

Distinct responses of soil microbial communities and nitrogen cycle genes to PBAT and PLA microplastics

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Research Article

Keywords: Biodegradable microplastics, microbial community structure, soil physicochemical properties, metagenomics

Posted Date: October 1st, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10709949/v1>

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Additional Declarations: No competing interests reported.

Abstract

This study compared the differential effects of two biodegradable microplastics, poly(butylene adipate-co-terephthalate) (PBAT) and polylactic acid (PLA), on soil nitrogen cycling and microbial communities. Results showed that PBAT and PLA exhibited distinct degradation behaviors under the same soil conditions. PBAT addition significantly increased soil pH and organic carbon concentration, while reducing ammonium and nitrate contents by 89.43% and 58.08%, respectively. In contrast, PLA decreased ammonium by 58.54% but increased nitrate by 20.53%. Metagenomic analysis revealed that PBAT suppressed nitrification genes *hao* and *amoCAB* by 45.83% and 87.78%, and enhanced denitrification genes *nirK* and *narGHI* by 50.88% and 84.08%. PBAT also induced stronger shifts in microbial community structure, with Pseudomonadota, Ascomycota, and Bacteroidota becoming dominant. For PLA, metagenomic sequencing further showed increased abundance of Planctomycetota, upregulation of *amoCAB* (4.35%) compensating for *Hao* reduction (9.17%), and enhanced *nxrAB* (35.28%) and *norBC* (38.05%). The nitrogen-fixing cluster *nifHDK* was markedly up regulated on PLA surfaces (165.79%) and in bulk soil (84.21%). This study elucidates the distinct mechanisms by which PBAT and PLA modulate soil nitrogen transformation. These findings highlight that biodegradable plastics are not uniformly safe and their environmental risks depend on polymer type, providing scientific references for the risk assessment of biodegradable plastic residues in agricultural soils.

1 Introduction

As a core component of Earth's surface ecosystems, soil supports plant growth, cycles biogenic elements like carbon, nitrogen and phosphorus, purifies pollutants, and serves as an ecological barrier, with soil health directly linked to food security, ecological balance and human well being (Kundu et al., 2025). However, rapid industrialization, urbanization and agricultural intensification have increased the variety and quantity of soil pollutants, placing soil under mounting environmental stress. Among these pollutants, plastic pollution has become a global concern due to its wide distribution, persistence and ecological risks (MacLeod et al., 2021). Beyond discarded plastic mulches and greenhouse films, plastic particles enter soils through fragmentation, wastewater irrigation, organic fertilizer application, atmospheric deposition, and agricultural tire wear, resulting in an average microplastic concentration of 1.7 mg/kg in farmland soils that continues to accumulate annually (Li et al., 2022; Bläsing et al., 2018; BÜKS et al., 2020). Owing to their chemical stability and high mobility, microplastics not only disrupt soil aggregates, reduce aeration, permeability and fertility, but also alter soil microbial community structure and function, thereby interfering with nutrient cycling and energy flow and posing a serious threat to soil ecosystem stability and sustainability (Yang et al., 2023; Zhou et al., 2021).

With worsening pollution caused by conventional plastics, biodegradable plastics have been widely adopted as environmentally friendly alternatives (Dai and Lan et al., 2025). Biodegradable plastics are currently extensively applied in agricultural mulch films, food packaging and disposable consumer goods. Global annual production of biodegradable plastics is projected to rise from 2.18 million tonnes in 2023 to 7.43 million tonnes by 2028, and these materials are regarded as one of the key approaches

for mitigating plastic pollution (European Bioplastics., 2023). However, the degradation efficiency of biodegradable plastics is strongly dependent on soil texture, pH and indigenous microbial communities. The degradation of biodegradable plastics can release additives such as plasticizers and generate biodegradable microplastics (BMPs), yet the environmental toxicity of these degradation products remains unclear, and a clear understanding is still lacking regarding the interactions between BMPs and coexisting soil contaminants such as heavy metals and pesticides, as well as the risks of combined pollution. Furthermore, inconsistent degradation performances among various BMP types as well as their underlying mechanisms altering microbial community functions, key enzyme activities and functional gene abundances are insufficiently explored.

Two types of biodegradable plastics widely used in agricultural production, namely PBAT and PLA, were selected as research subjects in this study. The degradation characteristics of the resulting BMPs in the soil environment were investigated, along with their effects on soil physicochemical properties, key enzyme activities, microbial community structure, and the abundance of nitrogen metabolism functional genes. This study further clarified the differential impacts of PBAT and PLA on soil functions, particularly the nitrogen cycling process, and elucidated the environmental behavior and potential ecological risks of these two BMPs in soil, thereby providing a solid theoretical foundation for the environmental safety assessment and scientific management of biodegradable microplastics.

2 Materials and Methods

2.1 Microplastics and Soil

PLA and PBAT microplastics (particle size: about 3–5 μm) were purchased from Jinheng Plastics Co., Ltd. (Dongguan, China). Soil samples were collected from agricultural fields in Houxi Town, Jimei District, Xiamen City, Fujian Province.

2.2 Experimental Design

Exogenous incorporation was adopted for PBAT and PLA microplastics amendment at a dosage of 5% (w/w, based on dry soil weight) (Fei et al., 2020). Specifically, 60 g of microplastics were homogeneously mixed into 1200 g of tested soil. Three replicates were set for each treatment and the control (CK, without BMPs). All treated and control soils were filled into flower pots with an inner diameter of 11 cm and a height of 10 cm, followed by 180 days of outdoor incubation under ambient conditions. Subsequently, soil and microplastic samples were collected. Microplastics were recovered by sieving, ultrasonically cleaned, and weighed.

2.3 Analytical Methods

2.3.1 Analysis of Soil Chemical Properties

Soil layers enriched with microplastics were sampled and sieved through a 60-mesh screen to remove coarse impurities and residual microplastics. Soil pH was determined in a 1:2.5 (w/v) soil water

suspension (Wang et al., 2020). Nitrate and ammonium N were extracted with 1 M KCl and quantified via a Shimadzu UV spectrophotometer (UV-1800, i2700) (Bao et al., 2000). Total organic carbon (TOC) was measured using a total organic carbon analyzer (TOC-L CPH, Shimadzu, Japan) (Wang et al., 2022).

2.3.2 Assessment of Soil Enzyme Activities

The activities of catalase, soil urease, and protease of soil were determined by spectrophotometry using commercial assay kits purchased from Hefei Lier Biotechnology Co., Ltd. (China) (Zhang et al., 2025).

2.3.3 Metagenomic Sequencing

Genomic DNA from plastic-attached and bulk soil was extracted with the Soil DNA Kit. The concentration and purity of DNA were quantified using a NanoDrop spectrophotometer. Metagenomic sequencing and subsequent bioinformatic analyses were performed on an Illumina NovaSeq HiSeq Xten platform (Illumina, USA) by Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China.

2.4 Data Processing

Normality of all datasets was verified via the Shapiro-Wilk test, and homogeneity of variances was assessed using the Levene test. Significant differences among treatments were analyzed by one-way analysis of variance (ANOVA).

3 Results and Discussion

3.1 Soil Physicochemical Properties

The effects of BMPs on soil pH are presented in Fig. 1. As shown in the analytical results, native soil without microplastic amendment (BC) exhibited an acidic pH of 5.785. A slight decline to 5.52 in soil pH was observed after PLA microplastic incorporation, while a remarkable rise to 6.10 was detected with PBAT amendment ($p < 0.05$). Furthermore, a higher degradation rate of 7.5% was detected for PBAT microplastics under soil conditions, while only a low degradation rate of 1.08% was recorded for PLA microplastics.

Soil total organic carbon (TOC) increased to 30.56 mg/L with PBAT supplementation, whereas concentrations of ammonium nitrogen ($\text{NH}_4^+\text{-N}$) and nitrate nitrogen ($\text{NO}_3^-\text{-N}$) declined by 89.43% and 58.08%, respectively. On the other hand, PLA amendment reduced the $\text{NH}_4^+\text{-N}$ content by 58.54% but increased the $\text{NO}_3^-\text{-N}$ concentration by 0.031 mg/L (Fig. 2). It is well established that carbon serves as the dominant elemental constituent of BMPs. As reported by Ya et al. (2022), microplastics added at 5% of soil dry weight can supply abundant carbon sources for the growth and reproduction of soil microorganisms. Furthermore, Wan et al. (2023) indicated that incorporated microplastics can act as substrates for carbon decomposition, where carbon containing microplastics are converted into dissolved carbon sources under the action of specific bacteria.

Regarding the underlying functional mechanisms, soil pH can be regulated by alkaline intermediate metabolites released during PBAT degradation. Specifically, the carbon source derived from PBAT stimulates microbial proliferation and immobilizes inorganic nitrogen, while simultaneously suppressing the activity of nitrifying bacteria. Consequently, this results in a synchronous reduction of both NO_3^- and NH_4^+ . In contrast, lactic acid produced from PLA degradation either preserves or further lowers soil acidity and activates nitrifying bacteria, thereby accelerating the transformation from NH_4^+ to NO_3^- . Therefore, despite the consumption of ammonium, nitrate accumulates markedly due to intensified nitrification, which may increase the risk of soil nitrogen leaching. This finding is consistent with the study by Chen et al. (2020), in which PLA significantly reduced the concentration of $\text{NH}_4^+\text{-N}$ while markedly increasing both $\text{NO}_3^-\text{-N}$ and $\text{NO}_2^-\text{-N}$ contents. Such changes can be attributed to the accelerated transformation from $\text{NH}_4^+\text{-N}$ to $\text{NO}_3^-\text{-N}$ promoted by microplastics in the soil.

3.2 Effects of Biodegradable Microplastics on Soil Enzyme Activities

Changes in major soil enzyme activities following the amendment of two BMPs are illustrated in Fig. 3. Soil enzyme activities were significantly affected by the addition of BMPs. Catalase, a critical enzyme involved in soil redox reactions, showed significantly elevated activity under both PBAT and PLA treatments compared with the control. Specifically, catalase activity increased from 6.02 U/g to 25.34 U/g in the PBAT amended soil, while a 98.29% rise was observed following PLA addition (Fig. 3a). Protease activities were also enhanced, with increases of 15.93% and 13.17% for PBAT and PLA, respectively (Fig. 3b). In contrast, no obvious differences in urease activity were detected between any BMP amended soils and the control, suggesting that BMPs exerted negligible regulation on urease, which is responsible for the initial transformation of soil organic nitrogen (Fig. 3c). Collectively, soil enzyme activities can be altered by BMPs incorporation, and the magnitude of enzyme promotion varies with microplastic type (PBAT or PLA).

Bioavailable carbon sources released during the degradation of BMPs in soil can supply energy and nutrients for microorganisms, thereby stimulating the synthesis and secretion of functional enzymes (Li et al., 2025). Similarly, the present study observed elevated protease activities in BMPs amended soils, which is consistent with this mechanism. Moreover, the comparable promotion effects on protease activity induced by PBAT and PLA may be attributed to the minor difference in total bioavailable carbon released from the two BMPs. Reactive oxygen species generated by fine microplastic particles induce microbial oxidative stress, which prompts microbes to synthesize catalase for the removal of excess reactive oxygen species (Lu et al., 2025). Such physiological response accounts for the remarkable rise in catalase activity observed in soils amended with BMPs in our study. In addition, the divergence in catalase activity between PBAT and PLA amended soils may stem from differences in the induction intensity of reactive oxygen species, which are caused by discrepancies in degradation rate of the two plastics.

3.3 Effects of Microplastics on Soil Microbial Community

To explore the succession of microbial community structure during the degradation of BMPs in soil, soil from control without BMPs (BC), bulk soil away from PBAT (PBAT-BS) and PLA (PLA-BS), and plastic attached surface soil (PBAT-PS, PLA-PS) were collected. Metagenomic sequencing was used to analyze the microbial community composition and changes in functional gene abundance. As shown in Table 1, the alpha diversity index of the non-adhering bulk soil after BMPs addition was significantly higher than that of the control without BMPs, and the microbial community structure in the plastisphere soil was more diverse, indicating that BMPs addition effectively enhanced the richness and diversity of the soil microbial community. Furthermore, the microbial community structure in both the PBAT-adhering soil and the non-adhering bulk soil was higher than that observed for PLA, suggesting that PBAT promotes the richness and diversity of soil microbial species to a greater extent than PLA addition.

As revealed by the cluster analysis in Fig. 4, all soil samples were clearly divided into two major clusters at the clustering level. Specimens of PBAT-PS and PBAT-BS were tightly clustered together, indicating a high similarity in their microbial community composition. PLA-PS and PLA-BS were firstly grouped with each other and then incorporated into the same large cluster together with the soil without BMPs amendment. The clustering results fully demonstrate that the introduction of the two BMPs reshapes the species composition and relative abundance of soil microbial communities to varying degrees. The microbial community under PLA addition shares high similarity with that of the soil without BMPs amendment, with no remarkable community shift observed. In contrast, obvious divergence in microbial community is induced by PBAT addition, forming a distinct community separation from both the soil without BMPs amendment and the PLA amended soils.

Moreover, phylum-level heatmap analysis clarified differences in microbial composition among soils amended with different BMPs. Compared with unamended soil, Pseudomonadota, Ascomycota and Bacteroidota became dominant in PBAT-BS, with relative abundance increases of 2.00%, 36.91% and 7.19%, respectively, while in PBAT-PS, Bacillota and Chloroflexota were highly colonized on the microplastic surface soil, showing relative abundance elevations of 7.68% and 7.04%. The capability of *Bacillus pumilus* (belonging to Bacillota) to degrade PBAT was verified by Muroi et al. (2017). Similarly, Chien et al. (2022) reported that Ascomycota exhibit prominent biodegradation potential toward the biodegradable plastic PBSA. The enrichment of these dominant phyla in PBAT-PS and PBAT-BS following PBAT addition provides microbiological confirmation of the biodegradation potential of PBAT. Meanwhile, the relative abundance of Acidobacteriota was significantly lower in PBAT amended soils than in soil without microplastic amendment and PLA amended soils, which can be attributed to the rise in soil pH during PBAT degradation. Such an elevated pH environment is unfavorable for the survival and proliferation of Acidobacteriota, thereby restricting its growth and relative abundance in the soil.

Table 1
Alpha diversity indices of soil microbial communities in response to different types of biodegrade microplastic additions

BC	Sobs	Ace	Chao 1	Shannon	Simpson
	27910	27910	27910	6.241156	0.012572
PBAT-BS	28965	28965	28965	6.556793	0.007777
PBAT-PS	29390	29390	29390	6.743364	0.006089
PLA-BS	28656	28656	28656	6.356742	0.009212
PLA-PS	29517	29517	29517	6.435998	0.008851

Variations in the relative abundance of soil microorganisms at the genus level after PBAT and PLA amendment are displayed in Fig. 5. As shown in Fig. 5, genera including *Bradyrhizobium*, *Nocardioides*, *Sphingomonas*, *Paraburkholderia* and *Streptomyces* were significantly enriched and became dominant in the soil with PBAT amendment. The abundances of *Bradyrhizobium*, *Paraburkholderia* and *Chitinophaga* were significantly increased in soil amended with 5% PBAT, and these genera can serve as core biomarkers of PBAT affected soil microbial communities (Liu et al., 2022). By contrast, the genus level distribution in PLA amended soil showed different patterns, where the relative abundance of *Chloracidobacterium* was significantly higher than in unamended soil, with an 8.00% increase in the plastisphere soil relative to the same unamended soil, while the abundances of *Sphingomonas* and *Sphingomicrobium* decreased. The relative abundances of most other genera remained comparable to those in unamended soil, indicating that the soil microbial community was relatively weakly disturbed by PLA. Collectively, these results suggest that changes in soil microorganisms are not only associated with the type of microplastics added but also regulated by the contact distance between soil and microplastics.

3.4 Analysis of Functional Genes Involved in Nitrogen Cycling

The abundance changes of genes encoding key enzymes involved in nitrogen fixation, nitrification, anammox, and denitrification were analyzed in PBAT and PHA amended soils using the KEGG nitrogen metabolism pathway (ko00910) as a functional annotation reference framework, aiming to elucidate the impact of BMPs on core soil nitrogen metabolism pathways at the molecular level.

Variations in the abundance of nitrogen metabolizing functional genes after PBAT and PLA supplementation are presented in Fig. 6. As shown in Fig. 6, the relative abundances of the *hao* gene and the *amoCAB* gene family related to nitrification were markedly reduced in soil with PBAT addition, accompanied by significant down regulation of corresponding metabolic pathways K10535, K10944, K10945 and K10946. The *hao* gene encodes hydroxylamine oxidase, a rate limiting enzyme indispensable for nitrification (Prommer et al., 2014). Correspondingly, the abundance of *hao* gene declined by 45.83% in PBAT attached plastisphere soil and by 64.17% in surrounding bulk soil. The

amoCAB gene family codes for the ammonia monooxygenase complex (AmoA/B/C), the core catalytic component of ammonia oxidation, and the abundance of this gene family decreased by 87.78% on PBAT surface soil and by 86.56% in non-attached bulk soil. Coordinated suppression of these genes jointly blocks soil nitrification and substantially hinders the transformation from NH_4^+ to NO_3^- within PBAT amended soil.

The abundances of core denitrifying functional genes (*nirK*, *nirS*, *narGHI*, and *napAB* gene families) were increased under PBAT amendment, which further upregulated denitrification-related metabolic pathways of K00370, K00371, K00374, K02567, K02568, K00368 and K15764 (Li et al., 2021). In PBAT attached plastisphere soil, the abundances of *nirK* and *nirS*, which encode copper containing nitrite reductase and cytochrome cd_1 type nitrite reductase respectively, increased by 50.88% and 227.27%, thereby enhancing the catalytic reduction of NO_2^- to NO. The *narGHI* gene abundance rose by 84.08% in plastisphere soil and 21.08% in bulk soil. For the *napAB* gene family encoding membrane-bound and periplasmic nitrate reductase, its abundance was enhanced by 117.07% and 101.20% in attached and bulk soil correspondingly.

Collectively, the elevated abundance of denitrifying genes driven by PBAT facilitates the coordinated reduction of NO_3^- , thereby decreasing soil nitrate concentration markedly. In addition, PBAT significantly upregulated *nirK* and *nirS* involved in anammox. Enhanced gene expression accelerates the coupled transformation between NH_4^+ and NO_2^- , ultimately resulting in a remarkable decline of ammonium content in PBAT amendment soil (Liu et al., 2026).

The abundance of the *hao* gene encoding hydroxylamine oxidoreductase declined by 9.17% relative to the soil without microplastic amendment following PLA addition, whereas the ammonia monooxygenase gene cluster *amoCAB* was increased by 4.35%. As the core gene governing the rate-limiting step of nitrification, *amoCAB* encodes ammonia monooxygenase (AMO), a key enzyme controlling ammonia oxidation rate. The significantly higher abundance of *amoCAB* compared with the *hao* gene guarantees sufficient enzyme activity and sustains normal nitrification, which constitutes the core molecular mechanism responsible for elevated concentration in PLA amendment soil.

Besides, obvious increases in the relative abundance of other nitrogen cycle functional genes were detected in the plastisphere soil of PLA. The *nxrAB* gene for nitrite oxidoreductase rose by 35.28%, further facilitating the progression of nitrification. Meanwhile, a 38.05% increment was observed for the nitric oxide reductase gene *norBC*, implying active intermediate denitrification metabolism in the soil attached to PLA. The key nitrogen fixing gene cluster *nifHDK* was extremely enriched both in PLA attached soil and surrounding bulk soil, with respective increases of 165.79% and 84.21% versus the control. Such variation demonstrates that PLA improves microhabitat conditions to enrich nitrogen fixing microbes and supplies an extra biological pathway for soil nitrogen input.

4 Conclusions

Soil nitrogen cycling and microbial communities were shaped by PBAT and PLA microplastics through opposing regulatory mechanisms at community and molecular functional levels. PLA promoted nitrification and nitrate accumulation, while PBAT suppressed nitrification and enhanced anammox and denitrification, reducing inorganic nitrogen pools. Functional gene analysis further confirmed such differential regulation at the molecular level. PBAT inhibited the expression of key nitrification genes but upregulated denitrification and anammox related functional genes. By comparison, PLA enriched core genes responsible for nitrification and nitrogen fixation to facilitate soil nitrogen oxidation and input. Both microplastics increased microbial diversity but induced distinct compositional shifts. PBAT favored Pseudomonadota, Ascomycota and Bacteroidota, whereas PLA targeted Acidobacteriota and Planctomycetota. These results reveal that BMPs are not ecologically interchangeable and their divergent effects on nitrogen transformation pathways, functional gene profiles, and microbial community assembly warrant differentiated risk assessments.

Declarations

Author Contribution

Y.C. Wu and B.J. Shu designed and performed the experiments, writing original draft. F.P. Xu, A.L. Yang and H.Y. Fu helped analysis all the data. Y.C. Wu was responsible for supervision and validation. All authors discussed and approved the manuscript.

Acknowledgments

This work was supported by Natural Science Foundation of Fujian Province, China (2025I0052, 2025Y4010).

Data Availability

All data supporting the findings of this study are available within the paper and its Supplementary Information. Metagenomic sequencing and subsequent bioinformatic analyses were performed on an Illumina NovaSeq HiSeq Xten platform (Illumina, USA) by Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China.

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Figures

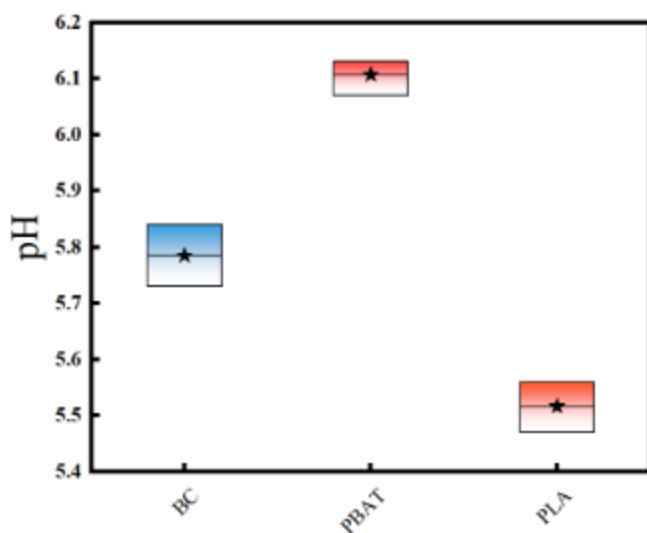


Figure 1

Effects of different types of microplastics on soil pH

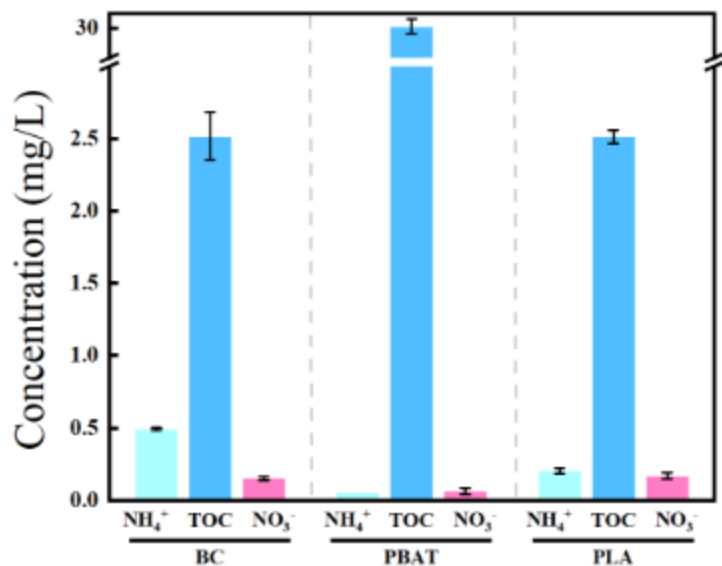


Figure 2

Effects of different types of biodegradable microplastics on concentrations of NH₄⁺-N, NO₃⁻-N and TOC in soil

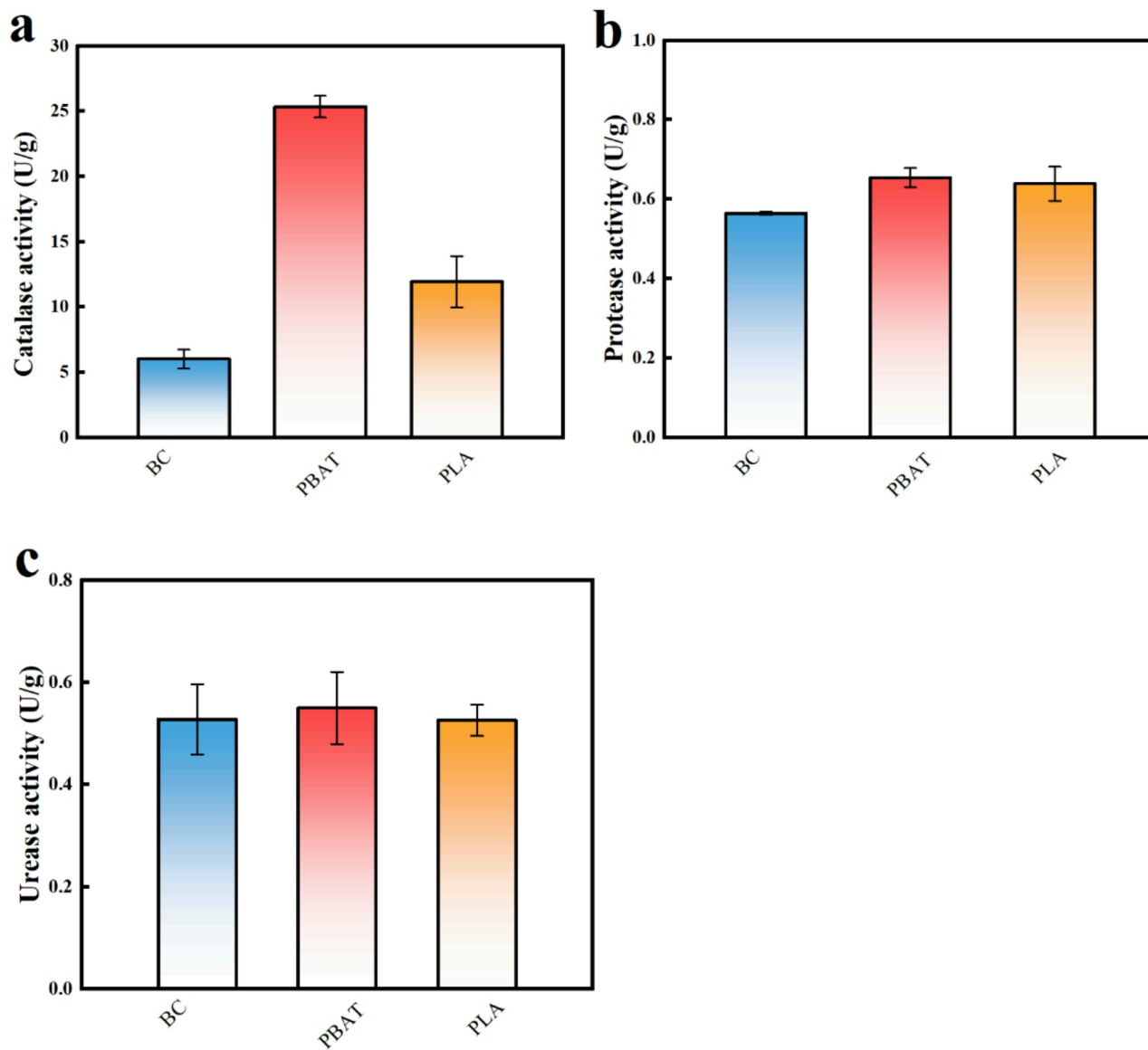


Figure 3

Effects of different types of microplastics on soil enzyme activities: (a) Catalase activity; (b) Protease activity; (c) Urease activity.

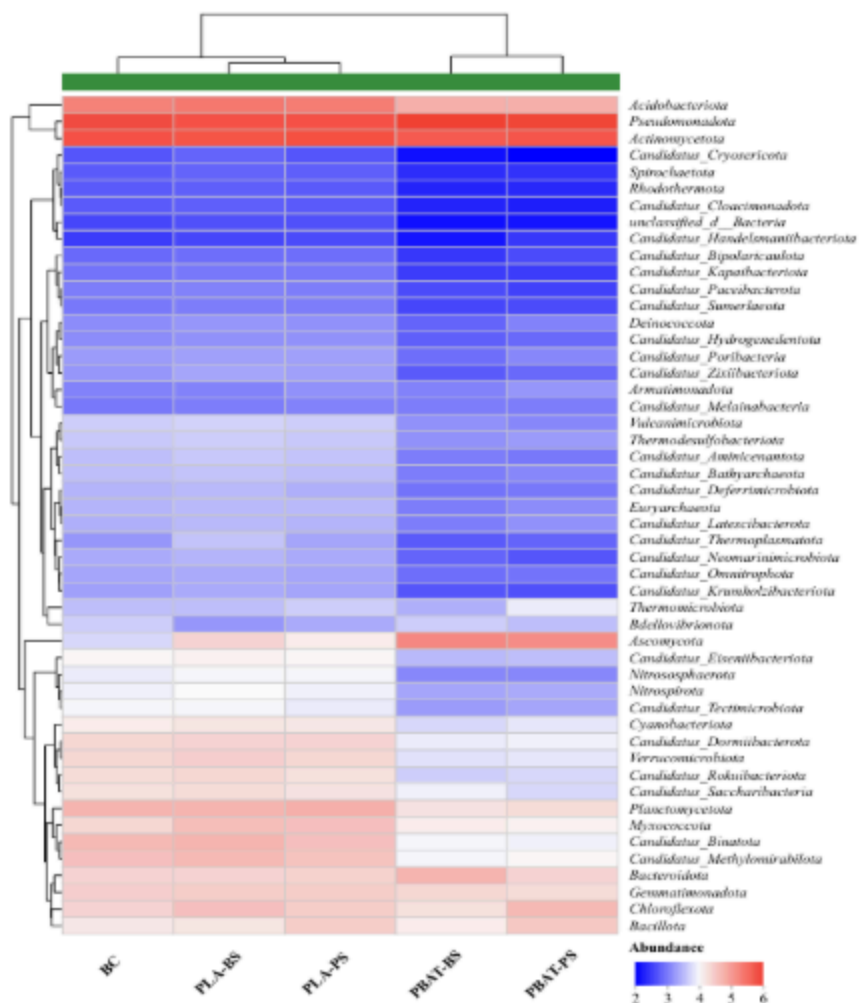


Figure 4

Heatmap analysis of microbial relative abundance at the phylum level

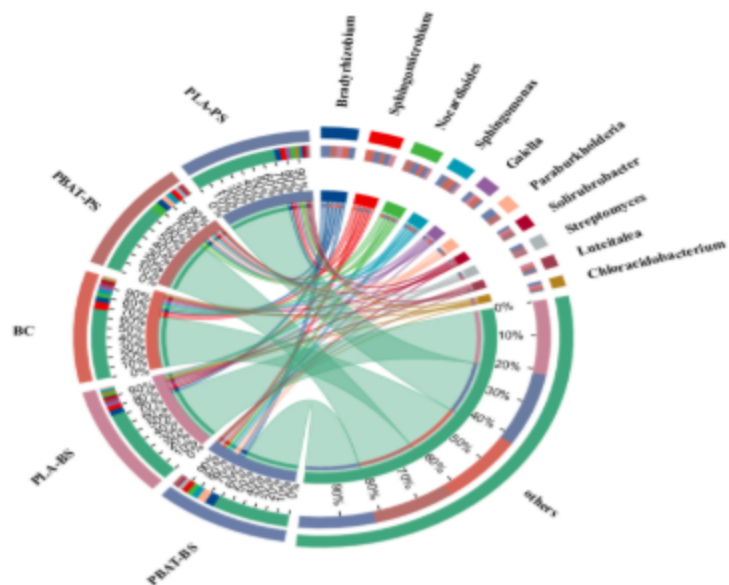


Figure 5

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Taxonomic composition of microbial communities at the genus level

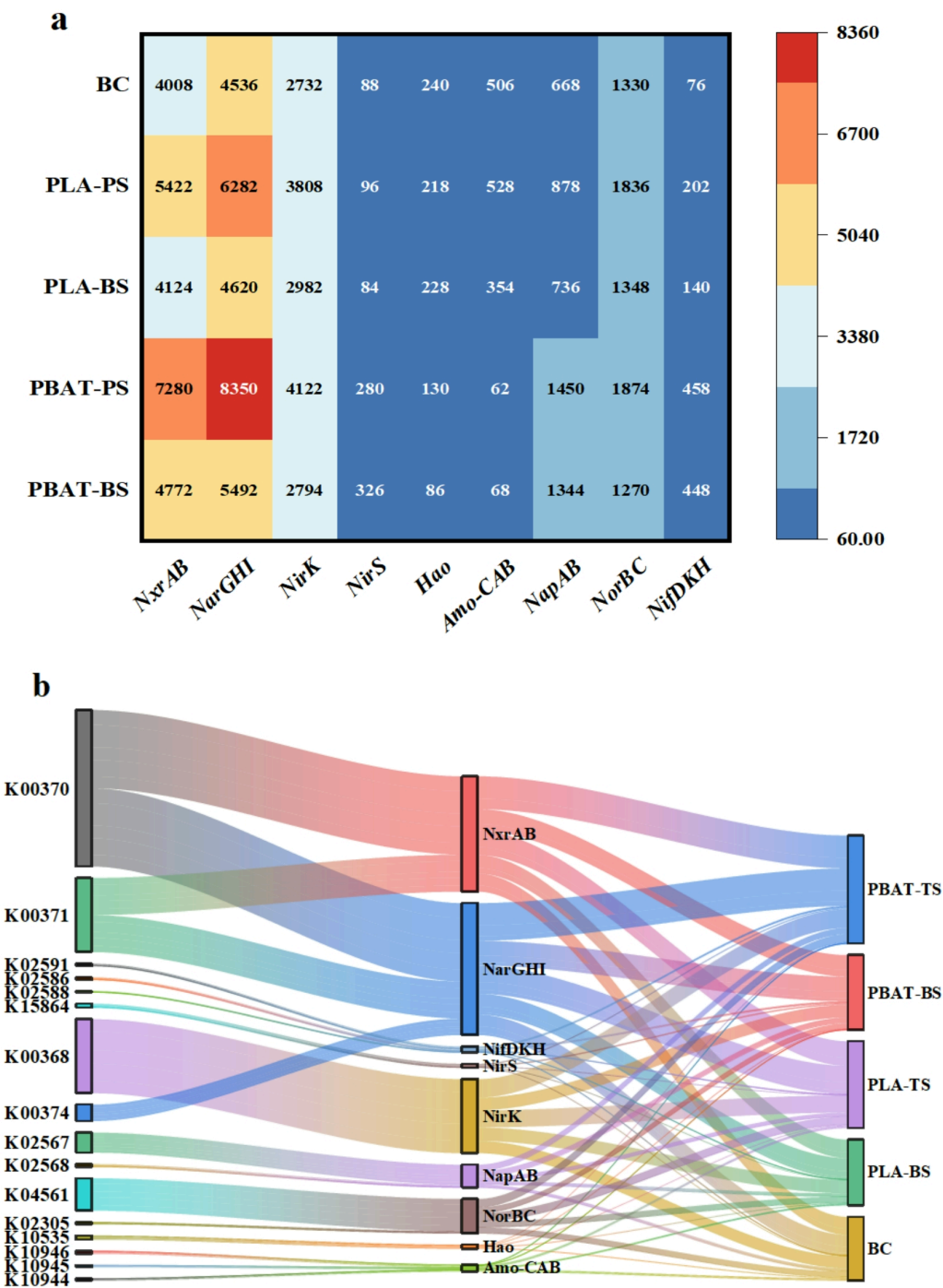


Figure 6

Nitrogen metabolism functional genes were analyzed in response to PBAT and PLA amendments. (a) Relative abundance of functional genes. (b) KO composition.