Soil Biology & Biochemistry 40 (2008) 1989–1998

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Soil solarization with biodegradable materials and its impact on soil microbial communities

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ARTICLE INFO

Article history:
Received 5 September 2007
Received in revised form 8 February 2008
Accepted 11 February 2008
Available online 6 May 2008

Keywords:
Biodegradable plastic materials
FDA
Fluorescent *Pseudomonas*
Fusarium oxysporum f.sp. *lycopersici*
Organic matter
PCR-DGGE
Sclerotinia minor

ABSTRACT

The application of soil solarization (SS), one of the most promising techniques for the control of soilborne pathogens, is seriously limited by the drawback regarding the disposal of the used plastic materials. A possible solution to this problem is the use of biodegradable plastics. The aim of this study was to make comparisons between the impact of SS performed with biodegradable materials and that of SS with plastic films and other pest management techniques (i.e. organic matter amendment, calcium cyanamide and Dazomet fungicide application) on crop productivity, soilborne disease incidence, weed suppression, and soil chemical (total N, NH_4-N, nitrate, available phosphorus, organic matter, hydrolysis of fluorescein diacetate) and microbial (cultivable *Pseudomonas*, DGGE fingerprinting of bacterial 16S- and fungal 28S rRNA gene fragments from total soil community DNA) parameters. We carried out field experiments in two types of soil with different textures (clay and sand) artificially inoculated with *Fusarium oxysporum* f.sp. *lycopersici* (vs. tomato) and *Sclerotinia minor* (vs. lettuce).

The temperature of soils covered with solarizing materials was always higher than that of bare soils, but plastic cover was more effective and consistent in rising soil temperature compared to biodegradable materials. Plant growth promotion by SS was limited, especially compared to Dazomet and organic matter applications, and a positive effect was observed only for lettuce in the clay soil. Differently, both plastic and biodegradable solarizing materials were effective in reducing lettuce drop caused by *S. minor*. Weed development was significantly suppressed by Dazomet application and SS with plastic film, while control with biodegradable materials was limited. SS had a variable and limited effect on chemical and microbial parameters, with a general tendency to reduce richness of bacteria and fungi. Dazomet caused the most pronounced reduction of the microbial community diversity in both soil types and a significant stimulation of the fluorescent *Pseudomonas* group. Organic amendment significantly enhanced the organic matter content, the hydrolysis of fluorescein diacetate and the *Pseudomonas* population. Among all measured soil parameters, the size of the fluorescent *Pseudomonas* population emerged as the most important factor affecting crop productivity.

The results of this experimentation show the potential of using biodegradable solarizing materials in place of plastic films, but also indicate the need for improving their properties to obtain performances comparable to those of other pest management techniques.

1. Introduction

The increasing concern about the impact of mineral fertilizers, fungicides and herbicides on the environment and human health requires the development of alternative agronomic techniques that may reduce the use of these products. This need is further emphasized by the occurrence in pests of resistance to fungicides, the breakdown of host resistance by natural populations (McDonald and Linde, 2002), and the phasing out of methyl bromide in 2005 for its negative impact on the ozone layer (Martin, 2003).

Among the alternative strategies, soil solarization (SS), which is a method used to increase soil temperature by using transparent plastic sheets over the soil to retain the sun radiation energy, seems one of the most promising techniques to control soilborne plant pathogens and weeds (Katan et al., 1976; Stapleton, 2000). In

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doi:10.1016/j.soilbio.2008.02.009

solarized soils, control of pests is imputable to multiple mechanisms which primarily involve thermal inactivation of plant pathogens, because of increased soil temperature under plastic films (Katan et al., 1976), or weakening of the pathogen propagules that become more susceptible to competition or antagonistic activity of the native soil microflora (Stapleton, 2000). Saprophytic microorganisms, including several antagonists, are usually more tolerant to heat than plant pathogens (Stapleton, 2000). SS has been proved to be effective in controlling populations of many important soilborne fungal pathogens such as *Verticillium dahliae*, the causal agent of vascular diseases of many plants (Pinkerton et al., 2000), certain *Fusarium* spp. that cause *Fusarium* root-rot and wilt in several crops (Bourbos et al., 1997; Tamietti and Valentino, 2006), and *Rhizoctonia solani* and *S. minor* that cause lettuce drop (Sinigaglia et al., 2001). In addition, like other soil-disinfestation techniques, SS often promotes plant growth by disease-independent mechanisms such as the improvement of soil structure (Chen et al., 1991), release of mineral nutrients (Chen et al., 1991; Grünzweig et al., 1999) and stimulation of plant growth promoting rhizobacteria (PGPR) (Gamliel and Stapleton, 1993). It is well known that SS with plastic films profoundly affects some soil chemical and microbiological parameters. For example, an increase of the NH_4-N and NO_3-N concentration in the top 15 cm of soil has been reported in several studies (Stapleton and DeVay, 1995; Grünzweig et al., 1999; Gelsomino et al., 2006). The concentration of soluble mineral nutrients, including calcium, magnesium, phosphorus, potassium, and others, increased sometimes, but frequently the results were not consistent (Chen et al., 1991; Grünzweig et al., 1999).

Although it is well recognized that SS affects a broad range of soil microrganisms, rather contrasting results were reported regarding the fate of soil microflora in response to SS. Both positive and negative effects on total bacterial and fungal populations have been found (Khaleeque et al., 1999; Barbour et al., 2002; Sharma et al., 2002). Solarization may increase many groups of bacteria like fluorescent *Pseudomonas* and *Bacillus* spp. in the bulk soil or rizosphere (Gamliel and Stapleton, 1993). However, recent studies, using denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S ribosomal RNA (rRNA) gene-coding fragments from soil-extracted DNA (Muyzer and Smalla, 1998), provided useful information on the effect of SS on the structure and diversity of soil microbial communities (Schönfeld et al., 2003; Gelsomino and Cacco, 2006). PCR-DGGE is a powerful method for assessing the structure of microbial communities in environmental samples (Muyzer and Smalla, 1998), without cultivation steps on cultural media. Following amplification, DGGE separates the products and allows the detection of a larger microbial community diversity compared to cultivation methods (Winding et al., 2005). The utilization of this approach for studying microbial ecology revealed the existence of a vast and previously unknown bacterial diversity (Felske et al., 1997). However, to be visible as a band on the gel, a species should represent at least 1% of the soil microbial community (Casamayor et al., 2000).

In addition to SS, alternative techniques to methyl bromide are based on the use of soil fumigants with a wide spectrum of actions such as Metham Sodium or Dazomet (Martin, 2003), the application of calcium cyanamide which has herbicidal and fungicidal properties (Bourbos et al., 1997), or soil amendments with organic matter (OM) (Hoitink and Boehm, 1999; Bonanomi et al., 2007). OM can control soilborne pathogens through several mechanisms such as the release of fungitoxic compounds, generation of fungistasis (Lockwood, 1977), or selective stimulation of soil microbes which are antagonists to pathogens (Hoitink and Boehm, 1999).

Despite its potential, an important limitation to the diffusion of the SS technique is the serious drawback regarding the disposal of used traditional plastic materials: plastic waste management, such as on-farm burning or land filling, has environmental and monetary costs for the farmers. A possible solution to this problem is the use of biodegradable plastics (Al-Kayssi and Al-Karaghouli, 2002), which gradually degrade when plowed-down due to the action of soil microorganisms. The use of biodegradable solarizing materials would eliminate the monetary costs for the farmer and reduce the environmental impact. Although there are some comparative studies between SS with biodegradable and plastic films (Russo et al., 2005), most of the research done deals with the effect of biodegradable materials on soil temperature. Little attention has been paid to their effects on crop productivity and on soil chemical and microbiological parameters.

The aim of this study was to investigate the impact of SS with biodegradable materials and plastic films, organic amendments and soil disinfestation with fumigants on crop productivity, soilborne disease incidence, weed suppression, and soil chemical and microbial parameters.

2. Materials and methods

2.1. Solarizing materials

SS was carried out by applying the following materials to soil: a) polyethylene plastic sheets POLYSOLAR (plastic sheet); b) starch based biodegradable film MaterBi (biodegradable film); and c) polysaccharides mixture based (1.5%) biodegradable spray material (biodegradable spray). Biodegradable film is a transparent film (thickness 30 μm) produced from a starch base by NOVAMONT (S.p.a. Novara, Italy). Biodegradable spray is a material obtained from a mixture of polysaccharides at a concentration of 1.5% and with the addition of fibres of cellulose for mechanical reinforcement produced by P.S.I. (Polysaccharide Industries AB, Sweden).

2.2. Field experiments

Experiments were carried out in Southern Italy (Salerno) during the 2005 cropping season at two sites with different soil types. The first was a clay soil (sand 45%, silt 21.5%, clay 33.5%, pH 8.21, organic matter 0.85%, total N 0.81 g/kg, C/N 6.1, total $CaCO_3$ 189 g/kg, available phosphorus (P_2O_5) 6.2 mg/kg, exchangeable potassium 0.46 meq/100 g, exchangeable magnesium 1.89 meq/100 g, exchangeable calcium 27.6 meq/100 g, exchangeable sodium 0.35 meq/100 g, EC 0.096 dS/m); the second was a sandy soil (sand 83.9%, silt 1.9%, clay 14.2%, pH 8.41, organic matter 0.55%, total N 0.65 g/kg, C/N 5, total $CaCO_3$ 140 g/kg, available phosphorus (P_2O_5) 48.1 mg/kg, exchangeable potassium 0.53 meq/100 g, exchangeable magnesium 0.96 meq/100 g, exchangeable calcium 15.5 meq/100 g, exchangeable sodium 0.17 meq/100 g, EC 0.188 dS/m).

Experimental plots consisted of three adjacent areas measuring 21 × 12 m, and treatments were arranged in a randomized block design with three replications. Plots (3 × 3 m) were separated by 1.0 m buffer areas. Seven soil treatments were made: (i) SS with plastic sheet; (ii) SS with biodegradable film; (iii) SS with biodegradable spray; (iv) soil amended with calcium cyanamide at a rate of 300 kg/ha (CaCN2); (v) soil amendment with *Medicago sativa* straw (straw); (vi) soil disinfestation with covered Dazomet at a rate of 50 g/m^2; and (vii) control as bare soil.

All treatments were applied to soil without pathogen inoculum and soil artificially inoculated with *F. oxysporum* f.sp. *lycopersici* (FOL) and *S. minor* (SM). FOL is the causal agent of the wilt disease of tomato and SM is the causal agent of soft rot of a wide range of hosts, including lettuce. For the artificial inoculum, common millet seeds, placed in 2-l flasks and imbibed with a Czapeck (OXOID) solution (1/10), were inoculated with FOL or SM previously cultured on PDA (Potato Dextrose Agar, DIFCO). Flasks were incubated for 21 days at 21 °C. The resulting FOL or SM millet inoculum was

air-dried for three days and added at a rate of 50 g/m^2 to the field plots seven days before soil treatments. This inoculation method proved to be effective in previous greenhouse experiments (data not shown). To avoid thermal stress to the fungal inoculum, the material was applied in the afternoon after 5:00 p.m. and manually incorporated by rake into the first 20 cm of soil. In the control, not-inoculated common millet was added to plots.

Before the application of solarizing materials, soil was milled (first 20 cm), levelled and subsequently brought to water field capacity through irrigation by aspersion. The SS with plastic films was carried out by mulching soils with plastic sheet (thickness 50 μm) in the period June–August for 63 days. Biodegradable film was applied as for plastic sheet, while biodegradable spray at a dose of 2 l/m^2, was sprayed with a gun connected to a compressor with an internal-combustion engine. During solarization soil temperatures were recorded at 2 and 20 cm deep in the untreated control and solarized plots by using thermocouples connected to a digital thermometer. Soil temperature was measured hourly during the whole day cycle six times during the solarization period (3, 14, 29 July and 3, 18 and 24 August). At the end of the solarization period, the solarizing materials were removed.

Soil amendments were carried out with air-dried straw (C/N ratio of 20) at rate of 500 g/m^2 equivalent to 12.5 g/m^2 of N. The straw was manually spread over the plots, and then incorporated into the soil by rototilling. The application of CaCN2 was done with the same procedure of straw at a rate of 30 g/m^2 equivalent to 6 g/m^2 of N. Finally, Dazomet, at a rate of 50 g/m^2, was incorporated by rototilling in the first 20 cm and soil covered with polyethylene sheets. Straw, CaCN2 and Dazomet have been applied at the beginning of the period of solarization.

2.3. Effects on crop productivity, disease incidence and weed suppression

At the end of the solarization period, 30 day-old seedlings of tomato (*Lycopersicon esculentum* cv. San Marzano) and lettuce (*Lactuga sativa* cv. Cambria) were planted on each plot. After 40 and 80 days of growth for tomato and lettuce, respectively, plants were harvested ($n = 30$ per plot) and their fresh weight measured. Disease severity of FOL on tomato and SM on lettuce was monitored at the end of the experiment counting the number of dead plants. Finally, after 100 days from the end of the soil treatments, the weed cover of each species was visually estimated using the abundance-dominance scale of Braun-Blanquet (1928).

2.4. Effects on soil chemical parameters

Immediately after the end of solarization, from each plot three composite soil samples each consisting of four different soil cores pooled together were randomly collected from the upper 20-cm layer. After air-drying (3 days) soil samples were sieved (mesh size 2 mm) and stored at 4 °C.

Soil was analyzed for total N, NH_4-N, nitrate, available phosphorus (Olsen method) and organic matter content. For all chemical analyses the official methods of the Italian National Society of Soil Science were used (Violante, 2000). Microbial activity was assessed with the Fluorescein Diacetate method (FDA) (Workneh et al., 1993).

2.5. Effects on soil microbiological parameters

Bacterial and fungal monitoring was done by using a multi-technique approach that combines both conventional cultivation-based methods and ribosomal RNA gene-based molecular analysis of soil community DNA (Liesack et al., 1997).

2.5.1. Pseudomonas enumeration

Pseudomonas Agar Base (OXOID) combined with *Pseudomonas* CFC supplement was chosen as medium for *Pseudomonas* enumeration. Ten grams of soil were transferred to a 250 ml flask with 90 ml of sterile distilled water containing 0.025% W/V of $Na_4P_2O_7$ to facilitate microbial release from the soil. Flasks were shaken for 30 min at 200 r.p.m. and then stored for 30 min to allow the sedimentation of soil particles. Aliquots of supernatants were serially diluted in Ringer solution 1/4× (OXOID) and each dilution was spread on the plate surface in triplicate. Plates were incubated at 20 °C for 24–48 h and colonies counted under UV-light. The results were expressed as CFU/g of dry soil.

2.5.2. DGGE analysis

DNA extraction was performed from 0.5 g of each soil by using the Fast DNA Spin kit for soil according to the manufacturer's instructions (BIO 101, Vista, CA, USA). The amount of DNA extracted from each soil was standardized by gel electrophoresis to obtain 10 ng of DNA template in each PCR mixture. Bacterial 16S rRNA gene fragments were amplified with primers 341f-GC and 534r which generated amplicons of about 194 bp (Muyzer et al., 1993). Amplifications were performed in a MyCycler thermocycler (Bio-Rad, Hercules CA 94547, USA) by using a touchdown temperature scheme as follows: 5 min at 94 °C, then 10 cycles of 1 min at 94 °C, 1 min from 65 °C to 55 °C (touchdown of 1 °C per cycle), and 3 min at 72 °C. Then, 25 additional cycles, each of 1 min at 94 °C, 1 min at 55 °C and 3 min at 72 °C were carried out. Finally, a time extension of 30 min at 72 °C was performed for eliminating artefacts in DGGE profiles (Janse et al., 2004). Each 50 μl mixture contained 1× PCR Buffer (Invitrogen; La Jolla, USA), 1.25 mM $MgCl_2$, 250 μM of each deoxynucleoside triphosphate, 0.1 μmol of each primer, 5 μg of bovine serum albumine and 5 U of *Taq* polymerase (Invitrogen; La Jolla, USA). Fungal 28S rRNA gene fragments were amplified with primers 403-f and 662-r (Sigler and Turco, 2002). Amplifications were performed by using a touchdown temperature scheme as follows: 5 min at 94 °C, 10 cycles of 30 s at 94 °C, 1 min from 60 °C to 50 °C (touchdown of 1 °C per cycle), and 2 min at 72 °C. Twenty additional cycles, each of 30 s at 94 °C, 1 min at 50 °C and 2 min at 72 °C were carried out. Finally, a time extension of 7 min at 72 °C was performed. Each 50 μl mixture contained 1× PCR Buffer (Invitrogen; La Jolla, USA), 1.25 mM $MgCl_2$, 250 μM of each deoxynucleoside triphosphate, 0.2 μmol of each primer, and 5 U of *Taq* polymerase (Invitrogen; La Jolla, USA). DGGE analyses were performed by using a DCode Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA, USA). Acrylamide gels (8% W/V) were prepared by means of a Model 475 Gradient Delivery System (Bio-Rad Laboratories) by using a denaturing gradient ranging from 30 to 60% (100% denaturant solution contained 7M urea and 40% deionized formamide). DGGE was performed with 1× Tris Acetate EDTA buffer at 60 °C and a constant voltage of 200 V for 4 h. After staining with ethidium bromide gels were observed by using an UV transilluminator. Banding patterns were photographed by using the Gel Doc 2000 documentation system (Bio-Rad Laboratories, Hercules, CA, USA).

2.6. Data analysis

Data were analysed statistically using analysis of variance (ANOVA). Two-way ANOVA was used to test the effects of soil type and soil treatments on crop productivity, disease incidence, weed suppression and soil chemical and microbiological parameters. The relationships between soil chemical and microbiological parameters and between these two types of parameters and crop growth were estimated using regression analysis. Significance was evaluated in all cases at $P < 0.05$.

Banding patterns of eubacterial and fungal DGGE were analyzed by Quantity One Image Analysis Software (Bio-Rad Laboratories, Hercules, CA, USA). After applying a rolling disc background subtraction (setting 8) and a sensitivity setting of 10, the software performed the analysis of each lane: a band was detected if it accounted for more than 0.5% of the total lane intensity. The program also provided the total band number and identification of bands occupying common positions in the lanes. The clustering of the patterns was performed by the Unweighted Pair Group method with Mathematical Average (UPMGA; Dice coefficient of similarity). Band richness from the DGGE profiles was used as a quantitative assessment of both bacterial and fungal species richness.

3. Results

3.1. Soil temperature during solarization

The temperature of soils covered with solarizing materials was always higher than that of bare soils. The highest values were recorded with the plastic sheet and the biodegradable film (Fig. 1). The biodegradable spray increased soil temperature, but was less effective compared to plastic sheet and biodegradable film (Fig. 1). The maximum temperatures in bare control, and covered soil with biodegradable spray, plastic sheet and biodegradable film were respectively 39.2, 52.2, 62.8 and 70.5 at 2 cm and 33.0, 33.8, 41.1 and 38.6 at 20 cm of depth. Biodegradable spray showed only limited evidence of biodegradation during solarization, which does not affect its solarizing capacity (data not shown). Differently, solarization with biodegradable film lasted only 20–25 days in both soil types because, after this period, the material was completely torn at the points where it was buried.

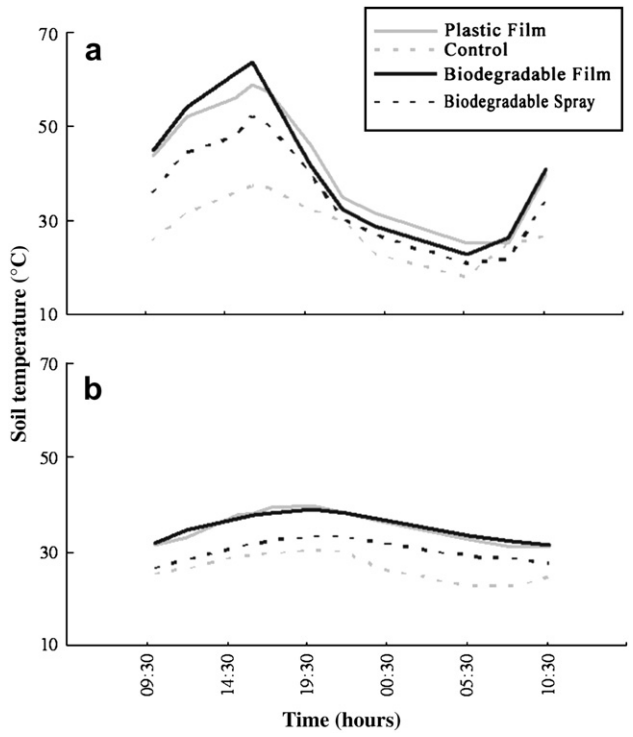

Fig. 1. Soil temperature variations measured at 2 (a) and 20 (b) cm depth during a summer day (14 July 2005) with different solarizing materials. The same dynamics has been recorded during the other days (data not shown).

3.2. Effects on crop productivity, disease incidence and weed suppression

SM and FOL inoculum did not affect plant growth (ANOVA, $P = 0.46$; data not shown). Soil treatments significantly influenced tomato growth in both soil types (Fig. 2a; ANOVA, $P < 0.01$ in both cases), but the interaction between soil type and treatments was not significant. Tomato growth was increased by the straw amendment and fumigation with Dazomet, and by plastic sheet solarization only in the sandy soil (Fig. 2a,b).

Soil treatments significantly affected lettuce growth in both soil types (Fig. 2b; ANOVA, $P < 0.01$ in both cases), and the interaction between soil type and treatments was significant (ANOVA, $P < 0.05$). Lettuce growth was significantly higher in the clay compared to the sandy soil (paired t-test: $P < 0.01$) and it was increased by plastic sheet and biodegradable film solarization and Dazomet fumigation in the clay soil, but only by Dazomet in the sandy soil (Fig. 2b). Lettuce mortality due to SM, as indicated by the presence of abundant sclerotia at the stem base, was significantly higher in the clay than in the sandy soil (paired t-test: $P < 0.01$). Plastic sheet and biodegradable film reduced lettuce mortality, compared to the control, in clay soil, as well as all treatments with the exception of Dazomet in the sandy soil (Fig. 3). FOL inoculum did not produce appreciable disease in tomato plants in all soil treatments (data not shown). We do not know the causes of this failure, but probably the climatic conditions (cold temperatures occurred during transplant) did not favour FOL infection.

Weed cover was significantly higher in the clay compared to the sandy soil (paired t-test: $P < 0.05$). Weed development was significantly suppressed, compared to the control, by plastic sheet

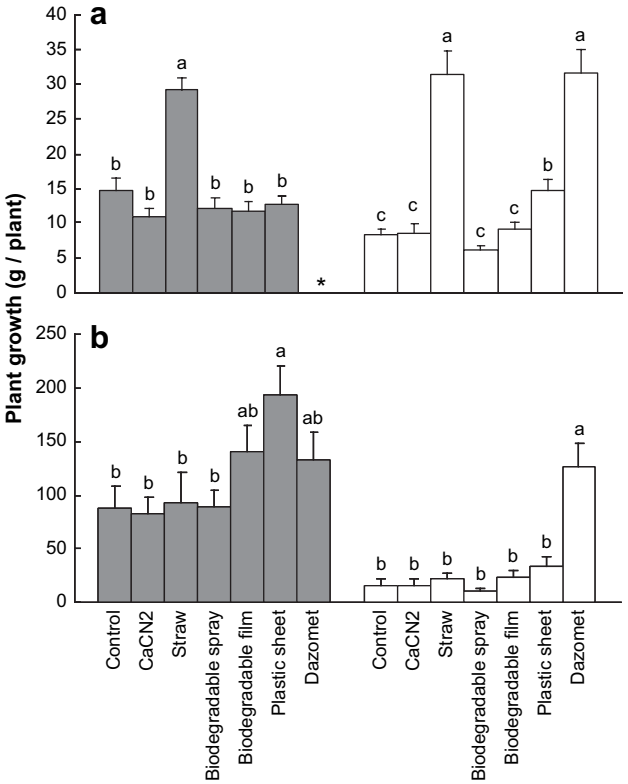

Fig. 2. Effect of soil treatments on growth of tomato (a) and lettuce (b) in the clay (grey bars) and sandy soil (open bars). Different letters indicate significant differences (comparison only within soil type; Duncan test, $P < 0.05$). Data are averages (+1SE) of three replicates. Data of tomato plants in the clay soil treated with Dazomet (*) were lost for technical problems.

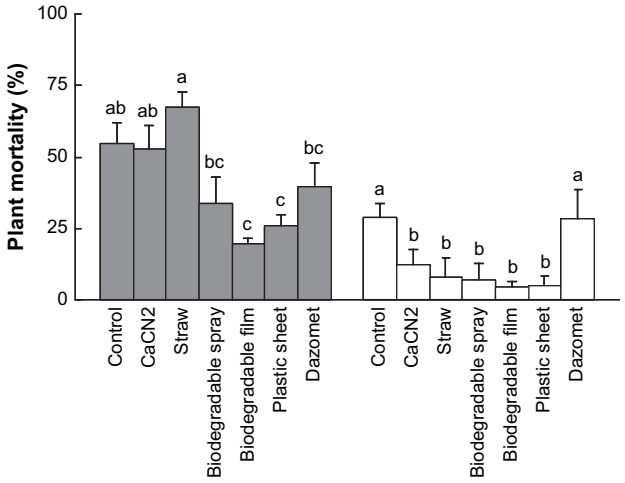

Fig. 3. Effect of soil treatments on lettuce mortality after 80 days of growth in the clay (grey bars) and sandy soil (open bars). Different letters indicate significant differences (comparison only within soil type; Duncan test, $P < 0.05$). Data are averages (+1SE) of three replicates.

and biodegradable film solarization and Dazomet fumigation in both soil types, whereas it was increased in the sandy soil by the straw amendment (Fig. 4). Plastic sheet and biodegradable film solarization and Dazomet fumigation strongly suppressed the development of *Hirschfeldia incana*, *Sonchus asper* and all the grass species in the clay soil. In the sandy soil, plastic sheet and biodegradable film solarization were able to control two of the three dominant weeds (*Senecio vulgaris* and *Veronica persica*), but were less effective, compared to Dazomet fumigation, in controlling *Cyperus rotundus*.

3.3. Effects on soil chemical parameters

All the soil chemical parameters analysed were significantly affected by the treatments in both soil types (one-way ANOVA: $P < 0.05$; Table 1), with the exception of nitrate nitrogen. Total nitrogen was increased by the amendment with straw in both soils and by the application of CaCN2 and Dazomet in clay soil and it was weakly decreased by solarization with biodegradable spray and

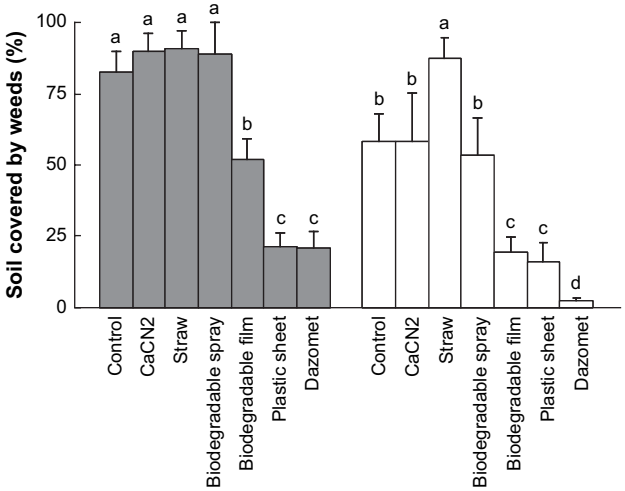

Fig. 4. Effect of soil treatments on weeds cover after 100 days from the treatments in the clay (grey bars) and sandy soil (open bars). Different letters indicate significant differences (comparison only within soil type; Duncan test, $P < 0.05$). Data are averages (+1SE) of three replicates.

biodegradable film in sandy soil (Table 1). NH_4-N was increased by the application of straw, CaCN2 and solarization with biodegradable film in the clay soil. In the sandy soil the most evident increase was due to the amendment with straw and the application of CaCN2 and Dazomet, while a less marked increase was recorded after the solarization with biodegradable film and plastic sheet. The soil concentration of nitrate nitrogen was always very low, without any differences among treatments (data not shown).

Available phosphorus in clay soil was reduced, compared to the control, by solarization with plastic sheet and biodegradable film and to a lesser extent by the application of CaCN2. In sandy soil available phosphorus was slightly increased only by the amendment with straw (Table 1).

Organic matter was significantly increased only by the soil amendment with straw in both soil types (Table 1). In addition, a weak but not significant decrease was recorded after the application of plastic sheet, biodegradable film and CaCN2 in both soils, with a clearer effect in the sandy soil.

Finally, FDA activity was not different between the two soil types (*t*-test: $P = 0.09$), and only the amendment with straw significantly increased this parameter in both soil types with values almost doubled compared to the non-amended control.

3.4. Effects on soil microbiological parameters

Since preliminary tests exhibited no significant influence of SM and FOL inoculum on the recovery of *Pseudomonas* spp. and on the diversity of DGGE banding patterns (results not shown), all the analyses were performed exclusively on soils without pathogen inoculum.

3.4.1. Pseudomonas enumeration

Population size of fluorescent *Pseudomonas* was significantly higher in the sandy soil (*t*-test: $P < 0.01$). Straw amendment and soil fumigation with Dazomet strongly increased the *P. fluorescens* in both soil types compared to the control (Fig. 5). SS affected *P. fluorescens* in a contrasting way: in the clay soil biodegradable film decreased the *P. fluorescens* number, while in the sandy soil *P. fluorescens* was decreased by biodegradable spray and increased by plastic sheet. Finally, the application of CaCN2 reduced the *P. fluorescens* in both soils compared to control (Fig. 5).

3.4.2. DGGE analysis

Denaturing gradient gel electrophoresis patterns of DNA from sandy and clay soils showed a considerable number of bacterial and fungal amplicons (Fig. 6). Band richness was significantly higher for fungi compared to bacteria, in both soils (*t*-test: $P < 0.001$ in both cases).

Band richness of bacteria from sandy and clay soils were not different (*t*-test: $P = 0.43$). All treatments decreased band richness of bacteria from the sandy soil compared to the control, with the exception of the CaCN2 application (Table 2). Band richness of bacteria from the clay soils showed a significant decrease with biodegradable film solarization and Dazomet sterilization, and an increase with straw amendment (Table 2). Since cluster analysis revealed high similarities between replicates of bacterial patterns (≥95% similarity, data not shown), we show only one replicate out of the three analyzed (Fig. 7a). The composition of the soil bacterial community was differently influenced by the treatments in each soil type and cluster analysis clearly separated sandy and clay soils, with the only exception of the biodegradable film treatments (cluster 3). In fact, biodegradable film solarization enabled the clustering of the sandy and clay soil samples together, overcoming the soil effect (cluster 3; Fig. 7a). Samples of Dazomet, CaCN2, biodegradable spray and plastic sheet grouped together with a similarity around 82% (cluster 1), while all the clay soil treatments

Table 1
Effect of soil treatments on total nitrogen, NH_4-N, available phosphorus and organic matter in the clay and sandy soils

	Clay soil				Sandy soil			
	Total N (g/kg)	NH_4-N (mg/kg)	P_2O_5 1 (mg/kg)	Organic matter (g/kg)	Total N (g/kg)	NH_4-N (mg/kg)	P_2O_5 (mg/kg)	Organic matter (g/kg)
Control	0.70 ± 0.02b	35.4 ± 1.15b	40.0 ± 6.51a	7.05 ± 1.03b	0.65 ± 0.02b	31.5 ± 1.01c	35.7 ± 2.08b	6.46 ± 0.47b
$CaCN_2$	0.84 ± 0.08a	61.6 ± 1.63a	23.8 ± 6.11b	6.89 ± 0.71b	0.66 ± 0.04b	38.8 ± 3.75b	32.7 ± 6.31b	6.11 ± 0.62b
Straw	0.90 ± 0.11a	60.8 ± 6.69a	38.5 ± 7.10a	8.86 ± 0.53a	0.78 ± 0.04a	47.1 ± 3.01a	48.4 ± 6.91a	8.41 ± 1.49a
Biodegradable spray	0.69 ± 0.06b	38.8 ± 1.05b	40.2 ± 5.47a	6.89 ± 0.61b	0.57 ± 0.02c	28.4 ± 1.04c	33.3 ± 3.68b	5.88 ± 0.38b
Biodegradable film	0.75 ± 0.08b	65.2 ± 2.47a	10.5 ± 1.48c	6.98 ± 0.15b	0.56 ± 0.02c	33.0 ± 2.40b	33.7 ± 3.69b	6.26 ± 0.85b
Plastic sheet	0.74 ± 0.04b	40.9 ± 0.68b	22.3 ± 3.55b	6.76 ± 0.46b	0.61 ± 0.12b	38.1 ± 2.63b	38.0 ± 7.35b	6.18 ± 0.39b
Dazomet	0.88 ± 0.05a	39.4 ± 1.15b	30.9 ± 7.30a	6.99 ± 0.89b	0.71 ± 0.14ab	46.8 ± 9.56a	34.1 ± 2.08b	6.12 ± 0.74b

Different letters indicate significant differences (comparison only within soil type; Duncan test, $P < 0.05$). Data are averages (±1SE) of four replicates.

except biodegradable film and CaCN2 clustered at 72% (cluster 2). Straw amendment in the sandy soil and CaCN2 application in the clay soil showed low similarities to any other treatment of each soil group.

Complex fungal community structure was found by analyzing DGGE profiles (Fig. 6). Fungal band richness of sandy soil was significantly greater than that of clay soil (*t*-test: $P < 0.05$). As observed for bacterial band richness, all soil treatments reduced fungal band richness compared to the control and Dazomet and straw treatments exhibited the greatest reduction (Table 2). No statistically significant differences were recorded for fungal band richness in the clay soils.

Fungal cluster analysis (Fig. 7b) clearly distinguished the two soil categories (10% similarity). Similarity values observed among the sandy soil samples were higher than those observed among the clay soil cluster (about 45% vs. 35%). However, replicates of each soil treatment were highly dissimilar and did not allow any separation among the treatment effects. Only replicates of Dazomet fumigation on sandy soils were grouped with a similarity around 60% (Fig. 7b).

3.5. Relation among soil variables, crop growth and lettuce drop

Tomato growth was positively related to the population size of *P. fluorescens* in both soil types (Fig. 8). Moreover, tomato growth was positively related to total nitrogen and NH_4-N in sandy soil (Pearson coefficient = 0.84 and 0.92, respectively; $P < 0.01$ in both cases) and to OM in clay soil (Pearson coefficient = 0.98; $P < 0.01$). Tomato growth was unrelated to bacterial band richness in both soil types, while a significant negative relationship was found with fungal band richness in the sandy soil (Pearson coefficient = − 0.82; $P = 0.022$). Lettuce growth was unrelated to all the chemical parameters monitored (data not shown), with the exception of a negative relationship with fungal band richness (Pearson coefficient = − 0.87; $P < 0.01$) and a positive relationship with *P. fluorescens* population in the sandy soil (Fig. 8). In addition, the incidence of lettuce drop was unrelated to all measured soil variables, including the FDA activity and fungal and bacterial band richness, both in the sandy and clay soils (data not shown).

Among the soil variables in the clay soil, only the NH_4-N was positively related to the FDA activity, and fungal band richness was positively related with bacterial band richness (Table 3). In the sandy soil several parameters were significantly related (Table 3): a positive relation was found between OM and FDA activity and available phosphorus, and between these latter variables. Total nitrogen was positively related to the NH_4-N and FDA activity. Finally, fungal band richness was negatively related to *P. fluorescens* and bacterial band richness was negatively related to P_2O_5, OM and FDA activity.

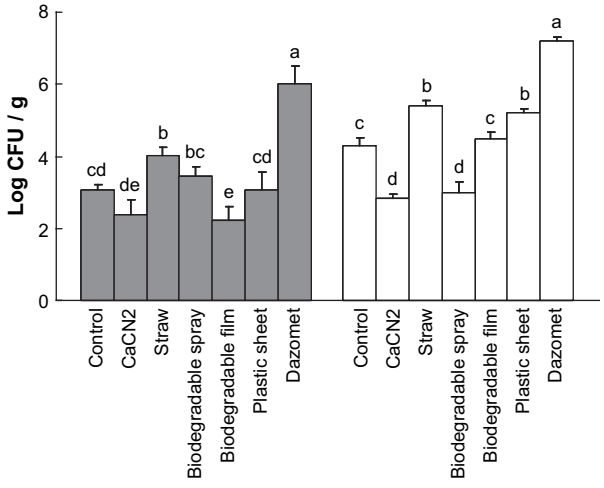

Fig. 5. Effect of soil treatments on the population size of *Pseudomonas fluorescens* in the clay (grey bars) and sandy soil (open bars). Different letters indicate significant differences (comparison only within soil type; Duncan test, $P < 0.05$). Data are averages (+1SE) of three replicates.

4. Discussion

4.1. Effects on crop productivity and disease incidence

Many studies provided evidence that SS with plastic films increases crop yields and allows the control of many soilborne pests and weeds (Stapleton and De Vay, 1995; Stapleton, 2000). In the present study, SS with plastic films, and to a lesser extent with biodegradable materials, was only partially effective in controlling soilborne pathogens and weeds. In addition, its positive effects on crop yields were limited in comparison to Dazomet fumigation and, in some cases, to amendment with straw. Previous studies related the positive effect of SS on crop yields to increases in NH_4^+-N or NO_3^--N (Chen et al., 1991; Patrício et al., 2006). According to these authors, the increase in NH_4^+-N is usually greater in soils with higher amounts of organic matter, because temperature enhancement increases soil organic N mineralization. Therefore, the limited increase of NH_4^+-N in both soil types recorded in our study could be due to the very low amount of OM in both soil types (<0.7%). NO_3^--N concentration in soils was always very low in both solarized and non-solarized plots (Table 1). Decreases in nitrifying bacteria due to SS have been reported and related to the accumulation of NH_4^+-N in soil (Chen et al., 1991). However, in the present work, NH_4^+-N accumulated in all treatments suggesting a very low bacterial nitrifying activity also in the untreated soil. The increased growth observed in solarized soils may be associated to the increase of *P. fluorescens* populations (Gamliel and Stapleton, 1993). Our results showed that SS has a variable but limited effect on *P. fluorescens* in comparison to other soil treatments. A negative effect of biodegradable spray and biodegradable film on *P. fluorescens* was

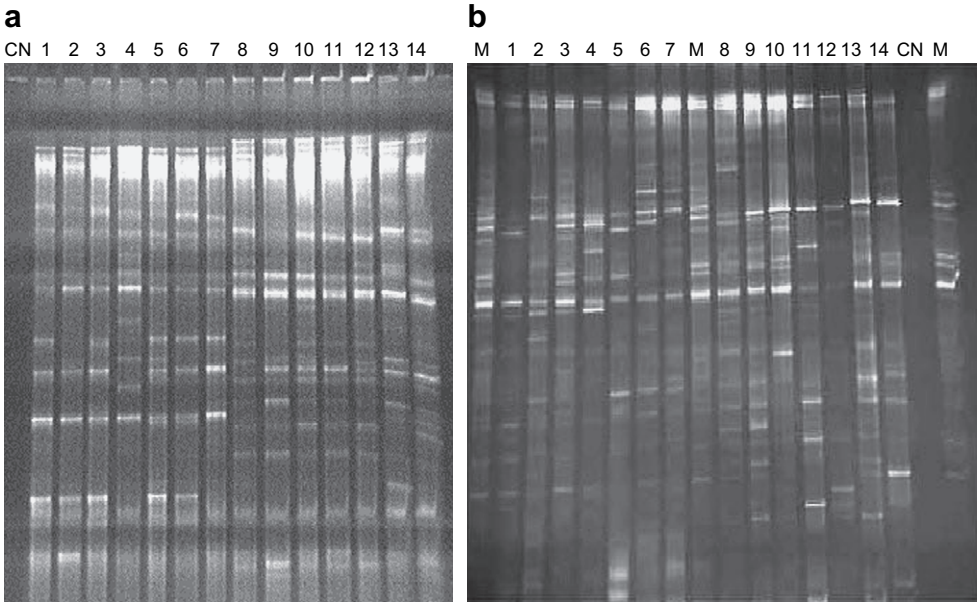

Fig. 6. DGGE pattern comparison of 16S rRNA (a) and 28S rRNA (b) amplified genes of clay (lane 1–7) and sandy (lanes 8–14) soils. Treatments were as follow: lanes 1 and 8, control soils; lanes 2 and 9, Dazomet treated soils; lanes 3 and 10, plastic sheet treated soils; lanes 4 and 11, CaCN2 treated soils; lanes 5 and 12, biodegradable spray treated soils; lanes 6 and 13, straw treated soils; lanes 7 and 14, biodegradable film treated soils. CN, negative control; M, markers.

recorded in the sandy and clay soil, respectively, while a positive effect of plastic sheet was found in the sandy soil. The observed variable effect of SS on *P. fluorescens* is in agreement with previous results that some bacteria of the fluorescent group decrease their population because are highly sensitive to SS. However, *P. fluorescens* are able to rapidly recolonize the soil after SS (DeVay and Katan, 1991; Stapleton and DeVay, 1984).

Amendment with straw significantly increased FDA activity, *P. fluorescens* population size, OM level and NH_4^+-N concentration in both soil types. Straw amendment has a contrasting effect on crop yields, with a strong positive effect on tomato and no effect on lettuce. Our data suggest that the positive effect of straw on tomato growth may be due to the increase of *P. fluorescens* rather than to the higher availability of mineral nitrogen. However, in a previous study, Mazzola et al. (2001) found an increase of *P. fluorescens* populations by amending with seed meal of *Brassica napus* at low dosages, but a dramatic decline, below the level of detection, when higher rates were applied. This study and our results suggest that the *P. fluorescens* response to organic amendments is dependent on the type of OM and their application rate.

Dazomet fumigation greatly improved the yield of both tomato and lettuce especially that of the latter species in the sandy soil, but had a limited influence on chemical parameters with only a slight positive effect on NH_4^+-N concentration. The positive effect of Dazomet on plant growth is well known and often has been related to elimination of soilborne pathogens (Martin, 2003). However, our experimental results suggest that the yield increase may depend on a change in the microbial community and, specifically, on the increase of *P. fluorescens* population. Dazomet fumigation increased population size of *P. fluorescens* by several orders of magnitude in both soil types. This is consistent with previous studies that reported a significant increase of *P. fluorescens* populations after soil fumigation (Miller et al., 1997; Toyota et al., 1999; Elliott and Des Jardin, 2001). This effect could be explained by hypothesizing that part of the microbial population is killed and used as substrate by *P. fluorescens*.

Table 2
Means of DNA-band number for bacterial and fungal PCR-DGGE in response to different treatments in the clay and sandy soils

	Clay soil		Sandy soil	
	Bacteria	Fungi	Bacteria	Fungi
Control	12.0 ± 0.6bc	19.0 ± 3.6a	14.7 ± 0.3c	27.0 ± 1.2c
CaCN2	13.0 ± 0.6cd	20.3 ± 1.5a	15.3 ± 0.3c	25.0 ± 3.1bc
Straw	14.0 ± 0.6d	19.0 ± 0.0a	8.7 ± 0.3a	19.7 ± 2.0b
Biodegradable spray	13.0 ± 0.0cd	17.3 ± 4.5a	13.0 ± 0.0b	23.3 ± 2.7bc
Biodegradable film	10.3 ± 0.3a	11.6 ± 2.3a	12.7 ± 0.3b	21.7 ± 3.0bc
Plastic sheet	11.0 ± 0.0ab	17.0 ± 1.5a	12.7 ± 0.9b	23.3 ± 0.7bc
Dazomet	10.3 ± 0.3a	15.3 ± 3.5a	13.0 ± 0.0b	13.0 ± 1.0a

In the columns, different letters indicate significant differences (Duncan test, $P < 0.05$).

In this context, it should be pointed out that crop yield was positively related to the *P. fluorescens* population size in both soil types. *P. fluorescens* are known as plant growth promoting rhizobacteria and they can improve plant mineral nutrition, release stimulatory compounds and act as biocontrol agents towards soilborne pathogens (Smith and Goodman, 1999; Lugtenberg et al., 2001). Although it is known that *Pseudomonas* spp. can improve plant mycorrhizal colonization (Frey-Klett et al., 2007), it seems unlikely that mycorrhizas did play a role in stimulating plant growth since solarization negatively affects the survival of such fungi (Al-Momani et al., 1988; Bendavid-Val et al., 1997).

Growth of both tomato and lettuce were negatively related to fungal richness in the sandy soil while no significant correlations were found with bacterial richness. These results do not support the hypothesis that microbial richness is directly and positively related to soil ecosystem function and fertility (Coleman and Whitman, 2005).

SS with plastic sheet, and to a lesser extend with biodegradable film and biodegradable spray, was efficient in reducing lettuce drop in both soil types, supporting previous results obtained in different environmental conditions (Sinigaglia et al., 2001; Patrício et al., 2006). The application of CaCN2 and straw was able to control lettuce drop only in the sandy soil and Dazomet fumigation only in the clay soil (Fig. 2). It is interesting to note that lettuce drop incidence was unrelated to FDA activity. Our results contrast with the evidence that this parameter is negatively related to the incidence of soilborne pathogens such as *Pythium* spp. (Chen et al., 1988; Craft

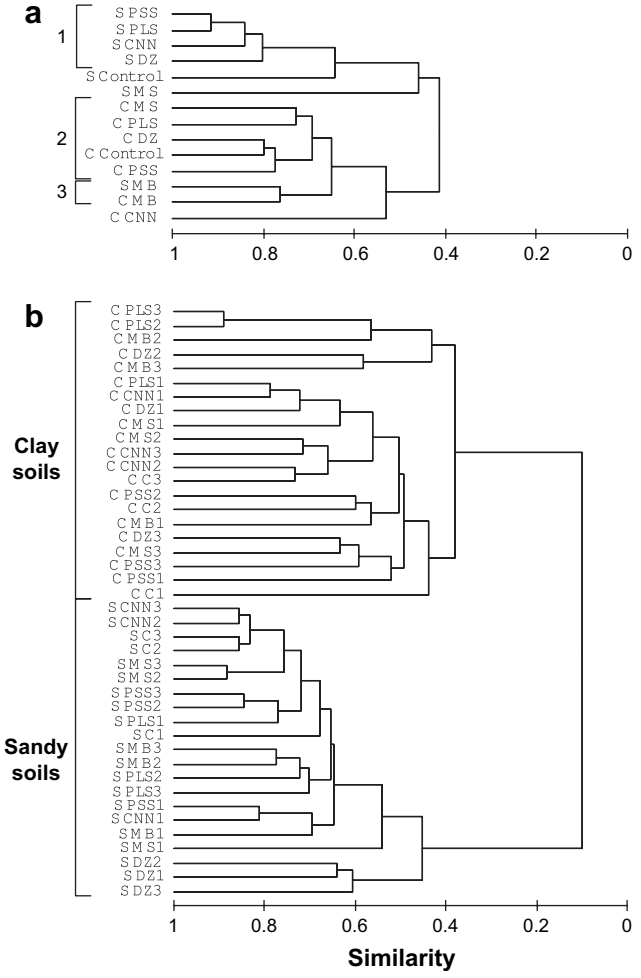

Fig. 7. Cluster analysis (UPGMA, Dice coefficient) of bacterial (a) and fungal (b) banding pattern of clay (capital letter C before the sign of the treatment) and sandy (capital letter S before the sign of the treatment) soils. Signs of the treatments are: C, control; CNN, $CaCN_2$; DZ, Dazomet; MB, biodegradable film; MS, Medicago sativa straw; PLS, plastic sheet; PSS, biodegradable spray material.

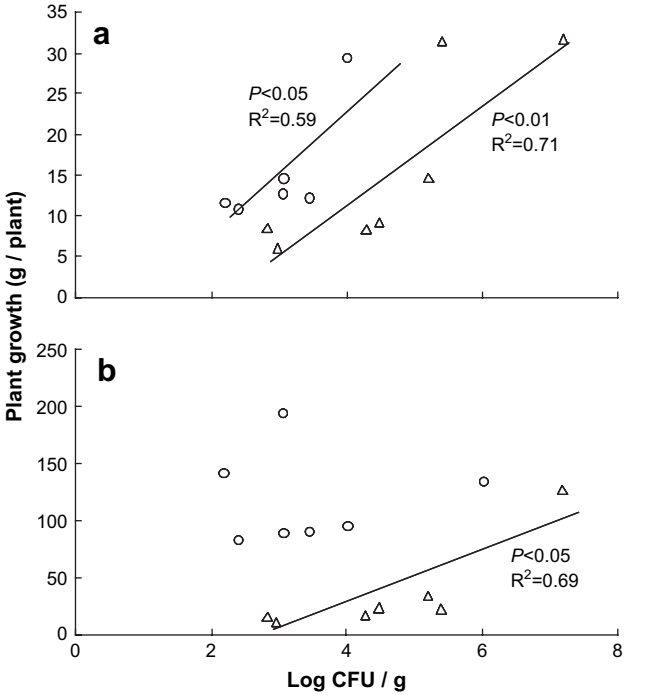

Fig. 8. Correlation between crop productivity (a = tomato, b = lettuce) and soil population size of *Pseudomonas fluorescens* (colony forming units/g = CFU/g) in the clay (circles) and sandy soil (triangles). The levels of statistical significance are indicated on each graph.

and Nelson, 1996), *Phytophthora cinnamomi* (Aryantha et al., 2000), *Pyrenochaeta lycopersici* (Workneh et al., 1993) and *Sclerotium rolfsii* (dos Santos and Bettiol, 2003). However, Yulianti et al. (2006) recently found that soil amendment with cruciferous plant residues increased soil FDA activity but, at the same time enhanced the incidence of *Rhizoctonia solani*.

4.2. Effects on soil microbiological and chemical parameters

DGGE analysis is a powerful and reliable method to compare the effect of different treatments on microbial community structure, although the DGGE banding pattern represents only the most abundant species in soil (Muyzer and Smalla, 1998). In our study, soil type was the major determinant of the composition and structure of the bacterial and fungal communities because it, more than soil treatments, determined the clustering into groups (Fig. 7). Costa et al. (2006a) stated that the sampling site is one of the main factors affecting the relative abundance and distribution of PCR-DGGE ribotypes. DGGE bacterial patterns among soil replicates were very similar, while fungal replicates were not related at all: cluster analysis was not able to differentiate among the treatments, as reported in other studies (Klamer et al., 2002; Costa et al., 2006b). According to Ranjard et al. (2003), the assessment of soil microbial community structure by the use of molecular techniques requires a satisfactory sampling strategy that takes into account the high microbial diversity and the heterogeneous distribution of microorganisms in the soil matrix. These authors stated that the sampling strategy should be different according to the objectives: large soil samples (≥1 g) for fungal community structures, while smaller soil samples (≥0.125 g) are sufficient for bacterial ones.

The impact of SS by traditional plastic on soil microbial communities has been previously studied (Culman et al., 2006). Gelsomino and Cacco (2006) stated that SS was the main factor inducing strong time-dependent population shifts in eubacterial community structure. However, to our knowledge this is the first report on the effect of solarization performed with biodegradable materials on microbial populations analyzed by DGGE. SS with both biodegradable and plastic films generally decreased fungal and bacterial band richness, with a more pronounced effect on bacteria. Among the solarizing treatments, biodegradable film showed the most negative effect on bacterial diversity in both soils and on fungal diversity in the sandy soil (Table 2). Biodegradable film treatments clustered together with the bacterial clay soil cluster. It is important to note that this was the only case in our study where the effect of the treatment overcomes the soil influence on the community structure. This could be related to the very strong, although temporally limited, soil heating observed with biodegradable film.

In our work, a single straw amendment significantly affected bacterial and fungal DGGE profiles, but the effect was strictly dependent on the soil type. Specifically, straw slightly increased bacterial richness in the clay soil, while a strong negative effect on both bacteria and fungi was recorded in the sandy soil. This fact was confirmed by the bacterial cluster analysis (Fig. 7a), which showed a low similarity among straw treated samples and all the other treatments. These microbial richness reductions appear surprising because organic amendments commonly are responsible for an increase of the microbial biomass and richness (Sun et al., 2004).

Table 3
Cross-correlation matrix between soil chemical and microbiological parameters measured in the clay and sandy soils (PF = *Pseudomonas fluorescens*; BR_F = fungal band richness; BR_B = bacterial band richness)

	Clay soil								Sandy soil							
	TotN	NH_4-N	P_2O_5	OM	FDA	PF	BR_F	BR_B	TotN	NH_4-N	P_2O_5	OM	FDA	PF	BR_F	BR_B
TotN	–	0.44	−0.01	0.58	0.41	0.57	0.17	0.19	–	**0.86**	0.69	**0.76**	**0.75**	0.41	−0.46	−0.51
NH_4-N	–	–	0.55	0.39	**0.84**	−0.47	−0.16	0.20	–	–	0.55	0.56	0.58	0.63	−0.71	−0.51
P_2O_5	–	–	–	0.38	−0.27	0.49	0.63	0.61	–	–	–	**0.95**	**0.90**	0.24	0.14	**−0.87**
OM	–	–	–	–	0.70	0.22	0.26	0.60	–	–	–	–	**0.97**	0.15	−0.14	**−0.82**
FDA	–	–	–	–	–	−0.26	−0.20	0.32	–	–	–	–	–	0.18	−0.24	**−0.85**
PF	–	–	–	–	–	–	−0.01	−0.15	–	–	–	–	–	–	**−0.87**	−0.48
BR_F	–	–	–	–	–	–	–	**0.77**	–	–	–	–	–	–	–	0.43
BR_B	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Values represent Pearson correlation coefficients, coefficients in bold indicate significant parameter at $P < 0.05$ in the regression analysis.

The loss of diversity following straw amendment could be due to the sharp increase of a few dominant microbial species, such as *P. fluorescens* (Fig. 5), that may rapidly exclude other species by competition. However, organic amendments have been reported to have either positive (Drinkwater et al., 1995; Sun et al., 2004) or no effect (Lawlor et al., 2000; Franke-Snyder et al., 2001) on microbial diversity.

Traditional methods have been previously used to study the effects of fungicides on soil microrganisms (Shukla and Mishra, 1996). In our work, Dazomet fumigation reduced both bacterial and fungal richness, with the most negative effect on fungi in the sandy soil. Dazomet replicates in the sandy soil cluster were the only ones that clustered together in the PCR-DGGE analysis performed with fungal primers, thus confirming the strong effect of the fumigation on the fungal community structure in this soil. The limited loss of diversity in the clay soil after Dazomet fumigation could be due to the presence of clay – humic complexes which may partially adsorb the fungicide thus reducing its negative effect on microbial populations. For example, Sigler and Turco (2002) analyzed the impact of the fungicide chlorthalonil on soil bacterial and fungal populations, by using a DGGE molecular approach, and found that after a single application, the community changes were less pronounced in soils with higher organic matter contents.

Soil organic C content was expected to diminish after solarization because of the heat-induced breakdown of soil organic resources and the enhanced microbial activity after heating. However, we found that total soil OM was not significantly changed by SS in agreement with previous studies (Chen et al., 1991; Stapleton et al., 1985; Gelsomino et al., 2006) that report a lack of significant differences of organic C amounts between non-solarized and solarized bare soils. In our study sites the OM level was already very low (<0.7%), suggesting that OM is present in a stabilized and recalcitrant form that is not susceptible to a rapid decomposition after soil heating by solarization.

4.3. Effects on weed suppression

Our results showing a positive effect of SS on weed suppression corroborate those of many other studies (Patrício et al., 2006; Culman et al., 2006). The strong reduction of weed cover observed with SS with plastic sheet was expected, considering the high soil temperatures recorded during the treatment in the surface soil layers. However, weed control with SS by biodegradable film was limited and almost absent with biodegradable spray. The limited effectiveness of SS with biodegradable materials are likely due to the short period of solarization with biodegradable film (20–25 days) and to the poor heating capability of biodegradable spray. Biodegradable film deterioration is due to both UV radiation and microbial decomposition, and as reported in a previous study (Russo et al., 2005) its life-span was shorter than one month. Differently, the biodegradable spray, although considerably resistant to deterioration, has a limited soil heating capability compared to biodegradable film and plastic sheet. The most effective treatment for weed control in both soil types was Dazomet fumigation, which is well know for its phytotoxicity (Slusarski, 1989; Gilreath and Santos, 2004).

4.4. Conclusions

The results of this experimentation show the potential of using the biodegradable solarizing materials in place of plastic films, but also, indicate the need for improving their technological properties (transparency and resistance to degradation) to obtain performances comparable to those of other pest management techniques.

Acknowledgements

We thank the farmer R. Pastore for conducting the experiments on his property and L. Cavaliere for technical assistance. The work was supported by the BIO.CO.AGRI. project (Life Environment 03/377).

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