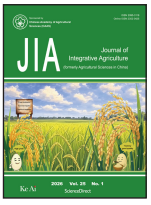
Journal of Integrative Agriculture 2026, 25(1): 313–325

Available online at ScienceDirect

Journal of Integrative Agriculture



RESEARCH ARTICLE

Maize–green manure intercropping improves maize yield and P uptake by shaping the responses of roots and soil

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Highlights

- Maize–green manure intercropping increases maize yield and grain P uptake.
- Intercropping increases the maize root length density and organic acid release rate.
- Intercropping increases the soil phosphatase activity and reduces soil pH.
- Intercropping converts non-labile P to mod-labile P and enriches beneficial bacteria in the soil.

Abstract

Green manuring is essential for improving soil quality and nutrient uptake. With the gradual depletion of phosphorus (P) resources, more attention is being paid to the role of green manures in cultivation systems, such as maize–green manure intercropping, to find possible pathways for enhancing soil P utilization. A maize–green manure intercropping experiment was started in 2009 to investigate the effects and mechanisms for enhancing P uptake and yield in maize. Three species of green manures (hairy vetch (HV), needle leaf pea (NP), sweet pea (SP)) and a sole maize treatment (CK) were used, resulting in four treatments (CK, HVT, NPT, and SPT) in the experiment. During 2020–2023, the intercropping treatments enhanced maize yields in 2020 and 2021, particularly in HVT with increases of 13.7% (1.96 t ha^{-1}) and 13.0% (2.13 t ha^{-1}) compared with CK, respectively. Grain P accumulation of maize was significantly higher in the intercropping treatments than CK in 2020, 2021, and 2023, and with an average increase of 10.6% over the four years (5.2% for NPT, 10.8% for SPT and 15.9% for HVT) compared with CK. Intercropping promoted maize growth with a greater root length density and a higher organic acid release rate. HVT changed the soil properties more dramatically than the other treatments, with increases in the acid phosphatase and alkaline phosphatase activities of 29.8 and 38.5%, respectively, in the topsoil (0–15 cm), while the soil pH was reduced by 0.37 units compared to CK (pH=8.44). Intercropping treatments facilitated the conversion of non-labile P to mod-labile P and stimulated the growth of soil bacteria in the topsoil. Compared with CK, the relative abundance of Gemmatimonadota, known for accumulating polyphosphate, and Actinobacteriota, a prominent source of bioactive compounds, increased significantly in the intercropping treatments, especially in HVT and SPT. A PLS-PM analysis showed that intercropping promoted soil P mobilization and the enrichment of beneficial bacteria by regulating maize root morphology and physiology. Our results highlight that maize–green manure intercropping optimizes root traits, soil properties and bacterial composition, which contribute to greater maize P uptake and yield, providing an effective strategy for sustainable crop production.

Keywords: green manure, root morphology, root exudate, soil P fractions, soil phosphatases, intercropping

1. Introduction

Phosphorus (P) is a non-renewable resource that is essential for plants (Filippelli 2008; Marschner 2012). Inputting P fertilizer into the soil is a common practice for increasing the concentration of available P in soil for plant growth. However, only about 20% of the P applied to the soil could be used by the plants in the first growing season (Yu *et al.* 2021), leading to the waste of the limited P resources and high potential

Received 29 November 2024; Received in revised form 5 January 2025; Accepted 26 January 2025; Available online 24 February 2025
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doi: 10.1016/j.jia.2025.02.051

environmental risks. Incorporating green manures into intensive cropping systems helps to improve soil properties and soil health (Ablimit *et al.* 2022; Ansari *et al.* 2022) to enhance agriculture sustainability and P management (Tonitto *et al.* 2006; Simpson *et al.* 2011; Damon *et al.* 2014).

Green manures, also known as cover crops, are an important method for improving the soil physical, chemical, and biological properties, as well as increasing the diversity of soil microorganisms (Hallama *et al.* 2019; Muhammad *et al.* 2021). The maize–green manure intercropping system is a common planting pattern in oasis irrigation areas in Northwest China. Hairy vetch (HV), needle leaf pea (NP), and sweet pea (SP) are the commonly used legume species of green manures that are incorporated into the maize–green manure intercropping system. Over a long period, this intercropping system has proven to be a sustainable cultivation method that can deliver yield advantages and increase soil fertility (Li *et al.* 2021; Xing *et al.* 2023). Greater P uptake and P use efficiency in maize have been observed in maize-legume intercropping systems due to changes in root architecture and rhizosphere exudate secretions (Shen *et al.* 2013; Li *et al.* 2014, 2024). Importantly, plant roots dominate these processes through the rhizosphere, the interface between roots and soil, which serves as the gateway for nutrients and water to enter the plant (Wang *et al.* 2022). Typical root morphological strategies include a higher root/shoot ratio, finer roots, more root branching, and greater specific root length (Postma *et al.* 2014; Wen *et al.* 2017; Haling *et al.* 2018). In addition, root exudates can directly or indirectly affect the availability of soil resources by altering soil chemical and biological processes, such as increasing the exudation of organic acid anions, phosphatases, and protons (Richardson *et al.* 2011; Wen *et al.* 2017, 2019; Pang *et al.* 2018; Chai and Schachtman 2022; Zhang K *et al.* 2024). Within intercropping systems, changes in root morphology effectively enhance crop P acquisition and yield production (Li *et al.* 2003; Gao *et al.* 2010; Brooker *et al.* 2015). For example, the root length of maize is significantly increased in a maize–faba bean intercropping system (Zhang D S *et al.* 2016). Physiological adjustments in plants have also been observed in intercropping systems. For example, reduced soil pH and increased acid phosphate activity were found when maize was intercropped with alfalfa (Sun *et al.* 2019) or soybean (Yang *et al.* 2022).

According to its ease of absorption by plants, the P in soil can be differentiated into labile P, moderately labile P (mod-labile P), and non-labile P fractions (Hedley *et al.* 1982; Redel *et al.* 2019). Plants exhibit distinct strategies to take up different soil P fractions in the intercropping systems (Damon *et al.* 2014; Liao *et al.* 2021). Thus, intercropping can facilitate access to different P fractions through various P-acquisition strategies, thereby enhancing P uptake and ultimately affecting the crop yield (An *et al.* 2023). On the other hand, research indicates that intercropping can also lead to changes in the soil microbial community composition based on variations in plant root morphology and physiology (Duchene *et al.* 2017; Zhang S *et al.* 2024). Soil microorganisms are regarded as the "second genome" of plants with a large variety and quantity, and they play an important role in the process of plant nutrient absorption (Berendsen *et al.* 2012). Soil microorganisms can form a reciprocal relationship with plants, directly or indirectly helping the plants absorb and utilize nutrient resources in the soil, thereby affecting plant growth and development (Zhang *et al.* 2017; Kumar and Dubey 2020). In addition, plant residue decomposition may also affect changes in soil properties, soil P fractions, and the microbial community (Damon *et al.* 2014; Hallama *et al.* 2019). Extensive research has revealed that the decomposition of green manure residues can stimulate the growth and P uptake of the main crop by increasing P availability through modifications in soil enzymes and microbial activity (Hallama *et al.* 2019; Rodrigues *et al.* 2021).

Despite these studies, the mechanisms linking plant root traits, soil P pools, soil bacteria, plant production, and P uptake in an intercropping system under field conditions remain largely unexplored. Understanding whether and how maize–green manure intercropping can benefit the P nutrition of maize is sorely needed. This study aims to elucidate whether a long-term maize–green manure intercropping system affects maize root traits and soil P fractions to improve P uptake for the maize, and whether soil microbial community composition and abundance respond to the long-term intercropping system.

2. Materials and methods

2.1. Experimental site and design

The field experiment was started in 2009 in Wuwei City, Gansu Province, China (38°37′N, 102°40′E). The soil was irrigated desert soil with a pH of 8.31 (1:2.5 soil/water), containing about 19.1 g kg^{-1} organic matter, 1.36 g kg^{-1} total N, 0.93 g kg^{-1} total P, 20.91 g kg^{-1} total K, 74.3 mg kg^{-1} alkali-hydrolyzable N, 17.92 mg kg^{-1} Olsen P, and 121 mg kg^{-1} available K. Four treatments were used in this experiment: sole maize (CK, without green manure), maize–HV intercropping then total HV plant return to the soil (HVT), maize–NP intercropping then total NP plant return to the soil (NPT), and maize–SP intercropping then total SP plant return to the soil (SPT). The maize and green manure were allowed to grow together for about two months, from the end of April to the end of June, after which the green manure was returned to the soil.

Each treatment had three replications with a randomized complete block design in the field. Each treatment plot was 19.8 m^2 (5.5 m×3.6 m). Maize was planted at a stand density of 82,500 plants ha^{-1}, with an inter-row distance of 0.4 m, and an inter-plant distance of 0.2 m. For the three species of green manures, the inter-row distance and inter-plant distance were both 0.2 m. Every intercropped plot included three intercropping strips, each of which consisted of two rows of maize and three rows of the green manure. All plots of all the treatments had the same amounts of fertilizers applied: 375 kg N ha^{-1}, 78.6 kg P ha^{-1}, and 80 kg K ha^{-1}.

2.2. Sampling and measurements

Soil sampling and measurements Soil samples from the 0–15, 15–30, and 30–45 cm layers were collected using an auger in April before the plants were sown in 2023. Five random cores were collected along a diagonal line across each plot and mixed through a 2 mm sieve. These soil

samples were then divided into three parts: one was air-dried for pH and P fraction determination; one was stored at 4°C for acid and alkaline phosphatase activity measurements; and one was stored at –20°C for microbial biomass P (MBP), microbial biomass C (MBC) and soil DNA extraction.

Soil pH was determined in a boiled deionized water extract (1:2.5) using a pH meter. MBP was determined after fumigation with $CHCl_3$ for 24 h and then extraction with 0.5 mol L^{-1} $NaHCO_3$ (Brookes *et al*. 1982). The 0–15 cm soil was assessed for its P pool according to the method from Hedley (1982) as modified by Tiessen and Moir (1993). Soil acid phosphatase (ACP) and alkaline phosphatase (AKP) activities were determined using the soil enzyme kit from Solarbio Science & Technology Co. (Beijing, China).

Maize grain sampling and measurements The maize grain yields were measured from 2020 to 2023. All the maize grain was collected from every plot (5.5 m×3.6 m= 19.8 m^2), dried and then weighed. Some maize grains from each sample were ground into powder and digested with 5 mL concentrated H_2SO_4 and 6 mL 30% v/v H_2O_2. Grain P concentration was analyzed by the molybdo-vanadophosphate method (Johnson and Ulrich 1959).

Maize root exudate collection and measurements Representative maize plants were selected randomly in each plot in June during the period that the maize was intercropped with green manures (maize was in the V8 phase, and green manure was in the full-bloom stage). Then the whole maize root system was dug out as far as possible with a shovel. Roots of the selected maize plants were washed with tap water to remove the soil. Then the roots were washed three times with sterile water and immersed in 300 mL of sterile water for 4 h with constant aeration using an air-pump (Tang *et al.* 2013). After exudate collection, 10 mL of the solution was stored at –20°C for organic acid anion (OA) analysis by HPLC, while 10 mL was used for dissolved organic C (DOC) concentration analysis by a Multi C/N 3100 TOC analyzer (Analytik Jena AG, Germany). After collecting the root exudate, the root samples were cut and separated from the shoot. The fresh weight of each root sample was measured for the later calculation of OA and DOC.

Maize root sampling and measurements of root morphological traits An auger sampling method was used to assess root length density and other root traits of the maize in different soil layers (Bohm 1979). Soil cores (10 cm diameter×15 cm depth) were collected and taken to a depth of 45 cm (separated into 0–15, 15–30, and 30–45 cm layers). The roots from each core were collected from the soil by passing it through a 2 mm-diameter mesh, followed by washing with running water in the lab. Then root systems were scanned with an Epson Perfection V700 dual lens scanning system at 400 dots per inch. Root images were analyzed with Win-RHIZO software (Regent Instruments Inc., Quebec, Canada) to obtain total root length and root diameter. Then, root dry weight was determined by weighing the oven-dried samples. Specific root length (SRL) was calculated by dividing the total root length by the dry weight of the roots (Wen *et al*. 2017). Root length density was determined by dividing the root length of each soil layer by the soil core volume (10 cm diameter×15 cm depth).

2.3. DNA extraction and high-throughput sequencing

Soil DNA was extracted using the Mo Bio Power Soil TM DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA). PCR amplification of the bacterial 16S rRNA gene V3–V4 region was performed using the forward primer 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and the reverse primer 806R (5′-GGACTACHVGGG TWTCTAAT-3′). PCR amplicons were purified with Vazyme VAHTSTM DNA Clean Beads (Vazyme, Nanjing, China) and quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). After the individual quantification step, amplicons were pooled in equal amounts, and pair-end 2×250 bp sequencing was performed using the IIIlumina NovaSeq platform with the NovaSeq 6000 SP Reagent Kit (500 cycles) at Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China). Microbiome bioinformatics was performed with QIIME2 2022.11 (Bolyen *et al.* 2018) according to the official tutorials (https://docs.qiime2.org/2022.11/tutorials/) with slight modifications. Briefly, raw sequence data were demultiplexed using the demux plugin followed by primer cutting with the cutadapt plugin (Martin 2011). Sequences were then quality filtered, denoised, and merged, and the chimeras were removed using the DADA2 plugin (Callahan *et al.* 2016).

2.4. Statistical analyses

Analysis of variance and least significant difference (LSD) analysis were performed with R 4.3.1 to test the differences in the effects of long-term maize–green manure intercropping on the indexes of maize yield, grain P uptake, root traits, soil P fractions, and soil properties. The alpha-diversity of the bacterial communities was estimated by the Shannon and Chao indexes based on the observed ASVs, and the beta-diversity was assessed by principal coordinate analysis (PCoA) based on the Bray-Curtis distances.

3. Results

3.1. Maize yield and grain P uptake

In 2020 and 2021, maize–green manure intercropping had a positive effect on maize yield compared to the CK, especially in the HVT treatment, with increases of 13.7% (1.96 t ha^{-1}) and 13.0% (2.13 t ha^{-1}), respectively (Fig. 1-A). In 2022 and 2023, no significant differences in yield were observed among the three intercropping treatments compared to CK (Fig. 1-A). In three of the four years (excluding 2022), intercropping, particularly HVT, increased the maize grain P concentration (Appendix A). The maize grain P accumulations in intercropping treatments were higher than CK in 2020, 2021 and 2023 (Fig. 1-B), with an average increase of 10.6% (5.2% for NPT, 10.8% for SPT and 15.9% for HVT) compared with CK during the four years. In addition, intercropping resulted in higher maize shoot P accumulation than CK from 2020 to 2023 (Appendix A).

3.2. Root morphology and exudate of the maize

The root length density of maize in 0–15 cm soil was affected by the intercropping system compared with CK, with

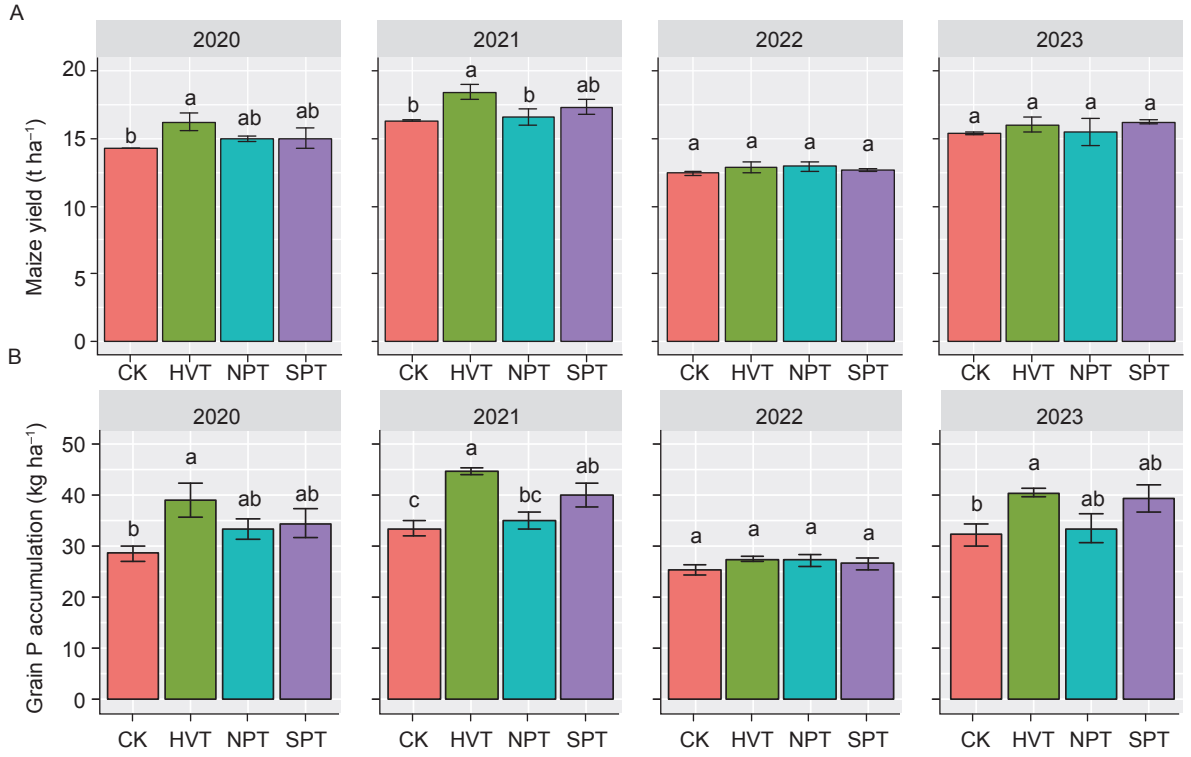


Fig. 1 Effects of intercropping on maize yield (A) and grain P accumulation (B) during the years 2020 to 2023. CK, sole maize without green manure; HVT, maize–hairy vetch intercropping; NPT, maize–needle leaf pea intercropping; SPT, maize–sweet pea intercropping. Each value is the mean±SE of three replicates. Different lowercase letters denote significant differences among treatments (P<0.05).

32.7 and 47.8% increases in HVT and NPT, respectively. Root length density in the soil layers of 15–30 and 30–45 cm showed no significant differences among the treatments (Fig. 2). Limited effects were produced by intercropping on average root diameter and no effects of intercropping on SRL of maize were observed in any of the three soil layers (Fig. 2-B and C).

The DOC release rate of maize was significantly higher in the intercropping treatments, especially in HVT and SPT (0.15 and 0.14 mg g^{-1} fresh weight (FW) h^{-1}, respectively), than in the CK treatment (0.06 mg g^{-1} FW h^{-1}) (Fig. 3-A). The total OA release rate of maize in the HVT (2.11 μmol g^{-1} FW h^{-1}) was more than twice as high as that of the CK (0.86 μmol g^{-1} FW h^{-1}) (Fig. 3-B). Seven types of organic acid anions including oxalic, malic, maleic, citric, succinic, fumaric, and T-aconitic were detected in this study. Principal component analysis (PCA) analysis showed that for the OA exudation, the CK treatment had a clear separation from the three intercropping treatments by PC1 (Fig. 3-C). Moreover, oxalic and succinic acids represented more than 90% of the total measured OA (Fig. 3-D).

3.3. Soil P fractions

Maize–green manure intercropping systems affected the soil P fractions (labile P, mod-labile P, and non-labile P pools) in the topsoil (Fig. 4-A). As shown by PCA analysis, a major difference in the soil P fractions was detected between the CK treatment and the intercropping treatments (HVT, NPT, and SPT) in the first principal components (PC1), representing 43.2% of the total variation (Fig. 4-A). The soil P fractions in the three intercropping treatments were not separated by either PC1 or PC2 (Fig. 4-A).

When comparing the percentages of P fractions under the different treatments, non-labile P (the sum of conc. HCl-Pi, conc. HCl-Po and Residual-P) accounted for the greatest proportion of total P, ranging from 65.0 to 67.6%, followed by mod-labile P pools (the sum of NaOH-Pi, NaOH-Po and 1 mol L^{-1} HCl-Pi), ranging from 19.5 to 25.2%, and the labile-P pools (the sum of Resin-P, $NaHCO_3$-Pi and $NaHCO_3$-Po) representing only 9.8–12.9% (Fig. 4-B). Compared to CK, the intercropping treatments increased the mod-labile P percentage but reduced the non-labile P proportions, particularly in the HVT treatment, followed by SPT (Fig. 4-B). In addition, the organic P pool (the sum of $NaHCO_3$-Po, NaOH-Po and conc. HCl-Pi) was the highest in HVT, followed by SPT, NPT and the last was CK (Fig. 4-C). No differences in the inorganic P pools (sum of Resin P, $NaHCO_3$-Pi, NaOH-Pi, 1 mol L^{-1} HCl-Pi and conc. HCl-Pi) were found among the four treatments (Fig. 4-C).

3.4. Soil pH and phosphatase activities

Intercropping effects on soil pH were observed in the topsoil (0–15 cm) but not in the 15–30 or 30–45 cm soil layers. In the topsoil, the pH under treatments with HVT and SPT were lower than the CK and NPT treatments (Fig. 5-A). Soil pH ranged from 8.44 (CK) to 8.07 (HVT), representing a reduction of 0.37 units (Fig. 5-A).

The activities of acid phosphatase (ACP) and alkaline phosphatase (AKP) in the three soil layers treated with HVT, NPT, and SPT were higher than in CK. Among all the

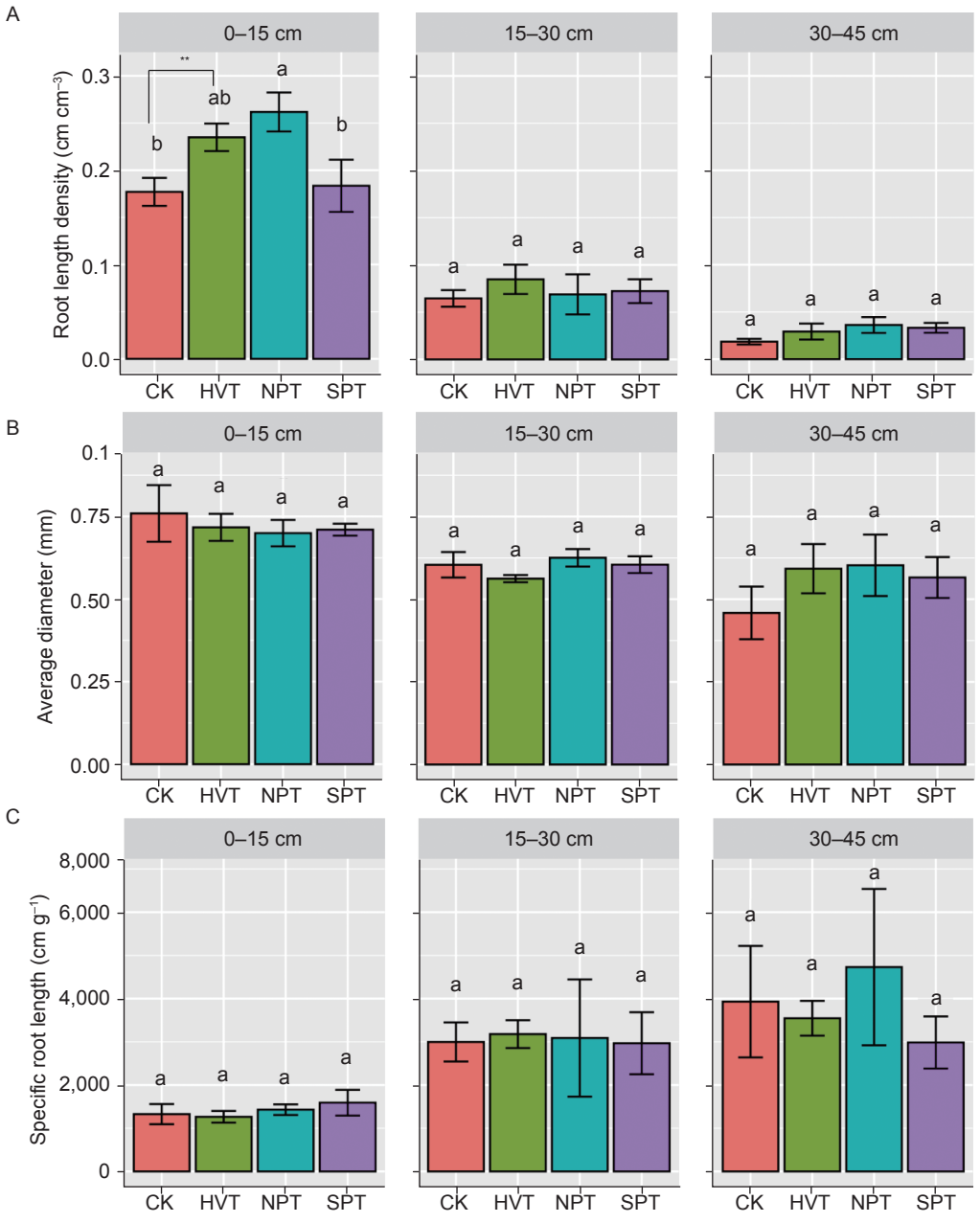


Fig. 2 Effects of intercropping on root length density (A), average diameter (B) and specific root length (C) of maize in different soil layers. CK, sole maize without green manure; HVT, maize–hairy vetch intercropping; NPT, maize–needle leaf pea intercropping; SPT, maize–sweet pea intercropping. Each value is the mean±SE of three replicates. Different lowercase letters denote significant differences among treatments (P<0.05). ** indicates significant differences between CK and HVT (t-test; P<0.05).

treatments, HVT ranked the highest while CK ranked the lowest in each soil layer. HVT increased ACP and AKP in the topsoil by 29.8 and 38.5%, respectively, compared to CK. In addition, phosphatase activities (ACP and AKP) in the topsoil were the highest among the three soil layers (Fig. 5).

3.5. Composition of soil bacteria

No significant differences were observed in α-diversity (Shannon and Chao indexes) among the treatments (Fig. 6-A and B). The principal coordinate analysis (PCoA) showed significant shifts in bacterial community composition among the treatments, in which the soil bacteria formed four distinct clusters and separated along the first coordinate axis (Fig. 6-C).

The relative abundances of the bacteria in topsoil (0–15 cm) at the phylum level showed that Proteobacteria was the most dominant bacterial phylum, accounting for close to or more than 25%, followed by Acidobacteriota and Gemmatimonadota (Appendix B). A heatmap of the dominant bacterial phyla (the relative abundance of the top 20 bacterial phyla) in the four treatments showed that Actinobacteriota, Gemmatimonadota, and Armatimonadota were higher in the HVT and SPT treatments than in NPT and CK (Fig. 6-D). Maize–green manure intercropping also stimulated the growth of soil microbial biomass (MBC) and increased the P content in the soil microbial biomass (Appendix C). The Mantel test results revealed that soil

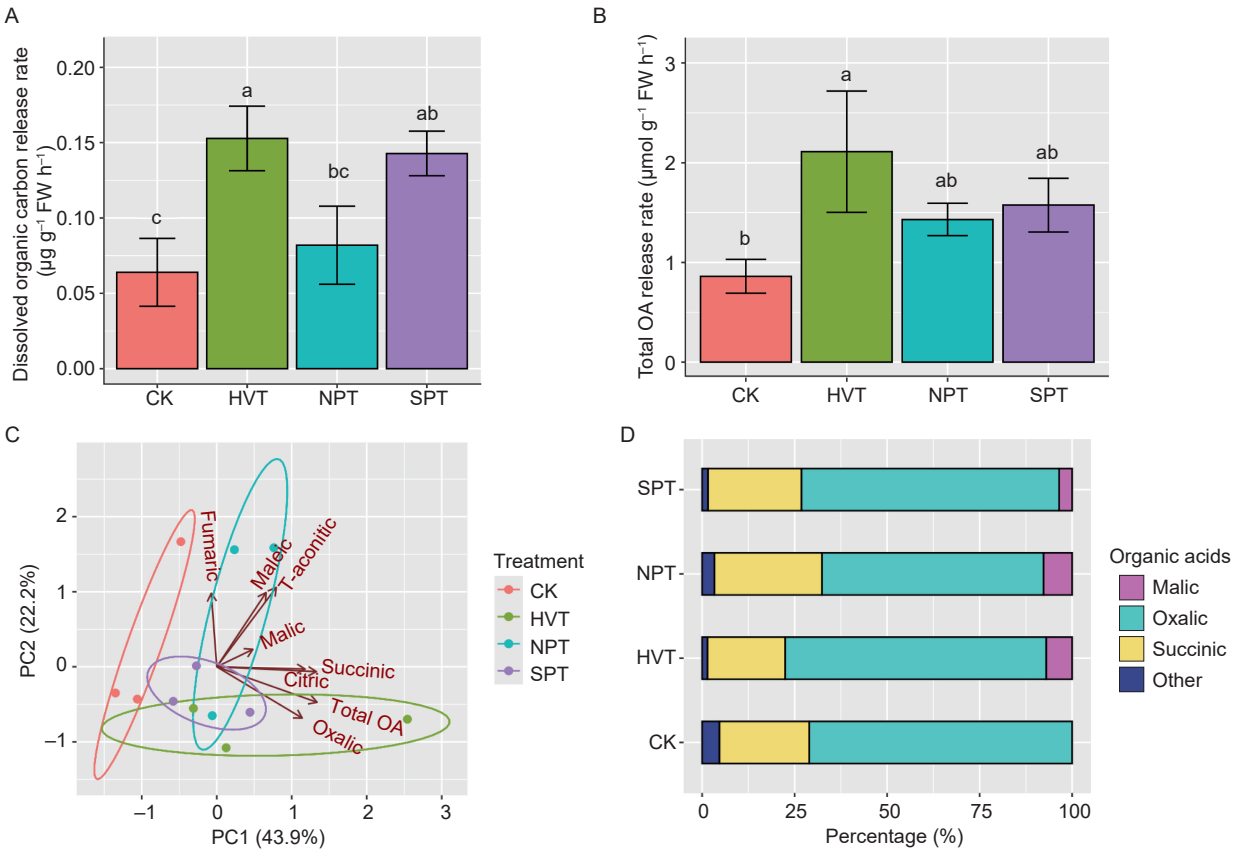
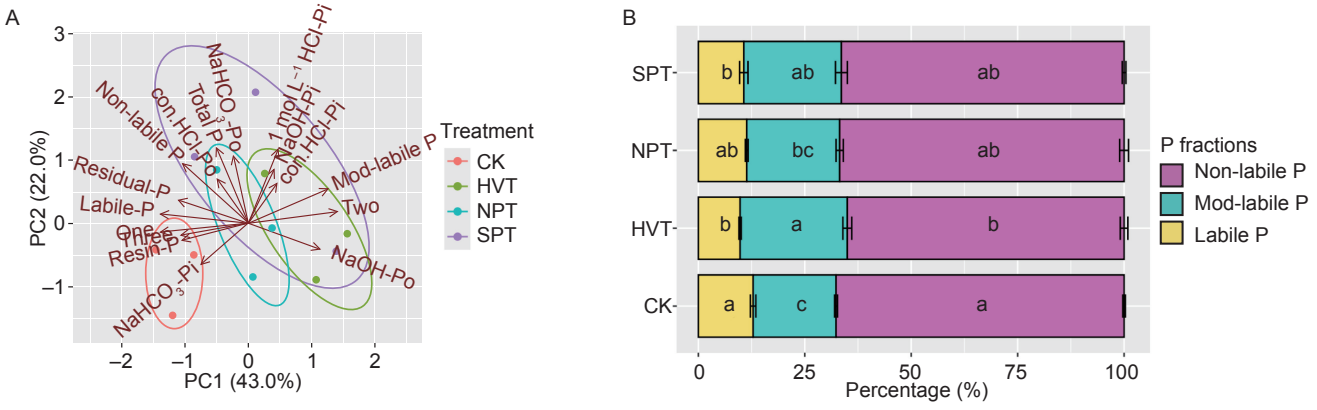


Fig. 3 Effects of intercropping on the dissolved organic carbon (DOC) release rate of maize (A), the total organic acid anion (OA) release rate (B), maize OA variation analyzed using principal component analysis (PCA) (C), and the proportions of various organic acids (the category of all acids with less than 3% is shown as "Other") (D). CK, sole maize without green manure; HVT, maize–hairy vetch intercropping; NPT, maize–needle leaf pea intercropping; SPT, maize–sweet pea intercropping. Each value is the mean±SE of three replicates. Different lowercase letters denote significant differences among treatments (P<0.05).



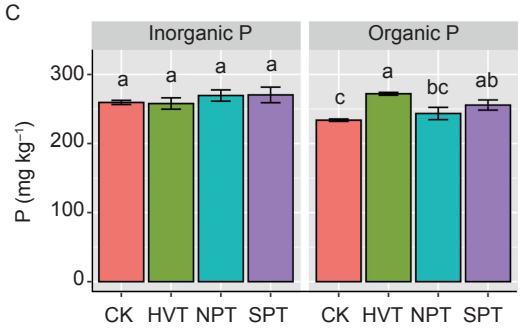


Fig. 4 Effects of long-term maize–green manure intercropping on soil P fractions analyzed by principal component analysis (PCA) (A), the proportions of soil P fractions classified as labile, mod-labile and non-labile P (B) and soil P pools classified as inorganic P and organic P (C). CK, sole maize without green manure; HVT, maize–hairy vetch intercropping; NPT, maize–needle leaf pea intercropping; SPT, maize–sweet pea intercropping; labile P pool, sum of resin-P, $NaHCO_3$-Pi and $NaHCO_3$-Po; mod-labile P pool, sum of NaOH-Pi, NaOH-Po and 1 mol L^{-1} HCl-Pi; non-labile P pool, sum of conc. HCl-Pi, conc. HCl-Po and residual-P; total P, sum of labile P, mod-labile P, and non-labile P; one, proportion of labile P to total P; two, proportion of non-labile P to total P; three, proportion of non-labile P to total P. Each value is the mean±SE of three replicates. Different lowercase letters denote significant differences among treatments (P<0.05).

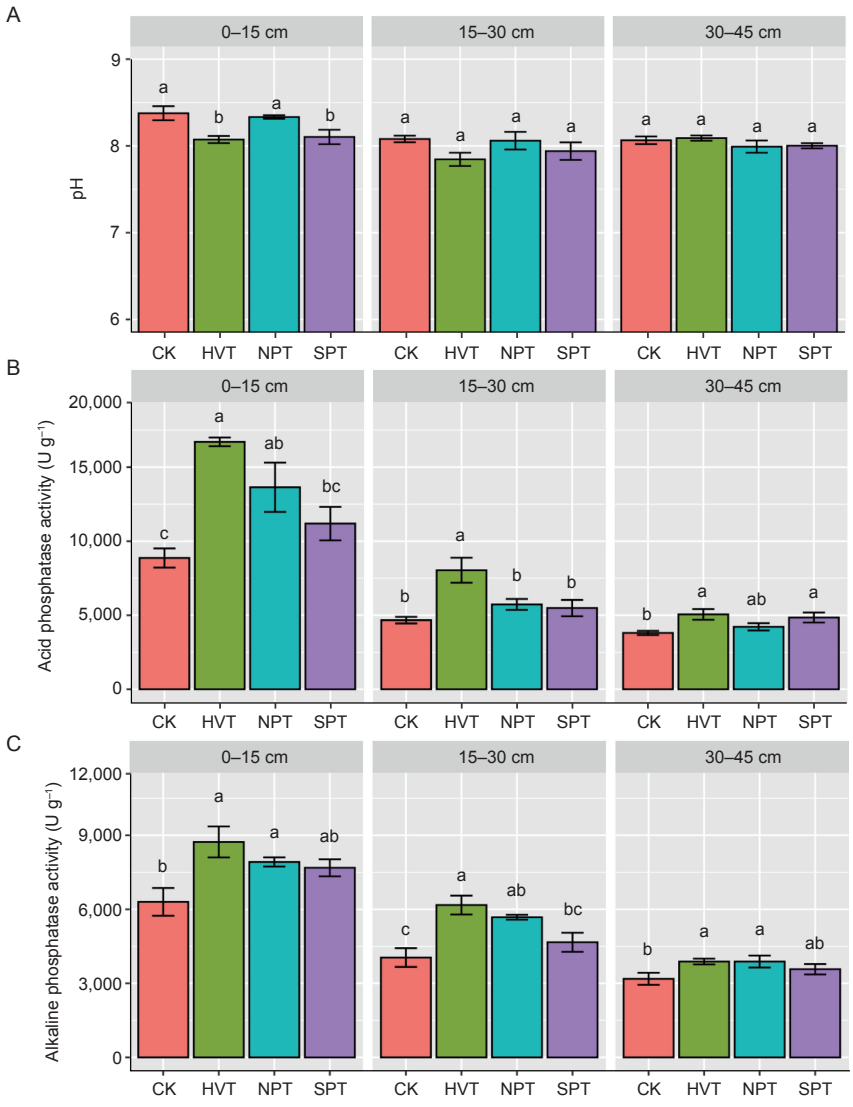


Fig. 5 Effects of maize–green manure intercropping on soil pH (A), soil acid phosphatase (ACP) (B) and soil alkaline phosphatase (AKP) (C) in three soil layers. CK, sole maize without green manure; HVT, maize–hairy vetch intercropping; NPT, maize–needle leaf pea intercropping; SPT, maize–sweet pea intercropping. Each value is the mean±SE of three replicates. Different lowercase letters denote significant differences among treatments (P<0.05).

pH, MBC, and total OA were each significantly correlated with bacterial community composition (Fig. 7).

3.6. PLS-PM analysis

The relationships between treatments, root morphology (root length density, average diameter, and SRL), root exudates (DOC and total OA), soil properties (soil pH, ACP, AKP, the proportions of labile P, mod-labile P and non-labile P, MBP, organic P, inorganic P and total P), soil bacteria (the first two axes of the PCoA), grain P (P concentration of grain) and maize yield (average yield from 2020 to 2023) were elucidated using PLS-PM analysis (Fig. 8-A; Appendix D). The four treatments had direct positive effects on root morphology (0.55), root exudates (0.64, P<0.05), soil properties (0.45), soil bacteria (0.78, P<0.05) and maize yield (1.06, P<0.05). Root exudates influenced soil properties with a direct effect of 0.47 (P<0.05), and root morphology had a direct negative effect on soil bacterial community composition (–0.58, P<0.05). Soil properties (–1.18, P<0.05), soil bacteria (–0.79, P<0.05), and grain P concentration (0.63, P<0.05) were closely correlated with maize yield. In addition, soil properties also directly affected grain P (1.28, P<0.05). The PLS-PM model explained 60% of the variance in maize yield.

4. Discussion

Overall, our results showed that maize–green manure intercropping systems were associated with significantly greater grain yield and P uptake than the sole maize by shaping the responses of maize root traits and influencing soil properties and bacterial communities (Fig. 8-B).

4.1. Root morphological and physiological responses of maize improved yield and P uptake in the maize–green manure intercropping systems

Plants can enhance P uptake by regulating root morphology

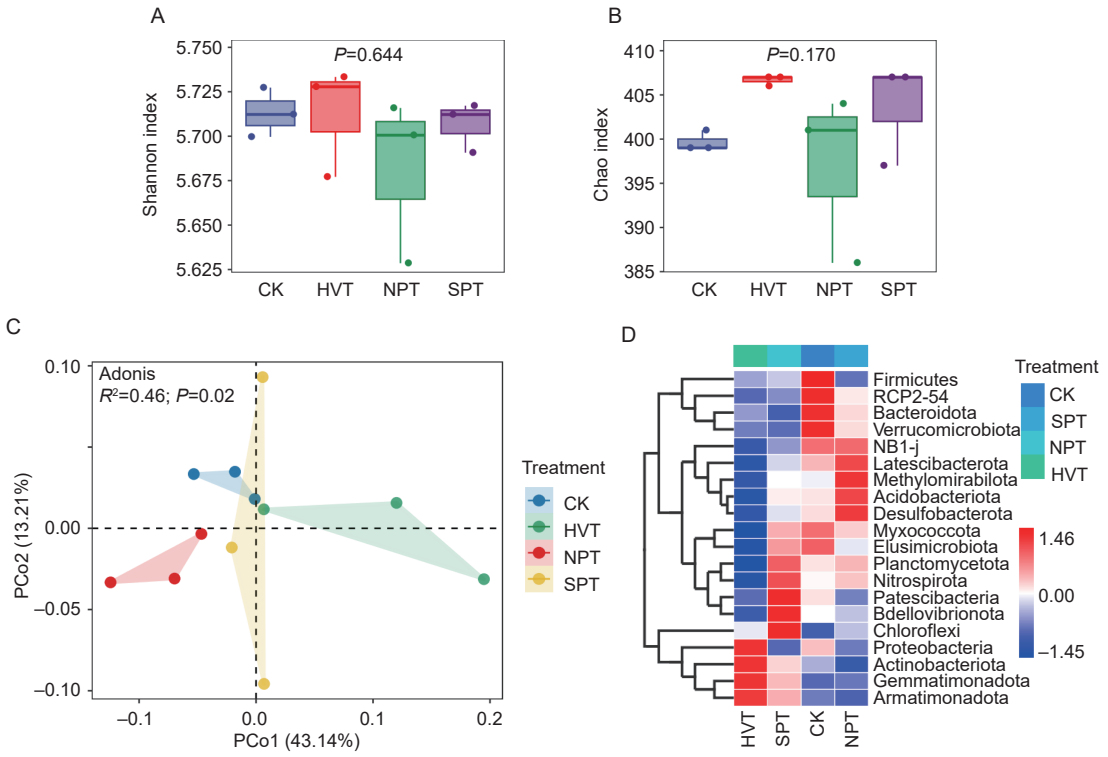


Fig. 6 Effect of maize–green manure intercropping on soil bacterial diversity in topsoil (0–15 cm). A, Shannon index. B, Chao index. The *P*-values indicate the results of Kruskal-Wallis tests among the treatments. C, principal coordinate analysis (PCoA) of the bacterial communities in the topsoil. D, relative abundances of bacterial community composition at the phylum level. CK, sole maize without green manure; HVT, maize–hairy vetch intercropping; NPT, maize–needle leaf pea intercropping; SPT, maize–sweet pea intercropping.

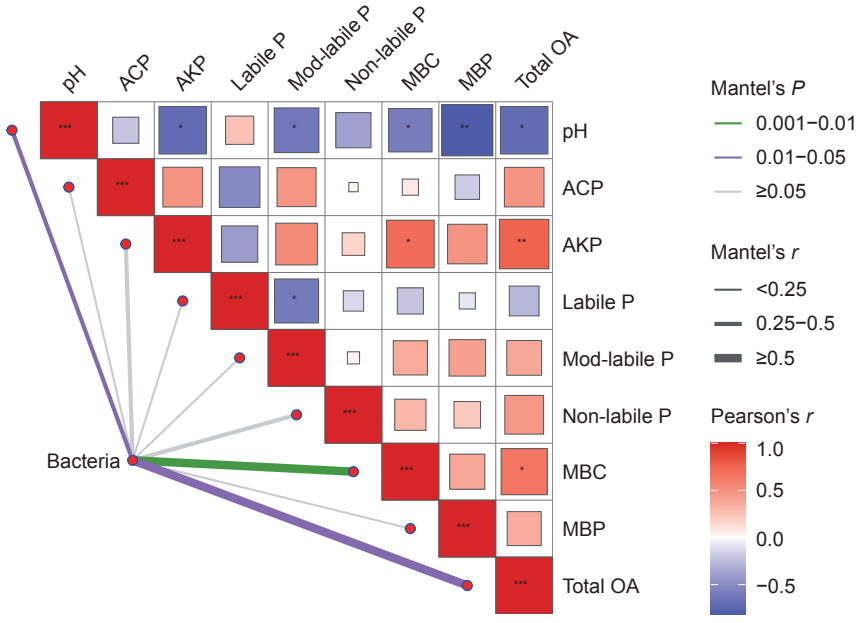


Fig. 7 Relationships between bacterial community composition and soil properties using the Mantel test. ACP, acid phosphatase; AKP, alkaline phosphatase; MBC, microbial biomass C; MBP, microbial biomass P; OA, organic acid anion. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

and physiological processes through the rhizosphere, where root-environment dynamics are critical for nutrient acquisition (Lyu *et al.* 2016; Wen *et al.* 2017, 2019; Wang *et al.* 2020; Lambers 2022; Zhang K *et al.* 2024). The roots of neighboring plants can directly or indirectly affect the root growth of target plants, as shown in a study where wheat root length density in the topsoil increased significantly when it was intercropped with maize, resulting in enhanced P uptake and higher yield (Li *et al.* 2006). Similarly, this study showed that maize–green manure intercropping significantly

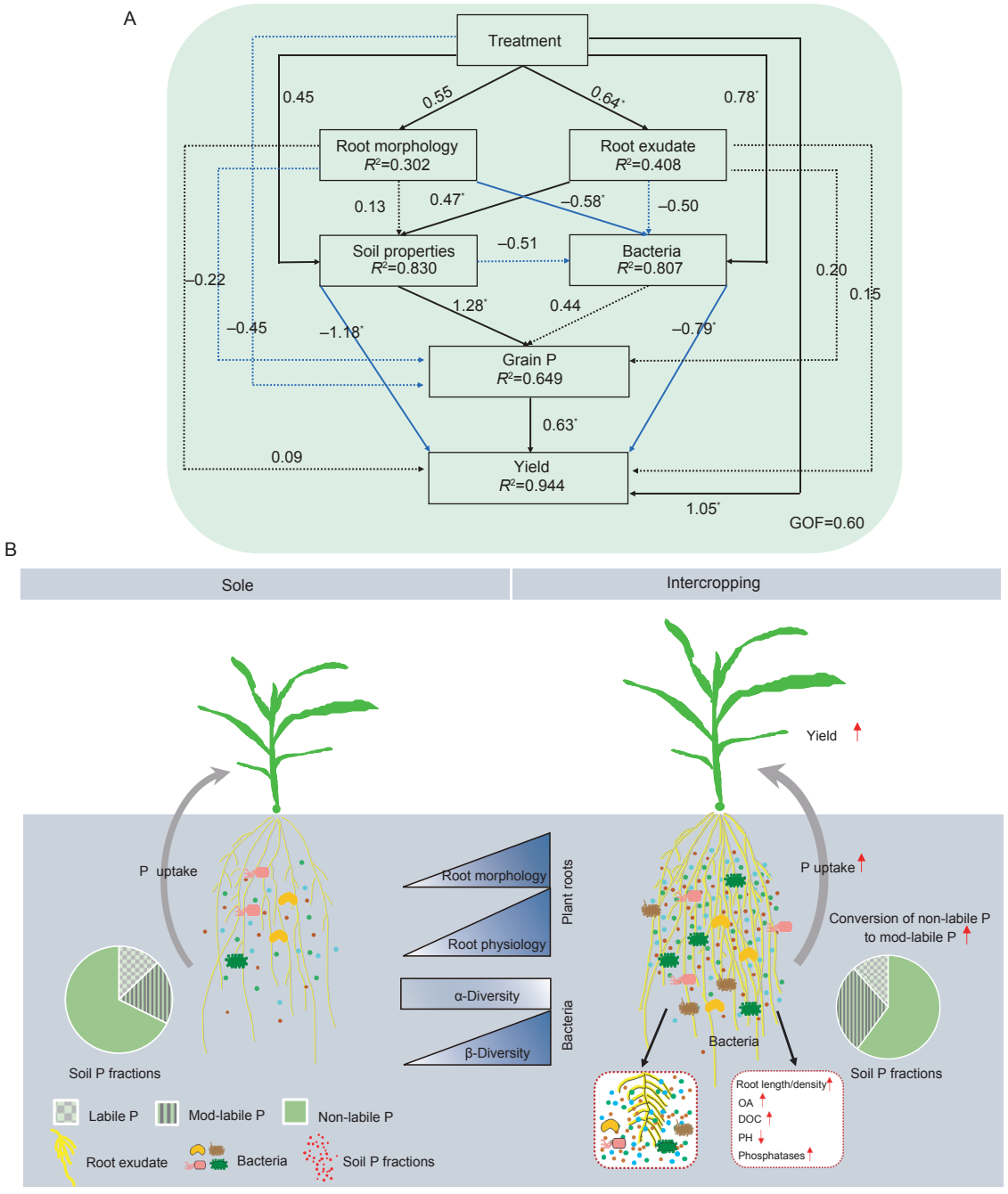


Fig. 8 PLS-PM analysis of the relationships between treatments, root morphology, root exudates, soil properties, soil bacteria, grain P and maize yield (A) and the conceptual model diagram (B). Root morphology includes root length density, average diameter, and specific root length. Root exudates include total organic acid anions (OA) and dissolved organic carbon (DOC). Soil properties include soil pH, soil acid phosphatase (ACP), soil alkaline phosphatase (AKP), soil labile-P percent, mod-labile P percent, non-labile P percent, microbial biomass P (MBP), inorganic P, organic P and total P. Bacteria refers to the first two axes of the PCoA. Grain P is the P concentration of grain and maize yield is the average yield from 2020 to 2023. Observed or latent variables are illustrated in the boxes. Positive and negative effects are represented by black and blue arrows, respectively. Path coefficients and coefficients of determination (R^2) are shown. Path coefficients that are not significantly different are shown as dashed lines; *, $P \leq 0.05$, **, $P \leq 0.01$, ***, $P \leq 0.001$. The GOF (goodness-of-fit) was used to assess the model.

increased maize root length density in the topsoil compared to CK (Fig. 2). These results were also consistent with previous studies, which showed a positive effect on maize root length in both maize–chickpea and maize–faba bean combinations, likely expanding the rhizosphere area and thereby enhancing P uptake (Li *et al.* 2004; Zhang *et al.* 2012; Lynch 2015).

Root exudates, especially organic acids, are crucial for mobilizing sparingly soluble inorganic and organic P by modifying the chemical environment in the rhizosphere, thus enhancing nutrient availability (Richardson *et al.* 2011; Wen *et al.* 2017, 2019; Pang *et al.* 2018; Zhang K *et al.* 2024). Our results also showed that maize in the intercropping systems exhibited stronger physiological responses than CK, particularly in the enhanced secretion of organic acids,

which improved soil P mobilization (Fig. 3). Organic acids play a pivotal role in mobilizing insoluble P by competing with P for adsorption sites on soil minerals, thereby promoting the dissolution and release of bound P (Jones *et al.* 2009; Shen *et al.* 2011; Wang and Lambers 2019). Indeed, we observed that the release rates of malic acid and citric acid by maize were positively correlated with increased mobilization of soil P, further supporting the role of organic acid exudates in enhancing soil P availability (Appendix E).

4.2. Soil P fractions were changed by maize–green manure intercropping systems

P is immobile, and it becomes concentrated in the topsoil over time by plant bioaccumulation and deposition (Richardson *et al.* 2011; Marschner 2012). Soil total P comprises various forms of P and reflects the size of the soil P pool and the potential P supply capacity (Qiao *et al.* 2018). In this study, the soil P fractions in topsoil revealed that maize–green manure intercropping promoted the conversion of non-labile P pools to mod-labile P pools in contrast to the CK treatment, especially in the HVT and SPT treatments (Fig. 4). In addition, the intercropping treatments achieved greater grain P accumulation, indicating that intercropping was more efficient at accessing soil P compared with CK. A previous study also showed that compared with sole maize, the maize-rape intercropping system reduced the soil non-labile P (An *et al.* 2023).

The mobilization of soil P can be partially explained by increases in the root exudate and organic acid release rate of maize in the intercropping system in this study (Figs. 3 and 4). In addition, the presence of green manure in the intercropping system played an important role in the interconversion of soil P fractions. These findings can be supported by a study from Brazil, which also reported that green manures often increase labile and mod-labile P pools while reducing Residual-P (Almeida and Rosolem 2016). Two main processes linked with green manures may be involved in explaining this phenomenon: i) During their growth, they can modify soil properties and mobilize P (Hallama *et al.* 2019); and ii) after decomposition, the P taken up by green manures may provide an easily accessible form of P in the soil (Eichler-Lobermann *et al.* 2008; Hallama *et al.* 2019). The P taken up by the green manure is protected from sorption in the soil or losses by erosion and leaching (Hallama *et al.* 2019). After green manure is returned and during the decomposition of green manure residues, the P taken up by green manure can become converted into more available forms in the soil (Eichler-Lobermann *et al.* 2008).

4.3. Maize–green manure intercropping reduced soil pH and increased phosphatase activities

Soil pH is one of the most important factors regulating P availability, and it reaches maximum values between 6 and 7 (Olsen and Khasawneh 1980). Therefore, in the alkaline soil at the beginning of this experiment (pH=8.31), reducing the pH value will increase the availability of soil P (Yang *et al.* 2014; Nobile *et al.* 2020). The relationship of mod-labile P with soil pH also proved that a lower soil pH promoted the expansion of mod-labile P pools (Appendix F). As in other studies (Vanzolini *et al.* 2017; Hansen *et al.* 2022), our results showed that compared to the original pH value and CK treatment, intercropping reduced soil pH, especially in the HVT and SPT treatments. Some previous studies reported different results, such as that green manure can increase soil pH (Tang and Yu 1999; Yan and Schubert *et al.* 2000; Yan *et al.* 2006), or no significant influence of green manures on soil pH (Ochiai *et al.* 2008). The varying results among these studies may be related to the green manure species, soil types, sampling times and other differences in the experimental systems (Haynes and Mokolobate 2001).

As previously reported, green manures increase the activity of phosphatases in topsoil (Zhang P *et al.* 2016; Wang *et al.* 2021). Our results showed that the phosphatases were not only affected by the presence of green manures in the topsoil, but they also significantly increased in the 15–30 and 30–45 cm soil layers compared with the CK treatment (Fig. 5). The highest activities of soil phosphatase observed in the topsoil may be attributed to the greater root activity in this soil layer, as well as the greater secretion capacity of roots (Jin *et al.* 2009). In addition, the incorporation of green manures into the topsoil can contribute to the enhancement of soil phosphatases by providing a source of energy and creating a favorable environment (Chen *et al.* 2023). The enhanced phosphatase activity serves as an indicator for an increased potential rate of organic P mineralization (Bergkemper *et al.* 2016; Hallama *et al.* 2019; Nakayama *et al.* 2021), and indeed, in our study, organic P was higher in the treatments with green manures than in CK (Fig. 4-C).

4.4. Maize–green manure intercropping affected the community composition of soil bacteria

Soil microorganisms represent an important participant that regulates various biological processes, especially nutrient cycling (Dubey *et al.* 2019). Studies have shown that soil microbial activity can be influenced under planting systems with green manure and associated with residue decomposition (Tsiafouli *et al.* 2015; Hallama *et al.* 2019; Kim *et al.* 2020; Rodrigues *et al.* 2021). Consistent with these studies, the treatments with green manure in this study did not impact the diversity of the soil bacterial community (Dell'Amico *et al.* 2008; Xu *et al.* 2024) but promoted changes in soil bacterial composition, with increased relative abundance of Actinobacteriota, Gemmatimonadota, and Armatimonadota (Fig. 6-D). As previously reported, Gemmatimonadota is a polyphosphate-accumulating bacterium which can grow better under green manure treatment (Wang *et al.* 2021). In one study, Gemmatimonadetes were enriched during the decomposition of organic residues, creating a healthy bacterial environment (Pang *et al.* 2017). Actinomycetes are a prominent source of bioactive compounds (enzymes, antibiotics, and plant growth modulators) that can facilitate plant growth promotion (Palaniyandi *et al.* 2013; Naylor *et al.* 2017; de Vries *et al.* 2020). Actinobacteria help mineralize organic P to inorganic P through phosphatase activity (Franco-Correa *et al.* 2010; Olanrewaju and Babalola 2019),

and the released P may be taken up by plants to enhance plant growth. Indeed, the result of PLS-PM in this study showed that changes in soil bacterial community composition could affect the maize yield (Fig. 8-A). Environmental factors, such as soil nutritional status, soil enzymes, and soil pH, play important roles in bacterial community composition (Prosser *et al*. 2007). The Mantel test results in this study also showed that soil pH was an important factor in explaining the bacterial community structure (Fig. 7). This result was in general agreement with many other studies that revealed a deterministic role of soil pH for the bacterial communities in the soil (Lauber *et al.* 2009; Sun *et al*. 2015).

The PLS-PM analysis revealed that soil properties had an indirect positive effect on both grain P concentration and maize yield (Fig. 8). Among the soil P traits, the percent of mod-labile P, sum percent of labile P and mod-labile P significantly promoted the grain P concentration, but the percent of non-labile P had a significant negative relationship with grain P concentration (Appendix E). These results suggested that the mobilization of non-labile P pools in the intercropping system was a key factor for increasing maize yield in this study. Furthermore, root exudates of citric and malic acids regulated the sum percent of labile and mod-labile P with an R-value of 0.71 (P<0.05) (Appendix E). Therefore, root exudates may have been the key factor driving the changes in soil P fractions, which then affected the P uptake and yield generation of maize in this study. However, this study found that the root morphology changes, but not root exudates, had a significant impact on soil bacteria (Fig. 8-A). In this study, root length density was significantly different among the four treatments, which may partially explain the results. Previous studies also support our results, in which microbial communities could be linked to various root morphological traits such as root length, biomass and branching intensity (Eisenhauer *et al.* 2017; Wang *et al.* 2017; King *et al.* 2021).

5. Conclusion

The results of this study across a 14-year field experiment with or without green manures intercropped with maize showed that compared with sole maize planting (CK), intercropping increased maize yield and grain P accumulation. The increased grain P uptake and maize yield were associated with changes in soil P fractions, especially the mod-labile P pools in the soil. Compared with CK, the better root morphological and physiological responses of maize under a system of intercropping with green manures promoted changes in the bacterial communities and soil properties (such as lower soil pH and higher soil phosphatases), thus promoting the absorption of P and the increase of maize yield. Our findings highlight the positive effects of intercropping with green manures on improving maize yield and enhancing the utilization of soil P.

Acknowledgements

This study was supported financially by the National Key Research & Development Program of China (2021YFD1700200), the National Natural Science Foundation of China (32402686), the Earmarked Fund for China Agriculture Research System (CARS-22), the Fundamental Research Funds for Central Non-profit Scientific Institution, China (1610132022013), the Science and Technology Innovation Project of Chinese Academy of Agricultural Sciences, and the China National Crop Germplasm Resources Platform for Green Manure (NICGR-2024-19).

Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

Appendices associated with this paper are available at https://doi.org/10.1016/j.jia.2025.02.051

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