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FULL PAPER

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Fungal content of ectomycorrhizal tips: comparison among 13 tree species

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Abstract To better understand soil carbon cycling in forest ecosystems, we studied the proportion of fungal sheath area (FSA) in the cross-sectional ectomycorrhizal area in 13 tree species. Ectomycorrhizal samples were collected from subalpine and temperate forests in Japan. The FSA values were in the range of 12% to 56% across all tree species, tree ages, and fungal species. In *Abies firma* and *Quercus serrata*, the FSA values were larger in mature trees than in seedlings, whereas no such differences were found in *Pinus densiflora* and *Fagus crenata*. In broad-leaved trees, because the plant tissue radii lay within a narrow range, the FSA was affected mainly by the fungal sheath thickness. In conifers, however, the plant tissue radii varied widely among genera, so the FSA was affected by both the plant tissue radius and the fungal sheath thickness. Our findings suggest that the fungal content of ectomycorrhizal tips differs among tree species and fungal species, so that both parameters must be considered in studies of forest carbon cycling. The estimates revealed that data gathering in each type of forest leads to more accurate estimates of the biomass of fungi in ectomycorrhizal tips.

Key words Biomass of ectomycorrhizal fungi · Forest carbon cycling · Fungal content of root · Fungal sheath thickness · Image analysis

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Introduction

Mycorrhizal fungi play an important role in forest carbon cycling, as a considerable amount of the carbon fixed by a host plant is consumed by the fungal partner (Rygiewicz and Anderson 1994; Hobbie 2006). In quantification of the role of mycorrhizal fungi in forest carbon cycling, fungal biomass is an important parameter. The product (fungal content of ectomycorrhizal tips × biomass of ectomycorrhizal tips) gives the biomass of ectomycorrhizal fungi in ectomycorrhizal tips.

Several methods have been used to quantify the fungal content of ectomycorrhizal tips (Harley and McCready 1952; Vogt et al. 1991; Nylund and Wallander 1992; Satomura et al. 2006a,b). Harley and McCready (1952) dissected ectomycorrhizal tips of *Fagus sylvatica* L. and found that the fungal sheath occupied about 40% of the tips by weight. This value has been widely used to calculate the fungal biomass of ectomycorrhizae, irrespective of plant species, fungal species, or climatic and soil conditions of study sites (Vogt et al. 1991; Ostonen and Lõmus 2003; Hobbie 2006). Vogt et al. (1991) used image analysis to measure the proportion of fungal sheath area in the cross-sectional ectomycorrhizal area (FSA) to estimate the fungal content of ectomycorrhizal tips on the assumption that the densities of fungal tissue and plant tissue are the same; they found that the FSA was about 40% in *Abies amabilis* (Dougl.) Forbes and 20% in *Pseudotsuga menziesii* (Mirb.) Franco ectomycorrhizae. Ostonen and Lõmus (2003) reported that the FSA ranged from 17.7% to 28.1% in *Picea abies* (L.) Karst ectomycorrhizal tips. The findings of these two studies suggest that Harley and McCready's 40% is not appropriate for all kinds of ectomycorrhizal trees.

Only limited data exist for the quantification of actual fungal biomass of ectomycorrhizal tips, and two major questions remain unclear. First, researchers need to elucidate how much variation occurs in the fungal content of ectomycorrhizal tips within the same plant species. Second, studies need to quantify the differences in the values of the fungal

content of ectomycorrhizal tips among plant species or fungal species.

In the present study, we sampled ectomycorrhizal tips of 13 tree species from subalpine, cool-temperate, and warm-temperate forests in Japan to measure the FSA values using an image analysis method. We investigated the effects of tree species, tree age, and fungal species on FSA as well. As in Vogt et al. (1991), we estimated the fungal content of ectomycorrhizal tips on the assumption that the densities of fungal tissue and plant tissue were the same. We discuss here the suitability of using 40% as the fungal content of ectomycorrhizal tips and note several important points for more accurate estimation of the fungal content of ectomycorrhizae.

Materials and methods

Sampling of ectomycorrhizal tree seedlings and soil cores containing ectomycorrhizal tips

Current-year to 1-year-old seedlings of 13 tree species and the root systems of mature trees of 4 species were sampled at seven sites in subalpine, cool-temperate, and warm-temperate forests in Japan (Table 1). Six conifer species were sampled: *Abies firma* Sieb. et Zucc., *Abies sachalinensis* (Schmidt) Masters, *Larix kaempferi* (Lamb.) Carrière, *Picea glehnii* (Fr. Schm.) Masters, *Picea jezoensis* (Sieb. et Zucc.) Carrière var. *hondoensis* (Mayr.) Rehder, and *Pinus densiflora* Sieb. et Zucc. Seven broad-leaved species were sampled: *Betula ermanii* Cham., *Betula platyphylla* Sukatchev var. *japonica* (Miq.) Hara, *Fagus crenata* Blume, *Quercus crispula* Blume, *Quercus glauca* Blume, *Quercus phillyraeoides* A. Gray, and *Quercus serrata* Thunb. ex Murray.

At each of the seven sampling sites, three seedlings of each species were dug up carefully, using a shovel, along with a soil block (~20 cm × 20 cm × 30 cm depth) to avoid damaging their root systems. The aboveground part of each seedling was removed. To sample mature trees, we targeted four sites without any ectomycorrhizal understory in *A. firma*, *P. densiflora*, *F. crenata*, and *Q. serrata* forests. Five soil cores (5-cm diameter and 10-cm depth from the surface of the mineral soil layer) were sampled randomly using a core sampler from a 10-m × 10-m quadrat at each site. Sampled materials were stored in plastic bags in a cooler, returned to the laboratory, and preserved at –80°C until use.

Sorting ectomycorrhizal tips

The frozen soil block samples and soil core samples were thawed at 4°C in a refrigerator for 3–6 h, soaked in 4°C water for 1–2 h, and washed thoroughly in a sieve with running tap water to reveal the root system. Ectomycorrhizal tips were picked out using forceps, and ectomycorrhizal formation on the root samples was confirmed under a dissection microscope (magnification 10–30×; model SZH, Olympus Optical, Tokyo).

Sectioning and measuring ectomycorrhizal tips and classifying morphotypes

Five unramified ectomycorrhizal tips from each seedling were selected arbitrarily and categorized into each morphotype (described in Table 4) on the basis of the fungal sheath surface structure, diameter of emanating hyphae, existence of clamp connections, and sheath color under an optical microscope (model BX-50; Olympus). From each mature tree sample, 15 ectomycorrhizal tips were selected arbitrarily. Each ectomycorrhizal tip was held in a segment of pith and sectioned transversely at a right angle to the ectomycorrhizal major axis by hand using a sharp razor. Preliminary observations under a light microscope revealed the consistency of the fungal sheath thickness from the apex to the proximal end of the tip in *P. densiflora* and *Q. serrata*, except at the root cap. Therefore, we used the section from the middle part of a mycorrhizal tip. The radius of each round sliced section and the fungal sheath thickness were measured as follows.

Two selected thin sections from each mycorrhizal tip were mounted on a glass slide in lactic acid. Under 200× magnification, the ectomycorrhizal radius (r, μm) was calculated from two centrosymmetrical cross-lines with diameters a and b using the formula $(a + b)/4$. Using the two centrosymmetrical cross-lines, we measured the fungal sheath thickness (t, μm) at four points on a section under 800× or 1000× magnification and averaged the values. The values of r and t in each ectomycorrhizal tip were obtained by averaging the values from the two sections. In each species, the r and t values of seedlings were obtained by averaging the values of 15 tips from three seedlings, except *P. glehnii* (10 tips from two seedlings). Using the same measurements, we also calculated the r and t values of each morphotype in each tree species by averaging the values of 2 to 14 tips from three seedlings. In each species, the r and t values of mature trees were obtained by averaging the values of 15 tips from a composite soil sample, except *P. densiflora* (25 tips from a composite soil sample).

Using these values, we calculated the proportion of the fungal sheath area in the cross-sectional ectomycorrhizal area (FSA, %) using the following equation:

$$\mathrm{FSA} = [1 - (t/r)^2] \times 100 \quad (1)$$

Statistical analyses

Differences in ectomycorrhizal radii, fungal sheath thicknesses, plant tissue radii, and FSA values among the 13 seedling species and the 4 mature species were analyzed according to Scheffé's F test based on one-way analysis of variance (ANOVA; level of significance = 0.05). Differences in these parameters among the ectomycorrhizal morphotypes were analyzed in the same way. For the species *A. firma*, *P. densiflora*, *F. crenata*, and *Q. serrata*, the effects of tree age (seedlings vs. mature trees) and tree species on the ectomycorrhizal radius, fungal sheath thickness, plant tissue radius, and the FSA were tested according to two-way ANOVA (level of significance = 0.05 with a post hoc test

Table 1. Tree species used and the environmental properties of sampling sites

Tree species	Sampling date	Sampling site properties	Site name (location/prefecture)	Coordinate		Elevation (m)	Climate[a]			AMeDAS station
				Latitude (N)	Longitude (E)		Division	Annual		
								Mean air temperature (°C)	Precipitation (mm)	
Seedlings										
Conifers										
Abies firma	6 Nov 02	Mature forest/clay loam	Yoshiwa/Hiroshima	34° 26′	132° 05′	820	Cool temperate	9.1	1886	Kake
Abies sachalinensis	13 Sep 02	Mature forest/on a fallen tree	Kamishihoro/ Hokkaido	43° 14′	143° 18′	720	Subalpine	2.7	887	Kamishihoro
Larix kaempferi	13 Sep 02	Open site/loam	Obihiro/Hokkaido	42° 55′	143° 13′	80	Cool temperate	6.7	948	Obihiro
Picea glehnii	13 Sep 02	Mature forest/on a fallen tree	Kamishihoro/ Hokkaido	43° 14′	143° 18′	720	Subalpine	2.7	887	Kamishihoro
Picea jezoensis	13 Sep 02	Mature forest/on a fallen tree	Kamishihoro/ Hokkaido	43° 14′	143° 18′	720	Subalpine	2.7	887	Kamishihoro
Pinus densiflora	12 Oct 03	Open site/sandy loam	Saijo/Hiroshima	34° 24′	132° 43′	200	Temperate	13.3	1485	Higashihiroshima
Broad-leaved trees										
Betula ermanii	13 Sep 02	Mature forest/on a fallen tree	Kamishihoro/ Hokkaido	43° 14′	143° 18′	720	Subalpine	2.7	887	Kamishihoro
Betula platyphylla	13 Sep 02	Open site/loam	Obihiro/Hokkaido	42° 55′	143° 13′	80	Cool temperate	6.6	948	Obihiro
Fagus crenata	4 Oct 02	Mature forest/loam	Yoshiwa/Hiroshima	34° 27′	132° 06′	660	Cool temperate	10.2	1886	Kake
Quercus crispula	14 Oct 02	Open site/loam	Yoshiwa/Hiroshima	34° 27′	132° 06′	660	Cool temperate	10.2	1886	Kake
Quercus glauca	19 Nov 02	Mature forest/loam	Fukutomi/Hiroshima	34° 30′	132° 49′	300	Warm temperate	12.0	1367	Kouchi
Quercus phillyraeoides	15 Nov 02	Open site/sandy loam	Kamagari/Hiroshima	34° 10′	132° 50′	5	Warm temperate	16.1	1014	Kure
Quercus serrata	19 Nov 02	Open site/loam	Saijo/Hiroshima	34° 24′	132° 43′	200	Warm temperate	13.3	1485	Higashihiroshima
Mature trees										
Conifers										
Abies firma	6 Oct 04	100–150 year forest/ clay loam	Yoshiwa/Hiroshima	34° 28′	132° 10′	930	Cool temperate	8.4	1886	Kake
Pinus densiflora	7 Oct 04	50–60 year secondary forest/sandy loam	Saijo/Hiroshima	34° 24′	132° 43′	200	Warm temperate	13.3	1485	Higashihiroshima
Broad-leaved trees										
Fagus crenata	6 Oct 04	100-year forest/clay loam	Geihoku/Hiroshima	34° 41′	132° 11′	990	Cool temperate	–	2352	Yawata
Quercus serrata	6 Oct 04	70–80 year secondary forest/ clay loam	Yoshiwa/Hiroshima	34° 29′	132° 08′	550	Cool temperate	10.9	1886	Kake

[a] Climatic data for each site were obtained from the nearest Automated Meteorological Data Acquisition System (AMeDAS) station of the Japan Meteorological Agency. The data are shown as the 26- to 30-year average values (until 2005). Annual mean air temperature at each sampling site was estimated from the AMeDAS data using a temperature lapse rate (0.65°C per 100m) based on the difference in elevation between each sampling site and the nearest AMeDAS station

by Scheffé's *F* test). Correlations between parameters (ectomycorrhizal radius, fungal sheath thickness, and FSA) for each tree type (broad-leaved or conifer) were analyzed using a Spearman's test. Analyses were performed using the software Stat View J-5.0 for Windows (SAS Institute, Cary, NC, USA).

Results and discussion

Comparison among 13 tree species using seedling data

Ectomycorrhizal radii (*r*) differed significantly among tree species (Table 2). The conifer species *A. firma*, *A. sachalinensis*, *L. kaempferi*, and *P. densiflora* had significantly greater ectomycorrhizal radii than those of all broad-leaved tree species. The average values were greater in conifers than in broad-leaved trees. These results matched those of Karizumi (1974), who reported a smaller fine-root diameter in broad-leaved trees than in conifers. Ectomycorrhizal radii in the same genus were similar; for example, the *r* values of *P. glehnii* (142.8–212.9 µm) and *P. jezoensis* (138.3–214.2 µm) were similar and overlapped those values reported for *P. abies* (157.9–211.3 µm; Ostonen and Lõmus 2003). The *r* values within genera of broad-leaved trees also overlapped widely.

Fungal sheath thickness (*t*) differed significantly among tree species and tended to be larger in conifers than in broad-leaved trees, although the ranges of *t* values in conifers and broad-leaved trees overlapped widely (see Table 2). Fungal sheath thickness showed similar values among the conifer trees in the same genus. For example, the *t* values of *P. glehnii* (17.7–29.1 µm) and *P. jezoensis* (14.0–28.2 µm) were similar to that reported for *P. abies* (16.5–29.0 µm; Ostonen and Lõmus 2003), and the *t* values of *A. firma* (17.2–30.1 µm) and *A. sachalinensis* (16.3–35.9 µm) also were similar. The ranges of fungal sheath thickness among the broad-leaved trees, however, tended to differ even within the same genus. For example, we measured *t* values of 16.6–24.4 µm in *B. ermanii* and 10.1–16.6 µm in *B. platyphylla*, whereas Agerer et al. (1996–2004) reported values of 18.8–25.0 µm in *Betula* species. In the genus *Quercus*, the fungal sheath thickness of four species also differed significantly (see Table 2).

Plant tissue radii (*r*–*t*) also differed significantly among tree species (see Table 2). Radii were similar within the allied conifer species and were markedly larger in conifers than in broad-leaved trees.

The FSA differed significantly among tree species (see Table 2). The average FSA values of conifers and broad-leaved trees were 23.2% and 26.4%, respectively, although the ranges of the FSA values of conifers and broad-leaved trees overlapped widely. The conifers in the same genus showed similar FSA values. In contrast, the allied broad-leaved trees did not always show similar FSA values: the FSA values of *B. ermanii* and *B. platyphylla* were significantly different, and the differences of the FSA values among the four *Quercus* tree species were also large (see Table 2).

Ectomycorrhizal radii and the plant tissue radii of conifers tended to be larger than those of broad-leaved trees. These values tended to differ markedly among conifer genera, but less so among broad-leaved genera. Although fungal sheath thickness was different among tree species, the ranges of *t* values overlapped across many tree species. The FSA values varied widely among broad-leaved trees, whereas the allied conifers showed similar values. The FSA values of broad-leaved trees tended to be higher in those of conifers.

Effect of tree age

Using data from *A. firma*, *P. densiflora*, *F. crenata*, and *Q. serrata*, we compared ectomycorrhizal radii, fungal sheath thickness, plant tissue radii, and FSA between seedlings and mature trees (see Table 2). Effects of tree age on ectomycorrhizal radius and plant tissue radius were not significant, whereas tree age did have a significant effect on fungal sheath thickness (Table 3). Fungal sheath thickness did not differ significantly between seedlings and mature *P. densiflora* and *F. crenata* trees (see Table 2). In *A. firma* and *Q. serrata*, however, fungal sheath thickness was significantly greater in mature trees than in seedlings.

The significant effect of tree age on FSA and fungal sheath thickness (see Table 3) suggests that the difference in FSA between seedlings and mature trees was mainly the result of differences in fungal sheath thickness among trees of different ages. The FSA values of *P. densiflora* and *F. crenata* did not differ significantly between seedlings and mature trees, whereas those of *A. firma* and *Q. serrata* did differ significantly and tended to be larger in mature trees (see Table 2). It is unclear why the effect of tree age on fungal sheath thickness and FSA differed among tree species; additional data are necessary to answer this question.

Effect of ectomycorrhizal morphotype

With the exception of *B. platyphylla*, all tree species were associated with two or more ectomycorrhizal morphotypes (Table 4). The effect of morphotype on the ectomycorrhizal radius was significant only in *Q. phillyraeoides*, and the effect on the plant tissue radius was significant in *B. ermanii* and *Q. phillyraeoides*. Morphotype had a significant effect on the fungal sheath thickness in 5 of the 13 tree species (*L. kaempferi*, *P. glehnii*, *P. densiflora*, *Q. glauca*, and *Q. phillyraeoides*; Table 4). The effect of a morphotype on FSA, however, was significant in only *P. densiflora* and *B. ermanii* (see Table 4). Although several tree species were associated with the same fungal species, i.e., *Cenococcum geophilum* Fr., the FSA (and we assume the fungal sheath thickness as well) differed among those tree species (Fig. 1).

Factors affecting the FSA

On the basis of Eq. 1, we would expect that (i) the FSA decreases with increasing ectomycorrhizal radius if the

Table 2. The mean, minimum, and maximum values of ectomycorrhizal radius (*r*), fungal sheath thickness (*t*), plant tissue radius (*r-t*), and fungal sheath area in the cross-sectional ectomycorrhizal area (FSA) of seedlings and mature trees of 13 tree species

Tree species	Ectomycorrhizal radius (μm)			Fungal sheath thickness (μm)			Plant tissue radius (μm)			FSA (%)		
	Average (SE)	Minimum	Maximum	Average (SE)	Minimum	Maximum	Average (SE)	Minimum	Maximum	Average (SE)	Minimum	Maximum
Seedlings												
Conifers												
Abies firma	251.9 (12.0)^{a}	209.1	329.6	23.6 (0.8)^{ab}*	17.2	30.1	228.3 (12.1)^{a}	184.3	306.3	18.3 (0.9)^{d}*	12.6	22.3
Abies sachalinensis	234.0 (12.5)^{ab}	108.4	308.5	25.2 (1.3)^{ab}	16.3	35.9	208.9 (11.9)^{ab}	92.1	279.3	20.8 (1.1)^{cd}	15.5	27.8
Larix kaempferi	225.5 (9.2)^{ab}	164.5	285.6	28.3 (1.1)^{a}	19.9	34.8	197.2 (8.9)^{ab}	134.3	255.1	23.8 (1.0)^{bcd}	17.7	33.3
Picea glehnii	164.6 (7.1)^{de}	142.8	212.9	23.3 (1.1)^{abc}	17.7	29.1	141.3 (6.9)^{de}	121.9	189.6	26.5 (1.2)^{abc}	20.7	32.1
Picea jezoensis	171.4 (5.7)^{cd}	138.3	214.2	22.3 (1.0)^{abc}	14.0	28.2	149.1 (5.3)^{cd}	118.8	190.2	24.3 (1.0)^{abcd}	19.3	31.4
Pinus densiflora	194.4 (5.8)^{bc}	156.8	245.4	27.4 (2.8)^{a}	15.2	47.5	167.1 (4.5)^{bc}	141.6	199.0	25.6 (2.0)^{abc}	14.7	38.7
Combined values of conifer seedlings	209.5 (5.1)			25.1 (0.7)			184.0 (5.0)			23.2 (0.6)		
Broad-leaved trees												
Betula ermanii	117.8 (2.3)^{ef}	105.2	133.9	20.1 (0.5)^{abc}	16.6	24.4	97.6 (2.2)^{e}	86.9	113.2	31.3 (0.7)^{a}	26.8	37.0
Betula platyphylla	116.8 (3.3)^{ef}	99.4	144.7	13.1 (0.6)^{d}	10.1	16.6	103.8 (3.1)^{e}	85.6	129.6	21.1 (0.8)^{bcd}	16.4	25.9
Fagus crenata	115.8 (2.8)^{ef}	101.4	139.6	17.4 (0.9)^{bcd}	12.1	24.8	98.4 (2.8)^{e}	84.2	120.7	27.8 (1.3)^{abc}	18.1	36.7
Quercus crispula	108.6 (4.3)^{f}	81.6	149.8	15.0 (1.0)^{cd}	10.9	25.1	93.7 (3.5)^{e}	70.6	124.7	25.4 (0.9)^{abcd}	21.1	31.8
Quercus glauca	139.5 (6.3)^{def}	103.9	210.4	21.3 (1.1)^{abc}	16.4	35.0	118.2 (5.6)^{de}	83.5	175.4	28.4 (1.1)^{ab}	21.1	35.4
Quercus phillyraeoides	126.6 (8.2)^{def}	90.5	202.7	21.2 (1.9)^{abc}	12.5	40.2	105.4 (6.5)^{de}	78.0	162.5	30.2 (1.1)^{ab}	25.3	37.4
Quercus serrata	126.3 (5.6)^{def}	96.9	167.7	13.8 (0.6)^{d}*	10.0	18.7	112.6 (5.2)^{de}	83.0	151.2	20.8 (0.8)^{cd}*	16.4	26.9
Combined values of broad-leaved tree seedlings	121.6 (2.1)			17.4 (0.5)			104.0 (1.8)			26.4 (0.5)		
Mature trees												
Conifers												
Abies firma	254.4 (6.2)^{a}	213.6	292.9	31.2 (2.1)^{a}*	19.5	45.8	223.2 (4.7)^{a}	194.1	250.5	22.8 (1.1)^{b}*	16.9	28.8
Pinus densiflora	197.6 (5.3)^{b}	147.9	255.3	25.7 (0.7)^{ab}	19.7	32.9	171.9 (5.2)^{b}	121.8	235.6	24.6 (0.8)^{b}	14.8	34.9
Broad-leaved trees												
Fagus crenata	122.2 (4.5)^{c}	95.6	161.9	16.7 (1.9)^{c}	10.4	32.3	105.5 (3.0)^{c}	85.2	130.5	24.7 (1.7)^{b}	17.0	38.0
Quercus serrata	124.0 (4.5)^{c}	95.6	173.1	23.5 (2.0)^{bc}*	9.8	40.0	100.5 (4.4)^{c}	78.9	152.1	34.0 (0.8)^{a}*	19.1	55.9

Values are means with SE in parentheses ($n = 15$ for all species except seedling *P. glehnii* [$n = 10$] and mature *P. densiflora* [$n = 25$])
Within each column of each tree age (seedlings or mature trees), different lowercase letters indicate significant differences among tree species using Scheffé's *F* test based on one-way ANOVA (level of significance = 0.05)
For four tree species (*A. firma*, *P. densiflora*, *F. crenata*, and *Q. serrata*), asterisks indicate significant differences between seedlings and mature trees of each species using Scheffé's *F*-test based on two-way ANOVA (level of significance = 0.05)

Table 3. Results of two-way ANOVA for the effect of tree age (seedlings and mature trees) and tree species (*Abies firma*, *Pinus densiflora*, *Fagus crenata*, and *Quercus serrata*) on the ectomycorrhizal radius (*r*), fungal sheath thickness (*t*), plant tissue radius (*r*-*t*), and FSA

Source of variance	Parameter (*P* values)			
	Ectomycorrhizal radius	Fungal sheath thickness	Plant tissue radius	FSA
Tree age	0.5957	0.0011	0.7534	0.0013
Tree species	<0.001	<0.001	<0.001	<0.001
Tree age × tree species	0.9297	0.0003	0.3564	<0.001

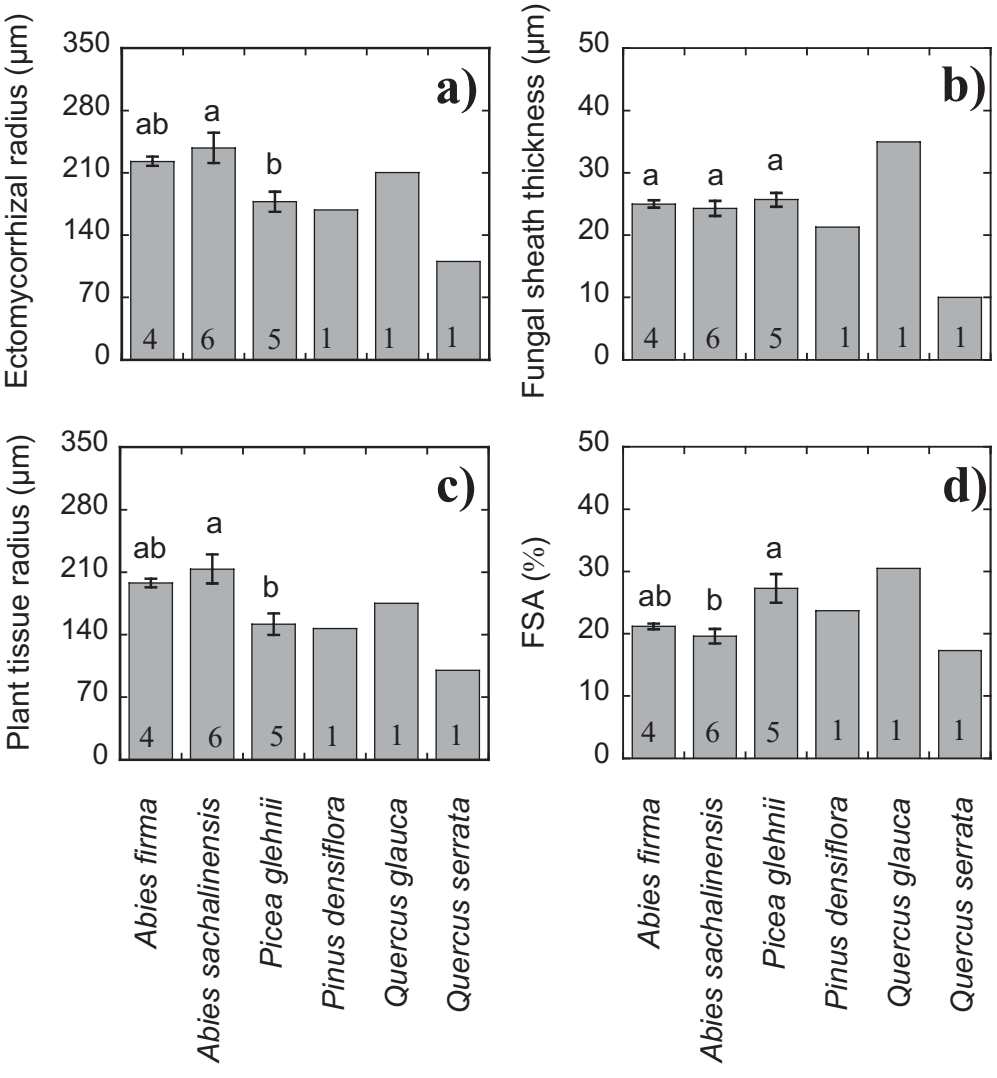

Fig. 1. Ectomycorrhizal radius (**a**), fungal sheath thickness (**b**), plant tissue radius (**c**), and fungal sheath area in the cross-sectional ectomycorrhizal area (FSA) (**d**) of *Cenococcum geophilum* Fr. mycorrhizae in six tree species. Different letters indicate significant differences among tree species (*Abies firma*, *Abies sachalinensis*, and *Picea glehnii*) by Scheffé's *F* test based on one-way analysis of variance (ANOVA) ($P < 0.05$). For the other tree species, the values have no replications and were not tested

fungal sheath thickness is constant, and (ii) the FSA increases with increasing fungal sheath thickness if the ectomycorrhizal radius is constant.

To assess the effects of ectomycorrhizal radius and fungal sheath thickness on the FSA, we analyzed the respective correlations of each component and FSA (Fig. 2). The FSA tended to increase with increasing fungal sheath thickness in both broad-leaved and coniferous trees (broad-leaved: $n = 105$, $r = 0.773$, $P < 0.0001$; conifers: $n = 85$, $r = 0.639$, $P < 0.0001$; Fig. 2b). These correlations did not conflict with expectation (ii). As shown in Fig. 2b, when fungal sheath thickness was assumed to be similar, the broad-leaved trees tended to have a larger FSA than conifers, which is attributable to the smaller plant tissue size (radius) in broad-leaved trees than in conifers. Among conifers, the FSA tended to decrease with increasing ectomycorrhizal radius ($n = 85$, $r = -0.510$, $P < 0.0001$; see Fig. 2a). This correlation did not conflict with expectation (i). In broad-leaved trees, however, there was no correlation between the FSA and the ectomycorrhizal radius ($n = 105$, $r = 0.023$, $P = 0.8175$). The ectomycorrhizal radius and fungal sheath thickness were correlated positively in broad-leaved trees ($n = 105$,

Table 4. Ectomycorrhizal radius (*r*), fungal sheath thickness (*t*), plant tissue radius (*r-t*), and FSA of each ectomycorrhizal morphotype in 13 tree species

Tree species (seedling)	Type no.	Color[a]	Sheath structure[b]	Clamp connections	Diameter of emanating hyphae (µm)	Ectomycorrhizal radius (µm)	Fungal sheath thickness (µm)	Plant tissue radius (µm)	FSA (%)	Number of tips observed	Identified as
Conifers											
Abies firma	Af1	BL	NS	Absent	3–4	223.0 (5.2)[a]	25.0 (0.6)[a]	198.0 (4.9)[a]	21.2 (0.5)[a]	4	*Cenococcum geophilum*
	Af2	PW	NS	Absent	1–2	260.6 (21.6)[a]	22.6 (1.8)[a]	238.1 (21.0)[a]	16.9 (1.4)[a]	6	Unidentified
	Af3	LB	NP	Exist	4–5	264.7 (23.9)[a]	23.8 (0.7)[a]	240.9 (24.4)[a]	17.8 (1.8)[a]	5	Unidentified
Abies sachalinensis	As1	BL	NS	Absent	3–4	238.1 (17.1)[a]	24.3 (1.2)[a]	213.8 (16.5)[a]	19.6 (1.2)[a]	6	*Cenococcum geophilum*
	As2	LB	NS	Absent	1–2	215.6 (24.8)[a]	23.3 (2.0)[a]	192.3 (23.7)[a]	21.3 (2.0)[a]	6	Unidentified
	As3	LB	NS	Exist	2–3	262.8 (10.4)[a]	30.8 (3.5)[a]	232.0 (13.8)[a]	22.3 (3.1)[a]	3	Unidentified
Larix kaempferi	Lk1	LB	NP	Exist	2–3	191.1 (9.3)[a]	23.2 (1.4)[b]	167.9 (9.4)[a]	22.9 (1.7)[a]	4	Unidentified
	Lk2	B	NS	Absent	2–3	237.0 (14.9)[a]	29.6 (0.9)[a]	207.3 (15.2)[a]	24.1 (1.9)[a]	7	Unidentified
	Lk3	PW	NP	Absent	1–3	239.9 (9.7)[a]	31.2 (1.7)[a]	208.7 (10.0)[a]	24.4 (1.6)[a]	4	Unidentified
Picea glehnii	Pg1	BL	NS	Absent	3–4	177.6 (11.3)[a]	25.7 (1.1)[a]	151.9 (12.1)[a]	27.3 (2.3)[a]	5	*Cenococcum geophilum*
	Pg2	PW	NS	Absent	1–2	151.6 (3.6)[a]	20.9 (1.0)[b]	130.7 (3.1)[a]	25.6 (1.0)[a]	5	Unidentified
Picea jezoensis	Pj1	PW	NS	Exist	1–3	156.5 (7.3)[–]	17.3 (1.5)[–]	139.2 (5.9)[–]	20.9 (0.8)[–]	2	Unidentified
	Pj2	LB	IS	Exist	2–4	173.6 (6.3)[–]	23.0 (1.0)[–]	150.6 (5.9)[–]	24.9 (1.0)[–]	13	Unidentified
Pinus densiflora	Pd1	BL	NS	Absent	3–4	168.3 –[–]	21.3 –[–]	147.0 –[–]	23.7 –[–]	1	*Cenococcum geophilum*
	Pd2	PW	NP	Exist	3–5	184.5 (9.8)[a]	17.6 (1.0)[b]	166.9 (9.8)[a]	18.3 (1.3)[b]	5	Unidentified
	Pd3	PW	IS	Absent	2–3	189.8 (4.5)[a]	23.8 (1.7)[b]	166.0 (4.9)[a]	23.5 (1.8)[b]	4	Unidentified
	Pd4	PW	FS	Absent	3–4	213.3 (9.5)[a]	41.2 (2.2)[a]	172.1 (8.5)[a]	35.0 (1.4)[a]	5	Unidentified
Broad-leaved trees											
Betula ermanii	Be1	PW	NS	Exist	3–4	121.4 (2.8)[a]	19.8 (0.7)[a]	101.6 (2.6)[a]	29.9 (1.0)[b]	8	Unidentified
	Be2	PW	IS	Exist	2–3	113.7 (3.4)[a]	20.5 (0.9)[a]	93.1 (2.8)[b]	32.9 (0.8)[a]	7	Unidentified
Betula platyphylla	Bp1	PW	NS	Exist	3–4	116.8 (3.3)[–]	13.1 (0.6)[–]	103.8 (3.0)[–]	21.1 (0.8)[–]	15	Unidentified
Fagus crenata	Fc1	PW	NP	Absent	4–6	118.2 (3.6)[a]	16.8 (1.0)[a]	101.4 (3.6)[a]	26.5 (1.5)[a]	10	Unidentified
	Fc2	LB	IS	Exist	3–5	106.3 (3.7)[a]	17.7 (1.6)[a]	88.6 (3.2)[a]	30.5 (2.2)[a]	3	Unidentified
	Fc3	B	NP	Absent	4–5	118.6 (2.5)[–]	20.0 (4.8)[–]	98.6 (2.2)[–]	30.6 (6.1)[–]	2	Unidentified
Quercus crispula	Qc1	PW	NS	Exist	1–2	105.7 (3.4)[–]	14.2 (0.8)[–]	91.5 (2.9)[–]	25.0 (0.8)[–]	14	Unidentified
	Qc2	LB	NP	Exist	3–4	149.8 –[–]	25.1 –[–]	124.7 –[–]	30.7 –[–]	1	Unidentified
Quercus glauca	Qg1	BL	NS	Absent	3–4	210.4 –[–]	35.0 –[–]	175.4 –[–]	30.5 –[–]	1	*Cenococcum geophilum*
	Qg2	PW	NS	Exist	4–5	136.8 (5.7)[a]	19.3 (0.6)[b]	117.5 (5.8)[a]	26.6 (1.5)[a]	9	Unidentified
	Qg3	B	RS	Exist	1–3	130.3 (4.7)[a]	22.2 (0.6)[a]	108.1 (4.6)[a]	31.2 (0.9)[a]	5	Unidentified
Quercus phillyraeoides	Qp1	LB	NS	Absent	3–4	108.4 (4.1)[b]	17.1 (1.0)[b]	91.4 (3.5)[b]	29.0 (1.2)[a]	10	Unidentified
	Qp2	LB	NP	Exist	2–4	162.8 (11.7)[a]	29.4 (2.9)[a]	133.5 (9.9)[a]	32.8 (2.0)[a]	5	Unidentified
Quercus serrata	Qs1	BL	NS	Absent	3–4	110.3 –[–]	10.0 –[–]	100.3 –[–]	17.3 –[–]	1	*Cenococcum geophilum*
	Qs2	PW	NP	Exist	1–2	127.5 (5.9)[–]	14.0 (0.6)[–]	113.4 (5.5)[–]	21.0 (0.9)[–]	14	Unidentified

Values are averages with SE in parentheses. –, no replicate

Different lowercase letters indicate significant differences among ectomycorrhizal morphotypes within each tree species (tested for $3 \leq n$)

[a] BL, black; PW, pale color or white; LB, light brown; B, brown

[b] Surface structure type of fungal sheath: NS, net synenchyma; NP, net prosynenchyma; IS, irregular synenchyma; FS, felt synenchyma; RS, regular synenchyma (Ingleby et al. 1990)

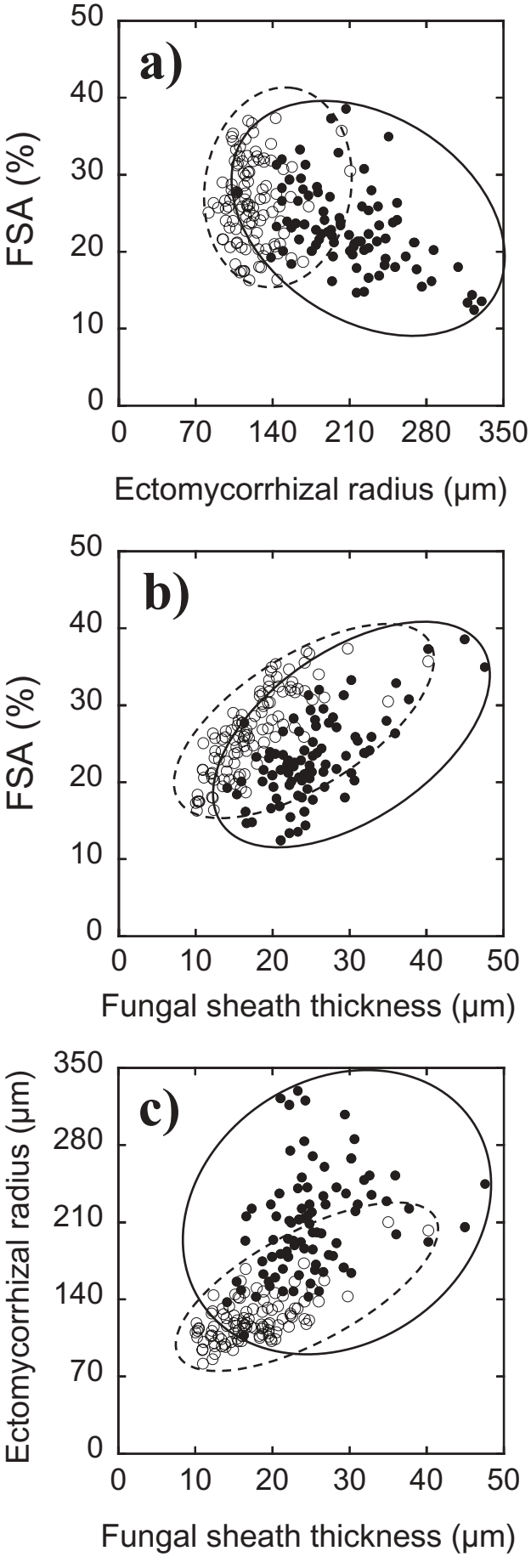

Fig. 2. Relationships between FSA and ectomycorrhizal radius (**a**), FSA and fungal sheath thickness (**b**), and fungal sheath thickness and ectomycorrhizal radius (**c**) of 13 tree species. Only the seedling data are shown. *Solid circles*, conifers; *open circles*, broad-leaved trees. *Solid and dotted circles* indicate distribution patterns of conifers and broad-leaved trees, respectively

$r = 0.639$, $P < 0.0001$), and more weakly in conifers ($n = 85$, $r = 0.306$, $P = 0.0044$; Fig. 2c).

On the basis of Eq. 1, we would also expect that (iii) the FSA decreases with increasing plant tissue radius if the fungal sheath thickness is constant. In this study, the ectomycorrhizal radius and plant tissue radius were strongly correlated ($n = 190$, $r = 0.995$, $P < 0.001$; figure not shown). Thus, the trend of the relationships of FSA and fungal sheath thickness versus plant tissue radius (data not shown) was similar to those of FSA and fungal sheath thickness versus ectomycorrhizal radius (Fig. 2a,c). FSA showed a negative correlation with plant tissue radius in conifers ($n = 85$, $r = –0.613$, $P < 0.0001$), but no significant correlation in broad-leaved trees ($n = 105$, $r = –0.190$, $P = 0.0526$). The plant tissue radius and fungal sheath thickness were correlated positively in broad-leaved trees ($n = 105$, $r = 0.459$, $P < 0.0001$), but no such correlation was observed in conifers ($n = 85$, $r = 0.181$, $P = 0.0969$).

Although several conifer species were associated with the fungal species *C. geophilum* and the fungal sheath thicknesses were similar across the plant species (Fig. 1c), the FSA values may differ because of the significant difference in plant tissue size, i.e., radius within the ectomycorrhizal tip (see Fig. 1a). Conifers show a large variation in plant tissue size (see Table 2), which is likely determined by plant genetics. The conifer plant tissue size and its variation are considerably larger than the thickness and variation of the fungal sheath. In the majority of conifers, there were no significant differences in the FSA across morphotypes, in spite of differences in the fungal sheath thickness (see Table 4). Therefore, in conifers, plant tissue size (radius) is the major factor determining the FSA.

Our results revealed that the FSA of broad-leaved trees is affected mainly by fungal sheath thickness, whereas that of conifers is weakly affected by fungal sheath thickness because of the larger plant tissue size. The fungal sheath thickness is partly affected by the fungal species composition, suggesting that the composition of the associated fungal species is important in assessments of the FSA in broad-leaved trees. In conifers, plant tissue size tended to differ among plant genera, which had a strong effect on the FSA, suggesting that the tree species as well as fungal species compositions of ectomycorrhizae must be considered. Although it is possible that the fungal sheath thickness of the same fungal species differs among host plant species, data on fungal species composition in each host plant and fungal sheath thickness in each type of ectomycorrhiza are scarce.

The effect of species composition of ectomycorrhizal fungi on the FSA is unclear. In general, it is known that the dominant ectomycorrhizal fungi differ among host plant species and/or plant age (Visser 1995; Kernaghan et al.

2003; Richard et al. 2005). Additional information about the effects of plant species and plant age on the development of the fungal sheath in consideration of the species composition of associated ectomycorrhizal fungi will provide a better understanding of the role of ectomycorrhizal fungi in forest ecosystem carbon cycling. In the image analysis used to determine the FSA, we measured the fungal sheath but not the Hartig net, which may have led to underestimates of the fungal content of ectomycorrhizal tips. Although estimation of the fungal content of ectomycorrhizal tips using the FSA has several limitations, our FSA estimates did reveal that data gathering in each type of forest leads to more accurate estimates of the biomass of fungi in ectomycorrhizal tips.

Quantifying the role of ectomycorrhizal fungi in forest carbon cycling

We studied the proportion of fungal sheath area in the cross-sectional ectomycorrhizal area (FSA) as an important parameter for the quantitative estimation of the role of ectomycorrhizal fungi in forest carbon cycling. On the assumption that the densities of plant tissues and fungal tissues are the same, we used FSA to calculate the fungal content of ectomycorrhizal tips, which ranged from 12.6% to 55.9%. The value of 40% fungal content reported by Harley and McCready (1952) and cited in various studies is relatively close to the upper value in our calculated range. Smith and Read (1997) suggested that Harley and McCready (1952) sampled only large ectomycorrhizae, probably those formed by *Lactarius subdulcis*. The fungal content of ectomycorrhizal tips in our study tended to differ among tree species (see Table 2). Therefore, when researchers estimate the fungal content of ectomycorrhizal tips, the conventionally cited value of 40% is not necessarily applicable to all types of forest ecosystems. For example, in a Japanese cool-temperate, broad-leaved, deciduous forest dominated by ectomycorrhizal broad-leaved trees such as *Q. crispula*, *B. ermanii*, and *B. platyphylla*, the biomass of ectomycorrhizal tips was estimated to be 88.7 $g\,m^{-2}$ (Satomura 2003). Using the value of fungal content of ectomycorrhizal tips of broad-leaved trees in this study (26.4%) gives 23.4 $g\,m^{-2}$ as the fungal biomass in ectomycorrhizal tips, whereas a 40% fungal content would give an estimate of 35.5 $g\,m^{-2}$. Likewise, based on the values in a *Pinus sylvestris* L. forest reported by Finlay and Söderström (1992, citing Persson 1978), the fungal content of ectomycorrhizal tips of pine trees in this study (25.6%) gives 468 $kg\,ha^{-1}\,year^{-1}$ as the annual production of fungi in the ectomycorrhizal tips, whereas a 40% fungal content would give 730 $kg\,ha^{-1}\,year^{-1}$. Therefore, when using the fungal content of ectomycorrhizal tips in the estimation of forest carbon dynamics, it is necessary to consider tree species composition as well as fungal species composition in each forest ecosystem.

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