T., R.L. Chaney, M.A. Adviento-Borbe, and B.A. Linquist. 2016. Alternate wetting and drying in high yielding direct-seeded rice systems accomplishes multiple environmental and agronomic objectives. *Agric. Ecosyst. Environ.* 229:30–39. doi:10.1016/j.agee.2016.05.020
- Latch, D.E., and K. McNeill. 2006. Microheterogeneity of singlet oxygen distributions in irradiated humic acid solutions. *Science* 311:1743–1747. doi:10.1126/science.1121636
- Leenheer, J.A., R.L. Wershaw, and M.M. Reddy. 1995. Strong acid, carboxyl group structures in fulvic acid from the Suwannee River, Georgia: I. Minor structures. *Environ. Sci. Technol.* 29:393–398. doi:10.1021/es00002a015
- Legorreta-Padilla, F.J. 2005. The impact of maize and soybean cropping systems on carbon and nitrogen dynamics in soil organic matter. Ph.D. diss., University of Nebraska, Lincoln.
- Lehmann, J., and M. Kleber. 2015. The contentious nature of soil organic matter. *Nature* 528:60–68.
- Lehmann, J., D. Solomon, J. Kinyangi, L. Dathe, S. Wirick, and C. Jacobsen. 2008. Spatial complexity of soil organic matter forms at nanometre scales. *Nat. Geosci.* 1:238–242. doi:10.1038/ngeo155
- Li, H.Y., and E.C. Minor. 2015. Dissolved organic matter in Lake Superior: insights into the effects of extraction methods on chemical composition. *Environ. Sci. Processes Impacts* 17:1829–1840. doi:10.1039/c5em00199d
- Linquist, B.A., M.M. Anders, M.A.A. Adviento-Borbe, R.L. Chaney, L.L. Nalley, E.F.F. da Rosa, and C. van Kessel. 2015. Reducing greenhouse gas emissions, water use, and grain arsenic levels in rice systems. *Glob. Change Biol.* 21:407–417.
- Lu, X.Q., J.V. Hanna, and W.D. Johnson. 2000. Source indicators of humic substances: An elemental composition, solid state ¹³C CP/MAS NMR and Py-GC/MS study. *Appl. Geochem.* 15:1019–1033. doi:10.1016/S0883-2927(99)00103-1
- MacCarthy, P. 2001. The principles of humic substances. *Soil Sci.* 166:738–751. doi:10.1097/00010694-200111000-00003
- Mahieu, N., D.C. Olk, and E.W. Randall. 2002. Multinuclear magnetic resonance analysis of two humic acid fractions from lowland rice soils. *J. Environ. Qual.* 31:421–430. doi:10.2134/jeq2002.4210
- Mao, J., D.C. Olk, X. Fang, Z. He, and K. Schmidt-Rohr. 2008. Influence of animal manure application on the chemical structures of soil organic matter as investigated by advanced solid-state NMR and FT-IR spectroscopy. *Geoderma* 146:353–362. doi:10.1016/j.geoderma.2008.06.003
- Massey, J.H., T.W. Walker, M.M. Anders, M.C. Smith, and L.A. Avila. 2014. Farmer adaptation of intermittent flooding using multiple-inlet rice irrigation in Mississippi. *Agric. Water Manage.* 146:297–304. doi:10.1016/j.agwat.2014.08.023
- McAdams, B.C., G.R. Aiken, D.M. McKnight, W.A. Arnold, and Y.P. Chin. 2018. High pressure size exclusion chromatography (HPSEC) determination of dissolved organic matter molecular weight revisited: Accounting for changes in stationary phases, analytical standards, and isolation methods. *Environ. Sci. Technol.* 52:722–730. doi:10.1021/acs.est.7b04401
- Neale, P.A., A. Antony, W. Gernjak, G. Leslie, and B.I. Escher. 2011. Natural versus wastewater derived dissolved organic carbon: Implications for the environmental fate of organic micropollutants. *Water Res.* 45:4227–4237. doi:10.1016/j.watres.2011.05.038
- Neale, P.A., B.I. Escher, K.U. Goss, and S. Endo. 2012. Evaluating dissolved organic carbon–water partitioning using polyparameter linear free energy relationships: Implications for the fate of disinfection by-products. *Water Res.* 46:3637–3645. doi:10.1016/j.watres.2012.04.005
- Niederer, C., R. Schwarzen, and K. Goss. 2007. Elucidating differences in the sorption properties of 10 humic and fulvic acids for polar and nonpolar organic chemicals. *Environ. Sci. Technol.* 41:6711–6717. doi:10.1021/es0709932
- Niemeyer, J., Y. Chen, and J.M. Bollag. 1992. Characterization of humic acids, composts and peat by diffuse reflectance Fourier transform infrared (DRIFT) spectroscopy. *Soil Sci. Soc. Am. J.* 56:135–140. doi:10.2136/sssaj1992.03615995005600010021x
- Nolan, B.T., R.W. Malone, J.E. Doherty, J.E. Barbash, L. Ma, and D.L. Shaner. 2015. Data worth and prediction uncertainty for pesticide transport and fate models in Nebraska and Maryland, United States. *Pest Manag. Sci.* 71:972–985. doi:10.1002/ps.3875
- Olk, D.C. 2006. A chemical fractionation for structure–function relations of soil organic matter in nutrient cycling. *Soil Sci. Soc. Am. J.* 70:1013–1022. doi:10.2136/sssaj2005.0108

- Olk, D.C., M.M. Anders, T.R. Filley, and C. Isbell. 2009. Crop nitrogen uptake and soil phenols accumulation under continuous rice cropping in Arkansas. *Soil Sci. Soc. Am. J.* 73:952–960. doi:10.2136/sssaj2008.0069
- Olk, D.C., K.G. Cassman, and T.W.M. Fan. 1995. Characterization of two humic acid fractions from a calcareous vermiculitic soil: Implications for the humification process. *Geoderma* 65:195–208. doi:10.1016/0016-7061(95)94048-9
- Olk, D.C., K.G. Cassman, E.W. Randall, P. Kinches, L.J. Sanger, and J.M. Anderson. 1996. Changes in chemical properties of organic matter with intensified rice cropping in tropical lowland soil. *Eur. J. Soil Sci.* 47:293–303. doi:10.1111/j.1365-2389.1996.tb01403.x
- Olk, D.C., M.C. Dancel, E. Moscoso, R.R. Jimenez, and F.M. Dayrit. 2002. Accumulation of lignin residues in organic matter fractions of lowland rice soils: A pyrolysis-GC-MS study. *Soil Sci.* 167:590–606. doi:10.1097/00010694-200209000-00004
- Olk, D.C., and E.G. Gregorich. 2006. Overview of the symposium proceedings, “Meaningful pools in determining soil carbon and nitrogen dynamics. *Soil Sci. Soc. Am. J.* 70:967–974. doi:10.2136/sssaj2005.0111
- Orlov, D.S. 1985. Humic acids of soils. Amerind, New Delhi.
- Perdue, E.M., and J.D. Ritchie. 2014. Dissolved organic matter in freshwaters. *Treatise Geochem.* 7:237–272. doi:10.1016/B978-0-08-095975-7.00509-X
- Peuravuori, J., T. Lehtonen, and K. Pihlaja. 2002. Sorption of aquatic humic matter by DAX-8 and XAD-8 resins: Comparative study using pyrolysis gas chromatography. *Anal. Chim. Acta* 471:219–226. doi:10.1016/S0003-2670(02)00931-5
- Peuravuori, J., P. Žbáňková, and K. Pihlaja. 2006. Aspects of structural features in lignite and lignite humic acids. *Fuel Process. Technol.* 87:829–839. doi:10.1016/j.fuproc.2006.05.003
- Piccolo, A. 2001. The supramolecular structure of humic substances. *Soil Sci.* 166:810–832. doi:10.1097/00010694-200111000-00007
- Piccolo, A., and G. Celano. 1994. Hydrogen bonding interactions between the herbicide glyphosate and water-soluble humic substances. *Environ. Toxicol. Chem.* 13:1737–1741. doi:10.1002/etc.5620131104
- Piccolo, A., G. Celano, and P. Conte. 1996. Adsorption of glyphosate by humic substances. *J. Agric. Food Chem.* 44:2442–2446. doi:10.1021/jf950620x
- Piccolo, A., G. Celano, and C. De Simone. 1992. Interactions of atrazine with humic substances of different origins and their hydrolysed products. *Sci. Total Environ.* 117–118:403–412. doi:10.1016/0048-9697(92)90106-3
- Plaza, C., and N. Senesi. 2009. The effect of organic matter amendment on native soil humic substances. In: N. Senesi et al., editors, *Biophysicochemical processes involving natural nonliving organic matter in environmental systems*. John Wiley & Sons, Hoboken, NJ. p. 147–181. doi:10.1002/9780470494950.ch4
- Porras, J., J.J. Fernandez, R.A. Torres-Palma, and C. Richard. 2014. Humic substances enhance chlorothalonil phototransformation via photoreduction and energy transfer. *Environ. Sci. Technol.* 48:2218–2225. doi:10.1021/es404240x
- Prietz, J., S. Müller, I. Kögel-Knabner, J. Thieme, C. Jaye, and D. Fischer. 2018. Comparison of soil organic carbon speciation using C NEXAFS and CP-MAS ¹³C NMR spectroscopy. *Sci. Total Environ.* 628–629:906–918. doi:10.1016/j.scitotenv.2018.02.121
- Remucal, C.K. 2014. The role of indirect photochemical degradation in the environmental fate of pesticides: A review. *Environ. Sci. Process. Impacts* 16:628–653. doi:10.1039/c3em00549f
- Ritchie, J.D., and E.M. Perdue. 2008. Analytical constraints on acidic functional groups in humic substances. *Org. Geochem.* 39:783–799. doi:10.1016/j.orggeochem.2008.03.003
- Schmidt, M.W.I., M.S. Torn, S. Abiven, T. Dittmar, G. Guggenberger, I.A. Janssens, M. Kleber, I. Kögel-Knabner, J. Lehmann, D.A.C. Manning, P. Nannipieri, D.P. Rasse, S. Weiner, and S.E. Trumbore. 2011. Persistence of soil organic matter as an ecosystem property. *Nature* 478:49–56. doi:10.1038/nature10386
- Schmidt-Rohr, K., J.-D. Mao, and D.C. Olk. 2004. Nitrogen-bonded aromatics in soil organic matter and their implications for a yield decline in intensive rice cropping. *Proc. Natl. Acad. Sci. USA* 101:6351–6354. doi:10.1073/pnas.0401349101
- Schnitzer, M., and S.U. Khan. 1972. Humic substances in the environment. Marcel Dekker, New York.
- Schnitzer, M., and C.M. Monreal. 2011. *Quo vadis* soil organic matter research? A biological link to the chemistry of humification. *Adv. Agron.* 113:143–217. doi:10.1016/B978-0-12-386473-4.00003-8
- Schnitzer, M., and S.I.M. Skinner. 1968. Alkali versus acid extraction of soil organic matter. *Soil Sci.* 105:392–396. doi:10.1097/00010694-196806000-00003
- Schulten, H.-R., and M. Schnitzer. 1997. Chemical model structures for soil organic matter and soils. *Soil Sci.* 162:115–130. doi:10.1097/00010694-199702000-00005
- Semenov, V.M., A.S. Tulina, N.A. Semenova, and L.A. Ivannikova. 2013. Humification and nonhumification pathways of the organic matter stabilization in soil: A review. *Eurasian Soil Sci.* 46:355–368. doi:10.1134/S106422931304011X
- Senesi, N. 1992. Binding mechanisms of pesticides to soil humic substances. *Sci. Total Environ.* 123–124:63–76. doi:10.1016/0048-9697(92)90133-D
- Senesi, N., and G. Brunetti. 1996. Chemical and physicochemical parameters for quality evaluation of humic substances produced during composting. In: M. De Bertoldi, P. Sequi, B. Lemmes, and T. Papi, editors, *The science of composting*. Chapman & Hall, London. p. 195–212. doi:10.1007/978-94-009-1569-5_20
- Senesi, N., and Y. Chen. 1989. Interactions of toxic chemicals with humic substances. In: Z. Gerstl et al., editors, *Toxic organic chemicals in porous media*. Springer, Berlin. p. 37–90. doi:10.1007/978-3-642-74468-6_3
- Senesi, N., V. D’Orazio, and T.M. Miano. 1995. Adsorption mechanisms of triazine and bipyridium herbicides on humic acids from hop field soils. *Geoderma* 66:273–283. doi:10.1016/0016-7061(94)00083-M
- Senesi, N., P. La Cava, and T.M. Miano. 1997. Adsorption of imazethapyr to amended and nonamended soils and humic acids. *J. Environ. Qual.* 26:1264–1270. doi:10.2134/jeq1997.00472425002600050010x
- Sheals, J., S. Sjöberg, and P. Persson. 2002. Adsorption of glyphosate on goethite: Molecular characterization of surface complexes. *Environ. Sci. Technol.* 36:3090–3095. doi:10.1021/es010295w
- Sierra, M.M.D., A.N. Fernandes, and B. Szpoganicz. 2004. Influence of amide linkages on acidity determinations of humic substances: Testing with model-mixtures. *Talanta* 62:687–693. doi:10.1016/j.talanta.2003.09.021
- Six, J., H. Bossuyt, S. Degryze, and K. Denef. 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. *Soil Tillage Res.* 79:7–31. doi:10.1016/j.still.2004.03.008
- Skene, T.M., P. Clarke, J.O. Skjemstad, and J.M. Oades. 1997. Browning reactions between *Eucalyptus* litter and different nitrogen sources. *Aust. J. Soil Res.* 35:1085–1091. doi:10.1071/S96116
- Solomon, D., J. Lehmann, J. Kinyangi, B. Liang, and T. Schafer. 2005. Carbon K-edge NEXAFS and FTIR-ATR spectroscopic investigation of organic carbon speciation in soils. *Soil Sci. Soc. Am. J.* 69:107–119. doi:10.2136/sssaj2005.0107dup
- Stevenson, F.J. 1982. Humus chemistry: Genesis, composition, reactions. John Wiley & Sons, New York.
- Stevenson, F.J. 1994. Humus chemistry: Genesis, composition, reactions, 2nd ed. John Wiley & Sons, New York.
- Sutton, R., and G. Sposito. 2005. Molecular structure in humic substances: The new view. *Environ. Sci. Technol.* 39:9009–9015. doi:10.1021/es050778q
- Swift, R. 1996. Organic matter characterization. In: D.L. Sparks, et al., editors, *Methods of soil analysis. Part 3. Chemical methods*. SSSA Book Series 5. SSSA, Madison, WI. p. 1011–1069.
- Swift, R.S., and A.M. Posner. 1972. Autoxidation of humic acid under alkaline conditions. *J. Soil Sci.* 23:381–393. doi:10.1111/j.1365-2389.1972.tb01669.x
- Thevenot, M., M.-F. Dignac, and C. Rumpel. 2010. Fate of lignins in soils: A review. *Soil Biol. Biochem.* 42:1200–1211. doi:10.1016/j.soilbio.2010.03.017
- Thurman, E.M., and R.L. Malcolm. 1981. Preparative isolation of aquatic humic substances. *Environ. Sci. Technol.* 15:463–466. doi:10.1021/es00086a012
- Tiberg, C., C. Sjöstedt, and J.P. Gustafsson. 2018. Metal sorption to Spodosol Bs horizons: Organic matter complexes predominate. *Chemosphere* 196:556–565. doi:10.1016/j.chemosphere.2018.01.004
- Tipping, E., S. Lofts, and J.E. Sonke. 2011. Humic ion-binding model VII: A revised parameterisation of cation-binding by humic substances. *Environ. Chem.* 8:225–235. doi:10.1071/EN11016
- Tisdall, J.M., and J.M. Oades. 1982. Organic matter and water soluble aggregates in soils. *J. Soil Sci.* 33:141–163.
- Trumbore, S.E., and S. Zheng. 1996. Comparison of fractionation methods for soil organic matter ¹⁴C analysis. *Radiocarbon* 38:219–229. doi:10.1017/S0033822200017598
- Uhle, M.E., Y.P. Chin, G.R. Aiken, and D.M. McKnight. 1999. Binding of polychlorinated biphenyls to aquatic humic substances: The role of substrate and sorbate properties on partitioning. *Environ. Sci. Technol.* 33:2715–2718. doi:10.1021/es9808447
- Ve, N.B., D.C. Olk, and K.G. Cassman. 2004. Characterization of humic acid fractions improves estimates of nitrogen mineralization kinetics for lowland rice soils. *Soil Sci. Soc. Am. J.* 68:1266–1277. doi:10.2136/sssaj2004.1266
- Vetter, T.A., E.M. Perdue, E. Ingall, J.-F. Koprivnjak, and P.H. Pfromm. 2007. Combining reverse osmosis and electrodialysis for more complete recovery of dissolved organic matter from seawater. *Separ. Purif. Tech.* 56:383–387. doi:10.1016/j.seppur.2007.04.012

- von Lützow, M., I. Kögel-Knabner, K. Ekschmitt, H. Flessa, G. Guggenberger, E. Matzner, and B. Marschner. 2007. SOM fractionation methods: Relevance to functional pools and to stabilization mechanisms. *Soil Biol. Biochem.* 39:2183–2207. doi:10.1016/j.soilbio.2007.03.007
- Waggoner, D.C., H. Chen, A.S. Willoughby, and P.G. Hatcher. 2015. Formation of black carbon-like and alicyclic aliphatic compounds by hydroxyl radical initiated degradation of lignin. *Org. Geochem.* 82:69–76. doi:10.1016/j.orggeochem.2015.02.007
- Waggoner, D.C., and P.G. Hatcher. 2017. Hydroxyl radical alteration of HPLC fractionated lignin: Formation of new compounds from terrestrial organic matter. *Org. Geochem.* 113:315–325. doi:10.1016/j.orggeochem.2017.07.011
- Waggoner, D.C., A.S. Wozniak, R.M. Cory, and P.G. Hatcher. 2017. The role of reactive oxygen species in the degradation of lignin derived dissolved organic matter. *Geochim. Cosmochim. Acta* 208:171–184. doi:10.1016/j.gca.2017.03.036
- Wang, C.-F., X. Fan, F. Zhang, S.-Z. Wang, Y.-P. Zhao, X.-Y. Zhao, W. Zhao, T.-G. Zhu, J.-L. Lu, and X.-Y. Wei. 2017. Characterization of humic acids extracted from a lignite and interpretation for the mass spectra. *RSC Advances* 7:20,677–20,684. doi:10.1039/C7RA01497J
- Wang, X., X. Guo, Y. Yang, S. Tao, and B. Xing. 2011. Sorption mechanisms of phenanthrene, lindane, and atrazine with various humic acid fractions from a single soil sample. *Environ. Sci. Technol.* 45:2124–2130. doi:10.1021/es102468z
- Wei-Haas, M.L., K.J. Hageman, and Y.P. Chin. 2014. Partitioning of polybrominated diphenyl ethers to dissolved organic matter isolated from arctic surface waters. *Environ. Sci. Technol.* 48:4852–4859. doi:10.1021/es405453m
- Wershaw, R.L. 1993. Model for humus in soils and sediments. *Environ. Sci. Technol.* 27:814–816. doi:10.1021/es00042a603
- Wickings, K., A.S. Grandy, S.C. Reed, and C.C. Cleveland. 2012. The origin of litter chemical complexity during decomposition. *Ecol. Lett.* 15:1180–1188. doi:10.1111/j.1461-0248.2012.01837.x
- Wingeyer, A.B., D.T. Walters, R.A. Drijber, D.C. Olk, T.J. Arkebauer, S.B. Verma, D.A. Wedin, and C.A. Francis. 2012. Fall conservation deep tillage stabilizes maize residues into soil organic matter. *Soil Sci. Soc. Am. J.* 76:2154–2163.
- Xing, B., and Z. Chen. 1999. Spectroscopic evidence for condensed domains in soil organic matter. *Soil Sci.* 164:40–47. doi:10.1097/00010694-199901000-00006
- Zech, W., N. Senesi, G. Guggenberger, K. Kaiser, J. Lehmann, T.M. Miano, A. Miltner, and G. Schroth. 1997. Factors controlling humification and mineralization of soil organic matter in the tropics. *Geoderma* 79:117–161. doi:10.1016/S0016-7061(97)00040-2
- Zhang, D., D. Hui, Y. Luo, and G. Zhou. 2008. Rates of litter decomposition in terrestrial ecosystems: Global patterns and controlling factors. *J. Plant Ecol.* 1:85–93. doi:10.1093/jpe/rtn002