Agronomic and environmental benefits of 're-using' a biodegradable mulching film for two consecutive lettuce cycles

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Highlights

- *Biodegradable mulching films (MB) showed good mechanical resistance in the medium-long term (2 consecutive lettuce cycles).*
- *Decrease in integrity and resistance to tearing became significant after 150-170 days.*
- *The effect of MB on lettuce yield quantity and quality was comparable with that using low-density polyethylene (LDPE) films.*
- *The effects of both mulching films on leaf nitrate content need further research in different pedoclimatic conditions.*
- *MB can be recommended since it reduces the economic and environmental costs of removal and disposal of LDPE films.*

Abstract

Biodegradable films are a valuable and sustainable alternative to plastic films for mulching soils since they avoid the environmental and economic problems related to plastic removal and disposal. Nevertheless, the fast degradation of such materials could make them unsuitable for mid- to long-term use. In a field experiment, the agronomic performance of a biodegradable mulching film (MB) was compared to that of conventional low-density polyethylene (LDPE) film for two consecutive lettuce cycles (winter and spring). In the conditions of this trial, MB showed good resistance to atmospheric agents, with a reduction of its integrity and mechanical properties only after six months. The effects on soil temperature and lettuce yield did not differ from those obtained with LDPE films. The effect on harvest timing was the same as that with LDPE in the spring cycle, while in the winter cycle, the harvest was delayed by about five days compared to LDPE. Mulching films reduced nitrate accumulation in leaves mainly during the winter cycle. However, the effect needs to be further explored with experiments in different pedoclimatic conditions that consider the effects of mulching on nitrification and nitrate-reductase activity that could be affected by changes in soil temperature and moisture.

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Key words: Mulching; biodegradable film; biodegradable film lifetime; polyethylene; lettuce; yield.

Acknowledgements: this work was financed by the project MIUR PON01_1966 'EnerbioChem' (Integrated agro-industrial chains with high energy efficiency for the development of eco-compatible processes of energy and biochemical production from renewable sources and for land improvement). We would like to thank Sabrina Nocerino and Roberto Maiello for their support in laboratory work.

Received for publication: 9 February 2022.
Revision received: 7 July 2022.
Accepted for publication: 11 July 2022.

Italian Journal of Agronomy 2022; 17:2061
doi:10.4081/ija.2022.2061

Introduction

The widespread use of plastics in many fields of human life, also thanks to the constant innovations of polymeric technology, explains their continuously growing production, which averaged almost 10% per year on a global basis, rising from around 1.3 million tons in 1950 to 367 million tons in 2020 (https://plasticseurope.org/).

Global consumption of plastics in agriculture hovers at around 6.5 million tons per year (Scarascia-Mugnozza *et al*., 2011). In the European Union (EU) in 2014, agricultural uses accounted for around 3.4% of plastics (Cassou, 2018), comprising greenhouse and tunnel covers and soil mulching, irrigation and drainage pipes, silage films, and other products (Vox *et al*., 2016).

The agricultural practice of mulching is widespread because it allows an increase in root zone temperature, influences physiological processes such as water and nutrient uptake (Ibarra-Jimenez *et al*., 2011; Diaz-Perez & Batal, 2002; Dodd *et al*., 2000), reduces evaporation losses (Wang *et al*., 2011), boosts productivity and qualitative crop performance, reduces the duration of the crop cycle, combats weeds (Anzalone *et al*., 2010; Coolong, 2010), and protects crops against pests, insects and pathogens (Cozzolino *et al*., 2020). Among the many types of plastic films, polyethylene (PE), especially that at low density (LDPE), is probably the most widely used for mulching. However, irrespective of the types of plastic materials, they often have a short duration due to their properties and to exposure to weather (solar radiation, temperature, rainfall, and wind) and/or to the installation mode (Picuno, 2014). The result is large-scale production of plastic waste, which has to be adequately managed to reduce its environmental impact (Briassoulis *et al*., 2014, 2013; 2012; Al-Maaded *et al*., 2012). Instead, they are often abandoned in fields or along watercourses, buried in the soil, taken to the landfill, or burnt in fields to avoid disposal costs. Therefore, plastic wastes represent an environmental, economic, and social problem that must be addressed by identifying sustainable alternatives. From this perspective, using biodegradable plastics represents a significant opportunity.

Research increasingly aims to create films based on biopolymers, such as starch, cellulose, and polylactic acid, derived from renewable resources, such as maize, potato, and rice (Scaringelli *et al*., 2016; Moreno and Moreno, 2008). At the end of their lifetime, biodegradable films can be incorporated directly into the soil or disposed of in a composting system where the films are degraded by soil microorganisms (Moreno and Moreno, 2008) which convert them into carbon dioxide, water, and biomass. While plastic requires many years to degrade, bio-films have very low degradation times, *i.e*., about 5-6 months after the end of the crop (Filippi *et al*., 2011).

Degradable plastic films degrade by the effects of sunlight (ultraviolet rays) and soil micro-organisms and ultimately decompose into water and carbon dioxide, which are harmless to the environment (Zhang *et al*., 2020). Environmental factors affect the degradation process of such films, and the degree of degradation can vary with different climates (Kasirajan and Ngouajio, 2012). Further, the degradation rate also depends on changing its ingredients or thickness (Braunack *et al*., 2015).

Lettuce (*Lactuca sativa* L.) is one of the most widely cultivated leafy vegetables. In 2013, the world production of lettuce was 24.6 million tons, mainly concentrated in China (13.5), the United States (3.6), India (1.1), Spain (0.9), and Italy (0.8) (Hernandez, 2022). In Italy, in 2021, the lettuce crop occupied 14,817 ha, yielding 318,000 tons (ISTAT, 2022). The nutritional value of lettuce is high since it is a source of secondary metabolites, such as vitamins, phenols, carotenoids, chlorophylls, and macro- and trace elements, which play a crucial role in human nutrition (Kim *et al*., 2016; Baslam *et al*., 2013). Production systems and agronomic practices significantly affect the yield and quality traits of the edible organs of lettuce (Rouphael *et al*., 2012). For example, mulching is widely used for lettuce because it leads to higher yields and early production (Malinconico, 2017).

Several studies have been carried out to test the agronomic response to biodegradable mulching of different crops: tomato (Moreno *et al*., 2009), strawberry (Costa *et al*., 2014), melon (Lopez *et al*., 2007), and many other vegetables (Waterer, 2010). However, little is known about the medium-term endurance and performance of biodegradable films. One key question is whether MB can be re-used for two short crop cycles.

This work aimed to evaluate the performance (endurance to weathering) of biodegradable films in the mid to long term, compared to LDPE, by testing two consecutive crop cycles (winter and spring) and assessing the effect of the different mulching films on the agronomic behaviour of lettuce.

Materials and methods

Experimental setting and design

The trial was carried out during winter 2013 and spring 2014 at a private farm of the cooperative ARCA 2010 in Acerra (N 40° 57’ 56.462’’; E 14° 25’ 50.213’’; 27 m asl), a typical horticultural area in Campania, southern Italy.

The experimental design was a factorial comparison between three soil mulching levels (M): i) soil covered by the black biodegradable film (MB12); ii) soil covered by commercial black low-density polyethylene (LDPE); iii) no mulching, bare soil (Control), over two consecutive cultivation cycles (winter, W, and spring, S) of *Lactuca sativa* L.

The treatments were arranged in a randomised block design with three replications for a total of nine experimental units (3M ×3 replications). Each block was 10.0 m long and 1.4 m wide.

At the beginning of the trial, three soil samples per treatment at 0-30 cm depth were collected; the soil was sandy-loam (USDA classification) of volcanic origin with a high content of organic matter, phosphorus, and potassium (Table 1).

Mulching films, plant materials, and cultural practice

The biodegradable film was a starch-based material (Mater-Bi) 12 microns thick. It consisted of destructured starch complexed with biodegradable polyesters (Bastioli, 1998) and marketed by Novamont S.p.A. (Novara, Italy) under the trade name Mater-Bi®. This product is compostable and certified ‘OK Biodegradable Soil’ by the Austrian certification institute TÜV as fully biodegradable and complies with the requirements related to the main regulations on biodegradation and environmental impact in force (European standards: UNI EN 13432: 2002, UNI EN 14995: 200). The LDPE film was a commercial plastic film 50 microns thick, commonly used by farmers for mulching soil.

The films were hand-placed on 11th December 2013, after hoses for drip irrigation were rolled out. The winter transplant was made on 19th December, and the spring transplant on 2nd May, both at the two true-leaf stage with a density of 8.2 plants per m^{-2}. For the winter cycle, the cultivar ‘*Bacolese*’ was chosen; it is a traditional cultivar of the Neapolitan area, very cold-resistant and thus suitable for open-air winter cultivation. For the spring cycle, the cultivar ‘*Forlina*’ was tested, which is a widely grown cultivar in the region suitable for spring cultivation.

Table 1. Physical and chemical soil properties.

Soil properties	Units	Mean values
Texture		
Coarse sand	%	39.9
Fine sand	%	22.2
Silt	%	22.3
Clay	%	15.6
N-total (Kjeldahl method)	g kg^{-1}	1.5
P_2O_5 (Olsen method)	mg kg^{-1}	216
K_2O (tetraphenylborate method)	mg kg^{-1}	2173
Organic matter (bichromate method)	%	2.2
pH		7.4
Electrical conductivity	dS m^{-1}	0.128

Because the soil had a high content of phosphorus and potassium, only nitrogen was added at the rate of 100 kg·ha^{-1} using ammonium nitrate (26%) three times: at transplant (40%) and then at 35 (30%) and 60 days (30%) after transplant (DAT), for the winter cycle, and at 20 and 40 DAT, for the spring cycle. Water needs of the plants were calculated using the Hargreaves formula and fully restored by irrigation. No pesticide applications were required to control pathogens and pests.

Soil temperature measurements

During the two cycles (winter and spring), probes (Vantage Pro2, Davis Instruments) were used to continuously monitor the temperature in the 0-20 cm soil layer and air temperature.

Marketable yield and sampling

The winter plants were hand-harvested on three occasions from 2nd to 18th April, while the spring plants were harvested twice on 10th and 18th June. At harvest, the diameter and fresh weight of the head and diameter and height of the stem were recorded; the marketable yield was also determined and expressed in tons per hectare. A representative sample for each treatment and replicate was oven dried at 70°C until a constant weight was reached to determine the dry matter. The dry samples were used to determine nitrate content in leaves.

Yield quality: nitrate content and colour parameters

On dried samples of leaves, nitrate content was determined from water extract based on the cadmium reduction method proposed by Sah (1994). The absorbance of the solution was determined at 550 nm wavelength with a Hach DR 2000 spectrophotometer (Hach Co., Loveland, CO); the values were then expressed as mg kg^{-1} of fresh weight (FW).

On ten entire fresh leaves for each experimental unit, a Minolta CR-300 Chroma Meter (Minolta Camera Co. Ltd., Japan) was used to measure the colour space parameters: L*, brightness, ranging from 0-black and 100-white; a*, chroma component ranging from –60 and +60, green and red respectively; b*, chroma component ranging from –60 and +60, blue and yellow respectively, following the *Commission Internationale de l'Eclairage* (CIELAB).

Mulching film measurement

The resistance of biodegradable films to atmospheric agents (rainfall, wind, *etc.*) was monitored during both cycles using the standard scoring performed by Novamont technicians. It provides a visual observation using a scale with a rating score from 1 (worst condition) to 9 (best condition). The scale evaluates the following parameters: degradation of the buried and unburied film, lesions, and resistance to tearing (Filippi *et al.*, 2011). These observations were made nine times during the two cycles (winter and spring).

Statistical analysis

Mulching film data were subject to one-way ANOVA analysis using the SPSS software package (SPSS version 22.0 for Windows, Chicago, IL, USA), comparing the different date samplings. Yield and its parameters were also analysed with the same software by ANOVA combined over two growing seasons. Means were separated according to the post-hoc Duncan Test (significance level 0.05). Data normality and homoscedasticity were verified with Levene's tests.

Results

Weather and soil temperature

The weather at the experimental site is typical of the Mediterranean area, with a mean annual temperature of 15.4°C and mean annual rainfall of 1214 mm (https://it.climate-data.org/europa/italia/campania/acerra-14146/). The lowest air temperature was recorded in December, but the coldest day was in February, albeit no lower than 4°C. Maximum air temperatures were constantly increasing, reaching 33°C in the first ten days of June (Figure 1). In the first cycle, the mean temperature was 10.9°C, while in the second, it was 20.6°C. Total rainfall was 891.6 mm, more than 50% of which occurred in the first growing period, almost 40% in the second, and the remaining part in the period between the two cycles (Figure 1). During the first cycle, two rainfall peaks exceeding 80 mm were recorded in the last decade of January and the first of February. By contrast, about 30% of the rainfall in the second cycle (343 mm) was concentrated in a windstorm event on 14th June (Figure 1).

In the top 0-20 cm soil layer, the mean soil temperatures were 13.7, 14.6, and 14.9°C for control, MB12, and LDPE, respectively (Figure 2). Therefore, the mulching films elicited a 7.9% increase compared to non-covered soil. The covered and non-covered soil difference was more significant in the spring cycle, about +1.8°C *vs* +0.8°C than in the winter cycle (Figure 2). Interestingly, the differences between the two mulching films were less marked in the first cycle; indeed, the LDPE resulted in a soil temperature 0.2°C higher than MB12, while in the second cycle, this difference was three times higher (0.6°C) (Figure 2).

Effect of mulching on winter lettuce yield and quality

Mulching statistically affected the marketable yield of winter lettuce, boosting it with an average increase of about 51% over the control (Figure 3). Although no significant differences were recorded between the two films, LDPE led to a 10% increase compared to MB12.

In addition, both mulching films reduced the duration of the crop cycle, but with a different effect: the plants grown on LDPE had the shortest cycle (less than 106 days) *vs* MB12 (110 days) and

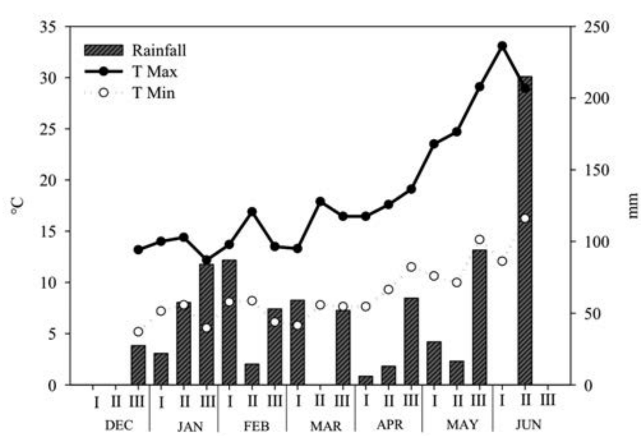

Figure 1. The trend of air temperature and rain during the two crop cycles.

the control (120 days) (Table 2). In addition, at harvest, the head fresh weight of control plants was significantly lower than that of MB12 and LDPE plants, which did not differ substantially, while the diameter of control plants, despite being the lowest, was not significantly different from that of MB12 (Table 2). Finally, both stem length and diameter of lettuces grown on mulching films were statistically higher than the control but without significant differences between the two films (Table 2).

The colour parameters of winter lettuce were significantly affected by mulching films; the brightness (L^*) of MB12 lettuces was significantly higher than that of the control and LDPE, which did not differ. By contrast, the opposite trend occurred in parameter a^* [green intensity, chroma component ranging from green (–60) to red (+60)] that was higher in the control and LDPE (Table 3). Finally, parameter b^* [chroma component ranging from blue (–60) to yellow (+60)] was not significantly affected by mulching films.

As regards the nitrate content in leaves, the values of the three treatments ranged between 1725.6 for LDPE and 1877.7 mg kg^{-1} for the control, which was significantly higher than the other two treatments (Table 3).

Effect of mulching on spring lettuce yield and quality

In the spring cycle, the effect of the biodegradable film on lettuce marketable yield was less marked compared to the winter cycle: LDPE showed the highest yield, with an 11.4% increase over the mean value of the other two treatments, even if no statistical differences were recorded between the three treatments (Figure 4).

As expected, the spring cycle was shorter than the winter cycle: 39 days for lettuces grown on mulching films and 47 for control plants, but without statistical differences between the three treatments (Table 4). Although LDPE showed higher values for all growth parameters (head weight and diameter, and stem length and diameter), statistical differences between the three treatments were recorded only for head diameter: LDPE was +11.3% higher than the mean value of MB12, and the control (Table 4).

Also, for the spring cycle, only brightness ($L^{\#}$) and green intensity ($a^{\#}$) were significantly affected by mulching films, and with the same trend recorded for winter lettuces: MB12 plants showed the highest brightness value and lowest component $a^{\#}$, and for both parameters it significantly differed from the other two treatments (Table 5). Finally, in this cycle, the nitrate content in control leaves was higher than in the other two treatments, albeit without statistical differences between them (Table 5).

Degradation of biodegradable film

The results of the statistical analysis regarding the lifetime parameters of the biodegradable film are reported in Table 6. As expected, the lifetime decreased during the two cycles. However, most interestingly, the degradation of buried film and resistance to tearing, only in the last observation, at 191 days after placing mulching film (DAPM), was statistically lower than all other

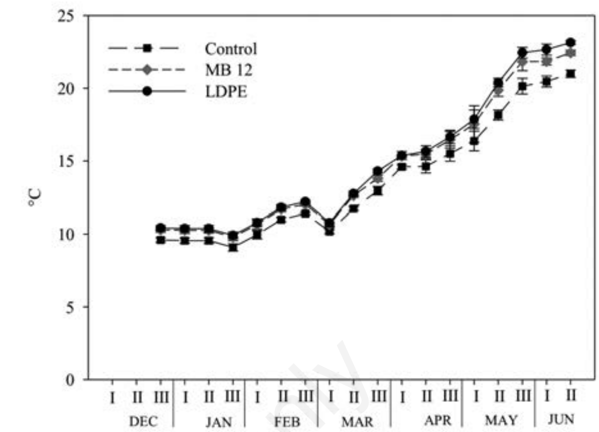

Figure 2. The trend of mean soil temperature under mulching treatments (bare soil, Control; soil covered by the black biodegradable film, MB12; soil covered by commercial black low-density polyethylene, LDPE) during the two crop cycles.

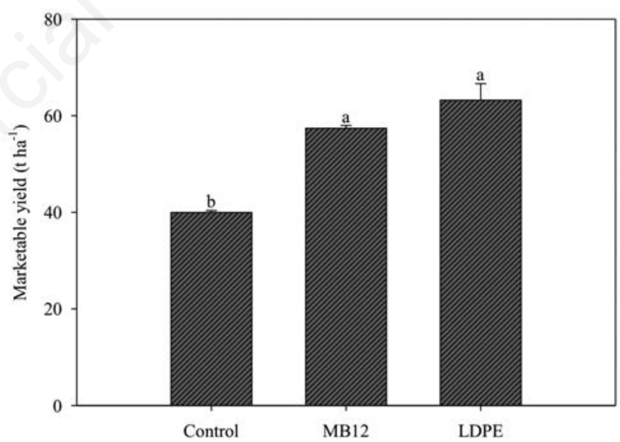

Figure 3. Effect of mulching films (bare soil, Control; soil covered by the black biodegradable film, MB12; soil covered by commercial black low-density polyethylene, LDPE) on marketable yield of winter lettuces. The vertical bars are the standard error; different letters indicate statistical differences according to the Duncan test (P≤0.01).

Table 2. Effect of mulching films on cycle duration, head weight and diameter collar diameter, and length of winter lettuces.

Treatments	Crop cycle	Head		Stem	
		Weight	Diameter	Length	Diameter
	Days	g	cm	cm	cm
Control	120.0±0.0^a	470.0±5.0^b	11.78±0.11^b	3.59±0.11^b	2.62±0.05^b
MB12	110.3±0.7^b	717.7±40.2^a	13.18±0.09^{ab}	4.72±0.24^a	2.85±0.11^a
LDPE	105.7±1.7^c	743.7±41.0^a	14.15±0.23^a	4.49±0.17^a	2.86±0.05^a
Significance	**	**	*	*	**

Control, bare soil; MB12, soil covered by the black biodegradable film; LDPE, soil covered by commercial black low-density polyethylene; *significant at P≦0.05; **significant at P≦0.01; $^{a-b}$different letters within each column indicate significant differences according to Duncan's test.

observations. Also, the part of the film not buried showed good resistance to atmospheric agents: the first statistical difference was observed at 168 DAPM. Finally, the number of lesions of the biodegradable film increased linearly during both cycles, but only in the last two measurements did it significantly different from the others (Figure 5 and Table 6).

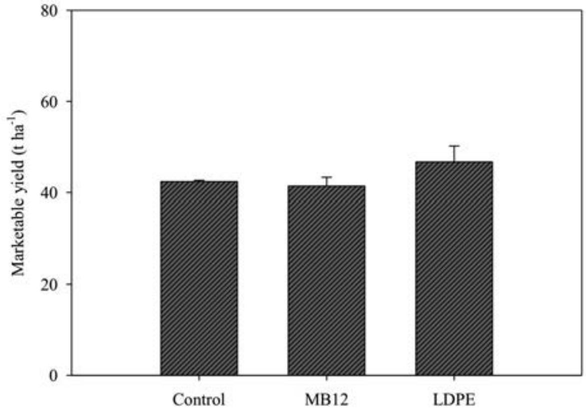

Figure 4. Effect of mulching films (bare soil, Control; soil covered by the black biodegradable film, MB12; soil covered by commercial black low-density polyethylene, LDPE) on marketable yield of spring lettuces. The vertical bars are the standard error.

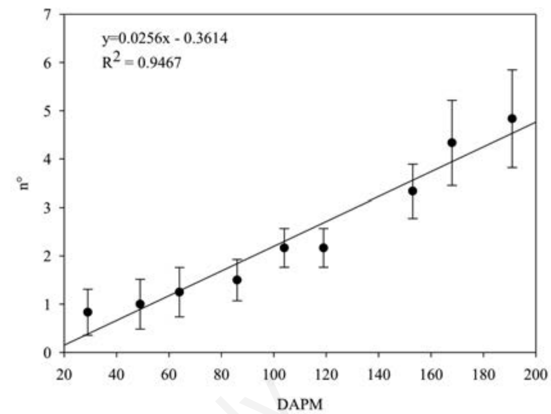

Figure 5. Linear regression between the number of lesions of biodegradable film and days after placing mulching films (DAPM). The vertical bars are the standard error.

Table 3. Effect of mulching films on colour parameters, and nitrate content of leaves of winter lettuces.

Treatments	L*	a*	b#	Nitrate mg kg^{-1} f.w.
Control	60.94±3.10^b	−13.96±0.57^a	33.14±2.60	1877.7±394.7^a
MB12	65.03±2.87^a	−11.45±0.50^b	33.55±2.15	1755.2±415.8^b
LDPE	59.46±3.20^b	−13.72±0.34^a	32.49±2.29	1725.6±338.0^b
Significance	**	**	ns	*

L*, lightness, ranging from 0=black to 100=white; a*, chroma component ranging from green (−60) to red (+60); b*, chroma component ranging from blue (-60) to yellow (+60); f.w., fresh weight; Control, bare soil; MB12, soil covered by the black biodegradable film; LDPE, soil covered by commercial black low-density polyethylene; ns, not significant; *significant at P≦0.05; **significant at P≦0.01; a-b different letters within each column indicate significant differences according to Duncan's test.

Table 4. Effect of mulching films on cycle duration, head weight and diameter, collar diameter and length of spring lettuces.

Treatments	Crop cycle	Head		Stem	
		Weight	Diameter	Length	Diameter
	Days	g	cm	cm	cm
Control	47.0±0.0	498.7±3.5	12.7±0.1^b	3.80±0.03	2.36±0.05
MB12	39.0±0.0	487.4±22.8	13.0±0.1^b	3.94±0.19	2.31±0.04
LDPE	39.0±0.0	549.5±41.4	14.3±0.2^a	4.29±0.47	2.44±0.01
Significance	ns	ns	*	ns	ns

Control, bare soil; MB12, soil covered by the black biodegradable film; LDPE, soil covered by commercial black low-density polyethylene; ns, not significant; *significant at P≦0.05; a-b different letters within each column indicate significant differences according to Duncan's test.

Table 5. Effect of mulching films on colour parameters, and nitrate content of leaves of spring lettuces.

Treatments	L*	a*	b*	Nitrate mg kg^{-1} f.w.
Control	50.55±4.30^b	−15.31±0.51^a	32.95±2.68	2377.3±89.3
MB12	59.17±4.14^a	−13.36±0.44^b	32.89±2.24	2356.8±184.7
LDPE	50.75±4.38^b	−15.60±0.59^a	32.87±2.19	1862.4±39.0
Significance	**	**	ns	ns

L*, lightness, ranging from 0=black to 100=white; a*, chroma component ranging from green (−60) to red (+60); b*, chroma component ranging from blue (−60) to yellow (+60); f.w., fresh weight; Control, bare soil; MB12, soil covered by the black biodegradable film; LDPE, soil covered by commercial black low-density polyethylene; ns, not significant; **significant at P≦0.01; a-b different letters within each column indicate significant differences according to Duncan's test.

Discussion

Biodegradable mulching films degrade rapidly and easily, making them unsuitable for mid- to long-term use. With the current research, we set out to test the performance of a biodegradable mulching film over two consecutive cycles of lettuce (winter and spring), comparing it with traditional LDPE and evaluating its resistance to atmospheric agents for the whole period, as well as its effect on yield and quality.

Our findings highlighted that both films led to the heating of the root zone soil (0-20 cm layer); the LDPE always reached higher temperatures, but in the winter cycle, the difference in heating between the two films was less marked compared to the spring cycle. Also, Lopez-Marin *et al*. (2011), in a study that compared different types of mulching films (traditional, biodegradable, and oxo-biodegradable), recorded the lowest soil temperatures under the biodegradable film, due to the composition of this material, which permits increasing gas exchange with the open air as a result of its higher permeability to water vapor, following Moreno *et al*. (2009). Indeed, Moreno *et al*. (2009) compared a black biodegradable film (Mater-Bi®), an aluminised photodegradable, and a black linear low-density polyethylene film (LLDPE) used on a tomato crop with a 143-day cycle (June-October). They reported that soil temperatures were always higher under LLDPE and lower under the biodegradable film, which, seven days after transplant, already showed the first cracks. However, it was able to ensure good soil cover until the tomato completely covered the mulch. At the end of the cycle, the covering capacity of tomato reduced the differences in temperature of the three films due to material composition. That said, with the biodegradable film, a similar yield, and quality of tomato was attained compared to LLDPE. In our study, the climatic conditions differed because the first cycle started in the winter and the mulches were more affected by weathering, especially precipitation. However, given that the degradation is mainly due to the effects of sunlight (ultraviolet rays), it is presumable that in the first months of the trial, the lower radiation affected the integrity of film to a lesser extent, which was compromised only during the second cycle with higher radiation and air temperatures. Also, in our previous research, comparing a 15 µ biodegradable film with LDPE in a spring cycle of courgettes, we found that the traditional plastic heated the soil more than the former (Di Mola *et al*., 2019). On the other hand, the polyethylene film absorbs more solar radiation and emits radiation, increasing soil temperature greatly (Gu *et al*., 2018; Moreno *et al*., 2016; Xiukang *et al*., 2015).

The reduced differences in heating capacity between the two films in the winter cycle could be due to the greater integrity of biodegradable film in the first cycle. Indeed, as stated above, MB12 showed no signs of degradation during the winter cycle. This is because the soil was perfectly covered by it thus, the film showed better thermic performance, allowing it to reach soil temperatures similar to those of LDPE. Instead, during the second cycle, the integrity of biodegradable film was lower, and since it failed to cover the soil completely, its thermic effect may have been lower. Indeed, the first signs of degradation were observed only after about 150-160 days from placing films, in mid-May, that is 15 days after spring transplant when the most significant differences were recorded.

However, only at the end of the second cycle did the biodegradable film show a substantially reduced resistance to tearing and a greater degradation of both the unburied and buried film: about 43.0%, 20.0%, 16.6%, respectively, less than the optimal value (9). Minuto *et al*. (2007) also reported good results for the 12 and 15 µ Mater-Bi® films in short crop cycles (3-5 months), highlighting a 15% reduction in mulched soil after 140 days and 60% and 26.6% of degradation, respectively, with respect to optimal conditions.

Mulching is a widely used agricultural practice in lettuce cultivation, mainly to improve earliness and quality, but also for weed control (Malinconico, 2017). Our findings highlighted a beneficial effect of the two mulching films (traditional-LDPE and biodegradable-MB12) on the marketable yield of lettuce only in the first cycle, with a 51% yield increase as compared to bare soil; in the second cycle (spring), the effect of mulching was less marked and not significant, with only a slight enhancement of lettuce yield grown on LDPE. In other field experiments, several authors also found a comparable yield and quality of summer lettuce grown on PE and biodegradable mulching films (12 and 15 µ) (Minuto *et al*., 2007; Miles *et al*., 2007). On the other hand, it is well known that the difference in yield due to soil temperature increase is more evident when the temperature is a limiting factor (Brown *et al*., 1992), as occurred in the winter cycle. Instead, when climatic conditions (at air and soil levels) are optimal, as well as in the spring cycle, the mulches do not affect crop yield, as also reported by other authors (Streck *et al*., 1995; Lorenzo *et al*., 2005).

Our findings are also close to the results of Cozzolino *et al*. (2020), who found that the marketable yield of lettuce (autumn

Table 6. Endurance of biodegradable film (degradation of buried film, unburied film, lesion number, and resistance to tearing) during the two cycles. The observations were made according to standard scoring made by Novamont technicians, based on visual observation, using a scale with a rating score from 1 to 9 (best conditions); only the actual lesions were counted for lesion number.

DAPM	Degradation of buried film	Degradation of unburied film	Lesions	Resistance to tearing
29	8.8±0.0^{a}	9.0±0.2^{a}	0.8±0.5^{c}	9.0±0.0^{a}
49	8.8±0.0^{a}	9.0±0.2^{a}	1.0±0.5^{c}	9.0±0.0^{a}
64	8.8±0.0^{a}	9.0±0.2^{a}	1.3±0.5^{c}	9.0±0.0^{a}
86	8.8±0.0^{a}	9.0±0.2^{a}	1.5±0.4^{bc}	9.0±0.0^{a}
104	8.8±0.0^{a}	9.0±0.2^{a}	2.2±0.4^{bc}	9.0±0.0^{a}
119	8.8±0.0^{a}	9.0±0.2^{a}	2.2±0.4^{bc}	9.0±0.0^{a}
153	8.8±0.2^{a}	8.8±0.2^{ab}	3.3±0.6^{ab}	9.0±0.0^{a}
168	8.8±0.3^{a}	8.4±0.2^{b}	4.3±0.9^{a}	8.9±0.1^{a}
191	7.5±0.3^{b}	7.2±0.2^{c}	4.8±1.0^{a}	5.1±0.6^{b}
Significance	***	***	***	***

DAPM, days after placing mulching films. ***Significant at P 0.001; $^{a-c}$different letters within each column indicate significant differences according to Duncan's test (P≤0.05).

cycle) grown on biodegradable mulching film had an intermediate value between the LDPE lettuce and plants cultivated on bare soil: the increase with LDPE was about 8% vs. 10% recorded in the first cycle of our test. Similarly, Lopez-Marin *et al*. (2011) reported that winter lettuces (January-April) grown on PE mulching achieved significantly higher yields than those grown on biodegradable and oxo-biodegradable films. The greater yield we recorded in LDPE reflects greater lettuce size (fresh weight and diameter) than the control lettuces in particular, especially in the winter cycle, as reported by Lopez-Marin *et al*. (2011).

Interestingly, both conventional and biodegradable mulching films reduced the crop cycle in both seasons. In the first cycle, LDPE resulted in a 5- and 10-day harvest earliness compared to MB12 and the control, respectively. By contrast, in the second cycle, both mulching films showed the same earlier production compared to bare soil (8 days). On the other hand, earliness is a key agronomic benefit for mulch technology (Malinconico, 2017), and it has been reported as an effect of biodegradable film as well in several crops (winter melon, Incalterra *et al*., 2004; tomato, Martin-Closas *et al*., 2008; muskmelon, D'avino *et al*., 2015; zucchini, Di Mola *et al*., 2019; perennial wall rocket, Caruso *et al*., 2019). This mulching effect constitutes a particularly interesting aspect for farmers who can obtain a higher price on the market for their early products. With the latter advantage, achieving high food quality is another crucial aspect since it drives consumer choice. Product quality can be evaluated based on different characteristics (nutritional, organoleptic, physical, *etc*.) (Gruda, 2005); among the quality traits of lettuce, colour, intended as brightness and green intensity, is a characteristic that can be readily evaluated by consumers, making it often one of the most significant drivers in the choice of products to buy. Biodegradable mulching significantly increased the brightness in both cycles, compared to LDPE and bare soil, but had the opposite trend for $a^{\#}$ colour parameter. Our results are partially (only for the $L^{\#}$ parameter) in accordance with the findings of Cozzolino *et al*. (2020).

By contrast, high nitrate content in lettuce leaves and in the other green leafy vegetables is a detrimental aspect of quality. Indeed, high nitrate values in leaves and their consequent entry into the human body through the diet have often been associated with the appearance of diseases, such as methemoglobinemia or the presence of carcinogenic compounds (*i.e*., nitrosamine) (Salehzadeh *et al*., 2020; Aires *et al*., 2013; Weitzberg and Lundberg, 2013; Tamme *et al*., 2010). Green leafy vegetables, including lettuce, are genetically predisposed to accumulate nitrate in leaf tissue. Nitrate accumulation in plant tissues stems from many factors, including environmental factors (light radiation) and agronomic factors (nitrogen fertilisation). In particular, nitrate accumulation in leaves is inversely correlated to light radiation (Fu *et al*., 2017; Non Renseigné *et al.*, 2007), which regulates nitrate reductase activity (Bian *et al*., 2020): high light radiation corresponds to high nitrate reductase activity and hence low nitrate content in plant tissues. Instead, nitrate accumulation is directly correlated to nitrogen fertilisation (doses and types of fertilisers; Colla *et al*., 2018; Abubaker *et al*., 2010; Porto *et al*., 2008; Stagnari *et al*., 2007; Goh and Akon, 1986): as the N dose increases, so does nitrate accumulation.

The nitrate content in leaves usually has a seasonal behaviour, with higher values in winter, but in our research, we found higher values in spring than in the winter cycle. However, this can be explained because we tested two different varieties, and it has been reported elsewhere that, for lettuce, the incidence of genotypic variation in nitrate accumulation is considerable (Burns *et al*., 2011, 2012; Lopez *et al*., 2014). In addition, also the shorter cycle recorded in spring could have reduced the time for nitrogen metabolism processes with consequent slightly higher accumulation in leaves.

Interestingly, in the winter cycle, the mulching reduced the nitrate content compared to bare soil, while no effects were recorded in the spring cycle. Our results are in contrast both with the findings of Cozzolino *et al*. (2020), according to whom nitrate values were lower in lettuce grown on bare soil, and with Wojciechowska *et al*. (2007), who reported that the nitrate content was higher in lettuce grown on black mulching film than lettuce grown on bare soil or white and transparent mulch. In the current research, our contrasting results could be explained by a combination of micro-climatic conditions and the indirect effect of mulching. It is widely reported that nitrate is usually higher in plants grown on mulch, primarily due to the absence of weeds competing for nitrogen (Dobrzański *et al*., 2004) and N leaching due to rainfall is reduced. At the same time, it is also well known that nitrate-reductase (NR) activity is increased by the presence of substrate (NO_3^-) (Lillo *et al*. 2004). In our conditions, we suggest that mulching films, thanks to the better micro-climate conditions (*i.e*., higher temperature), probably increased the root surface of lettuces as compared with the control plants, thereby increasing their capacity to uptake nitrogen, as also reported by Kumar and Dey (2011) in strawberry. Stimulating the activity of NR, could have reduced nitrate translocation to the leaves. On the other hand, it is worth pointing out that the differences in nitrate concentration were significant only in the winter cycle, probably because in the spring cycle, the generally higher temperatures reduced the effect mentioned above of mulching on root growth. That said, in both cycles, the nitrate values were within limits set by the European Community under Regulation No. 1258/2011.

Although we did not perform a cost analysis, given all the points mentioned above, it is worth making a brief economic consideration. In the literature, we found that Minuto *et al*. (2007) reported a cost analysis that compared PE to 12 μ and 15 μ Mater-Bi®. As a result, the cost of material was €639 and €700 per hectare for PE and MB12, respectively; to which, only for PE, the removal and disposal costs (10 and 50 €/ha) had to be added, making a total of €809 ha^{-1}. Therefore, the authors highlighted an interesting saving for MB12 compared to traditional film; instead, the trend was the opposite for MB15, whose costs were higher than those of PE (900 *vs* 809 €/ha).

Conclusions

Our findings highlighted the good resistance to atmospheric agents of the biodegradable mulching film also in the mid-long term, with a reduction of its integrity and mechanical properties only after about six months. In addition, the plants grown on this film achieved yields comparable to those obtained on traditional polyethylene without detrimental and significant effects on quality, especially regarding nitrate content in leaves and colour parameters. Finally, the biodegradable film resulted in an earlier production, which was the same as LDPE in the spring cycle, while in the winter cycle, the harvest was delayed *vis-à-vis* LDPE but earlier compared to bare soil. This is a particularly interesting trait for farmers since early production corresponds to higher prices.

Therefore, the lower cost of a thin biodegradable film than that of polyethylene suggests that farmers can profitably choose this more environmentally friendly alternative. Moreover, the film could give even better results under protected conditions where the effect of atmospheric agents is lower. Therefore, further research should be carried out to verify this hypothesis and also the effect of biodegradable film on other quality product traits. In particular, the effects of different mulching films on soil nitrification or nitrate-reductase activity have to be studied in depth to account for the different effects on nitrate accumulation in leaves.

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