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Effects of crop residue incorporation on soil-biodegradable mulch film degradation in a broccoli-sorghum rotation system

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Abstract

Soil-biodegradable mulch films are designed to be incorporated into the soil at the end of the crop cycle, to be degraded by soil microorganisms. Despite growing scientific interest, open-field studies about the fate of these mulch films in soils are rare, with most research conducted in laboratories. We performed an open-field trial to evaluate the effects of soil incorporation of both a combining a commercial soil-biodegradable mulch film and broccoli and sorghum crop residues over two cropping seasons. The mulch film was sampled after broccoli harvesting and placed inside mesh bags that were buried horizontally or vertically at different depths. Film biodegradation was assessed by direct (CO_2 emissions) and indirect indicators (film mass and area loss). Three experiments were conducted to evaluate (a) how incorporating crop residues affect mulch film degradation; (b) temporal changes of soil microbial activity after the incorporation of crop residues and mulch films; and (c) possible bias in the measurement of soil CO_2 emissions due to horizontal vs. vertical mesh bag orientation. Incorporation of crop residues enhanced mulch film degradation and increased the soil esterase activity. However, abundance of soil fungi and bacteria in bulk soil was not increased by mulch film presence. Mesh bag orientation did not alter the soil CO_2 efflux or affect the degradation degree of mulch films. Film in mesh bags buried at 10–15 cm depth showed greater degradation than those buried at 0–5 cm. Further in-field studies are necessary to evaluate the effects of soil incorporation of residue from different crops and impacts of other associated management practices such as various soil tillage regimes.

1 | INTRODUCTION

Mulching with plastic film is a well-established agronomic technique that increases crop yield by moderating soil temperature, increasing water use efficiency, controlling weeds, preventing disease, and reducing soil erosion and compaction (Chen et al., 2021; Martín-Closas et al., 2017; Sintim et al., 2021; Tofanelli & Wortman, 2020). Most of the mulch films currently used are based on polyethylene, a polymer that is not readily biodegradable and thus accumulates in the environment if not properly recycled (Narayan, 2017). Mulch films that are biodegradable in the soil environment have recently been developed (Francioni et al., 2022; Narayan, 2017; Sander, 2019). Soil-biodegradable mulch films are designed to be used by microorganisms as a source of energy, ultimately converting the film into CO_2, water, and new

Abbreviations: CFU, colony-forming unit; FILM, mulch film incorporation; IRGA, infrared gas analyzer; RES, crop residues incorporation; RMANOVA, repeated measure analysis of variance; SOIL, soil treatment (control, no film, no residue incorporation).

microbial biomass (Sander, 2019). This allows soil-biodegradable mulch films to be incorporated into the soil at the end of the cropping cycle, removing the need for the disposal of films by landfilling or incineration (Brodhagen et al., 2015; Choe et al., 2021; Francioni et al., 2022; Narayan, 2017).

Desirable characteristics of a soil-biodegradable mulch film include durability during the cropping cycle (i.e., the film must be easy to deploy and not fragment too much during the cropping cycle) and complete biodegradability over an acceptable time frame after being buried. Soil-biodegradable mulch films are being manufactured using blends of various polymers and additives, such as colorizers, that influence film's physical properties (Brodhagen et al., 2015; Francioni et al., 2022; Martín-Closas et al., 2017; Sander, 2019). While studies on soil-biodegradable mulch films are increasing, most of these studies have been conducted under controlled laboratory conditions (Francioni et al., 2022). Studies conducted in open-field conditions, where the effects of abiotic and biotic factors on the rate of biodegradation of buried mulch films are considered, are relatively scarce (Francioni et al., 2022; Griffin-LaHue et al., 2022). Among the few available open-field studies, some have assessed the degradation rates of mulch films under various pedo-climatic conditions (e.g., Sintim et al., 2020), while others compared degradation rates in laboratory vs. field conditions also addressing the issue of potential plastic accumulation over the years (Griffin-LaHue et al., 2022). In a recent study, it was highlighted that the combination of plowing and grubbing can accelerate the degradation rate of mulch film at the end of a zucchini cropping cycle (Bianchini et al., 2022). Other open-field studies have evaluated the degradation behavior of film of mulch films either before (Liu et al., 2022) or after their incorporation (Li et al., 2023), or have focused solely on their impact on crop yields and quality (Wang et al., 2021). Integration of results from laboratory-based and open-field studies would improve our understanding of the mechanics and dynamics of biodegradation of mulch films, as the former may not reflect actual biodegradation rates of mulch films under field conditions (Brodhagen et al., 2015; Griffin-LaHue et al., 2022). For example, crop residue incorporation can have a priming effect on metabolic activities of soil microorganisms, depending on residue quality and quantity (Shahbaz et al., 2017). Changes in the metabolism of microorganisms can be monitored by measuring enzymatic activities such as esterases that are associated with the biodegradation of polyester-based soil-biodegradable mulch films (Tsuboi et al., 2022) or by measuring soil CO_2 emissions (Basalirwa et al., 2020; Toderi et al., 2022; Yonemura et al., 2014). However, only a limited number of studies have investigated the interactions between soil-biodegradable mulch flms and soil organic matter (e.g., Guliyev et al., 2022), and very few explored their interactions with crop residues (e.g., Kapanen et al., 2008).

Core Ideas

- Crop residue incorporation resulted in greater mulch film degradation.
- Biodegradable mulch films and crop residue incorporation increased soil esterase.
- Mulch film presence in soil did not increase soil fungi and bacteria abundance.
- Mulch film biodegradation was not detected by direct CO_2 field measurements.
- Mulch film buried at 10−15 cm depth degraded more than that buried at 0−5 cm.

These studies were generally not conducted under open-field conditions.

Studies attempting to analyze the biotic and/or abiotic degradations of soil-biodegradable mulch films under open-field conditions are limited but increasing (Francioni et al., 2022). Examples include studies that have investigated the degradation of soil-biodegradable mulch films into macro and then microplastics followed by a quantification via chemical extraction (Li et al., 2023). Some other studies have placed mulch film samples in mesh bags, which allow the adhesion of soil to the film, ensuring the passage of water and microorganisms (Bianchini et al., 2022; Sintim et al., 2020; Tsuboi et al., 2022). In these studies, film area or mass loss, measured with infrared spectroscopy or nuclear magnetic resonance, was used to estimate degradation. It should be noted that such indirect indicators are not suitable for measuring the biodegradation level of mulch films, and that any claim of biodegradability must be supported by tracking the CO_2 evolved from the carbon of the tested material, following international standards or carbon labeling techniques (Sander, 2019). Nevertheless, for mulch films whose biodegradability has already been ascertained, such indirect indicators can be helpful in estimating the biodegradation potential of different soils, management practices, films, or combinations of these factors (Francioni et al., 2022). Biodegradation of mulch films (or of the polymers that compose them) is usually measured in incubation experiments under laboratory conditions. These biodegradation tests follow international standards (e.g., ISO 23517, 2021), with the CO_2 evolving from polymer carbon often measured by respirometers and plotted as a function of time (Ardisson et al., 2014). However, the CO_2 generated by the biodegradation of mulch films may be difficult to measure under open-field conditions, and attempts to measure it have not been reported. For soil CO_2 measurement, portable infrared gas analyzers (IRGA) are commonly used because they are relatively inexpensive and easy to operate (Francioni et al., 2020; Pumpanen et al., 2004). Portable IRGA can also

be used in the attempt to separate heterotrophic soil respiration (i.e., CO_2 emissions by soil microorganisms) from that emitted by the mineralization of mulch film carbon. However, considering that the amount of mulch film incorporated at the end of each cropping cycle is usually small (as is the amount of carbon to be mineralized), IRGA sensor might not be sufficiently sensitive to detect the CO_2 efflux. Moreover, mulch films are not likely to be evenly distributed in the soil, further limiting the utility of IRGA systems. The use of mesh bags would allow IRGA soil respiration chambers to be placed exactly above the buried mulch films. Moreover, since mesh bags are usually placed horizontally (e.g., Bianchini et al., 2022; Sintim et al., 2020; Tsuboi et al., 2022), the soil CO_2 efflux might be diverted and not be fully directed inside the IRGA chamber. This constraint could be overcome by the vertical orientation of the mesh bags, which could in turn decrease the accumulation of water on the film surface, limiting biotic and/or abiotic hydrolysis. Given the need to develop shared methodologies that could also be used for open-field trials, studies that clarify these aspects would be helpful but are currently lacking.

Considering the paucity of field studies available on soil-biodegradable mulch films, the effects of different management practices on their biodegradation rates, and the lack of reliable standard methodologies for open-field trials, this study had the following objectives:

1. investigate the effect of combining crop residue and soil-biodegradable mulch films on soil microbial activity via open-field experiment,
2. evaluate whether or not the CO_2 resulting from the biodegradation of mulch films can be directly measured under open-field conditions,
3. determine how mesh bag orientation (horizontal vs. vertical) influences soil CO_2 efflux and/or mulch film degradation.

2 | MATERIALS AND METHODS

2.1 | Experimental site

Field experiments were conducted at the National Institute for Agro-Environmental Sciences, Tsukuba, Japan (36°01′N,140°07′E). The soil is classified as Typic Hapludands (Soil Survey Staff, 2014) and is characterized by a light clay texture, a pH (H_2O) of 6.9, a bulk density of ∼0.69 g cm^{-3}, total carbon of 4.14%, organic carbon of 3.99%, and total nitrogen of 0.37%. The climate is mild, wet temperate and is characterized by cold, dry winters and hot, sunny summers. Precipitation is greatest during spring and fall (Yonemura et al., 2014). From 1991 to 2020, the average annual temperature was 14.3°C, and the annual rainfall was

1326 mm (Japan Meteorological Agency, AMeDAS system, Tateno observation site, Tsukuba). During the first monitoring period (March 2021–February 2022), the mean temperature was 14.9°C, and the cumulative rainfall was 1490 mm. During the second monitoring period (March 2022–February 2023), the mean temperature was 15°C, and the cumulative rainfall was 1207 mm (Weather Data Acquisition System of NARO, Kannondai, Tsukuba).

2.2 | Field management and experimental design

The experimental field had been used to cultivate wheat, soybean, Japanese mustard spinach, and sorghum for more than 10 years. On March 3, 2021, the experimental field was fertilized with N:P_2O_5:K_2O = 200:200:200 kg ha^{-1} and $CaCO_3$ = 1000 kg ha^{-1} and plowed to a depth of 0.15 m using a harrow (Kubota 34R + Kobashi M187TDX, Japan). On March 15, a Japanese commercial soil biodegradable mulch film (black, 135 cm wide, 18 μm thick) was deployed. Based on preliminary ^{1}H nuclear magnetic resonance using deuterated chloroform ($CDCl_3$) solution, the polymer composition was >50% poly(butylene terephthalate-*co*-adipate), combined with polybutylene succinate–type polymers and a very low ratio of polylactic acid. The mulch beds were 0.9-m wide, with a 0.8-m spacing between them. In total, there were 10 beds arranged along a 21-m lenght. The two outer beds and the 3 m on each end of the bed were designated as an edge-effect buffer zone. Each bed had 6-cm diameter holes arranged in two rows, with a 40-cm gap between rows and a 35-cm gap between holes. Broccoli [*Brassica oleracea* L. var. *italica*] cultivar 'Pixel' (Takii & Co.) was simultaneously transplanted into each hole. As a result, the broccoli plants were spaced at a density of 6.35 plant m^2 within the beds. On May 31, 2021, following the broccoli yield survey and harvesting, the eight remaining beds were divided in two parts, creating a total of 16 plots (each measuring 0.9 m × 7.5 m). These 16 plots were then randomly assigned to the following treatments: in 4 plots, both broccoli crop residue and mulch film were thoroughly handpicked out of the soil (SOIL); in 4 plots broccoli crop residue were thoroughly handpicked out of the soil and the mulch film was sampled (see section 2.5) and later buried again in mesh bags (SOIL + FILM); in 4 plots, the broccoli crop residue was left on the soil but the mulch film was removed (SOIL + RES); in 4 plots both crop residues and mulch film were left in the soil (SOIL + FILM + RES). Subsequently, harrowing was carried out in the order of SOIL, SOIL + FILM, SOIL + RES, and SOIL + FILM + RES. Each bed underwent harrowing twice, with special care taken to avoid mixing residue and film from one bed with those of another. Broccoli residue was incorporated at 9 ± 2.6 t ha^{-1} of dry matter (above + root biomass).

On August 26, 2021, the soil was plowed again, and sorghum [*Sorghum bicolor* (L.) Moench] cultivar Jambo (Snow Brand Seed Co.) was sowed on the same date (in rows with 60 cm between rows) in all plots. On October 29, 2021, sorghum was removed from SOIL and SOIL + FILM plots (both above- and belowground biomass) and plowed in as green manure in SOIL + RES and SOIL + FILM + RES plots. Sorghum was incorporated at 2.7 ± 0.6 t ha^{-1} of dry matter (above ground + root biomass). Dry matter incorporated was estimated in four random plots of 1 m by 1 m, by collecting above ground + root biomass of broccoli (five plants per plot) and sorghum (two lines of 0.5 m in each plot), which were then oven-dried at 70°C for 48 h.

In the second year of monitoring (March 2022–March 2023), the previously mentioned management practices were repeated with the same methods on the following dates: fertilization and soil plowing: March 7; mulch film deployment and broccoli transplanting: March 14; broccoli harvesting: 13–23 May; mulch film and/or residues removal: May 26; soil plowing, broccoli residues incorporation (4.2 ± 0.9 t ha^{-1} in SOIL + RES and SOIL + FILM + RES) and meshbag placement: June 1; sorghum sowing: August 30; and soil plowing, sorghum removal (in SOIL and SOIL + FILM) and sorghum incorporation (4.8 ± 0.8 t ha^{-1} of dry matter in SOIL + RES and SOIL + FILM + RES): October 25.

The repetition of the treatments in the second year was carried out in the same experimental plots used in the first year, thus generating an additive effect. The management practices and crop sequences are shown in Figure S1.

2.3 | Experiments

Three different experiments (A–C) were carried out to investigate our three aims (Table 1). Experiment A investigated the effect of incorporating crop residues on mulch film degradation. As a secondary aim, this experiment tested whether soil CO_2 emissions derived from mulch film biodegradation could be detected by portable IRGA systems. In each plot, soil CO_2 emissions were measured during 40 sampling dates. The degradation of mulch film in mesh bags was assessed on three occasions both in 2021 and 2022, ~3, 5, and 9 months after the burial of the mulch film and broccoli residues (a description of the methods used to measure film degradation in mesh bags and mesh bags placement is in Section 2.5). Soil esterase activity was measured in a 0.7- to 0.8-cm layer of soil immediately under the mulch films buried at 10 cm. Soil esterase activity was measured at 84, 149, and 280 days after broccoli residue incorporation in 2021, and at 89, 146, and 278 days after broccoli residue incorporation in 2022 (Figures S1 and S2).

Experiment B investigated temporal changes of soil microbial activity after the incorporation of crop residues with mulch films. Changes in pH, fungal and bacterial abundance, and esterase activity were measured at 0- to 15-cm depth in SOIL, SOIL + FILM, and SOIL + FILM + RES plots on the following sampling dates in 2021: 7 days before broccoli residue incorporation and 14, 28, 84, 147, 161, 182, and 280 days after broccoli residue incorporation. In 2022, the same investigation was conducted 9 days prior to the incorporation of broccoli residues, as well as at 6, 26, 82, 138, 159, 180, and 268 days after the incorporation of broccoli residues (Figures S1 and S2).

Experiment C investigated whether mesh bag orientation causes a bias in CO_2 measurement by IRGA systems. For this experiment, contrary to the ones already present for experiment A, mesh bags were placed horizontally in SOIL plots (see Section 2.5). As a secondary aim, Experiment C investigated whether different burial depths (5, 10, or 15 cm) affect the degradation rate of mulch films inside mesh bags. Soil CO_2 was measured on 14 sampling dates at roughly weekly intervals starting from 7 days after broccoli residue incorporation. The mesh bags were retrieved 87 days after the broccoli residue incorporation in 2021 (Figures S1 and S2).

2.4 | Soil CO$_2$ emissions monitoring

Soil CO_2 emission was measured in each plot using a portable CO_2 IRGA equipped with a soil respiration chamber (EGM-5 with SRC-1; PP-Systems). The soil respiration chamber was placed directly above the buried mesh bags for all plots of Experiments A and C (Figure S2). The measurements were performed in situ between 9:00 a.m. and 11:00 a.m. (Japan standard time). CO_2 emission measuring was performed over 189 days starting on the day of broccoli residue incorporation, at daily to biweekly intervals, with intensification after soil tillage or rainfall events (Figure S3).

2.5 | Mulch film degradation assessment

Mulch film used during the two broccoli growing seasons was retrieved on the same day the broccoli was harvested. The mulch film was cut into 7 cm × 5 cm pieces, weighed using an electronic balance (ML303E/02; Mettler-Toledo International Inc.), and placed inside polyethylene mesh bags with 4-mm diamond-shaped openings (Nihon Matai Co., Ltd.). In 2021, in each plot, nine mesh bags were buried at three depths (5, 10, and 15 cm, three replications per depth) and placed horizontally. In addition, in 2021, nine mesh bags were placed vertically and buried at three depths (0–5, 5–10, and 10–15 cm, three replications per depth) also in the four SOIL plots (see Experiment C, Section 2.3 and Figure S2). In 2022, mesh bags were horizontally placed and buried at a depth of

TABLE 1 Aims and factors considered in the three experiments and duration of each experiment.

Experiment	Aim	Response variable	Factors considered	Duration
A	Investigate the effect of incorporation of crop residues with mulch film; test the measurement of CO_2 emissions from the biodegradation of mulch films by portable infrared gas analyzer	Soil CO_2 emissions	Crop residues (Y/N); mulch film (Y/N); time (40 sampling dates)	One cropping season
		Mulch film degradation	Crop residues (Y/N); burial depth (three depths); time (three sampling dates)	Two cropping seasons
		Soil esterase activity	Crop residues (Y/N); mulch film (Y/N); Time (three sampling dates)	Two cropping seasons
B	Elucidate temporal changes of the soil microbial activity after the soil incorporation of crop residues with mulch film	Soil pH, fungal and bacterial abundance, esterase activity	Crop residues; crop residues + mulch film; soil (control); time (eight sampling dates)	Two cropping seasons
C	Clarify if the horizontal vs. vertical orientation of buried mesh bags affects soil CO_2 emissions and/or mulch film degradation; investigate if burial depth affects film degradation inside mesh bags	Soil CO_2 emissions	Mesh bag orientation (horizontal/vertical); time (15 sampling dates)	One cropping season
		Mulch film degradation	Burial depth (three depths); time (two sampling dates)	One cropping season

Abbreviations: N, no; Y, yes.

10 cm. The retrieval of mesh bags occurred on days 87, 149, and 280 after broccoli residue incorporation in 2021 and on days 89, 146, and 278 after broccoli residue incorporation in 2022 (Figure S1).

The film samples were gently cleaned in pure water and inserted into pre-weighed plastic laminating pouches (109 mm by 153 mm, 100-μm thickness; MSP-F109153N; Kokuyo, Co., Ltd.) and air-dried for two weeks at room temperature (around 25°C). After drying, the laminating pouches were heat-sealed using a laminating machine and weighed. The mass loss of each film sample was expressed as the proportion (%) of the remaining mass, calculated according to Equation (1):

$$\% \text{ remaining mass} = 1 - \left[(\text{initial weight} - \text{final weight}) \middle/ \text{initialweight} \right] \times 100. \quad (1)$$

After weighing, samples were scanned with an image scanner (Docu Centre-V 5080 N; Fuji Xerox). Each image was then processed using the ImageJ software (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, University of Wisconsin) following the method described by Bianchini et al. (2022). The area loss of each film sample was automatically calculated by the ImageJ software and was expressed as a proportion (%) of the remaining area.

2.6 | Soil pH and microbial activity indicators

Soil pH (H_2O) was determined in a 1:2.5 (w/v) aqueous solution. Viable counts of total bacteria and total fungi were determined on solid agarose media and measured as colony-forming units (CFUs) per soil suspension. One gram of each soil sample was added to 9 mL of distilled water. After shaking at 25°C for 10 min at 200 rpm, the solution was decimally diluted (10^{-1}–10^{-6}), and aliquots of the resulting solutions were plated on PTYG medium for bacteria and Martin medium for fungi (Martin, 1950). PTYG medium (L^{-1}) was composed of 0.25 g peptone, 0.25 g tryptone, 0.5 g yeast extract, 0.5 g glucose, 30 mg $MgSO_4 \cdot 7H_2O$, 3.5 mg $CaCl_2$ $2H_2O$, and 15 g agar with cycloheximide (final concentration, 150 μg mL^{-1}), which was added after autoclaving to inhibit the growth of eukaryotic microorganisms. The Martin medium (L^{-1}) was composed of 1 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 5 g peptone, 10 g glucose, 0.033 g Rose Bengal, and 20 g agar with streptomycin (final concentration, 50 μg mL^{-1}) added after autoclaving to inhibit the growth of bacteria. Plates were incubated at 25°C for 7 days; then, CFUs were counted.

The soil esterase activity of the soil samples was determined with pNP-valerate according to the method described by Tsuboi et al. (2018), but using ester substrate at 2.5 mmol L^{-1}, at the start of incubation.
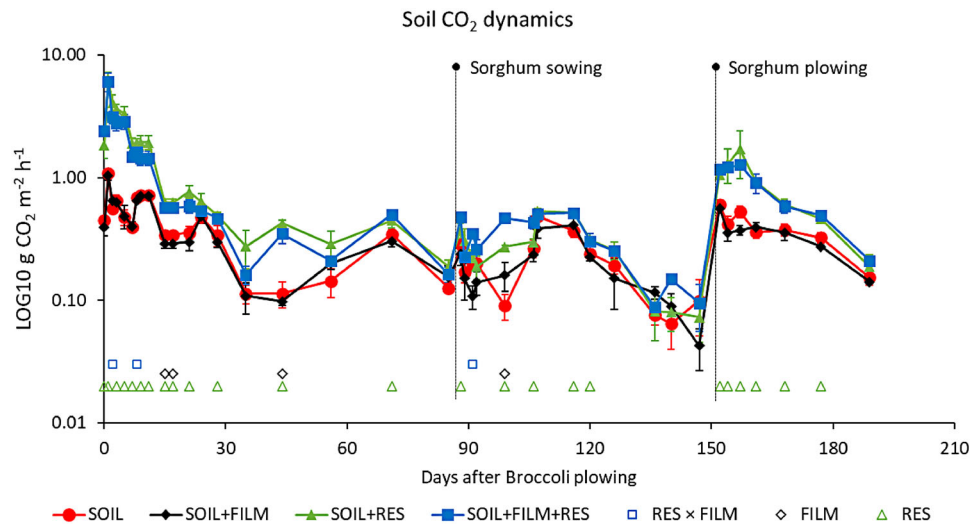
FIGURE 1 Soil CO_2 emissions dynamics over the monitoring period. Open symbols indicate a significant effect at $p < 0.05$ for mulch film (FILM, open black diamonds), crop residues (RES, open green triangles), and their interaction (RES × FILM, open blue squares). Bars indicate standard errors. The raw data (i.e., not LOG10-transformed) are provided in Figure S3.

2.7 | Statistical analysis

All data were analyzed using SPSS Statistics, version 25.0 (SPSS Inc., IBM) using the general linear model procedure. For all experiments, two- or three-way repeated-measures analysis of variance (RMANOVA) was used to determine the effects of sampling dates (within-factor) and interactions between factors (Table 1). For each experiment, a subsequent two-way analysis of variance (ANOVA) was used to determine differences within each sampling date. Pairwise differences between means were assessed with Tukey's honestly significant difference (HSD) test. The significance level for all analyses was set at $p = 0.05$.

When required, the data were transformed using the Box–Cox procedure to satisfy the assumption of normality and homoscedasticity, which were assessed through the inspection of box plots and Levene's test, respectively. In cases where sphericity was not met, the Greenhouse–Geisser correction was applied for the repeated-measure analysis.

3 | RESULTS

3.1 | Experiment A: Effect of incorporation of crop residues on mulch film degradation

3.1.1 | Soil CO_2 emissions

Soil CO_2 emissions showed strong temporal variability within each treatment and marked differences between treatments (SOIL = SOIL + FILM < SOIL + RES = SOIL + FILM + RES) (Figure 1). Peaks of soil CO_2 emissions were observed on the day following the incorporation of broccoli residue

(~6.45 and ~6.06 g CO_2 m^{-2} h^{-1} for SOIL + RES and SOIL + FILM + RES, respectively). On the same day, peaks of soil CO_2 emissions were also observed for treatments without the incorporation of crop residues, albeit of lower intensity (~1.08 and ~1.04 g CO_2 m^{-2} h^{-1} for SOIL and SOIL + FILM, respectively). Soil CO_2 emissions remained relatively high for all treatments for about a month, but with differences between treatments with and without the incorporation of crop residues. Subsequently, soil CO_2 emissions were relatively low for about 4 months, but with occasional differences between treatments with and without the incorporation of crop residues. Three days after sorghum residue was incorporated (day 151), a second soil CO_2 peak was observed for all treatments (~0.53, 0.37, 1.70, and 1.27 g CO_2 m^{-2} h^{-1} for SOIL, SOIL + FILM, SOIL + RES, and SOIL + FILM + RES, respectively). Soil CO_2 emissions then gradually decreased until the end of the monitoring period, with the highest values for the treatments with the incorporation of residues.

Overall, mean soil CO_2 emissions for SOIL and SOIL + FILM did not differ (~0.36 and 0.34 g CO_2 m^{-2} h^{-1}, respectively). Similarly, the mean CO_2 dynamics observed for SOIL + RES and SOIL + FILM + RES were not different (~1.11 and ~1.01 g CO_2 m^{-2} h^{-1}, respectively). The RMANOVA for the whole monitoring period highlighted an interaction between sampling dates and the presence of crop residues (Table S1). The two-way ANOVA, within each sampling date, showed differences in CO_2 emissions due to the effect of crop residues; notably, these were concentrated on dates immediately after the incorporation of broccoli and sorghum residues (Figure 1). On the contrary, differences in CO_2 emissions caused by the mulch film and/or its interaction with crop residues were only occasionally observed.
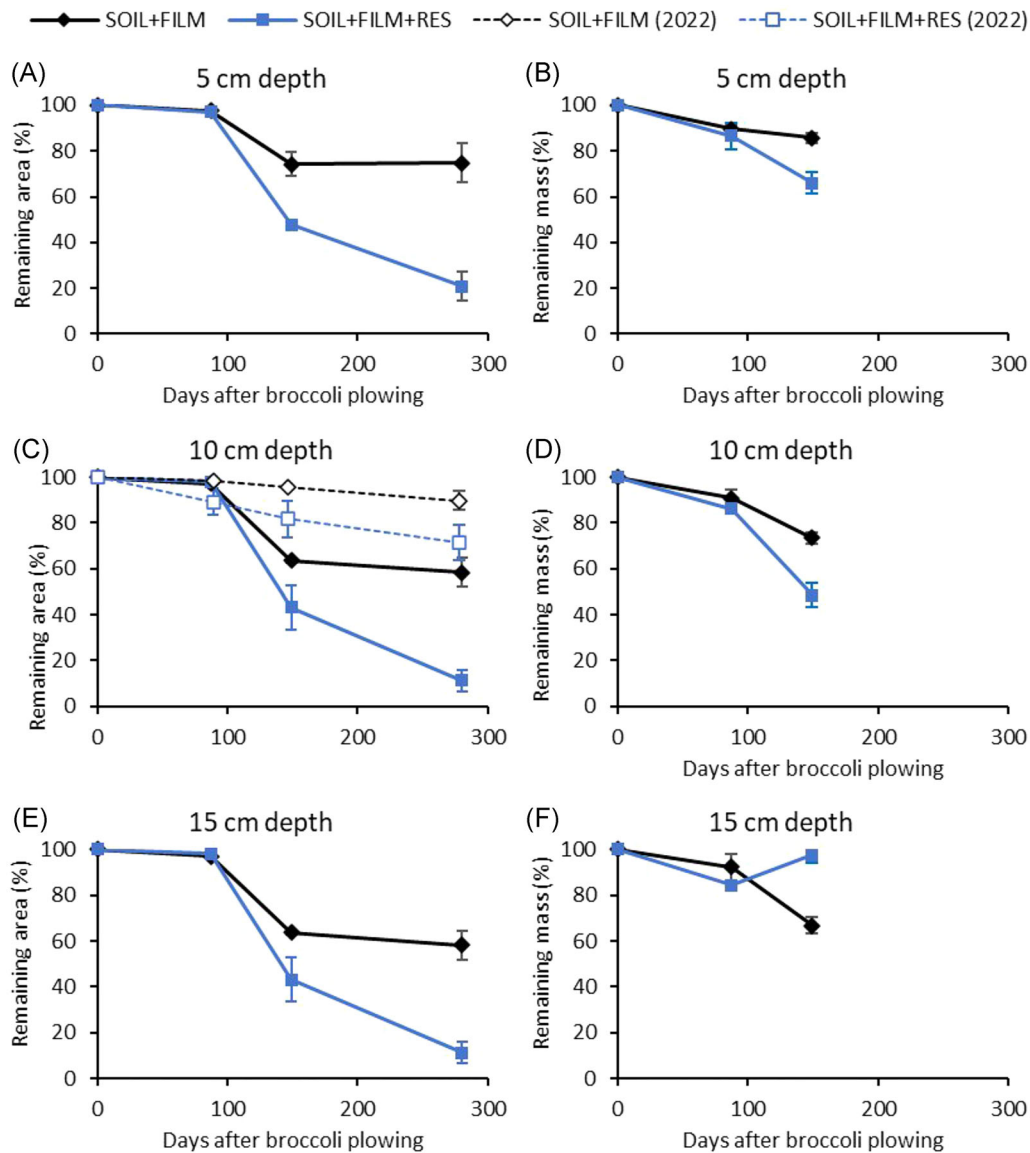
FIGURE 2 Mulch film degradation with and without crop residues (RES) and at different burial depths after 87 and 149 days. Mulch film degradation is expressed as % of remaining area and mass over time. The bars represent the standard error. In 2022, the mesh bags were only buried at a depth of 10 cm, and only the remaining area was evaluated.

3.1.2 | Mulch film degradation

In 2021, the degradation of mulch film was negligible after ~3 months in terms of both area and mass loss, regardless of crop residue presence/absence or burial depth (Figure 2). In 2021, differences in terms of area loss emerged after ~5 months, when both burial depth and the presence of crop residues had significant effects, but their interaction did not (Table S2). In 2022, the degradation rates of the mulch film were lower compared to 2021. Differences were observed between SOIL + FILM and SOIL + FILM + RES, which became evident after ~3 months and persisted throughout the monitoring period (Figure 2C).

In 2021, the dynamics of mass loss were similar to those of area loss (i.e., negligible after ~3 months and significant after ~5 months, regardless of crop residue presence/absence or burial depth). In 2021, it was not possible to measure the mass loss at 280 days because the retrieved film was so minimal that it could not be detected. Film mass loss was affected by a three-way interaction between sampling dates, the presence of crop residues, and burial depth (Table S3). At the end of the monitoring period in 2021, the mass lost from the film samples was ~29% for SOIL + FILM and ~25% for SOIL + FILM + RES, compared to the initial mass. It should be noted that at 15–cm depth in the SOIL + FILM + RES treatment, film mass increased instead of decreasing (Figure 2F).
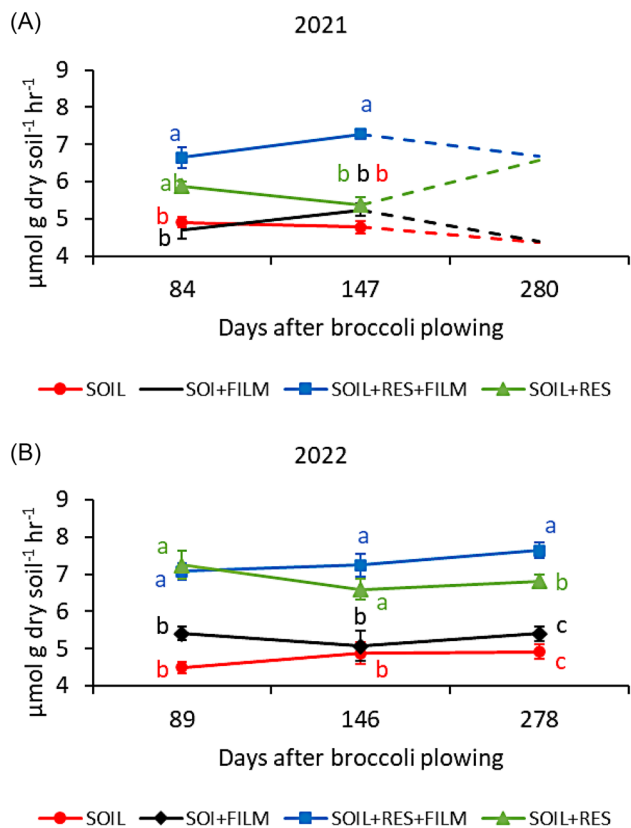
FIGURE 3 Soil esterase activity at a depth of 10 cm in Experiment A. Different letters denote significant differences at $p < 0.05$ (Tukey's honestly significant difference test) within each sampling date. The bars represent the standard errors. Dashed lines represent a potential trend in the dynamics of esterase activity, as two out of the four mesh bags could not be retrieved 280 days after broccoli plowing in 2021. RES, residues.

3.1.3 | Soil esterase activity

An interaction was observed between the presence of crop residues and mulch film in 2021 but not in 2022 ($p = 0.06$) (Table S4). In 2021, the mean soil esterase activity was highest for SOIL + FILM + RES ($\sim$6.9 μmol g dry soil^{-1} h^{-1}) and lowest for SOIL and SOIL + FILM ($\sim$4.9 μmol g dry soil^{-1} h^{-1} for both), with SOIL + RES showing intermediate values ($\sim$5.7 μmol g dry soil^{-1} h^{-1}) (Figure 3A). In 2022, the mean soil esterase activity was higher for SOIL + FILM + RES ($\sim$7.3 μmol g dry soil^{-1} h^{-1}) and SOIL + RES ($\sim$6.8 μmol g dry soil^{-1} h^{-1}) compared to SOIL + FILM ($\sim$5.3 μmol g dry soil^{-1} h^{-1}) and SOIL ($\sim$4.8 μmol g dry soil^{-1} h^{-1}) (Figure 3B).

Throughout the 2021 monitoring period, the soil esterase activity at 10-cm depth was consistently highest for SOIL + FILM + RES on both sampling dates (Figure 3A). After 84 days, both treatments with crop residues (SOIL + RES and SOIL + FILM + RES) showed greater soil esterase activity compared to the SOIL treatment. At the second sampling date

(147 days), the soil esterase activity was higher for SOIL + FILM + RES than any other treatments (Figure 3A). Unfortunately, two out of the four mesh bags could not be retrieved on the third sampling day (280 days). However, the available data suggest that esterase activity was once again higher in SOIL + RES and SOIL + FILM + RES compared to SOIL and SOIL + FILM.

Throughout the monitoring period in 2022, the soil esterase activity at a depth of 10 cm was consistently highest for both SOIL + RES and SOIL + FILM + RES, observed at both 89 and 146 days after broccoli plowing. On the final sampling date (278 days), SOIL + FILM + RES maintained higher soil esterase activity compared to SOIL + RES. In turn, SOIL + RES exhibited higher activity than both SOIL and SOIL + FILM, which had lower levels of esterase activity (Figure 3B).

3.2 | Experiment B: Soil microbial activity after incorporation of crop residues and mulch films

3.2.1 | Soil pH

On average, in 2021, soil pH was slightly higher for SOIL ($\sim$6.92) than SOIL + RES and SOIL + FILM + RES ($\sim$6.79 and $\sim$6.85, respectively). Similarly, in 2022, soil pH was on average higher for SOIL ($\sim$6.85) compared to SOIL + RES + FILM ($\sim$6.77) with SOIL + RES showing intermediate values ($\sim$6.8). In both 2021 and 2022, differences were attributable to the interaction between date and treatment (Tables S5 and S6).

Throughout the 2021 cropping season, soil pH tended to increase over time for all treatments (Figure 4A). After the incorporation of broccoli residue, pH was higher in SOIL than SOIL + RES and SOIL + FILM + RES, and this was observed for both cropping seasons. This significant difference was maintained for $\sim$30 days. Immediately after the incorporation of sorghum residue, pH was significantly higher in SOIL than in other plots.

Throughout the 2022 cropping season, soil pH exhibited a similar pattern to that observed in 2021. However, lower values were observed for SOIL + RES and SOIL + FILM + RES $\sim$1 month after the incorporation of broccoli residues (Figure 4B). Subsequently, there was an increasing trend in soil pH, with differences becoming evident about a month after burying the sorghum residues.

3.2.2 | Soil fungi

On average, in 2021, the soil fungal abundance was greater in SOIL + RES and SOIL + FILM + RES ($\sim$87 and $\sim$91 CFU mg dry soil^{-1}, respectively) compared to SOIL ($\sim$64
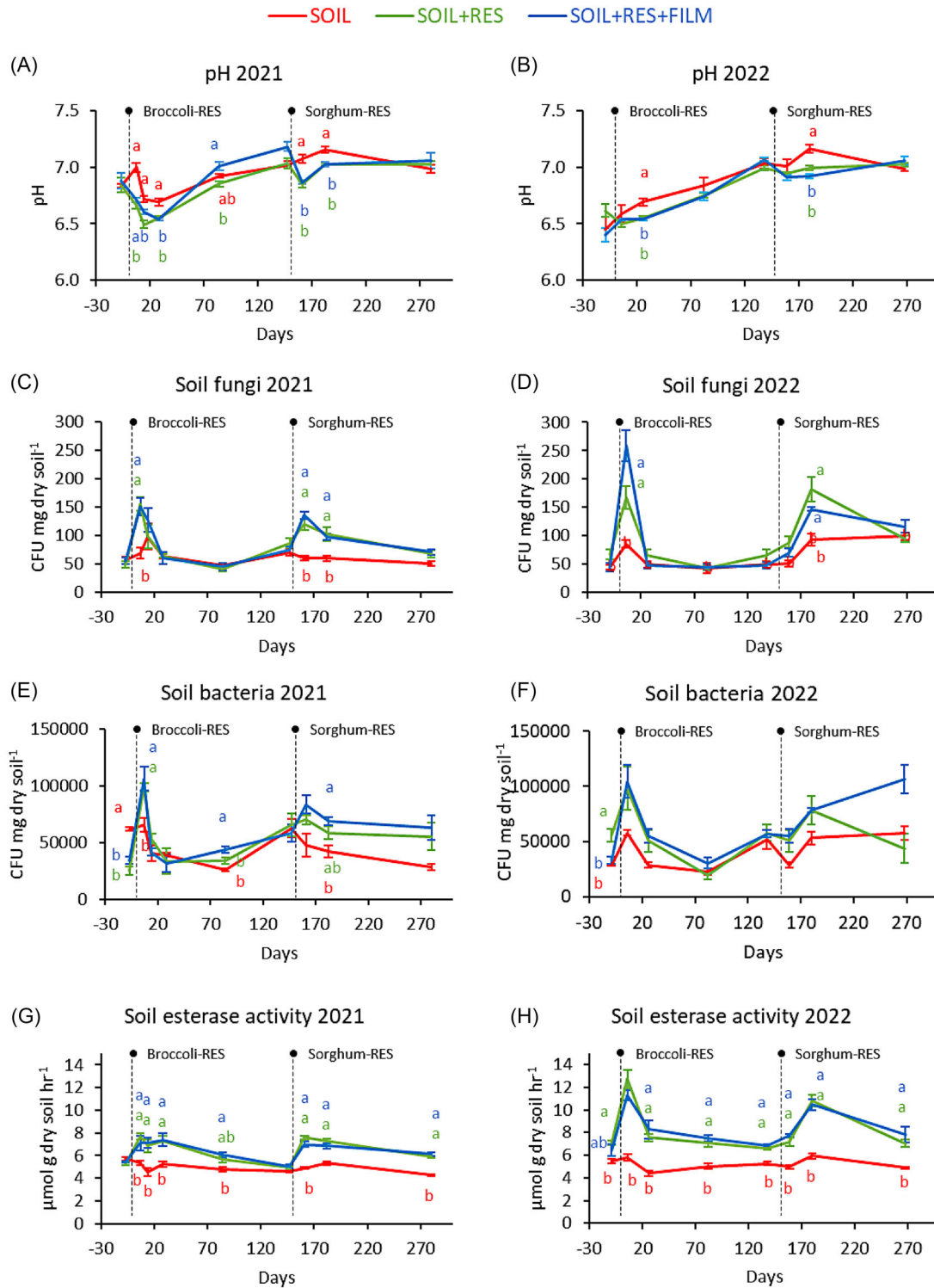
FIGURE 4 Soil pH, fungal abundance, bacterial abundance, and esterase activity dynamics over the monitoring period, measured at 0- to 15-cm depth. Different letters denote significant differences at $p < 0.05$ (Tukey's honestly significant difference test). Bars indicate standard errors. RES, crop residues incorporation.

CFU mg dry soil^{-1}). Similarly, in 2022, the fungal abundance was greater on average for both SOIL + RES and SOIL + FILM + RES (~96 and ~97 CFU mg dry soil^{-1}, respectively) compared to SOIL (~64 CFU mg dry soil^{-1}). The differences observed among treatments in both 2021 and 2022 were attributed to the effect of treatment, as no interaction was found between date and treatment (Tables S5 and S6).

Throughout the 2021 cropping season, fungal abundance showed two peaks after the incorporation of broccoli and sorghum residues (Figure 4C). About 1 month after incorporation of broccoli residue, fungal abundance dropped to relatively low levels for all treatments. After the incorporation of sorghum residue, the abundance of soil fungi was again higher for SOIL + RES and SOIL + FILM + RES compared to SOIL, with differences maintained for 1 month.

Throughout the 2022 cropping season, the fungal abundance showed similar trends to those observed in 2021, but with more pronounced peaks occurring immediately after burying broccoli residues and ~1 month after burying sorghum residues (Figure 4D). During both peaks, fungal abundance was higher in SOIL + RES and SOIL + FILM + RES compared to that observed in SOIL.

3.2.3 | Soil bacteria

On average, in 2021, bacterial abundance was higher for SOIL + RES (~5.5 × 10^4 CFU mg dry soil^{-1}) and SOIL + FILM + RES (~5.9 × 10^4 CFU mg dry soil^{-1}) compared to SOIL (~4.6 ×10^4 CFU mg dry soil^{-1}). On average, in 2022, bacterial abundance was higher in SOIL + RES + FILM (~6.4 × 10^4 CFU mg dry soil^{-1}) compared to SOIL (~4.1 × 10^4 CFU mg dry soil^{-1}). In 2021, an interaction was observed between the date of sampling and treatment. However, in 2022, the differences in bacterial abundance were attributed to the treatment (Tables S5 and S6).

In 2021, the dynamics of soil bacteria were similar to that observed for soil fungi. One week before the incorporation of broccoli residue, bacterial abundance was highest in SOIL plots (Figure 4E); 1 week after the incorporation of broccoli residue, bacterial abundance was higher in SOIL + RES and SOIL + FILM + RES than SOIL plots. Bacterial abundance was again higher for SOIL + FILM + RES compared to SOIL a few days prior to the sowing of sorghum, whereas SOIL + RES showed intermediate values. Bacterial abundance increased again after the incorporation of sorghum residue but with significant differences only between SOIL and SOIL + FILM + RES.

In 2022, the dynamics of soil bacteria were similar to those observed in 2021. However, no differences were found between treatments, except for the initial date where SOIL + RES exhibited higher bacterial abundance compared to the other two treatments (Figure 4F). Throughout the monitoring period, bacterial abundance tended to be higher for SOIL + RES and SOIL + FILM + RES, but these differences did not reach statistical significance (i.e., $p = 0.08$, 0.07, and 0.09 at day 26, 159, and 268, respectively).

3.2.4 | Soil esterase activity

On average, in 2021, the soil esterase activity was higher in SOIL + RES and SOIL + FILM + RES (~6.5 μmol g dry soil^{-1} h^{-1} for both) compared to SOIL (~5 μmol g dry soil^{-1} h^{-1}). Similarly, in 2022, the soil esterase activity was highest on average for SOIL + RES and SOIL + FILM + RES (~8.3 μmol g dry soil^{-1} h^{-1} for both). The differences observed in both 2021 and 2022 were attributed to the interaction between the date of sampling and the treatments (Tables S5 and S6).

In 2021, immediately after the incorporation of broccoli residue, the soil esterase activity was higher for SOIL + RES and SOIL + FILM + RES than SOIL and remained so throughout the monitoring period, except just before the sowing of sorghum, when differences only emerged between SOIL + FILM + RES and SOIL (Figure 4G).

In 2022, the soil esterase activity exhibited similar patterns to those observed in 2021. However, there were more pronounced differences between SOIL and the other two treatments. The SOIL + RES and SOIL + FILM + RES consistently showed higher esterase activity throughout the monitoring period, particularly during the peaks observed after residue incorporation (Figure 4H).

3.3 | Experiment C: Effects of mesh bag orientation

3.3.1 | Soil CO$_2$ emissions

Soil CO$_2$ emissions for horizontal and vertical mesh bags were comparable in the SOIL and SOIL + FILM plots of Experiment A (Figures S3 and S5). There were no significant differences in soil CO$_2$ emissions throughout the monitoring period, nor were there any interactions between sampling dates and vertical or horizontal orientation of mesh bags (Table S7).

3.3.2 | Mulch film degradation

No significant interaction emerged between mesh bag orientation and burial depth (5, 10, and 15 cm depth) for either film area or mass loss (Table S7). Instead, differences emerged for the depth of mesh bag burial, regardless of orientation (vertical vs. horizontal) (Table S7). After 87 days, mesh bags buried at 15 cm showed greater degradation (both area

and mass loss) than those buried at 5 cm (Figures S6 and S7).

4 | DISCUSSION

4.1 | Interaction between crop residues and mulch film

Soil CO_2 emissions were in the range of those reported in other studies conducted in similar pedo-climatic conditions (Yonemura et al., 2014). The peaks of soil CO_2 emissions observed immediately after the incorporation of broccoli residue were mainly due to the relatively low C:N ratio of the residues, which favored rapid degradation. These results are consistent with those of other studies that have also shown an effect on soil CO_2 and N_2O emissions soon after the incorporation of crop residues with low C:N ratios (Basalirwa et al., 2020; Toderi et al., 2022; Yonemura et al., 2014). Soil tillage also resulted in a soil–CO_2 emissions peak in all treatments, although a much smaller peak was observed in those without residue incorporation (Figure 1 and Figure S3). This increase in soil CO_2 emissions can be generally ascribed to improved soil aeration and rainfall penetration during the rainy season which favored the microbial activity and degradation of organic matter already present in soil, derived from previous crop management. However, the lack of differences in soil CO_2 emissions between SOIL and SOIL + FILM plots suggests that the IRGA system was not able to detect CO_2 emissions resulting from the biodegradation of mulch films, at least in this soil type (i.e., Typic Hapludands). This assumes that the mulch film had been at least partially biodegraded, that is, that at least some of the carbon in the mulch film had been transformed by microbes into CO_2 (Sander, 2019). Future studies are needed to clarify the biodegradation rate possible in this soil; incubation studies possibly coupled with in-field studies, such as the quantification of remaining polymers by using extraction techniques and nuclear magnetic resonance, would provide useful data (e.g., Nelson et al., 2020). Future studies could also test other soil CO_2 efflux monitoring techniques such as closed chambers in combination with a high-precision IRGA system (Basalirwa et al., 2020; Toderi et al., 2022; Yonemura et al., 2014).

Generally, when fresh organic matter such as crop residues is introduced into the soil, there is an increase in the soil organic matter decomposition rate, known as a priming effect. However, this priming effect depends on the quantity and quality (i.e., labile substance or C:N ratio) of the buried crop residues (Bending et al., 2002; Shahbaz et al., 2017). Our results suggest that the addition of crop residues may have accelerated the biodegradation of mulch film, potentially via a positive priming effect. However, in 2021, this effect only emerged after ~5 months of burial (Figure 2 and Figure S4).

Conversely, negligible degradation was observed after ~3 months of burial, even if the soil temperature and humidity conditions were probably optimal for microbial biodegradation. Various factors could account for the observed delay. First, the biodegradation of mulch films is an erosive process, and measuring area loss may not have detected decreases in film thickness (Brodhagen et al., 2015; Griffin-LaHue et al., 2022). Furthermore, the key biodegraders may have preferred more readily available carbon sources (i.e., broccoli residue) to the carbon of the mulch films and thus may have started the mulch film biodegradation process only after less-desirable sources had been consumed. Fungal and bacterial utilization of organic substrates depends on substrate complexity and nitrogen availability (Koranda et al., 2013). The mineralization of carbon in crop residues is correlated with factors such as the lignin content of residue, the carbon-to-nitrogen ratio, and the polyphenol-to-nitrogen ratio (Vanlauwe et al.,1996). Soil-biodegradable mulch films are typically made of aliphatic polyesters (such as polybutylene adipate-*co*-terephthalate) with long carbon chains in their structure. During the degradation of biodegradable plastics in an open environment, the release of low-molecular-weight organic compounds from the polymers through chemical, physical, and enzymatic activities represents a rate-limiting step, which occurs at a slower pace than the subsequent step. In the next phase, microorganisms assimilate these small molecules and break them down into water and CO_2 (Kitamoto et al., 2023). Furthermore, degradation is a multistep process involving colonization by fungi and/or bacteria, subsequent release of extracellular enzymes, and carbon assimilation, and this process takes time (Sander, 2019). It should also be noted that the indirect methods used here (i.e., area and mass loss) fail to distinguish whether and at what level abiotic or biotic hydrolysis occurred. Thus, future studies will be needed to determine the actual level of the biodegradation of mulch film, as the samples may have undergone only fragmentation and not biodegradation (Sander, 2019). In any case, area loss may be a better indirect indicator of biodegradation than mass loss, as the latter showed questionable results, in some cases appearing to increase over time (Figure 2F). Indeed, other studies have reported that area loss is a more reliable indicator than mass loss (e.g., Bianchini et al., 2022).

The high level of the soil esterase activity observed in soil under mesh bags buried at 10 cm throughout the two monitoring periods was attributed to the interaction between crop residues and mulch film (Figure 3). Extracellular esterases are key enzymes for the degradation of soil organic compounds, including crop residues as well as soil-biodegradable mulch films (Zumstein et al., 2017). Hydrolysis of ester bonds in the mulch film residues by esterases is an indicator of the abundance of key biodegraders that use the resulting monomers for their microbial activities (Yamamoto-Tamura

et al., 2015). The increase in the esterase activity of soil attached to the film may have been delayed in 2022 (Figure 3) because the film degradation was generally slower than that in 2021 (Figure 2C). Confirming that the interaction between crop residues and the mulch film increases esterase activity deserves greater attention in the future.

4.2 | Soil microbial activity after the incorporation of crop residues and mulch film

In the present study, soil pH tended to increase under all management practices but decreased sharply immediately after the incorporation of both residues in SOIL + RES and SOIL + FILM + RES plots (Figure 4A,B). However, no differences in soil pH were observed between SOIL + RES and SOIL + FILM + RES, suggesting that the incorporation of the mulch film has a negligible effect on the pH of this type of soil, at least over the short term.

Two peaks of fungal abundance were observed following residue incorporation, with very similar dynamics for SOIL + RES and SOIL + FILM + RES (Figure 4C,D). Fungi are key biodegraders of mulch films in upland soils because they have several advantages over bacteria, including lower sensitivity to soil–nitrogen limitations and the ability to use hyphae to colonize the film surface (Sander, 2019). Other studies have shown that fungi are primarily responsible for the degradation of soil-biodegradable mulch films (Yamamoto-Tamura et al., 2015) but also that biodegradation rates might differ by soil type and related fungi populations and community dynamics (Yamamoto-Tamura et al., 2020).

The abundance of soil bacteria showed similar dynamics to those of fungi, but CFUs were much higher (Figure 4E,F). In both 2021 and 2022, after the incorporation of sorghum residue, no differences emerged between SOIL + RES and SOIL (Figure 4E,F). Previous studies hypothesized that bacteria would play a minor role in the biodegradation of mulch films compared to fungi (Sander, 2019; Yamamoto-Tamura et al., 2015, 2020). Some recent studies suggest that interactions between fungi and bacteria in plastic biodegradation could be more complex than previously thought (Guliyev et al., 2022; Purahong et al., 2021). Future studies are needed to clarify which bacteria and fungi are responsible for the biodegradation of mulch films, as polymer breakdown may begin with a single microorganism or enzyme (e.g., esterase) and later require the activity of other organisms whose abundance differs spatially and might be influenced by farmers management practices (Brodhagen et al., 2015).

Soil esterase activity was highest in SOIL + RES and SOIL + FILM + RES plots following the incorporation of broccoli residue in both 2021 and 2022 (Figure 4G,H). This is consistent with the results of Experiment A (Figure 2) and suggests that film biodegradation may not have started immediately after burial, despite soil conditions that were probably very favorable for microorganisms. This hypothesis is supported by the low rate of biodegradation observed for mulch film samples inside mesh bags 84 days after burial in 2021 (Figures 2 and S4) and by the high level of esterase activity observed in Experiment A (Figure 3). A further confirmation of this hypothesis was the high level of soil–CO_2 emissions observed during the first monitoring period for Experiment A (Figure 1 and Figure S3) together with the high level of esterase activity in the bulk soil observed in Experiment B (Figure 4G,H). This was taken as a confirmation that soil microorganisms do not prefer mulch film as a primary source of carbon when other readily decomposable carbon sources such as broccoli residue are available (Basalirwa et al., 2020). Indeed, the mulch film only began to show degradation after 149 days of burial in 2021, when the broccoli residue was already mostly degraded (Figure 1). This supports the hypothesis that the incorporation of crop residues plays a fundamental role in the biodegradation of mulch films by promoting a positive priming effect and that this aspect deserves more attention in the future, especially in field trials.

4.3 | Effect of mesh bag orientation on film degradation

Soil CO_2 emissions did not differ between horizontal and vertical mesh bags (Figure S5). This result suggests that CO_2 efflux is not constrained by mesh bag orientation. The highest degree of degradation was observed for mesh bags buried at 15 cm, perhaps because the shallower soil layers had lower soil moisture, which could have decreased abiotic and/or biotic hydrolysis and microbial activity (Brodhagen et al., 2015; Choe et al., 2021; Zumstein et al., 2017). According to these results, the horizontal mesh bag orientation proposed in other studies (Bianchini et al., 2022; Sintim et al., 2020) would be preferable because it would maximize the surface area exposed to the infiltration of water after precipitation or irrigation, probably favoring film hydrolysis (Brodhagen et al., 2015; Choe et al., 2021).

5 | CONCLUSIONS

The incorporation of crop residues with soil biodegradable mulch film resulted in a significant increase in the activity of soil esterase, a key enzyme in the biodegradation of mulch films. The increase in the soil esterase activity of soil attached to the film when soil CO_2 emissions were low suggests that the mulch film started to biodegrade only after broccoli residue was mostly degraded. However, after ~5 months, the incorporation of crop residues with the mulch film resulted in greater degradation compared to mulch film buried

without residues. The incorporation of crop residues with the mulch film resulted in marked temporal variations in bulk soil pH, fungal and bacterial abundances, and esterase activity. However, the variation was comparable to that observed for the incorporation of crop residues alone in both years of monitoring.

The portable IRGA was not able to differentiate the flow of CO_2 emitted from the soil from that emitted by the soil and mulch film; thus, this technology seems poorly suited for open-field trials aimed at detecting CO_2 derived from mulch film biodegradation.

The horizontal or vertical orientation of mesh bags did not result in variation of soil CO_2 efflux or degradation degree of the mulch film. However, the burial of mesh bags at 10–15 cm depth resulted in greater degradation compared to those buried at 0–5 cm.

Future studies are necessary to evaluate the effects of the incorporation of different crop residues and the possible effects of different soil tillage regimes in different soils and across different seasons. All these effects should also be evaluated over the medium and long term.

AUTHOR CONTRIBUTIONS

Matteo Francioni: Conceptualization; data curation; formal analysis; investigation; methodology; visualization; writing—original draft. **Yuko Takada Hoshino**: Conceptualization; investigation; methodology; project administration; resources. writing—review and editing. **Ayaka Wenhong Kishimoto-Mo**: Conceptualization; investigation; methodology; project administration; resources; supervision; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT
The authors declare no conflicts of interest.

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REFERENCES
Ardisson, G. B., Tosin, M., Barbale, M., & Degli-Innocenti, F. (2014). Biodegradation of plastics in soil and effects on nitrification activity. A laboratory approach. *Frontiers in Microbiology*, 5, 1–7. https://doi.org/10.3389/fmicb.2014.00710

Basalirwa, D., Sudo, S., Wacal, C., Akae, F., Oo, A. Z., Koyama, S., Sasagawa, D., Yamamoto, S., Masunaga, T., & Nishihara, E. (2020). Assessment of crop residue and palm shell biochar incorporation on greenhouse gas emissions during the fallow and crop growing seasons of broccoli (*Brassica oleracea* var. *italica*). *Soil and Tillage Research*, 196, 104435. https://doi.org/10.1016/j.still.2019.104435

Bending, G. D., Turner, M. K., & Jones, J. E. (2002). Interactions between crop residue and soil organic matter quality and the functional diversity of soil microbial communities. *Soil Biology and Biochemistry*, 34(8), 1073–1082. https://doi.org/10.1016/S0038-0717(02)00040-8

Bianchini, M., Trozzo, L., D'Ottavio, P., Giustozzi, M., Toderi, M., Ledda, L., & Francioni, M. (2022). Soil refinement accelerates in-field degradation rates of soil-biodegradable mulch films. *Italian Journal of Agronomy*, 17(3). https://doi.org/10.4081/ija.2022.2044

Brodhagen, M., Peyron, M., Miles, C., & Inglis, D. A. (2015). Biodegradable plastic agricultural mulches and key features of microbial degradation. *Applied Microbiology and Biotechnology*, 99(3), 1039–1056. https://doi.org/10.1007/s00253-014-6267-5

Chen, N., Li, X., Šimůnek, J., Shi, H., Hu, Q., & Zhang, Y. (2021). Evaluating the effects of biodegradable and plastic film mulching on soil temperature in a drip-irrigated field. *Soil and Tillage Research*, 213, 105116. https://doi.org/10.1016/j.still.2021.105116

Choe, S., Kim, Y., Won, Y., & Myung, J. (2021). Bridging three gaps in biodegradable plastics: Misconceptions and truths about biodegradation. *Frontiers in Chemistry*, 9, 1–8. https://doi.org/10.3389/fchem.2021.671750

Francioni, M., Kishimoto-Mo, A. W., Tsuboi, S., & Hoshino, Y. T. (2022). Evaluation of the mulch films biodegradation in soil: A methodological review. *Italian Journal of Agronomy*, 17(3). https://doi.org/10.4081/ija.2021.1936

Francioni, M., Lai, R., D'Ottavio, P., Trozzo, L., Kishimoto-Mo, A. W., Budimir, K., Baldoni, N., & Toderi, M. (2020). Soil respiration dynamics in forage-based and cereal-based cropping systems in central Italy. *Scientia Agricola*, 77(3). https://doi.org/10.1590/1678-992x-2018-0096

Griffin-LaHue, D., Ghimire, S., Yu, Y., Scheenstra, E. J., Miles, C. A., & Flury, M. (2022). In-field degradation of soil-biodegradable plastic mulch films in a Mediterranean climate. *Science of The Total Environment*, 806, 150238. https://doi.org/10.1016/j.scitotenv.2021.150238

Guliyev, V., Tanunchai, B., Noll, M., Buscot, F., Purahong, W., & Blagodatskaya, E. (2022). Links among microbial communities, soil properties and functions: Are fungi the sole players in decomposition of bio-based and biodegradable plastic? *Polymers*, 14(14), 2801. https://doi.org/10.3390/polym14142801

ISO 23517. (2021). *Plastics—Soil biodegradable materials for mulch films for use in agriculture and horticulture—Requirements and test methods regarding biodegradation, ecotoxicity and control of constituents*. International Organization for Standardization.

Kapanen, A., Schettini, E., Vox, G., & Itävaara, M. (2008). Performance and environmental impact of biodegradable films in agriculture: A field study on protected cultivation. *Journal of Polymers and the Environment*, 16(2), 109–122. https://doi.org/10.1007/s10924-008-0091-x

Kitamoto, H., Koitabashi, M., Sameshima-Yamashita, Y., Ueda, H., Takeuchi, A., Watanabe, T., Sato, S., Saika, A., & Fukuoka, T. (2023).

Accelerated degradation of plastic products via yeast enzyme treatment. *Scientific Reports*, *13*(1). https://doi.org/10.1038/s41598-023-29414-1

Koranda, M., Kaiser, C., Fuchslueger, L., Kitzler, B., Sessitsch, A., Zechmeister-Boltenstern, S., & Richter, A. (2013). Fungal and bacterial utilization of organic substrates depends on substrate complexity and N availability. *FEMS Microbiology Ecology*, *87*(1), 142–152. https://doi.org/10.1111/1574-6941.12214

Li, S., Ding, F., Flury, M., & Wang, J. (2023). Dynamics of macroplastics and microplastics formed by biodegradable mulch film in an agricultural field. *Science of The Total Environment*, *894*, 164674. https://doi.org/10.1016/j.scitotenv.2023.164674

Liu, Q., Wang, Y., Liu, J., Liu, X., Dong, Y., Huang, X., Zhen, Z., Lv, J., & He, W. (2022). Degradability and properties of PBAT-Based biodegradable mulch films in field and their effects on cotton planting. *Polymers*, *14*(15), 3157. https://doi.org/10.3390/polym14153157

Martín-Closas, L., Costa, J., & Pelacho, A. M. (2017). Agronomic effects of biodegradable films on crop and field environment. M. Malinconico (Ed.), *Soil degradable bioplastics for a sustainable modern agriculture. Green chemistry and sustainable technology*. (pp. 67–104). Springer. https://doi.org/10.1007/978-3-662-54130-2_4

Martin, J. P. (1950). Use of acid, rose bengal, and streptomycin in the plate method for estimating soil fungi. *Soil Science*, *69*(3), 215–232. https://doi.org/10.1097/00010694-195003000-00006

Narayan, R. (2017). Biodegradable and biobased plastics: An overview. M. Malinconico (Ed.), *Soil degradable bioplastics for a sustainable modern agriculture. Green chemistry and sustainable technology*. Springer. https://doi.org/10.1007/978-3-662-54130-2_2

Nelson, T. F., Remke, S. C., Kohler, H.-P. E., McNeill, K., & Sander, M. (2020). Quantification of synthetic polyesters from biodegradable mulch films in soils. *Environmental Science & Technology*, *54*(1), 266–275. https://doi.org/10.1021/acs.est.9b05863

Pumpanen, J., Kolari, P., Ilvesniemi, H., Minkkinen, K., Vesala, T., Niinistö, S., Lohila, A., Larmola, T., Morero, M., Pihlatie, M., Janssens, I., Yuste, J. C., Grünzweig, J. M., Reth, S., Subke, J. A., Savage, K., Kutsch, W., Østreng, G., Ziegler, W., … Hari, P. (2004). Comparison of different chamber techniques for measuring soil CO_2 efflux. *Agricultural and Forest Meteorology*, *123*(3–4), 159–176. https://doi.org/10.1016/j.agrformet.2003.12.001

Purahong, W., Wahdan, S. F. M., Heinz, D., Jariyavidyanont, K., Sungkapreecha, C., Tanunchai, B., Sansupa, C., Sadubsarn, D., Alaneed, R., Heintz-Buschart, A., Schädler, M., Geissler, A., Kressler, J., & Buscot, F. (2021). Back to the future: Decomposability of a biobased and biodegradable plastic in field soil environments and its microbiome under ambient and future climates. *Environmental Science & Technology*, *55*(18), 12337–12351. https://doi.org/10.1021/acs.est.1c02695

Sander, M. (2019). Biodegradation of polymeric mulch films in agricultural soils: Concepts, knowledge gaps, and future research directions. *Environmental Science & Technology*, *53*(5), 2304–2315. https://doi.org/10.1021/acs.est.8b05208

Shahbaz, M., Kuzyakov, Y., Sanaullah, M., Heitkamp, F., Zelenev, V., Kumar, A., & Blagodatskaya, E. (2017). Microbial decomposition of soil organic matter is mediated by quality and quantity of crop residues: Mechanisms and thresholds. *Biology and Fertility of Soils*, *53*(3), 287–301. https://doi.org/10.1007/s00374-016-1174-9

Sintim, H. Y., Bandopadhyay, S., English, M. E., Bary, A., Liquet y González, J. E., DeBruyn, J. M., Schaeffer, S. M., Miles, C. A., & Flury, M. (2021). Four years of continuous use of soil-biodegradable

plastic mulch: Impact on soil and groundwater quality. *Geoderma*, *381*, 114665. https://doi.org/10.1016/j.geoderma.2020.114665

Sintim, H. Y., Bary, A. I., Hayes, D. G., Wadsworth, L. C., Anunciado, M. B., English, M. E., Bandopadhyay, S., Schaeffer, S. M., DeBruyn, J. M., Miles, C. A., Reganold, J. P., & Flury, M. (2020). In situ degradation of biodegradable plastic mulch films in compost and agricultural soils. *Science of the Total Environment*, *727*, 138668. https://doi.org/10.1016/j.scitotenv.2020.138668

Soil Survey Staff. (2014). Keys to soil taxonomy. *Soil Conservation Service*, *12*, 410. https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs142p2_051546.pdf

Toderi, M., D'Ottavio, P., Francioni, M., Kishimoto-Mo, A. W., Santilocchi, R., & Trozzo, L. (2022). Short-term response of soil greenhouse gas fluxes to alfalfa termination methods in a Mediterranean cropping system. *Soil Science and Plant Nutrition*, *68*(1), 124–132. https://doi.org/10.1080/00380768.2021.1983869

Tofanelli, M. B. D., & Wortman, S. E. (2020). Benchmarking the agronomic performance of biodegradable mulches against polyethylene mulch film: A meta-analysis. *Agronomy*, *10*(10), 1618. https://doi.org/10.3390/agronomy10101618

Tsuboi, S., Tanaka, T., Yamamoto-Tamura, K., & Kitamoto, H. (2018). High-throughput method for the evaluation of esterase activity in soils. *Journal of Microbiological Methods*, *146*, 22–24. https://doi.org/10.1016/J.MIMET.2018.01.009

Tsuboi, S., Yamamoto-Tamura, K., Takada, A., Yonemura, S., Hoshino, Y. T., Kitamoto, H., & Kishimoto-Mo, A. W. (2022). Selection of p-nitrophenyl fatty acid substrate suitable for detecting changes in soil esterase activity associated with degradation of biodegradable polyester mulch films: A field trial. *Italian Journal of Agronomy*, *17*(3). https://doi.org/10.4081/IJA.2022.2040

Vanlauwe, B., Nwoke, O. C., Sanginga, N., & Merckx, R. (1996). Impact of residue quality on the C and N mineralization of leaf and root residues of three agroforestry species. *Plant and Soil*, *183*(2), 221–231. https://doi.org/10.1007/bf00011437

Wang, Z., Li, M., Flury, M., Schaeffer, S. M., Chang, Y., Tao, Z., Jia, Z., Li, S., Ding, F., & Wang, J. (2021). Agronomic performance of polyethylene and biodegradable plastic film mulches in a maize cropping system in a humid continental climate. *Science of the Total Environment*, *786*, 147460. https://doi.org/10.1016/j.scitotenv.2021.147460

Yamamoto-Tamura, K., Hiradate, S., Watanabe, T., Koitabashi, M., Sameshima-Yamashita, Y., Yarimizu, T., & Kitamoto, H. (2015). Contribution of soil esterase to biodegradation of aliphatic polyester agricultural mulch film in cultivated soils. *AMB Express*, *5*(1), 10. https://doi.org/10.1186/s13568-014-0088-x

Yamamoto-Tamura, K., Hoshino, Y. T., Tsuboi, S., Huang, C., Kishimoto-Mo, A. W., Sameshima-Yamashita, Y., & Kitamoto, H. (2020). Fungal community dynamics during degradation of poly(butylene succinate-co-adipate) film in two cultivated soils in Japan. *Bioscience, Biotechnology and Biochemistry*, *84*(5), 1077–1087. https://doi.org/10.1080/09168451.2020.1713718

Yonemura, S., Nouchi, I., Nishimura, S., Sakurai, G., Togami, K., & Yagi, K. (2014). Soil respiration, N_2O, and CH_4 emissions from an Andisol under conventional-tillage and no-tillage cultivation for 4 years. *Biology and Fertility of Soils*, *50*(1), 63–74. https://doi.org/10.1007/s00374-013-0831-5

Zumstein, M. T., Rechsteiner, D., Roduner, N., Perz, V., Ribitsch, D., Guebitz, G. M., Kohler, H.-P. E., McNeill, K., & Sander, M. (2017). Enzymatic hydrolysis of polyester thin films at the nanoscale: Effects

of polyester structure and enzyme active-site accessibility. *Environmental Science & Technology*, *51*(13), 7476–7485. https://doi.org/10.1021/acs.est.7b01330

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.