GEOSCIENCE FRONTIERS 3(3) (2012) 351–356

available at www.sciencedirect.com

China University of Geosciences (Beijing)

GEOSCIENCE FRONTIERS

journal homepage: www.elsevier.com/locate/gsf

RESEARCH PAPER

Distinguishing ectomycorrhizal and saprophytic fungi using carbon and nitrogen isotopic compositions

Weiguo Hou [a,*], Bin Lian [b,], Hailiang Dong [a,c], Hongchen Jiang [a], Xingliang Wu [d]**

[a] *State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences, Beijing 100083, China*
[b] *State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550002, China*
[c] *Department of Geology and Environmental Earth Science, Miami University, Oxford, OH 45056, USA*
[d] *Guizhou Academy of Sciences, Guiyang 550001, China*

Received 13 September 2011; accepted 9 December 2011
Available online 16 December 2011

KEYWORDS
Ectomycorrhizal fungi;
Saprophytic fungi;
Stable isotopic compositions;
Ecological function

Abstract Ectomycorrhizal fungi, a group of widespread symbiotic fungi with plant, obtain carbon source from trees and improve plant mineral nutrient uptake with their widespread hyphal network. Ectomycorrhizal fungi can be used as inoculants to improve the survival rates of plantation. Saprophytic fungi use the nutrition from the debris of plant or animals, and it is difficult to distinguish the saprophytic and ectomycorrhizal fungi by morphological and anatomic methods. In this research, the differences of stable carbon and nitrogen isotopic compositions of these fungi were analyzed. The results showed that the abundances of ^{13}C of were higher than those of ectomycorrhizal fungi and the abundances of ^{15}N of saprophytic fungi were lower than those of ectomycorrhizal fungi. Such differences of stable carbon and nitrogen isotopic compositions between ectomycorrhizal fungi and saprophytic fungi can be ascribed to their different nutrition sources and ecological functions. These results collectively indicate that stable carbon and nitrogen isotopic compositions are an effective proxy for distinguishing between ectomycorrhizal and saprophytic fungi.

* Corresponding author.
** Corresponding author.
E-mail addresses: haital@126.com (W. Hou), bin2368@vip.163.com (B. Lian).

Peer-review under responsibility of China University of Geosciences (Beijing).
doi:10.1016/j.gsf.2011.12.005

Production and hosting by Elsevier

1. Introduction

Ectomycorrhizal fungi (EMFs) are an important part of forest ecosystem, and almost all trees can form symbiont–ectomycorrhizae with EMFs (Francis and Read, 1994; Markkola et al., 1996). To a great extent, a healthy and stable forest ecosystem rely on the ectomycorrhizal relationship and the community of EMFs for its function in nutrient element cycling (Haselwandter and Bowen, 1996; Lian et al., 2008). Many studies had proved that mycorrhizal fungi obtained carbon source from their vegetable partner (Hodge et al., 2001; Rosling et al., 2004; Hobbie, 2006). In return, EMFs will improve mineral nutrition uptake for plants by weathering on minerals and activating undissolvable nutrition, such as phosphorus (Leyval and Reid, 1991; Dixon and

Hiolhiol, 1992; Marschner and Dell, 1994; Hobbie et al., 2009). Ectomycorrhizal fungi also can assimilate nitric-, ammonium-, and protein-nitrogen with high-efficiency through their widespread hyphal network, providing nitrogen nutrition to plants (Bending and Read, 1996; Keller, 1996; Martin and Lorillou, 1997). EMFs can be used as inoculants to improve the survival rate of plantation (Amaranthus and Perry, 1987) and great economic values existed in the fruit bodies of EMFs, such as *Tuber magnatum*, *Tricholoma matsutake* (Wang and Hall, 2004).

Saprophytic fungi, the largest group of fungi, grow on dead organic matter such as fallen trees, cow patties, dead leaves, and even dead insects and animals. Saprophytic fungi play an important role in decomposition of organic matters and nutrition cycling, especially in the nitrogen cycling by excreting kinds of hydrolase, including proteinase, cellulase, laccase and so on (Mcmillan and Boynton, 1994; Hobbie et al., 1999; Baldrian and Valaskova, 2008; Dinis et al., 2009). EMFs and saprophytic fungi are involved in different ecological functions, but it is difficult to distinguish ectomycorrhizal sporocarps from sporocarps of saprophytic fungi using anatomical and taxonomic methods.

Molecular tools were largely used in identifying EMFs (Bruns and Gardes, 1993). By DNA sequencing targeting fungi partner from mycorrhizae, Bruns et al. (1998) identified a large number of EMFs, and assembled a sequence database for basidiomycetous EMFs. Lian et al. (2007) designed a pair of specific primers to identify *Boletus edulis*, a frequently occurring ectomycorrhizal fungus in Southwest China forest ecosystem. However, the strict methods for distinguishing EMFs are to obtain their marks, such as Internal Transcribed Spacer (ITS) sequences directly from ectomycorrhizas, or inoculate the EMFs to their symbiotic partner. By these ways, it is inevitable that some fungi are assigned to EMFs artificially.

Stable isotopic composition has been widely used in ecological and element cycling studies. The carbon and nitrogen stable isotopic compositions of plant can be affected by species, elevation, humidity, and other environmental factors (Piao et al., 2004; Yang et al., 2007). Taylor and Bruns (1997) found that non-photosynthetic orchid "cheated" carbon and nitrogen source from vicinal plant by mycorrhizal network traced by stable isotopic composition. Thus, due to different trophic manners, do the EMFs vary from saprophytic fungi in carbon and nitrogen isotopic compositions? Can such differences be used to distinguish the EMFs from saprophytic fungi? The aim of this study was to analyze the differences of the stable carbon and nitrogen isotopic compositions of EMFs and saprophytic fungi, and to examine the efficiency of the stable isotopic composition in distinguishing between saprophytic fungi and EMFs.

2. Materials and methods

2.1. Study site

Longli Planted Forest, located in the southwest part of Guizhou Province, China (Fig. 1), ranges in altitude from 1550 to 1700 m, and possesses an annual average temperature of 14.7 °C, a yearly average rainfall of 1100 mm, and relative humidity of above 80%. The weather is affected by north subtropical monsoon climate. The tree species are dominated by *Pinus massoniana* Lamb.

2.2. Materials

Samples were collected in June and August, 2008. All the samples were listed in Table 1. The sporocarps were identified by anatomic method according to a guideline (Wei, 1982). After brought to laboratory, the sporocarps were packed separately and dried at 50 °C for 24 h. Fine roots and ectomycorrhizas were rinsed by distilled water and were then dried at 50 °C for 24 h. The dried samples were ground into powder and then preserved in a dry place for stable isotopic composition analysis.

2.3. Stable isotopic composition analysis

For carbon isotopic composition analysis, 6–10 mg organic sample and 2 g CuO were put in a quartz tube of 25 cm in length and 9 mm in diameter. The tube was vacuumized and sealed with flame on a vacuum system. Then the sample was burnt at 850 °C for 5 h. By this way, the organic carbon was transferred to CO_2. The resulting CO_2 was collected with a vacuum system, and then the stable carbon isotopic composition was analyzed by a gas isotopic mass analyzer (MAT 252, Finnigan). Stable carbon isotopic composition is expressed by $\delta^{13}C(‰) = [(^{13}C/^{12}C_{sample} - {}^{13}C/^{12}C_{standard})/(^{13}C/^{12}C_{standard})] \times 1000$, where $^{13}C/^{12}C_{sample}$ and $^{13}C/^{12}C_{standard}$ are the ratios of the sample and the reference sample (PBD), respectively. For nitrogen isotopic composition analysis, 6–10 mg sample, 2 g CuO and 2 g Cu were added into a quartz tube. The tube was vacuumized and sealed with flame on a vacuum system. Then the sample was burnt at 850 °C for 5 h. The organic nitrogen was transferred to N_2. The stable nitrogen isotopic composition was analyzed by a gas isotopic mass analyzer (MAT 252, Finnigan). Stable nitrogen isotopic composition is expressed by $\delta^{15}N$ (‰) $= [(^{15}N/^{14}N_{sample} - {}^{15}N/^{14}N_{standard})/(^{15}N/^{14}N_{standard})] \times 1000$, where $^{15}N/^{14}N_{sample}$ and $^{15}N/^{14}N_{standard}$ are the ratios of the sample and the reference sample (atmospheric N_2), respectively.

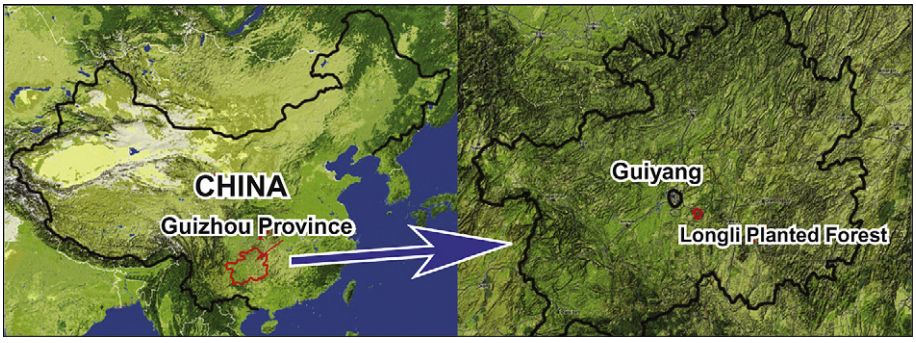

Figure 1 Location of Longli Planted Forest.