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

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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.

1. 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

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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. Materials and methods

2.1. Mesocosm setting up

The research was carried out in mesocosms, constituted by 14 pots 1 m 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 × 40 cm) of un-biodegradable (Unbiod, made by polyethylene) plastic constituted by little 16 squares (10 × 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. 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.

Fig. 1. Experimental mesocosms (left) and details of soils covered by un-biodegradable and biodegradable plastic sheets and uncovered soils (right).

Table 1
Monthly mean temperature and rainfall amount detected in downtown Naples (Italy) from January to August 2021 (http://www.ilmeteo.it/and www.anceca mpania.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

2.2. 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 × 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, 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 150 mM; $NaHCO_3$ 2.5 mM. 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 (PerkinElmer, 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 0.025 M 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 0.5 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 5000 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 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):

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

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

2.3. 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 ade4ˆ and Factoextra packages.

3. Results

3.1. 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). Moreover, soil respiration was, on average, 0.13 mg of CO_2 g^{-1} h^{-1} (Fig. 3), the mean values of hydrolase (HA),

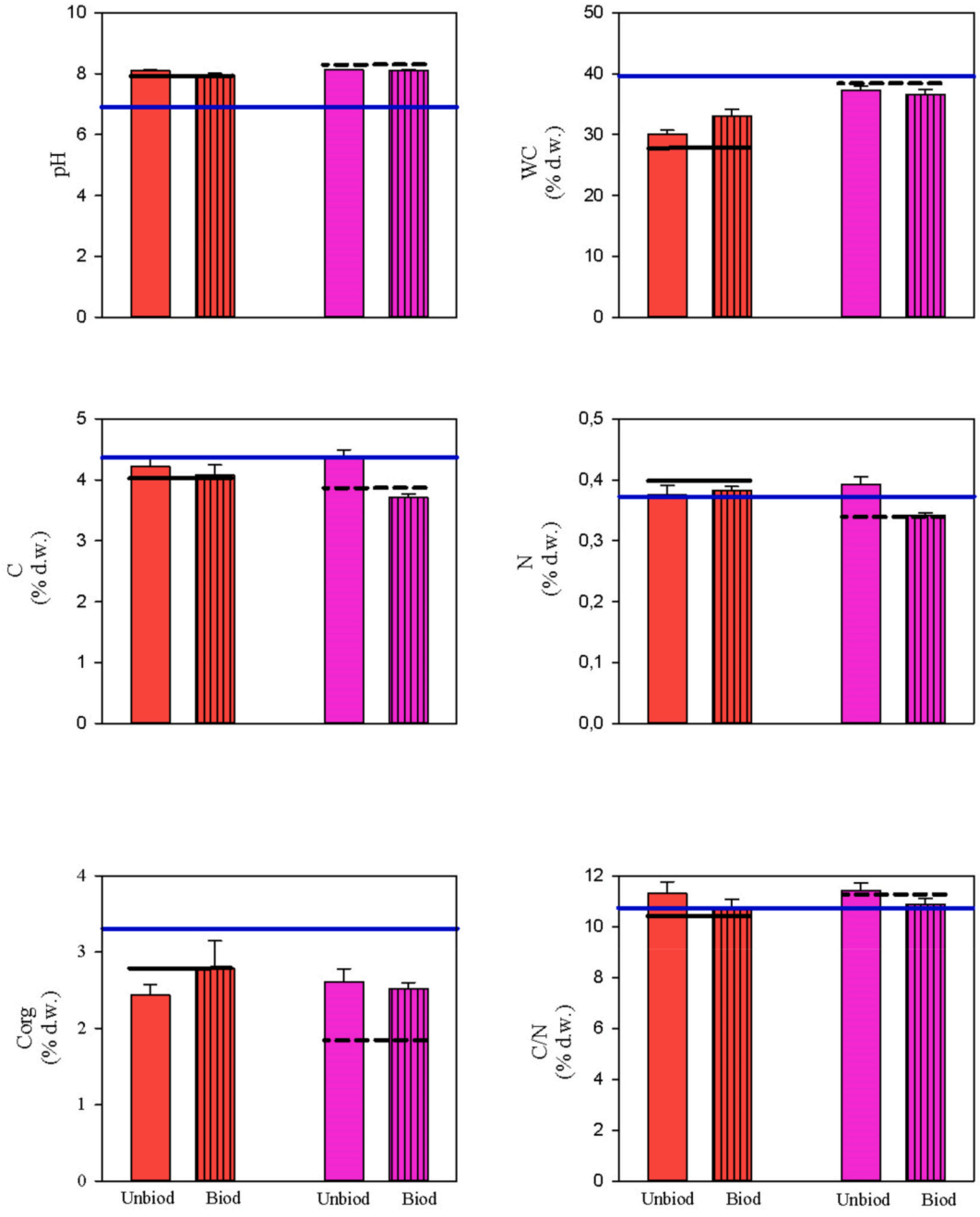

Fig. 2. Mean values (±s.e.; n = 5) of pH, water content (WC), concentration of total C and N and organic C (C_{org}), C/N ratios in soils covered by un-biodegradable (empty bars, n = 5) and biodegradable (filled bars, n = 5) plastic sheets collected three (pink) and six (violet) months after mesocosm 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. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

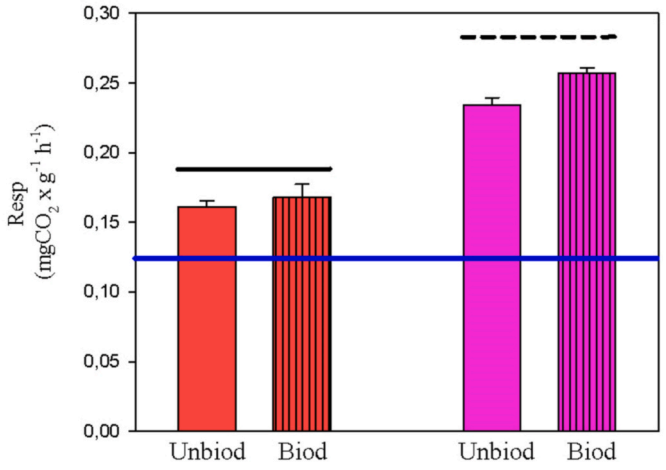

Fig. 3. Mean values (±s.e.; n = 5) of microbial respiration (Resp) in soils covered by un-biodegradable (empty bars, n = 5) and biodegradable (filled bars, n = 5) plastic sheets collected three (pink) and six (violet) months after mesocosm setting up. The mean values in soils at the beginning of the 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. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

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 × 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. 4), the effect percentage of phytotoxicity of *S. saccharatum* L was 19.1 and that of *L. sativum* was −19.1(Fig. 5).

3.2. 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, and did not statically differ among treatments with the exception of pH and water content (Table 1). In fact, soil pH showed statistically significant differences (Table 2) among Ctr (7.70), Unbiod (8.11) and Biod (7.97), and water content was statistically (Table 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. 3), and did not statistically vary among the treatments (Table 2).

The mean values of the enzymatic activities for each treatment are reported in Fig. 4. Among them, only DHA activity statistically differed among the treatments (Table 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. 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. 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. 5) that statistically differed only between Ctr and Unbiod (Table 2).

The mean values of the investigated abiotic properties of the soils collected at T2 are reported in Fig. 2. Among them, pH, concentrations of C, N and organic C showed statistically differences among treatments (Table 2). Particularly, pH was statistically higher at Unbiod (8.13) than at Ctr (8.03) (Table 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; Table 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; Table 2).

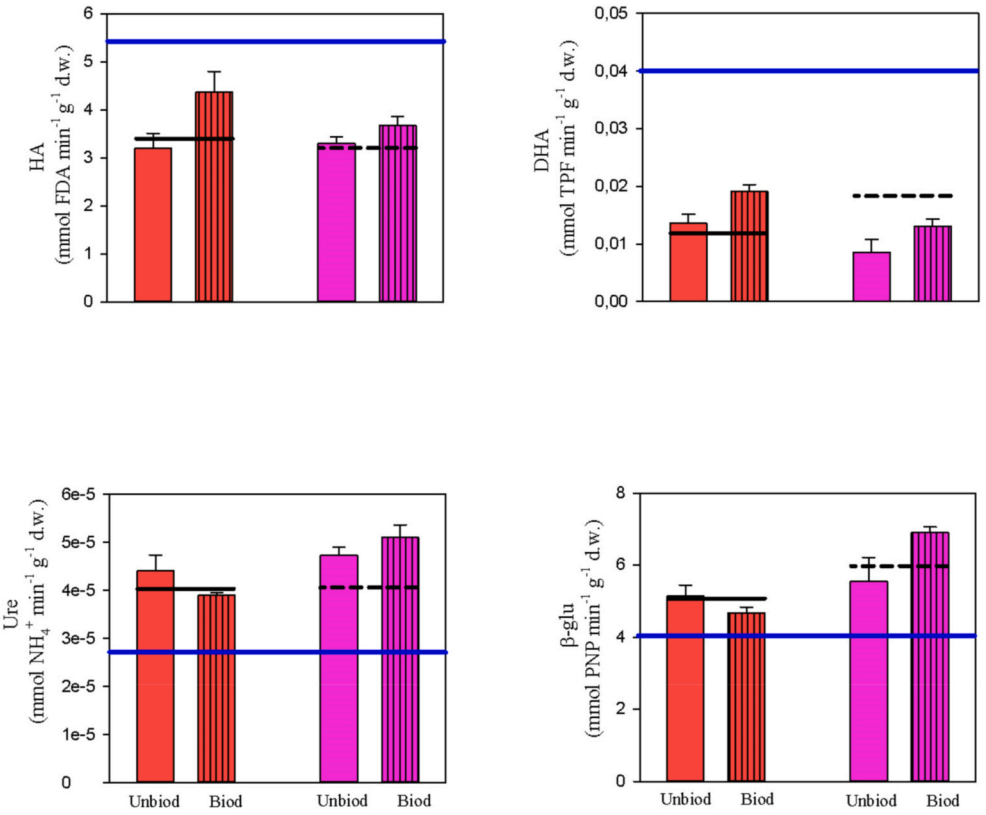

Fig. 4. Mean values (±s.e.; n = 5) of hydrolase activity (HA), dehydrogenase activity (DHA), urease activity (Ure) and β-glucosidase activity (β-glu) in soils covered by un-biodegradable (empty bars, n = 5) and biodegradable (filled bars, n = 5) plastic sheets collected three (pink) and six (violet) 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. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

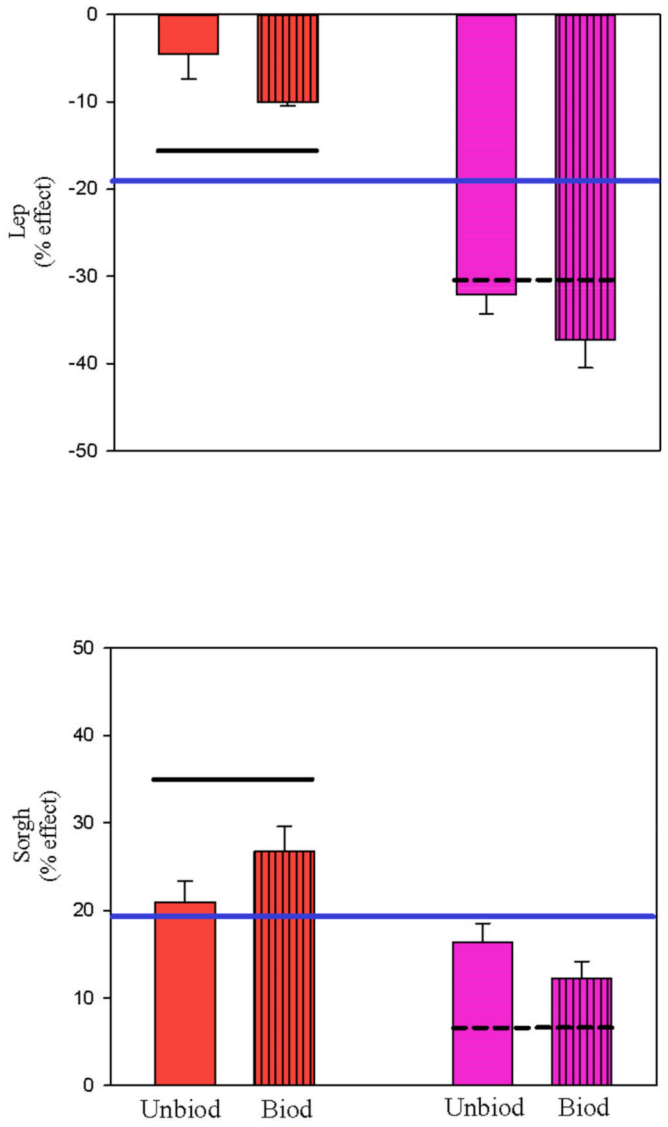

Fig. 5. Mean values (±s.e.; n = 5) of effect percentages of phytotoxicity through *Lepidium sativum* L. and through *Sorghum saccharatum* L. in soils covered by un-biodegradable (empty bars, n = 5) and biodegradable (filled bars, n = 5) plastic sheets collected three (pink) and six (violet) months after 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. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

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. 3; Table 2).

The values of the enzymatic activities are reported in Fig. 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. 4; Table 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. 5) that did not statistically differ among treatments (Table 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. 5) and statistically differed only between Ctr and Unbiod (Table 2).

Table 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: C_{org}; 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

NS: not significant; *P < 0.05; **P < 0.01.

3.3. 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; Table 3). In fact, pH statistically increased since T1 to T2 at Ctr and Biod (Fig. 2; Table 3); water content statistically increased since T1 to T2 at all the treatments (Fig. 2; Table 3); finally, N concentrations statistically decreased over the time at Biod (Fig. 2; Table 3).

Soil respiration statistically increased over the time at all the treatments (Fig. 3; Table 3).

Among the investigated enzymatic activities, only HA did not show statistically significant differences over the time at the treatments (Fig. 4). In fact, DHA statistically increased since T1 to T2 at Ctr and statistically decreased at Biod (Fig. 4; Table 3); URE statistically increased over the time at Biod (Fig. 4; Table 3); finally, β-glu statistically increased ad Ctr and Biod (Fig. 4; Table 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		Biod
	Ctr	Unbiod	
	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 **

NS: not significant; *P < 0.05; **P < 0.01; ***P < 0.001.

The phytotoxicity through *L. sativum* L. increased over the time for all the treatments (Fig. 5) with differences statistically significant at Biod and Unbiod (Fig. 5; Table 3); whereas, that through *S. saccharatum* L. decreased over the time for all the treatments (Fig. 5) with differences statistically significant at Ctr and Biod (Fig. 5; Table 3).

3.4. 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. 6). Soil water content, Resp, β-glu, and both the phytotoxicity assays explained the major part of the variance of the first axis (Fig. 6); whereas, DHA explained the major part of the variance of the second axis (Fig. 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. 6); whereas, Ctr separated by Unbiod and Biod along the second axis, as Ctr mainly located in the negative quadrants (Fig. 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. 6); whereas, T2 by all the remaining investigated properties (Fig. 6).

4. 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 because of 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

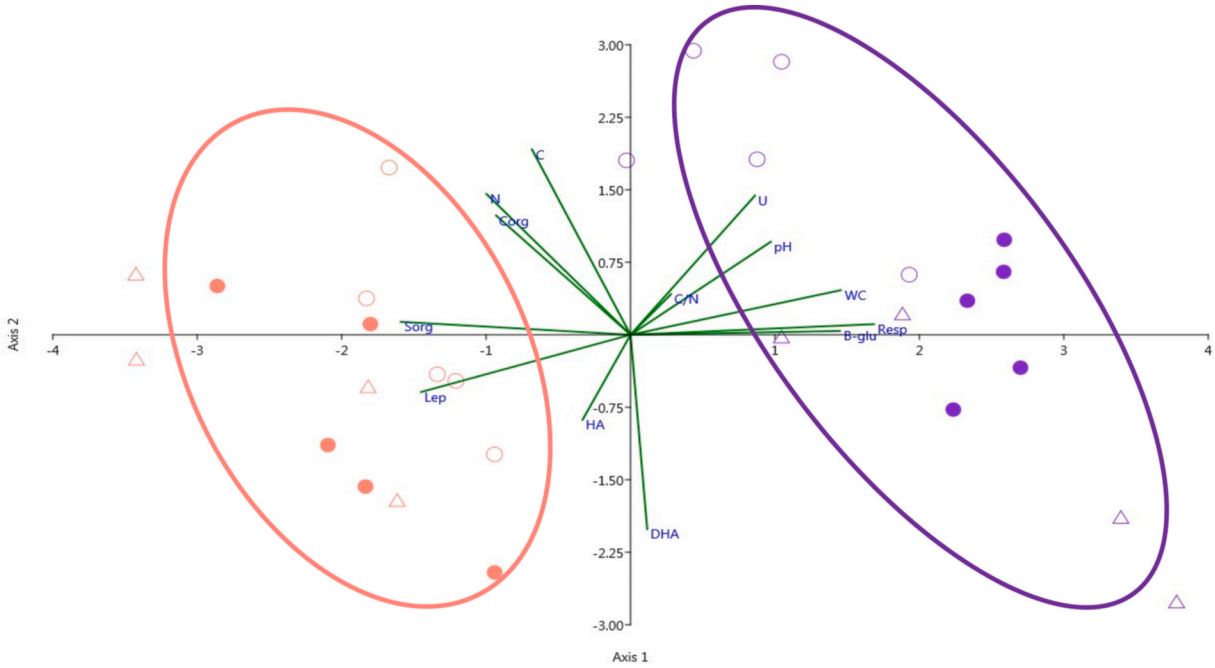

Fig. 6. Graphical display of the first two axes of the Principal Component Analysis on the soil abiotic (pH; water content: WC; organic Carbon: C_{org}; C and N contents; C/N ratios), biotic (Resp: microbial respiration; HA: hydrolase activity; DHA: dehydrogenase activity; Ure: urease activity; β-glu: 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. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

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; Luo 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; Lou et al., 2015).

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).

5. 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.

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.

Author statement

Giorgia Santini, **Valeria Memoli**, **Lucia Santorufo** and **Giulia Maisto**: Conceptualization; **Giorgia Santini, Sara acconcia and Mattia Napoletano**: Methodology; **Giorgia Santini and Lucia Santorufo:** Software; **Giorgia Santini**, **Valeria Memoli** and **Lucia Santorufo**: Validation; **Giorgia Santini**, **Sara acconcia, Mattia Napoletano Valeria Memoli** and **Lucia Santorufo:** Formal analysis; **Giorgia Santini**, **Valeria Memoli**, **Lucia Santorufo and Giulia Maisto**: Investigation; **Giulia Maisto:** Resources; **Giorgia Santini, Valeria Memoli and Lucia Santorufo**: Data curation; **Giorgia Santini and Giulia Maisto**: Writing—original draft preparation; **Giorgia Santini**, **Valeria Memoli**, **Lucia Santorufo** and **Giulia Maisto**: Writing—review and editing; **Giorgia Santini** and **Giulia Maisto**:Visualization; **Giulia Maisto**: Supervision; project ad-ministration; **Giulia Maisto** : Funding acquisition. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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