

Phosphorus availability-leaching trade-offs under long-term organic substitution: Synergistic microbial activation and adsorption capacity decline

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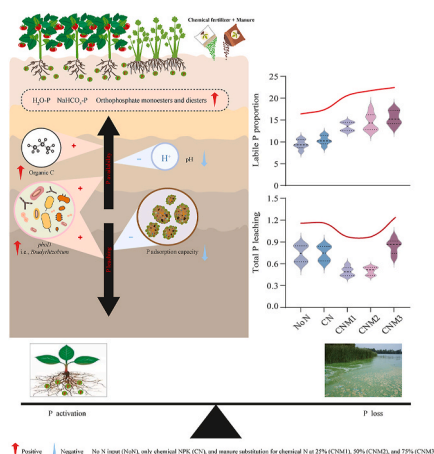
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HIGHLIGHTS

- Elevated manure substitution rates increased labile and moderately labile P content.
- Manure substitution enhanced labile P via organic C and *phoD* gene positive regulation.
- High amount manure substitution showed the lower microbial network connectivity.
- High manure substitute increased P loss by microbial activation and adsorption capacity decline.
- Indicating a ≤ 50 % substitution rate threshold is key for sustainable P management.

GRAPHICAL ABSTRACT



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ABSTRACT

Partial substitution of chemical fertilizers with manure enhances soil phosphorus (P) availability; yet, the trade-off between P supply and leaching risks under varying substitution rates remains unclear, particularly with regard to microbial-driven P transformation and soil P retention capacity. Here, we combined ³¹P NMR, sequential extraction, and microbiology techniques in a 15-years field experiment with an in-situ lysimeter to investigate P

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Sorption-desorption

³¹P-NMR

dynamics. Five treatments were applied: no N input (NoN), only chemical NPK (CN), and manure substitution for chemical N at 25 % (CNM1), 50 % (CNM2), and 75 % (CNM3), with equal NPK amounts. Compared to CN, manure substitution significantly increased the vegetable yield and P uptake, 15-year average yields of tomato and celery increased by 6.7 %–12.7 % and 10.5 %–15.4 %. Compared with NoN and CN, manure substitution increased the contents of soil labile and moderately labile P, orthophosphate monoesters and diesters, microbial biomass C and P, and alkaline phosphatase activity in both the tomato and celery seasons. CNM3 increased soil *phoD* and *Bradyrhizobium* abundance, sharply decreased P adsorption capacity, and increased total P leaching by 20.5 %–23.7 %, while CNM2 resulted in higher complexity and connectivity of *phoD*-harboring microbial networks and significantly decreased P leaching by 6.3 %–30 %. Manure substitution enhanced labile P by decreasing soil pH, promoting organic C accumulation, and upregulating the *phoD* gene. High manure substitution exacerbated P leaching predominantly by reducing P adsorption capacity and stimulating microbial mineralization of organic P. These findings provide new insights into understanding the roles of organic substitute strategies in soil P dynamics, and moderate amount substitution (≤ 50 %) enhances P availability while mitigating leaching, providing a critical strategy for sustainable P management.

1. Introduction

Phosphorus (P), as an essential mineral element, and its limitation is a widespread constraint on plant production (Vance et al., 2003; Liu et al., 2018). Applying phosphate fertilizer externally is crucial for agricultural productivity and ecosystem stability. Nevertheless, P applied to the soil is readily adsorbed and immobilized, making it challenging for crops to utilize (Ahmad et al., 2018; Hou et al., 2020). Consequently, the utilization efficiency of P fertilizers is low, at only about 20 % (Li et al., 2015). In order to maintain agricultural production, large amounts of P fertilizer are generally applied, causing P accumulation and increasing environmental risks (Fink et al., 2018; Lambers, 2022). Phosphate rock is a finite resource, with projections indicating potential depletion within the next 50–400 years (Cordell et al., 2009; Luo et al., 2024). Globally, the increase in food demand driven by population growth has resulted in the over-exploitation of phosphate resources, which not only depletes natural P reserves but also contributes to environmental issues (Cordell and White, 2014). There is an urgent need to explore sustainable P management practices to reduce P fertilizer use, minimize environmental risks, and enhance agricultural productivity (Zou et al., 2022; W.-P. Zhang et al., 2023). The partial substitution of chemical fertilizers with organic amendments reduces dependence on chemical fertilizers input while concurrently increasing organic matter content and beneficial microbial activity, fostering plant growth and sustaining soil health (Assefa and Tadesse, 2020; Dai et al., 2021; Yi et al., 2021). Recent studies have reported that partial substitution chemical fertilizers with organic materials, such as animal manure or crop straw, can improve soil P availability by releasing organic acids, boosting microbial activity, reducing P adsorption and fixation, and lowering the risk of P loss (Y. Zhang et al., 2023; Liu et al., 2024). However, is a higher substitution rate of organic matter always better? Research is still lacking on soil P transformation and P leaching risks associated with varying organic fertilizer substitution rates for chemical fertilizers. Elucidating these transformation mechanisms and associated environmental risks is fundamental to developing sustainable P management measures, especially for vegetable cultivation systems.

Organic fertilizer, particularly swine manure, has gained attention as a sustainable alternative to chemical fertilizers to enhance soil fertility and recycle agricultural waste (Yue et al., 2016; Komiyama and Ito, 2019; Sun et al., 2024). P derived from manure is predominantly present in organic forms and readily transformable inorganic forms. These forms undergo dynamic transformations in the soil, influenced by soil properties such as pH, mineralogy, organic carbon (C) content, and microorganisms (Huang et al., 2021; Sun et al., 2022). Numerous studies indicate that manure application improves P effectiveness by reducing P adsorption in soil (Yang et al., 2017), increasing phosphatase activity to hydrolyze organic P (Bi et al., 2020), and converting stable P to labile P (Zhang et al., 2021). Long-term manure application significantly modifies soil P biogeochemistry by altering turnover pathways and producing P species with varying bioavailability and environmental

mobility, processes that are also influenced by soil physicochemical and microbial properties. Consequently, the integrated application of chemical, microbiology, and spectroscopic techniques determines soil P speciation under long-term different fertilization and elucidates the effects of soil property factors on P transformation dynamics, offering a scientific basis for assessing both its bioavailability and environmental risks.

The role of microbial communities, especially *phoD*-harboring bacteria encoding alkaline phosphatase, is pivotal in regulating soil P cycling (Jiang et al., 2023). These microorganisms facilitate the breakdown of organic P and the dissolution of mineral-bound P, with these processes being significantly affected by organic amendments (Fraser et al., 2015; Xu et al., 2025). The application of manure enriches *phoD*-harboring populations by providing labile C and nutrients, thereby enhancing the mobilization of P through enzymatic activity (Chen et al., 2022). Therefore, replacing chemical fertilizers with manure may alter soil properties and environmental conditions, potentially influencing microbial functionality and P turnover. It is essential to conduct a systematic evaluation of *phoD*-harboring microbial communities and their relationship with P speciation under different levels of manure substitution to elucidate these complex interactions.

The dynamics of adsorption and desorption are crucial for regulating soil P retention and release, thereby impacting influencing crop nutrient availability and potential environmental losses (Nobile et al., 2018; Li et al., 2021). Research has shown that manure application can improve soil structure and increase P retention ability (Hua and Zhu, 2020), while existing research highlights that manure fertilization enhances P saturation, reducing its binding affinity and increasing the risk of soluble P leaching (Qin et al., 2020). Excessive manure application, especially when combined with intensive irrigation or rainfall, can saturate soil P sorption sites, destabilize P complexes originating from manure, and ultimately result in leaching. In developing sustainable agricultural strategies, it is crucial to evaluate the environmental consequences associated with the substitution of manure, particularly the increased risk of P leaching that is often associated with intensive water and fertilizer management in vegetable production. Most research primarily investigates the role of organic material application in facilitating P conversion and enhancing P availability by modulation of physical, chemical, and microbial properties. However, there is a notable lack of research examining the impact of varying substitution ratios of organic fertilizers on P speciation transformation, P availability, and leaching risk by synergistically regulating soil properties, adsorption and desorption characteristics, and P-mobilizing microorganisms. Furthermore, long-term experiments constitute an indispensable scientific basis for addressing sustainability challenges in agro-environmental fields by capturing impact across temporal scales.

Consequently, this study carried out a 15-year field trial of substituting chemical fertilizer by manure (same total N, P, and K input) at different gradient levels, aiming to i) elucidate the effects of long-term different amount manure replacement levels on the transformation

characteristics of P speciation in soil, ii) investigate the physicochemical and microbial processes involved in soil P dynamics, and iii) characterize P sorption-desorption properties and assess leaching risks. We posited the following hypotheses: (i) the substitution of chemical fertilizers with manure can enhance labile P and organic P contents, with greater substitution levels of manure resulting in more pronounced effects, and (ii) a higher degree of manure substitution also leads to increased P desorption and leaching. The study has the potential to elucidate the trade-offs between P availability and leaching within the context of an organic substitution strategy. Furthermore, it aims to precisely determine the optimal threshold for substitution rates, thereby offering a foundational basis for sustainable agricultural practices.

2. Materials and methods

2.1. Study design and sampling

The vegetable field was established in Tianjin, China (117°0'E, 39°13'N), with an autumn-winter season celery and spring-season tomato rotation in October 2009. Tomato were planted from March to June; fallowing or soil disinfection was performed from July to September (the soil is disinfected every two years); celery was planted from October to February of the following year. This study included five treatments with equal total amounts of N, P, and K applied: no N application (NoN), chemical N, P, and K (CN), and 25 % (CNM1), 50 % (CNM2), and 75 % (CNM3) of the chemical N replaced by manure N. All treatments were laid out in a randomized block design with three replicates. Each plot was 14.4 m² (2.4 m × 6.0 m) and segregated by PVC plates (105 cm in depth, with 100 cm underground and 5 cm above-ground) to avoid transversal migration of nutrients and water between plots. The specific rates of the fertilizers are displayed in Table 1. The chemical fertilizers included urea (46 % N), calcium superphosphate (12 % P₂O₅), diammonium phosphate (18 % N, 46 % P₂O₅), and potassium chloride (60 % K₂O). The organic fertilizer was commercial pig manure, which had the following properties: organic C content of 218 g kg⁻¹, total N content of 21.7 g kg⁻¹, total P content of 6.06 g kg⁻¹, and total K content of 13.5 g kg⁻¹. The soil in field was categorized as fluvo-aquic. Before trial, the soil (0–20 cm) properties were: bulk density 1.38 g cm⁻³, pH (H₂O) 7.9, organic carbon 15.3 g kg⁻¹, NO₃⁻-N 186 mg kg⁻¹, Olsen-P 145 mg kg⁻¹, and available potassium 404 mg kg⁻¹. The pan lysimeter was placed in each plot to gather leachate, the detailed layout

and description is shown in Fig. S1. The tomato planting density was 25000 plants ha⁻¹, and its row and plant spacing was 0.3 m × 0.6 m. The celery planting density was 330570 plants ha⁻¹, and its row and plant spacing was 0.15 m × 0.2 m. In the experiment, each plot was installed with a water meter to guarantee the accuracy of irrigation amounts.

Soil sample from the 0–20 cm depth was taken subsequent to the harvest of tomatoes and celery in June 2023 and February 2024, respectively. A portion of the sieved fresh samples was utilized to evaluate microbial biomass and extracellular enzymes activity. Another portion was extracted for DNA analysis and stored at –80 °C, while the third portion was air-dried for the assessment of P speciation and adsorption and desorption characteristics. Following soil sampling, the entire leachate was collected to quantify its volume and P concentration.

2.2. Sample analysis

The chemical P fractions were determined via the modified Hedley method (Hedley et al., 1982; Tiessen and Moir, 1993; Sui et al., 1999). Soil samples underwent sequential extraction using deionized water (H₂O-P), followed by 0.5 M NaHCO₃ (NaHCO₃-P), 0.1 M NaOH (NaOH-P), and 1 M HCl (HCl-P). The remaining P content was measured after digestion with H₂SO₄ and H₂O₂ (Fig. S2). Chloroform fumigation extraction was performed to measure the microbial biomass C (MBC) and P (MBP) (Brookes et al., 1982). Alkaline phosphatase (ALP, EC 3.1.3.1) activity was determined using soil-ALP kit (No. G0304F) (Zhang et al., 2022).

Soil samples (2 mm) were extracted with NaOH-Na₂EDTA solution and were measured using ICP-OES (McDowell et al., 2006). The extracts were freeze-dried to a fine powder, redissolved in NaOD and D₂O, and then transferred to a 5 mm NMR tube (Liu et al., 2019a; Zhang et al., 2021). NMR spectra were obtained on a Bruker Avance III 700 M spectrometer with a 14.5 μs pulse width, a 30 flip angle, 0.288 s acquisition time, 2 s pulse delay, at 25 °C, over 30,000 scans (about 20–24 h). Standards were added to the soil (in the CN treatment), including phytic acid, α- and β-glycerophosphate disodium salt hydrate, and phosphocholine chloride sodium salt, which were purchased from Sigma company (Xin et al., 2019). MestReNova version 9.0.1 was employed to calibrate the NMR spectra, with the orthophosphate peak adjusted to 6 ppm, and to determine the relative proportions and concentrations of each compound based on the integral areas. The orthophosphate monoesters and diesters were recalibrated by categorizing α-

Table 1
Annual carbon (C), nitrogen (N), phosphorus (P), and potassium (kg ha⁻¹) input in different fertilization treatment.

	C input				N input				P input				K input			
	PM	CF	PM	Total	CF	PM	Total		CF	PM	Total		CF	PM	Total	
Spring tomato seasonrowhead																
NoN	0	0	0	0	98	0	98	498	0	498			498	0	498	
CN	0	450	0	450	98	0	98	498	0	498			498	0	498	
CNM1	1130	337.5	112.5	450	66.6	31.4	98	427.9	70.1	498			427.9	70.1	498	
CNM2	2260	225	225	450	35.1	62.9	98	357.7	140.3	498			357.7	140.3	498	
CNM3	3391	112.5	337.5	450	3.7	94.3	98	287.6	210.4	498			287.6	210.4	498	
Autumn-winter celery seasonrowhead																
NoN	0	0	0	0	98	0	131	498	0	498			498	0	498	
CN	0	450	0	450	131	0	131	498	0	498			498	0	498	
CNM1	1130	337.5	112.5	450	99.6	31.4	131	427.9	70.1	498			427.9	70.1	498	
CNM2	2260	225	225	450	68.1	62.9	131	357.7	140.3	498			357.7	140.3	498	
CNM3	3391	112.5	337.5	450	36.7	94.3	131	287.6	210.4	498			287.6	210.4	498	

CF, chemical fertilizer; PM, pig manure; NoN, only chemical P and K; CN, chemical N, P, and K; 25 % (CNM1), 50 % (CNM2), and 75 % (CNM3) of the chemical N replaced by manure N.

and β -glycerophosphate as diesters, given their recognition as degradation products of diesters (Cade-Menun, 2015; Liu et al., 2019a).

The P sorption isotherms were determined as previously described (Yan et al., 2018). 1.00 g of air-dried soil sample was placed in a 50 mL centrifuge tube and 25 mL of KH_2PO_4 solution with varying P concentrations (1, 2, 5, 10, 20, 50, 100 mg L^{-1}) in 0.01 M CaCl_2 (pH 7) was added. The mixture was shaken, centrifuged, and measured to determine the equilibrium P concentration in the solution. Following the adsorption experiment, the P desorption experiment was initiated immediately. 20 mL of saturated NaCl solution were added to the centrifuge tube containing the soil sample to remove free P. Next, 25 mL of 0.01 M CaCl_2 solution was added, shaken, centrifuged, and measured to determine the P desorption. Langmuir and isotherms were used to calculate various P sorption parameters (Jalali and Jalali, 2016).

$$\frac{C}{Q} = \frac{1}{KQ_{\text{max}}} + \frac{C}{Q_{\text{max}}} \quad (1)$$

where C represents the equilibrium solution's P concentration (mg L^{-1}); Q denotes the soil's P adsorption capacity (mg kg^{-1}); Q_{max} is the maximum P adsorption capacity; and K is the adsorption affinity constant.

Based on the parameters derived from the Langmuir isothermal adsorption equation, the following indices were calculated: soil maximum buffering capacity (MBC, mg kg^{-1}) was calculated by $\text{MBC} = K \times Q_{\text{max}}$; P adsorption saturation (DPS, %) was calculated by $\text{DPS} = \text{Olsen-P}/Q_{\text{max}} \times 100 \%$.

Using the parameters of the Langmuir isothermal adsorption equation, the following indices were determined: the soil's maximum buffering capacity (MBC, mg kg^{-1}) as $\text{MBC} = K \times Q_{\text{max}}$, and the P adsorption saturation (DPS, %) as $\text{DPS} = \text{Olsen-P}/Q_{\text{max}} \times 100 \%$.

2.3. DNA extraction, sequencing, and gene quantification

DNA was extracted from 0.50 g soil samples using a FastDNA Spin kit. *phoD* amplicon sequencing was performed using the ALPS-F730/ALPS-1101 primer set (5'-CAGTGGGACGACCAGAGGT-3'/5'-GAGGCCGATCGGCATGTCG-3') to identify bacterial community encoding the *phoD* gene (Sakurai et al., 2008). The PCR mixture contained 5 μL of 2X ChamQ SYBR Color qPCR Master Mix, 0.4 μL each of forward and reverse primers (5 μM), 0.2 μL of 50X ROX Reference Dye 1, 1.0 μL of DNA template, and sterile water, totaling 10 μL . The PCR cycling conditions were: 95 $^{\circ}\text{C}$ for 3 min, followed by 35 cycles of 95 $^{\circ}\text{C}$ for 5 s, 58 $^{\circ}\text{C}$ for 30 s, and 72 $^{\circ}\text{C}$ for 1 min. After preparation, the 96-well plate was placed in the ABI 7300 Real-Time PCR System for amplification. A standard curve was generated based on the copy numbers and Ct values obtained from gradient amplification, with an R^2 of 0.9995 and 0.9989 and amplification efficiency of 97.6 % and 96.1 %, respectively. PCR products were confirmed by 2 % agarose gel electrophoresis, and target fragments were purified with a DNA gel extraction kit. A library was constructed using a commercial kit and sequenced on the Illumina HiSeq platform. The raw sequence data have been submitted to the Sequence Read Archive (SRA) database (PRJNA1214787).

Raw sequences were quality screened and trimmed, including steps for quality trimming, detection of singletons, and identification of chimeras. Sequences with 75 % similarity were grouped into OTUs using USEARCH v7.1 (Edgar, 2013; Tan et al., 2013; Wei et al., 2019). Tan et al. (2013) determined a 75 % similarity threshold, supported by correlation analysis between *phoD* and 16S rRNA genes, suggesting it can reliably estimate *phoD*-derived species-level OTUs (Fraser et al., 2015; Chen et al., 2019). Each OTU's representative sequence was categorized using BLAST and compared to the FunGene database (Fish et al., 2013).

2.4. Leachate analysis

The P fractions were measured as follows: dissolved inorganic phosphorus (DIP) was quantified in samples filtered through a 0.45 μm membrane. Total dissolved P (TDP) and total P (TP) were measured using filtered and unfiltered post-persulfate digestion. Dissolved organic P (DOP) was determined by subtracting DIP from TDP, and particulate P (PP) by subtracting TDP from TP (McDowell et al., 2021). The P leaching loss was calculated by multiplying the leachate volume by the P concentration.

2.5. Statistical analysis

A one-way ANOVA was performed using IBM SPSS Statistics to evaluate differences. Principal coordinate analysis (PCoA) was conducted using the "vegan" package in R (v.4.1.0) to assess the distribution of microbial communities. Permutational multivariate analysis of variance (PERMANOVA) was employed to assess the impacts of fertilization and season on microbial community. The co-occurrence network was constructed using Spearman correlations among OTUs (relative abundance $>0.05 \%$, $|r| > 0.6$ and $P < 0.01$) by the "psych" package. Gephi was used to visualize the network and compute topological parameters. The correlation analysis and random forest (RF) analysis was conducted using the "psych" and "randomForest" packages to evaluate the relationships among microbial taxonomy and P fractions. The "pls-pm" package, employing partial least squares path modeling (PLS-PM), was utilized to evaluate the impact of manure fertilization on the P dynamics in relation to soil properties.

3. Results

3.1. Vegetable yields, P uptake, and P leaching loss

Compared with the NoN, N application (CN, CNM1, CNM2, and CNM3) increased the 15-year annual yields of tomato from 2010 to 2023, with the average yield increasing by 10.6 %–24.6 %, and of celery from 2009 to 2023, with average yield increasing by 7.8 %–24.4 % (Fig. 1a and b). In the first three years, there was not much difference between the yields of CN and those of manure application (CNM1, CNM2, and CNM3). Three years later, compare to the CN, manure application increased the annual yields of tomato and celery from 2012 to 2023. Compare to the CN, manure application increased the 15-years average yields of tomato and celery by 6.7 %–12.7 % and 10.5 %–15.4 %, respectively. The P uptake amounts among the various treatments did not differ much in the first year (Fig. 1c and d). Compared with the NoN, N application increased annual P uptake of celery from 2010 to 2023 and tomato from 2011 to 2023. Compare to the CN, manure application increased the annual yields of tomato and celery from 2013 to 2023. Manure application increased 15-years average P uptake amount of tomato and celery by 7.2 %–24.6 % and 10.5 %–25.5 %, respectively.

The volume of leachate in CNM2 and CNM3 was significantly lower than that in other treatments during the tomato season, while the volume in CNM2 was significantly lower than that in the celery season (Fig. 1e and f). Compared to the NoN and CN, CNM1 and CNM2 significantly reduced the amount of TP leaching by 27.0 %–31.6 %, while CNM3 significantly increased the amount of TP leaching (20.5 %–23.7 %) by elevating DIP and DOP leaching during tomato season. There were no significant differences in TP leaching amount among the NoN, CN, and CNM3 treatments. Compared to the aforementioned treatments, CNM1 and CNM2 treatments significantly reduced P leaching by 34.4 %–39.3 % during the celery season. Compared with NoN and CN, CNM3 significantly increased the concentration of TP, DOP, and DIP, while CNM1 and CNM2 significantly decreased the DIP concentration in leachate during both the tomato and celery seasons (Fig. S4).

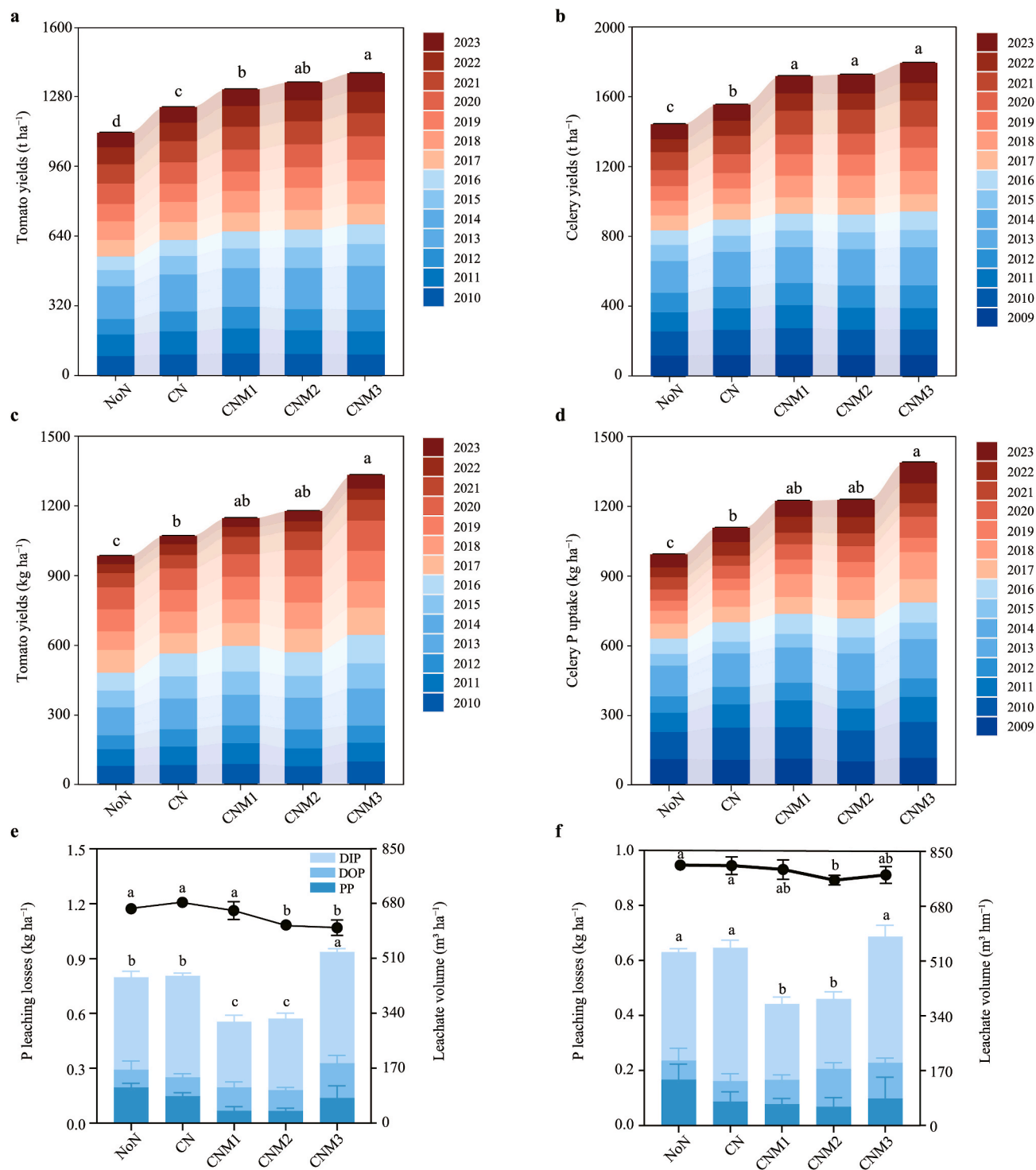


Fig. 1. Tomato (a and c) and celery (b and d) annual yields (t ha^{-1}) and P uptake amount (kg ha^{-1}) from 2009 to 2023. The volume of leachate, and the amount of P leaching in tomato (e) and celery (f) seasons. DIP, dissolved inorganic P; DOP, dissolved organic P; PP, particulate P. NoN, only chemical P and K; CN, chemical N, P, and K; 25 % (CNM1), 50 % (CNM2), and 75 % (CNM3) of the chemical N replaced by manure N. Different lowercase letters indicate significant differences among fertilization treatments ($P < 0.05$).

3.2. Chemical P fractions

In both tomato and celery seasons, higher manure substitution rates led to an increase in labile and moderately labile P proportions, while the proportion of stable P decreased (Table 2). Compare with the NoN and CN, CNM2 and CNM3 significantly enhanced the contents of $\text{H}_2\text{O-P}$ and $\text{NaHCO}_3\text{-Pi}$, manure application significantly improved the contents

of $\text{NaHCO}_3\text{-Po}$, CNM3 showed the highest content in both tomato and celery seasons. CNM2 and CNM3 significantly increased the content of NaOH-Pi in tomato season, and CNM2 significantly increased the content of NaOH-Pi in the celery season when compared to the NoN and CN. There was no significant difference in NaOH-Po among CN and manure application treatments. Compared to NoN and CN, manure application significantly reduced HCl-P and Residual-P contents (except for CNM3

Table 2
Soil phosphorus contents (mg kg⁻¹) from the chemical sequential fractionation.

Season	Treatment	H ₂ O-P	NaHCO ₃ -Pi	NaHCO ₃ -Po	NaOH-Pi	NaOH-Po	HCl-P	Residual-P	Labile P	Moderately labile P	Stable P
		(g kg ⁻¹)							(%)		
Tomato	NoN	43.2 b	160 d	91 b	89.1 c	278 b	936 b	1122 a	10.2 c	12.7 c	71.5 a
	CN	51.0 b	178 c	103 b	96.9 c	303 ab	1061 a	1008 b	11.1 c	13.4 c	69.4 b
	CNM1	51.7 b	191 bc	156 a	101.6 c	316 a	875 c	809 d	14.5 b	15.2 b	63.4 c
	CNM2	68.2 a	207 ab	167 a	126.4 b	321 a	783 c	821 d	16.3 a	16.6 a	59.4 d
	CNM3	73.6 a	218 a	182 a	149.7 a	329 a	749 c	888 c	16.9 a	17.1 a	58.3 d
Celery	NoN	37.2 c	145 c	75.6 c	189 b	87.8 d	1136 b	1147 a	8.69 c	9.35 d	77.1 a
	CN	43.0 bc	163 b	87.9 c	197 ab	113 cd	1261 a	1033 b	9.61 c	10.1 c	74.9 b
	CNM1	45.7 b	176 ab	141 b	192 b	136 bc	1042 c	959 c	12.6 b	11.4 b	69.9 c
	CNM2	54.2 a	182 a	142 b	216 a	151 ab	1033 cd	971 c	12.9 a	12.5 a	68.4 c
	CNM3	53.6 a	183 a	187 a	210 ab	179 a	966 d	1038 b	14.1 a	13.0 a	66.8 d

NoN, only chemical P and K; CN, chemical N, P, and K; 25 % (CNM1), 50 % (CNM2), and 75 % (CNM3) of the chemical N replaced by manure N. Different letters indicate significant ($P < 0.05$) differences among treatments.

in celery season).

3.3. P species by liquid-state ³¹P-NMR analysis

The ³¹P NMR spectra of solutions derived from soil samples collected during the tomato growing season are presented in Fig. S3. The peaks observed in the NMR spectrum were attributed to both organic P compounds (including orthophosphate monoesters, diesters, and phosphonates) and inorganic P (including orthophosphate, pyrophosphate, and polyphosphate). Peaks within the range of 2.5 and 7.5 ppm were amplified across all treatment spectra (Fig. 2) and identified as phytate (inositol hexakisphosphate, IHP), α- and β-glycerophosphate, and

choline phosphate, all of which fall within the orthophosphate monoesters category.

P extraction efficiency using the NaOH-EDTA method demonstrated a recovery range of 29.0 %–41.3 % relative to total soil P content under different treatments (Table 3). Compare to NoN and CN, manure application significantly increased the proportions and contents of NaOH-EDTA extracted P, with CNM3 showing the highest values. The dominant P species was inorganic P, accounting for 67.0 %–74.5 % of the total extracted P. Compare to the NoN and CN, manure application significantly decreased the proportions of inorganic P, while CNM2 and CNM3 significantly increased inorganic P content. Compare to the NoN and CN, manure application significantly enhanced the proportions and

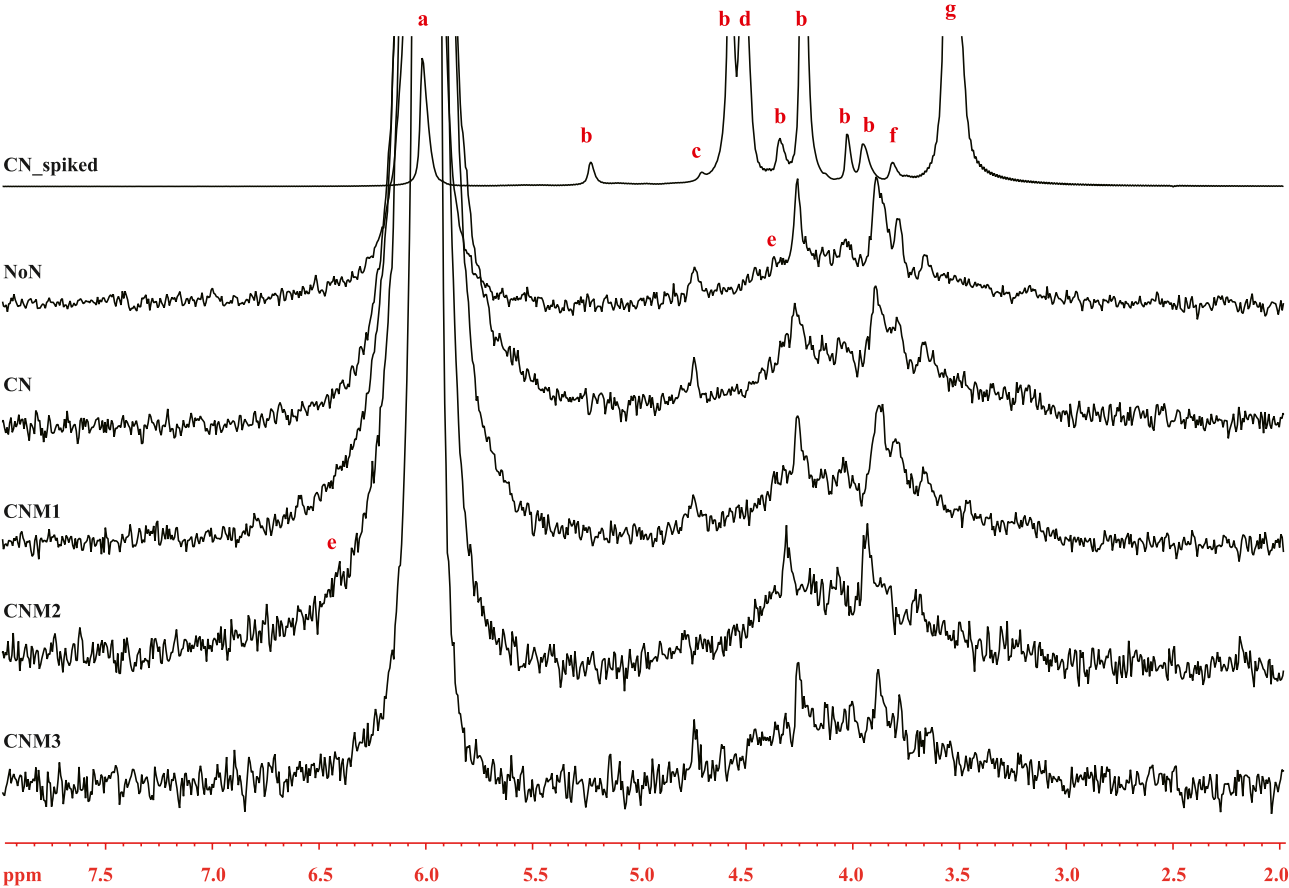


Fig. 2. Solution ³¹P nuclear magnetic resonance (NMR) spectra collected from the five treatments in tomato season soil sample and spiking experiments showing in the orthophosphate monoester region (7.0–3.0 ppm). NoN, only chemical P and K; CN, chemical N, P, and K; 25 % (CNM1), 50 % (CNM2), and 75 % (CNM3) of the chemical N replaced by manure N. a, orthophosphate, b, myo-Inositol hexakisphosphate, c, α-glycerophosphate, d, β-glycerophosphate, e, D-chiro-Inositol hexakisphosphate; f, choline phosphate, and g, scyllo-Inositol hexakisphosphate.

Table 3
Phosphorus form distributions and content (mg kg⁻¹) in soil determined by ³¹P-NMR spectra.

Treatment	NaOH-EDTA-P ^a	Inorganic P	Organic P	Monoester	Total IHP	Diester	Monoester (C) ^c	Diester (C)	M/D ratio	M/D ratio (C)
Proportion (%)										
NoN ^b	29.0 d	74.5 a	25.5 b	15.3 b	8.63 b	4.03 b	11.8 c	7.53 c	3.81 a	1.57 b
CN	29.7 d	73.3 a	26.7 b	18.2 b	11.2 b	4.06 b	14.7 c	7.56 c	4.51 a	1.94 a
CNM1	33.1 c	67.0 b	32.6 a	24.7 a	13.7 a	5.56 a	21.2 a	9.06 bc	4.59 a	2.37 a
CNM2	37.0 b	69.4 b	30.6 a	22.7 a	12.4 a	5.03 a	18.2 a	9.53 b	4.54 a	1.93 a
CNM3	41.3 a	69.8 b	30.2 a	20.8 a	11.2 b	6.03 a	15.7 b	11.2 a	3.49 a	1.41 a
Content (mg kg ⁻¹)										
NoN	835 d	622 c	213 b	128 b	33.7 c	33.7 c	98.6 b	62.9 d	-	-
CN	885 c	649 c	236 b	161 b	36.0 c	36.0 c	130 b	66.9 cd	-	-
CNM1	911 c	611 c	297 a	225 a	50.5 b	50.5 b	193 a	82.4 bc	-	-
CNM2	999 b	693 b	306 a	227 a	50.2 b	50.2 b	182 a	95.4 b	-	-
CNM3	1159 a	809 a	350 a	242 a	69.9 a	69.9 a	182 a	130 a	-	-

a: the proportion was the ratio NaOH-EDTA-P and total P (the total sum of P fractions contents); b: NoN, only chemical P and K; CN, chemical N, P, and K; 25 % (CNM1), 50 % (CNM2), and 75 % (CNM3) of the chemical N replaced by manure N. c: C denotes a correction for diester degradation products. Different letters indicate significant ($P < 0.05$) differences among treatments.

contents of organic P, including monoesters, monoester (C), and diesters. Compare to the NoN and CN, CNM1 and CNM2 significantly increased the proportions of IHP, and manure application significantly increased the content of IHP; CNM2 and CNM3 significantly improved the proportions and content of corrected diester (C). Compared to the NoN, N application treatments significantly increased the ratio of monoester (C) to diester (C).

3.4. Microbial biomass and ALP activity

Fertilization significantly altered the soil microbial biomass across both tomato and celery seasons (Fig. 3). Compared with the NoN, N application significantly increased the content of MBC. Compared to CN, manure application significantly enhanced MBC content, the CNM3 showed the highest value. In comparison to the NoN and CN, manure application significantly enhanced the content of MBP. MBC and MBP demonstrated an upward trend with the escalating application rate of

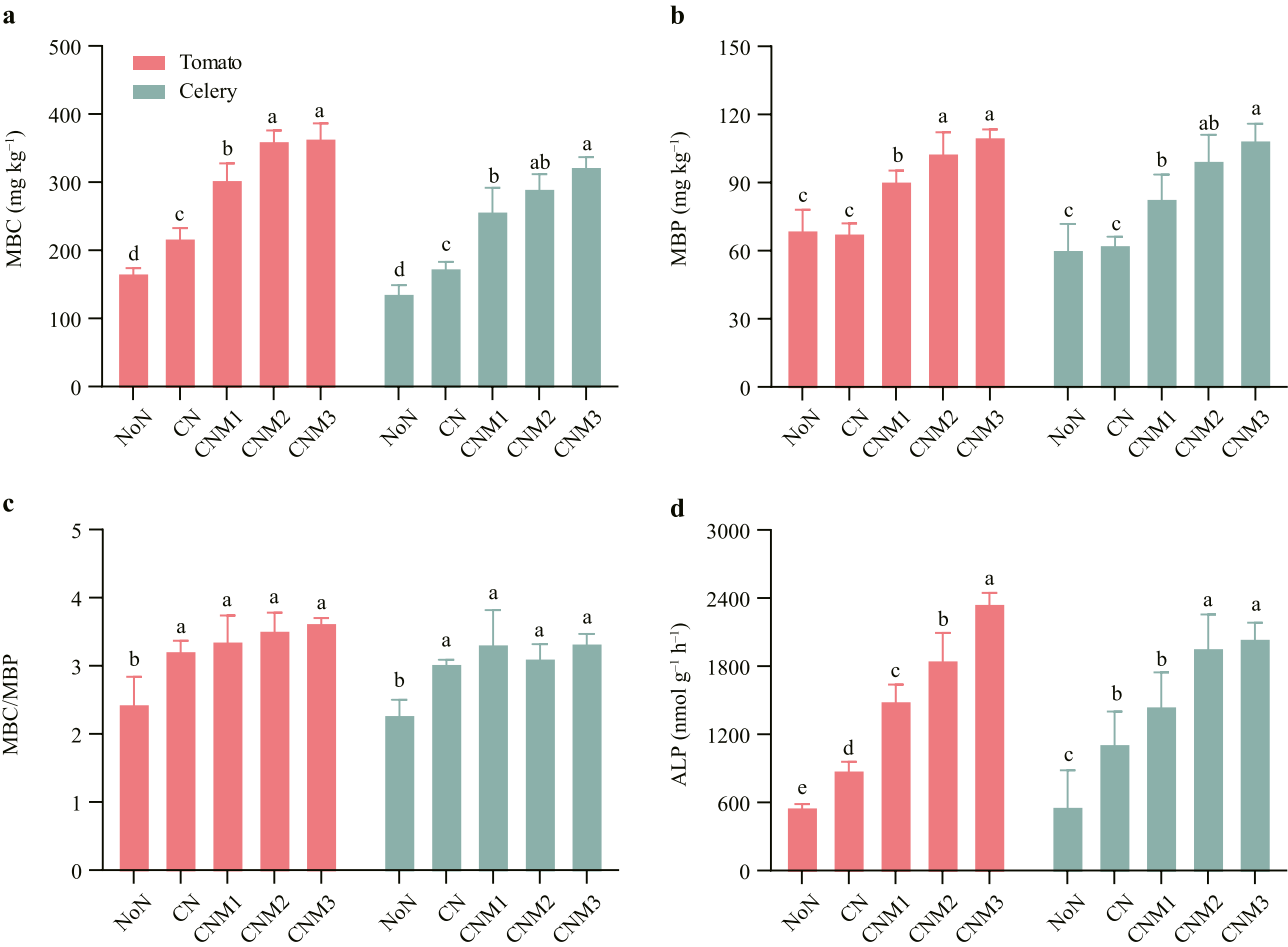


Fig. 3. Microbial biomass carbon (MBC) (a) and phosphorus (MBP) (b), the ratio of MBC/MBP (c) and the activity of alkaline phosphatase (ALP) (d) under different treatment in two season. NoN, only chemical P and K; CN, chemical N, P, and K; 25 % (CNM1), 50 % (CNM2), and 75 % (CNM3) of the chemical N replaced by manure N. Different lowercase letters indicate significant differences among fertilization treatments ($P < 0.05$).

manure. In comparison to the NoN, N application significantly elevated MBC/MBP ratio; there was no significant differences were observed among the other treatments during both tomato and celery seasons. Compared to the NoN, N application increased the activity of ALP. In comparison to the CN, manure application significantly enhanced ALP activity during tomato season, while CNM3 showed the highest ALP activity.

3.5. *phoD* abundance and harboring bacterial community structure

Compared to the NoN and CN, CNM2 and CNM3 significantly increased *phoD* gene abundance by 21.4 %–66.9 % and 70.0 %–148 %, respectively, with CNM3 showing the highest *phoD* gene abundance in both tomato and celery seasons (Fig. 4a). The PCoA results indicated a clear separation of the *phoD*-harboring microbial communities in tomato from celery season, and a clear separation in the NoN and CN from those in the CNM1, CNM2 and CNM3 (Fig. 4b). PERMANOVA analysis indicated that 72.8 % of the variation observed in the *phoD*-harboring microbial community could be attributed to season (19.0 %) and fertilization patterns (50.4 %).

In the tomato season, the most dominant genera were *Bradyrhizobium* (14.5 %–46.7 %), *Mesorhizobium* (17.6 %–27.2 %), *Rhizobium* (16.1 %–26.7 %), *Rhodoplanes* (2.6 %–16.4 %), and *Rhizobacter* (3.3 %–7.3 %) (Fig. 4c). Compared with the NoN and CN, manure application enhanced the relative abundance of *Bradyrhizobium* (21.5 %–121 %) and *Mesorhizobium* (3.02 %–54.7 %), while it reduced the relative abundance of *Rhizobium* and *Rhodoplanes*. In celery season, the most dominant genera belonged to *Bradyrhizobium* (18.0 %–42.4 %), *Rhizobium* (24.8 %–40.1 %), *Mesorhizobium* (19.6 %–26.5 %), and *Pseudomonas* (0.7 %–14.6 %) (Fig. 4d). Compared to the CN, manure application enhanced the relative abundance of *Bradyrhizobium* by 33.6 %–135.6 %, while it reduced the relative abundance of *Rhizobium* and *Mesorhizobium*. Spearman correlation showed that labile P (H_2O -P and $NaHCO_3$ -P) had significantly positive relationship with *Bradyrhizobium* and *Mesorhizobium*, and a negative relationship with *Rhizobium* (Fig. 4e). RF modeling revealed that soil P fractions were pivotal in explaining the observed variations among specific *phoD*-harboring bacterial taxa. The $NaHCO_3$ -Pi (43.7 %), $NaHCO_3$ -Po (28.4 %), $NaOH$ -Pi (82.7 %), and $NaOH$ -Po (87.4 %) was affected by *phoD*-harboring bacterial taxa.

Microbial networks were observed among the communities

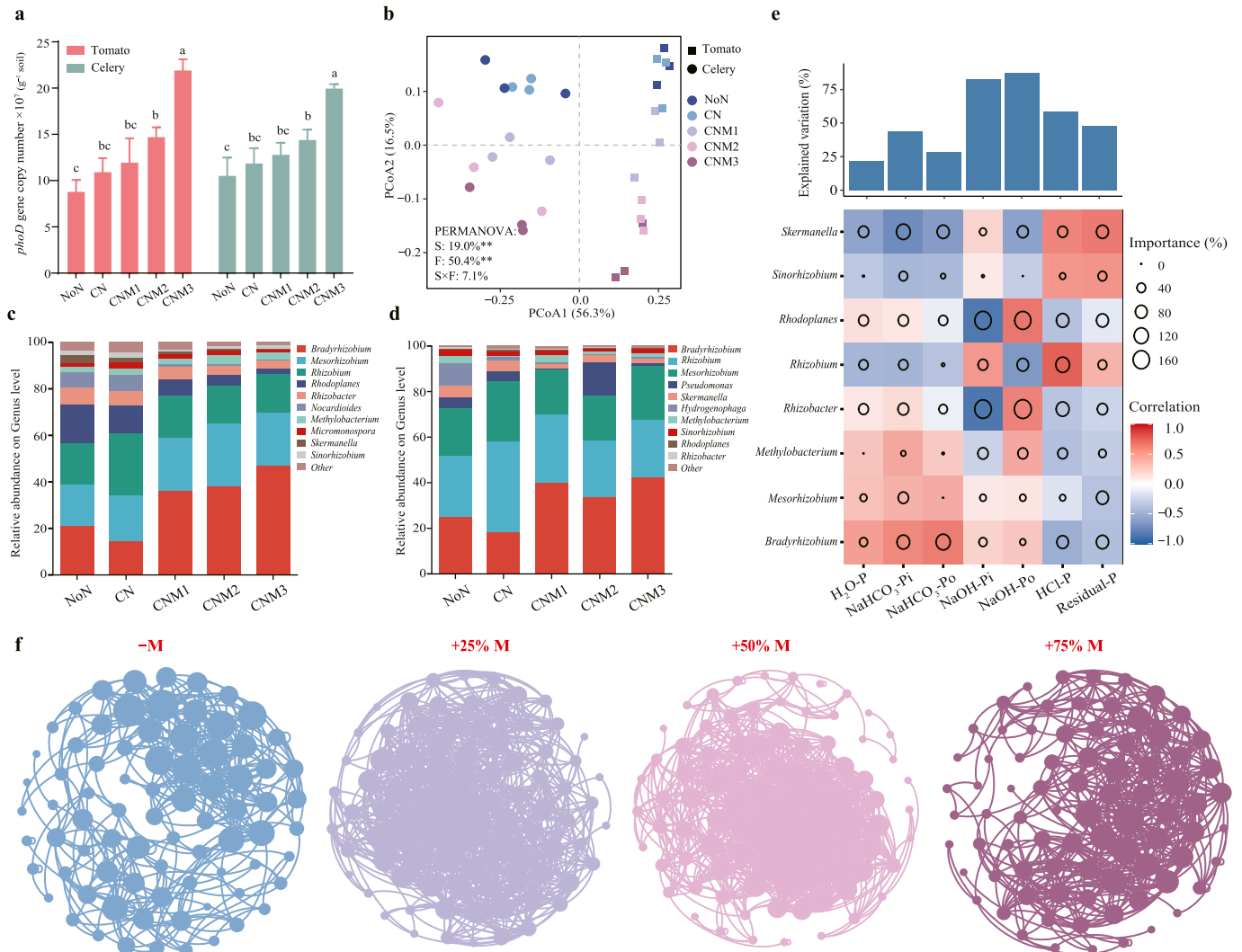


Fig. 4. The abundance of *phoD* gene (a). Principal coordinate analysis ordinations based on the Bray-Curtis dissimilarity matrices showing changes in the *phoD*-bacterial communities (b). Permutational multivariate analysis of variance (PERMANOVA) showing the effects of the S (season) and F (fertilization) on the *phoD*-bacterial communities (** indicated $P < 0.01$). Changes in the relative abundances of taxonomic (at the genus level) in tomato (c) and celery (d) season. Associations between the relative abundances of taxonomic and P fraction contents (e). Circle size represents the importance (%IncMSE) of explaining the variation in P fraction contents based on the random forest modelling. Colors represent Spearman correlations. Networks resulting from no manure (CN) and different manure application amount (f). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

harboring the *phoD* gene in –manure (CN) and +manure (CNM1, CNM2, and CNM3) (Fig. 4f). The complexity and connectivity of the co-occurrence network initially increased and then decreased as the application rate of manure was incrementally elevated (Table S1). The complexity and connectivity were relatively lower in CNM3.

3.6. P adsorption and desorption characteristics

As the P equilibrium solution concentration increased, the amount P adsorbed by the soil showed a trend of rapid initial rise followed by a gradual increase (Fig. 5a and b). The P sorption isotherms fit the Langmuir model well, with R^2 values ranging from was 0.990–0.998 (Table 4). Compared with the NoN and CN, manure application resulted in a reduction of the Qmax in soils during both the tomato and celery seasons. Compare with CN, manure application treatments decreased the K and MBC values; among them, CNM3 showed distinctly lower K and MBC values in both two seasons. Compared to NoN and CN, manure application increased the DPS. As manure application rate increasing, the DPS showed an upward trend in both tomato and celery seasons.

The P desorption rates across various fertilization treatments exhibited an initial rapid decline, followed by a gradual stabilization as P concentrations increased in soils during both the tomato and celery seasons (Fig. 5c and d). In comparison to the NoN and CN, manure application resulted in comparatively elevated desorption rates, with the CNM3 exhibiting significantly higher desorption rates than the other treatments.

3.7. Relationship among manure fertilization, soil properties, and P

The results of RF showed that soil physicochemical and microbial properties explained 80.1 % and 74.6 % of the variations in the labile P and leaching P, respectively (Fig. 6a and b). For individual soil properties on labile P, total organic C (TOC), *phoD* abundance, pH, ALP, and Qmax was significantly affected labile P, among which the relative importance of TOC was greatest (Fig. 6a). Furthermore, PLS-PM suggested that manure fertilization increasing labile P mainly resulted from the positive regulation by the soil TOC and *phoD* gene (Fig. 6c). The positive effect on labile P primarily attributed to $\text{NaHCO}_3\text{-Po}$ (with a loading value of 0.96). Manure fertilization had negative effects on pH, which in turn negatively influenced labile P. For individual soil properties affecting leaching P (Fig. 6b), the P sorption characteristics K and Qmax, *phoD* abundance, and pH significantly influenced the labile P, among which the relative importance of the K value was greatest. Furthermore, PLS-PM suggested that manure fertilization had a significant negative effect on the P sorption characteristics K and Qmax, and soil pH (Fig. 6d). The P sorption characteristic K negatively affected P leaching, while *phoD* gene showed a positive correlation.

4. Discussion

4.1. P availability rises with increased manure substitution

Optimizing fertilization is an important measure to improve the availability of P in the soil (Lan et al., 2012; Yang et al., 2025). Previous

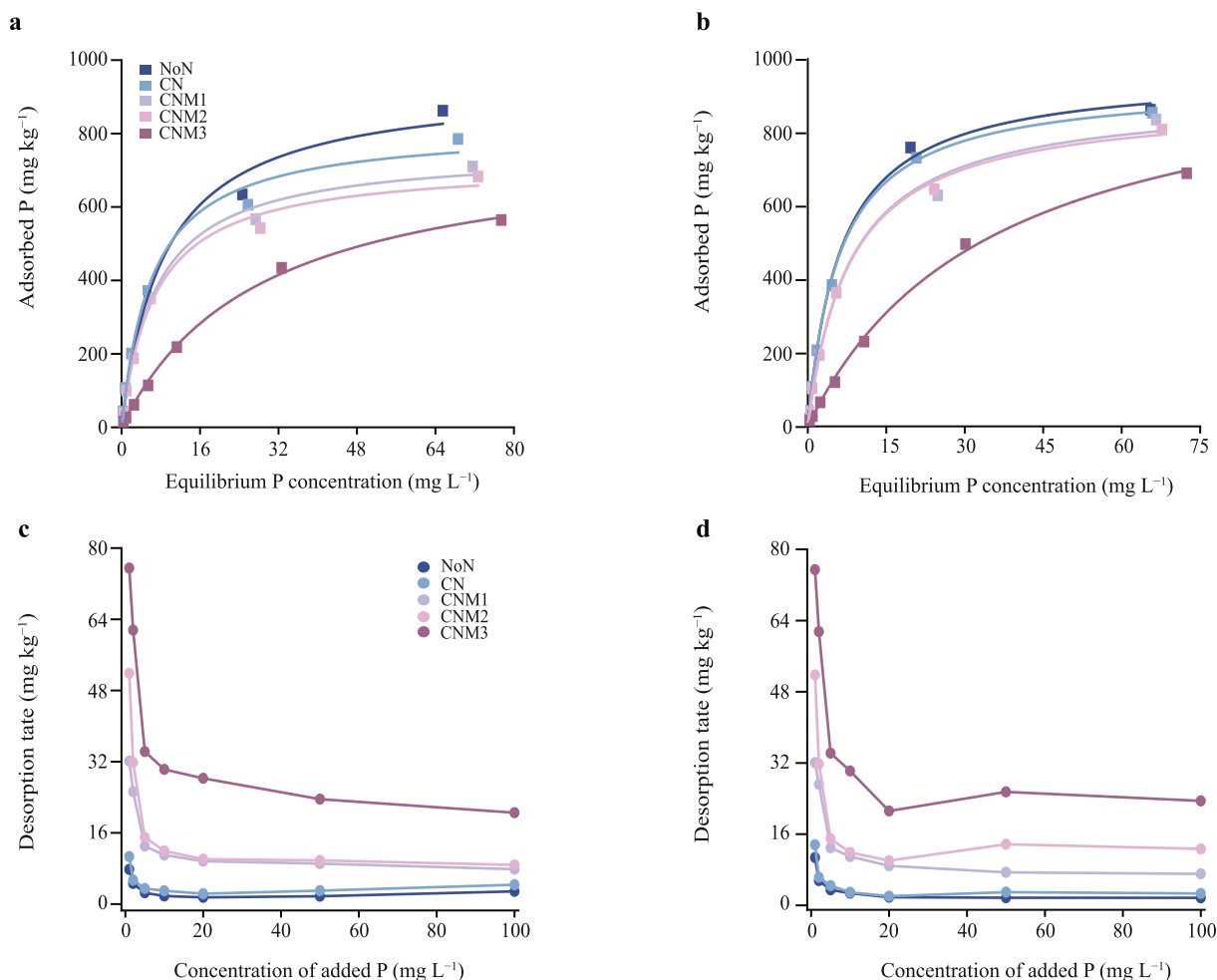


Fig. 5. Phosphate isothermal adsorption curves (a and b) and desorption rate (c and d) of different treatments soil in tomato (a and c) and celery (b and d) season. NoN, only chemical P and K; CN, chemical N, P, and K; 25 % (CNM1), 50 % (CNM2), and 75 % (CNM3) of the chemical N replaced by manure N.

Table 4
Phosphorus sorption characteristics of different treatments in two seasons.

Season	Treatment	Langmuir equation	R^2	Maximum adsorption (Qmax mg kg ⁻¹)	Adsorption constant(K)	Maximum buffering capacity (MBC mg kg ⁻¹)	Degree of phosphorus saturation (DPS %)
Tomato	NoN	C/Q = 0.0010C + 0.0094	0.991	936	0.114	106	7.71
	CN	C/Q = 0.0012C + 0.0076	0.990	817	0.160	131	9.87
	CNM1	C/Q = 0.0013C + 0.0093	0.995	755	0.142	107	13.2
	CNM2	C/Q = 0.0014C + 0.0095	0.994	720	0.146	105	16.8
	CNM3	C/Q = 0.0013C + 0.0367	0.998	779	0.035	27.3	19.3
Celery	NoN	C/Q = 0.0010C + 0.0066	0.997	964	0.158	152	3.76
	CN	C/Q = 0.0011C + 0.0065	0.997	934	0.165	154	4.74
	CNM1	C/Q = 0.0011C + 0.0090	0.992	901	0.122	110	5.75
	CNM2	C/Q = 0.0011C + 0.0090	0.998	887	0.125	111	6.79
	CNM3	C/Q = 0.0009C + 0.0344	0.998	836	0.028	29.0	6.92

NoN, only chemical P and K; CN, chemical N, P, and K; 25 % (CNM1), 50 % (CNM2), and 75 % (CNM3) of the chemical N replaced by manure N. Different letters indicate significant ($P < 0.05$) differences among treatments.

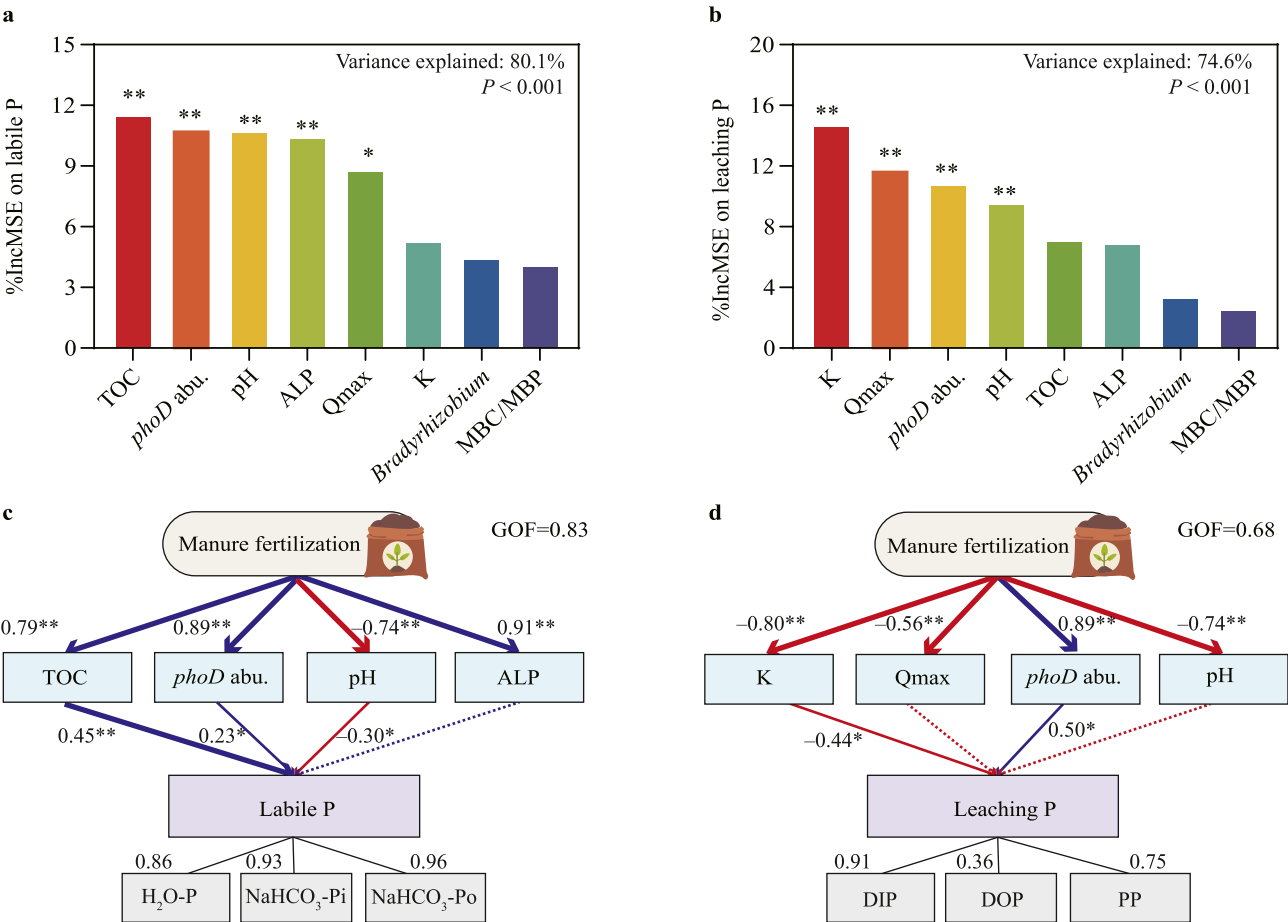


Fig. 6. The relative importance of soil physicochemical and microbial influencing factors in explaining variations in labile P (a) and leaching P (b) as predicted by random forest modeling. %IncMSE, the percentage increase in mean squared error. Partial least squares path model showing the influence paths of manure fertilization on soil labile P (c) and leaching P (d) through soil physicochemical and microbial properties. GOF, goodness-of-fit, was calculated to assess the model. Red and blue arrows denote positive and negative relationships, respectively. The numbers near the arrows are the standardized path coefficients, which were calculated after 1000 bootstraps. *, $P < 0.05$; **, $P < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

studies indicate that long-term manure addition increases labile P content in soils with various crops (Liu et al., 2020; Zhang et al., 2024). Similarly, our results showed that manure application increased both labile and moderately labile P under equal nutrient input conditions in vegetable cultivation (Table 2). An incubation study revealed that, with the same P input, soil available P was higher with high manure application (75 % organic P) compared to low manure application (Ahmad et al., 2018). Further, our study found that as manure substitution increased, the proportions of labile and moderately labile P rose, while the proportion of stable P declined. The main possible reasons for these results were: (1) the different content of inherent labile organic P present in manure input (Darch et al., 2014); (2) the quantity of organic C introduced into the soil varies with different manure replacement levels (Table S2), in which negatively charged organic molecules compete with inorganic P for sorption sites, preventing the formation of stable calcium-phosphate crystals and promoting the availability of P (Zhang et al., 2021); (3) organic acids and microbial metabolites from manure decomposition likely help dissolve stable P compounds, such as calcium and iron phosphates. They inhibit the crystallization of calcium-phosphate compounds and compete for soil P adsorption sites, thereby enhancing P availability (Nobile et al., 2020); (4) enhancing microbial activity promotes the decomposition of organic P. Notably, this study found that the increase in labile P resulting from manure substitution was mainly due to the positive regulation by the soil TOC and *phoD* gene (Fig. 6c), as well as the negative regulation by soil pH. In addition to the mechanisms mentioned above, soil organic C can provide energy to P-mineralizing microorganisms, thereby enhancing P availability. Furthermore, applying manure significantly reduced HCl-P content. This effect is due to organic acids from manure decomposition, as well as organic anions and protons from microbial metabolites, which help dissolve stable P species (Zhu et al., 2018; Zhang et al., 2024).

The findings of this study are consistent with previous research (Liu et al., 2020; Chen et al., 2023), demonstrating that the application of manure increases the levels of orthophosphate monoesters and monoester (C). However, varying rates of manure substitution did not yield significant differences, which is in agreement with the classification results of NaOH-Po content (Table 1). This phenomenon occurs due to the accumulation of orthophosphate monoesters in the soil over time, attributed to their strong adherence to soil mineral surfaces and resistance to degradation (Stutter et al., 2015). Diesters are easily degraded by microorganisms and have weak affinity for soil particles, which enhances the utilizability of P for plants (Hashimoto and Watanabe, 2014). A recent study suggested that, compared with no fertilization, the combination of organic and inorganic fertilizers reduced orthophosphate diester levels in newly reclaimed vegetable soils (Yang et al., 2024). Conversely, this study found that manure application treatments enhanced the proportion and content orthophosphate diesters, and a high amount manure substitution (CNM3) showed the highest content. These results may be attributed to manure containing more labile organic P, which is also released during organic matter breakdown (Shafqat et al., 2009; Liu et al., 2020). Moreover, our study found that the diester (C) was approximately twice as much as diester, due to higher content α -glycerophosphate, β -glycerophosphate, which were easily mineralizable by microbial cells (Li et al., 2022). IHP, as a relatively stable organic P pool, increased under manure application (Table 3), likely because manures are high in phytates (Turner et al., 2003).

MBP serves as a significant source of bioavailable P, as it quickly breaks down into accessible P when added to the soil as microbial remains, thereby enhancing the bioavailability of P in soil (Zhang et al., 2018). A prior study indicated that the incorporation of manure enhanced MBC and the MBC/MBP in paddy soil, thereby promoting the immobilization of P by microorganisms (Chen et al., 2022). This conclusion is inconsistent with our results, which show that, in comparison to the sole application of chemical fertilizers, the use of manure

did not result in an increase in the MBC/MBP, but enhanced the contents of MBC and MBP (Fig. 3). The possible reasons are as follows: (1) the application of manure enhances the availability of soil C to microorganisms, resulting in an elevated C metabolism, particularly in relation to high-activity organic C (Zhang et al., 2024); (2) in resource-rich settings, r-strategists such as *Proteobacteria* and *Actinobacteria* dominate the microbial community, which is marked by a low MBC/MBP ratio (Chen et al., 2024).

Recent studies have indicated that the prolonged application of chemical fertilizers or manure leads to a reduction in ALP activity and a decrease in the abundance of the *phoD* gene compared with no fertilization in rice-wheat rotation (Wang et al., 2022). This phenomenon occurs because the substantial influx of P inhibits the growth and reproduction of bacteria harboring the *phoD* gene. Compared to inorganic fertilization, organic-inorganic fertilization enhanced the abundance of *phoD* and ALP acidity (Bi et al., 2020). Similarly, this study found that, compared with single chemical fertilizer application, treatments in which 50 % and 75 % of manure N replaced chemical N showed significantly higher ALP activity (Fig. 3) and relative abundance of *phoD* (Fig. 4a). The rich organic matter and nutrients in soils treated with a high amount manure (substitute >50 %) may boost the variety and quantity of organic P mineralizers. This, in turn, significantly amplifies phosphatase activity, potentially leading to heightened competition among these mineralizers (Wan et al., 2020). In this study, in both the tomato and celery seasons, we found that *Bradyrhizobium* was main genus among *phoD*-harboring bacteria, and manure application treatments showed a higher relative abundance. This result also supports the conclusion that *Bradyrhizobium* (as N_2 -fixing members) is typically dominant in P-rich soils and is known to produce phosphatase or organic acids to enhance the P availability in soil (Erlacher et al., 2015; Long et al., 2018; Tao et al., 2021). Similar to the previous results, this study showed that manure combined with chemical fertilizer enhanced the complexity and connectivity of the microbial network compared to chemical fertilizer alone. Furthermore, our results found that the lower network complexity was found in CNM3. This indicates that high manure reduces microbial interactions due to uncoupling of microbial networks, thereby destabilizing soil functions and soil structure. The possible explanation is that the application of high quantities of manure, in conjunction with chemical fertilizers, introduces abundant organic matter and nutrients that may promote the proliferation of specific microbial taxa, consequently reducing the overall diversity of the microbial community (Hamm et al., 2016).

Taken together, the results presented here support our hypothesis that substitution of chemical fertilizers with manure can increase availability P levels, with higher manure substitution leading to stronger effects. These results reveal that the increased application of manure introduces a greater proportion of labile P and readily available P forms, such as orthophosphate monoesters, diesters, and MBP, into the soil. Concurrently, it modulates the soil's physical, chemical, and biological properties, adjusts soil pH, enhances organic matter content, reduces P adsorption, and elevates both the activity and potential of phosphatase, thereby further augmenting P availability. Further, the pattern and conclusions require further verification through field experiments on various crops to ensure their broader applicability in the development of fertilization theory.

4.2. P leaching increased with high manure substitution

K indicates the affinity between soil colloids and phosphate ions, while Q_{max} represents the quantity of available adsorption sites on soil colloids (Huang et al., 2014); higher values of both parameters reflect a stronger adsorption intensity. Numerous studies have demonstrated that the combined application of manure and chemical fertilizers decreases soil P absorption capacity (Yan et al., 2018; Qin et al., 2020). Notably, this study revealed that the high manure substitution resulted in sharp reduction in K and Q_{max} (Table 4), implying a higher loss risk. Further,

we found that a high amount of manure substitution increasing leaching P mainly resulted from the negative regulation by K (Fig. 6d). This may be ascribed to organic acids (e.g., citric and oxalic acids) introduced through manure decomposition, which may competitively occupy binding sites on iron/aluminum (hydr)oxides and clay surfaces, effectively blocking phosphate adsorption (Fei et al., 2020). This saturation effect is exacerbated by manure-induced pH shifts toward neutral conditions, which reduce the positive surface charge of soil colloids, thereby weakening their electrostatic attraction to anionic phosphate species (Nobile et al., 2020). Moreover, the high organic matter content under CNM3 enhances the dispersion of soil colloids, thereby facilitating the transport of adsorbed P to deeper soil layers or aquatic systems through colloidal mobility.

Organic fertilization is a vital agronomic practice that enhances nutrient availability and promotes crop growth, but the application of organic manure at elevated levels may also pose environmental pollution risks related to P (Ding et al., 2020; Yuan et al., 2024). Most of the previous studies were based on adding manure in addition to chemical fertilizer application, or manure replacement only took into account N, resulting in a high total P input (Liu et al., 2019b; Tiecher et al., 2020). In this study, the fertilizer treatments contain equivalent quantities of N, P, and K inputs (the recommended amounts based on yield targets and soil fertility), and different rates manure replaced chemical fertilizer. The leaching results indicated that, in comparison to the sole application of chemical fertilizers, substitution with a moderate amount ($\leq 50\%$) of manure resulted in a reduction of P leaching loss. Several studies have also supported that the applying an optimal amount of organic fertilizer can effectively reduce nutrient loss, as evidenced by both leaching and field experiments (Svanbäck et al., 2013; Hua and Zhu, 2020). However, the high amount manure substitution resulted in higher P leaching, mainly due to the high concentrations of DIP and DOP (Fig. S4). Moreover, this study found that high manure substitution exacerbated P leaching predominantly by reducing soil P adsorption capacity and stimulating microbial mineralization of organic P via upregulation of *phoD*. The main explanation was that higher labile P and desorption intensified the leaching of P and formed preferential flow pathways in vegetable fields (Kalkhajah et al., 2021). In summary, manure substitution fertilization enhances the bioavailability of P in the soil, thereby facilitating its uptake and accumulation by crops (Fig. 1). Nonetheless, it is important to acknowledge that high manure levels mainly boost P desorption and microbial activity, leading to increased levels of active and desorbed P and subsequently promoting P leaching.

Overall, an appropriate application of manure should be implemented to ensure optimal nutrient supply for plants and to avoid environmental risks. Under the conditions of this experiment, replacing 50 % chemical N with manure N is recommended, which resulted in higher vegetable yields and lower P leaching loss (Fig. 1). Moreover, this study found that soil organic C was predominant factor positively regulating labile P (Fig. 6). Therefore, integrating other exogenous high-C organic amendments (such as straw and biochar) is beneficial for enhancing soil organic C and optimizing soil P availability, while simultaneously mitigating the environmental impacts in agro-ecosystems. Utilizing long-term field data, this study is the first to quantitatively assess the trade-off between “P enhancement” and “environmental risk” associated with substituting chemical fertilizers with organic alternatives. It proposes a practical “safe substitution interval” ($\leq 50\%$) for effective management. The findings have universal implications for intensively farmed regions worldwide that aim to balance food security and the control of non-point source pollution.

5. Conclusion

In this study, we demonstrated that partial substitution of chemical fertilizers with manure significantly increased vegetable yields, enhanced soil P availability by promoting the accumulation of labile and moderately labile P fractions (including orthophosphate monoesters and

diesters), stimulated microbial activity (i.e., increased alkaline phosphatase activity, microbial biomass C and P, and *phoD* abundance), and drove organic P mineralization via keystone taxa such as *Bradyrhizobium*. CNM3 sharply decreased soil P adsorption capacity and increased total P leaching. CNM2 resulted in higher complexity and connectivity of *phoD*-harboring microbial networks, and significantly decreased P leaching loss. High manure substitution exacerbated P leaching predominantly by reducing soil P adsorption capacity and stimulating microbial mineralization of organic P via upregulation of *phoD*. These findings provide new insights into understanding the roles of organic substitute strategies in soil P biogeochemistry and loss risks, and moderate amount of substitution ($\leq 50\%$) enhances P availability while mitigating leaching, providing a critical strategy for sustainable P management.

CRediT authorship contribution statement

Yinjie Zhang: Writing – original draft, Software, Resources, Investigation, Funding acquisition, Formal analysis. **Wei Gao:** Writing – review & editing, Resources. **Shaowen Huang:** Writing – review & editing. **Chenyang Li:** Writing – review & editing, Software, Data curation. **Lantao Li:** Writing – review & editing. **Jiwei Tang:** Writing – review & editing. **Mingyue Li:** Writing – review & editing. **Peipei Li:** Writing – review & editing. **Yilun Wang:** Writing – review & editing, Supervision. **Chao Ai:** Writing – review & editing, Resources, 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jclepro.2025.146744>.

Data availability

Data will be made available on request.

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