RESEARCH ARTICLE

Effect of continuous application of bioorganic fertilizer on tobacco rhizosphere microecosystems

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Received: December 1, 2022; **accepted:** January 30, 2023.

To encourage the use of biological organic fertilizer in tobacco planting, a three-year field experiment was undertaken to examine the impact of a single kind of biological organic fertilizer on the microecosystems of tobacco rhizosphere. The soil physicochemical index, trace element index, and enzyme and microbial amount index were used to quantify the impact. Compared to traditional fertilization, biological organic fertilizer increased the level of organic matter, alkali-hydro nitrogen, and accessible phosphorus in all physical and chemical soil indexes, whereas the pH remained the same and the rapidly available potassium content decreased somewhat. In the soil trace element indexes, the contents of Mg, Cu, Mn, and Fe were increased annually by 4.53% to 9.42%, 1.45% to 14.12%, 5.22% to 11.07%, and 5.34% to 16.98%, respectively. In the soil enzyme indexes, the activity of urease, catalase, and sucrose invertase were increased, while acid phosphatase activity was decreased. Significant increases were observed in soil microbial carbon (MBC), soil microbial nitrogen (MBN), and soil microbiological phosphorus (MBP) in soil microbial biomass indexes. According to principal component analysis, the contribution rates of the aforementioned indexes to the micro-ecosystem of soil applying biological organic fertilizer were as follows: urease > organic matter > MBN > MBC > Mg > Cu > catalase > Mn > alkali-hydro nitrogen > invertase > Fe > acid phosphatase > rapidly available potassium (K) > available phosphorus (P) > pH > MBP. This study provided a significant scientific approach and theoretical foundation for the advancement of bio-organic fertilizer in the future.

Keywords: bioorganic fertilizer; soil microecosystem; soil properties; principal component analysis; continuous fertilization.

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Introduction

In pursuit of excessive economic rewards, the agricultural business has a tendency to boost crop output *via* higher fertilizer application and continuous cropping. This results in significant soil deterioration, a loss in crop quality, and crop damage from pests and diseases [1, 2]. To address these issues, investigations have been done on a global scale under the premise that soil deterioration is directly related to an imbalance in soil microecosystems [3, 4]. As a substitute for and supplement of chemical fertilizer, organic fertilizers provide nutrients for crop growth with a large number of organic matters and valuable trace elements vital for crops. Beyond that, organic fertilizers also enhance microorganisms' activities in the soil, thereby, accelerating the decomposition of nutrient elements and promoting plant growth [2]. Based on a previous study on tobacco bacterial wilt, the results demonstrated that bioorganic fertilizer might be used to enhance soil qualities, therefore, successfully avoiding and treating the illness and increasing tobacco yield and quality [5]. This strategy is thus of significant benefit and should be widely used. However, the effect of bioorganic fertilizer on the microecosystems of tobacco rhizosphere and the prevention of tobacco plant diseases have not been investigated yet.

In 2000, Nature magazine ran a cover story headlined "Ecology Goes Underground" [6], emphasizing that the ecology of the soil had become an active area of study. Soil microecosystems, an area of ecology that was created relatively late, is still in its infancy [7]. In ecology, microecosystems are described as the connection between normal microbiota and the host [8]. However, soil microorganisms are distinguished by tremendous quantity and complicated functioning and are evocatively referred to as "the dark matter of the earth" [9]. Even with the growing sophistication of sequencing technology, descriptive research on soil microorganisms is limited [10]. Relatively little is known about their function in soil microecosystems. A soil microecosystem is described as the ecological system in which plants, soil, microbes, and ambient circumstances interact. A soil microecosystem not only participates in the transfer, transformation, and exchange of matter and energy, but also resists external coercion *via* intricate interactions, hence encouraging plant growth [11, 12]. Changes in the physicochemical characteristics, biological properties, and microflora structure of soils owing to the interaction between plants, soils, and microbial systems have been the primary focuses of research on soil microecosystems [13, 14].

Numerous studies on a global scale have examined the impact of newly created bioorganic fertilizer in preventing and treating plant illnesses, boosting plant development, and enhancing plant quality [15, 16]. Furthermore, a number of research have evaluated the effects of bioorganic fertilizer on soil characteristics and microbiotas [17]. However, there are few research on the effect of continual bioorganic fertilizer application over the years on the microecosystems of plant rhizosphere. In this particular study, bioorganic fertilizer was administered to the same land for three consecutive years to determine the impact of bioorganic fertilizer on tobacco rhizosphere microecosystems. The purpose of this research was to give scientific evidence for altering fertilizer application and agricultural management practices in the event that bioorganic fertilizer becomes widespread in the future.

Materials and methods

Experiment location, soil conditions, and fertilizers

The location of the experimental field is Chengjiao Town, Puchen City, Fujian Province, China (117°51′ East, 27°30′ North). This area has a subtropical humid climate, ample rainfall (1,800 mm annually on average), abundant sunlight, and an annual average temperature of 18°C. The soil covering the experimental field has a light brown hue. The research period covered three consecutive years (2019, 2020, 2021) from February to July in each year. Two treatments were included in the study including conventional fertilization and bioorganic fertilization. The first trial performed in 2019 consisted of two randomized whole block treatments (RCBD). Each therapy was administered five times. There was a total of 10 experimental blocks, each measuring 132 m^2 (22 m × 5 m, with the guard rows of 3.6 m wide). A total of 220 tobacco plants (variety K326) were used in this study. Each block's method of fertilization was fixed. Prior to the experiment, soil was taken from the arable layer (depth: 5-20 cm) to establish its physicochemical baselines as the follows: pH was 5.13, the concentrations of organic matter, alkali-hydrolysable nitrogen (alkaline N), available phosphorus (P), and available potassium (K) were 28.15 g/kg, 90.29 mg/kg, 41.03 mg/kg, and 160.32 mg/kg, respectively. The particular fertilizer was supplied by Jinming Agricultural Supplies Co., Ltd. (Fuzhou City, Fujian, China) with the ratio of nitrogen, phosphorus, and potassium as 12.5:8.0:22.5 (w/w). The organic fertilizer was supplied by Xin-Shuguang Agricultural Development Co., Ltd. (Nanping City, Fujian, China), which was made by co-composting amino acid and cow manure at a ratio of 3:1 (w/w). During the bioorganic fertilizer composting process, the antagonistic microbe *Bacillus cereus* QJ-1 (China Center for Type Culture Collection, No. M2012271) that inhibits bacterial wilt was fed to the compost while the pile temperature was below 40°C. The ultimate concentration of viable microorganisms was at least 10^8 CFU/g.

Soil treatments and sample collection

The following treatments were applied in the experimental blocks.

(1) Conventional fertilization (C):

30 kg of special fertilizer, 25 kg of potassium nitrate (KNO_3), 1 kg of borax, and 120 kg of organic fertilizer were applied on the area of 667 m^2. The conventional fertilization in 2019, 2020, and 2021 were referred as C1, C2, and C3, respectively.

(2) bioorganic fertilizer (T):

The application of bioorganic fertilizer was conducted by applying 30 kg of special fertilizer, 25 kg of KNO_3, 1 kg of borax, and 120 kg of bioorganic fertilizer on the area of 667 m^2. The application of bioorganic fertilizer in 2019, 2020, and 2021 were referred as T1, T2, and T3, respectively.

The fertilizer was applied before sowing the tobacco plants and was administered in layers. The field management adhered to Shaowu City's technical standards for the cultivation of high-quality tobacco. Every year, soil samples were obtained during the robust development phase (65 days following seedling implantation). Briefly, two vigorously growing tobacco plants were selected at random from each treatment block. The topsoil of the tobacco rhizosphere was removed, and the tobacco plants were uprooted. The peripheral soil of the tobacco rhizosphere was shaken off, and the roots were cut 5 to 20 cm away from the root ends. Finally, the rhizosphere soil was collected.

Determination of the physicochemical properties of soil

The pH of the soil was evaluated by using LA-pH10 soil pH and moisture tester (Hach, Loveland, Colorado, USA). The contents of organic matter were determined by following the standard method recommended by the Ministry of Rural Agriculture of China (NY/T 1121.6-2006). Briefly, under heating conditions, the excessive potassium dichromate sulfuric acid solution was used to oxidize soil organic carbon. The surplus potassium dichromate was titrated with ferrous sulfate standard solution. The concentration of alkaline N was measured by using standard method recommended by the Ministry of Rural Agriculture of China (LY/T 1229-1999). The soil was treated with 1.0 mol/L NaOH to convert alkaline N into NH_3, which was absorbed by H_2BO_3 after diffusion, and then, was titrated by standard acid solution to calculate the contents of alkaline N in the soil. The concentration of available P was determined according to standard method recommended by the Ministry of Rural Agriculture of China (NY/T 1121.7-2006) by using acid ammonium fluoride and colorimetric method. The concentration of available K was determined by following standard method recommended by the Ministry of Rural Agriculture of China (NY/T 889-2004) through atomic absorption spectrophotometer.

Determination of the trace elements in soil

Trace element concentrations in soil were determined by using Shimadzu AA-680 atomic absorption spectrometry (Shimadzu, Kyoto, Japan) equipped with a hollow cathode lamp and a deuterium background corrector at respective wavelengths for exchangeable magnesium, diethylenetriamine pentaacetate (DTPA), acidtriethanolamine, available copper, ammonium acetate, available manganese, and available iron [18]. All experiments were performed triplicate and average of the results was reported.

Determination of soil enzymes

5 g of air-dried soil was used for the development of standard curve line. The concentrations of urease, invertase, acid phosphatase, and catalase were measured by using colorimetry [19], 3,5-dinitrosalicylic acid colorimetry [20], the nitrophenylcarbimide sodium phosphate method [19], and UV spectrophotometry [21], respectively.

Determination of soil microbial biomass

After fumigating the fresh soil sample with chloroform for 24 h, the dead cells of soil microorganisms were cracked, releasing microbial biomass carbon. A certain volume of 0.5 mol/L K_2SO_4 solution was added to the soil. Shimadzu TOC-500 total organic carbon analyzer (Shimadzu, Kyoto, Japan) was used to measure soil microbial biomass carbon (MBC) and chloroform fumigation-K_2SO_4 extraction. A FUTURA continuous flow analyzer (AMS-Alliance, Villeneuve-la-Garenne, France) were used to measure soil microbial biomass nitrogen (MBN) and chloroform fumigation-sodium bicarbonate extraction. The soil microbial biomass phosphorus (MBP) was measured by using colorimetry [22].

Statistical analysis

The data were calculated and statistically analyzed using Microsoft Excel and SPSS (version 15.0) (IBM, Armonk, New York, USA). The statistical significance of the results was determined by performing Fisher's protected least significant difference (LSD) test ($P < 0.05$).

Results and discussion

Effect of treatments on the physicochemical characteristics of soils

The physicochemical parameters of the soils exposed to treatments C and T were shown in Table 1. The results showed considerable improvement of soil physicochemical parameters comparing to that before treatments. The size and pattern of the improvement varied during the three years of the research period. The pH values of the soils under treatments C and T maintained roughly 5.55 and fluctuated minimally. Evidently, soil pH could only provide a fine adjustment (within a very narrow range) and a buffering effect [23]. The content of organic matter in soils under the treatments C and T increased continuously, where C3 treatment was 15.8% more than that of C1 treatment, while T3 treatment was 10.7% greater than that of T1 treatment. However, the concentration of soil organic matter in treatment T rose less than that in treatment C, although it was consistently greater than that in treatment C. A bioorganic fertilizer increases the soil's organic matter content and its availability of nutrients. The findings of this study were consistent with the other studies [24, 25]. It is evident that the content of nutrients in the soil increased as a result of the use of bioorganic fertilizer. As well, large quantities of microorganisms contained in bioorganic fertilizers can decompose and release nutrients into the soil [26], which are then absorbed and utilized by plants. In the present study, the content of available K decreased after continuous application of the bioorganic fertilizer, which was contrast to the results from Tian, *et al*. [27] and might be caused by the difference in the plants, soils, and bioorganic fertilizers. The content of alkaline N in the soils rose constantly in both C and T treatments with the treatment C substantially greater than treatment T. During the third year of the experiment, the content of alkaline N in the soil under treatment C3 was comparable to that under treatment T3 (142.20 mg/kg). Treatment C2 had the greatest concentration of accessible P in the soil (60.43 mg/kg) among treatments C1, C2, and C3. Treatment T1 had the greatest concentration of accessible P in the soil (69.79 mg/kg) comparing to treatments T2 and T3. The concentration of accessible K in the soil continued to grow under treatment C, reaching a high of 386.96 mg/kg in the third year. During the

Table 1. Effects of interventions on physicochemical soil properties.

	pH	Organic Matter (g/kg)	Alkaline N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)
C1	5.55 ± 0.02	35.19 ± 2.24	119.18 ± 7.31	49.59 ± 3.37	336.65 ± 16.10
C2	5.56 ± 0.03	39.61 ± 3.08	133.42 ± 8.04	60.43 ± 3.96	361.84 ± 18.11
C3	5.53 ± 0.05	40.75 ± 3.59	140.16 ± 9.66	47.18 ± 2.77	386.96 ± 20.47
T1	5.54 ± 0.04	40.60 ± 3.72	132.61 ± 8.26	69.79 ± 4.72	373.99 ± 21.71
T2	5.53 ± 0.03	42.24 ± 3.81	140.84 ± 8.35	64.65 ± 4.80	360.58 ± 18.64
T3	5.53 ± 0.03	44.95 ± 3.47	142.20 ± 9.27	65.36 ± 5.21	343.23 ± 17.61

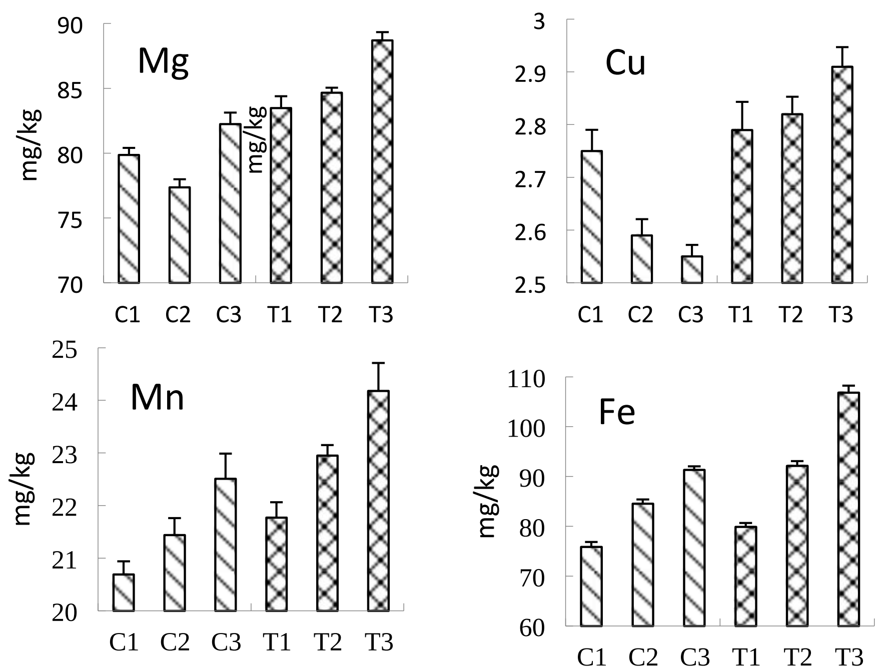

Figure 1. The effects of soil treatments on trace elements.

T3 period, the concentration of accessible K continued to decline to a minimum of 343.23 mg/kg. It was noteworthy that continuous application of the bioorganic fertilizer used in this study to a farmland might continuously result in a decrease of available K. This will adversely affect the quality of tobacco leaves and may even restrict their growth. This necessitates the application of additional potassic fertilizer.

Effect of the treatments on soil trace elements

Trace elements in the soil may affect the essential metabolic processes and enzyme activity of plants. Moreover, they affect soil fertility and nutrient absorption, and play a crucial role in the establishment of microecosystems in the rhizosphere [23]. Continual application of bioorganic fertilizer raised the concentration of trace elements including Mg, Cu, Mn, and Fe, in the tobacco plant's rhizosphere (Figure 1). During the three-year period, the treated soil had a greater concentration of the four types of trace elements than that in the control soil. The content of Mg, Cu, Mn, and Fe in the treated soil rose from 4.53% to 9.42%, 1.45% to 14.12%, 5.22% to 11.07%, and 5.34% to 16.98%, respectively, as compared to that of the control. The Mg content in the soil under treatment C first fell, and then, began to rise. The concentration of soil Mg in C3 was 2.98% more than that in C1. The concentration of soil Mg was increased during treatment T with the T3 being 6.25% greater than that in T1. Under treatment C, the concentration of Cu in the soil decreased with C3 treatment being 7.84% lower than that in treatment C1. Under the T treatment, the content of Cu in the soil increased. Specifically, the Cu content in the soil under treatment T3 was 4.30% greater than that under treatment T1. The contents of Mn in soils were both increased in the treatments C and T, with the treatment C3 being 8.79% greater than that under treatment C1. On the other hand, the Mn concentration in the soil under treatment T3 was 11.07% higher than that under treatment T1. The content of soil Fe was also increase in both treatment groups, while the treatment C3 was 20.39% greater than that under treatment C1, and treatment T3 was 33.70% higher than that under treatment T1. The results suggested that continuous application of bioorganic fertilizer to the soil increased the concentrations of trace elements such as Mg, Cu, Mn, and Fe. There was no special index created to evaluate trace elements in local soils. Therefore, it was not possible to verify whether the concentrations of Mg, Cu, Mn, and Fe were adequate. The concentration of trace elements in soil under continuous application of bioorganic fertilizer was higher than that in soil under conventional fertilization. There is a direct correlation between the trace elements in the soil and the growth and quality of tobacco plants. In addition, trace elements have a synergetic or antagonistic effect on soil nutrient absorption. Particularly, if the concentration of trace elements in the soil increases significantly, the synergetic or antagonistic effect may increase. Thus, if bioorganic fertilizer is continuously applied to a tobacco field for an extended period of time, it is recommended to reduce the application of trace elements moderately. However, this needs to be further investigated.

Effect of the treatments on the enzyme activity of the soil

The soil enzyme activity directly affects the conversion and synthesis process of organic materials in the soil [29]. It also affects the development and growth of plants. By influencing biomass, plants, and soil microorganisms, soil nutrients can affect the distribution of roots in the soil, thereby influencing the secretion of soil enzymes [30]. The catalase activities in soil treatments C1, C2, and C3 were 5.56 mg/g, 5.93 mg/g, and 5.44 mg/g, respectively, with an initial rise followed by a decline. The catalase activities in soil treatments T1, T2, and T3 rose consistently to 5.63 mg/g, 6.18 mg/g, and 6.35 mg/g, respectively (Figure 2). The findings indicated that continual application of bioorganic fertilizer improved catalase activity, facilitated catalase breakdown, and lowered catalase toxicity. The urease activities in both soil treatments C and T rose constantly. The activity of urease in soil treatment C3 was 16.00% more than that in soil treatment C1. In addition, the activity of urease in the treatment T3 was 36.67% more than that in the treatment T1 and was 41.38% greater than that in treatment C3. These findings indicated that continual application of bioorganic fertilizer promoted urease activity in the soil much more than conventional fertilizer (Figure 2).

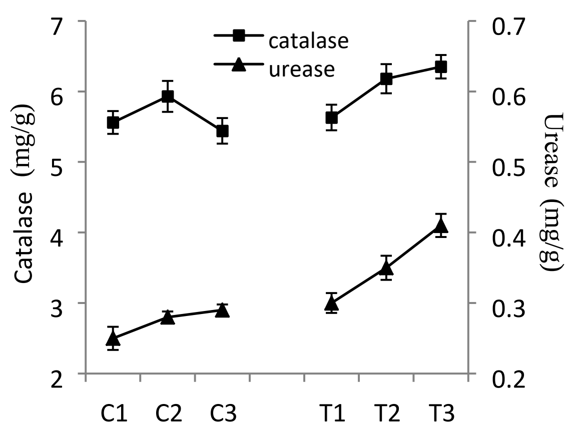

Figure 2. The effects of the treatments on soil catalase and urease activities.

The impacts of conventional fertilization and bioorganic fertilizer on the activities of acid phosphatase and invertase in the soil were shown in Figure 3. The activity of acid phosphatase in the soil treated with C first rose and then began to decline. The soils treated with

C1, C2, and C3 had acid phosphatase activities of 47.16 mg/g, 63.76 mg/g, and 58.04 mg/g, respectively. Under the treatment T, the activity of acid phosphatase in the soil reduced. The activities of acid phosphatase in the soil were 70.41 mg/g, 63.76 mg/g, and 58.04 mg/g under the treatments T1, T2, and T3, respectively. The administration of bioorganic fertilizer greatly boosted the activity of acid phosphatase. However, with continuing application of bioorganic fertilizer, the activity of acid phosphatase reduced somewhat. Under treatment C, there was a drop in the soil's invertase activity. Although the activity of invertase in the soil under treatment C2 was not considerably lower than that in the soil under treatment C1, the activity of invertase in the soil under treatment C3 declined dramatically by 27.07% comparing to that in the treatment C1. On the other hand, increased invertase activity in soil treated to the T treatment was observed. The activity of invertase in soil treated with T3 was 14.88% more than that in soil treated with T1 (Figure 3). The findings suggested that continual application of bioorganic fertilizer promoted invertase activity.

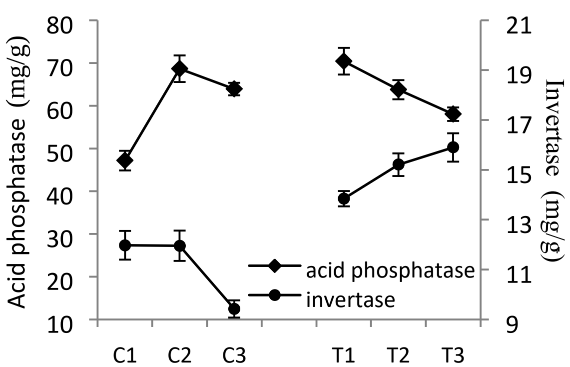

Figure 3. The effects of the treatments on the activities of acid phosphatase and invertase in the soil.

Bioorganic fertilizers have a considerable impact on soil enzyme activity. Zheng, *et al*. investigated the impact of biogas plant liquid fertilizer on soil enzyme activity [31]. The findings demonstrated that the activity of urease increased as the nitrogen content in liquid fertilizer rose. Li, *et al*. investigated the impact of the combined application of several kinds of bioorganic fertilizer and tobacco-specific chemical fertilizer on the enzyme activity of tobacco rhizosphere [32]. The data demonstrated that the application of bioorganic fertilizer greatly improved the soil enzyme activity. The application of bioorganic fertilizer boosted the activities of catalase and invertase, while decreasing the activity of acid phosphatase. Geng, *et al*. investigated the relationship between soil phosphatase activity and organic phosphorus fraction and concluded that acid phosphatase activity was significantly correlated with the concentration and fractions of organic carbon, nitrogen, and organophosphorus in the soil, as well as the pH of the soil [33]. Tao, *et al*. analyzed the changes in soil enzyme activity after the application of organic fertilizer as opposed to chemical fertilizer [34]. They hypothesized that the microbial biomass in the soil was directly proportional to the activity of soil enzymes, as soil enzymes were primarily created by the secretory action of microorganisms and plant roots. The application of bioorganic fertilizer alters not only the nutrient content of the soil, but also its microbial composition and biomass, thereby, influencing the activity of soil enzymes.

Impact of the treatments on the microbial biomass of the soil

Soil microbial biomass refers to the entire biomass of deactivated plants in the soil, comprising bacteria, fungus, algae, and protozoa, with an *in vitro* volume of less than 5×10^3 μm^3. The soil microbial biomass reflects the condition and functional changes of the soil microflora and represents the active portion of soil nutrients. Typically, it is used to assess the biological characteristics of soil [35]. In general, soil microbial biomass is divided into microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and microbial biomass phosphorus (MBP) categories. Table 2 showed that both conventional and bioorganic fertilization raised MBC, MBN, and MBP concentrations. Moreover, their attentiveness

Table 2. Treatment effects on the concentrations of MBC, MBN, and MBP.

	MBC (mg/kg)	MBN (mg/kg)	MBP (mg/kg)
C1	235.61 ± 9.72	40.33 ± 2.85	9.87 ± 0.63
C2	283.49 ± 10.56	44.67 ± 3.42	10.32 ± 1.06
C3	309.51 ± 10.83	46.10 ± 3.01	10.97 ± 0.90
T1	413.02 ± 14.51	53.86 ± 3.67	10.48 ± 0.81
T2	440.49 ± 14.87	54.04 ± 3.13	12.62 ± 1.68
T3	452.66 ± 18.19	56.45 ± 3.37	13.90 ± 1.62

continued to improve throughout the duration of the trial. The results were similar to the observations reported by Si, *et al*. [35] and Cao, *et al*. [36]. Carbon content of microbial biomass is an essential indicator of the magnitude of soil microbiota. After continual fertilizing, the concentration of MBC in soil treated with C3 was 31.36% more than that of soil treated with C1. In addition, MBC concentration in the soil under treatment T3 was 9.60% higher than that in the soil under treatment T1, and MBC concentration in the soil under treatment T was always greater than that in the soil under treatment C. Microbial biomass nitrogen is the total of soil organisms' *in vivo* nitrogen and is the most active organic nitrogen in the soil. After continual fertilization, the concentration of MBN in soil treated with C3 was 14.31% more than that of soil treated with C1. In addition, the concentration of MBN in the soil under treatment T3 was 4.81% greater than that in the soil under treatment T1, and it was 22.45% higher than that in the soil under treatment C3. Mineralizable organophosphorus, such as nucleic acid and phospholipid, and inorganic phosphorus were the primary constituents of MBP. The content of MBP in soil treatment C did not rise considerably despite constant fertilizing with the content of MBP in the soil treated with C3 was only 11.14% greater than that treated with C1. Nevertheless, the concentration of MBP in the soil under treatment T grew considerably, and the concentration of MBP in the soil during treatment T3 was 32.63% more than that in the soil under treatment T1. The use of bioorganic fertilizer considerably raised the concentration of MBC and MBN comparing to conventional fertilization. This is consistent with the results of Chen, *et al* [37]. With the continuing application of bioorganic fertilizer, the concentrations of MBC and MBN grew less than it would with conventional fertilization. This might be due to the fact that the application of bioorganic fertilizer resulted in a high concentration of soil nutrients and microorganism consumption saturation. The microflora structure and microbial biomass were thus typically constant. However, the concentration of MBP was much higher than that under conventional fertilization. This might be attributable to the capacity of soil microorganisms to ingest more accessible P following bioorganic fertilizer application.

Microecological indexes principal component analysis

The principal component analysis (PCA) is commonly employed to assess soil systems. It is widely believed that PCA can reduce errors caused by autocorrelation between variables, develop uncorrelated principal components, determine the score of each principal component, and provide the overall score through calculations, thereby, accurately evaluate soil systems [26]. Table 3 displays the findings of PCA on the microecological indexes of C and T soil treatments. In treatment C, four main principal components (PC) accounted for 83.799% of the experimental data provided a cumulative contribution of at least 80% and were used to analyze the data for the first 16 indexes. PC1 showed the largest variance contribution (37.955%) and was most strongly related to

Table 3. The results of the principal component analysis (PCA) of the microecological indexes.

Index	Treatment C				Treatment T			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
pH	0.386	0.119	0.126	**0.834**	0.514	-0.294	-0.541	0.303
Organic matter	**0.636**	0.113	0.534	-0.436	0.180	**0.762**	0.395	0.351
Alkaline N	**0.870**	0.096	0.332	0.064	-0.073	**0.803**	0.004	0.539
Available P	0.346	0.253	**0.853**	0.230	0.496	**-0.697**	0.370	0.163
Available K	**0.955**	0.116	0.172	0.006	0.512	**-0.660**	0.445	0.102
Mg	0.492	**0.756**	-0.029	-0.094	**0.913**	0.190	0.041	-0.205
Cu	0.458	-0.312	0.241	**-0.673**	**0.799**	0.083	0.179	0.082
Mn	-0.060	**0.827**	0.351	0.356	**0.844**	0.057	-0.061	-0.170
Fe	-0.085	**0.857**	0.051	0.229	**0.873**	-0.258	-0.195	0.017
Urease	0.054	0.442	-0.490	0.462	**0.904**	0.177	-0.005	0.200
Invertase	0.053	-0.481	-0.369	**-0.684**	**0.758**	0.291	-0.370	-0.307
Acid phosphatase	-0.055	**0.802**	-0.059	0.107	**-0.750**	-0.106	0.299	0.222
Catalase	0.354	0.117	**0.827**	0.155	**0.780**	0.257	-0.416	0.296
MBC	**0.872**	0.024	0.421	-0.088	0.379	**0.658**	0.510	-0.281
MBN	0.458	-0.280	**0.642**	-0.282	**0.615**	0.248	0.568	-0.074
MBP	**0.766**	-0.303	0.032	0.253	-0.355	**0.871**	-0.194	-0.214
Eigen value	6.073	4.417	1.507	1.411	6.966	3.753	2.002	1.022
Contribution (%)	37.955	27.604	9.421	8.820	43.540	23.456	12.512	6.389
Cumulative contribution (%)	37.955	65.558	74.979	83.799	43.540	66.996	79.508	85.896

soil accessible nutrients and microbial biomass. The indicators with a loading value greater than 0.6 included organic matter, alkaline nitrogen, available potassium, MBC, and MBP. In treatment T, the total contribution of the four main principal components was 85.896% with PC1's variance contribution accounting for 43.540% of the total. The indexes with a loading coefficient more than 0.6 included Mg, Cu, Mn, Fe, urease, invertase, acid phosphatase, catalase, and MBN. The majority of the indexes, including the trace elements in the soil and the enzymes, were tightly correlated with PC1.

To determine the relative significance of soil microecological indexes in the overall assessment, a weighted score was produced for each index. The greater the total weight score, the greater the significance of each indicator and the greater its impact on the whole soil microecosystem. Through dividing the loading coefficient of each index by the root of the Eigen value of the corresponding principal component, the linear combination coefficient and linear combination equation of each principal component were determined [38]. Using the variance contribution as the weight and plugging it into the equation yielded the weight coefficient and normalized weight coefficient. The weight coefficients of the soil microecological indexes were shown in Table 4. The normalized coefficients of weight were ordered. The arrangement of the microecological indexes of soil under treatment C based on their relative importance was as alkaline N > available K > available P > Mg > MBC > Mn > pH > catalase > invertase > organic matter > Fe > Acid phosphatase > MBP > urease > MBN > Cu. Similarly, the microecological indexes of soil under treatment T were arranged as urease > organic matter > MBN > MBC > Mg > Cu > catalase > Mn > Alkaline N > invertase > Fe > acid phosphatase > available K > available P > pH > MBP.

Table 4. Weight coefficient of the soil microecological indexes.

Index	Treatment C		Treatment T	
	Weight Coefficient	Normalized Weight Coefficient	Weight Coefficient	Normalized Weight Coefficient
pH	0.175	0.085	0.024	0.012
Organic matter	0.145	0.070	0.208	0.108
Alkaline N	0.211	0.102	0.139	0.072
Available P	0.202	0.098	0.047	0.024
Available K	0.210	0.102	0.059	0.030
Mg	0.198	0.096	0.191	0.099
Cu	-0.002	-0.001	0.190	0.098
Mn	0.182	0.089	0.151	0.079
Fe	0.144	0.070	0.112	0.058
Urease	0.075	0.037	0.213	0.110
Invertase	-0.160	-0.078	0.126	0.065
Acid phosphatase	0.120	0.058	-0.112	-0.058
Catalase	0.173	0.084	0.165	0.086
MBC	0.195	0.095	0.197	0.102
MBN	0.074	0.036	0.206	0.107
MBP	0.119	0.058	0.019	0.010

Conclusion

After the application of bioorganic fertilizer, the effects of soil physicochemical properties on the soil microecosystems were decreased with the alkaline N, available K, and available P decreased significantly. However, the impacts of soil enzyme activities and microbial biomass on soil microecosystems were increased with the urease, MBN, and MBC increased significantly. It must be investigated further on how these indexes contribute to the microecosystem and enhance their functions. The results of this study indicated that bioorganic fertilizer could increase the content of soil nutrients and microbial biomass, as well as promoted nutrient and signal exchanges between the root system, soil, and microorganisms in the microecosystems of tobacco rhizosphere, thereby, promoted the healthy growth of tobacco plants.

Acknowledgments

This research was financially supported by Development of Compound Microbial Agent and Application in Tobacco Production in China Tobacco Zhejiang Industrial Co., LTD (2021330000340172).

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