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Perspective

Humus: dark side of life or intractable 'aether'?

Johannes Lehmann and Markus Kleber, in a controversial 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 The first reason they invoked was that these notions were only instrumental in outdated soil analytical methods, being based on an alkaline extraction of soil organic matter (SOM). A second reason was that a wide array of soil analytical methods suggest a continuum from macromolecules synthesized by soil-dwelling organisms (plant roots, animals, microbes) and constitutive of their living and dead parts, to small organic molecules excreted by organisms or issued from the enzymatic degradation of macromolecules. A third reason was that too many 'humus' chemists still rely only on extraction procedures without due attention to the decomposer community. This urged these authors 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 CO2, adsorption and desorption to mineral surfaces. I think that their updated view of SOM, 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, I 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, as this has been fortunately suggested by Ohno et al. (2019) in conclusion to an overview of most recent developments in SOM chemistry.

Piccolo (2002) 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 SOM chemistry. The notion of 'supramolecular association', already highlighted based on 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 chemists, no one could describe a humus molecule with certainty. However, the supramolecular concept (small molecules linked by Van der Wals 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 them the ability to rapidly incorporate organic molecules (e.g. pesticides, proteins, sugars) and to intimately associate with minerals (e.g. clays, metal (oxyhydr)oxides) is remarkable (Livens, 1991; Senesi, 1992; Varadachari *et al.*, 1994, Lichtfouse *et al.*, 1995; Zang *et al.*, 2000). Molecular disorder operating during humus formation is opposed to molecular ordering in synthesis of proteins and other macromolecules by organisms. Of interest is that disordered regions of proteins 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).

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, this criticism concerns only the processing chain displayed in the central part of the SCM model, forgetting that on both sides of their scheme Lehmann and Kleber (2015) 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 (molecular marker or UV methods) overestimated it, and showed that black carbon and humic substances were in strong interaction through covalent and non-covalent linkages.

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 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 (2019) 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.

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-coloured 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. After more than a century of silence on this process, it has also been shown that humic substances are biologically active from a nutritional or physiological 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 faecal 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) comminute plant and fungal remains to an extent that only the greatest magnification of the light microscope can identify them. In contrast, bigger litter-consuming arthropods (millipedes, woodlice, fly larvae) accumulate gross fragments, visible to the dissecting microscope, in their faeces. Similar observations were made in mineral- organic horizons, where the intimate association of organic matter with minerals 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) showed in ultrathin sections that the so-called soil micro-aggregates are 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 SOM and silt- and clay-size mineral particles are controlled by the dynamics of earthworm populations. Topoliantz and Ponge (2003) showed that in tropical slash-and-burn cultivated fields, charcoal pieces are 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 for a long time the aim of soil micromorphologists (Kubiëna, 1938; Zachariae, 1965; Zaiets and Poch, 2016; Colombini et al., 2020). Knowledge on feeding and behavioural 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 near traces of their activity (Ponge 1990, 1991). By using total DNA as a tracer of biotic (mostly microbial) origin, Zaccone et al. (2018a) showed that most

SOM located between aggregates (free or associated with minerals) originated from soil organisms, either as excreta or living or dead bodies.

For a biologist, humus is thus made of plant, fungal and bacterial remains of a size varying from the micrometre to the millimetre, 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 nanometre to the micrometre (Foster, 1981). An increase in nanometre-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).

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 of decomposition products (Nykvist, 1963), compaction (Chauvel *et al.*, 1999), physicochemical protection (Balesdent *et al.*, 2000; Giannetta *et al.*, 2018), mechanical displacement along the soil profile and mixing (or not) with mineral matter (Lavelle *et al.*, 2016). The net result of this body of transformations, occurring over a large array of scales, is exemplified in the concept of humus form (Bal, 1970; Zanella *et al.*, 2018b). 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 humus horizons (Zanella *et al.*, 2018b), 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). At 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 (Chiou *et al.*, 1990) and strong affinity to mineral surfaces (Vermeer *et al.*, 1998). Some of the abovementioned biological processes contribute to degrading 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 (Zaccone *et al.*, 2018a). However, humus-forming processes where biological activity does not play an active role should be mentioned, too. This is the case of peat soils, where humification progresses at a very low rate due to inherent recalcitrance of organic inputs and anoxic environment (Zaccone *et al.*, 2018b), and of mor humus (also called "raw humus") accumulating in nutrient-poor terrestrial environments in the absence of notable faunal activity (Hempfling *et al.*, 1987).

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 does not fit to SOM complexity 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 SOM dynamics is urgently needed if we want to dispose of reliable models of carbon cycling and storage (Hedges *et al.*, 2000). Models of SOM dynamics proposed by Komarov *et al.* (2017) and Blankinship *et al.* (2018), including measurements of microbial and animal effect traits, indicative of their activity, are pivotal steps in this direction. We suggest speaking of humus as the 'dark side' of life, and not as an abiotic SOM component, 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).

Our knowledge of the large array of soil organic and mineral-organic components collectively called 'humus' could benefit from a tight cooperation between chemists and biologists. The concept of the soil as a collection of embedded aggregates, the basic unit being the microaggregate (Totsche *et al.*, 2018), is highly promising by allowing processes (e.g., stability, respiration, organic-mineral interactions, nutrient exchange) to be studied at the scale at which they occur in the soil ecosystem (Ponge, 2015). Another promising aspect of strong cooperation between chemists and biologists is the inclusion of biological processes in modelling SOM formation (Chertov *et al.*, 2017a, b; Blankinship *et al.*, 2018). Some burning questions such as the use of soil for mitigating climate warming by sequestering more atmospheric carbon (Lal, 2010) could be resolved by focusing our research effort on the interplay between biotic and abiotic compartments of the soil, with humus at the interface (Zanella *et al.*, 2018a).

As a conclusion, this short focus on humus and its various meanings (for the gardener, for the chemist, for the biologist) was intended to show that this complex matter (in both literal and figurative senses) could benefit from a better cooperation between all scientific disciplines devoted to soil studies. Rather than abandoning the term 'humus', as provocatively suggested by Lehmann and Kleber (2015), I propose considering humus as a prominent agent of measurable soil ecosystem services, including plant growth and fixation of atmospheric carbon, needing protection to the same extent as life, from which it might be considered as the 'dark side'.

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