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中文摘要

森林管理需考量生物多樣性保護與生態功能維持，其生態系中，營養循環的主要分解者為真菌，疏伐改變森林結構及環境，對真菌多樣性、群聚結構與生態功能的影響非常重要。本論文研究目的為瞭解疏伐對真菌族群、生態、多樣性和群聚結構的影響，包括一、疏伐強度對於土壤真菌遺傳多樣性與群聚結構之影響；二、疏伐對大型真菌多樣性與群聚結構的影響及其與環境因子的相關性；三、疏伐對優勢真菌族群遺傳之影響；四、扁枝瑚菌之內生真菌。第一項研究以變性梯度凝膠電泳分析紅檜與柳杉人工林土壤真菌多樣性和群聚結構，發現柳杉之真菌群聚結構在疏伐後 12-21 個月，處理間顯著不同，第 24 個月以後沒有差異；而紅檜林在疏伐後四年仍受疏伐效應影響。顯示土壤真菌群聚對疏伐擾動敏感，柳杉林在較短時間內恢復穩定。第二項研究以大型真菌的出菇及分布情形，反映疏伐及其後的環境改變對真菌有性生殖的影響。結果顯示，疏伐影響紅檜林和柳杉林內大型真菌多樣性和群落組成，群落組成的與相對濕度、森林光照度、樹冠層覆蓋度、土壤溫度、土壤含水量、pH 值、土壤質地等環境因子有關。柳杉林內的優勢真菌－扁枝瑚菌族群量受疏伐程度影響最明顯，疏伐程度越高，族群量越低。根據形態學和分子技術，確立與記述扁枝瑚菌(*Scytinopogon cryptomerioides* W.R. Lin & P.H. Wang., sp. nov.)為新種真菌。第三項研究監測該菌疏伐前後共七年的族群量變化，並研究族群量變化下的族群遺傳結構。結果顯示，扁枝瑚菌族群量在疏伐後立即減少，三年後開始回升。根據子實體樣本的 RAPD 指紋分析，發現該菌是高度遺傳多樣性的族群，其遺傳個體分佈範圍小，且生命週期短，顯示在白色扁枝瑚菌

的拓殖策略上，倚重有性生殖產生的擔孢子而非菌落的營養生長。白色扁枝瑚菌以擔孢子傳播與拓殖，疏伐降低其有性生殖出菇量，減少年度更新的菌落量，回復的族群為鄰近保留族群的擔孢子傳播拓殖形成。該菌可作為受疏伐影響的指標物種，林業管理應保護生態棲地與種源，使生物族群得以維持，以免滅絕。扁枝瑚菌的子實體中有炭角菌共棲，第四個研究調查共棲炭角菌的多樣性，及其在子實體中的分佈狀況，這是子囊菌內生於擔子菌之首次發現，也是真菌內生真菌的新發現。

關鍵字：柳杉、紅檜、森林經營管理、人工林、疏伐、炭角菌、白色扁枝瑚菌

Abstract

Forest management needs to consider biodiversity preservation and ecosystem function. Fungi are main decomposers of the nutrient cycle in forest ecosystem. Thinning changes forest structure and environments. It is an important issue to understand the changes of fungal diversity and community to forest thinning. The purpose of this dissertation was to know the impacts of thinning on fungal ecology and diversity from population to community level, including 1) impacts of thinning intensity on genetic diversity and community of soil fungi; 2) thinning effects on macrofungal diversity and community and the correlation between environments and fruiting; 3) thinning impacts on population genetic structure of a dominant fungus; 4) The endophytic fungi in basidiocarps of *Scytinopogon cryptomerioides*. In the first study, diversity and community of soil fungi in a *Chamaecyparis formosensis* and a *Cryptomeria japonica* plantation forest was investigated by denaturing gradient gel electrophoresis and plate count. Results showed that in the *C. japonica* plantation, soil fungal communities were different significantly among treatments in the first 21 months post-thinning; there were no significant differences afterwards. However, the soil fungal communities were still affected by thinning in *C. formosensis* plantation at the fourth year post-thinning. The soil fungi were sensitive to thinning disturbances, but they recovered and became stable after a relatively short period of time in *C. japonica* forest. In the second study, the phenology, diversity, and abundance of macrofungi were investigated to evaluate the influences of thinning and environments on sexual reproduction of fungi. Forest thinning affected macrofungal species richness observed but not abundance. Thinning influenced macrofungal diversity and community. The changes of macrofungal community in the *C. japonica* plantations

were significantly associated with relative humidity, light, canopy cover, soil water content, soil temperature, soil pH value and soil texture. The fruiting of a dominant fungal species, *Scytinopogon* sp., was significantly affected by thinning intensity. Based on morphological and molecular evidence, this coralloid fungus was confirmed and described as a new species *Scytinopogon cryptomerioides* W.R. Lin & P.H. Wang., from *C. japonica* plantation. In third study, its population fluctuations were monitored and population genetic structure was investigated during 7 years after thinning. Results showed that the population of *S. cryptomerioides* was reduced immediately after thinning and rose again after three years. According to the RAPD patterns, its population has high genetic diversity. Numerous short lifespan genets confirmed that the propagation of sexual basidiospores is more important than vegetative growth of its colony. Thinning decreased the numbers of fruiting bodies and inhibited the colonization. The recovery of the macrofungus population in thinned plots was by the basidiospores recruited from adjacent un-thinned shelter plots. To conserve the kind of fungal species, the habitats and minimum viable population have to be protected during forest managements. Xylariaceous fungi were found to be associated with the fruiting bodies of *S. cryptomerioides*. In the fourth study, I reported diversity of xylariaceous fungi and their distribution in *S.cryptomerioides* sporocarps.

Keywords: *Cryptomeria japonica*, *Chamaecyparis formosensis*, Forest management, Plantation, Selective thinning, *Xylaria*, *Scytinopogon*

Introduction

Taiwan is a rich forest region with about 2.10 million ha forest land occupying 58.53% of total land area (Taiwan Forestry Bureau, 1995). Among these area, 20% are plantations. Plantation played a very important role in timber products in the past (Taiwan Forestry Bureau, 2007). During stand developments, thinning treatments should be practiced in plantations. Over the past 15 yrs, forest management has become more complex and it has had to take into consideration a number of new concepts, including biodiversity preservation (Ehrlich and Wilson, 1991; Hunter and Gibbs, 2007), landscape-level management (Oliver, 1992), and global forest ecosystem management (Potvin et al., 1999). In addition to maximize commercial values, solving biodiversity problems caused by silvicultural practices has become one new aim (Reid and Miller, 1989; Plochman, 1992; Goodland, 1995; Perry, 1998).

Chamaecyparis formosensis Matsum. (Taiwan red cypress) and *Cryptomeria japonica* D. Don (Japanese cedar) are very important plantation species in Taiwan. *Chamaecyparis formosensis* is an endemic conifer that grows at elevations of 1500–2150 m in mountains of central Taiwan (Liu et al., 1988).

There were about 45,000 ha of *Cryptomeria japonica* plantations in mountainous regions (Taiwan Forestry Bureau, 1995) in Taiwan. *Cryptomeria japonica* was introduced from Japan in 1896 and was cultivated extensively for economic uses in 1911. The plantation forest in Taiwan entered the maturation stage. However, timbers of this tree species are no longer popular in Taiwan because of high cost and low economic values. A new management strategy to incorporate the needs of ecosystem functioning, recreation, conservation and educational purposes

is developed.

For forest managers, thinning is an effective option to create plant species diversity sooner and with more consistency in conifer plantation (Thomas et al., 1999; Bergstedit and Miberg, 2001; Utsugi et al., 2006). Thinning creates forest gaps and enhances forest regeneration. However, it is a disturbance to the organisms in the forest ecosystems (Tsui et al., 1998) and affects biodiversity (Kerr, 1999) and ecosystem function (Bengtsson et al., 2000). Understanding how organisms respond to the disturbance caused by forest thinning is important for developing forest management strategies.

Fungi, the major components of biodiversity, are essential for decomposition, nutrient cycling (Tate, 1995; Chapin et al., 2002,) and nutrient transport (Delvasto et al., 2006; Tortora et al., 2007) in the forest ecosystem. Fungi are decomposers. Fungi account for the majority of microbial biomass in forest soil and convert recalcitrant organic material into forms that other organisms can use (Dighton, 2003). Mycorrhiza, the symbiosis between plant roots and fungi, is one of the most ubiquitous mutualisms in terrestrial ecosystems. In addition, the fungi are also a major component of terrestrial food webs. Fungal mycelia serve as the primary carbon source in a number of soil food webs, and fungal fruiting bodies can serve as a significant food source for large vertebrates. Thus far, most reports on microorganisms have been restricted to bacteria, but I feel that it is equally important to know about the effects of thinning on fungi. As their habitats and the substrates they degrade are different (Dighton 2003), fungi and bacteria have different roles in nutrient cycling and ecosystem function. Fungal fruiting bodies appear in particular environments at particular times (Ingold and Hudson 1993). The distribution of fruiting body reflects the size of the underground fungal

colony (Bridge and Spooner, 2001) and presents fungal diversity. In order to provide science-based knowledge to developing a reasonable strategy, the large scale project to investigate the effect of different thinning practices in fungal population diversity, communities and ecosystem functions was conducted.

In chapter one, I investigated the diversity of microbes of a lightly thinned plot, a heavily thinned plot, and an unthinned control plot in a 30-year-old *Chamaecyparis formosensis*. The objectives of chapter one is the survey of effects of thinning and thinning intensity on the diversity, community, and function of macrofungi and soil microbes in a tropical forest.

However, due to the forest size and timing, there was no replicate and no baseline data before thinning in Chapter one. Moreover, I would like to know the link between the changes of macrofungi communities and environments.

In chapter two, instead of a *Chamaecyparis formosensis* forest, a *Cryptomeria japonica* forest was studied. The thinning programmed with plot replicates was organized. This chapter investigated the thinning effects on macrofungal diversity and community and identified environmental factors affecting fruiting by investigating the phenology, diversity, and abundance of macrofungi. In this context, there were three principle questions to answer. First, what is the effect of thinning on fungal diversity and community structure in the plantations? Second, what environmental factors affect the macrofungal community after thinning? Third, what are the dominant species and the thinning effect on their fruiting pattern? Potential indicator species for recovery of the environments from forest thinning were also investigated.

Soil fungi produced extracellular enzyme capable of breaking down

the recalcitrant components of plant materials. Beyond disturbance, temporal or seasonal effects also influence soil microorganisms. For example, the phospholipid fatty acid profiles of microbial communities have been reported to change in response to the change in seasons (Buckley and Schmidt, 2003; Hamel et al., 2006). Moreover, fungal communities associated with oak rhizospheres and grassland soils are known to be seasonally dynamic with temporal turnover (Kennedy et al., 2006; Voříšková et al., 2014).

Most studies of thinning impacts on soil microbes have compared microbial communities in forests 3 to 45 years post-thinning (Houston et al., 1998; Barbhuiya et al., 2004; Hannam et al., 2006; Maassen et al., 2006; Barbhuiya et al., 2008; Cookson et al., 2008; Chatterjee et al., 2008). Generally, long-term assessments have not focused on short-term and seasonal dynamics. Understanding the short-term effects of thinning on microbial communities in forest soils addresses an important knowledge gap. As soil fungi have a crucial role in the forest community structure and productivity, it is important to understand the response of these organisms to disturbances and their dynamics in the period that follows. In chapter three, I studied the response of soil fungi to thinning in a Japanese cedar (*Cryptomeria japonica*) forest plantation in central Taiwan. The objectives of this chapter were to: (1) investigate thinning effects on the soil microfungal population and communities in *C. japonica* plantations; (2) evaluate the changes corresponding to the thinning intensity; and (3) determine the duration of any thinning effects influenced on fungal communities in the soils of these forests. Plate counts and denaturing gradient gel electrophoresis (DGGE) were used as relatively simple methods to monitor the fluctuation of the soil fungal population and to describe the changes of their genetic diversity and

community after thinning. These methods were chosen because of their relative cost efficiency and their ability to detect major community shifts (e.g., Winder et al., 2013) without saturating the acquired datasets.

Conservation of fungi was aware in recent decades (Moore et al., 2001; Dahlberg et al., 2010; van der Linde et al., 2012), especially after obvious decline in the abundance of many taxa in Europe (Jansen and van Dobben, 1987; Lizon, 1993). The causes of the declines might be environmental stresses or disturbances, such as silviculture, nitrogen pollution and land use changes (Pilz et al., 2006; Dahlber et al., 2010). How interaction of population and disturbance affect species existence was a challenge for conservation managements (Reigada et al., 2015).

Most organisms suffer from natural or anthropogenic stresses or disturbances. These stresses or disturbances can shape biodiversity at the species and community levels (Allison and Martiny, 2008; Banks et al., 2011; Lin et al., 2015; Lotze et al., 2011) by affecting demographic parameters, including survival, dispersal or reproduction. Stress or disturbance not only influences biodiversity at the species and community levels but also drives the spatial and temporal patterns of genetic diversity (Banks et al., 2013).

Silviculture is an important disturbance to organisms in forest ecosystems and affects biodiversity and ecosystem function (Bengtsson et al., 2000). Many macrofungal species show a decrease of population after disturbances, such as silviculture, nitrogen pollution and land use changes (Pilz et al., 2006; Dahlber et al., 2010) and some of them show a recover of the macrofungal population after disturbance (Pilz et al., 2006). However, the specific processes and mechanism by which population recover from the disturbances are not well known.

Scytinopogon cryptomerioides is a fungal species that produced

coralloid fruiting bodies and only found in the *Cryptomeria japonica* forests in Taiwan (Lin et al., 2015). In chapter four, *Scytinopogon cryptomerioides* was described and illustrated herein as a new species based on morphological characteristics and DNA sequence data. The phylogenetic placement of *S. cryptomerioides* was inferred with the internal transcribed spacer (ITS) region and the large subunit DNA (LSU rDNA).

According to the results of chapter two, I found that the abundance of its sporocarps was clearly affected by thinning intensity in two years. In chapter five, I monitored the population dynamics of *S. cryptomerioides*, identified the genet of each sporocarp, and showed the temporal and spacial distribution of the genets. Specific processes by the population of *S. cryptomerioides* recovered could be observed. According to the data, the colonization strategy of *S. cryptomerioides* was inferred and the thinning influence on survival of *S. cryptomerioides* was evaluated. The results may be useful in understanding the mechanism of recovery and making recommendations in conservation managements for macrofungi.

In the context of ecological studies on *S. cryptomerioides*, I found that xylariaceous fungi were associated with the fruiting bodies of this fungus. In the last chapter, I reported the diversity of xylariaceous fungi and their distribution in *S. cryptomerioides* fruiting bodies. The ecology and interactions between the fungi were discussed.

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Chapter 1 – Effects of forest thinning on diversity and function of macrofungi and soil fungi

Introduction

Bacteria and fungi are the main decomposers in forests (Chapin et al., 2002; Tate, 1995). Saprotrophic fungi and bacteria account for 80 - 90% of the activities of all decomposers in the soil (Chapin et al., 2002). They play important roles in ecological processes and nutrient dynamics of a forest ecosystem (Delvasto et al., 2006; Tortora et al., 2007). Thus, knowledge of effects of disturbance on diversity and function of macrofungi and soil microorganisms is important for evaluating the stability and resilience of a forest ecosystem.

Thinning, a common forest management technique, improves the growth of the remaining trees (Grant et al., 2007) and enhances forest regeneration. However, it is a disturbance to the organisms in forest ecosystems (Tsui et al., 1998) and affects biodiversity (Kerr, 1999) and ecosystem function (Bengtsson et al., 2000).

Thinning was shown to change soil microbial communities measured by phospholipid fatty acid analysis (Maassen et al., 2006), DNA fingerprinting data (Smith et al., 2008) and community-level physiological profiles of the soil microorganisms (Cookson et al., 2008). However, some studies reported no effects of forest thinning on microbial biomass carbon, on soil respiration or on enzyme activity and communities (Grayston and Rennenberg 2006; Maassen et al., 2006). Therefore, the effects of forest thinning on soil microorganisms are still unclear.

In this study, we investigated the diversity of microbes of an unthinned control plot, a lightly thinned plot, and a heavily thinned plot.

There are three hypotheses possible: (1) Un-thinned plots exhibit the highest diversity because the disturbance was adverse to fungi. (2) Lightly thinned plots show the highest diversity, according to the intermediate disturbance hypothesis (Connell, 1978), moderate disturbance increase biodiversity. (3) Heavily thinned plots are most diverse because the treatment produces large amounts of substrates for saprotrophs. The objectives of this study is the survey of effects of thinning and thinning intensity on the diversity, community, and function of macrofungi and soil microbes in a temperate mountainous forest in tropical latitudes.

Materials and Methods

Research site

The study site was in the 121st and 123rd divisions of the Daan Creek Business District of the Dongshi Forest District Office, Taichung County, Taiwan. The area included plantations of 30-year-old *Chamaecyparis formosensis* Matsum. and an adjacent natural broadleaf forest. Elevations and slopes of the study site ranged from 1800 m to 2000 m and 20° to 25°. The mean annual temperature was approximately 12.4 ℃ and rainfall 4071 mm in 2006, according to the weather station in Taiwan. The study site included three 7.5 hectare plots: an unthinned control, a lightly thinned plot, and a heavily thinned plot; the remaining trees were 1500, 1000, and 825 per hectare, respectively. The thinning was performed in 2004. An adjacent natural broadleaf forest plot was investigated as the control.

Collection, identification and documentation of macrofungi

Fruitbodies (Basidiomycetes and Ascomycetes) were surveyed and collected from the 200 m transect of each plot in spring and autumn in 2006 and 2007. The annotation sheet of Lodge and Cantrell (1995) was modified to document the macro-morphological features of fresh fruitbodies. Species were identified morphologically. Fruitbodies were counted, fruiting seasons and locations were documented. Representatives of each species were photographed *in situ* and collected, dried at 50 ℃ for 1 or 2 days, and preserved at the Department of Life Science, Tunghai University, Taiwan.

Soil sampling

Three points at least 20 m apart were randomly selected in each plot, and four sampling sites were set in each direction from the centre point. In autumn 2008, a 100 g surface soil sample was collected from each sampling site; four individual samples were pooled. The experiment was conducted with the three mixed soil samples from each treatment. The samples were sieve through a 2 mm mesh to remove mesofauna, plant residue and stones. DNA was immediately extracted from the soil samples.

DNA extraction and PCR amplification from soil samples

A PowerSoil DNA Combo Kit (Mo Bio Laboratories, Inc. CA, USA) was used to extract DNA from 0.25 g of soil. The ITS rDNA region was amplified using a semi-nested PCR amplification method. In the first PCR, the primer pair ITS1F /ITS4 (Gardes and Bruns 1993; White et al., 1990) was used. Sample DNA was diluted ten times for PCR amplification. The 25 μl PCR mix consisted of 10 ng template DNA, 250

μM of each primer, 250 μM dNTPs, 1 U *Taq* DNA polymerase (Fermentas, USA), 2.5 mM $MgCl_2$, and 2.5 μl 10× buffer. The amplification protocol consisted of one denaturation at 94 °C for 5 min, 39 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, and extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min. These amplicates were used as template for the second PCR with primer pair ITS1F with a 5' 40-base GC-clamp /ITS2 (Gardes and Bruns 1993; White et al., 1990). The master mix was same as for the first PCR, the volume of the template DNA was 5 μl. The amplification program was as described above.

The 16S rRNA gene of bacterial communities in the soil samples were amplified by the primer pair 341f-GC /907r (Li et al., 2009). The PCR reagents were the same as described above. The PCR programs were 94 ℃ for 5 min, 35 cycles of 94 ℃ for 30 s, 55 ℃ for 1 min, and 72 ℃ for 1 min and a final step of 72 ℃ for 5 min. Agarose gel electrophoresis was used to evaluate the amount of PCR products.

DGGE analysis

PCR products were analysed with DGGE using the DCODE™ universal mutation detection system (Bio-Rad Laboratories, USA). For fungi, 8% polyacrylamide gels were prepared with a 20% to 45% vertical denaturing gradient. DGGE was executed at 70V for 16 h at 60 °C in 1× TAE buffer. For bacteria, we used 6% polyacrylamide gels with a 45% to 55% vertical denaturing gradient for DGGE. The electrophoresis was at 100 V for 12 h at 60 ℃ in 1× TAE buffer. The DGGE gels were stained with SYBR gold (Molecular Probes, USA) in the dark for 30 min at room temperature, rinsed once in deionised water, and then viewed under ultraviolet light.

Biolog assay

Ten grams of soil and 3 g of 3 mm diameter glass beads were added to 90 ml 0.85% NaCl in a flask and shaken one hour on a horizontal shaker at 140 rpm. Thirty ml soil suspension was centrifuged at 700 ×g for 10 min to remove soil particles. The suspension was diluted with 0.85% NaCl to a concentration of 10^{-3} g ml^{-1}. Biolog GN2 microplates (Biolog, CA, USA) were incubated in duplicates at 25 ℃ for 72 hours. Bacterial growth was measured at 590 nm using Biolog™ MicroStation™ System (Biolog™, USA).

Data analysis

Biodiversity indices and were estimated using the software PRIMER (Clark and Warwick 2001). The degree of similarity between the macrofungi associated with different plots was calculated by principle component analysis (PCA) using PRIMER (Clark and Warwick, 2001). DGGE profiles and Biolog™ data were used to calculate Bray-Curtic dissimilarity (Kerbs, 1989). The similarity matrix were analysed by multi-dimension scaling (MDS) and one-way analysis of similarity (ANOSIM) to determine the significance level by PRIMER (Clark and Warwick, 2001).

Results

Macrofungal communities and diversity

Five surveys from 2006 to 2008 yielded 6263 records of macrofungi comprising 142 species belonging to 63 families. The biodiversity indices including species richness and Shannon-Wiener index for macrofungi were higher in the broadleaf forest than in plantation plots (Table 1). In

the un-thinned plot we found the lowest fungal species counts but the highest total number of fruitbodies among the plantation treatments. The lightly thinned plot had the highest biodiversity indices among the plantation plots (Table 1).

In broadleaf forest more species and more fruitbodies can be found than the *Chamaecyparis formosensis* plantation. *Stereum ostrea* (Blume & T. Nees) Fr., *Trichaptum biforme* (Fr.) Ryvarden, *Mycena pura* (Pers.) P. Kumm., and *Daldinia eschscholzii* (Ehrenb.) Rehm were dominant in both, the plantation and broadleaf forest. Forty-eight species were found only in the plantation. Sixty-six additional species were found in the broadleaf forest, including ectomycorrhizal fungi, such as *Amanita rubrovolvata* S. Imai, *A. vaginata*, (Bull.) Lam., *Craterellus cornucopioides* (L.) Pers., *Cortinarius salor* Fr., *Lactarius camphoratus* (Bull.) Fr., *L. volemus* (Fr.) Fr., and *Russula senecis* S. Imai. The most common fungi in the research site were the wood inhabiting *Coprinellus disseminatus* (Pers.) J.E. Lange (641 records; un-thinned forest), *Dicephalospora rufocornea* (Berk. and Broome) Spooner (50 records; lightly thinned forest), *Psathyrella* sp. 1 (175 records; heavily thinned forest) and *Crepidotus variabilis* (Pers.) P. Kumm. (356 records; broadleaf forest).

Saprotrophic fungi were the main functional group in the study site (Table 1). The macrofungi found in the unthinned and lightly thinned plantations were all saprobes: most of them inhabited stumps and fallen branches; some of them were soil inhabitants (Table 1). Ectomycorrhizal and parasitic macrofungi were found in the heavily thinned plot and in the broadleaf forest (Table 1). In the heavily thinned plantation, an ectomycorrhizal *Amanita* sp. was recorded that was not symbiotic with *C.*

formosensis. Ectomycorrhizal Russulaceae and Amanitaceae were found in the broadleaf forest.

PCA of the macrofungal occurrence data clearly separated each macrofungal community (Figure 1). PC1 explained the 62.2% variability and separated the macrofungal communities of the broadleaf forest from those of the plantations (Figure 1). There were 70 species that were found only in the broadleaf forest. Eighteen species were found in plantations only. The macrofungal communities of the three plantation treatments were separated on PC2. PC2 accounted for the 33.9% variability (Figure 1). Thirteen species were found only in unthinned plantation. There were 18 species found only in the lightly thinned plantation. Twelve species were found only in the heavily thinned plantation. *Coprinellus disseminatus*, *C. micaceus* (Bull.) Vilgalys, Hopple and Jacq. Johnson, *Geastrum triplex* Jungh., *Scutellinia scutellata* (L.) Lambotte, *Pleurotus ostreatus* (Jacq.) P. Kumm., *Hypholoma fasciculare* (Huds.) P. Kumm., and *Oudemansiella mucida* (Schrad.) Höhn. were the dominant species in the unthinned plot, but could not be found in the thinned plots.

DGGE analysis of fungal and bacterial communities from soil samples

The PCR product of the soil fungal rDNA ITS region was about 700 bp long. The second PCR amplification generated ITS1 region products of 300 bp. DGGE profiles of each sample revealed 16 to 26 bands. MDS plot showed that the DGGE profile of soil fungi from each treatment clustered together (Figure 2a). The ANOSIM tests (Table 2) showed that there were significant differences in soil fungal communities constructed from the DGGE profiles ($P = 0.001$), but the pairwise tests of the treatments were not significantly different ($P = 0.1$). MDS plot and

ANOSIM tests constructed from the soil bacterial DGGE profiles showed the same trend with soil fungi (Figure 2b; Table 2). These results indicated that the soil fungal and bacterial communities were different among treatments.

Biolog assay with soil samples

MDS plot constructed from the Biolog assay showed that the microbial carbon use patterns of the soil samples collected from three sampling points at each treatment plot were scattered (Figure 2c). The ANOSIM tests (Table 2) showed that the microbial carbon utilisation patterns seen in the Biolog assay were not significantly different among the three treatments ($P = 0.175$).

Discussion

The fungal diversity was highly associated with the diversity of vascular plant (Packham et al., 2002). In this study, Fagaceae were dominant within the broadleaf forest and the diversity of plants was higher than in the plantations. Our data showed the macrofungal diversity was much higher in broadleaf forest than in the plantation and supported the evidence to prove the community of decomposers, including microbes, in the broadleaf forest is more divers than in a conifer forest (Brown et al., 2006).

The intensity of thinning also affected macrofungal diversity, as in relation to the heavily thinned plantation or the unthinned plantation, in the lightly thinned plantation a higher species was observable. According to the intermediate disturbance hypothesis (Connell, 1978), a high degree of diversity is maintained by moderate disturbance. In a less disturbed environment, the species with the strongest competitiveness will survive,

thereby excluding other species and progressing towards a community with low diversity. In a severely disturbed environment, only species with a high tolerance can survive. The detailed mode of disturbance influenced species communities and biodiversity (Armstrong, 1976). The here presented data confirm this hypothesis: light forest thinning yielded in higher macrofungal diversity.

The macrofungal, soil fungal and bacterial communities in the broadleaf forest were obviously different from those in the *C. formosensis* plantation. The litter qualities produced by different plant species were very different, which was a key factor influencing decomposer diversity, microbial community structure (Badejo and Tian, 1999; Ilieva-Makulec et al., 2006) and macrofungal community (Mullan-Fisher et al., 2002). Some macrofungal species were found in all plots, including *Stereum ostrea*, *Trichaptum bioforme*, *Mycena pura* and *Daldinia eschscholzii*. These species were also common in other forests (Ortega and Lorite, 2007). *Trametes versicolor* (L.) Lloyd could be found in both the unthinned and lightly thinned plantation forests but not in the heavily thinned forest, and the abundance of *T. versicolor* was higher in the unthinned plantation forest than in the lightly thinned plantation forest. *Galerina hypnorum* (Schrank) Kühner, *Dacrymyces chrysospermus* Berk. & M.A. Curtis, *Lepiota clypeolaria* (Bull.) P. Kumm., *Lycoperdon pyriforme* Schaeff., *Ramaria stricta* (Pers.) Quél., and *Megacollybia platyphylla* (Pers.) Kotl. & Pouzar were found in the thinned plantation forests but not in the unthinned plantation forest. They were found in the broadleaf forest and in the thinned plantation forests. Probably these species originally occurred in the broadleaf forest and re-colonised the plantations after thinning.

Thinning changes soil properties such as pH value, total nitrogen, and organic carbon, and it also influences microbial communities (Korb et al., 2001). After thinning, microbial carbon and soil nitrogen decreased (Grady and Hart, 2006), while the nitrogen mineralisation rate increased (Zhuang et al., 2005). The abundance of dominant fungal taxa was associated with nutrients conditions (Lauber et al., 2008). Nutrients and environmental conditions influenced decomposers and the macrofungal community as well. After thinning, biotic factors (e. g. vegetation) and abiotic factors (availability of nutrients) changed, and with them the macrofungal and soil microbial community.

A change in the structure of the soil microbial community was detected in this study, whereas the soil enzymes, measured by Biolog assay, were not altered in thinning treatments in the fourth year after thinning. Forest thinning altered the soil microbial community structure but maintained the ecological functions of the soil microorganisms due to functional redundancy (Allison and Martiny, 2008).

Forest thinning and forest type affected the diversity and community of the macrofungi and soil microbes. Thinning increased the diversity of saprotrophic macrofungi and higher macrofungal diversity can be maintained by light thinning. Thinning changed the structure of the soil microbial community of the plantation. There was no significantly thinning effect on the soil microbial function in the fourth year after thinning. This may be due to the functional redundancy of soil microbes.

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Table 1-1. The number of macrofungal species and fruitbodies, the biodiversity indices, and the number of macrofungal fruitbodies and of macrofungal species of different functional groups found in the different treatment plots.

		Unthinned	Lightly thinned	Heavily thinned	Broadleaf
Diversity	Numbers of species	26	39	32	93
	Numbers of Fruitbodies	1,010	438	784	4,031
	Species Richness index	3.61	6.25	4.65	11.1
	Pielou's Evenness index	0.51	0.81	0.77	0.76
	Shannon-Wiener index	1.65	2.97	2.66	3.43
Functional groups	Ectomycorrhizal	0/0	0/0	[a]2/1	22/11
	Parasitic	0/0	0/0	23/2	39/1
	Soil Saprotrophic	56/7	114/18	154/6	186/16
	Wood Saprotrophic	954/19	324/21	605/23	3,784/65

[a] macrofungal fruitbodies number/species number.

Table 1-2. Results of pair-wise ANOSIM tests of soil fungal and soil bacterial communities based on DGGE patterns and soil microbial functions using the Biolog assay between the different thinning treatments.

Pairwise test		soil fungi		soil bacteria		Biolog	
		R	*p*	R	*p*	R	*p*
Broadleaf	Unthinned	0.407	0.2	1	0.1	[a]n.d.	n.d.
Broadleaf	Lightly	0.444	0.1	0.148	0.2	n.d.	n.d.
Broadleaf	Heavily	0.556	0.1	0.37	0.2	n.d.	n.d.
Unthinned	Lightly	0.537	0.1	0.667	0.1	0.481	0.1
Unthinned	Heavily	0.963	0.1	0.963	0.1	0.185	0.3
Lightly	Heavily	0.926	0.1	-0.222	0.9	-0.037	0.7
Global		0.579	0.001*	0.478	0.007*	0.185	0.14

*The p-value was less than 0.05 that means significant differences

[a]n.d.: no data

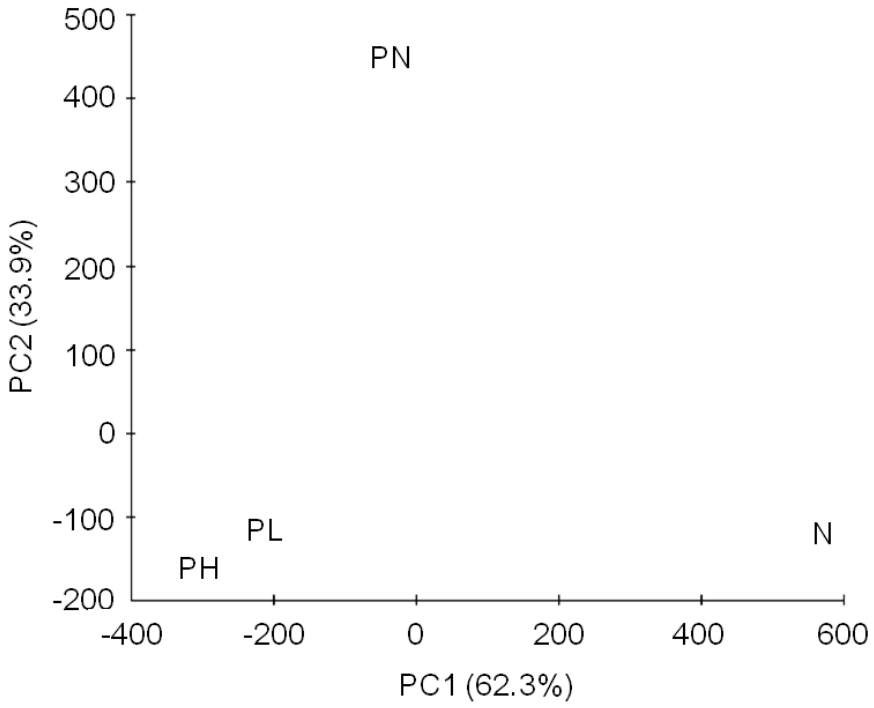

Figure 1-1. The Principal Components Analysis of macrofungal species components was done for samples from the natural broadleaf forest (N) and unthinned (PN), light thinned (PL), and heavy thinned (PH) plantation forests.

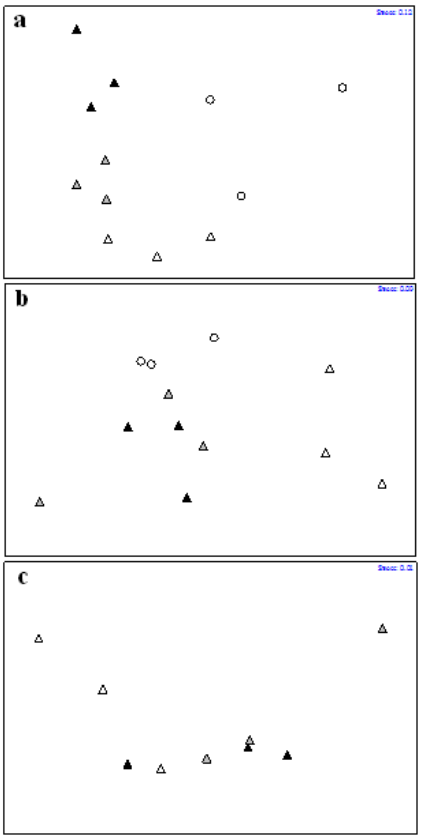

Figure 1-2. MDS ordination of soil microbial communities and functions from the broadleaf forest and the unthinned, lightly thinned, and heavily thinned plantation forests. **a**. soil fungal communities form DGGE profiles; **b**. soil bacterial communities form DGGE profiles; **c.** soil functions from Biolog assay. White circle (○), broadleaf forest; white triangle (△), unthinned plantation forest; gray triangle (◬), lightly thinned plantation forest; black triangle (▲), heavily thinned plantation forest.

Chapter 2 – The impacts of thinning on the fruiting of saprophytic fungi in *Cryptomeria japonica* plantations in central Taiwan

Introduction

Species diversity is increasingly considered a key function of the ecosystem (Scherer-Lorenzen et al., 2005). In recent decades, biodiversity conservation has been increasingly considered when conducting forest management operations (Bengtsson et al., 2000; Lindenmayer and Franklin, 2002). Thinning, a common forest management technique, improves the growth of the remaining trees and enhances forest regeneration (Grant et al., 2007). Thinning is nevertheless a disturbance to organisms in forest ecosystems and affects biodiversity and ecosystem function (Bengtsson et al., 2000). Studies regarding the impact of silvicultural systems on diversity and community have recently grown in number (e.g. Bonet et al., 2012; Luoma et al., 2004; Pilz et al., 2006; Seiwa et al., 2012; Teste et al., 2012; Yamashita et al., 2014). There has been lots of attention paid to soil arthropods and bacteria, and most of these studies focus on forests in the temperate zone (Luoma et al., 2004; Meyer et al., 2005; Pilz et al., 2006).

Fungi, a major component of biodiversity, are essential for decomposition, nutrient cycling (Chapin et al., 2002; Tate, 1995) and nutrient transport (Delvasto et al., 2006; Tortora et al., 2007) in the forest ecosystem. High fungal diversity is essential to support the stability and resilience of the forest ecosystem (Perry et al., 1989). Factors influencing the fungal community included vegetation (Ferris et al., 2000), environmental factors (Tedersoo et al., 2011), climate changes (Kauserud et al., 2008) and disturbance (Bonet et al., 2012; Luoma et al., 2004; Seiwa et al., 2012; Teste et al., 2012). Fungal species with different

environmental tolerances shift in abundance in response to changing environments.

Studies on the response of the macrofungal community to thinning in tropical areas were few (Brown et al., 2006; Lin et al., 2012), and most of the studies in tropical area (eg., Lin et al., 2012) fell short of the link of changing environments and macrofungal community. Thus, knowledge regarding the effects of disturbances on macrofungal diversity and community are important for evaluating the stability and resilience of a forest ecosystem.

There were about 45,000 ha of *Cryptomeria japonica* plantations in mountainous regions (Taiwan Forestry Bureau 1995) in Taiwan. This tree species was introduced from Japan in 1896 and was cultivated extensively for economic uses in 1911. The plantation forest in Taiwan entered the maturation stage. However, timbers of this tree species are no longer popular in Taiwan because of high cost and low economic values. Due to these reasons, a new management strategy to incorporate the needs of ecosystem functioning, recreation, conservation and educational purposes is developed. In order to provide science-based knowledge to developing a reasonable strategy, the large scale project to investigate the effect of different thinning practices in communities and ecosystem functions was conducted.

Macrofungi are major components of global biodiversity and control the rates of key ecosystem processes. Understanding how macrofungi respond to the disturbance caused by forest thinning is important for developing forest management strategies and biodiversity conservation. This study investigated the thinning effects on macrofungal diversity and community and identified environmental factors affecting fruiting by investigating the phenology, diversity, and abundance of macrofungi. In this context, there were three principle questions to answer. First, what is

the effect of thinning on fungal diversity and community structure in the plantations? Second, what environmental factors affect the macrofungal community after thinning? Third, what are the dominant species and the thinning effect on their fruiting pattern? Potential indicator species for recovery of the environments from forest thinning were also investigated.

Materials and Methods

Study site

The study site is located in Zen-Len area, Nantou County in central Taiwan. The site ranges from 23°28’ N to 23°55’ latitude and from 120°48’ E to 121°09’ E longitude. Elevation ranges from 1,300 to 1500 m. Average annual air temperature and rainfall recorded from nearby Sun Moon Lake Weather Station (23°53’N, 120°54’E) were 15.78℃ and 2,628 mm, respectively. Most rainfall at this site occurs during the summer, from June to September. Some rainfall also occurs during the spring, from March to May; there is no obvious dry season. The natural vegetation in this area was clear-cut about 35 years previous to the start of the study and replanted with Japanese cedar, *Cryptomeria japonica* (L. f.) D. Don. Two hundred and twenty-two species were classified to understory trees and shrubs, including 55 species of ferns, 138 species of dicotyledons and 27 species of monocotyledons. The dominant species are *Elatostemma lineolatum majus* and *Diplazium dilataum* (Hsieh, 2010).

Experimental design

Twelve 1-ha permanent plots (100 x 100 m) with northern and eastern aspects were established (Figure 1a) for long-term monitoring of biodiversity dynamics. Plot 1 to 5 and plot 12 had an easterly aspect while plots 6 to 11 had a northerly aspect. The twelve plots were divided

into three treatments of four plots, and each plot was randomly assigned a treatment of control, a 25% or a 50% thinning treatment (Figure 1a). Each plot was divided into one hundred 10 × 10 meter grids, using a theodolite. Thinning treatments consisted of tree removal in alternating quadrats as indicated in Figure 1b. In treatment plots with 25% thinning, trees in one 10-m quadrat with each 20-m quadrat were cut, while in 50% thinning plots, trees in two 10-m quadrats with each 20-m quadrat were thinned (Figure 1b). Thinning was performed during June to September of 2007. Average numbers of residual trees per plot (post-thinning) were 956 (control), 693 (25% thinning) and 476 (50% thinning) (I-Fan Sun, personal communication, September 23, 2009). The basal area in the control, 25% thinning and 50% thinning treatment before thinning was 58, 50.1 and 55.3 m^2/ha (Wang et al., 2010). After thinning, the basal area in the control, 25% thinning and 50% thinning treatment was 58, 43.1 and 24.9 m^2/ha (Wang et al., 2010). Only logged trunks with diameters >20 cm were removed from thinned plots for economic uses. Leaves, branches, and smaller trunks that were produced by thinning were left in the plots. To serve as a control for forest types, sampling plots were also established near the plantations in a natural broadleaf forest dominated by Lauraceae and Fagaceae.

Collection, identification and documentation of macrofungal species

In order to investigate macrofungal diversity and community in the plots, six 10-m diameter circular subplots were established in each plot (Figure 1c). Mature fruiting bodies in the subplots and on the transect line between the subplots were investigated from August 2006 to October 2010 once every two months during the fruiting season (March to October). Macro-morphological features of fresh sporocarps, such as size, shape and color, the substrates they grew and their ecology were noted

(Lodge and Cantrell, 1995). Morphological descriptions were compiled for each species to establish their identities. Sporocarp numbers, fruiting seasons and locations were also recorded. Voucher specimens of each species were photographed *in situ* and then collected. The macrofungi were dried at 40℃ for 1 or 2 days and preserved at the Department of Life Science, Tunghai University, Taiwan. Some collections were deposited in National Museum of Natural Science. To identify specimens, literatures including Chang et al. (2000), Chou and Chang (2005), Chou (2010), Corner (1950), Lassoe (1999), Ryvarden (1991), Tzean et al. (2010) were consulted.

Monitoring of environmental factors

The environmental factors in each study site plot, including temperature, water content, soil texture and pH, and temperature, relative humidity and light in the forest, were measured to identify the factors that influenced the differences in the macrofungal community. Hourly soil temperature was detected by T-type Thermocouple-type sensor (Omega Engineering Ltd, Stamford, USA) at soil depths of 10 and 20 cm. Soil water content was detected by a Soil Water Capacitance Probe (Sentek Pty Ltd, Stepney SA, Australia) at depths of 10 and 20 cm and data were recorded every 15 minutes. A soil:water ratio of 1:5 was used for the measurement of soil pH. Air-dried soils (< 2mm) were suspended in distilled water and dispersed by ultrasonication for 10 min. Dispersed soils were separated into clay, silt and sand fractions by sedimentation and centrifugation for the soil texture analysis (Jackson, 1979; Gee and Bauder, 1986). Air temperature and relative humidity in the forest were monitored by data loggers (HOBO Pro Series data logger, Onset, Bourne, MA). The data logger was placed at a height of 1.5 m and data were recorded every 5 min. Incident, light photosynthetically active radiation

(PAR) was measured and recorded at 5 min intervals at a height of 1.5 m with a quantum light sensor (LI-COR LI190SB-L Quantum Sensor, Lincoln, Nebraska, USA). We used the percentage of unthinned area to represent percent canopy cover. For example, the percentage canopy cover of the control plot, 25%-thinning plot and 50%-thinning plot was 100%, 75% and 50%, respectively.

Data analysis

Changes in fungal diversity and abundance over the course of the study were estimated by calculating the number of species and sporocarps and performing analysis of variance (ANOVA) using R software. Forest thinning and time series effects on diversity and abundance of different macrofungal functional groups were also analyzed by ANOVA. Construction of a data matrix based on diversity and abundance of macrofungal morpho-species, which were identified to family level, was followed by square root transformations. The binary matrix was used to calculate Bray-Curtis Similarity (Clark and Warwick, 2001) and to construct nonparametric multi-dimensional scaling (MDS) plots using Primer 6 software (version 6. 1. 15; Primer-E Ltd., United Kingdom). The PERMANOVA plug-in (version 1.0.5) of the Primer package was used to test observed differences statistically. A three-factor design permutational multivariate analysis of variance (PERMANOVA) was calculated with time, aspects and thinning treatments as the fixed factors to compare macrofungal community compositions over time. If there were significant differences for time, treatments, aspects or interactions, pairwise PERMANOVAs on the respective term for each level of the factor were performed on the respective term for each level of the factor. In all PERMANOVAs, a maximum of 999 random permutations was performed.

Redundancy analysis (Legendre and Anderson, 1999), as implemented in the ‘‘Vegan’’ package for R software (Oksanen et al., 2011) was used to analyze the relationships between environmental factors and macrofungal family profiles. Macrofungal family profiles were based on diversity and abundance and transformed by Hellinger distance (Rao, 1995). Air temperature, relative humidity, light, soil temperature and soil water contents were monitored as routine works every day. We only used the average data of sampling day and the data two weeks prior. The other environmental data, such as pH values and soil texture were monitored in each treatment. All the data were standardized and used to perform a redundancy analysis.

The abundance of dominant macrofungi was subjected by logarithmic transformation (base 10), and regression analysis was used to analyze the correlation between the abundance of dominant macrofungi and the environmental factors in plots by R.

Results

Macrofungal diversity

From August 2006 to October 2010, in twelve 1 ha plantation plots and a natural forest plot, a total of 158,037 fruiting bodies belonging to 610 taxa of macrofungi were observed and recorded. Among the bodies, 30% (184/610) of the taxa and 87% (138,079/158,037) of the fruiting bodies were identified to genus or species level, and 50 taxa were identified to family level. Although there were 376 unknown taxa, the majority of fruiting bodies were identifiable taxa. There were 376 unknown morpho-species accounting for only 10.77% of the fruiting bodies. In each plantation plot, 4 to 44 taxa and 26 to 42,986 sporocarps were recorded each year (Table 1; Table 2). Diversity of these macrofungi among plots varied greatly. In the natural forest plot, 34 to

66 taxa and 832 to 4,531 sporocarps were found each year. The natural forest had higher species diversity.

Spring, summer and autumn were the main fruiting seasons. From August 2006 to June 2007, June and October had 103 and 18 macrofungal species, the highest and lowest species richness, respectively. Before thinning, the most fertile month was September. Most macrofungal species were recorded as low frequency. Four hundred and eighty-six species appeared only once, 53 species appeared twice. The species were complete turnover from year to year and plot to plot. *Scytinopogon* sp., *Oxyporus cunneatus*, *Lepiota cygnea*, and *Polyporus tenuiculus* were widespread species, and *Scytinopogon* sp., *O. cunneatus*, and *P. tenuiculus* were also dominant fruiting species at this site.

Compared with data before thinning, the number of fungal species observed decreased in all treatments in the first year post-thinning. This trend was also present in the second and third years post-thinning. In order to observe thinning and time series effects, ANOVA was used to determine whether species and sporocarp numbers changed over time and whether treatments differed over time. Species number changed through time ($F = 8.1422$; $P = 0.007$) and significantly decreased in thinning treatments ($F = 7.0178$; $P = 0.002$). Species richness was higher in control plots than in the 25% and 50% thinning plots ($P < 0.05$; Table 1). Species richness was significantly higher before thinning ($P < 0.05$; Table 1). The number of sporocarps increased slightly in all treatments in the 3 years post-thinning (Table 2). However, the number of sporocarps was not significantly different between thinning treatments ($F = 0.5003$; $P = 0.6$) or years ($F = 2.8544$; $P = 0.09$).

The relationship between fruiting fungal species richness and environmental factors was analyzed by multiple-regression. Relative humidity proved to be a good estimator of the species richness ($R^2 = 0.52$;

Adjusted R^2 = 0.35). Regression analysis indicated that species richness was significantly and positively associated with relative humidity (F = 3.124; P = 0.01). Many macrofungal species was associated with higher relative humidity in the forest.

Functional groups of macrofungi

Table 3 and table 4 showed the diversity and abundance of saprotrophic macrofungi in the different treatments and time series. Saprotrophic macrofungi, including wood-inhabiting and soil-inhabiting, were dominant in the *C. japonica* plantations. Saprotrophs formed the main functional group of macrofungi in the *C. japonica* plantations.

Species richness of soil-inhabiting and wood-inhabiting saprotrophyic macrofungi observed in the plantations was significantly influenced by thinning (F = 7.6820; P = 0.001; F = 5.1797; P = 0.01). Richness and abundance of soil-inhabiting macrofungi were reduced by thinning but not influenced by time series. Control plots had significantly higher soil-inhabiting macrofungal species richness versus the 25%-thinning and 50%-thinning plots (P = 0.001; P = 0.007), but there was no difference between 25%-thinning and 50%-thinning plots (P = 0.56) (Table 3). Soil-inhabiting macrofungal abundance was significantly higher in control plots versus the 25%-thinning and 50%-thinning treatments (P = 0.002; P = 0.010) (Table 4). Species richness of wood-inhabiting macrofungi were decreased by thinning (F = 5.1797; P < 0.0098) and changed over time (F = 13.9539; P < 0.0006). The number of wood-inhabiting species was significantly lower in the 25%-thinning plots versus the control (P = 0.03) (Table 3). The number was also significantly higher post-thinning versus pre-thinning (P < 0.05) (Table 4).

The species and sporocarps of ectomycorrhizal fungi in the natural broadleaf forest were more abundant than in the plantations. Russulaceae and Amanitaceae were major ectomycorrhizal families in the natural broadleaf forest. However, natural forest had more saprophytic fruiting species than the plantation. Before thinning, the natural forest had 57 saprotrophic taxa and 9 ectomycorrhizal fungal species before thinning, while the control plots averaged 30 taxa (data not shown). The natural forest had almost twice the amount of saprophytic species.

Thinning influenced macrofungal community and environments

The macrofungal community based on morph-species profiles was influenced by the thinning treatments (Table 5). Multi-dimensional scaling (MDS) plots showed that macrofungal communities in the 50%-thinning treatments were distinct from the communities in the control, but there was greater overlap area between the 25%-thinning and 50%-thinning treatments (Figure 2). PERMANOVA pairwise comparisons also indicated that the macrofungal community from the 50%-thinning treatments was significantly different than that from control treatments ($P = 0.032$). There was no significant difference between other treatments and no interaction between years, aspects and thinning treatments (Table 5). The SIMPER test showed that *Oxyporus cuneatus* and *Scytinopogon* sp. were the main contributors of variation between the macrofungal communities in the control and 50%-thinning treatment, contributing 27.7 and 8.4% dissimilarity, respectively. The macrofungal species and abundance data matrix based on the family level were also used to test the effects of thinning levels on the macrofungal community. The same patterns were shown with the macrofungal community based on the level of morpho-species (data not shown).

To determine which environmental factors best explained the variation in macrofungal communities, we used redundancy analysis (RDA) to predict the principal coordinates (Figure 3) using a linear combination of several environmental factors (Table 6). The arrows indicate the size and direction of the coefficients of the environmental variables in the linear model (Figure 3). These environmental factors, including air temperature, relative humidity, light, canopy, soil water content, soil temperature, soil texture and pH, accounted for 38.37% of the variation of macrofungal communities. Relative humidity, light, canopy cover, soil water content, soil temperature, soil pH and soil texture (sand and silt) contributed significantly to explaining the variation in the macrofungal community (Fig 3; Table 7). Air temperature (F = 12.73; P =0.001) and light (F = 84.68; P <0.001) in the plantations significantly and negatively correlated with forest canopy cover. On the other hand, relative humidity (F = 10.11; P = 0.003) positively correlated with canopy cover.

Comparison of macrofungal species composition between investigated periods and aspects

A multi-dimensional scaling plot (Figure 2) showed that prior to thinning, the macrofungal communities cluster together, separately from others. PERMANOVA showed that the macrofungal community responded to a time effect (Table. 5) and pairwise comparisons showed that the macrofungal community differed significantly among years (for all comparsions, P < 0.01).

Aspect markedly influenced the macrofungal community (Table. 5). A multi-dimensional scaling plot generated from macrofungal morpho-species profiles showed that the macrofungal communities from northerly aspects clustered separately from those with easterly aspects

(Figure 4). *Scytinopogon* sp. was found only in plots with northerly aspects and *Oxyporus cuneatus* was more abundant in plots with northerly versus easterly aspects. The SIMPER test showed that *Oxyporus cuneatus* and *Scytinopogon* sp. was the main contributor, accounting for 28.6 and 8.9% of the variation in the macrofungal communities between the north and east aspects.

Environments influenced fruiting patterns of widespread species

Four widespread macrofungal species showed different population fluctuations after thinning. The wood-habitant saprophytes *Oxyporus cunneatus* and *Polyporus tenuiculus* seemed unaffected by thinning. *O. cunneatus* was widely distributed and fruited from March to October (Figure 5a). After thinning, the abundance of *O. cunneatus* macrocarps increased in thinned plots (Figure 5a); fruiting of *O. cunneatus* primarily correlated with light in the forest (F = 15.8456; P = 0.0004) and soil pH (F = 5.7191; P = 0.002). *Polyporus tenuiculus* was distributed widely, except for plot 2 (50% thinning treatment), and the fruiting season was from March to October (Figure 5b). Fruiting numbers of *P. tenniculus* were positively correlated with forest relative humidity (F = 4.6644; P = 0.04) and negatively associated with forest temperature (F = 5.8923; P = 0.02).

Lepiota cygnea, a soil-habitant, is distributed widely and did not fruit in the first year post-thinning in three treatments until October 2008 (Figure 5c). Its fruiting season was from March to October (Figure 5c), and its abundance correlated with light levels (F = 7.3225; P = 0.02) and soil pH (F = 7.9037; P = 0.01). The abundance of *Scytinopogon* sp. macrocarps was clearly affected by thinning intensity (Figure 5d). After thinning, the coral fungi fruited as before in the control plots, but declined in the plots which had 25% thinning and was not found at all in the plots

with 50% thinning during the first and second years post-thinning (Figure 5d). The fruiting of *Scytinopogon* sp. in 50%-thinned plots started to recover in the third year post-thinning and the abundance of its macrocarps was negatively correlated with thinning intensity ($F = 6.7844$; $P = 0.02$) and light levels ($F = 9.5173$; $P = 0.005$).

Discussion

Saprotrophic and symbiotic functional groups were recorded in this study. No ectomycorrhizal fungi have been reported as symbionts of *C. japonica*; the ectomycorrhizal fungi in the plantations were associated with other tree species, rather than *C. japonica*. Saprotrophic macrofungal diversity in the natural broadleaf forest was higher than in the conifer plantations examined in this study. It might due to the plant diversity of natural broadleaf forest was higher than the plantation (I-Fan Sun, personal communication, September 23, 2009). Higher vascular plant diversity supported higher macrofungal diversity (Packham et al., 2002).

With high frequency and comprehensive investigations in this study, we documented high diversity of saprophytic macrofungi. There were 4,000 vascular plant species in Taiwan. Based on the 1:6 vascular plant-fungus ratio (Hawksworth, 1991), 25,000 fungi species were estimated (Tzean et al., 2010). About 6,000 fungal species (24%) were documented (Tzean et al., 2010) in Taiwan. In our study, 30% fungal species which produced majority (87%) of fruiting bodies were identified. This is common for fungal surveys (Robinson and Tunsell, 2007; Robinson and Williams, 2011).

A forest thinning disturbance causes significant declines in the species richness of saprophytic macrofungi but does not affect sporocarp abundances. The great variations of fruiting number among plots within treatment could make it difficult to test thinning effects on abundance of

saprotrophic fungi. This study also confirmed the negative effects of silvicultural practices on saprophytic fungal diversity and observed results similar to those of Müller et al. (2007a, 2007b) and Kebli et al. (2012). The richness decline may be caused by the decrease of fruiting species or the disappearance of certain species.

Changes in environmental factors after thinning influenced fruiting species and fruiting patterns. For example, the fruiting of *Lepiota cygnea* was influenced by light and soil pH, whereas the fruiting of *Scytinopogon* sp. was influenced by light and thinning intensity. Thinning not only changed the microenvironments, in our observation, the practices also disturbed the understory and soil surface which affected the fruiting of soil-inhabiting fungi. In the 50%-thinned plots, the population of *Scytinopogon* sp. disappeared after the thinning treatment. Ultimately, a small population of *Scytinopogon* sp. appeared at new sites during the third year post-thinning. Both *Lepiota cygnea* and *Scytinopogon* sp. were soil-inhabiting macrofungal species. Thinning practices disturb the soil surface, compact the soil, change forest environments and soil characters, and all of these factors influence macrofungal survival, vegetative growth, and fruiting, decreasing the richness and abundance of soil-inhabiting macrofungi.

Our results also confirm that thinning decreased the species richness of observed wood-inhabiting macrofungi (Sippola et al., 2001; Josefsson et al., 2010; Kebi et al., 2012). New stumps and wood produced by thinning are presumed to be habitats with little-to-no competition, resulting in a patchwork distribution of new wood-inhabiting macrofungi (pioneer species). The majority of wood-inhabiting macrofungi, like *P. tenniculus* and *O. cunneatus*, fruited massively in this niche. These species are able to undergo rapid fruit-body formation as soon as a suitable substrate was available. However, the vegetative growth of a

minority of wood-inhabiting macrofungi continued for a long time until they occupied the substrate or after initial fruiting (Olsson et al., 2011; Otterson 2013). The fact that these species were not recorded might be due to fruiting related to suboptimal conditions and/or the need for a long timeframe, or alternatively abundance subsided post-thinning. Although thinning provided extra substrates for wood-inhabiting fungi, the wood-inhabiting macrofungal richness was diminished in the thinned plots with disappearance of minor species and the delay of fruiting. Lohmus (2011) also found that the number of polypore species declined during the first 20 years after cutting, rebounding afterward. The post-harvest time interval should rank among the most important factors determining the species richness of wood-inhabiting macrofungi. In the context of various forest management methods, the recovery of diversity of wood-inhabiting macrofungi merits future study.

Macrofungal communities are often affected by forest management activities, including clear-cutting (Lindner et al., 2006) and selective logging (Bader et al., 1995; Yamashita et al., 2014), and the environmental changes have been suggested to be an important factor that determines the biodiversity and community (Eveling et al., 1990; Peredo et al., 1983). Thinning has been reported to change the observed macrofungal community in *Cryptomeria japonica* and *Chamaecyparis formosensis* plantations (Lin et al., 2011). In this study, 50% thinning treatments changed the macrofungal community, which was correlated with the alteration of environmental parameters, including relative humidity, light and canopy in the forest and water contents, temperature, soil pH and texture. It is presumed that thinning decreases plantation canopy cover, causing an increase in air temperature, a decrease in relative humidity and an increase of light in the forest. Changes in these environmental regimes influenced mushroom fruiting.

Aspect was also an important factor controlling fruiting conditions for individual species (Bonet et al., 2004). Aspect can have very significant influences on microclimates. For example, slopes with western or southern aspects are warmer and dryer than eastern or northern aspects in the northern hemisphere (Bennie et al., 2006). Bertling and Cowan (1998) reported that in the southern hemisphere, daylight intensity declined on eastern aspects and increased on northern aspects as austral autumn progressed. Light has comprehensive effects on the formation of basidiomycete and ascomycete fruiting bodies; for example, it determines whether the fruiting bodies are produced, well as the development patterns (Morimoto and Oda, 1973) and numbers produced (Elliott, 1994; Moore et al. 2008). In this study, temperature and relative humidity were significantly higher in northerly aspects than in easterly aspects. Light was slightly higher in northerly aspects than in easterly aspects, although the difference was not significant. Moreover, aspect significantly influenced macrofungal community and *Scytinopogon* sp. distribution. Overall then, fruiting of macrofungi correlated with microclimates, which varied with aspect.

The temporal fluctuation in macrofungal communities is likely to be related to climatic variation (Matsuda and Hijii 1998; Lagana` et al. 2002; Straatsma and Krisai-Greilhuber 2003; Baptista et al., 2010) and macrofungal phenology. Annual rainfall in 2007 (before thinning), 2008 (the first year post-thinning), and 2009 (the second year post-thinning) in this study was 3,540, 3,177, and 1475 mm, respectively (http://www.cwb.gov.tw/V7/climate/dailyPrecipitation /dP.htm). Macrofungal communities in control plots fluctuated during the timeframe studied, possibly due to a variable weather conditions that included annual rainfall; the number of species found in this site varied

with mean yearly rainfall. Weather parameters clearly play a major role, affecting the periodicity and fluctuations of communities.

Although molecular methods could detect more taxa than fruiting body survey, the sporocarp communities were similar with the most abundant OTUs by 454 pyrosequence (Kubartova et al., 2012). That means changes in sporocarp communities post-thinning could reflect the population dynamics of the most abundant fungal taxa. This study was relied on sporocarps observations and the results represent the effect on fruiting of the fruiting fungi. It reflects both the reproductive activity and absence. Moreover, the molecular methods (eg., DGGE or next generation sequence) to confirm the observed trends and the genetic dynamics of dominant species population were worth of further investigation.

Currently in Taiwan, economic values of *C. japonica* plantation are no longer emphasized but their ecosystem functioning, recreation, conservation and educational values are incorporated in management purposes. Thinning had negative influences on observed macrofungal diversity. However, the gaps were created in the plantation forest post-thinning, seeds of native tree species (Fagaceae) from natural forest might be dispersaled and colonize in plantation forest. As a consequence, the appearance of ectomycorrhizal fungi could be predicable. Our results show that 25% thinning treatment had fewer impacts on observed macrofungal community and it's a better management method than 50%-thinning or clear-cutting for biodiversity preservation.

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Table 2-1. Number of macrofungal species (represented by fruiting bodies) in each plot in the *Cryptomeria japonica* plantations in Taiwan during investigated periods.

†Time	¶control					25% thinning treatments					50% thinning treatments					natural
	*P3	P6	P10	P12	means	P1	P4	P7	P11	means	P2	P5	P8	P9	means	forest
Before	41	32	16	30	30	17	28	31	24	25	29	44	28	29	33	66
1st yr	20	8	28	9	16	7	15	10	20	13	4	10	14	15	11	61
2nd yr	13	11	32	10	17	8	17	17	13	14	16	10	24	21	18	46
3rd yr	17	10	10	18	14	6	26	8	10	13	10	18	6	30	16	34

† Investigated periods, before: August 2006 to June 2007; 1st yr: 1st year post-thinning which is from March 2008 to October 2008; 2nd yr: 2nd year post-thinning which is from March 2009 to October 2009; 3rd yr: 3rd year post-thinning which is from March 2010 to October 2010

¶ Treatments, including control, 25% thinning and 50% thinning treatments. Each treatment included four plots.

*Plot number.

Table 2-2. Number of macrofungal sporocarps in each plot in the *Cryptomeria japonica* plantations in Taiwan during investigated periods.

†Time	¶control					25% thinning treatments					50% thinning treatments					natural
	*P3	P6	P10	P12	means	P1	P4	P7	P11	means	P2	P5	P8	P9	means	forest
Before	1,269	1,067	344	837	879	227	553	3,609	841	1,308	1,174	2,309	529	1,521	1,383	832
1st yr	725	2,204	1,690	4,762	2,345	26	732	2,755	5,545	2,265	250	98	3,691	3,144	1,796	898
2nd yr	312	42,986	6,185	1,576	12,765	245	2,563	2,638	647	1,523	484	77	10,880	3,867	3,827	3,545
3rd yr	2,794	6,678	3,888	4,725	4,521	210	1,029	2,152	894	1,071	141	746	1,810	10,802	3,375	4,531

† Investigated periods, before: August 2006 to June 2007; 1st yr: 1st year post-thinning which is from March 2008 to October 2008; 2nd yr: 2nd year post-thinning which is from March 2009 to October 2009; 3rd yr: 3rd year post-thinning which is from March 2010 to October 2010

¶ Treatments, including control, 25% thinning and 50% thinning treatments. Each treatment included four plots.

*Plot number.

Table 2-3. Number of saprotrophic species (represented by fruiting bodies) in each plot in the *Cryptomeria japonica* plantations in Taiwan during investigated periods.

†Time	§Functional group	¶control					25% thinning treatments					50% thinning treatments					natural forest
		*P3	P6	P10	P12	means	P1	P4	P7	P11	means	P2	P5	P8	P9	means	
Before	SS	16	10	3	7	9	5	7	7	4	6	10	12	14	12	12	21
	WS	25	22	13	23	21	12	21	24	20	19	19	31	14	17	20	36
1st yr	SS	7	3	7	3	5	1	6	2	3	3	1	2	2	1	2	15
	WS	13	5	21	6	11	6	9	8	16	10	3	8	12	14	9	36
2nd yr	SS	7	5	17	3	8	4	7	5	6	6	5	4	5	3	4	9
	WS	6	6	15	7	9	4	10	11	7	8	11	6	19	16	13	32
3rd yr	SS	9	5	4	4	6	1	7	3	4	4	0	6	1	10	4	9
	WS	8	5	6	13	8	4	19	5	6	9	10	12	5	19	12	17

† Investigated periods, before: August 2006 to June 2007; 1st yr: 1st year post-thinning which is from March 2008 to October 2008; 2nd yr: 2nd year post-thinning which is from March 2009 to October 2009; 3rd yr: 3rd year post-thinning which is from March 2010 to October 2010

§ Ecological group, SS: soil-inhabiting saprotroph; WS: wood-inhabiting saprotroph

¶ Treatments, including control, 25% thinning and 50% thinning treatments. Each treatment included four plots.

*Plot number.

Table 2-4. Number of saprotrophic sporocarps in each plot in the *Cryptomeria japonica* plantations in Taiwan during investigated periods.

†Time	§Functional group	¶control					25% thinning treatments					50% thinning treatments					natural forest
		*P3	P6	P10	P12	means	P1	P4	P7	P11	means	P2	P5	P8	P9	means	
Before	SS	113	797	157	124	298	46	40	949	167	301	62	59	165	270	139	339
	WS	1,156	270	187	713	582	181	513	2,660	674	1,007	1,112	2,247	364	1,251	1,244	478
1st yr	SS	15	122	622	148	227	7	55	103	17	46	1	6	5	9	5	58
	WS	710	2,082	1,068	4,614	2,119	19	677	2,652	5,526	2,219	249	92	3,686	3,135	1,791	690
2nd yr	SS	19	755	535	97	352	77	82	123	77	90	17	11	43	62	33	17
	WS	293	42,231	5,650	1,479	12,413	168	2,481	2,514	570	1,433	467	66	10,837	3,792	3,791	3,523
3rd yr	SS	27	414	394	15	213	2	113	28	94	59	0	13	1	34	12	44
	WS	2,767	6,264	3,494	4,580	4,276	207	916	2,124	800	1,012	141	733	1,809	10,767	3,363	4,445

† Investigated periods, before: August 2006 to June 2007; 1st yr: 1st year post-thinning which is from March 2008 to October 2008; 2nd yr: 2nd year post-thinning which is from March 2009 to October 2009; 3rd yr: 3rd year post-thinning which is from March 2010 to October 2010

§ Ecological group, SS: soil-inhabiting saprotroph; WS: wood-inhabiting saprotroph

¶ Treatments, including control, 25% thinning and 50% thinning treatments. Each treatment included four plots.

*Plot number.

Table 2-5. PERMANOVAs for the effects of time, aspect, thinning treatments and interactions on the macrofungal communities, based on macrofungal species and relative abundances in the *Cryptomeria japonica* plantations. A maximum of 999 permutations was possible.

Source	Degrees of freedom	Sum of squares	Pseudo-*F* ratio	Significance (*P*-value)
year	3	29375	3.9628	0.001
Thinning	2	7916	1.6018	0.034
Aspects	1	10493	4.2467	0.001
Year × thinning	4	8593	0.86942	0.721
Year × aspects	3	10597	1.4296	0.071
Thinning × aspects	2	6471	1.3094	0.127
Year × thinning × aspects	4	8308	0.84059	0.774
Residuals	26	64242		
Total	45	154980		

Table 2-6. Data corresponding to environmental variables used in redundancy analysis.

Time	Treatment	Temperature (℃)	Relative Humidity (%)	Light (m mole/sm^2)	[c]Canopy cover (%)	Soil water content (%)	Soil temperature (℃)	pH	Sand (%)	Silt (%)	Clay (%)
[a]Before thinning	[b]control	17.77	97.28	2,288.56	100%	10.26	11.62	4.27	75.0	12.5	12.5
		17.79	97.15	1,960.46	100%	17.01	16.88	4.05	74.0	16.0	10.0
		17.78	96.96	1,294.55	100%	11.94	14.38	4.24	77.0	14.0	9.0
1st year post-thinning	control	17.57	97.08	2,987.00	100%	20.29	16.98	4.27	75.0	12.5	12.5
	25%	17.77	95.85	7,028.43	75%	22.35	17.62	4.05	74.0	16.0	10.0
	50%	17.99	95.21	10,412.10	50%	23.38	17.50	4.24	77.0	14.0	9.0
2nd year post-thinning	control	18.12	97.19	2,202.52	100%	18.54	17.74	4.27	75.0	12.5	12.5
	25%	18.26	96.45	5,253.19	75%	20.23	18.33	4.05	74.0	16.0	10.0
	50%	18.48	95.90	9,839.95	50%	20.51	18.15	4.24	77.0	14.0	9.0

[a] Investigated periods, before thinning: August 2006 to June 2007; 1st year post-thinning: March 2008 to October 2008; 2nd year post-thinning: March 2009 to October 2009; 3rd year post-thinning: March 2010 to October 2010

[b]Treatments, including control, 25% thinning and 50% thinning treatments. Each treatment included four plots.

[c]Percentage of unthinned area was used to represented canopy cover.

Table 2-7. Results of redundancy analysis for influences of environmental factors on the macrofungal communities.

Environmental variables		r^2	Significance (*P*-value)
Temperature in forest		0.0935	0.223
Relative humidity		0.1627	0.040
Light		0.3255	0.002
Canopy		0.3227	0.002
Soil water contents		0.3522	0.001
Soil temperature		0.2135	0.016
Soil pH value		0.2593	0.012
Soil texture	sand	0.0024	0.959
	silt	0.1705	0.046
	clay	0.4018	0.046

Appendix 2-1. The identified soil- and wood-inhabiting fungi of the area

Ecological group	Scientific name	Ecological group	Scientific name	Ecological group	Scientific name	Ecological group	Scientific name	Ecological group	Scientific name
SS	*Agaricus praeclaresquamosus*	WS	*Pseudohydnum gelatinosum*	SS	*Lepiota* sp. 7	WS	*Trametes hirsuta*	WS	*Marasmiellus candidus*
SS	*Agaricus* sp.	SS	*Geastrum triplex*	SS	*Lepiota* sp. 8	WS	*Trametes versicolor*	WS	*Marasmiellus nigripes*
WS	*Auriclaria delicata*	WS	*Ascocoryne cylichnium*	SS	*Lepiota* sp. 9	WS	*Trichaptum biforme*	WS	*Marasmiellus ramealis*
WS	*Auricularia auricula*	SS	*Leotia lubrica*	SS	*Leucoagaricus bresadolae*	WS	*Tyromyces incarnatus*	WS	*Marasmius maximus*
WS	*Auricularia polytricha*	WS	*Elmerina cladophora*	SS	*Calvatia craniiformis*	WS	*Cymatoderma elegans*	WS	*Marasmius* sp. 1
SS	*Conocybe lactea*	SS	*Hygrocybe coccineocrenata*	SS	*Lycoperdaceae* sp.	SS	*Aleuria aurantia*	WS	*Marasmius* sp. 2
SS	*Scytinopogon* sp.	SS	*Hygrocybe* sp.	SS	*Lycoperdon perlatum*	WS	*Scutellinia scutellata*	WS	*Marasmius* sp. 3
WS	*Clavicorona pyxidata*	WS	*Hypocrea gelatinosa*	SS	*Dictyophora indusiata*	SS	*Ramaria stricta*	SS	*Mycena pura*
WS	*Coprinus disseminatus*	SS	*Lepiota acutesquamosa*	WS	*Steccherinum rhois*	WS	*Lycogala epidendrum*	WS	*Mycena* sp. 1
WS	*Coprinus micaceus*	SS	*Lepiota atrosquamulosa*	WS	*Lentinula edodes*	WS	*Cookeina insititia*	WS	*Mycena stylobates*
WS	*Coprinus* sp. 1	SS	*Lepiota cristata*	WS	*Panus fulvus*	WS	*Sarcoscypha coccinea*	WS	*Oudemansiella mucida*
WS	*Coprinus* sp. 2	SS	*Lepiota cygnea*	WS	*Pleurotus ostreatus*	WS	*Sarcoscypha humberiana*	WS	*Oudemansiella platyphylla*
SS	*Psathyrella candolleana*	SS	*Lepiota fusciceps*	WS	*Pleurotus* sp. 1	WS	*Galiella javanica* (*Trichaleurina javanica*)	WS	*Oudemansiella radicata*
SS	*Psathyrella* sp.	SS	*Lepiota praetervisa*	WS	*Pleurotus* sp. 2	WS	*Schizophyllum commune*	WS	*Oudemansiella* sp. 1
SS	*Psathyrella* sp. 1	SS	*Lepiota* sp. 1	WS	*Pluteus nigrofloccosus*	WS	*Dicephalospora rufocornea*	WS	*Oudemansiella* sp. 2
SS	*Psathyrella* sp. 2	SS	*Lepiota* sp. 10	WS	*Pluteus* sp. 1	WS	*Naematoloma fasciculare*	WS	*Oudemansiella* sp. 3
SS	*Psathyrella* sp. 3	SS	*Lepiota* sp. 11	SS	*Pluteus* sp. 2	WS	*Pholiota* sp.	WS	*Oudemansiella* sp. 4
SS	*Psathyrella velutina*	SS	*Lepiota* sp. 12	WS	*Antrodiella liebmannii*	SS	*Psilocybe cubensis*	WS	*Resupinatus trichotis*
WS	*Stereum ostrea*	SS	*Lepiota* sp. 13	WS	*Coriolopsis aspera*	WS	*Trichocoma paradoxa*	WS	*Xeromphalina campanella*
WS	*Xylobolus spectabilis*	SS	*Lepiota* sp. 14	WS	*Lenzites betulina*	SS	*Baeospora myosura*	WS	*Daldinia eschscholzii*

WS	*Galerina hypnorum*	SS	*Lepiota* sp. 15	WS	*Microporus affinis*	WS	*Campanella junghuhnii*	WS	*Daldinia* sp. 1
WS	*Galerina* sp.	SS	*Lepiota* sp. 16	WS	*Microporus xanthopus*	WS	*Collybia confluens*	WS	*Hypoxylon* sp. 1
WS	*Gymnopilus liquiritiae*	SS	*Lepiota* sp. 17	WS	*Oligoporus caesius*	SS	*Collybia* sp.	WS	*Hypoxylon* sp. 2
WS	*Gymnopilus* sp.	SS	*Lepiota* sp. 18	WS	*Oligoporus lowei*	WS	*Crinipellis stipitaria*	WS	*Xylaria allantoidea*
WS	*Gymnopilus* sp. 1	SS	*Lepiota* sp. 19	WS	*Oxyporus cunneatus*	WS	*Cyptotrama asprata*	WS	*Xylaria carpophila*
WS	*Gymnopilus* sp. 2	SS	*Lepiota* sp. 2	WS	*Polyporus arcularius*	WS	*Dictyopanus gloeocystidiatus*	WS	*Xylaria cubensis*
WS	*Crepidotus* sp. 1	SS	*Lepiota* sp. 20	WS	*Polyporus badius*	WS	*Filoboletus manipularis*	WS	*Xylaria melanaxis*
WS	*Crepidotus* sp. 2	SS	*Lepiota* sp. 3	WS	*Polyporus dictyporus*	WS	*Hohenbuehelia hobsoni*	WS	*Xylaria polymorpha*
WS	*Calocera cornea*	SS	*Lepiota* sp. 4	WS	*Polyporus tenuiculus*	WS	*Hohenbuehelia reniformis*	WS	*Xylaria* sp. 1
WS	*Calocera viscpsa*	SS	*Lepiota* sp. 5	WS	*Pycnoporus sanguineus*	SS	*Laccaria* sp. 1	WS	*Xylaria* sp. 2
WS	*Dacrymyces palmatus*	SS	*Lepiota* sp. 6	WS	*Skeletocutis stellae*	SS	*Laccaria* sp. 2	WS	*Xylaria* sp. 3

Ecological group, SS: soil-inhabiting saprotroph; WS: wood-inhabiting saprotroph

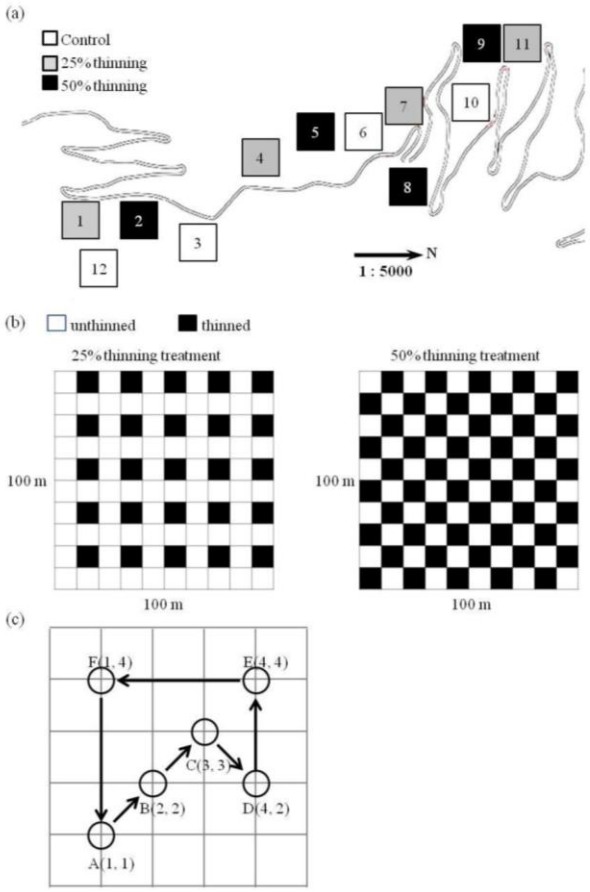

Figure 2-1. Map of twelve plots, experimental design and investigated subplots in central Taiwan. (a) Twelve 1-ha plots (100 x 100 m) with three treatments (control and two thinning intensities) replicated four-fold and distributed in the plantations of Japanese cedar (*Cryptomeria japonica*) in central Taiwan; (b) Thinned areas with plots corresponding to two thinning treatments are shown. The thinning treatments, including a 25% thinning treatment and a 50% thinning treatment, used the spacing thinning method. The plot was divided into 100 ten-meter grids. In the thinning treatment plots, trees in the dark ten-meter grids were thinned; (c) Six 10-m diameter circular subplots and transect lines were located in each 1-ha plot. Circles refer to subplots and arrows refer to transect lines. Macrofungal diversity was investigated in these subplots and along the transect lines from August 2006 to October 2009.

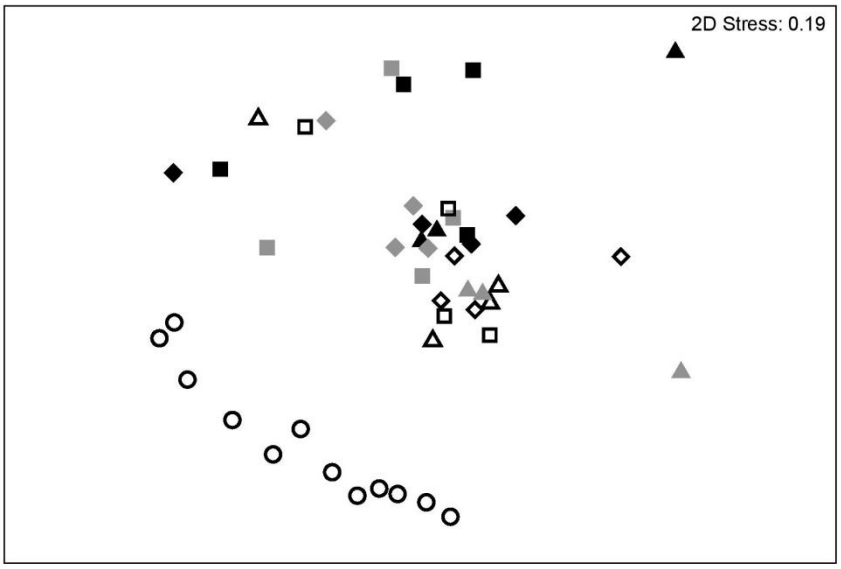

Figure 2-2. Multi-dimensional scaling ordination of macrofungal communities from thinning treatments among years. Symbols represent years: circles, pre-thinning; triangles, first year post-thinning; squares, second year post-thinning; diamond, third year post-thinning. Colors represent thinning treatments: white, gray and black represented control, 25% thinning and 50% thinning plots, respectively.

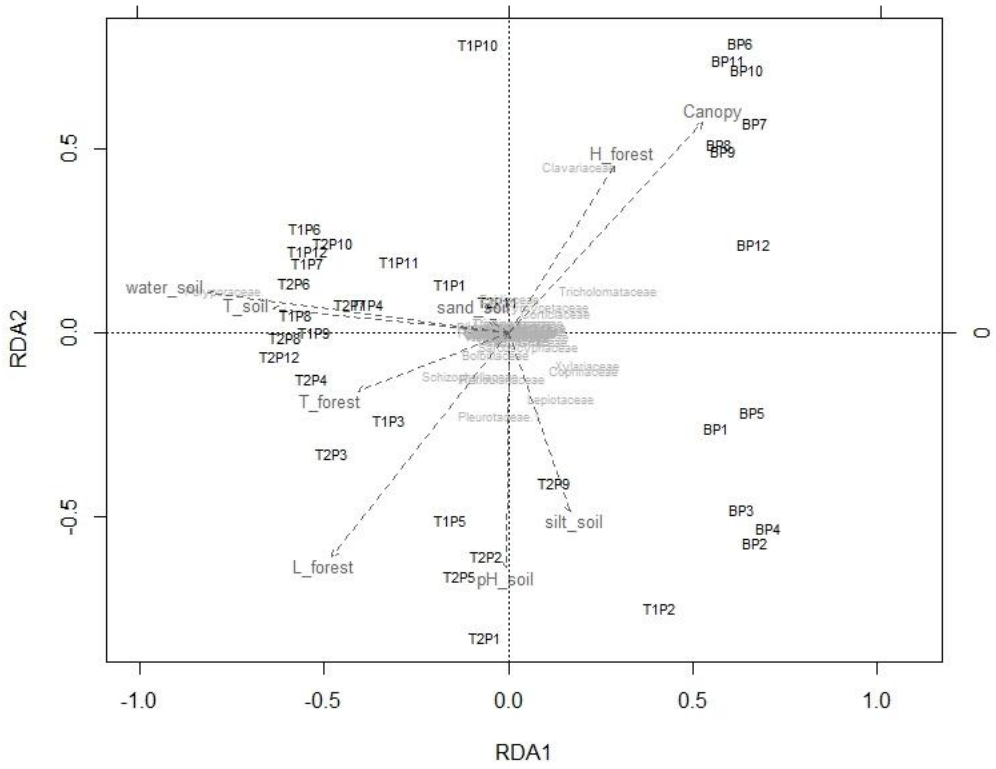

Figure 2-3. Redundancy Analysis of macrofungal communities and environmental factors instudied twelve plots from August 2006 to October 2009. Black letters refer to macrofungal communities from different years and plots. B: before thinning; T1: first year post-thinning; T2: second year post-thinning. P: number of plot. Blue arrows refer to environmental factors. T_forest: temperature in the forest; L_forest: light in the forest; water_soil: soil water content; T_soil: temperature in the soil; pH_soil: soil pH; H_soil: relative humidity in the soil. Red words refer to the macrofungal family.

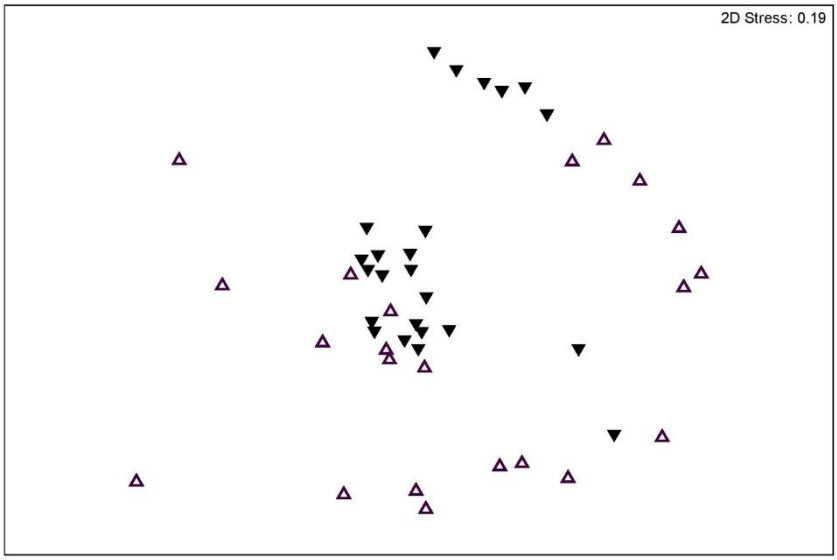

Figure 2-4. Multi-dimensional scaling ordination of macrofungal communities from north and east aspects: triangles represented macrofungal communities in sampling plots; white inverted triangles, east aspect; black inverted triangles, north aspect.

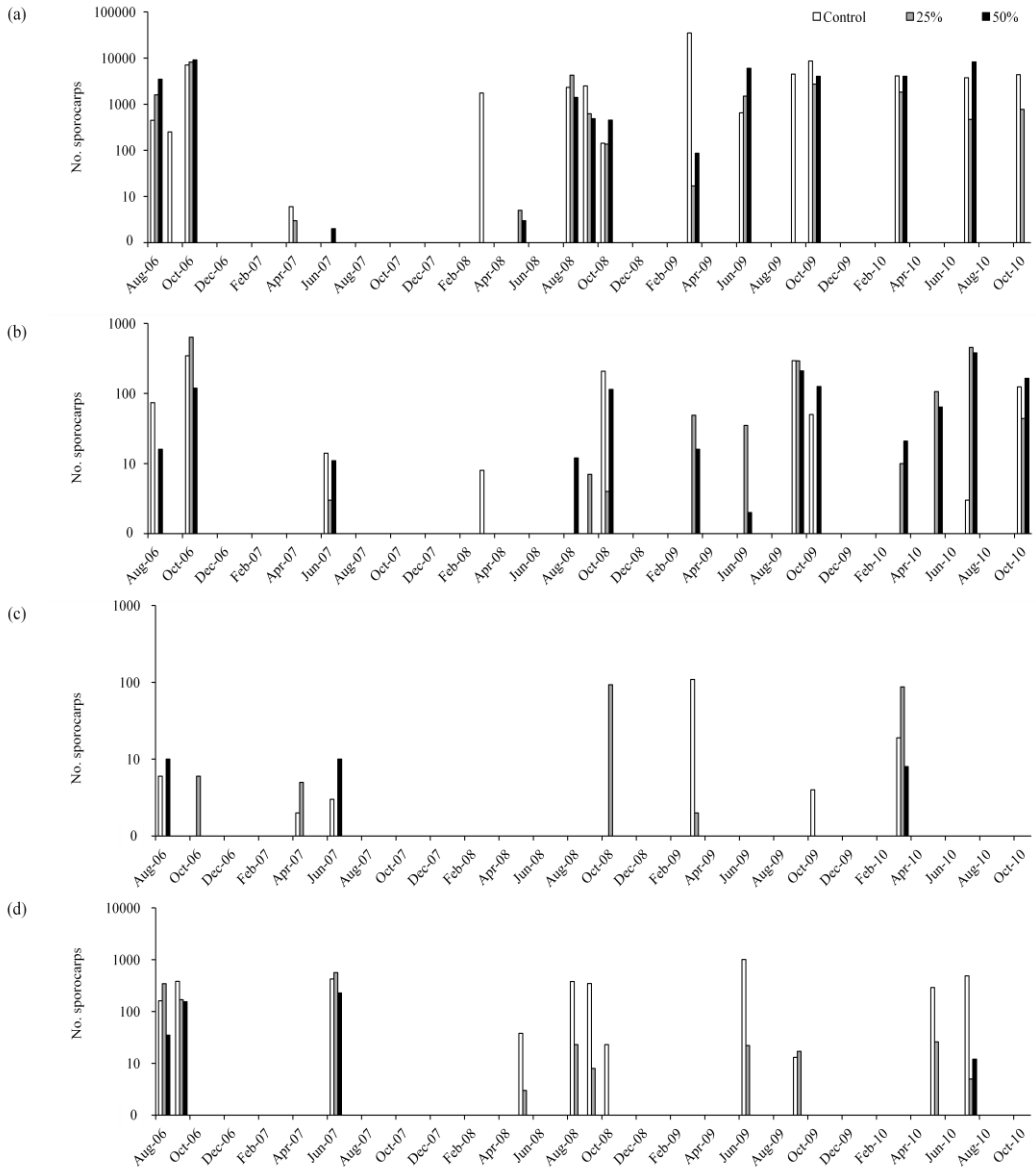

Figure 2-5. The temporal fruiting pattern of *Oxyporus cuneatus* (a), *Polyporus tenuiculus* (b), *Lepiota cygnea* (c), and *Scytinopogon* sp. (d)

Chapter 3 – Responses of soil fungal populations and communities to the thinning of *Cryptomeria japonica* forests

Introduction

Forest management activities, such as tree harvesting, forest thinning and clear-cutting, are key disturbances in many forest ecosystems. Thinning, a common way to manage forests, increases the tree stem diameter (Sullivan et al., 2002), crown size (Sullivan et al., 2001), and woody litter-fall (Covington, 1981); moreover, it enhances the growth rate of the remaining trees (Grant et al., 2007) and decreases their mortality rate (Brissette et al., 1999). However, thinning also influences the understory organisms in forest ecosystems (Bender et al., 1997; Tsui et al., 1998; Kerr, 1999) and thereby affects ecosystem function (Bengtsson et al., 2000).

Bacteria, fungi and other soil micro-organisms play important roles in ecological processes and the nutrient dynamics of forest ecosystems (Delvasto et al., 2006; Levy-Booth and Winder, 2010). Fungi account for the majority of microbial biomass in forest soil and convert recalcitrant organic material into forms that other organisms can use (Dighton, 2003). Regarding the impact of tree-thinning on these organisms, most studies have focused on the impacts of this major disturbance on soil bacteria or ectomycorrhizal fungi (Shaw et al., 2003); the effects of thinning on soil microorganisms appear to be complex. Thinning has been shown to significantly change soil microbial communities in a pine (*Pinus* spp.) forest (Maassen et al., 2006), a Jarrah (*Eucalyptus marginata*) forest (Cookson et al., 2008), and a *Chamaecyparis formosensis* forest (Lin et

al., 2011). In other studies, forest thinning was shown to have no significant effect on carbon associated with microbial biomass, enzyme activity, soil respiration (Maassen et al., 2006) or soil fungal communities (Houston et al., 1998). Grayston and Rennenberg (2006) found that the influence of heavy thinning on microbial biomass was spatially varied. Levy-Booth and Winder (2010) also found that the impact of thinning on free-living diazotrophic and denitrifying bacteria was spatially variable, making environmental trends for these microbial communities difficult to discern.

Beyond disturbance, temporal or seasonal effects also influence soil microorganisms. For example, the phospholipid fatty acid profiles of microbial communities have been reported to change in response to the change in seasons (Bossio et al., 1998; Bardgett et al., 1999; Buckley and Schmidt, 2003; Hamel et al., 2006). Moreover, fungal communities associated with oak rhizospheres and grassland soils are known to be seasonally dynamic with temporal turnover (Kennedy et al., 2006; Voříšková et al., 2014).

Most studies of thinning impacts on soil microbes have compared microbial communities in forests 3 to 45 years post-thinning (Houston et al., 1998; Barbhuiya et al., 2004; Hannam et al., 2006; Maassen et al., 2006; Barbhuiya et al., 2008; Cookson et al., 2008; Chatterjee et al., 2008). Generally, long-term assessments have not focused on short-term and seasonal dynamics. One study of short-term responses of soil decomposer communities in a boreal spruce (*Picea abies*) forest found that the microbial biomass was reduced by clear-cutting, with a corresponding structural change in the community as measured by

phospholipid fatty acid (PLFA) patterns. However, selective felling had no discernable impact (Siira-Pietikäinen et al., 2001).

Understanding the short-term effects of thinning on microbial communities in forest soils addresses an important knowledge gap. Fungi play important roles in recycling important chemical elements in terrestrial ecosystems, making nutrients available during critical plant growth phases and assisting in primary production. Beyond their role in nutrient cycling, fungi also participate in ecological food webs (Dighton, 2003) and are key determinants in the biodiversity of understory plant communities (Van der Heijden et al., 1998; Reynolds et al., 2003). Their activity may affect the success of seedling establishment and growth during the critical period following tree removal (Jones et al., 2003). As soil fungi have a crucial role in the forest community structure and productivity, it is important to understand the response of these organisms to disturbances and their dynamics in the period that follows.

We studied the response of soil fungi to thinning in a Japanese cedar (*Cryptomeria japonica*) forest plantation in central Taiwan. The objectives of this study were to: (1) investigate thinning effects on the soil microfungal population and communities in *C. japonica* plantations; (2) evaluate the changes corresponding to the thinning intensity; and (3) determine the duration of any thinning effects influenced on fungal communities in the soils of these forests. Plate counts and denaturing gradient gel electrophoresis (DGGE) were used as relatively simple methods to monitor the fluctuation of the soil fungal population and to describe the changes of their genetic diversity and community after thinning. These methods were chosen because of their relative cost

efficiency and their ability to detect major community shifts (e.g., Winder et al., 2013) without saturating the acquired datasets.

Materials and Methods

Study site

The study site is located in the Luan-Da forest management district, Nantou County, in central Taiwan, in a 40-year old plantation of *Cryptomeria japonica*. The site ranges from 23°28’ to 23°55’N latitude and from 120°48’ to 121°09’E longitude. The elevation ranges from 1,275 to 1,500 m. According to records from the nearby Sun Moon Lake Weather Station (23°53’N, 120°54’E), the average annual air temperature and rainfall are 19.2°C and 2,401.9 mm, respectively (Climate data from Sun Moon Lake Meteorological Observatory, http://www.cwb.gov.tw/V6/index.htm, accessed 20 January 2011). Most rainfall at the site occurs during the summer, from June to September. Some rainfall also occurs during the spring, from March to May; there is no obvious dry season.

Twelve 1-ha permanent plots (100 x 100 m) were established (Figure 1A) for the long-term monitoring of biodiversity dynamics. The twelve plots were divided into four blocks. The blocks had slightly different elevations on a slope. Each block had three plots, and each plot was randomly assigned a control (0%), 25% or 50% thinning treatment (Figure 1A). Each plot was divided into one hundred 10 x 10 m grids (Figure 1B). The x and y coordinates of the plot were staked at every 20-m and 10-m interval. Thinning treatments consisted of tree removal in alternating quadrats, as indicated in Figure 1B. In treatment plots with

25% thinning, trees in one 10-m quadrant within each 20-m quadrant were cut, while in the 50% thinning plots, trees in two 10-m quadrants within each 20-m quadrant were thinned (Fig 1B). Thinning was performed by chainsaws in August of 2007. Most slash and coarse woody debris was removed, and some minor fragmentary debris remained.

Soil sampling

Soil samples were collected in two ways. For the cultural analysis of fungal populations, soil samples were collected seasonally from January of 2008 to August of 2009. In each plot, 100 g of topsoil (15 cm depth, ca. 15 cm-diam.) samples from four 10×10 m random quadrants were collected using a trowel and pooled together. There was one mixed soil sample per plot, therefore each treatment provided four replicate soil samples. The soil samples were sieved with a 2-mm mesh and stored at 4℃ until analysis.

For the molecular analysis of the fungal communities in the soil, we selected three sampling sites (Figure 1C) in each plot. Each sampling site had four sample points (Figure 1C). Soil samples were collected from the topsoil of each sampling point and were mixed into one sample; this provided three replicate pooled soil samples from each plot. During the first year, subsequent to thinning (October of 2008 to August of 2009), soil samples were taken seasonally. To study the long-term impacts on the soil microbe communities, samples were also taken annually at the second (October of 2009) and third (October of 2010) year after thinning. Prior to DNA extraction, each soil sample was sieved and stored as described above.

Abundance of fungal populations

Sieved soil (10 g) was added to 90 ml of 0.1% water agar, which was then mixed well. The soil suspensions were serially diluted with 0.1% water agar. Diluted suspensions were spread on Rose Bengal agar (Cho et al., 2008). Each assessment was performed in triplicate. The plates were incubated at 25℃, and fungal colonies were counted after 5 days. Some colonies were examined with a microscope to visually characterize the most abundant types.

DNA extraction and amplification

DNA was extracted from 0.25 g of each soil sample using a PowerSoil® DNA Combo Kit (Mo Bio Laboratories, Inc. CA, USA). Fungal rDNA internally transcribed spacer (ITS) regions were amplified twice using a semi-nested PCR amplification method. In the first PCR amplification, fungal-specific ITS1F (Gardes and Bruns, 1993) and ITS4 (White et al., 1990) primers were used to amplify the ITS regions. PCR reactions were performed in a PCR thermocycler (Biometra, Gottingen, UK). The 25 μl PCR mixture included 10 ng of template DNA, 250 μM of each primer, 250 μM of dNTP, 1 U Taq DNA polymerase (Fermentas, USA), 2.5 mM $MgCl_2$ (Fermentas, USA), and 2.5 μl of 10x buffer (Fermentas, USA). The amplification protocol consisted of one denaturation step at 94 ℃ for 5 min, 39 cycles of denaturation at 94℃ for 1 min, annealing at 50℃ for 1 min, extension at 72℃ for 1 min, and a final extension at 72℃ for 5 min. The first-round PCR products were used as template DNA in the second PCR amplification, which used an

ITS2 primer (White et al., 1990) and an ITS1F fungal-specific primer (Gardes and Bruns, 1993) with a 40-base GC-clamp attached to the 5' end of the primer to aid in DGGE. The PCR mixtures and program were identical to the first PCR protocol. Agarose gel electrophoresis was used to evaluate and verify the quality and amount of PCR product.

DGGE analysis

To analyze the genetic diversity and structure of the soil fungal communities, all PCR products were analyzed with DGGE using the DCODE^{TM} Universal Mutation Detection System (Bio-Rad Laboratories Limited). The DGGE was performed using 8% polyacrylamide gels (40% acrylamide/bis-acrylamide [37:5:1] Bio-Rad stock solution) prepared with a 20% (1.4 M urea, 8% (v/v) formamide) to 45% (3.85 M urea, 22% (v/v) formamide) vertical denaturing gradient according to the manufacturer's suggested protocol. Electrophoresis was performed in 1X tris-acetate buffer at 60℃, using 70 volts for 16 hours. Subsequent to electrophoresis, the gel was stained with SYBR gold (Molecular Probes, USA) in darkness for 30 min at room temperature (ca. 20^{o}C), rinsed once in deionized water, and photographed under UV light.

Data analysis

Measurements of the abundance of fungi in soils were subjected to variance analysis (ANOVA) to determine the effects of forest thinning. Duncan's multi-range test determined the differences among the treatments. Microbial data were correlated with the physical soil properties and enzyme activities published by Chung et al. (2013). The

physical soil properties and enzyme activities were sampled at points corresponding to the microbial soil samples. Soil measurements included pH value, organic matter, total nitrogen, inorganic nitrogen and available phosphorus. The soil enzyme data included the activities of cellulase, glucosaminidase, acid phosphatase, arylsulfatase and dehydrogenase. Briefly, pH ranged from 3.89 to 4.14; organic matter content ranged from 21.2 to 34.1 (g/kg dry soil); total nitrogen ranged from 0.68 to 1.72 (g/kg dry soil), inorganic nitrogen ranged from 2.41 to 3.02 (g/kg dry soil); available phosphorus content ranged from 2.41 to 3.02 (g/kg dry soil); cellulase activity ranged from 0.11 to 0.32 (μmol/g); glucosaminidase activity ranged from 1.20 to 1.81 (μmol/g); acid phosphatase ranged from 21.94 to 30.38(μmol/g); arylsulfatase ranged from 7.85 to 10.61(μmol/g); and dehydrogenase ranged from 0.06 to 0.12(μmol/g). The relationships between the abundance of the soil fungi and the physiochemical soil properties were analyzed using a multiple linear regression model.

Image processing software (Qantity One, Bio-Rad, USA) was used to analyze and quantify the bands on the DGGE gels. The band intensity in each sample was recorded as a binary matrix. The binary matrix was used to calculate the Bray-Curtis Similarity Index (Clark and Warwick, 2001) and to construct nonparametric multi-dimensional scaling (MDS) plots using Primer 6 software (version 6. 1. 15; Primer-E Ltd., United Kingdom). The PERMANOVA plug-in (version 1.0.5) of the Primer package was used to statistically test any observed differences. A repeated measurement design permutational multivariate analysis of variance (PERMANOVA) was calculated using time (including seasons and years) and the thinning treatment as fixed factors and block as a random factor

to compare soil fungal community compositions over time. If there were significant differences for time, treatments, or interactions, pairwise PERMANOVA was performed on the respective term for each level of the factor. In all of the PERMANOVAs, a maximum of 9999 random permutations was performed.

Results

Populations of soil fungi

The study sites were thinned in October 2007. The fungal population in January 2008 and April 2008 was measured to be 1.1×10^5 to 2.1×10^5 and 4.7×10^4 to 1.1×10^5 colony forming units (CFU) g^{-1} of dry soil, respectively (Figure 2); thinned plots had significantly ($P = 0.045$) more fungal counts than the controls had. In August 2008, October 2008, January 2009, April 2009 and August 2009, the abundance of fungal populations ranged from 6.0×10^4 to 8.5×10^4, 9.3×10^4 to 1.4×10^5, 1.5×10^5 to 3.3×10^5, 1.6×10^5 to 3.0×10^5, 1.3×10^5 to 2.0×10^5 CFU g^{-1} dry soil, respectively (Figure 2). The abundance among the treatments from August 2008 to August 2009 did not show any significant differences ($P < 0.05$). Thinning increased the fungal population in the first six months after thinning. After 10 months, however, the differences in abundance among the treatments were not found to be significant ($P = 0.7$). Dominant fungi included *Penicilium* spp., *Fusarium* spp., *Trichoderma* spp., and Zygomycetes.

The relationships between the soil fungal population and the soil properties were analyzed by multiple-regression analysis. Organic matter and activity of cellulase proved to be good estimators of the soil fungal

population (R^2 = 0.801; Adjusted R^2 = 0.713). Regression analysis indicated that the population of soil fungi was significantly and positively associated with the amount of organic matter (β =1.92×10^5, $P < 0.001$) and the activity of cellulase (β =1.20×10^5, $P < 0.05$). Other factors, including water content, pH value, total nitrogen, inorganic nitrogen, available phosphorus content and soil temperature were not associated with the soil fungi population.

Community compositions of soil fungi

In MDS plotting, the compositions of fungal communities in the plantation soils were grouped separately according to corresponding thinning treatments, 12-21 months post-thinning (Figure 3, panels A-D); at 24 months post-thinning, there was no significant (P = 0.15) clustering of these communities (Figure 3, Panels E-F). PERMANOVA showed that the composition of the soil fungal community was significantly different (P = 0.0007) due to thinning treatments (Table 1). Pairwise comparisons of the DGGE gels showed that thinning levels significantly ($P < 0.05$) influenced the composition of fungal communities in all treatments 21 months post-thinning (data not shown); by 24 months post–thinning, however, there was no significant effect (P >0.1) of thinning on these communities in any of the thinning treatments (data not shown).

DGGE patterns were also analyzed to study the temporal (seasonal) dynamics of the soil fungal communities among different thinning treatments, using the MDS and PERMANOVA tests. Figure 4 shows the distinct seasonal variation of the fungal community composition in the control (Figure 4A), 25% thinning (Figure 4B), and 50% thinning

treatments (Figure 4C). The results of the PERMANOVA tests showed that the composition of the fungal communities in these soils varied significantly ($P = 0.0001$) among seasons (Table. 1), and pairwise comparisons showed that the seasonal effect was consistently observed throughout the study period, as there were significant ($P < 0.05$) differences in all pairwise tests among seasons. A season × treatment interaction was found for all treatments (Table 1). The 50% thinning treatment increased the variability of the fungal community between seasons. There was higher DGGE pattern dissimilarity between seasons in the 50% thinning treatment compared to the comparison among seasons in the control plots (Figure 5, Table 2).

The dissimilarity of the DGGE patterns among treatment pair comparisons in 2008 was higher than it was in 2009 or 2010 (Table 3), indicating that differences between treatment and control plots decreased by the second year post-thinning and that the influence of thinning on the fungal community had diminished.

Discussion

We evaluated the effects of thinning disturbances and seasons on the abundance of soil fungi and the structure of these communities in forest soils, using cultural methods and DGGE profiles. Thinning was found to increase the soil fungal populations within 6 months of the disturbance, and it was found to have changed the soil fungal community structure in the short term. Other studies have reported similar impacts, with thinning heightening soil respiration (Ohashi et al., 1999) and microbial biomass/activity (Grayston and Renneberg, 2006), and a changing

structure of soil microbe communities (Maassen et al., 2006). Green leaves contain nutrients that are more easily decomposed than fallen leaves (Girisha et al., 2003), providing extra available carbon, nitrogen, phosphorus and other nutrients for microbial growth (Ludovici and Kress, 2006). It has been reported that early colonizer taxa can readily respond to decomposable substrates from logging slash, leaf litter, fallen branches (Osono 2005; Rui et al., 2009) and dying roots. An alteration of carbon input resulted in composition shifts in the soil microbial communities and a regulation of belowground carbon flux (Churchland et al., 2013). In this study, thinning provided green fallen leaves, shoots, and dying and dead roots as nutritive substrates for soil saprotrophic microorganisms. The fungal population positively correlated with organic matter content, and this was reflected in correlation with cellulase activity.

In forest ecosystems, fungi convert recalcitrant organic material into forms that other organisms can use. In balsam fir stands, for example, thinning was reported to significantly increase organic matter decomposition, as indicated by mass loss of cellulose bags from 6 to 18 months post-thinning; increased fungal populations were found to be the cause of the increased cellulose decomposition (Thibodeau et al., 2000). In the *Cryptomeria japonica* plantation, thinning increased the fungal population in the first six months post-thinning, and the soil fungal abundance was significantly and positively associated with the amount of organic matter and the activity of the cellulase.

We presume that the return of fungal populations to their initial levels of abundance was caused by the depletion of the easily decomposable substrates provided by logging slash, dead stumps and roots. As the

substrate availability diminished, more oligotrophic taxa would have metabolized the remaining recalcitrant organic carbon pools and likely replaced the early colonizers taxa (Osono, 2005; Rui et al., 2009; Fierer et al., 2010). The shift in the soil fungal community could also have been compounded by other contributory factors, such as changes in temperature and humidity and the exchangeable cations in the soil within the local microenvironment. In other studies that were performed at the same study site, the air and soil temperature increased and the relative humidity decreased during the first year post-thinning; these thinning effects gradually diminished during the second year (Chen et al., 2010). Other researchers also found that the total number of exchangeable cations increased in the site soils during the first year post-thinning, and subsequently, the levels reverted to their original level after 16 months (Liu et al., 2010). Thinning also creates forest gaps and increases the available light to the forest. Tree cover has been reported to have an impact on the soil microbial community structure in forests, as it influences the microclimate and the physiochemical properties of the soil (Zhong and Makeschin, 2003; Connell et al., 2006; Fell et al., 2006; Maassen et al., 2006; Weng et al., 2007; Chatterjee et al., 2008; Kara et al., 2008).

The structure of the fungal communities in the plantation soils was also shown to be seasonally variable. Moreover, we found that thinning treatments emphasize the seasonal dynamics of the soil fungal community. Seasonal differences in these communities may be caused by seasonal variations in climate, soil properties, substrate availability, plant productivity and litter deposition throughout the year (Grayston et al.,

2001). As environmental changes increase, there are corresponding increases in the fluctuation of fungal community structure forest soils.

Forest ecosystems are complex. While fungal communities, including pathogens and mycorrhizal species, can be major drivers of plant community structure and forest biodiversity (Winder and Shamoun, 2006), it is also known that plant species diversity conversely influences fungal community structure (De Bellis et al., 2007). Changes in the fungal communities that were observed in this study might therefore also reflect changes in the composition of understory plant communities. According to a seedling survey performed in this study site, thinning provides niches for plant regeneration, wherein species richness and an abundance of native trees increased post-thinning (Sun et al., 2012). The increase and presence of native trees post-thinning has an impact on fungal communities.

Some studies have reported that thinning has no significant influence on the soil fungal biomass and activity from 4-45 years after thinning (Grady and Hart, 2006; Hannam et al., 2006; Maassen et al., 2006). Our results also show that thinning does not influence the soil fungal community in the long term. Clear-cut management decreases the biomass for fungi and other soil microbes (Baath et al., 1995; Mummey et al., 2010), and it has a long-term impact on lodgepole pine soil microbial communities (Chatterjee et al., 2008; Kennedy and Egger, 2010). Thinning, combined with prescribed burning, may also diminish the microbial populations (Switzer et al., 2012). In and of itself, green-tree retention has a less negative impact than clear-cutting, when species richness or abundance of ectomycorrhizal fungi and other taxa is

compared (Luoma et al., 2004; Rosenvald and Lohmus, 2008); green-tree retention has been shown to conserve the diversity of the mycorrhizal fungi (Outrebridge and Trofymow, 2004; Jones et al., 2008). Our results support the idea that thinning is a better management method than clear-cutting for biodiversity preservation and for preventing soil erosion.

After the thinning of the *Cryptomeria japonica* forests, the fungal populations increased and recovered after 6 months in plantation soils in Taiwan, whereas the soil respiration rates increased and recovered in the fourth year post-thinning in Japan (Ohashi et al., 1999). Taiwan is tropical, with higher average temperatures than Japan. Mycelial growth is higher in warmer and wetter environments (A'Bear et al., 2013). In tropical areas, plant residues therefore have faster decomposition rates compared to those in temperate areas, and thus, there is typically a more rapid return to nominal conditions after substrate pulses have been digested. This means that increases in fungal populations in response to thinning may be relatively short in comparison to responses in temperate forests.

In Finland, clear-cutting had an impact on the PFLA patterns of soil microbes, whereas selection-felling had no discernable effect (Siira-Pietikäinen et al., 2001). In the Finnish study, the soil microbial communities of the clear-cut plots changed during the first year, as indicated by PFLA patterns. It was also reported that the microbial biomass and basal respiration decreased during the second year. The impact of thinning was milder than that from clear-cutting; their microbial biomass and basal respiration decreased during the second year, but not significantly as in the clear-cut treatment. The study sites of Taiwan, Japan, and Finland were located approximately 23°N, 32°N, and

61ºN, respectively.

We presume that the effects of clear-cutting and thinning on soil microbial communities are slower in colder boreal forests and that the fluctuation would be smaller. In tropical regions, thinning had more rapid and short-term effects on the soil microbial community compared to the temperate and boreal regions. This has implications for forest management in post-thinning situations, particularly where there is a need for the recruitment or planting of new tree seedlings. Given that communities of soil fungi impact tree health and productivity, there is a need to understand how these dynamics might affect early seedling growth and productivity. Better understanding of these effects might then lead to improved sustainability of forestry operations.

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Table 3-1. Results of repeated measure design PERMANOVAs testing for the effects of the season, block, and thinning treatment as well as their interactions on the soil fungal community in a *Cryptomeria japonica* plantation in central Taiwan. A maximum of 999 permutations was possible.

Source	*df*	sum of squares	pseudo *F*-ratio	significance (*p*-value)
season	5	162980	11.937	0.0001
block	3	7829	4.084	0.0001
thinning	2	27366	7.796	0.0007
season x block	15	40975	4.275	0.0001
season x thinning	10	117830	7.835	0.0001
block x thinning	6	10532	2.747	0.0001
season x block x thinning	30	45138	2.355	0.0001
Residuals	143	91368		
Total	214	504670		

Table 3-2. Dissimilarity calculated from the binary matrices of the DGGE band intensity for the community of soil fungi occurring between two seasons in variously thinned plots of a *Cryptomeria japonica* plantation in central Taiwan.

Seasonal comparison	Thinning level		
	0% (control)	25%	50%
spring vs. summer	57.11[a]	58.98	63.32
spring vs. autumn	52.54	49.79	66.45
spring vs. winter	54.53	60.40	57.43
summer vs. autumn	52.43	47.75	50.34
summer vs. winter	57.81	54.52	62.32
autumn vs. winter	57.14	58.17	60.07

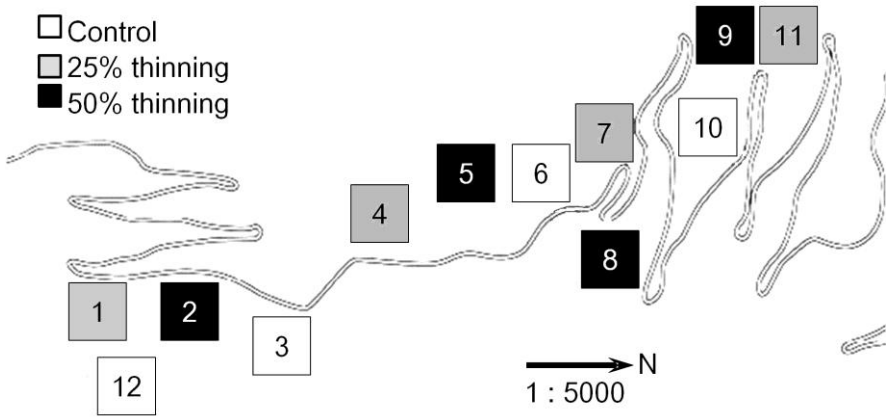
[a] Dissimilarity was calculated according to the method of Kerbs (1989).

Table 3-3. Dissimilarity calculated from the binary matrices of the DGGE band intensity for the community of soil fungi occurring in variously thinned plots of a *Cryptomeria japonica* forest in central Taiwan at yearly intervals subsequent to thinning.

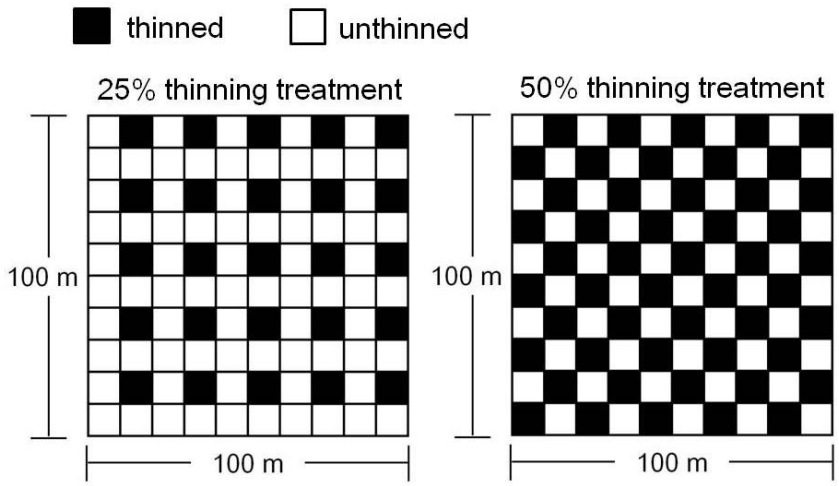
	Year		
Thinning comparison	2008	2009	2010
25% vs. 0%	50.33 [a]	16.46	12.54
50% vs. 0%	43.81	15.90	15.19
25% vs. 50%	36.55	14.54	18.61

[a] Dissimilarity was calculated according to the method of Kerbs (1989).

(A)



(B)



(C)

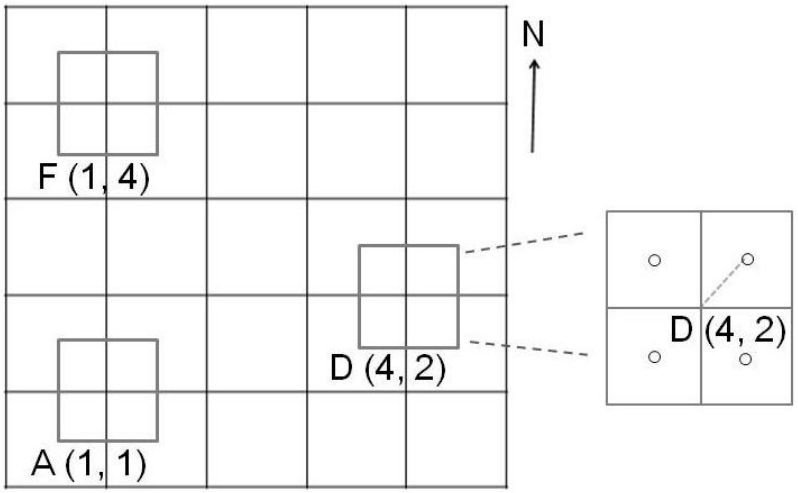


Figure 3-1. (A) Twelve 1-ha plots (100 x 100 m) with 3 treatments (control and two thinning intensities) of 4 replicates distributed in a

plantation of Japanese cedar, *Cryptomeria japonica* in central Taiwan. (B) Schematic of the thinning method comparing the arrangement of thinned (completely harvested) treatment areas corresponding to 25% thinning and 50% thinning treatments. The 100x100 m plots were divided into sections using a grid with 10-m intervals. In the thinned plots, all trees within a 10x10 m section (black quadrats in schematic) were thinned. (C) A schematic showing the arrangement of three sampling sites (A, D and F) within each 1-ha plot. There were four sampling points in each sampling site in the center of each of the four quadrants of the sampling site. A soil sample was taken from the organic soil layer at each of the four sample points. These four subsamples were mixed. Each plot contained three replicated soil samples. Soil samples were taken from all of the plots periodically starting in October of 2008.

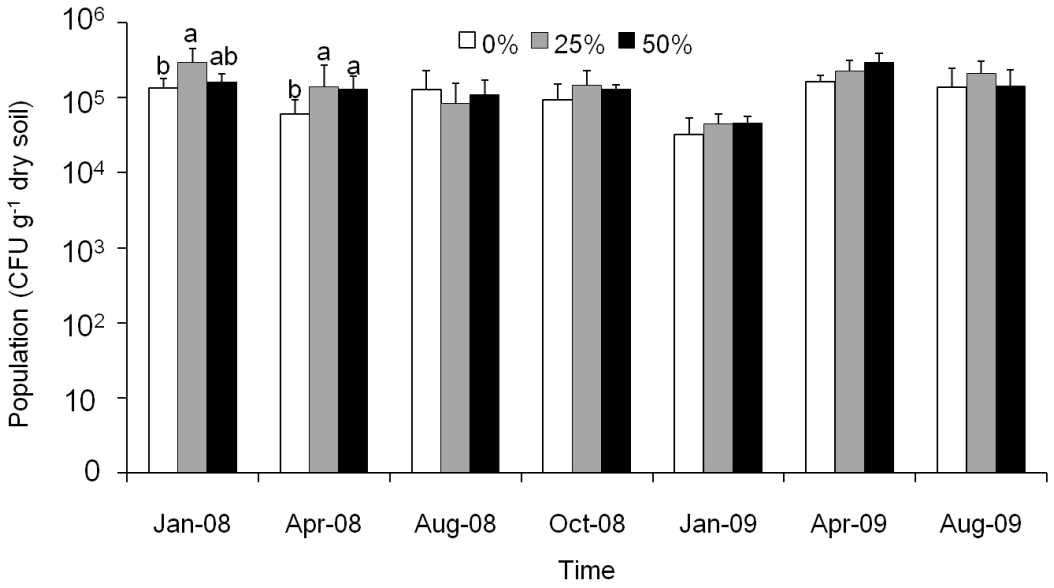


Figure 3-2. Abundance of fungal populations in soil samples collected in control, 25%, and 50% thinning treatment of *Cryptomeria japonica* plantation in central Taiwan from January 2008 to August 2009. Bars with the same letter are not significantly different according to Tukey's multi-range test ($P > 0.05$). In January 2008 and April 2008, thinned plots had significantly ($P = 0.045$) more fungal counts than the controls had.

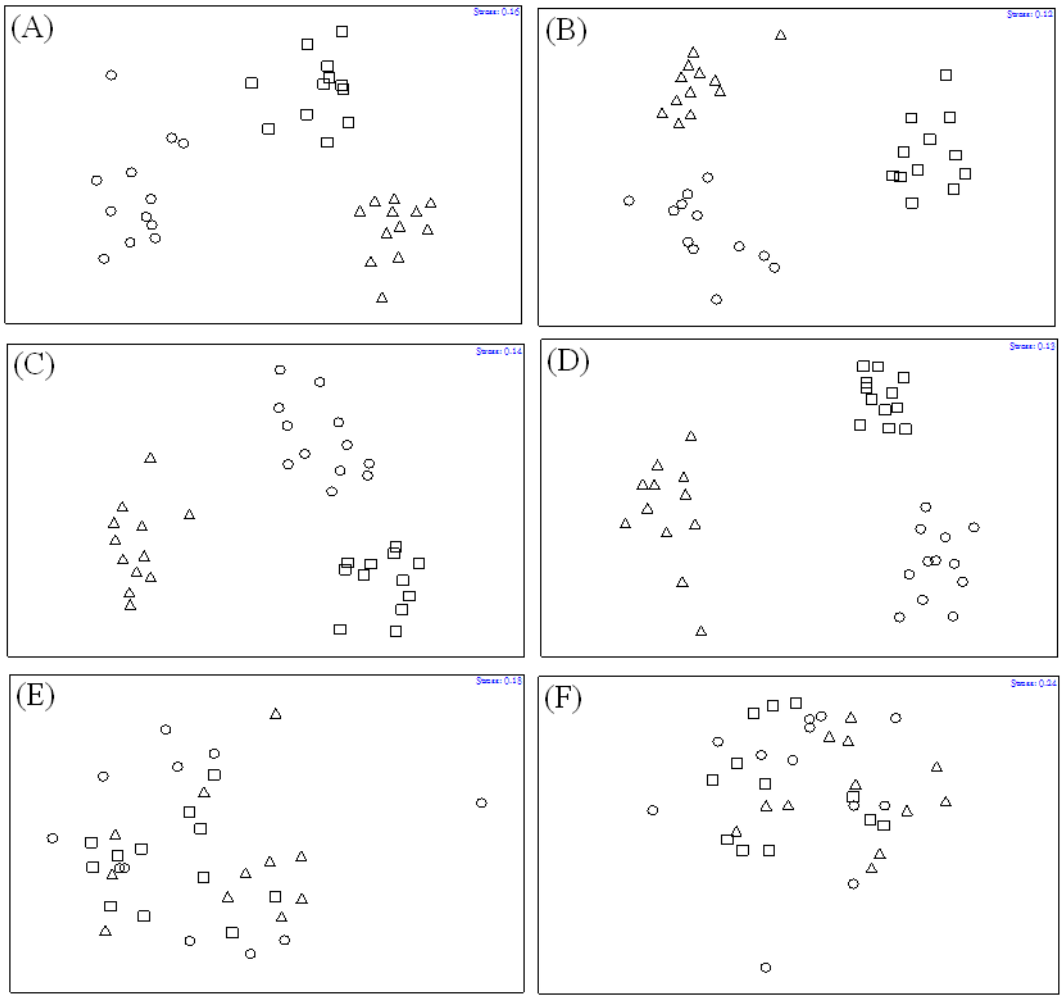


Figure 3-3. Multidimensional scaling of the DGGE profiles of soil samples collected from three thinning treatments in a *Cryptomeria japonica* plantation in central Taiwan. Symbols indicate different levels of thinning treatment: circles (○) = 0% thinning (controls); triangles (△) = 25% thinning; and squares (□) = 50% thinning. (A) Samples collected in October, 2008 (12 months post-thinning). (B) Samples collected in January 2009 (15 months post-thinning). (C) Samples collected in April 2009 (18 months post-thinning). (D) Samples collected August 2009 (21 months post-thinning). (E) Samples collected October 2009 (24 months post-thinning). (F) Samples collected October 2010 (36 months post-thinning).

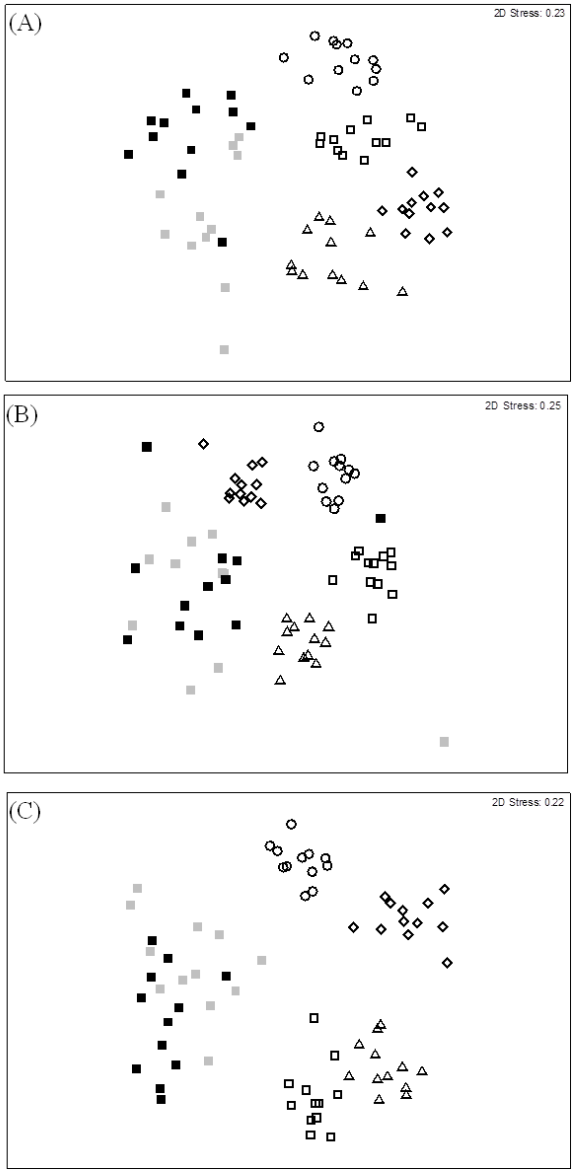


Figure 3-4. Multidimensional scaling of the seasonal DGGE profiles of soil samples collected from variously thinned plots in a *Cryptomeria japonica* plantation in central Taiwan. Symbols correspond to samples from four seasons: circle (○) = spring; triangles (△) = summer; squares (□) = fall; and diamonds (◇) = winter. Colors correspond to samples from different sampling years: white =2 008; light gray = 2009; black = 2010.(A) Samples from plots with 0% thinning (controls). (B) Samples from plots with 25% thinning. (C) Samples from plots with 50% thinning.

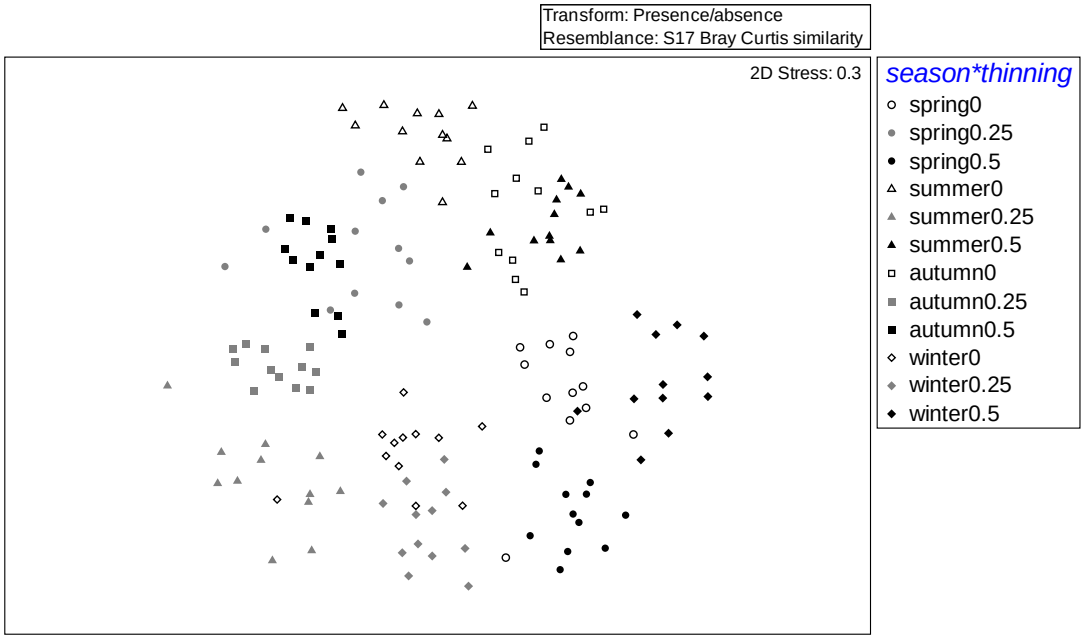


Figure 3-5. Multidimensional scaling of the seasonal DGGE profiles of soil samples collected from variously thinned plots of a *Cryptomeria japonica* plantation in central Taiwan during 2008. Symbols correspond to the sample season: circles (○) = spring; triangles (△) = summer; squares (□) = fall; and diamonds (◇) = winter. Colors correspond to samples from different thinning treatments: white = samples from control plots; light gray = samples from 25% thinned plots; black = samples from 50% thinned plots.

Chapter 4 – A new species of *Scytinopogon* from Taiwan

Introduction

Scytinopogon is a genus of clavarioid basidiomycetes, which was segregated from *Lachnocladium* by Singer (1945) based on the sub-fleshy basidiomes and verrucose-echinulate, angulate yellow spores. This genus contains several clavarioid species which are characterized by the richly branched, light colored basidiomata with flattened branches, a monomitic hyphal system with clamped, non-inflated, colorless hyphae, four-sterigmata basidia, and white to ochraceous, verrucose-echinulate, and angulate spores. Singer (1945) distinguished this genus from other similar genera by the consistency of its basidiomata and the color of the spores and designed *Scytinopogon pallescens* (Bres.) Singer [≡*Pterula pallescens* Bres.≡*Lachnocladium pallescens* (Bres.) Bres.] as the type species.

Corner (1950) suggested that *Scytinopogon* belongs to the ‘‘Thelephoroid-series’’ in the Clavariaceae. *Scytinopogon* has been included in several families, such as Thelephoraceae (Donk, 1964), Scytinopogonaceae (Jülich, 1981) and Gomphaceae (Maas Geesteranus, 1963). Most species of *Scytinopogon* have tropical-subtropical distribution (García–Sandoval et al., 2004; García-Sandoval and Cifuentes, 2005; Dutta et al., 2012; Zhang and Yang, 2003). We found and collected basidiomata of *Scytinopogon* in *Cryptomeria japonica* plantations in Taiwan. The phylogenetic placement of *Scytinopogon* sp. was inferred with internal transcribed spacer (ITS) region and ribosomal large subunit (rLSU) DNA (rDNA). Based on morphological characteristics and DNA sequence data, this *Scytinopogon* was described and illustrated herein as a new species.

Materials and methods

Morphological study

Specimens were collected from June to October in 2007 to 2011 and deposited at the Plant Collection of Life Science, Tunghai University and the herbarium of the National Museum of Natural Science (TNM), Taichung, Taiwan. Specimens were sliced by hand and soaked in drops of 5% KOH solution. Microscopic examinations were made at magnification up to X1,000 with bright field and phase contrast optics. Spores were mounted in Melzer's reagent to test for amyloidity. For spore measurement, 30 spores from five collections were studied. For the observation of basidiospores and basidia under a scanning electron microscope (SEM), tiny pieces of sporocarps from fresh specimens were fixed with 1.0% osmium tetroxide for 2 h, and then washed 3 times with deionized water for 15 min. After this fixation process, the specimens were dehydrated in graded ethanol (10%, 20%, 30%, 40%, 50%, 70%, 85%, 95%, 100%, 15 min each) and acetone, and critical-point dried. Samples were coated with gold in an E-1045 (Hitachi, Japan) and examined with a Hitachi S 4700 scanning electron microscope (Hitachi, Japan).

Molecular procedures and phylogenetic analysis

Total DNA was extracted from basidioma tissues by the CTAB method modified by Doyle and Doyle (1990) and described by Wang and Chang (2003). The internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) was amplified with the primers ITS5 and ITS4 (White et al. 1990). PCR reactions were performed in 25 μL reaction mixtures containing included 25 ng of template DNA, 250 μM of each primer, 250 μM of dNTP, 1 U *Taq* DNA polymerase (Fermentas, USA), 2.5 mM $MgCl_2$, and 2.5 μl 10x buffer. The amplification protocol consisted of one

denaturation at 94℃ for 2 min, 39 cycles of denaturation at 94℃ for 1 min, annealing at 56℃ for 20 seconds, and extension at 72℃ for 5 seconds, and a final extension at 72℃ for 2 min. DNA sequencing was performed at Mission Biotech, Taiwan, with the same primers.

Fungal rLSU region was amplified by primer pairs 5.8SR / LR7 (Vilgalys and Hester 1990). PCR reactions were performed in 25 μL reaction mixtures containing included 25 ng of template DNA, 250 μM of each primer, 250 μM of dNTP, 1 U *Taq* DNA polymerase (Fermentas, USA), 2.5 mM $MgCl_2$, and 2.5 μl 10x buffer. The amplification protocol consisted of one denaturation at 94℃ for 2 min, 35 cycles of denaturation at 94℃ for 1 min, annealing at 55℃ for 1 min, and extension at 72℃ for 1 min, and a final extension at 74℃ for 7 min. DNA sequencing was performed with primers LR5, LR16, LR0R, and LR3R (Moncalvo et al., 2000). Data were deposited in the GenBank databases (Accession nos. KF679498–KF679506 for ITS sequences, KR094135–KR094136 for LSU sequences). All DNA sequences generated in this study were provided along with additional sequences accessed from GenBank and used in phylogenetic analysis.

The sequence data of *Scytinopogon* used in this study were compared with other closely related species including *Ramaria*, *Typhula*, *Clavaria*, *Clavicorona*, *Clavulina*, *Clavulinopsis*, *Ramariopsis* and *Trechispora* from GenBank (http://www.ncbi.nlm.nih.gov/). Sequence data were initially aligned and subsequent manual adjustments made using BioEdit Sequence Alignment Editor (Hall 2011) and Clustal X 1.83 (Thompson et al., 1997) then improved and adjusted visually. Using the same aligned datasets, parsimony analysis was performed with the default settings and parsimony bootstrap values were generated with 1,000 replicate heuristic searches to estimate support for clade stability of the consensus tree with 1,000 replicates in PAUP (Phylogenetic Analysis Using Parsimony)

v4.0b10 (Swofford, 2003).

Results

Taxonomy

Scytinopogon cryptomerioides W.R. Lin & P.H. Wang., sp. nov.

MycoBank:

Etymology: *cryptomerioides* (Lat.), *cryptomeria*, the genus name of *Cryptomeria japonica*, referring to its habitat.

Holotypus: Taiwan, Nantou County, Zen-Len area, at 23.45 N 120.68 E, alt. 1500 m, 5 July 2009, 0906RK10-23; nLSU and ITS sequences generated from the holotype are KR094135 and KF679506, respectively.

Basidiomata (Fig 1) up to 50–110 mm, branched, tufts arising from a common stem or cluster of steas, flesh white, cream or tan on fresh, rather tough, subcoriaceous, brown on drying, solitary, gregarious, or caespitose on sandy loam soil. Stem up to 10–30 × 1–6 mm, thick, cylindric or sub-compressed. Branches dichotomous or polytomous, flattened, generally branched from the base. Spores (Fig 3A–3B) 4.0–6.0 × 3–3.5 μm, hyaline to white, angularly ellipsoid, echinulate or verrucose with acute warts or spines 0.5–0.7 μm long, slightly angular, inamyloid and cyanophilous. Hilar appendix small, obscured by spore ornamentation. Basidia formed laterally from generative hyphae ,with basal clamp (Fig 2). Basidia (Fig 3C–3E) 35–42 × 5.5–6 μm, short, clavate, finely granular-vacuolate, with four conical sterigmata (mostly 3–4 μm long). Cystidia none. Hyphae monomitic, hyaline, 1.9–2.8 μm wide, all with clamps.

Basidiospore development (Fig 4) starts with a spherical enlargement of the sterigma apex to form a basidiospore primordium 0.6–0.8 μm in diameter (Fig 4A). The basidiospore initial grows asymmetrically on its abaxial side. The spherical enlargements, the hilar appendices and the

ornamentations of the basidiospore are shown in Fig 3B–3C, the depression of the hilar appendices in Fig 4D.

Habitat and distribution: terrestrial, solitary, gregarious, or caespitose on sandy loam soil. In Taiwan, this taxon was collected on the earth in the forests dominated by *Cryptomeria japonica* at altitude of 600 – 2100 m.

Additional specimens examined: Taiwan, Nantou County, Zen-Len area, at 23°45’ N 120°68' E, alt. 1300-1500 m, 5 July 2009, 0906RK6-10; Taiwan, Hsinchu County, Guanwu, at 121°07'E, 24°31'N, alt. 2000-2250, 2 October 2010, 1010WRK-10.

Molecular phylogeny

The ITS dataset contained 55 sequences with 604 characters. In the maximum parsimony analysis, we obtained 213 equally most parsimonious trees (length = 2376, CI = 0.4621, RI = 0.8271, RCI = 0.3822). The phylogenetic tree from the maximum parsimony analysis is shown in Fig 5. *Ramaria botrytis* (AF377055) was the outgroup. *Typhula*, *Clavaria*, *Ramariopsis*, and *Clavulinopsis* were in a clade with high branch support (MP = 97%). *Scytinopogon cryptomerioides* was nested in several species of *Trechispora* with good branch support (MP = 72%).

The rLSU dataset contained 25 sequences with 553 characters. In the maximum parsimony analysis, we obtained ten equally most parsimonious tree (length = 913, CI = 0.6889, RI = 0.7633, RCI = 0.5259). The phylogenetic tree from the maximum parsimony analysis is shown in Fig 6. *Ramaria* was the most distinct genus. *Clavaria*, *Clavulina*, *Ramariopsis*, and *Clavulinopsis* were in a clade with high branch support (MP = 92%). *Scytinopogon cryptomerioides* was close to *Trechispora incisa* with good branch support (MP = 64%) and nested among *Trechispora* species together with *S. angulisporus* (MP = 100%). The *Scytinopogon*/*Trechispora* clade was separated from *Clavulina*

cinerea.

Discussion

The specimens of *Scytinopogon cryptomerioides* all have richly branched, light colored basidiomata with flattened branches, a monomitic hyphal system with clamped, non-inflated, colorless hyphae, four sterigmata basidia, and echinulate spores, and these features conforms to the concept of the genus *Scytinopogon* (Corner, 1950).

Scytinopogon pallescens (Bres.) Singer, the type species of the genus, has been considered conspecific with *S. angulisporus* (Pat. & Gaillard) Corner (Corner, 1950; Petersen, 1988). *Scytinopogon cryptomerioides* is different from *S. angulisporus* in both morphology and molecular phylogeny. Morphologically, the basidiomata of *S. angulisporus* (200 mm) were bigger than *S. cryptomerioides* (50–110 mm) and deeply immersed in the ground (Corner, 1950). The spore sizes of *S. cryptomerioides* (4.0–6.0 × 3–3.5 μm) were smaller than those of *S. angulisporus* (5.5–7.0 × 3.5 μm) and its wart size (0.5–0.7 μm) was larger than those of *S. angulisporus* (0.3–0.5 μm) (Corner 1950). Phylogenetically, there were 33 bp phylogenetically informative differences between *S. angulisporus* and *S. cryptomerioides*. Based on the description of *S. pallescens* described by Garcia-Sanoval et al. (2004), the shape of basidiospores in *S. pallescens* is subglobose to broadly ellipsoid basidiospores, their ornamentation is nodular-echinulate. Basidiospore shape and ornamentation differ from those of *S. cryptomerioides*.

Basidiomata of *S. cryptomerioides* are white, different from those of *Scytinopogon echinosporus* (Berk. & Broome) Corner being pallid or brownish at the base, pale purple or lilac towards the apex The spores of *S. echinosporus* (Corner, 1950; Zhang and Yang, 2003) are reniform, whereas the spores of *S. cryptomerioides* are ellipsoid.

Scytinopogon robustus (Rick) Corner has smaller basidiospores (4.2–5.6 × 2.8–4.2 μm) and bisporic or tetrasporic basidia (Garcia-Sanoval et al., 2004). *Scytinopogon chartaceum* (Pat.) R.H. Petersen has bigger basidiospore (6.1–6.5 × 3.2–4.0 μm) and shorter and wider basidia. The color of *Scytinopogon papillosus* Corner is gray-purple and it grows on woody substrates (Medel and Mata, 2011). *Scytinopogon dealbatus* (Berk.) Corner has smaller fruit-bodies (up to 70 mm) than *S. cryptomerioides* (up to 110 mm) and it grew on humus under Lecythidaceae trees (Petersen, 1988). *S. dealbatus* was the only species in this genus with gelatinous basidiomta (Petersen, 1988). The basidiomata of *S. scaber* (Berk. & M.A. Curtis) D.A. Reid were up to 2.7 cm high and smaller than those of *S. cryptomerioides*. *Scytinopogon scaber* has smaller and more rounded spores and a papillose hymenium.

Heretofore, the number of species in *Scytinopogon* was doubtful. The proposed names included *Scytinopogon angulisporus*, *S. dealbatus*, *S. pallescens*, *S. echinosporus*, *S. robustus*, *S. chartaceum*, *S. papillosus*, and *S. scaber*, but several names have been considered synonyms. A holonomic morphological description and treatment is need.

The members of *Scytinopogon* are characterized by richly branched, light colored basidiomata with flattened branches, monomitic hyphal systems with clamped, non-inflated, colorless hyphae, tetrasporic basidia, and echinulate spores. These characteristics are similar to those of the clavarioid genus *Ramariopsis* (García-Sandoval et al., 2004), whereas the microscopic characteristics, particularly echinulate spores conform to the corticioid genus *Trechispora* (Jülich, 1981; Larsson, 1992). A phylogenetic analysis of *Ramariopsis*, *Clavaria*, *Clavulinopsis*, and *Scytinopogon* based on 30 morphological characters showed the closed relationship between *Scytinopogon* and *Ramariopsis* (García-Sandoval and Cifuentes, 2005). However, Birkebak et al. (2013) found that

Scytinopogon clustered within *Trechispora* by rDNS LSU sequence analysis but with poor bootstrap support. Our study confirms the close relationship between *Scytinopogon* and *Trechispora* by the sequences of rDNA LSU and ITS region and that *Scytinopogon* is phylogenetically distinct from Clavariaceae.

Acknowledgements

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Figure 4-1. Basidiomata of *Scytinopogon cryptomerioides* and their habitat.

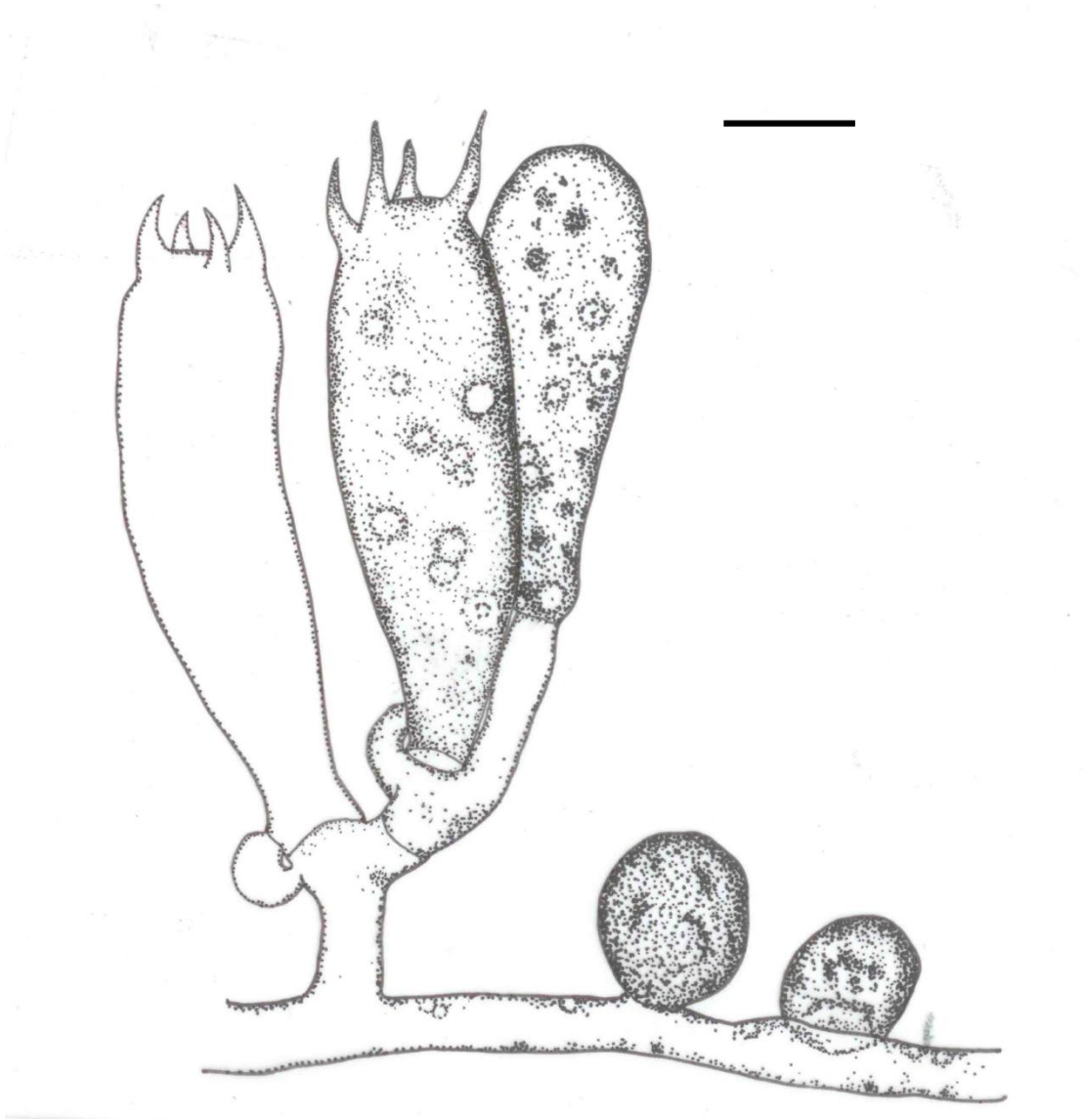

Figure 4-2 Basidia of *Scytinopogon cryptomerioides* (bar = 5μm).

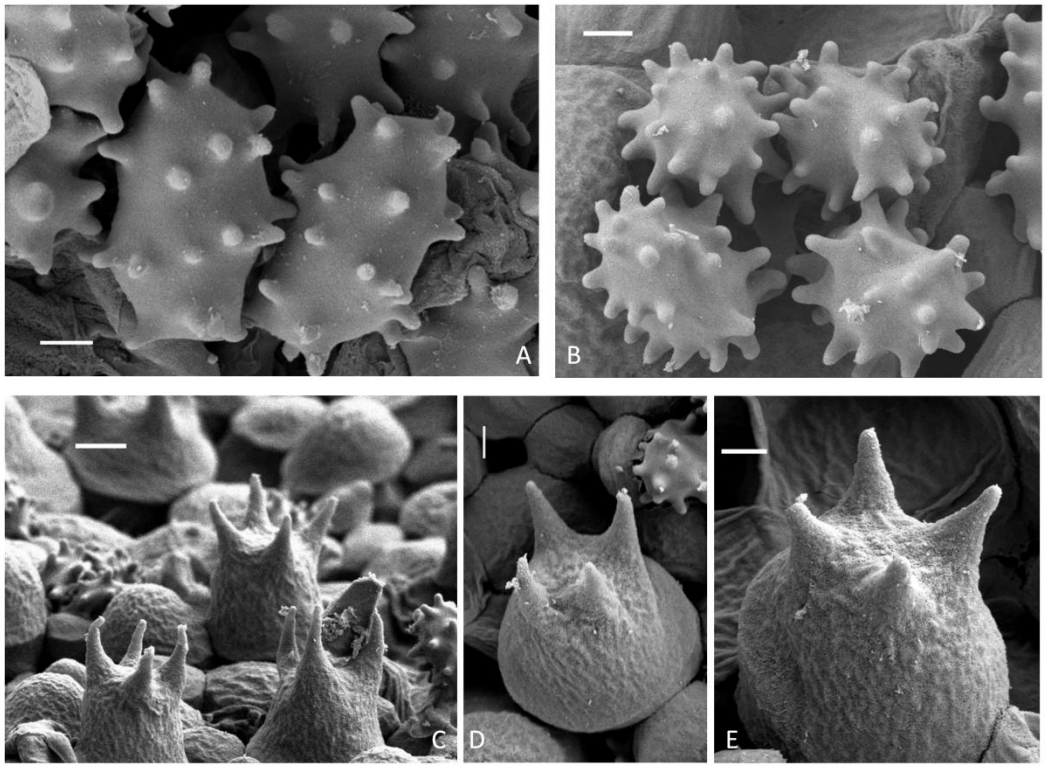

Figure 4-3 Scanning electron micrographs of *Scytinopogon cryptomerioides* (A) basidiospores (bar = 1μm); (B) basidiospores (bar = 2μm); (C) basidia (bar = 2 μm); (D) basidium (bar = 1 μm); (E) basidium (bar = 1 μm).

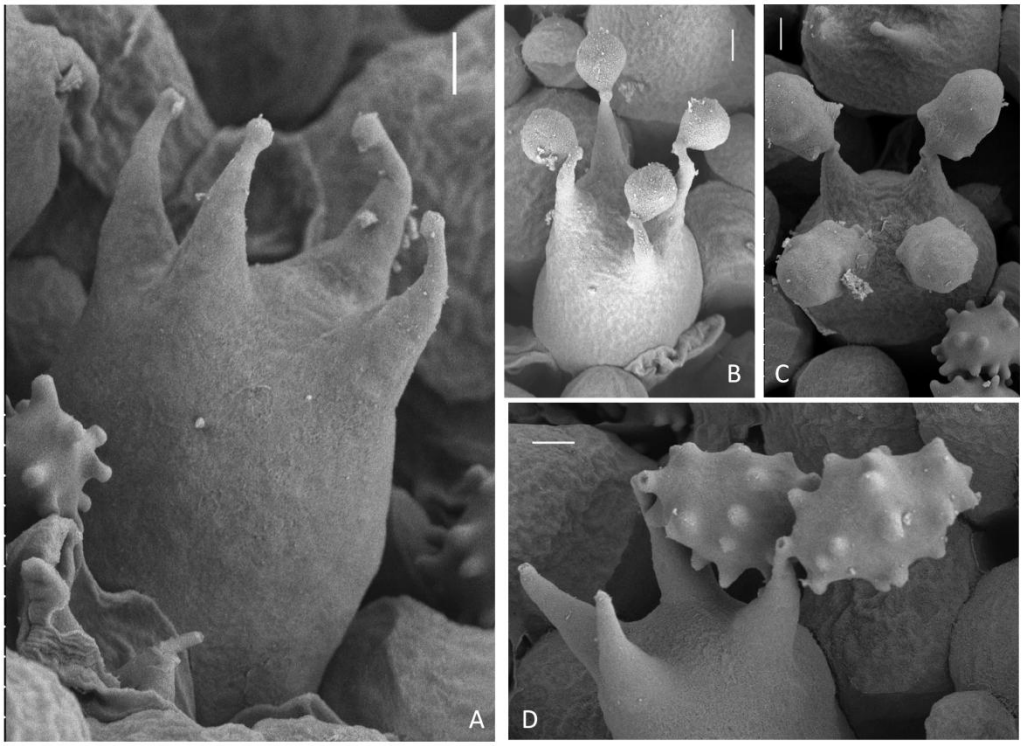

Figure 4-4 Development of basidiospores, (bar = 1 μm) (A) spherical enlargement of the sterigma apex; (B) spherical enlargement of the basidiospore; (C) formation of ornamentations; (D) depressions of the hilar appendices of spores.

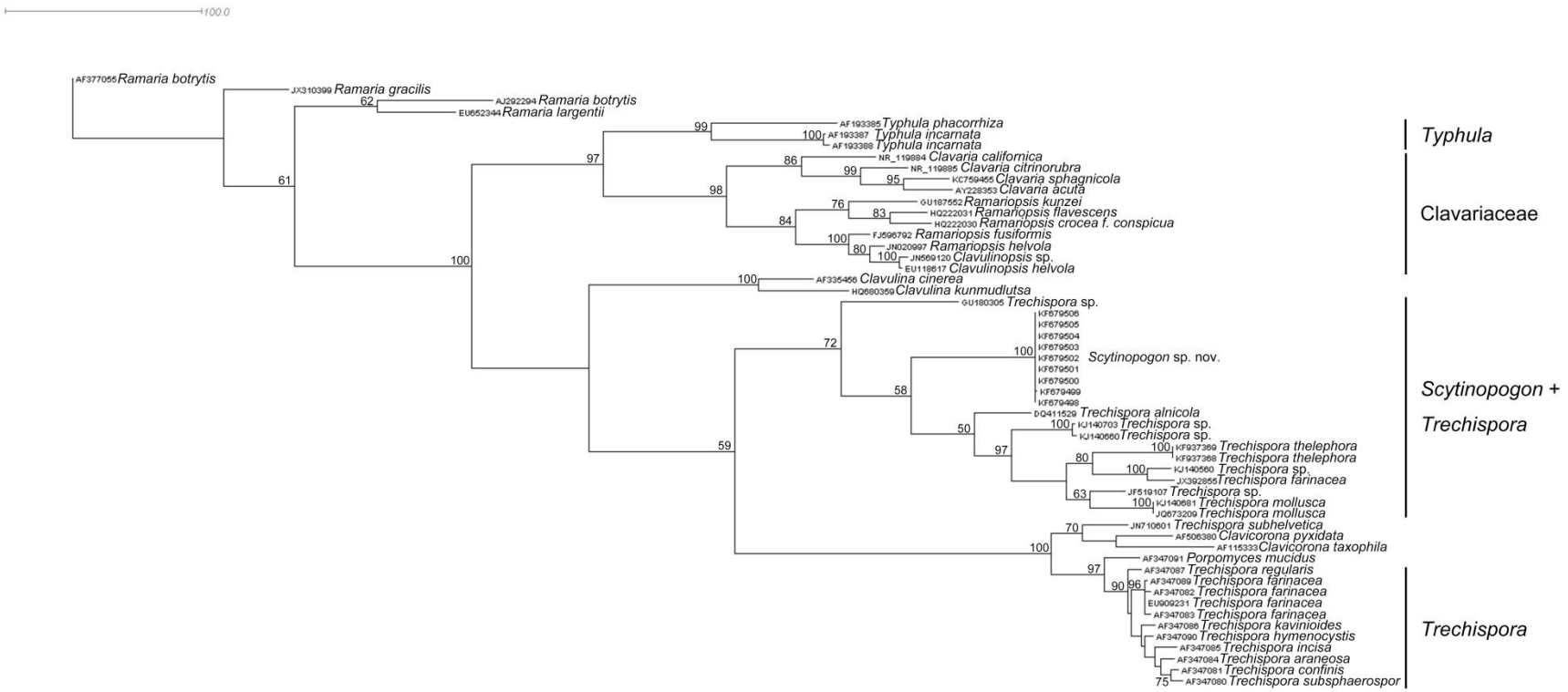

Figure 4-5 Maximum parsimony tree based on partial sequences of ITS gene of *Scytinopogon cryptomerioides* and allied species. Bootstrap values greater than 50% are shown above or below branches.

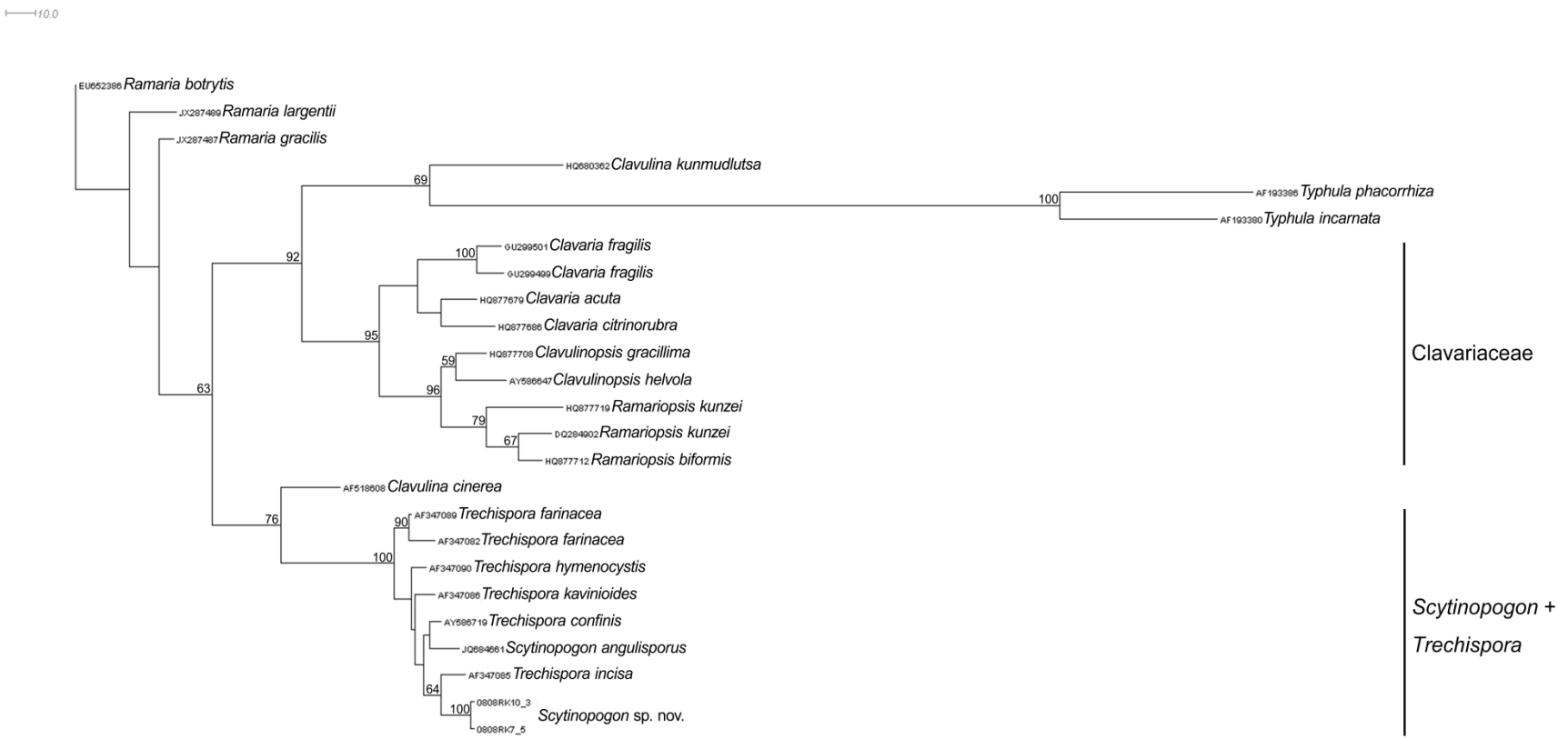

Figure 4-6 Maximum parsimony tree based on partial sequences of rLSU gene of *Scytinopogon cryptomerioides* and allied species. Bootstrap values greater than 50% are shown above or below branches.

Chapter 5 –Population genetic structure of a fluctuating population of a short lifespan and small genet macrofungus

Introduction

Fungi are major components for biodiversity and play important roles in ecological processes and the nutrient dynamics of terrestrial ecosystems (Delvasto et al., 2006; Levy-Booth and Winder, 2010). Conservation of fungi was aware in recent decades (Dahlberg et al., 2010; Moore et al., 2001; Van der Linde et al., 2012), especially after obvious decline in the abundance of many taxa caused by silviculture, nitrogen pollution and land use changes in Europe (Dahlberg et al., 2010; Fellner, 1993; Lizon, 1995; Pilz et al., 2006). Silviculture is an important disturbance to organisms in forest ecosystems (Bengtsson et al., 2000). Thinning altered the habitats and caused the decline or disappearance of macrofungal population (Egli et al., 2010; Lin et al., 2005; Ohenoja, 1988; Pilz et al., 2006).

In conservation status assessments, an important first step was to characterize patterns of genetic diversity within populations and genetic differentiation among populations (Allendorf and Luikart, 2007). Dispersal and colonization strategies affect fungal genetic diversity, contribute to population structures and are considered as important population biology. The colonization strategies of basidiomycetes have been inferred by depicting the size and distribution of fungal genets (Bendel et al., 2006; Dahlberg and Stenlid, 1994; Fiore-Donno and Martin, 2001; Kretzer et al., 2004). Based on Dahlberg and Stenlid's (1990) and Deacon and Fleming's (1992) concepts, fungal populations occurred as a few, large genets across an area, such as *Suillus bovinu*s (Dahlberg and Stenlid, 1994), and *Armillaria ostoyae* (Bendel et al., 2006). They were presumed to have longer life expectancies and invest

more resources to mycelium growth. Some fungal populations occurred as numerous genets of small spatial extent are presumed to have a short life span, frequently reproduce sexually and re-establish in new habitats by sexual spores (reviewed by Douhan et al., 2011), for example, *Laccaria amethystina* (Gherbi et al., 1999), *Cantharellus formosus* (Dunham et al., 2003) and *Tricholoma scalpturatum* (Carriconde et al., 2008).

Effective conservation for any biological organisms need a clear understanding of how the organism reproduces in nature and how the population copes with changed environments. In previous study, a new species, *Scytinopogon cryptomerioides* was reported and its fruiting bodies were impacted by thinning (Lin et al., 2015). Here we monitored the population dynamics of *S. cryptomerioides*, identified the genet of each sporocarps, and showed the temporal and spacial distribution of the genets. According to the data, the colonization strategy of *S. cryptomerioides* was inferred and the thinning influence on survival of *S. cryptomerioides* was evaluated. The results may be useful in making recommendations in conservation managements for macrofungi.

Materials and Methods

Study site and collection samples

The occurrence of *S. cryptomerioides* was recorded at Zen-Len area, Nantou County, in central Taiwan detailed in Lin et al. 2015. Briefly, this ecosystem is a 35-40-year-old *Cryptomeria japonica* plantation. Twelve 1-ha permanent plots (100 x 100 m) with northern and eastern aspects were established for the long-term monitoring of biodiversity dynamics (Lin et al. 2015). The twelve plots were randomly assigned a control, 25% or 50% thinning treatment. Thinning was performed by chainsaws in August of 2007. The population of *S. cryptomerioides* was monitored in

northerly six plots because its sporocarps were found only in plots with northerly aspects. There were six 10-m diameter circular subplots in each 1-ha plot for. Fruiting bodies of *S. cryptomerioides* in the subplots and on the transect line between the subplots were investigated, mapped and collected from 2006 to 2012 once every two months during the fruiting season (March to October).

DNA Extraction and Random Amplified Polymorphic DNA

Total DNA was extracted from tips of fresh sporocarps using a CTAB method (Dolye and Dolye 1990). Fungal tissues were crushed and homogenized using plastic pestle in 1.5 ml microtubes containing 500μL CTAB buffer and 5μL 1% 2-mercaptoethanol. The tubes were then incubated at 65℃ for 30 minutes. After centrifugation, an equal volume of dichloromethane/isoamylalcohol (24:1) was added for protein precipitation. After discarding protein, the DNA was precipitated with 0.6 vol of isopropanol, washed with 70% ethanol and 10mM ammonium acetate and resuspended in water. The purity and concentration of the DNA was determined by spectrophotometer.

RAPD reactions were carried out twice using primers 1-3 (5'-AGT CAGCCAC-3'), 1-7 (5'-GAAACGGGTG-3'), 1-13 (5'-CAGCACCCA C-3'), and B1F (5'-ACAAGCCAACGAGCATCACACA-3') that were selected for their ability to generate reproducible and polymorphic patterns on this sample set. RAPD amplification was performed using the following protocol. A 25 μl reaction mixture contained 0.2 μM of random primer (Biogene™, United state biological, USA), 2.0 units *Taq* DNA polymerase (Fermentas, USA), 100 μM of each of the four deoxynucleotides, in a PCR buffer, and 20 ng of fungal DNA. The amplification protocol consisted of one cycle of denaturation at 94 ℃ for 5 min, annealing at 40℃ for 30 sec, extension at 72℃ for 30 sec, 35

cycles of denaturation at 94℃ for 1 min, annealing at 40℃ for 30 sec, extension at 72℃ for 3 sec, and a final cycle of denaturation at 94 ℃ for 1 min, annealing at 58℃ for 20 sec, extension at 72℃ for 5 sec. The efficiency of amplification was monitored by running 5 μl of each reaction on a 1.5% agarose (Amresco, USA) gel at 100 V in Tris-borate-EDTA buffer. A 100-bp molecular weight ladder (Pharmacia, Freiburg, Germany) was used as the size standard. The gel was stained with ethidium bromide and visualised and photographed under ultraviolet light.

Data analysis

For RAPD analysis, each amplification reaction included a negative control and performed at least twice. Only distinct, clear-resolved and reproducible bands were recorded. To compare the various RAPD patterns, the presence/absence of each RAPD fragment was recorded by image processing software (Qantity One, Bio-Rad, USA). There was no differential weighting for band intensity or size. A binary matrix indicating the presence/absence of the DNA bands was analyzed. According to the RAPD patterns, the sporocarps were assigned as different genets.

RAPD products are mainly dominant markers. The assumption was made that the bands with the same sizes, which were present in the patterns generated by different sporocarps, represented products from equivalent loci. Each band was considered to be a locus with the dominant allele (Nybom and Bartish, 2000). The band was assumed to be monomorphic if it was detected in all the samples investigated. The percentage of polymorphic bands was calculated by dividing the number of polymorphic bands by the total number of bands surveyed. Shannon's index for genetic variation was analyzed by the GenAIEx 6.5 software

(Peakall and Smouse, 2012).

The analysis of molecular variance (AMOVA) was used to calculate the total genetic variation among the samples using the phi-statistic by the GenAIEx 6.5 software (Peakall and Smouse, 2006; 2012). Population differentiation was assessed from AMOVA using PhiPT index, the analogue of F_{ST} fixation index. Pairwise genetic distances between populations (Nei, 1973) were calculated using PopGene software. Based on the Nei's genetic distances, UPGMA (Unweighted Pair Group Method with Arithmetic Mean) was applied.

Results

Population fluctuations of *S. cryptomerioides*

Scytinopogon cryptomerioides fruited in 6 plots before thinning (2006, 2007). After thinning, the fungus fruited continuously in control plots (Table 1). From 2008 to 2012, the fruiting bodies decreased to 4%, 9%, 47%, 88% and 50% in 25% thinning plots compared with numbers of sporocarps in the same plot before thinning. In 50% thinning plots, no fruiting body was documented in 50% thinning plots during first and second year after thinning (2008 and 2009) (Table 1). From 2010 to 2012, the fruiting bodies recovered to 10%, 35% and 6% sporocarps compared with it before thinning (Table 1).

Genetic diversity

From 2006 to 2012, a total of 585 fruiting bodies were collected and including 67, 39, 244, 168, 67 fruting bodies from 2006, 2008, 2009, 2010 and 2012, respectively. Their RAPD patterns were analyzed. There were 73 polymorphic and 2 monomorphic scorable bands generated by four RAPD primers. The proportion of polymorphic bands from different years varied between 41.33% and 80.00%. Totally, the 97.33% bands

were polymorphic. A high level of polymorphism was observed. The Shannon's information index was 0.175 (Table 2). In addition, Shannon's information index ranged from 0.165 to 0.207 (Table 2). Shannon's information index was decreased from 2006, 2007 (before thinning) to 2012 (after thinning) (Table 2).

During 7-years' survey, RAPD characterization revealed a total of 473 distinct genets. Numbers of fruiting bodies per genet were low (1-6). Most genets were represented by a unique fruiting body and were not re-sampled across years.

Genetic differentiation among years

AMOVA analysis for *S. cryptomerioides* was used to assess the level of genetic variation among different sampling years by partitioning the total genetic variation into 2 components: among populations within regions, and among individuals within populations. The result of AMOVA showed that 20% of the total genetic variance (PhiRT) was found among populations from different years and 80% was obtained among individuals within population in the same year. The genetic differentiation (PhiPT) throughout the sampling years was 0.196 (lower than 0.25) that indicated a limited level of genetic differentiation among years. The higher pairwise population PhiPT values observed were between 2008/2012, 2009/2012, 2008/2012, and 2008/2010 populations, ranging from 0.269 to 0.367. Limited genetic differentiation was recorded when pairwise PhiPT was computed between 2 years (Table 3).

The temporal and spacial distributions of *S. cryptomerioides* genets

From 2006 to 2012, a total of 585 fruiting bodies were collected and their RAPD patterns were analyzed. According to the RAPD patterns, we identified 473 distinctive genets. Most genets were recorded once and

represented by a caespitose fruiting body, only few genets were found by several fruiting bodies (Table 2). Numbers of fruiting bodies per genet ranged from 1 to 9 and average is 1.24 (Table 2). Most genets were different among years, and 87.3% (413) genets were found only once. Sixty genets comprised more than one fruiting body or found more than once (Figure 1). Only six genets were found in two years (Figure 1a - 1e), they were Genet 179 (plot 10 in 2009 and 2012 (Figure 1b; Figure 1e)), Genet 204 (plot 7 in 2008 and plot 10 in 2009 (Figure 1b; Figure 1c)), Genet 210 (plot 6 in 2008 and plot 10 in 2009 (Figure 1b; Figure 1c)), Genet 243 (plot 6 in 2008 and plot 10 in 2009 (Figure 1b; Figure 1c)), Genet 8 (plot 6 in 2008, 2009 and plot 10 in 2009 (Figure 1b; Figure 1c)), and Genet 7 (plot 7 in 2009 and plot 6 and 7 in 2010 (Figure 1c; Figure 1e)). Seven genets found in different plots in the same year and 47 genets found in the same plots in the same year (Figure 1).

Cluster analysis

Figure 2 show the dendrogram of population genetic structure based on Nei's genetic distance. Twenty-three populations of *S.cryptomerioides* were divided into two groups based on investigated year. The populations from 2006 and 2009 were grouped together, respectively, as the first and second clusters. Another group consisted of populations from 2010 and 2012. The genetic structures from populations in the same year were more similar. The populations from the same plots but different years were distinct. The populations in plot 8 and plot 9 were not found in 2008 and 2009, and then re-found in 2010. The dendrogram showed that the re-found population in plot 8 and 9 in 2010 was more similar to other populations in 2010 genetically. That indicated that new genets most likely result from crossing among spores produced by fruiting bodies from adjacent area.

Discussion

RAPD patterns of *S. cryptomerioides* showed a high degree of polymorphism. The similar high levels of polymorphism were also found in *Pleurotus eryngii* (Ravash et al., 2010), *Lentinula edodes* (Xiao et al., 2010) and *Auricularia polytricha* (Du et al., 2011). These high levels of polymorphism were expected for a sexual and outcrossing species (James et al., 1999). Statistical analysis of the RAPD data showed that the variations among individuals within years were higher than the variations among populations from different years. The high levels of genetic variations result from different genets in the same year. The spores formed haploid colonies. Any two compatible haploid colonies recombined new genets. The high level of genetic variation is probably due to the compatibility of the colonies within populations.

The genet size of *S. cryptomerioides* was small. Its sporocarp genets differed among the years indicated that the colonies renewed yearly. They can't survive more than one year and they had short life span. The lack of large and persistent genets indicated that *S. cryptomerioides* invested less resource in mycelium extension and did not survive by underground colony. The data strongly suggested that sexual reproduction is the main contributor to the genetic structure of local population of *S. cryptomerioides*. *Scytinopogon cryptomerioides* was presumed to dispersal by basidiospores and established new colony each year according to Dahlberg and Stenlid (1990) and Deacon and Fleming (1992). Although some researches queried the assumption that small genets are short-lived because genets may not fruit each year (Selosse et al., 2001), or smaller genets often produce fewer fruiting bodies (Selosse et al., 2001). My results from long term investigation provided robust evidences.

Mycologists adopted Grime's C-S-R framework concepts (Grime, 1977) to classify fungi into functional types based on ecophysiology and reproductive biology (Andrews, 1992; Cooke and Rayner 1984). A fungus with ruderal strategy would reproduce primarily by basidiospores, resulting in numerous small genets at a given site. Therefore, my data suggested that *S. cryptomerioides* favored a ruderal ecological strategy. The species with ruderal strategy can cope with frequent disturbance but not high stress.

The most obvious result of this study was the population fluctuation among thinning intensity before and after treatment. Similarly, Pilz et al. (2006) observed a recovery of *Cantharellus formosa* yield during the sixth year after thinning, with low production during the first 3 years after thinning. However, they did not discuss the effects of environmental changes. The population fluctuation of *S. cryptomerioides* was negatively correlated with thinning intensity and light levels (Lin et al., 2015). Thinning lead to adverse environmental condition for *S. cryptomerioides*, such as lower humidity and higher light level and soil temperature (Lin et al., 2015). The adverse environmental conditions were stresses for *S. cryptomerioides*. *Scytinopogon cryptomerioides* which favored ruderal ecological strategy cannot cope with high stress. The adverse environmental conditions inhibit the colonization or fruiting of *S. cryptomerioides.* Therefore, the population declined immediately after thinning.

The recovery of population induced a new question whether genet recruitment originated from within population or from outside coming spores. According to the dendrogram, the recovered population was similar to the populations in the same year. For example, population in plot 9 in 2010 was more similar to other populations in 2010 genetically. That indicated that new genets most likely result from crossing among

spores produced by fruiting bodies from adjacent area. The weak level of genetic differentiation between years and the shorter genetic distance between populations in the same year also indicated that new genets most likely result from crossing among spores produced by fruiting bodies originally present in the population. Recovery of *S. cryptomerioides* population in thinning treatments was recruited by the basidiospores from adjacent plots.

A successful colonization is largely dependent on the species' ability to disperse and establish itself in a suitable habitat (Jönsson et al., 2008). The decline of subpopulations in thinning treatments might result from the unsuccessful dispersal or establishment in the disturbed habitats. However, the annual establishment of new colonies in the control treatments and the recovery of subpopulation in the thinning plots suggested that the dispersal in this area was not the limitation. Thus, we inferred that some spores were not able to establish a new colony successful in the thinning treatments because the adverse environmental conditions. The recovery of population relied on not only nearby viable spore banks but also improvement of environments. It took more than seven years to recover.

Conclusions

Scytinopogon cryptomerioides was found only in *C. japonica* forests in Taiwan. It is a small population. The changes of environments after thinning was stress for *S. cryptomerioides* and resulted in the decline of its population. The population of *S. cryptomerioides* started to recover at the third or fourth year after thinning. Sexual reproduction and recombination are common and important in *S. cryptomerioides* population. Maintaining a set of mature sporocarps and habitats is important for its subsequent reproduction and survival. To conserve rare

fungal species like *S. cryptomerioides*, minimum viable population and suitable habitats has to be protected during forest managements.

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Table 5-1. Numbers of sporocarps of *Scytinopogon cryptomerioides* from 2006 to 2012.

	0%			25%			50%		
	P6	P10	mean[a]	P7	P11	mean	P8	P9	mean
2006	886	282	584 (427)	984	0	492 (696)	272	281	277 (6)
2007	379	31	205 (246)	426	138	282 (204)	42	157	100 (81)
2008	307	609	458 (214)	34	0	17 (24)	0	0	0 (0)
2009	1,436	2,433	1,935 (705)	57	13	35 (31)	0	0	0 (0)
2010	561	97	329 (328)	337	29	183 (218)	30	10	20 (14)
2011	356	870	613 (363)	553	131	342 (298)	104	30	67 (52)
2012	145	206	176 (43)	220	166	193 (38)	20	4	12 (11)

[a] The values in parentheses brace denote the standard errors.

Table 5-2. Main characteristics of *Scytinopogon cryptomerioides* populations among years.

	No. of samples	Polymorphic bands No.	Percentage of polymorphic bands	No. of genets	Fruiting bodies / genet		Shannon's Information Index[a]
					Range	Mean	
2006	67	45	60.00%	61	1~3	1.10	0.206 (0.027)
2008	39	43	57.33%	35	1~3	1.11	0.207 (0.027)
2009	244	55	73.33%	194	1~9	1.26	0.174 (0.024)
2010	168	60	80.00%	128	1~8	1.31	0.165 (0.022)
2012	67	31	41.33%	61	1~2	1.10	0.121 (0.022)
Total	585	73	97.33%	473	1~6	1.24	0.175 (0.011)

[a] The values in parentheses brace denote the standard errors.

Table 5-3. Pairwise Population PhiPT calculated by RAPD data.

	2006	2008	2009	2010	2012
2006		0.001	0.001	0.001	0.001
2008	0.101		0.001	0.001	0.001
2009	0.115	0.075		0.001	0.001
2010	0.221	0.269	0.202		0.001
2012	0.276	0.367	0.278	0.111	

PhiPT Values below diagonal. Probability values based on 999 permutations is shown above diagonal.

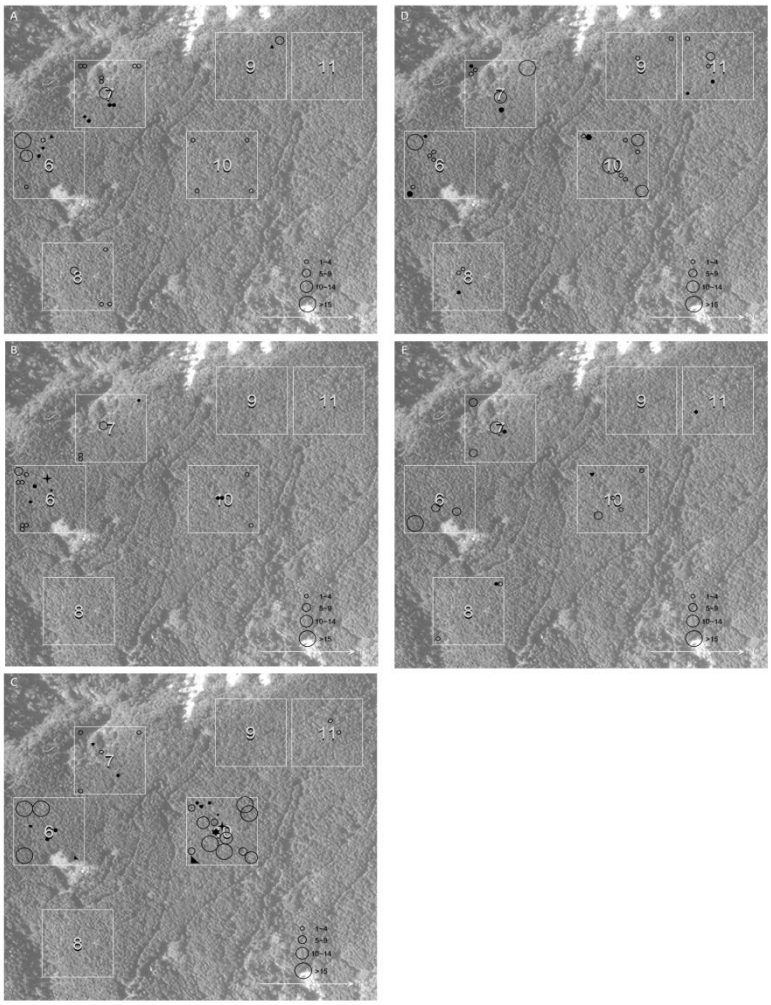

Figure 5-1. Spatial distribution of genets in 2006 (A), 2008 (B), 2009 (C), 2010(D), and 2012(E). Size of symbol means the population size. Open circles represented different genets. Closed circles represented the genets with more than one sporocarps in the same plots. Other closed symbols represented genets found more than once in different plots or different years.

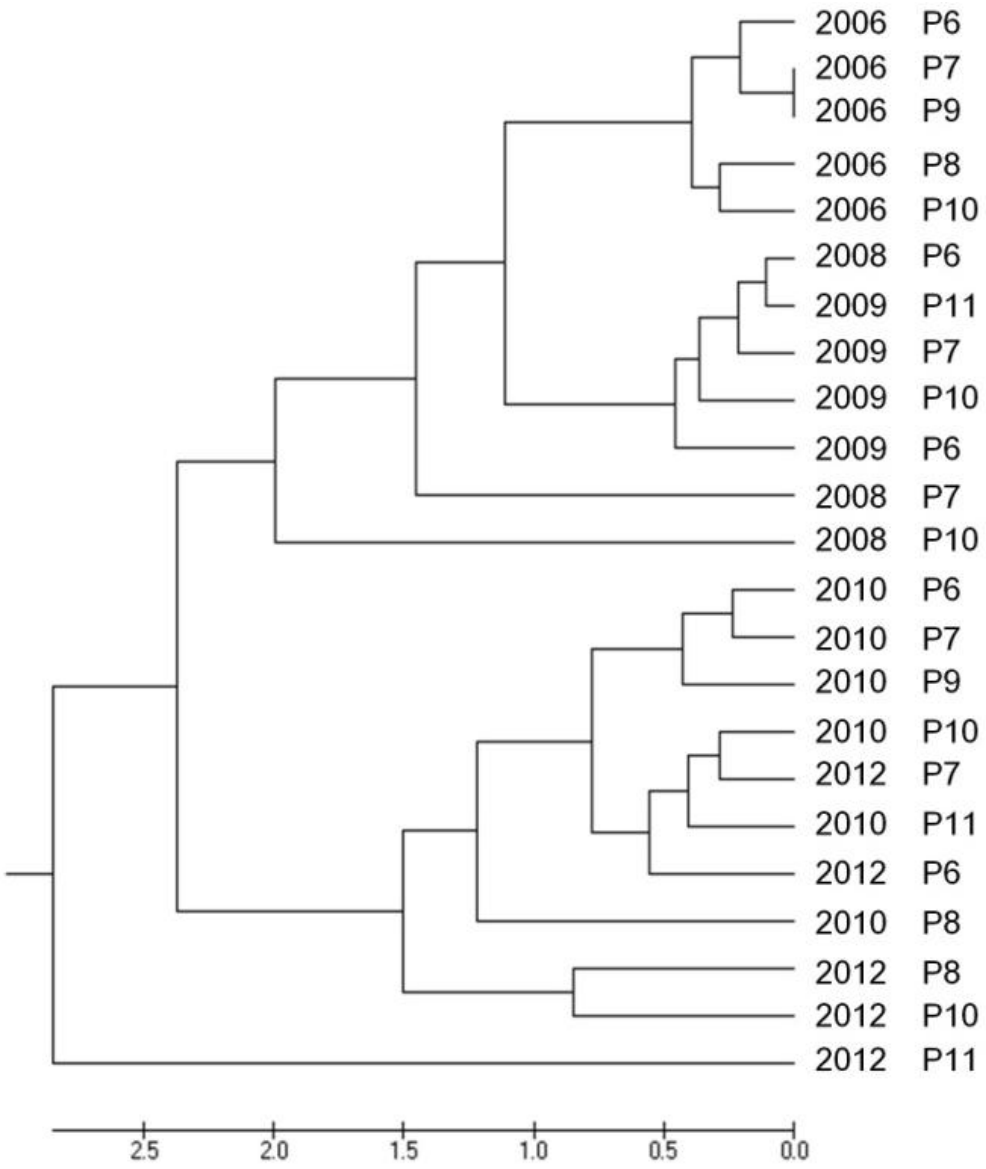

Figure 5-2. The UPGMA dendrogram of the 23 populations of *Scytinopogon cryptomerioides* based on Nei's genetic distance. The scale bar indicated the genetic distance.

Chapter 6 – Xylariaceous fungi were endophytic in coralloid basidiocarps?

Introduction

Fungicolous fungi indicated fungal species that regularly accompanied with other fungi (Jeffries, 1995). This term applied to any inter-fungal relationships, such as parasites, commensals or saprobionts (Kirk et al., 2008). The taxonomic spectrum of fungicolous fungi included almost all taxa of true fungi, such as Zygomycota, Ascomycota and Basidiomycota (Gam et al., 2004). Host range of the fungicolous fungi was broad, comprising representatives of all taxa of higher fungi (Gam et al., 2004) and different tissues including sporocarps, mycelia, sclerotia, and spores.

Fungal sporocarps were nutrient sources and niches for many microorganisms, such as filamentous fungi (Gams et al., 2004), yeasts (Yurkov et al., 2012) and bacteria (Barbieri et al., 2005). For example, *Tremella juniperina* is found on sporocarps of *Colpoma juniperi* (Jülich, 1983). Coral fungi are often colonized by *Mycogone calospora* (Gams, 1983) and *Helminthosphaeria clavariarum* (Samuels et al., 1997). *Trichoderma harzianum* colonized the sporocarps of *Agaricus bisporus* and have been responsible for their economic losses (Seaby, 1987). The fungi are not only present in decaying sporocarps but also associated with healthy fruiting bodies. The interaction between fungicolous fungi and their host can be recognized as neutralism, negative interactions and positive interactions (Cooke and Rayner, 1984).

Xylariaceous fungi play important roles in terrestrial ecosystems as saprophytes of plant debris or endophytes (Chen et al., 2013; Petrini and Petrini, 1985; Pinruan et al., 2007). The term “endophyte” was applied to that organisms lived inside healthy plant tissues and did not cause

obvious damages to the host (Wilson, 1995; Petrini and Petrini, 1985). Xylariaceous fungi were endophytes of the major plant groups, such as conifers, monocots, dicots, ferns, and lycopsida (Brunner and Petrini, 1992; Davis et al., 2003; Matsumura and Fukuda, 2013). Some xylariaceous fungi were associated with termites (Rogers et al., 2005; Ju and Hsieh, 2007). However, there is no report treated them as fungicolous or endophytic fungi of fungal sporocarps. In previous study, I found that *Scytinopogon cryptomerioides* distributed wildly in Japanese cedar plantations of central Taiwan and had a long fruiting season from April to October (Lin et al., 2015). In the context of ecological studies on *S. cryptomerioides*, I found that xylariaceous fungi were associated with the fruiting bodies of this fungus. Here I reported diversity of xylariaceous fungi and their distribution in the fruiting bodies of *S. cryptomerioides*. The ecology and interactions between the fungi were discussed.

Materials and Methods

Study site

The study was conducted at three separated area, two located in the central Taiwan, and one in north Taiwan. Zen-Len area ranges from 23°28’ N to 23°55’ N latitude and from 120°48’ E to 121°09’ E longitude. Elevation ranges from 1,300 to 1500 m. Average annual temperature and rainfall were 15.78℃ and 2,628 mm, respectively. Most rainfall at this site occurs during the summer, from June to September. Some rainfall also occurs during the spring, from March to May; there is no obvious dry season. The vegetation in this area was Japanese cedar, *Cryptomeria japonica* (L. f.) D. Don. Lienhuachih ranges from 500 to 900 m and flora here is a Lauro-Fagaceous forest (Su, 1984). The mean annual temperature and rainfall was 20.8℃.and 2285.0 mm with seasonality (Lu et al., 2008). More than half of the rain falls in between May and

September. Guanwu (121°07'E, 24°31'N) is a subtropical montane forest and ranges from 2000 to 2250 m. It was dominated by *C. japonica* with a few *Taiwania cryptomerioides*, *Chamaecyparis formosensis* and *Cunninghmia konishi*.

Isolation of xylariaceous fungi

Mature and healthy sporocarps of *Scytinopogon cryptomerioides* were collected from three study area. Sporocarps were cut into 1 cm pieces. The method of sterilization was modified from Guo et al. (2001). The sporocarp segments were surfaced sterilized with 1.05% Hydrogen peroxide for 1 minute and finally rinsed in sterile water for 45 seconds. The segments were dried with sterilized tissue paper and put onto potato dextrose agar. When hyphae grew out from the cross sections of the segments, the hyphal tip was isolated and purified on potato dextrose agar (difco) plate. The cultures were incubated at room temperature.

We also isolated xylariaceous fungi from some other substrates such as dead leaves, tree barks, plants and soil in the same niche. Isolation method was as described above.

DNA isolation and ITS amplification

Mycelia of xylariaceous fungi were harvested from the plates and stored at -20℃. Total DNA from mycelium was extracted by the CTAB method (Doyle and Doyle, 1990). The PCR amplification of the rDNA ITS region was undertaken using the universal primers ITS5 / ITS4 or primers ITS1 / ITS4 (White et al., 1990). PCR products were directly sequenced in ABI PRISM 3730 Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA).

Sequence Analysis

Sequence data for the ITS region were analyzed together with outgroup *Sordaria fimucola* (GenBank accession no. AY681188). Moreover, several species of *Xylaria*, 2 species of *Eutypella*, one species of *Hypoxylon* and *Nemania* were added in ITS analysis. These species from GenBank were analyzed with the isolates in this study (Table 1). Sequence data for the ITS region were initially aligned and subsequent manual adjustments made using BioEdit Sequence Alignment Editor (Hall, 2011) and Clustal X 1.83 (Thompson et al., 1997) then improved and adjusted visually. Using the same aligned datasets, parsimony analysis was performed with the default settings and parsimony bootstrap values were generated with 1,000 replicate heuristic searches to estimate support for clade stability of the consensus tree with 1,000 replicates in PAUP (Phylogenetic Analysis Using Parsimony) v4.0b10 (Swofford, 2003).

Specific primer design

Sequences of *Xylaria* spp. from GenBank were aligned with the same regions of xylariaceous fungal isolates and relative species and using Clustal X 1.83 (Thompson et al., 1997). Primers were designed from the variable regions of the ITS. To examine the specificity of the primers, Basic Local Alignment Search Tool (BLAST) from the National Center for Biotechnology Information was used to search for species with sequences homologous to the primers from GenBank. One oligonucleotide primer pairs, Xf1 (5’-GGGACATTCTGGGATGGGACA TCC-3’) and Xr2 (5’-ACACACAACACGGCCAGGGGAC-3’), targeting *Xylaria* spp. rDNA sequence were designed and these primers shared little or no homology with *S. cryptomerioides* rDNA sequences. Based on the sequence information, the primer pair was *Xylaria* genus-specific, and

the predicted amplification size of the *Xylaria* spp. product was about 400 - 500 bp. Primers were synthesized by Mission Biotech (Taipei, Taiwan).

Detection and isolation of *Xylaria* spp. from *Scytinopogon cryptomerioides* sporocarps

In order to know the distribution of *Xylaria*, 20 sporocarps of *S. cryptomerioides* were cut into 1-cm segments from top to bottom and were placed into 1.5-ml microcentrifuge tubes individually. Ten samples were analyzed by isolation and 10 for PCR detection. The isolation of *Xylaria* was as mentioned previously (modified from Guo et al., 2001). The presence of xylariaceous fungi was recorded. For PCR detection, DNA from specimens of 1-cm tissues was extracted by the CTAB method (Doyle and Doyle, 1990) and the PCR amplification with *Xylaria*-specific primer pair was used to detect the presence of *Xylaria*.

Results

Isolation

A total of 53 xylariaceous fungal isolates were isolated from fresh, healthy and intact sporocarps of *S. cryptomerioides* (Table 1). Xylariaceous fungi hyphae grew out from the cutting plan of the *Scytinopogon* sample on PDA plate in 24 – 48 hrs (Fig 1). The pure culture of these fungi formed white colony with radial hyphal strands and black pigments. After 2 to 4 weeks, stromata produced along rays. A total of 7 xylariaceous isolates belong to 5 *Xylaria* spp. were isolated from the plants near *Scytinopogon*'s sporocarps in plots. *Scytinopogon cryptomerioides* was non-culturable on 7 media (data not shown).

Diversity of xylariaceous fungi in sporocarps of *Scytinopogon cryptomerioides* and other substrates

The rDNA internal transcribed spacer (ITS) region sequences of about 600 bp were obtained from 53 xylariaceous fungal isolates. Using the BLAST function, the GenBank database was screened for ITS sequences of fungal taxa that closely matched ours. Twenty two reference sequences of 14 fungal species were obtained (Table 1).

According to ecological studies of endophytic fungi (Arnold et al., 2009; Okane et al., 2012), 90–95% ITS sequence similarity was often used as a species boundaries in fungi. In this study, 93% ITS sequence similarity was used to determine the species boundaries. The ITS-5.8S rDNA gene dataset contained 85 taxa with 754 characters. One hundred and ninety-two base pairs of ambiguous aligned regions were excluded from parsimony. The phylogenetic tree from the maximum parsimony analysis is shown in Fig 2. The newly isolated xylariaceous fungi were clustered into four clades. Clade A included 5 isolates of *Xylaria* sp. with high branch support (MP=100%). In Clade B, isolates ZLX7-3 and ZLX7-4 isolated from *S. cryptomerioides* sporocarps were clustered with *Nemania bipapillata* with strong bootstrap values (MP=100%) and ITS sequence similarity (93%). Clade C included two clusters; GWX-1 was identified as *H. monticulosum* with 97% ITS sequence similarity; isolates ZLX7-1 and ZLX7-2 had 95% ITS similarity to *Eutypella* sp., and they were different from reference sequence of *E. cerviculate*.

Clade D consisted of several species of *Xylaria* genus supported with strong bootstrap values, including *Xylaria* spp., *X. apoda*, *X. adscendens*, *X. bambuscola*, *X. curta*, *X.feejeensis*, *X. grammica*, *X. laevis*, *X. multiplex*, and *X.papulis*.

Two *X. apoda* isolates, 3 *X. bambusicola* isolates, 3 *X. bambusicola* isolates, 6 *X. grammica* isolates, 2 *X. papulis* isolates, 5 *X. multiplex*

isolates, 2 *X. feejeensis* isolates, 6 *X. laevis* isolates and 19 *X. curta* isolates were identified. Isolates ZLX10-1 was similar to *X. adscendens* with 85% ITS sequence similarity and grouped with *X. adscendens* with 77% branch support. This species was closed to *X. adscendens*.

According to the Blast results and phylogenetic analysis, 48 isolates of 14 species associated with *Scytinopogon* sporocarps were found in Zen-Len area, including *Eutypella* sp., *Nemania* sp., *N. bipapillata*, *X. adscendens*, *X. apoda*, *X. bambusicola*, *X. curta*, *X. feejeensis*, *X. grammica*, *X. laevis*, *X. multiplex*, *Xylaria papules*, and 2 *Xylaria* sp. (Table 1). Isolates of *Hypoxylon monticulosum* and *X. multiplex*, were found in Guanwu area; 3 isolates of *X. laevis* in Lienhuachih area (Table 2). In the same area, *Xylaria curta*, *X. feejeensis*, *X. papulis* and *X. grammica* were isolated from leaves of *Elatostema lineolatum*, and *X. laevis* was isolated from leaves of *Diplazium dilatatum* (Table 1). All species of *Xylaria* spp. were isolated from plants were found in the sporocarps of *S. cryptomerioides*. *Xylaria* spp. were not able to be isolated from 102 root, 42 bark and 42 dead leave samples of *C. japonica* and 45 soil samples.

Distribution of *Xylaria* spp. in *Scytinopogon cryptomerioides* sporocarps

The distribution of *Xylaria* in the sporocarps of *S. cryptomerioides* was investigated by isolation and PCR detection from the sporocarp segments. *Xylaria* was isolated in 70% sporocarps (N = 20). Both the isolation rates and detection rates in the lower part of the sporocarps were higher than in the upper part (Table 3).

Discussion

In this paper, we comfirmed that xylariaceous fungi were fungicolous

fungi associated with *S. cryptomerioides* and they grew in the tissues of basidiocarps and followed its growth. Most fungicolous fungi were reported on other fungi (Gams et al., 2004). For example, conidia of *Acremonium strictum* were observed on the stipe, pileus and gills of *Psilocybe fasciata* (He et al., 2006). Only two reports described observations of basidiomycetes hyphae in the ascocarp of truffles (Ceruti, 1988; Pacioni et al., 2007) and one reports isolated *Cryptococcus victoriae* from inside tissue of *Paxillus* (Yurkov et al., 2012).

It is worth to mention that no obvious damage or symptom were observed on the *Xylaria*-inhabiting *Scytinopogon* basidiocarps. These *Xylaria* spp. were not parasites. Other possible relationships between fungicolous fungi and host were competition, commensalism, or mutualism. *Xylaria* involved two competition cases. Species of *Pseudoxylaria* associated closely with *Termitomyces* species and termite nests; the growth of *Pseudoxylaria* was inhibited by *Termitomyces* species (Visser et al., 2011). *Coprinus comatus*, a commercialized edible mushroom, was suppressed by *Xylaria coprinicola* (Cui et al., 2008; Ju et al., 2011). In our case, xylariaceous fungi grew in the healthy sporocarps and its mycelium extended from the base to the top of sporocarps. *Scytinopogon cryptomerioides* grew in the soil. They were not competed for the same niche or resource. Xylariaceous fungi were not inhibited by *S. cryptomerioides*, and vice versa. Therefore, we rejected the hypothesis that they competed.

Another possibility was commensalism. In our study, xylariaceous fungi grew in the sporocarps of *S. cryptomerioides*. Xylariaceous fungi took advantage from *S. cryptomerioides*. Commensalism between xylariaceous fungi and *S. cryptomerioides* was confirmed. The situation that *Xylaria* species grew in the sporocarps was similar to the *Xylaria* endophytes which colonized plant tissues without apparent symptom or

disease. *Xylaria* kept dysgonic growth in the *S. cryptomerioides* sporocarps and did not cause the apparent symptom or disease. The sporocarps without *Xylaria* were not impacted in appearance as well. In this context, *Xylaria* inhabited in sporocarps to occupy the niches and take the preemptive opportunities to use host resource. The strategy was similar to endophytes which play a neutral role and lived as sit-and-wait saprotrophs spend a long time to occupy niches in the plant tissue and then become a saprotrophs when host dies (Herre et al., 2007; Van Bael et al., 2005).

Truffle-hosted fungi and *Tuber borchii* grew well and closely when co-culture and these truffle-hosted fungi might exert a positive or protective role in the sporocarps (Pacioni et al., 2007). *Scytinopogon cryptomerioides* was non-culturable and must have special nutrient demand. *Xylaria* spp. might play a particular role in the colonization, nutrition, growth and sporocarp-development of *S. cryptomerioides*. However, the benefits from xylariaceous fungi were not clear. Mutualism between *S. cryptomerioides* and xylariaceous fungi was need more evidence.

In this study, there were 15 species of Xylariales associated with *S. cryptomerioides* sporocarps. *Eutypella* genus belonged to Diatrypaceae; *Xylaria*, *Hypoxylon*, and *Nemania* genus belonged to Xylariaceae. *Xylaria* spp. were common endophytes and saprophytes which inhabited in conifers, monocots, dicots, and ferns (Brunner and Petrini, 1992). *Xylaria* endophytes have broad host range. *Xylaria feejeensis*, *X. laevis, X. curta, X. apoda*, *X. multiplex, X.papulis, X. grammica*, and *X. bambuscola* were endophytes associated with orchid, Annonaceae, Arecaceae, Asteraceae, Celastraceae, Myrsinaceae, Palmae, Pteridaceae, Rhamnaceae, Rubiaceae, and teak (Chen et al., 2013; Jiang et al., 2011; Okane et al., 2012; Govinda Rajulu et al., 2013). *Nemania bipapillata* was endophyte

associated with *Dendrobium* (Chen et al., 2013), Asteraceae (Okane et al., 2012) and Rubiaceae (Govinda Rajulu et al., 2013). *Xylaria curta*, *X. laevis*, *X. feejeensis*, *X. papulis* and *X. grammica* were isolated from fern and monocot in habitats of *S. cryptomerioides*. These *Xylaria* spp. may originate from these plants and establish the association with the sporocarps. Interestingly, these *Xylaria* species were not associated with termite nests. The interaction between them and the establishment mechanism of their commensalism were worth for further study.

Xylariaceous fungi grew up from the basal parts of *S. cryptomerioides* sporocarps. They were nondetectable in the apical segments (1-cm) of the tips of 585 sporocarps (data not shown). Very few tips of the sporocarps were colonized by xylariaceous fungi, which indicates that *S. cryptomerioides* sporocarps grew faster than their inhabiting xylariaceous species. Xylariaceous fungi established the commensalism relationship with *S. cryptomerioides* was a specially association. No xylariaceous species was isolated from other 33 coralloid Basidiomycetes (data not shown).

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Table 6-1. Fungal isolates recovered from sporocarps of *Scytinopogon cryptomerioides* in Taiwan. GenBank Accession number of the rDNA ITS sequence of isolates used in this study and the query coverage and max identity with their most closely related fungal ITS sequences in GenBank.

Isolates in this study	GenBank sequences			
	Blast result	Accession no.	Query cover	Max Identity
¶ZLX7-1	*Eutypella sp.*	JN637945	98%	99%
ZLX7-2	*Eutypella sp.*	FJ172283	98%	100%
GWX-1	*Hypoxylon monticulosum*	KJ774048	100%	99%
ZLX7-3	*Nemania bipapillata*	GU292818	99%	99%
ZLX7-4	*Nemania bipapillata*	GU292818	94%	97%
ZLX7-5	*Nemania* sp.	JX624281	94%	99%
ZLX10-1	*Xylaria adscendens*	GU322432	99%	93%
ZLX8-1	*Xylaria apoda*	GU322437	98%	99%
ZLX9-1	*Xylaria apoda*	GU322437	97%	99%
ZLX8-2	*Xylaria bambusicola*	JX256820	99%	96%
ZLX11-1	*Xylaria bambusicola*	EF026123	99%	97%
ZLX12-1	*Xylaria bambusicola*	EF026123	100%	96%
ZLX6-1	*Xylaria curta*	GU322444	99%	97%
ZLX6-2	*Xylaria curta*	GU322444	97%	99%
ZLX6-3	*Xylaria curta*	GU322444	97%	97%
ZLX6-4	*Xylaria curta*	GU322444	98%	99%
ZLX6-5	*Xylaria curta*	GU322444	98%	99%
ZLX7-6	*Xylaria curta*	JX256823	99%	97%
ZLX7-7	*Xylaria curta*	JX256823	99%	97%
ZLX7-8	*Xylaria curta*	JX256823	99%	97%
ZLX7-9	*Xylaria curta*	GU322444	100%	97%
ZLX7-10	*Xylaria curta*	GU322444	97%	97%
ZLX7-11	*Xylaria curta*	GU322444	97%	97%
ZLX7-12	*Xylaria curta*	GU322444	97%	97%
ZLX7-13	*Xylaria curta*	GU322444	99%	99%
ZLX9-2	*Xylaria curta*	GU322444	98%	98%
ZLX10-2	*Xylaria curta*	GU322444	98%	97%
ZLX10-3	*Xylaria curta*	GU322444	97%	97%
ZLX11-2	*Xylaria curta*	GU322444	97%	97%
ZLEX-1	*Xylaria curta*	GU322445	100%	99%
ZLEX-2	*Xylaria curta*	GU322444	100%	99%
ZLX10-4	*Xylaria feejeensis*	GU322453	97%	99%
ZLEX-3	*Xylaria feejeensis*	GU322454	100%	100%
ZLX10-5	*Xylaria grammica*	GU300097	100%	100%

ZLX10-6	*Xylaria grammica*	GU300097	100%	100%
ZLX10-7	*Xylaria grammica*	GU300097	100%	97%
ZLX7-14	*Xylaria grammica*	GU300097	99%	100%
ZLX7-15	*Xylaria grammica*	GU300097	95%	99%
ZLEX-4	*Xylaria grammica*	GU300097	100%	99%
LHCX-1	*Xylaria laevis*	GU324747	99%	99%
LHCX-2	*Xylaria laevis*	GU324747	96%	99%
LHCX-3	*Xylaria laevis*	GU324747	100%	99%
ZLX7-16	*Xylaria laevis*	GU324747	98%	99%
ZLDX-1	*Xylaria laevis*	GU324747	100%	99%
ZLDX-2	*Xylaria laevis*	GU324747	100%	99%
GWX-2	*Xylaria multiplex*	GU300099	99%	95%
ZLX6-6	*Xylaria multiplex*	GU300099	99%	95%
ZLX9-3	*Xylaria multiplex*	GU300099	98%	95%
ZLX9-4	*Xylaria multiplex*	GU300099	98%	95%
ZLX7-17	*Xylaria papulis*	GU300100	95%	99%
ZLEX-5	*Xylaria papulis*	GU300100	100%	99%
ZLX10-8	*Xylaria* sp.	HQ435666	98%	97%
ZLX10-9	*Xylaria* sp.	JX436805	98%	98%
ZLX10-10	*Xylaria* sp.	JX436805	99%	98%
ZLX6-7	*Xylaria* sp.	KM066560	94%	97%
ZLX7-18	*Xylaria* sp.	KM066560	93%	99%
ZLX7-19	*Xylaria* sp.	KM066560	95%	99%
ZLX7-20	*Xylaria* sp.	KM066560	95%	99%
ZLX8-3	*Xylaria* sp.	JX436805	100%	99%
ZLX8-4	*Xylaria* sp.	KC507251	99%	99%

[¶]ZLX indicated that the isolate was isolated from sporocarp of *Scytinopogon* in Zen-Len area; GWX indicated that the isolate was isolated from sporocarp of *Scytinopogon* in Guanwu; LHCX indicated that the isolate was isolated from sporocarp of *Scytinopogon* in Lienhuachih; ZLEX indicated that the isolate was isolated from leaves of *E. lineolatum* in Zen-Len area; ZLDX indicated that the isolate was isolated from leaves of *D. dilatatum* in Zen-Len area.

Table 6-2. Spatial distribution of xylariaceous fungi associated with *Scytinopogon cryptomerioides* sporocarps.

species/isolates	Zen-Len (n = 44)	Guanwu (n = 3)	Lienhuachih (n = 4)	Total
Eutypella sp.	1/2			1/2
Hypoxylon monticulosum		1/1		1/1
Nemania bipapillata	1/2			1/2
Nemania sp.	1/1			1/1
Xylaria adscendens	1/1			1/1
Xylaria apoda	1/2			1/2
Xylaria bambusicola	1/3			1/3
Xylaria curta	1/17			1/17
Xylaria feejeensis	1/1			1/1
Xylaria grammica	1/5			1/5
Xylaria laevis	1/1		1/3	1/4
Xylaria multiplex	1/4	1/1		1/5
Xylaria papulis	1/1			1/1
Xylaria sp.	2/8			2/8
Total	14/48	2/2	1/3	15/53

Table 6-3. *Xylaria* spp. were cultured/detected by PCR with its genus-specific primers from *Scytinopogon cryptomerioides* sporocarp samples. Xylariaceous fungi inhabited from the bases to tops of the sporocarps, but not in the tips.

Segment	Presence of xylariaceous fungi																			
	samples for Culture Isolation										samples for DNA detection									
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
g			-		-	-	-	-	-				-		-	-	-	-	-	
f		-	-	-	-	+	+	-	+	+		-	-	-	-	-	-	-	+	+
e	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	+	+	-	-	+
d	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	+	+	+	-
c	-	-	-	-	-	+	+	-	+	+	-	-	-	-	-	+	+	-	+	+
b	-	-	-	-	+	+	+	-	+	+	-	-	-	-	+	+	+	-	+	+
a	-	-	-	+	nd	nd	nd	+	+	+	-	-	-	+	+	+	+	+	+	+

+: positive result; -: negative result; nd: did not detect

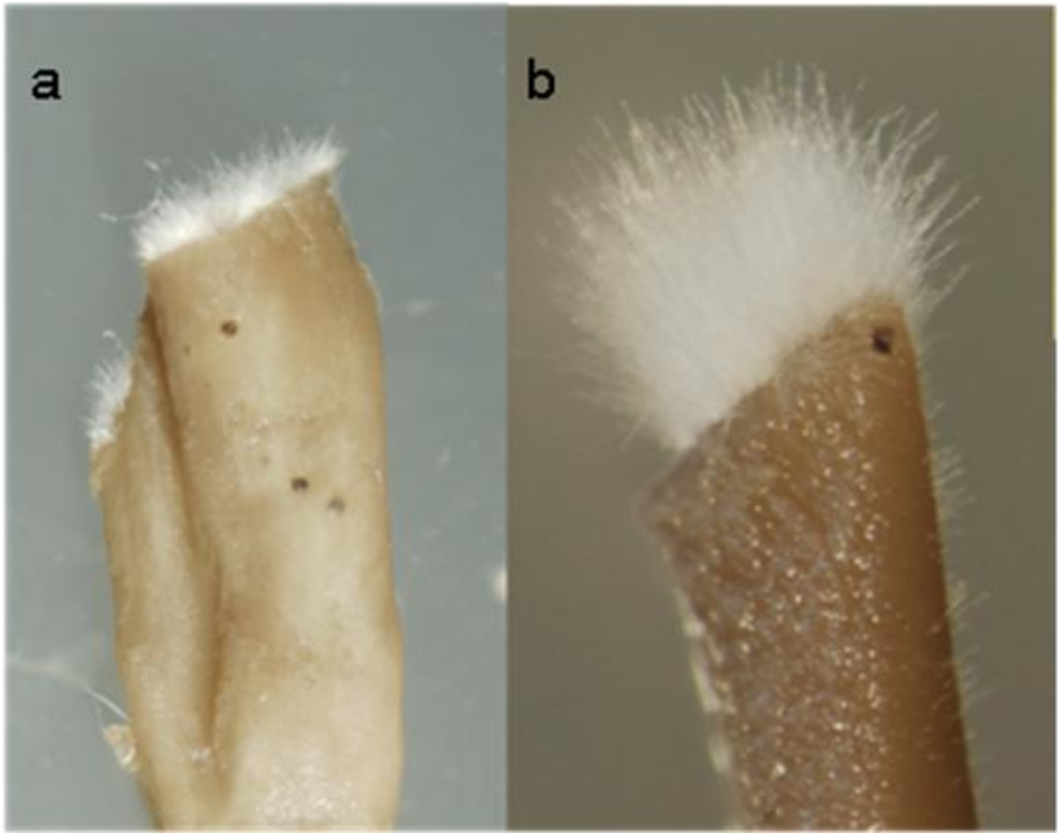

Figure 6-1. *Xylaria* hyphae grew out from the cross-section of *Scytinopogon cryptomerioides* sporocarps in (a) 24 – 48 hrs after isolation and 3 – 4 days after isolation.

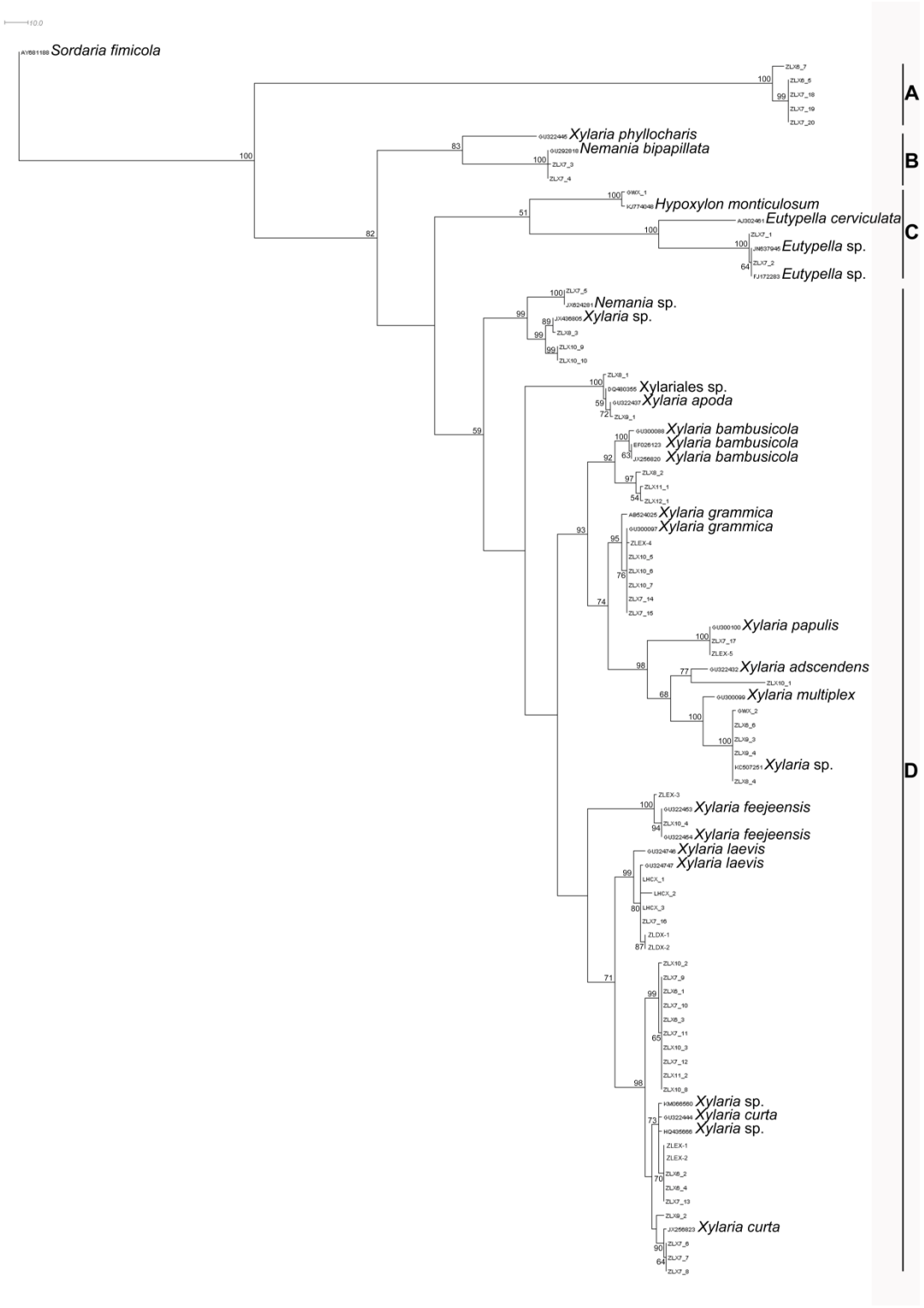

Figure 6-2. The tree generated from maximum parsimony analysis based on ITS-5.8S rDNA gene dataset. Bootstrap values greater than 50% are shown above or below branches.

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