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Prior Colonization Shapes Bacterial Communities on Tire Wear Particles in Agricultural Soil

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Abstract: Tire wear particles (TWPs) are an increasingly important source of microplastic contamination in agricultural soils, yet little is known about the processes governing bacterial colonization of their surfaces. We investigated how experimental pre-colonization and soil pretreatment influence TWP-associated bacterial communities across three independent 30-day greenhouse trials using agricultural soil collected adjacent to barley plots during the growing season. Initially uncolonized TWPs and TWPs pre-colonized with *Pseudomonas congelans* were incubated in non-autoclaved and autoclaved agricultural soil. Scanning electron microscopy, 16S rRNA gene amplicon sequencing, community-overlap analyses, and measurements of soil pH and carbon-to-nitrogen (C:N) ratios were used to characterize particle colonization, bacterial communities, and bulk soil responses. Treatment effects on bulk soil chemistry were limited, with no significant differences in C:N ratios and an overall pH effect in only one trial, for which adjusted pairwise comparisons were not significant. TWPs exhibited microbial attachment and biofilm-like material and supported bacterial communities that, despite sharing substantial proportions of taxa with surrounding soil, remained compositionally distinct. The genera enriched on particles varied among trials with prior colonization treatments and soil pretreatment, suggesting that colonization history and soil context contribute to variation in TWP-associated communities. Together, these findings indicate that TWPs support distinct, context-dependent bacterial communities despite limited changes in bulk soil chemistry. Further work is needed to determine whether this community variation affects soil processes and agricultural outcomes.

Keywords: microplastics, tire wear particles, biofilm, plastisphere, agricultural soils, soil ecology, pollution

Introduction

Agricultural soils are increasingly exposed to anthropogenic contaminants, including non-point source pollutants that are particularly challenging to track and regulate, such as microplastics (MPs) (1). MPs are plastic particles < 5mm in size that are pervasive in aquatic and terrestrial environments (2–4). Tire wear particles (TWPs) are a dominant, yet understudied source of environmental MPs (5,6). TWPs are unique in that they are generated by friction between the road surface and vehicle tires during driving (7). These particles vary widely in size, with a fraction occurring in the PM_{10} size range (≤10 μm) that can become airborne and undergo atmospheric transport (8–10).

TWPs can enter soil through multiple pathways, including road and stormwater runoff, atmospheric deposition, and land application of TWP-contaminated materials, such as biosolids (10–13). Agricultural soils near roadways are likely hotspots for TWP accumulation, particularly in intensively managed cropping systems where repeated atmospheric deposition and runoff inputs may contribute to long-term particle accumulation in surface soils (14–18). As global vehicle use continues to increase and transportation incorporates heavier hybrid and electric vehicles, TWP emissions are expected to rise (9,13).

Though MPs are known to act as dynamic substrates that reshape microbial habitats and ecological interactions within soils (4,5,19–23), little is known about TWP-associated microbial communities. MPs can create unique microbial niches, often referred to as the "plastisphere", where distinct microbial communities colonize plastic-associated surfaces differently from those in the surrounding environment (24–29). MPs can also alter soil physicochemical properties, enzyme activity, carbon and nitrogen cycling, and microbial diversity, with impacts varying according to polymer type, particle size, and environmental conditions (20,22,30–34). In agricultural systems, MP contamination has been associated with changes in nutrient cycling, plant performance, and microbial community composition surrounding the rhizosphere (15,19,21,30,33,35–37). TWPs are of particular concern because they contain complex mixtures of synthetic polymers, metals, and chemical additives that may further modify microbial activity and community composition (9,11,12,38,39).

TWP-associated biofilms have been shown to facilitate the horizontal transfer of antibiotic resistance genes (ARGs), highlighting their role as potential hotspots for microbial exchange (28,40,41). More broadly, plastisphere biofilms may promote ARG enrichment and dissemination by concentrating microorganisms, extracellular DNA, and co-selective contaminants (42). In addition, TWPs also contain and can transport a complex mixture of metals and tire-derived organic chemicals, including 6PPD, potentially altering their environmental fate and interactions with microbial communities in agricultural soils (23,40,43,44). More broadly, MPs can modify metal mobility and bioavailability in plant–soil systems, with effects depending on particle properties, soil geochemistry, and rhizosphere processes (45). Additionally, if certain microbial taxa preferentially colonize

TWPs, these organisms may also provide insight into potential biodegradation or bioremediation processes associated with tire-derived materials and contaminants (46). At larger scales, understanding the ecological behavior of TWPs may help inform environmental management strategies related to road runoff, soil health, and overall MP pollution mitigation in agroecosystems.

In this study, we investigated how TWPs with and without prior microbial colonization influenced bacterial communities and chemical properties in agricultural soil. Here, "prior colonization" refers to experimental pre-colonization of TWPs with *Pseudomonas congelans* before their introduction into agricultural soil. We conducted three independent 30-day greenhouse trials using soils collected adjacent to barley production plots in March, April, and May 2025. We hypothesized that (1) TWPs would support bacterial communities distinct from those in the surrounding soil; (2) TWP addition would decrease soil pH and increase the soil C:N ratio; and (3) prior colonization by *P. congelans* would modify these microbial and soil-chemical responses relative to uncolonized TWPs. Accordingly, our objectives were to (1) compare bacterial communities associated with recovered TWPs to those in the surrounding soil; (2) determine whether TWP addition altered soil bacterial community composition, pH, and C:N ratios; and (3) assess whether these responses differed between pre-colonized and uncolonized TWPs, among monthly soil collections, and between non-autoclaved and autoclaved soils. Together, these comparisons allowed us to evaluate how prior colonization and local soil conditions shape the development of TWP-associated bacterial communities in agricultural soil.

Methods

Tire Wear Particle Preparation

TWP samples were generated by Smithers, Inc. (Akron, Ohio) using a flat-track abrasion system with a sandpaper surface. The tire brand and manufacturer were not disclosed to the authors. To restrict the particles to the microplastic size range (<5 mm), TWPs were sequentially sieved through 5-mm and 1-mm sieves, retaining particles between 1 and 5 mm. The TWPs were then autoclaved under a gravity cycle for 30 minutes at 121°C to remove any living organisms from their surface. TWPs were stored in an autoclaved bottle until experimentation.

Soil Collection and Preparation

An overview of the workflow is illustrated in **Supplemental Figure 1**. Soil was collected monthly (March 2025 - May 2025) at Virginia Tech's Kentland Farm in Blacksburg, Virginia, USA, within 1 meter from barley research plots. Between the March and April soil collections, 28 g of USN (urea, ammonium, nitrate) and 0.9 oz of harmony pesticide was applied per acre to the barley plots. For each collection, approximately 40 kg of soil was brought back to the lab in 5-gallon buckets. Here, the soil was evenly spread out in closed, 70% ethanol disinfected snorkel cabinets, using the ventilation system intermittently to help aid in drying the soil. Soil moisture was monitored using a GS3 probe by Pro Check until soil moisture readings were stabilized, indicating equilibrium with the surrounding atmosphere. Between soil-moisture measurements, the soil was manually

homogenized using gloved hands. Gloves were disinfected with 70% ethanol between samples. Once the soil was dried, it was sieved through a 2mm sieve to ensure homogenization, removing any visible debris or vegetation. Once sieved, the soil was split in half, where one half was placed back into a bucket and stored at 4°C until use, and the second half was autoclaved in an autoclave bag under a gravity cycle for 30 minutes at 121°C to kill any living organisms. Half of the prepared soil was autoclaved prior to experimental assembly to alter the resident soil microbial community and evaluate its influence on subsequent TWP colonization. After soil was autoclaved, it was placed back into pre-sterilized snorkel cabinets with vents on and allowed to cool before being placed into a bucket within its autoclave bag and stored at 4°C until use.

Experimental Procedure

One gram of autoclaved TWPs were placed into sterile 250 mL Erlenmeyer flasks. Flasks were then filled with 50 mL Kings Broth (KB) media [0.9g Potassium Phosphate Monobasic, Anhydrous, (MP Biomedicals); 9.0 g Bacto™ Proteose Peptone, (Gibco Thermo Fisher cat# 211684); 9 g agar (ACROS organics); 6 mL Glycerol 99+%, extra pure, (Thermo Scientific)]. 15 of the 27 flasks per trial were then inoculated with *Pseudomonas congelans* 892 (47), a phyllosphere-associated bacterium part of the *Pseudomonas syringae* complex, at an OD_{600} of ~ 0.12 which represents ~ 8 x 10^6 cells when grown on an agar KB plate. Flasks were placed onto a rotating floor orbital shaker (Thermo MaxQ 2000) at 200 RPM at room temperature. Temperature of the room was monitored using a GoveeLife smart thermos-hygrometer. After 24 and 48 hours, all flasks

had KB media removed using a 25 mL serological pipet by allowing the TWPs to first settle to the bottom and pipetting the liquid off the top. Each flask with settled TWPs was rinsed by swirling with 20 mL of sterile 1X Phosphate-Buffered Saline (PBS) before being resuspended in 50 mL of fresh KB media. Flasks were placed back onto the orbital shaker at 200 RPM, room temperature. After 72 hours of incubation, all flasks had KB media removed and were rinsed with 20 mL 1X PBS, followed by a 20 mL sterile deionized (DI) water rinse. Three of the 15 flasks containing cultured TWPs were stored at 4°C after being rinsed and resuspended in 20 mL 1X PBS. After starting greenhouse experiments detailed below, these three flasks were rinsed with another 20 mL 1X PBS, and TWPs were placed into 50 mL Corning® centrifuge tubes with 5 mL 1X PBS. Tubes were then sonicated for 10 minutes at 20 kHz to dislodge adhered bacteria, and cell counts were estimated using serial dilution plating on KB plates.

Remaining flasks containing TWPs with no liquid, were then transported to the greenhouse along with the soil. For each monthly trial, soil collected in March, April, or May, had 1 kg of both autoclaved and non-autoclaved soil from the Kentland Farm collections transferred into 12 ceramic pots for each soil treatment. See **Table 1** for treatment groups. Autoclaved stainless steel tea balls (3 in x 3 in, Fu Store via Amazon, USA) were used to encapsulate TWPs. In brief, ~150 g of soil was added to the bottom of pots, before inserting an open sterile tea ball. The tea ball was then filled with alternating layers of TWPs and soil using a sterile scoopula. Each flask was then rinsed with 20 mL sterile water which was gently poured into the tea ball before it was clasped shut. The tea ball was then turned three times to help mix TWPs with the soil. After the addition of the tea ball to each pot, the remainder of the 1 kg of soil was placed on top of the tea ball

ensuring full coverage. Pots with soil were then brought to full saturation with sterile DI water and allowed to sit for one hour before taking initial soil moisture readings from six random pots.

Pots were randomly positioned in a single line along the greenhouse bench. Soil moisture was monitored every two days in six randomly selected soil-containing pots, with at least two pots sampled from each of the left, middle, and right portions of the bench. Eight additional pots contained tea balls holding TWPs without soil and served as the TWP-only air and Biofilm-TWP air treatments. These particles were sprayed three times with sterile DI water rather than brought to saturation. Soil-containing pots received 120–200 mL of sterile DI water to maintain moisture conditions. Soil moisture measurements were used to guide watering and were not included in downstream analyses. Sterile DI water was used throughout the incubations to minimize the introduction of exogenous microorganisms.

After incubating in the greenhouse for 30 days, all tea balls were collected into sterile foil containers and transported back to the snorkel cabinets where tea balls were left to air dry overnight. After the overnight drying period, all samples were stored at 4°C and subsets were taken out to remove TWPs. Tea balls containing soil and TWPs were weighed before TWPs were removed using sterile forceps. TWPs were placed into sterile 15 mL glass flacon tubes and capped. Once TWPs were removed from the tea balls, they were stored at 4°C until analyzed. After removing TWPs from any sample, the remaining tea ball soil was subdivided into small manilla envelopes and sterile 15 mL glass flacon tubes and stored at 4°C until analyzed.

Table 1. Summary of soil-microcosm treatments, including name, soil condition, and TWP preparation. Each treatment was established separately in autoclaved and non-autoclaved soil, n=4.

Treatment Name	Soil Condition	Initial TWP Preparation
Soil Control	Autoclaved and non-autoclaved soil	No TWPs
TWP-only	Autoclaved and non-autoclaved soil	Uncolonized (without prior bacterial colonization)
Biofilm-TWP	Autoclaved and non-autoclaved soil	Pre-colonized with *P. congelans*

Soil Chemical Analysis

For carbon and nitrogen analyses, three of the four biological replicates from each treatment were selected based on observations recorded during the incubation period. Replicates that maintained water and kept the tea ball fully buried were prioritized, whereas pots exhibiting poor water retention or partial emergence of the tea ball were excluded. After TWPs were removed, a 1 g soil subsample from each retained replicate (n = 3 per treatment) was analyzed separately for carbon and nitrogen concentrations

using an Elementar CNS analyzer (Elementar Americas, Ronkonkoma, NY, USA). Each monthly collection of the untreated soil source from Kentland Farm for each trial were also analyzed with the samples. Soil stored in small manilla envelopes were equilibrated to room temperature for ~ 2 hours and used to determine sample pH. Soil pH was measured for four replicate samples per treatment and one untreated source-soil sample (25 samples total). From each sample, 20 g of soil was transferred to a 250 mL glass beaker, combined with 50 mL of DI water, and stirred for at least 2 min to form a slurry before reading the pH with pH test strips (Ricca Chemical pH test strips, Cat. No. 8884-1). To independently assess whether TWPs altered solution pH in the absence of soil, TWPs were incubated in 20 mL ultrapure water at 2 mg, 20 mg, and 200 mg for 30 days. Following incubation, solution pH was measured using pH strips and a calibrated pH electrode (Fisher Scientific accumet basic AB15 pH meter).

Differences in soil pH and C:N ratio among treatments were evaluated separately by monthly trial and soil sterilization condition using Kruskal–Wallis tests in R version 4.5.2. Pairwise Wilcoxon rank-sum tests with Benjamini–Hochberg adjustment were used for post hoc comparisons. Statistical significance was evaluated at $\alpha = 0.05$.

Extraction and Purification of DNA

Microbial genomic DNA was extracted from 0.25 g of both soil and from recovered TWPs for each treatment using the DNeasy PowerSoil™ Pro Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocols and guidelines. The DNA concentration was then determined using a Qubit™ fluorometer with Quant-iT dsDNA HS Assay Kit

(Invitrogen, Carlsbad, CA, USA) and stored at 4°C in 0.1 mM EDTA until PCR analyses and amplicon sequencing. Triplicate samples from each treatment (both soil and TWPs) per trial were pooled using the samples with the best 260/280 ratios. Samples were pooled equally to either 10 ng/µL for non-autoclaved soil or 3 ng/µL for autoclaved soil in a total volume of 30 µL.

Endpoint PCR was conducted on extracted nucleic acid samples as a rapid quality-control step. The V4 region of the bacterial 16S rRNA gene was amplified using the Earth Microbiome Project 515F/806R primer pair (48,49). A ~302-bp fragment was generated using the forward primer 515F (5′-GTGYCAGCMGCCGCGGTAA-3′) and the reverse primer 806R (5′-GGACTACNVGGGTWTCTAAT-3′). Reactions were performed using HotStar Taq Plus DNA Polymerase (Qiagen, cat. no. 203643) in a total volume of 20 µL, containing 2 µL of template DNA and primers at a final concentration of 0.32 µM each. Thermal cycling conditions consisted of an initial denaturation at 95 °C for 5 min, followed by 32 cycles of denaturation at 95 °C for 30 s, annealing at 53 °C for 40 s, and extension at 72 °C for 1 min, with a final extension step at 72 °C for 10 min. PCR products were visualized on a 2% Tris–borate–EDTA (TBE) agarose gel with 50 bp DNA ladder (Invitrogen, cat. no. 10416014).

Microbial Community Analysis

Library preparation and amplicon sequencing of the bacterial 16S rRNA gene and initial sequence processing were performed by Molecular Research LP (MR DNA; Shallowater, TX, USA) using its standard 16S amplicon-sequencing and bioinformatics workflow. MR

DNA provided ZOTU (zero-radius operational taxonomic units) count tables and associated taxonomic assignments.

Following receipt of the processed data, control-associated background reads were subtracted from the corresponding experimental samples. The resulting count table was converted to relative abundance for community-composition and beta-diversity analyses. For alpha-diversity analyses, samples meeting the minimum sequencing-depth threshold were rarefied to 8,000 reads before observed ZOTU richness and Shannon diversity were calculated. Only curated samples passing all filtering criteria were retained for downstream analyses. All data processing and statistical analysis were conducted in R version 4.5.2.

To compare soil- and TWP-associated communities descriptively, the ten genera with the greatest summed relative abundance across matched TWP and surrounding-soil samples from all three trials, both soil pretreatments, and both TWP treatments were selected. This same set of genera was displayed in a consistent order across heatmaps to facilitate comparisons. Median relative abundance was calculated for each genus within each monthly trial, soil sterilization condition, TWP treatment, and sample matrix. Heatmap differences were calculated by subtracting the median relative abundance in each soil treatment from that in the corresponding TWP treatment; positive values therefore indicated greater relative abundance on TWPs, whereas negative values indicated greater relative abundance in soil. Due to sample pooling and limited replication within individual treatment-by-month combinations, these heatmaps were interpreted descriptively and were not subjected to formal differential-abundance testing. Differences in community composition were evaluated using PERMANOVA, and homogeneity of

multivariate dispersion was assessed using PERMDISP. Principal coordinates analysis (PCoA) was used to visualize patterns in Bray–Curtis community dissimilarity. To further evaluate the extent to which TWP-associated communities reflected surrounding soil assemblages, we also quantified the proportion of shared and unique ZOTUs between soil and TWP communities within each month and treatment.

SEM Imaging

TWPs from March and April for each treatment type were prepared for scanning electron microscopy (SEM) imaging by first rinsing the TWPs with 1X PBS to remove any residual soil. The TWPs were then placed into 5 mL of a 2.5% glutaraldehyde solution and incubated overnight at 4 °C. After incubation, TWPs were rinsed with 10 mL 1X PBS before being subjected to an ethanol dehydration series. The series was as follows: 30%, 50%, 70%, 80%, 90%, 100%, 100% incubating in each dilution for 5 minutes. TWPs were air dried in a laminar flow hood before being mounted on 1.25 mm SEM mounts and coated 20 nm thick with platinum palladium using the Cressington 208HR Sputter. Samples were then imaged using a JEOL IT500 SEM at 5.0kV, 50std.

Use of Generative AI

ChatGPT 5.0-5.6 was used to create R scripts to analyze and visualize data and to help with overall flow, organization, and editing of written drafts. All authors have read and revised all content and bear full responsibility for the final edited content.

Results

Microbial attachment on TWP surfaces

SEM was used to evaluate microbial colonization of TWP surfaces before and after soil incubation. Prior to incubation, TWPs exhibited irregular fragmented surfaces (**Figure 1a**). Following incubation, attached microbial cells, spore like shapes, and extracellular matrix-like material consistent with biofilm formation were observed on particle surfaces, for both uncolonized and biofilm-associated treatments (**Figure 1b, c**).

Soil pH and C:N responses

Changes in soil pH and C:N ratio across treatments and months are shown in **Figure 2**. Soil pH varied among treatments in March ($p = 0.04$); however, post hoc comparisons did not identify significant differences between specific treatment pairs after adjustment for multiple comparisons. No overall treatment effects on soil pH were detected in April ($p = 0.111$) or May ($p = 0.671$). Soil C:N ratio did not differ significantly among treatments in March ($p = 0.0665$), April ($p = 0.0794$), or May ($p = 0.561$).

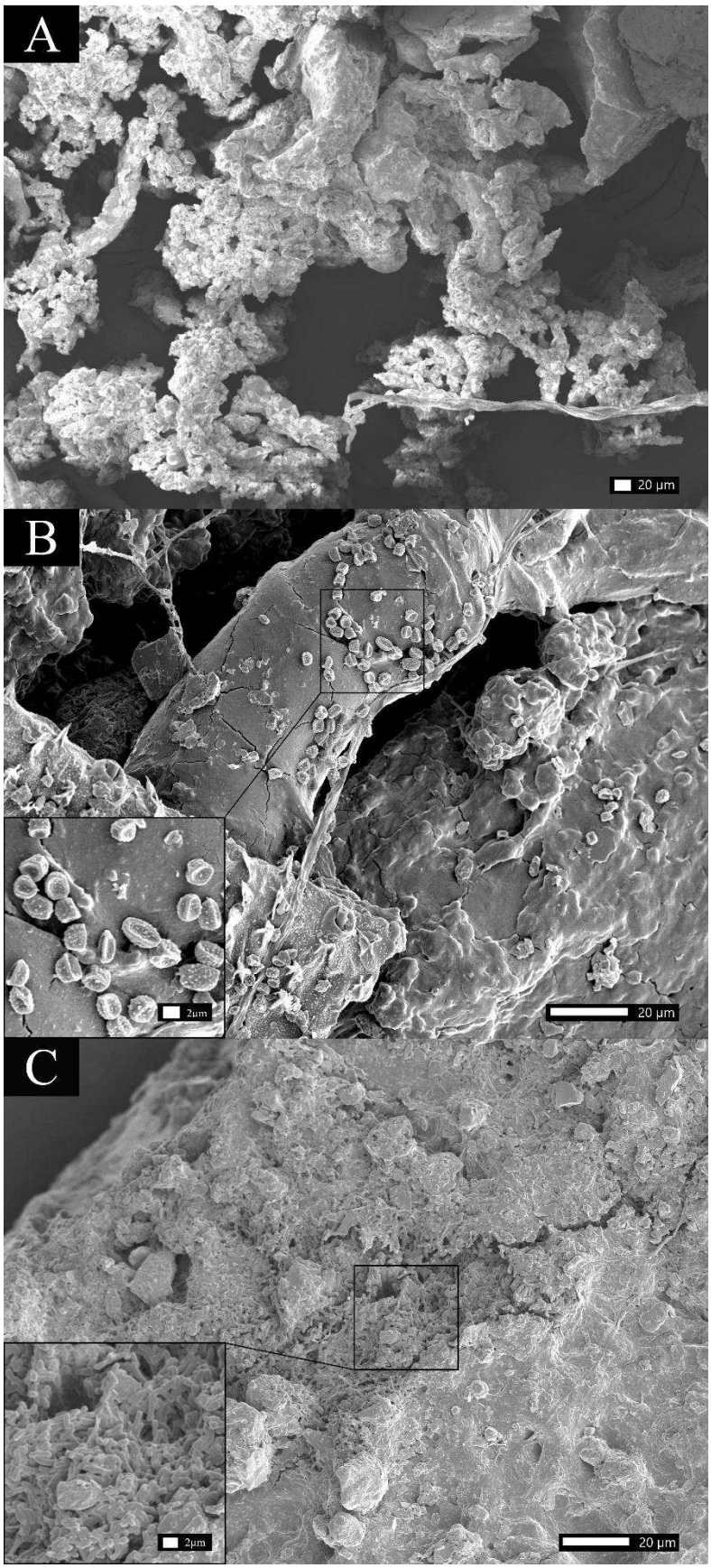


Figure 1. Surface morphology and microbial attachment on tire wear particles. SEM images of TWPs used in the experiments: (**a**) uncolonized TWP showing an irregular surface; (**b**) initially uncolonized TWP following 30 days of soil incubation, showing attached oval structures; (**c**) pre-colonized TWP following 30 days of soil incubation, showing numerous rod-shaped cells. Insets show enlarged views of the outlined regions in panels b and c.

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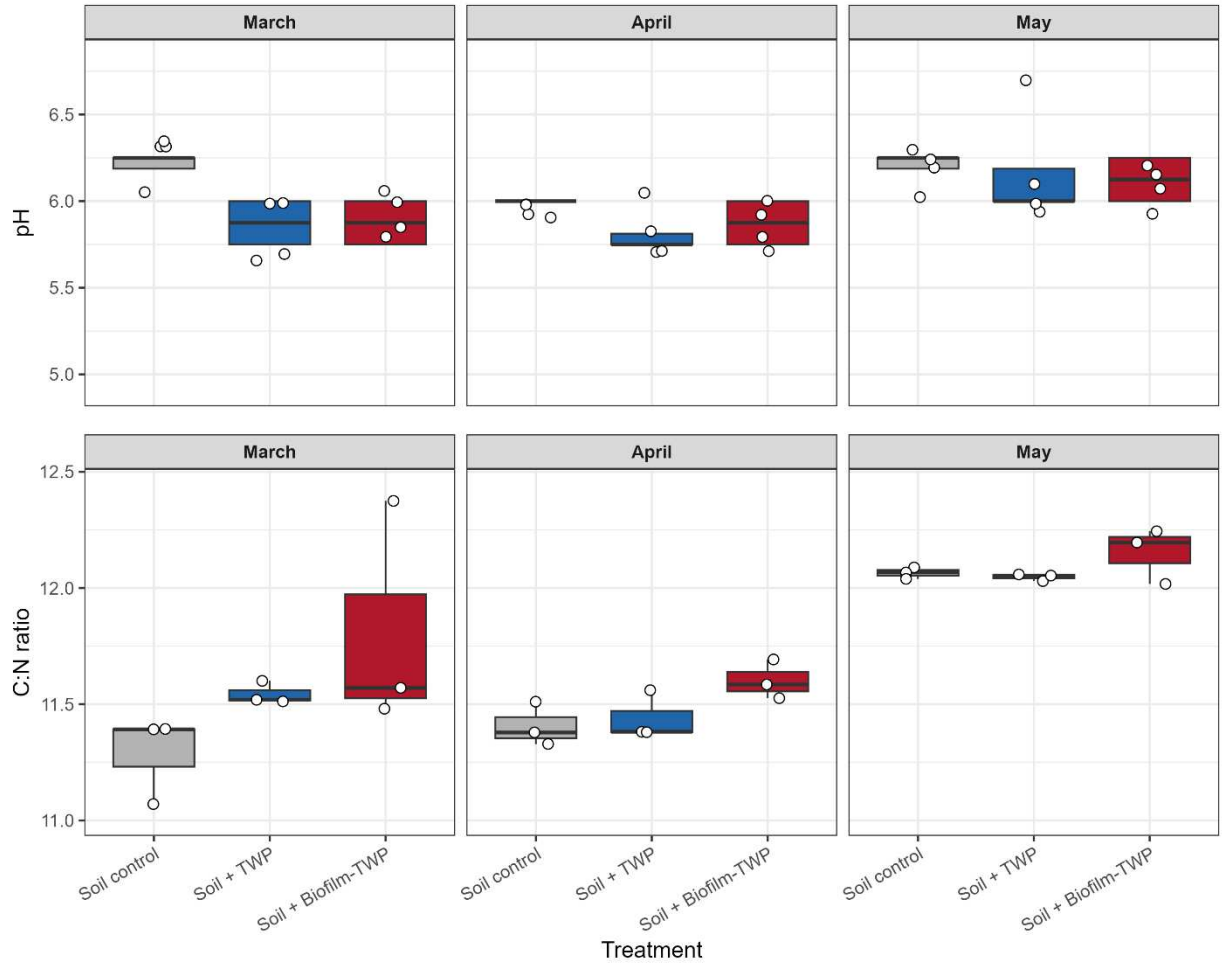


Figure 2. Soil pH (top, n=4 per treatment) and Soil C:N ratio (bottom, n=3 per treatment) among non-autoclaved treatments across March, April, and May. Treatments include Soil Control, Soil + TWP, and Soil + Biofilm TWP. Points represent individual measurements, boxes indicate the interquartile range, center lines represent medians, and whiskers extend to 1.5 x the interquartile range.

Bacterial community differences between TWPs and surrounding soil

Descriptive comparisons showed that several dominant genera, including *Massilia*, *Pseudomonas*, *Aquabacterium*, *Imtechium*, and *Noviherbaspirillum*, had higher median

313 relative abundances in TWP-associated communities than in the corresponding soil
314 treatment groups (**Figure 3**). These patterns occurred across multiple monthly trials,
315 although their magnitudes varied among months and treatments. PCoA of Bray–Curtis
316 dissimilarities similarly showed visual separation between soil- and TWP-associated
317 communities across the monthly trials (**Supplemental Figure S2**).

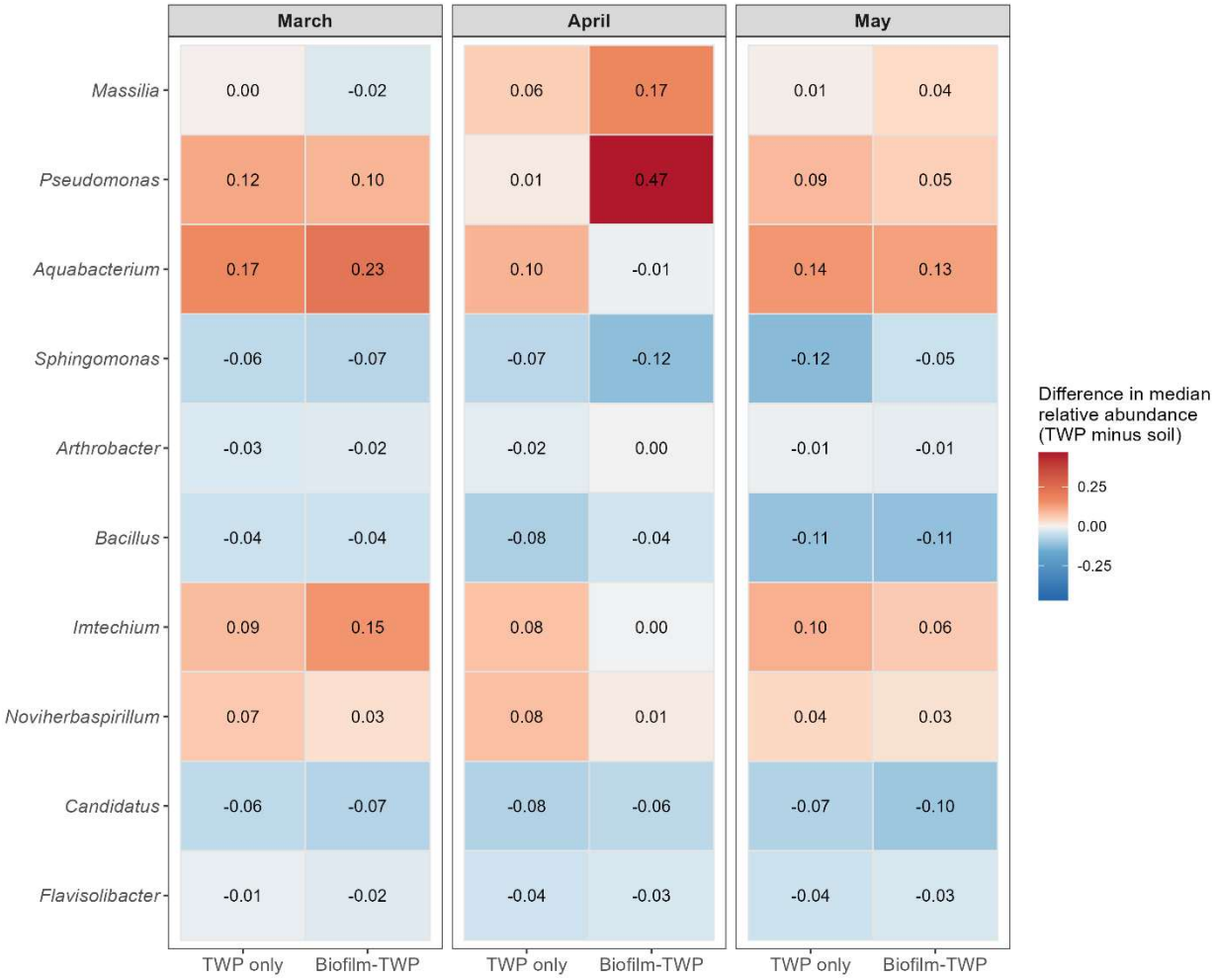


Figure 3. Differences in bacterial relative abundance between TWPs and surrounding non-autoclaved soil. Heatmap shows treatment-associated differences in median relative abundance between TWP-associated and surrounding soil communities for the global top 10 genera within systems across March, April, and May. Values represent the difference in median relative abundance calculated as TWP minus Soil for each genus and treatment combination. Positive values (red) indicate genera with relatively greater abundance in TWP-associated communities compared to soil, whereas negative values (blue) indicate relatively lower abundance in TWP-associated communities relative to soil. Columns represent TWP-only and Biofilm-TWP treatments, while rows correspond to dominant genera identified across all samples.

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Variation in TWP–soil differences among monthly trials

While several genera consistently exhibited enrichment on TWPs, the magnitude and direction of these shifts varied substantially across monthly sampling intervals (**Figure 4**). Both uncolonized and biofilm-associated TWPs exhibited dynamic month-to-month changes in relative abundance patterns, with pronounced shifts observed between March and April and April to May comparisons. Genera including *Pseudomonas*, *Aquabacterium*, *Massilia*, and *Imtechium* displayed highly variable enrichment patterns across intervals, indicating that TWP-associated community composition varied substantially with temporal changes in surrounding soil microbial communities. Biofilm-associated TWPs often exhibited larger temporal shifts in relative abundance patterns compared to uncolonized TWPs, suggesting that initial colonization history may further influence subsequent community assembly dynamics.

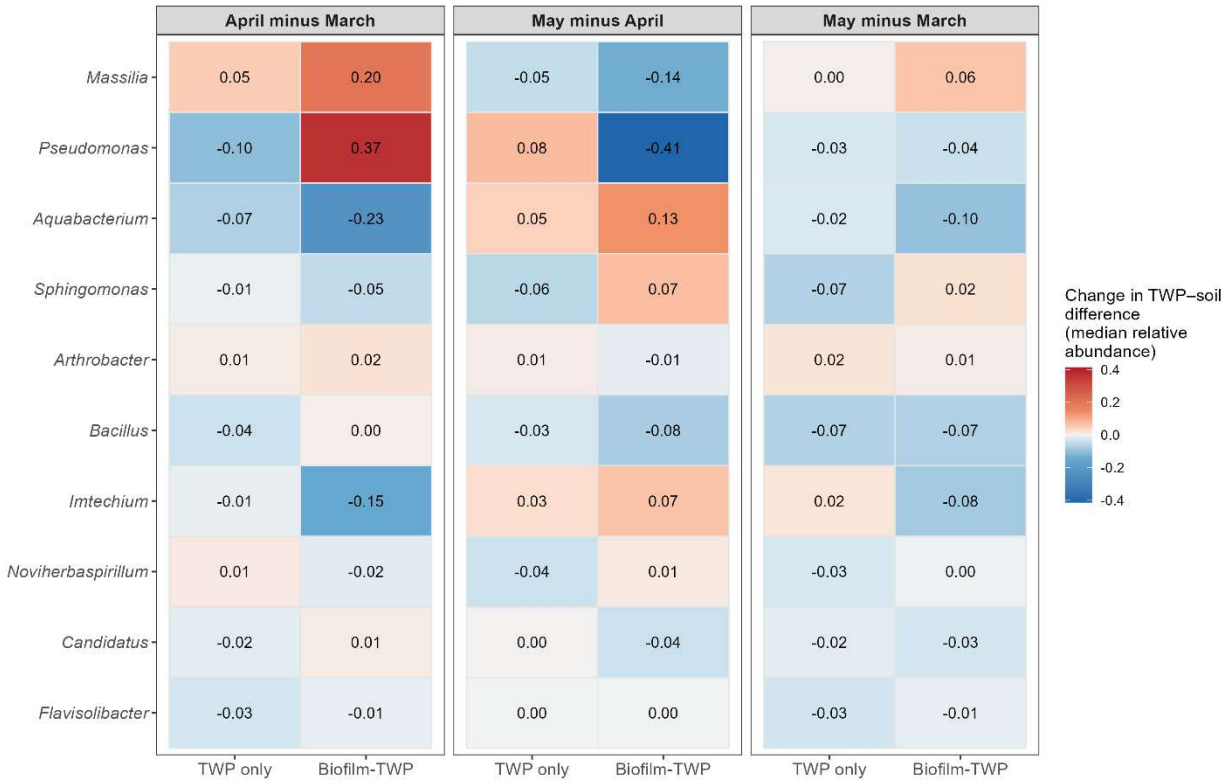


Figure 4. Changes in TWP–soil relative abundance differences among monthly trials. Heatmap shows temporal changes in TWP–soil median relative-abundance differences for the global top 10 genera across March, April, and May. Values represent the change in the median relative-abundance difference (TWP − Soil) between sampling periods, calculated as April − March, May − April, and May − March. Positive values (red) indicate that the relative enrichment of a genus on TWPs increased between the specified months, whereas negative values (blue) indicate that its relative enrichment on TWPs decreased. Columns represent TWP-only and Biofilm-TWP treatments, while rows correspond to the dominant genera identified across all samples.

Shared and unique taxa between TWPs and surrounding soil

Across all sampling periods, a substantial portion of ZOTUs were shared between TWP-associated and surrounding soil communities in both the TWP-only and Biofilm-TWP treatments (**Figure 5**). Shared ZOTUs consistently represented the largest proportion of

detected taxa, accounting for approximately 29–63% of total ZOTUs depending on month and treatment. Consistent with these patterns, the distribution of ZOTUs among the dominant bacterial genera was broadly similar between soil and TWP-associated communities (**Supplemental Figure S3 and S4**). Across all months, the dominant bacterial genera were largely conserved between soil and TWP-associated communities, with *Sphingomonas* consistently exhibiting the greatest ZOTU richness across treatments. Other abundant genera, including *Massilia*, *Bacillus*, *Flavisolibacter*, and *Arthrobacter*, were detected in both soil and TWP samples regardless of prior biofilm colonization or soil sterilization.

However, both treatments also maintained distinct fractions of unique taxa within the TWP-associated communities, with ZOTUs comprising approximately 12–24% of detected taxa across all months. Temporal variation in overlap patterns was also observed. In the TWP-only treatment, the proportion of shared ZOTUs, 40%, was greatest in April before declining in May due to increases in both soil-only and TWP-only taxa. In contrast, the Biofilm-TWP treatment exhibited a marked reduction in shared ZOTUs, 28%, during April, coinciding with a substantial increase in soil-only taxa. Despite these temporal differences, TWP-associated communities consistently retained distinct assemblages that differed from surrounding soil communities throughout the incubation period. Since TWP-associated communities appeared strongly influenced by surrounding soil composition and temporal dynamics, we next examined how plastisphere assembly responded following a major disturbance using autoclaved soils.

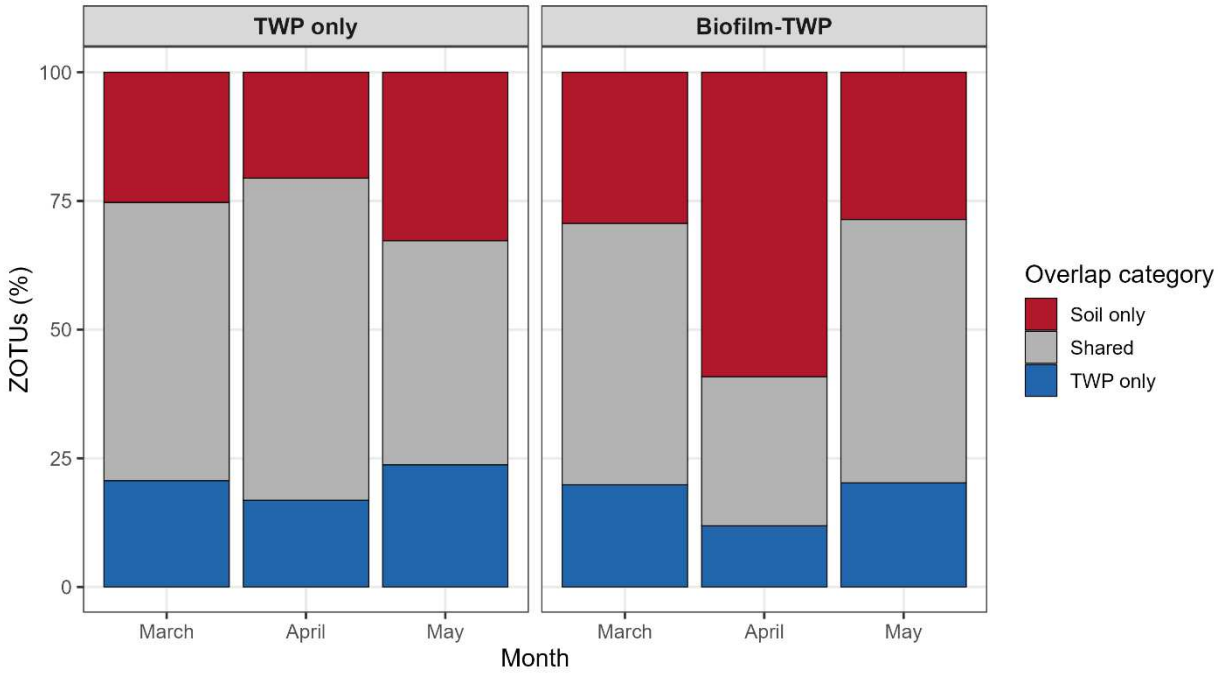


Figure 5. Shared and unique bacterial taxa between TWPs and surrounding non-autoclaved soil across March, April, and May. Stacked bars represent the % proportion of ZOTUs detected exclusively in soil ("Soil only"), exclusively in TWP-associated communities ("TWP only"), or shared between matrices ("Shared") within each month and treatment type. Panels correspond to TWP-only and Biofilm-TWP treatments. Overlap patterns varied across sampling periods, with shared ZOTUs generally comprising the largest proportion of detected taxa, although treatment-specific temporal shifts in unique and shared community fractions were observed.

TWP-associated bacterial communities in autoclaved soil

Autoclaved soils exhibited distinct TWP-associated community patterns compared to non-autoclaved treatments, with several genera displaying pronounced shifts in relative abundance across months (**Figure 6**). In contrast to the more variable patterns observed under non-autoclaved conditions, autoclaved treatments exhibited stronger enrichment of specific genera, particularly *Pseudomonas* and *Aquabacterium*, across multiple sampling periods. March biofilm-associated TWPs showed especially strong enrichment of *Pseudomonas*, while April and May treatments exhibited broader enrichment across

multiple genera including *Aquabacterium* and *Noviherbaspirillum*. Despite reduced background microbial complexity under autoclaved conditions, TWP-associated communities continued to exhibit temporal variability, indicating that microbial colonization and community restructuring on TWPs remained highly dynamic even following major disturbance to the surrounding soil microbiome.

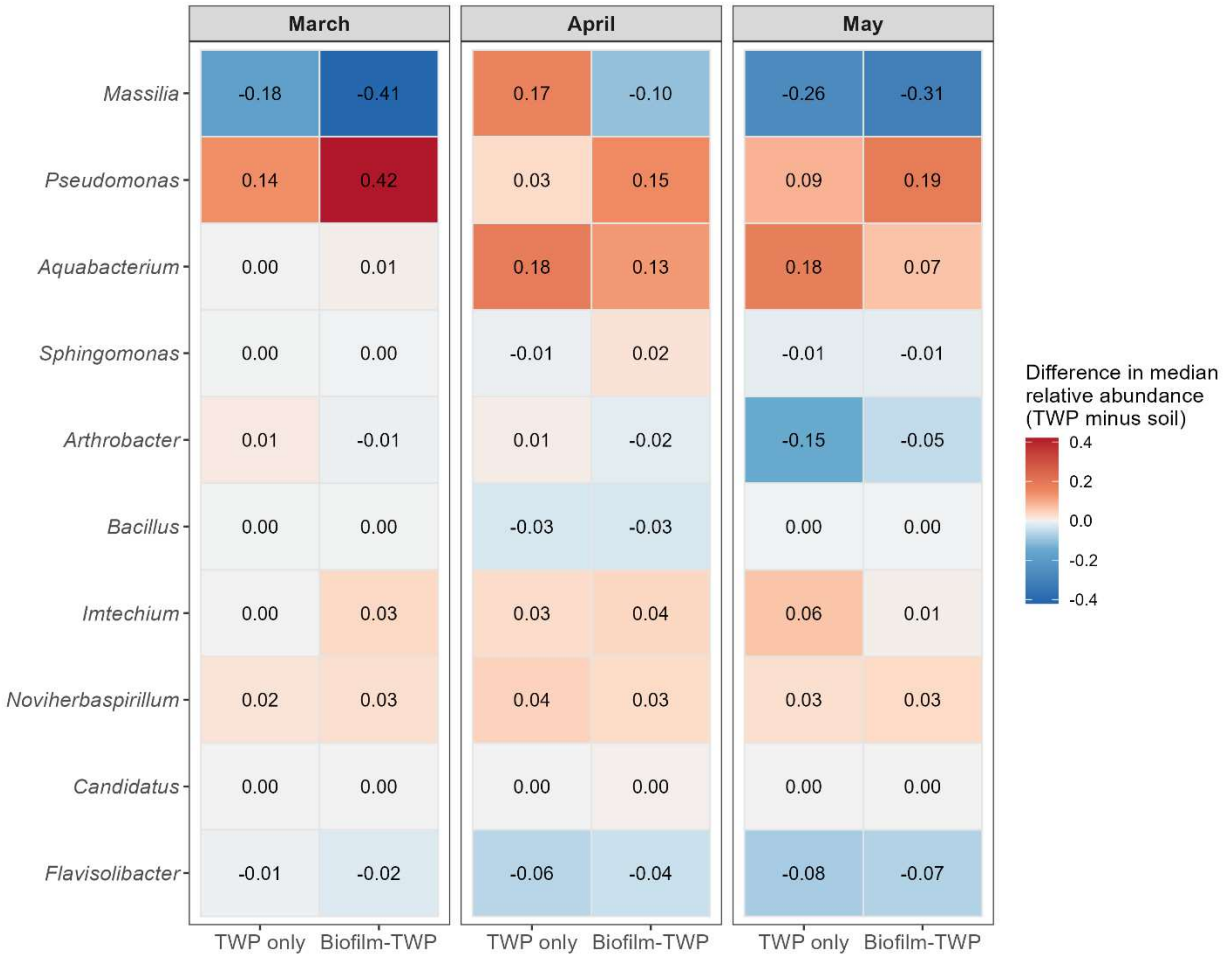


Figure 6. Differences in bacterial relative abundance between TWPs and surrounding autoclaved soil. Heatmap shows treatment-associated differences in median relative abundance between TWP-associated and surrounding soil communities for the global top 10 genera across March, April, and May. Values represent the difference in median relative abundance calculated as TWP minus Soil for each genus and treatment combination. Positive values (red) indicate genera with relatively greater abundance in TWP-associated communities compared to soil, whereas negative values (blue) indicate relatively lower abundance in TWP-associated communities relative to soil. Columns represent TWP-only and Biofilm-TWP treatments, while rows correspond to dominant genera identified across all samples. Patterns of relative enrichment and depletion varied across genera and sampling periods, indicating temporally dynamic responses of TWP-associated microbial communities within autoclaved soil systems.

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Discussion

Understanding how microbial communities develop on MPs in agricultural soils requires examining both particle characteristics and the conditions under which colonization occurs. This study addresses part of that knowledge gap by comparing bacterial communities associated with uncolonized and *Pseudomonas congelans*-pre-colonized TWPs and surrounding agricultural soil across three independent monthly trials in autoclaved and non-autoclaved soils. TWPs supported communities distinct from surrounding soil, with composition varying among trials and with prior colonization and soil pretreatment, while treatment effects on bulk soil pH and C:N were limited. These findings provide an initial basis for broader studies across crop species, soil types, and MP materials to determine how widely these community patterns occur and whether they have consequences for soil processes and plant performance.

SEM images showed microbial attachment and biofilm development on TWPs following the 30-day soil incubation. This extensive surface colonization is consistent with the concept of the soil plastisphere (27,50), in which plastic particles provide novel surfaces and niches for microbial attachment and biofilm development. TWP-associated bacterial communities were distinct from those surrounding soil. Similar differentiation has been reported across a variety of polymer types and environmental settings (50–54), including TWPs specifically, where bacterial communities diverged from surrounding soils (43). Together, these findings support that TWPs and other MPs can harbor microbial communities that differ in composition from those in the surrounding environment.

Despite pronounced microbial colonization of TWPs, changes in bulk soil chemistry were comparatively modest and varied among monthly incubations. An overall treatment effect on soil pH was detected in March (Kruskal–Wallis test), but no corrected pairwise Wilcoxon comparisons were significant. No significant overall treatment effects on soil pH were detected in April or May, and C:N ratios did not differ significantly among treatments in any monthly trial. Although C:N ratios did not differ significantly among treatments, examination of carbon and nitrogen concentrations separately indicated that their contributions to the observed numerical differences in C:N varied among monthly trials. These limited bulk-soil responses suggest that substantial microbial differentiation can occur on TWP surfaces without corresponding large or consistent changes in bulk soil chemistry over the 30-day incubation period.

Variable physicochemical responses to MPs and TWPs have similarly been reported across soil and sediment systems, with effects depending on particle composition, concentration, environmental conditions, and exposure duration (6,15,23,33,55–61). Such variability may reflect both differences among plastic materials and the capacity of soil to buffer particle-associated chemical inputs. In a separate water-only incubation, increasing TWP concentrations produced a progressive decline in solution pH, demonstrating that the particles were capable of altering aqueous chemistry in the absence of soil (**Supplemental Figure S5**). The comparatively limited pH response observed in soil may reflect soil buffering processes involving organic matter and mineral components (62,63), although buffering capacity was not measured in this study. In agricultural soils, these results highlight the value of examining particle-associated communities alongside bulk soil measurements, because pH and C:N responses alone

did not capture the compositional differences observed between TWP-associated and surrounding soil communities.

TWPs consistently supported bacterial communities distinct from surrounding soil, but the genera showing greater relative abundance on particles differed among the three independent monthly trials. Despite consistent preparation procedures, mean culturable bacterial loads recovered from Biofilm-TWPs before soil incubation were numerically lower in March (3.52×10^8 CFU/g TWP) than in April (7.15×10^8 CFU/g TWP) and May (7.90×10^8 CFU/g TWP). Differences in initial colonization density may have contributed to subsequent community variation in the Biofilm-TWP treatment, although this relationship was not directly tested. However, initial culturable bacterial load alone does not appear to explain the greater enrichment of *Pseudomonas* on Biofilm-TWPs relative to surrounding soil in April, as the mean initial load was numerically higher in May. Such variation in plastisphere community composition has been observed in plastisphere communities across aquatic and terrestrial systems, with community composition varying across environmental settings and over time (64–69). This variation is relevant to agricultural exposure because particles introduced at different times may encounter different soil communities and colonization conditions. The present trials demonstrate variation among collection periods, although they do not establish its seasonal drivers.

Overlap analyses indicated that TWP-associated communities shared substantial proportions of ZOTUs with surrounding soil while consistently retaining a fraction of particle-associated taxa. Likewise, dominant genera were broadly represented across both matrices, although their relative abundance and lineage-level richness differed between soil and TWP-associated communities. Together, these patterns suggest that

surrounding soil serves as an important reservoir of potential colonizers, while conditions on the TWP surface subsequently favor only a subset of the locally available community. This pattern is consistent with contemporary models of microbial community assembly, in which community composition emerges through interactions between deterministic environmental filtering and stochastic processes such as dispersal, ecological drift, and historical contingency (70–72). Environmental filtering can favor organisms possessing traits suited to a particular habitat (73), whereas the identity of organisms available to encounter that habitat depends on the surrounding species pool and dispersal history (74,75). Plastisphere studies increasingly support this combined framework. Polymer characteristics can impose deterministic selection in some systems (67,68,76–79), whereas stochastic dispersal and ecological drift may exert stronger influences in others (64,65).

The distinct communities observed on TWPs may partly reflect particle-associated chemical conditions, as tire-particle leachates have been shown to alter microbial community composition in coastal sediments (44). The TWPs used in this study contained abundant sulfur, zinc, silicon, calcium, and numerous trace metals (**Supplementary Figure S6**), and bacterial growth differed in association with several tire-derived elements (**Supplementary Figure S7**). TWPs are chemically heterogeneous materials containing inorganic fillers, vulcanization agents, metals, and other additives that can influence microbial growth and colonization (7,18,44,80). Although we did not directly test whether individual constituents drove community assembly, these observations provide one potential mechanism through which TWPs could impose environmental filtering on microorganisms recruited from surrounding soil.

Air-exposed TWPs also supported microbial assemblages distinct from those formed following soil incubation (**Supplementary Figure S8**). The presence of diverse taxa on particles maintained without soil under greenhouse air-exposure conditions suggests that microbial colonization may begin before TWPs enter agricultural soils. Strong enrichment of *Pseudomonas* on previously colonized particles further suggests that prior colonization may influence subsequent community composition. Evidence from aquatic systems supports this possibility: Song et al. (2022) found that a small fraction of particle-associated bacteria remained following transfer along a salinity gradient, despite substantial community turnover (64). Together, these observations raise the possibility that TWPs could enter agricultural soils carrying microbial communities that influence subsequent colonization, although microbial acquisition and persistence during environmental transport were not tested here.

Consistent with the air-exposure observations, experimentally altering the initial colonization state of TWPs changed subsequent community assembly after soil exposure. Although both pristine and previously colonized particles developed distinct bacterial communities, prior colonization altered the communities that subsequently established, indicating that the initial microbial biofilm influenced later recruitment. These findings are consistent with historical contingency and priority effects, whereby early colonizers can pre-empt resources, modify microhabitats, or alter species interactions and thereby influence the establishment of later-arriving organisms (81–84). For agricultural soils receiving TWPs, these findings identify the microbial colonization history of incoming particles as a factor worth considering when evaluating subsequent particle-associated community composition.

TWP-associated communities also differed between autoclaved and non-autoclaved soils, indicating that soil pretreatment influenced the communities developing on particles. Disturbance can remove established microbial interactions and alter the size and composition of the available species pool, allowing recolonization to proceed under fundamentally different ecological conditions (74,85). However, because autoclaving can also modify soil physicochemical conditions and nutrient availability, these differences cannot be attributed solely to changes in microbial source pools. These results motivate testing whether changes in resident communities associated with agricultural management also influence TWP colonization under field conditions.

Together, these experiments showed that TWPs supported bacterial communities distinct from surrounding soil, with community composition varying among monthly trials and with prior colonization and soil autoclaving. Repeated TWP inputs through atmospheric deposition and road runoff may create ongoing opportunities for plastisphere formation in agricultural soils (14,18,86). Previous studies have linked MP and TWP exposure to changes in carbon and nitrogen cycling, greenhouse gas emissions, and rhizosphere interactions, including reduced biological nitrogen fixation and increased fertilizer nitrogen losses (23,33,57,60). Other studies have reported enrichment of ARGs on TWPs in soil and enhanced horizontal gene transfer in aquatic TWP-associated communities (28,43), alongside plant uptake of tire-derived chemicals (87). Studies of other plastic materials in anaerobic digestion systems further indicate that polymer composition can influence plastisphere-associated antibiotic resistance profiles (88). These findings highlight the value of future studies testing whether variation in TWP-associated community composition affects soil processes, contaminant fate, or plant responses.

Several limitations should be considered when interpreting these findings. First, this study was conducted under controlled greenhouse conditions, with sterile DI water used to maintain soil moisture, allowing environmental variability and the introduction of exogenous microorganisms to be minimized. While this approach increased experimental control, field conditions are considerably more dynamic, with natural precipitation, fluctuating environmental conditions, and continual biological inputs that may influence plastisphere development differently (89). Second, our experiments examined a single agricultural soil and one TWP formulation. Because soil physicochemical properties, land management history, microbial source pools, and tire composition vary widely among agricultural systems, plastisphere assembly likely differs across environments and particle types (65,76,90). Finally, this study characterized bacterial community composition using DNA-based 16S amplicon sequencing following a 30-day incubation, providing insight into early bacterial community assembly but not the functional activity or metabolic potential of these communities, nor their interactions with other members of the soil microbiome, including fungi, archaea, protists, and viruses (89). Future studies integrating diverse soils, multiple TWP formulations, longer-term field experiments, and complementary approaches such as metagenomics, metabolomics, or transcriptomics will help determine how differences in plastisphere assembly translate into ecosystem function and agricultural outcomes.

Conclusion

Collectively, our findings demonstrate that TWPs function as dynamic microbial habitats whose community composition depends on particle properties, local microbial source pools, colonization history, and environmental conditions. Rather than supporting a single predictable microbiome, TWP-associated communities emerged through interactions between particle-associated selection and the biological context in which colonization occurred. Understanding how context-dependent plastisphere assembly translates into ecosystem function, plant performance, and contaminant dynamics will be essential for predicting the broader consequences of increasing TWP deposition in agricultural landscapes for soil health, food safety, and agricultural sustainability.

List of Abbreviations

ARGs	Antibiotic resistance genes
CFU	Colony-forming units
C:N	Carbon-to-nitrogen ratio
DNA	Deoxyribonucleic acid
dsDNA	Double-stranded DNA
EDTA	Ethylenediaminetetraacetic acid
KB	King’s broth
MPs	Microplastics
OD_{600}	Optical density at 600 nm

ARGs Antibiotic resistance genes

PBS Phosphate-buffered saline

PCoA Principal coordinates analysis

PCR Polymerase chain reaction

PM_{10} Particulate matter with an aerodynamic diameter ≤10 μm

rRNA Ribosomal ribonucleic acid

SEM Scanning electron microscopy

TBE Tris–borate–EDTA

TWPs Tire wear particles

ZOTUs Zero-radius operational taxonomic units

Supplemental Material

Supplemental data to this article can be found at:

Carpenter_et_al_TWPs_Soil_Supplemental_Material.docx

Declarations

Data Availability Statement

The datasets supporting the conclusions of this article are available in the Virginia Tech Data Repository via private link at https://figshare.com/s/51e3aa7ec4cd3fd242db. This dataset will be published and made publicly accessible via DOI upon manuscript acceptance.

Competing Interests

The authors declare that they have no competing interests.

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References

1. Rodriguez-Eugenio N, McLaughlin M, Pennock D. Soil Pollution: A Hidden Reality. 2018;142.

2. De Souza Machado AA, Kloas W, Zarfl C, Hempel S, Rillig MC. Microplastics as an emerging threat to terrestrial ecosystems. Glob Change Biol. 2018 Apr;24(4):1405–16. doi:10.1111/gcb.14020

3. Büks F, Kaupenjohann M. Global concentrations of microplastics in soils – a review. SOIL. 2020 Dec 17;6(2):649–62. doi:10.5194/soil-6-649-2020

4. Ilyas S, Mehnaz S, Machado S. Silent threats of 21st century: microplastic contamination of agroecosystems and its implications for the food chain toxicity. Environ Sustain. 2024 Sep 12;7(3):261–4. doi:10.1007/s42398-024-00325-9

5. Ding J, Lv M, Zhu D, Leifheit EF, Chen QL, Wang YQ, et al. Tire wear particles: An emerging threat to soil health. Crit Rev Environ Sci Technol. 2023 Jan 17;53(2):239–57. doi:10.1080/10643389.2022.2047581

6. Zeb A, Liu W, Ali N, Shi R, Zhao Y, Wang J, et al. Impact of Pristine and Aged Tire Wear Particles on *Ipomoea aquatica* and Rhizospheric Microbial Communities: Insights from a Long-Term Exposure Study. Environ Sci Technol. 2024 Dec 3;58(48):21143–54. doi:10.1021/acs.est.4c07188

7. Tamis JE, Koelmans AA, Dröge R, Kaag NHBM, Keur MC, Tromp PC, et al. Environmental risks of car tire microplastic particles and other road runoff pollutants. Microplastics Nanoplastics. 2021 Dec;1(1):10. doi:10.1186/s43591-021-00008-w

8. Rausch J, Jaramillo-Vogel D, Perseguers S, Schnidrig N, Grobéty B, Yajan P. Automated identification and quantification of tire wear particles (TWP) in airborne dust: SEM/EDX single particle analysis coupled to a machine learning classifier. Sci Total Environ. 2022 Jan;803:149832. doi:10.1016/j.scitotenv.2021.149832

9. Mayer PM, Moran KD, Miller EL, Brander SM, Harper S, Garcia-Jaramillo M, et al. Where the rubber meets the road: Emerging environmental impacts of tire wear particles and their chemical cocktails. Sci Total Environ. 2024 Jun;927:171153. doi:10.1016/j.scitotenv.2024.171153

10. Li X, Zhou S, Zhang T, Bai L, Chen F, Yan X, et al. Occurrence and environmental fate/behaviors of tire wear particles and their human and ecological health: an emerging global issue. Arch Toxicol. 2025 Aug 16. doi:10.1007/s00204-025-04147-4

11. Klöckner P, Seiwert B, Eisentraut P, Braun U, Reemtsma T, Wagner S. Characterization of tire and road wear particles from road runoff indicates highly dynamic particle properties. Water Res. 2020 Oct;185:116262. doi:10.1016/j.watres.2020.116262

12. Kang J, Liu X, Dai B, Liu T, Haider FU, Zhang P, et al. Tyre Wear Particles in the Environment: Sources, Toxicity, and Remediation Approaches. Sustainability. 2025 Jun 12;17(12):5433. doi:10.3390/su17125433

13. Foroutan H, Aryal A, Craine M, Rakha H. Projecting airborne tire wear particle emissions in the United States in the era of electric vehicles. Sci Total Environ. 2025 Mar;967:178848. doi:10.1016/j.scitotenv.2025.178848

14. Rødland ES, Heier LS, Lind OC, Meland S. High levels of tire wear particles in soils along low traffic roads. Sci Total Environ. 2023 Dec;903:166470. doi:10.1016/j.scitotenv.2023.166470

15. Ahmad T, Hussain H, Sabir A, Irfan M, Ghafar A, Jafir M, et al. Impact of Microplastic on RoadsideVegetable Cultivation: A Case Studyof Agricultural Farmland. Pol J Environ Stud. 2024 Sep 4. doi:10.15244/pjoes/188183

16. Chae E, Jung U, Choi SS. Types and concentrations of tire wear particles (TWPs) in road dust generated in slow lanes. Environ Pollut. 2024 Apr;346:123670. doi:10.1016/j.envpol.2024.123670

17. Kundel D, Wiget A, Fliessbach A, Bigalke M, Weber CJ. Tracks of travel: unveiling tire particle concentrations in Swiss cantonal road soils. Microplastics Nanoplastics. 2025 Mar 3;5(1):6. doi:10.1186/s43591-025-00112-1

18. Landebrit L, Guillon M, Muresan B, Poirier L, Gasperi J. Tire and road wear particles in soils: from roadside soil to widespread impregnation. Environ Res. 2026 Jun;300:124430. doi:10.1016/j.envres.2026.124430

19. De Souza Machado AA, Lau CW, Kloas W, Bergmann J, Bachelier JB, Faltin E, et al. Microplastics Can Change Soil Properties and Affect Plant Performance. Environ Sci Technol. 2019 May 21;53(10):6044–52. doi:10.1021/acs.est.9b01339

20. Zhao L, Zhao L, Cheng Z, Yao Y, Yuan C, Wang L, et al. LDPE microplastics affect soil microbial communities and nitrogen cycling. Sci Total Environ. 2021 Jun;773:145640. doi:10.1016/j.scitotenv.2021.145640

21. Guo A, Pan C, Su X, Zhou X, Bao Y. Combined effects of oxytetracycline and microplastic on wheat seedling growth and associated rhizosphere bacterial communities and soil metabolite profiles. Environ Pollut. 2022 Jun;302:119046. doi:10.1016/j.envpol.2022.119046

22. Jia X, Yao Y, Tan G, Xue S, Liu M, Tang DWS, et al. Effects of LDPE and PBAT plastics on soil organic carbon and carbon-enzymes: A mesocosm experiment under field conditions. Environ Pollut. 2024 Dec;362:124965. doi:10.1016/j.envpol.2024.124965

23. Wei Z, Ma X, Chai Y, Senbayram M, Wang X, Wu M, et al. Tire Wear Particles Exposure Enhances Denitrification in Soil by Enriching Labile DOM and Shaping the Microbial Community. Environ Sci Technol. 2025 Jan 21;59(2):1209–21. doi:10.1021/acs.est.4c09766

24. Zettler ER, Mincer TJ, Amaral-Zettler LA. Life in the “Plastisphere”: Microbial Communities on Plastic Marine Debris. Environ Sci Technol. 2013 Jul 2;47(13):7137–46. doi:10.1021/es401288x

25. Oberbeckmann S, Osborn AM, Duhaime MB. Microbes on a Bottle: Substrate, Season and Geography Influence Community Composition of Microbes Colonizing Marine Plastic Debris. Carter DA, editor. PLOS ONE. 2016 Aug 3;11(8):e0159289. doi:10.1371/journal.pone.0159289

26. Wang L, Luo Z, Zhen Z, Yan Y, Yan C, Ma X, et al. Bacterial community colonization on tire microplastics in typical urban water environments and associated impacting factors. Environ Pollut. 2020 Oct;265:114922. doi:10.1016/j.envpol.2020.114922

27. Rillig MC, Kim SW, Zhu YG. The soil plastisphere. Nat Rev Microbiol. 2024 Feb;22(2):64–74. doi:10.1038/s41579-023-00967-2

28. Jaffer YD, Abdolahpur Monikh F, Uli K, Grossart HP. Tire wear particles enhance horizontal gene transfer of antibiotic resistance genes in aquatic ecosystems. Environ Res. 2024 Dec;263:120187. doi:10.1016/j.envres.2024.120187

29. Pahlavan A. soil plastisphere: The nexus of microplastics, bacteria, and biofilms. InterPore J. 2024 Nov 27;1(3):IPJ271124-2. doi:10.69631/ipj.v1i3nr50

30. Zhu J, Liu S, Wang H, Wang D, Zhu Y, Wang J, et al. Microplastic particles alter wheat rhizosphere soil microbial community composition and function. J Hazard Mater. 2022 Aug;436:129176. doi:10.1016/j.jhazmat.2022.129176

31. Aralappanavar VK, Mukhopadhyay R, Yu Y, Liu J, Bhatnagar A, Praveena SM, et al. Effects of microplastics on soil microorganisms and microbial functions in nutrients and carbon cycling – A review. Sci Total Environ. 2024 May;924:171435. doi:10.1016/j.scitotenv.2024.171435

32. Han L, Chen L, Feng Y, Kuzyakov Y, Chen Q, Zhang S, et al. Microplastics alter soil structure and microbial community composition. Environ Int. 2024 Mar;185:108508. doi:10.1016/j.envint.2024.108508

33. Lo Porto A, Amato G, Gargano G, Giambalvo D, Ingraffia R, Torta L, et al. Polypropylene microfibers negatively affect soybean growth and nitrogen fixation regardless of soil type and mycorrhizae presence. J Hazard Mater. 2024 Dec;480:135781. doi:10.1016/j.jhazmat.2024.135781

34. Zou Z, Yu Q, Wang J, Liu X, Lynch I. Microplastic composition-dependent effects on N2O emissions driven by changes in soil N process and microbial communities. J Hazard Mater. 2025 Sep;495:139022. doi:10.1016/j.jhazmat.2025.139022

35. Zhang Z, Shi J, Yao X, Wang W, Zhang Z, Wu H. Comparative evaluation of the impacts of different microplastics on greenhouse gas emissions, microbial community structure, and ecosystem multifunctionality in paddy soil. J Hazard Mater. 2024 Dec;480:135958. doi:10.1016/j.jhazmat.2024.135958

36. Wu J yong, Gao J min, Pei Y zhi, Luo K yan, Yang W hao, Wu J cheng, et al. Microplastics in agricultural soils: A comprehensive perspective on occurrence, environmental behaviors and effects. Chem Eng J. 2024 Jun;489:151328. doi:10.1016/j.cej.2024.151328

37. Munhoz DR, Beriot N. Impacts of Nano- and Microplastic Contamination on Soil Organisms and Soil–Plant Systems. Microplastics. 2025 Oct 1;4(4):68. doi:10.3390/microplastics4040068

38. Mostoni S, Milana P, Di Credico B, D'Arienzo M, Scotti R. Zinc-Based Curing Activators: New Trends for Reducing Zinc Content in Rubber Vulcanization Process. Catalysts. 2019 Aug 2;9(8):664. doi:10.3390/catal9080664

39. Tunali M, Nowack B. Towards including soil ecotoxicity of microplastics and tire wear particles into life cycle assessment. Ecotoxicol Environ Saf. 2025 Sep;303:118856. doi:10.1016/j.ecoenv.2025.118856

40. Xu JY, Ding J, Du S, Zhu D. Tire particles and its leachates: Impact on antibiotic resistance genes in coastal sediments. J Hazard Mater. 2024 Mar;465:133333. doi:10.1016/j.jhazmat.2023.133333

41. Qin YF, Pang JY, Li Y, Liu FY, Shen LQ, Li HZ, et al. Tire Wear Particle Microbiome-Derived Extracellular Vesicles Function as Potent Vectors Amplifying Antimicrobial Resistance Risk in Soil Ecosystems. Environ Sci Technol Lett. 2026 May 12;13(5):684–90. doi:10.1021/acs.estlett.6c00328

42. Maryam B, Rehman Z, Erkan HS, Engin GO, Debik E, Iqbal M, et al. Hidden Interfaces of Antibiotic Resistance Genes: How Plastispheres Threaten Environmental Integrity, Health Security, and One Health Systems. Water Air Soil Pollut. 2026 Nov;237(21):1211. doi:10.1007/s11270-026-09896-1

43. Ding J, Zhu D, Wang Y, Wang H, Liang A, Sun H, et al. Exposure to heavy metal and antibiotic enriches antibiotic resistant genes on the tire particles in soil. Sci Total Environ. 2021 Oct;792:148417. doi:10.1016/j.scitotenv.2021.148417

44. Ding J, Meng F, Chen H, Chen Q, Hu A, Yu CP, et al. Leachable Additives of Tire Particles Explain the Shift in Microbial Community Composition and Function in Coastal Sediments. Environ Sci Technol. 2022 Sep 6;56(17):12257–66. doi:10.1021/acs.est.2c02757

45. You S, Ying K, Zhang S, Lai C, Ullah H, Ismail AM, et al. Microplastics-Mediated Behavior of Potentially Toxic Elements in Plant–Soil Systems: Adsorption, Bioavailability, and Phytotoxicity. Toxics. 2026 Aug 17;14(8):730. doi:10.3390/toxics14080730

46. Weyrauch S, Seiwert B, Voll M, Reemtsma T. Long term biodegradation study on tire and road wear particles and chemicals thereof. Sci Total Environ. 2025 May;975:179240. doi:10.1016/j.scitotenv.2025.179240

47. Clarke CR, Cai R, Studholme DJ, Guttman DS, Vinatzer BA. *Pseudomonas syringae* Strains Naturally Lacking the Classical *P. syringae hrp/hrc* Locus Are Common Leaf Colonizers Equipped with an Atypical Type III Secretion System. Mol Plant-Microbe Interactions®. 2010 Feb;23(2):198–210. doi:10.1094/MPMI-23-2-0198

48. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 2012 Aug 1;6(8):1621–4. doi:10.1038/ismej.2012.8

49. Walters W, Hyde ER, Berg-Lyons D, Ackermann G, Humphrey G, Parada A, et al. Improved Bacterial 16S rRNA Gene (V4 and V4-5) and Fungal Internal Transcribed Spacer Marker Gene Primers for Microbial Community Surveys. Bik H, editor. mSystems. 2016 Feb 23;1(1):e00009-15. doi:10.1128/mSystems.00009-15

50. MacLean J, Mayanna S, Benning LG, Horn F, Bartholomäus A, Wiesner Y, et al. The Terrestrial Plastisphere: Diversity and Polymer-Colonizing Potential of Plastic-Associated Microbial Communities in Soil. Microorganisms. 2021 Sep 3;9(9):1876. doi:10.3390/microorganisms9091876

51. Bandopadhyay S, Liquet Y González JE, Henderson KB, Anunciado MB, Hayes DG, DeBruyn JM. Soil Microbial Communities Associated With Biodegradable Plastic Mulch Films. Front Microbiol. 2020 Nov 13;11:587074. doi:10.3389/fmicb.2020.587074

52. Ya H, Xing Y, Zhang T, Lv M, Jiang B. LDPE microplastics affect soil microbial community and form a unique plastisphere on microplastics. Appl Soil Ecol. 2022 Dec;180:104623. doi:10.1016/j.apsoil.2022.104623

53. Luo G, Jin T, Zhang H, Peng J, Zuo N, Huang Y, et al. Deciphering the diversity and functions of plastisphere bacterial communities in plastic-mulching croplands of subtropical China. J Hazard Mater. 2022 Jan;422:126865. doi:10.1016/j.jhazmat.2021.126865

54. Zhang M, Zhao Y, Qin X, Jia W, Chai L, Huang M, et al. Microplastics from mulching film is a distinct habitat for bacteria in farmland soil. Sci Total Environ. 2019 Oct;688:470–8. doi:10.1016/j.scitotenv.2019.06.108

55. Leifheit EF, Kissener HL, Faltin E, Ryo M, Rillig MC. Tire abrasion particles negatively affect plant growth even at low concentrations and alter soil biogeochemical cycling. Soil Ecol Lett. 2022 Dec;4(4):409–15. doi:10.1007/s42832-021-0114-2

56. Azeem I, Adeel M, Shakoor N, Zain M, Bibi H, Azeem K, et al. Co-exposure to tire wear particles and nickel inhibits mung bean yield by reducing nutrient uptake. Environ Sci Process Impacts. 2024;26(5):832–42. doi:10.1039/D4EM00070F

57. Guo A, Pan C, Zhou X, Bao Y. Effects of co-exposure of antibiotic and microplastic on the rhizosphere microenvironment of lettuce seedlings. Sci Total Environ. 2024 Dec;954:175983. doi:10.1016/j.scitotenv.2024.175983

58. Zhou J, Feng W, Brown RW, Yang H, Shao G, Shi L, et al. Microplastic contamination accelerates soil carbon loss through positive priming. Sci Total Environ. 2024 Dec;954:176273. doi:10.1016/j.scitotenv.2024.176273

59. Lv M, Chen H, Liang Z, Sun A, Lu S, Ren S, et al. Stress of soil moisture and temperature exacerbates the toxicity of tire wear particles to soil fauna: Tracking the role of additives through host microbiota. J Hazard Mater. 2024 Dec;480:135995. doi:10.1016/j.jhazmat.2024.135995

60. Buchanan SW, Antunes PM, Sauvadet M, Prosser R, Dunfield K. Shape matters: microplastic fibers disrupt soil carbon cycling via shape-driven physical disturbance. Environ Toxicol Chem. 2025 Oct 6;vgaf247. doi:10.1093/etojnl/vgaf247

61. Zhang X, Chen Y, Ho CM, Feng W, Yu X. Mechanisms of Microplastic Effects on Carbon and Nitrogen Cycling in Aquatic and Terrestrial Ecosystems. Toxics. 2026 Jun 24;14(7):551. doi:10.3390/toxics14070551

62. Aitken R, Moody P, Mckinley P. Lime requirement of acidic Queensland soils. I. Relationships between soil properties and pH buffer capacity. Aust J Soil Res. 1990 Oct 1;28(5):695–701. doi:10.1071/SR9900695

63. Yang Y, Wang Y, Peng Y, Cheng P, Li F, Liu T. Acid-base buffering characteristics of non-calcareous soils: Correlation with physicochemical properties and surface complexation constants. Geoderma. 2020 Feb;360:114005. doi:10.1016/j.geoderma.2019.114005

64. Song J, Beule L, Jongmans-Hochschulz E, Wichels A, Gerdts G. The travelling particles: community dynamics of biofilms on microplastics transferred along a salinity gradient. ISME Commun. 2022 Dec 1;2(1):35. doi:10.1038/s43705-022-00117-4

65. Sun Y, Shi J, Wang X, Ding C, Wang J. Deciphering the Mechanisms Shaping the Plastisphere Microbiota in Soil. Poretsky R, editor. mSystems. 2022 Aug 30;7(4):e00352-22. doi:10.1128/msystems.00352-22

66. Tagg AS, Sperlea T, Labrenz M, Harrison JP, Ojeda JJ, Sapp M. Year-Long Microbial Succession on Microplastics in Wastewater: Chaotic Dynamics Outweigh Preferential Growth. Microorganisms. 2022 Sep 2;10(9):1775. doi:10.3390/microorganisms10091775

67. Shim KY, Yeo IC, Lee E, Kim HJ, Kim KE, Mo J, et al. Polymer type drives successional structural and functional organization of plastisphere microbial networks. Mar Environ Res. 2026 Sep;221:108271. doi:10.1016/j.marenvres.2026.108271

68. Zhao X, Li C, Lu J, Sun L, Horsburgh MJ, Sekar R. Early colonization and temporal development of biofilms on microplastics: Influence of polymer type, season, and surrounding land use. J Hazard Mater Plast. 2026 Jul;4:100074. doi:10.1016/j.hazmp.2026.100074

69. Zayen A, Sayadi S, Tedetti M, Taba H, Sammari C, Mhiri N, et al. Time-driven plastisphere: Temporal dynamics shape the marine microplastic microbiome under natural field conditions. Mar Environ Res. 2026 Nov;222:108390. doi:10.1016/j.marenvres.2026.108390

70. Van Der Gast CJ, Ager D, Lilley AK. Temporal scaling of bacterial taxa is influenced by both stochastic and deterministic ecological factors. Environ Microbiol. 2008 Jun;10(6):1411–8. doi:10.1111/j.1462-2920.2007.01550.x

71. Stegen JC, Lin X, Konopka AE, Fredrickson JK. Stochastic and deterministic assembly processes in subsurface microbial communities. ISME J. 2012 Sep 1;6(9):1653–64. doi:10.1038/ismej.2012.22

72. Tripathi BM, Stegen JC, Kim M, Dong K, Adams JM, Lee YK. Soil pH mediates the balance between stochastic and deterministic assembly of bacteria. ISME J. 2018 Apr 1;12(4):1072–83. doi:10.1038/s41396-018-0082-4

73. Martiny JBH, Jones SE, Lennon JT, Martiny AC. Microbiomes in light of traits: A phylogenetic perspective. Science. 2015 Nov 6;350(6261):aac9323. doi:10.1126/science.aac9323

74. Nemergut DR, Schmidt SK, Fukami T, O'Neill SP, Bilinski TM, Stanish LF, et al. Patterns and Processes of Microbial Community Assembly. Microbiol Mol Biol Rev. 2013 Sep;77(3):342–56. doi:10.1128/MMBR.00051-12

75. Ning D, Yuan M, Wu L, Zhang Y, Guo X, Zhou X, et al. A quantitative framework reveals ecological drivers of grassland microbial community assembly in response to warming. Nat Commun. 2020 Sep 18;11(1):4717. doi:10.1038/s41467-020-18560-z

76. Rohrbach S, Gkoutselis G, Hink L, Weig AR, Obst M, Diekmann A, et al. Microplastic polymer properties as deterministic factors driving terrestrial plastisphere microbiome assembly and succession in the field. Environ Microbiol. 2023 Dec;25(12):2681–97. doi:10.1111/1462-2920.16234

77. Zhang Z, Zhao J, Li K, Wang X, Xu H, Mao D, et al. "Tire plastisphere" in aquatic ecosystems: Biofilms colonizing on tire particles exhibiting a distinct community

structure and assembly compared to conventional plastisphere. J Hazard Mater. 2025 Feb;483:136660. doi:10.1016/j.jhazmat.2024.136660

78. Ren J, Ding Y, Huang Z, Yang S, Chen F, Ma J, et al. Surface dynamics of newly released microplastics during early-stage aging in coastal water: Physicochemical property alterations and microbial community successions. Environ Pollut. 2026 Dec;411:129266. doi:10.1016/j.envpol.2026.129266

79. Tumrani SH, Qi R, Lei J, He X, Soomro RA, Bux N, et al. Biodegradable polymer backbone shapes plastisphere assembly, enhancing antibiotic sorption and reshaping aquatic microbial communities. J Hazard Mater. 2026 Oct;517:143538. doi:10.1016/j.jhazmat.2026.143538

80. Li K, Chen Z, Hao W. Electrochemical-oxidative dualism: Decoupling the acute effects of lake water-aged tire wear particles on periphytic biofilm-mediated denitrification. Environ Pollut. 2025 Oct;383:126876. doi:10.1016/j.envpol.2025.126876

81. Vannette RL, Fukami T. Historical contingency in species interactions: towards niche-based predictions. Wootton T, editor. Ecol Lett. 2014 Jan;17(1):115–24. doi:10.1111/ele.12204

82. Fukami T. Historical Contingency in Community Assembly: Integrating Niches, Species Pools, and Priority Effects. Annu Rev Ecol Evol Syst. 2015 Dec 4;46(1):1–23. doi:10.1146/annurev-ecolsys-110411-160340

83. Hawkes CV, Keitt TH. Resilience vs. historical contingency in microbial responses to environmental change. Classen A, editor. Ecol Lett. 2015 Jul;18(7):612–25. doi:10.1111/ele.12451

84. Zee PC, Fukami T. Priority effects are weakened by a short, but not long, history of sympatric evolution. Proc R Soc B Biol Sci. 2018 Jan 31;285(1871):20171722. doi:10.1098/rspb.2017.1722

85. Shade A, Peter H, Allison SD, Baho DL, Berga M, Bürgmann H, et al. Fundamentals of Microbial Community Resistance and Resilience. Front Microbiol. 2012;3. doi:10.3389/fmicb.2012.00417

86. Yang L, Yang W, Li Q, Zhao Z, Zhou H, Wu P. Microplastics in Agricultural Soils: Sources, Fate, and Interactions with Other Contaminants. J Agric Food Chem. 2025 May 16;acs.jafc.5c03682. doi:10.1021/acs.jafc.5c03682

87. Castan S, Sherman A, Peng R, Zumstein MT, Wanek W, Hüffer T, et al. Uptake, Metabolism , and Accumulation of Tire Wear Particle-Derived Compounds in Lettuce. Environ Sci Technol. 2023 Jan 10;57(1):168–78. doi:10.1021/acs.est.2c05660

88. Jiang K, Pan X, Zhu S, Dang Z, Yang Z, Huang L, et al. Cellulose/polyester-blended microplastics amplify plastisphere pathogen and antibiotic resistome risks. Bioresour Technol. 2027 Jan;463:135752. doi:10.1016/j.biortech.2026.135752

89. Sun Y, Wu M, Zang J, Du L, Huang M, Chen C, et al. Plastisphere microbiome: Methodology, diversity, and functionality. iMeta. 2023 May;2(2):e101. doi:10.1002/imt2.101

90. Guo T, Huang S, Jin X, Xing M, Gustave W, Cai Y, et al. Interactive effects of soil characteristics and polymer types reveal patterns of denitrifying bacteria enrichment in the soil plastisphere. J Hazard Mater. 2025 Nov;499:140237. doi:10.1016/j.jhazmat.2025.140237

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