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Full Length Research Paper

Diversity of macrofungal community in Bifeng Gorge: the core giant panda habitat in China

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Macrofungi not only play an important role in pollution control and other environmental protection measures, but also an important resource in food and pharmaceutical industries. However, the diversity of the macrofungal community in the core habitat of the giant panda in Bifeng Gorge, China is still inadequate. In the current study, the macrofungal diversity in Bifeng Gorge was investigated using morphologic and molecular biological methods. The results show that 275 species were found which were classified into 122 genera from 52 families. Up to 54.55% of the species were classified into various families, including Russulaceae, Strophariaceae, Agaricacea, Tricholomataceae, Psathyrellaceae, Mycenaceae, Marasmiaceae and Polyporaceae. The microfungi thrive in different areas within the Bifeng Gorge, which could be roughly classified into six ecological communities, namely, evergreen broadleaf forest (I), *Cryptomeria fortunei* forest (II), *Metasequoia glyptostroboides* forest (III), bamboo forest (IV), mixed bamboo- broadleaf forest (V) and grassland (VI). Further studies revealed that the order of the richness index (*R*) was I > II > V > III > IV > VI, evenness index (*E*) and dominance index (*D*) were V > III > IV > I > II > VI, and species diversity index (*H'*) was I > V > III > IV > II > VI. The data suggests that despite the lagged sporophore peak compared with the highest temperature, macrofungal diversity was positively related to the temperature in the area.

Key words: Bifeng Gorge, biodiversity, macrofungi, vegetation type.

INTRODUCTION

Bifeng Gorge is the core habitat of the giant panda in China, located in the North Yucheng District in Ya'an, Sichuan, China. The area is a famous AAAA grade scenic tourist destination. It stands in the lower mountain zone of the Southwestern Qionglai Mountains with rolling hills and abundant creeks. Bifeng Gorge lies on the moist subtropical monsoon region in the mid-latitude inland where the main soil composition of the mountainous yellow soils and the formation is sandy clay rock of the Upper Cretaceous Guankou Formation (Tang et al., 2007). The main vegetation type is the partially humid subtropical evergreen broadleaf forest based on the *Castanopsis–Schima* forest and the Lauraceae forest (Ren and Liu, 2008), and at long intervals, the *Cryptomeria fortune* forest and bamboo, among others. Numerous macrofungi have very high value in research and development.

Macrofungal diversity is an important component of the global biodiversity, particularly community diversity, which is the essential part of fungal diversity. The total fungi were estimated to be more than 150 million, but only 7 million (4.6%) are known, including 1 million macrofungi (Hawksworth, 1991; Kohlmeyer and Volkmann-Kohlmeyer, 2001). The macrofungi differ because of the rich vegetation resources in China, but research on macrofungal diversity has lagged behind when compared with the studies on animal and plant diversity. Macrofungi are principal decomposers and play pivotal roles in the ecological balance (Malinowska et al., 2004; Guillén et al., 2009; Borovička et al., 2010; Gates et al., 2011),

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which could provide a favorable survival environment for pandas, and perhaps the biological diversity in the area because they can only live in the area. However, very little is known regarding the macrofungal diversity in the core habitat of the panda. Up to now, studies focused mainly on the cultivation and growth of macrofungi (Kalmiş and Sargin, 2004; Zhao et al., 2007; Israilides et al., 2008; Fazenda et al., 2008; Lee et al., 2009) and only occasionally on macrofungal diversity (Wang et al., 2009; Fan and Tolgor, 2010).

Moreover, the distribution of vegetation influences the frequency of visits of animals especially pandas, and both plants and animals influence the diversity of macrofungi (Harrington, 2003; Gabel and Gabel, 2007; Wei et al., 2008). Further studies are still necessary to analyze the macrofungal diversity in the different vegetation types in Bifeng Gorge. The present study, therefore, aims to reveal the relationship between the diversity of the macrofungal community and vegetation type, and the influence of monthly temperature on macrofungal quantity. That is, the current study hopes to provide a reference to macrofungi excavated and the survival environment of the panda, which is protected in this special ecological region.

MATERIALS AND METHODS

Sample arrangement

This experiment was established in six representative regions in Bifeng Gorge (102 °90 ′N, 29 °40 ′E, altitude 700 to 1971 m). The climate is moist subtropical monsoon in mid-latitude inland, with a mean annual temperature of 16.1°C and precipitation of 1732 mm. Five 20 × 20 m quadrats were set on each sample. The six samples are as follows:

Sample I, Evergreen Broadleaf Forest (altitude, 700 to 1250 m; pH, 4.25; moisture content, 51.51%; and soil organic matter, 6.10%): It is the most prevalent vegetation type in Bifeng Gorge. The major species are *Schima superba*, *Castanopsis fargesii*, *Quercus* sp., *Betula* sp., *Phellodendron chinense*, *Manglietia fordiana*, *Elaeocarpus chinensis*, and only occasionally, *Cunninghamia lanceolata*. The coverage of undergrowth vegetation area is small, mainly with tree layers, with a few ferns and weeds. The litter is thick.

Sample II, *C. fortunei* Forest (altitude, 1250 to 1450 m; pH, 4.47, moisture content, 34.54%, and soil organic matter, 5.03%): The largest plantation was restored from the early 1990s by planting *C. fortunei*. The main species is *C. fortunei* Hooibrenk and little *C. lanceolata*. Forest canopy density is high, with few ferns and mosses, and hardly any weeds.

Sample III, *Metasequoia glyptostroboides* Forest (altitude, 1450 to 1550 m; pH, 4.32; moisture content, 47.97%, and soil organic matter, 4.82%): The numbers are low, with plaque distribution around Bifeng Temple. The main species are *M. glyptostroboides* in the tree layers and *Camellia japonica* in the shrub layers. In the understory, the euphotic rate is extremely low, with only few ferns and mosses.

Sample IV, Bamboo Forest (altitude 1550 to 1650 m; pH, 4.69; moisture content, 32.52%; and soil organic matter, 1.51%): The difference in height distribution of bamboo was significant in Bifeng Gorge. Aside from the original bamboo, many other species were imported to feed the giant panda. The main species are *Indocalamus latifolius*, *Sasa pygmaea*, *Sinocalamus affinis*, *Phyllostachys* sp., *Fargesia* sp., *Indocalamus longiauritus*, *Dendrocalamopsis* sp., hybrid bamboo and so on. There are many herbs in this forest.

Sample V, Mixed Bamboo-Broadleaf Forest (altitude, 1650 to 1800 m; pH, 4.42; moisture content, 46.19%; and soil organic matter, 4.23%): It is composed of tall evergreen broadleaf trees and relatively low bamboo. The main species of evergreen broadleaf are the trees of the Fagaceae, Lauracea and Theacea family, of which *S. superba* and *C. fargesii* are common. It gives priority to *Phyllostachys* sp. and *Fargesia* sp. among the bamboo.

Sample VI, Grassland (altitude 1800 to 1950 m; pH, 5.64, moisture content, 26.49%; and organic matter in soil, 1.73%): The grassland is sparse and has been interfered with greatly by humans. The main species are *Oplismensus compositus* and *Torenia cordifolia*, among others.

Resources survey

Using connected detailed surveys and reconnaissance surveys, the macrofungi were surveyed in six samples from June 2007 to November 2009. Sporophores were photographed, and the growth locations, time, morphologic characteristics, quantity, altitude and vegetative types of macrofungi were recorded in detail for each quadrat. A frequency of occurrence (*F*) of up to 30% was considered dominant. F was computed using the following formula:

$$F\ (\%) = (n_i / N) \times 100\%$$

Where, n_i is the number of species; *i* and N is the total number of species in each type.

Identification

Macrofungal identification through the comparison of relevant information is essential. The identification of microfungal genera and species was carried out according to the macroscopic and microscopic characteristics of the specimens, as well as their characteristic responses to some chemical reagents (Wei, 1979; Huang, 1998; Mao, 1998, 2000; Liu, 2002; Lin et al, 2005; Yuan and Sun 2007; Diyabalanage et al., 2008; Ouzouni et al., 2009).

Molecular identification was performed using rDNA ITS (Moreau et al., 2006). The following procedure was as follows: (1) DNA was extracted from the sporophores with the improved two-step deposition method (Pan et al., 2011). (2) The rDNA sequences were amplified by polymerase chain reaction (PCR) using general primers ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and ITS5 (5′-GGAAGTAAAAGTCGTAACAAG-3′) in a 50 µl amplification system (40.5 µl of ultrapure water, 5.0 µl of 10× buffer, 1.0 µl of 10 mmol/L dNTP, 1.0 µl of ITS4, 1.0 µl of ITS5, 1.0 µl of template DNA, and 0.5 µl of *Taq* plus DNA polymerase) at 30 cycles per reaction (denaturation at 95°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min per cycle. In the first cycle, preheat denaturation was performed at 95°C for 3 min, and final extension was done at 72°C for 7 min). (3) The PCR amplicons were separated by agarose gel electrophoresis and sequenced by a biotechnology company. (4) The homology of the samples was compared with the present sequence (http://www.ncbi.nlm.nih.gov/blast.cgi).

Biodiversity measure

Several computational methods (Magnussen and Boyle, 1995; Borja et al., 2000; Yan et al., 2006; Gamito, 2010) in statistical

Table 1. Compositions of vegetation community and macrofungal species in Bifeng Gorge.

Forest community	Macrofungal species
Type I	Total 167 species; dominant species: *Clavulina cristata*, *Dicephalospora rufocornea*, *Hypoxylon fuscum*, *Lachnum abnorme*, *Marasmiellus candidus*, *Marasmiellus coilobasis*, *Marasmiellus* sp., *Marasmius androsaceus*, *Mycena abramsii*, *Panellus stypticus*, *Xylaria* sp.
Type II	Total 63 species; dominant species: *Armillariella tabescens*, *Gymnopus subnudus*, *Laccaria vinaceoavellanea*, *Mycena galericulata*, *Nidula niveotomentosa*, *Scleroderma verrucosum*, *Tricholomopsis sasae*
Type III	Total 32 species; dominant species: *Bisporella citrine*, *Lycoperdon ericaeum*, *Nidula niveotomentosa*, *Peziza sylvestris*
Type IV	Total 28 species; dominant species: *Lachnum abnorme*, *Lycoperdon polymorphum*, *Lysurus cruciatus*, *Psilocybe* sp.
Type V	Total 39 species; dominant species: *Dicephalospora rufocornea*, *Lycoperdon ericaeum*, *Marasmiellus candidus*, *Marasmiellus* sp., *Marasmius androsaceus*
Type VI	Total 6 species; dominant species: *Psilocybe* sp.

Type I: Evergreen broadleaf forest; Type II: *Cryptomeria fortunei* forest; Type III: *Metasequoia glyptostroboides* forest; Type IV: bamboo forest; Type V: mixed bamboo- broadleaf forest; Type VI: grassland.

ecology were applied, such as the Margalef's richness index (*R*), Simpson's dominance index (*D*), Shannon-Wiener species diversity index (*H'*), and the Pielou's evenness index (*E*). The computation formulas are as shown below:

Richness index (*R*) $$R=(S-1)/\ln N$$

Dominance index (*D*) $$D=1-\sum_{i=1}^{s} Pi^2 \quad (Pi = Ni/N)$$

Species diversity Index (*H'*) $$H'=-\sum_{i=1}^{S} Pi\ln Pi \quad (Pi = Ni/N)$$

Evenness index (*E*) $$E=H'/\ln S$$

Where, *S* is the total number of species in the quadrat of species *i*; *Ni* is the number of species *i* and *N* is the total number of *S* species.

Statistical analysis

All the data were subjected to one-way ANOVA to determine the significance of individual differences at the $p < 0.05$ level. Significant means were compared using the least significant difference (LSD) multiple comparisons test. All statistical analysis was conducted using the commercial statistical package SPSS 13.0.

RESULTS

Macrofungal Resources

According to the morphologic characteristics and sequence information, about 275 macrofungal are present in Bifeng Gorge. Based on the systematic classification in Ainsworth and Bisby's Dictionary of the Fungi (Kirk et al., 2008), the macrofungi were classified into 122 genera in 52 families. Escomycetes comprised 26 species in 14 genera from 10 families; basidiomycetes constituted 246 species in 105 genera from 39 families. In addition, myxomycetes comprised three species in three genera of three families. The 150 dominant species are the fungi in the following families: Russulaceae, Strophariaceae, Agaricacea, Tricholomataceae, Psathyrellaceae, Mycenaceae, Marasmiaceae and Polyporaceae, which summed up to 54.55% of all species in the region. In terms of genera, the 103 dominant fungal species were *Lactarius*, *Mycena*, *Russula*, *Amanita*, *Hypholoma*, *Marasmius*, *Xylaria*, *Psathyrella*, *Stropharia*, *Tricholomopsis*, *Agaricus*, *Lepiota* and *Marasmiellus*, which comprised 37.45% of all species in the region. Based on the distribution of macrofungal species, the species composition has significant differences among the different forest types (Table 1).

Macrofungal community diversity analysis in different forest types

Up to 275 macrofungal species are present in the six forest types based on survey and statistics. Based on the macrofungi sampled, richness index (*R*), dominance index (*D*), species diversity index (*H'*) and evenness index (*E*) were determined in the six types (Table 2).

Table 2. Contrast diversity indices in different forest types.

Index	Type I	Type II	Type III	Type IV	Type V	Type VI
R	14.2167[a]	7.8695[b]	4.5304[e]	4.0056[d]	5.4866[c]	0.9889[f]
D	0.9241[a]	0.6578[d]	0.8916[b]	0.8447[c]	0.9231[a]	0.3635[e]
H'	3.2771[a]	2.1026[e]	2.5544[c]	2.3734[d]	2.8536[b]	0.3921[f]
E	0.6651[d]	0.5001[e]	0.7370[c]	0.7123[b]	0.7736[a]	0.2189[f]

R: Margalef index; *D*: dominance index; *H'*: species diversity index; *E*: evenness Index. The lowercase letters mean significant difference at $p<0.05$ level (LSD test). Type I: Evergreen broadleaf forest; Type II: *Cryptomeria fortunei* forest; Type III: *Metasequoia glyptostroboides* forest; Type IV: bamboo forest; Type V: mixed bamboo- broadleaf forest; Type VI: grassland.

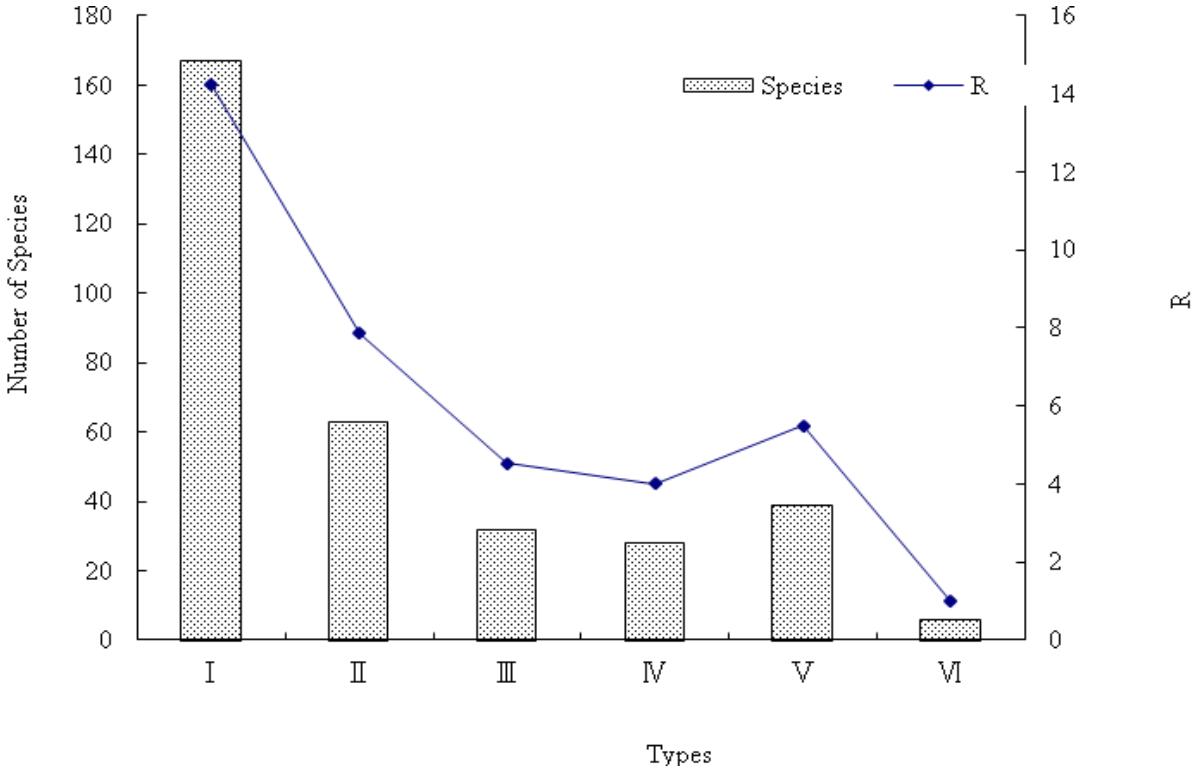

Figure 1. Species and richness index of macrofungi in different communities (R: richness index).

The order of richness index (*R*) is I > II > V > III > IV > VI, which is in accordance with the changes in macrofungal species among the different forest types (Figure 1). In type I, the richness index was maximal, which is partly attributed to the large canopy density, thick litter, abundant soil moisture and rich organic matter. Thus, type I forests are clearly suitable for macrofungal growth. In types II and V, the indices were intermediate. In the *C. fortunei* forest, the soil moisture was low, but the canopy density was above 0.95 to 1.00. Despite the wet understory, thick litter and rich organic matter, macrofungal species are relatively lower, resulting from a single tree species in this type of ecology. In the mixed bamboo-broadleaf forest type, because of large canopy density, moderate soil moisture and organic matter, macrofungal growth was inhibited by intense human disturbance. In both types III and IV, the indices are low because of serious human disturbance. In type VI, the index is lowest, which does not support macrofungal growth due to direct sunlight, plentiful evaporation on the ground, and the lowest moisture and organic matter content.

The order of the evenness index (*E*) and dominance index (*D*) are V > III > IV > I > II > VI, which differs from the richness index (*R*) (Figure 2). In types V, III and IV, the indices were high, although fewer macrofungal species were found, a large number of miniature sporophores were discovered, and were therefore, raised. Types I and II both had intermediate indices. The lowest was in type VI, with only six species. Additionally, the macrofungal occurrence quantities were unequal. For example,

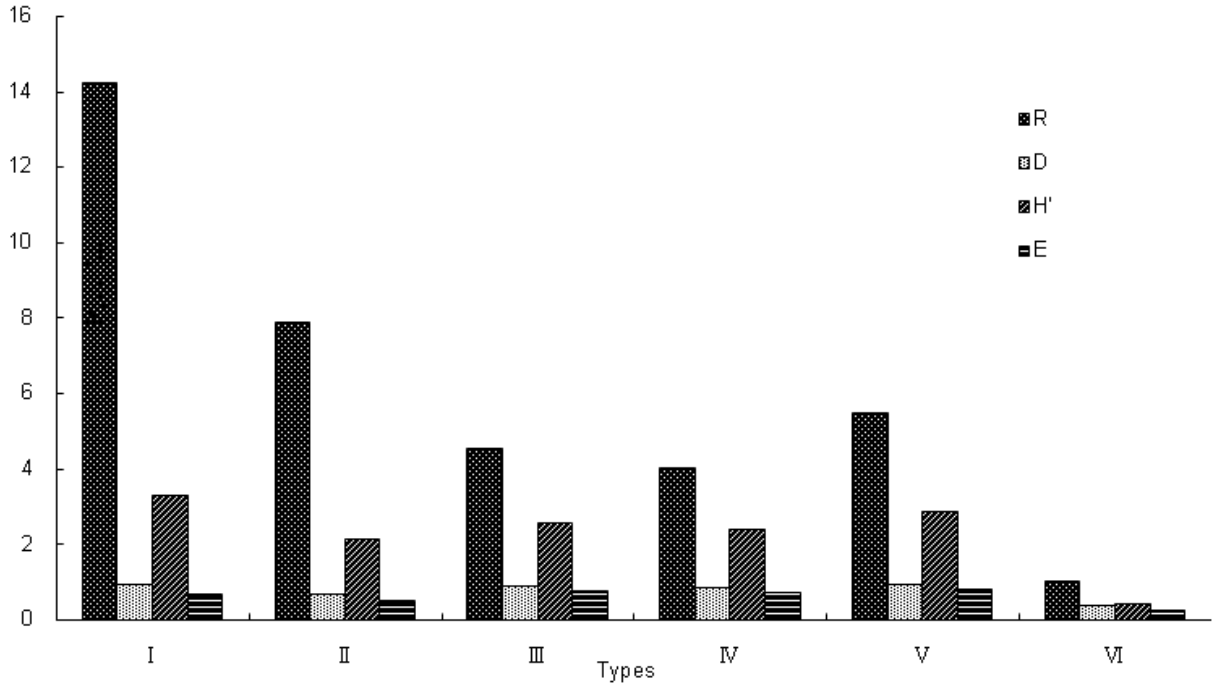

Figure 2. Diversity index in different communities (*R*: richness index, *D*: dominance index, *H'*: diversity index *E*: evenness index).

Psilocybe sp. was abundant, whereas the other species were very few.

The order of species diversity index (*H'*) was I > V > III > IV > II > VI (Figure 2). In the type I forest, the index is maximal, which is due to the suitability of this community for macrofungal growth (*R* is high); this reason is the same for types III, IV and V. In type II forests, the low *E* resulted in low *H'*. The index for the type VI forest was the lowest because both *R* and *E* were low.

Monthly temperature influence on macrofungal quantity

The macrofungi began to appear in April, which increased from April to August, decreased from August to November, and then peaked in August (Figure 3). The macrofungal species included miniature sporophores and annual Polyporaceae species were detected in April. The quantity and richness dropped more sharply in November than in October.

From April to July, the number of macrofungal species increased progressively following the increased temperature. The average maximum monthly temperature went up from 21.9 (April) to 29.9°C (July), and the minimum was from 14.3 to 22.7°C. The temperature from July to November gradually declined, so that the number of macrofungal species began to decrease in August, with a sharp decline recorded in November. The maximum quantity was recorded in August instead of July, where macrofungal growth exhibited a certain lag because of temperature. Moreover, the temperature from September to November was slightly lower than that in April to June, but the quantity increased.

DISCUSSION

Both vegetative communities and animals influence the macrofungal habitat in the forest ecosystem (Crabtree et al., 2010; Hustad et al., 2011), especially the core giant panda habitat, and in turn, the macrofungi are the essential decomposers that maintained regional ecological balance for plants and animals living. The diversity of the macrofungal community reflects the environmental conditions in the region. In the present study, the macrofungal diversity significantly differed among the six vegetation communities; the order of the species diversity was Type I > II > V > III > IV > VI. These findings show that species composition closely correlates with the vegetation communities, which affect the diversity and distribution of macrofungi. Moreover, among the different months, the species and quantity of macrofungi changed when the temperature changed. Although, temperature was highest in July, the microfungal quantity peaked in August, showing a certain lag in the macrofungal response to temperature. Generally, the quantity peaked in late summer to early autumn, which was same with the past studies of many scholars (Wu and Tan, 1993). The results indicated that the plants grew more luxuriant; the canopy

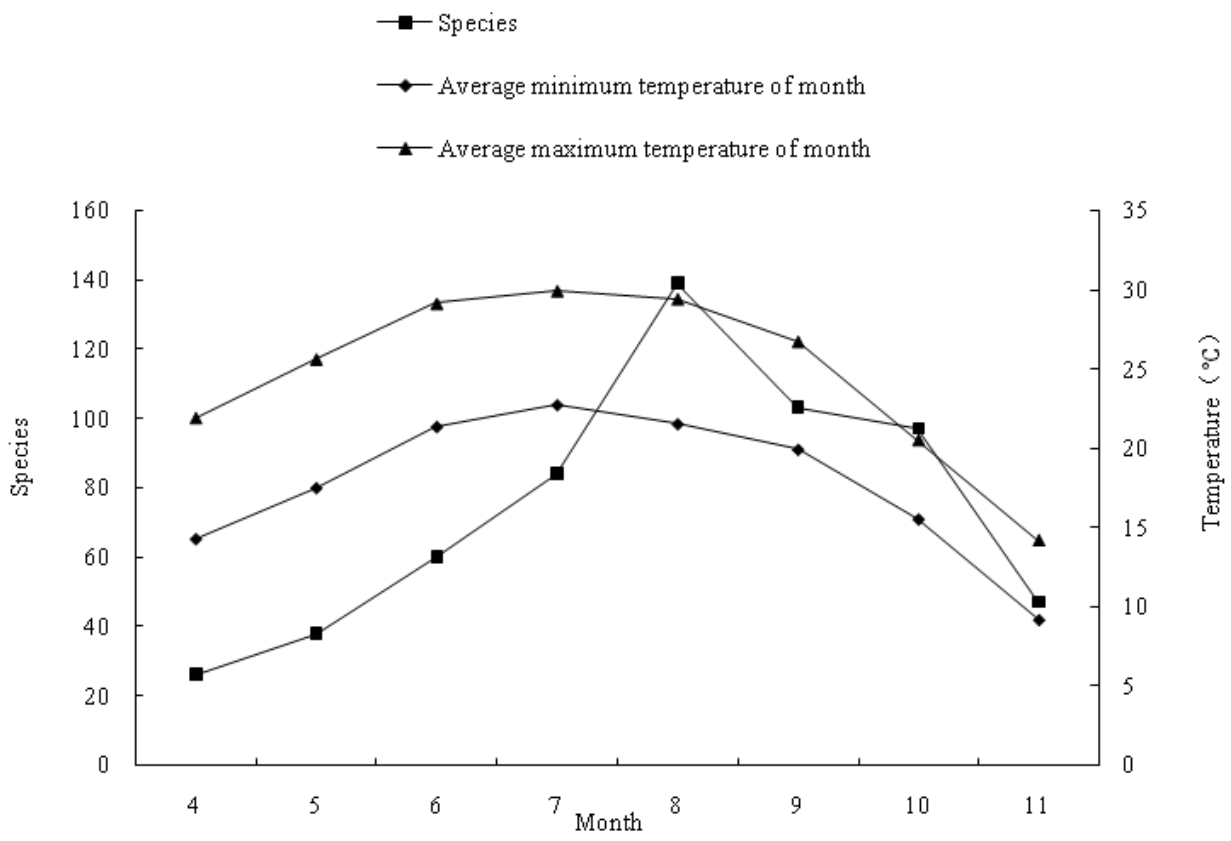

Figure 3. The relationship between temperature and macrofungal diversity in 2009.

density and relative humidity increased with the abundant precipitation in June and July. The temperature is more favorable for macrofungal growth in August, and the organic matter content increased after the litter decayed through fungal action, which provides a good environment for macrofungal growth.

In the current study, the macrofungal resources were seriously disturbed by humans in the area. When the wild edible fungi increased, the local inhabitants began to harvest them in Bifeng Gorge. A large number of tourists visited the area every month, and out of curiosity, they picked colorful macrofungi. To provide food for the pandas, many bamboo plants were planted in Bifeng Gorge, which were eventually felled by humans, thereby destroying the original vegetation and adversely influencing macrofungal growth.

To our knowledge, the macrofungal diversity in the core giant panda habitat is reported for the first time in the present study. The macrofungal species were abundant, among which saprophytic macrofungi are the majority; for example, *Lachnum abnorme*, *Dicephalospora rufocornea*, *Bisporella citrina*, *Stereum sanguinolentum*, *Pycnoporus sanguineus*, *Daedaleopsis confragosa*, *Polyporus squamosus*, *Phellinus adamantinus*, *Irpex lacteus* and *Marasmius*, which all play an important role in decomposing litter on the forest ground. On the other hand, a variety of edible and medicinal fungi remain to be developed and utilized such as *Cordyceps militaris*, *Cordyceps pruinosa*, *Armillaria mellea*, *Panellus stipticus*, *Laccaria vinaceoavellanea* and *Ganoderma lucidum*. These species may be rationally utilized by humans.

In conclusion, the research methods for evaluating macrofungal diversity are not yet fully developed (Tolgor and Li, 2000; Chai et al., 2010) and very few scholars have conducted research using specific methods that are not entirely suitable for the macrofungi in China. Therefore, the research methods for macrofungal diversity need further study and exploration. Moreover, the magnitude of occurrence of macrofungi is usually discontinuous in different seasons and different years of the same area (Gerben et al., 2001; Roberts et al., 2004; Baptista et al., 2010). Further studies are therefore needed to collect more data continuously through the years. Nevertheless, the results suggest that this approach lays the foundation for the recognition of the important role of macrofungi in the environment protected in this special ecological region.

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