



Study on the Self-Made Eco-Friendly Enzymes from Substandard Pear Fruits in Orchards and Their Application in Fruit Tree Cultivation

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Abstract. To explore the cultivation and management technology model for resource recycling in orchard ecosystems and promote the low-carbon green development of the forestry and fruit industry, this study focuses on the material circulation within the orchard ecosystem. Using substandard fruits from orchards and fresh soybean stems and leaves as raw materials to produce eco-friendly enzyme, the study set up different application concentration treatments based on field control experiments. The aim was to investigate the impact of the home-made eco-friendly enzyme on the growth of Jingbai pear tree leaves, fruit yield, and soil properties. The results indicate that compared with the water treatment, diluting the enzyme solution 50-fold and 100-fold significantly improved the chlorophyll content, leaf area, and individual plant yield of the pear trees. The chlorophyll content increased by 4.28% and 13.54%, the leaf area by 6.42% and 18.72%, and the individual plant yield by 72.00% and 54.23%, respectively, promoting leaf growth and increasing fruit yield. After treating the pear trees with 5 different dilutions, the relative soil moisture content increased, with the 50-fold diluted enzyme solution showing an increase of 12.8%. After treating the pear trees with the 50-fold diluted enzyme solution, the soil's total carbon, total nitrogen, available phosphorus, ammonium nitrogen, and nitrate nitrogen contents were the highest, reaching 38.20 g/kg, 3.00 g/kg, 88.60 mg/kg, 4.50 mg/kg, and 15.20 mg/kg, respectively. The 50-fold diluted enzyme solution also enhanced the diversity of microorganisms such as bacteria, fungi, and actinomycetes in the soil, with an increase of over 20.90%. Therefore, the application of 50-20fold diluted eco-friendly enzyme made from substandard orchard fruits as a fertilizer in fruit tree cultivation can significantly promote leaf growth, increase fruit yield, and improve soil mineral nutrition and microbial diversity.

Keywords: eco-enzyme; material composition; pear fruit yield; environmental soil; resource utilization

1 Introduction

China is a major fruit-producing country in the world. With the improvement of people's living standards, the cultivation area and yield of fruits in China have increased year by year. In 2022, the national orchard cultivation area reached 195 million mu, effectively ensuring the fruit demand of domestic residents. In the process of fruit tree production, a large number of low-quality defective fruits such as physiological fruit drop, diseased and insect-damaged fruits, and bird-pecked fruits are produced. Improper disposal can cause environmental pollution and the spread of diseases and pests^[1-2]. Therefore, the safe disposal and resource utilization of defective fruits and other waste in orchards have become a hot and difficult topic in the field of agricultural environmental science both domestically and internationally^[3-5]. Studies have shown that the fermentation broth of defective pears is rich in amino acids and other nutrients, and fermenting it into eco-enzyme is a good way of resource utilization^[2].

Eco-enzyme production is simple, safe, and environmentally friendly. It is rich in a large number of organic matter, mineral salts, organic acids, active enzymes, and anaerobic and facultative microorganisms. When applied in agricultural production, such as by spraying or irrigating the root soil of crops and fruit trees, it has outstanding effects like activating plant nutrition, improving soil, promoting plant growth, increasing yield, and enhancing quality. It has good agricultural value and prospects for promotion^[6-9]. Shi Lingshan^[10] and others found that applying self-made eco-enzyme diluted 900 times to crops maximized crop yield and quality, and also improved the chemical properties of the crop soil. Tao Jin^[11] and others discovered that treating chili peppers with a 750-fold diluted eco-enzyme solution not only increased the yield of chili peppers but also enhanced soil fertility. Studies by Nie Songqing and Zhang Rui^[12-13] and others indicate that applying the fermentation broth to crop soil through irrigation resulted in higher plant chlorophyll content and crop yield compared to other treatment groups. Huang Weijing^[14] and others found that treating fruit trees with eco-enzyme significantly improved tree growth and fruit quality. Liu Yanna^[15] and others demonstrated that applying the fermentation broth could significantly increase the growth of new shoots in pear trees. Eco-enzyme is rich in beneficial microorganisms, which play a positive role in promoting a healthy soil microecological environment, effectively balancing soil microbial diversity and making the soil more fertile^[16]. Numerous studies have shown that eco-enzyme has a significant effect when used in agricultural production, but there have been no reports on the technical model of circular utilization based on the orchard eco-system. Therefore, this study focuses on the material cycle of the orchard ecosystem, converting waste defective fruits into eco-enzyme, and explores its application in orchard cultivation. By investigating the technical model of resource circular utilization in cultivation management, it aims to provide data support and theoretical backing for the green and low-carbon development of the fruit and forestry industry.

2 Materials and Methods

2.1 Survey of the Experimental Site

The experiment was conducted in 2023 at the Mengwu Modern Agricultural Science and Technology Demonstration Park, located in Mengwu Village, Junzhuang Town, Mentougou District, Beijing. The coordinates are 116°07'39"E, 40°00'32"N, with an elevation of 167 meters. The park covers an area of 370 acres, surrounded by mountains and with a central open area, forming a typical small basin climate. The average annual temperature in the park is 14.6°C, with an average annual rainfall of 624.7 millimeter and an average annual sunshine duration ranging from 2600 to 2800 hours. These conditions provide an ideal ecological environment for the growth of Jingbai pears, making it the core production area for Jingbai pears in the Mentougou District.

2.2 Experimental Materials

2.2.1 Eco-enzyme. Pretreatment of raw materials: The substandard Jingbai pears collected from the orchard were crushed to less than 3 cm in diameter, and the soybean stems and leaves were cut into segments less than 5 cm in length. In a controlled greenhouse (with the temperature maintained at 25~35 °C), the waste materials, brown sugar, and water were mixed in a mass ratio of 3:1:10. The mixture was then placed in a 50 L airtight container. For the first 30 days of fermentation, the container was opened daily to release gas, after which the mixture was subjected to anaerobic fermentation for 3 months while avoiding light. Upon completion of the fermentation process, the pH of the ecoenzyme solution was stabilized between 3 and 4, resulting in the production of the ecoenzyme liquid.

2.2.2 Pear Trees. Four-year-old 'Jingbai' dwarf pear trees are selected for planting. The planting row spacing is approximately 4 meters, and the spacing between trees is about 0.5 meters, resulting in about 333 trees per mu.

2.3 Experimental Method

2.3.1 Test Design. Using 4-year-old dwarf and dense-planted Jingbai pear saplings as the experimental material, a 20-meter-long row of Jingbai pear trees was designated as one experimental plot. Each plot contained approximately 23 Jingbai pear trees with consistent growth conditions and load (all cultivation and management practices were consistent throughout the experiment, with the only variation being the experimental treatment). The experiment was repeated three times to ensure the reliability of the results. The experimental treatments included different dilutions of ecoenzyme (10x GE10, 50x GE50, 100x GE100, 200x GE200, 500x GE500) and a clear water control (CK). These were applied at five different growth stages: before bud break (March 20th), after flowering (April 20th), young fruit stage (May 20th), fruit expansion stage (June 20th, July 20th), with 2000 mL of diluted enzyme solution poured around each

tree. Normal water and fertilizer management were consistent for all experimental treatments. In October each year, organic fertilizer was applied in furrows at a rate of one ton per mu, and irrigation included winterizing water and reviving water. Additional watering was provided uniformly during drought periods.

2.3.2 Sample Collection. During the fruit harvest period (August 25th), pear fruit sampling and field measurements were conducted. For fruit yield determination, three representative trees were selected for whole-tree picking, and the yield per tree was recorded. For leaf sampling, leaves from the upper, middle, and lower parts of the tree canopy were selected for sampling and measurement, with five leaves collected from each part. Soil samples were collected from the soil layer around the pear tree roots at a depth of 0-20 centimeters using a 5-point sampling method. Each sample weighed approximately 1500 grams and was divided into two parts: one part was passed through a 2-millimeter sieve and then stored in a refrigerator at -80 °C for soil microbial diversity analysis; the other part was air-dried and sieved for the determination of soil physicochemical properties.

2.3.3 Enzyme Solution Component Detection. The pH value was measured using a pH meter (phs-2f model); enzyme activity was determined using an enzyme-linked immunosorbent assay (ELISA) kit; trace element content was measured according to the provisions of YS/T 587.5^[17]; organic acid content was determined in accordance with the relevant provisions of GB 5009.157-2016^[18]; organic matter content was measured according to NY/T 1976-2010^[19]; amino acid content was determined following the provisions of GB 5009.124-2016^[20]; the number of viable bacteria was measured according to the relevant provisions of GB 20287-2006/6.3.2^[21]; polyphenol content was determined according to the provisions of GB/T 31740.2-2015^[22]; and polysaccharide content was measured using high-performance liquid chromatography (HPLC).

2.3.4 Leaf and Fruit Yield Indicator Testing. Leaf area is measured directly using an electronic leaf area meter; chlorophyll content is measured directly on the leaves using a chlorophyll meter; the total weight of the fruits is weighed using an electronic scale, and the yield per plant is recorded.

2.3.5 Soil Physicochemical Property Testing. Soil moisture content is measured using the oven drying method, where soil samples are weighed and then placed in an oven to dry to constant weight. The soil moisture content is calculated by determining the weight difference before and after drying. Soil pH is measured using the water extraction potentiometric method (with a soil-to-water ratio of 2.5:1)^[23]. Available phosphorus is determined by the ammonium acetate extraction-flame photometer method^[23]. Total carbon and total nitrogen in the soil are measured using an elemental analyzer^[24]. Ammonium nitrogen and nitrate nitrogen in the soil are determined by the potassium chloride extraction-spectrophotometric method.

2.3.6 Soil Microbial Diversity Assessment or Soil Microbial Diversity Analysis. Microbial community diversity is analyzed by extracting phospholipid fatty acids from soil samples.

2.4 Data Processing

Data charts were plotted using Origin 2024 and Excel 2010 software; correlation and one-way ANOVA analyses were conducted using SPSS 27 software. The data analysis results represent the average of three replicate measurements.

3 Results and Analysis

3.1 Content of Major Material Components in Homemade Eco-Enzymes

Table 1 data shows that the fermentation broth pH is 3.7, and the pH range of successfully fermented eco-friendly enzymes is between 3 and 4, indicating that the fermentation broth has completed fermentation. The average content of organic acids, organic matter, trace elements, amino acids, polyphenols, and crude polysaccharides is 3.0, 27.6, 4.9, 17.6, 0.3, and 25.2 g/L, respectively. The number of effective viable bacteria reaches 3.6×10^7 CFU/mL. The protease activity and β -glucanase activity reach 3831.3 U/L and 65026.3 U/L, respectively, and their contents meet the agricultural and soil improvement standards specified in QB/T5323-2018.

Table 1. List of material composition content in the eco-enzyme

Indicators	Fermentation broth	Agricultural standards	Soil improvement standards
pH	3.7	≤ 4.5	≤ 7.5
Organic acids (g·L ⁻¹)	3.0	≥ 0.66	≥ 1
Organic matter (g·L ⁻¹)	27.6	≥ 5	≥ 1
Effective viable count (10 ⁷ CFU/mL)	3.6	$\geq 1 \times 10^7$	$\geq 1 \times 10^7$
Trace elements (g·L ⁻¹)	4.9	≥ 2	≥ 2
Amino acids (g·L ⁻¹)	17.6	≥ 10	≥ 10
Protease activity (U·L ⁻¹)	3831.3	≥ 100	—
β -Glucanase activity (U·L ⁻¹)	65026.3	≥ 200	—
Polyphenols (g·L ⁻¹)	0.3	≥ 0.1	—
Crude polysaccharides (g·L ⁻¹)	25.2	≥ 20	≥ 20

3.2 The Impact of Eco-Friendly Enzyme Application on the Growth of Jingbai Pear Leaves and the Yield of Pear Fruits.

The effects of applying different dilution multiples of fermentation broth on the growth of Jingbai pear leaves and the yield of pear fruits are shown in Figure 1. Compared to the CK treatment, the GE50, GE100, GE200, and GE500 treatments all significantly increased the leaf area of the pear trees ($P \leq 0.05$) (Figure 1A). The treatment effects

ranked as follows: GE100 > GE200 > GE50 > GE500 > CK > GE10. Compared to CK, the leaf area increased by 18.72% and 17.47% for the GE100 and GE200 treatments, respectively. The chlorophyll values after treatment with five different dilution multiples of enzyme solution were all significantly higher than those of the CK group ($P \leq 0.05$) (Figure 1B), with the treatment effects ranking as: GE200 > GE100 > GE10 > GE500 > GE50 > CK. Compared to the CK group, the chlorophyll values increased by 13.54% and 19.88% for the GE100 and GE200 treatments, respectively. The highest single-plant yield was observed in the GE50 treatment, followed by GE100 and GE200, both of which were significantly higher than the CK treatment ($P \leq 0.05$) (Figure 1C). Compared to CK, the GE50, GE100, and GE200 treatments increased the yield by 72.00%, 54.23%, and 45.14%, respectively. This indicates that the application of 50x, 100x, and 200x dilutions significantly promotes the fruit yield and leaf growth of pear trees.

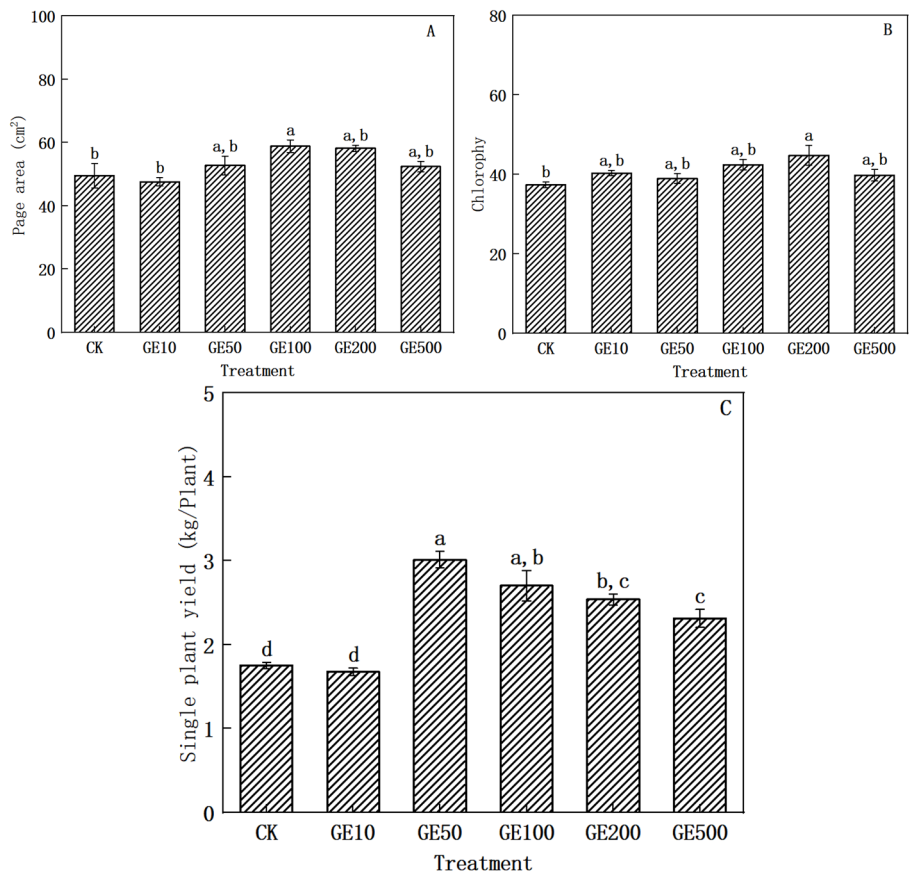


Fig. 1. Growth and yield differences of pear tree leaf with different treatme

3.3 The Impact of Eco-Enzyme Application on the Physical and Chemical Properties of ‘Jingbai’ Pear Orchard Soil

The physical and chemical properties of pear tree soil are crucial for the growth of pear trees. Enzyme treatments have significant effects on soil indicators such as relative moisture content, pH value, total carbon, total nitrogen, available phosphorus, ammoniacal nitrogen, and nitrate nitrogen.

3.3.1 Relative Moisture Content, pH. The effects of applying different dilutions of fermentation broth on the relative moisture content and pH of the soil of ‘Jingbai’ pear trees are shown in Figure 2. The soil relative moisture content in all enzyme treatment groups was significantly higher than that in the CK treatment ($P \leq 0.05$) (Figure 2A), with GE10 treatment showing the best effect. In terms of soil pH, there were significant differences between GE10 and GE50 treatments and the CK treatment ($P \leq 0.05$), but the difference in data values was small (Figure 2B), indicating a negligible impact on soil pH.

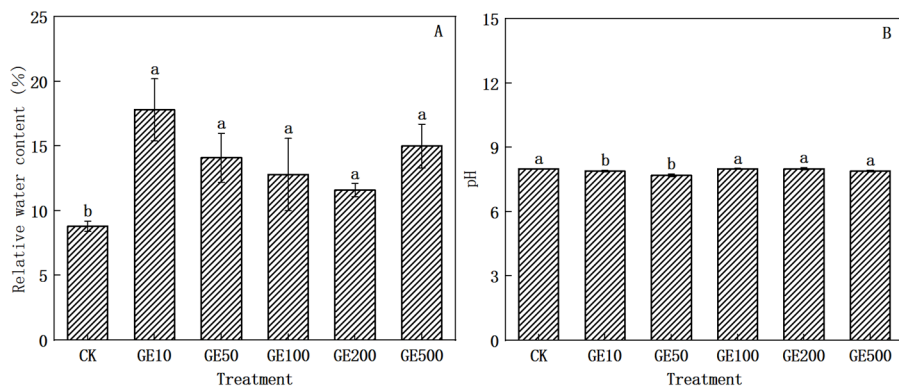


Fig. 2. Differences in relative water content and pH among different treatments

3.3.2 Total Nitrogen, Total Carbon, Available Phosphorus. The effects of applying different dilutions of fermentation broth on the total carbon, total nitrogen, and available phosphorus in pear tree soil are shown in Figure 3. After treatment with different dilutions of enzyme solution, the total carbon content was highest in the GE50 treatment, followed by the GE10 treatment, both significantly higher than other treatments ($P \leq 0.05$) (Figure 3A). Compared with the CK treatment, GE10 and GE50 treatments were 68.68% and 80.19% higher, respectively. The total nitrogen content in the soil was significantly higher in all five different dilution treatments than in the CK treatment ($P \leq 0.05$) (Figure 3B), with the highest value of 3.0 g/kg in the GE50 treatment. The available phosphorus content was significantly higher in the GE10 and GE50 treatments than in the other treatments ($P \leq 0.05$) (Figure 3C), with GE10 and GE50 treatments increasing by 62.13% and 65.30%, respectively, compared to the CK treatment.

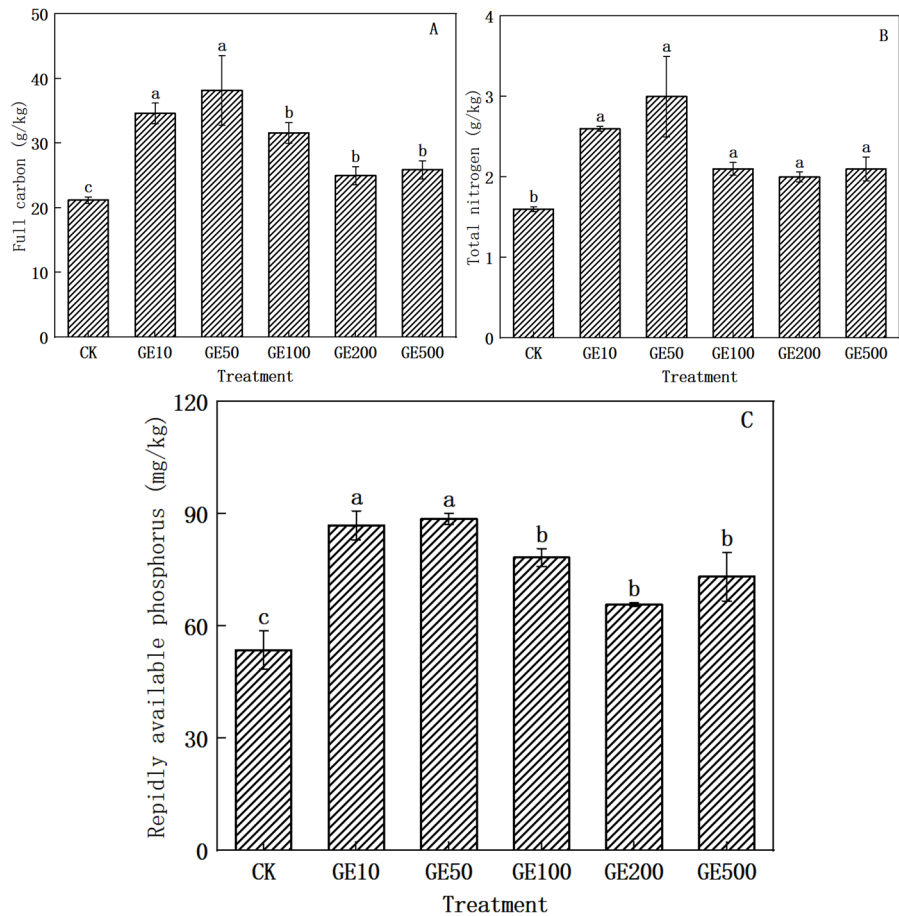


Fig. 3. Differences in soil total carbon, total nitrogen, and available phosphorus among different treatments

3.3.3 Nitrate Nitrogen, Ammoniacal Nitrogen. The effects of applying different dilutions of fermentation broth on the nitrate nitrogen and ammoniacal nitrogen in the soil of ‘Jingbai’ pear trees are shown in Figure 4. After treatment with different dilutions of enzyme solution, the ammoniacal nitrogen and nitrate nitrogen in all treatments were significantly higher than those in the CK treatment ($P\leq0.05$) (Figure 4A-B), with the highest values of ammoniacal nitrogen and nitrate nitrogen in the GE50 treatment being 4.5 mg/kg and 15.2 mg/kg, respectively.

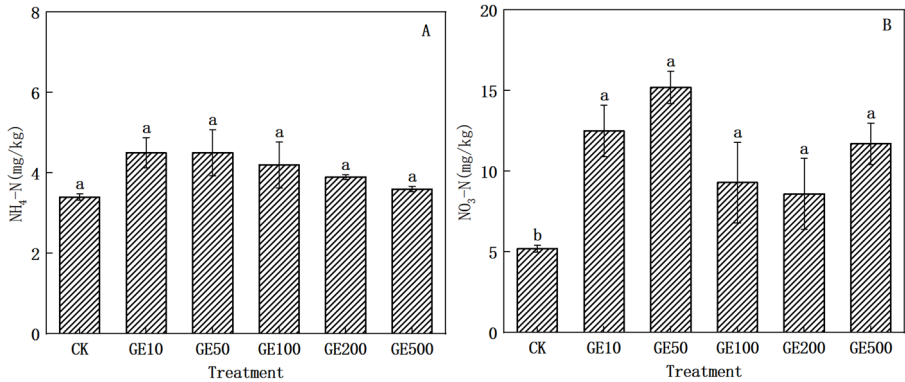


Fig. 4. Differences in ammonium and nitrate nitrogen of the soil from different treatments

In summary, compared with the CK treatment, after diluting the enzyme solution 10 times and 50 times, the soil moisture content, total carbon, total nitrogen, and nitrogen, phosphorus, and potassium contents were all increased, optimizing the soil properties and providing favorable conditions for plant growth.

3.4 The Impact of Environmental Enzyme Application on the Soil Microbial Diversity of ‘Jingbai’ Pear Orchards

Table 2 reflects the impact of applying different dilutions of fermentation broth on the microorganisms in the soil of ‘Jingbai’ pear trees. As shown in Table 2, after treatment with different concentrations of enzyme solution, the community diversity of Gram-negative bacteria, saprophytic fungi, and actinomycetes in all treatments was significantly higher than that in the CK treatment ($P<0.05$), with the GE50 treatment showing the highest values. Compared with the CK, the numbers of Gram-negative bacteria, saprophytic fungi, and actinomycetes increased by 0.61, 0.84, and 0.33 times, respectively. Thus, the application of environmental enzymes significantly enhanced the microbial diversity of Gram-negative bacteria, saprophytic fungi, and actinomycetes in the soil, with an increase of more than 20.90%, thereby improving the soil microecological environment.

Table 2. Effect of different treatments on the planting soil of pear tree

Treat- ment	TotalPL- FAs (nmol·g ⁻¹)	Bacterium (nmol·g ⁻¹)			Fungus (nmol·g ⁻¹)		ACT (nmol·g ⁻¹)
		GP	GN	Other bac- terium	SF	AMF	
CK	27.66±1.07 b	11.27±0.47 a	7.06±0.59 d	0.81±0.05 a	2.23±0.10 c	1.26±0.12 a	5.04±0.09 b
GE10	38.02±2.65 a	12.93±1.56 a	12.10±0.68 a	1.13±0.05 a	3.82±0.27 a	1.66±0.06 a	6.38±0.20 a

to- tal ni- tro ge n	- 0.2 00	0. 00 9	0.3 18	0.4 56	0.3 51	- 0.8 19 **	0.7 21 **	1								
Fu ll car bo n	- 0.1 38	0. 03 5	0.3 02	0.4 89 *	0.4 49	- 0.7 37 **	0.8 04 **	0.9 24 **	1							
To tal PL F As	0.6 12 **	0. 09 7	0.6 54 **	- 0.3 34	- 0.0 85	- 0.0 29	- 0.1 74	- 0.0 71	- 0. 09 3	1						
G P	0.4 01	0. 05 1	0.2 45	- 0.1 28	- 0.1 19	0.1 25	- 0.3 08	- 0.3 31	- 0. 35 4	0.7 62 **	1					
G N	0.6 15 **	0. 12 8	0.7 41 **	- 0.3 83	- 0.0 38	- 0.0 98	- 0.0 88	0.0 5	0. 04 5	0.9 45 **	0. 53 7*	1				
SF	0.6 43 **	0. 19 8	0.6 05 **	- 0.4 14	- 0.0 67	0.2 01	- 0.2 12	- 0.1 28	- 0. 08 5	0.8 02 **	0. 40 5	0.8 78 **	1			
A M F	0.3 48	0. 10 7	0.4 86 *	- 0.0 09	0.1 79	- 0.2 18	0.1 12	0.2 40	0. 19 5	0.6 37 **	0. 28 2	0.6 21 **	0. 43 8	1		
A C T	0.2 75	- 0. 10 3	0.5 89 *	- 0.2 29	- 0.1 27	- 0.3 22	0.0 68	0.2 40	0. 14 4	0.6 86 **	0. 42 9	0.6 06 **	0. 32 6	.6 41 **	1	
ot he r B	0.4 52	- 0. 14 6	0.3 56	- 0.3 77	- 0.2 44	- 0.0 92	- 0.1 45	- 0.0 83	- 0. 14 4	0.6 08 **	0. 35 0	0.6 19 **	0. 40 3	0. 39 1	0. 46 4	1

Note: * and ** represented the degree of correlation respectively, *represented $P \leq 0.05$, **rep-
resented $P \leq 0.01$.

4 Discussion

The study found that the environmental enzyme solution is rich in mineral elements, organic active ingredients, enzyme activity, and microbial content, which meets the standards of plant enzymes for use in agriculture and soil improvement as specified in QB/T5323-2018. When used as a fertilizer, it can increase the chlorophyll content in plant leaves, promote photosynthesis, thereby enhancing plant growth and increasing fruit yield^{[1],[25]}. Research has shown that applying plant-derived environmental enzyme nutrient solutions can effectively increase chlorophyll content, promote leaf growth, and enhance fruit yield, but only effective when applied at an appropriate concentration^[26]. Chlorophyll is a key substance affecting photosynthesis in plants; applying environmental enzymes can effectively increase chlorophyll content in plant leaves, improve photosynthetic rates, and promote leaf growth^[27-28]. Geng Jian and others^[29] found that treating pear trees with environmental enzyme solution significantly increased leaf area and chlorophyll content, promoting the growth of new shoots. Dai Mingliang and Liu Yan^[30-31] reported that treating pear trees with environmental enzymes significantly increased leaf chlorophyll content and area, leading to a notable increase in fruit yield. In this experiment, diluting the enzyme solution 100 times and 200 times before applying it to pear trees significantly increased leaf chlorophyll levels, promoted leaf growth, and enhanced fruit yield, which aligns with the findings of Wang Xiaoyue^[32] who observed a significant increase in chlorophyll levels in grape leaves treated with a 200-fold diluted enzyme solution, and the results of Sui Lili^[33] who achieved increased yields and income from fruit and vegetable crops treated with a 200-fold diluted plant enzyme solution.

Soil physical and chemical properties are fundamental to crop growth as they influence various aspects such as nutrient absorption, physiological metabolism, and root development, ultimately determining the growth status and final yield of crops. Environmental enzymes can improve soil fertility, increase soil moisture content, organic matter, and mineral elements, leading to significant improvements in soil physical and chemical properties^[10,34,35]. Liu Yan, Zeng Xibo, and Xi Zhenbang^[31,34,36] and others have treated pear trees with environmental enzyme solutions, which increased the soil moisture content and water use efficiency in the pear tree rhizosphere, while also significantly enhancing the content of available nitrogen, phosphorus, and potassium in the soil. In this experiment, treating pear trees with five different dilutions of enzyme solutions significantly increased the soil relative moisture content. Applying 10, 50, and 100 times diluted enzyme solutions to pear trees significantly increased the soil total carbon, total nitrogen, and available phosphorus content, which were significantly higher than the levels found in soils treated with environmental enzyme dilutions by Kang Liyun and Zhu Deyan^[37-38]. These results are similar to those of Pu Yanshuang and Tan Liting^[39-40], who found that applying a 100-fold diluted environmental enzyme solution significantly increased the total nitrogen, total phosphorus, and available phosphorus content in plant soils. In all treatments, the soil nitrate nitrogen and ammoniacal nitrogen content significantly increased, improving the nitrogen nutrition level of the plants, promoting leaf area growth, and increasing plant yield.

Microbial communities are currently the largest reservoir of biodiversity in the world, providing numerous ecosystem services to the soil and serving as important regulators of the structure of plant and agricultural ecological environments^[32]. Fan Heling and Zhao Zheng^[41-42] have shown that liquid agricultural enzymes can significantly affect soil microbial diversity. Yao Xiaoqin and Wang Xiaoyue^[42,32] and others have found that applying diluted enzyme solutions to the soil significantly increases the Ace and Chao indices of soil microbial communities, thereby enhancing the richness of the soil microbial community to some extent. Kang Liyun and Fu Qingming^[37,44] and others have treated farmland with enzyme solutions, resulting in a significant increase in the number of bacteria, fungi, and actinomycetes in the soil. In this study, the environmental enzyme solution used had a high viable count of 3.6×10^7 CFU/mL, with protease activity and β -glucosidase activity reaching 3831.3 U/L and 65026.3 U/L, respectively. Applying an appropriate dilution ratio of the environmental enzyme solution to pear trees can significantly increase the diversity of soil microorganisms such as Gram-negative bacteria, saprophytic fungi, and actinomycetes, promote the cycling and utilization of soil nutrients, optimize soil structure, enhance the stability of the soil microecosystem, and thus contribute to the healthy development of the ecological environment.

5 Conclusion

During the period from March to July each year, applying diluted ecoenzyme liquid at a rate of 50 to 200 times its original concentration for the treatment of pear trees in the late third week of each month can effectively promote the growth of pear tree leaves and increase chlorophyll content, thereby enhancing fruit yield. Ecoenzyme is rich in minerals, organic matter, enzyme activity, and trace elements. Its application in the growth of pear trees can also significantly improve the physical and chemical properties of the soil, and markedly increase the diversity of microorganisms such as Gram-negative bacteria, saprophytic fungi, and actinomycetes in the soil, thereby improving the soil microecological environment. This provides data support for the cultivation applications in orchards.

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2.Longitudinal Agricultural Non-Point Source Pollution Prevention and Control Plan Based on Water Quality Improvement Management in the Lower Reaches of the River and Sea Basin(42102018009)

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