Basidiomycetous Ectomycorrhizal Fungal Communities of Current-Year *Pinus Densiflora* Seedlings That Regenerated on Decayed Logs and on the Forest Floor Soil

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Abstract

Decayed pine logs killed by pine wilt disease (PWD) could be important seedbeds for many tree species in post-PWD forests in Japan. Although mycorrhizal symbionts are essential for seedling establishment on logs, ECM communities on decayed pine logs have not been studied yet. In this study, I measured the number and the properties of *Pinus densiflora* seedlings on pine logs and their associations with basidiomycetous ectomycorrhizal communities using rDNA sequence, and compared them with seedlings on the soil in a post-PWD *P. densiflora* stand in Japan. Pine seedling density, shoot dry mass, and specific root length (SRL) were significantly higher, but shoot/root ratio was lower in decayed logs than in soil. High water and nutrient contents of the white-rotted logs may partially contribute this. The ECM colonization rate was higher but the diversity of operational taxonomic units (OTUs) of basidiomycetous ECM was lower in decayed logs than in soil. In total, 21 basidiomycetes OTUs were detected, and *Amanita citrina* (Amanitaceae) was the most frequent species in decayed logs. Russulaceae and Thelephoraceae were the dominant ECM fungal families in the soil. Electrical conductivity negatively affected the OTU richness, and canonical correspondence analysis showed that the substrate water content, pH, and light conditions significantly affected the ECM fungal communities. *Tomentella* sp.2, Boletaceae sp.1, and *Rhizopogon luteolus* were associated with high pH and dry soil conditions, whereas *A. citrina* and *Lactarius* species were associated with low pH and wet log conditions. There results indicate that decayed logs of *P. densiflora* provide potential seedbeds for their juveniles, and *A. citrina* is the dominant basidiomycetous ECM fungi in the logs at this site.

Introduction

Tree seedling establishment on decayed fallen logs called “nurse logs” is an important mean of tree regeneration in various forest ecosystems (Harmon et al. 1986; Doi et al. 2008; Sanchez et al. 2009; Iijima and Shibuya 2010; Fukasawa 2012). Several advantages of seedling establishment on fallen logs have been reported, e.g., greater light availability (Harmon and Franklin 1989), reduced litter accumulation (Duchesneau and Morin 1999), reduced densities of soil pathogenic fungi (O’Hanlon-Manners and Kotanen 2004), lower root and shoot competition (Coomes and Grubb 2000), a relatively stable water content (Greene et al. 1999), and the high colonization capacity of mycorrhizal fungi (Tedersoo et al. 2008). Mycorrhizal symbiosis is an important nutritional strategy, particularly in nutrient-poor substrates (Read et al. 2004). In general, fallen logs contain less nutrients to support seedling growth compared with soil (Goodman and Trofymow 1998; Baier et al. 2006); thus, the mycorrhizal fungal communities that inhabit logs and their colonization of seedlings may be essential for seedling establishment, particularly during the colonization of highly decayed logs where mycorrhizal fungi dominate the fungal communities (Rajala et al. 2012). Although reports about mycorrhizal fungal communities within decayed logs have been increasing (Tedersoo et al. 2003, 2008, 2009a; Walker and Jones 2013; Walker et al. 2014), diver-

sity of mycorrhizal fungal in decayed logs has been poorly understood.

Japanese red pine (*Pinus densiflora* Sieb. et Zucc.) is a dominant canopy tree species in temperate secondary forests of Japan, while pine wilt disease (PWD) has killed many adult pine trees over recent decades (Takemoto and Futai, 2008). The post-PWD forest vegetation varies from *Pinus* to evergreen or deciduous *Quercus* stands, or plantations of *Cryptomeria japonica* and *Chamaecyparis obtusa*, depending on the climate and forest management regimes (Fujihara et al. 2002; Kato and Hayashi 2006, 2007), while a common feature is the deeply accumulated litter layer, due to the abandonment of coppice management including litter removal from forest floor for use as soil conditioner or fuel (Fujihara et al. 2002). Thick litter layer prevents regeneration of small-seeded tree species such as *P. densiflora*, and in such circumstances, decayed pine logs which massively accumulated on forest floor (Kato and Hayashi 2006) provide important microsites for small-seeded species (Fukasawa 2012). Thus, it is necessary to evaluate the pine logs as regeneration sites for pine seedlings and their ectomycorrhizal (ECM) associations within the logs, in order to find proper deadwood management for successful regeneration of pine trees. ECM community of *P. densiflora* forest is a well-researched area (Yamada and Katsuya 1996, 2001; Iwański and Rudawska 2007; Ma et al. 2010, 2012; Lee and Eom 2013), while ECM communities on decayed pine logs have not been studied yet.

The aims of the present study are (1) to compare pine seedling density and properties between logs and soil, and (2) to compare ECM communities on pine seedlings between logs and soil. Physicochemical properties of pine logs and soil were also compared and their relationships with seedling regeneration and ECM communities were discussed. In the present study, I focused on basidiomycetous ECM fungi because though they are only a part of the ECM communities, they include many functionally important species with ECM-forming capacities and the ability to decay organic compounds (Tanesaka et al. 1993). This dual functionality may be particularly important for colonizing and supporting seedling growth on logs. I expected that basidiomycetous ECM species with saprotrophic abilities such as Thelephorales, Atheliales, and Sebacinales are dominant on pine seedlings as suggested by Tedersoo et al. (2003, 2008, 2009a).

Materials and methods

Study site and sampling

The present study was conducted in a forest park called "Ikoi-no-mori" in Kurihara city (38°43.1′N, 141°00.2′E; 71 m a.s.l.), Miyagi Prefecture, Northern Honshu, Japan. The mean annual temperature for the period 2001-2010 at the nearest meteorological station in Tsukidate (38°44.1′N, 141°00.3′E; 25 m a.s.l.) was 11.3°C. The mean monthly temperature ranged from −0.6°C in January to 23.5°C in August. The mean annual precipitation was 1210.6 mm (Japan Meteorological Agency 2013). The study area was a plantation of *P. densiflora* with an approximate area of 2 km × 1 km, and it was surrounded by a mixture of paddy rice fields and residential districts. *P. densiflora* was the only canopy tree in the study area. The averages and standard errors for the tree density, basal area (BA), and diameter at breast height (DBH) were 7.3 ± 0.8 ha^{-1}, 33.8 ± 3.5 m^2 ha^{-1}, and 23.6 ± 0.8 cm, respectively (average ± SE of six 10 × 10 m plots for tree density and BA and of 44 pine trees within the plots for DBH). The forest floor was covered with a variety of vegetation, including dwarf bamboo (*Pleioblastus chino*), shrubs (*Ilex crenata, Rhododendron obtusum*, and *Viburnum dilatatum*), tree saplings and seedlings (*P. densiflora*, *Quercus serrata*, *Clethra barbinervis*, and *Eleutherococcus sciadophylloides*), herbs (*Thalictrum minus* var. *hypoleucum*, and *Sanguisorba officinalis*), and liana (*Akebia trifoliata*). The forest floor vegetation was annually cleared by cutting and the height was maintained at approximately 10–20 cm. PWD had severely affected the study area for a decade and dead pine trees were felled and cut to lengths of approximately 2 m, before piling up in mounds in the study area. Many of these mounds were observed to be in various stages of decay in the study area.

In October 2012, three mounds were selected and a 1 m × 1 m plot was established on each mound, which included the projecting areas of several logs. All of the logs in the selected mounds exhibited white rot and they were assigned to decay classes IV or V according to the classification of Fukasawa (2012), as follows: class IV wood was considerably decayed, it was penetrable with a knife to approximately 5–10 cm, bark was lost in most places, and the original log circumference had begun to disintegrate; class V

wood had disintegrated either to a very soft crumbly texture or was flaky and fragile, it was penetrable with a knife to >10 cm, and the original log circumference was barely recognizable or indiscernible. Three additional 1 m × 1 m plots were located on the ground adjacent to each of the three mound plots. Thus, three pairs of mound plots and ground plots were established. The numbers of *P. densiflora* seedlings were recorded in each plot. Photon densities were measured 15 cm above the substrates in each plot using a portable spectroradiometer (MS-720, EKO Instruments Co., Ltd., Tokyo, Japan). Because photon densities vary within a short-time scale, three measurements were obtained at intervals of several minutes within a cloudy day, which were averaged and used as the data for each plot.

In each plot, 10–12 current-year *P. densiflora* seedlings (66 seedlings in total) were sampled carefully to collect all of the roots, as far as possible. If sufficient seedlings were not collected within the ground plots, additional seedlings were taken from ground near the plots. The seedlings were taken to the laboratory and divided into aboveground shoots and underground roots. The shoots were oven dried to constant weight at 70°C and weighed. The intact root fresh weight and the overall root length were measured, and individual root samples were cut into two subsamples. The root lengths were measured using ImageJ with a scanned copy of the roots (Tajima and Kato 2013). One subsample was used to calculate a conversion coefficient for the fresh root weight to the dry mass at 70°C. Next, the dried weight of the intact whole root length was calculated based on the fresh weight of the intact root using the conversion coefficient. The shoot/root ratio (S/R) and the specific root length (SRL) were calculated using the following equations.

S/R = shoot dry mass/root dry mass

SRL (m g^{-1}) = root length/root dry mass

Another subsample was used to obtain mycorrhizal fungal colonization measurements and identification.

The substrate for seedling establishment (i.e., decayed log or soil) was sampled from each plot. Each substrate was sampled from three points in each plot, which were combined to form a composite sample. A sample from each plot was mixed well and divided into two subsamples; one was used to determine the water content after drying at 70°C, as the percentage of water weight relative to the dried substrate and the other was used for chemical analysis, after drying at 40°C and grinding using a laboratory mill, followed by sieving through a 2.0-mm mesh.

Mycorrhizal colonization rate and identification

For individual root subsamples, the ECM colonization rate (%) was measured as the percentage of ECM root tips relative to the total root tips (tip ratio). After obtaining the measurements, eight ECM root tips were sampled randomly from each subsample (528 root tips in total) and DNA was extracted from each root tip using a DNeasy 96 plant kit (Qiagen Sciences, Valencia, USA). The internal transcribed spacer (ITS) region of the basidiomycetous fungal rDNA was PCR-amplified using the primer pair ITS1F and ITS4B (Gardes and Bruns 1993). The PCR products were purified using Exo-Sap enzymes (Sigma, St Louis, MO, USA). Sequencing was performed using the same primer pair. An ITS region with a shared identity of 97% was used as the criterion for the molecular identification of species. The taxonomic interpretation depended on the percentage sequence identity at the genus (94%–96% similarity) or family (90%–93% similarity) level. Therefore, the units were identified at a mixture of species, genus, and family levels, which were represented uniformly as operational taxonomic units (OTUs) to simplify the analysis. BLAST searches were performed against the public sequence database at the National Centre of Biotechnology Information (NCBI) and UNITE to identify the ECM fungi. The occurrence of a fungal OTU in a seedling was scored as a binary variable, regardless of the number of root tips colonized by the OTU. The frequency (%) of each OTU in each substrate was calculated as the number of records for an OTU/total number of seedlings (N = 33) ? 100. The OTUs with frequencies >10 % in either of the two substrates were referred to as frequent OTUs.

Chemical analysis of substrates

The pH, electrical conductivity (EC), and the concentrations of cations (Na^+, NH_4^+, K^+, Mg^+, and Ca^+) and anions (F^-, Cl^-, NO_2^-, Br^-, NO_3^-, SO_4^{2-}, and PO_4^{3-}) were measured in the substrate samples (decayed log or soil) from each plot. Dried samples (10–50 g) were extracted using 25 mL of distilled water in a 50-mL polyethylene bottle with manual shaking for approximately 10 s. After leaving for 1 h, the pH of the supernatant was measured using a portable

pH meter (HORIBA, Kyoto, Japan). After obtaining the pH measurement, another 25 mL of distilled water was added to the bottle and the bottle was gently shaken using an automatic shaker for 1 h at 25°C. After shaking, EC was measured using a portable EC meter (HORIBA, Kyoto, Japan). The water extract was then filtered to remove any woody residues or soil particles, before the concentrations of cations and anions were measured using an ion chromatography system (ICS-1000/2000, DIONEX, CA, USA). The EC and ion concentrations were expressed as values per 1 g of dried sample.

Statistical analysis

The light conditions (photon density), water content, pH, and EC of the substrates, as well as the seedling density (no. m^{-2}), shoot dry mass, S/R, SRL, and the numbers and frequencies of ECM fungal OTUs were compared between the decayed log and soil samples using a generalized linear model with R version 2.14.2 (R development core team 2012).

To compare the accumulation and richness estimates for the OTUs in the decayed log and soil samples, rarefaction curves with 95% confidence intervals were calculated using EstimateS version 9 (Colwell 2013). The root systems of individual seedlings were used as the sampling units and they were sampled randomly without replacement.

A canonical ordination method was used to determine the relationships between the basidiomycetous ECM fungal community compositions and the substrate and seedling properties. A detrended correspondence analysis was performed as a preliminary analysis to check the length of the ordination axis, which showed that the length was > 4 SD, suggesting that the response curve could be unimodal. Thus, canonical correspondence analysis (CCA) was appropriate (Jongman et al. 1995). The occurrence data for fungal OTUs with > 10% frequency were binary transformed for each seedling and used as species data. One nominal variable (type of substrate: log or soil) and seven quantitative variables (light, water content, pH, and EC of substrates and shoot dry mass, S/R, and SRL of seedlings) were used as environmental variables. All of the quantitative variables were transformed into Z-scores using the following equation to eliminate the effects of different units:

$$Z = (x_0 - x_\times) / \mathrm{SD},$$

where x_0 is the value of variable x in a particular log and x' is the arithmetic mean of variable x in the sample.

All of the ordination analyses were performed using Canoco 4.5 (ter Braak and Šmilauer 2002), where the relationships between sets of environmental variables and ordination scores were plotted in ordination diagrams. In the diagrams, the arrows for the environmental variables depicted the direction and magnitude of the relationships among the environmental variables and the ECM fungal community. The biplots focused on the inter-species distance. We tested the explanatory variables using the automatic forward selection procedure provided by Canoco 4.5 (Monte Carlo permutation test with 9999 randomizations).

Results

Substrate properties

Table 1 shows the physicochemical properties of decayed logs and soil. The photon densities did not differ significantly between substrates. The water content was over 10 times higher in the decayed logs than the soil. The pH was lower in the decayed logs than the soil. EC was almost 50 times higher in the decayed logs than the soil. The concentrations of Na^+, NH_4^+, K^+, F^-, and Cl^- were greater in the decayed logs than the soil, whereas the concentrations of Mg^+, $Ca^{+,}$ Br^-, SO_4^{2-}, and PO_4^{3-} did not differ significantly between the substrates. The concentrations of NO_2^- and NO_3^- were too low to be detected.

Properties of *P. densiflora* seedlings and mycorrhizal colonization

The seedling density and shoot biomass of *P. densiflora* were significantly higher on decayed logs compared with the soil (Table 2). Very few individuals were observed in soil. S/R was significantly higher in soil than on decayed logs, whereas SRL was higher on decayed logs than in soil. The ECM fungal colonization rate was higher on decayed logs than soil, in terms of the tip ratio. Almost all of the root tips of the seedlings were colonized by ECM fungi on decayed logs.

In total, 21 OTUs of basidiomycetous ECM fungi were recorded from 528 randomly selected root tips (Table 3). Only six OTUs were detected on the decayed logs, which were dominated by Amanitaceae and Russulaceae, whereas all 21 OTUs were detected in soil, which were dominated by Russulaceae, Thelephoraceae, and Boletaceae (Table 4). In addition,

Table 1. Comparison of the qualities of the decayed log and soil substrates

	Log	Soil	P [a]
Photon density (μmol m^{-2} s^{-1})	35.3±2.9	41.5±2.9	n.s.
Water content (%)	439±68	32±11	**
pH	3.99±0.03	4.91±0.05	***
EC (μs cm^{-1})	50.7±9.1	0.9±0.2	**
Nutrients (mg L^{-1})			
Na^+	0.61±0.08	0.17±0.06	**
NH_4^+	1.37±0.27	0.40±0.10	*
NO_2^-	n.d.	n.d.	n.a.
NO_3^-	n.d.	n.d.	n.a.
K^+	6.13±1.85	0.21±0.01	*
Mg^+	0.39±0.21	0.07±0.01	n.s.
Ca^+	1.90±0.96	0.19±0.03	n.s.
F^-	1.25±0.08	0.37±0.06	***
Cl^-	2.04±0.18	0.40±0.06	**
Br^-	0.36±0.14	0.21±0.07	n.s.
SO_4^{2-}	0.39±0.07	0.46±0.07	n.s.
PO_4^{3-}	1.18±0.72	0.01±0.01	n.s.

Data represent average ± standard error (N = 3)
n.d. not detected
[a] GLM results *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, n.s. not significant, n.a. not applied

Table 2. Comparison of the properties of *Pinus densiflora* seedlings established on decayed logs and in soil

	Log	Soil	P [a]
Density (no. m^{-2})	75.0±17.4	0.3±0.3	***
Shoot biomass (mg)	47.7±3.5	30.7±2.7	***
S/R [b]	1.81±0.11	3.96±0.48	***
SRL (m g^{-1}) [c]	33.0±1.1	27.0±1.4	**
ECM tip ratio (%)	96.9±0.6	88.9±1.7	***

Data represent average ± standard error (N = 3 for density and 33 for other properties, except for S/R, see below)
[a] GLM results *** $P < 0.001$, ** $P < 0.01$
[b] Shoot to root ratio. Only data of seedlings without any root loss were used for analysis (N = 29 for decayed log and N = 11 for soil)
[c] Specific root length

38% and 28% of the total root tips of seedlings from decayed logs and soil, respectively failed to amplify (Table 4). Furthermore, 26% and 9% of the total root tips of seedlings on decayed logs and in soil, respectively, were not identified as known ITS sequences in the NCBI and UNITE databases. Among the identified fungi, *Amanita citrina*, Boletaceae sp.1, *Hygrophorus* sp., *Lactarius* sp., *Rhizopogon luteolus*, *Sebacina* sp., *Tomentella* sp.1, and *Tomentella* sp.2 were detected in > 10% of the seedlings on one or both of the substrates (Table 3). The differences in the substrates affected the ECM fungal diversity and composition. The number of OTUs per seedling was significantly lower on seedlings established on decayed logs compared with those in soil (Table 3). Based on the separation of the confidence intervals, the cumulative number of ECM fungi OTUs was significantly lower on seedlings established on decayed logs compared with those in soil (Fig. 1). The estimated total OTUs on logs and in soil were 6.5 and 23.9, respectively, according to Jacknife2, and 8.9 and 28.8, respectively, according to Chao2 (Table 3). Most of the OTUs showed no significant preferences for either of the two substrates, but Boletaceae sp.1,

Table 3. Frequencies (%) of basidiomycetous ectomycorrhizal (ECM) operational taxonomic units (OTUs) detected from *P. densiflora* seedlings established on decayed logs and in soil, and their sequence similarities and accession numbers

OTUs	Accession NO.	Frequcney (%)		*P* [a]	Most similar sequence in database			
		Log	Soil		Taxa	Accession NO.	% ID	Database
Frequent								
Amanita citrina	AB972821	39.4±26.9	23.0±12.2	n.s.	*Amanita citrina*	KF245908	99	NCBI
Boletaceae sp.1	AB972823	–	20.7±13.3	n.s.	Boletaceae sp.	HE814137	99	NCBI
Hygrophorus sp.	AB972829	–	16.7±16.7	n.s.	*Hygrophorus hypothejus*	UDB001579	95	UNITE
Lactarius sp.	AB972830	15.2±15.2	12.8±8.9	n.s.	*Lactarius zonarius*	UDB011468	95	UNITE
Rhizopogon luteolus	AB972831	6.1±3.0	18.5±5.5	n.s.	*Rhizopogon luteolus*	UDB015830	99	UNITE
Sebacina sp.	AB972836	–	17.2±9.6	n.s.	*Sebacina* sp.	HQ154376	97	NCBI
Tomentella sp.1	AB972838	3.0±3.0	20.0±15.3	+	*Tomentella* sp.	AY940642	97	NCBI
Tomentella sp.2	AB972839	–	21.8±13.2	n.s.	*Tomentella* sp.	EF655702	96	NCBI
Infrequent								
Atheliaceae sp.	AB972822	9.1	6.7	n.a.	Atheliaceae	UDB008299	98	UNITE
Boletaceae sp.2	AB972824	–	5.6	n.a.	Boletaceae	JQ991657	93	NCBI
Ceratobasidium sp.	AB972825	–	10.0	n.a.	*Ceratobasidium*	JQ991678	98	NCBI
Entoloma crassipes	AB972826	–	2.8	n.a.	E*ntoloma crassipes*	AB301603	99	NCBI
Entolomataceae sp.	AB972827	–	8.3	n.a.	*Entoloma*	UDB008232	89	UNITE
Gomphidius roseus	AB972828	–	3.3	n.a.	*Gomphidius roseus*	UDB011692	99	UNITE
Russula sp.1	AB972832	–	10.0	n.a.	*Russula* sp.	AB629047	100	NCBI
Russula sp.2	AB972833	–	3.3	n.a.	*Russula* sp.	JN129410	99	NCBI
Russula sp.3	AB972834	–	3.0	n.a.	*Russula* cf. *vesca*	EU567074	94	NCBI
Russulaceae sp.	AB972835	–	6.4	n.a.	Russulaceae sp.	AB636107	99	NCBI
Suillus bovinus	AB972837	3.0	3.3	n.a.	*Suillus bovinus*	UDB011438	99	UNITE
Tomentella sp.3	AB972840	–	3.0	n.a.	*Tomentella* sp.	GQ900537	99	NCBI
Tomentella sp.4	AB972841	–	6.4	n.a.	*Tomentella subclavigera*	UDB003303	96	UNITE
Number of OTUs per seedling		0.8±0.1	2.2±0.2	***				
Observed OTUs richness		6	21					
Eestimated OTUs richness [b]								
Jacknife 2		6.5	23.9					
Chao 2		8.9	28.8					

Data represent average ± standard error for frequent taxa (N = 3)

[a] GLM results *** $P < 0.001$, + $P < 0.1$, n.s. not significant, n.a. not applied

[b] EstimateS (Colwell 2013)

Table 4. Percentage occurrences of families of basidiomycetous ectomycorrhizal (ECM) fungi in randomly sampled root tips of current-year *Pinus densiflora* seedlings established on decayed logs and in soil

Family	Log	Soil
Amanitaceae	19.7	5.7
Atheliaceae	4.2	1.1
Boletaceae	–	7.6
Ceratobasidiaceae	–	2.7
Entolomataceae	–	6.8
Gomphidiaceae	–	0.4
Hygrophoraceae	–	3.8
Rhizopogonaceae	0.8	7.6
Russulaceae	10.2	11.0
Sebacinaceae	–	4.2
Suillaceae	0.4	0.8
Thelephoraceae	0.4	11.4
Unamplified	38.3	28.0
Unknown sequences	26.1	9.1

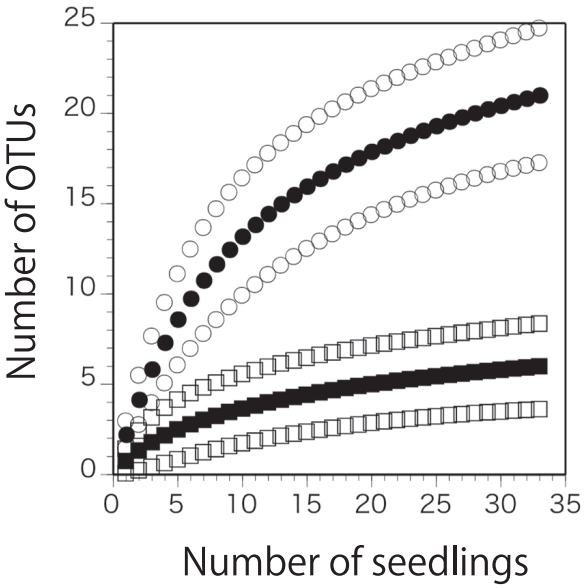

Figure 1. OTU accumulation curves of basidiomycetous ectomycorrhizal (ECM) fungi (filled symbols) and their 95% confidence intervals (open symbols). Square: decayed logs, circle: soil.

Hygrophorus sp., *Sebacina* sp., and *Tomentella* sp.2 were detected only in soil. Among the photon density, water content, pH, and EC, only EC significantly affected the observed OTU richness (estimated parameter = −0.61, $p = 0.047$).

Based on the CCA, the water content ($F = 4.89$, $p < 0.001$), light conditions ($F = 3.30$, $p = 0.002$), and pH ($F = 4.54$, $p < 0.001$) of the substrates affected the mycorrhizal communities significantly, in addition to the specific substrate ($F = 6.32$, $p < 0.001$) (Fig. 2). In particular, *Tomentella* sp.2, Boletaceae sp.1, and *R. luteolus* were associated with high pH and dry soil conditions, whereas *A. citrina* and *Lactarius* sp. were associated with low pH and wet log conditions. The shoot biomass, S/R, and SRL of seedlings were not significantly related to the ECM fungal communities.

Discussion

The numbers and shoot growth of current-year seedlings were greater on decayed logs than in soil at the study site. This may have been due partly to the high water and nutrient contents of logs compared with the soil at the study site. Previous studies have reported that decayed logs generally have poor nutrient levels compared with soil (Goodman and Trofymow 1998; Baier et al. 2006). However, Walker and Jones (2013) reported that the extractable ammonium content was higher in decayed logs than mineral soil in a clear-cut disturbed forest. Takahashi et al. (2000) reported that white-rotted logs contained a large amount of nutrients, with an EC value approximately 12 times greater than that of soil. White-rotted logs contain large amounts of delignified readily utilizable carbohydrate and high water content, stimulating the growth of bacterial communities, including nitrogen-fixers; thus, the white-rotted logs would contain large amounts of nitrogen (Jurgensen et al. 1989). This may be one reason why the white-rotted pine logs observed in this study had high nutrient levels. The low S/R and high SRL values of the pine seedlings growing on logs in the present study indicated the increased allocation to roots and the effective extension of the root lengths, respectively, demonstrating the effective absorption of nutrients in logs. This may appear to be inconsistent with the higher amounts of nutrients found in logs compared with the soil at this site, because it has been generally believed that C allocation to roots increases under nutrient poor conditions (Tilman 1988). A possible reason for this inconsistency may be the low pH of the logs, that stimulate C allocation to root and lateral root extension (Schindelbeck and Riha 1988; Narukawa and Yamamoto 2003; Doi et al. 2008; Six and Halpern 2008). Belowground competition with grass roots in soil also must be considered. However, it may not be the main factor determining root biomass allocation

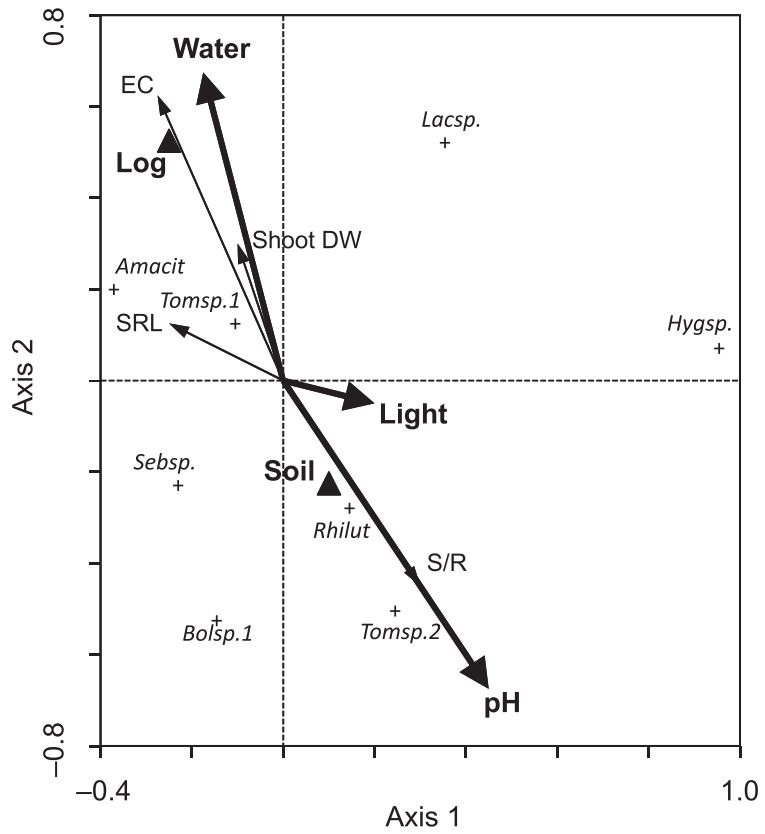

Figure 2. Diagram showing the canonical correspondence analysis ordination for the corresponding positions of frequent fungal OTUs (frequency > 10%) based on occurrence data from decayed logs and soil. The environmental variables are indicated by arrows (quantitative variables) and closed triangles (nominal variables). Quantitative variables with a significant effect are shown in bold (Monte Carlo permutation test, $p < 0.05$). Abbreviations for fungi: *Amacit*, *Amanita citrina*; *Hygsp*., *Hygrophorus* sp.; *Lacsp*., *Lactarius* sp.; *Rhilut*, *Rhizopogon luteolus*; *Sebsp*., *Sebacina* sp.; *Tomsp*.1, *Tomentella* sp.1; and *Tomsp*.2, *Tomentella* sp.2. Two axes explained 59.9% of the total variation in the species–environment relationship.

in the present study because previous experimental studies reported that the presence of root competition generally promote carbon allocation to root (Kolb and Steiner 1990), in contrast to the present study.

Increased biomass allocation to roots and effective extension of the root length inevitably affect the mycorrhizal colonization and vise versa. The basidiomycetous ECM colonization rate was high in seedlings on logs compared with those in soil, which is consistent with the previous studies (Vogt et al. 1995; Baier et al. 2006). The high demand for nutrients in poor nutritional conditions facilitates mycorrhization (Kazantseva et al. 2009), but this may not have been the case in the present study because the nutrient contents of the logs were higher than those of the soil. Alternative explanation seems to be the relatively good growth of seedlings on logs than soil supported by convenient water and nutrient content of logs promoted ECM colonization. Druebert et al. (2009) reported that seedlings with suppressed growth were less colonized by ECM fungi than well-growing individual even if the ECM propagule densities were set to a same level.

Substrate difference also affected species richness of ECM fungi. The observed low ECM diversity in logs was consistent with previous studies, but the causal mechanisms may differ slightly between this and previous studies. Previous studies suggest that the low nutritional status of decayed wood limits the colonization to the fungal taxa such as Thelephorales, Atheliales, and Sebacinales (Tedersoo et al. 2003, 2008, 2009a). Alternatively, in the present study, a possible explanation is that the high nutrient status limited the ECM diversity in decayed logs by stimulating some nutrient-demanding species (Rao et al. 1997; van der Heijden et al. 1999; Parrent et al. 2006;

Toljander et al. 2006; Kalliokoski et al. 2010) such as *Amanita* which dominated in logs in the present study. Among the nutrient ions measured, potassium concentration had the largest difference between logs and soil in the present study, and thus was supposed to be a main component of the difference in nutrient condition between logs and soil. Tedersoo et al. (2009b) reported that soil potassium concentration, rather than other elemental nutrients (nitrogen, phosphorus, calcium and magnesium), determine species diversity of ECM fungi associated with alders (Betulaceae) roots, but the reason was unclear.

Variables other than nutrient availability are also undoubtedly important determinants of fungal communities. I found that the substrate pH strongly affected the ECM fungal community, which is in agreement with other studies of soil ECM communities (van der Heijden et al. 1999; Toljander et al. 2006; Cox et al. 2010). Laboratory-based pure culture studies using *Amanita* and *Lactarius* species (which exhibited a preference for low pH in the present study) showed that their optimum hyphal growth occurred at relatively low pH values (pH 3–5; Jongbloed and Borst-Pauwels 1990). Theodorou and Bowen (1969) reported the hyphal growth of *R. luteolus* and its colonization of pine roots in soil at pH 5.0, supporting the ordination analysis of the present study that the occurrence of mycorrhiza of *R. luteolus* was closely associated with the soil pH gradient (i.e. pH = 4.9). The water content is another important factor that affecting fungal communities. A very low water content prevents hyphal growth by reducing the osmotic potential of hyphae (Griffin 1972), as well as reduces ECM fungal colonization of plant roots (Kennedy and Peay 2007), but a very high humidity level can prevent hyphal growth by decreasing the oxygen content (Hintikka and Korhonen 1970). In the present study, the water content of the decayed logs was over 10 times higher than that of the soil. However, it is not clear whether this high water content affected the oxygen demands of ECM fungi because the seedling roots were distributed only in the superficial regions of decayed logs where the oxygen content was not reduced greatly (Hintikka and Korhonen 1970). The root depths of the pine seedlings were not recorded in the present study, but Doi et al. (2008) reported that the seedling root depth of Pinaceae species was approximately 3 cm on logs in decay class IV. The light conditions also affect the ECM community structure by modifying plant carbon productivity (Druebert et al. 2009). The effect of light on the ECM community composition was significant in the present study, but it may not be an important determinant of the difference between substrates because the light conditions of the logs and soil were not significantly different.

Against my prediction, *A. citrina*, probably a species with little wood decay ability, was a dominant ECM symbionts on decayed logs. The relatively high nutrient content of the white-rotted logs may explain their dominance because species in the genus *Amanita* have been reported to prefer high nitrogen and phosphorus in soils (Cox et al. 2010; Reverchon et al. 2012). There have been a few reports of *Amanita* from decayed logs, but this genus has been detected frequently in the soil of mature pine stands in the Northern hemisphere (Yamada and Katsuya 1996; Rao et al. 1997; Gehring et al. 1998; Taylor and Bruns 1999; Parrent et al. 2006; Cox et al. 2010; Ma et al. 2012; Reverchon et al. 2012), as well as recently in the Southern hemisphere due to biological invasion (Pringle and Vellinga 2006). In contrast, the ECM fungi reported from boreal forests dominated by *Picea*, *Abies*, *Tsuga*, and *Pseudotsuga*, where the majority of the ECM fungal communities on decayed logs have been analyzed, rarely include *Amanita* (Tedersoo et al. 2003, 2008, 2009a; Toljander et al. 2006; Elliott et al. 2007; Kalliokoski et al. 2010; Walker and Jones 2013). Thus, it is possible that future studies on the ECM inventory in pine stands may detect *Amanita* on decayed logs with a high frequency.

In conclusion, this study showed that current-year *P. densiflora* seedlings colonized well on decayed logs and formed a seedling banks in a post-PWD pine stand in Japan. Seedling roots were highly colonized by ECM fungi, and their basidiomycetous ECM fungal communities were affected significantly by the physicochemical properties of the substrates. The dominance of *A. citrina* in the decayed logs may be attributable to the high nutrient contents of the white-rotted logs at this study site.

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