Into the functional ecology of ectomycorrhizal communities: environmental filtering of enzymatic

activities

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Running title: Functional ecology of ectomycorrhizal fungi

Type of article: Standard paper (plant-soil interactions)

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Abstract

1. Characterizing the ecological processes driving the assembly and functional composition of ectomycorrhizal (ECM) fungal communities is an area of active research.

2. Here, we applied a trait-based framework to address whether and how environmental filtering and niche differentiation influence the diversity of ECM fungal enzymatic activities in two oak-dominated forest ecosystems. We hypothesized that fungal enzymatic activities and ECM community assembly are influenced by the availability of targeted resources in the surrounding soil.

3. We pinpointed a major influence of environmental filtering on ECM fungal taxonomic and functional composition. Contrasted taxonomic composition among forest ecosystems was linked to broad interspecific trait variation and entailed contrasted functional responses at community-level.

However, intraspecific trait variation contributed to community response within ecosystems. We characterized a strong relationship between enzymatic activities and targeted resource availability in surrounding soil, suggesting a functional role of the enzymes for the acquisition of these resources. Conversely, we did not find a significant influence of niche differentiation in ECM community assembly.

4. *Synthesis*. Heterogeneous distribution of soil resources drives a community-level functional response and determines the functional and taxonomic mosaic of ECM communities in forest ecosystems.

Key Words: ectomycorrhizal fungi (ECM) / functional diversity / community assembly / RLQ analysis / environmental filtering / niche differentiation.

Introduction

Ectomycorrhizal (ECM) symbioses between fungi and plant roots are essential to forest ecosystem functioning (van der Heijden *et al.* 2015). Previous work has shown that abiotic and biotic factors drive variations in the taxonomic composition of ECM fungal communities (Koide *et al.* 2005; Genney *et al.* 2006; Pickles *et al.* 2010; Tedersoo *et al.* 2012; Anderson *et al.* 2014). In addition, rarer studies have addressed the signature of assembly processes on the functional composition of ECM communities (Koide *et al.* 2014), and in particular, the relative influence of small- and large-scale environmental heterogeneities on ECM fungal community assembly (Crowther *et al.* 2013; Talbot *et al.* 2014; Bahram *et al.* 2013, 2015). Previous works showed that enzymatic activities of ECM fungi are linked to resource availability at both specific and community scales (Burke *et al.* 2011; Talbot *et al.* 2013; Walker *et al.* 2014). Recent studies further evidenced a variation of enzymatic activities between taxa reflecting their ability to exploit different substrates (Baldrian *et al.* 2012; Phillips *et al.* 2014). However, the way community assembly processes affect variation in functional composition

within and among ECM fungal communities, over a hierarchy of environmental constraints, remains to be addressed.

Distinct signatures of community assembly processes can be detected from patterns of functional composition (Jung *et al.* 2010). Environmental filtering is a process whereby ecological constraints yield functional variation *among* communities along a gradient (Leps *et al.* 2011) and/or limits the range and functional variation of traits *within* communities (functional convergence; Grime 2006). By contrast, niche differentiation is a process whereby the coexistence of species with similar trait values is prevented, thus leading to increased trait divergence (Kraft *et al.* 2008) and/or increased evenness (Stubbs & Wilson 2004) within communities. Biotic interactions such as competition (Violle *et al.* 2011) and facilitation (Valiente-Banuet & Verdu 2007) can yield niche differentiation.

Resource acquisition is a major component of plant (Westoby 1998) and fungal (McGuire *et al.* 2010) ecology. Unlike litter-decomposing fungi, ECM fungi mostly depend on their plant hosts for carbon (C) supply. However, most ECM fungal lineages produce some extracellular and cell wall-bound hydrolytic and oxidative enzymes that degrade carbon (C-), nitrogen (N)- and phosphorus (P)- containing compounds in soil organic matter (SOM; Courty *et al.* 2010; Rineau *et al.* 2012). Enzymatic activities targeting distinct resources form a set of functional traits (Cullings & Courty 2010; Rineau & Courty 2011) that are directly involved in nutrient cycling within ecosystems (Courty *et al.* 2005; Pritsch *et al.* 2011). Enzymatic activities can be measured on excised ECM root tips, and this approach has revealed that co-existing ECM operational taxonomic units (OTU) vary widely in their ability to mobilize SOM nutrients (Courty *et al.* 2010). These variations are related to temporal dynamics of resource acquisition (Courty *et al.* 2010), to competitive interactions for resource acquisition (Koide *et al.* 2005), and to a range of abiotic and biotic variables (Courty *et al.* 2010; Jones *et al.* 2010; Walker *et al.* 2014). Although these results support the role of niche-based processes in the assembly of ECM fungal communities, how the availability of soil resources influences the functional diversity of ECM fungal communities is still little addressed.

Here, we test whether environmental filtering and niche differentiation constrain the distribution of enzymatic activities among and within ECM fungal communities (see Table 1 for the definitions of the key concepts and objectives of the present work). We investigated the functional composition of ECM fungal communities in two sites representing distinct oak-dominated forest ecosystems with highly diverse and patchily distributed ECM fungal communities (Richard *et al.* 2005; Courty *et al.* 2008). We characterized the enzymatic activities and taxonomy of ECM root tips making up the ECM fungal community found in a sampled soil core. We also analysed the physico-chemical characteristics of each soil core volume. We designed a hierarchical sampling scheme to examine the nature of functional variation between ECM soil core communities within and among the two sites (Fig. 1). First, we analysed the signature of environmental filtering yielding a variation in enzymatic activities among ECM soil core communities, and identified the contribution of interspecific and intraspecific trait variation to such functional variation. Second, we characterized the influence of functional convergence and niche differentiation on the distribution of ECM enzymatic activities within communities, by comparing it to appropriate null models (e.g., Kraft *et al.* 2008). Then, we addressed whether changing availability of alternative sources of C-, N- and P- in surrounding soil organic matter influenced ECM enzymatic activities in communities, using a multi-table ordination method (i.e. RLQ analysis; Doledec *et al.* 1996). Our basic hypothesis was that the distinct forest ecosystems and the variation in available resources across soil cores constitute environmental filters driving the variation of ECM enzymatic activities *among* communities, while local functional convergence and competitive interactions for shared resources should shape the distribution of these activities *within* communities (Fig. 1a). In addition, we addressed the enzymatic activities of other organisms in soil apart from the ECM root tips. We expected similar responses between soil and ECM activities (i) if the taxa present on root tips mostly contributed to enzymatic activities in surrounding soil, or (ii) if environmental filtering for resource acquisition applied to all the co-occurring organisms in a similar way. Alternatively, we expected niche differentiation to yield distinctive patterns of enzymatic expression among co-existing organisms.

Material and Methods

Study system

The research was performed in two 60-year-old oak forests located in North-east (Champenoux, 48°75′ N, 6°35′ E) and South (Puechabon, 43°44′ N, 3°35′ E) France (see Appendix S1 in Supporting Information). Champenoux is a temperate site where two deciduous oak species (*Quercus petraea* Liebl. and *Quercus robur* L.) co-dominate on luvic cambisols. Puechabon is a Mediterranean site where a single sclerophyllous oak (*Q. ilex* L.) grows on karstic soils. These sites thereby represent contrasted abiotic and biotic contexts for ECM community dynamics.

Hierarchical sampling scheme

Sampling was performed in September 2008 at Champenoux and November 2008 at Puechabon. The sampling periods correspond to acorn fall (a major phenological trait) and to the fruiting peak of ECM fungi in these forests. At each site, five 25×25 m plots of same age and same forest management history were selected at least 200 m apart for soil sampling. Four sampling locations were selected 5 m from the centre of each plot in the four cardinal directions (Fig. 1a). At each location, we sampled a soil core of 2 cm in diameter and 5 cm deep (15.7 cm^3). The 20 cores per site (4 cardinal points x 5 plots) were stored separately at 4°C and processed within five days after sampling (Pritsch *et al.* 2011).

Analysis of ECM root tips

Enzymatic activities of ECM root tips

Oak roots were soaked in tap water for 15 min before being gently washed. Root tips were observed in water under a stereomicroscope (x40) and the total number of ECM root tips was recorded in each soil core sample. ECM fungal communities made of more than two ECM root tips were detected from 34 out of the 40 soil cores. Each ECM root tip was collected and analysed using high-

throughput microplate assays as in Pritsch *et al.* (2011). Eight enzymatic activities (referred here as functional traits; See Appendix S2) were measured successively on each tip and considered as proxies of the ability of ECM fungi to exploit soil resources: phosphorus (acid phosphatase, PHO), chitin (N-acetylhexosaminidase, NAG), soil proteins (leucine aminopeptidase, LEU), various carbon hydrates including cellulose, hemicellulose and cellobiose (cellobiohydrolase, CEL; β-xylosidase, XYL; β-glucosidase, GLU; β-glucuronidase, GLR), and phenolic substrates (LAC). Enzymatic activities were expressed per unit time and per unit area to take into account tip size (projected area of the ECM root tip, in $pmol.mm^{-2}.min^{-1}$) as described in Pritsch *et al.* (2011), and were log-transformed to obtain an approximately Gaussian distribution.

Identification of ECM fungi

After measurement of enzymatic activities, each ECM root tip was kept individually at -20°C. Subsequently, fungal symbionts were identified by sequencing the ITS region of fungal ribosomal DNA. DNA was extracted using the REDExtract-N-Amp Plant PCR Kit (XNAP, Sigma, France) and the ITS was amplified as in Courty *et al.* (2008). Fungal ITS sequences were edited with Sequencher 4.9, corrected and identified using the BLAST algorithm and the UNITE database (http://unite.ut.ee/). Sequences with more than 97% similarity were ascribed to the same operational taxonomic unit (OTU), named after the nearest sequence recovered by BLAST. After identification, we averaged in each soil core community the eight enzymatic activities of all root tips belonging to the same taxon. We then analysed data on enzymatic activities per OTU and per soil core (Fig. 1a).

Analysis of the soil surrounding root tips

Soil enzymatic activity

Enzymes are also produced by the organisms living in the soil apart from ECM root tips, including saprotrophic fungal mycelia, bacteria, and ECM extramatrical mycelia. We hypothesized that this set of organisms contributes to soil resource recycling, and thus should likewise respond and interact

depending on resource availability. To test this hypothesis, we characterized and compared the enzymatic activities of ECM root tips and of the organisms in the surrounding soil. We therefore measured the previous eight enzymatic activities in the soil volume surrounding root tips for comparison with enzymatic activities of ECM root tips (see Appendix S3). They were also log-transformed to obtain an approximately Gaussian distribution.

Soil resources and physical context

Basic soil characteristics (i.e., pH, P and N contents) were measured at plot level (Fig. 1a). Furthermore, we applied the ^{13}C CPMAS NMR spectroscopy procedure (see Appendix S4) to measure the concentration of resources related to enzyme activities in each core (Fig. 1a): (i) polysaccharides, including cellulose and hemicellulose (O.Alk.C), (ii) lignin and recalcitrant C compounds (Methoxyl.C, Arom.C, Phen.C and Carbox.C), (iii) alkyl-compounds of lipids, waxes and cutins (Alk.C). A soil subsample was used to assess the concentration of glucosamine as a proxy of chitin concentration. Soil water content at the sampling date of each soil core was also measured based on soil weight difference before and after drying in an oven at 105°C for 12 h.

Statistical analyses

Functional variation of ECM fungal communities between and within sites

We measured the mean enzymatic activity of each ECM soil core community as $\overline{t_j} = \dfrac{1}{N} \sum_{i=1}^{N} t_{ij}$, where t_{ij} was the value of enzymatic activity *j* for OTU *i*, and *N* was the number of ECM fungal OTUs in the soil core community. $\overline{t_j}$ was therefore an index of the community-level functional response of fungi, which could vary across communities because of changing taxonomic composition and of intraspecific trait variation within taxa (Fig. 2). We performed the ANOVA of $\overline{t_j}$ between the two sites following the method of Leps *et al.* (2011), which allows separating the intraspecific (including both genotypic and phenotypic components) and interspecific sources of functional variation among

sites. A first ANOVA was performed on $\overline{t}_j$ values to represent the overall change of functional

composition between communities of the two sites ("Total" component). A second ANOVA was

performed on $\overline{t}_j^{fix}$ values calculated from the mean enzymatic activity t_s of each OTU s over all

cores, i.e., $\overline{t}_j^{fix} = \sum_{s=!}^{S} n_{sj} t_s$, where n_{sj} is the relative frequency of OTU s, and t_s is its mean enzymatic

value of OTU s. $\overline{t}_j^{fix}$ could only vary between communities of the two sites if the species composition

changed (see Fig. 2c), and the second ANOVA thus represented the contribution of interspecific trait

variation to changing functional composition between communities of the two sites ("Interspecific

component"). Finally, a third ANOVA was performed on $\overline{t}_j - \overline{t}_j^{fix}$ to represent departure from $\overline{t}_j^{fix}$

due to intraspecific variation ("Intraspecific component"). If significant, this ANOVA showed the

contribution of intraspecific trait variation to changing community composition. Fig. 2 illustrates

basic cases with different patterns of these three statistics between communities. In addition, we

included in the ANOVAs a nested effect of the variation of communities between plots within each

site, to further assess the functional variation of the assemblages within each forest ecosystem.

A subsequent objective was to assess the functional response of soil organisms to environmental

variation, apart from the response of root tips expressed in $\overline{t}_j$. We therefore performed the nested

ANOVA of soil enzymatic activities as for root tip activities. We then analysed the paired correlations

of $\overline{t}_j$ and of the corresponding enzymatic activity in soil, to characterize consistent or divergent

trends in ECM and soil functional responses among cores.

Functional variation within ECM soil core communities

We then assessed the functional structure of ECM communities using two indexes of functional

divergence $FDiv_j = \dfrac{1}{N} \sum_{i=1}^{N} \left(t_{ij} - \overline{t}_j \right)^2$, and functional evenness $FE_j = \overline{NND_j} \Big/ \sigma_{NND_j}$, where $\overline{NND_j}$

was the mean nearest neighbour distances (NND_j) of co-occurring OTUs for enzymatic activity j, and σ_{NND_j} was the corresponding standard deviation. *FDiv* is a primary component of functional diversity (Mason *et al.* 2005), which is expected to be smaller than random in communities when some environmental constraint limits the range of traits around some optimal value (functional convergence). Conversely, niche differentiation yields a more even distribution of traits than random when OTUs with more similar trait values are less likely to co-exist (Stubbs & Wilson 2004; Mason *et al.* 2005), which results in higher values of FE_j. We analysed $FDiv_j$ and FE_j for each enzymatic activity. We compared observed values of $FDiv_j$ and FE_j to those of a null model of community assembly (Fig. 1a, bottom), in order to detect the signatures of environmental filtering and niche differentiation within communities. These null models were designed to acknowledge the hierarchical structure of environmental variation in the sampling scheme (see Appendix S5). The *p*-values derived from this analysis were adjusted to take into account multiple testing (False Discovery Rate method, Pike 2011). All statistical analyses were carried out using the R software (R Development Core Team 2014).

Environmental drivers of functional community composition

We performed a RLQ analysis (Doledec *et al.* 1996; *rlq* function in R package ade4) to investigate the relationship between ECM enzymatic activities and resource concentrations in surrounding soil (Fig. 1b). The relationship was analysed through the presence-absence of ECM fungal OTUs per soil core community. The three-table approach thus integrated taxonomic, functional and environmental variation to identify synthetic components of environmental filtering among communities. We assessed the statistical significance of the link between traits and soil resources using the procedure *randtest.rlq* based on 999 permutations (Monte Carlo procedure; Dray & Legendre 2008). If the test was significant, the axes of the RLQ analysis represented functional syndromes of enzymatic activities depending on the availability of soil resources.

Results

The average number of ECM root tips per soil core was significantly higher at Champenoux than at Puechabon (20.5 *vs.* 6.2; See Table S1). The 532 ECM root tips successfully sequenced were ascribed to 104 unique OTUs (49 at Champenoux and 58 at Puechabon, 3 being present in both sites; see Tables S1 and S2), and we found 212 occurrences of the OTUs in the 34 selected soil cores (Fig. 1b). The richness of OTUs (rarefied to a standardized sample size of 121 root tips) was 1.8-fold lower at Champenoux while the average number of OTUs per soil core was 2.6-fold higher than at Puechabon (see Table S1). Three OTUs present at both sites represented 2.9% of all ECM fungal root tips (*Cenococcum geophilum*, *Tomentella atramentaria* and *T. badia*), but were not in all soil cores. *C. geophilum* was the most widespread species at both sites (present in 42% of the soil cores, i.e., 11 cores at Champenoux and 5 at Puechabon). Thelephoraceae and Russulaceae dominated communities of both sites (66 and 50% of total OTU number at Champenoux and Puechabon, respectively).

Functional variation of ECM fungal communities among and within sites

The ANOVA analysis showed that mean community enzymatic activities $\overline{t_j}$ per soil core were significantly higher at Champenoux, except for CEL (Table 2a, "Total" column). In any significant case, the variation was related to changing taxonomic composition (P < 0.01, "Interspecific component"), and the intraspecific component of the variation was not significant (P > 0.05, "Intraspecific component"), except for NAG (P = 0.02). However, we specifically analysed enzymatic activities of *C. geophilum*, the most widespread species at both sites, and found a significant decrease of enzymatic activities of *C. geophilum* between Champenoux and Puechabon, except for GLR, CEL and LAC (Fig. 3). This tendency was consistent with the whole community variation expressed by $\overline{t_j}$.

We found significant functional variation among plots within sites, for four enzymatic activities (XYL, CEL, GLU, NAG; $P < 0.05$ by ANOVA; Table 2a). Equivalent amount of variation was accounted among sites and among plots within sites (30.73% with 17% s.d. and 29.53% with 14% sd, respectively). Intraspecific trait variation contributed significantly to the total variation of these four activities ($P < 0.01$), while interspecific variation also contributed to the total variation for NAG and CEL ($P < 0.05$ but $P > 0.01$; Table 2a). We further performed separate analyses of the among-plot variation at Champenoux and Puechabon (Table S3) and found that the functional variation was only significant among plots at Champenoux.

Relationship between ECM and soil enzymatic activities

Soil enzymatic activities differed between soil cores of Puechabon and Champenoux, except for GLR ($P < 0.05$ by ANOVA; Table 2b). Remarkably though, three soil enzymatic activities (XYL, PHO, LEU) were significantly higher at Champenoux and four were significantly higher at Puechabon (CEL, GLU, NAG and LAC). Some soil activities (NAG, GLU) were negatively correlated with soil water content (Table S4). The activity of three soil enzymes (GLU, NAG and LAC; Table S4) positively correlated with SOM content. More variation was found among sites than among plots within sites (44.28% with 29.6% s.d. and 10.2% with 17.6% s.d., respectively).

Overall, we found significant correlation between ECM and soil enzymatic activities (Table 3 and Fig. 1b) for all the activities except CEL, but the correlations were negative for GLU, NAG and LAC. Therefore, there was no overall congruence of ECM and soil enzymatic activities.

Functional variation within ECM fungal communities

Functional divergence (*FDiv*) was calculated for each soil core community and for each enzymatic activity. Based on the null model performed separately at Puechabon and Champenoux (see Appendix S5), we found *FDiv* to be significantly smaller (adjusted $P \leq 0.05$) than random in 8.8% of the combinations [soil core x enzymatic activity]. Environmental filtering constrained the variation of

at least one of the eight enzymatic activities in 29.4% of the soil cores, and more often at Champenoux than at Puechabon (40% vs. 14.3%). This environmental filtering primarily concerned the exploitation of carbohydrates and chitin (CEL, GLR, LEU, NAG and XYL for which more than 9% of soil cores displayed environmental filtering). Separable signatures of environmental filtering are observable on Fig. 4 at Puechabon (filled circles) and Champenoux (empty circles). Even though enzymatic activities were lower overall at Puechabon (Fig. 4, x-coordinate), environmental filtering was less pronounced there than in communities featuring comparable mean enzymatic activities at Champenoux (Fig. 4, y-coordinate). This result is consistent with the ANOVA analysis that showed no significant variation among plots at Puechabon, while there was significant variation at Champenoux (see Table S3). When the ECM OTUs were randomized at plot level, only 1.29% of the [soil core x trait] combinations yielded FE significantly higher (adjusted $P \le 0.05$) than random. Therefore, we found less influence of niche differentiation than of environmental filtering on the functional composition of ECM communities.

Relationship between soil resources and ECM enzymatic activities

Champenoux soil was significantly richer in lignin and recalcitrant C compounds (Methoxyl.C, Arom.C, Phen.C and Carbox.C), and in alkyl-compounds (Alk.C; $P < 0.001$; Mann-Whitney test), while Puechabon soil was significantly richer in chitin ($P < 0.001$; Mann-Whitney test). Concentration of polysaccharides (O.Alk.C) did not differ between sites. The RLQ analysis evidenced an overall significant relationship between soil resources and ECM fungal enzymatic activities (randomisation test, $P < 0.001$). The first and the second RLQ axes respectively accounted for 90.95% and 7.53% of the overall relationship and thereby represented most of the overall variation. Each axis represented how syndromes of traits co-varied along an environmental gradient. Based on community scores, the first axis depicted the taxonomic, environmental and functional contrast among Puechabon and Champenoux communities, while the second axis represented remaining variation among communities within sites (Fig. 5a). The first axis was also related to the contrasted physical soil

characteristics between sites (pH and SOM Spearman ρ = -0.85 and -0.79, respectively, $P < 0.001$). In addition, both axes depicted a gradient of phosphorus in soil cores (Spearman ρ = 0.76 and 0.59, respectively, $P < 0.001$). The eight ECM enzymatic traits were positively related with axis 1 over a gradient of soil water content (Fig. 5b, abscissa). The driest plots (Mediterranean context, Puechabon) were the richest in chitin (Chitin = -0.77) and in alkyl-compounds (Alk.C = -0.70, Fig. 5b, orange arrows) compared to the moister soils, which were conversely enriched in recalcitrant C pools (Methoxyl.C = +0.84; Phen.C = +0.86). On axis 2 (Fig. 5b, ordinates), the ability to hydrolyse proteins (LEU = +0.29) and chitin (NAG = +0.14) was inversely related to the ability to hydrolyse polysaccharides (CEL = -0.39). The former strategy was favoured in soil cores rich in recalcitrant C compounds (Carbox.C = +0.14; Arom.C = +0.33), while the latter was favoured in soil cores more humid and richer in chitin, polysaccharides and alkyl-compounds. It is noteworthy that on both axes 1 and 2, the ability to hydrolyse chitin by ECM fungi (NAG) was negatively related to the soil chitin reservoir. Contrastingly, polyphenol concentration was positively correlated with laccase activity (LAC).

Discussion

We observed evidence of functional and taxonomic variation among and within communities in response to environmental heterogeneity in temperate and Mediterranean ecosystems. Our study provides two main insights into how and why enzymatic activities vary between and within ECM fungal communities. First, functional variation among the two sites was related to a broad change in taxonomic composition, while the functional variation among plots in the Champenoux site was mostly related to intraspecific variation in enzymatic activities. Second, the functional variation in enzymatic activities depended on resource concentrations in surrounding soil, supporting the hypothesis that the functional response of ECM communities relates to resource availability (Fig. 5).

Environmental constraints primarily drive functional variation within and between ECM communities

The two sites (Champenoux and Puechabon) represented contrasted forest ecosystems in terms of biotic and abiotic environment, so that variations in ECM enzymatic activities were expected in response to the variable environment. We also expected that local-scale assembly processes influenced the distribution of enzymatic activity within soil core communities (Fig. 1a, bottom). Significant differences in community-level enzymatic activities among and within sites (Table 2a) indeed showed that ECM fungal communities were structured by environmental heterogeneity at both spatial scales. We note here that the functional contrast between Puechabon and Champenoux was related to strong taxonomic change, as very few species were shared between Champenoux and Puechabon (Fig. 3). Such large difference in taxonomic composition was expected in contrasted bioclimatic and pedoclimatic contexts, because different host species generally shelter distinct ECM fungal communities (Morris *et al.* 2009). Different taxonomic composition still does not necessarily imply changing functional composition (e.g., Fig. 2a). Here the result means that different species identity is related to different functional properties, which suggests that fungi with different ecological niches are found at Champenoux and Puechabon. Conversely, variable functional properties of populations within species (intraspecific component) contributed more to the functional changes among plots at Champenoux. Therefore, we found a hierarchy of ecological processes where functional variation among ECM fungal communities was related to different taxonomic composition among contrasted forest ecosystems, and mostly to intraspecific variation at a more local scale (Table 2a, Table S3). Intraspecific functional variation has been recently recognized to be a key component of community dynamics (Bolnick *et al.* 2011), which has motivated much research in plant ecology (Albert *al.* 2011), but the contribution of individual variation in ECM symbioses remains neglected (Johnson *et al.* 2012): to our best knowledge, this is the first evidence of how the nested effects of inter- and intraspecific variation influence fungal community ecology. Although the approach does not distinguish genotypic and phenotypic

variations within OTUs, it basically shows that the functional composition of ECM root tips communities is influenced by variable environment.

A third level of the ecological hierarchy concerns functional trait variation *within* ECM fungal communities. We detected functional convergence for all the enzymatic activities, thereby revealing a constraint on the variation of enzymatic activities within communities. However, functional convergence was less frequent at Puechabon than at Champenoux. The Mediterranean climatic context of Puechabon, with typical summer drought, is expected to favour more stress-tolerant strategies than the temperate context of Champenoux. It may result in an overall environmental filtering of Puechabon communities, with functional convergence operating not only in local communities but also at a regional Mediterranean scale. Conversely, a release of the regional constraint can allow a variation of environmental filtering across communities depending on soil quality, as found at Champenoux (Fig. 4). In addition, we found a far weaker signal of niche differentiation based on the analysis of functional evenness. Our results thereby suggest that environmental filtering primarily influences variations of enzymatic activities both among and within ECM fungal communities.

Environmental drivers of ECM ecological strategies

Whenever significant functional variation was found among and within ECM communities regarding $\overline{t_j}$, $FDiv_j$ and/or FE_j, a subsequent issue was to identify the factors underlying such variation. We hypothesized that such community-level variation was primarily related to the availability of resources in surrounding soil. We then identified the environmental drivers underlying the functional variation of ECM fungal communities by performing a RLQ analysis relating functional trait variation to environmental variation through the changing community taxonomic composition (Thuiller *et al.* 2006; Raevel *et al.* 2012). We related the marked differences in functional and taxonomic composition between sites (Table 2a) to contrasted abiotic properties (soil water content, pH and phosphorus content) and soil resources (RLQ axis 1 on Fig. 4). The finding of a functional

response of ECM fungal communities to resource availability is congruent with previous studies. Sinsabaugh et al. (2009) and Schneider et al. (2012) indeed showed that the litter nutrient content and the stoichiometry of C:N:P affect activities of enzymes involved in SOM nutrient acquisition (GLU, NAG, LEU and PHO). We also found (Table S4) a negative correlation between soil water content and enzymatic activity regarding chitin (NAG) and cellulose (GLU but not XYL and CEL as in Brockett et al. 2012). The activity of three soil enzymes (GLU, CEL and NAG; Table S4) positively correlates with SOM content (Sinsabaugh et al. 2008), as in our study. Our study reveals a contrast between Champenoux and Puechabon regarding both phosphorus availability and PHO activity. Large spatial variations in PHO activities are indeed reported in temperate forest soils (Jones et al. 2010). Previous studies have also identified abiotic factors such as soil pH as primary environmental variables driving soil microbial activities (e.g. Kivlin & Treseder 2014); here the high XYL and PHO at Champenoux and the low GLU and NAG activities at Puechabon can be indirectly related to the differences in pH (4.6 versus 7.4 at Champenoux and Puechabon, respectively). The functional variation between the two sites was therefore likely to be an ecological response to a large-scale environmental contrast in soil water content, and in the nature and content of SOM. We further found that ECM fungal community assembly was functionally constrained within sites according to soil resources, especially at Champenoux (see Table S3a and RLQ axis 2 on Fig. 5). The spread of Champenoux communities on RLQ axis 2 reflected a trade-off between the ability to hydrolyse proteins and chitin on one hand, and the ability to oxidize phenols on the other hand, in response to variable SOM resources in soil. We found that intraspecific trait variation mostly contributed to the change of functional composition among communities at Champenoux (Table S3a), which underlines that functional plasticity can play a stronger role than taxonomic variation in the functional response of ECM communities at a local scale. Indeed the idea that functional and taxonomic diversity are not synonymous in microbial communities is now gaining momentum (e.g. Yuste et al. 2014).

Relationship between ECM enzymatic activities in root tips and soil enzymatic activities

Previous experiments suggested that ECM fungi and other fungi as saprobes are main actors of the SOM degradation in the ectomycorrhizosphere (Uroz *et al.* 2013). Enzymatic activities in the ectomycorrhizosphere in soil cores encompass all microbes potentially exploiting common resources. We found that environmental heterogeneity influences differently variations of ECM enzymatic activities in root tips and of soil enzymatic activities (Table 2a vs. 2b). Furthermore, although the correlation between ECM and soil enzymatic activities was sometimes positive and supported partial congruence as shown by Phillips *et al.* (2014), the significant negative relationships for GLU, NAG and LAC (Table 3 and see Fig. S1) suggest that ECM fungi only partly contribute to soil enzymatic activities. Other organisms (or even the extramatrical ECM mycelium) can therefore respond differently. In this regard, the negative correlations we found between ECM root tip and soil activities can reflect niche differentiation between organisms (or even between separate parts of ECM individuals). While niche differentiation was not found at the level of root tip ECM fungal communities, it could occur at a broader ecosystem level. Talbot *et al.* (2013) also reported that enzyme activity of ECM root tips and of bulk soil from the same soil cores did not correlate, suggesting that the ECM extramatrical mycelium contributes in a complementary way to nutrient cycling. Our results thus open perspectives towards enlarging the analysis of community assembly beyond the role of ECM fungi, by addressing the relative enzymatic activities of the other coexisting organisms and ECM fungus components (Talbot *et al*. 2014). However, we also note that using occurrences of OTUs in soil cores could not allow identifying their relative importance in surrounding soil. Further investigation of their relative volumes in soil would thus be needed to investigate the issue in greater details. Such methodological refinements will help better identify the functions of ECM fungi in a comprehensive functional landscape of fungi in soils (Anderson *et al.* 2014).

Conclusion

Our study provides evidence of the central role played by abiotic factors, particularly SOM composition, in the functional ecology of ECM communities. Among-community variation and local functional convergence in enzymatic activities were found within and among sites. We suggest that the assembly of ECM fungi on roots is constrained by soil resources, and that there are trade-offs in enzymatic activities both within and among OTUs, depending on variations in resource availability. Moreover, while we suggest that niche differentiation is not the primary mechanism of ECM community assembly, our results support functional differentiation of ECM root tips with other organisms in the surrounding soil. Finally, these results aggregate the fungal kingdom into the current and promising attempts to revisit community ecology using a functional, trait-based approach (McGill *et al.*, 2006). This opens novel perspectives for understanding the dynamics of coexistence within ECM fungal and soil microbial communities.

Data Accessibility

All data is present in the paper and its supporting information.

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Supporting information

Figure S1. Relationship between mean enzymatic activities of ECM communities (abscissa) and enzymatic activities of the surrounding soil, for each of the eight targeted activities as in Figure 3.

Table S1. Number of root tips and of OTUs in ECM communities at Champenoux (Ch) and Puechabon (Pu).

Table S2. Abundance (number of ECM root tips) of the 104 ECM operational taxonomic units (OTUs) found at Champenoux (Ch) and Puechabon (Pu) sites.

Table S3. Variation of the functional composition of ECM fungal communities among plots within each site.

Table S4.: Spearman correlation (p-values corrected for false discovery rate, with *p.adjust*) between soil characteristics and soil enzyme activities

Appendix S1. Study system

Appendix S2. Measurements of functional traits of ECM apices

Appendix S3. Measurement of soil enzymatic activity

Appendix S4. Soil nutrients analysis

Appendix S5. Null models of community assembly

Table 1. Glossary

Ectomycorrhizal root tip	Symbiotic structure linking soil filamentous fungi to short roots of most trees, including oaks, and involving diverse Basidiomycota and Ascomycota (Courty *et al.,* 2010; van der Heijden *et al.*, 2015). Their morpho-anatomy is variable, especially in the extension of the hyphal network surrounding root tips (= extramatrical mycelium; Agerer, 2001).
Enzymatic activity	As other fungi, ectomycorrhizal (ECM) fungi release extracellular enzymes to break down macromolecules—for example nucleic acids, phenols, and chitin. Activity of several enzymes can be directly measured on ECM root tips using standardized assays (Pritsch *et al.*, 2011).
Functional trait	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).
Environmental filtering	Represents the fact that only species that are ecologically adapted to the environment can be found in a given site, while other species cannot (Keddy, 1992; Lortie *et al.*, 2004). It thus predicts a reduction in the range of functional traits related to this adaptation (Grime, 2006).
Niche differentiation	Posits that species with identical or very similar niches cannot coexist due to biotic interactions. It thus predicts an underrepresentation of species with similar niches and similar functional traits in the community (Abrams 1983; McArthur and Levins 1967).
OTU: Operational **T**axonomic **U**nit	A proxy for delineating fungal species obtained using DNA-based methods applied on ectomycorrhizal root tips. By convention, fungal OTUs are defined for most ECM lineages according to ITS sequence dissimilarity at 3% cut-off value (Courty *et al.*, 2010).
RLQ analysis	This statistical analysis is based on separate multivariate analyses of three tables (Doledec *et al.*, 1996), where the rows of table R (here the SOM compounds) are related to rows of table L (the community composition), and columns of table L are related to rows of table Q (the enzymatic expressions). The objective of the RLQ analysis is to relate tables Q and R through their indirect link in L. It represents in the present study the functional response of enzymatic expressions to variable SOM composition. The analysis provides several axes that are independent and sorted in decreasing order of importance.

Table 2: Variation of the functional composition of ECM fungal communities (a) and of the organisms found in surrounding soil (b). Nested ANOVA analyses were performed to represent the variation of average enzymatic activities in communities between the Champenoux (Ch) and Puechabon (Pu) sites, as well as between plots within sites (nested predictor). The method of Leps *et al.* (2011) was used in (a) to assess the relative contribution of interspecific and intraspecific trait variation to the functional changes among communities (see Figure 2). Table (a) thereby includes p-values of these contributions and of the total variation between communities. The difference of enzymatic activities between sites is also provided (contrast Pu-Ch). Table (b) includes the p-values of the variation of enzymatic activities in soil between sites as well as between plots within sites. The difference of enzymatic activities between sites is also provided. Abbreviations: β-xylosidase (XYL), β-glucuronidase (GLR), cellobiohydrolase (CEL), β-glucosidase (GLU), N-acetylhexosaminidase (NAG), acid phosphatase (PHO), leucine aminopeptidase (LEU) and laccase (LAC). Enzymatic activities are expressed in pmol.min^{-1} per gram of soil, and in pmol.min^{-1} and per mm^2 of fungal sheath of ECM root tips. They are log-transformed and averaged in communities.

(a) Change in community-level ECM enzymatic activities between Puechabon and Champenoux, and

among plots within sites

Enzymatic activities	Variation between sites				Variation between plots within sites		
	Interspecific component	Intraspecific component	Total	Contrast Pu-Ch	Interspecific component	Intraspecific component	Total
XYL	**0.007**	0.26	**0.021**	-0.36	0.15	**<0.001**	**0.013**
GLR	**<0.001**	0.68	**<0.001**	-0.47	0.74	0.93	0.82
CEL	0.04	0.92	0.10	-0.80	**0.03**	**0.008**	**0.007**
GLU	**<0.001**	0.10	**<0.001**	-1.12	0.09	**<0.001**	**0.009**
NAG	**<0.001**	**0.02**	**<0.001**	-1.71	**0.01**	**<0.001**	**<0.001**
PHO	**0.001**	0.20	**0.002**	-1.03	0.10	0.05	0.09
LEU	**0.001**	0.15	**0.001**	-0.32	0.92	0.03	0.39
LAC	**<0.001**	0.64	**<0.001**	-1.03	0.20	0.13	0.26

(b) Change in soil enzymatic activities between Puechabon and Champenoux, and among plots within sites

Enzymatic activities	Variation between sites		Variation between plots within sites
	p-value	Contrast	p-value
XYL	**<0.001**	-1.28	0.32
GLR	0.31	-0.14	0.52
CEL	**0.01**	0.03	0.25
GLU	**<0.001**	0.77	0.30
NAG	**<0.001**	1.09	0.85
PHO	**0.01**	-0.42	0.14
LEU	**<0.001**	-2.38	**0.01**
LAC	**<0.001**	0.04	**0.003**

Table 3: Correlation between soil enzymatic activities and average ECM activities in soil cores. The Spearman ρ coefficient and corresponding p-value are provided for each activity.

	XYL	GLR	CEL	GLU	NAG	PHO	LEU	LAC
ρ	0.47	0.37	0.09	-0.35	-0.65	0.38	0.45	-0.33
p-value	**0.005**	**0.03**	0.60	**0.04**	**<0.001**	**0.03**	**0.007**	0.052

Figure legends

Figure 1. (**a**) Diagrammatic representation of the sampling design and of the related multiscale methodology. Colours indicate the four hierarchical sampling levels (site, plot, core, root tip).

(**b**) Schematic presentation of the RLQ analysis used to address the link between OTU traits (Q table) and local environment (R table) through the presence-absence of OTUs in local communities. Multivariate analysis of each table is performed prior to RLQ analysis (PCA = Principal Component Analysis, CA = Correspondence Analysis).

* Operational Taxonomic Units (OTUs) identified by a BLAST analysis of ITS sequences.

** Soil resources are concentrations of four categories of recalcitrant phenolic compounds (methoxyl C (Methoxyl.C), aromatic C (Arom.C), phenolic C (Phen.C) and carboxyl-C (Carbox.C)), CH_3-groups of lipids, waxes and cutins (alkyl C: Alk.C), polysaccharides including cellulose and hemicellulose (O-alkyl C: O.Alk.C), chitin (glucosamine: Chitin) and soil water content.

*** The enzymatic activities measured on ECM root tips (Myc) are: β-xylosidase (XYL), β-glucuronidase (GLR), cellobiohydrolase (CEL), β-glucosidase (GLU), N-acetylhexosaminidase (NAG), acid phosphatase (PHO), leucine aminopeptidase (LEU) and laccase (LAC).

Figure 2. Analysis of the variation of functional composition among communities, using the method of Leps *et al.* (2011). The panels exemplify different cases with functional and/or taxonomic changes among two communities, and the consequences on $\bar{t}_j$ and $\bar{t}_j^{fix}$ statistics. Different symbols represent distinct species, symbol size represents the trait value of an individual observed in a community. The colour represents the extent of intraspecific trait variation: black colour means that the observed individual value is greater than the mean species value, dark grey that it is equal to the mean, and light grey that it is smaller than the mean. Mean values vary across species (3 size groups each including 2 species). In (a), both taxonomic and functional compositions are different (different and smaller symbols in left community). Functional change is not due to different mean species

values (as $\bar{t}_1^{fix} = \bar{t}_2^{fix}$) but to intraspecific variation (different $\bar{t}_j - \bar{t}_j^{fix}$). In (b), functional and taxonomic compositions are also different, but the intraspecific component is the same, i.e., in both case we have the same colour composition in communities ($\bar{t}_1 - \bar{t}_1^{fix} = \bar{t}_2 - \bar{t}_2^{fix}$). Therefore only the variation of mean species values (interspecific variation) contributes to functional change in this case ($\bar{t}_1^{fix} \neq \bar{t}_2^{fix}$). In (c), the taxonomic composition is the same (same symbols), but the functional composition differs because of intraspecific variation (different $\bar{t}_j - \bar{t}_j^{fix}$). In (d), the two communities have the same functional composition (same symbol sizes), but changing taxonomic composition with interspecific variation of traits (different symbols and $\bar{t}_1^{fix} \neq \bar{t}_2^{fix}$). However, intraspecific variation (different $\bar{t}_j - \bar{t}_j^{fix}$) maintains identical functional composition despite the changing mean species values.

Figure 3. Variation in enzymatic activities of the most common fungus, *Cenococcum geophilum*, between Champenoux (Ch) and Puechabon (Pu) sites, with p-values (P) of the unpaired Wilcoxon test. The enzymatic activities are β-xylosidase (XYL), β-glucuronidase (GLR), cellobiohydrolase (CEL), β-glucosidase (GLU), N-acetylhexosaminidase (NAG), acid phosphatase (PHO), leucineaminopeptidase (LEU) and laccase (LAC).

Figure 4. Analysis of environmental filtering within ECM communities, based on the community-level variance (*FDiv*) of ECM enzymatic activities, at the Champenoux (red empty dots) and Puechabon (red filled dots) sites. Each dot represents an ECM fungal community (in a soil core). Mean trait values on the abscissa are standardized according to the mean and the standard deviation of each of the eight enzymatic activities, while the *FDiv* statistics on ordinates are standardized according to the 5% quantile of the null model and the mean enzymatic activity. Points under zero on ordinates are therefore lower than the 5% quantile.

Figure 5. Scores of (**a**) soil cores and (**b**) ECM enzymatic activities (continuous lines) and soil resources (dotted lines) on the two first axes of the RLQ analysis relating ECM enzymatic to soil resources. Enzymatic activities are β-xylosidase (XYL), β-glucuronidase (GLR), cellobiohydrolase (CEL), β-glucosidase (GLU), N-acetylhexosaminidase (NAG), acid phosphatase (PHO), leucineaminopeptidase (LEU) and laccase (LAC). Soil resources are concentrations of four categories of recalcitrant phenolic compounds (Methoxyl.C = methoxyl C, Arom.C = aromatic C, Phen.C = phenolic C and Carbox.C = carboxyl-C), CH_3-groups of lipids, waxes and cutins (Alk.C = alkyl C), polysaccharides including cellulose and hemicellulose (O.Alk.C = O-alkyl C), chitin (Chitin = glucosamine) and soil water content.
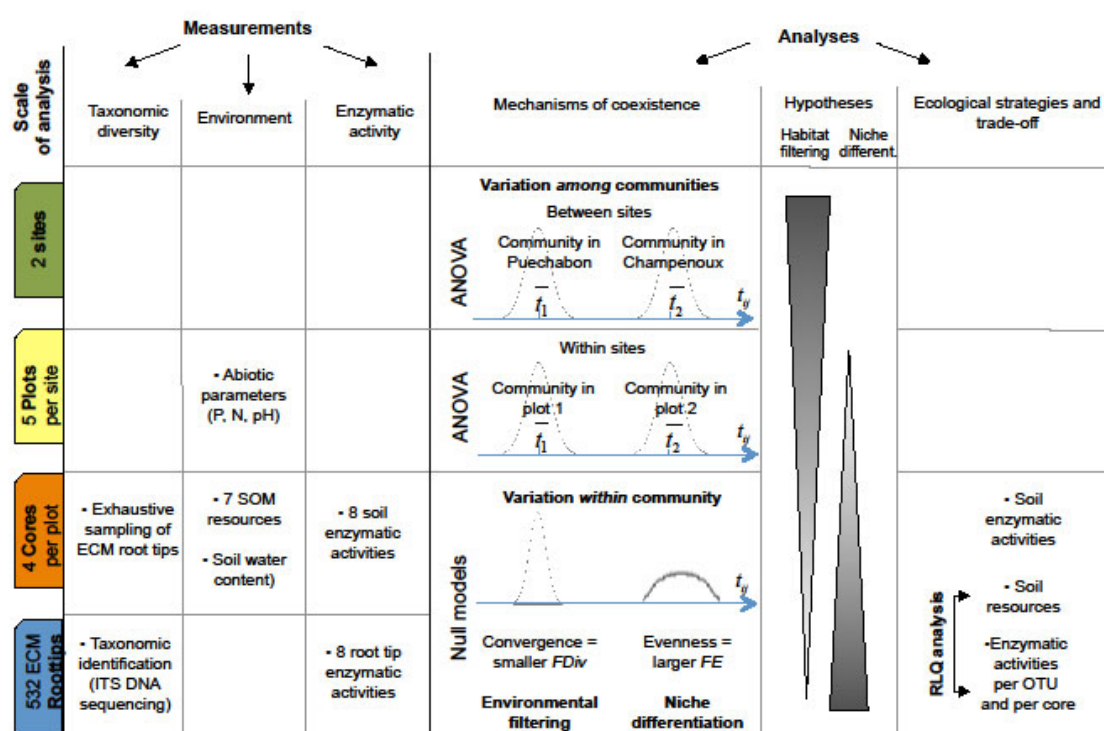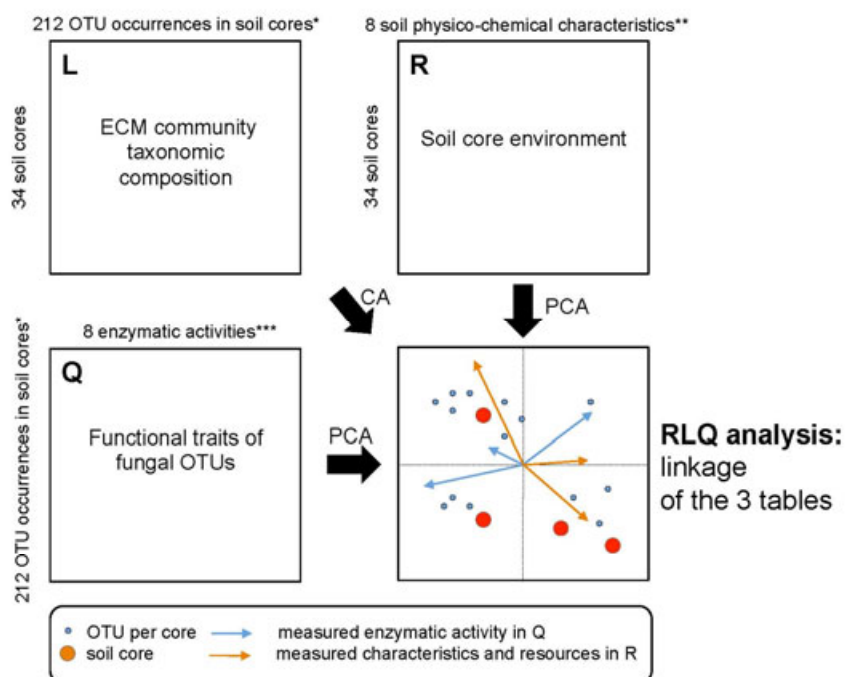

Figure 1a

Figure 1b

Figure 2

Fungal individuals with different trait values (varying symbol size)
and belonging to distinct species (varying symbol) differing in
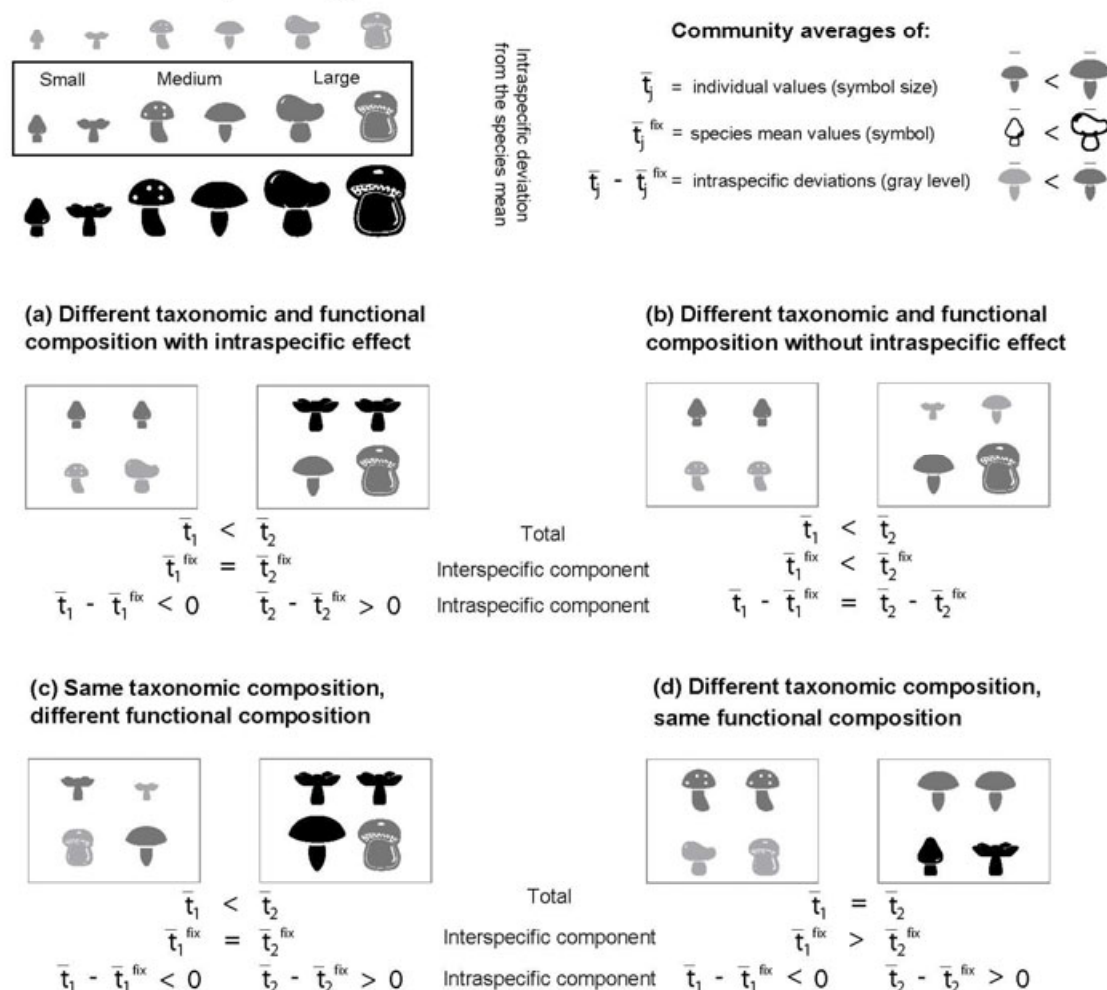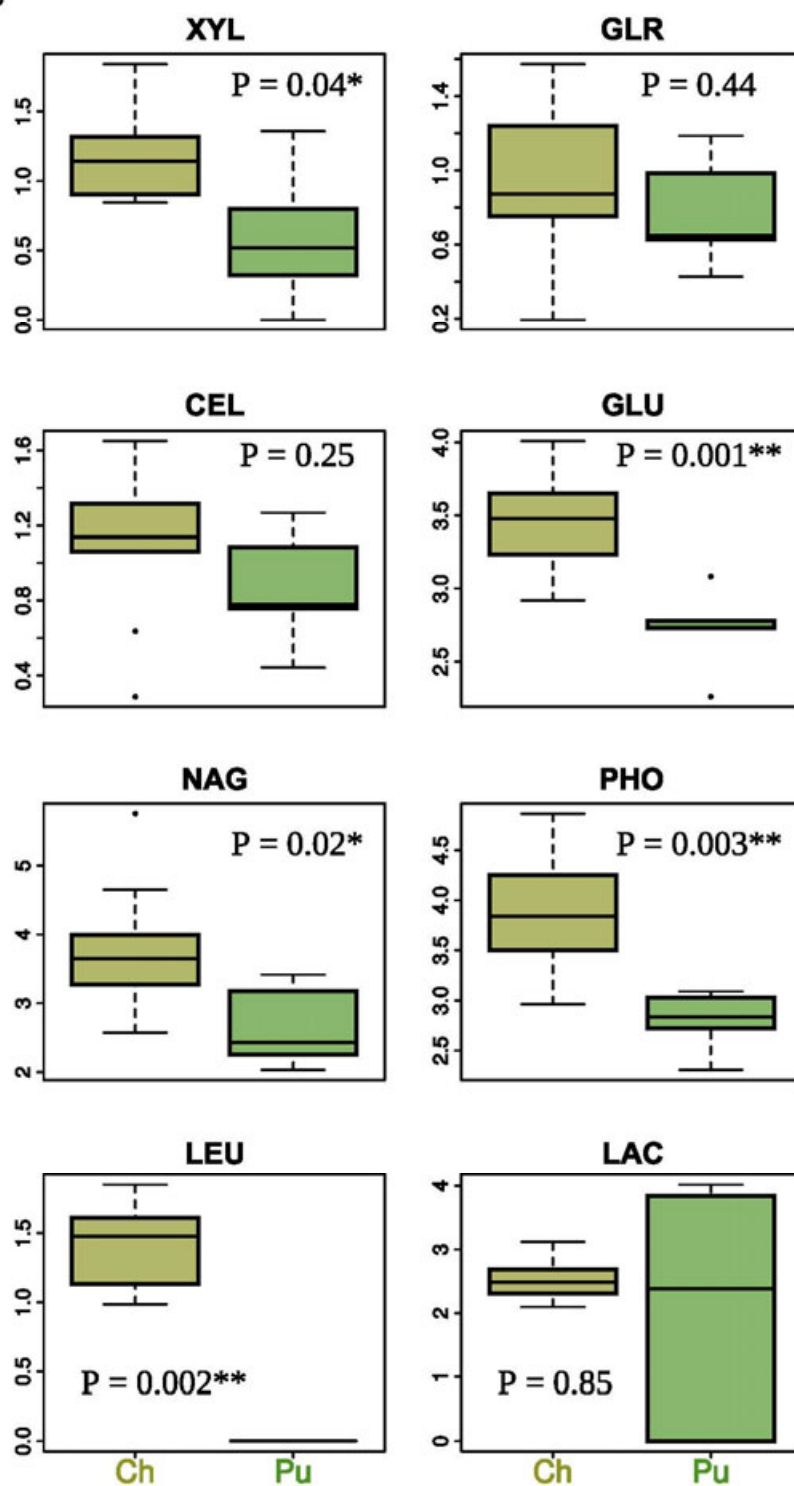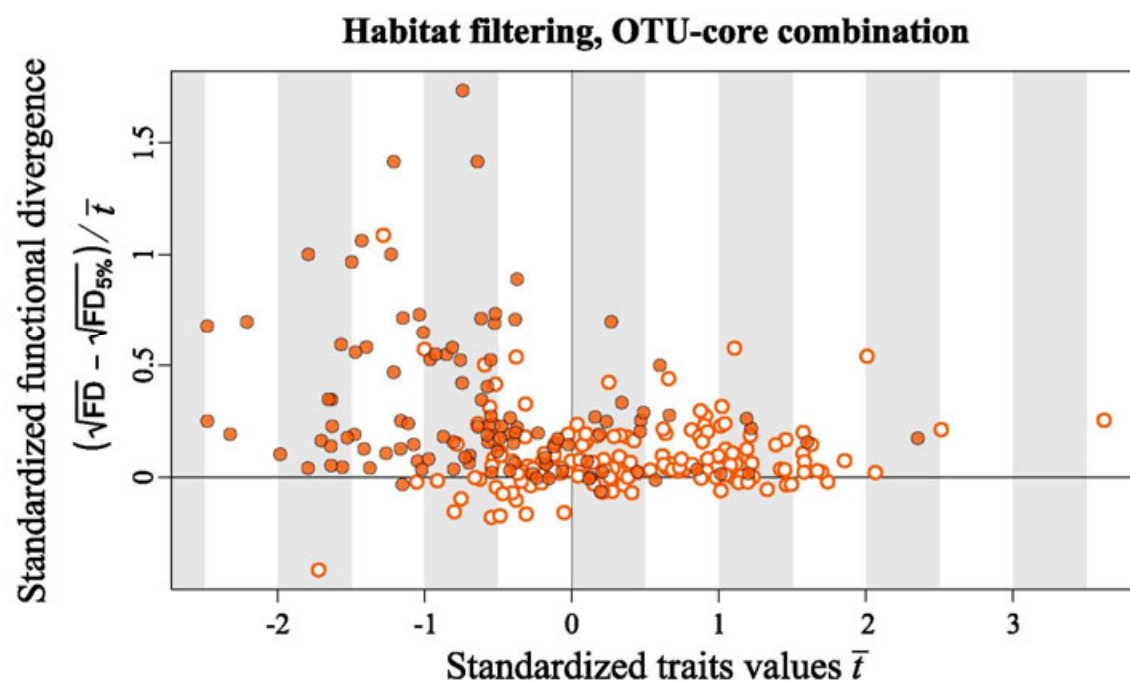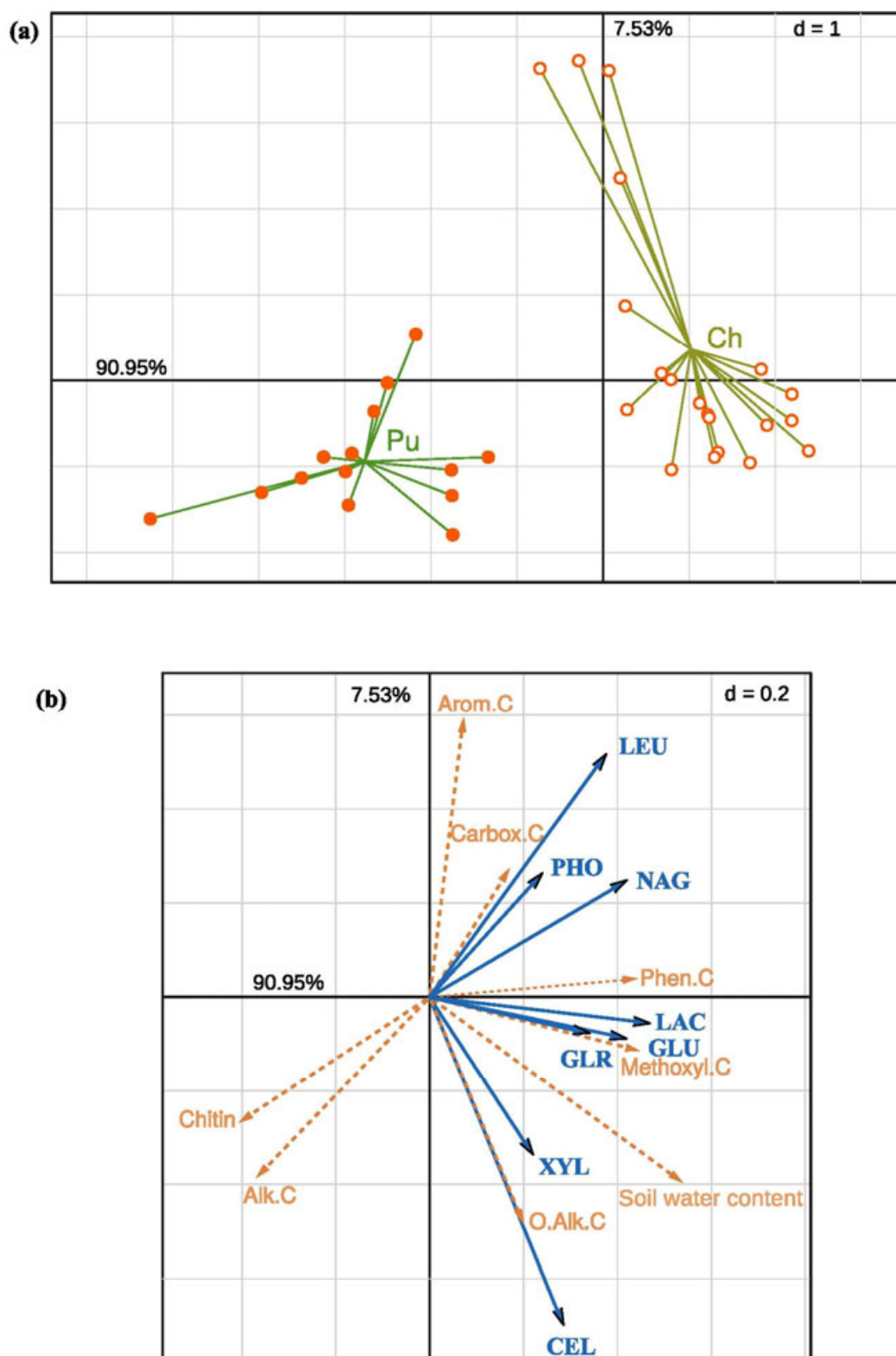terms of mean trait values (within rectangle)

Intraspecific deviation
from the species mean

Community averages of:

$\bar{t}_j$ = individual values (symbol size)

$\bar{t}_j^{fix}$ = species mean values (symbol)

$\bar{t}_j - \bar{t}_j^{fix}$ = intraspecific deviations (gray level)

**(a) Different taxonomic and functional
composition with intraspecific effect**

$$\bar{t}_1 < \bar{t}_2$$
$$\bar{t}_1^{fix} = \bar{t}_2^{fix}$$
$$\bar{t}_1 - \bar{t}_1^{fix} < 0 \qquad \bar{t}_2 - \bar{t}_2^{fix} > 0$$

**(b) Different taxonomic and functional
composition without intraspecific effect**

Total

Interspecific component

Intraspecific component

$$\bar{t}_1 < \bar{t}_2$$
$$\bar{t}_1^{fix} < \bar{t}_2^{fix}$$
$$\bar{t}_1 - \bar{t}_1^{fix} = \bar{t}_2 - \bar{t}_2^{fix}$$

**(c) Same taxonomic composition,
different functional composition**

$$\bar{t}_1 < \bar{t}_2$$
$$\bar{t}_1^{fix} = \bar{t}_2^{fix}$$
$$\bar{t}_1 - \bar{t}_1^{fix} < 0 \qquad \bar{t}_2 - \bar{t}_2^{fix} > 0$$

**(d) Different taxonomic composition,
same functional composition**

Total

Interspecific component

Intraspecific component

$$\bar{t}_1 = \bar{t}_2$$
$$\bar{t}_1^{fix} > \bar{t}_2^{fix}$$
$$\bar{t}_1 - \bar{t}_1^{fix} < 0 \qquad \bar{t}_2 - \bar{t}_2^{fix} > 0$$

Figure 3

Figure 4

Habitat filtering, OTU-core combination

Figure 5