Effects of Fertilization Depth on Vertical Distribution of Maize Root System and Rhizosphere Soil Fertility in Raw Soil (Postprint)

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Abstract

To investigate the effects of fertilization depth on aboveground productivity, root system, and rhizosphere soil fertility of maize (Zea mays L.) in raw soil, a two-year continuous experiment was conducted using loess parent material raw soil as the test soil. Employing the root tube soil column method and no fertilization as the control, we studied the effects of applying bio-organic fertilizer at different depths (0-20 cm, 60-80 cm, 100-120 cm, 140-160 cm, and 180-200 cm) on aboveground productivity, root weight, rhizosphere soil enzyme activities, and vertical distribution of rhizosphere soil nutrient contents. The results showed that: 1) Within the 0-200 cm soil profile, both aboveground productivity and total root weight of maize initially increased then decreased with increasing fertilization depth, reaching maxima of 52.3 g and 361.0 g, respectively, at the 100-120 cm depth. 2) The vertical distribution of root weight exhibited a 'T' -shaped pattern across all fertilization depths, with the 0-20 cm tillage layer accounting for approximately 50% of total root weight and decreasing significantly with depth ($P<0.05$). The 100-120 cm fertilization depth yielded the maximum total root weight and root weight in the 0-40 cm tillage layer (27.19 g). Root N, P, and K accumulation was moderate, averaging $6.60\ \mathrm{g \cdot kg^{-1}}$, $2.38\ \mathrm{g \cdot kg^{-1}}$, and $8.16\ \mathrm{g \cdot kg^{-1}}$, respectively. 3) Fertilization significantly enhanced rhizosphere soil enzyme activities and nutrient contents. At 60-80 cm depth, rhizosphere soil urease activity in the 0-200 cm layer ranged from 0.108-0.354 $\mathrm{mg(NH_3\text{-}N) \cdot g^{-1}(soil) \cdot 24h^{-1}}$. At 140-160 cm depth, rhizosphere soil sucrase activity and available phosphorus content were 12.9-19.6 $\mathrm{mg(glucose) \cdot g^{-1}(soil) \cdot 24h^{-1}}$ and 4.31-6.02 $\mathrm{mg \cdot kg^{-1}}$, respectively. At 180-200 cm depth, rhizosphere soil organic matter content ranged from 5.55-7.14 $\mathrm{g \cdot kg^{-1}}$. At depths <100 cm or >120 cm, rhizosphere soil alkaline phosphatase activity and alkali-hydrolyzable nitrogen content in the 0-20 cm layer were >0.497 $\mathrm{mg(phenol) \cdot g^{-1}(soil) \cdot 24h^{-1}}$ and

>25.4 mg·kg^{-1}, respectively. 4) Correlation analysis revealed close relationships among maize root weight, root NPK nutrition, rhizosphere soil enzyme activities, and rhizosphere soil NPK nutrition under different fertilization depths in raw soil. 5) Based on FACTOR process and CLUSTER analysis, the optimal fertilization depth range for improving the maize canopy-root-soil system in loess parent material raw soil was 60-160 cm. These results provide a novel approach for accelerating raw soil maturation through fertilization.

Full Text

Effect of Fertilization Depth on Maize Root and Rhizosphere Soil Fertility Vertical Distribution in Immature Loess Subsoil*

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Abstract

This study investigated the effects of fertilization depth on maize (Zea mays L.) productivity, root growth, and rhizosphere soil fertility in immature loess subsoil. Using a root-tube soil column method, a two-year experiment was conducted with loess parent material as the test soil. Bio-organic fertilizer was applied at different depths (0-20 cm, 60-80 cm, 100-120 cm, 140-160 cm, and 180-200 cm), with a no-fertilizer treatment as control. The results showed: (1) Across the 0-200 cm soil profile, maize productivity and total root weight initially increased then decreased with increasing fertilization depth, peaking at 100-120 cm depth (total root weight: 52.3 g; aboveground productivity: 361.0 g). (2) All fertilization treatments exhibited a "T-shaped" vertical root distribution, with approximately 50% of total root weight concentrated in the 0-20 cm surface layer, decreasing significantly with depth ($P<0.05$). The 100-120 cm fertilization depth produced both the maximum total root weight and the highest root weight in the 0-40 cm layer (27.19 g). Root N, P, and K accumulation were moderate, averaging 6.60 g·kg^{-1}, 2.38 g·kg^{-1}, and 8.16 g·kg^{-1}, respectively. (3) Fertilization significantly enhanced rhizosphere soil enzyme activities and nutrient contents. The 60-80 cm depth showed higher urease activity (0.108-0.354 mg(NH_3-N)·g^{-1}(soil)·24h^{-1}) throughout the 0-200 cm profile. The 140-160 cm depth increased sucrase activity and available phosphorus (12.9-19.6 mg(glucose)·g^{-1}(soil)·24h^{-1} and 4.31-6.02 mg·kg^{-1}, respectively). The 180-200 cm depth elevated organic matter content (5.55-7.14 g·kg^{-1}). Fertilization depths shallower than 100 cm or deeper than 120 cm resulted in higher alkaline phosphatase activity (>0.497 mg(phenol)·g^{-1}(soil)·24h^{-1}) and available nitrogen content (>25.4 mg·kg^{-1}) in the 0-20 cm rhizosphere. (4) Correlation analysis revealed significant relationships among root weight, root NPK nutrition, rhizosphere enzyme activities, and soil nutrients under different fertilization depths. (5) Based on FACTOR and CLUSTER analyses, the optimal fertilization depth range for improving the maize shoot-root-soil system in immature loess subsoil was 60-160 cm. These findings provide new insights for accelerating immature soil maturation through strategic fertilization depth management.

Keywords: Fertilization depth; Immature loess subsoil; Maize; Productivity; Root distribution; Rhizosphere soil fertility

Introduction

The Loess Plateau region of China suffers from severe soil erosion, resulting in thin surface tillage layers with low nutrient content. As agricultural intensification and management practices bring loess parent material from beneath the tillage layer to the surface, crop yields are inevitably affected. Fertilization represents the most fundamental technical measure for increasing crop yields and improving soil fertility. A critical research question for this region is how to resolve the relationship between fertilization and yield in loess parent material environments while promoting soil maturation through crop growth itself.

Previous research demonstrates that organic fertilizers, containing essential nutrients and abundant biologically active substances, can both provide nutrients for plant growth and improve soil conditions. Long-term application of organic and chemical fertilizers has shown sustained benefits for crop yield and soil organic carbon and nitrogen content. However, beyond certain yield levels, continued high-rate fertilization does not further increase crop production. While increased organic fertilizer application promotes soil organic carbon and nitrogen accumulation, equivalent increases in chemical fertilizer do not. Fertilization effectively improves nutrient content in lime concretion black soil, with organic manure showing stronger effects on organic matter, total nitrogen, and available potassium, while chemical fertilizers more effectively increase available phosphorus. Long-term fertilization, particularly with organic manure, significantly enhances active organic carbon and nitrogen in both surface and subsurface layers of black soil, increases urease, catalase, and sucrase activities (though not acid or neutral phosphatase), and promotes the formation of soil aggregates while reducing bulk density and increasing organic carbon storage in the 0-20 cm layer. Organic fertilizer substitution for chemical fertilizer not only maintains cotton yield in drip-irrigated fields in northern Xinjiang but also significantly improves soil enzyme activities and modulates microbial community structure. Continuous organic manure application is essential for maintaining or enhancing soil organic carbon levels, carbon pool management indices, and overall soil fertility.

Our previous research demonstrated that organic fertilizer application produces immediate effects on improving immature loess soil and achieving optimal millet yield, while robust crop root systems (such as maize) contribute to enhanced soil fertility. Maize (Zea mays) holds significant importance in industrial and agricultural production worldwide as a vital industrial raw material, excellent feedstock, and valuable economic crop, and is commonly cultivated in the Loess Plateau. Numerous studies have examined the effects of fertilizer type, application rate, and timing on maize yield, root growth, and soil fertility formation, with fertilization depths typically limited to surface broadcasting or 20 cm furrow application. While furrow application at 20 cm reduces nutrient loss and improves fertilizer use efficiency compared to surface broadcasting, whether this depth represents the optimal fertilization depth for maximizing crop yield and soil fertility remains uncertain. Building on our previous research, this study used maize as the test crop and Bete bio-organic fertilizer (a chicken manure compost) to investigate how fertilization depth affects maize root systems, rhizosphere enzyme activities, and nutrient content under simulated immature soil conditions. The objective was to determine the optimal fertilization depth for improving loess parent material soil fertility while maximizing maize yield, providing theoretical guidance for immature soil improvement.

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1. Materials and Methods

1.1 Study Area and Experimental Materials The experiment was conducted from April 2012 to December 2013 at the Loess Plateau Crop Research Institute of Shanxi Agricultural University. The research site is located in Taigu County, Shanxi Province (112°35 E, 37°25 N, altitude 800 m), within the Jinzhong Basin of central Shanxi. The region experiences a warm temperate continental climate with an average annual temperature of 9.8°C, annual precipitation of 456 mm, 2,600 sunshine hours annually, $ $10°C accumulated temperature of 3,520°C, and a frost-free period of 176 days, providing abundant light and heat resources. The climate is characterized by dry and windy springs, hot summers, concentrated rainfall in autumn, and cold, dry winters with minimal snowfall. From December 1 to early March (around the Awakening of Insects), the average monthly temperature is approximately -4.5°C. The main crops include winter wheat, spring maize, spring soybean, and cabbage, with one harvest per year. The soil type is calcareous cinnamon soil developed from loess parent material.

The maize variety used was ‘Jiyuan 1’ , a high-quality, high-yielding, disease-resistant, early-mid maturity cultivar provided by Yingxian Seed Company, Shanxi Province. The test soil was calcareous cinnamon soil developed from loess parent material, collected from below 3 m depth at a construction site on the Shanxi Agricultural University experimental farm. The soil was air-dried, sieved, and thoroughly mixed. The parent material had the following properties: total nitrogen 0.19 $g \cdot kg^{-1}$, available nitrogen 19.84 $mg \cdot kg^{-1}$, available phosphorus 2.98 $mg \cdot kg^{-1}$, available potassium 30.32 $mg \cdot kg^{-1}$, organic matter

1.17 g $\cdot$ kg^{-1}, pH 8.0. The native soil enzyme activities were: urease 0.29 mg $\cdot$ g^{-1}(soil) $\cdot$ 24h^{-1}, sucrase 1.27 mg $\cdot$ g^{-1}(soil) $\cdot$ 24h^{-1}, and alkaline phosphatase 0.71 mg $\cdot$ g^{-1}(soil) $\cdot$ 24h^{-1}.

1.2 Experimental Design and Methods Root-tube soil column method. Root tubes were specially constructed from rigid PE plastic (25 cm diameter × 200 cm height) with 1 cm wall thickness. Each tube was vertically cut into two halves, with steel plates and rings installed at both ends and the middle to allow reassembly and fixation. After assembly, tubes were placed vertically in a root chamber with the bottom sealed with thick plastic. The test soil was packed in 20 cm increments, with each layer weighing approximately 10 kg. For fertilization treatments, soil from the target depth was thoroughly mixed with fertilizer before packing.

The experiment included five fertilization depths: 0–20 cm, 60–80 cm, 100–120 cm, 140–160 cm, and 180–200 cm. Bete bio-organic fertilizer (a chicken manure compost containing $ $45% organic matter and $ 5{2}O{5}+K_{2}O)wasappliedat18gpertube(equivalentto3,200kg · ha^{-1}$). A no-fertilizer control (CK) was included. Each treatment was replicated three times. On April 20, 2012, tubes were thoroughly watered and three maize seeds were sown per tube. After emergence, one healthy seedling was retained per tube with regular watering.

Sampling method. At maize maturity, tubes were removed and aboveground parts were harvested to measure stem dry weight, total grain weight, and 100-grain weight. Tubes were then opened, and rhizosphere soil samples were carefully collected from layers at 0–20 cm, 20–40 cm, 40–80 cm, 80–120 cm, 120–160 cm, and 160–200 cm using sterilized toothpicks, placed in sterilized plastic bags for enzyme and nutrient analysis. After soil collection, remaining soil columns were washed to expose intact root systems, which were air-dried, photographed, then sectioned according to soil sampling depths, weighed, crushed, and bagged for root NPK content determination.

1.3 Measurement Methods Root and shoot weights were determined by direct air-drying and weighing.

Root NPK content was measured using combined plant NPK analysis. Root samples were digested with H_2SO_4-H_2O_2. Total nitrogen was determined by semi-micro Kjeldahl method, total phosphorus by vanadium-molybdate yellow colorimetry, and total potassium by flame photometry.

Soil enzyme activities were measured on air-dried and sieved samples: urease by indophenol colorimetry, alkaline phosphatase by disodium phenyl phosphate colorimetry, and sucrase by 3,5-dinitrosalicylic acid colorimetry.

Soil nutrients were measured on air-dried and sieved samples: organic matter by potassium dichromate volumetric method with external heating, available nitrogen by alkali-hydrolysis diffusion method, and available phosphorus by 0.5 $\mathrm{mol \cdot L^{-1}}$ $NaHCO_3$ extraction with molybdenum-antimony anti-colorimetry.

1.4 Data Analysis All data presented are from the 2012 experiment. Data were processed using Microsoft Excel and graphed with SigmaPlot 10.0. Statistical analysis was performed using SAS 9.1.3 software: ANOVA for variance analysis and multiple comparisons, CORR for correlation analysis, FACTOR for principal component analysis, and hierarchical cluster analysis. Results are expressed as mean ± standard error.

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2. Results

2.1 Effects of Fertilization Depth on Maize Aboveground Productivity The effects of fertilization depth on total root weight and aboveground productivity are shown in . Across the 0–200 cm soil profile, stem dry weight, total grain weight, 100-grain weight, and total root weight generally increased initially then decreased with increasing fertilization depth, with most indices showing significant differences ($P<0.05$) except total grain weight and total aboveground dry weight. Root weight showed the greatest response to fertilization depth (CV=25.4%), while total grain weight showed the least (CV=4.9%). In immature soil, maximum root weight and aboveground productivity occurred at 100–120 cm fertilization depth. The results also indicated that better root growth promoted higher aboveground productivity, suggesting that root growth regulation is a viable approach for improving productivity in immature soils. Notably, surface fertilization at 0–20 cm produced inferior results compared to the unfertilized control, with no significant differences between them.

2.2 Effects of Fertilization Depth on Vertical Distribution of Maize Root Weight The vertical distribution of maize root weight across 0–200 cm under different fertilization depths is presented in and . All fertilization treatments exhibited a "T-shaped" vertical root distribution, with maximum root weight in the 0–20 cm layer (approximately 50% of total root weight), decreasing significantly with depth ($P<0.05$). Within the 0–200 cm profile, vertical root distribution showed high variability (70.80%-159.46% CV), with the greatest variation at 180–200 cm depth and the least at 100–120 cm. Overall, fertilization reduced vertical distribution differences in the 0–160 cm range, with variation coefficients initially decreasing then increasing with fertilization depth.

Within individual soil layers, fertilization depth caused root weight differences of 27.35%-67.52%. Generally, root weight in all layers, particularly within 0-160 cm, increased initially then decreased with fertilization depth. Compared with the unfertilized control, fertilization at 0–80 cm significantly reduced both total root weight and root weight in the 0–20 cm layer. Based on total root weight and 0–20 cm root weight, the 100–120 cm fertilization depth was most beneficial for root growth.

Compared with the control, increasing fertilization depth from 0-20 cm to 140-160 cm significantly increased root weight and its proportion in middle and lower soil layers, demonstrating root gravitropism toward nutrients. The optimal fertilization depth for maize root growth was determined to be 100-120 cm.

2.3 Effects of Fertilization Depth on Vertical Distribution of Maize Root NPK Content Root NPK content vertical distribution under different fertilization depths is shown in . Within each fertilization treatment, root NPK content varied significantly with soil depth ($P<0.05$), except for total N at 180-200 cm depth and in the control. Coefficients of variation were 8.41%-30.76% for total N, 20.16%-46.43% for total P, and 20.37%-45.62% for total K, with P and K showing greater variation than N. Across fertilization depths within the same layer, variation coefficients were 7.61%-21.91% for N, 26.00%-58.38% for P, and 12.75%-43.84% for K, again with P and K showing greater variation.

Specifically, in the 0-20 cm layer, fertilization significantly reduced P accumulation compared with the control. With increasing fertilization depth, root N and P accumulation increased within the same layer, while K accumulation increased initially then decreased sharply. In all treatments, N and P contents were higher in roots below 20 cm than in surface roots, while K content was highest in the 0-20 cm layer, likely because the surface layer contained more support roots while absorptive roots were predominantly below 20 cm. Combined with root weight and productivity data, both excessive and deficient root NPK accumulation failed to produce maximum productivity and root weight.

2.4 Effects of Fertilization Depth on Vertical Distribution of Maize Rhizosphere Soil Enzyme Activity Rhizosphere soil urease, sucrase, and alkaline phosphatase activities under different fertilization depths are presented in . Background soil enzyme activities were 0.058 $\mathrm{mg(NH_3\text{-}N)\cdot g^{-1}(soil)\cdot 24h^{-1}}$ for urease, 0.270 $\mathrm{mg(phenol)\cdot g^{-1}(soil)\cdot 24h^{-1}}$ for alkaline phosphatase, and 7.2 $\mathrm{mg(glucose)\cdot g^{-1}(soil)\cdot 24h^{-1}}$ for sucrase. Key findings include: (1) All fertilization treatments significantly increased enzyme activities in the 0-20 cm layer compared with background soil ($P<0.05$). (2) Fertilization at 0-160 cm significantly increased urease activity across all layers compared with the control and 180-200 cm treatment ($P<0.05$). (3) Regardless of fertilization, all treatments showed highest enzyme activities in the 0-20 cm rhizosphere (except sucrase at 100-120 cm). (4) Enzyme activities in fertilized layers were significantly higher than in adjacent layers, mirroring root distribution patterns and indicating strong fertilization effects on root growth and rhizosphere enzyme activity. (5) Among the five depths, 60-80 cm was optimal for enhancing urease activity, followed by 140-160 cm and 0-20 cm. The 140-160 cm depth was most effective for sucrase activity, followed by 0-20 cm. Fertilization depths shallower or deeper than 100-120 cm favored alkaline phosphatase activity in the 0-20 cm layer. (6) Variation in enzyme activities due to fertilization depth reached approximately 60%, 20%, and 40% for urease, alkaline phosphatase, and sucrase, respectively. Variation due to soil depth within the same fertilization treatment was 13.6%-71.0% for urease, 25.0%-51.5% for alkaline phosphatase, and 7.8%-28.2% for sucrase. Most fertilization treatments reduced depth-related variation in urease but increased variation in alkaline phosphatase and sucrase.

Overall, except for urease activity at 180-200 cm (which was slightly lower than the control), all fertilization treatments significantly increased rhizosphere enzyme activities. The optimal depths were 60-80 cm for urease and 140-160 cm for sucrase.

2.5 Effects of Fertilization Depth on Vertical Distribution of Maize Rhizosphere Soil Nutrients Rhizosphere soil available nitrogen, available phosphorus, and organic matter contents under different fertilization depths are shown in . Background soil values were 10.0 $\mathrm{mg \cdot kg^{-1}}$ for available nitrogen, 1.56 $\mathrm{mg \cdot kg^{-1}}$ for available phosphorus, and 2.93 $\mathrm{g \cdot kg^{-1}}$ for organic matter. Key findings include: (1) All fertilization treatments significantly increased all three nutrients compared with background soil. (2) Available nitrogen in the 0-20 cm layer was significantly higher in all fertilization treatments than in the control ($P<0.05$). Available phosphorus and organic matter were also significantly higher in most layers ($P<0.05$). (3) Without fertilization, all three nutrients generally decreased with depth, but fertilization altered this trend, particularly increasing nutrients in or near the fertilized layer. (4) For available nitrogen, depths shallower or deeper than 100-120 cm increased 0-20 cm content, but showed no advantage below 20 cm compared with the control. For available phosphorus, all depths increased content throughout the profile, with 140-160 cm being most effective. For organic matter, all depths increased content, with 180-200 cm showing the best results. (5) Variation due to fertilization depth within the same layer was generally available nitrogen > available phosphorus > organic matter. Variation due to soil depth within the same treatment followed a similar pattern, with fertilization depth causing greater variation than soil depth.

In summary, organic fertilizer application promoted maize root growth, and the combined effects of organic fertilizer and maize roots enhanced rhizosphere available phosphorus and organic matter content in immature soil.

2.6 Correlation Analysis Among Root Weight, Root Nutrition, Rhizosphere Enzyme Activities, and Soil Nutrients Correlation analysis was performed among 10 indices: root weight, root NPK nutrition, rhizosphere urease, sucrase, alkaline phosphatase, and rhizosphere available nitrogen, available phosphorus, and organic matter across different fertilization depths and soil layers. Results are shown in . Root weight was significantly ($P<0.05$) or highly significantly ($P<0.001$) negatively correlated with root N and P nutrition, but highly significantly positively correlated with root K nutrition ($P<0.001$). Root weight was also highly significantly positively correlated with rhizosphere alkaline phosphatase activity ($P<0.001$), possibly due to phosphatase secretion by maize roots.

Root N and P nutrition were highly significantly positively correlated ($P<0.01$), while both were highly significantly negatively correlated with root K nutrition ($P<0.001$). Root N and P nutrition were significantly or highly significantly negatively correlated with all three rhizosphere enzyme activities, whereas root K nutrition was positively correlated with urease and alkaline phosphatase activities ($P<0.05$) but not significantly with sucrase ($P>0.05$). Additionally, root N and P nutrition were highly significantly negatively correlated with rhizosphere available phosphorus content ($P<0.01$). These results suggest that excessive root N and P accumulation may inhibit soil enzyme activity, while K accumulation may enhance enzyme activity through ion pump or activation mechanisms.

Increased rhizosphere urease activity promoted enhancement of the other two enzyme activities ($P<0.01$, $P<0.001$) and increased available phosphorus content ($P<0.05$). Increased alkaline phosphatase activity enhanced organic matter content, while increased sucrase activity improved available phosphorus content. Thus, the three enzyme activities were closely related to the three nutrient categories.

To identify the key factors influencing the root-soil system, the 10 indices were analyzed using SAS 9.1.3 FACTOR procedure . The primary factors affecting the maize root-soil system in immature soil were root weight, root N nutrition, root K nutrition, and rhizosphere phosphatase (Factor 1), followed by rhizosphere urease and sucrase (Factor 2), with available phosphorus belonging to Factor 3.

Based on factor importance and aboveground productivity indices (shoot dry weight, total grain weight, 100-grain weight), hierarchical cluster analysis was performed on the root-soil-productivity systems under different fertilization depths [Figure 1: see original paper]. The six treatments were first divided into two groups: four fertilization depths at 0–160 cm (Group I) and the 180–200 cm depth plus the unfertilized control (Group II). Group I was further subdivided into three categories: (a) 0–20 cm, (b) 60–80 cm and 140–160 cm, and (c) 100–120 cm. Based on these results, the suitable fertilization depth range for improving the maize root-soil system in immature loess subsoil was determined to be 60–160 cm.

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3. Conclusion and Discussion

Accelerating immature soil maturation to achieve immediate productivity in parent material soil through artificial fertilization that regulates interactions between soil and crop roots represents a crucial research topic for sustainable agriculture in the semi-arid Loess Plateau region. Previous research on the "fertilization-crop (root + productivity)-soil"relationship in loess parent material has focused primarily on conventional surface (0–20 cm) fertilization effects. Studies show that deep fertilization at 10–20 cm improves nutrient absorption efficiency compared with surface application, but whether deeper fertilization consistently enhances nutrient use efficiency, crop productivity, and soil fertility remains unclear.

This study investigated five fertilization depths (0-20 cm, 60-80 cm, 100-120 cm, 140-160 cm, and 180-200 cm) to examine their effects on maize productivity and vertical distribution of rhizosphere soil fertility in immature soil, providing theoretical guidance for soil improvement.

Miao et al. reported that without fertilization, maize root mass decreases exponentially from the soil surface downward (Y=A・e^(-BX)), with the greatest mass at the surface and decreasing amplitude at greater depths. They also found that fertilization at 20 cm promoted root penetration, 150-250 cm ultra-deep fertilization induced deep root growth, while 50 cm depth controlled root growth and created root clusters in the fertilized layer. Our study similarly found maximum root weight at 0-20 cm in the unfertilized control, decreasing with depth. All fertilization treatments showed T-shaped root distribution with approximately 50% of total root weight in the 0-20 cm layer. Compared with the control, fertilization at 0-80 cm significantly reduced total root weight and 0-20 cm root weight, possibly because shallow fertilization inhibited root extension—contrasting with Miao et al.' s findings. Deep fertilization induced downward root growth, consistent with their results. As fertilization depth increased from 0-20 cm to 140-160 cm, middle and lower layer root weight and proportions increased significantly, demonstrating root gravitropism toward nutrients. Maize, with its strong individual productivity, requires robust root support for aboveground growth. In the 0-120 cm range, stem dry weight, total grain weight, 100-grain weight, and total root weight all increased with fertilization depth, indicating that organic fertilizer promotes root growth and deep distribution, thereby enhancing aboveground productivity. Therefore, deeper fertilization encourages root growth into deeper soil layers, increasing total root mass and improving productivity. Both excessive and deficient root NPK accumulation failed to produce maximum root weight and productivity, with 100-120 cm identified as the optimal fertilization depth.

Soil enzymes in the maize rhizosphere are strongly affected by fertilization. Sun et al. reported that long-term combined NPK and organic fertilizer application significantly increased soil organic matter, total N and P, available nutrients, and enzyme activities. Our study found that deep fertilization produced higher enzyme activities than surface application. The 60-80 cm depth was optimal for urease activity, while 140-160 cm was best for sucrase activity, possibly because deep fertilization enhanced microbial activity in deeper soil layers.

Soil available nutrient content is a primary indicator of soil fertility, reflecting nutrient transformation capacity and management practices. Available nitrogen serves as an indicator of total available nitrogen supply, while phosphorus is a major element for crop growth. Our study found that all treatments significantly increased available nitrogen, phosphorus, and organic matter compared with background soil, demonstrating that fertilization and maize cultivation have ameliorative effects on immature soil. Without fertilization, all three nutrients decreased with depth, but fertilization altered this pattern, particularly increasing nutrients in or near the fertilized layer. Variation due to fertilization depth or soil depth generally followed the order: available nitrogen > available phosphorus > organic matter, indicating that fertilization profoundly affects rhizosphere nutrient content and soil fertility. Liu reported that long-term organic fertilizer application increases soil organic matter and various nutrients, improving soil structure and fertility, making fertilization a crucial agricultural practice for ensuring sustainable soil use. Appropriate fertilization depth is key to improving fertilizer efficiency.

In this study, increased rhizosphere urease activity promoted enhancement of other enzyme activities and available phosphorus content. Increased alkaline phosphatase activity enhanced organic matter content, while increased sucrase activity improved available phosphorus content. These relationships demonstrate close linkages among the three enzyme activities and three nutrient categories. Cluster analysis identified 60–160 cm as the suitable fertilization depth for improving the maize root-soil system in immature loess subsoil. Root biomass and plant nutrition are tightly associated with soil enzymes and nutrients. Increased root biomass enhances nutrient flow and distribution within roots while promoting rhizosphere enzyme activities and nutrient contents, ultimately improving soil fertility and ameliorating soil quality.

Given the large 40 cm interval between 0–20 cm and 60–80 cm depths compared with 20 cm intervals between other treatments, whether 20–60 cm depth might be superior to the identified 60–160 cm range requires further investigation, particularly with additional treatments at 20–40 cm and 40–60 cm. Additionally, current deep tillage-fertilization equipment typically reaches 40–60 cm maximum depth, raising questions about the feasibility of mechanized fertilization below 60 cm. For newly exposed immature soil, pre-plant fertilization followed by soil covering could be considered to achieve soil improvement and immediate productivity.

The maturation of loess parent material involves changes in physical structure and enhancement of nutrient content. Fertilization promotes robust root growth and frequent microbial activity, accelerating nutrient transformation and increasing plant-available nutrients. Post-harvest root residues decompose through microbial action, becoming nutrients for subsequent crops and thereby improving soil fertility. Therefore, appropriate fertilization that promotes deep root growth and enhances rhizosphere enzyme activities and nutrient contents is crucial for improving immature loess subsoil fertility.

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