Supplementary Materials

Materials and methods

Measurement of potential nitrification rate

The PNR_T was determined through the shaken-slurry method (Yao et al., 2011). Initially, 10 g of fresh soil was placed in a 250 mL triangular flask, and 100 mL of culture solution was added to each flask. The culture solution was a mixture containing 0.015 mol/L KH_2PO_4, 0.035 mol/L K_2HPO_4, and 0.0375 mol/L $(NH_4)_2SO_4$, and these reagents were purchased from Chongqing Chuandong Chemical, China. The bottles were sealed with plastic film with small air-permeable holes and placed in a shaker for incubation at 25℃ and 180 r/min for 24 h in the dark. During the incubation period, 10 mL soil suspension was aspirated at 2, 6, 12 and 24 h, and the NH_4^+-N content of the filtrate were determined after centrifugation and filtration. Taking the incubation time as the horizontal coordinate and the NH_4^+-N content in the extract as the vertical coordinate, soil PNR_T is the slope, which is the rate of depletion of NH_4^+-N content per unit of time. Soil PNR_T was calculated as shown in Eq. (S1). The same method was applied to determine PNR_{AOA}, with the difference that an additional 1.0 g/L ampicillin needed to be added to the medium to inhibit the activity of AOB (Zheng et al., 2014). The calculation is shown in Eq. (S2). The difference between the PNR_T and the PNR_{AOA} is the PNR_{AOB}, and the calculation is shown in Eq. (S3).

$$PNR_T = R_1 \cdot \frac{0.1+V}{m} \quad \text{(S1)}$$

$$PNR_{AOA} = R_2 \cdot \frac{0.1+V}{m} \quad \text{(S2)}$$

$$PNR_{AOB} = PNR_T - PNR_{AOA} \quad \text{(S3)}$$

where PNR_T is the total potential nitrification rate (mg N/(kg·h)), PNR_{AOA} is the potential nitrification rate of AOA (mg N/(kg·h)), and PNR_{AOB} is the potential nitrification rate of AOB (mg N/(kg·h)). R_1 is the rate of depletion of NH_4^+-N content (mg N/(L·h)), and R_2 is the rate of depletion of NH_4^+-N content after the addition of ampicillin (mg N/(L·h)). 0.1 is the volume of buffer (L), V is the volume of water in soil sample (L), and m is the dry weight of soil (kg).

Measurement of potential denitrification rate

The suspension culture method was used to determine soil PDR (Zhang et al., 2022). Initially, 10 g of fresh soil was placed in 250 mL triangular flask, and 100 mL of culture solution was added to each flask. The culture solution was a mixture containing 0.2 mol/L KH_2PO_4, 0.2 mol/L K_2HPO_4, and 0.03 mol/L KNO_3 (Chongqing Huihuang, China), 0.02 mol/L glucose (Chongqing Chuandong Chemical, China). The bottles were pre-filled with N_2 to ensure the anaerobic environment. Seal with a rubber stopper and place in an incubator at 20℃ for 72 h in the dark with oscillation. The supernatant was taken at 24-h intervals from 0 h and the NO_3^--N content were determined. Taking the incubation time as the horizontal coordinate and the NO_3^--N content in the extract as the vertical coordinate, soil PDR is the slope, which is the rate of depletion of NO_3^--N content per unit of time. Soil PDR was calculated as shown in Eq. (S4).

$$\mathrm{PDR} = \mathrm{R_3} \cdot \frac{0.1+\mathrm{V}}{\mathrm{m}} \qquad \text{(S4)}$$

where PDR is the potential denitrification rate (mg N/(kg·d)), R_3 is the rate of depletion of NO_3^--N content (mg N/(L·d)), 0.1 is the volume of buffer (L), V is the volume of water in soil sample (L), and m is the dry weight of soil (kg).

Measurement of phosphatase activity

Soil phosphatase activity (PAA) was determined by the colorimetric method of sodium benzene phosphate (Hou et al., 2023). 2–5 g of soil sample was weighed into a 200 mL triangular vial and 2.5 mL of toluene was added. After shaking gently for 15 min, 20 mL of 0.5% sodium benzoate phosphate (98%, Shanghai Tengzhun, China) was added, shaken carefully and put into a constant temperature incubator for 24 h at 37℃. Then 100 mL of 0.3% $Al_2(SO_4)_3$ solution (99%, Shanghai Macklin, China) was added to the culture solution and filtered. Aspirate 3 mL of the filtrate in a 50 mL volumetric flask and determine the phenol (PhOH) content at 660 nm by spectrophotometry (TU-1901, Beijing Puxi General Instrument, China). PAA is expressed as milligrams of phenol produced from the consumption of sodium benzene phosphate per unit of dry soil for 24 h of enzymatic reaction (mg PhOH/(g·d)). Soil PAA was calculated as shown in Eq. (S5). A substrate-free control should be made for each sample, replacing the substrate with an equal volume of pure water, and other operations are the same as in the sample experiments, so that any influence of the original ammonia in soil samples on the experimental results can be excluded. A soilless control was set up and other operations were the same as in the sample experiments to test the purity of the reagents and the decomposition of the substrate itself.

$$\mathrm{PAA} = \frac{(\mathrm{A_1}-\mathrm{A_2}-\mathrm{A_3})}{m} \qquad \text{(S5)}$$

Where PAA is the phosphatase activity (mg PhOH/(kg·d)), A_1 is the amount of PhOH produced in samples of the experimental groups (mg), A_2 is the amount of PhOH produced by the soil-free control (mg), A_3 is the amount of PhOH produced by the substrate-free control (mg), and m is the dry weight of soil (kg).

Table

Table S1 Soil physicochemical characteristics and activities at 0 d.

Indicator	Concentration
pH	6.86
OM	45.81 mg/g
WSA	19.30%
NH_4^+-N	37.61 mg/kg
NO_3^--N	6.03 mg/kg
PNR_T	0.05 mg N/(kg·h)
PNR_{AOA}	0.04 mg N/(kg·h)
PNR_{AOB}	0.02 mg N/(kg·h)
PDR	2.79 mg N/(kg·d)
Olsen-P	71.17 mg/kg
PAA	7.66 mg PhOH/(g·d)

Figure

Fig. S1 Scanning electron microscope (SEM) of polystyrene microplastics.

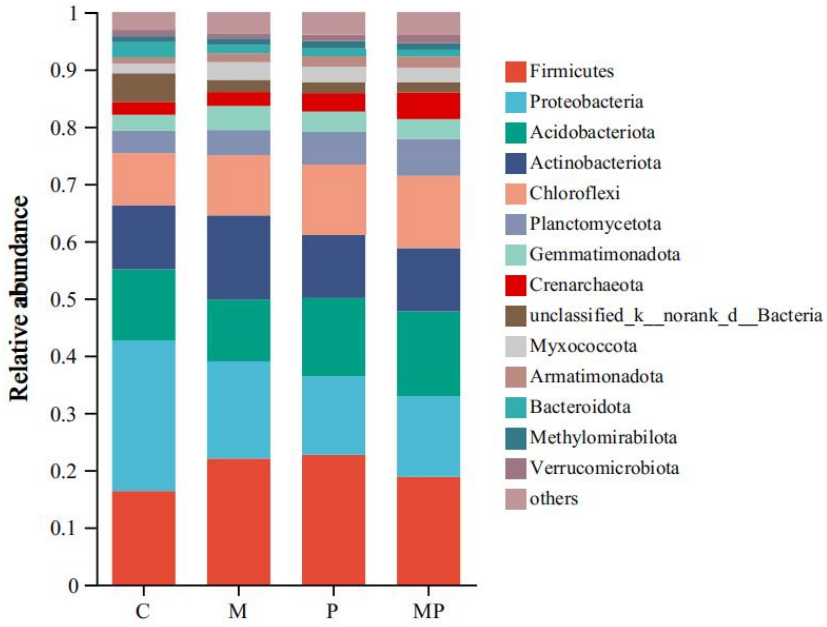

Fig. S2 Relative abundance of microbial community at the phylum level.

References of Supplementary Materials

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