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Global Meta-Analysis Integrated with Machine Learning Assesses Context-Dependent Microplastic Effects on Soil Microbial Biomass Carbon and Nitrogen

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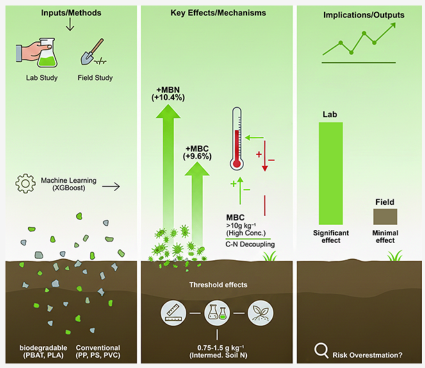
ABSTRACT: Microplastics (MPs) in soil can paradoxically stimulate microbial biomass in a highly context-dependent manner, potentially inducing decomposition and affecting carbon and nitrogen cycles. We conducted a global meta-analysis with 90 studies (710 observations of microbial biomass carbon (MBC), 354 of microbial biomass nitrogen (MBN)) integrated with machine learning to quantify MPs effects on soil microbial biomass. Field studies showed no significant effects, contrasting with controlled experiments where MPs increased MBC by 9.6% (95% CI: 7.2–11.9%) and MBN by 10.4% (6.8–14.0%). Biodegradable plastics (PBAT, PLA) induced stronger effects (36.1–67.6%) than conventional polymers (PE, PP, PS, PVC). Temperature emerged as the dominant factor, with a contrasting MPs effect on MBC (positive) and MBN (negative) at higher temperatures, suggesting potential decoupling of carbon and nitrogen cycles under warming conditions. Machine learning models (XGBoost, R^2 = 0.62) significantly outperformed linear regressions (R^2 = 0.02–0.05), revealing nonlinear responses and threshold effects. Stimulatory effects were most significant for medium-sized MPs (30–90 μm), at high concentrations (>10 g kg^{-1}), and in soils with intermediate fertility, highlighting context-dependent risks to soil carbon and nitrogen cycling.



KEYWORDS: *biodegradable plastics, carbon cycling, nitrogen cycling, soil microbial biomass, temperature effects*

1. INTRODUCTION

Microplastics (MPs), defined as plastic particles <5 mm, have emerged as significant contaminants in terrestrial ecosystems worldwide.[1,2] Studies suggest that annual plastic inputs to soils exceed those entering oceans by 4–23 fold, with agricultural lands receiving 63,000–430,000 tons of MPs annually through sewage sludge application, plastic mulch degradation, and atmospheric deposition.[3,4] MPs can persist for decades to centuries once it incorporated into soils, potentially affecting fundamental biogeochemical processes.[5,6]

Soil microbial biomass carbon (MBC) and nitrogen (MBN) are critical indicators of soil health, integrating microbial abundance, activity, and nutrient immobilization capacity.[7−9] MBC and MBN are sensitive to environmental perturbations and serve as indicators of soil quality degradation.[10,11] However, an increase in microbial biomass does not constantly benefit soil health or long-term carbon sequestration. While microbial residues can contribute to stable humus formation,[12] a rapid biomass increase often driven by labile carbon inputs like biodegradable microplastics may indicate a transient response.[13] This acceleration can trigger priming effects that decompose native soil organic matter,[14] potentially resulting in net carbon loss as carbon dioxide.[13] Moreover, the functional composition and turnover rate of microbial communities are essential factors.[12] MP-induced imbalances may disrupt nutrient cycling and decouple carbon and nitrogen processes,[15] finally threatening ecosystem stability. Therefore, understanding the context and consequences of the biomass increase is critical for accurate soil health assessment.

MPs may affect microbial biomass through multiple mechanisms: (1) providing unique surfaces for biofilm colonization, effectively expanding the soil habitat space;[16] (2) altering the soil physical structure, such as porosity, water retention, and aggregate stability;[17] (3) releasing plastic additives and degradation products that may serve as toxicants (e.g., bisphenol A);[18,19] and (4) influencing nutrient availability through surface adsorption or desorption processes.[20]

Despite growing research on MPs, their effects on soil MBC and MBN remain highly inconsistent across studies. Meta-analyses report divergent conclusions: Yan et al.[21] found no significant changes in MBC or MBN, whereas multiple studies

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reported significant increases, particularly in farmland soils (MBC +33.2% (percentage change), MBN +93.7%).[22] Positive effects were further suggested by Su et al.[23] (MBC +5.5% [CIs: 0.1−11.0%]), Zhao et al.[24] (MBC + 9.4% [2.0−16.2%], MBN + 25.9% [10.5−43.3%]), and Fan et al.[25] (MBC + 32.7% [24.6−41.3%], MBN + 57.6% [26.0−97.4%]). Notably, mixed responses occurred even within similar contexts: Liu et al.[26] reported MBC increased by 19.6% [0.9−42.0%] but no MBN change (−1.41% [−69.0−43.0%]), while Liu et al.[27] found MBC + 8.0% [0.9−15.4%] with neutral MBN effects (0.6% [−18.3−24.3%]). This variability likely results from complex interactions among MPs properties (polymer type, size, concentration), experimental conditions (duration, temperature), and soil properties (soil pH, organic matter content).[17,28−30] Moreover, most studies evaluate single factors under controlled conditions,[31] limiting extrapolation to field environments where multifactorial stressors coexist.

Conventional meta-analysis, while valuable for synthesizing central tendencies, often assume linear relationships and struggle to capture higher-order interactions or thresholds.[32] Machine learning (ML) provides a complementary framework by integrating nonlinear responses, high-dimensional feature spaces, and complex interactions without requiring a priori specification of functional forms.[33,34] Recent applications in environmental science have showed the utility of ML for predicting species distributions, contaminant fate, and ecosystem responses to global change.[35,36] These nonlinearities are ecologically critical because they suggest threshold behaviors. For instance, MPs may have minimal effects below certain concentrations but cause rapid ecosystem shifts above critical thresholds, similar to tipping points observed in other environmental systems. Despite this potential, ML remains underutilized for assessing and synthesizing MPs impacts on soil biota.

Here, we integrated quantitative meta-analysis with ML algorithms to develop a predictive insight into MPs effects on soil microbial biomass. Our objectives were to (1) quantify the overall magnitude and direction of MPs impacts on MBC and MBN through meta-analysis; (2) evaluate how experimental setup, MPs characteristics, and soil properties regulate these effects; (3) develop and compare ML models to identify primary drivers and their relative importance; and (4) illustrate nonlinear relationships and interaction effects through interpretable ML techniques. This work would provide a comprehensive assessment of MPs impacts on soil microbial biomass and suggests a framework for predicting responses under diverse environmental scenarios.

2. MATERIALS AND METHODS

2.1. Data Collection. All relevant peer-reviewed articles and dissertations on the effects of microplastics exposure on soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were comprehensively identified from the Web of Science (http://www.webofknowledge.com), Google Scholar (http://scholar.google.com), and the China National Knowledge Infrastructure (https://www.cnki.net) up to 8 February 2025. The search strategy employed terms such as (microplastic* OR "plastic microparticles") AND (soil) AND ("microbial biomass carbon" OR "microbial biomass C" OR MBC OR "microbial biomass nitrogen" OR "microbial biomass N" OR MBN).

For the selection of suitable publications, peer-reviewed articles were screened, and data were collected based on the following criteria: (1) at least one response variable must be reported, either microbial biomass carbon (MBC) or microbial biomass nitrogen (MBN); (2) the study design must include at least one control group and one treatment group, and in cases where multiple treatments are present, each treatment group must be individually compared with the control group; (3) for studies with identical experimental data published in multiple sources, those providing the most comprehensive information were included; (4) the study design should include at least three replications, and the sample size (n) for each treatment must be provided; and (5) the reported data must include means and their corresponding standard deviation (SD), and if the standard error (SE) is reported, it should be converted to the standard deviation (SD). Following this screening process, a total of 710 observations of MBC and 354 of MBN were extracted from the 90 included studies worldwide (Figures S1 and S2).

2.2. Data Compilation and Stratification. For all included studies, response variables (MBC, MBN) and potential regulator variables (e.g., experimental setup (ES), plant presence, nitrogen fertilization (NYN), exposure temperature (ET), exposure duration (ED), polymer composition (PT), microplastic type (MPT), microplastic size (MS), microplastic concentration (MPC), soil clay content (SCC), initial soil pH (ISpH), initial SOC (ISOC), and initial soil TN (ISTN)) were extracted using standardized protocols. Means, SDs, and sample sizes were obtained directly from text and tables or digitized from figures using GetData Graph Digitizer v2.26. SEs were converted to SDs using SD = SE × $\sqrt{n}$.

Regulator variables were classified into four categories, and continuous variables were categorized based on data distribution and ecological relevance (Table S1):

1 Experimental factors: setup (field, pot, incubation),[37] plant presence/absence,[28] nitrogen fertilization (yes/no),[38] temperature (°C),[39] and duration (days).[24]

2 Microplastic properties: polymer type (PBAT, PE, PLA, PP, PS, PVC),[39] degradability (biodegradable/conventional),[23] size (μm),[40] and concentration (g kg^{-1}, Table S1).[40]

3 Soil properties: clay content (%),[40] pH,[27] organic carbon (g kg^{-1}),[28] and total nitrogen (g kg^{-1}).[41]

2.3. Data Analysis. *2.3.1. Meta-Analysis.* The natural logarithm-transformed response ratio (ln(RR)) was utilized to evaluate the impact of MPs exposure on soil microbial biomass, which was defined as the ratio of variables between the microplastics treatment group and the control group (eq 1):[42]

$$\ln \mathrm{RR} = \ln(\overline{X_t}/\overline{X_c}) = \ln(\overline{X_t}) - \ln(\overline{X_c}) \quad (1)$$

where $\overline{X_t}$ and $\overline{X_c}$ are the means of the groups with and without microplastics addition, respectively.

Because multiple data points shared identical experimental or control settings, a variance-covariance (VC) matrix was employed to correct for interdependencies within the data set. Following the methodology of Lajeunesse[43] in analyses comparing experimental groups A and B to a common control (C), the VC matrix is constructed using the approach defined in eq 2[44]

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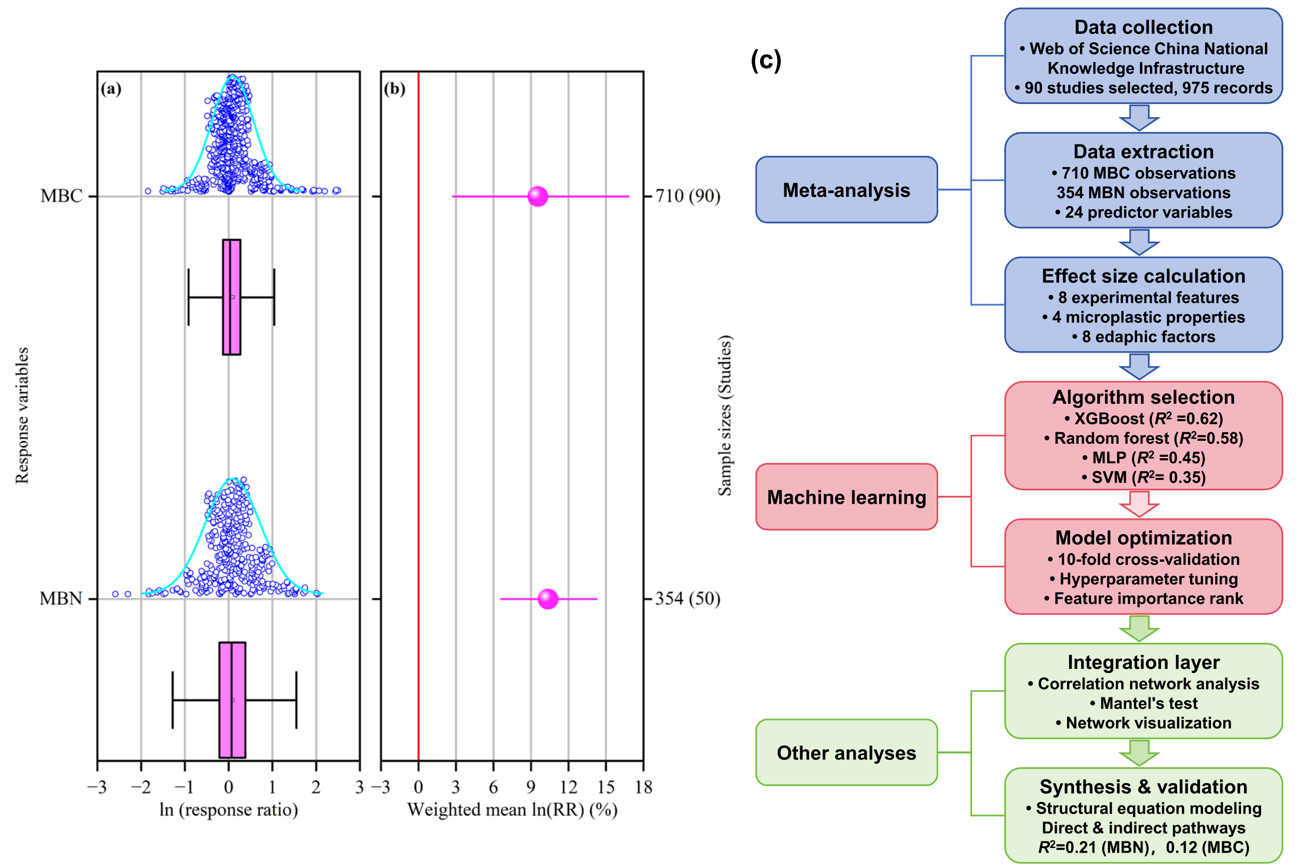

Figure 1. Distribution of ln (response ratio) (a) and weighted mean ln(RR) (b) associated with microplastics exposure on soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN). (c) Flowchart indicating data flow from meta-analysis to machine learning.

$$V^{\bar{X}_C} = \begin{bmatrix} \dfrac{(\mathrm{SD}_C)^2}{N_C \bar{X}_C^2} + \dfrac{(\mathrm{SD}_T^A)^2}{N_T^A (\bar{X}_T^A)^2} & \dfrac{(\mathrm{SD}_C)^2}{N_C (\bar{X}_C)^2} \\ \dfrac{(\mathrm{SD}_C)^2}{N_C (\bar{X}_C)^2} & \dfrac{(\mathrm{SD}_C)^2}{N_C \bar{X}_C^2} + \dfrac{(\mathrm{SD}_T^B)^2}{N_T^B (\bar{X}_T^B)^2} \end{bmatrix} \tag{2}$$

where SD_C, SD_T^A, and SD_T^B denote the standard deviations of the measured variable for the control group and MPs-treated groups A and B, respectively. Similarly, N_C, N_T^A, and N_T^B correspond to the sample sizes for the variable for the control group and MPs-treated groups A and B, respectively, whereas $\bar{X}_C$, $\bar{X}_T^A$, and $\bar{X}_T^B$ signify the mean values of the variable for the control group and MPs-treated groups A and B, respectively. To construct the variance-covariance matrix, a modified version of the "covariance_commonControl ()" function from the "metagear" package was utilized.[45]

When the 95% confidence interval (CI) excludes zero, the overall effect of MPs exposure on the response variable is deemed statistically significant. Conversely, the CI overlaps with zero, and the effect is viewed as nonsignificant. To simplify interpretation, the weighted RR (RR_{++}) and associated 95% CIs were expressed as percentage changes via the formula: effect sizes (%) = [exp(RR_{++}) − 1)] × 100.

Sensitivity analysis was performed to ensure the stability and credibility of meta-analytic findings for detecting study-specific influences. We conducted a leave-one-out approach using the "metafor" package[45] in *R* (v4.3.3), to assess publication bias, we first constructed funnel plots to visualize the distribution of effect sizes against their precision. We then applied Egger's regression test to statistically evaluate funnel plot asymmetry, where significant asymmetry indicates potential publication bias.[46] The results (Table S2 and Figure S3) suggested a balanced spread of observations across the funnel plots, supporting the absence of systematic bias. Furthermore, as a sensitivity analysis to evaluate the robustness of our results to potential publication bias, the trim-and-fill method (trimfill function) was applied to estimate the number of unpublished or omitted studies and adjust effect sizes accordingly, thereby enhancing the validity of the meta-analytic conclusions.[47] Comprehensive sensitivity analysis outcomes, including these adjustments, are provided in the supporting documentation (Figure S4).

2.3.2. Machine learning Analysis. Five ML algorithms were implemented: boosted regression trees (BRT), extreme gradient boosting (XGBoost), random forest (RF), multilayer perceptron (MLP), and support vector machines (SVM). Models were developed using the "gbm" (v2.1.8), "xgboost" (v1.7.3),[48] "randomForest" (v4.7.1),[49] "neuralnet" (v1.44.2),[50] and "e1071" (v1.7.13)[51] packages in R.

We screened completeness and imputed missing values ("mice" package (v3.14.0)), excluded variables with >20% missingness, and retained 710 MBC and 354 MBN observations for modeling (details are given in the Supporting Information).

Prior to modeling, predictor multicollinearity was assessed using variance inflation factors (VIF <5 retained). Data were split 80:20 for training:testing using stratified random sampling by study. Model hyperparameters were optimized through 10-fold cross-validation with grid search: BRT: tree complexity (3–

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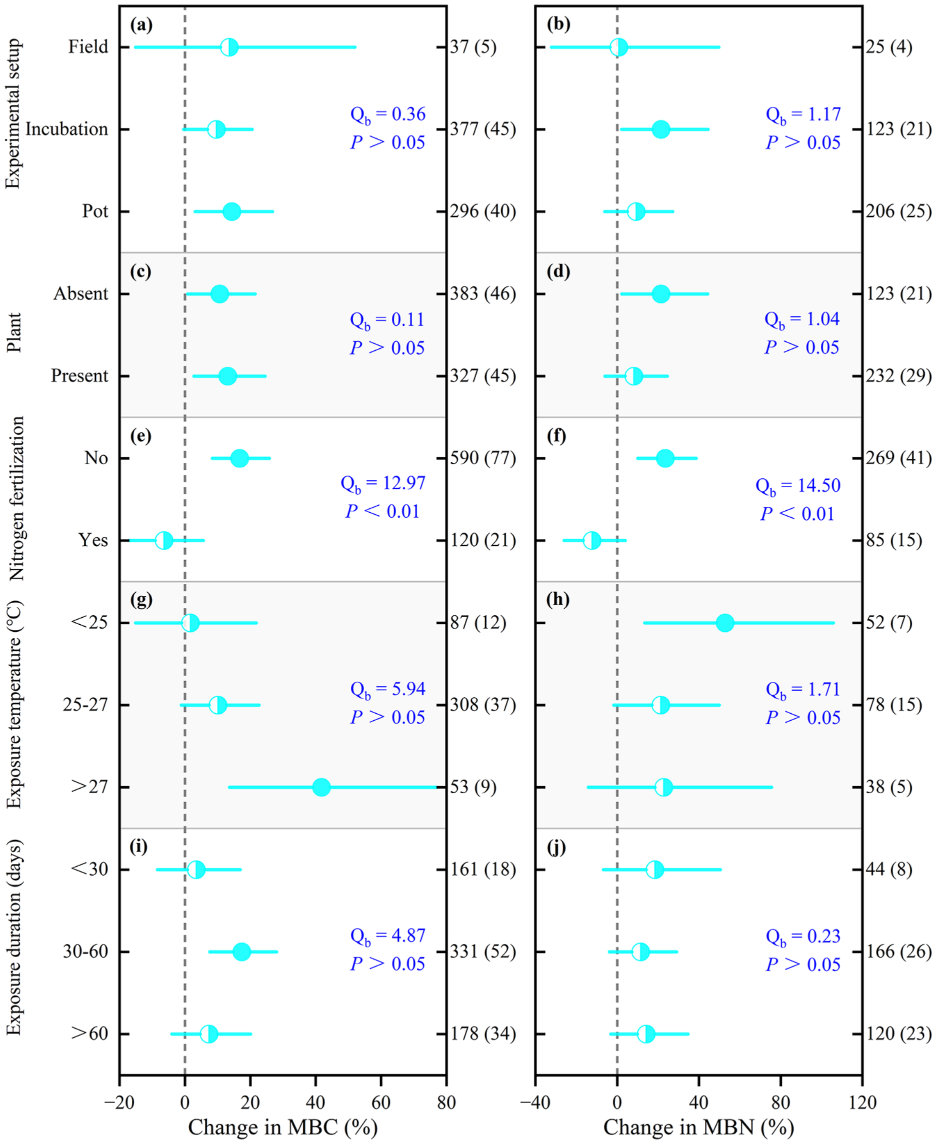

Figure 2. Responses of soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) to experimental conditions: a,b) experimental setup, c,d) plant presence, e,f) nitrogen fertilization, g,h) exposure temperature, and i,j) exposure duration. Circular markers indicate mean effects (weighted response ratio), with error bars showing 95% confidence intervals (CI). The vertical gray dashed line represents the zero-effect line. Half hollow and filled cyan circles denote neutral and positive effects, respectively. A significant effect is indicated when the 95% CI does not overlap with zero. The number of observations and paired studies is shown in brackets on the right.

7), learning rate (0.001−0.01), bag fraction (0.5−0.75); XGBoost: max depth (3−8), eta (0.01−0.3), subsample (0.5−1); RF: mtry (3−10), ntree (500−2000), nodesize (5−20). Model performance was evaluated using coefficient of determination (R^2), root-mean-square error (RMSE), and mean absolute error (MAE) on test data sets. Validation details of ML models are provided in the Supporting Information.

2.4. Model Interpretation and Statistical Analyses. Variable importance was quantified through (i) relative influence (sum of squared improvements) for BRT, (ii) gain importance (average gain across splits) for XGBoost, and (iii) permutation importance (accuracy decrease) for RF. Shapley additive explanations (SHAP) values were calculated using the "shapr" package v0.2.2 to determine feature contributions and directionality.[52] In simple terms, SHAP values show which factors most strongly influence predictions and whether they increase or decrease microbial responses, while partial dependence plots reveal how effects change across the range of each factor.

Partial dependence plots (1D and 2D) visualized marginal and interactive effects of predictors while averaging over other variables. Correlation networks were constructed for associations with $|r| > 0.3$ and $p < 0.05$ using Pearson correlations.

Structural equation modeling (SEM) using "piecewise SEM" v2.3.0[53] synthesized direct and indirect pathways linking experimental conditions, soil properties, and MPs characteristics to specific MBC and MBN responses. Predictors for the SEM were selected via PCA on raw-scale indices with high-loading, low-collinearity variables retained (details are given in the Supporting Information). Model fit was assessed using χ^2 tests, comparative fit index (CFI > 0.95), and root-mean-square error of approximation (RMSEA < 0.08).

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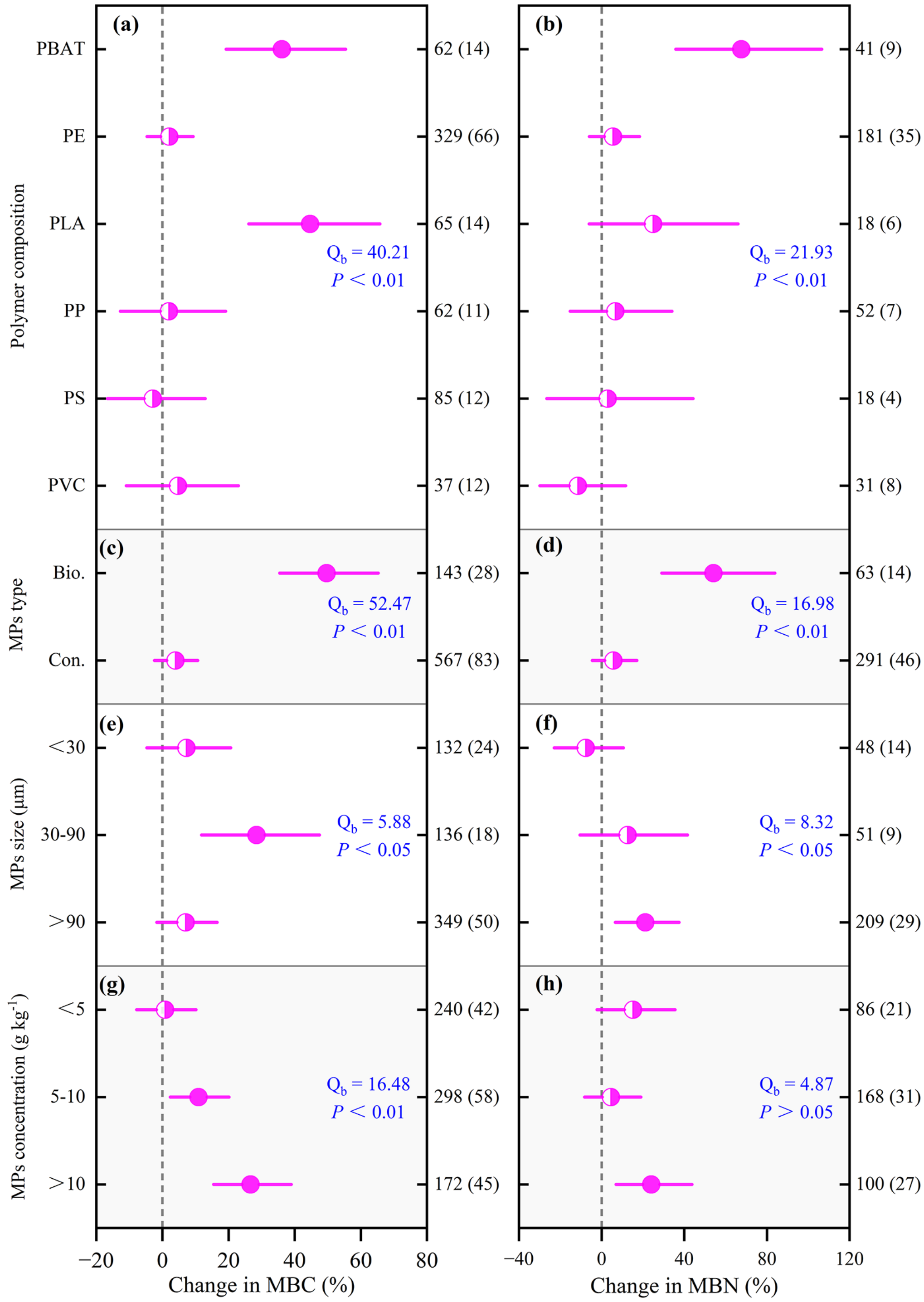

Figure 3. Responses of soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) to microplastic characteristics: a,b) polymer composition, c,d) microplastic type, e,f) microplastic size, and g,h) microplastic concentration. Circular markers indicate mean effects (weighted response ratio), with error bars showing 95% confidence intervals (CI). The vertical gray dashed line represents the zero-effect line. Half hollow and filled purple circles denote neutral and positive effects, respectively. A significant effect is indicated when the 95% CI does not overlap with zero. The number of observations and paired studies is shown in brackets on the right.

3. RESULTS

3.1. Overall Effect of Microplastics on Soil Microbial Biomass. The ln(RR) showed right-skewed distributions with medians above zero for both MBC and MBN (Figure 1a). MPs significantly increased soil MBC by 9.6% and MBN by 10.4% relative to the controls (Figure 1b).

3.2. Experimental Conditions Affect Microplastics Impact on Soil Microbial Biomass. The magnitude and direction of MPs effects varied significantly with the experimental conditions (Figure 2). Field studies showed no significant effects on either MBC or MBN (Figure 2a,b). In contrast, controlled environments yielded stronger responses: pot experiments increased MBC by 14.3% ($p < 0.01$) and laboratory incubations enhanced MBN by 21.5% ($p < 0.01$).

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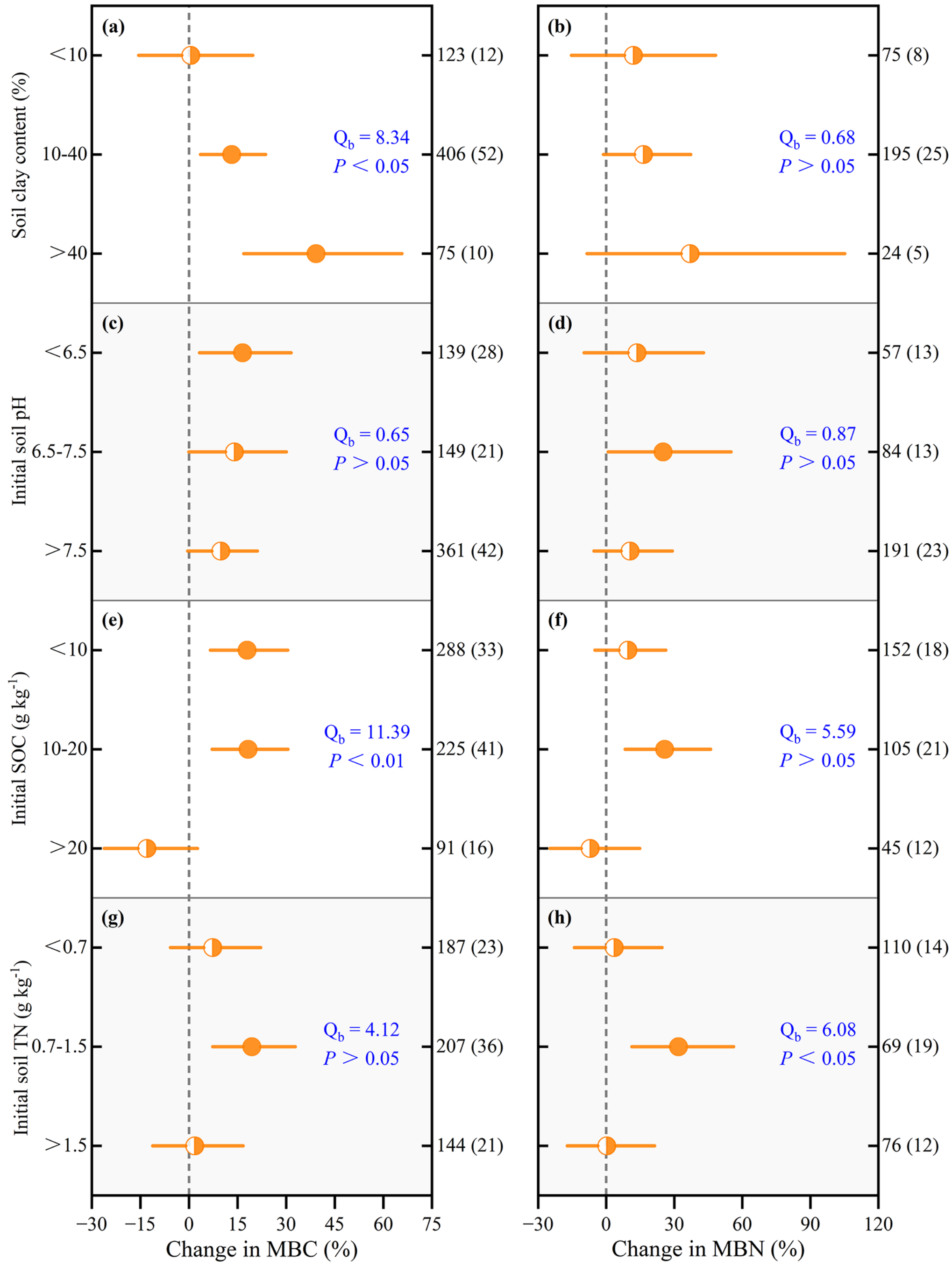

Figure 4. Responses of soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) to edaphic factors: (a,b) soil clay content, (c,d) initial soil pH, (e,f) initial SOC, and (g,h) initial soil TN. Circular markers indicate mean effects (weighted response ratio), with error bars showing 95% confidence intervals (CI). The vertical gray dashed line represents the zero-effect line. Half hollow and filled orange circles denote neutral and positive effects, respectively. A significant effect is indicated when the 95% CI does not overlap with zero. The number of observations and paired studies is shown in brackets on the right.

The following analyses include all studies (field, pot, and laboratory) unless otherwise specified, allowing an examination of how experimental conditions influence effect sizes. Plant presence did not significantly affect MPs effects on MBC (Figure 2c,d), with similar increases observed in the absence of plant (10.7%, $p < 0.01$) and in the presence of plant (13.1%, $p < 0.01$). However, MBN responses showed greater dependency on plant presence, increasing significantly only in the absence of plant (21.5%).

Nitrogen fertilization strongly regulated MPs impacts (Figure 2c,d). Without nitrogen addition, MPs increased MBC by 16.8% ($p < 0.01$) and MBN by 23.5% ($p < 0.01$). Conversely, when nitrogen fertilizer was applied, MPs had no significant effects on MBC or MBN.

Temperature was critical for regulating the effect of MPs on soil microbial biomass (Figure 2g,h). The effects of MPs on MBC increased with exposure temperature, becoming significantly positive (41.8%) when the exposure temperature exceeded 27 °C. In contrast, the impacts of MPs on MBN

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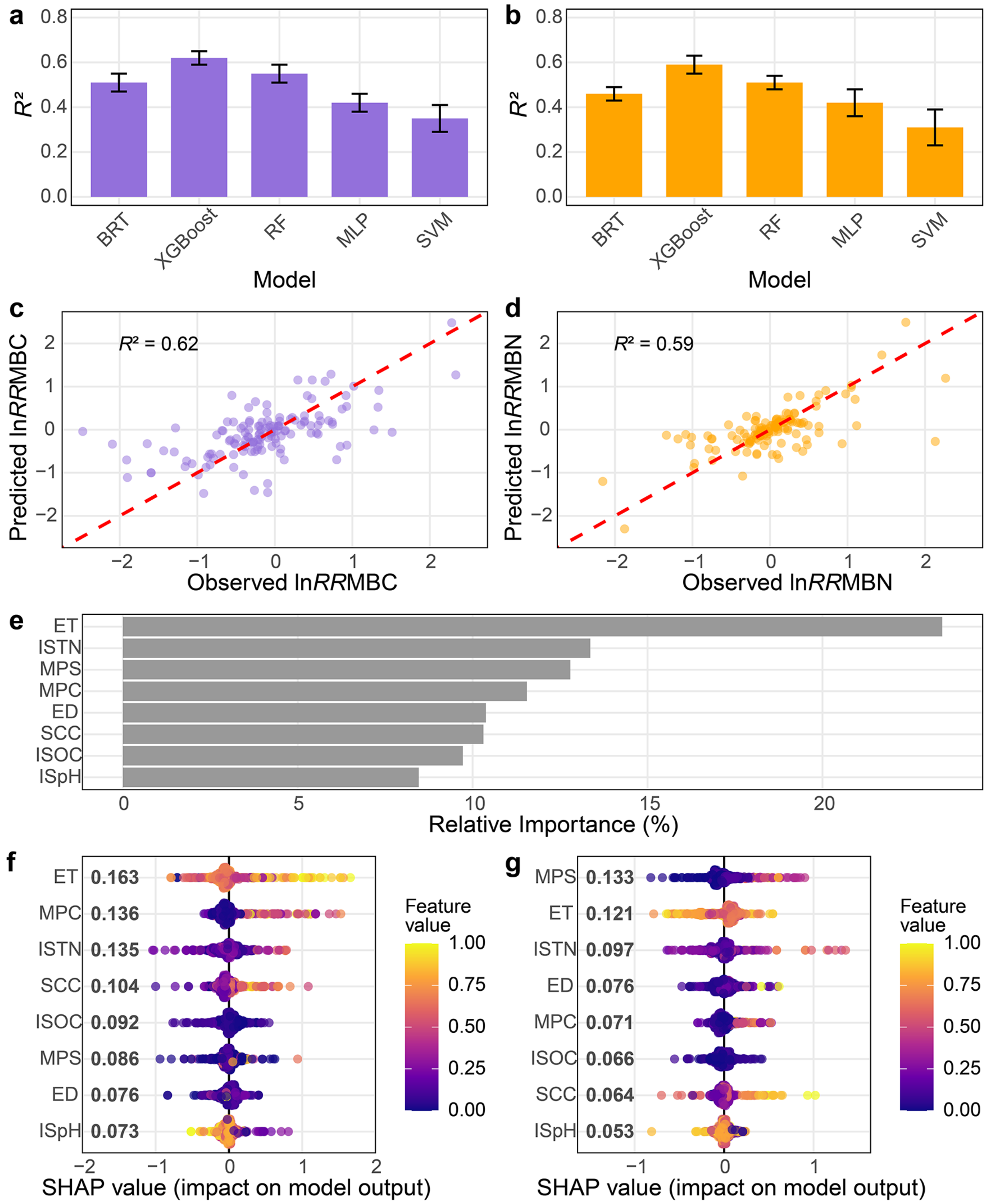

Figure 5. Machine learning model performance and feature importance analysis for predicting microplastic effects on soil microbial biomass. (a,b) Model performance comparison showing coefficients of determination (R^2) for five machine learning algorithms: boosted regression tree (BRT), extreme gradient boosting (XGBoost), random forest (RF), multilayer perceptron (MLP), and support vector machine (SVM) for MBC and MBN, respectively. Error bars represent standard deviations from 10-fold cross-validation. (c,d) Scatter plots comparing observed versus predicted values of response ratios for soil microbial biomass carbon (ln*RR*MBC) and nitrogen (ln*RR*MBN) using XGBoost model predictions ($n = 710$ for MBC; $n = 354$ for MBN). Dashed red lines indicate 1:1 relationships. (e) Relative importance (%) of predictor variables from XGBoost analysis for combined MBC and MBN responses. (f,g) SHAP (SHapley Additive exPlanations) summary plots displaying feature importance rankings and directional effects on model predictions for MBC and MBN, respectively. Points represent individual observations, with color gradients indicating feature values from low (blue) to high (red). Horizontal axis shows SHAP values representing impact magnitude and direction on model output. Abbreviations: SCC (soil clay content), ISpH (initial soil pH), ISOC (initial SOC), ISTN (initial soil TN), PT (Polymer composition), MPT (microplastics type), MPS (microplastics size), MPC (microplastics concentration), ES (experimental setup), ET (exposure temperature), ED (exposure duration), plant (plant absence or presence), and NYN (nitrogen fertilization).

declined with rising temperature, showing a significant positive effect (52.8%) when the exposure temperature was below 25 °C.

Exposure duration influenced MBC in a nonmonotonic pattern (Figure 2i,j). Compared to the control, the largest effect size (17.4%) for MBC was observed in experiments lasting 30–

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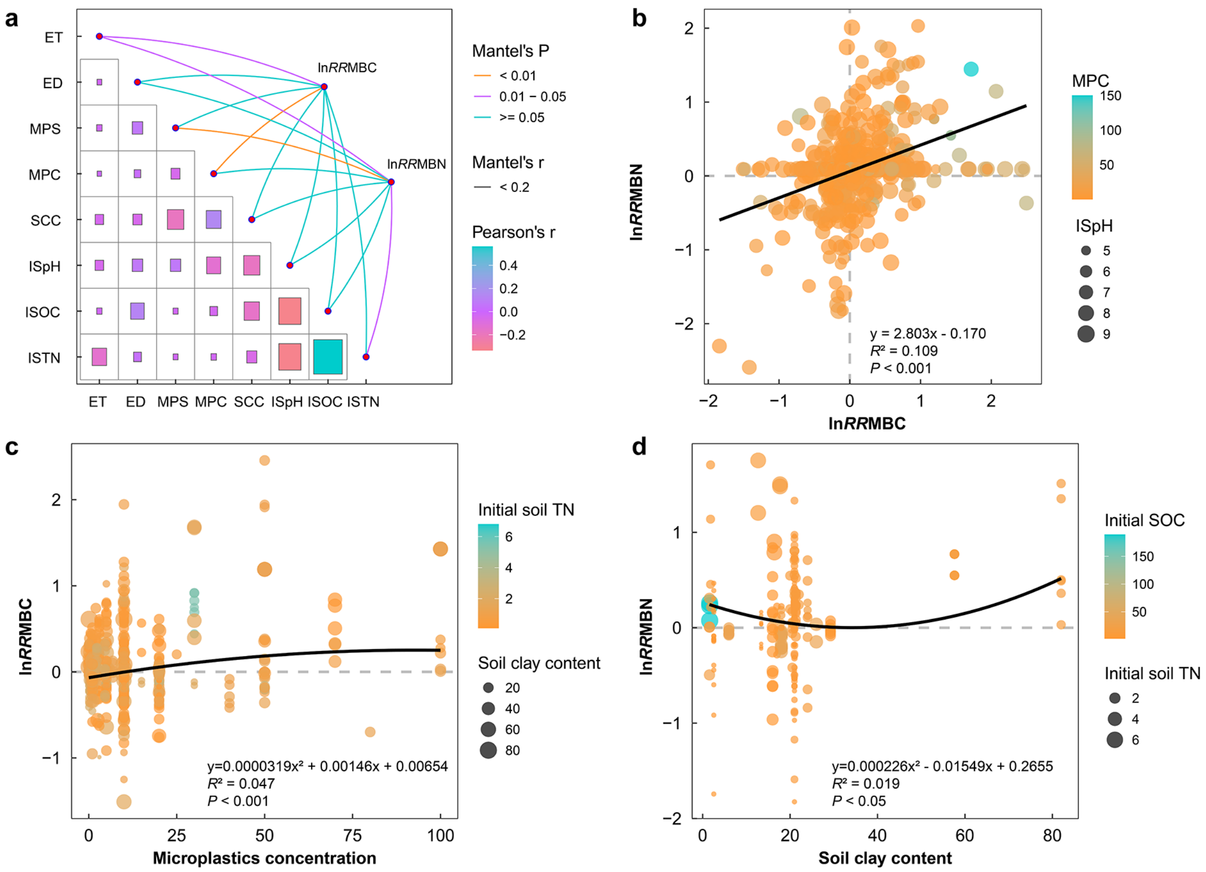

Figure 6. Correlation network and regression analyses of microplastic effects on soil microbial biomass. (a) Correlation network indicating relationships between response ratios of soil microbial biomass carbon (ln RRMBC) and microbial biomass nitrogen (ln RRMBN) and environmental parameters. Edge widths represent Mantel's *r* values, with edge colors indicating statistical significance ($p < 0.05$). The color scale represents Pearson's correlation coefficients for pairwise correlations between variables. (b) Regression analyses of ln RRMBC and ln RRMBN against key predictors identified by extreme gradient boosting analysis. Regressions show relationships between microbial biomass responses and (c) microplastic concentration (g kg^{-1}) and (d) soil clay content (SCC, %); other variables are also indicated, such as initial soil pH (ISpH), initial soil organic carbon (ISOC), and initial soil total nitrogen (ISTN). ET: exposure temperature; ED: exposure duration; MPS: microplastics size; MPC: microplastics concentration.

60 days, yet with nonsignificant trends in experiments shorter than 30 days or longer than 60 days (Figure 2i). For MBN, MPs showed nonsignificant regardless of exposure durations (Figure 2j).

3.3. Influence of Microplastics Properties on Soil Microbial Biomass. MPs properties significantly influenced the effect of MPs on soil microbial biomass (Figure 3). Among biodegradable polymers, PBAT had the largest increases in both MBC (36.1%, $p < 0.01$) and MBN (67.6%, $p < 0.01$). PLA only enhanced MBC (44.6%, $p < 0.01$). Conventional polymers generally did not significantly affect the effect of MPs on soil microbial biomass.

Significant differences were found in MBC and MBN responses between the two types of MPs (Figure 3c,d). MBC and MBN increased by 49.6% and 54.1%, respectively, following the addition of biodegradable MPs, while conventional MPs had nonsignificant effects on both MBC and MBN.

MPs size indicated different patterns for soil microbial biomass (Figure 3e,f). MPs of moderate size (30−90 μm) significantly increased MBC by 28.4%, whereas MBC showed nonsignificant responses to small (<30 μm) and large (>90 μm) MPs. Large (>90 μm) MPs significantly decreased MBN by 21.1%, while small and moderate-sized MPs had nonsignificant effects on MBN, respectively (Figure 3f).

A high MPs concentration (>10 g kg^{-1}) significantly increased MBC and MBN by 26.7% and 24.0%, respectively, whereas low concentrations (<5 g kg^{-1}) had nonsignificant effects on MBC and MBN (Figure 3g,h). In addition, moderate concentrations (5−10 g kg^{-1}) significantly increased MBC by 10.9%.

3.4. Influence of Edaphic Factors on Microplastics-Induced Shifts in Soil Microbial Biomass. MPs-induced changes in soil microbial biomass differed significantly across various edaphic factors (Figure 4). MPs exposed to soils with moderate clay content (10 ≤ SCC ≤ 40%) and high clay content (>40%) significantly increased MBC by 13.2% and 39.2%, respectively (Figure 4a). In contrast, nonsignificant effects of MPs on MBN were observed regardless of the soil clay content (Figure 4b).

MPs significantly increased MBC in acidic soils (ISpH < 6.5) by 16.5% and MBN in neutral soils (6.5 ≤ ISpH ≤ 7.5) by 25.1%, whereas MPs exposure to alkaline soils had non-significant effects on both MBC and MBN (Figure 4c,d).

MPs exposure significantly increased MBC by 17.9% and 18.3% in low ISOC (ISOC < 10 g kg^{-1}) and moderate ISOC (10 ≤ ISOC ≤ 20 g kg^{-1}) soils, respectively. A significant 25.8% increase in MBN occurred in moderate ISOC soils (10 ≤ ISOC ≤ 20 g kg^{-1}). In contrast, MPs addition to high ISOC soils

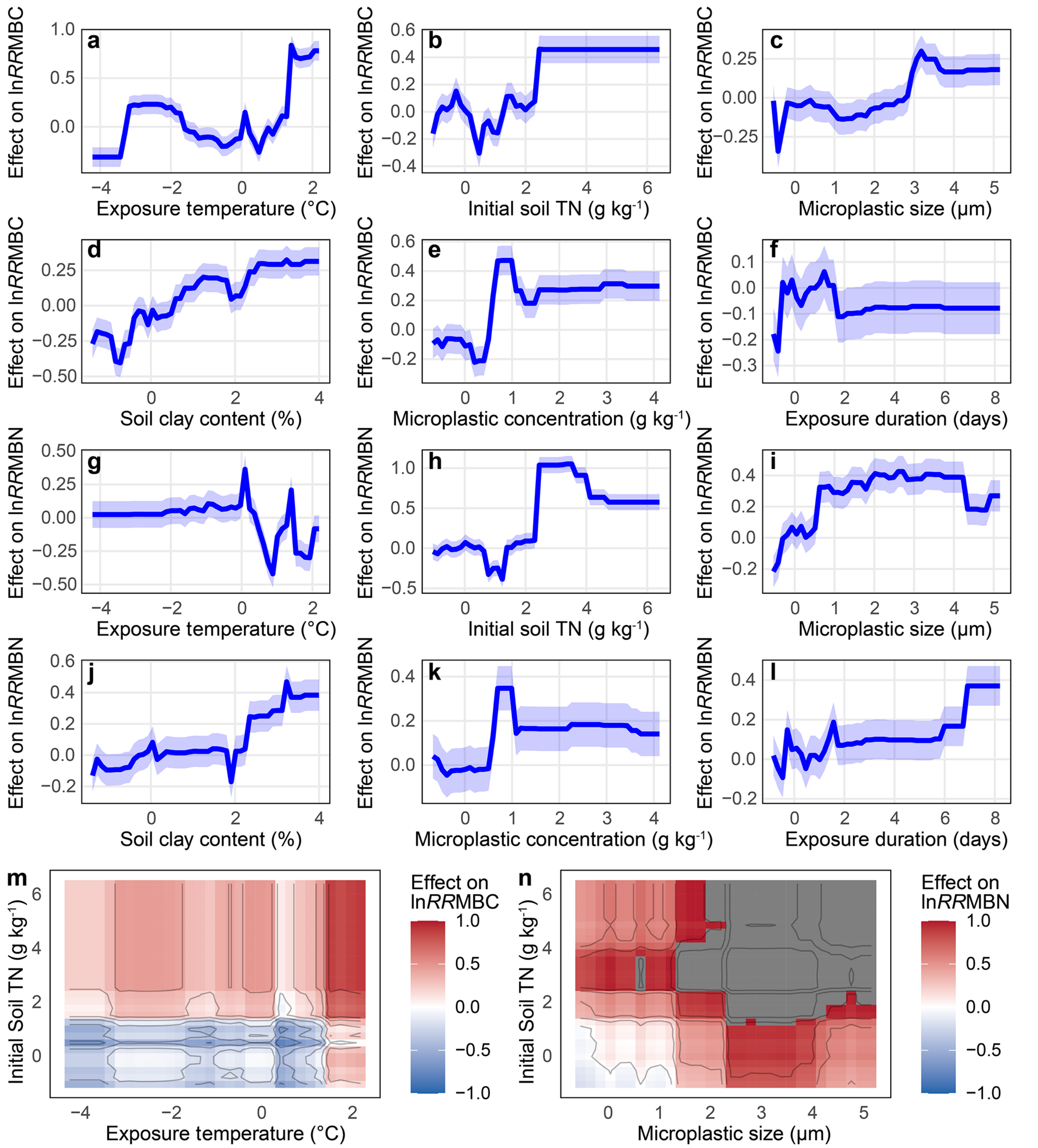

Figure 7. Partial dependence plots revealing nonlinear relationships and interaction effects between key predictors and soil microbial biomass responses to microplastics. (a−f) One-dimensional partial dependence plots for soil microbial biomass carbon (MBC) showing marginal effects of six key predictors: exposure temperature (ET), initial soil total nitrogen (ISTN), microplastic size (MPS), soil clay content (SCC), microplastic concentration (MPC), and exposure duration (ED). (g−l) Corresponding partial dependence plots for soil microbial biomass nitrogen (MBN) showing marginal effects of the same six predictors. Blue lines represent smoothed partial dependence functions with 95% bootstrap confidence intervals (n = 1000, shaded regions). *X*-axes display standardized values (mean = 0, SD = 1) used in model fitting. *Y*-axes show predicted ln(RR) values. (m) Two-dimensional partial dependence plot illustrating interaction effects between exposure temperature and initial soil total nitrogen on MBC response. (n) Two-dimensional partial dependence plot showing interaction between microplastic size and initial soil total nitrogen on MBN response. Color gradients represent predicted effect magnitudes, with warmer colors (red) indicating stronger positive effects and cooler colors (blue) indicating negative effects. Contour lines represent iso-response curves at 0.1 ln(RR) intervals. All plots derived from the XGBoost model.

(ISOC > 20 g kg^{-1}) had nonsignificant effects on both MBC and MBN (Figure 4e,f).

Compared with the control, both MBC and MBN significantly increased in soils with moderate ISTN (0.7 ≤ ISTN ≤ 1.5 g kg^{-1}), showing increases of 19.4% and 31.8%,

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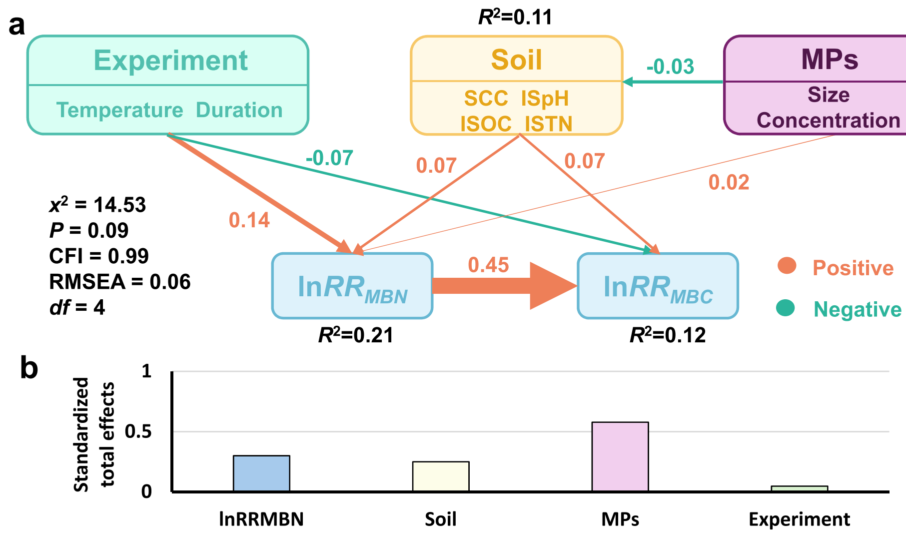

Figure 8. (a) Results of a piecewise structural equation model, demonstrating how experimental conditions (exposure temperature, duration), edaphic factors (soil clay content, initial soil pH, initial soil organic carbon, and initial soil total nitrogen concentrations), and microplastic properties (size, concentration) influence the response ratios of soil microbial biomass carbon (ln RRMBC) and microbial biomass nitrogen (ln RRMBN) under microplastic exposure and (b) standardized total effects resulted from the SEM. Orange and green arrows indicate significant positive and negative relationships ($p < 0.05$), respectively. Standardized path coefficients are shown next to arrows. Arrow thickness proportional to coefficient magnitude. R^2 values indicate proportion of variance explained.

respectively. In contrast, they had no significant response to MPs exposure in soils with low ISTN (ISTN < 0.7 g kg^{-1}) or high ISTN (ISTN > 1.5 g kg^{-1}, Figure 4g,h).

3.5. Primary Drivers of Soil Microbial Biomass Changes in Response to Microplastic Exposure. ML models suggested that ensemble methods outperformed other algorithms for predicting MPs effects (Figure 5a,b). XGBoost had the highest performance for both MBC (R^2 = 0.62) and MBN (R^2 = 0.59) (Figure 5a,b and c,d), followed by RF (R^2 = 0.55 and 0.51) and BRT (R^2 = 0.51 and 0.46). SVM (R^2 = 0.42 and 0.40) and MLP (R^2 = 0.35 and 0.31) showed inferior performance.

XGBoost feature importance analysis identified experimental (ET), soil factors (ISTN), and MPs characteristics (size and concentration) as primary drivers of MPs effects (Figure 5e). SHAP analysis further suggested that higher temperatures (>27 °C) and microplastic concentrations (>10 g kg^{-1}) consistently increased both MBC and MBN responses (Figure 5f,g). ISTN showed optimal effects at moderate concentrations (0.7 and 1.5 g kg^{-1}). Soil clay content showed positive associations with MBC responses but minimal influence on MBN.

Correlation network analysis indicated complex interactions among predictors and responses (Figure 6a). Strong positive correlations existed between ln RRMBC and ln RRMBN (r = 0.64, $p < 0.001$). Environmental parameters showed varying degrees of association: exposure temperature correlated positively with both responses ($r > 0.4$), while soil pH showed negative correlations.

Regression analyses confirmed nonlinear relationships between key predictors and microbial responses (Figure 6c,d). MPs concentration showed a relationship with ln RRMBC (R^2 = 0.05), with diminishing returns above 23 g kg^{-1}. Soil clay content indicated a threshold response with minimal effects below 20% and increasing positive effects thereafter (R^2 = 0.02). The interaction between ISTN and ISOC showed synergistic effects on soil microbial biomass.

Partial dependence plots further indicated nonlinear patterns and interaction effects (Figure 7). Exposure temperature showed distinct optima for MBC (~30 °C, Figure 7a) and MBN (15−20 °C, Figure 7a). MPs size suggested U-shaped relationships with both MBC and MBN, with minimum effects at intermediate sizes (30−90 μm; Figure 7c, i). Two-dimensional partial dependence plots revealed significant interactions (Figure 7m,n): the temperature effect on MBC was amplified at moderate ISTN concentrations, while MPs size effects on MBN were regulated by the ISTN content.

Structural equation modeling showed good fit (χ^2 = 14.53, p = 0.09, CFI = 0.99, RMSEA = 0.06) and explained 21% and 11% of variance in ln RRMBC and ln RRMBN, respectively (Figure 8a). Experimental temperature showed the strongest standardized total effect (0.45, Figure 8b), followed by the MPs concentration (0.07). Indirect effects through soil properties accounted for approximately 30% of total experimental condition effects.

4. DISCUSSION

4.1. Microplastics as Conditional Stimulators of Soil Microbial Biomass. Our meta-analysis shows that MPs generally enhance soil microbial biomass, increasing MBC by 9.6% and MBN by 10.4%, on average. This finding challenges prevailing narratives that characterize MPs primarily within an ecotoxicological framework[29,54] and aligns with emerging evidence of MPs as microbial substrates[1,24] or as factors of global change.[31] The stimulatory effects can be partially explained by multiple mechanisms. First, MPs provide extensive colonizable surfaces, with biofilm formation creating new ecological niches that expand the effective soil habitat.[1,17] Second, biodegradable polymers such as PBAT and PLA serve as direct carbon sources, feeding heterotrophic growth as evidenced by their 36−68% biomass increases.[28,55] Third, MPs may indirectly enhance microbial activity by affecting soil physical properties, including increased porosity and water retention in coarse-textured soils.[17,31] In addition, in the presence of plants, enhanced plant growth due to soil physical amelioration could result in increased root carbon inputs, in turn increasing substrate availability for microbes.[28] However, the

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high response variability (CIs ranging from −31% to +99%) highlights that MPs effects are highly context-dependent rather than universally beneficial or detrimental. This heterogeneity warrants moving beyond simple dose−response paradigms toward understanding the environmental conditions that amplify or mitigate MPs influences.[31]

4.2. Experimental Realism and the Laboratory-Field Divide. The contrast between nonsignificant field effects and significant laboratory/pot responses (14−22% increases) highlights an important challenge in MPs study: experimental artifacts may substantially overestimate real-world impacts. This discrepancy likely results from multiple factors operating in natural systems: (1) dilution effects, as field MPs concentrations typically remain orders of magnitude lower than experimental additions;[56] field studies normally applied MPs concentrations with a mean of 3.6 g kg^{-1} (range: 0.03−10.0 g kg^{-1}, Table S3); in contrast, laboratory experiments used a mean concentration of 14.9 g kg^{-1} (range: 0.01−150.0 g kg^{-1}), often 10−100-fold higher than field conditions; this concentration gradient could mostly explain the observed discrepancy, as our ML models identified threshold effects above 10 g kg^{-1}, a level rarely achieved under field conditions; (2) environmental buffering through diverse microbial communities with functional redundancy;[57] (3) competing stressors including temperature fluctuations, moisture variability, and predation that may overshadow MPs effects;[58] and (4) MPs aging and biofouling that affect surface properties and bioavailability over time.[1,6,59] Future MPs research should prioritize long-term field experiments that incorporate realistic exposure scenarios, weathered plastics, and natural stressors to improve the ecological relevance.

The significant stimulatory effects on microbial biomass observed at higher MPs concentrations (e.g., >10 g kg^{-1}) derive predominantly from controlled laboratory conditions that far exceed current environmental levels.[56] While these findings show potential mechanistic pathways, their direct ecological relevance remains uncertain.[59] Extrapolating these results to real-world scenarios should therefore be done with caution, as typical soil MPs concentrations are orders of magnitude lower and are embedded within complex environmental matrices that may buffer such responses.[60]

4.3. Temperature as a Dominant Regulator with Divergent Soil Microbial Biomass Effects. Temperature was the strongest predictor of MPs effect in our ML models, but with opposite effects on MBC and MBN. The intensification of MBC responses at higher temperatures (42% at >27 °C) likely reflects temperature-dependent acceleration of polymer hydrolysis and microbial metabolic rates.[61] Increased temperatures may also increase the bioavailability of plastic additives and oligomers through enhanced diffusion and solubility.[62]

Conversely, the decline in MBN responses at higher temperatures suggests differential thermal sensitivities between the carbon and nitrogen cycling communities. This divergence may result from (1) temperature-driven shifts toward copiotrophic bacteria that efficiently utilize labile carbon from MPs but have lower nitrogen demands;[63] (2) enhanced nitrogen mineralization at higher temperatures, reducing microbial nitrogen immobilization;[14] or (3) thermal stress on slower-growing microbes involved in nitrogen cycling, particularly nitrifiers and nitrogen-fixing bacteria.[64] These temperature dependencies have profound implications for predicting MPs impacts under climate change. Rising soil temperatures may amplify MPs effects on carbon cycling, potentially enhancing soil organic matter decomposition and carbon dioxide emissions.[14] Simultaneously, disrupted nitrogen cycling could impair soil fertility and plant nutrition, with cascading effects on ecosystem productivity.[65]

The divergence between ML predictions and meta-analysis results reflects methodological differences rather than contradictory findings. Meta-analysis examines univariate temperature effects by averaging across all other variables,[24] indicating MBN suppression at temperatures >27 °C. Conversely, ML models evaluate temperature effects within the full multivariate parameters,[34,36] identifying conditions where high temperatures can enhance MBN when coupled with optimal MPs concentrations (5−10 g kg^{-1}) and intermediate soil nitrogen (0.7−1.5 g kg^{-1}).

4.4. Nitrogen Availability Affects Microplastic Effects on Soil Microbial Biomass. The effects of MPs on soil microbial biomass were nonsignificant under nitrogen fertilization, providing mechanistic insights into MP−microbe−nutrient interactions. In nitrogen-limited soils, MPs may alleviate nutrient constraints through several pathways: (1) slow release of nitrogen-containing additives like plasticizers and stabilizers,[66] or from the polymer itself; (2) enhanced nitrogen retention through surface adsorption on charged MP surfaces;[20] or (3) stimulation of nitrogen-fixing bacteria in MP-associated biofilms.[67] When external nitrogen is added, these benefits disappear, and MPs may even compete with microbes for available nutrients. The high surface area and charge density of weathered MPs can adsorb significant amounts of ammonium and nitrate, potentially creating nutrient-depleted microsites.[68]

4.5. Size Matters: Effects of Microplastics on Soil Microbial Biomass. The size-dependent effects indicated unexpected nonmonotonic patterns that challenge assumptions about smaller MPs particles might yield greater impacts.[54] Medium-sized MPs (30−90 μm) mostly enhanced MBC, while large particles (>90 μm) specifically decreased MBN. This likely reflects trade-offs between surface area availability and physical disruption of soil architecture. Medium-sized MPs particles may represent a "Goldilocks zone" that maximizes colonizable surface area while maintaining favorable pore connectivity and water distribution.[1,31] MPs in this range can lodge within soil aggregates without severely disrupting their structure, creating protected microsites for microbial growth.[5,59] In contrast, very small MPs (<30 μm) may aggregate or clog micropores, reducing their effective surface area and limiting oxygen diffusion;[69] even smaller particles, in the nanosize range, likely primarily act through direct cytotoxicity. Large particles (>90 μm) appear particularly detrimental to nitrogen-cycling communities, possibly by physically disrupting fungal hyphal networks that play crucial roles in nitrogen immobilization and transfer.[70] The greater sensitivity of MBN to large particles may reflect the predominance of fungi in soil nitrogen cycling and their vulnerability to physical disturbance.[1,31]

4.6. Soil Properties Control on Microplastic−Microbe Interactions. The significant of MPs effects in high-clay soils (39% MBC increase at >40% clay) and at intermediate clay soils (10−40% clay) suggests edaphic controls on MP−microbe interactions.[30] Clay soils likely facilitate these interactions through (1) enhanced retention of MPs and their degradation products within organo-mineral complexes;[71] (2) maintenance of optimal moisture conditions in clay-MPs aggregates that support microbial activity;[69] and (3) increased contact between MPs and microbes in the confined spaces of clay-dominated pores.[1]

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The effect of MPs on soil microbial biomass was significant at moderate SOC (10−20 g kg^{-1}) and ISTN (0.7−1.5 g kg^{-1}), aligning with ecological theory predicting strongest responses to resource additions at intermediate resource availability.[72] In nutrient-poor soils, microbial communities may be too energy-limited to respond to MPs, while in nutrient-rich soils, additional carbon from MPs provides little marginal benefit. This pattern suggests that MPs may have the greatest impacts in moderately fertile agricultural soils, precisely where plastic inputs are highest.[4]

4.7. Machine Learning Unveils Hidden Complexities. The better performance of ensemble ML methods (R^2 = 0.59−0.62) compared to that of traditional linear approaches (R^2 = 0.02−0.05) suggests the value of accommodating nonlinear relationships in environmental data. Key insights from ML analyses include (i) identification of temperature, ISTN, and MPs concentration as primary drivers across models, providing evidence for their importance; (ii) suggestion of nonlinear patterns rather than monotonic effects for most predictors; and (iii) detection of significant interactions, particularly between temperature and soil nutrients.

The relatively modest variance explained (21% for MBC and 12% for MBN in SEM) indicates significant contributions from unmeasured factors. Critical missing variables likely include microbial community composition and diversity;[21,67] MPs weathering state and surface chemistry;[73] plant functional types and root exudate composition; and soil enzyme activities that mediate polymer degradation.[40,74]

SHAP analysis proved particularly valuable for mechanistic interpretation, revealing, for instance, that MPs concentration effects plateau above 50 g kg^{-1}, information critical for experimental design and risk assessment. The interpretability of ML models addresses common criticisms about "black box" algorithms in ecological applications.[34] While ML models identify strong predictive relationships and indicate important patterns, they do not establish causation. Controlled experiments are needed to verify the causal mechanisms suggested by these correlative patterns.

It is generally supported that ML has stronger predictive capabilities than process-based models.[34] The mechanisms in this study aim to enhance the understanding of the impact of MPs on MBC and MBN. Indeed, while ML excels in capturing complex patterns and predictions, integrating it with process-based models (e.g., biogeochemical simulators) could combine empirical data-driven insights with mechanistic explanations, such as modeling of MPs degradation rates and microbial turnover under varying temperatures.

4.8. Implications for Soil Health and Carbon Cycling, and Future Study. While increased microbial biomass might be beneficial, our results raise concerns about induced carbon cycling under MPs contamination. The 10% average increase in MBC, if accompanied by proportional increases in microbial activity, could enhance soil organic matter decomposition and carbon dioxide emissions.[14] With global soils containing approximately 1500 Pg of organic carbon, even modest increases in decomposition rates could significantly impact atmospheric carbon dioxide concentrations.[75]

The divergent temperature responses of MBC and MBN are particularly troubling, as they suggest that MPs could decouple carbon and nitrogen cycles under warming conditions. Such decoupling may lead to nitrogen limitation of plant growth despite abundant carbon, fundamentally changing ecosystem stoichiometry.[69] The decoupling of carbon and nitrogen cycling at high temperatures remains robust in both ML and meta-analysis, suggesting temperature as a primary driver that can override beneficial effects of other factors.

Biodegradable MPs, despite their environmental marketing, showed the strongest microbial stimulation (50−68% increases). While they are designed to decompose, their rapid effects on soil microbiota raise questions about unintended consequences. The carbon pulse from degrading MPs could prime the decomposition of native soil organic matter, potentially causing net carbon losses exceeding those from persistent conventional plastics.[14]

Our findings also highlight some future research directions; to bridge the laboratory−field divide and improve the ecological relevance, we recommend (1) standardizing experimental MPs concentrations to match environmental levels (0.01−1.0 g kg^{-1}) with high-concentration treatments reserved for mechanistic studies; (2) conducting multiseason field study that captures temporal dynamics and seasonal variations in microbial responses; and (3) developing dose−response curves spanning environmentally relevant to effect-threshold concentrations to establish no-observed-effect levels.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.5c12883.

> Validation details of machine learning models, the PRISMA flow diagram for the current meta-analysis; distribution of the sampling locations; publication bias tests; sensitivity analyses; factors and groupings within the meta-analysis; MPs concentration range according to the experimental setup; the 90 studies used in this paper; and the data set for the meta-analysis (PDF)

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