

Methods to study the role of ectomycorrhizal fungi in forest carbon cycling 1 : Introduction to the direct methods to quantify the fungal content in ectomycorrhizal fine roots

Takami Satomura^{1*}, Yasushi Hashimoto², Akihiko Kinoshita¹ and Takao Horikoshi³

¹Graduate School of Biosphere Science, Hiroshima Univ, Japan

²Agro-Environmental Science, Obihiro Univ. of Agriculture and Veterinary Medicine, Japan

³Graduate School of Integrated Arts and Sciences, Hiroshima Univ, Japan

Abstract: In terrestrial ecosystems, mycorrhizal fungi are considered to have important role on carbon cycling and its biomass has been studied to quantify their role. We noted why mycorrhizal fungi are important on carbon cycling and focused one of the key factors to assess how much mycorrhizal fungi contribute on the forest carbon cycling, i.e., the biomass of mycorrhizal fungi in ectomycorrhizal fine roots. Three direct methods to quantify the ectomycorrhizal fungi in ectomycorrhizal fine roots were introduced. In the pioneer studies, the value 40% was used as a fungal content in ectomycorrhizal fine roots in many types of forests. However, recent studies showed that the value 40% was not always suitable in various types of forest. Through this report, we emphasize the importance of the further data accumulations in this study field that would lead more precise estimation of forest carbon cycling.

Keywords: ergosterol, fine root, fungal biomass, fungal sheath, image analysis

森林の炭素循環における外生菌根菌の役割を研究する手法 1 : 外生菌根の菌類含量を定量化する直接的な手法についての序論: 里村 多香美 (広島大学大学院生物圏科学研究科), 橋本 靖 (帯広畜産大学畜産環境科学科), 木下 晃彦 (広島大学大学院生物圏科学研究科), 堀越 孝雄 (広島大学大学院総合科学研究科)

要 旨: 陸上生態系では、菌根菌は炭素循環において重要な役割を担っていると考えられており、菌根菌の役割を定量化するために菌根菌のバイオマスが研究されてきた。我々は、炭素循環においてなぜ菌根菌が重要なのかを明記し、菌根菌がどの程度森林の炭素循環に貢献しているか評価するための重要な要素の一つ、外生菌根に含まれる菌根菌を直接的に定量する方法について紹介する。先駆的な研究では、様々な森林において外生菌根の菌類含量の値として 40% が用いられている。しかしながら、近年の研究から 40% という値は様々な森林のすべてに適切な値とは限らないことが示された。この報告を通じて、外生菌根の菌類含量の値のデータを蓄積することの重要性を強調する。そのデータが蓄積されれば、森林の炭素循環の推定をより正確に示すことができる。

キーワード: エルゴステロール, 画像解析, 菌鞘(菌套), 菌類のバイオマス, 細根

Introduction

The majority of terrestrial plants have a symbiotic relation with fungi, i.e. mycorrhiza, within their roots of the finest diameter class (Harley and Harley 1987). Mycorrhizal symbiosis is chiefly characterized by the flow of water and inorganic nutrients from fungi to plants, and organic materials from plants to fungi (Smith and Read 1997) (Fig.1). The functions of mycorrhizal fungi, especially their roles in carbon dynamics, are

different from those of saprotrophic microorganisms that are related to the decomposition processes. A considerable amount of net photosynthate (~21%) could be allocated from plants to their fungal partners (mycorrhizal fungi) (reviewed by Hobbie 2006), which is used for the growth and maintenance of their symbiotic fungi (reviewed by Allen 1991). Therefore, mycorrhizal fungi are considered to be an important component in ecosystem carbon dynamics (Finlay and Söderström

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*連絡先 Field Science Center for the Northern Biosphere, Hokkaido University, Nishi 9-chime, Kita 9, Kita-ku, Sapporo 060-0809, Japan

Tel: +81-(0)11-706-3653 Fax: +81-(0)11-706-3450 E-mail: takami_satomura@ybb.ne.jp

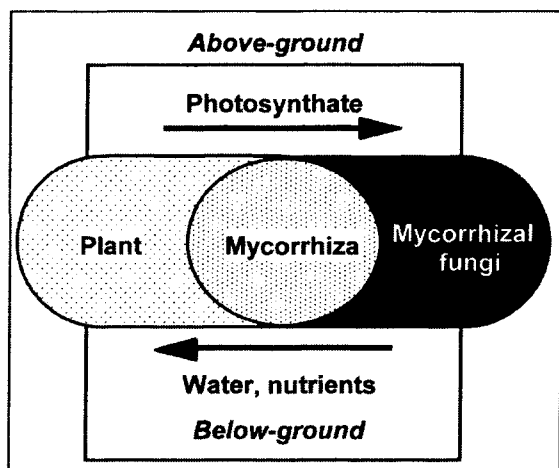


Fig.1 Schema of the mycorrhizal association. Mycorrhizal association is defined as a symbiotic relation between plant and fungi, which is chiefly characterized by a flow of water and nutrients from fungi to plant, and organic materials from plant to fungi. Mycorrhiza is defined by Smith and Read (1997) as 'a structure in which a symbiotic union between fungi and absorbing organs (roots) of plant confers increases of the fitness on one or both partners'. Each partner (plant or fungi) has individual specific organs in and/or on the ground.

1992).

In this article, we describe how carbon flows through mycorrhizal fungi in forest ecosystems. Key factors that are closely related to the biomass of mycorrhizal fungi in forest ecosystems are listed. We focus on the fungal biomass in mycorrhizal fine roots and three direct methods are introduced. At last, points of issues in this study field are clarified.

Characteristics of the mycorrhizal association in forest ecosystems

Mycorrhiza, which consists of plant fine roots and a fungal interface, was categorized 6 types according to the form of fungal mycelia in and around plant fine roots (Molina et al. 1992; Read 1998; Brundrett 2004). Two general types of the mycorrhiza are called arbuscular mycorrhiza and ectomycorrhiza. Arbuscular mycorrhiza is characterized by the arbuscules in root cortical cells (at the apoplast) and intercellular vesicles (Fig. 2). Ectomycorrhiza consist of a fungal sheath surrounding plant roots and a Hartig net that penetrates into the intercellular space of plant

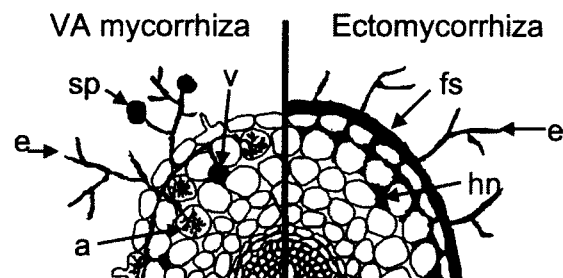


Fig.2 Schematic overview of the two major mycorrhizal types, i.e., vesicular-arbuscular mycorrhiza (VA mycorrhiza) and ectomycorrhiza. a, arbuscule; v, vesicle; sp, spore; e, external mycelium; fs, fungal sheath; hn, Hartig net. Each arbuscule seems to penetrate into the plant cell membrane; however, they penetrate into the cell wall but not into the cell membrane. In other words, each arbuscule is in the apoplastic zone.

cortical cells (Fig. 2).

In many forest ecosystems, the dominant trees forming the forest crown are ectomycorrhizal trees (Brundrett 2002). Although ectomycorrhizal tree species account for only ca. 10% of vascular tree species (Brundrett 2002; Harley and Harley 1987), they inhabit important niches in various forests: the conifers (e.g. trees in the genus *Pseudotsuga*, *Picea*, *Abies*, *Larix* and so on.) are widespread in sub-boreal zones, trees with commercial value (e.g. trees in the genus *Pinus*, *Fagus*, *Eucalyptus*, *Betula*, *Quercus* and so on.) in the temperate zones, and Dipterocarpaceae species, which form an emergency tree layer in Asian lowland tropical zones (cf. Molina et al. 1992). The majority of ectomycorrhizal plants are trees (Molina et al. 1992). Therefore, the plant-ectomycorrhizal association is important in forest ecosystems.

Carbon flow through mycorrhizal fungi in forest ecosystems

Carbon used for symbiotic fungal turnover is put into the soil as soil organic matter (Fig. 3). Carbon used for respiration of mycorrhizal fungi is released into the soil, and then escape to the air (Fig. 3). Mycorrhizal fungi have an invisible carbon pathway through soils. As you can see in Fig. 3, a lack of data about mycorrhizal fungi in carbon cycling of a natural ecosystem would lead to an underestimation of the carbon input to soil and lead to an overestimation of the decomposition of soil

organic matters. To more accurately estimate of soil carbon flow in various natural ecosystems, further quantitative studies on the contributions of mycorrhizal fungi to soil carbon flow are needed. The portion of NPP (net primary production) allocated to the root system (NPP_r) during a unit period (between time t1 to time t2) is given by the following equation (cf. Vogt et al. 1998 with some modification of the term):

$$NPP_r = B_{t2-t1} + M_{t2-t1} + D_{t2-t1} + E + R + Myc \dots (Eq.1)$$

where B_{t2-t1} is the change in live fine root biomass between time 1 and time 2, M_{t2-t1} is the change in dead root biomass between time t1 and t2, D_{t2-t1} is an estimate of root decay between time t1 and t2, E is the carbon loss due to exudation, R is carbon loss due to root respiration and Myc is the carbon allocation to the root-associated fungi, i.e. mycorrhizal fungi.

In order to follow the carbon dynamics through individual organisms, the following four questions need to be answered:

- Question I Where is the organism living?
- Question II How many (how much) organisms

are there?

- Question III How much carbon is contained in a unit biomass of the organism?
- Question IV How many times does the carbon in the organism turn over per unit of time?

Biomass data in natural ecosystems is one of the most fundamental components to assess the role of an organism in carbon dynamics. This fundamental concept has been applied to assess the mycorrhizal role in soil carbon dynamics. Question I about mycorrhizal association is answered by Johnson et al. (1999): The plant-fungal community, i.e., mycorrhizal association, can be divided into 4 parts as follows; 1) plant tissue, 2) fungal tissue (fruit body, sclerotium and spore, etc.), 3) plant-fungal interface (mycorrhiza) and 4) soil-fungal interface (external mycelium). A diagram expressing these four parts is shown in Fig. 4. Plant components are comprised of categories 1 and 3, and fungal components comprised of categories 2, 3 and 4. To answer question II, it is necessary to estimate the biomass of individual symbiotic partners, i.e. plant or mycorrhizal fungi, in each category. In previous

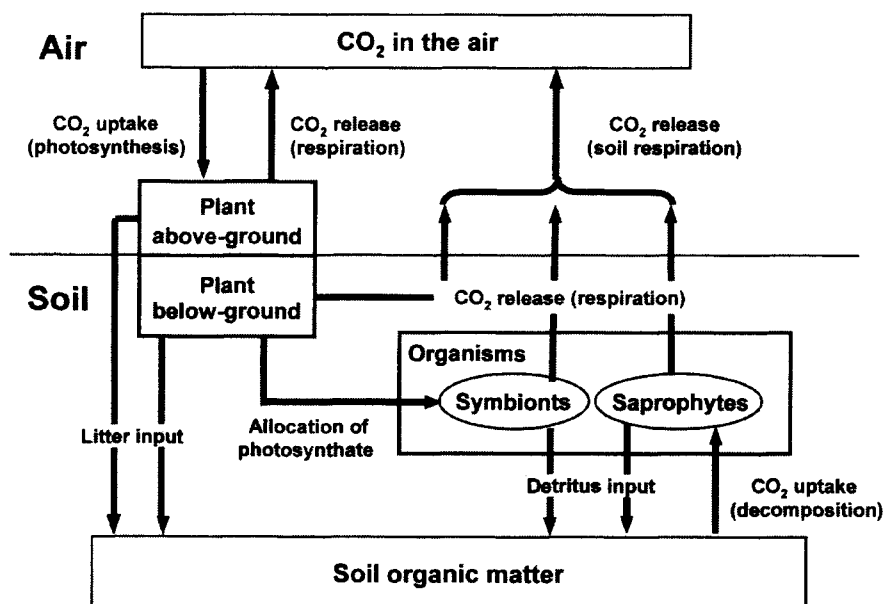


Fig. 3 Schematic diagram of carbon dynamics in forest ecosystems. Carbon dioxide in the air is assimilated by the plant photosynthetic activity and allocated to the above- and below-ground parts of the plant. Photosynthate is also allocated to plant-symbiotic fungi (mycorrhizal fungi). Dead parts of the plant are put into soil as 'litter'. Dead parts of the soil organisms (plant-symbiotic fungi and heterotrophic organisms) are put into the soil as detritus. Carbon dioxide is released from the soil as results of respiration by the plant, plant-symbiotic fungi and saprophytes. The respiration by plant and symbiotic fungi are not related to the decomposition, while respiration by saprophytes is closely related to the decomposition of soil organic matter.

studies, a common problem has been quantification of the role of mycorrhizal fungi in the latter two categories (categories 3 and 4) when we discuss the role of mycorrhizal fungi on carbon cycling in natural ecosystems, since they are difficult to pick up to count or weigh. Therefore, in the field study, data about the total biomass of mycorrhizal fungi are very limited despite their importance in carbon cycling. The data concerning about the questions III and IV are further limited. Even if we answer the latter two questions (III and IV) for some mycorrhizal fungi, it still would not be possible to determine the contribution of those fungi to carbon cycling in natural ecosystems without first answering question II.

Between the two fungal components (categories 3 and 4) in field conditions, quantitative estimation of the fungal component in the plant-fungal interface (category 3) is rather more reported than soil-fungal interface (category 4). It is necessary to determine the fungal content of ectomycorrhizal fine roots in order to consider the fungal component in the plant-fungal interface. The fungal component in the plant-fungal interface (category 3) is called 'intraradical mycelium' (e.g. Brundrett 2002) or 'internal mycelium' (e.g. Fujiyoshi et al.

2000; Wallander et al. 2001), contrasting the fungal component in the soil-fungal interface (category 4) termed 'extraradical mycelium' or 'external mycelium', respectively (Fig.4). In the ectomycorrhizal association, both the Hartig net and the fungal sheath are components of fungi in the plant-fungal interface (category 3) and they are regarded as components of the 'internal mycelium', while mycelia penetrate into the soil is a component of fungi in the soil-fungal interface (category 4) that is regarded as 'external mycelium'.

Factors closely related to the biomass of mycorrhizal fungi in forest ecosystems

At the natural stand level, the biomass of fungi in the plant-fungal interface has been estimated by multiplying the biomass of ectomycorrhizal fine roots by the fungal content (e.g. Fogel and Hunt 1979; Vogt et al. 1982; Kårén and Nylund 1996; Kårén and Nylund 1997; Satomura et al. 2003). The fungal content of ectomycorrhizal fine roots, i.e., mycorrhizal colonization intensity of ectomycorrhizal fine roots is closely related to the biomass of ectomycorrhizal fine roots (cf. Schneider et al. 1989). The amount of ectomycorrhizal fine roots and fungal content of them could be altered

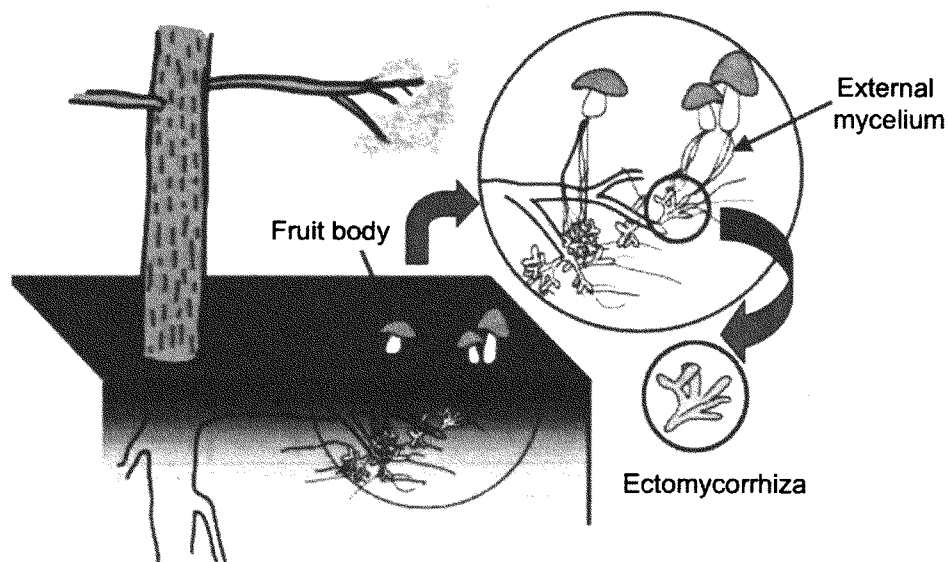


Fig. 4 Schematic diagram of a plant-ectomycorrhizal association. A parts of the plant fine roots is 'ectomycorrhiza' (plant-fungal interface) (category 3). Mycelia contained in the plant-fungal interface are regarded as 'internal mycelia'. Mycelia running out from the plant-fungal interface to the soil are regarded as 'external mycelia' of ectomycorrhizal fungi (soil-fungal interface) (category 4). Some of the ectomycorrhizal fungi produce 'fruit bodies' that is regarded as 'fungal tissue' (category 2). Non-mycorrhizal parts of the plants are regarded as 'plant tissue' (category 1).

by the photosynthetic activity of the plants, the carbon allocation strategy of plants (root carbon sink strength, root/shoot ratio, fine root/whole root biomass ratio), size of trees and tree density (cf. Anderson and Rygielwicz 1991; Curtis et al. 1996). Recently, Hobbie (2006) reviewed the pot culture studies of the ectomycorrhizal plants and found that the carbon allocation to the ectomycorrhizal fungi (% , proportion to plant total carbon fixation) is closely related to the carbon allocation to the below-ground parts of plants (% , proportion to plant total carbon fixation). Environmental factors, such as temperature, precipitation, CO₂ in the atmosphere, soil conditions would also affect all of the parameters that are related to the biomass of ectomycorrhizal roots, and thus environmental factors also relate to the biomass of mycorrhizal fungi (cf. Curtis et al. 1996; Stober et al. 2000; Rilling et al. 2002).

Direct methods for the estimation of fungal biomass in plant-fungal interface

In this section, we describe the methods to estimate the fungal content in roots and the obtained values by each method in the pioneer studies. There are three types of methods to estimate the fungal content of mycorrhizal fine roots: i) dissection method (Harley and McCready 1952), ii) round sliced section image analysis method (e.g. Vogt et al. 1982) and iii) biochemical indicator analysis method, in which ergosterol (e.g. Nylund and Wallander 1992) is used as an indicator.

Dissection method was used in Harley and McCready (1952). They peeled the fungal sheath like a banana (their method is clarified in Vogt et al. 1982). Image analysis method was firstly used in Vogt et al. (1982). They measured the areas occupied by plant and fungal tissues, calculated the fungal content on an area basis, applied the same content on a dry weight basis based on the hypothesis that both plant and fungi have similar densities. Biochemical indicator analysis method, i.e., ergosterol analysis method have been used in various studies (e.g. Nylund and Wallander 1992; Satomura et al. 2003). In this method, a fungal specific sterol, ergosterol, is extracted and quantified by HPLC system. The amount of the ergosterol is converted into fungal weight.

Hot topics in the study of fungal biomass in plant-fungal interface

One of the first of these studies (Harley and McCready 1952) found a 40% fungal content of beech (*Fagus* sp.) ectomycorrhizal fine root by a dissection method. Vogt et al. (1982) also obtained the same value (40% fungal content) at *Abies amabilis* stands by an image analysis of the round sliced sections of *A. amabilis* ectomycorrhizal root tips. However, the value of 40% fungal content may not always be suitable for whole ectomycorrhizal plants, since Vogt found 20% fungal content at a low altitude *Pseudotsuga menziesii* stand by an image analysis (Vogt et al. 1991). Furthermore, Kårén and Nylund (1996; 1997) found small fungal content of ectomycorrhizal fine roots (2.9–3.8%) at *Picea abies* stands using a fungal biochemical indicator, ergosterol. We also found small fungal content of ectomycorrhizal fine roots (1.2–6.9%) in a *Pinus densiflora* stand (Satomura et al. 2003). The value 40% was one of the controvertible topics in this study field (reviewed by Hobbie 2006). In the pioneer studies or reviews, in which the fungal biomass was estimated and/or discussed, have been applied the value 40% as a fungal content of ectomycorrhizal fine roots for several types of stand (e.g., Harley 1971; Finlay and Söderström 1992). However, the value 40% is likely to close to an upper limit in a fungal content of ectomycorrhizal fine roots. We found that the proportion of fungal sheath area to the cross-sectioned ectomycorrhizal fine root area ranged about 15–40% in various ectomycorrhizal tree species (Kinoshita, unpublished data). Consequently, the pioneer studies could overestimate the biomass of fungi in ectomycorrhizal roots as mentioned in Hobbie (2006). Now, we need to reexamine the values of fungal content of ectomycorrhizal fine roots in various plant-fungal combinations. Through these studies, we will know better about the role of ectomycorrhizal fungi in forest carbon cycling.

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