UC Berkeley
UC Berkeley Previously Published Works

Title

# Fungal ecological strategies reflected in gene transcription - a case study of two litter decomposers

Permalink
https://escholarship.org/uc/item/8mn549pp

Journal
Environmental Microbiology, 22(3)

ISSN
1462-2912

Authors
Barbi, Florian
Kohler, Annegret
Barry, Kerrie
et al.

Publication Date
2020-03-01

DOI
10.1111/1462-2920.14873

Supplemental Material
https://escholarship.org/uc/item/8mn549pp#supplemental

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

1 **Title**

2 **Fungal ecological strategies reflected in gene transcription – a**
3 **case study of two litter decomposers**

4

5 **Authors**

6 **Florian Barbi[1], Annegret Kohler[2], Kerrie Barry[3], Preetisri**
7 **Baskaran[4], Chris Daum[3], Laure Fauchery[2], Katarina Ihrmark[5], Alan**
8 **Kuo[3], Kurt LaButti[3], Anna Lipzen[3], Emmanuelle Morin[2], Igor V.**
9 **Grigoriev[3,6], Bernard Henrissat[7,8,9], Björn Lindahl[1], Francis Martin[2,10]**

10

11 [1]Swedish University of Agricultural Sciences, Department of Soil and Environment, Box
12 7014, SE-750 07 Uppsala, Sweden

13 [2]Université de Lorraine, INRA, UMR Interactions Arbres/Microorganismes (IAM), Laboratoire
14 d'excellence Recherches Avancés sur la Biologie de l'Arbre et les Ecosystèmes Forestiers
15 (LabEx ARBRE), Centre INRA Grand-Est, 54280 Champenoux, France.

16 [3]US Department of Energy Joint Genome Institute, Walnut Creek, CA 94598 USA

17 [4]Swedish University of Agricultural Sciences, Department of Ecology, Box 7044, SE-750 07
18 Uppsala, Sweden

19 [5]Swedish University of Agricultural Sciences, Department of Forest Mycology and Plant
20 Pathology, Box 7026, 750 07 Uppsala, Sweden

21 [6]Department of Plant and Microbial Biology, University of California Berkeley, Berkeley CA
22 94720

23 [7]CNRS UMR 7257, Aix-Marseille University, 13288, Marseille, France.

24 [8]INRA, USC 1408 AFMB, 13288, Marseille, France

25 [9]Department of Biological Sciences, King Abdulaziz University, Jeddah, Saudi Arabia

26 [10]Beijing Advanced Innovation Center for Tree Breeding by Molecular Design, Beijing
27 Forestry University, 35 Qinghua E Rd, Haidian Qu, Beijing Shi, China, 100083.

28

29 * Corresponding authors: Florian Barbi, Swedish University of Agricultural
30 Sciences, Department of Soil and Environment, Box 7014, SE-750 07 Uppsala,
31 Sweden. Phone: +46 18-672720. Mail: florian.barbi@slu.se
32 Björn Lindahl, Swedish University of Agricultural Sciences, Department of Soil and
33 Environment, Box 7014, SE-750 07 Uppsala, Sweden. Phone: +46 18-672720.
34 Mail: bjorn.lindahl@slu.se

35

36 **Running title**

37 Fungal ecology reflected in gene transcription

38

39 **Originality-Significance Statement**

40 The direct assessment of ecophysiological processes, such as decomposition of
41 organic matter, is a key to understand the role of microbial communities in their
42 environment. We addressed this challenge by comparing information given by
43 genomes *vs.* transcriptomes of two fungi during a litter decomposition
44 experiment, in parallel with a measure of organic matter chemical changes. Our
45 findings highlight that contrasting ecological strategies were reflected by
46 differences in expression of specific functional genetic markers, as well as
47 temporal changes in gene expression of different components of the decomposer
48 machinery, following the chemical changes in the substrate as decomposition
49 progressed. Consequently, we assume that targeting transcription ratios of

50 specific ‘keystone’ genes would be useful to assess fungal ecological strategies,
51 providing information about the dynamics of ecophysiological processes, such as
52 decomposition, at the ecosystem scale.
53
54
55
56 **Summary**

57 Microbial communities interplay with their environment through their functional
58 traits that can be as response or an effect to the environment. Here we explore
59 how a functional trait – the decomposition of organic matter, can be address
60 based on genetic markers and how the expression of these markers reflect
61 ecological strategies of two fungal litter decomposer *Gymnopus androsaceus* and
62 *Chalara longipes*. We sequenced the genomes of these two fungi, as well as their
63 transcriptomes at different steps of Pinus sylvestris needles decomposition in
64 microcosms. Our results highlighted that if the gene content of the two species
65 could indicate similar potential decomposition abilities, the expression levels of
66 specific gene families belonging to the glycosyl hydrolases (GH) superfamily
67 reflected contrasting ecological strategies. Actually C. longipes, the weaker
68 decomposer in this experiment, turned-out to have a high content of genes
69 involved in holocellulose decomposition but low expression levels, reflecting a
70 versatile ecology compare to the more competitive G. androsaceus with high
71 expression levels of keystone functional genes. Thus we established that
72 sequential expression of genes coding for different components of the
73 decomposer machinery indicated adaptation to chemical changes in the substrate
74 as decomposition progressed.

75

76

77 **Keywords**

78 Fungi, genomics/functional genomics/comparative genomics, Microbial ecology,

79 Transcriptomics, Ecophysiology

80

## 81 Introduction

82 To understand relationships between composition and function of microbial
83 communities it is essential to analyse how contrasting ecological traits and
84 strategies interplay with the environment (Koide *et al.*, 2014). Direct assessment
85 of ecophysiological traits of microorganisms colonizing natural substrates is a
86 major challenge, but indirect approaches based on gene content and expression
87 offer new ways forward. Here we explore how fungal ecological strategies can be
88 assessed based on genetic markers of functional traits (Kuske *et al.*, 2015;
89 Treseder and Lennon, 2015), using two fungal decomposers of needle litter with
90 contrasting ecological strategies as a model.

91

92 During the past decades the number of sequenced fungal genomes has increased
93 rapidly, especially due to the Community Science Program “1000 fungal
94 genomes” launched by JGI (Grigoriev *et al.*, 2014). Genomic information enables
95 comparative analyses of fungal species, in order to explain functional differences
96 related to the content of genes with specific functions among genomes (Eastwood
97 *et al.*, 2011; Floudas *et al.*, 2012; Talbot *et al.*, 2015; Martino *et al.*, 2018).
98 Organic matter decomposition is a good example where losses (Kohler *et al.*,

99 2015) and gains (Floudas *et al.*, 2012; Riley *et al.*, 2014) of plant cell-wall
100 degrading enzymes have been connected to the evolution of specialized
101 ecological groups. Divergent evolution of the genetic machinery of decomposition
102 has led to the distinction of fungal guilds within the general guild of saprotrophs,
103 such as the white-rot fungi capable of lignin degradation (Floudas *et al.*, 2012).
104 Consideration of different guilds of decomposers (*e.g.* opportunists, holocellulose
105 decomposers and lignin decomposers) may enable better prediction of
106 relationships between soil fungal communities, the chemical nature of organic
107 matter and the loss and stabilization of organic pools below ground (Moorhead
108 and Sinsabaugh, 2006; Talbot *et al.*, 2015; Bhatnagar *et al.*, 2018).
109
110 However, in relation to bacteria, fungi represent a narrow evolutionary branch,
111 and it seems plausible that ecological strategies among fungi largely reflect
112 differences in gene regulation rather than gene content. All genes involved in a
113 process may not be of the same importance, and ecological strategies could be
114 characterized by expression of certain keystone genes. Further, litter
115 decomposition is biochemically sequential, and a transcriptomic approach can
116 provide information about the genes expressed during different phases of the
117 decomposition process. Fungal gene expression in response to the chemical
118 composition of organic matter (Baldrian and López-Mondéjar, 2014), but also the
119 subsequent alteration of organic matter by fungal gene products should be
120 essential in defining the ecological strategy of fungal decomposers.
121
122 Fungal genes coding for enzymes involved in biochemical transformations during
123 decomposition are specified according to the CAZyme classification (Lombard *et*

124 *al.*, 2014). The CAZy database characterizes evolutionary distinct enzyme families
125 with respect to their biochemical properties and substrate specificities. Enzymatic
126 degradation during litter decomposition can be divided into three main processes
127 linked to the principal components of plant cell walls:

128 (1) **Cellulose** decomposition is a complex process resulting from the action of
129 a variety of glycoside hydrolases (GH) (Table1). Cellulases (primarily GH5-
130 5, GH6 and GH7) cleave cellulose chains, decreasing their length and
131 creating new chain ends. These enzyme can act on crystalline cellulose and
132 may either be binding-releasing enzymes that have to attach to the
133 cellulose via CBM for each hydrolytic cleavage or processive (non-
134 releasing) enzymes that generate the dimer cellobiose (cellobiohydrolases)
135 (Payne *et al.*, 2015). The 1,4-β-glucosidases (GH1 and GH3) then are able
136 to hydrolyze cellobiose into glucose. There is also an additional, oxidative
137 mechanism to cleave the cellulose internally, based on lytic polysaccharide
138 monooxygenases (LPMO), which belong the Auxiliary Activities (primarily
139 AA9) class of enzyme in the CAZyme database (Levasseur *et al.*, 2013).

140 (2) **Hemicelluloses** are branched polymers that, in addition to glucose, also
141 contain fucose, galactose, rhamnose, mannose, arabinose and xylose
142 (Sarkar *et al.*, 2009; Schädel *et al.*, 2010). Together these sugars form the
143 three main polysaccharides of hemicelluloses: xylan, xyloglucan and
144 galactomannan. Due to the molecular complexity of hemicelluloses,
145 several sets of enzymes are involved in their decomposition (Table1). The
146 involved gene families are polyspecific and contain enzymes that
147 potentially may target many different substrates. As for cellulose,
148 hemicellulose decomposition is initiated by endohydrolytic enzymes that

149 create new chain ends, enabling the subsequent action of processive
150 exohydrolases followed by monomer-releasing enzymes. **Pectins** are also
151 heteropolysaccharides that are degraded in a similar manner as
152 hemicelluloses, with pectin lyases playing a key role.

153 (3) **Lignin** is a phenolic polymer that is resistant to hydrolytic decomposition.
154 Breakdown of lignin, as well as other non-hydrolysable components,
155 require oxidative mechanisms. In particular, enzymes in the class II
156 peroxidase family (AA2) are able to attack a variety of chemical bonds in
157 an unspecific manner. Class II peroxidases include lignin peroxidases ,
158 manganese peroxidases (MnP) and versatile peroxidases (Martinez *et al.*,
159 2009; Hofrichter *et al.*, 2010). Of these, MnP are the most abundant in soils
160 (Kellner *et al.*, 2014) and act indirectly by oxidizing $Mn^{2+}$ to $Mn^{3+}$, using
161 $H_2O_2$ as electron acceptor. The $Mn^{3+}$, in turn, may oxidise a variety of
162 organic molecules. Multicopper oxidases (AA1), including laccases, use
163 molecular oxygen as electron acceptor and have also been proposed to
164 participate in lignin oxidation.

165

166 The primary steps of decomposition are extracellular processes responsible for
167 the depolymerisation of long polymers into low molecular weight compounds that
168 may be taken up by the fungi and used in intracellular metabolism. Part of the
169 acquired resources will then be used in catabolism to eventually be released in
170 the form of $CO_2$ during respiration via two steps of the citric acid cycle mediated
171 by isocitrate dehydrogenase (IDH) and the oxoglutarate dehydrogenase complex
172 (OGDC). Remaining resources may be used for anabolic purposes, building cell
173 components – primarily cell walls. The fungal cell wall is essentially composed by

174 two polymers; β-1,3-glucan, polymerized by glucan synthase (GT48), and chitin,
175 polymerized by chitin synthase (GT2) (Bowman and Free, 2006). The carbon (C)
176 use efficiency represents the proportion of acquired carbon that the organisms
177 use to build biomass (Manzoni *et al.*, 2018).
178
179 In this study we addressed how changes in chemical composition of *Pinus*
180 *sylvestris* needle litter were related to gene content and transcription during
181 decomposition by two litter decomposer fungi with contrasting ecological
182 strategies. We used the experimental material presented in Baskaran *et al.*
183 (2019) and chemical data therein, derived from $^{13}$C-PMAS-NMR spectroscopic
184 analyses. *Gymnopus androsaceus* (L.) Della Magg. & Trassin. and *Chalara*
185 *longipes* (Preuss) Cooke. are both common colonizers of pine litter but with
186 different ecological strategies and abilities regarding decomposition (Baskaran *et*
187 *al.*, 2019). *G. androsaceus* is an efficient decomposer with high ligninolytic
188 capacity (Boberg *et al.*, 2011), whereas *C. longipes* is characterized by stress
189 tolerance, endophytic capacity (Koukol, 2011) and lack of ligninolytic ability
190 (Boberg *et al.*, 2011). Baskaran *et al.* (2019) found that in the presence of *G.*
191 *androsaceus,* 40% of the needle litter mass was lost during 10 months of
192 incubation, but only 10 % in the presence of *C. longipes*. Further, *G. androsaceus*
193 was able to decompose non-hydrolysable constituents of the litter, including
194 aromatic and akyl C, whereas *C. longipes* only exploited the hydrolysable litter
195 fraction (mainly polysaccharide O-Alkyl C).
196
197 Here, we sequenced the genomes of the two fungi and analysed gene expression
198 during decomposition. We expected that the differences in decomposer capacity

199 and ecology would be reflected in the genomes, in the expression of genes
200 central for the decomposition machinery, as well as in the expression of genetic
201 markers related to C use efficiency. C use efficiency should decrease with time,
202 due to increased costs of mycelial maintenance and the more complex
203 decomposing machinery required as the substrate become increasingly
204 recalcitrant (Manzoni *et al.*, 2018). Further, C use efficiency was expected to be
205 lower for *C. longipes*, as its stress-tolerant mycelium should have a higher cost of
206 maintenance relative to its slow growth, compared with the rapidly growing *G.*
207 *androsaceus* (c.f. the C-S-R ecological strategies of Grime 1974 (Cooke and
208 Rayner, 1984)).
209
210 We hypothesized that:
211 (1) In the genomes, there would be a correlative link between the presence or
212 number of genes with a specific function, the biochemical action of a
213 fungus and its affiliation to a functional guild. While the gene diversity
214 among relevant GH families would be predictors of cellulose, hemicellulose
215 and pectin decomposition, presence of the AA1 and AA2 gene family
216 should be indicative of lignin decomposition.
217 (2) In the transcriptomes, genes coding for lignocellulolytic enzymes would be
218 expressed in sequence during the progressive decomposition of
219 hemicellulose, cellulose and lignin. The relative expression of genes coding
220 for hydrolases acting on long-chain substrates, including endohydrolases
221 and processive enzymes as well as the oxidative AA9) would be high
222 initially, promoting subsequent higher expression of genes coding for
223 enzymes that release monosaccharides. Oxidative enzymes (AA1 and AA2)

224 would primarily be expressed at late decomposition stages (Šnajdr *et al.*,
225 2011).
226 (3) We expected that *G. androsaceus*, growing and decomposing more rapidly,
227 would have a higher CUE than the slowly growing *C. longipes* (Manzoni *et*
228 *al.*, 2018) and hypothesised that this difference would be reflected in a
229 higher expression ratio of the anabolic enzymes GT48 and GT2 over the
230 catabolic enzymes IDH and OGDC. Further, we expected that CUE (as
231 indicated by the GT48 and GT2 to IDH and OGDC ratio) would decrease
232 with time and increasing recalcitrance of the substrate.
233

## 234 Materials and methods

### 235 Fungal strains and microcosms

236 Strains of *Gymnopus androsaceus* (isolate JB14) and *Chalara longipes* (isolate
237 BDJ) were obtained from the culture collection of the Department of Forest
238 Mycology and Plant Pathology at the Swedish University of Agricultural Sciences.
239 Details of the microcosm design are described in Baskaran *et al.* (2019). Briefly, a
240 total of 24 microcosms were filled with 85g of sand and 11g of dry Scots pine
241 needles (*Pinus sylvestris*) each and sterilised by gamma radiation. The needle
242 litter contained 0.42% N, 50.6% C, had a C:N-ratio of 120 and a lignin
243 concentration of approximately 25% (Boberg *et al.*, 2014). For each fungal
244 species, twelve microcosms were inoculated through the addition of single
245 needles that had been pre-colonized for 2 months on N-free agar cultures. After
246 inoculation, 20 ml of 100 mM $NH_4Cl$ solution were added to all microcosms, to
247 stimulate fungal growth. Sealed microcosms were incubated at 20 ºC and 4

248 replicates from each fungal species were harvested after 2, 5 and 10 months,
249 shock frozen in liquid nitrogen, ground using mortar and pestle and stored at -80
250 ºC.
251 A Bruker 500 MHz Avance III spectrometer equipped with a MAS probe was used
252 to acquire $^{13}$CP/MAS spectra (Schaefer and Stejskal, 1976), which were divided
253 into established spectral regions to quantify the relative contribution of O-Alkyl C,
254 Alkyl C, Aromatic C and Carboxyl C.
255
256 **DNA and RNA extraction for genome sequencing, assembly and**
257 **annotation.**
258 *C. longipes* BDJ was cultured in liquid Hagem medium for 30 days and *G.*
259 *androsaceus* JB14 was cultured in liquid medium with 1.75% malt and 0.25%
260 peptone for 16 days. Mycelium was freeze-dried and ground in a mortar and
261 pestle with sand, and 1-5 mL was used for DNA and RNA extraction. DNA was
262 extracted following a 3% CTAB-buffer/chloroform protocol (supplementary method
263 A). RNA was extracted with 2% CTAB-buffer/chloroform-isoamyl alcohol
264 (supplementary method B) and purified following the protocol of the RNeasy mini
265 kit (Qiagen).
266 *G. androsaceus* (https://genome.jgi.doe.gov/Gyman1/Gyman1.home.html) and *C.*
267 *longipes* (https://genome.jgi.doe.gov/Chalo1/Chalo1.info.html) were sequenced by
268 the U.S. Department of Energy Joint Genome Institute (JGI) using a combination of
269 Illumina fragment (270 bp insert size) and 4 Kbp long mate-pair (LMP) libraries,
270 and assembled using ALLPATHS-LG. *G. androsaceus* was improved with PacBio
271 and PBJelly. The genomes were annotated using the JGI annotation pipeline
272 (Grigoriev *et al.*, 2014) and are available via the JGI MycoCosm database

273 (jgi.doe.gov/fungi). Transcriptomes of the two species produced at JGI were
274 sequenced using Illumina, assembled using Rnnotator and used for genome
275 annotation (Kohler *et al.*, 2015).
276

277 **RNA extraction from microcosm, sequencing and data processing**

278 Total RNA was extracted from 800 mg of tissue per sample using the RNA
279 PowerSoil Total RNA Isolation Kit (Mobio now Qiagen). Quantification and integrity
280 check were conducted using an Experion Automated Electrophoresis Station (Bio-
281 Rad, Hercules, CA, USA). Preparation of libraries (IlluminaTruSeq Stranded mRNA)
282 and 2 x 125 bp Illumina HiSeq2500 sequencing was performed by the GeT
283 platform (Get-PlaGe GenoToul, Castanet-Tolosan, France) following their standard
284 protocol. Three replicates were sequenced except for *C. longipes* mycelium
285 harvested after 2 and 5 month. For these two time points the amount of fungal
286 material was only sufficient to extract good quality RNA for two replicates. Raw
287 reads were trimmed for low quality (quality score 0.05), Illumina adapters and
288 sequences shorter than 15 nucleotides and aligned to the respective reference
289 transcripts available at JGI (https://genome.jgi.doe.gov/programs/fungi/index.jsf)
290 using the CLC Genomics Workbench v9. The following CLC genomic workbench
291 parameters were used for read mapping: minimum length fraction 0.9, minimum
292 similarity fraction 0.8, Mismatch cost = 2, insertion cost = 3, Deletion cost = 3,
293 and the maximum number of hits for a read was set to 10. The unique and total
294 mapped reads number for each transcript were determined and then normalized
295 to RPKM (Reads Per Kilobase of exon model per Million mapped reads). Intact
296 pairs were counted as two, broken pairs as one. The complete data sets were
297 submitted to NCBI GEO as GSEXXX.

298

299 **Analysis of gene expression in microcosms**

300 Specific gene families were selected from the CAZyme database (Lombard et al.,
301 2014) or based on literature (Barbi *et al.*, 2014; Treseder and Lennon, 2015) In
302 the specific cases of the GH2 family, only beta-mannosidases were selected
303 based on JGI annotations (Table1). The number of genes in each selected gene
304 family were integrated across the two fungal genomes, and the normalized
305 number (RPKM) of sequenced transcripts were assessed. Statistical analyses were
306 performed using R (v3.3.1) with "vegan" packages (Oksanen *et al.*, 2019; R Core
307 Team, 2019). Changes in global patterns of gene expression during the
308 experiment were analysed by Principal Component Analysis (PCA) based on
309 mean-normalized expression values. Patterns of gene expression were related to
310 changes in chemical composition of the decomposing litter by fitting vectors of C
311 fraction ratios (Table 2) to the PCA. Specific correlations between expression
312 ratios of genes from specific families and time or C fraction ratios were analysed
313 *post-hoc* by linear regression.

314

315 ## Results

316 The 89 Mbp genome of *G. androsaceus* contained 29375 genes, of which 409
317 (1.4%) were identified as belonging to different GH families, while the 52 Mbp
318 genome of *C. longipes* contained a total of 19765 genes, of which 429 (2.2%)
319 were identified as GH genes
320 (https://genome.jgi.doe.gov/Gyman1/Gyman1.home.html, https://genome.jgi.doe.
321 gov/Chalo1/Chalo1.home.html) (Table S1).

322

323 The genome of *C. longipes* contained a somewhat higher number of genes coding
324 for β-glucosidases (GH1 and GH3) and cellulolytic enzymes (GH5_5, GH6, and
325 GH7) as that of *G. androsaceus* (56 vs. 52) (Fig. 1A; Table S1). *C. longipes* had 93
326 genes potentially involved in xylan decomposition (GH10, GH11, GH115, GH27,
327 GH3, GH35, GH36, GH43, GH51, GH54, GH62 and GH67), whereas *G. androsaceus*
328 had only 64. A high number of genes involved in hemicellulose decomposition
329 seems to be a common feature of Leotiomycetes, which in addition to litter
330 saprotrophs, such as *C. longipes*, also include endophytes and species forming
331 ericoid mycorrhiza (Fig. S1). *C. longipes* had 34 genes involved in galactomannan
332 decomposition (GH5_7, GH5_31, GH27, GH26, GH35 and GH36), whereas *G.*
333 *androsaceus* had only 24 genes. Both fungi contained a similar number of genes
334 involved in xyloglucan decomposition (GH12, GH27, GH29, GH31, GH35, GH36,
335 GH51, GH54, GH74 and GH95; 48 and 47 for *C. longipes* and *G. androsaceus*,
336 respectively) (Fig. 1C; Fig S2; Table S1). *C. longipes* had 46 genes involved in
337 pectin decomposition, whereas *G. androsaceus* had only 32 (Fig. 1E). Regarding
338 lignin breakdown, *C. longipes* had 28 genes annotated as multicopper oxidases,
339 whereas *G. androsaceus* had 32 (Fig. 1G). Moreover, no genes coding for versatile
340 or lignin peroxidase were identified in the genomes of any of the fungi, but the
341 genome of *G. androsaceus* contained 10 genes coding for class II peroxidases
342 (AA2) that were further classified as Manganese Peroxidases. *C. longipes*, similar
343 to other ascomycetes, had 4 class II peroxidases with missing key residues for Mn
344 oxidation (ExxxE and D) and these have no conserved tryptophan residue (Fawal
345 *et al.*, 2013). These enzymes have been classified as ascomycete class II
346 peroxidases or as “generic peroxidases” (Floudas *et al.*, 2012).
347

348 Transcriptomes were successfully sequenced from 9 microcosms with *G.*
349 *androsaceus* (3 from each harvest) and from 7 microcosms with *C. longipes* (2 x 2
350 months, 2 x 5 months and 3 x 10 months). In contrast to the lower number of GH
351 genes in the genome of *G. androsaceus*, the overall expression of β-glucosidases
352 and cellulase genes were 8 times higher for *G. androsaceus* than for *C. longipes*
353 (Fig. 1B), and genes coding for hemicellulases and pectin lyases were also more
354 highly expressed by *G. androsaceus* (Fig. 1D; Fig. 1F; Fig. S2). Among genes
355 involved in cellulose depolymerisation, *C. longipes* had 1.3 times more transcripts
356 coding for β-glucosidases (GH1 and GH3) than for cellulases (GH5_5, GH6 and
357 GH7), whereas *G. androsaceus* had higher relative expression of cellulases, with
358 an average GH1+3 to GH5_5+6+7 ratio of 0.17 (Fig. 1A).
359
360 **Gene expression during decomposition**
361 For *G. androsaceus,* changes in chemical composition of the litter followed a clear
362 temporal dynamic (Fig. 2A) from month 2 (top left) to month 10 (middle right)
363 (Baskaran *et al.*, 2019). The overall pattern of gene expression changed during
364 the progression of the experiment, as indicated by the clustering of
365 transcriptomes according to harvest time in the mean-normalized PCA, as well as
366 by the significant correlation between mass remaining and the PCA ordination
367 axes (*P*=0.016). Changes in gene transcription occurred in parallel with changes
368 in chemical composition of the litter, as indicated by the significant correlations
369 between different C fraction ratios, as analysed by NMR, and the PCA ordination
370 axes (Aromatic C to O-Alkyl C, *P*=0.015; Alkyl C to O-Alkyl C, *P*=0.026; Carboxyl C
371 to O-Alkyl C, *P*=0.032) (Fig. 2).
372

373 Expression of *G. androsaceus* genes coding for cellulolytic enzymes acting on long
374 chains (GH5_5, GH6 and GH7) increased during the experiment, whereas the
375 global expression level of β-glucosidase coding genes that release glucose (GH1
376 and GH3) was stable, and many β-glucosidase genes were more highly expressed
377 at month 2 and 5 compare to month 10 (Fig 2B; Fig S3A). The ratio of expression
378 between cellulases and β-glucosidases transcripts (i.e. the ratio of
379 GH5_5+GH6+GH7 to GH1+GH3) increased significantly with time (Fig. 3A)
380 ($R^2$=0.897, *P*=0.001) and was correlated with the Carboxyl C to O-Alkyl C ratio,
381 which is an indicator of increasing decomposition (*G. androsaceus* $R^2$=0.787,
382 *P*=0.001) (Fig. 3A).
383

384 Similarly, considering *G. androsaceus* genes coding for hemicellulases, we
385 observed that genes coding for monosaccharide releasing enzymes were
386 relatively more expressed during the early stages of the experiment, whereas
387 genes coding for long-chain acting enzymes were more highly expressed at later
388 stages (Fig. 2B and 2C). With the exception of one GH26 and one GH5_7, genes
389 coding for enzymes targeting general hemicellulose substrates and
390 galactomannan (i.e. GH2, GH26, GH27, GH35, GH5_7 and GH5_31) had their
391 maximum of expression at early stages. Pectinase encoding genes (GH28, GH55,
392 CE8 (CE=Carbohydrate Esterases) and PL1 (PL=Polysaccharide Lyases)) tended
393 to be highly expressed at intermediate stages of decomposition (Fig. S6). On the
394 contrary, enzymes targeting xylan and xyloglucan (i.e. GH10, GH11, GH12) were
395 mainly expressed at later stages (Fig. 2D).
396

397 Most of the *G. androsaceus* AA9 genes were highly expressed at month 2 but less
398 pronounced over time (Fig. 2B; Fig. 3C). AA2 genes had a maximum expression
399 level at month 5 (Fig. 2B). Although the correlation between the ratio of Aromatic
400 C to O-Alkyl C and the AA2 transcript expression level was only marginally
401 significant ($R^2$=0.296, p-value=0.129) (Fig. S5A), there was a trend that the lignin
402 to polysaccharides ratio decreased when transcription of AA2 genes was high
403 (Fig. 3B). AA1 genes, coding for multicopper oxidases, were expressed throughout
404 the experiment without a significant correlation ($R^2$=0.183, p-value=0.25) with
405 substrate chemical composition (Fig. S5C).
406
407 For *C. longipes* directional changes in gene expression levels were less obvious
408 (Fig. S4A; Fig. S6). Due to the lack of good replication for *C. longipes,*
409 interpretation of gene expression dynamics is ambiguous. Actually, neither mass
410 loss nor C fraction ratios were significantly correlated with the PCA ordination
411 (*P>0.5)*. Nonetheless, it appeared that genes coding for long-chain acting
412 enzymes (GH5_5, GH5_7, GH6, GH7, GH10, GH11) as well as LPMO (AA9) were
413 relatively more highly expressed at later stages, whereas genes coding for
414 monosaccharide releasing enzymes (GH1, GH3, GH2, GH27 and GH35) were more
415 expressed at early stages (Fig. S4B and S4C; Fig. S3B). Moreover, *C. longipes*
416 genes coding for ascomycetes class II peroxidases (AA2) were not significantly
417 expressed, and AA1 expression was much lower than for G. androsaceus (Fig.
418 1H).
419
420 It was visually obvious that *G. androsaceus* grew more vividly than *C. longipes*. It
421 also decomposed the organic matter four times more rapidly and the gradual

422 substitution of plant derived organic matter to organic matter derived from fungal
423 mycelium was indicated by a gradual increase in the ratio of Alkyl C to O-Alkyl C
424 in presence of *G. androsaceus* (Fig. 4) (Baskaran *et al.*, 2019). We used the
425 expression ratio of genes coding for the GT48 and GT2 families (β-1,3-glucan
426 synthase and chitin synthase) over the isocitrate dehydrogenase (IDH) and the
427 oxoglutarate dehydrogenase complex (OGDC) (responsible for $CO_2$ production in
428 the citric acid cycle) as an indicator of C use efficiency. This expression ratio was
429 3 times higher for *G. androsaceus* than for *C. longipes* and stable over the
430 duration of the experiment (Fig 3D).
431

432 **Discussion**

433 We related gene content and transcriptional patterns to chemical changes during
434 litter decomposition by two saprotrophic fungi with contrasting ecological
435 strategies. Contrary to our presupposition, the presence or diversity of functional
436 genes in the two genomes did not reflect the performance of the two fungi during
437 decomposition. Although *C. longipes* was a weak decomposer of holocellulose
438 compared to *G. androsaceus* (Baskaran *et al.*, 2019), we found that *C. longipes*
439 had a higher number of transcribed genes coding for enzymes involved in
440 cellulose, hemicellulose and pectin decomposition than *G. androsaceus* (Fig. 1;
441 Fig. S1; Fig. S2). However, the expression levels for genes considered as markers
442 for plant cell wall decomposition were considerably higher for *G. androsaceus*
443 than for *C. longipes*, with an almost 8 times higher average expression of genes
444 coding for cellulases and β-glucosidases and 9 times higher expression of
445 hemicellulose genes, as well as 4.5 time higher expression of pectinase genes
446 over the time course of the experiment (Fig. 1; Fig. S2). Thus, fungal gene

447 expression was a better predictor of cell-wall polysaccharides decomposition than
448 gene content, indicating that genomic information do not suffice to understand
449 functional differences between fungi of different ecological strategies.
450
451 In this context, one may consider the recent evolutionary history and larger
452 genomes of fungi relative to bacteria, which commonly have smaller genomes
453 that are highly optimized according to environmental selection pressure
454 (Martínez-Cano *et al.*, 2015). Compared to bacteria, fungi may be considered
455 metabolically similar, with sugars as their principal energy source. Thus,
456 ecological strategies among fungi primarily relate to differences in the way they
457 use extracellular processes and host interactions to acquire sugars. In some cases
458 systematic genomic differences have been identified, e.g. between mycorrhizal
459 and saprotrophic basidiomycetes (Kohler et al., 2015) or between different modes
460 of wood decomposition (Riley et al., 2014; Hori et al., 2018). In contrast, our
461 findings indicate that the distinct ecological strategies of two fungal litter
462 saprotrophs were largely regulated at the transcriptional level with a high degree
463 of genomic redundancy. Rather than indicating extensive decomposition
464 capabilities, the diverse set of genes involved in cell-wall polysaccharides
465 decomposition of *C. longipes* could reflect a high degree of ecological resilience
466 and flexibility. Generally, Leotiomycetes (to which *C. longipes* belongs) have a
467 broader tolerance to constrained nutrient availability and low pH than
468 Agaricomycetes (to which *G. androsaceus* belongs) (Sterkenburg *et al.*, 2015).
469 Further, *C. longipes* may colonise living needles as an endophyte (Koukol, 2011),
470 and this versatile ecology may be comparable to ericoid mycorrhizal
471 Leotiomycetes, which have been proposed to switch between biotrophism and

472 saprotrophism and also have a high content of genes involved in cell-wall
473 polysaccharide decomposition (Martino *et al.*, 2018) (Fig. S1).
474
475 In contrast to the stress tolerant, versatile strategy of *C. longipes*, the potent
476 decomposer capacity of the highly competitive *G. androsaceus* seems to be
477 related to high expression of a limited number of keystone genes. Its single gene
478 within the GH6 family, beneficial for cleavage of crystalline cellulose (Payne *et al.*,
479 2015), was highly transcribed at an almost 40 times higher rate than the three
480 GH6 genes of *C. longipes*, suggesting a keystone role of GH6 transcription for
481 cellulose decomposition (Fig. 1). The presence of 10 AA2 genes (MnP) in *G.*
482 *androsaceus* confirms the pivotal role of extracellular peroxidases for overall plant
483 cell-wall decomposition (Floudas *et al.*, 2012), particularly in boreal ecosystems
484 (Kyaschenko *et al.*, 2017; Stendahl *et al.*, 2017). While *G. androsaceus* was able
485 to cause significant mass loss of non-hydrolysable litter components, *C. longipes*,
486 with only four non-expressed genes coding for “generic” ascomycete class II
487 peroxidases and a low expression of AA1 genes (multicopper oxidases incl.
488 laccases), was unable to attack this fraction (Baskaran *et al.*, 2019).
489
490 We hypothesized that genes coding for plant cell wall decomposing enzymes
491 would be sequentially expressed as decomposition progressed, with long-chain
492 acting enzymes initiating the process, monosaccharide-releasing enzymes
493 increasing in relative importance with time, and oxidative enzymes primarily
494 being produced at late stages of decomposition. On the contrary, we found that
495 genes coding for enzymes acting on long chains (including the cellulases of GH6
496 and GH7 as well as endoxylanases, xyloglucan endoglucanases and

497 endomannanases belonging to the families GH10, GH11, GH12, GH5 and GH26)
498 were most expressed towards the end of the experiment, whereas genes coding
499 for monosaccharide-releasing β-glucosidases (GH1 and GH3), β-mannosidases
500 (GH2), galactosidases (GH 27 and GH35) and pectinases (GH28, GH55, PL1, CE8)
501 were expressed also during early stages of decomposition (Fig. 2, Fig. S3, Fig S6).
502 For both fungi the expression ratio of cellulases (GH5_5, GH6 and GH7) to β-
503 glucosidases (GH1 and GH3) increased over time with a higher ratio for *G.*
504 *androsaceus* (Fig. 3A). Further, the cellulases to β-glucosidases expression ratio
505 also increased as the Carboxyl C to O-Alkyl C ratio increased (Fig. 2; Fig. 3A),
506 which indicates loss of polysaccharides in relation to more stable compounds.
507 Thus, counterintuitively, production of long-chain acting enzymes seemed to
508 increase as the pool of hydrolysable polysaccharides was depleted. These results
509 partly agrees with the dynamics of enzyme activities in other decomposition
510 experiments (Šnajdr *et al.*, 2011; Presley *et al.*, 2018).
511
512 Based on these observations, we conceptualise that the polysaccharides of the
513 plant cell wall do not constitute a homogenous pool with respect to susceptibility
514 to enzymatic hydrolysis, but rather a spectrum from long unbranched molecules
515 to highly branched and cross-linked structures. Linear chains may be efficiently
516 hydrolysed by a minimum of internal chain cleavages, followed by rapid
517 depolymerisation by processive (non-releasing) enzymes, leading to ample
518 production of small polysaccharides. These, in turn, require high activity of
519 monosaccharide-releasing enzymes for sugars to become available for uptake. As
520 susceptible substrates are depleted, the proportion of branched and cross-linked
521 polysaccharides raises, and thus increasing the demand for internal cleavage and

522 chain-end formation. Further, cross-linking and branching efficiently disrupt the
523 processive mechanism of non-releasing hydrolases, leading to a lower production
524 of small polysaccharides (Yoshida *et al.*, 2008) (Fig. 5). Thus, the increasing
525 expression of genes coding for long-chain active enzymes with time should not be
526 interpreted as accelerating rate of decomposition, but rather as a response to
527 decreasing availability of susceptible substrates and a lower output of products
528 per enzymatic reaction event.

529

530 Another interpretation would be that crystalline cellulose – the primary target of
531 enzymes in the GH6 and GH7 families – is less readily hydrolysed than amorphous
532 cellulose and hemicellulose and thus remains until later stages of decomposition,
533 motivating late expression of GH6 and GH7 encoding genes. However, this theory
534 disagrees with our observations that genes coding for AA9 enzymes (LPMO) –
535 instrumental for the initiation of degradation of crystalline cellulose (Hu *et al.*,
536 2014) – were expressed primarily during early stages (for *G. androsaceus*),
537 whereas genes related to decomposition of xylan and xyloglucan – the main
538 hemicelluloses of the secondary cell wall of softwood (Shrotri *et al.*, 2017) – were
539 primarily expressed at later stages (Fig. 2D).

540

541 In light of our results we suggest that the expression ratio of GH5_5+GH6+GH7 to
542 GH1+GH3 could be used as a marker of declining substrate quality, with low
543 ratios indicating opportunistic use of more labile substrates, whereas a high ratio
544 indicate more efficient resource utilization, also targeting more recalcitrant
545 compounds.

546

547 In the case of *C. longipes,* which did not use oxidative mechanisms for
548 ligninolysis, the Aromatic C to O-Alkyl C ratio increased with time (Fig S5B) as
549 holocellulose was degraded. In contrast, in *G. androsaceus*, this ratio decreased
550 concurrently with an increase in AA2 gene expression from month 2 to month 5
551 (Fig 3B). Although the correlation was not clearly significant, we observed a trend
552 of lower Aromatic-C to O-Alkyl-C ratio when AA2 gene expression was higher (Fig
553 S5A). This may indicate that, after initial consumption of susceptible
554 polysaccharides, the proportion of lignin increases, and cross-linking between
555 polysaccharides and lignin (Iiyama *et al.*, 1994) may impede hydrolytic
556 depolymerisation (Yoshida *et al.*, 2008). This view is congruent with the
557 increasing ratio of Aromatic C over O-Alkyl C in *C. longipes*, but with the
558 ligninolytic *G. androsaceus* the chemical barrier to hydrolysis may have been
559 counter-acted by peroxidases making new polysaccharides susceptible to
560 depolymerisation. The relatively early expression of AA2 genes and many AA1
561 genes in *G. androsaceus* was in disagreement with our original hypothesis, as well
562 as with the result of Šnajdr *et al.* (2011), who observed increasing Mn-peroxidase
563 and laccase activity at late decomposition stages. Earlier expression of oxidative
564 enzymes during decomposition of pine needles may be explained by the high
565 proportion of O-Alkyl C in the non-hydrolysable pool (Baskaran *et al.*, 2019),
566 potentially cross-linked to lignin (Iiyama *et al.*, 1994). Moreover, sequential
567 patterns of decomposition observed in the field may also reflect the ecological
568 succession of fungi during litter decomposition, in which initial decomposition by
569 endophytic ascomycetes is replaced by the later action of basidiomycetes
570 (Voříšková and Baldrian, 2013).
571

572 The lytic polysaccharide monooxygenases (LPMO) of the AA9 family are,
573 supposedly, secreted during the early steps of the decomposition process
574 (Couturier *et al.*, 2015) and act in synergy with cellulases to enhance their
575 decomposition capacity (Harris *et al.*, 2010; Hu *et al.*, 2014; Couturier *et al.*,
576 2016). This idea is in agreement with our observations of *G. androsaceus*, where
577 AA9 gene expression declined as decomposition progressed and the more
578 efficient oxidative AA2 enzymes were induced (Fig. 2B; Fig. 3C). However, *C.*
579 *longipes* without expressed AA2 coding genes and low AA1 gene expression was
580 unable to attack lignin, and when susceptible polysaccharides were depleted,
581 expression of AA9 increased (Fig. 3C; Fig. S4B).
582
583 After uptake of decomposition products, carbohydrates are partitioned between
584 catabolic and anabolic metabolic pathways. The expression ratio of genes coding
585 for enzymes involved in the synthesis of the two principal components of the
586 fungal cell wall, namely β-1.3 glucan and chitin (i.e. GT48 and GT2) over genes
587 coding for central enzymes of the respiration (i.e. IDH and OGDC in the Citric acid
588 cycle) was much higher in *G. androsaceus* than *C. longipes* (Fig. 3D). This higher
589 expression ratio is indicative of a higher CUE for *G. androsaceus*, in line with our
590 initial hypothesis and the intense mycelial growth of *G. androsaceus* observed in
591 the microcosms (Fig. 4). *C. longipes* had a similar expression of genes associated
592 with $CO_2$ production, but lower expression of GT48 and GT2 genes, in line with its
593 meagre mycelial growth. However, contrary to our hypothesis the gene
594 expression indicator of CUE ratio remained constant over the experiment,
595 indicating that the partitioning of acquired resources between growth and
596 respiration was not altered by decreasing chemical quality of the substrate or

597 mycelial senescence. Thus, the increasing enzyme production in more recalcitrant
598 materials, as indicated by the upregulated transcription of CAZyme genes with
599 time, seems not to be associated with a major respiratory cost. However, the
600 markers we used to reflect respiration are putative, and careful analyses of gene
601 expression and $CO_2$ production in parallel are needed. Our observations may be
602 interpreted within the C-S-R theoretical framework (Grime, 1977; Cooke and
603 Rayner, 1984; Crowther et al. 2014), with *C. longipes* having stress-tolerant traits,
604 such as a versatile but inefficient decomposition machinery and low resource
605 allocation to mycelial growth. In contrast, the traits of *G. androsaceus* are
606 congruent with a C-strategy, with higher biomass production and efficient
607 exploitation of organic matter resources.
608
609 To conclude, expression analysis of specific genetic markers seems to be useful
610 to assess fungal ecological strategies, providing information about the dynamics
611 of ecophysiological processes, such as decomposition. By targeting transcription
612 ratios of specific 'keystone' genes at the ecosystem scale (*i.e.* meta-
613 transcriptomics), information about the interplay between the fungal communities
614 and their environment may be derived that could be used to decipher the role of
615 fungi as mediators of ecosystem responses to environmental change (Lindahl and
616 Kuske, 2013; Treseder and Lennon, 2015).
617

## 618 Acknowledgment

619 Thanks are due to Mats Sandgren for valuable comments and friendly review
620 of the manuscript.

621 This work was supported by The Swedish Research Council FORMAS (grant 2011-
622 1747 to B.L.), the Genomic Science Program, US Department of Energy, Office of
623 Science and the Laboratory of Excellence ARBRE (grant no. ANR-11-LABX-
624 0002_ARBRE). The research was also supported by the Institut National de la
625 Recherche Agronomique, University of Lorraine, Région Lorraine Research Council
626 and the European Fund for Regional Development. The work conducted by the
627 U.S. Department of Energy Joint Genome Institute, a DOE Office of Science User
628 Facility, is supported by the Office of Science of the U.S. Department of Energy
629 under Contract No. DE-AC02-05CH11231.

630

631 The authors have no conflict of interest to declare.

632

633 ## References

634 • Baldrian, P. and López-Mondéjar, R. (2014) Microbial genomics,
635 transcriptomics and proteomics: new discoveries in decomposition research
636 using complementary methods. *Applied Microbiology and Biotechnology*
637 98: 1531–1537.
638 • Barbi, F., Bragalini, C., Vallon, L., Prudent, E., Dubost, A., Fraissinet-Tachet,
639 L., et al. (2014) PCR Primers to Study the Diversity of Expressed Fungal
640 Genes Encoding Lignocellulolytic Enzymes in Soils Using High-Throughput
641 Sequencing. *PLoS ONE* 9: e116264.
642 • Baskaran, P., Ekblad, A., Soucémarianadin, L.N., Hyvönen, R., Schleucher,
643 J., and Lindahl, B.D. (2019) Nitrogen dynamics of decomposing Scots pine
644 needle litter depends on colonizing fungal species. *FEMS Microbiology*
645 *Ecology* 95:.

646 • Bhatnagar, J.M., Peay, K.G., and Treseder, K.K. (2018) Litter chemistry
647 influences decomposition through activity of specific microbial functional
648 guilds. *Ecological Monographs* 88: 429–444.
649 • Boberg, J.B., Finlay, R.D., Stenlid, J., Ekblad, A., and Lindahl, B.D. (2014)
650 Nitrogen and carbon reallocation in fungal mycelia during decomposition of
651 boreal forest litter. *PloS one* 9: e92897–e92897.
652 • Boberg, J.B., Ihrmark, K., and Lindahl, B.D. (2011) Decomposing capacity of
653 fungi commonly detected in Pinus sylvestris needle litter. *Fungal Ecology* 4:
654 110–114.
655 • Bowman, S.M. and Free, S.J. (2006) The structure and synthesis of the
656 fungal cell wall. *BioEssays* 28: 799–808.
657 • Cooke, R.C., (viaf)76376799 and Rayner, A.D.M. (1984) Ecology of
658 saprotrophic fungi, London : Longman.
659 • Couturier, M., Bennati-Granier, C., Urio, M.B., Ramos, L.P., and Berrin, J.-G.
660 (2016) Fungal Enzymatic Degradation of Cellulose. In, Soccol,C.R.,
661 Brar,S.K., Faulds,C., and Ramos,L.P. (eds), *Green Fuels Technology:*
662 *Biofuels*. Cham: Springer International Publishing, pp. 133–146.
663 • Couturier, M., Navarro, D., Chevret, D., Henrissat, B., Piumi, F., Ruiz-
664 Dueñas, F.J., et al. (2015) Enhanced degradation of softwood versus
665 hardwood by the white-rot fungus Pycnoporus coccineus. *Biotechnology for*
666 *biofuels* 8: 216–216.
667 • Crowther, T.W., Maynard, D.S., Crowther, T.R., Peccia, J., Smith, J.R., and
668 Bradford, M.A. (2014) Untangling the fungal niche: the trait-based
669 approach. *Front Microbiol* 5: 579–579.

670 • Eastwood, D.C., Floudas, D., Binder, M., Majcherczyk, A., Schneider, P.,
671 Aerts, A., et al. (2011) The Plant Cell Wall–Decomposing Machinery
672 Underlies the Functional Diversity of Forest Fungi. *Science* 333: 762.
673 • Fawal, N., Li, Q., Savelli, B., Brette, M., Passaia, G., Fabre, M., et al. (2013)
674 PeroxiBase: a database for large-scale evolutionary analysis of
675 peroxidases. *Nucleic acids research* 41: D441–D444.
676 • Floudas, D., Binder, M., Riley, R., Barry, K., Blanchette, R.A., Henrissat, B.,
677 et al. (2012) The Paleozoic Origin of Enzymatic Lignin Decomposition
678 Reconstructed from 31 Fungal Genomes. *Science* 336: 1715–1719.
679 • Grigoriev, I.V., Nikitin, R., Haridas, S., Kuo, A., Ohm, R., Otillar, R., et al.
680 (2014) MycoCosm portal: gearing up for 1000 fungal genomes. *Nucleic*
681 *Acids Research* 42: D699–D704.
682 • Grime, J.P. (1974) Vegetation classification by reference to strategies.
683 *Nature* 250: 26–31.
684 • Harris, P.V., Welner, D., McFarland, K.C., Re, E., Navarro Poulsen, J.-C.,
685 Brown, K., et al. (2010) Stimulation of Lignocellulosic Biomass Hydrolysis by
686 Proteins of Glycoside Hydrolase Family 61: Structure and Function of a
687 Large, Enigmatic Family. *Biochemistry* 49: 3305–3316.
688 • Hofrichter, M., Ullrich, R., Pecyna, M.J., Liers, C., and Lundell, T. (2010) New
689 and classic families of secreted fungal heme peroxidases. *Applied*
690 *Microbiology and Biotechnology* 87: 871–897.
691 • Hu, J., Arantes, V., Pribowo, A., Gourlay, K., and Saddler, J.N. (2014)
692 Substrate factors that influence the synergistic interaction of AA9 and
693 cellulases during the enzymatic hydrolysis of biomass. *Energy Environ Sci*
694 7: 2308–2315.

695 • Iiyama, K., Lam, TBT., and Stone, B.A. (1994) Covalent Cross-Links in the
696 Cell Wall. *Plant Physiol* 104: 315.
697 • Kellner, H., Luis, P., Pecyna, M.J., Barbi, F., Kapturska, D., Krüger, D., et al.
698 (2014) Widespread Occurrence of Expressed Fungal Secretory Peroxidases
699 in Forest Soils. *PLoS ONE* 9: e95557.
700 • Kohler, A., Kuo, A., Nagy, L.G., Morin, E., Barry, K.W., Buscot, F., et al.
701 (2015) Convergent losses of decay mechanisms and rapid turnover of
702 symbiosis genes in mycorrhizal mutualists. *Nature Genetics* 47: 410–415.
703 • Koide, R.T., Fernandez, C., and Malcolm, G. (2014) Determining place and
704 process: functional traits of ectomycorrhizal fungi that affect both
705 community structure and ecosystem function. *New Phytologist* 201: 433–
706 439.
707 • Koukol, O. (2011) New species of Chalara occupying coniferous needles.
708 *Fungal Diversity* 49: 75.
709 • Kuske, C.R., Hesse, C.N., Challacombe, J.F., Cullen, D., Herr, J.R., Mueller,
710 R.C., et al. (2015) Prospects and challenges for fungal metatranscriptomics
711 of complex communities. *Fungal Ecology* 14: 133–137.
712 • Kyaschenko, J., Clemmensen, K.E., Karltun, E., and Lindahl, B.D. (2017)
713 Below-ground organic matter accumulation along a boreal forest fertility
714 gradient relates to guild interaction within fungal communities. *Ecology*
715 *Letters* 20: 1546–1555.
716 • Levasseur, A., Drula, E., Lombard, V., Coutinho, P.M., and Henrissat, B.
717 (2013) Expansion of the enzymatic repertoire of the CAZy database to
718 integrate auxiliary redox enzymes. *Biotechnology for Biofuels* 6: 41.

719 • Lindahl, B.D. and Kuske, C.R. (2013) Metagenomics for Study of Fungal
720 Ecology. In, *The Ecological Genomics of Fungi*. John Wiley & Sons, Ltd, pp.
721 279–303.
722 • Lombard, V., Golaconda Ramulu, H., Drula, E., Coutinho, P.M., and
723 Henrissat, B. (2014) The carbohydrate-active enzymes database (CAZy) in
724 2013. *Nucleic Acids Research* 42: D490–D495.
725 • Manzoni, S., Čapek, P., Porada, P., Thurner, M., Winterdahl, M., Beer, C., et
726 al. (2018) Reviews and syntheses: Carbon use efficiency from organisms to
727 ecosystems – definitions, theories, and empirical evidence. *Biogeosciences*
728 15: 5929–5949.
729 • Martinez, D., Challacombe, J., Morgenstern, I., Hibbett, D., Schmoll, M.,
730 Kubicek, C.P., et al. (2009) Genome, transcriptome, and secretome analysis
731 of wood decay fungus Postia placenta supports unique mechanisms of
732 lignocellulose conversion. *Proceedings of the National Academy of Sciences*
733 106: 1954–1959.
734 • Martínez-Cano, D.J., Reyes-Prieto, M., MartÃnez-Romero, E., Partida-MartÃ-
735 nez, L.P., Latorre, A., Moya, A., and Delaye, L. (2015) Evolution of small
736 prokaryotic genomes. *Frontiers in Microbiology* 5:.
737 • Martino, E., Morin, E., Grelet, G.-A., Kuo, A., Kohler, A., Daghino, S., et al.
738 (2018) Comparative genomics and transcriptomics depict ericoid
739 mycorrhizal fungi as versatile saprotrophs and plant mutualists. *New*
740 *Phytologist* 217: 1213–1229.
741 • Moorhead, D.L. and Sinsabaugh, R.L. (2006) A THEORETICAL MODEL OF
742 LITTER DECAY AND MICROBIAL INTERACTION. *Ecological Monographs* 76:
743 151–174.

744 • Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn,
745 D., et al. (2019) vegan: Community Ecology Package.
746 • Payne, C.M., Knott, B.C., Mayes, H.B., Hansson, H., Himmel, M.E., Sandgren,
747 M., et al. (2015) Fungal Cellulases. *Chem Rev* 115: 1308–1448.
748 • Presley, G.N., Panisko, E., Purvine, S.O., and Schilling, J.S. (2018) Coupling
749 Secretomics with Enzyme Activities To Compare the Temporal Processes of
750 Wood Metabolism among White and Brown Rot Fungi. *Applied and*
751 *Environmental Microbiology* 84:.
752 • R Core Team (2019) R: A Language and Environment for Statistical
753 Computing.
754 • Riley, R., Salamov, A.A., Brown, D.W., Nagy, L.G., Floudas, D., Held, B.W., et
755 al. (2014) Extensive sampling of basidiomycete genomes demonstrates
756 inadequacy of the white-rot/brown-rot paradigm for wood decay fungi.
757 *Proceedings of the National Academy of Sciences* 111: 9923–9928.
758 • Sarkar, P., Bosneaga, E., and Auer, M. (2009) Plant cell walls throughout
759 evolution: towards a molecular understanding of their design principles.
760 *Journal of Experimental Botany* 60: 3615–3635.
761 • Schädel, C., Blöchl, A., Richter, A., and Hoch, G. (2010) Quantification and
762 monosaccharide composition of hemicelluloses from different plant
763 functional types. *Plant Physiology and Biochemistry* 48: 1–8.
764 • Schaefer, J. and Stejskal, E.O. (1976) Carbon-13 nuclear magnetic
765 resonance of polymers spinning at the magic angle. *J Am Chem Soc* 98:
766 1031–1032.

767 • Shrotri, A., Kobayashi, H., and Fukuoka, A. (2017) Chapter Two - Catalytic
768 Conversion of Structural Carbohydrates and Lignin to Chemicals. In,
769 Song,C. (ed), *Advances in Catalysis*. Academic Press, pp. 59–123.
770 • Šnajdr, J., Cajthaml, T., Valášková, V., Merhautová, V., Petránková, M.,
771 Spetz, P., et al. (2011) Transformation of Quercus petraea litter: successive
772 changes in litter chemistry are reflected in differential enzyme activity and
773 changes in the microbial community composition: Transformation of
774 Quercus petraea litter. *FEMS Microbiology Ecology* 75: 291–303.
775 • Stendahl, J., Berg, B., and Lindahl, B.D. (2017) Manganese availability is
776 negatively associated with carbon storage in northern coniferous forest
777 humus layers. *Scientific Reports* 7:.
778 • Sterkenburg, E., Bahr, A., Brandström Durling, M., Clemmensen, K.E., and
779 Lindahl, B.D. (2015) Changes in fungal communities along a boreal forest
780 soil fertility gradient. *New Phytologist* 207: 1145–1158.
781 • Talbot, J.M., Martin, F., Kohler, A., Henrissat, B., and Peay, K.G. (2015)
782 Functional guild classification predicts the enzymatic role of fungi in litter
783 and soil biogeochemistry. *Soil Biology and Biochemistry* 88: 441–456.
784 • Treseder, K.K. and Lennon, J.T. (2015) Fungal Traits That Drive Ecosystem
785 Dynamics on Land. *Microbiology and Molecular Biology Reviews* 79: 243–
786 262.
787 • Yoshida, M., Liu, Y., Uchida, S., Kawarada, K., Ukagami, Y., Ichinose, H., et
788 al. (2008) Effects of Cellulose Crystallinity, Hemicellulose, and Lignin on the
789 Enzymatic Hydrolysis of *Miscanthus sinensis* to Monosaccharides.
790 *Bioscience, Biotechnology, and Biochemistry* 72: 805–810.
791

792 ## Data accessibility

793 ***Gymnopus androsaceus*** genome:
794 https://genome.jgi.doe.gov/Gyman1/Gyman1.home.html
795 GenBank accessions: BioProject PRJNA234428, BioSample SAMN05660848,
796 Accession VKGB00000000.
797 ***Chalara longipes*** genome: https://genome.jgi.doe.gov/Chalo1/Chalo1.info.html
798 GenBank accessions: BioProject PRJNA213334, BioSample SAMN02745709,
799 Accession VKGA00000000.
800 Microcosms experiment transcriptome data sets: NCBI GEO as GSEXXX
801 *(Submission in progess)*
802
803
804 ## Author Contributions

805 FB, AKo, PB, BL, FM designed research; LF, PB, KI performed research; KI, LF, BH,
806 IG contributed new reagents or analytical tools; AKu, KL, CD, KB, BH, IG were
807 involved in genomes project and annotations; FB, AKo, EM, BL analyzed data; FB,
808 AKo, BL, FM wrote the paper.
809

810 **Table 1.** Characteristics of the selected CAZyme gene families.
811 For each CAZyme family information concerning the enzyme substrate,
812 mechanism, active site, 3D structure of catalytic domain and binding location are
813 from the CAZyme database (http://www.cazy.org/), CAZypedia
814 (http://www.cazypedia.org/index.php/Main_Page), ExplorEnz database
815 (http://www.enzyme-database.org/index.php), and JGI annotation
816 (https://jgi.doe.gov)

817 *GH2 beta-mannosidase were selected based on JGI annotations

818 **GH28 pectine lyases were selected based on JGI annotations

819 ***This family include exo- and endo-enzymes but a majority of the member are

820 exo-enzymes (CAZyme)

821 ****Including subfamilies AA1_1, AA1_2 and AA1_3

822

823 **Table 2.** Interpretation of $^{13}C$ CP/MAS-NMR spectroscopy

824 Specific C pools of chemical fractions of *Pinus sylvestris* needle litter are

825 interpreted in terms of organic matter compounds.

826

827

828 **Figure 1.** Gene content in genomes and global expression levels

829 **(A, C, E and G)** Bar plots indicating the number of genes present in the genomes

830 of *Gymnopus androsaceus* and *Chalara longipes*. **(B, D, F and H)** Bar plots

831 indicated the global expression levels (i.e. addition of the average of relative gene

832 expression levels for each month). **(A and B)** Selected gene families involved in

833 cellobiose and cellulose decomposition are indicated in light blue (GH1), blue

834 (GH3), light green (GH5 (subfamily 5)), green (GH6), dark green (GH7). **(C and B)**

835 Restricted number of relevant gene families coding for enzymes targeting a

836 specific substrate in hemicelluloses are indicated in cyan (GH10), blue (GH11),

837 purple (GH12), red (GH2), dark green (GH26), green (GH27), yellow (GH35) and

838 brown (GH5 (subfamilies 7 and 31)). **(E and F)** Selected gene families involved in

839 pectin decomposition are indicated in light blue (GH28*), darkblue (GH55), green

840 (CE8), yellow (PL1), orange (PL3_2). **(G and H)** Selected gene families coding for

841 multicopper oxidases are indicated in brown (AA1), darkred (AA1_1), red (AA1_2),
842 pink (AA1_3).
843 GH2*: Only beta-mannosidase. Based on JGI annotations. Transcript Id 916611,
844 962261 and 1012799 for *G. androsaceus*. JGI Transcript Id 193197, 345240,
845 354232, 396534, 406068, 470006, 493784 and 503773 for *C. longipes*.
846 GH28* For *G. androsaceus* the JGI Transcript Id 991351 is not annotated as
847 pectinase.
848
849
850 **Figure 2.**
851 Principal component analysis (PCA) ordination displaying *Gymnopus androsaceus*
852 overall pattern of gene expression during the experiment. Relative abundance of
853 expressed genes are mean-normalized to represent the dynamic of gene
854 expressions. Areas represent the global expression levels (i.e. sum of the average
855 for each of the 3 month) for the selected genes, at a logarithmic scale. Vectors
856 fitted the litter mass remaining and different carbon fractions of litter organic
857 matter to the PCA ordination. The different plots highlight **(A)** the transcriptomes
858 from month 2 (M2T1, M2T2, M2T3), month 5 (M5T1, M5T2, M5T3) and month 10
859 (M10T1, M10T2, M10T3), **(B)** expressed genes involved in cellobiose and cellulose
860 decomposition (monosaccharide releasing enzymes in blue, long-chain acting
861 enzymes in green and LPMO in red) and lignin decomposition (Class II peroxidases
862 in purple), **(C)** expressed genes coding for hemicellulases (monosaccharide
863 releasing enzymes in blue and long-chain acting enzymes in green), **(D)** the
864 different hemicelluloses (xylan and xyloglucan in blue, galactomannan in green
865 and all of them in red) targeted by the hemicellulases.

866

867

868 **Figure 3.**

869 **(A)** Relationship between the ratio GH5_5+GH6+GH7 to GH1+GH3 and the ratio
870 Carboxyl C to O-Alkyl C. **(B)** Evolution over months of the relative abundance of
871 expressed genes coding for class II peroxidases (AA2) in parallel with the ratio
872 Aromatic C to O-Alkyl C in presence of *G. androsaceus* (purple). **(C)** Evolution over
873 months of the relative abundance of expressed genes coding for lytic
874 polysaccharide monooxygenases (AA9). **(D)** Relationship between the relative
875 abundance of expressed genes coding for the β-glucan synthase (GT48) plus the
876 chitin synthase (GT2) and the genes coding for the oxoglutarate dehydrogenase
877 complex (OGDC) plus isocitrate dehydrogenases (IDH). Data obtained from litter
878 decomposition microcosms in presence of *Gymnopus androsaceus* (blue) and
879 *Chalara longipes* (red) at month 2 (empty circle), month 5 (circle with cross) and
880 month 10 (full circle). Lines represent fitted linear regressions with $P < 0.05$ (solid
881 line), $0.1 < P > 0.05$ (dashed line), $P > 0.1$ (dotted line).

882

883

884 **Figure 4.** Needle litter microcosms.

885 The pictures of the litter microcosms have been taken after 10 month in presence
886 of *Gymnopus androsaceus* (left side, blue) and *Chalara longipes* (right side, red).
887 The bar plots indicate the percentage of needle litter mass remaining and the
888 gradual substitution of plant derived organic matter to fungal derived organic
889 matter (i.e. ratio Alkyl C to O-Alkyl C) measured at month 2 (M2), 5 (M5) and 10
890 (M10) for both fungi.

891

892

893 **Figure 5.** Conceptual figure of plant polysaccharide pools hydrolysis over time.

894 Plant cell wall constitutes a spectrum from long unbranched molecules to highly

895 branched and cross-linked structures. **(Top frame)** At the beginning of the

896 decomposition process, long unbranched molecules are efficiently hydrolysed by

897 few long-chain acting enzymes and require high activity of monosaccharide-

898 releasing enzymes for sugars to become available for uptake. **(Bottom frame)** In

899 later stage of decomposition process, the higher proportion of branched and

900 cross-linked structures increase the demand for internal cleavage and chain-end

901 formation performed by long-chain acting enzymes an require equivalent or lower

902 activity of monosaccharide-releasing enzymes. Consequently with the progress of

903 decomposition the ratio of long-chain acting enzymes to monosaccharide-

904 releasing enzymes increase.

905

906

907 **Table S1.** CAZy assignments for the 2 fungi *Gymnopus androsaceus* and *Chalara*

908 *longipes*.

909

910 **Table S2.** *G. androsaceus* and *C. longipes* genomes information

911

912 **Figure S1.** Heatmap representing the degree of CAZYmes content similarity

913 between *Chalara longipes*, *Gymnopus androsaceus* and 28 other Ascomycota and

914 Basidiomycota published genomes. From left to right Cadospora sp. (Cadsp1)

915 END, Oidodendron maius (Oidma1) ERM, Phialocephala scopiformis (Phisc1) END,

916 Chalara longipes (Chalo1) SAP, Meliniomyces variabilis (Melva1) ERM, Armillaria
917 cepistipes (Armcep1) SAP, Gymnopus androsaceus (Gyman1) SAP, Gymnopus
918 luxurians (Gymlu1) SAP, Amanita muscaria (Amamu1) ECM, Paxillus involutus
919 (Paxin1) ECM, Laccaria bicolor (Lacbi2) ECM, Postia placenta (Pospl1) SAP, Serpula
920 lacrimans (Serla2) SAP, Heterobasidion annosum (Hetan2) P, Trametes versicolor
921 (Trave1) P, Phanerochaete chrysosporium (Phchr2) SAP, Schizophyllum commune
922 (Schco3) SAP, Pleurotus ostreatus (Pleos2) SAP, Coprinopsis cinerea (Copci1) SAP,
923 Sacharomyces cerevisiae (Sacce1) Y, Tuber melanosporum (Tubme1v2) ECM,
924 Terfezia boudieri (Terbo2) ECM, Morchella importuna (Morco1) SAP, Neurospora
925 crassa (Neucr2) SAP, Ascocoryne sarcoides (Ascsa1), Glarea lozoyensis (Glalo1)
926 SAP, Glonium stellatum (Glost2) SAP, Rhizoscyphus ericae (Rhier1) ERM,
927 Aspergillus nidulans (Aspni7) SAP, Cenococcum geophilum (Cenge3) ECM. Fungal
928 guilds: ericoid mycorrhiza (ERM), endophyte (END), Ectomycorrhiza (ECM),
929 saptrotroph (SAP), pathogen (P), yeast (Y).
930
931
932 **Figure S2.** Gene content in genomes and global expression levels
933 On the left: bar plots indicating the number of genes coding for hemicellulases
934 involved in xylan, xyloglucan and galactomannan decomposition, present in the
935 genomes of *Gymnopus androsaceus* and *Chalara longipes*. On the right: bar plots
936 indicating the global expression levels (i.e. addition of the average of relative
937 gene expression levels for each month) of genes coding for hemicellulases.
938
939

940 **Figure S3.** Dynamic of transcription for genes involved in cellobiose and
941 cellulose depolymerisation.
942 Relative abundance of expressed genes coding for monosaccharide releasing
943 enzymes (β-glucosidases GH1 in green and GH3 in yellow), genes coding for long-
944 chain acting enzymes (GH5_5 in black, GH6 in blue and GH7 in red), at month 2, 5
945 and 10, for **(A)** *Gymnopus androsaceus* and **(B)** *Chalara longipes* (right). Lines
946 represent fitted linear regressions with $P < 0.05$ (solid line), $0.1 < P > 0.05$ (dashed
947 line), $P > 0.1$ (dotted line).
948
949
950 **Figure S4.**
951 Principal component analysis (PCA) ordination displaying *Chalara longipes* overall
952 pattern of gene expression during the experiment. Relative abundance of
953 expressed genes are mean-normalized to represent the dynamic of gene
954 expressions. Areas represent the global expression levels (i.e. sum of the average
955 for each of the 3 month) for the selected genes, at a logarithmic scale. Vectors
956 fitted the litter mass remaining and different carbon fractions of litter organic
957 matter to the PCA ordination. The different plots highlight **(A)** the transcriptomes
958 from month 2 (M2T1, M2T2), month 5 (M5T1, M5T2) and month 10 (M10T1,
959 M10T2, M10T3), **(B)** expressed genes involved in cellobiose and cellulose
960 decomposition (monosaccharide releasing enzymes in blue, long-chain acting
961 enzymes in green and LPMO in red), **(C)** expressed genes coding for
962 hemicellulases (monosaccharide releasing enzymes in blue and long-chain acting
963 enzymes in green), **(D)** the different hemicelluloses (xylan and xyloglucan in blue,
964 galactomannan in green and all of them in red) targeted by the hemicellulases.

**Figure S5.**

**(A)** Relationship between the ratio Aromatic C to O-Alkyl C and the relative abundance of expressed genes coding for Class II peroxidases (AA2) for *Gymnopus androsaceus* at month 2 (empty circle), month 5 (circle with cross) and month 10 (full circle). **(B)** Evolution over month of the ratio Aromatic C to O-Alkyl C in presence of *Chalara longipes*. **(C)** Relationship between the relative abundance of expressed genes coding for multicopper oxidases (AA1+AA1_1+AA1_2+AA1_3) and the ratio Aromatic C to O-Alkyl C. Data obtained from litter decomposition microcosms in presence of *Gymnopus androsaceus* (blue) and *Chalara longipes* (red) at month 2 (empty circle), month 5 (circle with cross) and month 10 (full circle). Lines represent fitted linear regressions with $P < 0.05$ (solid line), $0.1 < P > 0.05$ (dashed line), $P > 0.1$ (dotted line).

**Figure S6.**

Principal component analysis (PCA) ordination displaying *Gymnopus androsaceus* and *Chalara longipes* overall pattern of gene expression during the experiment. Relative abundance of expressed genes are mean-normalized to represent the dynamic of gene expressions. Areas represent the global expression levels (i.e. sum of the average for each of the 3 month) for the selected genes, at a logarithmic scale. Vectors fitted the litter mass remaining and different carbon fractions of litter organic matter to the PCA ordination. The different plots highlight **(A)** *Gymnopus androsaceus* transcriptomes from month 2 (M2T1, M2T2),

990 month 5 (M5T1, M5T2) and month 10 (M10T1, M10T2, M10T3), **(B)** *Gymnopus*
991 *androsaceus* expressed genes involved in pectin decomposition (yellow and
992 green) and expressed genes coding for laccases (blue). **(C)** *Chalara longipes*
993 transcriptomes from month 2 (M2T1, M2T2), month 5 (M5T1, M5T2) and month 10
994 (M10T1, M10T2, M10T3), **(D)** *Chalara longipes* expressed genes involved in pectin
995 decomposition (yellow and green) and expressed genes coding for laccases (blue)
996