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FULL PAPER

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Dynamics of fruit-body production and mycorrhiza formation of ectomycorrhizal ammonia fungi in warm temperate forests in Japan

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Abstract To investigate the quantitative correlation between dynamics of ectomycorrhiza (ECM) formation and fruit-body production of the ammonia fungi, we treated forest soils in two ECM forests, a *Castanopsis cuspidata* forest and a *Quercus serrata* forest, in warm temperate Japan with urea to induce fruiting of ammonia fungi. We identified the ectomycorrhizae of two known species of ammonia fungi, *Alnicola lactariolens* and *Hebeloma vinosophyllum*, using morphological typing and PCR-RFLP. ECM initiation, increase, and subsequent decrease preceded the start, increase, and decrease of fruit-body production for each species. We also found many kinds of ECM fungi that did not develop fruit bodies after urea treatment during the observation period, which suggests that it is necessary to expand the definition of ammonia fungi from one that refers solely to the species reproducing on the ground to one which includes those in somatic forms in the soil.

Key words Ammonia fungi · Ectomycorrhiza formation · Fruit-body production · PCR-RFLP · Urea treatment

Introduction

In cool temperate areas of Europe and North America, a wide variety of studies on ectomycorrhizal (ECM) fungal communities has been conducted. Authors of these studies first considered fruit-body assemblages (Bills et al. 1986; Dighton et al. 1986; Arnolds 1988; Vogt et al. 1992; Keizer and Arnolds 1994; Jumpponen et al. 1999; Walker and Miller 2002) and then attempted to identify ectomycorrhizae in terms of fungal symbionts by morphological typing (Danielson and Pruden 1989; Baxter et al. 1999; Kranabetter et al. 1999; Massicotte et al. 1999) and by polymerase chain reaction-restriction enzyme fragment length polymorphism (PCR-RFLP) analysis (Gardes et al. 1991; Gardes and Bruns 1993, 1996; Dahlberg et al. 1997; Kårén et al. 1997; Gehring et al. 1998; Jonsson L. et al. 1999; Jonsson T. et al. 1999; Jonsson L. et al. 2000; Glen et al. 2001; Peter et al. 2001). Studies on changes in the fungal flora after N-application (N-fertilization) have also been conducted (Alexander and Fairley 1983; Termorshuizen 1993; Kårén and Nylund 1997; Boxman et al. 1998; Brandrud and Timmermann 1998; Jonsson et al. 2000; Peter et al. 2001) and revealed the effects of air pollution and acid rain containing NO_x on fungi in Europe. However, no study has investigated the phenological dynamics between fruit-body and ECM formation, especially on a local scale.

This study focuses on the dynamics and characteristics of both fruit-body production and ectomycorrhizal formation. "Dynamics" here means quantitative changes over time. By identifying fruit bodies and counting ECM tips, we investigated simultaneously the dynamics of fruiting aboveground and that of ectomycorrhizal formation belowground.

The ammonia fungi have been recognized as members of fungal communities composed of a wide variety of taxonomic groups that form after the application of ammonia or ammonia-forming nitrogenous compounds. We used urea treatment (Sagara 1975) to induce fruit-body production of ammonia fungi and to investigate the dynamics of fruit-body production and ECM formation simultaneously. The term ammonia fungi has referred solely to the fungal species reproducing on soil (Sagara 1975, p. 270): "a chemoecological group of fungi which sequentially develop reproductive structures exclusively or relatively luxuriantly on the soil after a sudden addition of ammonia, some other nitrogenous materials which react as bases by themselves or on decomposition, or alkalis." Although fruit bodies of

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ammonia fungi are rarely seen under natural conditions, they are not necessarily dependent on artificial disturbance for their growth and reproduction. They are considered to be latent inocula in soils (Sagara 1976b; Suzuki et al. 2002; Suzuki 2006) of forests in various areas and to be adapted to many kinds of local but frequent input disturbances, e.g., animal excretions, carcasses, or giant-hornet wastes (Sagara et al. 1985; Sagara 1989, 1995; Wang and Sagara 1997; Fukiharu et al. 2000a,b; Harmaja 2002; Kasuya 2002; Oda et al. 2002).

Sequential fruit-body production of the ammonia fungi in the field has been induced by applying a large amount of urea to forest soil (about 350 g N/m^2; Sagara 1975, 1976a; Fukiharu 1991; Fukiharu and Hongo 1995; Yamanaka 1995a–c; Fukiharu et al. 1997; Imamura 2001; Suzuki et al. 2002; Imamura and Yumoto 2004a). These studies proved that a limited number of fungal species (10–20 species) frequently develop a very large quantity of fruit bodies (maximum, 50 g/m^2 in dry weight; Sagara 1975, 1976a; Fukiharu 1991; Imamura and Yumoto 2004a) on small plots (experimental unit, 0.5 m^2) after the treatment. Especially, the dominant fungal species develop fruit bodies on almost all the treated plots (Fukiharu 1991; Imamura and Yumoto 2004a). With this method, we simplified the structure of the original ECM fungal community, which has high species diversity even on a local scale (Bruns 1995), and therefore we could experimentally examine the dynamics of the ammonia fungi.

Sagara (1995) applied the concept of succession to changes in fruiting species of the ammonia fungi and the abundance of their fruit bodies and defined two successional phases: early phase (EP) and late phase (LP). These phases are especially marked in ECM forests. The EP species are saprobic (SAP), and most LP species are ECM, based on taxonomic information obtained from urea treatment experiments on soil, without a host plant, in the laboratory, and in field experiments where penetration of host roots was blocked (Sagara 1975, 1995).

Identifications of ECM formed by LP species, however, have not been confirmed. LP species consist mainly of *Hebeloma* (Cortinariaceae) and *Laccaria* (Tricholomataceae) species, which are early-stage fungi in the sense of Deacon and Fleming (1992). They are also facultative ECM species, having weak activity of cellulolytic and ligninolytic enzymes (Enokibara et al. 1993; Yamanaka 1995b; Sponsathien 1998) and the ability to decompose macromolecular organic matter (Ohta 1997; Yamanaka 1999). Thus, this study aimed at the following two points: (1) detecting ectomycorrhizae formed by known LP ammonia fungi and (2) assessing the quantitative correlation between ECM formation and fruit-body production in the same LP species.

Materials and methods

Study sites

Experimental study sites were established in an evergreen broad-leaved forest dominated by *Castanopsis cuspidata* (Thunberg) Schottky (Site C) and in a deciduous broad-leaved forest dominated by *Quercus serrata* Murray (Site Q). Site C was located at Kamitakano-saimyoujiyama, Kyoto-shi, Kyoto Pref., Japan (35°4′ N, 135°48′ E, 150 m alt.), and Site Q was at Iwakura-hanazono-cho, Kyoto-shi, Kyoto Pref., Japan (35°4′ N, 135°48′ E, 154 m alt.). Distance between the two sites is about 1.5 km. The precipitation data at Kamigamo Experimental Forest Station of Kyoto University (35°4′ N, 135°46′ E, 150 m alt.) were used as the nearest records of Sites C and Q. Both the dominant species at Sites C and Q are ectomycorrhizal members of the Fagaceae (Maeda 1954; Harley and Harley 1987; Imazeki and Hongo 1987). *Castanopsis cuspidata* is a dominant species of climax forests, and *Q. serrata* is dominant in the secondary forests around Kyoto.

The forest understory at both sites consisted mostly of *Q. glauca* Thunberg, *Cleyera japonica* Sieb. et Zucc., *Eurya japonica* Thunberg, and *Camellia japonica* L. At Site C, some planted trees of *Cryptomeria japonica* (L. f) D. Don and *Chamaecyparis obtusa* (Sieb. et Zucc.) Endlicher joined the dominant trees in the canopy layer. Besides the dominant species mentioned above, only *Q. glauca* is ectomycorrhizal (Maeda 1954). Therefore, we established urea plots where no *Q. glauca* was present as canopy trees.

Experimental plot design

According to Sagara (1975, 1976a) and Imamura (2001), the unit area for an experimental urea plot is 0.5 × 1 m (parallel to the slope in the undulate topography in Japan); LP ammonia fungi fruit abundantly in almost all such plots. Sites C and Q were set up so that each had ten units to reduce the edge effects: one unit in the horizontal and ten units along the vertical slope direction; i.e., 0.5 m (horizontal) × 10 m (vertical). We divided each of the plots into five subplots (two units per subplot) 2 m long in the direction of the slope.

To induce fruiting of the ammonia fungi, granular urea fertilizer (N 46%; Kumiai Nyouso, National Federation of Agricultural Co-operative Associations, Tokyo, Japan) was scattered by hand onto the experimental plots on 15 June 2000. The amount of urea for 1 m^2 was 686 g/m^2 (320 g N), which is 10–100 times more than used in other N-application experiments mentioned above.

Collection of fruit bodies and soil samples

Sites were visited every 10 days through the observation period. The six species listed in Table 1 were expected to develop fruit bodies based on the results of previous studies (Fukiharu 1991; Sagara 1995; Imamura 2001; Imamura and Yumoto 2004a). All the matured fruit bodies occurring inside the subplots were recorded and harvested. The dry weight of the fruit bodies was measured after drying at 55°C for 1 week. The weight was cumulatively totaled over the term of soil sampling, i.e., 1 month (see following). Collection of fruit bodies was continued for 15 months, until the

Table 1. Specimens of fruit bodies used in polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of this study

Abbreviation	Scientific name	Collection data	
		Site	Date
Al	*Alnicola lactariolens*	C	2 Jul 2001
Hv	*Hebeloma vinosophyllum*	C1[a]	5 Jul 2001
Hs	*H. spoliatum*	Q	11 Jun 2001
Hr	*H. radicosoides*	C1[a]	5 Jul 2001
Lb	*Laccaria bicolor*	Q1[a]	17 Oct 2000
La	*L. amethystina*	Q-I[b]	26 Jun 1998

All the specimens were collected after urea treatment by Imamura in Kyoto, Japan, either in the present study or in others
[a] Imamura and Yumoto 2004a
[b] Imamura 2001

end of August 2001, when the second flush of fruiting of the LP/ECM ammonia fungi occurred.

ECM tips and plant roots were collected as a soil block with a surface of 5 × 5 cm and a depth of 5–10 cm, including the O and A layers. One soil block from each subplot (i.e., five blocks per experimental site) was collected from where no soil block had been collected before. Each soil block was taken at a point 10 cm away from either of the two edges in the vertical direction and more than 10 cm away from the horizontal edges of the plot. Intervals between sampling points in adjacent subplots were more than 2 m from each other in one sampling date. Sampling was carried out monthly at each experimental site from July 2000 to August 2001, except November 2000 (15 June, 26 July, 31 Aug., 29 Sept., 31 Oct., and 12 Dec. in 2000; 19 Jan., 20 Feb., 23 Mar., 30 Apr., 31 May, 30 June, 31 July, and 24 Aug. in 2001). To assess the untreated ECM condition of the soil, soil blocks were also collected 10 m apart from the urea plot at each site when urea was applied (15 June 2000). However, the control data were not collected continuously. The soil blocks were put in polyethylene bags and brought to the laboratory, where they were stored for about 1 month at 4°C until processed in the laboratory.

Separation and sorting of ECM tips and plant roots

Each soil block was washed with tap water using sieves (mesh size, 2 mm; 0.5 mm in diameter), and fine roots (≤2 mm in diameter) were picked out of the residue. The roots were sorted into three categories: living ECM tips, living fine roots without ECM tips, and dead fine roots. The latter two contained roots of non-ECM trees. Judgment of root senescence was made by visual estimation after Bauhus and Bartsch (1996): "live roots were intact, tough, and flexible, while dead roots were brittle and fractured easily." Dry weight of the living fine roots excluding ECM tips and that of the dead fine roots was chosen and was measured after 1 week of drying at 55°C to determine root phenology. Thereafter, we calculated the density (per cm^3) for each index for each subplot.

Sorting of living ECM tips into tentative morphotypes was done under a binocular microscope (20×) and numbered serially. Sorting was made for each soil block using three criteria: external color, shape, and existence of external hyphae. The number of ECM tips for each tentative morphotype was counted, and typical tips of each tentative morphotype were photographed. Microscopic observation in detail followed Ingleby et al. (1990). Typical ECM tips were mounted on glass slides stained in trypan blue (Nacalai Tesque, Kyoto, Japan). The rest of the tips were stored at –20°C for DNA extraction.

DNA analysis

The fruit bodies selected for PCR-RFLP are listed in Table 1. All the specimens were collected in the urea plots set up either in this study or in others (Imamura 2001; Imamura and Yumoto 2004a). The ECM morphotypes were selected for DNA extraction by referring to the macroscopic features of the ECM tips of *Hebeloma* and *Laccaria* species described by Brand (1988), Ingleby et al. (1990), Treu (1990), and Jakus and Magyar (1999). The characteristics that we considered most important were pale color, the existence of external hyphae emanating from or surrounding the mantle of the ECM tips, and the loose structure of the mantle. Eighty-one tentative morphotypes of a total of 314 were selected, using the foregoing criteria, for DNA extraction and analysis.

Identification of ECM morphotypes at the species level was made using the internal transcribe spacer (ITS) region. After a study of the literature (Gardes et al. 1991; Gardes and Bruns 1993; Yamada et al. 2001; http://plantbio.berkeley.edu/~bruns/picts/results/map.GIF) and preliminary experiments, two consecutive PCRs with two primer pairs, ITS1F (Fungal)/ITS4B (Basidio) in the first and ITS1F/ITS4 in the second, were executed for all the DNA extracts. These pairs are highly fungus specific and produce sufficient amplification from a few milligrams of ECM tips (Gardes et al. 1991; Gardes and Bruns 1993; Yamada et al. 2001). The DNA of each ECM morphotype was extracted and amplified following Gardes and Bruns (1993), with modification as follows: 1.25 units Taq DNA polymerase (Toyobo, Osaka, Japan) per 50 µl reaction; 30 or 35 amplification cycles (95°C for 30 s, 55°C for 30 s, 72°C for 90 s) after 95°C for 3 min. The Perkin Elmer GeneAMP PCR System 2400 was used in the amplification.

Four restriction enzymes were used: *Afa*I, *Alu*I, *Hae*III, and *Hin*fI (Takara Shuzo, Shiga, Japan). Aliquots of 2–4 µl amplified DNA were digested with these enzymes at 37°C for more than 5 h. The digested DNA was electrophoresed for 0.5–2 h on a 3%–3.2% agarose gel (#011-53; Nacalai Tesque, Japan) with a 100-bp DNA ladder (Bexel Biotechnology, Union City, CA, USA) in 0.5× TBE buffer. The gel was stained with 1 µg/ml ethidium bromide for 15 min, and the lengths of the DNA fragments were calculated with 1D Image Analysis Software, version 3.0 (Kodak Digital Science). The PCR-RFLP patterns of the fruit bodies were measured at least three times. The pattern of ECM morphotypes was determined by electrophoresis on side-by-side lanes with fruit-body DNA.

Data analysis

All the data were analyzed with StatView J-5.0 (SAS Institute, Cary, NC, USA). Data in 15 June 2000 (before urea treatment) were excluded in the analyses below. Significance of difference between study sites in fruit-body production and in density of roots and ectomycorrhizae was analyzed by analysis of variance (ANOVA). In each site, significance of temporal changes in the density of roots and ectomycorrhizae and in fruit-body production were analyzed by ANOVA with repeated measures and the Tukey–Kramer test. The correlation coefficient between monthly totaled precipitation and total ECM tip density, or the ECM tip density of particular species, was analyzed. On temporal changes of total ECM tips and ECM tip density and fruit-body production of two particular species, *A. lactariolens* and *H. vinosophyllum*, the effects of precipitation were analyzed by multiple linear regression analysis, for which precipitation was cumulatively totaled for preceding periods, i.e., about 1–15 days, 16–30 days, 31–45 days, and 46–60 days before each sampling date, because sampling periods were about 30 days. These four values were used in the multiple linear regression analysis as the independent variables.

Results

PCR-RFLPs and identification of ectomycorrhizae

The PCR procedure was successful on fruit bodies of the six LP species. The length of the PCR products was about 700+ bp in all the species. The RFLP patterns of the six LP species are shown in Table 2, which shows that all these species were clearly distinguished by the four enzymes. Although the RFLP patterns of *A. lactariolens* and *H. spoliatum* were similar, electrophoresis on 3% agarose gel enabled us to distinguish one from the other.

The PCR-RFLP procedure was successful for 55 of the 81 ECM morphotypes that we had selected. These morphotypes were classified into 28 RFLP patterns; i.e., Al, Hv, and 26 unidentified (Table 3). Among them, 14 RFLP patterns were found at Site C and 14 at Site Q. Each RFLP pattern was unique to only one of the two sites.

After side-by-side electrophoresis using the four enzymes, 20 types of the 55 ECM morphotypes were identified as *A. lactariolens* and 3 as *H. vinosophyllum*. *Alnicola lactariolens* tips were found at Site C and *H. vinosophyllum* tips at Site Q. Among them, 2 of the 20 *A. lactariolens* ECM morphotypes and 1 of the 3 *H. vinosophyllum* ECM morphotypes showed variations; i.e., *Alu*I digested a 530-bp fragment of *A. lactariolens* to 350 and 180 bp and a 190-bp fragment of *H. vinosophyllum* to 175 bp and shorter fragments, respectively (data not shown). RFLP patterns showing similarity to the other four LP species were not found among the 55 ECM morphotypes.

ECM morphotypes other than the foregoing two species were detected on few sampling dates in spite of the fact that the ectomycorrhizae of these two species were found repeatedly within these samplings. For example, RFLP taxon II was detected only twice in the earlier samples and taxon I only twice in the later samples (see Table 3).

The ectomycorrhizae of *A. lactariolens* were characterized by a light brown surface and infrequent branching in the whole mycorrhizal tip with white external hyphae. Microscopic observations revealed a loose, thin, net prosenchyma to net synenchyma mantle, abundant emanating hyphae with clamp connections, infrequent branching of hyphae, and no cystidia (Table 3, Figs. 1–3). The ectomycorrhizae of *H. vinosophyllum* were similar, but differed from those of *A. lactariolens* in having a more transparent, often paler surface (white or transparent light brown) and a looser mantle structure (net prosenchyma to net synenchyma) (Table 3, Figs. 4–6).

Dynamics of fruit bodies, plant roots, and ECM tips

Fruit bodies

Four species including *A. lactariolens* and *H. vinosophyllum* fruited at both sites (Table 4). *Laccaria bicolor* and *L. amethystina* did not fruit. The total dry weight of fruit bodies of the four species was not significantly different between sites (ANOVA, $F = 1.02$), although it was slightly greater at Site C. *Alnicola lactariolens* exhibited significant abundance at Site C ($F = 19.3$, $P = 0.002$) and *H. vinosophyllum* at Site Q ($F = 6.61$, $P = 0.03$), while *H. spoliatum* and *H. radicosoides* exhibited no significant differences between sites ($F = 4.25$,

Table 2. PCR-RFLP patterns (bp) of ectomycorrhizae (ECM) ammonia fungi distinguished using four enzymes

Fungal species	PCR products (bp)	Enzyme			
		*Afa*I	*Alu*I	*Hae*III	*Hin*fI
Al	720	Uncut	530, 190	450, 170, 100	385, 335
Hv	720	Uncut	530, 190	615, 105	385, 335
Hs	730	Uncut	540, 190	450, 180, 100	390, 340
Hr	725	465, 260	330, 205, 190	440, 180, 105	395, 265, 65
Lb	715	510, 205	395, 130, 115, and shorter[a]	Uncut	390, 325
La	715	Uncut	375, 130, 100, and shorter[a]	Uncut	380, 335

[a] Fragments were considered to have been digested (data not shown)

Table 3. Characteristics of ectomycorrhizal DNA extracted and analyzed in this study

Serial number of putative morphotype[a]	Site name	Sampling date	No. of collected tips	Proportion to the total ECM tips from each subplot (%)	Species identified or RFLP taxa[b]	External color	Macroscopic features		Microscopic features		
							External hyphae[c]	Branching[d]	Surface structure of mantle[e]	Clamp connections	Cystidia
4	C	26 Jul 2000	7	18.9	Al	Glossy brown, brown	–	IF	2–3	–	–
8	C	26 Jul 2000	15	46.9	II	Brown	++	?	3	–	–
19	Q	26 Jul 2000	5	100	III	Brown, pale orange	–	IF	3	–	–
30	C	31 Aug 2000	56	87.5	II	Brown	++	F	3	–	–
47	C	29 Sep 2000	52	37.7	Al	Glossy brown	++	IF	2–3	+	–
49	C	29 Sep 2000	42	44.2	Al*	Glossy brown	++	IF	2–3	+	–
58	Q	29 Sep 2000	54	64.3	IV	Pale brown	++	F	2–3	+	–
59	Q	29 Sep 2000	9	10.7	IV	Pale brown	++	IF	2–3	–	–
60	Q	29 Sep 2000	5	6.0	V	Brown	–	IF	3	–	–
61	Q	29 Sep 2000	12	14.3	IV	Brown	–	F	2	–	–
64	Q	29 Sep 2000	17	100	VI	Pale brown, white	–	F	3	–	–
71	C	31 Oct 2000	12	18.8	Al	Glossy brown	++	IF	2–3	+	–
77	Q	31 Oct 2000	3	42.9	VII	Dark brown	–	IF	2–3	–	–
78	Q	31 Oct 2000	4	100	Hv	Pale brown	+	IF	2	–	–
81	C	12 Dec 2000	14	26.4	Al	Glossy brown	++	IF	2–3	+	–
82	C	12 Dec 2000	37	69.8	Al	Glossy brown	++	IF	2–3	+	–
85	C	12 Dec 2000	8	19.0	Al	Glossy brown	++	IF	2–3	+	–
88	C	12 Dec 2000	16	41.0	Al	Glossy brown, brown	++	IF	2–3	+	–
91	C	12 Dec 2000	100	86.2	Al	Glossy brown, brown	++	IF	2–3	+	–
92	C	12 Dec 2000	9	7.8	Al	Glossy brown, brown	++	IF	2–3	+	–
93	C	12 Dec 2000	7	6.0	Al	Glossy brown	+	IF	2–3	+	–
100	Q	12 Dec 2000	19	90.5	VIII	Brown, ocher	+	IF	4	–	–
102	Q	12 Dec 2000	2	9.5	Hv*	Pale brown	+	IF	2	+	–
106	Q	12 Dec 2000	5	41.7	Hv	Pale brown	+	IF	2	+	–
109	Q	12 Dec 2000	26	24.3	IX	Dark brown	–	IF	3	–	–
111	Q	12 Dec 2000	3	2.8	X	Pale brown	++	IF	2–3	–	–
112	C	19 Jan 2001	3	13.0	XI	Pale brown	+	IF	3	+	–
132	C	20 Feb 2001	27	100	Al	Brown	++	IF	2–3	+	–
150	C	30 Apr 2001	15	71.4	Al*	Glossy brown	++	IF	2–3	+	–
152	C	30 Apr 2001	1	4.8	Al	Glossy brown, white	++	IF	?	+	–
159	C	31 May 2001	31	63.3	Al	Glossy brown	++	IF	2–3	+	–
161	C	31 May 2001	24	38.7	Al	Glossy brown	++	IF	2–3	+	–
164	C	31 May 2001	11	20.8	Al	Glossy brown	++	IF-F	2–3	+	–

Table 3. *Continued*

Serial number of putative morphotype[a]	Site name	Sampling date	No. of collected tips	Proportion to the total ECM tips from each subplot (%)	Species identified or RFLP taxa[b]	External color	Macroscopic features		Microscopic features		
							External hyphae[c]	Branching[d]	Surface structure of mantle[e]	Clamp connections	Cystidia
165	C	31 May 2001	81	35.1	XII	Brown	?	IF	3	–	–
166	C	31 May 2001	8	3.5	XIII	Brown, ocher	++ (brown)	IF	2	–	–
167	C	31 May 2001	123	53.2	XIV	Dark brown	++	F	2	–	–
168	C	31 May 2001	8	3.5	XV	Brown	-	IF	2–3	–	–
171	C	30 Jun 2001	21	77.8	XVI	Pale brown	++	IF	2–3	+	–
172	C	30 Jun 2001	6	22.2	XVI	Brown	++	IF	2–3	+	–
173	C	30 Jun 2001	94	81.0	XVII	Brown, pale brown	++	F	2	+	–
174	C	30 Jun 2001	13	11.2	XVIII	Dark brown	-	IF	3	+	–
176	C	30 Jun 2001	122	93.8	XIX	Brown, ocher	-	IF-F	3	–	–
177	C	30 Jun 2001	4	3.1	XVI	Brown	-	IF	3	–	–
179	C	30 Jun 2001	10	66.7	Al	Glossy brown	++	IF	2–3	+	–
180	C	30 Jun 2001	5	33.3	XX	Glossy brown	++	IF	2	+	–
181	C	30 Jun 2001	19	100	Al	Glossy brown, pale brown	++	IF	2–3	+	–
184	Q	30 Jun 2001	29	30.5	XXI	Dark brown, brown	-	IF-F	3	–	+
185	Q	30 Jun 2001	57	60.0	I	Brown, ocher	-	IF	3	–	+
186	Q	30 Jun 2001	9	9.5	XXII	Pale brown	++	IF	2–3	–	–
188	Q	30 Jun 2001	6	25.0	XXIII	Pale brown	++	IF	1–2	–	–
191	Q	30 Jun 2001	68	64.8	XXIV	Brown	-	IF-F	2	+	–
194	Q	30 Jun 2001	98	66.2	XXV	Pale brown	++	F	2–3	–	–
199	Q	31 May 2001	36	65.5	XXVI	Glossy brown	++	IF	2	+	–
203	Q	31 May 2001	76	80.0	I	Brown, ocher	-	IF	3	–	–

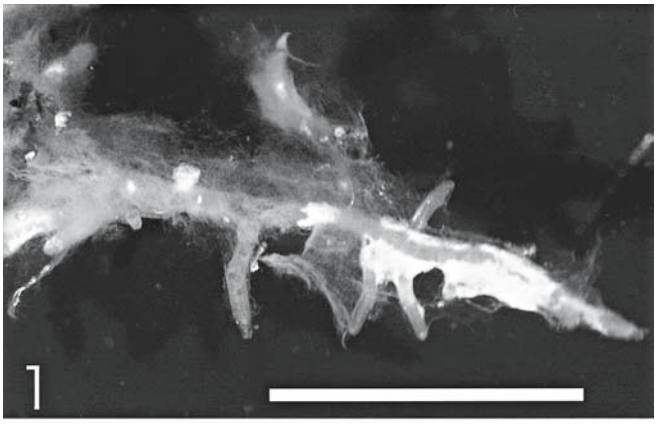
[a] Serial number represents 1 of the total 314 tentative morphotypes sorted out; among the 81 morphological types tested, 55 was successfully analyzed and listed

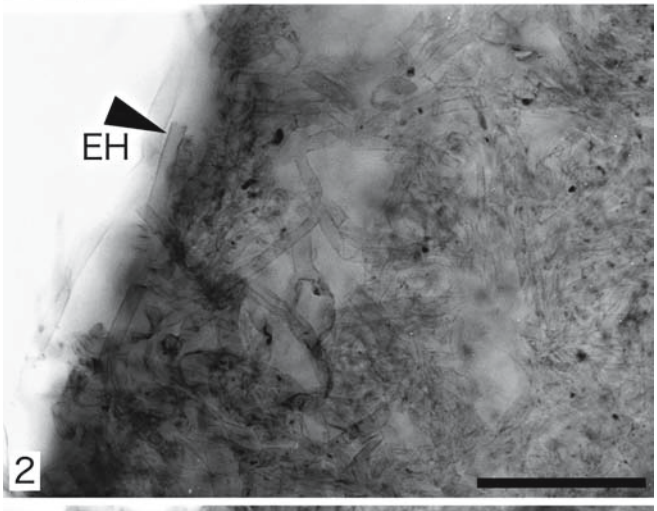
[b] The Greek numerals I–XXVI denote RFLP taxa, and Al and Hv denote *Alnicola lactariolens* and *Hebeloma vinosophyllum*, respectively; *PCR product digested by *Alu*I presented one more fragment than in other samples identified as Al or Hv; this should be an intraspecific variation, as mentioned by Kårén et al. (1997)

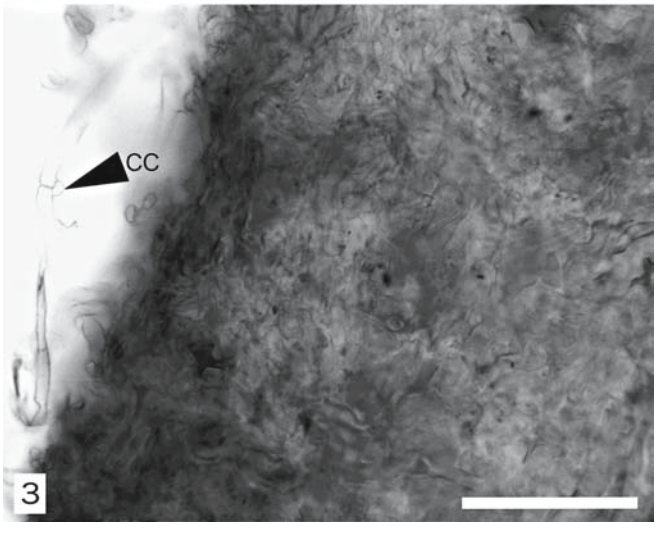
[c] ++ well developed, + present, –, absent

[d] F, frequent; IF, infrequent

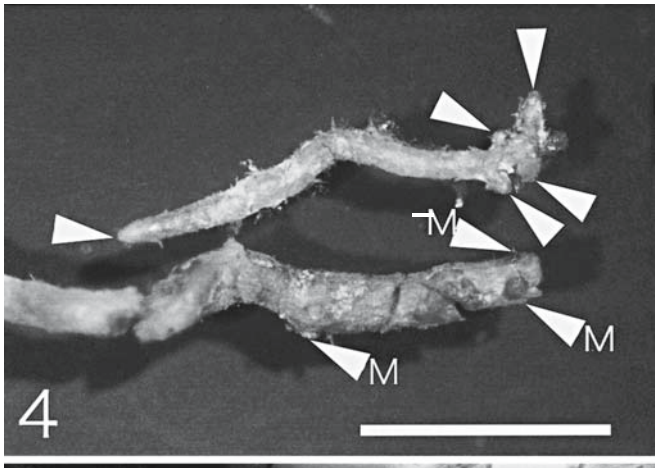
[e] Classification after Ingleby et al. (1990): 1, felt prosenchyma; 2, net prosenchyma; 3, net synenchyma; 4, irregular synenchyma

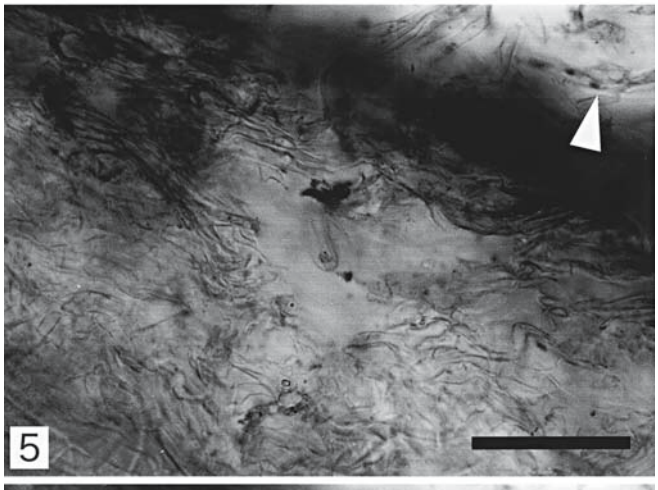


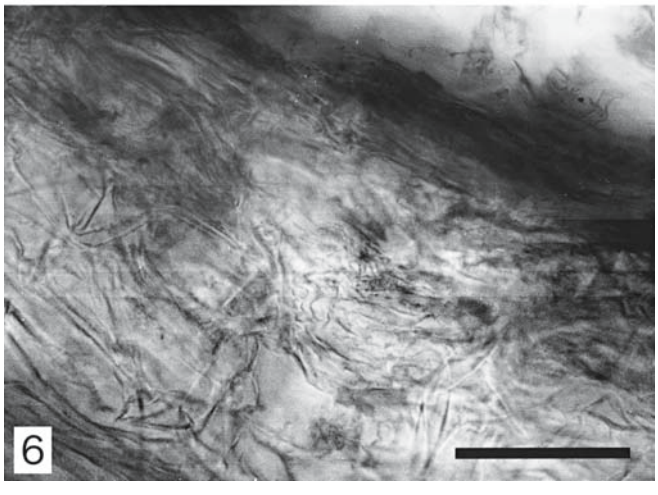




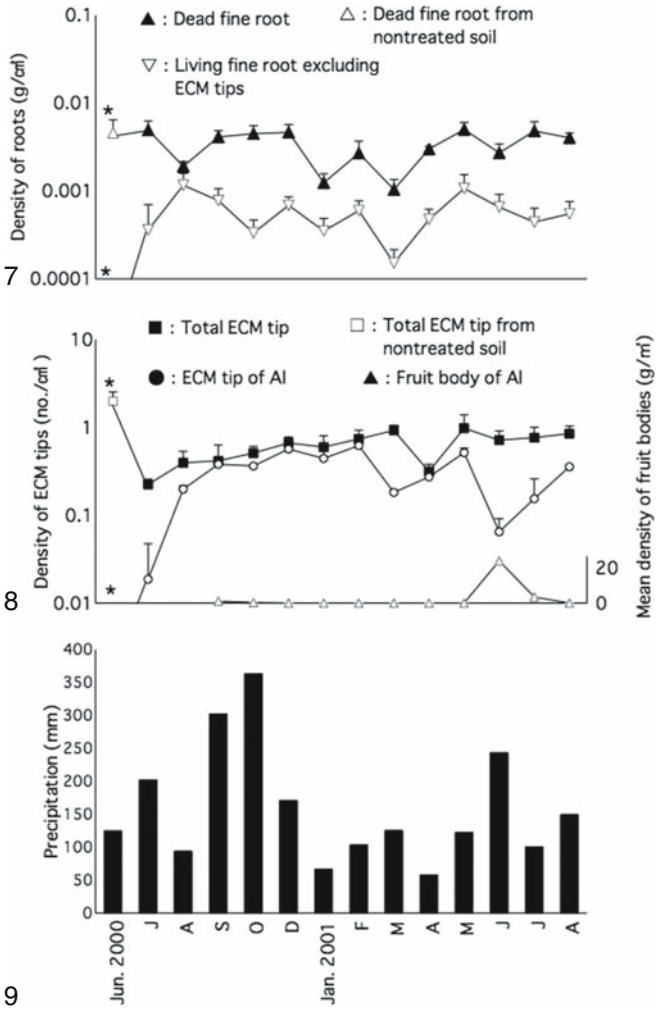
Figs. 1–3. Typical *Alnicola lactariolens* ectomycorrhiza. **1** Appearance of the mycorrhiza is characterized by light brown surface, infrequent branching, and white hyphae. **2** Mantle structure near the surface, showing a loose, thin, net prosenchyma to net synenchyma with emanating hyphae. *EH*, emanating hyphae. **3** Inner layer of mantle structure shown in **2**, forming a net synenchyma structure. *CC*, clamp connection. *Bars* **1** 2.5 mm; **2** 30 µm; **3** 30 µm



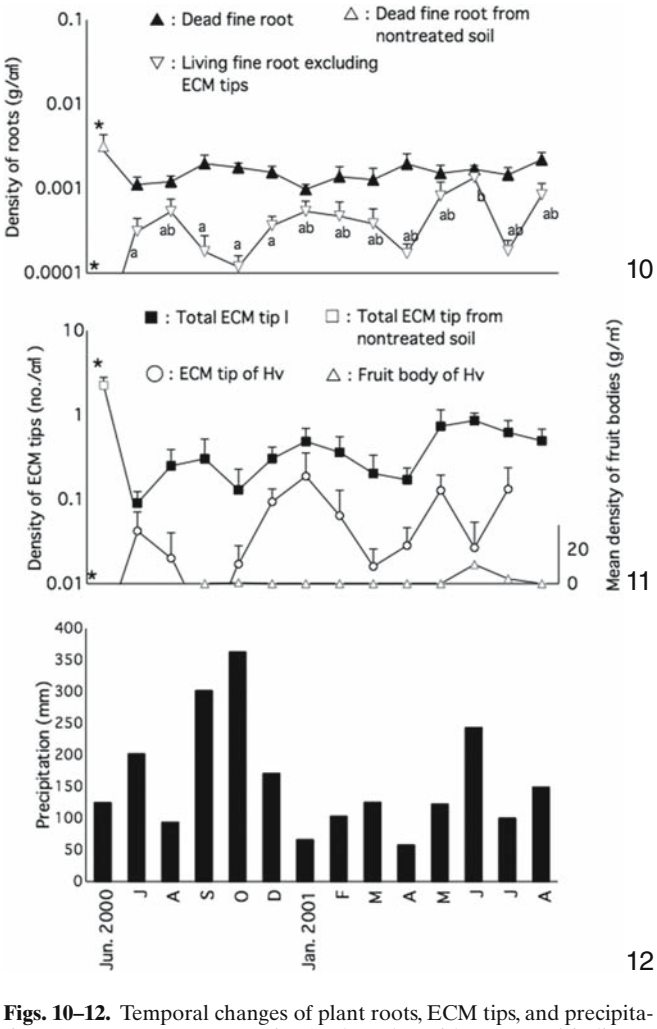




Figs. 4–6. Typical *Hebeloma vinosophyllum* ectomycorrhiza (ECM). **4** Appearance of the mycorrhiza characterized by white and transparent light brown surface and infrequent branching. *Arrowheads*, typical ECM tips; *M*, marks of ECM tips that have fallen away. **5** Mantle structure near the surface showing a loose, thin, net prosenchyma to net synenchyma with emanating hyphae. *Arrowhead*, emanating hyphae. **6** Inner layer of mantle structure shown in **5** forming a net-synenchyma structure. *Bars* **4** 2.5 mm; **5** 30 µm; **6** 30 µm



Figs. 7–9. Temporal changes of plant roots, ECM tips, and precipitation after urea treatment of Site C. The value with an *asterisk* indicates the data for nontreated soil. No data collection in November 2000. **7** Dead roots and living roots excluding living ECM tips (mean + SE). **8** Total ECM tips and *Alnicola lactariolens* (Al) ECM tips and fruit bodies (mean + SE). **9** Amount of precipitation



Figs. 10–12. Temporal changes of plant roots, ECM tips, and precipitation after urea treatment of Site Q. The value with an *asterisk* indicates the data for nontreated soil. No data collection in November 2000. **10** Dead and living roots excluding living ECM tips (mean + SE). The values followed by the same alphabet character did not significantly differ according to Tukey–Kramer at $P < 0.05$. **11** Total ECM tips and *Hebeloma vinosophyllum* (*Hv*) ECM tips and fruit bodies (mean + SE). **12** Amount of precipitation

$F = 2.96$, respectively). Ectomycorrhizae of *A. lactariolens* and *H. vinosophyllum* were uniquely found from Site C and Site Q, respectively, where the fruit bodies of each species were abundant. Fruit bodies of *A. lactariolens* and *H. vinosophyllum* comprised about 92% and 89% of the total dry weight of Site C and Site Q, respectively (Table 4).

The first appearance of fruit bodies in any of the four species occurred about 100 days after the urea treatment (September 2000), and the peak of fruiting was observed about 380 days after the treatment (late June 2001). However, *H. spoliatum* at Site C and *A. lactariolens* at Site Q were found only during the first fruiting period, and *H. radicosoides* at Site Q was found only during the second fruiting period, whereas the remaining species were found during both the first and second fruiting periods (see Table 4, Figs. 7–12).

Plant roots

The density of dead roots was significantly different between the two vegetation types ($F = 24.9, P < 0.01$) and was greater in Site C. Dead root density was significantly affected ($F = 3.08, P < 0.01$) and not affected ($F = 1.06$) by sampling date in Site C and Site Q, respectively. However, in Site C, no significant difference was found between any of two sampling dates by Tukey–Kramer test. The density of living roots excluding ECM tips was not significantly affected by vegetation type ($F = 0.40$), and was not significantly affected ($F = 1.50$) and was significantly affected ($F = 3.33, P = 0.01$) by sampling date in Site C and Site Q, respectively (Figs. 7, 10).

Table 4. Fruit-body production and ECM formation by each late phase (LP)/ECM ammonia fungus in each site

Species[a]	Order of flushes in which the species fruited		Total dry weight of fruit bodies (g)		Relative abundance of fruit body in each site		Relative abundance of ECM tips in each site (%)	
	Site C	Site Q	Site C	Site Q	Site C	Site Q	Site C	Site Q
Al	1st, 2nd	1st	144	2.17	91.6	2.60	50.3	nd
Hv	1st, 2nd	1st, 2nd	8.53	74.1	5.42	88.8	nd	14.6
Hs	1st	1st, 2nd	0.038	5.75	0.024	6.89	nd	nd
Hr	1st, 2nd	2nd	4.54	1.41	2.88	1.69	nd	nd

nd, not detected
[a] For abbreviations of species names, see Table 1

ECM tips

The difference in total-tip density was not significantly affected ($F = 3.03$) by the vegetation type. The total-tip density was not significantly affected by sampling date in both sites ($F = 1.00$ in Site C; $F = 1.79$ in Site Q) (Figs. 8, 11). The total-tip density was reduced just after the urea treatment at each site, but it gradually increased after the initial decrease (Site C: $r^2 = 0.086$; Site Q: $r^2 = 0.128$) (Figs. 8, 11). Total ECM tip density and precipitation were not significantly correlated (C: $r^2 = 0.024$; Q: $r^2 = 0.001$), and the multiple linear regression analysis showed significant effect only in Site Q ($F = 7.90$, $df = 4$). In Site Q, the standard regression coefficient was highest in the precipitation of 46–60 days (–0.62) and second highest in the precipitation of 0–15 days (0.47). Although, at Site C, it decreased unexpectedly in April 2001 from that of the previous sampling date by about 65%, no significant effect of the precipitation was found by these two analyses (Figs. 7–12).

By comparing the microscopic observations with the results of PCR-RFLP, the ectomycorrhizae of *A. lactariolens* were found uniquely at Site C and those of *H. vinosophyllum* at Site Q. Throughout the observation period, ECM tips of these species contributed as much as 50% and 15% of total ECM tips, respectively (see Table 4). ECM morphotypes other than these two species, e.g., RFLP taxa II, III, IV, VI, VII, VIII, and IX, comprised 10%–100% of the total ECM tips from each subplot (see Table 3).

The dynamics of fruit-body production and ECM formation of *A. lactariolens* and *H. vinosophyllum* at each site are shown in Figs. 8 and 11. The ECM tips of *A. lactariolens* were found only from Site C and were not affected by the sampling date ($F = 0.87$). The ECM tips of *H. vinosophyllum* were found only from Site Q and were not affected by the sampling date ($F = 0.84$).

Ectomycorrhizae of these species were detected first on the first sampling date, 30 days after the urea treatment. Then, ECM tips of *A. lactariolens* and *H. vinosophyllum* increased and comprised about 90% (September 2000–May 2001, except March 2001) and 20%–35% of the total ECM tips at the maximum (December 2000–May 2001, except March 2001) at Site C and Site Q, respectively. ECM tips of these fungi were detected from July 2000, earlier than their first fruiting period by about 60 days, i.e., from September 2000 (Figs. 8, 11). Ectomycorrhizae of *A. lactariolens* and *H. vinosophyllum* sharply decreased, by 88% and 79%, respectively, from the second as well as the maximum peak of their fruiting (30 June 2001) (Figs. 8, 11). After this initial decrease, they increased again, although the fruit-body production decreased by about 90% in *A. lactariolens* and 75% in *H. vinosophyllum*.

The ectomycorrhizae of *H. vinosophyllum* were not detected in September 2000, just before the first and small fruiting peak in this species. In March 2001, the ectomycorrhizae of *A. lactariolens* decreased after the previous sampling date by 70% (Fig. 8). In February and March 2001, the ectomycorrhizae of *H. vinosophyllum* also decreased after the previous sampling date by 62% and 85%, respectively (Fig. 11). The least amount of rainfall and an absence of fruit-body production were observed then (Figs. 8, 9, 11, 12), although the correlation coefficient between precipitation and ECM tip density was not significant (*A. lactariolens*: $r^2 = 0.029$; *H. vinosophyllum*: $r^2 = 0.20$). Neither the multiple linear regression analysis between ECM tips of each species of each site nor the precipitation of the four periods showed a significant effect in the two sites ($F = 0.54$ in Site C; $F = 1.18$ in Site Q; $df = 4$).

Discussion

ECM formation by LP ammonia fungi

We identified ectomycorrhizae of two of the known six LP species from the urea-treated soil by PCR-RFLP. This is the first study to identify ectomycorrhizae formed by any of the LP ammonia fungi. Variation in the external colors of Al ectomycorrhizae, from pale brown to brown, was observed macroscopically (Table 3). One of them, ECM 152, was partly white. External color variation is often observed and is thought to be related to aging of the mycorrhizae (Mehmann et al. 1995). By molecular identification, 2 of the 20 *A. lactariolens* ectomycorrhizae and 1 of the 3 *H. vinosophyllum* ectomycorrhizae were considered to

show intraspecific variation (Table 3) in the sense of Kårén et al. (1997). Although we do not have any further evidence confirming this variation as intraspecific, microscopic observations supported this result. Further investigations such as sequencing or inoculation should provide clarification.

Moreover, we found that at least a part of the LP ammonia fungi formed abundant ECM tips even in EP, possibly under N-rich and higher-pH conditions. These conditions have been shown to be favorable to their proliferation by Yamanaka (1995a). The present results are quite consistent with the predictions by Sagara (1975, 1995) and Fukiharu (1991), i.e., that LP species are ECM fungi. Although *Q. glauca* (evergreen) was growing as the subdominant and ectomycorrhizal tree in the experimental sites, almost all the ectomycorrhizae formed by these two fungi seemed to be hosted by the dominant tree of each site, *C. cuspidata* and *Q. serrata*, according to observation of the morphology of plant roots (data not shown).

Alnicola lactariolens fruited significantly more abundantly at Site C whereas *H. vinosophyllum* was more abundant at Site Q. Moreover, the primary fruit body-producing fungi formed the majority of ectomycorrhizae at each site. *Alnicola lactariolens* was considered the dominant ECM producer because it comprised 50% of the cumulative total over the observation period. *Hebeloma vinosophyllum* was considered as the dominant or the second to sixth predominant ECM producer because it comprised 15% of the cumulative total over the observation period.

In each sampling date, because *A. lactariolens* comprised 70%–90% of the ectomycorrhizae, this species was surely the dominant ECM producer at that time. In the same way, because *H. vinosophyllum* comprised about 35% of the ectomycorrhizae, during peak ECM production (January 2001), this species was probably the dominant or the second predominant ECM producer at that time despite the absence of its fruit bodies. Ectomycorrhizae of *A. lactariolens* were found only in Site C, where the majority of fruit bodies of this species appeared (Table 4). Similarly, ectomycorrhizae of *H. vinosophyllum* were found only in Site Q, where the majority of fruit bodies of this species appeared (Table 4). This result suggests that, for these two ECM species, aboveground and belowground abundance is positively correlated.

Despite the fact that we searched for and analyzed ECM tips having the characteristics of *Hebeloma* and *Laccaria* species among numerous ECM morphotypes, we did not find ectomycorrhizae of the other four LP *Hebeloma* and *Laccaria* species. The reasons may be that (1) ectomycorrhizae of these four species may have been very few or absent, as was represented by the less abundant fruiting of *H. spoliatum* and *H. radicosoides* and by the absence of *L. bicolor* and *L. amethystina* fruiting in this study; and (2) ectomycorrhizae of *H. spoliatum* and *H. radicosoides* might colonize and grow deeper in the soil, as suggested in fruit-body production by the literature (Imazeki and Hongo 1987; Sagara 1995; Sagara et al. 2000). Thus, to discuss ECM formation of these two species, a deeper sampling method should be applied.

Dynamics of plant roots and ECM tips

Damaging effect of ammonia

Because our data on untreated soils are unfortunately limited, the results of comparison between the urea-treated and untreated soils should be regarded cautiously. However, immediately after the urea treatment, the amount of living ECM tips decreased significantly. Similar observations were made by Sagara (1975), who also reported the death of fine roots following urea treatment. Yamanaka (1995c) reported that the ammonia released by urea treatment caused a significant decrease in soil bacteria and nematodes, paralleling the increase of the soil ammonia content after the treatment. Because the amount of N added to the soil at one time in this study was much larger than in other N-fertilization studies (Alexander and Fairley 1983; Termorshuizen 1993; Kårén and Nylund 1997; Boxman et al. 1998; Brandrud and Timmermann 1998; Jonsson et al. 2000; Peter et al. 2001), the destructive effects by ammonia were expected to be greater. Their studies, in which the form of N-compounds and the amount of N added to soil at one time varied greatly, differed on whether the density of ECM tips and roots increased or decreased after the treatment. Thus, based on our results and those reported in comparable studies, urea treatment not only increases the supply of nutrient but also creates a strong destructive disturbance (Sagara 1992) in the ECM fungal community.

Plant roots

Dead root density was relatively constant. The larger value of the density compared to living roots is the result of slow decomposition of the roots. Silver and Vogt (1993) reported that more than half the dead roots remained undecomposed in soil for 1 year. The same may apply to the presence of a rather constant amount of dead plant roots in our sites. On the other hand, the live root density (excluding ECM tips) in the treated soil fluctuated over time. Especially in Site Q, the density was significantly affected by sampling date, but not in Site C. The data might have been influenced by the root phenology, as suggested by Parrotta and Lodge (1991), as well as by the size and heterogeneity of the soil samples, although further investigation is needed. The density of dead roots was significantly higher at Site C and the density of ECM tips and fruit bodies tended to be higher at Site C than at Site Q, although the results were not significant. This fact may indicate differences between the two vegetation types in productivity of ectomycorrhizae, fruit bodies, or plant roots.

Dynamics of fruit bodies and ECM tips

The dominant species in fruit-body production in this study have been reported to be especially predominant in the plots treated in spring to summer (Fukiharu 1991; Imamura and Yumoto 2004a). The smaller amount of fruit-body pro-

duction by *H. spoliatum* and *H. radicosoides*, as observed here, also agrees with previous work (Fukiharu 1991; Imamura and Yumoto 2004a). The absence of fruit bodies of *L. bicolor* and *L. amethystina* in this study is also consistent with the view that they are less frequently seen in urea-treated plots than other LP species (Imamura 2001). Thus, experimental induction of the ammonia fungi is reproducible, and is reliable enough for referring to ECM tips. Consequently, we can now discuss the dynamics of the phenomena above- and belowground.

ECM formation by the two LP ammonia fungi, *A. lactariolens* and *H. vinosophyllum*, began only a month after urea treatment and preceded their fruit-body production. The peak and subsequent decrease of the ECM density also preceded that of fruit-body development of the two species, but the decrease of ECM density was not significant. This fact indicates that the occurrence of ECM formation and proliferation in advance of fruiting of these ECM fungi is necessary.

The correlation between precipitation and the density of total ECM tips or the ECM tips of particular species in each site was not significant by multiple linear regression analysis, except for total ECM tip density in Site Q. However, the density in Site Q was most correlated negatively with 46–60 days preceding rainfall and secondarily correlated positively with 0–15 days preceding rainfall, and therefore it is hard to determine which period of rainfall best explained the temporal change of the density. Because the temporal change of ectomycorrhizae with fruit-body production in particular species has never been investigated, phenology of plant roots, fruit bodies of ECM fungi, and ectomycorrhizae should be of more concern in future studies.

Previous studies dealing with N-application to the forest floor reported that ectomycorrhizae of fruit body-producing species occupied only a small proportion of ECM fungal community belowground (Brandrud and Timmermann 1998; Jonsson et al. 2000; Peter et al. 2001). On the other hand, the ectomycorrhizae of the fruiting basidiomycetes of the ammonia fungi comprised a great majority of the belowground ECM communities. At present, we think that this may be related to the extraordinarily abundant production of fruit bodies by the two species. The annual productivity of fruit bodies (15–40 g dry weight/m^2) we observed was markedly greater than that of fruit bodies observed in other N-application studies (e.g., 0.001–0.003 g/m^2 annual total; Termorshuizen 1993). The proliferation of ectomycorrhizae in urea-treated plots might have enabled a greater production of fruit bodies by these two ammonia fungi. The decrease in ECM-tip density that preceded the largest peak of fruit-body production by the two ammonia fungi may have been the result of translocation of material from ectomycorrhizae to fruit bodies, but we have no data to support this hypothesis.

Nonfruiting ECM fungi and expansion of the definition of the ammonia fungi

We found 26 ECM RFLP taxa that did not fruit among the total 55 tentative morphotypes using PCR-RFLPs (i.e., taking 2 RFLP patterns that did fruit from 28 total RFLP patterns). By microscopic observation, we also found a great variety of ECM morphotypes other than those from which we extracted DNA. These results are in accordance with the previous studies by Jonsson et al. (2000) and Peter et al. (2001), who reported from N-treated soils that the ECM fungi involved do not necessarily bear fruit bodies. Although the ECM tips of *A. lactariolens* or *H. vinosophyllum* comprised the majority in the urea-treated soil, nonfruiting ECM fungi were also present (see Table 3). Moreover, most of the varieties of ECM morphotypes detected in treated soils were not the same as those in untreated soils (see Table 5). Of the total 314 putative ECM morphotypes we detected in soils treated with urea, we examined 55 morphotypes by PCR-RFLP, and of these, about half (26 morphotypes) were nonfruiting. In addition to DNA-examined ECM morphotypes, some morphotypes comprised a greater portion of the total ECM tips and seemed to proliferate in the urea-treated soils (e.g., morphotype 287, 298, 326 in Table 5). Moreover, we detected a total 58 putative morphotypes in the untreated soils on 15 June 2000, and in 2001, a total of 37 and 56 morphotypes on 31 July and 24 August, respectively (Imamura, unpublished data). Although we did not classify these morphotypes in detail, no ectomycorrhizae of Al or Hv were detected in the untreated soils. Furthermore, there were no ECM morphotypes in common between the treated and the untreated soils, except for *Cenococcum geophilum* Fr. (Cg) (see Table 5; also see Imamura and Yumoto 2004b).

Thus, we propose the necessity of the expansion of the concept of the ammonia fungi to include nonfruiting ECM and saprobic fungi that proliferate after the addition of ammonia-releasing compounds. We could modify Sagara's (1975) definition to read, "a chemoecological group of fungi that sequentially develop reproductive structures or sequentially proliferate without developing reproductive structures, exclusively or relatively luxuriantly on the soil after a sudden addition of ammonia, some other nitrogenous materials that act as bases by themselves or on decomposition, or alkalis." However, to verify the expansion of the definition of the ammonia fungi, confirmation of the change in the fungal community is needed. The change must be investigated quantitatively, using pre- and posttreatment measures as well as comparisons between treated and adjacent untreated plots, with specific identification of fungi in ECM or hyphal form based on the original definition by Sagara (1975). If the concept is expanded, the term "ammonia fungi" comes to denote not a species assemblage as viewed from the basis of reproduction, but as a fungal community including vegetative stages.

Closing remarks

This is the first report on the dynamics of fruiting and mycorrhiza formation in a warm temperate area following addition of urea. This type of study has rarely been reported,

Table 5. Comparison of ECM tentative morphotypes between treated and nontreated soil sampled on 24 Aug 2001

Serial number of putative morphotype	Site name and treatment	No. of collected tips	Proportion to the total ECM tips from each subplot (%)	Species identified	External color	External hyphae	Branching	Surface structure of mantle[a]
287	CU	98	84.5	Al	Pale brown, white	+	IF-F	2–3
288	CU	18	15.5	NID	Yellowish-brown	+	IF-F	3
289	CU	28	51.9	Al	Pale brown, white	++	IF-F	2
290	CU	21	38.9	NID	Yellowish, glossy brown	–	IF-F	2–3
291	CU	6	11.1	Cg	Black	+ (black)	?	Star-like
292	CU	24	77.4	NID	Brown, white	+	IF	3
293	CU	3	9.7	NID	Glossy brownish-orange	–	IF	3
294	CU	4	12.9	Cg	Black	+ (black)	IF	Star-like
295	CU	48	57.1	NID	Dark brown	–	IF	3
296	CU	8	9.5	NID	Dark brown, white	+	IF	2
297	CU	26	31.0	NID	Black	++ (brown)	IF-F	4
297b	CU	2	2.4	Cg	Black	+ (black)	IF	Star-like
298	CU	54	81.8	NID	Glossy brownish-orange	–	IF	3
299	CU	12	18.2	Al	Pale brown, white	++	IF-F	2
315	CN	9	9.5	Cg	Black	++ (black)	IF	Star-like
316	CN	73	76.8	NID	Pale brownish orange, white	–	F	3
317	CN	2	2.1	NID	Glossy brown, white	–	?	1
318	CN	3	3.2	NID	Brownish-orange	+	IF	2–3
319	CN	2	2.1	NID	White, brown	+	IF	1
320	CN	6	6.3	NID	Dark brown	–	IF	1–2
321	CN	32	32.3	Cg	Black	++ (black)	IF-F	Star-like
322	CN	43	43.4	NID	Glossy brownish-orange	+	IF-F	3
323	CN	19	19.2	NID	Ocher, white	–	F	2–3
324	CN	5	5.1	NID	Dark reddish brown	–	IF	4
325	CN	14	7.6	Cg	Black	+ (black)	IF	Star-like
326	CN	154	83.2	NID	White, brownish-ocher	–	IF-F	3
327	CN	15	8.1	NID	Brownish-ocher	++	IF-F	3–4
328	CN	2	1.1	NID	Dark brown, white	–	IF-F	3
329	CN	25	16.3	Cg	Black	+ (black)	IF-F	Star-like
330	CN	107	69.9	NID	Glossy yellowish-brown	–	F	2–3
331	CN	7	4.6	NID	Dark, reddish brown	–	IF	3
332	CN	14	9.2	NID	Brown, white	–	F	3–4
333	CN	17	17.2	Cg	Black	+ (black)	?	Star-like
334	CN	20	20.2	NID	White, pale brown	–	F	3
335	CN	50	50.5	NID	Glossy brownish-orange	–	IF	3–4
336	CN	12	12.1	NID	Black-dark brown	–	IF-F	3

Sampling of nontreated soil was conducted in the same way as that on 15 June 2000 (see text); only *Alnicola lactariolens* fruited in the treated plot

Cenococcum geophilum Fr. was identified according to Ingleby et al. (1990)

Al, *Alnicola lactariolens*; Cg, *C. geophirum* Fr.; NID, not identified; C, *Castanopsis* forest; U, urea treated; N, nontreated; ++ well developed; +, present; –, absent; F, frequent; IF, infrequent

[a] Classification after Ingleby et al. (1990): 1, felt prosenchyma; 2, net prosenchyma; 3, net synenchyma; 4, irregular synenchyma

even in the cool temperate areas of Europe and North America where the ECM fungal communities have been well studied. Ectomycorrhizae of two fungal species were studied that comprised a large portion of the ectomycorrhizae in soils in fruit bodies aboveground. For the two fruiting species, initiation, increase, and subsequent decrease of their ectomycorrhizae preceded the initiation, increase, and decrease of their fruit-body production, respectively. Whether these same ectomycorrhizal types were also present in untreated soils is still unclear. To clarify how ectomycorrhizae form and proliferate after urea treatment and during their fruit-body production in soils and how the fungal communities overlap between urea-treated and untreated soils, we should investigate the untreated soils adjacent to the treated plots and the untreated soils apart from the plots in addition to the treated soils. In the future, we may elucidate stimuli that initiate fruit-body production, nutrient transfer between fungi and plants, and nutrient allocation between mycorrhizae and fruit bodies.

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