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Dynamics of soil specific enzyme activities and temperature sensitivities during grassland succession after farmland abandonment

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ABSTRACT

The influence of plant secondary succession on soil enzyme activities has been increasingly recognized recently. However, the characteristics of specific enzyme activities and temperature sensitivity (Q_{10}) in the rhizosphere remain elusive. We collected rhizosphere and bulk soil samples from a secondary successional series (0, 7, 12, 17, 22, and 32 years after farmland abandonment, and a natural grassland reference) in a typical semi-arid ecosystems on the Loess Plateau of China. The potential activity of soil enzymes, including β-1,4-glucosidase (BG), β-1,4-N-acetylglucosaminidase (NAG), leucine aminopeptidase (LAP), and alkaline phosphatase (AP), was assayed at 37, 20 and 4 °C and expressed as activity per unit of soil organic carbon (specific enzyme activity). Q_{10} values were calculated at 20–37 °C and 4–20 °C, respectively. The specific enzyme activities of BG, NAG, LAP and AP in bulk soil increased to a maximum at the 17-year site and then significantly decreased in older grasslands. In the rhizosphere, specific enzyme activities increased and then decreased along the successional gradient with significantly higher value at the 7-year site and the 17-year site. The Q_{10} values of BG activity in bulk soil and the four enzyme's activities in rhizosphere soil were changed significantly along the successional gradients. In general, the Q_{10} values at the two temperature ranges differed in bulk soil, but not in the rhizosphere soil. Along the plant successional gradient, the rhizosphere effects of specific enzyme activities gradually changed to negative values in the oldest sites. Our results indicate that plant secondary succession had different impacts on the enzyme activity and characteristics in the rhizosphere and bulk soil, and highlights the importance of temperature sensitivity and rhizosphere effects of enzyme activity along plant secondary successional gradients.

1. Introduction

Secondary succession following agricultural abandonment is an effective method for controlling soil erosion and restoring ecological functions of degraded ecosystems (Lozano et al., 2014; Zhang et al., 2017). Cessation of agricultural practices can result in a rapid re-establilshedment of natural vegetation with considerable changes in plant biomass production and simultaneously alters soil physical, chemical, and microbiological properties (Zhang et al., 2016; Zhao et al., 2019). Soil microbiological properties, such as soil enzyme activities, play a fundamental role in soil organic matter decomposition and mineralization, and in nutrient cycling (Burns et al., 2013; Xiao et al., 2020a). Soil enzyme activities are a result of soil physicochemical properties and microbial community characteristics, and have often been proposed as an early and sensitive indicator of soil restoration processes in degraded ecosystems (Wang et al., 2011; Raiesi and Salek-Gilani, 2018).

Soil enzyme activities can be expressed as either absolute activity (per unit of oven-dried soil), or specific activity (per unit of soil organic carbon or microbial biomass carbon) (Raiesi and Beheshti, 2014; Xiao et al., 2019). Numerous studies have evaluated the variation in absolute activities of soil enzymes during plant secondary succession (Jiang et al., 2009; Wang et al., 2011). For example, on the Loess Plateau of China, An et al. (2009) reported that the soil enzyme activities increased along a re-vegetation chronosequence up to 23 years, and then remained stable. Zhang et al. (2015a) found that the extracellular enzyme activities were progressively enhanced along a 50-year chronosequence of restoration on land that underwent desertification. Usually, the increased trend of

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soil enzyme activities with plant recovery was positively correlated with increased soil organic matter content and/or microbial biomass (Zhang et al., 2012; Li et al., 2015; Cui et al., 2019). Thus, this may mask the actual variation of soil enzyme activity during plant secondary succession.

Information about the specific enzyme activities would be useful to decouple the changes in soil enzyme activities from the changes of soil organic matter or microbial biomass (Zhang et al., 2015b; Xiao et al., 2019). However, much less information is available on the changes of specific activities of soil enzymes along plant secondary successional gradients (Raiesi and Salek-Gilani, 2018), especially in the rhizosphere zone of plant species, where soil enzyme activities are different due to the influence of different root exudates and diverse rhizospheric microorganisms in plant species rhizosphere (Song et al., 2019). Rhizosphere enzyme activities perform essential roles in soil carbon and nutrient cycling and the establishment of plant species (Cesco et al., 2012). These important functions have led to considerable interest in understanding the soil specific enzyme activities in the rhizosphere during plant secondary succession.

Soil enzyme activity is the rate limiting step in the decomposition and mineralization of soil organic matter (Allison and Vitousek, 2005). There are several factors influencing soil enzyme activity of which temperature and substrate availability are the most important (Stone et al., 2012; Wallenstein et al., 2009; Jarosch et al., 2019). Current soil enzyme methods (e.g. Saiya-Cork et al., 2002) measure potential enzyme activities, which are indicative of overall enzyme concentrations at certain temperatures and sufficient substrate. Thus, they do not provide insight into the actual rate of enzyme-catalyzed reactions under field conditions (Wallenstein and Weintraub, 2008).

Many researchers have focused on temperature sensitivity (assessed by Q_{10} value) of soil enzymes in relation to C, N and P acquisition to capture the response of enzyme activities to temperature (German et al., 2012; Menichetti et al., 2015; Allison et al., 2018). The temperature sensitivity of soil enzymes is critical for predicting daily, seasonal and annual variation of carbon and nutrient cycling (Ali et al., 2015). Generally, high temperatures generate lower Q_{10} value (Razavi et al., 2016; Schipper et al., 2014). However, the temperature sensitivity of enzymatic reactions is also associated with factors such as temperature range, enzyme and substrate availability, and enzyme-substrate complex formation rate (Razavi et al., 2016; Jarosch et al., 2019).

Plant secondary succession significantly altered plant species composition and diversity patterns (Sun et al., 2017), hence contribute to different quality and quantity of soil organic matter input and the soil aggregate microstructure (Novara et al., 2013; Zhao et al., 2017; Xiao et al., 2020b), which may contribute to the variation and distribution of soil enzymes through the formation of enzyme-clay or enzyme-humus complexes. Thus, because enzyme diffusion, availability, and the formation of the enzyme-substrate complex varies with temperature, the temperature sensitivity of soil enzymes would be affected. In the rhizosphere, increased microbial activity due to root exudates and other rhizodeposits, and the direct release of enzymes by different plant roots or by lysis of root cells causes diverse soil enzyme composition and activity when compared with bulk soil (Koranda et al., 2011; Wang et al., 2019). These further affected the temperature sensitivity of soil enzymes. A deep understanding of the temperature sensitivity of soil enzymes at different temperature ranges in the rhizosphere and bulk soil would help to further recognize the variation of soil enzyme characteristics during plant secondary succession restoration.

In the present research, we measured the activities of four enzymes at three assay temperatures in the rhizosphere and bulk soils collected along a plant secondary successional series on the Loess Plateau of China. We aimed to investigate the changes of specific enzymatic activities and temperature sensitivity in bulk and rhizosphere soil during plant secondary succession. We hypothesized that the rhizosphere and bulk soil would have different specific enzyme activities and temperature sensitivities along the plant secondary successional gradient, and that secondary succession would influence rhizosphere effects on specific enzyme activities and temperature sensitivities.

2. Materials and methods

2.1. Study site

The study site is located in a typical hilly gullied region of the northern Loess Plateau in the middle reaches of Yellow River in Zhifanggou watershed (36°42′–36°46′N, 109°13′–109°16′E, 1041.5–1425.7 m.a.s.L.), Ansai County, Shaanxi Province, China. The area is characterized by a temperate semiarid climate with a hot and humid summer, and a cold and dry winter. The annual mean temperature is 8.8 °C, and annual mean precipitation is 510 mm, most of which occurs between July and September. The soil, which originates from the parental material of wind-deposited loess, belongs to calcaric cambisols, according to the FAO-UNESCO soil classification system. The soils are calcareous with low soil organic matter, total nitrogen, and phosphorus content, and are thus extremely susceptible to soil erosion. The Chinese Government implemented the "Green for Grain" Project to control soil and water loss and improve watershed conservation in the 1980s. Many croplands were gradually abandoned for secondary succession to reestablish natural grasslands. This was one of the most effective methods for soil erosion control and improvement of the area's ecological condition. After more than 30 years of vegetation restoration, a chronosequence of grassland secondary succession series had been established in this area.

2.2. Sample collection

The "space" for "time" substitution method, which is a commonly used method for evaluating variation in soil physical and biochemical properties during plant restoration, was used to study the changes of specific activity and temperature sensitivity of enzymes in the bulk and rhizosphere soil along a secondary succession series. Six sloped farmland sites that had been abandoned for 0, 7, 12, 17, 22, and 32 years and a natural grassland that had been restored for more than 50 years were selected as a secondary succession series. The sloped farmland was fertilized annually with 6.0 t ha^{-1} goat manure, 60 kg ha^{-1} nitrogen (urea; $CO(NH_2)_2$) and 45 kg ha^{-1}phosphorus pentoxide (P_2O_5). Those study sites had similar slope aspects, gradients, and elevations, plus similar farming practices before they were abandoned for recovery. In each study site, the dominant plant species and other companion species were identified. After farmland abandoned for plant secondary succession, *Artemisia capillaris* was the dominant plant species at the 7- and 12-year site, with *Heteropappus altaicus*, *A. sacrorum*, and *Lespedeza davurica* as companion species. Then, *A. sacrorum* replaced *A. capillaris* as the dominant plant species, with *Stipa bungeana*, *L. davurica* and *Bothriochloa ischaemum* as the main companion species. The characteristics of the seven study sites are shown in Table 1.

Three 100 m × 100 m sampling plots were established at random locations in each of the study sites. The sampling plots were approximately 300–500 m distance. We collected rhizosphere and bulk soil samples in September 2016, when the plant biomass peaked and the rhizosphere effects tend to be most pronounced (Dijkstra et al., 2006). In each sampling plot, several dominant plant species and their roots were extracted for rhizosperic soil collection. After shaking off the loosely adhering soil, that remaining tightly attached to the roots was collected and thoroughly homogenized to constitute a rhizosphere soil sample.

At the same time, several soil cores were collected at random locations from the interspace of the plant species in each sampling plots, and homogenized to constitute a bulk soil sample. The field-moisture soil samples were sieved through a 2-mm mesh to remove visible plant roots, residues, and other large debris. Sieved soil samples were divided into two subsamples:(1) one used for soil chemical properties analysis after air-drying and being sieved through a 0.25 mm mesh, and (2) another

Table 1
Summary of study site characteristics along the secondary successional gradient.

Study site	Coordinates	Slope aspect	Slope gradient (°)	Elevation (m)	Dominant species	Companion species
Farmland	36°44′9″N, 109°14′35″E	E15°N	25	1274	*Seteria italica* + *Glycine* max	*Salsola collina*
7-year	36°44′47″N, 109°15′12″E	W10°N	20	1303	*Artemisia capillaries*	*Heteropappus altaicus, Salsola collina*
12-year	36°44′02″N, 109°16′31″E	E40°N	26	1276	*Artemisia capillaries*	*Artemisia sacrorum, Lespedeza davurica, Heteropappus alticus*
17-year	36°44′09″N, 109°16′14″E	E25°N	28	1307	*Artemisia sacrorum*	*Stipa bungeana, Artemisia capillaries, Heteropappus alticus, Lespedeza davurica*
22-year	36°44′05″N, 109°16′27″E	E10°N	30	1267	*Artemisia sacrorum*	*Stipa bungeana, Potentilla tanacetifolia, Heteropappus alticus*
32-year	36°44′15″N, 109°15′55″E	E16°N	30	1246	*Artemisia sacrorum*	*Lespedeza davurica, Potentilla tanacetifolia, Vicia sepium*
Natural grassland	36°44′59″N, 109°16′28″E	E34°N	26	1183	*Artemisia sacrorum*	*Bothriochloa ischaemum, Lespedeza davurica, Potentilla tanacetifolia*

stored at −80 °C for analysis of enzymatic activities.

2.3. Soil chemical properties analysis

Soil organic carbon (SOC) and total nitrogen (TN) were measured using the dichromate oxidation ferrous sulfate titration method and the semi-micro-Kjeldahl method, respectively (Nelson and Sommers, 1996; Bremner, 1996). Total phosphorus (TP) was measured using the molybdenum antimony blue colorimetry method (Olsen and Sommers, 1982). The chemical properties of rhizospehere and bulk soil were shown in Table 2.

2.4. Soil enzyme activity and temperature sensitivity

The potential activities of four enzymes involved in C, N, and P cycling were determined: β-glucosidase (BG), C-acquiring enzyme; N-acet-ylglucosaminidase (NAG) and L-leucine aminopeptidase (LAP), both N-acquiring enzymes; and phophatase (AP), a P-acquiring enzyme. Enzyme activities were assayed using a protocol modified from Saiya-Cork et al. (2002). For each sample, a 1.0 g dry mass of fresh soil was combined with 100 mL of 50 mM Tris-base buffer in a beaker and homogenized to a uniform suspension using a blender for approximately 1 min. The pH of the Tris-base buffer was experimentally varied to approximate the pH of soil samples. The soil suspension (200 μL) and 200 μM substrates specific to each enzyme (Table 3; 50 μL) were dispensed into the wells of a black 96-well microtiter plate. We included six replicates for each soil sample, a negative control, and a quench standard. The microtiter plates were incubated in the dark for 4 h (the optimal duration of the assay determined prior to experiment) at three different temperatures: 4, 20, and 37 °C. Fluorescence values were measured using a Synergy^{TM4} microplate reader (BioTek, USA) with 365-nm excitation and 450-nm emission filters. The unit of the enzyme activities was expressed as nmol h^{-1} g^{-1} dry soil. Soil enzyme activities were normalized by the magnitude of the SOC content, and the unit was expressed as nmol h^{-1} mg^{-1} SOC.

The three incubation temperatures were used to estimate two temperature sensitivity (Q_{10}) values in case there was a significant difference between Q_{10} among temperatures. The Q_{10} value of each enzyme activity was calculated as $Q_{10}=(E(T2)/E(T1))^{(10/[T2-T1])}$, where E(T1) and E(T2) are the enzyme activities at temperatures T1 and T2, respectively (Khalili et al., 2011; Li et al., 2020a).

Table 3
Extracellular enzymes assayed in the rhizosphere and bulk soil, their enzyme commission number (EC), and corresponding substrate.

Enzyme	Substrate	EC
β-glucosidase	4-MUB-β-D-glucoside	3.2.1.21
N-acetyl-glucosaminidase	4-MUB-N-acetyl-β-D-glucosaminide	3.2.1.30
L-leucine aminopeptidase	L-Leucine-7-amido-4-methylcoumarin	3.4.11.1
Phosphatase	4-MUB-phosphate	3.1.3.1

2.5. Statistical analysis

The rhizosphere effect (RE) of the four enzyme activities and Q_{10} value was calculated as:

$$\text{Rhizosphere effect} = (C_R - C_B)/C_R$$

Where C_R and C_B are the parameter values in rhizosphere and bulk soil, respectively.

Two-way analysis of variance (ANOVA) was conducted to test the effects of factors (successional stages, temperatures) and their interactions (successional stages × temperatures) on rhizosphere and bulk soil specific enzyme activities, Q_{10} values, and the rhizosphere effects. Then, successional stages effects of this variables were assessed separately for each temperatures using one-way ANOVA. Statistical significance was determined at the 95% level ($P < 0.05$) with the Duncan post-hoc test. Paired sample t-tests were used to detect significant differences between the Q_{10} values at 20–37 °C and those at 4–20 °C. In addition, 95% confidence intervals for rhizosphere effects were also calculated. If the confidence interval did not contain zero, the rhizosphere effect was considered to be statistically different from zero. A positive or negative

Table 2
Soil chemical properties in bulk and rhizosphere soil along the secondary successional gradient.

Study site	Bulk soil			Rhizosphere soil		
	SOC (g/kg)	TN (g/kg)	TP (g/kg)	SOC (g/kg)	TN (g/kg)	TP (g/kg)
Farmland	5.22 ± 0.18	0.45 ± 0.01	0.49 ± 0.02	5.69 ± 0.44	0.57 ± 0.02	0.55 ± 0.02
7-years	3.33 ± 0.49	0.21 ± 0.02	0.53 ± 0.03	3.85 ± 0.15	0.38 ± 0.03	0.57 ± 0.02
12-years	3.37 ± 0.57	0.20 ± 0.05	0.57 ± 0.03	4.22 ± 0.31	0.39 ± 0.03	0.57 ± 0.07
17-years	2.69 ± 0.34	0.22 ± 0.05	0.61 ± 0.03	3.99 ± 0.41	0.41 ± 0.02	0.63 ± 0.06
22-years	3.20 ± 0.07	0.27 ± 0.03	0.55 ± 0.02	5.68 ± 0.70	0.48 ± 0.06	0.58 ± 0.02
32-years	5.01 ± 0.43	0.48 ± 0.03	0.49 ± 0.01	7.42 ± 0.37	0.61 ± 0.02	0.55 ± 0.05
Natural grassland	5.49 ± 0.31	0.50 ± 0.01	0.50 ± 0.03	7.65 ± 0.72	0.62 ± 0.03	0.53 ± 0.03

rhizosphere effect indicates a higher value in the rhizosphere or bulk soil, respectively. All statistical analyses were performed using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). SigmaPlot 12.0 was used to create the graphs.

3. Results

3.1. Soil specific enzyme activities in bulk and rhizosphere soil

The BG, NAG, LAP, and AP enzyme activities in both bulk and rhizosphere soil were significantly affected by plant successional stages and temperatures ($P < 0.05$; Table 4). All soil enzyme activities decreased with decreasing incubation temperatures. The BG, NAG, LAP, and AP enzyme activities at 37, 20, and 4 °C in both bulk and rhizosphere soil were significantly different along the plant secondary successional gradient ($P < 0.05$; Table 5). In the bulk soil, the BG, NAG, LAP, and AP enzyme activities at 37, 20, and 4 °C first increased and then decreased along plant successional stages, with the highest value at the 17-year site, except for the BG enzyme activity at 4 °C, and LAP enzyme activity at 20 °C (Fig. 1). In the rhizosphere soil, the BG, NAG, LAP, and AP enzyme activities at 37, 20, and 4 °C increased to their highest values at the 7-year site, and then tended to decrease over the course of secondary succession, but with a higher value at the 17-year site (Fig. 2).

3.2. Soil enzyme temperature sensitivity in bulk and rhizosphere soil

The temperature sensitivity (Q_{10}) value of BG activity in bulk soil and Q_{10} value of BG, NAG and LAP activities in rhizosphere soil were significantly affected by successional stages, and the Q_{10} value of all the four enzyme activities in both bulk and rhizosphere soil were significantly affected by temperature ranges ($P < 0.05$; Table 4). In bulk soil, the Q_{10} value of BG and LAP activities at 20–37 °C and the Q_{10} value of BG activity at 4–20 °C differed along the plant secondary successional gradient ($P < 0.05$; Table 5). The Q_{10} value of BG activities at 20–37 °C first significantly increased and then decreased with plant secondary succession (Fig. 3). The Q_{10}value of BG activities at 4–20 °C first decreased and then increased with plant secondary succession. The Q_{10} values of enzyme activities were different at different temperature ranges. For example, the Q_{10} value of BG activity at 20–37 °C in the bulk soil was lower than that at 4–20 °C along the plant secondary succession, except for at the 12-year site. In addition, the Q_{10} value of AP activity at 20–37 °C was significantly higher than that at 4–20 °C along the plant secondary successional gradient, except for in the farmland and 22-year sites.

In the rhizosphere soil, the Q_{10} value of the four enzyme activities at both 20–37 °C and 4–20 °C were significantly different along secondary successional gradients ($P < 0.05$) (Table 5). The Q_{10} value of BG activities at 20–37 °C significantly increased and then decreased along the secondary successional gradient, with highest value at 22-year site (Fig. 4). And, the Q_{10} value of BG activities at 4–20 °C first significantly decreased after farmland abandoned, and then the value had no significant difference along the secondary successional gradients. The Q_{10} values of NAG, LAP and AP activities at both 20–37 °C and 4–20 °C varied with plant secondary succession. In most cases, the Q_{10} value did not differ between the two temperature ranges.

3.3. Rhizosphere effects of soil specific enzyme activity and temperature sensitivity

The rhizosphere effects of BG, NAG, LAP, and AP activities were significantly affected by successional stages, and the rhizosphere effects of BG and NAG activities were significantly affected by incubation temperatures ($P < 0.05$; Table 4). In general, the rhizosphere effects of most enzyme activities at different incubation temperatures were significantly different along plant secondary successional gradients ($P < 0.05$; Table 6). In farmland and the 7-year site, there were no significant rhizosphere effects of the four enzyme activities, while the rhizosphere effects changed to negative effects with plant secondary succession at the three temperatures (Fig. 5).

The rhizosphere effects of the Q_{10} value of the four enzyme activities were significantly affected by temperature ranges ($P < 0.05$; Table 4). The rhizosphere effects of the Q_{10} value of BG and AP activities at the two temperature ranges had significant differences along plant secondary successional gradients ($P < 0.05$) (Table 6). Specifically, the Q_{10} value of BG activities at 20–37 °C showed positive rhizosphere effects,

Table 4
Results of two-way ANOVA of plant successional stages, temperatures and their interactions on soil specific enzyme activities, specific enzyme temperature sensitivities and rhizosphere effects of soil specific enzyme activities.

Enzyme parameters		Successional stages		Temperatures		Successional stages × Temperatures	
		F	*P*	*F*	*P*	*F*	*P*
Bulk soil	BG	8.900	**0.000**	318.813	**0.000**	4.654	**0.000**
	NAG	17.882	**0.000**	63.029	**0.000**	0.740	0.706
	LAP	26.894	**0.000**	136.552	**0.000**	2.847	**0.006**
	AP	46.872	**0.000**	6.120	**0.005**	0.255	0.993
	Q10 value of BG	7.828	**0.000**	394.083	**0.000**	14.905	**0.000**
	Q10 value of NAG	1.870	0.121	55.582	**0.000**	1.492	0.217
	Q10 value of LAP	1.118	0.377	39.659	**0.000**	1.866	0.122
	Q10 value of AP	0.875	0.526	102.145	**0.000**	1.346	0.270
Rhizosphere soil	BG	45.423	**0.000**	559.619	**0.000**	15.072	**0.000**
	NAG	54.278	**0.000**	64.064	**0.000**	4.804	**0.000**
	LAP	53.108	**0.000**	91.757	**0.000**	5.759	**0.000**
	AP	72.717	**0.000**	3.369	**0.044**	1.086	0.396
	Q_{10} value of BG	5.875	**0.000**	40.625	**0.000**	18.733	**0.000**
	Q_{10} value of NAG	5.510	**0.001**	29.113	**0.000**	4.090	**0.005**
	Q_{10} value of LAP	2.669	**0.036**	30.299	**0.000**	14.271	**0.000**
	Q_{10} value of AP	1.443	0.234	16.796	**0.000**	5.047	**0.001**
Rhizosphere effects of BG		35.235	**0.000**	47.923	**0.000**	10.679	**0.000**
Rhizosphere effects of NAG		8.937	**0.000**	4.107	**0.023**	0.609	0.822
Rhizosphere effects of LAP		18.082	**0.000**	0.873	0.425	0.642	0.794
Rhizosphere effects of AP		11.627	**0.000**	0.680	0.512	0.485	0.912
Rhizosphere effects of Q_{10} (BG)		4.412	**0.003**	133.054	**0.000**	4.065	**0.005**
Rhizosphere effects of Q_{10} (NAG)		1.755	0.145	5.415	**0.027**	0.894	0.513
Rhizosphere effects of Q_{10} (LAP)		1.364	0.263	6.739	**0.015**	4.431	**0.003**
Rhizosphere effects of Q_{10} (AP)		2.054	0.091	11.484	**0.002**	8.835	**0.000**

Bold text indicates $P < 0.05$.

Table 5
Results of one-way ANOVA of plant secondary succession on soil specific enzyme activities at different incubation temperatures and on Q_{10} value at two temperature ranges in both bulk and rhizosphere soil.

Enzyme		37 °C		20 °C		4 °C		Q_{10} at 20–37 °C		Q_{10} at 4–20 °C	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Bulk soil	BG	7.951	**0.000**	4.115	**0.014**	20.170	**0.000**	9.018	**0.000**	11.541	**0.000**
	NAG	4.416	**0.009**	13.181	**0.000**	12.809	**0.000**	1.799	0.171	0.766	0.608
	LAP	12.040	**0.000**	12.463	**0.000**	5.547	**0.003**	3.100	**0.038**	1.223	0.352
	AP	20.832	**0.000**	15.734	**0.000**	12.513	**0.000**	0.786	0.595	1.215	0.355
Rhizosphere soil	BG	23.062	**0.000**	29.911	**0.000**	20.155	**0.000**	10.288	**0.000**	12.989	**0.000**
	NAG	18.068	**0.000**	27.929	**0.000**	31.455	**0.000**	5.858	**0.003**	3.489	**0.025**
	LAP	18.266	**0.000**	39.087	**0.000**	13.388	**0.000**	8.679	**0.000**	8.353	**0.000**
	AP	41.514	**0.000**	35.504	**0.000**	13.416	**0.000**	3.914	**0.017**	3.150	**0.036**

Bold text indicates $P < 0.05$.

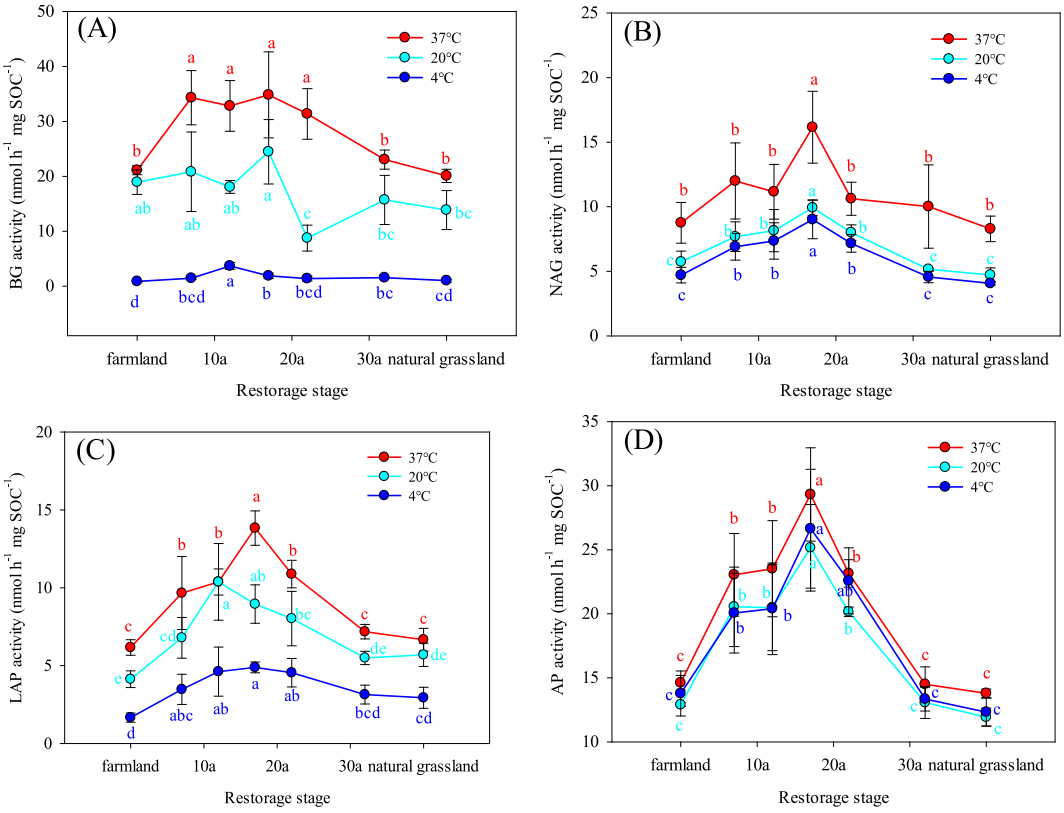

Fig. 1. Dynamics of soil specific enzyme activities in bulk soil at different successional stages. Different lowercase letters with the same color indicate significant differences among study sites ($P < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

while the Q_{10} value of BG activities at 4–20 °C showed negative rhizosphere effects (Fig. 6). In most cases, the Q_{10} value of NAG, LAP, and AP activities at the two temperature ranges had no significant rhizosphere effects; the exceptions were for the Q_{10} values of NAG activities at 20–37 °C at the 12-year site and in natural grassland, the Q_{10} value of LAP activities at the two temperature ranges at the 17-year site and the Q_{10} value of AP activities in both temperature ranges at the 12-year site.

4. Discussion

4.1. Soil specific enzyme activities during plant secondary succession

Using soil specific enzyme activities to decouple the changes in soil enzyme activities from the variation in soil organic carbon, we found that bulk soil specific enzyme activities related to C, N, and P cycling increased after the succession of abandoned farmland for 17 years (Fig. 1). This result indicates that the accumulation of soil organic carbon was less than for the soil enzymes during the early stages of plant succession. After farmland abandonment, cessation of organic fertilizer input significantly decreased the available nutrients for plant uptake; more enzymes were produced by soil microbial communities to decompose plant litter and other organic matter to meet the demand of plant growth. However, after 17 years of succession, the specific enzyme activities decreased significantly, which indicated that the accumulation of soil organic carbon was greater than changes in enzyme activities after the transformation of dominant plant species along secondary succession series. Similarly, Allison et al. (2007) and Raiesi and Salek-Gilani (2018) reported that the specific activity of C-, N-, and P-cycling enzymes decreased with successional age. The lower ratios of enzyme activities:SOC following plant succession may indicate a decline of enzyme synthesis of release by microorganisms, which could be metabolically less active (Katsalirou et al., 2010). Specific enzyme activities were more strongly associated with the nutrient status and microbial activity of the secondary successional series, and thus might be a more suitable indicator of plant secondary succession.

Rhizosphere is the interface between plant living roots and soil

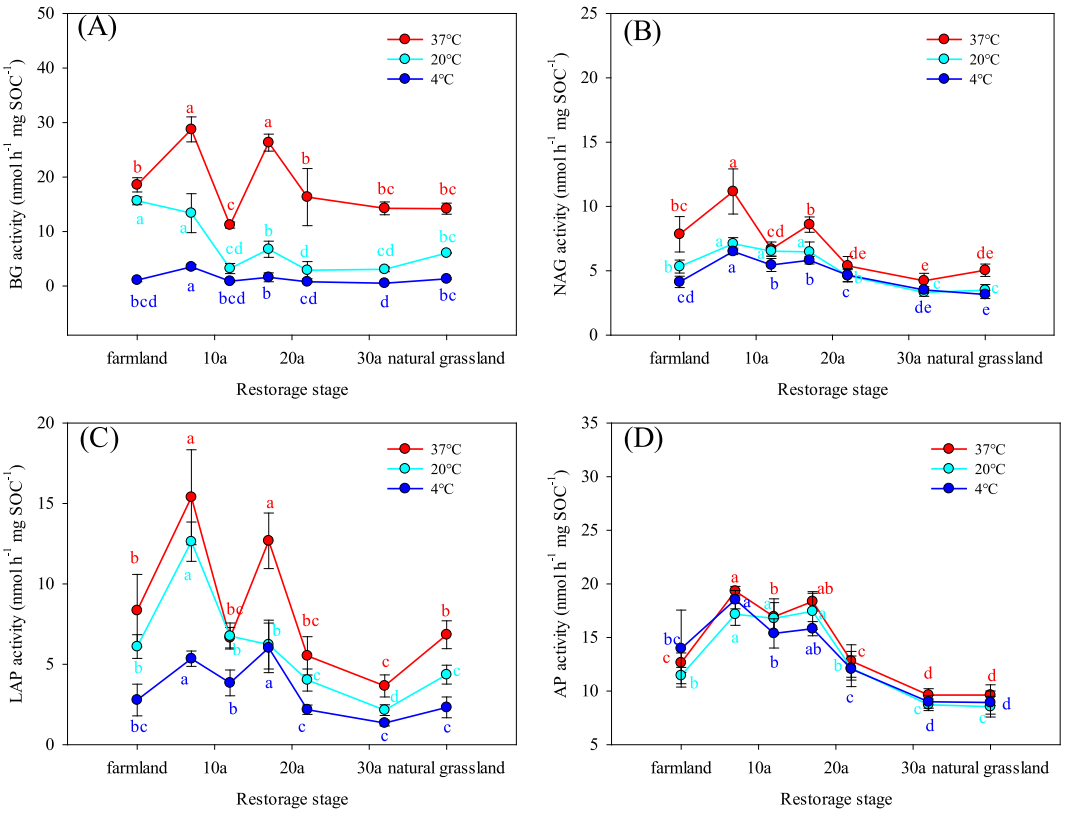

Fig. 2. Dynamics of soil specific enzyme activities in rhizosphere of dominant plant species at different successional stages. Different lowercase letters with the same color indicate significant differences among study sites ($P < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

where communications and interactions among a myriad of microorganisms and invertebrates affect carbon and nutrient cycling, plant growth and tolerance to various environmental factors (Philippot et al., 2013; Ahkami et al., 2017; Kuzyakov and Razavi, 2019). In our study, the specific enzyme activities in the rhizosphere were significantly higher at 7-year and 17-year old sites, when *A. capillaris* and *A. sacrorum* became the dominant plant species, respectively (Table 1). When one plant species first emerged and occupied the ground, the enzyme activities of the rhizosphere significantly increased to provide more available nutrients for plant growth, which benefits plants occupying the ground and those competing with other plant species (Zhou and Staver, 2019). Increased enzyme activities might be due to (1) the increased root exudates and other rhizodeposits stimulated by microbial activity, and thus increasing the production of extracellular enzymes; and (2) direct release of enzymes by plant roots or by lysis of root cells (Razavi et al., 2016; Xiao et al., 2017; Kuzyakov and Razavi, 2019). Meanwhile, when plant species become the dominant, the soil micronutrient and nutrient status improves and promotes their persistence. Subsequently, soil structure was obviously improved and more organic carbon was stored in the soil aggregates, thus decreasing the specific enzyme activities (Cheng et al., 2015; Raiesi and Salek-Gilani, 2018).

Compared with bulk soil, the rhizosphere soil had significantly higher soil organic carbon, microbial activity and absolute enzyme activities (Xiao et al., 2017; Yang et al., 2017). Many studies reported positive rhizosphere effects of absolute enzyme activities during vegetation restoration (Zhang et al., 2012; Yang et al., 2017). Meanwhile, we found that the rhizosphere effects of specific enzyme activities gradually became negative with plant succession, which was mainly due to the higher soil organic carbon content in the rhizosphere soil (Table 2).

Plants release about one third of their photosynthetic products in the form of root exudates and other rhizodeposits into the soil (Kuzyakov et al., 2003). Those root exudates had great effects on soil organic carbon and nutrient accumulation, especially in the rhizosphere soil, decreasing rhizosphere specific enzyme activity. The gradually decreased rhizosphere effects of specific enzyme activity indicated that the soil microbial communities suffer less carbon and nutrient limitation in the rhizosphere soil than bulk soil. In consequence, the plant species composition and diversity pattern became more stable with plant secondary succession.

4.2. *Soil enzyme temperature sensitivities during plant secondary succession*

Soil enzyme temperature sensitivity is an important control of *in situ* enzyme activity (Koch et al., 2007; Allison et al., 2018). Previous studies showed that enzyme activities are less temperature-sensitive, with Q_{10} values < 2 (Koch et al., 2007; Ge et al., 2017). We also found that the Q_{10} values of specific enzyme activities were<2, except for BG activity, which is related to organic carbon degradation and transformation. Carbon was the main energy source for soil microbial community growth and reproduction (Soong et al., 2020). The relatively higher Q_{10} value of BG activity indicated that carbon cycling enzymes' activity during plant secondary succession was more sensitive to temperature than the activities of enzymes involved in the cycling of other nutrients (N and P), especially at lower temperatures. We also showed that the Q_{10} value of soil specific enzymes varies along plant secondary succession series, suggesting that different isoenzymes were contributing to enzyme activity for different plant species composition along succession stages. This is likely due to different soil microbial community composition in different plant species along the succession series, but could also be due to different isoenzymes, if the same microbes are transcribing alternative genes (Wallenstein et al., 2009; Zhang et al., 2016; Liu et al., 2020). For example, Zheng et al. (2019) and Li et al. (2020b) reported distinct soil microbial community composition among different successional

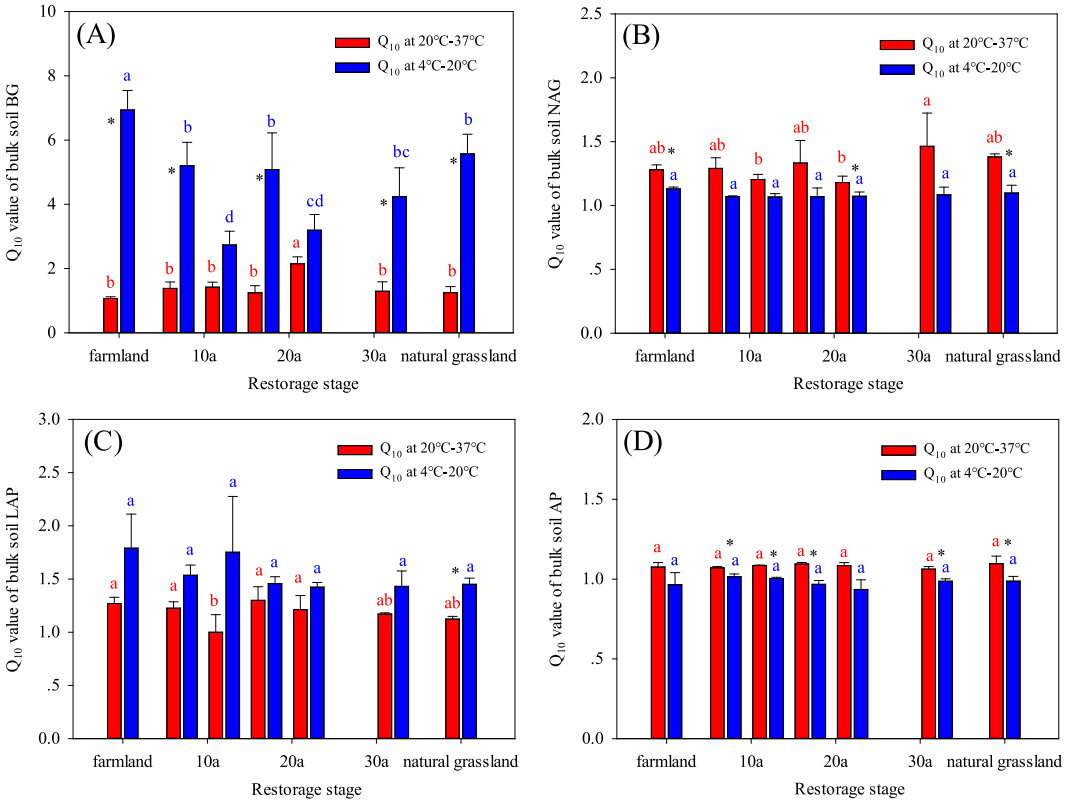

Fig. 3. Dynamics of soil enzyme temperature sensitivity (Q_{10}) in bulk soil at different successional stages. Different lowercase letters with the same color indicate significant differences among study sites ($P < 0.05$). * indicate significant difference between the two Q_{10} values at the same study sites ($P < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

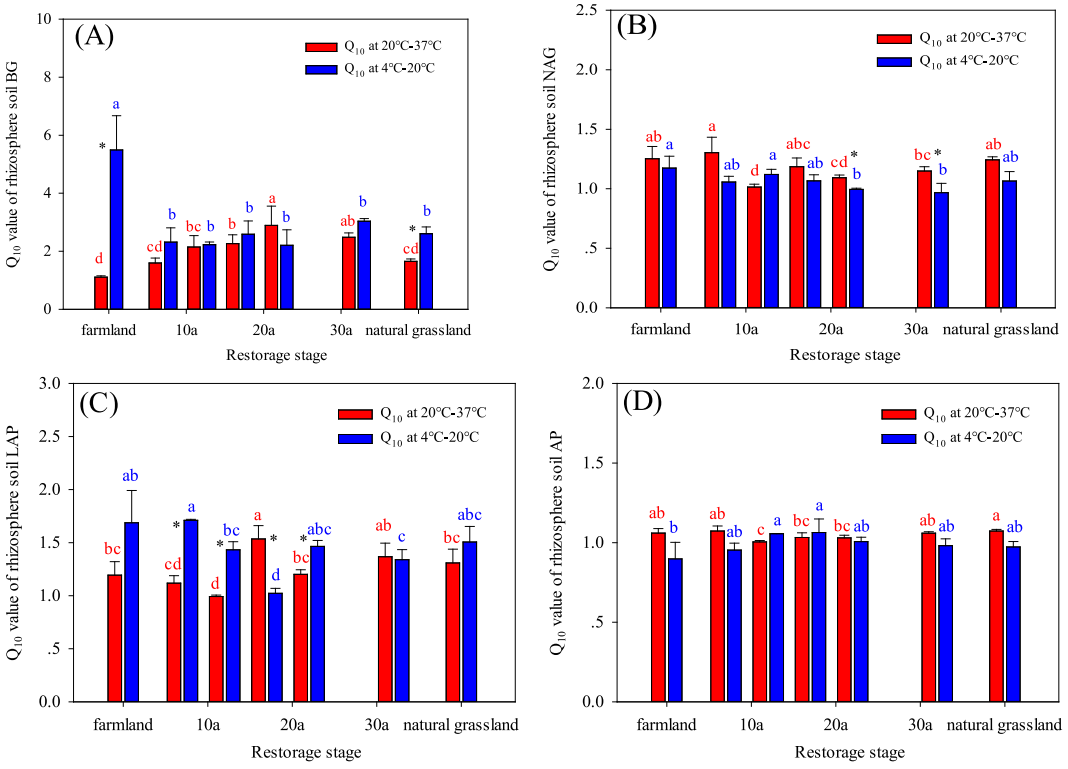

Fig. 4. Dynamics of soil enzyme temperature sensitivity (Q_{10}) in the rhizosphere of dominant plant species at different successional stages. Different lowercase letters with the same color indicate significant differences among study sites ($P < 0.05$). * indicates significant differences between the two Q_{10} values at the same study sites ($P < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 6
Results of one-way ANOVA of rhizosphere effects of soil specific enzyme activities at different incubation temperatures and on Q_{10} values at two temperature ranges along plant secondary successional gradient.

Enzyme	37 °C		20 °C		4 °C		Q_{10} at 20–37 °C		Q_{10} at 4–20 °C	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
BG	7.244	**0.001**	9.404	**0.000**	23.86	**0.000**	4.289	**0.012**	4.005	**0.015**
NAG	3.412	**0.027**	4.653	**0.008**	2.299	0.094	1.202	0.361	1.601	0.219
LAP	6.076	**0.003**	25.126	**0.000**	3.475	**0.026**	3.288	**0.031**	2.661	0.062
AP	3.769	**0.019**	2.485	0.075	6.109	**0.003**	3.584	**0.023**	5.884	**0.003**

Bold indicate $P < 0.05$

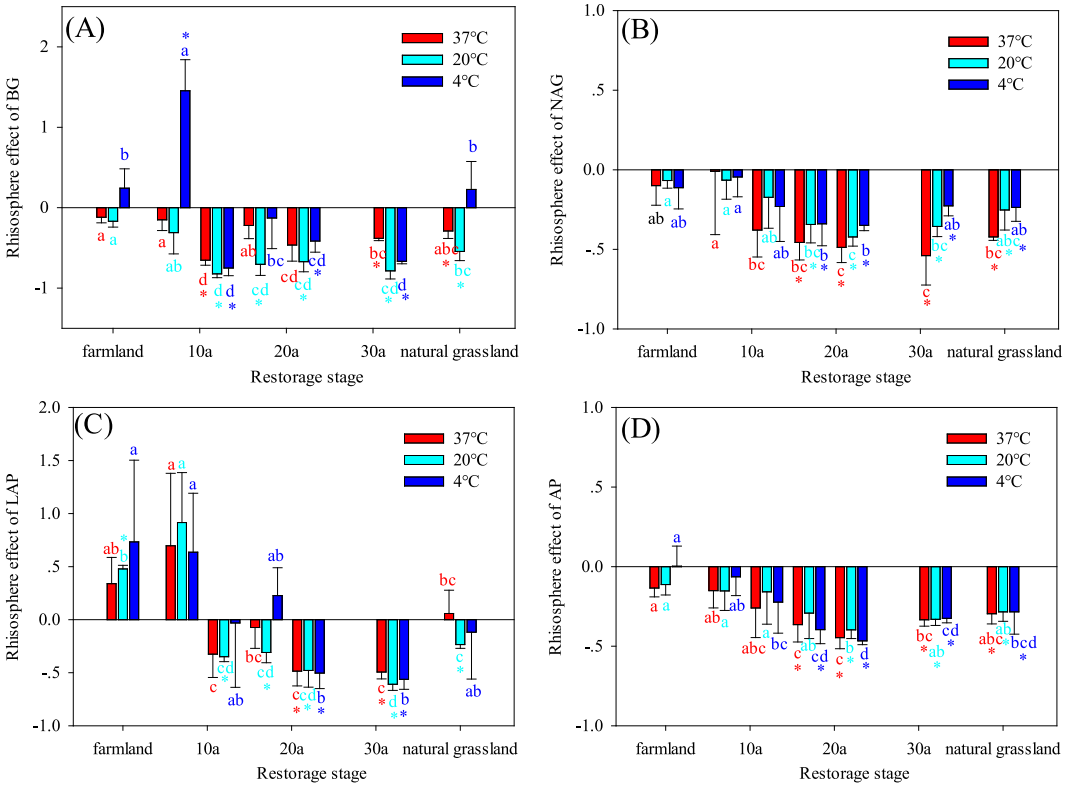

Fig. 5. Rhizosphere effects of soil specific enzyme activities at different successional stages. Different lowercase letters with the same color indicate significant differences among study sites ($P < 0.05$). * indicates significant rhizosphere effect ($P < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

stages. Hu et al. (2019) found that the abundances of most C, N, and P cycling genes increased and then decreased along a desert revegetation chronosequence. The variation of soil microbial community composition and/or functional genes would both contribute to different isoenzymes, thus causing the variation of soil enzyme temperature sensitivity. Bulk and rhizosphere soil both exhibited significant different soil microbial community composition due to changes in plant root exudates and soil nutrient status (Liu et al., 2018). Based on high-throughput sequencing, Song et al. (2019) further found faster succession of the microbial community in the rhizosphere than in bulk soil. And the rhizosphere soil had much lower molecular and labile carbon substrates from plant root exudation, which was more easily degraded by soil enzymes, no matter how the temperature changes. Thus, in most cases, the Q_{10} value of specific enzyme activity in the rhizosphere soil differed little between different temperature ranges.

The degradation of organic matter via multiple enzymatic reactions resulted from the combined effects of soil enzyme activity, organic matter quality and quantity, and temperature (Arndt et al., 2013). Compared with bulk soil, rhizosphere soil had relatively higher microbial diversity and a large amount of organic matter, especially easily degradable organic matter (Toberman et al., 2011; Wang et al., 2019). While bulk soil had relatively higher specific enzyme activity to satisfy the nutrient demand of microbial community. Thus, soil enzyme reaction rates under different temperature ranges showed different response characteristics in rhizosphere and bulk soil. During plant secondary succession, the input of root exudates and plant litter quantity and quality, as well as soil microbial community and composition significantly changed (Zhang et al., 2016; Liu et al., 2020), thus contributing to the variation of rhizosphere effects of enzyme temperature sensitivity. Further research should be conducted to evaluate the diversity of soil isoenzymes in both rhizosphere and bulk soil, so we can better understand the carbon and nutrient cycling driven by soil enzymes during plant secondary succession.

Over all, the present research evaluated four specific enzyme activities (BG, NAG, LAP, and AP) associated with C, N, and P cycling in both bulk and rhizosphere soil along a secondary succession series. Those four enzymes are selected because they are frequently linked to the microbial metabolic processes and generally used to assess the investment of microbial community in the acquisition of the limiting elements of C, N and P (Sinsabaugh et al., 2008; Peng and Wang, 2016). Many previous studies used those four enzyme activities to identify the status of natural vegetation restoration (Knelman et al., 2015; Li et al., 2020b; Xiao et al., 2020a). Also, some other studies included more C-acquiring enzymes (such as cellobiosidase and peroxidase) and N-acquiring

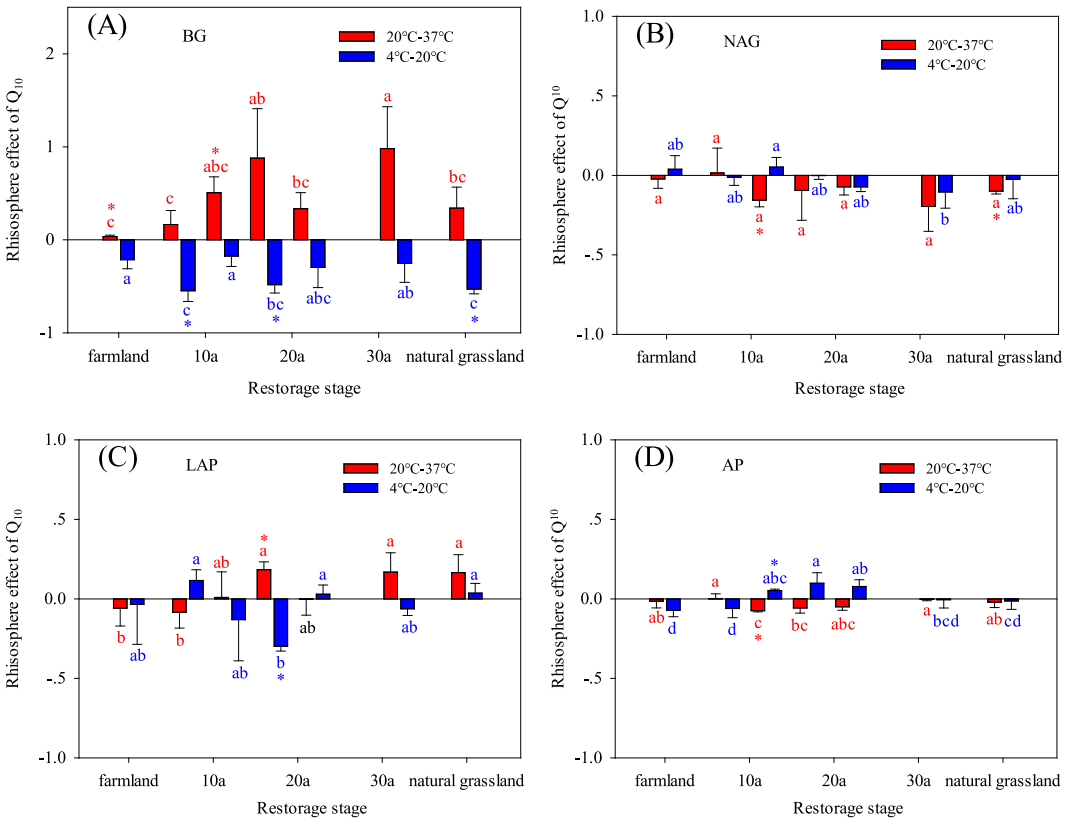

Fig. 6. Rhizosphere effects of soil enzyme temperature sensitivity (Q_{10}) at different successional stages. Different lowercase letters with the same color indicate significant differences among study sites ($P < 0.05$). * indicates significant rhizosphere effect ($P < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

enzymes (urease) to elucidate the dynamics of soil enzyme activity during plant succession (Jiang et al., 2019; Li et al., 2020c). More researches should be conducted to determine which soil enzymes were more effective indicators of soil restoration processes in degraded ecosystems. This study evaluated rhizosphere soil specific enzyme activities and temperature sensitivities along the plant secondary successional gradient, but more study is need to illustrate the relationship between soil specific enzyme activities and nutrient elements transformation for further revealing the biogeochemical cycling during plant secondary restoration process.

5. Conclusions

Plant secondary succession has a great impact on soil extracellular enzyme activities. We evaluated the specific activity and temperature sensitivity of four enzymes in the rhizosphere and bulk soil along a gradient of secondary succession grassland on the Loess Plateau of China. The specific activity of enzymes first increased and then decreased along the successional gradient in both bulk and rhizosphere soil. The rhizosphere effect of specific enzyme activity decreased along the successional gradient. The influence of plant succession on temperature sensitivity of soil specific enzymes was mainly observed in the rhizosphere soil. However, the temperature sensitivity at different temperature ranges mainly showed significant differences in bulk soil. Plant succession obviously changed the specific enzyme activity in both bulk and rhizosphere soil, while plant root exudates probably play an important role in mediating the soil enzyme activity and temperature sensitivity in the rhizosphere and the magnitude of rhizosphere effects. Further study is still required to understand the enzyme characteristics in the rhizosphere to better understand carbon and nutrient cycling in the restoration of ecosystems.

Declaration of Competing Interest

None.

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