



Article Coordination of Root Traits and Rhizosphere Microbial Community in Tea (*Camellia sinensis* L.) Plants under Drought and Rehydration

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Abstract: Soil drought and rehydration have an immense impact on plant physiology and productivity, whereas the response of plant-microbe interactions to varied water availability remains largely elusive. In this study, two tea (Camellia sinensis L.) cultivars, Longjing43 and Yingshuang, were subjected to drought followed by rehydration. Soil drought significantly induced the elongation of taproots in the Yingshuang cultivar after two weeks of drought. Moreover, the four-week drought significantly reduced the root dry mass and root nitrogen, phosphorus, and potassium concentrations in both tea cultivars. Two-week rehydration recovered the root potassium concentration in the two tea cultivars, revealing the rapid response of root potassium levels to water conditions. Drought and rehydration also resulted in shifts in rhizosphere microbial diversity. A four-week drought reduced microbial alpha diversity in Longjing43 but not in the Yingshuang cultivar, and rehydration was effective in restoring alpha diversity in Longjing43. The rhizosphere microbial community tended to recover to the initial stages after rehydration in Longjing43 but not in the other cultivar. In addition, 18 microbial genera were identified as the featured microbial taxa in response to varied water availability, and a rare genus Ignavibacterium was significantly increased in the Longjing43 cultivar by rehydration after a four-week drought. Furthermore, root nitrogen, phosphorus, potassium levels, and dry mass were positively correlated with the microbial alpha diversity, while the taproot length was negatively correlated, suggesting the crucial role of plant-microbe interactions in response to drought and rehydration. Moreover, the root phosphorus concentration and taproot length also had significant effects on microbial beta diversity, further confirming their effects on the community structure of the rhizosphere microbiome. Overall, this study provides insights into the effects of drought on plant-microbe interactions in the rhizosphere of tea plants. These findings are important for harnessing the roles of the tea rhizosphere microbiome under drought.

Keywords: tea (Camellia sinensis); drought; roots; rhizosphere; plant-microbe interaction

1. Introduction

Tea (*Camellia sinensis* L.), a woody crop, is one of the most popular beverages in the world. Tea is widely cultivated in tropical and subtropical regions [1] but can acclimate to various climates [2]. However, tea production is facing a major challenge due to the increasing frequency and severity of drought stress, which can affect the growth, yield, and quality of tea products [1]. The effects of soil drought can be passed to leaves and thereby affect the flavor, aroma, and nutrients of tea products [1,3]. With projected future scenarios of increased drought severity, drought-related damage to tea plants has become more common and unexpected in recent years [4]. Therefore, we need to further understand the impact of drought on tea plants and its potential consequences for ecosystem services.



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Drought not only modifies plant physiology but also results in diverse ecological effects in the microbial communities in the rhizosphere [5]. The rhizosphere microbiome is a complex community of bacteria, fungi, archaea, and viruses that live at the interface between plant roots and soil [6]. The rhizosphere microbiome has immense effects on plant health, growth, development, and stress tolerance by modulating nutrient acquisition, the immune system, and secondary metabolism [7]. Drought can reduce the diversity and abundance of plant-associated microorganisms, especially those that depend on plantderived resources, such as root exudates and litter [8]. A study found that the microbial biomass and activity decreased and the composition of microbial communities shifted during periods of scant rainfall in Mediterranean-type ecosystems, which further impacted the carbon cycling in the soil and plant nutrition acquisition [9]. The assembly of the rhizosphere microbiome is strongly influenced by root morphological, physiological, and biochemical traits [6]. Under drought conditions, root traits are useful predictors of plant responses to drought since roots are in direct contact with the soil environment and are essential for absorbing nutrients and water [10]. Recent studies have revealed that root traits are significantly affected by the rhizosphere microbiome under drought. Soil drought can alter root traits such as root exudation and various morphological traits, and these adaptions can alter the discharge of carbon and other nutrients to the rhizosphere and thereby influence the rhizosphere microbial communities [11]. On the other hand, microorganisms can also produce stress-tolerant compounds or enzymes, such as antioxidant enzymes, that enhance plant resilience under drought conditions [11,12]. However, studies are lacking in identifying the response of microbes to drought and their interactions with root traits.

Under drought conditions, plants can seek help from microbial communities through complicated signaling pathways under drought [7]. Increasingly, studies have reported the resistance and resilience of rhizosphere soil microbial communities to drought [13]. The microbiome may improve plant resistance to drought through reactive oxygen species cleavage [14], nutrient acquisition [15], and hormone production [16]. Drought can also affect the communication and signaling between plants and microorganisms, such as through volatile organic compounds and secondary metabolites [13]. These molecules can mediate the recognition, attraction, colonization, and mutualism of plant–microbe interactions under drought conditions. Moreover, studies on rehydration after drought have revealed the persisting effect of droughts on plant–microbe interactions. However, the results remain controversial. For sorghum, it was reported that the enrichment of monoderm bacteria under drought was reduced with rehydration [17]. However, in another study, several endospheric Actinobacteria microbes were induced by drought and their abundance persisted after rehydration [18]. Therefore, more evidence is needed to assess the effects of rehydration on plant–microbe interactions after drought.

Tea plants maintain intricate interactions with the surrounding microorganisms, which have developed during the long history of tea breeding [19] and contribute to tea quality and flavor [20]. However, the effects of drought stress on below-ground microbiomes remain largely elusive. In this study, we aimed to investigate how drought stress affected tea roots and the rhizosphere microbiome in two tea cultivars, Yingshuang (YJ) and Longjing43 (LJ). We also applied machine learning approaches to identify the featured microbial taxa of drought stress and post-drought recovery. Furthermore, we explored the correlations between root traits and microbial diversity. The results revealed the distinct responses of microbial taxa to drought stress and the coordination between root traits and the rhizosphere microbiome in tea plants. These findings provide new insights into plant–microbe interactions under varied soil water availability.

2. Materials and Methods

2.1. Plant Materials and Sampling

Two tea (*C. sinensis*) cultivars, Loingjing43 (LJ) and Yingshuang (YS), were planted in the greenhouse of Lishui University, Lishui City, Zhejiang Province, China. These two elite cultivars were bred in Zhejiang and have wide plantation areas for green tea production [21,22]. Two-year-old tea seedlings with an average height of 25–30 cm were transferred to pots with soil from a tea garden (28.51° N, 119.86° E). The soil had been used for tea production for more than three decades and the average pH was 4.7 (determined with a PHS-3C pH meter, Leici, Shanghai, China). The seedlings were grown under a rain shelter and irrigated regularly for 15 days before treatment. The whole treatment lasted for six weeks with four weeks of drought and two weeks of rehydration. For each of the tea cultivars, 30 pots were irrigated with 500 mL of water every other day throughout the treatment (CK). Drought treatment (DT) was conducted on 30 pots by reducing the irrigated water to 20% of the level of the control treatment (100 mL). After two-week and four-week treatments, respectively, three replicates of each treatment (RT) was conducted by irrigating the droughted seedlings with 500 mL of water every other day.

For each of the biological replicates, three seedlings were randomly selected and grouped. The top ~5 cm of soil was removed. Tea roots were manually shaken to remove soil loosely attached to the roots and transferred to a 100 mL phosphate-buffered saline solution (Macklin, Shanghai, China). The washed-off soil was then centrifuged at $3000 \times g$ to collect the rhizosphere soil [19]. Roots were continuously rinsed with deionized water three times and dried at 70 °C for 48 h. Soil samples were stored at -80 °C before DNA extraction.

2.2. Root Trait Determination

Taproot length was measured with a ruler. Root dry mass was determined with a scale (Sartorius, Goettingen, Germany). The root sample was crushed and digested with H_2SO_4 ; the nitrogen (N) concentration was determined with a flow injection analyzer [23], the phosphorus (P) concentration was determined with the molybdenum antimony colorimetric method [24], and the potassium (K) concentration was determined with a flame spectrophotometer [25]. The roots were digested with HNO_3 - $HClO_4$ (10:1, v/v), and the calcium (Ca) concentration was determined with inductively coupled plasma atomic emission spectrometry [26].

2.3. Microbial Amplicon Sequencing and Analyses

About 0.5 g of rhizosphere soil was used for DNA extraction with a PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA). The V5-V7 regions of bacterial 16S rRNA genes were amplified with a pair of primers (799F and 1193R) [27]. An Illumina NovaSeq platform (Personal Bio, Shanghai, China) was used to perform 250-bp pairend amplicon sequencing. The raw reads were quality-controlled and processed with the UPARSE pipeline [28]. The operational taxonomic units (OTUs) were clustered at 97% similarity. The OTUs were annotated to phylum, class, order, family, and genus taxa with the 16S rRNA reference database RDP Version 16 [29]. With R software, the OTU count table was used to build phyloseq objects with the phyloseq v1.42.0 R package [30] and normalized with the DESeq2 v1.38.3 R package [31]. The weighted UniFrac dissimilarities and alpha diversity (Shannon index) were extracted from the phyloseq objects.

2.4. Statistics

The mean values of root traits, microbial alpha diversity, and microbial composition were compared between treatments with a one-way ANOVA and Tukey's test. The microbial dissimilarity was visualized with principal coordination analysis (PCoA). Linear regressions were performed with the 'lm' function in R. To disclose the relationships between root traits and the microbial community, the root traits were regressed to microbial dissimilarity matrices with the multivariate distance matrix regression (MDMR) analysis in the MDMR v0.5.0 package for R [32], and the significance of each of the root traits was evaluated with the analytical *p*-values [33]. Random forest classification was performed in the randomForest v4.7-1.1 R package to identify the featured microbial taxa by employing the experimental treatments as predictors and microbial relative abundance as features.

A ten-fold cross-validation was conducted to determine the optimal number of microbial taxa in the classification model [34]. The importance scores of the identified microbial taxa were ranked.

3. Results

3.1. Root Traits under Drought Conditions

Tea seedlings were treated with four weeks of drought. The root chemical composition was altered by drought treatment (Figure 1). A four–week drought significantly reduced root N, P, and K levels in the two tea cultivars (Figure 1a–c). Noticeably, two-week rehydration restored the levels of root K concentration. The root Ca concentration was less affected by drought compared with the other elements (Figure 1d).



Figure 1. Root traits of two tea (*Camellia sinensis* L.) cultivars under drought and rehydration. Two tea cultivars, Longjing43 (LJ) and Yingshuang (YS), were treated with a four-week drought (DT) followed by two-week rehydration (RT). DT and well-watered (CK) plants were sampled at two and four weeks, respectively. (**a**–**f**) Root nitrogen (N, mg/g), phosphorus (P, mg/g), potassium (K, mg/g), and calcium (Ca, mg/g) concentrations; taproot length (RL, cm); and root dry mass (DM, g) were determined (n = 3). Mean values were compared with a one-way ANOVA, and the letters on the boxes indicate significant differences among all experimental treatments based on Tukey's HSD.

The droughted YS plants showed longer taproots than the well-watered plants after two weeks of treatment, but the difference in the root length was not observed in the LJ cultivar (Figure 1e). Root dry mass was markedly reduced compared with the control treatments in both the LJ and YS cultivars (Figure 1f).

3.2. Shifts in Rhizosphere Microbial Diversity under Drought

Microbial alpha diversity was evaluated in the LJ and YS cultivars. A four-week drought reduced rhizosphere alpha diversity in LJ but not in the YS cultivar (Figure 2a,b). A two-week rehydration recovered the alpha diversity in the rhizosphere of the LJ cultivar (Figure 2a).



Figure 2. Rhizosphere microbial alpha diversity of two tea cultivars. LJ (**a**) and YS (**b**) rhizosphere microbial communities were examined with 16S rRNA amplicon sequencing. Alpha diversity was estimated based on the Shannon index. Control (CK) and drought (DT) treatments were sampled at two and four weeks of treatment, respectively. A two-week rehydration (RT) was conducted followed by a drought. Mean values were compared with a one-way ANOVA (n = 3), and the letters on the boxes indicate significant differences among all experimental treatments based on Tukey's HSD.

To explore the microbial community structure upon soil drought over the sampling period, we performed ordination based on weighted UniFrac dissimilarity (beta diversity) (Figure 3). Compared with the well-watered plants, a four-week drought resulted in distinct rhizosphere microbial communities in both the LJ and YS cultivars. Interestingly, the microbial community of the LJ cultivar tended to recover to the initial stages after a two-week rehydration, but this trend was not obvious in the YS cultivar.



Figure 3. Rhizosphere microbial community structure. Microbial community structure was analyzed based on the weighted UniFrac dissimilarity of the LJ (yellow color) and YS (blue color) cultivars and visualized with the principal coordination analysis (PCoA). Conrol (CK, open symbols), and drought (DT, filled symbols) treatments were sampled at two (squares) and four (circles) weeks of treatment, respectively, followed by a two-week rehydration (RT, triangles).

3.3. Featured Microbial Taxa under Drought and Rehydration

The random forest method was employed to identify the microbial genera responding to drought treatment. With a ten-fold cross-validation, the machine learning model identified 18 microbial genera as the most important taxa in response to the drought treatment (Figure 4a).



Figure 4. Featured microbial genera in response to water availability. (a) The rank of feature importance in the random forest model. The relative abundance of the 18 microbial genera were compared with a one-way ANOVA across treatments, and the significant comparisons ($p \le 0.05$) in the LJ (b) and YS (c) cultivars are shown. The values of the microbial relative abundance were \log_{10} transformed. The letters in the blocks indicate the results of Tukey's test for each microbial genus across all treatments. The asterisks on the left of the heatmaps indicate the significant differences compared between the treatments for each microbial genus (one-way ANOVA, $\alpha = 0.05$). * $p \le 0.05$, ** $p \le 0.01$. CK: control treatment, DT: drought treatment, RT: rehydration treatment.

The relative abundance of the 18 genera was compared across the experimental treatments in the LJ and YS cultivars, respectively (Figure 4b,c). Six microbial genera exhibited significantly different compositions across treatments in the LJ cultivar (Figure 4b). The four-week drought resulted in significant declines in the relative abundance of *Taonella* (mean value = 0.1% in all LJ plants) and *Clostridium_XI* (0.3%) genera; moreover, the relative abundance of a rare taxa *Ignavibacterium* (0.02%) was significantly increased in the LJ cultivar with rehydration compared with the plants under a four-week drought (Figure 4b). In contrast, the relative abundance of eight genera differed between treatments in the YS cultivars (Figure 4c). After four weeks of treatment, droughted plants harbored significantly more abundant *Hyphomicrobium* (0.3%) and *Bosea* (0.02%) genera; the following rehydration treatment did not change the relative abundance of the two genera (Figure 4c).

3.4. Associations between Tea Root Trait and Rhizosphere Community

Microbial alpha diversity was linearly regressed to root traits to discover the affecting factors of the microbial community. The root N, P, K concentrations and dry mass exhibited significantly positive correlations with the Shannon index, whereas the tap root length was negatively correlated with the Shannon index (Figure 5).



Figure 5. Relationships between rhizosphere microbial alpha diversity and root traits. (**a**–**f**) Microbial Shannon index was linearly regressed to root nitrogen (N, mg/g), phosphorus (P, mg/g), potassium (K, mg/g), and calcium (Ca, mg/g) concentrations; taproot length (RL, cm); and root dry mass (DM, g). The adjusted R-squared and *p* values are shown, and regression lines were added if $p \le 0.05$.

Using the permutations method, we also examined the effects of root traits on microbial beta diversity (Figure 6). The six determined root traits together explained 32.9% of the variance in microbial dissimilarity (MDMR method, p < 0.001). Among them, root P concentration ($R^2 = 0.05$, p = 0.01) and taproot length ($R^2 = 0.06$, p = 0.04) exhibited significant effects on microbial beta diversity, while no significance was detected for other traits.



Figure 6. Effects of root traits on microbial beta diversity. MDMR analysis was employed to examine the effect sizes of individual root traits and overall effect (omnibus effect). The *p*-values ($-\log_{10}$ transformed) are shown, and the significant level of $\alpha = 0.05$ is represented with a dashed vertical line. Root nitrogen (N, mg/g), phosphorus (P, mg/g), potassium (K, mg/g), and calcium (Ca, mg/g) concentrations; taproot length (RL, cm); and root dry mass (DM, g).

4. Discussion

In this study, we showed that drought stress affects both root characteristics and rhizosphere microbial populations in two tea cultivars. Several root characteristics were shown to be closely related to the alpha and beta diversities of rhizosphere microbial populations. Furthermore, we discovered a distinct collection of microbial genera that reacted to drought and rehydration treatments. The two tea cultivars differed in their responses of the microbial community to drought and rehydration, indicating a genotype-specific response to varying water availability. These findings add to the knowledge of how drought affects plant–microbe interactions at the rhizosphere interface.

4.1. Changes in the Rhizosphere Microbial Community under Drought and Rehydration

In this study, we demonstrated that drought affects the microbial community structure (beta diversity) of the two examined tea cultivars. It is well acknowledged that the rhizosphere microbiome is shaped by both plant and soil characteristics [35,36]. The decrease in soil moisture directly affects the survival and activity of microorganisms in the rhizosphere environment, which disrupts microbial communities and alters their composition. Moreover, the chemical composition and the morphological traits of tea roots exhibited significant changes under drought in our results (Figure 1), which may have contributed to the shifts in the rhizosphere microbial community by altering the availability of nutritional resources for the microbes. As consistently shown in other studies, drought leads to changes in diverse plant physiological and biochemical processes, and thereby, has a direct or indirect impact on the rhizosphere microbial community [10,37]. For instance, drought modifies the quantity or composition of root deposition into the surrounding soil [38]. The deposition of root litter serves as nutritional resources for microorganisms, and thus alterations due to drought can influence microbial communities.

Interestingly, the microbial beta diversity tended to recover to the well-watered stage after rehydration in the LJ tea cultivar but not in the other cultivar (Figure 3). This may have been derived from the cultivar-specific response to drought and rehydration, which was consistent with another study showing the differential rhizosphere microbial communities in two sugarcane varieties under drought stress [39]. Similarly, the microbial alpha diversity also recovered to the well-watered stage in the LJ cultivar (Figure 2), indicating the effective recovery after a two-week rehydration in the LJ cultivar. However, the microbial beta diversity in the LJ cultivar was still separated from the control treatment, possibly suggesting a persisting effect of drought on the rhizosphere microbial community. In parallel with the changes in the rhizosphere microbiome during the two-week rehydration, most of the determined root traits of the two tea cultivars remained unchanged, except for the root K concentration, compared with the tea plants droughted for four weeks (Figure 1); these results also confirmed the persisting effect of drought on tea plants. The root K concentration dropped under drought conditions due to the reduced K mobility and the inhibition of root membrane K transporters [40]. Further analysis in this study also showed the relationship between root K concentration and microbial alpha diversity (Figure 5). Therefore, the changes in the rhizosphere microbial community after rehydration may have been partially derived from the recovery of root physiology including root K homeostasis. Other studies have also reported that microbial communities can rapidly recover from abiotic and biotic stresses, but the community structure may not return to the initial stage [41]. For instance, prolonged drought alters the resilience and temporal dynamics of the root microbiota in rice plants, with specific taxonomic groups showing enrichment during drought and persisting effects on the recovery response of plants [18]. Plants may recruit specific microbes to jointly cope with the challenge of limited water availability as a "cry for help" mechanism [42]. Thus, deciphering the changes in the rhizosphere microbial community can provide clues to promote plant health under drought and rehydration.

4.2. Specific Microbial Taxa in Response to Water Availability

The identification of featured microbial taxa in the tea rhizosphere provided strong evidence for microbial adaptation to drought and rehydration. Using a random forest method, 18 microbial genera were identified as the featured taxa in response to the experimental treatments (Figure 4a). Further comparisons across the experimental treatments indicated that these taxa differentially responded to soil water availability. Interestingly, the *Ignavibacterium* genus showed a very low abundance (0.02%) in the rhizosphere soil of the LJ cultivar but was induced by the rehydration treatment after drought stress (Figure 4b). The Ignavibacterium genus is involved in denitrification [43], and this biogeochemical process is inhibited by soil drought [44]. Thus, the rehydration treatment may have affected the abundance of Ignavibacterium by inducing denitrification activities in the rhizosphere soil of the LJ cultivar. In the YS cultivar, two genera of *Hyphomicrobium* and *Bosea* were induced by the four-week drought compared with the well-watered plants (Figure 4c), whereas their role under drought has not been reported to the best of our knowledge. The observed changes in the compositions of microbial genera in response to drought and rehydration likely represented microbial shifts in the whole community [45]. A few studies have also employed machine learning methods for the identification of featured microbial taxa in response to drought [18,46]. The employment of machine learning approaches increased the efficiency in handling high-dimensional microbial data [46]. However, one drawback of this study was the lack of follow-up studies validating the roles of the identified microbial taxa in response to drought. Part of the reason can be attributed to the unavailability of cultured microbial strains. Therefore, further efforts should be made, including the separation of microbes from the rhizosphere soil and the co-culture with tea plants under drought and rehydration.

Noticeably, a few microbial genera identified above exhibited differential abundances when comparing the control treatments at two and four weeks in both the LJ (*Actinoplanes* and *Hyphomicrobium*) and YS (*Terriglobus*, *Gp1*, and *Motilibacter*) cultivars. The microbial

diversity and composition in the rhizosphere are heavily shaped by the root activities, which change over plant growth periods [47,48]. Thus, our results possibly reflected the effect of plant growth on the rhizosphere microbiome.

4.3. The Associations between Root Traits and The Rhizosphere Microbial Community

Root morphological and chemical traits exhibited wide associations with tea rhizosphere microbial communities. These results partially explained the source of variation in the rhizosphere microbial community under varied water availability and suggested the feasibility of trait-based approaches in the study of drought-induced ecological effects in the holobiont consisting of plants and microbiome [49]. Numerous studies have quantified the impact of drought on root traits [18,50,51]. Trait-based models have also helped to simulate the interactions between plants and the environment. At the community level of the microbiome, microbial species diversity and community dissimilarity were useful in quantitating microbial variation since these indices take into account both microbial complexity and the ease-of-use principle. Promisingly, several studies have also employed trait-based approaches to build links between the microbiome and plant traits [52–54].

In our results, the rhizosphere microbial alpha and beta diversities showed differential interactions with the morphological and chemical traits. We found that the rhizosphere microbial alpha diversity could be predicted with root N, P, and K concentrations and dry mass (Figure 5). The plant rhizosphere is attractive to microbes in the surrounding soil since it provides a nutrient-rich niche for microbes [55]. Therefore, our results likely revealed the coordination of root traits and the rhizosphere microbial community under drought. Consistently, other studies have also reported that plant root traits and the microbial community collectively responded to drought [18,39]. The interaction between root traits and microbial diversity has important implications for the role of plant-microbe interactions in response to drought stress. The changes in the root traits and the structure of the rhizosphere microbial community can enhance plant performance under drought and hence promote plant health and tolerance [39]. In contrast, root length was negatively correlated with microbial diversity (Figure 5). The elongation of roots under drought has been observed as a responsive strategy in different species [56]. Longer roots are favorable for plants obtaining water from deeper soil. However, root dry mass was reduced by drought and thus the elongated roots resulted in no increase in nutrient input for the rhizosphere microbes.

In association with the microbial beta diversity, we showed that root P concentration and root length were the dominating root traits (Figure 6). Phosphorus is a limiting factor for both plants and microbes in diverse soils, and thus plants may compete with microbes for P, especially under limited nutrient conditions [57]. Under drought conditions, P movement is restricted by reduced water availability and the hampered transporters in roots [58]. The forms and availability of P can be affected by soil microbial activities [59], which are also affected by drought. Plants can adjust their growth, morphology, and physiochemical characteristics during drought, and the modified root traits may absorb more water and nutrients including P [58]. Plant adaption to drought can be further enhanced by microbial activities [60]. Therefore, our results highlighted the crucial role of P in mediating plant–microbe interactions under drought in the rhizosphere, which should be considered in future studies aiming at harnessing the rhizosphere microbiome for drought-resilient agricultural practices [11].

5. Conclusions

In this study, we examined the impact of drought and rehydration on both the root characteristics and rhizosphere microbial community of two tea cultivars. Several root characteristics were tightly correlated with microbial diversity in the rhizosphere. Moreover, the responses of the microbial community to drought and rehydration treatments differed between the two tea cultivars, indicating a genotype-specific response to water availability. Overall, these findings contribute to our understanding of how drought affects plant–soil interactions in the rhizosphere, and the results highlighted the interactions between root traits and the rhizosphere microbiome under drought and rehydration.

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