Insight into application of phosphate-solubilizing bacteria promoting phosphorus availability during chicken manure composting

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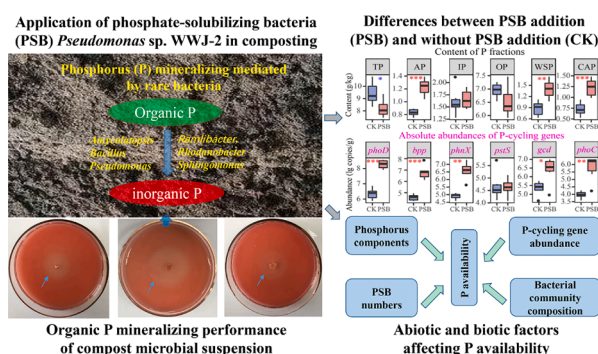[c] *State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550081, PR China*
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HIGHLIGHTS

- PSB addition notably increased available phosphorus from 0.83 to 1.23 g kg^{-1}.
- PSB addition significantly increased abundances of phosphorus-cycling genes.
- PSB inoculation notably affected compost bacterial community composition.
- Stochastic and homogenizing processes affected more on bacterial community assembly.
- Rare bacteria potentially mediated organic phosphorus mineralization.

GRAPHICAL ABSTRACT

Application of phosphate-solubilizing bacteria (PSB) *Pseudomonas* sp. WWJ-2 in composting

Organic P mineralizing performance of compost microbial suspension

Differences between PSB addition (PSB) and without PSB addition (CK)

Abiotic and biotic factors affecting P availability

ARTICLE INFO

ABSTRACT

Understanding ecological roles of phosphate-solubilizing bacteria (PSB) is important to optimize composting systems. Illumina MiSeq sequencing, gene quantitation, and statistical analyses were employed to explore ecological mechanisms underlying available phosphorus (AP) facilitation during composting with the inoculation of PSB *Pseudomonas* sp. WWJ-22. Results displayed that the inoculation of PSB significantly increased AP from 0.83 to 1.23 g kg^{-1}, and notably increased abundances of phosphorus-cycling genes as well as numbers of PSB mineralizing phytate and lecithin. The PSB addition significantly affected compost bacterial community composition, and phosphorus factions and phosphorus-cycling genes independently explained 25.4 % and 25.0 % bacterial compositional dissimilarity. Stochastic and homogenizing processes affected more on bacterial community assembly, and rare bacteria potentially mediated organic phosphorus mineralization. These results emphasized that phosphorus fractions, PSB number, phosphorus-cycling gene abundance, and bacterial community composition contributed differently to phosphorus availability. Findings highlight ecological roles of exogenous PSB during chicken manure composting.

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1. Introduction

The social development with continuous prosperity brings about massive organic solid wastes (OSWs), including kitchen wastes, animal manures, and crop residuals (Liang et al., 2022). Irrational treatments of these OSWs result in potential ecological risks, including breeding mosquitos and producing unpleasant smell (Tan et al., 2022). The recycling of OSWs meets the demand of worldwide-proposed sustainable economy because useful materials and energy can be retrieved and reused (Shah et al., 2022). Composting, regarded as an environment-friendly solution, appeals global attentions to handle agricultural (Cheng and Wan, 2022) and municipal (Zhang et al., 2021a) OSWs. Prior studies report that composting can promote availability of nutrients including carbon, nitrogen, and phosphorus (Chen et al., 2022; He et al., 2022).

Phosphorus is non-renewable resource and phosphorus is in great demand in society (Li et al., 2019). Agricultural and municipal OSWs contain plentiful organic phosphorus (OP), such as phospholipids, phytates, and nucleic acids (Grigatti et al., 2019). The transformation of insoluble OP to soluble inorganic phosphorus (IP) (e.g., hydrophosphate) needs functions of microorganism-produced enzymes including phosphatase, phytase, and nuclease (Villamizar et al., 2019). Microorganism-synthesized organic acids, such as lactic acid and succinic acid, can solubilize the insoluble IP (Beheshti et al., 2021). Microorganisms play important roles in mineralizing OP and solubilizing IP during composting (Sarr et al., 2020; Ma et al., 2020). Therefore, it is essential to harvest abundant AP from OSWs during composting by applying functional microorganisms.

The application of exogenous PSB can promote compost phosphorus availability (Zhang et al., 2021a). For example, the additions of PSB *Bacillus* sp. *P6* (Zhang et al., 2021a) and *Bacillus subtilis* (Mohamed et al., 2022) to kitchen waste increase the content of AP. The consortium of PSB *Pseudomonas* sp. PSBR12 and *Bacillus* sp. BACBR01 can enhance phosphorus availability during sugarcane waste composting (Estrada-Bonilla et al., 2017). The PSB *Pseudomonas fluorescens* facilitate AP level during composting with cow dung and waste paper (Lukashe et al., 2019). The application of PSB distinctly changes microbial community structure and phosphorus fractions after composting (Zhang et al., 2021a; Chen et al., 2022). Phosphorus-cycling genes (e.g., *phoD*, *phnX*, and *pstS*) mediate phosphorus turnover by producing phosphorus-cycling enzymes (Grafe et al., 2018), and phosphorus-cycling genes present dynamic changes during composting with chicken manure (Wan et al., 2020a) or sorghum straw (Sarr et al., 2020). However, it remains largely unclear whether the inoculation of PSB can distinctly affect the abundances of phosphorus-cycling genes.

Riding on technology blooms in terms of microbial community analyses, many studies have investigated community composition of microorganisms (Chen et al., 2022; Mohamed et al., 2022), absolute abundances of phosphorus-cycling genes (Sarr et al., 2020; Wan et al., 2020a), and colony numbers of culturable PSB (Wei et al., 2016) during composting. However, it remains largely unknown which core bacterial species mediating phosphorus availability and how bacteria responding to compost environmental change. Additionally, linkages among microbial community composition, phosphorus-cycling gene abundance, phosphorus fractions, and PSB number are rarely reported. To uncover these mysteries, chicken manure composting was conducted with the application of isolated PSB *Pseudomonas* sp. WWJ-22 (Chen and Wan, 2023). Research targets are to (i) investigate differences in phosphorus components and phosphorus-cycling gene abundance between PSB addition and without PSB addition, (ii) decipher core bacterial species mediating phosphorus transformation, and (iii) reveal factors affecting phosphorus availability. Considering PSB inoculation affects phosphorus fractions (Zhang et al., 2021a; Chen et al., 2022), one reasonable hypothesis was that the inoculation of PSB *Pseudomonas* sp. WWJ-22 would notably change abundances of phosphorus-cycling genes and numbers of PSB. This study will reveal ecological mechanisms behind phosphorus fractionation and phosphorus availability during manure composting and might be helpful to optimize composting by introducing PSB.

2. Materials and methods

2.1. Compost substrates and experiment design

The raw compost substrates of chicken manure and vegetable wastes were collected from Bachu Vegetable Base located in Yichang city, China. The chicken manure was naturally dried and crushed, and the dehydrated vegetable wastes were chopped into 2-cm-length pieces (Wan et al., 2020a). The carbon-to-nitrogen (C/N) ratios of dry vegetable wastes and chicken manure were 69.5 and 10.1, respectively, and vegetable wastes were mixed with chicken manure at a ratio of 1:3 (weight/weight) to set the initial C/N ratio of 25. The initial physico-chemical properties of compost mixture were pH of 6.7, total organic carbon of 50.9 %, total nitrogen of 2.15 %, C/N of 23.7, total phosphorus (TP) of 11.86 g kg^{-1}, AP of 0.51 g kg^{-1}, and moisture of 60 %.

Small-scale composting was conducted in summer season, and room temperature was recorded every day. Compost mixture were added to the full of 12 buckets with 7-L volume, and each six buckets were separately inoculated with strain WWJ-22 seed cultures (EX group) and sterile water (CK group) at a volume-to-volume of 1 %. The strain WWJ-22 were cultured in LB medium, collected by centrifuging, washed by sterile water, and then resuspended in sterile water to prepare seed cultures (OD$_{600}$ = 1). Compost temperatures at three depths (i.e., 5, 10, and 15 cm) were measured every day, and compost temperature was regarded as the average of temperatures at three depths. Composts were turned and mixed every ten days by using sterile iron scoop, and simultaneously moisture was adjusted to 60 % by spraying sterile water. After composting for 50 days, composts were collected from each buckets by using spoon to determine phosphorus fractions and molecular analyses.
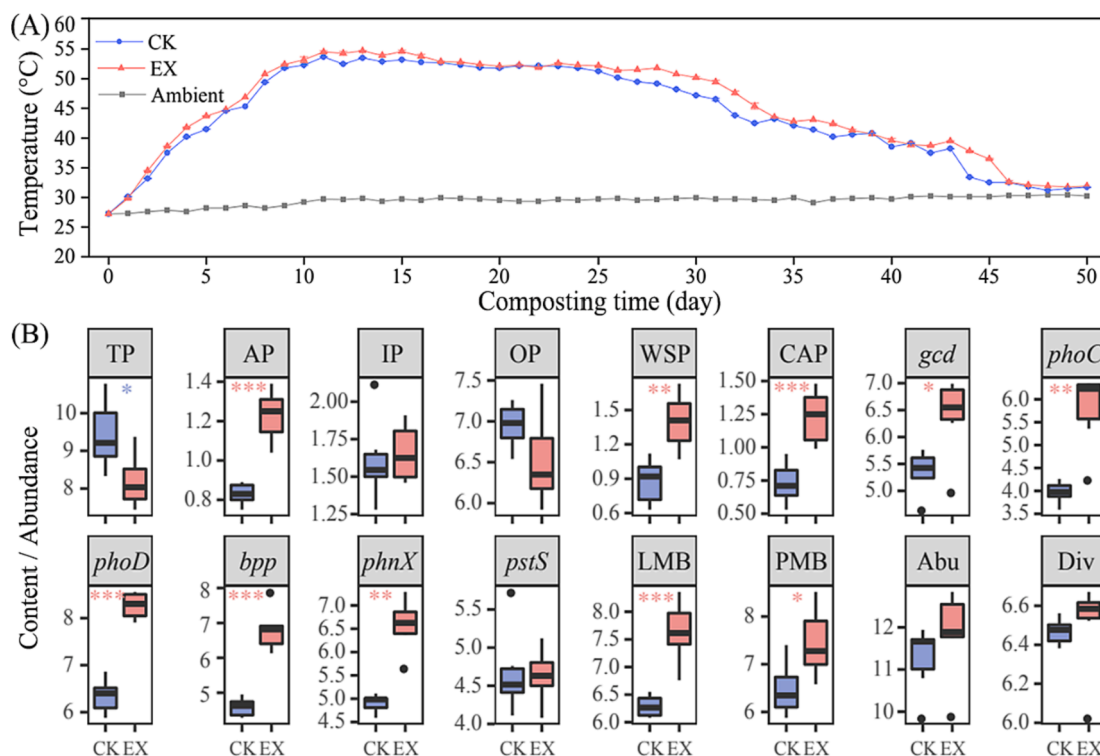
2.2. Determination of phosphorus fractions and PSB numbers

Compost AP, TP, IP, water soluble phosphorus (WSP), and citric acid phosphorus (CAP) were extracted using 0.5 mol/L of NaHCO$_3$, concentrated HClO$_4$-H$_2$SO$_4$, 1 mol/L hydrochloric acid, deionized water, and 2 % citric acid, respectively. The content of these phosphorus fractions were assayed by applying the molybdate-blue colorimetric method (Jackson, 1958). The content of OP is the difference between TP and IP.

The NBRIP medium containing lecithin or phytate was used to estimate numbers of lecithin-mineralizing bacteria (LMB) or phytate-mineralizing bacteria (PMB). The NBRIP contains 10 g/L of glucose, 0.25 g/L of MgSO$_4$·7H$_2$O, 0.1 g/L of (NH$_4$)$_2$SO$_4$, 5 g/L of MgCl$_2$·6H$_2$O, 0.2 g/L of KCl, 5 g/L of soybean lecithin or sodium phytate, 0.2 g/L of cycloheximide, and 18 g/L of agar. The colony numbers of the culturable LMB and PMB were counted after plates incubated for 3 days.

2.3. Molecular analysis of phosphorus-cycling genes and bacterial community

Universal primers were used to amplify phosphorus-cycling genes and bacterial 16S rRNA gene. The phosphorus-cycling genes included IP-solubilizing gene (i.e., *gcd*), OP-mineralizing genes (i.e., *phoC*, *phoD*, *bpp*, and *phnX*), and IP transport gene (i.e., *pstS*) (Sarr et al., 2020; Wan et al., 2020b). Bacterial 16S rRNA gene targeting V3-V4 region was amplified by applying universal forward primer 338F (5′- ACT CCT ACG GGA GGC AGC A-3′) and reverse primer 806R (5′-GGA CTA CHV GGG TWT CTA AT-3′) (Mori et al., 2013). The raw sequencing data generated from Illumina MiSeq platform were purified to gain non-singleton, chimera-free, and denoised sequences by applying the QIIME2 pipeline and Vsearch. The clean sequences were designated as operational taxonomic units (OTUs) using the 97 % similarity threshold against the

Fig. 1. Effects of the inoculation of PSB *Pseudomonas* sp. WWJ-22 on compost abiotic and biotic properties. (A) Changes in compost and ambient temperature during 50 days. (B) Differences in content of phosphorus fractions, abundances of phosphorus-cycling genes, numbers of PSB, bacterial abundance and diversity between CK and EX. Units for parameters: g kg^{-1} (TP, AP, IP, OP, WSP, and CAP), log$_{10}$ copies g^{-1} (*gcd*, *phoC*, *phoD*, *bpp*, *phnX*, *pstS*, and Abu), and log$_{10}$ cfu g^{-1} (LMB and PMB). Abbreviations: LMB, lecithin-mineralizing bacteria; PMB, phytate-mineralizing bacteria; Abu, bacterial abundance; and Div, bacterial diversity represented by Shannon-Wiener index. Asterisks above columns represent significance (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

SILVA v132 reference. The raw sequencing datasets were deposited in NCBI under accession number PRJNA915782.

2.4. Data analyses

Student's t test ($p < 0.05$) was applied to estimate significant difference in data (e.g., phosphorus content and gene abundance) when data obeyed the normal distribution. Spearman's correlations were used to evaluate relationships between phosphorus fractions and phosphorus-cycling genes. Venn diagram, nonmetric multidimensional scaling (NMDS), and analyses of similarity (ANOSIM) were employed to estimate community similarity between CK and EX (Chen and Wan, 2023). Redundancy analysis (RDA) was employed to investigate influences of phosphorus fractions and phosphorus-cycling genes on bacterial community composition. Mantel correlogram was used to estimate phylogenetic signals and null model was employed to determine relative contributions of ecological processes (Stegen et al., 2012, 2013). Structural equation modeling (SEM) was used to explore relationships between components (e.g., AP, PSB number, and bacterial community composition). The first axis of the principal component analysis (Chen and Wan, 2023), occupying 83.08 %, 81.28 %, and 56.36 % of the total variation, was applied as an agent to represent phosphorus fractions (i. e., TP, IP, OP, WSP, and CAP), gene abundance, and bacterial community structure, respectively.

3. Results and discussion

3.1. Dynamic changes in compost temperature

The ambient temperature presented small fluctuation with range from 27.2 to 30.4 °C (Fig. 1A). No significant difference in compost temperature was found between CK and EX in each day ($p > 0.05$),

which agrees with a finding for kitchen waste composting (Zhang et al., 2021a). The compost temperature of CK and EX groups displayed similar change trend, including temperature increase from day 0 to day 10 (CK, 52.3 °C; EX, 53.2 °C), temperature stability from day 10 to day 25 (CK, 51.3 °C; EX, 52.2 °C), and temperature decrease from day 25 to day 50 (CK, 31.7 °C; EX, 31.9 °C). The temperature shift is in consistent with temperature change during traditional composting, comprising heating, thermophilic, and cooling periods (Wan et al., 2020a; Zhang et al., 2021a; He et al., 2022). The highest compost temperature was 53.7 °C for CK group and 54.6 °C for EX group (Fig. 1A). Prior studies have reported that the highest compost temperature is 55 °C for food waste composting (He et al., 2022), 60 °C for kitchen waste composting (Zhang et al., 2021a), 61 °C for chicken manure composting (Wan et al., 2020a), and 72 °C for pig manure composting (Awasthi et al., 2022). The complete composting needed 50 days, which differs from kitchen waste composting occupying 30 days (Zhang et al., 2021a) and pig manure composting taking 26 days (He et al., 2022). The divergences in the highest compost temperature and completion time are mainly attributable to composting substrates and manners. The stability of compost temperature and decomposition of plant residues are important indicators for the completion of composting (Wei et al., 2018; Wan et al., 2020a).

3.2. Addition of PSB enhancing phosphorus availability and PSB numbers

Significantly higher content (g kg^{-1}) of AP (CK, 0.83; EX, 1.23), WSP (CK, 0.88; EX, 1.40), and CAP (CK, 0.73; EX, 1.24) were found in EX group than that in CK group ($p < 0.01$ or $p < 0.001$; Fig. 1B). However, TP was notably higher in CK group (9.64 g kg^{-1}) than that in EX group (8.25 g kg^{-1}) ($p < 0.05$). There were no dramatic differences in content (g kg^{-1}) of IP (CK, 1.61; EX, 1.66) and OP (CK, 6.95; EX, 6.52) between CK and EX ($p > 0.05$). These results indicated that the inoculation of
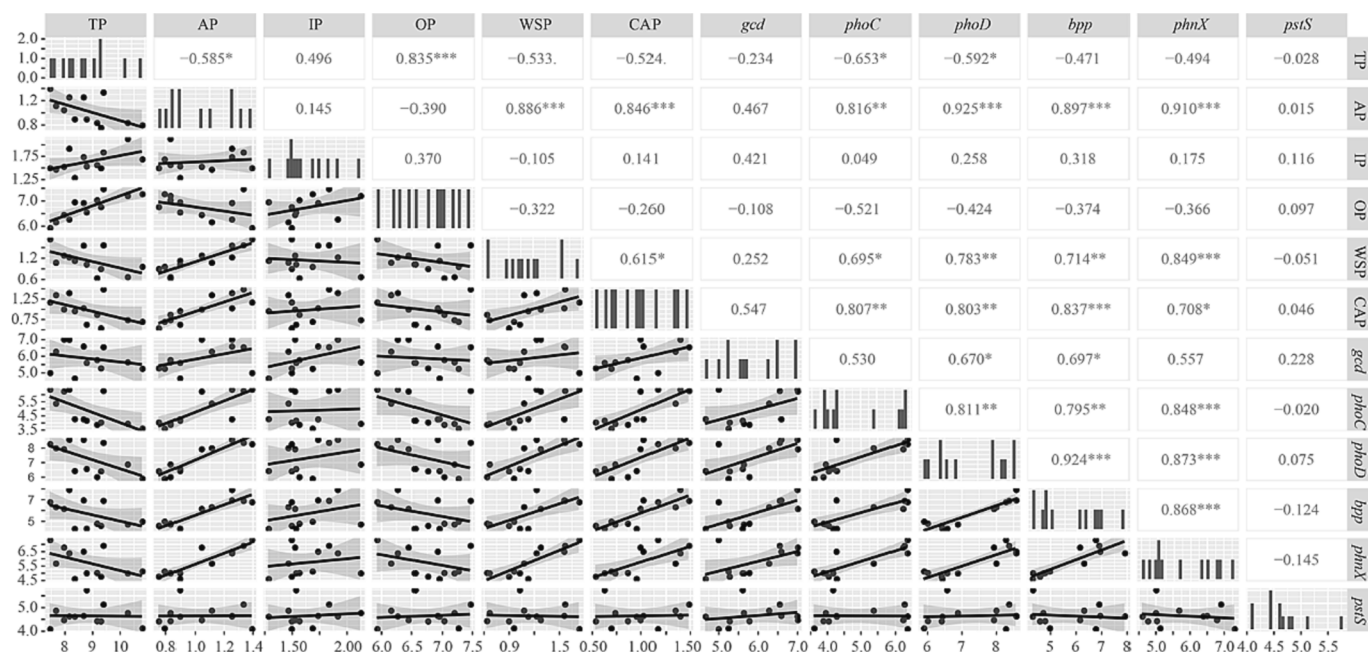
Fig. 2. Spearman's correlations between phosphorus fractions and phosphorus-cycling genes. Asterisks close to numerical values denote significance (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

Table 1
Spearman's correlations between biotic components and phosphorus fractions and phosphorus-cycling genes, and Mantel's tests showing effects of phosphorus fractions and phosphorus-cycling genes on bacterial community composition. Asterisks close to numerical values represent significance (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

| Factors	LMB number	PMB number	Bacterial abundance	Bacterial diversity	Bacterial composition
TP	−0.531	−0.385	−0.262	0.225	0.067
AP	0.768**	0.675*	0.347	−0.130	0.437**
IP	0.184	0.390	−0.120	0.347	−0.194
OP	−0.370	−0.421	−0.094	0.455	0.315
WSP	0.710**	0.510	0.462	−0.226	0.509**
CAP	0.572	0.520	0.248	0.153	0.045
gcd	0.754**	0.543	0.330	0.457	0.186
phoC	0.726**	0.442	0.413	−0.063	0.211*
phoD	0.853***	0.754**	0.259	0.115	0.127
bpp	0.812***	0.731**	0.388	0.111	0.143
phnX	0.890***	0.515	0.446	−0.089	0.474**
pstS	−0.047	0.298	−0.126	0.250	0.037

strain WWJ-22 can affect phosphorus fractionation and enhance phosphorus availability, which is similar to findings for sugarcane waste composting with *Bacillus* sp. BACBR01 inoculation (Estrada-Bonilla et al., 2017) and kitchen waste composting with *Bacillus* sp. inoculation (Zhang et al., 2021a; Mohamed et al., 2022). AP was significantly negatively correlated with TP (Fig. 2), which differs from prior findings for composting with sorghum straw (Sarr et al., 2020) or kitchen waste (Zhang et al., 2021a). The divergence might be greatly due to divergences in substrate properties and composting conditions. The numbers of LMB (CK, 1.94×10^6 cfu g^{-1}; EX, 4.28×10^7 cfu g^{-1}) and PMB (CK, 2.99×10^6 cfu g^{-1}; EX, 2.78×10^7 cfu g^{-1}) were significantly higher in EX than that in CK ($p < 0.05$ or $p < 0.001$; Fig. 1B). The results indicated that the inoculation of strain WWJ-22 increased abundances of LMB and PMB. Abundances of PSB for solubilizing IP fluctuates during composting (Lukashe et al., 2019; Wan et al., 2020a; Zhang et al., 2021a), whereas numbers of PSB for solubilizing OP are rarely reported in composting systems. The numbers of PMB and LMB were significantly positively correlated with AP (Table 1), which agrees with a prior finding for vermicomposting with *Pseudomonas fluorescens* inoculation

(Lukashe et al., 2019). The abundant PSB is the promise of production of small molecular organic acids (e.g., citric acid) and extracellular enzymes (e.g., phosphatase and phytase) (Wei et al., 2018; Lukashe et al., 2019), which in turn solubilize IP and/or OP. It has been reported that microbial inoculation to new conditions (e.g., composts and soils) accompany death of exogenous cells (Chaín et al., 2020). Therefore, the inoculation of *Pseudomonas* sp. WWJ-22 enhancing phosphorus availability might via two pathways: (i) contribution of partial dead *Pseudomonas* sp. WWJ-22 to AP and (ii) AP generation mediated by active PSB. Future study will investigate the survival rate of PSB *Pseudomonas* sp. WWJ-22 to highlight ecological roles of inoculated PSB.

3.3. Increases in abundances of phosphorus-cycling genes

Absolute abundances (copies g^{-1}) of *gcd* (CK, 2.25×10^5; EX, 2.43×10^6), *phoC* (CK, 9.30×10^3; EX, 6.19×10^5), *phoD* (CK, 2.22×10; EX, 1.86×10^8), *bpp* (CK, 3.88×10^4; EX, 6.46×10^6), and *phnX* (CK, 8.04×10^4; EX, 3.77×10^6) were notably higher in EX than that in CK ($p < 0.05$ or $p < 0.01$ or $p < 0.001$; Fig. 1B). However, the absolute abundance (copies g^{-1}) of *pstS* was slightly higher in EX group (4.71×10^4) than that in CK group (4.27×10^4) ($p > 0.05$). Phosphorus-cycling genes (e.g., *phoD*, *pstS*, and *phoX*) are reported during composting (Sarr et al., 2020; Wang et al., 2022; Chen and Wan, 2023), but effects of PSB inoculation on abundances of phosphorus-cycling genes are rarely investigated in terms of composting systems. The inoculation of PSB *Acinetobacter pittii* gp-1 (Wan et al., 2020c) and *Raoultella* sp. SQP80 (Li et al., 2022) can enhance abundances of phosphorus-cycling genes (e.g., *phoD*, *phoA*, and/or *gcd*) of soil microbial communities. The decisive role of PSB in enhancing abundances of phosphorus-cycling genes might mainly arise from inoculated PSB harboring these phosphorus-cycling genes. Additionally, the inoculation of PSB can affect endogenous bacterial community and therefore enhance abundances of phosphorus-cycling genes (He and Wan, 2021). Significant correlations were found between phosphorus fractions and phosphorus-cycling genes (Fig. 2). For example, AP was dramatically related to *phoC* (r = 0.816, $p < 0.01$), *phoD* (r = 0.925, $p < 0.001$), *bpp* (r = 0.897, $p < 0.001$), and *phnX* (r = 0.910, $p < 0.001$). Close linkage between AP and *phoD* gene abundance has been reported during pig manure composting (Chen and Wan, 2023), sorghum straw composting (Sarr et al., 2020), and in long-term
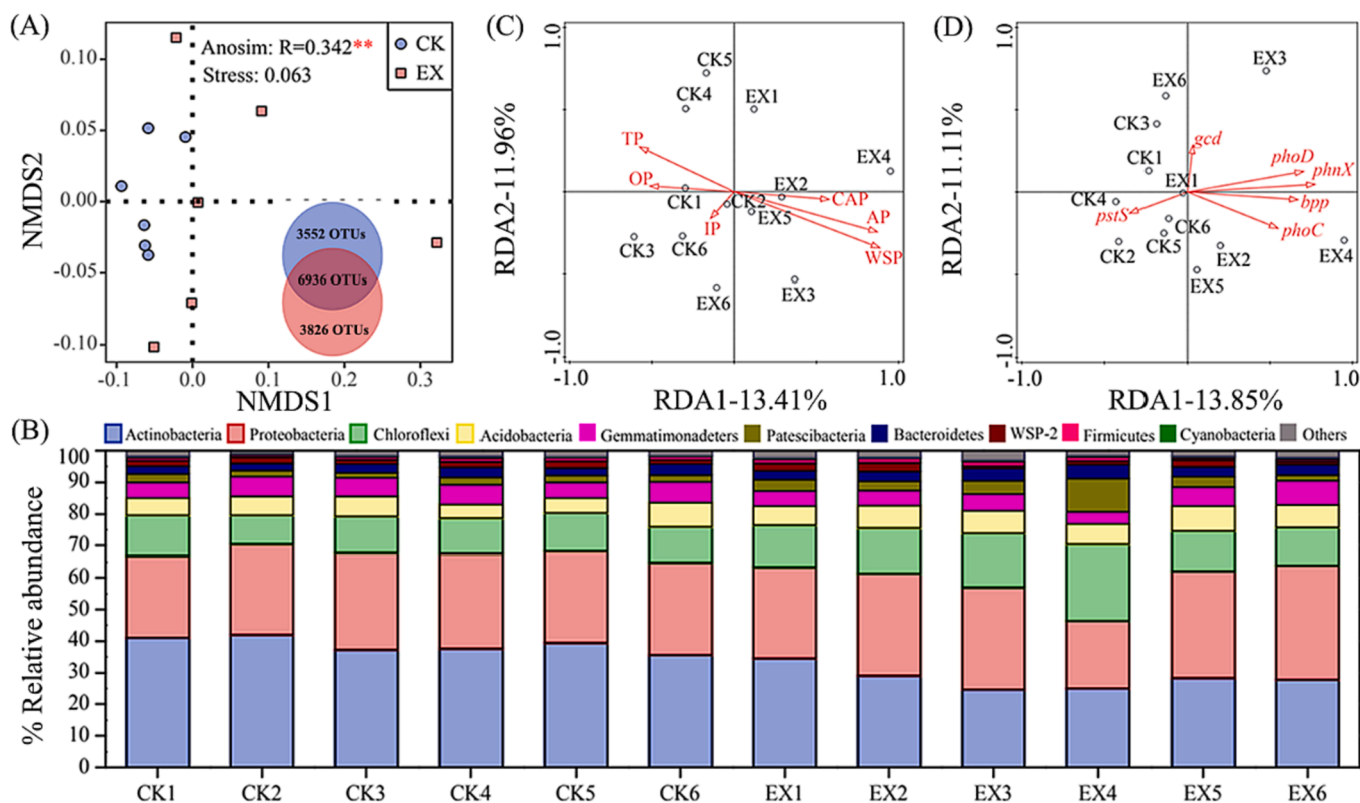
Fig. 3. Effects of the inoculation of PSB *Pseudomonas* sp. WWJ-22 on compost bacterial community composition. (A) NMDS plot and Venn diagram reflect bacterial compositional dissimilarity between CK and EX. Asterisks denote significance (**, $p < 0.01$). (B) Relative abundances of top 10 bacterial phyla across 12 compost samples. RDA shows effects of phosphorus fractions (C) and phosphorus-cycling genes (D) on bacterial community structure.

fertilized soils (Hu et al., 2018). Understanding distribution patterns of phosphorus-cycling genes in composts is critical to estimate phosphorus turnover.

3.4. PSB addition affecting bacterial abundance and community composition

Bacterial abundance and diversity (Shannon-Wiener index) were slightly higher in EX group rather than CK group (Fig. 1B). Phosphorus fractions and abundances of phosphorus-cycling genes were weakly correlated with bacterial abundance and diversity ($p > 0.05$; Table 1). A
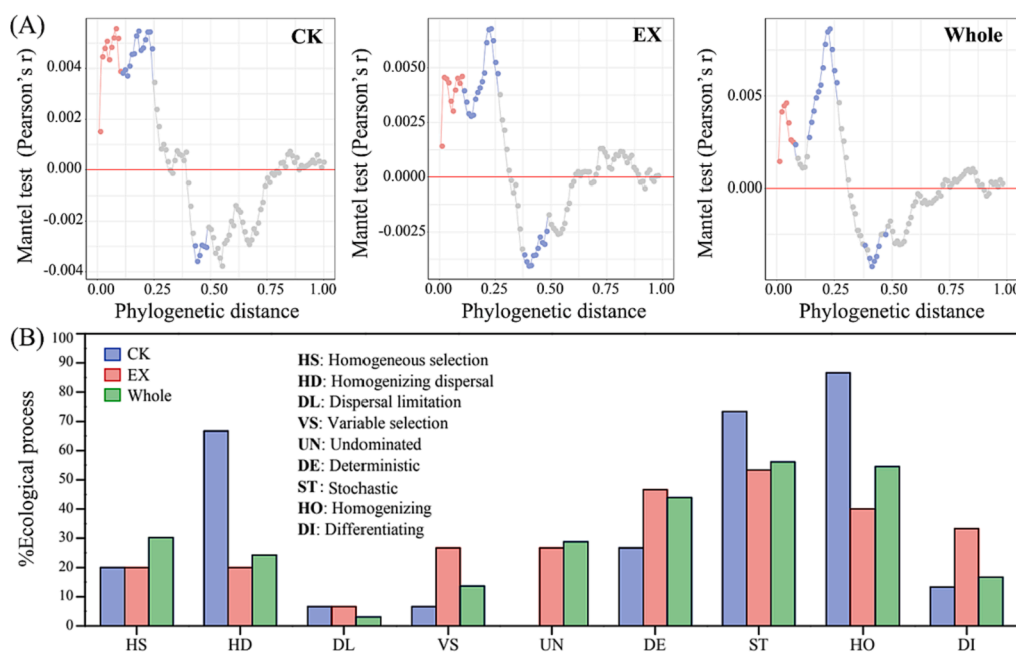
Fig. 4. Ecological community assembly based on the null model. (A) Mantel correlograms reveal whether significant phylogenetic signals of bacteria community emerge at short phylogenetic distances along environmental gradients. Each point represents the mantel correlation coefficient of the given range in phylogenetic distances. Red, blue, and grey symbols denote highly significant ($p < 0.01$), significant ($p < 0.05$), and insignificant ($p > 0.05$) correlations, respectively. (B) Relative contributions of ecological processes to bacterial community assemblies in CK, EX, and whole samples. Deterministic = Variable selection + Homogeneous selection; Stochastic = Dispersal limitation + Homogenizing dispersal + Undominated processes; Differentiating = Variable selection + Dispersal limitation; Homogenizing = Homogeneous selection + Homogenizing dispersal.
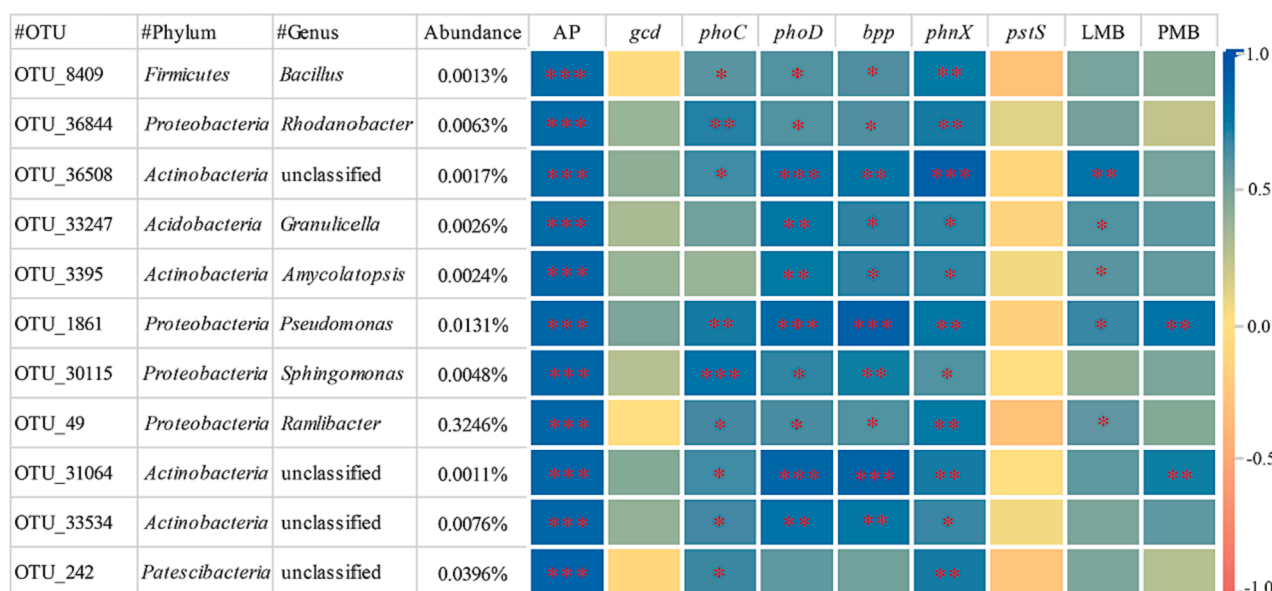
#OTU	#Phylum	#Genus	Abundance	AP	gcd	phoC	phoD	bpp	phnX	pstS	LMB	PMB
OTU_8409	*Firmicutes*	*Bacillus*	0.0013%	***		*	*	*	**			
OTU_36844	*Proteobacteria*	*Rhodanobacter*	0.0063%	***		**	*	*	**			
OTU_36508	*Actinobacteria*	unclassified	0.0017%	***		*	***	**	***		**	
OTU_33247	*Acidobacteria*	*Granulicella*	0.0026%	***			**	*	*		*	
OTU_3395	*Actinobacteria*	*Amycolatopsis*	0.0024%	***			**	*	*		*	
OTU_1861	*Proteobacteria*	*Pseudomonas*	0.0131%	***		**	***	***			*	**
OTU_30115	*Proteobacteria*	*Sphingomonas*	0.0048%	***		***	*	**	*			
OTU_49	*Proteobacteria*	*Ramlibacter*	0.3246%	***		*	*	*	**		*	
OTU_31064	*Actinobacteria*	unclassified	0.0011%	***		*	***	***	**			**
OTU_33534	*Actinobacteria*	unclassified	0.0076%	***		*	**	**	*			
OTU_242	*Patescibacteria*	unclassified	0.0396%	***		*			**			

Fig. 5. Spearman's correlations between relative abundances of 11 rare bacterial species and AP, phosphorus-cycling genes, and PSB number. The relative abundances of 11 rare bacterial species were notably positively correlated with AP (correlation coefficient > 0.8, significance $p < 0.001$). Asterisks in boxes denote significance (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

total of 14,314 OTUs emerged in all samples, and CK and EX shared 6,936 OTUs (Fig. 3A). The NMDS plot displayed separated bacterial community between CK and EX, and ANOSIM identified notable divergence in bacterial community structure between CK and EX (R = 0.342, $p < 0.01$; Fig. 3A). The OTUs were mainly identified as *Actinobacteria* (24.64–42.02 %), followed by *Proteobacteria* (21.29–36.12 %), *Chloroflexi* (9.09–24.18 %), *Acidobacteria* (4.46–7.73 %), *Gemmatimonadetes* (3.85–7.68 %), *Patescibacteria* (1.64–10.56 %), *Bacteroidetes* (2.28–4.51 %), WPS-2 (0.75–2.71 %), *Firmicutes* (0.84–1.43 %), and *Cyanobacteria* (0.20–0.62 %) (Fig. 3B). Significantly higher relative abundances of *Chloroflexi* and *Patescibacteria* were found in EX rather than CK ($p < 0.05$), whereas *Actinobacteria* was more abundant in CK ($p < 0.001$). Phosphorus fractions (Fig. 3C) and phosphorus-cycling genes (Fig. 3D) explained 25.37 % and 24.96 % bacterial compositional dissimilarity, respectively. According to consequences of Mantel's tests, AP (r = 0.437, $p < 0.01$), WSP (r = 0.509, $p < 0.01$), *phoC* (r = 0.211, $p < 0.05$), and *phnX* (r = 0.474, $p < 0.01$) displayed potentially notable influences on bacterial community structure (Table 1).

The results mentioned above indicated that the inoculation of strain WWJ-22 significantly affected bacterial community composition, which is similar to findings for kitchen waste composting (Zhang et al., 2021a; Mohamed et al., 2022). Additionally, inoculation of PSB *Acinetobacter pittii* gp-1 (He and Wan, 2021) and *Raoultella* sp. SQP80 (Li et al., 2022) can affect soil bacterial community structure. This might partially arise from the death of partial inoculated bacteria (Chaín et al., 2020), which supply extra nutrients and therefore in turn affect microbial community structure. In addition, the inoculation of PSB can affect microbial interaction (Zhang et al., 2021a; Mohamed et al., 2022), and interaction pattern (e.g., cooperation and competition) potentially influence microbial community structure. Understanding bacterial community structure is important to optimize composting condition via introducing specific microorganisms.

### 3.5. Addition of PSB decreasing stochasticity of bacterial community assembly

Significant phylogenetic signals of bacteria in CK, EX, and whole samples displayed at short phylogenetic distances (Fig. 4A). Except homogenizing dispersal (66.7 %) of bacterial community assembly for CK group (Fig. 4B), homogeneous selection (20.0–30.3 %), dispersal

limitation (3.0–6.7 %), variable selection (6.7–26.7 %), and "undominated" processes (0–28.8 %) displayed limited effects on community assemblies of bacteria in CK, EX, and whole samples. Consequently, stochastic (53.3–73.3 %) and homogenizing (40.0–86.7 %) processes showed larger influences on bacterial community assembly than deterministic (26.7–46.7 %) and differentiating (13.3–33.3 %) processes. Besides, stochasticity influenced more on community assembly of bacteria in CK rather than EX.

Stochastic processes dominate community assembly of OP-mineralizing bacteria during swine manure composting (Chen and Wan, 2023). Additionally, stochastic processes also determine community assemblies of bacteria in plateau soils (Kang et al., 2022) and temperate grassland soils (Richter-Heitmann et al., 2020). Nutrient-rich conditions tend to generate stochasticity-dominated community assembly of microorganisms, whereas nutrient-insufficient conditions tend to induce determinism-dominated community assembly (Chase, 2010). Spatial scale and environmental heterogeneity also shape the balance between stochasticity and determinism relating to microbial community assembly (Shi et al., 2018; Huber et al., 2020). Understanding microbial community assembly during composting is important because ecological assembly processes can compulsorily couple community structure and ecosystem functioning (Feng et al., 2018).

3.6. Biotic and abiotic factors affecting phosphorus availability

Relative abundances of 11 bacterial species were positively correlated with AP (correlation coefficients > 0.8, $p < 0.001$; Fig. 5). Most of these bacteria were rare taxa (relative abundance < 0.1 %), and were mainly identified as *Amycolatopsis* (OTU_3395), *Bacillus* (OTU_8409), *Granulicella* (OTU_33247), *Pseudomonas* (OTU_1861), *Ramlibacter* (OTU_49), *Rhodanobacter* (OTU_36844), and *Sphingomonas* (OTU_30115). Some PSB belong to *Bacillus* (Zhang et al., 2021a; Mohamed et al., 2022), *Pseudomonas* (Djuuna et al., 2022; He and Wan, 2022), *Ramlibacter* (Zhang et al., 2021b), and *Sphingomonas* (Damo et al., 2022). These bacterial species were correlated differently with phosphorus-cycling genes and PSB (i.e., LMB and PMB). For instance, species OTU_1861 was notably positively correlated with *phoC* (r = 0.743, $p < 0.01$), *phoD* (r = 0.838, $p < 0.001$), *bpp* (r = 0.907, $p < 0.001$), *phnX* (r = 0.773, $p < 0.01$), LMB (r = 0.677, $p < 0.05$), and PMB (r = 0.791, $p < 0.01$) (Fig. 5). Prior studies report that rare bacteria mediate
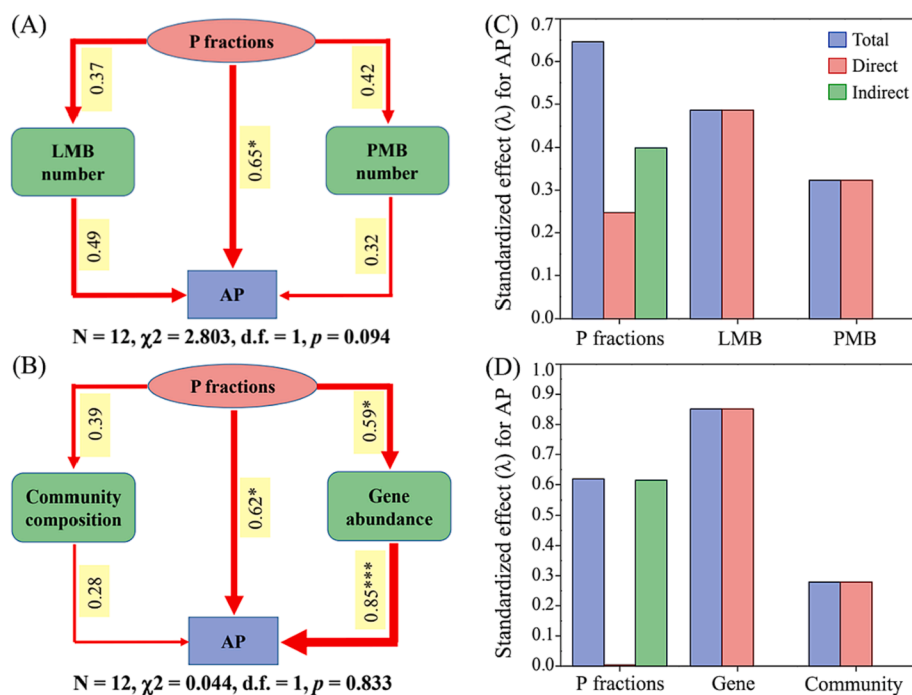
Fig. 6. Abiotic and biotic factors on phosphorus availability. (A) SEM reveals potential linkages among phosphorus fractions (i.e., TP, IP, OP, WSP, and CAP), LMB, PMB, and AP. (B) SEM signifies potential linkages among phosphorus fractions, bacterial community composition, phosphorus-cycling genes, and AP. The numerical values and width of arrows represent the strengths of the standardized path coefficients. (C) Effects of phosphorus fractions, LMB, and PMB on phosphorus availability in direct and indirect ways. (D) Effects of phosphorus fractions, bacterial community composition, and phosphorus-cycling genes on phosphorus availability in direct and indirect manners. Abbreviations: Community, bacterial community composition; Gene, phosphorus-cycling gene abundance; P, phosphorus; N, sample numbers; d.f., degree of freedom. Asterisks close to numerical values denote significance (*, $p < 0.05$; ***, $p < 0.001$).

OP utilization (Wei et al., 2019; Chen and Wan, 2023). Future study will verify these findings via introducing target bacteria especially PSB.

Potential effects of phosphorus fractions (i.e., TP, IP, OP, WSP, and CAP), PSB number (i.e., PMB and LMB), phosphorus-cycling gene abundance, and bacterial community structure on phosphorus availability were estimated by using SEM (Fig. 6). Phosphorus fractions showed significantly positive effect on AP (Fig. 6A, 6B). Phosphorus fractions displayed slightly effects on LMB and PMB, which in turn weakly influenced AP (Fig. 6A). The model fitted the data well, as denoted by the non-significant $\chi2$ test (N = 12, $\chi2 = 2.803$, d.f. = 1, $p = 0.094$). Phosphorus fractions showed notable influence on phosphorus-cycling genes, which in turn dramatically affected AP (Fig. 6B). However, phosphorus fractions showed slight impact on community structure, which in turn weakly affected AP. The model matched the data well (N = 12, $\chi2 = 0.044$, d.f. = 1, $p = 0.833$). Phosphorus factions showed larger indirect effect on AP than direct effect (Fig. 6C, 6D). LMB, PMB, community structure, and phosphorus-cycling genes only displayed direct effects on AP. Prior studies report that abiotic and biotic factors potentially affect PSB number, phosphorus-cycling gene abundance, and AP in agro-ecosystems (Hu et al., 2018; He and Wan, 2022). The coupling of carbon, nitrogen, and phosphorus cycles is reported in different ecosystems (Mooshammer et al., 2017; Li et al., 2018), and therefore carbon and nitrogen content potentially affect phosphorus transformation and availability (Liu et al., 2021). Future study will investigate influences of carbon- and nitrogen-cycling genes on phosphorus availability. Understanding abiotic and biotic factors on AP is important to enhance compost phosphorus availability (Wan et al., 2020a; Cheng and Wan, 2022).

4. Conclusions

The study reported the inoculation of PSB *Pseudomonas* sp. WWJ-22 significantly increased available phosphorus, abundances of phosphorus-cycling genes, and numbers of PSB for utilizing lecithin and phytate. Organic phosphorus-mineralizing genes potentially affect available phosphorus. The inoculation of PSB *Pseudomonas* sp. WWJ-22 notably affected compost bacterial community composition, and phosphorus fractions and phosphorus-cycling genes were responsible for bacterial community structure. Stochastic and homogenizing processes mainly affected bacterial community assembly. Rare bacteria were related closely to organic phosphorus mineralization, and mediated phosphorus availability. These findings provide new insights into application of PSB facilitating phosphorus availability during animal manure composting.

CRediT authorship contribution statement

Qiusheng Wu: Investigation, Methodology. **Wenjie Wan:** Methodology, Writing – original draft, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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