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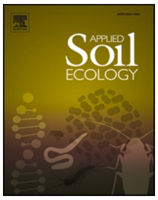

Research paper

Fertilizer enriched bio-based mulch films increase nitrogen and phosphorus availability and stimulate soil microbial biomass and activity

Sara Paliaga[a,d,*], Luigi Badalucco[a], Veronica Concetta Ciaramitaro[b], Delia Francesca Chillura Martino[b], Antonio Gelsomino[c], Ellen Kandeler[d], Sven Marhan[d], Vito Armando Laudicina[a,e]

[a] *University of Palermo, Department of Agricultural, Food and Forest Sciences, Palermo 90128, Italy*
[b] *University of Palermo, Department of Biological, Chemical, and Pharmaceutical Sciences and Technology, Palermo 90128, Italy*
[c] *Mediterranean University of Reggio Calabria, Department of Agriculture, Reggio Calabria 89122, Italy*
[d] *University of Hohenheim, Institute of Soil Science and Land Evaluation, Soil Biology Department, Stuttgart 70599, Germany*
[e] *NBFC, National Biodiversity Future Center, Palermo 90133, Italy*

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ABSTRACT

Plastic mulch films are widely used in agriculture to increase crop productivity and to control weeds, but their non-biodegradable nature is causing many negative effects, such as environmental pollution and land degradation. These drawbacks are favoring the development of biodegradable alternatives. Recently, innovative bio-based mulch films composed of carboxymethyl cellulose (CMC), chitosan (CS) and sodium alginate (SA) have been developed. These films have also been enriched with monoammonium phosphate (MAP) to be potentially released during their degradation thus supplying N and P to soil. This study aims to evaluate the impact of N and P enriched bio-based films on N and P pools dynamics and microbial biomass, activity and community structure. For this purpose, two types of films, both with and without MAP-enrichment, were mixed with the soil at 0.1 % (*w*/w) ratio to simulate field conditions. Soil samples were analyzed at 30, 60, 90 and 120 days after film application to assess changes in above variables. The results showed that MAP-enriched films significantly increased the concentrations of available nitrate and phosphate by up to 76 % and 72 %, respectively. All four film types increased microbial biomass C and N, while enhanced β-glucosidase and *N*-acetyl-β-d-glucosaminidase activities indicated some biodegradation of CMC and CS. The degradation of the biopolymers was further confirmed by lipase activity, which was on average 79 % higher in the film-amended soils. Moreover, films influenced the microbial community structure, favoring the growth of bacteria, particularly Gram positive, over fungi. Overall, these results suggest that these innovative bio-based films are promising candidates for sustainable agricultural practices.

1. Introduction

Many millions of tonnes of plastic are produced worldwide, with almost sixty million produced in Europe alone. In 2019, the agricultural sector consumed over 7 million tonnes of plastic, accounting for 2 % of the global plastic production (Vox et al., 2016). In particular, a significant contributor to plastic pollution in agricultural soils is the use of mulch films that improve crop growth and yield, due to several benefits, such as weed control (Hayes et al., 2019; Paliaga et al., 2023a), increased soil temperature (Gao et al., 2019), and reduced soil water evaporation (Zhao et al., 2023).

The most used plastic films are made of low-density polyethylene (LDPE), due to its high chemically inertness, favorable mechanical and thermo-optical properties, as well as ease of processing (Yoon et al., 2012). However, due to their resistance to microbial degradation, LDPE films need to be disposed of at the end of their service life to prevent soil contamination (Briassoulis et al., 2013; Hou et al., 2019). The resulting pollution from LDPE films is estimated to be up to 200 kg ha^{-1}, affecting soil quality, crop development and yield (D'Avino et al., 2015; Razza et al., 2018).

In response to the problems posed by non-biodegradable plastic waste and in line with the goals of the circular bioeconomy, the

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* Corresponding author at: University of Palermo, Department of Agricultural, Food and Forest Sciences, Palermo 90128, Italy.
E-mail address: sara.paliaga@unipa.it (S. Paliaga).

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development of biodegradable mulch films (BDMFs) has gained ground in recent years (Sanchez-Hernandez et al., 2020). Unlike LDPE films, BDMFs can be buried in the soil at the end of their life cycle, where soil microorganisms facilitate their biodegradation (Kyrikou and Briassoulis, 2007; Lucas et al., 2008). This degradation process leads to the formation of natural inorganic by-products (CO_2 and water) and organic compounds (even plant and microbial biomass), thereby reducing the generation of hazardous waste (Chen et al., 2020; Aleksanyan, 2023). Currently, many commercially available BDMFs are petroleum-based, often made of synthetic polymers such as polycaprolactone (PCL) or poly(butylene-adipate-*co*-terephthalate) (PBAT). While these materials are biodegradable under certain conditions, their reliance on fossil fuels for production raises sustainability concerns. In contrast, bio-based polymers are derived from renewable resources, such as plant, algae, and animal biomasses. These polymers are produced by polymerization of monomers extracted directly from natural materials, such as cellulose, or synthesized by microorganisms, e.g. polyhydroxyalkanoates (PHAs) (Merino et al., 2019). Among bio-based polymers, those derived from plants —such as starch and cellulose— are considered the most sustainable due to their abundance and biodegradability (Mitrus et al., 2010).

BDMFs can affect soil properties both during their use, by altering the soil microclimate and thus nutrient availability and microbial community structure, and at the end of the crop cycle when they are buried into the soil (Bandopadhyay et al., 2020). Although the amount of BDMFs relative to soil volume is quite small, when buried, they provide a carbon supply that can be utilized by soil microorganisms as substrate, unbalancing the microbial C/N ratio, influencing their growth, microbial community structure, and activity (Rillig et al., 2019), thus impacting nutrient cycling and availability to plant roots, i.e. the overall soil fertility (Bastida et al., 2008; Giacometti et al., 2013).

Several studies have shown that burying BDMFs increases soil microbial biomass, respiration, and enzyme activities (Moreno and Moreno, 2008; Yamamoto-Tamura et al., 2015) and also causes changes in soil microbial community structure. Mazzon et al. (2022) found that the application of 1 % (*w*/w) of the bio-based film Mater-Bi® (Novamont S.p.A.), to loamy and sandy soils increased microbial biomass C (MBC) and N (MBN), and enzymatic activities in one-year laboratory experiment, while film doses up to 0.1 % had no effect. In addition, Moreno and Moreno (2008) reported that after one year, MBC was 23 % higher in soils treated with starch-based MFs than those treated with LDPE films. Several studies have also found a shift in the microbial community structure with fungi being favored over bacteria after incorporation of BDMFs derived from chemical modification of citric acid fermentation waste (Ma et al., 2016; Muroi et al., 2016). In addition, several studies have evaluated the effect of biodegradable films on soil enzyme activities in recent years, as their dynamics provide an early indication of changes in fundamental soil biochemical processes and thus represent a useful tool for assessing and monitoring soil quality (Kandeler and Dick, 2006). For example, Gao et al. (2021) showed that, using films based on polyester polylactic acid (PLA) and poly(butylene-adipate-*co*-terephthalate) (PBAT) for two consecutive years, increased soil β-glucosidase and β-1,4-*N*-acetylglucosaminidase activities by 38 % and 36 %, respectively, while L-leucine aminopeptidase activity did not. On the other hand, Yu et al. (2023), by adding another type of PBAT/PLA film to bulk soil at different weight ratios, found that it had no or a negative effect on enzymatic activities related to the carbon and nitrogen cycles.

The conflicting results reported in the literature can be attributed to several factors, including the different compositions of biodegradable films, such as the types and proportions of polymers, as well as the amounts added to soil and the presence or absence of additives and plasticizers. In addition, the intrinsic characteristics of the starting soil, such as pH, organic matter content, microbial community structure, and nutrient availability, can influence the outcomes.

Currently, the main natural polymers used to produce biodegradable films are starch, cellulose, alginate, chitosan, and glucomannan (Lan et al., 2018; Riseh, 2023). By combining these biopolymers, it is possible to formulate multifunctional and biodegradable composites materials. Recently, Ciaramitaro et al. (2024) have developed new bio-based films from carboxymethyl cellulose, chitosan, and sodium alginate linked in different weight ratios using the solvent casting method. Moreover, these films were enriched with monoammonium phosphate ($NH_4H_2PO_4$) to release N and P, as well as C into the soil during decomposition, thus acting as a nutrient source for microorganisms and plants. How these innovative N- and P-enriched cellulose-chitosan-based films affect soil nutrient dynamics and microbial biomass is of great interest for evaluating their potential as alternatives to conventional plastic mulch films.

The aim of this work was to assess the extent to which the degradation of these innovative films after burial in the soil affected available N and P dynamics, microbial biomass and activity, and community structure. Therefore, this study aims to provide valuable information on the suitability of these films as sustainable alternatives to conventional plastic mulch films. Four types of mulch films were prepared with a 1:1 or 17:3 mass ratios of chitosan to cellulose, both with and without the addition of 90 %, relatively to film dry weight, of $NH_4H_2PO_4$. Based on previous evidence, we hypothesized that incorporation of these innovative films in soil could stimulate microbial biomass and activities but also alter the composition of the microbial community. As a consequence, the addition of MAP to mulch films could affect differently to previous research the soil functioning even due to possible effects on the availability of mineral N and available P.

2. Materials and methods

2.1. Soil and mulch films

The soil was collected from the topsoil (0–20 cm depth) of a citrus orchard at the Department of Agricultural, Food, and Forestry Sciences, University of Palermo, Italy (38°10′66.6"N, 13°35′03.9″E). After the sampling, the soil was air-dried and sieved at <2 mm. Before starting the experiment, soil was amended with 1.5 % (*w*/w) of mature manure and wetted up to 50 % of water holding capacity; then, pre-incubated at 20–22 °C in the dark for 14 days to restore soil microbial community. The main soil characteristics are reported in Table 1.

The biodegradable mulch films were produced in the laboratory by using the solvent casting technique described by Ciaramitaro et al. (2024). Briefly, two dispersions were prepared: the first was obtained by dissolving 1.5 g of carboxymethyl cellulose (CMC; Mw 75–150 kDa, CAS 9005-38-3) in 100 mL of distilled water. The second was obtained by dissolving 1.5 g of chitosan (CS; Mw 150–700 kDa, degree of acetylation >75 %, CAS 9012-76-4), 1.5 g of sodium alginate (SA; average Mw ~250 kDa, degree of substitution 0.9, CAS 9004-32-4) and 2 g of glycerol in 100 mL of a 2.0 % (*v*/v) aqueous acetic acid solution (CS/SA). All the reagents used for producing the films were purchased from Merck Life Sciences S.r.l. (Milan, Italy). To obtain a bioplastic films, the two dispersions were mixed with two different mass ratios CS/SA:CMC, 1:1 or 17:3, and the obtained dispersions were immediately spread within a plastic Petri dish (8 cm in diameter), and left at room temperature for drying (Fig. S1). From now on, the films will be referred to as 1:1 or 17:3, depending on the mass ratio of the dispersions used. Generally, after 24 h (the drying period slightly varied depending on air temperature and humidity), the films were manually peeled off from Petri dishes. Further bio-based mulch films enriched with monoammonium phosphate ($NH_4H_2PO_4$ – MAP) were prepared as described above, by adding MAP to the CMC dispersion at 90 % relative to the dry weight of film. More details about the preparation of the BDMFs are reported in Ciaramitaro et al. (2024).

2.2. Incubation experiment

The incubation experiment was conducted in microcosms consisting of 2 L plastic bottles, each containing 500 g of pre-wetted soil, in which

Table 1
Main characteristics of the soil used for experiment.

Parameter	Clay (%)	Silt (%)	Sand (%)	pH (H_2O)	pH (KCl)	EC (dS m^{-1})	TOC (g kg^{-1})	TN (g kg^{-1})	Available P (mg kg^{-1})
Value	6	14	80	7.1	6.2	0.6	49	3.9	68.5

pH (H_2O), in distilled water; pH (KCl), in 1 M KCl (1:2.5, w/v); EC, electrical conductivity; TOC, Total Organic Carbon; TN, total nitrogen; Available P, available phosphorus.

0.1 % by weight of the films, cut into square pieces (5 × 5 mm), were buried to simulate field conditions after one cultivation cycle. The soil treatments were as follow: CTR, control soil without added films; 1:1, soil added with 1:1 film; 1:1 + MAP, soil added with 1:1 film enriched with MAP; 17:3, soil added with 17:3 film; 17:3 + MAP, soil added with 17:3 film enriched with MAP. Each treatment was replicated four times, with one microcosm per replicate. The soil incubation period lasted 120 days, during which soil samples were taken from the same bottle at 30, 60, 90, and 120 days. Fresh subsamples were analyzed for ammonium (NH_4^+-N), nitrate (NO_3^--N), and available P, as well as soil microbial biomass C and N (MBC and MBN, respectively). Additionally, an aliquot of 15 g of soil was collected and immediately frozen (−22 °C) for subsequent determination of the main microbial groups and enzyme activities.

2.3. Soil chemical analysis

Soil pH and electrical conductivity were determined in 1:2.5 (*w*/*v*) soil:distilled water suspension by a pHmeter (FiveEasy, Mettler Toledo Spa, Milan, Italy) and a conductometer (HI5321, Hanna Instruments Italia srl, Padua, Italy), respectively.

NH_4^+-N and NO_3^--N concentrations were determined on soil extracts obtained by shaking 15 g of fresh soil with 60 mL 0.5 M K_2SO_4 (1:4 w/v) for 45 min on a horizontal shaker (70 rpm). Soil suspensions were filtered through Whatman 42 paper and extracts analyzed by the Spectroquant® Nitrate and Ammonium tests using a spectrophotometer (UVmini-1240, Shimadzu Italia srl, Milan Italy). Available P was determined using the colorimetric Olsen method with sodium bicarbonate extraction at pH 8.5, using the same spectrophotometer after the formation of a blue complex (Murphy and Riley, 1962).

2.4. Soil biochemical analyses

Soil microbial biomass C and N (MBC and MBN, respectively) were determined by the fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987) as described by Paliaga et al. (2023b). Briefly, soil aliquots of 15 g were fumigated with alcohol-free chloroform in vacuum desiccators for 24 h in the dark. After the chloroform was removed by repeated evacuations, soil samples were extracted with 60 mL 0.5 M K_2SO_4 for 45 min on a horizontal shaker (70 rpm). Non-fumigated soil samples were similarly extracted and used as controls. All soil suspensions were then filtered through Whatman 42 paper, and the obtained extracts were analyzed for total organic C (TOC) by the acid-dichromate oxidation method (Walkley and Black, 1934) and for total N by the Kjeldahl method (Bremner, 1996). MBC and MBN were estimated as the differences in TOC and total N extracted between fumigated and non-fumigated samples, respectively, multiplied by a conversion factor of 2.64 (k_{EC}) for MBC (Vance et al., 1987) and 2.22 (k_{EN}) for MBN (Jenkinson, 1988).

Potential activity of the soil enzymes β-glucosidase (β-glu; EC 3.2.1.21), β-xylosidase (Xyl; EC 3.2.1.37), *N*-acetyl-glucosaminidase (N-Ac; EC 3.2.1.52), acid Phosphatase (Phos; EC 3.1.3.2) and Lipase (Lip; EC 3.1.1.3) were measured using fluorometric and photometric methods (German et al., 2011; Marx et al., 2001). Lipase activity was determined with a modified protocol according to Cooper and Morgan (1981), as described in Schöpfer et al. (2022). Briefly, 1 g soil samples of each treatment were suspended in 50 mL of H_2O and disaggregated by sonication (UP200S ultrasonic processor, Hielscher Ultrasonics GmbH, Teltow, Germany) for 120 s at 50 J s^{-1}. Three aliquots (50 μL) of each soil suspension were placed in 96-well microplates. Next, 50 μL of buffer solution - either 0.1 M MES (2-morpholinoethanesulfonic acid; pH 6.1) for β-glu, Xyl, N—Ac and Phos or 0.1 M Tris-HCl (Tris(hydroxymethyl) aminomethane hydrochloride; pH 7.8) for lipase analysis - and 100 μL of 1 mM substrate solution linked to fluorescent 4-methylumbelliferone (4-MUF) were added.

Fluorescence was measured spectroscopically at 360/460 nm (excitation/emission) after 0, 30, 60, 120, and 180 min, or 0, 30, 60, 90 and 120 min for lipase, using a fluorescence microplate reader (Synergy HTX, Agilent Technologies). At the same time, a standard plate with concentrations of 0, 0.5, 1, 2.5, 4, and 6 μM 4-MUF was measured. During measurements, the samples were kept at 30 °C. Enzyme activities, expressed in nanomoles per hour per gram, were determined using linear correlation between fluorescence intensity and enzymatic activity of the standards. The specific enzymes activities were calculated by dividing total enzyme activities by MBC, as described by Waldrop et al. (2000).

Phospholipid fatty acids of microbial cell membranes (PLFA) were extracted and analyzed according to the modified Bligh and Dyer method as reported by Frostegård et al. (1991). Lipids were extracted with a mixture of chloroform, methanol, and 0.15 M citrate buffer solution, pH 4 (1:2:0.8 *v*/v/v), then separated on a silica column into neutral lipids, glycolipids, and phospholipids. Then, the latter were methylated and resulting methylated fatty acids (FAMEs) separated and quantified by gas chromatography. Tetracosanoic acid (C24:1) methyl ester was used as internal standard for FAMEs quantification. The extracted FAMEs were analyzed on an Agilent 8860 gas chromatograph equipped with a 5977b mass selective detector (MSD) (Agilent, USA). Peaks were identified by comparison with retention times of bacterial methyl ester mix (BacMIX, Sigma-Aldrich, USA) as standard. Fatty acids (FAs) with <14 or >19 carbon atoms were neglected as originating from non-microbial sources. The fatty acids i15:0, a15:0, i16:0, 16:1ω7, i17:0, cy17:0, cy19:0 represented bacteria, while 18:2ω6,9 corresponded to fungi (Joergensen, 2022). The FAs i15:0, a15:0, i16:0, i17:0; a 17:0 represented Gram-positive bacteria (Gram+), while cy17:0 and cy19:0 Gram-negative bacteria (Gram-).

2.5. Statistical analyses

Results are given as arithmetic means of 4 replicates ($n = 4$) and are expressed on dry soil weight basis at 105 °C. Before performing parametric statistical analyses, normal distribution and variance homogeneity of the data were checked by Kolmogorov–Smirnoff goodness-of-fit and Levene's tests, respectively. Data were analyzed by using the repeated measures procedure in the general linear model. One-way analysis of variance (ANOVA) was performed for each sampling day (CTR, 1:1, 1:1 + MAP; 17:3; 17:3 + MAP as factors) for all tested variables. Significant statistical differences among treatments at a given sampling day were assessed by Tukey test ($p < 0.05$). Furthermore, a correlation analysis (Table S1) was performed between the main microbial groups (bacteria, Gram-positive bacteria, Gram-negative bacteria, and fungi) and the chemical and biochemical properties of the soil, using data from the last sampling day, i.e., after 120 days of incubation. All statistical analyses were carried out using SPSS 13.0 (IBM, USA).

3. Results

3.1. Soil pH and electrical conductivity

Soil pH, as measured in a soil:water suspension, indicated the soil actual acidity. It was not significantly influenced by the addition of all films during the 120-day incubation, varying between 6.8 and 7.2 (data not shown). Also the soil electrical conductivity did not vary significantly after the films addition, compared to control, as it ranged between 0.6 and 0.7 dS m^{-1} (data not shown).

3.2. Dynamics of mineral N

Both NH_4^+-N and NO_3^--N were significantly influenced by time and by the interaction time x treatment (Table 2). Regardless of the treatments, throughout the 120-d incubation, NH_4^+-N decreased, and NO_3^--N increased (Fig. 1). NH_4^+-N concentration during the incubation did not show significant differences among treatments except at day 30, when NH_4^+-N was the highest in soil with both films without MAP-enrichment. In particular, the maximum increase (76 %), compared to the control, was recorded in soil added with 17:3 film. By contrast, with 17:3 + MAP film, the lowest NH_4^+-N concentration was observed, compared to all remaining treatments. Moreover, at day 30, NH_4^+-N in soil with both 1:1 films did not differ from that of the control.

During the incubation, NO_3^--N concentration was 28 to 41 % higher in soils with MAP-enriched films compared to control and the two treatments with not MAP-enriched films. In turn, these last three treatments (control and not MAP-enriched films) did not differ among them during the incubation.

3.3. Dynamics of available P

Available P increased over time in soil added with both MAP-enriched films, with no difference between them. This increase was threefold higher than that of control and soil with not enriched films, which remained equal among them throughout the incubation. The maximum concentration of available P was reached at day 60 and afterwards it kept constant until the end of the incubation (Fig. 2).

Table 2

Results of repeated measures analysis showing within-subject (TIME) and interaction (TIME*TREATMENT) effects across a 120-day incubation period, with sampling at four intervals and five treatments applied. Significant between-subject effects (TREATMENT) indicate differences across treatments. Reported value are Fisher value (F). Level of significance: n.s., not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

	Tests of Within-Subjects Contrasts		Tests of Between-Subjects Effects
	TIME	TIME*TREATMENT	TREATMENT
NH_4^+-N	973.3 ***	12.9 **	19.6 ***
NO_3^--N	600.3 ***	5.5 *	29.5 ***
Available P	548.5 ***	62.0 ***	687.3 ***
MBC	15.5 **	4.0 *	7.2 **
MBN	49.7 ***	3.1 n.s.	2.4 n.s.
MBC/MBN	19.1 **	6.9 **	0.9 n.s.
β-glucosidase	0.0 n.s.	3.8 *	3.0 n.s.
β-Xylosidase	0.0 n.s.	3.6 *	1.3 n.s.
N-acetyl-glucosaminidase	0.1 n.s.	3.6 *	1.2 n.s.
Acid Phosphatase	3.0 n.s.	4.6 *	1.3 n.s.
Lipase	25.6 ***	5.5 *	429.8 ***
Total PLFAs	612.5 ***	3.6 *	51.6 ***
Fungi/Bacteria	227.6 ***	13.6 ***	5.8 **
Gram+/Gram-	12.9 **	2.6 n.s.	0.3 n.s.

3.4. Dynamics of microbial biomass C and N

Microbial biomass C and N showed a sharp increase up to day 30 in all treatments, then gradually declined over time. Soil with films generally exhibited higher MBC compared to control (Fig. 3). In particular, at day 30, MBC was, on average, 24 % higher in soils added with films than the control, except for 1:1 + MAP treatment where MBC was similar. The greatest MBC enhancement was recorded for the 17:3 + MAP treatment. At the end of incubation (day 120), soil added with the two MAP-enriched films showed 35 % higher MBC values than the control, whereas the two films without MAP had 25 % higher MBC than the control. In contrast, soil MBN was affected only by the time and no differences were recorded between the different treatments, except for soil added with 17:3 + MAP film, where after 30 days of incubation MBN was 40 % higher than control. However, regardless of the treatment, MBN decreased linearly from day 30 to the end of incubation, returning to its initial level. The changes of MBC and MBN during the incubation period affected the MBC/MBN ratio. Up to 90 days the MBC/MBN ratio ranged from 6 to 8.2 with no differences between the treatments, while at day 120, the ratio significantly increased to about 9.3 in soils added with MAP-enriched films in comparison to the other treatments.

3.5. Enzyme activities

On day 30, β-Glu activity was on average 32 % higher in soil added with films compared to the control. On day 120, the highest β-Glu activity was in soil added with 17:3 film (Fig. 4). Xyl activity remained relatively stable over time, and did not show any significant differences. On day 120, in soils with both 17:3 films, whether enriched with MAP or not, N—Ac increased by 29 % compared to the control and 1:1 films. Phos activity showed significant differences between treatments only at the first two sampling days: at day 30, soils added with films, regardless of MAP enrichment, showed higher Phos activity compared to the control, while at day 60, soil added with 1:1 + MAP film showed the lowest Phos activity (significant time x treatment interaction). Both total and specific lipase activity was always higher in soils added with films compared to the control (Fig. 4 and S2). Furthermore, on day 120, lipase activity in soil with non-enriched films increased compared to soils with MAP-enriched films. Except for lipase, no treatments effects were found for the specific enzyme activities (Fig. S2).

3.6. Dynamics of the main microbial groups

The total PLFAs content showed a decreasing trend between day 30 and 90 (Fig. 5), similar to that of MBC. Indeed, there was a strong positive correlation between MBC and PLFAs ($R^2 = 0.783$, $p < 0.001$; Fig. S3).

In particular, throughout the entire incubation period, the total PLFAs of soils added with films were higher than those of the control (from 26 to 42 %). The addition of films to the soil decreased the fungi/bacteria ratio during the whole incubation period, with the most significant difference on day 60. The Gram+/Gram- ratio remained relatively stable and similar among the five treatments until day 60. However, from day 90 onwards the highest ratio was found in soil added with 1:1 + MAP, while at day 120 the lowest ratio occurred in the control, with the remaining 4 treatments being similar on both incubation days. Significant positive correlations were observed between the different microbial groups and the concentration of nitrate and available P, with the strongest positive relationships found for available P ($p < 0.01$; Table S1). Among the enzyme activities, β-Glu, N—Ac and Lipase showed significant positive correlations with bacteria.

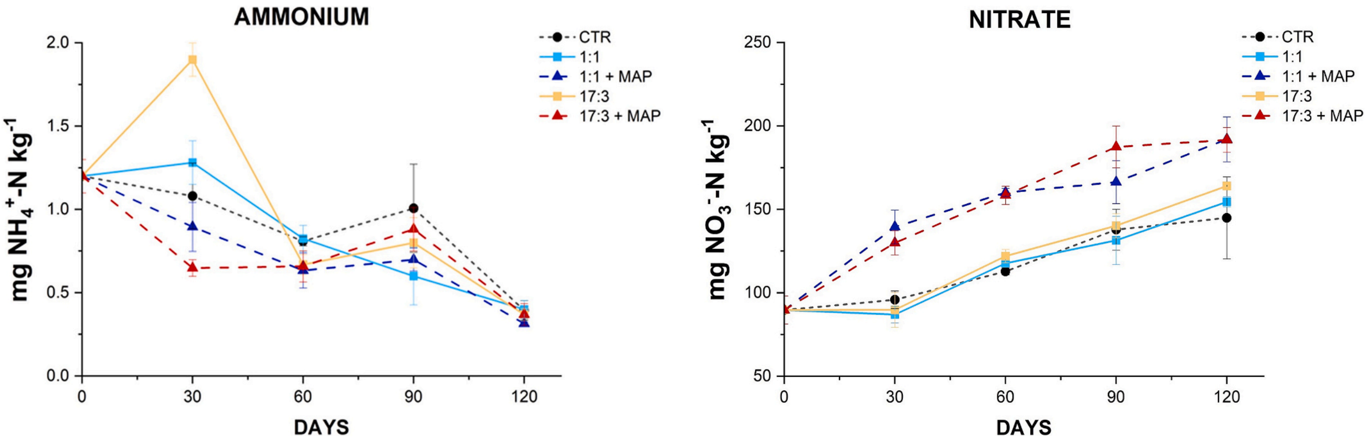

Fig. 1. Ammonium (NH_4^+-N) and Nitrate (NO_3^--N) concentration in soil with and without addition of films: 1:1 or 17:3 both with and without the MAP-enrichment, over 120 days of incubation.

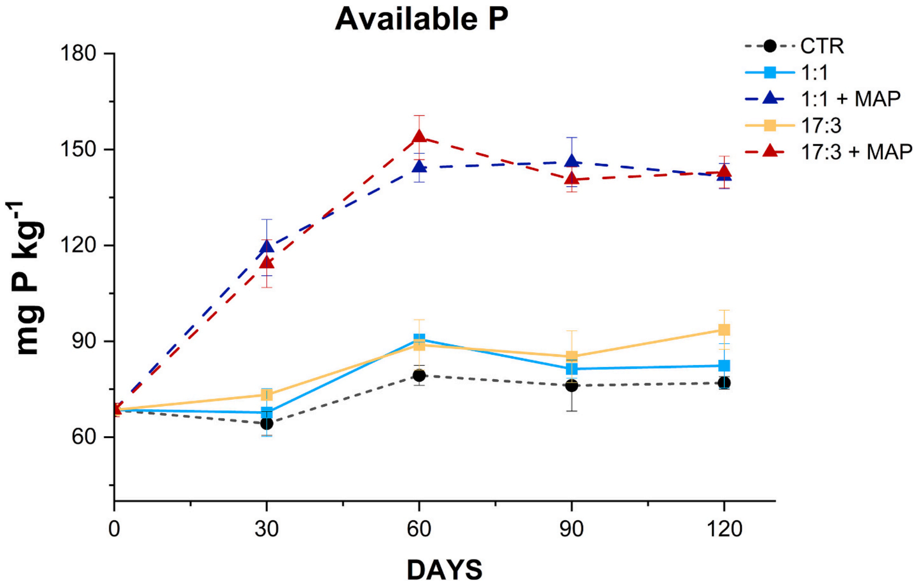

Fig. 2. Available P concentration in soil with and without addition of films 1:1 or 17:3 both with and without the MAP-enrichment, over 120 days of incubation.

4. Discussion

4.1. *Soil nutrients dynamics following film addition*

Incorporation of the innovative bio-based mulch films, particularly those enriched with MAP, significantly affected soil nutrients dynamics, by enhancing nitrification and increasing available P levels (Figs. 1 and 2). The sharp increase in NO_3^--N in soil MAP-enriched treatments indicates a strong stimulation of nitrifying microbial activity, likely due to the combined high availability of NH_4^+ and P. Indeed, it is known that addition of ammonium to soil may favor the nitrification process by enhancing its availability for nitrifying bacteria (Zhao et al., 2007), as also confirmed by the positive correlation between bacteria, both Gram-positive and -negative, and nitrate concentration after 120 days of incubation (Table S1). Furthermore, DeForest and Otuya (2020) reported that synthetic P fertilizers could increase soil nitrification process by up to 12 times. However, generally the increase in microbial nitrifiers following the application of synthetic fertilizers persists only temporarily (Quemada et al., 2019). Although the nitrification process generally decreases soil pH due to the release of H^+ ions, the lack of pH variation in soils treated with MAP-enriched films is likely due to the soil buffering capacity, due to organic and inorganic colloids (Brady and Weil, 2008). Also, the increase in nitrification was favored by the soil pH remaining around neutrality, i.e. the optimal environmental condition for nitrifying bacteria (Ayiti and Babalola, 2022). The increase of NO_3^--N in soil, if not excessive, is beneficial for plant growth, as nitrate is more mobile than ammonium and, therefore, the most available form of inorganic N that plants can uptake for a better growth and productivity. Additionally, being P often immobilized in soil and unavailable to plants, its improved availability due to MAP-enriched films can further support plant development, as P is essential for energy transfer, photosynthesis, and the synthesis of nucleic acids. (Ellsworth et al., 2015). In detail, available P increased during the first 60 days of incubation, before gradually stabilizing. This pattern suggests an increased release of P from the film in the early stages of contact with the soil, followed by an equilibrium between nutrient release and microbial uptake.

4.2. *Soil microbial biomass dynamics and community shifts after films addition*

The addition of innovative bio-based mulch films affected microbial biomass growth and activity, as well as caused changes in microbial community structure.

The rapid increase in MBC and MBN just after the addition of the

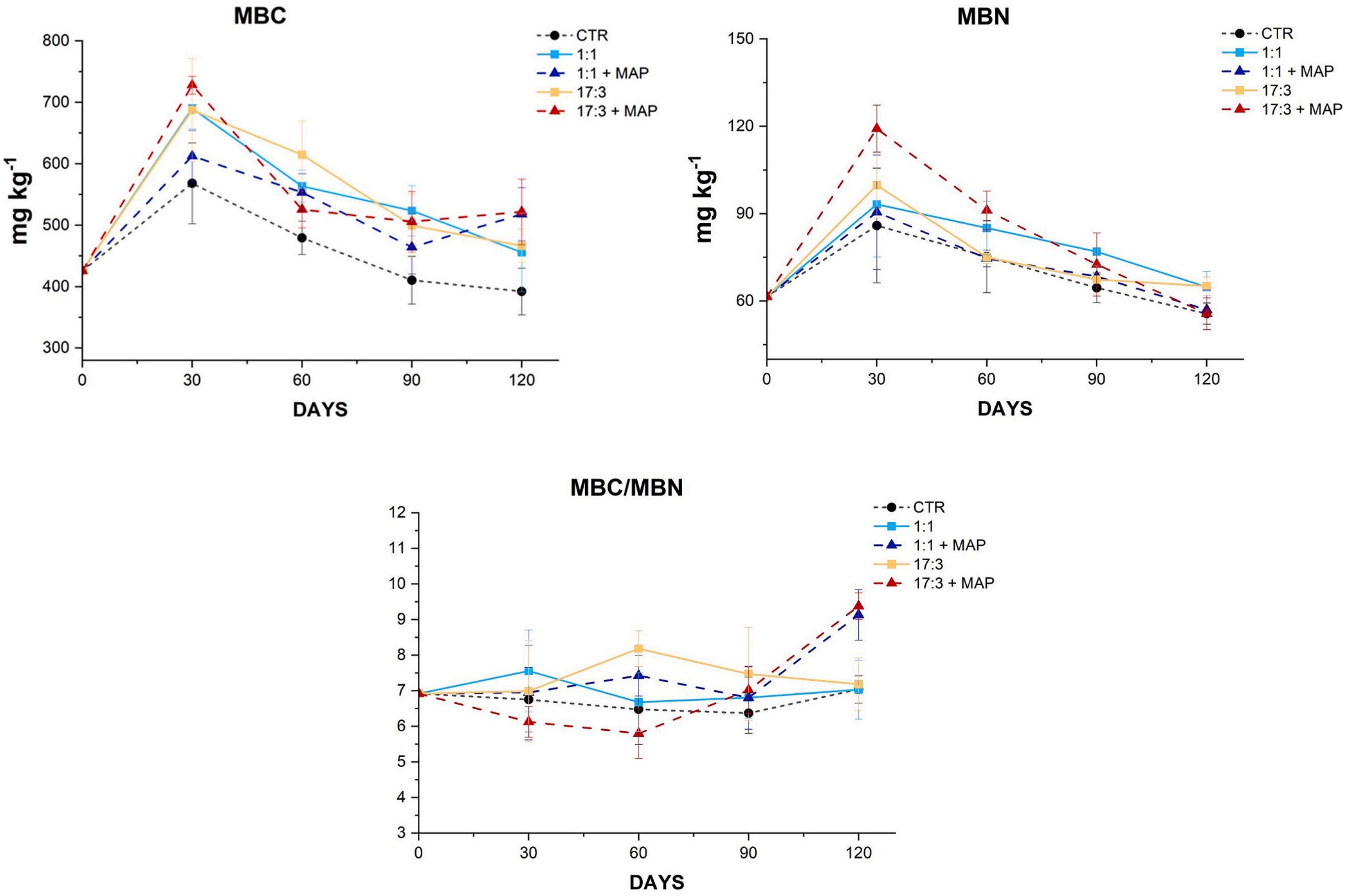

Fig. 3. Soil microbial biomass C (MBC) and N (MBN) and MBC/MBN ratio in soil with and without addition of films 1:1 or 17:3 both with and without the MAP-enrichment, over 120 days of incubation.

films to soil suggests that their C input, although small compared to the total soil volume, may have stimulated microbial growth and consequent activity. The rising of soil microbes in agricultural soils is usually C-limited, and several studies have monitored the responses of soil microbes to these small C inputs, revealing that the effects of biodegradable plastic mulch on soil health were mostly positive (Bandopadhyay et al., 2018; Sintim et al., 2019). Furthermore, the addition of N derived from chitosan, the main polymer in the film, may also have stimulated microbial growth, overcoming one of the main problems associated with the use of bio-based films, namely the imbalance in C/N stoichiometry originated from the addition of films with low N contents (Mazzon et al., 2022). Conversely, the addition of MAP-enriched films to soil increased the MBC/MBN ratio, but only at the end of the incubation period. This was likely also due to P released from the films, which increased the available P in the soil (Fig. 2) and, in turn, promoted an increase in MBC due to the high C availability (initial TOC content of 4.9 %).

It is also important to highlight that, given the initial soil pH (actual acidity) and its stability throughout the incubation period, P precipitation or fixation is expected to be very low, thus preventing the released P from becoming unavailable (Brady and Weil, 2008). However, this favorable condition may not be generalizable to all soil types, and a considerably variable amount of film-released available P is likely to occur depending on chemical and mineralogical soil properties. This remark was further supported by the positive correlation between available P and both bacterial and fungal abundance. The increase of bacteria, particularly Gram+, compared to fungi, in film-treated soils can be attributed to different ecological roles and metabolic capacities of these microorganisms. Gram+ bacteria are well adapted to decay complex organic molecules, such as the polymers that constitute the film (Fanin et al., 2019). Fungi, on the other hand, generally prefer acidic soils and are more efficient in decomposing complex and recalcitrant organic matter, such as lignin, thus being less competitive in environments rich in simpler substrates (Martínez-García et al., 2018).

However, it is important to consider that changes in microbial community structure are closely related to soil type and to initial microbial community. In fact, there are several studies that disagree with our results, where conversely an increase in fungal biomass at the expense of bacterial biomass has been reported following incorporation of biodegradable films into the soil (e.g. Ma et al., 2016; Muroi et al., 2016). In contrast, Li et al. (2014) investigated microbial responses in multiple locations and with different biodegradable mulch films, showing an enrichment of fungi at one location and of Gram+ bacteria at another. This underscores that microbial responses to biodegradable mulch films could be not univocal and influenced not only by composition of the film itself but also by soil type, environment and/or management.

4.3. *Enzyme activities as indicators of film degradation*

When biopolymer films undergo biodegradation, a complex sequence of processes leads to the breakdown of polymer chains and the final transformation into smaller compounds. At early stages, water permeates within the film, allowing enzymes and microorganisms to diffuse into the polymer matrix. Soil microorganisms mediate this degradation by secreting specific enzymes, and subsequently using the C and N derived from films as matter and energy source (Ng et al., 2018; Rillig et al., 2021). Each polymer component degrades through distinct enzymatic pathways. For example, cellulose degradation involves three

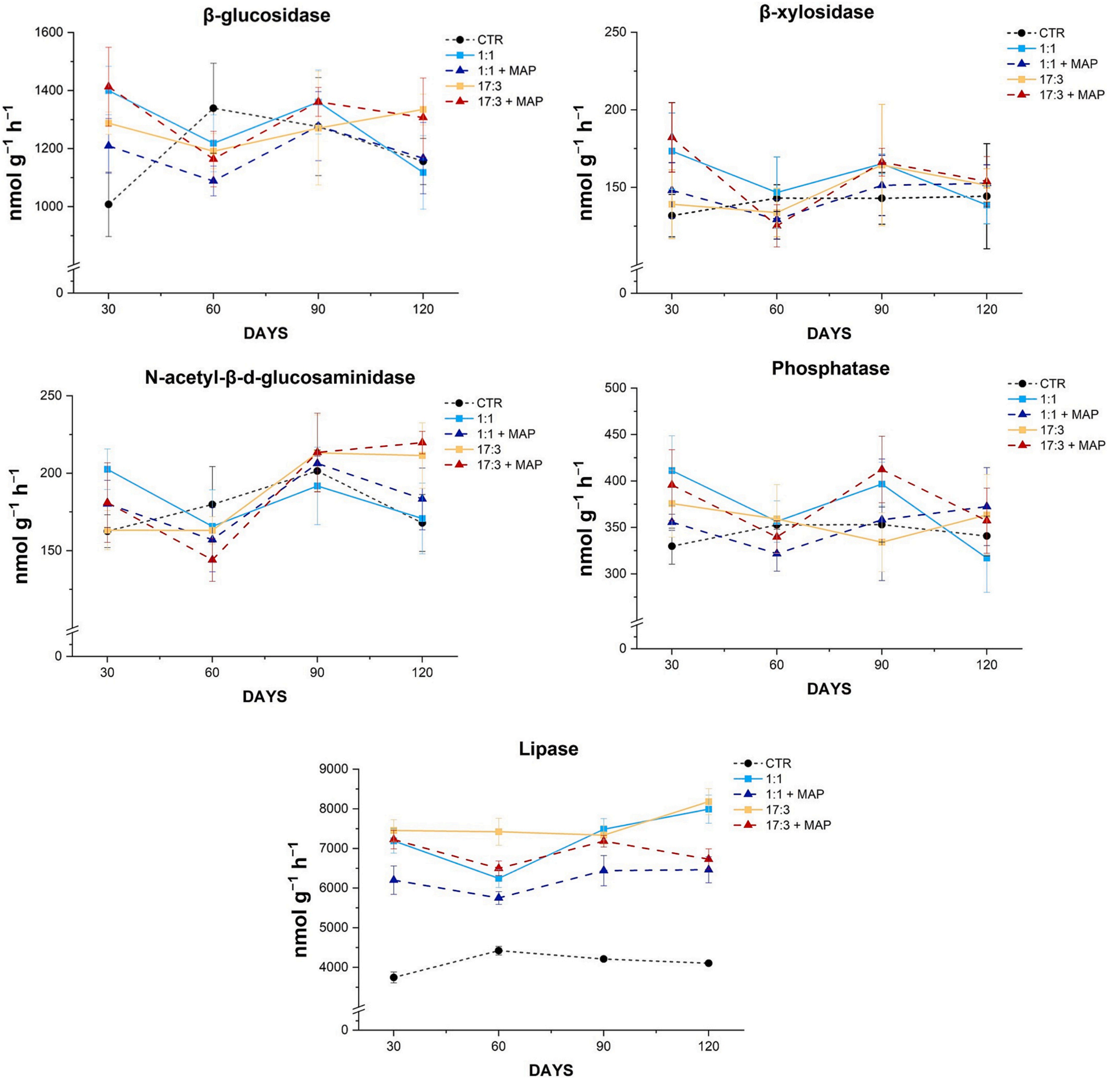

Fig. 4. Soil enzymatic activities in soil with and without addition of films 1:1 or 17:3 both with and without the MAP-enrichment, over 120 days of incubation.

main enzyme systems: *endo*-β-1,4-glucanase, cellobiohydrolase and β-glucosidase, that act synergistically to hydrolyze glycosidic bonds (Davies and Henrissat, 1995; Datta, 2024). In particular, β-Glu is mainly involved in the final stage of degradation of C-substrates as glucosidases hydrolyze the degradation products of amylase and cellulase (Deng and Popova, 2011; Piotrowska-Długosz, 2014; Kandeler, 2024). The increase in β-Glu activity in film-added soils, in our study especially observed at day 30 of incubation, indicates that the supply of biopolymers through the film stimulated the secretion of this enzyme and theirsubsequent degradation. On the other hand, chitosan degradation follows a different pathway, involving an initial random cleavage of β-1,4-glycosidic bonds (depolymerization), followed by hydrolysis of *N*-acetyl bond (deacetylation). The resulting oligosaccharides are further degraded into individual monomers, such as D-glucosamine or *N*-acetyl-glucosamine, which are in turn hydrolyzed by the N—Ac enzyme (Tronsmo and Harman, 1993; Wrońska et al., 2023). Thus, the increased N—Ac activity in soils with 17:3 films can be attributed to their greater chitosan content compared to 1:1 films.

Similarly, elevated lipase activity in film-treated soils compared to control, suggests enzymatic cleavage of polymer ester bonds, which formation derive from carboxylic and alcoholic groups (Marten et al., 2005; Treichel et al., 2010; Lee et al., 2015). It is well-established that lipases play a crucial role in the degradation of biodegradable polyester-based films, such as aliphatic polylactic acid (PLA), polyhydroxybutyrate (PHB), and aromatic poly(butylene co-adipate terephthalate) (PBAT) (Agarwal, 2020). So far, until now no study had examined the degradation of films composed of CS, CMC, and SA (either combined or individually) by lipase. However, considering here the structure of the film components, it is possible to hypothesize that lipase breaks the ester bonds in the chains of CMC and SA (Fig. 6). It is

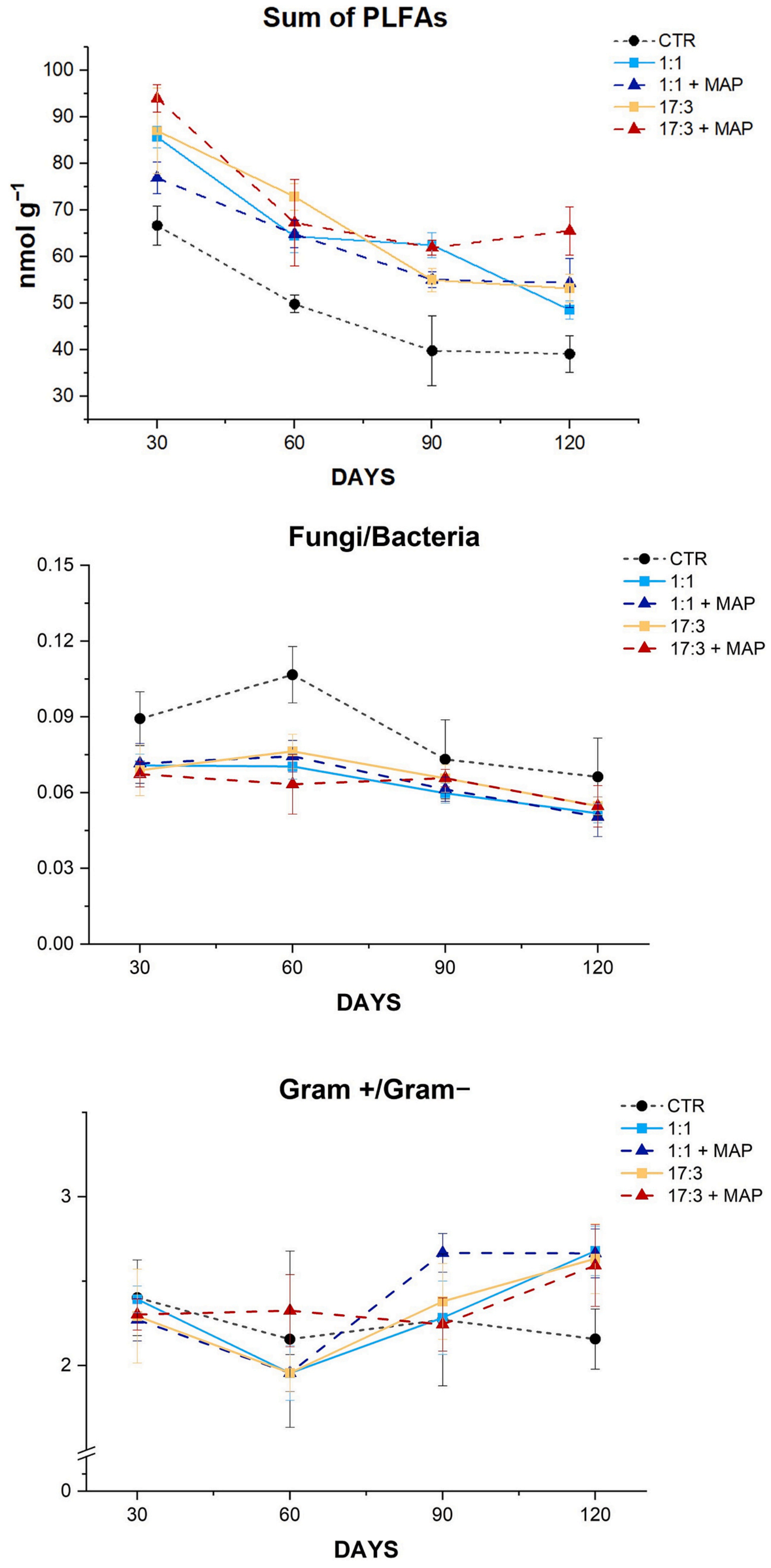

Fig. 5. Total phospholipid fatty acids (PLFAs) of microbial cell membranes, fungi/bacteria ratio, and Gram-positive/Gram-negative bacteria ratio (Gram+/Gram-) in soil with and without the addition of films 1:1 or 17:3, both with and without MAP-enrichment, over 120 days of incubation.

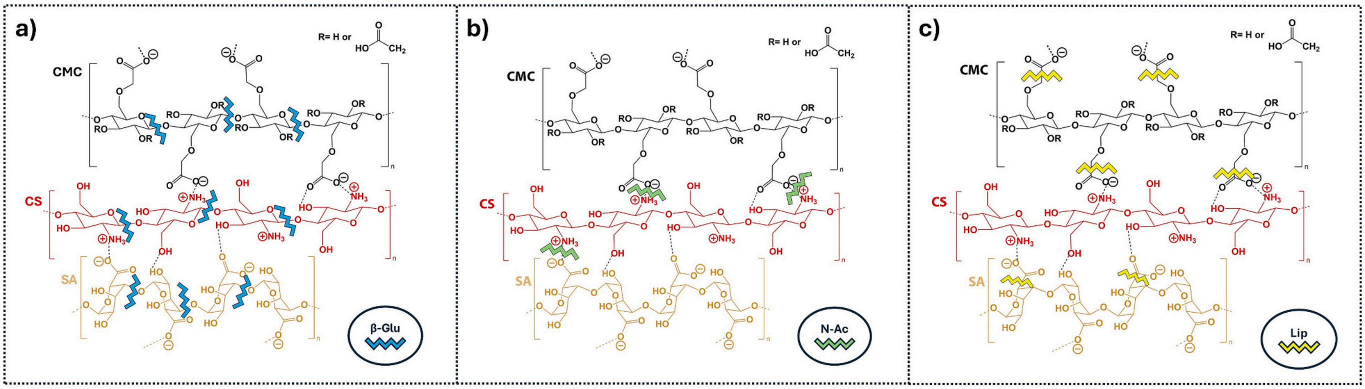

Fig. 6. Schematic representation of the structure of CS, SA, and CMC-based films, depicted in red, yellow, and black, respectively, along with the potential breakage sites mediated by the following enzymes: (a) β-glucosidase, (b) *N*-acetyl-β-D-glucosaminidase, and (c) lipase. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

important to note that, unlike other cellulose derivatives, CMC is soluble in water, which plays a key role in its enzymatic degradation. For example, in their study on the biodegradation of PLA/cellulose acetate polymer blends by lipase, Calil et al. (2007) found that degradation by lipase was inversely proportional to the cellulose acetate content. This result was likely due to the hydrophobic nature of cellulose acetate, which may hinder efficient hydrolysis. The lower lipase activity observed in soils with MAP-enriched films at the end of the incubation period is likely due to the reduced amounts of polymers added into soil. This is because the enriched films consisted of approximately 47 % MAP, and thus the total weight of polymers added into soil was about half that of the unenriched film treatment. Additionally, the higher lipase activity was positively correlated with the abundance of Gram-positive bacteria (Table S1), suggesting that these microorganisms may be key contributors to lipase secretion for subsequent polymer degradation. The lack of differences in Xyl among the different treatments suggests that the presence of these innovative films, whether enriched with MAP or not, does not affect this enzymatic activity. In fact, Xyl is an enzyme involved in the cleavage of hemicelluloses, particularly xylans, into xylose units (Warren, 1996). The film components do not interact directly with Xyl nor are they a substrate for it. The small but significant decreases in Phos activity at days 30 and 60 of incubation in the presence of MAP were likely due to the release of soluble phosphate which, as widely reported in literature, is able to inhibit Phos enzyme through feedback by end-product (Nannipieri et al., 2011). Since generally the specific enzyme activities did not differ significantly across treatments, we assumed that microorganisms produced these enzymes at a constant rate (Raiesi and Beheshti, 2014). Nevertheless, the specific lipase activity was increased after the addition of the biopolymers, thus suggesting an induced microbial lipase secretion by some biofilm by-product from the initial degradation by extracellular enzymes (Burns et al., 2013), thus triggering its complete biopolymer decay.

5. Conclusion

This study highlights that innovative bio-based mulch films, especially MAP-enriched ones, can enhance soil fertility by increasing NO_3^--N and available P, essential for plant growth and crop productivity. In addition, the films improved soil biochemical quality by increasing microbial biomass and enzyme activities, particularly that of lipase. This increase may result from microbial enzyme secretion induced by early by-products of film degradation. The films also influenced microbial community structure, favoring Gram+ bacteria over fungi, likely also due to the soil neutral pH, that is optimal for bacteria, throughout the whole incubation after films additions. Although the tested bio-based mulch films are promising for sustainable agriculture, further studies are needed to assess their effects across different soil types and under varying temperature and humidity conditions.

CRediT authorship contribution statement

Sara Paliaga: Writing – original draft, Formal analysis, Data curation, Conceptualization. **Luigi Badalucco:** Writing – review & editing, Supervision, Conceptualization. **Veronica Concetta Ciaramitaro:** Validation, Formal analysis, Conceptualization. **Delia Francesca Chillura Martino:** Validation, Supervision, Conceptualization. **Antonio Gelsomino:** Validation, Supervision, Conceptualization. **Ellen Kandeler:** Writing – review & editing, Supervision, Data curation, Conceptualization. **Sven Marhan:** Writing – review & editing, Validation, Supervision, Methodology, Formal analysis, Conceptualization. **Vito Armando Laudicina:** Writing – review & editing, Supervision, Resources, Project administration, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Sara Paliaga reports financial support was provided by Italian ministry of universities and research. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2025.106159.

Data availability

Data will be made available on request.

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