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Effects of three different microplastics on wheat growth and nitrogen utilization

Xiaohua Hao

394782029@qq.com

Xinzhou Normal University

Xingyu Xie

Xinzhou Normal University

Article

Keywords: microplastics (MPs), nitrogen absorption, biomass, wheat, seedlings

Posted Date: November 6th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11329089/v1

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Additional Declarations: No competing interests reported.

Abstract

As a new type of pollutant, microplastics exist widely in agricultural soil and may pose a threat to crop growth and food safety. As an important crop, wheat plays an important role in the development of agricultural economy. The effects of three different types of microplastics, polyethylene (PE), polyvinyl chloride (PVC) and polyethylene terephthalate (PET) on the growth and nitrogen uptake and utilization of wheat were investigated. The results showed that PE treatment significantly increased the total biomass of wheat by 16% compared to the control group on day 30, while PVC and PET treatment also showed a positive effect on day 72, with a total biomass increase of 16% and 12%, respectively. In terms of nitrogen absorption, PET treatment significantly increased the total nitrogen content of wheat leaves and roots, and the 15N utilization rate of wheat leaves increased by 28% on day 58, and the 15N utilization rate of wheat roots increased by 16% on day 72. PE and PVC treatments significantly increased the total nitrogen content of wheat root by 24% and 18%, respectively, on day 72. PE, PVC and PET treated wheat stem total nitrogen content decreased significantly. In addition, the addition of microplastics also had an impact on the abundance of soil nitrogen conversion functional genes, some microplastics treatment promoted the abundance of soil denitrification genes, while the impact on the abundance of ammonia oxidation genes was small. On the 72nd day, PVC treatment increased the soil nirK gene abundance by 240%, and PET treatment increased by 231%. These results indicated that microplastics could promote wheat growth and nitrogen utilization to some extent, and provided scientific basis for understanding the role of microplastics in agro-ecosystem.

Introduction

Microplastics refer to a new type of pollutant less than 5mm, which can enter the farmland soil through a variety of ways, such as the use of domestic sewage containing microplastics for irrigation, the application of compost and sludge containing plastic residues, and the plastic debris remaining in the soil after the decomposition of agricultural film[1]. These microplastics accumulate in the soil, affecting soil structure and function, reduce soil permeability, hinder the development of plant root systems, and even lead to the imbalance of soil microbial communities, thus affecting the normal growth of crops[2]. Food chain transfer microplastics are not only found in the soil, but can also be absorbed by crops, especially those plants with strong water-absorbing properties, such as rice, wheat and vegetables. Microplastics enter the body through the roots of plants and may be transferred to edible parts, which are then transmitted to humans and other animals through the food chain, increasing the risk of consuming microplastics and potentially threatening food safety and human health[3].

The impact of microplastics on crops is manifold. On the one hand, microplastics can promote crop growth.Previous study showed that polystyrene can promote the growth of root biomass of scallion, and PE and PET (average length 5000 μ m, average diameter 8 μm) had no significant effect on the growth of scallion[4]. High-density polyethylene significantly promoted maize root biomass increase, lower than 0.1% and 1% (mg · kg^{-1}) PLA promotes an increase in plant biomass[5]. On the other hand, microplastics

also have an inhibitory effect on plant growth. For example,PET caused the decrease of root fresh weight, PVC caused the decrease of shoot fresh weight, and polypropylene caused the decrease of fresh weight in root and shoot parts, while PET and PVC caused the decrease of root and shoot, respectively[6]. However,the accumulation of PVC severely inhibited the growth and metabolism of rice,10,50 and 100 $mg \cdot L^{-1}$ PE of 13 μm inhibited soybean growth at a high concentration (500 $mg \cdot L^{-1}$) The strongest inhibition on seedlings[7]. Therefore, there is no clear conclusion on the effects of microplastics, particle size and concentration of microplastics on different crops.

Nitrogen is one of the most important nutritional elements for plant growth. Microplastics can affect the growth of crops by affecting the absorption and utilization of nitrogen. Some studies have shown that microplastics can change soil characteristics to increase the water holding capacity and stimulate the activity of enzymes, and then stimulate the absorption of water and nutrients by roots[8]. For example, the results of study are 0.01 to 10 $mg \cdot L^{-1}$ Polymicroplastic styrene nanoplastics of 1 significantly increased nitrogen uptake in wheat. In contrast,adding microplastics to Chinese cabbage would reduce the chlorophyll content in Chinese cabbage leaves, affecting photosynthesis and further affecting nitrogen absorption[9]. The potted corn experiment showed that polyester microplastic fiber inhibited the absorption of nitrogen through the root growth of corn. In recent years, research on terrestrial microplastic pollution has been increasing[10]. However, it is not clear how microplastics affect the nitrogen absorption and growth process, and affect the growth of crops[11].

At present, the effect of microplastics on wheat growth is not clear. Therefore, in this study, three different kinds of microplastics (PE, PVC and PET) were tested on the growth of microplastics and the absorption and utilization of microplastics (root, stem and leaf biomass of wheat seedlings)^{15}N utilization rate, carbon and nitrogen content), the research results can help to provide a deeper understanding of the impact of microplastics on crops.

Materials and Methods

Test design

The test site is located in the greenhouse of Xinzhou Normal University, with average annual temperature of 19℃ and average annual precipitation of 1000 mm. In this experiment, CK treated cultivated soil without microplastics; PE, PVC and PET were evenly mixed with microplastics corresponding to particle size of 250 μm, with a total of 12 basins. Each basin of 8kg of dry weight was used for cultivation, with microplastics added to 3% of the soil dry weight. After the soil and microplastics are fully mixed, they are placed in a stainless steel basin 38 cm long, 28 cm wide and 16 cm high. Two drainage holes with the diameter of 2 cm are opened at the bottom of the stainless steel basin to prevent water accumulation in the basin floor. After full watering, stand for a week for the soil to stabilize, and then sow 6 wheat seeds in each basin. For about 10 d, two wheat seedlings with consistent growth were retained in each pot, and then grown 2d later ^{15}N marker, according to 25$mg \cdot m^{-2}$ ^{15}N addition, for each pot of wheat seedlings

$^{15}NH_4{}^{15}NO_3$ The solution was carried out ^{15}N mark to remove weeds in the basin in real time. Each organ and soil of wheat seedlings were sampled at treatments 30 d, 58 d and 72 d, with three replicates for each treatment.

Sample collection

Plant samples at 30 d, 58 d and 72 d were collected, roots, stems and leaves, washed, finished with oven and dried at 60℃ for the determination of wheat plant sample biomass (roots, stems and leaves) and carbon and nitrogen content. Soil samples were collected together with plant samples, selected within each basin, and drilled using an inner diameter stainless steel soil drill. After soil collection, the impurity was removed by screening, fully mixed and divided into two parts. One part was air-dried to determine the soil physical and chemical properties, and the other was stored at 80℃ for quantitative analysis of functional genes related to soil nitrogen transformation.

Determination and calculation of plant samples

Weigh the root, stem and leaf and calculate the biomass of the whole plant. Organ samples of cucumber were ground with ball mill and the carbon and nitrogen content was determined by elemental analysis-Iope MS (Flash-2000Delta V ADVADTAGE, Germany) ^{15}N abundance.^{15}N utilization was calculated as described in reference[12].

Total$^{15}N = W \times N \times {}^{15}N\% \times 100$

Total ^{15}N represents the total accumulation amount ($mg \cdot m^{-2}$), and W indicates the plant dry biomass ($mg \cdot m^{-2}$), N indicates the total nitrogen content (%).

^{15}N= plants^{15}N accumulation / input fertilizer^{15}N quantity ×100

^{15}N indicates the plants ^{15}N utilization rate (%), and the plants ^{15}N accumulation indicates the in vivo plants ^{15}N total amount, put into the fertilizerThe ^{15}N quantities indicate the imposed ^{15}N fertilizer.

Determination of soil samples

Soil moisture content was determined by aluminum box drying method, pH of soil by potential method (soil:water = 1:2.5); nitrate nitrogen by potassium chloride extraction violet-visible spectrophotometer (UV-Vis spectrophotometer); ammonium nitrogen by potassium chloride, sodium bicarbonate for effective phosphate-hydrochloric acid extraction, molybdenum and antimony resistance[13].

Functional genes associated to soil nitrogen transformation were determined by quantitative fluorescence PCR. That is, the sample DNA was extracted using the Fast DNA™ Spin Kit for Soil (MP

Biomedicals, USA) DNA extraction kit. The extracted DNA was analyzed by 1% (mass concentration) agarose gel electrophoresis for DNA integrity and DNA concentration and purity using NanoDrop™ 2000 (Thermo Scientific, USA) and stored at 20℃ after qualification. Quantitative PCR reactions using SYBR Green as a fluorescent dye (TaKaRa, Japan). The concentrations of the target genes (AOA-amoA, AOB-amoA, nirK, nirS, and nosZ) were subjected to a 10-fold serial gradient dilution to generate a standard curve. The standard curve was established by linking each target gene into the plasmid separately, followed by plasmid DNA extraction, determining the concentration and calculating the copy number, as described in reference[14].

Data analysis and processing

The difference of different treatments for the same sampling time was compared by Duncan, and the figure was plotted with Origin 2021.

Results and analysis

Effect of microplastic addition on wheat growth

As shown in Fig. 1, PE treatment increased 16% ($P < 0.05$) at 30d, with no significant effect on 58 d and 72d. PVC treatment increased 14% ($P < 0.05$) at 30d and 16% ($P < 0.05$) at 72 d, with no significant effect on the 58d. PET treatment increased 16% ($P < 0.05$) at 30d and 12% ($P < 0.05$) at 72 d, with no significant effect on the 58 d. PE, PVC and PET treatments increased wheat leaf biomass at 72 d ($P < 0.05$) by 11%, 16% and 17%, respectively. PE and PET increased by 14% at 30d ($P < 0.05$) and 14%, respectively, and PVC and PET at 70 d (12% at $P < 0.05$) and 10%, respectively. PE treatment increased wheat root biomass at 30d ($P < 0.05$), and PE and PVC treatment at 72d ($P < 0.05$) by 19% and 17%.

Effects of microplastic addition on nitrogen absorption and utilization in wheat

As shown in Fig. 2, PE treatment increased nitrogen uptake in wheat plants by 17% at 30 d ($P < 0.05$). PVC treatment increased nitrogen uptake in wheat plants by 11% at 58 d ($P < 0.05$). PET treatment increased nitrogen uptake at 30 d, 58d, and 72 d ($P < 0.05$) by 27.9%, 18.6%, and 20%, respectively. As shown in Fig. 3, PET treatment at 30 d and 58 d made the wheat leaves ^{15}N utilization increased ($P < 0.05$) by 14.5% and 28%, respectively, and PVC treatment made wheat leaves at 30 d, 58 d and 72 d ^{15}N utilization increased ($P < 0.05$) by 11%, 11% and 10%, respectively. PE treatment at 58 d and 72 d ^{15}N utilization increased ($P < 0.05$) by 18% and 24%, respectively. PVC treatment at 72 d ^{15}N utilization increased ($P < 0.05$) by 18%. PET treatment made wheat roots at the 72 d ^{15}N utilization increased ($P < 0.05$) by 16%.

Effect of microplastic addition on total carbon and nitrogen content, soil physical and chemical content and nitrogen transformation function genes in wheat organs

As shown in Fig. 4, PE, PVC and PET treatments increased the whole carbon content of wheat leaves at 30 d ($P < 0.05$) by 10.2%, 15%, 12.7%,10.2%, 13.5% and 13.5% at 58d, and 10.3%, 12.8%, and 13.6% at 72 d. PE treatment increased the whole carbon content at 30 d, 58 d, and 72 d ($P < 0.05$) by 16.9%, 13.7%, and 20.6%, respectively. PVC Treatment increased the whole carbon content of wheat leaves at 30 d, 58 d and 72 d ($P < 0.05$) by 21.4%, 18.8% and 21.3%, respectively. As shown in Fig. 4,PET treatment increased the total nitrogen content in wheat leaves by 21.6%, 22.3%, and 21% on days 30, 58, and 72, respectively ($P < 0.05$), and increased the total nitrogen content in wheat roots by 27.2%, 41.5%, and 21.4%, respectively ($P < 0.05$). PE treatment increased the total nitrogen content in wheat roots by 32.6% and 21.4% on days 58 and 72, respectively ($P < 0.05$). PVC treatment increased the total nitrogen content in wheat roots by 12.3%, 16.5%, and 14.2% on days 30, 58, and 72, respectively ($P < 0.05$).

PE and PET treatment causes the soil nitrate nitrogen on the 58 d (NO_3^--N) The content increased by 56.9% and 6.9% (Table 1).PE treatment significantly reduced the abundance of *AOA-amoA* functional genes in 30 d soil by 12% and significantly increased the abundance of *AOB-amoA* functional genes by 12.4%. PVC Treatment significantly reduced the abundance of *AOA-amoA* by 21% and 22.6% at 30 d and 58 d, respectively, but significantly increased 72 d soil *AOA-amoA* functional genes by 26% and 27%, respectively, and significantly reduced *AOB-amoA* by 26%. PET treatment significantly reduced the abundance of soil *AOA-amoA* functional genes at the 30 d by 24%. PVC Treatment increased soil *nirK* gene abundance at 72 d ($P < 0.05$ by 240%). PET treatment increased the soil *nirK* gene abundance ($P < 0.05$) at 30 d by 101%, 130% and 231%, respectively. PVC Treatment increased the abundance of soil *nirS* genes at 72 d ($P < 0.05$) by 440.7%. PVC Treatment showed 17% soil *nosZ* gene abundance at 72 d inhibition ($P < 0.05$). PET treatment significantly decreased ($P < 0.05$) soil *nosZ* gene abundance at 30 d and 72 d by 31.2% and 20%, respectively, in No 30 d, significant soil *nosS* gene abundance at 58 d and 72 d were 61.1%, 105% and 93%

Table 1
Three effects of different microplastic additions on soil physical and chemical properties

physicochemical property	duration /d	PE	PVC	PET
pH	30	5.72 ± 0.05	5.62 ± 0.03	5.65 ± 0.03
	58	6.08 ± 0.05	5.99 ± 0.1	6.02 ± 0.05
	72	5.9 ± 0.05	5.89 ± 0.05	5.9 ± 0.05
ω(AP) /mg·kg^{-1}	30	33.29 ± 6.62	24.16 ± 7.03	24.16 ± 3.31
	58	35.32 ± 6.56	26.59 ± 6.39	26.59 ± 6.2
	72	24.58 ± 9.75	24.77 ± 8.65	32.34 ± 1.95
ω(NO^{3-}-N)/mg·kg^{-1}	30	2.9 ± 0.25	4.48 ± 0.83	4.48 ± 0.34
	58	2.13 ± 0.16	1.77 ± 0.12	1.77 ± 0.07
	72	1.62 ± 0.11	1.69 ± 0.12	1.68 ± 0.08
ω(NH^{4+}-N)/mg·kg^{-1}	30	16.11 ± 2.34	14.59 ± 0.44	14.59 ± 0.97
	58	48.14 ± 6.11	32.59 ± 11.78	32.59 ± 4.05
	72	16.91 ± 1.27	13.58 ± 3.4	13.58 ± 0.71

Note: the value is the mean ± standard deviation, n = 4; CK: control group, PE: 3% polyethylene, PVC: 3% PVC, PET: 3% polyethylene diester, pH: acid-base index, AP: effective phosphorus; NO_3^- -N: Soil nitrate nitrogen, and NH_4^+ -N: soil ammonium nitrogen

Conclusion

This study examined the effects of three widely distributed microplastics—polyethylene (PE), polyvinyl chloride (PVC), and polyethylene terephthalate (PET)—on wheat growth, nitrogen uptake and utilization, carbon and nitrogen allocation in plant tissues, soil physicochemical properties, and the abundance of key functional genes involved in soil nitrogen transformation. Samples were collected and analyzed at 30, 58, and 72 days after sowing to capture dynamic changes during wheat seedling development.

Results demonstrated that all three microplastic treatments significantly promoted wheat growth and biomass accumulation. Compared with the control group, PE application significantly increased total wheat biomass by 16% at 30 days. PVC significantly enhanced total biomass by 14% at 30 days and 16% at 72 days, while PET increased total biomass by 16% at 30 days and 12% at 72 days. At the organ level, PE, PVC, and PET significantly elevated leaf biomass at 72 days by 11%, 16%, and 17%, respectively. PE and PVC also markedly improved root biomass at 72 days by 19% and 17%, respectively. These observations confirm that microplastics can effectively stimulate both aboveground and belowground growth in young wheat plants.

Microplastics also significantly enhanced nitrogen absorption and ^{15}N use efficiency in wheat, with clear differences among polymer types. PET exerted the most stable and pronounced promotion, significantly increasing total plant nitrogen uptake by 27.9% at 30 days, 18.6% at 58 days, and 20% at 72 days. PE significantly boosted nitrogen uptake by 17% at 30 days, and PVC increased uptake by 11% at 58 days. In terms of ^{15}N utilization, PET significantly raised leaf ^{15}N use efficiency by 14.5% at 30 days and 28% at 58 days, and root ^{15}N efficiency by 16% at 72 days. PE and PVC increased root ^{15}N utilization by 24% and 18% at 72 days, respectively. Notably, all three microplastics consistently reduced total nitrogen content in wheat stems throughout the experiment, indicating that microplastics redirect nitrogen partitioning within plants, favoring leaves and roots over stems.

Furthermore, microplastics significantly altered carbon and nitrogen accumulation and distribution in wheat tissues. PE, PVC, and PET all increased total carbon content in leaves at 30, 58, and 72 days. PE and PVC also enhanced carbon accumulation in stems and roots. For nitrogen, PET consistently and significantly increased total nitrogen levels in both leaves and roots across all sampling times. PE and PVC significantly increased root total nitrogen by 24% and 18% at 72 days, respectively. These findings indicate that microplastics regulate carbon and nitrogen metabolism in wheat, supporting nutrient retention in photosynthetic and absorptive organs.

Microplastics had minimal effects on soil pH and available phosphorus but significantly influenced soil inorganic nitrogen fractions and nitrogen-cycling functional genes. PE and PET increased soil nitrate-nitrogen (NO_3^--N) by 56.9% and 6.9% at 58 days, respectively. For functional genes, microplastics exerted weak effects on ammonia-oxidizing genes AOA-amoA and AOB-amoA, but strongly promoted denitrification genes. At 72 days, PVC increased nirK abundance by 240% and nirS by 440.7%, while PET increased nirK by 231%. Meanwhile, PVC and PET significantly suppressed nosZ gene abundance. These results suggest that microplastics primarily modulate soil nitrogen cycling by enhancing denitrification rather than nitrification.

Overall, 3% application of PE, PVC, and PET microplastics significantly promotes wheat growth, improves nitrogen uptake and utilization, modifies carbon and nitrogen allocation in plant tissues, and reshapes the abundance of soil nitrogen-transforming functional genes. Among the three polymers, PET displays the most comprehensive and persistent stimulatory effects. This study clarifies short-term impacts of common microplastics on wheat performance and soil nitrogen cycling, providing essential data and a theoretical foundation for assessing ecological risks of microplastic contamination in agricultural soils and understanding nutrient dynamics under microplastic exposure.

Discussion

Effect of microplastic addition on wheat growth

In this study, the three microplastic treatments promoted the growth of wheat (Fig. 1), which is consistent with the addition of microplastic would promote the biomass of potted wheat[15].

Microplastics promote wheat growth because microplastics can change soil properties to some extent and increase the soil microbial activity[16]. Studies have shown that microplastic addition can improve the physical and chemical properties of soil, promote the growth of root and nutrient absorption, so as to promote the growth of crops[17]. For example, the addition of different types of microplastics promoted plant growth overall (fiber treatment increased by 27%, film treatment by 60%, foam treatment by 45%, and debris treatment by 54%)[18]. However, some studies have pointed out that the addition of microplastics with different particle sizes reduced the biomass of cabbage in the first and second quarters, but had no significant impact on the biomass of cabbage in the third quarter[19], and the adaptability of microplastics with different particle sizes and crops to microplastics may be the reason for the inconsistent response of cabbage to microplastics[20].

Microplastic addition partly increased the nitrogen content of wheat plants, and the reason for this result may be that increased wheat chlorophyll promotes photosynthesis and the demand for nitrogen, thus increasing the nitrogen content of wheat plants[21]. Rice hydroponic test results showed that 100 to 500 mg L^{-1} The addition of tire grinding particles increased the chlorophyll content of rice, thus promoting the photosynthesis and plant growth of rice. However, some studies have also shown that the size and concentration of microplastic ethylene-vinyl acetate copolymer, linear low density polyethylene and polymethyl methacrylate particles have no effect on the chlorophyll content of soybean leaves, High concentration (200 mg L^{-1}) polystyrene microspheres decreased chlorophyll b content, and different types and concentrations of microplastics will affect plant photosynthesis[22]. Therefore, the type, concentration and soil type of microplastics will have an uncertain impact on crop growth, and the relationship between microplastics and crop growth still needs to be further studied.

Effect of microplastic addition on nitrogen absorption and utilization in wheat

Nitrogen is an essential nutrient element for plant growth[23]. In this study, the addition of three microplastics promoted the uptake and utilization of nitrogen in wheat to some extent (Fig. 2 and Fig. 3).The addition of microplastics can promote the absorption of nutrients in wheat, which is consistent with the results of this study [40]. Related studies show that microplastic particles in soil can change the properties of soil to promote nitrogen uptake by crops[24]. At the same time, microplastics can stimulate the activity of enzymes in the soil, activate the soil organic nitrogen reservoir, and promote the accumulation of soluble nitrogen in the soil[25]. For example, microplastics can promote the growth of plant roots and the activity of rhizosphere microorganisms, thus facilitating the absorption of nutrient elements. Similarly,study indicated that nitrogen cycling in sediments is affected by different microplastics, NH_4^+ concentrincreased with PVC treatment time, while NO_3^- or NO_2^- gradually decreases, thereby promoting plants to NH_4^+ uptake and utilization by plants, while polyurethane foam and PLA are the opposite to PVC treatment[26].

Microplastics can also affect the expression of soil functional genes and nitrogen cycle process by affecting soil microbial communities and enzyme activities, and then affecting nitrogen uptake by plants[27]. In this study, microplastic addition had inconsistent effects on soil nitrogen conversion functional genes, and microplastic addition could promote the abundance of soil denitrification conversion functional genes, but the addition of 0.05% microplastic in red soil inhibited the abundance of soil denitrification conversion functional genes.The study explored different 0.5% and 5% ($mg \cdot kg^{-1}$) PLA microplastics can promote *AOB-amoA* abundance overall. However, it has also been shown that low-density polyethylene (2%) can occasionally inhibit the abundance of the ammonia-oxidized archaea *AOA-amoA* gene, and subsequently inhibit the nitrification rate .

The PVC observation experiment in water sediment found that the abundance of denitrification genes is reduced and the denitrification rate is lower[28]. Therefore, the influence of microplastics on nitrogen cycle and soil nitrogen conversion function genes are influenced by many factors, such as soil type, microplastic concentration and addition amount. How microplastics affect the soil nitrogen cycle process and then affect the nitrogen absorption by plants is not clear in this study, and more studies need to demonstrate it.

Declarations

Funding :

No funding was received for this manuscript

Author Contribution

Hao Xiaohua wrote the main manuscript text and Xie Xingyu prepared all figures . All authors reviewed the manuscript

Acknowledgement

I do not have anyone to acknowledge

Data Availability

All data during this study are included in this manuscript.

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Figures

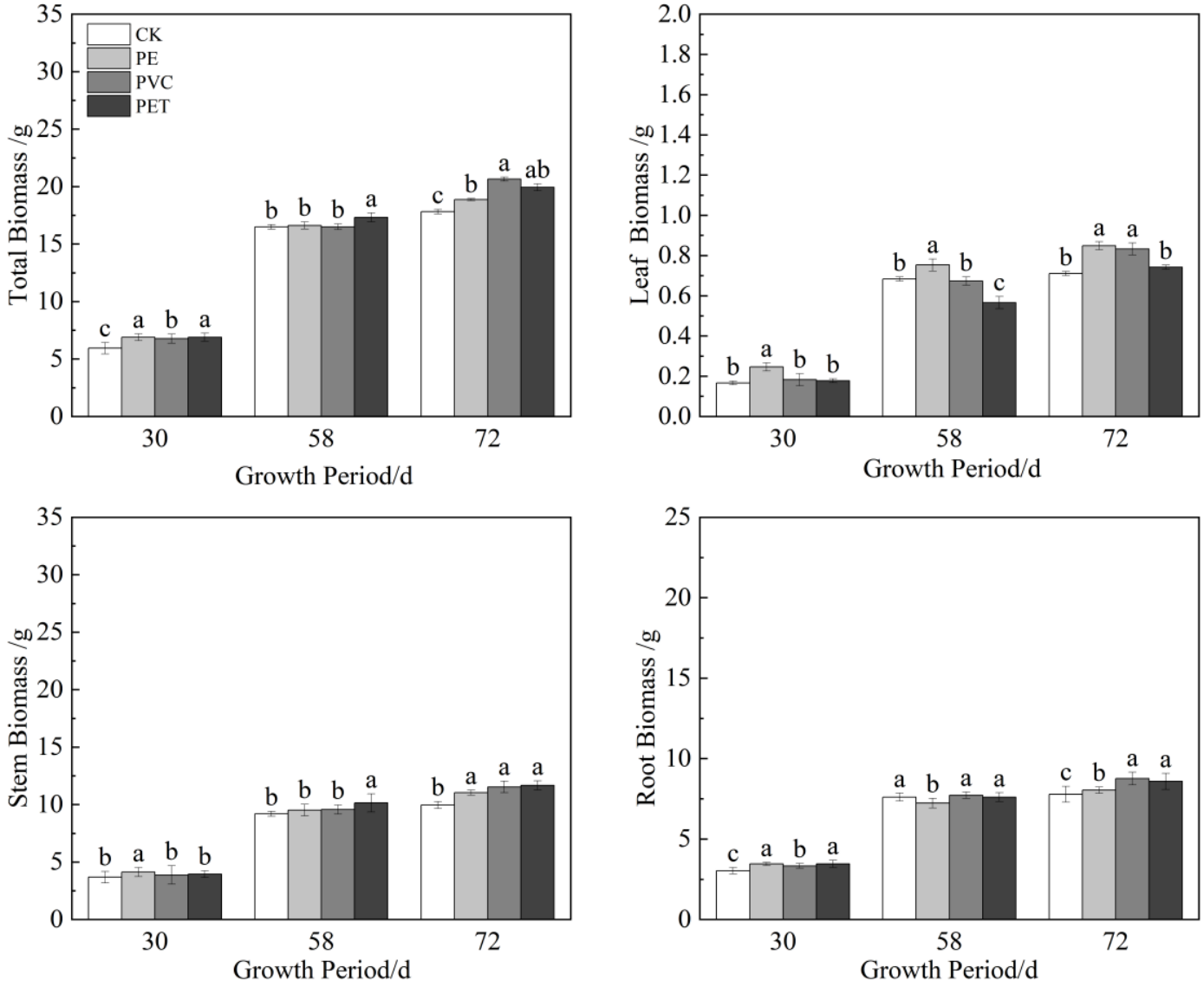


Figure 1

Effect of microplastic treatment on the total wheat biomass and the biomass of each organ

CK: Control group; PE: 3% polyethylene, PVC: 3% polyvinyl chloride, PET: 3% polyethylene terephthalate;

Different lowercase letters indicate a significant difference between the treatments (P <0.05);

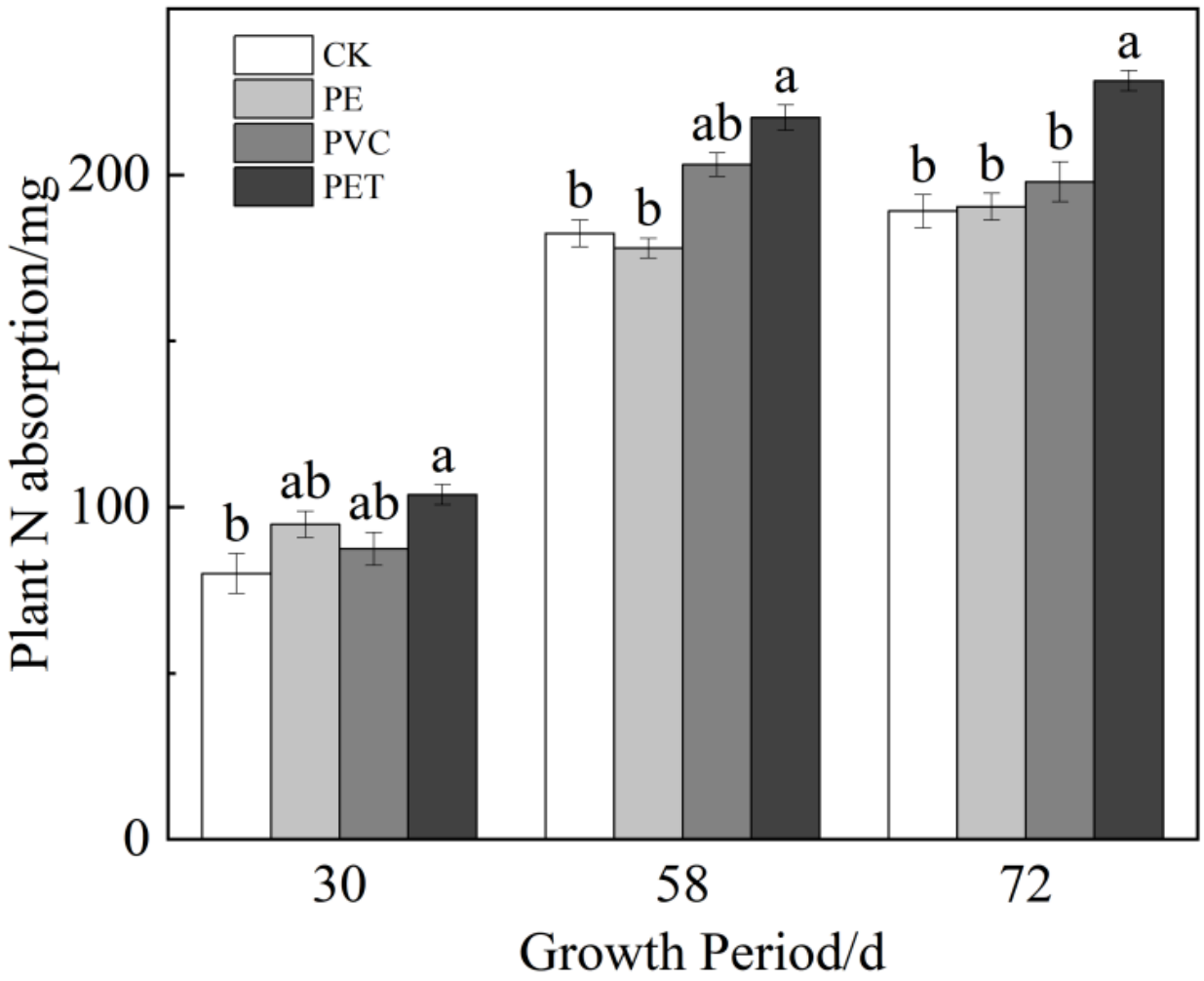


Figure 2

Effects of microplastic treatment on nitrogen uptake in wheat plants

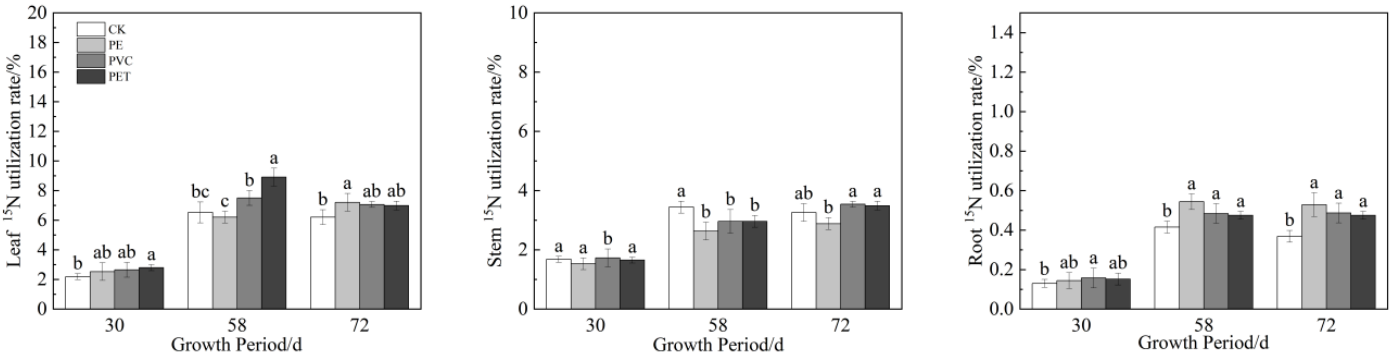


Figure 3

Microplastic treatment for wheat the effect of the ^{15}N utilization rate

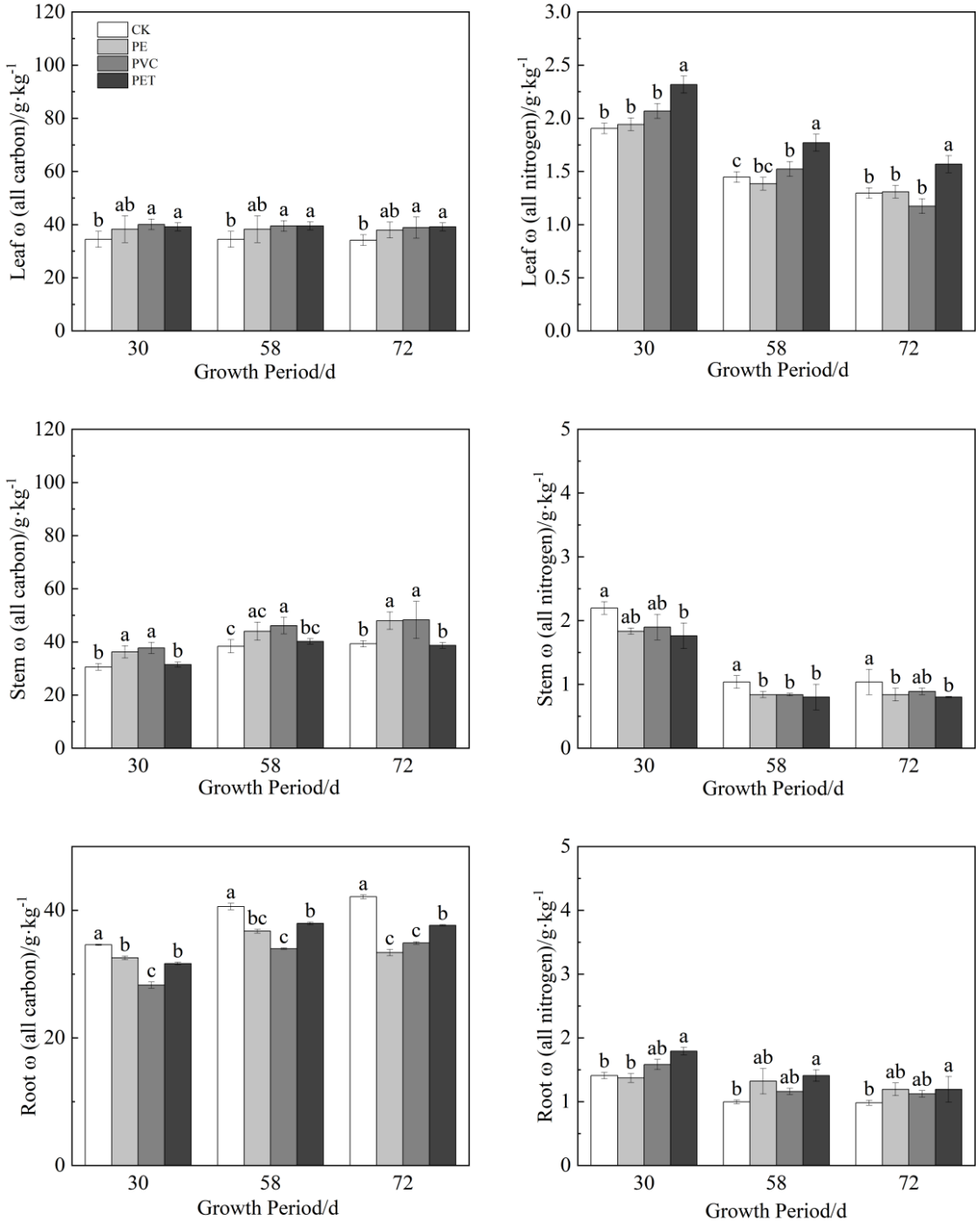


Figure 4

Effect of microplastics on carbon and nitrogen content in all wheat organs

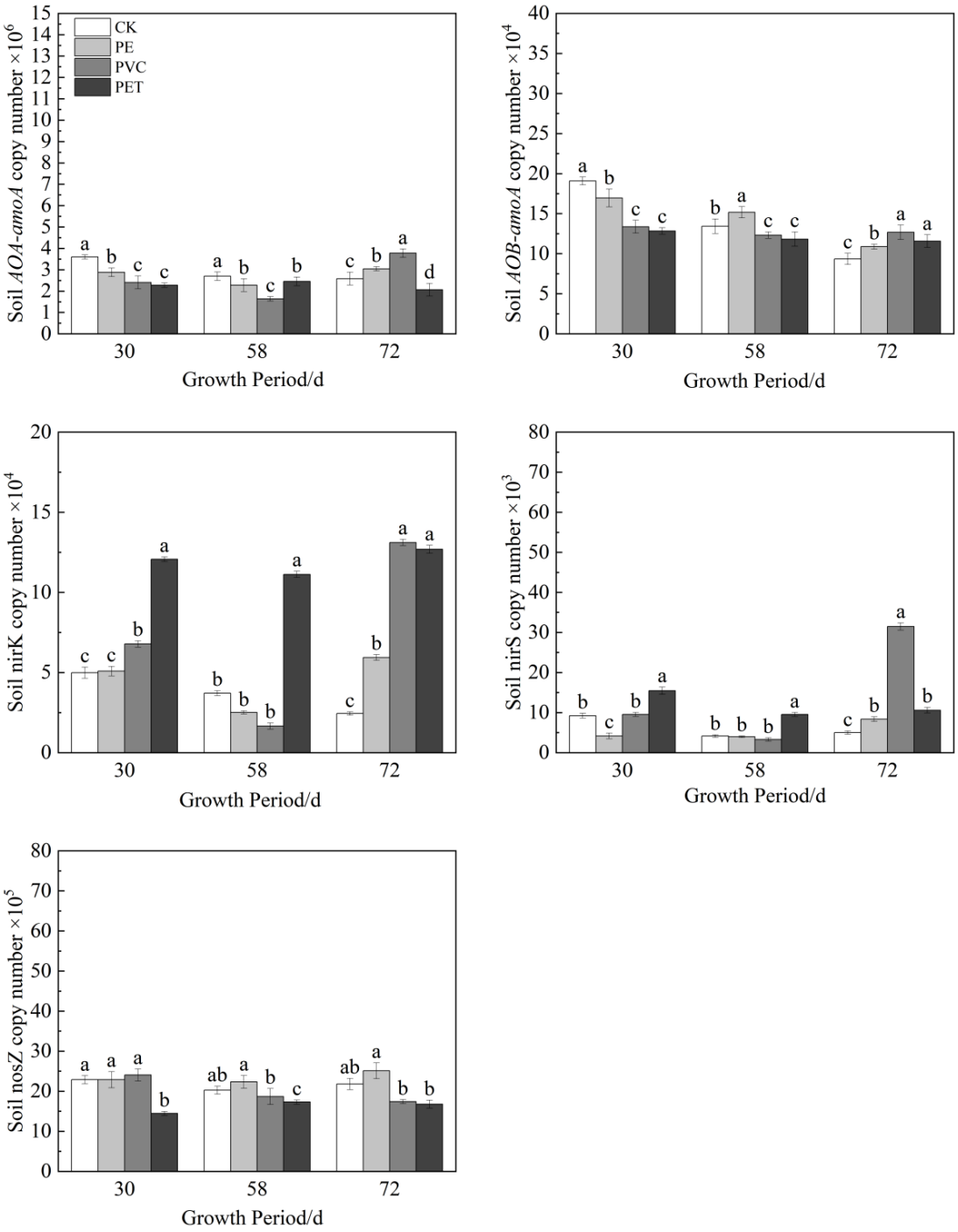


Figure 5

Effect of microplastic treatment on the abundance of functional genes for soil nitrogen transformation