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Spatial distribution of Beta glucan containing wild mushroom communities in subtropical dry forest, Thailand

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Abstract In addition to their use as food, mushrooms have been gaining importance in medicinal practices. Beta glucan, known as a modulator of the immune system, is one of the bioactive compounds of interest. This study explores the relationship between environmental variables and spatial distribution of mushroom communities containing beta-glucan using Canonical Correspondence Analysis. Mushroom samples were collected for study from 125 sampling points within three sites in seven subtypes of subtropical dry forest along elevational gradients with a variety of ecosystems and climates from Thung Salaeng Luang National Park in the lower North of Thailand. Most mushrooms in the family Russulaceae, growing in deciduous dipterocarp forests and mixed deciduous forests with bamboo in the northern and the central-western parts of the park, and Polyporaceae, found in dry evergreen forests, were found to have relatively high beta glucan content. Mushroom communities could be categorized into five groups by cluster analysis using the Sorensen (Bray-Curtis) distance technique with remaining information of 75%; the groups were named according to altitude and forest type. It was found that the high beta glucan content mushroom community consisting of *Pycnoporus cinnabarinus*, *P. coccineus,* and *P. sanguineus* mostly occurred in the highland dry evergreen forest habitat. It is also shown that the occurrence of high beta glucan content mushroom communities is correlated with the specific habitat characteristics of high altitude, high crown cover percentage and high rainfall.

Keywords Spatial distribution · Mushroom · Beta Glucan · Canonical correspondence analysis · Subtropical dry forest · Thung Salaeng Luang National Park

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Introduction

For centuries, mushrooms have been known throughout the world to possess numerous health benefits. In eastern culture, both edible and non-edible varieties have been used as dietary nutraceuticals and in traditional medicine (Ng 1998). This is due to their various bioactive components with potential health promoting functions including immunomodulators in cancer therapy, resistance to bacterial, viral, fungal and parasitic infections, and reduction of blood cholesterol and blood glucose. (Gunde-Cimerman 1999; Wasser 2002). Beta glucans, with beta (1-3), beta (1-4) and beta (1-6) glucosidic linkages, are one of the key functional components that impart some of the healthy properties of mushrooms. In particular, beta glucans enhance the function of macrophages and the host's resistance to microbial infections, stimulate the immune system, and are known as the primary components responsible for mushrooms' physiological effects (Manzi and Pizzoferrato 2000; Wasser 2002; Cheung 1998; Diyabalanage et al. 2008).

A number of researchers have worked on edible wild and commercial mushrooms regarding their nutrient quantity, nutraceutical properties and other medicinal qualities as therapeutic alternatives with antioxidant, antimicrobial and anti-inflammatory activities. (Cheung 1998; Manzi and Pizzoferrato 2000; Bobek et al. 2001; Mau et al. 2001; Mau et al. 2002). These papers covered species of the

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genera Lentinus, Hericium, Grifola, Flammulina, Pleurotus, Tremella, Boletus, Agrocybe and Leucopaxillus which occur in forest areas from the most diverse climates around the world (Gunde-Cimerman 1999; Kues and Liu 2000; Manzi et al. 2004; Carbonero et al. 2006; Barros et al. 2007; Barros et al. 2008). Interspecific differences in health beneficial functions of various species of mushrooms were examined by Rosado et al. (2002) and Rhee et al. (2008). However, the literature contains little information concerning the ecology of such mushrooms, particularly of the beta-glucan containing species. Since habitat characteristics of different species are influenced by their environment, we hypothesized that environmental factors may significantly correlate with the presence of mushroom species rich in beta-glucan. The aim of this study was therefore to investigate the correlation of environmental variables with spatial distribution of mushroom species with high beta-glucan content and to provide information on beta-glucan content in wild mushrooms occurring in subtropical dry forest with different ecosystems and climate.

Materials and methods

Study area

Thung Salaeng Luang National Park is the largest protected area for forest resource conservation and tourism in the Lower North of Thailand, located at 16°25′ to 16°57′N and 100°37′ to 101°00′E. It covers approximately 1,670 km^2 (167,000 ha). The topography varies from about 60 m above mean sea level in a flat area in the western part to 1,100 m in a hilly and mountainous area on the southeastern edge (Fig. 1). The local climate is classified as tropical wet and dry or savanna climate or Köppen's Aw (Land Development Department 1998). The mean annual temperature is 27°C, the mean rainfall is about 1,257 mm per year with mean annual evaporation of 1,695 mm, 6 to 8 months of drought are observed in a year (November to April), and the mean annual humidity is 71% (data averaged from 1972 to 2008). The national park consists of seven subtypes of subtropical dry forest, altogether covering approximately 125,647.56 ha (1,256.48 km^2) or 75.24% of the total national park area in year 2004. The elevational gradient contains ecotypes ranging from low altitude flatland savanna and dry evergreen forest, to upland mixed deciduous forest and deciduous dipterocarp forest, and finally tropical pine forest in the highest mountain areas. Some of the forest areas in this national park have long been disturbed by several factors such as forest and agricultural fire, illegal logging and forest product harvesting by local communities (Royal Forest Department 2000). Typic Kandiustults based on the USDA classification system (Soil Survey Staff 1999) or Red Yellow Podzolic in the Thai soil system (Land Development Department 1998) dominate the soil types in the study area.

Site attributes

The study was undertaken in three localities (Fig. 1) within Thung Salaeng Luang National Park. Ten line transects were selected to study plant populations and biodiversity. The habitats and characteristics of study sites are described in Table 1. The first study site is located in the north part of the National Park and covers an area of 9.5 km^2 with altitude varying from 320 to 560 m over the generally flat and hilly terrain. Vegetation is predominantly deciduous dipterocarp forest, generally of *Dipterocarpus tuberculatus* Roxb., *Shorea siamensis* Miq., *Dipterocarpus obtusifolius* Teijsm. ex Miq., *Xylia xylocarpa* (Roxb.) W.Theob., *Pterocarpus macrocarpus* Kurz or combinations thereof, whereas, the understory is dominated by *Arundinaria pusilla* A.Chev. & A.Camus and *Coelorachis striata* (Nees) A. Camus.

The second study site is of approximately 42.13 km^2 and located in the central-west part of the National Park. Upland and high mountains dominate the regional landscape, with the elevation ranging from 300 m in the lower part of Rom Klao administrative village to about 960 m at the summit of Thung Neon Son plateau. The geology is comprised of shale and sandstone. The wide variation of topography and altitude results in a high diversity of forest ecology; there is dry evergreen forest, mixed deciduous forest with bamboo, pine forest, and grassland and savanna. *Michelia champaca* L., *Sandoricum koetjape* Merr., *Dipterocarpus alatus* Roxb. & G.Don are the dominant species in dry evergreen forest. In mixed deciduous forest located between 310 and 550 m, the dominant species are *Pterocarpus macrocarpus* Kurz, *Lagerstroemia floribunda* Jack, *Cratoxylum sumatranum* (Jack) Blume, and *Afzelia xylocarpa* (Kurz) Craib. This forest type is associated with bamboo (*Bambusa natans* Wall. Ex Munro and *Oxytenanthera albociliata* Munro) at every altitude (Royal Forest Department 2000). At higher altitudes over 900 m, areas are covered by a tropical pine species *Pinus merkusii* Jungh. & de Vriese, commonly in association with native hardwoods such as *Castanopsis acuminatissima* (Blume) Rehder while the undergrowth consists of *Equisetum debile* Roxb., *Utricularia delphinoides* Thorel ex Pellegr. and *Burmannia coelestris* D. Don. Grassland and savanna are found at altitudes ranging from 310 to 350 m in areas which had been cultivated, particularly with upland rice and maize, for at least 10 years; the vegetation here is dominated by *Imperata cylindrica* (L.) P. Beauv. and *Sorghum halepense* (L.) Pers. as well as other herbs such as *Eupatorium odoratum* L. and *Albizia procera* (Roxb.) Benth.

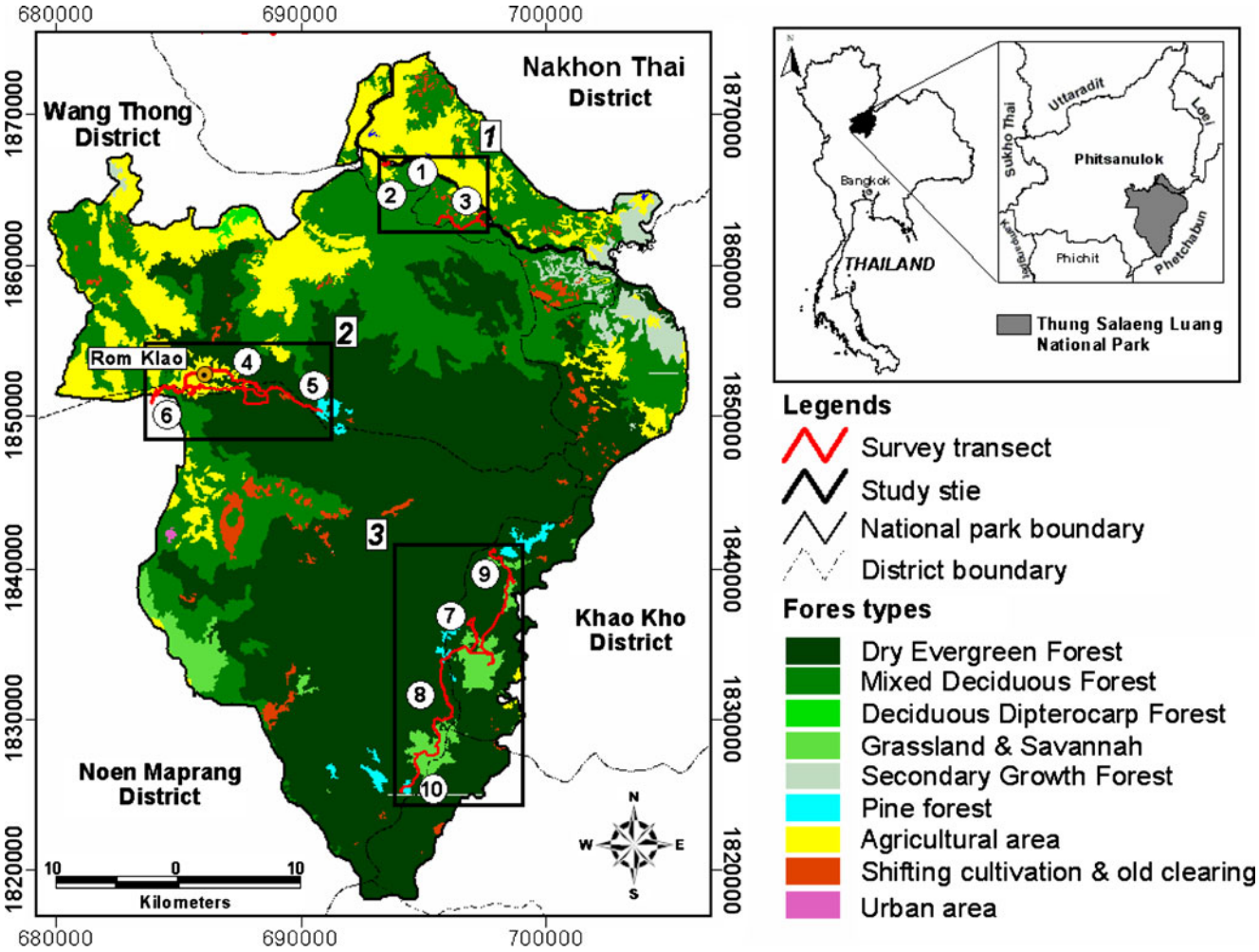

Fig. 1 The location of the study site in Thung Salaeng Luang National Park

The third study site is located in the southern region of the National Park and covers an area of 182.64 km^2, with a range of altitude from 680 to 1 020 m. This region is also dominated by various forest ecosystems; tropical pine forest (*Pinus merkusii* Jungh. & de Vriese), deciduous dipterocarp forest (dominated by *Dipterocarpus obtusifolius* Teijsm. ex. Miq. and *Aporusa villosa* Baill.), dry evergreen forest dominated by Fagaceae (*Castanopsis acuminatissima* (Blume) Rehder and *Quercus ramsbottomii* A. Camus). Besides those, grassland and savanna dominated by. *Carex stramentita* Boot and *Imperata cylindrica* (L.) P. Beauv. exists here. The upper tropical pine forests are found primarily on the northern and southern sections where the altitude is between 680 and 750 m. and 850 to over 1,000 m, respectively (Fig. 1). A combination of tropical pine forest, dry evergreen forest and deciduous dipterocarp forest types can be found in the northeast area, in which *Pinus merkusii* Jungh. & de Vriese, *Dipterocarpus obtusifolius* Teijsm. ex. Miq., and *Aporusa villosa* Baill. are the dominant vegetation. Grassland and savanna commonly occur at middle elevations (750 to 850 m in the central section), and are surrounded by dry evergreen forest as a result of long-term agricultural cultivation by nearly settlements in the past hundred years. *Aporusa villosa* and *Dillenia obovata* (Blume) Hoogland are the dominant tree species mainly distributed in the large area of savanna flatland (7.42 km^2).

Chemicals

Lichenase [EC3.2.1.73], 1,000 U/ml, beta glucosidase, [EC 3.2.1.21] 40 U/ml, and glucose standards were purchased from Megazyme Int. (Ireland Ltd). The glucose assay kit was a product of Sigma (St. Louis, MO, USA). All chemicals used in this study were of analytical reagent grade.

Sample collection and measurement of environmental variables

Elevational gradients are useful for assessing environmental characteristics to which species respond, because changes

Table 1 Habitats study sites and their characteristics

Site no.	Transect no.	Length (km)	Elevation (m)	Slope (degree)	Rainfall[a] (mm)	Temperature[b] (°C)	Forest type[c]	Geology
1	1	2.59	380–410	0–18	1,240–1,250	27.2–27	DDF	Brown, reddish-brown micaceous sandstone; pale brown micaceous shale, siltstone, and conglomerate (Cretaceous: Korat Group, Khok Kruat Formation)
	2	2.42	320–440	0–15	1,240–1,250	27.5–26.9	DDF	Brown, reddish-brown micaceous sandstone; pale brown micaceous shale, siltstone, and conglomerate (Cretaceous: Korat Group, Khok Kruat Formation)
	3	6.12	420–560	0–20	1,255–1,270	27–26.3	DEF	Brown, reddish-brown micaceous sandstone; pale brown micaceous shale, siltstone, and conglomerate (Cretaceous: Korat Group, Khok Kruat Formation)
2	4	2.49	460–540	0–26	1,250–1,255	26.8–26.4	GL&SVN	White to light brown quartz sandstone; siltstone, and shale (Jurassic: Korat Group, Sao Khua Formation)
	5	6.04	480–960	0–39	1,250–1,255	26.7–24.3	DEF, MDFB	Section 1: Reddish brown siltstone, mudstone, sandstone, and shale (Jurassic: Sao Khua Formation)
							PF, MDF	*Section 2:* White, pale orange, yellowish brown, pebble sandstone intercalated with shale and conglomerate (Cretaceous: Phu Phan Formation)
	6	12.36	300–560	1–28	1,250–1,260	27.6–26.3	DEF, MDF, MDFB, GL&SVN	Purplish-red siltstone, fine grained sandstone, shale, and conglomerate (Jurassic: Korat Group, Phu Kradung Formation)
3	7	5.83	680–760	0–36	1,300–1,310	25.7–25.3	DEF, and GL&SVN	Reddish brown siltstone, mudstone, sandstone, and shale (Jurassic: Korat Group, Sao Khua Formation)
	8	15.68	700–920	0–16	1,300–1,315	25.6–24.5	GL&SVN, DEF, DEFF, PF	Reddish brown siltstone, mudstone, sandstone, and shale (Jurassic: Korat Group, Sao Khua Formation)
	9	10.47	680–740	0–22	1,300–1,310	25.7–25.4	DDF, DEF, PF, GL&SVN	Reddish brown siltstone, mudstone, sandstone, and shale (Jurassic: Korat Group, Sao Khua Formation)
	10	4.37	880–1,020	0–18	1,305–1,310	24.7–24	GL&SVN, DEF, PF	White to light brown quartz sandstone; siltstone, and shale (Jurassic: Korat Group, Phra Wihan Formation)

[a] GIS data derived from spatial interpolation of yearly mean climate data; [b] data range based on the mean temperature recorded by the nearest meteorology station to the study sites at elevation 450 m assuming an average decreasing air temperature gradient of 5°C/km elevation; [c] forest type: *DEF* dry evergreen forest; *DEFF* dry evergreen forest dominated by Fagaceae; *MDF* mixed deciduous forest; *MDFB* mixed deciduous forest with bamboo; *DDF* deciduous dipterocarp forest; *PF* tropical pine forest; *GL&SVN* tropical grassland & savanna

in elevation result in predictable variation in both abiotic factors such as temperature and precipitation as well as in vegetational composition (Willig et al. 2009). Thus, the collection procedure of wild mushrooms and the plant diversity investigation in this study were designed based on forest types and elevation gradients using the distance sampling method (Barry and Welsh 2001). Mushroom samples were collected from ten surveyed transects within each particular forest type at a different height along an altitude gradient 260 to 1,050 m. In addition, plots of 40 m×40 m were set at the points where mushrooms were found to investigate the quantitative biodiversity variation of plant species. The number of sampling points in each transect depended upon the points that mushroom were found within the sampling plot. The total numbers of sampling points for mushroom collection and sampling plots were turned out to be 125 points and 67 sampling plots in ten transects, respectively. The biophysical environment of the studied surveyed track and biodiversity in the sampling plots including tree height, diameter at breast height (DBH) for each tree, plot location and current condition of habitat together with environmental variables

were also recorded. The percentage of crown cover was visually estimated in a 40 m×40 m sampling plot. Shannon-Wiener index (H) (Hayat et al. 2010) was calculated by the following equation:

$$H = -\text{sum}(P_i \log[P_i])$$

where P_i is the proportion of each species in the sample.

Mushroom sample preparation and beta glucan determination

The 32 mushroom species were collected from Thung Salaeng Luang National Park, Thailand during November, 2008–September, 2009. Mushroom specimens were identified at the herbarium of the Department of National Parks, Wildlife and Plant Conservation of Thailand. The identification of the specimens was also performed according to Chandrasrikul et al. (2008), TISTR (2007), Royal Institute of Thailand (1996), and Imazeki et al. (1988). The mushroom specimens are extant in the herbarium for later revision and further study. Mushroom samples were dried in a hot air oven at 45°C for 24 h. They were then ground to pass through a 100 mm screen. Samples were kept in a low humidity area at room temperature before analysis.

Beta glucan contents were determined according to the method of McCleary and Holmes (1985), slightly modified for mushroom analysis as reported by Manzi and Pizzoferrato (2000). Ground mushroom samples were hydrolysed with lichenase (10 U/ml) and the assay was carried out at 45°C for 60 min. Sodium phosphate (20 mM, pH 6.5) was prepared to dissolve the lichenase. Oligosaccharides obtained from digestion by the lichenase were then separated from solid residue and other beta-linked saccharides by centrifugation. The filtrate was then further cleaved to glucose by adding beta-glucosidase dissolved in 50 mM sodium acetate buffer (pH 4.0). The concentration of this enzyme in the sample mixture was 0.8 U/ml. and the reaction was performed at 45°C for 30 min. The amount of released free glucose was determined spectrophotometrically at 540 nm using a glucose assay kit containing glucose oxidase/peroxidase reagent. Glucose is oxidized to gluconic acid and hydrogen peroxide by glucose oxidase and the colored product is then formed in the presence of peroxidase.

Data analysis

Canonical Correspondence Analysis (CCA) was used to analyze the relationship between distribution of wild mushrooms and environmental variables. Ordination was undertaken within the PC-ORD for Windows program, version 4.17 (McCune and Mefford 1999). CCA is a direct gradient analysis technique that relates community variation (composition and abundance) to environmental variation, enabling the significant relationship between environmental variables and community distribution to be determined. CCA assumes that meaningful environmental variables have been identified and measured. This is not always the case, with the result that the variation attributable to factors not included in the ordination is lost (Vogiatzakis et al. 2003). Therefore, the CCA results were also compared with results from detrended correspondence analysis (DCA) (Hill 1979). CCA axes were evaluated statistically with a Monte Carlo permutation test (P=0.01).

Because the inclusion of a moderately to strongly inter-correlated group of variables in the ordination may yield unreliable results (Ter Braak 1986), the variables employed were tested first for correlation using the Pearson correlation coefficient (Vogiatzakis et al. 2003). Variables not following the normal distribution such as altitude, were log transformed. ArcSin transformation was used for the crown cover data which is expressed in percentage. Thirty two species of wild mushroom were used in both classification and ordination analysis (Table 2).

Results

Wild mushroom collection and beta glucan content

The survey of three study sites resulted in 32 species of wild mushrooms being found at several stages of development in different habitats depending not only on the forest types but also on the altitude and season. In the rainy season, among all study sites the highest mushroom diversity was found in mixed deciduous forest (14 species dominated by *Amanita caesarea* (Scop.) Pers., *Amanita hemibapha* (Berk. & Broome) Sacc., *Lentinus squarrosulus* Mont. and *Termitomyces microcarpu*s (Berk. & Broome) R. Heim), followed by deciduous dipterocarp forest, dry evergreen forest, pine forest, mixed deciduous forest with bamboo, dry evergreen forest dominated by Fagaceae, and grassland and savanna, respectively (Table 2). Mushrooms were rarely found in dry dipterocarp forest, pine forest, and grassland and savanna during winter (November–February) and summer (March–May). Furthermore, only two species of mushrooms (*Russula alboareolata* Hongo and *Russula densifolia* Secr. ex Gillet were found in grassland and savanna of the central-western part (Transect No. 6) during the rainy season. The survey result showed the dominant families of mushrooms in the deciduous dipterocarp forest were Russulaceae and Amanitaceae (such as *Russula delica* Fr., *Russula cyanoxantha* (Schaeff.) Fr., *R. alboareolata*, *A. caesarea*, and *A. hemibapha*).

Beta glucan content determined in all mushroom species in these survey transects was in a broad range (0.001–

Table 2 Name of mushroom species, habitat, and beta glucan content

Site No.	Transect No.	Family	Mushroom species	Abbreviation	Beta glucan Content (g/100 g)	Forest type[a]
1	1	Lyophyllaceae	*Termitomyces fuliginosus* R. Heim	T. fuli	0.01	DDF
		Boletaceae	*Boletus colossus* R. Heim	B. col	0.03	DDF
		Russulaceae	*Russula densifolia* Secr. ex Gillet	R. dens	0.25	DDF
			Russula cyanoxantha (Schaeff.) Fr.	R. cyano	0.29	DDF
			Russula alboareolata Hongo	R. albo	0.42	DDF
			Russula emetica (Schaeff.) Pers.	R. eme	0.10	DDF
			Russula delica Fr.	R. del	0.38	DDF
		Polyporaceae	*Microporus xanthopus* (Fr.) Kuntze	M. xan	0.001	DDF
			Pycnoporus cinnabarinus (Jacq.) P. Karst.	P. cin	0.35	DDF
		Amanitaceae	*Amanita hemibapha* (Berk. & Broome) Sacc.	A. hem	0.05	DDF
			Amanita princeps Corner & Bas	A. prin	0.09	DDF
			Amanita caesarea (Scop.) Pers.	A. cae	0.04	DDF
	2	Lyophyllaceae	*Termitomyces fuliginosus* R. Heim	T. fuli	0.01	DDF
		Boletaceae	*Boletus colossus* R. Heim	B. col	0.03	DDF
			Boletus edulis Bull.	B. edu	0.01	DDF
			Heimiella retispora (Pat. & C.F. Baker) Boedijn	H. reti	0.19	MDFB
		Russulaceae	*Russula densifolia* Secr. ex Gillet	R. den	0.25	DDF
			Russula cyanoxantha (Schaeff.) Fr.	R. cyano	0.29	DDF
			Russula alboareolata Hongo	R. albo	0.42	DDF
			Russula emetica (Schaeff.) Pers.	R. eme	0.10	DDF
			Russula delica Fr.	R. del	0.38	DDF
		Polyporaceae	*Microporus xanthopus* (Fr.) Kuntze	M. xan	0.001	DDF
			Pycnoporus cinnabarinus (Jacq.) P. Karst.	P. cin	0.35	DDF
		Amanitaceae	*Amanita hemibapha* (Berk. & Broome) Sacc.	A. hem	0.05	DDF
			Amanita princeps Corner & Bas	A. prin	0.09	DDF
			Amanita caesarea (Scop.) Pers.	A. cae	0.04	DDF
	3	Russulaceae	*Russula densifolia* Secr. ex Gillet	R. dens	0.25	MDFB
			Russula emetica (Schaeff.) Pers.	R. eme	0.10	DDF
		Cortinariaceae	*Cortinarius claricolor* var. *turmalis* (Fr.) Quadr	C. clar	0.13	DEF
		Polyporaceae	*Microporus xanthopus* (Fr.) Kuntze	M. xan	0.001	DEF
2	4	Polyporaceae	*Microporus xanthopus* (Fr.) Kuntze	M. xan	0.001	DEF
	5	Russulaceae	*Russula densifolia* Secr. ex Gillet	R. dens	0.25	MDFB
		Polyporaceae	*Microporus xanthopus* (Fr.) Kuntze	M. xan	0.001	DEF
	6	Russulaceae	*Russula densifolia* Secr. ex Gillet	R. dens	0.25	MDFB, GL&SVN
			Russula alboareolata Hongo	R. albo	0.42	GL&SVN
			Russula delica Fr.	R. del	0.38	MDFB
		Lyophyllaceae	*Termitomyces tylerianus* Otieno	T. tyle	0.12	MDFB
			Termitomyces microcarpus (Berk. & Broome) R. Heim	T. micro	0.08	MDF
			Termitomyces eurhizus (Berk.) R. Heim	T. eur	0.07	MDF
		Polyporaceae	*Polyporellus varius* (Pers.) P. Karst.	P. var	0.02	MDF, DEF
			Pycnoporus cinnabarinus (Jacq.) P. Karst.	P. cin	0.35	DEF
			Pycnoporus coccineus (Fr.) Bondartsev & Singer	P. coc	0.45	DEF
			Lentinus squarrosulus Mont.	L. squa	0.02	MDF
			Daedaleopsis confragosa (Bolton) J. Schröt	D. con	0.03	MDF
			Pycnoporus sanguineus (L.) Fr.	P. san	0.35	DEF
		Amanitacea	*Amanita princeps* Corner & Bas	A. prin	0.09	MDF
			Amanita caesarea (Scop.) Pers.	A. cae	0.04	MDF

Table 2 (continued)

Site No.	Transect No.	Family	Mushroom species	Abbreviation	Beta glucan Content (g/100g)	Forest type[a]
			Amanita hemibapha (Berk. & Broome) Sacc.	A. hem	0.05	MDF, PF
			Amanita virgineoides Bas	A. vir	0.01	MDF, PF
		Agaricaceae	*Agaricus silvaticus* Schaeff.	A. sil	0.03	DEF
			Chlorophyllum molybdites (G. Mey.) Massee	C. moly	0.03	MDF
		Ganodermataceae	*Ganoderma lucidum* (Curtis) P. Karst.	G. luci	0.33	MDF
			Amauroderma rugosum (Blume & T. Nees) Torrend	A. rug	0.04	DEF
		Suillaceae	*Suillus bovinus* var. *bovinus* (Pers.) Kuntze	S. bov	0.01	MDF, PF
		Suillaceae	*Suillus bovinus* var. *bovinus* (Pers.) Kuntze	S. bov	0.01	MDF, PF
		Cortinariaceae	*Cortinarius claricolor* var. *turmalis* (Fr.) Quadr	C. clar	0.13	DEF
		Tricholomataceae	*Clitocybe suaveolens* (Schumach.) P. Kumm.	C. sua	0.09	MDF
		Sclerodermataceae	*Scleroderma verrucosum* (Bull.) Pers.	S. ver	0.09	MDF, PF
		Boletaceae	*Heimiella retispora* (Pat. & C.F. Baker) Bodeijn	H. reti	0.19	MDFB
3	8	Marasmiaceae	*Lentinula edodes* (Berk.) Pegler	L. edo	0.34	DEFF
		Russulaceae	*Russula alboareolata* Hongo	R. albo	0.42	PF
		Polyporaceae	*Microporus xanthopus* (Fr.) Kuntze	M. xan	0.001	PF
	9	Russulaceae	*Russula cyanoxantha* (Schaeff.) Fr.	R. cyano	0.29	PF
		Polyporaceae	*Pycnoporus cinnabarinus* (Jacq.) P. Karst.	P. cin	0.35	DEF
			Pycnoporus coccineus (Fr.) Bondartsev & Singer	P. coc	0.45	DEF
	10	Russulaceae	*Russula alboareolata* Hongo	R. albo	0.42	PF
		Polyporaceae	*Pycnoporus sanguineus* (L.) Fr.	P. san	0.35	DEF
			Pycnoporus sanguineus (L.) Fr.	P. san	0.35	DEF

[a] Forest type: *DEF* dry evergreen forest; *DEFF* dry evergreen forest dominated by Fagaceae; *MDF* mixed deciduous forest; *MDFB* mixed deciduous forest with bamboo; *DDF* deciduous dipterocarp forest; *PF* tropical pine forest; *GL&SVN* tropical grassland & savanna

0.45%). Some members of the Boletaceae, namely *Boletus colossus* R. Heim and *Boletus edulis* Bull., which had beta glucan content of only 0.03% and 0.01% respectively, were only found in upland deciduous dipterocarp forest at an altitude range of 312–365 m. However *Heimiella retispora* (Pat. & C.F. Baker) Boedijn, in the Boletaceae, containing higher beta glucan content (0.19%) occurred in mixed deciduous forest with bamboo at higher altitude (440–543 m). *Lentinula edodes* (Berk.) Pegler with high beta glucan content of 0.34% was only found in high altitude dry evergreen forest dominated by Fagaceae at about 890 m, but only rarely. The beta glucan content was mostly high in the genus Russula, ranging from 0.10% to 0.42%, which is commonly found in deciduous dipterocarp forest and mixed deciduous forest with bamboo of the north and the central-west parts, respectively. Interestingly, *R. alboareolata* which contains the highest beta glucan (0.42%), among in the Russulaceae, could be found in both deciduous dipterocarp forests (altitude 378–476 m) and pine forests (altitude 875–905 m). Additionally, *Pycnoporus coccineus* (Fr.) Bondartsev & Singer, *Pycnoporus cinnabarinus* (Jacq.) P. Karst., and *Pycnoporus sanguineus* (L.) Fr. showed a rather high beta glucan content (0.45, 0.35 and 0.35%, respectively) and they were mostly found in highland dry evergreen forest at an altitude range of 702–832 m. This suggests that the environmental factors, particularly forest type and altitude may contribute to the distribution of mushroom species.

Species ordination

The eigenvalues in an ordination analysis represent the relative contribution of each axis to the explanation of the total variation in the data. The CCA eigenvalues of the first three axes were 1.0, 0.73, and 0.53, respectively (Table 3). The eigenvalues obtained were rather different from the ones derived from DCA. The first three CCA axes explained 7.3% of the variance in the species data. Since the canonical coefficients define the ordination axes as linear combinations of the environmental variables, and the intraset correlations are the correlation coefficient between the environmental variables and these ordination axes, these coefficients indicate that the first axis is the beta glucan content, the second CCA axis is highly related to the percentage of crown cover, and the third axis is associated with the value of the Shannon-Wiener index and the

Table 3 Summary table of statistical analysis for comparing the CCA ordination with DCA result

	Monte Carlo test		
	Axis 1	Axis 2	Axis 3
CCA			
Eigenvalues	1.00	0.730	0.530
Variance explained (%)[a]	3.2	2.4	1.7
Cumulative% explained	3.2	5.6	7.3
Pearson correlation (Species-Environment)	1.00	0.855	0.728
Kendall (Rank) correlation (Species-Environment)	0.969	0.641	0.523
DCA			
Eigenvalues	1.00	0.99	1.00

[a] Cumulative variance in species data (P=0.01)

percentage of crown cover (Table 4). Moreover, the signs and relative magnitudes of the intraset correlations and of the canonical coefficients were standardized. The result shows that the important environmental variables for predicting mushroom species distribution were beta glucan content, Shannon-Wiener index value and crown cover percentage. In addition, the canonical coefficients gave rather different information compared to the intraset correlations (Table 4). This is probably due to some environmental variables being correlated with each other (Ter Braak 1986).

The mushroom species distribution pattern in Thung Salaeng Luang National Park is displayed in an ordination diagram with 125 sampling points, with wild mushroom species represented by points, and environmental variables represented by arrows (Fig. 2). The beta glucan content vector shown in the CCA ordination diagram is not an environmental variable for presenting the pattern of mushroom species distribution. However, the ordination diagram indicates that the highest beta glucan content was found in *P. coccineus* (0.45 g/100 g). Interestingly, this mushroom species was only found in dry evergreen forest. Other mushroom species with high beta glucan content include *Ganoderma lucidum* (Curtis) P. Karst., *L. edodes*, *P. cinnabarinus*, *P. sanguineus*, *R. alboareolata*, *R. cyanoxantha*, and *R. delica*. Several low beta glucan content mushroom species were *A. hemibapha*, *A. caesarea*, *Agaricus silvaticus* Schaeff., *Amanita princeps* Corner & Bas, *Amanita virginioides* Bas., *Amauroderma rugosum* (Blume & T. Nees) Torrend, *B. edulis*, *Boletus colossus* R. Heim, *Chlorophyllum molybdites* (G. Mey.) Massee, *Daedaleopsis confragosa* (Bolton) J. Schröt, *L. squarrosulus*, *Microporus xanthopus* (Fr.) Kuntze, *Polyporellus varius* (Pers.) P. Karst., *Termitomyces eurrhizus* (Berk.) Heim, and *Termitomyces fuliginosus* R. Heim. The content of beta glucan found in mushrooms therefore depends on species, not forest type. However other environmental variables such as rainfall and crown cover percentage may influence the distribution of mushroom species. This agrees with the high value of intraset correlation coefficient associated with axis 2 shown in Table 4.

Mushroom community type

From the cluster analysis using Sorensen (Bray-Curtis) distance technique with remaining information of 75%, the 125 mushroom sampling points in Thung Salaeng Luang National Park could be categorized into 5 groups (Fig. 3). These mushroom communities are named according to altitude and forest type as follows:

Group 1, Upland DDF-MDFB This community type is found at altitudes ranging from 378 to 476 m in the deciduous dipterocarp forest (DDF) and mixed deciduous forest with bamboo (MDFB). The Shannon-Wiener Index, crown cover, and rainfall were in the ranges of 2.203–5.030, 29.02–75.81%, and 1,245–1,305 mm, respectively. The beta glucan content found in this community showed quite a wide range (0.001–0.42%). There are 13 mushroom species belonging to this community, namely *A. rugosum*, *A. silvaticus*, *B. colossus*, *Clitocybe suaveolens* (Schumach.) P. Kumm, *G. lucidum*, *R. alboareolata*, *R. cyanoxantha*, *R. delica*, *Russula emetica* (Schaeff.) Pers., *Suillus bovinus var. bovinus* (Pers.) Kuntze, *T. microcarpus*, *T. fuliginosus*, and *Termitomyces tyleranus* Otieno. Among these species, *B. colossus*, *R. cyanoxantha*, *R. alboareolata,* and *R. emetica* were mostly found in deciduous dipterocarp forest and *Termitomyces*

Table 4 Canonical coefficients and intraset correlations of environmental variables with the first three axes of CCA

Variables	Canonical coefficients (Standardized)			Correlations coefficients (intraset)		
	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3
Beta glucan content	1.00	0.381	0.074	−1.00	0.00	0.00
Altitude	0.000	1.875	0.653	−0.104	0.220	0.203
Shannon-Wiener Index	0.000	0.051	1.102	0.062	−0.233	0.846
Crown cover (%)	0.000	−0.170	−0.237	−0.155	−0.348	0.814
Rainfall	0.000	−1.92	−0.128	−0.284	−0.242	0.148

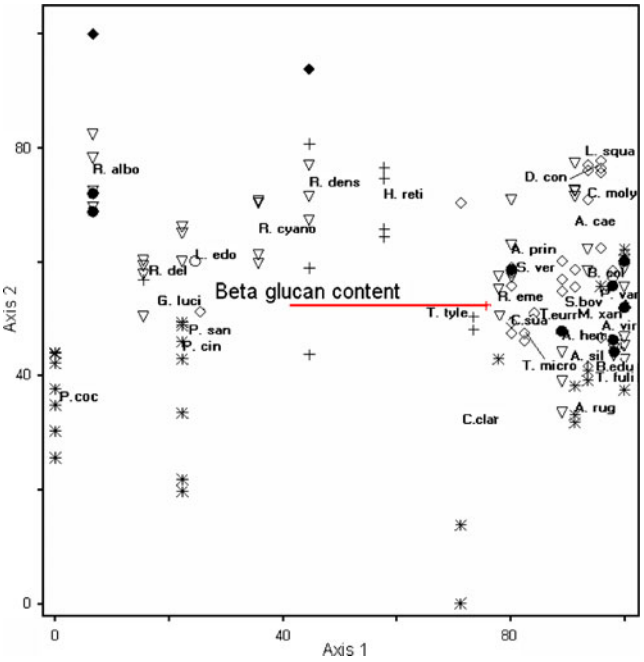

Fig. 2 The distribution of 32 species of wild mushroom collected during year 2008–2009 in Thung Salaeng Luang National Park. Canonical Correspondence Analysis (CCA) ordination diagram of Axis 1 and 2 with wild mushrooms showing variation in beta Glucan content (vector) and forest types. (*), dry evergreen forest; (◇), dry evergreen forest dominated by Fagaceae; (+), mixed deciduous forest; (∇), mixed deciduous forest with bamboo; (●), deciduous dipterocarp forest; (♦), tropical pine forest; (○), tropical grassland & savanna. Only the variables with a correlation coefficient higher than 0.5 are presented. For species abbreviations see Table 2

microcarpus (Berk. & Broome) R. Heim was only found in mixed deciduous forest.

Group 2, Upland DDF-DEF This mushroom community is comprised of five species. The area where this group is found is classified as upland (312–365 m). *A. hemibapha*, *B. edulis*, *Cortinarius claricolor* var. turmalis (Fr.) Quadr., *P. cocci-neus*, and *T. fuliginosus* which comprise this group were found either in deciduous dipterocarp forest (DDF) or dry evergreen forest (DEF), except *P. cinnabarinus* which grew in both forests. The lowest detected beta glucan content was 0.01% whereas the highest was 0.45% (*w*/*w*). The minimum rainfall was similar to that of Group 1 (1,245 mm), while the maximum rainfall was slightly lower (1,265 mm). The Shannon-Wiener Index and crown cover percentage were found to be 3.415–5.378 and 40.55–75.81%, respectively.

Group 3, Upland MDF This community includes 13 species and grows between 440 and 543 m. The ranges of the Shannon-Wiener Index and crown cover percentage were broad, while annual rainfall is similar to that of Group 2 community (1,245–1,270 mm). *A. princeps*, *C. molybdites*, *D. confragosa*, *Heimiella retispora* (Pat. & C.F. Baker) Boedijn, *L. squarrosulus*, and *R. densifolia* presented mainly in mixed deciduous forest (MDF). Contradictorily, *A. caesarea*, *A. hemibapha*, *A. virginioides*., *M. xanthopus*, *P. varius*, and *T. eurrhizus* were found, but rarely, in dry evergreen forest, mixed deciduous forest with bamboo, deciduous dipterocarp forest and savanna. The beta glucan content range was exactly equivalent to that of Group 1.

Group 4, Highland PF-DEF (Fagaceae) All five of the sampling plots in this community type happened to be exclusively in the southern part of the national park where the altitude ranged from 875 to 905 m. *M. xanthopus* and *R. alboareolata* commonly appeared in pine forest and highland dry evergreen forest, whereas in this study *L. edodes* could only be found in dry evergreen forest dominated by Fagaceae. The sampling plots in highland pine forest (PF) had a Shannon-Wiener Index between 1.577 and 3.425, whereas dry evergreen forest dominated by Fagaceae had the value of 2.203. Mean annual rainfall of this community type was relatively high in comparison with Groups 1, 2 and 3 at 1 305 mm.

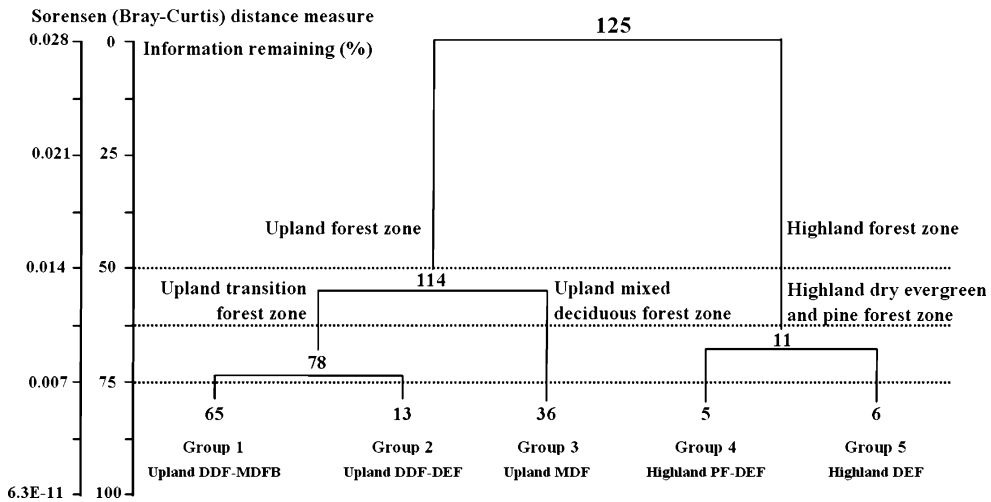

Fig. 3 Dendrogram derived from hierarchical cluster analysis of 125 mushroom sampling plots found in Thung Salaeng Luang National Park. The analysis is based on Sorensen (Bray-Curtis) distance measure with farthest neighbor linkage method

Group 5, Highland DEF This community type was the site of six plots in the highland region in the southern part of the National Park (altitude of 702 to 832 m). Only three mushroom species belong to this group: *P. cinnabarinus, P. coccineus* and *P. sanguineus*. *P. coccineus* was found in highland dry evergreen forest with high percentage of crown cover (82.28%) and annual average rainfall of 1,310 mm. There was only one plot where *P. cinnabarinus* was present in highland dry evergreen forest at the attitude of 702 m with Shannon-Wiener Index of 3.452. *P. sanguineus* appeared rarely in highland dry evergreen forest at the attitude of 810 m.

The relationship of mushroom community type and environmental variables

In comparing ordination diagrams with different presenting axes, Axis 1–2 and Axis 1–3 revealed the important variables influencing the community distribution (Table 5). Altitude and rainfall are the main correlates of the first CCA axis (r=0.953 and r=0.959, respectively). Apart from beta glucan content, altitude also shows the highest correlation (r=0.293) to Axis 2 compared to other environmental variables. Axis 3 is strongly correlated with crown cover percentage (r=0.670). Since the percentage of cumulative explanation was quite high (49.8%), the interpretation of the result was meaningful (Table 6).

The five community groups can be fairly distinguished by the ordination diagram presenting Axis 1 and Axis 2 (Fig. 4). The community types of Highland DEF and Highland PF-DEF (Fagaceae) shown in the right side of the plot are well separated. While, the scattered groups in the left side of the plot show the overlap of communities found in Upland MDF, Upland DDF-MDFB and Upland DDF-DEF groups. They are associated negatively with altitude and rainfall. However it can be seen clearly that Highland DEF and Highland PF-DEF (Fagaceae) have positive association with altitude and rainfall. Mushrooms constituting the Highland PF-DEF (Fagaceae) community could mostly be found in the highest altitude region.

The ordination diagram presenting Axis 1 and Axis 3 can clearly separate the mushroom communities of Highland PF-DEF (Fagaceae) and Highland DEF (Fig. 5). Mushrooms in Highland PF-DEF (Fagaceae) community shown in the upper right part of the plot are strongly positively associated with crown cover percentage, rainfall and altitude. In contrast, the Highland DEF in the lower right part of the plot illustrates a negative association with crown cover percentage, but positive association with rainfall and altitude. However the relative correlation of environmental variables and mushrooms communities of Upland MDF, Upland DDF-MDFB and Upland DDF-DEF cannot clearly be explained by the biplot of Axis 1–3.

Table 6 Summary table of statistical analysis for the CCA plot ordination

	Monte Carlo test		
	Axis 1	Axis 2	Axis 3
CCA			
Eigenvalues	0.963	0.632	0.383
Variance explained (%)[a]	24.3	15.9	9.7
Cumulative% explained	24.3	40.2	49.8
Pearson correlation (Species-Environment)	0.98	0.801	0.619
Kendall (Rank) correlation (Species-Environment)	0.582	0.581	0.103
DCA			
Eigenvalues	0.989	0.998	0.048

[a] Cumulative variance in species data (P=0.01)

Discussion

Collection and beta glucan content of wild mushrooms

The sampling design and method for classifying wild mushroom community types in this study were not based on the quadrat method because the quadrats of 40 m×40 m used for studies on vegetation diversity and surrounding conditions could not ensure the inclusion of wild mushrooms. This was probably caused by the seasonal growing conditions of the mushroom community. Several kinds of

Table 5 Canonical coefficients and intraset correlations of variables with the first three axes used in plot-variable ordination analysis

Variables	Canonical coefficients (Standardized)			Correlations coefficients (intraset)		
	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3
Beta glucan content	0.053	−0.021	0.184	0.246	−0.303	0.413
Altitude	0.453	2.034	−0.163	0.953	0.293	−0.031
Shannon-Wiener Index	−0.085	−0.143	−1.727	−0.432	0.195	0.270
Crown cover (%)	−0.075	0.542	2.185	−0.006	0.153	0.670
Rainfall	0.540	−2.077	−0.649	0.959	−0.164	0.122

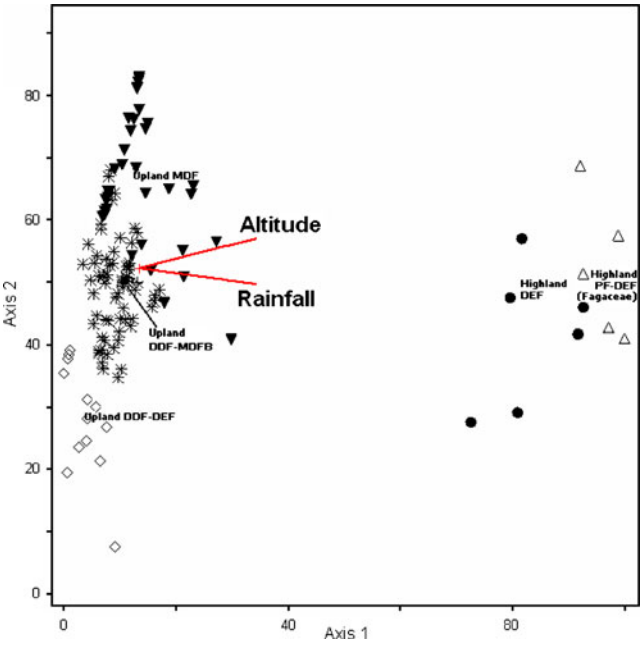

Fig. 4 CCA plot ordination of axis 1 and 2 with the mushroom community types derived from hierarchical cluster analysis. (*), Group1: Upland DDF-MDFB; (◇), Group 2: Upland DDF-DEF; (▼), Group 3: Upland MDF; (△), Group 4: Highland PF-DEF (Fagaceae); (●), Group 5: The highland DEF. Only the variables with a correlation coefficient higher than 0.5 are presented

wild mushrooms could be found only in particular forest types and associated with certain hosts or base materials, but others could grow in a variety of habitat types. This study attempted to reduce the level of unbalanced sampling design caused by the two major environmental gradients, namely forest type and altitude. The 125 sampling points in 67 plots from ten transects of three localities were randomly surveyed for collecting mushroom samples.

Mushrooms in the families Russulaceae and Polyporaceae in this study were mostly found to have relatively high beta glucan content. Similarly to our findings high beta glucan content was reported in *Russula virescens* and *Lentinus* spp. which belong to the family Polyporaceae (Sun et al. 2009; Wasser 2002). In contrast, all mushroom species in the family Amanitaceae analyzed in this study showed beta glucan content lower than 0.1% (*w*/*w*). However, beta-glucan content ranging from 0.001 to 0.45% (*w*/*w*) was found in various mushroom species occurring in different types of forest. Particularly, mixed deciduous forest and deciduous dipterocarp forest were found to have the most diverse species comparing to other forest types in this study. Most mushrooms in the family Russulaceae could grow in deciduous dipterocarp forest and mixed deciduous forest with bamboo in the north and the central-west parts respectively, while Polyporaceae were found in dry evergreen forest. This might suggest that dry evergreen forest and deciduous dipterocarp forest are the most potent sources of mushrooms rich in beta glucan.

In addition, it was found that *P. coccineus* showed the highest content of beta glucan (0.45 g/100 g) among collected wild mushrooms even though its uses in human nutrition have not been reported yet. *R. alboareolata*, *R. delica*, *R. cyanoxantha*, and *R. densifolia*. have popularly been consumed by local inhabitants of the lower North of Thailand. Some other edible mushrooms including *A. princeps*, *B. colossus*, *L. squarrosulus*, *T. fuliginosus*, and *B. edulis* possess low quantities of beta glucan. However, their nutritional composition and other therapeutic effects (Kues and Liu 2000; Kalac 2009) provide significant health benefits in human diets.

Species ordination

Canonical Correspondence Analysis (CCA) is a direct gradient analysis (ordination) technique used for multivariate analysis of ecological community data (Palmer 1993; McCune 1997). The result of species ordination showed that the variance of the mushroom species explained by the three CCA axes was 7.3%. This very low value could be attributed to high noise levels typical of species-abundance data (Ter Braak 1986). There are several ways that noise can be generated in an ecological data set. It can result from

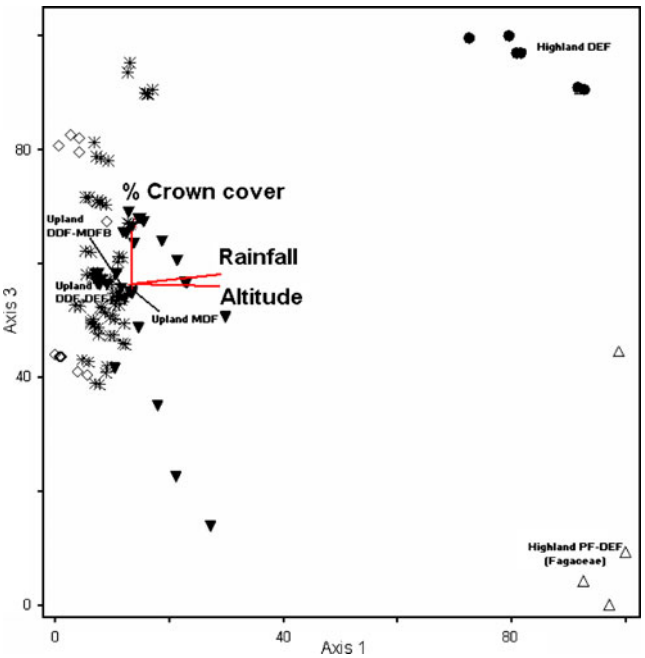

Fig. 5 CCA plot ordination of axis 1 and 3 with the mushroom community types derived from hierarchical cluster analysis. (*), Group1: Upland DDF-MDFB; (◇), Group 2: Upland DDF-DEF; (▼), Group 3: Upland MDF; (△), Group 4: Highland PF-DEF (Fagaceae); (●), Group 5: The highland DEF. Only the variables with a correlation coefficient higher than 0.5 are presented

measurement error, inadequate sampling intensity, or probably most importantly, stochastic variations of true abundance around the mean or ideal distribution (Gauch 1982a, b; Palmer 1993). The meaningfulness of the environmental variables used in this study was tested by comparing the eigenvalues obtained from CCA and DCA (Table 3). The eigenvalues of the second and the third CCA axes were lower than that of the DCA axes, indicating that the important explanatory site variables were included in the analysis. This result supports the idea based on a previous study by Palmer (1993) in which CCA as an explanatory technique led to a reasonable interpretation of important gradients in a few dimensions.

Several primary environmental factors such as vegetation, soil characteristics, forest stand, and microclimate were found contributing to wild mushroom habitat and significantly correlated with their distribution (Bergemann and Largent 2000). Thus it can be considered that the low value of variance explained in this study was probably due to inadequate environmental factors in the analysis. However to improve the species ordination and contribution to the variance explained, other important variables such as soil properties, characteristics of mushroom hosts and vegetation, as well as local environmental conditions should be additionally included. Importantly, even though the species-environment correlations are very high at the first three axes (Table 3), it should not be interpreted literally as a measure of the strength of the relationship between species and the environment. A similar study conducted by McCune (1997) has suggested applying a randomization test for statistical significance.

In this study, pairs of environmental variables including Shannon-Wiener Index-Crown cover percentage (r= 0.822) and altitude-rainfall (r=0.875), were still retained in the analysis even though they show high inter-correlation. It could be seen that the signs of altitude and rainfall were different in the intraset correlation at Axis 2 (Table 4). Moreover, the Shannon-Wiener index and crown cover percentage showed relatively high correlation with Axis 2 and 3 (Table 4). The influence of these two pair variables on the correlations of the environmental variables and ordination axes were proved by CCA ordination (Axis 2 and 3) which showed fair differentiation of the tropical pine forest, mixed deciduous forest with bamboo, and deciduous dipterocarp forest from the other forest types (Fig. 6).

It can be concluded that CCA was an appropriate ordination technique for this study to analyze the distribution of wild mushrooms associated with important environmental factors since unusual sampling designs were employed and there were situations where not all factors known to determine species composition were identified.

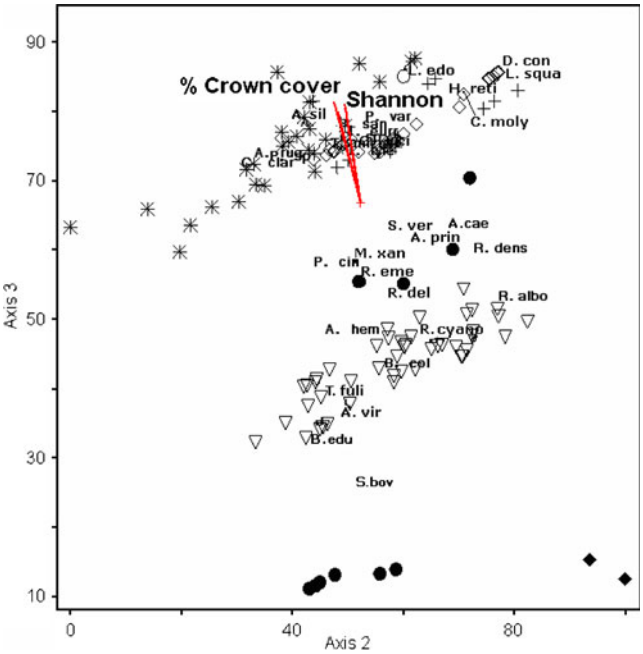

Fig. 6 Ordination diagram of Axis 2 and 3 based on CCA of wild mushroom species with respect to two environmental variables (Shannon-Wiener index and crown cover percentage) on seven different forest types. (*), dry evergreen forest; (◇), dry evergreen forest dominated by Fagaceae; (+), mixed deciduous forest; (▽), mixed deciduous forest with bamboo; (●), deciduous dipterocarp forest; (♦), tropical pine forest; (○), tropical grassland & savanna. Only the variables with a correlation coefficient higher than 0.5 are presented. For species abbreviations see Table 2

Mushroom community type-environmental relationships

The classification of mushroom community types derived from cluster analysis and corroborated by CCA results distinguish mushroom communities more effectively (Figs. 4 and 5). In particular, the CCA ordination diagram of the first three axes clearly showed the separation of mushroom community types Group 4 and Group 5. In contrast, there was a certain degree of overlapping between communities in Group 1, 2 and 3 as shown in plot ordination of both Axis 1–2 and Axis 1–3.

The vector of crown cover percentage could not be seen as an important factor in plot ordination of Axis 1–2, but it was important in the plot of Axis 1–3. Plot ordination of Axis 1 and 3 therefore revealed that crown cover percentage, rainfall and altitude were the most important factors for clear separation of mushroom community Group 4 and Group 5. It can be seen that Group 4 and Group 5 both appear at high altitude with high rainfall, whereas Group 5 was present in an area with high percentage in crown cover but Group 4 could grow in low crown cover percentage. Considering beta glucan content, all mushroom species in Group 5 contain quite high beta glucan amounts (0.35–0.45%) and are mostly found in high land with high rainfall and crown cover percentage.

However some beta glucan containing species belonged to other mushroom groups that grow at lower altitude and rainfall. Thus it is not clear that altitude and rainfall solely affect the beta glucan content of mushrooms but they do strongly influence the mushroom community type. This is supported by a number of studies revealing the relationship between environmental factors and distribution of various plant communities including mushrooms (Wiensczyk and Berch 2001; Dreisbach et al. 2002).

Our study thus concludes that the highest probability of finding mushrooms with high beta glucan content will be in a habitat characterized by high altitude with high crown cover percentage and rainfall. This finding supports the idea that promoting the sustainable use of wild mushrooms as nutritious foods and pharmaceutical necessitates the wise management of mushroom habitat.

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