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Effect of Polyvinyl Chloride Microplastics on Bacterial Community and Nutrient Status in Two Agricultural Soils

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Abstract

Knowledge of the influence of microplastics on soil microbiome and nutrients is important for understanding the ecological consequences of microplastic pollution in terrestrial ecosystems. In this study, we investigated whether polyvinyl chloride (PVC) microplastic pollution at environmentally relevant concentrations would affect soil bacterial community and available nitrogen/phosphorus content. The results showed that although PVC microplastics at 0.1% and 1% levels did not have a significant effect on overall bacterial community diversity and composition in soil over the course of 35 days, a number of bacterial genera were significantly reduced or enriched by the presence of microplastics. Potentially due to their effect on certain functional groups, microplastics caused a significant change in soil available P content. It is noteworthy that, depending on soil type, pollution level and plasticizer presence, contrasting effects of microplastics may be observed. Further research is definitely warranted to gain a clearer picture of the threats posed by microplastic pollution in soil environments.

Keywords Microplastic · Soil · Bacterial community · Available phosphorus · Plasticizer

Microplastic pollution and related ecological risks have recently become a global concern. Microplastics are generally defined as plastic particles with a size of < 5 mm (Cole et al. 2011). As high amounts of plastic wastes are released into the environment each year, microplastics are now widely detected in various environmental matrices, such as the open ocean, inland freshwaters, aquatic animals, soil and sludge (Cole et al. 2011; Corradini et al. 2019). Studying microplastics in soil is of great importance, since soils are important sink for microplastics and they provide a wide range of ecosystem services that are essential for life (Barrios 2007; Rochman 2018). Generally, microplastic concentrations ranging from 0.54 to 55.50 mg/kg have been reported in farmland or natural reserve soils (Scheurer and Bigalke 2018; Zhang et al. 2018a), while in heavily impacted industrial soils it could reach a high level of 6.7% (Fuller and Gautam 2016). Considering that soil pollution is likely to continue or become more severe in the future, it is imperative to investigate how microplastics would influence soil biota and the biogeochemical processes occurring in it.

Available data indicate that the presence of microplastics may affect the composition and metabolic activity of soil microorganisms. For instance, Fei et al. (2020) found that addition of 1%–5% polyethylene (PE) or 5% polyvinyl chloride (PVC) microplastics induced a decline in the richness and diversity of soil bacterial community and significantly affected the abundance of several bacterial groups such as the family *Burkholderiaceae*. Ren et al. (2020) reported that 5% PE microplastics reduced N_2O emission and showed selective effects on soil microbes in fertilized soil. Mechanisms underpinning the changes observed is not clear yet, but could be the results of direct and indirect impacts on soil microbes. Direct impacts are adaptation of microorganisms to the presence of microplastics as a physical vector and/or chemical contaminant, while indirect effects are changes in soil structure (such as soil texture, bulk density and water stable aggregates) that could ultimately lead to modified microbial communities (de Souza Machado et al. 2018).

The above studies added valuable insights into the impacts of microplastics on soil ecosystems. However, usually high levels of microplastics were used in these studies

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and few examined how the threats associated with microplastics differed in different types of soil. Moreover, in many cases microplastics are a mixture of polymers and chemical additives such as plasticizers, which are used to improve the flexibility and durability of plastic products (Zhang et al. 2018b). Plasticizers are generally not chemically bound to the polymers and thus can be slowly released into the surrounding environment (Paluselli et al. 2019). Previous research has shown that the presence of plastic additives may increase the toxicity of microplastics to lugworms or play an important role in determining the impacts on sludge anaerobic digestion (Browne et al. 2013; Wei et al. 2019). Therefore, it is also necessary to compare the impacts of plasticized and unplasticized microplastics on soil microbiota.

In this study, PVC microplastics were selected, as they are commonly detected in soil environments (Ding et al. 2020), they may contain significant amount of plasticizers (Paluselli et al. 2019) and their ecological risks have not been fully understood. Acidic and neutral soils were used to represent arable soils commonly found in China. PVC microplastics were added to soil at the doses of 0.1% and 1% by weight to simulate in-situ farmland soil contamination conditions. The main aims of this study were: (1) to assess the effects of PVC microplastics on soil bacterial community and available nutrient content, and (2) to explore how soil type, microplastic concentration and plasticizer presence would influence the interaction of microplastics with soil microbial communities.

Materials and Methods

Soils used in this study were collected from agricultural fields. The acidic soil was obtained from Yingtan, Jiangxi Province while the neutral soil was from Suzhou, Jiangsu Province. They were classified as red and paddy soils, respectively, according to Chinese soil classification. Soils were air-dried and sieved through a 2 mm mesh before use. The basic characteristics of soils are shown in Table 1.

PVC films with or without plasticizer di (2-ethylhexyl) phthalate (DEHP) were prepared according to the method of Takehisa et al. (2005). DEHP was chosen as the target plasticizer as it is widely used in flexible PVC products (such as agricultural films and table cloths). Briefly, PVC pellets (Aladdin Biochemical Technology Co. Ltd, Shanghai, China) and DEHP dissolved in solvent tetrahydrofuran were transferred into glass petri dishes with a pipette. After solvent evaporation, PVC films were formed. Then, PVC films were rinsed with distilled water and gently air dried. PVC microplastics were obtained by shredding PVC films into small pieces using a grinder under liquid nitrogen conditions. Microplastics with the size of < 0.9 mm were used in this experiment. Determination of DEHP content in PVC microplastics was carried out by acetone:hexane extraction and a gas chromatography–mass spectrometry analysis procedure as described by Zhu et al. (2018a). Plasticizer in PVC products generally ranges from 0% to 50% (0–500 mg g^{-1}) (Cao 2010). In this study, the initial DEHP concentration in PVC film/microplastics was set to be 10 mg g^{-1} (determined concentration 9.6 ± 0.5 mg g^{-1}) to mimic the situation that microplastics found in the environment are very likely to contain less additives than unaged plastics.

Soil microcosms were prepared in glass beakers, each containing 50 g of soil (dry weight) with or without PVC microplastics. For each soil type, five treatments were included: unamended soil (control), soil amended with 0.1% unplasticized or DEHP plasticized PVC microplastics (PVC0.1 and PVC0.1_D), and soil amended with 1% unplasticized or plasticized microplastics (PVC1 and PVC1_D). All treatments were prepared in triplicate and incubated in the dark at 25.0 ± 1.0 ℃ in a temperature-controlled chamber. Soil moisture was maintained at 70% of maximum water holding capacity during the incubation. After 35 days, samples were taken for soil nutrient content and bacterial community analyses.

Available nitrogen was extracted from 5 g of fresh soil samples with 20 mL 2 M KCl solution at 200 rpm for 1 h. NH_4^+–N and NO_3^-–N in the extracts were analyzed colorimetrically using the indophenol blue method (Mulvaney 1996). Soil available phosphorus was determined by NH_4F–HCl (acidic soil) or $NaHCO_3$ (neutral soil) extraction followed by the molybdenum blue colorimetric method according to Chinese Agricultural Standard NY/T 1121.7-2014.

Total genomic DNA was extracted from 0.5 g frozen soil using FastDNA Spin Kit for Soil (MP Biomedicals, USA). The V4–V5 regions of bacterial 16S rRNA gene was amplified using the primer set of 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′). The polymerase chain reaction protocol used was as follows: 95 °C for 3 min, followed by 28 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s and a final extension at

Table 1 Basic physical and chemical properties of the two soils used

Soil type	pH	Organic matter (g kg^{-1})	Total nitrogen (g kg^{-1})	Total phosphorus (g kg^{-1})	Soil texture (%)		
					Sand	Silt	Clay
Red soil	4.9	10.1	0.7	0.6	19.7	41.7	38.6
Paddy soil	7.4	22.6	1.3	0.7	9.1	69.2	21.8

72 °C for 10 min. Purified amplicons were sequenced on an Illumina MiSeq platform (Majorbio Bio-pharm Technology Co., Ltd, Shanghai, China). Raw sequence data were deposited into the NCBI Sequence Read Archive database (accession number: SRP260786).

Sequence analysis was performed using Quantitative Insights Into Microbial Ecology toolkit (QIIME version 1.9.1). Clean reads were clustered into operational taxonomic units (OTUs) at identity threshold of 97% using USEARCH version 7.0, and taxonomic analysis was carried out based on the Silva release 132 database. The abundance of bacterial taxon was presented as the percentage of sequences relative to the total sequences within a soil sample. Chao1, ACE and Shannon index were calculated to assess the variation of community richness and diversity across samples. Bacterial community profiles were visualized using non-metric multidimensional scaling (NMDS) ordination plots based on Bray–Curtis distance; the significance of differences among treatments was determined using analysis of similarity (ANOSIM). Nonparametric Kruskal–Wallis test was used to identify bacterial taxa significantly affected by microplastics.

Data were means (± standard error) of triplicates. Statistical analysis was carried out using SPSS version 16.0 (SPSS Inc, Chicago, USA). One-way analysis of variance (ANOVA) followed by a least significant difference (LSD) multiple comparison procedure were used to evaluate the significance of the effect of microplastics on soil nutrient content and community diversity indices; if homogeneity of variance assumption was not met, Dunnett T3 multiple comparison test was then applied. The statistical significance level was set at $p = 0.05$.

Results and Discussion

Available nitrogen and phosphorus contents are important indicators of soil fertility and soil quality. PVC microplastics were found to have a minimal effect on available nitrogen content in the two soils examined (Fig. 1a–b). A significant effect was only observed under high pollution level in the paddy soil, with NO_3^-–N content being 10%–13% ($p < 0.05$) lower in soils amended with 1% PVC microplastics (plasticized or unplasticized) relative to the control.

Available phosphorus was more affected by microplastic presence than available nitrogen, as significantly ($p < 0.05$) greater changes were observed for available P. In the red soil, addition of DEHP plasticized PVC microplastics induced an increase in available P content, although unplasticized microplastics did not (Fig. 1c). Due to the addition of 0.1% and 1% DEHP plasticized microplastics, available P concentration increased from 10.6 ± 0.2 mg/kg to 18.0 ± 0.8 mg/kg ($p < 0.05$) and 14.7 ± 1.5 mg/kg, respectively. In the paddy soil, a negative effect of PVC microplastics on available P content was observed (Fig. 1c). Addition of 1% unplasticized PVC microplastics significantly reduced available P content from 38.4 ± 2.5 mg/kg to 26.9 ± 1.3 mg/kg ($p < 0.05$). Surprisingly, the decrease induced by plasticized PVC microplastics was less obvious in comparison to the unplasticized counterparts, suggesting that in some cases plasticizer presence may be able to mitigate the negative impact of microplastics on P cycling.

Currently, very few studies have examined the influence of microplastics or phthalate plasticizers on phosphorus cycling. Previously, a positive effect of 28% polypropylene (PP) microplastics and no effect of 2% polylactic acid (PLA) microplastics on soil available/inorganic P were observed (Chen et al. 2020; Liu et al. 2017). Herein, we reported a potential impact of 0.1%–1% PVC microplastics on P transformation in soil, showing the importance of studying risks from microplastic pollution at more environmentally relevant concentrations. Increase in available P content could be due to microbe-mediated solubilization of inorganic P and mineralization of organic P while a decrease may be caused by a less efficient solubilization or mineralization process (Qu et al. 2020; Satyaprakash et al. 2017). The mechanisms of P transformation in microplastic amended soils and whether the effects are long-lasting, require further investigation.

The contrasting effects of microplastics on nutrient content in the two soils examined, indicate that soil origin/type is a critical factor to be considered when examining and predicting the influence of microplastics on soil nutrient cycling. The red soil was an acidic soil with relatively low organic matter and high clay content, while the paddy soil was a neutral soil with relatively high organic matter and silt content. In addition, the two soils probably harbored distinct microbial communities. The discrepancies in soil physiochemical and biological parameters may have an important effect on the interaction of microplastics with soil components, thus leading to different outcomes. Soil type-dependent effects of a disturbance (e.g., biochar and organic pollutant) have also been reported in many previous studies (Smider and Singh 2014; Zhu et al. 2018b). Moreover, the inconsistent effects of unplasticized and plasticized PVC microplastics, indicate that the presence of plasticizer which could slowly migrate from the inner part to the surface of microplastics is factor that should not be neglected. Plastic additive bisphenol A from microplastics has been reported to greatly affect methane production from waste activated sludge digestion (Wei et al. 2019).

Bacterial communities in soil play essential role in the decomposition of organic matter and nutrient cycling. In this study, addition of PVC microplastics at a dosage of 0.1% or 1% showed no significant effect on soil bacterial community richness and diversity as indicated by Chao1 and ACE

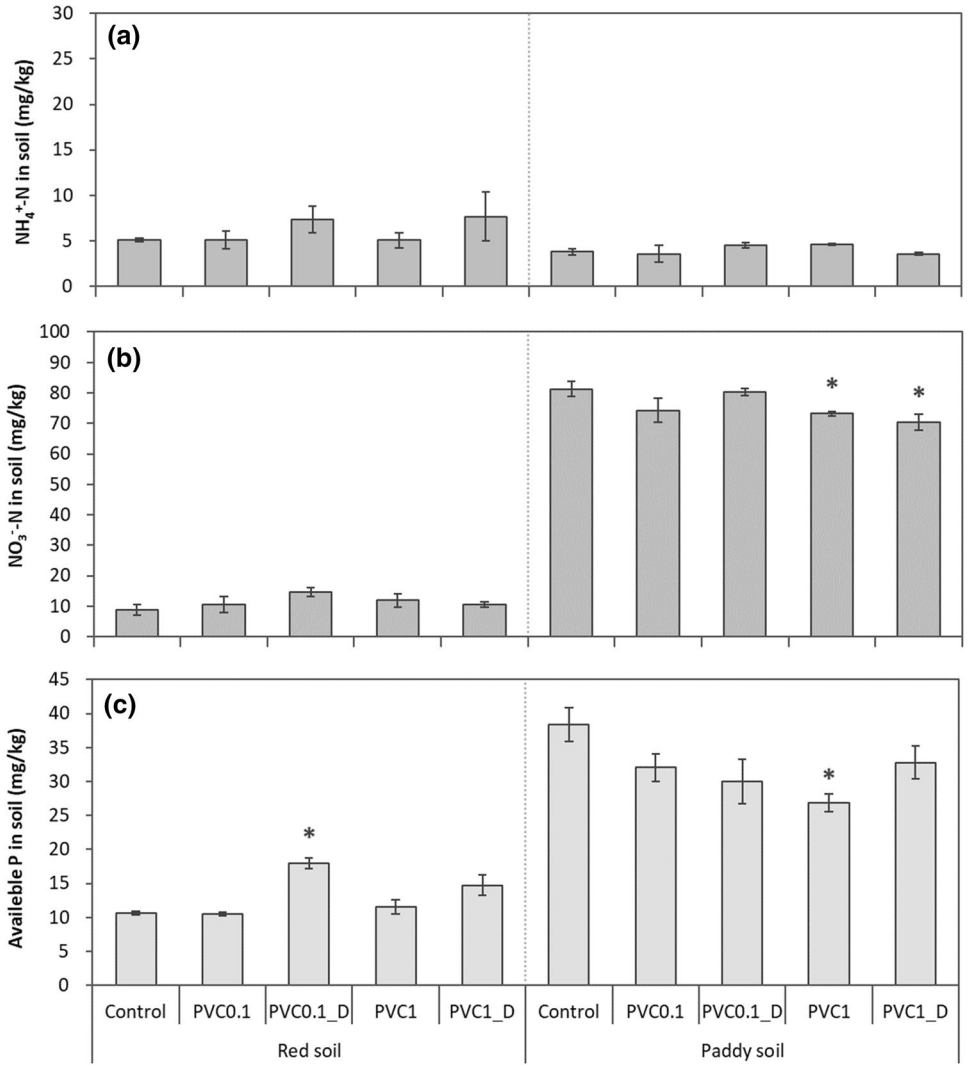

Fig. 1 Effect of microplastics on ammonium nitrate (**a**), nitrate nitrogen (**b**) and available phosphorus (**c**) in the two soils examined. "*" indicates a significant difference compared to the control at $p < 0.05$

estimators and Shannon diversity index (Table S1). This agrees with the finding by Fei et al. (2020) that bacterial community richness and diversity in an acid cropland soil was not significantly impacted by 1% PVC microplastics.

Then, bacterial community compositions in different treatments were analyzed. Bacterial communities in the two soils used varied greatly. While the acidic red soil was dominated by the phyla *Chloroflexi* (relative abundance of 30.7% in the control soil), *Proteobacteria* (23.0%), *Acidobacteria*, WPS-2 and *Actinobacteria*, the neutral paddy soil was composed mainly of *Proteobacteria* (49.7%), *Bacteroidetes*, *Acidobacteria*, *Gemmatimonadetes*, *Chloroflexi* and *Firmicutes* (Fig. S1). Microplastic amended soils and the control soil had similar bacterial communities at phylum level, indicating a negligible effect of 0.1%–1% PVC microplastics on bacteria at high taxonomic level. At OTU level, samples tended to cluster based on microplastic concentration (Fig. 2). However, ANOSIM analysis showed that the difference between microplastic treatment and the control was not statistically significant in both soils (e.g., 1% PVC vs. control in the paddy soil, $p = 0.098$). Similarly, previous studies also demonstrated that PVC or PLA microplastics at up to 2% did not trigger significant change in overall bacterial community structure in soil (Chen et al. 2020; Fei et al. 2020; Judy et al. 2019). Taken together, these results suggest that PVC microplastics at a dose higher than 1% may be needed to induce a significant effect on soil bacterial community as a whole (Fei et al. 2020).

Although no significant effect on overall bacterial community was observed, PVC microplastics significantly altered the abundance of some bacterial taxa (Fig. 3). This information would be useful for identifying microplastic-associated microorganisms and predicting changes in soil metabolic potential. In the red soil, the relative abundance of the genera *Ramlibacter*, *Bradyrhizobium*, *Luteimonas* and norank_o_SBR1031 was significantly increased due to addition of 1% DEHP-plasticized PVC microplastics. In the study by Fei et al. (2020), members from the family

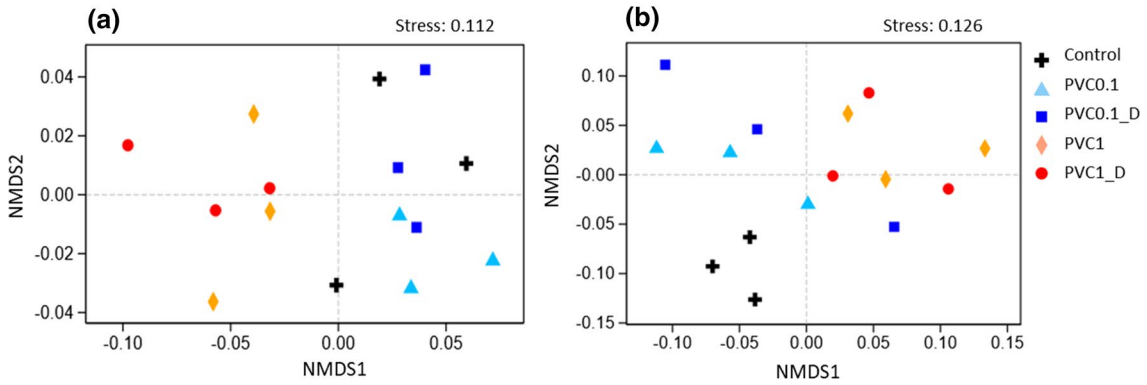

Fig. 2 NMDS plot illustrating shifts in overall bacterial community structure due to microplastic presence in red soil (**a**) and paddy soil (**b**)

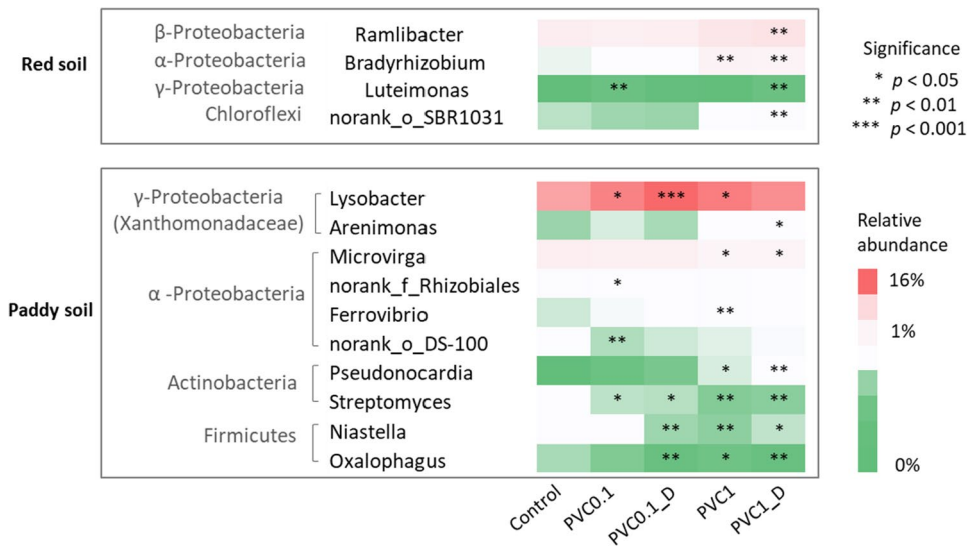

Fig. 3 Bacterial genera significantly affected by PVC microplastics in the two soils examined. Only the genera within the top 50 most abundant ones in each soil are shown

Intrasporangiaceae (belonging to the phylum *Actinobacteria*) were enriched by PVC microplastics in an acid farmland soil. This was not observed in the present study, possibly because although both soils were acidic, they differed in geographic source and microbial composition. The positive effect on *Ramlibacter* and *Bradyrhizobium* observed in the current study was consistent with the finding by Chen et al. (2020) and Zhu et al. (2018b, 2020) that microplastic or DHEP may stimulate members from these two genera. *Ramlibacter* members (class *β-Proteobacteria*) are frequently detected in various soil habitats, with versatile catabolic potentials such as utilizing hydroxybenzoate and biomineralizing Ca-phosphates (Liang et al. 2012; Skouri-Panet et al. 2018), while *Bradyrhizobium* (class *α-Proteobacteria*) has been extensively studied due to their ability to induce nitrogen-fixing nodules on legumes (Msaddak et al. 2017).

Compared to the red soil, more bacterial genera were affected by microplastic presence in the paddy soil. It was likely due to a visually closer contact of microplastics with soil particles in the latter soil, which had a higher organic matter and silt content. In the paddy soil, addition of PVC microplastics caused a general increase in the relative abundance of *Lysobacter* and a general decline in the relative abundance of *Streptomyces*, *Niastella* and *Oxalophagus*. Microplastics at high level also showed a significant positive effect on *Pseudonocardia* and a negative effect on *Microvirga*. The enrichment of *Lysobacter* (class *γ-Proteobacteria*) and *Pseudonocardia* (phylum *Actinobacteria*) may be related to their ability to degrade polymers, as they have been reported to be capable of degrading polyhydroxyalkanoate (PHA) (Volova et al. 2017) and polyethylene glycol (PEG) (Eubeler et al. 2010). It was surprising that *Streptomyces* (phylum *Actinobacteria*) was reduced due to microplastic presence, since members from this genera are well-known for their capacity to degrade natural/synthetic polymers and complex organic materials, such as PHA, PE and lignin (Abraham et al. 2017; Volova et al. 2017). Actually, in another study, the relative abundance of *Streptomyces* was also found to be lower in PE microplastic-amended soil (1.45%) as compared to the unamended soil (0.53%) (Huang

et al. 2019). The influence of microplastics on *Streptomyces* members deserves further research. *Microvirga* species have been commonly found to be N-fixing root nodule bacteria (Msaddak et al. 2017), while *Oxalophagus* members are linked to the assimilation of oxalate (Cornick and Allison 1996); thus, a decline in the abundance of these two genera indicated relevant processes might be impacted due to the presence of PVC microplastics.

Correlation of soil available P content with specific bacterial taxa was anlyzed to provide linkage between microbial community and soil nutrients. In the red soil the relative abundance of OTUs 2241, 2159 and 25 rised significantly after addition of 0.1% or 1% DEHP-plasticized PVC microplastics while no such an increase was observed due to addition of unplasticized microplastics (Fig. 4). The sum of relative abundance of these three OTUs was significantly positively correlated with soil available P content (Pearson's correlation coefficient 0.917, $p = 0.014$), suggesting that modification of OTUs 2241, 2159 and 25 might be accounting for elevated available P in the red soil. Similarly, in the paddy soil, available P content was found to be sinificantly correlated with the sum of relative abundance of OTUs 64, 562 and 592 (Pearson's correlation coefficient 0.937, $p = 0.019$) (Fig. S2). Although most of these OTUs were rare speicies (< 1%), they were not deemed to be redundant (Hol et al. 2010).

OTUs 224, 2159, 25, 64, 562 and 592 were identified as unclassified TK10, unclassified B12-WMSP1 (class *Ktedonobacteria*), *Haliangium*, *Bacillus*, *Oligoflexus*, and unclassified Subgroup_18, respectively. Among them, *Bacillus* is commonly reported as phosphate-solublizing bacteria and several *Bacillus* species (e.g., *Bacillus megaterium, B. circulans and B. subtilis*) have been identified as effective P solubilizers (Satyaprakash et al. 2017). In addition, the class *Ktedonobacteria* was found to contain genes involved in inorganic P solubilization (Dai et al. 2020), and a *Oligoflexus strain* was reported to contain phosphonate utilization genes (Nakai et al. 2016). Therefore, members from these six OTUs may be involved in phosphorus cycling in the two soils examined, and PVC microplastics might have influenced soil available P content via affecting these bacterial taxa.

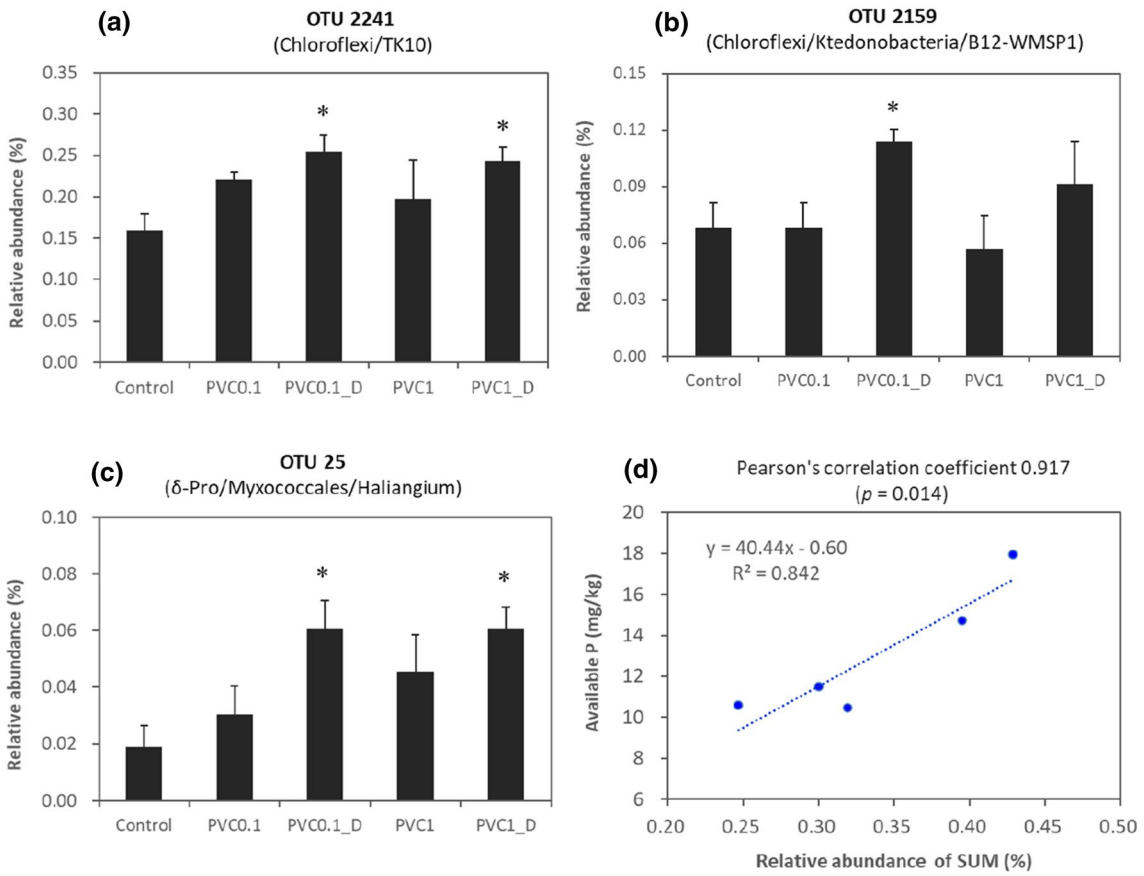

Fig. 4 Effect of PVC microplastics on the relative abundance of OTU 2241 (**a**), OTU 2159 (**b**) and OTU 25 (**c**) in the red soil, and correlation of available P content and the sum of relative abundance of OTUs 2241, 2159 and 25 (**d**). "*" indicates the difference is significant when compared with the control ($p < 0.05$)

Findings from this study tend to confirm that PVC microplastics at ≤ 1% may not be sufficient to induce significant changes in overall bacterial community diversity and composition. However, changes in soil nutrients and the relative proportion of specific bacterial taxa due to the presence of microplastics (such as the genera *Lysobacter* and *Streptomyces* as well as possible P-solublizing species), indicate that attention is still required to monitor the threats posed by microplastics at environmentally relevant concentrations. In this study, microplastic addition showed a positive effect on soil available P content in the acidic red soil but a negative effect was observed in the neutral paddy soil. No clear trend was found regarding other factors such as plasticizer presence and pollution level under studied conditions. Overall, the results demonstrated that PVC microplastics have a complex effect on soil microorganisms and nutrient cycling processes. More research focusing on the factors determining the toxicity/threats of microplastics is needed to gain a better understanding.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest to this work.

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