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Nine years of grazing and fertilization shape dynamics of soil phosphorus fractions in Karst pasture ecosystems

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ABSTRACT

Understanding soil phosphorus (P) cycling is critical for sustaining grassland productivity and soil health, especially in P-limited karst ecosystems. This study evaluated the long-term effects of three pasture management strategies — grazed-abandoned pasture (three years grazing followed by six years abandonment), continuous grazing without fertilization, and grazing with NPK fertilization—compared to unused land, in a subtropical karst region of Guizhou, China. Using a randomized complete block design established in 2012 (n = 4), soil samples were collected across five depths (0–45 cm) in 2012, 2015, and 2021 to assess changes in P fractions and related soil properties. Grazed-abandoned pasture had higher C/P and N/P ratios but lower soil organic matter than other treatments in the 0–10 cm soil. The effect of fertilized-grazed pasture on bioavailable P, active Po, and secondary mineral P shows an interannual cumulative effect; these effects shifted down the soil profile during the experiment. Grazed-abandoned pasture reduced total soil P and shifted P composition by increasing resin-extractable P at the expense of mineral and active organic P, maintaining available P. High acid and alkaline phosphatase activities in grazed-abandoned pasture supported organic P conversion and microbial biomass P. Grazed pasture did not alter the size or composition of P fractions but had lower MBP due to slower microbial P turnover. Fertilized-grazed pasture increased mineral and occluded P fractions (10,7–21 % of TP) and primarily supplied plants through NaHCO3-Pi and NaOH-Pi, with 1 M HCl-Pi. Thus, fertilization combined with grazing proved most effective in enhancing soil P availability and supporting long-term soil fertility, while abandonment may reduce P sustainability in karst grasslands. These findings provide important insights for optimizing pasture management in fragile subtropical ecosystems.

1. Introduction

Phosphorus (P) availability limits productivity in subtropical and tropical ecosystems (Du et al., 2020), and P availability also plays a role in ecosystem carbon (C) storage capacity (Peñuelas et al., 2012). Phosphorus availability in soil is influenced by adsorption-desorption, precipitation, dissolution of native P minerals, organic phosphorus mineralization, and transport (Helfenstein et al., 2024), and P in subtropical areas is primarily controlled by Fe and Al hydro(oxides) minerals (Wang et al., 2022). Microorganisms regulate the mineralization of organic phosphorus (via phosphatases), dissolution, and transport processes (Bünemann, 2015; De Araújo et al., 2015). Climate change (Mou

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et al., 2020), agricultural management practices (Azene et al., 2022; Katsalirou et al., 2016; Li et al., 2024), soil pH (Hou et al., 2018), organic matter content, phosphatase enzyme activity (Wan et al., 2020), and carboxylates secreted by plants (Sugihara et al., 2016) influence the transformation and bioavailability of P fractions. Most current studies discussed the relationship between phosphorus components and microorganisms, enzyme activity, or plants from the perspective of Hedley chemical fractionation or focused solely on P availability, such as Olsen P (Guan et al., 2023). Re-classification of P components based on biological availability was done (Hou et al., 2016), however, limited attention was given to interannual variation. Understanding the dynamics of soil P fractions and their composition is crucial for optimizing nutrient management in agroecosystems.

Fertilization and grazing are two primary management practices that influence P cycling in grassland ecosystems (Biassoni et al., 2023; Katsalirou et al., 2016; Damian et al., 2020; Li et al., 2009), and P cycling varies widely across temporal and spatial scales (Sun et al., 2023; D'Angioli et al., 2022). Grazing exclusion is used as a restoration strategy in temperate arid and semi-arid grasslands (Su et al., 2017), alpine meadows (Ma et al., 2016), and temperate wet meadows (Oelmann et al., 2017). However, the plant succession following grazing abandonment can promote complex alterations in soil P patterns (Oelmann et al., 2017) through changes in plant community composition, soil physical properties, and microbial activity. Despite the importance of these management practices, understanding how the combined effects of grazing and fertilization influence soil P content and dynamics remains limited.

The southwestern karst region of China has a hilly structure, creating a fragile and vulnerable ecology, posing challenges to agricultural and economic sustainability (Li et al., 2018). The region's water and heat resources offer potential for agro-grassland production, which could alleviate local food security concerns and ecological stress. Recent studies have investigated the impacts of management practices on C and nitrogen (N) cycling in karst pastures (Qin et al., 2023; Yang et al., 2023), with some studies of soil total P and phosphatase activity (Wang et al., 2018; Chen et al., 2017). However, a comprehensive understanding of the conversion dynamics between various P fractions and their driving factors in subtropical karst regions that are more vulnerable to soil P, N, or combined N-P limitations than other global regions is unclear (Du et al., 2020; Hou et al., 2021).

Here, we studied the long-term impacts of three pasture management practices (fertilized grazed, unfertilized grazed, and grazing exclusion) on the size and composition of soil P fractions in a karst ecosystem. Our objectives were to i) quantify the effects of grazing and fertilization on the size and composition of soil P fractions, ii) evaluate the relationships between P fractions and key environmental variables in the karst subtropical region, and iii) assess how grazing and fertilization influence grassland ecosystem services linked to soil P fractions and soil properties. We hypothesized that 1) fertilization would increase the size of labile P fractions and alter the relative proportions of P fractions, favoring more plant-available forms; 2) grazing would accelerate P cycling but potentially decrease total P fractions compared to grazing exclusion; 3) the combination of grazing and fertilization would balance distribution of P fractions, optimizing P availability for plant growth while minimizing potential environmental risks. This study would provide crucial insights into P dynamics under various management regimes in karst ecosystems, with broader implications for mitigating P limitations for forage production.

2. Materials and methods

2.1. Experimental area

This study was conducted at the grassland-livestock system coupling research station of Guizhou University (27° 23′N, 105° 52′E; altitude 1760 m) in Dafang, Bijie City, Guizhou Province, southwest China. This is a typical karst region with a subtropical monsoon climate. The average annual temperature for the study period was 12 ℃, and the average annual precipitation was 1064 mm (Fig S1). The dominant soil types are Ali-Perudic Argosols, and humic Alisol (clayic, endostagnic), according to WRB. The locally prevailing flora consists mainly of shrubs and warm-season grasses (Qin et al., 2023). The clay content is 28 %, silt is 61 %, and sand is 11 %.

The grasslands were established in the northern and central regions of the research area; the total area amounted to 80 ha in late 2012. Native vegetation (e.g., *Rosa omeiensis*, *Macrothelypteris oligophlebia*) was removed using prescribed burning, mowing, and tilling. Immediately after that, we planted a combination of legumes and grasses, including perennial ryegrass (*Lolium perenne*), duck grass (*Dactylis glomerata*), white clover (*Trifolium repens*), and red clover (*Trifolium pratense*). The sowing rate was 18, 9, 7.5, and 4.5 kg ha^{-1}, respectively. The unused region, totaling 11.5 ha, was in the southern part of the experimental site and maintained the original native vegetation composition. Electric fences enclose the entire experimental area. The basic soil conditions are shown in Table S1.

2.2. Experimental design

The experimental design is a randomized complete block design, including four treatments (Fig. S2): i) unused land, where grazing or fertilization activities were prohibited; ii) grazing-abandoned pasture, the area underwent rotational grazing management from 2012 to 2015, followed by a cessation of grazing activities from 2015 to 2021; iii) grazed pasture, the practice of implementing rotational grazing was conducted from 2012 to 2021; iv) fertilized grazed pasture, combined use of rotational cattle grazing and chemical fertilizer application from 2012 to 2021. The experimental unit areas (100 × 100 m) were randomly selected within each treatment, with three blocks corresponding to each.

The total annual compound fertilizer of N:P_2O_5:K_2O = 15:15:15 (N fertilizer was from $(NH_4)_2SO_4$) was applied at 300 kg ha^{-1}. The timing of fertilizer application was set according to the dominant forage species' spring peak growth (March) and the typical stockpiling season according to local practices (September). The details of grazing management practices were described in Qin et al. (2023). Throughout the experimental period from 2012 to 2021, no anthropogenic activity was done within the experimental area except for the management protocols.

2.3. Soil sampling and analysis

From each treatment, soil samples were collected in the three-unit areas (100 × 100 m) in August 2012, 2015, and 2021. Five soil samples were randomly carried out using an 8-cm diameter corer in five soil depths: 0–5, 5–10, 10–20, 20–30, and 30–45 cm. Soil from the same depth and same unit area of each treatment was combined to remove large stones and plant roots. The composite soil samples were transported to the laboratory for subsequent analysis.

Soil pH was measured as water: soil ratio of 5:1 (V/M) by a pH meter (Thomas, 1996). The metal ring method determined bulk density (BD) (Blake and Hartge, 1986). Total N (TN) was obtained by the Kjeldahl method (Hanon-K1100, Hyene Future Technology Group Co., Ltd., China) with titration (Bremner and Tabatabai, 1972). Soil organic C (SOC) was determined using a SKALAR carbon analyzer (Primacs ATC 100-IC-E, Netherlands).

The Mo-Sb colorimetric method was employed to analyze the total P (TP), and the $NaHCO_3$ extraction/Mo-Sb colorimetric method was utilized for the measurement of Available P (AP) (Kuo, 1996). Microbial biomass P (MBP) was determined using the chloroform fumigation method (Brookes et al., 1982). The chemical sequential fractionation method established by Hedley et al. (1982) and modified by Tiessen and Moir (1993) was employed to extract Soil P fractions. In brief, this method employs a series of progressively potent chemical extractants to segregate inorganic and organic P fractions. The extractants used sequentially were deionized water, 0.5 M $NaHCO_3$, 0.1 M NaOH, 1 M HCl, concentrated HCl, and concentrated H_2SO_4. The obtained product resin-P, $NaHCO_3$-P_i and total $NaHCO_3$-P, NaOH-P_i and total NaOH-P, 1 M HCl-Pi, conc.HCl-P_i and total conc.HCl-P, and residual P were obtained in turn. The organophosphates (P_o) in the different extracts ($NaHCO_3$-P, NaOH-P, and conc.HCl-P) was calculated by subtracting P_i from the total P. The Soil P fractions were redefined as five categories based on their bioavailability, including bioavailable P (resin-P and $NaHCO_3$-P_i), active P_o ($NaHCO_3$-P_o and NaOH-P_o), secondary mineral P (NaOH-P_i), primary mineral P (1 M HCl-P_i, conc.HCl-P_i and total conc. HCl-P), and occluded P (residual P) (Hou er al., 2016; Tian et al., 2022a; Zhang et al., 2023).

The topsoil (0–10 cm) P fraction was calculated by P fraction of 0–5, 5–10 cm, and BD (g cm^{-3}). The subsoil (10–45 cm) P fraction was calculated by P fraction of 10–20, 20–30, 30–45 cm, and BD (g cm^{-3}). Soil C to P ratio (C/P) was calculated by TP (g kg^{-1}) and SOC (g kg^{-1}). Soil N to P ratio (N/P) was calculated by TN (g kg^{-1}) and TP (g kg^{-1}). Soil organic matter (SOM) was calculated by SOC (g kg^{-1}) with a coefficient of 1.724 (Brady and Weil, 1996).

The analysis of soil enzyme activity focused specifically on alkaline phosphatase (AKP) and acid phosphatase (ACP). The testing was conducted using standard kits (Quanzhou Ruixin Biotechnology Co., Ltd.). The detailed protocols for AKP and ACP can be found in Acosta-Martínez and Ali Tabatabai (2011).

2.4. Data analysis

To assess the effects of management patterns on soil P fractions and other properties, we use linear mixed-effects models (MEMs). In these models, the fixed effect was treatment, and the random effect was the plot. We used the natural log-transformed response variables and the Fligner–Killeen test to test the homogeneity of model residuals. When the given variable had significant differences among treatments at a significance level of 0.05, Tukey's test was used for a post-hoc test ($\alpha = 0.05$). To assess the effects of soil depth and management time on soil physicochemical properties and soil enzyme activities, we employed univariate analysis of variance (ANOVA) with a significance level set at $p < 0.05$. Tukey's test was conducted for mean separation. The relationships between MBP, AKP activity, ACP activity, and bioavailable P fractions were assessed using linear regression. Redundancy analysis (RDA) and hierarchical partitioning analysis were employed to determine the contribution of soil environmental variables and plant functional groups to the bioavailable P fractions.

The partial least squares path model (PLS-PM) was employed to examine the influence of soil P fractions and their ecosystem services from grazing management and fertilization. P fractions and other indicators can be categorized into different groups based on their association with ecosystem service provision. We categorize the ecosystem service functions of grasslands into three types (Garland et al., 2021; Richter et al., 2024): i) provisioning, including food production, primary production, and rapid supply of P; ii) regulating, including water purification, erosion regulation, and decomposition processes; iii) supporting: soil P storage capacity and nutrient cycling. We calculated the crude protein yield of the grasses based on the dry matter yield and the crude protein content of the different plant function groups.

The analyses were conducted utilizing R software (version 4.3.2), with the lme4 package for MEMs, with the "ggplot2" package for linear regression, the "vegan" package for RDA, and the "rdacca.hp". package for hierarchical partitioning (Lai et al., 2022), and the "plspm" package for PLS-PM.

3. Results

3.1. Soil chemical, and biological properties

In 2015, the C/P and N/P ratios in grazed pasture were higher than in controlled grazing with fertilization in the 0–5 cm horizon (Fig. 1). SOM content in 2021 exceeded 2012 levels in controlled grazing with fertilizer in the 0–30 cm. In 2021, C/P ratios were higher than in other years in grazing-abandoned pasture (0–45 cm), grazed pasture, and controlled grazing with fertilizer (10–30 cm), but lower than 2012 in controlled grazing with fertilizer (0–5 cm). The N/P ratios of 2021 were higher than those of other years in grazing-abandoned pasture (0–5 cm) and grazed pasture (5–10 cm).

In 2021, in the 0–5 cm, the C/P and N/P ratios in grazing-abandoned pasture were higher than in controlled grazing with fertilizer (Fig. 1, $p < 0.05$). The lowest SOM content was found in unused land in the 0–5 cm ($p < 0.05$). At 5–10 cm depth, SOM contents in grazed pasture and controlled grazing with fertilizer were 12–15 % higher than in the other two treatments ($p < 0.05$). At 10–30 cm depth, grazing-abandoned pasture had 30–40 % lower SOM than controlled grazing with fertilizer and grazed pasture ($p < 0.05$). In the 10–20 cm, the C/P ratio in unused land was 33–43 % lower than in the other three treatments ($p < 0.05$). N/P ratios in grazed pasture and controlled grazing with fertilizer were 24 % higher than in unused land ($p < 0.05$).

In 2021, TP in controlled grazing with fertilizer (0–5 cm), AP in grazing-abandoned and grazed pasture, and MBP in controlled grazing with fertilizer (10–45 cm) were higher than in other years (Fig. 1 and Fig. 2, $p < 0.05$). However, TP in the grazing-abandoned pasture (5–45 cm) was lower than in other years. MBP in 2015 was lower than in other years in grazing-abandoned pasture (0–5 cm) but higher than in 2012 and lower than in 2021 in grazed pasture (Fig. 1, 0–5 cm). AP in the 0–10 cm for controlled grazing with fertilizer was 66–88 % higher than in unused land ($p < 0.05$). MBP in the 5–10 and 20–30 cm were 2–4 times higher than in grazed pasture ($p < 0.05$). AP in grazed pasture was lower than in unused land and grazing-abandoned pasture across 0–10 cm ($p < 0.05$). MBP in controlled grazing with fertilizer was 25–28 % lower than in grazed pasture across 0–10 cm ($p < 0.05$). Alkaline phosphatase activity in the grazing-abandoned pasture was 10–12 % higher in the 10–30 cm and 15 % higher in the 0–30 cm (Fig. 2, $p < 0.05$). ACP activity in the entire profile ($p < 0.05$) in grazing-abandoned pasture was 1–2 times higher than in other treatments.

3.2. Distribution of soil total phosphorus and fractions

In 2015, $NaHCO_3$-P_i and $NaHCO_3$-P_o in controlled grazing with fertilizer were 25–30 % higher in the 0–5 cm (Fig. 3, $p < 0.05$). NaOH-P_i (0–5 cm) was greater in controlled grazing with fertilizer (Fig. 4, $p < 0.05$). HCl- P_o and residual P in the 0–5 cm were 25 % higher, and residual-P was greater than in unused land in the 5–10 cm and 20–30 cm (Fig. 5, $p < 0.05$). In the 0–10 cm topsoil, bioavailable P accounted for 3.7 % of TP in unused land, 11 % in grazing-abandoned pasture, 4.3 % in grazed pasture, and 4.1 % in controlled grazing with fertilizer (Fig. 6). Active Po constituted 65 % of TP in unused land and 70 % in subsoil (10–45 cm). Controlled grazing with fertilizer had 17.5 % and 11 % of TP, and secondary and primary mineral P was 13 % (Fig. 6). Resin-extractable P_i in the grazing-abandoned pasture was 20 % higher than in other treatments in the 0–5 cm and 10–20 cm (Fig. 3, $p < 0.05$). $NaHCO_3$-P_i in controlled grazing with fertilizer was 86 % higher than in the grazing-abandoned pasture in the 5–10 cm (Fig. 3, $p < 0.05$). NaOH-P_i (0–45 cm), and NaOH-P_o (0–5 and 20–45 cm) in controlled grazing with fertilizer were higher than in the grazing-abandoned pasture (Fig. 4, $p < 0.05$), while the grazing-abandoned pasture had 15–20 % lower contents ($p < 0.05$). HCl-P_i (0–5 and 20–45 cm) was higher in controlled grazing with fertilizer and unused land than in grazing-abandoned and grazed pastures (Fig. 5). HCl-P_o (0–30 cm) in controlled grazing with fertilizer was higher than unused land in the

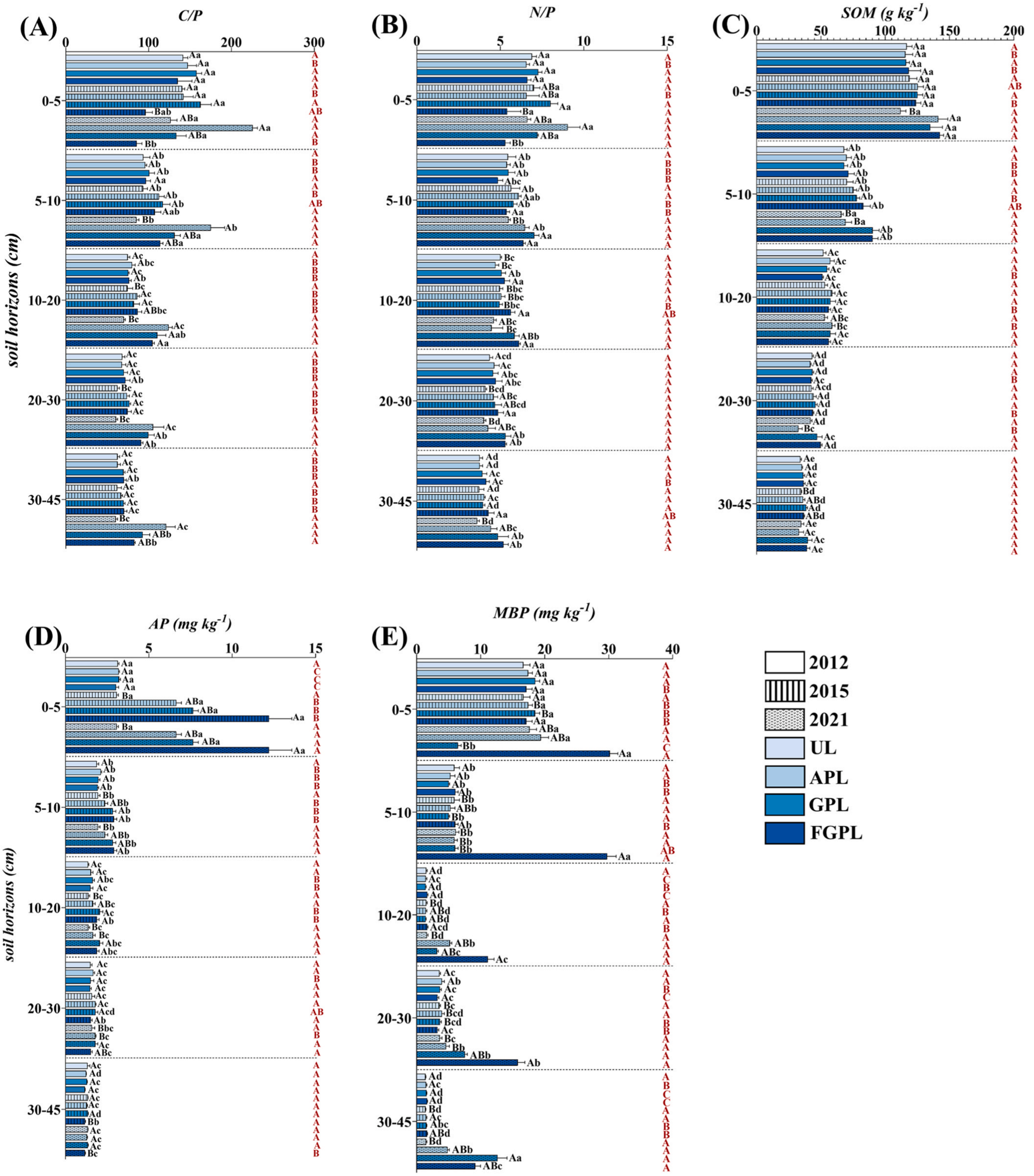

Fig. 1. The concentration of A) carbon phosphorus ratio (C/P), B) nitrogen phosphorus ratio (N/P), C) soil organic matter (SOM), D) available phosphorus (AP), E) microbial biomass phosphorus (MBP), alkaline phosphatase activity (AKP), acid phosphatase activity (ACP) at different layers in the unused land (UL), abandoned grazing pastureland (APL), grazing pastureland (GPL), fertilized grazing pastureland (FGPL) treatments in 2012, 2015 and 2021. Different capital letters (in black color) indicate significant differences between different treatments in the same year and soil layer, and different lowercase letters indicate significant differences between different soil layers in the same year and treatment. The different capital letters (in red color) indicate significant differences between different year in the same treatment and layer.

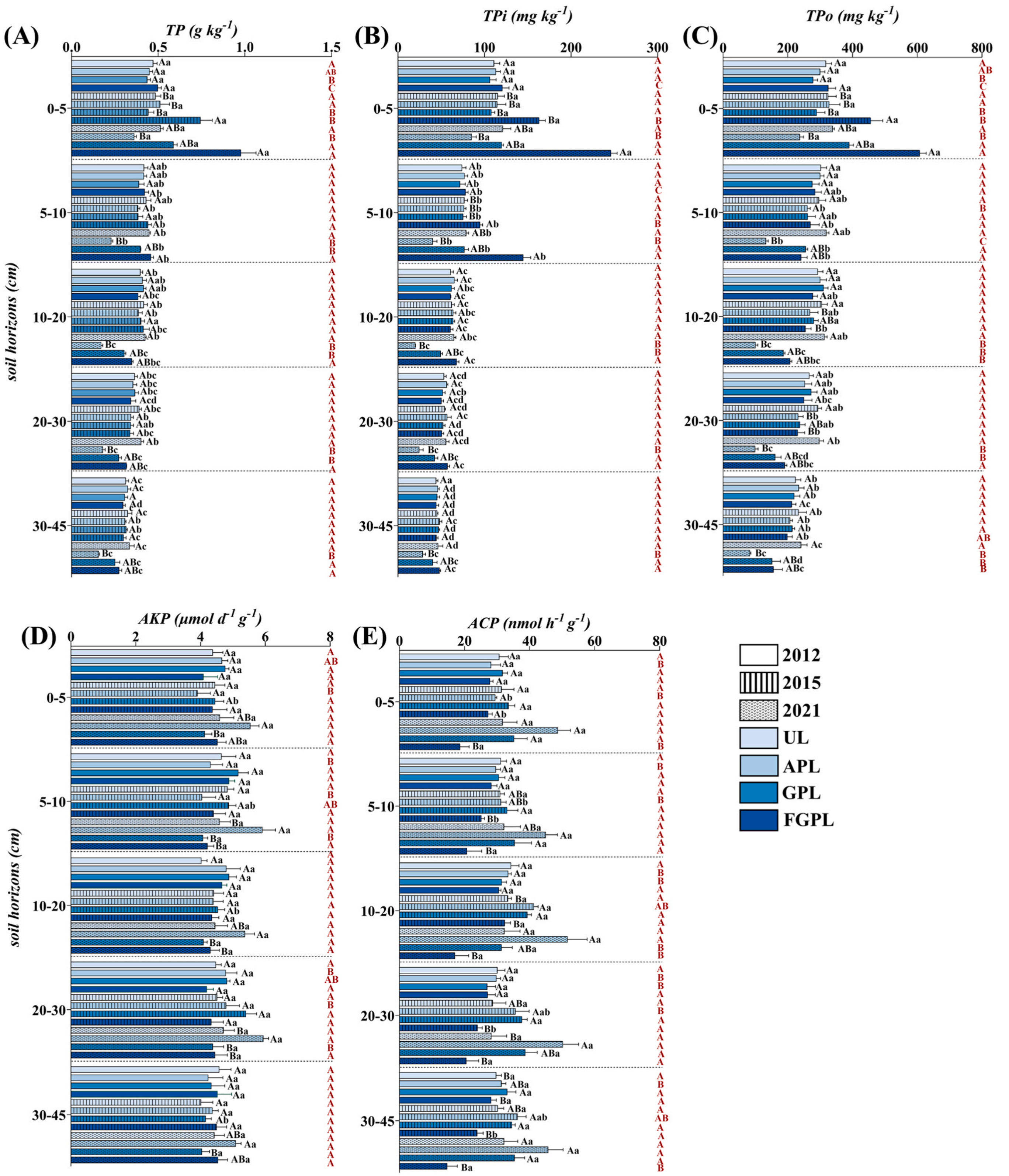

Fig. 2. The concentration of A) total P (TP), B) total inorganic phosphorus (TPi), C) total organic phosphorus (TPo), D) alkaline phosphatase activity (AKP), E) acid phosphatase activity (ACP) at different layers in the unused land (UL), abandoned grazing pastureland (APL), grazing pastureland (GPL), fertilized grazing pastureland (FGPL) treatments in 2012, 2015 and 2021. Different capital letters (in black color) indicate significant differences between different treatments in the same year and soil layer, and different lowercase letters indicate significant differences between different soil layers in the same year and treatment. The different capital letters (in red color) indicate significant differences between different year in the same treatment and layer.

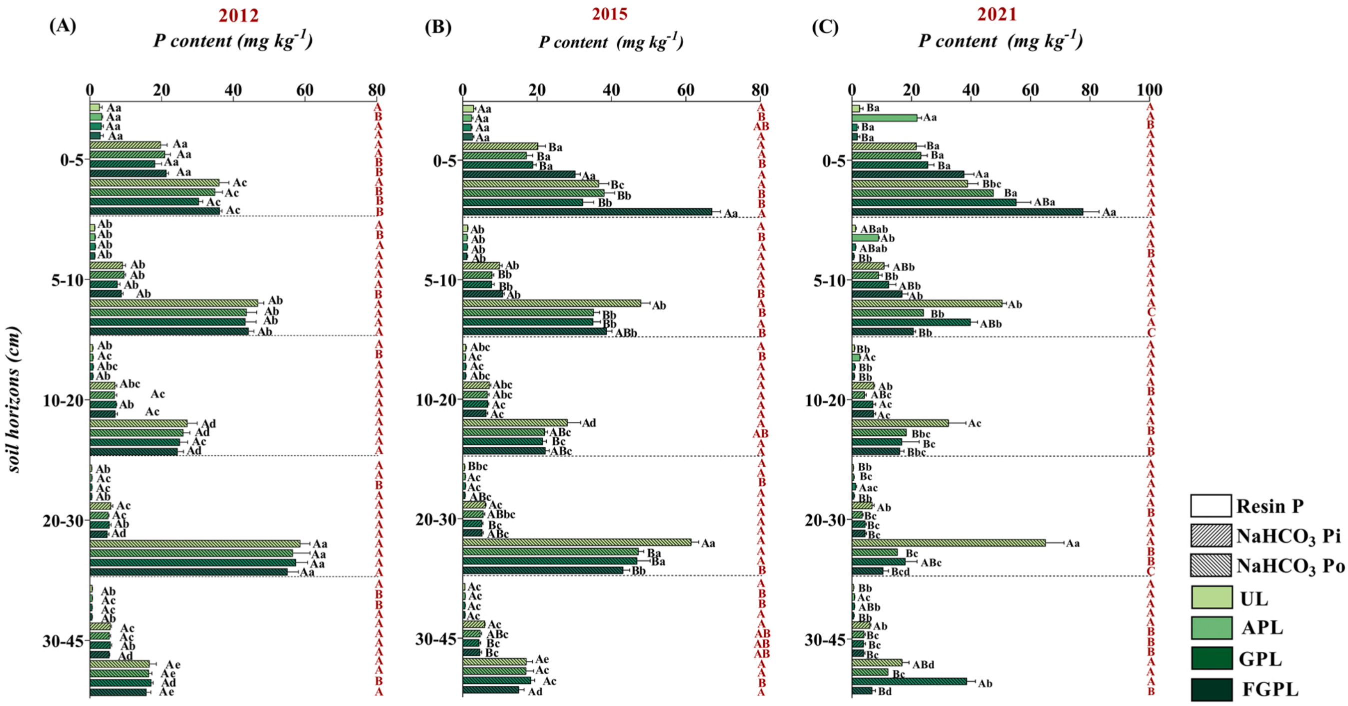

Fig. 3. The concentration of resin-Pi, $NaHCO_3$-Pi, $NaHCO_3$-Po at 0–5, 5–10, 10–20, 20–30 and 30–45 cm in the unused land (UL), abandoned grazing pastureland (APL), grazing pastureland (GPL), fertilized grazing pastureland (FGPL) treatments in A) 2012, B) 2015 and C) 2021. Different capital letters (in black color) indicate significant differences between different treatments in the same year and soil layer, and different lowercase letters indicate significant differences between different soil layers in the same year and treatment. The different capital letters (in red color) indicate significant differences between different year in the same treatment and layer.

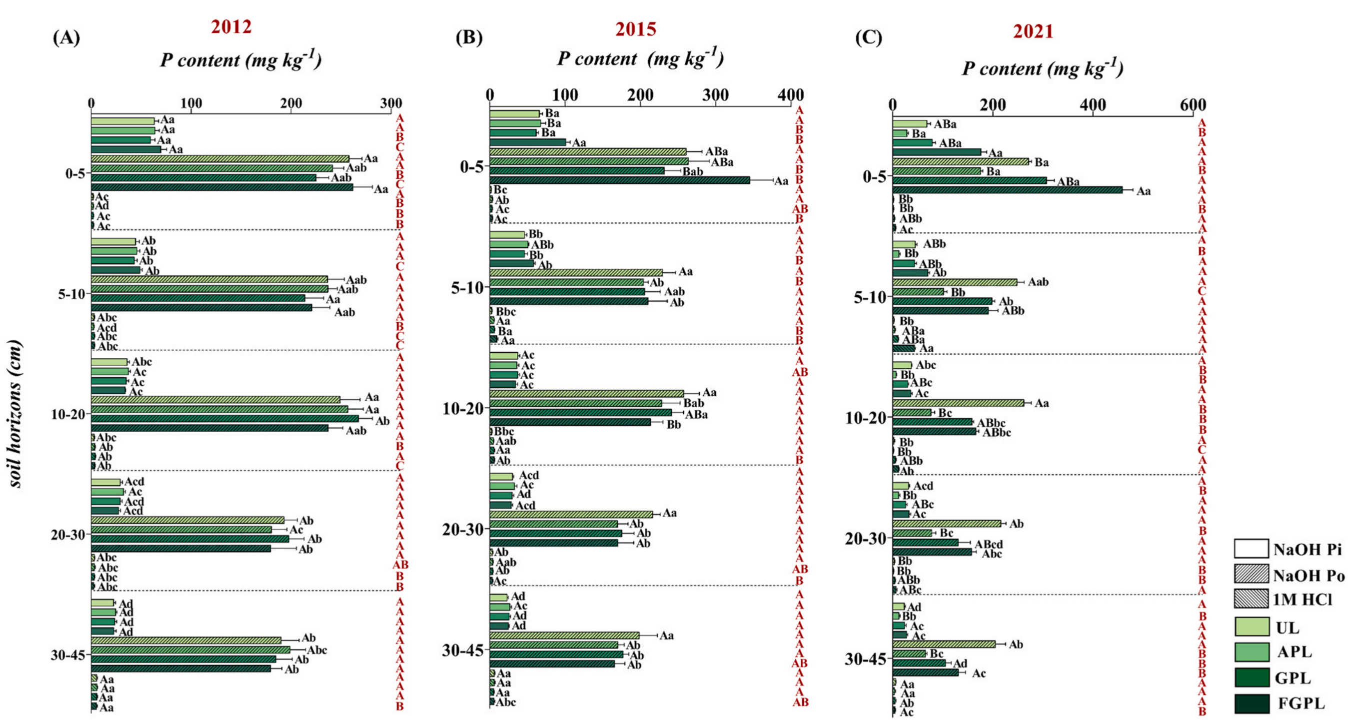

Fig. 4. The concentration of NaOH-Pi, NaOH-Po, 1 M HCl-Pi at 0–5, 5–10, 10–20, 20–30 and 30–45 cm in the unused land (UL), abandoned grazing pastureland (APL), grazing pastureland (GPL), fertilized grazing pastureland (FGPL) treatments in A) 2012, B) 2015 and C) 2021. Different capital letters (in black color) indicate significant differences between different treatments in the same year and soil layer, and different lowercase letters indicate significant differences between different soil layers in the same year and treatment. The different capital letters (in red color) indicate significant differences between different year in the same treatment and layer.

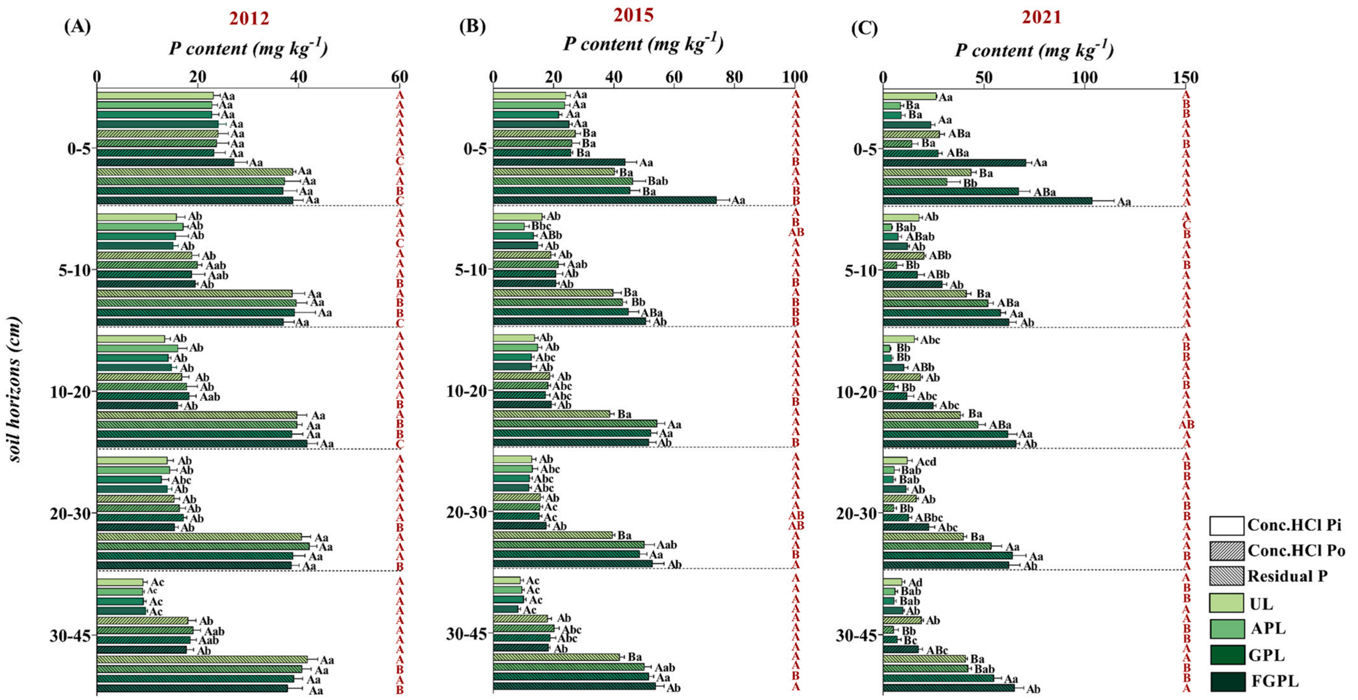

Fig. 5. The concentration of conc.HCl-Pi, conc.HCl-Po and residual-P at 0–5, 5–10, 10–20, 20–30 and 30–45 cm in the unused land (UL), abandoned grazing pastureland (APL), grazing pastureland (GPL), fertilized grazing pastureland (FGPL) treatments in A) 2012, B) 2015 and C) 2021. Different capital letters (in black color) indicate significant differences between different treatments in the same year and soil layer, and different lowercase letters indicate significant differences between different soil layers in the same year and treatment. The different capital letters (in red color) indicate significant differences between different year in the same treatment and layer.

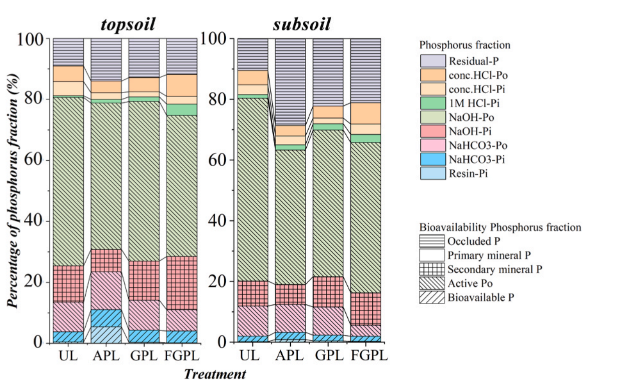

Fig. 6. The proportion of resin-Pi, $NaHCO_3$-Pi, $NaHCO_3$-Po NaOH-Pi, NaOH-Po, 1 M HCl-Pi, conc.HCl-Pi, conc.HCl-Po and residual-P in total P at topsoil (0–10 cm) and subsoil (10−45) cm in the unused land (UL), abandoned grazing pastureland (APL), grazing pastureland (GPL), fertilized grazing pastureland (FGPL) treatments in 2021.

0–5 cm and 30–45 cm ($p < 0.05$). Residual-P in controlled grazing with fertilizer was higher than in grazing-abandoned in the 0–5 cm and 30–45 cm ($p < 0.05$).

In 2021, TP and TP_i contents were 99–169 % higher in controlled grazing with fertilizer than in grazing-abandoned pasture in the 0–10 cm (Fig. 2, $p < 0.05$). In contrast, TP and TP_i in grazing-abandoned pasture were 20 % and 18 % lower than those in unused land in the 5–45 cm and controlled grazing with fertilizer in the 0–45 cm, respectively ($p < 0.05$). TP_o contents in controlled grazing with fertilizer were 22 % higher in the 0–5 cm. Unused land had 1.3–2.1 times higher P_o than grazing-abandoned pasture in the 5–45 cm ($p < 0.05$). Resin-P_i was higher than in 2012 in grazed pasture, and $NaHCO_3$-P_i and $NaHCO_3$-P_o were greater in grazed pasture than in other years (Fig. 3, $p < 0.05$). $NaHCO_3$-P_o in grazing-abandoned pasture and controlled grazing with fertilizer decreased by 5–10 cm over time. From 2012–2015, NaOH-P_i (0–10 cm), NaOH-P_o (0–5 cm), and 1 M HCl-P_i (5–20 cm) increased in controlled grazing with fertilizer (Fig. 4). However, in 2021, NaOH-P_i (0–10 cm) and NaOH-P_o (0–10 cm) were lower in grazing-abandoned pasture than in previous years. HCl-P_o (0–30 cm) and residual-P (0–45 cm) were higher in 2021 than in 2012 controlled grazing with fertilizer.

3.3. Relationship between phosphorus fractions and environmental factors

Linear regression showed correlations between P fractions and MBP (Fig. 7). Bioavailable P, secondary mineral P, and primary mineral P increased with MBP in unused land, grazing-abandoned pasture, and controlled grazing with fertilizer ($p < 0.001$). Active P_o had increased with MBP in grazing-abandoned pasture ($p < 0.01$).

RDA revealed that the first axis explained 95.5 % of the variation in P fractions (Fig. 8). Hierarchical partitioning analysis showed that soil MBN accounted for 24.4 %; SOM accounted for 23.1 % of the variation in P fractions ($p < 0.001$). The AP content contributed 19.4 % of the explained variance ($p < 0.01$), while C/P contributed 9.4 % and MBP contributed 5.9 % ($p < 0.05$). PLS-PM indicated that decomposition processes and grazing management increased P cycling ($p < 0.001$; Fig. 9). Grazing and P cycling capacity decreased soil P storage ($p < 0.001$). Fertilization increased bioavailable P ($p < 0.01$), which in turn had a positive effect on grassland production ($p < 0.001$).

4. Discussion

4.1. Effects of grazing and fertilization on soil P fractions

Grazing abandonment reduced total P content in the 0–45 cm soil layer compared to grazed and fertilized–grazed pastures (Fig. 2), consistent with findings from temperate and alpine ecosystems (Hou

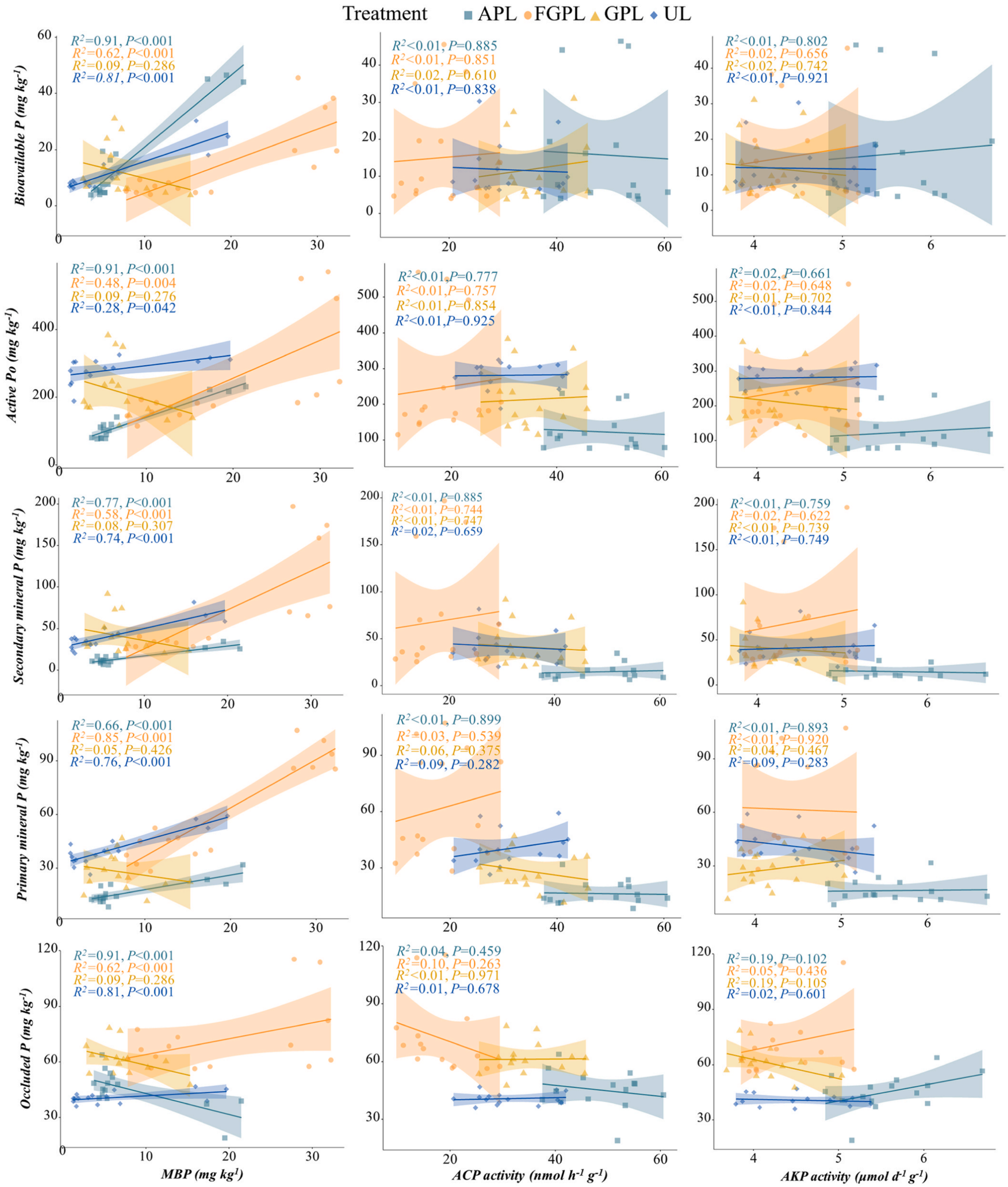

Fig. 7. Responses of microbial biomass phosphorus (MBP), soil acid phosphatase (ACP) activity, soil alkaline phosphatase activity (AKP) to soil phosphorus fractions in the unused land (UL), abandoned grazing pastureland (APL), grazing pastureland (GPL), fertilized grazing pastureland (FGPL) treatments in 2021. All regressions should be checked for normality of residuals.

et al., 2020; Yang et al., 2023). This decline can be attributed to three main factors: i) The absence of exogenous inputs such as cattle dung and fertilizers in the grazing-abandoned pasture. Grazing has been shown to increase SOM (Segoli et al., 2015; Mosier et al., 2021), a pattern also observed in our study (Fig. 1). This increase is likely due to the high N and P content in cattle manure (Sitters and Venterink, 2021) and the benefits of rotational grazing practices (Mosier et al., 2021). ii) Following grazing cessation, vegetation in abandoned pastures was

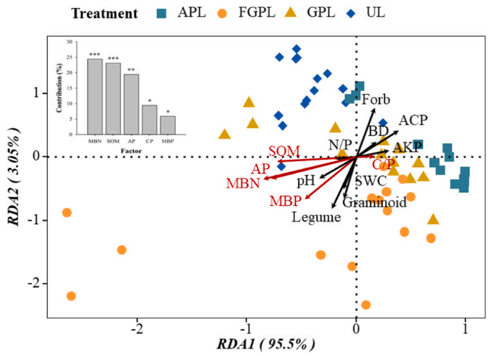

Fig. 8. RDA analysis and the hierarchical partitioning analysis (the left corner) of environmental factors and P fractions in the unused land (UL), abandoned grazing pastureland (APL), grazing pastureland (GPL), fertilized grazing pastureland (FGPL) treatments in 2021. The angles between the response and explanatory variables represent their correlations, and the acute and obtuse angles represent positive and negative correlations, respectively. The amounts of variance explained for RDA 1 and 2 are in brackets. The red colour indicate the factors, which were significant contribution of the P fraction variation, * ($P < 0.05$), ** ($P < 0.01$), and *** ($P < 0.001$). Notes: soil water content (SWC), microbial biomass N (MBN), the plant function group proportion (Legume, Graminoid, Forb).

dominated by native shrubs and low-productivity grasses, leading to lower-quality litter inputs (Knapp et al., 2011). iii) The lack of physical disturbance from large herbivores, such as trampling, may have reduced litter decomposition rates and, consequently, P inputs (Wei et al., 2021; Jiang et al., 2024). These explanations are further supported by observed interannual decreases in soil TP, total Pi, and total Po across the 0–45 cm profile in grazing-abandoned pastures (Fig. 1). Livestock grazing plays a key role in stimulating soil P cycling by returning organic matter and enhancing microbial activity, particularly in surface layers (Liu et al., 2021). However, its long-term impact on TP appears limited to the 0–5 cm soil depth (Fig. 2), likely due to P's sedimentary behavior and the slow downward movement of organic forms within the soil profile (Katsalirou et al., 2016).

Notably, following the adoption of modified management practices in 2015, abandoned pastures maintained similar levels of available P despite having lower total P content compared to grazed pastures. In addition to the changes in P dynamics described earlier, the high phosphatase activity and MBP were measured for abandoned pasture. These factors enhanced the mineralization of organic P (Katsalirou et al., 2016), thereby sustaining available P to support plant growth during ecological succession (D'Angioli et al., 2022). In contrast, the fertilization + grazing increased TP content compared to grazed pasture (Fig. 2), due to the combined effects of mineral fertilizer and manure inputs. These inputs not only boosted P availability but also promoted the retention of additional P within the soil matrix and enhanced soil P cycling (Gu et al., 2023), as evidenced by high available P and MBP levels. Across all treatments, NaOH-extractable Po dominated the P fractions (Fig. 6), consistent with prior studies showing that most organic P remains in stable forms, making it less available for plant uptake (Chen et al., 2015). Grazing increased the contents of bioavailable P, active Po, and secondary mineral P compared to ungrazed land (Figs. 3, 4, 5); however, it did not alter the relative proportions of these fractions. This suggests that grazing stimulated P release without significantly changing overall P partitioning. The low MBP observed in grazed pastures indicates that plants may be more efficient than microorganisms in utilizing available P (Fig. 1; Sun et al., 2023). Under

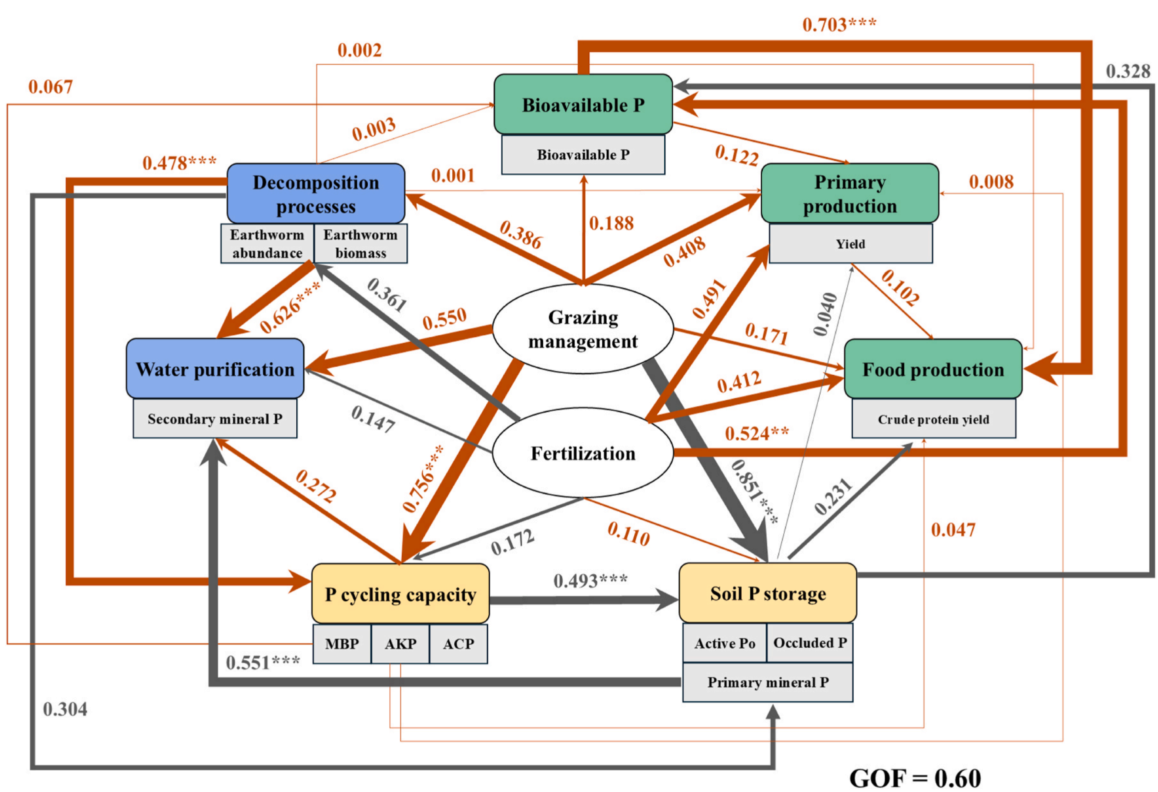

Fig. 9. The partial least squares path model (PLS-PM) to describing the effects of grazing management and fertilization on ecosystem service provision link with soil phosphorus fractions and other grassland plants or soil characteristics in 2021. The green, yellow and blue latent variable represents the provisioning, supporting and regulating function, respectively. Models with different structures were assessed using the Goodness of Fit (GOF) statistic, a measure of the overall prediction performance. The orange lines indicate a positive effect, the grey lines indicate a negative effect. The values are the path coefficients. Path coefficients that differed significantly from 0 were indicated by * ($P < 0.05$), ** ($P < 0.01$), and *** ($P < 0.001$). Notes: MB*P*: Microbial biomass phosphorus; AKP: alkaline phosphatase; ACP: acid phosphatase.

long-term grazing and fertilization, primary mineral P and occluded P increased in the 5–30 cm soil horizon (Figs. 3, 4, 5). This pattern aligns with findings from tropical and subtropical grasslands, where phosphate fertilizers tend to be immobilized into stable forms through adsorption to mineral colloids (Damian et al., 2020), a process further supported by the measured levels of Fe and Al oxides (Fig. S4).

From an interannual perspective, particularly within the 0–10 cm soil horizon, fertilization had a cumulative effect on total P, P_o, active P_o, secondary mineral P, primary mineral P, and occluded P fractions. While mineral fertilizers are known to enhance soil P cycling, their effects are typically limited to inorganic P forms (McLaren et al., 2020; Jing et al., 2021). However, this study's combined influence of grazing inputs and mineral fertilizers increased in TP and various P fractions consistently, suggesting improved long-term P bioavailability. Although the presence of Fe and Al oxides promoted the formation of complex phosphorus compounds (Ando et al., 2022), the accumulation of bioavailable P was not hindered. Due to limited direct evidence on the long-term dynamics of soil P fractions under chemical fertilization, further monitoring is necessary to fully assess the cumulative impacts of fertilization and grazing on soil P availability and transformation.

Phosphatase activity was lower in the controlled grazing + fertilization compared to other treatments, while MBP was higher (Fig. 2, $p < 0.05$). This indicates that microbial communities remained active, and P was more abundant, but nutrient cycling may have slowed due to the accumulation of stable P forms. Grazing, in contrast, stimulated faster nutrient cycling by promoting biomass turnover and excreta deposition, which contributed to increased occluded P (Sun et al., 2023). Despite this, both primary mineral P and occluded P were still mobilized in the controlled grazing + fertilization, due to the presence of legumes and their ability to mobilize recalcitrant P (Ma et al., 2016; Rose et al., 2016; Sugihara et al., 2016; Tian et al., 2022b).

4.2. Relationships between soil P fractions and environmental variables

This study found that long-term grazing abandonment pasture increased C/P and N/P ratios in the 0–20 cm soil horizon (Fig. 1) due to vegetation succession processes that enhanced organic C accumulation. Meanwhile, the limitation for N and P increased because of intensive uptake of these elements by plants or leaching (Zeng et al., 2017). Although abandonment increased SOM in the surface horizon, this increase was not accompanied by a proportional rise in soil P content, suggesting an imbalance in nutrient cycling.

Fertilization under controlled grazing did not alter soil C/P and N/P ratios compared to grazed pastures ($p > 0.05$), although a slight reduction was observed in the 0–5 cm horizon (Fig. 1). Differences in nutrient mobility may explain this pattern—P accumulated in surface horizons, while N was leached. Fertilization may alleviate nutrient imbalances common in P-limited ecosystems, supporting SOM accumulation (Tao et al., 2021). It also appears to sustain a positive interaction among soil C, N, and P, thereby preventing growth-limiting deficiencies of individual nutrients (Reichert et al., 2023). In grazing-abandoned pastures, available P increased in the 0–20 cm soil depth due to litter accumulation and organic matter stabilization during secondary succession. Stabilized SOM provided binding sites for phosphate, reducing adsorption losses and maintaining P availability (Azene et al., 2022). However, MBP levels were low in grazed pastures ($p < 0.05$), potentially due to lower soil pH and high bulk density (Figs. 1, 7, S3; Liu et al., 2021). Thus, microbial activity played a key role in sustaining P cycling, particularly in systems with limited external P inputs (e.g., grazing-abandoned and unused land) or high fertilizer input (e.g., controlled grazing + fertilizer) (Fig. 7). Hierarchical partitioning analyses identified MBN, SOM, available P, and MBP as the primary drivers of P fraction transformations, emphasizing the central role of microbial processes in mediating P dynamics across pasture management regimes (Fig. 8; Zhang et al., 2023; Katsalirou et al., 2016).

4.3. Modulation of P fractions transformation under pasture practices

P fraction dynamics in pasture ecosystems are governed by complex biochemical interactions among soil, vegetation, and livestock (Zhang et al., 2023). Grazing promoted P cycling by enhancing microbial activity through feces and urine deposition, accelerating nutrient turnover (Katsalirou et al., 2016). Additionally, grazing indirectly stimulated decomposition processes, further increasing P availability.

Fertilization strongly influenced primary productivity more than grazing alone, aligning with evidence that soil fertility underpins livestock production (Waller et al., 2001). Fertilization enhanced bioavailable NaOH-P_i and 1 M HCl-P_i fractions in the 0–10 cm soil horizon, improving P cycling and plant accessibility (Fig. 9; Sun et al., 2022; Damian et al., 2020). The beneficial impacts of grazing on P fraction dynamics were revealed with a temporal lag compared to the more immediate effects of fertilization. Low nutrient inputs and limited microbial activity in grazed pastures restricted P cycling and failed to meet the increased nutrient demand of aboveground biomass, resulting in negligible effects observed in the early phase (2015) (Song et al., 2023). In contrast, the combination of grazing and fertilization enhanced soil bioavailable P and active Po within three years. These improvements were sustained through 2021, with no observed decline in bioavailable P.

With the management duration, the positive effects of combined fertilization and grazing on $NaHCO_3$-P_i shifted within the soil profile, due to interactions between soluble SOM and inorganic P, which could promote seasonal variation in available P dynamics (Hagedorn et al., 2015). In contrast, resin-P_i was less responsive to management time, due to its rapid plant uptake and sensitivity to soil solution chemistry, making temporal trends difficult to detect (Hedley et al., 1982).

Although grazing enhanced P cycling, it depleted long-term soil P reserves. Path analysis indicated a negative effect of active P cycling capacity on soil P storage (Song et al., 2023). Fertilization mitigated this effect by sustaining productivity over time (McLaren et al., 2020). These findings highlighted the importance of integrating grazing and fertilization to maintain balanced nutrient cycling, enhance P bioavailability, and preserve essential ecosystem services in pasture systems.

This study advanced understanding of P cycling in fragile ecosystems of the subtropical Karst region and offered preliminary evidence that grazing abandonment may threaten long-term P availability. However, we would like to address several limitations of the obtained results. i) The lack of a fertilized–non-grazed control treatment restricts the ability to fully disentangle the individual and interactive effects of grazing and fertilization. ii) The absence of data on plant exudate composition (particularly from legumes) and microbial community structure limits our understanding of the underlying biological mechanisms driving P transformations. Future research should include additional treatments and focus on plant–microbe interactions using microbial sequencing and detailed analyses of root exudates to elucidate P cycling processes better.

5. Conclusion

This study evaluated the effects of grazing, fertilization, and their interaction, as well as the management duration on soil P fractions in a karst pasture ecosystem in subtropical areas. Our results showed that grazing-abandoned pasture promoted P limitation and decoupling of N and P in the surface soil (0–5 cm), resulting in increased availability of resin-P_i but a reduced total P, active P_o, and mineral P preservation. Grazing enhanced active P_o while decreasing primary mineral P, though it was constrained by limited organic P mineralization due to microbial and enzymatic limitations. In contrast, fertilization combined with grazing increased TP, bioavailable P, active P_o, and secondary mineral P. An accumulative effect was observed as management duration increased. Controlled grazing with fertilizer proved more effective in maintaining long-term P cycling and ecosystem sustainability than grazed pasture. Grazing-abandoned pasture could lead to detrimental long-term impacts on soil health and productivity while promoting short-term P availability.

CRediT authorship contribution statement

Wangfei Qin: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Rui Dong:** Validation, Software, Resources, Investigation, Data curation. **Xinyao Gu:** Software, Resources, Investigation. **Shaokun Hu:** Software, Resources, Investigation. **Song Cui:** Writing – review & editing, Formal analysis, Conceptualization. **Xuechun Zhao:** Methodology, Investigation, Data curation. **Jihui Chen:** Methodology, Investigation. **Yinglai Shi:** Validation, Data curation, Investigation. **Chao Chen:** Software, Resources. **Yuan Li:** Writing – review & editing, Methodology, Funding acquisition, Formal analysis. **Narasinha Shurpali:** Writing – review & editing. **Mikko Järvinen:** Writing – review & editing. **Anna Gunina:** Writing – review & editing, Methodology. **Yingwen Yu:** Writing – review & editing, Formal analysis. **Zhou Li:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2025.109826.

Data availability

Data will be made available on request.

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