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The diversity of coprophilous fungi from Dahuadian and Zhongdian grasslands, Yunnan, China

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Abstract – The coprophilous fungi on cattle dung (*Bos taurus*) were investigated at Dahuadian and Zhongdian grasslands, in Yunnan Province, China. Fifty dung samples from Dahuadian and 50 from Zhongdian were collected and examined for fungi. A total of 61 species were recorded in this study (47 species from Dahuadian and 27 from Zhongdian) including 15 new records for mainland China. Species occurrence, species frequency, species richness, Shannon-Weiner index (H'), species evenness (E) and Sørensen's index of similarity (S') from the two grasslands were calculated. The fungal diversity from Dahuadian (altitude: 2940 meters) was higher than that from Zhongdian (altitude: 3300 meters). The proportion of ascomycetes (70%) to basidiomycetes (19%) from Zhongdian was much larger than the proportion of ascomycetes (54%) to basidiomycetes (15%) from Dahuadian. The reason for differences in fungal communities are discussed.

Ascomycetes / Basidiomycetes / Coprophilous fungi / Fungal community / Fungal diversity / Hyphomycetes / Zygomycetes

INTRODUCTION

Coprophilous fungi are an important component of the ecosystem, responsible for recycling the nutrients in animal faeces (Richardson, 2001). Animal dung is easy to collect and excellent for ecological study and therefore many mycologists have been attracted to study dung fungi (Webster, 1970).

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Cain (1934) studied the coprophilous sphaeriales from 400 collections in Ontario, and many new taxa were discovered. Lundqvist (1972) reported 18 genera and 100 species of Nordic Sordariaceae (mostly coprophilous), while Angel & Wicklow (1975) examined 93 dung samples from four animal species from semi-arid grasslands in Colorado and found 37 species. Angel & Wicklow (1983) investigated the coprophilous fungal communities in semi-arid to mesic grasslands and found that the composition of the fungal community varied according to the age of the dung collected. Caretta *et al.* (1998) identified 59 coprophilous species from Kenya and Wang (2000a) reported 90 ascomycetes from dung samples in Taiwan. Richardson (2001) recorded fungi developing on dung samples from a wide range of locations, and highly significant differences were found among the mycota of different dung types, from different latitudinal ranges, and those collected at different seasons. Hu *et al.* (2006) reported on the coprophilous genera *Podospora* and *Schizothecium* from mainland China, while Jacobs and Botha (2008) reported a new coprophilous species from Southern Africa. Kruys & Ericson (2008) reported the coprophilous mycota in the boreal forest of Sweden, and found that there was a strong positive relationship between the total number of ascomycete species and the number of plant species foraged by the herbivores.

Most of the previous studies on coprophilous fungi concentrated on differences of hosts and geographic distribution, or were descriptive. There is little information on the effect of altitude on the distribution of coprophilous fungi. There have also been few studies of coprophilous fungi in mainland China (Hu *et al.*, 2006). In the present study, we establish the diversity of dung fungi at two sites, Dahuadian and Zhongdian grasslands, and provide data on the distribution of coprophilous fungi in relation to altitude.

MATERIALS AND METHODS

Study sites

Dahuadian grassland (25º53'N, 100º00'E, altitude 2940 m) is located in Dali County, West of Yunnan Province, China. The animals on the grasslands are mostly cattle (*Bos taurus*). The cattle were hurded to the pasture by the farmers to feed during the daytime, and returned to the barn at night. This area is surrounded by mountains, which are covered with bush as well as established forests. The soil contains sufficient water and the annual precipitation is about 1846.4 mm. The average annual temperature is 8.2ºC. The main plant cover comprisies *Corydalis balfouriana*, *Cardamine calcicola*, *Rodgersia henrici*, *Geranium forrestii*, *Daphne* spp., *Potentilla fulgens*, *Duchesnea indica, Astragalus sinicus, Rhododendron decorum*, *Pyrola forrestiana*, *Achillea wilsoniaana, Carduus acanthoides, Cirsium griseum, Taraxacum mongolicum, Primula pseudodenticulata, Roscoea tibetica, Juncus bufonius, Dactylis glomerata, Cyperus difformis* and *Eulalia speciose*.

Zhongdian grasslands (26°51'-28°52'N, 99°20'-100°19'E, altitude about 3300 m) is located in DeQin County, Northwest of Yunnan Province, China. It is a cold highland. The average annual temperature is 5.4°C; the lowest temperature was –27.4°C (December 27th, 1982); the highest temperature 25.1°C (August 3rd, 1977); the average temperature in the hottest month 13.3°C; and the average

temperature in the coldest month is –3.8°C. The annual rainfall is 617.6 mm while the annual sunshine is 2203 hours. The average annual relative humidity is 70%. The pasture mostly comprises *Iris* spp., *Primula* spp., *Pedicularis* spp., *Ligularia* spp., *Aster* spp., *Rheum* spp., *Sambucus* spp., Liliaceae, Gentianaceae, Leguminosae and Gramineae.

Sample collection and incubation

Fifty dung samples were collected respectively from Dahuadian and Zhongdian in December 2004. Samples were recently dropped and unweathered. The samples were gently air dried and stored in sealed plastic bags until incubation. Then the dung samples were placed on a filter paper in Petri dishes ("moist chamber") and slightly wetted with tap water (Lundqvist, 1972), and incubated under ambient light and at room temperature (ca. 18-26°C). Samples were examined every week during the incubation period until no new fungi appeared (usually after 48 days). The fruiting bodies were picked off with a sterilized needle or forceps. Identification was made based on living specimens mounted in water using refs. Dry specimens and permanent slides of some species were also prepared and are held in Yunnan University Herbarium (YNU).

Ecological analysis

The species-area curves were plotted for the two collections to examine the sample size (Begon *et al*., 1993). The number of species, the occurrence and the frequency (F) were recorded and calculated. To compare the fungal communities between the two sites, species richness and species abundance were calculated. Shannon-Weiner index (H') was applied to evaluate the diversities of coprophilous fungi, including species richness and evenness (E). Sørensen's index of similarity (S') was plotted to evaluate different fungal communities and expressed with values between 0 (no similarity) and 1 (absolute similarity). The above data are calculated using the following formulas:

$$H' = -\sum_{i=1}^{s} Pi \log_e Pi, \text{ where } Pi = N_i / N$$

$$E = e^{H'} / S$$

$$S' = \frac{2c}{a + b}$$

Ni = the individual number of *i*th species;
N = the individual number of all species;
Pi = the proportion of *i*th species;
$Log_e Pi$ = the natural logarithm of Pi;
S = species number;
a = total number of species from site 1;
b = total number of species from site 2;
c = number of common species to both sites.

RESULTS

Sample size

Species cumulative curves reached asymptote (Fig. 1) at about 30 samples and therefore 50 samples provided a reasonable estimate of coprophilous fungal communities at Dahudian and Zhongdian.

Fungal communities in Dahuadian pasture and Zhongdian pasture

Sixty-one taxa were recorded from the Dahuadian and Zhongdian pastures (Table 1) including 15 species new for mainland China (newly recorded species are marked with an *). They comprised 35 ascomycetes, 8 basidiomycetes, 14 anamorphic taxa and 4 zygomycetes. Fourty-seven taxa were identified from Dahuadian and 27 from Zhongdian; their frequency of occurrence is listed in Table 1. The species richness, species richness per sample and diversity index were calculated and are listed in Table 2.

The coprophilous fungi community in Dahuadian comprised 25 ascomycetes, 7 basidiomycetes, 12 anamorphic taxa and 3 zygomycetes. The most common genera from Dahuadian were *Podospora*, *Saccobolus*, *Schizothecium*, *Coprinus* and *Mucor*. Dominant species were *Schizothecium curvuloides* (85%), *Mucor* sp. 1 (68%) and *Saccobolus citrinus* (40%). The most common ascomycetes were from Ascobolaceae, Lasiosphaeriaceae, and Sporormiaceae; the most common basidiomycetes were from Coprinaceae; the most common zygomycetes were from Mucoraceae. There were 12 anamorphic taxa and most were not common.

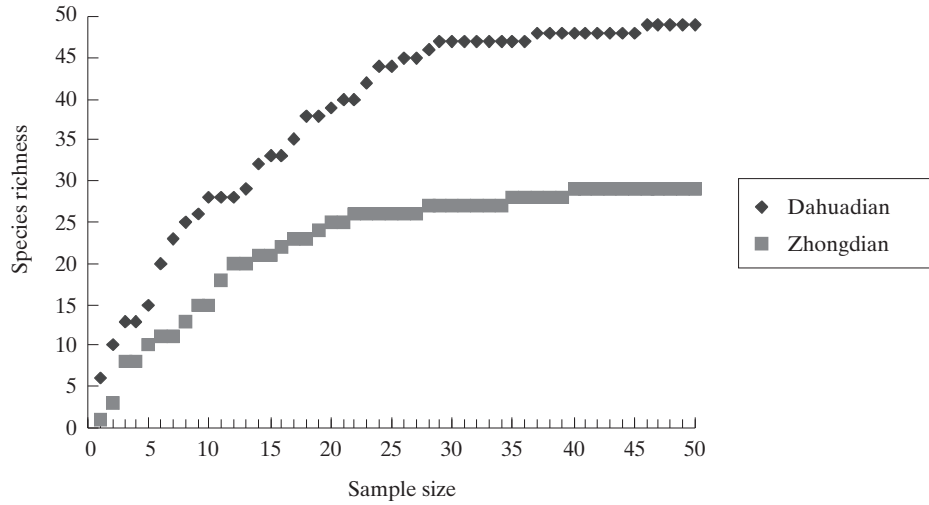

Fig. 1. Sample size.

Table 1. Fungal list (*the new records of mainland China)

Fungi	*Occurrence*		*Relative frequency*	
	Dahuadian	*Zhongdian*	*Dahuadian*	*Zhongdian*
Ascomycetes				
Ascobolaceae				
**Saccobolus citrinus* Boud. & Torrend	20	18	0.082	0.161
**Saccobolus glaber* (Pers.) Lambotte	1		0.004	
**Saccobolus minimus* Velen.	1		0.004	
Coniochaetaceae				
Coniochaeta discospora (Auersw. ex Niessl) Cain		5		0.045
Lasiosphaeriaceae				
**Apiosordaria verruculosa* (C.N. Jensen) Arx & W. Gams		1		0.009
Arnium arizonense (Griffiths) N. Lundq. & J.C. Krug		5		0.045
**Cercophora mirabilis* Fuckel		4		0.036
**Cercophora acanthigera* (Berk. & Broome) N. Lundq.	2		0.008	
**Cercophora areolata* N. Lundq.	2		0.008	
**Cercophora californica* (Plowr.) N. Lundq.	1		0.004	
**Cercophora septentrionalis* N. Lundq.	8		0.033	
Podospora anserina (Rabenh.) Niessl		1		0.009
Podospora argentinansis (Speg.) Mirza, J. H. and Cain, R. F.	11	5	0.045	0.045
Podospora communis (Speg.) Niessl	4	2	0.016	0.018
Podospora fimiseda (Ces. & DeNot.) Niessl	15		0.061	
Podospora pleiospora (Winter) Niessl	2	1	0.008	0.009
Podospora pyriformis (Bayer) Cain	4	3	0.016	0.027
Podospora sp. 1		2		0.018
Podospora sp. 2		1		0.009
Schizothecium aloides (Fuckel) N. Lundq.	2	2	0.008	0.018
Schizothecium curvuloides (Cain) L. Cai	40		0.164	
Schizothecium dakotensis (Griff.) N. Lundq.	1	8	0.004	0.071
Schizothecium miniglutinans (J.H. Mirza & Cain) N. Lundq.	8	15	0.033	0.134
Zygospermella insignis (Mouton) Cain		5		0.045
Pezizaceae				
Iodophanus carneus (Pers.) Korf	1		0.004	
Pyronemataceae				
**Coprobia granulata* (Bull.) Boud.	1		0.004	
Coprotus aurora (Cr. & Cr.) Thind & War.	1		0.004	
Coprotus granuliformis (P. Crouan & H. Crouan) Kimbr.	2		0.008	
Coprotus sp.		2		0.018
**Coprotus trichosurus* Bell & Kimbr.	1		0.004	

Table 1. Fungal list (*the new records of mainland China) *(continued)*

Fungi	*Occurrence*		*Relative frequency*	
	Dahuadian	*Zhongdian*	*Dahuadian*	*Zhongdian*
Sporormiaceae				
Preussia sp. 1	1		0.004	
**Sporormiella australis* (Speg.) Ahmed & Cain		2		0.018
**Sporormiella intermedia* (Auersw.) Ahmed & Cain	11	9	0.045	0.08
**Sporormiella longispora* (Cain) Ahmed & Cain	1		0.004	
**Sporormiella octomera* (Auersw.) Ahmed & Cain	2		0.008	
Basidiomycetes				
Bolbitiaceae				
Conocybe pubescens (Gillet) Kühner	2	1	0.008	0.009
Coprinaceae				
Coprinus miser P. Karst.	1	6	0.004	0.054
Coprinus patouillardii Quél.	14	2	0.057	0.018
Coprinus poliomallus Romagn.	4		0.016	
Coprinus sp. 1	8		0.033	
Coprinus stercoreus Fr.	1		0.004	
Strophariaceae				
Psilocybe coprophila (Bull.) P. Kumm.	1	3	0.004	0.027
Psilocybe sp.		1		0.009
Coelomycetes				
Coelomycete sp. 1	6		0.025	
Hyphomycetes				
Arthrobotrys oligospora Fresen.		1		0.009
Chrysosporium merdarium (Ehrenb.) J.W. Carmich.	4		0.016	
Hyphomycete sp. 1-6	9		0.032	
Monacrosporium ellipsosporum (Preuss) R.C. Cooke & C.H. Dickinson		6		0.054
Penicillium sp. 1	2		0.008	
Penicillium sp. 2	2		0.008	
Phoma sp. 1	8		0.033	
Verticillium sp. 1	2		0.008	
Zygomycetes				
Mucoraceae				
Mucor mucedo Fresen.	2		0.008	
Mucor sp. 1	34		0.139	
Rhizopus sp. 1	1		0.004	
Rhizopus sp. 2		1		0.009
Total	244	112	1	1

Table 2. Species richness, species richness per sample and diversity index

Sampling sites	*Dahuadian Pasture*	*Zhongdian Pasture*
Altitude	2940 m	3300 m
Sample size	50	50
Number of ascomycetes	25	19
Number of basidiomycetes	7	5
Number of anamorphic fungi taxa	12	2
Number of zygomycetes	3	1
Average number of taxa per sample	4.88	2.24
Unique species	34	14
Overlapping species in two sites	13	
Five most common species	*Schizothecium curvuloides* *Mucor* sp.1 *Saccobolus citrinus* *Podospora fimiseda* *Coprinus patouillardii*	*Saccobolus citrinus* *Schizothecium miniglutinans* *Sporormiella intermedia* *Schizothecium dakotensis*
Species richess	47	27
H’	3.128	2.904
S’	0.351	

The coprophilous fungi community in Zhongdian comprised 19 ascomycetes, 5 basidiomycetes, 2 anamorphic taxa and 1 zygomycete. The most common genera from Zhongdian were *Saccobolus*, *Schizothecium*, *Podospora*, and *Sporormiella*. Dominant species were *Saccobolus citrinus* (36%) and *Schizothecium miniglutinans* (30%), *Sporormiella intermedia* (18%). The most common ascomycetes were from Ascobolaceae, Lasiosphaeriaceae, and Sporomiaceae; the most common basidiomycetes were from Coprinaceae.

Difference in fungal communities with altitude

The fungal communities identified at the two sites are compared in Table 2 and differences between the two sites are significant. Many more taxa were identified from Dahuadian as compared to Zhongdian (47 species vs. 27 species). The fungal diversity from Dahuadian was higher than that from Zhongdian (Shannon-Weiner indices: 3.128 vs. 2.904). Evenness of Zhongdian was higher than at Dahuadian (0.676 vs. 0.486). Thirteen species overlapped between the two sites (S’ = 0.351). Of the five most common species from the two sites, only one species (*Saccobolus citrinus*) overlapped.

DISCUSSION

Fungal communities

The number of taxa identified (61) from the two grasslands were mostly ascomycetes (57.4%), which is in agreement with previous studies (Caretta *et al.*, 1994; Richardson, 2001). Anamorphic taxa were the second largest group (22.9%), followed by basidiomycetes (13.1%) and zygomycetes (6.6%).

Ascomycetes

Ascomycetes are the largest group of fungi (Kirk *et al*., 2001), and a large proportion of coprophilous species belong to this group. They include discomycetes, plectomycetes and pyrenomycetes (Bell, 1983), although this terminology is rather dated. Coprophilous ascomycetes have several adaptive characters for survival on dung (Bell, 1983), which includes dark spores, phototropism, and sticky appendages. *Podospora* species were very common, and 8 species were recorded in this study. *Podospora fimiseda* (30%) was the third most common species in Dahuadian. There has been much confusion concerning the genera *Podospora* and *Schizothecium*. Lundqvist (1972) divided them to two genera and stated that *Schizothecium* was characterized by and distinguished from *Podospora* principally by the swollen agglutinated hairs or prominent protruding peridial cells, lack of interascal filiform paraphyses, early spore septation, and long persistent, plasma-filled pedicels. Although most mycologists have not accepted *Schizothecium* as a genus (Krug & Khan, 1989; Bell & Mahoney, 1995; Wang, 2000b), Cai *et al*. (2005) re-evaluated the genus using molecular data and found that *Schizothecium* species constitute a natural grouping and merit generic rank. *Schizothecium* was the most common genus in our study. *Schizothecium curvuloides* (80%) was the most common species at Dahuadian, *Schizothecium miniglutinans* (30%) was the second most common species at Zhongdian, and *Schizothecium dakotensis* (16%) was the fourth most common species at Zhongdian.

Saccobolus was also a common genus at the study sites. Among the five most common species in the two pastures, only *Saccobolus citrinus* was found at both Dahuadian and Zhongdian. The other common genera were *Cercophora* (5 species), *Coprotus* (4 species) and *Sporormiella* (4 species).

Basidiomycetes

Of all the 8 Basidiomycete species, 5 species were from the genus *Coprinus*. Most previous studies on coprophilous fungi show that *Coprinus* was the dominant agaric developing on dung (Bell, 1983; Richardson, 2001), and this was confirmed in our study. *Coprinus miser* is widespread on dung following incubation (Richardson, 2000); this taxon occurred at both sites in this study, but it was not the most frequent species. The most frequent agaric was *C. patouillardii*. *Conocybe* and *Psilocybe* were also recorded but were not common.

Anamorphic taxa

Most anamorphic taxa could not identified to species level. *Arthrobotrys oligospora* and *Monacrosporium ellipsosporum* are nematode trapping fungi (Li *et al*., 2000) and were recorded only from Zhongdian.

Zygomycetes

Pilobolus is a common coprophilous genus but we did not observe any species of *Pilobolus* in this study. There is no explanation for this, but the fact that it took five days to transport the dung to Kunming may be the reason, as *Pilobolus* is an early colonizer of dung (Bell, 1975; McCarthy, 2000; Dickinson, 1977; Nagy & Harrowerr, 1979). Only *Mucor* and *Rhizopus* species were recorded in our study.

Difference in fungal communities with altitude

The diversity of coprophilous fungi can be affected by many factors, such as temperature, humidity, light, host, latitude, longitude and altitude. The affect of latitude on coprophilous fungi has previously been investigated (Cain, 1934; Lundqvist, 1972; Richardson, 2001). However, few studies have examined the affect of altitude on dung fungi. The two sites studied were at a similar geographic latitude (25°53'N, 100°00'E vs. 26°51'-28°52'N, 99°20'-100°19'E). Samples collected from the two sites were cattle dung and samples were incubated in the same way. Dahuadian and Zhongdian are at high altitude (above 2900 meters), but Zhongdian is higher and colder than Dahuadian (3300 m vs. 2940 m). The index of fungal diversity from Dahuadian was much higher than that from Zhongdian, and more species were recorded from Dahuadian than from Zhongdian (Table 2). Species evenness at Zhongdian was much higher than that from Dahuadian (Table 2) indicating that there were less dominant species from Zhongdian. In conclusion we identified different fungal communities from dung from the two sites and this is probably due to the temperature difference. The average annual temperature of Zhongdian (5.4ºC) is much lower than that of Dahuadian (8.2ºC).

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