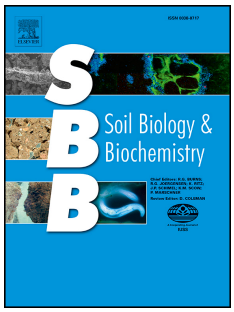

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Metagenomics and stable isotope probing reveal the complementary contribution of fungal and bacterial communities in the recycling of dead biomass in forest soil

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Abstract

Forest soils represent important terrestrial carbon (C) pools, where C is primarily fixed in plant biomass and then is incorporated in the biomass of fungi and bacteria. Although classical concepts assume that fungi are the main decomposers of the recalcitrant organic matter within plant and microbial biomass, whereas bacteria are considered to mostly utilize simpler compounds, recent studies have shown that fungi and bacteria overlap in substrate utilization. Here, we studied the microbial contribution to the recycling of dead biomass by analyzing the bacterial and fungal communities in soil microcosms supplemented with ^{13}C-labeled biomass of plant, fungal, and bacterial origin using a combination of DNA-stable isotope probing and metagenomics. Both fungi and bacteria contributed actively to the degradation of complex components of plant and microbial biomass. Specific families of carbohydrate-active enzymes (CAZyme) were involved in the degradation of each biomass type. Moreover, the analysis of five bacterial metagenome-assembled genomes indicated the key role of some bacterial genera in the degradation of plant biomass (*Cytophaga* and *Asticcacaulis*) and microbial biomass

(*Herminiimonas*). The enzymatic systems utilized by bacteria are highly complex and complementary but also highly diverse among taxa. The results confirm the importance of bacteria, in addition to fungi, as decomposers of complex organic matter in forest soils.

Keywords: decomposition, forest soil, microbial community, CAZyme, SIP-metagenomic, dead biomass

1. Introduction

Forests represent some of the most important carbon (C) pools and sinks on Earth. Since nearly half of the C stored in these ecosystems is contained in soils, understanding the processes involved in C cycling in forest soils is essential in the current context of global climate change (Pan et al., 2011). Microorganisms are the main players involved in the recycling and turnover of soil organic matter. As such, they contribute largely to the C flow in this habitat and have the potential to influence the feedback between climate and the global C cycle (Schimel and Schaeffer, 2012). Therefore, predicting how forests will respond to future environmental conditions is impossible without understanding the roles of soil microbes in C cycling (Graham et al., 2016; Trivedi et al., 2013).

The major sources of forest soil C are comprised of the C allocated by tree roots into soil and of the C contained in the dead plant biomass in the forms of litter and dead wood. This dead plant biomass is composed mostly of cellulose, hemicelluloses and lignin, forming a complex and recalcitrant matrix (Bomble et al., 2017). Microbial biomass represents another important pool of organic matter whose fate in the soil is far less understood. Forest soils are rich in ectomycorrhizal (ECM) and saprotrophic fungi and the decomposition of dead mycelia represents an important process for the cycling of C and other nutrients in these ecosystems (Ekblad et al., 2013; Fernandez and Koide, 2014). Dead fungal biomass is composed mainly of polysaccharides that can make up 80-90% of the total cell wall, but it also contains lipids and mannoproteins (Baldrian et al., 2013b; Fesel and Zuccaro, 2016; Free, 2013). The

main components of the polysaccharide fraction include chitin, a polymer of *N*-acetylglucosamine units, different types of beta- and alpha-glucans, glucomannans and galactomannans. Dead bacterial biomass is considered to be equally abundant in forest soils, showing higher turnover rates than fungal biomass (Gunina et al., 2017). The composition of cell walls is highly diverse in bacteria (Silhavy et al., 2010). Peptidoglycan (PG), a polymer of *N*–acetylglucosamine and *N*-acetylmuramic acid units connected to chains of amino acids, is a major and universal component of bacterial cell walls (Egan et al., 2017; Scheffers and Pinho, 2005). In gram-positive bacteria, PG is densely functionalized with other polymers. Cell-wall glycopolymers such as teichoic, teichuronic and teichulosonic acids, which are attached either to the PG or to membrane lipids, are the most abundant (Brown et al., 2013; Schaffer and Messner, 2005; Weidenmaier and Peschel, 2008). Apart from PG and cell-wall glycopolymers, the bacterial cell wall includes proteins, glycosyl 1-phosphates and other sugar-containing polymers such as arabinogalactan, lipomannan and lipoarabinomannan (Hamedi and Poorinmohammad, 2017). Furthermore, gram-negative bacteria contain lipopolysaccharides and lipoproteins in their outer membranes (Silhavy et al., 2010). Many bacteria also produce a range of chemically different extracellular polysaccharides, which can be utilized as C sources by other microorganisms in soils (Bazaka et al., 2011; Mishra and Jha, 2013; Wang et al., 2015).

The turnover of carbohydrates in plant and microbial biomass can be tracked by analyzing the microbial enzymes that take part in the C turnover—the carbohydrate-active enzymes (CAZymes) (Žifčáková et al., 2017). CAZymes, classified into a hierarchy of families based on their structure and function, act on oligosaccharides, polysaccharides and glycoconjugates (Lombard et al., 2014). Among the CAZYmes, glycoside hydrolases (GHs), which hydrolytically cleave the glycosidic bonds within carbohydrates or between a carbohydrate and a noncarbohydrate moiety, are the most important in decomposition. In this sense, cellulases, β-glucosidases and hemicellulases such as endoxylanases, β-xylosidases, xyloglucanases, endomannanases, mannosidases, fucosidases, and arabinosidases from several GH

families are the main enzymes that degrade plant biomass (Bomble et al., 2017). Beside them, lytic polysaccharide monooxygenases (LPMOs), classified as enzymes with auxiliary activities (AA) in the CAZy database, have also been found to play an important role in the degradation of cellulose (Vaaje-Kolstad et al., 2017). In addition, several AA families including peroxidase, oxidoreductase and laccase activities participate either directly or indirectly in the degradation of lignin (Levasseur et al., 2013). Finally, carbohydrate esterases (CEs) from several families participate in the decomposition of hemicelluloses. In the case of fungal biomass, chitinases and N-acetylglucosaminidases from three GH families are involved in the degradation of chitin, and glucanases from several GH families, which degrade glucans, are highlighted as main players involved in its degradation. The lysozymes and PG lytic transglycosylases are important enzymes involved in the degradation of PG in bacterial biomass. Catalytically active CAZYmes may contain carbohydrate-binding modules (CBMs), which are essential for effective hydrolysis because they mediate binding to cellulose, xylan, chitin or other carbohydrates (Donohoe and Resch, 2015). Many GH families include enzymes that are structurally similar but have wider substrate specificity, and associating one family to the degradation of one type of compound is not always easy (Nguyen et al., 2018). Moreover, the complex, diverse and not fully characterized composition of dead biomass in forest soils, especially in the case of fungal and bacterial biomass, may entail the implication of more CAZyme families than those currently proposed.

For a long time, fungi were assumed to be the major decomposers of complex organic matter in forest soils due to their filamentous nature, which allows them to colonize substrates efficiently, their ability to produce a rich battery of extracellular enzymes and their limited requirements of N, which is rather rare in cell wall biopolymers. This assumption led to underestimation of the role of bacteria in decomposition, and bacteria were typically expected to target simple substrates (de Boer et al., 2005; Rousk and Frey, 2015). Different studies have indicated that bacteria play a more important role in the transformation and mineralization of organic matter and contribute significantly to decomposition in

forest soils (Eichorst and Kuske, 2012; Stursova et al., 2012; Verastegui et al., 2014). The high percentage of bacteria that potentially decompose cellulose found in forest soil and the high frequency of genes involved in the degradation of structural plant polysaccharides found in bacterial genomes support that the involvement of bacteria in plant biomass decomposition is relatively common (Berlemont and Martiny, 2015; López-Mondéjar et al., 2016a; Wilhelm et al., 2019). In addition, analyses of forest soil metatranscriptomes show significant contribution of bacteria to CAZyme production (Hesse et al., 2015; Lladó et al., 2019; Žifčáková et al., 2017). Moreover, Brabcova et al. (2016) showed that decomposing mycelium in forest soil presents hotspots of bacterial abundance, maintaining bacterial over fungal decomposers. In our previous work, we demonstrated that both fungi and bacteria are involved in the assimilation and mineralization of C from complex sources existing in soil. In addition, we showed that fungi may be better suited for the utilization of plant biomass, whereas most bacteria prefer microbial biomass (López-Mondéjar et al., 2018).

The aim of this study was to describe the enzymatic toolbox used for the decomposition of various biomass types by forest soil bacteria and fungi. To accomplish this, we prepared soil microcosms with the addition of ^{13}C-labeled biomass of plant, fungal, and bacterial origin. We used DNA-SIP and metagenomics to analyze the enzymatic tools of microbial decomposers. We hypothesized that although the CAZyme pool will be different for each type of biomass, fungi and bacteria will encode similar CAZyme families involved in the degradation of biomass of the same origin. Additionally, in line with the preference of fungi for plant biomass, we hypothesized that the number of fungal CAZymes involved in the degradation of plant biomass will be higher than the number of those targeting microbial biomass. Importantly, this study also provides a comprehensive answer about the CAZyme families involved in the degradation of various biomass types, including the involvement of minor CAZy families that might have been overlooked so far.

2. Material and Methods

119 *2.1. Sample collection*

120 Soil was collected from the organic horizon of a sessile oak (*Quercus petraea*) forest in the Xaverovský
121 Háj Natural Reserve in the Czech Republic. Previously, this site has been studied with respect to the
122 composition of microbial communities and their seasonal changes and the activity of extracellular
123 enzymes related to the decomposition process (Šnajdr et al., 2008, Baldrian et al., 2010; Baldrian et al.,
124 2013a, Voříšková et al., 2014, López-Mondéjar et al., 2015). This study used the samples collected
125 previously, which were incubated as described in the study of López-Mondéjar et al. (2018). Briefly, soil
126 was sieved and preicubated at 10 °C for 48h and allocated in 100-ml flasks containing 5 g of soil and 0.08
127 g of different ^{13}C-labeled substrates: ^{13}C-glucose (99 atom% ^{13}C), ^{13}C-cellulose (from *Zea mays*, 97 atom%
128 ^{13}C), ^{13}C-hemicellulose (from *Zea mays*, 97 atom% ^{13}C), ^{13}C-plant biomass (from ground maize leaves, 97
129 atom% ^{13}C), ^{13}C-bacterial biomass (from *Streptomyces* sp. PR6, prepared by cultivation in media with ^{13}C-
130 glucose as sole C source) and ^{13}C-fungal biomass (from *Phanerochaete velutina* PV29, prepared by
131 cultivation in media with ^{13}C-glucose as sole C source). Microcosms were slightly moistened with water
132 to reach 60% water content and incubated at 10 °C in the dark for 3 weeks (21 days). After this, three
133 microcosms per treatment were harvested and the material was frozen at -80 °C, freeze-dried and
134 stored at -40 °C.

135 *2.2. DNA extraction and sequencing*

136 DNA was extracted with the FastDNA Spin Kit for Soil (MP Biomedicals) in triplicate and purified with the
137 GeneCean Turbo Kit. Three micrograms of total DNA was used for isopycnic centrifugation in a cesium
138 trifluoroacetate (CsTFA) solution to separate the labeled and unlabeled fractions, as shown in López-
139 Mondéjar et al. (2018). After centrifugation, the labeled fractions representing the ^{13}C-DNA were pooled
140 for each microcosm. Due to the small amount of DNA recovered after centrifugation, the labeled DNA
141 from triplicates from the same substrate was also pooled, and the total DNA was used for metagenome

142 sequencing. DNA libraries were prepared using the KAPA Hyper Prep Kit (Roche) according the
143 manufacturer's instructions. Metagenome libraries were sequenced on an Illumina HiSeq 2000 to
144 generate 250-base paired-end reads. In total, eight metagenomes were sequenced, including the ^{13}C-
145 labeled DNA isolated after 21 days of incubation of soil with ^{13}C-substrate (glucose (GL), cellulose (CE),
146 hemicellulose (HE), plant biomass (PB), bacterial biomass (BB) and fungal biomass (FB) and the DNA
147 from the control microcosms (with no substrate addition) at both 0 (C1) after 21 (C2) days of incubation.

148 *2.3. Metagenome assembly*

149 Reads from all the metagenome libraries were processed together in the same way as originally
150 described in Žifčáková et al. (2016). Briefly, the reads were quality trimmed by removing adapters,
151 filtered by base call quality, and normalized. Errors were trimmed by removing low abundance
152 fragments of high coverage reads. The paired-end assembly of the remaining reads was performed with
153 the Velvet assembler (v 1.2.10) (Zerbino and Birney, 2008) using odd k-mer lengths ranging from 33 to
154 63. Resulting assembled contigs were merged using CD-HIT v4.6 (Li and Godzik, 2006; Fu et al., 2012)
155 and minimus2 Amos v3.1.0 (Sommer et al. 2007). Sequence data of all contig sequences (whole
156 metagenome) were deposited in the MG RAST database under the dataset number mgs446518.

157 Annotation of contigs was performed using both MG RAST (Meyer et al., 2008) and an in-house fungal-
158 predicted protein database (FPPD), as described by Žifčáková et al. (2017). The taxonomic classification
159 for each contig was retrieved from that of the two databases which showed lower bitscore values of the
160 best hit.

161 *2.4. CAZyme annotation*

162 The annotation of CAZymes in the metagenome contigs was carried out after gene calling using the
163 pipeline dbCAN (Yin et al., 2012), followed by manual curation considering the alignments to the
164 sequences in the CAZy database (February 2018) (Table 1). To assess the abundance in the

metagenome, individual sequence reads from each sample were mapped onto contigs identified as CAZyme using bowtie 2.2.1 (Langmead et al., 2009), with the default settings of end to end alignment—sensitive. Data were expressed as: per base coverage = read count × read length/contig length to calculate gene abundance, as previously described (Žifčáková et al., 2017). R software (RCoreTeam, 2019) was used for statistical analysis. Differences in gene abundance in each metagenome and the controls were tested using the exact Fisher test (Gharechahi and Salekdeh, 2018). Differences at $P < 0.05$ were considered statistically significant.

2.5. Recovery and analysis of metagenome assembled genomes (MAGs)

The recovery of metagenome-assembled genomes (MAGs) was performed as follows. First, quality-controlled reads (see earlier) were assembled into scaffolds using IDBA-UD (Peng et al., 2012). Second, the scaffolds were assigned as eukaryotic or prokaryotic using the EUKREP pipeline (West et al., 2018). No significant number of scaffolds was assigned as eukaryotic; hence, we did not attempt to recover the eukaryotic bins. Prokaryotic scaffolds were binned using ABAWACA (https://github.com/CK7/abawaca), Maxbin2 (Wu et al., 2015), MetaBAT2 (Kang et al., 2019), and CONCOCT (Alneberg et al., 2014) and further refined with the DAS Tool refinement method (Sieber et al., 2018). Completeness and contamination values for each bin were determined using CheckM (Parks et al., 2015). Bins were considered MAGs when the quality score was above 50, as defined by Parks and collaborators (Parks et al., 2017). Briefly, this quality score is defined by the percentage of completeness of a bin minus five times the percent contamination. The taxonomy was assigned to MAGs using GTDB version v0.2.2 (Parks et al., 2018) and the Microbial Genomes Atlas (MiGA) webserver (Rodriguez et al., 2018). MAGs were compared with all available genomes of bacteria belonging to the same family using *anvi'o v5.5* (Eren et al., 2015) and the average nucleotide identity (ANI) was calculated. Average amino acid identity (AAI) was computed using the CompareM (v0.0.23) AAI workflow (D. H. Parks, unpublished materials,

188 https://github.com/dparks1134/CompareM). The CAZyme content of these genomes was predicted
189 using dbCAN2 (Zhang et al., 2018) followed by manual curation using the CAZy database.

190 **3. Results**

191 *3.1. Total diversity of the CAZyme pool in the metagenome*

192 In total, 132,197 CAZymes were identified from the 7.2 million predicted proteins of the whole
193 metagenome (1.84% of genes), of which 26.0% and 42.6% were assigned to bacteria and fungi,
194 respectively, and the rest (31.4%) were unassigned. The bacterial CAZymes belonged to 233 families,
195 including 95 GHs, 4 AAs, 14 CEs, 17 Polysaccharide Lyases (PLs), 49 CBMs and 54 GlycosylTransferases
196 (GTs), and the fungal CAZymes were assigned to 209 families, including 84 GHs, 10 AAs, 11 CEs, 9 PLs, 32
197 CBMs and 63 GTs (Fig. S1, S2, S3 and S4). Bacterial GHs accounted for 9.1% of all CAZymes with the
198 highest diversity in the families GH13 (amylase/α-glucosidase/trehalase), GH109 (α-N-
199 acetylgalactosaminidase), GH3 (β-glucosidase), GH23 (lysozyme/ peptidoglycan lytic transglycosylase)
200 and GH15 (glucoamylase/glucodextranase). Fungal GHs accounted for 16.2% of all CAZymes. Similar to
201 bacteria, GH3, GH13 and GH15 were the most diverse families, in addition to GH43 (β-
202 xylosidase/endoxylanase) and GH31 (α-glucosidase/ α-xylosidase). The number of fungal genes encoding
203 AAs and CEs was twice as high (1.7 and 8.5%, respectively) as bacterial genes (0.5 and 4.5%), but similar
204 for PLs (0.3 vs 0.4%), GTs (7.7 vs 8.1%) and CBMs (2.7 vs 2.7%).

205 In general, most of the fungal CAZymes were assigned to Ascomycota (55.9%), Basidiomycota (21.2%)
206 and Mucoromycota (9.3%). Among them, Ascomycota showed a higher number of contigs encoding
207 GHs, AAs, CBMs, CEs, PLs and GTs (Fig. 1). In the case of bacteria, the contigs encoding CAZymes were
208 mainly assigned to Proteobacteria (28.4%), Actinobacteria (25.7%), Acidobacteria (20.3%) and
209 Bacteroidetes (16.4%). These four phyla also showed the highest numbers of CAZymes of all six classes
210 (Fig. 1).

3.2. CAZyme pools in the fungal and bacterial communities

Relative abundances of genes assigned to CAZyme families in the ^{13}C-enriched metagenomic DNA from microcosms containing ^{13}C-supplemented substrates were compared with the abundance in the metagenome of the control samples individually. In total, the percentage of CAZyme families that were significantly increased in at least one of the ^{13}C-supplemented treatments was higher in fungi (69%) than in bacteria (49%) (Fig. 2, Fig. 3). The increase in the relative abundance of certain CAZy families may indicate a relative increase in the abundance of microorganisms utilizing carbon from certain sources. When ^{13}C-cellulose was added to the soil, more CAZyme families were enriched in the fungal community than in the bacterial community (24 vs 18, respectively). On the other hand, the bacterial community showed more CAZyme families increasing upon the addition of ^{13}C-fungal biomass (52 vs 39) and ^{13}C-bacterial biomass (52 vs 37). For ^{13}C-plant biomass and ^{13}C-hemicellulose, the numbers were similar for bacteria and fungi; 52 and 53 in plant biomass, 67 and 69 in hemicellulose, respectively.

Both fungal and bacterial communities were enriched in numerous CAZymes known to be involved in the degradation of plant, fungal and bacterial biomass (Fig. 2, Fig. 3). The CAZyme pool in the plant and microbial dead biomass metagenomes was composed of numerous well-known fungal- and bacterial-encoded CAZyme families including cellulases, glucosidases, xylanases, xylosidases, mannosidases, galactosidases, glucanases, xyloglucanases, glucuronidases, chitinases, hexosaminidases, lysozymes, acetyl xylan esterases and CBMs binding several polysaccharides.

3.3. CAZyme families involved in the degradation of plant, fungal and bacterial biomass

Although many of the CAZyme families were significantly increased in all of the ^{13}C metagenomes (Fig. 2, Fig. 3), several of them were only enriched in a specific type of dead biomass (Fig. 4, Fig. 5). In general, the fungal community contained a richer set of CAZyme families that were specifically increased upon the addition of ^{13}C-labeled plant biomass and its components. The bacterial community also contained

several CAZyme families that increased after the addition of plant biomass, but this community was particularly rich in CAZymes that increased after the addition of ^{13}C-labeled bacterial and fungal biomass.

Regarding the fungal CAZymes involved in the degradation of plant biomass, we found several families encoding enzymes that degrade cellulose and hemicelluloses (Fig. 4). In this sense, CAZyme families containing β-glucosidases (GH1 and GH3), endoglucanases (GH5, GH9, and GH45), cellobiohydrolases (GH7), endoxylanases (GH10 and GH11), other hemicellulases (GH62, GH67 and GH131), LPMOs and oxidases (AA1, AA7 and AA9), and CBM families binding to plant biomass components (CBM1, CBM6, CBM20, CBM21) increased in abundance when plant biomass, cellulose or hemicellulose were added to soil. Interestingly, we found other families that were not related to the degradation of plant biomass and their components, but encoding chitinases, glucanases, chitosanases or glucanosyltransferases (e.g. GH72, GH75, GH79) and binding to chitin (CBM5 and CBM18). In the bacterial community, plant biomass amended soil was enriched in different CAZy families than those of fungi (Fig. 5). Although most of the families are also known to be involved in plant polysaccharide degradation, such as those encoding endoglucanases (GH5 and GH9), β-glucosidases (GH116), endomannanases (GH26), endoxylanases (GH30), xyloglucanases (GH44 and GH115), acetyl xylan esterases (CE2, CE4, CE7, CE8, CE14 and CE15) and CBMs binding cellulose (CBM3), the rest of the CAZymes belonged to families that have not been shown to participate in plant biomass degradation. The families GH4 (α-galactosidase/ 6-phospho-β-glucosidase/ maltose-6-phosphate glucosidase), GH109 (α-N-acetylgalactosaminidase), AA6 (1,4-benzoquinone reductase) and the domains CBM32 (binding to D-galactose and N-acetyl-D-galactosamine) and CBM50 (binding to N-acetylglucosamine) were abundant in the metagenome.

Meanwhile, most of the fungal CAZymes enriched in ^{13}C-bacterial biomass were assigned to families with potential activity in bacterial biomass degradation. The roles of several families of bacterial CAZymes in the decomposition of bacterial biomass are still unclear (Fig. 4, Fig. 5). Regarding the

degradation of ^{13}C-fungal biomass, both bacterial and fungal CAZymes belonged to several families with uncertain roles in the process, such as the family GH32, whose abundance increased in both the fungal and bacterial communities.

3.4. Bacterial taxa and CAZymes degrading the main types of dead biomass in forest soil

Six MAGs were obtained from the metagenomes, but only 5 of them showed acceptable quality for further analyses (Table S1). Four MAGs belonged to Proteobacteria, and the other two belonged to Bacteroidetes. Since databases only classified the MAGs up to the family level, we compared MAGs with the available genomes in the respective family. The results showed that MAG_2 and MAG_6 were most similar to *Asticcacaulis benevestitus* DSM 16100 (76.3% and 75.9% ANI, respectively), *Asticcacaulis biprosthecium* C19 (75.11% and 75.0% ANI, respectively) and *Asticcacaulis excentricus* CB 48 (74.58% and 74.15% ANI, respectively) (Fig. S5). MAG_3 showed the highest similarity to *Cytophaga aurantiaca* DSM 3654 (77.25% ANI) and to *Cytophaga hutchinsonii* ATCC 33406 (75.67% ANI) (Fig. S6). MAG_4 and MAG_5 showed the highest similarity to *Herminiimonas arsenicoxydans* DSM 17148 (73.35% and 73.52% ANI, respectively) and to *Collimonas fungivorans* NCCB 100033 (73.06% and 73.07% ANI, respectively) (Fig. S7). The same similarity results were found when calculating the AAI values for all the MAGs (Supplementary File S1).

MAG_2, MAG_3 and MAG_6 showed the highest abundance on ^{13}C-plant biomass, while MAG_4 and MAG_5 were most abundant in the ^{13}C-bacterial biomass metagenome (Fig. S8). These results corresponded with the composition of their CAZyme gene sets (Table S2). For example, MAG_3 presented numerous genes of families involved in plant biomass degradation, such as endoglucanases (GH9: 5 genes, GH5: 4 genes), hemicelluloses (GH26: 2 genes, GH30: 2 genes, GH44: 1 gene, GH74: 2 genes) and xylan esterases (CE2: 1 gene, CE4: 6 genes and CE14:1 gene). The CAZyme composition of the other two MAGs abundant in plant biomass amended soil was different. Both MAG_2 and MAG_6 also

encoded genes of the GH9 (one gene each) and GH5 (two genes each) families and CEs, although to a lesser extent than MAG_3. However, both genomes contained several genes belonging to the hemicellulolytic families, such as GH39 (1 and 2 genes), GH42 (1 and 2 genes), GH51 (3 genes), GH67 (1 gene) and GH115 (2 genes), which were not detected in MAG_3. MAG_4 and MAG_5 increased upon bacterial biomass addition, and none of the CAZyme families with known involvement in bacterial biomass degradation were found, with the exception of GH108 (lysozyme) in MAG_4. On the other hand, MAG_4 and MAG_5 contained genes from the families GH36 (α-galactosidase), GH73 (peptidoglycan hydrolase with endo-β-N-acetylglucosaminidase specificity) and GH94 (phosphorylases), which were highly enriched in the ^{13}C-bacterial biomass metagenome (approximately 2 and 5 times higher abundance than in ^{13}C-plant biomass (Fig. 3). Interestingly, other families only found in the ^{13}C-bacterial biomass metagenome, such as GH39 (β-xylosidase/ α-L-arabinofuranosidase) and GH105 (unsaturated glucuronyl/galacturonyl hydrolase), were present in the genomes of MAG_2 and MAG_6, despite these bins were not abundant in the BB metagenome.

4. Discussion

Using DNA-SIP and metagenomics, our study revealed that fungi and bacteria that utilize C from plant and microbial biomass possess numerous CAZymes involved in the degradation of both types of biomass. This finding supports the recent view that the roles of fungi and bacteria as primary consumers of complex substrates are important (Kramer et al., 2016; Rousk and Frey, 2015; Žifčáková et al., 2017).

Despite the possibility that fungal CAZyme predictions in metagenomes are underestimated due to introns in sequences, which make gene calling less reliable (Fierer et al., 2012; Pold et al., 2016), we still found that the number of CAZYmes assigned to fungi was larger than that of bacteria. The presence and abundance of numerous well-known families encoding cellulases and hemicellulases in bacteria confirm

304 their role in the degradation of plant-derived biomass. These results add to the list of previous evidence
305 on the active contribution of bacteria in the decomposition of cellulose and hemicellulose (López-
306 Mondéjar et al., 2016a; Stursova et al., 2012; Wilhelm et al., 2019) and confirm that the increase in
307 labeled bacterial biomass after incubation is due to the actual degradation of plant biomass by
308 hydrolytic enzymes and not just a result of mutualistic feeding by cross-feeders in SIP experiments
309 (López-Mondéjar et al., 2018).

310 Contrary to our first hypothesis, the pool of CAZymes for degrading the diverse types of biomass was
311 distinct in fungi and bacteria. In this sense, some CAZyme families seem to be specific for the
312 decomposition of plant biomass and its components in fungi and others in bacteria. While bacteria
313 contain more esterases, fungi possess more oxidases, laccases and monooxygenases. The presence of
314 these complex fungal enzymatic systems for degrading plant biomass, which include not only GHs but
315 also LPMOs, may explain why fungi seem better suited to utilize plant-derived compounds (López-
316 Mondéjar et al., 2018). Bacteria contained more CBMs than fungi, which points at the importance of
317 substrate binding. Unlike fungi that produce mostly extracellular enzymes, cell-bound enzymes play a
318 more important role in the degradation of organic matter in soil bacteria (Lasa et al., 2019). Therefore,
319 the presence of CBMs in bacterial lytic enzymes allows the direct association of the bacterial cell with
320 the target polysaccharide, improving the efficacy of the lytic enzymes and increasing the competitive
321 exclusion of non-cellulolytic opportunists (Donohoe and Resch, 2015). Interestingly, after the addition of
322 plant biomass and its components, fungi also showed high abundance of specific CBM families assigned
323 to chitin- and glucan-binding (typical fungal cell wall components), which, however, were not increased
324 in the fungal biomass metagenome. Moreover, the addition of these plant-derived substrates also
325 increased the abundance of GHs from families potentially encoding chitinases, glucanases, chitosanases
326 and glucanosyltransferases (such as GH18, GH71, GH72 and GH75). The abundance of fungal enzymes
327 that target and bind to fungal cell wall components may indicate a strong competition among fungi for

plant-derived substrates. These competitive fungal-fungal interactions for resources among different taxonomic groups often occur in soils (Boddy and Hiscox, 2016; Woodward and Boddy, 2008), which is especially important between different fungal guilds (Fernandez and Kennedy, 2016). The fact that these families were not increased in the fungal biomass metagenome may be explained by the differences in the attractivity of the living and dead fungal biomass as a target for attack, such as the nutrient content or the presence of secondary compounds (Fernandez et al., 2016) and by the reportedly minor role of fungi in the decomposition of dead fungal biomass (Brabcova et al., 2016; López-Mondéjar et al., 2018).

In accordance with our second hypothesis, we demonstrated that fungal communities encode more specific CAZymes that degrade plant biomass, while bacterial communities are richer in CAZymes that target microbial biomass. As mentioned above, when cellulose was added to soil more fungal CAZymes were increased, including not only endoglucanases as in the bacterial community but also LPMOs and CBMs for cellulose binding. Similar results were presented by Žifčáková et al. (2017), who found that fungi are the major producers of CAZymes involved in lignocellulose degradation in spruce forests, while the transcription of CAZymes involved in the degradation of bacterial and fungal cell walls was increased in bacteria. Our results are in accordance with previous studies supporting the role of fungi as the agents primarily responsible for the transformation of plant-derived carbon in terrestrial ecosystems and reinforce the evidence that bacteria are the main decomposers of mycelia in litter and soils (Bhatnagar et al., 2018; Brabcova et al., 2016; Tláskal et al., 2016; Voříšková et al., 2014). Microbial biomass represents a more readily decomposable substrate than lignocellulose, and bacteria have been shown to dominate the initial phase of dead fungal biomass decomposition (Brabcová et al., 2018). As noted previously, the different nitrogen content between microbial and plant biomass could explain the high abundance of bacteria in the decomposition of microbial biomass (López-Mondéjar et al., 2018). Bacteria contain more N in their biomass than fungi, and specialization to the decomposition of N-rich biomass may reflect their higher nutritional demand (Wallenstein et al., 2006).

Linking genes to environmental processes is vital for understanding the role of microbes in ecosystems (Graham et al., 2016). In the case of CAZyme families, it is possible to link genes with targeted substrates and thus with a specific part of the soil decomposer food web (Nguyen et al., 2018). The links between the CAZyme families, catalytic functions and substrates demonstrated here, were, however, not always entirely clear. While as much as 70% of the CAZyme families that were enriched after addition of plant-derived biomass are known to target plant biomass components, in the case of fungal or bacterial biomass, the percentage of CAZyme families known to target them was smaller. Despite some CAZyme families showing unique substrate specificity, most families have wide substrate specificity; moreover, this substrate specificity is often unknown for several families (Nguyen et al., 2018). Although gene enrichment after substrate addition may indicate the involvement of a gene product in the processing of the substrate, none of the two highly abundant MAGs on bacterial biomass contained any of the known BB-specific genes, while they contained other families such as GH73 and GH108, both encoding lysozymes. Although these two families were enriched in bacterial biomass, they were not specific and appeared on plant and fungal biomass amended soil. Previous studies using SIP showed that the degradation of some bacterial components in soil is mainly carried out by bacteria with low identities to known species, mostly from uncultured bacteria from the *Planctomycetes*, *Armatimonadetes* and the Candidate Phyla Radiation (CPR) groups (Wang et al., 2015). Most of these taxa are still poorly represented in the databases with only a low number of available sequences, which may hinder the biochemical characterization of the genes potentially involved in the degradation of microbial biomass (Lloyd et al., 2018).

After demonstrating that bacteria incorporate significant amounts of C from dead plant biomass (López-Mondéjar et al., 2018), the enrichment of bacterial CAZyme families targeting plant biomass that we showed here confirms that bacteria indeed contribute to plant biomass degradation. Two potentially different enzymatic systems for the degradation of plant biomass were observed across MAGs: the first

was represented by *Cytophaga* (MAG_3) and the second was represented by *Asticcacaulis* (MAG_2 and MAG_6). Although some of the genes encoding hydrolytic enzymes were common, the genome of *Cytophaga* contained more genes potentially encoding endoglucanase, endomannanase, endoxylanase and xyloglucanase activities. *Asticcacaulis* contained fewer endoglucanases, but more genes encoding debranching hemicellulases, such as glucuronidases, α-fucosidases, β-galactosidases, α-L-arabinofuranosidase, β-glucosidases and β-xylosidases. In line with these differences, *Asticcacaulis* was more abundant on hemicellulose. *Cytophaga* is a common cellulolytic soil bacterium that has a unique mechanism for cellulose degradation (Lopez-Mondejar et al., 2019). As previously found by other authors, the recovered genome did not contain any cellobiohydrolases or LPMOs, showing that the system for cellulose degradation used by this bacterium is still poorly understood (Wang et al., 2017; Zhu et al., 2016). *Asticcacaulis* spp. have been isolated from soils, and their utilization of cellulose has been demonstrated using SIP (Eichorst and Kuske, 2012; Stursova et al., 2012). It was recently demonstrated that *Asticcacaulis* assimilated ^{13}C from both cellulose and hemicellulose added to forest soil, indicating its high abundance and its ability to adhere to lignocellulose, suggesting its role in the decomposition of cellulose and hemicellulose (Wilhelm et al., 2019). Our previous results also showed that the genera *Asticcacaulis* and *Cytophaga* are specialists utilizing plant-derived compounds. Both genera were abundant in the bacterial community when ^{13}C-plant biomass or ^{13}C-cellulose was added to soil, while *Asticcacaulis* was also abundant in samples with ^{13}C-hemicellulose (López-Mondéjar et al., 2018). The presence of structurally variable enzymatic systems for decomposing cellulose and hemicellulose among different cellulolytic bacteria has been previously reported in several taxa abundant in forest soils (López-Mondéjar et al., 2016a; López-Mondéjar et al., 2016b). Here, the complementary nature of the two enzymatic systems of these abundant and specialist bacterial taxa, one targeting the backbones and the other targeting the branches of lignocellulose, confirm that in natural environments, plant biomass is degraded by the cooperation of complex microbial communities

400 rather than by a single species, as previously assumed (Cragg et al., 2015, Cavaliere et al., 2017, López-
401 Mondéjar et al., 2019).

402 Our results also indicate the importance of *Herminiimonas* in the degradation of dead microbial
403 biomass, which is used by this genus as a C source (López-Mondéjar et al., 2018). To date, only few
404 species of this genus have been described, mainly those isolated from water but also from urban and
405 contaminated soils (Koh et al., 2017; Sahin et al., 2010). Previous studies showed that *Herminiimonas* is
406 abundant in forest soils (Barta et al., 2017), where it accumulates C from cellulose and plant biomass
407 (López-Mondéjar et al., 2018; Stursova et al., 2012). This ability can be explained by the presence of
408 several genes involved in plant biomass degradation in the MAGs related to *Herminiimonas*, although in
409 smaller numbers than in the MAGs related to *Cytophaga* and *Asticcacaulis*. For example, three potential
410 endoglucanases from families GH9 and GH5 were found both in MAG_4 and MAG_5 but no
411 endoxylanases or other hemicellulose backbone-degrading enzymes. However, these genomes encoded
412 numerous genes from the families GH23, GH24, GH73 and GH108, potentially encoding lysozymes for
413 degrading bacterial cell walls, which may explain their enrichment after bacterial biomass addition.
414 Moreover, both MAGs contained genes encoding a β-1,2-glucanase from the family GH144, which is
415 involved in the degradation of glucans naturally present in some bacteria (Abe et al., 2017). Recently,
416 *Herminiimonas* has been reported to be involved in the degradation of mycelial necromass in forest soil
417 (Sukdeo et al., 2019). However, the analysis of our two genomes revealed only one (in MAG_5) and two
418 (in MAG_4) genes encoding for chitinases and no *N*-acetylglucosaminidases for chitin utilization;
419 moreover, none of the CAZy families enriched on fungal biomass were present. In accordance with our
420 previous results, *Herminiimonas* appears to be a generalist decomposer that is able to use substrates of
421 various origins (López-Mondéjar et al., 2018).

422 In summary, the current study confirms that both fungi and bacteria are involved in the recycling of
423 dead biomass in forest ecosystems and in the assimilation and mineralization of C of both plant and

424 microbial origin. In addition to the proven role of fungi in the decomposition of plant biomass, members
425 of the bacterial community appear to be important players in the degradation of plant-derived
426 compounds by using structurally variable enzymatic systems. Moreover, the complementary nature of
427 these systems, targeting the cellulose and hemicellulose backbones (such as *Cytophaga*) or
428 preferentially focused on the disassembly of hemicellulosic branches (such as *Asticcacaulis*), supports
429 the existence of different ecological niches for specialist cellulolytic bacteria and their potential synergy
430 in the decomposition of complex polysaccharides. Bacteria are also relevant members of the community
431 involved in the degradation of microbial dead biomass in forest soil, using enzymes that are still
432 unknown. Unlike plant biomass, composed mainly by cellulose, hemicellulose and lignin, the
433 composition of microbial biomass appears to be much more diverse and complex. The degradation of
434 this pool of various polysaccharides (glucans, mannans, chitin, or galactans) and glycoconjugates
435 (glycopolymers, glycosyl phosphates or glycoproteins) composing microbial cell walls has not yet been
436 properly addressed and deserves further attention. The importance of microbial biomass, its pool size
437 and turnover rates in forest soils are also still difficult to quantify (Brabcová et al., 2018; Ekblad et al.,
438 2013), and understanding the fate of dead microbial biomass in soils and its importance in C cycling
439 deserves future attention as well. The results of this study indicating specific roles of microbial taxa in C
440 cycling improves our potential to develop models of C cycling in terrestrial ecosystems and to improve
441 the predictions about the fate of C in soils.

442

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449

450 **Figure legends**

451 **Figure 1.** Diversity of genes in the whole metagenome encoding CAZymes assigned to bacterial (A) and
452 fungal (B) phyla. GH: Glycoside Hydrolases, AA: Auxiliary Activities, CBM: Carbohydrate-Binding
453 Modules, CE: Carbohydrate Esterases, PL: Polysaccharide Lyases, GT: GlycosylTransferases.

454 **Figure 2.** Relative abundance of genes assigned to CAZyme families of fungi in the ^{13}C-incorporating
455 microbial community compared to the control. The values indicate fold enrichment in the ^{13}C-DNA of
456 each treatment / total DNA of control without substrate addition. Genes significantly more abundant in
457 treatment than in control are shown in yellow-red, genes significantly less abundant are shown in light
458 and dark blue, white squares indicate no significant difference between control and treatment.
459 Abbreviations indicate ^{13}C substrate addition of FB: fungal biomass; BB: bacterial biomass; PB: plant
460 biomass; CE: cellulose; and HE: hemicellulose. Only CAZy families with relative abundance significantly
461 different from control in at least one of the substrates are shown. Families containing enzymes
462 degrading cellulose or hemicellulose have a green mark, fungal biomass blue and bacterial biomass
463 purple (source: www.cazy.org).

464 **Figure 3.** Relative abundance of genes assigned to CAZyme families of bacteria in the ^{13}C-incorporating
465 microbial community compared to the control. The values indicate fold enrichment in the ^{13}C-DNA of
466 each treatment / total DNA of control without substrate addition. Genes significantly more abundant in
467 treatment than in control are shown in yellow-red, genes significantly less abundant are shown in light
468 and dark blue, white squares indicate no significant difference between control and treatment.
469 Abbreviations indicate ^{13}C substrate addition of FB: fungal biomass; BB: bacterial biomass; PB: plant

biomass; CE: cellulose; and HE: hemicellulose. Only CAZY families with relative abundance significantly different from control in at least one of the substrates are shown. Families containing enzymes degrading cellulose or hemicellulose have a green mark, fungal biomass blue and bacterial biomass purple (source: www.cazy.org).

Figure 4. Fungal CAZyme families enriched after addition of each one of the different ^{13}C substrates: in cellulose, in plant biomass or hemicellulose, in bacterial biomass, in fungal biomass and in both types of microbial biomass and their known catalytic properties. Abundance represents the total number of CAZymes of that family found in the whole metagenome. In bold, families showing activities directly involved in the degradation of that substrate.

Figure 5. Bacterial CAZyme families enriched after addition of each one of the different ^{13}C substrates: in cellulose, in plant biomass or hemicellulose, in bacterial biomass, in fungal biomass and in both types of microbial biomass and their known catalytic properties. Abundance represents the total number of CAZymes of that family found in the whole metagenome. In bold, families showing activities directly involved in the degradation of that substrate.

Table legend

Table 1. List of the main CAZyme families encoding the enzymatic activities involved in the degradation of several compounds presented in plant and microbial biomass according to CAZy (http://www.CAZy.org).

Supplementary figures

Fig. S1. Diversity of GH families in the whole metagenome. Data show the numbers of CAZyme genes in each family. Bacterial CAZymes are in red, fungal CAZYmes are in blue. Families containing genes involved in the degradation of components of the plant biomass are marked in green, for fungal biomass in blue and for bacterial biomass in purple (source: www.cazy.org).

493 Fig. S2. Diversity of AAs (A), CEs (B) and PLs (C) families of in the whole metagenome. Data show the
494 numbers of CAZyme genes in each family. Bacterial CAZymes are in red, fungal CAZYmes are in blue.
495 Families containing genes involved in the degradation of components of the plant biomass are marked
496 in green, for fungal biomass in blue and for bacterial biomass in purple (source: www.cazy.org).

497 Fig. S3. Diversity of CBM families in the whole metagenome. Data show the numbers of CAZyme genes
498 in each family. Bacterial CAZymes are in red, fungal CAZYmes are in blue. Families containing genes
499 involved in the degradation of components of the plant biomass are marked in green, for fungal
500 biomass in blue and for bacterial biomass in purple (source: www.cazy.org).

501 Fig. S4. Diversity of GT families in the whole metagenome. Data show the numbers of CAZyme genes in
502 each family. Bacterial CAZymes are in red, fungal CAZYmes are in blue.

503 Fig. S5. Comparison of MAG_2 and MAG_6 with the genomes of the members of the family
504 Caulobacteraceae.

505 Fig. S6. Comparison of MAG_3 with the genomes of the members of the family Cytophagaceae.

506 Fig. S7. Comparison of MAG_4 and MAG_5 with the genomes of the members of the family
507 Oxalobacteraceae.

508 Fig. S8. Relative abundance of MAG sequences in the metagenomic DNA. Color codes indicate the
509 relative abundance of MAGs across samples. Abbreviations: C1 (control at t=0), C2 (control at t=3), FB
510 (fungal biomass), BB (bacterial biomass), PB (plant biomass), CE (cellulose), HE (hemicellulose), GL
511 (glucose).

512 Supplementary Table S1. Taxonomy and properties of the six metagenome assembled genomes
513 recovered from the metagenomes generated in this study. Taxonomy and closest hit were generated by
514 using GTDB, MiGA and average nucleotide identity (ANI) comparison with available genomes in NCBI.
515 Selected genomic characteristics were generated by CheckM.

Supplementary Table S2. List of CAZymes (GHs, AAs, CEs, CBMs and PLs) encoded by MAGs. In color the families which increased their abundance after addition of: plant biomass or their components (in green), bacterial biomass (in purple), fungal biomass (in blue), or any type of microbial biomass (in pink).

Supplementary File S1. Amino acid identity (AAI) similarity between the MAGs and other genomes from the same bacterial family. Bacterial genomes were obtained from NCBI. AAI was computed using the CompareM AAI workflow.

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Table 1. List of the main CAZyme families encoding the enzymatic activities involved in the degradation of several compounds presented in plant and microbial biomass according to CAZy (http://www.CAZy.org).

Origin	**Compound**	**CAZyme families**
Plant biomass	cellulose	GH1(β-glucosidase), GH3 (β-glucosidase), GH5 (β-glucosidase/endoglucanase), GH6 (cellobiohydrolase), GH7 (reducing end-acting cellobiohydrolase), GH8 (endoglucanase/endoxylanase), GH9 (endoglucanase), GH12 (endoglucanase), GH45 (endoglucanase), GH48 (reducing end-acting cellobiohydrolase/endoglucanase), GH116 (β-glucosidase), AA9 (lytic polysaccharide monooxygenase), and AA10 (lytic polysaccharide monooxygenase)
	hemicellulose	GH2 (β-galactosidase/β-glucuronidase), GH10 (endoxylanase), GH11 (endoxylanase), GH16 (xyloglucanase/endoglucanase), GH26 (endomannanase), GH30 (endoxylanase/ β-1,6-glucanase/β-xylosidase), GH36 (α-galactosidase), GH39 (β-xylosidase/α-L-arabinofuranosidase), GH43 (β-xylosidase/endoxylanase), GH44 (xyloglucanase/endoglucanase), GH51 (α-L-arabinofuranosidase), GH52 (β-xylosidase), GH54 (α-L-arabinofuranosidase), GH62 (α-L-arabinofuranosidase) GH67 (xylan α-1,2-glucuronidase), GH74 (xyloglucanase), GH95 (α-L-fucosidase/α-L-galactosidase), GH115 (xylan α-1,2-glucuronidase), GH120 (β-xylosidase),GH131 (exo-β-1,3/1,6-glucanase/endo-β-1,4-glucanase) and CE1, CE2, CE3, CE4, CE5, CE6, CE7, CE12, CE15, CE16 (acetyl xylan esterases)
	lignin	AA1 (laccase), AA2 (peroxidase), AA3 (oxidase), AA4 (oxidase), AA5(oxidase), AA6 (1,4-benzoquinone reductase),
Fungal biomass	chitin	GH18 (chitinase), GH19 (chitinase), GH20 (N-acetyl β-glucosaminidase) and AA11 (lytic polysaccharide monooxygenase)
	glucans	GH17 (endo-1,3-β-glucanase), GH55 (exo-β-1,3-glucanase/endo-1,3-β-glucanase), GH64 (endo-1,3-β-glucanase), GH81 (endo-1,3-β-glucanase), and GH128 (endo-1,3-β-glucanase)
Bacterial biomass	peptidoglycan	GH22 (lysozyme), GH23 (lysozyme/peptidoglycan lytic transglycosylase), GH24 (lysozyme), GH25 (lysozyme), GH73 (peptidoglycan hydrolase with endo-β-N-acetylglucosaminidase specificity), GH102 (peptidoglycan lytic transglycosylase), GH103 (peptidoglycan lytic transglycosylase), GH104 (peptidoglycan lytic transglycosylase) and GH108 (lysozyme)

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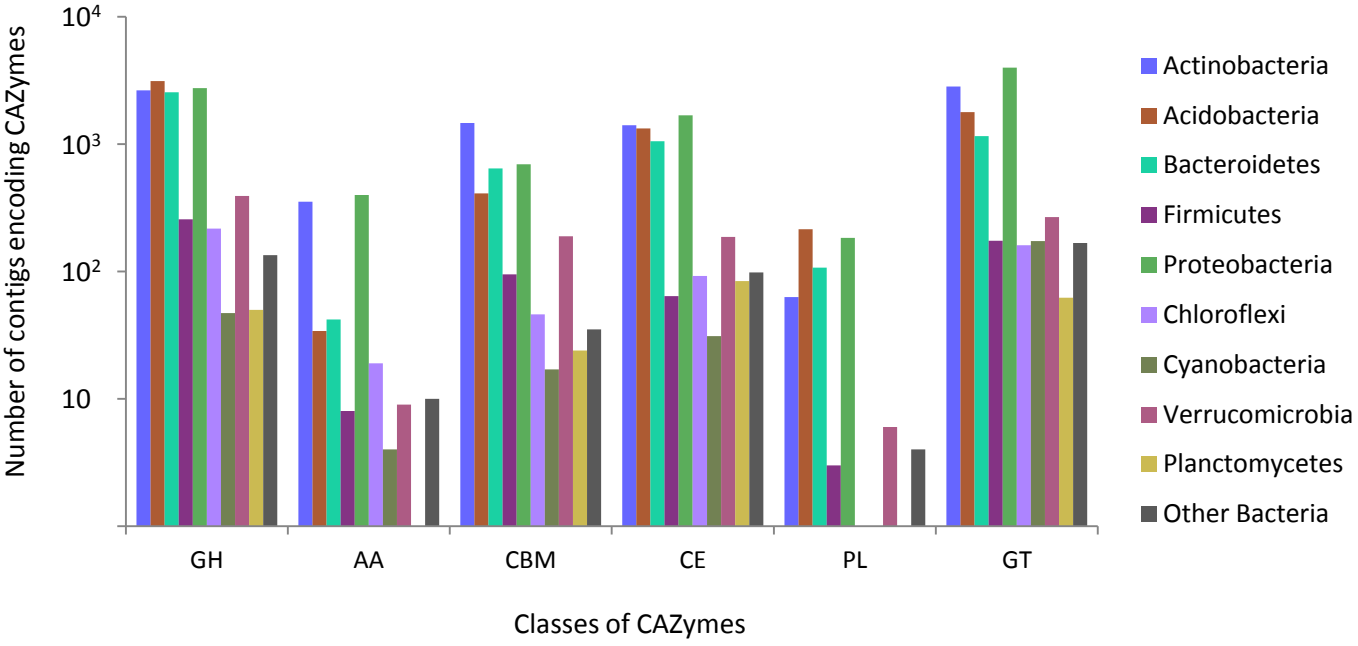
Number of contigs encoding CAZymes
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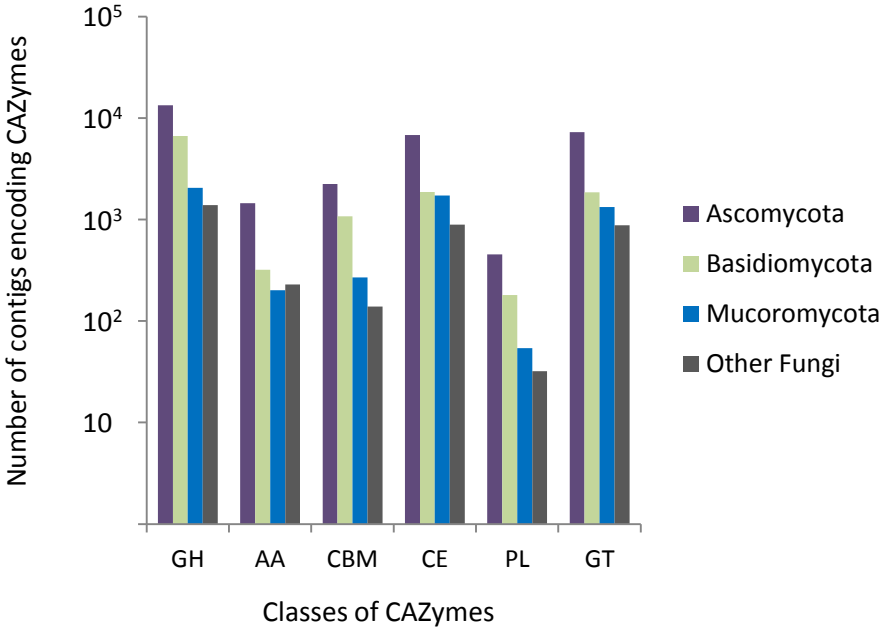
Bacteroidetes
Firmicutes
Proteobacteria
Chloroflexi
Cyanobacteria
Verrucomicrobia
Planctomycetes
Other Bacteria

B


Number of contigs encoding CAZymes
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10⁴
10³
10²
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GH
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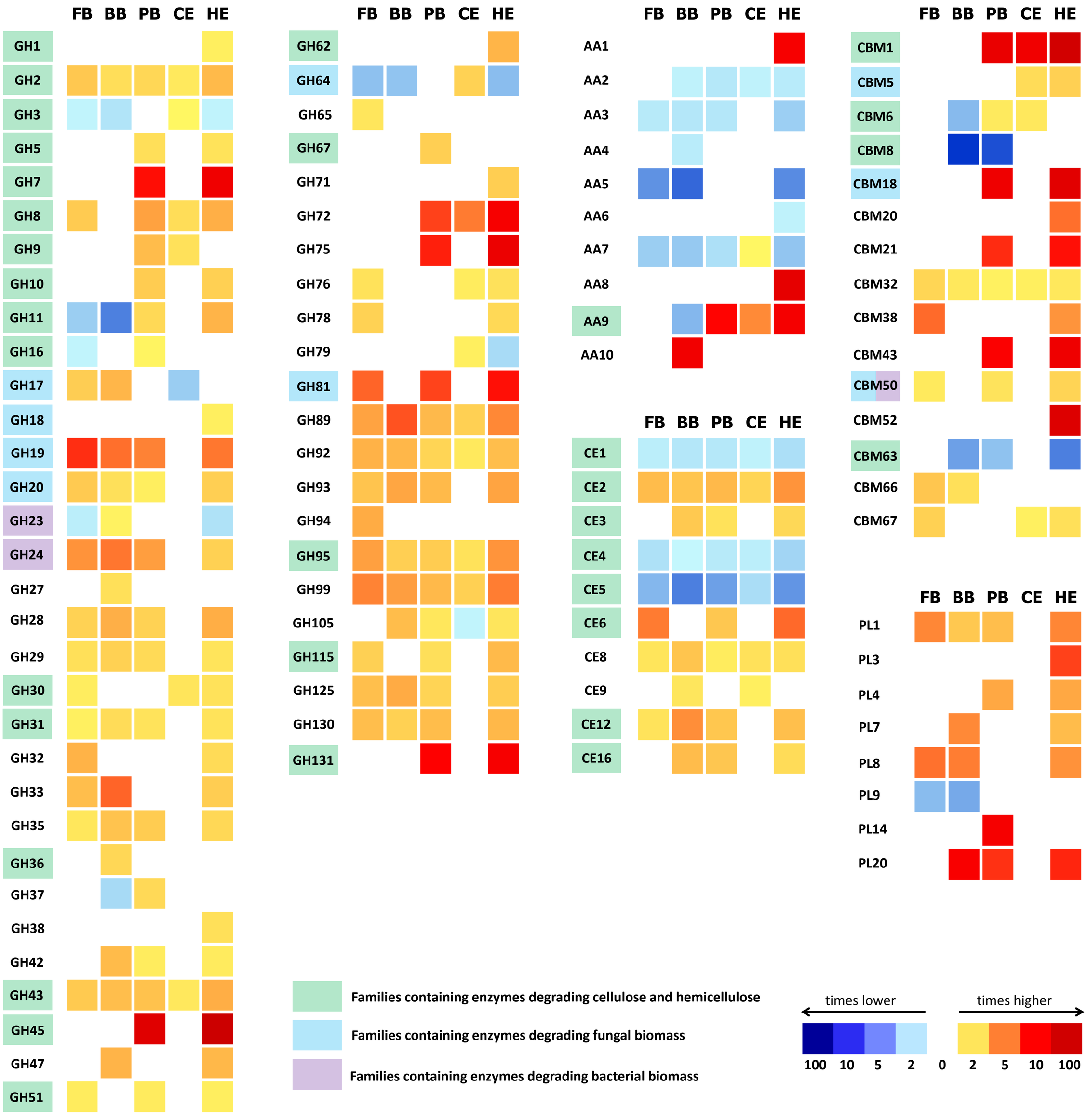
Classes of CAZymes
Ascomycota
Basidiomycota
Mucoromycota
Other Fungi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Families containing enzymes degrading cellulose and hemicellulose
Families containing enzymes degrading fungal biomass
Families containing enzymes degrading bacterial biomass
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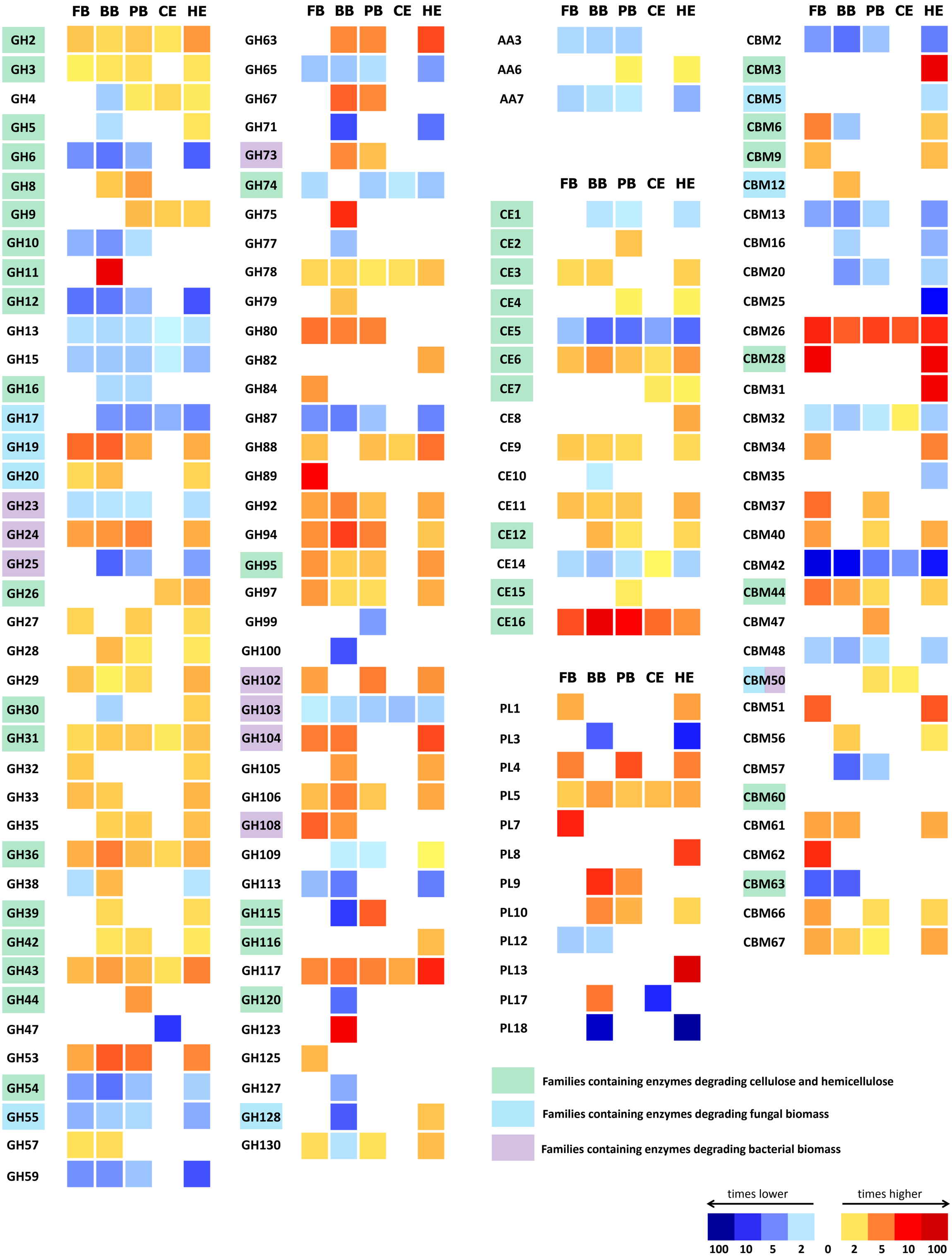
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CBM67
Families containing enzymes degrading cellulose and hemicellulose
Families containing enzymes degrading fungal biomass
Families containing enzymes degrading bacterial biomass
times lower
times higher
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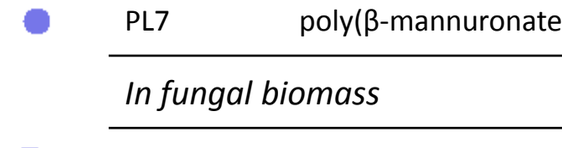
Abundance	Family	Activity
	In cellulose	
	GH3	**β-glucosidase (EC 3.2.1.21)**
	GH9	**endoglucanase (EC 3.2.1.4)**
	GH64	**β-1,3-glucanase (EC 3.2.1.39)**
	GH72	β-1,3-glucanosyltransglycosylase (EC 2.4.1.-)
	GH79	β-glucuronidase (EC 3.2.1.31)
	AA7	**glucooligosaccharide oxidase (EC 1.1.3.-); chitooligosaccharide oxidase (EC 1.1.3.-)**
	AA9	**LPMOs**
	CBM1	**Cellulose-binding function**
	CBM5	Chitin-binding described
	CBM6	**Cellulose-binding function**
	CBM18	Chitin-binding function
	In plant biomass or hemicellulose	
	GH1	**β-glucosidase (EC 3.2.1.21), β-xylosidase (EC 3.2.1.37), β-mannosidase (EC 3.2.1.25)**
	GH5	**endoglucanase (EC 3.2.1.4), endoxylanase (EC 3.2.1.8), licheninase (EC 3.2.1.73)**
	GH7	**endoglucanase (EC 3.2.1.4), endo-β-1,3-1,4-glucanase (EC 3.2.1.73)**
	GH10	**endoxylanase (EC 3.2.1.8)**
	GH11	**endoxylanase (EC 3.2.1.8)**
	GH16	**endo-1,3-β-glucanase (EC 3.2.1.39); endo-1,3(4)-β-glucanase (EC 3.2.1.6), xyloglucanase (EC 3.2.1.151)**
	GH18	chitinase (EC 3.2.1.14); lysozyme (EC 3.2.1.17); endo-β-N-acetylglucosaminidase (EC 3.2.1.96)
	GH38	α-mannosidase (EC 3.2.1.24)
	GH45	**endoglucanase (EC 3.2.1.4)**
	GH62	**α-L-arabinofuranosidase (EC 3.2.1.55)**
	GH67	**α-glucuronidase (EC 3.2.1.139); xylan α-1,2-glucuronidase (EC 3.2.1.131)**
	GH71	α-1,3-glucanase (EC 3.2.1.59)
	GH75	chitosanase (EC 3.2.1.132)
	GH131	**broad specificity exo-β-1,3/1,6-glucanase with endo-β-1,4-glucanase activity (EC 3.2.1.-)**
	AA1	**Laccase-like multicopper oxidase (EC 1.10.3.-)**
	AA8	Iron reductase domain
	PL3	**pectate lyase (EC 4.2.2.2)**
	PL4	**rhamnogalacturonan endolyase (EC 4.2.2.23)**
	PL14	alginate lyase (EC 4.2.2.3); exo-oligoalginate lyase (EC 4.2.2.26)
	CBM20	**Starch-binding function**
	CBM21	**Starch-binding function**
	CBM43	**β-1,3-glucan binding function**
	CBM52	**β-1,3-glucan binding function**
	In bacterial biomass	
	GH23	**lysozyme type G (EC 3.2.1.17)**
	GH27	**α-galactosidase (EC 3.2.1.22); α-N-acetylgalactosaminidase (EC 3.2.1.49), β-L-arabinopyranosidase (EC 3.2.1.88)**
	GH36	**α-galactosidase (EC 3.2.1.22), α-N-acetylgalactosaminidase (EC 3.2.1.49)**
	GH47	**α-mannosidase (EC 3.2.1.113)**
	AA10	**LPMOs**
	PL7	poly(β-mannuronate) lyase / M-specific alginate lyase (EC 4.2.2.3)
	In fungal biomass	
	GH32	Invertase (EC 3.2.1.26); endo-inulinase (EC 3.2.1.7), exo-inulinase (EC 3.2.1.80), fructosyltransferase (EC 2.4.1.-)
	GH65	α,α-trehalase (EC 3.2.1.28)
	GH78	α-L-rhamnosidase (EC 3.2.1.40); rhamnogalacturonan α-L-rhamnohydrolase (EC 3.2.1.174)
	GH94	**cellobionic acid phosphorylase (EC 2.4.1.321)**
	CBM38	**Inulin-binding function**
	In both microbial biomasses	
	GH17	**glucan 1,3-β-glucosidase (EC 3.2.1.58), β-1,3-glucanosyltransglycosylase (EC 2.4.1.-)**
	GH33	**sialidase or neuraminidase (EC 3.2.1.18)**
	PL8	hyaluronate lyase (EC 4.2.2.1), xanthan lyase (EC 4.2.2.12); chondroitin ABC lyase (EC 4.2.2.20)
	CBM66	Binding to fructoside residue of fructans



Abundance	Family	Activity
	In cellulose	
	GH4	6-P glucosidase (EC 3.2.1.122); α-glucosidase (EC 3.2.1.20); α-galactosidase (EC 3.2.1.22); 6-P-β-glucosidase (EC 3.2.1.86)
	GH9	**endoglucanase (EC 3.2.1.4)**
	GH26	**β-mannanase (EC 3.2.1.78); exo-β-mannanase (EC 3.2.1.100); β-1,3-xylanase (EC 3.2.1.32); endo-β-1,3-1,4-glucanase (EC 3.2.1.73)**
	CE7	**acetyl xylan esterase (EC 3.1.1.72)**
	CE14	**Deacetylase (EC 3.5.1.-)**
	CBM32	Binding to galactose, lactose, polygalacturonic acid and LacNAc
	CBM50	Found in enzymes cleaving either chitin or peptidoglycan
	In plant biomass or hemicellulose	
	GH5	**endoglucanase (EC 3.2.1.4), endoxylanase (EC 3.2.1.8), licheninase (EC 3.2.1.73)**
	GH30	**endo-β-1,4-xylanase (EC 3.2.1.8); β-glucosidase (3.2.1.21); β-glucuronidase (EC 3.2.1.31); β-xylosidase (EC 3.2.1.37)**
	GH44	**endoglucanase (EC 3.2.1.4); xyloglucanase (EC 3.2.1.151)**
	GH82	I-carrageenase (EC 3.2.1.157)
	GH109	α-N-acetylgalactosaminidase (EC 3.2.1.49)
	GH115	**xylan α-1,2-glucuronidase (3.2.1.131); α-(4-O-methyl)-glucuronidase (3.2.1.-)**
	GH116	**β-glucosidase (EC 3.2.1.21); β-xylosidase (EC 3.2.1.37)**
	GH128	β-1,3-glucanase (EC 3.2.1.39)
	AA6	1,4-benzoquinone reductase (EC. 1.6.5.6)
	CE2	**acetyl xylan esterase (EC 3.1.1.72)**
	CE4	**acetyl xylan esterase (EC 3.1.1.72); chitin deacetylase (EC 3.5.1.41); peptidoglycan GlcNAc deacetylase (EC 3.5.1.-)**
	CE8	**pectin methylesterase (EC 3.1.1.11)**
	CE15	**4-O-methyl-glucuronoyl methylesterase (EC 3.1.1.-)**
	CBM3	**cellulose-binding function**
	CBM31	**Binding to β-1,3-xylan**
	CBM47	Fucose-binding activity
	In bacterial biomass	
	GH11	endo-β-1,4-xylanase (EC 3.2.1.8); endo-β-1,3-xylanase (EC 3.2.1.32)
	GH38	**α-mannosidase (EC 3.2.1.24); mannosyl-oligosaccharide α-1,2-mannosidase (EC 3.2.1.113)**
	GH39	β-xylosidase (EC 3.2.1.37)
	GH75	chitosanase (EC 3.2.1.132)
	GH79	**β-glucuronidase (EC 3.2.1.31), hyaluronoglucuronidase (EC 3.2.1.36); heparanase (EC 3.2.1.166)**
	GH105	unsaturated rhamnogalacturonyl hydrolase (EC 3.2.1.172)
	GH123	β-N-acetylgalactosaminidase (EC 3.2.1.53); glycosphingolipid β-N-acetylgalactosaminidase (EC 3.2.1.-)
	PL17	alginate lyase (EC 4.2.2.3); oligoalginate lyase (EC 4.2.2.26)
	CBM12	Chitin-binding function
	CBM56	β-1,3-glucan binding function
	In fungal biomass	
	GH32	invertase (EC 3.2.1.26); endo-levanase (EC 3.2.1.65); exo-inulinase (EC 3.2.1.80); endo-inulinase (EC 3.2.1.7)
	GH84	**N-acetyl β-glucosaminidase (EC 3.2.1.52); hyaluronidase (EC 3.2.1.35);**
	GH89	α-N-acetylglucosaminidase (EC 3.2.1.50)
	GH125	**exo-α-1,6-mannosidase (EC 3.2.1.-)**
	PL1	pectate lyase (EC 4.2.2.2); exo-pectate lyase (EC 4.2.2.9); pectin lyase (EC 4.2.2.10)
	PL7	poly(β-mannuronate) lyase / M-specific alginate lyase (EC 4.2.2.3); α-L-guluronate lyase / G-specific alginate lyase (EC 4.2.2.11)
	CBM6	**Cellulose-binding function and also bind β-1,3-glucan, β-1,3-1,4-glucan, and β-1,4-glucan**
	CBM9	cellulose-binding function
	CBM28	**Binding to non-crystalline cellulose, cellooligosaccharides, and β-(1,3)(1,4)-glucans**
	CBM34	Granular starch-binding function
	CBM51	**Binding to galactose**
	CBM62	**Binding to galactose moieties found on xyloglucan, arabinogalactan and galactomannan**
	In both microbial biomasses	
	GH20	**β-hexosaminidase (EC 3.2.1.52); lacto-N-biosidase (EC 3.2.1.140)**
	GH33	**sialidase or neuraminidase (EC 3.2.1.18); anhydrosialidase (EC 4.2.2.15)**
	GH57	α-amylase (EC 3.2.1.1); α-galactosidase (EC 3.2.1.22); amylopullulanase (EC 3.2.1.41); 4-α-glucanotransferase (EC 2.4.1.25)
	GH108	**N-acetylmuramidase (EC 3.2.1.17)**
	CE3	acetyl xylan esterase (EC 3.1.1.72)
	CBM61	**β-1,4-galactan binding function**

2500 1000 500 250 100 50 25 10

Research highlights:

- Both fungi and bacteria actively degrade complex plant and microbial biomass
- The pool of CAZymes was distinct in fungi and bacteria
- Fungal communities encode more specific CAZymes that degrade plant biomass
- Bacterial communities are richer in CAZymes that target microbial biomass
- Bacteria use structurally variable but complementary enzymatic systems

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: