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Long-Term Effects of Plastic Mulch in a Sandy Loam Soil Used to Cultivate Blueberry in Southern Portugal

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Abstract: Numerous plastic products are used in agriculture, including containers, packaging, tunnels, drip irrigation tubing, and mulches. Large amounts of plastics are used as mulches on the soil surface for vegetable and fruit production (tomato, cucumber, watermelon, strawberry, and vine) to reduce weed competition, increase water and fertilizer use efficiency, and enhance crop yield. Portugal uses around 4500 t/year of polyethylene to cover approximately 23,000 ha of agricultural land, and only a small amount is recovered for recycling or secondary uses because of issues of contamination with the soil, vegetation, pesticides, and fertilizers. Cleaning and decontaminating polyethylene mulch are costly, and commercial technology is often not accessible or economical. Most plastic mulch is composed of polyethylene that degrades slowly and produces a large quantity of residues in the soil, with a negative impact on 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, organic carbon concentrations, electric conductivity, and microbial activity were significantly reduced in the planting row compared with the bare soil without mulching. Furthermore, the presence of plastic mulch did not negatively affect the presence of nematodes, and the number of 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



Citation: Pedra, F.; Inácio, M.L.; Fareleira, P.; Oliveira, P.; Pereira, P.; Carranca, C. Long-Term Effects of Plastic Mulch in a Sandy Loam Soil Used to Cultivate Blueberry in Southern Portugal. *Pollutants* **2024**, *4*, 16–25. <https://doi.org/10.3390/pollutants4010002>

Academic Editors: Raimundo Jimenez-Ballesta and Mauro Marini

Received: 1 November 2023

Revised: 14 December 2023

Accepted: 5 January 2024

Published: 11 January 2024



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1. Introduction

There are a multitude of uses for plastic products in agriculture (plasticulture), including containers, pots, packaging, tunnel coverings, drip irrigation tubing, and plastic mulch. Large amounts of plastics are used as mulches applied to the soil surface in vegetable and fruit production (e.g., tomato, cucumber, watermelon, strawberry, vine, and young orchards) to reduce weed competition, increase water and fertilizer use efficiency and soil temperature (depending on the type and color of mulch), 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 and other organic residues). Colored plastic mulches are widely accepted and utilized by farmers to solve above-lined problems (weed, soil, temperature, and soil water control) [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]. This plastic degrades in soil and produces a large quantity of residues in soil, with a negative impact on the environment [4,6]. Around 25.8 Mt of plastic residues are produced yearly in Europe [7], but the amount of plastic mulch residues that remains in the soil in Europe is based on experts' opinion, rather than

collected data. France, Spain, Germany, Italy, and the UK are the main European users of plastic mulch [8]. Portugal uses around 4500 t/year of plastic mulch 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 contamination with the soil, vegetation, pesticides, and fertilizers [1]. To comply with European legislation and the circular economy transition for agricultural plastics, it is necessary to facilitate plastic recycling and promote the use of materials with a less negative impact on the environment, such as biopolymers.

Berries have gained popularity over the last decade among consumers, especially blueberries [9]. 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 in vitamin C, minerals, and a number of antioxidants [10]. Portuguese blueberry production is mainly exported to The Netherlands, France, Spain, Belgium, the UK, and Germany [11]. Blueberries are commonly eaten fresh, but they can also be baked in a variety of pastries. Micro- and nanoplastics (MPs and NPs) in soil, i.e., plastic residues smaller than 5 mm and smaller than 1 mm in size, respectively, can not only limit crop productivity but also negatively impact fruit quality and increase the concentration of food safety hazards, thus raising concerns regarding their potential health risks for humans [12]. Studies on the presence of MPs and NPs in fruits are scarce. Oliveri Conti et al. [12] produced probably the first study that shows MPs are able to penetrate the fruits' (pear and apple) plant cells based on their size and type. Oliveri Conti et al. [12] hypothesized that MPs may be absorbed due to the similarity of carbon nanomaterials and translocated into plant tissues, similarly to the modality of uptake of carbon nanomaterials. This uptake is inversely proportional to particle size. Soil moisture, in particular, and weed competition for water and nutrients may have a tremendous negative effect on blueberry fruit yield [13]; therefore, mulching is a common agricultural practice to reduce these abiotic and biotic plant stresses.

Environmental impacts of blueberry production and studies on this topic in Europe are scarce [10], including long-term (>4-year) studies on the effects of plastic mulch on soil characteristics. The aims of the present work were to assess, for the first time, the impact of a long-term production system using green high-density plastic (HDPE) mulching (a) on chemical and biological properties of topsoil used to cultivate blueberry (*Vaccinium virgatum* Aston.) in southern Portugal, and (b) on the accumulation of phthalate esters (PAEs), namely di(2-ethylhexyl) phthalate (DEHP), in cultivated soil. We hypothesized that permeable green plastic (100% HDPE) mulch of weft 50×70 with 97 g m^{-2} used for blueberry cultivation will not degrade significantly in 10 years under the semi-arid condition of southern Portugal and may improve the soil's chemical and biological properties, as Lalitha et al. [14] mentioned.

2. Material and Methods

2.1. Environmental Conditions and Experimental Layout

This study was run at the Fataca Experimental Farm, Odemira, in southern Portugal, Lat: 37.5903, Long: -8.7403 , and about 20 ha. The region offers environmental conditions favorable for berry cultivation. A 30-year period annual temperature in the region varied from a minimum of $7 \text{ }^\circ\text{C}$ to a maximum of $31 \text{ }^\circ\text{C}$ (average of $19 \text{ }^\circ\text{C}$). Rainfall varied from 3 mm in July and August to 75 mm in December (average annual rainfall of 437 mm).

Two-year-old blueberry plantlets (*Vaccinium virgatum* Aston cv. Centra Blue) were transplanted from a nursery to the field in the summer of 2013 and maintained for 10 years. 'Centra Blue' (Figure 1B), 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)" [15].



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

The cultivated soil was amended with composted pine bark to improve the soil structure and organic matter concentration. In the open field, inter-row spacing was 2.50 m, and plantlets were spaced 0.80 m apart in the row. Planting rows were covered with a permeable green high-density polyethylene (100% HDPE, weft 50×70 , 97 g m^{-2}) mulch (Figure 1A) to control weed emergence and conserve soil moisture. The green 100% HDPE mulch was designed to alter the microclimate at the plant and soil levels, light absorption, soil water permeability, and crop physiology. According to the manufacturer, this fabric structure is a resistant ($85 \pm 0.05 \text{ Kg/5 cm}$; ISO 5081) and relatively dense weft that allows the passage of water ($12 \pm 2 \text{ L m}^{-2} \text{ s}^{-1}$; ISO 811) and air to the soil but blocks light passage, therefore preventing weeds from growing. HDPE mulch could last on the soil surface for 10 years (the manufacturer indicated at least a 5-year duration). In each planting row, two lines for drip fertigation were used, with emitters of 2.0 L h^{-1} placed each 30 cm. Fertigation was applied according to the plant's needs, i.e., ammonium, potassium, phosphorus, and magnesium sulphate. The electric conductivity (EC) of the fertigation solution was set to a maximum of 1.2 mS cm^{-1} . A contiguous bare soil, without soil management, mulching, or planting, was separated from the cultivated and covered soil with plastic mulch by about 5 m and used as a control treatment (CK).

The experimental layout included two treatments: a CK soil with no planting (bare soil) without soil management and with no mulching, and a cultivated soil with blueberry 'Centra Blue' covered with green high-density polyethylene (100% HDPE) mulch for 10 years and 3 replicates (5 plants each). Both treatments were compared for soil chemical and biological characteristics.

2.2. Soil Sampling and Chemical and Biological Determinations

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

In each fresh soil sub-sample, dehydrogenase activity was evaluated through spectrophotometric quantification of the triphenyl formazan formed from the reduction of 2,3,5-triphenyltetrazolium chloride using a modification of the method of Casida et al. [16], as described by Menino et al. [17]. Nematodes were extracted from 500 mL fresh sub-samples using the tray method [18]. 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.

In each dried soil sub-sample, the pH was determined in a 1:2.5 soil:water ratio, and the electric conductivity (EC) was measured through potentiometry. Total nitrogen (N) was determined through dry combustion, and organic carbon (C) was estimated through sodium dichromate digestion and measured through UV-Vis spectrophotometry. Extractable P and K were determined using the Egnér–Riehm method [19], exchangeable bases were extracted using a 1M ammonium acetate ($\text{CH}_3\text{COONH}_4$) solution at pH 7.0 [20], micronutrients (Cu, Zn, Fe, and Mn) were extracted according to Lakanen and Ervio [21], and extracted boron (B) was estimated using the hot water method. The di(2-ethylhexyl) phthalate (DEHP) was determined through GC-MS.

2.3. Statistical Analysis

Chemical properties and enzymatic activity in the topsoil of planting rows covered with green 100% HDPE mulch and after 10 years of cultivation of blueberry were compared with a bare control soil (non-mulching and without planting) with three replicates through one-way Analysis of Variance (ANOVA) using *Statistica* version 12 (Stat Soft, Inc. Tulsa, OK, US). For significant differences, means were separated using Tukey's honestly significant difference (HSD) test at $p < 0.05$. For nematode communities, their relative proportion was compared for both soil treatments. The values in tables are presented as means, coefficients of variation, and standard deviations in Figure 2 ($n = 3$).

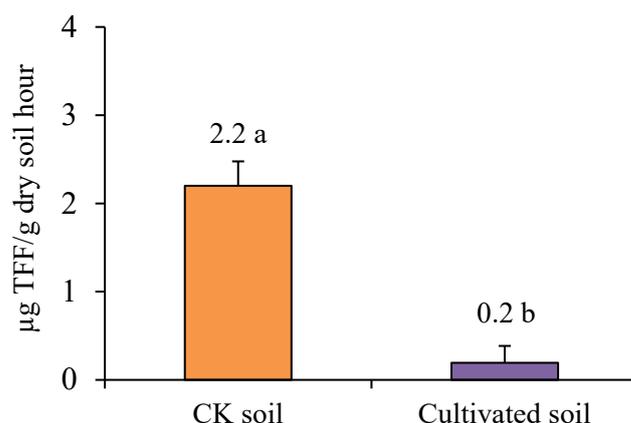


Figure 2. Comparison of soil dehydrogenase activity after 10 years of cultivation of blueberry ‘Centra Blue’ covered with green HDPE mulch at the Fataca Experimental Farm at Odemira in the south of Portugal. Values above columns and error bars represent, respectively, the means and standard deviations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

3. Results and Discussion

3.1. Changes in the Soil’s Chemical Composition

After 10 years cultivation of blueberry ‘Centra Blue’ in the open field using a conventional green plastic (100% HDPE) mulch in soil, the chemical properties were compared (Tables 1 and 2) to the CK (bare) soil.

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

Treatment	pH(H ₂ O)	EC	Total N	Org. C	Ext. P	Ext. K	Ca	K	Mg	Na	CEC
		mS cm ⁻¹	g kg ⁻¹		mg kg ⁻¹				cmol(+) kg ⁻¹		
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-year-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 according to Tukey's HSD test, at $p < 0.05$.

Table 2. Changes in some micronutrients in soils (0–20 cm depth) after 10 years of 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 ⁻¹				
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-year-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 according to Tukey's HSD test, at $p < 0.05$.

In the soil, the chemical indicators, like the organic C, total N, and EC, were significantly reduced in the planting row covered with plastic mulching in the long term comparing to the CK soil without cultivation or mulching (Table 1). Indicators of soil quality are defined as those soil properties that display the greatest sensitivity to changes in soil function [22]. Despite the soil amendment with pine bark compost, organic C in cultivated soil (10.03 g kg⁻¹) was low for optimal blueberry growth (optimal value of 23 g C kg⁻¹ [23]), even lower than the concentration in the CK soil (Table 1). Domagała-Świątkiewicz and Siwek [24] observed an opposite result in soil for raspberry cultivation covered with a black polypropylene (PP) non-woven mulch, i.e., an increase in soil organic C. A similar trend occurred for the total soil N, with a significantly lower value in the long-term-cultivated soil with mulching (0.84 g kg⁻¹) compared to the uncovered CK soil (1.08 g kg⁻¹), where herbaceous vegetation was growing naturally. Nevertheless, blueberries have a relatively low N requirement and thrive well on organic fertilizer, which was added as a pine bark compost in the present study. The C/N ratio in both soil treatments was similar (11.9), and it was appropriate for mineralization to occur. The lowest organic C and total N concentrations in the cultivated soil covered with 100% HDPE mulch could be explained by a faster mineralization rate due to possibly higher soil temperature and moisture content due to the mulching effect. The EC of 0.07 mS cm⁻¹ in the planting row with mulching, which was significantly lower than that of 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 of cultivated soil (5.7) did not differ significantly from that of bare soil (5.8) and was above the optimum range of values (4.0–5.5) for blueberry growth [24,26] but slightly lower than that of the CK soil (Table 1). Ochmian et al. [23] and Yang et al. [25] found that when the soil pH exceeds that range of values, 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 [26] confirmed that blueberry plants are very sensitive to the soil's pH and the N fertilizer source.

After 10 years of cultivation of blueberries using permeable green plastic (100% HDPE) mulch, concentrations of micronutrients in the soil at a 0–20 cm depth, i.e., extractable copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), and B, were lower than those in CK

(bare) soil, particularly Zn and Mn (Table 2). Nevertheless, in both soils, extractable Cu was in the medium range of values for agricultural soils, and extractable Fe was very high. The extractable Zn concentration in the control soil was in the medium range but decreased to a low value in cultivated soil with mulching. Extractable Mn was very low, which is good for agricultural purposes and, together with a 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 plants should be further monitored; furthermore, pH > 5.5 may induce leaf Fe chlorosis [27]. Finally, extractable B had a low concentration in the CK (bare) soil and decreased to a very low level in planting rows with plastic mulching in cultivated soil. 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 ($\text{pH}_{(\text{H}_2\text{O})} \leq 5.5$) with low B availability. According to the literature [28,29], 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 plants (leaf and fruit) is recommended.

In agreement with our hypothesis, the cultivated soil did not show contamination with di(2-Ethylhexyl) phthalate ($<2.7 \text{ kg DEHP kg}^{-1}$ dry matter) after using the green 100% HDPE mulch for a 10-year period, similarly to the control (bare) soil ($<2.8 \text{ kg DEHP kg}^{-1}$ dry matter). The type of mulch used was of long-life span and was not degradable for at least 10 years (the manufacturer reported a minimum UV stability of 5 years, i.e., a stabilizer was added to the material for protection from UV light degradation). This finding agrees with Pérez et al. [9], who found 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 the Soil's Enzymatic Activity

Dehydrogenases are intracellular oxidoreductase enzymes that are involved in microbial respiration metabolism. The activity of dehydrogenases is an appropriate, crucial, and responsive soil biological indicator that responds immediately to changes in soil systems, cropping, and conditions [30]. The soil dehydrogenase activity (DHA) has been recognized as an indicator of the metabolic status of the soil microbiota and of viable microbial activity [30]. DHA depends on the same factors that affect the abundance and activity of microorganisms. In the present study, DHA in soil collected at a 0–20 cm depth at the end of spring and after 10 years of cultivation of blueberry using a permeable green 100% HDPE mulch was significantly lower than that of the CK (bare) soil (Figure 2), thus aligning with the differences in organic C concentration in both soils. Soil organic C is a strong determinant of the soil's enzymatic activities, including dehydrogenase, because higher organic C represents more substrate for microbial energy with the consequent increase of microbial biomass and enzyme production [31]. The highest DHA in bare soil could also indicate a possible negative effect of the planting row covered with green plastic mulch on the overall soil microbial activity, thus agreeing with Moreno et al. [32] and their findings related to organic pepper cultivation using black PE film. They explained this effect with reference to a higher soil temperature and a certain reduction of gas interchange between the soil and the atmosphere under plastic mulching. However, the present plastic material allows some gas exchange. The possibility of other factors (e.g., pH and essential nutrient availability in the limited root zone) related to blueberry cultivation may negatively influence the soil's microbial activity and cannot be ruled out.

3.3. Effect of Long-Term Cultivation with Green HDPE Mulch on Nematode Communities in the Soil

Although this is a preliminary study, shifts in the nematode community after 10 years of cultivation of blueberry 'Centra Blue' in the open field covered with green 100% HDPE mulch were observed in comparison with the CK (bare) soil (Figure 3). The Rhabditida

(bacterial feeders) showed a four-fold increase in cultivated soil compared with the CK soil. This might be explained by the previous soil organic amendment with composted pine bark and possibly higher temperature and moisture caused by the plastic mulch. The presence of more bacterivorous nematodes in cultivated soil indicates that organic matter mineralization/nitrification was taking place, thus reducing the total organic C and total N in the 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 the large differences in the relative abundance of the two main trophic groups, the populations of fungivorous nematodes (*Aphelenchus* sp.) differed less between the two soil treatments, probably because this group of nematodes is less responsive to soil modifications [34]. Dorylaimida (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 negatively affect 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 [35,36]. Some authors have found that the nematode community is one of the best biological indicators [35–38].

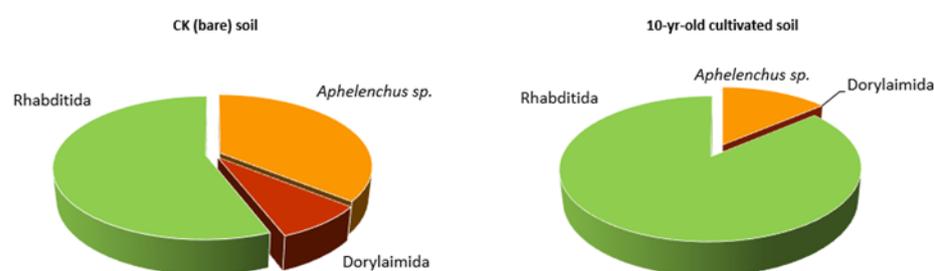


Figure 3. Relative proportion of nematodes per 100 mL of CK (control) soil (no mulching and no planting) and soil with 10 years of cultivation of blueberries ‘Centra Blue’ covered with green 100% HDPE mulch at the Fataca Experimental Farm at Odemira in the south of Portugal: *Aphelenchus* sp. = fungivorous;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

4. Conclusions

This is a former open-field study on the effects of long-term cultivation of perennial plant species, blueberry ‘Centra Blue’, covered with a permeable green high-density plastic mulch in a semi-arid environment in the south of Portugal. This study revealed that the present mulching system generated unique soil conditions mirrored by variations in specific soil parameters at a 0–20 cm depth. 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 this plastic mulching reduced the soil’s chemical quality in comparison with the control soil without planting, soil management, or mulching. The most relevant chemical indicators for land-use change, like the organic carbon and total nitrogen concentrations, pH, and electric conductivity, were significantly reduced in planting rows covered with high-density polyethylene mulch. Manganese and boron were depleted from cultivated soil, and their concentration in fruits should be further controlled because these micronutrients are important for blueberries’ nutrition.

The 100% HDPE mulch is a promising weed barrier as it conserves soil moisture and promotes plant growth and yield. This fabric mulch was not visually degraded after 10 years and did not produce macroplastic (visible) residues in the soil. The present findings highlighted, for the first time, that no relevant phthalate or heavy metals were released in covered soil with 100% HDPE mulch, thus showing its reduced soil contamination potential. This positive effect confirms our hypothesis, and this type of mulch can apparently be used in the long term without soil contamination.

The biological quality of the cultivated soil requires further development because the results of the soil’s enzyme activity and the nematode community evaluations were

apparently contradictory. On the one hand, the negative effect of blueberry cultivation with plastic mulching on the overall soil microbial activity indicates a shift in the soil's microbial communities, thus favoring groups that are less active in terms of oxidative metabolism. On the other hand, the rise in bacterivorous nematodes reflects an increase in the soil's bacterial populations, which are normally major contributors to soil microbiological activity. Clearly, more studies are needed to elucidate this situation.

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 bioindicators makes ecological sense as they represent a central position in the soil's food web and are related to ecological processes, such as organic matter mineralization/nitrification, farming practices, and plant growth.

Further long-term studies comparing different mulches and including biomaterials should be put in place to confirm the present findings, the presence of microplastics in the soil and in plants, and their 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 the ecosystem, climate, and soil practice (mulching).

Author Contributions: All authors contributed to this study under the field conditions and for manuscript preparation. Conceptualization of this study was performed by F.P., M.L.I., P.F., P.O., P.P. and C.C.; the methodology was planned by P.O. and C.C.; the formal analysis and measurements were carried out by F.P., M.L.I. and P.F.; writing the original draft preparation was performed by C.C., M.L.I. and P.F.; writing, reviewing, and editing were undertaken by all authors; and supervision was undertaken by C.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by *Plano de Recuperação e Resiliência (IE10 – Excelência da Organização da Produção)* through the project “*Agri-Plast-Organização e Inovação para a Redução de Plásticos Agrícolas*” [PRR-C05-i03-I-000167 LA10.2 and PRR-C05-i03-I-000167 LA10.5], and by *Fundação para a Ciência e a Tecnologia* (Portugal) through the R&D Unit “*GREEN-IT—Bioresources for Sustainability*” [UIDB/04551/2020, DOI: 10.54499/UIDB/04551/2020 and UIDP/04551/2020, DOI: 10.54499/UIDP/04551/2020] and LS4FUTURE Associated Laboratory [LA/P/0087/2020, DOI: 10.54499/LA/P/0087/2020].

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Acknowledgments: The authors acknowledge the Fataca Experimental Farm and BerrySmart company for supporting the experimental field work and materials.

Conflicts of Interest: The authors declare no conflicts of interest.

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