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Letter to the Editor

Humus: Dark side of life or intractable “aether”?

Dear Editor,

Johannes Lehmann and Markus Kleber, in a controversial paper entitled “The contentious nature of soil organic matter” (Lehmann and Kleber, 2015), suggested the abandonment of the term “humus” and other associated terms such as “humic”, “humin”, “humified”, “humification”. The first reason they cited was that these labels are only instrumental in outdated soil analytical methods, which are based on alkaline extraction of soil organic matter (SOM). The second reason was that a wide array of soil analytical methods suggests a continuum from macromolecules synthesized by soil-dwelling organisms (plants, animals, and microbes) and released from their living and dead parts to small organic molecules excreted by organisms or issued from the enzymatic degradation of macromolecules. The third reason was that too many “humus” chemists still rely only on extraction procedures, without due attention to the decomposer community. These reasons prompted the authors to propose a model for the fate of organic debris, called the “soil continuum model (SCM)”, which considers biopolymers of various sizes, monomers, processes of aggregate formation and destruction, transformation of residues to carbon dioxide, adsorption and desorption of organic matter to mineral surfaces. I believe that their updated view of SOM, rejecting the chemical sense appropriated to the word “humus”, is received amiably since soil biology has currently become an unavoidable component of “soil science”. However, a further step should be taken towards clarifying our understanding of the humus concept before rejecting it from the field of science, as has been suggested by Ohno *et al.* (2019) in their conclusion to an overview of the most recent developments in SOM chemistry.

Piccolo (2002) described 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 is independent of previous ones and opens up new avenues in 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 elude chemical description. Even if unit components of humus have been explored for a long time by soil chemists, no one

can describe a humus molecule with certainty. 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 dotted 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 the 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) points to the postulated ability of humus to store recently added atmospheric carbon and thus help mitigate climate change (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 of plant and animal residues to biopolymers and then to monomers and finally to carbon dioxide. However, this criticism concerns only the processing chain displayed in the central part of the SCM model, overlooking that on both sides of their scheme, Lehmann and Kleber (2015) showed that the molecules derived from the degradation of organic debris and the organic debris itself contribute to the formation and destruction of aggregates and the adsorption and desorption processes involving mineral surfaces. The notion of “black carbon” (Goldberg, 1985) was also discussed in detail in Gerke’s review, claiming that humic aromatic structures are derived from fire-affected organic matter and thus should not be termed humus. Gerke explained why the methods used to quantify black carbon (molecular marker or UV methods) overestimated it and showed that black carbon and humic substances interacted strongly through covalent and non-covalent linkages.

Baveye and Wander (2019) also responded to Lehmann and Kleber (2015). They showed that despite the turmoil

in the soil scientific community caused by Lehmann and Kleber's proposal to reject "humus", this term continues to be largely used by scientists, with an ever-increasing number of publications citing it routinely. They also showed that the "new" SCM model was not new and had been 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 purely chemical assessment of humus was a dead end, Baveye and Wander (2019) pleaded for multidisciplinary research on humus, indicating that this notion was not a prerogative of chemists. From their point of view, microbiology and agronomy have their say, too. We also acknowledge and highly recommend involving zoology as well.

Commonly, non-chemists use the term "humus" to designate any 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 soil is known to improve water retention (Giusquiani *et al.*, 1995), nutrient retention and exchange (Steiner *et al.*, 2008), and heat capture (Pinamonti, 1998) and protect soil from erosion (Bazzoffi *et al.*, 1998), among other ecosystem services. Humic substances have been recently shown to be 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).

However, what is humus to a biologist? When observation moves from the naked eye to the microscopic level of organic and mineral-organic horizons, the biological nature of humus is revealed. Ponge (1984, 1985, 1988, 2016) showed that, by scrutinizing a small volume of pine litter at varying stages of decomposition, most plant (pine and moss) remains were processed by microbes and animals, turning to "black matter" composed of faecal pellets, in which minute plant, fungal, and bacterial remains were clearly visible under a light microscope. The most minute arthropods (*e.g.*, springtails, mites) and annelids (*e.g.*, earthworms and enchytraeids) comminute plant and fungal remains to such an

extent that they can only be identified using the maximum magnification of a light microscope. In contrast, larger litter-consuming arthropods (*e.g.*, millipedes, woodlice, fly larvae) accumulate gross fragments, visible under a dissecting microscope, in their faeces. Similar observations were made in mineral-organic horizons, where the intimate association of organic matter with minerals can be unravelled. A large amount of debris, either of plant or microbial origin, can be easily identified in organic-mineral assemblages by transmitted electron microscopy (Foster, 1988; Saur and Ponge, 1988). Previously, Tisdall and Oades (1982) showed that in ultrathin sections, the so-called soil microaggregates are quiescent microbial colonies embedded in clay sheets. Bernier and Ponge (1994) showed that the links between the amorphous (non-recognizable using a light microscope) part of SOM and the silt- and clay-sized 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 and ground into 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 a long-standing aim of soil micromorphologists (Kubiëna, 1938; Zachariae, 1965; Zaiets and Poch, 2016; Colombini *et al.*, 2020). Knowledge of the 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 (*e.g.*, 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). 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) originates from soil organisms, either as excreta or living or dead bodies.

For a biologist, humus comprises plant, fungal, and bacterial remains varying from micrometers to millimeters in size and "amorphous" matter where partly degraded plant and microbial cell pieces varying from nanometers to micrometers in size can be discerned by transmission electron microscopy (Foster, 1981). An increase in nanometer-sized electron-dense particles can be observed during plant cell wall degradation (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 regard, unfortunately, over the last 30 years, "modern" techniques of organic matter analysis, such as 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), have taken precedence over soil imaging, while adapting the scale of observation to the studied process should

be 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 soil, as viewed by the biologist, is mainly a physical process, including comminution (Mori *et al.*, 2009), leaching of decomposition products (Nykqvist, 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 these transformations, occurring over a large range of scales, is exemplified in the concept of humus form (Bal, 1970; Zanella *et al.*, 2018b). This physical transformation of organic matter is mainly affected 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 (Garvín *et al.*, 2000) enzymatic degradations, 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 (Powelson *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 those are humus. Most properties humus confers to the soil ecosystem (Ponge, 2015) are linked to its high surface area for nutrient exchange and water retention (Chiou *et al.*, 1990) and its strong affinity to mineral surfaces (Vermeer *et al.*, 1998). Some of the abovementioned biological processes contribute to the degradation of organic matter (until respired as carbon dioxide), whereas others stabilize it in various forms, *e.g.*, deep carbon by roots (Kell, 2011) or earthworms (Shuster *et al.*, 2001), clay-humus assemblages by earthworms (Sullivan 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 for peat soils, where humification progresses at a very low rate due to the inherent recalcitrance of organic inputs and the anoxic environment (Zaccone *et al.*, 2018b), and for 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 to be (Gerke, 2018). The recognition of the biological nature of humus would allow 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 close cooperation between chemists and biologists. The concept of soil as a collection of embedded aggregates, the basic unit being the microaggregate (Totsche *et al.*, 2018), is highly promising, because it allows 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 soil compartments, with humus at the interface (Zanella *et al.*, 2018a).

In conclusion, this short focus on humus and its various significances (for the gardener, for the chemist, and 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, requiring as much protection as life, from which it might be considered as the “dark side”.

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