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Posted Date: 3 November 2023

doi: 10.20944/preprints202309.1304.v2

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## Article

# Long-Term Effects of Plastic Mulch in a Sandy-Loam Soil Cultivated with Blueberry in Southern Portugal

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**Abstract:** Agriculture uses plastic products for containers, packaging, tunnels, drip irrigation tubing, mulches. Large amounts of plastics are used as mulches on the soil surface for vegetable production (tomato, cucumber, watermelon, strawberry, vine) to reduce the weed competition, increase water and fertilizer use efficiency and enhance crop yield. Portugal uses around 4,500 t/year of polyethylene plastic to cover approximately 23,000 ha of agricultural land and only a small amount is recovered for recycling or secondary uses because of dirt with soil, vegetation, pesticide and fertilizers. Cleaning and decontaminating polyethylene mulch are costly and commercial technology is often not accessible nor economically viable in the current economic Portuguese situation. Most plastic mulch is composed of polyethylene that degrades slowly and produces a large quantity of residues in soil with negative impact in the environment. In the present study, the effects of long-term cultivation of blueberry using green 100% high density polyethylene mulch, in the south Portugal were evaluated for soil chemical and biological changes. High density green plastic mulch did not contaminate the topsoil with di(2-ethylhexyl) phthalate and heavy metals, but total nitrogen and organic carbon concentrations, electric conductivity and microbial activity were reduced in the planting row compared with bare soil, without mulching. Furthermore, the presence of plastic mulch did not affect negatively the presence of nematodes, and the Rhabditida (bacterial feeders) increased in the planting and covered row.

**Keywords:** green high-density polyethylene mulch; microbial activity; nematode community; open-field; soil chemical composition; topsoil; *Vaccinium virgatum* Aston cv. Centra Blue

## 1. Introduction

There are a multitude of uses for plastic products in agriculture (plasticulture) including containers, pots, packaging, tunnel coverings, drip irrigation tubing and mulch films. Large amounts of plastics are used as mulches applied on the soil surface in vegetable production (e.g., tomato, cucumber, watermelon, strawberry, vine, young orchards) to reduce the weed competition, increase water and fertilizer use efficiency and soil temperature, and enhance crop yield, especially in arid and semi-arid environments [1–3]. The two main sources of mulch materials used are synthetic sources (polypropylene and polyethylene) and organic sources (derived from organic by-products, organic residues). Colored plastic mulches are widely accepted and utilized by farmers to solve above-lined problems [2] but residual plastics can affect soil organisms and various physicochemical properties of soil and plant performance [2,4]. Often, plastic mulch is composed of polyethylene, PE [5] that degrades slowly in soil, with an incomplete recovery producing a large quantity of residues left in soil with negative impact in the environment [4,6]. Around 25.8 Mt of plastic residues are yearly generated in Europe [7], but the percentage of plastic mulch that remains in the soil in Europe is based on experts' opinion, rather than collected data. France, Spain, Germany, Italy and UK are the main European users of plastic mulch [8]. Portugal uses around 4,500 t/year of plastic to cover

approximately 23,000 ha of agricultural land, and only a small part of this is recovered for recycling or secondary uses because of issues of dirt contamination with soil, vegetation, pesticides, fertilizers [9,10]. To comply with the European legislation and the circular economy transition for agricultural plastics it is necessary to take the necessary measures to facilitate the plastics recycling and promote the use of materials with less negative impact in the environment, such as the biopolymers.

Berries have gained popularity over the last decade, especially blueberries [11]. Blueberry (*Vaccinium* sp.) is a perennial flowering plant with blue or purple berries. It belongs to the Ericaceae Family, prized for their sweet edible fruits rich of vitamin C, minerals, and a number of antioxidants [11]. Portuguese blueberry production is mainly exported to The Netherlands, France, Spain, Belgium, UK and Germany [12]. Blueberries are commonly eaten fresh, but can be baked in a variety of pastries. Micro- and nanoplastics (MPs and NPs) in soil, i.e., plastic residues lower than 5 mm and lower than 1 mm size, respectively can not only limit crop productivity but also negatively impact fruit quality and enhance the concentration of food safety hazards, thus raising concerns for their potential health risks for humans [13]. As to our best knowledge, studies on the presence of MPs and NPs in fruits are scarce. Oliveri Conti et al. [14] is probably the first study referring that MPs are capable to penetrate the fruits (pear, apple) plant cell based on their size and type. Oliveri Conti et al. [14] hypothesized that MPs may be absorbed due to the similarity of carbon nanomaterials and translocated in plant tissues similarly to the modality of uptake of carbon-nanomaterials. This uptake is inversely proportional to particles size. Soil moisture, in particular, and weeds competition for water and nutrients may have a tremendous effect on blueberry fruit yield [15], therefore mulching is a common agricultural practice to reduce these abiotic and biotic stresses.

Environmental impacts derived from blueberry production have scarcely been analyzed and studies on this topic in Europe are rare [11], particularly the long-term (>4 years) studies on the effects of plastic mulch on soil characteristics. The aims of present work were to assess for the first time the impact of a long-term production system using a green high-density plastic (HDPE) mulching *a)* on chemical and biological properties of topsoil cultivated with blueberry (*Vaccinium virgatum* Aston.) in Southern Portugal, and *b)* on the accumulation of phthalate esters (PAEs), namely the di(2-ethylhexyl) phthalate (DEHP) in cultivated soil. We hypothesized that permeable green plastic (100%HDPE) mulch of weft 50x70 with 97 g m<sup>-2</sup> used for blueberry cultivation will not degrade significantly in 10 years under the semi-arid condition of Southern Portugal and can contribute to improve soil chemical and biological properties, as Lalitha et al. [16] mentioned.

## 2. Material and methods

### 2.1. Environmental conditions and experimental layout

The study was run at Fataca Experimental Farm, Odemira, in Southern Portugal, Lat: 37.5903, Long: -8.7403. The region is representative of this crop cultivation. A 30-year period annual temperature in the region varied from a minimum of 7 °C to a maximum of 31 °C (average of 19 °C). Rainfall varied from 3 mm in July and August to 75 mm in December (average annual rainfall of 437 mm).

Two-years-old blueberry plantlets (*Vaccinium virgatum* Aston cv. Centra Blue) were transplanted from nursery to the field in summer 2013 and maintained for 10 years. 'Centra Blue', a cross between 'Centurion' and 'Rahi', is a new rabbiteye cultivar (*Vaccinium ashei* J.M Reade syn. = *V. virgatum* Aiton) developed by "The Horticulture and Food Research Institute of New Zealand Limited (HortResearch)" [17].

The cultivated soil was amended with composted pine bark to improve soil structure and organic matter concentration. In the open-field, inter-row spacing was 2.50 m and plantlets were 0.80 m spaced in the row. Planting rows were covered with a permeable green high density polyethylene (100%HDPE, weft 50x70, 97 g m<sup>-2</sup>) mulch (Figure 1A) to control weeds emergence and conserve soil moisture. HDPE mulch could last on the soil surface for 10 years. In each planting row, two lines for drip fertigation were used, with emitters of 2.0 L h<sup>-1</sup> placed each 30 cm. Fertigation was applied according to plant's needs, i.e., ammonium, potassium, phosphorus and magnesium sulphate.

Electric conductivity (EC) of fertigation solution was set to a maximum of  $1.2 \text{ mS cm}^{-1}$ . A contiguous bare soil, without mulching and planting, was separated from cultivated soil, covered with plastic mulch, by about 5 m, and was used as a control treatment (CK).

The experimental layout included two treatments: a control (bare) soil with no planting and no mulching, and a cultivated soil with blueberry 'Centra Blue' covered with green high density polyethylene (100%HDPE) mulch for 10 years, and three replicates (5 plants each). Both treatments were compared for soil chemical and biological characteristics.



**Figure 1.** Soil covered with conventional green high-density plastic mulch (A) in blueberry (*Vaccinium virgatum* Aiton cv. Centra Blue) plantation (B), at Fataca Experimental Farm (Odemira, in the south Portugal).

## 2.2. Soil sampling and chemical and biological determinations

In June 2023, during the vegetative phase of 10-yr-old blueberry plants, soil samples were taken at 0-20 cm depth, for both treatments and each replicate (in the planting row of cultivated soil), using a stainless steel auger. Fresh soil sub-samples were sieved ( $<2 \text{ mm}$ ) to evaluate the enzymatic activity and the population of nematodes; other sub-samples were air-dried and sieved ( $<2 \text{ mm}$ ) to evaluate the changes of chemical composition after 10 years cultivation of blueberries, using green HDPE mulch on the planting row.

In each dried soil sub-sample, pH was determined in 1:2.5 soil:water ratio and the electric conductivity (EC) was measured by potentiometry. Total nitrogen (N) was determined by dry combustion, and organic carbon (C) was estimated by sodium dichromate digestion and measured by UV-Vis spectrophotometry. Extractable P and K were determined by Egnér-Riehm method [18], exchangeable bases were extracted by 1M ammonium acetate ( $\text{CH}_3\text{COONH}_4$ ) solution at pH 7.0 [19], micronutrients (Cu, Zn, Fe, Mn) were extracted according to Lakanen and Ervio [20], and extracted boron (B) was estimated by hot water method. The di(2-ethylhexyl) phthalate (DEHP) was determined by GC-MS.

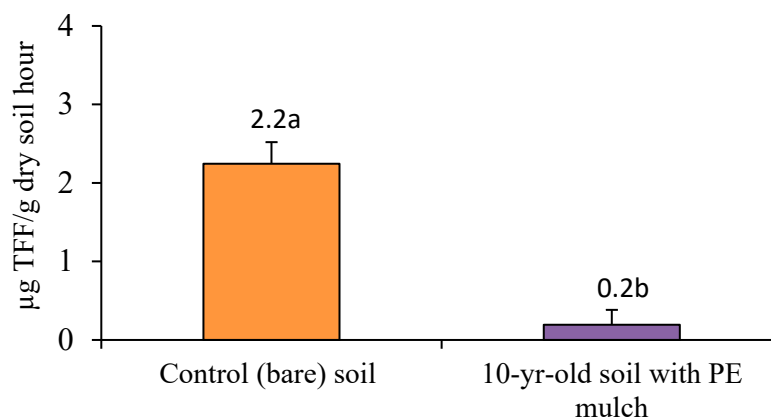
In each fresh soil sample, dehydrogenase activity was evaluated by spectrophotometric quantification of the triphenyl formazan formed from reduction of 2,3,5-triphenyltetrazolium chloride, using a modification of the method of Casida et al. [21], as described by Menino et al. [22]. Nematodes were extracted from 500 mL subsamples using the tray method [23]. The suspensions were observed under a stereomicroscope (Nikon SMZ1500, Tokyo, Japan), and specimens of each trophic group (plant parasites, bacterial feeders, fungivores, omnivores and predators) were counted, followed by observation using a brightfield light microscope (Olympus BX-51, Hamburg, Germany) for identification.

## 2.3. Statistical analysis

Changes of chemical properties, enzymatic activity and nematode communities in the topsoil of planting rows, covered with green 100%HDPE mulch and after 10-years cultivation of blueberry, were evaluated and compared with a bare control soil (non-mulching, without planting,) with three replicates, by one-way Analysis of Variance (ANOVA) using the *Statistica* version 12 (Stat Soft, Inc.).



For significant differences, means were separated by Turkey's honestly significance (HSD) test at  $p < 0.05$ . Values in tables were presented as means and coefficient of variation, and standard deviation in Figure 2 ( $n=3$ ).



**Figure 2.** Comparison of soil dehydrogenase activity after 10 years' cultivation of blueberry 'Centra Blue' covered with green HDPE mulch, in Fataca Experimental Farm, at Odemira, in the south Portugal. Values above columns and error bars represent, respectively, the mean and standard deviation of three replicate samples. Different letters next to values above columns express significant differences according to Tukey's HSD test at  $p < 0.05$ . TFF, triphenyl formazan.

### 3. Results and discussion

#### 3.1. Changes in soil chemical composition

After 10 years cultivation of blueberry 'Centra Blue' in the open-field, using a conventional green plastic (100%HDPE) mulch, some effects in soil chemical properties were observed (Tables 1 and 2) compared to the control (CK) bare soil. The green 100%HDPE mulch, of weft  $50 \times 70$  with  $97 \text{ g m}^{-2}$  was designed to alter the microclimate at the plant and soil levels and was mainly formulated with role in light absorption, soil water permeability and crop physiology. According to manufacturer, this fabric structure is a resistant ( $85 \pm 0.05 \text{ Kg/5 cm}$ ; ISO 5081) and a relatively dense weft that makes passage of water ( $12 \pm 2 \text{ L m}^{-2} \text{ s}^{-1}$ ; ISO 811) and air to soil, but blocks light passage preventing weeds from growing.

In the soil, the organic C, total N and EC, the best chemical indicators of soil quality were significantly reduced in the planting row covered with plastic mulching in the long-term, comparing to bare soil without cultivation and no mulching (Table 1). Indicators of soil quality are defined as those soil properties that display the greatest sensitivity to changes in soil function. Despite the soil amendment with pine bark compost, organic C in cultivated soil ( $10.03 \text{ g kg}^{-1}$ ) was low for an optimal blueberry growth (optimal value of  $23 \text{ g C kg}^{-1}$ ; [24]), even lower than the concentration in CK soil (Table 1). Domagała-Świątkiewicz and Siwek [25] observed an opposite result on raspberry cultivation soil covered with a black polypropylene (PP) non-woven mulch, i.e., an increase of soil organic C. A similar trend occurred for total soil N, with a significantly lower value in the long-term cultivated soil with mulching ( $0.84 \text{ g kg}^{-1}$ ) compared to uncovered CK soil ( $1.08 \text{ g kg}^{-1}$ ). Nevertheless, blueberries have a relatively low N requirement and thrive well on organic fertilizers, added as a pine bark compost in the present study. The C/N ratio in both soil treatments was similar (11.9), and was appropriate for mineralization to occur. The lowest organic C and total N concentrations in present cultivated soil covered with 100%HDPE mulch could be explained by a faster mineralization rate due to a possible higher soil temperature and moisture content by mulching effect. The present reduction of EC to  $0.07 \text{ mS cm}^{-1}$  in planting row with mulching, significantly lower than the CK soil, was more favorable for plant growth, and had the same response as in raspberry soil using a polylactic acid-based (PLA) biopolymer [25]. The pH-value in cultivated soil (5.7) did not differ significantly from control soil and was above the optimum range of values (4.0-5.5) for blueberry

growth [24,26], but slightly lower than the CK soil (Table 1). Ochmian et al. [26] and Yang et al. [26] referred that when the soil pH exceeds that range of values, the blueberries may present adverse symptoms, such as nutrients deficiency, delay of flower bud differentiation and flowering, growth inhibition, low photosynthetic intensity, and reduced fruit yield and quality (e.g., increase of titratable acid concentration). Strik [27] confirmed that blueberry plants are very sensitive to soil pH and N fertilizer source.

**Table 1.** Changes in studied soil (0-20 cm depth) chemical characteristics after 10 years cultivation of blueberry 'Centra Blue' in Fataca, Odemira, in the south Portugal (mean $\pm$ CV, n=3).

Treatment	pH <sub>(H2O)</sub>	EC	Total N	Org. C	Ext. P	Ext. K	Ca	K	Mg	Na	CEC
		mS cm <sup>-1</sup>	g kg <sup>-1</sup>		mg kg <sup>-1</sup>				cmol(+) kg <sup>-1</sup>		
CK soil	5.8 $\pm$ 0.03a	0.10 $\pm$ 0.28a	1.08 $\pm$ 0.05a	12.9 $\pm$ 0.16a	57.47 $\pm$ 0.23a	104.3 $\pm$ 0.15a	1.40 $\pm$ 0.09a	0.23 $\pm$ 0.42a	0.40 $\pm$ 0.30a	0.07 $\pm$ 0.55a	3.40 $\pm$ 0.28a
10-yr-old cultivated soil with HDPE mulch	5.7 $\pm$ 0.15a	0.07 $\pm$ 0.20b	0.84 $\pm$ 0.04b	10.03 $\pm$ 0.10b	69.57 $\pm$ 0.04b	83.02 $\pm$ 0.25a	0.87 $\pm$ 0.46a	0.19 $\pm$ 0.38a	0.57 $\pm$ 0.32a	0.04 $\pm$ 0.26a	3.33 $\pm$ 0.20a

CK= control (bare) soil; CV= coefficient of variation; n= number of observations; PE= polyethylene; EC= electric conductivity; Org. C= organic carbon; Ext.= extractable; CEC= cation exchange capacity; in each column, means with different letters are significantly different for Turkey's HSD test, at p<0.05.

After 10 years' cultivation of blueberries using permeable green plastic (100%HDPE) mulch, concentrations of micronutrients in soil at 0-20 cm depth, i.e. the extractable copper (Cu), zinc (Zn), iron (Fe), manganese (Mn) and B were lower than those in control (bare) soil, particularly Zn and Mn (Table 2). Nevertheless, in both soils, the extractable Cu was in the medium range of values for agricultural soils and the extractable Fe was very high. As to the extractable Zn, concentration in the control soil was in the medium range but decreased to a low value in cultivated soil with mulching. The extractable Mn was very low, which is overall good for agricultural purposes and together with soil pH of 5.7-5.8 is not toxic for blueberries. But Fe and Mn are important nutrients for blueberry (*Vaccinium* sp.) fruits and their concentrations in plant should be further monitored, also because pH>5.5 may induce leaf Fe chlorosis [28]. Finally, the extractable B had a low concentration in the control (bare) soil, and decreased to a very low level in planting rows with plastic mulching. Boron is an essential micronutrient, required for physiological and biochemical processes in fruit crops such as blueberry, a plant species well adapted to acidic soils (pH<sub>(H2O)</sub>≤ 5.5) with low B availability. According to the literature [29,30], the critical range of low and high B levels in plant tissues is narrow, and B requirement is highly variable among plant species and genotypes, with an optimum value for one cultivar being either insufficient or toxic for other species or cultivars. There is little information regarding deficiency and excess of B in fruit plants grown in acidic soils like the blueberry, therefore under the present conditions the evaluation of nutritional status of plant (leaf, fruit) is recommended.

**Table 2.** Changes in some micronutrients in soils (0-20 cm depth) after 10 years' cultivation of blueberries 'Centra Blue' in Fataca, Odemira, south Portugal (mean $\pm$ CV, n=3).

Treatment	Ext. Cu	Ext. Zn	Ext. Fe	Ext. Mn	Ext. B
			mg kg <sup>-1</sup>		
CK soil	2.3 $\pm$ 0.10a	2.4 $\pm$ 0.42a	227 $\pm$ 0.10a	6.0 $\pm$ 0.28a	0.21 $\pm$ 0.05a
10-yr-old cultivated soil with green HDPE mulch	1.2 $\pm$ 0.29b	0.8 $\pm$ 0.15a	207 $\pm$ 0.14a	<2.5 $\pm$ ndb	<0.20 $\pm$ ndb

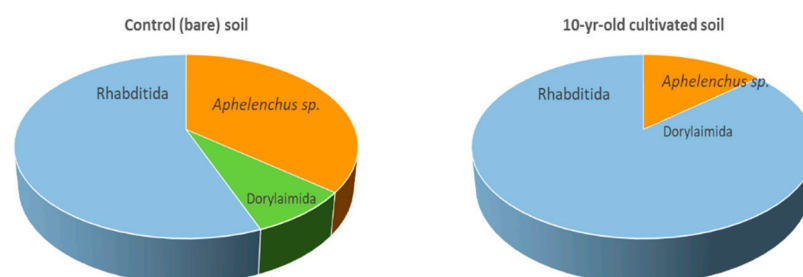
CK= control (bare) soil; CV= coefficient of variation; n= number of observations; PE= polyethylene; Ext.= extractable; nd= not determined; in each column, means with different letters are significantly different for Turkey's HSD test, at p<0.05. In agreement with our hypothesis, the cultivated soil did not show contamination with di(2-Ethylhexyl) phthalate (<2.7 kg DEHP kg<sup>-1</sup> dry matter) by using the green 100%HDPE mulch for a 10 years period, similar to the control (bare) soil (<2.8 kg DEHP kg<sup>-1</sup> dry matter). The type of mulch used was of long-life span and was not degradable for at least 10 years (manufacturer reported a minimum UV stability for 5 years, i.e. a stabilizer was added to the material for protection from UV light degradation). This finding agrees with Pérez et al. [11] who referred that this type of material has an average lifetime of about 10 years.

### 3.2. Effect of long-term cultivation with green HDPE mulch on soil enzymatic activity

Dehydrogenases are intracellular oxidoreductase enzymes that are involved in microbial respiration metabolism. The activity of dehydrogenases is among the most appropriate, crucial and responsive soil biological indicators, responding immediately to changes in soil systems, cropping and conditions. The soil dehydrogenase activity (DHA) has been recognized as an indicator of the metabolic status of the soil microbiota and of the viable microbial activity [31]. DHA depends on the same factors that affect the abundance and activity of microorganisms. In the present study, DHA in soil collected at 0-20 cm depth at the end of spring and after 10 years' cultivation of blueberry using a permeable green 100%HDPE mulch was significantly lower than the bare soil (Figure 2), aligning with the differences in organic C concentration in both soils. Soil organic C is a strong determinant of soil enzymatic activities, including dehydrogenase, since higher organic C represents more substrate for microbial energy with the consequent increase of microbial biomass and enzyme production [32]. The higher DHA in bare soil could also indicate a possible negative effect of planting row covered with green plastic mulch on the overall soil microbial activity, agreeing with Moreno et al. [33] for organic pepper cultivation, using black PE film. They explained this effect by a higher soil temperature and a certain reduction of gas interchange between the soil and the atmosphere under plastic mulching. Nevertheless, the possibility that other factors related to blueberry cultivation may negatively influence soil microbial activity cannot be ruled out.

### 3.3. Effect of long-term cultivation with green HDPE mulch on nematodes communities in soil

Although this is a preliminary study, shifts of nematode community after 10 years' cultivation of blueberry 'Centra Blue' in the open-field covered with green 100%HDPE mulch were observed in comparison with uncultivated (bare) soil (Figure 3). The Rhabditida (bacterial feeders) showed a 4-fold increase in cultivated soil compared with the control soil. This huge increase in bacterial-feeder nematodes was probably due to previous soil organic amendment with composted pine bark, and a possible higher temperature and moisture caused by the plastic mulch. The presence of higher values of bacterivorous nematodes in cultivated soil indicated that the organic matter mineralization/nitrification was taking place, reducing total organic C and total N in soil, in agreement with Table 1. According to Öztürk [33], free-living bacterivorous nematodes play an essential role in the mineral cycle and can feed on many bacterial species, including plant pathogens. Despite differences in the relative abundance of the two main trophic groups, the populational effectiveness of fungivorous nematodes (*Aphelenchus* sp.) did not differ between the two soil treatments, probably because this group of nematodes is less responsive to soil modifications [35]. Dorylamida (omnivore nematodes) were negligible, especially in cultivated soil, and predators were not found in any treatment. The presence of a green HDPE mulch did not affect negatively the presence of nematodes, but these results need further confirmation. Moreover, the nematode community structure must be studied using different indices in order to better assess any change due to soil disturbance [36,37].



**Figure 3.** Relative proportion of nematodes per 100 mL of bare (control) soil (no mulching, no planting) and soil with 10 years' cultivation of blueberries 'Centra Blue' covered with green 100%HDPE mulch, in Fataca Experimental Farm, at Odemira, in the south Portugal: *Aphelenchus* sp. = fungivorous; Dorylamida = omnivorous; Rhabditida = bacterivorous.

#### 4. Conclusions

This is a former open-field study on the effects of a long-term cultivation of a perennial plant species, the blueberry 'Centra Blue', covered with a permeable green high-density plastic mulch ( $50 \times 70$  and  $97 \text{ g m}^{-2}$ ) in a semi-arid environment in the south of Portugal. This study revealed that mulching system apparently generated unique soil conditions, mirrored by variations in specific soil parameters. The green polyethylene mulch controlled the emergence of natural vegetation and did not increase soil salinity in the topsoil (0-20 cm). However, long-term cultivation with plastic mulching tended to reduce the soil chemical quality comparing with bare soil without planting and non-mulching. The most relevant chemical indicators for land-use change like the organic carbon and total nitrogen concentrations, pH and the electric conductivity which were reduced in planting rows with high density polyethylene mulch. Manganese and boron were depleted from soil and their concentration in fruits should be further controlled since these micronutrients are important for blueberries nutrition.

The 100%HDPE mulch behaved as a promising weed barrier, conserving soil moisture and promoting plant growth and yield (data not shown). This fabric mulch was not degraded in 10 years and did not produce macroplastic residues in soil. Present findings also highlighted for the first time that no relevant phthalate and heavy metals concentrations were released in covered soil with 100%HDPE mulch.

Biological quality in present cultivated soil requires further developments since the enzymatic activity and nematodes community appear contradictory trends. A negative effect of plastic mulching on the overall soil microbial activity was observed. Considering the results of both nematology and enzyme evaluations, it can be hypothesized that the main responsible for dehydrogenase activity in bare soil were fungi. In cultivated soil, fungal populations may have suffered an imbalance for some reason, either lowering their total abundance or favoring fewer active groups in terms of oxidative metabolism, with a concomitant increase in bacterial populations. Soil dehydrogenase activity and nematode community analyses are valuable tools for assessing the impact of land management practices on soil biology, soil health and overall soil condition. The use of nematodes as soil bio indicators makes ecological sense as they represent a central position in the soil food web and are related to ecological processes such as organic matter mineralization/nitrification, cultural practices and plant growth. Some authors found the nematode community the best biological indicator [36–39].

Further studies in the long-term, comparing different mulches and including biomaterials, should be put in place to confirm present findings, and the presence of microplastics in soil and plant, and the effects on plant performance. In addition, the diversity of nematodes should be further defined through the identification of key taxa, correlation of key taxa to disturbance, and calibration of indices relative to ecosystem, climate and soil practice (mulching).

**Author Contributions:** All authors contributed for the study, under the field condition, and for manuscript preparation. Conceptualization of the study was by Filipe Pedra, Maria de Lurdes Inácio, Paula Fareleira, Pedro Oliveira, Pablo Pereira and Corina Carranca; the methodology was planned by Pedro Oliveira and Corina Carranca; formal analysis and measurements were carried out by Filipe Pedra, Maria de Lurdes Inácio and Paula Fareleira; writing the original draft preparation was done by Corina Carranca, Maria de Lurdes Inácio and Paula Fareleira; writing, reviewing and editing were taken by all authors; supervision was taken by Corina Carranca.

**Acknowledgments:** Authors acknowledge the Fataca Experimental Farm and BerrySmart company, for supporting the experimental field work and materials. Authors also acknowledge the PRR project: "Agri-Plast-Organização e Inovação para a Redução de Plásticos Agrícolas" and the strategic project UIDB/04551/2020 "GREEN-IT: Bioresources for Sustainability" for funding the study.

**Conflicts of Interest:** The authors declare no conflict of interest.



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