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# Nano-Iron Oxide ($Fe_3O_4$) Mitigates the Effects of Microplastics on a Ryegrass Soil–Microbe–Plant System

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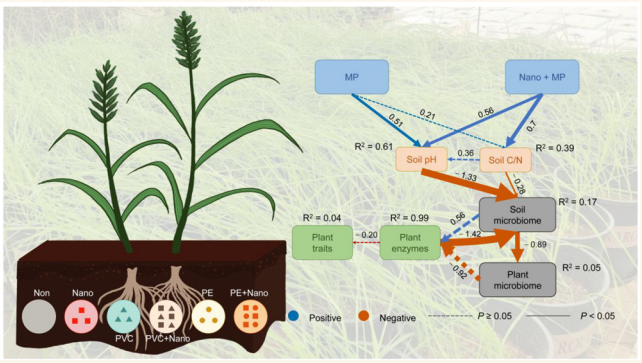
**ABSTRACT:** To understand microplastic–nanomaterial interactions in agricultural systems, a randomized block 90-day pot experiment was set up to cultivate ryegrass seedings in a typical red sandy soil amended with compost (1:9 ratio). Polyvinyl chloride (PVC) and polyethylene (PE) microplastic (MP) contaminants were added into pot soils at 0.1 and 10%, whereas nano-$Fe_3O_4$ (as nanoenabled agrochemicals) was added at 0.1% and 0.5% in comparison with chemical-free controls. The combination of nano-$Fe_3O_4$ and MPs significantly increased the soil pH (+3% to + 17%) but decreased the total nitrogen content (−9% to − 30%; $P < 0.05$). The treatment group with both nano-$Fe_3O_4$ and PE had the highest total soil C (29 g $kg^{-1}$ vs 20 g $kg^{-1}$ in control) and C/N ratio (13 vs 8 in control). Increased rhizosphere nano-$Fe_3O_4$ concentrations promoted ryegrass growth (+42% dry weight) by enhancing the chlorophyll (+20%) and carotenoid (+15%) activities. Plant leaf and root peroxidase enzyme activity was more significantly affected by nano-$Fe_3O_4$ with PVC (+15%) than with PE (+6%). Nano-$Fe_3O_4$ significantly changed the ryegrass bacterial community structure from belowground (the rhizoplane and root endosphere) to aboveground (the phylloplane). Under MP contamination, the addition of nano-$Fe_3O_4$ increased bacterial diversity (+0.35%) and abundance (+30%) in the phylloplane and further intensified the connectivity of ryegrass aboveground bacterial networks (positive association increased 17%). The structural equation model showed that the change in the plant microbiome was associated with the rhizosphere microbiome. Overall, these findings imply the positive influences of nano-$Fe_3O_4$ on the soil–microbe–plant system and establish a method to alleviate the harmful effects of MP accumulation in soils.




**KEYWORDS:** *grass, microbiome, microplastics, nanoparticles, soils, ryegrass*

## INTRODUCTION

The numerous applications of plastics, mainly due to their flexible surfaces and lightweight nature, have greatly boosted plastic production since 1950 to the current manufacture of more than 12.5 million tons annually.[1,2] The prevalent use of plastics is projected to generate around 12 billion tons of plastic debris by 2050, and this is likely to lead to severe environmental issues.[3,4] Microplastic (MP) particles of synthetic organic polymers with sizes <5 mm have emerged as dangerous pollutants since 1980, and much attention has been paid to the MPs and their relevant health issues.[5,6] MPs with different shapes and morphologies such as pellets, fibers, foams, and films have been reported to spread in the atmosphere, terrestrial, aquatic, and soil environments, posing a threat to living beings.[7,8] Therefore, MP contamination has been intensifying/escalating rapidly, gaining a lot of research attention and generating the need to understand the impact of MPs on terrestrial environments, especially agriculture. Although the use of plastic in agriculture initially promoted food security worldwide, today it is well-known that it has left a pollution legacy, as MPs threaten food production systems.[9]

The loading of polyethylene (PE) in the soil is extremely common due to the extensive use of PE mulch in agriculture.[10] PE raw material is preferably used to produce mulch that is difficult to degrade, causing PE accumulation.[11,12] A growing body of evidence has become available that shows that PE MPs

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Table 1. Physicochemical Properties of Ryegrass Rhizosphere Soils with Different Microplastic (PVC and PE) and/or Nano-$Fe_3O_4$ Additions[a]

| treatment | pH | total C (g $kg^{-1}$) | total N (g $kg^{-1}$) | C:N ratio | organic matter (g $kg^{-1}$) | $Fe_2O_3$ (mg $kg^{-1}$) |
|---|---|---|---|---|---|---|
| control | 6.53 ± 0.02d | 19.97 ± 0.65bc | 2.45 ± 0.21a | 8.20 ± 0.83e | 45.6 ± 3.7c | 208 ± 2.4a |
| nano-$Fe_3O_4$ | 6.74 ± 0.08c | 20.20 ± 0.09b | 2.06 ± 0.03bc | 9.79 ± 0.17d | 52.8 ± 10.7b | 207 ± 4.2a |
| PVC | 7.10 ± 0.05b | 18.99 ± 0.58c | 2.03 ± 0.05c | 9.37 ± 0.31d | 34.2 ± 2.7d | 147 ± 3.8c |
| PVC + nano-$Fe_3O_4$ | 7.06 ± 0.09b | 19.84 ± 0.4bc | 1.83 ± 0.04d | 10.86 ± 0.34c | 41.6 ± 7.6c | 117 ± 10.1d |
| PE | 7.16 ± 0.07b | 20.53 ± 0.28b | 1.70 ± 0.03d | 12.06 ± 0.10b | 58.9 ± 4.6ab | 195 ± 4.0b |
| PE + nano-$Fe_3O_4$ | 7.67 ± 0.12a | 29.09 ± 0.92a | 2.23 ± 0.08b | 13.06 ± 0.10a | 74.1 ± 3.2a | 184 ± 1.7b |

[a]Note: the significance tests among chemical treatments are based on the least significant difference (LSD) test ($n = 4$, $P < 0.05$).

may alter the physical and chemical conditions of soil.[13] In particular, several soil properties that are indicators of soil health, such as the pH, organic carbon, aggregation, aeration, organic matter (OM), water retention capacity, and nutrient (nitrogen (N) and phosphorus (P)) content and availability, are affected.[13,14] PE MPs are also known to reduce seed germination by mechanically covering the pore of the seed.[15] Moreover, the bioavailability of heavy metals such as Cd after soil contamination with PE reduces root growth and arbuscular mycorrhizal fungi symbiotic associations with plants.[16] Soil biota are indicators of plant health and nutrient cycling and have a significant impact on soil ecosystem services. Recent studies have reported that PE MPs have the potential to alter microbial community composition and activities as well as the stability of microfood webs.[17] Similarly, polyvinyl chloride (PVC) is one of the most commonly detected MPs in soil environments,[18] and it has also substantial impacts on microbial abundance (such as Burkholderiaceae) and network stability, with wide implications for crop growth.[19,20] Such impacts on soil microbes are due to either changes in soil properties or the release of impurities and chemical additives from plastics.[21] Hence, the loading of PE and PVC MPs represents a major challenge for agroecosystems. Importantly, the concentration of these MPs in soil is most likely to have substantial impacts on the soil microbes and plant growth. For instance, the growth of romaine lettuce was found to decrease due to PE contamination at the rates of 0.005%, 0.025%, and 0.1% in the soil, although the impact of 0.1% was the greatest.[22] A PE content of 1% in soil has been shown to negatively affect the above- and belowground compartments of wheat plants during both vegetative and reproductive growth.[23] In a recent experiment aimed at investigating the effects of MPs on plant performance, PE and PVC each at 0.2% and 1% concentrations appeared to affect the growth and reproduction biomass of native and invasive *Phytolacca* species.[24] In addition, a 0.1% content of high-density PE in soil can reduce the total biomass of perennial ryegrass through the alteration of soil stability components such as pH, organic matter, and water-stable aggregates.[25] Thus, MP contamination in grasslands represents an important concern within agricultural production systems.[26] However, information regarding the impacts of relatively higher doses of PE and PVC on the agroecosystem is scarce. In addition, extremely limited knowledge exists on how to protect the functioning of these ecosystems in cases whereby the PE and PVC concentrations in soil exceed the current stock.

To meet the global food production demand, using nanoparticles (NPs) that promote plant growth and help crops cope with environmental stress is becoming common in agriculture.[27] NPs can enhance plant disease resistance,[28] increase plant rhizosphere N-fixing bacteria,[29] and stimulate N fixation and plant growth.[30] In terms of plant protection and fertilization, NP use has contributed tremendously to sustainable nanoenabled agricultural development.[31] Among nanomaterials, the use of $Fe_3O_4$ is common because its positive influence has been reported in many crops.[32] For example, the application of 500 mg $L^{-1}$ of $Fe_3O_4$ significantly promoted the fresh weight of barley leaves by 19% and roots by 88%[33] while the application of $Fe_3O_4$ (100 mg $L^{-1}$) was shown to promote maize plant growth.[34] The positive effects of $Fe_3O_4$ on soil microbes have also been reported, where for instance applying magnetic $Fe_3O_4$ NPs at the rates of 0.04%, 0.08%, and 0.26% could facilitate soil carbon (C) and N cycling by changing the bacterial community structure.[35] In particular, the abundance of N-fixation-related bacteria Bradyrhizobiaceae and iron-redox bacteria Sediminibacterium were noted to decline while the proliferation of *Duganella* and *Nocardioides* bacteria was observed. Additionally, nano-$Fe_3O_4$ application at the rate of 0.01% significantly increased the populations of carbon-cycling bacteria, *Nocardioides*, *Chitinophaga sancti*, *Pantoea*, and *Rhizobium* from 0.14 to 0.96, which constituted an average of 0.58% in relative abundance.[34] However, the use of $Fe_3O_4$ (0.2%) induced significant decline in the microbial biomass by up to 55%, 36% of mineral N content, and 125% reduction in N mineralization efficiency of sandy soil mainly due to the presence of labile Fe in the microbial biomass.[36] Thus, understanding the effects of $Fe_3O_4$ on both plants and microbes in soils and the relevant mechanisms is crucial for risk evaluation and sustainable agriculture practices. Practically, the amount of nanoscale Fe can be scaled up and produced in an economically feasible manner for that level of amendment on a per acre basis. Furthermore, $Fe_3O_4$ has the potential to magnetize MPs via surface adsorption[37] and this can facilitate the removal of MPs, especially from water bodies. Similarly, in soil environments, applied $Fe_3O_4$ can bind with PE and PVC and may reduce the negative effects of these MPs on plants and soil microbial life. However, empirical evidence of these beneficial effects associated with $Fe_3O_4$ is scarce. Consequently, studies evaluating interactions between MPs and $Fe_3O_4$ are important to achieving resilience against the adverse effects of MPs on the soil−plant system. This study evaluates the integrated responses of soil microbes and plant systems when exposed to both low and extremely high levels of MP contamination accompanied by nano-$Fe_3O_4$ addition using soil planted with ryegrass (*Lolium perenne*), a common perennial grass used in agriculture during the fallowing stage. The present study hypothesized that PVC and PE would adversely alter plant growth and physiological traits, as well as the soil physicochemical and biochemical properties, while the coapplication of nano-$Fe_3O_4$ and MPs would alleviate these effects. The findings will enhance the understanding of microplastic−nanomaterial interactions and provide important


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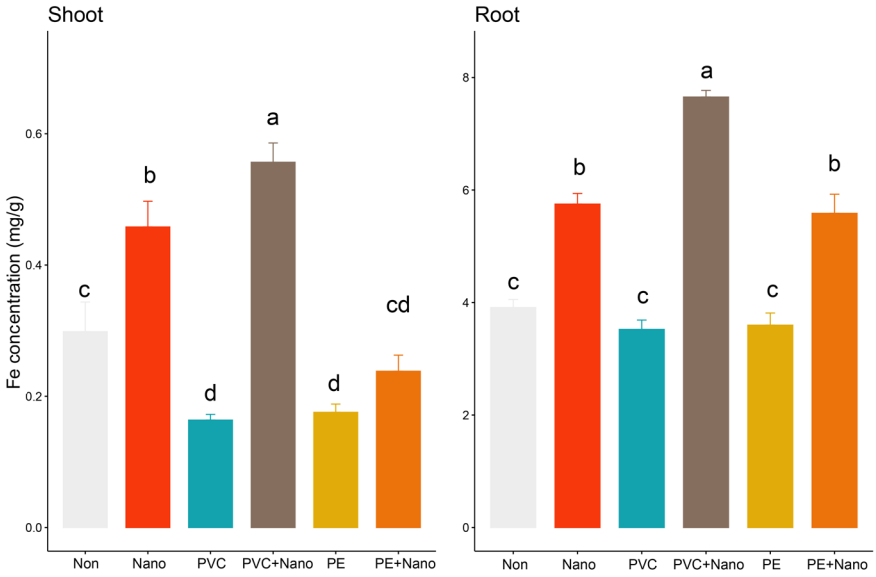


**Figure 1. Effects of the addition of nano-$Fe_3O_4$ and microplastics (poly(vinyl chloride) (PVC) and polyethylene (PE)) on the Fe uptake of ryegrass shoots (a) and roots (b). Values are the means (SE) ($n$ = 12); values followed by different letters are significantly different at $P$ < 0.05 (analysis of variance (ANOVA), least significant difference (LSD) test).**

information for the development of sustainable nanoenabled strategies to alleviate microplastic contamination in agricultural systems.

## RESULTS AND DISCUSSION

**Rhizosphere Soil Physicochemical Properties.** Nano-$Fe_3O_4$ and MP addition, either individually or in combination, significantly increased the soil pH (+3% to + 17%) but decreased (−9% to −30%; $P$ < 0.05) the soil total N content (Table 1).

The combined application of PE and nano-$Fe_3O_4$ had the highest pH, total C, and C/N ratio, followed by the application of PE alone. However, the unamended control displayed the maximum total N. It was found that adding MPs (PE and PVC) and nano-$Fe_3O_4$ increased the ryegrass rhizosphere soil pH. The increased pH was in accordance with Yang's research,[38] regardless of plastic type, low-density PE or high-density PE. Such effects can be explained by the alteration of the soil acid−base equilibrium via changing the competitive sorption of hydrophobic organic compounds between MPs and soil OM.[39] Previous studies have shown that soil pH changes can affect soil nutrient mobility.[40] In this study, under the presence of nano-$Fe_3O_4$ and MPs, the increased soil pH might stimulate ryegrass N absorption, leading to a significant decrease (−9 to −30%) in the rhizosphere total N contents. However, the effects of nano-$Fe_3O_4$ on soil N (or soil C) varied with the MP type. This is because MPs made from various polymers with different properties have different effects on soil properties and biota.[41] The structural aspect of MPs that affects chemical sorption is the glass transition temperature ($T_g$). Due to the pore-filling process of organic chemicals into PVC (glassy polymers, $T_g > T_{ambient}$), a nonlinear sorption isotherm was observed on PVC, which differed from the linear sorption isotherm for PE (rubbery polymers, $T_g < T_{ambient}$).[39] As a common soil component, $Fe_2O_3$ has a large proportion of colloid-based fractions. This was true in the red soil tested in the current study, which had the highest content of $Fe_2O_3$ (208 mg $kg^{-1}$; Table 1). However, under the presence of MPs (either PE or PVC), the content of $Fe_2O_3$ decreased significantly ($P$ < 0.05), which could be explained by the effects of MP-mediated pH and ionic composition changes on the sorption/desorption of soil OM on Fe compounds.[42] As a key soil characteristic influencing Fe chemistry and behavior, OM influences the stability of Fe and redox processes associated with electron transfer processes.[43] When applying nano-$Fe_3O_4$ to MP-contaminated soils, the OM showed an increase but the content of $Fe_2O_3$ was decreased (Table 1). The increased soil OM (from 46 to 53 g $kg^{-1}$) under the addition of nano-$Fe_3O_4$ could be associated with the adsorption of fulvic acid (one of the humic substances) on the surfaces of $Fe_3O_4$ NPs through chemical reactions.[44] Nanomaterials are known to have a wide range of potential applications in agriculture, including providing efficient nutrient delivery, crop protection strategies, and responsive phytohormones.[45,46] In comparison to chelated Fe, the nano-Fe was the most efficient source of Fe and it can enhance Fe solubility and its subsequent uptake by the plant (either by roots or leaves) due to its smaller particle size and larger surface area.[47] For example, foliar application of nano-Fe can lead to greater growth than conventional Fe chelates.[48] Research works have indicated the positive and enhancing effects of nano-Fe fertilization on plant growth and yield.[30]

**Plant Fe Uptake, Plant Growth, and Physiological Traits.** The uptake of Fe was over 15 times higher in the ryegrass shoots (~5 mg $g^{-1}$) than in roots (~0.3 mg $g^{-1}$), indicating that Fe was mainly transferred and stabilized in plant shoots. Compared to the control, MPs decreased the Fe content in shoots (−76%, $P$ < 0.05) and roots (−8%, $P$ < 0.05) (Figure 1), showing that MPs could decrease the available Fe concentration in the rhizosphere and Fe uptake in the aboveground parts of plants. This should be associated with the negative effects of MPs in belowground soil environments such as decreased pH, reduced soil stability, and significantly decreased microaggregates (<63 $\mu$m),[25] and the variations in soil physicochemical characteristics and structure could further impair plant Fe acquisition systems and the major components


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**Table 2. Effects of the Nano-$Fe_3O_4$ and Microplastic (Polyvinyl Chloride (PVC) and Polyethylene (PE)) Additions on the Growth and Physiological Traits of Ryegrass**

| treatment (w/w soil) | root length (cm) | dry weight (g) | Fv/Fm | chlorophyll A (mg $g^{-1}$) | chlorophyll B (mg $g^{-1}$) | carotenoids (mg $g^{-1}$) |
|---|---|---|---|---|---|---|
| control | 18.62 ± 3.47a | 0.17 ± 0.13a | 0.79 ± 0.06a | 1.4 ± 0.24abc | 0.4 ± 0.07a | 0.32 ± 0.05ab |
| 0.01% $Fe_3O_4$ | 20.84 ± 3.00a | 0.26 ± 0.10a | 0.87 ± 0.08a | 1.47 ± 0.19abc | 0.39 ± 0.06a | 0.34 ± 0.04ab |
| 0.05% $Fe_3O_4$ | 25.3 ± 7.27a | 0.37 ± 0.13a | 0.74 ± 0.02a | 1.73 ± 0.34ab | 0.48 ± 0.12a | 0.39 ± 0.06ab |
| 1% PVC | 22.42 ± 6.2a | 0.32 ± 0.13a | 0.78 ± 0.08a | 1.88 ± 0.31a | 0.55 ± 0.12a | 0.47 ± 0.13a |
| 1% PVC + 0.01% $Fe_3O_4$ | 23.26 ± 7.9a | 0.22 ± 0.10a | 0.75 ± 0.07a | 1.62 ± 0.38abc | 0.50 ± 0.14a | 0.39 ± 0.09ab |
| 1% PVC + 0.05% $Fe_3O_4$ | 26.5 ± 13.9a | 0.26 ± 0.16a | 0.80 ± 0.06a | 1.49 ± 0.04abc | 0.42 ± 0.02a | 0.35 ± 0.01ab |
| 10% PVC | 28.0 ± 5.38a | 0.28 ± 0.11a | 0.83 ± 0.09a | 1.31 ± 0.30bc | 0.46 ± 0.18a | 0.30 ± 0.07b |
| 10% PVC + 0.01% $Fe_3O_4$ | 28.8 ± 4.14a | 0.41 ± 0.22a | 0.77 ± 0.09a | 1.44 ± 0.11abc | 0.41 ± 0.07a | 0.33 ± 0.02ab |
| 10% PVC + 0.05% $Fe_3O_4$ | 27.1 ± 9.22a | 0.41 ± 0.04a | 0.73 ± 0.01a | 1.48 ± 0.18abc | 0.45 ± 0.06a | 0.34 ± 0.04ab |
| 1% PE | 23.75 ± 1.19a | 0.30 ± 0.09a | 0.77 ± 0.10a | 1.42 ± 0.45abc | 0.41 ± 0.09a | 0.32 ± 0.12ab |
| 1% PE + 0.01% $Fe_3O_4$ | 31.20 ± 5a | 0.35 ± 0.09a | 0.78 ± 0.06a | 1.42 ± 0.11abc | 0.41 ± 0.02a | 0.33 ± 0.02ab |
| 1% PE + 0.05% $Fe_3O_4$ | 22.90 ± 4.47a | 0.32 ± 0.11a | 0.77 ± 0.06a | 1.27 ± 0.16bc | 0.37 ± 0.03a | 0.30 ± 0.03b |
| 10% PE | 24.10 ± 5.6a | 0.34 ± 0.16a | 0.74 ± 0.01a | 1.15 ± 0.22c | 0.35 ± 0.05a | 0.27 ± 0.06b |
| 10% PE + 0.01% $Fe_3O_4$ | 23.50 ± 2.78a | 0.25 ± 0.05a | 0.79 ± 0.10a | 1.34 ± 0.42abc | 0.39 ± 0.09a | 0.31 ± 0.1b |
| 10% PE + 0.05% $Fe_3O_4$ | 31.40 ± 10.9a | 0.40 ± 0.13a | 0.85 ± 0.10a | 1.65 ± 0.29abc | 0.48 ± 0.11a | 0.39 ± 0.06ab |

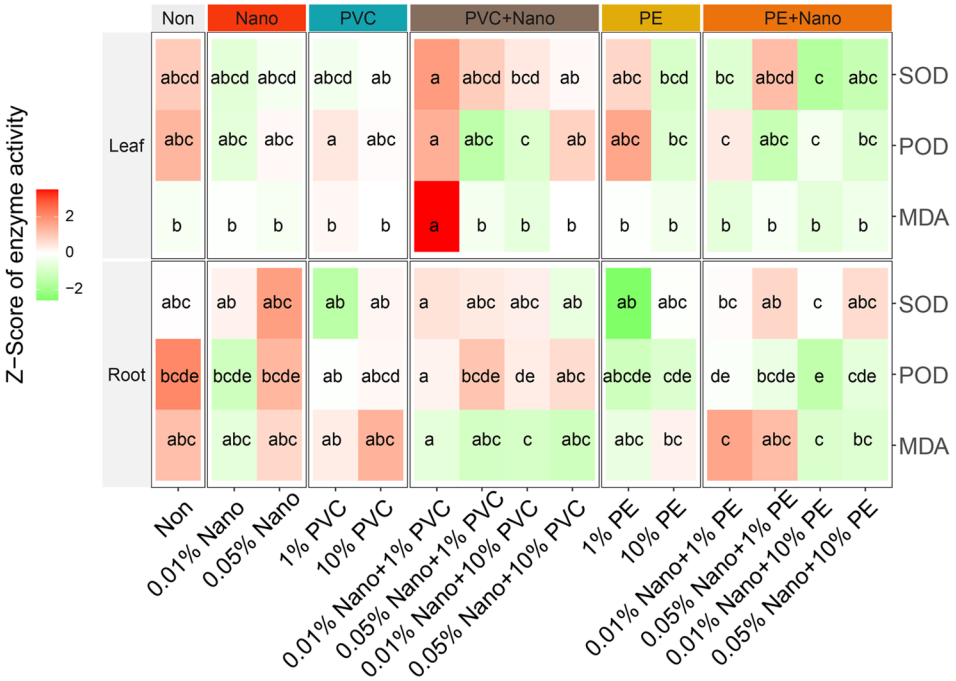


**Figure 2. Effects of the addition of nano-$Fe_3O_4$ and microplastics (poly(vinyl chloride) (PVC) and polyethylene (PE)) on the malondialdehyde (MDA), superoxide dismutase (SOD), and peroxidase (POD).**

that regulate Fe uptake systems.[49] However, when nano-$Fe_3O_4$ was applied to MP-contaminated soils, the Fe uptake was significantly increased in the roots (+116% in the PVC treatment; +30% in the PE treatment; Figure 1). The observed result reflected the positive effect of nano-$Fe_3O_4$ in mitigating the negative effects of MPs on plant Fe uptake and subsequent growth. Such positive effects can be attributed to (i) the strong adherence of nano-$Fe_3O_4$ on the MPs surface which weakens MP's ecotoxicology on plants[37,50] and (ii) the positive effects of nano-$Fe_3O_4$ in facilitating plant rhizosphere nutrient cycling[35] and increasing plant growth promoting microbes.[51]

Photosynthesis is a key physiological process that is affected by different plant stresses. The addition of nano-$Fe_3O_4$ and/or MPs exerted weak stresses on ryegrass, as shown by a slight decrease (−3% on average) of the Fv/Fm (a chlorophyll fluorescence parameter reflecting the maximum quantum efficiency of photosystem II (PSII) photochemistry) in the majority of treatments (10 of 14).

There were strong increases in ryegrass root length (+21.6%; 20.8−25.3 cm) and dry weight (+42.3%; 0.26−0.37 g) when the concentration of plant rhizosphere nano-$Fe_3O_4$ increased from 0.01 to 0.05%. This can be explained by (i) the effect of nano-$Fe_3O_4$ on plant growth promotion via improved soil N-


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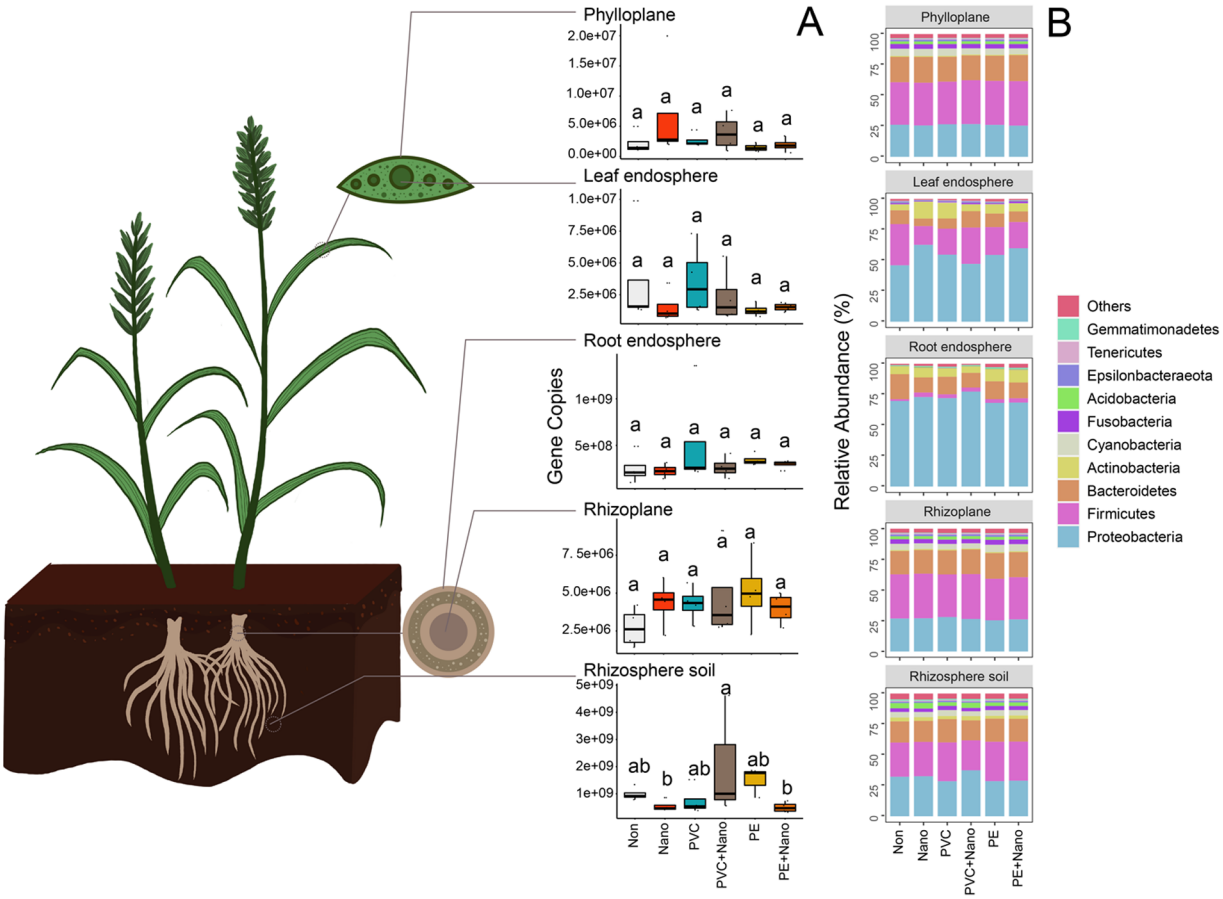


**Figure 3. Effects of the nano-$Fe_3O_4$ and microplastics (polyvinyl chloride (PVC) and polyethylene (PE)) on the ryegrass bacterial gene copy numbers (A) and the relative abundance of endophytic bacterial phyla (B) in the above- and belowground compartments. Relative abundances of uncultured and unclassified/unidentified taxa are grouped as "Others". Values are the mean values ($n$ = 4). Different letters indicate significant differences at $P < 0.05$ based on the least significant difference (LSD) test.**

use efficiency,[32] (ii) the nanozyme-like attribute of nano-$Fe_3O_4$, which reduced plant hydrogen peroxide content under an increased nano-$Fe_3O_4$ dose,[33] and (iii) the uptake and translocation of nano-$Fe_3O_4$ by roots and leaves, which enhanced plant growth without phytotoxic effects.[33] Furthermore, nano-$Fe_3O_4$ enhanced the absorption and transfer of light energy and chlorophyll in the aboveground grass, as indicated by the increases in chlorophyll A (+18%; from 1.47 to 1.73 mg/g), chlorophyll B (+23%; from 0.39 to 0.48 mg/g), and carotenoids (+15%; from 0.34 to 0.39 mg/g), although the increases were not statistically significant (Table 2). Moreover, these physiological indicators reflected the strengthening of plant photosynthesis and the carbon−oxygen cycle, which can be explained by the function of nano-$Fe_3O_4$ in increasing the abundance of plant rhizosphere bacterial taxa associated with growth promotion and carbon cycling.[51] The effects of nano-$Fe_3O_4$ on the rhizosphere soil properties (pH and total C and N contents) of ryegrass were affected by the MP features, which could be associated with their varied effects (due to shape, size, and polymer type) on the soil pH, bulk density, and nutrient retention.[21,52] Similarly, the results showed that the effect of nano-$Fe_3O_4$ on improving the aboveground biomass of ryegrass was also highly associated with the MP type and dose. For example, the treatments receiving high doses (10%) of PVC and nano-$Fe_3O_4$ had a stronger ryegrass growth-promoting effect than those receiving low doses (1%) of PVC and nano-$Fe_3O_4$, and vice versa for the interaction between PE and nano-$Fe_3O_4$, suggesting the diverse effects of MPs (varying based on type and dose) and nano-$Fe_3O_4$ on ryegrass growth.

High doses of PVC can improve the soil's physical structure,[21] which may increase the contact area between Fe particles and the plant rhizosphere by boosting the diffusion of nano-$Fe_3O_4$. The improved soil porosity could further accelerate soil enzyme activities involved in C, N, and P cycling, as shown by similar studies conducted on MP types such as polypropylene[53] and polyethylene.[19] The regulation of soil porosity on enzymes may increase the uptake of nutrients by ryegrass and stimulate grass aboveground biomass and photosynthesis (as shown by increased chlorophyll A and carotenoid contents under high-dose PVC application). Furthermore, the results on plant enzymes also showed that ryegrass leaf and root peroxidase activities were significantly increased (+25%) with the addition of high-dose PVC and nano-$Fe_3O_4$.

**Plant Enzymes.** The enzyme activities varied significantly for nano-$Fe_3O_4$ and MP additions at different concentrations (Figure 2). The maximum increase in root superoxide dismutase (SOD), peroxidase (POD), and malondialdehyde (MDA) activities occurred with the addition of 0.01% nano-$Fe_3O_4$ and 1% PVC, and this was followed by PVC application alone. In the leaves, the SOD, POD, and MDA activities were


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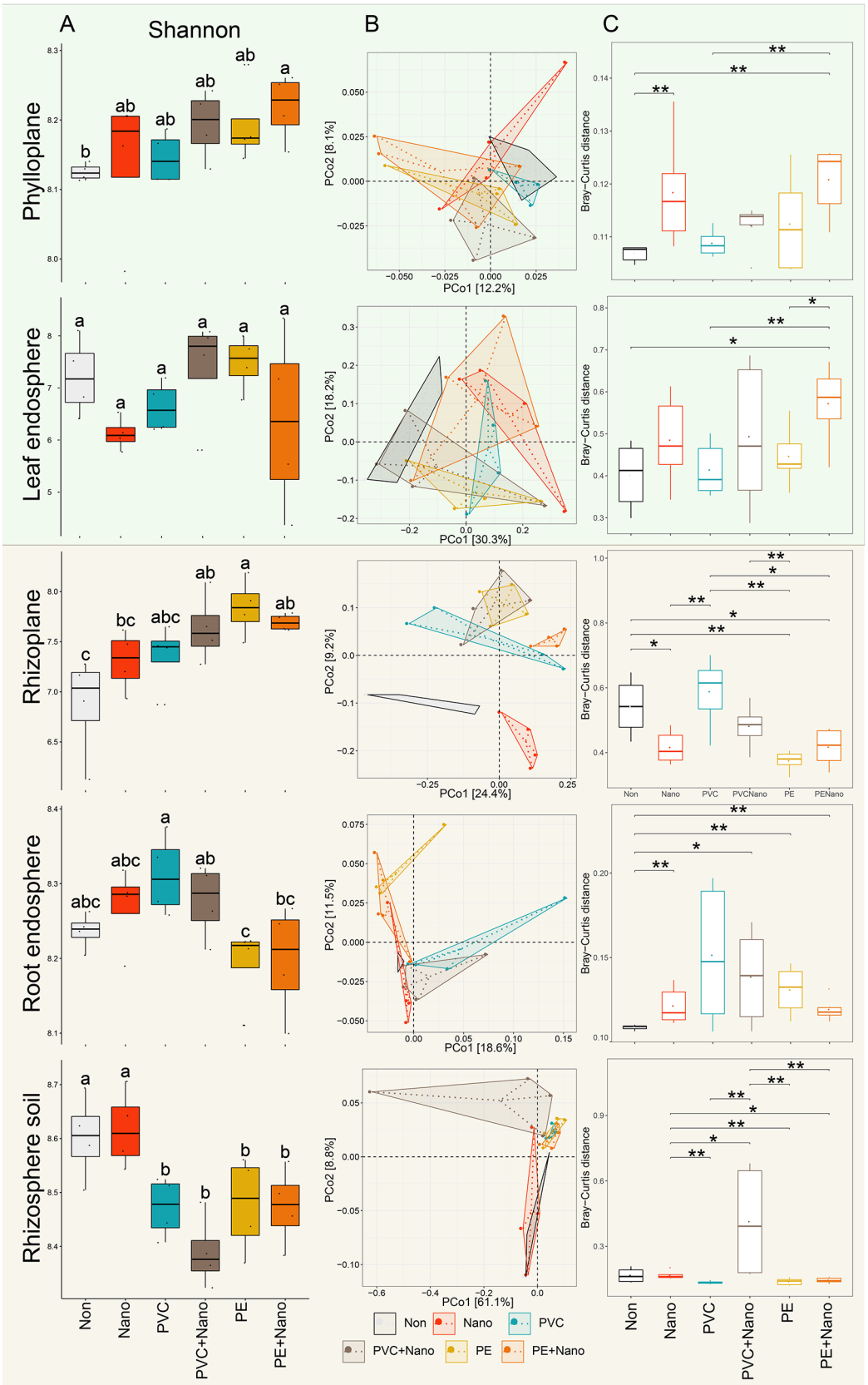


**Figure 4. Effects of the nano-$Fe_3O_4$ and microplastics (polyvinyl chloride (PVC) and polyethylene (PE)) on ryegrass bacterial Shannon diversity (A), community in principal coordinate analysis (PCoA) (B), and community-based Bray−Curtis distance (C). Significant differences between treatments were detected using Tukey's multiple comparison test. *$P < 0.05$, **$P < 0.01$.**



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highest with the addition of 0.01% nano-$Fe_3O_4$ and 1% PVC. Under the single nano-$Fe_3O_4$ treatments, ryegrass leaf and root enzyme activities, including SOD, POD, and MDA activities, increased with the increase in the nano-$Fe_3O_4$ concentration (0.01 vs 0.05%) (Figure 2). At the 1% (w/w) PVC condition, the increase of the nano-$Fe_3O_4$ concentration (from 0.01% to 0.05%) generally reduced the ryegrass leaf and root enzyme activities (Figure 2); however, at the high PVC concentration (10%), the accumulation of nano-$Fe_3O_4$ significantly increased ryegrass POD activity (+164% in leaves, +16.3% in roots) (Figure 2). These results indicated that the positive effect of nano-$Fe_3O_4$ application on ryegrass enzymes was diminished in soils containing 1% PVC. In contrast, in treatments receiving high doses of PVC (10%), the plant oxidative stress reaction (as revealed by POD activity) could be further increased.

In comparison, under the two levels of PE, the effect of nano-$Fe_3O_4$ accumulation increased the ryegrass SOD in both leaves and roots. However, for the other enzymes, the effect of nano-$Fe_3O_4$ was not significant with PE when compared to that with PVC treatments (Figure 2). In plants, both enzymatic and nonenzymatic antioxidants play an important role in neutralizing reactive oxygen species (ROS) to avoid possible oxidative damage.[54] Generally, excessive ROS levels can prompt cell membrane lipid peroxidation, which is indicated by an increased content of MDA, one of the final products of membrane lipid peroxidation. Therefore, MDA is used to indicate the extent of lipids resulting from oxidative stress.[55] In the present study, the accumulation of nano-$Fe_3O_4$ significantly increased plant antioxidant enzymes, which was in line with Cao et al.,[30] who demonstrated that nano-$Fe_3O_4$ activated the antioxidative system in plants. This could be associated with the uptake of NPs within plants because advanced nanotechnologies (i.e., inductively coupled plasma mass spectrometry and X-ray fluorescence imaging) have shown a clear size-dependent transport/uptake of NPs in plants.[56] Overall, the beneficial effects of nano-$Fe_3O_4$ on plants were size- and concentration-dependent; the smallest Fe yielded the highest growth promotion.[30] In the present study, the contents of the main root antioxidant enzymes, SOD and POD, with the addition of 0.05% nano-$Fe_3O_4$ were higher than those under 0.01% nano-$Fe_3O_4$. This suggested that 0.05% nano-$Fe_3O_4$ caused an oxidative stress reaction in the ryegrass roots. Furthermore, the MDA content in ryegrass leaves treated with 0.01% nano + 1% PVC was higher than that in other PVC + nano treatments. It is believed that this result is due to the root system absorbing 0.01% of the NPs and transporting them to the leaves.

**Bacterial Community Composition and Structure.** The bacterial community size (absolute content) and composition across the compartments are given in Figure 3. In various compartments, namely, the leaf endosphere, phylloplane, root endosphere, and rhizoplane, the absolute bacterial abundance was not significantly affected by MP pollution either alone or in the presence of nano-$Fe_3O_4$ (Figure 3A). However, PVC with nano-$Fe_3O_4$ and PE alone increased the absolute bacterial abundance in the rhizosphere. In general, Proteobacteria was dominant in the leaf endosphere (contributing an average of 46% of the total bacterial abundance) and root endosphere (70%) after the addition of MPs either alone or in combination with nano-$Fe_3O_4$ (Figure 3B). However, the abundance of Bacteroidetes (~12 to 20%) and Firmicutes (13 to 33%) increased over 2-fold in the exterior ryegrass areas (the phylloplane and rhizoplane) and the rhizosphere. Moreover, the effects of nano-$Fe_3O_4$ and MP addition (PVC and PE) were substantial at the bacterial family level, as detected using random forest analysis with bootstrapping. The numbers of significantly changed taxa were 9 in the phylloplane, 10 in the rhizoplane, 26 in the leaf endosphere, 33 in the root endosphere, and 238 in the rhizosphere (Figure S1 and Figure 3). The addition of nano-$Fe_3O_4$ significantly ($P < 0.05$) increased the abundances of Nocardioidaceae and Pseudonocardioidaceae in the leaf endosphere, Micavibrionaceae in the root endosphere, Microbacteriaceae and uncultured Planctomycetes in the rhizoplane, and Chthoniobacteriaceae, Acidobacteriaceae subgroup I, and Frankiaceae in rhizosphere soil (Figure S1 and Figure 3).

The Shannon diversity indices showed that the addition of nano-$Fe_3O_4$ alone had the highest bacterial diversity in the rhizosphere soil, followed by the control (no chemical addition) (Figure 4A). However, PVC and PE addition with or without nano-$Fe_3O_4$ increased the bacterial diversity in the rhizosphere to a lesser extent. In the root endosphere, the maximum increase in bacterial diversity occurred after sole PVC or PVC with nano-$Fe_3O_4$ addition.

For the rhizoplane, the bacterial diversity was at its maximum when soils were amended with PE. There were no significant differences in bacterial diversity among PE, PVC with nano-$Fe_3O_4$, and PE with nano-$Fe_3O_4$. However, in the phylloplane, nano-$Fe_3O_4$ addition with PE increased the ryegrass bacterial diversity.

The similarity of the bacterial community composition was analyzed using principal component analysis (PCoA) (Figure 4B). There was a clear separation of community composition between the control (no chemical addition) and other treatments for the rhizosphere, root endosphere, and rhizoplane. The community composition with nano-$Fe_3O_4$ addition formed clusters and was distinctly separated from the clusters of the control, sole MP addition, and the combination of different MPs with nano-$Fe_3O_4$. However, the bacterial community composition was not separated for all the treatments in the leaf endosphere and phylloplane.

Compared to the control, nano-$Fe_3O_4$ significantly ($P < 0.05$) changed the bacterial community composition from belowground (rhizoplane and root endosphere) to the aboveground phylloplane (Figure 4C). Moreover, PVC with nano-$Fe_3O_4$ significantly altered ($P < 0.05$) the bacterial community. In contrast, PE with nano-$Fe_3O_4$ changed the bacterial community composition in the leaf endosphere. When added individually, the three exogenous chemical additives increased the ryegrass endophytic bacterial community size, especially in the rhizoplane. This could be associated with enhanced soil macroporosity, which would facilitate plant root penetration,[21] increasing the colonization and reproduction of rhizoplane bacteria. For the belowground compartment (the rhizoplane and root endosphere), the addition of nano-$Fe_3O_4$ with MPs (PVC and PE) decreased the root bacterial abundance. The results were in line with the single effects of MPs on soil bacterial diversity and richness,[57] implying that the commonly used nanomaterial may not neutralize the negative influence of MPs in soil.[58] However, when moving toward the aboveground, the endophytic bacterial abundance was increased in the phylloplane. Nano-$Fe_3O_4$ mediated the effects of MPs on ryegrass bacterial abundance, and such an effect was highly related to plant compartments. However, the implications of the compartment effect have not yet been elucidated. Under the PVC treatment, nano-$Fe_3O_4$ addition


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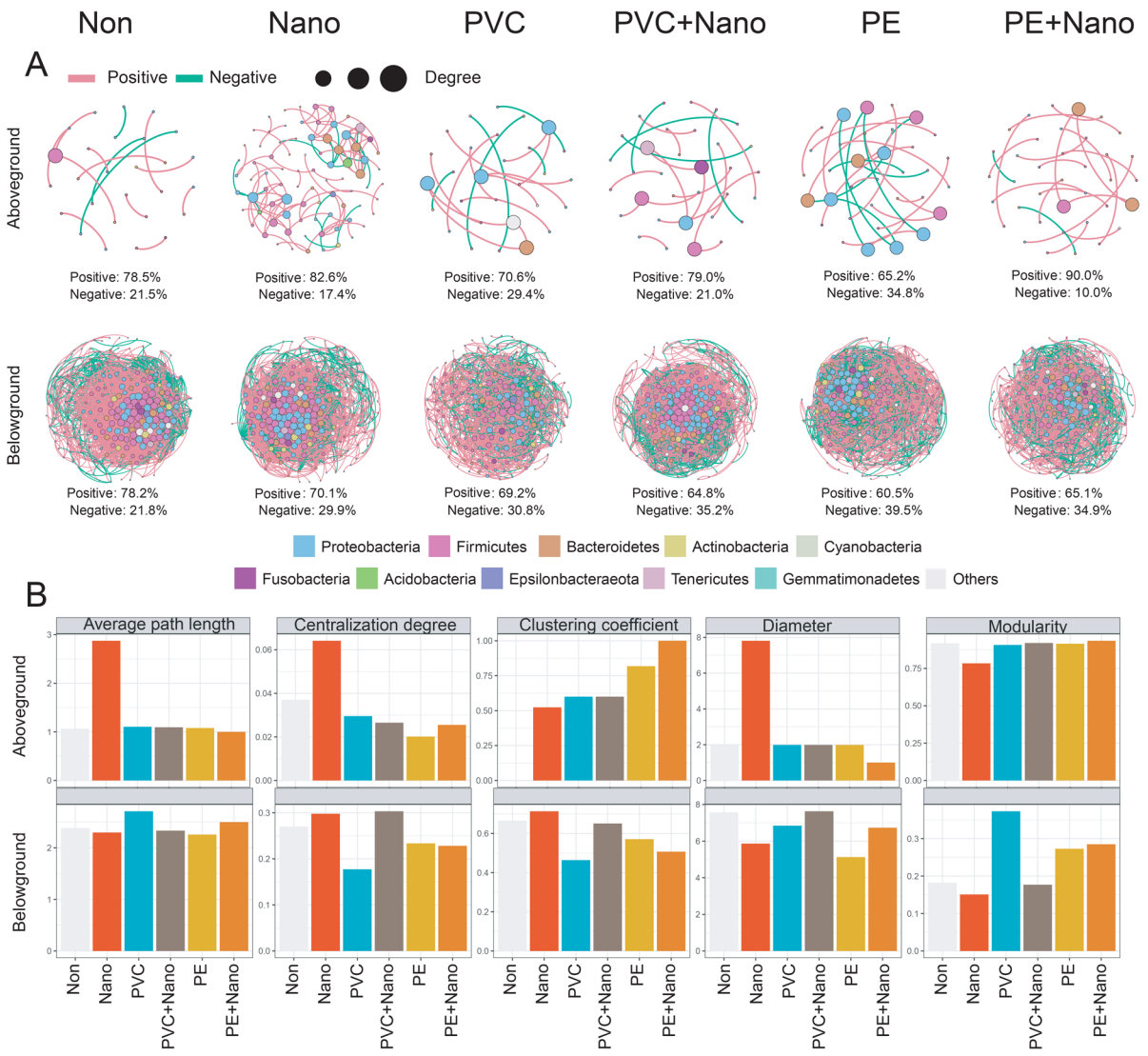


**Figure 5. Effects of nano-$Fe_3O_4$ and microplastics (poly(vinyl chloride) (PVC) and polyethylene (PE)) on the above- and belowground ryegrass bacterial network interactions (A) and topological parameters (B). Nodes represent genera, and the colors of nodes indicate various major phyla. Solid lines represent relationships among bacterial genera, and a module is a cluster of highly interconnected bacterial genera. "Non" represents the control (no chemical addition treatment).**

increased (~10%) the relative abundance of Proteobacteria in ryegrass roots. Many classes of Proteobacteria are eutrophic groups.[59] The abundance of Proteobacteria in the leaf and root endosphere of ryegrass may be closely related to their roles in promoting access to N and P, regulating the immune system, and enhancing plant resistance to pathogenic bacteria.[60,61] There were significant increases of Bacteroidetes (~0.6 times in relative abundance) and Firmicutes (~1.5 times) in the compartments of ryegrass exposed to external environments such as the leaf surface, root surface, and rhizosphere. This selective accumulation of microbes could be related to their functions in environmental stress and disease resistance.[62] Under different chemical treatments, nano-$Fe_3O_4$ addition increased the abundance of Proteobacteria, especially the Nocardioidaceae and Pseudonocardioidaceae in the leaf endosphere of ryegrass compared with the control, which may be linked to the function of Nocardioidaceae in degrading a variety of organic compounds, including aromatic and polyaromatic pollutants and toxic chemicals.[63]

Due to the differences in MP type and concentration, MPs can increase,[64] decrease,[19] or neutrally influence[65] the Shannon diversity of soil microbial communities.

Under the existence of either PVC or PE in ryegrass soils, the addition of nano-$Fe_3O_4$ mediated the ryegrass bacterial microbiome in a different way: the plant bacterial community size and diversity were all enhanced in the phylloplane but weakened in the rhizoplane and rhizosphere soil.

In the ryegrass leaf endosphere, the bacterial diversity was significantly increased with PVC (+19%) and decreased with PE (−7%) under the addition of nano-$Fe_3O_4$. The plant microbiome is mainly derived from soils and is gradually enriched and filtered in various compartment niches.[66] Therefore, the observed effect of nano-$Fe_3O_4$ on the ryegrass root and leaf bacterial community structure can be mainly ascribed to its influence on the rhizosphere soil microbiome. This argument was also supported by the results from the structural equation model (SEM) shown in Figure 6. Moreover, the results demonstrated that the effect of nano-


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$Fe_3O_4$ addition on the ryegrass bacterial community diversity and structure varied under the PVC and PE treatments. This is likely associated with the difference in the polymer backbone structure of the two investigated MPs, as the polymer chemical is an essential factor in modulating the responses of the rhizosphere soil bacterial community functions, such as carbohydrate metabolism, amino acid metabolism, and lipid metabolism.[67] In terms of MP types, this study found that PE-contaminated soils had greater plant rhizosphere bacterial diversity than that of PVC-contaminated soils. This was consistent with the study by Song et al., who compared the effects of different MP types (biodegradable polylactide and PVC) on plant (rice) rhizosphere bacterial communities.[20] In the high-dose MP treatments (10% w/w), there were distinct effects of MP types on the plant rhizosphere bacterial communities (including diversity, community, and biomass),[20] although there were differences in plant species (rice versus grass) and soil type (paddy field versus upland soils). The present work further explored the effect of MPs on plant tissue bacterial community structure and found a significant difference in the rhizoplane. The rhizoplane is a hotspot for interactions between plants and MPs because MPs associate with plant roots through attachment to root cap cells, regardless of the MP size (either nano- or microsized MPs).[68] Moreover, the effects of MPs on the plant microbiome occur not only in plant roots but also in the plant phyllosphere, which may be highly associated with the translocation/uptake of MPs from plant roots to aboveground tissue, where MPs are mostly aggregated on cell walls and in intercellular regions.[69]

**Bacterial Co-occurrence Network.** In terms of competitive/cooperative relationships, the above- and belowground ryegrass bacterial microbiome exhibited co-occurrence patterns, with positive correlations accounting for >60% of potential interactions observed in the co-occurrence networks of all treatments (Figure 5A). In the aboveground parts of the plant, the positive associations in the ryegrass bacterial microbiome were strengthened after the addition of nano-$Fe_3O_4$ with PVC (from 70.6 to 79%) and PE (from 65.2 to 90%). Compared with the control with no chemical addition, other treatments exhibited reduced belowground bacterial positive interactions, as shown by the percentage of positive links among the total interactions (78.2% in the control compared to an average of 65.8% in other treatments). After PE addition, the bacterial association was the least (60.5%); however, combining PE with nano-$Fe_3O_4$ increased bacterial interactions to 65% (Figure 5A).

A set of network topological parameters showed that the ryegrass bacterial network complexity differed above and belowground in response to the addition of MPs and nano-$Fe_3O_4$ (Figure 5B). The addition of nano-$Fe_3O_4$ caused the aboveground bacterial network to become more complex, featuring a higher average path length and a greater degree of centralization between nodes. The mediation of nano-$Fe_3O_4$ on MP was stronger for PVC than for PE. For example, compared to the addition of PVC, the PE and nano-$Fe_3O_4$ treatment increased the aggregate structure of the belowground ryegrass bacterial network, as shown by the clustering coefficient and centralization degree between nodes. The average centralization degrees for the networks in all treatments were distributed according to the power-law distributions, demonstrating a nonrandom co-occurrence pattern. Changes in the ryegrass bacterial network structure could further affect network organizational principles such as modularity. Thus, the nano network had the highest average path length and diameter, but the highest modularity was observed in the PE + nano treatment. Previous studies reported the negative effects of MPs on the soil microbiome, especially for bacterial communities.[57] Under the presence of MPs, $Fe_3O_4$ NPs not only induced variation in bacterial community structure but also had strong effects on co-occurrence networks: the plant rhizosphere bacterial co-occurrence relationships were strengthened by the addition of NPs. The strengthened microbial functionality resilience represents a potential of NPs for mitigating the negative effects of MPs. In the present study, the addition of pure MPs induced negative effects on the plant bacterial microbiome. This was reported to be true even in larger soil microfood networks, including soil bacteria, fungi, protists, and nematode communities.[70] In agreement with Liu et al., in the present work MPs decreased the stability of microbial- and microfood networks,[70] with smaller MPs having stronger negative effects than larger MPs. For ryegrass in this study, a minor dose effect was observed for both the addition of NPs and MPs, which could singly and jointly increase plant growth. However, a previous study detected a strong MP dose effect in maize.[71] The dose effect is most likely related to the plant type and plant traits. For example, the MP dose effect was not obvious for the ryegrass growth, but it was obvious for ryegrass physiological and enzyme activities, which might have profound ecological impacts on plant fitness, resulting in uncertain consequences for ecosystems.[71] In the present study, the addition of pure $Fe_3O_4$ NPs showed a positive dose effect on the plant microbiome through an increase in bacterial keystone taxa and community associations. This could be explained by the following advantages of Fe nanomaterials: (i) Fe NPs have great potential due to their high adsorption capacity and reactivity and (ii) nano-Fe facilitates rhizosphere microbial changes, particularly in terms of the relative abundances of dominant genera.[16]

In the present study, network analysis further confirmed the negative influence of MPs in terms of undermining negative and positive cohesion not only in soil but also in the plant bacterial microbiome, indicating the destabilization of microbial communities.[72] Furthermore, the present results showed that nano-$Fe_3O_4$ addition could mitigate the stress caused by MP accumulation, improving ryegrass aboveground bacterial abundance and creating communities dominated by positive associations. The phenomenon may be linked with the accumulation of the beneficial phyllosphere microbiome, including N-fixing bacteria and pathogen-resistant microbes, in improving plant performance.[73] Exogenous disturbances such as MP pollutants lead to changes in the soil C:N ratio, as indicated by the SEM (Figure 6), which are necessary resource limitations for microbes.

**Combined Effects of Nano-$Fe_3O_4$ and MPs on Ryegrass Microbiome.** The SEM showed that 22% of the ryegrass bacterial microbiome (17% for the rhizosphere soil microbiome) was explained by the selected key edaphic variables (Figure 6). The ryegrass rhizosphere bacterial microbiome was directly controlled by the soil indices, mainly the pH ($R^2$ = 0.61). the C/N ratio ($R^2$ = 0.39), and plant enzymes (SOD, POD, and MDA; $R^2$ = 0.99), while nano-$Fe_3O_4$ and MPs affected the soil microbiome through soil properties. The SEM analyses supported the observation that nano-$Fe_3O_4$ and MPs had direct and positive correlations with the soil pH and the C/N ratio (Table 1 and Figure 6). The


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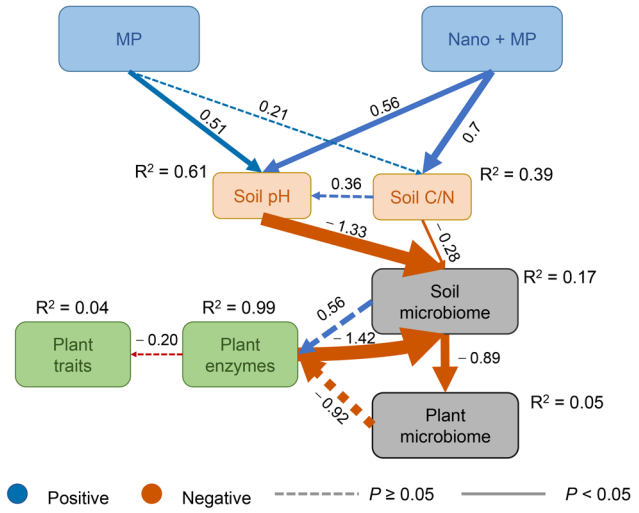


**Figure 6. Structural equation model (SEM) showing the effects of nano-$Fe_3O_4$ and microplastics (MPs) on ryegrass rhizosphere soil properties (pH and C/N ratio), plant traits and enzymes, and plant and soil microbiomes. The numbers adjacent to the dashed (nonsignificant influence; $P > 0.05$) and solid (significant influence; $P < 0.05$) arrows are the standardized path coefficients. Arrow width indicates coefficient strength. Blue and red lines indicate positive and negative coefficients, respectively. $R^2$ indicates the proportion of variance in the ryegrass microbiome explained by the model.**

SEM results further indicated that pH had a significant ($P < 0.05$) and negative ($r = -1.33$) effect on variations in the soil bacterial microbiome, which was in accordance with the findings of previous studies. pH has been confirmed to be a predictor of the soil bacterial community.[74] The change in the plant microbiome was directly dependent on its rhizosphere soil bacterial microbiome ($R^2 = 0.05$), with 89% of the variation explained (Figure 6). Such findings emphasize the critical roles of soil in shaping the plant microbiome. In the controlled pot experiment, it was observed that nano-$Fe_3O_4$ mitigated the effect of MPs on the plant microbiome through the pathway of the rhizosphere soil properties to the plant rhizosphere and phyllosphere microbiome. As an open system, the phyllosphere is subjected to complex environmental perturbations, such as alterations in temperature, water availability, and light availability, which impact the crosstalk between plants and their microbiomes.[75] In the short term (years to decades), the adaptation of plants to environmental change is mainly driven by the plant microbiome.[76] In the present study, it was observed that the change in the plant microbiome was mainly dependent on its rhizosphere microbiome. However, the rhizosphere in natural soil is heterogeneous. The soil heterogeneity in a field scenario might weaken the observed significant trends from the homogenized system. Thus, subsequent long-term field experiments with considerations of single factors (environment or chemical) and their combined influences can be a promising way to capture detailed variations due to environmental and anthropogenic impacts.

The SEM results showed that the iron oxide NPs changed the plant rhizosphere soil properties and increased bacterial abundance. Plants form complex interaction networks with diverse microbiomes. Human-induced variation in soil environments shapes the nearby soil microbiome, and then, through the cross-talk mechanism of plant microbiome functionality (i.e., nutrient signaling and immune signaling), these changes in soil properties can finally affect the phyllosphere microbiome.[75] The connection between the phyllosphere microbiome and plant physiological activity can be explained by (i) alterations in the composition and activities of plant microbiomes that affect host functions[76] and (ii) the functional traits of the phyllosphere microbiome that mediate the hydraulic activation of stomata relevant to the foliar water uptake pathway.[75] The SEM in this work indicates significant microbial connectivity from the rhizosphere to the phylloplane. This association among different plant tissues can be largely attributed to the horizontal transmission of the microbiome, from the soil to the aboveground parts of the plant.[75] In addition to the bottom-up (soil−phyllosphere) effect, the foliar application of iron oxide NPs can also exert top-down effects through activating the antioxidative system, upregulating cytokinin synthesis, and promoting plant nutritional quality.[30] In terms of nanoenabled plant protection, the results showed a positive effect of nano-$Fe_3O_4$ from plant belowground soils. Moreover, Fe-based NP products can be applied by spraying them on the top of the soil as nanocarriers of biopesticides, which can weaken the toxicity of pesticides to soil microbiota.[77] NPs can also be applied as a foliar spray to enhance crop nutritional quality. These findings reflect the potential of NPs as an ecofriendly, high-efficiency, and sustainable plant protection strategy. With the development of advanced technologies, including inductively coupled plasma mass spectrometry, sensing techniques, and X-ray fluorescence imaging, research can deepen the understanding of the effects of NPs on plant−microbiome systems at the nanoscale—the scale of function in biology. It is anticipated that nanotechnologies coupled with metadata analytics could promote needs in plant microbiome research by analyzing microbial interactions at the relevant spatial (plant tissue) and temporal (plant growth) scales. On the other hand, MPs can concentrate in arable soils through plastic film breakage and atmospheric deposition. Under the long-term action of natural factors such as sun rays, rain, wind, and biodegradation, plastic waste would be disintegrated and form smaller MPs.[78] PE and PVC MPs have relatively high specific surface areas and hydrophobicity. The hydrophobic surface of MPs can be magnetized via binding nanoparticles.[79] Thus, in soil aqueous solution, numerous nano-$Fe_3O_4$ could be adhered on the MP surfaces under agricultural irrigation or humid conditions.

The PE and PVC MPs feature high mobility and strong affinity toward nano-$Fe_3O_4$ and other pollutants such as heavy metals and organic pollutants.[37,80] The MP-adsorbed chemicals can be cotransported in soil, which would change the environmental fate and bioavailability of pollutants. Meanwhile, these chemicals can migrate downward in soil alongside MPs. In the process of practical application, pristine nano-$Fe_3O_4$ and MPs get added to soil but they can change dramatically over time, due to surface geochemical processes. The aging process can impact the MP mobility, adsorption capacity, and release of the associated contaminants derived from the MPs.[37,80] In order to uncover the effects of aging on the release behavior of endogenous chemicals, the study on the release of chemicals from MPs and their interactions with nano- $Fe_3O_4$ should be strengthened. Our results showed nano-$Fe_3O_4$ mitigates the negative effects of MPs on the soil−microbe−plant system. By the application of exogenous nano-$Fe_3O_4$, massive MPs and their adhering pollutants can be


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separated and removed by an environmentally friendly magnetic method.[37,81] This is a promising way to mitigate the negative effects of MPs on agroecological systems.

## CONCLUSION

Understanding how MP pollution impairs soil function and plant productivity is a priority topic within the general food security agenda. In this study, the use of nanoiron oxide soil amendments as a mechanism for mitigating plant stress effects arising from PE- and PVC-based MPs was explored in an experimental trial with *Lolium perenne*. The major strength of the present work is the comprehensive consideration of the plant microbiome, which spans the rhizosphere through the phylloplane. The addition of nano-$Fe_3O_4$ and MPs significantly affected the ryegrass rhizosphere environment and its surrounding soil microbiome, and such effects ultimately changed the plant microbiome. This study provides the evidence that nano-$Fe_3O_4$ can mitigate the negative effects of MPs on the ryegrass microbiome by (i) improving soil health (as revealed by the main soil quality indicators of the soil total carbon and C/N ratio (ii) alleviating plant physiological stress and oxidative damage, (iii) promoting the bacterial abundance on leaf and root surfaces, especially taxa (such as Bacteroidetes and Firmicutes) that defend against environmental stresses, and (iv) strengthening the positive associations of the phyllosphere microbiome for potential pathogen resistance. In agricultural soils, MPs have high mobility and a hydrophobic surface with strong affinity for nanoparticles, and they can be magnetized via binding nanoparticles. Since the aging process can impact MP mobility and adsorption capacity, future investigations should focus on the plastic weathering and the interaction between MPs and nanomaterials. In total, the findings deepen the understanding of the impacts of MPs and the potential for nanomaterials to play a protective role and modulate the plant microbiome.

## MATERIALS AND METHODS

**Experimental Soil.** Red soil is one of the most widespread soil types in China, with a total area of 56.9 million hectares. The soil was classified as a typical red sandy with a soil pH of 6.5 ± 0.01, 19.97 ± 0.65 g $kg^{-1}$ total carbon content, 2.45 ± 0.21 g $kg^{-1}$ total N content, and 1.016 g $cm^{-3}$ bulk density. Approximately 200 kg of MP-free homogeneous red soil was used for all of the pot experiments. Details on the soil texture, sampling site location, land-use background, and soil pretreatment can be found in the Supporting Information (SI). The soil pH of the suspension was measured using a pH meter (1:2.5 w/v soil-to-water ratio, Mettler-Toledo, Switzerland). Soil total carbon (TC) and total N (TN) were determined using an elementary analyzer-stable isotope ratio mass spectrometer (EI-IRMS; Elementar Vario PYRO cube and Isoprime100). The quality control (QC) of the soil pH test was achieved by performing replicate analysis of 10 samples in each batch test. The replicate results were set to fall within 0.1 pH unit, and analysis of one internal standard for every batch analysis was carried out. The acquired results were plotted in a quality control chart (QC chart) and monitored for Out of Warning Signals (±2sd). The QC of soil TC and TN data was assured by assessing each sample in 3 replicates to monitor the deviation under 5%. Blank samples (free of carbon and nitrogen), standard reference materials (China national certified reference soil: GSBZ50013-88), and duplicate samples were also applied in each batch of soil samples to control the quality of soil TC and TN.

The iron oxide content ($Fe_2O_3$) of soil samples was determined by converting the total Fe content into $Fe_2O_3$ content with the equation total $Fe_2O_3 \times 0.6993$ = total Fe. Soil total Fe content was determined by the inductively coupled plasma-atomic emission spectrometry (ICP-AES) method after microwave digestion. The quality of total Fe content measured by the ICP-AES method was controlled by (i) testing all the samples 3 times (replicates to make sure the absolute deviation under the tolerance of China national standard (LY/T 1253-1999)), (ii) checking the internal standard (China national certified reference material (CRM): GBW (E) 070045) after every 10 samples (deviation less than 5%), (iii) interelement and background correction check sampling at the beginning, end, and at periodic intervals of every 10 samples throughout the sample run to ensure that deviation would within control limits, and (iv) plotting the results in a QC chart to ensure the linearity.

**Experimental Setup.** The pot experiment was laid out in a randomized block design in a greenhouse under natural light conditions at the KIB using five replications ($n$ = 5). The position of each pot was changed three times during the experiment to homogenize the environmental conditions. In the greenhouse, the air temperature averaged 22.5 °C and the relative humidity averaged 40%. Pots (20 × 15 cm) were filled with a mixture of soil and compost at a ratio of 9:1 (total weight 2 kg) according to Freiberg et al.[82] The compost used was commercial organic fertilizer specifically designed for pot plant growth (Gro-Rich COM, The Richlawn Company Organix Supply, USA). The treatments consisted of three exogenous chemical additives: PVC (Chemi Shanghai Aladdin Biochemical Technology Co. Ltd.; Chemical Abstracts Service number (CAS number 9002-86-2); $(C_2H_3Cl)_n$; 1.38 g/$cm^3$; size ∼100 μm), polyethylene (PE; Shanghai Aladdin Biochemical Technology Co. Ltd.; CAS number 25322-68-3; $HO(CH_2CH_2O)_nH$; 1.27 g/$cm^3$; size ∼75 μm; scanning electron micrographs and Fourier transform infrared (FTIR) spectra of PE and PVC particles are shown in Figure S4), and nano-$Fe_3O_4$ (20 nm in diameter; Shanghai Macklin Biochemical Technology Co. Ltd.; purity 99.5%; detailed dynamic light scattering (DLS) analysis and transmission-electron micrographs (TEM) of nano-$Fe_3O_4$ are shown in Figure S5). In total, 3 levels of nano-Fe concentrations (0%, 0.01%, and 0.05%) × 5 plastic treatments (0%, 1% PVC, 10% PVC, 1% PE, and 10% PE) = 15 treatments were used (Table S2). The 5 high dosing treatments (A, Control; C, 0.05% Nano; G, 10% PVC; I, 10% PVC + 0.05% Nano; M, 10% PE; O, 10% PE + 0.05% Nano) were selected for soil and microbial analyses. The details of MP pretreatment can be found in the SI. Soil OM was measured based on the national standard method (LY/T 1237-1999), whereas the Fe concentrations in both shoots and roots of ryegrass samples were measured using the ICP-AES method. The quality control of plant Fe concentrations was the same as that detecting Fe concentration in soils as described previously.

Perennial ryegrass (*Lolium perenne*) was chosen as a model species on the basis that it is the most widely grown cool-season grass worldwide and has been used widely for pot experiments.[83] The seeds were obtained from the Faculty of Animal Science and Technology, Yunnan Agricultural University. Around 500 ryegrass seeds were surface sterilized with 10% NaClO (5 min) and 75% ethanol (2 min) and then thoroughly rinsed with sterile water. The rinsed seeds were then germinated in sterilized sand trays, and 20 seedlings of similar size were subsequently transplanted into each pot. Deionized water (50 mL) was used to irrigate the pots every week to maintain the 50% water-holding capacity, while pesticides and fungicides were not used during the 90 day experimental period. The Fe concentrations in both shoots and roots in ryegrass samples were measured by using the ICP-AES method.

**Plant Physiological Analysis.** We collected fresh leaf samples and dark-stored them at 18 °C for chlorophyll analysis. A spectrophotometer was used to measure the chlorophyll A, chlorophyll B, and carotenoid concentrations (SPAD-502; Konica Minolta Sensing, Inc., Osaka, Japan). The chlorophyll concentrations were calculated by the following equations and expressed as the amount of chlorophyll $g^{-1}$ dry biomass:[25]

$$\text{chlorophyll A: } 11.93 \times \lambda_{664\text{nm}} - 1.93 \times \lambda_{647\text{nm}}$$

$$\text{chlorophyll B: } 20.36 \times \lambda_{667\text{nm}} - 5.5 \times \lambda_{664\text{nm}}$$


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The maximum quantum efficiency of photosystem II phytochemistry (Fv/Fm) was measured instantly on the adaxial ryegrass surface, according to Sharma et al.[84] Roots were manually collected and washed to remove the adhesion of soil particles. The plant height, root length, and fresh weight were measured, whereas the dry weight was determined by oven drying the samples at 50 °C for 12 h.[25] For plant chlorophyll A, chlorophyll B, carotenoid concentrations, and Fv/Fm, the QC contained the following steps: (i) calibrate spectrophotometers regularly using appropriate standards, (ii) include samples with internal standards and blank samples (solvent only) in each batch of plant samples, and (iii) analyze duplicate plant samples to assess the precision and repeatability. The activities of superoxide dismutase (SOD) and peroxidase (POD) in leaves and roots were determined by following the method of Li et al.[85] For SOD, POD, and MDA activity measurement in leaf and root samples, the QC contained the following steps: (i) test and analyze duplicate plant samples (3 times) to assess the precision and repeatability of the enzyme activity determination, (ii) use certified reference materials with known enzyme activity levels to validate the accuracy, and (iii) create control charts to monitor the performance of the testing method over time.

**Plant Microbiome DNA Extractions.** Destructive samplings were conducted at the plant maturing stage (80 days after sowing). For the rhizosphere, the soil adhering to the root was gently shaken off and collected (10 g) in a sterile tube.[66] The roots and leaves were rinsed three times with sterile water. Root and leaf samples (50 g) were placed into a centrifuge tube (50 mL) containing buffer (1 M Tris-HCl, 0.5 M $Na_2EDTA$, 1.2% CTAB, pH = 8) and then centrifuged (5 min at 4000*g*). The vortexed liquid was filtered (0.22 $\mu$m) to collect microbes from phylloplane and rhizoplane samples. MP Fast DNA spin kit (MP Biomedicals, Solon, OH, USA) was used to collect the microbial cells and extract epiphytic DNA based on the method of Ruiz-Perez et al.[86] Before subsequent endophytes (leaf endosphere and root endosphere) DNA extraction, the disinfection was verified (from the same leaves and roots) by adding the 100 $\mu$L last rinse sterile water to the PDA, LB, and Gao's No. 1 culture media.[66,86] Sterilized leaves and roots were pulverized using a Mixer Mill (MM400, Retsch, Germany), and endophytic DNA was extracted using a Power Soil DNA kit (MoBio, Carlsbad, CA, USA). PowerSoil DNA isolation kit was also used to extract DNA from rhizosphere soils (0.5 g). The DNA quality was checked by agarose gel (2%) electrophoresis. The DNA concentration and purity were measured using a NanoDrop 2000C spectrophotometer (Thermo Scientific, Wilmington, USA.).

**Quantitative PCR and Illumina Amplicon Sequencing.** Quantitative real-time PCR (qPCR) was conducted using primer pairs 338F/806R to quantify gene copy numbers of bacterial communities from five compartment niches of ryegrass. The qPCR reaction mix contained qPCR Supermix (5 $\mu$L), primer (0.5 $\mu$L), template DNA (1 $\mu$L), and $ddH_2O$ (3 $\mu$L). To estimate bacterial gene abundances, a standard curve was generated with a 10-fold serial dilution of a plasmid template in which the target gene amplified from the sample had been ligated to the T-vector (pMD18-T). Fluorescence intensities were detected in an Analytikjena-qTOWER2 instrument (Analytik, Jena, Germany) with the following cycling conditions: 94 °C for 3 min, 39 cycles of 20 s at 94 °C, 63 °C 30 s, and 72 °C 30 s. Each plant and soil DNA (biological replicates) was subjected to three independent qPCR runs (technical replicates), and the final gene copy number (copies/$\mu$L) was calculated from the equation

$$\begin{aligned}\text{gene copy number} = {} & \text{plasmid concentration of standard} \\ & \times 6.02 \times 10^{23} \\ & /(\text{length of target segment} + \text{vector}) \\ & \times 1 \times 10^{9} \times 660\end{aligned}$$

For the microbial DNA, Illumina amplicon sequencing of V3−V4 (hypervariable region of the bacterial 16S rRNA) was performed using the primer pairs 338F/806R.[87] PCR reaction mix contained $ddH_2O$ (10 $\mu$L), primer (10 $\mu$M), High GC Enhancer (10 $\mu$L), dNTP (10 $\mu$L), Q5 High-Fidelity DNA Polymerase (0.2 $\mu$L), and template DNA (60 ng). PCR thermal cycling conditions were as follows: 95 °C for 5 min (initial denaturation), 15 cycles of 60 s at 95 °C, 50 °C 60 s, and 72 °C 60 s, concluded with a final extension for 7 min at 72 °C. Amplicons were purified with VAHTSTM DNA Clean Beads, and DNA concentrations were measured with a Nanodrop 2000 instrument (Thermo Scientific, Wilmington, DE, USA) and quantified by QuantiT dsDNA HS Reagent. Purified amplicons were combined in equimolar concentrations and sequenced (2 × 250 paired ends) on the Illumina PE250 platform. Obtained sequences were demultiplexed on the QIME2 platform and stitched using FLASH2. DADA 2 software was used to denoise and dereplicate reads. The remaining reads were denoted as amplicon sequence variants (ASVs) by searching effective reads against the SILVA-based bacteria reference alignment (version 128). To minimize the impact of read count variation from different samples, the number of sequences for each sample was then normalized, randomly subsampling them to the sample with the minimum read count. All sequence data have been deposited in the Sequence Read Archive under accession number PRJNA916371.

**Statistical Analysis.** Soil and plant traits were presented as the mean standard error. Analysis of variance (one-way ANOVA) with an associated least significant difference (LSD) test at a 5% probability level was used to test the significant difference among treatments for plant physiological and bacterial communities among plant compartments and/or chemical treatments. All of the statistical analyses were performed in the R environment (version 3.60). Alpha-diversity difference of plant bacterial communities was estimated using the Shannon diversity index based on ASVs. Plant bacterial community structure (beta-diversity) was evaluated by pairwise Bray−Curtis distances and visualized using principal coordinate analysis (PCoA) plots. Permutational multivariate analysis of variance (PERMANOVA) was applied to test the significant differences of the ryegrass-associated bacterial community in different compartments using the Adonis function of the R package *vegan* v2.6-1. The random forest analysis by bootstrapping and the nonparametric test was applied to identify the significantly different biomarkers at the family level of different Nano and MP additions. Mean Decrease Gini was selected as the indicator value in the analysis using the "trans_diff class" function of the R package *microeco* v0.90. Co-occurrence network analyses of bacterial taxa from aboveground (leaf endophyte and phylloplane) and belowground (root endosphere, rhizoplane, and rhizosphere soil) were examined separately in the R environment with *igraph*, *psych*, and *microeco* packages,[88] following the protocols described by Barberan et al.[89] To reduce the network's complexity, we only examined strong interactions between different genera with $p < 0.05$ and Spearman's $p > 0.80$, and the $p$ values were adjusted using the Benjamini−Hochberg (FDR) method. All strong correlations identified from a pairwise comparison of genus abundance formed a correlation network in which the node represented bacterial genus taxa, and the edge represented a strong and significant correlation between the nodes. The network was visualized in Gephi (version 0.9.2; https://gephi.org/) with a Fruchterman−Reingold layout algorithm. The network complexity was defined by a series of topological parameters (number of nodes and edges, average path length, network diameter, centralisation degree, average degree, clustering coefficient, and modularity) calculated in R package *igraph*. Structural equation modeling (SEM) analysis was conducted using the *lavaan* R package (version 0.60) to evaluate the direct and indirect effects of MP and nano-$Fe_3O_4$ addition on plant and soil microbiomes. We considered a hypothesized conceptual model (Figure S3) that included all reasonable pathways. The vector "MP" indicated the treatment with PE and PVC addition ("0" for nonaddition and "1" for PE or PVC addition). The vector "MP + nano-$Fe_3O_4$" indicated the treatments with PE/PVC and Nano addition ("0" for nonaddition and "1" for PE + Nano or PVC + nano-$Fe_3O_4$ addition). The vector "Soil microbiome" indicated the Shannon index of the soil bacterial communities from different treatments. All variables besides "MP" and "MP + Nano" were


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standardized by log10 transformation to improve normality in R. A principal component analysis (PCA) was applied to simplify the variables of "Plant microbiome", "Plant enzymes", and "Plant traits" subjected to SEM. Specifically, "Plant microbiome" was represented by the PCA axis (PCA1), explaining 35% of the variation in the Shannon index of rhizoplane, root endosphere, leaf endosphere, and phylloplane bacterial communities. "Plant enzymes" was represented by the PCA1, explaining 37% of the variation in the three leaf and root enzyme activities. "Plant traits" was represented by the PCA1 explaining 49% of the variation in plant height, root length, fresh weight, dry weight, aboveground biomass, chlorophyll A content, chlorophyll B content, chlorophyll, and carotenoid content. Then, we sequentially eliminated nonsignificant pathways unless the pathways were biologically informative.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsnano.3c05809.

Permutational multivariate analysis of variance in soil bacterial communities, overall experimental design, random forest analysis of significant taxonomical changes in plant, conceptual model of structural equation model, background of agricultural soil, microplastic pretreatment and dosing section, and plant physiological analysis (PDF)

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### Notes

The authors declare no competing financial interest.

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