

Assessment of Olsen Phosphorus Extraction for Predicting Plant P Availability under Agroecological Soil Management

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Abstract

Background and Aims

Bioavailability of phosphorus (P) in agroecological systems is a major constraint where synthetic fertilizer input is limited. Conventional soil P tests may not accurately reflect plant-available P under such conditions. This study evaluated the Olsen method as a predictor of P availability in lettuce (*Lactuca sativa*L.) under minimum and no-till systems combined with microbial inoculation.

Methods

A field experiment was conducted in Gödöllő, Hungary, using a randomized complete block design with four treatments: loosening with or without microbial inoculation, no-tillage with or without inoculation, each with three replications. After harvest, soil and plant samples were analyzed for content using Olsen extraction and total P determination. Soil physicochemical properties including pH, CaCO_3 , moisture, penetration resistance, infiltration, organic matter, and micronutrients were also measured. Statistical analyses included ANOVA, regression models, PCA, PERMANOVA, Spearman correlations, and Partial Least Squares Regression (PLSR).

Results

Plant phosphorus concentration was significantly affected by treatment, with the highest values under loosening combined with microbial inoculation. Olsen-extractable phosphorus was a strong predictor of plant P uptake, explaining 94.5% of variation together with treatment effects. Soil pH, CaCO_3 , and penetration resistance negatively influenced both Olsen P and plant uptake, while Mg, Zn, organic matter, and soil moisture showed positive effects. Soil compaction strongly limited phosphorus uptake.

Conclusion

The Olsen P method proved more reliable for estimating plant-available phosphorus than conventional extraction approaches. No-till helped preserve soil structure and microbial integrity, while loosening improved short-term nutrient accessibility but disrupted biological networks. Microbial inoculation further enhanced phosphorus uptake efficiency.

INTRODUCTION

Phosphorus (P) is an essential macronutrient for plant growth, and it limits the crop productivity, particularly in agroecological systems where the application of synthetic fertilizer is avoided (Nicksy and Entz 2021). Although soils often contain substantial total P reserves, the majority exist as insoluble mineral complexes or stabilized organic forms, leaving only a small fraction readily available for plant

uptake (A. McConnell et al. 2020; Bhat et al. 2017). Consequently, plant-available P is governed mainly by dynamic interactions among microbial activity, root-induced processes, and soil physicochemical conditions such as pH, moisture, and mineral composition (Bi et al. 2018; Nyamaizi et al. 2022).

Accurate assessment of the bioavailable P pool remains a major challenge. Conventional extraction methods, such as ammonium lactate, may not reliably reflect plant-accessible phosphorus, particularly in calcareous or biologically active soils where fixation processes dominate (Braun et al. 2019; Raus and Bolohan 2026). In contrast, the Olsen method, based on sodium bicarbonate extraction, is widely recognized for targeting the labile inorganic P fraction and providing a more realistic estimate of plant-available P across a range of soil conditions (Matar et al. 1988; Braun et al. 2019). This distinction is especially relevant in agroecological systems, where reduced tillage and microbial inoculants are used to enhance natural nutrient mobilization (Li et al. 2024).

Numerous methods exist to estimate plant-available P, with Olsen-P (0.5 M NaHCO_3 , pH ~ 8.5) being widely used for calcareous or neutral soils, Bray-1 for acidic to mildly acidic soils, Mehlich III for multi-element extraction, and Colwell and AB-DTPA for situational assessments. The choice of methods depends on soil pH, mineralogy, and the crop system. Across the literature, Olsen-P is often used as a proxy for plant-available P in field soils and experimental plots. In some natural/semi-natural soils, multiple methods were compiled to map plant-available P globally, illustrating method-dependent variability but a general agreement that plant-available P spans orders of magnitude and is governed by parent material and total soil P content (Luo et al. 2025).

Experimental evidence shows that plant-available P correlates with shoot P uptake and biomass, but the relationship is not always linear, particularly when P is not limiting. In field or pot studies with P supply, plants increase tissue P concentration with increasing soil P, yet biomass responses can plateau or depend on species' traits and nutrient-use strategies, indicating species-specific P-use efficiency and the potential for luxury uptake without growth gains at high P supply (Moeneclaey et al. 2021).

Soil management practices further influence phosphorus dynamics by altering both biological activity and soil structure. No-tillage systems tend to promote surface P accumulation and microbial activity, while soil loosening can reduce compaction and improve aeration, facilitating root growth and nutrient access (Cai et al. 2014; Hu et al. 2025). Moreover, microbial inoculants, particularly phosphate-solubilizing and plant-growth-promoting bacteria, can enhance P availability through biochemical mechanisms such as organic acid production and enzymatic mineralization (Rodríguez and Fraga 1999; Cheng et al. 2023). However, the combined effects of these practices on soil P fractions and plant uptake, alongside interacting soil properties (e.g., organic matter, CaCO_3 , moisture, and penetration resistance), remain insufficiently understood under field conditions (Tyler 2021).

Mechanical soil disturbance through conventional tillage (CT), such as intensive moldboard ploughing, redistributes soil phosphorus (P) throughout the ploughing layer while exposing previously protected organic matter to microbial attack (Abdi et al. 2014; Wei et al. 2019; Sun et al. 2022). Conversely, conservation tillage (CST) or no-till (NT) systems significantly alter vertical P distribution, leading to

pronounced P stratification characterized by the enrichment of inorganic and organic P fractions in the 0–5 cm surface layer (Zhang et al. 2026). Long-term field trials indicate that NT management supports higher surface acid phosphatase activity, which accelerates the turnover of organic P pools relative to tilled systems (Abdi et al. 2014). A global meta-analysis further quantifies that NT practices can result in a remarkable 60% increase in organic P fractions, particularly $\text{NaHCO}_3\text{-Po}$, compared to CT (Zhao et al. 2024). While intensive ploughing redistributes P to deeper profiles to alleviate surface enrichment, it may concurrently increase the risk of particulate P leaching (Wei et al. 2019). Ultimately, CST optimizes root architecture by promoting deeper penetration into the 20–30 cm layer, thereby enhancing subsurface nutrient acquisition and overall P use efficiency (Zhang et al. 2026).

Multiple studies underscore P's importance in leafy greens like lettuce. Phosphorus dynamics from soil to plant involve root proliferation in P-enriched zones, yet lettuce's shallow root system in soilless or greenhouse systems reduces P uptake sensitivity, emphasizing balanced nutrition over excess application (Shen et al. 2011; Baslam et al. 2011).

In plants, in general, beyond structural roles, P promotes robust root development, enhances flower and seed formation, and improves crop quality by strengthening stalks and boosting disease resistance (Shen et al. 2011). Phosphorus deficiency often appears as stunted growth, purplish leaves, and poor seed set, while adequate supply accelerates maturity and yield, though excess can tie up micronutrients like iron and zinc. In high-impact reviews, P signaling pathways regulate stress responses and nutrient homeostasis, linking low P to altered hormone balances like auxin and cytokinin (Shen et al. 2011).

Optimizing P nutrition is crucial amid global depletion risks, with research emphasizing efficient uptake via mycorrhizae and solubilizing microbes to sustain productivity. Balanced application, typically 20–60 kg/ha depending on soil, maximizes returns without environmental runoff (Pang et al. 2024). While phosphorus plays a role in plant energy metabolism, genetic fidelity, and developmental signaling, its application in lettuce cultivation demands precision to mitigate deficiency without courting excess, aligning with sustainable agronomic paradigms. Integrating microbial inoculants, organic amendments, and calibrated fertilization emerge as an optimal strategy to harness P efficiency, fortifying yield resilience amid soil depletion and climatic pressures. Future investigations should prioritize genotypic screening for P-use efficiency alongside precision delivery systems, ensuring lettuce production remains viable in resource-scarce contexts while minimizing environmental phosphorus losses (Baslam et al. 2011; Pang et al. 2024; Zulfiqar et al. 2025).

Soil inoculation with phosphate-solubilizing bacteria (PSB), primarily belonging to the *Bacillus* and *Pseudomonas* genera, represents a critical biological strategy for enhancing phosphorus bioavailability in agroecosystems (Song et al. 2022). These microbial biofertilizers mobilize P from recalcitrant pools through the secretion of low-molecular-weight organic acids such as citric and oxalic acids, which acidify the rhizosphere and chelate metal cations (Nassal et al. 2017). PSB inoculation also significantly elevates soil acid phosphatase activity, facilitating the mineralization of organic P into plant-available orthophosphate (Song et al. 2022). Recent evidence suggests that specific strains, such as *Priestia*

aryabhatai, can increase labile organic P fractions by up to 8.4% while facilitating the conversion of low-activity mineral P into bioavailable forms (Zhao et al. 2025). Beyond biochemical solubilization, synthetic microbial communities can optimize root traits, increasing root surface area, and up-regulating P-transporter genes to maximize acquisition (Li et al. 2023). Integrating PSB with organic amendments like vermicompost further synergizes these processes, significantly improving plant height, root development, and total P uptake (Nassal et al. 2017).

Phosphorus-solubilizing microorganisms (PSM) and phytases can mobilize organic and inorganic P, increasing the pool of plant-available P in soils, particularly in weathered or high-P-fixation soils. Inoculation with PSM can enhance P availability and plant uptake, offering a biologically based avenue to improve P-use efficiency and reduce reliance on chemical fertilizers (Dahlia et al. 2025).

Although the Olsen method is increasingly recognized as a reliable indicator of plant-available phosphorus, it has so far been mainly validated in conventional farming systems. It remains less well understood how well it captures the complexity of agroecological systems, where phosphorus dynamics may be simultaneously influenced by reduced soil disturbance practices, diverse crop rotation, microbial inoculation, and the interactions of soil physical, chemical, and biological factors. This study addresses this research gap by evaluating the suitability of the Olsen method for predicting plant phosphorus uptake in lettuce (*Lactuca Sativa* L.) under different tillage and microbial treatments without phosphorus fertilization, and by identifying the key soil factors that influence phosphorus availability in such complex agroecological settings.

Based on the identified knowledge gap, this study formulates the following hypotheses to evaluate the effects of soil management practices and microbial inoculation on phosphorus dynamics, and to assess the predictive performance of the Olsen phosphorus extraction method under agroecological field conditions.

H1: Loosened soil improves soil function and enhances plant phosphorus uptake compared to no-till soil management.

H2: Application of microbial inoculants positively influences soil function and promotes the uptake of phosphorus and other essential nutrients by plants.

H3: The Olsen P extraction method provides a more accurate prediction of plant-available phosphorus than alternative phosphorus extraction methods.

H4: Combined application of microbial inoculants and soil loosening enhances plant phosphorus uptake and soil function.

MATERIALS AND METHODS

Experimental Site and Soil Characteristics

The research was conducted at the SZIA Agroecological Garden in Gödöllő, Hungary (47.5941° N, 19.3593° E), a dedicated facility within the Hungarian University of Agriculture and Life Sciences (MATE) designed for the long-term study of agroecological practices. According to the WRB classification (2015), the soil is classified as an Anthrosol, developed on sandy parent material under mesic temperature and ustic moisture regimes. The soil profile is characterized predominantly by sandy loam and loamy sand textures, with sand content increasing from 65% in the topsoil to more than 80% in the deeper horizons. The topsoil horizons (0–40 cm) contain relatively higher soil organic matter (3.0–3.5%) and slight carbonate content (1.3–3.1% CaCO₃), while the subsoil is carbonate-free and low in organic matter. Soil pH values are neutral to slightly alkaline throughout the profile (pH(H₂O) 7.2–7.4). The low clay content (approximately 10–14%) and dominance of coarse-textured fractions indicate limited nutrient retention capacity, which is particularly relevant for evaluating soil phosphorus (P) supplying capacity during the experiment. (See more details in Figure A1 of the Supplementary files).

Experimental Design and Field Management

The field experiment was established in April 2023. The study area was divided into twelve plots (80 cm × 300 cm), separated by buffer beds to avoid cross-contamination between microbial treatments. The experiment followed a randomized complete block design (RCBD) with a split-plot arrangement and three replicates per treatment.

Four treatment combinations were applied throughout the experiment:

A–: loosening without microbial inoculation

B–: no-tillage without microbial inoculation

A+: loosening with microbial inoculation

B+: no-tillage with microbial inoculation

The spatial arrangement of the plots is shown in Fig. 1.

The experiment followed a three-year crop rotation: potato (*Solanum tuberosum*) in 2023, pea (*Pisum sativum*) in 2024, and lettuce (*Lactuca sativa*) in 2025. The rotation was designed to represent the management practices of a market-oriented organic vegetable garden under field conditions. All planting materials originated from organic seed sources.

A summary of the cultural management practices and operations applied during the experimental years is presented in Table 1. Although the experiment was conducted as a multi-year trial, the present study focuses on the 2025 lettuce growing season (Year III), to evaluate the cumulative effects of three consecutive years of tillage and microbial inoculation treatments on phosphorus-supplying capacity.

Table 1
Chronological summary of plot management and field operations (2023–2025)

Operation	Year I (2023): Potato	Year II (2024): Pea	Year III (2025): Lettuce
Soil loosening (on selected plots)	Broad fork April 20	Broad fork March 28	Broad fork April 13
Planting / Micobiological inoculation (on selected plots)	April 20 <i>Pseudomonas putida</i> , <i>Azotobacter chroococcum</i> , <i>Bacillus circulans</i> , <i>Bacillus megaterium</i> L. species to enhance nutrient mobilization	March 28 <i>Rhizobium leguminosarum</i> , <i>Ensifer kummerowiae</i> , <i>Pseudomonas putida</i> , <i>Pseudomonas fluorescens</i> , <i>Bacillus megaterium</i> , <i>Bacillus subtilis</i> L to facilitate biological nitrogen fixation	April 13 <i>Pseudomonas putida</i> , <i>Pseudomonas fluorescens</i> , <i>Bacillus megaterium</i> , <i>Bacillus subtilis</i> . L strains to support rapid vegetative growth and root development
Mulch application	-	March 20	April 15
Plant protection	No protection	No protection	April 24 Ferramol (iron (III) phosphate) applied for slug control
Weeding	April 20; May 07; May 28; June 11; July 18	March 28; May 19	April 13; May 25
Irrigation	Manual irrigation applied uniformly across all plots throughout the growing season	Manual irrigation applied uniformly across all plots throughout the growing season	Manual irrigation applied uniformly across all plots throughout the growing season
Harvesting	July 28	June 16	June 17

A central component of the experimental design was the application of crop-specific microbial inoculants supplied by Phylazonit ([http 1](http://www.phylazonit.com)), formulated to account for both crop requirements and prevailing soil conditions. These inoculants consisted of beneficial bacterial strains adapted to temperate agroecosystems and were applied uniformly across treatments at a concentration of 10^9 CFU cm^{-3} directly into the planting zone during seedbed preparation. The detailed composition of the inoculants for each crop is provided in Table 1.

Crop management followed agroecological and organic-compliant practices throughout the study period. No additional fertilizers were applied during the experimental years, with nutrient supply relying exclusively on microbial inoculation. In the second and third years, mulch was introduced as a soil conservation practice to support weed suppression and moisture retention. The only plant protection intervention was Ferramol Schneckenkorn (iron(III) phosphate), an organic-certified molluscicide applied specifically for slug control. In the no-tillage treatment, soil disturbance was limited exclusively to

manual weeding operations, while in the soil loosening treatment, a broad fork was used and soil disturbance was likewise restricted to weeding activities.

Data Collection and Analysis (Soil and Plant Sampling, Field Measurements, Laboratory Analysis)

Soil chemical and nutrient analysis

After lettuce harvest, soil samples were collected from 12 experimental plots using a random zigzag pattern to capture spatial variability. Sampling was conducted at crop-specific root depths (up to 40 cm), with five subsamples per plot combined into a homogenized composite sample.

This study focuses on soil phosphorus, and analyzed P fractions using two different methods: the Ammonium Lactate (AL) extraction and the Olsen method. The AL method measures the readily available and reserve phosphorus in the soil, while the Olsen method was used post-harvest to measure plant-available phosphorus.

All other soil properties were analyzed at the accredited MATE Central Testing Laboratory using standard protocols (detailed in Table A1, Supplementary Files):

- Soil Properties and Acidity: Measured soil plasticity (Arany index), calcium carbonate (CaCO_3), total organic matter, total water-soluble salts, and pH [both $\text{pH}(\text{H}_2\text{O})$ and $\text{pH}(\text{KCl})$].
- Primary Nutrients: Measured potassium ($\text{K}_2\text{O-AL}$), sodium (Na-AL), and mineral nitrogen [$(\text{NO}_2^- + \text{NO}_3^-)\text{-N}$].
- Secondary and Micronutrients: These included magnesium (Mg), sulfate (SO_4^{2-}), and EDTA-extractable copper (Cu), manganese (Mn), and zinc (Zn).

Plant sampling and laboratory analysis

Following sample collection, lettuce quality and nutrient content were evaluated. Laboratory analyses were performed at the accredited MATE Central Testing Laboratory using standardized protocols, as detailed in Table A2 (Supplementary Files). Specifically, the research examined the following parameters: moisture, phosphorus (P), calcium (Ca), potassium (K), magnesium (Mg), and iron (Fe) contents.

Soil physical properties assessment

Three physical properties were measured in this study: soil penetration resistance, soil moisture content, and soil infiltration rate.

To measure soil penetration resistance at four specific depth intervals (0–10 cm, 10–20 cm, 20–30 cm, and 30–40 cm), we have used a penetrometer (AGRETO) with a color-coded scale (green for low, yellow for moderate, and red for high resistance) measurement range of 0–400 PSI ([http 2](http://2)). The readings were taken at 3 random places of each plot, accounting for total 36 readings per treatment.

Soil moisture (SM) was determined using the gravimetric method (Haokip et al. 2025). Briefly, soil samples were collected with an auger from four depth intervals (0–10, 10–20, 20–30, and 30–40 cm), immediately weighed in the field to obtain fresh mass, and stored in sealed zip-lock bags to minimize moisture loss. Samples were then oven-dried at 105°C until constant weight was achieved. Soil moisture content was calculated as the percentage of water loss relative to the oven-dry soil mass.

Soil infiltration rate was evaluated using the single ring infiltrometer method adapted from Reynolds et al. 2002. Measurements were conducted at two time points (before planting and after harvest) at three locations within each plot, resulting in a total of nine replicate per treatment. Briefly, a metal single-ring infiltrometer was inserted vertically into the soil to a uniform depth of 40 cm across all plots, after which specific volume of water was applied and infiltration was recorded at 1, 2, 3, 4, 5, 10, 15, 20, 25, and 30 min intervals. The volume of infiltrated water at each interval was used to fit infiltration curves and assess soil cumulative infiltration rate (SCI).

Statistical Analysis

All analyses were performed using R (version 4.4.3) (R Core Team, 2024). Normality was assessed with the Shapiro-Wilk test using the stats package and homogeneity of variance was evaluated using Levene's test using the car package (Fox et al. 2019). If assumptions were not met ($p < 0.05$), the data underwent log-transformation ($\log(x + 1)$). Treatment effects were examined through one-way ANOVA (aov) and Kruskal-Wallis tests (kruskal.test) when assumptions were violated. Linear associations among phosphorus fractions were assessed using linear regression (lm) and ANCOVA (lm), both using the stats package. The multivariate structure was examined through principal component analysis (prcomp) using the stats package and PERMANOVA (adonis2) using the vegan package Oksanen et al., 2001, while dispersion was assessed using betadisper function. Partial least squares regression (PLSR) was performed using the pls package to determine significant predictors of Olsen P, total P, and plant P, utilizing cross-validation to assess the model (Wakeling & Morris, 1993). The correlation between all variables were evaluated using Spearman's rank correlation (cor) using the stats package with significance determined using the Hmisc package (Frank E Harrell Jr 2003). P-values were modified using the Benjamini-Hochberg method (Benjamini and Hochberg 1995).

RESULTS

The treatment effects on plant and soil parameters

To address the first and second hypotheses (H1 and H2), treatment effects on plant and soil parameters were evaluated. The only variable significantly influenced by treatment was plant phosphorus ($p = 0.023$),

with the highest values recorded under loosening along with microbial inoculation (A+: 3016.67 ± 175.59), surpassing those in A-, B-, and B+ (Fig. 2a). No other soil physicochemical and physical properties showed significant differences across treatments ($p > 0.05$), though variations in mean values suggested consistent patterns (SP Table 2). Organic matter, total nitrogen, and total phosphorus were typically greater in no-tillage, especially in B-, while Olsen-P was highest in B- and lowest in B+. Conversely, microbial treatments, particularly B+, generally exhibited elevated CaCO_3 and Mg levels compared to non-inoculated treatments (Fig. 2b, 2c; SP Table 2). Soil moisture was slightly elevated with no-tillage, whereas soil penetration resistance was highest in A- and lowest in B+ (SP Table 2). Soil pH and plasticity showed minimal variation among treatments. Multivariate analyses indicated that treatments had little impact on overall soil characteristics; this was additionally confirmed by PERMANOVA (999 permutations), which showed no significant treatment effects ($R^2 = 0.2$, $F = 0.652$, $p = 0.849$). Principal component analysis (PCA) indicated that the initial two components accounted for 61.6% of the variation (PC1 = 44.2%, PC2 = 17.4%) without a distinct separation between treatments (Fig. 2d).

Olsen P outperforms total P

To test the third and fourth hypotheses (H3 and H4), the relationships between soil phosphorus fractions and plant phosphorus uptake were evaluated. The correlation between soil phosphorus fractions (Total P, Olsen P) and plant phosphorus (Plant P) were evaluated through linear regression models (Table 2). When modeled as simple linear relationships, neither Olsen P nor total soil P significantly forecasted Plant P (Olsen P: $R^2 = 0.106$, $p = 0.302$; total P: $R^2 \approx 0.0004$, $p = 0.95$). To address experimental structure, two linear regression models incorporating treatment as a categorical variable were applied (Table 2). Within this framework, the model that includes Olsen P and treatment accounted for 94.3% of the variance in Plant P ($R^2 = 0.9425$, $p < 0.001$), with Olsen P recognized as a very significant predictor ($p < 0.001$). Both A+ and B+ treatments significantly enhanced plant P uptake (Fig. 3a). In comparison, the relevant model that included total P and the treatment accounted for considerably less variation ($R^2 = 0.7208$, $p = 0.041$), and total P was not significant ($p = 0.33$). This indicates that total soil P does not significantly aid in predicting Plant P (Fig. 3b). Apart from that, simple linear regression was utilized to assess the relationship between Olsen P and total P, revealing no significant correlation ($R^2 = 0.1085$, $p = 0.296$), indicating that total P does not sufficiently represent plant-available P (Fig. 3c). In general, the findings indicate that Olsen P is a robust and dependable indicator of plant P, while total P does not effectively reflect plant-available phosphorus across different treatments.

Table 2
Statistical Summary of Linear Models Evaluating the Influence of Olsen P, Total P, and Treatment on Plant Phosphorus Dynamics

Model	R ²	Adj. R ²	F-statistic	Significant Predictors
PlantP ~ OlsenP	0.106	0.016	1.183	None
PlantP ~ TotalP	0.0004	-0.100	0.004	None
OlsenP ~ TotalP	0.109	0.019	1.218	None
PlantP ~ OlsenP + Treatment	0.943	0.910	28.69***	OlsenP, Treatment A+, B+
PlantP ~ TotalP + Treatment	0.721	0.561	4.518*	Treatment A+

Key drivers of soil and plant phosphorus dynamics

To test the fourth hypothesis (H4), Partial Least Squares Regression (PLSR) analysis was applied to identify the distinct yet interconnected factors influencing soil phosphorus fractions (Olsen P and Total P) and plant P uptake in relation to soil chemical, physical, and biological characteristics. Olsen P was mainly influenced by soil chemical and physical factors, with pH acting as the most significant negative factor, followed by CaCO₃, Mg, soil penetration resistance, and SO₄. The results show that plant-available phosphorus is restricted in more alkaline, calcareous, and physically limited soil conditions. Conversely, Zn served as the most potent positive driver, trailed by Total P, moisture, and organic matter (Fig. 3d). In contrast, soil organic matter and structural properties primarily governed Total P. Soil plasticity (assessed via Arany) appeared as the most significant negative factor, succeeded by negative impacts of pH and CaCO₃. The results indicate that lower long-term phosphorus accumulation occurs in more alkaline and carbonate-rich environments. The positive effects of organic matter, Mg, Zn, SO₄, SCI, and soil penetration resistance suggest that Total P storage is strongly related to the buildup of organic matter and the stability of the soil matrix (Fig. 3e).

Plant P uptake demonstrated the most comprehensive response, significantly and positively influenced by Olsen P (the primary factor), Zn, and Mg, with further reinforcement from Soil Cumulative Infiltration (SCI), showcasing reliance on both easily accessible P sources and general soil fertility. Nonetheless, Plant P was heavily restricted by soil penetration resistance (the primary negative factor), followed by Total N and pH, emphasizing that soil physical compaction and chemical limitations greatly hinder P uptake despite adequate soil P availability (Fig. 3f).

Integrated soil property networks

Hierarchical clustering of Spearman correlations indicated a distinct multivariate structure among phosphorus fractions and soil properties and supported the proposed hypothesis (Fig. 4). A robust fertility-related cluster was found, with organic matter, Total P, and Total N showing strong intercorrelation ($\rho = 0.83\text{--}0.87$) and a positive relationship with moisture ($\rho = 0.80$), signifying linked soil carbon nutrient dynamics. This cluster displayed significant negative correlations with pH ($\rho = -0.60$ to -0.87), indicating the difference between alkaline soil conditions and those rich in organic matter. A

second geochemical category featuring CaCO_3 , Zn, and SO_4 displayed a robust internal structure, with Zn showing a significant negative correlation with pH ($\rho = -0.80$), suggesting common influences related to soil chemistry and mineral connections. Soil physical and hydrological functions, indicated by soil penetration resistance and cumulative infiltration (SCI), created an intermediate cluster that showed a moderate association with fertility and chemical variables ($\rho = 0.30$ to 0.67). Phosphorus fractions took up transitional roles within this structure. Olsen P had a positive correlation with Zn ($\rho = 0.72$) and a negative correlation with pH ($\rho = -0.55$), while demonstrating weaker associations with organic matter ($\rho = 0.30$) and Total P ($\rho = 0.33$). Conversely, Total P showed a strong correlation with organic matter ($\rho = 0.87$) and hydrological indicators of soil structure like SCI ($\rho = 0.79$). Plant P exhibited low overall connectivity, showing only slight positive correlations with Zn ($\rho = 0.39$) and Olsen P ($\rho = 0.27$) and negative connections with soil penetration resistance ($\rho = -0.35$), suggesting that plant absorption is more limited by physical accessibility rather than by the bulk nutrient reserves in the soil.

DISCUSSION

The present study evaluated the capacity of the Olsen method to reflect plant-available phosphorus in an agroecological system producing lettuce. Our finding is that Olsen-P, when considered together with tillage and microbial management practices, substantially outperformed Ammonium Lactate-extractable Phosphorus in explaining the variation in lettuce tissue phosphorus concentration. Our result emphasizes that a biologically meaningful extraction must be interpreted within the management context if soil P tests are to reliably guide nutrient stewardship in organic, reduced-input systems.

Effects of soil loosening and microbial inoculation on soil function and plant phosphorus uptake

The results showed that treatment significantly affected only plant phosphorus (Plant P), with the highest value observed under soil loosening combined with microbial inoculation (A+), thereby supporting H4. All other soil physico-chemical and physical parameters were not significantly influenced by treatments ($p > 0.05$), although trends suggested higher organic matter, total N, and total P under no-tillage systems, and reduced soil penetration resistance under microbial treatments, thereby supporting H4. Multivariate analyses (PCA and PERMANOVA) confirmed minimal overall treatment effects on soil properties. Microbial inoculation likely enhanced rhizosphere activity, increasing P solubilization and uptake without substantially altering total soil nutrient pools. Meanwhile, the lack of significant differences in soil properties may reflect short-term experimental duration, inherent soil buffering capacity, or spatial variability masking treatment effects. Reduced penetration resistance in inoculated treatments could be attributed to improved soil aggregation mediated by microbial exudates.

The enhanced P-uptake observed in this study aligns with studies that show the positive role of microbial inoculation in plant nutrient uptake. Through solubilization and mineralization processes, microbial communities mobilize phosphorus, thereby improving plant uptake efficiency without significantly altering bulk soil P pools (Jatana et al. 2021). Additionally, field evidence demonstrates that

inoculation with phosphate-solubilizing bacteria (PSB) improves available P and crop performance. Phosphate-solubilizing bacteria (PSB) release organic acids and phosphatases that solubilize insoluble P forms, increasing P_i in the rhizosphere and enabling plant uptake. Isolates capable of P solubilization span diverse taxa, including *Pseudomonas* and *Acinetobacter* species, with reported solubilization ranging from tens to hundreds of $mg\ L^{-1}$ under various pH regimes (Yaghoubi Khanghahi et al. 2020).

However, the combination of loosening and inoculation created the most favorable conditions for root proliferation and P acquisition, as loosening alleviates physical constraints on root access to nutrient-rich microsites, while introduced phosphate-solubilizing bacteria (PSB) and plant-growth-promoting rhizobacteria actively mobilize recalcitrant P pools (Menezes-Blackburn et al. 2017). Different research has confirmed that the combination of the PSB/PGPR consortium and loosening creates favorable soil conditions, which helps to improve aeration and enhance phosphorus mobility and nutrient solubilization, in turn allowing the microbes to efficiently mobilize recalcitrant phosphorus pools for the plant growth (Gaspareto et al. 2023; Majed et al. 2023).

The elevated organic matter and total P observed under no-till (especially B-) are consistent with well-documented P stratification, where labile organic P accumulates in the top 0–5 cm but becomes spatially segregated from the bulk of the root system later in the season. A long-term study confirmed that phosphorus stratification occurs over time, mainly in the 0- to 5-cm topsoil layer, irrespective of fertilizer management (Costa et al. 2010). No-till systems, especially when combined with crop residues, typically enhance soil organic matter (SOM), soil structure, and improve microbial habitat, enabling greater PSB activity and, consequently, increased P solubilization and availability. The findings of this study align with field evidence, which indicates that no-till with residue retention often yields higher P availability at shallow depths and increased phosphorus use efficiency (PUE) when coupled with PSB inoculation, compared to conventional tillage alone (Costa et al. 2010). Additionally, the findings of this study confirm the synergistic relationship between microbial inoculation and conservative tillage. In previous studies, inoculation with PSB under NT or reduced-till systems has been associated with increases in available P and yield (Padbhushan et al. 2023; Kumar et al. 2023). It could, therefore, be suggested that microbial inoculation, particularly when combined with soil loosening, primarily enhances plant phosphorus mobilization and uptake rather than altering soil properties. This underscores the importance of biological processes over short-term physicochemical changes in driving nutrient acquisition.

Olsen P as a key predictor of plant-available phosphorus

Our results demonstrate that soil phosphorus quantified using the Olsen method is a markedly stronger predictor of lettuce tissue P content than total soil P, thereby supporting H3. When phosphorus metrics were evaluated without accounting for experimental structure, neither Olsen P nor total P significantly explained variation in plant P concentration (Table 2). However, once the experimental structure was incorporated, Olsen P emerged as a highly significant predictor of plant P ($p < 0.001$; Table 2), and the corresponding model explained 94.3% of the variance in plant P ($R^2 = 0.9425$). In contrast, total P remained non-significant ($p = 0.33$; Table 2) and explained substantially less variance (Table 2). Partial

Least Squares Regression (PLSR) consistently identified Olsen-P as the primary direct predictor of plant phosphorus uptake (Fig. 3), confirming its suitability as an indicator of plant-available phosphorus under agroecologically managed field conditions. This finding aligns with previous literature reporting that Olsen-P more accurately reflects labile phosphorus pools than conventional extraction methods, particularly in low-input, biologically active systems (Braun et al. 2019). The findings also indicate that Olsen P captures functionally relevant phosphorus pools directly linked to plant uptake, whereas total P does not adequately reflect plant-available phosphorus, in line with current agronomic consensus (Jalali et al. 2025; McDowell et al. 2026).

The superior performance of Olsen P method can be attributed to its ability to operationally target the labile inorganic phosphorus fraction that governs soil–solution equilibrium and root uptake. The bicarbonate extractant minimizes interference from calcium, iron, and aluminum, thereby approximating the chemical environment encountered by plant roots, particularly in neutral to calcareous soils. In contrast, total P measurements integrate stable mineral phosphates and organic P forms that are largely unavailable to plants within a single growing cycle. This mechanistic distinction explains why total P often shows weak or non-existent correlations with crop P uptake, especially in soils with high sorption capacity or accumulated legacy P. Recent syntheses confirm that Olsen P more closely reflects the dynamic phosphorus pool accessible to crops across contrasting soil chemistries (Recena et al. 2015; McDowell et al. 2026).

Our findings align closely with previous studies on lettuce and other vegetable crops, which consistently report stronger relationships between Olsen P and plant P uptake or yield than with total soil P. Leafy vegetables exhibit high phosphorus demand and shallow rooting systems, making them particularly sensitive to short-term P availability rather than total P reserves. Reviews of critical soil phosphorus thresholds show that Olsen P is the most commonly calibrated soil test for vegetables and provides more consistent tissue P and yield predictions than total P or alternative indices, supporting its widespread agronomic use (Ibrahim et al. 2021; Jalali et al. 2025).

Despite its strong performance, Olsen P does not fully capture biological contributions to phosphorus acquisition, particularly where organic P mineralization, rhizosphere enzyme activity, or mycorrhizal associations play an important role. Soil physical constraints, such as compaction, may further limit root access to available P pools, emphasizing that Olsen P should be interpreted alongside physical and biological indicators. Nevertheless, from an agronomic perspective, our results reinforce Olsen P as the preferred diagnostic indicator for phosphorus availability in lettuce production, supporting more precise fertilizer management, improved phosphorus use efficiency, and reduced risk of P accumulation compared with reliance on total soil phosphorus alone (Jalali et al. 2025; McDowell et al. 2026).

Phosphorus availability and uptake are primarily controlled by interacting soil properties rather than total P

Our results demonstrate that soil phosphorus dynamics and plant phosphorus uptake are governed by a tightly interconnected network of chemical, physical, and biological soil properties, rather than by total

soil phosphorus content alone.

In line with H3, soil chemical (e.g., pH and CaCO_3 , Zn, Mg), physical (penetration resistance, soil moisture, infiltration), and biological-related properties (organic matter) all influenced both soil P availability and plant P uptake. Among chemical soil constraints, pH was the strongest negative driver of Olsen-P, followed by CaCO_3 , suggesting strong phosphorus immobilization under alkaline and calcareous conditions. These results are consistent with known mechanisms of reduced phosphorus solubility at elevated soil pH. In addition, soil penetration resistance exerted a strong negative effect, suggesting that physical compaction limits root access to phosphorus pools that would otherwise be available. Together, these findings highlight that plant-available phosphorus is simultaneously constrained by chemical fixation and by physical limitations in soil structure.

In contrast, positive drivers of Olsen-P- including Zn, Mg, soil moisture, and organic matter- point to the importance of biologically active and well-functioning soils in sustaining phosphorus availability. Although Zn–P antagonism is well documented under conditions of excessive phosphorus supply, the positive association observed here under agroecological conditions without synthetic fertilizer inputs and with enhanced microbial activity reflects improved overall soil fertility and biological functioning rather than a direct synergistic interaction between Zn and P (Nadeem et al. 2024).

Organic matter and soil moisture likely enhance phosphorus cycling by increasing microbial activity and improving nutrient turnover, while micronutrients may indicate a generally more balanced and fertile soil environment that supports nutrient mobilization. Overall, these results suggest that phosphorus availability is not determined by a single nutrient pool but by multiple interacting soil fertility processes.

Plant phosphorus uptake exhibited the most complex response. It was most strongly and positively driven by Olsen-P, followed by Zn, Mg, and soil cumulative infiltration (SCI), emphasizing the importance of both readily available phosphorus pools and overall soil functionality. However, plant uptake was most strongly constrained by soil penetration resistance, followed by total nitrogen and pH, indicating that physical limitations and broader nutrient imbalances can override sufficient labile phosphorus availability. This highlights that effective phosphorus acquisition is strongly dependent on soil physical accessibility. The negative association between total nitrogen and plant P uptake may reflect indirect, system-level interactions, where higher organic nitrogen levels are associated with increased microbial immobilization and greater soil physical resistance, rather than a direct inhibitory effect of nitrogen on phosphorus uptake (Ibrahim et al. 2021; Nadeem et al. 2024).

Soil and Plant Phosphorus Dynamics largely rely on the integrated soil property

The complex physicochemical interactions within soil largely govern soil functioning and nutrient dynamics. In the present study, soil pH emerged as one of the most influential regulators of total nitrogen, soil organic matter, and Olsen P. This observation is consistent with previous findings showing that plant nutrient absorption is generally highest at pH 6.5–7.0 (Penn and Camberato 2019).

Soil pH, however, is strongly influenced by soil structural conditions. Compaction-induced reductions in air-filled porosity can alter redox conditions and increase CO₂ accumulation, thereby shifting rhizosphere pH dynamics (Hinsinger 2001). Consistent with this mechanism, our results identified soil cumulative infiltration, penetration resistance, and soil moisture as major drivers of soil pH.

Beyond its influence on pH, soil structure directly regulates phosphorus mobility and availability. Because phosphate ions move primarily through diffusion over millimetre-scale distances, increases in bulk density and reductions in pore continuity restrict diffusive P transport to the root surface (Shierlaw and Alston 1984; Cornish et al. 1984). In the current study, mechanical loosening reduced bulk density and penetration resistance while increasing microporosity and infiltration capacity. These changes modified the physical environment governing phosphorus diffusion, root exploration, and nutrient acquisition. Collectively, these mechanisms help explain why Olsen P, bulk density, porosity, and pH together accounted for plant P uptake more effectively than any individual factor alone (Achat et al. 2016).

Microbial inoculants also operated within this structurally mediated framework rather than independently of it. The effectiveness of microbial inoculants is strongly influenced by pore architecture, soil moisture, and pH conditions (Richardson and Simpson 2011). In our study, the synergistic response observed most prominently in the A+ treatment likely resulted from the interaction between improved soil structural conditions created through loosening and enhanced microbial phosphorus mobilisation (Fig. 3a, Table 2). Thus, the combined effects of physical soil modification and microbial activity produced greater benefits than either factor acting independently (Richardson and Simpson 2011).

Soil available P is also closely linked with other nutrients. It is influenced by macronutrients such as nitrogen and micronutrients including Zn, Cu, and S (Alloway 2008; Vitousek et al. 2010).

In the present study, Olsen P and Zn showed a strong positive correlation (Fig. 4). This finding contrasts with the well-established observation that phosphorus (P) and zinc (Zn) are often negatively related in soils (Graham and Rengel 1993). This antagonism is commonly attributed to the precipitation of insoluble zinc phosphate compounds under high P availability (Recena et al. 2021). However, positive relationships between P and Zn can occur under biologically active conditions. In our study, this pattern is likely driven by microbial processes. The inoculated bacteria (*Pseudomonas putida*, *Pseudomonas fluorescens*, *Bacillus megaterium*, and *Bacillus subtilis*) are known to solubilise both phosphate and zinc compounds. They produce organic acids and reduce rhizosphere pH, which enhances the release of both nutrients from insoluble soil pools (Upadhyay et al. 2025). This results in simultaneous increases in P and Zn availability. Our findings are consistent with research of Tang et al. 2025, who also reported a positive association between Zn and available P in agricultural soils.

Therefore, it is important to consider the complex interactions within the soil matrix when designing microbial inoculants or implementing new management strategies in organic agroecosystems. Although our study validates this interconnection, further research is needed to explore these relationships across diverse ecosystems and management practices.

CONCLUSION

This study demonstrates that phosphorus bioavailability in agroecological lettuce systems is governed by a complex interaction of soil chemical, physical, and biological attributes shaped by management practices. The Olsen P method proved to be the most reliable indicator of plant-available phosphorus, outperforming conventional extraction techniques, the total P method by capturing the dynamic equilibrium between labile inorganic pools and biologically mediated organic transformations. Its sensitivity to microbial activity and soil structure makes it particularly suited for low-input, biologically active systems where nutrient cycling depends on microbial and root interactions rather than intensive fertilization.

No-till management preserved soil aggregation and microbial integrity, creating conditions that enhanced phosphorus mobilization through biological pathways and increased plant-available phosphorus. In contrast, soil loosening increased total soil phosphorus but did not improve plant phosphorus uptake.

Microbial inoculation further amplified phosphorus uptake by reinforcing the functional connectivity among soil biota, organic matter, and mineral interfaces. Collectively, these findings highlight that sustainable phosphorus management in agroecological systems requires integrating biological and physical soil processes rather than relying solely on chemical indices. Future research should refine predictive models linking Olsen P with microbial functional traits and explore adaptive nutrient strategies that maintain soil fertility while minimizing external phosphorus inputs.

DECLARATIONS

ETHICS DECLARATION

Consent statement

Not applicable.

Health and safety statement

All laboratory and field procedures were conducted in compliance with standard institutional health and safety guidelines.

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

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Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were primarily performed by Jana Budimir-Marjanović and Apolka Uji, with support from the co-authors.

Specific contributions include:

- Statistical Analysis & Results: Shamsul Islam Shipar
- Data Collection & Analysis Support: Alhaji Alusine Kebe, Gideon Adu Donyina, Pias Roy, and Daniel Brhane
- Manuscript Preparation: The first draft was collaboratively written by all authors, with everyone providing comments on subsequent versions.

All authors read and approved the final manuscript.

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Data Availability

The datasets generated and analyzed during the current study are available from the authors on reasonable request.

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Figures

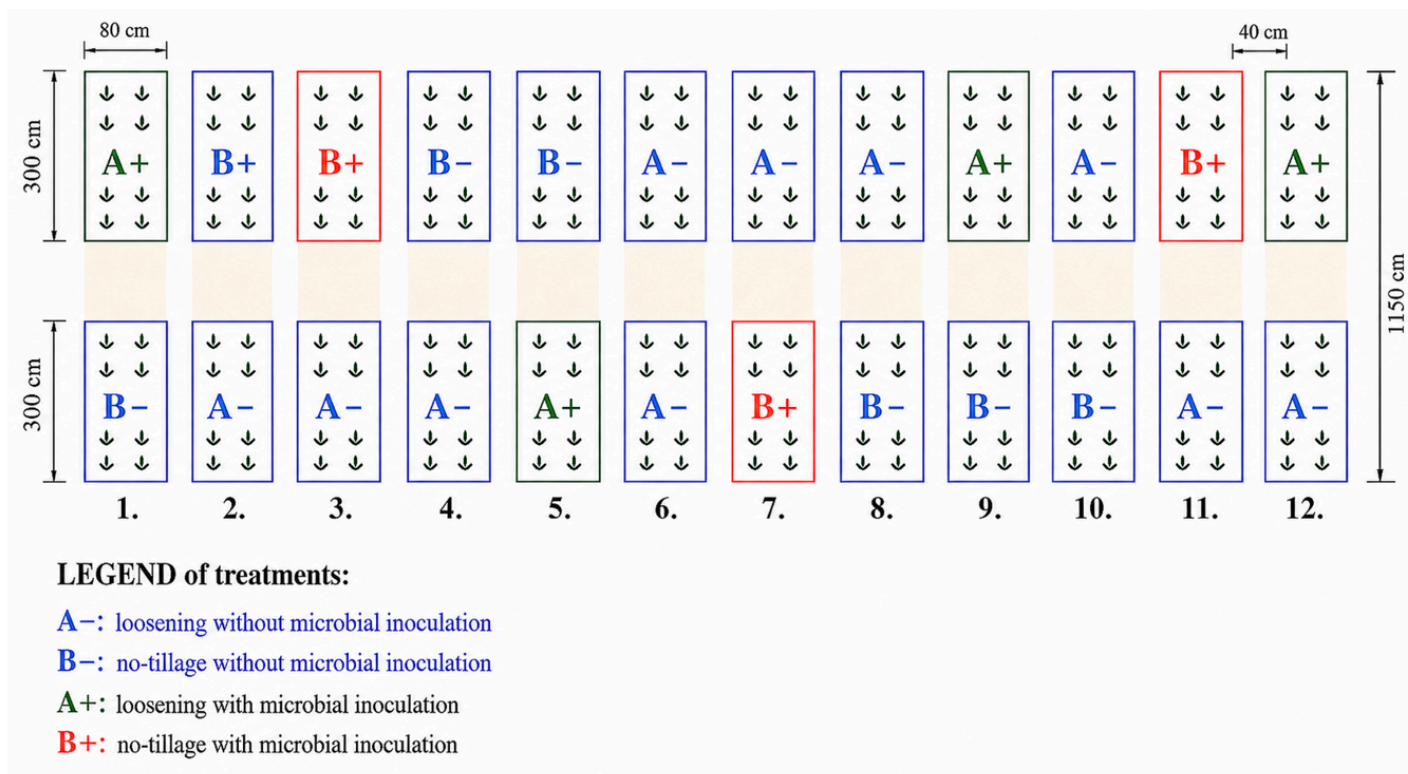


Figure 1

Spatial arrangement of the experimental field plots

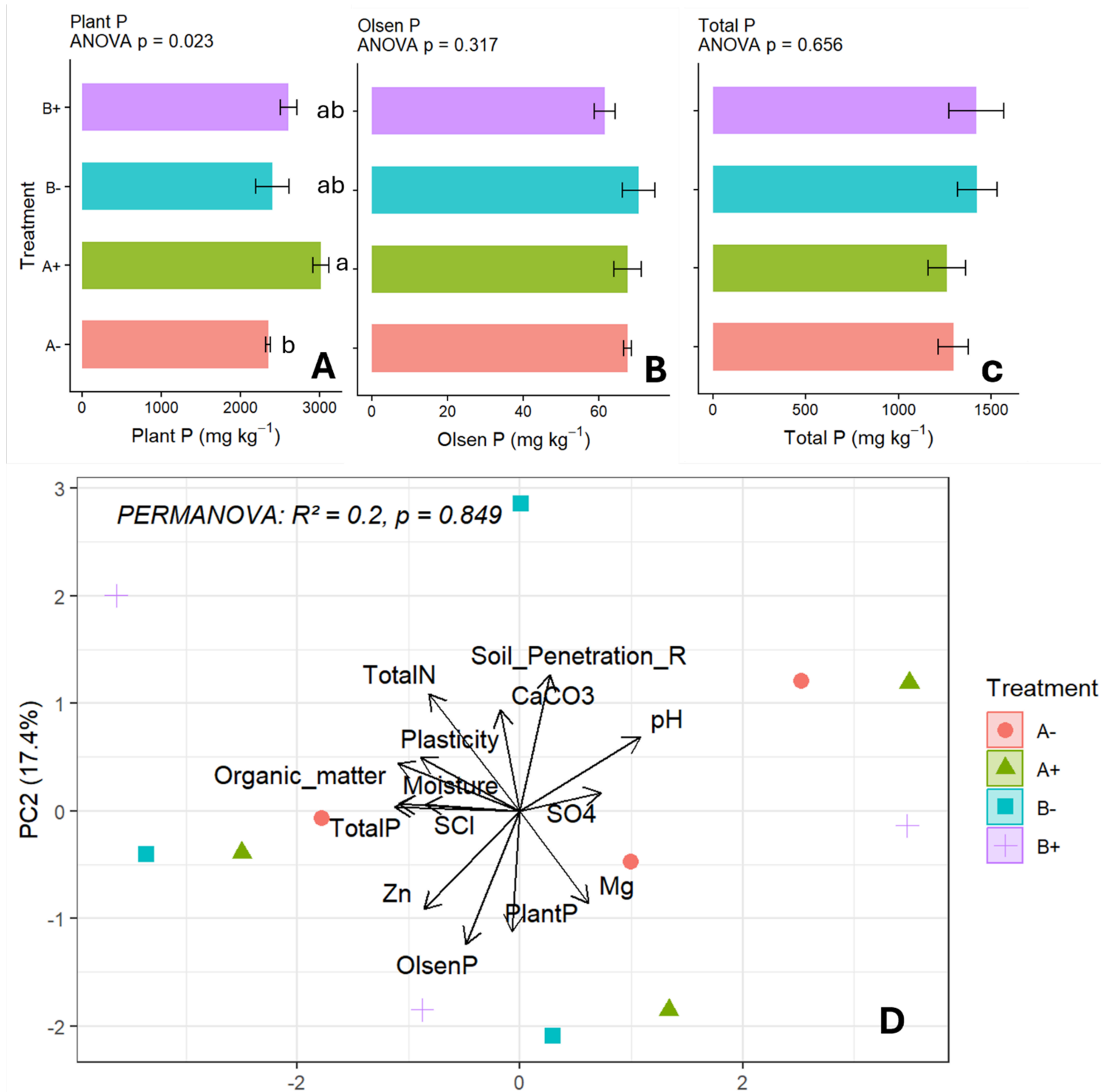


Figure 2

Effects of soil tillage and microbial inoculation on phosphorus dynamics in lettuce and soil. (a) Phosphorus content in dry lettuce (Plant P), (b) Olsen-extractable phosphorus in soil (Olsen P), and (c) total phosphorus in soil (Total P) across four treatments: A- (loosening without microbes), B- (no tillage without microbes), A+ (loosening with microbes), and B+ (no tillage with microbes). Bars represent mean $\pm$ standard error. ANOVA p-values are indicated for each variable; where significant, Tukey's HSD letters denote differences among treatments (treatments sharing the same letter are not significantly different). (d) Principal

component analysis (PCA) biplot showing relationships among soil chemical and physical properties (vectors) and treatment groups (points), with PERMANOVA results and associated p-value.

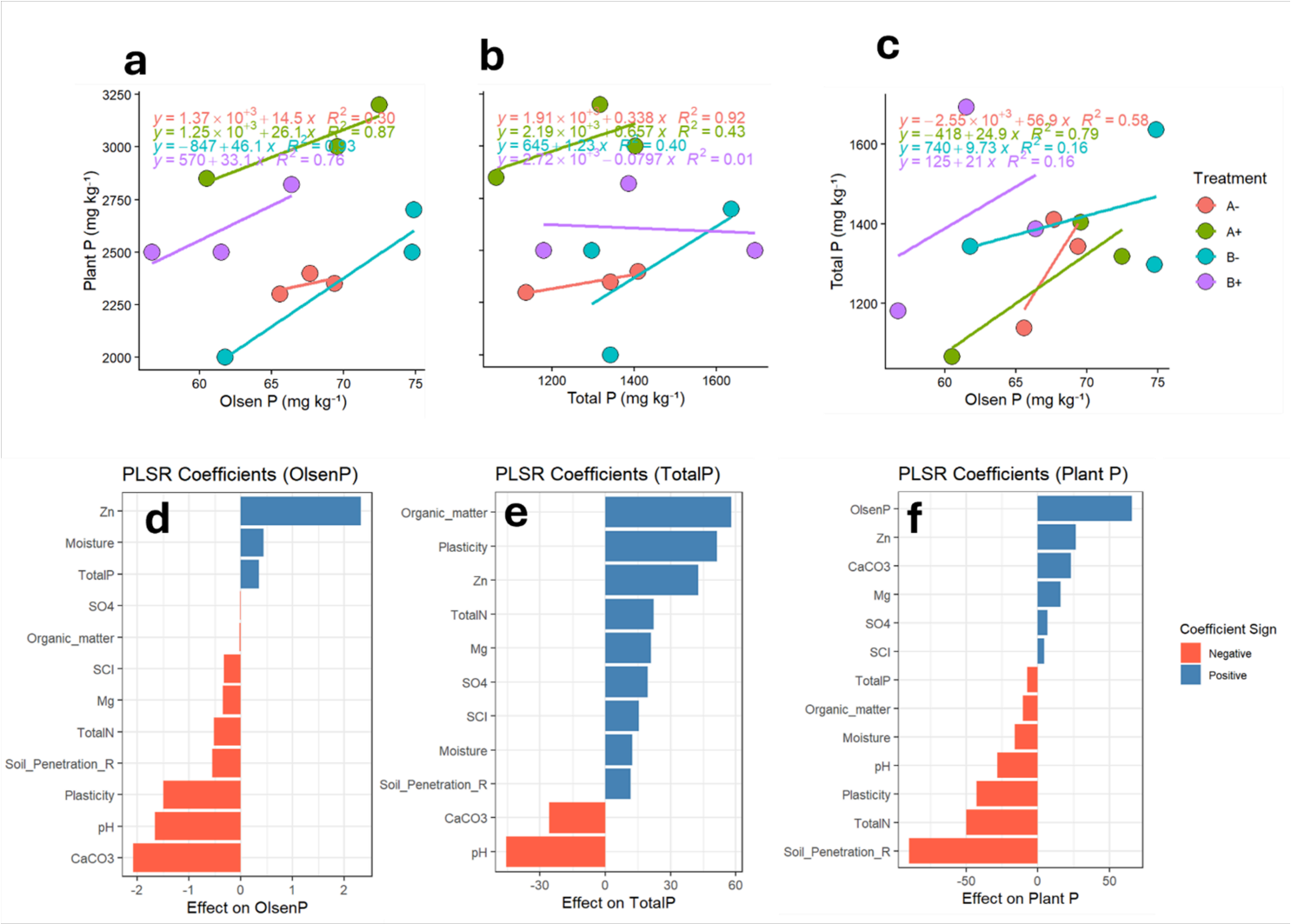


Figure 3

Relationships between soil phosphorus fractions and plant phosphorus uptake under different treatments, along with Partial Least Squares Regression (PLSR) analyses. (a) Relationship between Olsen P and Plant P, (b) Total P and Plant P, and (c) Olsen and Total across treatments (A-: loosening without microbes, A+: loosening with microbes, B-: no tillage without microbes, B+: no tillage with microbes). (d–f) PLSR coefficients showing the relative influence of soil physicochemical properties on (d) Olsen P, (e) Total P, and (f) Plant P. Positive (blue) and negative (red) bars indicate the direction and magnitude of variable effects.

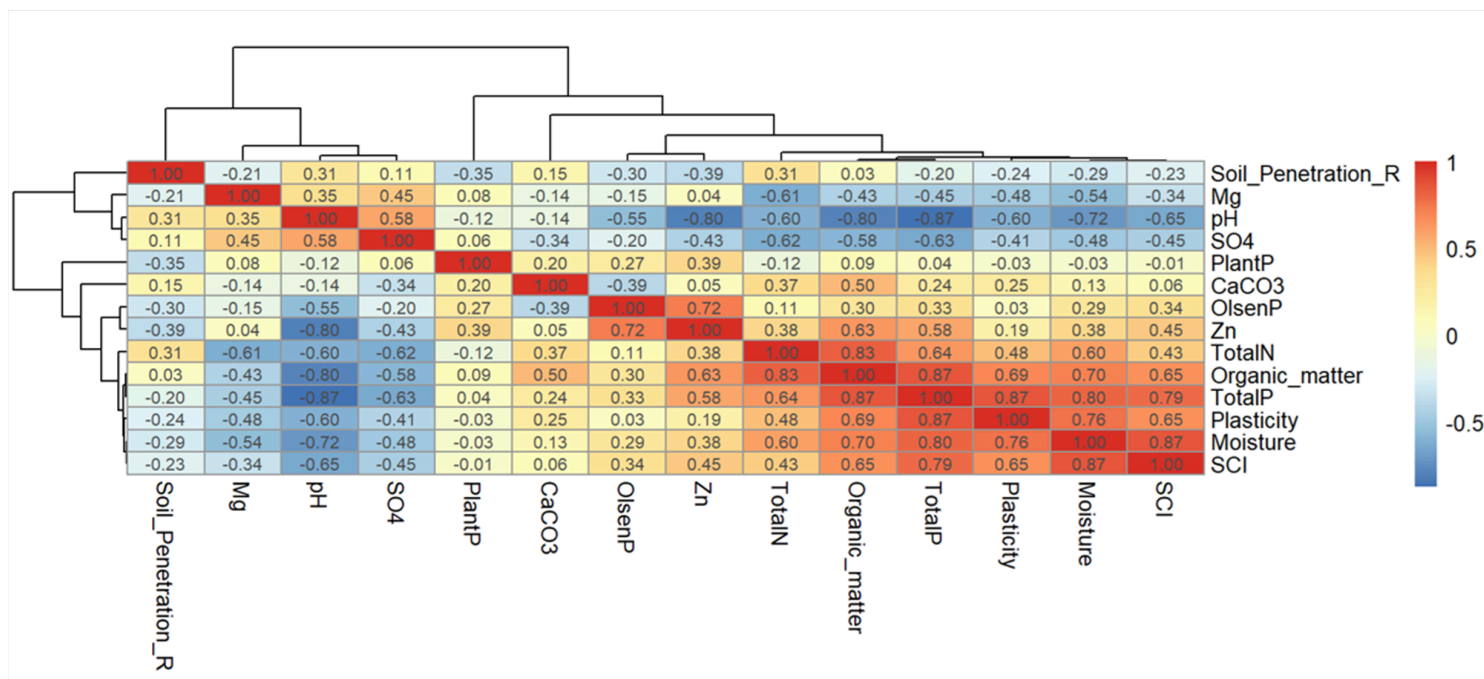


Figure 4

Correlation heatmap with hierarchical clustering of soil physical, chemical, and fertility-related variables, including soil penetration resistance (Soil_Penetration_R), Mg, pH, SO₄, plant-available P (PlantP), CaCO₃, Olsen-P, Zn, total N (TotalN), organic matter, soil total P (TotalP), plasticity, moisture, and soil cumulative infiltration (SCI). Dendrograms indicate similarity-based clustering among variables.

Supplementary Files

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