ARTICLE | Article in Press

Biochar promotes the dissolution of inorganic inactive phosphorus by strengthening phoD-harboring bacterial communities during rice stover and sheep manure co-composting

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Received: 11 June 2026 / Accepted: 2 September 2026

Abstract

Inorganic phosphorus (P) predominantly exists as insoluble fractions in natural soil environments, particularly in calcareous soils where calcium-bound stable P and occluded P represent major non-labile P pools. This study investigated the regulatory effect of biochar amendment on the transformation and fractionation of inorganic P during rice stover and sheep manure co-composting. Via high-throughput sequencing of the alkaline phosphatase gene (phoD), cultivable microbial isolation and bacterial ecological network analysis, the ecological function of phoD-harboring bacterial communities in mediating the solubilization of inorganic non-labile P was systematically elucidated. The results demonstrated that biochar addition effectively facilitated the dissolution of occluded P (O-P) and calcium-bound stable P (Ca_{10}-P), while simultaneously elevating the richness, diversity and metabolic activity of phoD-harboring bacterial assemblages. Biochar amendment also optimized the topological structure of phoD-harboring bacterial networks, manifested as enhanced network complexity, interspecies connectivity and community stability. Five culturable phosphate-solubilizing bacterial strains affiliated to Escherichia, Pseudomonas, Brucella, Cellulosimicrobium and Bacillus were screened and identified, all possessing the capacity to decompose recalcitrant inorganic P fractions. Redundancy analysis (RDA) indicated that pH, total organic carbon (TOC) and alkaline phosphatase (ALP) activity constituted the dominant abiotic drivers shaping phoD-harboring bacterial community assembly and P fraction evolution. Structural equation modeling (SEM) further verified that the synergistic regulation of pH, TOC and phoD-harboring microbiota collectively accelerated the solubilization of inorganic non-labile P in biochar-amended compost. This work clarifies the microbial mechanism underlying biochar-modulated P activation, providing a theoretical basis for efficient P recycling and availability improvement in calcareous agroecosystems.

Keywords Phosphorus fractionation · phoD-harboring bacterial community · Ecological co-occurrence network · Phosphate-solubilizing bacteria · Composting · Biochar

https://doi.org/10.1038/s41598-026-70373-0

Published online: 15 September 2026

scientific reports

Introduction

Phosphorus is an essential macronutrient sustaining soil biological metabolism and ecosystem function. Despite its high total abundance in soil matrices, P bioavailability is severely constrained by low solubility under neutral to alkaline conditions, particularly in calcareous soils enriched with carbonate minerals[1,2]. In calcareous soils, inorganic P primarily exists as calcium phosphate minerals and occluded P (O-P), both of which are classified as non-labile P pools with extremely low plant availability[3,4]. Chemical P fertilizers are conventionally applied to alleviate soil P deficiency, yet the non-renewable nature and uneven global distribution of phosphate rock reserves inevitably restrict the long-term sustainability of conventional fertilization regimes[5,6]. Therefore, activating recalcitrant inorganic P pools and improving P use efficiency have become critical challenges for sustainable agricultural development.

Agricultural residual wastes including crop straw and livestock manure represent abundant secondary P resources. Phosphorus in these organic wastes primarily occurs as phytate, phospholipid and nucleic acid forms[7]. Statistical estimation indicates that China annually generates approximately 1 billion tons of crop residues containing 2.56 million tons of total P, while livestock and poultry waste output reaches 4238 million tons with a P load of 1.9 million tons[8,9]. Livestock manure generally contains higher proportions of labile P fractions such as water-soluble Pi and sodium bicarbonate-extractable Pi, which are prone to leaching loss and pose potential eutrophication risks under improper disposal[10,11]. Therefore, enhancing the bioavailability and cyclic utilization of P in agricultural wastes is critical for mitigating global P resource scarcity, reducing agricultural non-point source pollution, and controlling P leaching losses from compost-amended soils.

Composting serves as a feasible bioconversion technology for organic solid waste recycling, wherein indigenous microorganisms decompose complex organophosphorus compounds into low-molecular-weight intermediates, facilitate humification and release inorganic plant-available P[12,13]. In compost matrices, inorganic P is categorized into exchangeable P, Al-bound P, Fe-bound P, Ca-bound P and occluded P (O-P). Labile P mainly consists of dicalcium phosphate (Ca_2-P) adsorbed on the surface of clay minerals and metal oxide-hydroxide colloids, which can be directly assimilated by microorganisms and plants[3,4]. Among various P fractions, octacalcium phosphate (Ca_8-P), Al-P and Fe–P are classified as moderately labile P, capable of interconverting with exchangeable P pools[14,15]. By contrast, O-P is encapsulated by Fe/Al oxide-hydroxide coatings and remains biologically recalcitrant under conventional conditions, whereas reductive microenvironments can trigger its release[15,16]. Both tricalcium phosphate (Ca_{10}-P) and O-P belong to stable non-labile P fractions with extremely low plant availability[3]. Previous studies have documented that composting progression tends to reduce readily available P (Olsen-P, citrate-soluble P and water-soluble P) while accumulating moderately labile and recalcitrant P fractions[7]. Nevertheless, raw material composition and composting parameters can reshape P fraction distribution and modulate P bioavailability[17].

Microorganisms act as the core biotic drivers of P transformation during composting. Phosphate-solubilizing microorganisms secrete extracellular phosphatases, phytases and low-molecular-weight organic acids to mineralize organophosphorus substrates and solubilize mineral-bound insoluble P[18,19]. Alkaline phosphatase (ALP), predominantly synthesized by phoD-harboring bacteria, is the pivotal hydrolase mediating organophosphorus dephosphorylation in compost ecosystems[20–22]. The structural composition, abundance and metabolic activity of phoD-harboring bacterial communities therefore exert deterministic effects on P fraction turnover. Recent evidence reveals that rare ALP-producing bacteria can enhance organophosphorus mineralization and improve P availability during manure composting[12]. Furthermore, phoD-harboring microbiota can elevate microbial biomass P, water-soluble P and citric acid-soluble P pools in organic waste composting systems[13]. Importantly, beyond direct enzymatic hydrolysis of organic P, phoD-harboring bacteria can also indirectly promote the dissolution of inorganic non-labile P through multiple pathways: (i secretion of low-molecular-weight organic acids (e.g., gluconic acid, citric acid that chelate $Ca2^+$, $Fe3^+$ and $Al3^+$ ions and lower ambient pH, thereby destabilizing calcium phosphate and oxide-encapsulated P,(ii modification of redox microenvironments via respiratory

metabolism, which can reduce $Fe3^+$ to $Fe2^+$ and trigger the release of O-P; and (iii synergistic interactions with other functional microbes that collectively enhance P solubilization[18,23]. These indirect mechanisms potentially link phoD-harboring bacterial communities to the activation of recalcitrant inorganic P pools.

Biochar is a porous carbonaceous material derived from biomass pyrolysis under oxygen-limited conditions, characterized by large specific surface area, strong adsorption capacity, high cation exchange capacity and abundant surface functional groups[24,25]. Biochar incorporation can substantially modify compost pH and regulate microbial-mediated P transformation. Existing research has confirmed that phoD-harboring bacteria dominate the regulation of Hedley P fractionation in biochar-amended compost[26]. However, the intrinsic mechanism by which phoD-harboring bacterial communities modulate recalcitrant inorganic P fractions in calcareous soil-compost systems remains poorly understood[3]. Based on previous findings, we propose the core hypothesis that biochar amendment can enhance the diversity and metabolic activity of phoD-harboring bacterial communities, which in turn promote the dissolution of inorganic non-labile P (O-P and Ca_{10}-P) through both direct enzymatic pathways and indirect microenvironmental modification (organic acid secretion, pH regulation, and redox alteration). This study established a control composting group (CK, without biochar) and a 10% biochar-amended treatment group (TB) using rice stover and sheep manure as raw materials. The 10% biochar addition ratio was selected based on previous studies demonstrating that this dosage effectively optimizes compost physicochemical properties and microbial community structure without causing excessive alkalization[27]. The objectives were to: (1 clarify biochar-induced variations in inorganic P fractions, particularly the dissolution of non-labile P (O-P and Ca_{10}-P during composting,(2 elucidate biochar effects on the composition, diversity and interspecific interaction of phoD-harboring bacterial communities; (3 isolate and identify culturable phoD-harboring strains with non-labile P-solubilizing capability and explore their topological roles in microbial ecological networks; and (4 identify key biotic and abiotic pathways regulating the dissolution of inorganic non-labile P. The findings aim to deepen the mechanistic understanding of P bioconversion in biochar-amended compost and provide theoretical support for the efficient recycling of agricultural organic P resources.

Materials and methods

Experimental setup and sample collection

The composting experiment was conducted at the experimental base of Heilongjiang Bayi Agricultural University (125°16′E, 46°59′N) with a total composting duration of 59 days. Rice stover and sheep manure were mixed at a dry weight ratio of 1:3 to establish the initial compost substrate, with an adjusted initial C/N ratio of 25:1 and moisture content maintained at approximately 65%. The basic physicochemical properties of the raw materials are presented in Table 1. Two experimental treatments were designed: blank control (CK, no biochar addition) and biochar amendment (TB, 10% biochar addition based on dry substrate weight). The 10% biochar addition ratio was selected based on previous studies demonstrating that this dosage effectively optimizes compost physicochemical properties, enhances microbial community diversity, and promotes P transformation without causing excessive alkalization that might inhibit microbial activity[27]. Each treatment was set up in three biological replicates. Compost piles were constructed in trapezoidal morphology with dimensions of 2.5 m (length) × 1.5 m (width) × 1.0 m (height). Daily pile temperature was recorded at 15:00 at three depths (20 cm, 50 cm, and 80 cm)

Table 1 Basic physicochemical properties of raw feedstocks.

Feedstock name	TOC g/kg	N g/kg	P2O5 g/kg	H2O %	pH	EC mS/cm
rice stover	404.21	22.33	16.54	15.42	7.08	2.94
sheep manure	283.85	26.01	13.41	67.63	7.038	4.43
biochar	54.63	1.2	8.76	0.09	8.57	5.61

throughout the composting period, and manual turning was implemented on day 7, 22 and 42 to optimize aeration and mitigate temperature stratification.

Representative compost samples were collected at five typical composting stages: initial stage (day 0), warming stage (day 2), thermophilic stage (day 10), cooling stage (day 49) and maturation stage (day 59). A five-point sampling method was adopted to collect subsamples at 10 cm, 30 cm and 50 cm depth of each pile, which were fully homogenized to form composite samples of approximately 500 g. Each composite sample was divided into three subsamples: the first was preserved at − 80 °C for bacterial DNA extraction and phoD-harboring strain isolation; the second was stored at − 20 °C for subsequent ALP activity determination; the third was air-dried naturally and sieved for the analysis of compost physicochemical properties and P fractionation. Sample labels were designated as CK0, CK2, CK10, CK49, CK59 for the control group and TB0, TB2, TB10, TB49, TB59 for the biochar treatment group.

Determination of compost physicochemical properties

Compost pH and electrical conductivity (EC) were measured in a water-to-solid suspension ratio of 2.5:1 using a calibrated pH meter and conductivity meter, respectively[28]. Total organic carbon (TOC) was quantified via potassium dichromate external heating oxidation method. Total nitrogen (TN) was determined by sulfuric acid digestion combined with Kjeldahl nitrogen determination apparatus, and the C/N ratio was calculated as the mass ratio of TOC to TN. Available P (AP) was extracted by sodium bicarbonate solution and measured via ascorbic acid-molybdenum blue colorimetric method[18]. Nitrate nitrogen (NO_3^--N and ammonium nitrogen (NH_4^+-N were extracted with 2 M KCl solution and determined by colorimetric assay. Redox potential (Eh was directly detected using a portable redox potential meter. Alkaline phosphatase (ALP activity was assayed using a commercial soil ALP detection kit (Solarbio Life Sciences, Beijing, China,Cat. No. BC0280 following the manufacturer's standard protocol. Briefly, ALP catalyzes the hydrolysis of p-nitrophenyl phosphate to produce p-nitrophenol, which exhibits a characteristic absorption peak at 405 nm under alkaline conditions; ALP activity was calculated based on the absorbance value and expressed as units per gram of dry compost.

Inorganic phosphorus fractionation

Sequential chemical fractionation procedures were employed to separate five inorganic P fractions adapted for calcareous compost samples[3,4]: dicalcium phosphate (Ca_2-P), octacalcium phosphate (Ca_8-P), aluminum-bound P (Al-P), iron-bound P (Fe–P), occluded P (O-P), and tricalcium phosphate (Ca_{10}-P). A total of 0.500 g sieved compost sample (100-mesh) was placed in 50 mL centrifuge tubes, with graded extractants added sequentially: 0.25 M $NaHCO_3$ (pH 7.5) for Ca_2-P, 0.5 M NH_4OAc (pH 4.2) for Ca_8-P, 0.5 M NH_4F (pH 8.2) for Al-P, and mixed 0.1 M NaOH–0.1 M Na_2CO_3 for Fe–P extraction. Each extraction system was shaken at 25 °C for 1 h and centrifuged at 6000 rpm for 15 min to collect supernatant. Occluded P was extracted by adding 0.3 M sodium citrate and 0.5 g sodium dithionite, water-bath heating at 90 °C for 15 min, followed by neutralization with 0.5 M NaOH. Residual Ca_{10}-P in the sediment was extracted with 0.5 M H_2SO_4 at room temperature for 1 h. The concentration of P in all extracted supernatants was determined using phosphomolybdate blue colorimetry.

High-throughput sequencing of phoD-harboring bacterial community

Total microbial genomic DNA was extracted from frozen compost samples using Omega Soil DNA Isolation Kit according to the kit instructions. The phoD functional gene fragment was amplified using specific primer pairs phoD-F733 (TGGGAYGATCAYGARGT) and phoD-R1083 (CTGSGCSAKSACRTTCCA) (Chen et al.2019)[27]. Purified PCR amplicons were sequenced on the Illumina MiSeq PE300 platform. Raw sequencing reads were quality-filtered and trimmed using Trimmomatic v0.36 and Pear v0.9.6, with sequences shorter than 120 bp discarded. Paired-end sequences were assembled via Flash v1.20, and chimeric sequences were removed using

UCHIME. High-quality clean reads were clustered into operational taxonomic units (OTUs) at 97% sequence similarity using Vsearch v2.7.1, and taxonomic annotation was performed against the NCBI Nucleotide database. All raw sequencing data were deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1073698.

Isolation and identification of phoD-harboring phosphate-solubilizing bacteria

Pikovskaya selective medium (pH 7.0) containing insoluble $Ca_3(PO_4)_2$ as the sole P source was used to screen culturable strains capable of solubilizing non-labile inorganic P[29]. Homogenized composite compost samples from five composting stages were diluted with sterile water at a 1:9 ratio, incubated in a constant-temperature shaker at 30 °C and 170 rpm for 30 min. Gradient dilution was performed to obtain dilution gradients of $10^{-}3$, $10^{-}4$, $10^{-}5$ and 10^{-6}, which were uniformly spread onto Pikovskaya agar plates and incubated at 28 °C for 7 d. Isolated colonies with obvious transparent solubilization halos were repeatedly streaked to obtain pure cultures. Purified strains were subjected to 16S rRNA gene sequencing using universal primers 27F and 1492R, and taxonomic identification was conducted via sequence alignment against the KEGG database[30].

Statistical and bioinformatics analysis

All data were presented as mean ± standard error of three biological replicates. One-way analysis of variance (ANOVA) combined with Duncan's multiple range test ($p < 0.05$) was performed using SPSS 25.0 for significance analysis between treatments. Duncan's multiple range test was selected as the post-hoc comparison method because it is widely used in agricultural and environmental studies, provides appropriate control of Type I error for multiple pairwise comparisons, and is particularly suitable when comparing all treatment means simultaneously with balanced sample sizes (Duncan, 1955). Graphical visualization was completed using GraphPad Prism 8.0.1. Community alpha diversity indices (Chao1, Shannon, Species richness), Venn diagram and PCoA analysis based on Bray–Curtis distance were generated via ImageGP platform. Bacterial co-occurrence ecological networks were constructed based on Spearman correlation coefficients ($|r| \geq 0.8$, $p < 0.05$) using the ggClusterNet package in R. Network topological parameters including node number, edge number, modularity, closeness, betweenness, robustness and vulnerability were calculated and visualized via Gephi 0.9.2. Random forest regression was applied to screen key environmental factors driving phoD-harboring bacterial community variation, with variable importance quantified by the percentage increase in mean squared error (%IncMSE). Redundancy analysis (RDA) was conducted using Canoco 5.0 to quantify the correlation between environmental variables, phoD-harboring microbiota and P fractions. Structural equation modeling (SEM) was constructed via IBM AMOS 23.0 to dissect the direct and indirect regulatory pathways of abiotic factors and microbial community on non-labile P transformation. Model fit was evaluated using chi-square ($\chi 2$), comparative fit index (CFI), root mean square error of approximation (RMSEA), and standardized root mean square residual (SRMR).

Results

Temperature dynamics during composting

The temperature dynamics of both CK and TB treatments throughout the 59-day composting period are presented in Fig. S1. The slightly lower peak temperature in TB may be attributed to the porous structure of biochar enhancing heat dissipation. Both treatments satisfied the hygienic requirements for composting, with temperatures exceeding 50 °C for more than 5 consecutive days, ensuring effective pathogen inactivation.

Physicochemical properties of compost

The basic physicochemical properties of compost samples at five stages are summarized in Table S1. In both treatments, pH showed an initial increase followed by a gradual decline, with TB consistently exhibiting higher pH values than CK due to the alkaline nature of biochar. At the maturation stage, pH values were 8.32 ± 0.08 in CK and 8.67 ± 0.11 in TB. TOC content decreased continuously during composting as organic matter was decomposed, with final TOC concentrations of 268.45 ± 12.36 g/kg in CK and 253.72 ± 10.89 g/kg in TB. EC increased initially and then stabilized, reflecting the release of soluble salts during organic matter decomposition. The C/N ratio decreased from approximately 25:1 to 15–17:1 by the end of composting, indicating satisfactory compost maturity. NO_3^--N content increased significantly during the thermophilic and cooling stages, while NH_4^+-N decreased gradually, consistent with nitrification processes. Available P content showed an initial increase followed by a decline in both treatments, with TB maintaining higher AP levels during the middle and late stages of composting.

Variation and distribution characteristics of phosphorus fractions

Dynamic changes of individual phosphorus fractions

At the initial and warming stages, Ca_2-P content in biochar-amended compost (TB) was significantly higher than that in the control (CK) ($p < 0.05$), which could be attributed to the inherent labile P released from biochar matrix (Fig. 1A). From the thermophilic stage onward, Ca_2-P concentration in TB decreased markedly relative to CK ($p < 0.05$). Two underlying mechanisms accounted for this variation: biochar addition elevated compost pH, inducing the precipitation of labile P with $Ca2^+$, $Fe2^+$, $Fe3^+$ and $Al3^+$ ions and reducing P solubility[31],meanwhile, biochar stimulated microbial metabolic activity, accelerating the assimilation and consumption of labile P by indigenous microorganisms[12,26,32].

The concentrations of Ca_8-P, Al-P and Fe–P in both CK and TB increased continuously throughout composting, consistent with previous findings on organic waste composting[7,33]. At the initial stage, TB exhibited significantly higher Ca_8-P, Al-P and Fe–P levels than CK ($p < 0.05$). At maturation, Al-P content remained higher in TB, whereas Ca_8-P and Fe–P concentrations were significantly lower than CK ($p < 0.05$), which was closely associated with biochar-induced elevation of compost ambient pH (Fig. 1B–D).

The temporal variation trends of O-P and Ca_{10}-P in both treatments followed an initial decline followed by a subsequent accumulation during cooling and maturation stages (Fig. 1E–F). Except for the initial day, O-P content in TB was consistently and significantly lower than CK throughout composting ($p < 0.05$). Particularly at maturation, O-P in TB was 15.8% lower than CK. Biochar amendment reduced compost redox potential, forming relatively strong reductive microenvironments that dissolved Fe/Al oxide-hydroxide encapsulation on O-P surface and promoted its release[15,16].

Relative to CK, Ca_{10}-P content in TB decreased by 33.4%, 21.8%, 19.1% and 8.9% at warming, thermophilic, cooling and maturation stages respectively, all reaching statistical significance ($p < 0.05$), indicating that biochar significantly activated the most stable Ca_{10}-P fraction. This phenomenon was closely linked to the enhanced metabolic activity of phoD-harboring bacterial communities in biochar-amended compost, which corroborated prior research conclusions[27].

Proportional distribution of phosphorus fraction groups

Labile P proportion gradually decreased while moderately labile P proportion increased with composting progression in both treatments; non-labile P proportion initially declined and rebounded afterward, reaching the minimum at the thermophilic stage. This pattern implied that high microbial activity during the thermophilic phase effectively triggered the dissolution of non-labile P. At the thermophilic stage, TB showed a 12.3% reduction in

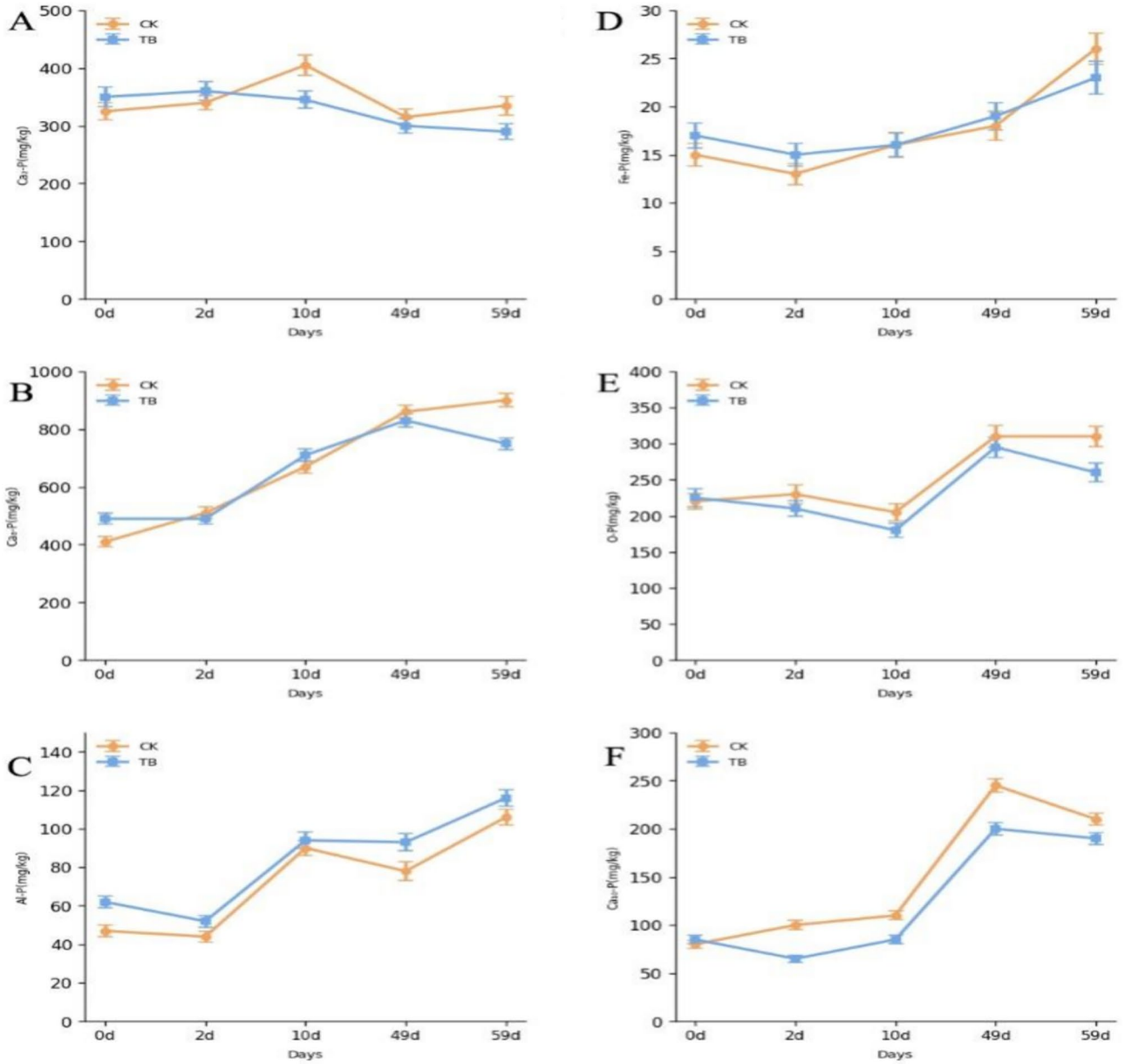

Fig. 1 Dynamic variations of different inorganic P fractions and their proportional distribution during composting. (**A**) Ca_2-P (dicalcium phosphate); (**B**) Ca_8-P (octacalcium phosphate); (**C**) Al-P (aluminum-bound phosphorus); (**D**) Fe–P (iron-bound phosphorus); (**E**) O-P (occluded phosphorus); (**F**) Ca_{10}-P (tricalcium phosphate). CK: no biochar control group; TB: 10% biochar amendment group. Values represent mean ± standard error (n = 3). Asterisks (*) indicate significant differences between CK and TB at the same composting stage ($p < 0.05$).

labile P proportion and an 11.3% increment in moderately labile P proportion compared with CK. The non-labile P proportion in TB was 12.1%, 11.9% and 6.3% lower than CK at warming, thermophilic and cooling stages, respectively, which was attributed to the stronger functional activity of phoD-harboring bacteria in TB. At maturation, the proportional difference of Ca_{10}-P between two treatments diminished, primarily due to the pH elevation induced by biochar amendment.

Among all P fractions, Ca_2-P represents the most active inorganic P pool, capable of transforming into Ca_8-P and further converting to Al-P, Fe–P or O-P[3]. Occluded P can be liberated under reductive conditions via the dissolution of Fe/Al oxide-hydroxide protective films[15,16]. Ca_{10}-P is dominated by insoluble calcium phosphate

minerals, which are chemically stable under neutral-alkaline conditions but can be solubilized under acidic microenvironments[15,16].

Structural composition and metabolic activity of phoD-harboring bacterial communities

Compost material transformation is fundamentally modulated by microbial community succession, and any shift in community structure directly reshapes substrate degradation and nutrient turnover processes[34]. In this study, high-throughput sequencing of the phoD functional gene was performed to characterize the alkaline phosphatase-harboring bacterial community. A total of 1,286,432 high-quality sequences were obtained across all samples, with an average of 42,881 sequences per sample. These sequences were clustered into 2,347 operational taxonomic units (OTUs) at 97% sequence similarity. The Venn diagram showed that 1,246 OTUs were shared by CK and TB, with 478 unique OTUs in CK and 639 unique OTUs in TB (Fig. 2A), demonstrating that biochar addition significantly enriched the endemic taxa of phoD-harboring bacteria.

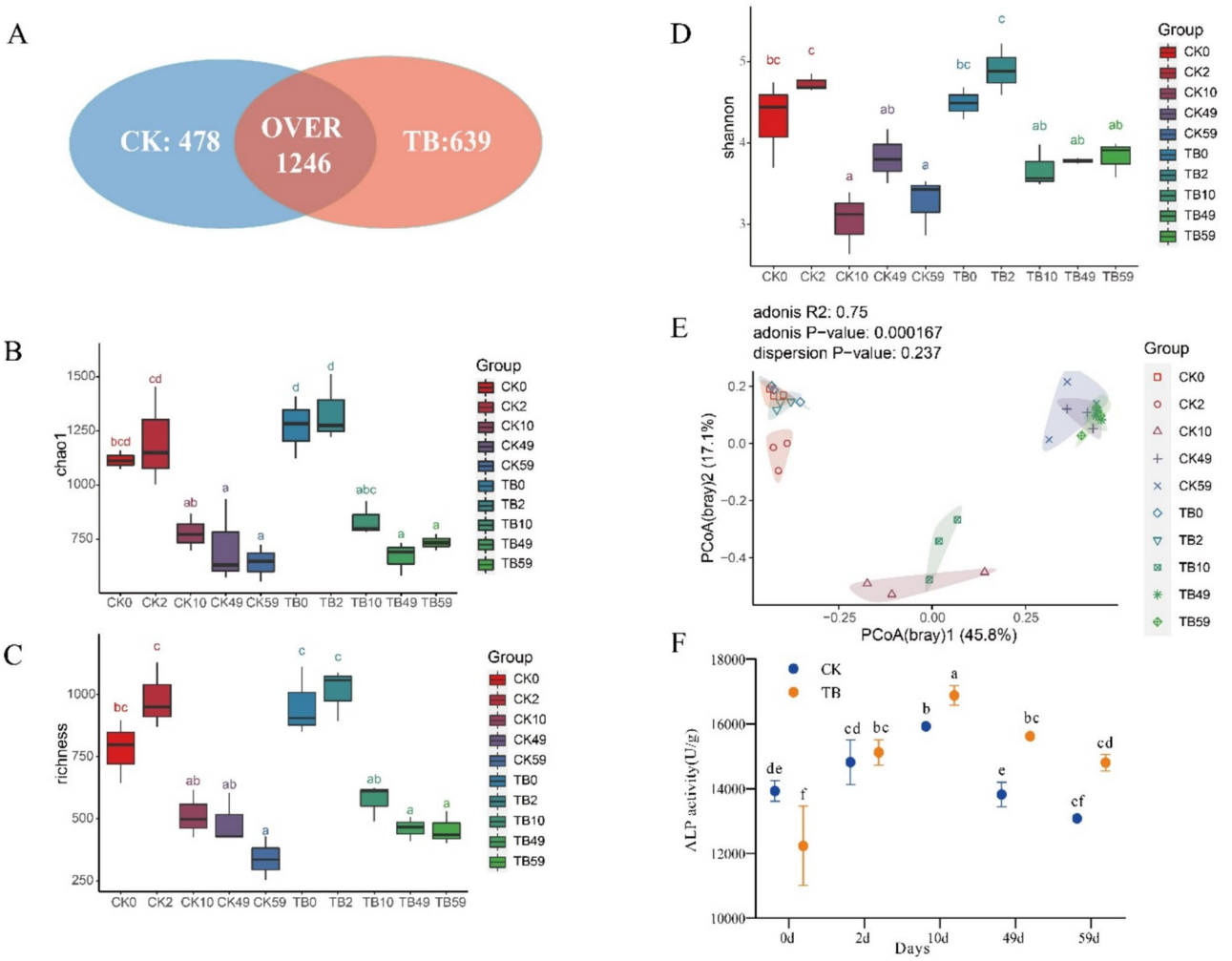

Fig. 2 Community diversity structure of phoD-harboring bacteria and dynamic changes of ALP activity across composting stages. (**A**) OTU Venn diagram; (**B**) Chao1 index; (**C**) Species richness; (**D**) Shannon diversity index; (**E**) PCoA clustering analysis based on Bray–Curtis distance; (**F**) Variation of alkaline phosphatase (ALP) activity. CK: control group without biochar; TB: biochar treatment group. Values represent mean ± standard error (n = 3). Asterisks (*) indicate significant differences between CK and TB at the same composting stage ($p < 0.05$).

Higher Chao1, species richness and Shannon indices in TB confirmed that biochar improved the species richness and community diversity of phoD-harboring microbiota (Fig. 2B–D). This could be explained by biochar's porous structure improving compost aeration and permeability, as well as its large specific surface area providing ecological niches and attachment sites for microbial colonization and proliferation[32,35,36].

PCoA analysis based on Bray–Curtis distance revealed obvious community differentiation between CK and TB (Fig. 2E). The first two principal coordinates explained 62.9% of total community variation. Samples from initial and warming stages clustered closely, while thermophilic, cooling and maturation stages formed discrete clusters, indicating that composting stage was the primary factor driving phoD-harboring bacterial community succession. Complete separation of CK and TB samples at the thermophilic stage further verified that biochar amendment profoundly altered the assembly pattern of phoD-harboring microbiota[26,37].

Alkaline phosphatase (ALP) activity in both treatments presented a trend of initial increase followed by decrease. CK exhibited higher ALP activity at the initial stage, while TB maintained significantly higher ALP activity from warming to maturation, especially at thermophilic, cooling and maturation stages ($p < 0.05$) (Fig. 2F). This verified that biochar addition persistently enhanced the functional activity of phoD-harboring bacterial communities during composting[26].

Interspecific interaction and network characteristics of phoD-harboring bacterial communities

Co-occurrence ecological networks were constructed based on the top 500 OTUs to characterize the interspecific interaction of phoD-harboring bacteria (Fig. 3A–B). Network complexity is positively correlated with node quantity and closeness centrality, and negatively correlated with betweenness centrality[38]. The network of CK contained 486 nodes and 5,553 edges, while TB network possessed 492 nodes and 5,379 edges. TB exhibited higher average closeness and lower average betweenness than CK, demonstrating that biochar optimized the topological complexity of phoD-harboring bacterial networks (Fig. 3C–D).

Network modularity exceeding 0.4 in both treatments indicated high modular structural differentiation[37,39]. Positive correlations accounted for 100% and 99.8% of total edges in CK and TB networks, respectively. The slight reduction of positive correlation proportion in TB implied intensified interspecific competition among phoD-harboring bacteria, which strengthened the intrinsic stability of the community network[40].

Network robustness and vulnerability reflect community resistance to environmental disturbance[41]. After random and targeted node removal, TB network showed slower species decay slope and significantly lower vulnerability than CK (Fig. 3H–J), indicating that biochar enhanced the anti-interference capability of phoD-harboring bacterial assemblages[42]. Additionally, TB had lower average clustering coefficient and average path length (Fig. 3F–G), implying tighter interspecies connection and faster material and information transmission within the network, along with higher sensitivity to ambient environmental fluctuation[43].

Isolation, identification and network functional role of phoD-harboring phosphate-solubilizing bacteria

Via Pikovskaya selective medium screening based on transparent solubilization halo characteristics, five pure phoD-harboring bacterial strains with non-labile P-solubilizing capacity were obtained, taxonomically affiliated to Escherichia, Pseudomonas, Brucella, Cellulosimicrobium and Bacillus. Among them, Pseudomonas and Bacillus are well-documented core phosphate-solubilizing taxa with multiple ecological functions including plant growth promotion and disease resistance enhancement[44,45]. The relative abundance of these five genera accounted for 8.7% and 12.3% of total phoD-harboring bacteria in CK and TB, respectively, with biochar amendment significantly increasing their abundance ($p < 0.05$). Correlation analysis revealed that the relative abundance of these PSB genera was significantly positively correlated with ALP activity ($r = 0.78$, $p < 0.01$) and negatively correlated with Ca_{10}-P content ($r = -0.72$, $p < 0.01$), suggesting their important role in inorganic P activation.

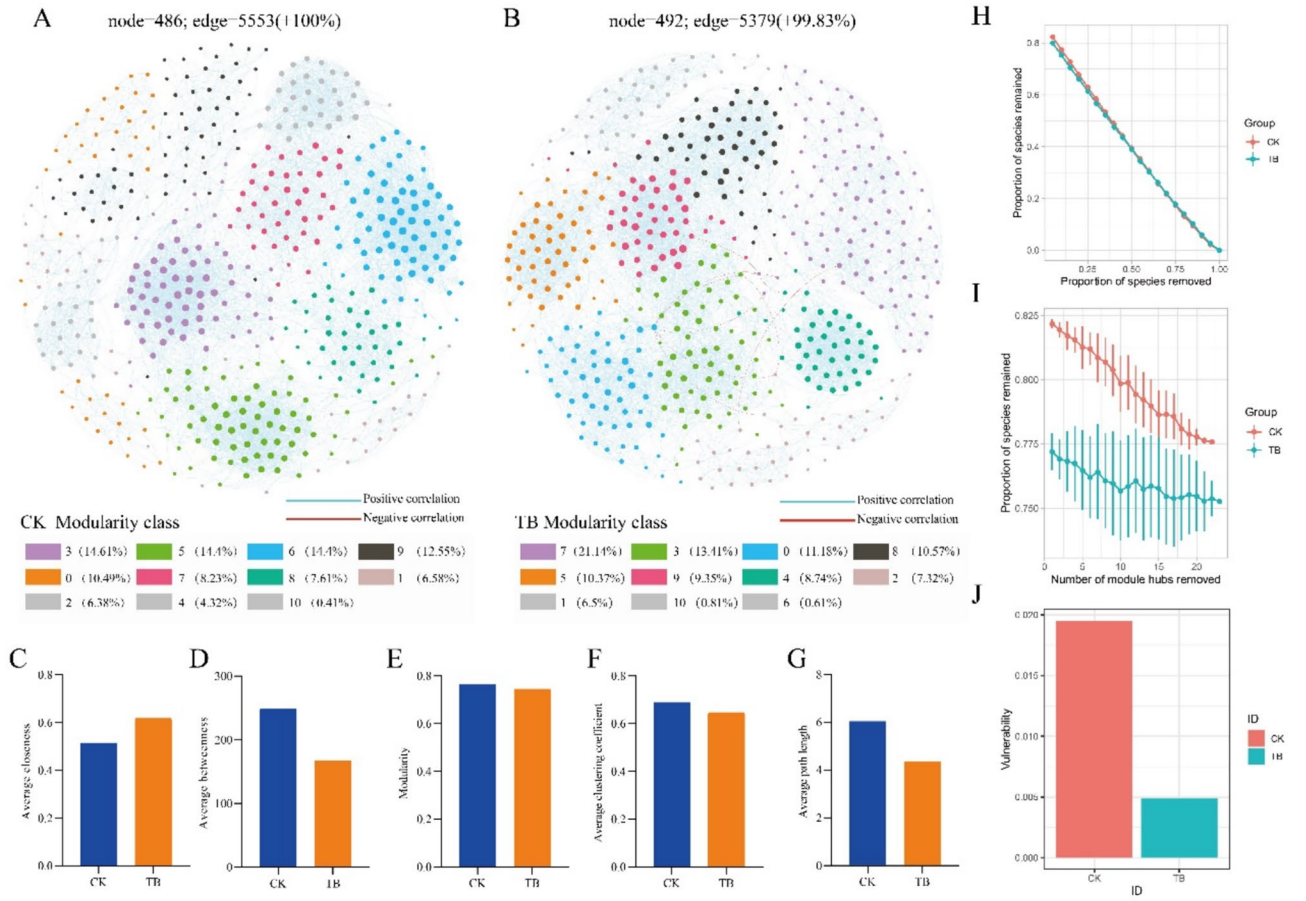

Fig. 3 Topological characteristics of phoD-harboring bacterial ecological networks based on Spearman correlation. (**A**) Network of CK group; (**B**) Network of TB group; (**C**) Average closeness centrality; (**D**) Average betweenness centrality; (**E**) Modularity; (**F**) Average clustering coefficient; (**G**) Average path length; (**H**) Random removal robustness; (**I**) Targeted removal robustness; (**J**) Network vulnerability. Nodes represent individual OTUs; node size indicates network degree; blue and red lines represent positive and negative correlations, respectively. CK: control group; TB: biochar treatment group.

After removing all OTUs affiliated to the five identified genera, network module composition and stability were compared. Only four similar modules remained between CK and TB after node deletion, accompanied by a sharp increase in unique modules (Fig. 4A–B). Network vulnerability of TB increased by 67.0% after node removal, while CK network vulnerability was less affected (Fig. 4C). These results confirmed that the screened phoD-harboring P-solubilizing bacteria were the key hub taxa maintaining network structure stability. Biochar addition reinforced the ecological function of these core taxa, thereby improving the resistance of phoD-harboring bacterial networks to environmental perturbation.

Correlation between environmental factors, phoD-harboring bacterial community and phosphorus fractions

Random forest regression analysis was employed to identify the key environmental factors driving phoD-harboring bacterial community variation. The results showed that EC (%IncMSE = 11.53), TOC (%IncMSE = 11.40), and C/N ratio (%IncMSE = 10.24) were the top three most important environmental factors, followed by pH (%IncMSE = 9.90), ALP activity (%IncMSE = 9.90), and NO_3^--N (%IncMSE = 8.61) (Fig. 5A). TN (%IncMSE = 4.09), AP (%IncMSE = 4.81), and NH_4^+-N (%IncMSE = 4.93) had relatively lower importance. These results indicated

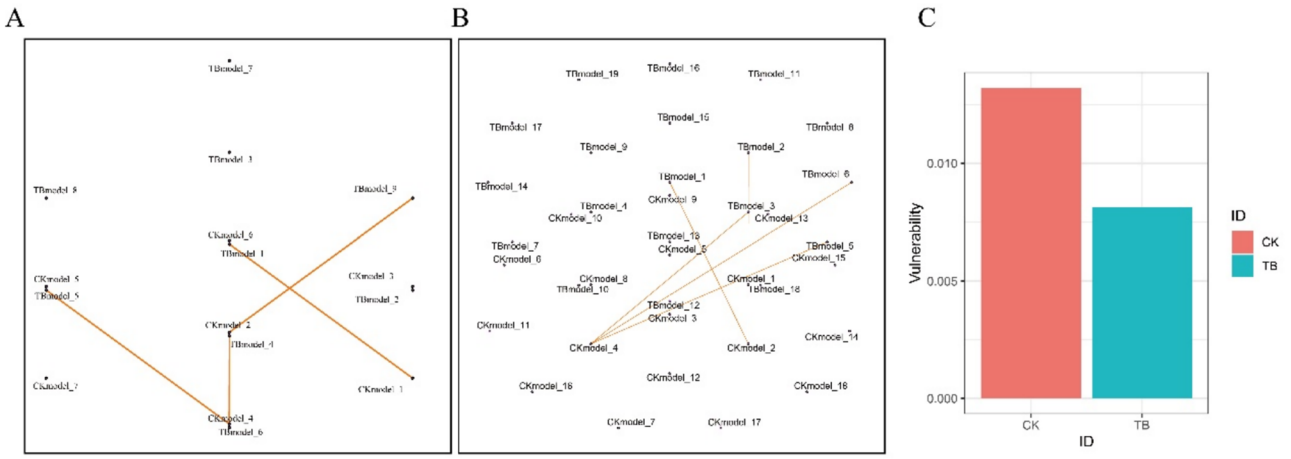

Fig. 4 Variations of ecological network modules and stability before and after core functional node deletion. (**A**) Module similarity comparison between CK and TB original networks; (**B**) Module distribution after core node removal; (**C**) Network vulnerability variation after node deletion. CK: control group; TB: biochar treatment group.

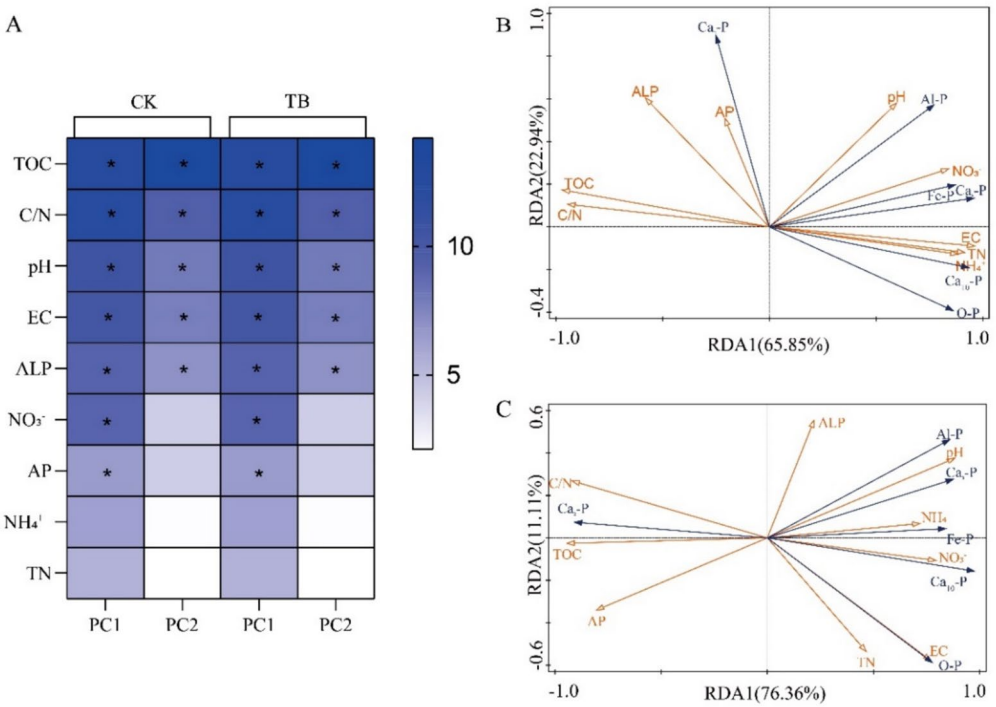

Fig. 5 Correlations among environmental factors, phoD-harboring bacterial community and P fractions. (**A**) Random forest analysis showing the relative importance of environmental factors (%IncMSE); (**B**) RDA ordination of environmental factors and P fractions in CK; (**C**) RDA ordination of environmental factors and P fractions in TB. TOC: total organic carbon; TN: total nitrogen; C/N: TOC/TN ratio; EC: electrical conductivity; ALP: alkaline phosphatase activity; AP: available phosphorus; RDA: redundancy analysis.

that compost nutrient status (TOC, C/N) and physicochemical properties (EC, pH) were the dominant abiotic drivers shaping phoD-harboring bacterial community structure.

Redundancy analysis (RDA) further confirmed that TOC, pH and ALP activity were the three core variables controlling P fraction transformation (Fig. 5B–C). Carbon and nitrogen provide energy and nutrient substrates for

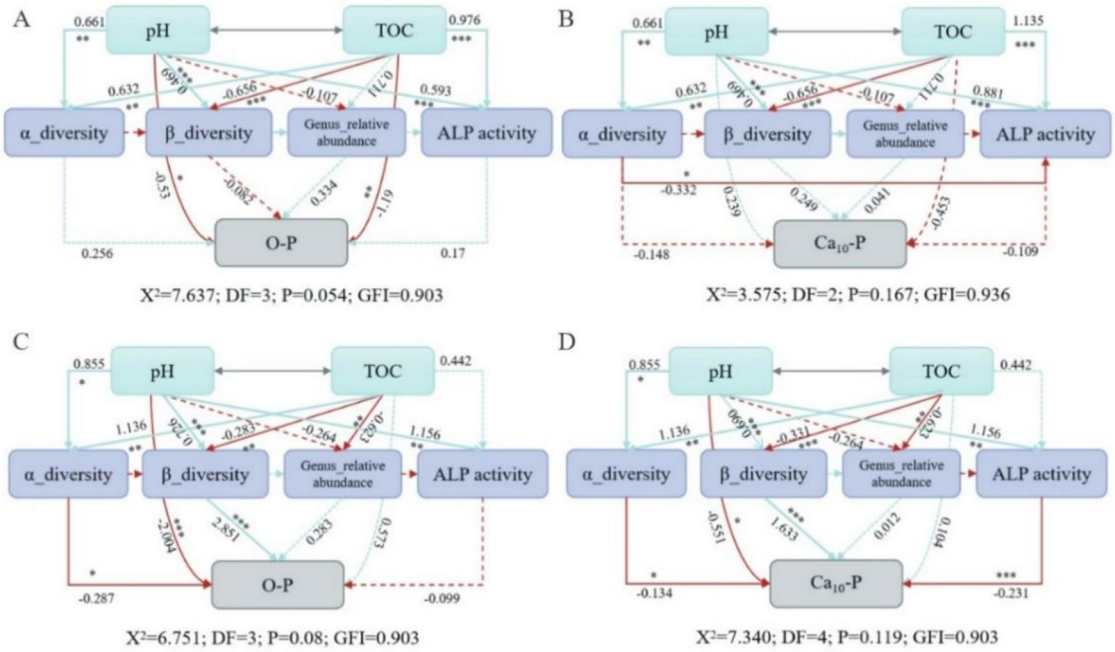

Fig. 6 Structural equation models (SEM) revealing the regulatory pathways of abiotic factors and phoD-harboring microbiota on non-labile P fractions. (**A**) O-P regulatory pathway in CK; (**B**) Ca_{10}-P regulatory pathway in CK; (**C**) O-P regulatory pathway in TB; (**D**) Ca_{10}-P regulatory pathway in TB. Solid lines indicate significant correlations, dashed lines indicate non-significant correlations; numbers on arrows represent standardized path coefficients. *p < 0.05, **p < 0.01, ***p < 0.001. Model fit statistics: CFI > 0.95, RMSEA < 0.08, SRMR < 0.05. CFI: comparative fit index; RMSEA: root mean square error of approximation; SRMR: standardized root mean square residual.

microbial growth; sufficient TOC and TN supply can enhance microbial biomass and community activity, further accelerating P fraction turnover[46].

In the CK group, TOC, pH and ALP activity contributed 65.4%, 16.6% and 3.5% to P fraction variation, respectively. TOC and ALP activity were positively correlated with Ca_2-P and negatively correlated with other P fractions, while pH showed positive correlation with all P fractions, with stronger correlation to moderately labile P (Al-P, Fe–P, Ca_8-P). In the TB group, the contribution rates of TOC, pH and ALP activity were 70.9%, 8.7% and 3.2%. TOC was strongly positively correlated with Ca_2-P; pH and ALP activity were positively correlated with moderately labile P; pH presented positive correlation with non-labile P, while ALP activity was negatively correlated with O-P and positively correlated with Ca_{10}-P.

Biotic and abiotic regulatory pathways of non-labile P dissolution

Structural equation modeling (SEM) was constructed to quantify the direct and indirect effects of abiotic factors (pH, TOC), phoD-harboring bacterial community diversity (α-diversity, β-diversity), genus composition and ALP activity on O-P and Ca_{10}-P transformation (Fig. 6A–D). The model fit indices indicated good model performance: comparative fit index (CFI) > 0.95, root mean square error of approximation (RMSEA) < 0.08, and standardized root mean square residual (SRMR) < 0.05 for all four models.

In both treatments, pH was significantly positively correlated with community α-diversity, β-diversity and ALP activity, and weakly negatively correlated with genus relative abundance. TOC was positively correlated with α-diversity and ALP activity, and significantly negatively correlated with β-diversity. The correlation between TOC and genus relative abundance differed between treatments, showing weak positive correlation in CK and highly significant negative correlation in TB.

In the CK system, O-P transformation was directly negatively regulated by pH and TOC; α-diversity, genus abundance and ALP activity exerted weak positive regulation, while β-diversity showed weak negative regulation. Ca_{10}-P in CK was weakly positively modulated by pH, β-diversity and genus abundance, and weakly negatively modulated by TOC, α-diversity and ALP activity. In the TB system, O-P was negatively regulated by pH and α-diversity and positively regulated by β-diversity; Ca_{10}-P was negatively regulated by pH, α-diversity and ALP activity and positively regulated by β-diversity. Meanwhile, pH and TOC could indirectly regulate non-labile P fractions via mediating bacterial community diversity and ALP activity. The standardized total effect of ALP activity on Ca_{10}-P dissolution in TB (−0.42) was substantially higher than in CK (−0.13), highlighting the enhanced microbial regulatory role under biochar amendment.

Collectively, non-labile P transformation in the control compost was predominantly controlled by abiotic factors (pH and TOC). By contrast, biochar addition enabled the co-regulation of pH, TOC and phoD-harboring bacterial community, wherein phoD-harboring microbiota and ALP activity became the core driving forces promoting the solubilization and activation of inorganic non-labile P.

Discussion

Biochar amendment reshapes phoD-harboring bacterial communities and enhances inorganic P solubilization

The present study demonstrated that biochar addition significantly enhanced the richness, diversity and metabolic activity of phoD-harboring bacterial communities during composting, which is consistent with previous findings[26]. The porous structure and large specific surface area of biochar provide favorable microhabitats for microbial colonization, protecting bacteria from environmental stress and facilitating intercellular communication (Lehmann et al., 2011). Moreover, biochar can serve as a slow-release nutrient source, supplying essential minerals that support microbial growth and metabolism. The increased ALP activity observed in TB treatment further confirmed that biochar stimulated the functional expression of phoD-harboring bacteria, which is crucial for organic P mineralization and subsequent inorganic P transformation.

Mechanisms underlying enhanced dissolution of inorganic non-labile P by phoD-harboring bacteria

A key finding of this study is that biochar-amended phoD-harboring bacterial communities promoted the dissolution of both O-P and Ca_{10}-P, which are considered recalcitrant inorganic P pools. While ALP primarily catalyzes the hydrolysis of organic P esters, our results suggest that phoD-harboring bacteria can indirectly facilitate inorganic P solubilization through multiple pathways. First, many phoD-harboring bacteria, particularly Pseudomonas and Bacillus species, are known to secrete low-molecular-weight organic acids such as gluconic acid, citric acid, and oxalic acid[23]. These organic acids can chelate $Ca2^{+}$, $Fe3^{+}$ and $Al3^{+}$ ions, lowering the ambient pH and destabilizing calcium phosphate minerals and oxide-encapsulated P. Second, the respiratory metabolism of phoD-harboring bacteria can create localized reducing microenvironments, which promote the reduction of $Fe3^{+}$ to $Fe2^{+}$ and the subsequent release of O-P bound to iron oxides. Third, the enhanced microbial biomass and activity may increase the mineralization of microbial biomass P upon cell death, releasing available P that was previously immobilized. These indirect mechanisms collectively contribute to the observed dissolution of inorganic non-labile P in biochar-amended compost.

Ecological significance of phoD-harboring bacterial co-occurrence networks

The co-occurrence network analysis revealed that biochar amendment optimized the topological structure of phoD-harboring bacterial networks, enhancing network complexity, connectivity and stability. From an ecological

perspective, a more complex and connected network indicates stronger interspecific interactions and greater functional redundancy, which can enhance community resilience to environmental disturbances[38]. The higher modularity in TB suggests that biochar promotes the formation of functional modules, where specialized bacterial groups collaborate to perform specific ecological functions such as P solubilization. The identification of five culturable PSB genera as key hub taxa further highlights the ecological importance of these functional groups in maintaining network integrity. The significant increase in network vulnerability after removing these hub taxa demonstrates their keystone role in the phoD-harboring bacterial community. Biochar appears to strengthen the ecological function of these core taxa, thereby improving the overall resistance and functional stability of the P-cycling microbial network.

Synergistic regulation of abiotic factors and biotic communities on P transformation

The SEM results provided important insights into the relative contributions of abiotic and biotic factors to non-labile P transformation. In the control compost, non-labile P dynamics were predominantly driven by abiotic factors (pH and TOC), reflecting the strong chemical control on P solubility. However, with biochar amendment, the regulatory pathway shifted toward a synergistic abiotic-biotic system, wherein phoD-harboring microbiota and ALP activity became the core driving forces promoting inorganic P activation. This shift can be attributed to biochar's dual role: (i) directly modifying compost pH and nutrient availability, and (ii) indirectly stimulating microbial P-cycling functions. The enhanced indirect effect of pH on P fractions via microbial pathways in TB underscores the importance of microbially mediated P transformation under biochar amendment. These findings are consistent with the emerging paradigm that biochar modulates soil P cycling primarily through microbial pathways rather than direct chemical effects[31].

Implications for agricultural P management and future perspectives

The findings of this study have important implications for sustainable P management in calcareous agricultural systems. By enhancing the dissolution of recalcitrant inorganic P pools, biochar-amended compost can potentially increase P use efficiency and reduce reliance on chemical P fertilizers. However, several limitations should be acknowledged. First, the composting experiment was conducted under controlled conditions; field-scale validation is needed to assess the practical applicability of these findings. Second, while we identified five culturable PSB genera, the majority of phoD-harboring bacteria remain uncultured, and their specific functional roles in P cycling warrant further investigation using metagenomic and metatranscriptomic approaches. Third, the long-term fate of activated P in soil–plant systems and its impact on crop P uptake remain unclear. Future research should integrate multi-omics techniques with field experiments to fully decipher the coupled abiotic-microbial mechanisms underlying biochar-mediated P activation and its agronomic effectiveness.

Conclusions

This study systematically investigated the regulatory effects of biochar amendment on inorganic P fraction transformation and phoD-harboring bacterial communities during rice stover and sheep manure co-composting. The main conclusions are as follows:

(1) Biochar addition effectively promoted the dissolution of inorganic non-labile P fractions, including occluded P (O-P) and calcium-bound stable P (Ca_{10}-P), while simultaneously increasing the content of moderately labile P pools (Ca_8-P, Al-P, Fe–P).

(2) Biochar amendment significantly enhanced the richness, diversity and metabolic activity of phoD-harboring bacterial communities, and optimized the topological structure of bacterial co-occurrence networks with improved complexity, connectivity and stability.
(3) Five culturable phosphate-solubilizing bacterial genera (Escherichia, Pseudomonas, Brucella, Cellulosimicrobium and Bacillus) were identified as key hub taxa maintaining network structure stability and driving inorganic P solubilization.
(4) Structural equation modeling revealed that non-labile P transformation in control compost was predominantly abiotic-driven (pH and TOC), whereas biochar amendment induced a synergistic abiotic-biotic regulatory system with phoD-harboring microbiota and ALP activity as core drivers of inorganic P activation.

These findings advance our mechanistic understanding of biochar-modulated P bioconversion and provide a theoretical basis for improving P recycling efficiency in calcareous agroecosystems through biochar-amended composting.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-026-70373-0.

Acknowledgements The authors thank the Key Laboratory of Low-carbon Green Agriculture in Northeastern China for providing the basic experimental platform.

Author contributions Fengzhen Fu: Methodology, Writing – original draft, Formal analysis. Liqin Zhao: Conceptualization & Data curation. Bowen Fan: Writing – review & editing, Methodology & Visualization. Ning Wang: Writing – editing, Supervision & Validation. Fengjun Yang: Project administration, Methodology & Funding acquisition.

Funding This work was supported by the Provincial-Academy Technology Cooperation Project in Heilongjiang (Grant No.YS20B16), the Doctoral Scientific Research Foundation of Heilongjiang Bayi Agricultural University (Grant No.XYB-2020-04) and the Heilongjiang Provincial Postdoctoral Research Initiation Grant(Grant No.2032030207). The funders did not and will not have a role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data availability All raw sequencing data generated in this study were deposited in the NCBI Sequence Read Archive (SRA) repository under accession number PRJNA1073698. Other analysed datasets are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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