Analysis of factors affecting the activity of several major soil enzymes

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Abstract: Soil enzymes are a general term for a class of active substances that are free or bind to enzymatic action in the soil. Soil enzymes are closely related to microorganisms in the soil, and their activity influencing factors are also diverse. In order to study the influencing factors of several major soil enzymes, this paper determined the main physical and chemical properties, microbial quantity and soil urease, sucrase, dehydrogenase and cellulase activities of a tomb in a tomb, and analyzed soil properties, microbial biomass and The correlation between enzyme activity and the main factors affecting the activity of several enzymes were studied by single factor experiments, which provided a reference for the study of soil enzyme activity. The results showed that the factors related to soil enzyme activity were: total phosphorus content, organic matter content, electrical conductivity and fungal number, and organic matter content was the key influence factor. The addition of exogenous nitrate nitrogen, glucose and humic acid promoted urease, sucrase, cellulase and dehydrogenase activities, respectively.

1. Materials and methods

1.1 Collection of soil samples

Overview of the sampling area: The sampling site is located in the Fufeng and Lushan areas of Baoji City, Shaanxi Province. It has a warm and semi-humid climate with uniform precipitation and high vegetation coverage. The terrain gradually decreases from north to south. At that time, the main soil was black loess soil located 1 meter below the current farming layer. From the color of the soil, it should be a semi-humid grassland.

1.2 Instruments and reagents

Instruments: steam sterilization pot, biochemical incubator, constant temperature oscillator, ultraviolet spectrophotometer, oven, pH meter, electric thermostatic water bath, electronic balance;

Reagents: The reagents used in the experiments were all pure or analytically pure, and the experimental water was ultrapure water.

1.3 Experimental methods

The physical and chemical properties of the soil were determined as follows: pH was determined by potentiometry according to NY/T1121.2-2006; organic matter content was determined by potassium dichromate titration according to GB 9834-88; soil total phosphorus was used according to GB 9837-88. Determination by color method; available phosphorus is determined according to HJ 704-2014 using sodium bicarbonate extraction-molybdenum antimony anti-spectrophotometry; ammonia nitrogen is determined by spectrophotometry according to HJ 634-2012 using potassium chloride solution; water content is based on NY/T52 -1997 was determined by the drying method; the conductivity was measured using a conductivity meter.

Method for determining the amount of microorganisms: The microorganisms were separated and counted by a plate dilution method.

The soil urease was determined by sodium phenolate-sodium hypochlorite colorimetric method, the invertase was determined by DNS colorimetry, the dehydrogenase was determined by TTC spectrophotometry, and the cellulase was determined by DNS colorimetry.

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2. Analysis of experimental results

2.1 Experimental results and discussion

2.1.1 Soil physical and chemical properties

The test results are shown in Table 1:

Table 1 Determination of soil physical and chemical properties at each sampling point

Sampling point	pH	Effective phosphorus (mg/kg)	Total phosphorus (mg/kg)	Total phosphorus (mg/kg)	Organicmatter content (%)	Volume of water (%)	Conductivity (us/cm)
Southeast point of Che Ma Keng	7.79	19.46	631.12	220.96	0.828	3.84	21.2
WestNorth point of Che Ma Keng	7.86	18.60	530.72	190.79	0.478	4.36	21.5
NorthEast point of Che Ma Keng	7.10	20.87	638.76	110.29	0.510	4.01	20.6
East wall point of CheMaKeng	7.98	18.10	428.31	188.29	0.836	3.24	20.2
West wall point of Che Ma Keng	7.94	10.29	511.45	201.63	0.644	2.35	24

According to the soil nutrient grading standard, after analyzing the physical and chemical properties of the soil, the pH value of the experimental soil is between 7.04 and 8.51, which is neutral to alkaline. This is because the precipitation in the north is low, the evaporation is high, and the groundwater is evaporated. The salt is left in the soil, not like the iron-aluminum in the soil of the southern soil, and the soil leaching is more serious, the salt base is not saturated, which causes the black soil to be alkaline; the lowest value of soil available phosphorus is 10.29mg/kg. The highest value is 20.87mg/kg, except for the second grade (>20mg/kg) in the northeast corner of Che Ma Hang. The remaining soil samples were all three grades (10mg/kg~20mg/kg); the highest total phosphorus content was 638.76mg/kg, and the lowest value was 418.31mg/kg, all of which were intermediate (0.44%~0.66%); the organic matter content was Between 1.26% and 4.36%, it is six (<6%), which is relatively poor. This is due to the fact that it has been buried in the grave for thousands of years, with less biological activity and no organic matter-rich substances such as exogenous plant remains. The water content is below 5%, which is dry soil. On the one hand, the climate in the north itself is dry, on the other hand, it is weathered for a long time after excavation, and it is not planted. Lower water content also results in less water-soluble ions, so the soil conductivity value is lower.

2.1.2 Soil enzyme activity measurement

The experimental results are shown in the following table:

Table 2 Results of soil enzyme activity determination at each sampling point

	Urease (μg/(g*d))	Sucrase (mg/(g*d))	Dehydrogenase (μg/(g*d))	Cellulase (μg/(g*d))
North East Poin of Che Ma Keng	97	1.54	0.49	10
West North point of Che Ma Keng	96	1.13	0.33	8
South East point of Che Ma keng	80	0.64	0.38	11.06
East point	89	0.88	0.29	11.77
West point	95	0.92	0.31	8.59

The soil enzyme activities measured this time are relatively low, because after thousands of years of burial, soil nutrients are already quite poor. Among them, the dehydrogenase activity is low, because there are many copper products in the horse-horse pit, and copper can react with the final product of the dehydrogenation reaction, resulting in a low measured value [19]. Cellulase can decompose cellulose in wood. The rich wood products in the pit are almost completely decayed when unearthed, and the cellulose content is very low, so the cellulase activity is low.

2.2 Analysis of results

2.2.1 Relationship between soil enzymes and soil physical and chemical properties

Table 3 Correlation between soil physical and chemical properties and enzyme activity at each sampling point

	pH	Available phosphorus (mg/kg)	Total phosphorus (mg/kg)	Ammonia nitrogen (mg/kg)	Organic matter content (%)	water content (%)	Conductivity (us/cm)
Urease	-0.825**	0.446	-0.218	0.961**	0.334	-0.204	-0.494*
Sucrase	0.487	0.065	0.211	0.813**	0.452	0.19	0.077
Enzyme	-0.317	0.458	0.827**	0.144	0.235	0.413	-0.191
Cellulose	-0.337	0.476	-0.052	-0.407	0.498*	0.003	-0.715*

From Table 3, urease activity was negatively correlated with soil pH, total phosphorus content and conductivity, which was significantly correlated with pH and conductivity, indicating that the lower the pH, the smaller the conductivity, the stronger the urease activity; meanwhile, the urease activity Significantly positively correlated with ammonia nitrogen content. Insulin activity is significantly positively correlated with pH and ammonia nitrogen content. In addition, the activity of sucrase is mainly affected by organic matter, because organic matter is the main carbon source of soil microbial activity [20], while invertase and soil carbon The cycle is closely related; dehydrogenase is not only positively correlated with total phosphorus content, but also effective phosphorus has a positive effect on dehydrogenase activity. Dehydrogenase cannot exist in the soil in a complex form, so its correlation with other environmental factors is not significant. Cellulase was positively correlated with organic matter content and negatively correlated with conductivity.

2.3 Analysis of the response of several soil enzymes to environmental factors by different single factor experiments

(1) Effects of different pH on soil enzymes:

It can be seen from Fig. 1 that pH has a significant effect on urease, dehydrogenase and cellulase, but the effect on invertase is not obvious.

The optimal active pH point of urease is about 7.0, the dehydrogenase is 8.0~8.5, and the cellulase is about 6.5. A lot of data indicate that urease is more active in acidic to neutral [25-26], which is roughly consistent with the results of this study. On the one hand, pH affects the enzyme activity by affecting the dissolution of soil organic carbon, and low pH increases the biotoxicity of the exchangeable ions in the soil solution, thereby reducing the carbon released into the soil environment [27].On the other hand, soil acidity changes the dissociation state of the enzyme. When the environment changes, the activity of the adsorbed enzyme is smaller than that of the free enzyme. The increase in soil pH reduces cellulase activity. This is because cellulase decomposes wood, wood is rich in tannin and is acidic, so its optimum pH is low.

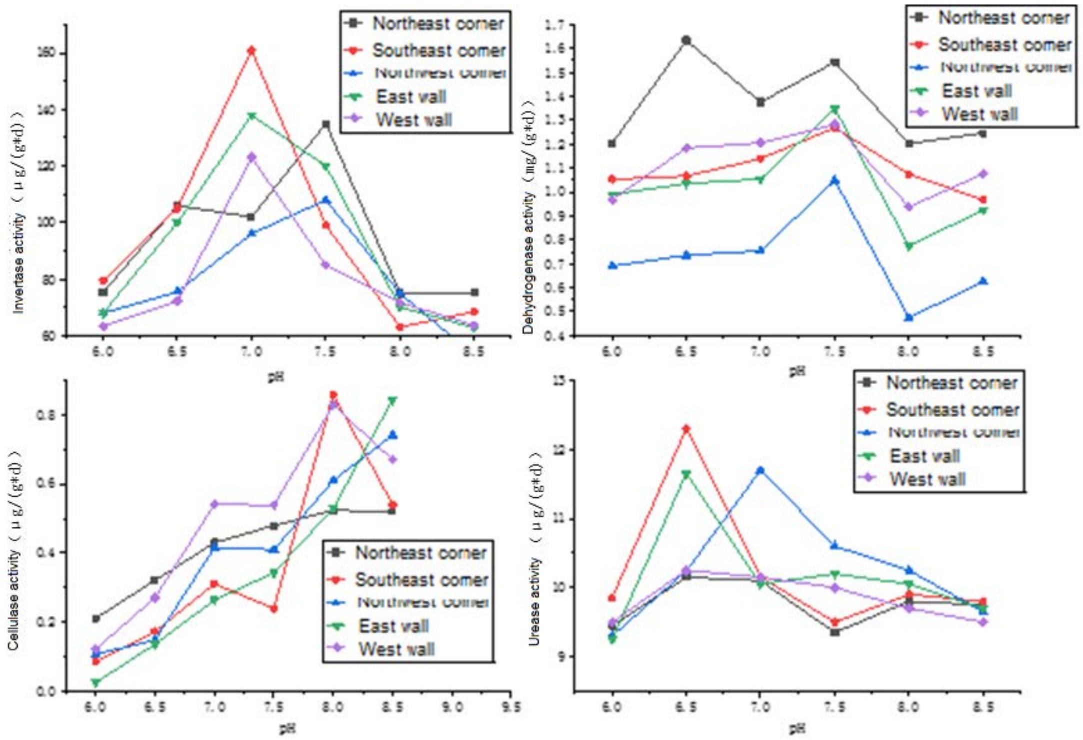

Figure 1 Effect of pH on enzyme activity

(2) Effects of different humic acid concentrations on soil enzymes:

It can be seen from the figure that with the addition of humic acid, the invertase activity is changed from a sharp increase to a stationary state, while the cellulose and dehydrogenase are gradually increased, and the urease activity is significantly inhibited. The optimum humic acid concentration point for sucrase appeared at 0.01%, while dehydrogenase and cellulase appeared at 0.015%. At the optimal humic acid concentration point, the sucrase activity increased by 30%~78%, the dehydrogenase activity increased by 70%~218%, and the cellulase activity increased by 22.9%~40.7%.

Soil organic matter is mainly decomposed by exogenous enzymes. In general, humic acid has a strong promoting effect on the activity of these enzymes. As a kind of organic matter, the ability of humic acid to adsorb enzyme is greater than mineral, enzyme and soil. The combination of organic matter or cosmid enhances the stability of the enzyme and prevents modification by proteases or passivators [32]. It can be seen that the promoting effect of humic acid on dehydrogenase is very strong, which is consistent with the research conclusions of previous researchers, and with the increase of the amount of humic acid added, the effect first increases and then weakens. In addition, humic acid also activates soil nutrients through the combination of elements such as nitrogen and phosphorus, improves soil nutrient availability and sustained release properties, thereby promoting enzyme activity; while urease activity is significantly reduced due to humic acid The unsaturated bond contained inhibits the oxidation of urease-reactive functional groups and sequesters urease sulfhydryl groups in the soil, thereby reducing the rate of urea hydrolysis. Meanwhile, Dong et al. showed that humic acid can reduce the hydrolysis of urea to ammonia in soil. And buffering the pH of the soil to inhibit the activities of urease-producing microorganisms such as ammoniated bacteria and ammoniated archaea.

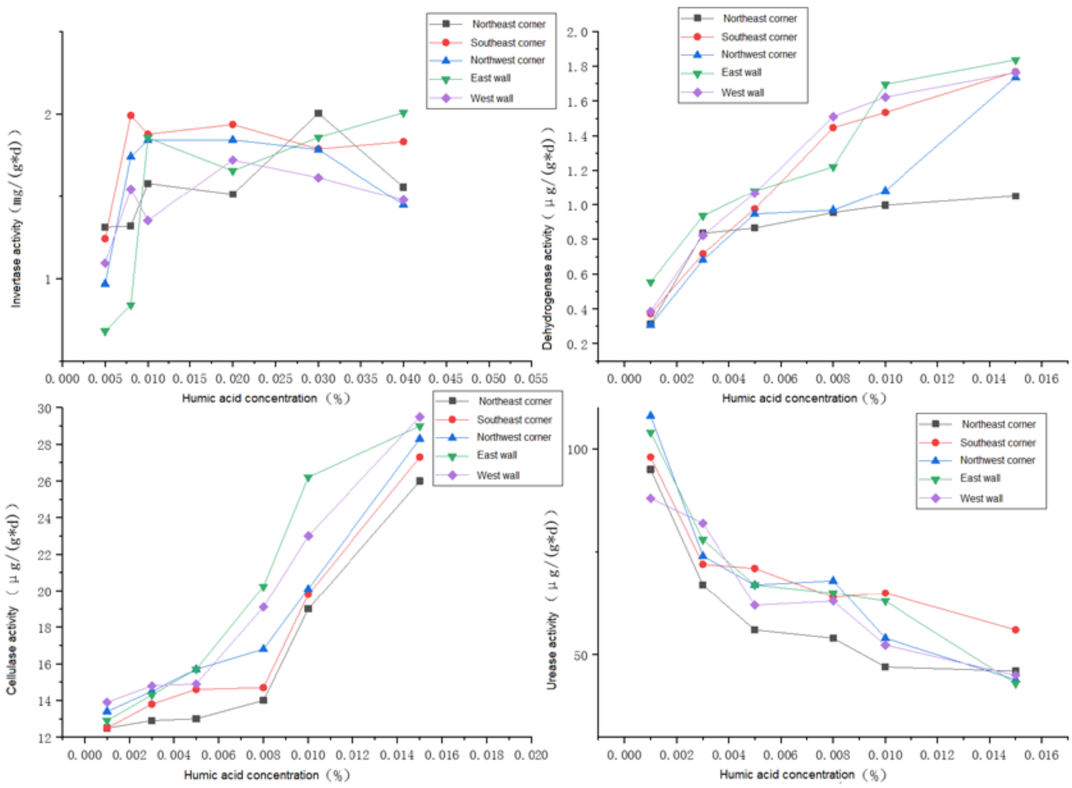

Figure 2 Effect of humic acid on enzyme activity

(3) Effects of different nitrogen source types and concentrations on soil enzymes:

The addition of nitrate nitrogen significantly promoted the activity of urease and dehydrogenase, while the cellulase showed a stable performance in the early stage, only a transient peak appeared at the concentration of 0.01%, and then decreased, and the invertase added to the nitrate nitrogen. No obvious performance. At the optimum concentration, urease activity increased by 36.8%~91.8%, dehydrogenase activity increased by 42.35%~106%, and cellulase activity increased by 0.4%~13.8%.

The addition of ammonium nitrogen caused a decrease in urease and cellulase activity, and the change in sucrase was not obvious. The activity increased at the concentration of 0.01%, and then decreased, indicating that 0.01% is the optimum concentration of sucrase, while dehydrogenase The corresponding change of ammonium nitrogen is steadily rising, and will not continue to change until 0.01%~0.015%. At the optimum concentration, the dehydrogenase activity increased by 30% to 72.6%, and the invertase activity increased by 41.6% to 58.2%.

Ammonium sulfate can reduce the activity of urease, while potassium nitrate can increase the activity of urease to some extent and the continuous input of exogenous nitrogen can promote the increase of soil cellulase activity, which confirms the conclusions of predecessors. Among them, the promotion of potassium nitrate is stronger than that of ammonium sulfate, because the presence of inorganic nitrogen can promote the decomposition of organic matter and the mineralization of the original organic matter in the soil.

Nitrogen application not only increased the activity of cellulase and dehydrogenase by increasing the number of soil actinomycetes and fungi, but also changed the composition of large aggregates in the soil. The soil colloid accumulated and adsorbed stable enzymes in the dry soil. Exogenous nitrogen input also affects soil enzyme activity by affecting soil pH. Nitrogen input increases NH_4+ and NO_3- content in soil. On the one hand, when these ions are absorbed, soil acidity is enhanced. On the other hand, NH4+ can also replace soil colloid surface. Salt-based ions, due to charge balance, increase H+ and aggravate soil acidification. At the same time, the nitrification of ammonium nitrogen is also an important cause of soil acidification. The reaction equation is: $NH_4{+}+2O_2=NO_3{-}+H_2O+2H^+$.

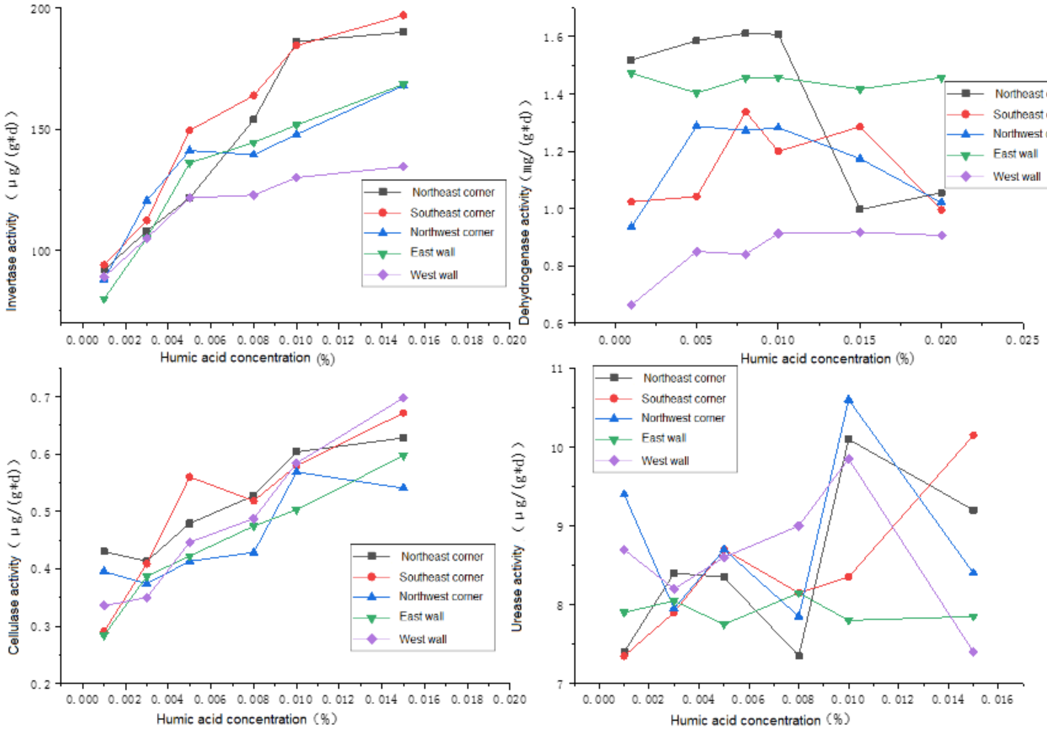

Figure 3 Effect of nitrate nitrogen on enzyme activity

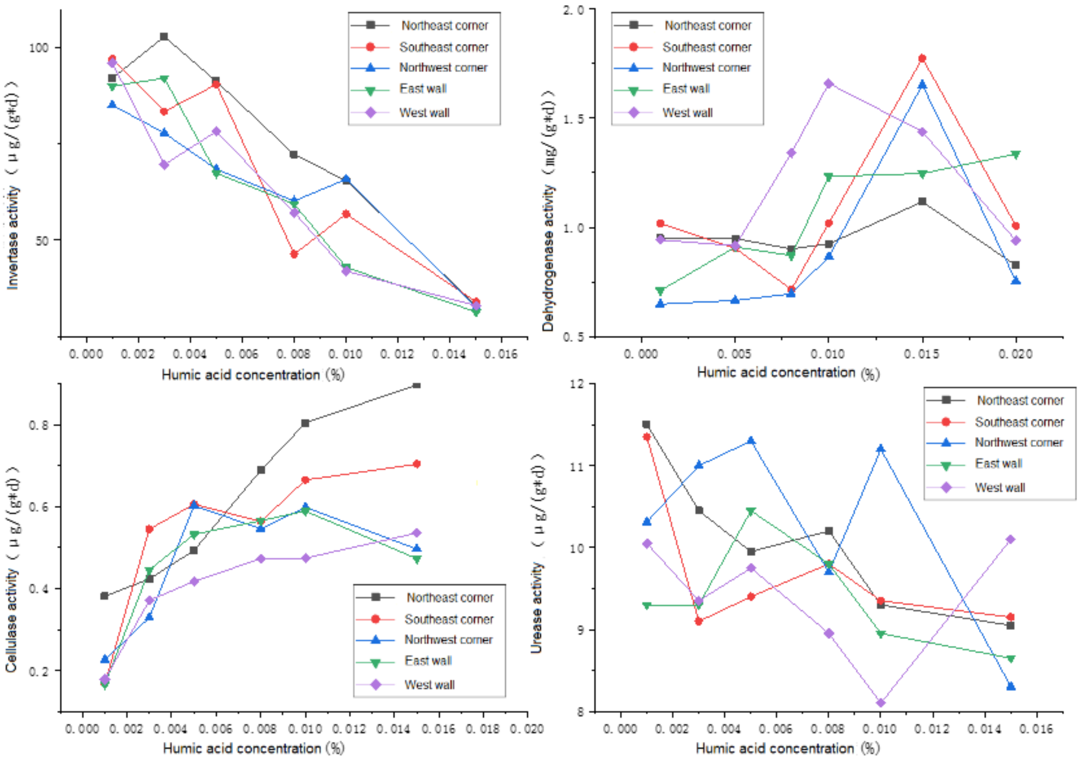

Figure 4 Effect of ammonium nitrogen on enzyme activity

3. Conclusion

(1) Urease activity was negatively correlated with soil pH, total phosphorus content and conductivity, and positively correlated with ammonia nitrogen content; sucrase activity was positively correlated with pH, ammonia nitrogen content and organic matter content; dehydrogenase and total phosphorus, available phosphorus The content was significantly positively correlated; cellulase was positively correlated with organic matter content and negatively correlated with

conductivity.

(2) The addition of exogenous amino acids, glucose and humic acid promoted the activities of urease, sucrase, cellulase and dehydrogenase, respectively.

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