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Soil Biochemical Indicators to Monitor the Impact of Microplastics on Soil Functionality in Terrestrial Ecosystems

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Abstract: The present paper introduces soil as a complex system, so a multidisciplinary approach is needed to study not only the composition, abundance, and transport of microplastics (MPs) in terrestrial ecosystems but also soil properties and processes involved in their degradation and/or interaction with soil polyphasic matrix. Despite many researchers focusing their studies on the impact of MPs on the terrestrial ecosystem over the past years, little has been done about the use of biochemical indicators to study their effect on soil functionality.

Keywords: Biochemistry · Eco-physiological indices · Enzyme activity · Microbial biomass · Soil chemistry

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1. Microplastics in Terrestrial Ecosystems

A survey performed on Sept 22nd, 2023 on Scopus by using the keywords 'microplastics' and 'soil' in the title field returned 647 articles. Among these, 98 are reviews, confirming the great interest raised by this topic within the scientific community over the last five years. When adding the keyword 'soil biochem*' only 50 articles were obtained (Fig. 1, Fig. 2). When replacing 'soil biochem*' with 'soil microbial biomass' or 'soil enzymatic activity' only 42 and 20 articles were obtained, respectively. Finally, when adding 'metabolic quotient' only one article was obtained. This result shows that the use of biochemical indicators to assess soil quality in the presence of MPs is scarce, in particular the use of microbial quotients.

For this reason, the aim of this paper is to discuss the role of soil biochemical indicators as a tool to assess the impacts of microplastic pollutants on soil functions mainly related to nutrient storage and cycling.

Global plastic production has increased rapidly since the end of World War II, rising from 1.5 million tons in 1950 to 367 million tons in 2020 and will reach 33,000 million metric tons of plastic waste by 2050.[1] Microplastics, plastic particles <5 mm, mainly including polyethylene (PE), high-density polyethylene (HDPE), low-density polyethylene (LDPE), polyethylene tereph-

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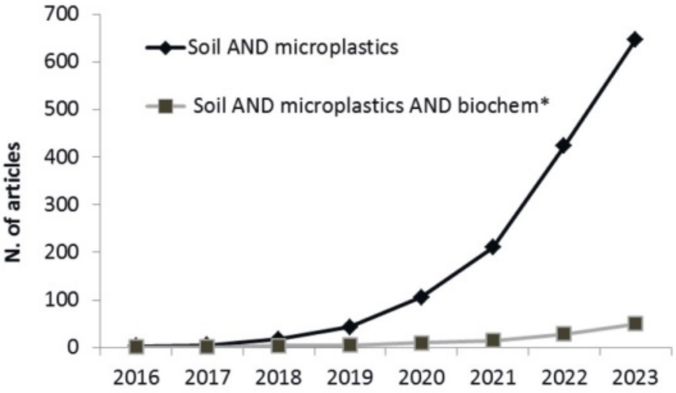

Fig. 1. Bibliographic search performed on Sept 22th, 2023 on the Scopus platform.

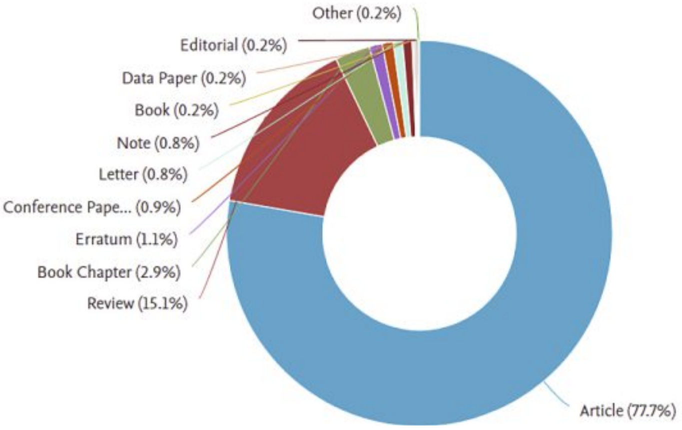

Fig. 2. Type of publications obtained from the bibliographic search performed on Sept 22nd, 2023 on the Scopus platform.

thalate (PET), polyvinyl chloride (PVC), polypropylene (PP), and polystyrene (PS), encompass characteristics of small size, large surface area, and high hydrophobicity. Soil MPs mainly include PE, PP, and PS, mostly in the forms of fibres and fragments. MPs deposition in terrestrial ecosystems is much higher than in marine ecosystems,[2] and mulching and sludge operations are the main sources of MPs in the soil.[3] When these materials reach the soil, especially if in the form of small particles, they become part of a complex mixture of organic matter and minerals as the plastic's surface is negatively charged and interacts with positively charged particles or ions.[4] As a consequence plastic materials can change the soils' physical and chemical properties, such as soil structure, porosity, pH, nutrient availability,[5] extractable ions, dissolved organic matter, water holding capacity, aggregate stability, and bulk density, therefore influencing the habitat of soil (micro)organisms and ultimately plant growth.[6] In addition, microplastics can adsorb pollutants through hydrogen bonding and electrostatic interactions, such as heavy metals, organic pollutants, pathogens, and resistance genes.[7]

2. Soil is a Complex Dynamic System

Soil is the most complicated biomaterial on the planet other than perhaps humans themselves.[8] This complexity lies not only in its various properties and different constituents but starts from its many definitions. Soil definition has changed over time and mainly among the different disciplines whose interests converge on this natural resource.[9] Hartemink lists more than 45 different definitions taken from soil science textbooks from 1900 to 2014.[9] During the XIXth century soil definitions considered the developments in agricultural chemistry (or geology) and soil was supposed to be only a production factor. Conversely, during the last century, soil science achievements, and the recognition of the many ecological functions performed by soil, brought additional definitions pointing to its central role in terrestrial ecosystem balance and sustainability (*e.g.* the recent concept of soil security[10]). Many different disciplines and competences look at soils from various perspectives; they range from agriculture to engineering, from landscape architecture to geology, from microbiology to mineralogy, from economics to archaeology. Therefore, not all soil features and current issues may be properly addressed. Soil complexity lies also in its composition and specific properties. Soil is a multiphase system where the solid, liquid, and gaseous components are tightly interrelated, and exchange matter and energy. Soil complexity is furthermore represented by the physical, chemical, and biological properties that give life to an extremely dynamic system where numerous processes occur. The final aspect to be taken into account is the fact that soil is a non-renewable resource on a human time scale, thus the knowledge of its current health status and the potential to carry out its numerous ecological functions is imperative.

Among the many threats to soil quality and health, pollution is one of the most worrying due to its rapid spread across all natural matrices (air, water, and soil) and its potential impact on the environment and animal/human health. Contaminants and/or pollutants may be of organic or inorganic origin, natural or xenobiotics, and may reach the soil through different pathways such as water, air, wastes, dumps, sludges, *etc*. Their fate, in terms of mobility/retention and potential toxicity, depends on many factors that imply the knowledge of their specific chemical composition and the behavior and interactions occurring with the soil components. As mentioned above, since soil is an extremely dynamic system where different transformation processes take place leading towards an equilibrium state, it is very difficult and site-specific to evaluate the potential hazard of certain pollutants on soil quality and health. The transformations include physical, chemical, and biological processes such as precipitation and dissolution, oxidation and reduction, biosynthesis and degradation, polymerization and hydrolysis, adsorption and desorption, *etc*. The soil reaction (pH) and the extent of solid phase electrical charges drive the intensity and type of these processes, in particular, they may strongly influence the fate of many pollutants, including MPs. Indeed, once on the soil surface, MPs can follow different transport routes: (i) horizontal distribution at the surface by runoff/erosion[11] and (ii) vertical distribution along the soil profile, reaching deeper soil horizons[12] through leaching processes or transported by soil organisms such as earthworms.[13] The fragmentation of MPs to nanoparticles (NPs; < 100 nm) in the soil system may affect nutrient storage, cycling, and carbon processing,[14–16] which are mostly regulated by three mechanisms of organic molecule stabilization in the soil:[17] (i) organo-mineral interactions, (ii) physical protection through occlusion into soil aggregates and, (iii) partial polymer degradation followed by polymerization and condensation reactions generating complex macromolecules resistant to microbial degradation.

2.1 *Soil Organo-mineral Interaction of Microplastics*

In the environment, chemical, physical, and photodegradation can cause modifications to plastics and subsequently lead to changes in the physicochemical properties of MPs. Under environmental weathering, the appearance of carbonyl functional groups leads to an overall negative surface charge of the plastic particles.[18,19] Charges on MPs may lead to ionic interactions with contaminant cations and anions. The interaction between MPs and clay minerals such as chlorite, kaolinite, montmorillonite, illite, and iron and aluminum oxides was studied in aquatic environments by Wang and coauthors.[20] Moreover, Dong *et al.*[21] and Hou *et al.*[22] have shown that humic acid (HA) and fulvic acid (FA) are able to enhance the stability as well as the migration capability of plastics by binding to them and causing electrostatic or steric repulsion. Through the formation of heteroaggregates, iron oxides such as hematite and goethite could also alter the transport/retention behaviors of plastics in saturated porous media.[23] A recent study reported that kaolinite particles could also alter the transport behavior of plastics by adsorbing onto their surfaces.[24]

2.2 *Soil Chemistry and Biochemistry of Microplastic Degradation and Transformation Processes*

Once in the soil, plastic particles slowly degrade into microplastics. Since they are difficult to degrade, they gradually accumulate in the environment. Plastic polymers are large molecules that have both crystalline and amorphous regions, and the latter gives the polymers flexibility. The rate of polymer biodegradation depends on several factors including chemical structure, molecular weight, and degree of crystallinity. Highly crystalline polymers like polyethylene (95%), are rigid with a low capacity to resist impacts. Polyethylene terephthalate (PET) based plastics possess a high degree of crystallinity (30–50%), which is one of the main reasons for their low rate of microbial degradation. In fact, they are expected to take more than 50 years for complete degradation in the terrestrial environment and hundreds of years in the oceans, due to low temperature and oxygen availability.

The microplastics in soil can mainly follow four degradation pathways: i) biodegradation, ii) photodegradation, iii) chemical degradation, and iv) thermal degradation.

i) The biodegradation process is the mineralization of plastic particles by microorganisms through the formation of biofilms on their surface, the destruction of their main skeletal structure, and the depolymerization of side chains under the action of specific enzymes to produce oligomers, dimers, and monomers. The soil meso- and macro-fauna are also included within biotic degradation agents. Studies have shown that some insects, including some invertebrates and social insects, are able to chew and feed on plastic products and use them as the sole source of carbon, converting microplastics into CO_2 and H_2O by physical means such as biting, chewing, or digesting and a series of biochemical processes.[25]

ii) Photodegradation is a crucial step in the disintegration of polymers in the presence of sunlight. Protracted sunlight irradiation, with UV light being the key element affecting this degradation pathway, can cause the creation of free radicals, oxygen inclusion, hydrogen abstraction, and scission or cross-link of chemical chains,[26] as well as morphological characteristics, such as flakes and cracks.[27]

iii) MPs are chemically broken down by reactive oxygen species (ROS). Photodegradation and chemical degradation of microplastics have been less studied compared to biodegradation, and advanced oxidation processes are currently the most used processes for the chemical degradation of microplastics.[28] Hakkarainen *et al.* showed that photodegradation processes and chemical degradation contributed to subsequent biodegradation and that plastics were destroyed by UV light to produce products that could be further used by microorganisms.[29]

iv) MP also degrades due to high temperatures.

Abiotic degradation normally occurs before biotic degradation in nature.[30]

3. Monitoring the Effect of Microplastics on Soil Functionality

Soil functionality is tightly linked to the concept of soil quality.[31] Monitoring soil quality and health has become imperative since the introduction of these new concepts in the 1990s. A list of indicators, belonging to three distinct categories, physical, chemical and biological, was proposed in 1994 by Doran and Parkin as a minimum data set.[31] Since then, a wide number of indicators and bioindicators have been validated as reliable tools under different land uses, soil threats and degradation factors. In particular, bioindicators, being strongly related to the soil living fraction, have been found to be particularly effective as early warnings of degradation processes, extremely sensitive and easy to use in a large scale and diverse experimental set-up. Bioindicators such as microbial biomass, in terms of size and diversity, and its metabolic activity, such as respiration and enzymatic activities involved in various nutrient cycles, allow the assessment of soil quality and health.[32] Soil enzymatic activities, in particular, inform on proper soil functioning, specifically nutrients cycling and storage, in response to certain stress conditions.[33] The use of fluorogenic substrates and microplate fluorimetric technique make this approach extremely effective, rapid, and sensitive, able to process a great number of samples.[34,35] As reported by Sajjad *et al.*,[36] different types of MPs may show diverse impacts on soil enzymatic activity involved in P, N, and C cycles, either enhancement or inhibition. For this reason, it is recommended to use enzyme activity as a class of sensitive indicators linked to nutrients cycling. When dealing specifically with soil C cycling, which may be impacted by MPs, a wide range of bioindicators may be suggested ranging from potential microbial respiration[37] to measuring the efficacy in using C-substrates (CLPP-MicroResp and Biolog techniques).[38–40] These techniques have proven to be particularly effective in providing information on soil microbial functional diversity,[40] another important soil function to be monitored under MPs pollution and current environmental conditions. These bioindicators may be further integrated by different ecophysiological indexes (metabolic, microbial, and mineralization quotients) that are extremely sensitive to perturbations affecting either nutrients (C and N) organic forms availability or microbial performances, in particular those provoked by pollutants[17,41] or environmental changes.[42] In the literature concerning MPs effects on soil, many studies have been published on the effects on soil properties (biological and chemical) but very few have focused on the transformations underlying these processes. In particular, Zhang *et al.*[43] reported a higher metabolic quotient at 1.0% MP concentration suggesting a stress condition for microbes and increase in CO_2 emission induced by this dose of MPs. Therefore, the use of biochemical indicators and of the ecophysiological indexes seems promising to fill this knowledge gap.[43]

4. Conclusions

The effect of MP pollution on soil ecosystem requires diverse approaches starting from a deep knowledge of soil properties and processes. The use of biochemical indicators is particularly recommended as they are sensitive tools providing early warning on changes in nutrient cycling, carbon storage, biodiversity level, and, in general, on soil function. Microbial eco-physiological indexes, derived from biochemical indicators such as soil microbial biomass and enzymatic activities, may further inform on stress conditions for soil microorganisms that may arise from MP soil contamination. The identified scientific gaps will be useful to microbiologists, hydrologists, ecologists and finally policymakers for monitoring microplastic pollution and to consider possible environmental strategies to prevent and or contain MP hazards.

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