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Substitution of Inorganic Fertilizer with Organic Fertilizer Influences Soil Carbon and Nitrogen Content and Enzyme Activity under Rubber Plantation

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Abstract: Conventional fertilization practices can lead to many ecological problems, such as nutrient imbalance, soil acidity, and reduced soil fertility, in natural rubber plantations. To address these challenges, a field investigation was strategically carried out to substitute inorganic fertilizer with organic fertilizer, consisting of six treatments: no fertilization (CK), inorganic fertilizer (NPK), 25% replacement of inorganic through organic (25% manure (M)), 50% replacement of inorganic through organic (50% manure (M)), 75% replacement of inorganic through organic (75% manure (M)), and 100% organic fertilizer (100% manure). The soil physicochemical properties (soil organic carbon (SOC), total nitrogen (TN), mineral nitrogen (N), ammonium nitrogen (NH4+-N), and nitrate nitrogen (NO_3^--N) , C:N, pH, and the carbon- and nitrogen-converting enzymes β -1,4-glucosidase (BG), N-acetylglucosaminidase (NAG) and L-leucine aminopeptidase (LAP) were all determined. The partial substitution of inorganic fertilizer with organic fertilizer (i.e., 75% M at surface soil layer) showed higher SOC (14.52 g·kg⁻¹), TN (1.06 g·kg⁻¹), N (20.07 mg·kg⁻¹), C:N (14.63), NH₄⁺-N (10.63 mg·kg⁻¹), and NO₃⁻⁻N (11.06 mg·kg⁻¹) than NPK and CK. This increase in physicochemical properties after partial replacement of inorganic with organic fertilizer resulted from higher carbon and nitrogen enzyme activities (BG (143.17·nmol·g⁻¹·h⁻¹), NAG (153.96 nmol·g⁻¹·h⁻¹), and LAP $(153.48 \text{ nmol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}))$ compared to NPK and CK. Further, the Pearson correlation and redundancy analysis (RDA) analyses confirmed a significant positive correlation between SOC, N, and soil enzymes. This study presents a new strategy for assessing the impact of partially replacing inorganic fertilizer with organic fertilizer in rubber plantations in tropical regions, mainly by modifying the soil nutrient composition.

Keywords: organic fertilizer substitution; soil fertility; soil carbon; mineral nitrogen; soil enzymes; rubber plant

1. Introduction

Natural rubber (*Hevea brasiliensis Willd. ex A. Juss*) is of great economic importance in tropical regions. Plantations of natural rubber have recently expanded quickly across mainland Southeast Asia and are projected to increase by 8.5 million hectares in the next ten years [1,2]. In rubber agroforestry, the soil tends to be acidic, with low organic matter concentration and soil fertility [2–4]. Chemical fertilizers are frequently applied to improve soil fertility and increase rubber yield [5]. However, the excessive use of these fertilizers can cause environmental issues such as nutrient imbalance, nitrogen leaching, soil acidification, and low soil fertility [2]. Therefore, there is a need to find a viable approach that might mitigate the adverse effects of chemical fertilizers on the ecosystems, potentially leading to enhanced nutrient accumulation in rubber plantations.



Citation: Yang, Q.; Li, J.; Xu, W.; Wang, J.; Jiang, Y.; Ali, W.; Liu, W. Substitution of Inorganic Fertilizer with Organic Fertilizer Influences Soil Carbon and Nitrogen Content and Enzyme Activity under Rubber Plantation. *Forests* **2024**, *15*, 756. https://doi.org/10.3390/f15050756

Academic Editor: Choonsig Kim

Received: 29 March 2024 Revised: 22 April 2024 Accepted: 22 April 2024 Published: 25 April 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). In many agricultural systems, organic fertilizers are often used as a substitute for inorganic fertilizers to maintain soil organic carbon levels and increase soil nitrogen, phosphorus, and potassium availability [6,7]. According to Salehi et al. (2017) [8], adding both organic and inorganic fertilizers increased soil SOC by 2.45% and total nitrogen (TN) content by 3.27% when compared to the control. However, organic and inorganic fertilizations were reported to result in differences in soil SOC and other soil physicochemical properties, probably because fertilization type, application time, and climate can induce contrasting soil physicochemical properties. In fact, a recent study in a subtropical climate demonstrated that substituting chemical fertilizer (NPK) and control (no fertilization) [5]. Another study found that after 30 years of co-application of organic and inorganic fertilizer in semi-arid regions, the soil SOC content improved, but no positive effect was observed on soil N, P, or K [9]. However, in tropical areas of Hainan Island, China, where annual mean temperature and precipitation are high, the impact of substituting inorganic fertilizer with organic fertilizer with organic fertilizer with organic fertilizer is used to result or soil physicochemical properties, SOC, and nitrogen content remains unclear.

Soil enzymatic activity is crucial for assessing the relationship between soil nutrients, especially changes in soil organic carbon content and composition [5,10,11]. Recent research indicates that the SOC mineralization process may be influenced by specific enzymes involved in carbon cycling, such as β -1,4-glucosidase (BG), and cellulase (CEL) [2,12]. Other studies have also shown that the use of fertilizers can cause significant alterations in enzymatic activity and nutrient levels in soil [2,5,13]. However, it is still not fully understood whether the variations in enzyme activity due to the use of organic and inorganic fertilizers are closely connected to changes in soil nutrient concentration in rubber plantations.

China is the sixth-largest natural rubber producer globally, with a total output of 0.65 million metric tons, and Hainan Island is the primary site in China for natural rubber cultivation [14,15]. Crucially, the use of inorganic fertilizers has resulted in significant soil nutrient depletion, reduced soil enzymatic activity, and land degradation in rubber plantations. However, the effects of the partial replacement of inorganic fertilizer with organic fertilizer on soil SOC, nitrogen content, and the enzymes associated with nutrient cycling in rubber plantations on Hainan Island (which is characterized by high annual temperatures and precipitation) is not fully understood. Therefore, this study aimed to (1) elucidate the influence of partial substitution of inorganic fertilizer with organic fertilizer on soil organic carbon (SOC) and nitrogen (N) properties and SOC- and N-converting enzyme activity and (2) examine the relationship between SOC, nitrogen characteristic, and soil enzyme activity. This study findings will enhance rubber plantation management through the optimization of fertilization applications, leading to improved environmental quality and more sustainable agriculture.

2. Materials and Methods

2.1. Study Area Overview

The research site is located at Zhubi farmland, Baisha County, Hainan Island, China (19°23'13.83" N, 109°17'35.82" E, 114 m above sea level) in a rubber plantation field. The climate in this area is characterized as a tropical monsoon climate with a period of rainfall occurring from May to October, followed by a dry period from November to April. The annual average temperature ranges from 21 to 29 °C, with an average rainfall of 1725 mm. As per the USA Soil Classification System, the soil is categorized as Ultisol and is produced from granite brick red soil [16]. The soil composition of the rubber plantation consists of 43.71% sand, 8.28% silt, and 48.01% clay. The basic chemical and physical characteristics are shown in Table 1.

Soil Depth (cm)	0–10	10–20	20-30	30–40
SOC $(g \cdot kg^{-1})$	16.23	15.89	13.66	11.95
TN $(g \cdot kg^{-1})$	0.91	0.61	0.69	0.73
NH_4^+ -N (mg·kg ⁻¹)	10.37	8.56	9.65	7.37
$NO_3^{-}-N$ (mg·kg ⁻¹)	10.10	5.96	4.94	5.16
pH	5.69	5.83	5.78	5.80
BG (nmol·g ^{-1} ·h ^{-1})	138.85	142.69	146.85	139.98
NAG (nmol·g ^{-1} ·h ^{-1})	144.55	136.31	144.16	145.48
LAP (nmol·g ⁻¹ ·h ⁻¹)	162.14	153.92	144.71	139.16

Table 1. Basic soil conditions of different soil layers in rubber plantation.

2.2. Experimental Design

In 2004, a clone PR-107 rubber tree was planted at the research site with a recommended density of 3×7 m, equal to 480 plants per hectare. Six fertilization treatments with three replications were set up in a randomized complete block design for this study: (1) CK: no fertilization as control; (2) NPK: 100% chemical fertilizer application; (3) 25% M: 25% manure, 75% chemical fertilizer; (4) 50% M: 50% manure, 50% chemical fertilizer; (5) 75% M: 75% manure, 25% chemical fertilizer; and (6) M: 100% manure application. There were a total of 18 plots chosen for the fertilization treatments. Each plot had an area of 667 m² and was separated from the others by a 20 m transition zone. The manure comprised cow dung with 5.76 g·kg⁻¹ N, 0.29 g·kg⁻¹ P, and 0.27 g·kg⁻¹ K. Using the local conventional farming practices, we applied 1.0 kg of chemical fertilizer containing 15% N as urea (46% N), 9% P as single superphosphate (12% P₂O₅), and 6% K as potassium chloride (60% K₂O). All treated organic fertilizers were applied at the same time at the beginning of the experiment in April 2021 and all chemical fertilizers were applied in April, June, and September, in accordance with 2:1:1. The total amount of fertilizer applied to each fertilization pit each year is shown in Table 2.

Treatment —	Total Amount of Fertilizer (kg)				
	Urea	Ca(H ₂ PO ₄) ₂ ·H ₂ O	KC1	Organic Fertilizer	
NPK	1.79	1.36	0.18	0.00	
25% manure	1.34	1.27	0.16	35.78	
50% manure	0.90	1.18	0.15	71.57	
75% manure	0.45	1.10	0.13	107.35	
100% manure	0.00	1.01	0.12	143.13	
CK	0.00	0.00	0.00	0.00	

Table 2. Fertilization treatment plan in rubber plantation.

2.3. Soil Sample Collection and Analysis

In April 2022, five samples were collected from each plot and mixed thoroughly to make a composite sample from a depth of 0–10, 10–20, 20–30, and 30–40 cm using an augur (5 cm in diameter). The samples were then transported and stored in the laboratory using ice bags. The soil was air-dried and sieved through 0.149 mm mesh in order to determine SOC and other soil physical and chemical properties. The second part was kept at 4 °C to determine soil moisture content, ammonium nitrogen (NH₄⁺-N), nitrate nitrogen (NO₃⁻-N) content, and soil enzyme activities of β -1,4-glucosidase (BG), N-acetylglucosaminidase (NAG) and L-leucine aminopeptidase (LAP).

The SOC was quantified using the dichromate oxidation method [16]. A pH electrode (PHS-3E, INESA, Shanghai, China) was used to measure the pH by suspension in water with a 1:2.5 ratio. The soil moisture content was determined by the drying method, the total nitrogen content of the soil was extracted by the semi-micro-Kelvin method and determined by a fully automated flow analyzer, and the content of ammonium nitrogen and nitrate nitrogen in the soil were extracted by KCl and determined by automatic flow

analyzer (Proxima1022/1/1, Alians Scientific Instruments, Paris, France) [17]. Using the DeForest (2009) [18,19] method, soil enzyme activity BG, NAG, and LAP were measured fluorometrically in 300 mL 96-well polystyrene microplates.

2.4. Statistical Analysis

The normality and homogeneity of variances were evaluated prior to data analysis using the Shapiro–Wilk test (p > 0.05) and Levene test (p > 0.05), respectively. After fulfilling these two criteria, significant differences in SOC, pH, nitrogen properties, and soil enzyme activity were examined through one-way ANOVA using the SPSS 25 statistical software (IBM Crop., Chicago, IL, USA). The statistical significance was evaluated using Tukey's test with a fixed significance level of p > 0.05. Pearson correlation analysis and Redundancy analysis (RDA) were performed in Origin and R-Studio [20], and all the figures were created using Origin 2021 (Origin Lab. Crop, Vienna, Austria).

3. Results

3.1. Fertilization Influence on Soil Physiochemical Characteristics

In the rubber plantation, there were significant variations in soil physicochemical characteristics among the different fertilization applications (Figure 1). The SOC exhibited a notable difference. The highest SOC occurred for 100% manure, followed by 75% manure, and then 50% manure, for all depths (Figure 1a). For example, the higher SOC for 100% manure displayed as 14.96, 11.36, 11.14, and 11.05 g kg⁻¹ at 0–10, 10–20, 20–30, and 30–40 cm, respectively. The lower SOC of CK ranged from 8.04 to 13.55 $g \cdot kg^{-1}$ over all four depths. The differences in SOC also depended on the soil depth, and the most evident differences of SOC among fertilization treatments occurred at 20–30 cm depth. In addition, there were notable differences in soil total nitrogen (TN) between fertilization treatments, as shown in Figure 1b. The 100% M-treated plot displayed the highest TN, followed by 75% M and 50% M, at all depths. For example, the TN of 100% M treatment varied between 0.86 and 1.02 g·kg⁻¹, 75% M varied between 0.79 and 1.01 g·kg⁻¹, 50% M varied between 0.74 and 1.06 g·kg⁻¹, 25% M varied between 0.71 and 1.05 g·kg⁻¹, NPK varied between 0.68 and 1.02 g·kg⁻¹, and CK varied between 0.65 and 1.02 g·kg⁻¹. The TN among fertilization treatments also depended on soil depth; the most visible difference among fertilization treatments occurred in 20–30 and 30–40 cm depths. There was also a significant difference in C:N, although there was no significant difference in pH (Figure 1c,d).

Various levels of replacement of inorganic fertilizer with organic fertilizer resulted in contrasting soil nitrogen mineralization at different soil depths. (Figure 2). The soil mineral nitrogen (N) was significantly different from the higher N observed in NPK treatment, followed by 25% M and then CK, at all depths (Figure 2a). For example, the lower mineral nitrogen of 100% M was found to be 19.95 mg·kg⁻¹ at 0–10 cm, 16.49 mg·kg⁻¹ at 10–20 cm, 13.25 mg·kg⁻¹ at 20–30 cm, and 12.01 mg·kg⁻¹ at 30–40 cm. The higher N of NPK treatment ranged from 13.4 to 23.09 mg·kg⁻¹. The most significant differences in N content with fertilizer application were observed at depths of 10–20 and 20–30 cm. There were also significant differences in NH⁺₄-N and NO⁻₃-N between fertilization applications, as shown in Figure 2b,c. Unlike mineral nitrogen order among fertilization applications, the 100% M, 75% M, 50% M, and 25% M had higher NH⁺₄-N than NPK and CK at all depths (Figure 2b). There were also significant differences in NO⁻₃-N among fertilization treatments (Figure 2c). For example, the NO⁻₃-N of 100% M treatment ranged from 5.16 to 10.1 mg·kg⁻¹, 75% M ranged from 5.41 to 11.15 mg·kg⁻¹, 50% M ranged from 4.18 to 11.06 mg·kg⁻¹, 25% M ranged from 3.97 to 9.44 mg·kg⁻¹, NPK ranged from 3.81 to 8.84 mg·kg⁻¹, and CK ranged from 2.46 to 6.2 g·kg⁻¹.



Figure 1. Partial substitution of inorganic fertilizer with organic fertilizer: effect on (**a**) soil organic carbon, (**b**), soil total nitrogen, (**c**) C:N, and (**d**) soil pH under rubber plantation.



Figure 2. Partial substitution of inorganic fertilizer with organic fertilizer: effect on (**a**) soil mineral nitrogen, (**b**) ammonium nitrogen, and (**c**) nitrate nitrogen under rubber plantation.

3.2. Fertilization Effect on Soil Enzyme Activity

The activities of soil carbon and nitrogen-converting enzymes were significantly different between fertilization treatments (Figure 3). The soil enzyme BG was significantly different among fertilization treatments. The highest level was recorded for 75% manure, followed by 50% manure, and the lowest for NPK and CK treatments at all depths (Figure 3a). The difference in BG enzyme also depended on soil depth. For example, at 0–10 cm depth, among fertilization treatments, it followed the order 50% manure (143.17) > 25% manure (140.30) > 75% manure (138.85) > 100% manure (138.27) > NPK (143.17) > CK (125.82 nmol·g⁻¹.h⁻¹). Similarly, the soil enzyme NAG was significantly different. The highest NAG was observed for 75% manure, followed by 50% manure, 25% manure, 100% manure, NPK, and then CK (Figure 3b). The differences in NAG also depended on the soil depth, and the most evident differences in NAG among fertilization treatments occurred at 0–10 cm depth, following the order 50% manure > 25% manure > 75% manure > 100% manure > NPK > CK. There were also significant differences in LAP enzyme activity among fertilization treatments (Figure 3c). The LAP enzyme showed a consistent trend in the other soil layers.



Figure 3. Partial substitution of inorganic fertilizer with organic fertilizer: effect on soil enzyme activities of (**a**) β -1,4-glucosidase (BG), (**b**), acetylglucosaminidase (NAG), and (**c**) L-leucine aminopeptidase (LAP) under rubber plantation.

3.3. Relationship between Soil Physiochemical Characteristics and Soil Enzyme Activity

The Pearson correlation analysis further indicated the fertilization effect on soil physiochemical characteristics, soil carbon, nitrogen content, and soil enzyme activity at various soil depths (Figure 4). For example, at 0–10 cm depth, the SOC was significantly positively correlated with C:N and pH, while negatively correlated with mineral nitrogen, nitrate nitrogen, and enzyme activity (BG, NAG, and LAP). Redundancy analysis (RDA) was performed using carbon and nitrogen converting enzymes (BG, NAG, and LAP) as dependent variables and soil physicochemical properties (SOC, TN, N, C:N, pH, NH₄⁺-N, and NO₃⁻-N) as independent variables (Figure 5). For example, in the 0–10 cm soil layer, after the partial substitution of inorganic fertilizer with organic fertilizer, the influence of soil physicochemical properties on three soil activities followed an order NO₃⁻-N > NH₄⁺-N > pH > C:N > SOC > AN > TN. RDA-1 explained 92.29% and RDA-2 explained 5.48% of the total variations (Figure 5a). The same trends can be observed for other soil layers.







Figure 5. Redundancy analysis (RDA) showing effects of soil carbon and soil nitrogen on soil enzyme activities under partial substitution of inorganic fertilizer with organic fertilizer. Red solid arrows indicate the significant effects of explanatory variables on response variables (blue arrows).

4. Discussion

4.1. Partial Substitution of Inorganic Fertilizer with Organic Fertilizer: Impact on Soil Physicochemical Characteristic

The implementation of management practices can have a substantial influence on the physical and chemical characteristics of soil and significantly influence its overall quality [21]. Our study revealed that partially replacing inorganic fertilizer with organic fertilizer makes it possible to maintain or even enhance the soil C content compared to both the sole use of inorganic fertilizer and control. Previous studies have shown that balanced fertilization is a sustainable strategy for preserving soil organic carbon (SOC) content [5,20]. Using organic amendments can improve soil nutrient availability and increase organic carbon content. Moreover, they can enhance soil structure, decrease soil erosion, and lower nutrient losses through runoff [22]. Furthermore, the use of both organic and inorganic fertilizers resulted in an increase in the nitrogen content of the soil [23,24]. In this study, soil pH was slightly increased by partially replacing inorganic fertilizer with organic fertilizer because the organic fertilizers contain manure, which may increase the soil pH due to the alkaline nature of manure [25]. The correlation analysis and redundancy analysis (RDA) also showed that SOC had a positive correlation with soil pH (Figure 4) and that pH plays a significant role in organic and inorganic fertilizer application.

Furthermore, this study indicates that substituting inorganic fertilizer with organic fertilizer can significantly increase soil TN and N concentrations. Hence, in comparison to inorganic fertilizer, organic fertilizers promote nitrogen accumulation in the soil and improve its capacity for nitrogen storage [5,23,25]. Fertilization had a significant impact on the C:N ratio in this study, consistent with prior research indicating that applying urea to soil with a high C:N ratio can reduce the retention of plant litter as soil organic carbon (SOC) and result in the depletion of existing SOC [26,27]. The soil with a high C:N ratio (<25) signifies that organic matter accumulates in the soil faster than it is being decomposed. However, in the current study, the soil C:N ratio varied between 12 and 15.7, suggesting that the soil organic matter has thoroughly degraded [5]. In addition, the partial replacement of conventional N fertilizers with organic fertilizer increased the content of available nitrogen, nitrate, and ammonia in this study. Firstly, organic fertilizers (such as manure and compost) containing organic matter serving as a substrate for microbial activity contribute to increased nitrogen availability in soil by releasing NH⁺₄-N during microbial decomposition, which undergoes a nitrification process to convert into $NO_{3}^{-}N$, thereby increasing the availability of both forms of nitrogen in the soil [28,29]. Secondly, due to extreme weather conditions (high temperatures and rainfall) in the Hainan region of southern China, nitrogen undergoes rapid conversion in the soil, resulting in the loss of NH_4^+ and NO_3^- through ammonia volatilization and nitrate leaching [2]. Thirdly, organic fertilizers lead to enhanced soil structure and water retention capacity, which might create a condition that is favorable for nitrification processes and promote the accumulation of nitrate and ammonia ions in the soil [29].

4.2. Partial Substitution of Inorganic Fertilizer with Organic Fertilizer: Impact on Soil Enzyme Activity

The study of soil nitrogen and carbon-converting enzymes and their changes under the partial substitution of inorganic fertilizer with organic fertilizer is essential for understanding soil health and nutrient flow in agroecosystems. According to the findings of this study, the partial substitution of inorganic fertilizer with organic fertilizer significantly increased the soil enzyme activity relative to sole NPK and CK (Figure 3), consistent with the previous study [2,5]. Organic fertilizers contribute to improved plant growth and development by improving root biomass and exudates, which in turn enhance soil enzyme activity [6]. Organic fertilizers can help adjust the C:N ratio to provide a more suitable environment for microorganisms. Nevertheless, organic fertilizers have a more significant impact on soil extracellular enzyme activity. This is probably due to exogenous organic materials stimulating C-related enzymes that speed up the degradation of cellulose, hemicellulose, and lignin [30]. Organic fertilizers can supply soil enzyme substrates, leading to increased enzyme activity and providing protection against carbon and nitrogen depletion [27].

Soil pH influences enzyme activity and biosynthesis by altering microbial composition and nutrient availability [31]. Our study revealed a substantial positive correlation between soil pH and all soil enzymes in the surface soil (Figure 4). Partially substituting inorganic fertilizer with organic fertilizer may have caused an elevation in soil pH in the acidic tropical soil, thereby increasing BG, NAG, and LAP activities in the soil [2,31]. Furthermore, the partial replacement of inorganic fertilizer with organic fertilizer can enhance the synthesis of enzymes that degrade carbon and acquire nitrogen compared to sole nitrogen fertilizer. The soil enzymes BG, NAG, and LAP exhibited a strong positive relationship with SOC and TN, indicating their essential function in breaking down proteins [13,32]. Considering that proteins make up 60% of the total nitrogen in plant and microbial cells, LAP may be the critical factor in nitrogen mineralization [33].

5. Conclusions

Our study explored the influence of the partial substitution of inorganic fertilizer with organic fertilizer on soil physicochemical characteristics (SOC and nitrogen concentrations) and soil enzyme activity under natural rubber plantations. The results clearly indicate that utilizing organic fertilizer to partially replace chemical fertilizer has a significant impact on the nutrient content. The influence is primarily achieved through alteration in SOC and nitrogen concentrations, nitrogen mineralization, and soil enzyme activity. Notably, at 0–10 cm depth, the 50% manure and 75% manure treatments significantly increased the SOC (7.15% and 3.46%, respectively), total nitrogen (3.92% and 0.98%, respectively), and mineral nitrogen (6.46% and 11.49%, respectively), as well as soil enzyme activity—BG (18.55%) and 27.33%, respectively), NAG (11.16% and 26.54%, respectively), and LAP (17.83% and 18.82%, respectively) compared to CK. The results showed that in a rubber plantation on Hainan Island, replacing inorganic fertilizer with organic fertilizer could help preserve soil organic carbon stability, nitrogen concentration, and enzyme activity. We recommend that the use of 50% or 75% organic fertilizer with inorganic fertilizer could be an optimal strategy to enhance nutrient availability for sustainable agricultural production on Hainan Island. Therefore, more attention should be paid to the dynamics of soil nutrient and enzyme activities in rubber plantation soil profiles under different climatic conditions after the co-applications of inorganic fertilizer and manure.

Author Contributions: Q.Y.: writing—original draft, data curation; J.L.: writing—original draft, data curation; W.X.: data curation; J.W.: data curation; Y.J.: data curation, writing—review and editing; W.A.: writing—review & editing, supervision, methodology, funding acquisition, conceptualization, data curation; W.L.: writing—review and editing, supervision, methodology, funding acquisition, conceptualization, conceptualization. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Key Research and Development Project of Hainan Province (No. ZDYF2022XDNY181), the National Natural Science Foundation of China (No. 42367034 and 32160291), and the National Key Research and Development Program of China (No. 2018YFD0201105).

Data Availability Statement: The data presented in this study are available upon request to the corresponding author.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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