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Impacts of Partial Substitution of Chemical Fertilizer with Organic Manure on the Kinetic and Thermodynamic Characteristics of Soil β–Glucosidase

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Abstract: To study the characteristics of the β–glucosidase enzymatic reaction in wheat field soil under the condition of reducing the application of chemical fertilizer, five fertilization treatments were established, including no fertilizer (CK), chemical fertilizer (F), organic fertilizer (OF), 25% organic fertilizer plus 75% chemical fertilizer (25% OF), and 50% organic fertilizer plus 50% chemical fertilizer (50% OF). The activity of β–glucosidase and its kinetic and thermodynamic characteristics were analyzed by using microplate p–nitrophenol colorimetry. The results showed that the V_{max} values of soil β–glucosidase in the organic substitution of chemical fertilizer treatment were higher than those in the chemical fertilizer and no fertilizer treatments, and the K_m values were lower than those in the chemical fertilizer and no fertilizer treatments at the different growth stages. The V_{max} value in the 25% OF treatment was the highest at the jointing stage and that of the OF treatment was the highest at the booting stage; the K_m value in the 50% OF treatment was the lowest at the different growth stages. Compared with the chemical fertilizer and no fertilizer treatments, the application of organic fertilizer effectively reduced thermodynamic parameters such as E_a, Q_{10}, ΔH, ΔG, and ΔS at the jointing and booting stages of wheat. The thermodynamic parameters in the 25% OF treatment were the lowest at the jointing stage and those in the OF treatment were the lowest at the booting stage. A reasonable amount of organic fertilizer is more beneficial to enzymatic reactions and improves the soil quality and the ability to supply nutrients to wheat cultivation.

Keywords: organic fertilizer; reducing chemical fertilizer in wheat; β–glucosidase in soil; enzyme kinetics; enzyme thermodynamics

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1. Introduction

The application of fertilizer is an important way to increase crop yields and improve crop quality [1]. However, in recent years, the excessive application of chemical fertilizer in pursuit of high yields has become increasingly serious, resulting in a series of problems, such as soil nutrient imbalance, low fertilizer utilization, and agricultural nonpoint source pollution, which seriously restricts the sustainable development of Chinese agriculture and will inevitably result in crop yield reduction in the long run. In view of this situation, we could use organic substitutes for fertilizer reduction on the basis of ensuring crop yield and food security, appropriately reducing chemical fertilizer application, improving physical and chemical properties by applying a certain proportion of organic fertilizer to the field, improving the activity of soil nutrients, and effectively reducing the environmental pollution and damage due to excessive chemical application, which could improve the quality and yield of crops.

β–glucosidase activity has been shown to be very sensitive to external environmental stress [2], respond rapidly to changes in land management, and has been proposed as an indicator to evaluate soil quality status because it provides an early indication of

changes in soil organic matter status and turnover [3]. Several studies have shown that *β*–glucosidase (EC 3.2.1.21) is the most abundant and easily detected of the several enzymes involved in cellulose degradation in soil, is rarely limited by substrates, and is an essential enzyme in the carbon cycle [4]. It plays a major role in the final rate–limiting step of soil cellulose degradation by catalyzing the cleavage of cellobiose, and the reaction product glucose may serve as an important energy source for soil microbial growth [5,6]. Therefore, *β*–glucosidase is the main enzyme detected in soil and its activity is an ideal indicator for characterizing soil organic matter turnover and soil fertility levels.

Currently, most studies on enzyme activity have focused on potential enzyme activity at saturated substrate concentrations and suitable temperatures. The response of potential enzyme activity to fertilization measures is helpful in order to understand the specific results of enzymatic reactions and reflect the quality and nutrient supply of soil after fertilization. However, it is impossible to explain the reasons for the change in enzyme activity based on the mechanism of enzymatic reaction, and it is difficult to describe the influencing factors of enzyme activity. In contrast, enzyme dynamic and thermodynamic parameters not only truly reflect the effect of the substrate concentration and temperature changes on enzyme activity in soil, but they also reflect the degree of affinity and process of enzymatic reactions between enzymes and substrates, soil colloids, etc. [7]. This can explain the mechanistic process and energy change characteristics of enzymatic reactions as a whole. Therefore, it is important to carry out research on the characteristics of the changes in enzymes' kinetic and thermodynamic parameters in fertilized soils to reveal the mechanism of enzyme activity changes [8].

Enzyme dynamics and thermodynamics have been applied in the study of the soil enzyme properties of contaminated soils, such as pesticides and heavy metals [9,10], but there are fewer studies on soil enzyme kinetics and thermodynamics under the organic manure substitution of chemical fertilizers.

The growth period of spring wheat in Northeast China is short and the critical periods, such as the jointing stage and booting stage, require large amounts of nutrition and are most significantly affected by fertilizer application. *β*–glucosidase activity can detect changes in soil management over a relatively short time period (two years) and is relatively stable with the seasons [3], which is beneficial as an indicator of soil quality. Therefore, in this work, *β*–glucosidase involved in the organic carbon cycle was selected as the study object. The aims were (i) to study *β*–glucosidase activity in the soil at the jointing and booting stages of wheat, (ii) to examine dynamic and thermodynamic change characteristics, and (iii) to evaluate the relationship between enzymes' kinetic and thermodynamic parameters and soil chemical properties.

2. Materials and Methods

2.1. Experimental Design and Soil Sampling

The experimental area was located in Nenjiang County of Northeast China (N 49°33′46″, E 125°27′38″). The soil type in the area is dominated by Phaeozems, according to the WRB for soil resources [11]. The cropping pattern was soybean–wheat rotation, and the current crop was spring wheat, which is grown once a year. The tested soil samples came from a positioning experiment of organic manure substitution for chemical fertilizer reduction in wheat cultivation in this area. The experiment had a completely randomized block design with three replicates. Five fertilization treatments were set up, namely, no fertilizer (CK), single application of chemical fertilizer (F), organic fertilizer (OF), 25% organic fertilizer and 75% chemical fertilizer (25% OF), and 50% organic fertilizer and 50% chemical fertilizer (50% OF). Except for the control treatment (CK), the total fertilizer amounts in the F, OF, 25% OF, and 50% OF treatments were the same, and the basic amount of organic fertilizer (N 2.33%, P_2O_5 1.85%, and K_2O 2.35%) was 30 tons per hectare, while the other treatments were supplemented with urea (N 46%), calcium superphosphate (P_2O_5 18%), and potassium chloride (K_2O 60%), applied in autumn. Soil samples were collected from the surface layer (0–15 cm) at the jointing stage (19 June 2019) and booting stage (8 July 2019) of wheat. After

the samples were collected, sand, gravel, roots, and plant residues in the soil samples were removed, passed through 2-mm sieves, and stored at 4 °C to study the soil enzyme activity and enzymatic characteristics. The field experiment started in 2018. The basic physical and chemical properties of the tested soil samples before implementation in 2017 are listed in Table 1.

Table 1. Basic physical and chemical characteristics of tested soils.

pH	Organic Matter (g kg^{-1})	Total Nitrogen (g kg^{-1})	Available Nitrogen (mg kg^{-1})	Available Phosphorus (mg kg^{-1})	Available Potassium (mg kg^{-1})
5.67	41.2	2.20	189	23	201

Note: Soil pH values were measured at 1:2.5 soil-to-water ratio.

2.2. Determination of β–Glucosidase Activity

The activity of *β*–glucosidase was determined via microplate p–nitrophenol colorimetry [12,13]: a 0.1-g soil sample was weighed in a 1.5-mL centrifuge tube, and 0.4 mL of 0.1 mol L^{-1} modified universal buffer (MUB) (pH = 6) was added with a pipette gun to stabilize the pH of the solution. After oscillating with a vortex oscillator version QL–905 (Kylin-Bell Lab Instruments Co., Ltd., Haimen city, Jiangsu, China) for several seconds, p–nitro–*β*–glucoside was added. After thorough shaking, the enzyme was cultured in a constant–temperature water bath. After 1 h, 0.4 mL of a tris hydroxymethyl aminomethane (THAM) solution (pH = 12) was added to terminate the enzymatic reaction. Then, 0.1 mL of 0.5 mol L^{-1} $CaCl_2$ was added, and the sample was shaken and centrifuged at 12,000 rpm for 10 min. A total of 250 µL of the micro–supernatant was absorbed into a microplate. The enzyme activity was measured by a BIO–RAD iMark^{TM} microplate (Bio–Rad Benchmark Hercules, CA, USA) at 405 nm and expressed as the number of micromoles of p–nitrophenol produced per kg of soil after 1 h. Each sample was analyzed 3 times, and substrate and soilless treatments after enzyme inactivation were set as controls.

2.3. Kinetic and Thermodynamic Analysis of β–Glucosidase

2.3.1. Kinetics of the Enzymatic Reaction

Enzyme kinetics were evaluated at different concentrations (1, 2, 5, 10, 12.5, 17.5, 20, and 25 mmol L^{-1}) of p–nitro–*β*–glucoside at a culture temperature of 37 °C. The remaining experiments were carried out according to the general method of enzyme activity determination described in Section 2.2, and the enzyme kinetic parameters V_{max} and K_m were calculated according to the corresponding substrate concentrations.

Linear transformation of the Michaelis–Menten equation included (1) Lineweaver–Burk transformation, $1/V = 1/V_{max} + (K_m/V_{max})(1/[S])$; (2) Hanes–Wolf transformation, $[S]/V = K_m/V_{max} + (1/V_{max})[S]$; and (3) Eadie–Hofstee transformation, $V = V_{max} - K_m(V/[S])$, where [S] is the substrate concentration (mmol L^{-1}) and V is the enzymatic reaction rate [µmol $(kg\ h)^{-1}$].

2.3.2. Thermodynamics of the Enzymatic Reaction

Enzyme thermodynamics were established at different incubation temperatures (10, 20, 30, and 40 °C), 25 mmol L^{-1} p–nitro–*β*–glucoside was selected, and the remaining parameters were determined according to the general method of enzyme activity calculation in Section 2.2. According to the enzyme activity measured at the corresponding temperatures, the thermodynamic parameters, such as the activation energy (E_a), the temperature coefficient (Q_{10}), the enthalpy change (ΔH), the free energy change (ΔG), and the entropy change (ΔS) were obtained.

The Arrhenius equation can also be expressed in logarithmic form as $\ln K = (-E_a/R)(1/T) + \ln A$, where E_a is the activation energy (J mol^{-1}), K is the enzyme activity at temperature T, R is the universal gas constant, i.e., 8.314 J $(mol\ K)^{-1}$, T is the Kelvin temperature, 273 + t(K), and A is the frequency factor. A linear regression equation of lnK as a function of 1/T and A was obtained by fitting, and E_a values could be calculated from the intercept and slope of the linear relationship.

The temperature coefficient (Q_{10}) can be obtained with the following equation: $Q_{10} = \exp[10E_a/RT(T + 10)]$. The activation enthalpy change ΔH can be calculated according to the following equation: $\Delta H = E_a\text{-}RT$. The activation energy change ΔG can be calculated according to the following equation: $\Delta G = RT\ln(RT/NhK)$. The activation entropy ΔS can be calculated according to the following equation: $\Delta S = (\Delta H - \Delta G)/T$, where ΔH is the activation enthalpy change (kJ mol^{-1}), ΔS is the activation entropy change [J $(mol\ K)^{-1}$], ΔG is the activation energy change (kJ mol^{-1}), N is the Avogadro constant (6.023×10^{23} mol^{-1}), h is the Planck constant (6.626×10^{-34} J S^{-1}), T is the Kelvin temperature, 273 + t(K), R is the universal gas constant 8.314 J $(mol\ K)^{-1}$, and K is the enzymatic reaction rate.

2.4. Statistical Analysis

SPSS version 23.0 (IBM Corp; Armonk, NY, USA) was used to analyze the data. Single–factor analysis of variance (ANOVA) was used to evaluate the significance of the soil enzymes' kinetics parameters under the different treatments. The Duncan method was used to achieve multiple comparisons ($p < 0.05$) and SigmaPlot version 12.5 (Systat Software Inc, San Jose, CA, USA) was used for plotting. Redundancy analysis (RDA) was applied to evaluate the relationship between soil enzyme dynamics and thermodynamic parameters and soil factors using Canoco version 5.0(Microcomputer Power, Ithaca, NY, USA) for Windows.

3. Results

3.1. Kinetic Characteristics of β–Glucosidase in Soil

Figure 1 shows the effect of the substrate concentration on *β*–glucosidase activity in soil under different treatments at the jointing and booting stages of wheat. At the same culture temperature, the enzymatic reaction rate of *β*–glucosidase increased with increasing substrate concentration, and the increase range decreased with increasing substrate concentration and finally reached a maximum at a substrate concentration of 25 mmol L^{-1}. At the same substrate concentration, the *β*–glucosidase activity in the organic manure substitution of chemical fertilizer treatment was higher than that in the no fertilizer treatment at the different growth stages, while the enzyme activity in the chemical fertilizer treatment was always lower than that of the no fertilizer treatment. The *β*–glucosidase activity in the 25% OF treatment was the highest at the jointing stage, and that of the OF treatment was the highest at the booting stage. In summary, the activity of *β*–glucosidase in soil could be improved by applying a specific substitution proportion of organic fertilizer at the different growth stages, but chemical fertilizer alone often caused the opposite effect. Regarding the organic fertilizer treatment, with an increasing proportion of organic–manure–substituted chemical fertilizer, the *β*–glucosidase activity in soil first decreased and then increased.

The activity of *β*–glucosidase in soil follows the Michaelis–Menten equation. According to the influence of the concentration in the substrate and the data obtained from the above three linear transformations of the Michaelis–Menten equation, the V_{max} and K_m of *β*–glucosidase in this system were calculated. K_m ranged from 2.02 to 3.63 mmol L^{-1}, V_{max} ranged from 609.7 to 1061.4 μmol $(kg\ h)^{-1}$, and V_{max}/K_m ranged from 287.7 to 320.3×10^{-3} L $(kg\ h)^{-1}$ among the five treatments at the jointing stage. K_m ranged from 2.31 to 3.63 mmol L^{-1}, V_{max} ranged from 834.7 to 1055.6 μmol $(kg\ h)^{-1}$, and V_{max}/K_m ranged from 262.6 to 361.5×10^{-3} L $(kg\ h)^{-1}$ among the five treatments at the booting stage.

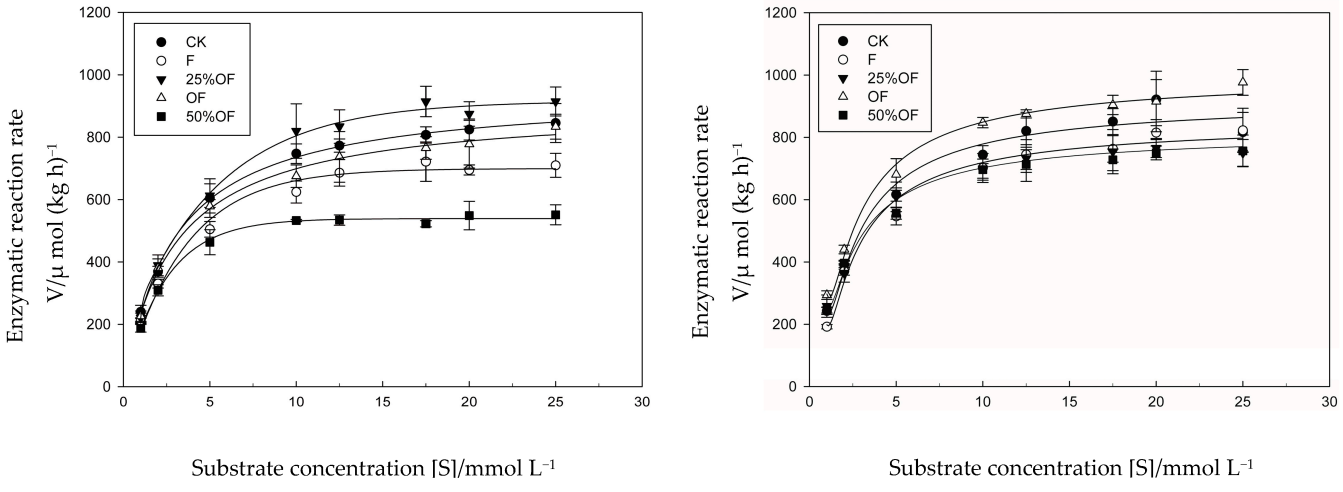

Figure 1. Curve of enzymatic reaction rate of *β*–glucosidase versus substrate concentration on jointing stage (**a**) and booting stage (**b**) of spring wheat. Data represent mean ± SD (n = 3). CK indicates no fertilizer treatment. F indicates single fertilizer treatment. 25% OF indicates 25% organic fertilizer plus 75% chemical fertilizer treatment. OF indicates organic fertilizer treatment. 50% OF indicates 50% organic fertilizer plus 50% chemical fertilizer treatment.

According to the results in Table 2, under the same culture conditions, the K_m value of *β*–glucosidase in the organic substitution of chemical fertilizer treatment was significantly lower than that of the no fertilizer treatment at the jointing and booting stages of wheat, and the K_m value in the 50% OF treatment was the lowest. The K_m value of *β*–glucosidase in the chemical fertilizer treatment was lower than that of the no fertilizer treatment only at the jointing stage, and it failed to reach significance. Therefore, compared with that of the no fertilizer and chemical fertilizer treatments, the *β*–glucosidase in soil treated with organic fertilizer had a stronger affinity for the substrate and provided more advantages in the formation of the enzyme–substrate complex, and the effect was strong.

Table 2. Kinetic parameters of *β*–glucosidase in soil under different fertilization treatments.

Treatment	V_{max} [μmol (kg h)$^{-1}$]		K_m (mmol L^{-1})		V_{max}/K_m [10^{-3} L (kg h)$^{-1}$]	
	Jointing Stage	Booting Stage	Jointing Stage	Booting Stage	Jointing Stage	Booting Stage
CK	924.39 ± 26.50 b	998.16 ± 3.19 ab	2.88 ± 0.12 b	2.99 ± 0.17 b	320.3 ± 3.95 a	334.38 ± 19.72 c
F	825.84 ± 12.98 c	950.61 ± 45.96 b	2.87 ± 0.04 b	3.63 ± 0.33 a	287.69 ± 5.7 a	262.63 ± 11.02 d
25% OF	1061.4 ± 56.47 a	840.58 ± 12.54 c	3.63 ± 0.4 a	2.33 ± 0.05 c	293.7 ± 17.54 a	361.31 ± 4.87 b
50% OF	609.67 ± 19.13 d	834.71 ± 2.38 c	2.02 ± 0.34 c	2.31 ± 0.05 c	307.03 ± 44.95 a	361.53 ± 7.66 b
OF	906.53 ± 4.43 b	1055.57 ± 55.56 a	2.99 ± 0.10 b	2.69 ± 0.20 b	303.07 ± 8.98 a	393.42 ± 7.93 a

Note: OF (organic fertilizer), F (chemical fertilizer), 25% OF (25% organic fertilizer plus 75% chemical fertilizer), 50% OF (50% organic fertilizer plus 50% chemical fertilizer), CK (no fertilizer). Data are means (n = 3) ± SD (Standard Deviation); The different letters in the same column indicate a significant difference among groups by one–way ANOVA (Duncan, $p < 0.05$).

In addition, compared with that of the no fertilizer treatment, the V_{max} value of *β*–glucosidase in the chemical fertilizer treatment was decreased at the different growth stages, while the V_{max} value in the organic substitution of chemical fertilizer treatment was increased. The highest V_{max} value was found in the 25% OF treatment at the jointing stage and the highest V_{max} value was found in the OF treatment at the booting stage. This indicated that the application of organic fertilizer increased the content of soil-active *β*–glucosidase and enhanced the ability of the enzyme–substrate complex to form products.

The relationships of V_{max}/K_m between the fertilizer treatments and the no fertilizer treatments were not the same at the different growth stages. At the jointing stage, the V_{max}/K_m of *β*–glucosidase in the organic and chemical fertilizer treatments were lower than that of the no fertilizer treatment, and the V_{max}/K_m in the chemical fertilizer treatment was the lowest. At the booting stage, the V_{max}/K_m of *β*–glucosidase in the organic fertilizer treatments was significantly higher than that of the no fertilizer treatment, and the V_{max}/K_m of *β*–glucosidase in the chemical fertilizer treatment was significantly lower than that of the no fertilizer treatment. The performance of the chemical fertilizer treatment was the worst among the fertilizer treatments, and the application of organic fertilizer improved the catalytic capacity of *β*–glucosidase to a certain extent.

In conclusion, compared to the large–scale application of chemical fertilizers in agricultural production, the application of chemical fertilizers partially substituted with organic manure promotes a more stable combination of enzymes and substrates, the decomposition of intermediate complexes into products occurs faster, and the catalytic rate of enzymes is higher.

3.2. Thermodynamic Characteristics of β–Glucosidase in Soil

Temperature is also an important factor affecting change in the enzymatic reaction rate. According to the temperature response curve of soil *β*–glucosidase, it can be seen from Figure 2 that there was a linear positive correlation between lnK and T among the different treatments at the jointing and booting stages of wheat. At the same substrate concentration, *β*–glucosidase activity in the soil of each treatment gradually increased with an increasing culture temperature in the temperature range of 10~40 °C.

The dependence of the enzymatic reaction rate on the temperature below the enzyme denaturation temperature can be expressed by the Arrhenius equation. For many chemical reactions, E_a values range from 50 to 100 kJ mol^{-1}. However, in enzyme–catalyzed reactions, the E_a values are generally lower than those seen in non–enzyme–catalyzed reactions [14], with a concentrated distribution at approximately 50 kJ mol^{-1}. The effect of temperature on the catalytic reaction of *β*–glucosidase can also be expressed by the temperature coefficient Q_{10}, and the mean value of the temperature coefficient Q_{10} ranges from 1.8 to 2.4 at soil temperatures of 10, 20, 30, and 40 °C.

The activation energy E_a represents the amount of energy required for the enzyme to form a complex with the substrate during the enzymatic reaction. According to the Arrhenius empirical equation, the higher the activation energy is, the slower the enzymatic reaction rate. At the different growth stages of wheat, the E_a values of *β*–glucosidase in the organic substitution of chemical fertilizer treatment were significantly lower than those of the no fertilizer and chemical fertilizer treatments, with the lowest E_a value in the 25% OF treatment at the jointing stage and the lowest E_a value in the OF treatment at the booting stage. The application of organic fertilizers in corresponding amounts during the different growth periods of wheat reduced the energy barrier that the enzyme reaction needs to overcome, which tended to facilitate enzymatic reaction.

The temperature coefficients Q_{10} of the soil *β*–glucosidase in different treatments gradually decreased with an increasing incubation temperature and reached the minimum value at 40 °C. For the CK, F, 25% OF, 50% OF, and OF treatments, the measured soil temperature coefficients Q_{10} ranged from 1.918 to 2.212, 1.857 to 2.127, 1.743 to 1.967, 1.968 to 2.283, and 2.031 to 2.372, respectively, at the jointing stage of wheat, and the soil temperature coefficients Q_{10} at the booting stage ranged from 1.999 to 2.327, 1.898 to 2.185, 1.976 to 2.294, 1.917 to 2.212, and 1.866 to 2.140, respectively. The temperature coefficients of the same enzyme under the different treatments varied, but the differences were not significant. The fertilization method did not change the effect of temperature on the rate of enzymatic reaction to a large extent.

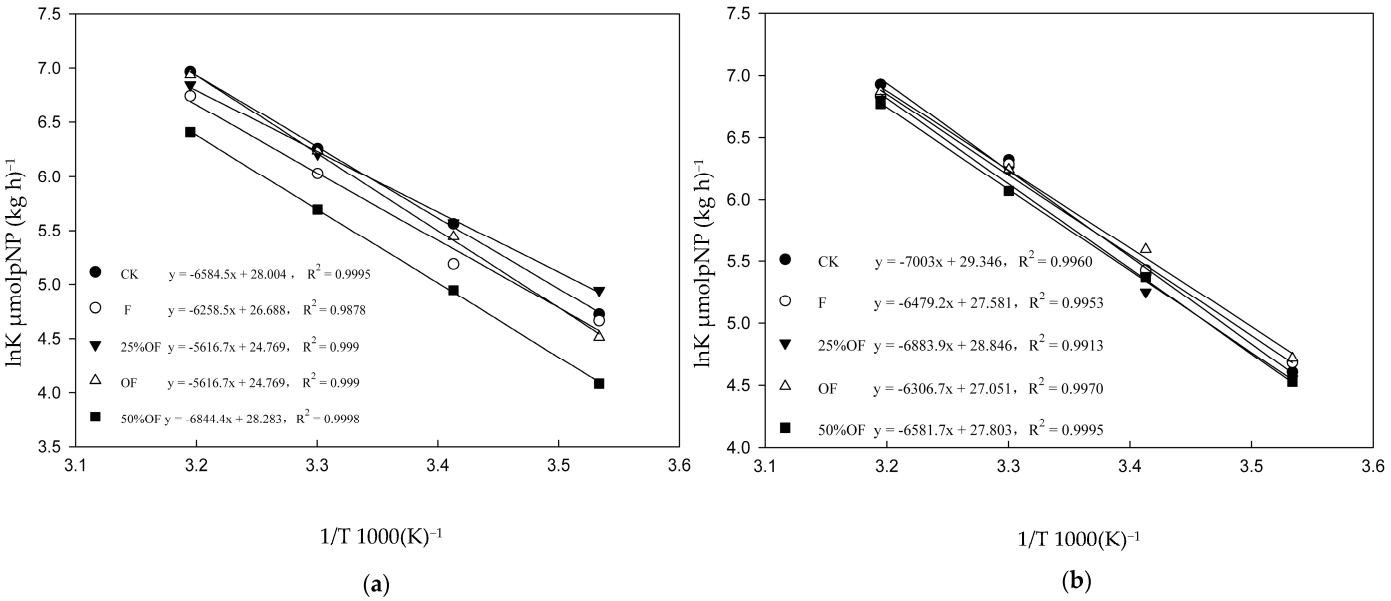

Figure 2. Linear transformation plots of the Arrhenius equation for *β*–glucosidase activity in soils on jointing stage (**a**) and booting stage (**b**) of spring wheat at temperatures ranging from 10 to 40 °C. CK indicates no fertilizer treatment. F indicates single fertilizer treatment. 25% OF indicates 25% organic fertilizer plus 75% chemical fertilizer treatment. OF indicates organic fertilizer treatment. 50% OF indicates 50% organic fertilizer plus 50% chemical fertilizer treatment.

Entropy change (ΔS) is a measure of the disorder of the reaction system. The smaller the ΔS value, the greater the directional order of the reactants in the enzyme–active center. The minimum value of ΔS was mostly found at 40 °C among all treatments at the different growth stages of wheat, which indicated that the enzymatic reaction of each treatment had the highest order and the deepest reaction degree under this culture temperature. At the same temperature, the ΔS value of *β*–glucosidase in the organic substitution of chemical fertilizer treatment was lower than that of the chemical fertilizer and no fertilizer treatments. The lowest ΔS value was found in the 25% OF treatment at the jointing stage and the lowest ΔS value was found in the OF treatment at the booting stage. Fertilization reduced the degree of disorder of the enzymatic reaction system, and the effect of organic substitution of chemical fertilizer treatment was the most significant.

The enthalpy change (ΔH) is usually used to characterize the energy obtained externally when the active site of the enzyme is complementary to the reactants. A high activation enthalpy change indicates that the formation of transition states has strong tension, distortion, and even bond breaking, which leads to a reduction in the reaction rate and the need for more energy supply to maintain the reaction. As shown in Table 3, the ΔH value of *β*–glucosidase in the organic substitution of chemical fertilizer treatment was lower than that of the chemical fertilizer and no fertilizer treatments at the same temperature. Similar to E_a and ΔS, the ΔH value of *β*–glucosidase in the 25% OF treatment was the lowest at the jointing stage, and the ΔH value in the OF treatment was the lowest at the booting stage. Therefore, the complementary energy required to carry out the soil enzymatic reaction under the organic substitution of chemical fertilizer treatment was relatively low, the tension between the enzyme and the substrate in forming the complex was also low, and the enzymatic reaction was easier to carry out.

Table 3. Thermodynamic parameters of *β*–glucosidase in soil under different fertilization.

Determination Stage	Treatment	t (°C)	E_a kJ mol^{-1}	Q_{10}	Mean	ΔG kJ mol^{-1}	Mean	ΔH kJ mol^{-1}	Mean	ΔS J (mol K)$^{-1}$	Mean
Jointing stage	CK	10 °C	53.56	2.212	2.058	56.77	57.03	51.26	51.14	−19.47	−19.76
		20 °C		2.099		56.85		51.18		−19.38	
		30 °C		2.002		57.16		51.09		−20.03	
		40 °C		1.918		57.33		51.01		−20.17	
	F	10 °C	50.91	2.127	1.986	56.90	57.57	48.60	48.48	−29.32	−30.48
		20 °C		2.024		57.75		48.52		−31.49	
		30 °C		1.935		57.72		48.44		−30.64	
		40 °C		1.857		57.89		48.36		−30.46	
	25% OF	10 °C	45.69	1.967	1.850	56.26	57.00	43.38	43.26	−45.49	−46.08
		20 °C		1.883		56.82		43.30		−46.14	
		30 °C		1.808		57.28		43.22		−46.38	
		40 °C		1.743		57.63		43.14		−46.30	
	50% OF	10 °C	55.67	2.283	2.118	58.24	58.47	53.37	53.25	−17.20	−17.50
		20 °C		2.162		58.33		53.29		−17.19	
		30 °C		2.058		58.55		53.21		−17.62	
		40 °C		1.968		58.75		53.13		−17.97	
	OF	10 °C	58.27	2.372	2.193	57.25	57.25	55.97	55.84	−4.53	−4.705
		20 °C		2.241		57.14		55.88		−4.29	
		30 °C		2.128		57.21		55.80		−4.65	
		40 °C		2.031		57.39		55.72		−5.35	
Booting stage	CK	10 °C	56.96	2.327	2.155	57.04	57.18	54.66	54.54	−8.39	−8.848
		20 °C		2.201		57.25		54.58		−9.13	
		30 °C		2.093		57.00		54.50		−8.26	
		40 °C		1.999		57.42		54.42		−9.61	
	F	10 °C	52.70	2.185	2.035	56.86	57.20	50.40	50.28	−22.82	−23.21
		20 °C		2.075		57.17		50.32		−23.40	
		30 °C		1.980		57.09		50.24		−22.60	
		40 °C		1.898		57.67		50.16		−24.02	
	25% OF	10 °C	55.99	2.294	2.127	57.16	57.42	53.69	53.57	−12.26	−12.92
		20 °C		2.171		57.60		53.61		−13.61	
		30 °C		2.066		57.20		53.53		−12.12	
		40 °C		1.976		57.73		53.45		−13.68	
	50% OF	10 °C	53.54	2.212	2.058	57.21	57.50	51.23	51.11	−21.13	−21.41
		20 °C		2.099		57.31		51.15		−21.01	
		30 °C		2.002		57.63		51.07		−21.64	
		40 °C		1.917		57.83		50.99		−21.84	
	OF	10 °C	51.30	2.140	1.996	56.77	57.08	49.00	48.88	−27.48	−27.52
		20 °C		2.035		56.78		48.92		−26.84	
		30 °C		1.944		57.19		48.83		−27.58	
		40 °C		1.866		57.57		48.75		−28.17	

Note: CK indicates no fertilizer treatment. F indicates single fertilizer treatment. 25% OF indicates 25% organic fertilizer plus 75% chemical fertilizer treatment. OF indicates organic fertilizer treatment. 50% OF indicates 50% organic fertilizer plus 50% chemical fertilizer treatment.

The free energy change (ΔG) reflects the free energy required to bring the reactants to the transition state. ΔG represents the relationship between enthalpy change and entropy change, which is derived from state parameters, such as ΔH and ΔS. As shown in Table 3, the difference in ΔG among the different treatments was not significant. Compared with the no fertilizer treatment, the ΔG value of soil *β*–glucosidase in the organic substitution of chemical fertilizer treatment decreased at the different growth stages, while the ΔG value in the chemical fertilizer treatment increased year–over–year. The 25% OF treatment attained the lowest ΔG value at the jointing stage and the lowest ΔG value at the booting stage was observed for OF treatment. The above results indicate that soil *β*–glucosidase consumes less energy to form an activated complex with the substrate and has a higher possibility of carrying out enzymatic reactions in the organic fertilizer substitution treatment.

3.3. Redundancy Analysis

The effects of soil total nitrogen, organic carbon, alkali–hydrolyzable nitrogen, and the C/N ratio on soil *β*–glucosidase's kinetic and thermodynamic parameters were investigated by computational redundancy analysis (RDA) [15]. These soil factors exhibited redundant effects in explaining changes in soil enzymes' kinetic and thermodynamic pa-

rameters. The effects of soil factors on soil enzymatic properties were different at the different growth stages of wheat. Figure 3a shows the effects of soil properties on soil enzymes' kinetic and thermodynamic parameters at the jointing stage of wheat. RDA1 and RDA2 explained 98.35% and 1.19% of the variation, respectively, and these soil properties cumulatively explained 99.5% of the soil enzymes' kinetic and thermodynamic properties. As shown in the figure, the β–glucosidase kinetic parameter K_m was negatively correlated with total nitrogen, alkali–hydrolyzable nitrogen, organic carbon, and C/N ratio to different degrees; V_{max} was negatively correlated with total nitrogen, alkali–hydrolyzable nitrogen, and organic carbon, and had little correlation with C/N ratio; V_{max}/K_m was positively correlated with organic carbon, alkali–hydrolyzable nitrogen, and C/N ratio, and negatively correlated with total nitrogen. The thermodynamic parameters of the enzymes (E_a, Q_{10} and ΔS) were positively correlated with soil total nitrogen, alkali–hydrolysable nitrogen, organic carbon, and the C/N ratio. ΔG was positively correlated with soil total nitrogen, alkali–hydrolysable nitrogen, and organic carbon, and negatively correlated with C/N ratio. Figure 3b shows the effects of soil properties on the enzymes' kinetic and thermodynamic parameters at the booting stage of wheat. RDA1 and RDA2 explained 76.74% and 20.10% of the soil enzymatic properties, respectively, which was 96.8% overall. The results showed that the enzyme kinetic parameter K_m was negatively correlated with organic carbon, alkali–hydrolyzable nitrogen, and the C/N ratio, and positively correlated with total nitrogen. V_{max} was almost linearly negatively correlated with organic carbon and the C/N ratio, had some positive correlation with alkali–hydrolyzable nitrogen, and was almost linearly positively correlated with total nitrogen. V_{max}/K_m was positively correlated with alkali–hydrolysable nitrogen, but was not significantly correlated with total nitrogen, the C/N ratio, or organic carbon. The thermodynamic parameters of the enzymes (E_a, Q_{10}, ΔG, and ΔS) were positively correlated with the C/N ratio and organic carbon, and were negatively correlated with total nitrogen and alkali–hydrolyzable nitrogen.

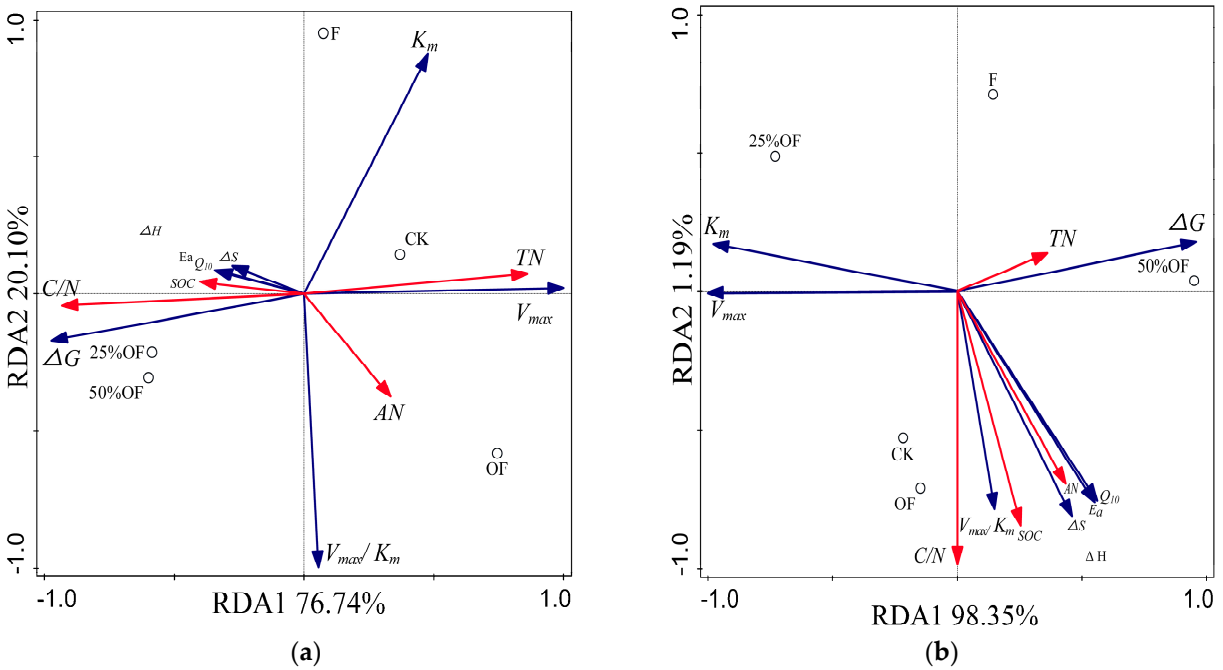

Figure 3. Redundancy Analysis (RDA) of relationships between soil properties and kinetic and thermodynamic parameters of soil β–glucosidase at the jointing stage (**a**) and booting stage (**b**) of spring wheat. Note: TN total nitrogen; AN alkali–hydrolyzable nitrogen; SOC organic carbon; C/N ratio of carbon to nitrogen.

4. Discussion

4.1. Kinetic Characteristics of Soil Enzymes

The relationship between the enzymatic reaction rate and substrate concentration at the different growth stages showed a rectangular hyperbola. With increasing substrate concentration, the enzymatic reaction rate correspondingly increased until reaching a maximum value, which was basically consistent with the Michaelis–Menten equation. Based on this observation, the kinetic parameters K_m, V_{max}, and V_{max}/K_m were derived.

The present study found that there were significant differences in the K_m values of soil *β*–glucosidase among fertilization treatments at different growth stages, which was consistent with the studies of Tan et al. (2018) [16], indicating that the enzyme kinetic parameter K_m was sensitive to the response of various treatments. However, Marx et al. (2001) [17] and Moscatelli et al. (2012) [12] reported that there was no difference in the K_m value of soil *β*–glucosidase among different fertilization treatments and concluded that different treatments yielded no significant effect on the K_m of soil *β*–glucosidase. The results of studies on the effects of fertilization on soil enzymes are often contradictory, which is related to differences in soil types, fertilization patterns, fertilization amounts, crop types and periods, and experiment durations.

Agricultural management practices, such as fertilization, can affect the physical location of enzyme molecules and the diffusion and availability of substrates. In this case, different fertilization treatments may affect the possibility of enzyme–substrate interactions and the induction of enzyme synthesis. K_m is used as an indicator of enzyme adsorption or accessibility level [18]. Many studies have found that the application of organic fertilizers enhanced the adsorption and immobilization of *β*–glucosidase by soil solids, resulting in a significant increase in the K_m value of the enzyme [14,19]. This is consistent with the study results of Liu et al. (2019) [20], who found that organic fertilizer treatment changed the quantity and quality of soil organic matter and increased the content of soil humus and organic–inorganic complexes. These substances have a large specific surface area and abundant active functional groups, which readily immobilize *β*–glucosidase by covalent binding, possibly causing conformational changes in the enzyme structure or reducing accessibility of the substrate to the enzyme active site, thus resulting in a significant decrease in the affinity of enzymes to substrates.

However, based on the experimental results, the K_m value of soil enzymes in the organic substitution of chemical fertilizer treatment was significantly lower than that of the chemical fertilizer and no fertilizer treatment, and there was a positive correlation between substrate affinity and soil organic carbon content. There are two possible explanations for this result. First, it could be that the application of organic fertilizers did not enhance the adsorption and immobilization of soil *β*–glucosidase. Second, soil *β*–glucosidase was not adsorbed by soil carriers in a way that blocked the enzyme–active site or modified conformational structure of the enzyme under the organic substitution of chemical fertilizer treatment. Consequently, the lower K_m value may indicate that there were few or no conformational changes in the soil enzymes' structure, and the accessibility of the enzyme to its substrate increased [21]. Studies have shown that despite enzyme adsorption, immobilized enzymes in the soil substrate have a strong affinity for substrates (exhibit lower K_m values than the free enzyme), and it was found that the soil carrier does not immobilize the enzyme through covalent bonds, but rather adsorbs it by weak intermolecular forces or physically retains the enzyme molecule encapsulated in the carrier [22]. This type of immobilization does not significantly affect the natural conformation of the enzyme and could obtain the optimal conditions for positive interaction between the enzyme and the free diffusion of the substrate molecule [23]. Whether this is the type of immobilization taking place with the organic substitution of chemical fertilizer treatment in this experiment needs to be further investigated.

A relatively wide range of K_m values (2.02~3.63) was observed in soils with different fertilization treatments, suggesting that the location and pool of *β*–glucosidase in soil may be different. The K_m values of soil enzymes represent the average of all isoenzymes from different sources that contribute to the total activity measured [12]. The application of organic fertilizers may change the microbial community structure and cause changes in soil enzyme affinity [24], which is consistent with the studies of Tian et al. (2020) [25] and Fontaine and Barot (2005) [26].

The change in enzyme intrinsic properties (K_m) may be related to changes in the dominant species of microorganisms, and the decrease in the K_m value may indicate the change in the functional structure of the microbial community toward population dominance, with a higher substrate affinity and substrate utilization efficiency accompanied by the expression of isozymes with the same function but different conformations and structures. Therefore, the application of organic fertilizer may promote microorganisms to synthesize and secrete isoenzymes with a high substrate affinity to maintain the catalytic characteristics of enzymes [27].

There was a more significant difference in the V_{max} of the soil enzyme among the different treatments in this experiment, which is consistent with the research results of Knight and Dick (2004) [28] and Moscatelli et al. (2012) [12]. The V_{max} of *β*–glucosidase was more sensitive to different soil management practices and was superior to K_m in detecting the effect of each treatment on *β*–glucosidase activity.

The V_{max} parameter could be used as a substitute index for the concentration of active enzymes and the total amount of enzymes [28]. In this study, it was found that the V_{max} value of *β*–glucosidase in the organic substitution of chemical fertilizer treatment was significantly higher than that of the no fertilizer and chemical fertilizer treatments at the different growth stages, and the increased V_{max} values reflected the higher production and activity of soil *β*–glucosidase after partial replacement of chemical fertilizer by the organic fertilizer. It is widely accepted that *β*–glucosidase is mainly synthesized and released by soil microorganisms [29]. Microbial activity and biomass directly or indirectly determine the potential for enzyme synthesis and production, and changes in soil enzyme concentration levels (V_{max}) induced by applied fertilizers may be correlated with soil microorganisms. Chen et al. (2020) [30] and Zhou et al. (2019) [31] found that high nutrient additions from chemical fertilizers may lead to the saturation of available nutrients in soil, and microorganisms did not need to obtain available nutrients from the soil by secreting large amounts of enzymes, which reduced the demand of microorganisms for enzyme synthesis, thus inhibiting the catalytic activities of enzymes related to carbon, nitrogen, and phosphorus cycles to a certain extent. Therefore, in this study, the application of chemical fertilizer alone may have reduced the nutrient demand of soil microbial biomass and slowed the turnover and activity of microorganisms, which in turn led to a reduction in soil *β*–glucosidase activity. However, reasonable application of organic fertilizer can significantly increase the amount of soil enzymes and improve enzyme activity. Chen et al. (2020) [30] proposed that the application of reduced chemical fertilizer combined with organic fertilizer could ensure that more microorganisms entered the treated soil, which could provide a large number of substrates, such as carbohydrates, that could be easily utilized by microorganisms at the same time, and the organic fertilizer itself is rich in enzyme substances. Furthermore, this provided abundance of nutrient sources and metabolic substrates for enzyme–producing microorganisms significantly increased the activity and biomass of microorganisms stimulating *β*–glucosidase synthesis, which in turn could support the production and release of enzymes into the soil environment, thus contributing to a significant enhancement in enzyme yield and activity.

Substrate availability may also be the main factor driving the production and enzymatic reaction rates of *β*–glucosidase in the soils under each treatment. The application of organic fertilizer may be accompanied by the adsorption of substrates into the soil solid phase while promoting the adsorption of enzymes, increasing the contact between the enzyme and the adsorbed substrate through co–localization, resulting in greatly enhanced

hydrolysis as the effective substrate concentration increases. On the other hand, substrate adsorption on the surface of the solid phase, such as soil humus, may establish a substrate concentration that stimulates enzyme production, which could also explain the increase in soil enzyme production and enzyme activity under the organic substitution of chemical fertilizer treatment.

Notably, the higher the organic matter content, the higher the total amount, and catalytic activity of soil enzymes is not always the case. This experiment found that there was a negative correlation between V_{max} and soil organic carbon content at the different growth stages, which may indicate that the application of organic fertilizer affected enzyme concentration and activity more by changing the quality and type of soil organic matter and other soil properties than the amount of organic matter. The exact reason for this needs to be further investigated.

In addition, the specificity constant (Ka), namely the ratio of V_{max} to K_m, was used to compare the catalytic properties of the same enzyme among the different fertilization treatments as a whole. The V_{max}/K_m value of soil β–glucosidase in the organic substitution of chemical fertilizer treatment was higher than that of the chemical fertilizer treatment at the different growth stages, and the V_{max}/K_m value in the chemical fertilizer treatment was the lowest. The reason for this is that, on the one hand, a significant fraction of the enzyme activities measured in soil originate from immobilized enzymes adsorbed into soil organic matter or clay. This was confirmed by the studies of Busto and Perez–Mateos (1995) [32], who extracted humus from soil and found that the extract accounted for up to 50% of the total soil β–glucosidase activity. The immobilized form not only contributes significantly to total enzyme activity, but also improves the stability of the enzyme in the soil matrix and maintains its activity. Hayano and Katami (1977) [5] reported that β–glucosidase could stabilize humus and clay colloids in soil and could maintain most of its activities without interference from proteolytic enzymes or other factors, which was also illustrated by the research results of Wang et al. (2019) [33]. Organic matter provides abundant binding sites or complex sites for adsorbed enzymes, which ensures the effectiveness of enzymes. Combined with this experiment, the application of a certain proportion of organic fertilizer increased the content of clay colloid and humus in the tested soil and improved the ability of the soil to stabilize and protect enzymes [34].

On the other hand, the application of organic fertilizer to the soil can not only provide sufficient enzymatic substrates and abundant nutrients for the corresponding enzymes, but it can also increase soil microbial biomass, resulting in more nutrients needed by microorganisms to complete metabolic activities. Moreover, this can stimulate the reaction catalyzed by β–glucosidase, thus contributing to improvements in enzyme activity and catalytic efficiency. However, the application of high amounts of chemical fertilizer might reduce the pH value of soil, promote the accumulation of salts in soil, and have a certain inhibitory effect on enzyme activity [35]. In conclusion, organic matter can not only provide sites for the stabilization and protection of enzymes and improve the stability of enzymes in soil, but it can also enhance the catalytic activity of enzymes. Compared with the application of chemical fertilizer alone, the utilization of organic fertilizer significantly improved the catalytic performance of β–glucosidase, which was supported by the positive correlation between soil β–glucosidase V_{max}/K_m and organic carbon content.

4.2. Thermodynamic Characteristics of Soil Enzymes

Increasing the temperature accelerates the collision and decomposition of enzymes and substrates, ensuring more complete substrate transformation and resulting in high–speed enzymatic reactions [36]. The soil kinetics assay set the culture temperature at 37 °C, as this temperature is considered the physiologically optimal temperature for most microorganisms [37].

Among the established assay methods, 10 °C, 20 °C, 30 °C, and 40 °C were selected as culture temperatures for consistency with recognized classical soil enzyme thermodynamic determination methods. In addition, studying the effect of the culture temperature on

enzyme activity not only provides the optimal temperature range for enzyme activity, but also provides thermodynamic parameters, such as the activation energy and temperature coefficient [14]. The temperature dependence of *β*–glucosidase was further evaluated based on the temperature coefficient and activation energy of the enzyme to predict the response of the enzyme activity to global warming [38–40].

The temperature coefficient (Q_{10}) is a multiple of the rate constant increase when the temperature rises by 10 °C [38], and the Q_{10} value is not constant and varies depending on the activation energy (E_a) of the enzyme in the soil [41]. The sensitivity of the enzyme–catalyzed reaction to temperature changes increased with an increasing Q_{10}. This test found that during the growth period of wheat, especially at the gestational panicle stage, the Q_{10} of the soil enzyme in the organic substitution of chemical fertilizer treatment was relatively low, and the thermal stability was higher than that of the no fertilizer and chemical fertilizer treatments. The activation energy (E_a) represents the minimum energy required for an enzyme to bind to a substrate and yield a transition complex, the magnitude of which is an intrinsic factor in determining the reaction rate. Surprisingly, few studies have been conducted to determine the E_a of soil enzymes, especially hydrolytic enzymes. More studies have been conducted on pure soil enzymes, but these studies do not indicate how soil fertilization may alter E_a [42]. This study filled this gap and found that the E_a value order between different treatments at each growth stage was consistent with that of Q_{10}. The E_a value in the organic substitution of chemical fertilizer treatment was lower than that of the no fertilizer and single–application fertilizer treatments, and the treatment with the highest enzyme activity attained the lowest activation energy, which revealed the activity mechanism of the soil enzyme from a thermodynamic perspective. The lower E_a value indicated that the enzyme and substrate reaction required less energy to initiate at the beginning and in the transition state, resulting in a higher activity of *β*–glucosidase, which was supported by higher V_{max} [21].

The response of enzymatic reactions to temperature involves a number of complex factors, depending on the structure of the enzyme and its interaction with other low–molecular weight and polymer substances emerging during the reaction [43]. Thus, the differences in the enzyme thermodynamic parameters Q_{10} and E_a observed between the different fertilization treatments may be related to the presence of different isoenzymes produced in the soil and enzyme interaction with compounds in the reaction environment [44].

Yan et al. (2010) [14] found that the thermodynamic parameters E_a and Q_{10} of immobilized *β*–glucosidase were decreased compared with those of the free enzyme in soil. The temperature sensitivity and activation energy of the enzyme in the organic substitution of chemical fertilizer treatment were lower, which may be partly because *β*–glucosidase was readily immobilized and adsorbed on the humus or clay colloid and protected by it under this treatment, resulting in higher thermal stability of the enzyme. Some studies suggest that the decrease in the E_a value with the addition of organic fertilizer may also be due to the high affinity and efficient interaction between the enzyme and substrate [45]. However, this statement itself has limitations and does not fully explain the experimental results that have emerged. More research is needed to understand the relationship between the E_a value and the K_m constant of *β*–glucosidase in the organic substitution of chemical fertilizer treatment under different soil types.

Another explanation is that the organic fertilizer treatment alters the community composition and structure of soil microorganisms, which implies a shift in dominant microbial populations, accompanied by the release of a different set of isozymes, resulting in reduced sensitivity to temperature changes [46]. Since we did not measure the microbial community composition in this study, we can only speculate that changes in the microbial community are a key factor affecting the observed thermodynamic parameters of *β*–glucosidase. This aspect still needs further exploration.

In addition, the application of organic fertilizer may change the physicochemical properties of soil. There are few reports on the effects of soil physicochemical properties on Q_{10} and E_a, and it is difficult to quantify how much of the observed temperature sensitivity changes can be attributed to changes in soil chemical conditions, especially changes in the soil pH, which should be studied further. The temperature sensitivity of enzymes has important ecological significance for predicting the response of enzymes to global warming, and the decrease in the dependence of soil *β*–glucosidase on temperature after the application of organic fertilizer may lead to a decrease in soil carbon loss under global warming.

Second, the Q_{10} of the enzyme under different soil fertilization treatments was not the same, but the difference was not large. For example, the soil temperature coefficient Q_{10} at the jointing stage of wheat ranged from 1.918–2.212, 1.857–2.127, 1.743–1.967, 1.968–2.283, and 2.031–2.372, which is very close to the research results of Frankenbergerjr and Tabatabai (1991) [47]. The Q_{10} values of enzymes in different soils usually vary between 1.64 and 2.27. The E_a value of the test soil ranged from 45~59 kJ mol^{-1}, but the difference between the different treatments was not large, which is consistent with the change trend of the temperature coefficient Q_{10}. The difference was not significant, indicating that the response mechanism of enzymes to temperature under the different treatments was consistent, and the correlation with land fertilization management was not notable. This is consistent with the views of Chen et al. (2018) [48]. Generally, the thermodynamic properties of soil enzymes usually depend on the intrinsic properties of enzymes, and other factors (such as the amount of organic fertilizer added) can only affect the direction of the thermodynamic response of enzymes.

Entropy change ΔS is one of the important factors limiting the occurrence of enzyme reactions, which is related to the degree of order of the enzyme–substrate activation complex; the smaller the ΔS value of the enzyme system is, the higher the order of the substrate in the active part of the enzyme. The ΔS values of *β*–glucosidase in all treatments were negative, indicating a high spontaneity of the substrate and enzyme to achieve the transition state [49]. There was no obvious relationship between the size order of the ΔS values and the corresponding enzyme activities between each treatment, but the minimum value of ΔS at each growth stage corresponded to the same treatment, similar to the maximum value of enzyme activity, which is consistent with the Q_{10} and E_a sequences. The ΔS value of the 25% OF treatment at the jointing stage was the lowest, while the value of the OF treatment at the gestational panicle stage was the lowest. ΔS can affect the activity characteristics of enzymes to a certain extent, but it cannot determine the final magnitude of enzyme activity. The lower ΔS value indicated that the soil *β*–glucosidase was more likely to show directional complementarity with the reactants, and the enzymatic reaction tended to be stronger.

Enthalpy ΔH is another important parameter restricting the occurrence of enzymatic reactions and is often used to indicate the energy obtained from the outside when the enzyme activity center is complementary to the substrate. The ΔH value of *β*–glucosidase in each treatment was different and positive at the different growth stages, which reflected that the enzymatic reaction can be carried out by absorbing energy from the outside, which is a characteristic of energy complementarity. The higher the ΔH value is, the larger the number of stretching, compression, and even breaking occurrences of chemical bonds reaching the transition state [50]; thus, more energy is required to form the product [51]. The low enthalpy and negative entropy values of the organic fertilizer treatments indicate the formation of a more efficient and ordered transition state complex between the enzyme and substrate. The ΔH of each treatment was consistent with the variations in E_a, ΔS, and Q_{10} and exhibited little correlation with the change characteristics of the enzyme activity.

ΔG combines the contributions of enthalpy and entropy and is an indicator of the feasibility and difficulty of reactions [52]. The positive or negative values of ΔG in the system can be used to determine whether the reaction can proceed spontaneously or not. The ΔG values of *β*–glucosidase in all treatments were positive, indicating that the process

of *β*–glucosidase forming an activated complex in soil could not be spontaneous, and the transition state could only be achieved by external energy. The average ΔG value at the jointing stage showed the order of 50% OF > F > F > CK > 25% OF, and at the gestational panicle stage, the order of 50% OF > 25% OF > F > CK > OF was observed, which is exactly the opposite of the size order of the enzyme activity during each period. The smaller ΔG is, the less energy is required for enzymes and substrates to form intermediate complexes, the easier it is to carry out enzymatic reactions, and the higher the enzyme activity is. Contrary to the variation trend of V_{max}, the enzymes' kinetic characteristics were verified from the perspective of activation–free energy.

Thermodynamic parameters, such as E_a, ΔH, ΔG, and ΔS, are related and complementary, which jointly affect characteristics of enzyme activity. The study of thermodynamic properties revealed that the application of a reasonable proportion of organic fertilizers at different growth stages not only reduced the activation energy required for enzymatic reactions and the energy obtained from the outside world when enzymes are bound to the substrate, but also enhanced the directional order of the substrate in the active part of the enzyme, which is more conducive to the occurrence and progress of enzymatic reactions. However, the results of this experiment showed that the thermodynamic parameters of *β*–glucosidase at different growth stages were positively correlated with soil organic carbon and total nitrogen, which may indicate that the application of organic fertilizer did not promote enzymatic reaction by improving the physical and chemical properties of the soil; the specific reasons need to be further explored.

5. Conclusions

Fertilization significantly affects the characteristic parameters of enzyme kinetics. At the different growth stages, the V_{max} of *β*–glucosidase in the organic substitution of chemical fertilizer treatment was higher than that of the single fertilizer and no fertilizer treatments, and the K_m was lower than that of the chemical fertilizer and no fertilizer treatments. The application of organic fertilizer improved the affinity between the enzyme and substrate and the ability of the enzyme–substrate complex to form products, which essentially increased the catalytic ability of soil enzymes and then facilitated the occurrence of enzymatic reactions.

The soil enzyme thermodynamic parameters (E_a and Q_{10}) in the organic substitution of chemical fertilizer treatment were much lower than those of the no fertilization and chemical fertilizer treatments. The E_a and Q_{10} in the 25% OF treatment were the lowest at the jointing stage, and those in the OF treatment were the lowest at the booting stage, which indicated that the application of organic fertilizer increased the thermal stability of *β*–glucosidase and reduced its sensitivity to increasing temperature. It also indicated that the application of organic fertilizer created more favorable conditions for the occurrence and progression of enzymatic reactions under the premise of providing equal nutrients via fertilization, and the intensity and efficiency of enzymatic reactions were significantly improved.

Nevertheless, more studies are needed to confirm whether the effect of the organic substitution of chemical fertilizer treatment on *β*–glucosidase is consistent with other carbon cycle enzymes or soil types, in order to more conclusively demonstrate the underlying mechanism of enzyme response to organic fertilizer application.

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