The effects of different human disturbance regimes on root fungal diversity of *Rhododendron ovatum* in subtropical forests of China

<table>
<thead>
<tr>
<th>Journal:</th>
<th>Canadian Journal of Forest Research</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID</td>
<td>cjfr-2016-0388.R1</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Article</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>05-Dec-2016</td>
</tr>
</tbody>
</table>
| Complete List of Authors: | Zhang, Yanhua; Shaoxing University, College of life science  
                             Ni, Jian; Shaoxing University,  
                             Tang, Fangping; Shaoxing University  
                             Jiang, Lifen; University of Oklahoma,  
                             Guo, Tianrong; Shaoxing University,  
                             Pei, kequan; Institute of Botany Chinese Academy of Sciences,  
                             Sun, Lifu; Shaoxing University, College of life sciences  
                             Liang, Yu; Institute of Botany Chinese Academy of Sciences |
| Keyword: | Rhododendron ovatum, fungal diversity, human disturbance, subtropical forests, land use |
The effects of different human disturbance regimes on root fungal diversity of *Rhododendron ovatum* in subtropical forests of China

Yanhua ZHANG*, Jian NI*, Fangping TANG¹, Lifen JIANG³, Tianrong GUO¹,
Kequan PEI², Lifu SUN¹,³**, Yu LIANG²**

² K.Q. Pei, Y. Liang**: State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China.
³ L.F. Jiang. Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK 73019, USA.

*These authors contribute equally to the paper.

**Corresponding authors: Lifu Sun (e-mail: sunlifu@usx.edu.cn) and Yu Liang (e-mail: coolrain@ibcas.ac.cn).

Funding: This study was supported by the National Natural Science Foundation of China (31170469 and 31170495) and Technology division of Shaoxing (2013B70040). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.
Abstract: Ericoid mycorrhizal associations are symbiotic relationship between soil fungi and ericaceous plants. Diversity of fungi associated with hair roots of ericaceous plants may vary as a result of frequent disturbances by human activities. The fungal diversity and communities associated with hair roots of *Rhododendron ovatum* was investigated along a human disturbance gradient in subtropical forests of China. 900 fungal operational taxonomic units (OTUs) were determined by the high-throughput sequencing, including different phyla such as Ascomycota, Basidiomycota, Zygomycota, Chytridiomycota, and Glomeromycota. The dominant phylum in *Cunninghamia lanceolata* plantation (PLF) and old growth forest (OGF) was Ascomycota, while Basidiomycota was the dominant phylum in secondary forests. The indicator species analyses showed that more pathogenic indicator fungi appeared in the disturbed forests, whereas more putative ericoid mycorrhizal fungi existed in the old growth forests. Principal Component Analysis also showed that the fungal communities in the hair roots of *R. ovatum* were distinct between natural forests and plantations, suggesting that the fungal communities associated with hair roots of *R. ovatum* after logging were resilient and could recover to pre-disturbance status. The results of envfit analysis showed that performance of host plants rather than accompanying plant community and soil parameters of plots was the key determinant of root-associated fungal community of *R. ovatum*.

Key words: *Rhododendron ovatum*, fungal diversity, human disturbance, subtropical forests, land use
1. Introduction

Ericaceae is a large plant family, being distributed widely in heathlands (Toberman et al. 2008; Tejesvi et al. 2010), and most forest ecosystems, such as boreal coniferous forests (Perotto et al. 2002; Ishida and Nordin 2010), temperate forests (Frak and Ponge 2002; Bougoure et al. 2007), Mediterranean forests (Perotto et al. 2002), subtropical forests (Zhang et al. 2009; Sun et al. 2012), tropical or neotropical forests (Setaro and Kron 2011; Bruzone et al. 2014). Root-associated fungi, especially ericoid mycorrhizal (ERM) fungi can help host plants to obtain N and P from soils (Persson et al. 2003; Kai and Caroline 2007) and adapt to harsh and nutrient-poor environments very well (Cázares and Trappe 2005). Some ERM fungal species such as *Oidiodendron maius* (Martino et al. 2002; Vohnik et al. 2005; Mark 2006) and *Rhizoscyphus ericae* (syn. *Hymenoscyphus ericae*) (Read 1996; Berch et al. 2002; Ishida and Nordin 2010) have been often observed in hair-roots of ericaceous plants based on isolation, culture and morphological identification. More and more fungi are found in the roots since molecular biotechnology is applied, especially the application of high-throughput sequencing (Lentendu et al. 2011; Davey et al. 2012; Oja et al. 2015). These fungi co-exist in the same roots and consist of the fungal communities, including not only Ascomycota and Basidiomycota (Berch et al. 2002; Bougoure et al. 2007; Sun et al. 2012), but also other phyla (Allen et al. 2003; Wurzburger et al. 2011). Among the fungal communities, there might be ectomycorrhizal fungi (ECMF), arbuscular mycorrhizal fungi (AMF), and other non-mycorrhizal fungi such as dark septate endophytes (DSE), saprophytes and pathogens, as well as some fungi with

Forest ecosystems are frequently disturbed by human activities, such as clear-cutting, selective-cutting, and land use changes. Both host plants and their fungal partners may be affected by human disturbance, and the diversity and community of soil fungi (McGuire et al. 2014), AMF (Gavito et al. 2008; Stürmer and Siqueira 2011; Glinka and Hawkes 2014), ECMF (Gebhardt et al. 2007), and ERMF (Hazard et al. 2014) would also change with vegetation degradation under human disturbances. McGuire et al. (2014) compared fungal diversity along a disturbance gradient in Southeast Asia tropical forests and found soil fungal richness was highest in secondary forests rather than oil palm plantations and primary forests, which seems to support the “Intermediate Disturbance Hypothesis” (IDH). IDH states that the highest species richness would be obtained at the intermediate disturbance level, where organisms of early and late successional stages are allowed to coexist (Connell 1978). It is generally believed that fungal diversity appears to be related to plant diversity, and decreased fungal diversity is expected to be observed in disturbed forests following the loss of plant diversity (Tsui et al. 1998). However, the results of comparative studies on mycorrhizal fungal diversity between secondary and primary forests indicate decreased (Gavito et al. 2008), increased (Stürmer and Siqueira 2011) or non significant AMF diversity (Lekberg et al. 2012) under human disturbances. The responses of root associated fungi to human disturbances and the underlying mechanisms are still poorly understood.
Rhododendron ovatum is an evergreen shrub or a small tree, native in subtropical forests of China (flora of china). It is distributed widely in the subtropical zone with altitude ranging from 500 to 1200 m, and has been introduced to many arboretums and botanic gardens all over the world because of its beautiful flowers. While R. ovatum can grow in many subtropical forests, its population is sensitive to forest management practices such as clear-cut and planting of economic tree species (Table S1). Since ERM fungi are essential for survival and growth of ericaceous plants, researches on fungal diversity of R. ovatum will be helpful in understanding the adaptation mechanisms of R. ovatum to human disturbances.

In the present study, diversity and community compositions of root-associated fungi of R. ovatum in old growth forests, secondary forests and plantations were determined by high-throughput sequencing. We proposed the following hypotheses: (1) root-associated fungal diversity and fungal community structure of R. ovatum will change along with the human disturbance gradient; and (2) Fungal communities in roots of R. ovatum would be related to performance of host plant, composition of neighbor plants, and abiotic environmental factors.

2. Materials and Methods

2.1 Study sites

The study site is located at Gutianshan National Nature Reserve (GNNR), Zhejiang Province in East China. Annual mean temperature is 15.3°C and annual precipitation
ranges from 1793 to 1960mm. The typical vegetation in the study site is subtropical evergreen broad-leaved forest (Zhang et al. 2011), in which *Castanopsis eyrei* (ECM plant) and *Schima superba* (AM plant) are the dominant trees.

Twelve 1-ha (100m×100m) plots were established with different disturbance history: old growth forests (OGF), secondary forests harvested once (SEC I), secondary forests harvested twice (SEC II), and *Cunninghamia lanceolata* (AM plant) plantations (PLF). SEC I was clear-cut approximately 50 years ago, while SEC II was clear-cut about 50 years ago and then select-cut about 20 years ago. PLF were planted about 20 year ago after clear-cut of secondary forests. OGF are undisturbed forests that did not experience tree-felling during the last 100 years (Yu et al. 2011). The distances between the 12 plots ranged from ca 200 m to 5.4 km (Zhang et al. 2016).

### 2.2 Sampling procedure

Hair roots of four individuals of *R. ovatum* were sampled in March of 2012 from each plot. In total, root samples from 48 individuals were collected (12 plots × 4 samples per plot). Hair roots were retrieved from soils at four directions around the trunk of each *R. ovatum* individual. The roots were washed carefully after 1 h soaking in sterile water. Hair roots were then cut into 1 cm segments and 20 hair root segments were selected randomly from each *R. ovatum* individual. Each root segment was put into a centrifuge tube and preserved in 70% alcohol at -70 °C before DNA extraction.
2.3 DNA extraction, PCRs and MiSeq analysis

DNA was extracted from hair roots of *R. ovatum* following the protocol of Zhang et al. (2016). Hair root segments were put into a sterile centrifuge tube containing 20 µl 2×CTAB extraction buffer solution (2%CTAB, 100mM Tris-HCl pH8.0, 20mM EDTA pH8.0, 1.4M NaCl), and ground with a plastic pestle on ice. A 630µl aliquot of 2×CTAB extraction buffer solution was added into the centrifuge tube. Samples were warmed at 65 ºC for 1 h, and then shaken for 10 min. A 630 µl aliquot of chloroform/isoamyl alcohol (24:1) was added into the tubes. After being fully mixed, the samples were centrifuged at 13,201×g for 8 min at room temperature. The supernatant was transferred into a new centrifuge tube and an equal volume of chloroform/isoamylol (24:1) was added. After centrifuging at 13,201×g for 8min again, the supernatant was transferred into a new centrifuge tube and DNA was precipitated with a double volume of 100% alcohol at 4 ºC for 1h. After centrifuging at 17,968×g for 8 min, the supernatant was discarded. DNA precipitate was washed twice using 70% ethanol, dried in a vacuum desiccator, and dissolved in 30ml sterile ddH$_2$O at 4°C. DNA samples were stored at -20 ºC prior to downstream analyses.

The ITS1 region of fungi was amplified by PCR (95ºC for 2min, followed by 25 cycles at 95ºC for 30s, 55ºC for 40s, and 72ºC for 50s and a final extension at 72ºC for 5min) using primers 1723F 5’-barcode-CTTGGTCATTTAGAGGAAGTAA-3’ and 2043R 5’-GCTGCGTTCTTCATCGATGC-3’ (Mello et al, 2011), where barcode is an eight-base sequence unique to each sample. PCR reactions were performed in triplicate 20µl mixture containing 4µl of 5× FastPfu Buffer, 2µl of 2.5mM dNTPs,
0.8µl of each primer (5µM), 0.4µl of FastPfu Polymerase, and 10ng of template DNA. Amplicons were purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) and pooled in equimolar and paired-end sequenced (2 × 250) on an Illumina MiSeq platform adopting the standard protocols.

Raw fastq data were demultiplexed and quality-filtered using QIIME (ver 1.7) with the following criteria: (i) these reads were truncated at any site receiving an average quality score <20 over a 10bp sliding window, discarding the truncated reads that were shorter than 50bp; (ii) exact barcode matching, 2 nucleotide mismatch in primer matching, reads containing ambiguous characters were removed; (iii) only sequences that overlapped by longer than 10bp were assembled according to their overlap sequence. Reads that could not be assembled were discarded.

Open reference OTU picking was done with pick_open_reference_otus.py using the default uclust method and ITS 12-11 dataset (97% similarity cutoff was used, alpha release, download from web site of QIIME http://qiime.org/home_static/dataFiles.html), and singletons and doubletons and sequences with length less than 200bp were removed during OTU picking. The phylogenetic affiliation of each sequence was analyzed by RDP Classifier (http://rdp.cme.msu.edu/) against ITS 12-11 dataset using confidence threshold of 80%. Diversity analyses were performed by running a workflow on QIIME, with the script core_diversity_analyses.py and single_rarefaction.py in Qiime was used to generate OTU table with even reads of 1,400 in each root sample.
2.4 Statistical analysis

One-way ANOVA was carried out to test the difference of total area at breast height and mean DBH per individual of *R. ovatum* and soil parameters between four forest types using SPSS (ver. 16.0, SPSS Inc.). Indicator species analysis was performed using out_category_significance.py in Qiime to determine the indicator fungal species showing significant preference to specific forest types. Principal Component Analysis (PCA) was conducted using the rda function in the R package “vegan” to determine the community structure of root-associated fungi (Dixon 2003). “Envfit” function was used to identify the main factors influencing root-associated fungal community. PCAs were also performed using “rda” function for plant community and soil parameters of plots, and the first two components (PC1 and PC2) of plant community and PC1 of soil parameters were evaluated using “envfit”. “Anosim” function in the R package “vegan” was used to evaluate the impacts of forest type on community composition of root-associated fungi. Fungal families were grouped to four different functional groups according to functional annotations of Tedersoo et al. (2014), i.e., ERMF, ECMF, AMF, and plant pathogen (PAT). Since many unidentified species of Helotiales have been frequently observed in roots of ericaceous plants and are usually considered as ERM fungi, we group unidentified Helotiales as ERMF in the present study.

3. Results
3.1 Changes of *R. ovatum* populations and soil properties along the disturbance gradients

Based on the latest data of regular investigation from GNNR, both diameter at breast height (DBH) and total area at breast height of *R. ovatum* were much higher (P<0.01) in OGF than those in disturbed forests (Table S1). Soil properties of different forests are shown in Table 1. A typical acidic soil was found in our sites, with pH ranging from 4.73 to 4.77, but pH was not significantly different among all forests. Soil nutrient analysis showed that soil organic carbon (SOC) and soil total nitrogen (STN) in OGF were significantly higher than those in the other three forest types. Significant differences of NO$_3^-$-N and available phosphorus (AP) were also found between OGF and SEC II. Soil total phosphorus (STP) and NH$_4^+$-N were not significantly different among the forests along the disturbance gradients.

3.2 Fungal OTUs observed in hair roots of *R. ovatum*

Rarefaction curves of fungal OTUs estimated by Chao1 in hair roots of *R. ovatum* are shown in Fig. 1. Estimated fungal OTU richness (Chao1) at a sample depth of 1,400 reads was 294, 232, 221 and 373 in OGF, SECI, SECII, and PLF, respectively. Reduced fungal richness was observed in two secondary forests (SECI and SECII) and PLF had the highest fungal richness in roots of *R. ovatum* (Fig. 1).

The Venn diagram shows the number of specific and shared OTUs of the forests with different human disturbances (Figure 2). When comparing two secondary forests with old growth forest, we found that clear-cut only or clear-cut combined with...
select-cut reduced the number of habitat specific fungi (125 and 144 vs. 164, Fig. 2).

In *Cunninghamia* plantation, however, there were more forest-type-specific fungi (241, Fig. 2). There were 41 fungal OTUs observed in all four forest types.

### 3.3 Fungal community structure in the forests along the human disturbance gradients

Community compositions of root-associated fungi of *R. ovatum* are shown in Fig. 3. Ascomycota and Basidiomycota were the two dominant phyla in all four types of forests, accounting for 87.0%-99.1% of the total reads. Zygomycota was 0.45%-11.96% of total reads, and Glomeromycota and Chytridiomycota together accounted for less than 0.3% of the total reads. Common classes in those forests were Leotiomycetes, Sordariomycetes, Dothideomycetes, and Eurotiomycetes in Ascomycota, and Agaricomycetes in Basidiomycota. Moreover, as shown in Figure 3, the proportions of common classes in SEC I and SEC II were similar.

Results of Principal Component Analysis (PCA) are shown in Figure 4 to distinguish community structure of root-associated fungi of *R. ovatum* in forests with different human disturbances. OGF, SECI and SEC II were well separated from PLF by the PCA.

### 3.4 Frequency and relative abundance of fungal families with different functions

Frequency rate and relative abundance of fungal families of four functional groups are shown in Fig. 5. Seven out of nine ERMF families had a frequency rate over 0.833 (10/12 plots), and Sebacinaeae, Unidentified Helotiales, Myxotrichaceae,
Dermateaceae, and Vibrisseaceae had a relative abundance >1%. In the functional group of ECMF, Russulaceae was observed in all plots with a relative abundance of 8.9%. Gloniaceae had a high frequency rate but low relative abundance and Cortinariaceae had a relative abundance of 1.4% but occurred in only 7 of 12 plots. Fungal families of AMF had low relative abundance and low frequency rate. Fungal families harboring pathogens had relatively low relative abundance and only Diaporthaceae had a relatively high abundance (1.6%). Nectriaceae and Amphisphaeriaceae were two pathogen-harboring fungal families with frequency rate > 80%.

3.5 Indicator fungal species of forests with different human disturbances

There were 38 fungal OTUs that showed significant preference to human disturbances (Table 2). The number of indicator species for OGF, SECI, SECII, and PLF were 16, 3, 1 and 18, respectively. Of the indicator fungal species in OGF, Helotiales, Herpotrichiellaceae (Asc), and Sebacinaceae (Bas) are typical ERMF taxa and were often observed in roots of ericaceous plants. Thelephoraceae and Russula were typical ECM fungal taxa, and Penicillium and Talaromyces were saprotrophic fungal genera (Phuwiwat and Soytong 2001). For the indicator species in PLF, Ilyonectria liriodendri and Plectosphaerella cucumerina were plant pathogens; Cryptosporiopsis ericae, Lecanicillium psalliotae, Xenochalara juniper and four OTUs from the genus Penicillium were saprotrophic fungi according to functional annotations of fungal genera (Tedersoo et al. 2014).

3.6 Determinant factors of root-associated fungal community
The results of “Anosim” showed a significant impact of forest type on community composition of root-associated fungi of *R. ovatum* (P=0.045). Mean DBH of *R. ovatum* had significant effects on root-associated fungal community of *R. ovatum* (Table 3). Both diversity and composition of plant community and edaphic parameters had no significant effects on root-associated fungal community of *R. ovatum* (Table 3).

4. Discussion

4.1 Diversity of total fungal OTUs associated with hair roots of *R. ovatum*

Diverse fungal taxa were observed in hair roots of *R. ovatum* in the present study, and both Ascomycota and Basidiomycota were dominant phyla. We observed 900 fungal OTUs in our study sites by high throughput sequencing, which were much more than those observed from 12 to 35 fungal taxa associated with roots of *Rhododendron* species by using traditional molecular methods (Zhang *et al.* 2009; Tian *et al.*, 2011; Sun *et al.*, 2012) in subtropical climate zones of China.

Since it is impossible to identify ERMF according to only ITS sequences without mycorrhizal structures, only some fungal taxa observed in roots of ericaceous plants are often proposed as putative ERM fungi, e.g. Helotiales (Zhang *et al.* 2009; Sun *et al.* 2012), Sebacinales (Selosse *et al.* 2007), and *Oidiodendron* of Onygenales (Vohnik *et al.* 2005). In this study, we found numerous OTUs, including putative and possible ERMF, ECMF, AMF, saprotrophic fungi, pathogens, and other endophytes (DSE), which were frequently observed in plant roots (Smith *et al.* 1995; Vohnik *et al.* 2005;
Diverse fungal taxa were also found associated with ericaceous plants (Wurzburger et al. 2011; Gorzelak et al. 2012; Zhang et al. 2016), and ecological functions of these co-occurring fungi could be investigated in further studies.

### 4.2 Distributions of fungal taxa and functional groups

Fungal communities of *R. ovatum* in four forest types displayed different proportions of fungal phyla and classes (Fig. 3), of which SEC I and SEC II had similar proportion of Ascomycota (39.38% and 40.66%) and Basidiomycota (58.67% and 53.72%). Since OGF had more Ascomycota (57.09%) and less Basidiomycota (38.2%), it seemed that forest logging might provide the chances for Basidiomycota fungi to establish in the soils and to affect the competition between main fungal taxa.

The proportion of our dominant phyla is different from that in roots of *Calluna vulgaris* and *Vaccinium myrtillus* across heathland to native Scots pine forest vegetation gradient (Bougoure et al. 2007), where ascomycetes were 2.4 times more frequent than basidiomycetes in the fungal community.

Our results showed that putative and possible ERM fungal orders had both high relative abundance and high frequency (Fig. 5), implying these fungi may be relatively resistant to human disturbances and essential for survival of *R. ovatum*. It is interesting that a typical ECM fungal order, Russulales, were also associated with roots of *R. ovatum* in all plots with high abundance (Fig. 5). It has been noticed that ECM fungal taxa could also be found in roots of some ericaceous plants (Allen et al.)
2003; Smith et al. 1995; Wurzburger et al. 2012; Zhang et al. 2016), indicating that
they may form symbiotic structures with both ERM and ECM host plants.

Human disturbance may also affect different fungal groups, and the sensitivity of
ECM fungi, AM fungi, and soil fungi to disturbances may be quite different (Carney
and Matson 2006; Gebhardt et al. 2007; Lentendu et al. 2011; Fichtner et al. 2014;
McGuire et al. 2014; Glinka and Hawkes 2014). From our results of indicator species
analysis, some fungal taxa showed significant preferences for old growth forests, e.g.
Herpotrichiellaceae, *Plectosphaerella cucumerina*, Thelephoraceae, and *Russula*.
Those fungal taxa were usually considered as ERM and ECM fungi in previous
studies (Smith et al. 1995; Berch et al. 2002; Allen et al. 2003; Tian et al. 2011;
Wurzburger et al. 2011). Preferences of ERM and ECM fungi for old growth forests
were also observed in studies on root-associated fungi of other ericaceous plants
(Zhang et al., 2016), which may be due to the dominance of ERM and ECM host
plants.

There were some indicator species for PLF in the present study that were usually
considered as pathogens, such as *Cryptococcus*, *Gloeosporium*, *Ilyonectria*, and
*Lecanicillium* (Zare and Gams 2001; Cho et al. 2003; Heitman et al. 2010; Pathrose et
al. 2014). The much pathogenic indicators in PLF may be due to the relatively lower
ERM colonization in PLF, since extensive ERM colonization could enhance pathogen
resistance of plants (Grunewaldt-Stöcker et al. 2013). We should also notice that these
putative pathogens (i.e. *Ilyonectria liriodendra* and *Plectosphaerella cucumerina*)
might be “real” pathogens for *R. ovatum*, since the status of these species as plant
pathogen mainly based on observations in agricultural fields rather than natural forests (Carlucci et al. 2012; Pathrose et al. 2014; Pétriacq et al. 2016).

4.3 Impacts of human disturbances and determinants of root-associated fungal community of \textit{R. ovatum}

Our results showed that the highest fungal richness associated with hair roots of \textit{R. ovatum} is observed in PLF and that more specific fungal species are present in PLF and OGF, which does not agree with the “Intermediate Disturbance Hypothesis” for AMF observed by McGuire et al. (2014). The reason of why high fungal richness appeared in PLF might be that the introduction of AM host plant \textit{C. lanceolata} in PLF enhances the opportunities for AMF and other fungal taxa distinct from native soil fungi to colonize roots of \textit{R. ovatum}. Lindahl et al. (2010) also found that opportunistic saprotrophic fungi would be induced and enhanced by human disturbances.

Host identity may also affect the responses of root-associated fungal community to land use changes. For example, Hazard et al. (2014) studied the fungi associated with two ericaceous plants in three land use types and found that fungal diversity measured by T-RFs in roots of \textit{Vaccinium macrocarpon} differed significantly between different land use types while fungal diversity in roots of \textit{Calluna vulgaris} was not significantly different between land use types.

Some previous studies indicated that although the fungal richness did not show significant changes, the fungal community composition still varied along the
disturbance gradient (Stürmer and Siqueira 2011), and that disturbed and undisturbed habitats were often characterized by different fungal groups as indicators (Moora et al. 2014). Our results from PCA (Fig. 4) allowed us to distinguish community structure of root-associated fungi of *R. ovatum* in forests with different human disturbances, with OGF, SECI and SEC II well separated from PLF by the PCA, suggesting that both types of secondary forests may harbor fungal communities similar to those from the old growth forests rather than PLF. This trend is similar to the results of soil fungal community by McGuire et al. (2014) and endophyte community associated with hair roots of *Vaccinium carlesii* by Zhang et al. (2016).

While performance of host plant, neighbor plant community and soil parameters may affect root-associated fungal communities, our results showed that performance of host plant rather than neighbor plant community and soil parameters determined root-associated fungal community of *R. ovatum* (Table 3). Some studies on diversity and composition of root-associated fungal communities have shown that soil properties may act as important factors in determining these fungal communities (Frak and Ponge 2002; Toberman et al. 2008; Wurzburger et al. 2011; Fujimura and Egger 2012). Our recent studies also showed that root-associated fungal community of *Vaccinium carlesii* (Zhang et al., 2016) was determined by both accompanying plant community and soil parameters, while root-associated fungal community of *V. mandarinorum* (Zhang et al., unpublished data) was only determined by PC1 of accompany plant community. These results indicated that the mechanisms underlying
that root-associated fungal community may be quite different for different plant species.

5. Conclusions

In summary, 900 fungal OTUs associated with hair roots of *R. ovatum* were obtained from four forest types by high-throughput sequencing in this study, mainly including putative ERM fungi, typical ECM fungi, saprobes and putative pathogens. The dominant phylum in PLF and OGF was Ascomycota, accounting for 75.82% and 57.09% respectively, while Basidiomycota was the most dominant phylum in the two secondary forest types (SEC I and SEC II, 58.67% and 53.72%, respectively). The indicator fungal species showed that more putative pathogens preferred disturbed forests and more putative ERM fungi preferred old growth forests. Results of PCA showed that the fungal communities in hair roots of *R. ovatum* along the human disturbance gradient were profoundly distinct between plantations and other forest types. Our results also showed that performance of host plant rather than neighbor plant community and soil parameters determined root-associated fungal community of *R. ovatum* (Table 3).

Acknowledgments

We thank Dr. Xiaojuan Liu for providing climatic information of the sampling sites, and Yefei Jin and Li Han for preparing the material and experiments.
Reference


doi:10.1111/j.1365-294X.2007.03540.x


doi:10.1016/j.soilbio.2013.06.012

doi:10.3767/003158512X638251.


Table 1. Soil parameters of four forest types (i.e., old growth forest (OGF), secondary forest with once or twice cuts (SEC I and SEC II), and plantation (PLF)) in the study site.

<table>
<thead>
<tr>
<th>Forest type</th>
<th>pH</th>
<th>SOM (g/kg)</th>
<th>STN (g/kg)</th>
<th>STP (g/kg)</th>
<th>NH$_4^+$-N (mg/kg)</th>
<th>NO$_3^-$-N (mg/kg)</th>
<th>AP (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OGF</td>
<td>4.77±0.03a</td>
<td>115.0±18.0a</td>
<td>3.18±0.51a</td>
<td>0.19±0.04a</td>
<td>41.28±5.74a</td>
<td>2.09±0.60b</td>
<td>6.19±1.21a</td>
</tr>
<tr>
<td>SEC I</td>
<td>4.77±0.02a</td>
<td>66.9±7.3b</td>
<td>1.97±0.19b</td>
<td>0.17±0.02a</td>
<td>30.29±2.37a</td>
<td>3.22±0.15ab</td>
<td>4.26±0.85ab</td>
</tr>
<tr>
<td>SEC II</td>
<td>4.73±0.02a</td>
<td>68.8±2.4b</td>
<td>1.89±0.10b</td>
<td>0.14±0.01a</td>
<td>28.57±0.75a</td>
<td>3.66±0.27a</td>
<td>2.85±0.21b</td>
</tr>
<tr>
<td>PLF</td>
<td>4.75±0.03a</td>
<td>74.3±4.3b</td>
<td>2.12±0.16b</td>
<td>0.20±0.01a</td>
<td>33.05±4.47a</td>
<td>2.47±0.59 ab</td>
<td>4.32±0.19ab</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Index</th>
<th>Identified name</th>
<th>Group</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dothideomycetes 1 (Asc)</td>
<td>OGF</td>
<td>0.0184</td>
</tr>
<tr>
<td>2</td>
<td>Helotiales 1 (Asc)</td>
<td>OGF</td>
<td>0.0168</td>
</tr>
<tr>
<td>3</td>
<td>Herpotrichiellaceae 1 (Asc)</td>
<td>OGF</td>
<td>0.0001</td>
</tr>
<tr>
<td>4</td>
<td>Herpotrichiellaceae 2 (Asc)</td>
<td>OGF</td>
<td>0.0002</td>
</tr>
<tr>
<td>5</td>
<td>Herpotrichiellaceae 3 (Asc)</td>
<td>OGF</td>
<td>0.0003</td>
</tr>
<tr>
<td>6</td>
<td>Herpotrichiellaceae 4 (Asc)</td>
<td>OGF</td>
<td>0.0203</td>
</tr>
<tr>
<td>7</td>
<td>Herpotrichiellaceae 5 (Asc)</td>
<td>OGF</td>
<td>0.0427</td>
</tr>
<tr>
<td>8</td>
<td>Penicillium sp1 (Asc)</td>
<td>OGF</td>
<td>0.0382</td>
</tr>
<tr>
<td>9</td>
<td>Russula sp1 (Bas)</td>
<td>OGF</td>
<td>0.0149</td>
</tr>
<tr>
<td>10</td>
<td>Sebacina sp1 (Bas)</td>
<td>OGF</td>
<td>0.0121</td>
</tr>
<tr>
<td>11</td>
<td>Talaromyces mimosinus (Asc)</td>
<td>OGF</td>
<td>0.0043</td>
</tr>
<tr>
<td>12</td>
<td>Thelephoraceae 1 (Bas)</td>
<td>OGF</td>
<td>0.0033</td>
</tr>
<tr>
<td>13</td>
<td>unidentified Ascomycota</td>
<td>OGF</td>
<td>0.0141</td>
</tr>
<tr>
<td>14</td>
<td>unidentified Ascomycota</td>
<td>OGF</td>
<td>0.0297</td>
</tr>
<tr>
<td>15</td>
<td>unidentified Ascomycota</td>
<td>OGF</td>
<td>0.0420</td>
</tr>
<tr>
<td>16</td>
<td>unidentified fungus 1</td>
<td>OGF</td>
<td>0.0066</td>
</tr>
<tr>
<td>17</td>
<td>Cryptococcus flicatus (Bas)</td>
<td>SEC I</td>
<td>0.0086</td>
</tr>
<tr>
<td>18</td>
<td>Gloeosporium sp1 (Asc)</td>
<td>SEC I</td>
<td>0.0123</td>
</tr>
<tr>
<td>19</td>
<td>unidentified ERM fungus (Asc)</td>
<td>SEC I</td>
<td>0.0436</td>
</tr>
<tr>
<td>20</td>
<td>unidentified fungus 2</td>
<td>SEC II</td>
<td>0.0306</td>
</tr>
<tr>
<td>21</td>
<td>Cryptosporiopsis ericae (Asc)</td>
<td>PLF</td>
<td>0.0438</td>
</tr>
<tr>
<td>22</td>
<td>Helotiales 2 (Asc)</td>
<td>PLF</td>
<td>0.0068</td>
</tr>
<tr>
<td>23</td>
<td>Ilyonecchia liriodendri (Asc)</td>
<td>PLF</td>
<td>0.0021</td>
</tr>
<tr>
<td>24</td>
<td>Lecanicillium psalliotae (Asc)</td>
<td>PLF</td>
<td>0.0252</td>
</tr>
<tr>
<td>25</td>
<td>Penicillium sp2 (Asc)</td>
<td>PLF</td>
<td>0.0135</td>
</tr>
<tr>
<td>26</td>
<td>Penicillium sp3 (Asc)</td>
<td>PLF</td>
<td>0.0032</td>
</tr>
<tr>
<td>27</td>
<td>Penicillium sp4 (Asc)</td>
<td>PLF</td>
<td>0.0267</td>
</tr>
<tr>
<td>28</td>
<td>Penicillium raphiae (Asc)</td>
<td>PLF</td>
<td>0.0210</td>
</tr>
<tr>
<td>29</td>
<td>Plectosphaerella cucumerina (Asc)</td>
<td>PLF</td>
<td>0.0051</td>
</tr>
<tr>
<td>30</td>
<td>Sebacinae 1 (Bas)</td>
<td>PLF</td>
<td>0.0116</td>
</tr>
<tr>
<td>31</td>
<td>Sebacinae 2 (Bas)</td>
<td>PLF</td>
<td>0.0135</td>
</tr>
<tr>
<td>32</td>
<td>unidentified endophyte</td>
<td>PLF</td>
<td>0.0018</td>
</tr>
<tr>
<td>33</td>
<td>unidentified fungus 3</td>
<td>PLF</td>
<td>0.0035</td>
</tr>
<tr>
<td>34</td>
<td>unidentified fungus 4</td>
<td>PLF</td>
<td>0.0297</td>
</tr>
<tr>
<td>35</td>
<td>unidentified fungus 5</td>
<td>PLF</td>
<td>0.0307</td>
</tr>
<tr>
<td>36</td>
<td>unidentified fungus 6</td>
<td>PLF</td>
<td>0.0001</td>
</tr>
<tr>
<td>37</td>
<td>unidentified fungus 7</td>
<td>PLF</td>
<td>0.0462</td>
</tr>
<tr>
<td>38</td>
<td>Xenochalara juniperi (Asc)</td>
<td>PLF</td>
<td>0.0213</td>
</tr>
</tbody>
</table>
Table 3 Correlations of microbial community composition with factors of host plant, plant community and abiotic environments

<table>
<thead>
<tr>
<th></th>
<th>RDA1</th>
<th>RDA2</th>
<th>$r^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean DBH of host plant</td>
<td>0.214</td>
<td>-0.977</td>
<td>0.573</td>
<td>0.026</td>
</tr>
<tr>
<td>Plant richness of plot</td>
<td>0.564</td>
<td>0.826</td>
<td>0.053</td>
<td>0.785</td>
</tr>
<tr>
<td>Total area at breast height of plot</td>
<td>0.958</td>
<td>0.286</td>
<td>0.067</td>
<td>0.733</td>
</tr>
<tr>
<td>PC1 (19.9%) of plant community</td>
<td>0.187</td>
<td>-0.983</td>
<td>0.015</td>
<td>0.938</td>
</tr>
<tr>
<td>PC2 (15.2%) of plant community</td>
<td>-0.140</td>
<td>-0.990</td>
<td>0.283</td>
<td>0.223</td>
</tr>
<tr>
<td>PC1 (97.9%) of soil parameters</td>
<td>-0.996</td>
<td>-0.089</td>
<td>0.016</td>
<td>0.909</td>
</tr>
</tbody>
</table>
Figure legends

Fig. 1 Rarefaction curves of fungal OTUs estimated by Chao1 in roots of *R. ovatum* in forests with different human disturbances, i.e., old growth forest (OGF), secondary forest I (SECI), secondary forest II (SECII), and plantation (PLF).

Fig. 2 Venn diagram showing specific and shared OTUs of forests with different human disturbances i.e., old growth forest (OGF), secondary forest I (SECI), secondary forest II (SECII), and plantation (PLF).

Fig. 3 Proportion of main fungal taxa associated with roots of *R. ovatum* in forests with different human disturbances, i.e., old growth forest (OGF), secondary forest I (SECI), secondary forest II (SECII), and plantation (PLF).

Fig. 4 Principal Component Analysis (PCA) of root-associated fungi of *Rhododendron ovatum* in forests with different human disturbances, i.e., old growth forest (OGF), secondary forest I (SECI), secondary forest II (SECII), and plantation (PLF).

Fig. 5 Frequency rate and relative abundance of fungal families in roots of *Rhododendron ovatum*. 
Fig1

297x210mm (150 x 150 DPI)
Fig 2

124x116mm (150 x 150 DPI)
Fig3

Proportion of main fungal taxa

Forest types
Table S1 Total area at breast height and mean DBH per individual of *R. ovatum* in forests with different human disturbances (i.e., old growth forest (OGF), secondary forest I (SEC-I), secondary forest II (SEC-II), and plantation (PLF)).

<table>
<thead>
<tr>
<th>Forest type</th>
<th>Total area at breast height (m$^2$ ha$^{-1}$)</th>
<th>Mean DBH per individual (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OGF</td>
<td>1.01±0.09 a</td>
<td>6.10±0.80 a</td>
</tr>
<tr>
<td>SEC-I</td>
<td>0.23±0.12 bc</td>
<td>3.32±0.15 b</td>
</tr>
<tr>
<td>SEC-II</td>
<td>0.42±0.07 b</td>
<td>3.63±0.14 b</td>
</tr>
<tr>
<td>PLF</td>
<td>0.07±0.05 c</td>
<td>2.33±0.13 b</td>
</tr>
<tr>
<td>F-value</td>
<td>24.03</td>
<td>14.76</td>
</tr>
<tr>
<td>$P$</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>