Biodiversity of arbuscular mycorrhizal fungi in a tropical rainforest of Xishuangbanna, southwest China

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The tropical rainforests of Xishuangbanna in southwestern China are located at the northern margin of the tropical rainforests of Southeast Asia. They harbour a high diversity of animals and plants. We investigated the diversity of arbuscular mycorrhizal fungi in soil under trees in these forests in order to establish if these fungi are also highly diverse. One hundred and eighteen rhizosphere soil samples were collected from a tropical rainforest in Xishuangbanna, and 525 arbuscular mycorrhizal fungal spores (or sporocarp) samples were obtained using the wet-sieve method. Twenty-seven species of arbuscular mycorrhizal fungi were identified from the collections. The species of arbuscular mycorrhizal fungi were of the genera *Acaulospora* (9 species), *Gigaspora* (1 species), *Glomus* (13 species), *Sclerocystis* (3 species) and *Scutellospora* (1 species). *Acaulospora* and *Glomus* were dominant at the study site. The arbuscular mycorrhizal fungi spore density ranged from 25 to 2550 per 100 g soil (average 675), and the species richness of arbuscular mycorrhizal fungi ranged from1-7 (average 4.4). Although tropical rainforests support a high diversity of plants, their associated symbiotic fungi are not as diverse as we had expected, possibly because arbuscular mycorrhizal fungi are not specific to their host plants.

Key words: rainforest, arbuscular mycorrhizal fungi

Introduction

Arbuscular mycorrhizal fungi constitute an important component of the soil microbial community and are extremely successful fungi that form mutualistic symbioses with about two thirds of all plant species (Trappe, 1987). They improve plant nutrition and promote plant diversity (Van der Heijden *et al*., 1998), help to control pests and fungal pathogens (Azcon-Aguilar and Barea, 1996) and affect the fitness of plants in polluted environments (Hildebrandt *et al*., 1999). They can even alter the folia chemistry and

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influence the life history traits of lepidopteran herbivores (Goverde *et al*., 2000). In nutrient-poor soil of the humid tropics, many plants are obligately or ecologically dependent on arbuscular mycorrhizal fungi (Gemma *et al*., 2002). Therefore, it is important to study the biodiversity of arbuscular mycorrhizal fungi as this can improve understanding of tropical forest functioning, plant succession and reforestation in disturbed areas. Tropical rainforests harbour a high diversity of animals and plants and there has been much research on the diversity of large plants and animals in these habitats. However, little attention has been paid to the diversity and roles of microfungi, including arbuscular mycorrhizal fungi in this ecosystem (Hsu and Agoramoorthy, 2001; Zhao *et al*., 2001).

Tropical rainforests are important habitats for arbuscular mycorrhizal fungi (Read, 1994). The tropical rainforests of Xishuangbanna, southwest China are located at the north margin of the tropical zone of Southeast Asia. Since this is a type of transitional vegetation (from tropical to subtropical zone), tropical and subtropical flora coexist; and the habitat contains more species diversity than tropical rainforests of Southeast Asia (Jin and Ou, 1997). It has been found that 434 plant species are condensed in 40000 m^2 (Jin, 1997). Arbuscular mycorrhizal fungi are believed to be obligate biotrophs that are wholly or partly dependent on the plant partner for their carbon supply (Hodge *et al*., 2001). In this study we wanted to establish if there is also a high diversity of arbuscular mycorrhizal fungi in the soil under this diverse vegetation and what types of arbuscular mycorrhizal fungi are found in this habitat. In this way we can contribute data to the global debate on fungal diversity (Aptroot, 2001; Hawksworth 2001; Hyde 2001).

Materials and methods

One-hundred and eighteen soil samples were collected from different plant rhizospheres to a depth of 5-30 cm from a tropical rainforest in (21°45'N, 101°02'E) in January 2000 (dry season). The samples (about 500g for each) were air-dried for 2 weeks and stored in sealed plastic bags at 4ºC for up to 6 months until samples could be treated.

The soil samples were wet-sieved for spores using the method described by An *et al*. (1990). Twenty grams of soil was independently suspended in 250 ml of water, stirred with a magnetic stirrer for 10 minutes and the suspension sieved. Spores and debris were collected on 40 µm, 70 µm, 100 µm and 150 µm sieves with tap water, filtrated onto a filter paper, then placed in a 9 cm Petri-dish for examination under a binocular stereomicroscope. Arbuscular mycorrhizal fungi spores were counted in the four sieve samples. Some spores

were tightly grouped in sporocarps and it was difficult to count the number of spores per sporocarp, so to simplify this procedure, we referred to a sporocarp as one spore.

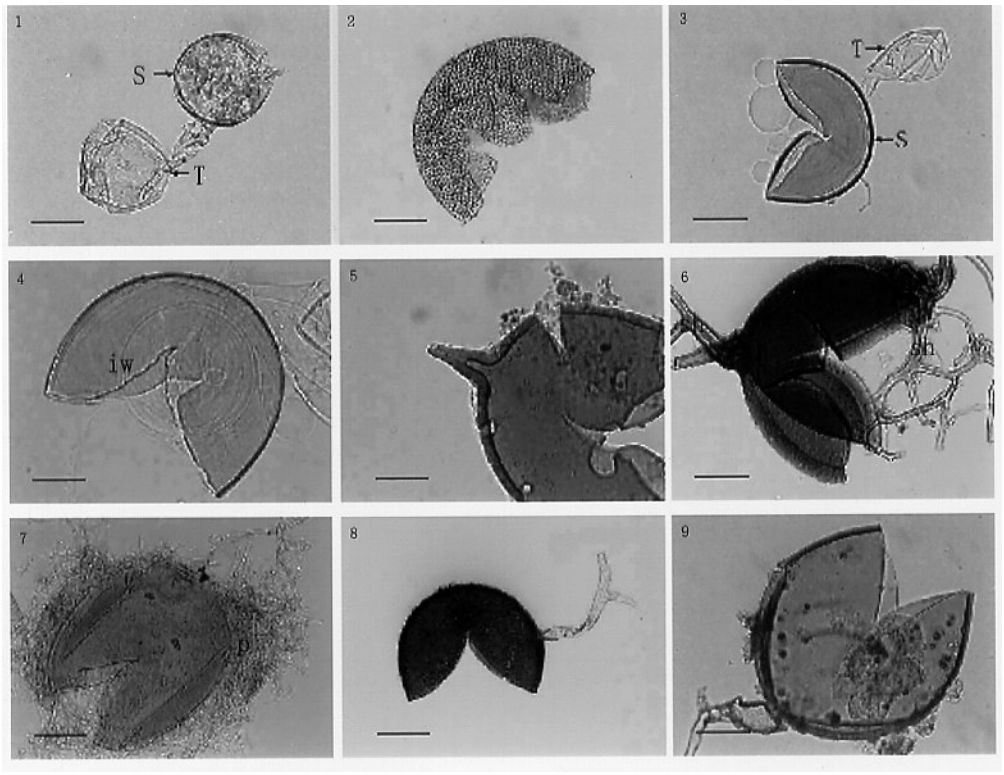
Each type of spore was mounted in water, lactophenol, PVA and Melzer's reagent respectively (Morton, 1988; Morton and Benny, 1990) for identification. The identification was based on spore colour, size, surface ornamentation and wall structure with reference to the descriptions and pictures provided by the International Collection of Vesicular and Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu) and originally published species descriptions.

Some spore specimens could not be identified to species as only a few spores were isolated, or the spores lacked distinguishable, fine taxonomic characters. These spores were statistic in spore density (spores in 100 g soil), and not statistic in species richness (species numbers in each soil sample). The occurrence frequency of each identified species was calculated by the number of occurrences of a species, divided by the total number of identified spore samples.

Results and discussion

Arbuscular mycorrhizal fungi species and their occurrence frequencies

Five-hundred and twenty-five arbuscular mycorrhizal fungal spore (or sporocarp) samples were wet-sieved from the 118 soil samples, from which 27 species of arbuscular mycorrhizal fungi were identified. The morphological characters of some identified arbuscular mycorrhizal fungi were illustrated in Figures 1-9. The species diversity of arbuscular mycorrhizal fungi was not as high as we had previously thought, as 27 species of arbuscular mycorrhizal fungi were far less than the 118 host plants examined. This low diversity of arbuscular mycorrhizal fungi species has also been reported elsewhere (Walker *et al*., 1982; Bever *et al*., 1996; Sanders *et al*., 1996). Although arbuscular mycorrhizal fungi have an ancient origin and a long coevolutionary history with plant roots (Brundrett, 2002), their asexual reproductive mechanism (Sanders *et al*., 1996) and divergence from the same ancestor appear to have restricted these monophyletic fungi (Schübler *et al*., 2001) from developing high species diversity. Arbuscular mycorrhizal fungi are not thought to be host specific (Mosse, 1973). This means that one species of arbuscular mycorrhizal fungi can form mutualistic associations with many plant species, which ensures that although the species diversity of arbuscular mycorrhizal fungi is low, most



Figs. 1-9. Various arbuscular mycorrhizal fungi from a tropical rainforest in Xishuangbanna. **1, 2.** *Acaulospora scrobiculata*. **1.** Spore (S) and the soporiferous saccule (T). **2.** The pitted spore wall. **3, 4.** *Acaulospora laevis*. **3.** Spore (S) and the soporiferous saccule (T). 4. Inner membranous wall (iw). **5.** *Glomus mosseae*, spore and funnel-like subtending hypha. **6.** *Glomus multicaule*, crushed spore and subtending hyphae (sh). **7.** *Glomus monosporum*, one sporocarp contains one spore, the peridium (p) of the sporocarp was interwoven by hyphae. **8.** *Glomus constrictum*, spore and constricted subtending hypha. 9. *Scutellospora heterogama*, spore and the suspensor-like cell. Bars = 50 μm.

plants in the tropical rainforest of Xishuangbanna were colonized by arbuscular mycorrhizal fungi (Zhao *et al*., 2001).

The identified species of arbuscular mycorrhizal fungi belonged to the genera of *Acaulospora* (9 species), *Gigaspora* (1 species), *Glomus* (13 species), *Sclerocystis* (3 species) and *Scutellospora* (1 species). The occurrence frequency of the five genera was 47.81%, 0.76%, 48.19%, 2.47% and 0.76%, respectively (Table 1). The results indicated that *Acaulospora* and *Glomus* were the dominant genera, and *A. denticulata*, *A. foveata*, *A. spinosa*, *A. tuberculata*, *G. claroideum*, *G. clarum*, *G. constrictum* and *G. monosporum* were the dominant species in the tropical rainforest of Xishuangbanna (Table 1). The fact that *Acaulospora* and *Glomus* are dominant genera in

Table 1. Identified arbuscular mycorrhizal fungi and their occurrence frequencies

No.	Arbuscular mycorrhizal fungi	Occurrence times	Occurrence frequency (%)
	Acaulospora	**251**	**47.81**
1	*A. bireticulata* Rothw. & Trappe	19	3.62
2	*A. denticulata* Sieverding & Toro	87	16.57
3	*A. foveata* Trappe & Janos	22	4.19
4	*A. laevis* Gerdemann & Trappe	4	0.76
5	*A. mellea* Spain & Schenck	4	0.76
6	*A. morrowae* Spain & Schenck	2	0.38
7	*A. scrobiculata* Trappe	16	3.05
8	*A. spinosa* Walker & Trappe	64	12.19
9	*A. tuberculata* Janos & Trappe	33	6.29
	Glomus	**253**	**48.19**
10	*G. aggregatum* Schenck & Smith	3	0.57
11	*G. claroideum* Schenck & Smith	37	7.05
12	*G. clarum* Nicol. & Schenck	21	4.00
13	*G. constrictum* Trappe	29	5.52
14	*G. etunicatum* Becker & Gerd.	4	0.76
15	*G. fasciculatum* (Thaxter) Gerd. & Trappe	13	2.48
16	*G. geosporum* (Nicol. & Gerd.) Walker	8	1.52
17	*G. leptotichum* Schenck & Smith	4	0.76
18	*G. microcarpum* Tul. & Tul.	3	0.57
19	*G. monosporum* Gerd. & Trappe	112	21.33
20	*G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe	5	0.95
21	*G. multicaule* Gerd. & Bakshi	10	1.90
22	*G. rubiforme* (Trappe & Gerd.) Almeida & Schenck	4	0.76
	Gigaspora	**4**	**0.76**
23	*G.. gigantea* (Nicol. & Gerd.) Gerd. & Trappe	4	0.76
	Sclerocystis	**13**	**2.47**
24	*S. clavispora* (Trappe) Almeida & Schenck	3	0.57
25	*S. coremioides* Berk. & Broome	8	1.52
26	*S. sinuosa* (Gerd. & Bakshi) Almeida & Schenck	2	0.38
	Scutellospora	**4**	**0.76**
27	*S. heterogama* Walker & Sanders	4	0.76
Total: AMF = 27 species		**525**	**100**

Xishuangbanna must be related to their sporogenous characteristics. It has been found that *Acaulospora* and *Glomus* species usually produce more spores than *Gigaspora* and *Scutellospora* species in the same environment (Bever *et al*., 1996). Because of their smaller spore size, *Acaulospora* and *Glomus* species

require a short time to produce spores than *Gigaspora* and *Scutellospora* species (Hepper, 1984). Humid tropical ecosystems are sustained by a rapid material recycling (Read, 1994). Fungi with a rapid sporogenous cycles may be better adapted to this ecosystem (Hepper, 1984).

Spore density and species richness of arbuscular mycorrhizal fungi

The distribution of the 27 identified species of arbuscular mycorrhizal fungi in the 118 soil samples, the spore density (spores/100g soil) and the species richness per soil sample is given in Table 2. Fungal spore density ranged from 25 to 2550 per 100 g dry soil ($\bar{x}$ = 675) and species richness ranged from 1-7 ($\bar{x}$ = 4.4). The spore density was usually positively related to the species richness. The distribution of arbuscular mycorrhizal fungal species was relatively even, with 86% samples in the range of 3-6 (4.4 ± 1.3). The spore density was uneven, with 74% samples in the range of 235-1015 (675 ± 440). The unevenness of spore density must be due to their different ability of sporulation of the different species of arbuscular mycorrhizal fungi (Bever *et al*., 1996).

Table 2. Arbuscular mycorrhizal fungi species distribution, spore density (SD) and species richness (SR).

Soil sample	Arbuscular mycorrhizal fungi	SD	SR	Soil sample	Arbuscular mycorrhizal fungi	SD	SR
01	9* 19 25	105	3	60	2 9 12 19	550	4
02	2 8 18 19	555	4	61	7 19 25	450	3
03	2 19	150	2	62	2 3 11 19	430	4
04	3 8 19	475	3	63	2 9 19 22	265	4
05	2 4 8 16 19	1195	5	64	1 2 7 8 11 14	1075	6
06	7 13	105	2	65	2 11 13 19	625	4
07	8 19 21	115	3	66	1 2 7 8 13 19	470	6
08	2 11 19 21	595	4	67	2 9 17 19	290	4
09	2 3 8 15 19 21 26	1265	7	68	8 9 12 19	320	4
10	1 8 9 11 19	420	5	69	2 13 19 20	395	4
11	2 8 9 11 19	1095	5	70	2 8 11 19 27	930	5
12	2 3 8 11 19 21 26	2550	7	71	2 7 8 19 20 21	1595	6
13	2 9 19	345	3	72	2 3 8 19 20	835	5
14	2 8 9 10 12 19	670	6	73	2 7 9 13 19 21 24	1330	7
15	2 8 19	270	3	74	6 8 13 19 23	1225	5
16	1 2 9 12 19	830	5	75	3 8 10 12 15 19 23	1240	7
17	2 3 19	470	3	76	2 9 12	155	3
18	1 8 11 16 19	955	5	77	2 3 8 11 12 19	900	6
19	1 3 8 13 19	1015	5	78	2 3 8 13 15 19	1280	6
20	2 17 19 25	275	4	79	1 3 8 11 19	895	5

Table 2. (continued).

Soil sample	Arbuscular mycorrhizal fungi	SD	SR	Soil sample	Arbuscular mycorrhizal fungi	SD	SR
21	2 5 9 13 19 22	1560	6	80	8 9 11 19	390	4
22	1 2 7 19	535	4	81	1 2 8 10 19	475	5
23	5 8 15 19	400	4	82	7 15 19	90	3
24	2 7 12 16 19 27	815	6	83	8 9 16 19	310	4
25	2 13 19 21	500	4	84	1 2 8 14 19	775	5
26	5 8 11 19	520	4	85	1 2 8 13 19	960	5
27	2 16 19	165	3	86	2 8 9 13 15 19	985	6
28	2 12 13 19	235	4	87	2 17 19 21 27	625	5
29	2 9 16 19	760	4	88	2 7 8 11 12 13 19	1270	7
30	2 19	85	2	89	1 2 11 19 22	725	5
31	2 11 13 19 25	500	5	90	2 8 11 19 21	915	5
32	8 11 13 19 25	635	5	91	2 8 9 11 15 19	1445	6
33	2 5 8 12 18 19	880	6	92	3 8 11 19	595	4
34	1 8 16 19	655	4	93	3 8 12 19	470	4
35	2 8 19 24	535	4	94	2 9 14 19	610	4
36	2 7 8 11 13 19	725	6	95	2 19	430	2
37	2 8 9 11 19 20	1175	6	96	2 3 8 12 19 21	1225	6
38	2 8 16 19	750	4	97	2 4 9 11 12 19	1125	6
39	2 8 11 19 27	580	5	98	1 2 3 17 19	555	5
40	2 3 12 13 19	680	5	99	1 2 9 12 13 19	1675	6
41	1 2 8 11 13 19	1015	6	100	2 8 13 19	450	4
42	3 8 9 13 15 19 23	2110	7	101	2 8 9 12 19	860	5
43	1 2 8 11 19	690	5	102	3 4 8 15 19	1340	5
44	2 8 11 19	420	4	103	2 11 19	215	3
45	6 7 9 19	225	4	104	2 8 9 11 13	655	5
46	1 2 19 22	405	4	105	2 8 15 19	785	4
47	3 8 11 19	530	4	106	2 19	85	2
48	2 19	95	2	107	19	30	1
49	8	45	1	108	3 8 12 19 23	625	5
50	2 7 12 18 19	920	5	109	2 3 19	235	3
51	2 19	25	2	110	2 7 13 19	345	4
52	2 19	145	2	111	2 9 11 19 25	665	5
53	8 9 19	470	3	112	2 15 19	275	3
54	2 7 11 13 24	1100	5	113	2 8 11 19	725	4
55	2 7 9 11 13 19	1270	6	114	2 9 11 13 19	820	5
56	2 9 12 19	650	4	115	2 4 8 11 15 19 20	945	7
57	2 19 25	130	3	116	2 8 12 19	550	4
58	1 3 8 11 13 19	1005	6	117	2 8 9 13 19	805	5
59	8 9 14 19	465	4	118	2 15 19 25	475	4
Total: soil samples =118; Average spore density = 675 ± 440; Species richness = 4.4 ± 1.3							

*Numbers in this column refers to the codes of arbuscular mycorrhizal fungi species in Table 1.

There are many factors that could affect spore density and species richness in a given host rhizosphere. Values for arbuscular mycorrhizal fungal spore density associated with different plants at different sites has varied greatly in previous reports (Walker *et al*., 1982; Sylvia, 1986; Koske, 1987). Seasonality, edaphic factors, host-dependence, age of the host plants, the sporulation abilities of arbuscular mycorrhizal fungi, and the dormancy and the distribution patterns of arbuscular mycorrhizal fungal spores in the soils, have been reported previously (Walker *et al*., 1982; Sylvia, 1986; Koske, 1987; Gemma and Koske, 1988; Bever *et al*., 1996; Zhao, 1999). Guadarrama and Alvarez-Sanchez (1999) reported that disturbance, but not seasonality, affects the abundance and richness of mycorrhizal spores in a tropical wet forest in Mexico. The results in this research support our previous suggestions (Zhao *et al*., 2001) that the uneven spatial distribution (clumped distribution) of arbuscular mycorrhizal fungal spores and the complex below ground structure of tropical rainforests are major factors that affect the spore density.

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