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Effect of tea soil acidification on the diversity and function of fungi community

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Summary

Soil fungi play an important role in the process of planting tea tree. However, effects of acidification on the diversity and function of the fungi community in tea rhizosphere soil have been rarely reported. In this study, tea rhizosphere soils with pH of 3.3, 4.7, 5.3 and 6.4 were investigated for diversity and function of fungal communities through T-RFLP technology. The results showed that the abundance and diversity of fungi increased significantly with the decrease of pH value of rhizosphere soil. The results of significance analysis showed that 38 T-RFs fragments were significantly correlated with pH value, among which 32 were negatively correlated with pH value and 6 were positively correlated with pH value. After database comparison, 23 fungi were identified and classified according to their nutritional patterns, which can be divided into four types, including pathotroph, symbiotroph, saprotroph and unknown, accounting for 36.85%, 7.89%, 15.79% and 39.47%, respectively. At pH value of 3.3, the fungus abundance reached the maximum value. In conclusion, acidification leads to changes in the structure and diversity of the fungi community in tea rhizosphere soil, specifically, a significant increase in the number and species of fungi, of which the pathotroph type is the largest. This study provides an important theoretical basis for controlling fungal diseases of tea tree in acidified tea plantations.

Keywords: tea tree; rhizosphere soil; pH value; fungi community structure; T-RFLP

Introduction

As one of the most important cash crops in China, tea has a long history of cultivation (Jia et al., 2019). A good soil ecosystem ensures the healthy growth of tea trees. Soil bacteria, fungi and other microorganisms are an important part of the soil ecosystem. Fungi not only degrade complex compounds better than bacteria, but also play an important role in rhizosphere soil nutrient cycling. Wang et al. (2016) found that a highly significant correlation exists between soil fertility and changes in the number of soil fungal population in tea plantations. Wu et al. (2011) found that factors such as soil pH and available phosphorus affect the number of soil fungi and bacteria. Lin et al. (2012, 2013) found that soil pH showed a downward trend with increasing age of planted tea trees and eventually severe soil acidification, and further determined soil microbial community diversity using T-RFLP technique. In conclusion, tea tree root secretions accumulate in the soil, leading to soil nutrient imbalance and abnormal soil acidity, which eventually leads to an increase in the number of inter-root harmful microorganisms (Jia et al., 2019; Wang et al., 2016).

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Terminal restriction fragment polymorphism (T-RFLP) is a comprehensive molecular fingerprinting technique with high resolution and good reproducibility (Sun et al., 2009). With the continuous improvement of experimental techniques, it has been widely used in the analysis of microbial communities.

On the basis of previous studies (Wang et al., 2018a), the present study classifies and identified fungi in the rhizosphere soil of tea plants with different acidity, and further elaborates the interaction between various fungi and soil acidity to provide some theoretical basis for the restoration of soil acidification in tea plantations.

Material and methods

Materials

The research was carried in Long Juan township, Anxi county, Quanzhou city, Fujian province (East Longtitude, 117°93´, Northern Latitude, 24°97´). On the basis of previous research (Wang et al., 2018a, 2018b), four tea plantations with different acidity in the same area, labeled S1, S2, S3 and S4, with a total area of about 500 m^2, were selected as the study sites and planted with 4-year-old Tieguanyin tea trees.

The soil pH values of the four tea plantations were 3.3, 4.7, 5.3 and 6.4, respectively. Their basic physicochemical indexes are shown in Tab. 1. The sampling method for rhizosphere soil of tea plants was as follows: six tea plants were randomly selected from each tea plantation, the impurities on the soil surface were removed, tea plants were dug up and rhizosphere soil of tea leaves was collected. The sample size of each sample was about 1 kg, and each sample had 3 replicates.

T-RFLP analysis of soil fungi

CTAB – protease K – liquid nitrogen freezing-thawing method was used to extract total microbial DNA from tea rhizosphere soil with different acidity (Jia et al., 2019). The PCR reaction procedure was: pre-denaturation at 94 °C for 3 min, denaturation at 94 °C for 45 s, annealing at 51 °C for 60 s, extension at 72 °C for 60 s, and the above steps account for 30 cycles, finally extension at 72 °C for 10 min. The total volume of the PCR reaction system was 25 μL, and the reaction system contain 12.5 μL 2×Taq Master Mix, 1μL of each ITS1-FAM and ITS4 primers (10 μmol/L), 1 μL DNA template, 9.5 μL ddH_2O. At the end of PCR, 5μL amplification product was detected by 2% agar-gel electrophoresis, and about 600 bp of PCR product were recovered using UNIQ-10 column DNA gel recovery kit for digestion.

The amplification products were digested with *Hinf* 1 incision enzyme. The enzyme digestion system comprises 1 μL *Hinf* 1, 2 μL 10×Buffer, 7 μL ddH_2O, and 10 μL PCR products. The configured enzyme digestion system was placed at 37 °C water bath for 5 hours. The product after enzyme digestion was desalted, and combined with

Tab. 1: Basic physicochemical indexes of tea plantation soils with different acidity.

Sample	Total nitrogen (g/kg)	Total phosphorus (g/kg)	Total potassium (g/kg)	Available nitrogen (mg/kg)	Available phosphorus (mg/kg)	Available potassium (mg/kg)
S1	2.63±0.14	1.35±0.12	1.76±0.05	27.64±0.15	77.45±1.23	305.26±4.35
S2	2.61±0.16	1.38±0.03	1.72±0.08	27.17±0.31	78.04±1.05	301.37±3.72
S3	2.58±0.19	1.34±0.08	1.73±0.12	27.16±0.23	77.17±1.16	303.26±2.38
S4	2.64±0.13	1.39±0.04	1.69±0.09	27.48±0.22	77.59±0.87	300.57±4.47

Note: Different lowercase letters indicate the significant difference at $P < 0.05$ levels among soil of different sample.

sample buffer, standard Marker (Genscan-500, Applied Biosystems). The above mixture was denatured at 96 °C for 4 minutes, then quickly transferred onti ice, and then determined by the ABI sequencing automatic analyzer (Model 3130 Applied Biosystems).

Data analysis

The sequencing results were analyzed with GeneMarker V1.2 software, and the parameters were set according to the SoftGenetics Application Note July, 2006. The length of T-RFs obtained from T-RFLP was compared with Ribosomal Database Project II and the results of DICKIE et al. (2002) and BUCHAN et al. (2003) to identify the corresponding microbial species. The abundance percentage (Ap) of each T-RF is calculated according to the formula Ap = ni/N * 100, where ni represents the peak area of the distinguished T-RF and N represents the sum of all t-RF peak areas (HORSWELL et al., 2002). Circos plot analysis was carried out by the method of analyzing online on http://mkweb.bcgsc.ca/tableviewer/, consulting KRZYWINSKI et al. (2009). Secondly, the function of the microbes corresponding to each T-RF were compared using the FUNGuild version 1.0 database (NGUYEN et al., 2016). The database included three nutrient models, namely, symbionts, humus and pathogens. FUNGuild assigns function according to the matches at the genus and species level, together with confidence levels including *highly probable*, *probable*, and *possible*. Functionality prediction was achieved according to the assessments given in existing research. EXCEL was used for data classification and variance analysis, and DPS data processing system was used for significance analysis.

Results and discussion

Structural diversity analysis of fungal communities in tea rhizosphere soils

In this study, the T-RFLP technique was used to analyze the changes in the number and species of fungi in tea rhizosphere soil with different pH values (Fig. S1, S2, S3 and S4). The fragments of T-RFs of soil fungi and their abundance were obtained by T-RFLP analysis (Tab. S1). Analysis results of Circos plot showed that the number and abundance of T-RFs fragments of fungi in tea rhizosphere soil showed a decreasing trend with the increase of pH value (3.3 ~ 6.4) (Fig. 1). It can be seen that the species and abundance of soil fungi increase with the increase of soil acidity. Further analysis showed

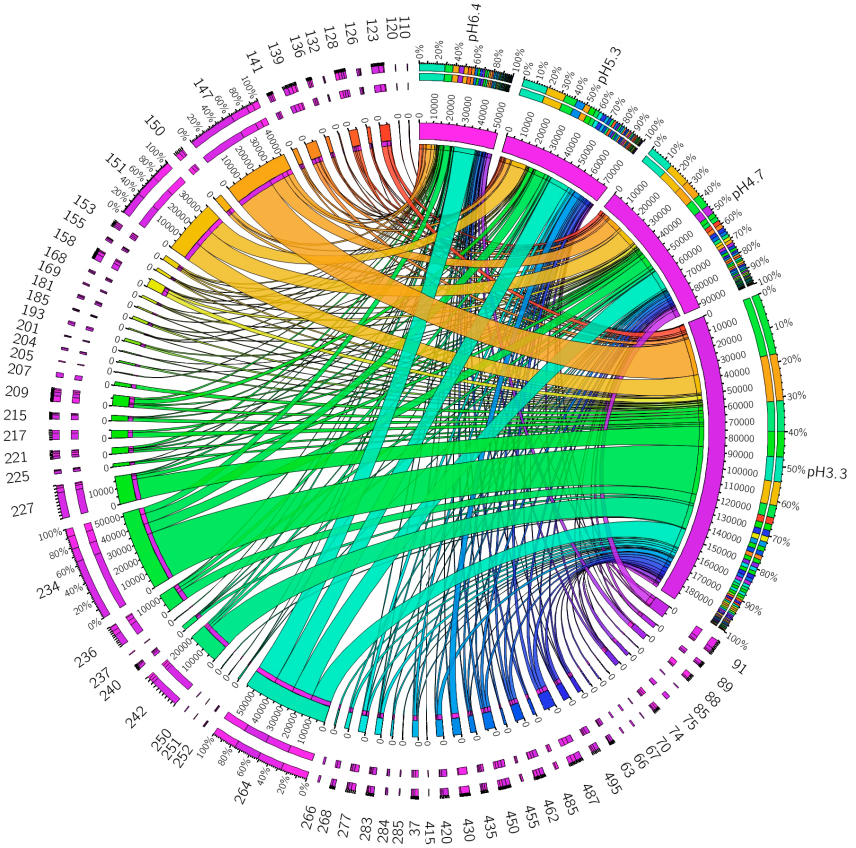

Fig. 1: The Circos plot representing the changes of T-RFs fragments of fungi in rhizosphere soil of tea trees at different soil pH values.

that when the pH value of rhizosphere soil was 3.3, the dominant T-RFs fragments in soil fungi were 147 (13.5%) and 234 (17.6%), When the pH value was 4.7, the dominant T-RFs fragment was 147 (10.4%), 151 (13.2%) and 264 (15.0%), When the pH value was 5.3, the dominant T-RFs fragment was 151 (14.5%) and 264 (18.6%). At 6.4 of pH value, the dominant T-RFs fragment was 264 (28.3%). It can be seen that as soil acidity increased, the structure of rhizosphere soil fungi community changed significantly.

Correlation analysis between pH value of tea rhizosphere soil and T-RFs fragment of fungi

The results of correlation analysis showed that 38 fungal T-RFs fragments were significantly correlated with the pH value of tea rhizosphere soil, among which 32 T-RFs fragments were significantly negatively correlated and 6 T-RFs fragments were significantly positively correlated (Tab. 2). Further analysis on the 32 T-RFs fragments found, nine in all soil, six in soil with pH 3.3, 4.7 and 5.3, 8 in soil with pH 3.3 and 4.7, and nine only in soil with pH 3.3 (Fig. S5, Fig. S6). Among the six T-RFs fragments with significant negative correlation, four were detected in soil with pH 6.4, 1 was in soil with pH 6.4 and 5.3, and 1 was in all soil (Fig. S5, Fig. S6). The results of the T-RFs fragment area analysis showed that the total T-RFs fragment area and the negative correlation T-RFs fragment area showed a significant decreasing trend with the increase of the pH value of tea rhizosphere soil, while the T-RFs fragment area with positive correlation showed a significant increasing trend (Fig. 2). It can be seen that, as soil acidity increased, the structure of fungi community in tea rhizosphere soil changed, and the number of fungi changed significantly.

According to the research of many scholars and the standard of "environmental and technical conditions of tea producing areas" of the Ministry of Agriculture, China, the pH range between 4.5 and 5.5 is moderate, and out of this range, the conditions are unsuitable for tea plant (Wang et al., 2020c; Mehra and Baker, 2017; Nejatolahi et al., 2014). Wang et al. (2018b) found that soil acidity in tea plantations was significantly associated with the yield and quality of tea leaves. As soil pH value decreased, the yield and quality of tea leaves also decreased. Wang et al. (2018a) found that as pH of tea rhizosphere soil decreased, the number of culturable microbe present a download trend, specially, the number of bacteria decreased and the number of fungi increased. Then analysis of PFLA and qRT-PCR technology also proved that lower pH can cause the rise of fungi in the number. In this study, as tea rhizosphere soil

Tab. 2: Correlation analysis of T-RFs fragment area and soil pH value.

T-RFs fragment (bp)	Correlation coefficient	T-RFs fragment (bp)	Correlation coefficient	T-RFs fragment (bp)	Correlation coefficient	T-RFs fragment (bp)	Correlation coefficient
37	0.300	136	0.782	207	-0.847	268	-0.930*
63	-0.949*	139	-0.524	209	-0.241	277	-0.205
66	-0.947*	141	-0.254	215	-0.957*	283	-0.842
67	-0.947*	147	-0.939*	217	-0.942*	284	-0.942*
70	-0.947*	150	0.841	221	-0.264	285	-0.947*
74	0.941*	151	-0.936*	225	-0.872	415	0.931*
75	-0.937*	153	-0.207	227	-0.935*	420	-0.978*
85	-0.937*	155	-0.758	234	-0.932*	430	0.313
88	0.808	158	-0.945*	236	-0.964*	435	0.394
89	-0.585	168	-0.506	237	0.941*	450	-0.614
91	-0.420	169	0.107	240	-0.895	455	0.642
110	-0.947*	181	-0.997**	242	-0.937*	462	-0.964*
120	-0.947*	185	0.330	250	-0.947*	485	0.931*
123	-0.969*	193	-0.931*	251	0.951*	487	-0.938*
126	-0.871	201	-0.934*	252	0.612	495	-0.972*
128	0.761	204	-0.933*	264	0.731		
132	-0.942*	205	0.941*	266	-0.947*		

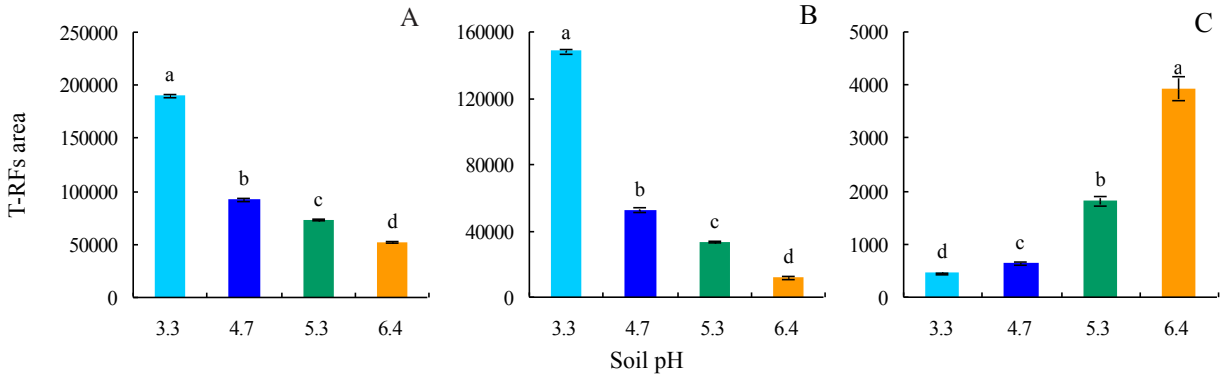

Fig. 2: The area of T-RFs in soils with different pH values was significantly correlated with pH values.
Note: A: T-RFs significantly correlated with pH; B: T-RFs significant negative correlation with pH value; C: T-RFs significantly positively correlated with pH value. The lowercase letters represent a significant level at $P < 0.05$.

acidity increased, the species and abundance of fungi in soil increased, and the dominant fungus population changed with soil acidity (Fig. 1, Tab. S1). The results confirmed that the increase of soil acidity could change the number of fungi in soils, its diversity and community structure, and that the number of fungi and the diversity of community structure increased with soil acidity.

Analysis of fungal species and trophic mode in rhizosphere soil of tea tree

According to the database comparison and analysis of fungal T-RFs fragments, a total of 23 species of fungi were identified, and 15 fungal T-RFs fragments could not be identified temporarily due to the limitation of the database (Tab. 3). According to the nutritional model of fungi, it can be divided into four different types, including pathotroph, symbiotroph, saprotroph and unknown. Among them, there were 14 pathtroph fungi (36.85%), three symbiotroph fungi (7.89%), six saprotroph fungi (15.79%), and 15 unknown fungi (39.47%), respectively (Fig. 3). The analysis results of fungus abundance of different nutritional models showed that the abundance of the four nutritional models all showed a significant decreasing trend with the increase of the pH value of tea rhizosphere soil (Fig. 3). At soil pH of 3.3, the abundance of the four nutritional models were maximum. In brief, as soil acidity increased, the number of fungi in tea rhizosphere soil increased, and manifested in the significant increase in the number of pathotroph fungi.

Pathotroph fungi mainly attack host plant cells to obtain nutrients for reproduction, leading to crop disease and stunted growth (Anthony et al., 2017). Symbiotroph fungi play a certain role in the growth of crops and can promote the absorption of nutrients by crops (Rouphael et al., 2015; Igiehon et al., 2017). The propagation of saprotroph fungi depends on the humus in soil. The large amount of humus produced by crops after aging is conducive to the propagation of saprotroph fungi, while the increase in the number of saprotroph fungi can inhibit the propagation of symbiotroph fungi and promote the propagation of pathotroph fungi (Schmidt et al., 2019; Dai et al., 2018; Toropova et al., 2018). In this study, the proportion of pathotroph, symbiotroph, and saprotroph fungi in tea rhizosphere soil was 36.85%, 7.89%, 15.79%, respectively. At soil pH of 3.3, Pathotroph, symbiotroph and saprotroph fungi had the highest abundance (Tab. 3, Fig. 3). So, as soil acidity decreased, the number of pathotroph-type fungi in soil increased, and tea tree becomes more susceptible to pathogenic organisms, and the growth of tea tree is blocked, and the aging is accelerated. The growth of saprotroph fungi in tea rhizosphere soil was accelerated because of the senescence of tea tree. Normally, accelerating breeding of saprotroph fungus affect symbiotroph fungi reproduction, however, this study found that the number of three symbiotroph fungi increased, especially fungi with 234 bp of T-RFs fragment length. At soil pH of 3.3, its abundance accounted for 98% of total symbiotroph fungus, and after database comparison, the above fungus is *Suillus intermedius*. It has been reported that catechin and epicatechin gallic acid salts facilitate the reproduction of *Suillus intermedius*, thereby increase its number (Koide et al., 1998). Tea leaves shed by tea tree contain large amounts of catechins and their components. And under the attack of Pathotroph fungus, tea tree senescence is accelerated, further in-

Tab. 3: The T-RFs fragments with significant correlation to soil acidity and their corresponding fungi and trophic mode.

T-RFs fragment (bp)	Organism name	Family	Class	Trophic mode
74	*Phaeosphaeria spartinicola*	Phaeosphaeriaceae	*Dothideomycetes*	Pathotroph
75	*Cercospora asparaagi*	Dematiaceae	*Fungi Imperfecti*	
85	*Fusarium oxysporum*	Tuberculariaceae	*Fungi Imperfecti*	
110	*Amanita muscaria* var. *formosa*	Amanitaceae	*Agaricomycetes*	
123	*Tylopilus felleus*	Boletineae	*Agaricomycetes*	
132	*Bionectria* spp.	Bionectriaceae	*Sordariomycetes*	
147	*Verticillium dahliae*	Plectosphaerellaceae	*Sordariomycetes*	
158	*Alternaria tenuissima*	Dematiaceae	*Fungi Imperfecti*	
215	*Boletus pallidus*	Boletineae	*Agaricomycetes*	
217	*Penicillium oxalicum*	Discellaceae	*Fungi Imperfecti*	
227	*Amanita citrina*	Amanitaceae	*Basidiomycetes*	
266	*Cladosporium perangustum*	Cladosporiaceae	*Dothideomycetes*	
285	*Cylindrocarpon radicicola*	Moniliaceae	*Hyphomycetes*	
415	*Amanita brunnescens*	Amanitaceae	*Agaricomycetes*	
70	*Boletus* spp.	Boletaceae	*Agaricomycetes*	Symbiotroph
120	*Cortinarius c*	Cortinariaceae	*Agaricomycetes*	
234	*Suillus intermedius*	Suillaceae	*Agaricomycetes*	
151	*Polyporus sulphurea*	Pokyporaceae	*Agaricomycetes*	Saprotroph
242	Saprotroph 8	Unknown	Unknown	
268	*Chaetomium globosum*	Chaetomiaceae	*Sordariomycetes*	
284	*Aspergillus calidoustus*	Aspergillaceae	*Eurotiomycetes*	
420	Saprotroph 6	Unknown	Unknown	
462	*Collybia dryophila*	Tricholomataceae	*Hymenomycetes*	
63, 66, 67, 181, 193, 201, 204, 205, 236, 237, 250, 251, 485, 487, 495	Unknown	Unknown	Unknown	Unknown

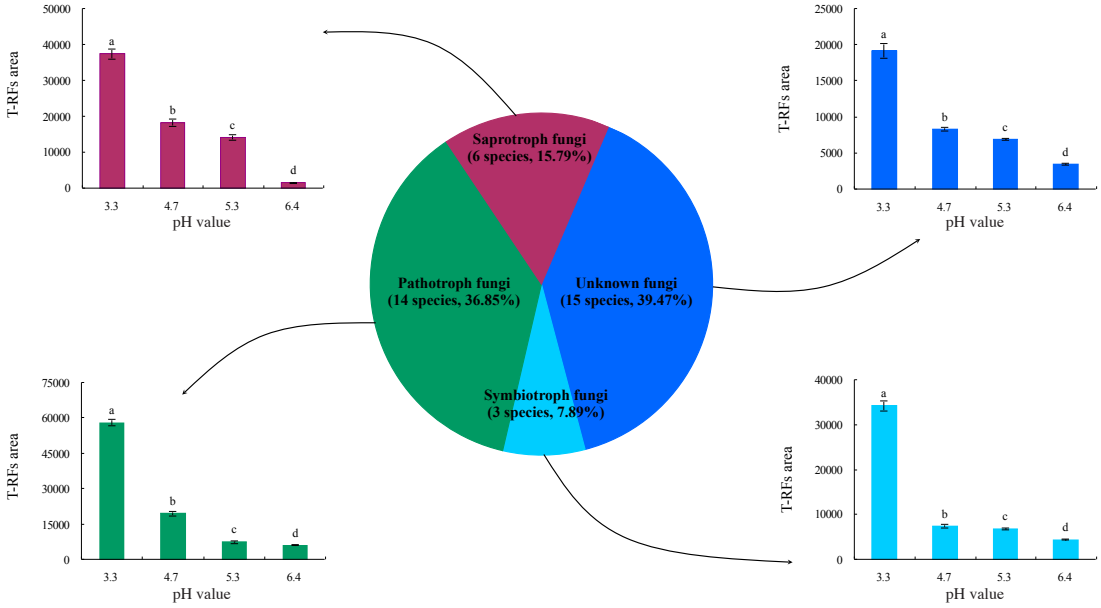

Fig. 3: The proportions of fungi in different trophic mode and their T-RFs fragments area distribution in soil at different pH values.

creasing tea leaves shed in the soil. Then saprotroph fungi accelerate the decomposition of residue and release catechins and theirs components, thereby promote the reproduction of *Suillus intermedius*. Secondly, it was found that pathotroph fungi identified in soil had the most species and the greatest abundance in this study, and the abundance of the 14 pathotroph fungi increased with the increasing of soil acidity, except *Amanita brunnescens* (Tab. 3, Fig. S5, Fig. S6). Reportedly harm of *Phaeosphaeria spartinicola*, *Cercospora asparagi*, *Fusarium oxysporum*, *Bionectria spp.*, *Alternaria tenuissima* to plants, mainly manifested as infecting plants in the form of a pathogen, and decomposing plants' roots, finally plants withered and died (Elmer, 2016; Hay et al., 2017; Anjos et al., 2019; Melo et al., 2014; Liu et al., 2019). Hazards of *Cladosporium perangustum*, *Cylindrocarpon radicicola*, *Verticillium dahliae* to plants, showed up as infecting plants and secreting toxic substances to inhibit the growth of plants (Liu et al., 2017; Khan et al., 2019; Kombrink et al., 2017). However, *Amanita muscaria* var. *formosa*, *Tylopilus felleus*, *Boletus pallidus*, *Penicillium oxalicum*, *Amanita citrina*, *Amanita brunnescens* did not infect plants in the form of pathogen, but mainly inhibited plant growth by secreting toxic substances (Wu et al., 2019; Grzybek et al., 1990; Rodriguez-Ramirez et al., 2010; Shi et al., 2017; Loizides et al., 2018; Mighell et al., 2019). Thus, with the increasing of tea rhizosphere soil acidity, saprotroph fungi in rhizosphere soil proliferated rapidly. Some of the saprotroph fungi secreted toxic substances to inhibit tea growth and reduced the resistance of tea trees (Wang et al., 2018a). After that, some of saprotroph fungi quickly infected tea tree, entered into tea tree tissue and secreted toxic substances to poison tea tree, which inhibited tea growth and reduced its ability to resist pathogenic bacteria. On this basis, another part of the saprotroph fungi rapidly infected tea trees, and caused root rot, then reduced water absorption capacity, finally root rot and blight of tea trees occurred, and seriously hindered tea growth, thus tea yield and quality declined (Wang et al., 2020a, 2020b).

Conclusion

The number and species of fungi in tea rhizosphere soil increased significantly with the intensification of acidity, and the dominant fungal populations changed. The acidity significantly affects the structural diversity of fungi community in the soil. Secondly, the identified fungi in the soil can be divided into four types according to their nutritional patterns, pathtroph, symbiotroph, saprotroph and unknown, accounting for 36.85%, 7.89%, 15.79% and 39.47%, respectively, and the number of pathtroph fungi accounted for a large proportion. In the soil with lowest pH, fungus abundance reached the maximum. This study provided a theoretical basis for soil restoration of acidified tea plantation.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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Supplementary material

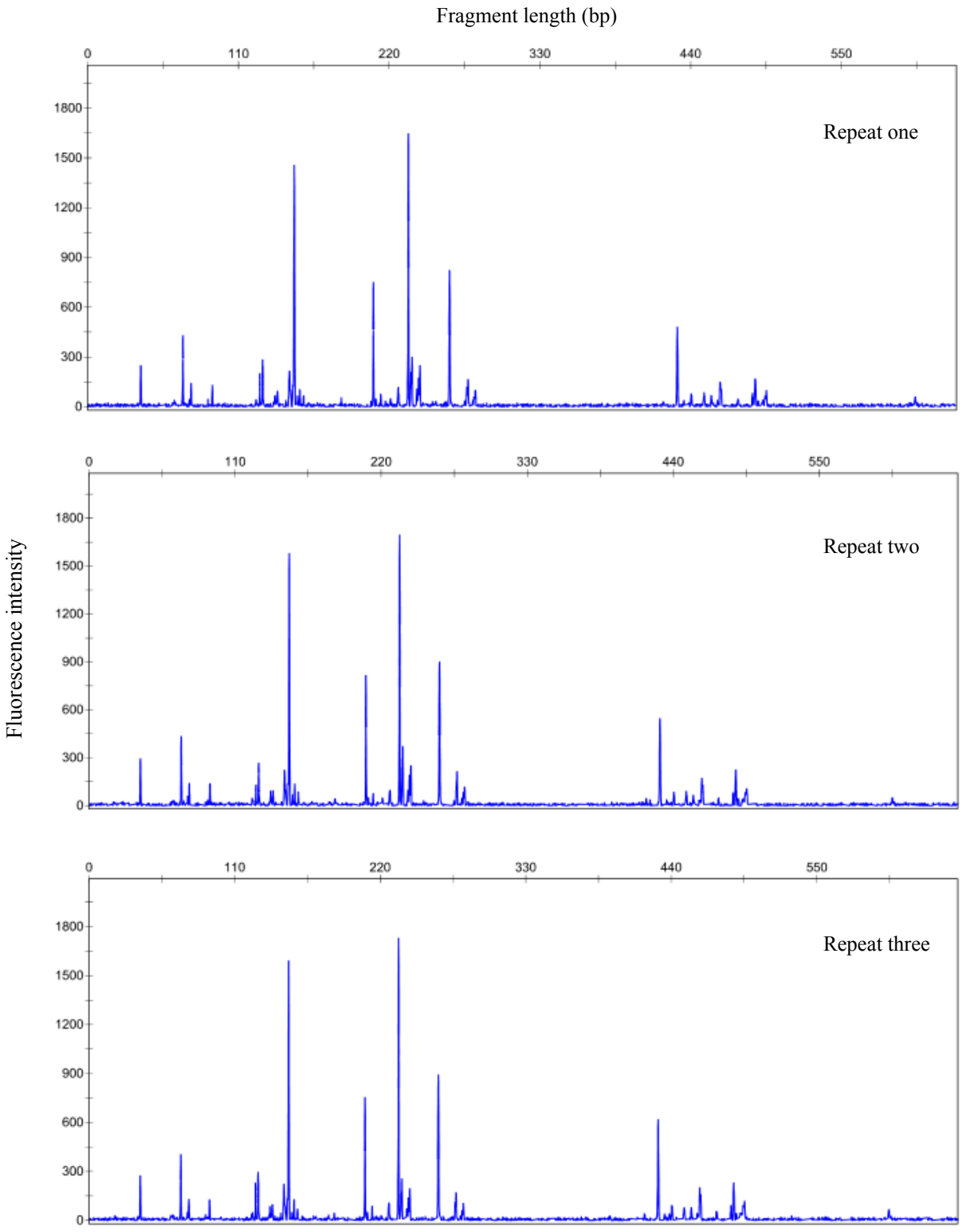

Fig. S1: T-RFLP chromatogram of fungi in rhizosphere soil of tea tree with pH value of 3.29

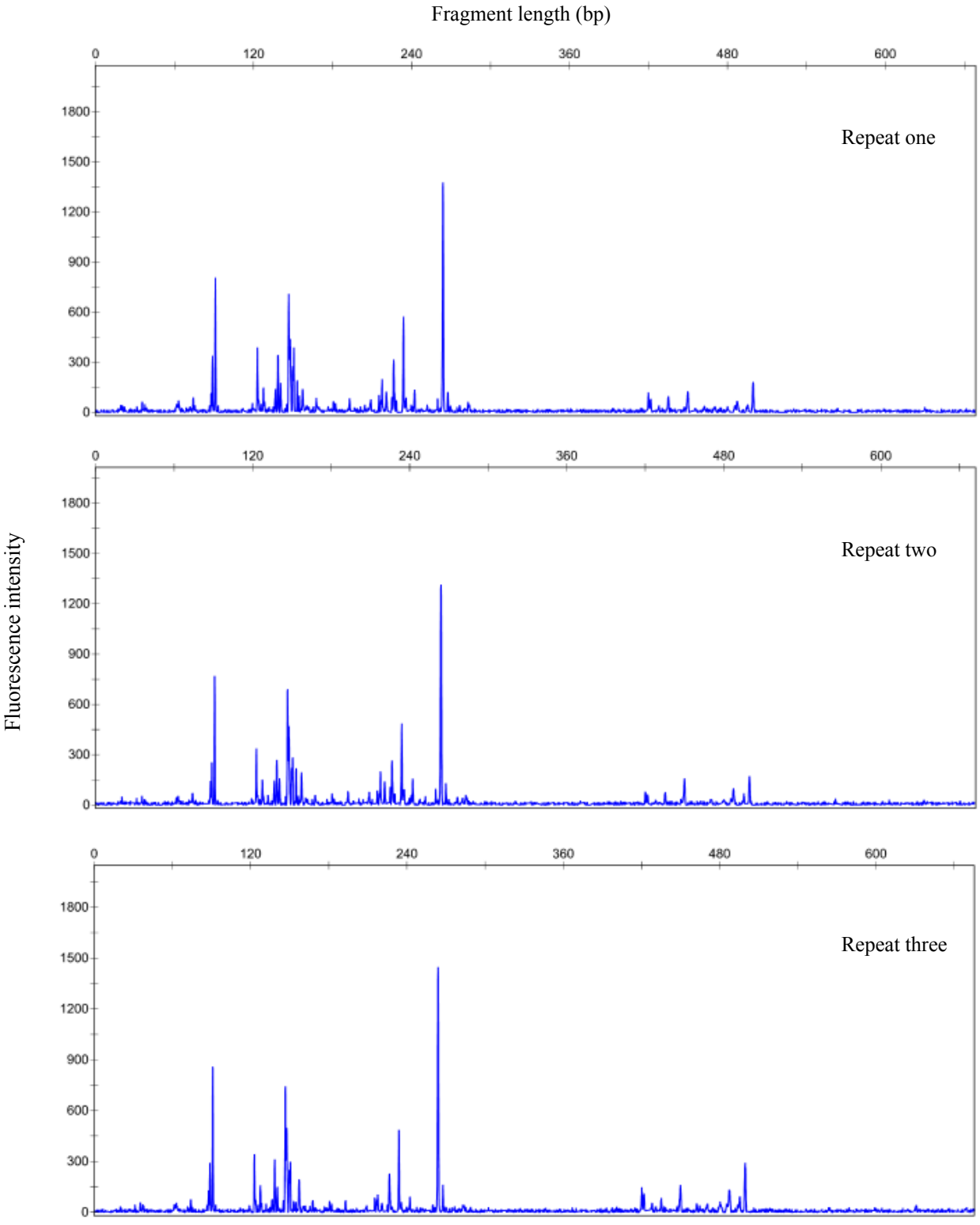

Fig. S2: T-RFLP chromatogram of fungi in rhizosphere soil of tea tree with pH value of 4.72

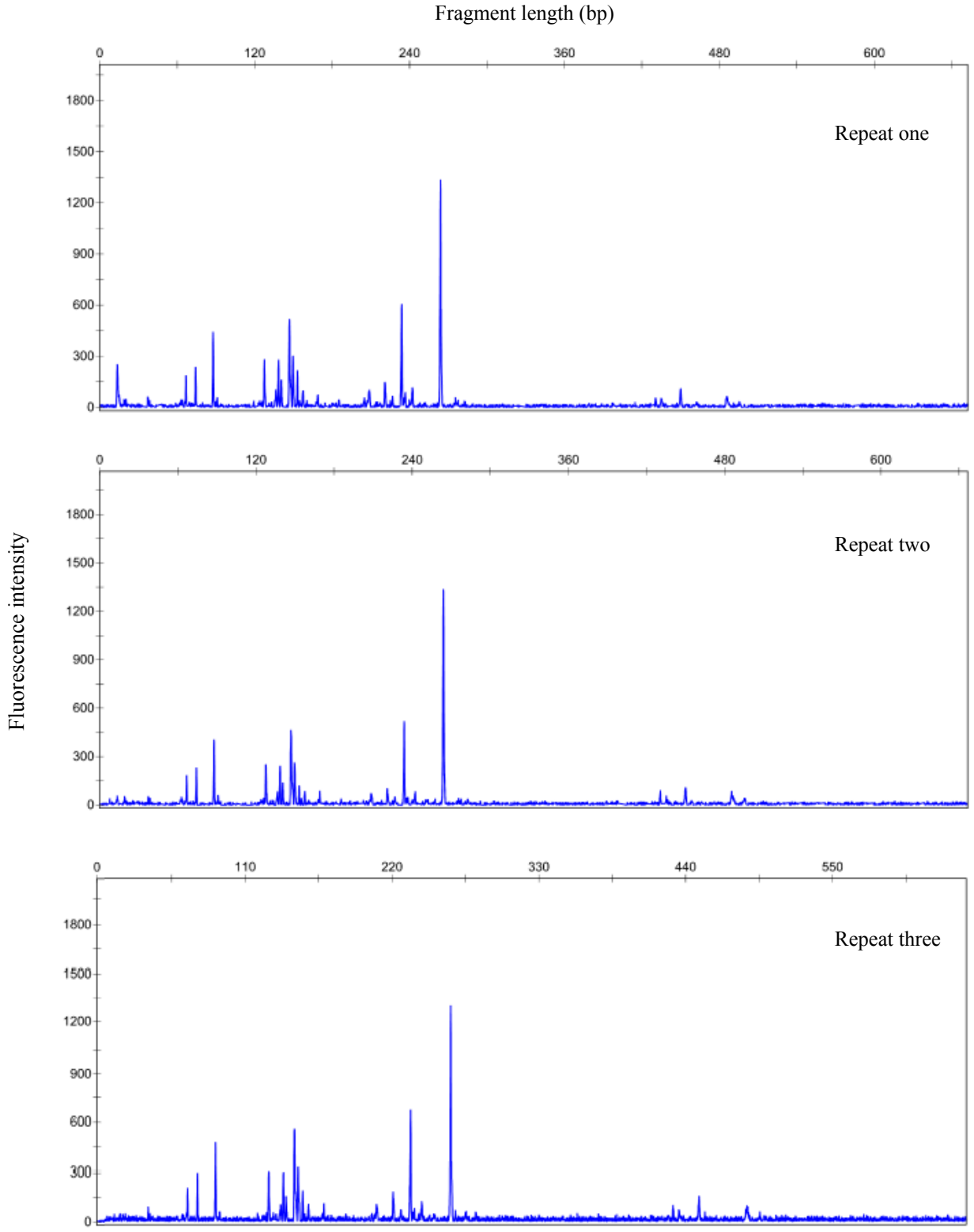

Fig. S3: T-RFLP chromatogram of fungi in rhizosphere soil of tea tree with pH value of 5.32

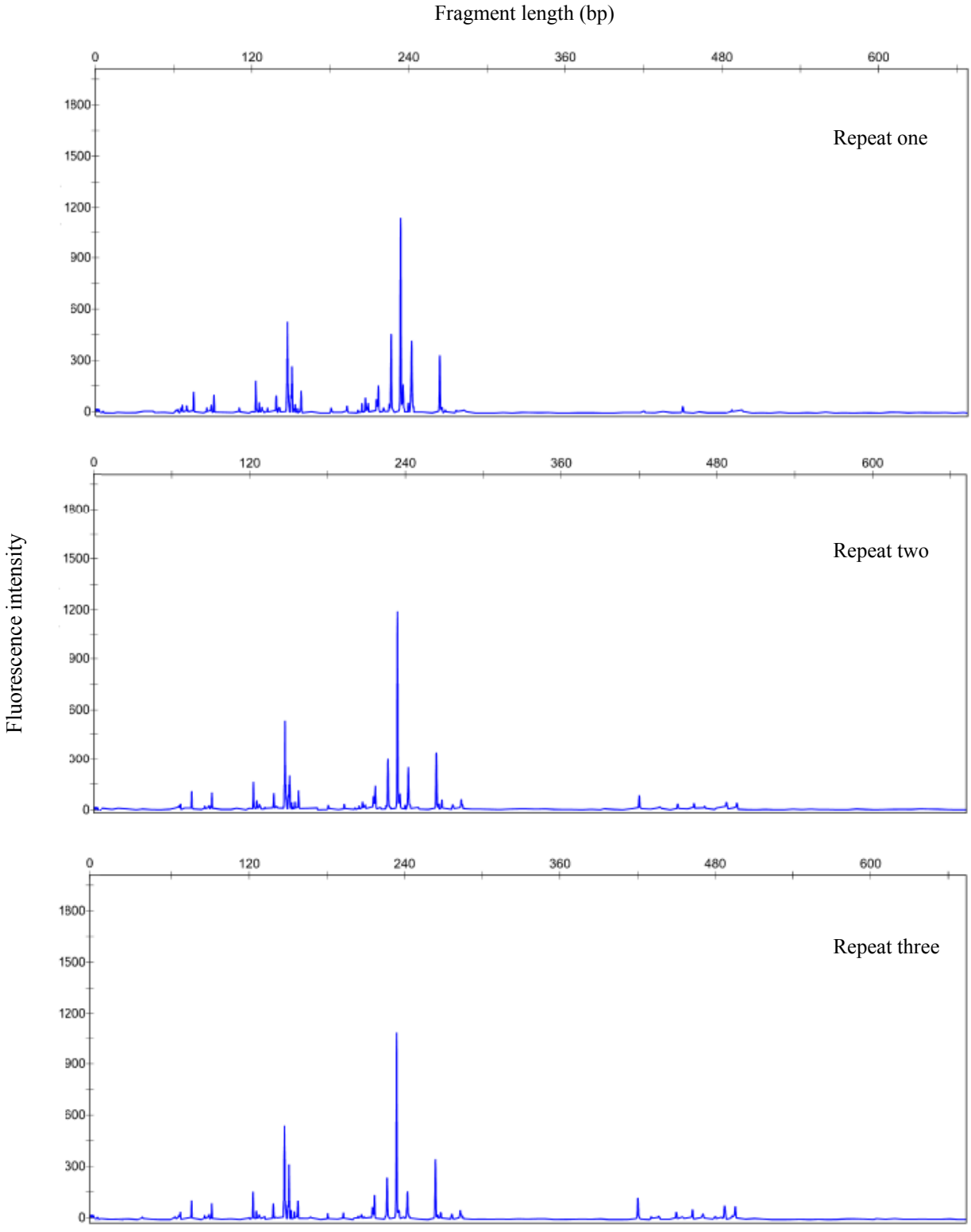

Fig. S4: T-RFLP chromatogram of fungi in rhizosphere soil of tea tree with pH value of 6.38

Tab. S1: Effects of different acidity on the abundance of T-RFs fragments in rhizosphere soil fungi of tea tree

T-RFs fragment	T-RFs fragment abundance of soil fungi at different pH values			
	pH 3.29	pH 4.72	pH 5.32	pH 6.38
37	547±54	960±102	1724±71	705±8
63	1220±50	1131±37	305±35	202±25
66	816±37	ND	ND	ND
67	886±52	ND	ND	ND
70	505±40	ND	ND	ND
74	442±18	621±53	1117±43	1291±58
75	2235±60	187±7	ND	ND
85	625±54	ND	ND	ND
88	ND	803±74	214±13	2651±23
89	898±60	1710±185	275±17	
91	1918±41	4984±589	758±50	317±4
110	331±11	ND	ND	ND
120	276±36	ND	ND	ND
123	3742±169	2326±187	587±47	ND
126	1112±55	314±47	575±54	203±32
128	1240±40	1077±40	1373±32	1956±67
132	558±32	258±3	ND	ND
136	ND	980±44	731±86	859±28
139	2381±11	2269±166	858±63	1809±22
141	1014±6	1086±81	ND	952±50
147	25577±686	9600±260	3423±16	3387±148
150	ND	1729±49	793±17	2339±37
151	12864±175	12160±283	10590±180	ND
153	996±64	1005±59	376±59	993±45
155	780±17	361±34	660±24	254±8
158	3031±32	1752±104	532±25	493±46
168	563±23	693±21	ND	323±18
169	401±28	ND	ND	530±58
181	1018±67	525±37	408±5	ND
185	275±20	ND	345±46	365±13
193	1268±42	746±64	ND	ND
201	477±13	153±15	ND	ND
204	947±35	292±7	ND	ND
205	ND	ND	ND	435±101
207	2541±29	ND	ND	
209	2123±38	892±31	3005±64	1077±99
215	2381±76	650±54	489±7	ND
217	3542±41	1473±54	ND	ND
221	1464±19	1023±40	411±40	1353±39
225	1514±60	482±59	ND	221±13
227	14042±511	2517±89	1115±77	490±46
234	33403±1683	7337±199	6748±85	4383±179

Table S1 continue

236	6644±176	2337±126	2445±161	452±105
237	ND	ND	ND	403±
240	1585±87	482±57	720±92	317±36
242	16577±857	1785±78	1489±5	1047±21
250	429±64	ND	ND	ND
251	ND	ND	ND	259±9
252	ND	399±30	301±16	253±37
264	13642±238	13772±630	13578±50	14656±948
266	1021±28	ND	ND	ND
268	1721±69	1027±121	ND	ND
277	1504±62	430±70	2279±105	682±12
283	2135±47	471±20	949±112	441±81
284	938±3	436±34	ND	ND
285	427±44	ND	ND	ND
415	ND	ND	ND	242±45
420	2824±87	1129±96	473±47	
430	ND	ND	4821±163	632±39
435	554±23	932±51	1258±105	734±58
450	1923±52	2018±75	1070±12	1446±105
455	ND	ND	853±66	476±13
462	2387±59	1575±46	1556±37	418±29
485	ND	ND	690±54	1300±51
487	3328±7	2130±86	2176±85	ND
495	2120±23	966±7	858±8	408±51
Total	189711±1403	91984±1428	72930±533	51754±1291

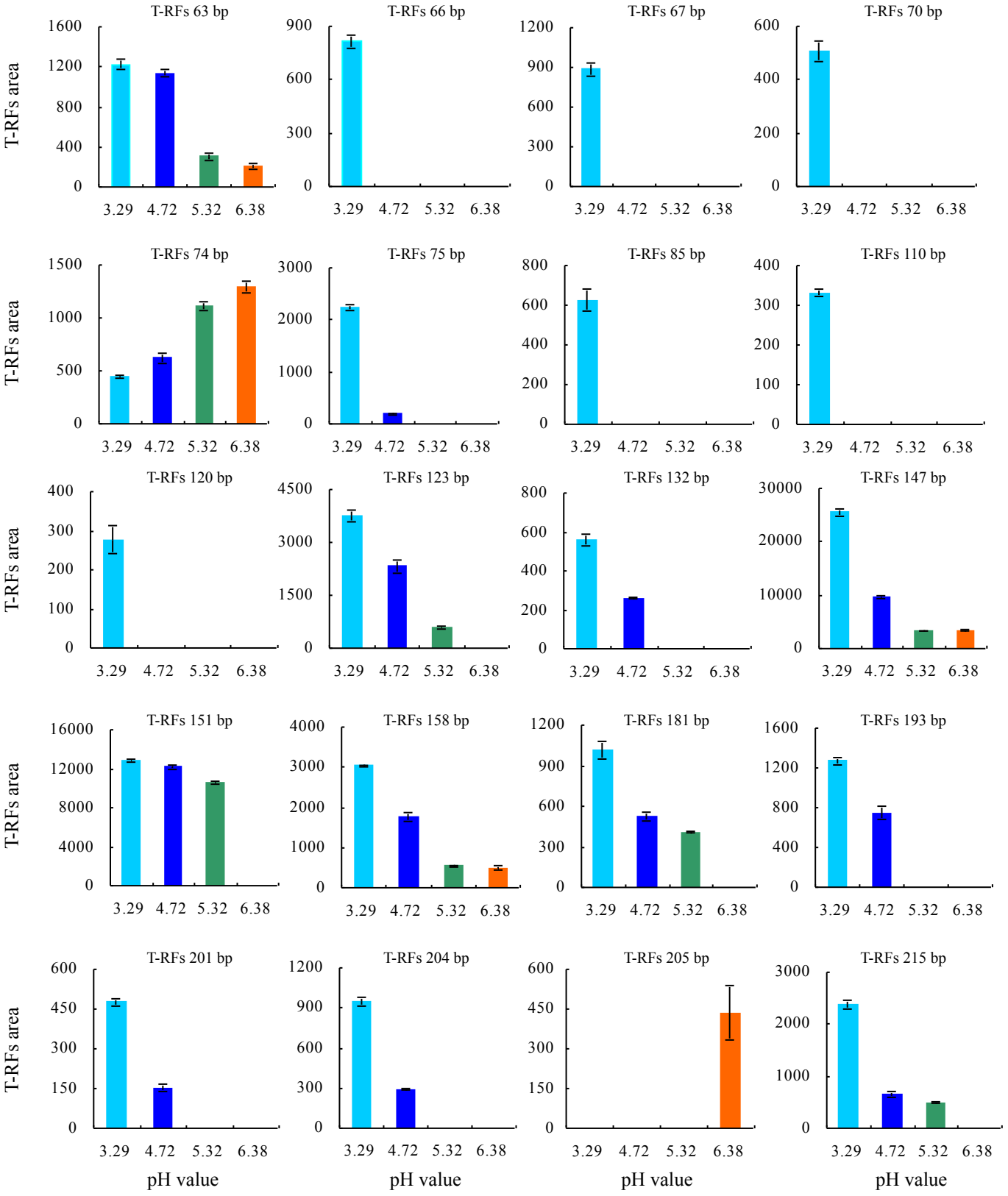

Fig. S5: T-RFs fragment area of fungi in rhizosphere soil of tea tree with different pH values

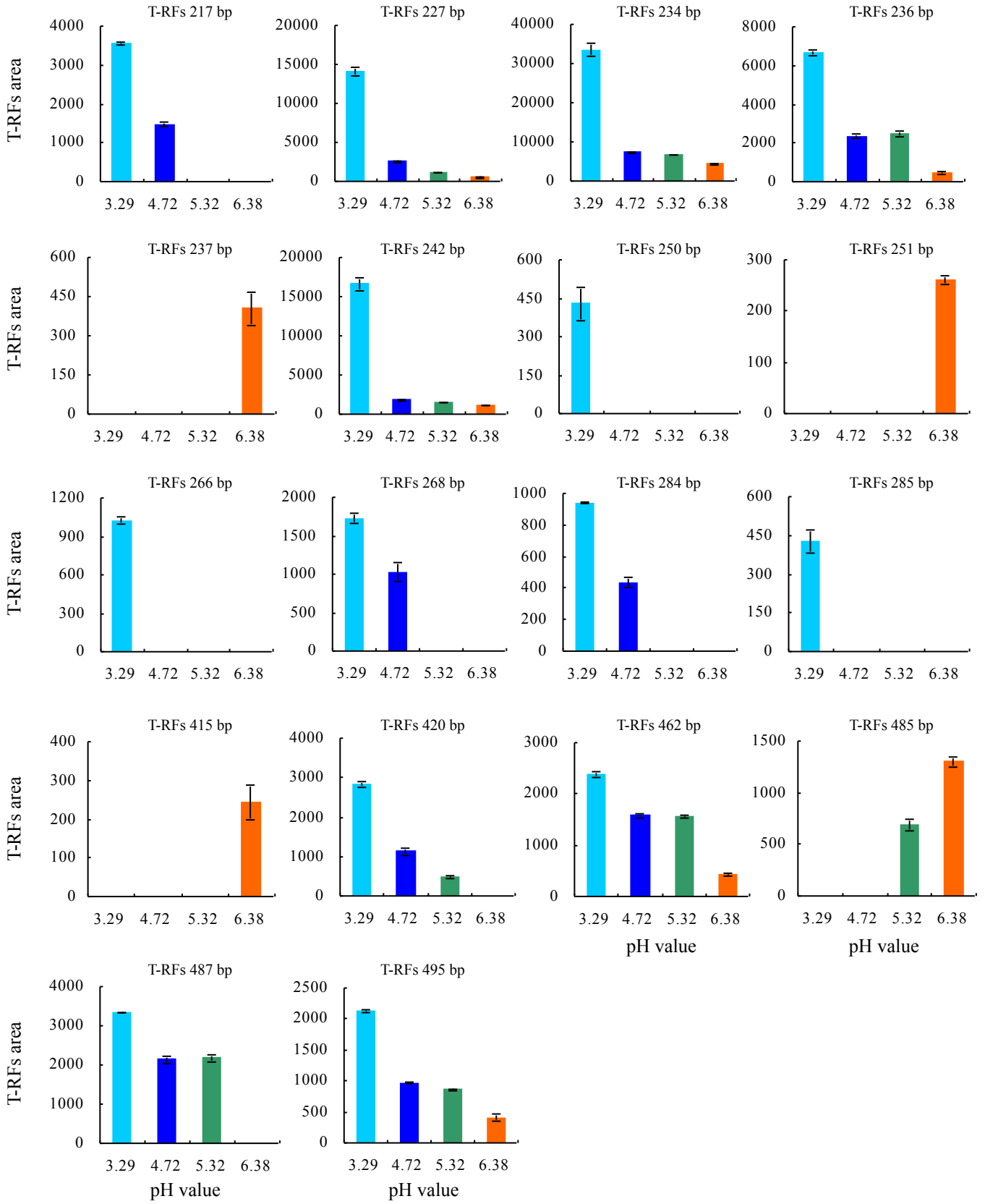

Fig. S6: T-RFs fragment area of fungi in rhizosphere soil of tea tree with different pH values