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Effects of microplastics on soil quality

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ABSTRACT

Plastic pollution, especially caused by poorly managed agricultural practices is gaining increasing interest precisely because of its link to human nutrition. Certainly, agricultural mulching has many advantages but one of the risks is that mulches subject to wear and tear can release microplastics into the soil that accumulate over time. The effects of microplastics on soil have recently been investigated but many questions remain associated with the effects of bioplastics as an environmentally sustainable alternative to conventional plastics. This PhD thesis seeks to address this knowledge gap through a mesocosm study. The first chapter of the thesis analyses the effects of conventional plastics and bioplastics after six months of exposure on the chemical and physical characteristics of soil. In particular, the availability of nutrients and total metals in soils was investigated in order to understand the risk also associated with bioplastics. Subsequently, in the second chapter, the biotic properties of the soil such as enzyme activities, microbial respiration and phytotoxicity are also investigated. Finally, in the third chapter, the aspect of molecular ecology is investigated in order to understand whether these two types of plastics have influenced microbial and micro-arthropod communities over time. Finally, in the fourth chapter, the effects of microplastics generated by conventional and biodegradable plastic mulches on some properties of soils and on the growth of spinach (*Spinacia oleracea* L.) are analyzed.

GENERAL INTRODUCTION

Soil can be seen as a true living organism, whose function is strictly dependent on its quality, which is optimal when the biotic and abiotic components are in balance (Halvorson et al., 1997). As an extremely variable entity, characterized by a wide range of properties, to define the concept of soil "quality" is not trivial. The concept of soil quality is linked to the functions that can be performed and to the benefits that humans can derive from it (Costanza et al, 1997).

Soil is responsible for the degradation of organic matter, provides food and supports biomass production, contributes to water filtration, allows the anchoring of overlying vegetation, supports the nutrient cycle, has a high biodiversity and is the support for culturally valuable infrastructure and buildings (Cachada et al., 2018). The importance of soil for ecosystem integrity and human health makes it crucial to preserve its quality for sustainability.

Soil functions can be affected by various anthropogenic pressures, such as inappropriate agricultural and forestry practices, industrial activities, tourism, urbanization, industrialization and construction works.

Anthropogenic pressures contribute to changes in the chemical content of soils. Plastics and their sub-products are the most common among the anthropogenic organic contaminants and the increase in its production increases the resulting waste (Hu and Palic, 2020). Plastic is a large synthetic polymer (Laskar and Kumar, 2019) and is one of the compounds that has been observed to have the greatest environmental spread, due to its extensive application in industry and everyday life (*i.e.* packaging, construction, personal care products, materials used in agriculture, household products). This is due to characteristics such as versatility, stability, durability and low production costs, which have caused demand for plastic products to grow exponentially over the past 40 years (Yang et al., 2021). The limited and inappropriate disposal methods applied to plastic waste have led to a visible accumulation of debris in the environment (Wang et al., 2020).

The input of plastics into the terrestrial environment can occur through processes associated with sewage sludge application and composting (Huerta Lwanga et al., 2017; Li et al., 2019), irrigation (Blasing and Amelung, 2018), agricultural mulching (Zhang et al., 2020), littering (Akdogan and Guven, 2019) and atmospheric deposition (Allen et al., 2019). In particular, urban and agricultural soils prone pollutants as they are frequently exposed to anthropogenic activities.

The use of plastics in agriculture has increased considerably in recent decades in order to improve crop productivity and to reduce food loss (FAO and UNEP, 2021). Application of plastic mulches was shown to suppress weed growth, prevent soil erosion and increase soil temperature resulting in an improved crop quality and production (Zhang et al., 2013; Blaise et al., 2021). Despite these potential advantages, the widespread, long-term use and weathering of plastic mulches combined with a lack of systematic collection may cause plastic accumulation in the soil (Steinmetz et al., 2016). Perhaps, at the short-term, the pollution deriving by plastic mulches outweighs the benefits associated with it.

Polyethylene (PE), polyvinyl chloride (PVC) and polypropylene (PP) are the plastic material conventionally used for agricultural mulch (Hayes et al., 2012). Methods of removing plastic films from fields at the end of the crop growing season are laborious and it is almost impossible to extract all the small fragments of the mulch film (Changrong et al., 2014). Furthermore, there is no practical or cost-effective way to recycle contaminated plastic mulches from the soil (Changrong et al., 2014). These residual plastic films cause the accumulation of macro- (> 5mm) and microplastics (MPs: 100μm - 5mm) into the soil. MPs have recently been recognized as toxic pollutants in agroecosystems (Khalidh et al., 2023). In particular, PE has been documented to be a major source of MPs in agricultural soils (Kasirajan and Ngouajio, 2012; Blasting and Amelung, 2018; Wang et al., 2021).

Environmentally friendly biodegradable plastics, such as Mater-bi®, polylactic acid (PLA), starch, cellulose and polyhydroxyalkanoate (PHA) appear as promising substitutes for conventional non-degradable plastics (Qin et al., 2021). The advantage of using biodegradable mulches in agriculture primarily relies on their rapid degradation rate based on their structural and surface characteristics that allow the attack of enzymes. Through conversion to CO_2, water and biomass, these polymers are catabolized by soil microbiota (Brodhagen et al., 2015). However, it cannot be guaranteed that biodegradable mulch films will indeed degrade in the field within a 24-month time frame as environmental conditions, such as the microbial community present, temperature, and moisture content, vary from soil to soil, and are also dependent on climatic conditions (Sintim et al., 2020). However, fragmentation of bioplastic mulches can also occur under a range of environmental conditions with the subsequent release of micro-bioplastics into agricultural soils (Li et al., 2014; Qin et al., 2021). Since micro-bioplastics can be used as an exogenous carbon source, providing selective niches for soil microorganisms (Zhou et al., 2021), they are expected to influence soil microbial community composition to a larger extent than conventional MPs (Qi et al., 2020, Wang et al., 2022).

MPs from mulch films can penetrate along the soil column. The horizontal and vertical migration of MPs in the soil is certainly influenced by certain biotic and abiotic activities, such as tillage, leaching and bioturbation. The main driving forces behind the biological, physical and chemical transformation of MPs in soil are mechanical stresses, certain biological factors and oxidation processes (Zhao et al., 2022). Previous studies have confirmed that MPs can potentially influence the physical and chemical properties of soil directly or indirectly (Khalid et al., 2020). Regardless of whether these soil alterations are negative or positive, they would certainly affect the rhizosphere (Khalid et al., 2023) and on key ecological functions performed by soil-dwelling microorganisms (Rillig et al., 2017; Seeley et al., 2020).

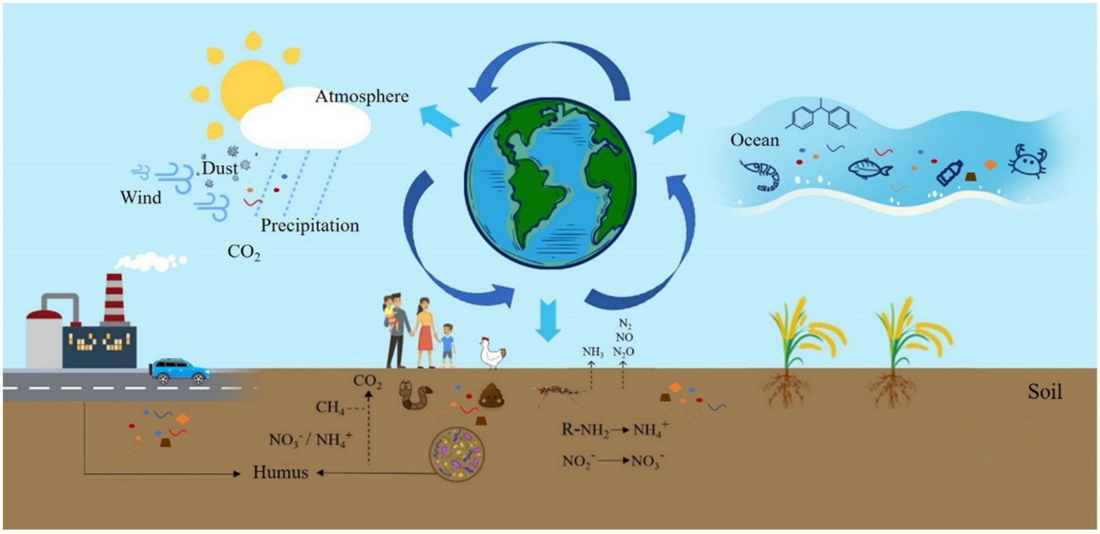

Fig.1 Microplastics and their biochemical processes in soils. Black arrows illustrate the movement of carbon and nitrogen. Source from Huang, Wang and Yin et al. 2022.

In particular, MPs can cause alterations in soil stability, soil porosity, water-holding capacity and bulk density (de Souza Machado et al., 2018). In turn, MPs influence pH that is one of the most important properties of soil, as it influences the availability of nutrients for plant roots and microorganisms. Some studies report an increase in soil pH in the presence of MPs (Lozano et al., 2021; Yang et al., 2021); by contrast, others report a decrease (Boots et al., 2019). However, soil pH is a factor causing ageing of MPs, which, in turn, release organic components (Piccardo et al., 2020).

In soils with a significant number of MPs, the release of dissolved organic carbon compounds could alter the C:N ratio, influencing the uptake of elements (Lozano et al., 2021, Yan et al., 2021). Some studies have reported the uptake of heavy metals, such as Pb, Cd, Cr, As, Co, Ni and Zn, on MPs (Guo et al., 2020, Zou et al., 2020). The interaction between metals and MPs depends on the polymer type, metal and compatibility. MPs vary greatly in their surface characteristics, such as morphology, particle size, roughness and degree of ageing (Godoy et al., 2019, Mao et al., 2020).

Nowadays, it is widely recognized that enzymatic activities, quickly responding to modification of soil abiotic properties, can be considered good predictors of soil quality (Memoli et al., 2019, 2021). Some of them, such as hydrolase, dehydrogenase, β-glucosidase and urease, are involved in litter degradation and are used as indirect measurements of soil nutrient cycles (Miralles et al., 2012; Woli´nska and Stępniewska, 2012; Zorzona et al., 2006). MPs can result in both inhibition (Dong et al. 2021) and potentiation by promoting the dissolution of organic matter (Liu et al. 2017) of some enzyme activities, the results are still controversial. A useful tool for assessing the integrated effects of different soil properties are ecotoxicological and, in particular, phytotoxicological assays (Manzo et al., 2010; Memoli et al., 2018), precisely because the accumulation of MPs in the soil is also responsible for the release of harmful additives (Halden, 2010) and the uptake of a range of toxic substances, which worsen the overall soil quality (Zhang et al., 2015). Through binding to organic matter and microbial secretions, MPs can affect different aspects of the soil microbial community, such as its composition and biomass (Li et al., 2019; Ren et al., 2020), and the soil micro- and meso-fauna community, such as their growth and metabolism (Buks et al., 2020; Wang et al., 2021). As soil biota participates directly or indirectly in the decomposition of organic matter, MPs may also affect soil nutrient cycling (de Souza Machado et al., 2018). MPs have been reported to influence the diversity and richness of microorganisms negatively (Feng et al., 2022) and positively (Yi et al., 2021), or have no significant effect at all (Sun et al., 2022). The surface morphologies of MPs change over time due to their degradation and provide more pockets, crevices or cavities for resident microbial communities. MPs derived from plastic mulch from a cotton field revealed unique microbial communities that were significantly different from adjacent soil particles (Zhang et al., 2019). It was also found that the microbes present on MPs are mostly Proteobacteria, Actinobacteria and Bacteroidetes that degrade plastics. The substrates present on MPs recruit taxa involved in plastic degradation and are known as special microbial accumulators (Zhang et al., 2019).

Soil fauna is of increasing interest to the scientific community as a symptom of soil quality (Cao et al., 2017). Earthworms, ecosystem engineers, placed in contact with MPs deriving by PE showed significant reductions in their biomass (Boots et al., 2019). This obviously led to the realization that ingestion of MPs by soil fauna had negative impacts on their fitness, growth and survival (Cao et al., 2017). Moreover, mesofauna, moving vertically and horizontally, inevitably results in the displacement of plastic fragments in the soil (Rillig et al., 2017).

Plants play an important role in the Earth's ecosystem and when MPs enter the soil, they can directly affect their growth, development and stress responses (Meng et al., 2021; Pignattelli et al., 2021). Recent studies, through the analysis of physiological indicators and plant growth, discuss the effects that different MPs can have on roots, stems, leaves and other plant organs (Gao et al., 2019; Li et al., 2020). Current research has also found that the effects of MPs on plants are not all negative and sometimes, have no significant effects (Judy et al., 2019). Therefore, the direct and indirect effects of MPs on the soil-plant system have become the focus of recent researches (Allouzi et al., 2021). Furthermore, the interaction of virgin and aged microplastics with other pollutants in the soil cannot be ignored (Fu et al., 2021; Mao et al., 2020; Wang et al., 2020). MPs have hydrophobic surfaces and large surface areas that could adsorb a variety of organic and inorganic pollutants, increasing their local concentration in soil or having combined effects on plants (Tourinho et al., 2019; Gao et al., 2021; Hu et al., 2020; Wang et al., 2021; Xu et al., 2021a).

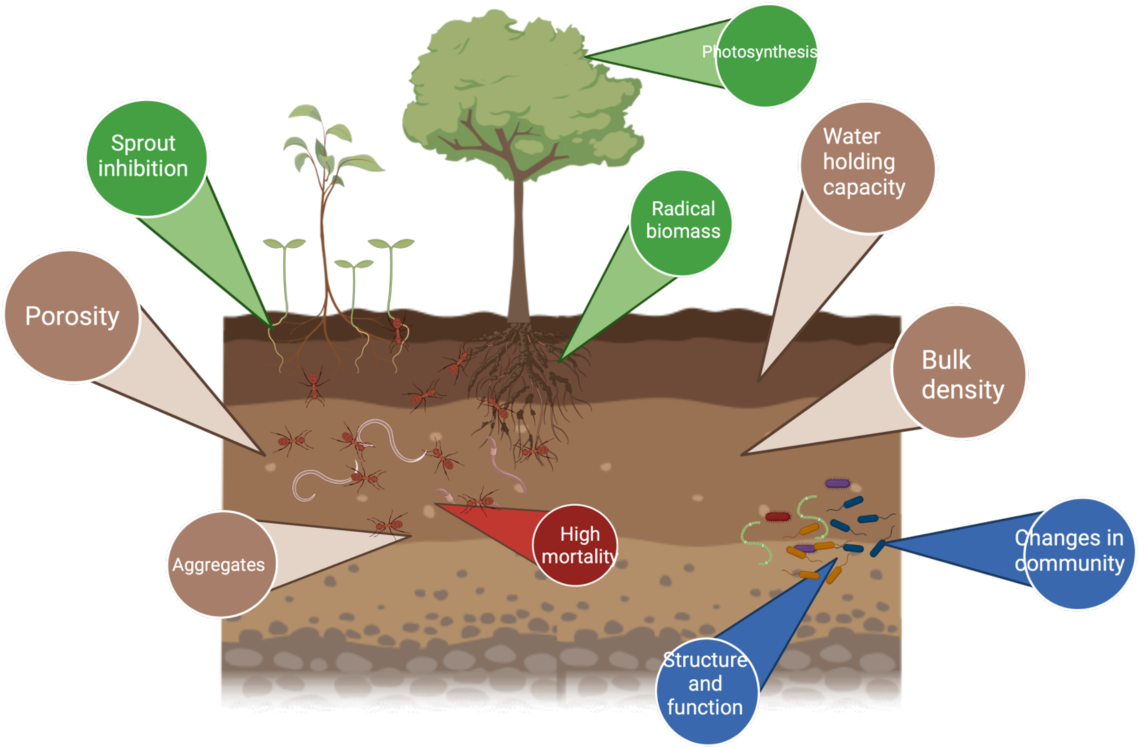

Fig. 2 Effects of microplastics (MPs) on terrestrial ecosystem

AIMS AND STRUCTURE OF THE THESIS

This thesis has been organized as a sequence of four chapters reporting already published papers and manuscripts submitted for publication to scientific journals. The common thread that binds all the chapters is the need to fill the current knowledge gap about the impact of plastic on soil quality. Moreover, it was also investigated if the presence of bioplastics exerts impacts lower than those caused by conventional plastics on soil quality. To achieve the aims, the research activities were performed in mesocosm trials and were tested the effects of conventional unbiodegradable and biodegradable agricultural mulches on soil abiotic and biotic properties, as mulches are the main sources of plastics in soils.

Chapter 1: In the first chapter of the thesis, the manuscript entitled "Does the Element Availability Change in Soils Exposed to Bioplastics and Plastics for Six Months?" is presented, published in the International Journal of Environmental Research and Public Health (Santini, G.; Maisto, G.; Memoli, V.; Di Natale, G.; Trifuoggi; M.; Santorufo, L. 2022), https://doi.org/10.3390/ijerph19159610. The aim of the work was to assess the impact of conventional plastic and bioplastic sheets on the total and available concentrations of elements (Al, Ca, Cu, Fe, K, Mg, Mn, Na, Ni, Pb and Zn) in soil. To achieve the objective, the research was conducted in mesocosm trials in which conventional plastic and bioplastic sheets were placed on the soil and the effects were evaluated six months after exposure. This work was carried out in collaboration with the Department of Chemical Sciences of the Federico II University of Naples (Italy).

Chapter 2: In the second chapter of the thesis, the manuscript entitled "Un-biodegradable and biodegradable plastic sheets modify the soil properties after six months since their applications" is presented, published in Environmental Pollution journal (Santini G.; Acconcia S.; Napoletano M.; Memoli V.; Santorufo L.; Maisto G., 2022), https://doi.org/10.1016/j.envpol.2022.119608.

The present research aimed to contribute in increasing the current knowledge about the effects of the use of plastics mulches on soil properties. Particularly, the research aimed to evaluate the effects, over the time, of un-biodegradable and biodegradable plastic sheets on soil abiotic properties, enzymatic activities and phytotoxicity. To achieve the aims, the research was performed in mesocosm trials and the effects were evaluated after three and six months since mesocosm setting up. The hypotheses behind the aims were: *(i)* the presence of un-biodegradable plastic sheets on soils causes variations in the investigated abiotic soil properties, enzymatic activity and soil phytotoxicity; *(ii)* the presence of un-biodegradable and biodegradable plastic sheets differently affects the investigated soil properties; *(iii)* longer exposure time to plastic sheets cause changes in soil properties of greater extent.

Chapter 3: In the third chapter of the thesis, the manuscript entitled "Microbiome dynamics of soils covered by plastic and bioplastic mulches" [redacted] (Santini, G.; Probst, M.; María Gómez-Brandón, M.; Manfredi, C.; Ceccherini, M.T.; Pietramellara, G.; Santorufo, L.; Maisto, G. 2023). [redacted]

[redacted]

This work was carried out in collaboration with the animal ecology group of the University of Vigo (Spain), the department of microbiology of the University of Innsbruck (Austria), the department of Agricultural, Food, Environmental and Forestry Sciences and Technologies of the University of Florence (Italy) and the department of chemical sciences of the University Federico II of Naples (Italy).

Chapter 4: In the fourth chapter of the thesis, the manuscript entitled "Metal Release from Microplastics to Soil: Effects on Soil Enzymatic Activities and Spinach Production" is presented, published in International journal of Environmental Research and Public Health (Santini, G.; Memoli V.; Vitale E.; Di Natale G.; Trifuoggi M.; Maisto, G. Santorufo, L.; 2023), https://doi.org/10.3390/ijerph20043106. The present research aimed to fill the current gap about the impact of microplastics on soil properties and on crop growth. Moreover, a comparison of these impacts between soil contaminated by conventional (PE-MPs) and biodegradable microplastics (Bio-MPs). To achieve the aims, the research was performed in pots filled with horticultural soils contaminated by PE-MPs and Bio-MPs at three different percentages (0.5 %, 1% and 2% v/v) where individuals of spinach plants were grown.

This work was carried out in collaboration with the department of chemical sciences of University of Naples Federico II.

CHAPTER 1

Does the element availability change in soils exposed to bioplastics and plastics for six months?

International Journal of *Environmental Research and Public Health*

MDPI

Article

Does the Element Availability Change in Soils Exposed to Bioplastics and Plastics for Six Months?

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1.1 Abstract

Plastic sheets are widely used in farming soil to improve the productivity of cultures. Due to their absorption capacity, plastic sheets can alter element and metal content in soils, and in turn affect soil properties. The use of biodegradable films is an attractive ecosustainable alternative approach to overcome the environmental pollution problems due to the use of plastic films but their impacts on soil are scarcely studied. The aim of the research was to evaluate the impact of conventional plastic and bioplastic sheets on total and available concentrations of elements (Al, Ca, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, and Zn) in soils. The research was performed in mesocosm trials, filled with soil covered by conventional plastic and bioplastic sheets. After six months of exposure, soils were characterized for pH, water content, concentrations of organic and total carbon and total nitrogen, and total and available Al, Ca, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, and Zn element concentrations. The results highlighted that soils covered by bioplastic sheets showed higher total and available concentrations of elements and higher contamination factors, suggesting that bioplastic sheets represented a source of metals or a less-effective sink to these background metals in soils, compared to conventional plastic ones.

Keywords: microplastic; metal contamination; polyethylene; biodegradable plastic; soil contamination

1.2 Introduction

The huge production of plastic materials has caused a widespread dispersion of plastic waste into the environment, forming debris of microplastics (MP) with size ranging from 0.1 to 5 mm [1,2]. As MPs have long persistence and slow degradation, they are ubiquitous in the environment and are recognized as emergent pollutants [3], that can cause serious hazards to organisms. The presence of MPs and their effects have been widely investigated in the aquatic environment, but the research in the terrestrial environment is incomparably lacking.

Agricultural soils can be polluted by MPs owing to intensive human activities such as application of sewage sludge and compost and fragmentation of plastic mulches [4]. As agroecosystems provide food, MPs in soils could cause unknown effects on farm ecosystems and food security, posing serious risks for human health [5]. Considering the risks microplastics pose to the ecosystem through the food chain, it is essential to understand the behavior of microplastics in the agricultural soil systems. The effects and the fate of MPs in soils are still controversial. It is well known that MPs can change soil porosity, water retention, and bulk density [6]. Moreover, they can be also responsible for the release of harmful additives [7] that can worsen the overall soil quality [8,9].

Polyethylene-MPs are the main kind of MPs in soil environments [10] and are also the main material of agricultural film, which is widely utilized in farming soil [11,12]. MPs directly or indirectly affect soil ecosystem functions and microbial communities [13–15], changing soil structure [6,16], soil pH [17–19], and increased soil aggregation [20]. Moreover, MPs can alter nutrient and metal content in soils [21,22], by absorbing some contaminants, such as Zn [23,24]. In addition, MPs, by modifying the soil abiotic properties can indirectly influence the chemical forms and bioavailability of heavy metals [11,25,26]. Therefore, MPs are an important factor governing the transformation of heavy metal speciation in soil. Although some studies have shown that MPs can adsorb heavy metals [23,27,28], potential changes in the chemical speciation of heavy metals triggered by MP contamination have scarcely been studied. Moreover, soil is a matrix with diverse microenvironments rather than a homogenous matrix [29], and this can lead to components and environments in different soil fractions responding differently to changes in the external environment. This response mechanism warrants investigation given its expected importance in guiding soil management.

The use of biodegradable films is an attractive eco-sustainable alternative approach to overcome the environmental pollution problems due to the use of plastic films [30]. Biodegradable films have already been tested as soil mulching films on several crops, such as zucchini squash [31], tomato [32], strawberry [33], lettuce [34], pepper, eggplant, musk melon, and sweet corn [35]. However, the impact of biodegradable plastic application to soil is not deeply investigated, and their effects on soil characteristics and element bio-availability are scarcely studied. Current studies on the differences between the adsorption capacities of bioplastics and conventional plastics for chemical pollutants have not yet reached an unambiguous conclusion.

Therefore, the aim of the present research was to evaluate the impact of conventional plastic and bioplastic sheets on total and available concentrations of elements (Al, Ca, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, and Zn) in soils. To achieve the aim, the research was performed in mesocosm trials in which conventional plastic and bioplastic sheets were placed on soils and the effects were evaluated after six months since exposure.

1.3 Materials and Methods

1.3.1 Mesocosm setting up

The experiment was performed in mesocosms. Ten pots, of one meter in diameter, were filled to 40 cm of height with limestone debris of different granulometry (1–4 cm diameters) picked up in a quarry located near Caserta. Contextually, in November 2020, forest surface soil was collected inside the Natural Reserve of Astroni. Approximately 50 kg of soil were placed on the limestone debris of each of the 10 pots for 30 cm of height.

In December 2020, a sheet (40 × 40 cm) of conventional plastic (made by polyethylene) constituted by little 16 squares (10 × 10 cm) was placed on the surface of the soil of five pots; whereas a sheet of bioplastic (made from polysaccharide complexes) of the same size was placed on the surface of the soil of five other pots. The choice of the materials was made according to the widespread plastic and emergent bioplastic mulches used in southern Italy in agriculture. The mesocosms were left outdoors on the terrace of the Department of Biology of the University of Naples Federico II.

1.3.2 Sampling and Analyses

In January 2021, before the placement of sheets of plastics (T0), surface soils (0–10 cm) were collected from each of the 10 pots, sieved (mesh: 2 mm) and characterized for pH, water content, concentrations of organic and total carbon, and total nitrogen (Table 1). Moreover, total and available element concentrations (Al, Ca, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, and Zn) were evaluated.

Table 1.1 Mean values ± s.e. (n = 14) of soil properties (pH; water content: WC (% d.w.); C total concentration (% d.w.); N total concentration (% d.w.); organic carbon concentration: Corg (% d.w.); C/N ratio (% d.w.)) at the beginning of the experiment (T0).

Abiotic Properties	Mean Values ± s.e.
pH	7.4 ± 0.07
WC	39.4 ± 0.63
C	4.2 ± 0.10
N	0.4 ± 0.01
C_{org}	3.2 ± 0.05
C/N	10.8 ± 0.64

Six months (T2: July 2021) after the mesocosm was set up, cores of soils were collected under a little square of the plastic sheet (10 × 10 cm) from each of the 10 pots by a sampler (10 cm Ø) from the upper 10 cm layer. The soil samples were analyzed for the same properties detected at T0.

The total concentrations of Al, Ca, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, and Zn were measured in dried soil samples (80 °C), pulverized by an agate mortar (Fritsch Analysette Spartan 3 Pulverisette 0), and digested by hydrofluoric acid (50%) and nitric acid (65%) in a ratio of 1:2 (v:v) in a microwave oven (Milestone-Digestion/Drying Module mls 1200). According to the method of Lindsay and Norwell [36], the available fractions of Al, Ca, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, and Zn were extracted. In brief, 25 mL of pentacetic diethylentriaminic acid (DTPA), CaCl2, and triethanolamine (TEA) solution at pH 7.3 ± 0.05 was added to 12.5 g of oven-dried soil samples (75 °C) to measure the Al, Cu, Fe, Mn, Ni, Pb, and Zn fractions. Whereas the availability of Na, Mg, K, and Ca was evaluated by BaCl2 and TEA pH 8.1 [36]. The soil suspensions were stirred for 2 h and filtered through a Whatman 42 filter. Element concentrations in the digests and extracts were measured by inductively coupled plasma mass spectrometry (ICP-MS Aurora M90, Bruker, Germany).

1.3.3 Assessment of Soil Metal Contamination

The Contamination Factor (CF) index was calculated in order to evaluate the contamination extent of the investigated soil for each metal (Al, Cu, Fe, Mn, Ni, Pb, and Zn); whereas the Pollution Load Index (PLI) was calculated in order to evaluate the metal whole and integrated contamination extent of the investigated soils.

The CF was calculated as reported below [37,38]:

$$CF = \frac{C}{Bn}$$

where C represents the concentration of the metal in soil samples and Bn represents the background value of the same metal in soils of the Campania Region [39]. Luo et al. [37] have distinguished the CF into four classes: CF < 1, low contamination factor; 1 ≤ CF < 3, moderate contamination factors; 3 ≤ CF < 6, considerable contamination factors; and C ≥ 6, very high contamination factor.

The PLI was calculated as reported below [38,40]:

$$PLI = \sqrt[n]{\prod_{i=1}^{n} CF}$$

where n is the metal and CF is the contamination factor. Banu et al. [41] have distinguished the PLI into two classes: PLI < 1, no pollution and PLI > 1, pollution.

1.3.4 Statistical Analyses

The normality of the dataset distribution passed (Wilk–Shapiro test for α = 0.05; n = 14); therefore, parametric tests were performed.

Student t-tests were performed to evaluate the differences in metal concentrations and in the calculated CF and PLI in soils exposed to conventional plastic and bioplastic and was considered significant at least for $\alpha < 0.05$.

The statistical analyses were performed by using the R 4.0.3 programming environment. The graphs were created using the SigmaPlot12 software (Jandel Scientific, San Rafael, CA, USA).

1.4 Results

1.4.1 Soil Total Metal Concentrations

The total concentrations of the investigated metals at the beginning of experiment (T0) and those in soils covered by both types of plastics are reported in Figure 1.1. The comparison between soils covered by bioplastic and conventional plastic highlighted that the concentrations of K, Mn, Na, Ni, Pb, and Zn did not significantly vary (Figure 1.1); by contrast, those of Al, Ca, Fe, Mg, and Cu were significantly higher in soils covered by bioplastic (70.8, 62.4, 31.5, 10.1 mg g^{-1} d.w., and 121 μg g^{-1} d.w., respectively, for Al, Ca, Fe, Mg, and Cu) than in those covered by conventional plastic (57.5, 37.2, 26.1, 7.5 mg g^{-1} d.w., and 101 μg g^{-1} d.w., respectively, for Al, Ca, Fe, Mg, and Cu) (Figure 1.1).

The Contamination Factors (CFs), reported in Table 1.2, were statistically higher in soils covered by bioplastic for Al, Fe, and Mg than in those covered by conventional plastic.

The PLI values were 1.11 and 1.26, respectively, for soils covered by conventional plastic and bioplastic, with no significant differences between the treatments (Table 1.2).

Table 1.2 Mean values of the Contamination Factor (CF) and, in bold, of the Pollution Load Index (PLI) for the investigated soil covered by plastic and bioplastic. Asterisks indicate significant differences between plastic and bioplastic (t-test; p < 0.05).

CF		
Metals	**Plastic**	**Bioplastic**
Al	1.47	1.81 *
Ca	2.12	2.51
Cu	0.62	0.74
Fe	1.10	1.33 **
K	1.55	1.58
Mg	1.29	1.74 *
Mn	0.96	1.02
Na	2.57	2.69
Ni	0.78	1.11
Pb	1.05	0.98
Zn	1.68	1.28
PLI	**1.11**	**1.26**

* $p < 0.05$; ** $p < 0.01$.

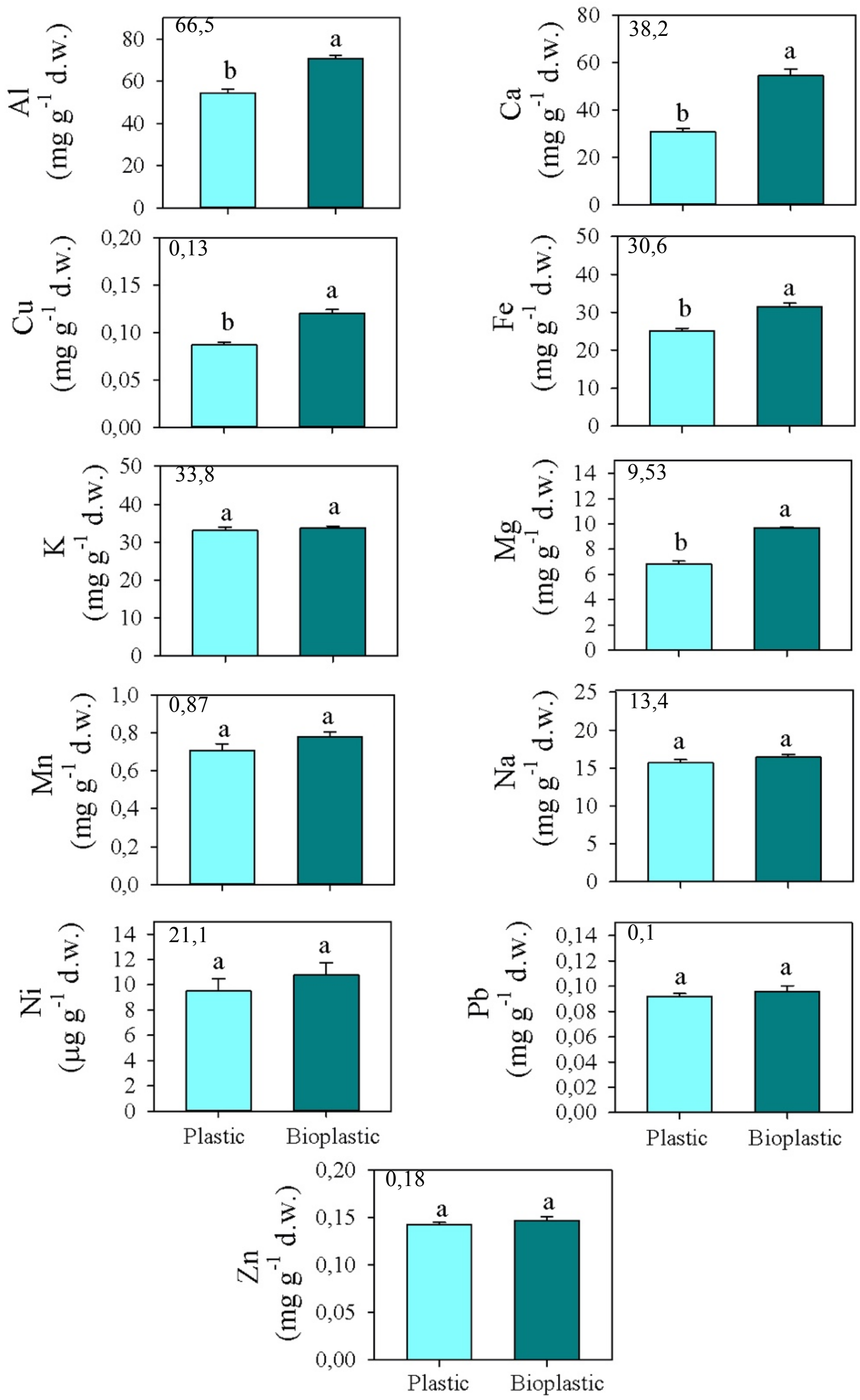


Figure 1.1 Mean values (±s.e.) of total Al, Ca, Cu, Fe, K, Mg, Mn, Na, Pb, and Zn (expressed as mg g^{-1} d.w.), and Ni (expressed as μg g^{-1} d.w.) concentrations measured in soils at T0 (top left) and after six months of exposure to plastic (light blue) and bioplastic (dark blue). Different small letters indicate statistically significant differences between soils covered by plastic and bioplastic, respectively (one-way ANOVA; $p < 0.05$).

1.4.2 Soil Available Fractions

The available fractions of the investigated metals at the beginning of experiment are reported in Table S1.1, and those in soils covered by both types of plastic are reported in Figure 1.2. The comparison between soils covered by bioplastic and conventional plastic highlighted that the availabilities of Fe, Mg, Mn, Na, Ni, and Zn were significantly higher in soils covered by bioplastic (0.45, 0.79 mg g^{-1} d.w., and 34.1, 7.26, 0.26, 28.5 μg g^{-1} d.w., respectively, for Mg, Na, Fe, Mn, Ni, and Zn) than in those covered by conventional plastic (0.392, 0.69 mg g^{-1} d.w., and 29.8, 6.31, 0.182, 27.4 μg g^{-1} d.w., respectively, for Mg, Na, Fe, Mn, Ni, and Zn) (Figure 1.2). By contrast, the availability of Pb was significantly higher in soils covered by conventional plastic (8.09 and 7.24 μg g^{-1} d.w., respectively, in soils covered by conventional plastic and bioplastic) (Figure 1.2).

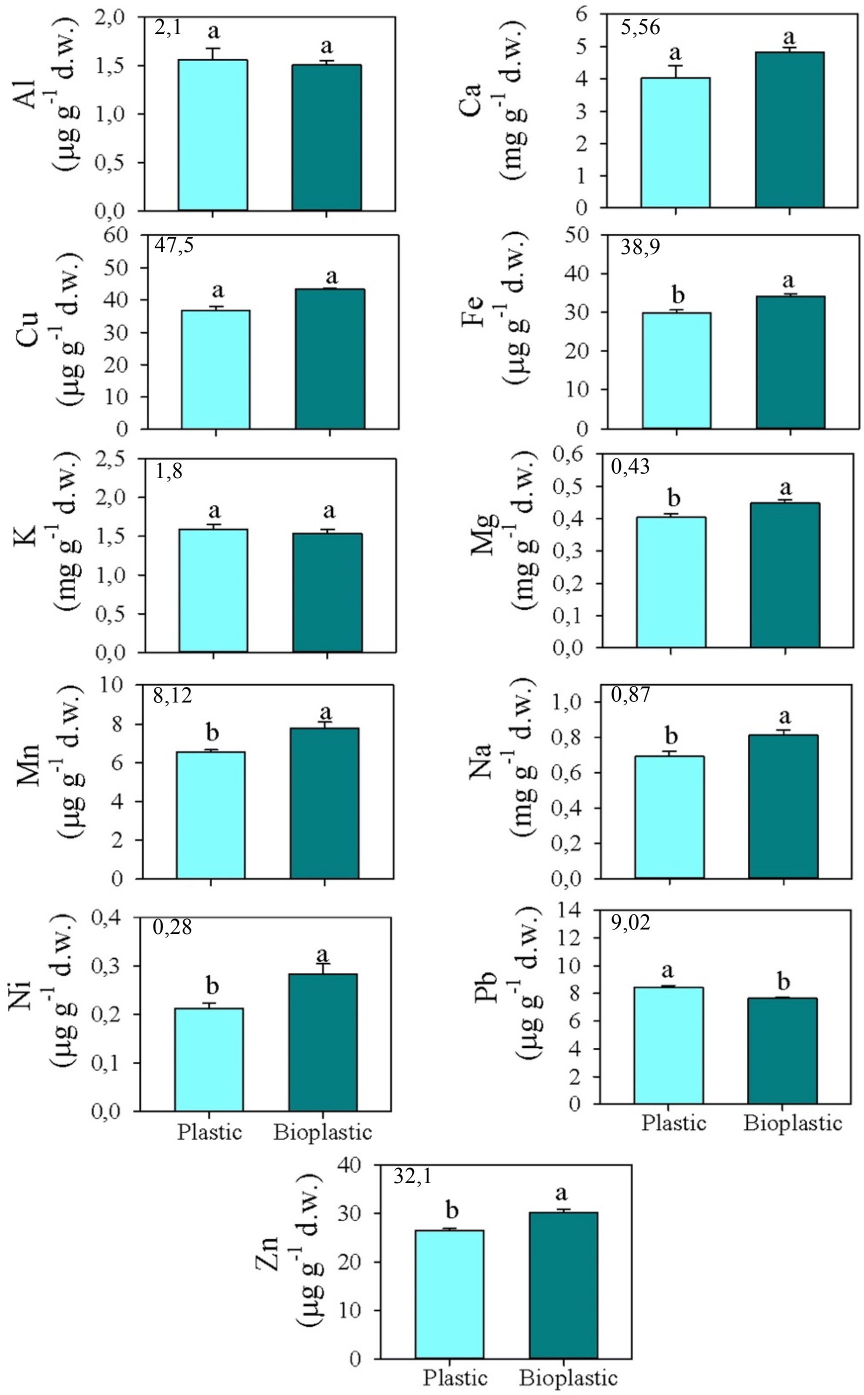


Figure 1.2 Mean values (± s.e.) of available Al, Cu, Fe, Mn, Pb, and Zn (expressed as μg g^{-1} d.w.), and Ca, K, Mg, and Na (expressed as mg g^{-1} d.w.) concentrations measured in soils at T0 (top left) and after six months of exposure to plastic (light blue) and bioplastic (dark blue). Different small letters indicate statistically significant differences between soils covered by plastic and bioplastic, re-spectively (one-way ANOVA; $p < 0.05$).

1.4.3 Percentages of Metal Availability with Respect to Total Concentration

The percentages of metal availability with respect to total concentration of Al, Ca, Cu, Mg, and Pb were significantly higher in soils covered by conventional plastic (0.003, 13.1, 42.2, 5.91, 9.23%, respectively, for Al, Ca, Cu, Mg, and Pb), than in those covered by bioplastic (0.002, 8.83, 36.1, 4.61, 8.00%, respectively, for Al, Ca, Cu, Mg, and Pb); by contrast, that of Zn was higher in soils covered by bioplastic (20.6 and 18.5%, respectively, for soils covered by bioplastic and conventional plastic) (Table 1.3).

Table 1.3 Mean values of ratios (expressed as percentage) of elements available and total concen-tration measured after six months of exposure. Asterisks indicate statistically significant differ-ences between soils covered by plastic and bioplastic, respectively (t-test; p < 0.05).

Metals	Plastic	Bioplastic
Al	0.003	0.002 **
Ca	13.1	8.83 **
Cu	42.2	36.1 *
Fe	0.12	0.11
K	4.80	4.56
Mg	5.91	4.61 **
Mn	0.93	1.00
Na	4.41	4.96
Ni	2.23	2.64
Pb	9.23	8.00 *
Zn	18.5	20.6 **

* $p < 0.05$; ** $p < 0.01$

1.5 Discussion

The findings highlighted that the presence of bioplastic sheets on soils caused significant increases in total and available concentrations of most of the investigated metals. This is corroborated by the observed capability of plastics to hold metals [27,42], which may be added as pigments or heat stabilizers during different phases of their production [43]. Moreover, the capability of plastics to hold metals depends also on their characteristics, such as pore filling, hydrophobic interactions, hydrogen bonds, electrostatic interactions, van der Waals forces, and specific surface area [23,44].

The significantly higher total concentrations of Al, Ca, Cu, Fe, and Mg in soils covered by bioplastic than conventional plastic sheets likely can be due to the nature of the bioplastic itself. In fact, the used bioplastic sheets, constituted by polysaccharide com-plexes such as amylose and amylopectin, likely showed many O-ligands to link metal ions [45]. Bioplastics, because of their crystallization characteristics and carrier adsorption, have stronger adsorption capacity of metals than conventional plastic [10,46].

The higher degradability of bioplastic sheets would seem to be responsible for the greater availability of Fe, Mg, Mn, Na, Ni, and Zn observed in soils covered by bioplastic than by conventional plastic sheets. In fact, the used bioplastic sheets, rich in organic carbon compounds, represent an important resource for the soil-dwelling microorganisms [47,48]. The formation of fragments of bioplastic sheets, due to biological activity, increased the surface of contact with soils, enhancing the further physical and/or chemical fragmentation of the sheet itself [10]. Although the availability of most of the investigated elements were higher in soils covered by bioplastic sheets, Pb availability was meaningfully higher in soils covered by conventional plastic sheets.

Likely, this could be due to the release from conventional plastic sheets of petroleum-based compounds added during their production [43].

Despite bioplastic being widely regarded as an environmentally friendly substitute for conventional plastic, its effects on metal accumulation in soils remain largely unknown. Unfortunately, the PLI (greater than 1) highlighted that the bioplastic sheets represented sources of metal contamination for the soils to the same extent as the conventional plastic sheets [23]. Moreover, bioplastic was responsible for the greater accumulation of Al, Fe, and Mg, as the contamination factors for these elements were significantly higher than in soils covered by the conventional plastic sheets.

The percentage of the available fraction of the element in relation to its total concentration showed significant differences in soils exposed to the investigated sheets. These percentages calculated for Al, Ca, Cu, Mg, and Pb were significantly higher in soils exposed to conventional plastic sheets, suggesting that these sheets decreased the adsorption capability of elements to soil and increased their desorption [49]. Instead, the stronger adsorption capacity of bioplastic sheets [10] increased their degradation rate, causing higher total and available concentrations of the elements. The lack of significant differences for the other investigated elements could be due to the fact that, likely, they are linked in chemical complexes that need a longer time to be exchanged.

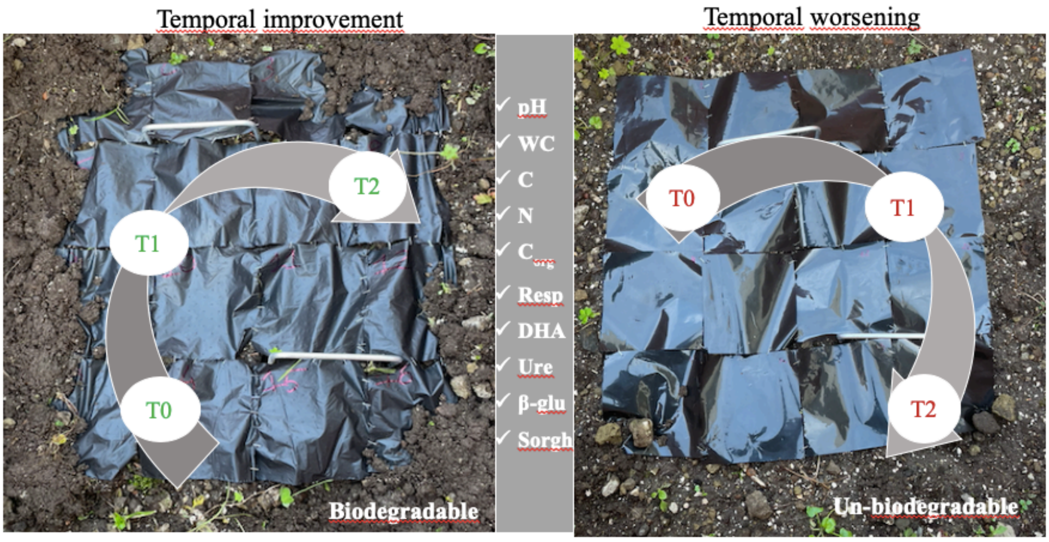
1.6 Conclusions

The overall comparison between soils covered by conventional plastic and bioplastic sheets highlighted that soils covered by the former showed higher ratios between available and total concentrations of elements, whereas those covered by the latter showed higher total and available concentrations of elements and higher contamination factors. Both the kinds of plastic sheets caused soil metal accumulation as the pollution load indices were greater than 1.

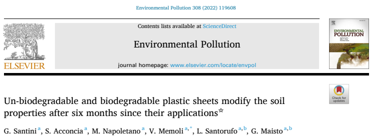
Thereby, the findings suggest that bioplastic sheets represented a source of metal contamination or a less-effective sink to these background metals, comparable to the conventional plastic ones.

Further investigations are needed to evaluate the effects of metal accumulation in soils due to longer periods of plastic sheet exposure.

CHAPTER 2



Un-biodegradable and biodegradable plastic sheets modify the soil properties after six months since their applications



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Un-biodegradable and biodegradable plastic sheets modify the soil properties after six months since their applications☆

G. Santini [a], S. Acconcia [a], M. Napoletano [a], V. Memoli [a,*], L. Santorufo [a,b], G. Maisto [a,b]

2.1 Abstract

Nowadays, microplastics represent emergent pollutants in terrestrial ecosystems that exert impacts on soil properties, affecting key soil ecological functions. In agroecosystems, plastic mulching is one of the main sources of plastic residues in soils. The present research aimed to evaluate the effects of two types of plastic sheets (un-biodegradable and biodegradable) on soil abiotic (pH, water content, concentrations of organic and total carbon, and total nitrogen) and biotic (respiration, and activities of hydrolase, dehydrogenase, β-glucosidase and urease) properties, and on phytotoxicity (germination index of *Sorghum saccharatum* L. and *Lepidium sativum* L.). Results revealed that soil properties were mostly affected by exposure time to plastics rather than the kind (un-biodegradable and biodegradable) of plastics. After six months since mesocosm setting up, the presence of un-biodegradable plastic sheets significantly decreased soil pH, respiration and dehydrogenase activity and increased total and organic carbon concentrations, and toxicity highlighted by *S. saccharatum* L. Instead, the presence of biodegradable plastic sheets significantly decreased dehydrogenase activity and increased organic carbon concentrations. An overall temporal improvement of the investigated properties in soils covered by biodegradable plastic sheets occurred.

Keywords: soil abiotic properties; respiration; enzymatic activities; phytotoxicity


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2.2 Introduction

Microplastics (MPs), constituted by particles with size lower than 5 mm, are among the main global environmental pollutants that, recently, have been extensively detected in both aquatic and terrestrial ecosystems. Plastic mulching, sewage and sludge applications, wastewater irrigation and atmospheric transport are considered to be the major source (Chae and An, 2018; de Souza Machado et al., 2018) of MP pollution in soils.

Plastic mulches are widely used for their benefits as they increase soil temperature and moisture, improving crop yield and quality. Traditionally, the widespread use of un-biodegradable plastic (*i.e.*, polyethylene) has caused MP accumulation in soil (Li et al., 2022). Recently, to avoid that, biodegradable plastic (*i.e.*, Mater-bi®) mulches, providing agronomic improvements comparable to those deriving by the un-biodegradable ones (Tofanelli and Wortman, 2020), have been used.

The interest in soil accumulation of MPs is due to their effects on the soil abiotic properties and on the key ecological functions performed by soil-dwelling microorganisms (*i.e.*, nutrient cycles, organic matter decomposition and enzymatic activity). Particularly, MPs can cause changes in soil porosity, water holding capacity and bulk density (de Souza Machado et al., 2018), and, attaching to organics and microbial secretions, can modify the microbial community structure (Rillig et al., 2017; Seeley et al., 2020).

Nowadays, it is widely recognized that enzymatic activities, quickly responding to modification of soil abiotic properties, can be considered good predictors of soil quality (Memoli et al., 2019, 2021). Some of them, such as hydrolase, dehydrogenase, β-glucosidase and urease, are involved in litter degradation and are used as indirect measurements of soil nutrient cycles (Miralles et al., 2012; Wolińska and Stępniewska, 2012; Zorzona et al., 2006). By now, the effects of MPs on soil enzymatic activities are still controversial. In fact, despite of findings of Dong et al. (2021) that report inhibition of some enzymatic activities, those of Liu et al. (2017) report that MPs can enhance the enzymatic activity, favoring organic matter dissolution.

MP accumulation in soils is also responsible for the release of harmful additives (Halden, 2010) and the absorption of a variety of toxic substances, that worse the overall soil quality (Zhang et al., 2015). In this framework, ecotoxicological assays and, particularly, the phytotoxic ones (Manzo et al., 2010; Memoli et al., 2018) are useful tools to evaluate the integrated effects of several soil properties on its quality. In fact, plant seeds are sensitive to the conditions of both soil matrix and aqueous phase and are informative of short- and long-term effects (Manzo et al., 2008).

The present research aimed to contribute in increasing the current knowledge about the effects of the use of plastics mulches on soil properties. Particularly, the research aimed to evaluate the effects, over the time, of un-biodegradable and biodegradable plastic sheets on soil abiotic properties, enzymatic activities and phytotoxicity. To achieve the aims, the research was performed in mesocosm trials and the effects were evaluated after three and six months since mesocosm setting up. The hypotheses behind the aims were: H1) the presence of un-biodegradable plastic sheets on soils causes variations in the investigated abiotic soil properties, enzymatic activity and soil phytotoxicity; H2) the presence of un-biodegradable and biodegradable plastic sheets differently affects the investigated soil properties; H3) longer exposure time to plastic sheets cause changes in soil properties of greater extent.

2.3 Materials and Methods

2.3.1 Mesocosm setting up

The research was carried out in mesocosms, constituted by 14 pots one meter in diameter, filled for about 40% of the total height (40 cm) with limestone debris of different granulometry (1-4 cm diameter) picked up in a quarry located near Caserta. In November 2020, soil collected in the Natural Reserve of Astroni was placed on the limestone debris. The amount of soil put into each pot was 50 kg and filled 30 cm of height.

In January 2021, a sheet (40 x 40 cm) of un-biodegradable (Unbiod, made by polyethylene) plastic constituted by little 16 squares (10 x 10 cm) was placed on surface soil of five pots; whereas, a sheet of biodegradable (Biod, Mater-bi®) plastic of the same size was placed on surface soil of other five pots. No sheets of plastic were placed in the last four pots, considered as a control (Ctr). The mesocosms (Fig. 2.1) were left outdoors on the terrace of the Department of Biology of the University of Naples Federico II and exposed to weather conditions and not irrigated. Monthly rainfall and mean temperature for the period January - August 2021 (http://www.ilmeteo.it; www.ancecampania.it) are reported in Table 1.

Table 2.1 Monthly mean temperature and rainfall amount detected in downtown Naples (Italy) from January to August 2021 (http://www.ilmeteo.it/ and www.ancecampania.it).

	Rainfall (mm)	Temperature (°C)
January	6.70	9.27
February	5.14	10.4
March	2.97	11.3
April	1.04	13.5
May	1.05	18.4
June	0.07	24.6
July	0.94	26.6
August	1.14	27.2

Fig. 2.1 Experimental mesocosms (left) and details of soils covered by un-biodegradable and biodegradable plastic sheets and uncovered soils (right).

2.3.2 Sampling and analyses

In January 2021, before the placement of sheets of plastics (T0), surface soils (0 – 10 cm) were collected from each of the 14 pot, sieved (mesh: 2 mm) and characterized for pH, water content, concentrations of organic and total carbon, and total nitrogen. Moreover, respiration and four enzymatic activities (hydrolase, dehydrogenase, β-glucosidase and urease) were evaluated. Finally, the phytotoxicity of the soils was

evaluated through the evaluation of seed germination and root elongation of *Sorghum saccharatum* L.

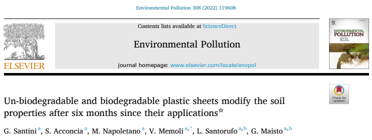
After 3 (T1: April 2021) and 6 (T2: July 2021) months since the mesocosm setting up, cores of soils (0 – 10 cm) were collected (10 cm Ø) under a little square of plastic sheet (10 x 10 cm), randomly selected among the sixteen ones, posed at the top of the ten pots (five covered by un-biodegradable sheets and five covered by biodegradable sheets). Soil cores were also collected from the four control mesocosms. The soil samples were analyzed for the same properties detected at T0.

The sieved (< 2 mm) soil samples were characterized for pH, WC, organic C (C_{org}) concentrations and total C and N concentrations. Soil pH was measured, by an electrometric method, in a soil : distilled water (1 : 2.5 = v : v) suspension; WC was determined gravimetrically by drying fresh soil at 105 °C until constant weight; C_{org} was measured by a CNS Analyzer (Thermo Finnigan) on soil samples previously treated with HCl (10 %) to exclude carbonates; C and N concentrations were evaluated on oven-dried (105 °C) and grounded (Fritsch Analysette Spartan 3 Pulverisette 0) soil samples by a CNS Analyzer (Thermo Finnigan, Italy). Details for the above described analyses are reported in Memoli et al. (2018).

The biological analyses (*i.e.*, microbial respiration, hydrolase, dehydrogenase, β-glucosidase and urease activities) were performed on soil samples stored at 4 °C within three days since sampling and on triplicate.

The microbial respiration (Respiration) was measured using MicroResp® (Macaulay Scientific Consulting, Aberdeen, UK) assays (Campbell et al. 2003). Four replicates of fresh sample, corresponding to approximately 0.30 g dry weight, were incubated in a 96 - deep well Microplate (Fisher Scientific E39199, Illkirch France).

After pre-incubation between 3 and 5 days at 25 °C in the dark, each deep well plate was covered with a detection plate, using a silicone gasket (MicroResp™, Aberdeen,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UK). The detection plate was prepared with cresol red gel 10 g L^{-1} (purified agar) according to the following concentrations: Cresol red 37.2 µmol L^{-1}; KCl 150mM; $NaHCO_3$ 2.5mM. After 3-5 days of pre-incubation, the deep well plate and the detection plate were clamped and incubated for a further 6 h. The optical density at 590 nm (OD_{590}) was measured for each detection well at time zero (before exposure of the deep well plate to the detection plate) and time six (after 6 h of incubation) using a Victor 1420 Multilabel Counter (Perkin Elmer, Massachusetts, USA). Final OD_{590} were normalized using OD_{590} at time zero and converted to mg of CO_2 respired g^{-1} of h^{-1} sample.

Hydrolase activity (HA) was determined by adding 7.5 mL of 60 mM potassium phosphate (pH 7.6) and 0.100 mL of fluorescein diacetate (FDA) to 1 g of fresh soil. The reaction mixture was incubated at 30 °C for 20 min. At the end of incubation, the fluorescein was extracted with 7.5 mL of acetone and centrifuged at 5000 rpm for 5 min. The absorbance of the supernatant was measured at 490 nm and the results were expressed as mmol of FDA produced for 1 g of dry soil in 1 min (Adam and Duncan, 2001).

Dehydrogenase activity (DHA) was determined by adding 1 mL of 1.5% 2,3,5-triphenyltetrazolium chloride (TTC) dissolved in 0.1 M Tris-HCl buffer (pH 7.5) to 1 g of fresh soil. The reaction mixture was incubated at 30 °C for 24 h in the dark. At the end of incubation, the triphenylformazan (TPF) was extracted with 8 mL of acetone, and the extract was centrifuged at 3500 rpm for 15 min. The absorbance of the supernatant was measured at 546 nm and the results were expressed as mmol of TPF produced for 1 g of dry soil in 1 min (Memoli et al., 2018).

β-glucosidase activity (β-glu) was determined by adding 4 mL of modified universal buffer (MUB) pH 6 and 1 mL of 0.025M p-nitrophenyl β-D-glucopiranoside (PNP) to 1 g of soil. The mixture was then incubated at 37°C for 1 h, after which the enzymatic reaction was stopped by cooling on ice for 15 min. Then, 1 mL of 0.5M $CaCl_2$ and 4 mL of 0.1M Tris-hydroxymethilaminomethane-sodium hydroxide (THAM-NaOH) pH 12 was added. In the control, the substrate was added before the addition of $CaCl_2$ and NaOH. The absorbance of the supernatant was measured at 420 nm and the results were expressed as mmol of PNP produced for 1 g of dry soil in 1 min (Tabatabai and Bremner, 1969; Tabatabai, 1982).

Urease activity (Ure) was determined by adding 0.5 ml of urea (0.1 M) and 4 ml of borate buffer (0.1 M pH 8.8) to 1 g of fresh soil. The solution was incubated at 37 °C for 2 h and then 10 ml of potassium chloride in hydrochloric acid (KCl 1.35 M in 0.1 M HCl) was added. The samples were shaken for 30 min and then centrifuged at 5000 rpm for 10 minutes. The extract was taken from each sample, to which 2.5 ml of buffer, 4 ml of salicylate and 2.5 ml of hypochlorite were added. The samples were incubated again at 37 °C for 30 min. The absorbance of the supernatant was measured at 660 nm and the results were expressed as mmol of NH_4^+ produced for 1 g of dry soil in 1 min (Kandeler and Gerber, 1988; Alef and Nannipieri, 1995).

The phytotoxicological assays were performed according to EPA (1996) using *Sorghum saccharatum* L. and *Lepidium sativum* L. as test organisms and performed on fresh and sieved (2 mm) samples. Ten seeds were placed in Petri dishes containing an amount of fresh soil equivalent to 10 g of oven-dried soil, subsequently saturated with water. Standard soil (OECD, 1984) and $K_2Cr_2O_7$ were used as negative and positive controls, respectively. After incubation in darkness (72 h, at 25 °C), the number of germinated seeds and root elongations were measured.

To express the two endpoints simultaneously, the germination index (IG) was calculated using the following:

$$IG = L\,x\,n \tag{1}$$

where L is the average root length and n is the average number of germinated seeds.

The results were expressed as percentage of effect compared to the negative control (K):

$$\% \, Effect = \frac{(IGK - IGsample)}{(IGK)} \, x \, 100 \qquad (2)$$

Positive and negative values of the effect percentages indicate inhibition and biostimulation, respectively.

2.3.3 Statistical analyses

Normal data distribution and homogeneity of variance were verified by Shapiro-Wilks and Levene Median test, respectively.

The effects of substrate type (Ctr, Unbiod and Biod) on biological and chemical parameters were assessed through one-way analysis of variance (ANOVA) combined with post hoc comparison tests (pairwise Student-Newman Keuls test or Fisher LSD method).

The temporal effects on biological and chemical parameters were assessed through the unpaired t-test.

Computations were made with Sigma-Stat 3.0 software and graphical displays with Sigma-Plot 9.0 software (Jandel Scientific, USA).

A Principal Components Analysis (PCA) was performed on soil properties to evaluate the soil sample distributions according to the sampling times (T1 and T2) and to identify the main properties driving the temporal distribution. The PCA was conducted using the Past 4.0 software.

The ellipses were added manually to highlight the PERMANOVA results. PERMANOVA analyses (Adonis function – pairwise.perm.manova test for $P < 0.05$) was carried out on the selected soil abiotic properties and all the soil biotic properties. Permanova was performed using the R 4.0.3 programming environment with ade4^ and Factoextra packages.

2.4 Results

2.4.1 Soil properties at T0

At T0, the mean value of soil pH was 7.37 and that of water content was 39.4. The mean concentrations of total and organic C were, respectively, 4.15 and 3.21 % d.w., that of N was 0.38 % d.w., and the C/N ratio was 10.8 (Fig. 2.2). Moreover, soil respiration was, on average, 0.13 mg of CO_2 g^{-1} h^{-1} (Fig. 2.3), the mean values of hydrolase (HA), dehydrogenase (DHA), urease (Ure) and β–glucosidase (β-glu) were, respectively, 5.06 mmol of FDA $min^{-1} g^{-1}$ d.w., 0.03 mmol of TPF min^{-1} g^{-1} d.w., 2.68 x 10^{-5} mmol of NH_4^+ min^{-1} g^{-1} d.w., and 4.06 mmol of PNP min^{-1} g^{-1} d.w. (Fig. 2.4), the effect percentage of phytotoxicity of *S. saccharatum* L was 19.1 and that of *L. sativum* was -19.1 (Fig. 2.5).

2.4.2 Comparison of soil properties among treatments at T1 and T2

The mean values of the investigated abiotic properties of the soils collected at T1 are reported in Fig. 2.2, and did not statically differ among treatments with the exception of pH and water content (Table 2.1). In fact, soil pH showed statistically significant differences (Table 2.2) among Ctr (7.70), Unbiod (8.11) and Biod (7.97), and water content was statistically (Table 2.2) lower at Ctr (27.8 % d.w.) than at both Unbiod (30.1 % d.w.) and Biod (33.0 % d.w.).

Soil microbial respiration showed mean values of 0.19, 0.16, and 0.17 mg CO_2 g^{-1} h^{-1}, respectively, for Ctr, Unbiod, Biod (Fig. 2.3), and did not statistically vary among the treatments (Table 2.2).

The mean values of the enzymatic activities for each treatment are reported in Fig. 2.4. Among them, only DHA activity statistically differed among the treatments (Table 2.2). In fact, it was statistically higher at Biod, with values of 0.02 mmol TPF min^{-1} g^{-1} d.w., than at both Unbiod and Ctr with values of 0.01 and 0.01 mmol TPF min^{-1} g^{-1} d.w. (Fig. 2.4).

Soil phytotoxicity through *L. sativum* L. showed effect percentages of -16.5, -4.55 and -10.1 %, respectively, at Ctr, Unbiod and Biod (Fig. 2.5) that did not statistically differ among treatments; whereas, soil phytotoxicity through *S. saccharatum* L. showed effect percentages of 35.7, 20.9 and 26.7, respectively, at Ctr, Unbiod and Biod (Fig. 2.5) that statistically differed only between Ctr and Unbiod (Table 2.2).

The mean values of the investigated abiotic properties of the soils collected at T2 are reported in Fig. 2.2. Among them, pH, concentrations of C, N and organic C showed statistically differences among treatments (Table 2.2). Particularly, pH was statistically higher at Unbiod (8.13) than at Ctr (8.03) (Table 2.2); C and N concentrations were statistically higher at Unbiod (4.49 % d.w. and 0.39 % d.w., respectively) than at Biod (3.71 % d.w. and 0.34 % d.w., respectively), and C concentrations were also statistically higher at Unbiod (4.49 % d.w.) than at Ctr (3.71 % d.w.) (Fig. 2.2; Table 2.2); finally, organic carbon concentrations were statistically higher at both Unbiod (0.03 % d.w.) and Biod (0.02 % d.w.) than at Ctr (0.02 % d.w.) (Fig. 2.2; Table 2.2).

Soil microbial respiration with values of 0.27 mg CO_2 g^{-1} h^{-1} at Ctr was statistically higher than at Unbiod (0.23 mg CO_2 g^{-1} h^{-1}) as well as that at Biod (0.26 mg CO_2 g^{-1} h^{-1}) was statistically higher than at Unbiod (Fig. 2.3; Table 2.2).

The values of the enzymatic activities are reported in Fig. 2.4. Among them, only DHA showed differences statistically significant with values higher at Ctr (0.02 mmol TPF min^{-1} g^{-1} d.w.) than at both Unbiod (0.01 mmol TPF min^{-1} g^{-1} d.w.) and Biod (0.01 mmol TPF min^{-1} g^{-1} d.w.) (Fig. 2.4; Table 2.2).

Soil phytotoxicity through *L. sativum* L. showed effect percentages of -32.2, -32.1 and -37.2 %, respectively, at Ctr, Unbiod and Biod (Fig. 2.5) that did not statistically differ among treatments (Table 2.2); whereas, soil phytotoxicity through *S. saccharatum* L. showed effect percentages of 6.65, 16.3 and 12.3, respectively, at Ctr, Unbiod and Biod (Fig. 2.5) and statistically differed only between Ctr and Unbiod (Table 2.2).

Table 2.2 T-values (One way ANOVA) of statistically significant differences of soil properties (pH; water content: WC; C total concentration; N total concentration; organic carbon concentration: Corg; microbial respiration: Resp; dehydrogenase activity: DHA; phytotoxicity of Sorghum saccharatum L.: Sorgh) among treatments (control soil: Ctr; soils covered by unbiodegradable sheets: Unbiod; soils covered by biodegradable sheets: Biod) at each time since mesocosms setting up (after three months: T1; after six months: T2).

		Treatment within Time			
		T1		T2	
		Ctr	Unbiod	Ctr	Unbiod
pH	Unbiod	- 6.26 *	-	- 2.79 *	-
	Biod	- 3.21 *	39 *	NS	NS
WC	Unbiod	- 2.88 *	-	NS	-
	Biod	- 3.74 **	NS	NS	NS
C	Unbiod	NS	-	- 2.49 *	-
	Biod	NS	NS	NS	4.66 **
N	Unbiod	NS	-	NS	-
	Biod	NS	NS	NS	40 **
C_{org}	Unbiod	NS	-	- 2.48 *	-
	Biod	NS	NS	- 2.66 *	NS
Resp	Unbiod	NS	-	30 *	-
	Biod	NS	NS	NS	- 3.62 **
DHA	Unbiod	NS	-	3.65 **	-
	Biod	- 3.32 *	- 2.75 *	3.06 *	NS
Sorgh	Unbiod	2.97 *	-	- 3.19 *	-
	Biod	NS	NS	NS	NS

2.4.3 Comparison of the soil properties over the time for each treatment

Over the time, slight differences were detected for all the investigated soil abiotic properties, with the exception of pH, water content and N concentrations (Fig. 2.2; Table 2.3). In fact, pH statistically increased since T1 to T2 at Ctr and Biod (Fig. 2.2; Table 2.3); water content statistically increased since T1 to T2 at all the treatments (Fig. 2.2; Table 2.3); finally, N concentrations statistically decreased over the time at Biod (Fig. 2.2; Table 2.3).

Soil respiration statistically increased over the time at all the treatments (Fig. 2.3; Table 2.3).

Among the investigated enzymatic activities, only HA did not show statistically significant differences over the time at the treatments (Fig. 2.4). In fact, DHA statistically increased since T1 to T2 at Ctr and statistically decreased at Biod (Fig. 2.4; Table 2.3); URE statistically increased over the time at Biod (Fig. 2.4; Table 2.3); finally, β-glu statistically increased ad Ctr and Biod (Fig. 2.4; Table 2.3).

The phytotoxicity through *L. sativum* L. increased over the time for all the treatments (Fig. 2.5) with differences statistically significant at Biod and Unbiod (Fig. 2.5; Table 2.3); whereas, that through *S. saccharatum* L. decreased over the time for all the treatments (Fig. 2.5) with differences statistically significant at Ctr and Biod (Fig. 2.5; Table 2.3).

Table 3. T-values (unpaired t-test) of statistically significant differences of soil properties (pH; water content: WC; N concentration; microbial respiration: Resp; dehydrogenase activity: DHA; urease activity: Ure; β-glucosidase activity: β-glu; phytotoxicity of Lepidium sativum L.: Lep; phytotoxicity of Sorghum saccharatum L.: Sorgh) between times (after three months: T1; after six months: T2) for each treatment (control soil: Ctr; soils covered by unbiodegradable sheets: Unbiod; soils covered by biodegradable sheets: Biod).

	Time whitin Treatment		
	Ctr	Unbiod	Biod
	T1 vs T2	T1 vs T2	T1 vs T2
pH	- 4.13 **	NS	16.5 *
WC	10 *	- 7.39 ***	- 2.40 *
N	NS	NS	5.25 **
Resp	10 *	- 10.61 ***	- 8.84 ***
DHA	- 2.95 *	NS	3.42 **
Ure	NS	NS	15 **
β -glu	10 *	NS	- 9.92 ***
Lep	NS	7.69 ***	40 **
Sorgh	5.63 **	NS	4.20 **

2.4.4 Effects of treatments and time on soil abiotic and biotic properties

The PCA, performed on all the investigated soil properties, highlighted that the first two axes accounted, respectively, for 36% and 16% of the total variance (Fig. 2.6). Soil water content, Resp, β-glu, and both the phytotoxicity assays explained the major part of the variance of the first axis (Fig. 2.6); whereas, DHA explained the major part of the variance of the second axis (Fig. 2.6). The treatments clearly separated according to the time, as they located along the first axis with values of T1 in the negative quadrants and those of T2 in the positive ones (Fig. 2.6); whereas, Ctr separated by Unbiod and Biod along the second axis, as Ctr mainly located in the negative quadrants (Fig. 2.6). The treatments at T1 were affected by phytotoxicity through both the tested organisms, total C and N concentrations, organic C contents, and HA (Fig. 2.6); whereas, T2 by all the remaining investigated properties (Fig. 2.6).

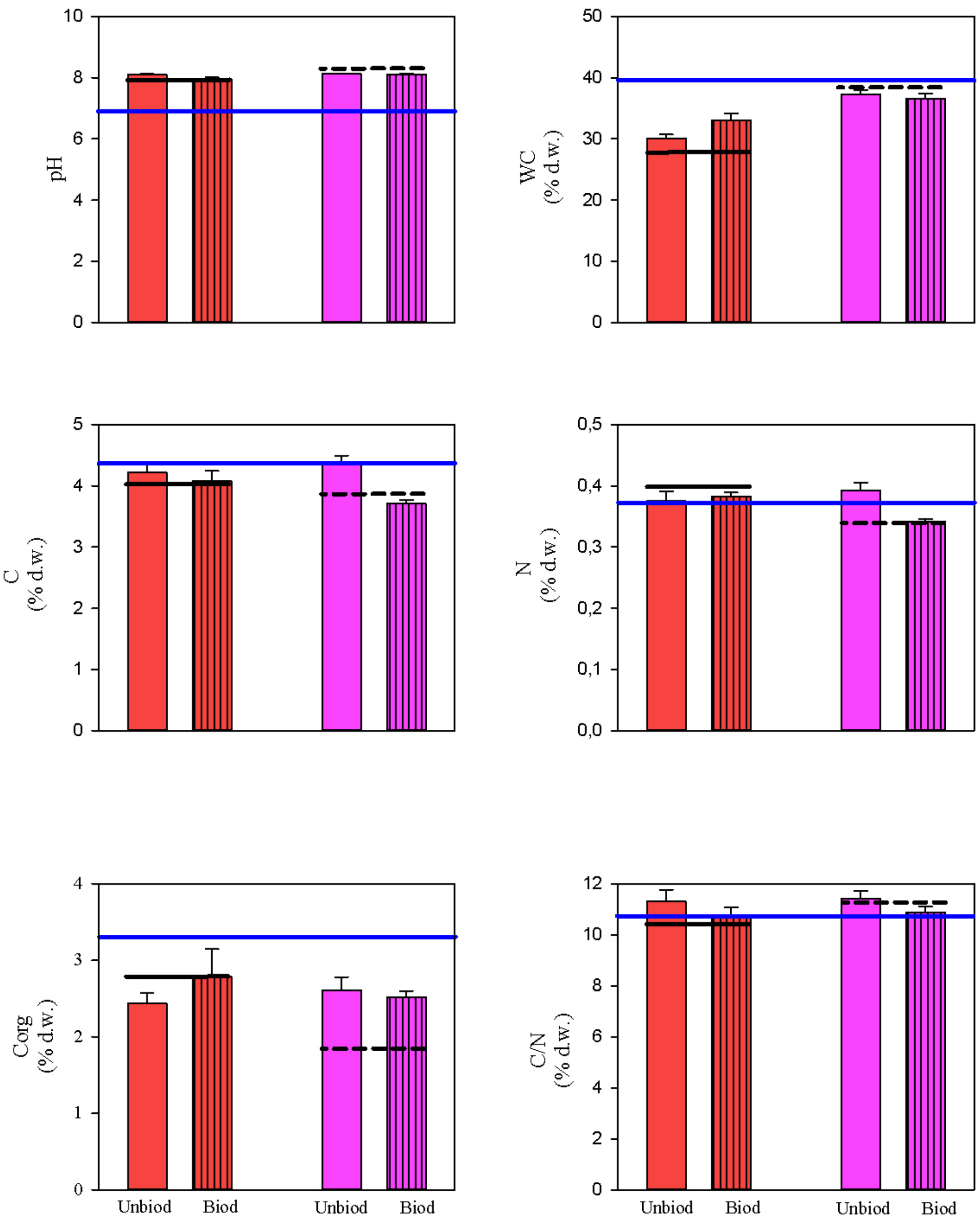

Fig. 2.2 Mean values (± s.e.; n = 5) of pH, water content (WC), concentration of total C and N and organic C (C_{org}), C/N ratios in soils covered by un-biodegradable (empty bars, n = 5) and biodegradable (filled bars, n = 5) plastic sheets collected three (pink) and six (violet) months after mesocosm setting up. The mean values in soils at the beginning of the mesocosm setting up (blue line, n = 14) in uncovered soils after three month (black line, n = 4) and six (dashed line, n = 4) are also reported.

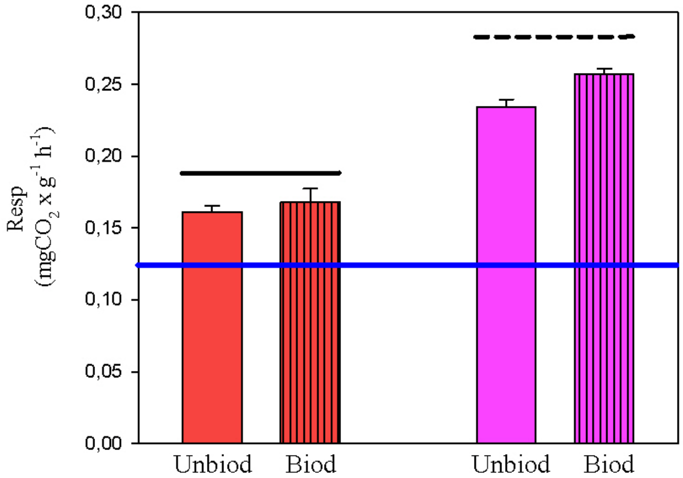

Fig. 2.3 Mean values (± s.e.; n = 5) of microbial respiration (Resp) in soils covered by un-biodegradable (empty bars, n = 5) and biodegradable (filled bars, n = 5) plastic sheets collected three (pink) and six (violet) months after mesocosm setting up. The mean values in soils at the beginning of the mesocosm setting up (blue line, n = 14) in uncovered soils after three month (black line, n = 4) and six (dashed line, n = 4) are also reported.

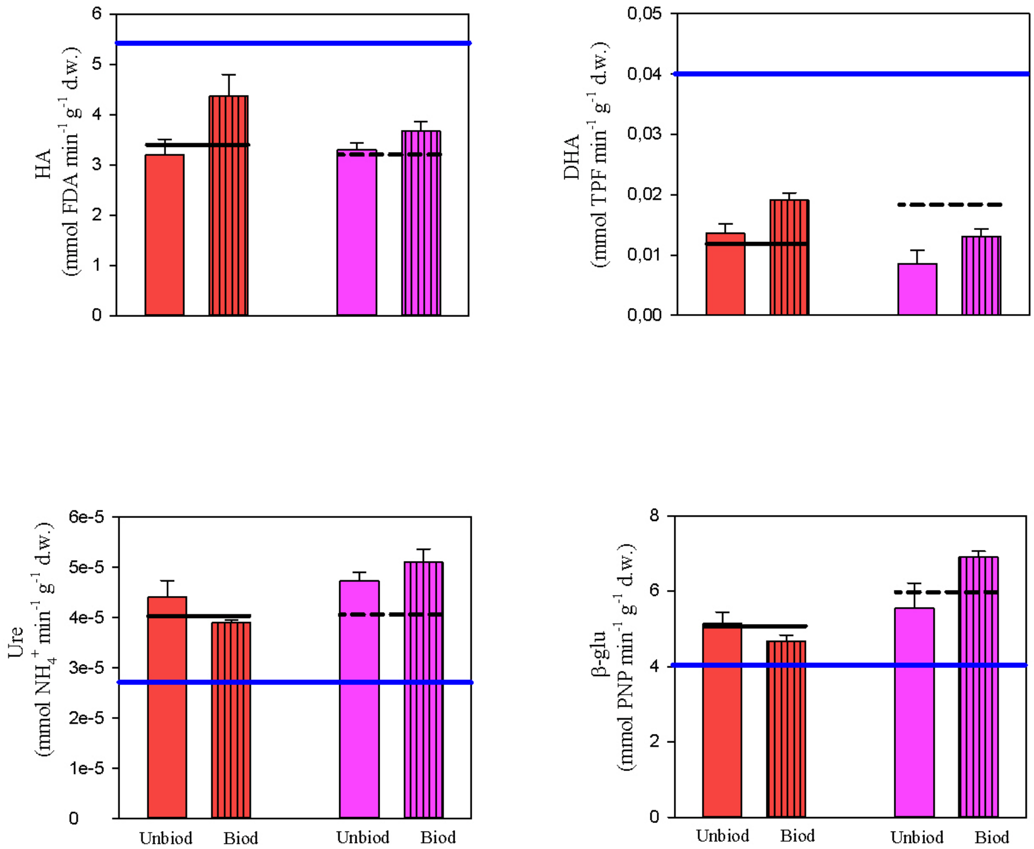

Fig. 2.4 Mean values (± s.e.; n = 5) of hydrolase activity (HA), dehydrogenase activity (DHA), urease activity (Ure) and β-glucosidase activity (β-glu) in soils covered by un-

biodegradable (empty bars, n = 5) and biodegradable (filled bars, n = 5) plastic sheets collected three (pink) and six (violet) months after mesocosm setting up. The mean values in soils at the beginning of the mesocosm setting up (blue line, n = 14) in uncovered soils after three month (black line, n = 4) and six (dashed line, n = 4) are also reported.

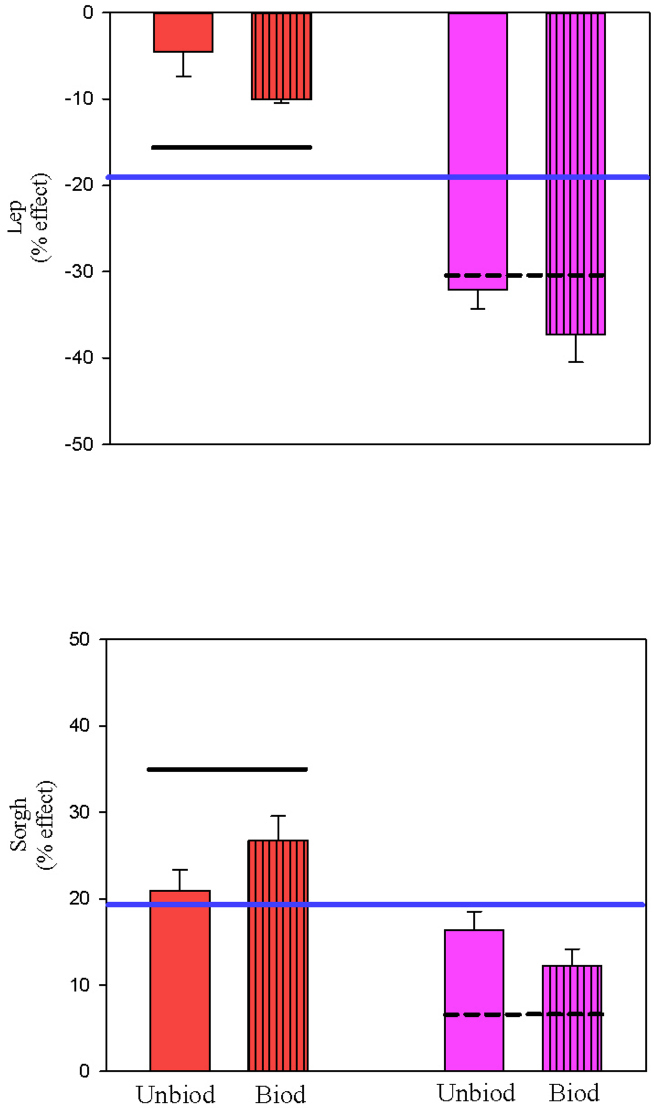

Fig. 2.5 Mean values (± s.e.; n = 5) of effect percentages of phytotoxicity through Lepidium sativum L. and through Sorghum saccharatum L. in soils covered by un-biodegradable (empty bars, n = 5) and biodegradable (filled bars, n = 5) plastic sheets collected three (pink) and six (violet) months after mesocosm setting up. The mean values in soils at the beginning of the mesocosm setting up (blue line, n = 14) in uncovered soils after three month (black line, n = 4) and six (dashed line, n = 4) are also reported.

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Un-biodegradable and biodegradable plastic sheets modify the soil properties after six months since their applications☆

G. Santini [a], S. Acconcia [a], M. Napoletano [a], V. Memoli [a,*], L. Santorufo [a,b], G. Maisto [a,b]

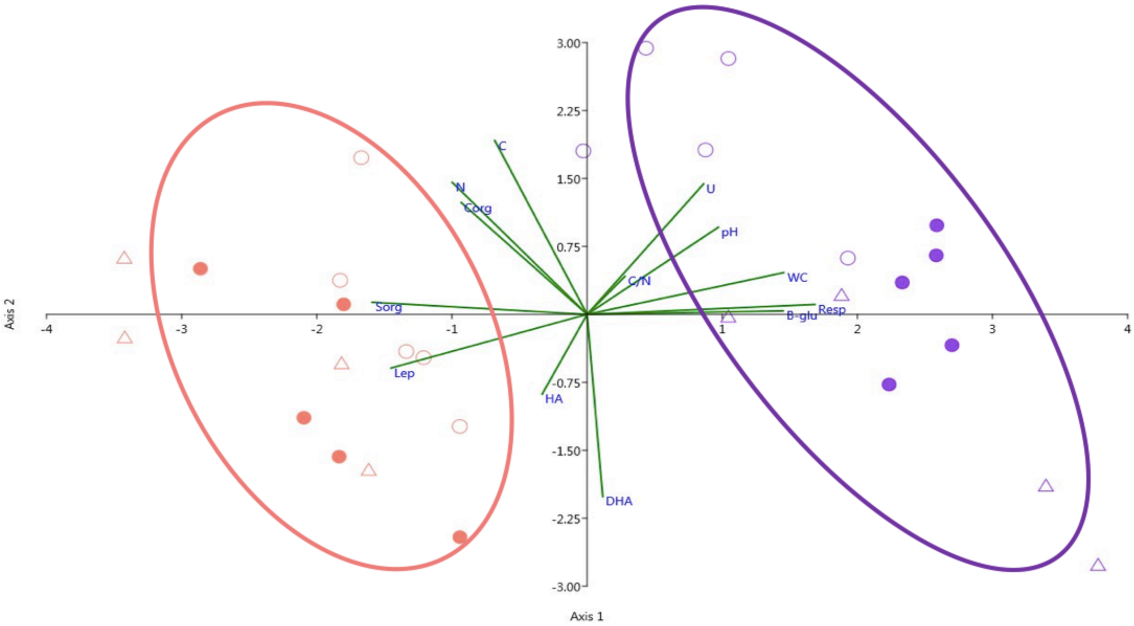


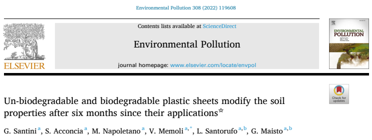
Fig. 2.6 Graphical display of the first two axes of the Principal Component Analysis on the soil abiotic (pH; water content: WC; organic Carbon: C_{org}; C and N contents; C/N ratios), biotic (Resp: microbial respiration; HA: hydrolase activity; DHA: dehydrogenase activity; Ure: urease activity ; β-glu: β-glucosidase activity) and ecotoxicological (Lep: *Lepidium sativum* L. ; Sorgh: *Sorghum saccharatum* L.) properties measured in soils (control: triangle; with un-biodegradable plastic sheets: un-filled circles; with biodegradable plastic sheets: filled circles) after three (pink) and six (violet) months since mesocosm setting up.

2.5 Discussion

All the obtained results together with the output of the PCA, performed on the dataset of all the investigated soil properties, highlighted that soil properties were mostly affected by exposure time to plastics rather than the kind (un-biodegradable and biodegradable) of plastics. In fact, the PCA clearly separated all the treatments between T1 and T2 along the first axis. Moreover, the main responsible for the separation of the treatments over the time would seem to be soil water content, microbial respiration and β-glu activity, properties that were correlated to the first axis of the PCA. Soil water content and microbial respiration were the properties that increased (approximately, 1.5-fold) for the greatest extent since T1 and T2 at both Unbiod and Biod treatments. Instead, β-glu activity would seem to meaningfully increase over the time only at Ctr and Biod. As temporal variations were observed in both uncovered and covered soils, it can be supposed that the climatic conditions influenced the biological activities (Santorufo et al., 2014; Memoli et al., 2021). However, the impact of the two kinds of plastics cannot be neglected, as a lot of significant differences were observed between Biod and Unbiod.

The lower water content at T1 than at T2 in soils covered by both the mulches and in uncovered soils could be due to water loss for gravity, due to the greatest amount of water likely reached before the soil collected in April, occurring after three rainy months. During the period April-July the rainfalls were scarce and, likely, water was held by soil for capillarity. Moreover, the results of soil water content agreed with those reported by Bandopadhyay et al. (2018) who found that plastic mulches (un-biodegradable and biodegradable) have a fundamental role in regulating water retention, especially at brief-time, as they reduce the vapor flux between surface soil and atmosphere. However, at T1, significant differences in water contents appeared also between Unbiod and Biod, with values higher at the latter. Un-biodegradable plastic mulches typically increase the water-use efficiency by 20–60% due to reduced evaporation (Qin et al., 2015, Zribi et al., 2015). During specific microclimatic conditions, the water retention could be enhanced by biodegradable mulches that thanks to their high permeability, promote the gradual disintegration (Moreno and Moreno, 2008) and the stabilization of soil aggregates (Six et al., 2004). This, in turns, increase soil water retention (Domagała-Świątkiewicz and Siwek, 2013; Mbah et al., 2010) and reduce of vertical water transport (Sharma et al., 2009). The lack of differences in retaining water in soils under un-biodegradable and biodegradable plastic sheets, after six months since the mesocosm setting up, agrees with the results reported in various studies for long period of exposure to plastic mulches (Han et al., 2013; Li et al., 2013).

The gradual reduction of rainfall from January (but also from November, not shown data) to April could explain the consequent increase of air availability in soils over the



time. In fact, it is well known that water and air are competitors for soil pores. The incremented air availability could explain the higher soil respiration at T1 than at T0. As observed for soil water content, also microbial respiration, with similar values among treatments at the beginning of experiment, significantly increased over the time, although with minor extent in soils under un-biodegradable plastic sheets. An integrated role of climatic conditions and presence of plastic sheets in regulating the microbial respiration can be supposed. In fact, some researchers (Li et al., 2004; Zhou et al., 2012; Lou et al., 2015) report for soils under plastic mulches higher microbial respiration due to the increased temperature; by contrast, other researchers (Frey et al., 2008; Moreno and Moreno, 2008; Sintim et al., 2021) report lower microbial respiration. The negative impact of un-biodegradable plastic sheets on soil microbial activity was highlighted also by the lack of significant temporal increases of β-glu activity at Unbiod differently from that occurred at Ctr and Biod. By contrast, the greatest temporal increase of β-glu activity was detected at Biod (approximately, 1.5-fold higher than T1) as compared to Ctr (approximately, 1.2-fold higher than T1). This suggests a progressive input of carbon compounds, deriving by the transformation of the biodegradable plastic sheets in fragments, resources of the β-glu activity (Zhou et al., 2021).

At longer exposure time (T2), the presence of un-biodegradable plastic sheets significantly affected both soil total and organic carbon concentrations as compared to the un-covered soils. This behavior could be associated with the nature of plastic that prevents carbon exchange with the atmosphere, causing C storage and soluble organic carbon depletion (Zhou et al., 2012; Li et al., 2013). Instead, the presence of biodegradable plastic sheets enhanced the organic carbon concentrations although the total concentration slightly varied as compared to the un-covered soils. Thus, could be due to the further input of organic matter deriving by the sheets that can be biodegraded (Ding et al., 2021).

With a reduced extent, also soil pH, N concentrations, DHA, Ure, and phytotoxicity meaningfully changed over the time, although different temporal trends were observed according to the treatments. The significant higher pH observed after three months since mesocosm setting up in soils covered by un-biodegradable and biodegradable plastic sheets as compared to that at Ctr, likely, depended on the sudden changes in soil structure (Steinmetz et al., 2016). Moreover, the significant temporal increases of pH in soils under biodegradable plastic sheets could be due to a better soil aeration and porosity (Zhao et al., 2021). However, it cannot be excluded a role of the microclimatic conditions in controlling the temporal pH variations in soils covered by the un-biodegradable plastic sheets that better isolated the soils (Zhang et al., 2019). The significant pH variations as well as the microclimatic conditions could be responsible for the of increased microbial activities (Lammel et al., 2018). The presence of biodegradable plastic sheets may also be considered responsible for the signficant decreases in N concentrations. This hypothesis is also corroborated by the highest soil Ure activities (involved in the N cycle) that likely were stimulated by the great soil aeration and by the further input of organic resources deriving by the biodegradable plastic sheets (Lalitha et al., 2010).

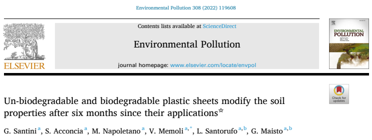
DHA was the only investigated soil property that showed a specific behavior. In fact, at T1, DHA was statistically lower at Ctr than at Unbiod and Biod; conversely, at T2, it was statistically higher at Ctr. Therefore, it can be hypothesized that the presence of plastic sheets created a micro-environmental condition responsible for the sudden stimulation of the microbial activity (Gao et al., 2019). Instead, a longer period of exposure to plastic sheets caused a noticeable inhibition of the DHA activity. As dehydrogenase activity, oxidizing the soil organic matter, is a well-known biological indicator of overall microbial respiration (Wolińska and Stępniewska, 2012), it can be supposed an overall worsening of the quality of soils covered by both the types of plastic sheets (Memoli et al., 2021).

Changes in the investigated soil properties did not exert significant impacts on germination of *L. sativum* L. for all the treatments and at the two-time samplings. Instead, the germination of *S. saccharatum* L. appeared significantly inhibited in soils under Unbiod, exceeding the values of – 30 % and suggesting that the soil changes caused ecotoxicity for this species that appeared more sensitive than *L. sativum* L. This hypothesis agrees with the results obtained in previous studies regarding the effects of fires, tourism, inorganic and organic contamination on soil quality (Memoli et al., 2019).

2.6 Conclusion

The obtained results revealed that soil properties were affected more by the exposure time to plastics than by the kind (un-biodegradable and biodegradable) of plastics. In particular, water content, respiration and phytotoxicity through *L. sativum* L. increased over the time in soils covered by both un-biodegradable and biodegradable plastic sheets.

However, the kind of plastic sheets also played a role in influencing soil properties, as many differences were found between un-biodegradable and biodegradable plastic sheets. After six months since mesocosm setting up, the soils covered by un-biodegradable plastic sheets showed significant lower pH values, higher concentrations of total and organic carbon, lower values of respiration and DHA, and higher toxicity through *S. saccharatum* L. as compared to the uncovered soils. Instead, the soils covered by biodegradable plastic sheets showed significant higher organic carbon concentrations and lower DHA as compared to the uncovered soils.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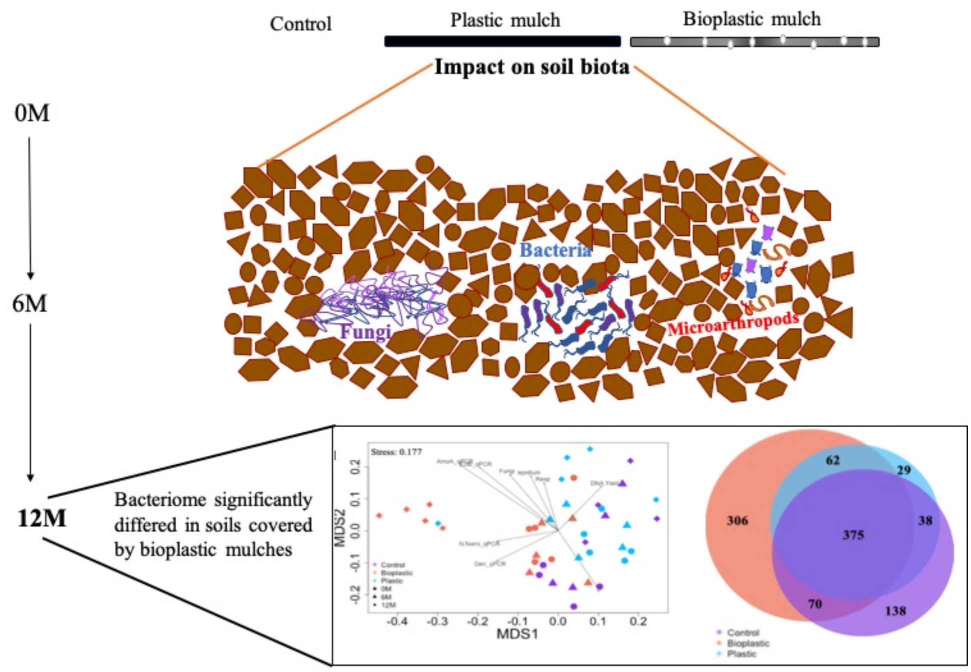
Un-biodegradable and biodegradable plastic sheets modify the soil properties after six months since their applications☆

G. Santini[a], S. Acconcia[a], M. Napoletano[a], V. Memoli[a,*], L. Santorufo[a,b], G. Maisto[a,b]

Almost all the investigated soil properties improved over the time in soils covered by biodegradable plastic sheets, whereas they slightly varied over the time in soils covered by un-biodegradable plastic sheets.

CHAPTER 3



Microbiome dynamics of soils covered by plastic and bioplastic mulches (Santini et al. submitted to [redacted]

3.1 Abstract

In recent decades, the use of plastic mulch in agriculture has largely increased to meet the growing demand for food. Despite their potential benefits, it is not still known the impact of mulches on soil microbiome. In this mesocosm study, we compared the effects of polyethylene (Plastic) and Mater-bi® (Bioplastic) mulches on the soil physical-chemical properties ([redacted]), biological properties [redacted]

Keywords: Mulching practices, Soil microbiome, Microarthropod community, Nitrogen cycle.

3.2 Introduction

The use of plastics in agriculture has increased considerably in recent decades aiming at improving crop productivity and ultimately reducing food losses (FAO and UNEP, 2021). Application of plastic mulches was shown to suppress weed growth, prevent soil erosion and increase soil temperature resulting in an improved crop quality and production (Hill et al., 1982; Lamont, 2005; Zhang et al., 2013; Blaise et al., 2021). Despite these potential advantages, the widespread, long-term use and weathering of plastic mulches combined with a lack of systematic collection and management may cause their accumulation in the soil (Steinmetz et al., 2016), and subsequently, the accumulation of macro- (> 5mm) and microplastics (100µm < MPs < 5mm).

The release and accumulation of MPs has been associated with changes in soil porosity, water retention and bulk density (de Souza Machado et al., 2018). Moreover, through binding to organic matter and microbial secretions, MPs can affect different aspects of the soil microbial community, such as its composition and biomass (Li et al., 2019; Ren et al., 2020), and the soil micro- and meso-fauna, such as their growth and metabolism (Buks et al., 2020; Wang et al., 2021). As soil biota participates directly or indirectly in the decomposition of organic matter, MPs may also affect soil nutrient cycling (de Souza Machado et al., 2018). In agroecosystems, nitrogen deficiency is often a limiting factor for microbial and plant growth, and the availability of ammonia and nitrate is considered a bottleneck for the activity of most of the organisms present (Lehtovirta-Morley, 2018). The presence of MPs in agricultural soils can have an impact on microbial groups involved in some steps of N cycle as previously shown by Seeley et al. (2020). With regards to the nutrient turnover, several key indicators of soil quality include urease (mineralization) as well as dehydrogenase, -glucosidase and hydrolase activities (oxidation). These enzymes are sensitive to environmental changes and, in turn, they may affect soil C and N contents (Adetunji et al., 2017; Feng et al., 2019).

Polyethylene is the plastic material conventionally used for agricultural mulch (Hayes et al., 2012) and documented to be a major source of MPs in agricultural soils (Kasirajan and Ngouajio, 2012; Blasting and Amelung, 2018; Wang et al., 2021). A reduction in both microbial activity and richness has been reported in response to the addition of polyethylene to the soil (Fei at al., 2020; Shi et al., 2022). Environmentally friendly biodegradable plastics, such as Mater-bi® appear as promising substitutes for conventional non-degradable plastics (Qin et al., 2021). The advantage of using biodegradable mulches in agriculture primarily relies on their rapid degradation rate based on their structural and surface characteristics that allow the attack of enzymes (Brodhagen et al., 2015). Through conversion to CO_2, water and biomass, these polymers are catabolized by soil microbiota (Brodhagen et al., 2015). However, fragmentation of bioplastic mulches can also occur under a range of environmental conditions with the subsequent release of micro-bioplastics into agricultural soils (Li et al., 2014; Qin et al., 2021). Since micro-bioplastics can be used as an exogenous carbon source, providing selective niches for soil microorganisms (Zhou et al., 2021), they are expected to influence soil microbial community composition to a larger extent than conventional MPs (Qi et al., 2020, Wang et al., 2022). Nonetheless, previous research has shown that the effect of micro-bioplastics on the soil ecosystem was similar to that of conventional MPs (Green et al., 2015; Green, 2016; González-Pleiter et al., 2019; Shruti and Kutralam-Miniasamy, 2019; Zuo et al., 2019). Notwithstanding, current knowledge about the effects of bioplastics and their degradation products on soil microbial and microarthropod communities is still scarce (Santini et al., 2022; Huang et al., 2023). These discrepancies call for further studies evaluating the impact of conventional plastics and bioplastics on the soil properties, with a special emphasis on the microbiological component. [redacted]

3.3 Material and Methods

3.3.1 Experimental set-up and sample collection

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3.3.3 Soil physical-chemical and biological analyses

The soil pH was measured by an electrometric method in a distilled water suspension (1:2.5, v:v). Water content (WC) was determined gravimetrically by drying fresh soil at 105 °C until constant weight. Organic total carbon (C_{org}) was measured by a CNS Analyzer (Thermo Finnigan, Italy) on soil samples previously treated with HCl (10%) to exclude carbonates. Total C and N concentrations were measured from oven-dried (105 °C) and grounded (Fritsch Analysette Spartan 3 Pulverisette 0) soil samples by CNS Analyzer (Thermo Finnigan, Italy).

Microbial respiration (Resp) was assessed using MicroResp® assays (Macaulay Scientific Consulting, Aberdeen, UK) (Campbell et al. 2003). Five technical replicates of each biological soil sample (circa 0.3 g dry weight) were incubated in a 96-deep well microplate (Fisher Scientific E39199, Illkirch France) as previously shown in Santini et al. (2022).

Hydrolase activity (HA) was determined by adding 7.5 mL of 60 mM potassium phosphate (pH 7.6) and 0.1 mL of fluorescein diacetate (FDA) to 1 g of fresh soil. The reaction mixture was incubated at 30 °C for 20 min. At the end of incubation, the fluorescein was extracted with 7.5 mL of acetone and centrifuged at 5,000 rpm for 5 min. The absorbance of the supernatant was measured at 490 nm and the results were expressed as mmol of FDA produced for 1 g of dry soil in 1 min (Adam and Duncan, 2001). Dehydrogenase activity (DHA) was determined by adding 1 mL of 1.5% 2,3,5-triphenyltetrazolium chloride (TTC) dissolved in 0.1 M Tris-HCl buffer (pH 7.5) to 1 g of fresh soil. The reaction mixture was incubated at 30 °C for 24 h in the dark. At the end of incubation, the triphenylformazan (TPF) was extracted with 8 mL of acetone, and the extract was centrifuged at 3,500 rpm for 15 min. The absorbance of the supernatant was measured at 546 nm and the results were expressed as mmol of TPF produced for 1 g of dry soil in 1 min (Memoli et al., 2018). β-glucosidase activity (β-glu) was determined by adding 4 mL of modified universal buffer (MUB) pH 6 and 1 mL of 0.025 M p-nitrophenyl β-D-glucopiranoside (PNP) to 1 g of fresh soil. The mixture was then incubated at 37 °C for 1 h, after which the enzymatic reaction was stopped by cooling on ice for 15 min. Then, 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.1 M Tris-hydroxymethilaminomethane-sodium hydroxide (THAM-NaOH) pH 12 was added. In the control, the substrate was added before the addition of $CaCl_2$ and NaOH. The absorbance of the supernatant was measured at 420 nm and the results were expressed as mmol of PNP produced for 1 g of dry soil in 1 min (Tabatabai and Bremner, 1969; Tabatabai, 1982). Urease activity (Ure) was determined by adding 0.5 ml of urea (0.1 M) and 4 ml of borate buffer (0.1 M pH 8.8) to 1 g of fresh soil. The solution was incubated at 37 °C for 2 h and then 10 ml of potassium chloride in hydrochloric acid (KCl 1.35 M in 0.1 M HCl) was added. The samples were shaken for 30 min and then centrifuged at 5,000 rpm for 10 min. The extract was taken from each sample, to which 2.5 ml of buffer, 4 ml of salicylate and 2.5 ml of hypochlorite were added. The samples were incubated again at 37 °C for 30 min. The absorbance of the supernatant was measured at 660 nm and the results were expressed as mmol of NH_4^+ produced for 1 g of dry soil in 1 min (Kandeler and Gerber, 1988; Alef and Nannipieri, 1995).

The phytotoxicological assays were performed according to EPA (1996) using *Sorghum saccharatum* L. and *Lepidium sativum* L. as test organisms and performed in fresh and sieved (2 mm) soil samples. Ten seeds were placed in Petri dishes containing an amount of fresh soil equivalent to 10 g of oven-dried soil, subsequently saturated with water. Standard soil (OECD, 1984) and $K_2Cr_2O_7$ were used as negative and positive controls, respectively. After incubation in darkness (72 h, at 25 °C), the number of germinated seeds and the root elongation were measured as described in Santini et al. (2022).

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Table 3.1 [redacted]

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Fig. 3.3 [redacted]

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Fig. 3.5 [redacted]

3.5 Discussion

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3.6 Conclusions

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CHAPTER 4

Metal Release from Microplastics to Soil: Effects on Soil Enzymatic Activities and Spinach Production

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Article

Metal Release from Microplastics to Soil: Effects on Soil Enzymatic Activities and Spinach Production

Giorgia Santini [1], Valeria Memoli [1,*], Ermenegilda Vitale [1], Gabriella Di Natale [2], Marco Trifuoggi [3], Giulia Maisto [1,4] and Lucia Santorufo [1,4]

4.1 Abstract

Microplastics (MPs) represent emergent pollutants in terrestrial ecosystems. Microplastics can cause release of metal and damages to crop quality. The present research aimed to evaluate the effects of Mater-bi (Bio-MPs) and polyethylene (PE-MPs) MPs at different concentrations on soil properties and on growth of *Spinacia oleracea* L. Plants were grown in 30 pots filled with soil mixed with 0.5, 1 and 2% d.w. of Bio-MPs and PE-MPs and in 5 pots filled only with soil, considered as controls (K). At the end of vegetative cycle, the spinach plants were evaluated for the epigeal (EPI) and hypogeal (HYPO) biomasses, and the ratio HYPO/EPI was calculated. In the soil, the total and the available fractions of Cr, Cu, Ni and Pb and the hydrolase (HA), β-glucosidase (β-glu), dehydrogenase (DHA) and urease (U) activities were evaluated. Results revealed that the addition of Bio-MPs increase soil total Cr, Cu and Pb and available Cu concentrations, and the addition of PE-MPs increases Pb availability. In soil contaminated by both Bio-MPs and PE-MPs, Ha and β-glu activities were stimulated, whereas DHA activity was reduced. The HYPO and HYPO/EPI biomasses were reduced only in soils contaminated by the 2% of Bio-MPs.

Keywords: Agroecosystem; Microplastics; bio-microplastics; enzymatic activities, soil.


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Metal Release from Microplastics to Soil: Effects on Soil Enzymatic Activities and Spinach Production

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4.2 Introduction

Vegetables are at the base of the human diet as they contain healthy compounds and guarantee human wellbeing [1]. Among the most consumed leafy vegetables worldwide, spinach (*Spinacia oleracea* L.) is easy to grow, has a short growing period and is rich in bioactive compounds that work as reactive-oxygen species scavengers, modulate the expression of genes involved in human metabolism, inflammation, proliferation and provide antioxidant defense [2]. To provide the human population with sufficient vegetables all year round, it is necessary to apply agricultural management promoting and maximizing vegetable production [3].

Plastic mulching is a widespread application in agriculture because it creates soil conditions that favor vegetable growth [4]. For this reason, recently, the use of plastic mulches has increased rapidly worldwide in order to meet the growing demand for food [5]. Unfortunately, plastic mulches are very often left on soil for decades and their improper management causes their degradation [6] in small fragments: microplastics (MPs). Microplastic fragments, especially those that are very tiny, can be absorbed by microorganisms and by plant roots, entering into the food web [7].

Polyethylene (PE), because of its durability, is the most common type of plastic mulch used in agriculture. Recently, in order to mitigate the adverse effects of conventional MPs, biodegradable plastic mulches have been used as they degrade more rapidly than conventional PE film [8], guaranteeing comparable agricultural benefits [9].

Moreover, both conventional and biodegradable MPs can serve as carriers of heavy metals that are added during the production processes to improve the specific performance, functionality and aging properties of the end products [10]. During the weathering and fragmentation processes of plastic sheets, metals can be released leading to the contamination of the soil system [11].

Intensive cultivation and agricultural management have resulted in certain soil problems, among which is soil metal accumulation [12]. Total metal concentration does not reflect metal bioavailability and can exert adverse effects on soil microbial activity [13] and plant growth [14]. In fact, metals present in bioavailable form directly affect plant growth, physiology and development, as plants can easily absorb these elements from soil [15]. Previous research highlighted that in soils contaminated by Cu and Pb an inhibition of seed germination, root proliferation and plant biomass occurred [16].

As with plants, soil microorganisms are also vulnerable to the available fraction of heavy metals that affect their growth and activities [17]. In particular, many soil enzymes (such as hydrolase, dehydrogenase, β-glucosidase and urease) are used as indicators of heavy metal contamination since they quickly respond to changes of soil condition [18]. For example, Hu et al. [19] proposed the use of dehydrogenase as an indicator of microbial activity in soils contaminated by heavy metals.

The effects of heavy metals on soil functioning and plant growth have been recognized [14,20], but the combined effects of metal and MP pollution on microbial activity and plant development are scarcely known. Therefore, the present research aimed to fill the current gap about the impact of MPs and metals on soil activities and on plant growth. Moreover, a comparison of these impacts between soil contaminated by conventional (PEMPs) and biodegradable microplastics (Bio-MPs) was assessed. To achieve the aims, the research was performed in pots filled with horticultural soils contaminated by PE-MPs and Bio-MPs at three different percentages (0.5%, 1% and 2% v/v) where individual spinach plants were grown.

4.3 Materials and Methods

2.1 Experimental design

A total of 35 pots with a diameter of 15 cm were set up, each filled with approximately 350 g of horticultural soil (EUROTERRIFLORA s.r.l.) (Table 4.1).

Table 4.1 Properties of horticultural soil (EUROTERRIFLORA s.r.l.) used to perform the experiment with MPs and Spinach (Spinacia oleracea L.).

Horticoltural soil properties	
pH	7
Electrical conductivity	0.8 dS/m
Carbon	30 %
Dry bulk density	360 kg/m^3
Total porosity	80 % (v/v)

Five pots were filled only with horticultural soil and used as controls (K), fifteen were treated with microplastics (MPs) from polyethylene (PE-MPs) at different percentages 0.5, 1 and 2% of soil dry weight (for 5 pots each), and fifteen were treated with MPs fro Mater-bì® (Bio-MPs) at the same percentages of PE-MPs (Fig. 4.1). The chosen percentages are reported in the literature as the most used to highlight the impacts of MPs on soil properties and plants [7,21].

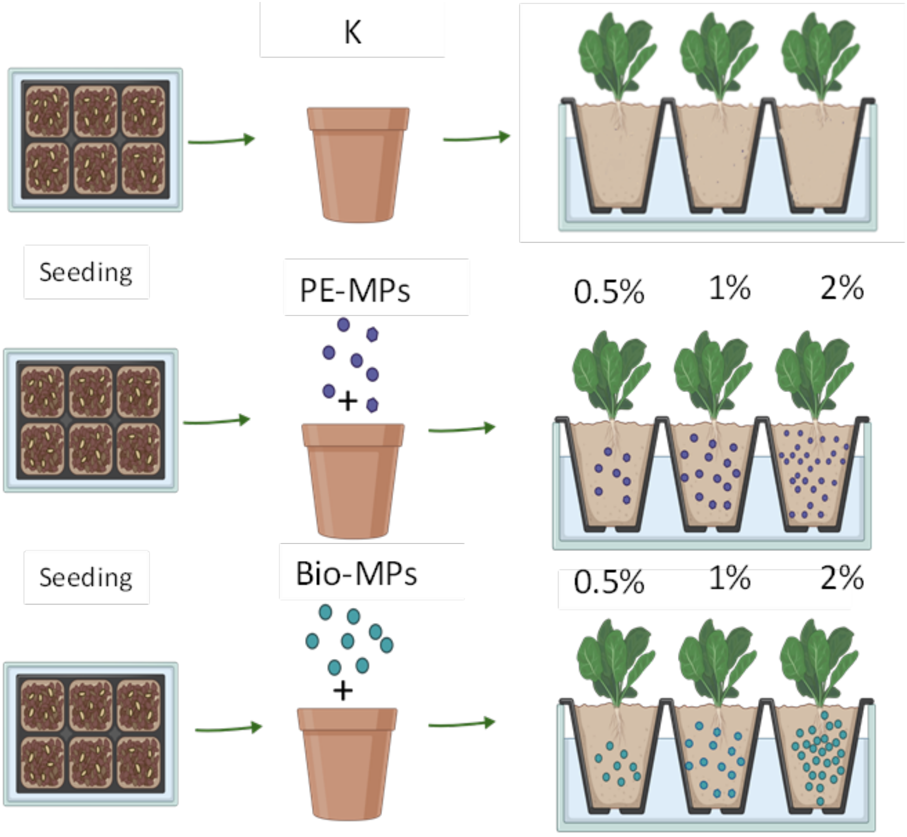

Fig. 4.1 Experimental set up performed with horticultural soil used as control (K), mixed with conventional microplastics (PE-MPs) and with Mater-bì® microplastics (Bio-MPs) at different percentages (0.5, 1 and 2% of soil dry weight).

Fragments of PE and Mater-bì® were generated in the laboratory from agricultural mulch sheets using a liquid nitrogen grinder. Grinding cycles were performed from 5 to 10 times to achieve microplastic sizes ranging from 20 μm to 5 mm in diameter (Fig. 4.2 A, B).

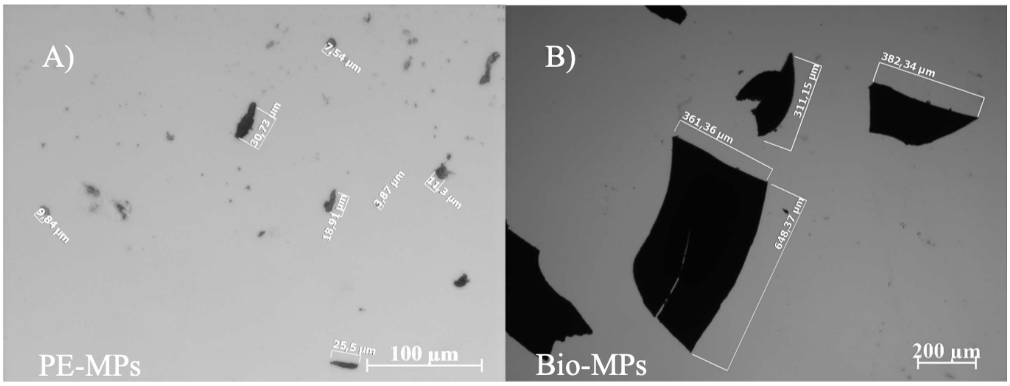

Figure 4.2 Images showing a) conventional (PE) and b) Mater-bì® (Bio) microplastic (MPs) fragments, obtained to perform the experiment.

Previously, forty seeds of Spinacia oleracea L. (spinach) of the "matador" variety were germinated in the dark at a room temperature of 24 ± 1 °C. Then, the seedlings were transplanted in pots as described above and grown in a greenhouse at the same environmental conditions: PPFD of 900 ± 100 µmol (photons) m^{-2} s^{-1} at the top of the canopy, photoperiod of 12 h, temperature of 26 ± 1 °C, relative humidity of 55–60%. Plants were regularly watered and followed until the end of the vegetative cycle. Spinach was chosen for its importance at social and economic levels. In fact, it is widely cultivated in southern Italy as it is at the base of the human Mediterranean diet.

2.2 Sampling and analyses

The soil and plant sampling were carried out at the end of plant vegetative cycle. In each pot, the spinach plants were collected removing the soil from the roots and separating the epigeal (EPI) from the hypogeal (HYPO) portions. Contextually, soil samples (0–10 cm) were collected from each pot.

The total concentrations and available fractions of Cr, Cu, Ni and Pb were measured according to Memoli et al. (2017) [22] and measured by inductively coupled plasma mass spectrometry (ICP-MS Aurora M90, Bruker, Billerica, MA, USA).

Hydrolase activity (HA) was determined by adding 7.5 mL of 60 mM potassium phosphate (pH 7.6) and 0.100 mL of fluorescein diacetate (FDA) to 3 g of fresh soil. The details of the method were reported in Adam and Duncan (2001) [23].

Dehydrogenase activity (DHA) was determined by adding 1 mL of 1.5% 2,3,5-triphenyltetrazolium chloride (TTC) dissolved in 0.1 M Tris-HCl buffer (pH 7.5) to 1 g of fresh soil according to Memoli et al. (2018) [17].

β -glucosidase activity (β-glu) was determined by adding 4 mL of modified universal buffer (MUB) pH 6 and 1 mL of 0.025M p-nitrophenyl β-D-glucopiranoside (PNP) to 1 g of soil according to Tabatabai and Bremner (1969) and Tabatabai (1988) [24,25].

Urease activity (U) was determined by adding 0.5 mL of urea (0.1 M) and 4 mL of borate buffer (0.1 M pH 8.8) to 1 g of fresh soil according to Kendeler (1988) and Alef and Nannipieri (1995) [26,27].

The biomass of EPI and HYPO portions was determined on oven-dried plant samples at 75 °C for 48 h and expressed in grams of dry weight (d.w.) per plant. The ratio of HYPO/EPI for all experimental conditions was also calculated.

2.3 Statistical analyses

In order to verify the normal data distribution and homogeneity of variance, the Shapiro–Wilks and Levene Median tests were assessed, respectively.

The differences in soil element concentrations (total and available), in soil enzymatic activities (HA, DHA, β-glu and U) and in plant biomasses (EPI, HYPO, HYPO/EPI), between K and the different percentages (0.5, 1 and 2% d.w.) of PE-MPs and Bio-MPs were assessed through one-way analysis of variance (ANOVA) combined with post hoc comparison tests (pairwise Student–Newman–Keuls test or Fisher LSD method).

The differences in soil element concentrations (total and available), in soil enzymatic activities (HA, DHA, β-glu and U) and in plant biomasses (EPI, HYPO, HYPO/EPI), among the percentages (0.5, 1 and 2% d.w.) inside the same treatment (PE-MPs or Bio-MPs) were assessed through one-way analysis of variance (ANOVA) combined with post hoc comparison tests (pairwise Student–Newman–Keuls test or Fisher LSD method).

The differences in soil element concentrations (total and available), in soil enzymatic activities (HA, DHA, β-glu and U) and in plant biomasses (EPI, HYPO, HYPO/EPI), between the same percentage (0.5, 1 and 2% d.w.) of PE-MPs and Bio-MPs were assessed through the t-test.

A Principal Components Analysis (PCA) was performed on soil and plant properties to evaluate the treatment distribution (K, PE-MPs and Bio-MPs) and to identify the main properties driving the distribution. The PCA was conducted using the Past 4.0 software. The PERMANOVA analyses (Vegan package, Adonis function—pairwise.perm.manova test for $p < 0.05$) were carried out on the selected soil and plant properties to highlight the significant differences among treatments (K, PE-MPs and Bio-MPs).

The statistical analyses and the PERMANOVA analyses were performed using the R 4.0.3 programming environment and graphical displays with Sigma-Plot 9.0 software (Jandel Scientific, San Rafael, CA, USA).

4.4 Results

4.4.1 Soil metal total concentrations

Total concentrations of Cr, Cu, Ni and Pb in control (K) and in soils contaminated with different percentages (0.5, 1 and 2% d.w.) of conventional microplastics (PE-MPs) and biodegradable microplastics (Bio-MPs) are reported in Figure 4.3. Soil total Ni concentra-tions in both PE-MPs and Bio-MPs did not statistically vary as compared to K (Fig. 4.3). Instead, soil total concentrations of Cr, Cu and Pb in 2% Bio-MPs (Cr:

28.7 μg g-1 d.w.; Cu: 46.9 μg g-1 d.w.; Pb: 21.6 μg g-1 d.w.) were significantly ($P < 0.05$) higher than in K (Cr: 19.9μg g-1 d.w.; Cu: 35.6 μg g-1 d.w.; Pb: 16.2 μg g-1 d.w.) as well as soil total concen-trations of Cu in 0.5% Bio-MPs (51.5 μg g-1 d.w.) were significantly ($P < 0.01$) higher than in K (Fig. 4.3).

Total concentrations of the investigated metals did not statistically vary among the different percentages within the same treatment (PE-MPs or Bio-MPs).

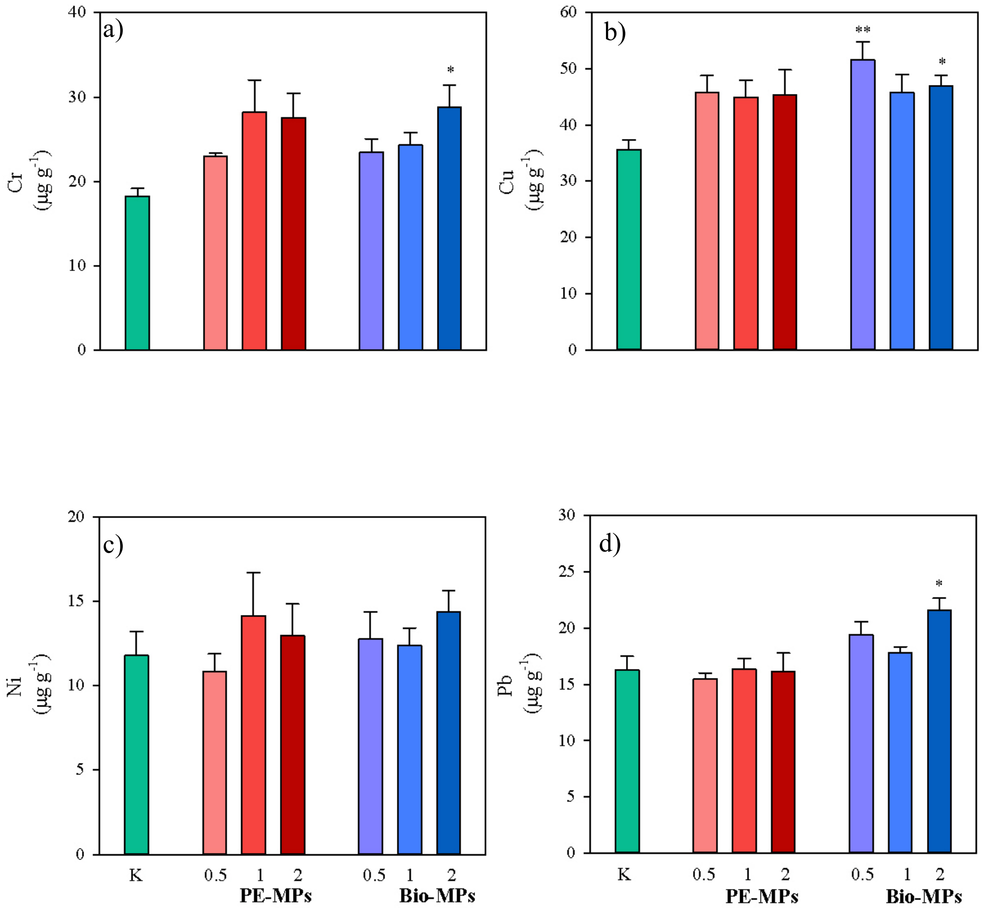

Figure 4.3 Mean values (±s.e.) of total (a) Cu, (b) Cr, (c) Ni and (d) Pb concentrations measured in soil without microplastics (K), mixed with Polyethylene (PE-MPs) and Mater-bì® (Bio-MPs) microplastics at different concentrations (0.5, 1 and 2% d.w.). Asterisks indicate significant differences between soils mixed with microplastics and control respectively (one-way ANOVA; $p < 0.05$).

Yet, the total concentrations of the investigated metals in soils contaminated with the same percentage did not statistically vary between the two treatments (PE-MPs vs Bio-

MPs), except for Pb that was significantly ($P < 0.05$) higher in 2% PE-MPs than 2% Bio-MPs (Table 4.2).

Table 4.2 Significant differences in total and available Cu, Cr, Ni and Pb concentrations within the pots with the same Polyethylene (PE) and Mater-bi® (Bio) microplastic concentrations (T-test; *** p< 0.001; ** p < 0.01; * p < 0.05).

	PE-MPs *vs* Bio-MPs		
	0.5%	1%	2%
Cr_{Tot}	n.s.	n.s.	n.s.
Cu_{Tot}	n.s.	n.s.	n.s.
Ni_{Tot}	n.s.	n.s.	n.s.
Pb_{Tot}	n.s.	n.s.	*
Cr_{Av}	n.s.	n.s.	n.s.
Cu_{Av}	***	***	**
Ni_{Av}	***	*	n.s.
Pb_{Av}	n.s.	**	**

4.4.2 Soil available fractions

The available fractions of Cr, Cu, Ni and Pb in control (K) and in soils contaminated with different percentages (0.5, 1 and 2% d.w.) of conventional microplastics (PE-MPs) and biodegradable microplastics (Bio-MPs) are reported in Figure 4.4. Soil Cr and Ni availabilities in both PE-MPs and Bio-MPs did not significantly vary as compared to K (Fig. 4.4). Instead, soil Cu availabilities were significantly ($P < 0.001$) higher in all Bio-MPs treatments (0.5%:12.5 μg g-1 d.w.; 1%: 14.2 μg g-1 d.w.; 2%:15.9 μg g-1 d.w.)

than in K (1.39 μg g-1 d.w.) (Fig. 4.4) and also soil Pb availabilities were significantly (P < 0.05) higher in 2% PE-MPs (3.23 μg g-1 d.w.) than in K (2.18 μg g-1 d.w.) (Fig. 4.4).

Metal availabilities did not significantly vary among the different percentages within the same treatment (PE-MPs or Bio-MPs).

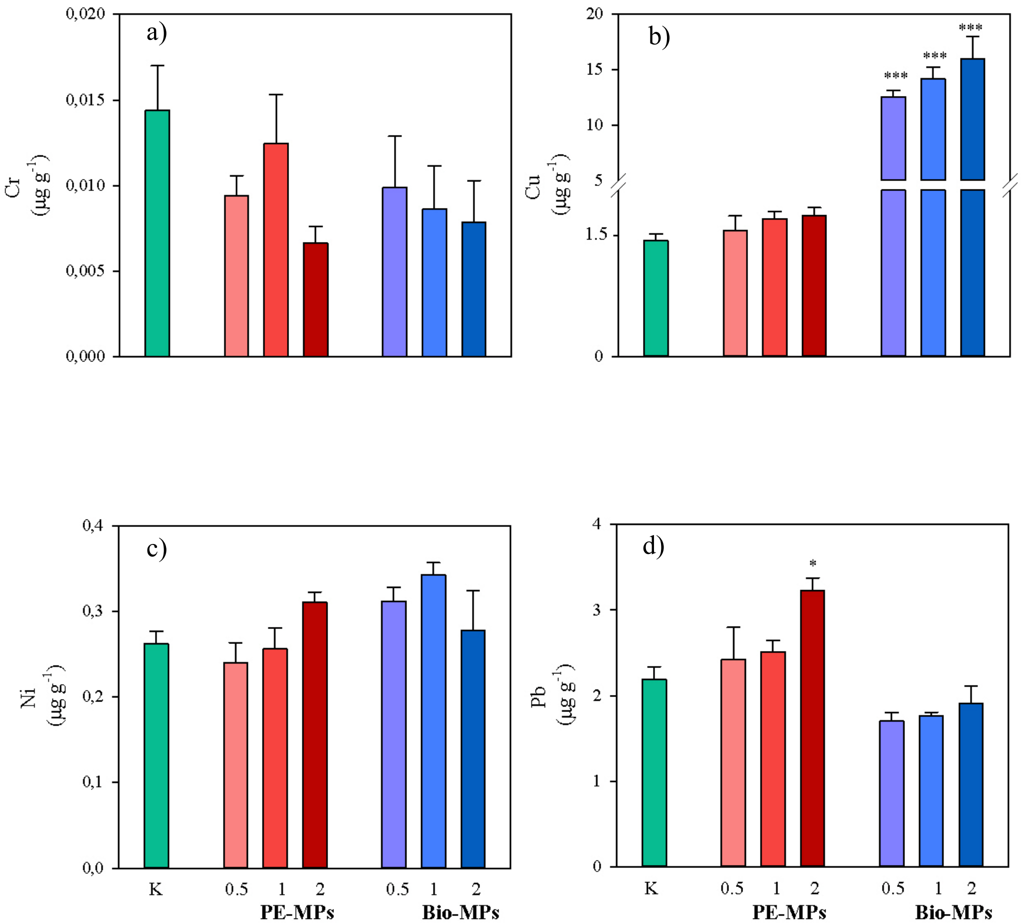

Figure 4.4 Mean values (±s.e.) of available (a) Cu, (b) Cr, (c) Ni and (d) Pb concentrations measured in soil without microplastics (K), mixed with Polyethylene (PE-MPs) and Mater-bì® (Bio-MPs) microplastics at different concentrations (0.5, 1 and 2% d.w.). Asterisks indicate significant differences between soils mixed with microplastics and control respectively (one-way ANOVA; p < 0.05).

The comparison of metal availabilities in soils contaminated with the same percentage of PE-MPs and Bio-MPs highlighted that Cr did not significantly vary (Table 2). Instead, Cu availabilities in all the percentage Bio-MPs were significantly (P

< 0.001 for 0.5% and 1% and P < 0.01 for 2%) higher than in PE-MPs (Table 4.2); Ni availabilities in 0.5% and 1% Bio-MPs were significantly (P < 0.001 and P < 0.05, respectively) higher than in PE-MPs (Table 4.2). By contrast, Pb availabilities in 1% and 2% PE-MPs were significantly (P < 0.05) higher than in Bio-MPs (Table 4.2).

4.4.3 Ratio of metal availability with respect to total concentration

The ratios between the availability and the total concentration for Cr, Cu, Ni and Pb are reported in Table 4.3. The ratios of all the metals for PE-MPs as well as those of Cr and Ni for Bio-MPs did not significantly vary as compared to K (Table 4.3). Instead, the ratios of Cu for all the percentage Bio-MPs were significantly (P < 0.05) higher than in K (Table 4.3) and those of Pb for 0.5% and 2% Bio-MPs were significantly (P < 0.05) lower than in K, and those of Pb for 2% PE-MPs were significantly (P < 0.05) higher than in K (Table 4.3).

The ratios between the availability and the total concentration for the investigated metals did not significantly vary among the different percentages within the same treatment (PE-MPs or Bio-MPs).

Table 4.3 Mean values (±s.e.) of available fraction and total concentrations ratios of Cu, Cr, Ni and Pb calculated in soil without microplastics (K), mixed with Polyethylene (PE) and Mater-bì® (Bio) microplastics at different concentrations (0.5, 1 and 2 %). Asterisks indicate significant differences between soils mixed with microplastics and control re-spectively (one-way ANOVA; p < 0.05).

	K	PE-MPs			Bio-MPs		
		0.5%	1%	2%	0.5%	1%	2%
Cr	0.10	0.04	0.07	0.03	0.04	0.04	0.03
Cu	3.93	3.26	3.76	3.92	24.7*	31.4*	34.2*
Ni	2.38	2.26	2.10	2.60	2.73	2.83	2.05
Pb	13.7	14.5	15.8	20.9*	8.90*	9.95	8.99*

The ratios in soils contaminated with the same percentage did not significantly vary between the two treatments (PE-MPs vs Bio-MPs) for Cr and Ni (Table 4.4). Instead, the ratios for Cu were significantly higher in Bio-MPs than in PE-MPs for all the percentages ($P < 0.01$ for 0.5% and $P < 0.001$ for 1% and 2%); whereas the ratios for Pb were significantly higher in PE-MPs than in Bio-MPs for 1% and 2% ($P < 0.01$) (Table 4.4).

Table 4.4 Significant differences in available fraction and total concentrations ratios of Cu, Cr, Ni and Pb within the pots with the same Polyethylene (PE) and Mater-bì® (Bio) microplastic concentrations (T-test; *** p< 0.001; ** p < 0.01; * p < 0.05).

	PE-MPs *vs* Bio-MPs		
	0.5%	1%	2%
Cr	n.s.	n.s.	n.s.
Cu	**	***	***
Ni	n.s.	n.s.	n.s.
Pb	n.s.	**	**

4.4.4 Enzymatic activities in soil contaminated with PE-MPs and Bio-MPs at different percentages

The soil enzymatic activities, hydrolase (HA), dehydrogenase (DHA), β-glucosidase (β-glu) and urease (U), were reported in Figure 4.5.

Urease in both PE-MPs and Bio-MPs did not significantly vary as compared to K (Fig. 4.5). Instead, HA and β -glu in 2% PE-MPs (HA: 10.5 mmol FDA $min^{-1}g^{-1}$ d.w and β -glu: 23.8 mmol PNP $min^{-1}g^{-1}$ d.w) were significantly ($P < 0.05$) higher than in K (HA: 8.84 mmol FDA min-1g-1 d.w and β -glu: 17.8 mmol PNP $min^{-1}g^{-1}$ d.w) (Fig. 4.5); by contrast, DHA in all the percentage PE-MPs (0.5%: 0.06 mmol TPF min-1g-1 d.w.; 1%: 0.06 mmol TPF min-1g-1 d.w.; 2%: 0.05 mmol TPF $min^{-1}g^{-1}$ d.w.) were significantly ($P < 0.01$) lower than in K (0.139 mmol TPF $min^{-1}g^{-1}$ d.w.) (Fig. 4.5). Moreover, HA in 0.5% and 2% Bio-MPs (0.5%: 10.9; 2%: 10.4) were significantly ($P < 0.05$) higher than in K (Fig. 4.5); β-glu in 1% and 2% Bio-MPs (1%: 21.6 mmol PNP $min^{-1}g^{-1}$ d.w; 2%: 25.6 mmol PNP $min^{-1}g^{-1}$ d.w) were significantly (P < 0.05 and P <

0.001, respectively) higher than in K (Fig. 4.5); DHA in all the percentage Bio-MPs (0.5%: 0.10 mmol TPF $min^{-1}g^{-1}$ d.w.; 1%: 0.07 mmol TPF $min^{-1}g^{-1}$ d.w.; 2%: 0.10 mmol TPF $min^{-1}g^{-1}$ d.w.) were significantly ($P < 0.05$) lower than in K.

The enzymatic activities DHA and U in soils did not significantly vary among the different percentages within the same treatment (PE-MPs or Bio-MPs) except for HA for PE-MPs and β-glu for Bio-MPs that were higher at the increase of the percentage of MPs (Fig. 4.5).

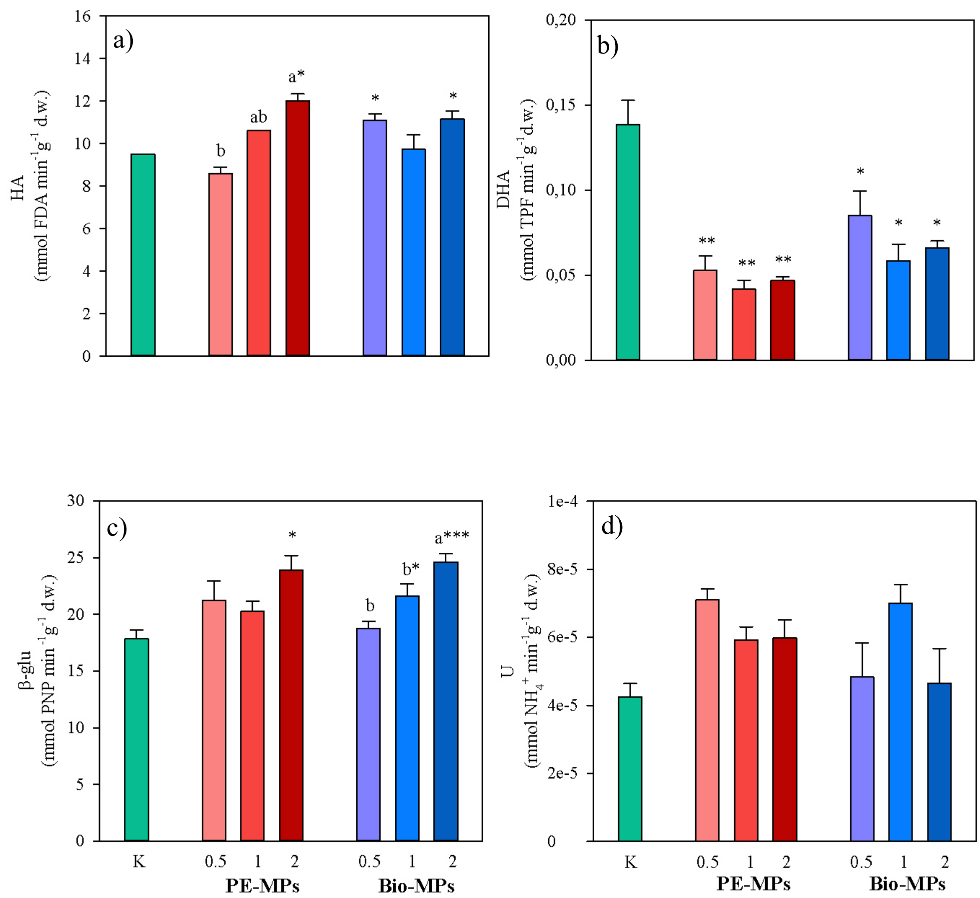

Figure 4.5 Mean values (±s.e.) of (a) hydrolase (HA), (b) Dehydrogenase (DHA), (c) β-glucosidase and (d) Urease (U) activities measured in soil without microplastics (K), mixed with Polyethylene (PE-MPs) and Mater-bì® (Bio-MPs) microplastics at different concentrations (0.5, 1 and 2 %). Asterisks indicate significant differences between soils mixed with microplastics and control respectively (one-way ANOVA; $p < 0.05$). Different small letters

indicate significant differences among the percentages of the same treatment (one-way ANOVA; $p < 0.05$).

The enzymatic activities in soils contaminated with the same percentage did not significantly vary between the two treatments (PE-MPs vs Bio-MPs).

4.4.5 Biomasses of plants

The values of plant epigeal (EPI) and hypogeal (HYPO) biomasses and their ratios (HYPO/EPI) measured at the end of the vegetative cycle of plants grown on control soils and in soils contaminated with different percentages of conventional microplastics and biodegradable microplastics are reported in Figure 4.6.

The comparison of the biomasses of plants grown on K, PE-MP and Bio-MP soils highlighted that both EPI and HYPO biomasses as well as the HYPO/EPI of plants grown on soils contaminated by PE-MPs did not significantly vary as compared to those of plants grown on K (Figure 4.6); instead, they were significantly ($p < 0.05$) lower in plants grown on 2% Bio-MPs (EPI: 15.0 g; HYPO: 1.2 g; HYPO/EPI: 0.1 g) than in K (EPI: 26.1 g; HYPO: 5.1 g; HYPO/EPI: 0.2 g) (Figure 4.6).

The comparison of the biomasses of plants grown on soils at different percentages PE-MPs highlighted that no statistical differences were found. Instead, EPI, HYPO and HYPO/EPI were significantly lower at the increase in the percentage of Bio-MP (Figure 4.6).

The comparison of EPI and HYPO biomasses and the HYPO/EPI ratio of plants grown at the same percentage between the two treatments (PE-MPs vs. Bio-MPs) highlighted that no significant differences were found (Figure 4.6).

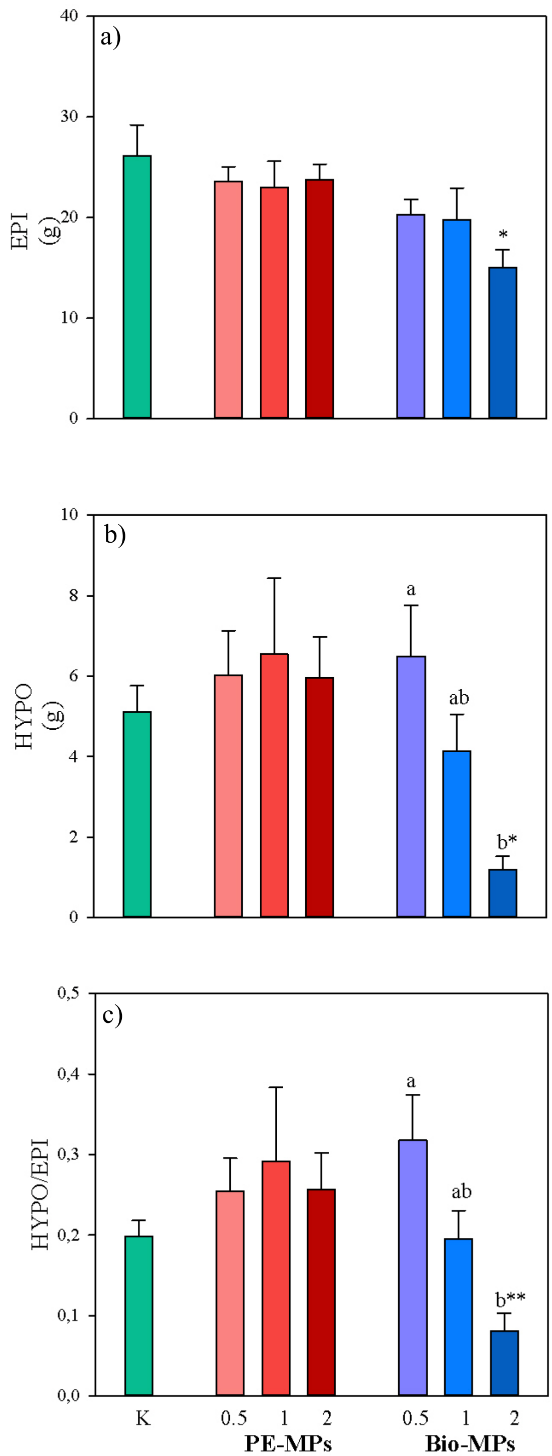

Figure 4.6 Mean values (±s.e.) of (a) Epigeal (EPI), (b) Hypogeal (HYPO) and (c) hypogeal and epigeal ration (HYPO/EPI) of Spinach plants grown in soil without microplastics (K), mixed with Polyethylene (PE) and Mater-bì® (Bio) microplastics at different concentrations (0.5, 1 and 2 %). Asterisks indicate significant differences between soils mixed with microplastics and control respectively (one-way ANOVA; $p < 0.05$). Different small letters indicate significant differences among the percentages of the same treatment (one-way ANOVA; $p < 0.05$).

4.4.6 Correlations between soil abiotic and biotic parameters in soil contaminated with PE-MPs and Bio-MPs

The correlations performed to evaluate the significance of the relationships between the enzymatic activities in soils or the plant biomasses and the soil metal concentrations highlighted that, for soils contaminated with PE-MPs, both β-glu and plant epigeal biomass were positively correlated to Ni availability (Fig. 4.7).

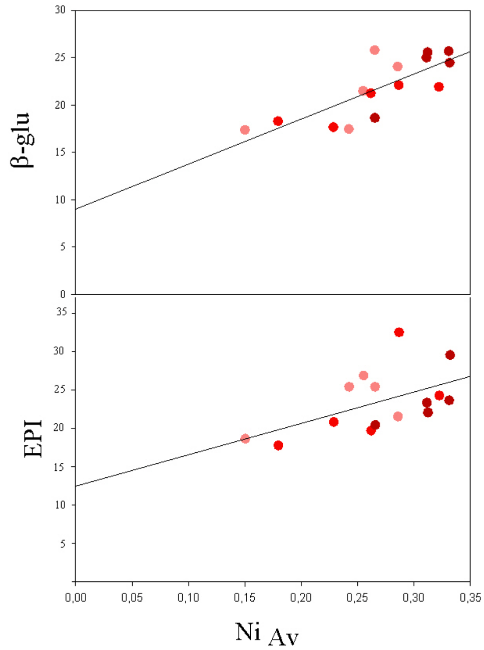

Figure 4.7 Regression lines (Spearman's correlations) between a) β-glucosidase activity (β-glu) and b) epigeal biomass (EPI) of Spinach plants with Ni available fractions in soils contaminated by PE-MPs (0.5%: light red bar; 1%: red bar and 2%: dark red bar).

4.4.7 Principal component analyses on soil parameters

The PCA, performed on all the investigated soil and plant properties, highlighted that the first two axes accounted, respectively, for 20% and 17% of the total variance (Fig.

4.8). The available fraction of Cu, EPI, HYPO and HYPO/EPI explained the major part of the variance of the first axis (Fig. 4.8); whereas, the available fractions of Ni and Pb, the total metal concentration of Cr, DHA and β-glu explained the major part of the variance of the second axis (Fig. 4.8). The first axis clearly separated PE-MPs and Bio-MPs, as the former located along the negative part of the axis and the latter along the positive one (Fig. 4.8). The second axis clearly separated K soils as they located along the negative part of the second axis (Fig. 4.8). The PERMANOVA analyses highlighted that soil treated with Bio-MPs were significantly different ($P < 0.05$) from K and PE-MPs soils.

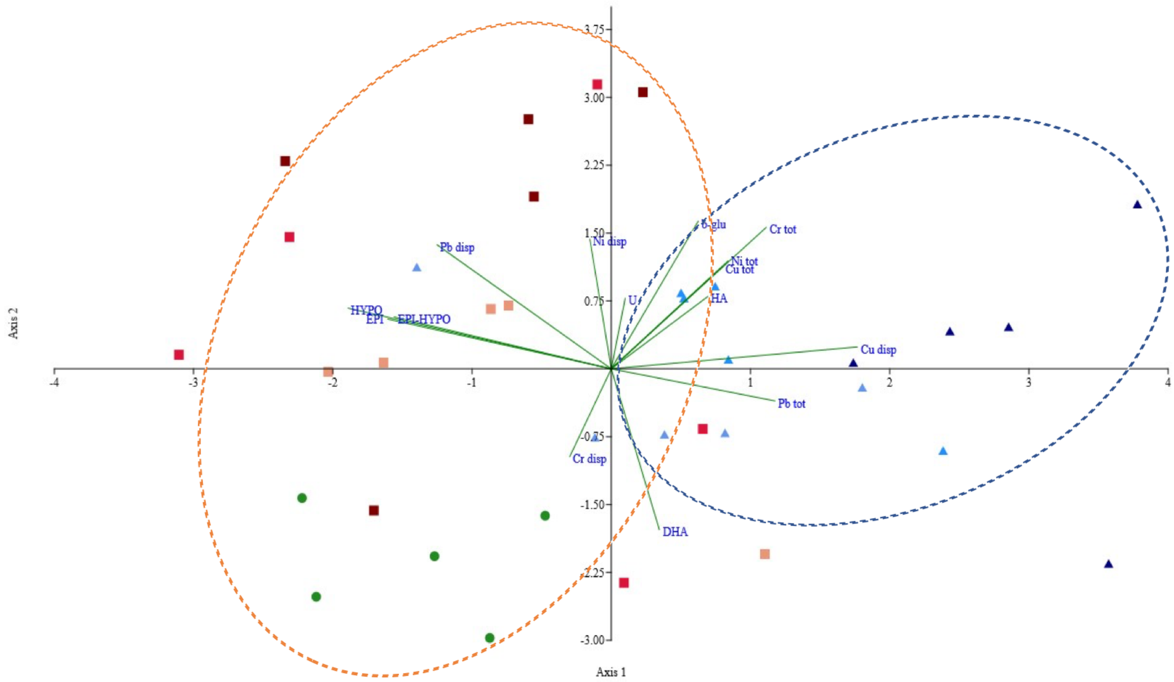

Figure 4.8 Graphical display of the first two axes of the Principal Component Analysis (PCA) on the soil total and available Cu, Cr, Ni and Pb concentrations, enzymatic activities (HA, DHA, β-glu and U) and plant biomasses in soil without microplastics (green dots), mixed with 0.1, 1 and 2% d.w. of Polyethylene (orange, red and dark red squares, respectively) and of Mater-Bì (cyan, blue and dark blue triangles, respectively) microplastics. Significant differences among the treatments were shown by confidence ellipses (Permanova analysis $p < 0.05$).

4.5 Discussion

The present research highlighted that the addition of conventional microplastics (PE-MPs) and biodegradable microplastics (Bio-MPs) to soils caused variations in the total and available fraction of the investigated metals (Cu, Cr, Ni and Pb) that often were also significant. The findings agree with those reported by several researchers [28–30] who found that MPs can affect the speciation, transformation and bioavailability of heavy metals such as Zn, Cu, Ni, Cd, Cr, As and Pb.

Contrarily from what happened in soils contaminated by PE-MPs, those highly (2%) contaminated by Bio-MPs caused significant increases in total concentrations of Cu, Cr and Pb. This could be due to the release in soils of harmful additives, containing metals used during the production of bioplastic films [31–33]. Bioplastics have stronger metal adsorption capacities than conventional plastics, due to their crystallization and carrier adsorption characteristics [11]. In addition, this phenomenon becomes more marked at the highest concentrations of biodegradable microplastics, because of the high contents of fragments that increase the contact surface area between soil and biodegradable microplastics. Finally, the phenomenon of metal adsorption to soil particles cannot be neglected.

Although the soils contaminated by Bio-MPs showed higher total concentrations of Cr, Cu and Pb, as compared to K, only the Cu availability significantly increased. In fact, Cu can lead to chemical speciation through physical, chemical and biological interactions with soil components [34]. By contrast, although the soils contaminated by PE-MPs did not show significant differences in total concentrations of the investigated metals, as compared to K, an increase in Pb availability was observed. It can be supposed there is a release of petroleum-based compounds containing Pb by conventional plastic films [30]. The different behaviors of Cu and Pb were confirmed by the calculated ratios between the availability and the total concentration of these metals that were, respectively, higher in soils contaminated by Bio-MPs and PE-MPs.

Among the investigated soil enzymatic activities, only U did not appear to be affected by MPs, whereas HA and β-glu were stimulated and DHA reduced by the presence of both PE-MPs and Bio-MPs (although not at all the tested percentages). The effects of microplastics on microbial activity are highly variable and dependent on the kind and concentration of microplastic [35]. The observed stimulation of HA and β-glu agrees with several pieces of research [36,37] and could be due to the possible release of dissolved carbon from plastic films in soil [38]. This hypothesis is corroborated by the increase in β-glu, using carbon compounds as a substrate [39], already at 1% Bio-MPs. Instead, the reduction of the intracellular enzyme DHA in both PE-MP- and Bio-MP-contaminated soils suggests an overall stress condition for microbial metabolism [40,41].

The effect of MPs on the investigated crop was limited to highest percentages of Bio-MPs that inhibited plant growth, as significant reductions of both HYPO and EPI biomasses were observed. The findings agree with those reported by various researchers who found negative dose-effect impacts on plant growth [7,42,43] due to MPs. Also, Qi et al. [44] found that starch-based Bio-MPs had a negative effect on wheat biomass compared to PE-MPs. The decrease in HYPO biomass and the HYPO/EPI ratio at the increase in percentage of Bio-MPs suggests that these plastics hinder the movement of water and nutrients in soil, limiting their absorption and utilization, with a negative consequence on plant root growth [45,46].

In PE-MP-contaminated soils a key role was played by Ni availability, which enhanced EPI biomass and β-glu activity. The lowest Ni availability, compared to those measured in Bio-MP-contaminated soils, suggests that this metal is present in concentrations essential for crop growth and for maintaining its health [47].

An overall evaluation, considering the investigated soil properties and the crop biomasses, highlighted a clear separation of Bio-MP-contaminated soils from both

PEMPs and K. Bio-MP-contaminated soils, especially at 2%, were characterized by high Cu availability and reduced crop production, indicating its role in metal contamination increase and the inhibition of plant biomasses.

4.6 Conclusion

The findings contributed to highlight differences in soil properties and crop production after soil MP contamination. In particular, the addition of PE-MPs did not cause variations in soil total metal concentrations as compared to K, whereas the addition of Bio-MPs caused the increase in total Cr, Cu and Pb concentrations. Notwithstanding, in soils contaminated by PE-MPs higher Pb availability was observed, and in soils contaminated by Bio-MPs only the Cu availability significantly increased as compared to K.

Extracellular enzymatic (HA and β-glu) activities were stimulated in MP-contaminated soils, especially at 2%, whereas the intracellular one (DHA) was reduced. Finally, the HYPO and HYPO/EPI biomasses were reduced only in soils contaminated by the highest percentage Bio-MPs.

Based on the obtained data, it can be concluded that Bio-MPs more than PE-MPs contribute to a major metal release in soils and have a negative impact on spinach biomass.

The present research highlighted a negative role of Bio-MP presence in soils, but further studies in open fields are required to clarify the effects of Bio-MPs on soil properties and plant growth.

4.7 Acknowledgments

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GENERAL CONCLUSIONS

In the recent decades, plastic pollution is one of the hazards most discussed by the scientific community although only a few studies have been focused in terrestrial ecosystems. Plastic, an emerging pollutant, can degrade and form small particles (MPs) that can disperse into the whole environment causing serious dangerous on organisms. In the terrestrial environments, mulching is one of the most source of plastics in soils. Unfortunately, by now the effects of plastics on soil quality have been poorly addressed and the available findings are often controversial. In this framework, the thesis research, performed in mesocosm trails, has contributed to increase the current knowledge about the impact of conventional plastic mulches on soil abiotic and biotic properties. Moreover, the thesis research has also evaluated if the use of bioplastic mulches reduced the negative effect on soil quality as compared to conventional ones.

After six months of exposure to both conventional and biodegradable plastic mulches, soils properties significantly changed. In particular, the presence of conventional plastic mulches caused a significant decrease of pH values and a significant increase of total and organic carbon concentrations. Instead, the presence of biodegradable plastic mulches caused a significant increase only of organic carbon concentrations (that was also higher than that measured in soils covered by conventional plastic mulches). Moreover, the presence of both conventional and biodegradable plastic mulches increased the soil metal contamination. In fact, the metal contamination factor in soils was higher than 1 and higher than that calculated for uncovered soils. However, differences occurred between soils covered by conventional and biodegradable plastic mulches regarding to metal accumulation. In fact, soils covered by biodegradable plastic mulches showed higher total and available metal concentrations; whereas those covered by conventional plastic mulches showed higher ratios between available and total concentrations of metals. Finally, also the biotic properties of soils were impacted by the presence of plastic mulches. Particularly, the presence of both plastic mulches impacted the dehydrogenase activity that was lower than that measured in uncovered soils. Moreover, the presence of conventional plastic mulches reduced the microbial respiration and enhanced the ecotoxicity as compared to the uncovered soils.

[redacted]

This thesis research also aimed to contribute to the effects of soil contamination with microplastics from plastic and bioplastic mulches. In particular, the research revealed a negative role of the presence of microplastics generated by biodegradable mulch. contamination with microplastics generated by biodegradable mulch caused an increase in total Cr, Cu and Pb concentrations. However, in soils contaminated with microplastics generated with the conventional plastic mulch, an increased availability of Pb was observed, whereas in soils contaminated with microplastics generated by the biodegradable mulch only the availability of Cu increased significantly compared to uncontaminated soils. The activities of hydrolase and beta-glucosidase were stimulated in microplastics-contaminated soils, especially at 2%; whereas that of dehydrogenase was inhibited. Finally, the epigeal and hypogean biomasses of spinach were only inhibited in soils contaminated by the highest percentage of microplastics generated by the biodegradable mulch.

FUTURE INVOLVEMENTS

The data reported in the thesis highlighted that, after one year of exposure to conventional and bioplastic mulches, the differences in the abiotic and biotic properties gradually became wider between soil covered by the two types of mulches. Therefore, further investigations will be performed in order to highlight the long-term effects. Moreover, the content of soil MPs as well as changes in specific functional groups of chemical compounds and their impacts on soil quality will be evaluated.

The thesis research has highlighted the impact of plastic mulches on soil at mesocosm scale. Although, the findings provide useful information to contribute in filling the current gaps about the issue, they are scarcely useful to define conclusions about the impact on real conditions. Therefore, the impact of conventional and biodegradable plastic mulches will be evaluated in the field and compared with those conducted in mesocosm trials.

The results achieved from the thesis research highlighted the impacts of microplastics generated by plastic mulches on certain soil characteristics and spinach crop production. Although the data provide useful information on the effects of microplastics, further studies will be needed to investigate further impacts on soil properties and further on different plant crops, investigate ecophysiological parameters that could be altered by the presence of these contaminants.

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Supplemental Materials

Chapter 1:

Table S1.1 Mean values (± s.e.) of Total Al, Ca, Cu, Fe, K, Mg, Mn, Na Pb and Zn (expressed as mg g^{-1} d.w.), Ni (expressed as µg g-1 d.w) concentrations and available Ca, K, Mg and Na (expressed as mg g^{-1} d.w.), and Al, Cu, Fe, Mn, Ni, Pb and Zn (expressed as µg g^{-1} d.w) measured in soils at the beginning of experiment (T0).

	Total	Available
Al	66.5	2.07
Ca	38.2	5.56
Cu	0.135	47.5
Fe	30.6	38.9
K	33.8	1.80
Mg	9.53	0.433
Mn	0.870	8.12
Na	4.41	0.875
Ni	21.1	0.285
Pb	0.1	9.02
Zn	0.183	32.1

Chapter 3:

Table S3.1 [redacted]

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