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Research

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 in which they occur, ERM plant survival relies on nutrient mobilization from soil organic matter (SOM) by their fungal partners. The characterization of the fungal genetic machinery underpinning both the symbiotic lifestyle and SOM degradation is needed to understand ERM symbiosis functioning and evolution, and its impact on soil carbon (C) turnover.

• We sequenced the genomes of the ERM fungi *Meliniomyces bicolor*, *M. variabilis*, *Oidiodendron maius* and *Rhizoscyphus ericae*, and compared their gene repertoires with 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 to those of ectomycorrhizal symbionts. The fungal genes most highly upregulated in symbiosis are those coding for fungal and plant cell wall-degrading enzymes (CWDEs), lipases, proteases, transporters and mycorrhiza-induced small secreted proteins (MiSSPs).

• The 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.

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 mycorrhizal (ERM) fungi involve 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 *c.* 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 *c.* 117 million yr (Myr) (Schwery *et al.*, 2015), whilst diversification of the ERM-forming lineages might date back to 90–75 million yr ago (Ma), during angiosperm radiation in the Late Cretaceous (Nixon & Crepet, 1993; Carpenter *et al.*, 2015). The ERM symbiosis is hypothesized to have evolved within that

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time frame, and to be the most recent of all mycorrhizal types (Brundrett, 2002).

ERM habitats are usually characterized 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 *c.* 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 Perotto *et al.*, 2012) as they contribute to the mobilization of nutrients from complex organic matter 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; Weiß *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 unnamed 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). *Meliniomyces 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* have been 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 depolymerization and degradation of plant and fungal cell wall polymers, organic phosphorus forms and complex aliphatic compounds, such as polyphenols and tannic acid (for a summary, see Smith & Read, 2008). 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 1.5–47% 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 whether 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 as follows. Did the ERM habit arise in Leotiomycetes together with the appearance of the host plants? 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? Do ERM fungi have a specific genomic 'signature' as for ECM fungi (Kohler *et al.*, 2015)?

Materials and Methods

Fungal strains

The 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 as that which led to the first formal description of the *R. ericae* species after the production of ascomata in culture. We also included, for comparison, the as yet unpublished genome of the saprotrophic Leotiomycetes *Amorphotheca resinae*. The 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 (insert size, 270 bp) 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 *A. 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). The transcriptomes of all four species were sequenced using Illumina, assembled using RNNOTATOR (Martin *et al.*, 2010) and

used for genome annotation (for further details, see Supporting Information Methods S1). 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 Randomized Axelerated Maximum Likelihood (RAxML) program (Stamatakis, 2006). Ultrametric trees were calculated from the maximum likelihood (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 Ma.

Comparative genomic analyses and annotation of functional categories

Our comparative analyses focused on repeated elements (REs), 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 (GHs), glycosyl transferases (GTs), polysaccharide lyases (PLs), carbohydrate esterases (CEs), redox enzymes that act in conjunction with CAZymes (auxiliary activities, AAs), carbohydrate-binding modules (CBMs) and enzymes distantly related to plant expansins (EXPNs) – 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 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 v.4, 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 the 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 *Vaccinium myrtillus* were obtained *in vitro* as described in Kohler *et al.* (2015). Fifteen plates, each containing 10 plants, were analyzed for each treatment. RNA extraction and sequencing (RNA-Seq), and the 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* respectively.

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 was illustrated 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 transcript homologs in the two different fungal genome 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 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 the differences between mycorrhizal and non-mycorrhizal plant fresh biomass was statistically evaluated by ANOVA with Tukey's *post hoc* test ($P < 0.05$).

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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 with 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 mycorrhizal (ORM) fungi), 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 *c.* 148 Ma, whereas the MRCA of ERM fungi was estimated to have occurred *c.* 118 Ma (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 REs 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) were also very similar, although the *M. variabilis* genome encoded a much larger set of unspecific and salicylate monooxygenases which 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. Leotiomycete-specific proteins with

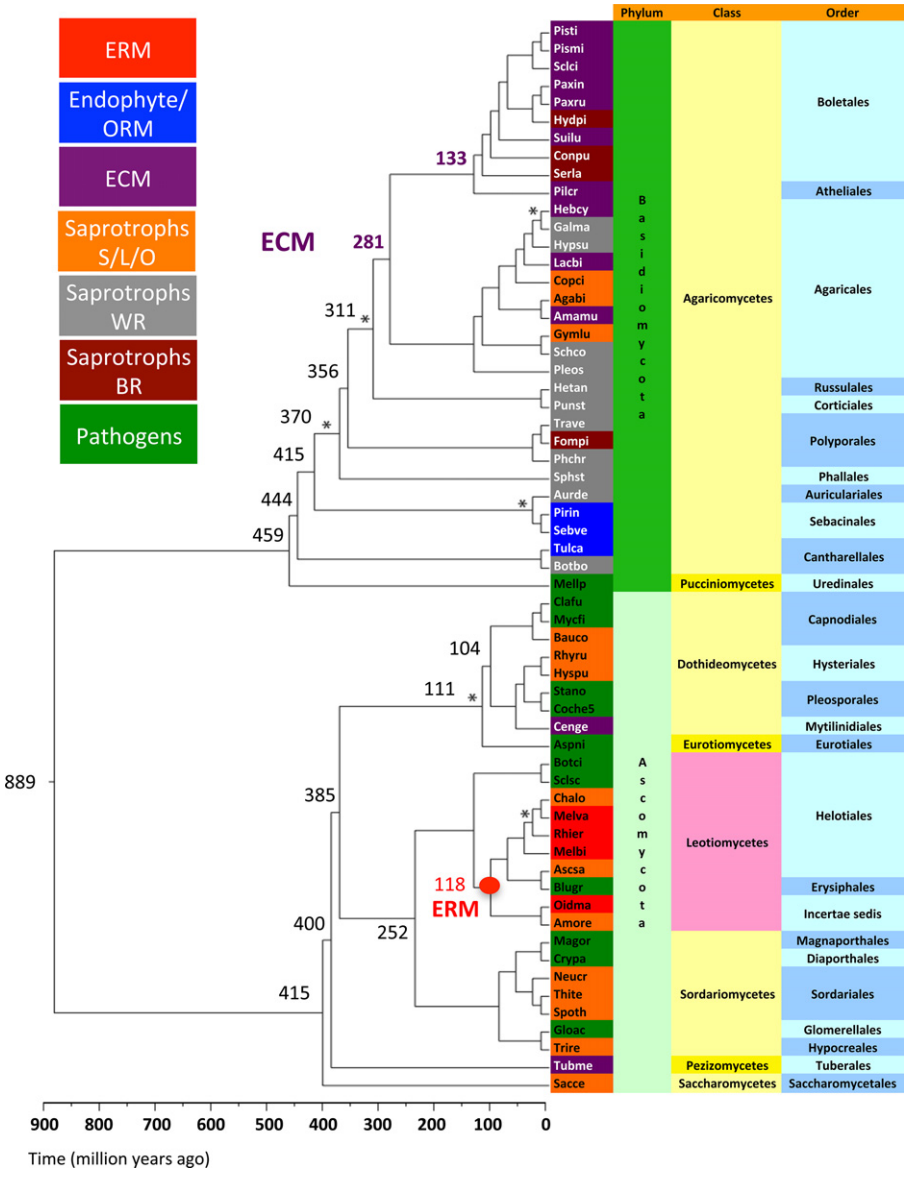


Fig. 1 Phylogenetic and molecular clock tree. Phylogenetic tree constructed with 199 core gene representatives out of the 246 single-copy families (deposited in FUNYBASE, Marthey *et al.*, 2008). Branch confidence values were obtained from 500 bootstrapped topologies, and only nodes receiving less than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

known domains included 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, $P < 0.05$) coded for proteins involved in self/non-self recognition (heterokaryon incompatibility proteins), nutrient uptake/exchange (major facilitator superfamily (MFS) and sugar transporters), detoxification of environmental pollutants and/or stress response (cytochrome P450, ankyrin repeats, β-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 (Tables S5–S7; Figs 2, S3, 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 ERM fungi, pathogens and soil/litter saprotrophs, or ECM fungi, ORM fungi, and white and brown rot fungi (Fig. 2). Overall, ERM genomes encoded 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 contained nearly twice the median number of lipase genes as any other type of fungus (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 contained 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 for pectin lyases (PL1).

ERM genomes contained a significantly greater number of CAZyme genes than did ECM genomes (Table S5). They encoded a greater 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 with 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 were 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).

The 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 (Methods S1) showed that two of them (*arp1* – scytalone dehydratase and *abr1* – brown 1) were significantly enriched in ERM fungi compared with fungi with different ecological strategies (Table S9). Three more genes (*alb1* – PKS; *arp2* – 1,3,6,8-tetrahydroxynaphthalene (THN) reductase; *ayg1* – yellowish-green) were significantly more enriched in ERM than in ECM, white and brown rot fungi (Table S9). Compared with the other fungal groups (Table S5), ERM fungi showed 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 were characterized by a rich set of CAZymes (Fig. S5), they were discriminated by their secreted CAZyme 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 with 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, such as CBM1 and CBM6, were more represented in *O. maius.* However, *O. maius* contained less CAZymes degrading chitin, which were 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-degrading 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 the 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

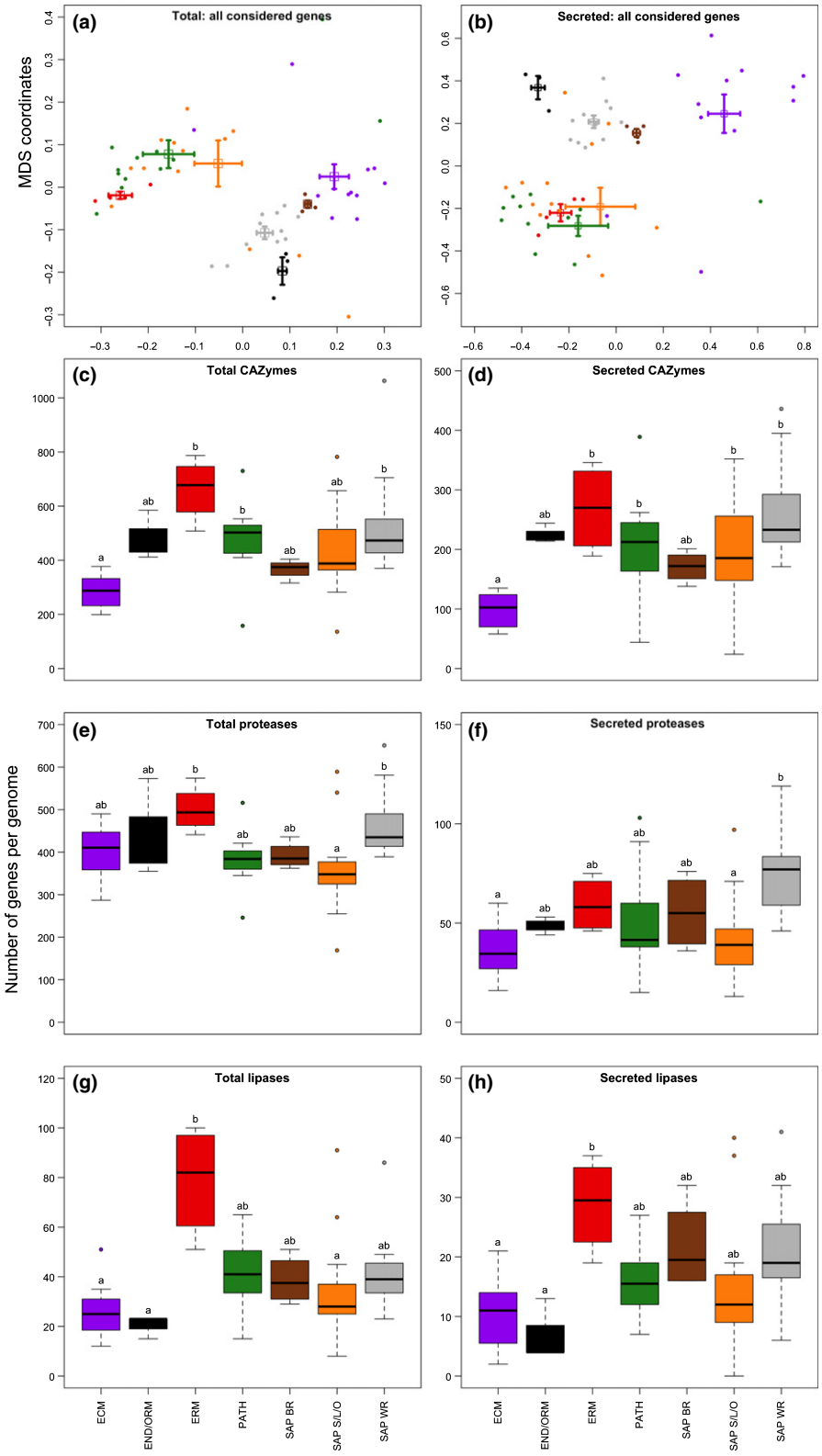


Fig. 2 Multidimensional scaling (MDS) ordinations and gene counts for Carbohydrate-Active enZymes (CAZymes), proteases and lipases in the 60 genomes. Effect of lifestyle on the number of genes involved in soil organic matter (SOM) decomposition (CAZymes, proteases and lipases). Lifestyles are color-coded as follows: purple (ectomycorrhizal fungi (ECM); $n = 12$), black (endophytes (END)/orchid mycorrhizal fungi (ORM); $n = 3$), red (ericoid mycorrhizal fungi (ERM); $n = 4$), green (pathogens (PATH); $n = 12$), orange (saprotrophs (SAP) soil, litter, organic matter (S/L/O); $n = 14$), brown (SAP brown rot (BR); $n = 4$) and gray (SAP white rot (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 ± SE 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 plots (c–h), lifestyles with different letters are significantly different at $P < 0.05$ (modified one-way ANOVA using MULTCOMP in R). See Table S1 for full names of species and lifestyles.

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 (*c.* 6%) *O. maius* genes, 545 (*c.* 3%) *M. bicolor* genes and 481 (*c.* 3%) *R. ericae* genes were either mycorrhiza-specific (i.e. no detectable expression in free-living mycelium) or upregulated (fold change > 5; $P < 0.05$ Baggerley's test) in symbiotic roots (Tables S10–S13).

The three ERM fungi showed a similar pattern in the percentage of upregulated CAZymes, lipases, proteases and also transporters and small secreted proteins (SSPs) which have important

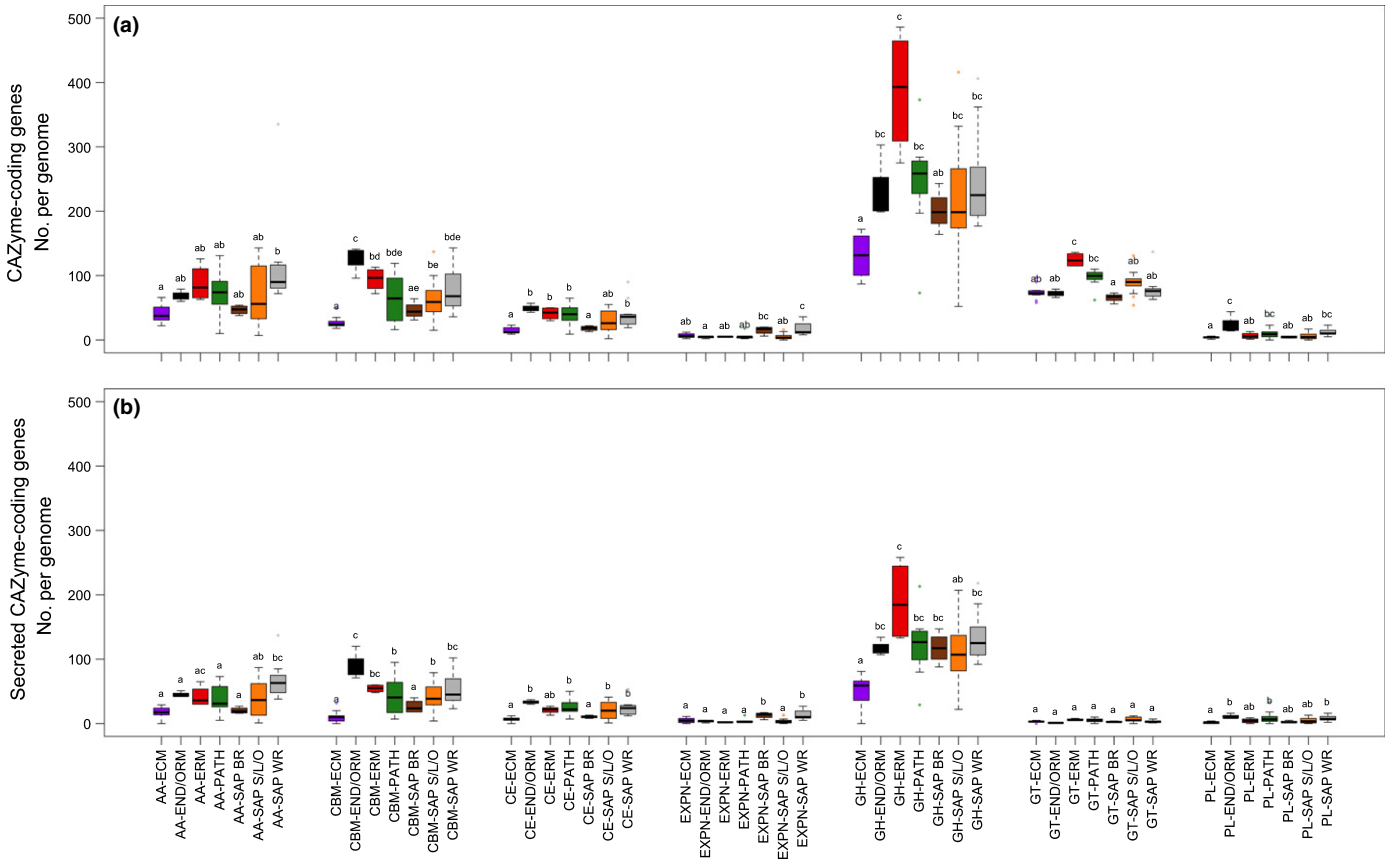


Fig. 3 Gene counts for total and secreted Carbohydrate-Active enZyme (CAZyme) coding genes. Effect of lifestyle on the number of genes belonging to the different CAZyme families, coding for (a) total or (b) secreted enzymes. Lifestyles are color-coded as follows: purple (ectomycorrhizal fungi (ECM); $n = 12$), black (endophytes (END)/orchid mycorrhizal fungi (ORM); $n = 3$), red (ericoid mycorrhizal fungi (ERM); $n = 4$), green (pathogens (PATH); $n = 12$), orange (saprotrophs (SAP) soil, litter, organic matter (S/L/O); $n = 14$), brown (SAP brown rot (BR); $n = 4$) and gray (SAP white rot (WR); $n = 11$). Plots show the average distributions of gene 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 using MULTCOMP in R, performed on each individual CAZyme subcategory). GH, glycoside hydrolases; GT, glycosyl transferases; PL, polysaccharide lyases; CE, carbohydrate esterases; AA, auxiliary activity enzymes; CBM, carbohydrate-binding modules; EXPN, enzymes distantly related to plant expansins. See Table S1 for full names of species and lifestyles.

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 upregulated 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, GH95), whereas the upregulated CAZymes with the highest transcript levels mainly act on cellulose or contain 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 upregulated in symbiosis, representing 44% of the secreted proteins upregulated in symbiosis and 65% of the total upregulated CAZymes (Fig. S6; Table S10). For some secreted CAZyme families, most or all members were upregulated in symbiosis, such as acetyl xylan esterase (CE1; three out of four, 3/4), 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).

Meliniomyces bicolor and *R. ericae* showed a similar pattern. In *M. bicolor*, of the 14% CAZyme genes upregulated 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). Upregulation in symbiosis was observed for most members of some secreted CAZyme 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 upregulated 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 Twenty-one percent of the *O. maius* genes coding for secreted proteases were upregulated in symbiosis (Table S10), with aspartic proteases (A1), glutamic proteases (G01) and subtilisins (S53) being the most highly upregulated (Table S11). The proportion of upregulated 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 upregulated, together with a

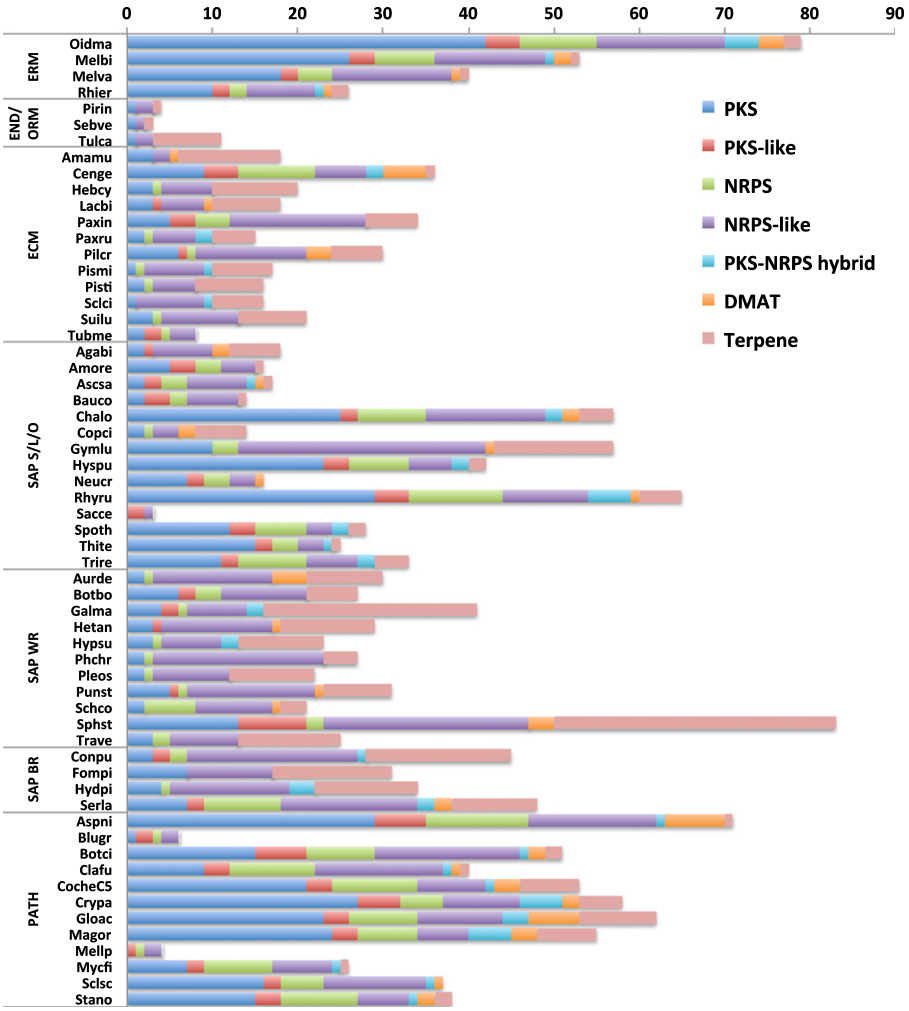


Fig. 4 Genes coding for secondary metabolism enzymes. Number of genes coding for secondary metabolism enzymes predicted for each of the 60 fungal species using a pipeline based on the Smurf method (Peter *et al.*, 2016), given as a bar chart. PKS, polyketide synthase; NRPS, nonribosomal peptide synthase; DMAT, aromatic prenyltransferase; ECM, ectomycorrhizal fungi; ERM, ericoid mycorrhizal fungi; 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.

carboxypeptidase (S10) (Tables S12, S13). Several lipase genes (mostly coding for carboxylesterase B) were upregulated in mycorrhizal roots colonized by *O. maius, M. bicolor* and *R. ericae* (Tables S7, S11–S13).

Transporters Genes coding for membrane transporters belonging to the MFS, and amino acid and ion permease families, were significantly upregulated during symbiosis (Tables S11–S13).

Mycorrhiza-induced SSPs (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 coded for taxonomically conserved genes involved in core metabolism (e.g. nitrogen and carbon assimilation, membrane transport) in both saprotrophic and symbiotic fungi (Kohler *et al.*, 2015), whereas a substantial proportion of genes (7–38%) coded 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–S13).

Most homologs of upregulated 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 coded mainly for CAZymes and proteins involved in primary metabolism, cellular processes and signaling (Fig. 7a; Table S11). However, a substantial proportion (13%) of the *O. maius* upregulated genes was restricted to this species (cluster II), with no homologs in other species. These taxonomically restricted genes mainly encoded 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-upregulated *O. maius* genes sporadically found in other Leotiomycete genomes (Fig. 7a).

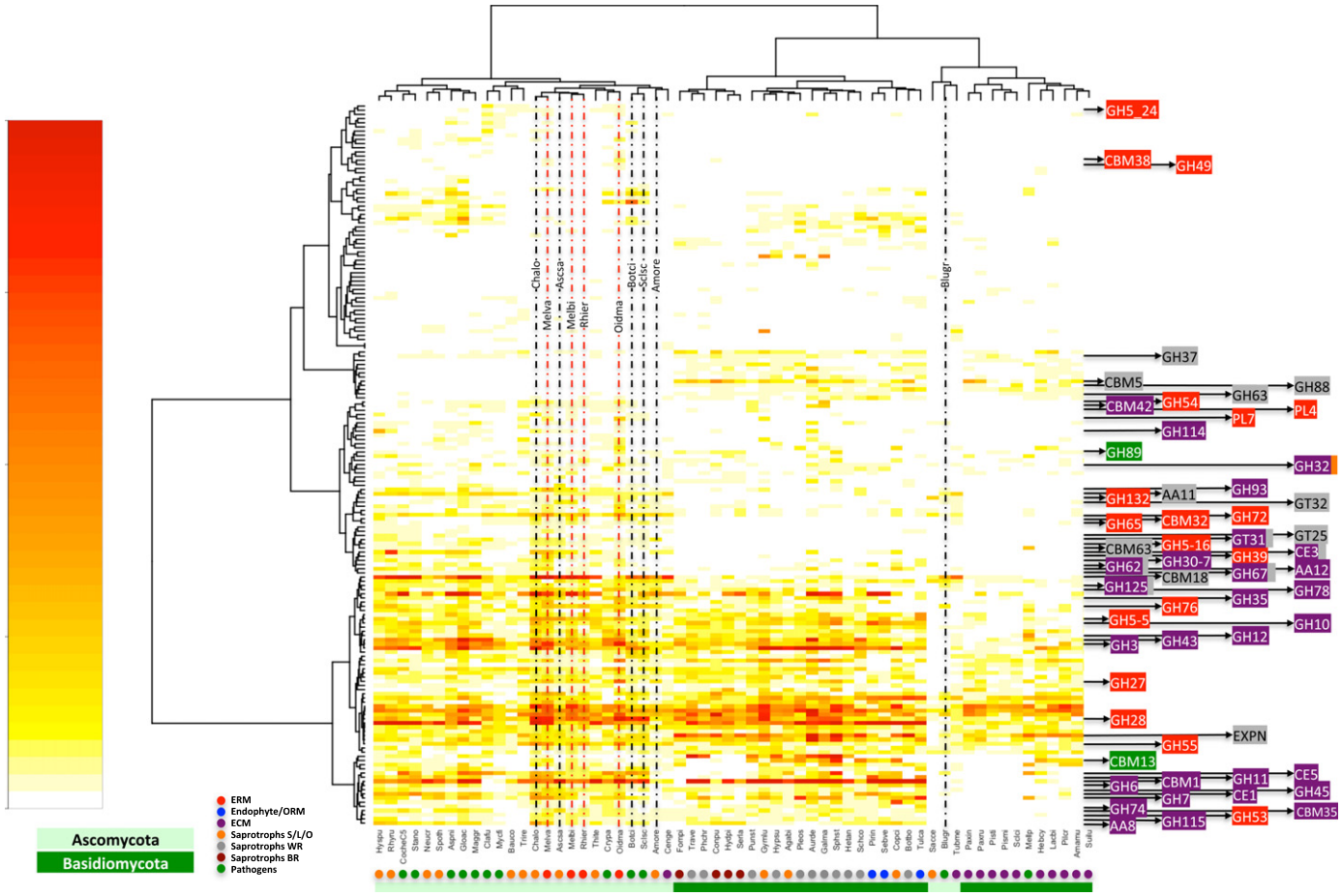


Fig. 5 Double hierarchical clustering of the secreted Carbohydrate-Active enZyme (CAZyme) coding gene numbers in 60 genomes. A double hierarchical clustering of the number of secreted CAZyme coding genes for each of the 60 fungal species was performed using the GENESIS software (Sturn *et al.*, 2002).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

Not surprisingly, a high proportion of symbiosis-upregulated genes in *M. bicolor* and *R. ericae* (*c.* 85% for *M. bicolor* and *c.* 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* (Fig. 7b,c).

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

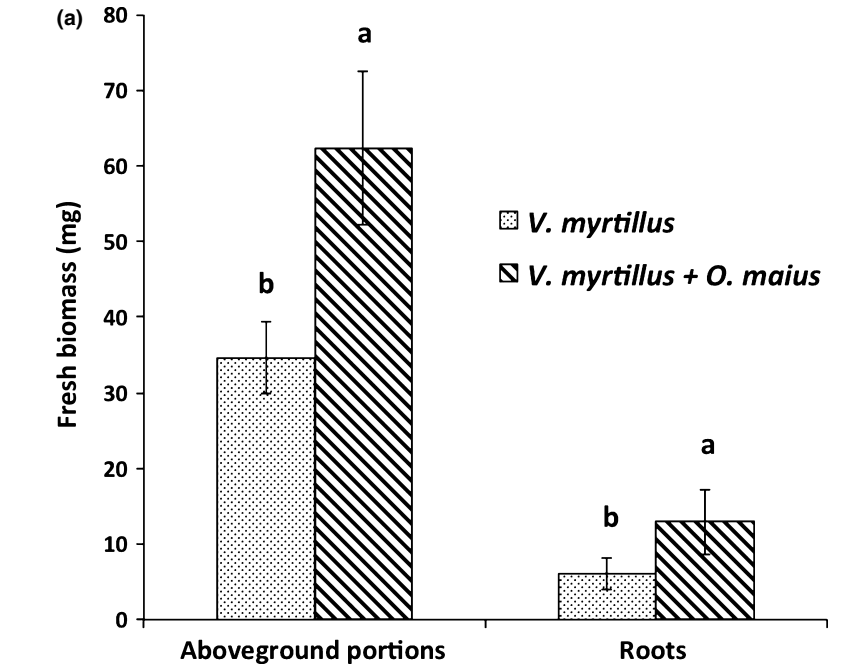
For *R. ericae*, 61% of homologs of the symbiosis-upregulated genes (clusters I, II and III) were also conserved in several Leotiomycetes, except *B. graminis*. The proportion of taxonomically restricted symbiosis-upregulated genes was low (*c.* 10%, cluster VI), and these genes mainly coded for MiSSPs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

To assess whether 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 *c.* 118 Myr. This is the same age as the Ericaceae family (*c.* 117 Myr) recently estimated by Schwery *et al.* (2015). Thus, our fungal phylogenomic reconstruction provides further



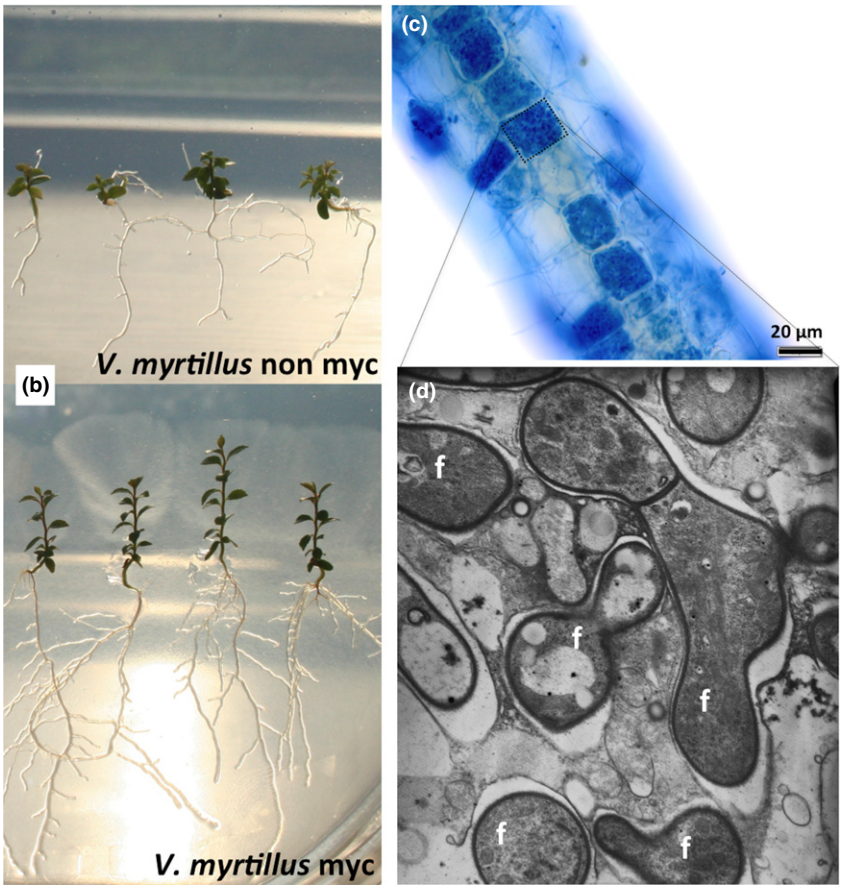


Fig. 6 *In vitro* mycorrhization of *Vaccinium myrtillus* by *Oidiodendron maius*. (a) Biomass of aboveground and root portions of nonmycorrhizal (dotted bars) or mycorrhizal (hatched bars) *V. myrtillus* plants. Significantly different values by ANOVA ($P < 0.05$) are indicated by different letters. Error bars indicate ± SD. $n = 15$. (b) Nonmycorrhizal (non myc) and mycorrhizal (myc) *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*: fungal hyphae (f) surrounded by the plant plasma membrane and forming coils inside the plant cell are visible (courtesy of R. Balestrini).

evidence that the ERM symbiosis is the youngest mycorrhizal symbiosis. Indeed, the origin of the arbuscular mycorrhizal (AM) symbiosis has been dated back to 450 Ma (Redecker *et al.*, 2000), whereas the origin of the ECM symbiosis in the Pinaceae has been placed between *c.* 270 and 130 Ma (see Martin *et al.*, 2016). Schwery *et al.* (2015) set the diversification of the ERM-forming lineages of the Ericaceae at *c.* 90–75 Ma, with contemporary dominant ERM plant species being even younger (45.6 Myr for present-day Vaccinieae and 22.3 Myr for *Dracophyllum* in the Styphelioideae). Considering possible dating errors (see Schwery

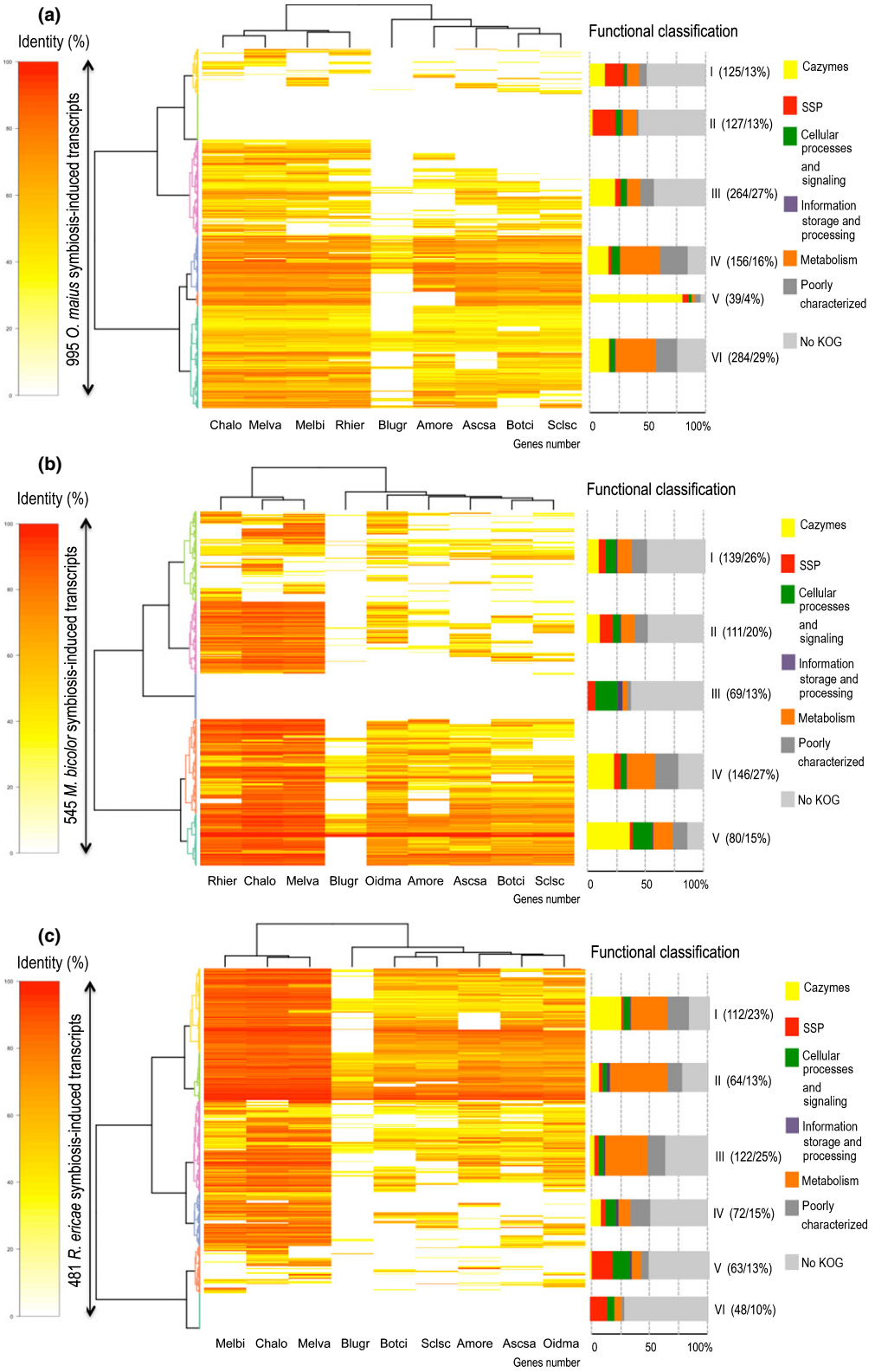


Fig. 7 Sequence conservation and functional analysis of the ericoid mycorrhizal (ERM) symbiosis-induced genes. Homologs of symbiosis-upregulated genes from (a) *Oidiodendron maius*, (b) *Meliniomyces bicolor* and (c) *Rhizoscyphus ericae* in the genomes of saprotrophic and pathogenic Leotiomycetes. The 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; SSP, small secreted protein. See Table S1 for full names of species and lifestyles.

et al., 2015), our results suggest two possible scenarios: the ancestral ERM fungal and plant partners diversified simultaneously, or 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 as a result of 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 (Rice & Currah, 2005, 2006; Smith & Read, 2008). Our survey of the gene repertoires of *O. maius*, *R. ericae*, *M. bicolor* and *M. variabilis* confirms 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 β-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 with that of fungi with different lifestyles, the most striking differences were found relative to ECM fungi. The greater 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 differ significantly from those of 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 (four to five 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 nitrogen 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). The 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 CAZyme 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 contribute significantly to the mobilization 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 greater 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 with 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 those 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 that of most of the sequenced soil saprotrophs and plant pathogens. Given the more recent appearance of 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.

In addition to being true endomycorrhizal symbionts of ericaceous hosts, forming typical mycorrhizal structures and promoting plant growth and reciprocal resource exchange (Kosola *et al.*, 2007; Grelet *et al.*, 2009; 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 which may reflect their polyphyletic origin, but may 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 colonizing co-occurring 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). *Oidiodendron 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 large number of genes involved in secondary metabolism; such as PKS and PKS-like genes (Fig. 4). PKSs 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, 2012).

A common genomic trait of fungal root endophytes is that transition from saprotrophytism to endophytism, similar to transition to ERM, did not involve PCWDE gene 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 REs 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 with saprotrophs and plant pathogens. *Chalara 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 REs 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). Similar 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; Weiß *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 the light of these considerations, we cannot exclude the possibility that the first interactions of ERM fungi with Ericaceae, *c.* 118 Ma, 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 the expression of several genes coding for secreted CAZyme isoforms during symbiosis with *V. myrtillus*. The most upregulated CAZyme genes coded for secreted PCWDEs targeting cellulose, pectin and hemicellulose. Only four, one and six CAZyme genes were downregulated 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 AM fungi (Bonfante *et al.*, 1990), β-1,4-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 *c.* 5 wk (Rice & Currah, 2006), after which mycorrhizal root cells first, and then the intracellular fungal hyphae, degenerate (Smith & Read, 1997). Seven to nine percent of secreted proteins upregulated in symbioses were lipases and proteases. Altogether, these enzymes could promote the recycling of degenerating plant and fungal materials through decomposition and solubilization of carbon and nitrogen compounds from senescing tissues, providing an efficient adaptive mechanism to low nutrient environments.

The expanded gene family (Table S4b) most represented in the symbiosis-upregulated transcriptome was the MFS. Nutrient exchange is at the core of both saprotrophic and mycorrhizal functioning, and expansion of this gene superfamily in ERM fungal evolution was probably 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.

The 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 AM and ECM 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 AM 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 the 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 with both 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 which may be involved in the manipulation of 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.L., 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 the experiments or sequencing, and collected, analyzed or interpreted the data; B.D.L., S.W.S. and J.W.S. gave access to nonpublished genomes; E. Martino, G-A.G., F.M.M. and S.P. wrote the manuscript.

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References

Abuzinadah RA, Read DJ. 1989. The role of proteins in the nitrogen nutrition of ectomycorrhizal plants. V. Nitrogen transfer in birch (*Betula pendula*) grown in association with mycorrhizal and nonmycorrhizal fungi. *New Phytologist* **112**: 61–68.

Alberton O, Kuyper TW, Richard C, Summerbell RC. 2010. Dark septate root endophytic fungi increase growth of Scots pine seedlings under elevated CO_2 through enhanced nitrogen use efficiency. *Plant and Soil* **328**: 459–470.

Allen TR, Millar T, Birch SM, Berbee ML. 2003. Culturing and direct DNA extraction find different fungi from the same ericoid mycorrhizal roots. *New Phytologist* **160**: 255–272.

Almario J, Jeena G, Wunder J, Langen G, Zuccaro A, Coupland G, Bucher M. 2017. Root-associated fungal microbiota of nonmycorrhizal *Arabis alpina* and its contribution to plant phosphorus nutrition. *Proceedings of the National Academy of Sciences, USA* **114**: E9403–E9412.

Averill C, Turner BL, Finzi AC. 2014. Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. *Nature* **505**: 543–545.

Balestrini R, Bonfante P. 2014. Cell wall remodeling in mycorrhizal symbiosis: a way towards biotrophism. *Frontiers in Plant Science* **5**: article 237.

Baral H-O, Krieglsteiner L. 2006. *Hymenoscyphus subcarneus*, a little known bryicolous discomycete found in the Bialowieza National Park. *Acta Mycologica Warszawa* **41**: 11–20.

Berch SM, Allen TR, Berbee ML. 2002. Molecular detection, community structure and phylogeny of ericoid mycorrhizal fungi. *Plant and Soil* **244**: 55–66.

Bergero R, Perotto S, Girlanda M, Vidano G, Luppi AM. 2000. Ericoid mycorrhizal fungi are common root associates of a Mediterranean ectomycorrhizal plant (*Quercus ilex*). *Molecular Ecology* **9**: 1639–1649.

Bonfante P, Vian B, Perotto S, Faccio A, Knox JP. 1990. Cellulose and pectin localization in roots of mycorrhizal *Allium porrum*: labeling continuity between host cell wall and interfacial material. *Planta* **180**: 537–547.

Bougoure DS, Cairney JWG. 2005. Assemblages of ericoid mycorrhizal and other root-associated fungi from *Epacris pulchella* (Ericaceae) as determined by culturing and direct DNA extraction from roots. *Environmental Microbiology* **7**: 819–827.

Britton T, Anderson CL, Jacquet D, Lundqvist S, Bremer K. 2007. Estimating divergence times in large phylogenetic trees. *Systematic Biology* **56**: 741–752.

Brundrett MC. 2002. Coevolution of roots and mycorrhizas of land plants. *New Phytologist* **154**: 275–304.

Cairney JWG, Meharg AA. 2003. Ericoid mycorrhiza: a partnership that exploits harsh edaphic conditions. *European Journal of Soil Science* **54**: 735–740.

Calvo AM, Cary JW. 2015. Association of fungal secondary metabolism and sclerotial biology. *Frontiers in Microbiology* **6**: 62.

Cantarel BL, Coutinho PM, Rancurel C, Bernard T, Lombard V, Henrissat B. 2009. The Carbohydrate-Active EnZymes database (CAZy): an expert resource for glycogenomics. *Nucleic Acids Research* **37**: D233–D238.

Carpenter RJ, Macphail MK, Jordan GJ, Hill RS. 2015. Fossil evidence for open, Proteaceae-dominated heathlands and fire in the Late Cretaceous of Australia. *American Journal of Botany* **12**: 2092–2107.

Cerniglia CE, Sutherland JB. 2010. Degradation of polycyclic aromatic hydrocarbons by fungi. In: Timmis KN, McGenity TJ, van der Meer JR, de Lorenzo V, eds. *Handbook of hydrocarbon and lipid microbiology*. Berlin, Germany: Springer, 2080–2110.

Chambers SM, Curlevski NJ, Cairney JWG. 2008. Ericoid mycorrhizal fungi are common root inhabitants of non-Ericaceae plants in a south-eastern Australian sclerophyll forest. *FEMS Microbiology Ecology* **65**: 263–270.

Clemmensen KE, Bahr A, Ovaskainen O, Dahlberg A, Ekblad A, Wallander H, Stenlid J, Finlay RD, Wardle DA, Lindahl BD. 2013. Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science* **339**: 1615–1618.

De Bie T, Cristianini N, Demuth JP, Hahn MW. 2006. CAFE: a computational tool for the study of gene family evolution. *Bioinformatics* **22**: 1269–1271.

Edmonds P, Cooney JJ. 1967. Identification of microorganisms isolated from jet fuel systems. *Applied Microbiology* **15**: 411–416.

Eijkelkamp BA, Pederick VG, Plumptre CD, Harvey RM, Hughes CE, Paton JC, McDevitt CA. 2016. The first Histidine Triad Motif of PhtD is critical for zinc homeostasis in *Streptococcus pneumoniae. Infection and Immunity* **84**: 407–415.

Eisenman HC, Casadevall A. 2012. Synthesis and assembly of fungal melanin. *Applied Microbiology and Biotechnology* **93**: 931–940.

English AC, Richards S, Han Y, Wang M, Vee V, Qu J, Qin X, Muzny DM, Reid JG, Worley KC *et al.* 2012. Mind the gap: upgrading genomes with Pacific Biosciences RS long-read sequencing technology. *PLoS One* **7**: e47768.

Enright AJ, Van Dongen S, Ouzounis CA. 2002. An efficient algorithm for large-scale detection of protein families. *Nucleic Acids Research* **30**: 1575–1584.

Fernandez CW, Langley JA, Chapman S, McCormack ML, Koide RT. 2016. The decomposition of ectomycorrhizal fungal necromass. *Soil Biology and Biochemistry* **93**: 38–49.

Fesel PH, Zuccaro A. 2016. Dissecting endophytic lifestyle along the parasitism/mutualism continuum in Arabidopsis. *Current Opinion in Microbiology* **32**: 103–112.

Freeze HH, Haltiwanger RS. 2009. Other classes of ER/Golgi-derived glycans. In: Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P, Bertozzi CR, Hart GW, Etzler ME, eds. *Essentials of glycobiology, 2nd edn.* Cold Spring Harbor, NY, USA: Cold Spring Harbor Laboratory Press, chapter 12. [WWW document] URL https://www.ncbi.nlm.nih.gov/books/NBK1947/ [accessed 16 March 2017].

Gnerre S, Maccallum I, Przybylski D, Ribeiro FJ, Burton JN, Walker BJ, Sharpe T, Hall G, Shea TP, Sykes S *et al.* 2011. High-quality draft assemblies of mammalian genomes from massively parallel sequence data. *Proceedings of the National Academy of Sciences, USA* **108**: 1513–1518.

Grelet GA, Johnson D, Paterson E, Anderson IC, Alexander IJ. 2009. Reciprocal carbon and nitrogen transfer between an ericaceous dwarf shrub and fungi isolated from *Piceirhiza bicolorata* ectomycorrhizas. *New Phytologist* **182**: 359–366.

Grelet GA, Johnson D, Vrålstad T, Alexander IJ, Anderson IC. 2010. New insights into the mycorrhizal *Rhizoscyphus ericae* aggregate: spatial structure and co-colonization of ectomycorrhizal and ericoid roots. *New Phytologist* **188**: 210–222.

Grigoriev IV, Nikitin R, Haridas S, Kuo A, Ohm R, Otillar R, Riley R, Salamov A, Zhao X, Korzeniewski F *et al.* 2014. MycoCosm portal: gearing up for 1000 fungal genomes. *Nucleic Acids Research* **42**: D699–D704.

Hacquard S, Kracher B, Hiruma K, Munch PC, Garrido-Oter R, Thon MR, Weimann A, Damm U, Dallery J-F, Hainaut M *et al.* 2016. Survival trade-offs in plant roots during colonization by closely related beneficial and pathogenic fungi. *Nature Communications* **7**: 11362.

Hambleton S, Sigler L. 2005. *Meliniomyces*, a new anamorph genus for root-associated fungi with phylogenetic affinities to *Rhizoscyphus ericae* (= *Hymenoscyphus ericae*), Leotiomycetes. *Studies in Mycology* **53**: 1–27.

Hardoim PR, van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A, Döring M, Sessitsch A. 2015. The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiology and Molecular Biology Reviews* **79**: 293–320.

van der Heijden MGA, Martin FM, Selosse MA, Sanders IR. 2015. Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytologist* **205**: 1406–1423.

Hobbie EA, Hobbie JE. 2008. Natural abundance of ^{15}N in nitrogen-limited forests and tundra can estimate nitrogen cycling through mycorrhizal fungi: a review. *Ecosystems* **11**: 815–830.

Hoegger PJ, Kilaru S, James TY, Thacker JR, Kües U. 2006. Phylogenetic comparison and classification of laccase and related multicopper oxidase protein sequences. *FEBS Journal* **273**: 2308–2326.

Johnson NC, Graham JH, Smith FA. 1997. Functioning of mycorrhizas along the mutualism–parasitism continuum. *New Phytologist* **135**: 1–12.

Kerley SJ, Read DJ. 1995. The biology of mycorrhiza in the Ericaceae. XVIII. Chitin degradation by *Hymenoscyphus ericae* and transfer of chitin-nitrogen to the host plant. *New Phytologist* **131**: 369–375.

Kerley SJ, Read DJ. 1997. The biology of mycorrhiza in the Ericaceae. XIX. Fungal mycelium as a nitrogen source for the ericoid mycorrhizal fungus *Hymenoscyphus ericae* and its host plants. *New Phytologist* **136**: 691–701.

Kernaghan G, Patriquin G. 2011. Host associations between fungal root endophytes and boreal trees. *Microbial Ecology* **62**: 460–473.

Kloppholz S, Kuhn H, Requena N. 2011. A secreted fungal effector of *Glomus intraradices* promotes symbiotic biotrophy. *Current Biology* **21**: 1204–1209.

Kohler A, Kuo A, Nagy LG, Morin E, Barry KW, Buscot F, Canbäck B, Choi C, Cichocki N, Clum A *et al.* 2015. Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. *Nature Genetics* **47**: 410–415.

Koizumi T, Nara K. 2017. Communities of putative ericoid mycorrhizal fungi isolated from alpine dwarf shrubs in Japan: effects of host identity and microhabitat. *Microbes and Environments* **32**: 147–153.

Kosola KR, Workmaster BAA, Spada PA. 2007. Inoculation of cranberry (*Vaccinium macrocarpon*) with the ericoid mycorrhizal fungus *Rhizoscyphus ericae* increases nitrate influx. *New Phytologist* **176**: 184–196.

Koukol O. 2011. New species of *Chalara* occupying coniferous needles. *Fungal Diversity* **49**: 75–91.

Kron KA, Judd WS, Stevens PF, Crayn D, Anderberg AA, Gadek P, Quinn CJ, Luteyn JL. 2002. Phylogenetic classification of Ericaceae: molecular and morphological evidence. *Botanical Review* **68**: 335–423.

Lallemand F, Gaudeul M, Lambourdière J, Matsuda Y, Hashimoto Y, Selosse M-A. 2016. The elusive predisposition to mycoheterotrophy in Ericaceae. *New Phytologist* **212**: 314–319.

Leonowicz A, Cho N-S, Luterek J, Wilkolazka A, Wojtas-Wasilewska M, Matuszewska A, Hofricheter M, Wesenberg D, Rogalski J. 2001. Fungal laccase: properties and activity on lignin. *Journal of Basic Microbiology* **41**: 185–227.

Li H, Benedito VA, Udvardi MK, Xuechun Zhao P. 2009. TransportTP: a two-phase classification approach for membrane transporter prediction and characterization. *BMC Bioinformatics* **10**: 418.

Marthey S, Aguileta G, Rodolphe F, Gendrault A, Giraud T, Fournier E, Lopez-Villavicencio M, Gautier A, Lebrun MH, Chiapello H. 2008. FUNYBASE: a FUNgal phYlogenomic dataBASE. *BMC Bioinformatics* **27**: 456.

Martin F, Kohler A, Murat C, Veneault-Fourrey C, Hibbett DS. 2016. Unearthing the roots of ectomycorrhizal symbioses. *Nature Reviews Microbiology* **14**: 760–773.

Martin J, Bruno VM, Fang Z, Meng X, Blow M, Zhang T, Sherlock G, Snyder M, Wang Z. 2010. Rnnotator: an automated *de novo* transcriptome assembly pipeline from stranded RNA-Seq reads. *BMC Genomics* **11**: 663.

Martino E, Turnau T, Girlanda M, Bonfante P, Perotto S. 2000. Ericoid mycorrhizal fungi from heavy metal polluted soils: their identification and growth in the presence of zinc ions. *Mycological Research* **104**: 338–344.

Massicotte HB, Melville LH, Peterson RL. 2005. Structural characteristics of root–fungal interactions for five ericaceous species in eastern Canada. *Canadian Journal of Botany* **83**: 1057–1064.

Nixon KC, Crepet WL. 1993. Late Cretaceous fossil flowers of ericalean affinity. *American Journal of Botany* **80**: 616–623.

Oksanen J, Kindt R, Legendre P, O'hara B, Simpson GL, Solymos P. 2009. Vegan: community ecology package. *R package version 1.15.4.* [WWW document] URL https://cran.r-project.org/web/packages/vegan/index.html [accessed 10 March 2016].

Pearson V, Read DJ. 1973. The biology of mycorrhiza in the Ericaceae. *New Phytologist* **72**: 1325–1331.

Pellegrin C, Morin E, Martin FM, Veneault-Fourrey C. 2015. Comparative analysis of secretomes from ectomycorrhizal fungi with an emphasis on small-secreted proteins. *Frontiers in Microbiology* **6**: 1278.

Peng R-H, Xiong A-S, Xue Y, Fu X-Y, Gao F, Zhao W, Tian Y-S, Yao Q-H. 2008. Microbial biodegradation of polyaromatic hydrocarbons. *FEMS Microbiology Reviews* **32**: 927–955.

Perotto S, Martino E, Abbà S, Vallino M. 2012. Genetic diversity and functional aspects of ericoid mycorrhizal fungi. In: Hock B, ed. *The Mycota, IX: fungal associations, 2nd edn.* Berlin, Germany: Springer, 255–285.

Perotto S, Peretto R, Faccio A, Schubert A, Bonfante P, Varma A. 1995. Ericoid mycorrhizal fungi: cellular and molecular basis of their interactions with the host plant. *Canadian Journal of Botany* **73**: 557–568.

Peter M, Kohler A, Ohm RA, Kuo A, Krützmann J, Morin E, Arend M, Barry KW, Binder M, Choi C *et al.* 2016. Ectomycorrhizal ecology is imprinted in the genome of the dominant symbiotic fungus *Cenococcum geophilum*. *Nature Communications* **7**: 12662.

Piercey MM, Thormann MN, Currah RS. 2002. Saprobic characteristics of three fungal taxa from ericalean roots and their association with the roots of *Rhododendron groenlandicum* and *Picea mariana* in culture. *Mycorrhiza* **12**: 175–180.

Plett JM, Martin F. 2015. Reconsidering mutualistic plant–fungal interactions through the lens of effector biology. *Current Opinion in Plant Biology* **26**: 45–50.

Ploner A. 2015. Heatplus: Heatmaps with row and/or column covariates and colored clusters. *R package version 2.24.0.* [WWW document] URL https://github.com/alexploner/Heatplus [accessed 20 December 2016].

Price AL, Jones NC, Pevzner PA. 2005. *De novo* identification of repeat families in large genomes. *Bioinformatics* **1**: i351–i358.

Qian XM, El-Ashker A, Kottke I, Oberwinkler F. 1998. Studies of pathogenic and antagonistic microfungal populations and their potential interactions in the mycorrhizoplane of Norway spruce (*Picea abies* (L.) Karst.) and beech (*Fagus sylvatica* L.) on acidified and limed plots. *Plant and Soil* **199**: 111–116.

Read DJ. 1974. *Pezizella ericae* sp. nov., the perfect state of a typical mycorrhizal endophyte of ericaceae. *Transactions of the British Mycological Society* **63**: 381–383.

Read DJ. 1996. The structure and function of the ericoid mycorrhizal root. *Annals of Botany* **77**: 365–374.

Read DJ, Leake JR, Perez-Moreno J. 2004. Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. *Canadian Journal of Botany* **82**: 1243–1263.

Read DJ, Stribley DP. 1973. Effect of mycorrhizal infection on nitrogen and phosphorus nutrition of ericaceous plants. *Nature* **244**: 81–82.

Redecker D, Kodner R, Graham LE. 2000. Glomelean fungi from the Ordovician. *Science* **289**: 1920–1921.

Rice AV, Currah RS. 2001. Physiological and morphological variation in *Oidiodendron maius*. *Mycotaxon* **79**: 383–396.

Rice AV, Currah RS. 2005. *Oidiodendron*: a survey of the named species and related anamorphs of *Myxotrichum*. *Studies in Mycology* **53**: 83–120.

Rice AV, Currah RS. 2006. *Oidiodendron maius*: saprobe in sphagnum peat, mutualist in ericaceous roots? In: Shulz BJE, Boyle CJC, Sieber TN, eds. *Microbial roots endophytes*. Berlin, Germany: Springer-Verlag, 227–246.

Rice AV, Tsuneda A, Currah RS. 2006. *In vitro* decomposition of *Sphagnum* by some microfungi resembles white rot of wood. *FEMS Microbiology Ecology* **56**: 372–382.

Rodriguez RJ, White JF, Arnold AE, Redman RS. 2009. Fungal endophytes: diversity and functional roles. *New Phytologist* **182**: 314–330.

Ryberg M, Matheny PB. 2012. Asynchronous origins of ectomycorrhizal clades of Agaricales. *Proceedings of the Royal Society B* **279**: 2003–2011.

Sadeghi M, Dehghan S, Fischer R, Wenzel U, Vilcinskas A, Kavousi HR, Rahnamaeian M. 2013. Isolation and characterization of isochorismate synthase and cinnamate 4-hydroxylase during salinity stress, wounding, and salicylic acid treatment in *Carthamus tinctorius*. *Plant Signaling & Behavior* **8**: e27335.

Schild DE, Kennedy A, Stuart MR. 1988. Isolation of symbiont and associated fungi from ectomycorrhizas of Sitka spruce. *European Journal of Forest Pathology* **18**: 51–61.

Schlegel M, Münsterkötter M, Güldener U, Bruggmann R, Duò A, Hainaut M, Henrissat B, Sieber CMK, Hoffmeister D, Grünig CR. 2016. Globally distributed root endophyte *Phialocephala subalpina* links pathogenic and saprophytic lifestyles. *BMC Genomics* **17**: 1015.

Schulz B, Boyle C. 2005. The endophytic continuum. *Mycological Research* **109**: 661–686.

Schwery O, Onstein RE, Bouchenak-Khelladi Y, Xing Y, Carter RJ, Linder HP. 2015. As old as the mountains: the radiations of the Ericaceae. *New Phytologist* **207**: 355–367.

Selosse M-A, Dubois M, Alvarez N. 2009. Do Sebacinales commonly associate with plant roots as endophytes? *Mycological Research* **113**: 1062–1069.

Selosse M-A, Setaro S, Glatard F, Richard F, Urcelay C, Weiss M. 2007. Sebacinales are common mycorrhizal associates of Ericaceae. *New Phytologist* **174**: 864–878.

Setaro S, Weiß M, Oberwinkler F, Kottke I. 2006. Sebacinales form ectendomycorrhizas with *Cavendishia nobilis*, a member of the Andean clade of the Ericaceae, in the mountain rain forest of southern Ecuador. *New Phytologist* **169**: 355–365.

Smith SE, Read D, eds. 1997. Ericoid mycorrhizas. In: *Mycorrhizal symbiosis, 2nd edn.* London, UK: Academic Press, 323–346.

Smith SE, Read D, eds. 2008. Ericoid mycorrhizas. In: *Mycorrhizal symbiosis, 3rd edn.* New York, NY, USA: Academic Press, 389–418.

Spanu PD, Abbott JC, Amselem J, Burgis TA, Soanes DM, Stuber K, Loren Ver, van Themaat E, Brown JK, Butcher SA *et al.* 2010. Genome expansion and gene loss in powdery mildew fungi reveal tradeoffs in extreme parasitism. *Science* **330**: 1543–1546.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**: 2688–2690.

Sturn A, Quackenbush J, Trajanoski Z. 2002. Genesis: cluster analysis of microarray data. *Bioinformatics* **18**: 207–208.

Tedersoo L, May TW, Smith ME. 2010. Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. *Mycorrhiza* **20**: 217–263.

Tedersoo L, Pärtel K, Jairus T, Gates G, Põldmaa K, Tamm H. 2009. Ascomycetes associated with ectomycorrhizas: molecular diversity and ecology with particular reference to the Helotiales. *Environmental Microbiology* **11**: 3166–3178.

Thormann MN. 2001. *The fungal communities of decomposing plants in southern boreal peatlands of Alberta, Canada*. PhD thesis, University of Alberta, Edmonton, AB, Canada.

Thormann MN, Currah RS, Bayley SE. 2002. The relative ability of fungi from *Sphagnum fuscum* to decompose selected carbon substrates. *Canadian Journal of Microbiology* **48**: 204–211.

Tisserant E, Malbreil M, Kuo A, Kohler A, Symeonidi A, Balestrini R, Charron P, Duensing N, Frei dit Frey N, Gianinazzi-Pearson V *et al.* 2013. Genome of an arbuscular mycorrhizal fungus provides insight into the oldest plant symbiosis. *Proceedings of the National Academy of Sciences, USA* **110**: 20117–20122.

Trong S, LaButti K, Foster B, Han C, Brettin T, Lapidus A. 2009. GapResolution: a software package for improving Newbler genome assemblies. In: *Proceedings of the 4th Annual Meeting on Sequencing Finishing, Analysis in the Future, 35, Report Number: LBNL-1899E Poster.* [WWW document] URL http://www.lanl.gov/conferences/finishfuture/2009index.shtml [accessed 26 October 2016].

Tsuneda A, Thormann MN, Currah RS. 2001. Modes of cell-wall degradation of *Sphagnum fuscum* by *Acremonium* cf. *curvulum* and *Oidiodendron maius*. *Canadian Journal of Botany* **79**: 93–100.

Tsuzuki S, Handa Y, Takeda N, Kawaguchi M. 2016. Strigolactone-induced putative secreted protein 1 is required for the establishment of symbiosis by the arbuscular mycorrhizal fungus *Rhizophagus irregularis*. *Molecular Plant–Microbe Interactions* **29**: 277–286.

Villarreal-Ruiz L, Anderson IC, Alexander IJ. 2004. Interactions between an isolate from the *Hymenoscyphus ericae* aggregate and roots of *Pinus* and *Vaccinium*. *New Phytologist* **164**: 183–192.

Villarreal-Ruiz L, Neri-Luna C, Anderson IC, Alexander IJ. 2012. *In vitro* interactions between ectomycorrhizal fungi and ericaceous plants. *Symbiosis* **56**: 67–75.

Vohník M, Mrnka L, Lukešová T, Bruzone MC, Kouhout P, Fehrer J. 2013. The cultivable endophytic community of Norway spruce ectomycorrhizas from

microhabitats lacking ericaceous hosts is dominated by ericoid mycorrhizal *Meliniomyces variabilis*. *Fungal Ecology* **6**: 281–292.

Vrålstad T, Fossheim T, Schumacher T. 2000. *Piceirhiza bicolorata* – the ectomycorrhizal expression of the *Hymenoscyphus ericae* aggregate? *New Phytologist* **145**: 549–563.

Wang Z, Binder M, Schoch CL, Johnston PR, Spatafora JW, Hibbett DS. 2006. Evolution of helotialean fungi (Leotiomycetes, Pezizomycotina): a nuclear rDNA phylogeny. *Molecular Phylogenetics and Evolution* **41**: 295–312.

Wei X, Chen J, Zhang C, Pan D. 2016. A new *Oidiodendron maius* strain isolated from *Rhododendron fortunei* and its effects on nitrogen uptake and plant growth. *Frontiers in Microbiology* **7**: 1327.

Weiß M, Waller F, Zuccaro A, Selosse M-A. 2016. Sebacinales – one thousand and one interactions with land plants. *New Phytologist* **211**: 20–40.

Zhang N, Wang Z. 2015. Pezizomycotina: Sordariomycetes and Leotiomycetes. In: McLaughlin DJ, Spatafora JW, eds. *Systematics and evolution: the Mycota VII part B, 2nd edn.* Berlin/Heidelberg, Germany: Springer-Verlag, 57–88.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Fig. S1 Genome size, gene number and repeated elements (REs) in the 60 fungi analysed.

Fig. S2 Orthology assignment of the 25 Ascomycete genomes.

Fig. S3 Total and secreted CAZymes (Carbohydrate Active enZymes) 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 (Carbohydrate Active enZyme) coding gene numbers in the 60 genomes.

Fig. S6 Ericoid fungi symbiosis-induced genes.

Fig. S7 Sequence conservation and functional analysis of *Oidiodendron maius* symbiosis-induced genes in the 59 genomes.

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 Leotiomycetes and ericoid fungi-specific proteins and protein families; TOP 14 ericoid fungi expanded families (CAFE analysis) as compared with the other 16 genomes

Table S5 Total and secreted CAZymes (Carbohydrate Active enZymes) in the 60 fungi with Mann–Whitney test analyses and Bonferroni correction

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 with the other 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-upregulated gene categories in ericoid fungi

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

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

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

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

Table S15 Comparison of total and secreted CAZyme (Carbohydrate Active enZyme), protease and lipase coding gene numbers between ericoid fungi and ectomycorrhizal ascomycetes, and between ectomycorrhizal basidiomycetes and ectomycorrhizal ascomycetes, with *t*-test analyses

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

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See also the Commentary on this article by Selosse *et al.*, **217**: 968–972.