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Rineau, Courty. Secreted enzymatic activities of ectomycorrhizal fungi as a case study of functional diversity and functional redundancy. Annals of Forest Science, 2011, 68 (1), pp.69-80. 10.1007/s13595-010-0008-4 . hal-00930663

HAL Id: hal-00930663

https://hal.science/hal-00930663

Submitted on 11 May 2020

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Annals of Forest Science (2011) 68:69–80
DOI 10.1007/s13595-010-0008-4

ORIGINAL PAPER

Secreted enzymatic activities of ectomycorrhizal fungi as a case study of functional diversity and functional redundancy

François Rineau · Pierre-Emmanuel Courty

Received: 12 March 2010 / Accepted: 7 June 2010 / Published online: 22 February 2011
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Abstract
• ***Introduction*** Many studies deal with taxonomic diversity (TD) or estimation of functional traits of ectomycorrhizal communities. However, to our knowledge, none of them has ever tried to calculate the functional diversity (FD) of those communities, which is of critical importance for the diversity and reliability of nutrient supply to the tree.
• ***Objectives*** Here, we propose to evaluate the functional diversity of ECM communities in their potential ability to mobilise nutrients from organic matter, in two different forest stands.
• ***Results*** Results show that in both forest stands:

– TD is strongly correlated with FD.
– ECM species are functionally redundant within the communities for their abilities to secrete organic matter degrading enzymes.
– The functional traits (enzymatic activities) of the studied ectomycorrhizal communities are not related within genera, nor within families or phylogenetically related groups of taxa.
– Some species show a wide phenotypic plasticity for their enzymatic profiles.

• ***Conclusion*** This study provides the first evidence of functional redundancy in ectomycorrhizal communities. More studies are now needed to evaluate the robustness of these results at wider ecosystem scales and using other relevant functional traits.

Keywords Ectomycorrhiza · Functional diversity · Functional redundancy · Functional plasticity · Functional traits · Taxonomic diversity

Handling Editor: Jean Garbaye

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1 Introduction

Ectomycorrhizal fungi (ECMf) are dominant members of the soil microbial community in temperate and boreal forests. At the level of each individual ectomycorrhiza (ECM), crossing fluxes take place: photosynthates allocated to the root contribute to fungal growth and respiration, while water and nutrient solutes are extracted from the soil and channeled to the root through the extraradical mycelium that forages the mineral and the organic fraction of the soil (Smith and Read 2008). However, ECMs should not be considered as a simple association between an ECMf and an individual root tip. ECMs are composite and complex organs formed by the root, the main ECM fungus and other associated organisms as bacteria or other fungi. But, for the sake of convenience, we will here use as a convention the terms *population* and *community* of ECMs according to the fungal taxa associated with the roots (Courty et al. 2010a).

ECM mycelia colonize the upper horizons of soils of temperate and boreal forests, where mineral and organic matter are intermingled and where nutrient cycling is most intense. The structure and the diversity of ECM communities has been extensively studied across a wide range of forest types (e.g., Courty et al. 2008; Tedersoo et al. 2010) as well as its response to seasons, tree phenology, host genotype, soil chemistry, or environmental disturbances (e.g., Parrent et al. 2006; Courty et al. 2007, 2010b, 2011; Dickie et al. 2009; Rineau and Garbaye 2009a), but the

structure–function relation in these communities has been uncompletely addressed (Leake et al. 2002). Studies of ecological preferences and resilience of ECMf species according to disturbances are rare (Elmqvist et al. 2003; Folke et al. 2004). Moreover, the functional organization of ECMf species (functional redundancy, functional complementarity) is also of interest to understand how ECMf communities impact on ecosystem functions (Peay et al. 2008; Courty et al. 2010a). To assess this functional organization, functional traits should be measured. Functional traits are measurable properties (i.e., morphological, physiological characters), which define the performances of the organisms (i.e., growth, reproduction, survival; Violle et al. 2007). Functional traits are usually measured at the individual level and used comparatively across species (McGill et al. 2006). In plants, functional traits as potential photosynthetic rate or leaf mass per area are usually measured (Wright et al. 2004). Functional diversity (FD) has been defined as the value and the range of functional traits of the organisms present in a given ecosystem (Diaz and Cabido 2001). The ecological literature about the measurement of functional diversity, which captures how functional trait composition varies among assemblages, has grown during the last decade (i.e., Thompson et al. 2005; Barnett and Beisner 2007). Functional diversity can be expressed as the measurement of the branch length of a dendrogram based on similarities between functional traits (Petchey and Gaston 2002, 2009; Cianciaruso et al. 2009).

Among their functions, ECMf mobilize nutrients from organic compounds by secreting oxidative and hydrolytic enzymes (Read and Perez-Moreno 2003; Courty et al. 2009, 2010b; Pritsch and Garbaye 2010). The recent development of new methods for determining the potential enzymatic activity profiles of individual ECMs makes possible to decipher trait diversity of ECM communities (Courty et al. 2005). Activities of extracellular enzymes can be considered as functional traits to study functional diversity and resilience of ectomycorrhizal community (Cullings and Courty 2009). The first applications of this novel approach (e.g., Courty et al. 2005, 2007; Buée et al. 2007; Rineau and Garbaye 2009b; Jones et al. 2010) have revealed that the activity profile of ECMs formed by the same species may change significantly according to ever-changing ecological conditions (i.e., seasons, short-term climatic events, tree phenology, accidental or man-made disturbances such as soil amendment).

However, none of these authors has ever tried to evaluate the functional diversity of these communities. Here, we propose to evaluate the functional diversity of ECM communities according to their potential ability to degrade organic matter, in two different forest stands where the taxonomical structure has already been extensively studied (Courty et al. 2008; Rineau and Garbaye 2009a).

2 Material and methods

2.1 Forest stand

Experimental site of Champenoux This site (48°75′N, 6°35′ E, altitude 250 m, North-Eastern France) is a 100-year-old oak forest with a continuous canopy and a hornbeam understory (*Quercus petraea* (Mattuschka) Liebl., *Quercus robur* Ehrh., and *Carpinus betulus* L.). The luvic cambisol (pH [H_2O], 4.6) has a loamy texture in the A_1 (0–5 cm; P, 0.3 gkg^{-1}; total N, 1.9 gkg^{-1}; and C-to-N, 14.6) and A_2 horizons (P, 0.4 gkg^{-1}; total N, 2.35 gkg^{-1}; and C/N, 8.55; Courty et al. 2008). The forest floor is flat with scarce vegetation (oak seedlings, *Convallaria majalis* L., *Deschampsia cespitosa* L.).

Experimental site of Humont This site (48°00′N, 6°29′E, altitude: 570 m, Vosges forest, north-eastern France) consists of moderately declining stands of 35-year-old Norway spruce (*Picea abies*) and 60-year-old beech (*Fagus sylvatica*). The allocrisol (typic dystrochrept) is formed on sandstone. A liming treatment was carried out by helicopter in 1991 with 757 kg/ha of $CaCO_3$ and 380 kg/ha of $MgCO_3$. Nowadays, liming has restored tree health, mineral nutrition, and vegetation diversity, has shifted humus type from moder to oligomull or from dysmoder to oligomull, in the beech and spruce stands, respectively. It has also strongly enhanced colonization by earthworms as shown by the abundance of mounds. The present soil chemical properties in the treated and untreated plots have been published previously (Rineau et al. 2010).

2.2 Root sampling and sample processing

Experimental site of Champenoux Six soil cores (10 cm in diameter and 25 cm deep) were sampled monthly from July 2004 to September 2005 according to the pattern described in Courty et al. (2008). Each soil core was taken at least 1.5 m from oak trees. They were immediately transported to the nearby laboratory and processed within 10 days after sampling. The soil cores were sliced into two samples: the top (0–5 cm), corresponding to the A_1 horizon enriched in organic matter and containing densely packed fine roots, and the 5–25-cm layer corresponding to the top of the A_2 horizon.

Experimental site of Humont We chose, separately for spruce and beech, two pairs of 96 m^2 plots (12.5×8 m), each pair with one plot in the limed area and the other plot outside (untreated area). The plot couples were set in areas homogeneous in terms of topography, stand age, density, and sylviculture (data not shown). They contained ten and 13 trees for beech and spruce, respectively. Twenty soil cores (4 cm diameter, 18 cm deep, 225 cm^3, 2×2.5 m apart from

each other on a 5×4 grid) were collected in each plot, before leaf fall on October 2007 and 2008, and before bud break, on May 2007. Estimation of abundances of each morphotype has been measured on each soil core at each sampling date (n=20 for abundance estimation), while enzymatic activities were measured on five pooled soil cores per treatment at each sampling date (n=20/5=4 for functional trait estimation).

The following procedure was common in both studies. All soil cores were stored at 4°C and processed within 10 days after sampling. Roots were soaked in tap water for 15 min before being gently washed. Fine roots were observed in water with a stereomicroscope (×40). Initially, population of ECM morphotypes (ectomycorrhizae sharing common morphological features) were characterized according to Agerer (1987–1998) and provisionally classified at the genus level (e.g., *Russula*, *Lactarius*, *Cortinarius*, *Boletus*, *Tomentella* spp.) or as an "unidentified morphotype". For each encountered morphotype, a few tips (3–5) were kept at −20°C in Eppendorf tubes. Subsequently, the fungal symbionts of the frozen root tips were identified by sequencing the ITS region of its ribosomal DNA. The procedure is detailed in Courty et al. (2008). The description of the ectomycorrhizal fungal community and the estimation of the ECM abundance were described for the experimental site of Champenoux (Courty et al. 2008). Concerning the site of Humont, the analysis of the taxonomical diversity was published only for the sampling period of October 2007 (Rineau and Garbaye 2009a).

2.3 Enzymatic activity profiling of ECMs

Experimental site of Champenoux Fourteen ECM tips of each morphotype (chosen among the most turgid and healthy-looking ones, from a pooled sample of those of the six soil cores in which this ECM type was present) were used to determine their potential enzymatic activities, using the high-throughput photometric and fluorimetric microplate assays described by Pritsch et al. (2004) and Courty et al. (2005), and applied in previous studies (Buée et al. 2007). Each well of the 96-well micro-titration plate contained a single ECM tip. Six activities were first sequentially measured on seven tips: acid phosphatase (EC 3.1.3.2), β-glucosidase (EC 3.2.1.3), N-acetylhexosaminidase (EC 3.2.1.14), glucuronidase (EC 3.2.1.31), xylosidase (EC 3.2.1.37), and cellobiohydrolase (EC 3.2.1.91). Then, the seven remaining tips were used to measure leucine aminopeptidase (EC 3.4.11.1) and laccase (EC 1.10.3.2) activities.

Experimental site of Humont In each plot, the 20 soil cores have been randomly pooled into four composite samples (i.e., pseudoreplicates) obtained by mixing together the content of five cores. Enzymatic activities were measured on all ECM species presenting more than seven healthy root tips after pooling, in order to have enough ECM material for the enzymatic assays. Here, the high-throughput assay described by Pritsch et al. (2004) and Courty et al. (2005) was modified. The eight enzymatic activities previously described were sequentially measured on the seven selected tips. The analysis of the enzymatic activities was published only for the sampling period of October 2007 (Rineau and Garbaye 2009b) without any calculation of functional diversity.

In both studies, the standard deviation (n=7) for each value was around 15% for very low activities and below 10% for medium and high activities.

2.4 Expression of results and data analysis

When enzymatic assays were over, ECM tips were transferred into a fresh clear microplate containing 100 µl of water per well, and the projection surface area of each ECM tip was determined using the automated image analysis software WINRHIZO 2003b (Regent instruments, Inc., Canada; Buée et al. 2005). All measured activities

Table 1 Effects of soil amendment or soil horizon on ECM taxonomic diversity (Shannon index) and functional diversity. The TD was calculated as $H' = -\sum((Ni/N) \times \log 2(Ni/N))$, where Ni was the abundance of the species i and N the total number of individuals

Site	Humont						Champenoux
Tree species	Spruce			Beech			Oak
Date	Autumn 2007	Spring 2008	Autumn 2008	Autumn 2007	Spring 2008	Autumn 2008	July 2004–September 2005
Taxonomic diversity (TD)	NS	(−) (p<0.01)	(−) (p<0.001)	NS	(−) (p<0.03)	(−) (p<0.01)	(−) (Courty et al. 2008)
Functional diversity (FD)	NS	NS	NS	NS	NS	NS	(−) (p<0.05)

The FD was calculated as the total branch length of a minimum spanning tree based on ECM species dissimilarity in their eight potential enzymatic activities, according to Petchey and Gaston (2002)

For FD, n=4 (Humont) or n=15 (Champenoux); For TD, n=20 (Humont)

(−) significantly lower diversity in the limed plots (Humont) or in the A_2 horizon (Champenoux)

were normalized per square millimeter of surface area of individual ECM tips and expressed in picomole per square millimeter per minute (pmol mm^{-2} min^{-1}). Such enzymatic assays actually measure the total secreted potential activity of the ECM considered as a mixed organ composed of plant and fungal tissues and associated bacteria. All these components potentially contribute to the measured enzymatic activities. Here, we did not try to separate the activities due to each partner, because the mobilization of nutrients by ECMs and its benefits to the tree result from all these activities together (Courty et al. 2005). Moreover, this method measures potential activities on ECM root tips only

Table 2 Average and maximal contributions (in percent) of the different taxonomic groups to the functional diversity of ECM communities in Humont (Table 2a) and Champenoux (Table 2b)

a) Humont

Horizon					Beech Limed	Beech Untrd	Spruce Limed	Spruce Untrd	Max	Average	t-test
Ascomycetes			15%	*Cenococcum geophilum*	21%	15%	16%	13%	34%	15%	
				Fagirhiza sp.			10%	15%	15%	12%	
				Tuber sp.			7%	7%	7%	7%	
Basidiomycetes	Agaricales	Amanitaceae	9%	*Amanita rubescens*			4%	10%	14%	9%	
		Cortinariaceae	13%	*Cortinarius sp.*	7%			13%	22%	11%	
				Cortinarius sp 2	36%			12%	36%	20%	
		Hygrophoraceae	11%	*Hygrophorus olivaceoalbus*	10%	16%		7%	16%	11%	
		Tricholomataceae	15%	*Laccaria amethystina*			20%	11%	34%	14%	
				Laccaria sp.				16%	16%	16%	
	Thelephorales	Thelephoraceae	14%	*Tomentella sp.*				7%	7%	7%	
				Tomentella sp 2			14%	7%	14%	11%	
				Tomentella sp 3			16%	10%	28%	13%	
				Tomentella sublilacina	24%	13%	15%	12%	34%	15%	
	Boletales	Boletaceae	13%	*Xerocomus pruinatus*	18%	15%	12%	10%	35%	14%	
				Boletale *sp 1*				10%	12%	10%	
				Boletale *sp 2*		15%		7%	19%	13%	
				Boletale *sp 3*				8%	9%	8%	
	Russulales	Russulaceae	14%	*Russula cyanoxantha*		13%	10%	9%	14%	10%	
				Russula ochroleuca	17%	17%	16%	8%	34%	16%	
				Russula sp			18%	14%	24%	17%	
				Russula sp 2			18%	7%	28%	15%	
				Russula xerampelina				7%	7%	7%	
		Lactariaceae	13%	*Lactarius rostratus*	16%	17%		11%	34%	15%	
				Lactarius sp.			10%	12%	17%	11%	
				Lactarius sp 2			14%	7%	14%	10%	
				Lactarius subdulcis			9%	12%	17%	11%	
	Cantharellales	Clavulinaceae	20%	*Clavulina cristata*	25%		19%	12%	36%	20%	
Unidentified			15%	UECM sp 1	22%	18%		7%	36%	19%	
				UECM sp 10		25%		7%	25%	16%	
				UECM sp 12		17%		7%	17%	12%	
				UECM sp 14		17%		7%	22%	14%	
				UECM sp 18		17%		7%	17%	12%	
				UECM sp 2	19%	16%	17%	7%	33%	16%	
				UECM sp 20	12%	14%		7%	15%	11%	
				UECM sp 22			9%	7%	9%	8%	
				UECM sp 23				7%	7%	7%	
				UECM sp 3			16%	11%	24%	14%	
				UECM sp 4			10%	7%	10%	8%	
				UECM sp 5			18%	11%	23%	14%	
				UECM sp 6			7%	7%	7%	7%	
				UECM sp 7			12%	7%	12%	10%	
				UECM sp 8		12%		7%	15%	10%	
				UECM sp 11	19%	19%		7%	34%	18%	

The color of each table cell depends on the contribution of each ECM species or each group of ECM species to the FD (*white* low contribution; *dark* high contribution)

The contribution to the FD was calculated as the percentage of FD lost when the species was omitted from the community in the dataset

The contributions have been calculated for each date and each replicate

Here, we present the average contribution of each species or group of species in each plot (Humont: average contribution in each plot: Beech Limed, Beech Untreated, Spruce Limed, Spruce Untreated, for mixed three sampling dates and four replicates) or in each horizon (Champenoux: average contribution in each of the two soil horizons for mixed 15 sampling dates)

The average contribution for the whole sampling season, as well as the maximal punctual contribution recorded (in a specific sample at a specific date), are also presented

A *t* test was performed on the contributions of ECM species between limed and untreated plots (Humont) and between horizons 1 and 2 (Champenoux).

Significant differences ($p<0.05$) are indicated with an asterisk

Table 2 (continued)

b) Champenoux					Species	Maximum		Date of maximum		Average		
			H1	H2	Horizon	H1	H2	H1	H2	H1	H2	ttest
Ascomycetes			5%	15%	*Cenococcum geophilum*	9%	21%	Nov-04	Sep-04	5%	16%	*
					Tuber borchii	7%	22%	May-05	Jul-04	5%	15%	*
					Peziza depressa	8%	4%	Jan-05	Mar-05	4%	4%	
Basidiomycetes	*Agaricales*	*Cortinariaceae*	5%	10%	*Cortinarius rouxii*	6%	14%	Jan-05	Mar-05	3%	14%	
					Cortinarius anomalus	15%	14%	Oct-04	Nov-04	6%	9%	
					Cortinarius casimiri	12%	11%	Aug-05	Jul-05	4%	6%	
					Cortinarius olivaceofuscus	23%	35%	Aug-04	Aug-04	11%	20%	
					Cortinarius rufus	7%	4%	Feb-05	Mar-05	3%	4%	
					Hebeloma sacchariolens	5%	4%	Mar-05	Mar-05	2%	4%	
					Inocybe petiginosa	7%	4%	May-05	Mar-05	3%	4%	
		Tricholomataceae	7%	4%	*Laccaria amethystina*	20%	4%	Aug-04	Mar-05	8%	4%	
					Laccaria laccata	8%	4%	Jul-04	Mar-05	5%	4%	
		Amanitaceae	4%	6%	*Amanita rubescens*	5%	8%	Jan-05	Feb-05		6%	
		Entolomataceae	5%	4%	*Entoloma nitidum*	7%	4%	Jul-04	Mar-05	5%	4%	
	Thelephorales	*Thelephoraceae*	6%	9%	*Telephora terrestris*	7%	4%	Jul-04	Mar-05	5%	4%	
					Tomentella atramentaria	12%	4%	Dec-04	Mar-05	5%	4%	
					Tomentella badia	9%	22%	Jul-04	Aug-05	5%	13%	*
					Tomentella badialateritia	6%	4%	Mar-05	Mar-05	2%	4%	
					Tomentella botryoides	6%	4%	Apr-05	Mar-05	3%	4%	
					Tomentella bryophila	6%	4%	Jan-05	Mar-05	3%	4%	
					Tomentella ellisii	8%	16%	Feb-05	Feb-05	3%	10%	
					Tomentella fuscoresinosa	8%	4%	Jan-05	Mar-05	4%	4%	
					Tomentella lateritia	27%	18%	Sep-05	Oct-04	8%	11%	
					Tomentella lilacinogrisea	9%	15%	Jul-05	Feb-05	5%	9%	
					Tomentella stuposa	6%	4%	Jun-05	Mar-05	2%	4%	
					Tomentella sublilacina	27%	19%	Sep-05	Jul-04	9%	14%	
					Tomentellopsis echinospora	4%	4%	Jan-05	Mar-05	2%	4%	
	Boletales	*Boletaceae*	6%	13%	*Boletus aestivalis*	10%	4%	Sep-04	Mar-05	4%	4%	
					Leccinum carpini	13%	15%	Aug-05	Jul-05	6%	11%	
					Leccinum crocipodium	15%	21%	Aug-05	Jul-04	8%	13%	
					Xerocomus chrysenteron	16%	21%	Oct-04	May-05	8%	14%	*
					Xerocomus pruinatus			Jan-05	-			
		Rhizopogonaceae	3%	6%	*Rhizopogon sp*	7%	8%	Apr-05	Sep-05	3%	6%	
	Russulales	*Russulaceae*	5%	11%	*Russula atropurpurea*	5%	20%	Feb-05	Jan-05	3%	14%	*
					Russula cyanoxantha	7%	11%	Jul-04	Sep-05	7%	11%	*
					Russula delica	12%	19%	Dec-04	May-05	6%	13%	*
					Russula laurocerasi	6%	4%	May-05	Mar-05	2%	4%	
					Russula nigricans	12%	12%	Aug-05	Aug-05	6%	8%	
					Russula ochroleuca	9%	14%	May-05	May-05	4%	10%	*
					Russula rosea	7%	4%	May-05	Mar-05	3%	4%	
		Lactariaceae	9%	16%	*Lactarius chrysoreus*	13%	10%	Jul-05	Apr-05	7%	7%	
					Lactarius quietus	28%	35%	Sep-05	Aug-04	10%	18%	*
	Atheliales	*Atheliaceae*	10%	15%	*Byssocorticium atrovirens*	30%	35%	Sep-05	Aug-04	11%	17%	
					Piloderma croceum	13%	4%	Oct-04	Mar-05	7%	4%	
	Cantharellales	*Clavulinaceae*	6%	9%	*Clavulina cristata*	14%	15%	Dec-04	Jun-05	6%	9%	

and does not take into account the extraradical mycelium, which can be important for some long-distance exploration type ECMf species (Agerer 2001).

The Shannon and Wiener index was used as an estimator of the taxonomic diversity (TD) of the ECMf communities: $H' = -\sum((Ni/N) \times \log 2(Ni/N))$, where Ni was the abundance of the species i and N the total number of individuals.

All activity profiles obtained in both studies were analyzed together in the same initial matrix. Here, as variables were following a log-normal distribution, variable transformation was straightforward. Variable was transformed in two ways. First, due to unavoidable fluctuation in data production, some activities were negative. They have been corrected to zero. Second, the transformed variable z is produced from the actual value y through $z = Log(1 + y)$. The constant 1 is required as many actual values are equal to zero. It has been tested visually that transformed variables follow a Gaussian law, i.e., are unimodal with humped shape. This is qualitatively the case, which is not too much of an inconvenience, as tests are known to be robust. This initial matrix was then centered and reduced. A linear model was then applied to compare all activity profiles.

2.5 Calculation of functional diversity

After the enzymatic tests, we ended up with initial matrices of species × 8 mean enzymatic activities. In Humont, we had 48 initial matrices, one matrix per pseudo-replicate, treatment, and date (four pseudoreplicates, four treatments, three dates). For the experimental site of Champenoux, we had 30 initial matrices, one per date, and soil horizon (15 dates, 2 horizons). A distance matrix of enzymatic activities between species was then calculated for each initial matrix

and a minimum spanning tree built based on each distance matrix. The FD was calculated as the total branch length of the tree, according to Petchey and Gaston (2002). We then ended up with 48 values of FD in Humont and 30 in Champenoux. Significant differences in FD and TD between limed and untreated plots (site of Humont), and between the A_1 and the A_2 horizon (site of Champenoux) were assessed using a student *t* test. Correlations between the TD and the FD per pseudo replicate (Humont) or soil core (Champenoux) in both sites were calculated with the Spearman's Rho coefficient. Significant differences in FD and TD between limed and untreated plots (site of Humont), and between the A_1 and the A_2 horizon (site of Champenoux) were assessed using a student *t* test. Correlations between the TD and the FD per soil core in both sites were calculated with the Spearman's Rho coefficient. No significant correlation between the abundance of a species and its activities was found.

In order to identify structure in the activity profiles, a hierarchical clustering based on a species–species Euclidean distance matrix was built. Ten clusters representing the main profile patterns were designed using *k*-means clustering. The number of occurrences of each species in each of the ten clusters was reported. The frequency corresponding to the number of clusters where a species was present was also given.

Statistical analyses were performed using the R software (http://www.r-project.org; Ihaka and Gentleman 1996; R development core team 2006).

3 Results

In the ECMf community of Humont, liming had no significant effect on FD (Table 1) whatever the date, the tree species, or the treatment. However, TD was significantly lower in the limed plots for the two sampling dates of 2008. The number of replicates used to calculate TD (20) and FD (4) could explain the difference in the significance of the results (at the same dates, FD appeared also lower in the limed plots). In the ECMf community of Champenoux, the FD and TD were significantly higher in the A_1 horizon than in the A_2 horizon.

The contribution of each ECM species or each group of species to the FD of the community was evaluated as the percentage of FD loss when the species or the group of species was removed from the dataset (Table 2). The contribution to FD was different in the two sites regarding TD. In Champenoux, the contribution of *Thelephoraceae* (6% and 9% in the A_1 and A_2 horizon, respectively) and *Russulaceae* (5% and 11% in the A_1 and A_2 horizon, respectively) to FD were low regarding to their high number of species (13 species of *Thelephoraceae* and 7 species of *Russulaceae*). In Humont, the contribution of the four main groups (*Thelephoraceae*, *Boletaceae*, *Russulaceae*, *Lactariaceae*) was similar (around 13–14%) regarding to their similar contribution to TD (four to five species). In average, the contribution of each ECMf species to FD was similar in Humont (between 9% and 20%) and Champenoux (between 3% and 20%). In both sites, the maximal contribution (i.e., maximal contribution of a given species at a given date in a treatment) was similar: 36% in Humont (*Cortinarius* sp2) and 35% in Champenoux (*Cortinarius olivaceofuscus*, *Lactarius quietus*, and *Byssocorticium atrovirens*). In the A_1 horizon of the Champenoux site, the maximal contributions were found for *L. quietus* (30%), *B. atrovirens* (28%), *Tomentella sublilacina* (27%), and *Tomentella lateritia* (27%) in September 2005. In the A_2 horizon of the Champenoux site, the maximal contributions were found for *L. quietus* (35%), *B. atrovirens* (35%), and *C. olivaceofuscus* (35%) in August 2004. In average, three species (*L. quietus*, *B. atrovirens*, and *C. olivaceofuscus*) were the most active in the A_1 horizon (10%, 11%, and 11%, respectively) and in the A_2 horizon (18%, 17%, and 20% respectively). We consider those species as major species. In average, the contribution was significantly different between the two horizons for many species of Russulales (*L. quietus*, *Russula ochroleuca*, *Russula delica*, *Russula cyanoxantha*, *Russula aurantiaca*), one *Boletale* species (*Xerocomus chrysenteron*), one *Tomentella* species (*Tomentella badia*) and two species of Ascomycetes (*Cenococcum geophilum, Tuber borchii*; Table 2b).

In the two studies, TD and FD were significantly linearly correlated ($p<0.001$), and the correlation slopes and the Spearman's Rho were quite similar (0.91 and 0.79 for the sites of Champenoux and Humont respectively; Fig. 1). In

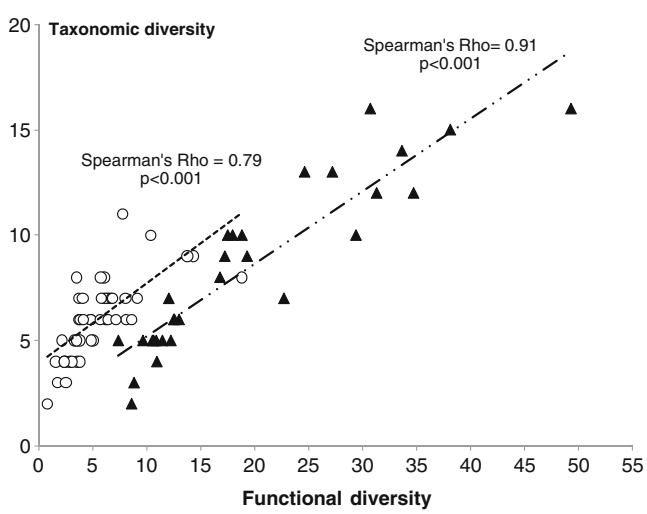
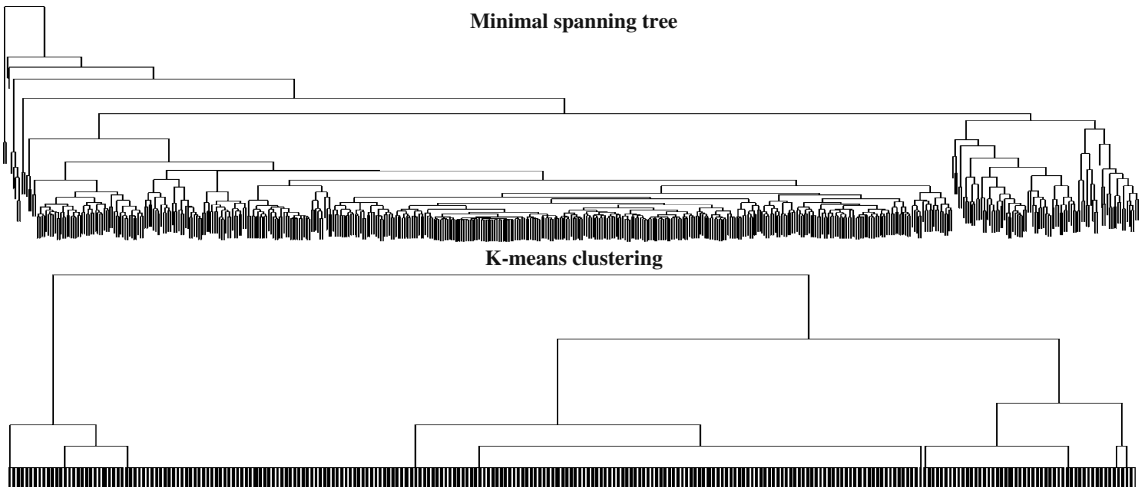


Fig. 1 Relationships between measured FD and TD per soil core in the two studied sites. *Empty circles* Humont (48 points: 3 sampling dates×4 treatments×4 replicates), *black triangles* Champenoux (30 points: 15 dates×2 horizons)

order to describe the diversity of activity patterns of ECMf species, a hierarchical clustering was done (Fig. 2). The activity patterns were distributed in ten groups (clusters). The number of activity profiles per group was from 2 (cluster 9) to 210 (cluster 10). The clusters 1 and 9 grouped activity profiles (6 and 2, respectively) with the highest activities for all enzymes. The clusters 6 and 10 grouped the activity profiles (129 and 207, respectively) with the lowest activities for all enzymes. The cluster 3, 5, and 8 grouped activity profiles (30, 33, and 61, respectively) with high activity of one enzyme (laccase, leucine aminopeptidase, and chitinase, respectively). The clusters 2, 4, and 7 grouped activity profiles (33, 21, and 8, respectively) with medium-range activity.

Activity profiles from many species were distributed in many clusters (Table 3): i.e., *Xerocomus pruinatus* (29 activity profiles distributed in eight clusters), *L. quietus* (27 activity profiles distributed in seven clusters), *C. geophilum* (50 activity profiles distributed in six clusters), and *T. sublilacina* (45 activity profiles distributed in five clusters). Moreover, even if activity profiles were distributed in many groups, most of the activity profiles belonged to one or two groups: i.e., *X. pruinatus* (29 activity profiles, 11 in cluster 5, and 6 in cluster 6), *L. quietus* (27 activity profiles, 9 in cluster 5), *C. geophilum* (50 activity profiles, 15 in cluster 6, and 28 in cluster 10), and *T. sublilacina* (45 activity profiles, 25 in cluster 10, and 9 in cluster 6). However, some species as *B. atrovirens* and UECM sp.1, were common (17 and 20 activity profiles respectively) but distributed in three clusters only and mainly in one (Cluster 10, with 12 and 11 activity profiles respectively). Finally, no clustering of ECMs



Enzyme	Cluster 1	2	3	4	5	6	7	8	9	10
Beta Glucosidase	0.2	0.8	-0.3	2.9	-0.1	-0.1	0.6	0.6	7.6	-0.6
Cellobiohydrolase	2.3	0.7	-0.4	1.8	0.7	-0.1	1.4	0.2	7.5	-0.6
Glucuronidase	6.5	0.9	-0.1	0.3	-0.2	0.2	0.0	-0.3	5.2	-0.4
Xylosidase	0.3	1.7	0.0	1.8	-0.2	-0.1	0.7	0.0	7.5	-0.5
Laccase	1.6	0.1	2.6	0.0	-0.3	-0.2	0.1	0.1	0.5	-0.3
Chitinase	0.3	0.7	-0.3	2.4	-0.1	-0.3	1.2	1.0	6.3	-0.6
Leucine Aminopeptidase	0.1	0.5	-0.1	0.9	2.0	-0.2	3.8	-0.2	0.4	-0.5
Acid Phosphatase	0.5	0.8	0.0	0.2	0.0	0.0	4.8	0.1	4.8	-0.5
Size	6	33	30	21	33	129	8	61	2	207
Nb of species	6	20	18	15	14	44	6	37	2	51

Fig. 2 Clustering of ECMf species based on the similarities in their potential enzymatic activity profiles. Clustering of ECMf species based on their enzymatic activities was done by comparing similarities in their functional traits (eight enzymatic activities). Data coming from both sites (Humont, Champenoux), all sampling dates (3 for Humont, 15 for Champenoux), all treatments (four for Humont: Beech limed, Beech Untreated, Spruce limed, Spruce Untreated, two for Champenoux: Horizon A_1 and Horizon A_2) and all replicates (Humont: n=4) were pooled altogether. Then a minimal spanning tree ("Minimal spanning tree") was computed from a clustering of the species–species Euclidean distance matrix. The tree was then split into ten clusters according to the *k*-means method following algorithm of Hartigan and Wong ("K-means clustering"). The center-reduced average enzymatic activities (centroids) of each cluster are presented in the table below the tree. The whiter the cell is, the higher the average potential activity in the cluster (lowest activities are in black, highest ones in white). The size of each cluster and the number of species present in each cluster are reported on the 2 bottom rows. The size of a cluster is the number of enzymatic profiles, an enzymatic profile being measurement of eight enzyme activities for 1 species at a given date (Humont: 3; Champenoux: 15) in a given replicate (Humont: 4) of a given treatement (Humont: 4; Champenoux: 2)

Table 3 Frequency and occurrence of each ECMf species in the ten clusters defined by *k*-means method from the minimal spanning tree presented in Fig. 3

Species	Cluster										Frequency	Occurrence
	1	2	3	4	5	6	7	8	9	10		
Xerocomus pruinatus		2		2	11	6	1	1	1	5	8	29
Lactarius quietus	1	1	2	4	9	5		2		3	8	27
Xerocomus chrysenteron	1		2	2	2	2				3	7	15
Cenococcum geophilum		1	2		1	15		3		28	6	50
Russula ochroleuca	1	4	3			11		2		9	6	30
Russula cyanoxantha		1	3	1		5			1	3	6	14
Lactarius subdulcis			2	1	1	2		3		2	6	11
Russula delica	1	1	2			1		2		2	6	9
Tomentella sublilacina	1		6			9		4		25	5	45
Clavulina cristata		2		1		10		2		17	5	32
Cortinarius sp.		3	1			3	1			4	5	12
Tomentella lateritia					1	1	1	1		7	5	11
UECM sp.3		2				3	1	2		3	5	11
Cortinarius casimiri		1	2		1			1		3	5	8
Lactarius chrysoreus			1	1	1	3				2	5	8
Byssocorticium atrovirens				1		2		2		12	4	17
Lactarius rostratus		3				3		1		7	4	14
Cortinarius anomalus			3			1	1			3	4	8
Russula nigricans		1				4		2		1	4	8
Russula atropurpurea					1	1		2		3	4	7
Leccinum carpini					1	2		2		1	4	6
Cortinarius olivaceofuscus		1	1					1		2	4	5
UECM sp.5		1		1		1				2	4	5
UECM sp.1		3				6				11	3	20
Laccaria amethystina						1		3		8	3	12
Amanita rubescens						2		3		3	3	8
Tomentella badia			1			2				5	3	8
Tuber borchii				1		3		2			3	6
Tomentella lilacinogrisea					1	4				1	3	6
Leccinum crocipodium				2		2		1			3	5
Russula sp.				1				1		2	3	4
UECM sp.2		1				2				1	3	4
Tomentella sp.3									3	4	2	7
Cortinarius roux						2				2	2	4
Tomentella atramentaria						1				2	2	3
Russula sp.2									2	1	2	3
Piloderma croceum						1				2	2	3
UECM sp.20						1				2	2	3
Hebeloma sacchariolens					1					1	2	2
Hygrophorus olivaceoalbus								1		1	2	2
Laccaria laccata			1					1			2	2
Entoloma nitidum								1		1	2	2
Thelephora terrestris					1			1			2	2
Tomentella ellisii				1						1	2	2
Tomentella fuscoresinosa					1					1	2	2
Tomentella sp.2			1					1			2	2
Boletale sp.1		1						1			2	2
Boletale sp.2		1								1	2	2

Table 3 (continued)

Species	Cluster										Frequency	Occurrence
	1	2	3	4	5	6	7	8	9	10		
Rhizopogon sp						1				1	2	2
Russula rosea						1		1			2	2
Fagirhiza sp.		2									1	2
Cortinarius sp.2						2					1	2
Tomentella botryoides										2	1	2
Lactarius sp.								2			1	2
UECM sp.14										2	1	2
UECM sp.8						2					1	2
Peziza depressa						1					1	1
Tuber sp.				1							1	1
Inocybe petiginosa								1			1	1
Laccaria sp.			1								1	1
Tomentella badialateritia										1	1	1
Tomentella bryophila										1	1	1
Tomentella stuposa						1					1	1
Tomentellopsis echinospora										1	1	1
Boletales p.3								1			1	1
Boletus aestivalis										1	1	1
Russula laurocerasi	1										1	1
Russula xerampelina						1					1	1
Lactarius sp.2								1			1	1
UECM sp.10										1	1	1
UECM sp.12						1					1	1
UECM sp.18								1			1	1
UECM sp.22		1									1	1
UECM sp.4				1							1	1
UECM sp.6						1					1	1
UECM sp.7						1					1	1

Data coming from both sites (Humont, Champenoux), all sampling dates (3 for Humont, 15 for Champenoux), all treatments (4 for Humont: Beech limed, beech Untreated, Spruce limed, Spruce Untreated, 2 for Champenoux: Horizon A1 and Horizon A2), and all replicates (Humont: $n=4$) were pooled altogether

The number of occurrences of each species in each of the ten clusters is reported. Occurrences ≥5 are highlighted in *bold*

The frequency column is the number of the clusters where this species is present

Species names highlighted in *bold* are the species chosen for the Fig. 3

species based on the similarities of their activity profiles throughout different conditions was shown according to taxonomy.

Four dendrograms comparing activity profiles of the most characteristic species were built: *C. geophilum* and *T. sublilacina* were the two common and abundant species in both sites, *X. pruinatus* was one of the most abundant and the most active species in Humont, and *L. quietus* was the most abundant and active in Champenoux. The dendrograms showed that the activity profiles obtained under different conditions were mixed together, without any site or treatment structuring (Fig. 3).

4 Discussion

In forest ecosystems, ECMf species respond differently to environmental factors (e.g., drought stress, tree decline, fire). Response diversity has been defined in Ecology as the diversity of responses to environmental change among species contributing to the same ecosystem function (Elmqvist et al. 2003). It has also been proposed that the diversity of response to disturbance is critical to ecosystem resilience (Chapin et al. 1997; Norberg et al. 2001). In this context, such ecological concepts can be applied to ECMf communities to explore

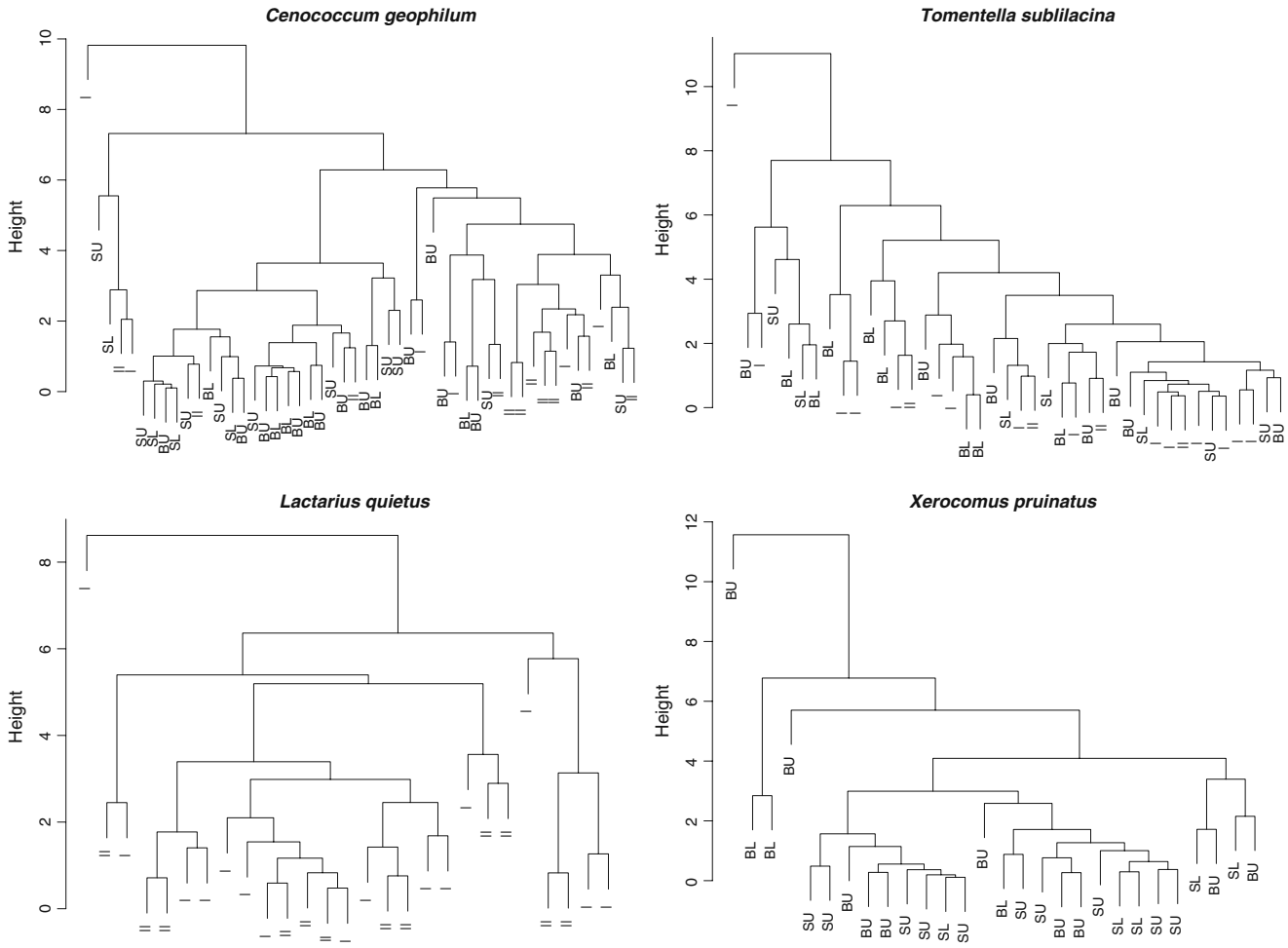


Fig. 3 Dendrogram comparing enzymatic profiles of the most characteristic species (*C. geophilum* and *T. sublilacina* for their abundance in both sites, *X. pruinatus* for its high enzymatic activities and high frequency in Humont and *Lactarius quietus* for its high enzymatic activities and its high frequency in Champenoux). Legends: | horizon 1; || horizon 2 (Champenoux); *B* Beech; *S* Spruce; *L* limed; *U* untreated (Humont)

how their functional diversity is important to maintain the stability and the functioning of forest ecosystems in changing conditions.

4.1 Taxonomic diversity, major, and minor species

The high TD of the two ECMf communities described here is similar to that from previous studies (e.g., Tedersoo et al. 2010) and is changing with seasons, soil horizons, and local soil conditions. In both sites, three ECMf species were significantly more abundant than others: *L. quietus*, *T. sublilacina*, and *C. geophilum*, in the site of Champenoux (Courty et al. 2008); *X. pruinatus*, *C. geophilum*, and *T. sublilacina* in the site of Humont (Rineau and Garbaye 2009a). However, these most abundant ECMf species were not mainly contributing to the activity of the whole community at each date of sampling; no relation between the abundance of an ECMf species and its activities was found. This temporal heterogeneity of the ECMf community, due to many interspecific interactions (Legendre and Fortin 1989; Pickles et al. 2010) can increase the probability of having ECMf species mobilizing nutrients of different types at different times, and can also limit competition with other organisms. So, ECMf species are in turn considered either as major species (high impact on a function) or minor species (low impact on a function; Walker et al. 1999).

4.2 Redundancy and response diversity of functional groups

In our study, a functional group was considered as a collection of species that perform similar ecological roles (i.e., high activity for the eight enzymes) rather than on phylogenetic relationships (Diaz and Cabido 2001; Lavorel et al. 1997; Naeem and Wright 2003). Here, after clustering ECMf activity profiles, ten functional groups were distinguished. Activity profiles of one ECMf species can be

distributed in many groups (i.e., eight for *L. quietus*). So, ECMf species within a functional group can belong to several functional groups or shift between groups, which depicts a high functional plasticity at the species level. This high functional plasticity could be an explanation for their widespread distribution (i.e., *C. geophilum*, *T. sublilacina*, Tedersoo et al. 2010) in different ECM communities and also a trait of their capability of adaptation to disruption or disturbances.

In our study, we have shown that TD was strongly correlated to functional diversity (FD) and that many ECMf species with similar ecological effects co-occured in the same ECMf community. This implies that a high diversity of ECMf species were functionally redundant for their capability to mobilize nutrients from organic compounds (Lawton and Brown 1993; Walker et al. 1999; Bolger 2001). This is supported by the study of Nygren et al. (2007) in which these authors have addressed intra- and interspecific functional differences or similarity of ECMf species in extracellular protease activity. This functional redundancy provides ecological insurance against changes of soil conditions and host parameters (Diaz and Cabido 2001; Loreau et al. 2001; Elmqvist et al. 2003) to keep efficient mobilization and transfer of nutrients from ECMf to the plant. This is also in agreement with a previous study done by Courty et al. (2007) in the same site of Champenoux. These authors have shown that some enzymatic activities of *L. quietus* were highly correlated to host parameters as the bud break index. However, the co-occurrence of species in an ECMf community with similar ecological effects does not necessarily imply that these species are functionally redundant (Lawton and Brown 1993). Redundancy is also a way to buffer processes and to maintain the reliability of the forest ecosystem rather than a superfluous repetition (Walker et al. 1999; Chapin et al. 2000). Anyway, we have measured eight functional traits only related to the mobilization of nutrients from organic matter. Thus, this experimental bias could explain the functional redundancy observed. That is why, even if the ECMf species studied here may have overlapping functions, they may each contribute differently to the mobilization process by a complementary effect (Naeem et al. 1999). We believe that facilitation is occurring because the different ECMf species may contribute differently to mobilize different resources by secreting substrate-specific enzymes, not detectable in the microplate assay used here. Moreover, at a species level, functional diversity is also determined by the fungal and the host genetic diversity (Courty et al. 2011).

Acknowledgments The authors thank the European Science foundation for supporting the exploratory workshop "Diversity and Function in Ectomycorrhizal communities" (Nancy (F), December 2009). Francois Rineau acknowledges the FUNDIV project (ANR-06-BDIV-06) for fundings and scientific discussions for the Humont sampling site. Pierre-Emmanuel Courty gratefully acknowledges the Swiss National Science Foundation. We thank Odile Thiéry for language corrections and helpful comments on the manuscript.

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