EFFECTS OF RAINFALL EXCLOSURE ON THE ECTOMYCORRHIZAL FUNGI OF A CANOPY TREE IN A TROPICAL RAINFOREST

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YAMASHITA S, HIROSE D & NAKASHIZUKA T. 2012. Effects of rainfall exclosure on the ectomycorrhizal fungi of a canopy tree in a tropical rainforest. We expected ectomycorrhizal (ECM) fungal community structure on mature tree to be affected by drought. A rainfall exclosure treatment was conducted to study changes in the ECM community of a single tree at Lambir Hills National Park, Malaysia. A vinyl sheet of conical shape covered the entire rooting area of a *Dryobalanops aromatica* from 22 November 2008 until 7 December 2008. Three root samples each were collected from both the tree that was treated with rainfall exclosure sheet and a control tree nearby before and after the treatment. ECM samples were identified using molecular technique. The ECM species abundance (mean number of species per sample) on the rainfall exclosure tree decreased from 8.0 ± 1.0 to 4.7 ± 0.6, whereas species abundance on the control tree was similar. Non-metric multidimensional scaling (NMDS) showed that the species composition of ectomycorrhizas differed between samples collected before and after treatment. Changes in species composition before and after treatment were caused by difference in tolerance to reduction in rainfall among fungal species and by spatiotemporal variation in species composition of ectomycorrhizas.

Keywords: 28S rDNA D1/D2 region, Dipterocarpaceae, field experiment, Lambir Hills National Park, non-metric multidimensional scaling, Borneo

YAMASHITA S, HIROSE D & NAKASHIZUKA T. 2012. Kesan halangan hujan terhadap kulat ektomikoriza pokok kanopi di dalam hutan hujan tropika. Kami menjangka yang struktur komuniti kulat ektomikoriza (ECM) pada pokok matang dipengaruhi oleh kemarau. Perubahan komuniti ECM sebatang pokok di Taman Negara Bukit Lambir, Malaysia dikaji dengan mengasaskan eksperimen halangan hujan. Sekeping vinil berbentuk kon menutup seluruh kawasan pengakaran sebatang pokok *Dryobalanops aromatica* dari 22 November 2008 hingga 7 Disember 2008. Tiga sampel akar masing-masing diambil daripada pokok yang ditutup dengan kepingan vinil dan pokok kawalan yang tumbuh berhampiran, sebelum dan selepas rawatan. Sampel ECM dikenal pasti menggunakan teknik molekul. Kelimpahan spesies ECM (purata bilangan spesies setiap sampel) pada pokok yang menerima halangan hujan berkurang daripada 8.0 ± 1.0 menjadi 4.7 ± 0.6 manakala kelimpahan spesies pokok kawalan adalah sama. Penskalaan pelbagai dimensi bukan metrik (NMDS) menunjukkan yang komposisi spesies ECM berbeza antara sampel yang diambil sebelum dan selepas rawatan. Perubahan komposisi spesies sebelum dan selepas rawatan disebabkan oleh perbezaan toleransi spesies kulat terhadap pengurangan air hujan dan juga variasi ruang dan masa bagi komposisi spesies ECM.

INTRODUCTION

Ectomycorrhizal (ECM) fungi provide water and nutrients to host plants in exchange for photosynthates (Smith & Read 2008). This mutualistic relationship often occurs in dominant trees in a forest ecosystem, such as members of the Pinaceae, Betulaceae, Fagaceae and Dipterocarpaceae (Smith & Read 2008). Dipterocarpaceae is one of the most dominant families in South-East Asia (SEA) in terms of number of individuals and basal area (Roubik et al. 2005). Its seedlings have around 60 species of ECM symbionts (Alexander & Lee 2005).

In SEA, drought can trigger general flowering (Sakai et al. 2006). Interactions between trees and ECM fungi might affect the tree physiology related to this general flowering, as suggested in

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Korup National Park, south-western Cameroon, Africa (Newbery et al. 2006).

Previous experiments on the effects of drought on ECM community structure on seedlings have shown that drought changes the species composition (Shi et al. 2002, Cavender-Bares et al. 2009). We, thus, expected that ECM species composition and species abundance would be affected by drought stress. We conducted a rainfall exclosure treatment with a mature tree of a dominant canopy species, *Dryobalanops aromatica* (Dipterocarpaceae), and observed the ECM fungal communities before and after the rainfall exclosure.

MATERIALS AND METHODS

The study site was located at the Lambir Hills National Park, Sarawak, Malaysia (4°12' N, 114° 01' E, 150 to 250 m asl). The park covers an area of 6949 ha and its vegetation is classified as lowland mixed dipterocarp forest. Within this forest, more than 1100 tree species have been found in 52 ha of the forest (Roubik et al. 2005).

We chose two adult trees of *D. aromatica*, which formed the canopy layer at the study site, growing 20 m apart on level ground. This species is most abundant in both number of individuals (3% of the total) and basal area (6.9% of the total) in the 52 ha plot (Roubik et al. 2005). The diameter at breast height (dbh) of the tree subjected to drought treatment was 107 cm and that of the control was 95 cm. To exclude rain from the treated tree, we hung a vinyl sheet on a wooden frame from the centre of the tree to 15 m out to cover the entire rooting area of the tree. The sheet was suspended more than 0.5 m above the forest floor and formed a conical shape. The sheet excluded rainfall entirely from 22 November 2008 until 7 December 2008. Daily change in precipitation from 21 October till 10 December 2008 is shown in Figure 1 (T Kumagai, unpublished).

We followed randomly selected main roots from each tree until we found a branching coarse root. We then traced the branched fine roots (0–1 mm in diameter) that led back to the main root through the coarse root by gently removing the soil and litter layer if needed. We collected three 30 cm lengths of coarse roots with fine roots from each tree between 10 and 15 November and used these to determine the ECM community before the drought treatment (Figure 1). We collected another three lengths from each tree between 3 and 7 December 2008 and used these to determine the effects of drought treatment. On each sampling date, we chose sampling points 2 to 5 m from the trunk. The effect of spatial heterogeneity was not properly controlled.

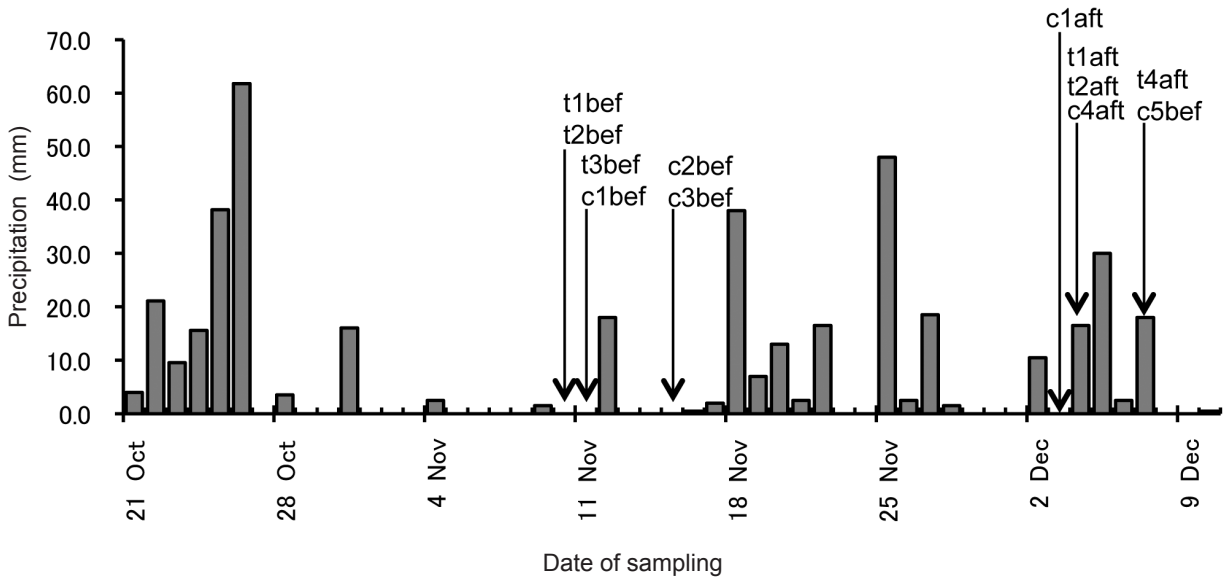

Figure 1 Daily changes in precipitation during the study period; rainfall exclosure started from 22 November 2008; t = treated tree; c = control tree; numbers following t and c represent root number, different numbers indicate samples taken from different main roots; bef = before drought treatment, aft = after drought treatment

Roots were stored in a refrigerator at around 7 °C with their original soil for no more than three days. The roots were washed in a sieve (mesh size 0.045 mm) under running water to remove the soil. After cleaning, 10 fine roots measuring 5 cm sections were removed from each coarse root. Under a dissecting microscope, all root tips with ECM were morphologically sorted out from each fine root. Ectomycorrhizal root tips were collected and sorted by the structure and colour of their external hyphae and fungal mantle according to Ingleby et al. (1990). Root tips of each morphological type were counted as index of abundance of ECM species. A total of 10 fine roots from each coarse root were pooled as a single sample. The total number of root tips of each morphotype from the 10 fine roots of one coarse root was used as index of abundance of the morphotype.

One root tip was randomly chosen from each ECM morphological type and used for DNA analysis. DNA was extracted using the modified CTAB method described by Matsuda and Hijii (1999). Sequences of the 28S rDNA D1/D2 region were obtained using the LR0R/LR3 (Vilgalys & Hester 1990) or the ITS 1f/LR3 primer pair (Gardes & Bruns 1993). This region was chosen owing to its high conservativeness, which helps taxonomic placement. The polymerase chain reaction (PCR) was performed using a HotStarTaq Plus Master Mix. Each PCR tube contained a 50 µL mixture comprising 16 µL distilled water, 25 µL HotStarTaq Plus Master Mix (Qiagen), 3 µL template DNA, 5 µL CoralLoad Concentrate (Qiagen) and 0.5 µL each primer (final concentration 0.25 µM). Each DNA fragment was amplified in a PCR thermal cycler under the following thermal cycling conditions: 5 min at 94 °C, 35 cycles of 30 s at 94 °C, 30 s at 58 °C, 1 min at 72 °C and a final 10 min at 72 °C. The reaction mixture was then cooled for 5 min at 4 °C.

The PCR products were purified with a PCR purification kit. The purified products were sequenced by Macrogen Japan Inc in a PTC-225 Peltier thermal cycler using ABI PRISM bigdye terminator cycle sequencing kits with AmpliTaq DNA polymerase. The fluorescence-labelled fragments were purified from the unincorporated terminators using an ethanol precipitation protocol. The samples were then resuspended in formamide and electrophoresised in an ABI 3730xl sequencer. The sequences were deposited in the DNA Database of Japan (DDBJ accession numbers AB546550 to AB546597, http://www.ddbj.nig.ac.jp/index-e.html). The sequences were compared with those of known species using the Basic Local Alignment Search Tool (BLAST) and the closest fungal taxon for each isolate was identified.

Species abundance (mean number of ECM species per sample) was calculated for each tree before and after rainfall exclosure. Species abundance was compared between the treated tree and control as well as between before and after treatment by means of two-way ANOVA.

Non-metric multidimensional scaling (NMDS) was conducted to compare species composition among sample units. NMDS does not assume a linear relationship between variables and is suited to data with numerous zero values (McCune & Grace 2002). The Bray–Curtis coefficient was used as distance measure. PC-ORD software (McCune & Mefford 2006) was used in slow and thorough autopilot mode (maximum number of iterations = 500). The best dimension to represent the data was identified by calculating the average stress scores for 250 runs of real data and compared with randomised data. This recommended a two-dimensional solution with final stress value of 16.2 (Monte Carlo randomisation test, $p < 0.012$). This implies that our result was usable (McCune & Grace 2002). We included ECM species on ≥10 root tips in this analysis with log (number of root tips + 1) transformation.

RESULTS

A total of 98 to 504 root tips per sample were collected (Table 1). Average number of root tips per sample was 269.2 ± 126.3 (mean ± SD). From a total of 3230 root tips, 46 ECM morphotypes were recorded: 36 from drought-treated tree and 29 from control tree (results not shown). DNA analysis of the 46 morphotypes revealed a total of 22 species from the Ascomycota, Basidiomycota and Zygomycota (Tables 2 and 3). Of the nine Ascomycota found on the drought-treated tree before treatment, only two were present after treatment (Table 2). On the other hand, the species composition in the Russulales was almost similar between trees on each sampling occasion, although the species composition changed before and after treatment.

The number of fungal species differed significantly between trees ($p = 0.046$) and

between dates (p = 0.046). There was significant (p = 0.046) interaction between sampling date and tree (Figure 2; for the whole model, df = 11, F = 5.55, p = 0.023). The average number of ECM species on the treated tree decreased from 8.0 ± 1.0 to 4.7 ± 0.6 (mean ± SD) after the treatment (Figure 2). On the other hand, the average of control tree did not change before and after treatment.

In the NMDS diagram, axis 1 explained 35.9% of the total variance of species data while axis 2, 37.4% (Figure 3). The species composition clearly differed between samples collected before and after treatment. For example, *Tomentella* sp. 1 was plotted around t1bef, and Boletaceae sp. 1 around t2bef, c2bef and c3bef. *Russula* sp. 1 was plotted around t4aft, c4aft and c5aft. Tricholomataceae sp. 1 was plotted around t1aft and t2aft. The degree of difference in ECM species composition was larger in samples after treatment compared with those before treatment.

DISCUSSION

In this study, the species abundance decreased after the rainfall exclosure. In a temperate beech forest, drought intensity was negatively correlated with the number of ECM morphotypes, suggesting that some fungal species had superior drought tolerance (Buée et al. 2005). In a subarctic region, drought treatment significantly influenced colonisation by one of three ECM species (Nilsen et al. 1998). In our study, some ECM species such as Rhytismatales sp. 1, Pezizomycotina sp. 2 and Boletaceae sp. 1 might be sensitive to rainfall exclosure because they were observed only in samples obtained before treatment. The tolerance to drought is likely to differ between fungal species and such differences can explain the drought-related decrease in the species abundance on the treated tree because fungi with low tolerance to drought may go extinct locally and the species abundance will decrease.

Some studies have revealed the effect of drought on ECM species composition (Izzo et al. 2005, Cavender-Bares et al. 2009). However, we could not clearly show this effect because we observed similar ECM species compositions on both trees after rainfall exclosure. This suggested that the same factors affected both trees during the treatment. One possible explanation is the existence of a common mycorrhizal network (Selosse et al. 2006) that connects the trees. Such a network may carry water to a tree under drought stress from other trees with better access to water (Egerton-Warburton et al. 2007). Since we did not dig a trench to separate the two trees and sever any mycelial connection below the ground, it was possible that the control tree supplied water to the treated tree or that drought affected the control tree. In addition, it could be possible that the control tree was affected by the treatment itself because the two trees were close to each other. However, we did not observe the main root of the control tree extend to the

Table 1 Numbers of root tips with ectomycorrhizas collected from each root sample

Sample code	No. of root tips
t1bef	135
t2bef	400
t3bef	504
t1aft	281
t2aft	129
t4aft	311
c1bef	98
c2bef	232
c3bef	244
c1aft	421
c4aft	298
c5aft	177

t = treated tree, c = control tree; numbers following t and c represent root numbers, different numbers indicate samples taken from different main roots; bef = before drought treatment, aft = after drought treatment

Table 2 Mean number (± standard deviation) of root tips with ectomycorrhizas of each species on the fine roots of the trees

Taxon	Abbreviation	Treatment		Control	
		Before	After	Before	After
Ascomycota					
Chaetothyriales					
Chaetothyriales sp. 1	Chae 1	14.0 ± 24.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Eurotiales					
Eurotiales sp. 1	Euro 1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.3 ± 5.8
Trichocomaceae sp. 1	Tric 1	5.7 ± 6.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Helotiales					
Helotiales sp. 1		0.0 ± 0.0	2.0 ± 3.5	0.0 ± 0.0	0.3 ± 0.6
Sordariales					
Sordariomycetidae sp. 1	Sord 1	8.6 ± 12.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Sordariomycetidae sp. 2	Sord 2	54.0 ± 93.5	30.0 ± 46.0	0.0 ± 0.0	0.0 ± 0.0
Rhytismatales					
Rhytismatales sp.1	Rhyt1	33.7 ± 31.7	0.0 ± 0.0	60.3 ± 65.6	0.0 ± 0.0
Rhytismatales sp.2	Rhyt 2	17.0 ± 16.1	2.0 ± 3.5	2.7 ± 2.1	69 ± 62.6
Xylariales					
Xylariaceae sp. 1		0.3 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Others					
Pezizomycotina sp. 1		1.7 ± 2.9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Pezizomycotina sp. 2	Pezi 2	14.7 ± 24.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Basidiomycota					
Agaricales					
Tricholomataceae					
Tricholomataceae sp. 1	Tril 1	0.0 ± 0.0	6.3 ± 8.5	0.0 ± 0.0	0.0 ± 0.0
Boletales					
Boletaceae					
Boletaceae sp. 1	Bole 1	80.7 ± 139.7	0.0 ± 0.0	59.7 ± 52.0	0.0 ± 0.0
Polyporales					
Polyporales sp. 1		1.7 ± 2.9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Hymenochaetales					
Hymenochaetaceae					
Coltriciella sp. 1	Colt 1	0.0 ± 0.0	0.0 ± 0.0	11.3 ± 19.6	8.0 ± 13.6
Russulales					
Russulaceae					
Russula sp. 1	Russ 1	0.0 ± 0.0	94 ± 67.1	0.0 ± 0.0	202.7 ± 78.5
Russula sp. 2	Russ 2	2.7 ± 2.5	0.0 ± 0.0	10. 7 ± 12.2	0.0 ± 0.0
Russula sp. 3	Russ 3	0.0 ± 0.0	1.7 ± 2.9	0.0 ± 0.0	1 ± 1.7
Russula sp. 4	Russ 4	106.7 ± 112.5	101.3 ± 58.8	35.3 ± 58.6	10 ± 11.5
Russula sp. 5	Russ 5	0.0 ± 0.0	3.0 ± 5 .2	0.0 ± 0.0	0.0 ± 0.0
Thelephorales					
Thelephoraceae					
Tomentella sp. 1	Tome 1	5.0 ± 6.1	0.0 ± 0.0	11.3 ± 19.6	0.0 ± 0.0
Zygomycota					
Endogonales					
Endogonaceae					
Endogone sp. 1	Endo 1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	4.3 ± 7.5
Total		1039	721	574	896

Table 3 List of ectomycorrhizal fungi and BLAST match information

Taxon	Sample name	DDBJ accession no.	Bp	BLAST match taxon	Accession number	Score (expected value)	Query coverage %	Sequence similarity %
Boletaceae sp. 1	LAN21	AB546550	601	*Alpova diplophloeus*	DQ989497	850 (0.0)	97	93
Boletaceae sp. 1	LAN134	AB546559	598	*Alpova diplophloeus*	DQ989497	856 (0.0)	97	93
Chaetothyriales sp. 1	LAN192	AB546589	555	*Capronia semiimmersa*	FJ358226	959 (0.0)	100	97
Coltriciella sp. 1	LAN-31	AB546552	620	*Coltriciella dependens*	AM412254	966 (0.0)	100	94
Coltriciella sp. 1	LAN211	AB546560	621	*Coltriciella dependens*	AM412254	968 (0.0)	100	94
Endogone sp. 1	LAN-72	AB982123	656	*Endogone lactiflua*	DQ273788	542 (1e-150)	83	84
Eurotiales sp. 1	LAN741	AB546593	598	*Thermoascus aurantiacus*	EU021617	697 (0.0)	100	88
Helotiales sp. 1	LAN992	AB546596	586	*Coleophoma empetri*	FJ588252	889 (0.0)	100	94
Rhytismatales sp.1	LAN183	AB546588	586	*Rhytismataceae* sp.	HM595596	966 (0.0)	100	96
Rhytismatales sp.2	LAN-17	AB546578	574	*Hypoderma vincetoxic*	HM123406	992 (0.0)	94	99
Rhytismatales sp.2	LAN1113-1	AB546597	585	*Hypoderma vincetoxic*	HM123406	990 (0.0)	92	99
Rhytismatales sp.2	LAN133-1	AB546585	583	*Hypoderma vincetoxic*	HM123406	987 (0.0)	92	99
Rhytismatales sp.2	LAN181	AB546587	582	*Hypoderma vincetoxic*	HM123406	963 (0.0)	92	99
Rhytismatales sp.2	LAN262	AB546590	584	*Hypoderma vincetoxic*	HM123406	989 (0.0)	92	99
Rhytismatales sp.2	LAN321	AB546591	583	*Hypoderma vincetoxic*	HM123406	987 (0.0)	92	99
Rhytismatales sp.2	LAN742	AB546594	574	*Hypoderma vincetoxic*	HM123406	992 (0.0)	94	99
Rhytismatales sp.2	LAN743	AB546595	584	*Hypoderma vincetoxic*	HM123406	989 (0.0)	92	99
Pezizomycotina sp. 1	LAN31	AB546579	564	*Pseudeurotium zonatum*	AF096198	542 (1e-150)	93	85
Pezizomycotina sp. 2	LAN102	AB546584	573	*Phacidiopycnis* sp.	HM595597	580 (2e-162)	98	85
Polyporales sp. 1	LAN34	AB546556	607	*Perenniporia detrita*	FJ393866	861 (0.0)	99	92
Russula sp. 1	LAN711	AB546563	627	*Russula densifolia*	AF325304	981 (0.0)	96	95

(continued)

Bp = base pairs of 28SrDNA D1-D2 region used to BLAST search

Table 3 (continued)

Taxon	Sample name	DDBJ accession no.	Bp	BLAST match taxon	Accession no.	Score (expected value)	Query coverage %	Sequence similarity %
Russula sp. 1	LAN712	AB546564	622	*Russula ingwa*	EU019919	1053 (0.0)	98	97
Russula sp. 1	LAN713	AB546565	622	*Russula ingwa*	EU019919	1053 (0.0)	98	97
Russula sp. 1	LAN733	AB546566	628	*Russula densifolia*	AF325304	983 (0.0)	96	95
Russula sp. 1	LAN745	AB546567	629	*Russula densifolia*	AF325304	979 (0.0)	96	95
Russula sp. 1	LAN752	AB546568	617	*Russula densifolia*	AF325304	989 (0.0)	98	95
Russula sp. 1	LAN764	AB546569	617	*Russula densifolia*	AF325304	976 (0.0)	98	95
Russula sp. 1	LAN765	AB546570	627	*Russula ingwa*	EU019919	1053 (0.0)	97	97
Russula sp. 1	LAN792	AB546571	630	*Russula densifolia*	AF325304	981 (0.0)	96	95
Russula sp. 1	LAN793-1	AB546572	200	*Russula densifolia*	FJ845430	346 (9e-92)	100	98
Russula sp. 2	LAN593-1	AB546561	628	*Russula nauseosa*	AF506462	963 (0.0)	100	94
Russula sp. 3	LAN-12	AB546551	624	*Russula favrei*	EF530944	994 (0.0)	100	95
Russula sp. 4	LAN12	AB546553	622	*Russula* sp.	EU598161	996 (0.0)	100	95
Russula sp. 4	LAN121-1	AB546557	627	*Russula* sp.	EU598161	1003 (0.0)	100	95
Russula sp. 4	LAN813	AB546573	626	*Russula* sp.	EU598161	1009 (0.0)	100	95
Russula sp. 4	LAN832	AB546574	627	*Russula* sp.	EU598161	998 (0.0)	100	95
Russula sp. 5	LAN922	AB546576	631	*Russula foetens*	AF325299	974 (0.0)	96	95
Sordariomycetidae sp. 1	LAN101	AB546583	576	*Calosphaeria barbirostris*	EF577059	791 (0.0)	100	91
Sordariomycetidae sp. 1	LAN35	AB546581	572	*Calosphaeria barbirostris*	EF577059	795 (0.0)	100	91
Sordariomycetidae sp. 1	LAN36	AB546582	564	*Calosphaeria barbirostris*	EF577059	780 (0.0)	100	91
Sordariomycetidae sp. 2	LAN342	AB546592	574	*Calosphaeria barbirostris*	EF577059	784 (0.0)	100	91
Tomentella sp. 1	LAN23	AB546555	604	*Tomentella botryoides*	AY586717	1066 (0.0)	100	98
Trichocomaceae sp. 1	LAN22	AB546554	606	*Crinipellis* sp.	AB512366	1038 (0.0)	93	99
Tricholomataceae sp. 1	LAN892	AB546575	617	*Hydropus* sp.	AF261369	928 (0.0)	97	94
Tricholomataceae sp. 1	LAN953	AB546577	620	*Hydropus* sp.	AF261369	933 (0.0)	97	94
Xylariaceae sp. 1	LAN33	AB546580	565	*Xylaria* sp.	AB512403	1038 (0.0)	100	99

Bp = base pairs of 28SrDNA D1-D2 region used to BLAST search

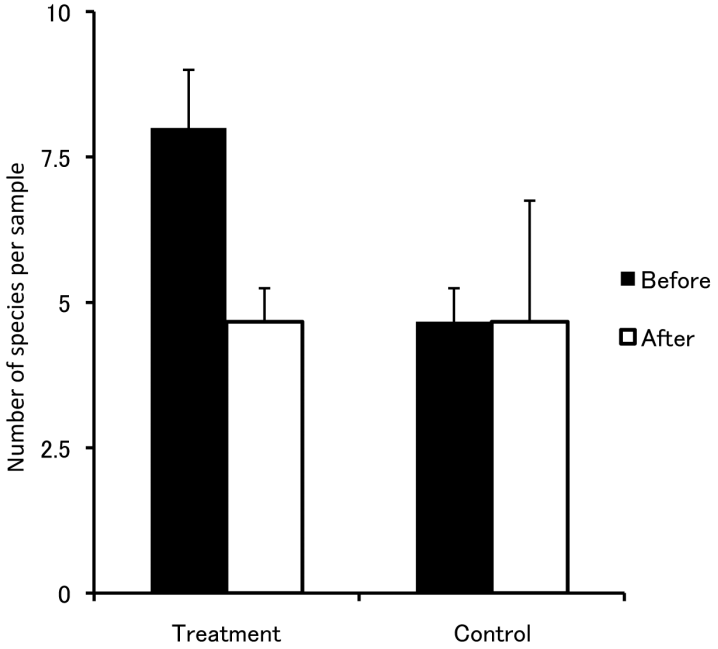

Figure 2 Numbers of ectomycorrhizal fungal species on each tree before and after drought treatment; error bars indicate standard deviation (n = 3)

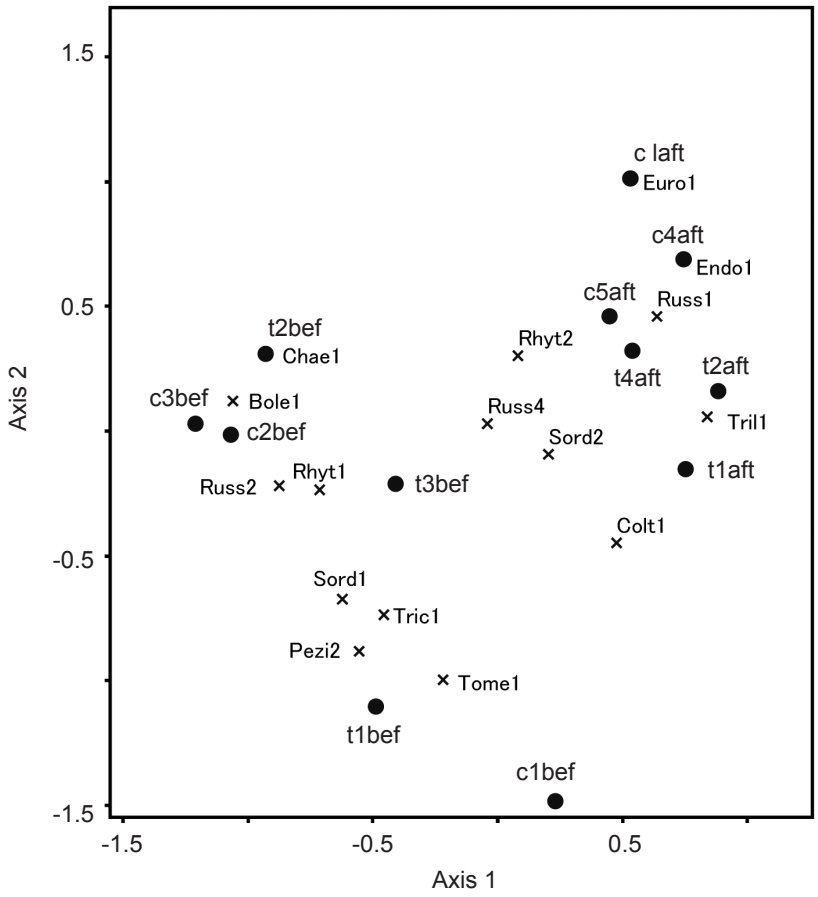

Figure 3 Ordination diagram of root samples (filled circles) and ECM species (crosses) along non-metric multidimensional scaling axes 1 and 2; t = treated tree; c = control tree; numbers following t and c represent root numbers, different numbers indicate samples taken from different main roots; bef = before drought treatment, aft = after drought treatment

rainfall exclosure. Thus, the effect on control tree should be very small.

Differences between trees in their response to environmental factors might also explain the observed pattern. ECM species richness associated with *Picea abies* seedlings was reported to be positively related to the growth rate and size of the host tree (Korkama et al. 2006). We chose two trees that were close together, but the treated tree was slightly larger than the control tree, and this might explain the difference in the number of species before treatment. However, the species composition and species abundance after treatment did not differ greatly between the two trees, suggesting that differences in the tree characteristics were less important.

ECM community in this study changed within 20 to 30 days as shown in Koide et al. (2007) and Courty et al. (2008). However, due to only two sampling occasions and uncontrolled effect of spatial heterogeneity, we could not evaluate effects of spatial and temporal variations of ECM community separately. We could not tell whether ECM community was restored after the rainfall exclosure or not.

In tropical Asia, studies of ECM fungi have been limited to descriptions based on morphological traits (Lee et al. 1997) and molecular techniques (Sirikantaramas et al. 2003), and to a few experimental studies such as a study of the relationship between ECM fungi and the intensity of shade (Tennakoon et al. 2005, Saner et al. 2011). At the Lambir Hills National Park, the fungal community structure differs among soil types (Peay et al. 2010). In the present study, the community structure of ECM fungi on an individual tree was found to change over a short period in a small area.

Studies of ECM fungi on mature trees in the tropical rainforest encounter many obstacles. One of the biggest problems is the collection of sufficiently large and representative sample to permit statistical analysis. In this study, we collected root samples directly from mature trees, as Lilleskov et al. (2002) did. However, this method is labour intensive and time consuming due to the difficulty of searching and collecting samples. We could not collect enough samples to conduct appropriate statistical test as suggested by Underwood (1992). Thus, our study is best considered as a case study based on a single individual. Molecular techniques may make it possible to obtain more samples (Horton & Bruns 2001, Saari et al. 2004) if the target tree species is abundant and its fine roots are easily found. However, technical problems such as the difficulty of building water-exclusion enclosure in a tropical rainforest remain to be solved (Nepstad et al. 2002).

In this study, we attempted to conduct drought experiment on the community structure of ECM fungi on mature tree in relation to general flowering. In the Bornean tropical rainforest, severe drought associated with El Niño events can trigger general flowering (Sakai et al. 2006). It is possible that this drought stress may cause changes in the dominance of particular ECM fungi (i.e. an increase in the abundance of those that tolerate drought). This phenomenon may be related to the general flowering phenomenon, if the dominance of particular species of ECM fungi encourages synchronisation of nutrient uptake by host trees at the stand level. Furthermore, the rapid accumulation of phosphorus in trees after fruit maturation (Ichie et al. 2005) suggests that host trees need to sustain ECM fungi not only during drought (Shi et al. 2002) but also thereafter.

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