Comparative genomics and transcriptomics depict ericoid mycorrhizal fungi as versatile saprotrophs and plant mutualists

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Summary

- Some soil fungi in the Leotiomycetes form ericoid mycorrhizal (ERM) symbioses with Ericaceae. In the harsh habitats where they occur, ERM plants survival relies on nutrient mobilisation from soil organic matter (SOM) by their fungal partners. Characterization of the fungal genetic machinery underpinning both symbiotic lifestyle and SOM degradation is needed to understand ERM symbiosis functioning and evolution, and its impact on soil C turnover.
- We sequenced the genomes of the ERM fungi *Meliniomyces bicolor, M. variabilis, Oidiodendron maius* and *Rhizoscyphus ericae* and compared their gene repertoires to those of fungi with different lifestyles (ecto- and orchid mycorrhiza, endophytes, saprotrophs, pathogens). We also identified fungal transcripts induced in symbiosis.
- The ERM fungal gene contents for polysaccharide-degrading enzymes, lipases, proteases, and enzymes involved in secondary metabolism are closer to those of saprotrophs and pathogens than of ectomycorrhizal symbionts. The fungal genes most highly upregulated in symbiosis are those coding for fungal- and plant-cell wall degrading enzymes (CWDE), lipases, proteases, transporters and mycorrhiza-induced small secreted proteins (MiSSPs).
- ERM fungal gene repertoire reveals a capacity for a dual saprotrophic and biotrophic lifestyle. This may reflect an incomplete transition from saprotrophy to the mycorrhizal habit, or a versatile life strategy similar to fungal endophytes.

Key words

Comparative genomics, Ericaceae, ericoid mycorrhizal fungi, Leotiomycetes, transcriptomics

Introduction

Mycorrhizal symbioses have arisen repeatedly during plant evolution and are a key innovation influencing plant diversification (Tedersoo *et al*., 2010; Van der Heijden *et al*., 2015; Martin *et al*., 2016). Ericoid mycorrhiza (ERM) involves several soil fungi and the youngest lineage of a single monophyletic plant family, the Ericaceae. The remaining Ericaceae taxa encompass subfamilies displaying morphologically diverse mycorrhizal associations (Lallemand *et al*., 2016). Together, there are ~4400 recorded species of ericaceous trees and shrubs worldwide, distributed from arctic to temperate and tropical regions (Kron *et al*., 2002). The latest age estimate for the whole Ericaceae family is ~117 My (Schwery *et al*., 2015), whilst diversification of the ERM-forming lineages might date back to 90–75 Mya, during angiosperm radiation in the Late Cretaceous (Nixon & Crepet, 1993; Carpenter *et al.* 2015). The ERM symbiosis is hypothesized to have evolved within that time frame, and to be the most recent of all mycorrhizal types (Brundrett, 2002).

ERM habitats are usually characterised by acidic soils low in nutrients and high in recalcitrant polyphenolic compounds, where decomposition and soil organic matter (SOM) turnover are slow (Cairney & Meharg, 2003). SOM accumulation in these ecosystems is significant, as they hold ~20% of the earth's terrestrial soil carbon stocks (Read *et al*., 2004). In these harsh environments, ERM fungi are instrumental in plant survival (see in Perotto *et al*., 2012) as they contribute to the mobilisation of nutrients from complex organic matter and to transfer them to the host plant (Read & Stribley, 1973). In these habitats, ERM fungi are also key players in soil carbon cycling (Clemmensen *et al*., 2013; Averill *et al*., 2014): up to 50% of carbon assimilated by the host plant can be allocated to ERM fungi (Hobbie & Hobbie, 2008), and ERM fungi contain high levels of recalcitrant carbon compounds (Read *et al*., 2004).

Fungi known to form ERM symbioses include Ascomycetes in the Leotiomycetes and some Basidiomycetes in the Serendipitaceae (Setaro *et al*., 2006; Selosse *et al*. 2007; Weiβ *et al*., 2016). Among the Leotiomycetes, the helotiacean *Rhizoscyphus ericae* (Zhuang & Korf) was the first ERM fungal species to be isolated (Pearson & Read, 1973). It was recently transferred to the *Pezoloma* genus but this transfer is questionable, being based on incidental ecological reports (Baral & Krieglsteiner, 2006) with no strong taxonomical or morphological support. Vrålstad *et al*. (2000) first coined

the term "*R. ericae* aggregate" to accommodate several un-named root-isolated taxa with close taxonomic affinities to *R. ericae*, some of which were later described by Hambleton & Sigler (2005) within the new genus *Meliniomyces*. In particular, *M. variabilis* forms ERM associations with several species of Ericaceae and is endophytic in ectomycorrhizal (ECM) roots of Northern temperate conifers (Grelet *et al*., 2010; Vohník *et al*., 2013). *M. bicolor* can form both ERM with ericaceous species and ECM (morphotype *Piceirhiza bicolorata*) with temperate forest trees (Villarreal-Ruiz *et al*., 2004; Grelet *et al*., 2009). The other well-studied ERM fungus is *Oidiodendron maius* (Barron), a species belonging to Myxotrichaceae, recently moved to the Leotiomycetes (Wang *et al*., 2006) and found to form ERM with several Ericaceae (Read, 1996; Allen *et al*. 2003; Bougoure & Cairney 2005). Like *M. variabilis*, *O. maius* is also commonly isolated from roots of other plants (Bergero *et al*., 2000; Kernaghan & Patriquin, 2011) as well as from peat, soil, and decaying organic matter throughout temperate ecosystems including peatlands, forests, and heathlands (Rice & Currah, 2006).

Both *R. ericae* and *O. maius* were shown to degrade *in vitro* a variety of complex soil organic sources including tannic acid, cellulose, pectin and chitin (Kerley & Read, 1995, 1997; Rice & Currah 2001, 2005; Thormann *et al*., 2002). They secrete a wide range of enzymes involved in the depolymerisation and degradation of plant and fungal cell wall polymers, organic phosphorus forms and complex aliphatic compounds such as polyphenols and tannic acid (see Smith & Read, 2008 for summary). More strikingly, *O. maius* can decompose *Sphagnum* moss, whose cell walls are chemically analogous to wood (Tsuneda *et al*., 2001). Different isolates of *O. maius* caused mass losses of 1.5% to 47% and eroded all *Sphagnum* cell wall components simultaneously, in a manner similar to wood decomposition by white rot fungi (Rice *et al*., 2006). The *O. maius* genome contains genes coding for a rich repertoire of polysaccharide-degrading enzymes (Kohler *et al*., 2015), providing a genetic basis for SOM decomposition. It is unknown if this rich repertoire is shared by other ERM fungi, and how these genes are regulated when ERM fungi grow within host plant cells.

In this study we sequenced the genomes of *R. ericae, M. bicolor* and *M. variabilis*, and compared their gene repertoires with the genomes of *O. maius*, six saprotrophic or pathogenic Leotiomycetes, and 50 other Ascomycetes and Basidiomycetes representing different life strategies. We further compared the transcriptomes of *O. maius, M. bicolor*

and *R. ericae* in free-living mycelia and in symbiotic root tissues to identify symbiosis-related genes, focusing on genes putatively involved in plant cell wall polysaccharide decomposition. Our main questions were the following: (a) Did the ERM habit arise in Leotiomycetes together with appearance of the host plants? (b) Is the rich complement of genes responsible for SOM degradation in *O. maius* a common feature of ascomycetous ERM fungi, and are these genes expressed in symbiosis? (c) Do ERM fungi have a specific genomic 'signature' as for ECM fungi (Kohler *et al*., 2015)?

Materials and Methods

Fungal strains

Isolation and identification of the four ERM isolates are described in Martino *et al*. (2000) for *Oidiodendron maius* (MUT1381/ATCC MYA-4765), Grelet *et al*. (2009) for *Meliniomyces variabilis* (UAMH11265/ICMP18552), and *Meliniomyces bicolor* (UAMH11274/ICMP18549), and Read (1974) for *Rhizoscyphus ericae* (UAMH7375/ICMP18553). *R. ericae* UAMH7375 is the same strain that led to the first formal description of the *R. ericae* species after production of ascomata in culture. We also included for comparison the as yet unpublished genome of the saprotrophic Leotiomycetes *Amorphotheca resinae*. Isolation and description of the sequenced strain ATCC 22711 are described in Edmonds & Cooney (1967).

Genome sequencing, assembly, annotation, and data access

The nuclear genomes of *M. bicolor*, *M. variabilis* and *R. ericae* were sequenced using a combination of Illumina fragment (270 bp insert size) and 4 Kbp long mate-pair (LMP) libraries and assembled using ALLPATHS-LG (Gnerre *et al*., 2011). The genome of *M. bicolor* was then further improved by closing gaps with Pacific Biosciences (PacBio) reads using PBJelly (English *et al*., 2012). The genome of *Amorphotheca resinae* was sequenced using a combination of 454 (Roche) standard and LMP libraries, assembled using Newbler (2.5-internal-10Apr08-1) (Roche) and further improved by closing 328 gaps with gapResolution (Trong *et al*., 2009). Transcriptomes of all four species were sequenced using Illumina, assembled using Rnnotator (Martin *et al*., 2010) and used for genome annotation (see SI – Methods S1 for further details). All four genomes were

annotated using the JGI Annotation pipeline and made available *via* the JGI MycoCosm database (jgi.doe.gov/fungi; Grigoriev *et al*., 2014). The data were also deposited at DDBJ/EMBL/GenBank under the following BioProject/GenBank Accessions: *M. bicolor* E: PRJNA196026/LXPI00000000; *M. variabilis* F: PRJNA200595/LXPR00000000; *R. ericae* UAMH 7357*:* PRJNA263050/LYBP00000000, *A. resinae* ATCC 22711: PRJNA207866/MADK00000000. Genomes from other fungi were downloaded from the JGI MycoCosm database (http:jgi.doe.gov/fungi; Grigoriev *et al*., 2014).

Phylogenetic tree

A phylogenetic tree was constructed with 199 core gene representatives out of 246 single-copy families (Marthey *et al*., 2008). We aligned each corresponding protein sequence with 60 orthologous sequences using CLUSTAL omega, extracted the conserved blocks from each alignment with Gblocks, and concatenated all the blocks in one sequence per species. Bootstrap analysis and tree inference were carried out with the RAxML (Randomized Axelerated Maximum Likelihood) program (Stamatakis, 2006). Ultrametric trees were calculated from the ML tree generated above using the *pathd8* method (Britton *et al*., 2007). The molecular clock was calibrated using the Pezizomycotina node estimated in Kohler *et al*. (2015) at 400 Mya.

Comparative genomic analyses and annotation of functional categories

Our comparative analyses focused on repeated elements, carbohydrate-active enzymes (CAZymes), lipases, proteases, secreted proteins, and additional gene categories involved in secondary metabolism. RepeatScout (Price *et al*., 2005) was used to identify *de novo* repetitive DNA in the genome assembly as reported in Peter *et al*. (2016). CAZymes – glycoside hydrolases (GH), glycosyl transferases (GT), polysaccharide lyases (PL), carbohydrate esterases (CE), redox enzymes that act in conjunction with CAZymes (Auxiliary activities, AA), carbohydrate-binding modules (CBM) and enzymes distantly related to plant expansins (EXPN) – were identified using the CAZy database (www.cazy.org) annotation pipeline (Cantarel *et al*., 2009). To compare the distribution of genes encoding CAZymes in the various genomes, we applied hierarchical clustering of the number of genes for each of the 60 species using the

Genesis software (Sturn *et al*., 2002). The Euclidian distance was used as the distance metric and a complete linkage clustering was performed. Proteases were identified using the MEROPS peptide database (http://merops.sanger.ac.uk) and lipases using the Lipase engineering database (www.led.uni-stuttgart.de). Secreted proteins were identified using a custom pipeline including SignalP v4, WolfPSort, TMHMM, TargetP, and PS-Scan algorithms as reported in Pellegrin *et al*. (2015). Genes and gene clusters involved in secondary metabolism were predicted for the 60 species using a pipeline based on SMURF (Peter *et al*., 2016). Potential transporters were predicted using TransportTP online tool (http://bioinfo3.noble.org/transporter/) (Li *et al*., 2009).

MCL/CAFE analyses

Multigene families were predicted on a subset of 20 genomes using the MCL algorithm (Enright *et al*., 2002) with an inflation parameter set to 3.0. Multigene families were analyzed for evolutionary changes in protein family size using the CAFE program (P<0.001) (De Bie *et al*., 2006). CAFE estimates for each branch in the tree whether a protein family has not changed, expanded or contracted.

RNA-Seq

Mycorrhizal roots of *V. myrtillus* were obtained *in vitro* as described in Kohler *et al*. (2015). Fifteen plates, each containing ten plants, were analyzed for each treatment. RNA extraction and sequencing (RNA-Seq), and identification of mycorrhiza-induced transcripts in *M. bicolor* and *R. ericae* were performed as described for *O. maius* in Kohler *et al*. (2015). The complete data sets have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession numbers GSE63947, GSE107845 and GSE107647 for *O. maius*, *M. bicolor* and *R. ericae* respectlively.

Double hierarchical clustering analysis

Homologs of symbiosis-upregulated genes from *O. maius, R. ericae* and *M. bicolor* in the other Leotiomycete genomes and from *O. maius* in 59 genomes of saprotrophic, mycorrhizal, pathogenic and endophytic fungi were identified and their distribution illustrated by using heatmaps. The predicted protein sequences of symbiosis-

upregulated genes were queried using BLASTP (e-value $1e^{-5}$) against the different sets of gene repertoires to find homologs. Proteins were considered as homologs of symbiosis-regulated transcripts if they showed 70% coverage over the regulated sequence and at least 30% amino acid identity. Heatmaps were produced using double-hierarchical clustering matrices (euclidian distance metric and ward clustering method) of symbiosis-upregulated transcripts homologs in the two different fungal genomes sets. Data were visualized and clustered using R (package HeatPlus) (Ploner, 2015).

Statistical analyses

We performed unconstrained ordination analyses in R of gene counts for all CAZymes, lipases and proteases. We used a non-metric multidimensional scaling (NMDS) approach (function metaMDS in package Vegan, Oksanen *et al.*, 2009; see SI – Methods S1). The non-parametric Mann-Whitney U test with Bonferroni adjustment for multiple testing was used to identify gene families enriched in ERM fungi. The significance of differences between mycorrhizal and non-mycorrhizal plant fresh biomass was statistically evaluated by ANOVA with Tukey's post hoc test (P<0.05).

Results

Phylogeny and genomic features of sequenced ERM fungi

We generated and assembled the draft genomes of *R. ericae, M. bicolor* and *M.variabilis*, and compared them to those of *O. maius* (Kohler *et al*., 2015), six other Leotiomycetes (three soil saprotrophs – *A. resinae, Ascocoryne sarcoides* and *Chalara longipes* – and three plant pathogens – *Blumeria graminis, Botrytis cinerea* and *Sclerotinia sclerotiorum*) and 50 additional taxonomically and ecologically distinct fungi, including other mycorrhizal fungi (ECM and orchid mycorrhiza, ORM), endophytes, soil saprotrophs, white and brown rot fungi, and pathogens (Table S1). A phylogenetic tree constructed using 199 core orthologous single-copy genes highlights the taxonomic relationships of these 60 fungi (Fig. 1). As expected, the four ERM fungi clustered in the Leotiomycetes. The most recent common ancestor (MRCA) of the Leotiomycetes was estimated to have occurred ~148 Mya, while the MRCA of ERM fungi was estimated to have occurred ~118 Mya (Fig. 1).

The genome size (from 46 Mbp for *O. maius* to 82 Mbp for *M. bicolor*) and the number of predicted genes (from 16,703 for *O. maius* to 20,389 for *M. variabilis*) of the four ERM fungi fell within the range of other Ascomycetes (Fig. S1, Table S2). The percentage of repeated elements (RE) ranged from 4.6% for *M. variabilis* to 26.6% for *M. bicolor* (Fig. S1b, Table S3). The four ERM species showed similar numbers of common and clade-specific genes to other Leotiomycetes (Fig. S2). Gene distributions in the KEGG pathways, KOG and Gene Ontology categories (available through the *R. ericae* genome portal at the JGI MycoCosm database) are also very similar, although the *M. variabilis* genome encodes a much larger set of unspecific and salicylate monooxygenases that may be involved in aromatic compound metabolism (Peng *et al*., 2008).

Predicted proteins specific to the Leotiomycetes and to ERM fungi are listed in Table S4a. Leotiomycetes-specific proteins with known domains are enzymes involved in detoxification (e.g. glutathione S-transferase) and regulation of gene expression and development (e.g. methyltransferases, zinc finger transcriptional factors and deoxycytidylate deaminases). Proteins specific to ERM fungi included enzymes involved in resistance to environmental stress, such as isochorismate synthase (Sadeghi *et al*., 2013) and histidine triad domain-containing proteins (Eijkelkamp *et al*., 2016).

Gene families significantly expanded in the four ERM genomes (MCL-CAFE analysis; Mann-Whitney test $P<0.05$) code for proteins involved in self/non-self recognition (heterokaryon incompatibility proteins), nutrient uptake/exchange (Major Facilitator Superfamily and sugar transporters), detoxification of environmental pollutants and/or stress response (cytochrome P450, ankyrin repeats, beta-ketoacyl synthases, and carboxylesterases) (Table S4b).

ERM fungi resemble saprotrophs and pathogens in their repertoire of degrading enzymes

We compared the distribution of genes coding for the degradation of polysaccharides, proteins and lipids (Table S5, S6 and S7; Fig. 2, S3 and S4). Lifestyle had a significant effect on gene content and distribution ($P<0.001$). Gene repertoires fell into two broad groups ($P<0.01$) including (1) ERM fungi, pathogens and soil/litter saprotrophs or (2) ECM, ORM, white and brown rot fungi (Fig. 2). Overall, ERM genomes encode a

higher median number of secreted and total CAZymes than the other fungi, although this difference was consistently significant only between ERM and ECM genomes (Fig. 2). Interestingly, ERM fungi contain nearly twice the median number of lipase genes as any other type of fungi (Fig. 2), mostly coding for secreted carboxylesterases (GGGX) (Table S7). The total number of protease genes was less affected by lifestyle, except for a lower median protease gene count in soil saprotrophs (Fig. 2).

ERM genomes contain the highest number of CAZyme genes encoding GHs and GTs (Fig. 3, Table S5). Although GTs are predicted to be secreted enzymes, they remain in the endoplasmic reticulum (Freeze & Haltiwanger, 2009). There were no specific patterns in the ERM genomes for genes encoding CEs, EXPNs, CBMs and redox enzymes that act in conjunction with CAZymes (AAs) (Fig. 3), whereas genes encoding PLs were mostly absent, except pectin lyases (PL1).

ERM genomes contain a significantly higher number of CAZyme genes than ECM genomes (Table S5). They encode a higher set of genes coding for lignocellulose oxidoreductases, such as laccases (AA1), cellobiose dehydrogenases (AA3), and lytic polysaccharide monooxygenases (LPMOs) involved in the cleavage of chitin (AA11) and cellulose (AA9). Compared to ECM or white/brown rot fungi, iron reductases (AA8) and quinone-dependent oxidoreductases (AA12) acting on cellulose were significantly enriched in ERM fungi (Mann-Whitney U test; Table S5). Seventeen families of secreted CAZymes were significantly enriched in ERM fungi compared with all other fungi (Table S8); they are involved in the degradation of cellulose (GH5_5 and GH5_16), hemicellulose and/or pectin (GH27, GH28, GH53, GH54), but also β-1,3-glucans (GH55, GH72, GH132) and mannans (GH76) (Table S8).

Secretion of secondary metabolites is important for fungal survival in competitive environments and in fungal-plant interactions (Calvo & Cary, 2015). The number of polyketide synthase (PKS) and PKS-like genes was strikingly higher in the *O. maius* genome than in any other sequenced fungi (Fig. 4). The two *Meliniomyces* species were among the top 20 fungi for the number of secondary metabolite coding genes, whereas *R. ericae* featured a lower gene number (Fig. 4).

Melanin allows fungi to tolerate environmental stress and makes their biomass recalcitrant to degradation (Fernandez *et al.,* 2016). The analyses of five genes linked to melanin biosynthesis (SI-Methods S1) showed that two of them (*arp1* - scytalone

dehydratase and *abr1* - brown 1) are significantly enriched in ERM fungi compared to fungi with different ecological strategies (Table S9). Three more genes (*alb1* - polyketide synthase; *arp2* - 1,3,6,8-tetrahydroxynaphthalene (THN) reductase; *ayg1*- yellowish-green) are significantly more enriched in ERM than in ECM, white and brown rot fungi (Table S9). Compared to the other fungal groups (Table S5), ERM fungi have a significantly higher number of AA1, multicopper oxidases (MCOs). MCOs are involved in melanin biosynthesis (Hoegger *et al*., 2006), but also in lignin degradation (Leonowicz *et al*., 2001).

ERM fungi display different substrate preference

Although ERM fungi are characterized by a rich set of CAZymes (Fig. S5), they are discriminated by their secreted CAZymes repertoire (Fig. 5). In particular, *O. maius* clustered with a general soil saprotroph and a pathogenic Ascomycete, neither belonging to the Leotiomycetes, whereas members of the *R. ericae* aggregate (*M. variabilis*, *M. bicolor* and *R. ericae*) clustered with two saprotrophic Leotiomycetes, *C. longipes* and *A. sarcoides* (Fig. 5). These differential repertoires of secreted CAZymes suggest that ERM fungi preferentially decompose different carbon compounds. For example, compared to the *R ericae* aggregate, *O. maius* contained a higher set of CAZymes degrading mainly hemicelluloses and pectins (e.g. GH2, GH27, GH79) (Table S5) and cellulose-binding domains as CBM1 and CBM6 are more represented in *O. maius.* On the other hand *O. maius* contained less CAZymes degrading chitin, which are all present in the *R. ericae* aggregate species (e.g. CE4, AA7 and the chitin-binding domains CBM18 and CBM50) (Table S5). In addition, *Meliniomyces* genomes contained a larger set of genes coding for secreted lignocellulose degradating enzymes (e.g., laccases – AA1, cellobiose dehydrogenases – AA3 – and galactose oxidase – AA5) than *O. maius* and *R. ericae* (Table S5).

Gene expression profiles during ERM symbiosis

To investigate expression of fungal genes during symbiosis, *V. myrtillus* seedlings were inoculated with the four ERM fungi. Typical fungal coils were observed in the *V. myrtillus* root epidermal cells inoculated with *O. maius, M. bicolor* and *R. ericae,* but not with *M. variabilis*. Mycorrhizal plants also showed an increased biomass. The

effects of mycorrhizal inoculation on growth and root phenotype are shown for *O. maius* only (Fig. 6).

Transcriptome analysis showed that 995 (~ 6%) *O. maius* genes, 545 (~ 3%) *M. bicolor* genes and 481 (~ 3%) *R. ericae* genes were either mycorrhiza-specific (i.e. no detectable expression in free living mycelium) or up-regulated (fold change >5; $P<0.05$ Baggerley's test) in symbiotic roots (Tables S10, S11, S12, S13).

The three ERM fungi showed a similar pattern in the percentage of up-regulated CAZymes, lipases, proteases, and also transporters and small secreted proteins (SSPs) which have important roles in symbiotic interactions (Fig. S6). Overall, *O. maius* displayed a higher number and percentage of symbiosis-regulated genes in most categories (Table S10).

CAZymes. Of the 27% CAZyme genes up-regulated in *O. maius* during symbiosis (Table S10), the most highly induced genes (Table S11) code for secreted enzymes targeting pectin (PL1, CE8, GH28) and hemicellulose (GH27, GH43 and GH95), whereas the up-regulated CAZymes with the highest transcript levels mainly act on cellulose or contained a cellulose-binding domain (e.g. GH7-CBM1, GH5, GH10-CBM1, GH6-CBM1, GH62-CBM1) (Table S11). Almost 40% of the secreted CAZymes in the *O. maius* genome were up-regulated in symbiosis, representing 44% of the secreted proteins up-regulated in symbiosis and 65% of the total up-regulated CAZymes (Fig. S6 and Table S10). For some secreted CAZyme families, most or all members were up-regulated in symbiosis, such as acetyl xylan esterase (CE1; 3 out of 4), pectin methylesterase (CE8; 4/4), acetylesterase (CE16; 3/4), endo-β-1,4-glucanase (GH7; 4/5), endoxylanases (GH11; 7/8), and β-galactosidase (GH35, 5/6) (Table S5).

M. bicolor and *R ericae* showed a similar pattern. In *M. bicolor*, of the 14% CAZyme genes up-regulated during symbiosis (Table S10), the most highly induced genes (Table S12) coded for secreted enzymes targeting hemicellulose (GH43, GH35, GH54) cellulose (GH5) and pectin (GH28, PL4). Up-regulation in symbiosis was observed for most members of some secreted CAZymes families, such as pectin methylesterase (CE8; 4/5) and xyloglucan β-1,4-endoglucanase (GH12; 3/4). In *R. ericae*, of the 10% CAZyme genes up-regulated in symbiosis (Table S10), the most highly induced genes coded for secreted enzymes targeting pectin (GH28), cellulose (GH45, GH7-CBM1), hemicellulose (GH12) and starch (GH31) (Table S13).

Proteases and lipases. 21% of the *O. maius* genes coding for secreted proteases were up-regulated in symbiosis (Table S10), with aspartic proteases (A1), glutamic proteases (G01) and subtilisins (S53) being the most highly up-regulated (Table S11). The proportion of up-regulated secreted proteases was lower in *M. bicolor* (6%) and *R. ericae* (11%) (Table S10). In these species aspartic proteases (A1) and glutamic proteases (G01) were the most highly up-regulated, together with a carboxypeptidase (S10) (Tables S12 and S13). Several lipase genes (mostly coding for carboxylesterase B) were up-regulated in mycorrhizal roots colonized by *O. maius, M. bicolor* and *R. ericae* (Tables S7, S11, S12, S13).

Transporters. Genes coding for membrane transporters belonging to the major facilitator superfamily (MFS), and amino acid and ion permease families were significantly up-regulated during symbiosis (Tables S11, S12, S13).

MiSSPs. The percentage of MiSSPs, out of the total SSP-coding genes found in the ERM fungal genomes, ranged between 10% (for the two *R. ericae* aggregate fungi) to 20% for *O. maius* (Table S10).

Conservation of mycorrhiza-upregulated genes in ERM and Leotiomycete genomes

In ECM fungi, most symbiosis-upregulated genes code for taxonomically conserved genes involved in core metabolism (e.g. N and C assimilation, membrane transport) in both saprotrophic and symbiotic fungi (Kohler *et al*., 2015), while a substantial proportion of genes (7–38%) code for species-specific genes with unknown function (e.g. MiSSPs). We identified homologs of the symbiosis-upregulated genes of *O. maius, R. ericae* and *M. bicolor* by BLASTP queries in the other ERM genomes and in six genomes of saprotrophic or pathogenic Leotiomycetes (Fig. 7, Tables S11, S12, S13).

Most homologs of up-regulated genes identified in the three ERM fungi were also found in the saprotrophic fungus *C. longipes*. About half (49%) of the 995 symbiosis-upregulated *O. maius* genes were conserved (>40% sequence identity) in the gene repertoires of other Leotiomycetes (clusters IV, V and VI), with the exception of the pathogenic *Blumeria graminis,* known for its highly compact genome (Spanu *et al.*, 2010) (Fig. 7a). These conserved symbiosis-upregulated genes code mainly for CAZymes and proteins involved in primary metabolism, cellular processes and

signaling (Fig. 7a; Table S11). On the other hand, a substantial proportion (13%) of the *O. maius* up-regulated genes was restricted to this species (cluster II), with no homologs in other species. These taxonomically restricted genes mainly encode MiSSPs, proteins with no known KOG domains and a few metabolic components (e.g. MFS transporters, zinc finger C2H2-type transcription factors, CAZymes) (Fig. 7a; Table S11). Cluster I contains symbiosis up-regulated *O. maius* genes sporadically found in other Leotiomycete genomes (Fig. 7a).

Not surprisingly, a high proportion of symbiosis-upregulated genes in *M. bicolor* and *R. ericae* (~85% for *M. bicolor* and ~90% for *R. ericae*) were also highly conserved (>60% sequence identity) in closely phylogenetically-related species of the *R. ericae* aggregate, as well as in the saprotroph *C. longipes* (Figs. 7b and 7c).

Most homologs (87%) of the *M. bicolor* symbiosis up-regulated genes were also conserved in at least one of the sequenced Leotiomycete genomes (clusters I, II, IV and V), and taxonomically restricted symbiosis up-regulated genes (13%, cluster III) mainly code for proteins with no known conserved KOG domains, including MiSSPs, and components of the signaling pathways (Fig. 7b and Table S12). Several *M. bicolor* symbiosis up-regulated genes had no homologs in the gene repertoire of the congeneric species *M. variabilis* (Fig. 7b).

For *R. ericae*, 61% of homologs of the symbiosis up-regulated genes (clusters I, II and III) were also conserved in several Leotiomycetes, except *B. graminis*. The proportion of taxonomically restricted symbiosis up-regulated genes was low (~10%, cluster VI), and these genes mainly code for MiSSPs and proteins with no known conserved KOG domains (Fig. 7c; Table S13).

To assess if the transcripts conserved across all Leotiomycetes were also conserved across other fungal groups, we also blasted the 995 symbiosis-upregulated genes from *O. maius* against the entire gene repertoire of all other 59 fungal genomes included in this study. We found similar transcript distribution patterns between conserved and taxonomically restricted genes (Fig. S7, Table S14).

Discussion

ERM symbiosis is the youngest mycorrhizal type

Our phylogenomic analysis placed the MRCA of the sequenced ERM fungi at ~118 My. This is the same age as the Ericaceae family (~117 My) recently estimated by Schwery *et al*. (2015). Thus, our fungal phylogenomic reconstruction provides further evidence that the ERM symbiosis is the youngest mycorrhizal symbiosis. Indeed, the origin of the AM symbiosis has been dated back to 450 Mya (Redecker *et al*., 2000), whereas origin of the ECM symbiosis in the Pinaceae has been placed between about 270 and 130 Mya (see in Martin *et al*., 2016). Schwery *et al*. (2015) set the diversification of the ERM-forming lineages of the Ericaceae about 90–75 Mya, with contemporary dominant ERM plant species being even younger (45.6 My for present-day Vaccinieae and 22.3 My for *Dracophyllum* in the Styphelioideae). Considering possible dating errors (see Schwery *et al.*, 2015), our results suggest two possible scenarios: 1) the ancestral ERM fungal and plant partners diversified simultaneously, or 2) ERM fungi existed, in a different niche, before the diversification of the ERM-forming plant lineage.

Several authors (see Smith & Read, 2008) have proposed that the ERM symbiosis evolved under pressure to adapt to carbon-rich, nutrient-deficient soils with a high content of recalcitrant organic compounds. Carpenter *et al.* (2015) further suggested that the appearance of Australian sclerophyllous Ericaceae was concomitant with the massive loss of soil phosphorus due to increased fire frequency. Under these environmental conditions, a dual saprotrophic/mutualistic habit of the ERM fungal symbionts may have provided their hosts with greater ecological flexibility.

ERM genomes contain a rich repertoire of genes coding for degradative enzymes

The ability of ERM fungi (in particular *R. ericae* and *O. maius*) to degrade a wide range of complex substrates has been extensively explored (Smith & Read, 2008; Rice & Currah, 2005, 2006). Our survey of the gene repertoires of *O. maius, R. ericae*, *M. bicolor* and *M. variabilis* confirmed that their ability to digest most organic compounds found in SOM (including recalcitrant ones) is explained by the rich and varied repertoire of genes coding for CAZymes and other degradative enzymes, such as lipases and proteases.

All ERM fungi are well equipped with secreted CAZymes involved in the degradation of plant and fungal cell wall components, such as cellulose, hemicellulose,

pectins, chitin and ß 1,3 glucans. In addition, genes coding for lignocellulose-degrading enzymes were identified in all ERM genomes, providing genetic support for the observation that *O. maius*, and to a lesser extent *R. ericae* and *M. variabilis*, can decompose *Sphagnum* moss (Piercey *et al.,* 2002), a rare, but crucial, trait in the fungal kingdom (Thormann, 2001). Monooxygenases are also abundant in ERM fungi and may be involved in the biodegradation of complex polyaromatic organic molecules (Cerniglia & Sutherland, 2010).

When the gene repertoire of ERM fungi was compared to fungi with different lifestyles, the most striking differences were found with ECM fungi. The higher number of CAZyme and lipase coding genes identified in ERM fungi (all Ascomycetes) may partly mirror their different phylogenetic position from sequenced ECM fungi (mostly Basidiomycetes). However, the profiles of CAZyme, protease and lipase coding genes for ECM ascomycetes (i.e. *Cenococcum geophilum* and *Tuber melanosporum*) did not significantly differ from ECM basidiomycetes, yet differed (P<0.05) from those of ERM fungi, with the exception of total proteases (Table S15). This indicates that lifestyle is more important than phylogeny in shaping the fungal genomes.

Although most degradative enzymes were common to all four ERM fungal genomes, *O. maius* seemed to be better equipped to attack cellulose (e.g. with almost three times the number of genes containing the cellulose binding domain CBM1) and pectin, whereas *M. bicolor, M. variabilis* and *R. ericae* featured a much higher (4 to 5 times) number of secreted proteins containing the chitin binding domain CBM18, as well as enzymes involved in chitin degradation (e.g. CE4, AA7) absent in *O. maius*. Chitin contributes almost half the total N in the litter layer of heathland soils and *R. ericae* can readily degrade this polymer (Kerley & Read 1997).

These differential repertoires of degrading enzymes may reflect the different phylogenetic position of ERM fungi in the Leotiomycetes, with *M. bicolor*, *M. variabilis* and *R. ericae* belonging to the Helotiaceae (Vrålstad *et al*., 2000), and *O. maius* belonging to the Myxotrichaceae (Rice & Currah, 2006). Different gene repertoires may also mirror different habitat preferences of ERM fungi, as shown in Japan, where communities of putative ERM fungi differed among microhabitats (Koizumi & Nara, 2017). Notwithstanding its large CAZymes arsenal, *M. bicolor* is the

ERM strain with the lower percentage of secreted CAZymes (Fig. S3). This observation could partly support its double ERM and ECM nature.

ERM fungi significantly contribute to the mobilisation and accumulation of soil carbon in ERM habitats not only because of their role in decomposition, but also because their fungal biomass is rich in recalcitrant carbon compounds (Read *et al*., 2004), as supported by the higher number of genes coding for melanin metabolism.

ERM fungi: recently recruited or highly versatile mycorrhizal partners?

ECM fungi evolved multiple times from saprotrophic fungi (Ryberg & Matheny, 2012) and transition to the mycorrhizal habit coincided with an extensive loss of genes coding for plant cell wall degrading enzymes (PCWDEs), a genomic hallmark of ECM fungi (Martin *et al.*, 2016). In contrast to ECM fungi, all ERM fungi, regardless of their taxonomic position, feature a large set of CWDEs, specifically those involved in the degradation of hemicelluloses, pectins, glucans and mannans. The ERM CAZyme and lipase gene profiles differ from that of ECM fungi, including the two ECM ascomycetes considered in our analyses (*C. geophilum* and *T. melanosporum*). The decay apparatus of ERM genomes is even greater than most of the sequenced soil saprotrophs and plant pathogens. Given the more recent appearance of the ERM symbiosis, it is tempting to speculate that ERM fungi have retained this efficient saprotrophic arsenal because, unlike ECM symbionts, they are still in a transitional evolutionary stage between saprotrophy and mutualism.

Besides being true endomycorrhizal symbionts of ericaceous hosts, forming typical mycorrhizal structures, promoting plant growth and reciprocal resource exchange (Grelet et al., 2009; Kosola *et al*., 2007; Villarreal-Ruiz *et al*., 2012; Wei *et al*., 2016), ERM fungi also occur as root endophytes in other plant species.

Fungal endophytes are a ubiquitous, highly diverse group, comprising both Ascomycetes and Basidiomycetes. They have been recognized as fundamental components of many ecosystems (Rodriguez *et al*., 2009; Hardoim *et al*., 2015). Like mycorrhizal fungi, fungal endophytes may behave as mutualistic symbionts or as latent pathogens along a mutualism–antagonism continuum that may reflect their polyphyletic origin, but also depend on host and environmental conditions (Johnson *et al*., 1997; Schulz & Boyle, 2005; Hacquard *et al*., 2016).

ERM fungi, in particular *M. variabilis* and *O. maius*, have been found as co-associated endophytes in the root tips of ECM plants (Bergero *et al*., 2000; Tedersoo *et al*., 2009; Grelet *et al*., 2010; Kernaghan & Patriquin, 2011; Vohník *et al*., 2013), and colonising co-occuring ECM and neighboring non-ECM species (Chambers *et al*., 2008).

Although the ecophysiological role of ERM fungi as endophytes is unclear, Abuzinadah & Read (1989) showed that *Oidiodendron* enhances the growth of *Betula pendula* on a medium containing proteins as sole nitrogen source. Similarly, *M. variabilis*, formerly known as the "Variable White Taxon" (Hambleton & Sigler, 2005), increased Scots Pine biomass under elevated CO_2 (Alberton *et al*., 2010). *O. maius* isolated from ECM tips inhibits *in vitro* root pathogens such as *Phytophthora cinnamomi* and *Heterobasidium annosum* (Schild *et al*., 1988; Qian *et al*., 1998). This antagonistic activity may rely on the production of secondary metabolites. Interestingly, *O. maius* features a high number of genes involved in secondary metabolism; such as polyketide synthase (PKS) and PKS-like genes (Fig. 4). PKS play important functions in fungal biology being involved in the production of several secondary metabolites, including pigments (melanin), toxins, antibiotics and signaling molecules (Eisenman & Casadevall, 2011).

A common genomic trait of fungal root endophytes is that transition from saprotrophytism to endophytism, similar to transition to ERM, did not involve PCWDE genes loss (Fesel & Zuccaro, 2016). This is observed for endophytes belonging to the Helotiales (Leotiomycetes). For example, the widely distributed root endophyte *Phialocephala subalpina* shares several genomic features with ERM fungi, including expansion of PCWDE families acting on pectin, hemicellulose, cellulose and lignin, a low abundance of repeated elements and a large number of genes coding for key secondary metabolite enzymes (Schlegel *et al*., 2016). Almario *et al*. (2017) also reported a larger set of CAZyme encoding-genes in the genomes of two beneficial helotialean endophytic fungi, compared to saprotrophs and plant pathogens. *C. longipes*, a saprotrophic helotialean fungus with an endophytic phase (Koukol, 2011), also shares many of these features: numerous genes coding for PCWDEs, lipases and secondary metabolite enzymes, very few repeated elements, and many homologs of ERM symbiosis-induced genes. Intriguingly, there are also ecological and genomic features

shared between ERM fungi and Sebacinales. These Basidiomycetes include species displaying transitions from saprotrophy to endophytism and to mycorrhizal nutrition (Weiß *et al*., 2016). Some Sebacinales subclades also form ERM associations with Ericaceae plants (Berch *et al.*, 2002; Selosse *et al*., 2007). Similarly to ERM fungi, genomic studies in the Sebacinales have revealed a rich array of PCWDEs, supporting their known saprotrophic abilities *in vitro* (Kohler *et al*., 2015; Weiβ *et al*., 2016). As suggested for the Sebacinales (Selosse *et al*., 2009; van der Heijden *et al*., 2015), endophytism could be a 'waiting room' leading to mycorrhizal symbiosis (Weiß *et al*., 2016). In light of these considerations, we cannot exclude that the first interactions of ERM fungi with Ericaceae, about 118 Mya, may have been as endophytes, and that the mycorrhizal lifestyle evolved later.

Thus, the genomic features of ERM fungi reflect their ecological flexibility, capable of forming mycorrhizal and endophytic associations and, for *O. maius* at least, living as saprotrophs in substrates rich in organic matter (Rice & Currah, 2006). This ecological strategy sets ERM fungi well apart from ECM fungi, which specialized as plant symbionts by losing their degradative ability during the evolution of symbiosis.

Fungal gene expression in symbiosis

All three ERM fungi increased expression of several genes coding for secreted CAZyme isoforms during symbiosis with *V.myrtillus*. The most up-regulated CAZyme genes coded for secreted PCWDEs targeting cellulose, pectin and hemicellulose. Only four, one and six CAZyme genes were down-regulated in symbiosis (fold change <-5, P<0.05) for *O. maius*, *M. bicolor* and *R. ericae,* respectively. Several hypotheses may explain this pattern. To establish intracellular structures inside the epidermal root cells of ericaceous hosts (Massicotte *et al*., 2005), fungi may use secreted PCWDEs to penetrate the thick outer plant cell wall. In addition, PCWDEs may influence cell–cell interactions by altering the symbiotic plant-fungus interface formed by the invagination of the plant membrane (Balestrini & Bonfante, 2014). For example, whereas cellulose and other plant cell wall components have been identified in the interface formed around intracellular arbuscular mycorrhizal fungi (Bonfante *et al*., 1990), β1,4 glucans were missing in the ERM plant-fungus interface (Perotto *et al*., 1995), and their absence may reflect the sustained expression of secreted PCWDEs in symbiosis.

Sustained expression of PCWDEs in symbiosis might also contribute to closing the life cycle of an infected cell. Indeed the plant-fungus interface is thought to last about five weeks (Rice & Currah, 2006), after which mycorrhizal root cells first, then the intracellular fungal hyphae degenerate (Smith & Read, 1997). Seven to nine percent of secreted proteins up-regulated in symbioses were lipases and proteases. Altogether, these enzymes could promote the recycling of degenerating plant and fungal materials through decomposition and solubilisation of C and N compounds from senescing tissues, providing an efficient adaptative mechanism to low nutrient environments.

The expanded gene family (Table S4b) most represented in the symbiosis up-regulated transcriptome was the Major Facilitator Superfamily (MFS). Nutrient exchange is at the core of both saprotrophic and mycorrhizal functioning, and expansion of this gene superfamily in ERM fungi evolution was likely advantageous. By contrast, the expression of most members of other expanded families (e.g. *HET* genes) was not regulated in symbiosis, suggesting that these genes may be more relevant to saprotrophic growth.

Symbiosis-upregulated genes of ERM fungi include a cluster of taxonomically restricted genes specific to each ERM fungal species that contain a high proportion of effector-like MiSSPs. Characterized MiSSPs dampen plant defense reactions in arbuscular mycorrhizal and ectomycorrhizal symbioses (Kloppholz *et al*., 2011; Plett & Martin, 2015; Tsuzuki *et al,* 2016). The percentage of MiSSPs (10–20% of total SSPs) in ERM falls in a range similar to that found for ECM fungi (4–21%; Kohler *et al*., 2015) and for the arbuscular mycorrhizal fungus *R. irregularis* (19%, Tisserant *et al*., 2013).

In conclusion, our study describes the genetic machinery underpinning the extremely versatile nutrition mode of ERM fungi in the Leotiomycetes. This class of Ascomycetes includes fungi displaying different lifestyles, including plant pathogens, plant endophytes and saprotrophs (Zhang & Wang, 2015), and evolution of ERM fungi from any of these guilds is plausible. The results from this work and from Schlegel *et al*. (2016) suggest a closer relationship between ERM fungi and saprotrophic fungi, but genome sequencing of additional ERM isolates will help to verify this hypothesis.

The phylogenetic distance between *O. maius* and ERM fungi in the *R. ericae* aggregate suggests that the ERM habit evolved independently multiple times. However,

common traits of ERM fungi are their ecological plasticity and their ability to interact both with recalcitrant organic substrates as saprotrophs, and with living plants as biotrophs. The genetic bases of this dual life strategy are their large array of degradative secreted enzymes, often richer and more varied than that of soil saprotrophs and wood decayers, and a wide set of MiSSPs that may be involved in manipulating the host plant response.

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Author contributions

F.M.M. is leading the Mycorrhizal Genomics Initiative. E. Martino, E. Morin, G-A.G., C.V-F., A. Kohler, I.V.G., F.M.M. and S.P. planned and designed the research; E. Martino, E. Morin, G-A.G., A.Kuo, A.Kohler, S.D., K.W.B., N.C., A.C., R.B.D., M.H., R.C.K., K.LaB., E.A.L., A.L., H-R.K., J.M., C.M., R.A.O., M.W., C.V-F., B.H., I.V.G., F.M.M. and S.P. performed experiments or sequencing, collected, analysed or interpreted the data; B.D.L., S.W.S. and J.W.S. gave access to non published genomes; E. Martino, G-A.G., F.M.M. and S.P. wrote the manuscript.

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Figures

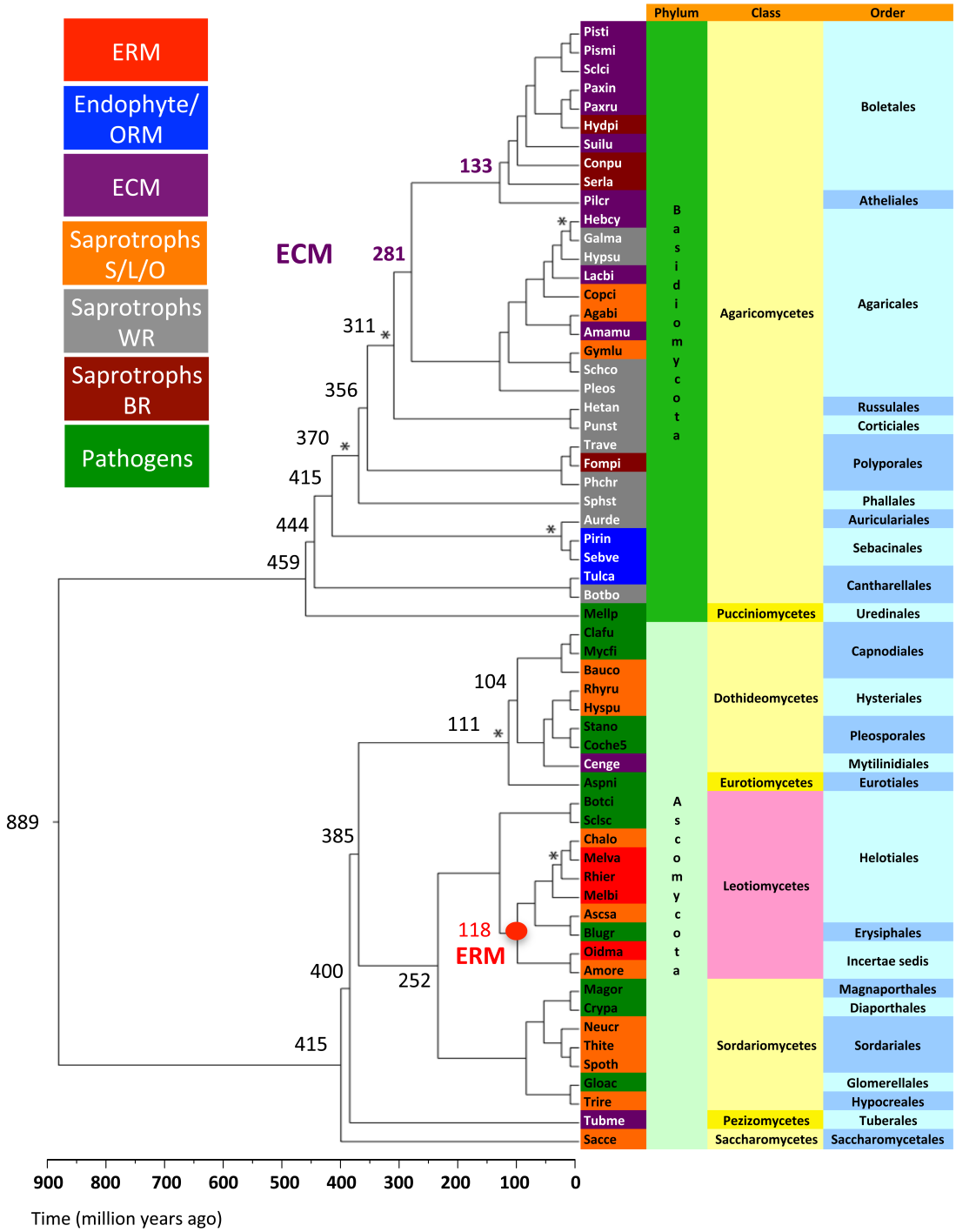

Fig. 1. Phylogenetic and molecular clock tree. Phylogenetic tree constructed with 199 core gene representatives out of the 246 single-copy families (deposited in the FUNYBASE, Marthey *et al*., 2008). Branch confidence values were obtained from 500 bootstrapped topologies and only nodes receiving less than maximal support are indicated with asterisks. Numbers in the tree correspond to My of nodes. The molecular clock was calibrated using the Pezizomycotina node estimated at 400 Mya (Kohler *et al*., 2015). The red dot in the tree corresponds to the ericoid fungal species separation. ECM, ectomycorrhizal, ERM, ericoid mycorrhizal, ORM, orchid mycorrhizal fungi; S/L/O, soil, litter, organic matter; WR, white rot; BR, brown rot. See Table S1 for full names of species and lifestyles.

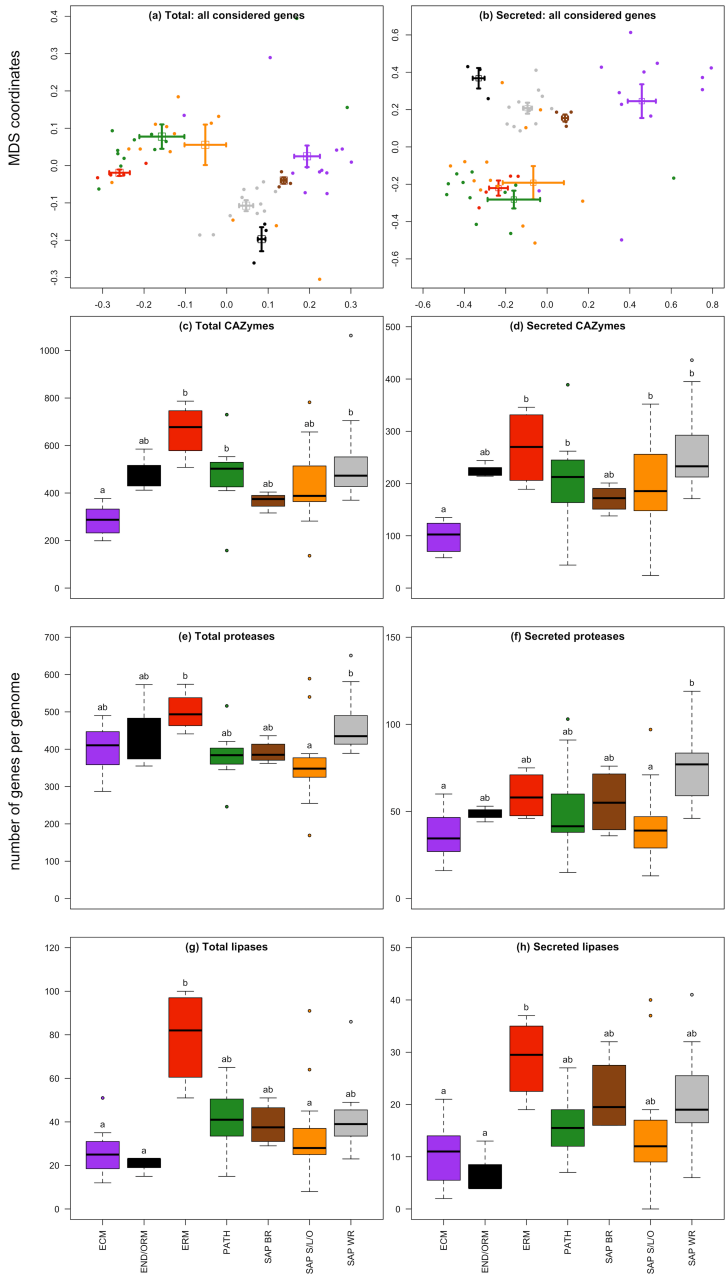

Fig. 2. MDS ordinations and gene counts for CAZymes, proteases and lipases in the 60 genomes. Effect of lifestyle on the number of genes involved in SOM (soil organic matter) decomposition (CAZymes - Carbohydrate-Active EnZymes, proteases and lipases). Lifestyles are color coded as follows: purple (ECM; n=12), black (END/ORM; n=3), red (ERM; n=4), green (PATH; n=12), orange (SAP S/L/O; n=14), brown (SAP BR; n=4) and grey (SAP WR; n=11). Plots (a) and (b) illustrate the MDS ordinations for all gene counts (total or secreted only, including all counted CAZyme, protease and lipase coding-genes), square symbols and lines indicate means and SEM for each lifestyle. Each individual genome is marked as a closed circle. Plots (c)–(h) show the average distributions of genes counts (minimum, first quartile, median, third quartile, and maximum) and outliers per gene category per lifestyle. In plot (c)-(h), lifestyles with different letters are significantly different at $P<0.05$ (modified one-way ANOVA analyses using MULTCOMP in R). ECM, ectomycorrhizal, ERM, ericoid mycorrhizal, ORM, orchid mycorrhizal fungi; END, endophytes; PATH, pathogens; SAP, saprotrophs; S/L/O, soil, litter, organic matter; WR, white rot; BR, brown rot. See Table S1 for full names of species and lifestyles.

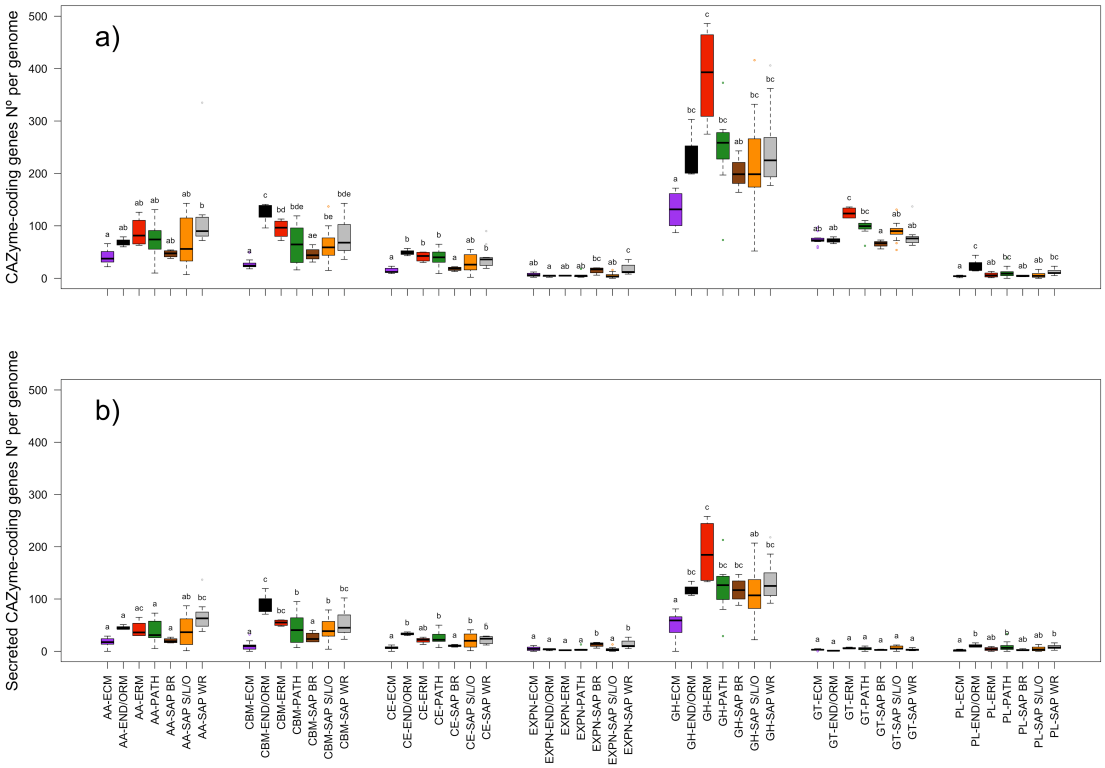

Fig. 3. Gene counts for total and secreted CAZyme coding genes. Effect of lifestyle on the number of genes belonging to the different CAZyme (Carbohydrate-Active EnZymes) families, coding for total (a) or secreted (b) enzymes. Lifestyles are color coded as follows: purple (ECM; n=12), black (END/ORM; n=3), red (ERM; n=4), green (PATH; n=12), orange (SAP S/L/O; n=14), brown (SAP BR; n=4) and grey (SAP WR; n=11). Plots show the average distributions of genes counts (minimum, first quartile, median, third quartile, and maximum) and outliers per CAZyme gene family per lifestyle. Lifestyles with different letters are significantly different at $P<0.05$ (modified one-way ANOVA analyses using MULTCOMP in R, performed on each individual CAZymes sub-category). GH, glycoside hydrolases; GT, glycosyl transferases; PL, polysaccharide lyases; CE, carbohydrate esterases; AA, Auxiliary Activities enzymes; CBM, carbohydrate-binding modules; EXPN, enzymes distantly related to plant expansins. ECM, ectomycorrhizal, ERM, ericoid mycorrhizal, ORM, orchid mycorrhizal fungi; END, endophytes; PATH, pathogens; SAP, saprotrophs; S/L/O, soil, litter, organic matter; WR, white rot; BR, brown rot. See Table S1 for full names of species and lifestyles.

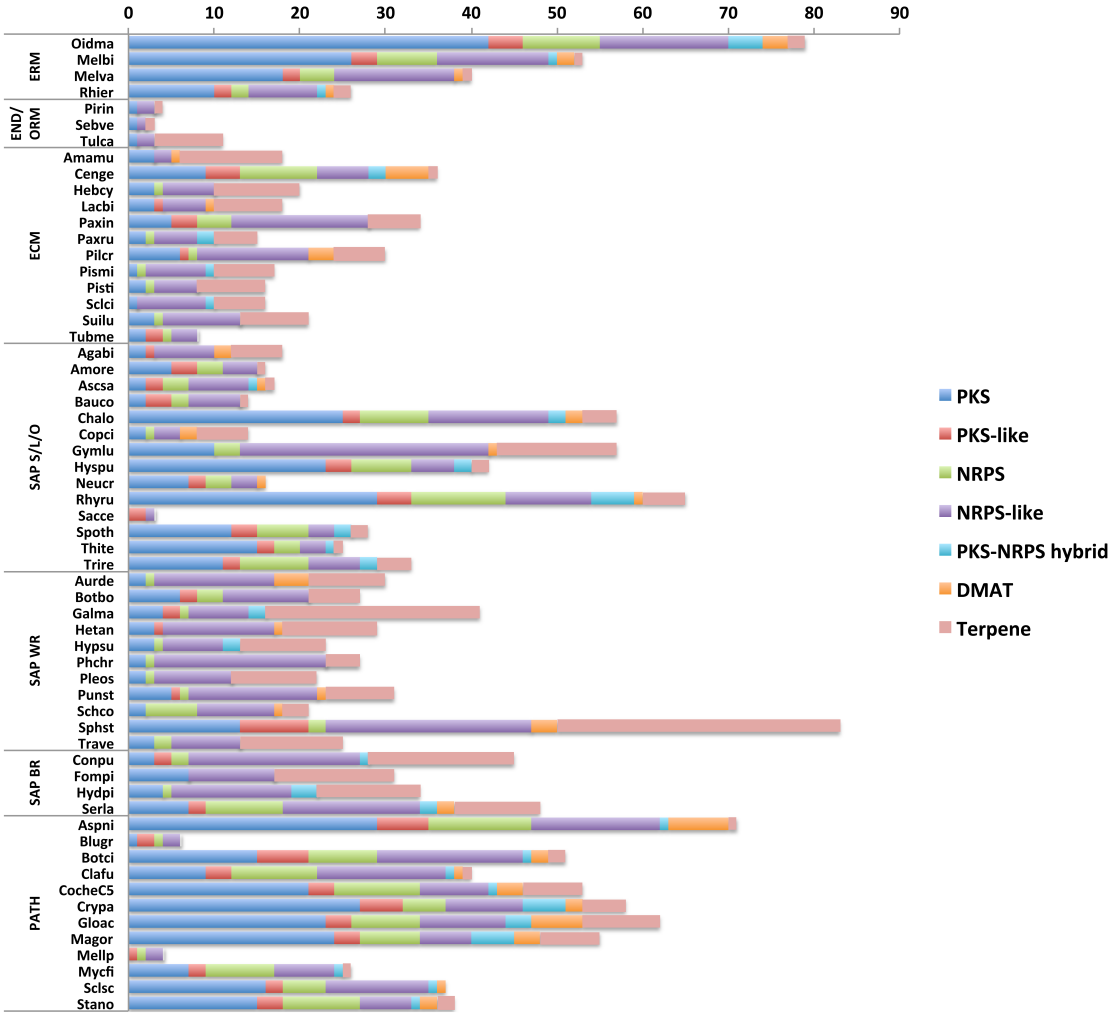

Fig. 4. Genes coding for secondary metabolism enzymes. Number of genes coding for secondary metabolism enzymes predicted for each of the 60 fungal species using a pipeline based on the SMURF method (Peter *et al*., 2016) is given as bar chart. PKS, polyketide synthase; NRPS, nonribosomal peptide synthase, DMATS, aromatic prenyltransferase. ECM, ectomycorrhizal, ERM, ericoid mycorrhizal, ORM, orchid mycorrhizal fungi; END, endophytes; PATH, pathogens; SAP, saprotrophs; S/L/O, soil, litter, organic matter; WR, white rot; BR, brown rot. See Table S1 for full names of species and lifestyles.

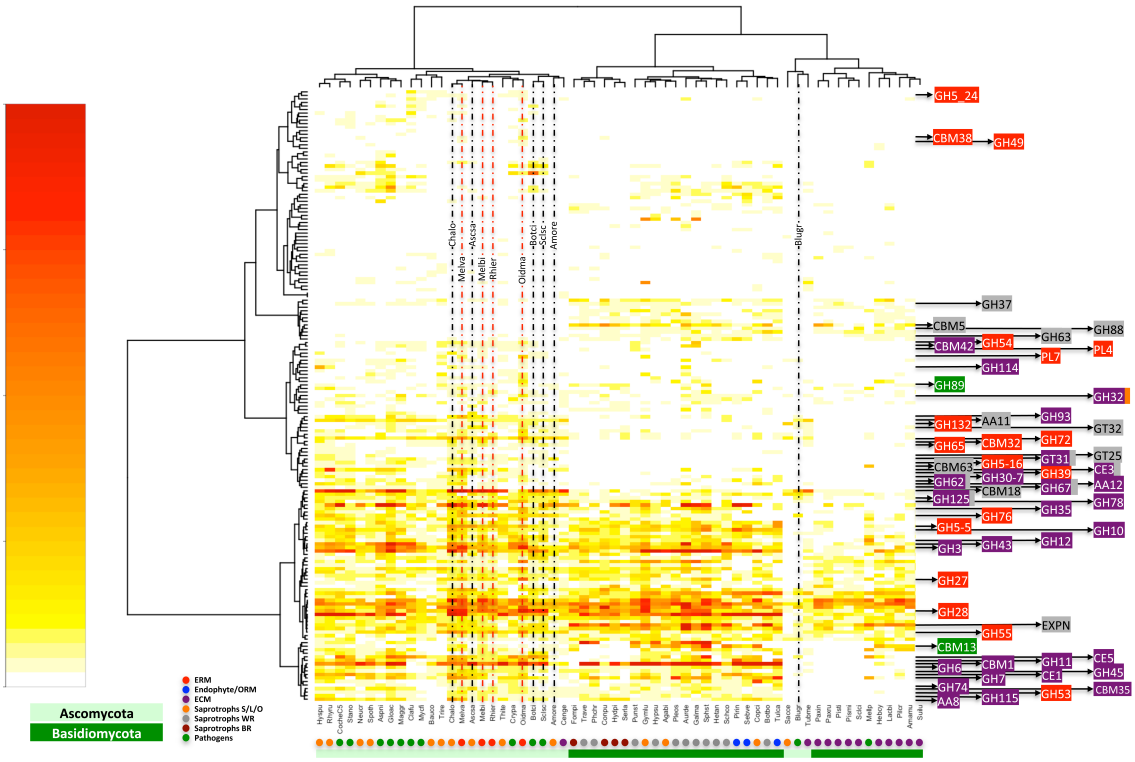

Fig. 5. Double hierarchical clustering of the secreted Carbohydrate-Active EnZyme (CAZyme) coding gene numbers in 60 genomes. A double hierarchical clustering of the number of secreted CAZyme coding genes for each of the 60 fungal species was performed using the Genesis software (Sturn *et al*., 2002). The Euclidian distance between gene counts was used as distance metric and a complete linkage clustering was performed. Relative abundance of genes is represented by a color scale (on the left), from the minimum (white) to the maximum (red) number of copies per species. On the right, CAZyme names were color-coded as in Table S5 to indicate significantly enriched CAZyme classes in ERM fungi (Mann Whitney test with Bonferroni correction): red, against all; pink, against all other Leotiomycetes; purple, against ECM fungi; grey, against white and brown rot fungi; orange: against saprotrophs S/L/O; green, against pathogens. Double-colored cases means significantly enriched CAZyme classes against two different fungal groups. Species marked by dotted lines are Leotiomycetes (in red, ERM fungi; in black, other fungi). ERM, ericoid mycorrhizal, ECM, ectomycorrhizal, ORM, orchid mycorrhizal fungi; S/L/O, soil, litter, organic matter; WR, white rot; BR, brown rot. See Table S1 for full names of species and lifestyles.

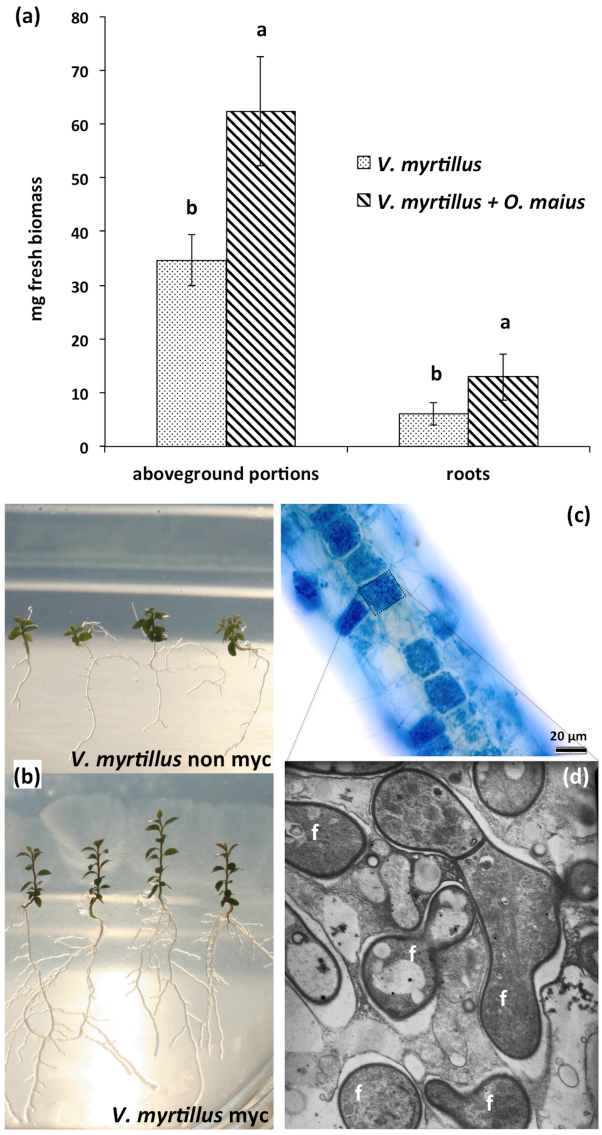

Fig. 6. *In vitro* mycorrhization of *Vaccinium myrtillus* by *O. maius*. (a) Biomass of aboveground and root portions of non mycorrhizal (dotted bars) or mycorrhizal (hatched bars) *V. myrtillus* plants. Significantly different values by ANOVA ($P<0.05$) were indicated by different letters. Error bars indicate standard deviation. n=15. (b) Non mycorrhizal and mycorrhizal *V. myrtillus* plants in Petri plates. (c) Ericoid fungal coils in the root epidermal cells, as observed by light microscopy (cells containing fungal coils are dark blue). (d) Ultrastructure of an epidermal cell of *V. myrtillus* where the fungal hyphae (f) surrounded by the plant plasma membrane and forming the coil inside the plant cell are visible (courtesy of R. Balestrini).

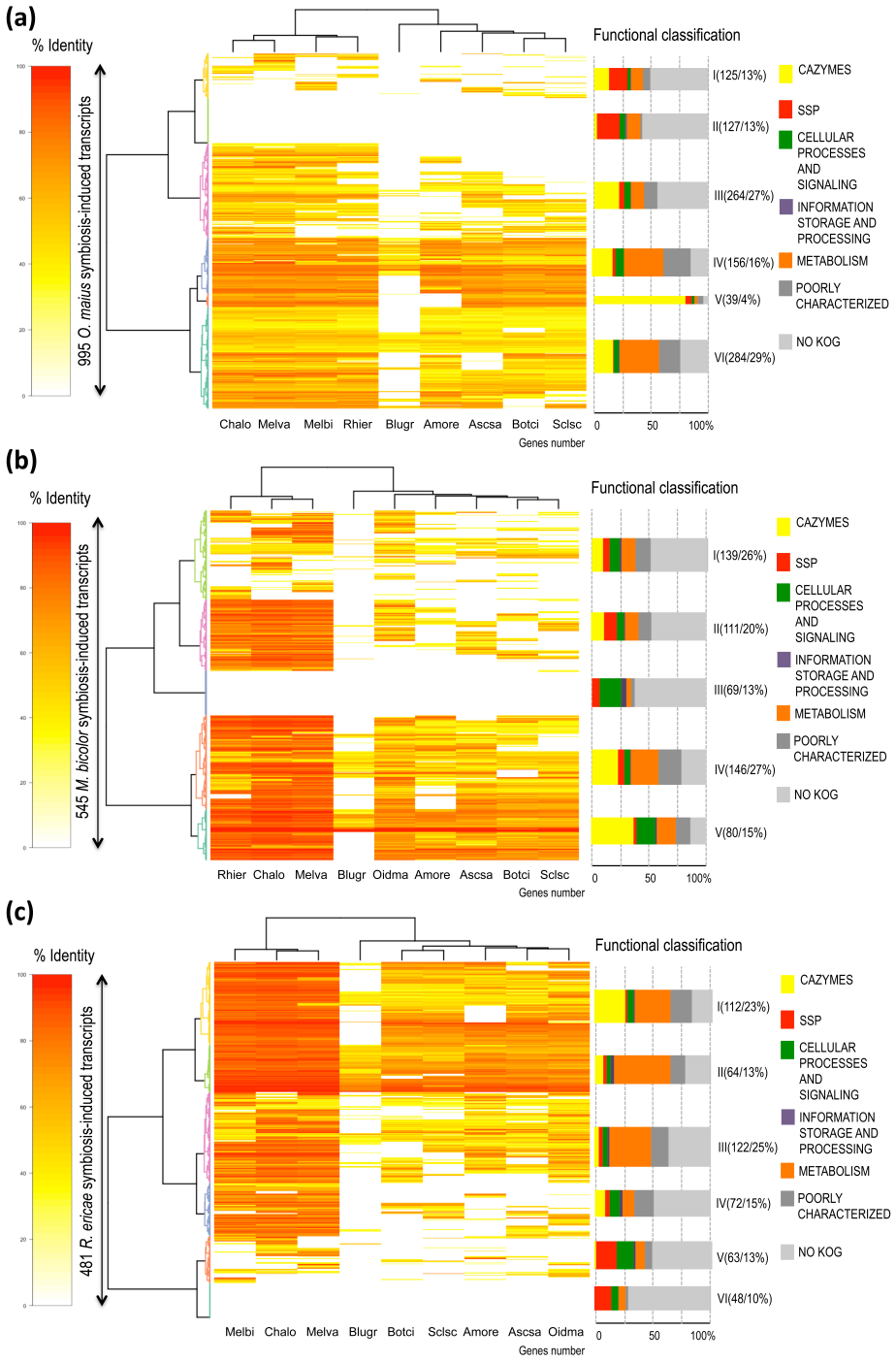

Fig. 7. Sequence conservation and functional analysis of the ERM symbiosis-induced genes. Homologs of symbiosis-upregulated genes from (a) *O. maius*, (b) *M. bicolor* and (c) *R. ericae* in the genomes of saprotrophic and pathogenic Leotiomycetes. The heatmaps represent a double hierarchical clustering of symbiosis-upregulated genes for the three ericoid fungi (rows, fold change >5, false discovery rate–corrected $P<0.05$; Tables S11-S13) based on their percentage sequence identity (color scale at left) with their homologs (if any) in selected fungal species (columns). Right of heatmap, the percentages of putative functional categories are given for each cluster as bargrams and the number and percentage of genes in each cluster are shown. In (a) genes of cluster II are *O. maius*–specific genes, in (b) genes of cluster III are *M. bicolor*–specific genes, in (c) genes of cluster VI are *R. ericae*–specific genes. CAZymes, Carbohydrate Active enZymes; SSPs, Small Secreted Proteins. See Table S1 for full names of species and lifestyles.

Supporting Information

Methods S1. Additional information for genome/transcriptome sequencing and assembly; genes linked to melanin synthesis; multivariate statistical analyses.

Figures

Fig. S1. Genome size, gene number and repeated elements (RE) in the 60 fungi analysed.
Fig. S2. Orthology assignment of the 25 Ascomycetes genomes.
Fig. S3. Total and secreted CAZymes in the 60 genomes.
Fig. S4. Total and secreted proteases and lipases in the 60 genomes.
Fig. S5. Double hierarchical clustering of the total CAZyme coding gene numbers in the 60 genomes.
Fig. S6. Ericoid fungi symbiosis-induced genes.
Fig. S7. Sequence conservation and functional analysis of *O. maius* symbiosis-induced genes in the 59 genomes.

Tables

Table S1. List of the 60 fungi utilized for comparative analyses.
Table S2. Genomic features of ericoid mycorrhizal fungal genomes.
Table S3. Genome coverage of the different repeated sequences.
Table S4. (a) Leotiomycetes and ericoid fungi specific proteins and protein families; (b) TOP 14 ericoid fungi expanded families (MCL-CAFE analysis) as compared to other 16 genomes.
Table S5. Total and secreted CAZymes (Carbohydrate Active enZymes) in the 60 fungi with Mann-Whitney test analyses and Bonferroni correction. Sheet 3 reports total and secreted CAZyme coding-gene numbers used to prepare Fig. S3. These numbers do not correspond to those reported in sheets 1 and 2, where each CAZyme module was considered separately.

Table S6. Total and secreted proteases in the 60 fungi with Mann Whitney test analyses and Bonferroni correction.

Table S7. Total and secreted lipases in the 60 fungi, with Mann Whitney test analyses and Bonferroni correction

Table S8. Secreted CAZymes (Carbohydrate Active enZymes) significantly enriched in the ericoid fungi as compared to the others ecological strategies (as identified by Mann Whitney test and Bonferroni correction).

Table S9 Genes linked to melanin synthesis in the 60 fungi, and Mann Whitney test with Bonferroni correction.

Table S10. Summary of the total symbiosis up-regulated gene categories in ericoid fungi.

Table S11. List of the 995 mycorrhizal-induced genes in *O. maius* and their assignment to the clusters in Fig. 7a.

Table S12. List of the 545 mycorrhizal-induced genes in *M. bicolor* and their assignment to the clusters in Fig. 7b.

Table S13. List of the 481 mycorrhizal-induced genes in *R. ericae* and their assignment to the clusters in Fig. 7c.

Table S14. List of the 995 mycorrhizal-induced genes in *O. maius* assigned to the clusters mentioned in Fig. S7.

Table S15 Total and secreted CAZyme (Carbohydrate Active enZymes), protease and lipase coding-gene numbers comparison between between ericoid fungi and ectomycorrhizal ascomycetes, and between ectomycorrhizal basidiomycetes and ectomycorrhizal ascomycetes, with T-test analyses.