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Microplastic effects on soil nitrogen cycling enzymes: A global meta-analysis of environmental and edaphic factors

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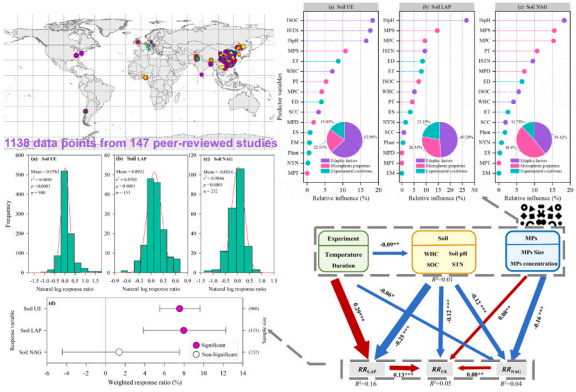
HIGHLIGHTS

- MPs enhance soil urease (UE) and leucine aminopeptidase activities.
- Biodegradable MPs affect soil enzymes more than conventional MPs.
- Soil pH and clay content affect the impact of MPs on enzyme activities.
- Acidic soils enhance UE activity with MPs exposure, neutral soils reduce it.
- MPs increase enzyme activity most in soils with 10–40 % clay content.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastic accumulation in soil ecosystems poses significant environmental concerns, potentially impacting nitrogen cycling processes and ecosystem health. This meta-analysis of 147 studies (1138 data points) assessed the impact of microplastics (MPs) on soil nitrogen-acquisition enzymes. We found that MPs exposure significantly increased soil urease (UE) and leucine aminopeptidase activities by 7.6 % and 8.0 %, respectively, while N-acetyl-β-D-glucosaminidase activity was not significantly affected. Biodegradable MPs showed more pronounced effects compared to conventional MPs. Enzyme activities were influenced by MPs properties (e.g.,

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polymer type, size, concentration), experimental conditions (e.g., field or laboratory setting, temperature, nitrogen fertilization), and soil properties (e.g., clay content, pH, organic carbon, total nitrogen). For instance, acidic soils enhanced UE activity, while neutral soils reduced it. These findings emphasize the complex interactions between MPs and soil ecosystems, highlighting the need for context-specific environmental management strategies and policy-making approaches to mitigate the impacts of MPs pollution on soil health.

1. Introduction

The pervasive accumulation of microplastics (MPs; particles size < 5 mm) in soil ecosystems due to inadequate waste management poses significant environmental challenges. MPs result from the degradation of larger plastic materials and infiltrate soil systems through various pathways, including agricultural practices, sewage sludge application, and atmospheric deposition [1–3]. Environmental factors such as ultraviolet radiation, mechanical stress from agricultural activities, and wind erosion facilitate the breakdown of plastics into MPs [4,5]. These particles have the potential to alter soil structure, disrupt microbial community dynamics, and interfere with nutrient cycling processes, potentially leading to adverse effects on agricultural productivity and ecosystem health [2,6]. Terrestrial environments may accumulate higher concentrations of MPs compared to marine ecosystems, underscoring the urgent need for comprehensive research on soil MPs pollution [7–9].

Nitrogen (N) is an indispensable macronutrient for plant growth and plays a crucial role in shaping soil microbial communities, ecosystem productivity, and nutrient cycling [10,11]. The increasing concern over MPs pollution has spurred research into its effects on terrestrial ecosystems, particularly its impact on soil N cycling [1,12,13], leading researchers to investigate its effects on soil N cycling [14,15]. Central to these investigations are soil N-acquisition enzymes, particularly urease (UE), leucine aminopeptidase (LAP), and β-1,4-N-acetylglucosaminidase (NAG) [16–18]. UE, present in both intracellular and extracellular forms, catalyzes the hydrolysis of urea into ammonium, a key step in soil N mineralization [19]. LAP, an extracellular enzyme, hydrolyzes leucine and other hydrophobic amino acids from the N-terminus of polypeptides, facilitating protein degradation and nutrient cycling [20,21]. NAG, involved in the breakdown of chitin, a major component of fungal cell walls and insect exoskeletons, hydrolyzes N-acetylglucosamine residues from β-1,4-glycosidic bonds, contributing to the degradation of chitinous materials in soil ecosystems [20,21]. These enzymes serve as critical indicators for assessing the impact of soil pollutants on N cycling [22].

Recent studies have revealed complex and contradictory effects of MPs on soil N-acquiring activities. For instance, study have reported that polyethylene 2,5-furan-dicarboxylate (PEF) and polyethylene terephthalate (PET) MPs at 2 % soil concentration significantly reduced UE activity [23], while polypropylene (PP) MPs and polylactic acid (PLA) above 1 % concentration enhanced it [24,25]. Similarly, LAP activity showed varying responses, with some studies reporting increased activity in forest soils exposed to polyethylene (PE) MPs [26], while other studies found decreased LAP activity with PE and PP MPs [27]. Conflicting results were also observed for NAG activity, with some studies indicating a decrease [28], while others suggesting an increase upon exposure to PE MPs [29], and still others show no response to PE MPs [30].

These varying findings likely stem from the complex interplay of multiple factors, including MP properties such as polymer type [31–33], biodegradability [34–36], size [17,37], and concentration [38,39], as well as experimental conditions, including setup, exposure medium, temperature [35], N fertilization, plant involvement, and exposure duration [38,40]. Additionally, edaphic factors such as water holding capacity, soil clay content [35,41], initial soil pH [41–43], soil organic carbon (SOC) concentration [35,41,43], and soil total N (TN) concentration [41,43] can modulate the response of soil N-acquiring activities to MPs expose.

Previous meta-analyses have investigated the complex relationships between MPs and soil UE activity, yielding divergent results [15,44–46]. For instance, some meta-analyses reported significant positive effects of MPs on soil UE activity [15,46], while others suggested non-significant impacts [44,45]. Similarly, the impact of MPs on LAP activity has been explored through various studies [26,47,48] and meta-analyses [15,46], with results ranging from markedly positive influences to significantly adverse effects. These discrepancies likely arise from differences in the collected sample data (n = 9–39). To date, no global synthesis has been conducted on the influence of MPs on soil NAG activity.

Given the expanding body of research on the impact of MPs on soil N-acquiring enzymes, a thorough and up-to-date meta-analysis is essential. This meta-analysis aimed to evaluate the impacts of MPs on soil N-acquiring enzymes and the underlying mechanisms governing these effects on a global scale. We hypothesized that MPs exposure differentially affects the activities of soil UE, LAP, and NAG, with responses modulated by MP properties, experimental conditions, and edaphic factors. Leveraging a comprehensive dataset of 1138 observations from 147 peer-reviewed studies, our investigation intended to: 1) quantify global trends in soil UE, LAP, and NAG enzyme activities following MP exposure; 2) evaluate the influences of MP properties (e.g., polymer type, size, concentration), experimental parameters, and soil properties on N-acquiring enzyme activities; 3) identify key factors and potential interaction effects that shape soil N-acquiring enzyme responses to MP contamination. This meta-analysis synthesized the current body of knowledge, aiming to guide future research in this rapidly evolving field and to inform strategies that could mitigate the impacts of MPs pollution on soil ecosystems and agricultural productivity.

2. Materials and methods

2.1. Literature search

This meta-analysis drew upon data from peer-reviewed articles published up to May 28, 2024, detailing the effects of MPs on soil N-acquisition enzymes across various regional environments. We conducted a comprehensive search using the following databases: Web of Science (Core Collection) (http://webofknowledge.com), China National Knowledge Infrastructure (CNKI) (https://www.cnki.net), and Google Scholar (http://scholar.google.com). Our search strategy employed keywords and phrases including microplastics*, nanoplastic*, “plastic fragment”, “plastic debris”, microfiber, microbeads, and soil or sediment, and specific enzyme-related terms such as urease, UE, leucine aminopeptidase, LAP, β-1,4-N-acetylglucosaminidase, NAG, or N-acquisition enzyme.

2.2. Criteria for data collection

To ensure the integrity and relevance of our meta-analysis, we established specific criteria for literature selection and data extraction. Studies were included if they met all of the following conditions: (1) studies involved soil or sediment that had been exposed to MPs; (2) study reported at least one type of soil N-acquisition enzyme activity: UE, LAP, or NAG; (3) concentration of MPs used in the study was clearly specified; (4) experimental design included at least one treatment group with MPs and a control group without MPs; (5) results provided paired means, along with either standard deviations (SDs) or standard errors

(SEs), based on a minimum of three replicates for both the experimental and control groups; (6) initial environmental and edaphic factors were identical between the experimental and control groups to ensure comparability.

Data management protocols: 1) we excluded data from groups where MPs were mixed with other contaminants such as pesticides, herbicides, heavy metals, and acid rain; 2) studies that included various soil sampling locations, experimental setups, exposure media, temperatures, durations, N fertilization, plant involvement, polymer types, MP types, biodegradation, concentrations, sizes, water-holding capacities, soil clay content, initial soil pH, initial SOC, and initial soil TN were treated as separate observations; 3) when multiple publications presented similar findings from the same experiment, we included data only from the most recent publication.

After this rigorous screening process, we identified 147 papers that encompass a total of 1138 observations (Fig. 1). Details of the PRISMA flowchart guidelines are provided in the supporting information (Fig. S1) [49]. The complete list of the 147 papers is included in the supporting information (Notes S1).

2.3. Data extraction and classification

From each study, we extracted data on soil UE, LAP, and NAG activities, as well as the sample sizes of both MPs treatment and control groups. Additional information collected included soil sampling locations, experimental setup, exposure medium, exposure temperature, exposure duration, N fertilization, plant involvement, polymer type, MPs type, MPs biodegradation, MPs concentration, MPs size, water-holding capacity, soil clay content, initial soil pH, initial SOC, and initial soil TN. Data were extracted directly from tables and textual content, while figures were digitized using GetData Graph Digitizer software (version 2.26, available at http://getdata-graph-digitizer.com/download.php). In instances where data were not immediately accessible from the published literature, we contacted the authors directly to obtain the necessary information.

To facilitate data interpretation, we categorized the independent variables (Table 1). These categories included polymer type [50], MPs type [51], MPs biodegradation [52], MPs size [50], experimental setup [53], exposure medium [54], exposure temperature [50], N fertilization [55], plant involvement [44], initial soil pH [56], initial SOC [13], and initial soil TN [57]. We further classified the exposure duration, MPs concentration, water-holding capacity, and soil clay content according to the range and characteristics of the data from the studies included in our meta-analysis. These classifications enabled a more comprehensive analysis of the effects of these variables on the activities of soil N-acquisition enzymes such as UE, LAP, and NAG.

2.4. Data calculation

When source references reported only soil organic matter (SOM) values, we transformed SOM (g kg^{-1}) into soil organic carbon (SOC) (g kg^{-1}) using a conventional conversion factor of 0.58 [58]. For soil pH determined with $CaCl_2$, we applied the conversion formula $pH[H_2O] = 1.65 + 0.86 \times pH[CaCl_2]$ [59]. In cases where only the standard error (SE) was provided instead of the standard deviation (SD), we calculated the SD using the formula SD = SE × n^0.5, where n represents the sample size [15].

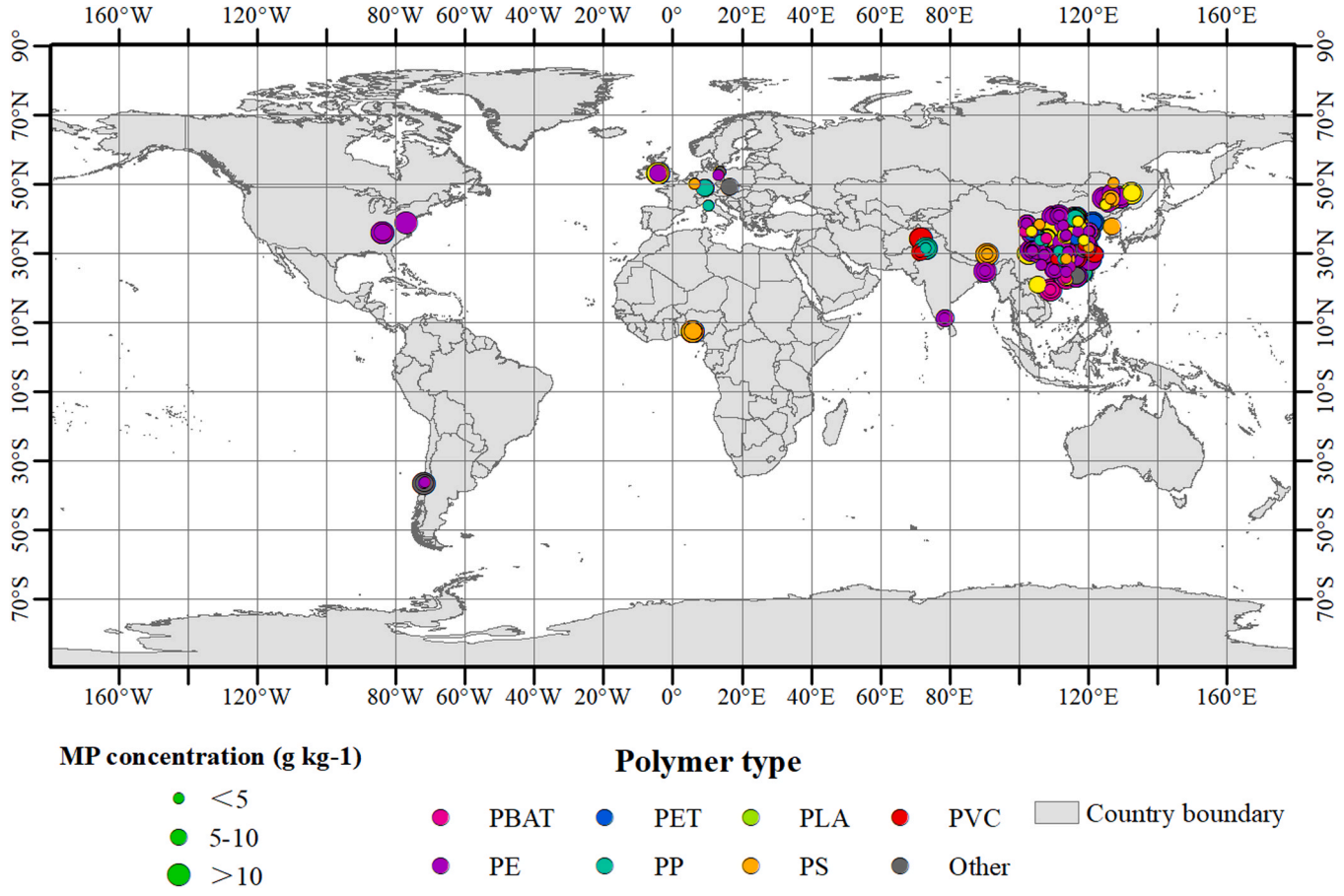

Fig. 1. Distribution of the data points used in this study with geographical coordinates in word map.

Table 1
Variables and subgroups in the meta-analysis.

Moderators	Subgroups			
Polymer type[a]	PBAT	PE	PET	PLA
	PP	PS	PVC	
Microplastics type	Aged	Virgin		
Microplastics biodegradation[b]	Bio.	Con.		
Microplastics size (μm)	< 30	30–90	> 90	
Microplastics concentration (g kg^{-1})	< 5	5–10	> 10	
Experimental setup	Field	Incubation	Pot	
Exposure medium	Soil	Sediment		
Experimental temperature (℃)	< 25	25–27	> 27	
Nitrogen fertilization	Yes	No		
Plant involvement	Presence	Absence		
Exposure duration (day)	< 30	30–60	> 60	
Water - holding capacity (%)	< 55	55–65	> 65	
Soil clay content (%)	< 10	10–40	> 40	
Initial soil pH	< 6	6–7	> 7	
Initial SOC (g kg^{-1})	< 10	10–20	> 20	
Initial soil TN (g kg^{-1})	< 0.75	0.75–1.5	> 1.5	

Note:.
[a] Polymer type: PBAT refers to polyadipate/butylene terephthalate; PE denotes Polyethylene; PET stands for polyethylene terephthalate; PLA signifies polylactic acid; PP is an acronym for polypropylene; PS represents polystyrene; PVC means polyvinyl chloride.
[b] Microplastics type: Bio. indicates biodegradable microplastics, while Con. denotes conventional microplastics.

2.5. Meta-analysis

We applied the natural log-transformed (ln) response ratio (*RR*) to quantify the effects of MPs exposure on soil N-acquisition activities. The *RR* was calculated as follows (Eq. (1)) [60]:

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

here, $\overline{X_t}$ and $\overline{X_c}$ represent the means of the variables under MPs treatment and control groups, respectively.

Given that certain observations were subjected to identical control or experimental conditions, we employed a variance-covariance (VC) matrix to adjust for interdependencies among the data points [61]. The VC matrix accounts for the violation of the independence assumption, which occurs when multiple treatments share a common control or experimental condition. To address this, impacts were pooled using an appropriate variance-covariance matrix. When observations are independent, the matrix is simplified to include only variances on the diagonal.

According to Lajeunesse [61], when comparing two experimental conditions (A and B) against a common control condition (C), the variance-covariance matrix is structured as follows using Eq. (2) [62]:

$$V^{\overline{X}_C} = \begin{bmatrix} \frac{(SD_C)^2}{N_C\overline{X}_C^2} + \frac{(SD_T^A)^2}{N_T^A(\overline{X}_T^A)^2} & \frac{(SD_C)^2}{N_C(\overline{X}_C)^2} \\ \frac{(SD_C)^2}{N_C(\overline{X}_C)^2} & \frac{(SD_C)^2}{N_C\overline{X}_C^2} + \frac{(SD_T^B)^2}{N_T^B(\overline{X}_T^B)^2} \end{bmatrix} \quad (2)$$

here, SD_T^A, SD_T^B, SD_C represent the standard deviation of the variable of interest in the MPs treatment A, B, and control groups, respectively, and N_T^A, N_T^B, N_C represent the sample sizes of the variable of interested in the MPs treatment A, B, and control groups, respectively, while $\overline{X}_T^A$, $\overline{X}_T^B$, $\overline{X}_C$ represent the means of the variable of interested in the MPs treatment A, B, and control groups, respectively.

For the formulation of the variance-covariance matrix, we used a refined version of the covariance_commonControl() function from the "*metagear*" package [63]. In analyzing moderator effects, we adjusted for the structure of shared control or experimental treatments by generating a specific variance-covariance matrix for each moderator. Using a mixed-effects model framework, we employed the rma.mv() function from the "*metafor*" package in R [64], with REML as the estimation technique. To address variability between studies, we included a random effect for each study and factored in the corresponding sampling variances. For assessing the overall effect, a mixed model was executed with only a fixed intercept and the random study effect.

Using a random-effects model, we analyzed the association between the logarithmically transformed response ratios of soil enzymes involved in N acquisition (UE, LAP, and NAG) and various factors such as experimental setup, exposure medium, experimental temperature, N fertilization, plant involvement, exposure duration, polymer type, MPs type, MPs biodegradation, MPs size, MPs concentration, water-holding capacity, soil clay content, initial soil pH, initial SOC, and initial soil TN. When examining each categorical variable, the overall heterogeneity (Q_T) of the group categories was divided into two components: between-group heterogeneity (Q_M) and within-group heterogeneity (Q_E). The Q_M test evaluated if the responses showed significant variation between subgroup categories, with group variations considered significant at a Q_M *p*-value of less than 0.05 [65].

If the 95 % confidence interval (CI) values included zero, the influence of MPs addition on the activity levels of soil N-acquisition enzymes was considered statistically insignificant. Otherwise, non-overlap with zero indicated a significant effect. To convey the response more succinctly, the weighted *RR* (RR_{++}) was transformed into a percentage change as follows: percentage change = [exp(RR_{++}) - 1)] × 100 %.

2.6. Sensitivity analysis

Sensitivity analysis is crucial for assessing the influence of individual studies on the outcomes of a meta-analysis and for confirming the reliability of the results. We conducted sensitivity analysis using the "*metafor*" package in R (v4.3.3) [64]. This package allows for the sequential exclusion of studies through its sensitivity function, enabling the detection of any significant impacts on the overall effect size.

To assess publication bias, we created funnel plots using the funnel function. Asymmetry in these plots may suggest potential bias, which we further scrutinized using Egger's regression test via the regtest function [66]. The observations on the funnel plots (Fig. S2) were distributed symmetrically, indicating the absence of publication bias.

Additionally, we employed the trim-and-fill method using the "*trimfill*" function to estimate and impute the number of missing studies. This method adjusts the effect sizes to provide a more accurate representation of the evidence [67]. Detailed results of the sensitivity analysis are presented in the supporting information (Fig. S3).

2.7. Statistical analysis

To enhance the understanding of the importance of explanatory variables regarding their effects on *RR* of soil UE, LAP, and NAG activities, we employed a boosted regression tree (BRT) approach. This approach was used to rank the contributions of various variables, including MPs properties (e.g., polymer type, type, biodegradation, size, and concentration), experimental conditions (e.g., experimental setup, exposure medium, temperature, N fertilization, plant involvement, and exposure duration), and edaphic factors (e.g., water holding capacity, soil clay content, initial soil pH, initial SOC, and initial soil TN). We utilized the "*gbm*", "*dismo*", and "*sp*" packages in R (v4.3.3) to perform the BRT analyses [68], specifying model parameters as follows: tree complexity of 5, shrinkage rate of 0.005, and subsampling rate of 0.75 [69].

Using the "*vegan*" package in R (v4.3.3) [68], we conducted Mantel tests to perform correlation analyses, determining the relationships between the *RR* of soil UE (RR_{UE}), LAP (RR_{LAP}), and NAG (RR_{NAG}) and several determinants. These determinants included experimental conditions (e.g., exposure time and temperature), MPs properties (e.g., size and concentration), and edaphic factors (e.g., soil clay content, initial pH, initial SOC, and initial soil TN). Polynomial regression was

employed to assess the relationships between initial soil TN, soil pH, and the activities of RR_{UE}, RR_{LAP}, and RR_{NAG}.

A piecewise structural equation modeling (SEM) framework was used to identify how MPs exposure might affect the activity of soil enzymes involved in N acquisition, indicated by the RR_{UE}, RR_{LAP}, and RR_{NAG}. The model considered the effects of microplastics properties, experimental conditions, and edaphic factors. To simplify variable selection and interpretation before constructing the SEM, we utilized principal component analysis (PCA). Variables with high absolute loadings on the first principal components were specifically chosen for the SEM. PCA processed all indices in their raw form. The SEM's performance was evaluated using the Akaike information criterion (AIC) and Fisher's C statistic, with model selection based on the lowest AIC. All statistical analyses were performed using R version 4.3.3 [68].

3. Results

3.1. Microplastics increased soil UE and LAP but not NAG activities

The Gaussian equation was applied to adjust the frequency distributions of RR_{UE}, RR_{LAP}, and RR_{NAG} in response to microplastics exposure (Fig. 2a–c). The normal distribution observed in these histograms indicates the absence of publication bias in the datasets of soil UE, LAP, and NAG activities influenced by MPs exposure.

A comprehensive analysis encompassing all data points revealed that MPs significantly affected the activities of soil N-acquiring enzymes, particularly UE and LAP. MP treatment resulted in a significant increase in these enzymatic activities, with UE of a 7.6 % increase and LAP of 8.0 % increase compared to control treatments (Fig. 2d). However, the impact on NAG activities was not significant, with a slight mean decrease of 1.4 %.

3.2. Influence of microplastics properties on soil N-acquisition activities

The effects of MPs on soil enzymes responsible for N acquisition were influenced by their specific properties (Fig. 3). In particular, regarding polymer types, MPs of PBAT, PLA, and PP significantly increased soil UE activity by 36.9 %, 10.3 %, and 15.1 %, respectively (Fig. 3a). Additionally, PBAT were associated with a significant increase in soil LAP activity, 79.3 % (Fig. 3b). A significant improvement in soil NAG activity was observed for PLA (75.4 %), whereas PS resulted in a significant reduction (42.2 %; Fig. 3c).

Biodegradable MPs caused significant increases in soil UE, LAP, and NAG activities by 14.0 % (Fig. 3g), 55.6 % (Fig. 3h), and 41.5 % (Fig. 3i), respectively. In contrast, conventional MPs did not significantly affect the three types of soil N-acquisition activities (Fig. 3g–i).

A significant increase in soil UE activity was observed only when the MPs concentration was above 10 g kg^{-1} (increase of 7.3 %), whereas concentrations at or below 10 g kg^{-1} had no significant influence on soil UE activity (Fig. 3m). Furthermore, soil LAP activity showed a minimal but not significant improvement with increasing MPs concentration (Fig. 3n). Similarly, soil NAG activity exhibited a small but statistically insignificant change with increasing MPs doses (Fig. 3o).

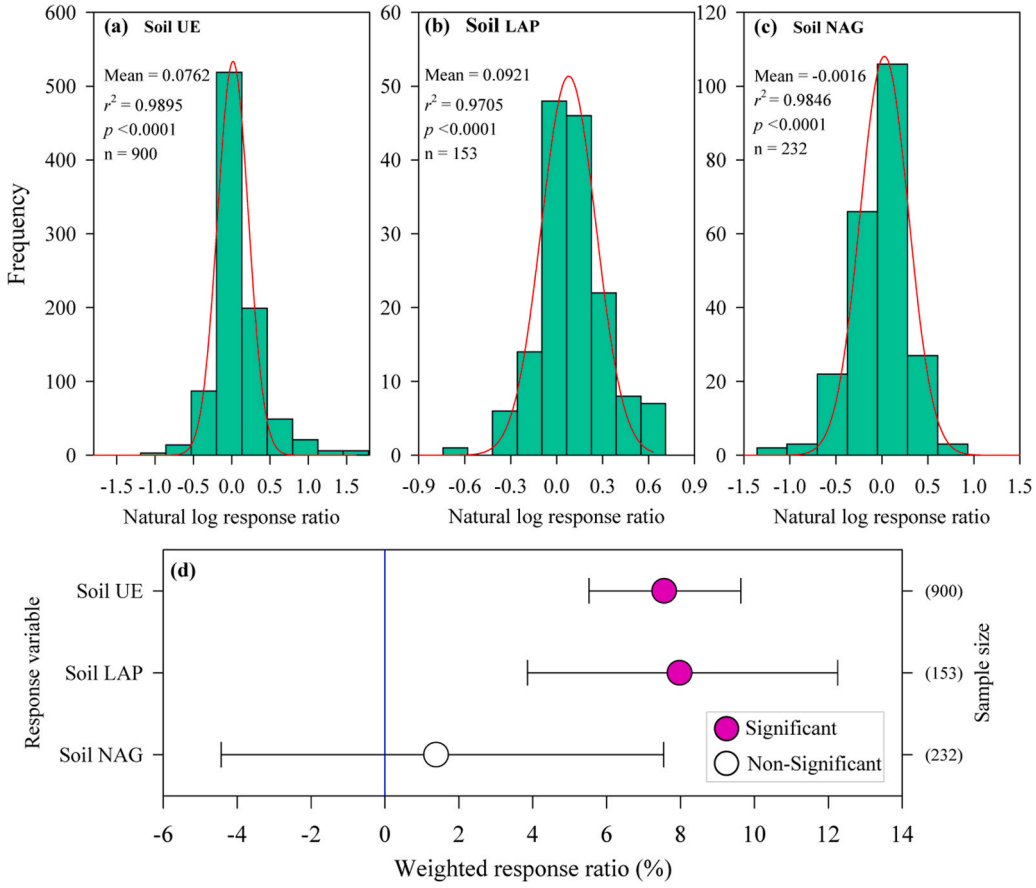

Fig. 2. Distribution of the response ratios (*RR*) for soil a) UE, b) LAP, and c) NAG activities under microplastics exposure. The red curves represent the Gaussian distribution model fitted to the frequency data. d) depicts the cumulative effect of microplastics on the activities of nitrogen-acquiring enzymes in soil. The blue vertical line represents a state of no change in soil N-acquisition activities. The circles and error bars indicated weighted effect sizes and the corresponding 95 % CIs of microplastics influences, respectively. Significant microplastic impact is indicated when the 95 % CI does not include zero. Sample sizes (n) for each enzyme are provided in parentheses.

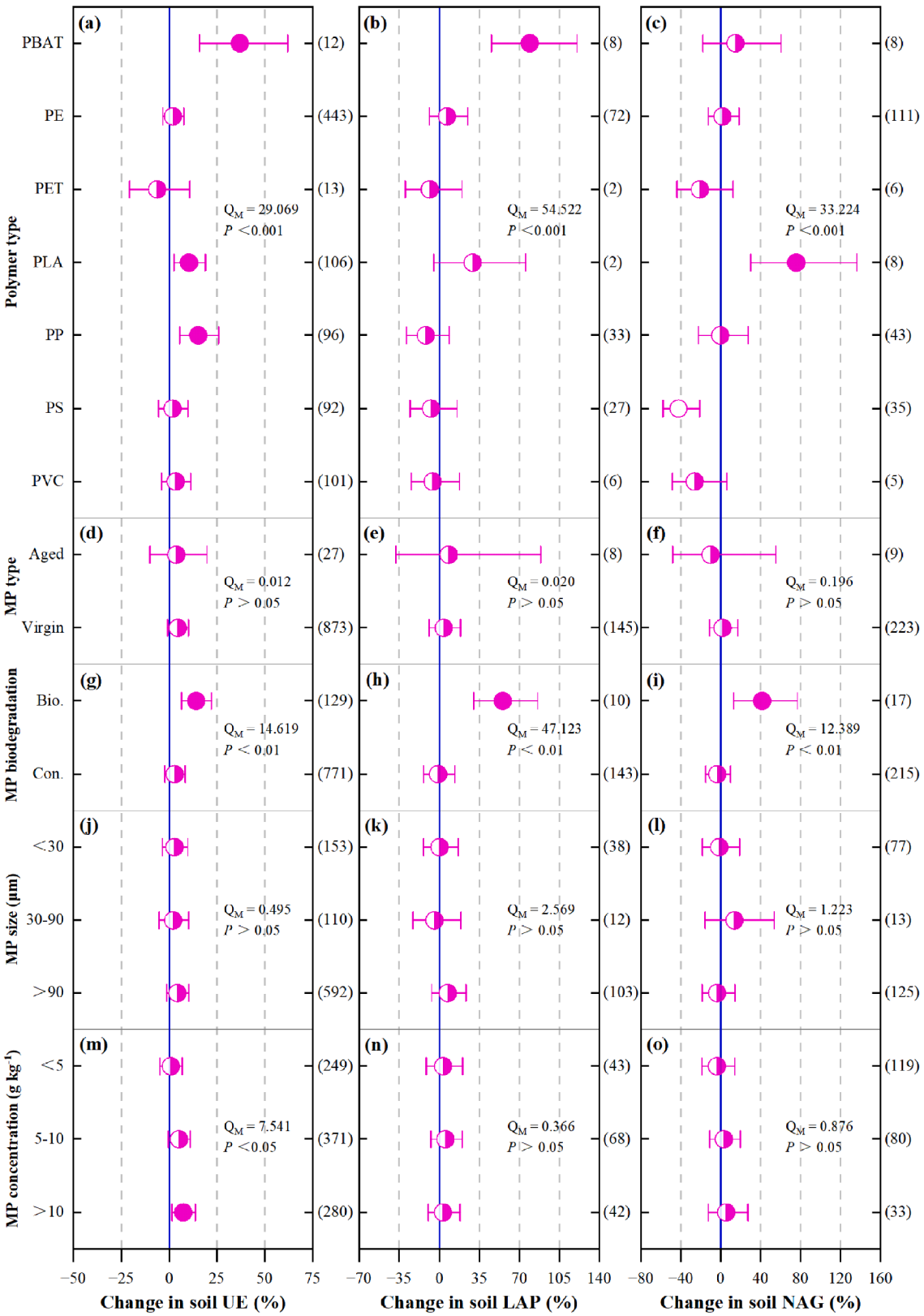

Fig. 3. Effects of microplastic characteristics on urease (UE), leucine aminopeptidase (LAP), and β-1,4-N-acetylglucosaminidase (NAG) activities: a–c) polymer type, d–f) microplastic type, g–i) microplastic biodegradability, j–l) microplastic size, and m–o) microplastic concentration. Circular markers represent mean effect sizes, with whiskers indicating 95 % confidence intervals. The blue vertical line denotes no change in enzyme activity. Hollow, outlined, and filled purple circles signify neutral, negative, and positive microplastic effects, respectively. Sample sizes (n) are provided in brackets. Statistically significant differences between groups ($p < 0.05$) are indicated.

3.3. Experimental conditions regulate microplastics impact on soil N-acquisition activities

The impact of microplastics on the activities of soil enzymes involved in N acquisition was significantly influenced by the experimental conditions (Fig. 4). In particular, soil UE activity significantly reduced following MPs exposure in field conditions, whereas exposure in incubation and pot environments did not yield significant impacts on UE activity (Fig. 4a). The impact of MPs on soil LAP and NAG activities showed no significant changes across the three experimental conditions (Fig. 4b–c).

At temperatures ranging from 25 °C to 27 °C, there was a significant increase in soil UE activity by 9.3 % (Fig. 4g). Conversely, at temperatures below 25 °C, MPs addition to soil led to a significant decrease in LAP activity, with a reduction of 34.7 % (Fig. 4h). Soil NAG activity was not significantly changed by MPs exposure across the evaluated temperature ranges (Fig. 4i).

N input did not lead to a significant change in soil UE activity affected by MPs exposure. However, in the absence of N fertilizer, there was a significant enhancement of soil UE activity in response to MPs exposure (Fig. 4j). The activity of LAP and NAG enzymes in response to MPs exposure was not significantly changed by N fertilization, regardless of its application (Fig. 4k–l).

MPs exposure significantly increased soil UE activity by 7.1 % in the absence of plants, but did not significantly reduce it in the presence of plants (2.0 % reduction; Fig. 4e). Soil LAP and NAG activities showed no significant response to MPs exposure, regardless of the presence of plants (Fig. 4n–o).

Soil UE activity was significantly increased by 6.4 % with MPs exposure lasting between 30 to 60 days. Conversely, exposure durations below 30 days and above 60 days had neutral impacts on soil UE activity (Fig. 4p). MPs exposure resulted in insignificant effects on LAP activity regardless of the duration of exposure (Fig. 4q). However, significant variations were observed in NAG activity in response to MPs exposure among the three evaluated durations (Fig. 4r). Specifically, NAG activity was significantly higher with 30–60 days of exposure compared to durations longer than 60 days. Additionally, MPs exposure for durations shorter than 30 days did not produce a significant increase of NAG activity.

3.4. Influence of edaphic factors on microplastics-induced shifts in soil N-acquisition activities

Significant variations were observed in the effects of microplastics on soil enzymes involved in N acquisition across different soil properties (Fig. 5). MPs exposure led to a significant enhancement of 62.7 % in UE activity in soils with 10–40 % clay content. However, in soils with less than 10 % or more than 40 % clay content, MPs induced a negative but non-significant change in soil UE activity (Fig. 5d). The presence of MPs did not cause significant changes in soil LAP and NAG activities, regardless of soil clay contents (Fig. 5e–f).

MPs exposure significantly increased soil UE activity by 27.7 % in soils with pH < 6. Conversely, for soils with pH values between 6 and 7, MPs exposure caused a significant decrease in soil UE activity by 14.5 % (Fig. 5g). MPs exposure did not result in significant variations in soil LAP and NAG activities, irrespective of initial soil pH (Fig. 5h–i).

MPs exposure to soil with initial SOC contents below 10 g kg^{-1} resulted in a significant 18.9 % increase in soil UE activity. In contrast, MPs exposure to soil with initial SOC contents above 20 g kg^{-1} led to a significant 17.0 % decrease in soil UE activity (Fig. 5j). Additionally, a significant increase of 13.9 % in soil LAP activity was observed for initial SOC contents below 10 g kg^{-1} (Fig. 5k). There were no significant changes in soil NAG activity due to MPs exposure, regardless of initial SOC contents (Fig. 5l).

MPs exposure within the range of 0.75 to 1.5 g kg^{-1} of initial soil TN resulted in a significant enhancement of soil UE activity by 11.9 % (Fig. 5m). Soil LAP activity showed a significant increase of 11.9 % for soils with initial TN below 0.75 g kg^{-1} and a 12.9 % increase for soils with initial TN above 1.5 g kg^{-1} when exposed to MPs (Fig. 5n). MPs exposure did not lead to significant changes in NAG activity, regardless of initial TN levels (Fig. 5o).

3.5. Principal regulators of soil N-acquisition activities following exposure to microplastics

The *RR*s of soil N-acquisition activities showed significant correlations with various drivers (Fig. 6). Specifically, the RR_{UE} was significantly correlated with the MPs concentration. The RR_{LAP} exhibited an extremely significant correlation with the initial SOC content. Additionally, the RR_{NAG} was significantly correlated with the soil water-holding capacity.

Based on the outcomes indicating the relative significance of determinants, the primary factors affecting soil UE activity were initial SOC, initial soil TN, and initial soil pH (Fig. 7a). For soil LAP and NAG activities, the crucial determinants were similar: initial soil pH, MPs size, and MPs concentration (Fig. 7b–c). Among the various contributing factors categorized into three groups, edaphic factors had the most significant impact on the variability of soil UE activity, accounting for the largest proportion of the relative influence (RI = 63.0 %). MPs properties ranked second in influence (RI = 22.0 %), and experimental conditions constituted the remaining share (RI = 15.0 %) (Fig. 7a). For soil LAP activity, edaphic factors remained predominant (RI = 49.3 %). MPs properties held a more significant share than in UE activity (RI = 28.5 %), while experimental conditions had a lesser impact (RI = 22.2 %) (Fig. 7b). In contrast, for soil NAG activity, MPs properties were the most influential, driving a considerable portion of the response variance (RI = 48.8 %). Edaphic factors remained influential but had a reduced effect (RI = 39.4 %), and experimental conditions had the least impact (RI = 11.8 %) (Fig. 7c).

Regression analysis indicated a pronounced quadratic correlation between the *RR* of soil UE activity and the initial soil TN content (Fig. 8a). Despite this significant relationship, the initial soil TN explained only 3 % of the variability in soil UE activity. Additionally, a quadratic relationship was discerned between the *RR* of soil LAP activity and soil pH (Fig. 8b), and it was a more substantial explanatory variable, accounting for 13 % of the variance in LAP activity. Furthermore, the analysis revealed a quadratic relationship between the *RR* of soil NAG activity and soil pH (Fig. 8c), but soil pH had minimal explanatory power for NAG activity, explaining only 4 % of its variance.

The SEM suggested the significant role of experimental conditions (exposure temperature and duration), edaphic factors (water-holding capacity, initial soil pH, initial SOC, and initial soil TN), and MPs properties (size and concentration) on changes in soil N-acquisition enzymatic activities (UE, LAP, and NAG) under MPs exposure (Fig. 9). These factors collectively accounted for 5 %, 16 %, and 4 % of the variance in the *RR* of soil UE, LAP, and NAG activities.

4. Discussion

Our study provides critical insights into how MPs affect soil N-acquisition activities, highlighting the complex interplay between MP attributes, experimental conditions, and soil properties. The findings emphasize that MPs can significantly alter soil enzymatic activities, with potential implications for soil health and nutrient cycling. The observed variability in enzymatic responses highlights the importance of considering multiple factors when assessing the environmental impact of MPs. For instance, the significant effects of biodegradable MPs suggest that promoting the use of such materials could mitigate some adverse impacts of conventional plastics. However, the complex interactions between MPs and soil properties, such as pH and SOC content, indicate that context-specific management strategies are necessary.

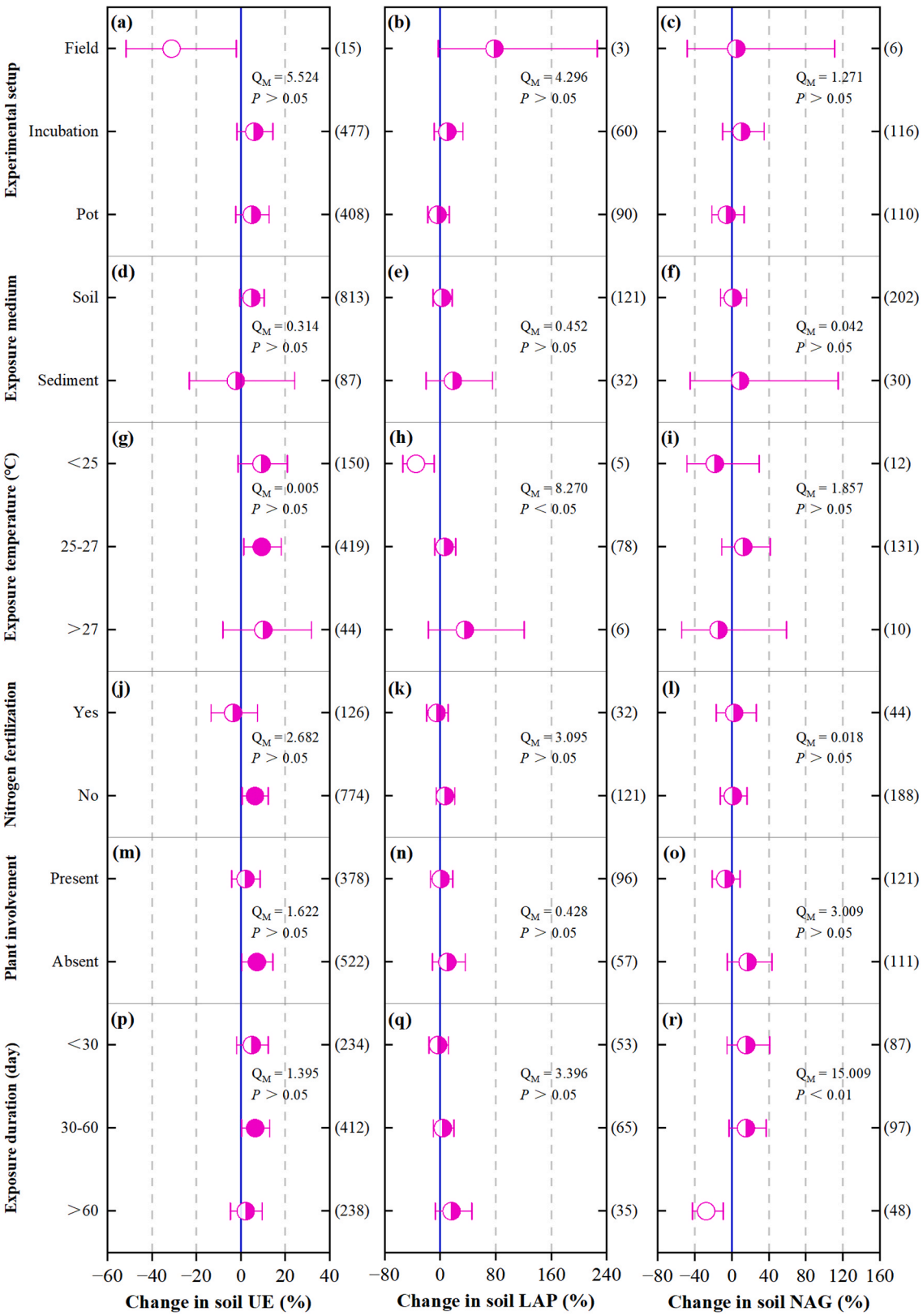

Fig. 4. The responses of urease (UE), leucine aminopeptidase (LAP), and β-1,4-N-acetylglucosaminidase (NAG) activities to various experimental conditions: a–c) experimental setup, d–f) exposure medium, g-i) exposure temperature, j–l) nitrogen fertilization, m–o) plant presence, and p–r) exposure duration. Dots represent mean effect sizes, with lines indicating 95 % confidence intervals. The blue vertical line denotes no change in enzyme activity. Hollow, outlined, and filled purple circles signify neutral, negative, and positive microplastic effects, respectively. Sample sizes (n) are provided in brackets. Statistically significant differences between groups ($p < 0.05$) are indicated.

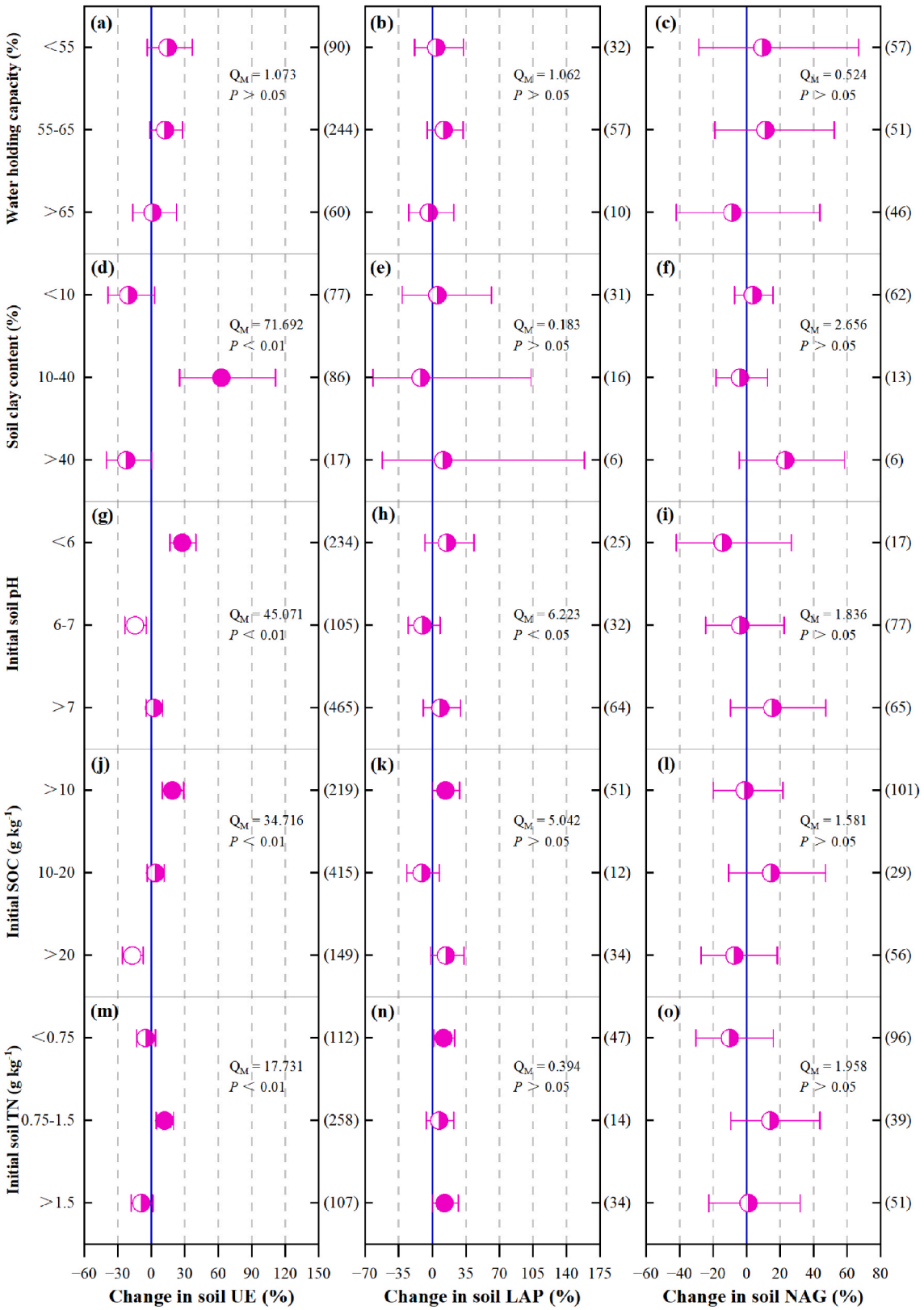

Fig. 5. The influence of edaphic factors the impact of microplastics on urease (UE), leucine aminopeptidase (LAP), and β-1,4-N-acetylglucosaminidase (NAG) activities: a–c) water holdind capacity, d-f) soil clay content, g–i) initial soil pH, j–l) initial soil organic carbon (SOC), and m–o) initial soil TN. Dots represent mean effect sizes, with lines indicating 95 % confidence intervals. The blue vertical line denotes no change in enzyme activity. Hollow, outlined, and filled purple circles signify neutral, negative, and positive microplastic effects, respectively. Sample sizes (n) are provided in brackets. Statistically significant differences between groups ($p < 0.05$) are indicated.

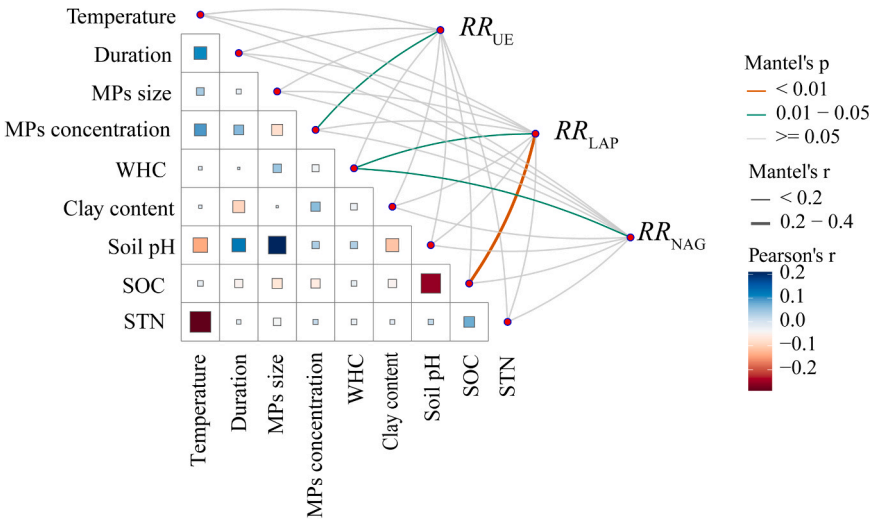

Fig. 6. Correlations between response ratios of soil urease (RR_{UE}), leucine aminopeptidase (RR_{LAP}), and β-1,4-N-acetylglucosaminidase (RR_{NAG}) activities and various factors. These factors include experimental conditions (exposure duration and temperature), microplastic properties (size and concentration), and edaphic factors (water-holding capacity, soil clay content, initial soil pH, initial SOC, and initial soil TN). Edge widths represent Mante's *r* values, with edge colors indicating statistical significance. The color scale represents Pearson's correlation coefficients for pairwise correlations between variables.

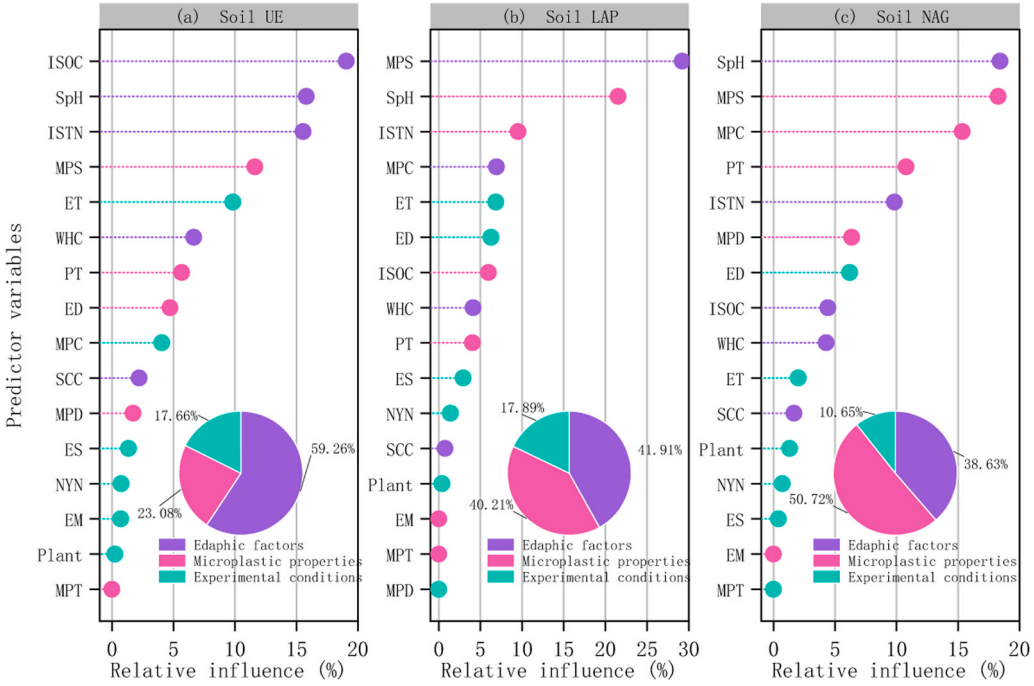

Fig. 7. The results of boosted regression tree (BRT) analyses, showing the relative contribution (%) of various predictor variables in modulating the impacts of microplastics on soil a) urease (UE), b) leucine aminopeptidase (LAP), and c) β-1,4-N-acetylglucosaminidase (NAG) activities. Predictor variables include microplastic properties, experimental conditions, and edaphic factors. Abbreviations: ISOC (initial SOC), ISTN (initial soil TN), ISpH (initial soil pH), MPS (microplastics size), ET (exposure temperature), WHC (water-holding capacity), PT (polymer type), MPC (microplastics concentration), ED (exposure duration), SCC (soil clay content), MPD (microplastics biodegradation), ES (experimental setup), EM (exposure medium), NYN (nitrogen fertilization), MPT (microplastics type).

4.1. General effect of microplastics on soil N-acquisition activities

The overall findings indicate that MPs substantially enhanced UE and LAP activities, while their effect on NAG activity was not statistical significance (Fig. 2d). This enhancement may be attributed to the chemical composition or physical presence of MPs, which could potentially stimulate microbial activity and enzyme production [70,71]. This enhancement could be attributed to several mechanisms: 1) alteration of soil physicochemical properties: MPs may modify soil structure, potentially creating microenvironments that favor microbial growth and enzyme production; these changes could increase water retention and alter aeration, impacting microbial communities and enzyme activities [2,5]; 2) microbial community shifts: MPs could selectively promote microorganisms capable of producing UE and LAP, leading to increased enzyme activities [72]; 3) interaction with enzymes: the surface properties of MPs might stabilize enzymes, prolonging their activity in the soil matrix [26,37,43]. MPs could act as physical supports that protect enzymes from degradation or denaturation, especially under varying environmental conditions.

In contrast, the impact on NAG activities was less significant. This

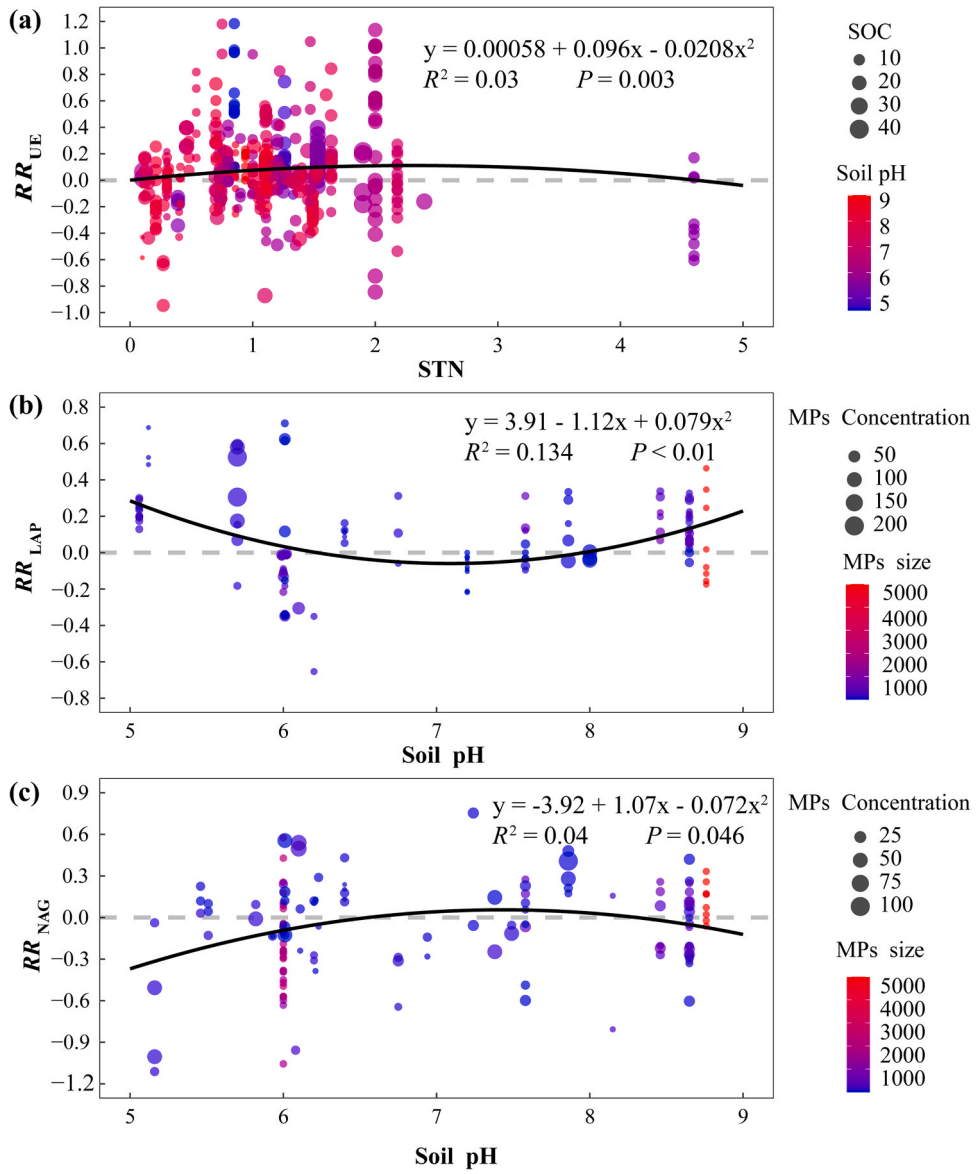

Fig. 8. The relationships between response ratios of a) soil urease (RR_{UE}), b) leucine aminopeptidase (RR_{LAP}), and c) β-1,4-N-acetylglucosaminidase (RR_{NAG}) activities and initial soil total nitrogen (STN), and initial soil pH under microplastics (MPs) exposure. Dot sizes represent initial soil organic carbon (SOC) or MPs concentration, while color gradients indicate initial soil pH or MPs size.

discrepancy might be due to several factors: 1) enzyme-specific sensitivity: NAG may be less responsive to MP-induced changes in the soil environment compared to UE and LAP; 2) substrate availability: if MPs affect the availability of NAG substrates differently from UE and LAP substrates, this could explain the varying responses; 3) microbial community dynamics: MPs might preferentially stimulate microorganisms that produce UE and LAP over those producing NAG [16]; and 4) structural complexity: NAG may have a more complex tertiary structure that could be more susceptible to denaturation or loss of functionality in the presence of MPs, leading to a diminished or inconsistent response. The lack of a significant effect on NAG activity underlines the need for further investigation to fully understand these mechanisms.

4.2. *Effects of microplastic attributes on soil N-acquisition activities*

The complex interplay between the MPs properties and soil enzymatic responses was evident. Polymer type emerges as a significant factor, with PBAT, PLA, and PP demonstrating a considerable capacity to increase soil UE activity by 36.9 %, 10.3 %, and 15.1 %, respectively (Fig. 3a). This variability suggests that chemical composition and structure substantially affect the interaction between MPs and soil enzymes, potentially influencing microbial metabolism and N mineralization processes [73,74]. Biodegradable MPs like PBAT and PLA might release compounds during degradation that stimulate microbial activity and enzyme production, leading to more pronounced effects compared to conventional plastics [73,74].

The significant increase of soil LAP activity in response to PBAT (Fig. 3b), indicates a possible synergistic effect between this polymer and the enzymatic machinery of the soil [75]. Conversely, the significant reduction in soil NAG activity upon exposure to PET (Fig. 3c) suggests that not all MPs uniformly increase enzymatic activities; some may exert inhibitory effects, potentially disrupting soil microbial function and N cycling [76,77]. These differential effects suggest the importance of considering specific polymer types in environmental impact assessments of MPs. Biodegradable MPs induced significant increases in soil UE, LAP, and NAG activities (Fig. 2g–i), which underscores the pivotal role of MP degradability in modulating enzymatic responses [73], implying that the rate and extent of MP breakdown in the soil are key determinants of

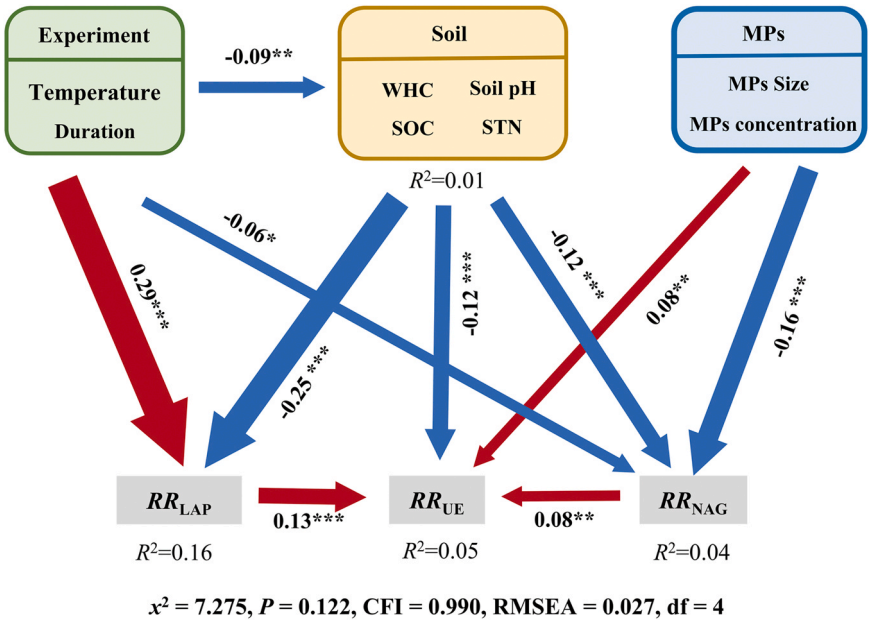

Fig. 9. The results of a piecewise structural equation model, demonstrating how experimental conditions (exposure temperature, duration), edaphic factors (water holding capacity, initial soil pH, soil organic carbon, and initial soil total nitrogen concentrations), and microplastic properties (size, concentration) influence the response ratios of a) soil urease (RR_{UE}), b) leucine aminopeptidase (RR_{LAP}), and c) β-1,4-N-acetylglucosaminidase (RR_{NAG}) activities under microplastic exposure. Red and blue arrows indicate significant positive and negative relationships ($p < 0.05$), respectively. Standardized path coefficients are shown next to arrows, with varying widths reflecting significance levels (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). The percentage (R^2) associated with response variables represents the proportion of variance explained by other variables.

their environmental impact [78,79].

The concentration-dependent effects of MPs reveal a threshold concentration for soil UE activity above which a significant increase is observed (10 g kg^{-1}; Fig. 3m). This suggests a potential saturation point beyond which the soil enzymatic response plateaus, highlighting the necessity for dose-response studies to better understand the ecological risks associated with MP pollution. The minimal and non-significant improvement in soil LAP activity and the negligible shift in soil NAG activity with increasing MP doses (Fig. 2n–o) suggest that the relationship between MPs concentration and enzymatic activity may not be linear [47,80]. Other factors, such as polymer type and environmental conditions, may affect this relationship, indicating a need for multifactorial studies to disentangle these effects.

4.3. Role of experimental setup in mediating microplastic effects

Our findings indicate a stark contrast in the response of soil UE activity to MP exposure across different conditions. Notably, a reduction in UE activity was observed under field conditions, while incubation and pot environments remained relatively unaffected (Fig. 4a). This suggests that the complex environmental factors present in field settings, such as diverse microbial communities and fluctuating environmental conditions, may interact with MPs in ways that are not replicated in controlled experimental setups [81]. Field conditions introduce a range of biotic and abiotic interactions that could influence the stability and activity of soil enzymes. Confounding factors, such as variable soil water contents, natural vegetation, and organic matter inputs, might also play a role in these differential responses.

The influence of experimental temperatures on soil N-acquisition activities further highlights the significant impact of MP exposure. An optimal temperature range of 25–27 °C significantly increased soil UE activity by 9.3 % (Fig. 4g), potentially due to the enhanced kinetic activity of UE enzymes at these temperatures [82,83]. However, temperatures below 25 °C led to a substantial decrease in LAP activity by 34.7 % (Fig. 4h), indicating that temperature sensitivity may be a key factor in modulating LAP enzymatic responses to MPs [18]. The resilience of soil NAG activity across the evaluated temperature ranges (Fig. 4i) implies that this enzyme may be less influenced by thermal variations post-MP exposure [84]. Enzyme kinetics could play a role here, as temperature variations might affect enzyme-substrate interactions differently depending on the specific characteristics of each enzyme.

Application of N fertilizers did not significantly alter soil UE activity affected by MPs. Interestingly, the substantial enhancement of soil UE activity was observed in the absence of N fertilizer application (Fig. 4j), suggesting that MPs may stimulate enzymatic activity under N-limited conditions [72]. This finding implies that MPs could influence N cycling dynamics, particularly in agricultural soils where fertilizer use is prevalent. The lack of N input may create a condition where microbial activity is driven by the need to mineralize organic N, potentially amplified by the presence of MPs.

The presence or absence of plants also influenced the response of soil UE activity to MPs, with a significant increase in the absence of plant (Fig. 4e). This suggests that plants may mitigate the impact of MPs on soil UE activity, potentially through competition for N resources or alterations in the rhizosphere [50]. Plants can influence microbial community composition and function through root exudates and nutrient uptake, which may buffer the effects of MPs. The lack of significant response in LAP and NAG activities to MP exposure, irrespective of plant presence (Fig. 4n–o), indicates that these enzymes might be less influenced by plant-microbial interactions, or that the regulatory effect of MPs on these enzymes is dominant.

4.4. Influence of soil properties on microplastic effects

Our study reveals that soil properties significantly modulate the effects of MPs on nitrogen-acquisition activities of soil enzymes. Soil clay content emerged as a crucial factor, with MPs exposure leading to a significant enhancement in soil UE activity in soils containing 10–40 % clay (Fig. 5d). Soils with intermediate clay content provide a larger surface area for microbial colonization and enzyme adsorption, enhancing enzyme stability and activity. This intermediate clay range

also likely reflects a more balanced soil texture, where sand and silt particles are present in significant amounts, promoting both effective water retention and good gas exchange. High clay content might hinder enzyme diffusion, while low clay content might not provide sufficient adsorption sites [85,86]. However, soils with clay content outside this range did not exhibit significant changes in UE activity, possibly indicating a threshold effect of soil texture on MP-soil interactions.

Initial soil pH also emerges as a pivotal factor, with acidic soils (pH < 6) showing a significant increase in UE activity upon MP exposure, while neutral soils (pH 6–7) experienced a decrease (14.5 %) (Fig. 5g). This pH-dependent response may be attributed to the altered solubility of MP particles and their subsequent bioavailability to soil microbes [79,87]. MPs in acidic soils might enhance enzyme activity by creating favorable conditions for microbial growth, whereas in neutral soils, the interaction might inhibit enzyme function. The interaction between pH and MPs could also influence the charge and binding affinity of enzymes, further affecting their activity.

The role of SOC is highlighted by findings indicating that soils with an initial SOC below 10 g kg^{-1} exhibit significant increases in UE and LAP activities when exposed to MPs (Figs. 4j and 4k). Conversely, soils with higher initial SOC contents show a decrease in UE activity. This suggests that SOC content may affect the bioavailability and effects of MPs, potentially influencing microbial activity and N mineralization processes [87,88]. Lower SOC contents might limit nutrient availability, making the additional input from MPs more impactful, whereas higher SOC contents might buffer the effect of MPs, leading to reduced enzyme activity. SOC can interact with MPs by adsorbing or complexing with them, potentially reducing their availability to interact with enzymes or microbes directly.

Initial soil TN contents also modulate the response of soil enzymes to MPs, with an optimal range of 0.75–1.5 g kg^{-1} resulting in a significant enhancement of UE activity (Fig. 5m). Soils with TN contents outside this range show an increase in LAP activity, indicating that N availability may play a role in the response of soil N-acquisition enzymes to MP exposure [34,41]. The varying TN contents might influence microbial community composition and function, thereby affecting enzyme activities. N availability might modulate the competition between MPs and natural organic matter for microbial degradation, influencing the overall impact on enzyme activities.

4.5. Study limitations, further research, and implications

Our meta-analysis on the effects of MPs on soil N-acquisition activities provides valuable insights but has limitations. The variability in experimental conditions and soil types across studies introduces inconsistencies, potentially limiting result generalizability. The focus on short-term effects and specific MPs attributes and soil properties may overlook other influential factors and long-term impacts. Additionally, the predominance of laboratory-based studies may not fully represent real-world soil environments.

To address these limitations, future research should develop standardized protocols for MPs studies in soil, conduct long-term experiments, and investigate combined effects of MPs with other soil contaminants. Expanding field-based research is crucial to validate laboratory findings under realistic conditions. Studies exploring the influence of varying environmental factors, such as climate change and agricultural practices, will enhance our understanding of MP-soil enzyme interactions. In-depth mechanistic studies are also needed to elucidate how MPs affect soil enzyme activities at the molecular level.

Our findings suggest that MPs pollution may alter soil N cycling, affecting agricultural productivity and ecosystem health. This highlights the need for improved waste management practices and the development of biodegradable alternatives to conventional plastics. The varying effects of different MPs types and concentrations underscore the importance of targeted regulations on plastic production and disposal. Lastly, our study provides a foundation for more focused research on specific MPs types and soil conditions, enabling a nuanced understanding of MPs impacts on soil ecosystems and informing effective mitigation strategies.

5. Conclusions

Our meta-analysis demonstrates that MPs have complex, context-specific interactions with soil N-acquisition enzyme activities, influenced by various MPs attributes, experimental conditions, and soil properties. Specifically, urease and leucine aminopeptidase activities increased by 7.6 % and 8.0 %, respectively, upon MPs exposure, while N-acetyl-β-D-glucosaminidase activity remained unaffected. Biodegradable polymers, such as PBAT and PLA, exhibited more pronounced effects than conventional plastics, highlighting the role of chemical properties and biodegradability in shaping soil enzyme responses. Experimental factors, including incubation periods, temperature, N fertilization, and environmental settings, significantly modulated MPs impacts on enzyme activities. Furthermore, soil-specific characteristics such as pH, organic carbon content, and clay content were critical regulators of enzyme responses. For instance, acidic soils (pH < 6) enhanced urease activity, whereas neutral soils (pH 6–7) reduced it, and soils with intermediate clay content (10–40 %) provided optimal conditions for increased enzyme activity.

These findings highlight the need for context-specific assessments to accurately determine the ecological impacts of MPs across diverse environments. The study's reliance on short-term, controlled experiments and the lack of standardized methodologies are notable limitations. Future research should prioritize long-term field studies to assess chronic MPs exposure, explore synergistic effects with other soil contaminants, and establish standardized protocols for evaluating MPs impacts. The ecological safety and long-term behavior of biodegradable MPs also require further study to determine their suitability as sustainable alternatives. Our findings are vital for guiding environmental management and policy-making toward sustainable practices that protect soil health and ecosystem functioning. Although promoting biodegradable MPs and tailored management strategies could help mitigate adverse effects, caution is needed given the uncertainties surrounding their long-term impacts.

Environmental Implication

This study reveals that microplastics (MPs) significantly alter soil nitrogen-acquisition enzyme activities, particularly enhancing urease and leucine aminopeptidase in soils. The findings underscore the critical role of soil properties, such as pH and clay content, in modulating these effects. Promoting biodegradable MPs and adopting site-specific management strategies could mitigate the adverse impacts of MPs on soil health and nutrient cycling. This research provides essential insights for environmental policy-making and sustainable soil management practices, addressing the growing concern of MPs pollution in terrestrial ecosystems.

CRediT authorship contribution statement

Yangzhou Xiang: Designed research, Collected data, Formal analysis, Visualization, Writing – original draft, Funding acquisition. **Bin Yao:** Designed research, Methodology, Supervision, Writing – original draft. **Josep Peñuelas** and **Jordi Sardans**: Designed research, Writing – review & editing, Discussion of methods and results, Funding acquisition. **Luca Nizzetto, Rui Li,** and **Mari Räty:** Writing – review & editing. **Ying Liu:** Collected data, Data curation, Writing – original draft, Funding acquisition. **Yang Luo:** Formal analysis, Data curation. **Jian Long:** Project administration, Writing – review & editing, Funding acquisition. **Yuan Li:** Writing – original draft, Visualization, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2024.136677.

Data availability

Data has been included in the Supplementary materials.

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