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ミニレビュー
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Methods to study the role of ectomycorrhizal fungi in forest carbon cycling 3: Quantification of the amount of carbon consumed by ectomycorrhizal fungi in a Japanese red pine forest

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Abstract: We estimated the role of mycorrhizal fungi quantitatively in a Japanese red pine (*Pinus densiflora*) forest. We directly estimated several parameters to calculate the amount of carbon consumed by mycorrhizal fungi, such as the biomass of fungi in ectomycorrhizal fine roots and fine root biomass, and drew other parameters from the literature. Our study site, a Japanese red pine forest, was characterized by a very small ectomycorrhizal fine root biomass (only 91.0 g m^{-2}) and small fungal content in ectomycorrhizal fine roots (2.2%) compared with the literature data. The ectomycorrhizal fine root biomass has a greater influence than the fungal content of ectomycorrhizal fine roots on the difference in fungal biomass in ectomycorrhizal fine roots among forests. The total biomass of ectomycorrhizal fungi in ectomycorrhizal fine roots and in soil was estimated to be only 10.0 g m^{-2}. However, the total amount of carbon consumed by the production-death decomposition cycle of ectomycorrhizal fungi was estimated to be 117.0 g C m^{-2} $year^{-1}$, which corresponds to about 24% of carbon release from soil as soil respiration. Our estimation reconfirmed the importance of ectomycorrhizal fungi in forest carbon cycling. The carbon consumed by ectomycorrhizal fungi is not negligible, even in a stand having a very small biomass of ectomycorrhizal fungi.
Keywords: ergosterol, fine root, fungal biomass, mesh bag method, turnover

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3: アカマツ林における外生菌根菌に消費される炭素量の定量化の試み: 里村 多香美(広島大学大学院生物圏科学研究科), 橋本 靖(帯広畜産大学環境総合科学講座), 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: 生態系の炭素循環における菌類の役割の重要性が認識されているにも関わらず, 野外条件下で菌根菌に分配される炭素量の推定値は数えるほどしか報告されていない。生態系の炭素循環における菌類の役割について定量的な値を用いて議論するため, アカマツ林で外生菌根菌に分配される炭素量を概推した。直接得られなかった土壌中の外生菌根菌バイオマス, 細根(菌根を含む)と外生菌根菌のターンオーバーの値は, 文献値を参照した。その結果, アカマツ林の外生菌根菌のバイオマスの総量はわずか10.0 g m^{-2} であると推定され, 細根のバイオマスが少ないことが大きく影響していると考えられた。この林分では菌根菌の生成と枯死サイクルによって年間に消費される炭素は117.0 g C m^{-2} $year^{-1}$ と推定され, 土壌からの炭素の放出の約24%に相当した。アカマツ林は細根のバイオマス, 細根中の菌類含有量, 外生菌根菌のバイオマスが共に文献値よりも非常に低いという特徴があった。森林タイプ間の外生菌根菌のバイオマスの違いに大きく影響を及ぼしているのは, 細根中の菌類含有量の差異よりも細根のバイオマスの差異であった。外生菌根菌のバイオマスが小さい森林においても, 菌根菌の生成・枯死サイクルによって消費される炭素量は無視できないことが改めて確認された。
キーワード: エルゴステロール, 細根, 菌類バイオマス, メッシュバック法, ターンオーバー

Introduction

We detailed methods to study the role of ectomycorrhizal fungi in forest carbon cycling in previous papers in this series (Satomura et al., 2006a, 2006b). The biomass of fungi in ectomycorrhizal fine roots (plant-fungal interface),

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as well as the biomass of ectomycorrhizal fungi in soil (called external mycelium; soil-fungal interface), the biomass of fungal tissues such as fruiting bodies and sclerotia, and their turnover are needed in order to quantify the role of ectomycorrhizal fungi in forest carbon cycling (for details see Satomura et al., 2006a). The importance of mycorrhizal fungi in terrestrial ecosystem carbon dynamics is widely recognized, but reports concerning the stand-level quantitative estimation of their role are limited. Investigations for improving the methodology are necessary. Pioneer studies to estimate the total amount of carbon consumed by ectomycorrhizal fungi (e.g. Finlay and Söderström, 1992; Wallander et al., 2001) gave valuable estimates for modeling carbon cycling in forests at the local and global scale, even though the estimates are rough. In Asian forests, however, no one has attempted to estimate it.

In this article, we compare the biomass and fungal contents of ectomycorrhizal fine roots between forests using literature data. Based on our data and assumptions in pioneering studies, we report the first estimate of the amount of carbon consumed by ectomycorrhizal fungi in an Asian forest.

Variation in fungal biomass in ectomycorrhizal fine roots among forests

Few attempts have been made to quantify the role of ectomycorrhizal fungi in forest carbon dynamics. In stand-level estimates, the biomasses of ectomycorrhizal fungi in fine roots differ among forest types (Table 1). The highest value (1000.9 g m^{-2} ground area) was obtained from a *Pseudotsuga menziesii* forest (Fogel and Hunt, 1979), and the lowest value (2.0 g m^{-2}) in a *Pinus densiflora* forest (Satomura et al., 2003). The fungal contents of ectomycorrhizal roots and the ectomycorrhizal fine root biomass, two factors used to calculate the biomass of fungi in ectomycorrhizal fine roots, were much higher in the *P. menziesii* forest than in the *P. densiflora* forest (Table 1). Judging from the results in Table 1, the ectomycorrhizal fine root biomass has a stronger effect than fungal content of ectomycorrhizal fine roots on the difference in fungal biomass in ectomycorrhizal fine roots. The fungal content of ectomycorrhizal fine roots in the *P. menziesii* forest (40%) was an order of magnitude greater than that in the *P. densiflora* forest (2.2%), while the ectomycorrhizal fine root biomass in the *P. menziesii* forest (2502.3 g m^{-2}) was two orders of magnitude greater than that in the *P. densiflora* forest (91.0 g m^{-2}). Fine root biomass (all types of

Table 1 Variations in the biomass and content of root components among the sites studied in the literature.

Parameters	*Pinus densiflora* forest	*Picea abies* forest	*Abies amabilis* forest		*Pseudotsuga menziesii* forest
			23-year-old	180-year-old	
Climate	Warm-temperate zone	Boreal zone	Cool-temperate zone	Cool-temperate zone	Cool-temperate zone
Forest type	Needleleaf evergreen forest	Needleleaf evergreen forest	Needleleaf evergreen forest	Needleleaf evergreen forest	Needleleaf evergreen forest
Total root biomass (g m^{-2})	3932.0	-	2369.0	14067.0	7431.2
Ectomycorrhizal fine root biomass (g m^{-2})	91.0	560.0	113.0	185.0	2502.3
(Non-mycorrhizal fine root biomass (g m^{-2}))	-	-	(839.0)	(1441.0)	-
Ectomycorrhizal fine root / total root (%)	2	-	5	1	34
Ergosterol content of ectomycorrhizal fine root (μg g^{-1} root dw)	43.1 - 321.0	166.0	-	-	-
Total amount of ergosterol in ergosterol fine root (mg m^{-2})	8.2	93.0	-	-	-
Fungal biomass in ectomycorrhizal fine root (g m^{-2})	2.0	15.1	68.0	111.0	1000.9
Fungal content of fine root (%)	2.2	2.9	40	40	40
Method to obtain the fungal biomass and fungal content in ectomycorrhizal fine roots (conversion factor)	Ergosterol method (4.10)	Ergosterol method (5.7)	Root section image analysis	Root section image analysis	Dissection method
Reference	Satomura et al. (2003)	Kårén and Nylund (1996)	Vogt et al. (1982)	Vogt et al. (1982)	Fogel and Hunt (1979)

Ergosterol method: the ergosterol concentration of ectomycorrhizal fungi (mg g^{-1} fungal d.w.) is used as a conversion factor to calculate the fungal biomass and fungal content in ectomycorrhizal fine roots from the ergosterol content of ectomycorrhizal fine roots. Root section image analysis: areas of plant and fungal tissues are obtained on the assumption that both tissues have similar densities. Dissection method: fungal sheath is peeled like a banana and weighed. See Satomura et al. (2006a, 2006b) for the detailed methodology. -, data not available.

fine roots, including ectomycorrhizal fine roots and arbuscular mycorrhizal fine roots) tends to be smaller in Japanese forests dominated by the genus *Pinus* or *Larix* than in European and North American forests (Noguchi, personal communication). In *Abies amabilis* forests, the biomass of fungi in ectomycorrhizal fine roots, the fungal content of ectomycorrhizal roots, and ectomycorrhizal fine root biomass showed intermediate values (Vogt et al., 1982; Table 1). The values in a *Picea abies* forest (Kårén and Nylund, 1996) were rather close to the values in a *P. densiflora* forest (Table 1).

Biomass of ectomycorrhizal fungi in soil

Wallander et al. (2001, 2004) reported a pioneering stand-level estimation of the biomass of ectomycorrhizal fungi in soil using ergosterol analysis. Ergosterol is a common sterol in higher fungi such as the Ascomycetes and Basidiomycetes (Weete and Gandhi, 1996), and an ergosterol analysis of a soil sample reveals all functional types of fungi (e.g., heterotrophic, ectomycorrhizal) by measuring the total amount of ergosterol derived from all types of fungi. Wallander et al. (2001) separated out the ectomycorrhizal fungi by putting nutrient-poor sandy soil into mesh bags and incubating them *in situ* in soil from a *P. abies* forest on the assumption that the mycelia of the mycorrhizal fungi alone can penetrate into the soil in the mesh bags (the carbon isotope ratio of the mycelia extracted from the mesh bags supported this assumption). After a time, they analyzed the ergosterol content of the incubated sandy soil. The biomass of ectomycorrhizal fungi in the soil was about four times that in the fine roots. In other words, a large proportion of the biomass of ectomycorrhizal fungi (about 80%) existed in the soil in the form of external mycelia. This value was within the range of the proportion of external mycelium to total fungal biomass in ectomycorrhizal fine roots and in soil (2-87%) reported from laboratory experiments (Colpaert et al., 1992; Wallander and Nylund, 1992; Ekblad et al., 1995).

How much do mycorrhizal fungi contribute to forest carbon dynamics?

How much of a plant's fixed carbon is consumed by its fungal partner (mycorrhizal fungi)? We attempted to estimate the value in a *P. densiflora* forest (Satomura et al., 2003), making the following assumptions (Table 2): (i) turnover of fine roots (0-2 mm in diameter) is 0.8 $year^{-1}$ (Gill and Jackson, 2000), (ii) the biomass of ectomycorrhizal fungi in soil is four times that in mycorrhizal fine roots (Wallander et al., 2001), (iii) the turnover time of mycelia is 1 week during the 6-month growing season for ectomycorrhizal fungi (turnover is 26 $year^{-1}$) (Finlay and Söderström, 1992), and (iv) carbon content is 45% in both fine roots and the mycelia of mycorrhizal fungi. Based on these assumptions, we estimated the total biomass of fungi in ectomycorrhizal fine roots and soil to be only 10.0 g m^{-2} (Table 2). The productions of plant tissue in ectomycorrhizal fine roots, fungi in ectomycorrhizal fine roots, and ectomycorrhizal fungi in soil are estimated to be 71.2, 52.0, and 208.0 g m^{-2} $year^{-1}$, respectively (Table 2). The amounts of carbon consumed by fine roots, ectomycorrhizal fungi in fine roots, and ectomycorrhizal fungi in soil are estimated to be 32.0, 23.4, and 93.6 g C m^{-2} $year^{-1}$, respectively (Table 2). The total amount of carbon consumed by the ectomycorrhizal fungi in a year (117.0 g C m^{-2} $year^{-1}$) corresponds to about 24% of the annual carbon emission from soils (soil respiration) in a *P. densiflora* forest (487.0 g C m^{-2} $year^{-1}$) (Nakane et al., 1984). The study forest, a *P. densiflora* forest, is characterized by a very small biomass of ectomycorrhizal fine roots and their fungal partners (91.0 and 10.0 g m^{-2}, respectively), compared with the total biomass of the belowground parts of plants

Table 2 Values in the calculations of carbon consumed by ectomycorrhizal fine roots and ectomycorrhizal fungi in a Japanese red pine forest.

Component	Biomass (g DW m^{-2} ground area)	Turnover ($year^{-1}$)	Production (g DW m^{-2} ground area $year^{-1}$)	Carbon content of each parameter (% biomass)	Consumed carbon (g C m^{-2} ground area)
Ectomycorrhizal fine roots	91.0	0.8	123.2	45	55.4
Plant tissue in ectomycorrhizal fine roots	89.0	0.8	71.2	45	32.0
Fungi in ectomycorrhizal fine roots	2.0	26	52.0	45	23.4
Ectomycorrhizal fungi in soil	8.0	26	208.0	45	93.6
Ectomycorrhizal fungi total	10.0		260.0		117.0

at the study site (3932.0 g m^{-2}; Table 1; Satomura et al., 2003). However, the estimated productions of ectomycorrhizal fine roots and their fungal partners are considerable, reconfirming their importance in forest carbon dynamics. Neglecting the turnover of carbon through mycorrhizal fungi would significantly affect estimates of carbon dynamics in a forest ecosystem.

Further considerations

To quantitatively evaluate the role of ectomycorrhizae in carbon dynamics, it is necessary to determine not only the biomass but also the turnover of ectomycorrhizal fungi. But little is known about turnover. Data are also needed on the carbon content of mycelia and fruiting body production of ectomycorrhizal fungal species. Normally, fungal fruiting body production has been evaluated in Japan as the number of fruiting bodies (e.g., Shimono, 1988; Fujita, 1989). However, to quantify the carbon consumed by the production of fruiting bodies by ectomycorrhizal fungi, mass-based (weight-based) data are needed (Vogt et al., 1992).

Mycorrhizal fungi connect plant individuals and even species through the external mycelia in the soil (Simard et al., 2002; Simard and Durall, 2004). In some cases, the carbon fixed by one plant appears to be allocated to other plants through this hyphal network (e.g., Miller and Allen, 1992; Leake, 1994, 2004; Simard et al., 2002). This complicates the analysis of carbon movement in the soil. Further studies of carbon movement through plant-fungal-plant combinations are needed.

Many questions remain about the role of ectomycorrhizal fungi in forest carbon dynamics. Much more quantitative data collected in various types of forest under diverse environments is needed to answer questions about the role of mycorrhizal fungi in forest carbon dynamics. Many methodological problems must be solved to obtain all the required data.

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Erratum

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

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Parts of the caption of **Fig.3** were lacked as print error. Please note that there is another error in **Fig.3**: the carbon transfer process in the decomposition, "CO_2 uptake" should be "Carbon uptake".

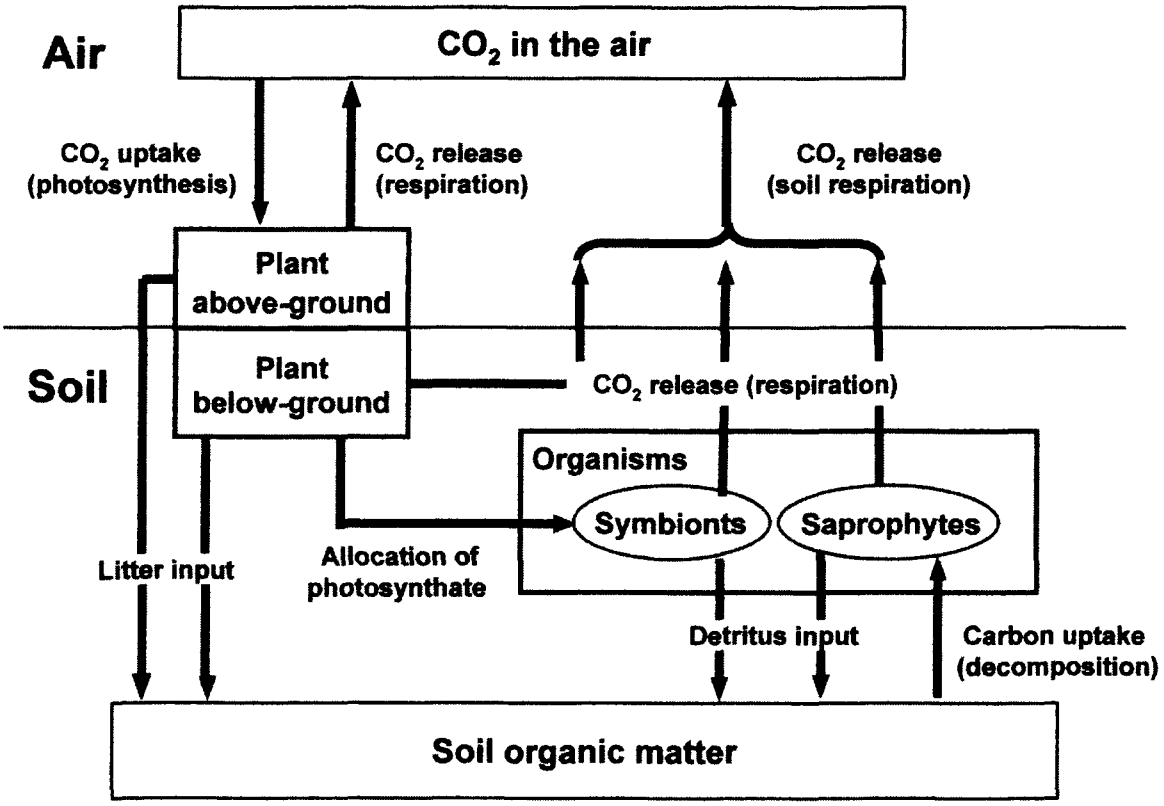

Fig.3 Schematic diagram of carbon dynamics in forest ecosystems. Carbon dioxide in the air is assimilated by the plant photosynthetic activity and allocated to the above- and below-ground parts of the plant. Photosynthate is also allocated to plant-symbiotic fungi (mycorrhizal fungi). Dead parts of the plants 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 the plant and symbiotic fungi are not related to the decomposition, while respiration by saprophytes is closely related to the decomposition of soil organic matter.