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# Humus: dark side of life or intractable ‘aether’?

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## Abstract

Does humus exist or is it just a view of the mind as some authors claimed it? I suggest, based on the observation of the activity of soil organisms, that humus should be considered as the ‘dark side’ of life and not as an intractable chemical component of soil organic matter. Most properties humus confers to the soil ecosystem are linked to high surface area for nutrient exchange and water retention and strong affinity to mineral particles. Comminution and transport of organic matter along the soil profile, together with intimate blending with mineral particles, take a prominent part in ecosystem services provided by humus. Microscopy, coupled with thorough examination of humus profiles, may help to reveal the biological origin of humus and the chain of processes by which living matter is transformed and recycled within the soil ecosystem.

## Keywords

Soil, Humus, Biology, Chemistry, Microscopy

## Introduction

Four years ago Johannes Lehmann and Markus Kleber, in a controversy paper entitled “The contentious nature of soil organic matter” (Lehmann and Kleber 2015), suggested to abandon the term ‘humus’, together with correlated terms like ‘humic’, ‘humin’, ‘humified’, ‘humification’, etc. The first reason they invoked was that these notions were only instrumental in outdated methods of soil analysis, being based on an alkaline extraction of soil organic matter and its further precipitation in the form of a dark substance upon acidification. A second reason was that modern, non-extractive methods of soil analysis suggest a continuum from macromolecules synthesized by soil-dwelling organisms (plant roots, animals, microbes) and constitutive of their living then dead parts, to small organic molecules excreted by organisms or issued from the enzymatic degradation of macromolecules. A third reason was that the decomposer community was largely ignored from ‘humus chemists’ who relied only on extraction procedures without any attention to the functions ensured by soil organic matter. This urged them to propose a model for the fate of organic debris, called the “soil continuum model” (SCM), speaking of biopolymers of various sizes, monomers and associated processes of aggregate formation and destruction, transformation from residues to CO<sub>2</sub>, adsorption and desorption to mineral surfaces. We think that their updated view of soil organic matter, rejecting the chemical sense still given to the word ‘humus’, is warmly welcomed now that soil biology has become an unavoidable component of ‘soil science’. However, we also think that a further step should be taken for a clear understanding of the humus concept, before rejecting it from the field of science.

## Recent developments in humus chemistry

The most comprehensive and updated view of humus from chemical sense seems to be that of Piccolo (2002). This author describes humic substances as “supramolecular associations of self-assembling heterogeneous and relatively small molecules deriving from the degradation and decomposition of dead biological material.” This definition has nothing to do with previous ones and open new avenues

to the chemistry of soil organic matter. The notion of ‘supramolecular association’, already highlighted on the base of previous experiments by Piccolo and Conte (2000), explains why humic compounds extracted by classical analytical methods resist so much to chemical description. Even if unit components of humus have been searched for a long time by soil organic chemists, not one was able to describe with certainty a humus molecule. The most reliable reason is that humus molecules do not exist, as convincingly claimed by Lehmann and Kleber (2015). However, the supramolecular concept (small molecules linked by Van der Waals forces and hydrogen bonds) allows explaining that organic matter, not only in soils but also in sediments and even in atmospheric aerosols (Kiss et al. 2003), may upon degradation become self-reassembled in molecular clouds doted of original properties. Among these properties, the ability to rapidly incorporate organic molecules (e.g. pesticides, proteins, sugars) and to intimately associate with minerals (e.g. clays, metals) is remarkable (Livens 1991, Senesi 1992, Varadachari et al. 1994, Lichtfouse et al. 1995, Zang et al. 2000). Molecular disorder operating during the formation of humus is opposed to molecular ordering in the synthesis of proteins and other macromolecules by organisms. Of interest is the fact that disordered regions of proteins are those which allow them to interact with their binding partners, like humus does (Turoverov et al. 2010). The strong capacity of humic substances to incorporate and sequester extraneous organic molecules (Spaccini et al. 2002) point to the postulated ability of humus to store recently added atmospheric carbon and thus fight against climate warming (Lal et al. 2007).

In a recent review on our present-day knowledge on humic substances Gerke (2018) criticized the SCM model proposed by Lehmann and Kleber (2015), arguing that they did not take into consideration the polymerizing and further reacting ability of humic substances, restricting their model to decomposition processes and to the shift from plant and animal residues to biopolymers then to monomers then at last to carbon dioxide. However, it is clear that this criticism concerns only the processing chain displayed in the central part of the SEM model, forgetting that on both parts of it Lehmann and Kleber show that molecules derived from the degradation of organic debris, and organic debris themselves contribute to the formation and destruction of aggregates and to adsorption and desorption processes in which mineral surfaces are involved. The notion of ‘black carbon’ (Goldberg 1985) was also discussed in detail in Gerke’s review, because it has been claimed that humic aromatic structures were derived from fire-affected organic matter and thus should not be termed humus. Gerke explained why the methods used to dose black carbon (BPCA marker or UV methods) overestimated it, and showed that black carbon and humic substances were in strong interaction through both covalent and non-covalent linkages.

In an appealing paper Baveye and Wander (2019) also replied to Lehmann and Kleber (2019). They showed that despite the turmoil in the soil scientific community caused by Lehmann-Kleber’s proposal to reject ‘humus’, this term continues to be largely used by scientists, with a still increasing number of publications citing it routinely. They also showed that the ‘new’ SCM model was in fact not new, and well under the seminal views elaborated more than 80 years ago by Waksman (1936). This author defined humus as consisting “of certain constituents of the original plant material resistant to further decomposition, of substances undergoing decomposition, either by processes of hydrolysis or by oxidation and reduction, and of various compounds synthesized by microorganisms.” Following Waksman’s idea that a pure chemical assessment of humus was a dead end, Baveye and Wander pleaded for a multidisciplinary research on humus, meaning that this notion was not a prerogative of chemists. From their point of view microbiology but also agronomy have their say, too. We acknowledge and warmly recommend adding zoology, too.

### **The biological meaning of humus**

Commonly, non-chemists use the term ‘humus’ to designate every kind of organic matter which cannot be assigned by the naked eye to recognizable plant or animal debris, either in the form of dark-colored deposits of fine organic matter (in superficial humus layers, below the litter) or mixed with mineral matter deeper in the soil (Zanella et al. 2011). This highly transformed organic substrate is the target of well-managed composting processes (Sugahara and Inoko 1981) and is used to amend the

soil for agricultural or horticultural purposes under the name of ‘compost’ (e.g. vermicompost). The application of humified matter to the soil is known to improve water retention (Giusquiani et al. 1995), nutrient retention and exchange (Steiner et al. 2008), heat capture (Pinamonti 1998), and to protect soil from erosion (Bazzoffi et al. 1998), among other ecosystem services. It has also been shown, after more than a century of silence on this process, that humic substances are biologically active from a nutritional point of view. They can be taken up by plants to be assimilated as extra carbon and nitrogen sources (Näsholm et al. 2009) and display nutrient-capture and growth-promoting hormone-like properties (Nardi et al. 2002), soil and roots being involved in a win-win feedback mediated by positive interactions (Nardi et al. 2017).

But what is humus for a biologist? When passing from the naked eye to the microscopic observation of organic and mineral-organic horizons, the biological nature of humus is revealed. Ponge (1984, 1985, 1988, 2016) showed, by scrutinizing a small volume of pine litter at varying stages of decomposition, that most plant (pine and moss) remains were processed by microbes and animals, turning to ‘black matter’ made of fecal pellets in which minute plant, fungal and bacterial remains were clearly visible under the light microscope. The most minute arthropods (springtails, mites), as well as annelids (earthworms, enchytraeids) were able to comminute plant and fungal remains to an extent that only the greatest magnification of the light microscope was able to identify them, while bigger litter-consuming arthropods (millipedes, woodlice, fly larvae) accumulated gross fragments, visible to the dissecting microscope, in their faeces. Similar observations were made in mineral-organic horizons, where the intimate association of organic with mineral matter can be disentangled. A lot of debris, either of plant or microbial origin, can be easily identified in organic-mineral assemblages under transmitted electron microscopy (Foster 1988, Saur and Ponge 1988). Previously Tisdall and Oades (1982) had shown in ultrathin sections that the so-called soil micro-aggregates were in fact quiescent microbial colonies embedded in clay sheets. Bernier and Ponge (1994) showed that links between the amorphous (non-recognizable to the light microscope) part of soil organic matter and silt- and clay-size mineral particles were controlled by the dynamics of earthworm populations. Topoliantz and Ponge (2003) showed that in tropical slash-and-burn cultivated fields pieces of charcoal were ingested, ground in tiny particles in the muscular gizzard and mixed with mineral matter by earthworms. Such observations of biological contributions to humus formation are not new, being since a long time the aim of soil micromorphologists (Kubiëna 1938, Zachariae 1965, Zaiets and Poch 2016). However, knowledge on feeding and behavioral habits of soil organisms, together with plant anatomy, allows much more plant and microbial material to be observed and identified and much more structures (aggregates, coatings) to be assigned to the activity of animals and microbes, in particular when soil organisms can be observed and identified in the immediate vicinity of traces of their activity (Ponge 1990, 1991).

For a biologist, humus is thus made of plant, fungal and bacterial remains of a size varying from the micrometer to the millimeter, and of ‘amorphous’ matter in which transmission electron microscopy still allows to discern partly degraded plant and microbial cell pieces of a size varying from the nanometer to the micrometer (Foster 1981). An increase in nanometer-sized electron-dense particles can be observed as a degradation stage of plant cell walls (Messner et al. 1985, Saur and Ponge 1988). These particles could be considered with caution as ‘true’ humic substances, the existence of which is still debated (Schmidt et al. 2011). In this respect it is a pity that during the last 30 years ‘modern’ techniques of organic matter analysis, e.g. stable isotopes (Briones et al. 1999, Nguyen Tu et al. 2011), high-resolution molecular techniques (Lynch et al. 2004), and more recently metabolomics (Swenson et al. 2015), took precedence over soil imaging, because adapting the scale of observation to the studied process is a basic requirement of the search for causal relationships in complex systems (Coleman et al. 1992, Chapura 2009).

## **Reconciling biological and chemical views about humus**

How to reconcile the view of the biologist with the most recent developments in humus chemistry? The transformation of organic matter in the soil, as viewed by the biologist, is mainly a physical

process, embracing comminution (Mori et al. 2009), leaching (Nykqvist 1963), compaction (Chauvel et al. 1999), physical protection (Balesdent et al. 2000), displacement along the soil profile and mixing (or not) with mineral matter (Lavelle et al. 2016), the net result of this transformation being exemplified in the concept of humus form (Bal 1970, Zanella et al. 2018). This physical transformation of organic matter is mainly effected by saprophagous animals (Wolters 2000) and to a more limited extent by microbial (Tisdall and Oades 1982) and abiotic processes (Denef et al. 2001). To these physical transformations, visible to the naked eye in the formation of the so-called humus horizons (Zanella et al. 2018), are superimposed microbial (Keeler et al. 2009) and to a lesser extent faunal (Garvin et al. 2000) enzymatic degradation, resulting in the formation of easily leached (Allison and Vitousek 2004) or metabolized small molecules (Tian et al. 2010). In the same time every soil-dwelling organism elaborates its own biomass (Powlson et al. 1987), which is in turn processed along soil trophic networks (Lueders et al. 2006) or accumulates as more or less degraded dead bodies (Kallenbach et al. 2015). All that is humus, most properties it confers to the soil ecosystem (Ponge 2015) are linked to high surface area for nutrient exchange and water retention (Laird et al. 2010) and strong affinity to mineral particles (Vermeer et al. 1998). Some of the abovementioned processes contribute to degrade organic matter (until respired as carbon dioxide) while others stabilize it under various forms, e.g. deep carbon by roots (Kell 2011) or earthworms (Shuster et al. 2001), clay-humus assemblages by earthworms (Scullion and Malik 2000) or bacteria (Six et al. 2004).

All models proposed by soil chemists cope with this view as far as they do not give precedence to a pure chemical formulation of humus which is, to our opinion, a complete waste of time given the complexity of soil organic matter even at the smallest scale (Lehmann et al. 2008). It has been claimed that most properties given to the soil by organic matter cannot be deduced from its molecular composition (Schmidt et al. 2011), and thus that a better knowledge of the environment and of the organisms which contribute to the dynamics of soil organic matter is urgently needed if we want to dispose of reliable models of carbon cycling and storage (Hedges et al. 2000). We suggest speaking of humus as the ‘dark side’ of life, and not as the abiotic component of soil organic matter, as most authors suggest it be (Gerke 2018). The recognition of the biological nature of humus would allow a better assessment of its origin, dynamics and emergent properties (Ponge 2005), like a step has been taken in soil science when the direct role of soil organisms in mineral weathering has been universally acknowledged (Neilands 1995, Jongmans et al. 1997).

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