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Soil nutrients and enzyme activities based on millet continuous cropping obstacles

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In order to evaluate the effects of continuous cropping of millet on soil nutrients and soil enzyme activities, the present study was based on four treatments of 2 years of continuous cropping (T1), 3 years of continuous cropping (T2), 4 years of continuous cropping (T3) and rotational cropping (CK), based on 4 years of no fertilizer positioning experiments, and the soil nutrients, soil enzyme activities and millets yields were determined, respectively. The results showed that with the increase of continuous cropping years, the millet yield decreased and was significantly lower than that of rotating with legume crops, and compared with CK, the yields of T1, T2 and T3 treatments were reduced by 8.92%, 13.73% and 37.60%, respectively; the soil nitrogen and phosphorus contents were reduced, the quick-acting potassium content did not change obviously, and the soil pH was increased; Soil urease, alkaline phosphatase, sucrase and catalase activities generally showed a decreasing trend and the decrease was more significant with the increase in the number of years of continuous cropping. Therefore, in order to maintain the soil fertility and increase the millet yield, it is necessary to practice crop rotation and stubble reversal between millets and leguminous crops such as kidney beans, and to apply certain fertilizers.

Keywords Millet, Continuous cropping obstacles, Soil enzyme, Soil nutrient, Rotational cropping

Millet is a drought-resistant, barren and adaptable crop, which is the main food crop in the north of China, mainly distributed in the arid and semi-arid areas in the northwest, north and northeast of China[1]. Restricted by natural conditions, millets are mostly annual crops and rarely fertilized, with a low level of yields, generally 1750–3200 kg/hm^2, and low planting efficiency. The reason for this is that, in addition to factors such as mixed varieties and degradation, sloppy farming management, etc[2]., the continuous cropping obstacle may also be an important factor.

Continuous crop disorder refers to the phenomenon that when crops of the same species or family are cultivated continuously in the same piece of land, even under normal cultivation and management conditions, there will be weakening of growth, lower yield, lower quality, and serious pests and diseases[3]. The causes of continuous cropping disorders are complex, such as the accumulation of soil-borne pathogens, the autotoxic effect of plant root secretions, changes in the soil microbiota and soil physicochemical properties, and the decline in soil fertility[4–7]. China and foreign countries have conducted research on the continuous cropping disorder of many field crops, and some studies believe that the change of soil enzyme activity is the main reason for the crop to produce the continuous cropping disorder, for example, studies have shown that with the increase of continuous cropping of maize, cotton and other crops, their soil alkaline phosphatase activity decreased[8], and the activity of neutral phosphatase decreased and then increased[9]; and the inter-root soil urease activity decreased year by year in the continuous cropping of crops such as diabase, cucumber, peanut and other crops[10,11]. Some studies have also suggested that the barriers to continuous cropping are related to self-toxic substances in plant root secretions[12], and the phenomenon of self-toxicity has been found to exist to a certain extent in grasses such as maize, sorghum and rice, and legumes such as soybeans and peas. In contrast, there are few reports on changes in soil properties and grain yields under continuous cropping of millets and other miscellaneous crops. Therefore,

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the aim of this paper was to investigate the changes in soil nutrients, enzyme activities and grain yield under continuous cropping of millets in different years through a locational experiment.

Materials and methods
Overview of the study area
The experimental site was located near the experimental station of Yangjuangou in Yan'an, Shaanxi Province (Fig. 1), which has a semi-humid and semi-dry early continental monsoon climate. The average annual temperature is 9 ℃, the average precipitation is 562.1 mm, the average annual evaporation is 1579.7 mm, the distribution of precipitation is uneven, and the average precipitation from June to September accounts for about 70% of the annual precipitation. The land in the gully is mostly reclaimed as farmland, crops are cooked once a year, water resources are relatively scarce, there is no irrigation, and the soil type is loess soil. The basic physicochemical properties of the soil before sowing in 2020 were as follows: total nitrogen (TN) 1.08 g/kg, available nitrogen (N) 85.3 mg/kg, total phosphorus (TP) 0.15 g/kg, available phosphorous (AP) 12.88 mg/kg, available potassium (AK) 206.74 mg/kg, and pH 8.3.

Design of the experiment
A continuous locational trial was conducted from 2020–2023 with four treatments, treatment T1 was a 2-year millet cropping (2022 and 2023, planting one season of millet each year), T2 was a 3-year cropping (2021–2023, planting one season of millet each year), T3 was a 4-year cropping (2020–2023, planting one season of millet each year), the control (CK) was a field rotated with millet-buckwheat-kidney bean-millet during 2020–2023. The millet variety was "Jingu 29", the millet variety was Xinong 9940, and the kidney bean variety was small black kidney bean. The experimental plot area was 12 m^2 (3 m × 4 m), with three replications, and was planted in mid- to late-April, with traditional tilling without fertilizer, no irrigation during the whole growing stage, and conventional field management. the upper part of the crop was harvested in mid- to late-October, and the rest of the straw and stubble were left to decompose naturally in the ground.

Soil sample collection
In 2023, soil samples were collected at sowing, seedling, flowering, filling, mature and post-harvest stages, and the post-harvest samples were used for the determination of soil physical and chemical properties, while the rest of the samples were used for the determination of soil enzyme activities. Each plot was collected by the cross five-point method, the inter-root soil of 0–20 cm crops was collected and mixed well, brought back to the laboratory, natural air drying, after removing debris with the "four-partitioning method"[13], that is, mixing the soil samples well and spreading them into squares of equal thickness, dividing the samples into four by using the four-partitioning device on the median line, and then taking the diagonal soil samples, which resulted in 2 kg of soil samples, if there were less than 2 kg of soil samples, the soil sample would be divided into four. If the

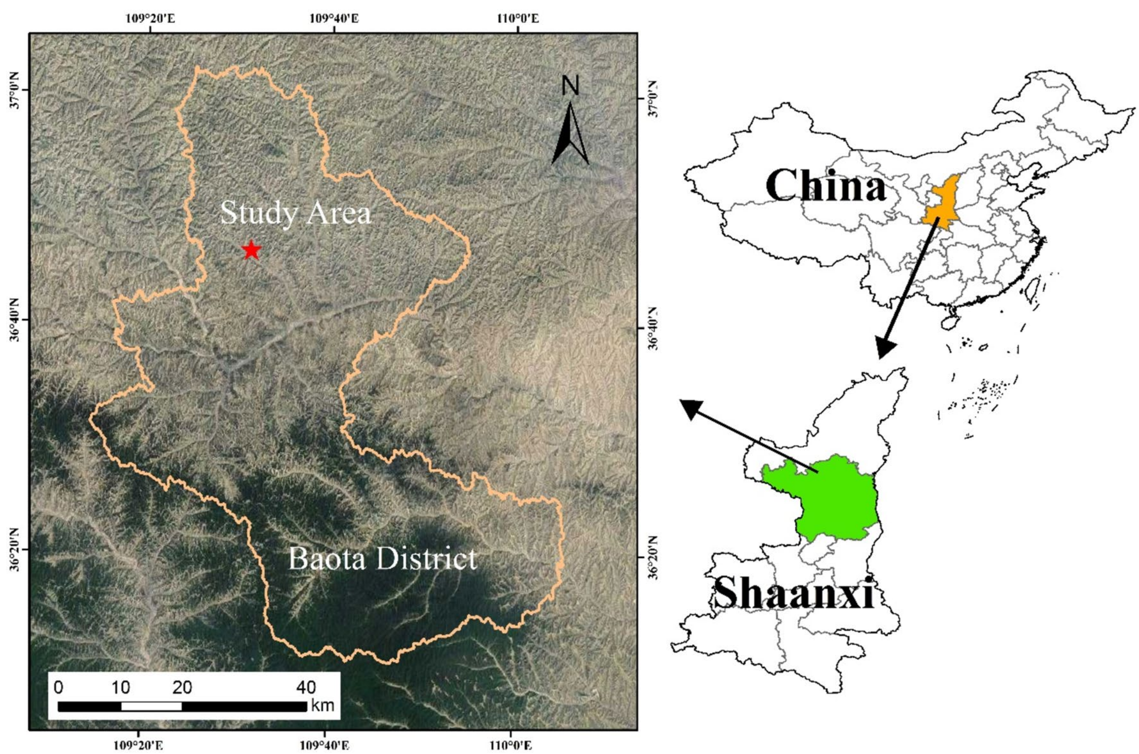

Figure 1. Geographical location of test area. The figure was drawn by ArcGIS10.8 (Esri China Information Technology Co. Ltd. http://my.ally.net.cn/index.php?c=com_index&m=yp&userid=10876).

soil sample is less than 2 kg, mix the rest of the sample evenly and repeat the above method until it reaches 2 kg. After that, the 2 kg soil samples were ground through 1 ~ 2 mm sieve and stored at 4 ℃ for the determination of soil physical and chemical properties and soil enzyme activity indexes.

Measurement methods

Determination of basic physical and chemical properties of soil: TN was determined by Kjeldahl digestion-continuous flow analyzer[14]; AN was determined by 1.0 mol·L^{-1} KCl leaching—AA3 continuous flow analyzer, and the AN measured was ammonium nitrogen[15]; TP was determined by H_2SO_4-$HClO_4$ digestion and molybdenum antimony sulfate colorimetric method; AP was determined by 0.5 mol·L^{-1} $NaHCO_3$ leaching and molybdenum antimony antimony sulfate colorimetric method[16]; AK was determined by 1.0 mol·L^{-1} NH_4OA_c leaching-flame photometric method[17]; pH was determined by 1:2.5 soil–water ratio leaching-potentiometric method[18].

Determination of soil enzymes: Urease was determined by sodium phenol-sodium hypochlorite colorimetric method[19], and the enzyme activity was expressed as the milligrams of NH_3-N in 1 g of soil after 24 h. Alkaline phosphatase(ALP) was determined by sodium phenyl disodium phosphate colorimetric method[19], and it was expressed as the milligrams of P_2O_5 released from 1 g of soil after 24 h. Sucrase activity was determined by the colorimetric method of 3,5-dinitrosalicylic acid[20], and it was expressed as the milligrams of glucose produced from 1 g of soil after 24 h. Catalase activity was determined by ultraviolet spectrophotometry[21], and it was expressed as milligrams of hydrogen peroxide decomposed by 1 g of soil after 20 min.

Data processing and analysis

All data were collated and analyzed using Microsoft Excel and SPSS (Statistical Product and Service Solutions) 17.0 software https://www.ibm.com/cnzh/spss?utm_content=SRCWW&p1=Search&p4=0&p5=1) , Origin 2021(https://www.originlab.com/) for graphing, and Duncan's New Compound Extreme Difference Test for multiple comparisons.

Ethical approval

The use of plants in the present study complies with international, national and/or institutional guidelines.

Results and analysis

Effect of continuous cropping on millet yield

The millet yield decreased significantly with increasing years of continuous cropping, with 8.92%, 13.73% and 37.60% yield reduction in T1, T2 and T3 treatments, respectively, compared with CK (Fig. 2), and the yield reduction increased with increasing years of continuous cropping.

Effect of continuous cropping on inter-root soil chemical properties

It can be seen from Table 1, the contents of TN, AN, TP and AP decreased with the increase of millet cropping years, but there was no significant difference in the content of TP among treatments with different cropping years ($P > 0.05$), and the difference of AN among treatments with different cropping years was significant ($P < 0.05$). The contents of TN, AN and AP of CK treatment were significantly higher than that of T2 and T3 treatments, but the difference with T1 treatment was not significant ($P > 0.05$); the AK content of CK treatment was slightly lower than that of T1 treatment ($P > 0.05$), and significantly lower than that of T2 and T3 treatments ($P < 0.05$). pH increased with the increase of years of continuous cropping of millets, and the difference was significant.

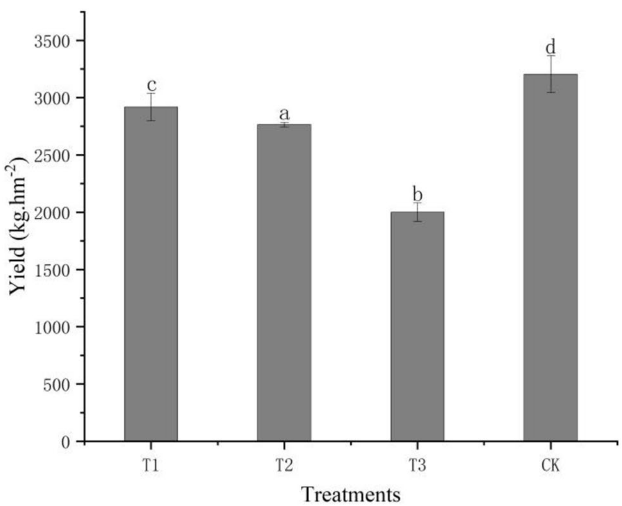

Figure 2. Effects of continuous cropping on yields of millet.

Treatment	TN/(g kg^{-1})	AN/(mg kg^{-1})	TP/(g kg^{-1})	AP/(mg kg^{-1})	AK/(mg kg^{-1})	pH
Tl	0.96 ± 0.05ab	78.01 ± 1.89a	0.07 ± 0.02a	9.89 ± 0.67ab	166.45 ± 7.80ab	8.3a
T2	0.89 ± 0.0lbc	65.50 ± 2.10b	0.04 ± 0.01a	7.23 ± 0.25b	175.78 ± 5.40c	8.4b
T3	0.78 ± 0.03c	58.03 ± 2.05c	0.04 ± 0.01a	4.08 ± 0.54c	172.23 ± 4.50bc	8.6c
CK	l.01 ± 0.06a	80.63 ± 1.50a	0.08 ± 0.01a	10.79 ± 0.71a	162.34 ± 4.51a	8.4b

Table 1. The basic properties of rhizosphere soils under different continuous cropping years.

Effect of crop succession on soil enzyme activity

Soil urease activity in millets of different cropping years and rotations showed a trend of increasing and then decreasing with the fertility period (Fig. 3), the urease activity of the CK treatment continued to increase up to the filling stage, while that of the continuous cropping treatments began to decrease after the flowering stage. The urease activity of the T3 treatment was significantly lower than that of the other treatments ($P < 0.05$), and the CK treatment was significantly higher than that of the continuous cropping treatments ($P < 0.05$) at all fertility periods. Except for the T2 treatment which was slightly higher than T1 at the sowing stage, the effects of the treatments on soil urease at other reproductive stages were as follows: CK > Tl > T2 > T3.

Soil ALP activity under different treatments showed the same trend, with the advance of fertility, ALP activity of millets with different years of continuous cropping and rotational cropping showed a first increase and then a decrease (Fig. 4). The T1 treatment was higher than the CK treatment at all fertility periods, while the T3 treatment was lower than the CK treatment at all fertility periods. With the increase of continuous cropping years, the soil ALP activity showed a gradual decreasing trend, that is, Tl > T2 > T3.

It can be seen from Fig. 5, the trend of sucrase activity in Tl and T2 treatments was more or less the same as that in CK, showing a first decline and then increase, and then decline after the flowering stage; the sucrase activity in T3 treatment was different from that in CK, showing a decreasing trend all the time. The differences between T2 and T3 treatments were significant ($P < 0.05$) compared to CK treatment in all fertility periods. The average soil sucrase activity during the whole fertility period of millets was in the order of T1:47.58 mg·g^{-1}·24 h^{-1}, T2:35.32 mg·g^{-1}·24 h^{-1}, T3:30.68 mg·g^{-1}·24 h^{-1}, CK:48.31 mg·g^{-1}·24 h^{-1}, and soil sucrase activity gradually decreased with the increase of years, and all of them were lower than the CK treatment.

It can be seen from Fig. 6, from sowing to maturity, soil catalase activity varied among different treatments: T1 and CK treatments showed the same trend of change, with an increase in the early stage and a decrease since the flowering stage; T2 and T3 treatments showed an increase in catalase activity during the seeding stage and then a decreasing trend until the maturity stage, with a slight increase. There was no significant difference ($P > 0.05$) between T1 and CK during the whole millet fertility period, and soil catalase activity was higher in T1 and CK than in T2 and T3, and the difference was especially significant at the flowering stage, which decreased by 25.14% in T2 and 36.22% in T3 relative to CK.

Discussion

Effect of continuous cropping on basic physicochemical properties of inter-root soils of millets

Continuous cropping alters the soil microenvironment and has a certain effect on soil nutrients[22]. It is now widely recognized that the malignant change in soil physical and chemical properties is one of the main factors contributing to the occurrence of crop continuous cropping disorders[23,24]. Some studies have shown that with

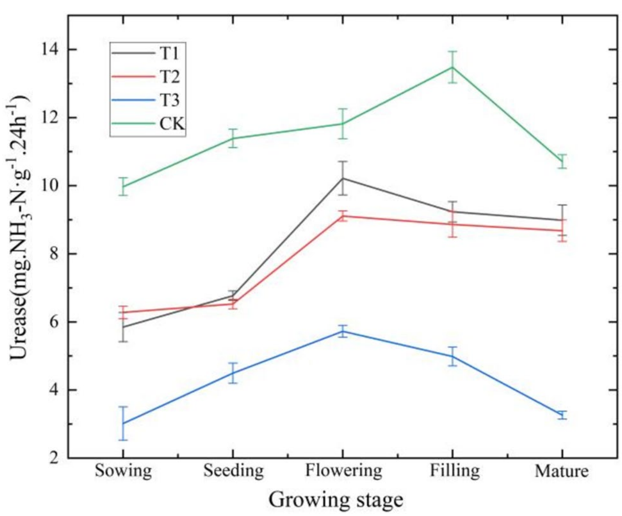

Figure 3. Effects of continuous cropping on urease activities in soil.

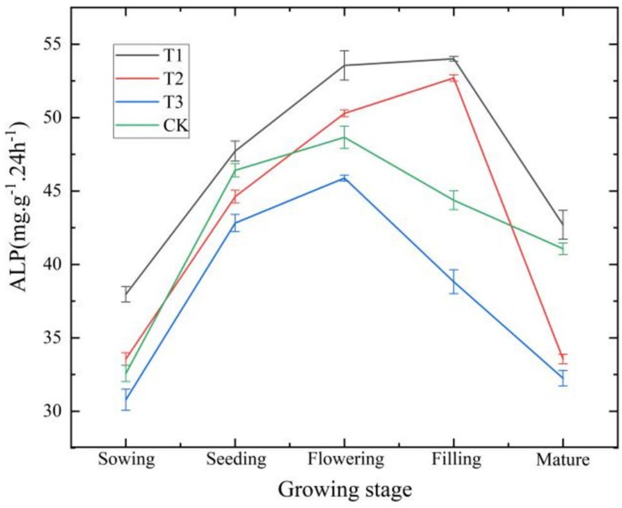

Figure 4. Effects of continuous cropping on alkaline phosphatase activities in soil.

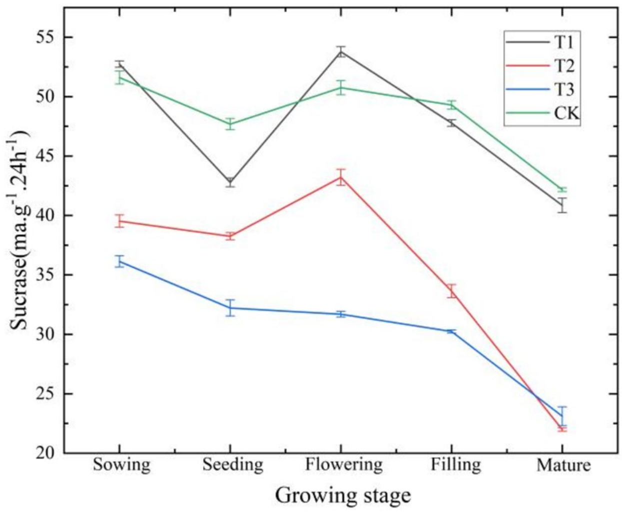

Figure 5. Effects of continuous cropping on sucrase activities in soil.

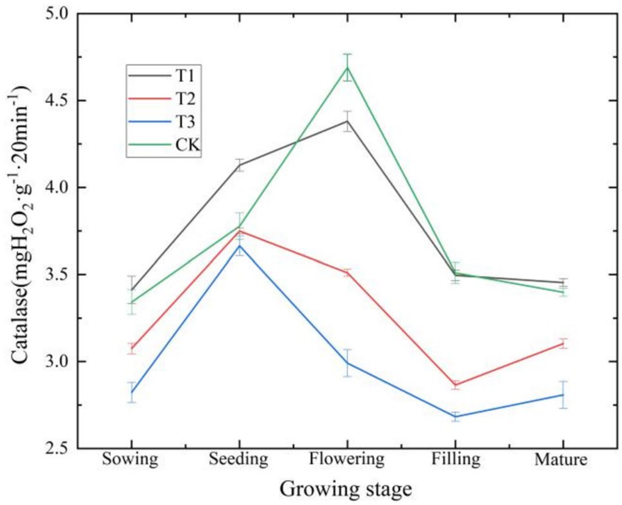

Figure 6. Effects of continuous cropping on catalase activities in soil.

the increase in the number of years of continuous cropping of millets, the AK content of soil was significantly higher than that of the control rotation, and the soil pH of rotated millet fields was significantly lower than that of continuous cropping[25]. The results of the this paper also indicate that, without fertilization, the content of AK 3 years (T2) and 4 years (T3) millet crop was significantly higher than that of crop rotation (CK), AK includes water-soluble and exchangeable potassium, and the accumulation of AK in the soil produces a change in the osmotic pressure of the soil environment, which affects the uptake of K^+ as well as other nutrients by the crop, thus affecting the growth and development of the crop[26]; meanwhile millet continuous cropping increased soil pH, the pH increased from 8.3 to 8.6 after 4 a, which may be related to the uptake of acidic anionic elements by the millet root system and root secretions[27]; the content of N and P in millet continuous cropping soils showed a tendency to decrease, and N decreased more quickly, due to the presence of leguminous plants in the CK treatment of the rotation, which reduced soil N consumption to some extent, and the soil N content of the CK treatment was relatively higher than that of the continuous crop treatment.

Soil nutrients are the ability of soil to provide nutrition for plant growth and to coordinate nutritional and environmental conditions, and are a comprehensive expression of various basic properties of the soil, and tillage practices, fertilization systems, and planting patterns all have an impact on soil nutrients[28]. Abundant N, P, and K elements in the soil is a prerequisite for achieving high crop yields[29]. Different crops have different preferences for the absorption and utilization of soil nutrients, and their root systems have different impacts on the physical and chemical properties of the soil; therefore, crop rotation of different crops can well coordinate the changes in soil physical and chemical properties and nutrients, and improve the effective utilization of soil nutrients. However, continuous cropping will consume a huge amount of a certain nutrient element in the soil, while absorbing and utilizing less of certain elements, leading to the accumulation of the seed element and affecting the overall fertility level of the soil[30].

Effect of continuous cropping on inter-root soil enzyme activities in millets

Soil enzymes mainly come from plant root secretions and soil microbial activities, and participate in various soil biochemical processes, which are sensitive indicators for soil environmental quality monitoring[31]. Soil urease is a kind of specialized amidase, which can promote the hydrolysis of peptide bonds, and urease activity reflects the nitrogen supply status of soil to a certain extent[32]. This study showed that soil urease activity decreased year by year with the increase of years of continuous cropping. Continuous cropping reduces its activity probably because soil urease is mainly derived from microorganisms[33], and the microbial populations of soil under continuous cropping for many years have changed, the urease activity is reduced. The urease activity of the CK treatment of the control rotation was higher than that of the continuous cropping treatment in all fertility periods, which may be due to the fact that the previous crop of the CK treatment was kidney bean, and the nitrogen fixation of legumes increased the soil N content, according to the relevant research, the N content in the soil was positively correlated with the soil urease activity[34].

Soil ALP activity characterizes the fertility status of soil, especially phosphorus, and is a key enzyme in determining soil phosphorus conversion[30]. In this study, the soil ALP activity showed a gradual decreasing trend with increasing years of continuous cropping in millet, in which T3 was significantly lower compared with T1, while the mean values of ALP activity in both T1 and T2 treatments were greater than those in CK treatment during the whole fertility period. Short-term continuous cropping of millets had little effect on soil ALP activity, while long-term continuous cropping significantly reduced ALP activity.

Sucrase is an enzyme that catalyzes the hydrolysis of sucrose into glucose and fructose, which can increase the soluble nutrients in the soil and improve the biological activity of the soil, reflecting the degree of soil maturation[31]. This study showed that continuous cropping of millets reduced soil sucrase activity, and the longer the years of continuous cropping the lower the activity, which is consistent with the findings of Bai Yanru[35] on potato. The reduction of sucrase activity makes the soluble nutrients in the soil decrease, and the soil maturation degree decreases, which is not favorable to the growth and development of the crop, which may be one of the reasons for the decrease of millet yield in continuous cropping.

Soil catalase catalyzes the decomposition of hydrogen peroxide to form water and oxygen, relieving the toxic effects of hydrogen peroxide on organisms and soil due to plant or microbial metabolism[30]. This study showed that continuous cropping reduced catalase activity, slightly in T1 and more significantly in T2 and T3 compared to CK, which is in agreement with the results of Gao Yang[27] et al. on continuous cropping of wheat. With the inhibition of hydrogen peroxide hydrolysis by long-term continuous cropping in millet, the toxic effect of hydrogen peroxide in the inter-root soil was aggravated, which may be one of the reasons for the occurrence of crop failure in millet, and the inhibitory effect of short-term continuous cropping on the activity of catalase was not significant; crop rotation can effectively enhance the oxidative capacity of the soil, and increase the ability of hydrogen peroxide decomposition[31].

Continuous cropping Obstacle is the result of integrated soil-microbe-plant-climate interactions, and there is also a mutual utilization relationship between the reaction sites and products of various soil enzymes[36]. Therefore, only from the soil enzyme activity of a single index change is still very difficult to fully explain the possible occurrence of continuous cropping obstacles from the mechanism, and the study of a single factor is difficult to fully and completely explain the situation of field experiments, long-term continuous cropping of millet on soil microbial taxa, soil physical and chemical properties and soil enzyme activity of the impact of further in-depth research is still needed.

Fertilization of millets cultivation

This study showed that under millet continuous cropping in the arid northwest region, without fertilizer application, millet yield decreases year by year, indicating that fertilizer application is still required to maintain soil fertility and increase yield considering the depletion of nutrients in the soil. In contrast, millets yields under the rotation treatment were significantly higher than the continuous treatment, which was related to the legume crop brassica in the rotation, whose root nitrogen-fixing rhizobacteria fixed a certain amount of nitrogen from the atmosphere to replenish the soil, thus increasing millet yields[37]. Therefore, crop rotation with leguminous crops should be practiced as much as possible in millet production.

Conclusions

(1) Continuous cropping of millets reduces yields, and the longer the duration of continuous cropping, the more obvious the yield reduction: compared with the CK treatment, the T1, T2 and T3 treatments reduced yields by 8.92%, 13.73% and 37.60%, respectively, and the magnitude of the reduction increased with the increase in the number of years of continuous cropping.
(2) Continuous cropping had a significant effect on soil nutrients, the content of N and P decreased, in which the content of AN decreased most significantly ($P < 0.05$), the content of AK changed insignificantly ($P > 0.05$), and soil pH increased.
(3) Under continuous cropping conditions, the activities of soil urease, ALP, sucrase and catalase showed a tendency to decrease year by year, and with the increase in the number of years of continuous cropping, the decrease was more significant, and the T3 treatment was significantly lower than that of other treatments ($P < 0.05$).
(4) In order to maintain the ground strength and increase the yield of millets, it is necessary to practice crop rotation and stubble reversal between millets and leguminous crops such as kidney beans, and to apply certain fertilizers.

Data availability

The datasets generated and analysed during the current study are not publicly available due this experiment was a collaborative effort, the trial data does not belong to me alone but are available from the corresponding author on reasonable request.

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Author contributions

The manuscript was reviewed and approved for publication by all authors. P.Z. conceived and designed the experiments. P.Z., L.X. and Y.S. performed the experiments, analyzed the data, S.G. drew the figures, wrote the paper. P. Z. and P.Z. revised the paper.

Competing interests

The authors declare no competing interests.

Additional information

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