IMPROVEMENTS IN THE GROWTH, QUALITY, AND YIELD OF WUYI ROCK TEA (*CAMELLIA SINENSIS*) AFTER BREEDING EARTHWORMS IN SITU IN TEA GARDENS IN CHINA

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Abstract. This study aimed to evaluate in situ the effects of earthworms on the growth, quality, and yield of the tea plant, *Camellia sinensis* (L.) O. Kuntze. Our results show that the incorporation of earthworms and organic waste in the soil significantly enhanced both the tea bud density and hundred-bud weight for both summer and autumn tea, but did not significantly influence the number of leaves per shoot or single leaf area. Furthermore, earthworms enhanced the quality of summer and autumn tea, in terms of significantly higher amino acid content, lower polyphenol and caffeine content, and maintenance of the total water extract content. Principal component analysis found that the four groups of samples were well separated at the first principal component and that free amino acids and polyphenols had the greatest effect between the treatment and the control group. Furthermore, these two parameters were also the primary reasons for differences between summer and autumn teas under the same treatment. Therefore, in situ earthworm breeding could be a promising alternative to chemical fertilizers and assist in the sustainable, pollution-free production of tea since it can improve both the quality and yield of tea and eliminate considerable amount of organic waste.
Keywords: *earthworm inoculation, organic fertilization, summer tea, autumn tea, quality and production*

Introduction

Tea, one of the world's most popular drinks, contains numerous bioactive ingredients which are responsible for its pleasurable taste and health-promoting benefits (Wei et al., 2018). The tea plant, *Camellia sinensis* (L.) O. Kuntze, originates from southwest China and its leaves have been used to make many kinds of tea for thousands of years. Today, it is cultivated all over the world (Kingdom-Ward, 1950; Taniguchi et al., 2014). Over the past decade, the worldwide production of tea has steadily increased, with the total cultivated area in 2020 being 66% greater than in 2010, and has achieved 5.3 million tons of tea from 3.5 million ha across more than 50 tea-cultivating countries (www.fao.org/faostat). This yield increase can be attributed to many factors, including the use of modern technical equipment, improvements in agronomic practices, adaptation of inorganic fertilizers and pesticides, huge capital investment, and the use of newly developed clones with higher production capabilities (Giri, 1995). However, intensive agriculture is often criticized for its detrimental influences on the environment and human health, particularly through its use of chemical fertilizers (Bertrand et al., 2015). These fertilizers can damage the soil structure and reduce soil fertility by decreasing the nitrogen and carbon content (Ngo et al., 2012). Furthermore, chemical fertilizers have adverse effects on animal and human health due to their heavy metal content which can pass through the food chain (Vu et al., 2007). As a result, interest in organic fertilizers is growing, and proper organic amelioration involving composting, vermicomposting, and in situ earthworm breeding have become promising biological avenues for enhancing the growth, quality and production of tea.

Most tea growing regions are in hilly terrain. The soil in these regions this is often vulnerable to constant leaching from heavy rainfall, adversely affecting its biological and physicochemical parameters (Giri, 1995). Such effects can include reduced organic matter content, decreased water-holding capacity, acidification, lower cation exchange, soil surface compaction, soil erosion, nutrient leaching, and loss of important organisms from the soil (Giri, 1995). The incorporation of compost with the soil is considered a form of sustainable agriculture, contributing to improvements in soil fertility and plant nutrition (Caravaca et al., 2002). The presence of earthworms is of great interest since, as an important functional group in soil, they are marvelous in their ability to improve its structure, microbe diversity, and physicochemical properties and help plant development by their peculiar feeding, burrowing, and casting activities (Edwards and Bohlen, 1996). Earthworms have been present in soils for several hundred million years and constitute more than 80% of the soil invertebrate biomass in most terrestrial ecosystems (Lavelle and Spain, 2001); therefore, earthworms very likely co-evolved with plants (Bertrand et al., 2015). The positive impact of earthworms on plant growth and health has been known for over a century, with works dating as far back as the late 1800s (Darwin, 1881). Indeed, the effect that earthworms exert on primary yield has been fully explored both in the laboratory and the field, with some investigations taking place over several years (Giri, 1995; Blanchart et al., 1997). However, there are two previous studies which found that earthworms could have an unfavorable impact on plant growth (Brown et al., 1999, 2004). The authors found that in 4% of 246 assays (performed in tropical countries) there was a 20% decrease in crop production correlating with the occurrence of earthworms, and that several environmental determinants like soil type, soil texture and carbon content could account for variation in the productive response of the plants.

In China, the application and promotion of earthworms in tea plantations is low in comparison to the other major tea-growing country, India. Earthworms are only used over 0.4% China's tea-growing area (Kamel et al., 2002; Zhang et al., 2018), heavily concentrated in southern China such as Guangdong and Hainan Province (Tang et al., 2008, 2011, 2016; Zhuo et al., 2007, 2017). Until now, rare work has been undertaken in the other main tea-growing areas such as Fujian, Yunnan and Sichuan Provinces.

The present study focuses on Wuyi Mountain, situated in the north of Fujian Province. It is a UNESCO World Heritage site famous for oolong tea; the consumption of this tea has increased substantially worldwide in recent years due to its favorable taste and pleasant aroma (Chen et al., 2011). It is widely accepted that abundant earthworm populations are beneficial to agricultural production; however, these earthworm-mediated ecological services may not always enhance the performance of a particular cropping system because the effects are both species- and site-dependent (Bertrand et al., 2015). Therefore, the purpose of our research was to determine in situ the effects of earthworm breeding in a tea plantation on Wuyi Mountain, measured in terms of the growth, quality, and yield of oolong tea. We thus considered their use as a potential organic fertilizer as a substitute for chemical fertilizer to improve tea quality and yield.

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Materials and methods

Test site description

The research was conducted from March to September 2019 at the Tea Science and Education Park of Wuyi University (27°43’38”N and 118°0’2”E) which covers an area of 0.4 ha (width 80 m × length 50 m, *Fig. 1*). The area is located at northern Fujian Province, China and characterized by a subtropical monsoon climate with an average annual precipitation of 1926.9 mm; most of the rainfall is concentrated in the wet season between March and June. The relative humidity (RH) of the experimental site is moderately high, ranging from 64 to 85%, and the mean temperature was 22.6 °C for whole year (Municipal Government of Wuyishan, 2019). Weeds were manually removed using a hand hoe wherever needed. The initial properties of the soil were investigated and are shown in *Table 1*.

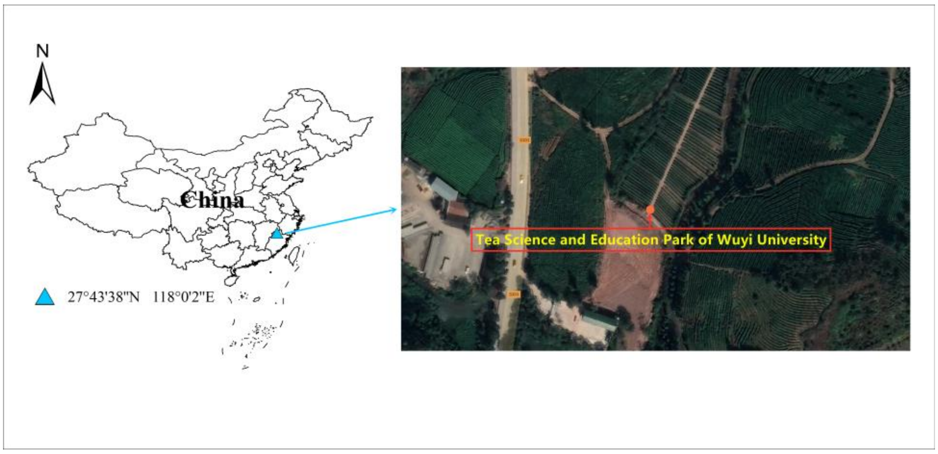

Figure 1. *Map of study area*

Table 1. *Initial chemical properties of the soil at the experimental site*

Index	Initial status of soil
pH value	4.05
Available N (mg kg^{-1} soil)	20.17
Available P (mg kg^{-1} soil)	10.85
Available K (mg kg^{-1} soil)	53.98
Organic carbon (%)	1.74

Earthworms and materials

In the present experiment, five-year-old *C. sinensis* cv. Shuixian tea plants were used. Based on our previous field, the predominant indigenous earthworm species for the local tea gardens is *Eisennia foetida* Savigny (unpublished data); this species was reared under laboratory conditions in accordance with the procedure by Dai (2014). The organic fertilizer used in the experiment was cow dung, which was naturally decomposed for one month prior to the experiment; the water and total nitrogen content was about 50% and 1%, respectively. Tea leaf litter was collected from tea garden ground and naturally dried in the field, with a total nitrogen content was about 2%.

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Experimental design and treatments

The experimental area was divided into six plots, each covering an area of 5 × 5 m. To minimize the effects caused by other plots and surroundings, each plot was separated by concrete boards made from calcium silicate, and were 0.5 m apart. The boards were placed 0.6 m deep into the soil with 0.2 m of the board showing above the ground in order to prevent the escape of earthworms and fertilizer runoff. The treatment group plots contained organic fertilizer mixed with composting species of *E. foetida*, and the control group plots contained organic fertilizer alone without any earthworms. There were three replicates of each treatment and control plot, and these six plots were randomly arranged in the experimental area (*Fig. 2*). The field pattern of in situ earthworm breeding is shown in *Figure 3*. Finally, tea leaf litter and a sun shading net were placed upon the bed, and organic waste and water were provided regularly in order to create a better environment for the development and reproduction of the earthworms. The introduction of earthworms and fertilizers to the beds was performed in early March. Field management practices such as watering and artificial weeding were used for all plots in the tea garden to ensure the normal activity of earthworms. No chemical pesticide was applied during the experiment.

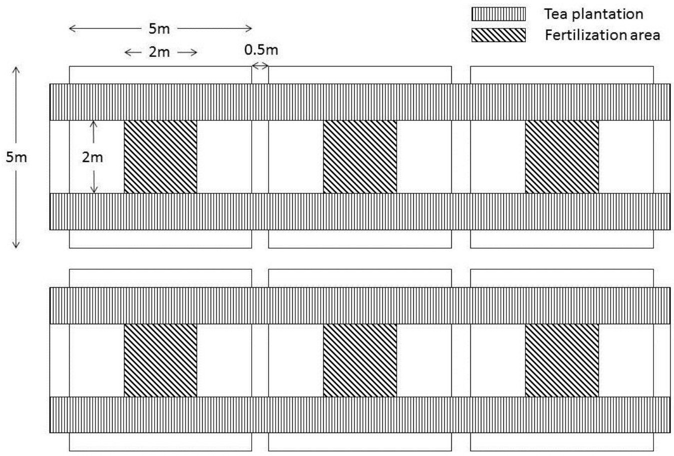

Figure 2. *Diagram of the experimental area. Treatment and control groups were randomly arranged into six plots*

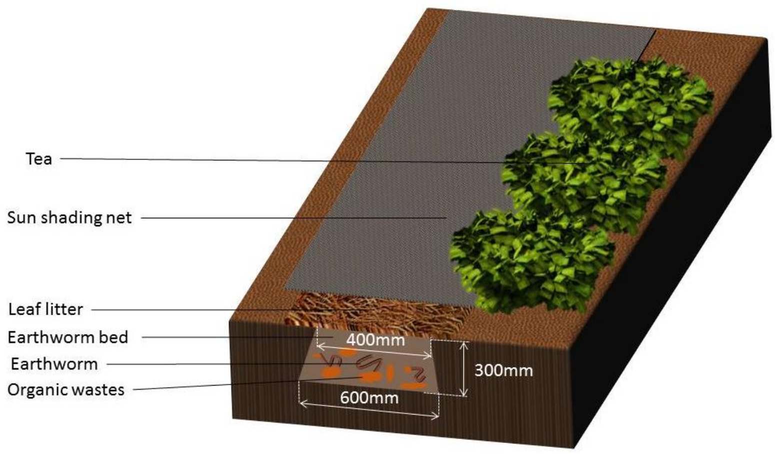

Figure 3. *The field pattern of in situ earthworm breeding in each plot*

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Earthworm bed (length: 6 m, surface width: 0.4 m; bottom width: 0.6 m; height: 0.3 m) was prepared approximately 0.5 m away from the tea plantation for each plot. For each bed, 5 kg/m^3 of organic waste, derived from the process of manufacturing beer, was supplied to the bottom of the bed. In the treatment plots, 20 g of *E. foetida* were introduced into the central of bed.

Assessments

Determination of the growth indicators of tea

Several months after introducing the earthworms, tea leaves that collected in early June (from 1st to 5th June) were named summer tea, while tea leaves that collected in early September (from 1st to 5th September) were named autumn tea. The number of leaves on mature shoots, leaf area, one-hundred-bud weight, and bud density were evaluated during the summer (summer tea) and autumn (autumn tea) tea-picking season as mentioned below. All measurements were performed in triplicate for each plot.

Leaf number and area: Twenty mature shoots were randomly picked from each plot and the number of leaves on each shoot were calculated. The width and length of the leaves were gauged and the area of one leaf was calculated according to the following formula: length × width × 0.7 (Zhang and Wang, 2006).

Bud density: The number of buds in a 0.1 × 0.1 m area wooden-frame was counted.

One hundred bud weight: One hundred tea buds were randomly selected from each plot and then weighed in total.

After the above assessments, fresh leaves were collected fixed at a temperature of 105 °C for 1 h, followed by drying at 80 °C to a constant weight. They were then ground, screened using a 60-mesh sieve, and refrigerated at -20 °C prior to the tea quality assessments.

Determination of the quality of tea leaves

For both summer and autumn tea-picking season, the total mass of water extracts, polyphenols and free amino acid were assessed in accordance with the National Standards of People’s Republic of China. All measurements were performed in triplicate for each plot.

Assessment of tea water extract

The tea leaves were first ground and approximately 2 g of leaf sample (measured with an analytical balance accurate to 0.0001 g) was placed in an aluminum box; the initial weight of each tea leaf sample was recorded as m_0. The samples were then placed in an electric drying oven (DH-9146A, Suzhou Weir Experimental Supplies Co., Ltd, PR China), held at 120 °C for 60 min, and then transferred to a drier (IPC250-1, Hebei Rixiang Experimental Supplies Co., Ltd, PR China) for cooling to room temperature. The weight of the dried tea sample was recorded as m_1. The dried tea sample was then soaked in 300 mL of boiling distilled water (100 °C) for 45 min using a water bath, and manually shaken every 10 min. The leach liquor was then immediately subjected to decompression filtration and the tea residue was washed multiple times using a small amount of 100 °C distilled water. The tea residue was transferred to a new aluminum box and placed in an oven for drying at 120 °C for 60 min, removed for 60 min cooling,

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again placed in the oven at 120 °C for 60 min, and finally transferred to the drier for cooling to room temperature. The weight of this treated tea residue was recorded as m_2. The content of water extract was calculated using the following formula (GB/T 8305-2013, 2013):

$$(1-\frac{m_2}{m_0 \times m_1})\times 100\%$$

Assessment of polyphenol content

Tea samples of 0.2 g were blended in 5 mL of 70% (v/v) methanol preheated to 70 °C. The polyphenol content was determined using Folin-Ciocalteu reagent and checked with a spectrophotometer (UV2900, Sunny Optical Technology Co., Ltd., China) at 765 nm. Gallic acid was used as the standard (GB/T 8313-2008, 2008).

Assessment of total free amino acid content

The free amino acid content was assessed by combining 1 mL of tea infusion, 0.5 mL of phosphate-buffered solution (pH 8.0) and 0.5 mL of 2% ninhydrin solution including 0.8 mg/mL of tin chloride in a 25-mL volumetric flask. The optical density of the solution was determined using the UV2900 spectrophotometer at 570 nm (GB/T 8314-2013, 2013).

Assessment of caffeine content

The process for extracting caffeine from tea samples was similar to that for free amino acids. The caffeine content was measured according to the method described by Wang et al. (2011) using a HPLC system (SPD-20A, Shimadzu, Japan) with a UV detector (LC-20AT, Shimadzu, Japan), RPC18 column (5 um, 250 mm × 4.6 mm i.d.) and analytical software.

Assessment of soluble sugar content

The soluble sugar content was determined based on the procedure proposed by Zhou et al. (2017). In order to extract the sugar content, tea leaf samples each weighing 0.3 g were soaked in 450 mL of boiling (100 °C) distilled water and kept at this temperature for 45 min in a water bath. The whole 450 mL of samples were then centrifuged for 4 min at 3500 × *g*. After collecting the tea infusion by vacuum suction filtration, water was added to make a final volume of 500 mL. The soluble sugar content was measured against a standard curve of glucose solution with concentrations ranging from 50 to 1000 ug/mL.

Statistical analysis

Statistical analysis was performed using SPSS Statistics 17.0 (SPSS, Inc., Chicago, Illinois, USA); significant differences between the means of replicates were evaluated by an independent sample t-test with a significance level of $P \leq 0.05$. Graphs were created using GraphPad Prism 7.0 (GraphPad Software, San Diego, CA, USA). SIMCA-P (Sartorius Stedim Data Analytics AB, Umeh, Sweden) was used for principal component analysis.

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Results

Tea growth and yield

As shown in *Figure 4*, the addition of earthworms to the soil significantly enhanced the tea bud density and hundred-bud weight; these positive effects were observed both in summer and autumn tea (bud density: summer tea, F = 0.582, df = 4, t = 4.243, P = 0.013; autumn tea, F = 2.882, df = 4, t = 5.588, P = 0.005; hundred-bud weight: summer tea, F = 0.542, df = 4, t = 4.243, P = 0.023; autumn tea, F = 3.152, df = 4, t = 7.102, P = 0.002). However, for both summer and autumn tea, leaf number per shoot and single leaf area were not significantly different between the control and treatment groups (leaf number per shoot: summer tea, F = 0.571, df = 4, t = 1.044, P = 0.492; autumn tea, F = 1.96, df = 4, t = 0.64, P = 0.234; single leaf area: summer tea, F = 0, df = 4, t = 0.766, P = 0.487; autumn tea, F = 1.469, df = 4, t = 2.553, P = 0.292).

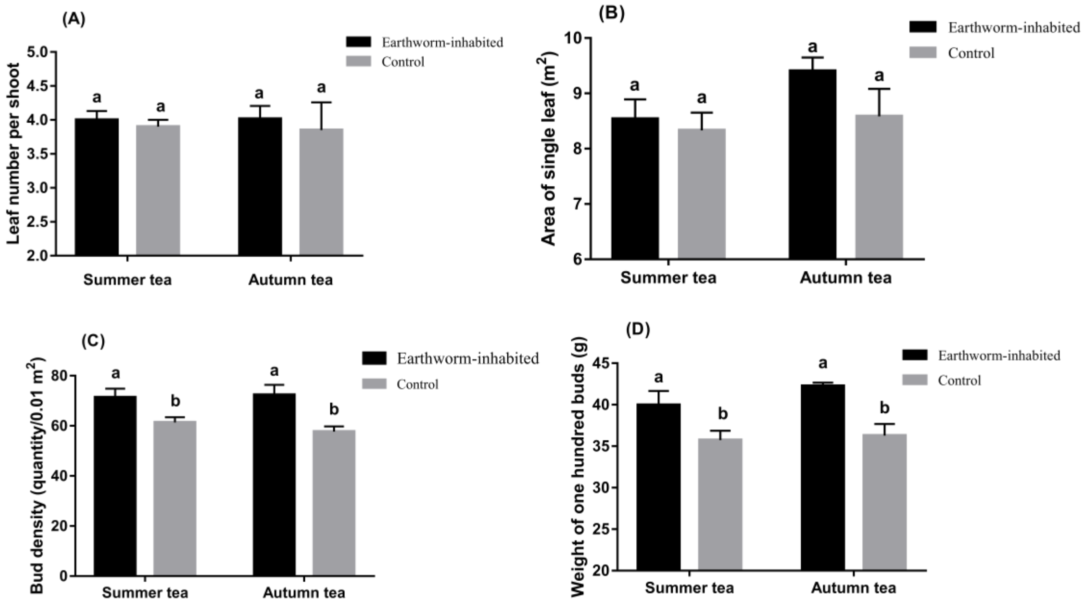

Figure 4. *Growth indicators of summer and autumn tea for the treatment and control groups. Data are shown as mean ± SD. Different letters above the bars indicate significant differences (independent sample t-test, P < 0.05)*

Tea quality

As shown in *Figure 5A* and *B*, there were no significant differences between the control and treatment group on water extract content or soluble sugar for summer and autumn tea (water extracts: summer tea, F = 9.119, df = 4, t = -2.789, P = 0.051; autumn tea, F = 5.841, df = 4, t = -0.74, P = 0.5; soluble sugar: summer tea, F = 0.87, df = 4, t = 3.611, P = 0.404; autumn tea, F = 0.447, df = 4, t = 0.683, P = 0.54). As shown in *Figure 5C*, in situ earthworm breeding significantly increased the total free amino acid content for both summer and autumn tea (summer tea, F = 0.086, df = 4, t = -5.124, P = 0.007; autumn tea, F = 0, df = 4, t = -2.99, P = 0.041). As shown in *Figure 5D*, *E* and *F*, the polyphenol to amino acid ratio and the content of caffeine and polyphenols for summer and autumn tea were significantly less in the experimental group (caffeine: summer tea, F = 9.799, df = 4, t = -3.771, P = 0.035; autumn tea, F = 9.728, df = 4, t = -

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2.557, P = 0.036; polyphenols: summer tea, F = 0.015, df = 4, t = -4.792, P = 0.001; autumn tea, F = 2.761, df = 4, t = -4.57, P = 0.002; polyphenol to amino acid ratio: summer tea, F = 1.307, df = 4, t = -5.265, P = 0.006; autumn tea, F = 1.865, df = 4, t = -4.431, P = 0.011).

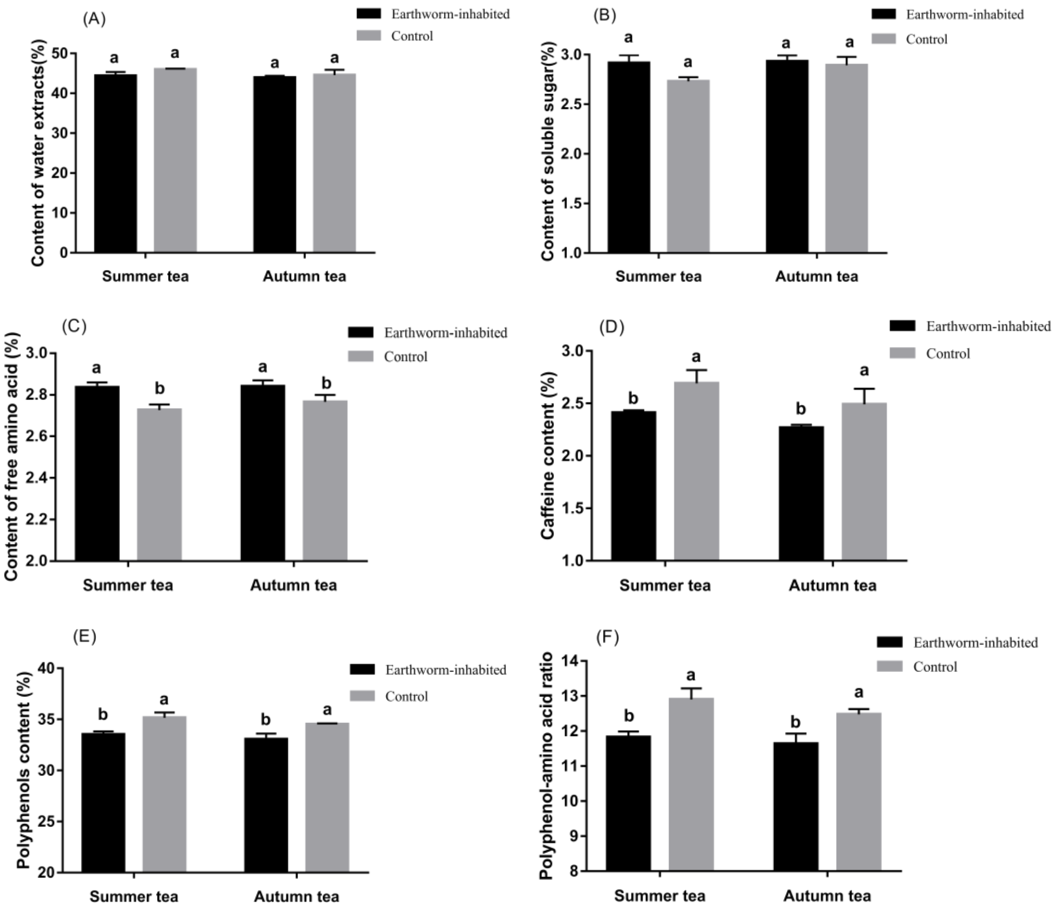

Figure 5. *Quality indicators of summer and autumn tea for the treatment and control groups. The quality parameters includes water extract content (A), soluble sugar content (B), free amino acid content (C), caffeine content (D), polyphenol content (E) and polyphenol to amino acid ratio (F). Bars show mean ± SD and the same letters above the bars indicate no significant differences (independent sample t-test, P < 0.05)*

Principal component analysis

From the score graph (*Fig. 6A*), the four sample groups are well separated for the first principal component with a 65.8% model contribution rate. For the first principal component, treated autumn tea, treated summer tea, control autumn tea and control summer tea were in order from the negative to the positive direction. For the second principal component, the separation of the four sample groups was not clear, with a 14.3% model contribution rate.

From the perspective of load graph (*Fig. 6B*), the parameters that contributed more to the first principal component are FA (free amino acid), HB (hundred bud weight), BD (bud density), C (caffeine), P-A (polyphenol to amino acid ratio) and P (polyphenols).

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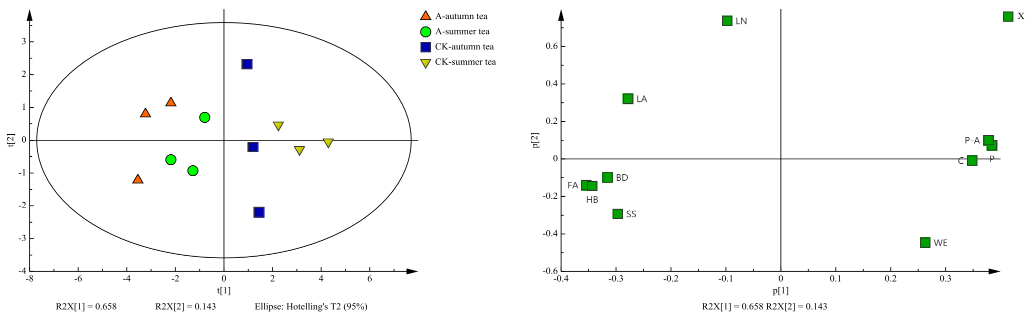
The parameters that contributed more to the second principal component are LN (leaf number), WE (water extracts), LA (leaf area) and SS (soluble sugar). The differences between the control group and treatment group mainly came from FA, HB, C, P-A and P; of these FA and P had more significant roles between control and treatment group, meanwhile these two parameters made the great contribution to different season tea under the same treatment.



A **B**

Figure 6. *Principal component analysis for yield and quality components of tea treated with in situ earthworm breeding. LN, leaf number; LA, leaf area; BD, bud density; FA, free amino acid; HB, hundred-bud weight; SS, soluble sugar; P-A, polyphenols-amino acid; P, polyphenols; C, caffeine; WE, water extracts*

Discussion

Water extracts, free amino acid, polyphenols, caffeine and soluble sugar content are very important indices of tea quality. Indeed, the flavor of a particular tea comes from the balance of these components in the tea leaves (Chen et al., 2010). During the present field evaluation, it was found that in situ earthworm breeding significantly enhanced the tea quality because it significantly increased the content of free amino acids, reduced the content of caffeine and polyphenols, and maintained the total content of water-soluble substances. Lower caffeine and polyphenol contents in particular are good indicators for tea quality since high levels of these components tend to make tea infusions bitter and astringent (Zhang and Wei, 2012). Our results are consistent with other studies on the effect of earthworms on tea plants in other tea-producing regions of China (Tang et al., 2011; Zhou et al., 2017).

Amino acids are the products of nitrogen metabolism in tea plants, which are synthesized in the roots and transported to the young shoots. Tea plants growing in fertile soil and with well-developed roots can produce more amino acids which generally have a fresh and slightly sweet taste. Moreover, they are important ingredients that contribute to the pleasant aroma of tea. Thus, a greater free amino acid content in the plant indicates that the infusion will have a superior aroma and taste (Tang et al., 2011). In our study, the free amino acid content for both summer and autumn tea were increased in the presence of earthworms. Based on our unpublished data, the results demonstrated that inoculating the soil with earthworms significantly enhanced soil fertility, which contributed to the tea plant absorbing more nitrogen from the soil. The term “water extracts” refer to total amount of water-soluble ingredients in tea and contains the following nine groups: alkaloids, polyphenols, free amino acids and proteins, lipids, carbohydrates, vitamins, pigments, minerals, and aromatic compounds.

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These are positively related to the quality of the tea infusion, considering factors such as color, aroma, persistence, and taste (Chen et al., 2010), where persistence is an explanation of the number of one tea can be brew. In this study, although some quality indices were lower in earthworm-treated groups, the content of water-soluble ingredients were not significantly different between the treatment and control groups. The positive correlation between earthworm and plant growth is not systematic, but the primary reason may be the occurrence of earthworm casts, which are also known as vermicompost. The positive effects of vermicompost application for increasing crop quality have been investigated in other horticultural settings (Xiang et al., 2016).

In our study, in situ earthworm breeding significantly promoted tea plant growth and yield; this treatment led to a higher bud density and heavier hundred bud weight, while buds were generally utilized to produce tea and their weight was considered as the one of important quality parameter of tea. Our findings are in-line with other research on the effect of earthworms on tea plants in other tea-producing regions of China (Tang et al., 2008; Zhuo et al., 2007) or in other above-ground plant communities such as *Carica papaya* L., *Plantago lanceolata* L. and *Trifolium repens* L. (Xiang et al., 2016). The following mechanisms may account for these results.

First, earthworm activities can improve the soil structure (e.g. increase porosity) and nutrient content, therefore supplying a better medium for root development; their actions change the soil’s biological, chemical, and physical characteristics which helps the plant to develop (Lee, 1985). A previous study found that soil aggregation and water infiltration were significantly influenced by earthworm communities (Derouard et al., 1997). The function of earthworms in increasing plant yield and growth relies on the synchronization and synlocalization of their activities with the sphere and period of active root development and nutrient requirement. Most earthworm species release considerable quantities of assimilable nutrients in their fresh casts that can be utilized by plants (Lavelle et al., 1992). Moreover, earthworm activities intensely promote soil mineralization, releasing significant amounts of nitrogen (N), phosphorous (P), and potassium (K); an increase in these nutrients may stimulate plant growth and thus enhance the tea yield (Chaoui et al., 2003). In our previous study (unpublished data), we found that the nutrient content of the soil (measured as available N, P, and K) was greater in earthworm-inoculated plots was higher than in the control. This demonstrated that earthworm inoculation results in better conditions for tea plant growth.

Secondly, plant-growth hormones contained within the earthworm casts can regulate tea plant growth. Several studies have suggested that regulators such as auxins, gibberellins, and cytokinins (Krishnamoorthy and Vajrabhiah, 1986; Grappelli et al., 1987; Tomati et al., 1990) and plant-growth-regulating materials such as humic acids (Atiyeh et al., 2002) are present in the casts and can result in better plant growth for many crops (Atiyeh et al., 2002). Our research revealed that in situ earthworm breeding in a tea garden can contribute to enhanced plant growth. Other mechanisms by which earthworms can improve plant growth incorporate interactions with other organisms: (1) earthworms could be beneficial for pest biocontrol, and (2) earthworms could help to spread symbionts via their colonization (Bertrand et al., 2015).

The earthworms in our study were added to the sample plots only once and at a density of only 8 g/m^2. We therefore hypothesize that increasing the earthworm density could have a positive effect on tea leaf yield and quality; this should be examined in a further study. Furthermore, since the price of earthworms is low, agricultural management costs could be decreased by using this method rather than chemical

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fertilizers. However, a drawback is that we found the preparation of earthworm beds in the field to be complicated. Finally, we did not investigate whether this technique is appropriate for other types of tea garden, if other earthworm species are also beneficial, or if this technique maintains the growth and yield performance long-term. These issues require further exploration.

Conclusion

Tea is one of the most well-known and popular drinks found all over the globe. However, the heavy use of chemical fertilizers to achieve higher yields is contributing to serious environmental problems. Whilst organic fertilizers can be used, their application is greatly limited in tea gardens due to problems with low efficiency and effective duration. The present study investigated the effects of in situ earthworm breeding on the growth, yield and quality of tea plants. We demonstrated that earthworm breeding in soil offers the best growth medium for the tea plant and conferred higher leaf quality and total bud yield. The incorporation of earthworm and organic waste in soil could be a potential organic substitute for chemical fertilizers since it not only improves the yield and quality but also decreases (through the action of the earthworms) the amount of organic waste. Therefore, it is advised that the ecological function of the earthworm should be taken into account and intensifies research of the earthworms in other tea produced regions so as to harness its potentials to improve plant performance in the studied regions especially now organic farming is the order of the day. Additionally, extra long-term field investigations will be essential to adequately understand the influences of earthworms on tea quality and yield.

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Author contributions. DH and PMC led the project and designed and conceived the study. PMC, YZS, FHM and YCH prepared the manuscript. DH, KQY and LL performed the data analysis. DH, FHM, PMC and HMZ performed sampling and experiments. All authors revised and approved the final manuscript.

Conflict of interests. The authors declare that they have no competing interests.

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