



Outlining the Potential Role of Humic Products in Modifying Biological Properties of the Soil—A Review

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There is a growing awareness among farmers about the importance of soil for sustaining crop production and soil health. Marked interests in “environmentally-friendly” soil fertilization in agriculture has attracted significant attention in the last decades. A wide range of commercial humic products (HPs) are used as soil supplements to improve the nutrient utilization efficiency of plants as well as to increase crop production. However, the implications of increased HPs use for soil biology are being questioned, but a comprehensive review on this topic is lacking. The aims of this review are to understand behavior of HPs applied to soil, with a special focus on environmental protection issues, and to summarize the available data how HPs may influence the soil microbial communities, including fungi and bacteria strains. The interaction between HPs and soil inhabitants is highly complex and is controversial to a certain extent for several reasons. First, applying HPs may promote specific bacteria communities (*Acidobacteria*, *Actinobacteria*, *Bacterioidetes*, etc.) rather than other microbiome species. Soil enzymes such as laccase, peroxidase and dehydrogenase were seemingly affected as well. Applying HPs may result in the microbial respiration of CH₄, preventing the production of CO₂ from microbial respiration. These results would potentially lead to an overall decrease in the microbial respiration of CH₄, which is associated with a deficit in CO₂. Furthermore, HPs can control the nematode population in different soils. Finally, applying HPs in terms of physiological functioning can also have various consequences for different groups of soil fungi. However, the conditions that promote specific effects (chemical composition of HPs, environmental conditions, etc.) have yet to be investigated. Given the surveyed data, we conclude that there are gaps in the current knowledge of this topic. We propose an integrated approach including a targeted research involving not only species of plants but also the integrated chemical, toxicological and biological analyses as a useful approach of soil health protection.

Keywords: humic substances, microbial activity, mesofauna, bacteria, fungi, bioassay, biodiversity, toxicity

INTRODUCTION

Healthy and fertile soil are fundamental for food security and agricultural sustainability, yet it remains a challenge due to poor management and environmental changes (Doran and Safley, 1997; Moebius-Clune, 2016). Increasingly, soils become degraded and marginal for production. This situation is exacerbated by the need to increase food production to feed an increasing global population. Consequently, there is a common interest in the development and implementation of sustainable agricultural practices (Quintero-Angel and González-Acevedo, 2018; Gazzola et al., 2019). Such practices need to maintain the ability of the soil to produce food, while also delivering other ecosystem services such as regulation and storage of nutrients and carbon.

Among many different carbon-based substances for soil health improvement, humic-based soil amendments are accessible due to the industrial request for efficient and environmentally friendly soil supplements. Nowadays, the HPs market size is estimated to cross 1 billion US\$ by 2024 (Pulidindi and Pandey, 2017) because they are using worldwide as soil conditioners and in cropland agriculture (Olk et al., 2018). According to Billingham (2012) humic products (HPs) are materials “that are often sold as soil amendments with or without accompanying plant nutrients.” Industrial technologies of the production of HPs differ from research analytical techniques used for the isolation of humic substances (HS). In general, they are manufactured by alkaline extraction from various organic matter resources, including lignite, peat, composts and organic waste materials (Perminova et al., 2005), but the resulted HPs show various properties (Yakimenko and Terekhova, 2011; Yakimenko et al., 2018). Practically, commercial HPs usually contain between 15 and 85% of HS (Yakimenko and Terekhova, 2011; Lamar et al., 2014).

In the context of potential effects of HPs on living organisms, there is a promising concept of emergent properties. According to this, the complexity of HPs as a whole substances is essential for their performance in compare with information about their separate components (Yakhin et al., 2016). In plant science, HPs are considered belonging to plant biostimulants, which are defined as “any substance or microorganism applied in minor quantities to plants with the aim of enhancing nutrition efficiency, abiotic stress tolerance, and crop quality traits, regardless of nutrients content” (Calvo et al., 2014; Canellas et al., 2015; Shah et al., 2018). A later definition underlines that a biostimulant is “a formulated product of biological origin that improves plant productivity as a consequence of the novel or emergent properties of the constituents, and not as a sole consequence of the presence of known essential plant nutrients, such as plant growth regulators, or plant protective compounds” (du Jardin, 2015; Yakhin et al., 2016). The benefits of applying HPs to enhance crop productivity have been well documented in the literature (Albiach et al., 2001; Quilty and Cattle, 2011; Tan, 2014). The positive effect of HPs on plant growth is commonly associated not only with the direct interaction of HS molecules with the plant root (“hormone-like activity”) and the activation of physiological processes in the plant, but

also through exhibiting multiple indirect effects. For example, HPs buffer pH, increase water retention and mobilize nutrients availability. In respect to plants, both stimulating and inhibiting effects of HPs are illustrated in some comprehensive reviews (Canellas and Olivares, 2014; Rose et al., 2014; Nardi et al., 2016; Shah et al., 2018). At the same time, HPs may be promising remediation agents to degraded lands, due their ability to improve physical, chemical and biological properties of soil (Piccolo and Stevenson, 1982; Khaled and Fawy, 2011; Pukalchik M. et al., 2017; Tregubova et al., 2017).

However, the general picture of HPs influencing soil health is neither unambiguously positive nor simple. Besides the effects on plant productivity, the ubiquitous use of HPs in agriculture may bring specific changes to the soil food chain and microbial community. Being biostimulants, HPs can provide either stimulating or toxic effects on microorganisms, fungi and soil fauna. When applied as soil amendments, HPs provide a certain influence on the biological activity of the soil as a whole. For example, Kulikova et al. (2005) emphasized that the complexity of HPs structure and the initial experimental conditions bring a broad range of observed biological effects, which may promote the toxicity of HPs. Understanding of their potential effects on soil biota is of particular importance for soil health since it is mostly affected as a result of the field application of HPs. Moreover, HPs can exert the dose-toxicity effect phenomenon, which would result from an increase in application rates and may induce soil toxicity on living organisms, thereby damaging the environment (Guo et al., 2019).

Overall, the issue of HPs safety with respect to soil health should not be dismissed, yet should always be considered when applying amendments to the HPs. The main objective of this literature review concerns the analysis of peer-reviewed data on the role of HPs in biological and ecotoxicological soil properties. The aim of the review is to understand the behaviors of HPs applied to the soil, with a special focus on the protection of soil health and key ecosystem health indicators.

EFFECTS OF HUMIC PRODUCTS ON SOIL BIOTA

Effects on Soil Microbial Activity and Functional Characteristics of Soil

Soil heterotrophic organisms play a crucial role in decomposing the organic matter, retaining C and N in their biomass and releasing CO₂, CH₄, NO₂ back to the atmosphere (Le Mer and Roger, 2001; Trumbore, 2006; Zhu et al., 2013). Due to their gradual decomposition kinetics, inputs of HPs continuously provide nutrients utilized by microbial communities (Murphy et al., 2004). For that reason, HPs tend to act as slow-release biostimulants for microorganisms. **Table 1** provides selected examples of how HP impacts specific soil microbial and enzyme activities, with associated soil processes given and a link to the impact on soil health (**Table 1**).

The introduction of HPs in soils induced considerable cascading effects on soil C and N transformation processes. The

TABLE 1 | Impacts of HPs on key biological processes in soil (C and N, enzyme activities) and functional effects on soil health.

Soil health indicator	HPs influence	Soil health impact	References
CH ₄ production	↑	+ OM anaerobic decomposition	Tan et al., 2018
Soil respiration (CO ₂ production)	↓ a ↑ b, c	- OM mineralization +OM mineralization	a. Tan et al., 2018 b. Gorlenko et al., 2012 c. Maji et al., 2017
Microbial biomass (soil biomass carbon)	↑ c ↑ ↓ d	+ C regulation ± C regulation	c. Maji et al., 2017 d. Pukalchik M. et al., 2017
Microbial biomass (Microbial quotient)	↑ b ↓ d	- microbial community + microbial community	b. Gorlenko et al., 2012 d. Pukalchik M. et al., 2017
Nitrification	↑	+ N-regulation and plant growth	Dong et al., 2009
Microbial biomass N	↑	+ N regulation	Maji et al., 2017
Soil Enzyme Activity:			
Oxidoreductases (laccase, peroxidase, dehydrogenase, protease)	↓	- OM oxidation	Mato et al., 1971; Pflug, 1980; Pflug and Ziechmann, 1981; Cozzolino and Piccolo, 2002; Jorobekova et al., 2005; Eichlerová et al., 2012; Dou et al., 2018
Oxidoreductases (catalase)	↑	+ aerobic microbial activity and soil fertility	Sellamuthu and Govindaswamy, 2003; Li et al., 2013
Oxidoreductases (urease)	↑	+N- cycling and plant growth	Sellamuthu and Govindaswamy, 2003; Li et al., 2013; Pukalchik M. A. et al., 2017
Oxidoreductases (acid phosphatase)	↑	+ P- cycling and soil fertility	Pukalchik M. A. et al., 2017
Extracellular enzyme (carboxypeptidase A, pronase, chymotrypsin A, trypsin)	↓	- C-cycling	Butler and Ladd, 1971; Malcolm and Vaughan, 1979; Rostovshikova and Korneeva, 1999; Liu et al., 2015

↑ = increase; ↓ = decrease; + = positive impact; - = negative impact.

mechanism of the influence of HPs may be linked with redox-active functional groups of HPs, which can be readily oxidized and subsequently act as electron donors for bacterial respiration, with subsequent changes in CO₂ and CH₄ production (Lovley et al., 1999; Coates et al., 2002). For example, Tan et al. (2018), following the application of three different HPs, highlighted CH₄ emissions in paddy and wetland soils, thus decreasing CO₂ emissions. According to those authors, that effect would account for not only for the ability of HPs to accept electrons but also trigger the anaerobic oxidation of CH₄. Quite the opposite results were published by Gorlenko et al. (2012), where the application of 0.1, 0.5, and 1 g•kg⁻¹ of commercial HPs, manufactured from brown coal, markedly increased emissions of CO₂ from a model soil mixture. Only small doses of 0.1 g/kg HPs exhibited a positive trend for the soil microbial community, considering indicators such as biodiversity, metabolic activity, and stability of the soil microbial community. The highest applications induced a decrease in microbial biodiversity during the 45 day experiment. Maji et al. (2017) showed that applying HPs through enriched vermicompost (comprising 39.9% of HP, application dose is 9 g•kg⁻¹ in soil) promoted soil CO₂ respiration, microbial biomass carbon, and microbial biomass nitrogen values, unlike a traditionally used vermicompost and an NPK-fertilizer. Moreover, the highest rhizosphere fungal and bacterial populations were detected in the HPs-enriched vermicompost treatment. In our recent studies, we exploited Cu, Zn, Cd and Cr-contaminated soils in pilot greenhouse

experiments and observed a positive short-term effect (0–60 days) produced by a commercial Na-humate (0.05, 0.5 and 1%) on a microbial metabolic quotient and microbial biomass carbon (Pukalchik M. et al., 2017).

The application of HPs has substantial impacts on soil biochemical processes, in particular on soil enzymes (see **Table 1**). The addition of HPs may affect the supply of C and N to plants and thus the processes of organic matter decomposition in soil. According to Wallenstein and Burns (2011), the following parts of microbial cells are mostly subjected to interactions with HPs: cytoplasm of microbial cells and the outer surface of the cells. The HPs may affect fungal spores, protozoal cysts and bacterial spores, which would constitute enzyme-substrate complexes and co-polymerized complexes with soil organic matter; they could subsequently be absorbed on the surface of clay minerals. Therefore, HPs can be involved in a plethora of processes and interactions within soil extracellular enzyme complexes.

On the one hand, HPs can be degraded by enzymes in the course of oxidative catalysis. In case the redox potential of laccases is too small (4,500–800 mv) to oxidize the C-C bonds in the HPs directly, HPs oxidize a variety of organic molecules in the soil, creating extracellular diffusible radicals which can depolymerize HPs (Brenelli et al., 2018). The redox potential of peroxidase is up to 1,490 mv. Moreover, peroxidases include Fe-containing groups that use H₂O₂ as an electron acceptor, enabling them to directly oxidize C-C bonds (Wang et al., 2012).

Dehydrogenases that transfer hydride groups from a substrate to an acceptor such as NAD⁺ play a key role in the decomposition and mineralization of aromatic compounds (Bugg et al., 2011). The activity of these oxidative enzymes usually decreases upon addition of HPs. The reduction of laccase activity in the presence of HP was observed by Eichlerová et al. (2012); according to Dou et al. (2018), HP-induced inhibition may be linked with the oxidation of phenoxyl radicals. The peroxidase activity may be inhibited by (i) competitive interactions between the donor substrate NADH in peroxidase and HP (Pflug, 1980) and (ii) the fact that hydrogen bonds may confer conformational rigidity to humic associations and depress molecular reactivity (Cuzzolino and Piccolo, 2002). The decrease in dehydrogenase activities was due to the competition of the forward reaction enzyme-HP (Pflug and Ziechmann, 1981). Applying HPs may also inhibit the activity of proteases in the soil through non-specific mechanisms. According to Jorobekova et al. (2005), enzyme-inhibitor complexes are formed by electrostatic, hydrophobic and other types of intermolecular interactions. A strong linear correlation between oxidase activities and the HPs phenolic content, as well as the C:H ratio and C and N content were shown by Mato et al. (1972). The recent data regarding the correlation between HPs and the activity of the soil enzyme pool suggested that HPs increased the catalase and urease activities in soil (Sellamuthu and Govindaswamy, 2003; Li et al., 2013). Our recent study, which involved a model of artificial soil and HPs of different origin, has also shown that HPs can impact the activities of acid phosphatase and urease while having no influence on soil dehydrogenase activity (Pukalchik M. A. et al., 2017).

On the other hand, HPs may be involved in specific mechanisms of binding reactions, which hamper access to the substrate for the enzymes active site. As a result, there was an observed decrease in activities of the different extracellular enzymes (Butler and Ladd, 1971; Vaughan and Ord, 1980; Allison, 2006). According to Butler and Ladd (1971), the content of carboxyl groups was responsible for carboxypeptidase A, pronase, chymotrypsin A and trypsin inhibitions in the soil. In case of phosphatase, a decrease in enzymatic activity did not correlate with C, H, and N ratios, and the content of ash and even carboxyl and phenol in groups (Malcolm and Vaughan, 1979). Still, low molecular weight components of HPs were particularly active (Mato et al., 1972; Nardi et al., 2007), while high molecular weight components had residual activity (Butler and Ladd, 1971). The data above evidence that molecular weight conditions influence the biological behavior of these substances. The inhibiting effect of HPs may be linked to hydrophobicity properties (Liu et al., 2015). The results on which the hypothesis was advanced had been published earlier by Rostovshikova and Korneeva (1999), who showed that molecular and hydrophobicity fractions of humic acids in HPs have the most considerable influence on enzyme hydrolysis.

Effects on Diversity of Soil Microbial Community

HP's amendments usually affect the bacterial community more than soil fungi or actinomycetes (Dong et al., 2009; Puglisi

et al., 2009). In a series of column experiments in which the 16S rRNA gene hybridization method was used, Van Trump et al. (2011) showed that *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* were greatly affected by the addition of HPs. In a recent study, Yuan et al. (2017) obtained similar results in a model based on experiment with biofilms from HPs on the electrodes. The added layer of HPs specifically promoted *Proteobacteria*, *Firmicutes*, and *Bacteroidetes*. According to Schoebitz et al. (2016), adding 15 g of HP from Leonardite shale to blueberry seedlings growing in andosol (TOC 5.77 g•kg⁻¹, pH 6.74) did not change the fungal communities in the rhizosphere, yet altered the bacterial population. The opposite effect was observed by Sellamuthu and Govindaswamy (2003), who examined the changes in the microbial community growing in a sandy clay loam soil. The soil had been amended with four rates (10, 20, 30, and 40 kg•ha⁻¹) of K-enriched HP addition in a 30 day pot experiment sown with sugarcane. For all HP-treatments, no effect was recorded in the bacteria population, whereas there was a substantial increase in the total amount of fungi and actinomycetes. Interestingly, the two higher rates accentuated these effects; the total amount of fungi in the rhizosphere increased up to 150% with 30 kg•ha⁻¹ HPs as compared to the control group, while the 30 kg•ha⁻¹ HP-induced variable increased only 76%. This finding could be explained by just a few microbial groups (e.g., bacteria). Nevertheless, more research needs to be conducted in this area before forming any conclusions.

Effects on Soil Invertebrates

The application of HPs to soil systems also affects mesofauna. Among all the effects upon soil fauna, the interaction of earthworms and nematodes with HPs appears to be the best-studied. Earthworms evidently ingest HPs particles. The passage of HPs through the digestive tract of the earthworm *Eisenia fetida* and *Eisenia andrei* influenced the average molecular weights of the HPs, particularly causing them to decrease (Tikhonov et al., 2011). Other authors have proposed that HPs may successfully mitigate pollutant-induced toxicity on these species. Shen et al. (2015) used cultivated soil spiked with Ni (100 and 1 mg/kg) and deltamethrin, examining remediation activities of HPs (0, 0.5, 1, and 3%) on the *E. fetida* oxidative and DNA damages over the course of the 48 day test. Any increase in the density of the earthworm would lead to an improvement of soil health. The presence of HPs successfully mitigated the damage inflicted by nickel and deltamethrin on DNA, proteins and lipid membranes. However, the damage caused by the lipid membrane was prevented most effectively when the content of HPs was 0.5 or 1%, while damage to DNA was prevented most effectively with 3% of HP. However, the 3% of HPs also negatively affected lipid membranes of earthworms. In addition, He et al. (2017) detected a protective effect of HPs from Ni contamination of *Enchytraeus crypticus* in a model soil system. The most prominent decrease in sub-lethal concentration of Ni for *E. crypticus* was observed after 4, 7, and 14 days of exposure. The preceding authors proposed that HPs could mitigate and delay the disruption of ion-regulatory mechanisms (e.g., plasma Na⁺), contributing to reduced physiological stress. In a recent

study, 0.1 and 1% of HPs successfully promoted the *E. fetida* survival rate and cocoon production in a 60 day greenhouse experiment (Pukalchik et al., 2018).

Data on the response of nematodes to HPs is limited. Some studies reported the possibilities of HPs to control nematode populations in soils for agriculture purposes to prevent plant roots. For instance, (Kesba and Al-Shalaby, 2008) tested commercial HPs with and without nutrients suppliers against the root-knot nematode in sandy loam, sandy and clay soils. They observed that HP supplemented with micro-elements (Fe, Mn, Cu) was notably better than pure HP and HP+NPK in reducing the number of surviving juveniles, achieving the highest percentage of nematode inhibition (49.2%). Jothi and Poornima (2017), reported that $2 \text{ g} \cdot \text{kg}^{-1}$ of HPs in soil reduced the nematode population 2-fold in a clay loam soil. Similar results were reported by Seenivasan and Senthilnathan (2017), who observed a marked drop of the nematode population *Meloidogyne incognita*. The soil density, egg population and reproduction rate dropped by 53.5–56.7%, 61.9–63.8%, and 55.7–56.6%, respectively, when exposed to the commercial humic product (0.04, 0.08, 0.2, and 0.4% in a sandy loam soil). This study highlighted that HPs can act as an effective agent for biological control. They also stated that HPs under the field conditions may cause drastic changes in the soil food web; the complexity of those processes require detailed investigations.

Effects of Humic Products on Pure Cultures of Bacteria and Fungi

Apart from the physical and chemical components, the soil system includes complex fungal and bacterial communities. As such, the biological effects of each species on the environment are difficult to specify. So that, artificial liquid media for growing individual species is usually used to elucidate the interaction between HPs and fungal and bacterial metabolisms, as well as the biological effects produced by HPs themselves (Crowther

et al., 2018). Recently, some works analyzed the influence of HPs on pure cultures of microorganisms in artificial media have received attention while explaining interactions. Overall, HPs produce both beneficial and non-beneficial effects on the microorganisms isolated from soil habitats and cultivated on different artificial media, such as Czapek's medium with sugars (Tikhonov et al., 2010; Fedoseeva et al., 2018), potato dextrose agar (PDA) (Pascual et al., 2002; Loffredo et al., 2007, 2008; Loffredo and Senesi, 2009) and under other artificial conditions.

Humic products produce an stimulatory effect on microorganisms (Kirschner et al., 1999; Gryndler et al., 2005; Tikhonov et al., 2010; Kanaparthi and Conrad, 2015). Major direct effects of HP on microorganisms include the stimulation of biomass growth, which is due to the biosynthetic activity that regulates the metabolism of a cell (Kirschner et al., 1999; Kulikova et al., 2005; Tikhonov et al., 2010). The HPs stimulated the growth of the intestinal bacteria strains *Agromyces* spp., *Aminobacter vorans*, *Arthrobacter* spp., *Bacillus* spp., *Kocuria palustris*, *Microbacterium* sp., *Nocardioides* spp., *Oxalobacter* sp., *Pseudomonas* spp., *Rhodococcus* sp., *Sphingopyxis* spp., and *Streptomyces* spp. (Tikhonov et al., 2010). HPs from peat promoted the growth of hydrocarbon-oxidizing *Acinetobacter* sp. bacteria (Tikhonov et al., 2010). Kanaparthi and Conrad (2015) showed that HPs isolated from a lake sediment significantly enhanced the growth and activity of autotrophic, nitrate-dependent-oxidizing *Thiobacillus* sp. microorganisms. HPs extracted from soil stimulated root colonization by extra radical mycelium of the mycorrhizal fungus (Gryndler et al., 2005). The addition of HPs activate molecular signaling for the transition from growth forms to dormant forms (such as spores and cysts) in the growth media, while improving the development of bacteria in the soil (Stevenson et al., 2004). Furthermore, HPs could be utilized by humus-reducing microorganisms as terminal acceptors supporting their microbial growth (Zhao et al., 2017).

TABLE 2 | Functional groups and elements of HPs and effects on biological characteristics of microorganisms.

Functional groups and elements of HSs	Biological effects	References
BIOELEMENTS		
H	Inhibition of fungal mycelial growth; activation of sclerotia formation	Loffredo et al., 2007, 2008
C	Inhibition of fungal mycelial growth	
N	Inhibition of fungal mycelial growth; activation of sclerotia formation	Loffredo et al., 2008
O	Activation of sclerotia formation	
FUNCTIONAL GROUPS		
Hydrophilic components (not only but especially): alkyl C–O (50–110), carboxyl–C (160–190) and ketone–C (190–230)	Activation of primary solute transport systems, such as plasmalemma and vacuolar H ⁺ -ATPases and tonoplast H ⁺ -PPase	Canellas et al., 2002; Zandonadi et al., 2007; Zancani et al., 2009
Phenols	Antioxidant and anti-inflammatory properties of HSs Inhibition of fungal mycelial growth; activation of sclerotia formation	Aeschbacher et al., 2012; Klein et al., 2018; Loffredo et al., 2008; de Melo et al., 2016
Carboxylic acids	Antioxidant and anti-inflammatory properties of HSs Inhibition of conidial germination and mycelial growth	Siddiqui et al., 2009; Aeschbacher et al., 2012; de Melo et al., 2016; Klein et al., 2018
Quinones	Formation of reactive oxygen species and fungicidal/bactericidal properties	de Melo et al., 2016

The degree of decomposition of HPs positively correlated with the accumulation of fungal biomass (Fedoseeva et al., 2018).

A number of studies reported that HPs considerably inhibit the growth of different fungal and bacteria species (Pascual et al., 2002; Gryndler et al., 2005; Loffredo et al., 2007, 2008; Fedoseeva et al., 2009; Loffredo and Senesi, 2009) (Table 2). The HPs extracted from soil decreased the populations of saprophytic microorganisms, such as the fungi *Paecilomyces lilacinus* and an unidentified actinomycete in the cultivation system (Gryndler et al., 2005). To confirm those results, the mycelial growth and conidial germination of two forms of pathogenic *Fusarium oxysporum* were investigated in the soil and composts (Loffredo et al., 2007). Furthermore, the growth and sclerotial formation of pathogenic *Sclerotinia sclerotiorum* and two antagonistic *Trichoderma* species (Loffredo et al., 2008), as well as the mycelial growth of pathogenic strains from *Pythium ultimum* and *F. oxysporum* (Loffredo and Senesi, 2009), were assessed.

In general, HPs exhibit a stimulatory effect on antagonistic species, such as those representatives of plant pathogens. However, the effect on *T. harzianum* was less sensitive than *T. viride* in the presence of HPs in the growth medium (Loffredo et al., 2007, 2008). Furthermore, *A. alternata* was sensitive to some HS types extracted from cambisols (Moliszewska and Pisarek, 1996). However, Loffredo et al. (2008) observed that various environmentally safe humic fractions and bioactive natural organic molecules should be considered as a possible alternative to the large number of inorganic and organic chemical fungicides that control the phytopathogenic fungi in the soil. Moreover, fungicide activity exhibited by HPs against phytopathogenic fungi *Botrytis cinerea*, *Rhizoctonia cerealis*, *Fusarium graminearum*, and *Phytophthora infestans* was confirmed with field research (Wu et al., 2016). However, should be considered as an alternative, Fedoseeva et al. (2017) found that sporulation of phytopathogenic *A. alternata* was stimulated by HP from lignosulfonate. A wide variety of the observed effects may account for the multiple functions of HPs and the variability of soil microorganisms. Several mechanisms that are stimulated by HPs or inhibited by microorganisms are not fully understood, although a series of studies have shown the existence of a close connection between chemical and functional properties of HPs and the effects that they produce.

Quite often, artificial media fails to reproduce the endogenous abiotic and biotic conditions required for the microbial growth. However, this is a vital prerequisite for comprehensively studying the chemical, physical and biological processes of the environment where microbes live. It is important to apply this knowledge to the interaction of the aforementioned factors with the growth of microorganisms (Pham and Kim, 2012).

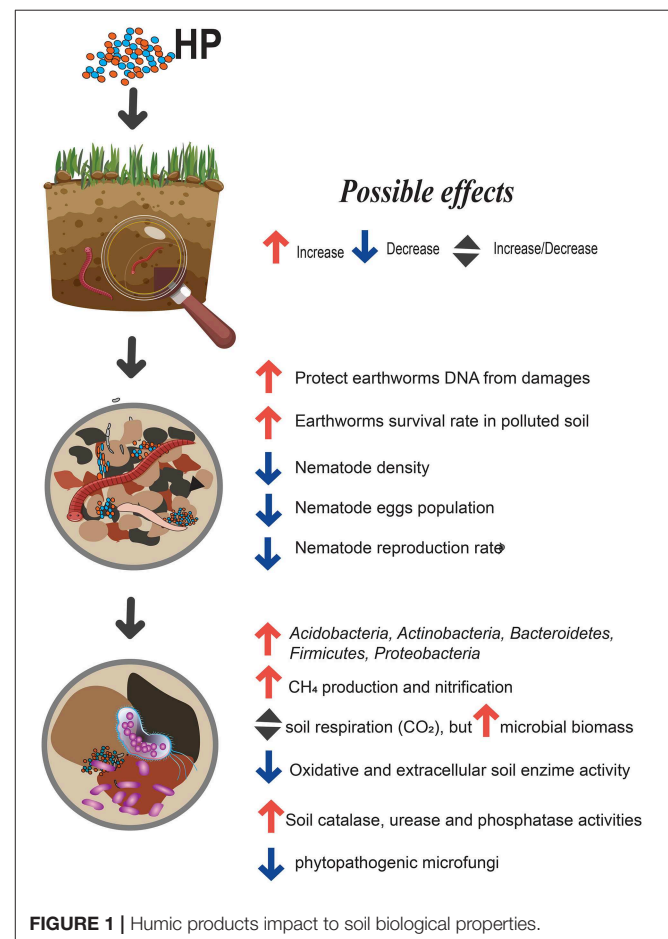
PROBABLE ACTION PATH-WAYS OF HPS AT THE CELLULAR LEVEL

HPs can influence various cell components such as plasma membrane, mitochondria and DNA containing components. The physiological influence of HPs is determined by their heterogenic composition that vary according to the origin,

process of obtainment, and functional groups present in their structures, such as quinones, phenols, carboxylic acids, and hydrophilic groups (Zancani et al., 2009; de Melo et al., 2016). The physiological influence of HPs on plant growth was correlated mostly with specific bioactive molecules content than with their size (Canellas et al., 2010). However, these authors mark that “molecules may be dynamically released from humic superstructures and exert their bioactivity weaker is the humic conformational stability as that obtained in the separated size-fractions.”

Some studies reveal that the response of certain physiological groups of HPs exhibit micellar-like properties and act as one kind of surfactants (Visser, 1985; de Melo et al., 2016; Xie et al., 2017). HPs could form micelle-like structures in neutral to acidic conditions due to their amphiphilic character (de Melo et al., 2016). According to Xie et al. (2017) the treatment of bacterial surfaces by the enhanced sorption properties of HAs promotes their interactions with bacteria. The core results demonstrated that the presence of humic acids significantly affected bacterial transport and deposition due to changes in the physicochemical properties of bacteria (Yang et al., 2016).

Quinones are responsible for the formation of reactive oxygen species in HPs, which are useful for wound healing and have fungicidal/bactericidal properties (de Melo et al., 2016).



Furthermore, phenols and carboxylic acids are responsible for various other functions, such as antioxidant and anti-inflammatory properties of HSs (Aeschbacher et al., 2012; de Melo et al., 2016; Klein et al., 2018). In particular, the presence of phenolic groups in HPs provides antioxidant properties due to their free radical scavenging capacity (de Melo et al., 2016). Stress factors can induce excessive production of reactive oxygen species, resulting in the disintegration of lipid membrane components due to lipid peroxidation. Therefore, the protective effect of HSs is considered to be due to the presence of phenolic fragments, which provide antioxidant activity (Kulikova et al., 2018). This response of HSs appears to be comparable to those of natural reactive oxygen species-neutralizing antioxidants, such as flavonoids, phenolic acids and stilbenes (Fujita et al., 2006).

HPs are able to activate primary solute transport systems, such as plasmalemma and vacuolar H⁺-ATPases and tonoplast H⁺-PPase (Zandonadi et al., 2007). In the presence of HPs, and particularly the most hydrophilic fraction, other biochemical plant parameters were modified. In that study, Glu-6-P and ATP concentrations, SAP activity (evaluated as phytate hydrolysis), total cellular P and the basal oxygen consumption by suspension cells all increased (Zancani et al., 2009). HPs were found to promote cell energy as inducers of plasma membrane H⁺-ATPase synthesis (Canellas et al., 2002). This enzyme cleaves ATP molecules and generates the electrochemical gradient that provides energy to secondary cell transporters (Morsomme and Boutry, 2000).

Moreover, HPs can efficiently facilitate extracellular electron transfer with the participation of out-membrane cytochrome c (Cyt c), which interacts with humic acid via electrostatic or hydrogen-bonding interactions to form organic complexes (Tang et al., 2014). Additionally, as a ubiquitous biogeochemical, HPs can induce the DNA-methylation in the cladocerans (Menzel et al., 2011). DNA-methylation is one pathway of epigenetic programming of gene expression and can alter the stress response, presumably including exposure to synthetic xenobiotic chemicals.

Therefore, we can resume that, according to their heterogenic composition, HPs can express various properties at the cellular and organismal level (Table 2). Despite the contrasting data, it is evident that antibacterial and antifungal effects are more likely to have quinone groups. Numerous attempts have been done to reveal the structure-property-function relationship in humic substances to explain their biological activity

(Muscolo et al., 2006; Garcia et al., 2016; Shah et al., 2018).

However, a main driving force of the influence of HPs to different organisms are not revealed. Consequently, the contemporary concept of HPs as a biostimulants can better explain their effects on living organisms.

CONCLUSIONS

Soil is the main target for HP application, given their well-known role as soil supplements and plant biostimulants. Overall, the majority of papers reported a positive impact on crop yields when HPs applied at recommended field-application rates. Meanwhile, the other side of this coin is the unpredictable influence of HPs to soil health and non-targeted organisms, such as soil microorganisms and mesofauna. In general, most studies suggest that the impact of HP's application on soil biological indicators are minor and temporary (Figure 1). Here, we present comprehensive review about the influence of HPs on C and N cycling, enzyme activities and growth and reproduction of earthworm and nematode populations. Stimulatory effect on microbial biomass, as well as inhibitory effects on different fungal and bacteria species were also pointed. Although HPs can be grouped according to chemical composition, source of HPs and formulation process, this does not guarantee that they will have similar impacts on soil organisms. Finally, we support the idea to attribute HPs into the class of biostimulants and to recognize that "this classic reductionist biology/chemistry may indeed be insufficient to explain their biological complexity" (Yakhin et al., 2016). One of the main matter that have to be improved in the assessment of HPs is the development of a detailed procedure, which will include not only plant species, but also various indicators of soil quality and different bioassay tests (for example, invertebrates, soil bacteria and fungi, etc.).

AUTHOR CONTRIBUTIONS

All authors contributed equally to the development and writing of the manuscript and MP led the writing team.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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