

## Article

# Employment of Biodegradable, Short-Life Mulching Film on High-Density Cropping Lettuce in a Mediterranean Environment: Potentials and Prospects

Marco Pittarello , Maria Teresa Rodinò , Rossana Sidari , Maria Rosaria Panuccio , Francesca Cozzi, Valentino Branca, Beatrix Petrovičová and Antonio Gelsomino \* 

Department of Agricultural Sciences, Mediterranean University of Reggio Calabria, Feo di Vito, 89124 Reggio Calabria, Italy; marco.pittarello@unirc.it (M.P.); mariateresa.rodino@unirc.it (M.T.R.); rossana.sidari@unirc.it (R.S.); mpanuccio@unirc.it (M.R.P.); cozzifrancesca1@gmail.com (F.C.); valentino.branca@unirc.it (V.B.); beatrix.petrovicova@unirc.it (B.P.)

\* Correspondence: agelsomino@unirc.it

**Abstract:** Biodegradable mulch films were developed over the last decades to replace polyethylene, but their short durability and higher costs still limit their diffusion. This work aimed to test an innovative composite mulching film constituted by a mixture of carboxymethyl cellulose, chitosan and sodium alginate, enriched or not with an inorganic N- and P-source to help the microbial breakdown in soil. The trial was carried out using outdoor mesocosms cultivated with lettuce plants with high-density planting. Commercial Mater-Bi<sup>®</sup> and a polyethylene film were taken as control treatments. Air temperature and humidity monitored daily during the 51 d cropping cycle remained within the ideal range for lettuce growth with no mildew or fungi infection. Visible mechanical degradation of the experimental biopolymers occurred after 3 weeks; however, Mater-Bi<sup>®</sup> and polyethylene remained unaltered until harvest. Chemical soil variables (TOC, TN, CEC, EC) remained unchanged in all these, whereas the pH varied. The yield, pigments, total phenols, flavonoids and ROS scavenging activity of lettuce were similar among treatments. Despite their shorter life service (~3 weeks), polysaccharide-based mulching films showed their potential to protect lettuce plants at an early stage and provide yield and nutraceutical values similar to conventionally mulched plants, while allowing a reduced environmental impact and disposal operations.

**Keywords:** bio-based polymers; chitosan; sodium alginate; carboxymethyl cellulose; lettuce; nutraceutical values; high-density planting



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## 1. Introduction

The mulching practice through plastic films in agriculture has been widely adopted all over the world over the past several decades: it helps increase soil temperature and stimulate soluble nutrient release, preserve soil moisture and reduce water loss, control weeds and protect soil from erosion [1]. The result is a higher crop yield [2].

In the European Union, approximately 1.74 million tons of plastics were used in 2018 for the agricultural sector, that is between 3 and 4 percent of the total European plastic converter demand of 51.2 million tons FAO [3]. The most used plastic polymer for soil mulching is black polyethylene (PE), which is considered the standard in agriculture due to its cheapness and good performance in terms of strength, transparency, insulation and water resistance. However, PE mulch films are not biodegradable, and due to the high cost

for their proper disposal, they are frequently being buried in soil after their life service has expired. Therefore, they remain in the environment for a time that largely exceeds a human life [4]. The soil burial of PE films brings about disintegration/fragmentation, the release of microplastics into environmental compartments (soil, water, air) and their consequent entering into the food chain which negatively affects not only soil quality and crop yield [5,6], but also animal and human health [7,8]. Zhang et al. [9] reported that in agricultural soil up to 9708 particles of plastics/kg of soil can be found. Furthermore, polyethylene fragments release phthalates [10] and contribute to the spread of pesticides into the soil [11]. Last but not least, being a low density PE and a non-renewable fossil-based industrial product, its use is rising and increasing public concerns for its wide impact on environmental resources [3].

To avoid the high costs of proper polyethylene disposal in terms of both time and expenses [12,13], and to make the burial of mulching films at the end of their life cycle feasible, while minimizing microplastics' accumulation in agricultural soils, biodegradable mulching films have been developing for many years. They can be buried safely because after the physical degradation/disintegration has taken place, the microbial soil community is able to fully biodegrade the residual products into harmless carbon dioxide, water and biomass [14].

The most common, and consequently the most easily available and cheap, naturally existing polymers are polysaccharides, widely present in plants and animals and frequently produced by algae and bacteria. In this class of naturally occurring biomolecules, it is possible to find chitin, cellulose and alginate: they consist of chains of monosaccharides linked by glycosidic bonds.

Carboxymethyl cellulose (CMC) is a water-soluble, non-toxic polymer obtained by the reaction between cellulose and chloroacetate, which is able to form films characterized by moderate physical strength and good flexibility; however, some researchers are concerned about the need to improve CMC mechanical properties by adding graphene [15,16].

Chitosan (CS) is a derivative of chitin, the second most abundant biopolymer in nature after cellulose, and it can form non-toxic, biodegradable and water-resistant films [17].

Sodium alginate (SA) is a biodegradable biopolymer extracted from algae [18]. Although sodium alginate films are water-soluble, this characteristic can be faded away by crosslinking with  $\text{CaCl}_2$ , making them usable as a component of mulching films [19].

Despite previous advantages, these biodegradable mulch constituents show sensitivity to moisture and brittleness due to their mechanical behavior related to water adsorption and retention [20]. Indeed, the strong presence of hydroxyl and carboxyl groups in CMC causes a great susceptibility to water [21], while CS films are affected by low mechanical strength [22].

The mechanical limits of CMC, CS and SA can be overcome by mixing them in proper ratios [23]. The combination CMC-CS positively affects tensile strength and water resistance [24]. Kulig et al. [25] reported an improvement in flexibility and the thermal stability by CS-SA blending. A further strategy to enhance the required polymers' mechanical properties is adding crosslinkers, such as  $\text{CaCl}_2$ , that act on the interconnections of non-covalent bonds and the entanglement of molecular chains, forming ionic bonds with the carboxylic groups ( $-\text{COO}^-$ ) of polysaccharides [26,27]. On the other hand, an excessive  $\text{CaCl}_2$  concentration causes an increase in the brittleness of the film [28]. To further enhance the structural flexibility and the mobility of polymers chains, plasticizers like glycerol are therefore recommended [29]. Unaffordable high costs are among the factors limiting the employment of biodegradable mulch films [30]. An example is given by one of the more successful biodegradable biopolymers, compliant to the European standard EN 13432 [31], known as Mater-Bi<sup>®</sup> (Novamont s.p.a, Novara, Italy). This polymer is resistant

to photodegradation, and, based on information released by the Italian patents database (<http://brevettidb.uibm.gov.it>, accessed on 26 May 2025), the main components of the Mater-Bi<sup>®</sup> formula are polymerized aliphatic and/or aromatic polyesters, at least a vegetal polyphenol and up to 10% in its weight of additives such as plasticizer agents, pigments and surfactants.

Depending on the country, amount and employment, Mater-Bi<sup>®</sup> plastic film can cost, in the European market, from 4.70 to 5.50 EUR/kg versus 0.5 to 1.5 EUR/kg of polyethylene. In the US market, depending on the roll dimension and film thickness, biodegradable mulch films cost between USD 212 and USD 250 per roll, whereas the cost of polyethylene mulches ranges from USD 135 to USD 154 per roll [13].

In order to achieve a decisive cost cut off, more simple production processes, including a low number of cheap and easily accessible materials, such as the CMC, SA and CS described above, together with the adoption of specific crop management practices can represent a more economically and technically feasible strategy among those being developed.

Indeed, although biodegradable mulch films must be strong enough to be laid out on the field, according to European standards EN 17033 [32], the requirement of a durability covering for the entire crop cycle could be not so necessary in some cases, opening the possibility of the employment of cheaper and more simple, in terms of components, short-life degradable mulch films for a specific step of a crop cycle and/or for short crop cycles.

The employment of a planting density higher than that commonly considered as optimal for lettuce cropping [33] is still debated as a possible way to grow several varieties of *Lactuca sativa* L. It is important to note that while Tarara [34] underlined the beneficial effects of less-dense plant spacing in terms of the quality of the product, Fekadu et al. [35] reported a higher fresh leaf yield per unit area, although the fresh leaf weight per plant decreased. Yet, Jenni et al. [36] found that the combination of higher density and white mulching increased the head weight, while the percentage of the marketable heads was like that of low-density cropping.

Given this premise, the focus of this study was to assess the performances of an innovative CMC-CS-SA mulch film, enriched or not with the monoammonium phosphate (MAP,  $\text{NH}_4\text{H}_2\text{PO}_4$ ) provided as a fertilizer/microbial activity primer, on soil, and plants' responses during an entire lettuce (*Lactuca sativa* L. var. *Romana*) crop cycle grown in soil mesocosms under field conditions. The two versions of the experimental mulcher (with and without MAP) evaluated only the mechanical resistance and the consequent effects on the mulching action. The experimental mulching film, named BPFM (BioPolymer for Mulching Films), was pre-tested in lab conditions showing fair/good performances related to EN 17033 standards [32]. It was then tested in field conditions in a Mediterranean environment without any crops, showing a 15/20 day long effective mulching action before a clear mechanical structural collapse as briefly reported in Materials and Methods. This work is embedded in a wider project focused on the effects of the degradation of the experimental bio-based mulcher once buried. The aspects related to the response of the microbial soil community and N and C cycling will be considered in future papers.

Based on the field pre-test performances, due to the simple composition of this polymer, including the absence of coloring, and its potential low cost once produced at an industrial scale, the main aim of this work was to test the effects on a lettuce yield. The nutraceutical quality of a short mulching action by a white biodegradable film on the early stages of lettuce growth was compared to a mulching action covering the entire crop cycle with black-colored Mater-Bi<sup>®</sup> and black-colored polyethylene (PE) mulching films in a Mediterranean environment, taking into account both the positive and negative effects of combined early mulching and high crop density.

## 2. Materials and Methods

### 2.1. Mulching Films

The experimental bio-based and biodegradable mulch film (BPMF) was prepared in the laboratory by the researchers from the University of Palermo (Italy) by using the solvent casting technique as reported by Ciaramitaro et al. [37]. Briefly, dispersion A (CMC) prepared by dissolving 6.75 g of CMC (1.5% *w/v*) in 450 mL of deionized water was mixed with dispersion B (CS/SA) prepared by dissolving 6.75 g of CS (1.5% *w/v*), 6.75 g of SA (1.5% *w/v*) and 9 g of glycerol (2.0% *w/v*; used as plasticizer agent) in 450 mL of a 2.0% *v/v* aqueous acetic acid solution. The CMC and the CS/SA suspensions were mixed at a 1:1 mass weight ratio, with the mixed solution gently poured over a flat and clean glass area and left drying at room temperature. The MAP-enriched bioplastic film (BPMF + MAP) was produced the same way as above, with the addition of 5.0 g of monoammonium phosphate (MAP) to the CMC dispersion (25% salt percentage relative to the 20 g mass weight of the dry polymer). This MAP addition, initially set at a 90% salt percentage, was then lowered to increase the tensile strength of the film. The thickness of the resulting film, albeit irregular, was approximately 50  $\mu\text{m}$ . No colorants were added. All the reagents used for BPMF preparation were purchased from Merck Life Science Srl (Milan, Italy).

The commercial biodegradable (according to DIN EN 17033:2018 [32]) and compostable (according to EN 13432 [31]) black-colored mulch film used in the mesocosm experiment (Mater-Bi<sup>®</sup>) was an ECOPAC BIO BLACK film (thickness 15–18  $\mu\text{m}$ ), kindly provided by Guarniflon SpA PATI division (San Zenone degli Ezzelini, Treviso, Italy), and it was made of Mater-Bi grade EF04P converted into a film with the addition of black masterbatch (FDM 324006 BK BIOE MASTERBATCH provided by AVIENT).

A commercial, black-colored non-degradable polyethylene (PE, 50  $\mu\text{m}$  thickness) mulch film was purchased from Si.Sac. S.p.a. (Ragusa, Italy). The film PE was removed at the end of the crop cycle without being buried into the soil.

### 2.2. Soil and Substrates

The soil used for the mesocosms' filling was a Calcaric-Fluvi Cambisol [38] collected from the surface (0–20 cm) Ap horizon of a citrus orchard located in the Sibari plain (Northern Calabria Region, sampling date October 2022). After coarse sieving (4 mm), soil was thoroughly mixed with commercial perlite (Agrilit<sup>®</sup>3, purchased from Perlite Italiana s.r.l., Milan, Italy) to prepare an 80/20 (*v/v*) soil/perlite mixture. Soon after mixing, the mixture was amended with an equivalent amount of 1 kg dry weight of municipal solid waste compost (TerrasanaBio<sup>®</sup>, Calabria Maceri S.p.A., Rende, Cosenza, Italy). Major characteristics of soil, perlite and compost are reported in Tables A1–A3, respectively (Appendix A). The choice to carry on the trial using outdoor mesocosms characterized by a soil type different from that of the experimental location was driven by the need to guarantee a good degree of soil fertility.

### 2.3. Plant Material

Lettuce (*Lactuca sativa* L. var. *Romana*) was purchased from Fratelli Ingegnoli (Milan, Italy), germinated and grown under greenhouse conditions for four weeks before transplanting.

### 2.4. Chemicals

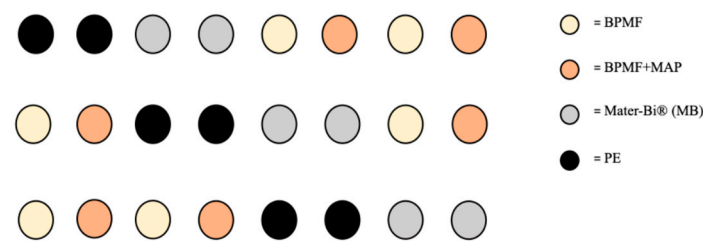
Analytical standards 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), rutin, gallic acid, DPPH and ethanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Folin–Ciocalteu's reagent, NaOH and AlCl<sub>3</sub> were purchased from Carlo Erba (Cornaredo, Milan, Italy). All chemicals were of analytical grade.

### 2.5. Mesocosms and Experimental Set-Up

The experimental plan consisted of twenty-four mesocosms filled with the compost-amended soil/perlite mixture and buried up to the top in three 40 cm deep dugouts, leaving their surface unburied. Outdoor mesocosms were located close to the Department of Agricultural Sciences at the Mediterranean University of Reggio Calabria (38°07'13.6" N, 15°40'10.3" E, 150 m a.s.l.). The experimental site was surrounded by a protective enclosure to avoid wild animal grazing. The experimental area was 24 m<sup>2</sup> wide, and the buckets were placed in 3 lines of eight buckets each. The buckets were distanced at 30 cm from one another.

The experimental design was built by dividing the twenty-four buckets in four theses characterized by two versions of BPFM and the other two plastic mulchers, with six replicates for each one: (1) white BPFM; (2) white BPFM + MAP; (3) black Mater-Bi® (MB); (4) conventional non-degradable black polyethylene (PE), which was considered the control because of its well-known performances in several decades of employment.

The theses replicates were distributed through a semi-randomized geometric distribution as shown in Figure 1.



**Figure 1.** Experimental design: bucket placement and theses distribution.

The pre-trial focused on the mechanical resistance of BPFM was carried on as follows: the BPFM disks (six added with MAP and six without MAP) were placed on the top of the bucket covering the entire mesocosm surface. The test was 8-months long, crossing all the seasons from May 2023 to January 2024, and included three consecutive mulching cycles: at the end of each one, the films were buried, and after 1 week, they were replaced with new ones. The remaining twelve buckets were mulched with six MB and six PE disks, in agreement with the experimental design, to avoid different management before the beginning of the main experiment. Each mulching cycle was on average 70 days  $\pm$  7. The water supply consisted of 5 L of tap water per bucket every 20 days plus rainfall.

Differently from PE and MB, BPFM and BPFM + MAP, both in cold and hot seasons, started quickly to show physical changes in two steps: the quick water accumulation and loss led at first from a white/semi-transparent to an opaque white color, then to a crinkling of the structure followed by a breakage (Figure 2). Therefore, the phenomenon must be intended not as chemical or bio-chemical degradation but only as a result of mechanical stress. The entire process made clear the inefficacy of the mulching action after about 20 days.

### 2.6. Mesocosm Soil Analysis

On 10 April 2024, five days before the start, and on 5 June 2024, the day of the end of the main trial, twenty-four composite soil samples, each consisting of three samples pooled together, were surface (0–10 cm) collected per mesocosm, air dried, sieved at <2 mm and then stored before analysis.

The soil texture was determined by the Bouyoucos method [39], while the chemical properties were determined as described in [40] (Appendix A, Tables A1 and A2): the pH was potentiometrically measured in a 1:2.5 (*w/v*) soil-to-0.01 M CaCl<sub>2</sub> solution mixture

(pH); electrical conductivity was measured at 25 °C in a 1:2 (*w/v*) soil-to-water ratio ( $EC_{1:2}$ ). Total organic C (TOC) and N (TN) were analyzed by an elemental analyzer LECO CN628 (LECO Corporation, St. Joseph, MI, USA). TOC/TN provided the C/N ratio (C/N). The cation exchange capacity (CEC) was determined accordingly by the Mehlich method [41].



**Figure 2.** The BPMF mechanical collapse process: at the beginning of the pre-trial (a) and after 3-week exposure to field conditions (b).

### 2.7. Main Experiment

The trial started on 15 April 2024: in the twenty-four buckets, seventy-two 30-day old lettuce seedlings were transplanted, that is three seedlings per mesocosm, 15 cm apart from each other and 10 cm apart from the bucket border to avoid potential leaves burning and guarantee the highest feasible plant density that resulted in 19 seedling/m<sup>2</sup>. After those twelve BPMF disks, six BPMF and six BPMF + MAP were drilled with three 6 cm diameter holes according to the seedling's placement.

Six Mater-Bi<sup>®</sup> disks of the same size of BPMF were cut out and drilled in correspondence to the seedling's position. The same procedure was carried out to obtain six black-colored 50 µm-thick polyethylene drilled disks. All the mulching film disks were placed on the same day as the seedling transplanting.

All these were irrigated with 5 L of tap water two times a week, and supplied two times during the entire crop cycle (on 29 April and 16 May 2024) with 2 g per plant of a commercial Ca(NO<sub>3</sub>)<sub>2</sub> (1.2% NH<sub>4</sub><sup>+</sup>-N, 14.4% NO<sub>3</sub><sup>-</sup>-N, 26.6% CaO) (Agrisol, Siriac, Ragusa, Italy), with inorganic fertilizers supplying in total 0.048 g of NH<sub>4</sub><sup>+</sup>-N, 0.560 g of NO<sub>3</sub><sup>-</sup>-N and 0.761 g of Ca per lettuce plant.

On 19 May 2024, an antifungal treatment (propamocarb hydrochloride) was supplied by spraying Previcur Flex (Bayer) both on the soil and leaf surface.

During the field trial, the air temperature and humidity of the site were recorded daily through the meteorological station located at San Brunello in Reggio Calabria, Italy (<https://wheather.com>).

On 3 June 2024, the lettuce was harvested, leaving in the soil only the root systems to avoid any unnecessary perturbation to the mesocosms. For each bucket, all three plants were immediately weighed to record the fresh weight. One plant per bucket was oven dried at 60 °C for 48 h to record the dry weight. From the remaining two plants, three or four intermediate leaves, avoiding the oldest and the youngest ones, were stored at −20 °C for further analysis.

### 2.8. Chlorophylls and Carotenoids' Determination

Chlorophylls and carotenoids were determined as described in Papalia et al. [42]. Briefly, after a gentle crumbling in small pieces, 50 mg of fresh leaf tissue was mixed with 2.5 mL of absolute ethanol and left in the dark at 4 °C for 24 h. After that, the samples were centrifuged for 10 min at 7000× *g*. Chlorophyll a and b, and carotenoids' content were determined spectrophotometrically at 649, 665 and 470 nm, respectively, employing Lichtenthaler's equation. Absorbance, like for all of the following colorimetric assays, was taken by a double ray spectrophotometer Perkin Elmer Lambda 35 (Perkin Elmer, Shelton, CT, USA). Chlorophylls and carotenoid content were expressed as mg kg<sup>-1</sup> FW.

### 2.9. Determination of Total Phenols, Total Flavonoids and Radical Scavenging Activity

Ethanolic extracts were prepared according to Muscolo et al. [43] with the following modifications: frozen (−20 °C) pieces of lettuce leaves were dehydrated for 96 h through a freeze dryer Alpha 1-2 LDplus (Martin Christ GmbH, Osterode am Harz, Germany), and then were gently (to avoid increasing the temperature and phenols' degradation) crumbled. An amount of 500 mg of each sample was extracted with absolute ethanol (10 mL) (1:20, *w/v*) under continuous stirring at room temperature for 90 min. The process was repeated once. Pooled extracts were centrifuged at 2365× *g* for 15 min, and the supernatants were filtered with Whatman™ n. 42 filter papers. The final volume of the two extractions was stored at −20 °C.

The total phenols were spectrophotometrically determined following the Folin–Ciocalteu assay with some modifications: in a 5 mL Eppendorf tube, 2.5 mL of ultrapure deionized water, 1 mL of extract, 500 µL of Folin–Ciocalteu reagent and last, to start the reaction, 1 mL of 0.33 M NaOH were added. The mixture was left to incubate at room temperature in the dark for two hours. The absorbance of the samples was recorded at 725 nm. A calibration curve was constructed with gallic acid, and the results were expressed as µg of gallic acid equivalent (GAE) g<sup>-1</sup> DW.

The total flavonoid content in extracts was measured spectrophotometrically by mixing 1 mL of extract with 1 mL of 0.15 M AlCl<sub>3</sub> ethanolic solution, as described in [43]. After 15 min of incubation at room temperature, the absorbance was measured at 430 nm. The flavonoid content was calculated from a calibration curve of rutin and expressed as µg rutin equivalent (RUE) g<sup>-1</sup> DW.

The DPPH radical scavenging assay was performed following the method described by Muscolo et al. [43] with some modifications: the DPPH concentration in the cuvette was chosen to give absorbance values close to 1.0. In a 5 mL Eppendorf tube, 100 µL of ethanolic extract was added to 2.9 mL of 0.063 M DPPH. Immediately after the extract addition, the reaction mixture absorbance was spectrophotometrically measured at 517 nm: this value worked as the “control”, and it was thought to reduce as much as possible the differences in composition between the control and incubated mixture in comparison with a pure DPPH solution. After 30 min of incubation at room temperature in the dark, a second absorbance reading was taken at 517 nm. The inhibition *I* (%) of radical scavenging activity was calculated as  $I (\%) = [(A_0 - A_S) / A_0] \times 100$ , where *A*<sub>0</sub> is the absorbance of the control (value at *T*<sub>0</sub>) and *A*<sub>S</sub> is the absorbance of the sample after 30 min of incubation. Results were expressed as µmol of the Trolox equivalent (TE) g<sup>-1</sup> DW.

### 2.10. Statistics

A Shapiro–Wilk normality test and Levene's test for the homogeneity of variance were performed. Depending on the test results, a one-way ANOVA plus Tukey *post hoc* test or Welch one-way ANOVA plus Games–Howell *post hoc* test was applied to the plant parameters' dataset. The soil dataset corresponding to the beginning and the end

of the mesocosm trial was analyzed with a *t*-test for independent samples to compare each soil parameter at the beginning and at the end of the experiment, while potential differences among the theses within the same sampling time were analyzed through a one-way ANOVA. All analyses were performed by the open-source software Jamovi 2.5.1 (the Jamovi project, 2024).

### 3. Results

#### 3.1. BPFM Degradation

Differently from polyethylene and Mater-Bi<sup>®</sup> mulch films (Figure 3A,B), BPFM and BPFM + MAP started quickly to change color, from a semi-transparent white to an opaque white after the first irrigation and/or the rainfall event. The rapid imbibition and the following water loss caused a partial crumpling of the film structure, in agreement with the high degree of swelling found by Ciaramitaro et al. [37], progressively reducing the covered soil area. Indeed, an acceptable mulching function was maintained until day 22 of the lettuce life cycle, when seedlings suddenly accelerated their growth to cover the entire bucket surface in a few days, replacing the BPFM as the “auto-mulcher”. The crushed BPFM structure finally started to fragmentate itself, favoring the final fragments’ burial. On the contrary, both MB and PE (Figure 3C,D) maintained their unaltered color and mechanical properties. MB only apparently showed a crumpling phenomenon, due to its lower thickness (15 µm vs. 50 µm) and weight and the windy condition of the experimental site. No fragments were lost by MB and PE during the experimental period.



**Figure 3.** Different stages of the lettuce during the growing cycle and the mulch film physical degradation process. Treatments are as follows: (A) BPFM; (B) BPFM + MAP; (C) MB; (D) PE. The red line in (A,B) indicates the soil area remained covered by the mulching film.

### 3.2. Soil Chemical Variables

Total organic carbon (TOC), total nitrogen (TN) and, consequently, the C/N ratio (Table 1) remained stable across the experimental period. In comparison with the soil not supplied with perlite and compost (Table A1), the mesocosm data showed a depletion in TOC and TN probably due to the perlite addition and consequent increase of volume, and the mechanical harvest of some weeds during the period October 2022–May 2023, with the removal of some nutrients. Furthermore, the soil disturbance during the sampling and mesocosm loading could have increased the C and N mineralization process, thus priming the organic matter mineralization process.

**Table 1.** Variations in major chemical (TOC, TN, C/N, pH, EC<sub>1:2</sub>, CEC) properties (mean ± standard deviations,  $n = 3$ ) of mesocosm soils under differing treatments (BPMF, BPMF + MAP, MB and PE as in M&M) at the beginning ( $T_b$ ) and at the end ( $T_e$ ) of the lettuce growing cycle.

Thesis	TOC		TN		C/N	
	$T_b$	$T_e$	$T_b$	$T_e$	$T_b$	$T_e$
	$\text{g kg}^{-1}$					
BPMF	18.50 ± 1.12	18.55 ± 1.94	1.99 ± 0.08	1.94 ± 0.24	9.30 ± 0.22	9.56 ± 0.59
BPMF + MAP	17.92 ± 1.26	17.82 ± 1.16	1.87 ± 0.13	1.86 ± 0.14	9.58 ± 0.29	9.58 ± 0.35
MB	17.72 ± 1.57	17.28 ± 0.64	1.81 ± 0.12	1.78 ± 0.09	9.77 ± 0.27	9.68 ± 0.23
PE	17.86 ± 2.09	16.57 ± 0.51	1.86 ± 0.25	1.63 ± 0.15	9.57 ± 0.23	10.10 ± 0.85
	pH		EC <sub>1:2</sub>		CEC	
	$\mu\text{S cm}^{-1}$					
	$\text{cmol kg}^{-1}$					
BPMF	7.05 ± 0.07 <sup>b</sup>	7.79 ± 0.05 <sup>a</sup>	319.1 ± 60.2	287.1 ± 47.1	19.74 ± 7.36	24.72 ± 6.36
BPMF + MAP	7.20 ± 0.12 <sup>b</sup>	7.88 ± 0.06 <sup>a</sup>	260.4 ± 50.3	286.3 ± 36.6	18.52 ± 7.17	25.11 ± 4.15
MB	7.28 ± 0.04 <sup>b</sup>	7.92 ± 0.04 <sup>a</sup>	266.5 ± 51.4	279.4 ± 34.8	23.21 ± 4.98 <sup>b</sup>	28.20 ± 0.75 <sup>a</sup>
PE	7.23 ± 0.09 <sup>b</sup>	7.82 ± 0.12 <sup>a</sup>	282.4 ± 69.5	248.7 ± 36.8	22.23 ± 5.62	24.82 ± 5.45

When present, different letters within each treatment indicate significant differences among sampling times (Tukey's HSD at  $p < 0.05$ ).

The electrical conductivity (EC) and the cation exchange capacity (CEC) (Table 1) did not show any significant difference between the beginning ( $T_b$ ) and the end of trial ( $T_e$ ), whereas pH showed a slight increase, probably due to the Ca contained in the fertilizer and added two times during the experiment. No significant differences were found among the theses within the same sampling time, confirming no roles of different mulch films in soil conditioning during the crop cycle.

### 3.3. Plant Yield and Biochemical Parameters

All the 72 plants grew in optimal environmental conditions in terms of air temperature and air humidity (Figures 3, A1 and A2) that were recorded in a range between 9 °C at 8:00 p.m. at the end of April and a peak of 27 °C recorded on May 18 at 2:00 p.m. However, the maximum air temperature remained below 25 °C for the greater part of the crop cycle. Air humidity, although it showed negative peaks around 40%, stayed for the great majority of the days in the range of 60–80%.

Among the plant parameters, chlorophyll a and b, total phenols and radical scavenging activity (Table 3) did not show significant differences among the theses. Moreover, MB showed the lowest fresh weight (FW), while there were no differences among BPMF, BPMF + MAP and PE (Table 2). No differences in dry weight were found (Table 2). On the opposite end, MB showed a higher value in carotenoids and flavonoids, whereas there were no differences in lettuce plants grown under BPMF, BPMF + MAP and PE (Table 3).

**Table 2.** Variations in fresh and dry weight (mean  $\pm$  standard deviations,  $n = 3$ ) of lettuce plants grown in mesocosm soils under different mulching conditions (BPMF, BPMF + MAP, MB and PE as in M&M).

Thesis	Fresh Weight	Dry Weight
	G	
BPMF	371.31 $\pm$ 112.52 <sup>a</sup>	23.56 $\pm$ 9.10
BPMF + MAP	296.57 $\pm$ 113.51 <sup>ab</sup>	21.82 $\pm$ 7.22
MB	209.23 $\pm$ 51.12 <sup>b</sup>	23.51 $\pm$ 11.89
PE	247.33 $\pm$ 69.38 <sup>ab</sup>	23.87 $\pm$ 5.61

When present, different letters in a column indicate significant differences among treatments (Tukey's HSD at  $p < 0.05$ ).

**Table 3.** Variations in biochemical parameters (mean  $\pm$  standard deviations,  $n = 3$ ) of lettuce plants grown in mesocosm soils under different mulching conditions (BPMF, BPMF + MAP, MB and PE as in M&M).

Thesis	Chlorophyll a	Chlorophyll b	Carotenoids	Total Phenols	Total Flavonoids	Radical Scavenging Activity
		mg kg <sup>-1</sup> FW		$\mu$ g GAE g <sup>-1</sup> DW	$\mu$ g RUE g <sup>-1</sup> DW	$\mu$ mol TE g <sup>-1</sup> DW
BPMF	36.33 $\pm$ 11.02	18.71 $\pm$ 9.48	4.84 $\pm$ 1.14 <sup>b</sup>	141.20 $\pm$ 28.40	1.72 $\pm$ 0.39 <sup>ab</sup>	0.55 $\pm$ 0.02
BPMF + MAP	35.60 $\pm$ 12.91	17.43 $\pm$ 9.04	4.31 $\pm$ 1.33 <sup>b</sup>	126.55 $\pm$ 47.53	1.56 $\pm$ 0.68 <sup>b</sup>	0.51 $\pm$ 0.05
MB	40.50 $\pm$ 8.67	17.42 $\pm$ 3.78	7.16 $\pm$ 0.71 <sup>a</sup>	190.15 $\pm$ 32.13	2.54 $\pm$ 0.49 <sup>a</sup>	0.51 $\pm$ 0.05
PE	31.15 $\pm$ 4.16	13.44 $\pm$ 1.63	5.54 $\pm$ 0.79 <sup>ab</sup>	165.39 $\pm$ 26.37	2.08 $\pm$ 0.26 <sup>ab</sup>	0.53 $\pm$ 0.02

When present, different letters in a column indicate significant differences among treatments (Tukey's HSD at  $p < 0.05$ ).

## 4. Discussion

### 4.1. BPMF Degradation and Soil Parameters

The experimental mulching film showed the well-known problems of each component [20–24]. Nevertheless, the rapid opacifying behavior, once wet, and the conservation of the structure integrity, although the beginning of crinkling, allowed for the protection of the seedlings from weeds during the early stages of growth.

Despite the differences in mulching films' soil coverings, the main soil variables remained stable in all four theses in the short period (~4 weeks) corresponding to the crop cycle, except for the soil reaction, which was probably affected by the compost alkaline reaction. Indeed, the pH increase was roughly +0.60 for all the treatments, achieving values close to that of the compost (Appendix A, Table A3). Compared with the pristine agricultural soil characteristics, in mesocosm soils, only TOC lowered from 2.8 to 1.8%, but remained, like other parameters, in a range typical of a good agricultural cambisol/luvisol [44–46].

### 4.2. Yield and Nutraceutical Parameters

Plants in field conditions grew in similar ranges of temperature and humidity as those settled in the greenhouse by Sytar et al. [47]: 18–21 °C with 60% humidity. The fresh weights of our high-density experimental design (19 plants/m<sup>2</sup>) agreed with those recorded by Yordanova and Nikolov [48], who carried a trial with 16 plants/m<sup>2</sup>; whereas, the fresh weight values were lower than the 700 g reported by Saleh et al. [49] with a planting density of 8 plants/m<sup>2</sup>. Fekadu and coworkers [35] found that a higher planting density increased the total fresh leaf yield per unit area but reduced the fresh leaf weight per plant: the highest fresh leaf yield was obtained at 12.5 plants/m<sup>2</sup> from the Great Lakes variety, while the Rsk-3 variety recorded its highest fresh leaf yield at 16.7 plants/m<sup>2</sup>. This finding indicates that, depending on the variety, employing a higher density than that considered the ideal could be a feasible solution. Furthermore, the dry weights were in

line with those reported by Riga and Benedicto (2017) [50] who, although using a different variety, found a range of dry biomass of 18–28 g kg<sup>-1</sup> with a water loss close to 95% (*w/w*). The differences among the theses were significant only in fresh weights and only between BPFM and MB, but the interpretation of these data was rather difficult considering there were no differences among the BPFM + MAP and the other comparative mulches, nor between BPFM and BPFM + MAP. Furthermore, dry weights confirmed that the whole growth performances of all tested plants were similar.

Carotenoids and polyphenols' content results were, respectively, up to ten times and fifty times lower than those recorded by Kim et al. [51] in lettuce var. *Romana*; whereas, Cano and Arnao [52] found a polyphenols' concentration roughly ten times higher than our one (their results were expressed in mg per g of fresh weight instead of dry weight). On the opposite end, other experiments conducted in microcosms under greenhouse conditions and hydroponics showed, when compared with our findings, average values ten to twenty times lower in chlorophylls [53] and five times lower in chlorophyll a, up to 100 times lower in chlorophyll b and up to twenty times lower in carotenoids [54]. Kang and Saltveit [55] found in lettuce var. *Romana* a polyphenol content roughly 70 times lower than that found in our experiment, even though their analyses involved lettuce plants taken from a commercial source, a fact that could not exclude an ongoing initial phenols' degradation.

The radical scavenging activity was low in all the theses without any differences: Kang and Saltveit [55] reported values 2.5 times higher in percentage compared to the control in leaves subjected to heat shock, while Liu et al. [56] showed a percentage of the DPPH radical quenched around the 70% for lettuce var. *Romana* that is in agreement with our results (60% in average: data shown as  $\mu\text{mol}$  equivalents of Trolox). Mahmoudi et al. [57] reported, under hydroponics conditions, a total flavonoid content about forty times higher than that found in our experiment and, like carotenoids, it increased in the case of NaCl toxicity.

The similar values among the theses in chlorophylls, total phenols and radical scavenging activities with slight, although significant, differences in flavonoids and carotenoids between MB and the other theses indicate a substantial absence of stressing conditions and micro-environmental differences among BPFM, BPFM + MAP and PE, while MB plants could have responded to a light heat stress due to the lower fresh weight that means lower water accumulation.

Furthermore, the lack of differences in yield in terms of dry weight suggested the following: (i) the different mulching films did not substantially affect the crop cycle; (ii) the high plant density could have led to lower nutraceutical parameters. Indeed, carotenoid biosynthesis depends on light intensity, and differences in carotenoid contents among lettuce varieties could be related to the head structure [58,59]. Although the Romaine variety is a loose-leaf head that allows for an easy light penetration, the high density of our trial could be the reason for the non-optimal light catching and the consequent low carotenoids and, probably, phenols' concentrations. It needed to take into account that the literature gives contradicting results depending not only on the differences among cultivars and sub-varieties, but also on the different experimental conditions. (iii) The mesocosm trial showed that, under Mediterranean conditions not needing a strong soil warm-up, a short-life (~3 weeks) bio-based mulching film can guarantee seedlings at early stages of their development sufficient protection against weeds and a regulation of temperature at the soil surface due to the imbibition of the composite CMC-CS/SA mulching polymer with reduced water loss through evapotranspiration; (iv) the air humidity range was lower than the 98–100% considered the optimum for fungi *Sclerotinia sclerotiorum* proliferation as reported by Clarkson et al. [60], and no mildews were found in this experiment, suggesting

that the Mediterranean climate allows high-density lettuce planting with a reduced risk of fungal diseases linked to high air humidity.

## 5. Conclusions

Agricultural mulching is an important practice to guarantee protection against weed competition, a stable upper soil temperature and to avoid excessive soil water loss. PE and MB-based polymers help achieve these targets. However, the first one is fossil-derived, non-biodegradable and non-renewable, thus requiring an additional cost for proper disposal after its life service. The second one is biodegradable and compostable and it can be soil-incorporated at the end of its life cycle; however, it is also more expensive, as most biodegradable mulches are.

The BPMF, constituted of a reduced number of easily available and economically affordable components, effectively maintained its mulching action for the first 3 weeks of the lettuce life cycle. Despite the different plastic color (milky white vs. black), it did not negatively affect the crop yield and the nutraceutical values of lettuce plants compared to those treated with Mater-Bi<sup>®</sup> and PE.

In a Mediterranean environment, the lettuce var. *Romana* can grow in an ideal range of air temperature and air humidity, and, under a regular water supply, a high crop density helps plants to control weed competition in the second half of their life cycle when the mulching film has become ineffective. Furthermore, irrespective of the high planting density, no fungi and mildews attacks were found.

Due to contradicting results found in the literature about the crop density and nutraceutical values of the lettuce, further investigation is required to refine this potential agricultural strategy that could give access to cheap and less durable biodegradable films to a new market niche and introduce a win–win strategy in intensive horticulture, namely, cutting down expenses for Mater-Bi<sup>®</sup> purchase and PE disposal without negatively affecting the environmental quality and soil health.

Despite their service life being shorter (~3 weeks) than that of the commercial mulching films actually on the market, the innovative composite polysaccharide-based mulching films have shown to promote comparative yields and competitive nutraceutical features when tested in a lettuce cropping system; therefore, they offer great potential for further developments.

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## Abbreviations

The following abbreviations are used in this manuscript:

BPMF	Biopolymer for mulching films
MAP	Monoammonium phosphate
MB	Mater-Bi®
PE	Polyethylene
CMC	Carboxymethyl cellulose
CS	Chitosan
SA	Sodium alginate
DPPH	$\alpha,\alpha$ -diphenyl- $\beta$ -picrylhydrazyl
TOC	Total organic carbon
CEC	Cation exchange capacity
EC	Electrical conductivity
TN	Total nitrogen

## Appendix A

**Table A1.** Major physicochemical properties of the Calcaric-Fluvi Cambisol used for preparing the 80/20 (*v/v*) soil/perlite mixture loaded in the mesocosms.

Soil Variable	Value
Sand ( $\text{g kg}^{-1}$ )	324
Silt ( $\text{g kg}^{-1}$ )	651
Clay ( $\text{g kg}^{-1}$ )	25
Texture (according to USDA)	Silt loam
pH <sub>CaCl2</sub>	7.15
Total CaCO <sub>3</sub> ( $\text{g kg}^{-1}$ )	52.6
Active CaCO <sub>3</sub> ( $\text{g kg}^{-1}$ )	13.3
EC <sub>1:2</sub> ( $\mu\text{S cm}^{-1}$ )	291.50
TOC ( $\text{g kg}^{-1}$ )	28.83
TN ( $\text{g kg}^{-1}$ )	2.19
C/N	13.2
CEC ( $\text{cmol kg}^{-1}$ )	20.4
Ca <sup>2+</sup> ( $\text{mg kg}^{-1}$ )	3100
Mg <sup>2+</sup> ( $\text{mg kg}^{-1}$ )	390
K <sub>exch</sub> ( $\text{mg kg}^{-1}$ )	564
Na <sup>+</sup> ( $\text{mg kg}^{-1}$ )	52
Ca/Mg	4.8
Mg/K	2.2
Base saturation (%)	99.6

**Table A2.** Major mineralogical and physicochemical properties of the Agrilit®3 (Perlite Italiana Srl, Milan, Italy) used for preparing the 80/20 (*v/v*) soil/perlite mixture loaded in the mesocosms.

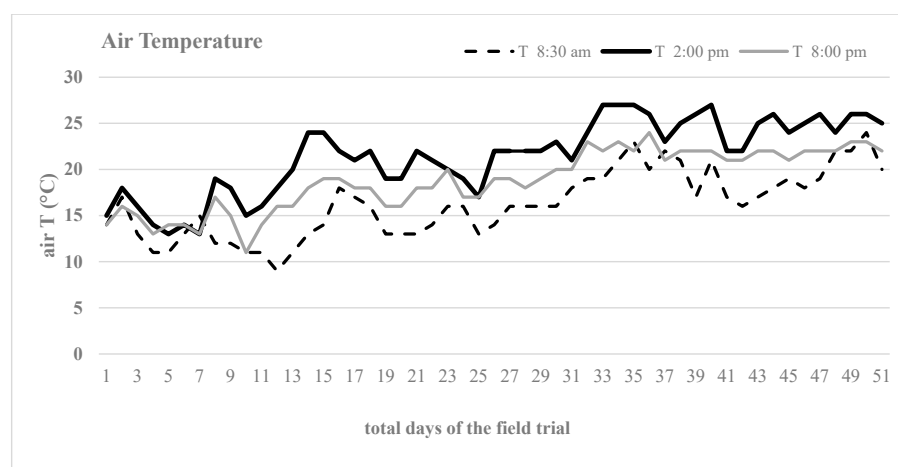
Variable	Value
<i>Mineral components</i>	
SiO <sub>2</sub> (%)	74–78
Al <sub>2</sub> O <sub>3</sub> (%)	11–14
Fe <sub>2</sub> O <sub>3</sub> (%)	0.5–1.5
Na <sub>2</sub> O (%)	3–6
K <sub>2</sub> O (%)	2–4
CaO (%)	1–2

Table A2. Cont.

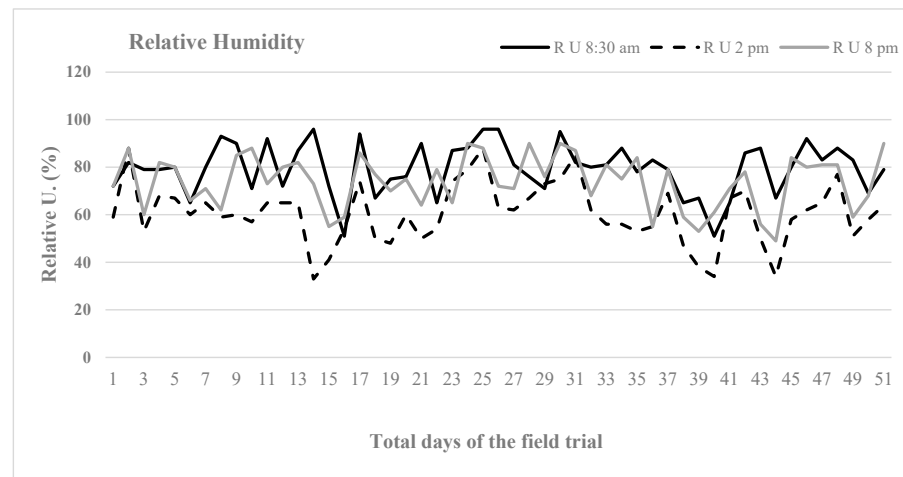
Variable	Value
MgO (%)	0–0.5
<i>Physical properties</i>	
Particle size (mm)	2–6
Bulk density ( $\text{kg m}^{-3}$ )	$(70\text{--}90) \pm 10$
pH <sub>H2O</sub>	6.5–7.0
EC ( $\mu\text{S cm}^{-1}$ )	20
CEC ( $\text{cmol kg}^{-1}$ )	0.79
Total porosity (% <i>v/v</i> )	95.88
Air porosity (% <i>v/v</i> )	80.20
Water-holding capacity (% <i>v/v</i> )	15.70
Easily available water (% <i>v/v</i> )	1.46
Available water (% <i>v/v</i> )	0.91
Unavailable water (% <i>v/v</i> )	13.33
Internal drainage ( $\text{cm s}^{-1}$ )	>1

**Table A3.** Chemical, biochemical and microbiological properties of the compost obtained after a 3-month accelerated composting process of municipal solid waste (Calabra Maceri & Servizi S.p.a., Rende, Cosenza, Italy). The composted material was used for amending the soil/perlite mixture during the mesocosms' filling.

Variable	Value
pH <sub>H2O</sub>	$8.5 \pm 0.6$
EC <sub>1:10</sub> ( $\mu\text{S cm}^{-1}$ )	$370 \pm 40$
TOC ( $\text{g kg}^{-1}$ )	$220 \pm 20$
TN ( $\text{g kg}^{-1}$ )	$19 \pm 2$
C/N	$12 \pm 2$
C <sub>humic+fulvic</sub> (%)	$8.4 \pm 1.0$
Germination index (%)	$93 \pm 10$
Cd ( $\text{mg kg}^{-1}$ )	$0.63 \pm 0.18$
Cr <sub>VI</sub> ( $\text{mg kg}^{-1}$ )	<0.1
Hg ( $\text{mg kg}^{-1}$ )	$0.19 \pm 0.07$
Ni ( $\text{mg kg}^{-1}$ )	$28 \pm 5$
Pb ( $\text{mg kg}^{-1}$ )	$32 \pm 10$
Cu ( $\text{mg kg}^{-1}$ )	$81 \pm 14$
Zn ( $\text{mg kg}^{-1}$ )	$221 \pm 49$
<i>Salmonella</i> (MPN 25 $\text{g}^{-1}$ )	absent
<i>Escherichia coli</i> (CFU $\text{g}^{-1}$ )	<100



**Figure A1.** Trend of air temperature recorded at three moments during the day across the lettuce crop cycle (15 April 2024–3 June 2024).



**Figure A2.** Trend of relative humidity recorded at three moments during the day across the lettuce crop cycle (15 April 2024–3 June 2024).

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