J Polym Environ (2008) 16:109–122
DOI 10.1007/s10924-008-0091-x

ORIGINAL PAPER

Performance and Environmental Impact of Biodegradable Films in Agriculture: A Field Study on Protected Cultivation

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Published online: 3 May 2008

Abstract The performance, the degradability in soil and the environmental impact of biodegradable starch-based soil mulching and low tunnel films were assessed by means of field and laboratory tests. The lifetime of the biodegradable mulches was 9 months and of the biodegradable low-tunnel films 6 months. The radiometric properties of the biodegradable films influenced positively the microclimate: air temperature under the biodegradable low tunnel films was 2 °C higher than under the low density polyethylene films, resulting in an up to 20% higher yield of strawberries. At the end of the cultivation period, the biodegradable mulches were broken up and buried in the field soil together with the plant residues. One year after burial, less than 4% of the initial weight of the biodegradable film was found in the soil. According to ecotoxicity tests, the kinetic luminescent bacteria test with *Vibrio fischeri* and the *Enchytraeus albidus* ISO/CD 16387 reproduction potential, there was no evidence of ecotoxicity in the soil during the biodegradation process. Furthermore, there was no change in the diversity of ammonia-oxidizing bacteria in the soil determined on the basis of the appearance of *amoA* gene diversity in denaturing gradient gel electrophoresis.

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Keywords Starch based material · Radiometric properties · Biotest · Microbial community · Ammonia-oxidizing bacteria

Introduction

Plastic films made of low-density polyethylene (LDPE) are commonly used in agriculture as coverings for greenhouse or low tunnel and for soil mulching in order to increase the yield and quality of horticultural products. The purpose of the greenhouse plastic covering is, in addition to protection of the plants from atmospheric agents, to increase the greenhouse internal air temperature, thus lengthening the market availability of the products. The plastic films used for soil mulching reduce water and pesticide consumption, soil-borne pathogens and protect the cultivation area against erosion; black mulching films reduce the growth of weeds.

The annual consumption of plastic films for greenhouses, low tunnels and mulching is about 1.3 million tonnes world-wide [1]. During exposure in the field, the plastic films are subjected to degradation resulting from solar radiation, wind, hail, snow, high air temperature and relative humidity and thermal cycling, as well as the chemical products used during cultivation [2–5]. As a consequence, the plastic films become brittle and their useful lifetime is reduced. The lifetime of the plastic films ranges from a few months to 3–4 years depending on the thickness of the material and on the stabilising additives that protect the film from the ultra-violet (UV) fraction of solar radiation [4, 5]. After use, the plastic is classified as waste and can be disposed of in many different ways. The plastic waste can be transported to landfills, collected and recycled, or burned in incineration plants to produce

energy. The recovered plastics are heavily contaminated with soil and biological waste, making the recycling process expensive and time-consuming [6]. Owing to the challenging disposal problems, plastic waste is often left on the side of the field or burned in uncontrolled conditions. Moreover, because plastics are of fossil fuel origin and their use is expanding rapidly, especially among the large developing countries such as China and India, this clearly puts pressure on the already limited non-renewable resources [7].

In order to increase the sustainability of agricultural practices and to overcome the disposal problems with conventional plastics, films based on biodegradable and renewable agricultural raw materials can nowadays be used to an ever-increasing extent [8–14]. At the end of their lifetime, biodegradable films can be disposed of directly in the soil or in a composting plant together with the normal organic waste stream. This appears to be a suitable solution especially for biodegradable mulching films as the films can be broken up and ploughed into the soil at the end of their lifetime. When biodegradable plastics degrade in soil, there should be no formation of toxic residues [15]. At the same time, the functionality of biodegradable films must be guaranteed during their use in field with the same cultivation techniques currently used with LDPE-based films.

The use of biodegradable films in agriculture is at the research stage [16–22] and more information is needed about their performance during the planting to harvesting stages, and about their possible environmental impact. Biodegradable materials must have physical properties comparable to those of LDPE plastic films in order for them to be suitable for crop protection. Among the physical properties, the radiometric characteristics of the covering films play an important role in determining the microclimate under the protected area and in regulating plant growth [23]. The increase in the air temperature inside a greenhouse or low tunnel compared to the external air temperature, known as the 'greenhouse effect', is achieved by allowing solar radiation to pass through the film whilst reducing the convective and radiative losses of energy. This is strongly dependent on the radiometric properties of the covering material, such as its transmissivity [23].

In addition to having good physico-mechanical properties and being suitable for the target applications, such material should be biodegradable to harmless end products in compost or in the soil [24]. In agricultural applications, like mulching, the biodegradable materials are in direct contact with the soil. In order to cause no undesirable effects to the performance of agricultural soil, there should be no accumulation of harmful substances from the biodegradable materials used. Determination of the ecotoxicological properties is included in the evaluation of the environmental impact of biodegradable films [24]. In addition to conventional toxicity studies, soil health can also be studied using molecular biological methods [25]. Ammonia-oxidizing bacteria (AOB) play an important role in the global nitrogen cycle as well as in agricultural soils. The diversity of AOB in soil has been used as an indicator of soil health by evaluating the appearance of the *amoA* gene. The *amoA* gene encodes the α-subunit of ammonia monooxygenase, which catalyzes the first step in the conversion of ammonia to nitrite, and the conversion of ammonia to hydroxylamine [26–29].

The aim of the present research was to evaluate the performance and the environmental impact (with respect to ecotoxicity in the soil) of the starch-based biodegradable films used for crop protection. A full-scale field experiment was conducted in Southern Italy using biodegradable films for soil mulching and for low tunnel covering in the cultivation of strawberry plants. The behaviour and radiometric properties of the biodegradable materials were compared with the LDPE plastic films commonly used in protected cultivation. Attention was focused on the environmental impact of the biodegradable mulching films, which remained for 9 months in contact with the soil and which were ploughed into the soil at the end of cultivation. The environmental impact was evaluated by conducting ecotoxicity tests and assessing possible changes in the AOB population. The ecotoxicity tests applied were the kinetic luminescent bacteria test for acute toxicity and *Enchytraeus albidus* for the reproduction potential of the soil fauna.

Materials and Methods

Experimental Field and Sampling

Evaluation of the performance of the starch-based biodegradable films and of their environmental impact was based on experiments carried out at the "Pantanelli" Agriculture Experimental Station of the University of Bari at Policoro (Matera), Italy. The latitude of the site was 40°13′ N, longitude 16°40′ E and altitude 31 m. A full-scale field experiment was conducted from autumn 2001 to summer 2003 on a flat area of 5,100 m^2. The soil characteristics of the cultivation site are given in Table 1. The trial was carried out using three biodegradable black mulching films (M1, M2 and M3) and two biodegradable transparent low tunnel films (L1 and L3) in a protected strawberry cultivation. Two non-biodegradable LDPE-based films, a black mulching film (M0) and a transparent low tunnel film (L0), were used as a conventional practice reference.

The biodegradable films were manufactured in cooperation by three industrial companies. The starch-based raw material, under the trade name Mater-Bi, made of

Table 1 Soil properties of the experimental field

Soil textural classes	45.94% clay 39.25% silt 14.81% sand	[30]
pH in H_2O	7.60	[31]
pH in KCl	7.12	[31]
Conductivity of saturated soil paste	1.22 mS/cm	[30]
Total $CaCO_3$ concentration	74.15 g/kg	[32]
Active $CaCO_3$ concentration	51.07 g/kg	[32]
Organic matter	32.11 g/kg	[30]
Organic matter Loss of weight on ignition (LOI)	7.0%	Dry soil at 550 °C for 15 h
Total nitrogen concentration	1.75 g/kg	[33]
Assimilable phosphorus concentration	34.25 mg/kg	[34]
Exchangeable potassium concentration	313 mg/kg	[30]
C/N ratio	10.64	[30]
Cationic exchange capacity	23.44 meq/100 g	[30]
Water total holding capacity	56.28%	[30]
Dry weight	83.9%	Overnight at 105 °C

destructurised starch complexed with biodegradable polyesters, was supplied by Novamont S.p.A (Novara, Italy) [35]. The extrusion was made by Pati S.p.A (San Zenone degli Ezzelini, Treviso, Italy) for the M1, M2 and L1 films, and by Plastika Kritis S.A (Heraklion, Crete, Greece) for the M3 and L3 films. The L1 and L3 low tunnel films, with a thickness of 60 μm, were UV stabilised. L1 was stabilised by adding 1.8% of additive, i.e. 2,000 ppm of triazine UV absorber in ethylene vinyl acetate (EVA) polymer carrier with 7% of vinyl acetate, while L3 was produced by adding 1% of masterbatch with UV stabiliser. The M1 and M2 mulching films, with a thickness of 50 μm and 30 μm, respectively, contained 32,000 ppm of carbon black. The M3 mulching film, with a thickness of 25 μm, contained 9% of carbon black masterbatch. The M0 LDPE commercial mulching film (thickness of 50 μm) and the L0 LDPE commercial low tunnel film (thickness of 60 μm) were supplied by PATI S.p.A.

Eight different combinations of the film materials were studied: M1-L1, M2-L1 and M3-L3 with mulching and low tunnel biodegradable films; M0-L0 with LDPE materials; M1-L0, M2-L0, M3-L0 and M0-L1 with biodegradable and LDPE films. Each material combination, covering a soil surface of 10 m × 1 m, was replicated three times (a, b, and c) in a randomized block design in the experimental field (Fig. 1).

The mulching films were installed mechanically in September 2001; low tunnel films were mounted on low

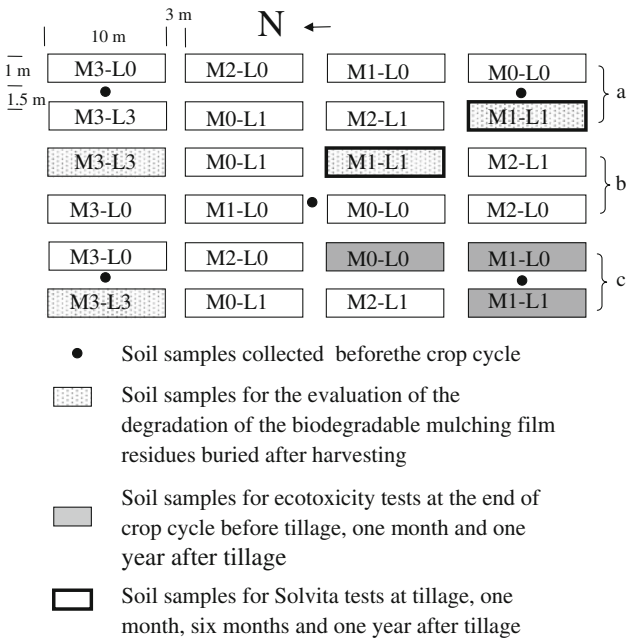

Fig. 1 Layout of the soil sampling locations in the experimental field

steel arches in order to cover the strawberry plants growing in mulched soil in January 2002 (Fig. 2). The longitudinal axis of the low tunnels was north-south oriented. In July 2002, after the strawberries were harvested, the biodegradable low tunnel films were dismantled and moved away from the cultivation area. The biodegradable mulching films were tilled together with soil and plants by a milling machine in order to break up the mulching biodegradable films and to bury them, thus accelerating the degradation process. The LDPE mulching and low tunnel films were removed and disposed of at a plastic waste collection centre. Afterwards the experimental area was left uncultivated in order to permit soil sampling for

Fig. 2 Low tunnel and mulching films in the experimental protected strawberry cultivation

monitoring biodegradation and ecotoxicity, as well as any possible changes in the microbial diversity of AOB.

During the test period, both the weather conditions and the microclimatic parameters under the soil and the low tunnels were recorded by a data logger with sensors. The data logger (Tecno El, Rome, Italy) measured the parameters with a 60 s frequency and stored the hourly average values. Air temperature and relative humidity were collected at the site. Besides the air temperature and relative humidity inside the low tunnels, the soil temperature under the mulching films at 20 cm depth was also measured and recorded. PT100 platinum resistance thermometers (Tecno El, Rome, Italy) were used for measuring the air and soil temperature. Both dry and wet bulb temperatures were measured in order to obtain the relative air humidity.

During strawberry harvesting, which took place from April to June 2002, agronomic analyses were carried out in order to record the marketable yield.

Degradation and Microbial Activity

The degradation of the mulching films residues in the soil was investigated after the cultivation period. Soil samples were taken periodically from 1 m^2 of soil surface in the M1-L1a, M1-L1b, M3-L3b, M3-L3c blocks (Fig. 1) at a depth of 0.20 m, which was the depth to which the digging tools of the milling machine penetrated. The soil samples were sieved through a 1.8 cm mesh, placed on the ground at an angle of 45°. The residues of biodegradable film that did not pass through the mesh were collected and weighted.

In order to define soil microbial activity affecting the degradation process, Solvita™ Soil Life Tests (Woods End Research laboratory, Inc.) that measures the soil microbial respiration rate were performed. The Solvita™ Soil Life Test was carried out in the field using fresh soil samples from the M1-L1a and M1-L1b blocks (Fig. 1) at tillage, and 1 month, 6 months and 1 year after tillage. The results are expressed as numbers ranging from 5 (very high soil respiration activity) to 0 (no soil respiration activity).

Radiometric Tests

The radiometric properties of the films were analysed as follows. Total and direct transmissivity in the wavelength range 200–2,500 nm were measured with a Perkin–Elmer UV-VIS-NIR Lambda 950 spectrophotometer (Norwalk, USA). An integrating sphere was used as receiver of the Lambda 950 spectrophotometer to evaluate total transmissivity. The diffuse transmissivity was evaluated by subtracting the direct transmissivity from the total transmissivity. The transmissivity coefficients were calculated as weighted average values of the transmissivity over the wavelength interval 300–2,500 nm for the solar range, and 400–700 nm for the photosynthetically active radiation (PAR) range, using the spectral distribution of the terrestrial solar radiation as weighting function [23]. The transmissivity coefficient in the solar wavelength range represents the fraction of solar radiation passing through the material: the higher the value of the solar transmissivity coefficient, the higher the temperature rises inside the low tunnel.

The transmissivity in the long wave infrared radiation (LWIR) range between 2,500 nm and 25,000 nm was measured by a Perkin–Elmer FT-IR 1760 X spectrophotometer. The LWIR transmissivity coefficient was calculated as the average value over the wavelength interval 7,500–12,500 nm, at which bodies at ambient temperature have the maximum energy emission as expressed by the Planck's spectral distribution of emissive power [23, 36].

Ecotoxicity and Environmental Impact

From autumn 2001 to summer 2003 soil samples were collected in the experimental area in order to study the effect of the usage and biodegradation of the mulching films on soil ecotoxicity and microbial diversity. Soil samples were collected in the field from the M0-L0c, M1-L1c and M1-L0c blocks as shown in Fig. 1. Sampling was performed before starting the crop cycle, at the end of the cultivation period before tillage (28/06/2002), 1 month after tillage (09/09/2002), and about 1 year after tillage (08/07/2003). At each sampling time, a composite sample consisting of five randomly selected soil cores were taken down to a depth of 0.2 m over an area of 10 m^2 and mixed to obtain samples for the ecotoxicity tests and microbial community analysis. Samples for the ecotoxicity tests were selected as follows: M0 and L0 served as a reference for conventional practice, M1 was the thickest of the biodegradable mulching films and L1 had the highest UV stabilizer content. The samples were stored at −18 °C. Dry weight and organic matter content of all the soil samples were determined using the methods described in Table 1.

The Flash test is a kinetic application of the luminescent bacteria test and is especially tailored for determining the toxicity of solid and coloured samples [37, 38]. It was used to measure the acute toxicity of soil samples collected during the course of the field experiment. The test organism *Vibrio fischeri* was treated as instructed in BioTox™ Kit (Aboatox Oy, Finland). The inhibition of light production was determined on a 1251 Luminometer (Bio-Orbit, Turku, Finland). Kinetic measurement was carried out according to Lappalainen et al. [37] with soil sample concentrations of 100 g/L. Luminescence was measured kinetically throughout the 30 s exposure time, and the peak luminescence value was obtained during the first 5 s after

adding the bacterial suspension to the sample. In addition, the level of luminescence was recorded after 30 min exposure time. The results were calculated as instructed in ISO/CD 21338 [38] and expressed as inhibition percentages.

The ISO/CD 16387 [39] standard method measures the response of an encytraeid worm (*Enchytraeus albidus*), also known as the plant root worm to chemicals. Mortality and reproductive potential of the test organism are used to assess the toxic effect. Carbendazim was used as a reference chemical. According to the standard, the test is performed in an artificial soil substrate. However, the test substrate had to be modified in order to be able to test field soil. Field soil was mixed with standard soil in the ratio 3:5 (dry weight). The results were expressed as juveniles produced per adult. Two separate tests were performed. In the first test the soil samples tested were as follows: before starting the crop cycle (START) and 1 month after tillage. The samples taken 1 year after tillage were tested in the second test.

Ammonia Oxidizers by PCR-DGGE

Changes in the microbial population of ammonium oxidizers were studied by the polymerase chain reaction-denaturing gradient gel electrophoresis method (PCR-DGGE). Total DNA extraction from 0.5 g of soil and purification for the PCR-detecting *amoA* gene were performed according to Stephen et al. [40]. Primers detecting *amoA* (*amoA*-1FGC and *amoA*-2RTC) producing a 490 bp product in PCR were adopted from Rotthauwe et al. [29], Oved et al. [28], and Nicolaisen et al. [27] with slight modifications. GC clamp (GC: 5′-CCGCCGCGCGGCG GGCGGGGCGGGGGCACGGGG-3′, [41]) was added to the forward primer in order to be able to perform DGGE. *Nitrosomonas europaea* served as a positive control in PCR and DGGE.

PCR analysis was conducted as follows: initial denaturation of DNA at 94 °C for 5 min and amplification in 35 cycles at 94 °C (30 s), 59 °C (2 min) and 72 °C (40 s) in 50 μL reaction volume with Dynazyme II using Mastercycler gradient (Eppendorf, Germany). The structure of the ammonium oxidizer community was analyzed by the DGGE method. PCR products were loaded on 8% polyacrylamide gel with a denaturing gradient from 30% to 70% (100% denaturant = 7 M urea, 40% [vol/vol] formamide). Electrophoresis was run in 0.5 × TAE buffer (20 mM Tris acetate, 0.5 mM EDTA, pH 8) at 60 °C for 17 h according to Muyzer et al. [41] on the Decode universal mutation detection system (Biorad, CA, USA). The gel was stained with SYBR Green I (BioWhitaker Molecular Application, USA) and analysed with the Gel DocTM2000 gel documentation system (Biorad, CA, USA). Dominant bands representing a range of different AOB species were cut from the acrylamide gel, DNA was diluted to water and reamplified as described above. The PCR product was purified with QIAquick PCR Purification Kit (Qiagen, Germany). ABI Prism BigDay TERMINATOR v3.1 Cycle Sequencing Kit (PE, Applied Biosystems, UK) was used to prepare the sample for sequencing carried out on an ABI Prism 310 genetic analyzer (Applied Biosystems, USA). The sequences were studied by comparing them with the GenBank sequences using the Blast search [42]. The studied sequences were also aligned with ClustalW, WWW Service at the European Bioinformatics Institute (http://www.ebi.ac.uk/clustalw, Rodrigo Lopez, Services Program). The sequences recovered from DGGE bands were submitted to GenBank under the accession numbers EU076732, EU076733 and EU076734.

Results

Performance

Lifetime of the Films

During experimental cultivation of strawberry plants the biodegradable films showed sufficient mechanical resistance to guarantee material functionality in providing crop protection from planting to harvesting. The M1, M2 and M3 black biodegradable mulching films remained almost intact throughout the entire 9-month cultivation period. The buried edges of the films functioned satisfactorily to hug the soil bed. In addition, the biodegradable mulching films controlled weeds, reduced water evaporation from the soil, and kept the strawberry fruits clean as well as the LDPE mulching films. The transparent low tunnel biodegradable films lasted for the required 6 months, protecting crop from atmospheric agents such as wind, rain, hail and snow. The materials also retained sufficient mechanical strength to allow the normal practice of manually opening and closing the low tunnels for horticultural operations and for natural ventilation in relation to the agronomical thermal requirements.

Radiometric Properties and Microclimate

The transmissivity coefficients of the tested films in the solar, PAR and LWIR ranges are shown in Fig. 3. The mulching materials, i.e. the M1, M2 and M3 biodegradable films, had a PAR total transmissivity coefficient lower than 0.01% and were opaque, like the M0 LDPE film, in this wavelength range. Of the low tunnel covering materials, the L0 LDPE film had the highest solar total transmissivity coefficient of 91.12%. The L1 and L3 biodegradable films had a coefficient of 80.81% and 81.21%, respectively. The

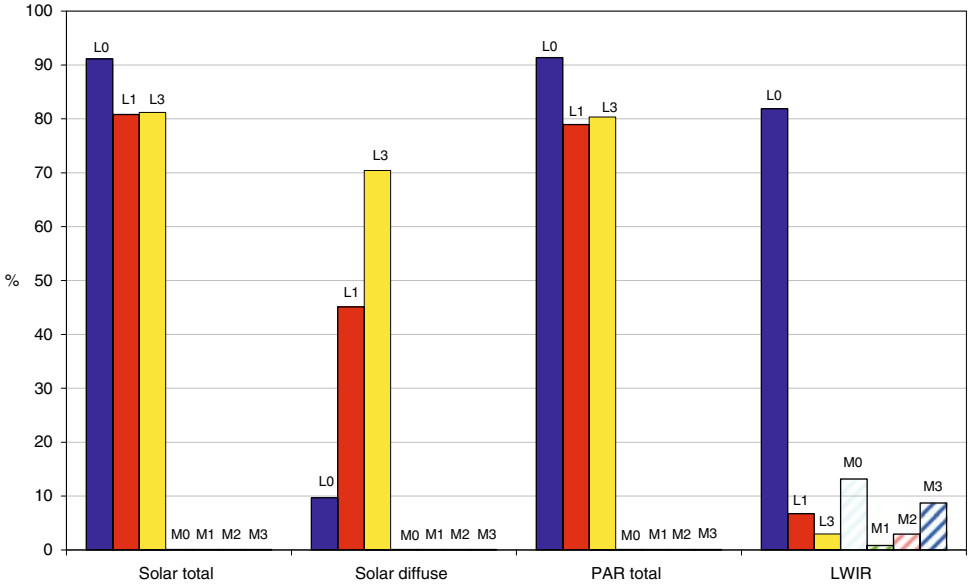

Fig. 3 Transmissivity coefficients of the films used on the experimental field in the solar, PAR and LWIR range; PAR, photosynthetically active radiation; LWIR, long wave infrared radiation

diffuse fraction of the transmitted solar radiation of the L1 and L3 biodegradable films was higher (45.13% and 70.42%, respectively) than that of the L0 LDPE film (9.68%). The different diffusive capacity of the low tunnel films was also reflected in the transmissivity curves of the L0 LDPE film and of the L3 biodegradable film (Fig. 4). There were also differences in the long wave infrared transmissivity between the biodegradable films and the LDPE film (Fig. 5). The LWIR transmissivity coefficient was 6.74% for the L1 film, 2.98% for the L3 film, and 81.88% for the L0 LDPE film.

There were differences in the air temperature under the low tunnels constructed of the LDPE films, M0-L0, and of the biodegradable films, M1-L1, M2-L1 and M3-L3. Figure 6 shows the average values of the maximum, minimum and mean (denoted afterwards simply as maximum, minimum and mean) daily soil temperature under the mulching films and of the temperature and relative humidity of the air under the low tunnels. The temperature of the uncovered soil and external air temperature, as well as the relative humidity, are presented for comparison. The average value of the daily soil temperature (Fig. 6a) was measured during the cultivation period. The air temperature (Fig. 6b) and relative humidity (Fig. 6d) were recorded from 25th of March to 2nd of April 2002, when the low tunnels were closed. Figure 6c shows the air temperature when the low tunnels were open from 5th to 24th of April 2002.

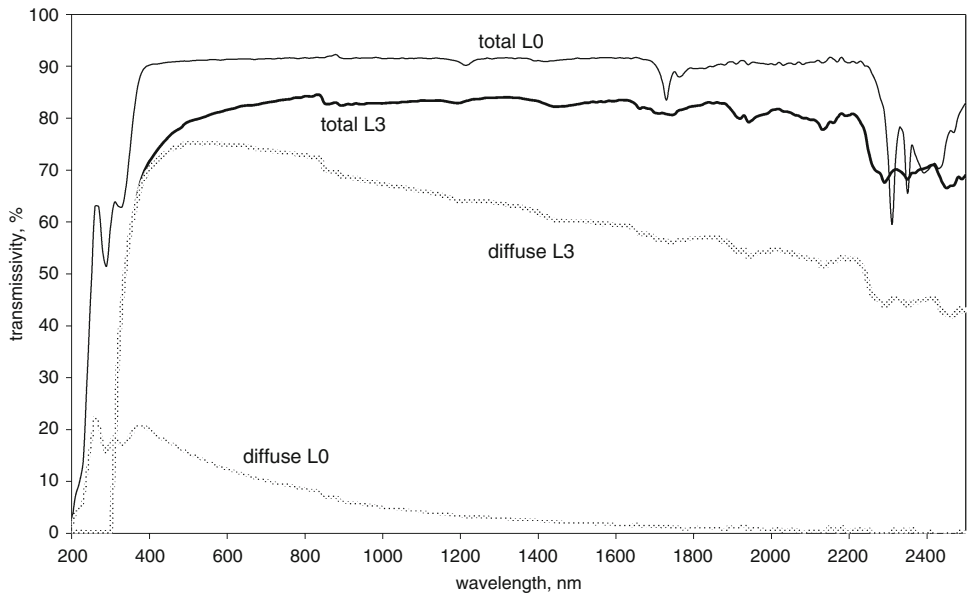

Fig. 4 Total and diffuse transmissivity as a function of the wavelength of the L0 LDPE film and of the L3 biodegradable film in the solar wavelength range 200–2,500 nm

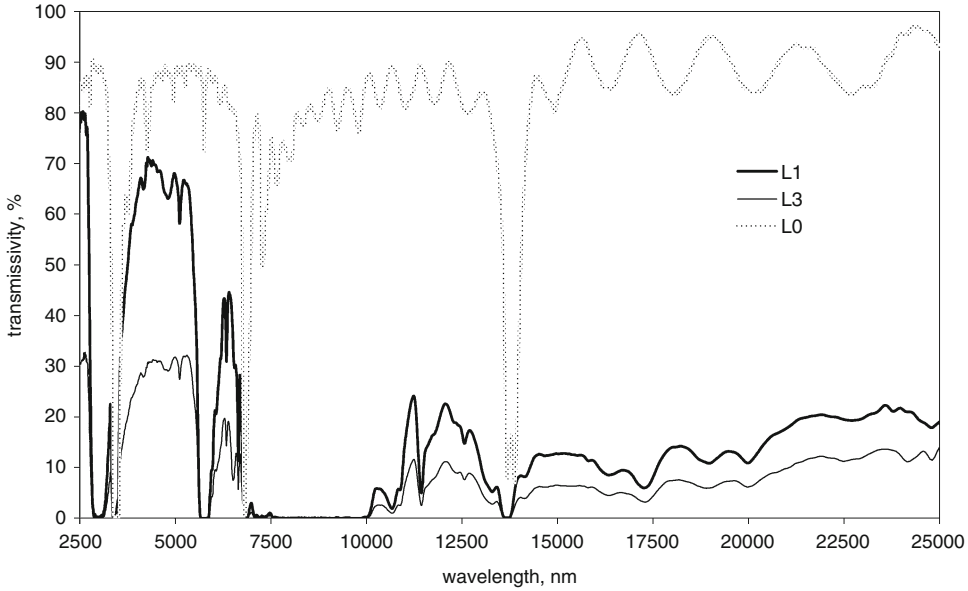

Fig. 5 Transmissivity as a function of the wavelength of the L1 and L3 biodegradable films and of the L0 LDPE film in the long wave infrared range 2,500–25,000 nm

Mean soil temperature ranged from 13.4 °C under the M0 mulching film of M0-L0 to 14.4 °C under the M2 mulching film of M2-L1 (Fig. 6a). The uncovered soil recorded a value of the mean temperature equal to 14.0 °C. When the low tunnels were closed, the highest mean air temperature (11.9 °C) occurred inside the low tunnels constructed of M2-L1 and the lowest (9.9 °C) under the M0-L0 (Fig. 6b). The diurnal maximum air temperature ranged from 23.6 °C for M2-L1 to 21.9 °C for M0-L0. The highest nocturnal minimum air temperature (4.9 °C) also

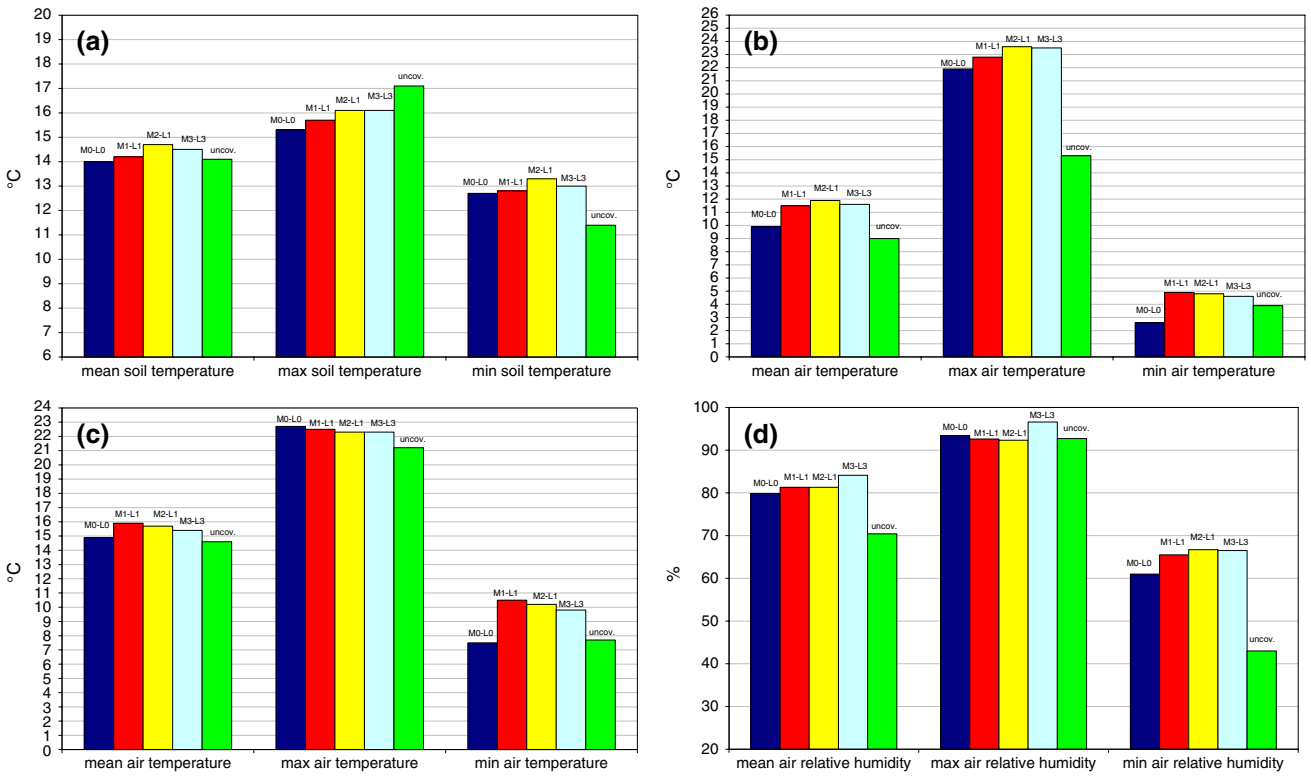

Fig. 6 Average values of the mean, maximum and minimum daily: soil temperature under the mulching films and of the bare soil (uncovered) during the cultivation period (**a**); air temperature inside the low tunnels kept closed and outside (uncovered) from 25/3/2002 to 2/4/2002 (**b**); air temperature inside the low tunnels kept open and outside (uncovered) from 5/4/2002 to 24/4/2002 (**c**); relative humidity inside the low tunnels kept closed and outside (uncovered) from 25/3/2002 to 2/4/2002 (**d**); max, maximum; min, minimum

occurred under the biodegradable combination, M1-L1, and the lowest (2.6 °C) under M0-L0. The temperature of 2.6 °C under M0-L0 was lower than that of the external air, which had an average minimum value of 3.9 °C. In the open low tunnels, slight differences were recorded for the maximum air temperatures while the minimum air temperatures ranged from 7.5 °C for M0-L0 to 10.5 °C for M1-L1 (Fig. 6c).

There were slight differences in relative humidity inside the low tunnels (Fig. 6d). The mean relative humidity was the lowest (79.8%) under M0-L0, and the highest (84.1%) under the biodegradable films (M3-L3).

Agronomic analyses of the earliness of the first harvest day and the marketable total yield were carried out on the strawberry cultivation. The total yields were higher with the biodegradable materials than with the LDPE films: 21.5 t/ha for M1-L1, 19.2 t/ha for M2-L1, 18.2 t/ha for M3-L3, and 17.8 t/ha for M0-L0. There were also significant differences between the earliness of the first harvest day. By 23 April 2002, M3-L3 had produced 35.7% of the total yield, M2-L1 31.2%, M1-L1 25.1% and M0-L0 only 20.8%.

Degradation Rate of the Biodegradable Films in the Soil

The organic matter content of the test field soil was relatively low (Table 1). In the beginning of the experiment the organic matter content (determined as loss of weight on ignition, LOI) was 7%, and varied from 6.4% to 7.5% on the M0-L0c, M1-L0c and M1-L1c blocks, at the end of the crop cycle, 1 month after tillage and 1 year after tillage. Other soil properties depicting soil fertility are presented in Table 1.

Solvita™ Soil Life Tests were performed to determine the microbial activity in the soil. At tillage, the Solvita test value was 4, i.e. the soil had an active population of microorganisms. One month after tillage the value decreased to 3.5, 6 months after tillage to 3 and 1 year after tillage to 2.5, when the soil had only marginal biological activity.

The amount of mulching film residues in the soil were determined in order to assess the time frame necessary for degradation of the buried biodegradable film residues after tillage. One month after tilling the films into the soil, there was 44% and 50% of the initial weight of the film laid on the soil on the M1 and M3 test blocks, respectively. M1 degraded faster than M3. After 3 months only 17% of the initial weight of M1 was present in the soil, but 36% of M3. The amount of both M1 and M3 residues remaining in the soil showed a relative decrease over time: 12 months after tillage the amount of film residues present in the soil was less than 4% of the initial weight of the film. After 12 months sampling it was impossible to separate out the mulching film residues in the soil due to their reduced size.

Ecotoxicity and Environmental Impact

Ecotoxicity

Soil samples collected from the field experiment were tested for toxicity by the Flash test using two exposure times of 30 s and 30 min, and on the basis of *Enchytraeidae* reproduction. With a 30 s exposure time in the Flash test, the soil samples taken before the start of the field experiment and at the end of the cultivation period gave a slight toxic response of 13% and 12%, respectively. However, there were no differences in the inhibition % between the soil samples collected from the M0-L0c, M1-L0c and M1-L1c blocks. With the 30 min exposure time and the same soil samples, the slight toxic response was less than 5%. Furthermore, there was no toxic response in the soil samples taken 1 month after tillage of the biodegradable mulching films into the soil (Fig. 7). In contrast, the samples activated the light production of the test organism. After 30 min exposure time, the light production increased by one half. In the soil samples taken 1 year after tillage, light induction was no longer so intense, only 16%, 12% and 10% in M0-L0, M1-L0 and M1-L1, respectively. Tilling the biopolymers into the soil did not increase light induction compared to that for the control test plot without polymers.

The reproduction of *Enchytraeidae* was evaluated in the soil samples collected from the experimental field after strawberry cultivation with biodegradable materials and LDPE as mulching. The results are presented as the number of juveniles per adult, and are compared to the number of juveniles produced in standard soil (Fig. 8). Carbendazim (1.2 mg/kg dry weight) served as a reference chemical. The soil was tested before the field experiment and 1 month and 1 year after tillage. In addition to the reference, only the soil sample taken from the M0-L0c block after 1 month of tillage had a statistical difference (*t*-test, 0.04) in the

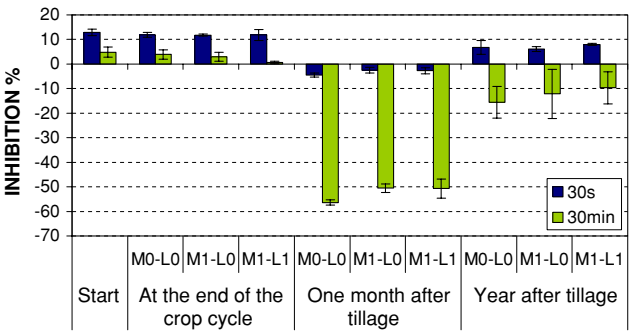

Fig. 7 Inhibition % in the Flash test on soil samples taken at four different times from the M0-L0c, M1-L0c and M1-L1c blocks; start of the field experiment, at the end of the crop cycle, 1 month after tillage, and 1 year after tilling the films into the soil

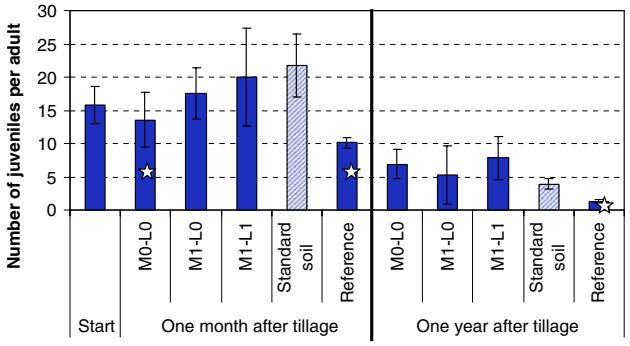

Fig. 8 Number of juveniles in the plant root worm test in soil samples from the M0-L0c, M1-L0c and M1-L1c blocks at the start of the field experiment, 1 month after tillage, and 1 year after tilling the films into the soil (star represents statistical difference (*t*-test 0.05) from standard soil

number of juveniles compared to the standard soil. The juvenile production in the reference sample (10 juveniles per adult) was 47% of the number of juveniles in the standard soil. Fifteen juveniles were produced per adult in M0-L0c, which corresponded to a reduction of 38% compared to that in the standard soil. However, there were no differences in reproduction between the soil samples taken before and after the field experiment. There were also no differences in juvenile production in the soil samples in which the biodegradable films had been buried in the soil after the cultivation period. In the second test with soil samples collected 1 year after tillage, the level of reproduction of juveniles in the standard soil was 82% lower than that in the first test. However, the differences in juvenile production between the test soils were not statistically significant.

Ammonium Oxidizer Diversity

The diversity of ammonium oxidizers was studied by determining the appearance of the *amoA* gene. The DGGE profiles depicting AOB diversity were not affected by the mulching film materials (Fig. 9), and there were only some slight differences during the cultivation period. There was only one major band detected in the soil before the field experiment and the situation remained relatively constant during the whole experiment, except for a number of stronger bands in some of the replicates. The bands sequenced (1–7) from the soil samples collected 1 year after tillage were very similar to each other. Readable sequences were obtained from bands (1), (4), and (5–7). When band (1) was compared to the sequence data in the GenBank sequences using the Blast search, the closest match (99%) was found with the uncultured ammonia-oxidizing bacterium clone VCASc4 (AY256351) (Table 2). Similarity with the cultured ammonium oxidizers *Nitrosospira* sp. PJA1 (DQ228457.1), *Nitrosovibrio* sp. RY3C (DQ228466.1) and *Nitrosolobus multiformis* (U91603.1, NMU91603), was only 94–95%. Sequences from bands (6) and (7) were exactly the same as those for band (1). Band (4) differed from band (1) only by 1%. However, sequence analysis showed that the similarity of band (5) with all the other bands sequenced was only 95%, as well as was the closest similarity match with sequences derived from the GenBank.

Discussion

The performance of the biodegradable films, as well as their degradation in the soil and ecotoxicity and environmental impact, were evaluated in the field experiment for the cultivation of strawberry plants under low tunnel and with mulching film. The functionality of the films was evaluated by analysing their useful lifetime, radiometric performance and influence on the microclimate, agronomic results, and the degradation rate of the biodegradable film residues buried after harvesting. The environmental impact of this innovative agricultural practice was investigated by evaluating the ecotoxicity of the field soil on the basis of the kinetic luminescent bacteria test and *Enchytraeus albidus* reproduction potential. The diversity of ammonium oxidizing bacteria depicting soil health was also evaluated.

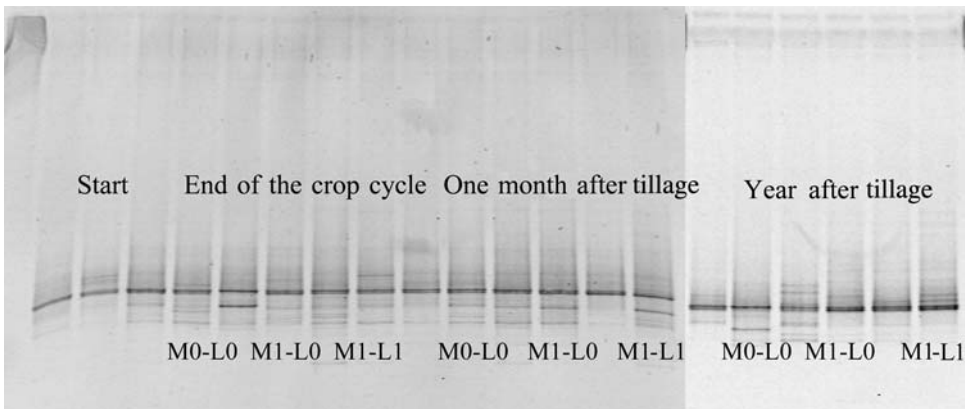

Fig. 9 Ammonium oxidizer community in soil samples collected from the M0-L0c, M1-L0c and M1-L1c blocks at the start of the field experiment, 1 month after tillage, and 1 year after tilling the films into the soil. Sequenced bands are marked as 1–7

Table 2 Sequence similarity between the dominant 16S rDNA-bands and the GeneBank sequences

Band	Closest relative	Identities	Exp	Source
1 (6 and 7)	Uncultured AOB clone VCASc4	99% (417/419)	0	AY256351
	Nitrosospira sp. PJA1	95% (405/423)	0	DQ228457.1
	Nitrosovibrio sp. RY3C	95% (403/423)	0	DQ228466.1
	Nitrosolobus multiformis	94% (399/423)	0	U91603.1 [26]
4	Uncultured bacterium clone PWT1-82	99% (406/409)	0	AY944217 soil
5	Uncultured bacterium clone PWT1-82	95% (394/412)	0	AY944217 soil

The field experiment showed that the lifespan of the biodegradable films was sufficient for strawberry plant cultivation: the black mulch films retained their mulching effect for 9 months, and the transparent low tunnel films on steel arches lasted for 6 months. The lifespan of the biodegradable films was found to be sufficient for the Mediterranean area, and even longer than the previously tested similar type of Mater-Bi based films, the lifespan of which varied from 2 to 5 months [43]. The performance of kraft paper impregnated with vegetable oil-based resins has been tested for mulching purposes as an alternative to LDPE films. This product withstood field conditions for an even shorter period of time, only 8–12 weeks [44].

Solar radiation in the PAR wavelength range is necessary for photosynthesis, which is the fundamental process in crop production [45]. The transmissivity coefficient in the PAR range, i.e. the fraction of solar PAR radiation transmitted by the covering material, is of considerable importance for crop growth: the mulching films used to reduce the growth of weeds must be opaque to PAR radiation. The M1, M2 and M3 biodegradable black mulching materials were opaque in the PAR range. They had PAR total transmissivity coefficients lower than 0.01%, which fulfil the requirements of the standard concerning LDPE agricultural black mulching films [46]. The field experiments clearly showed that M1, M2 and M3 inhibited weed growth as well as M0 LDPE film, thus satisfying one of the main purposes of mulching.

The radiometric tests on the low tunnel films showed that the biodegradable films had a lower solar total transmissivity, a higher solar diffuse transmissivity, and a lower LWIR transmissivity than the L0 LDPE film (Fig. 3). The solar total transmissivity coefficients of the L1 and L3 biodegradable films, about 80%, were comparable with the coefficients of the anti-fogging (82.8%) and diffuse (79.8%) LDPE films used for greenhouse covering reported by Pearson et al. [47].

The average daily minimum air temperature inside the closed low tunnels, showed that the experimental area covered with the biodegradable films had a higher air temperature than using the LDPE films (M0-L0) (Fig. 6b). The minimum values, which occurred during the night, were more than 2 °C higher under the L1 and L3 biodegradable low tunnel films than under the L0 low tunnel film. This is due to the very low LWIR transmissivity coefficient of the biodegradable low tunnel films compared to that of the L0 LDPE film, which has a LWIR transmissivity coefficient higher than 80% (Fig. 3). The indoor greenhouse air temperature rises along with a decrease in the LWIR transmissivity coefficient of the low tunnel material. This phenomenon is more evident during the night when the exchange of LWIR radiation energy plays an important role. The LWIR transmissivity coefficient of the L1 (6.74%) and L3 (2.98%) biodegradable films was lower than that of the best thermic LDPE and EVA films for greenhouse covering, which have a LWIR transmissivity coefficient higher than 12% [23].

Only minor differences were recorded during the day among the biodegradable films and the L0 LDPE film: the increase in the maximum daily air temperatures under the biodegradable films, compared to M0-L0, ranged from 0.9 °C to 1.7 °C (Fig. 6b). These small differences were due to the higher solar total transmissivity coefficient of the L0 film (Fig. 3), which allowed the passage of a larger fraction of solar energy, thus compensating during the day for the higher long wave infrared radiative losses.

In the open low tunnels, slight differences were recorded for the maximum air temperatures, which were affected by ventilation (Fig. 6c). Higher differences were pointed out mainly during the night, when the biodegradable films recorded higher minimum air temperature, having a lower LWIR transmissivity coefficient, compared to the LDPE films.

There were only minor differences in the soil temperature measured under the mulching films because they were opaque to solar radiation and the soil was not heated directly by solar radiation. The soil temperatures under the biodegradable films were warmer than that under the M0-L0 film combination (Fig. 6a) due to their lower LWIR coefficients (Fig. 3).

Under the L0 LDPE low tunnel film the diurnal minimum relative humidity was lower than in the low tunnel

covered by the biodegradable films (Fig. 6d). This is most likely due to the higher permeability of the biodegradable films to water vapour compared to the LDPE film [48]. The difference in relative humidity did not have any adverse effect on the crops.

Plant growth and yield were positively influenced by the microclimate produced by the biodegradable films compared with those obtained with the LDPE films. The low tunnels formed with biodegradable films maintained a higher internal air temperature for the duration of the cultivation period (Fig. 6b). As a result, the strawberry plants protected with the biodegradable films produced more and earlier berries than the plants protected with the LDPE films. In addition, the solar radiation was more uniformly scattered under the low tunnels covered with biodegradable films which had higher solar diffuse transmissivity coefficients, thus having a positive effect on plant growth and also reducing the incidence of scorch [47].

After giving a good performance in strawberry cultivation, the residues of the biodegradable film buried in the field soil degraded in an acceptable time, leaving less than 4% of the initial weight of the installed film 12 months after tillage.

Soil microorganisms are responsible for nutrient cycling and the decomposition of organic material such as the biodegradable materials in the soil. The properties of biodegradable material, as well as the environmental conditions, affect the degradation rate of polymers in soil [49–53]. The Solvita test, which measures microbial activity in the soil, showed that there was an active microbial population present in the experimental field and degraded the biopolymer mixed into the soil after cultivation. Microbial activity showed a decreasing trend during degradation of the biopolymer. Decrease in microbial activity may also depend on the environmental conditions (e.g. soil temperature, pH, availability of nutrients) [20, 53].

The degradation rate of individual biopolymers in the soil varies considerably depending on the properties of the polymer and the environmental conditions [54]. The biodegradation of biodegradable mulching films in field soil is reported in Briassoulis [20] and Schettini et al. [55]. The degradation of other types of biodegradable materials has also been studied in the soil environment [56]. When the degradation of three commercial biodegradable plastics, poly(3-hydroxybutyrate) (PHB), Sky-Green® (SG) an aliphatic polyester, and Mater-Bi® (MB) was studied in a soil burial test, the order of biodegradation was PHB > SG > MB. In farm soil at 28 °C, the degradation for PHB was 41% in 25 days, for SG 65% and for MB 27% in 55 days [55]. PHB and SG had a relatively similar degradation rate as the M1 and M3 mulching films, while the degradation of MB was slower. The behaviour of LDPE in soil has also been investigated. Ohtake et al. [57] studied the biodegradation of LDPE films in agricultural fields. According to their results, LDPE film was partially degraded after 3.5 years in the soil. However, there has been a lot of controversial discussion on the biodegradation potential of polyethylene in soil. Kyrikou and Briassoulis [53] cover the issue of stability, the low rate of (bio)degradation, and possible fragmentation of LDPE in soil environment. In our study the LDPE films were collected after use and disposed of at a plastic waste collection centre.

After verifying the biodegradability of the material by means of a standard test, the toxicity in the degradation environment, compost or soil should also be tested. Toxic degradation products can be released during the biodegradation process. Linking the ecotoxicological evaluation to biodegradation studies facilitates the interpretation of possible toxic responses [58].

The standard luminescent bacteria test ISO 11348 [59] is widely used for evaluating the toxicity of environmental samples. Since the standard test was primarily intended for measuring aquatic samples, new versions of the test have been developed for solid and coloured environmental samples [37, 60]. One of these developed methods, the Flash test, has been found to produce comparable results to conventional tests for the toxicity of soil samples [61]. The Flash test has been also used as a method for evaluating the toxicity of soil and compost samples in studies with the biodegradation of biopolymers [58, 62]. These studies showed that the Flash test is suitable for testing the toxicity of solid samples during and after the degradation of a range of biopolymers. The results obtained in our study indicated that the films did not cause any environmental risk to the agricultural soil. The slight toxic response found in the soil at the end of the crop cycle, around 10% inhibition of the light production by *Vibrio fischeri*, could not have originated from the biopolymers because the same reduction was detected before the biopolymers came into contact with the soil. Inhibition might be induced by the use of pesticides and fungicides. However, the increase in luminescence in the soil samples taken 1 month after tillage is probably caused by nutrient availability in the soil. This phenomenon is often seen with compost samples containing large amounts of nutrients and substrate [63]. One year after tillage, when the amount of film residues left in the soil was only 4%, the soil samples did not induce a toxic response in any of the samples tested. As the Solvita test showed, microbial activity decreased as the amount of film in the soil diminished. Furthermore, the activation of light production diminished due to the decrease in easily available nutrients in the soil.

Contradictory results have been obtained for the numbers of *Enchytraeidae* in agricultural soil subjected to

different agricultural management systems. In organically managed agricultural soil, the number of *Enchytraeidae* was higher than in conventionally managed soil [64]. In contrast, Springett et al. [65] reported that the number of *Enchytraeidae* was higher in soil subjected to normal farming, and Palojärvi et al. [66] did not find any differences between organically managed and conventionally managed soil.

The number of *Enchytraeidae* in the soil might be affected by the amount of organic matter [66]. In our study, after the cultivation period the biodegradable film in the soil acted as an organic substrate for soil microorganisms and soil fauna, and provided better growing conditions for *Enchytraeidae* than when no film was tilled into the soil. The phenomenon was detected 1 month after tillage when there was plenty of substrate for the microorganisms, but not 1 year after tillage when most of the biopolymers were degraded.

Toxicity of a polymer can be derived from additives in the polymer structure, even when the amount in the polymer product is very low, as shown with lactic acid based poly(ester-urethanes) by Tuominen et al. [62]. In this case, the toxicity of the polymer was removed when the additive was changed. In our study, most of the film (96%) had been degraded 1 year after tilling the mulching films into the soil. The toxicity tests used in our study, i.e. the Flash test and *Enchytraeidae* reproduction, showed no evidence of a toxic impact in the soil induced by these biopolymers. However, dilution factor of the biopolymer residues when mixed into the soil has to be recognised. On this account, it is recommended that the toxicity of biopolymers should be studied after and during the standard laboratory scale biodegradation test with relatively high polymer concentration [62].

Even if the connection between biodiversity and soil functioning is still only partly understood, microbial diversity is an extremely useful tool when studying soil health [25]. In our study, in which microbial community analysis was performed by PCR-DGGE in order to determine changes in the microorganisms reflecting soil health in relation to the agricultural management system, no evidence was found of changes in the population of ammonium oxidizing bacteria. Like in our field study, Phillips et al. [67] found no differences in the community structure of AOB derived from agronomic soil subjected to different types of fertilization or cultivation. In contrast, the studies carried out by Norton et al. [26] on *amoA* diversity reported different types of AOB present in agricultural soil treated with nitrogen fertilizer, composted dairy waste or liquid dairy waste. In their studies, in which the effect of agronomic treatment on the composition of AOB was studied, *Nitrosospira* and *Nitrosomonas* were the dominating AOB species [26, 67]. The major band (1) that was found to be a dominating species in our field study had a similarity of 95% with *Nitrosospira*. The closest relatives were uncultured clones of AOB, and only two different types of sequences were detected in our soil samples.

Conclusion

The ecological impact of the raw material resources used and the fate of the product when it enters the waste stream have to be determined when a product is designed [68]. Replacing conventional plastics with biodegradable materials in agricultural applications does not reduce the amount of waste, but it does provide the opportunity to choose an alternative waste treatment strategy, i.e. organic recovery. In this study, the use of biodegradable films for soil mulching and low tunnel covering was proposed as a sustainable solution for the disposal and management of agricultural plastic waste.

The experimental trial illustrated how biodegradable films possess the functionalities needed for agricultural applications during the whole experimental crop cycle. In fact, the biodegradable films had radiometric properties and a useful lifetime fully comparable with the low density polyethylene films currently in use. Moreover, when the biodegradable materials were buried in the soil after the cultivation period, they degraded within a reasonable time frame. Furthermore, there was no indication of ecotoxicity or reduced soil quality resulting from the use of biodegradable films. It is especially important to evaluate the ecotoxicity of biodegradable materials designed for mass applications that are in direct contact with the food chain. During the use and degradation of biodegradable materials, which are designed to support sustainability, there should be no release of toxic breakdown products that could persist and accumulate in the environment.

Biodegradable films with a thickness and width suitable for soil mulching and low tunnel covering were tested in this research. Future development of the research will be addressed at producing films with a greater width, up to 8–10 m, to be used as covering for larger tunnels or greenhouses. Industrial production and broader adoption of biodegradable films for agriculture would enhance the protection of the landscape in rural areas against pollution, and increase the use of renewable non-oil raw materials such as starch.

Acknowledgments The work has been carried out under the project "Environmentally friendly mulching and Low tunnel cultivation-BIOPLASTICS" funded by the European Commission (EC RTD QLRT, Contract n° QLK5-CT-2000-00044). The responsibility for designing and carrying out this investigation was shared equally among the authors: the radiometric and microclimate aspects were studied by Schettini and Vox, and the soil ecotoxicity and microbial community aspects by Kapanen and Itävaara. Marjo Öster and Asta

Pesonen at VTT are thanked for their skilful laboratory work in the ecotoxicological and molecular analyses, and Michele Cosmo at the Department PROGESA for his co-operation in the spectrophotometric measurements.

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