Ecological stoichiometry-based study on carbon, nitrogen, and phosphorus nutrient limitation of different land use patterns in the Yellow River Delta

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Abstract

The conversion of land uses was frequent in the saline-alkali reclamation region of the Yellow River Delta (YRD), China. However, the knowledge of the combined effects of carbon (C), nitrogen (N), and phosphorus (P) stoichiometry for soil and enzyme under different land use patterns is limited. We investigated soil C, N, P stoichiometry and soil extracellular enzyme activities of three representative land use patterns (Alfalfa artificial grassland, AG; wheat-maize rotation field, WM; native grassland, PC). The results showed that the average soil stoichiometry of AG, WM, and PC was 31.32: 1.74: 1, 34.05: 1.46: 1, and 26.58: 1.14: 1 respectively, indicating that the level of C and N in the YRD was low. The AG was beneficial to promote the mineralization of C, N, and P, but its effect on the sequestration of C and P was not as good as WM. The average enzyme stoichiometry of AG, WM, and PC was 0.96: 0.76: 1, 0.94: 0.74: 1, and 0.86: 0.73: 1 respectively, which deviated from 1: 1: 1, and were significantly correlated with soil stoichiometry, indicating that enzyme stoichiometry in the YRD was nutrient dependent rather than homeostasis. Meanwhile, according to the vector analysis of enzyme stoichiometry, PC could alleviate C limitation of soil microorganisms. Collectively, land use changes affected C, N, P stoichiometry, and should further change the biochemical cycle and microbial nutrient limitation. Therefore, we argued that conversion and proper conduct of land should be cautions for the degraded land restoration and ecological security, and develop perennial legume forage and conservation tillage for crops should be taken for soil healthy and sustainable development.

1. Introduction

Ecological stoichiometry provides a tool to investigate the interdependency and balance of nutrient elements (Michał et al., 2020), which reflects the nutrient limitation of organisms, especially carbon (C), nitrogen (N), and phosphorus (P). Soil extracellular enzyme activity is one of the important factors affecting ecological stoichiometry. Recent studies also emphasized that enzyme stoichiometry should be incorporated into biochemical models (Nannipieri et al., 2018). Soil microorganisms drive global nutrient cycling by producing a variety of extracellular enzymes (Singh and Kumar, 2021). Meanwhile, sinsabaugh et al. (2008) found that the logarithm transformed enzymes activities for β-1,4-glucosidase (βG), β-1,4-N-acetyl-glucosaminidase (NAG) + L-leucine aminopeptidase (LAP), and acid phosphatase (ACP) tend to be 1: 1: 1 at the global scale. Furthermore, soil enzyme stoichiometry is consistent on global and regional scales, so many studies predict the variation of soil microbial resource allocation by comparing the regional enzyme stoichiometry with global average levels (He et al., 2020), and guide the healthy development of agriculture in response to global changes.

The change of land use pattern is one of the important factors affecting the steady state of ecological stoichiometry (Gao et al., 2014). Firstly, different land use patterns lead to different fertilization rates, which will directly change the stoichiometric ratio of soil C, N, and P. Some results showed that long-term N addition could significantly improve the total nitrogen content and N: P ratios (Li et al., 2021), and high N input could change the soil community structure and microbial biomass (Muhammad et al., 2021). Secondly, the litter and root exudates of different vegetation types are different, which leads to changes in ecological stoichiometry. Many studies have found that litter changes the soil enzyme stoichiometry due to the alteration of C input (Liu et al., 2021a). Moreover, the root exudates significantly affected the stoichiometry of rhizosphere soil enzymes, resulting in the change of stoichiometric steady state (Xiao et al., 2021). Thirdly, different aboveground vegetation has different nutrient requirements, which affect the soil nutrient cycle (Zhang et al., 2020). For example, Cui et al. (2019) found that in natural grassland, shrubland, woodland, and cropland, the microbial community in natural grassland was the least limited by C and P.

The Yellow River Delta (YRD) is a modern sedimentary plain formed by the deposition of a large amount of sediment carried by the Yellow River (Li et al., 2014). It is one of the largest coastal saline-alkali lands in the warm temperate zone of China and has the characteristics of shallow groundwater, poor soil texture, and high evaporation-precipitation ratio (Cui et al., 2021). In recent decades, a large area of wilderness has been cultivated to promote the development of agriculture. However, the land use changed frequently due to the secondary salinization and human disturbance, resulting in serious soil quality reduction and land degradation. Studies had shown that under the combined long-term effects of sedimentation, reclamation, and fertilization, the average soil C: N: P ratio in the YRD is 64.5: 2: 1, which led to the common limitation of N and P in the local soil (Meng et al., 2021). Therefore, the study of ecological stoichiometry in the YRD is of great significance to guide the rational land use in the saline-alkali reclamation region. Although previous studies have revealed the soil stoichiometry at the regional scale of YRD, there are few studies on combined effect of soil C: N: P ratio and enzyme stoichiometry under different land use patterns. In this study, three representative land use patterns were selected to analyze the nutrient elements, enzyme activity, and stoichiometric characteristics, in order to provide a theoretical basis for the rational land use and sustainable development of agricultural ecosystem in the YRD.

2. Materials and methods

2.1. Site description

The study field was located in the reclamation region of the YRD (37°54'19"-37deg57'46"N, 118deg40'52"-118deg43'02"E), Dongying City, Shandong Province, China. The region belongs to the continental monsoon climate zone in warm temperate, with an annual average temperature of 12.1degC and annual average precipitation of 690 mm, mostly concentrated in summer, accounting for 63.9% of the total annual precipitation. The annual sunshine time is 2571–2865 h and the annual average frost-free period is 210 days. As a piece of young land in the YRD, the soil is mainly silt particle type and soil texture is light loam. The soil types are saline soil and fluvo-aquic soil. The average pH is 8.31 and the average salt content is 5.84 g kg^{-1} (0-20cm of soil layer) (Wang et al., 2021b). The soil had a poor fertilizer conservation due to short development time. But the sense data and vector data for more than 30 years about land use change have shown that the area of new reclaimed land for forage land and arable increased fast (Zhang et al., 2021a). The comprehensive agriculture was planned to develop in the YRD by Chinese central government due to the anthropogenic expansion (Zhang et al., 2011). Therefore, land use conversion was frequent and secondary salinization was occurred seriously due to human activities, which threated the food production and environment safety.

The native representative species in the study site consist of*Phragmites communis* (Cav.) Trin. ex Steud. and *Suaeda salsa* (L.) Pall.. The large part with native vegetation has been reclaimed for gaining new cultivated land in 2001 (Jiao et al., 2014). The new cultivation vegetation mainly consists of wheat (*Triticum aestivum* L.) and maize (*Zea may* L.), cotton (*Gossypium*spp), and alfalfa (*Medicago sativa* L.). The wheat-maize rotation is a main cultivated model with conventional tillage in the saline-alkali reclamation region. The winter wheat was sown in early October and harvested in early June next year, and the fertilizer amounts of N, P_2O_5, and K_2O were 240 kg hm^{-2}, 120 kg hm^{-2}, and 90 kg hm^{-2}, respectively. The summer maize was sown in mid-June and harvested in late September, and the fertilizer amounts of N, P_2O_5, and K_2O were 225 kg hm^{-2}, 75 kg hm^{-2}, and 90 kg hm^{-2}, respectively. The cotton with 85% coverage was applied with basal fertilizer and after fertilizer ($CO(NH_2)_2$: $(NH_4)_2HPO_4$ = 3: 1; 600 kg hm^{-2}) every year. The alfalfa as leguminous forage is the important green forage source harvested four times in late May, early July, late August, and early October each year, respectively, and did not receive any fertilization. The conversion between cotton and alfalfa happened frequently due to the secondary salinization and waterlogging.

2.2. Experimental design and sampling method

We selected three predominant land use patterns discussed above (alfalfa artificial grassland: AG, wheat-maize rotation field: WM, and native grassland: PC). Each land use pattern was located in three representative sample sites with relative flat surface and had the same physiographical units and slope gradients, and established nine 20 x 20 m^2 plots in each land use type in spring 2011. In total, we determined 3 representative sample sites and 9 plots as true replicated in each land use. The native grassland with more than 30-45 m width was located beside the reclamation field, respectively, and the distance between each plot exceeded the spatial correlation of most soil physicochemical and microbial characteristics (< 13.5 m) (Jiao et al., 2019). The cultivation history of three land use types and soil basic physiochemical properties in 0-20 cm depth when plots were established in spring 2011 were shown in Table 1 and Table 2, respectively.

A field survey was conducted in mid-June 2020 (after the winter wheat was harvested). We selected 5 quadrats of 1mx1m in each plot for soil sample collection according to the "S" shape sampling method, and five soil sampling drills were located at the center and the four diagonal corners of each 1mx1m quadrat at three depths (0-20cm, 20-40cm, 40-60cm) by using a drill (8 cm-diameter). All the soil samples at the same layer of each quadrat were mixed to create a representative soil sample. Overall, 81 composite soil samples were collected, representing three land uses, three depths, and nine replicates. The mixed soil samples were taken back to the laboratory. After removing roots and stones, the samples were divided into two parts. One part was stored in the refrigerator at -80 for the enzymatic activity, ammonium nitrogen (NH_4^+-N), and nitrate nitrogen (NO_3^--N). The other part of soil samples was screened with 0.15 mm and 2 mm sieves respectively after dried and crushed for soil properties determined. Soil bulk density (BD) was collected at the intermediate position of 0-20, 20-40, and 40-60cm soil layer of each quadrat using a cutting ring with 5.0 cm inner diameter and 100 cm^3 volume. The undisturbed soil was collected in 0-20, 20-40, and 40-60cm soil layer of each quadrat with aluminum box for the content of water stable aggregate (WSA).

2.3. Laboratory analysis

The BD was determined by drying method of oven-dried at 105 for 24 h to constant weight. The contents of WSA were determined by wet sieving method using soil aggregate analyzer (TTF-100, Shunlong experimental instrument factory, Shanghai, China). The enzymatic activities were determined by Deforest method for β-1,4-glucosidase (βG), β-1,4-N-acetyl-glucosaminidase (NAG), L-leucine aminopeptidase (LAP), and acid phosphatase (ACP) (Deforest, 2009). The contents of NH_4^+-N and NO_3^--N in soil were determined by continuous flow injection analyzer (AA3-A001-02E, Germany), after extracting 3 g of fresh soil sample with 25 ml 1 mol L^{-1} KCl solution for 30 min. Soil hydrogen ion concentration (pH) (water-soil ratio of 2.5:1) was determined by pH meter (Sartorius PB-10, Germany). Soil electrical conductivity (EC) (water-soil ratio of 5:1) was determined by conductivity meter (DDS-307, Shanghai, China). Soil total carbon (TC) contents were determined by element analyzer (ECS4024, Italy), after wrapping 0.2 g of dry soil sample screened 0.15 mm sieve with tin paper. The contents of soil organic carbon (SOC) and soil total nitrogen (TN) were determined by potassium dichromate external heating method and Kjeldahl method, after weighing 0.1 g and 1 g of dry soil sample screened 0.15 mm sieve, respectively. Soil total phosphorus (TP) contents after digesting 0.5 g of dry soil sample screened 0.15 mm sieve by $HClO_4$-H_2SO_4 and adjusting pH and soil available phosphorus (AP) contents after extracting 2 g of dry soil sample screened 2 mm sieve with 40 ml 0.5 mol L^{-1} $NaHCO_3$ solution for 30 min were determined by Mo-Sb colorimetric method with ultraviolet spectrophotometer (UV-5500, Shanghai, China) at 700 nm wavelength (Zhang et al., 2019c).

2.4. Statistical analysis

Soil C: N, C: P, and N: P were calculated by formula (1) (2) (3).

$Soil\ C : N\ ratio = TC \div TN(1)$

$Soil\ C : P\ ratio = TC \div TP(2)$

$Soil\ N : P\ ratio = TN \div TP(3)$

Enzyme C: N, C: P, and N: P were calculated by formula (4) (5) (6).

$Enzyme\ C : N\ ratio = \ln \beta\Gamma \div \ln \mathrm{NAG}(4)$

$Enzyme\ C : P\ ratio = \ln \beta\Gamma \div \ln \mathrm{AP}(5)$

$Enzyme\ N : P\ ratio = \ln \mathrm{NAG} \div \ln \mathrm{AP}(6)$

The vector analysis of enzyme activity is used to test the relative nutrient limitation. The relatively long vector length indicates the greater carbon limitation of soil microorganism, and the vector angle $< 45°$ or $> 45°$ indicates the relative degree of N or P limitation of soil microorganism, respectively. The vector length (VL) and vector angle (VA) are calculated by formula (7) (8) (Bai et al., 2021).

$VL = \left[(Enzyme\ C : N\ ratio)^2 + (Enzyme\ C : P\ ratio)^2\right]^{\frac{1}{2}}(7)$

$VA = Degrees\left\{ATAN2\left[(Enzyme\ C : P\ ratio), (Enzyme\ C : N\ ratio)\right]\right\}(8)$

Excel 2016 was used for data processing, SPSS 23 was used for one-way ANOVA and LSD significance test ($\alpha = 0.05$), and origin 2018 was used for chart drawing. Redundancy analysis (RDA) was used to analyze how enzyme activity and enzyme stoichiometry changed with soil physicochemical properties and soil stoichiometry, and the significance of these factors was tested by Monte Carlo permutation (permutation = 499). RDA analysis charts were drawn by Canoco 5.0 software.

3. Results

3.1. Effects of different land use patterns on soil physical and chemical properties

Soil physical properties were significantly affected by land use patterns (Table 3). Compared with PC, AG and WM significantly reduced soil BD and increased the contents of WSA (> 0.25 mm). The WSA contents of AG were mainly concentrated in > 0.5 mm, accounting for 69.51%, 74.20%, and 73.58% of the total WSA content in 0-20, 20-40, and 40-60 cm soil layers, respectively. The WSA contents of WM were mainly concentrated in 2-0.25 mm and accounted for 63.68%, 60.88%, and 66.25% of the total WSA content in 0-20, 20-40, and 40-60 cm soil layers, respectively. The WSA contents of PC were concentrated in > 2 mm and $<$ 0.25 mm.

Soil pH and EC increased with soil depth (Table 4). Compared with PC, AG and WM increased soil pH in 0-20 cm layer and decreased soil pH in 40-60 cm layer. AG increased EC in 0-20 cm soil layer and decreased EC in 40-60 cm soil layer, while EC in WM was higher than that in other land use patterns. The contents of SOC, NH_4^+-N, NO_3^--N, and AP decreased with soil depth, and these contents of AG and WM were significantly higher than that of PC. Compared with WM, AG was more helpful to increase the contents of NH_4^+-N and NO_3^--N in soil. NH_4^+-N contents in 20-40 and 40-60 cm soil layers and NO_3^--N contents in 0-20 cm soil layer of AG were significantly higher than those of WM and increased by 9.58%, 13.36%, and 15.06% respectively.

3.2. Effects of different land use patterns on soil stoichiometry

The total carbon contents of each soil layer were WM > AG > PC (Fig. 1). The total nitrogen contents decreased with the deepening of the soil layer, and the total nitrogen contents of each soil layer were AG > WM > PC, and the differences were significant. The total phosphorus contents of AG and WM were significantly higher than PC and increased by 47.57% and 54.60% respectively in 0-20cm soil layer, while they were not significant in 20-40 and 40-60 cm soil layer.

The soil C:N ratios in 0-20 and 40-60 cm soil layers were WM > PC > AG, while those in 20-40 cm soil layer were PC > WM > AG (Fig. 1). The soil C:P ratios of each land use pattern were not significant in 0-20 cm soil layer, while they showed WM > AG > PC in 20-40 and 40-60 cm soil layers. The soil N:P ratios of AG were significantly higher than those of WM and PC in each soil layer. The mean soil stoichiometric ratios of AG, WM, and PC were 31.32: 1.74: 1, 34.05: 1.46: 1, and 26.58: 1.14: 1, respectively.

3.3. Effects of different land use patterns on soil enzyme activity and enzyme stoichiometry

The enzyme activities of βG and NAG decreased with the deepening of the soil layer (Fig. 2). In 0-20 and 20-40 cm soil layer, the βG enzyme activities of AG and WM were significantly higher than those of PC. The NAG enzyme activities of AG in all soil layers were higher than that of WM and PC. ACP enzyme activities in 0-20 and 20-40 cm soil layers were AG > WM > PC, but in 40-60 cm soil layer, WM were significantly higher than other land use patterns. The enzyme activities of LAP in each soil layer were WM > AG > PC, and increased with the deepening of the soil layer.

The enzyme C: N ratios of different land use patterns in different soil layers were greater than 1:1 (Fig. 2). The enzyme C: N ratios of PC were lower than those of other land use patterns in 0-20 and 20-40 cm soil layers, and the same was true for AG in 40-60 cm soil layer. The enzyme N:P ratios were less than 1:1. The enzyme N:P ratios of WM in 0-20 cm soil layer and AG in 40-60cm soil layer were higher than those of other land use patterns. The enzyme C:P ratios were less than 1:1 except for the WM in 20-40 cm soil layer. The enzyme C:P ratios were WM > AG > PC in 0-20 and 20-40 cm soil layers and AG > PC > WM in 40-60 cm soil layer. The mean enzyme stoichiometric ratios of AG, WM, and PC were 0.96: 0.76: 1, 0.94: 0.74: 1, and 0.86: 0.73: 1, respectively. In 0-20 and 20-40 cm soil layers, the VL values of PC were significantly lower than those of other land use types. The VA of each land use pattern was more than 45°.

3.4. Linkages between soil CNP stoichiometry and enzyme stoichiometry

There was a negative linear correlation between enzyme C: N ratio and soil C: N ratio in 0-20 and 20-40 cm soil layers ($p < 0.01$), but a positive linear correlation in 40-60 cm soil layers ($p < 0.05$) (Fig. 3). Enzyme C: P ratio had a negative linear correlation with soil C: P ratio in 0-20 and 40-60 cm soil layers ($p < 0.01$), but a positive linear correlation in 20-40 cm soil layer ($p< 0.01$). Enzyme N: P ratio had a negative linear correlation with soil N: P ratio in 0-20 cm soil layer ($p < 0.01$), but a positive linear correlation in 20-40 and 40-60 cm soil layers ($p < 0.01$).

In AG (Fig. 4a), RDA analysis showed that enzyme activity (βG, NAG, and ACP) and enzyme stoichiometry (enzyme C: P and N: P ratio) were mainly associated with soil nutrient elements (TC, TN, TP, SOC, NH_4^+-N, NO_3^--N, and AP, positively) and soil stoichiometry (soil C: N and C: P ratio, negatively). Among them, TP and NH_4^+-N had significant effects on biological factors ($p < 0.01$), indicating that they were the main factors driving AG enzyme activity and enzyme stoichiometry variation. In WM (Fig. 4b), RDA analysis showed that enzyme activity (βG and NAG) and enzyme stoichiometry (enzyme C: P and N: P ratio) were mainly related to soil nutrient elements (TN, TP, SOC, NH_4^+-N, NO_3^--N, and AP, positively) and soil stoichiometry (soil C: N, C: P, and N:P ratio, negatively). Among them, TP and WSA (2-1 mm) had significant effects on biological factors ($p < 0.01$). In PC (Fig. 4c), RDA analysis showed that enzyme activity (βG, NAG, and ACP) and enzyme stoichiometry (enzyme C: P and N: P ratio) were mainly related to soil nutrient elements (TC, TN, TP, SOC, NH_4^+-N, NO_3^--N, and AP, positively) and soil stoichiometry (soil C:P and N:P ratio, positively). Among them, NO_3^--N had significant effects on biological factors ($p <$ 0.01). Meanwhile, the enzyme C:P and N:P ratios were negatively associated with enzyme C:N ratios in all land use patterns.

4. Discussion

4.1. Effects of different land use patterns on soil physical and chemical properties

Different land use patterns, due to differences in fertilization, vegetation types, and tillage methods, will lead to significant changes in soil physicochemical properties. In our study, AG and WM significantly increased WSA (> 0.25 mm) content compared with PC, and WSA (> 0.25 mm) was considered to play an important role in soil stability (Zhang et al., 2021b). This is because AG and WM have more litter, root biomass, and root exudates, which can provide important binders for soil aggregates and promote the formation of soil macroaggregates (Hazra et al., 2019). The application of chemical fertilizer has little effect on the size distribution of soil aggregates (Liao et al., 2021), while long-term cultivation and frequent disturbance will directly reduce the stability of WSA (Li et al., 2009), which also explains the reason why the WSA (> 0.25 mm) content of AG is higher than that of WM.

The research showed that soil salinity in the YRD was affected by groundwater depth, soil evaporation, soil water holding capacity, and vegetation canopy density (Zhang et al., 2019a). The soil salinization degree in this study increased with the deepening of soil layer, which was due to the increase of rainfall in summer, increasing salt leaching (Xie et al., 2011). Meanwhile, the soil salinity of AG and WM was higher than that of PC, which was due to the salt enrichment of rhizosphere soil caused by the "salt island" effect of alfalfa and the "fertilizer island" effect of wheat and maize in the face of the poor environment (Li et al., 2019c). The soil salinity of AG was less than that of WM, which was due to the higher ground coverage and canopy density of alfalfa, which reduced soil evaporation and inhibited the accumulation of salt on the soil surface (Xia et al., 2019).

The effects of different land use patterns on soil nutrient cycle are also different. The results showed reasonable N management and straw returning could promote soil C sequestration in wheat-maize rotation (Zhao et al., 2021). In this study, the WM was more conducive to increasing soil TC content compared with AG, while cutting alfalfa did not increase soil TC content (Bell et al., 2012). The AG was more conducive to the increase of SOC content, which was due to its higher content of macroaggregates, and the C in macroaggregates was easier to be decomposed by microorganisms (Bhattacharyya et al., 2021). In this study, AG significantly increased the contents of TN, NH_4^+-N, and NO_3^--N in soil compared with WM and PC, which was consistent with the previous research conclusions (Li et al., 2019b). Leguminous forage contributed 40-70kg N hm^{-2} per quarter (Sanginga, 2003), and continuous alfalfa cropping could promote N mineralization and improve N availability (Li et al., 2019b). Contrary to the trend of N in this study, the contents of TP and AP in AG were lower than that in WM. This is because leguminous forage such as alfalfa need more P than traditional crops. After all, P plays an important role in the energy transformation of rhizobia (Wang et al., 2021a). The research showed that soil P content decreased with the increase of alfalfa continuous cropping years (Jiang et al., 2006).

4.2. Effects of different land use patterns on soil stoichiometry

Soil C: N: P ratio is an important index that reflecting the vegetation nutrient limitation and biochemical cycle. The C: N ratio is used to characterize the mineralization degree of organic matter, and low C: N ratio (< 25) indicates that mineralization of soil organic matter is greater than fixation (Muhammad et al., 2021). And C: P ratio is used to measure P availability. The lower C: P ratio is, the higher P availability is (Zhang et al., 2019e). Meanwhile, soil C: P ratio is negatively correlated with soil C emissions (Liu et al., 2021b). In this study, the C: N ratios of AG were lower than that of WM and PC in all soil layers, and the C: N ratios of WM in 40-60cm soil layer and PC in 20-60cm soil layer were higher than 25. The soil C: P ratios were WM > AG > PC in 20-60cm soil layers. The results indicated that AG was beneficial to microbial decomposition of soil organic matter and improvement of soil P availability, but it also increased the risk of C emissions, while WM was beneficial to soil C sequestration in 40-60cm soil layer. In addition, PC was beneficial to soil C sequestration in 20-60cm soil layer, which was caused by no disturbance of PC to soil (Topa et al., 2021). Soil N: P ratio can be used as an effective index to measure plant nutrient limitation. With the increase of soil N: P ratios, the limitation of P on plant growth also increases (Xiao et al., 2021). In this study, soil N: P ratios were AG > WM > PC and were less than 14. The results showed that when the soil N: P ratio was less than 14, there was nitrogen limitation in plants (Xi and Jiang, 2019). Therefore, the aboveground vegetation growth of the three land use patterns in the YRD was limited by N, and AG was more limited by P than WM and PC.

In this study, the average soil stoichiometric ratios of AG, WM, and PC were 31.32: 1.74: 1, 34.05: 1.46: 1, and 26.58: 1.14: 1, respectively. Compared with the soil stoichiometry in the world of grassland (C: N: P=169: 12: 1) (Cleveland and Liptzin, 2007) and farmland (C: N: P=64: 5: 1) (Zheng et al., 2021), it showed remarkably narrow C: N: P ratios in the YRD, which was due to lower C and N levels. Meanwhile, there was little difference between AG and WM, which indicated that the fixation effect of AG on soil C was far lower than expected. This is due to a large amount of human disturbance in AG, such as mowing, which will lead to the loss of soil C (Wang et al., 2020). Compared with the average soil stoichiometry of China (C: N: P=60: 5: 1) (Tian et al., 2010), AG, WM, and PC showed lower C and N contents, while the N content of AG was closer to the average level of China. Therefore, N limitation is a common problem in the YRD. Among the three land use patterns, AG is more conducive to alleviate the N limitation.

4.3. Effects of different land use patterns on soil enzyme activity and stoichiometry

Land use patterns have different effects on soil enzyme activities due to different planting systems and management measures. Some studies showed that the SOC content and βG activity increased gradually with the decrease of soil disturbance and the settlement of plants, especially leguminous crops (Yu et al., 2017). Similar conclusions exist in this study, the βG enzyme activity of AG was higher than that of WM and PC. Meanwhile, the βG enzyme activity of WM was higher than that of PC, which was due to the C source provided by straw returning in wheat maize rotation (Chen et al., 2021). The sequence of NAG activity was similar to that of BG, *i.e.* , AG > WM > PC. The increase of NAG activity may be due to the increase of soil N availability. According to the theory of resource allocation, soil extracellular enzyme activities are usually significantly positively correlated with nutrient availability before reaching the response boundary of microbial disturbance or fluctuation to nutrient availability (Sinsabaugh et al., 2008; Zhang et al., 2019c). In this study, the LAP activity of WM was significantly higher than that of AG and PC, which may be due to the hydrolysis of cellulose in wheat and corn straw to produce glucose. Some studies have shown that the increase of glucose content would drive the change of soil microbial community (Zwetsloot et al., 2020), and increase the activities of LAP and ACP (Zhang et al., 2019d). This also explained the significant increase of ACP activities of AG in 0-20cm soil layer and WM in 40-60cm soil layer, in which AG was due to the higher βG activity led to the increase of glucose content at the end of hydrolysis, while WM increased the glucose content due to a large amount of carbon source provided by straw returning.

Soil enzyme stoichiometry is used to indicate the nutritional needs of microorganisms and converges to 1:1:1 globally (Sinsabaugh et al., 2008). According to the viewpoint that microorganisms optimize the allocation of resources to obtain the most limited resources, the greater the investment in enzyme activity of C, N, or P, the greater the demand for this nutrient element (Bai et al., 2021). In this study, the enzyme C: N ratios were greater than 1. The enzyme N: P and C: P ratios were less than 1. According to the vector analysis of enzyme stoichiometry, the VL value of PC in 0-40 cm soil layer were significantly lower than that of other land use types, and the VA value of each land use type in each soil layer was greater than 45 °. These results showed that PC was less limited by C, and the microorganisms in each land use pattern were limited by P than N. This may be because PC has no human disturbance for a long time. According to the homeostasis theory of enzyme stoichiometry, soil microorganisms can promote the equilibrium and homeostasis of enzyme stoichiometry (Xiao et al., 2020), thus reducing the C limitation of PC microorganisms. Soil P mainly comes from weathering of primary minerals and cannot be acquired largely from soil (Zhang et al., 2019b). Moreover, in saline-alkali soil, the adsorption of calcium and magnesium ions and the consumption of plants will lead to the decline of P bioavailability (Zhang et al., 2019c). This also explains why soil microorganisms in the YRD were generally limited by P.

4.4. Linkages between soil stoichiometry and enzyme stoichiometry

In this study, the average enzyme stoichiometry of AG, WM, and PC was 0.96: 0.76: 1, 0.94: 0.74: 1, and 0.86: 0.73: 1, respectively, which deviated from 1: 1: 1. Moreover, the enzyme stoichiometry and soil stoichiometry showed the significant linear correlation in each soil layer. These results indicated that enzyme stoichiometry of land use patterns in the YRD was not homeostasis, but nutrient dependent (Qiu et al., 2021). According to RDA analysis, the main driving factors of community variation in AG, WM, and PC were TP, TP and WSA (2-1 mm), and NO_3^--N, respectively, and these factors were positively correlated with the enzyme activities (βG, NAG, and ACP) and enzyme C: P and N: P ratios, but negatively correlated with enzyme C: N ratios. Studies have shown that extracellular enzyme activity is mainly determined by the number of available substrates and the microbial biomass for extracellular enzyme synthesis (Blagodatskaya et al., 2014). The increase of TP provided sufficient substrate for ACP. Meanwhile, the fungal biomass would affect the microbial biomass involved in extracellular enzyme synthesis, and the fungal biomass was easily affected by tillage (Li et al., 2019a). Therefore, the main driving factors of community variation in WM and AG were TP and WSA (2-1 mm), and TP, respectively. According to the viewpoint that microorganisms optimize resource allocation to obtain the most limited resources (Bai et al., 2021; Zhang et al., 2019d). According to RDA analysis, when P was no longer the nutrient limiting factor of microorganisms with the increase of P content, microorganisms would promote the synthesis of βG and NAG enzymes, thus increasing the enzyme C: P and N: P ratios. However, the increase of enzyme C: P and N: P ratios would lead to the decrease of enzyme C: N ratio, which would cause the N limitation of microorganisms. This is consistent with the conclusion of Yang et al. (2020), who found that the nutrient limitation of soil microorganisms changed from P limitation to N limitation with the plant restoration process to about 15 years. It also explained that NO_3^--N was the main factor driving the variation of PC community without human disturbance for a long time. Therefore, although soil microorganisms in the YRD were generally limited by P, the microbial nutrient limitation would change from P limitation to N limitation over time. Our results should contribute to the development of soil improvement strategies in the YRD.

5. Conclusions

In this paper, we studied the compound effects of soil C: N: P ratio and enzyme stoichiometry under different land use patterns in the YRD, and better revealed the biochemical cycle and microbial nutrient limitation of YRD. The results showed that: (1) AG and WM could promote the formation of soil macro-aggregates ($>$ 0.25mm). AG was beneficial to promote the mineralization of C, N, and P, but its effect on the sequestration of TC and TP was not as good as WM. (2) According to the soil C: N: P ratios, the levels of soil C and N in the YRD were low, and AG could alleviate the limitation of aboveground vegetation growth by N. (3) According to the enzyme stoichiometric, soil microorganisms were generally limited by P in the YRD, while PC could reduce the C limitation of soil microorganisms due to less soil disturbance. Accordingly, we suggest that human disturbance, such as mowing and soil disturbance, should be minimized in the agricultural production of YRD, to alleviate C restriction. Reasonable planting of alfalfa can alleviate N limitation. Meanwhile, we should increase the input of soil P and carry out reasonable N management to deal with the possible N limitation. In general, ecological stoichiometry provided a theoretical basis for the rational land use in the YRD.

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Declarations

The authors declare that they have no known conflict of interest that may affect the work reported in this paper.

CRediT authorship contribution statement

Baishu Kong: Conceptualization, Data Curation, Formal Analysis, Investigation, Writing-Original Draft Preparation, Writing-Review & Editing. Shuying Jiao: Funding Acquisition, Project Administration, Resources, Supervision, Writing-Review & Editing. Yongqiang Li: Investigation, Writing-Review & Editing.

Lianhui Shi: Investigation, Resources. Chuanrong Li: Investigation. Yuwen Shen: Funding Acquisition. Sen Jia: Investigation. Chunyu Fu: Investigation. Taochuan Zhu: Investigation.

Data availability

The datasets generated during and/or analyzed during the current study are available on the Dryad Digital Repository. https://doi.org/10.5061/dryad.wm37pvmph

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Table 1 Cultivation history of different land use patterns

Land use patterns	Cultivation and reclamation history
AG	Reclaimed from natural land in 2001. Alfalfa was planted for four years in 2001-2004. Cotton was plan
WM	Reclaimed from natural land in 2001. Then wheat-maize rotation was carried out from 2001 to 2020.
PC	No reclaimed site (native vegetation) of *Phragmites communis* (Cav.) Trin. ex Steud. and *Suaeda sals*

Note: AG: alfalfa artificial grassland. WM: wheat-maize rotation field. PC: native grassland.

Table 2 The basic soil physiochemical properties in 0-20 cm depth of three land use patterns in 2011

Land use patterns	Soil particle size (%)	Soil particle size (%)	Soil particle size (%)	pH	EC (µs cm^{-1})	SOC (g kg^{-1})	TN (g kg^{-1})	AP (mg kg^{-1})
	Sand	Silt	Clay					
AG	87.12	10.43	2.54	8.15	357.56	8.53	1.23	22.16
WM	83.46	13.65	3.10	8.13	380.87	7.86	1.14	18.77
PC	77.57	18.92	3.51	7.85	341.35	7.43	0.76	6.43

AG: alfalfa artificial grassland. WM: wheat-maize rotation field. PC: native grassland. The particle size ranges of sand, silt, and clay are 2-0.02 mm, 0.02-0.002 mm, and < 0.002 mm, respectively. pH: hydrogen ion concentration. EC: soil electrical conductivity. SOC: soil organic carbon. TN: total nitrogen. AP: available phosphorus.

Table 3 Effects of different land use patterns on soil physical properties

Soil layer (cm)	Land use patterns	BD (g cm^{-3})	Mass fraction of WSA (%)	Mass fraction of WSA (%)	Mass fraction of WSA (%)	Mass fraction of WSA (%)	Mass fraction of WSA (%)
			> 2 mm	2-1 mm	1-0.5 mm	0.5-0.25 mm	< 0.25 mm
0-20	AG	1.12±0.15b	27.52±7.31a	21.93±1.82a	20.07±2.92b	12.21±5.78b	18.28±5.17b
	WM	1.12±0.13b	11.88±5.44b	16.75±3.41b	26.11±2.77a	20.82±3.90a	24.43±2.56b
	PC	1.46±0.04a	20.88±10.10ab	14.70±2.64b	12.87±2.10c	13.47±3.03b	38.07±5.44a
20-40	AG	1.12±0.02b	36.48±13.18a	22.33±5.00a	15.39±5.16b	10.44±6.10b	15.36±3.06c
	WM	1.16±0.03b	16.12±9.11b	15.25±2.44b	24.83±5.94a	20.81±5.82a	23.00±4.61b
	PC	1.43±0.04a	19.62±5.21b	12.50±2.73b	14.10±2.37b	13.26±6.11ab	40.52±2.82a
40-60	AG	1.10±0.06b	33.18±14.38a	21.33±3.91a	19.07±8.36b	12.36±4.34a	14.06±4.62b
	WM	1.11±0.05b	16.33±6.26b	21.55±5.31a	28.05±4.11a	16.65±2.86a	17.42±3.23b
	PC	1.47±0.02a	18.35±6.25b	16.25±6.09a	14.65±4.17b	14.83±1.12a	35.92±9.58a

Note: Different letters indicate significant differences among different land use patterns in the same soil layer ($p < 0.05$). AG: alfalfa artificial grassland. WM: wheat-maize rotation field. PC: native grassland. BD: soil bulk density. WSA: soil water stable aggregate.

Table 4 Effects of different land use patterns on soil chemical properties

Soil layer (cm)	Land use patterns	pH	EC (µs cm^{-1})	SOC (g kg^{-1})	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	AP (mg kg^{-1})
0-20	AG	8.51±0.08a	343.11±84.83ab	11.13±1.29a	13.12±0.96a	28.57±3.10a	32.27±10.51a
	WM	8.52±0.15a	428.12±104.06a	9.98±0.75a	12.21±1.34a	24.83±1.13b	27.95±10.46a
	PC	8.37±0.14b	297.65±50.71b	6.47±1.55b	5.22±0.38b	10.75±0.43c	6.85±2.88b
20-40	AG	8.53±0.12a	479.02±115.97a	9.23±1.02a	11.21±1.03a	23.59±3.07a	19.97±8.48a
	WM	8.56±0.06a	511.89±73.86a	7.57±0.66a	10.23±0.37b	20.96±3.33a	17.15±7.00a
	PC	8.64±0.13a	425.34±85.05a	4.64±1.23b	4.63±0.81c	7.60±0.31b	3.60±0.80b
40-60	AG	8.56±0.06ab	535.49±43.56b	7.50±1.35a	9.67±0.57a	17.12±0.65b	10.44±3.28a
	WM	8.50±0.07b	619.75±75.88a	6.11±0.89a	8.53±0.33b	18.75±1.67a	9.04±2.92a
	PC	8.64±0.14a	561.75±105.17ab	4.89±0.70b	3.65±0.87c	6.35±0.44c	3.13±0.55b

Note: Different letters indicate significant differences among different land use patterns in the same soil layer ($p < 0.05$). AG: alfalfa artificial grassland. WM: wheat-maize rotation field. PC: native grassland. pH: hydrogen ion concentration. EC: soil electrical conductivity. SOC: soil organic carbon. NH_4^+-N: ammonium nitrogen. NO_3^--N: nitrate nitrogen. AP: soil available phosphorus.

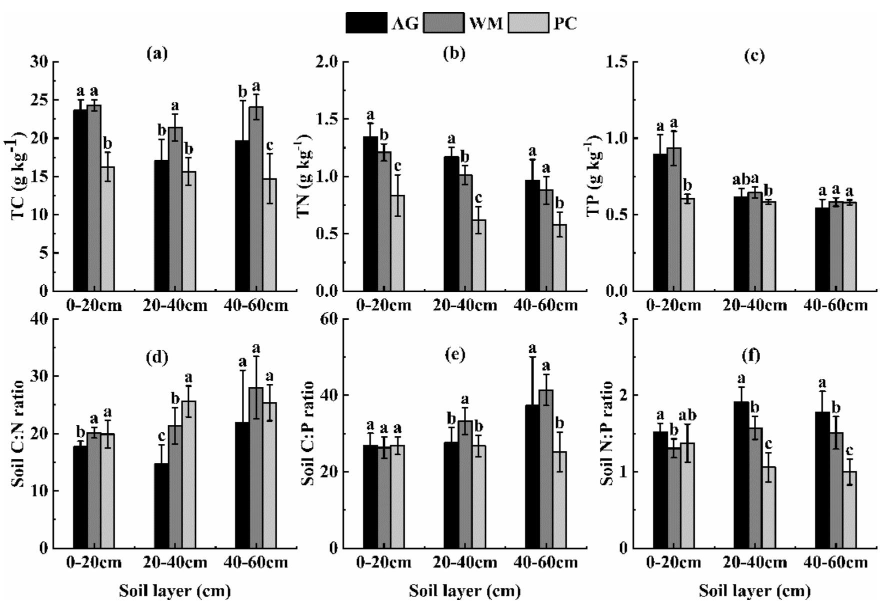


Fig. 1 Effects of different land use patterns on TC (a), TN (b), TP (c), C: N ratio (d), C: P ratio (e), and N: P ratio (f). Different letters indicate significant differences among different land use patterns in the same soil layer ($p < 0.05$). TC: soil total carbon. TN: soil total nitrogen. TP: soil total phosphorus. C: carbon. N: nitrogen. P: phosphorus. AG: alfalfa artificial grassland. WM: wheat-maize rotation field. PC: native grassland.

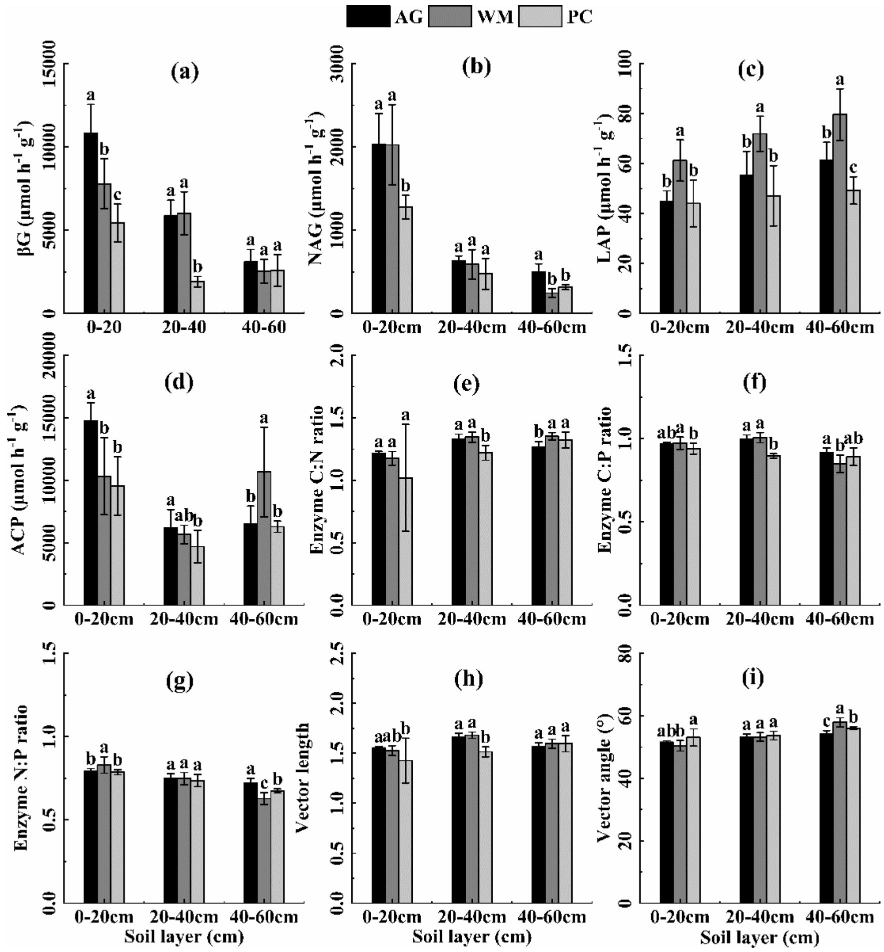


Fig. 2 Effects of different land use patterns on βG (a), NAG (b), LAP (c), ACP (d), enzyme C: N ratio (e), enzyme C: P ratio (f), enzyme N: P ratio (g), vector length (h) and vector angle (i). Different letters indicate significant differences among different land use patterns in the same soil layer ($p< 0.05$). βG: β-1,4-glucosidase. NAG: β-1,4-N-acetyl-glucosaminidase. LAP: L-leucine aminopeptidase. ACP: acid phosphatase. C: carbon. N: nitrogen. P: phosphorus. AG: alfalfa artificial grassland. WM: wheat-maize rotation field. PC: native grassland.

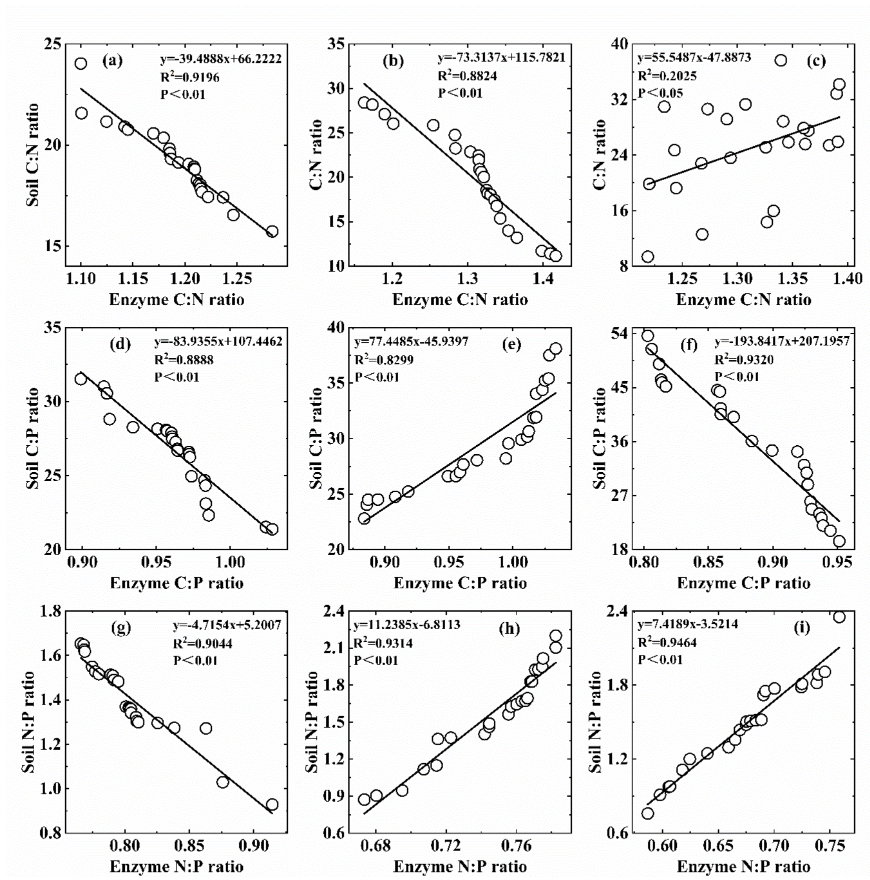
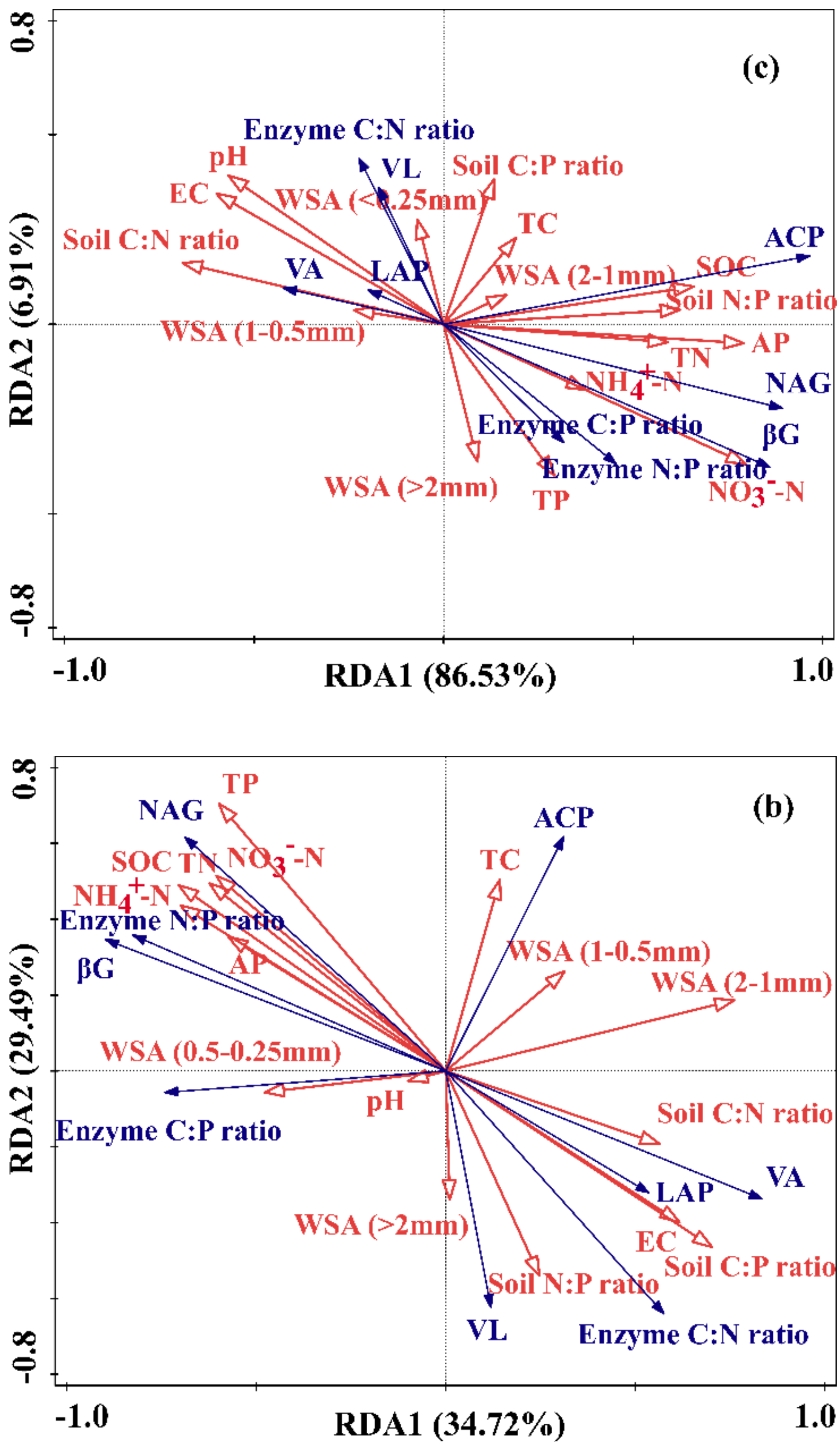


Fig. 3 Link between soil stoichiometry and enzyme stoichiometry in different land use patterns. The fitting curves of C:N ratio and enzyme C:N ratio at 0-20cm, 20-40cm and 40-60cm were shown as (a), (b) and (c) respectively. The fitting curves of C:P ratio and enzyme C:P ratio at 0-20cm, 20-40cm and 40-60cm were shown as (d), (e) and (f) respectively. The fitting curves of C:N ratio and enzyme C:N ratio at 0-20cm, 20-40cm and 40-60cm were shown as (g), (h) and (i) respectively. C: carbon. N: nitrogen. P: phosphorus.



(c)
RDA1 (86.53%)
RDA2 (6.91%)
Enzyme C:N ratio
VL
pH
EC
Soil C:P ratio
WSA (<0.25mm)
TC
Soil C:N ratio
VA
LAP
ACP
SOC
WSA (2-1mm)
Soil N:P ratio
WSA (1-0.5mm)
AP
TN
NH4+-N
NAG
Enzyme C:P ratio
βG
Enzyme N:P ratio
WSA (>2mm)
TP
NO3--N
(b)
RDA1 (34.72%)
RDA2 (29.49%)
TP
NAG
ACP
SOC
TN
NO3--N
TC
NH4+-N
Enzyme N:P ratio
βG
AP
WSA (1-0.5mm)
WSA (2-1mm)
WSA (0.5-0.25mm)
pH
Enzyme C:P ratio
Soil C:N ratio
VA
LAP
WSA (>2mm)
EC
Soil C:P ratio
Soil N:P ratio
VL
Enzyme C:N ratio

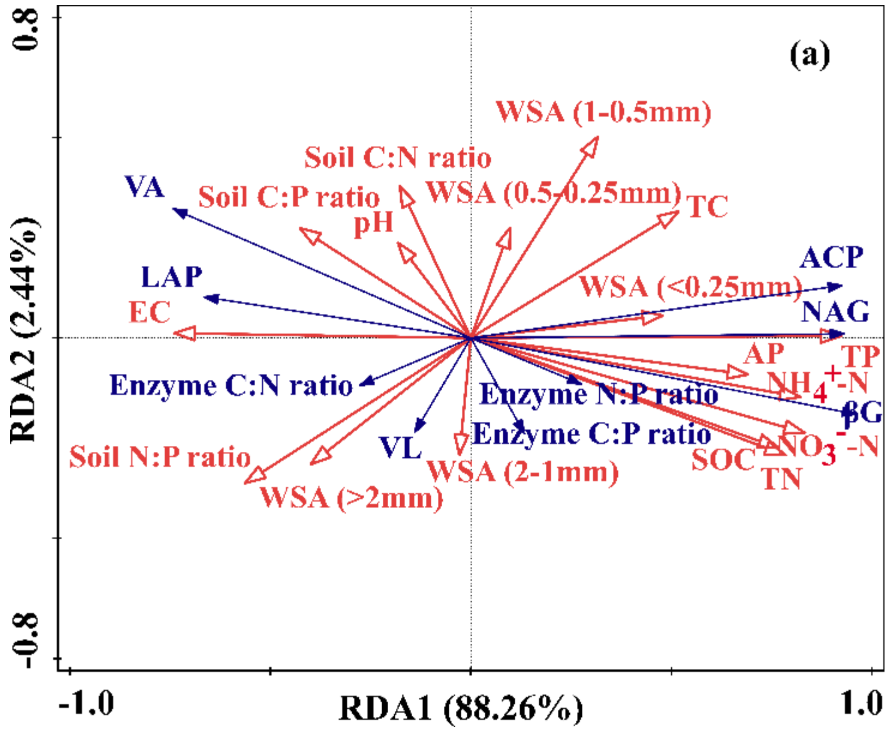


Fig. 4 Redundancy analysis (RDA) showing the relationships between enzyme activity and enzyme stoichiometry (blue arrows) with soil physicochemical properties and soil stoichiometry (red arrows) in different land use patterns. (a): The relationships in alfalfa artificial grassland (AG). RDA analysis explained 90.80% of the total variation in AG, RDA axis 1 and axis 2 explained 88.26% and 2.44% of the variation respectively. TP and NH_4^+-N had significant effects on biological factors ($p < 0.01$), and the explanatory degrees of biological factors were 75.4% and 7.4% respectively. (b): The relationships in wheat-maize rotation field (WM). RDA analysis explained 64.50% of the total variation in WM, RDA axis 1 and axis 2 explained 34.72% and 29.49% of the variation respectively. TP and WSA (2-1 mm) had significant effects on biological factors ($p < 0.01$), and the explanatory degrees of biological factors were 27.1% and 18.3% respectively. (c): The relationships in native grassland (PC). RDA analysis explained 93.80% of the total variation in PC, RDA axis 1 and axis 2 explained 86.53% and 6.91% of the variation respectively. NO_3^--N had significant effects on biological factors ($p < 0.01$), and the explanatory degree of biological factors was 56.00%. WSA: soil water stable aggregate. pH: hydrogen ion concentration. EC: soil electrical conductivity. TC: total carbon. TN: total nitrogen. TP: total phosphorus. SOC: soil organic carbon. NH_4^+-N: ammonium nitrogen. NO_3^--N: nitrate nitrogen. AP: soil available phosphorus. βG: β-1,4-glucosidase. NAG: β-1,4-N-acetyl-glucosaminidase. LAP: L-leucine aminopeptidase. AP: acid phosphatase. VL: vector length. VA: vector angle. C: carbon. N: nitrogen. P: phosphorus.