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Short communication

Biodegradable mulching vs traditional polyethylene film for sustainable solarization: Chemical properties and microbial community response to soil management

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ABSTRACT

Soil solarization is usually performed with polyethylene plastic films, which are often disposed of by taking them to landfills, burying them in soil, burning them or occasionally recycling them, and these approaches have a great impact on the environment. Therefore, the use of biodegradable films seems to be an interesting eco-sustainable alternative to traditional films. The effect of soil solarization carried out by using biodegradable mulch or traditional polyethylene plastic film was determined under greenhouse conditions. The response of the soil was assessed by chemical determinations and microbiological culture-dependent and culture-independent approaches to evaluate the microbial biodiversity, biological status and quality of the soil. The biodegradable film avoided a high ammonia concentration in the soil, thanks to both lower soil water content and slightly lower temperature than polyethylene film, and these conditions probably have been optimal for growth of nitrifying bacteria, which were more efficient in BIO, as highlighted not only by lower ammonia value but also by higher nitrate value. Both films did not affect organic matter and total nitrogen content. Moreover, the modifications of the environmental and ecological conditions associated with the different film covers applied to the soils affected prokaryotic and eukaryotic populations, leading to the establishment of a new dominant microbial community. Interestingly, microbiological analyses highlighted a different behavior modulated with the two films indicating different times of recovery post stress.

Overall, the results highlighted the potential of biodegradable film that appears to be a suitable replacement for traditional polyethylene plastic film for soil solarization, with great environmental benefits.

1. Introduction

Soil temperature affects several physical, chemical and biological processes, such as evaporation, uptake of nutrients and water, decomposition of organic matter by microbes, seed germination and seedling emergence (Al-Shammary et al., 2016). Following the phasing-out of methyl bromide and severe restrictions for the use of alternative fumigants imposed by European Community (2037/2000), the sustainable heat-based approach for the eradication of fungi, weeds, and nematodes was re-evaluated (Castello et al., 2017). Among several approaches, soil solarization is a cheap technique (Chellemi et al., 1997) that involves low-risk management for farmers that could also boost crop yield (Culman et al., 2006). This technique is usually accomplished by covering the soil with a transparent plastic film for a variable period, even up to 6 weeks or more (McGovern and McSorley, 1997). In a covered moist soil, the high temperature (45–55 °C) at a 5 cm soil depth causes the death of several soil-borne plant pathogens, such as *Verticillium dahliae* (Pinkerton et al., 2000), *Fusarium* spp. (Tamietti and Valentino, 2006), *Rhizoctonia solani* and *Sclerotinia minor* (Sinigaglia et al., 2001), and resulted in a decrease in nematodes (Stapleton and Heald, 1991; McGovern et al., 2002). Moreover, solarization also increases the soil moisture percentage (Sofi et al., 2014), promoting the breakdown of organic matter supplied with soil improvers with consequent accumulation of volatile compounds toxic to many pathogens (Oka et al., 2007), as well as increasing N-NO_3 and N-NH_4 concentrations (Birthisel et al., 2019). Soil solarization is usually performed with

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plastic films such as polyethylene (PE), especially low-density polyethylene (LDPE), but they have a large environmental impact since their decomposition takes approximately 100 years. Their disposal is conducted through burying, burning, and recycling (Ren, 2003; Kyrikou and Briassoulis, 2007), although recycling is often difficult and expensive because of soil contamination. Mormile et al. (2017) report the global use of plastic films is 3.9 million tons, of which 16% in Europe and almost half (1.8 million tons) are used for mulching, including solarization. The use of biodegradable films is an attractive eco-sustainable alternative approach to overcome the environmental pollution problems due to the use of plastic films (Tsia et al., 2009) because after their use, they can be degraded progressively in the soil without releasing toxic residues into the environment (Castronuovo et al., 2005). Biodegradable films have already been tested as soil mulching films on several crops, such as zucchini squash (Di Mola et al., 2019), tomato (Moreno et al., 2009), strawberry (Costa et al., 2014), lettuce (Cozzolino et al., 2020), pepper, eggplant, muskmelon and sweet corn (Waterer, 2010). Although the cost of biodegradable films is usually higher than that of PE films (Sarnacke and Wildes, 2008), the cost is offset by the absence of removal and disposal costs (Malinconico et al., 2008). Although solarization is considered an effective method for reducing soil-borne pathogens and improving the emergence and growth of crop plants, it could affect soil microbial equilibrium (Balakrishna et al., 2015). Temperature gradients established during soil solarization could determine a shift in soil microbiota, although these impacts have not yet been well quantified (Fernández-Bayo et al., 2017). Several reports have described the influence and alteration of microbial communities in soils treated by solarization, but their results were not consistent (Yokoe et al., 2015). It has been reported that this practice seems to have a minimal effect on beneficial microorganisms, such as *Bacillus* spp., Actinomycetes and fluorescent pseudomonads, and arbuscular mycorrhizal (AM) (Bonanomi et al., 2008). Conversely, a reduction in AM fungi and colonization were found in solarized soil by Schreiner et al. (2001). Balakrishna et al. (2015) reported that although AM significantly decreased in solarized soils, an increase in soil microbial biomass was observed. These contrasting reports indicate a need for further studies to better understand the impact of different films on soil microbial communities since soil microbiota play a fundamental role in maintaining biological equilibrium and fertility (Ventorino et al., 2019).

On the basis of these considerations, the aim of this research was to compare the effect of a biodegradable mulching film and a traditional LDPE plastic film on soil chemical properties with soil solarization in greenhouse conditions. Their effect on soil microbial communities was also assessed by culture-dependent and culture-independent approaches to evaluate the microbial biodiversity, biological status and quality of the soils.

2. Materials and methods

2.1. Experimental conditions

The experiment was carried out in the summer 2017 at the experimental field of the Department of Agricultural Sciences in Portici (Naples, Italy; latitude 40°49′N; longitude 14°20′E), in a polyethylene greenhouse. A completely randomized block design with three replicates was used to compare two different mulching films for solarization: a traditional transparent LDPE (60 μ thickness) and a transparent biodegradable film-PC17T6/35, starch-based monolayer film treated with a stabilizer of natural origin (BIO with a 35 μ thickness; Novamont S.p.A.), with bare soil (control). The films were manually placed on 22 June 2017; at the same time, the temperature probes were installed to continuously measure the soil temperature at two depths (7.5 and 15.0 cm); three probes per each depth and treatment were installed. The films were removed after one month, but the whole trial lasted 70 days so that the trend in the chemical and microbiological soil proprieties could be monitored for the successive 40 days film removal. During this period, no soil tillage neither irrigation was applied.

2.2. Soil measurements

Before placing the mulching films, soil sampling was conducted at 0–20 cm for physical and chemical characterizations; the soil was a sandy loam soil (USDA classification) with a pH of 6.94, EC of 0.6 dS m^{-1}, total nitrogen of 0.115% and a high content of organic matter (2.2%), phosphorus (87 ppm) and potassium (1811 ppm). Every fifteen days, the soil was sampled to determine the water content, nitrogen (nitrate, ammonia and organic N) content and organic matter. The soil moisture was measured by the gravimetric method. For the chemical analyses, soil samples were oven-dried at 40 °C. The nitrate-nitrogen ($N\text{-}NO_3$) and ammonia-nitrogen ($N\text{-}NH_4$) contents were determined on the water extract of the dried soil samples based on the cadmium reduction method proposed by Sah (1994), measuring the absorbance of solutions at wavelengths of 500 and 425 nm, respectively, with a spectrophotometer Hach DR 2000 (Hach Co., Loveland, CO); the final results were expressed in mg kg^{-1}. The organic nitrogen was determined by the Kjeldahl method (Bremner, 1965), and the organic matter was determined by the Walkley and Black (WB) method (Walkley and Black, 1934).

2.3. Microbiological analysis

Soil samples (0–20 cm depth) were collected according to Romano et al. (2020) before placing the mulching films and every 15 days.

For microbiological counting, decimal suitable dilutions (1:10) of the soil samples were prepared as previously reported (Ventorino et al., 2014). The samples were characterized for total heterotrophic aerobic bacteria on plate count agar (PCA, Oxoid, Milan, Italy), for fungi on malt extract agar (Oxoid) supplemented with chloramphenicol (100 mg L^{-1}) and for actinomycetes on starch casein agar containing cycloheximide (100 mg L^{-1}) (Parillo et al., 2017; Ventorino et al., 2018). For molecular analysis, total microbial DNA was extracted using a FastDNA SPIN Kit for Soil (MP Biomedicals, Illkirch Cedex, France) according to the manufacturer's instructions. The primers V3f-GC and V3r (Muyzer et al., 1993) were employed for prokaryotic analysis using PCR mixtures and conditions previously described (Ventorino et al., 2017). The primers NL1-GC (Kurtzman and Robnett, 1998) and LS2 (Cocolin et al., 2000) were used to analyze the eukaryotic population using PCR mixtures and conditions according to Fiorentino et al. (2018). DGGE analyses were performed using a polyacrylamide gel using a Bio-Rad DCode Universal Mutation System (Bio-Rad Laboratories, Milan, Italy) as previously described by Ventorino et al. (2016).

2.4. Statistical analysis

Data of the chemical parameters and microbial counting were statistically analyzed by one-way ANOVA and Duncan's post hoc test for pairwise comparison of means ($P < 0.05$) using the SPSS 21.0 software package.

DGGE bands were analyzed by Phoretix 1 advanced version 3.01 software to perform a cluster analysis as described by Ventorino et al. (2013). The method described by Saitou and Nei (1987) was used to obtain the correlation matrix of the band patterns that was analyzed using the average linkage method in the cluster procedure of Systat 5.2.1 to estimate the percentage of similarity (S) of the microbial communities.

3. Results and discussion

3.1. Soil temperature

In the soil covered by mulching films (LDPE and BIO films) a higher temperature than control soil was recovered during the solarization

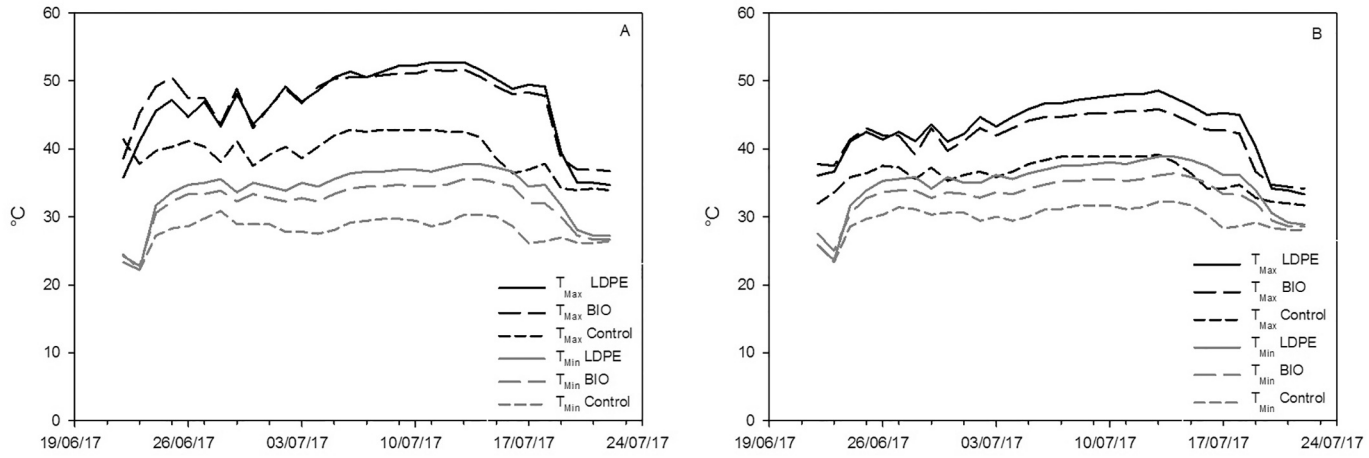

Fig. 1. Trend in the maximum and minimum temperature at 0–10 cm (A) and 10–20 cm (B) during the test. LDPE, traditional, transparent low-density polyethylene; Bio, transparent biodegradable film; and Control, bare soil.

(Fig. 1). The solarization effect on soil temperature was recorded especially in the topsoil layer. However, no differences in the maximum temperature between LDPE (46.7 °C on the average) and BIO (47 °C on the average) treatments was observed in the 0–10 cm layer. Instead, in the 10–20 cm layer the maximum temperatures were 43.2 °C and 41.9 °C (on the average), for LDPE and BIO films, respectively. Regarding minimum temperatures, although both films warmed up the soil with respect to control (about +4.8 °C and +4.1 °C for 0–10 and 10–20 cm depth, respectively), a difference between the two films was also noted. In fact, the soil covered by LDPE was averagely heater than soil covered by BIO film (+1.7 °C at both depths).

In the period following solarization, the soil temperatures of the three treatments didn't show differences being 30.8, 31.1 and 30.9 °C for LDPE, BIO and control, respectively.

During the solarization period, the air temperature under greenhouse was 20.8 °C and 52.7 °C, for minimum and maximum, respectively; instead the minimum and maximum temperatures out of greenhouse were 19.6 °C and 35.5 °C, respectively.

Also in terms of number of hours of warm up, the effect of solarization was evident (Table 1). The soil temperatures at the two depths (0–10 and 10–20 cm) have been grouped into 4 ranges (36–40, 41–45, 46–50, and 51–55 °C), and for each range, the number of hours was categorized into two periods of the test: the first 15 days of solarization, and the second 15 days of solarization. Temperatures under 36 °C was not considered because they are not effective in the pathogens control (Gamliel et al., 2000; Gelsomino and Cacco, 2006). The bare soil (control) didn't record hours over 45 °C, and already in the range 41–45 °C at the 10–20 cm depth no hours were recorded. In particular, during the whole period of solarization, the number of hours in the range 36–40 °C was higher in both films than bare soil (245, 207 and 190 at 0–10 cm, and 294, 307, and 173 at 0–10 cm, respectively for LDPE, BIO and control) (Table 1). In the range 41–45 °C, differences between covered and non-covered soil were more evident: 54 h on total period for bare soil, and 190, and 138 for conventional and biodegradable films, respectively. Heat hours in the range 46–50 °C were recorded in both films (Table 1). However, although at 0–10 cm layer the number of hours in this range was similar (130 vs. 129, for LDPE and BIO), at 10–20 cm layer a marked difference was observed (65 vs. 9, for LDPE and BIO). Finally, in the range 51–55 °C, LDPE had a slightly higher number of hours in the 0–10 cm layer than biodegradable film (Table 1).

Our results are consistent with the findings reported by Scopa et al. (2008), who found that at a 10 cm soil depth, the number of hours with a temperature between 51 and 60 °C was higher for LDPE than for biodegradable film, 127 and 13, respectively for LDPE and BIO. Notably, in the second period of solarization for the three ranges higher, the number of hours was always higher than those recorded for the first period, due probably to the higher air temperatures. The number of hours at high temperatures (> 40 °C) could be sufficient to control several pathogenic organisms and probably affect the activity, ecology and dynamics of the whole soil biota, as also reported by other authors (Gamliel et al., 2000; Gelsomino and Cacco, 2006), who observed the same effect with 22, 23, and 14 days of temperatures between 40 and 54 °C. High temperatures also promote the breakdown of organic matter, supplied to soil, with consequent accumulation of volatile compounds damaging many soil-borne pathogens (Oka et al., 2007).

Table 1
Numbers of hours in each temperature (T) range per treatment during the first 15 days and the second 15 days of solarization at 0–10 cm and 10–20 cm soil depth.

Range T (°C)	Soil depth (cm)	Numbers of hours					
		Control		LDPE[a]		BIO[b]	
		0–15 d	16–30 d	0–15 d	16–30 d	0–15 d	16–30 d
36–40	0–10	102	88	122	123	85	122
	10–20	61	112	174	120	140	167
41–45	0–10	11	43	102	88	73	65
	10–20	0	0	93	150	70	130
46–50	0–10	0	0	41	88	57	73
	10–20	0	0	2	63	0	9
51–55	0–10	0	0	0	31	0	23
	10–20	0	0	0	0	0	0

[a] LDPE = traditional transparent low density polyethylene.
[b] BIO = transparent biodegradable film.

3.2. Chemical soil properties

In addition to the soil temperature increase, the solarization also determines an increase of soil moisture (Sofi et al., 2014), so altering the micro-climate soil conditions. In fact, an increase in water content was mainly recorded in covered soil 16.5% (mean value of two films) with respect to bare soil (15.4%; Fig. 2). However, although the control showed always the lowest soil moisture percentage, during the whole experimental period (about two months) it had a decreasing trend in all treatments (Fig. 2); notably, the differences between covered and non-covered soil have been greater after mulching films removal. Probably this behavior was due to the higher water accumulation in the solarized soil and to a consequent slower loss by evaporation, as highlighted by the different slope of curves in the Fig. 2.

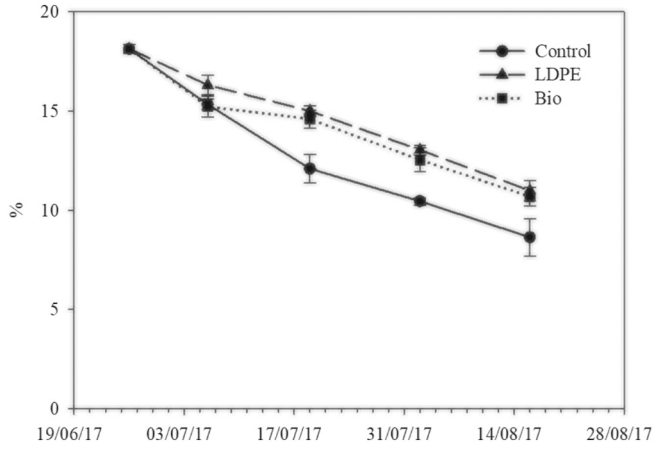

Fig. 2. Trend in the soil moisture percentage during the test period. LDPE, traditional, transparent low-density polyethylene; Bio, transparent biodegradable film; and control, bare soil.

The N-NO_3 soil content increased in all treatments until the film removal, when the increases were 93.3, 100.9% and 84.5% compared to the values of the first sampling for PE, BIO, and control, respectively (Fig. 3a). After this date, the soil N-NO_3 content showed a slight decrease, but however, at the end of the experiment the N-NO_3 values were +84.4%, +56.3 and +64.1%, for BIO, LDPE, and control, respectively, over the corresponding initial values.

The soil N-NH_4 content had a different trend; it was constant in the control with a mean value of 0.67 mg kg^{-1}, in contrast, it increased in the soils covered by the two films, with a peak at day 15, when the LDPE reached a higher value than BIO, 10.43 mg kg^{-1} and 5.01 mg kg^{-1}, respectively (Fig. 3b). Then, the N-NH_4 content decreased, but at a different rate for LDPE and BIO, which reached 3.97 mg kg^{-1}, and 2.98 mg kg^{-1}, respectively. However, at the end of experiment, the NH_4 content in the soil covered with the two films was +89.1% over bare soil. Also Birthisel et al. (2019) found that available nitrogen (NO_3 and NH_4) increased during and after solarization. The increase in N-NH_4 in solarized soil could be due to several causes: 1) the higher percentage water content in covered soil, especially in LDPE, established anaerobic conditions that could have inhibited the growth and activity of nitrifying bacteria with N-NH_4 accumulation in the soil (Fiorentino et al., 2016); 2) the soil temperature increase under mulching films could have caused the death of nitrifying bacteria, which have an optimal growth temperature around 30 °C, and below or over this threshold they limit the growth, stopping it at 45 °C (Neufeld et al., 1986).

Finally, the solarization did not affect the organic matter and total nitrogen content in the soil (Fig. 4a and b): both parameters were about constant during the whole experimental period and without differences between them. Similar findings about total organic matter content were found by Thuriès et al. (2000), Bonanomi et al. (2008) and Morra et al. (2018) which did not found differences in total nitrogen content between bare soil and soils covered with plastic and biodegradable films.

3.3. Microbial enumerations

As shown in Tables 2, 3 and 4, both prokaryotic and eukaryotic populations significantly decreased by 0.5–1 log CFU g^{-1} over time in the control soil, whereas the LDPE and BIO treatments affected the three microbial groups differently.

Although a constant reduction in the heterotrophic aerobic bacteria was observed at 15 and 30 days of solarization, decreasing from approximately 7 to 6 log CFU g^{-1} (corresponding to a 86–87% of survival rate) in both covered soils, a significant increase up to 6.81 ± 0.02 log CFU g^{-1} was observed with the BIO at the end of the experiment, corresponding to approximately 40 days after film removal (Tables 2), indicating a shorter recovery phase post thermic stress in BIO-treated soil than LDPE-treated soil. This result could be due to the higher number of hours with a temperature > 37 °C reached in the treated soils, especially with LDPE, than in the control (Table 1). The significant reduction in heterotrophic bacteria at the end of the experiment with respect to that at the beginning could be due to a low abundance of thermotolerant species. Due to spore-mediated thermotolerance, Bacilli abundance was positively affected by solarization in the long and short term (Kaanan et al., 2018). Similarly, Balakrishna et al. (2015) observed an increase in microbial biomass after solarization and assumed that it was related to the presence of bacterial populations such as spore-forming Bacilli, which can survive for long periods at high temperatures. However, although Bacilli are able to quickly recolonize treated soils and become the dominant Gram-positive bacteria after solarization, Bacilli could decrease by as much as 86% (Stapleton and De Vay, 1984).

Actinomycetes showed a similar behavior in LDPE soil, decreasing by 1 log CFU g^{-1} at 30 days of solarization (corresponding to a survival rate of approximately 77%) and significantly increasing at the end of the experiment (survival rate equal to 85%; Table 3). In the BIO soil, actinomycetes had a survival rate of approximately 76% at day 30 (4.48 ± 0.08 log CFU g^{-1}), and this rate remained constant until the end of the experiment (4.54 ± 0.18 log CFU g^{-1} and a survival rate of 76.96%; Table 3). However, since actinomycetes are heat-tolerant bacteria, their

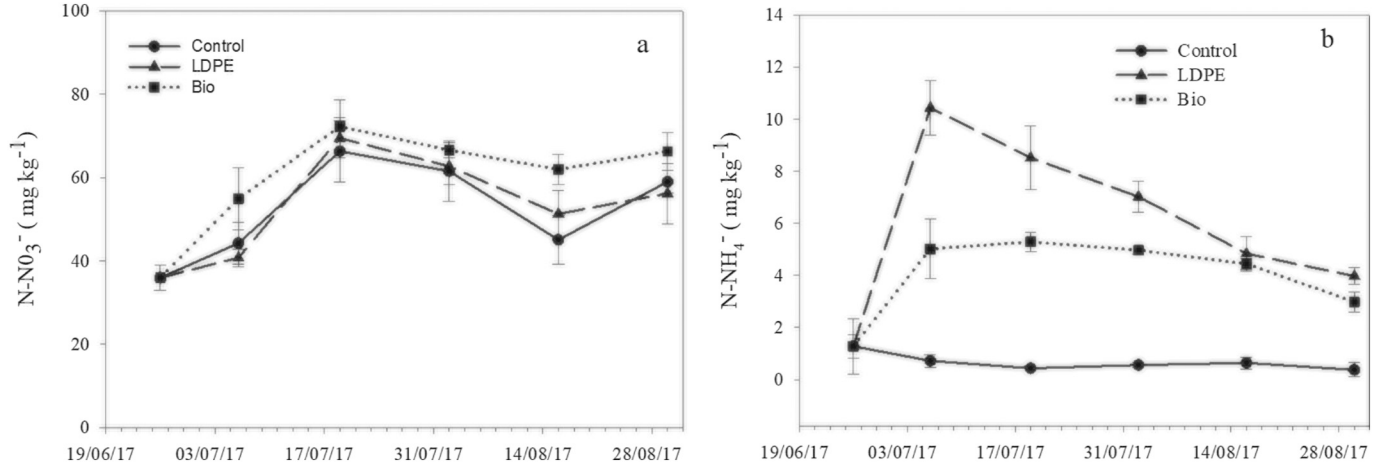

Fig. 3. Trend in nitrate (a) and ammonia nitrogen (b) during the test period. LDPE, traditional transparent low-density polyethylene; Bio, transparent biodegradable film; and control, bare soil.

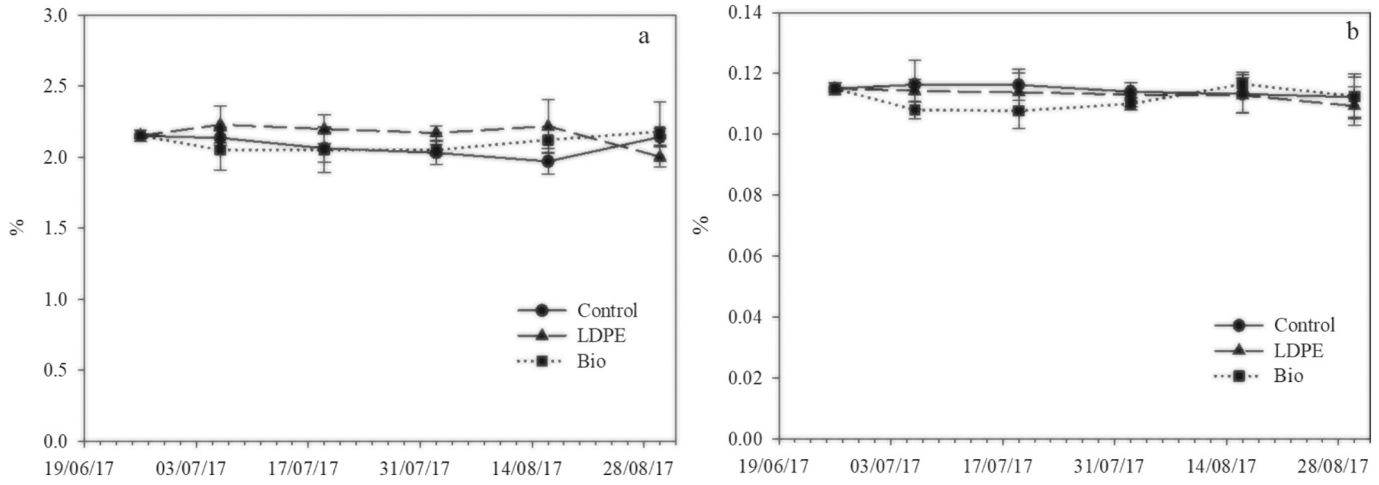

Fig. 4. Trend in organic matter (a) and total nitrogen (b) during the test period. LDPE, traditional, transparent low-density polyethylene; Bio, transparent biodegradable film; and control, bare soil.

Table 2
Enumerations (log CFU g^{-1}) and survival rate (%) of total heterotrophic aerobic bacteria in control soils (NP), in solarized soils with the transparent biodegradable film-PC17T6/35 (BIO) and in solarized soils with the traditional transparent low density polyethylene (LDPE).

Time	NP		BIO		LDPE	
	log CFU g^{-1}†	Survival rate	log CFU g^{-1}†	Survival rate	log CFU g^{-1}†	Survival rate
22/06 (0)	7.06 ± 0.14^{ab}	100	7.22 ± 0.33^{a}	100	7.04 ± 0.10^{ab}	100
06/07 (15 d)	6.84 ± 0.15^{bcd}	96.94	6.90 ± 0.14^{bc}	95.48	6.76 ± 0.08^{cd}	96.09
19/07 (30 d)	6.42 ± 0.14^{ef}	90.92	6.24 ± 0.09^{f}	86.33	6.17 ± 0.10^{f}	87.61
30/08 (40 d)	6.60 ± 0.05^{de}	93.53	6.81 ± 0.02^{bcd}	94.26	6.41 ± 0.15^{ef}	91.11

† The values represent the means ± SD of three replicates. Different letters after the values indicate significant differences ($P < 0.05$).

Table 3
Enumerations (log CFU g^{-1}) and survival rate (%) of actinomycetes in control soils (NP), in solarized soils with the transparent biodegradable film-PC17T6/35 (BIO) and in solarized soils with the traditional transparent low density polyethylene (LDPE).

Time	NP		BIO		LDPE	
	log CFU g^{-1}†	Survival rate	log CFU g^{-1}†	Survival rate	log CFU g^{-1}†	Survival rate
22/06 (0)	6.12 ± 0.01^{a}	100	5.89 ± 0.03^{b}	100	5.89 ± 0.13^{b}	100
06/07 (15 d)	5.56 ± 0.07^{c}	90.78	5.30 ± 0.07^{d}	89.94	5.34 ± 0.17^{d}	90.66
19/07 (30 d)	4.91 ± 0.17^{e}	80.20	4.48 ± 0.08^{f}	75.91	4.53 ± 0.06^{f}	76.98
30/08 (40 d)	4.98 ± 0.16^{e}	81.37	4.54 ± 0.18^{f}	76.96	5.00 ± 0.15^{e}	84.90

† The values represent the means ± SD of three replicates. Different letters after the values indicate significant differences ($P < 0.05$).

Table 4
Enumerations (log CFU g^{-1}) and survival rate (%) of mould and yeast in control soils (NP), in solarized soils with the transparent biodegradable film-PC17T6/35 (BIO) and in solarized soils with the traditional transparent low density polyethylene (LDPE).

Time	NP		BIO		LDPE	
	log CFU g^{-1}†	Survival rate	log CFU g^{-1}†	Survival rate	log CFU g^{-1}†	Survival rate
22/06 (0)	4.89 ± 0.20^{a}	100	4.55 ± 0.42^{ab}	100	4.47 ± 0.26^{b}	100
06/07 (15 d)	4.56 ± 0.29^{ab}	93.26	4.18 ± 0.32^{bc}	91.92	3.84 ± 0.26^{c}	85.99
19/07 (30 d)	4.14 ± 0.10^{bc}	84.77	4.03 ± 0.08^{c}	88.65	4.01 ± 0.13^{c}	89.81
30/08 (40 d)	4.22 ± 0.10^{bc}	86.37	4.26 ± 0.08^{bc}	93.75	4.01 ± 0.10^{c}	89.83

† The values represent the means ± SD of three replicates. Different letters after the values indicate significant differences ($P < 0.05$).

significant reduction over time in the BIO soil and non-covered control soil was not due to the temperature, which reached its highest values in the LDPE-treated soil, but to other environmental factors such as changes in soil properties, nutrient availability and microbial competitiveness (Gelsomino and Cacco, 2006). In fact, a previous study reported that actinomycetes were less affected by solarization than other bacteria, revealing a reduction of up to 58% in solarized soil (Stapleton and De Vay, 1984). Moreover, the behavior of actinomycetes in the BIO soil was interesting, and their steady presence in the soil was a positive achievement. In fact, they belong to one of the dominant prokaryotic taxon living in the soil, which contains many beneficial microbes with different plant growth promoting activities, and is known to exert useful effects on several crop plants (Jog et al., 2012, 2014; Gopalakrishnan et al., 2014). Therefore, the presence of actinomycetes indicated a high biological fertility potential of the agricultural soils because of the improvements in the fitness and growth of crops and their function as antagonists to phytopathogens (Ventorino et al., 2019).

Finally, in the soils covered with the BIO film, filamentous fungi and yeast loads remained constant over time, ranging from 4.03 to 4.55 log CFU g^{-1} (Table 4), whereas a significant decrease from 4.7 to 3.8–4.0 log CFU g^{-1}, corresponding to an approximately 86–89% survival rate, was detected when soils were covered with the LDPE film for 15 or 30 days

(Table 4). This behavior could be linked to lower soil temperatures in BIO (Table 1). A similar general microbial trend was observed by Gupta et al. (2017), who detected a significant reduction in fungal and bacterial populations due to solarization under protected conditions, recording the maximum reduction (also up to 82%) in the population of actinomycetes. However, soil type was found to influence the efficiency of solarization that, in turn, affected microbial communities living in the soil (Khlaif, 2003).

3.4. Prokaryotic and eukaryotic populations in soil

PCR-DGGE was employed to obtain a qualitative fingerprint of the bacterial and fungal communities due to the effect over time of the cover films on microbial soil diversity. The main results indicated that solarization treatment was the major determinant of the composition and structure of the prokaryotes and eukaryotes because it, more than sampling time, determined the clustering into groups (Figs. 5 and 6). The DGGE patterns of prokaryotes in the soil samples shown in Fig. 5 were very complex, producing 42–57 bands. DGGE profiles indicated that solarization did not reduce the richness of bacterial populations since the number of DGGE bands did not differ among the treatments. Although Schönfeld et al. (2003) also observed no significant difference in the bacterial DGGE band patterns between the solarized and control soils, others (Gelsomino and Cacco, 2006; Yokoe et al., 2015) reported a reduction in the number of DGGE bands by solarization. These strong differences in the effect of solarization on microbial community composition could be due to the differences in the soil type and conditions, especially soil temperatures during solarization (Yokoe et al., 2015) or to the different solarization time (also up to 72 days as by Gelsomino and Cacco, 2006). Statistical analysis of the position and intensity of the bands allowed the classification of three major clusters clearly associated with the cover films applied to the soils (Cluster 1: control soil NP, Cluster 2: soil treated with BIO film, and Cluster 3: soil treated with LDPE film) in which slight changes within the prokaryotic populations were observed (similarity level from 93/94 to 98% in the BIO and LDPE soils and from 86 to 97% in the control soil). Clusters 2 and 3, comprising covered-treated soils, were very similar, demonstrating a similarity of 89%, while Cluster 1 had a similarity as high as 76% with the assembly of these two groups (Fig. 5). Gelsomino and Cacco (2006) indicated that soil solarization was the main factor inducing population shifts in the eubacterial DGGE profiles, indicating strong changes in the community structure. It was interesting to note that within each of the major clusters delineated by the solarization treatment, the subgroupings of the prokaryotes were always similar and determined by the sampling time. The bacterial shift over time could have been correlated to the variations in the temperature values recorded during the experiment (Fig. 1; Table 1).

Eukaryotic populations showed relatively simple profiles generating 25–47 bands (Fig. 6), especially at the beginning of the experiment, from which 25, 33 and 38 different bands were enumerated in BIO, NDPE and

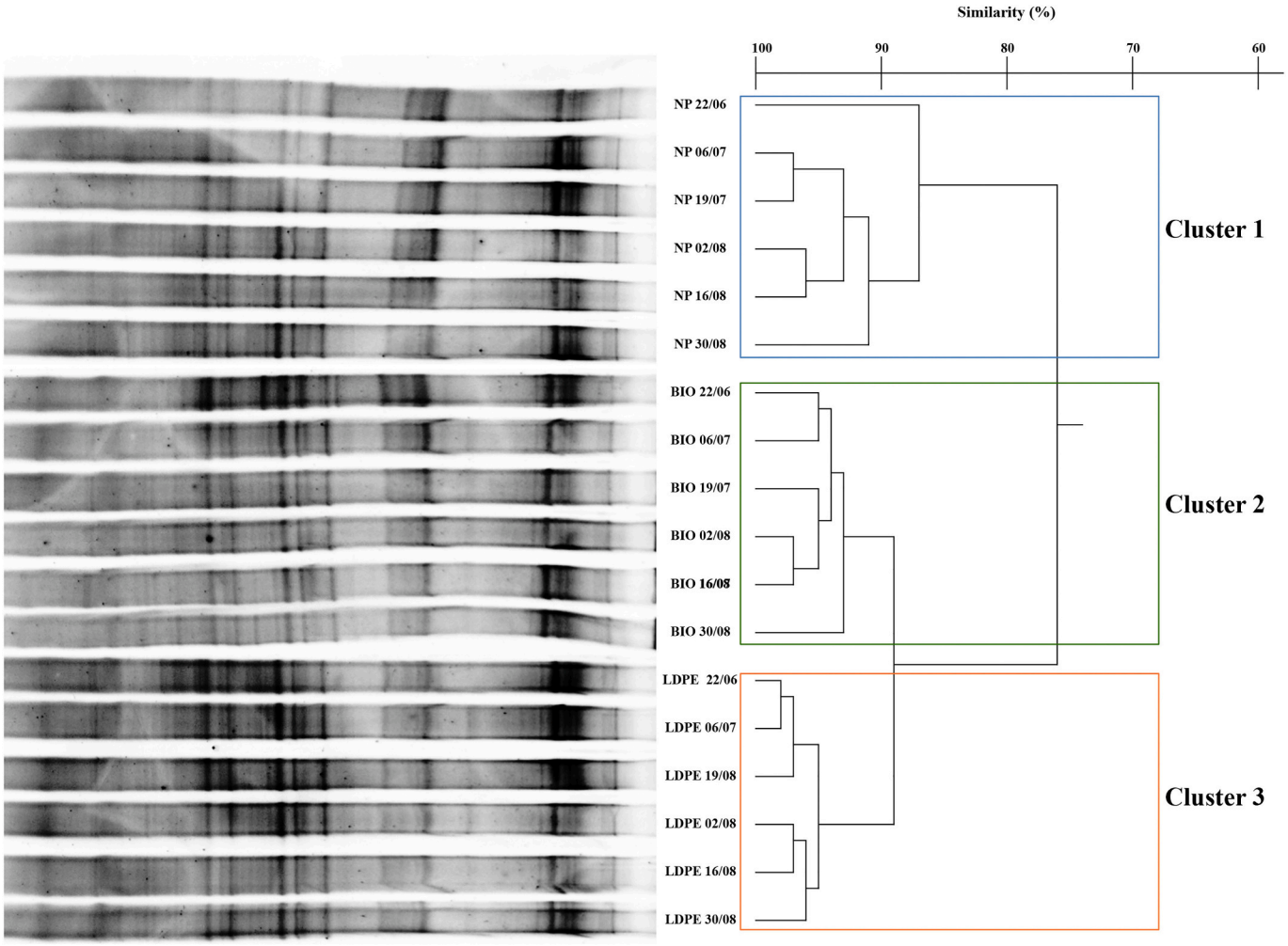

Fig. 5. DGGE profiles and dendrogram showing the degree of similarity (%) of the PCR-DGGE profiles of the prokaryotes in the soil samples. NP, non-solarized control soil; BIO, solarization with the transparent biodegradable film-PC17T6/35; and LDPE, solarization with the traditional, transparent low-density polyethylene. The number after the treatment indicates the sampling date.

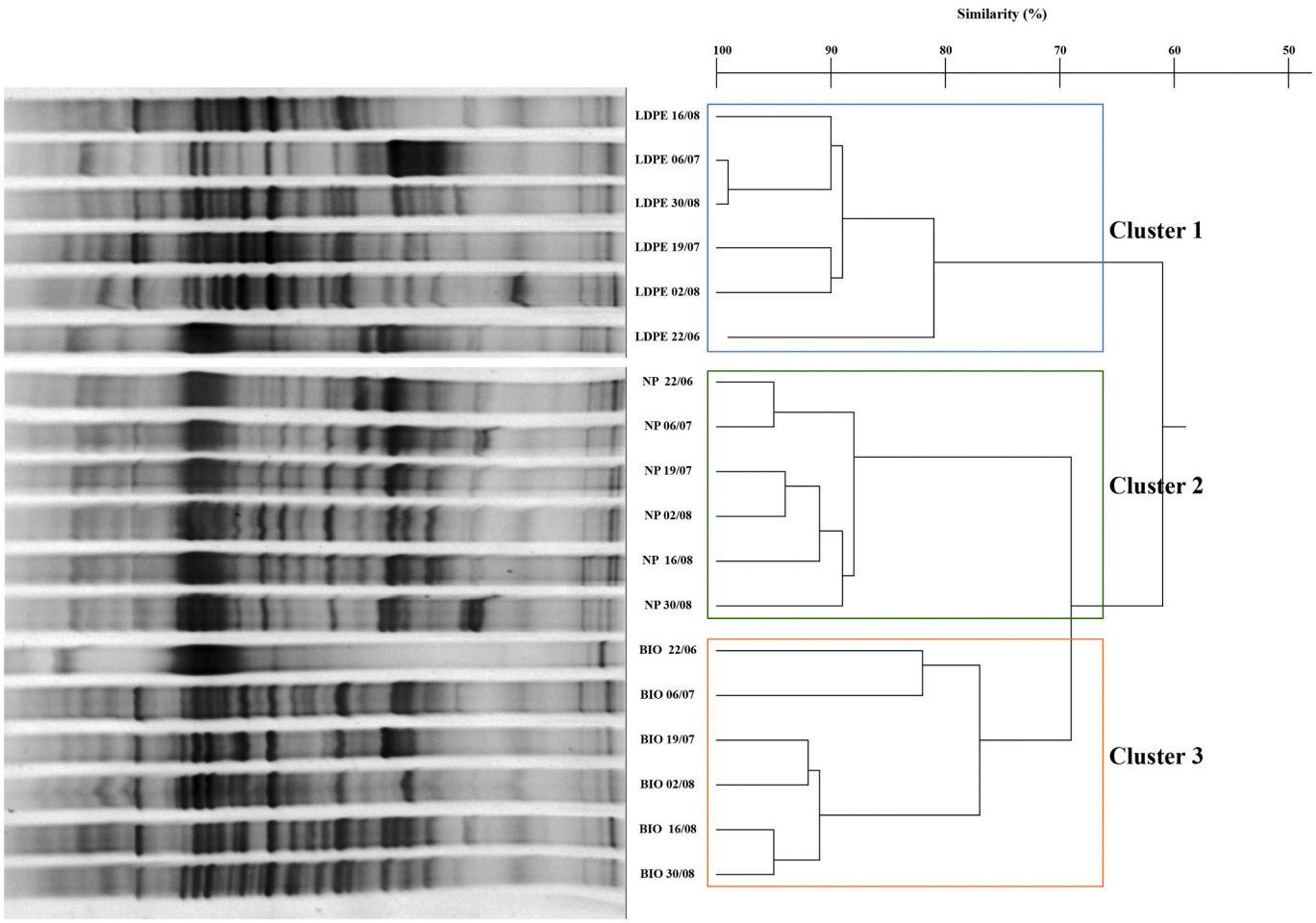

Fig. 6. DGGE profiles and dendrogram showing the degree of similarity (%) of the PCR-DGGE profiles of the eukaryotes in the soil samples. NP, non-solarized control soil; BIO, solarization with the transparent biodegradable film-PC17T6/35; and LDPE, solarization with the traditional, transparent low-density polyethylene. The number after treatment indicates the sampling date.

PE, respectively. However, the number of bands increased over time in all soils, although in the BIO-treated soil, the lowest fungal biodiversity was observed. Similar to the prokaryotic results, statistical analysis of the fungal DGGE profiles determined that there were three major fungal groups associated with the cover films that were applied to the soils: Cluster 1: soil treated with LDPE film, Cluster 2: control soil NP, and Cluster 3: soil treated with BIO film (Fig. 6). In particular, Cluster 1 showed a similarity level of 61% with Clusters 2 and 3, while Cluster 2 showed a similarity as high as 69% with Cluster 3. Similar to the trend in prokaryotic soil populations, within each of the major clusters of the BIO and control soils (Clusters 2 and 3), eukaryotic diversity exhibited subgroups determined by the sampling time (Fig. 6). As for prokaryotic populations, the subgroups related to sampling time and temperature in the eukaryotes are a clear evidence of changes indexes linked to recovery post stress. However, in Cluster 1, representing the LDPE-treated soil, fungal biodiversity was not determined by sampling time. The shift in microbial populations and, in some cases, the increase in microbial richness could have been due not only to direct thermal effects but also to other environmental and ecological factors, such as changes in soil properties and microbial habitats, an increase in nutrient availability, a reduction in microfaunal predators and the competitiveness of the dominant microbial species (Gelsomino and Cacco, 2006). The change in the microbial populations led to the establishment of a new balance among prokaryotic and eukaryotic populations and to the development of a change in the microbial community diversity.

4. Conclusions

The biodegradable film used in this work led to a soil temperature slightly lower than that recorded in soil covered with conventional films, but both films did not affect organic matter and total nitrogen content. The behavior of the two films was different with respect to the available nitrogen ($N\text{-}NH_4$ and $N\text{-}NO_3$): the biodegradable film avoided a high ammonia concentration in the soil, thanks to both lower soil water content and slightly lower temperatures than polyethylene film, and these conditions probably have been optimal for the growth of nitrifying bacteria which were more efficient in BIO, as highlighted not only by lower ammonia value but also by higher nitrate value. Moreover, the thermal effect as well as other environmental and ecological factors, such as changes in soil properties and microbial habitats, affected the prokaryotic and eukaryotic populations that were associated with the different cover films applied to the soils. In fact, microbial counts highlighted a different behavior modulated with the two films indicating that the time needs the microbial community to recover from the thermic stress induced by solarization was shorter in BIO-treated soil than LDPE-treated soil. Further, the changes in prokaryotic and eukaryotic populations determined by the sampling time and temperature are interesting indexes of changes linked to recovery post stress. This change led to the establishment and development of a new dominant microbial community.

Therefore, under our conditions, the biodegradable film appears to be a suitable replacement for traditional PE plastic film for soil solarization, with great environmental benefits.

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Declaration of competing interest

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

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