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ORIGINAL PAPER

Influence for Soil Environment by Continuing use of Biodegradable Plastic

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Abstract The influence on soil environment by continuing use of the biodegradable plastic films (biodegradable mulching films) in farmland was investigated. The difference was not seen in the amount of soil bacteria between mulching film plowing sections and non-plowing sections. The total bacteria amount did not increase by the effect of plowing the biodegradable mulching film. Poly-(butylene succinate and adipate) (PBSA) and poly-(ε-caprolactone) (PCL) decomposing bacteria did not increase in PBSA and PCL mulching film plowing sections comparing polyethylene covering section (PE) and no-film section. Polylactic acid (PLA) decomposing bacteria were not detected in all sections. Total denaturing gradient gel electrophoresis (DGGE) band patterns did not show a clear transition of the bacterial community structure in both the cultivating and promoting sections. In usual usage condition of the biodegradable plastic films, it was hardly influence to the soil environment such as bacterial community structure in farmland.

Keywords Bacterial decomposition · Bacterial community structure · Biodegradable mulching film

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Introduction

The synthetic plastics have high performance and stability. On the other hand, it is serious problem that they remain undecomposed in the landfills, draft as floatage garbage on ocean, etc. To solve the serious problem, biodegradable plastics are accepted to the society. They are decomposed by the bacteria in the soil environment and reduced to the soil environment [1, 2]. For these reasons, the quantity consumed of biodegradable plastics increases every year. However, the influence on the soil bacteria in the degradative process in those natural environments is not clear. Especially, the biodegradable plastic films (biodegradable mulching films) are covered to the field every cultivation in farmland. They are broken up and plowed into the field after cultivation. Therefore, it is worried that bacteria decomposing biodegradable mulching film increase in the field and the soil bacteria community structure destroys [3].

The influence on soil environment by continuing use of the biodegradable mulching film in farmland was investigated in this study.

Method

Cultivation Schedule using Biodegradable Mulching Films

The biodegradable mulching films were used from June 2006 to October 2008 continuously in the university farm of Osaka Prefecture University (Fig. 1). The latitude of the site was 34 °32′N and longitude 135 °30′E. It was harvested by the rotation of crops system that combined the sweet potato, the radish, and the onion. After harvesting, biodegradable mulching films were broken up and plowed

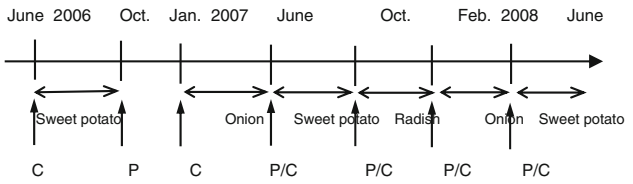

Fig. 1 Cultivation schedule using biodegradable mulching films. *C* covering of mulching films, *P* plowing of mulching film, *P/C* plowing and covering of mulching film

into the field (plowing of five times in total). Moreover, biodegradable mulching films were broken up and plowed into the field for ten times without cultivation as a promoting section. Fertilizers were added at every cultivation [$N:P_2O_5:K_2O = 10:6.3:8.8$ (g/m^2)]. They were added to promoting sections at same time. At the radish cultivation, insecticides were used.

Mulching Films

The three kinds of biodegradable mulching films [PBSA (Kiemaru, Unyck, Tokyo, Japan), PCL film containing starch (Eco-green-mulch II, Taiyo-kogyo, Tokyo, Japan), and PLA (Terramac, Unitika, Osaka, Japan)] were used in this study. The undecomposed polyethylene mulching film [PE (Noupori, Taiyo-kogyo, Tokyo, Japan)] was used for the comparison. All mulching films were 0.02 mm thickness. The black mulching films containing carbon black as additive were supplied to the experiment.

Measurement of Soil Bacteria

The soil contacting with the mulching film (surface) and the soil which contains from surface to 10 cm depth of a ridge (whole) were sampled regularly. The sampling soils were suspended to distilled water with ultrasonication (3 times for 1 min at intervals of 3 min, Branson, 2210 J-MTH type). The supernatant were inoculated onto nutrient agar medium (meet extract 5 g, peptone 10 g, NaCl 5 g and agar 15 g per liter, pH7.0) and incubated for 2 days at 25 °C. The raised colonies were counted.

Measurement of Biodegradable Plastic Decomposing Bacteria

The regularly sampling soils were suspended to distilled water with ultrasonication. The supernatant were inoculated onto emulsion agar medium containing 0.1% biodegradable plastic. They were incubated for 7 days (PBSA and PCL) or 14 days (PLA) at 25 °C. The colonies which have clear haro by decomposition were counted [1]. Three types of biodegradable plastics (PBSA (3001, Showa High Polymer, Tokyo, Japan), PCL (Mn = 40,000, Wako Chemicals, Tokyo, Japan) and PLA (Mn = 2,000, Polyscience, USA)) were used.

Nucleic Acid Extraction

Nucleic acid extraction from soil was carried out according to the procedure described by Miller et. al. [4] with some modifications. The soils containing 0.5% sodium dodecyl sulfate solution were homogenized with Bead Beater. They kept at 70 °C for 20 min. Chlorofolm-isoamylalcohol was used for DNA extraction. The obtained DNA was then concentrated by ethanol precipitation and dissolved in TE buffer (10 mmol/l Tris–HCl (pH8.0), 1 mmol/l EDTA).

PCR Amplification of 16s rDNA

The variable V3 region of the 16s rDNA was enzymatically amplified in the PCR [5] with primers to conserved regions of the 16s rRNA genes [6]. The nucleotide sequences of the primers were as follows: 341F-GC, 5′-CGCCCGCCG CGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCT ACGGGAGGCAGCAG-3′ and 534R, 5′-ATTACCGCGG CTGCTGG-3′ [7]. Combination of these primers generated a PCR fragment of about 200 bp suitable for subsequent DGGE analysis. Each amplification reaction mixture (20 µl) consisted of 1 µl sample DNA (4–20 ng), 2 µl of 10 × PCR buffer, 0.125 mM deoxynucleotide triphosphate (dNTP) mix, 1.25 mM $MgCl_2$, 0.5U of LA *Taq* DNA polymerase (TaKaRa Bio, Otsu, Japan), and each primer at the concentration of 5 µM. A touchdown program [7] was implemented as follows: After pre-incubation at 94 °C for 5 min, 30 cycle were performed at 94 °C for 30 s, the annealing temperature for 30 s, and 72 °C for 1 min. In the first 20 cycles, the annealing temperature was decreased by 0.5 °C from 61 °C to 52 °C every cycles. The annealing temperature was 52 °C in the last 10 cycles with PCR thermal cycler. The PCR product was analyzed on 15 g/l agarose gels containing 50 g/l of 20× TAE buffer (40 mmol/l Tris–acetate, 1 mmol/l EDTA) by applying ethidium bromide (0.5 µg/ml) staining. The gel was photographed under ultraviolet (UV) light to ascertain the successful amplification.

DGGE Analysis

PCR samples were loaded onto 8% polyacrylamide-bisacrylamide (29:1) denaturing gels with a gradient from 35% to 55% in 1× TAE. One hundred percent of denaturant corresponded to 7 M ures and 40% (v/v) foramide. Electrophoresis was performed at 80 V at 60 °C for 170 min. After electrophoresis, the gels were stained with 0.5 µg/ml ethidium bromide solution and photographed under UV light.

Results and Discussion

Annual Transition of Amount of Soil Bacteria

The biodegradable mulching films are decomposed by soil bacteria in farmland. Therefore, it was expected that the activity of microorganism including the biodegradable plastic decomposing bacteria became active, and the amount of soil bacteria on surface increased compared with that of the whole. In addition, it was expected that the amount of soil bacteria of more biodegradable plastic plowing sections as PBSA and PCL increased comparing those of PLA. However, the difference was not seen compared with the surface and the whole at the cultivation section in the amount of soil bacteria (Fig. 2, 3). The difference by the kind of films was not also seen (Fig. 2, 3).

In the promoting sections, the biodegradable mulching films were plowed into the field twice volume comparing the cultivating section. However, the difference was not seen in the amount of soil bacteria in the cultivating section (whole) (Fig. 3) and the promoting section (whole) (Fig. 4).

No film section had slightly few bacteria comparing other sections in cultivation section (whole). On the other hand, no film section had slightly many bacteria comparing other sections in promoting section (whole). PCL plowing section had slightly many bacteria comparing other sections in cultivation section. This might be due to the starch component in PCL film. The amount of soil bacteria tended to decrease in cultivating section (whole) in the summer season. The difference by the season was few in the promoting section.

Thus, the difference by the surface soil and the whole soil, the kind of film, and the plowing amount of film was hardly observed in the amount of soil bacteria. Moreover, even if plowing the biodegradable films to the field after cultivation, the total bacteria amount did not increase.

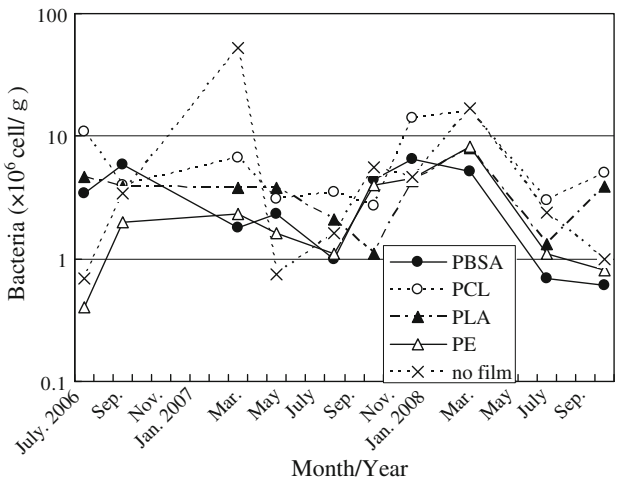

Fig. 2 Soil bacteria (cultivating section, *surface*)

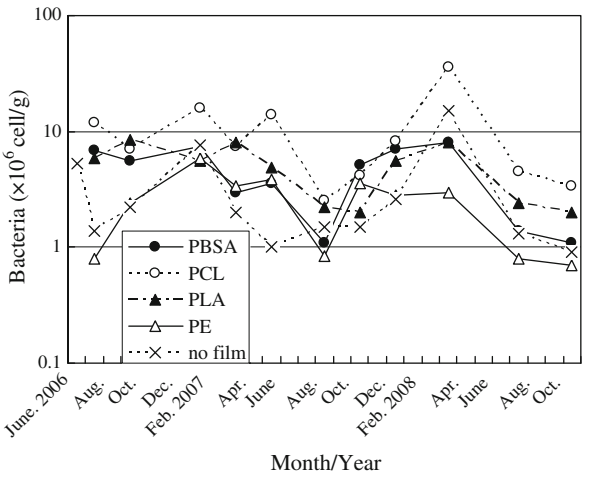

Fig. 3 Soil bacteria (cultivation section, *whole)*

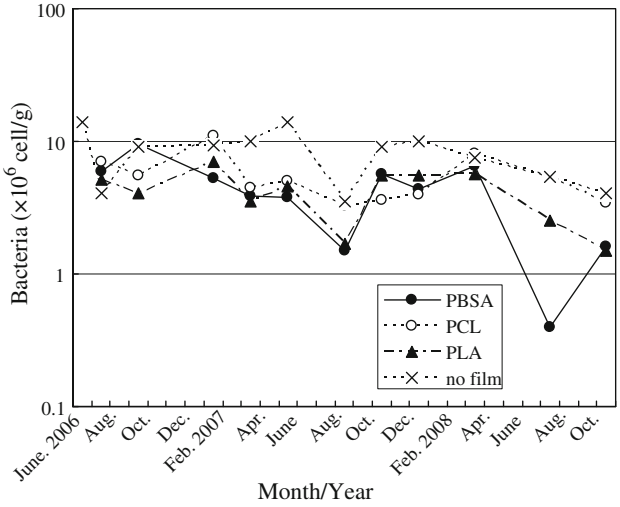

Fig. 4 Soil bacteria (promoting section, *whole*)

Transition of Amount of Biodegradable Plastic Decomposing Bacteria

The amount of PBSA decomposing bacteria was not different in PBSA plowing section, PE covering section, and no film section at all. In addition, the amount did not increase at promoting section. The amount of decomposing bacteria did not increase by the effect of plowing mulching film (Table 1). It was similar about the amount of PCL decomposing bacteria in PCL plowing section (data not shown).

PLA decomposing bacteria were not detected in neither the cultivation section nor the promotion section [1, 8].

The detected biodegradable plastic decomposing bacteria were about 10^3–10^4 cells/g, and the bacteria in the field were 10^6–10^7 cells/g. It is thought that about 1% of the soil bacteria in the field took part in the decomposition of biodegradable mulching film.

Table 1 PBSA decomposing bacteria (cell/g)

Month/year	Cultivating section		Cultivating section		Cultivating section		Promoting section	
	PBSA		PE		No film		PBSA	No film
	Surface	Whole	Surface	Whole	Surface	Whole	Whole	Whole
June 2006						1.3×10^3		1.3×10^3
July	4.0×10^3	1.0×10^3	0.5×10^3	1.0×10^3	ND	ND	0.5×10^3	ND
Sep.	0.5×10^3	0.5×10^3	0.5×10^3	0.5×10^3	ND	ND	0.5×10^3	ND
Jan. 2007		1.0×10^3		0.5×10^3		0.5×10^3	ND	1.0×10^3
Mar.	0.5×10^3	1.5×10^3	ND	ND	ND	0.5×10^3	2.0×10^3	1.5×10^3
May	ND	1.5×10^3	1.0×10^3	0.5×10^3	ND	ND	3.0×10^3	1.5×10^3
Aug.	0.5×10^3	ND	0.5×10^3	ND	ND	ND	0.5×10^3	4.5×10^3
Oct.	8.5×10^3	7.0×10^3	2.5×10^3	0.5×10^3	3.0×10^3	4.0×10^3	6.5×10^3	6.5×10^3
Dec.	ND	1.5×10^3	5.0×10^3	3.5×10^3	1.0×10^4	0.5×10^3	0.5×10^3	3.0×10^3
Mar. 2008	5.5×10^3	7.5×10^3	1.0×10^3	5.5×10^3	2.5×10^3	2.5×10^3	1.0×10^3	2.5×10^3
July	ND	1.5×10^3	2.0×10^3	0.5×10^3	2.5×10^3	1.0×10^3	ND	1.0×10^3
Oct.	ND	0.5×10^3	1.0×10^3	ND	0.5×10^3	ND	1.7×10^3	0.5×10^3

Identification limit, 0.5×10^3 cell/g; ND, Not detected

DGGE Analysis of Bacterial Community Structure

The total bacteria amount did not increase by plowing the biodegradable mulching film to the field. The amount of biodegradable plastic decomposing bacteria did not also increase by the effect of plowing mulching film to the field. However, there is a possibility that the bacteria community structure containing anaerobic bacteria, no-colony forming

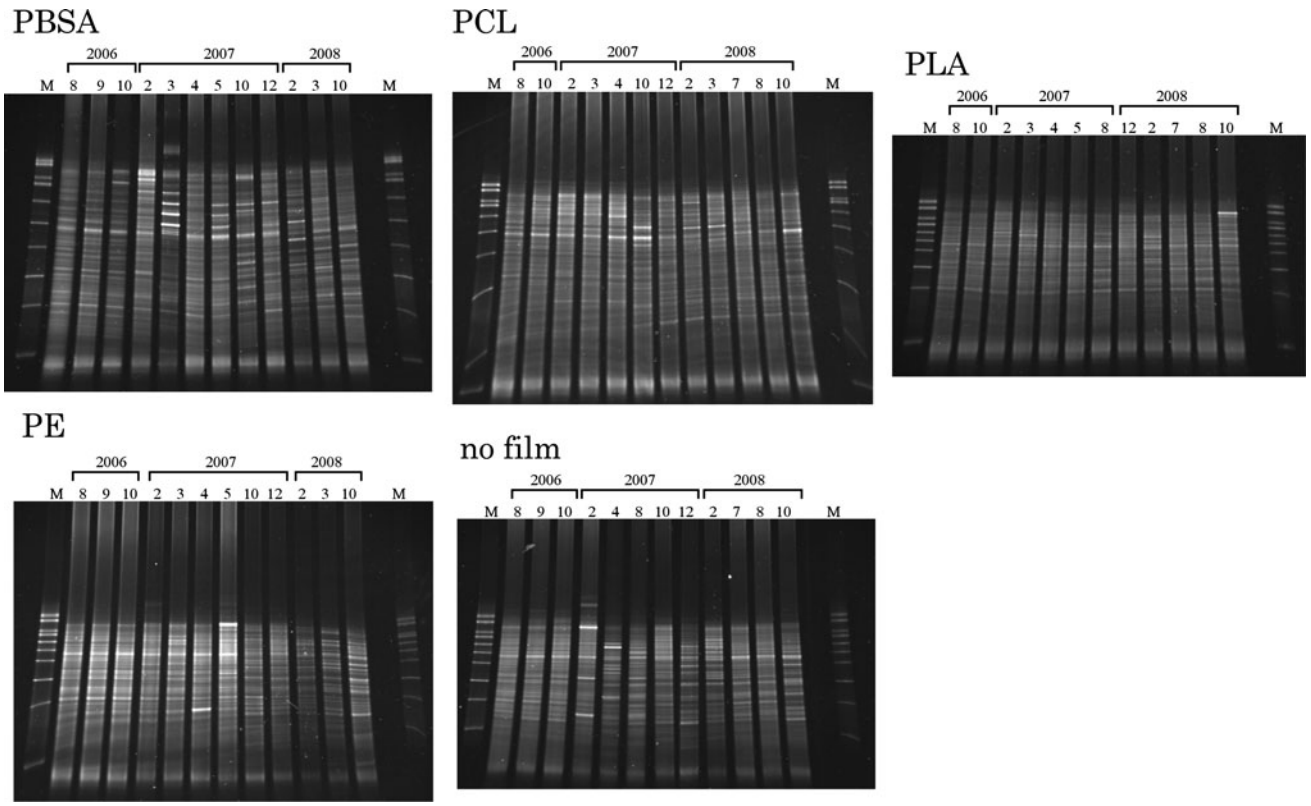

Fig. 5 DGGE band profiles (cultivating section, *whole*). The data of from Aug. 2006 to Oct. 2008 were shown. *M* DGGE marker

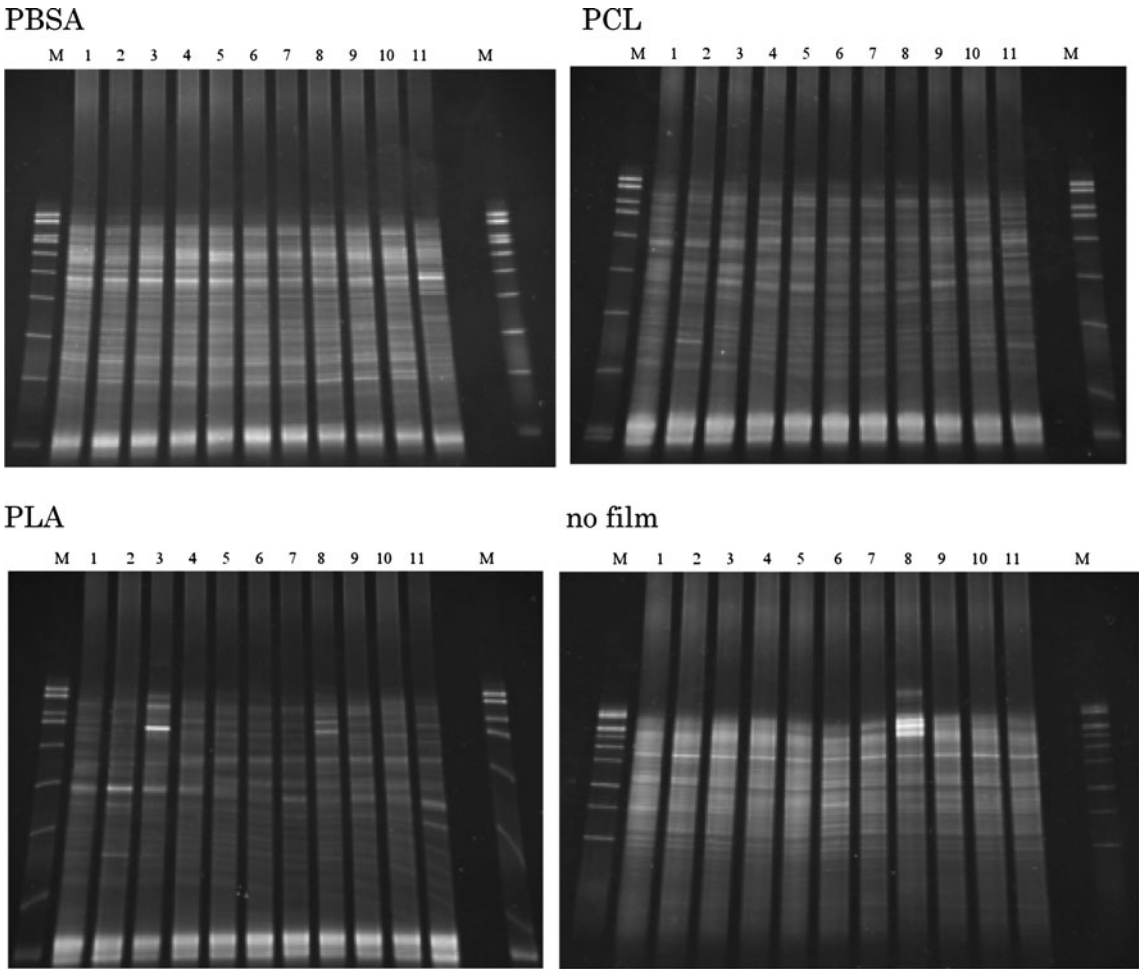

Fig. 6 DGGE band profiles (promoting section, *whole*). *1* Aug. 2006 *2* Oct. 2006 *3* Jan. 2007 *4* Mar. 2007 *5* May 2007 *6* Aug. 2007 *7* Oct. 2007 *8* Dec. 2007 *9* Mar. 2008 *10* July 2008 *11* Oct. 2008 *M* DGGE marker

bacteria, and soil fungi has changed. DGGE analysis was performed to confirm the transition of the bacterial community structure.

In cultivating sections, some bands appeared or disappeared unperiodically in all sections (Fig. 5). In promoting sections, flat bands were considered (Fig. 6). The difference might be due to outward factors by harvesting. Though some appearing or disappearing bands were observed unperiodically, a clear transition of the bacterial community structure from Aug. 2006 to Oct. 2008 was not seen both the cultivating section and the promoting section from total DGGE band patterns.

In the beginning, it was thought that the activities of the biodegradable plastic decomposing bacteria became to high level by plowing mulching film, the soil bacterial community structure changed, and new bacteria community structure were set up. However, bacteria communities did not changed by plowing biodegradable mulching films in practice.

Influence of Biodegradable Plastic Films Plowing

At first, it was estimated that the specific bacteria that would decompose mulching film increased and these bacteria gradually had priority in the soil plowing biodegradability mulching film continuously. However, plowing the biodegradable plastics were not most influences for the amount of soil bacteria, biodegradable plastics decomposing bacteria, and bacteria community structure in neither cultivating section nor promoting section. It is because the weight of mulching film plowing into the field was about 0.01% for the that of the soil, the amount did not relate to the increasement of decomposing bacteria actually.

There was no difference between the production of the crops body (sweet potato, onion, and radish) by using biodegradable mulching films and that by using PE film. (data not shown) Moreover, it was not observed that the crops body had wound or blanching by films remaining in the field after plowing.

PBSA and PCL films were hardly decomposed at covering period to the field. They were gradually decomposed by plowing into the field. When PLA film was broken up and plowed into the field, it was physically splintered gradually. However, it was hardly decompose in the field, and tended to accumulate in the field gradually. Moreover, PLA decomposing bacteria was not detect in this field. Therefore, to promote decomposition of PLA is great problem in the case of PLA film using.

In conclusion, if the biodegradable mulching films were used a usual usage manner and frequency in the farmland and plowed into the field afterward, soil environment do not have stress hardly and it was hardly influence to the soil environment such as soil bacteria community structure.

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