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Research

Diversity of genomic adaptations to the post-fire environment in Pezizales fungi points to crosstalk between charcoal tolerance and sexual development

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Summary

- Wildfires drastically impact the soil environment, altering the soil organic matter, forming pyrolyzed compounds, and markedly reducing the diversity of microorganisms. Pyrophilous fungi, especially the species from the orders Pezizales and Agaricales, are fire-responsive fungal colonizers of post-fire soil that have historically been found fruiting on burned soil and thus may encode mechanisms of processing these compounds in their genomes.
- Pyrophilous fungi are diverse. In this work, we explored this diversity and sequenced six new genomes of pyrophilous Pezizales fungi isolated after the 2013 Rim Fire near Yosemite Park in California, USA: *Pyronema domesticum*, *Pyronema omphalodes*, *Tricharina praecox*, *Geopyxis carbonaria*, *Morchella snyderi*, and *Peziza echinospora*.
- A comparative genomics analysis revealed the enrichment of gene families involved in responses to stress and the degradation of pyrolyzed organic matter. In addition, we found that both protein sequence lengths and G + C content in the third base of codons (GC3) in pyrophilous fungi fall between those in mesophilic/nonpyrophilous and thermophilic fungi.
- A comparative transcriptome analysis of *P. domesticum* under two conditions – growing on charcoal, and during sexual development – identified modules of genes that are co-expressed in the charcoal and light-induced sexual development conditions. In addition, environmental sensors such as transcription factors STE12, LreA, LreB, VosA, and EsdC were upregulated in the charcoal condition.
- Taken together, these results highlight genomic adaptations of pyrophilous fungi and indicate a potential connection between charcoal tolerance and fruiting body formation in *P. domesticum*.

Introduction

Forest fires are increasing in frequency in the western United States and other parts of the world due to a lack of fire suppression strategies, global warming, and drought (Belval *et al.*, 2017; Chen, 2022). Wildfires drastically impact the soil environment. At the soil surface, with temperatures of 220–450°C, the fire causes the soil organic matter to undergo significant changes (Fernández *et al.*, 1997; Santín *et al.*, 2016), such as an increase in black carbon and pyrolyzed organic matter (PyOM) – a mix of polyaromatic hydrocarbons (Almendros *et al.*, 1992; Bird *et al.*, 2015). At 5 cm depth, soil temperatures usually do not rise higher than 150°C, and soils remain at an ambient temperature below 15–30 cm unless there are large piles of fuel (Bruns *et al.*, 2020). Above 120°C, there is an increased release of soluble sugars due to the pyrolysis of microbial biomass and plant litter (Knicker *et al.*, 2005; Bruns *et al.*, 2020). Present in lower concentrations in the soil, carbohydrates are denatured to a greater degree than waxes and cutins, and the abundance of short-chain lipids increases (Mainwaring *et al.*, 2013; Chen *et al.*, 2020).

Along with abiotic changes in the soil, the biomass of soil microorganisms reduces markedly after fire (Dove & Hart, 2017;

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Pressler *et al.*, 2019; Fox *et al.*, 2022). Most soil microorganisms are killed in the temperature range of 70–80°C, and the disruption of cellular components, including membrane lipids, nucleic acids, and proteins, occurs just above 100°C (Cerda, 2009). Therefore, most microorganisms combust after fire (combustion level depends on fire intensity and duration) in the top 5 cm of soil, and their abundance can decrease by half in the 5–10 cm of soil directly below the surface layer (Prieto-Fernández *et al.*, 1998; Knicker *et al.*, 2005). This killing effect of the heating of the soil creates a predicable necromass zone (Bruns *et al.*, 2020) that is rich in sugars, small peptides, amino acids, lipids and fatty acids, Krebs cycle intermediates, and any other minor nutrients and easily mineralizable forms of carbon newly released from the lysed membranes of the pyrolyzed microbial necromass. In general, fire-heated soils exhibit a more significant reduction in the fungal population than the bacterial population (Bååth *et al.*, 1995; Pressler *et al.*, 2019). This reduction opens up the soil environment to colonization by a group of fungi adapted to rapidly colonizing such environments.

Pyrophilous fungi have historically only been found fruiting on burned soil, and they are known fire-responsive fungal colonizers of post-fire soil (Bruns *et al.*, 2020). Many of these fungi are Ascomycota of the Pezizales order (El-Abyad & Webster, 1968; Hughes *et al.*, 2020), although some Basidiomycota are also represented in the pyrophilous guild (Steindorff *et al.*, 2021). These fungi are known to fruit in a predictable order after fire, and observations of this phenomenon have been made at various sites world-wide (Petersen, 1970; Warcup, 1990; Bruns *et al.*, 2020; Fischer *et al.*, 2021). It has been suggested that the post-fire succession of pyrophilous fungi is a result of competitive interactions in which the species that appear later dominate earlier colonizers (Wicklow & Hirschfield, 1979).

In this work, we discuss the diversity of pyrophilous fungi based on published literature, provide the first report of six genomes of pyrophilous Pezizales fungi: *Pyronema domesticum*, *Pyronema omphalodes*, *Tricharina praecox*, *Geopyxis carbonaria*, *Morchella snyderi*, and *Peziza echinospora*, and compare the genetic content and structure of these fungi to those of other relevant ecologically defined fungal groups. *Wilcoxina mikolae* was also identified in our work, but we used the genome sequenced in a different study (Miyauchi *et al.*, 2020). In addition, we compare the transcriptome of *P. domesticum* under two different conditions: growing on charcoal, and during light-induced sexual development. In our previous work studying convergent features of Basidiomycetes pyrophilous fungi (Steindorff *et al.*, 2021), we found an enrichment/expansion of developmental genes and carbohydrate-active enzyme (CAZy) families involved in the degradation of plant biomass. Here we show that pyrophilous fungi from both Ascomycota and Basidiomycota share genomic features such as elevated G + C content at the third codon position (GC3) and increased average protein sequence length, and are enriched in CAZymes (lytic polysaccharide monooxygenases (LPMOs), xylanases, glucanases, and esterases); however, there are differences between the two, since they colonize the soil at different time periods following fire events.

Materials and Methods

Isolation of pyrophilous fungi and nucleic acid extraction

We collected the six pyrophilous fungi in this study as ascocarps from the surface of burned soil in the Stanislaus National Forest at the site of the Rim Fire of August 2013. The fungi samples were collected starting in November 2013, after the first rainfall (*Pyronema domesticum* (Sowerby) Sacc., *Pyronema omphalodes* (Bull.) Fuckel, *Wilcoxina mikolae* (H.E. Wilcox, Chin S. Yang & Korf), and *Tricharina praecox* (P. Karst.) Dennis), and through April/May of 2014 (*Peziza echinospora* (P. Karst.), *Geopyxis carbonaria* (Alb. & Schwein.) Sacc., and *Morchella snyderi* (M. Kuo & Methven)). The soil type in that area is in the Holland series, which consists of very deep, well-drained soils formed in material weathered from granitic rock (https://casoilresource.lawr.ucdavis.edu/gmap/), and the pine-needle duff layer was completely burned off during the fire. Live tissue was isolated from surface-sterilized (using H_2O_2) pieces of fruiting bodies, which were grown on cornmeal yeast malt (CMYM) agar medium with antibiotics (50 mg l^{-1} streptomycin sulfate and 50 mg l^{-1} chloramphenicol), and replated onto CMYM medium without antibiotics. The samples were grown in Petri dishes on cellophane over CMYM agar medium at 25°C for 3–14 d, depending on the growth rate, and were allowed to experience light/dark cycles. The samples were ground in liquid nitrogen and frozen at −80°C.

DNA was extracted using the Qiagen Monarch Genomic DNA Purification Kit (Qiagen). DNA fragments were size-selected using the AMPure XP (> 10 kbp) bead cleanup kit (Beckman Coulter, Indianapolis, IN, USA) (*T. praecox*, *G. carbonaria*, *M. snyderi*, and *P. echinospora*) or Blue Pippin (> 7 kbp) (Sage Science Inc., Beverly, MA, USA) (*P. domesticum*, *P. omphalodes*). RNA extraction was performed using the 'Fleming method' (Fleming *et al.*, 1998; Sessitsch *et al.*, 2002), modified using a solution of 50% dH_2O and 50% Buffer RLT from the Qiagen RNeasy Mini Kit (Qiagen) as a lysis buffer and precipitated using lithium chloride.

Genome sequencing and assembly

All genomes were sequenced using the Pacific Biosciences (PacBio, Menlo Park, CA, USA) platform at the US Department of Energy Joint Genome Institute (JGI). Unamplified libraries were generated using the PacBio standard template preparation protocol for creating > 10 kb libraries. Five micrograms of gDNA (10 µg for the Blue Pippin protocol) was used to generate each library, and the DNA was sheared using g-Tubes (Covaris, Woburn, MA, USA) to generate sheared fragments of > 10 kb in length. The sheared DNA fragments were then prepared using the PacBio SMRTbell Template Preparation Kit. The fragments underwent DNA damage repair – their ends were repaired so that they were blunt-ended and 5′ phosphorylated. Pacific Biosciences hairpin adapters were then ligated to the fragments to create the SMRTbell template for sequencing. The SMRTbell templates were then purified using exonuclease treatments and size-selected using AMPure PB beads. For the Blue Pippin protocol, the SMRTbell templates

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were size-selected using the Sage Science BluePippin instrument with a 4 kb or 7 kb lower cutoff, depending on DNA quality. In both protocols, PacBio Sequencing Primer was then annealed to the SMRTbell template library, and sequencing polymerase was bound to them using Sequel Binding Kit 2.0. The prepared SMRTbell template libraries were then sequenced on a PacBio Sequel sequencer using v.3 sequencing primer, 1 M v.2 SMRT cells, and v.2.0 (2.1 for the Blue Pippin protocol) sequencing chemistry with 6- and 10-h sequencing movie run times.

The genomes were assembled using Falcon v.1.8.8 (Chin *et al.*, 2016). For the two *Pyronema* species, we used the bbduck.sh script in BBtools to remove bacterial contaminants (*Paenibacillus* sp., which are often present in fungal samples), relying on *k*-mer matching (*k*=25 mm=f mkf=0.05) (https://sourceforge.net/projects/bbtools/). The mitochondria were assembled separately from the Falcon pre-assembled reads (preads), using an in-house tool (assemblemito.sh) to filter the preads, and polished with 'Arrow' in SMRTLink v.5.0.1.9578 (https://github.com/PacificBiosciences/GenomicConsensus). A secondary Falcon assembly was generated using the mitochondria-filtered preads, improved with FinisherSC v.2.1 (Lam *et al.*, 2015), and polished with 'Arrow'.

Transcriptome sequencing and assembly for genome annotation

For all transcriptomes combined across growth conditions (cellophane over CMYM agar medium at 25°C for 3–14 d, depending on the growth rate, under light and dark conditions) and used to support genome annotation in this study, stranded cDNA libraries were generated using the Illumina (San Diego, CA, USA) Truseq Stranded RNA LT Kit. mRNA was purified from 1 µg of total RNA using magnetic beads containing poly-T oligos, and was fragmented and reversed transcribed using random hexamers and SSII (Invitrogen) followed by second-strand synthesis. The fragmented cDNA was treated with end-pair, A-tailing, adapter ligation, and eight cycles of polymerase chain reaction (PCR). The prepared library was quantified using KAPA Biosystems' (Wilmington, MA, USA) next-generation sequencing library quantitative polymerase chain reaction (qPCR) kit and run on a Roche LightCycler 480 real-time PCR instrument. The quantified library was then multiplexed with other libraries, and the pool of libraries was then prepared for sequencing on the Illumina HiSeq sequencing platform utilizing a TruSeq paired-end cluster kit, v.4, and Illumina's cBot instrument to generate a clustered flow cell for sequencing. Sequencing of the flow cell was performed on the Illumina HiSeq 2500 sequencer using HiSeq TruSeq SBS sequencing kits, v.4, following a 2 × 150 bp indexed run recipe. For *G. carbonaria*, the pool of libraries was prepared for sequencing on the Illumina NovaSeq 6000 sequencing platform using NovaSeq XP v.1 reagent kits, S4 flow cell, following a 2 × 150 bp indexed run recipe. Using BBDuk (https://sourceforge.net/projects/bbmap/), raw reads were evaluated for artifact sequence by kmer matching (kmer = 25), allowing one mismatch, and the detected artifact was trimmed from the 3′ end of the reads. RNA spike-in reads, PhiX reads, and reads containing any Ns were removed. Quality trimming was performed using the 'Phred' trimming method set at Q6. Finally, following trimming, reads under the length threshold were removed (minimum length 25 bases or 1/3 of the original read length – whichever is longer). Filtered Fastq files were used as input for *de novo* assembly of RNA contigs. Reads were assembled into consensus sequences using Trinity (v.2.3.2) (Grabherr *et al.*, 2011). Trinity was run with the --normalize_reads (*in-silico* normalization routine) and --jaccard_clip.

Genome annotation and principal component analysis

All genomes were annotated using the JGI Annotation Pipeline (Grigoriev *et al.*, 2014), which combines several gene predictions and annotation methods with transcriptomics data and integrates the annotated genomes into MycoCosm (https://mycocosm.jgi.doe.gov), a web-based fungal resource for comparative analysis (Grigoriev *et al.*, 2014). Completeness of genome annotation was assessed using Busco v.4.0.6 (Simão *et al.*, 2015), with the ascomycetes_odb10 database. Functional annotation was done using Interproscan v.5.35–74.0. Carbohydrate-active enzymes were annotated using the CAZy annotation pipeline (Lombard *et al.*, 2014). Gene count heatmaps were created using the 'heatmap.2' function of R/gplots, with *z*-score normalization. Hierarchical clustering with Euclidean distance and average-linkage clustering was carried out on copy-number using the 'hclust' function in R. Phylogenetic principal component analysis (phylo-PCA) on CAZyme and Pfam copy numbers was performed on the phylogenetic size-corrected dataset, followed by PCA using the 'phyl.pca' function from Phytools (Revell, 2009). A copy number matrix normalized by proteome size and the species tree were used as input data. Independent contrasts were calculated under the Brownian motion model and the parameter mode 'cov'.

Comparative genomics and gene family analyses

We used OrthoFinder v.2.5.1 (Emms & Kelly, 2015) to identify orthogroups for the 18 Pezizales species retrieved from Mycocosm (Grigoriev *et al.*, 2014) (Supporting Information Table S6). Markov clustering was applied to generate a network graph with Mcl v.14.137 and create initial clusters with a default inflation factor ($I = 1.5$). OrthoFinder generated a multiple sequence alignment (MSA) for all orthogroups with Mafft v.7.312 (Katoh & Standley, 2013), inferred a gene tree with FastTree v.2.1 (Price *et al.*, 2010), and used them to refine the orthogroups. To build the species tree, we used 2057 single-copy orthogroups from OrthoFinder. The protein sequences in each orthogroup were aligned using Mafft (---maxiterate 1000 --globalpair) and trimmed using TrimAl v.1.4.rev22 (-gt 0.3). All the filtered MSAs were concatenated. FastTree inferred the species tree (-spr 4 -mlacc 2 -slownni -gamma). We used Computational Analysis of Gene Family Evolution (Cafe) v.4.2.1 (De Bie *et al.*, 2006) to study the gene expansions of the orthogroups along with the evolutionary history. Cafe requires two main inputs: the gene counts in the orthogroups and an ultrametric species tree. We directly used the gene counts from OrthoFinder. Only the gene families

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for which $P < 0.01$ were considered for further analysis. The Viterbi method was applied to them to compute branch-specific *P*-values to detect rapid expansions and contractions at each node. The enrichment of Pfam domains and orthologous clusters was tested using a two-tailed Fisher's exact test (FET) and corrected for multiple testing using the Benjamini–Hochberg method in PYTHON v.3.6 using the modules PANDAS v.1.1.5, NUMPY v.1.20.2 and SCIPY v.1.5.2. Calculations of protein length and GC3 content, and Mood's median nonparametric test (Brown & Mood, 1951), were performed using an in-house PYTHON script.

Differential expression analysis

In addition to the transcriptomics experiments described earlier and used only for genome annotation, we performed differential gene expression analysis using previously published data for two species: *P. domesticum* and *P. omphalodes*. The FASTQ files were downloaded from GenBank – Bioproject PRJNA662999. *Pyronema domesticum* was grown for 4 d with different 1.5% agar plate treatments: sucrose (20 g l^{-1}) minimal medium, water agar, pyrolyzed soil from Illilouette Creek Basin (10 g l^{-1}), or 750°C-burned white-pine char (10 g l^{-1}). Three independent replicates were sequenced for each sample (Fischer *et al.*, 2021). The second dataset described the transcriptional profile of *P. omphalodes* (syn. *Pyronema confluens*) during its white-light-induced sexual development (Traeger *et al.*, 2013) – Bioproject PRJNA177769: the samples sex-dev (sexual development – GSM1020388 and GSM1020389) and veg (growth in darkness, no sexual development – GSM1020390 and GSM1020391). In both experiments, the fungi were grown for 4 d on agar plates, RNAs were isolated from surface mycelium/fruiting bodies, and two independent biological replicates were sequenced. Quality trimming was performed using TRIMMOMATIC v.0.36 (Bolger *et al.*, 2014) (ILLUMINACLIP:TruSeq3-PE.fa:2:30:10:2:keepBoth Reads LEADING:15 TRAILING:15 SLIDINGWINDOW:4:15 MINLEN:50). Filtered reads from each library were aligned to the reference genome using HISAT2 v.2.1.0 (Kim *et al.*, 2019), and 'featureCounts' was used to generate the raw gene counts. DESEQ2 v.1.18.1 (Love *et al.*, 2014) was subsequently used to determine differentially expressed genes in the charcoal, soil, and water conditions in comparison to sucrose as a control, and sex-dev to veg as a control. A gene is considered differentially expressed when the adjusted *P*-value < 0.05.

The gene co-expression network was calculated across expression profiles for the six samples (charcoal, soil, water, sucrose, sex-dev, and veg) using the R package WGCNA (Langfelder & Horvath, 2008). After filtering genes out due to low expression across > 95% of all conditions, 9759 genes were used in correlation analysis. The correlations were scaled using soft power of 9, assuming a scale-free network. Hierarchical clustering was applied to identify co-expressed gene modules with a minimum cluster size of 30 genes. The network was visualized using CYTOSCAPE. Scaled correlations between gene pairs > 0.2 are represented as edges in the network. Functional enrichment for the gene clusters was performed using gene ontology (GO) assignments. The *P*-values for enrichment were calculated using the one-tailed hypergeometric test and adjusted for multiple hypothesis testing using the Benjamini–Hochberg correction.

Results

Phylogeny and functional annotation of pyrophilous fungi

The genomes of six pyrophilous species were sequenced using long-read PacBio technology, assembled into 41–701 scaffolds with lengths ranging from 38–117 Mbp, and annotated with predicted 10 413–13 093 gene models. BUSCO (Simão *et al.*, 2015) analysis showed 96.3–98.2% completeness using the Ascomycetes dataset (Table 1).

We reconstructed the phylogeny from 2057 single-copy orthologs (1011 944 amino acid positions) using 18 representative Pezizales taxa and *Trichoderma reesei* (Hypocreales) as the outgroup (Fig. 1a). With high bootstrap support (all nodes 100%), the tree shows a topology consistent with the Pezizales order. In our study, five of the seven pyrophilous fungi belong to the Pyronemataceae family, and the other two, *P. echinospora* and *M. snyderi*, to the Pezizaceae and Morchellaceae families, respectively. Regarding genomic metrics among the Pyronemataceae fungi, only *W. mikolae* showed a larger genome size (117.29 Mb) with 55.8% of repetitive content, comparable with other ectomycorrhizal truffle genomes (Fig. 1b). *Tricharina praecox* showed the smallest genome within this dataset (38.36 Mb), 6.9% of which consisted of repetitive elements. Other than these two cases, pyrophilous assemblies showed similar metrics of size and numbers of repeats compared to their close relatives.

In the pangenome distribution (Fig. 1c), the homologous gene set that was found in all 19 species (blue in the bar plot in Fig. 1c) showed a low variance (mean ± SD = 4145 ± 131) among all genomes. The number of unique genes positively correlates with proteome size ($R^2 = 0.88$, $P < 0.05$). Pyrophilous proteomes showed fewer unique genes compared to nonpyrophilous proteomes (2376 ± 800 and 3949 ± 2409, respectively; Wilcoxon $P = 0.0562$); however, this finding was not statistically significant. The functional annotations normalized by the proteome size (Fig. 1d) show that the underlying nutritional mode (saprotroph and ectomycorrhizal) is the main driving force of the functional distribution.

Period of post-fire soil colonization is reflected in pyrophilous genomes

The soil colonization after fire events happens in succession – not all of the species grow simultaneously (Bruns *et al.*, 2020; Hughes *et al.*, 2020). We considered pyrotolerant fungi opportunists that occupy, at a later stage than pyrophilous fungi, substrates and habitats made available by fires (Fox *et al.*, 2022). This colonization process is represented in Fig. 2, in which the species used in this work are divided into two groups: four 'early colonizers' (Pyrdom1, *P. domesticum*; Pyrom1, *P. omphalodes*; Tripra1, *T. praecox*; Wilmi1, *W. mikolae*), which appear in the first couple of months after the fire event, and three 'late colonizers' (Pezech1,

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Table 1 Summary statistics for pyrophilous genomes.

Species	Assembly size (Mbp)	No. of contigs	Coverage	N50	L50 (Mbp)	No. of genes	Busco (%)
Pyronema domesticum, CBS 144463	58.39	78	74.15×	13	1.47	11 812	97.10
Pyronema omphalodes, CBS 144459	61	260	70.21×	30	0.71	10 940	97.10
Tricharina praecox, CBS 144465	38.36	104	54.25×	12	1.007	10 413	95.60
Geopyxis carbonaria, CBS 144460	49.45	116	289.9×	21	0.91	11 663	97.30
Morchella snyderi, CBS 144464	54.77	81	160.8×	15	1.25	11 100	98.20
Peziza echinospora, CBS 144458	63.77	241	161.4×	15	1.19	10 991	96.30
Wilcoxina mikolae, CBS 423.85	117.29	1604	95×	67	0.48	13 093	96.40

Busco, percentage of complete Busco models using the Ascomycetes dataset. N50, count of smallest number of contigs whose length sum makes up half of genome size. L50, weighted median statistic such that 50% of the entire assembly is contained in scaffolds equal to or larger than this value. *Wilcoxina mikolae* was sequenced in a different study (Miyauchi *et al.*, 2020).

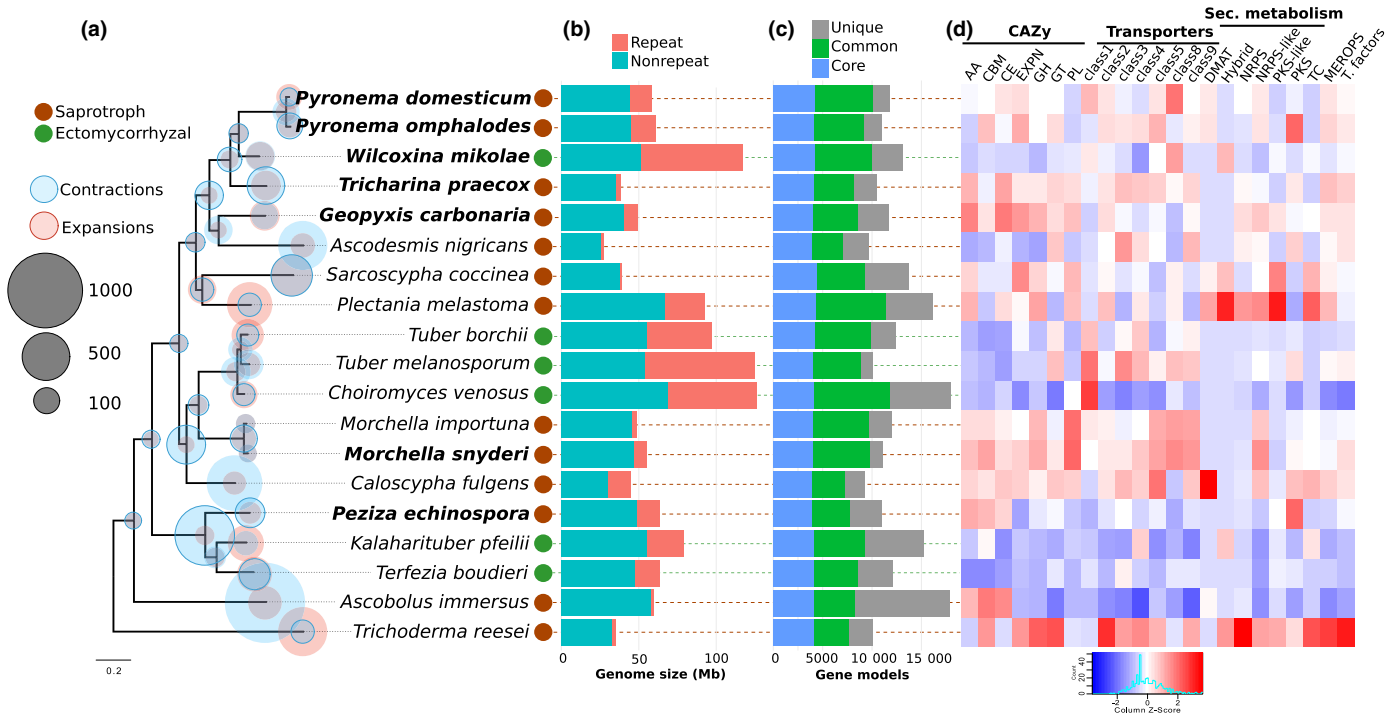

Fig. 1 Genome features of fire-associated and other Pezizales fungi. (a) Maximum likelihood tree showing the phylogenetic relationships based on 2057 single-copy orthologs and their respective nutritional mode. Pyrophilous species are represented in bold. All support values are 100%. Circles in the nodes represent all family-wide gene expansions (light red) and contractions (blue) according to Cafe (De Bie *et al.*, 2006). (b) Genome size in Mbp (1000 000 base pairs), showing the distribution of repeats and nonrepeat content. (c) Gene model counts of each genome are divided into core genes (present in all genomes), common genes (present in two or more genomes), and unique genes (exclusively found on that genome). (d) Column *z*-score heatmap of functional annotations normalized by total proteome size.

P. echinospora; Geocar1, *G. carbonaria*; Morsny1, *M. snyderi*), which appear after the transition shown in Fig. 2. An exception is the conifer ectomycorrhizal *W. mikolae* (Fig. 2), which is present in the early stages of post-fire colonization (Bruns *et al.*, 2020; Miyauchi *et al.*, 2020) but disappears rapidly due to the death of its plant host.

In order to find species-specific genomic features, we performed an enrichment analysis of Pfam domains of unique genes (shown in grey in the barplot in Fig. 1c) on each pyrophilous species individually ($P < 0.05$, Fisher's exact test, Benjamini–Hochberg adjusted *P*-values, abbreviated as FET). This analysis showed only one enriched domain, the collagen triple helix repeat (PF01391) in *P. omphalodes* (Table S1). All species-wide enrichment tests identified ankyrin-related domains (PF12796, PF13606, PF13637, and PF13857).

To infer enriched functional gene families in pyrophilous fungi, we analyzed all Pfam domain counts and homologous protein groups generated with Markov Clustering Algorithm (Mcl) across 19 Pezizomycetes genomes. Among the Pfam domains in early and late colonizers, 11 and 3 were overrepresented, respectively, and 9 were underrepresented in both cases (FET, $P < 0.05$) (Table S2). In the early colonizers, the most overrepresented domains were CBM14 (PF01607), arthropod defensin (PF01097), collagen triple helix repeat (PF01391), STAND

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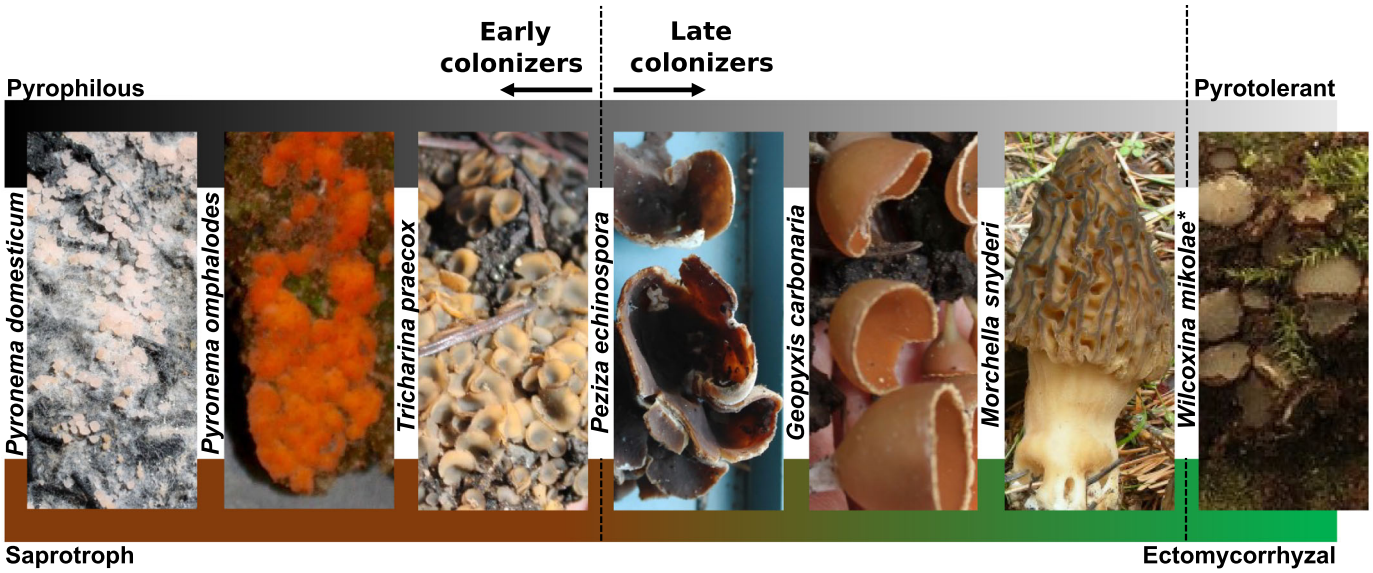

Fig. 2 Early and late pyrophilous colonizers. The brown–green bar shows the underlying lifestyle of these species in a mix of saprotrophic and ectomycorrhizal fungi. Similarly, the black–grey bar shows a gradient of pyrophily (i.e. fungi that are present in the early stages of post-fire soil colonization), and pyrotolerance (i.e. fungi that colonize the soil months after fire events). *Wilcoxina mikolae** is an exception to this trend and is considered an early colonizer since it was found in the first 2 wk after fire (Bruns *et al.*, 2020).

proteins (PF17111), and SUN β-glucosidase (PF03856). In the late colonizers, the cellulose-binding module 1 – CBM1 (PF00734) and AA9 – formerly GH61 (PF03443) were overrepresented. There were no overlaps between overrepresented Pfam domains, suggesting the colonization stage may be correlated with phylogenetic proximity and consequently with the species' Pfam contents. The same enrichment analysis using MCL counts (Table S3) revealed 316 and 171 enriched clusters in early and late colonizers, respectively, and the majority of clusters showed no functional annotations (early – 77%, late – 89%). The clusters uniquely enriched for the early colonizers included three CAZymes clusters (CBM14, cutinase CE5, and SUN β-glucosidase GH132) and other clusters with functional annotations such as STAND proteins (PF17111), methyltransferase (PF13489), ankyrins (PF12796), fungal lectin (PF07938), heterokaryon incompatibility (HET) (PF06985), CFEM (PF05730), arthropod defensin (PF01097), BTB/POZ (PF00651), F-box (PF00646), cysteine-rich secretory protein (PF00188), transcription factors (PF00172, PF00096), proteases (PF00082, PF00026), protein kinase (PF00069), p450 (PF00067), and heat-shock proteins (PF04119, PF00012). Interestingly, clusters containing hsp70 (PF00012), STAND proteins (PF17111), and ankyrins (PF12796) were enriched in both early and late colonizers (Table S2). In late colonizers, we found enriched CAZymes, an LPMO (AA9) and CMB1.

The nodes of the phylogenetic tree in Fig. 1(a) show the number of gene families expanded and contracted (family-wide $P \leq 0.01$) using the program CAFE (De Bie *et al.*, 2006). Among the pyrophilous fungi, only *P. domesticum* and *G. carbonaria* showed higher ratios of expansions : contractions (4.19 and 1.22, respectively). For the rapidly evolving families (Viterbi $P \leq 0.01$) in the pyrophilous genomes (Table S4), only *P. omphalodes* showed a ratio of expansions : contractions < 1, totaling 114 expanded and 56 contracted families. Among the expanded gene families, we found CAZymes and other enzymes potentially involved in the degradation of PyOM, such as LPMO (AA9 – Pezech1), gluco/chitooligosaccharide oxidase (AA7_dist – Pezech1), endoglucanase (GH6-CMB1 – Pezech1), GMC oxidoreductase (AA3_2 – Pyrdom1, Pyrom1), cutinase (CE5 – Tripra1), cytochrome p450 (Tripra1, Pyrdom1), endo-β-1,4-glucanase (GH7 – Wilmi1), and xylanase (GH10-CBM1 – Wilmi1).

Pyrophilous fungi have a larger protein sequence length and GC3 content

Since both Basidiomycetes and Ascomycetes are found fruiting in post-fire environments (Bruns *et al.*, 2020), we hypothesize that some genomic features and adaptations might be shared across these distantly related fungi. Compared to closely related fungi belonging to the same class (Table S5), we found that the median protein sequence length of the whole proteome in pyrophilous species is significantly larger ($P = 0.028$ for Agaricales; $P = 0.031$ for Pezizales) than that in nonpyrophilous fungi (Fig. 3a) using Mood's median nonparametric test (Brown & Mood, 1951). We also found that the median protein sequence length for pyrophilous species lies between that of nonpyrophilous and thermophilic species. Since thermophiles evolved to live at higher temperatures (Berka *et al.*, 2011), we hypothesize that this feature might be related to survival under the higher temperature conditions below the soil during fire, helping the pyrophilous fungi to be the first colonizers.

We also compared the GC content in the third base of codons (GC3) to assess whether there is a difference between pyrophilous, nonpyrophilous, and thermophilic species, as found by Berka *et al.* (2011) for mesophiles vs thermophiles. Among

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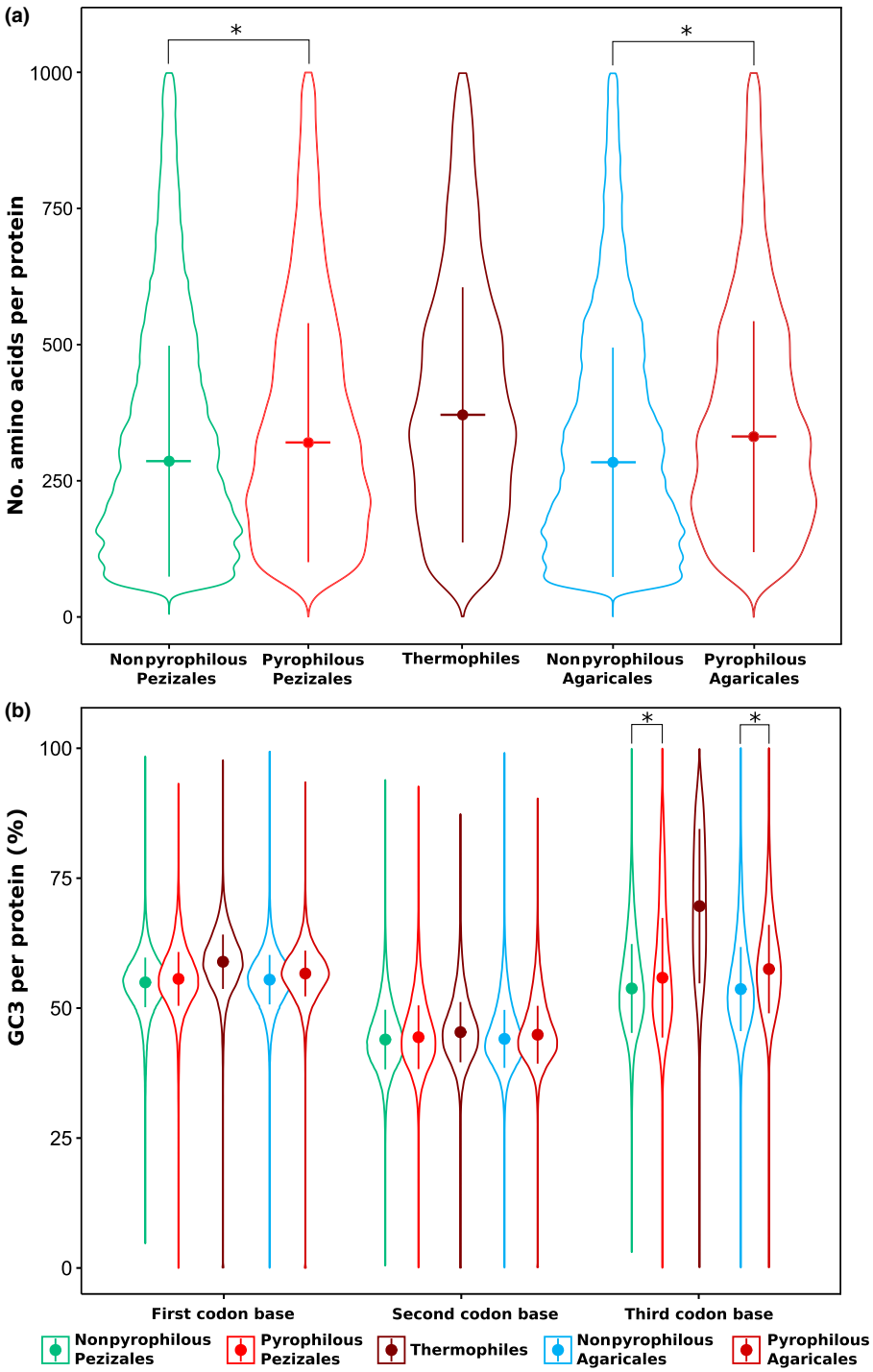

Fig. 3 Protein size distribution and G + C content at the third codon position (GC3) for pyrophilous, nonpyrophilous, and thermophilic fungi. (a) For nonpyrophilous Pezizales and Agaricales (Supporting Information Table S5), we performed Mood's median nonparametric test (*, $P < 0.05$). The circle and horizontal bar represent the median, and the vertical bar represents the median absolute deviation (MAD). (b) GC content of each position by coding sequence (CDS). Mood's median nonparametric test was used to establish the significance level (*, $P < 0.05$). The thermophile species used in this analysis were *Myceliophthora thermophila*, *Thermoascus aurantiacus*, *Thermomyces lanuginosus*, and *Thielavia terrestris*. It is important to note that there are no known basidiomycetes thermophiles (Morgenstern *et al*., 2012).

the genomes of pyrophilous Pezizales species, the median GC3 content was 55.84 ± 11.5% (median ± MAD) (Fig. 3b), significantly higher than in nonpyrophilous Pezizales (53.7 ± 8.5%) ($P = 0.016$), but lower than in thermophiles (69.62 ± 14.85%). For the Agaricales genomes, the difference between pyrophilous and nonpyrophilous species was also significant ($P = 0.035$). Interestingly, the fungus *Schizophyllum commune*, which was not known to be related to the fire response, exhibited a high GC3 content (70.69 ± 9.14%). However, it was found growing on the charred bark of fire-killed hardwood (H. J. Simpson, pers. obs.; https://www.inaturalist.org/observations/31012075), implying that the trend described here exists outside of the species under study, and may be shared by fungi not yet known to be related to the pyrophilic lifestyle.

Carbohydrate-active enzymes and stress-related gene families are enriched families in pyrophilous fungi

To perform an in-depth analysis of different fungal lifestyles, we selected 124 Dikarya genomes representing the broad ecological

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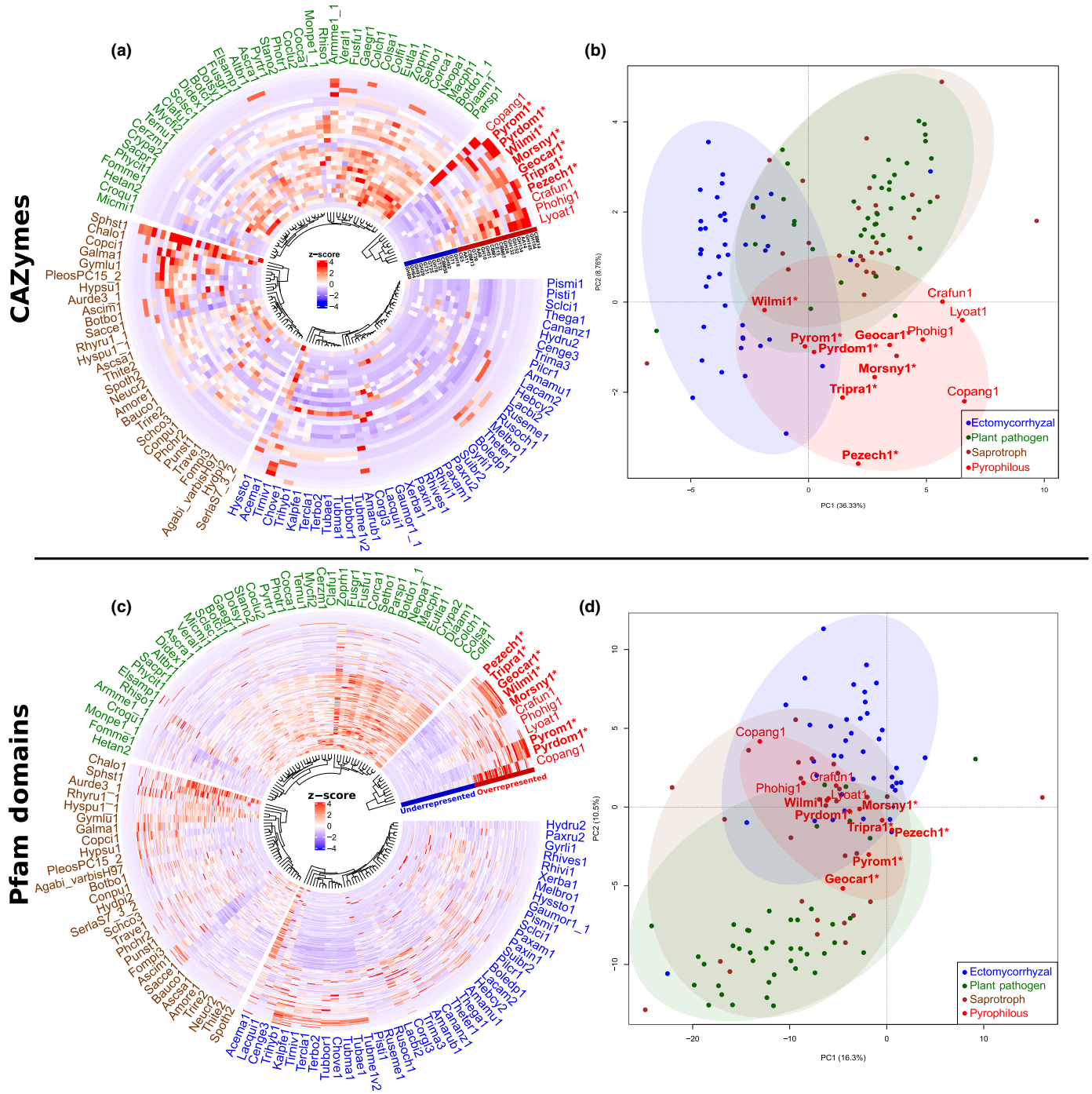

Fig. 4 Enriched Pfam domains and carbohydrate active enzymes (CAZymes). (a, c) The heatmap shows the overrepresented (red bar) and underrepresented (blue bar) gene families. The Pfam domains and CAZyme counts were normalized and displayed as *z*-scores. (b, d) Phylogenetic principal component analysis (phylo-PCA; Revell, 2009) of overrepresented and underrepresented families, showing a separation of pyrophilous species in terms of CAZymes, but not in terms of Pfam domains. In all four figures, pyrophilous ascomycetes are shown in bold and indicated by an asterisk (*).

classification according to MycoCosm groups (https://mycocosm.jgi.doe.gov): ectomycorrhizal, plant pathogens, saprotroph, and pyrophilous (Fig. 4; Table S6).

Regarding the CAZymes, we found that 18 families were overrepresented in pyrophilous vs nonpyrophilous fungi (FET, $P \leq 0.05$) and 12 were underrepresented (Fig. 4a). Among the overrepresented families, 11 are potentially related to the degradation of partially or fully pyrolyzed plant biomass, such as LPMOs (AA9, AA11, and AA14), xylanase (GH10, and GH51), β-1,3-glucanases (GH132, and GH152), endo-β-1,4-mannanase (GH134), and acetyl xylan esterase/cutinase (CE5). Interestingly, the AA11 and CE5 families are primarily overrepresented in pyrophilous Pezizales and are potentially crucial for early and late colonizers. The phylogenetic phylo-PCA based on CAZymes

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clearly separates pyrophilous from other lifestyles and Pezizales from Agaricales with the first principal component (PC1), showing that despite being found in the same environment, CAZymes are not fully shared by both phyla (Fig. 4b). For instance, chitinases (GH18) were underrepresented in pyrophilous Pezizales (Fig. 4a, in bold) but overrepresented in pyrophilous Agaricales (Steindorff *et al.*, 2021). In addition, the enriched families in pyrophilous fungi separated the plant pathogens and ectomycorrhizal fungi, showing an overlap between essential CAZymes in all these lifestyles (Fig. 4b).

We performed the same analysis using Pfam domains and found 78 overrepresented and 98 underrepresented entries (Fig. 4c). In this case, we can see a clear separation between pyrophilous Pezizales and Agaricales fungi. Pfam domains prevalent in Pezizales genomes show functions involved in defense against microbial attack and stress, such as chitin binding peritrophin-A (PF01607 or CBM14), arthropod defensin (PF01097), tocopherol cyclase (PF14249), ricin-type beta-trefoil lectin (PF00652), heat shock protein 9/12 (PF04119), fungal fucose-specific lectin (PF07938) and fungalysin metallopeptidase (PF02128). The phylo-PCA (Fig. 4d) does not show a separation of pyrophilous fungi but still has a strong signal separating plant pathogens and ectomycorrhizal fungi. Saprotrophy, the main pyrophilous underlying lifestyle, is more functionally diverse and scattered over the plot.

Charcoal modulates developmental genes in *P. domesticum*

Pyronema spp. are known to form abundant fruiting bodies on burned soil a few weeks after large forest fires (Bruns *et al.*, 2020), and *P. omphalodes* (syn. *P. confluens*) is an established model organism for the analysis of cell biology and fruiting body development in filamentous fungi (Traeger *et al.*, 2013). Therefore, we hypothesized that exposure to the products of pyrolysis could trigger sexual development in these fungi. To check this, we have performed a gene co-expression analysis of the two available datasets (see the Materials and Methods section). A study by Fischer *et al.* (2021) focused on the metabolic pathways used by *P. domesticum* to utilize charcoal as its sole carbon source. Since the two strains are very closely related, we found direct orthologs for 73.2% of *P. confluens* and 90% of *P. domesticum* genes using bidirectional BLAST hits.

The PCA plot shown in Fig. 5(a) shows that *P. domesticum* sampled while growing on charcoal or during sexual development induced by white light exhibited the most divergent expression profiles among the samples. Since the sexual development (sex-dev) and vegetative growth (veg) samples are from different *Pyronema* species and experiments, this separation is expected. For this reason, we used normalized expression for all downstream analyses. Among the genes differentially expressed on charcoal vs sex-dev, 372 were upregulated and 282 were downregulated (Fig. 5b). The upregulated genes with known functions include oxidoreductases (cupredoxin, laccase, cytochrome P450), transcription factors, RNA metabolism-related proteins, and membrane transporters (carbohydrates, ammonium, amino acids, peptides) (Table S7), similar to the truffle (Pezizales) fruiting body transcriptome (Murat *et al.*, 2018). Also, among the induced genes were CAZymes potentially involved in cell wall modification, such as β-glucanases (GH16, GH132), chitin-binding modules (CBM18), an LPMO (AA9), cellulase (GH5_49), glucooligosaccharide oxidase (AA7), and a ferroxidase (AA1_2). Interestingly, acetyl xylan esterase/cutinase (CE3, CE5) was also upregulated in both conditions and expanded in pyrophilous Agaricales (Steindorff *et al.*, 2021).

To understand how the genes regulated in both the charcoal and sex-dev conditions are correlated, we constructed a co-expression network with weighted gene co-expression analysis (WGCNA) (Langfelder & Horvath, 2008), revealing two major groups of genes in the network structure and 14 modules with correlated co-expression profiles calculated across conditions (Fig. 5c; Table S8). The gene modules, on average, contained 697 genes and ranged in size from 140 to 3811 genes. We calculated functional enrichment using GO terms for each module and investigated those modules with commonly upregulated genes in the charcoal and sex-dev conditions. In Fig. 5c the blue module, which contains 57.5% (214 of the 372) of the upregulated genes common in the two conditions and 65.5% (913 of 1394) unique in sex-dev (Fig. 5b), showed no significant GO enrichment (Table S8). The magenta module, which contains 191 exclusively charcoal-induced genes, and 33 upregulated genes in both charcoal and sex-dev, showed enrichment of aromatic amino acid biosynthesis (GO:0009073), response to oxidative stress (GO:0006979), alcohol metabolic process (GO:0006066), and defense response (GO:0006952) (Fig. 5d). The tan, black, turquoise, and brown modules, which contain a mix of upregulated genes in the charcoal and sex-dev conditions (Table S8), showed the enrichment of genes involved in transport, protein/amino acid biosynthesis, signal transduction, and cell communication. A similar pattern was found when we compared all conditions (water, soil, charcoal, and sex-dev), and looking at all intersections in the Venn diagram shown in Fig. S1, charcoal and sex-dev share more genes when compared to other conditions.

Taking all this information together, we can see a trend of charcoal modulating genes and categories involved in fruiting body development. To conduct an in-depth investigation of these genes, we used a list of genes involved in sexual reproduction in *Saccharomyces cerevisiae*, *Aspergillus nidulans*, and *Ustilago maydis* (Mondo *et al.*, 2017) (Fig. 6; Table S9).

Ortholog clustering (Fig. 6a) revealed that these genes are conserved across Pezizales genomes, with 71% of them present in all 19 species analyzed in this study. Interestingly, only one gene was significantly upregulated in both the charcoal and sex-dev conditions: the homolog of a transcription factor, HMG, from *A. nidulans* (AN3667), which is involved in nutrient-sensing in this fungus (Muthuvijayan & Marten, 2004). The transcription factor STE12 was also upregulated in the charcoal condition; STE12-like proteins regulate diverse functions across the fungal kingdom, such as growth, virulence, sex, dimorphism, and asexual development, responding to cues to initiate developmental transitions (Hoi & Dumas, 2010; Fischer & Glass, 2019). In

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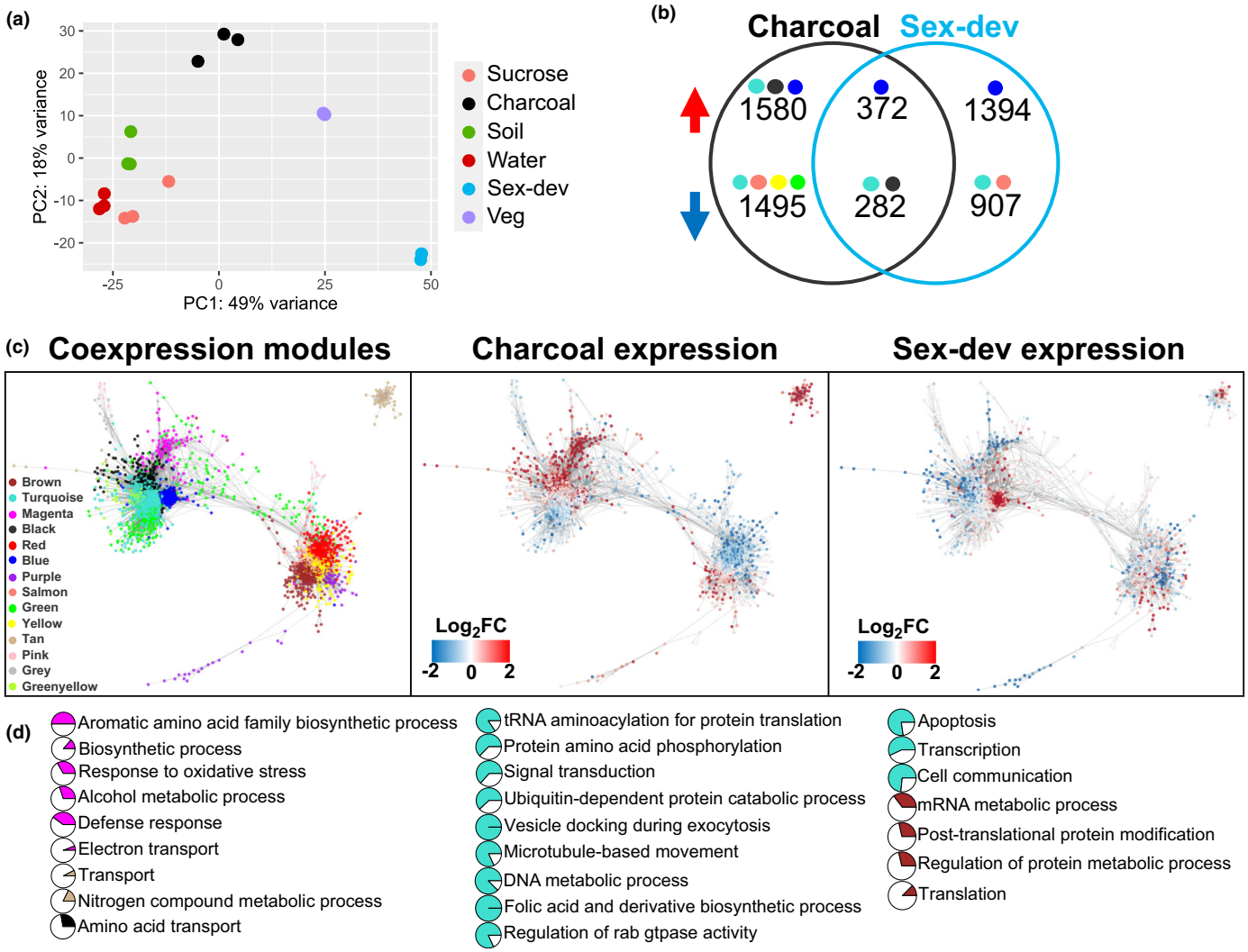

Fig. 5 (a) A principal component analysis of the gene expression data colored by condition, with each circle representing a biological replicate. (b) Venn diagram of differentially expressed genes of *Pyronema domesticum* in response to charcoal and *Pyronema omphalodes* during sexual development. The top row (red arrow) represents the number of upregulated genes, and the bottom row (blue arrow), represents the number of downregulated genes. Colored circles represent the modules, with at least 100 genes belonging to the co-expression module given in (c). (c) Gene co-expression network calculated using weighted gene co-expression network analysis (WGCNA), with genes colored according to module (left), average fold change in the charcoal samples (middle), and average fold change in the sexual development samples (right). (d) Enriched gene ontology (GO) terms in biological processes ($P \leq 0.01$) for modules containing genes commonly upregulated in the charcoal and sexual development conditions. The coloring corresponds to the modules, and the pie charts represent the genes assigned to each GO term in the given module, as a proportion of those of the whole genome.

S. cerevisiae the STE12 gene codes for a sequence-specific DNA binding transcription factor that activates the transcription of mating-specific genes and the response to pheromones (Merlini *et al.*, 2013). In *Neurospora crassa*, the STE12 homolog is not essential for mating cell fusion, but it is essential for wild-type-like growth, somatic cell fusion, protoperithecium development, ascus development, and ascospore germination (Fischer & Glass, 2019). Among the environmental sensor genes, we can see a clear difference in the gene expression in the charcoal condition (Fig. 5b), with four genes significantly upregulated only in this condition: the blue light-sensing genes LreA and LreB (Purschwitz *et al.*, 2008), the light/dark response regulator VosA (Ni & Yu, 2007), and the early sexual developmental gene EsdC (Han *et al.*, 2008). Even though the growth in the charcoal, soil, water, and sucrose conditions followed the same light cycle, we still see a difference in expression, suggesting that these genes might be a developmental trigger in conditions other than light.

Discussion

To examine the link between genomic features of pyrophilous fungi and the role they play in post-fire soil ecology, we started focusing on the ascomycetes fungi found at the Rim Fire in the Stanislaus National Forest. Then, we sequenced and annotated six novel genomes of pyrophilous Pezizales fungi (*P. domesticum*, *P. omphalodes*, *G. carbonaria*, *T. praecox*, *P. echinospora*, *M. snyderi*) and compared them with 12 other Pezizales genomes. In addition, we performed a comprehensive comparison using 124

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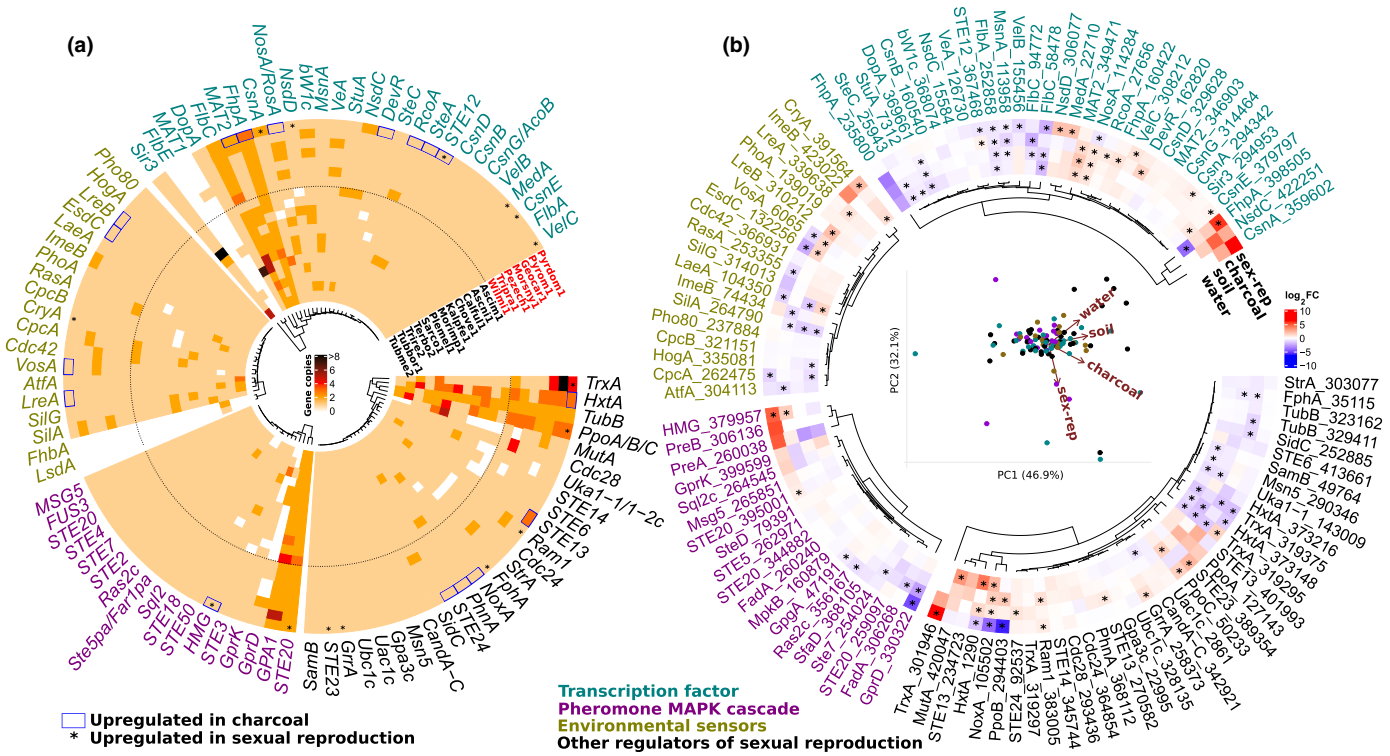

Fig. 6 Conservation and expression of developmental genes in *Pyronema domesticum*. (a) Hierarchical clustering of orthologous clusters of developmental genes in Pezizales fungi. Pyrophilous fungi are shown in red. (b) Normalized gene expression ($\log_2$ fold change) of developmental genes in *P. domesticum*. The asterisk (*) represents differentially expressed genes (adjusted $P \leq 0.05$). Gene names and protein IDs are colored according to the aspect of sexual reproduction they are involved in: beige, environmental sensors; light blue, transcription factors; purple, pheromone mitogen-activated protein kinase (MAPK) cascade. Shown in the middle is a principal component analysis (PCA) biplot for the expression values of developmental genes.

Dikarya genomes, including pyrophilous Agaricales genomes (Steindorff *et al.*, 2021) (Table S6). Even though most pyrophilous fungi analyzed in this study belong to the Pyronemataceae family, they colonize the post-fire soil in different periods (Bruns *et al.*, 2020; Hughes *et al.*, 2020). Hence, to make an intra-Pezizales enrichment comparison, we separated the data into early and late colonizers. *Pyronema*, *Tricharina*, and *Wilcoxina* species (Fig. 1) increase in prevalence soon after fire and rapidly decline within weeks. The colonization is then followed by other pyrophilous species (Bruns *et al.*, 2020). Also, the drastic environmental changes triggered by the fire – heat, pH, and chemical composition – might induce spore germination and the appearance of fruiting bodies (El-Abyad & Webster, 1968; Petersen, 1970; Fox *et al.*, 2022).

One would expect the early colonizers of the necromass zone to be 'r-selected', benefitting from the open niche and undergoing rapid growth, initially with little competition. This idea was expanded into the chemical substrates available in the necromass zone in a study by Bruns *et al.* (2020), who speculated that the survival of heat tolerant, rapidly growing, pyrophilous fungi would allow them to capture the labile compounds released from the organisms that had been killed by the heat. However, Fischer *et al.* (2021) showed that *Pyronema* species, which are the most dominant early post-fire colonist, also have the ability to metabolize more recalcitrant forms of highly aromatic carbon. Our future investigations will address whether other pyrophilous fungi share this ability.

The gene content and enrichment of gene families and functional domains suggest an inferior competitive ability of early pyrophilous colonizers. Specifically, we found that they have lower numbers of secondary metabolite clusters and other parasitic signatures such as chitinases and proteases. The only potentially defense-related expanded families found mainly in the Pyronematacae family are the CBM14 (peritrophin-A – PF01607) and arthropod defensin (PF01097). The CBM14 domain is mainly present in Metazoa, especially in insects and fungi. It is found in 224 genomes on the MycoCosm portal, with 173 belonging to Eurotiomycetes, 27 to Mucoromycota, 7 to Dothideomycetes, 7 to Pezizomycetes, 5 to Zoopagomycota, 4 to Leotiomycetes, and 1 to Agaricomycetes; Only Pyronemataceae have > 6 copies of this gene (Table S10). A similar distribution was found for the arthropod defensin. The expansion and involvement in sexual reproduction have already been described in *P. confluens* (Traeger *et al.*, 2013). Here we found that this expansion is a Pyronemataceae feature accentuated in the *Pyronema* genus. Among the 16 copies of CBM14 genes in *P. domesticum*, half were upregulated in the sex-dev condition, but only two in the charcoal condition – similar to the findings for the water and soil conditions (Table S7) – revealing that fewer copies are activated during stress and low nutrient situations – opposite to the findings for white-light exposure in a richer medium (Traeger *et al.*, 2013). Both CBM14 and arthropod defensin might be involved in defense mechanisms in arthropods and are not likely acquired by horizontal gene transfer. Since these

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domains are found in early diverging fungi such as Mucoromycota, Zoopagomycota, and Chytridiomycota (Table S10; Figs S2, S3), if this horizontal transfer happened, it was in the early steps of fungal evolution, being retained in a few clades (including Pezizales), and in low copy numbers in Eurotiomycetes.

We compared some genome features of pyrophilous fungi with those of phylogenetically related nonpyrophilous and thermophilic fungi; we found that the median protein sequence length for pyrophilous fungi lies between the values for nonpyrophilous and thermophilic fungi. A similar pattern was found for G + C content in the third base of the codon (GC3) (Fig. 3). The intermediate position of these sequence features might confer on pyrophilous fungi an increased protein thermotolerance and thus an increased ability to survive fire events compared to nonpyrophilous fungi.

Within the pyrophilous Agaricales fungi, we found the expansion of several gene families involved in fruiting body development (Steindorff *et al.*, 2021), a pattern we have not found in Pezizales fungi. Instead, we found the pyrophilous Pezizales fungi mainly contained expansions of CAZymes (LPMOs – AA9, AA11, and AA14; glycosyl hydrolases – GH10, GH51, GH132, GH152, GH134; cutinase – CE5), defense and stress-related families (Fig. 4), which points towards the degradation of recalcitrant material rather than easily accessible organic matter. For the enrichment analysis we considered all pyrophilous fungi as the test group, but the gene counts are not evenly distributed (Fig. 4). Since the heatmap color scale in Fig. 4 represents a *z*-score, it appears that some species have low counts, but in reality it means that they are on the lower side of the distribution.

The development of mature fruiting bodies follows specific genetically encoded programs that determine the species-specific morphologies (Krizsán *et al.*, 2019). To examine whether charcoal could induce sexual development in *P. domesticum*, the first responder found at the Rim Fire site in 2013 and in experimental pyrocosms (Bruns *et al.*, 2020), we compared transcriptome datasets from two studies where *P. domesticum* was grown on charcoal (Fischer *et al.*, 2021) and sexual reproduction was induced by white/blue light (Traeger *et al.*, 2013). We found some modules containing genes induced in both the charcoal and sexual reproduction conditions through co-expression analysis, and they were enriched in GO terms like amino acid biosynthesis, response to oxidative stress, and defense response. Looking more closely into genes known to be involved in sexual reproduction in *A. nidulans*, *S. cerevisiae*, and *U. maydis* (Mondo *et al.*, 2017), we found that their homologs are highly conserved in Pezizales and not commonly induced in response to charcoal and during sexual development, except the *Pyronema*-specific HMG transcription factor (Fig. 6). Four environmental sensors were upregulated only in the charcoal condition, suggesting that the hydrophobic environment might be an essential activator of sexual development during the growth of these fungi on charcoal. The addition of charcoal to induce mating in smut fungi is a procedure that has been used for decades (Garrido *et al.*, 2004; Elías-Villalobos *et al.*, 2015); one hypothesis is that the charcoal mimics a hydrophobic environment similar that associated with the cutin in plant leaves (Elías-Villalobos *et al.*, 2015) and by eliminating inhibitory compounds present in the substrate (De Groot *et al.*, 1998). Interestingly, cutinase (CE5) is an expanded family in pyrophilous fungi and is differentially expressed on charcoal. Since they are not plant pathogens, and this strain of *P. domesticum* can consume charcoal as a carbon source (Fischer *et al.*, 2021), this family might be involved in the degradation of hydrophobic products of combustion.

In summary, we performed a comparative genomics analysis of pyrophilous fungi and we found the enrichment of gene families involved in response to stress and degradation of PyOM. Also, we found that the protein length and GC3 content of pyrophilous fungi lie between those of nonpyrophilous and thermophile fungi. The transcriptome analysis found overlapping modules of genes co-expressed in charcoal and light-induced sexual development conditions in *P. domesticum*. In addition, known environmental sensors such as LreA, LreB, VosA, and EsdC were upregulated in the charcoal condition, relative to other conditions. This study analyzed a representative subgroup of known pyrophilous fungi. However, there is still a large diversity of these specialized groups, which can drive other genome projects to provide more data to understand their evolution and role in recovering the biocapacity of soils.

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Author contributions

TDB and IVG designed the research and secured funding; AC, KS and HL sampled, cultured, and isolated the DNA/RNA for sequencing; MSF and MT performed RNA-Seq; AL, GH, MY, BA, JP, KL, SC, VN and IVG sequenced, assembled, and annotated the genomes; BH and ED predicted CAZyme genes in the analyzed genomes; ASS, KS, HJS and JSS performed comparative analyses and bioinformatics; ASS, KS, AC, TDB and IVG wrote the paper. All authors read and commented on the manuscript.

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Data availability

Genome assemblies and annotations are available at MycoCosm (https://mycocosm.jgi.doe.gov) (Grigoriev *et al.*, 2014) and have been deposited at DDBJ/ENA/GenBank under the following accessions: *Pyronema domesticum*, CBS 144463 (JAJJXL0000 00000); *Pyronema omphalodes*, CBS 144459 (JAJJXM00000 0000); *Geopyxis carbonaria*, CBS 144460 (JAJJXG000000000); *Tricharina praecox*, CBS 144465 (JAJJXF000000000); *Morchella snyderi*, CBS 144464 (JAJJXB000000000); *Peziza echinospora*, CBS 144458 (JAJLQT000000000).

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Gene co-expression network and Venn diagram of differentially expressed genes in all conditions.

Fig. S2 Phylogenetic tree of 401 proteins containing the Pfam domain CBM_14 (PF01607).

Fig. S3 Phylogenetic tree of 272 proteins containing the Pfam domain Defensin_2 (PF01097).

Table S1 Fisher's exact test (FET) for unique genes in pyrophilous Pezizales genomes.

Table S2 Pfam enrichment in 19 Pezizomycetes genomes.

Table S3 Orthologous group enrichment in 19 Pezizomycetes genomes.

Table S4 Fast-evolving gene families that are expanded and contracted in pyrophilous genomes, identified using Cafe v.4.2.1.

Table S5 List of genomes used for protein length and GC3 analysis.

Table S6 Full list of genomes used in this study, and Fisher's exact test of Pfam and carbohydrate-active enzyme (CAZyme) counts for pyrophilous and nonpyrophilous genomes.

Table S7 *Pyronema domesticum* expression profile.

Table S8 Genes present in each co-expression module and gene ontology enrichment analysis.

Table S9 Reproductive proteins across fungi.

Table S10 Counts and phylogenetic trees for the CMB14 and Defensin_2 domains (Figs S2, S3).

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