

# Ecological studies of ectomycorrhizal fungi: an analysis of survey methods

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Received: 20 April 2010 / Accepted: 22 July 2010  
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**Abstract** In this paper, by reviewing ecological studies of ectomycorrhizal fungi where both fruiting bodies and mycorrhizal root tips were simultaneously surveyed, we investigate whether the diversity data obtained by the two methods leads to similar conclusions about the underlying ecological processes of interest. Despite discrepancies in identifying species, we found that both survey methods identified similar responses by ectomycorrhizal fungal communities to experimental manipulations, successional changes and environmental disturbances (exceptions are short-term or low-level disturbances). By analysing the results of the reviewed studies, we found a positive relationship to exist between fungal species richness and (i) the host plant age and (ii) the number of putative host plant species, independently of the applied survey method. Of the methodological variables, only the number of soil samples (for the below-ground approach) and the duration of the study (for the above-ground approach) have a significant effect on the EMF species richness, with species richness increasing with both. Our investigation also shows

that in 73% of the reviewed studies (27 out of 37) a greater species richness was found by fruiting body surveys than by methods based on sampling of the root tips. Based on these findings, we argue for the continuation of fruiting body surveys in order to gain rapid and still valuable information on ecosystems over a wide spatial and temporal range and strongly recommend their use in long-term ecosystem monitoring projects.

**Keywords** Fungal diversity · Ecosystem processes · Monitoring · Sampling methods · EMF community · Above-ground and below-ground responses · Bioindication

## Introduction

Ecosystem change caused by human activities is one of the pivotal issues in ecological research (Staddon et al. 2002). Ectomycorrhizal (EM) fungi are potentially excellent indicators of the effects of these activities due to the large number of species, their specialised life style, and their important ecological function. Mycorrhizal fungi mediate the interaction between plants and the soil and have important roles in nutrient cycling and the development of soil structure (Read et al. 2004). Thus, studies of ectomycorrhizal fungal (EMF) communities can reveal the direct and indirect (e.g. via the host plant or the soil microbial community) impacts of an environmental factor on the EMF community itself and also on the ecosystem in which it occurs (Staddon et al. 2002). EMF community structure is investigated often and has become an important topic in ecological research (van der Heijden et al. 1998; Copley 2000; Heckman et al. 2001; Högberg et al. 2001; Stinson et al. 2006). Until recently, the applied methodologies have been based on all developmental stages of EM fungi. These stages include (i) the spore-producing epigeous

**Electronic supplementary material** The online version of this article (doi:10.1007/s13225-010-0052-2) contains supplementary material, which is available to authorized users.

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(above-ground) or hypogeous (underground) fruiting bodies (the sporocarps); (ii) the mycorrhizal stage, which is the substrate-exchanging interface on the fine root system of the plant hosts and (iii) the mycelium, which is responsible for exploring the soil for nutrients and water. This balanced approach has, however, recently been abandoned due to the advent of molecular biology-based fungal identification methods. Nowadays, as a result, ecological studies of fungal communities focus almost exclusively on the mycorrhizal stage. One of the main reasons for this shift in methodology is the fact that in the same habitat the results of sporocarp surveys can differ considerably from the results obtained by underground sampling of EMF communities (Gardes and Bruns 1996). While molecular methods are fully accepted, sporocarp surveys are often interpreted as representing only an unpredictable sub-sample of the whole underground community, yielding hard-to-interpret data (Dahlberg et al. 1997; Gehring et al. 1998; Horton and Bruns 2001; Anderson and Cairney 2007; Lilleskov and Parrent 2007). This widespread belief has led to a decline in the number of studies using fruiting body surveys. However, other studies show that all three of the above methods (sporocarp, root tip, and mycelium sampling) used for investigating the EMF community have their own limitations and benefits (Buscot et al. 2000; Horton 2002; Allen et al. 2003; Schmidt and Lodge 2005; Koide et al. 2005; Avis et al. 2006; Dickie and FitzJohn 2007), so it is not so surprising that they can provide strikingly different results (e.g., Peintner et al. 2007). As a consequence, it is very difficult to get a complete picture of the EMF community from either above- or below-ground approaches alone.

While detailed biodiversity information on EMF communities has its value, a more important question concerns what this information can tell us about the functioning of the ecosystems in which they occur. Therefore, it would seem worthwhile to assess whether or not the data obtained by the different methods lead to similar conclusions about the underlying ecological processes of interest than concentrating on the exact details of species composition. To facilitate the investigation of this problem, we review those studies in which both the above-ground (fruiting bodies) and below-ground (i.e. mycorrhizas on the plant root system) survey methods were simultaneously applied to study EMF communities. In addition, we discuss the advantages and disadvantages of the methods applied to EMF community research.

### Comparison of studies of the EMF community

By using several search engines and our own database of articles, we found 34 studies in which EMF communities

were investigated by using both above-ground and below-ground sampling simultaneously (Tables 1 and 2, and Table Suppl.1). Both the goals of these studies and their methodologies vary greatly (Table 1, Suppl.1). We were, however, able to classify the studies into two main categories: studies in the first category simultaneously sampled both the above-ground and below-ground aspects of an EMF community in a given habitat in order to compare their compositions and so to assess the efficiency of different methods, while the second category contains studies which collected above- and below-ground EMF community data to investigate how they changed in response to varying environmental factors or to determine their suitability as an indicator for environmental change.

### Studies on EMF community composition

When the purpose of the studies was merely to determine the structure (species richness, abundances, composition, diversity or distribution) of the above-ground and below-ground aspects of EMF communities, the two different approaches usually found different community structures (Table 2 and Table Suppl.1; Gardes and Bruns 1996; Dahlberg et al. 1997; Pritsch et al. 1997; Dunstan et al. 1998; Jonsson et al. 1999b; van der Heijden et al. 1999; Yamada and Katsuya 2001; Taylor 2002; Avis et al. 2003; Valentine et al. 2004; Richard et al. 2004; Fujimura et al. 2005; Chen et al. 2007; Riviere et al. 2007; Smith et al. 2007b; Nieto and Carbone 2009; Palmer et al. 2009). However, the opposite (i.e., finding similar structures) also occurs. For instance, Nara et al. (2003a, b) found that the species composition of the underground EMF community corresponded closely with that of the sporocarp community in an early primary volcanic desert on Mont Fuji. Similarities in above and below-ground sampling results were also found in some well-studied taxa, such as *Paxillus involutus* (Laiho 1970), *Suillus grevillei* (Zhou et al. 2001), *Hebeloma cylindrosporum* (Guidot et al. 2002), *Russula* species (Avis et al. 2003; Matsuda and Hijii 2004; Palmer et al. 2009), *Suillus pictus* (Hirose et al. 2004; Kikuchi and Futai 2003), *Pisolithus microcarpus* (Ducousso et al. 2004), *Tylospora fibrillosa* (Carfrae et al. 2006), *Tricholoma matsutake* (Lian et al. 2006) and four hypogeous genera (Luoma et al. 1997 cf: Valentine et al. 2004). These studies have found considerable overlap between the spatial distribution and the biomass of genets of sporocarps and those of mycelium or mycorrhizas on the fine roots on the same site.

### Studies of EMF diversity vs. environmental variables

Despite our rapidly-increasing knowledge of EMF community ecology, we still need to learn more about the relationship between fruiting bodies and underground

mycorrhizas at a given site in order to gain a better insight of EMF community functioning. To advance our understanding it would be important to investigate whether the sampling of the different parts of the fungal individuals (mycorrhizas vs. sporocarps) can identify the same underlying ecological processes occurring in the ecosystem. The accumulation of multiple datasets from around the world (albeit mainly from the northern hemisphere) has enabled us to address this question. In the following sections, we briefly review articles investigating the correlation between EMF diversity and environmental variables.

### *Nitrogen surplus*

From the results of field investigations where nitrogen (N) was experimentally supplied or it heavily polluted the experimental sites (N is the main limiting factor for plants in many soil habitats), we can conclude that the time frame used to investigate changes in EMF community maybe a significant factor. Short-term studies found that the addition of surplus nitrogen does not affect the species richness or underground diversity of EMF communities, whereas sporocarp surveys show that the diversity of the EMF community was drastically reduced (Kårén and Nylund 1997; Jonsson et al. 2000). Interestingly, Peter et al. (2001a) reported the same for the first year of N addition, but after 2 years of further N supply significant changes were observed. The abundance of species that form large sporocarps (cap diameter about 5–10 cm) decreased on the fine roots in the underground community while the abundance of species with no or resupinate sporocarps increased. In a similar, but long-term experiment, Lilleskov et al. (2001, 2002a) demonstrated that species richness based on both sporocarp surveys and sampling of the fine roots decreased along an increasing anthropogenic N deposition gradient. They also reported that, compared to the relatively rapid decline of sporocarp abundance and diversity in response to surplus N, the response of the underground community is slower. In a field study conducted in Minnesota (US), Avis et al. (2003) measured the diversity, structure, and composition of EMF communities in a 16 year N-addition (fertilization) field experiment. Total sporocarp species richness was reduced by more than 50% in the fertilization treatments and the evenness and diversity also decreased. In addition, they found that different species responded differently to the treatment and so the composition of the above-ground EMF community differed across the fertilization treatments. The below-ground response was similar at the scale of plots of land, but it vanished at smaller spatial scales (Avis et al. 2003). Lilleskov et al. (2002b) also noted that EMF taxa can differ in response to N deposition. A similar result was found by Carfrae et al. (2006), who demonstrated that after

a 3-year nitrogen, sulphur and acidity treatment, N deposition suppressed the appearance of EM fungi, producing larger sporocarps in young plantations (Carfrae et al. 2006). There were generally fewer sporocarps and lower EMF sporocarp diversity under the N treatment plots, while the number of mycorrhizal root tips was greatest. In contrast, species richness and diversity were also the lowest in these plots. Kårén and Nylund (1997) and Wiklund et al. (1995) found the opposite; thus the biomass of the fine roots and the number of mycorrhizal root tips were halved following fertilization with both N and sulphur, while the EMF species richness and diversity on the roots were not changed. They reported a 50% reduction in sporocarp number and species richness of mycorrhizal species during the N fertilization experiment. To summarise the results of the above fertilisation studies, we can conclude that the above-ground response of the EMF community to N surplus is detectable sooner than is the case for below-ground changes.

### *Chronosequence studies*

Despite the rather general finding that the data obtained for EMF species compositions derived by sporocarp and fine root sampling overlap only slightly, in the following chronosequence studies the same relationship between age and the EMF community structure emerges independently of the applied methods (sporocarp or mycorrhizal sampling). For instance, Peter et al. (2001b) showed that sites of different spatial structure and stand age (35, 100–200 years old) can be differentiated by either sampling method. Peter et al. (2001b) argued that the differing histories of the sites can be a possible explanation for these findings. Similarly, Visser (1995) pointed out that both fruiting body and root tip assessments revealed a distinct sequence of mycorrhizal fungi related to stand age after regeneration following wildfire disturbance (6, 41, 65 122 years ago). It was possible to categorize them into early-stage, multi-stage and late-stage fungi (Visser 1995). Another group of researchers have demonstrated, both by sporocarp survey and root tip sampling, a progressive increase in species richness of mycobionts along chronosequences of Sitka spruce forests (6, 12, 30, 40 years), although the number of species encountered was low at all ages (Palfner et al. 2005). Gebhardt et al. (2007) attempted to characterize the diversity and succession of EMF sporocarps and root tip morphotypes of red oak growing on forest reclamation sites. Both methods showed that each site (5, 21, 33, 43, and 46 years old) exhibited stand-specific EMF communities with low similarity to other stands. The total number of EM species obtained by either sporocarp survey or by sampling the root system was the highest in the 46 year-old undisturbed stand. On the other

**Table 1** Applied methods of EMF community studies (above- and below-ground surveys)

Country, Reference	Forest type	Duration of the study	Age	Area represented	Area sampled for sporocarps	No. of sporocarp surveys (year)	Sporocarp biomass assessment	No. of soil samples (year)	Soil sample size in cm (dmxdp or lwxwd)	No. of root tips for PCR/morph.
Sweden Kärrén and Nylund (1997); Wiklund et al. 1995 <sup>1</sup>	<i>Picea abies</i>	1989–1993	30	2,025 m <sup>2</sup>	ng. (larger)	ng.	number	60 (1992–1993)	5xdown to the mineral layer	106
Canada (Alb.) Visser 1995 <sup>2</sup>	<i>Pinus banksiana</i>	1988–1991	6, 41, 65, 122	>2 ha	1 ha	4 (1988–1991)	number	24 (1988–1991)	5.5×20	cc. 4,800 morph.
USA (California) Gardes and Bruns 1996 <sup>3</sup>	<i>Pinus muricata</i>	1991, 1992, 1994	40	ng.	75.56 m <sup>2</sup>	cc. 24 (1991–1993)	number and dry mass	31 (1991), 4 (1994)	10×25, 10×40, 4×40	ng.
Sweden Dahlberg et al. 1997 <sup>4</sup>	<i>Picea abies</i>	1986–1993	100	19 ha	100 m <sup>2</sup>	26 (1986–1990, 1992)	number and Ohenoja's factor	10 (1993)	ng. 100 cm <sup>3</sup> down to the mineral soil	99
Germany Pritsch et al. 1997 <sup>5</sup>	<i>Alnus glutinosa</i> , <i>Alno-Fraxinetum</i>	n.g.(3 year)	60	1 ha	1 ha, 2.5 ha, 3 ha	cc. 18 (1 years)	number	216 (3 years)	10×5 from the 1 ha plots	ng. morph.
Australia Dunstan et al. 1998 <sup>6</sup>	<i>Pinus radiata</i> , <i>Pinus pinaster</i>	1996–1997	22	2 ha	2 ha	15 (1996–1997)	ng.	28 (ng)	6×18	ng. morph.
USA (Arizona) Gehring et al. 1998 <sup>7</sup>	<i>Pinus edulis</i> , <i>Pinus ponderosa</i> , <i>Quercus gambelii</i>	1992–1995	matured	20 km <sup>2</sup>	6×0.5 km <sup>2</sup>	ng. (1992–1995)	ng.	300 (1995 Apr.)	25–30×10–20	272
Sweden Jonsson et al. 1999 <sup>8</sup>	<i>P. sylvestris</i> , <i>P. abies</i> , <i>B. pendula</i>	1995–1996	1–241	8 stands	2–3×200 m <sup>2</sup> /stands	6 (1995–1996)	number	80 (1995–1996)	2.8×15	881
Sweden Jonsson et al. 1999 <sup>9</sup>	<i>Pinus sylvestris</i>	1986–1995	200–400	3 stands, 1 ha/stands	10×0.9 m <sup>2</sup> (9 m <sup>2</sup> )	8 (1991–1993)	number and Ohenoja's factor	25, 171 seedlings (1995, 1996)	2.8×15	464
Sweden Jonsson et al. 1999 <sup>9</sup>	<i>Picea abies</i>	1985–1992, 1995, 1996	47	2,700 m <sup>2</sup>	3×900 m <sup>2</sup> (2,700 m <sup>2</sup> )	ng.	ng.	15 (1996)	10×10×ng.	75
Netherlands van der Heijden et al. 1999 <sup>11</sup>	<i>Salix repens</i>	1990–1995	early and late stage of succession	16 stand	500 m <sup>2</sup>	8 (1990–1992)	number	10 (pooled)×16 (1993) 16×2 (1994–1995)	5×10 15×10×15	ng. morph.
Sweden Jonsson et al. 2000 <sup>12</sup>	<i>Picea abies</i> , <i>Pinus sylvestris</i>	1992–1996	70–90	ng.	1,300 m <sup>2</sup>	cc. 18 (1992–1996)	number and Ohenoja's factor	80 (1994), 25 (1992–1995)	2.8×15, 3×1	315
Ecuador Chapela et al. 2001 <sup>13</sup>	<i>Pinus radiata</i>	1996–1997	>10	11 plots	5×100 m <sup>2</sup> (500 m <sup>2</sup> )	3 (1996–1997)	number, fresh and dry mass	3×11 (1996–1997)	15×30	ng.
USA (Alaska) Lilleskov et al. 2001, 2002 <sup>14</sup>	<i>Picea glauca</i> , <i>Betula kenaiica</i> , <i>Populus spp.</i>	1968–1995	110–150	ng.	15×503 m <sup>2</sup>	cc. 24 (1993–1995)	number	135 (1995 Sept.)	ng.	ng.
Japan Yamada and Katsuya 2001 <sup>15</sup>	<i>Pinus densiflora</i> , <i>Quercus sp. Castanea arenata</i>	1992–1994	45	625 m <sup>2</sup> (25 m×25 m)	ng.	3×cc. 30 (1992–1994)	number	2 (1993 and 1994)	20×40×10	4,655 morph.
Switzerland Peter et al. 2001 <sup>16</sup>	<i>Picea abies</i>	1997–1999	100–200, 35	2,200 m <sup>2</sup>	2,200 m <sup>2</sup>	ng. (1997–1999)	number	106 (1997)	8×5	458
Switzerland Peter et al. 2001 <sup>17</sup>	<i>Picea abies</i>	1997–1999	35	1,100 m <sup>2</sup>	1,100 m <sup>2</sup>	cc. 100 (1994–1999)	number	1,536 (1997–1999)	sort piece of fine roots from 5 cm deep	1,536
Sweden Taylor 2002 <sup>18</sup>	<i>Pinus sylvestris</i>	2000	50	2,700 m <sup>2</sup>	2,700 m <sup>2</sup> (30 m×30 m)	5 (2000)	number	30 (2000)	2.8xdown to the mineral layer	5,371 morph.
USA (Minst.) Avis et al. 2003 <sup>19</sup>	<i>Quercus macrocarpa</i> , <i>Q. ellipsoidalis</i>	1983–2002	50–150, 35–70	9 plot (20 m×50 m)	400 m <sup>2</sup> /plot	3×13 (2000–2002)	number	2×144 (2000–2001)	2.8×20	1,031
Japan Nara et al. 2003a,b <sup>20</sup>	<i>Salix reinii</i>	2000–2001	early primary succession	5.5 ha	5.5 ha	34 (2000–2001)	mean dry mass of 20 sporocarp of each species	72 (2001)	10×10×10	1,242

Japan Hirose et al. 2004 <sup>21</sup>	<i>Pinus pentaphylla</i> var. <i>himekomatsua</i>	2000–2002	5	20×24 m and 6×16 m	ng. (2000–2002)	number and dry mass	3.5 (2002)	5×15	242
USA (Sth.Oreg.) Valentine et al. 2004 <sup>22</sup>	<i>Quercus garryana</i>	2000–2002	n.g.	58 ha	ng.	number	19 seedlings soil core (2000–2001)	2.8×2.5	700
French (Corsica) Richard et al. 2004, 2005 <sup>23</sup>	<i>Quercus ilex, Arbutus unedo</i>	1999–2002	1, 2–10, 170	15 ha	6,400 m <sup>2</sup> (160 m×40 m)	number	2×30 seedlings / saplings; 230 soil cores	10×10×20	558
USA (Oregon) Fujimura et al. 2005 <sup>24</sup>	<i>Pinus ponderosa</i>	1998	100–200	cc. 40 ha	24×240 m transect	number	32 (1998 June)	5×15	cc.91
UK (England) Palfner et al. 2005 <sup>25</sup>	<i>Picea sitchensis</i>	2000–2001	6, 12, 30, 40	500 ha	ng.	number	40 cores + 25 seedlings (2000–2001)	4.5×12	morph. 118,000
UK (Scotland) Carfrae et al. 2006 <sup>26</sup>	<i>Picea sitchensis</i>	1999	13	1.5 ha (20plots)	1.5 ha	ng.	80 (1999 August)	5.2×10	ng. (morph.)
China (South) Chen et al. 2007 <sup>27</sup>	<i>Eucalyptus</i> sp.	ng.	1–7	155×50 ha	155×100 m <sup>2</sup>	number	155×5 (1 time)	Deepness: 10–30 cm (cc.500 g)	ng. (morph.)
Germany Gebhardt et al. 2007 <sup>28</sup>	<i>Quercus rubra</i>	2002–2003	5, 21, 33, 43, 46	100,000 ha	5×720 m <sup>2</sup>	ng.	160 cores + 32 seedlings (2002, June–2003, May)	5×15, 10×15	490
USA (California) Peay et al. 2007 <sup>29</sup>	<i>Pinus muricata</i>	2005–2006	10	500 ha	6,330 m <sup>2</sup> 10 min/occasion	number and Program Estimates	114 (2005)	2.5×30.5	570
Guinea (West) Riviere et al. 2007 <sup>30</sup>	<i>Caesalpinaceae, Phyllanthaceae</i>	ng.	native rainforest	ng.	3×20 m	ng.	4 (4 years)	ng. (under sporocarps and other places)	100
USA (Calf.) Smith et al. 2007 <sup>31</sup>	<i>Quercus douglasii</i>	2000–2005	cc.50	1,024 m <sup>2</sup> (32×32 m)	12.56 ha (cc. 200 m radius)	number	59 + 35 (2003–2004)	11.5×8.5	9,400
Czech Republic Peter et al. 2008 <sup>32</sup>	<i>Picea abies</i>	1999–2000	matured	75 ha	3×25 ha	number	3×100, 3×75 (2000)	Deepness: 5 cm, 4.5×30	236
Spain Nieto and Carbone 2009 <sup>33</sup>	<i>Pinus pinaster</i>	2007–2008	5	ng.	700 m <sup>2</sup>	number	45 saplings (2007)	deepness 10–20 cm	107 (18,463 root tips)
USA (Wisconsin) Palmer et al. 2009 <sup>34</sup>	<i>Castanea dentata</i>	2001–2005	cc.100	cc.38 ha	cc.38 ha	number	cc.45 (2005)	25×25×25	233

ng. not given, *Ohenoja's factor* Ohenoja's species-specific conversion factor for estimating fungal biomass (Ohenoja et al. 1993), *morph.* mycorrhizas on root tip were identified using morphological traits only, numbers in superscript (1–34) mark references used in Table 2

**Table 2** Survey results of EMF community studies (above- and below-ground surveys)

Root tips survey results			Sporocarp survey results	
The most abundant species	Number of species or RFLP or sequence type	No. of explicit identified species	Number of species	The most abundant species
<i>Tylospora fibrillosa</i> , <i>Piceirhiza bicolorata</i> <sup>1</sup>	21	9	64	<i>Lactarius necator</i> , <i>L. rufus</i> , <i>L. theiogalus</i> , <i>Boletus edulis</i>
<i>Suillus brevipes</i> <sup>2</sup>	12	7	15	<i>Inocybe</i> spp., <i>Suillus brevipes</i>
<i>Suillus brevipes</i> , <i>Russula</i> spp., <i>S. tomentosus</i> <sup>2</sup>	20	13	34	<i>Cortinarius</i> spp., <i>Bankera Juligineo-alba</i> , <i>Russula</i> spp.
<i>Russula</i> spp., <i>Suillus brevipes</i> , <i>Cortinarius</i> spp. <sup>2</sup>	25	14	41	<i>Suillus tomentosus</i> , <i>Cortinarius</i> spp., <i>Russula</i> spp.
<i>Russula</i> spp., <i>Micelium radicans</i> , <i>Tricholoma</i> spp. <sup>2</sup>	27	14	34	<i>Suillus tomentosus</i> , <i>Cortinarius</i> spp., <i>Russula</i> spp.
<i>Russula amoenolens</i> , <i>Tomentella sub-lilacina</i> , <i>Russula brevipes</i> , <i>R. xerampelina</i> , <i>A. franchetii</i> <sup>3</sup>	20	9	10	<i>Suillus pungens</i> , <i>Amanita franchetii</i> , <i>Russula xerampelina</i>
<i>Piloderma croceum</i> , <i>Cenococcum geophilum</i> , <i>Tylospora fibrillosa</i> , <i>Russula decolorans</i> <sup>4</sup>	25	12	48	<i>Cortinarius malachius</i> , <i>C. paleaceus</i> , <i>C. traganus</i> , <i>C. brunneus</i> ,
<i>Naucoria</i> sp., <i>Lactarius</i> sp., <i>Paxillus rubicundulus</i> , <i>Alnirhiza</i> sp. <sup>5</sup>	16 (1 plot)	8	28 (from 3 plots)	<i>Naucoria striatula</i> , <i>Cortinarius bibulus</i>
<i>Thelephora terrestris</i> , 'Bcb' type, <i>Hebeloma crustuliniforme</i> <sup>6</sup>	6	4	9	<i>Rhizopogon roseolus</i> , <i>R. vulgaris</i> , <i>Hebeloma crustuliniforme</i> , <i>Suillus granulatus</i>
RFLP type 10,12,3, <i>Tricholoma terreum</i> , <i>Lactarius deliciosus</i> <sup>7</sup>	51	7	22 (13)	<i>Tricholoma terreum</i> , <i>Lactarius deliciosus</i>
RFLP-taxon X1, <i>Cenococcum geophilum</i> , <i>Suillus variegatus</i> , <i>Cortinarius</i> group1. <sup>8</sup>	135	11	66	<i>Suillus variegatus</i> , <i>Cortinarius obtusus</i> s.1, <i>C. semisanguineus</i>
RFLP-taxon 1, <i>Suillus variegatus</i> , <i>Piceirhiza bicolorata</i> , <i>Cortinarius</i> groups <sup>9</sup>	43 RFLP, 20 morph.	14	62	<i>Lactarius rufus</i> , <i>Suillus variegatus</i> , <i>Cortinarius traganus</i> , <i>Russula paludosa</i>
<i>Thelephora terrestris</i> , <i>Tylopilus felleus</i> , <i>Tylospora fibrillosa</i> <sup>10</sup>	16	9	28	<i>Hygrophorus olivaceoalbus</i> , <i>Russula ochroleuca</i> , <i>Paxillus involutus</i> , <i>Hebeloma fragilipes</i> , <i>Lactarius theiogalus</i>
<i>Hebeloma</i> sp., <i>Inocybe</i> sp., ITE5, <i>Laccaria</i> sp., <sup>11</sup>	15	7(genus)	78	<i>Hebeloma leucosarx</i> , <i>H. pusillum</i> , <i>Laccaria laccata</i> , <i>Paxillus involutus</i>
<i>Cenococcum geophilum</i> (20%), <i>Phialocephala fortinii</i> , <i>Piloderma croceum</i> <sup>12</sup>	52	10	111	<i>Cortinarius obtusus</i> , <i>Cantharellus tubaeformis</i>
<i>Suillus luteus</i> , <i>Thelephora terrestris</i> , <i>Rhizopogon vulgaris</i> <sup>13</sup>	3	3	3	<i>Suillus luteus</i> , <i>Thelephora terrestris</i> , <i>Rhizopogon vulgaris</i>
<i>Piloderma byssinum</i> , <i>Amphinema byssoides</i> , <i>Cortinarius</i> subgenus <i>telamonina</i> <sup>14</sup>	30	12	144	<i>Tricholoma inamoenum</i> , <i>Cortinarius idahoensis</i> complex, <i>C. brunneus</i> , <i>Boletus subtomentosus</i> var, <i>subtomentosus</i> , <i>Lactarius rufus</i> , <i>Russula abietina</i>
<i>Lactarius theiogalus</i> , <i>Paxillus involutus</i> <sup>14</sup>	9	7	14	<i>Lactarius theiogalus</i> , <i>Laccaria bicolor</i> , <i>L. laccata</i> , <i>Paxillus involutus</i> , <i>Hygrophorus olivaceoalbus</i> , <i>Lactarius olivaceo-umbrinus</i> , <i>Russula betularum</i>
Morphotype 51, 28, 6, 15, 54, 35, <i>Cenococcum geophilum</i> , <i>Russula</i> sp.1. <sup>15</sup>	28	6	40	<i>Coltricia cinnamomea</i> , <i>Inocybe</i> sp. (sec. <i>Depauperatae</i> ), <i>Inocybe umbratica</i> , <i>Russula</i> spp.
<i>Tylospora fibrillosa</i> , <i>T. asterophora</i> , <i>Cenococcum geophilum</i> , RFLP48, RFLP2, RFLP3, RFLP50 <sup>16</sup>	79 RFLP, 36 morph.	28	128	<i>Cortinarius brunneus</i> , <i>Russula ochroleuca</i> , <i>Russula laricina</i>

**Table 2** (continued)

Root tips survey results			Sporocarp survey results	
The most abundant species	Number of species or RFLP or sequence type	No. of explicit identified species	Number of species	The most abundant species
RFLP 1 ( <i>Thelephoraceae</i> ), <i>Tylospora asterophora</i> , <i>Russula laricina</i> , RFLP 2–4, <i>Hygrophorus pustulatus</i> <sup>17</sup>	68	17	25 (23)	<i>Russula laricina</i> , <i>Clavulina cristata</i> , <i>Hygrophorus pustulatus</i> , <i>Amanita aff. submembranacea</i> , <i>Inocybe grammata</i>
ng. <sup>18</sup>	37	19(species or genus)	56	<i>Cortinarius sp.</i> (42.3%) <sup>11</sup>
<i>Coenococcum geophilum</i> , <i>Cortinarius subg. telamonia</i> , <i>Russula aff. amoenolens</i> , <i>Tomentella sp.</i> <sup>19</sup>	72	38	59	<i>Lactarius camphoratus</i> , <i>R. aff. amoenolens</i> , <i>L. laccata</i> , <i>Inocybe sp.</i> , <i>Boletus nobilissimus</i> , <i>Hydnum zonatum</i> , <i>Lyophyllum cf. decastes</i>
<i>Laccaria laccata</i> , <i>Inocybe lacera</i> , <i>L. amethystina</i> , <i>L. murina</i> , <i>Scleroderma bovista</i> <sup>20</sup>	21	12	23	<i>Laccaria laccata</i> , <i>Hebeloma mesophaeum</i> , <i>Scleroderma bovista</i> , <i>L. amethystina</i> , <i>L. murina</i>
<i>Suillus pictus</i> , <i>Cenococcum geophilum</i> <sup>21</sup>	ng.	ng.	17	<i>Suillus pictus</i> , <i>Strobilomyces confusus</i> , <i>Lactarius chrysorrheus</i> , <i>Tylopilus castaneiceps</i>
<i>Cenococcum geophilum</i> , <i>Tuber sp.</i> <sup>22</sup>	39	7	>100	<i>Inocybe geophylla</i> , <i>Russula spp.</i> , <i>Boletus satanas</i>
<i>Cenococcum geophilum</i> , <i>Russula decipiens</i> , <i>R. acrifolia</i> , <i>Inocybe spp.</i> , <i>Thelephoraceae</i> , <i>Sebacinaceae</i> <sup>23</sup>	140	46	166	<i>Laccaria laccata</i> , <i>Inocybe tigrina</i> , <i>Lactarius chrysorrheus</i>
<i>Wilcoxina rehmi</i> , <i>Geopora cooperi</i> <sup>24</sup>	6	2	10 (genus)	<i>Tricharina</i> , <i>Anthracobia</i> , <i>Morchella</i> , <i>Peziza</i>
<i>Tylospora fibrillosa</i> , <i>Lactarius rufus</i> , “ <i>Piceirhiza sulfo-incrustata</i> ”(not identified), <i>Russula emetica</i> , <i>Hymenoscyphus ericae</i> , <i>Dermocybe crocea</i> <sup>25</sup>	13	11	8	<i>Russula emetica</i> , <i>Lactarius rufus</i> , <i>Dermocybe crocea</i> , <i>Russula ochroleuca</i>
<i>Lactarius rufus</i> , <i>Cortinarius spp.</i> , <i>Tylospora fibrillosa</i> , <sup>26</sup>	7	ng.	6	<i>Tylospora fibrillosa</i> , <i>Lactarius rufus</i> , <i>Inocybe spp.</i> , <i>Laccaria spp.</i>
<i>Scleroderma sp.</i> , <i>Laccaria sp.</i> , <i>Cenococcum sp.</i> <sup>27</sup>	7	1	15	<i>Scleroderma cepa</i> , <i>Pisolithus sp2</i> , <i>S. polyrhizum</i> , <i>S. citrinum</i> , <i>Laccaria laccata</i> , <i>Russula aeruginea</i>
<i>Cenococcum geophilum</i> , <i>Boletus aestivalis</i> , <i>Tricholoma muricatum</i> , <i>Tuber spec. 01</i> , <i>Laccaria amethystina</i> <sup>28</sup>	61	17	10	<i>Scleroderma citrinum</i> , <i>Boletus edulis</i> , <i>Amanita muscaria</i>
ng. <sup>29</sup>	28	12	36 (genus) 19 (species)	<i>Suillus pungens</i> , <i>Russula occidentalis</i>
Russulaceae, Amanitaceae, boletoids <sup>30</sup>	55	ng.	119	Russulaceae, Amanitaceae, boletoids, Sclerodermataceae
<i>Thelephoraceae</i> , <i>Pyronemataceae</i> , <i>Cortinariaceae</i> <sup>31</sup>	92	42	108	<i>Tomentella</i> , <i>Russula</i> , <i>Inocybe</i>
<i>Tylospora fibrillosa</i> , <i>Russula emetica</i> , <i>Tylospora asterophora</i> , <i>Thelephora terrestris</i> <sup>32</sup>	40 (15, 20, 30)	24	45 (5,27, 33)	<i>Clavulina cristata</i> , <i>Russula emetica</i> , <i>Cortinarius flexipes</i> , <i>Cortinarius sp.</i> , <i>Lactarius rufus</i>
<i>Tomentella sublilacina</i> , <i>Thelephora terrestris</i> , <i>Russula drimeia</i> , <i>Suillus bovinus</i> , <i>Paxillus involutus</i> <sup>33</sup>	17	9	9	<i>Suillus bovinus</i> , <i>Paxillus involutus</i> , <i>Thelephora terrestris</i> , <i>Xeroocomus badius</i> , <i>Scleroderma verrucosum</i>
<i>Russula pectinatoides</i> , Pezizales, <i>Thelephoraceae</i> , <i>Sebacinaceae</i> , <i>Scleroderma areolatum</i> <sup>34</sup>	46	28	99	Russulaceae, Boletales, Cortinariaceae, Tricholomataceae, Amanitaceae, Pezizales

Numbers in superscript (1–34) identify references in Table 1

ng. not given

hand, Richard et al. (2004, 2005) found that the age of *Quercus ilex* did not strongly shape the EMF diversity and composition below the ground. However this study was not conducted in stands of different ages, but in a single 170-year old stand with seedlings and older saplings. Accordingly, the sporocarp survey method was not applicable here because, as a result of the large coverage of the mycelium, it could not provide accurate and locally information about specific hosts. The results of fruiting body surveys have shown that species richness decreases as the number of layers of vegetation increases; unfortunately, this phenomenon was not investigated by the below-ground survey (Richard et al. 2004, 2005). Sporocarp inventory also indicated preferential fruiting of some fungal species near either *Q. ilex* or *Arbutus unedo*, and the below-ground sampling also found that the two EM hosts shared few EM species (only 12.9% of the taxa were shared, Richard et al. 2004). In a field study conducted in the Netherlands, above- and below-ground EMF association of *Salix repens* communities was investigated in relation to soil chemistry in a succession of dune ecosystems (van der Heijden et al. 1999). The study demonstrated that both the above- and below-ground fungal community data support the classification of the 16 field sites into four habitat categories derived by soil chemistry attributes (pH and moisture). These habitat categories are best interpreted as successional stages of this dune ecosystem. These successional studies indicate that when sufficient time is available for EMF communities to adapt to changing environments, both methods can draw the same conclusions.

#### *Studies with other abiotic factors*

It has also been investigated whether the EMF communities respond to abiotic environmental conditions other than increased N level, such as soil type, moisture level or nutrients, a question that can be closely related to the investigation of succession. In a study carried out in several Pinyon pine forests, sporocarp censuses and EMF root tip patterns showed the same relationship with the nutrient and the moisture level (Gehring et al. 1998). The EMF species richness was not correlated with measures of ecosystem productivity. The investigated two soil types had similar numbers of EMF species but their composition was different. In a Swedish study, the EMF community structure in spruce stands treated for more than 10 years with different levels of dolomite lime were compared (Jonsson et al. 1999c). Despite the differences in fungal taxa found above- and below-ground, tests between treatments using either fruiting bodies or root tip sampling data revealed a similar shift in community structure. In another study, Jonsson et al. (1999a) found that low-intensity wildfire did

not affect the average number of species per stand as determined by sporocarp surveys. Similarly, no significant differences were found between the number of restriction fragment length polymorphism (RFLP)-taxa on the fine root system in the controls (insulated stands) and on the stands exposed to fire. The underground EMF community was, however, less evenly distributed in the burnt stands than in the controls. To the contrary, Fujimura et al. (2005) found that several months after a low-intensity fire, five genera (*Anthracobia*, *Morchella*, *Peziza*, *Scutellinia* and *Tricharina*) of post-fire *Pezizales* were observed by fruiting body survey, while no root tips colonized by any species of post-fire *Pezizales* fruiting at the site were found. This suggests that fungi may switch from mutualism in stable forests to saprotrophism after a disturbance, or could indicate a methodological inefficiency of the root tip sampling procedure. Future studies are needed to clarify this. In a field study by Chapela et al. (2001) conducted in new plantations of pines in paramo grasslands in Ecuador, the effects of the introduction of exotic *P. radiata* pines and their accompanying EM fungi were examined. They found an extreme reduction in the number of species in the EMF community. Only three species (*Suillus luteus*, *Thelephora terrestris* and *Rhizopogon vulgaris*) were found in an established plantation. This reduction in species was not limited to estimates based on fruiting bodies but was also confirmed using DNA identification methods on EM root-tips. In a field investigation in the Czech Republic, Peter et al. (2008) obtained information about the EMF community in a heavily-damaged spruce forest and assessed whether missing EMF partners could contribute to the observed lack of regeneration. The EM species richness on the roots of adult trees was significantly lower in the heavily-damaged site than in the other two sites. The fruiting body survey found an even more drastic decline in EMF species richness.

#### *Studies with biotic factors*

Several studies have examined how habitat size and isolation affect the richness of EM fungi. Peay et al. (2007) found that island size had a strong effect on EM assemblage structure. Total species richness increased significantly with island area, independently of the approach used. Distance from the closest EMF colonist alone was a poor predictor of species richness, having a negative correlation with species number only on the largest islands. Durall et al. (1999) examined the effects of small forest gaps and partial cutting on EM mushroom diversity and biomass in order to provide suggestions for forest management on both optimal timber and edible mushroom harvesting. They found that sporocarp species richness along 100 m

long transects decreased as gap area increased. In 1995, the gap area threshold (the gap area at which species richness decreased substantially) was estimated to be between 214 and 950 m<sup>2</sup>, whereas in 1996 it was between 629 and 950 m<sup>2</sup>. EM richness on seedling root tips also decreased slightly with increasing distance from the edge of the intact forest. The maximum richness was found to be 7 m or less from the forest edge for both tree species investigated.

### Why is the overlap between the species composition yielded by the above-and below-ground surveys so small?

In the following section we consider several reasons that can cause a discrepancy between the two types of surveys.

#### Sporadic fruiting, different sexual-asexual mating behaviour

A frequently mentioned weakness of sporocarp surveys is the sporadic, unpredictable fruiting of a particular species. Because of this stochasticity, many species might be missed in a fruiting body survey (Gehring et al. 1998). Molecular data indicate that some EMF species do not sporulate very often, because the importance of sporulation for propagation may be much less than has been previously assumed (Sanders 2004) or sporulation might depend upon environmental conditions. Studies have revealed that sporocarp production is strongly affected by prevailing weather conditions, so many researchers suggest at least 5–10 years of study are needed to get useful information about the community (Hering 1966; Arnolds 1988). This seems too long for most ecological studies and might be avoidable or reduced by a well-designed sampling procedure (e.g., Feest 1999). On the other hand, if we were able to follow this irregular pattern of fruiting, it might be a good indicator of ecosystem activity. Changing weather conditions are not the only contributors to sporadic fruiting. For example, Gardes and Bruns (1996) did not record large changes in species diversity among three fruiting seasons even though the annual average of rainfall varied substantially over the same seasons. Peter et al. (2001b), during a 3 year study, obtained similar results: the 10 most abundant species did not change for the duration of the study, despite considerable variations in weather. Gehring et al. (1998) suggested that sporocarp surveys are unlikely to be informative in arid areas where fruiting is infrequent and potentially biased towards only a few species; however, this is dependent on the goal of the study. For example, if one is seeking the most active EMF species of the habitat, sporulating data can provide very useful information. Furthermore, one can reduce or elimi-

nate the effect of unpredictable weather conditions on the sporadic fruiting of the fungi. A possible way is to use saprobic community data as a control. Many studies have found no changes in the saprobic community structure during experiments where the disturbance was applied directly to the immediate environment of EM fungi (e.g., Peter et al. 2001a). On the other hand, weather conditions can instantly affect both types of fungal community (Wiklund et al. 1995). Thus, following the change in proportion of sporocarps of mycorrhizal species compared to those of saprobic species in the fungal community could reveal changes in the relevant EM environment at an early stage. In addition to the weather, the production of sporocarps by EM fungi has also been closely linked to the supply of photosynthate to the root systems (Högberg et al. 2001), which is strongly dependent on, and hence indicative of, the host plant's condition. It also seems that in unfavourable conditions, for many EMF species giving up fruiting (i.e. trading-off reproduction for survival) is one of the first steps in rearranging their resource allocation strategy (e.g., Last et al. 1979; Kuikka et al. 2003), and this can cause an immediate shift in the community assembly as assessed by sporocarp survey. Consequently, despite its unpredictable nature, a sporocarp community survey can give extra information not only about the attributes of the fungal communities but also about the underlying functional processes of the ecosystem.

To identify the key environmental factors affecting the species richness of the EMF communities, we analysed the results of the reviewed studies by non-parametric statistical tests (Spearman rank correlation, Kruskal-Wallis test and Wilcoxon paired rank test). As expected, there was a strong positive significant relationship between the host plant age and (i) the sporocarp species richness (Spearman rank correlation,  $r_s=0.63$ ,  $p<0.001$ ,  $n=36$ ) and (ii) the mycorrhizal species richness on the roots ( $r_s=0.52$ ,  $p=0.001$ ,  $n=35$ ), respectively. In addition, studies found significantly more fungal symbionts by sporocarp survey from forests which have more than one putative host plant species compared to forests with one putative host (Wilcoxon rank sum test,  $W=74.5$ ,  $p=0.018$ ,  $n=38$ ) and the same trend also appears for the underground mycorrhizal species richness ( $W=91$ ,  $p=0.087$ ,  $n=37$ ). This suggests that EMF species richness might correlate with host plant diversity, which can indicate a strong relationship between the two trophic levels. Nonetheless, forest type (deciduous, pine or mixed) has no influence on the EMF species richness either above- or below-ground (Kruskal-Wallis test,  $\chi^2=3.72$ ,  $df=2$ ,  $p=0.156$ ;  $\chi^2=2.61$ ,  $df=2$ ,  $p=0.272$ ). It should be emphasized that results from the different methodological approaches found similar relationships between the examined environmental variables.

## Survey methodologies and sampling efforts

The most abundant EMF species recorded by the two sampling methods often differ. However, as Table 1 shows, most studies (17 out of 32) estimate only the sporocarp biomass from their number, which is not the best indicator of biomass (Tóth and Feest 2007). Where the biomass was measured more directly (only 4 studies) by weighing the gathered and dried fungal fruiting bodies, the overlap were larger between the above- and below-ground survey methods (Hirose et al. 2004; Chapela et al. 2001; Nara et al. 2003a, b; Gardes and Bruns 1996). A further discrepancy between the two sampling procedures can be caused by the fact that in most studies there are significant differences between the sampling effort for the two types of survey. Often, a short period of sampling is considered to be enough to provide a valid estimate of the EMF community on the root system. Sporocarp samplings lasted for 3.054 years on average ( $SD=1.77$ ,  $n=37$ ) in the reviewed studies, whereas sampling of the root tips was significantly shorter (1.97 years on average,  $SD=1.18$ ,  $N=36$ , Wilcoxon paired rank sum test,  $W=919$ ,  $p=0.004$ ), and is often done only once (e.g., Dahlberg et al. 1997; Jonsson et al. 1999c; Taylor 2002; Fujimura et al. 2005; Chen et al. 2007). Koide et al. (2007) revealed that temporal partitioning can occur among the species of EM fungi in a community and because of this seasonality many species can be missed by the short and infrequent sampling of the root tips. Sometimes, the two different sampling surveys were carried out in different years (e.g., van der Heijden et al. 1999; Valentine et al. 2004) or not even on the same plots (e.g. Dahlberg et al. 1997; Table 1). These sampling inconsistencies can also contribute to the differences in the results. Additionally, Taylor (2002) demonstrated that species with a low abundance in the mycorrhizal community of the root system will usually be omitted from the sample because of the very patchy and stochastic occurrence of these species; hence, the results could be highly influenced by sample size. The number of soil samples taken range between 2 and 1,536, with an average of 156.6 ( $SD=278.6$ ,  $n=37$ ) per study (Table 1). This is significantly higher than the number of sporocarp surveys (mean is 22.75,  $SD=25.4$ ,  $n=32$ , range 1–100; Wilcoxon paired rank sum test  $W=188.5$ ,  $p<0.001$ ). Soil depth can also significantly influence the observed species composition and species richness (Fransson et al. 2000; Rosling et al. 2003; Hirose et al. 2004). In the reviewed studies, the maximum depth of the samples varied between 1 cm and 40 cm (mean depth is 17.63 cm,  $SD=8.18$ ,  $n=30$ ); moreover, it is often varied, even during the same study (e.g., Gardes and Bruns 1996; van der Heijden et al. 1999; Jonsson et al. 2000; Table 1). Rosling et al. (2003) demonstrated that the organic layer is most intensively

exploited by the fine roots, but considerable numbers of mycorrhizal roots occur in the mineral horizon (deeper region) as well, and different types of fungi occupy different parts of a soil profile. This could contribute to the mismatch between the results obtained by below-ground sampling and sporocarp survey.

By analysing the results of the reviewed studies (Spearman rank correlation), we found that among the methodological variables (sample depth, number of soil sample, duration of the study, sample volume and number of root tips collected for PCR), only the number of the soil samples had a significant effect on the EMF species richness found on the fine roots in the samples ( $r_s=0.40$ ,  $p=0.014$ ,  $n=36$ ). This means that studies taking more soil samples have found significantly higher species richness on the fine roots, while the other variables had no significant effect on the underground EMF species number. In addition, in the case of sporocarp surveys, the duration of the study had a significant positive effect on the sporocarp species richness ( $r_s=0.41$ ,  $p=0.011$ ,  $n=37$ ), while the number of surveys or the size of the sampled area had no considerable effect on the observed sporocarp species richness.

## Numerous unidentified RFLP type

Another reason for the different results could be the numerous unidentified RFLP types found when analysing the root tip samples. Currently, the major problem with the molecular ITS-RFLP approach when used alone is that the number of unidentified types typically remains high (on average 55.8%,  $SD=21.5\%$ ,  $n=36$ , Table 2). This high level of inefficiency is caused by (i) the limited number and (ii) the narrow phylogenetic coverage of the named internal transcribed spacer (ITS) DNA sequences available on GenBank, (iii) the size estimates for fragments vary considerably and (iv) significant intraspecific variation exists across large geographical scales (Kårén et al. 1997). Many recent studies (Kårén et al. 1997; Buscot et al. 2000; Sanders 2004) have noted that without knowing the true extent of the genetic variation within an individual, among individuals of the same species and among different species, it is very difficult to know exactly what the sequence diversity in a sample actually means. Smith et al. (2007a) examined intraspecific and intra-sporocarp ITS variation by DNA sequencing from sporocarps and pooled roots from 68 species of EM fungi collected at a single site in a *Quercus* woodland. They detected significant ITS variation within 27 species, roughly 40% of the taxa examined. Although intraspecific ITS variation was generally low (0.16–2.85%, mean=0.74%), it was widespread within this fungal community. Aanen et al. (2000, cf. Buscot et al. 2000), also pointed out that in the case of *Hebeloma mesophaeum*, it was the differences between the homolo-

gous chromosomes in the two nuclei of the dikaryotic hyphae which caused most of the sequence variation. Recent publications have warned of misleading sequence information contained within GenBank, and hence, increased potential for misidentifying unknown species using BLAST searches (Douglas et al. 2005). It is also estimated that c. 70% of the taxonomic diversity of the fungal herbarium collections is not yet represented in GenBank (Brock et al. 2009). These weaknesses will probably be minimised or eliminated in the near future. Our investigations revealed that more recent studies found a higher number of explicitly identified species ( $r_s=0.35$ ,  $p=0.046$ ,  $n=32$ ) and also there was a trend of obtaining higher species richness ( $r_s=0.31$ ,  $p=0.079$ ,  $n=33$ ) for below-ground sampling, while there was no correlation between species richness and the date of the study for above-ground samplings ( $r_s=0.06$ ,  $p=0.738$ ,  $n=32$ ). These findings might indicate that the applied root tip sampling methods are continually improving. Currently, however, most EMF community studies of the fine root system still need to simultaneously carry out fruiting body surveys to obtain species names. Horton (2002) stated that species richness is higher when analysing root tip data than when analysing sporocarp data. However, in the reviewed papers, a sporocarp survey appears to be a more accurate assessment of species richness, since investigations indicate that estimates of the number of symbionts appear to be much higher when based on observations of fruiting bodies than of mycorrhizae (sporocarp species richness median = 35, mycorrhizal species richness median = 27,  $n=37$ , Wilcoxon paired rank sum test,  $W=164.5$ ,  $p=0.008$ ) and also yielded more explicitly identified species than root tip sampling (explicitly identified mycorrhizal species richness on roots median = 11,  $n=35$ , Wilcoxon paired rank sum test,  $W=8.5$ ,  $p<0.001$ ). We found that in 73% of the reviewed studies (27 out of 37) a greater species richness was found from fruiting body samples than by methods based on sampling the root tips (Table 2). It should be mentioned that in many cases the identification of species based on fruiting body morphology is far from straightforward, especially in the so-called problematic genera such as *Cortinarius*, *Inocybe* and *Russula*, whose identification at species level require highly trained experts.

#### Species with inconspicuous sporocarp

Another cause of the difference in results obtained by the different sampling methods could be the colonisation of a large proportion of the root system by species without conspicuous sporocarps or with underground sporocarps (23–41%, Jonsson et al. 2000; 51.1%, Smith et al. 2007b). More than the half of the reviewed papers (56.25% or 18 out of 32, for 6 not applicable, Table 2) found that species with no or inconspicuous sporocarps were dominant

on the fine roots. *Cenococcum geophilum*, an asexually-reproducing species, is a representative of this group. Around 30% of the studies found that *C. geophilum* was among the three most abundant below-ground species (Table 2). The reason for this could be that this species has easily distinguishable mycorrhizal morphological traits, but it is hard to distinguish their condition (dead or alive), and it also has a wide ecological and geographical distribution (Douhan and Rizzo 2005). Obviously, species with inconspicuous sporocarps will be easily missed by above-ground sporocarp surveys, especially as most EMF community studies based on fruiting body surveys do not collect resupinate (inconspicuous) and hypogeous (underground) sporocarps. More careful studies indicate that this can be a serious problem. A study (Kõljalg et al. 2000) of EMF communities in Swedish boreal forests demonstrated that the proportion of tomentelloid fungi (taxa with resupinate sporocarps) in the underground EM community exceeded 1–8%. Therefore this taxon has considerable importance in EMF communities in boreal forests. In a more recent study, due to exhaustive sporocarp collecting methods, Smith et al. (2007b) found that besides the many species that form epigeous sporocarps (55.1%), 26.8% of the collected species produced hypogeous sporocarps and 18.5% of the species produced resupinate sporocarps. This means that nearly half of the collected species (44.9%) had inconspicuous fruiting bodies, which could have been easily overlooked during a less careful survey. Because they had surveyed not only the conspicuous epigeous sporocarps, the level of correspondence between fruiting bodies and EMF root surveys shifted from c. 20% to c. 45%.

#### Ectomycorrhizal or saprotrophic?

Several studies revealed that many fungal species have unsettled trophic status, i.e. EM fungi can occupy a large portion of the biotrophy (parasitic)–saprotrophy continuum (e.g., Lillekov et al. 2002b; Koide et al. 2008). Some EM fungi can utilize cellulose and other complex carbon sources to a limited extent and genes for lignicolytic activities appear to be widespread in EM fungi (Chen et al. 2001). Truffles seem to move along differential nutritional strategies (saprotrophic, endophytic and symbiotic) depending on the environmental phase of their life cycle (Murat et al. 2005). Fujimura et al. (2005) proposed a similar suggestion for the case of post-fire *Pezizales* fungi (see above). However, Taylor and Alexander (2005) concluded that there is no unequivocal evidence that any ECM fungus can complete its life cycles in the absence of a host. Furthermore, Hibbett et al. (2000) suggested that mycorrhizae are highly variable, evolutionarily dynamic, associations and it is not clear that the families of macrofungi, as currently envisaged, represent entirely natural groupings. Contemporary isotope studies

examining the natural abundance of stable isotope ratios of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  have revealed that families such as *Tricholomataceae* and *Cortinariaceae* contain large numbers of saprotrophs as well as EM taxa (Taylor et al. 2003; Trudell et al. 2004). Many genera known as EM are not monophyletic, and therefore scaling up from species to genus level introduces errors. Rinaldi et al. (2008) emphasise that although numerous genera have been proposed as being EM, but in a number of studies evidence for the hypothesized EM habit is lacking. Care must thus be used when compiling list of EM and saprotrophic fungi in community-level studies on the basis of published information only. The doubtfulness of the trophic status (actual and species level) of the sampled specimen can also contribute to the discrepancy between the results of the different survey methods.

### Cryptic species

Species concepts have been discussed by many authors (e.g., Taylor et al. 2000) and there are several prevalent species concepts that are currently in use in fungal systematics. These are the morphological species (species with distinct morphological characters), phylogenetic species (genetic and evolutionary distinctiveness represented by each terminal taxon in phylogenetic trees) and Operational Taxonomic Unit (OTU, based on 99% sequence similarity) (Taylor et al. 2000). Among the higher fungi, many reproductively isolated cryptic species exist that are morphologically difficult to distinguish owing to a lack of taxonomically useful morphological characters (Sato and Murakami 2008). This feature has already been demonstrated even within the most commonly known taxa (e.g., *Amanita muscaria* (Geml et al. 2006); *Cenococcum geophilum* (Douhan and Rizzo 2005)) and can result in the recording of several species in the root tip sampling instead of one. To detect cryptic species, one needs a very accurate sequence analysis; using only RFLP typing is not enough (Sato and Murakami 2008). However, this discrepancy can be avoided or minimised by analysing the sequences not only of the EM on the root tips, but of the fruiting bodies as well.

### Discussion

The aim of this paper was to elucidate how different results are obtained by the different sampling methods used for investigating EMF communities and how this phenomenon can affect the findings of ecological investigations. The review of studies simultaneously using different survey methods revealed that at present, EMF community research is an eclectic assemblage of different methodological approaches and research perspectives that make the

generalisation of their results difficult and could potentially lead to incorrect conclusions about ecosystem processes and the applied methods (Dahlberg et al. 1997; Gehring et al. 1998; Horton and Bruns 2001; Anderson and Cairney 2007; Lilleskov and Parrent 2007). We found that community studies of EM fungi can find contradictory results in determining EMF community composition at a site depending on the applied methods. So bending an one time assessed EMF diversity to a certain functionality not a reliable way to help to describe ecosystem processes. However, despite the discrepancies in community composition findings, our most vital result is that the majority of the studies that applied both above- and below-ground sampling methods to investigate changes in the habitats detected a similar relationship between environmental variables and the fungal community by either method (Table Suppl.1). This means that sporocarp data provided the same general conclusions as root tip data about changes in the EMF community; this shows the validity of the application of both survey methods in fungal ecological studies. Exceptions to this are those short-term studies where apparently the mycorrhiza have not had enough time to respond to the disturbance (e.g., fertilisation, defoliation, girdling experiments). This highlights the importance of the time frame used to assess changes in the fungal community.

### Response speed of EMF communities

It has been demonstrated previously that changes in the environment could cause a rapid decline in sporocarp production. For example, Högberg et al. (2001) investigated the response in sporocarp production after the flow of photosynthates from foliage to roots was terminated by girdling trees at chest height. Two months after the initiation of early girdling (in spring) the sporocarps had been virtually eliminated by the girdling procedure. In August they observed a more rapid response of 2–3 days. Similarly, in the study by Last et al. (1979) a decline of sporocarp number was profound after the defoliation of trees, whereas the mycorrhizal level of the fine root system had not changed. They found that sporocarp production ceased almost immediately (within 2 days) after defoliation. During the investigation, they surveyed sporocarps of mycorrhizal fungi newly-produced within the different periods of observation. Trees, common hosts of mycorrhiza, seem to respond within hours/days to, for example, elevated levels of  $\text{CO}_2$ , initiating the response of the underground symbionts. As EM fungi are heavily dependent on current of assimilates (as been shown in both laboratory and field experiments: Söderström and Read 1987; Lamhamedi et al. 1994; Högberg et al. 2001) physiological responses by EM fungi to changes in carbon supply are therefore likely to take place shortly after the plant responses. Shifts in community composition, however, may take longer to be noticeable

(Fransson et al. 2001). It has been shown that considerable changes in the functional activity (e.g., modified enzyme activity profiles [François and Garbaye 2009], changes in the activity of fruiting body formation [Last et al. 1979]) of the EM fungi occur early on, but these are undetectable by root tip sampling, making it difficult to detect the early effects of a disturbance. But if the disturbance is long-lasting or strong enough, the EMF community composition will slowly change. The delayed response at the mycorrhizal root level, however, raises the question of which environmental variable affects the mycorrhiza. Studies have already demonstrated the dynamic nature of the EMF community both above- and below-ground even in the absence of large-scale disturbances (Izzo et al. 2005). Thus, preliminary knowledge about the characteristic setting of the EMF community structure is required in order to detect environmental changes. After a baseline survey (we recommend one or two seasons), monitoring of fruiting body production could provide a very early signal to detect unfavourable processes due to the high sensitivity of sporocarp formation (Last et al. 1979; Högberg et al. 2001). Therefore, sporocarp community surveys can provide extra information not only about the attributes of fungal communities but also about the underlying functional processes of their ecosystems. In addition, a thorough fungal sporocarp survey, including EM and saprobic species, can also provide new insights by introducing the possibility of evaluating the relative contributions of symbiotic and saprobic components of the microflora. Following the changes in this ratio could reveal disturbances more precisely in the relevant ectomycorrhizal environment at an early stage.

#### Key environmental factors and methodological constraints

The main objective of ectomycorrhizal ecological studies is to identify key environmental factors affecting the species richness and diversity of EMF communities. We analysed the results of papers examining mycorrhizal communities by sporocarp and root tip inventory, looking for any trends. We found a significant and strong positive relationship between fungal species richness and (i) the host plant age and (ii) the number of putative host plant species, independently of the applied survey methods. This suggests that EMF species richness might correlate with host plant diversity, which can indicate a strong relationship between the two trophic levels. This is a concordance with van der Heijden et al. (1998) result, as they have demonstrated that mycorrhizal fungal diversity determines plant biodiversity. Of the methodological variables, only the number of soil samples (for the below-ground approach) and the duration of the study (for the above-ground approach) had a significant effect on the EMF species richness, both correlating positively with it. These results support Taylor's (2002) finding that the survey results of EMF species richness

found on root tips could be strongly influenced by the sample size. In the case of sporocarp surveys, we can assume that environmental factors will be more variable during long-term studies, because the time frame is wider, and more species meet their environmental demand for fruiting body formation. Species differ in their environmental sensitivity and increasing number of fruiting species might indicate the strong influence of environmental variability as a factor in the sporocarp formation of EMF community.

Despite the high environmental sensitivity of sporocarp production, our investigation shows that in 73% of the reviewed studies (27 out of 37) greater species richness was found by fruiting body surveys than by methods based on sampling of the root tips. ITS-RFLP and/or sequencing allow for efficient diagnostics of genetic groups (species delimitations) and are good at estimating species richness, although diversity data without species names are less useful, especially when one can connect functionally distinctive features to the species, such as species with numerous, short-lived (1–2 yr.) small genets such as *Hebeloma cylindrosporum*, *Laccaria amethystina*, *Amanita franchetii* and *Russula cremoricolor* or species with few, long-lived, relatively large genets (e.g., *Suillus* ssp. and *Cortinarius* ssp. (Redecker et al. 2001)). There is also high variation between fungal species in the benefits they provide to their hosts (Morgan et al. 2005). Knowledge of explicit species names also opens up new possibilities to investigate EMF communities, such as analysis of the constitution and liaisons of the phylogenetic diversity of the community (e.g., Hibbett et al. 2007; Riviere et al. 2007) and broadens our knowledge of the evolutionary processes that form EMF communities (Faith 1992; Vamosy et al. 2009). Based on these findings, we argue for the continuation of fruiting body surveys in order to gain rapid and still valuable information on ecosystems over a wide spatial and temporal range; in addition, we strongly recommend their use in long-term ecosystem monitoring projects.

It is important to emphasise that by sampling the above-ground fungal community it is possible to obtain an accurate picture of ectomycorrhizal communities and detect changes, providing valuable information for forest biodiversity and conservation or silvicultural management without excessive effort and cost. Epigeous sporocarps are much easier and cheaper to sample and identify than mycorrhizas (training an individual to identify most EMF species can actually be costly and time consuming, but fortunately in many regions numerous people already do this), therefore it is useful to know that they are good indicators of the potential activity of fungal communities. As it is difficult to gain information about the physiological processes and functionality of EM fungi in the field, it seems unwise to neglect fruiting bodies. The identification process maybe accelerated by using purpose-built computer

programs, for example the *MatchMaker: Mushrooms of the Pacific Northwest* (Gibson and Gibson 2008). These new tools have received less attention, although they provide a strikingly different identification method compared to books using dichotomous keys. Collectors, or data obtained from public markets can also provide useful information about spatial and temporal changes in the macrofungal community both locally and at larger scales. In contrast, the molecular approach can be beyond the reach of many researchers because of the expensive equipment, supplies, and training. But once a laboratory is established, the method can be applied to all taxa across regions.

#### The benefits of combining above- and below-ground methods

Our limited information about how ecological variables influence the investment between sexual and asexual reproduction of different EMF species also suggests a need for studies that address this question. It would be useful to define environmental and inherent factors that influence the mating and sporulating processes in the field and give meaning to the observed EMF community structures. A combination of the methods (sporocarp survey and root tip/mycelium sampling) is the only way to demonstrate which fungi are reproducing in a particular environment, as opposed to which fungi are present but cannot reproduce (sexually). The differences between the results of the above- and below-ground surveys (if genuine and not due to a methodological bias) could expand our knowledge about the population dynamic strategy of the species. Genets of many EM fungi are relatively small in size (Redecker et al. 2001), and in some EMF species, rapid genet turnover is apparent (Guidot et al. 2004). This strongly indicates the importance of spores (fruiting bodies) for the development and maintenance of EMF populations because in that case spore-mediated regeneration is the only way to produce new genets. Studies have also shown that habitat fragmentation and isolation affect the EMF richness and assemblage structure (Peay et al. 2007). This indicates that at least some members of the EMF community maybe directly limited by restricted dispersal ability and species vary the investment between dispersal forms (fruiting body formation/asexual propagulums). In a study by Peay et al. (2007) those species that occurred widely and colonized even small islands tended to be those that invested the most in dispersal structures relatively to vegetative structures.

#### Conclusions and perspectives

We conclude that the integration of sporocarp and molecular analyses of EMF communities is important in order to

document more completely the community assemblage and to reveal mechanisms (evolutionary and ecological) which are significant in structuring these communities. To monitor environmental changes, the detection of variations in the ecosystem must be prompt. In response to the global decline of biodiversity, policy-makers require a rigorous, relevant, and comprehensive suite of biodiversity indicators which helps them track changes over time, assess the impacts of policy and management responses, and to identify priorities for action (Walpole et al. 2009). Monitoring sporocarp production may seem outdated, but it has proved to be an excellent and rapid indicator of the changes in ecosystems and readily applicable in many locations over wide areas. Thus, we strongly recommend applying this method in long-term ecosystem monitoring projects. This would allow us to expand our limited information, which is currently obtained mostly by a few research groups from several coniferous forests in the northern hemisphere. Finally, a real but not easily measurable disadvantage of neglecting sporocarp surveys is that researchers spend less time in the field and so have fewer observations of fungi in their natural environment; thus, despite the many advantages of “peeping in to the black box” (Horton and Bruns 2001), there is a growing gap between living fungi and us.

**Acknowledgement** We would like to thank Steven L. Stephenson and Tamás Székely, jr. for the corrections they made to the manuscript. We also grateful to Alan Feest for useful discussions.

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