Original Research Article

Unveiling the spatial architecture of biodegradable polyester plastisphere in soil and its implications for organic matter composition

Jana Šerá[1], Václav Pecina[2], Vendula Mašláňová[1], Martin Brtnický[3], Adéla Baťová[1], Jiří Holátko[3], Tereza Hammerschmiedt[3], Veronika Kučabová[1], Ondrej Malíček[3], Markéta Kadlečková[4], Jiří Kučerík[3*], Marek Koutný[1*]

[1] Department of Environmental Protection Engineering, Faculty of Technology, Tomas Bata University in Zlín, Nad ovčírnou 3685, 760 01, Zlín, Czech Republic

[2]Department of Forest Ecology, Faculty of Forestry and Wood Technology, Mendel University in Brno, Zemedelska 3, 613 00 Brno, Czech Republic

[3]Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Faculty of AgriSciences, Mendel University in Brno, Zemedelska 1, 613 00 Brno, Czech Republic

[4]Centre of Polymer Systems, University Institute, Tomas Bata University in Zlín, Tr. T. Bati 5678, 760 01 Zlín, Czech Republic

E-mail mkoutny@utb.cz, jiri.kucerik@mendelu.cz

Abstract

Biodegradable plastics (BPs) are increasingly presented as sustainable alternatives to conventional plastics; however, their ecological effects on soils are poorly understood. BPs can alter soil microbiomes and nutrient cycling; yet, the extent, dynamics, and effects of the plastisphere on soil organic matter (SOM) after biodegradation remain underexplored. This study characterized the microbial plastisphere of three BPs, poly-3-hydroxybutyrate (PHB), poly(butylene succinate-co-adipate) (PBSA), and polybutylene adipate terephthalate (PBAT), and measured polymer biodegradation to link microbial dynamics with material breakdown. Metagenomics, scanning electron microscopy, and thermogravimetric analysis showed that these polymers formed structured plastispheres that significantly altered microbial communities and SOM. Each polymer hosted a distinct plastisphere; bacterial communities diverged more strongly between polymers than fungal ones. Functional profiling revealed shifts in nitrogen metabolism and ecological strategies at BP surfaces, including a decrease in nitrifiers and an increase in parasitic/pathogenic fungi. The plastisphere extended up to 1.25 mm for bacteria and 2.75 mm for fungi. PHB plastispheres were enriched in *Comamonadaceae*, *Oxalobacteriaceae*, and *Rhodocyclaceae*; PBAT favored *Xanthobacteraceae* and

Burkholderiaceae; *Xanthomonadaceae* colonized all BPs. Fungal communities were dominated by *Nectriaceae*, *Herpotrichiellaceae*, and *Aspergillaceae*, and their composition changed over time. BP exposure reduced SOM, most strongly for PHB and PBSA, and to a lesser extent for PBAT, suggesting a positive priming effect. Overall, BP degradation promoted nitrogen-limited conditions and host-dependent microbial strategies. Although plastisphere communities showed signs of stabilization after 350 days, full recovery of microbial composition and SOM may require longer, indicating potential long-term impacts of BPs on soil ecosystems. These results underscore that BPs can alter soil microbial ecology and organic matter turnover, highlighting the need for further long-term studies.

Keywords: Plastisphere, Soil microbiome, Poly-3-hydroxy butyrate, Poly(butylene succinate-co-adipate), Polybutylene adipate terephthalate, Soil organic matter

1. Introduction

1.1 What is plastisphere?

The plastisphere, first described for marine plastics Zettler et al., 2013, now encompasses distinct microbial ecosystems that assemble on plastic surfaces across environments, including soils(Rüthi et al., 2020; Rillig et al., 2024).In soils, the plastisphere includes the surface-attached biofilm and the immediately adjacent community that is influenced by the presence of the plastics and differs from the bulk soil communities. So far, as the focus has been on the immediate surface of the plastic soil, plastispheres are seen as dominated by biofilm-forming taxa (Li et al., 2024; Shi et al., 2022), with polymer chemistry and degradability shaping the community structure (Ju et al., 2021; Peng et al., 2022).PBSA plastispheres frequently host *Burkholderia*, *Roseateles,* and fungi such as *Talaromyces*, *Tetracladium,* and *Cladosporium*(Ahmad et al., 2019; Purahong et al., 2021; Tanunchai et al., 2023a). Nitrogen-fixing bacteria, such as Bradyrhizobium, Burkholderiaceae, Rhodopseudomonas, and Mycobacterium, enriched in PBSA plastispheres, suggesting a role in nitrogen metabolism (Zhou et al., 2021a; Feng et al., 2022). PHB attracts groups such as *Chitinophagaceae*, *Comamonadaceae*, and *Oxalobacteraceae,* as well as associated fungi like *Herpotrichiellaceae* and *Bionectriaceae* (Nagarajan et al., 2014; Zhang et al., 2022). PBAT (and PBAT/PLA blends) have been shown to harbor yet other communities, including *Bradyrhizobium* and various

saprophytic fungal families, such as *Phiomyceceae* and *Aspergillaceae* (Ahmad et al., 2019; K. Li et al., 2024).

Recent evidence suggests that the plastisphere has both spatial and (Deng et al., 2025) temporal dimensions that strongly influence soil organic matter (SOM) (Zhang et al., 2023) dynamics. Spatially, as aforementioned, plastisphere represents a microsite of enhanced microbial biomass (Shi et al., 2022; C. Li et al., 2024), in which bacterial communities exhibit greater temporal dynamics and play a more active role in plastisphere development and nutrient transformation than fungi (Deng et al., 2025). The succession of microorganisms during the colonization and degradation of biodegradable polymers follows a distinct pattern. The initial colonizers generally consist of fast-growing, opportunistic bacteria such as *Pseudomonas* and *Bacillus*, which rapidly form biofilms and begin degrading accessible polymer components (Ju et al., 2021). In intermediate stages, a shift towards a more diverse microbial community can be expected, including *Actinobacteria*, which are capable of degrading more complex molecules (Bandopadhyay et al., 2020). Late stages are characterized by specialized degraders, such as *Aspergillus* species (Ruthi et al., 2020). These changes modulate SOM cycling by stimulating its carbon mineralization, nitrogen immobilization, and priming effects (Palucha et al., 2024a), which are believed to depend on the degradability rate of biodegradable plastic (Zhang et al., 2023).

1.2 Plastisphere shapes the soil environment

Functionally, plastispheres on biodegradable plastics act as transient carbon "hotspots", where extracellular depolymerization releases oligomers and monomers that fuel particle-centered food webs. Biofilm microenvironments also promote the partitioning of aerobic versus fermentative metabolism and taxon selection. For example, on PBSA, the plastisphere has been described as taxonomically and functionally distinct and including inter-kingdom relationships between fungi (*Tetracladium* spp.) and N-fixing bacteria. Its assembly is further modulated by soil type and climatic conditions, underscoring environmental control over polymer-specific microbiomes (Purahong et al., 2021; Tanunchai et al., 2023). Inputs of PHB-type polymers also restructure soil communities: PHB microplastics suppress bacterial diversity and activity, and alter metabolic pathways in the rhizosphere, with measurable knock-on effects on plant–microbe interactions. Soil texture also influenced PHB degradation kinetics and the microbiome shifts, revealing trade-offs between nutrient availability and plant growth (Brown et al., 2023; Brtnicky et al., 2025). In contrast, slowly degrading PBAT increased the dissolved organic carbon and microbial biomass, alternated enzyme activities and bacterial–fungal networks, and

accelerated decomposition of native soil organic matter (SOM, a positive priming effect) while altering SOM molecular signatures (e.g., lignin- and amino-sugar-derived pools), with soil properties such as pH strongly shaping degradation rates and biogeochemical responses (Jia et al., 2024; Hua et al., 2025).

Key drivers of plastisphere composition and impact include intrinsic polymer properties (chemical composition, surface energy, hydrophobicity, specific surface area and inherent biodegradability), particle size/age and environmental controls (temperature, moisture, nutrients, texture, pH and the resident microbiota) (Miao et al., 2020; Wang et al., 2022a). BPs typically support thicker, more active biofilms than recalcitrant plastics, with consequences for soil structure, water dynamics, and aggregation that may feed back on microbial processes and plant growth (De Souza MacHado et al., 2018; Tian et al., 2022).

1.3. Research gap and opportunities in soil plastispehere interactions

Despite rapid advances, substantial gaps remain in understanding of soil plastispheres. Key unknowns include the spatial extent of plastisphere effects beyond the particle surface and the compositional and functional gradients of microbial communities at increasing distances from particles; most work to date has sampled only the immediate particle surface and usually at a single time point. Equally poorly constrained is the temporal evolution of plastisphere structure and function, i.e., how community composition and metabolic activity develop, persist, or dissolve over weeks, months, and possibly seasons and years. Additional deficiencies include the degree to which native taxa are displaced or functionally reconfigured, polymer-specific, and particle-size effects, the influence of particle weathering and concentration, and the net balance between soil organic carbon sequestration and loss in BP-contaminated soils (Sun et al., 2024). These uncertainties are especially consequential in agroecosystems, where even slight shifts in microbial carbon and nutrient dynamics can significantly impact soil health and crop productivity (Wang et al., 2022).

1.4. Objectives of the present study

Accordingly, this study aims to address some of the knowledge gaps about soil plastispheres by characterizing their spatial reach, temporal dynamics, and functional consequences during the biodegradation of representative biodegradable polymers. Specifically, we aim to (i) quantify the spatial extent of plastisphere influence from the immediate particle surface into surrounding soil on millimeter scale and determine how microbial community composition, and functional potential change with distance; (ii) follow plastisphere succession through time to

resolve trajectories of community assembly, eventual succession and decline; and (iii) test whether polymer identity and degradation rate (PHB, PBSA — relatively fast; PBAT — slower) produce differences in taxonomic and functional assembly and in downstream effects on microorganisms and soil organic matter (SOC) dynamics.

The core of our approach is the use of amplicon metagenomics, which enables the processing of many samples corresponding to spatial and temporal points in our study. The latter methodology is supported by respirometry, providing a macroscopic timeframe for the processes, and thermogravimetry to characterize SOC turnover.

2. Materials and methods

2.1 Polymer materials and soil used in experiments

PHB Enmat Y3000 (TianAn, China), PBSA 4042D (NatureWorks, USA), and PBAT (BASF, Germany) films (100 μm thick) were prepared via compression molding. The carbon content in the polymers was determined using a Flash 1112 elemental analyzer (Thermo Scientific, Waltham, MA, USA), with the results showing carbon percentages of 55.6%, 56.9%, and 62.3% for PHB, PBSA, and PBAT, respectively. Crystallinity was previously determined using differential scanning calorimetry, yielding values of 66.2%, 37.5%, and 7.2% for PHB, PBSA, and PBAT, respectively.

The soil used in this study, Modal Cambisol from the top layer of the freshly tilled field (Bohuňovice, Czech Republic; N 49°39.919', E 17°16.381'), was air dried and sieved through a 2 mm mesh size before the experiments. Soil pH and dry weight were measured according to ISO 10390:2005 and ISO 11465:1993 standards, while organic carbon and total nitrogen contents were determined according to methods ISO 10694:1995 and ISO 13878:1998, respectively, using the Vario MACRO cube (Elementar Analysensysteme GmbH, Langenselbold, Germany). The soil had a pH of 7.2, an organic carbon content of 11.5 $g.kg^{-1}$, and a total nitrogen content of 1.8 $g.kg^{-1}$.

These materials and soil were used for four separate experiments, specifically a (I) polyester biodegradation experiment, (II) soil incubation experiment for metagenomic analysis, (III) soil incubation experiment for microscopic observation of the polymer surface, and (IV) soil incubation experiment for thermogravimetric analysis. Soil incubation experiments were conducted separately; however, all incubation experiments had the same soil and general conditions.

2.2 Polyester biodegradation experiment

A polyester biodegradation experiment was conducted as part of the study to investigate the progression of polymer biodegradation in the soil to elucidate the relationship between biodegradation, the development of the plastisphere in soil, and the impact of biodegradation on the plastisphere surroundings.

Two experimental setups were established in bioreactors consisting of 500 mL glass biometric flasks, following a modified respirometric test based on (Šerá et al., 2022) and aligned with ISO 17556:2019 standard. In the first setup, bioreactors underwent completely static incubation, simulating the conditions of the metagenomic experiment described below. In the second setup, the content was agitated at one- or two-week intervals, as is standard in biodegradation tests, to achieve maximum rates of polymer carbon mineralization.

The bioreactors, equipped with septum-sealed caps, were prepared with 15 g of soil, 50 mg of powder polymer sample, and 5 mL of sterile potable water for the static incubation setup. Five g of perlite was included for the agitated setup, and the sterile potable water volume was increased to 10 mL. This experimental procedure was optimized throughout many iterations and validated against the ISO 17556 standard by an accredited laboratory with an identical set of samples (Šerá et al., 2020). A negative control (without polymer), representing soil respiration, was included and also served as a blank to calculate the net degree of material mineralization. A positive cellulose control (as required by ISO 17556:2019) was used to validate the test system's biodegradation potential. Triplicates were prepared for each variant and positive control, along with four blanks. The flasks were stored at 25°C in the dark. To determine CO_2 concentration, the headspace gas was sampled from the bioreactors weekly through the septum using a gastight needle and directed via a capillary tube into a gas analyzer (UGA 300, Stanford Instruments, USA). CO_2 measurements were taken weekly during the initial phase of the experiment and approximately every 14 days during the later phase.

The static incubation experiment was conducted for 400 days to assess long-term biodegradation under natural, undisturbed soil conditions, allowing for a gradual microbial response and biodegradation. In contrast, the weakly agitated incubation experiment lasted 240 days, as mixing and perlite enhanced oxygen availability and microbial activity, potentially accelerating polymer degradation, making a shorter duration sufficient to capture biodegradation dynamics. The cumulative CO_2 produced during the experiment was compared to a theoretical total quantity ($ThCO_2$), providing insight into the extent of polymer biodegradation under each condition. The theoretical production of CO_2 (represented by $ThCO_2$) from complete substrate breakdown was calculated as follows:

199 $ThCO_2 = w_{sample} \times TC \times (44/12)$ (Eq. 1)

200 where:

201 $ThCO_2$ represents the amount of theoretical CO_2 production from total substrate breakdown
202 (mg), w_{sample} is the weight of the tested sample (mg), TC denotes the percentage of organically
203 bound carbon in the tested material, 44 is the molecular weight of CO_2, and 12 is the molecular
204 weight of carbon.

205 The net degree of a material mineralization (D), reduced by the endogenic CO_2 production of
206 soil obtained from blank experiments (m_{blank}), expressed as a percentage of theoretical CO_2
207 production, was calculated using the formula:

208 $D = [(m_{sample} - m_{blank})/ThCO_2] \times 100\ CO_2$ (Eq. 2)

209 where:

210 m_{sample} is the quantity of CO_2 produced from the breakdown of the tested films (mg), m_{blank} is
211 the quantity of CO_2 produced from the endogenous respiration of microorganisms in the blank
212 (mg).

213 These calculations provided a quantitative measure of CO_2 production relative to the theoretical
214 maximum, thereby indicating the extent of mineralization achieved during polymer degradation
215 in soil.

216 2.3 Microscopic observation of the polymer surface

217 Scanning electron microscopy (SEM) was performed using a Phenom Pro Desktop SEM
218 (ThermoFisher Scientific, Waltham, MA, USA) to examine surface alterations and biofilm
219 formation on polyester samples incubated in soil for up to 350 days. Soil incubations for this
220 analysis followed the procedure described in Section 2.2. Briefly, air-dried and sieved soil (30
221 g) was placed in sterile polyethylene containers together with polyester film samples (0.5 × 0.5
222 cm, 100 μm thick). Each flask also received 10 mL of sterile potable water to adjust moisture
223 content, and the incubation was conducted under static conditions at 25 °C in the dark to prevent
224 photodegradation. The water content was periodically monitored and adjusted to maintain
225 stable moisture levels.

226 Polymer fragments were retrieved at four time points: 40, 120, 200, and 350 days. After each
227 incubation period, the samples were carefully removed from the container, gently rinsed with
228 sterile potable water to remove loosely attached soil particles, and air-dried at room
229 temperature. Prior to SEM analysis, the dried samples were sputter-coated with a gold/platinum
230 alloy to ensure conductivity. Imaging was conducted at an acceleration voltage of 10 kV using
231 backscattered electron mode. To obtain representative surface morphology data, fragments

from each time point were imaged in at least three different areas. The most representative images were selected for publication to illustrate changes in surface texture and biofilm development over time.

2.4 Metagenomic analysis

2.4.1 Experimental arrangement and conditions

The experiment was conducted in 250 mL polyethylene rectangular boxes. The soil's actual moisture content was determined and brought up to 50% by adding sterile potable water. In each box, the soil was spread, and a 100 µm-thick film (1.5 x 5 cm) of a particular polymer was inserted vertically into the center of the box along its shorter side. A 5 mm thick layer of soil separated the plastic film from the bottom of the box, and a similar layer of soil was placed above the plastic film. The headspace above the soil was 50 mm high to provide adequate aeration, and the boxes were closed with lids to prevent desiccation.

Individual boxes were prepared for each plastic type and each designated time point (3, 6, 12, 20, 40, 80, 120, 200, and 350 days). The incubations in closed boxes at 25°C were aerated every seven days, weighed, and rehydrated as needed to maintain a stable moisture content throughout the experiment. During each aeration event, the incubation bottles were weighed, and sterile potable water (boiled and cooled) was added when necessary to maintain a stable moisture content throughout the experiment period. Moisture content was therefore controlled gravimetrically. At specified time points, thin aluminum separators were vertically inserted into the soil, allowing sampling of the soil at three-distance intervals from the plastic film (Figure 1). Layer A consisted of fragments of the polymer film itself (100 µm), while the adjacent soil layers were designated as layer B (0 to 2.5 mm from the polymer film), layer C (2.5 to 5.0 mm), and layer D (5.0 to 7.5 mm). The choice of distances was made based on the technical limitations of the methodology, soil granularity, and existing literature data (Suarez et al., 2019).

Multiple polymer fragments with a limited amount of attached soil particles (layer A) and small amount of soil (from layers B, C, and D) were collected from each layer, individually thoroughly homogenized, and 250 mg of the resulting composite samples were subjected to DNA isolation using the PowerSoil Pro kit (MoBio, USA). DNA was extracted from soil samples using the DNeasy PowerSoil Pro Kit (Qiagen, Germany) following the manufacturer's instructions without modification. Briefly, 0.25 g of soil was added to a PowerBead Pro tube containing lysis buffer, and cells were disrupted by bead beating for 10 min at room temperature. The lysate was then clarified by centrifugation (13,000 × g, 1 min), and inhibitor

removal technology reagents were applied according to the protocol. DNA binding to the silica spin column was carried out under high-salt conditions, followed by two washing steps with ethanol-based buffers. Finally, DNA was eluted in 100 µL of elution buffer and stored at –20 °C until further analysis.

This setup allowed for the metagenomic analysis of microbial communities and plastisphere development at the material surface and in varying proximities to the plastic material. Moreover, each sample's homogenization helped obtain an average sample and smooth out eventual local deviations.

Figure 1. Experimental setup of the soil incubation experiment in plastic boxes.

2.4.2 DNA processing and sequencing

Isolated DNA was used to amplify specific regions of bacterial and fungal rRNA genes via PCR, targeting the bacterial 16S rRNA gene (V3–V5 regions) and the fungal ITS2 region. For bacterial amplification, primers F357 (5′-CCTACGGGAGGCAGCAG-3′) and R926 (5′-CCGYCAATTYMTTTRAGTTT-3′) were used, while fungal amplification employed primers ITS3F (5′-GCATCGATGAAGAACGCAGC-3′) and ITS4R (5′-TCCTCCGCTTATTGATATGC-3′). Primers were synthesized with universal Illumina overhang adapter sequences, enabling binding to the Illumina flowcell. In a second PCR amplification step, dual indices were added using indexed primers provided by the manufacturer (Metabion, Germany), following the standard Illumina protocol (Illumina, 2013). The first PCR amplification was performed in 25 cycles to amplify the target regions, while the second PCR, used for attaching Illumina sequencing adapters, was carried out in 15 cycles.

PCR products were evaluated by agarose gel electrophoresis, quantified using the AccuGreen™ High Sensitivity dsDNA Quantitation Kit (Biotium, Inc., CA, USA), and purified with NucleoMag NGS Clean-up and Size Select (MACHEREY-NAGEL, Germany). The purified products were then pooled to create a sequencing library. Sequencing was performed on an Illumina MiSeq platform (Illumina, Inc., CA, USA) with the reagent kit v2, producing paired-end 250 nt reads. Sequencing was outsourced to an external laboratory (SEQme s.r.o., Czech Republic). The data were submitted to the NCBI BioProject database under the accession number SAMN47885978.

296 2.4.3 Bioinformatic processing and statistical analysis of next-generation sequencing data
297 Data processing and statistical analyses were conducted using R software (version 3.6.1; R Core
298 Team, 2020). Sequencing data from the soil microbiome were processed with the DADA2
299 v1.26.0 pipeline (Callahan et al., 2016). Taxonomic assignment for bacterial sequences was
300 performed using the SILVA 132 SSU NR 99 reference database (Quast et al., 2013), while
301 fungal sequences were classified based on the UNITE 8.3 reference database (Kõljalg et al.,
302 2019). During processing, data from 6 out of 230 bacteria and fungi sample sequencing results
303 were excluded due to their abnormal dominant taxa composition; these time points are missing
304 from the results. Additional details can be found in the supplementary materials.
305 The resulting taxa count datasets were further processed and visualized using the Phyloseq
306 v1.42.0 (McMurdie and Holmes, 2013) and MicroEco v1.6.0 (Liu et al., 2021) R packages.
307 Analysis of variance (ANOVA), diversity indexes, and principal coordinates analysis (PCoA)
308 were performed using the inbuild functions of the MicroEco R package (Liu et al., 2021)with
309 the confidence level set to $\alpha = 0.05$, unless stated otherwise. Functional traits of the microbial
310 communities were assigned using the FAPROTAX v1.2.10 and FungalTraits v0.0.3 databases
311 and their associated software tools (Cao et al., 2022), enabling functional characterization of
312 the bacterial and fungal communities in the soil.

313 2.5 Thermogravimetric analysis
314 2.5.1 Experimental arrangement and conditions
315 Thermogravimetry (TG) was employed to investigate changes in SOM within the soil in
316 incubations with polymers during biodegradation. Separate incubations were established
317 explicitly for TG analysis because of the larger amount of soil necessary for the method. For
318 the experiment, 40 g of soil was incubated in individual containers and mixed with 200 mg of
319 tested polyester films (0.5 × 0.5 cm, 100 µm thick) and 15 ml of sterilized potable water. All
320 incubations were maintained at 25°C and regularly aerated. The water content was periodically
321 monitored and adjusted to maintain stable moisture levels. Soil samples were collected on days
322 6, 20, 80, and 300, corresponding approximately to 40, 120, and 350 days of SEM and
323 metagenomic analysis. The control on day 6 represented a condition with an established contact
324 between plastic and soil.
325 After sampling, visible polymer fragments were removed using tweezers, while a small portion
326 of non-visible polyester fragments remained in the soil and were analyzed together with the soil
327 samples. The soil from each incubation was then homogenized using a mortar and pestle, and
328 3 g of homogenized soil was taken from each incubation for TG analysis. Prior to the analyses,

soils were air dried at laboratory temperature and equilibrated in a sealed container at a relative humidity of 43±2%, controlled by a saturated solution of K_2CO_3, at a temperature of 20±2°C for a week (Kučerík et al., 2020). The analyses were performed using the thermogravimetric analyzer TGA 550 (TA Instruments, New Castle, Delaware, USA). Approximately 200 mg of homogenized sample was placed into an alumina pan and inserted into the autosampler of the analyzer, which maintained a humidity similar to that of the desiccator. The reactive atmosphere (the air supplied to the oven) was also enriched to a humidity of 43±2% to ensure consistent conditions during the measurement (Kučerík et al., 2020). The temperature program set a heating rate of 5°C per minute from laboratory temperature to 650°C under a dynamic air atmosphere (90 mL min^{-1}). The soils were analyzed in triplicate.

2.5.2 Evaluation of thermogravimetric results

TG categorizes soils into several zones based on the temperature range in which they are thermally oxidized. Initially, moisture evaporation occurs between 30°C and 200°C (Tokarski et al., 2020). Above 200°C, TG provides information about the quality of SOM and its content. In particular, the labile fraction of SOM is reflected as a thermal mass loss between 200 and 300°C, stabilized fractions (such as aggregates) are reflected by thermal mass loss from 300 to 450°C and the stable organic clay complexes degrade at the temperatures from 450 to 600°C (Kučerík et al., 2018; Tokarski et al., 2020). Accordingly, total SOM content corresponds to the thermal mass loss from 200–600°C.

3. Results

3.1 Biodegradation of polymers and their surface biodeterioration

The biodegradation experiment was performed to provide the time frame for the subsequent investigation of the microbial plastisphere development and dynamics. Moreover, the experiment was done under both dynamic conditions, including mixing and optimal aeration of the reactor, to observe a maximum rate of mineralization, and also under static conditions, which were more realistic and closer to the experiments established for the plastisphere sampling.

The results of biodegradation experiments represented by carbon mineralization curves revealed noticeable differences in biodegradation rates among the polymers (Figure 2). Cellulose (positive control) reached nearly complete carbon mineralization with a final value

of almost 90%. PHB showed high biodegradation rates, achieving also approximately 90% in a dynamic experiment and 70% in a static experiment. PBSA showed even more substantial mineralization, with dynamic conditions reaching over 90% mineralization by 240 days and static conditions reaching similar values after 350 days. PBAT exhibited limited degradation, in both dynamic and static conditions, achieving about 30% mineralization by the end of the experiments. Under dynamic conditions, carbon mineralization of all polymers proceeded faster, with the difference bigger for fast-degrading polymers PHB and PBSA.

Figure 2. Carbon mineralization in soil over time within the static (closed circles) and agitated (open circles) incubation. Error bars represent variability between replicates as twice the standard deviation.

Surface deterioration and microbial colonization on the polymer films were observed using SEM (Figure 3) in separate incubations under static conditions. Small cracks appeared after 40 days of incubation in all three polymers. While no clear microscopic evidence of microbial growth was detected on PBSA and PBAT at this stage (Figure 3g, 3l), some bacterial cells were observed on the PHB surface (Figure 3b). After 120 days, fungal filaments and bacterial cells became visible on the exposed spherulites of the PHB film (indicated by arrowheads, Figure 3c), while microbial colonization on PBSA and PBAT remained limited (Figure 3h, 3m).
Significant bacterial and fungal growth accompanied by the formation of dense biofilms was only evident after 200 days on the surfaces of PHB and PBSA (Figure 3d, 3i), with fungal growth being particularly prominent. These observations align with the mineralization trends of PHB and PBSA (Figure 2). The SEM analysis confirms a gradual transition towards degradation of these polymers with extensive surface deterioration (Figure 3d, 3i) in accordance with the biological degradation process, which, for the same period, shows carbon mineralization in static incubation of around 40–50%. Fungi filaments were also observed on PBAT, though with limited biodeterioration (Figure 3n), as confirmed by mineralization trends (Figure 2).
After 350 days of incubation, the microbial consortium on the surfaces of PBSA and PHB films appeared to shift predominantly towards bacterial species, including Actinobacteria colonies (Figure 3e, 3j), and the surfaces exhibited significant structural disruption. In contrast, the PBAT sample developed deep cracks, with fungal filaments following their pattern (Figure 3o). These results also correspond to polymer biodegradation represented by carbon mineralization (Figure 2).

Figure 3. Development of PHB, PBSA and PBAT surface morphology at various stages of static incubation in soil during 350 days (a-o) visualized through scanning electron microscopy (SEM). Magnification: 3000x. The scale corresponds to 20 µm. Spherulites (Sph), bacterial cells (Bac), fungal hyphae (Hyp), and actinomycete filaments (Act) are pointed out by yellow arrows and indicated with the abbreviations in the brackets.

3.2 Microbial community composition responses to the polymers

The results of the microbial community response to the polymers describe its development in the range from the polymer surface (layer A) to a distance of 7.5 mm over 350 days. The aim was to reconstruct the plastisphere development in terms of extent and time frame.

3.2.1 Development of plastisphere around PHB

Alpha diversity of the bacterial community in the PHB A layer was significantly lower ($\alpha = 0.05$) compared to the other layers (Figure S1). Shannon and Chao1 indexes recorded an overall lower number of taxa, including rare taxa, respectively. Simpson index indicated that PHB biodegradation promoted the dominance of certain individual taxa. The influence of PHB was, to a lesser extent, reflected in the B layer. In contrast, alpha diversity in the C layer remained unaffected. For the fungal community, alpha diversity indexes affected by large data variance (Figure S5) did not show significant differences in the diversity between layers. However, they suggested a gradual decrease in taxa richness from the D to the A layer.

Marked shifts in bacterial community composition were observed around PHB (Figure 4a, S2, S3, S4). Layers A and B exhibited distinct temporal changes, indicating a dynamic succession of bacterial communities, whereas layers C and D remained relatively stable throughout the experiment. This pattern is further illustrated in the PCoA plot (Figure 4b), where layers C and D cluster closely together, while layer A is clearly distinct, with layer B positioned between A and C/D. The findings suggest that the polymer's influence on bacterial communities diminishes rapidly with distance but profoundly impacts its immediate surroundings. The effect of PHB on fungal community composition was also significant ($\alpha = 0.05$) in layers A and B (Figure 4c, S6, S7). Still, it was not as pronounced as in the case of bacteria, as also well documented by PCoA (Figure 4d).

Selected taxa showing significant differences in their Ras ($\alpha = 0.05$) as a function of their distance from the polymer surface were identified (Figure S3, S7), and their temporal dynamics in each distance interval were further analyzed (Figure 4a, c).

Figure 4. Dynamics of relative abundance (RA) of selected taxa in the distance intervals (A to D) from the PHB polymer surface over time (a, c), and principal coordinates analysis (PCoA) comparing the overall microbial community composition across these intervals (b, d). The data represent bacterial and fungal communities during PHB biodegradation in soil. The presented taxa were selected based on their significantly different RA between the layers (Figure S3, S7; $\alpha = 0.05$). Statistical details of the PCoA are presented in Table S1 of the Supplementary data file.

Among bacteria, PHB surface represented by layer A was colonized mainly by *Oxalobacteraceae*, *Comamonadaceae*, and *Rhodocyclaceae* (Figure S2, S3). The rapid growth of these groups on the PHB surface probably allowed their expansion into layer B. These families exhibited high dynamics in their RAs within the first 120 days of the experiment (Figure 4a), during which they usually reached their highest values. *Comamonadaceae* were the first colonizers, showing the fastest initial growth on the PHB surface. However, around day 40, a notable fluctuation occurred when the RAs of these three dominant taxa suddenly declined, only to rebound shortly thereafter. During this period, anaerobic taxa such as *Sporomusaceae* (Figure 4a) and *Clostridiaceae* (Figure S4) appeared briefly, suggesting a time and space-localized reduction in oxygen availability. After this anaerobic episode, *Xanthomonadaceae* RA peaked (Figure S4).
In contrast, the RAs of *Nitrososphaeraceae*, *Nitrosomonadaceae*, and *Nitrospiraceae* were suppressed by the presence of PHB (Figure S2, S3) on the polymer surface (layer A), and the influence of PHB was negatively reflected in the case of *Nitrososphaeraceae* and *Nitrosomonadaceae* also in layer B (Figure S2, S3). Furthermore, *Nitrososphaeraceae* were declining with time and the progressing biodegradation (Figure S2).

In the fungal community, *Nectriaceae* dominated the PHB surface (Figure S6, S7), initially present at around 5% RA but peaking at 70% by day 120 before declining to near initial levels by the experiment's end (Figure 4c). Other fungal families were only marginally affected by the presence of PHB (Figure S6); for example, *Mrakiaceae* showed limited colonization of the PHB surface, and *Aspergillaceae* was detected as dominant at day 200 in the A layer only. However, groups such as *Pyronemataceae* and *Clavicipitaceae* (Figure 4c) exhibited sharp transient PHB-related increases, with *Pyronemataceae* peaking around day 40 and *Clavicipitaceae* around day 20, likely before being outcompeted by *Nectriaceae*.

3.2.2 Development of plastisphere around PBSA

The microbial community's response to PBSA was similar to that of PHB in many aspects. The alpha diversity shifts in the bacterial community in PBSA presence (Figure S8) were even more restricted to the A layer; however, total community richness expressed by the Chao1 index was the same in all layers. In the fungal community (Figure S11), the alpha diversity Shannon index showed the presence of a small group of dominant taxa close to the polymer surface.

Like PHB, PBSA also influenced bacterial community composition in layers A and B as these followed a distinct spatial pattern in PCoA (Figure 5b), with clearly separated A-layer communities from those in layers C and D, with layer B forming a transition zone. PCoA of the fungal community (Figure 5d) showed a similar result with less separation between A and more distant layers.

The biodegradation of PBSA in soil was accompanied by specific shifts in microbial community composition, despite some general similarities with PHB. In the PBSA environment, *Comamonadaceae* showed strong enrichment in layer A during the active degradation phase (Figure 5a, S9, S10) and also expanded in layer B (Figure S3, S10). However, other microbial features diverged. Notably, *Oxalobacteriaceae*, dominant in PHB degradation, were only sparsely present here. In contrast, *Xanthobacteraceae*, which were suppressed during PHB degradation (Figure S2), played an important role in PBSA decomposition (Figure S7, S8). Additionally, the transient oxygen depletion indicated in PHB (through the temporary rise of anaerobic taxa) was not apparent in PBSA, suggesting different oxygen dynamics near the polymer surface. It should be acknowledged that the described oxygen depletion was proposed based solely on taxonomic evidence, without additional independent evidence.

At the beginning of PBSA degradation, there was also a sharp decline in the relative abundance of previously dominant bacterial taxa in layer A (Figure S10), indicating a disruption of the existing community. This shift likely altered local niche conditions and allowed *Xanthobacteraceae* to rise in subsequent stage (Figure 5a). A similar trend was observed for microbial taxa involved in nitrogen cycling. Specifically, *Nitrososphaeraceae* and *Nitrosomonadaceae* were initially suppressed in layers A and B during the first 20 days of PBSA incubation (Figure 5a), then gradually recovered in layer B but remained low in layer A. Another marked distinction was that PHB degradation involved several taxa with comparable relative abundances (each ~15%, Figure S2), while PBSA degradation was dominated by a single group reaching ~25% RA (Figure S9). These findings suggest that although the overall

biodegradation trajectories of PBSA and PHB were similar, distinct microbial taxa were likely responsible for their breakdown.

Together, these results show that although the overall biodegradation pattern of PBSA resembled that of PHB, distinct microbial responses and succession patterns developed in the plastisphere surrounding PBSA.

The fungal community exhibited a distinct response to PBSA degradation compared to PHB. Alpha diversity analysis showed that the Shannon index in layer A (at the polymer surface) was significantly lower ($\alpha = 0.05$) than in the surrounding soil layers, indicating a dominance of a few taxa during active biodegradation (Figure S11). Members of the family *Nectriaceae* were strongly enriched during the initial phase of PBSA degradation, suggesting their early involvement in polymer colonization and breakdown (Figure 5b). Their relative abundance, however, declined rapidly after approximately 20 days, coinciding with a shift in community composition. Around day 80, *Herpotrichiellaceae* became the dominant fungal group. Later, between days 120 and 200, *Bionectriaceae*, *Neopyrenochaetaceae*, and *Aspergillaceae* successively increased in abundance, indicating a dynamic succession pattern of fungal taxa during ongoing polymer degradation (Figure 4, S10). By day 350, the fungal community in layer A showed a shift back toward its initial composition (Figure S10), indicating a partial recovery of the original community structure following the main degradation phase.

Figure 5. Dynamics of relative abundance (RA) of selected taxa in the distance intervals (A to D) from the PBSA polymer surface over time (a, c), and principal coordinates analysis (PCoA) comparing the overall microbial community composition across these intervals (b, d). The data represent bacterial and fungal communities during PBSA biodegradation in soil. The presented taxa were selected based on their significantly different RA between the layers (Figures S10, S13; $\alpha = 0.05$). Statistical details of the PCoA are presented in Table S1 of the Supplementary data file.

3.2.3 Development of plastisphere around PBAT

For PBAT environment, alpha diversity variations in the bacterial community (Figure S14) were similar to those observed for PBSA. The evenness of the community was distorted by the dominance of a few taxa in layer A, as reflected by the significant decreases in the Shannon and Simpson indexes ($\alpha = 0.05$). However, the fungal community's alpha diversity (Figure S17) exhibited a distinct pattern different from PHB and PBSA. A significant decline ($\alpha = 0.05$) in

all three indexes was observed in the A layer, suggesting that fungi played a crucial role in PBAT biodegradation.

Compared to previous polymers, the effect of PBAT on the microbiome was narrowly concentrated in layer A, as evident from the PCoA results for both bacterial and fungal communities (Figure 6b, d).

The composition of the bacterial community and the course of its development were similar to those in the case of PBSA. In the PBAT-affected environment, *Comamonadaceae* dominated the A layer and maintained a consistent presence throughout the experimental period (Figure 6a, S15). This suggests that *Comamonadaceae* played a key role in PBAT degradation. This group also had a similar importance in PBSA and PHB degradation, however, here, its high RA was strictly limited to layer A, which was also reflected in the PCoA (Figure 6b). *Xanthobacteraceae* were present from the beginning, peaking around day 40 (Figure 6a, S15), similar to the environment affected by PBSA, only about 40 days earlier. Another successful taxon that was able to colonize the PBAT surface was *Burkholderiaceae* (Figure S15), whose RAs, after the initial boost, decreased consistently until day 120, after which they began to grow again (Figure 6a).

Also, in this case, *Nitrososphaeraceae*, *Nitrosomonadaceae, and Nitrospiraceae* were significantly suppressed in layer A (Figure S16, $\alpha = 0.05$). However, *Nitrososphaeraceae* were temporarily enriched in layers C and D around day 40 (Figure 6a), likely due to ammonia-N mobilization driven by high metabolic microbial activity near PBAT indicated by the peak RAs in PBAT-degrading groups *Comamonadaceae* and *Xanthobacteraceae* (Figure 6a), and utilization of the ammonia-N in layers C and D.

Among fungi, *Nectriaceae* showed a distinct PBAT-related peak around day 12 (Figure 6c, S18), similar to its pattern in PBSA (Figure 5c, S12), with *Bionectriaceae* and *Neopyrenochaetaceae* becoming prominent in later stages (Figure 6c, S18), mirroring PBSA degradation dynamics. Conversely, common fungi groups of B, C, and D layers, such as *Mrakiaceae* and *Mortierellaceae,* were unsuccessful in colonizing PBAT (Figure S18, S19).

Fungal community changes in the presence of PBAT were primarily confined to layer A (Figure S18, S19). However, *Nectriaceae* results indicate possible gradual expansion into more distant layers over time (Figure 6c), indicating a progressive outward migration of fungal activity as the experiment progressed. Additionally, the results for *Pleosporaceae* (Figure 6c) suggest that a transient boom of this group in layers A and B between days 40 and 80 was followed by its penetration further from the PBAT within layer C until day 120.

Figure 6. Dynamics of relative abundance (RA) of selected taxa in the distance intervals (A to D) from the PBAT polymer surface over time (a, c), and principal coordinates analysis (PCoA) comparing the overall microbial community composition across these intervals (b, d). The data represent bacterial and fungal communities during PBAT biodegradation in soil. The presented taxa were selected based on their significantly different RA between the layers (Figure S16. S19; $\alpha = 0.05$). Statistical details of the PCoA are presented in Table S1 of the Supplementary data file.

3.2.4 Comparison of microbial communities among polymers

The results indicate that distinct microbial communities are responsible for the biodegradation of each polymer despite many similarities. To further investigate these differences, we conducted a taxonomic and functional analysis of the microbiome, comparing microbial communities and their functional traits in the A-D layers (Figure 7).

According to the bacterial community PCoA (Figure 7a), PHB-related communities in A layer were distinct from those associated with PBSA and PBAT, which clustered more closely together. This suggests that bacterial communities participating in PHB biodegradation are more material-specific, differing significantly from those on the synthetic polyesters PBSA and PBAT ($p.adjusted_{PHBvsPBSA} = 0.015$; $p.adjusted_{PHBvsPBAT} = 0.015$). In contrast, fungal communities exhibited weaker separation (Figure 7c), with communities from all three materials clustering more closely, indicating lower material-specificity among fungi in layer A.

To assess the energy metabolism and ecological strategies of bacterial and fungal communities in the A layers, representing the community most influenced by the polymer and the biodegradation process, and the D layers, the most distant from the polymer surface and serving here as a natural state, we utilized the Faprotax and FungalTraits metabolic databases (Figure 7b,d). The bacterial community in both layers (Figure 7b) was predominantly composed of chemoheterotrophs, with a slightly greater tendency towards aerobic chemoheterotrophy in the A layer.

Notably, nitrogen utilization pathways differed between the A and D layers. In the A layer, nitrification and aerobic ammonia oxidation were reduced, likely due to the availability of simpler carbon substrates, reduced oxygen levels, and potentially limited nitrogen sources resulting from extensive nitrogen fixation in the rapidly growing biomass. Pathways associated with rapid biomass growth, such as assimilative nitrate reduction, ureolysis, and nitrogen

fixation, were enhanced. This suggests that nitrogen was a limiting factor for microbial metabolism during intensive polymer biodegradation, resulting in higher carbon availability. These nitrogen-related functional shifts were observed across all materials, including both fast-degrading polymers (PHB, PBSA) and more slowly degrading PBAT. Even the relatively lower influx of organic carbon from PBAT degradation appeared sufficient to drive these effects.
In the fungal community, the D layer contained a higher proportion of saprotrophs, while the A layer exhibited an increased presence of parasitic and pathogenic fungi (e.g., fungal parasites and pathotrophs). This shift may reflect the accumulation of bacterial and fungal biomass in layer A, creating an environment that favors parasitic and pathogenic lifestyles due to the increased availability of host organisms.

Figure 7. Taxonomic (a, c) and functional (b, d) analysis of the plastisphere microbiome. (a, c) PCoA based on taxonomic profiles comparing the A layers of all tested polymers. (b, d) Functional comparison of the A and D layers for each polymer. Statistical details of the PCoA are presented in Table S1 of the Supplementary data file.

3.3 Dynamics of SOM

Results from TG records (Figure S20) are presented in Figure 8. Firstly, comparing mass losses in the 200–300°C range (labile organic matter) shows a similar trend for all the polymers (Figure 8a). Specifically, there was a slight increase in mass losses after 20 days, followed by a decrease after 80 and 300 days. The most significant decrease was observed for PHB, while PBSA and PBAT exhibited a similar pattern.

Figure 8. Content of soil organic matter fractions in the close vicinity of a plastic and their development in time, labile fraction (a), stabilized fraction (b), stable fraction (c), and total organic matter (d).

On the contrary, the plastics displayed distinct behavior in the temperature range of 300–450°C (Figure 8b). In particular, PHB showed a steady decrease throughout the entire time interval, while PBSA began to decrease between 20 and 80 days. PBAT showed no relevant change in this temperature range. Similarly, in the 450–600°C range (Figure 8c), a relevant decrease was observed only for PHB, with the most pronounced decrease occurring between days 20 and 80.

The total SOM content reflected by mass loss between 200–600°C did not change for PBAT (Figure 8d). Conversely, for PHB and PBSA, the pattern was similar, with a decrease observed across the entire time interval, most notably after the 20th day.

4. Discussion

4.1 Rate of biodegradation and polymer properties

The comparative analysis revealed clear differences in biodegradability among the three polymers: PBSA showed the fastest mineralization, followed closely by PHB, and PBAT degraded markedly more slowly. This pattern aligns with known structural drivers. Aliphatic polymers are generally more susceptible to enzymatic hydrolysis than polymers containing aromatic moieties; the terephthalate units in PBAT likely hinder enzymatic access and slow depolymerization due to steric and electronic effects (Marten et al., 2005). Contrary to common expectations, crystallinity appeared to be a secondary influence in our experiment. Highly crystalline PHB (66.2%) degraded rapidly. In comparison, low-crystallinity PBAT (7.2%) degraded slowly, indicating that chemical composition (notably the presence of aromatic domains) can outweigh crystalline fraction as a control on biodegradability (Herzog et al., 2006). SEM micrographs supported these conclusions, showing pronounced surface erosion and biofilm formation on PHB and PBSA, but much slower deterioration of PBAT (Figure 3) (Stloukal et al., 2012; Purahong et al., 2021), consistent with earlier reports of accelerated PBSA surface erosion under soil burial conditions.

Dynamic incubation further emphasized these differences. Aeration significantly enhanced the mineralization of PHB and PBSA but only marginally affected PBAT. The stronger stimulation of PHB degradation under dynamic conditions may reflect the ecology of PHB-synthesizing and -degrading bacteria, which are widespread in soils (Wang and Bakken, 1998; Volova et al., 2017). Their dual role, as both producers and consumers of PHB, could provide an ecological advantage under aerated conditions where rapid turnover of storage polymers is feasible. Oxygen depletion during fast PHB mineralization has also been observed in soils (Zhou et al., 2021b; Brown et al., 2023; Trojan et al., 2024).

4.2. Microbial community structure and dynamics

4.2.1. General patterns

Biodegradable polymers supported thicker plastispheres and higher microbial biomass than non-biodegradable counterparts, consistent with earlier studies (Mercier et al., 2017; Bandopadhyay et al., 2020; Odobel et al., 2021; Wicaksono et al., 2022). However, diversity consistently declined at the polymer–soil interface (layer A), with Shannon and Simpson indices indicating strong dominance of a few fast-growing copiotrophs. This pattern likely reflects competitive exclusion rather than stress-induced suppression: organisms capable of exploiting polymer-derived carbon rapidly proliferated, displacing oligotrophs less adapted to carbon-rich conditions (Hibbing et al., 2010). Similar reductions in plastisphere diversity relative to bulk soil have been documented in multiple environments (Sun et al., 2022).

The enrichment of copiotrophic taxa suggests that BPs serve as selective hotspots for microbial growth. Yet, the concomitant reduction of nitrifiers (*Nitrosomonadaceae*, *Nitrososphaeraceae*, *Nitrospiraceae*) points to altered nitrogen cycling near the polymer surface (Figure S2, S3, 5a, S16, 6a). This agrees with evidence that rapid polymer degradation can generate oxygen- and nutrient-limited microenvironments (Brown et al., 2023). Interestingly, no distinction was observed between fast-biodegrading polymers (PHB, PBSA) and slower-degrading PBAT; even the lower carbon release rate from PBAT biodegradation was sufficient to drive these effects. Several studies report that PBAT and other biodegradable polymers alter soil N partitioning and microbial N-processing (reduced nitrifier abundance/activity and elevated denitrification or N-fixation), consistent with the idea that even modest organic carbon input from slowly degrading polymers is sufficient to stimulate heterotrophic growth and shift N-acquisition pathways (Han et al., 2021; Zhang et al., 2024).

4.2.2. Bacterial communities

Distinct successional trajectories were observed for each polymer. PHB (Figure 4a, S2, S3), and PBSA (Figure 5a, S9, S10) shared enrichment of *Comamonadaceae* during active degradation, whereas PHB uniquely supported *Oxalobacteriaceae*, a group linked to SOM turnover. PBSA instead favored *Xanthobacteraceae*, previously associated with polyester and PAH degradation (Sun et al., 2022)(Sun et al., 2022)(Sun et al., 2022). PBAT (Figure 6a, S15) promoted *Comamonadaceae* and *Xanthomonadaceae*, i.e., taxa which were also reported in hydrocarbon degradation (Bodor et al., 2024).

Transient oxygen depletion was evident during PHB degradation, indicated by temporary proliferation of anaerobic taxa (*Clostridiaceae, Sporomusaceae*, Figure 4a, S4), followed by

pathogenic copiotrophs (*Xanthomonadaceae*; Bayer-Santos et al., 2019). In contrast, PBSA biodegradation, though rapid, did not show similar hypoxic episodes, which suggests subtle differences in polymer–microbe–oxygen interactions. It appears that fungi played a more significant role in PHB biodegradation than in the case of PBSA. Their greater metabolic capacity and a larger spatial footprint potentially caused the anerobic species to be transiently detected in the PHB plastisphere.

Burkholderiaceae, known for nitrogen fixation (Bartholomäus et al., 2024), were enriched particularly on PBAT surfaces. Their prevalence highlights that polymer degradation may interact with nitrogen acquisition pathways, with implications for soil fertility.

4.2.3. Fungal communities

Fungal assemblages exhibited clear successional shifts. Early colonizers (*Nectriaceae*, *Clavicipitaceae*) were gradually replaced by *Herpotrichiellaceae*, *Aspergillaceae*, and *Bionectriaceae*, consistent with niche partitioning driven by resource fluxes. This temporal sequence resembles classical decomposition dynamics in natural substrates such as leaf litter (Voriskova and Baldrian, 2013).

Such dynamics support the view that in plastisphere consortia, not all members degrade plastics but interact through a food web (Kirstein et al., 2019)). This stage-specific hierarchy likely arises from metabolic complementarity and competitive exclusion. For example, early-dominant taxa such as *Nectriaceae* or *Clavicipitaceae* were gradually replaced by *Herpotrichiellaceae*, *Aspergillaceae*, and *Bionectriaceae*, suggesting a temporal resource-driven niche partitioning. These successional shifts likely reflect both metabolic complementarity, where late colonizers exploit by-products of early degraders, and competitive exclusion in resource-rich plastisphere environments. This supports the existence of a dynamic, stage-specific colonization hierarchy.

Unlike bacteria, fungal communities showed less polymer specificity, with taxa clustering more closely across PHB, PBSA, and PBAT. This suggests fungi act as secondary colonizers, not only degrading PHB, but also recycling bacterial necromass and by-products (Mehmood et al., 2022). In addition, the frequent detection of pathotrophs in A layers supports recent concerns that the plastisphere may act as a niche for potential fungal pathogens (Sathicq et al., 2021; Trojan et al., 2024).

Such shifts in bacterial and fungal assemblages suggest that the type of dominant degraders may directly influence how polymer biodegradation feeds back on SOM dynamics.

715

716 4.3 Spatial extent of the plastisphere

717 Our results indicate that bacterial communities were most strongly altered in the A and B
718 layers, corresponding to a plastisphere thickness of ~1.25 mm, whereas fungal communities
719 extended up to the C layer (~2.75 mm). This broader fungal influence may result from hyphal
720 networks that connect the plastisphere with bulk soil, contrasting with the localized bacterial
721 response (Frey et al., 2003). Such a specification of the plastisphere extent placed at the exact
722 center of the given layer is somewhat arbitrary and is guided by the resolution of this study.
723 Soil is a naturally granular matrix where the micro sampling is limited by the grain or soil
724 aggregate size. In this experiment, the soil was sieved through a 2-millimeter screen, which
725 shows that the grain size was close to the employed resolution. Still, we believe that a better
726 spatial resolution can be achieved with a modified sampling method, which is currently under
727 development.

728 The volume fraction of soil occupied by plastisphere can be calculated by assuming a few
729 simplifications (Figure 9). We assume that a 50 µm thick plastic film fragmented into square
730 microparticles with side lengths of 5.0, 1.0, and 0.2 mm, evenly distributed in the soil. The
731 modeling suggested that fragmentation into smaller microparticles greatly expands the
732 proportion of soil volume influenced by plastisphere processes. Even at low concentrations,
733 micro-sized PB fragments could affect large soil fractions, particularly for fungi. While this
734 scenario may overestimate field conditions and is highly sensitive to real-world factors, like
735 soil heterogeneity, uneven fragment-size distribution, or fragment aggregation, it underscores
736 the ecological importance of particle size: smaller fragments create disproportionately larger
737 interactive surfaces, and may accelerate carbon turnover, but also increase transient ecological
738 impacts (Wang and Chen, 2023). In the case of the real-life highly uneven distribution of plastic
739 particles, higher microplastic soil concentrations can be tolerated.

740 Such mm-scale plastisphere zones are in line with recent conceptual and experimental studies
741 describing sharp gradients in microbial activity around plastics (Li et al., 2023). Furthermore,
742 the different spatial footprints of bacterial and fungal plastispheres imply that the extent of SOM
743 alteration may also depend on how far degradation-driven processes are spread into the bulk
744 soil.

745

746 Figure 9. Modeling the fraction of the plastisphere volume from the total soil volume for
747 different sizes of square-shaped microplastics from a 50 µm thick film for bacteria and fungi
748 communities. The model assumes a hypothetical 50 µm thick film fragmented into square

microparticles with side lengths of 5.0, 1.0, and 0.2 mm, evenly distributed in the soil (X-axis). The corresponding plastisphere volume fractions were then calculated (Y-axes).

4.4 The influence of BPs on SOM dynamics

Building on the differences in degradation rates (section 4.1), the associated microbial successions (section 4.2), and the spatial reach of plastisphere processes (section 4.3), we next address how these factors jointly shape soil organic matter (SOM) dynamics. In fact, the interaction between biodegradation mode and microbial community structure emerged as a key driver of priming effects on SOM. While designing the experiment for SOM analysis, we chose arelatively high initial polymer concentration with the expectation that the whole or a substantial volume of the soil would be a part of the plastisphere and would be affected. Later, this assumption was confirmed by the metagenomic experiment (Fig. 9).

The influence of the plastisphere on SOM remains ambiguous, with both positive and negative priming effects possible. Positive priming, i.e., co-mineralization of native SOM, may occur when polymer degradation products stimulate microbial co-metabolism, thereby accelerating carbon loss (Wang et al., 2022b). This was observed in this work as total SOM content (200–600°C, Figure 8D) decreased under PHB and PBSA, but remained unchanged under PBAT.

TG data showed that a pronounced SOM reduction occurred between days 20 and 80, coinciding with the proliferation of fast-growing bacterial families such as *Comamonadaceae*, *Oxalobacteraceae*, and *Xanthobacteraceae* (Figures 4–6). These copiotrophic taxa, known for their rapid exploitation of labile carbon and secretion of depolymerizing enzymes, likely drove the observed bacterial-driven co-mineralization of native SOM. Thus, the timing of the microbial "boom phase" closely matched the period of steepest SOM decline, linking microbial succession directly to carbon loss. In addition, this microbial bloom was accompanied by a decline in nitrifying taxa (*Nitrososphaeraceae*, *Nitrosomonadaceae*, *Nitrospiraceae*) and a shift toward N-mining behavior, which is a well-known microbial response to C enrichment and N limitation. Under such conditions, heterotrophic bacteria produce N-hydrolases (proteases, chitinases, ureases) to access N bound in organic molecules (Geisseler et al., 2010). In our study, the rise of *Comamonadaceae*, *Rhodocyclaceae*, and *Xanthobacteraceae*, taxa often annotated to carry protease or urease genes, suggests a potential for N-mining under C-enrichment. In particular, *Comamonadaceae* strongly supports the soil C and N mineralization. (Wu et al., 2021).

In fact, *Variovorax paradoxus* (*Comamonadaceae*) can secrete extracellular proteases, and several genera in this family (e.g., *Delftia*) show urease activity. In addition, *Nectriaceae,* a

fast-growing and diverse fungal family that proliferated extensively in the PHB plastisphere, belongs among active saprotrophs capable of degrading biomass residues (Habtewold et al., 2020; Rissi et al., 2024). Many taxa within this family are capable of producing cellulases, glucosidases, and chitinases, triggering a positive priming effect (Brandt et al., 2018).

On the contrary, in *Rhodocyclaceae*, diazotrophic *Azoarcus* encodes urease genes and fixes nitrogen, indicating potential for urea utilization and N buffering; however, direct demonstrations of extracellular protease secretion in this family under soil conditions are scarce. In *Xanthobacteraceae* (Oren, 2014), *Xanthobacter autotrophicus* carries a complete ureABC operon and is capable of dissimilatory nitrate/nitrite reduction. Simultaneously, many *Burkholderia* (*Burkholderiaceae* and *Xanthobacteraceae)* species are known as diazotrophs and denitrifiers (Estrada-De Los Santos et al., 2001; Oren, 2014), which may help buffer transient N deficits in C-rich environments.

In addition, microplastics with higher biodegradability were shown to exert stronger impacts on soil microbiota (Rüthi et al., 2020) and a positive priming effect (Zhang et al., 2023), which aligns with our data supporting the view that faster-degrading polymers (PHB, PBSA) promoted positive priming effects, whereas PBAT has a negligible impact.

The link between polymer biodegradability and SOM was also evident from mass loss patterns of individual SOM fractions. Changes in these fractions (200–300°C range, Figure 8A) revealed that PHB and PBSA initially increased the labile SOM pool, most likely due to the release of monomers and microbial biomass turnover (Palucha et al., 2024b). This was followed by a decline, indicating co-mineralization of native SOM. This trend was most pronounced for PHB, consistent with the dominance of fast-growing bacteria and nutrient immobilization within the plastisphere (Rillig et al., 2024). In contrast, PBSA and especially PBAT showed a less steep decline, suggesting the stabilization of SOM fractions due to a greater fungal contribution linked with their capacity to translocate nutrients (Frey et al., 2003). Modeling results from chapter 4.3 further support this interpretation, showing a more extended outreach of the fungal community in the plastisphere compared to bacteria.

This explanation is supported by the chemical composition of hyphae, mainly chitin, degrading between 300–450°C (Dassanayake et al., 2018). In this temperature range, TG analysis showed no statistically significant change for PBAT (Figure 8B), primarily due to the slow fungal degradation mechanism. PBSA exhibited a smaller reduction, with the largest loss observed in PHB. Additionally, the 300–450°C range in TG records reflects the formation of aggregates, with fungi significantly contributing to macroaggregate formation (Dassanayake et al., 2018).

The positive priming effect is generally associated with enhanced microbial growth, increased enzyme activity, and shifts in community structure (Guliyev et al., 2023). While some studies attribute stronger priming to bacteria (Deng et al., 2021) and others to fungi (Xiao et al., 2022), its magnitude appears substrate-dependent, with labile compounds favoring bacterial-driven priming and more complex substrates favoring fungi (Chao et al., 2019).

Results presented here confirm this pattern, as PBAT, which degraded slowly and mainly by fungi, did not induce a priming effect. In contrast, the faster, bacteria-driven degradation of PBSA and PHB enhanced SOM loss (Figures 2, 3). This aligns with reports of PHB reducing total SOM(Palucha et al., 2024b) and PBSA causing C loss through priming modulated by N availability (Guliyev et al., 2023). In contrast, PBAT has been linked to nutrient limitation and shifts in dissolved organic matter chemistry (Chen et al., 2024). However, its slow degradation favors the negative priming effect, i.e., carbon stabilization and sequestration (Liu et al., 2025). The continuation of SOM depletion observed after 300 days had slowed, suggesting a "legacy effect" of microbial activation. During the earlier degradation phase (days 20–80), induction of N-mining and extracellular enzyme production likely triggered sustained decomposition of native SOM. After this phase, the metabolically active microbial consortia, dominated by *Actinobacteria* and filamentous fungi such as *Bionectriaceae* and *Aspergillaceae*, continued to utilize SOM as an alternative carbon and nitrogen source. This self-perpetuating microbial demand, stimulated by polymer inputs, explains the persistence of positive priming at the end of the incubation. Similar legacy effects have been observed following pulses of labile carbon input in soils, where residual enzymatic activity and altered stoichiometric demands maintain enhanced SOM turnover. These findings reveal a clear temporal coupling between microbial succession and SOM dynamics in which rapid bacterial proliferation during PHB and PBSA degradation drives SOM depletion through enhanced enzyme activity and N mining, whereas the gradual, fungus-dominated degradation of PBAT promotes SOM stability. This implies that the direction and magnitude of the priming effect (and consequently the long-term ecological footprint of biodegradable plastics) depend on the interplay between polymer chemistry, microbial guild composition, and the timing of microbial succession.

4. Conclusions

This study demonstrates that biodegradable polyesters (PHB, PBSA, PBAT) generate distinct, spatially structured plastisphere communities and measurably alter the composition of proximate soil organic matter. Bacterial assemblages were tightly constrained to the immediate

polymer vicinity (≈1.25 mm), whereas fungal influence extended farther (≈2.75 mm). PHB and PBSA, the more rapidly mineralized materials, produced the largest shifts in community composition and the greatest reductions in labile and stabilized SOM pools. PBAT yielded subtler changes consistent with its slower degradation. At the functional level, plastisphere communities displayed reduced representation of canonical nitrifiers and an enrichment of pathways and taxa associated with rapid biomass production, ureolysis and nitrate reduction; concurrently, parasitic/pathotrophic fungi increased in relative abundance near polymer surfaces.

Mechanistically, our results indicate that polymer chemistry and biodegradation kinetics, rather than crystallinity, are primary determinants of plastisphere composition and downstream soil effects. Fast carbon release from PHB and PBSA appears to drive local nitrogen limitation, transient oxygen depletion, and successional cascades, first by fast-growing, polymer-utilizing bacteria and subsequently by fungi that exploit accumulated necromass and degradation by-products. Bacterial responses were more material-specific (suggesting selective depolymerase-mediated processes), while fungal communities behaved more as generalist recyclers, supporting a staged, resource-driven colonization model. The differing extents of the plastisphere for fungi and bacteria can also be directly linked to their ecological roles in polymer degradation. Fungi, as broad metabolic generalists, can utilize a range of polymer breakdown products and extend their hyphae into soil microhabitats beyond the immediate polymer–soil interface. This exploratory growth allows fungal metabolic influence to disperse more widely, which corresponds to the larger plastisphere observed around the polymers. In contrast, bacteria tend to act as biochemical specialists that require close physical association with polymers to access oligomers and monomers released during degradation.

These findings suggest that biodegradable polymers can have persistent, host-dependent impacts on soil microbial ecology and carbon–nitrogen cycling that extend beyond visible polymer fragments and may produce positive priming of native SOM. Given that some alterations persisted after 350 days, ecological recovery may be slow and also depend on particle size and fragmentation.

5. Declaration of competing interest

The authors 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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