The contentious nature of Soil Organic Matter – what is it and why should we care what it is?

Markus Kleber & Johannes Lehmann
Defining soil organic matter

Long known: topsoils contain an amorphous, darkish organic material that is strongly related to soil fertility

Interest in Soil Organic Matter origin and function probably as old as farming lifestyle
Organic Matter Decomposition and Soil Fertility

Also long known: To enhance fertility, soil organic matter must decompose!


Tull observed that tillage enhanced decomposition of soil organic matter, which in turn promoted plant growth and fertility.
By the early 1980’s, interest in soil organic matter had started to move beyond agricultural applications:

1. The broader scientific community becomes aware of a significant increase in atmospheric CO$_2$

2. Budget calculations reveal soil carbon to be the largest of the fast cycling carbon pools in the Biosphere (the others are oceans, vegetation and atmosphere)

3. Computers become powerful enough to simulate future trends in global climate

4. Novel methodologies and supramolecular chemistry cast doubts on the polymeric macromolecular nature of soil organic matter, thereby questioning the inherent stability of “humic” matter
Realisation: $\text{CO}_2$ accumulating in the atmosphere

$\rightarrow$ $\text{CO}_2$ (and $\text{CH}_4$) concentrations in the atmosphere are **rising**
CO$_2$ in the atmosphere and soil C are linked!

human activity increases CO$_2$ content in atmosphere

More CO$_2$ in atmosphere means more heat is retained = higher temperature

warmer temps induce decomposer microorganisms to be more active and decompose more SOIL ORGANIC MATTER and thus produce (respire) more CO$_2$ and other trace gases


Scheffer, M; Brovkin, P & PM Cox. 2006. Positive feedback between global warming and atmospheric CO$_2$ concentration inferred from past climate change. GEOPHYSICAL RESEARCH LETTERS, VOL. 33, L10702, 2006
Three major soil carbon – global climate feedbacks

Permafrost thawing

Priming effects of C additions to subsoils

“Heimann & Reichstein 2008 Nature

“terrestrial ecosystems will provide a positive feedback on climate in a warming world”
Three major soil carbon – global climate feedbacks

- Permafrost thawing
- Excess N - inputs

Heimann & Reichstein 2008 Nature

→ Calls to stabilize or, where possible, increase soil carbon levels!
Isn’t there an easy fix?

When there is a lack of carbon in soil, can we not just put some carbon back in - preferentially a type of carbon that does not decompose easily?
The problem: ecosystems rely on carbon turnover!

“Soil organic matter is far more than a potential tank for impounding excess CO$_2$; it is a relentless flow of C atoms, through a myriad of streams—some fast, some slow—wending their way through the ecosystem, driving biotic processes along the way.”

Remember Jethro Tull!

“A relentless flow of Carbon”
“...in our new-found zeal for locking up C, do we overlook a fundamental precept: that organic matter has most benefit, biologically, when it decays?”

Sequestration or flux management?

“...when we aim to regain some of the C lost, we may need new ways of thinking about soil C dynamics, and tuning them for the services expected of our ecosystems.”

To develop those new ways of thinking...

...we need to understand: what exactly is soil organic matter?

- Is the historic view of the **stable Humus**
  (= organic matter as a “recalcitrant” product of secondary)
  **still defensible**?

- or should we rather conceptualise soil organic matter as a “continuous flow of C-atoms” in the sense of Janzen?
To sort this out, let's start at the beginning:

In almost any soil, we observe an amorphous, darkish organic material.

We cannot say much about its properties by just looking at it.

We have to have a conceptual model of what this “darkish amorphous material” actually is, in order to understand its role in terrestrial biogeochemistry.
The accepted truths as postulated in our textbooks:

“Soil organic matter (SOM) derives from dead plant parts in the litter layer”

“A fraction of SOM persists because it can resist decomposition.”

“The process which converts litter into resistant or ‘recalcitrant’ soil organic matter was called **humification**”

The product of humification = presumably ‘stable’ organic matter = is called **humus**
Photosynthesis

\[ H_2O; CO_2 \]

Plant respiration

Harvest

Surface litter

Decomposition

Soil organic matter

"Stable" Humus
The paradigm of secondary synthesis = humification

The ecologically relevant part of soil organic matter ("humus") is the product of **secondary synthesis**, also called "humification"

Assumption: organic fragments in various stages of decomposition are synthesized into **new materials**, called humic substances
In search of the “humic” molecule

The polymer concept implies that “humic molecules” have a certain, identifiable molecular structure(s)

Many such structures have been proposed (= modeled), such as the Schulten/Schnitzer model shown here

but to date there is no report of the isolation of a unique “geopolymer” from soils

(= a molecule whose properties are independent of the extraction procedure)

Model affects assumptions about mechanisms

Traditional models (i.e. Stevenson 1985) assume the main organic counterparts for mineral organic interaction to be **macromolecular polymers**:


“It becomes more and more apparent that humic substances are not single molecules but rather associations of molecules...”

Schnitzer and Khan 1978, page 46

But:

“Humic substances are huge molecules...with molecular weights varying from 2000 to 300 000 g mol\(^{-1}\). They are the organic materials most resistant to microbial attack.”

Brady and Weil 2008, page 510
Scepticism

There were sceptics from the beginning:

Van Bemmelen (1830-1911): “The determination of that part of the soil humus, which is soluble in dilute alkalies... can teach us very little. Such simple procedures are entirely unsatisfactory in order to judge the value of the humus.

Nobel Laureate Selman Waksman (Waksman 1936) writes: “One may feel justified in abandoning without reservation the whole nomenclature of “humic acids”. These labels designate not definite compounds, but merely certain preparations, which have been obtained by specific procedures.
A growing concern

If the humic polymer model is not correct,

much of the traditional understanding of the mechanisms of carbon turnover and carbon “stabilization” in soils is most likely incorrect as well

So where are we today?
The evolution of the „New View“

Over the last 3-4 decades, the scientific community started to question the humus model of soil organic matter. There were a number of reasons to do so:

a) nobody had managed to elucidate the “humification” process beyond conflicting theories

b) the advent of new analytical techniques, especially NMR, allowed for direct observation of organic matter, avoiding the artifacts of alkaline extraction

c) the evolution of a whole new discipline of chemistry (Supramolecular Chemistry, Nobel prize 1987) provided new mechanistic explanations* for observed phenomena

The new View: a Continuum of decomposition fragments
The „Continuum Model“: there is no secondary synthesis!
The evidence supports the continuum model


“Surprisingly,

nearly all of the NMR signals in traditional Humic Substance fractions could be assigned to intact and degrading biopolymers.”

“We therefore suggest that the vast majority of operationally defined humic material in soils is a very complex mixture of microbial and plant biopolymers and their degradation products but not a distinct chemical category”
Direct search finds no evidence for “humic substances” in soil

The investigation of Lehmann et al. 2008:

→ If there are such things as humic substances, we should be able to observe and identify them with advanced spectromicroscopic techniques, such as in STXM/ NEXAFS*

*Scanning Transmission X-ray Microscopy / Near Edge X-ray Absorption Fine Structure Spectrometry

OM chemistry of alkaline extracts = Humic Substances

C-1s NEXAFS spectrum of alkaline extract or “humic substance”:

three distinct signals at

- 285.0 (aromatic C)
- 286.7 (phenolic C)
- 288.6 (amid and carboxyl C)

The “real” OM chemistry across microaggregate

Spectral signature of “native” OM is much different from that of the alkaline extract (= the alleged humic substance)!
Persistence does not correlate with “recalcitrance”

Assumptions about organic matter dynamics that are based on an alleged inherent molecular recalcitrance of “humic” substances (such as the Carbon – Quality-Temperature or CQT-theory) have no robust mechanistic foundation.

<table>
<thead>
<tr>
<th>Soil Type</th>
<th>Turnover Time (years)</th>
<th>Aromaticity (NEXAFS)</th>
<th>Thermal “Lability” (DSC)</th>
<th>Acid / Aldehyde (CuO)</th>
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<tr>
<td>Oxisol</td>
<td>107</td>
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Implications for soil carbon management

“Humic Polymer” versus “Continuum Model”

large, covalently bonded “humic” polymers made during “humification”

supramolecular aggregates of degradation fragments

Polymers with strong covalent bonds formed by secondary synthesis

Fragments self-assemble through weak, non-covalent bonds

**implies** molecular stability and inherent resistance to decomposition, also called “recalcitrance”

does NOT provide a structural argument for inherent stability against decomposition
Management and policy depend on the SOM - model

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Water holding capacity, ion exchange capacity, and soil physical structure are critical for healthy soils. According to the traditional “humic” model, soils will be “healthy” when they are saturated with stable, recalcitrant “humus.” In contrast, the evidence-based “continuum” model suggests that soils will be “healthy” and fertile when we ensure constant production of reactive, assimilable, and labile carbon.
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<td>misinterpretes reactivity of aqueous NOM based on a physically non-existing proxy = humic substances</td>
<td>accept chemical diversity of aqueous carbon forms and the need to investigate dissolved organic compounds independent of alkaline extraction</td>
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Conclusions

There is strong evidence suggesting that the historic “humus” model of the physical and chemical nature of Soil Organic Matter is critically flawed.

If we are serious about handing functional agroecosystems down to the generations to come, we must turn to modern, evidence based concepts, and abandon the operational proxies of the past.
Thank you for your attention!
Spares
The competing “Continuum Model”

- Soil organic matter (SOM)
  - (1) Partially degraded plant litter
  - (2) Living and dead microorganisms
  - (3) Organics attached to mineral surfaces
  - (4) Thermally altered materials (black carbon)
  - (5) Metal-organic complexes/precipitates

- Organic matter in aqueous phase (DOM)
  - (1) Truly dissolved DOM
  - (2) Colloidal DOM

Piccolo 2000; Wershaw 2004; Sutton and Sposito 2005
The way forward

Old: Humic Polymer
- large polymer
- polyanion
- N in heterocycles
- random coil
- nonhumics excluded
- formed by "secondary synthesis"

New: Molecular aggregate
- low molecular mass fragments
- amphiphilic
- N in amide groups
- Supramolecular aggregate
- biomolecules included
- most carboxylate groups form during oxidative depolymerisation
What is the difference

**Progressive Decomposition**
- Carbon input (plant litter/dead organisms)
  - enzymatic oxidation and depolymerization
  - assimilable C, soluble and MW < ≈ 600 Da
  - microbial uptake
  - microbial biomass, CO₂

**Selective Preservation**
- Carbon input (plant litter/dead organisms)
  - “labile” (carbohydrates, protein)
  - fast enz. oxidation & depolymerization
  - assimilable C, soluble and MW < ≈ 600 Da
  - microbial uptake
  - microbial biomass, CO₂

**Humification/Secondary Synthesis**
- Carbon input (plant litter/dead organisms)
  - “recalcitrant” (lignin, lipids, char)
  - slow enz. oxidation & depolymerization
  - enzymatic oxidation and depolymerization
  - monomers
  - secondary synthesis aka “humification”
  - polymeric macromolecules, resistant to decomposition = humic substances
  - “stable Humus”
Large organic debris, biopolymers

Exo-enzymatic oxidation and depolymerization

‘assimilable’ fragmentation Products
(< 600-800 Da = small enough for cell wall passage)

Assimilation and respiration

→ obeys second law of thermodynamics = energy must be dissipated and disorder must be increased
Large organic debris, biopolymers

HN (NOSC = -0.08)

HA (NOSC = 0.04)

FA (NOSC = 0.35)

“Secondary synthesis”

‘assimilable’ fragmentation Products
(< 600-800 Da = small enough for cell wall passage)

Exo-enzymatic oxidation and depolymerization

Assimilation and respiration

$\rightarrow$ requires energy input = not clear where this energy is sourced and what the ecological benefits of such an energy investment would be

“Energy-rich compounds”

Nominal oxidation state of the Carbon (NOSC)

“Energy-poor”

CO$_2$, HCO$_3^-$

“Secondary synthesis” ('humification') Model
## Contradicting definitions for „humic substances“

Table 1: Selection of definitions for "humic substances" to illustrate the extent of semantic variation in the use of the term. For more examples consult Table S1 in the supplement.

<table>
<thead>
<tr>
<th>Community</th>
<th>Bottom line</th>
<th>Definition verbatim</th>
<th>Reference</th>
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<tr>
<td>General Public</td>
<td>all-inclusive term for non-living organic materials in the environment</td>
<td>&quot;humic substances are the major organic constituents of soil (humus), peat, coal, many upland streams, dystrophic lakes and ocean water&quot;</td>
<td><a href="http://en.wikipedia.org/wiki/Humic_acid">http://en.wikipedia.org/wiki/Humic_acid</a></td>
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<tr>
<td>Aquatic chemistry</td>
<td>a significant fraction of the bulk of organic matter in moist soils and waters</td>
<td>&quot;Humic substances may be described as polymers (300 – 30000 molecular weight) containing phenolic OH and carboxylic groups with a lower number of aliphatic groups&quot;</td>
<td>Stumm and Morgan 1996</td>
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<td>Soil Science, traditional</td>
<td>a generic term to describe colored organic material or its fractions on the basis of solubility characteristics</td>
<td>&quot;A series of relatively high-molecular-weight, yellow to black colored substances formed by secondary synthesis reactions&quot;</td>
<td>Stevenson 1994</td>
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<tr>
<td>Environmental Science</td>
<td>attempt to constrain molecular properties of alkaline extracts</td>
<td>&quot;humic substances are collections of diverse, relatively low molecular mass components forming dynamic associations stabilized by hydrophobic interactions and hydrogen bonds&quot;</td>
<td>Sutton and Sposito 2005</td>
</tr>
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<td>Soil Science, modern</td>
<td>effort to reduce variations in the use of the term</td>
<td>Organic molecules with chemical structures which do not allow them to be placed into the category of non-humic biomolecules</td>
<td>Baldock and Broos 2011</td>
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The classic view (as it continues to appear in textbooks)

**Soil organic matter**

- Removal of dissolved organic carbon and macroorganic matter by water extraction and particle size and density fractionation

**Humus**

- Dissolved organic carbon < 0.45 μm
- Plant litter, free and occluded particulate carbon, and charcoal and charred residues

**Humic substances**

- Fractionation on the basis of extractability in alkali and solubility on acidification of alkaline extracts

- Nonextractable in alkali
  - Humic acid fraction
  - Humin
- Alkali extractable acid insoluble
  - Fulvic acid fraction
- Alkali extractable acid soluble

**Nonhumic biomolecules of plant and microbial origin**

- Carbohydrates
  - Hydrolysis
  - Hexoses
  - Pentoses
  - Uronioacids
  - Amino sugars

- Proteinaceous structures
  - Hydrolysis
  - Amino acids

- Lignin residues
  - Thermochemicalysis
  - A range of phenylpropanoid monomeric structures

- Aliphatic structures
  - Extraction
  - A range of fatty acid structures

---

Baldock and Nelson, 2000, Handbook of soil science
1987: Nobel Prize for Chemistry awarded to Donald Cram, Jean-Marie Lehn and Charles Pedersen for their contributions to supramolecular chemistry

Supramolecular chemistry is the science of the formation of complex, functional structures by molecular self-assembly through noncovalent interactions.

Molecular self-assembly is exhibited in the self-assembly of lipids to form the membrane, the formation of double helical DNA through hydrogen bonding of the individual strands, and the assembly of proteins to form quaternary structures.
Supramolecular assemblies

Micelle

Liposome

Phospholipid bilayer
This condition is called **amphiphilicity**.

Low molecular mass „component“ molecules have, at the same time, **hydrophilic** (polar or ionic) molecular segments and **hydrophobic** molecular segments.

This condition is called **amphiphilicity**.
Definitions

**hydrophilic:**
‘Water loving’. The capacity of a molecular entity or of a substituent to interact with polar solvents, in particular with water, or with other polar groups.
hydrophobic: the tendency of hydrocarbons (or of lipophilic hydrocarbon-like groups in solutes) to form intermolecular aggregates in an aqueous medium, and analogous intramolecular interactions. Water loving'. The capacity of a molecular entity or of a substituent to interact with polar solvents, in particular with water, or with other polar groups.
amphiphilic:
term used to describe a compound containing a large organic cation or anion which possesses a long unbranched hydrocarbon chain. The existence of distinct polar (hydrophilic) and nonpolar (hydrophobic) regions in the molecule promotes the formation of micelles in dilute aqueous solution
component molecules

draw hydrophilic exterior

behave in aqueous environments like surfactants and form *micelle* – like structures

hydrophobic core
The first application of the new idea to soil organic matter.
Molecular aggregate model: the former 'humic' substances are (i) low molecular mass components...where the unaltered portion will be relatively nonpolar.

...while carboxyl groups are formed during oxidative depolymerization.

derived from the decomposition of biomolecules
Recognizable biomolecules may be associated with ‘molecular aggregates’

Xinfeng Gao and T. C. Wong, "A Molecular Dynamics Investigation of the Structure and Dynamics of ACTH (1-10) peptides partitioned in a Dodecylphosphocholine (DPC) Micelle", *Biopolymers*, 58, 643-649, 2001
2D-NMR does not find „humic“ polymers

(A) biopolymers, lignin (grey), amylopectin (red), albumin (blue), and cuticle (green)
2D-NMR does not find „humic“ polymers

(A) biopolymers, lignin (grey), amylopectin (red), albumin (blue), and cuticle (green)

(B) all biopolymers illustrated in black
2D-NMR does not find „humic“ polymers

(A) biopolymers, lignin (grey), amylopectin (red), albumin (blue), and cuticle (green)

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(C) IHSS Humic Acid extract from peat
2D-NMR does not find „humic“ polymers

(A) biopolymers, lignin (grey), amylopectin (red), albumin (blue), and cuticle (green)

(B) all biopolymers illustrated in black

(C) IHSS Humic Acid extract from peat

(D) biopolymers (black) overlaid on IHSS humic acid (green)
Synchrotron spectromicroscopy (Myneni 1999):

Apparent “structure” of humic acid is controlled by pH, ionic strength, cation species!

pH = 3.0
NaCl = 1.0 M
C = 1500 mg L\(^{-1}\)
Synchrotron spectromicroscopy (Myneni 1999):

Apparent “structure” of humic acid is controlled by pH, ionic strength, cation species!

pH = 3.0  
NaCl = 1.0 M  
C = 1500 mg L\(^{-1}\)

pH = 9.0  
NaCl = 0.5 M  
C \(\approx\) 1500 mg L\(^{-1}\)
Synchrotron spectromicroscopy (Myneni 1999):

Apparent “structure” of humic acid is controlled by pH, ionic strength, cation species!

- **pH = 3.0**
  - NaCl = 1.0 M
  - C = 1500 mg L$^{-1}$

- **pH = 4.0**
  - CaCl = 0.018 M
  - C = 1000 mg L$^{-1}$

- **pH = 9.0**
  - NaCl = 0.5 M
  - C ≈ 1500 mg L$^{-1}$
Synchrotron spectromicroscopy (Myneni 1999):

Apparent “structure” of humic acid is controlled by pH, ionic strength, cation species!

- pH = 3.0, NaCl = 1.0 M, C ≈ 1500 mg L⁻¹
- pH = 4.0, CaCl = 0.018 M, C = 1000 mg L⁻¹
- pH = 4.0, Fe³⁺ = 0.001 M, C = 100 mg L⁻¹
- pH = 9.0, NaCl = 0.5 M, C ≈ 1500 mg L⁻¹
Schulten and Schnitzer 1997
Conceptual approach:

- perform alkaline extraction on soil sample to extract humic acid
- obtain bulk C-1s NEXAFS spectrum for the isolated humic fraction
  - perform STXM on intact soil microaggregate
  - generate map of C-1s spectral information for microaggregate
- use principal component analysis to distinguish spectral signatures by location within the microaggregate
  - compare spectra from microaggregate with spectrum of the isolated humic fraction
- accept or reject hypothesis (= soil organic matter has same chemistry as humic acid)
What can be observed

A closer look at the litter layer (the presumable source) suggests a hypothesis:

“Litter is transformed into a **residue** that appears to have survived or “resisted” decomposition.”
How did this concept evolve?

The traditional concept of humus evolved out of early attempts to

a) extract humus matter from soils and

b) use chemical treatments to synthesize humus-like materials from organic precursor materials
Achard performs the first “alkaline extraction”

The traditional alkaline extraction

Soil organic matter
extract with alkali

- insoluble residue
  HUMIN
- soluble
  treat with acid
  (precipitated)
  HUMIC ACID
  (not precipitated)
  FULVIC ACID
Three Observations:

1. Organic matter (peat) did not at all dissolve in water.

2. Organic matter did not dissolve in water, when $H^+$ (= acid) was added to the system

3. Organic matter soluble **only** when $OH^-$ (= base) was added to the system

4. In the order of half of the organic matter did not respond to either acid or base

**Inference:**
Adding base to the system did something to the organic matter which made it soluble in water.
Making an ion by adding base

Adding base to the system did something to the organic matter which made it soluble in water:

Adding base ionizes (parts of) organic matter
It can now attract polar water molecules and become dissolved
No ionization with water or acid

Achards experiment:
Adding acid had no effect

Adding acid produced no charge in the organic matter

Without charge, OM has no means to attract the water molecules
Much (about 50%) of the organic matter shows no response

**Achards experiment:**
Neither acid nor base had an effect on about half of the OM

Soil organic matter contains large amounts of compounds that cannot be ionised
Alkaline extraction separates organic matter in two functionally different parts:

(i) a portion that can be ionized at high pH values and

(ii) a part that is neither polar nor ionizable, and, for that reason, not extracted.

→ Neither humic nor fulvic substances adequately represent the whole of soil organic matter