Effects of forest thinning on diversity and function of macrofungi and soil microbes

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Forest management activities influence forest structure and development, and thereby influence biodiversity. We investigated diversity and function of macrofungi and soil microbes in a *Chamaecyparis formosensis* forest with light and heavy thinning treatments and control plots, and we compared them with an adjacent broadleaf forest. Principal component analysis was used to separate macrofungal species components exposed to thinning treatments and those in the broadleaf forest. The macrofungal diversity in the broadleaf forest was higher than in the plantations, and the diversity of fungi from the lightly thinned plantation was higher than those from the heavily thinned and unthinned plantations. Denaturing gradient gel electrophoresis profiles showed that soil fungal and bacterial communities were significantly different among the treatments. However, the carbon utilisation patterns of soil microbes did not differ significantly among treatments. Thinning increased the diversity of saprotrophic macrofungi and changed the soil microbial community of the plantation. This finding might be due to the functional redundancy of soil microbes, as there was no significant thinning effect on the soil microbial function through the fourth year after thinning.

Keywords: fungal diversity, forest thinning, *Chamaecyparis formosensis*, Taiwan

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).

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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 & 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 tropical forest.

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 °C 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 & 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 °C 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 & 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 & 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 °C for 5 min, 35 cycles of 94 °C for 30 s, 55 °C for 1 min, and 72 °C for 1 min and a final step of 72 °C 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 °C 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 °C 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 & Warwick 2001). The degree of similarity between the macrofungi associated with different plots was calculated by principle component analysis (PCA) using PRIMER (Clark & 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 & 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 (Tab. 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 (Tab. 1).

Tab. 1. Macrofungal species and fruitbody counts and biodiversity indices; macrofungal fruitbody and of species counts of different functional groups found in the different treatment plots.

		Unthinned	Lightly thinned	Heavily thinned	Broadleaf
Diversity	Species counts	26	39	32	93
	Fruitbody counts	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[a]	Ectomycorrhizal	0/0	0/0	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 fruitbody counts/species counts.

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. & 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 (Tab. 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 (Tab. 1). Ectomycorrhizal and parasitic macrofungi were found in the heavily thinned plot and in the broadleaf forest (Tab. 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 (Fig. 1). PC1 explained the 62.2% variability and separated the macrofungal communities of the broadleaf forest from those of the plantations (Fig. 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 (Fig. 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 & 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.

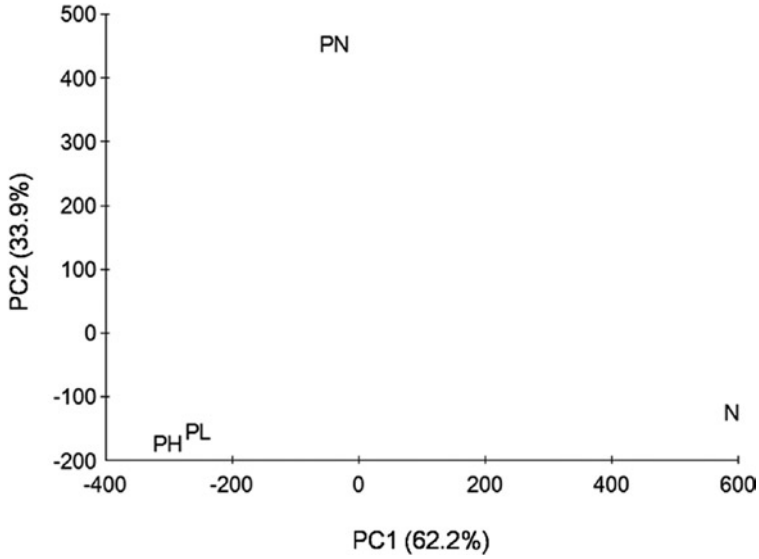

Fig. 1. The Principal Components Analysis of macrofungal species components was done for samples from the natural broadleaf forest (N) and unthinned (PN), light thinned (PL), and heavy thinned (PH) plantation forests.

MDS plot showed that the DGGE profile of soil fungi from each treatment clustered together (Fig. 2a). The ANOSIM tests (Tab. 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 (Fig. 2b; Tab. 2). These results indicated that the soil fungal and bacterial communities were different among treatments.

Tab. 2. 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	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

* Significant differences: $p < 0.05$; n.d.: no data

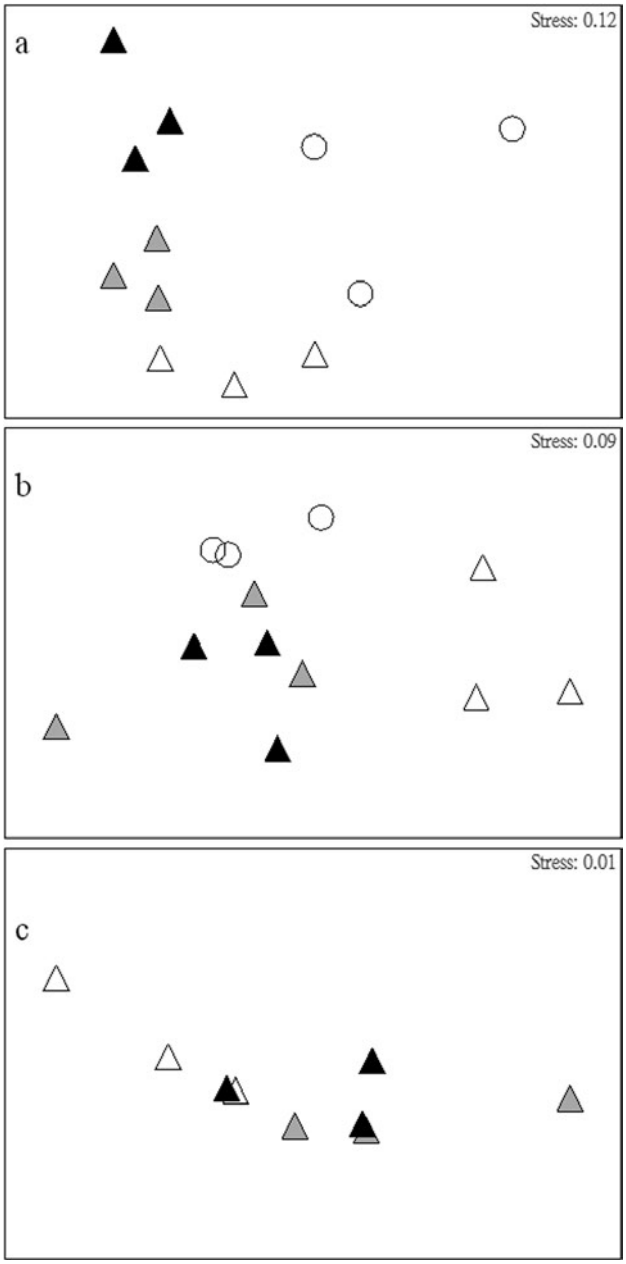

Fig. 2. MDS ordination of soil microbial communities and functions from the broadleaf forest and the unthinned, lightly thinned, and heavily thinned plantation forests. **a.** soil fungal communities form DGGE profiles; **b.** soil bacterial communities form DGGE profiles; **c.** soil functions from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

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 (Fig. 2c). The ANOSIM tests (Tab. 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 & 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 & 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 & 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 & Martiny 2008).

Forest thinning and forest type affected the diversity and community of the macrofungi and soil microbes. Thinning increased the diversity of saprotrophic macrofuni 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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