

Effects of Biochar-Based Fertilizer Application on Phosphorus Characteristics in Karst Limestone Soil: Postprint

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Abstract

A pot experiment was conducted using karst mountainous lime soil from Guizhou Province as the research subject to investigate the effects of biochar-based fertilizers prepared from biochar (rice husk charcoal), pig manure compost, and NPK fertilizer on various soil phosphorus fractions, alkaline phosphatase activity, and black locust seedling biomass. The experiment included 10 treatments: CK, M, F, MF, RH1MF, RH2MF, RH4MF, RH8MF, RH4M, and RH4F (where CK denotes control, M denotes compost, F denotes chemical fertilizer, RH denotes rice husk charcoal, and the numerals indicate the proportion of biochar in the biochar-based fertilizer calculated on a biochar-soil mass ratio basis). Results demonstrated that biochar-based fertilizer application significantly enhanced total phosphorus, available phosphorus, organic phosphorus, and microbial biomass phosphorus contents in karst lime soil, as well as black locust seedling biomass, with the high biochar application rate treatment (RH8MF) exhibiting superior effects. Furthermore, the four different phosphorus fractions showed extremely significant positive correlations with each other ($P < 0.01$). Soil alkaline phosphatase activity peaked under medium biochar application rate treatments (RH2MF and RH4MF), increasing by 82.7% and 63.4% compared to CK, respectively. In conclusion, biochar-based fertilizer application, particularly at higher biochar application rates, can substantially improve phosphorus status in karst lime soil, mitigate low phosphorus conditions, and enhance the efficacy of ecological restoration in artificial young forest stands in karst mountainous regions.

Full Text

Preamble

Effects of Biochar-Based Fertilizer on Phosphorus Content of Karst Calcareous Soil

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Abstract

This study investigated the effects of biochar-based fertilizer (produced from rice-husk-derived biochar, pig manure compost, and NPK fertilizer) on various soil phosphorus fractions, soil alkaline phosphatase activity, and black locust seedling biomass using a pot experiment with karst calcareous soil from Guizhou Province. Ten treatments were established: CK (control), M (compost), F (NPK fertilizer), MF (compost + NPK), RH1MF (1% rice-husk biochar + compost + NPK), RH2MF (2% rice-husk biochar + compost + NPK), RH4MF (4% rice-husk biochar + compost + NPK), RH8MF (8% rice-husk biochar + compost + NPK), RH4M (4% rice-husk biochar + compost), and RH4F (4% rice-husk biochar + NPK), where the numbers represent the biochar-to-soil mass ratio in the biochar-based fertilizer.

Results showed that biochar-based fertilizer application significantly increased soil total phosphorus, available phosphorus, organic phosphorus, microbial biomass phosphorus, and seedling biomass, with the highest biochar application rate (RH8MF) showing the strongest effects. The four different phosphorus fractions exhibited highly significant positive correlations with each other ($P < 0.01$). Soil alkaline phosphatase activity was highest under medium biochar application rates (RH2MF and RH4MF), being 82.7% and 63.4% higher than CK, respectively. In conclusion, biochar-based fertilizer application, particularly at higher biochar rates, significantly improved phosphorus content in karst calcareous soil and ameliorated the low-phosphorus status, thereby enhancing ecological restoration outcomes in young artificial forests in karst mountainous areas.

Keywords: calcareous soil; biochar-based fertilizer; phosphorus; alkaline phosphatase

Introduction

Guizhou Province contains the world's most extensively developed continuous karst landscape, with karst outcrop areas covering 61.9% of the province's to-

tal land area. The ecological environment is extremely fragile. Soil erosion in karst regions accelerates the leaching of nutrient elements such as nitrogen and phosphorus, making rocky desertification and soil loss the primary ecosystem degradation problems, which in turn accelerate soil impoverishment. Previous research indicates that karst mountainous soils are alkaline calcareous soils with high calcium ion content, where phosphorus exists primarily as insoluble calcium phosphate salts, becoming a major limiting factor for soil nutrients and vegetation restoration.

While quick-release chemical fertilizers are widely used in farmland and forest lands to rapidly increase available nutrient content and improve seedling survival and growth, long-term application can lead to soil quality degradation and reduced soil enzyme activity. Organic fertilizer application, by contrast, sustainably increases available nutrient content and promotes soil enzyme activity and nutrient cycling. Biochar, used as a soil amendment and fertilizer slow-release carrier, has been widely applied in agricultural and forestry soil improvement. Studies show that biochar can increase soil organic matter content, improve water retention, reduce nitrogen and phosphorus losses, and enhance available phosphorus content. However, research on biochar application in karst calcareous soils, particularly regarding phosphorus dynamics, remains scarce.

This study employed pot experiments to investigate the effects of biochar-based fertilizers on different phosphorus fractions and alkaline phosphatase activity in karst calcareous soil, analyzing correlations among indicators to elucidate soil phosphorus cycling mechanisms and provide reference for restoration of young forest lands in karst mountainous areas.

1. Materials and Methods

1.1 Soil and Fertilizers

The experimental soil was collected from a Grain-for-Green project site in Baiyan Town, Puding County, Guizhou Province (105°27' -105°58' E, 26°9' -26°31' N). Puding County is a typical karst region with calcareous soil and an average soil thickness of 25 cm. Soil samples were taken from the surface cultivation layer (0-20 cm), air-dried, passed through a 2 mm sieve, and sterilized. Basic physicochemical properties were: pH = 7.72, bulk density = 1.48 g/cm³, total phosphorus = 502.96 mg/kg, available phosphorus = 5.40 mg/kg, organic phosphorus = 319.45 mg/kg, organic matter content = 14.44 g/kg, and microbial biomass phosphorus = 1.37 mg/kg.

The biochar was rice-husk-derived, purchased from Anhui Baierfu Biotechnology Co., Ltd. The bio-organic fertilizer was pig manure compost from Shanghai Shike Biological Technology Co., Ltd. The NPK fertilizer contained 12.2% N, 46.7% P O₅, and 0% K₂O. Biochar-based fertilizer was laboratory-prepared using a solid-liquid adsorption method: biochar, NPK fertilizer, and compost were dissolved in ultrapure water, thoroughly mixed, equilibrated for 24 h, and oven-dried to constant weight. Nutrient contents are shown in Table 1.

Physical and chemical properties of rice-husk-derived biochar and pig manure compost

1.2 Experimental Design

The pot experiment was conducted at Xiashu Forest Farm, Nanjing Forestry University in May 2016. Black locust (*Robinia pseudoacacia*) seedlings were grown and transplanted into pots (height 18 cm, diameter 15 cm) containing 2 kg of soil. Ten treatments were established: (1) CK (no amendment), (2) M (compost only), (3) F (NPK only), (4) MF (compost + NPK), (5) RH1MF (1% biochar + compost + NPK), (6) RH2MF (2% biochar + compost + NPK), (7) RH4MF (4% biochar + compost + NPK), (8) RH8MF (8% biochar + compost + NPK), (9) RH4M (4% biochar + compost), and (10) RH4F (4% biochar + NPK). All treatments received equal nitrogen amounts. Fertilizer types and quantities are listed in Table 2.

Types and quantities of fertilizers in different fertilizer treatments

Soil samples were collected from the pot surface layer in October 2016 after six months of growth.

1.3 Measurement Indicators and Methods

- **Total phosphorus:** Determined by alkali fusion-molybdenum antimony spectrophotometry
- **Available phosphorus:** Determined by NaHCO₃ extraction-molybdenum antimony spectrophotometry
- **Organic phosphorus:** Determined by ignition method using 1 mol/L H₂SO₄; final result was the difference between phosphorus extracted from ignited and non-ignited soil
- **Microbial biomass phosphorus:** Determined by chloroform fumigation extraction
- **Alkaline phosphatase activity:** Determined by disodium phenyl phosphate colorimetry

1.4 Data Processing

Data were analyzed using SPSS 19.0 and Excel 12.0. One-way ANOVA was performed, and Duncan's method was used for multiple comparisons.

2. Results

2.1 Effects of Biochar-Based Fertilizer on Different Phosphorus Fractions in Calcareous Soil

All biochar-based fertilizer treatments significantly increased soil total phosphorus content compared to CK ($P < 0.05$). The ranking of total phosphorus content was: MF > RH8MF > RH4M > RH2MF > RH4MF > RH4F > F

> RH1MF > M > CK, with increases of 45.0%, 44.3%, 40.6%, 39.9%, 34.8%, 32.7%, 31.7%, 25.3%, and 20.7%, respectively. The high biochar rate treatment (RH8MF) significantly increased soil total phosphorus, and biochar combined with compost or NPK was more effective than applying compost or NPK alone.

[Figure 1: see original paper] Effects of different fertilizer treatments on total phosphorus content in calcareous soil

Available phosphorus content also increased significantly under all biochar-based fertilizer treatments ($P < 0.05$). The ranking was: RH8MF > RH4MF > RH2MF > RH4M > RH1MF > MF > RH4F > M > F > CK. Available phosphorus increased with biochar application rate, with RH4MF, RH4M, and RH4F showing 45.8% and 21.9% increases compared to MF, demonstrating that biochar promotes phosphorus transformation and increases available phosphorus.

[Figure 2: see original paper] Effects of different fertilizer treatments on available phosphorus content in calcareous soil

Soil organic phosphorus content was significantly higher in all treatments compared to CK ($P < 0.05$). The ranking was: RH8MF > M > RH2MF > RH1MF > RH4MF > RH4M > MF > F > RH4F > CK, with increases of 43.6%, 27.3%, 27.3%, 26.2%, 25.1%, 24.1%, 22.2%, 14.1%, and 4.8%, respectively. The RH8MF treatment showed the greatest increase, and biochar combined with both compost and NPK was superior to biochar combined with either alone.

[Figure 3: see original paper] Effects of different fertilizer treatments on organic phosphorus content in calcareous soil

Microbial biomass phosphorus content was significantly higher under all biochar-based fertilizer treatments compared to CK ($P < 0.05$). The ranking was: RH8MF > RH4MF > RH4M > RH4F > M > RH2MF > F > MF > RH1MF > CK. Microbial biomass phosphorus increased with biochar application rate, and biochar combined with compost and NPK was more effective than biochar combined with either alone.

[Figure 4: see original paper] Effects of different fertilizer treatments on microbial biomass phosphorus content in calcareous soil

2.2 Effects on Alkaline Phosphatase Activity

Alkaline phosphatase activity increased under all biochar-based fertilizer treatments compared to CK, with significant differences observed in all treatments except RH1MF ($P < 0.05$). The ranking was: RH2MF > MF > RH4MF > RH8MF > RH4M > RH4F > M > F > RH1MF > CK. Medium biochar rate treatments (RH2MF, MF, RH4MF) showed the strongest promotion, with increases of 82.7%, 75.3%, and 63.4% over CK, respectively. High biochar rates appeared to inhibit alkaline phosphatase activity. The RH4MF treatment showed

better promotion than RH4M or RH4F, indicating that compost and NPK combined application was superior to single applications.

[Figure 5: see original paper] Effects of different fertilizer treatments on alkaline phosphatase activity in calcareous soil

2.3 Effects on Black Locust Seedling Biomass

All fertilization treatments increased seedling biomass compared to CK, with significant differences in aboveground and total biomass among treatments ($P < 0.05$). Biochar combined with compost showed the best effect on increasing seedling biomass. The RH4M treatment produced maximum aboveground, belowground, and total biomass, with increases of 275.2%, 139.8%, and 204.8%, respectively. Seedling biomass increased with biochar application rate, indicating that high biochar rates promote plant growth.

Effects of different fertilization treatments on black locust seedling biomass

2.4 Correlation Analysis Among Different Phosphorus Fractions

Correlation analysis revealed highly significant positive correlations among total phosphorus, available phosphorus, organic phosphorus, and microbial biomass phosphorus ($P < 0.01$). The correlation between available phosphorus and microbial biomass phosphorus was strongest ($r = 0.774$). Black locust total biomass showed significant positive correlations with all four phosphorus fractions ($P < 0.05$) and with alkaline phosphatase activity.

Correlation analysis among different indicators

3. Discussion

Phosphorus is an essential nutrient for plant growth, typically comprising 0.02–0.2% of soil content. This study demonstrates that biochar-based fertilizer significantly increases total phosphorus in karst calcareous soil, consistent with findings by Guan Lianzhu et al. that biochar combined with chemical fertilizer increases soil total phosphorus more than chemical fertilizer alone. This may be because biochar itself contains phosphorus, and higher application rates introduce more phosphorus into the soil.

High biochar rate treatments (RH8MF) significantly increased soil organic phosphorus, which constitutes 20–80% of total phosphorus. Biochar's porous structure provides habitat for soil microorganisms, increasing microbial biomass phosphorus which can serve as a source of organic phosphorus. The highly significant positive correlation between microbial biomass phosphorus and available phosphorus suggests that phosphorus-solubilizing microorganisms promote transformation of organic phosphorus to available forms.

Available phosphorus, the fraction directly absorbable by plants, represents a small portion of total phosphorus but determines plant growth potential. This

study shows that available phosphorus increased with biochar application rate, likely because biochar adsorbs phosphorus and reduces leaching losses. Biochar may also alter phosphorus availability through anion exchange or by affecting cations that react with phosphorus, reducing soil phosphorus adsorption and increasing availability. Biochar can mineralize its own organic phosphorus and activate sparingly soluble phosphorus through complexation and reduction processes.

The RH4MF treatment (biochar + compost + NPK) was more effective than single applications, possibly because organic phosphorus in compost is easily mineralized, and organic ions from compost compete for soil phosphorus adsorption sites, increasing phosphorus availability. Increased soil phosphorus content directly enhanced black locust seedling biomass, with significant positive correlations between biomass and all phosphorus fractions.

Soil phosphatase catalyzes organic phosphorus mineralization, and its activity reflects the capacity to transform organic to available phosphorus. The highly significant positive correlation between alkaline phosphatase and available phosphorus aligns with other studies. Medium biochar rates (RH2MF, RH4MF) produced the highest enzyme activity, while high rates were inhibitory, possibly because biochar's alkaline nature raises soil pH, which strongly affects phosphatase activity.

4. Conclusion

Application of biochar-based fertilizer produced from biochar, compost, and NPK significantly increased total phosphorus, available phosphorus, organic phosphorus, microbial biomass phosphorus, and black locust seedling biomass in karst calcareous soil, with high biochar rates showing superior effects. The four phosphorus fractions were significantly positively correlated, with the strongest correlation between available phosphorus and microbial biomass phosphorus ($r = 0.774$), indicating that soil microorganisms substantially influence available phosphorus content. Medium biochar rate treatments (RH2MF and RH4MF) most effectively promoted alkaline phosphatase activity. High-rate biochar-based fertilizer can significantly improve phosphorus nutrient status and promote seedling growth, thereby enhancing ecological restoration of young artificial forests in karst mountainous areas.

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