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Responses of Soil Carbon and Nitrogen Nutrients and Extracellular Enzymes to Drought Stress under Different Tea Plantation Ages: Postprint

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Abstract

Frequent summer drought events resulting from global climate warming are increasingly impacting the productivity of tea plantation ecosystems. As tea constitutes a major economic crop in southern China, investigating alterations in soil nutrients, ecological enzyme activities, and microbial ecology across tea plantations of varying planting ages under drought conditions holds significant scientific importance. This study selected soils from three tea plantations with different planting ages (10a, 30a, and 50a) and adjacent barren soil in the Jingshan Tea Plantation, Yuhang District, Hangzhou City, as research objects. We examined temporal variations in soil carbon and nitrogen nutrients (dissolved organic carbon DOC, total organic carbon TOC, microbial biomass carbon MBC, microbial biomass nitrogen MBN, ammonium nitrogen NH4-N, nitrate nitrogen NO3-N) and extracellular enzyme activities (carbon-related extracellular enzymes: -glucosidase BG; nitrogen-related extracellular enzymes: N-acetylglucosaminidase + leucine aminopeptidase NAG+LAP) prior to treatment and on days 7 and 14 under contrasting moisture regimes (drought group: 30% WFPS (water-filled pore space); control group: 55% WFPS), aiming to elucidate the effects of moisture conditions on soil ecosystems of different planting ages. The results revealed that soil carbon pools and both carbon- and nitrogen-related extracellular enzyme activities in tea plantations initially increased then decreased with planting age, peaking at 30a, whereas soil nitrogen pool nutrients exhibited a consistent increasing trend with planting age. Drought treatment significantly increased soil TOC, NH4-N, and NO3-N contents while concurrently decreasing soil MBC content and the activities of BG and NAG+LAP. Notably, the 30a planting age soils exhibited the highest levels of DOC, MBN, NO3-N, and both carbon- and nitrogen-related extracellular enzymes both before and after treatment. Furthermore, the magnitude of variation in soil DOC,

TOC, MBC, and MBN contents between drought and control groups was relatively modest for the 30a treatment, suggesting that soils approximately 30 years of planting age possess a robust microecological environment and strong resistance to environmental changes.

Full Text

Preamble

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The Response of Soil Nutrients (Carbon and Nitrogen) and Extracellular Enzyme Activities to Drought in Various Cultivation Ages from Tea Orchards

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Abstract

Frequent summer droughts caused by global climate change have negatively affected the productivity and quality of tea orchard soils. Tea (*Camellia sinensis*) is an important cash crop in southern China, making it essential to examine soil nutrients, enzyme activity, and microbial community structural shifts under such drought conditions across various cultivation ages. This study utilized a pot incubation method to investigate soils from tea orchards of 0, 10, 30, and 50 years cultivation age. The soils were incubated at 25°C under two water treatments: 30% and 55% water-filled pore space (WFPS). Throughout the incubation period, soil samples were taken at 0, 7, and 14 days to measure soil carbon, nitrogen content, and extracellular enzyme activities.

Prior to incubation, nitrate nitrogen, ammonia nitrogen, microbial nitrogen, and total organic carbon in the soil increased with cultivation age. Microbial carbon and extracellular enzyme activities (-1,4-glucosidase activity related to soil carbon, and N-acetylglucosaminidase and L-leucine aminopeptidase activity related to soil nitrogen) were highest at the 30-year cultivation age. Under drought conditions, soil samples displayed increased extracellular enzyme activities, soil organic carbon, soil nitrate nitrogen, and ammonia nitrogen contents, while soil microbial carbon and extracellular enzyme activities declined. Soils from the 30-year cultivation age exhibited relatively high amounts of dissolved organic carbon, microbial nitrogen, nitrate nitrogen, and soil extracellular enzyme activities throughout all incubation periods. Drought conditions had a significant influence on the contents of soil nitrate nitrogen, ammonia nitrogen, total organic carbon, and microbial carbon. Furthermore, correlation analysis of soil carbon and nitrogen related biochemical properties indicated that carbon and nitrogen soil nutrients affected one another.

The results indicated that 30-year cultivation of tea trees had a positive effect on the accumulation of soil nutrients, but tea tree cultivation for almost 50 years produced an inferior micro-ecological environment. Thus, to improve the soil environment in tea orchards, measures such as balanced fertilization and green cover should be considered.

Keywords: tea orchards; cultivation age; soil nutrients; extracellular enzyme; drought

Introduction

Soil serves as the medium for tea growth, and soil quality directly affects tea yield and quality. While tea orchards apply large amounts of chemical nitrogen fertilizer to increase tea production, this practice also intensifies soil acidification, which gradually reduces soil nutrient availability with increasing cultivation age. To analyze soil environmental quality, an increasing number of studies integrate soil nutrient and enzyme activity analyses to evaluate soil quality. Soil carbon and nitrogen content are important indicators of soil quality, with significant implications for soil productivity, sustainable utilization, and environmental protection. Soil extracellular enzymes and microbial biomass play crucial roles in the decomposition of organic residues and nutrient cycling, serving as effective indicators for evaluating soil microbial decomposition capacity, fertility, and ecological stability.

Different cultivation ages have varying effects on soil nutrients. Research on artificial ecological forests in the Minjiang River area found that soil organic carbon and nitrogen storage generally increased with stand age. However, other studies have shown that different tea cultivation ages lead to different soil physicochemical properties, resulting in variations in soil carbon, nitrogen nutrients, and extracellular enzyme activities. After planting tea trees in barren land, soil microbial biomass carbon and enzyme activity showed a trend of first increasing and then decreasing with cultivation age. The quality management of tea orchard soils and planting duration have become primary research foci in tea orchard ecosystems.

Climate change studies indicate that the frequency of extreme summer climates in China is increasing, with average summer temperatures rising annually. As one of China' s important economic crops, tea is significantly affected by these changes. Sustained high temperatures and drought in southern summers lead to low tea yield and poor quality. Research has shown that drought reduces soil material mobility, thereby decreasing microbially available resources by limiting substrate diffusion and inducing microbial physiological stress, which negatively impacts soil microbial biomass and diversity. Other studies indicate that under global warming, soil net nitrification rates are suppressed during drought, reducing soil nitrate nitrogen content. Soil microbial activity and enzyme activity decrease, hindering the supply of nitrogen and carbon sources needed by crops. Soil carbon cycling is affected, and different tea cultivation ages with different ecological environments may respond differently to drought events. However, the responses of soil carbon and nitrogen nutrients and extracellular enzyme activities to drought stress across different tea cultivation ages remain unclear.

This study compared tea orchard soils of different cultivation ages (10, 30, and 50 years) and barren soil under different water conditions (30% WFPS and 55% WFPS) to examine changes in soil carbon and nitrogen nutrients and extracellular enzyme activities before and after treatment. The objective was to clarify how soil quality indicators change over time and under drought stress, promoting comprehensive evaluation of soil quality across different tea cultivation ages. The experimental results will provide a theoretical basis for promoting the development of tea orchards under global climate change conditions and safeguarding tea orchard soil ecosystem health, yield, and quality.

1. Experimental Site and Materials

The test soils were collected from the Yuhang Jingshan Tea Science Base of Zhejiang University, located in Jingshan Town, Yuhang District, Hangzhou (30°23 N, 119°53 E). The base has similar soil parent material and fertilization conditions across different tea orchards. Soils from tea orchards with cultivation ages of 10–12 years (Y10), 28–30 years (Y30), and 48–50 years (Y50) were selected as research objects, along with barren land (CK) near the tea orchards. In May, surface soil (0–10 cm) was collected from each plot after removing surface debris and plant roots. The same soil samples were mixed uniformly and passed through a 2 mm sieve. One portion was stored for physicochemical index determination, while the remaining soil was used for subsequent simulation experiments. The basic soil properties are shown in Table 1.

Soil properties

2. Experimental Treatments

The experiment was conducted through indoor soil incubation. Fresh soil (300 g) was placed in incubation pots (7.5 cm diameter × 10 cm height) with gauze wrapped at the bottom to prevent soil loss. The pots were placed in a constant temperature incubator at 25°C for two weeks. Two soil moisture levels were maintained: 30% WFPS (drought) and 55% WFPS (control). Sterile pure water was injected daily with a syringe to adjust soil moisture. Soil samples were destructively collected at three sampling times (0, 7, and 14 days) and divided into two portions for relevant index determination.

3. Measurement Methods

Soil total organic carbon (TOC) and dissolved organic carbon (DOC) were measured using a Multi N/C 2100 total organic carbon analyzer. Soil ammonium nitrogen (NH -N) and nitrate nitrogen (NO -N) were determined by indophenol blue colorimetry and UV dual-wavelength colorimetry, respectively. Soil microbial biomass carbon (MBC) and nitrogen (MBN) were measured by chloroform fumigation followed by extraction. Extracellular enzyme activities were determined using microplate fluorometric methods. The substrates for -glucosidase (BG), N-acetylglucosaminidase (NAG), and leucine aminopeptidase (LAP) were purchased from Sigma. Fluorescence intensity was detected using a multifunctional microplate reader (MD5, Molecular Devices) at 365 nm excitation and 460 nm emission. Enzyme activity calculations followed DeForest (2009). Each sample was replicated three times.

4. Data Processing and Analysis

Experimental data were processed using Excel 2007 and Origin 8.5. Repeated measures ANOVA in SPSS 16.0 was used to test the effects of soil moisture, tea cultivation age, and sampling time on soil chemical properties and extracellular enzyme activities. Pearson correlation analysis was used to test correlations between soil indicators.

1. Soil Carbon Pool Characteristics Across Different Tea Cultivation Ages Before and After Water Treatment

The content of dissolved organic carbon (DOC) in tea orchard soils was significantly greater than in barren soil. Drought treatment significantly increased soil DOC content across all soil types. The order of DOC content was Y10 Y30 > Y50 > Y0, with Y10 and Y30 being significantly higher than Y50 and Y0 ($P < 0.05$). Soil total organic carbon (TOC) content increased with cultivation age, with Y10, Y30, and Y50 showing increases of 86.93%, 95.78%, and 58.96%, respectively, compared to Y0.

Soil microbial carbon (MBC) content increased first and then decreased with cultivation age, with the order Y30 > Y10 > Y50 > Y0 ($P < 0.05$). After 14 days of incubation, MBC content in Y10 and Y30 soils under drought treatment was significantly higher than the control group, with increases of 106.67% and 85.39%, respectively. However, MBC content in Y50 soil under drought treatment was significantly lower than the control group, with a decrease of 44.62%.

-glucosidase (BG) activity increased first and then decreased with cultivation age. Before water treatment, BG activities in Y10 and Y30 soils were 2.92 and 4.19 times that of Y0, respectively. After water treatment, BG activity decreased substantially, with drought group activity significantly lower than the control group.

[Figure 1: see original paper] Variations of DOC and TOC with different tea cultivation ages incubation at 30% and 55% WFPS
[Figure 2: see original paper] Variations of MBC and activities of BG with

different tea cultivation ages incubation at 30% and 55% WFPS

2. Soil Nitrogen Pool Characteristics Across Different Tea Cultivation Ages Before and After Water Treatment

Before treatment, soil nitrate nitrogen (NO -N) and ammonium nitrogen (NH -N) contents increased with cultivation age. NO -N content ranged from 34.21–80.27 mg/kg, while NH -N content ranged from 3.72–43.41 mg/kg. After 7 days of incubation, NO -N and NH -N contents showed an increasing trend, with drought groups significantly higher than control groups. After 14 days, NO -N content was significantly lower than the control group, while NH -N content remained significantly higher.

Before water treatment, soil microbial nitrogen (MBN) content increased significantly with cultivation age. After water treatment, MBN content decreased significantly. The nitrogen-related enzyme activity (NAG+LAP) increased first and then decreased with cultivation age, with the highest activity at 30 years. Before water treatment, NAG+LAP activities in Y10 and Y30 soils were 21.79 and 16–17 nmol/g/h, respectively. After water treatment, the trend remained unchanged, but drought group activity was lower than the control group.

[Figure 3: see original paper] Variations of soil ammonia and nitrate nitrogen with different tea cultivation ages incubation at 30% WFPS and 55% WFPS
[Figure 4: see original paper] Soil MBN content and activities of NAG+LAP with different tea cultivation ages incubation at 30% and 55% WFPS

3. Relationships Between Soil Carbon and Nitrogen Pool Characteristics

The correlation analysis of soil carbon and nitrogen biochemical properties is shown in Table 2. All carbon and nitrogen indicators were significantly correlated with pH ($P < 0.01$), indicating that pH is a limiting factor for soil nutrients in tea orchards. NH -N was extremely significantly correlated with NO -N, TOC, MBC, and DOC ($P < 0.01$). NAG+LAP was extremely significantly correlated with TOC and BG ($P < 0.01$), while BG was extremely significantly correlated with DOC, TOC, and MBC ($P < 0.01$). This demonstrates that drought has a consistent inhibitory effect on carbon- and nitrogen-related enzymes, thereby affecting soil carbon and nitrogen nutrient content. Simultaneously, soil carbon and nitrogen pool nutrients influence each other, with changes in one directly affecting the other.

Pearson' s correlation coefficients among soil carbon and nitrogen related biochemical properties

3. Discussion

This study showed that before water treatment, soil NH -N, NO -N, and MBN increased with cultivation age, indicating that tea cultivation and management practices in the study area led to accumulation of soil carbon and nitrogen pool nutrients. Previous studies have reported that orchard soils show similar patterns, likely due to long-term fertilizer input causing soil nutrient accumulation. However, tea orchard soil MBC decreased with cultivation age, likely because severe soil acidification in long-term tea plantations reduces soil microbial activity and thus decreases organic mineralization capacity. NAG+LAP activity increased first and then decreased with cultivation age. Studies have shown that urease, sucrase, and phosphatase activities also exhibit trends of first increasing then decreasing with cultivation time, possibly due to low microbial biomass and severe acidification in long-term tea soils, which limit microbial enzyme production. Soil pH affects enzyme activity by influencing ionization-induced enzyme conformational changes and substrate availability.

Appropriate tea cultivation can enrich soil nutrient content and enhance biochemical processes in the tea orchard ecological environment. This study further demonstrates that different cultivation age soils respond differently to drought. Water treatment time, cultivation age, and their interactions directly affect tea orchard soil carbon and nitrogen pool nutrient contents and carbon- and nitrogen-related extracellular enzyme activities. After water treatment, drought group soils showed higher TOC, DOC, NH -N, and NO -N contents than control groups, possibly because drought reduced soil microbial activity, thereby decreasing microbial utilization of soil inorganic nutrients and affecting the release patterns of carbon and nitrogen oxidation and decomposition. Some bacteria may also synthesize carbohydrates under drought conditions. Another reason for lower NO -N content in control groups may be that denitrification is more complete in moist soils, releasing more NO -N to the atmosphere. However, Wang et al. and Schindlbacher et al. found that soil NO -N concentration increases accompanied by NH -N concentration decreases, which contradicts our results. This discrepancy may be due to continued soil mineralization under drought conditions, but with reduced ammonia-oxidizing archaea and bacteria abundance inhibiting the conversion of ammonium nitrogen to nitrate nitrogen.

After water treatment, soil NO -N content significantly decreased, possibly because strong soil acidity inhibited nitrifying bacteria activity. The significant negative correlation between NH -N and NO -N contents after water treatment suggests that NH -N may be mineralized and converted to NO -N. The smallest changes in DOC, MBN, and NO -N contents before and after treatment in 30-year soils indicate that soils of this age maintain higher activity. NAG+LAP activity showed greater variation before and after treatment, with activity in drought groups lower than controls. Kotroczó et al. also found that phosphatase activity was higher in soils with higher moisture content. Therefore, we can infer that different cultivation age soils have different micro-ecological environments, leading to different enzyme activity responses to moisture.

Studies show that phosphatase activity is related to soil temperature and moisture, so enzyme activity responses to different moisture levels vary. Soil carbon and nitrogen pools are significantly correlated, and drought treatment affects soil carbon and nitrogen transformation by reducing microbial decomposition and utilization. The inhibitory effect of drought on microbial nutrient utilization is greater than its inhibitory effect on active nutrient generation. Soils with 30-year cultivation age not only have relatively high nutrient content but also show stronger tolerance to environmental stress under drought conditions. Thus, tea cultivation within a certain timeframe is beneficial for soil nutrient accumulation and establishment of soil ecological environment diversity. However, when cultivation age is too long, soil quality decreases and acidification becomes a limiting factor for soil nutrient biochemical processes, thereby reducing resistance to external stress.

4. Conclusion

Tea orchard soils have higher nutrient content than barren soils. With increasing cultivation age, carbon pool nutrient content and carbon- and nitrogen-related extracellular enzyme activities show a trend of first increasing then decreasing, reaching maximum values at 30 years. Nitrogen pool nutrients show an increasing trend with cultivation age. Drought has significant effects on soil carbon and nitrogen pool nutrients and carbon- and nitrogen-related extracellular enzyme activities, increasing soil organic carbon content while decreasing soil microbial biomass and extracellular enzyme activities. The 30-year cultivation age soil shows the smallest variation in dissolved organic carbon, total organic carbon, and microbial carbon and nitrogen contents compared to control groups, indicating that drought group soils of this age have relatively high dissolved organic carbon and stronger resistance to environmental changes. Therefore, the biological and biochemical environment of tea orchard soils should be considered, and measures such as reasonable fertilization and green cover should be adopted to improve the tea orchard soil environment.

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