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1 **Title**

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3 communities in sapwood and heartwood

4

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16

17 **Running title:** Sapwood and heartwood select different bacterial communities

18

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22

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25 **Abstract**

26 Wood decay is an important process in forest ecosystems, which relies on wood  
27 chemical properties and the action of a complex community of decomposers. While  
28 the important role of fungi in this process is recognized, our knowledge concerning  
29 the colonization of decaying wood by bacteria, their relative distribution as well as  
30 their potential functional roles remain under-investigated. In this context, our aim was  
31 to characterize how the bacterial communities are structured at both taxonomic and  
32 functional levels along the soil-wood continuum, using oak discs positioned on the  
33 forest floor for nine months. Using a combination of culture-dependent and -  
34 independent methods associated to a physical measure of wood decay, we  
35 evidenced that at the middle stage of decay investigated, the heartwood- and  
36 sapwood-inhabiting bacterial communities significantly differed from one another in  
37 term of richness and taxonomic composition, but also from those of the bulk soil. The  
38 functional screening revealed low metabolic potentials and a higher frequency of  
39 cellulose decomposing bacteria in wood than in the bulk soil, suggesting an  
40 adaptation of these communities to this habitat and to the physical-chemical  
41 conditions occurring in decaying wood. Together, our data evidence that the  
42 colonization of decaying wood by bacteria is based on a deterministic process linking  
43 extrinsic and intrinsic factors.

## 44 1. Introduction

45 Forest ecosystems, due to the large area they cover on earth (up to 31% of land  
46 area in 2015), provide major ecosystem services. One of the most important is their  
47 capacity to stock carbon (C) and to act as a C sink (Pan et al., 2011; Seidl et al.,  
48 2014). In addition, trees accumulate inorganic nutrients taken from the soil in their  
49 biomass. Even if a significant C fraction is directly transferred to the surrounding  
50 environment through belowground allocation and root exudation (Litton et al., 2004;  
51 Hobbie, 2006), a major transfer comes from the seasonal litter deposition and after  
52 the death of trees or harvesting, during the wood decay. Consequently, decaying  
53 wood strongly impacts nutrient cycling and plays an important role in term of organic  
54 and inorganic nutrients recycling (Augusto et al., 2015; Ricker et al., 2016). In  
55 temperate regions, woody debris can be represented by logs, stumps or fine  
56 branches and can reach 2-65 m<sup>3</sup> ha<sup>-1</sup> in managed forests where wood is harvested,  
57 while it can reach up to 1200 m<sup>3</sup> ha<sup>-1</sup> in non-managed forests (Hahn and  
58 Christensen, 2005; Stokland et al., 2012). This material is a specific habitat for  
59 various organisms, such as insects and fungi, providing them an important source of  
60 cellulose, lignin and hemicellulose (Stokland et al., 2012; Seibold et al., 2015;  
61 Baldrian, 2017). However, the nutrients entrapped in the wood structure are not  
62 directly accessible to the wood biota, due to the recalcitrance of wood to  
63 decomposition. Indeed, the high content of lignin and the low nutrient content (mainly  
64 nitrogen (N) and phosphorus (P)) are not favorable to microbial development  
65 (Meerts, 2002). In addition, presence of toxic wood extractives and low pH conditions  
66 prevent wood microbial decomposition. All these parameters vary according to the  
67 wood species and the stage of decay, with some wood easily degradable (e.g. *Acer*  
68 sp. or *Tilia* sp.) and other more recalcitrant (i.e., *Quercus* sp. or *Acacia* sp.) (Weedon

69 et al., 2009; Rajala et al., 2012; Augusto et al., 2015).

70 A large body of literature suggests that fungi but also insects are the main  
71 decomposers of decaying wood. Insects, due to their capability of altering wood  
72 structure by tunneling and fragmentation and of decomposing wood through  
73 enzymatic digestion, are important engineers of early stage decay (Baldrian et al.,  
74 2016; Ulyshen, 2016). Fungi are well known for their ability to produce a large panel  
75 of extracellular lignocellulolytic enzymes to decompose the lignin and then the  
76 cellulose (Schneider et al., 2012; Mathieu et al., 2013; Baldrian et al., 2016; Noll et  
77 al., 2016). In comparison, less attention has been paid to wood-inhabiting bacteria,  
78 while they are known to colonize wood since the early steps of decomposition  
79 (Clausen, 1996; Blanchette, 2000; Kielak et al., 2016). Based on the analysis of the  
80 bacterial communities occurring at different stages of decomposition of pine-wood,  
81 Kielak et al. (2016) proposed that bacterial community assembly may be the result of  
82 a stochastic process at the initial stages of dead wood decomposition, and on the  
83 contrary determined by the wood properties at advanced stages. It was also reported  
84 that decaying bacteria communities could be diverse, potentially active at  
85 decomposing dead wood and capable of interacting with fungi (Valášková et al.,  
86 2009; Hervé et al., 2016; Johnston et al., 2016; Baldrian, 2017). Among the different  
87 studies performed on these communities, a dominance of *Proteobacteria*,  
88 *Actinobacteria*, *Bacteroidetes* and *Acidobacteria* was reported, suggesting an  
89 adaptation to the wood environment (Valášková et al., 2009; Hervé et al., 2014; Sun  
90 et al., 2014; Kielak et al., 2016; Rinta-Kanto et al., 2016). Noticeably, some bacterial  
91 classes or even genera were shown to differ according to the tree species (Prewitt et  
92 al., 2014; Moll et al., 2018) and/or the stage of decay (Hoppe et al., 2015). The  
93 potential structuring effect of the wood compartments (heartwood (HW), the dead

94 inner part of wood; sapwood (SW), the living outermost part of wood) on the bacterial  
95 communities was also investigated, but such studies remain scarce (Zhang et al.,  
96 2008; Moll et al., 2018). However, most of these studies described the community  
97 composition, while the relative role of the bacterial communities in the decomposition  
98 of wood is still largely underexplored.

99 Most of the current knowledge on the ability of bacteria to decompose wood  
100 comes from the analysis of soil bacteria and on their capacities to hydrolyse cellulose  
101 (Nicolitch et al., 2017), hemicelluloses (Lee et al., 2010), lignin (Větrovský et al.,  
102 2014) or even pectin (Clausen, 1996). For instance, these functional abilities were  
103 found in culturable representatives of *Acidobacteria* (*Silvibacterium bohemicum*)  
104 (Lladó et al., 2016), of *Actinobacteria* (e.g. *Streptomyces*) (Nicolitch et al., 2016) or of  
105 *Proteobacteria* (e.g., *Burkholderia*) (Hervé et al., 2016). Together, these results  
106 suggest that the soil may act as a reservoir of bacteria capable of adapting to woody  
107 debris and of participating to wood decomposition. Comparatively, few functional  
108 analyses have been done on bacteria directly collected *in situ* from decaying wood  
109 (Murray and Woodward, 2007; Valášková et al., 2009; Vorob'ev et al., 2009).  
110 Consequently, determining whether bacteria are functionally structured according to  
111 the stage of wood decay, the wood compartment or according to the tree species are  
112 questions of importance in microbial ecology. As bacteria are not considered as the  
113 main actors of wood decay in the early stages of decomposition, we investigated the  
114 taxonomic and functional distribution of the bacterial communities at middle stage of  
115 wood decay and across a soil-wood continuum. At this stage of decay, we  
116 hypothesized that i) the direct role and the structure of the bacterial communities vary  
117 according to the compartments (*i.e.*, bulk soil (BS) *versus* HW *versus* SW), and ii) the  
118 two main wood compartments (HW and SW) select different bacterial communities

119 according to their own properties (i.e., nutritive content, wood biopolymers). To test  
120 these hypotheses, we combined metabolic and functional bioassays, with a culture-  
121 independent approach on oak wood samples incubated nine months on a forest floor.

122

## 123 **2. Materials and Methods**

### 124 **2.1. Experimental site and sampling description**

125 The present study was performed in the forest experimental site of Champenoux  
126 (north-eastern of France; Lat: 48.718°; Long: 6.347°; Alt: 248 m; 0.5 ha of surface).  
127 The site is characterized as an oak forest aged of 110 years old where *Quercus*  
128 *petraea* (*Q. petraea*) and *Carpinus betulus* represent the dominant tree species,  
129 followed by some *Fagus sylvatica* and *Quercus robur* trees. The soil is classified as  
130 an acidic luvic cambisol (pH-H<sub>2</sub>O = 4.6 in the A1 horizon, 0-5 cm) and is  
131 characterized by a mull-type humus and a loamy texture. The site is flat with  
132 understory vegetation (i.e. oak and hornbeam seedlings, *Convallaria majalis* L.,  
133 *Deschampsia cespitosa* L.).

134 At this site, four living oak trees of similar age (ca. 110 years old; 42 cm  
135 diameter), spatially distant from 5 to 20 m, were selected. Oak was selected, as it  
136 represents a major tree species in Europe and its coverage is expected to increase  
137 with changes in temperatures and precipitations due to climate change (Hanewinkel  
138 et al., 2013). An area of 25 m<sup>2</sup> (~5 m x ~5 m) was delimited around each oak tree,  
139 and the litter was removed. Then, twenty oak discs were placed around each oak  
140 tree directly on the surface of the soil and spatially separated from one another by a  
141 distance of approximately 50 cm, giving a total of 80 wood discs. The wood discs  
142 came from a sessile oak tree (*Q. petraea*) aged of approximately 48 years and cut off  
143 in January 2015 from a neighboring forest plot and then stored and air-dried in the

144 laboratory protected from light and moisture for six months. Their average diameter  
145 without the bark was 14.5 cm and their average thickness 2 cm. They were placed  
146 directly in contact with the soil and incubated nine months in natural conditions to  
147 enable wood decomposition (Fig. SI-1).

148 After a nine months incubation period, sampling was performed (April 2016) to  
149 focus on the middle stage of wood decay and on the associated bacterial  
150 communities. For each tree area and each oak disc, SW and HW as well as the soil  
151 (BS) in contact with the wood discs were sampled. SW and HW samples were  
152 recovered by drilling using a sterilized 10-mm drill bit. For each tree area, the  
153 sawdust of the twenty oak discs was recovered from the two wood compartments  
154 (HW and SW). The samples were then pooled to obtain four composite HW sawdust  
155 samples ( $n=20$  per composite samples) and four composite SW sawdust samples  
156 ( $n=20$  per composite samples). To compare the wood-inhabiting bacterial community  
157 structures and functions, with those of the adjacent soil, 5 g of BS samples  
158 underlying each wood-decaying disc (5 cm depth corresponding to the A1 horizon)  
159 were sampled. The BS samples were then pooled for each tree area, leading to four  
160 composite BS samples ( $n=20$  per composite samples). Each sample was stored in  
161 sterile containers and once at the laboratory directly analyzed.

## 162 **2.2. Soil chemical analyses**

163 For each replicate tree area, the chemical properties of the four BS samples have  
164 been determined. Total N and C contents, both obtained after combustion at 1000°C,  
165 and P content, were determined according to methods published by Olsen (1954),  
166 Duchaufour and Bonneau, (1959) and Duval, (1963). The cation exchange capacity  
167 (CEC) was determined according to the cobalti-hexamine method, which is based on  
168 the titration of the cobalti-hexamine chloride soil extract. Exchangeable cations (Ca,



169 Mg, Na, K, Fe, Mn and Al) and protons were extracted using cobaltihexamine and  
170 determined by inductively coupled plasma spectrometry-atomic emission  
171 spectrometry (ICP-AES) for cations and by potentiometric measurement using 0.05  
172 M KOH for protons. The pH was determined by the water method using a soil/water  
173 ratio of 1:5 (w/v).

### 174 **2.3. Wood decaying stage and chemical analyses**

175 The variation of density of the SW and HW was assessed during the wood decay  
176 directly on specific oak discs incubated on the experimental site. For each replicate  
177 tree area, one oak disk at T0 and five oak disks at T3, T6 and T9 months were  
178 considered. A Pilodyn penetrometer with a 3 mm pin was used to measure  
179 penetration depth (i.e., the density) (Mäkipää and Linkosalo, 2011; Fundova et al.,  
180 2018). For each sampling time and oak disc, three measures were performed for  
181 both the SW and HW samples. Results were expressed as a mean percentage of  
182 penetration depth for each wood compartment at each time point.

183 For each tree area and each wood compartment, the chemical properties of the  
184 samples have been determined (four SW and four HW sawdust samples). All the  
185 samples were dried at 65°C during 72h, homogenized and then sub-sampled for the  
186 different analyses described in the supplementary section. Briefly, C and N were  
187 analyzed through total combustion. Nutrients (i.e., P, K, Ca, and Mg) were analyzed  
188 after wet mineralisation. Quantification of the cellulose was obtained by successive  
189 separations with different solvents using a Soxhlet extractor. Quantification of the  
190 lignin content in the wood samples was performed with the Klason method. Finally,  
191 the pH was determined for each sample.

### 192 **2.4. Collection of bacterial strains**

193 For each sample (four BS, four SW and four HW), 1 g of sawdust (SW or HW) or  
194 BS was added to 20 mL of sterile distilled water and vortexed three times during 90  
195 s. After a serial dilution in sterile distilled water, a volume of 0.1 mL from the dilutions  
196  $10^{-2}$  to  $10^{-5}$ , was then spread in triplicate onto 1/10 diluted Tryptic Soy Agar (TSA)  
197 medium (Tryptic Soy Broth (TSB) from Difco, 3 g.L<sup>-1</sup> and agar 15 g.L<sup>-1</sup>) containing  
198 cycloheximide (100 µg.L<sup>-1</sup>, final concentration). The pH of the medium was adjusted  
199 at 5 to fit with the pH found in oak sawdust and soil. All plates were then incubated at  
200 25°C for 5 days. After incubation, the number of colony-forming units (CFU).g<sup>-1</sup> from  
201 sawdust or soil samples was determined using the appropriate dilutions ( $10^{-3}$  and  $10^{-4}$   
202 according to the sample considered) permitting to quantify the densities of  
203 culturable bacteria in each compartment. For each compartment (BS, HW and SW)  
204 and replicate, and considering the dilution giving a total of 30 colonies per Petri dish,  
205 all the bacterial colonies were recovered. Bacterial isolates were then purified by  
206 three successive platings on 1/10 diluted TSA at pH 5 to obtain pure cultures. All the  
207 bacterial isolates were cryopreserved at -80°C in 35% glycerol. The bacterial  
208 collection was composed of 308 bacterial isolates that were distributed as follow: 117  
209 BS isolates, 107 SW isolates and 84 HW isolates.

## 210 **2.5. Metabolic and functional bioassays performed on bacterial isolates**

211 The metabolic potential of each bacterial isolate was determined using Biolog  
212 microplates, which contain ninety-five different C sources. The GN2 microplates were  
213 selected in our study because they contain relevant substrates related to wood  
214 derivatives (ie, cellulose) or fungal metabolites (trehalose). The screening was  
215 performed according to the manufacturer's instruction. After 48 h incubation at 25°C,  
216 the absorbance was measured at 595 nm using an iMark microplate reader (Bio-Rad,  
217 USA) (see supplementary section for detailed information).

218 The capacity of each bacterial isolate to degrade organic matter and specific  
219 compounds found in wood (cellulose, lignin, xylan and chitin) as well as their ability to  
220 recover iron through the production of siderophores was evaluated. Five different  
221 bioassays allowing to measure: i) their cellulolytic ability (carboxy-methyl-cellulose  
222 (CMC) assay), ii) their lignolytic ability (Remazol brilliant blue R (RBBR) assay), iii)  
223 their xylanolytic ability (xylan assay); iv) their chitinolytic ability (chitin assay) and v)  
224 their ability to mobilise iron (the CAS (chromoazurol-S assay), were used. Each  
225 bioassay is based on a glosed medium allowing the measure of a discoloration  
226 halo. When compatible with the bioassay, the pH of the medium was adjusted to 5 to  
227 stay close to the pH of the wood and the soil. Composition of each medium and the  
228 experimental procedure are fully described in the supplementary section.

## 229 ***2.6. Molecular identification of bacterial strains and phylogenetic*** 230 ***analyses***

231 A fragment of the 16S rRNA gene was amplified using the universal set of primers  
232 pA and 907r (Edwards et al., 1989; Lane, 1991). The polymerase chain reaction  
233 (PCR) was performed in a total reaction volume of 50  $\mu$ L containing 20  $\mu$ L of master  
234 mix (PCR Taq PCR Master Mix, Qiagen), 2  $\mu$ L of each primer (10  $\mu$ M) and 2  $\mu$ L of  
235 bacterial cells inoculum. PCR conditions were the following: 94°C for 4 min, then 30  
236 cycles of 30 s denaturation at 94°C, 60 s annealing at 53°C, 90 s extension at 72°C,  
237 and a final extension step of 10 min at 72°C. PCR products were then purified using  
238 the QIAquick PCR purification kit (Qiagen) and concentration determined using a  
239 Nanodrop-1000 spectrometer (NanoDrop Technologies, Wilmington, DE, USA). The  
240 Sanger sequencing reactions were performed by MWG Eurofin Operon (Ebersberg,  
241 Germany) using the primer 907r. All the sequences were identified using the  
242 nucleotide BLAST program (Altschul, 1997). Sequence alignment and distance tree

243 were constructed using SeaView (version 4.7) (Gouy et al., 2010) and by using  
244 neighbor-joining algorithms with the Kimura two-parameter correction. Distance trees  
245 were constructed and bootstrap values were obtained using 1,000 replicates. Trees  
246 were generated using the Dendroscope 3 software (version 3.6.3) (Huson and  
247 Scornavacca, 2012).

248 The 16S rRNA Sanger sequences generated for this study were submitted to the  
249 Genbank database of NCBI under the accession numbers MN193104 - MN193411.

### 250 ***2.7. Illumina 16S high-throughput sequencing and data processing***

251 Total DNA was extracted from 0.2 g of soil or oak sawdust (SW and HW) using  
252 the Fast DNA SPIN kit for soil extraction kit (MPbiomedical, Illkirch, France) following  
253 manufacturer's instructions with slight modifications including a step to grind the  
254 samples using a Retsch MM301 mixer (Retsch GbmH, Haan, Germany) and three  
255 additional washings with guanidine thiocyanate (5.5 M, pH 7) before to add the  
256 binding matrix. This step allows to efficiently denature endogenous nucleases and  
257 would facilitate the separation of proteins from the related sugars or polyols (Cox,  
258 1968; Mason et al., 2003). To prepare the amplicon libraries for the paired-end  
259 Illumina sequencing, the primers 515f and 806r targeting the V4 region have been  
260 used (Caporaso et al., 2011). The sample multiplexing, the paired-end Illumina  
261 sequencing using 2x250 bp Miseq v3 and the de-multiplexing of the sequences were  
262 performed at GeT-PlaGe (Genotoul, Toulouse, France) according to the  
263 manufacturer's instructions.

264 Raw sequences processing was performed using FROGS (Find Rapidly OTU with  
265 Galaxy Solution) pipeline (Escudié et al., 2018). Paired sequences were merged into  
266 contigs with flash (Magoc and Salzberg, 2011), cleaned with cutadapt (Martin, 2011)  
267 and chimeras were removed with VSEARCH (Rognes et al., 2016). The clustering to

268 generate Operational Taxonomic Units (OTU) was then performed with SWARM  
269 algorithm (Mahé et al., 2014) using a local clustering threshold (97% similarity) and  
270 the taxonomic affiliation of each OTU was based on the reference databases NCBI  
271 Blast+ on SILVA123 16S (Camacho et al., 2009, Quast et al., 2013). Finally, a table  
272 presenting the number of 16S rRNA sequences assigned to each OTU as well as  
273 their taxonomic affiliation was generated. OTUs belonging to *Eukaryota* or *Archaea*,  
274 chloroplasts or mitochondria were discarded as well as contaminant OTUs generated  
275 by the extraction and amplification steps. All the singletons were removed for the  
276 OTUs analyses. Finally, the number of sequences per sample ranged from 17,806 to  
277 39,132 giving a total of 334,380 sequences and 3,079 OTUs. OTU analyses were  
278 then performed using packages in R with a random subsampling to normalize each  
279 sample with the smallest number of sequences. The bacterial richness and alpha-  
280 diversity (Chao1, ACE, Shannon and inverse Simpson indexes) were calculated  
281 based on OTU relative abundance. The Illumina 16S rRNA sequences generated for  
282 this study were submitted to the Sequence Read Archive (SRA) of NCBI under the  
283 accession number PRJNA55031.

284 To determine both sequence homology and the relative environmental  
285 representativeness of the bacterial isolates recovered in our study, the Sanger 16S  
286 rRNA gene sequences were compared to the Illumina 16S rRNA gene sequences  
287 using the Basic Local Alignment Search Tool (BLAST) algorithm (Altschul et al.,  
288 1990). The Illumina 16S rRNA gene sequences of each compartment were used as  
289 database and an alignment of the Sanger 16S rRNA gene sequences was performed  
290 with NCBI-Blast+ using an e-value cutoff of  $1 \times 10^{-5}$ . This analysis was done on the 253  
291 bp overlapping between the Sanger and Illumina 16S rRNA gene sequences.

## 292 **2.8. Statistical analyses**

293 All statistical analyses were performed using R 3.2.3 software (R Core Team,  
294 2015). The effect of the compartment (i.e., BS vs HW vs SW) was tested on the  
295 chemistry data and the bioassay data using an analysis of variance (one-way  
296 ANOVA,  $P < 0.05$ ), followed by a Tukey test. Wood density differences between the  
297 two oak wood compartments (SW and HW) was determined by a t-test. For the  
298 culture-dependent approaches, a  $\text{Chi}^2$  test ( $P < 0.05$ ) analysis was performed to  
299 determine whether the frequency of distribution of the bacterial isolates according to  
300 their efficacy depended on their ecological origin.

301 For the Illumina 16S rRNA gene sequences and the related OTUs, the analyses  
302 were performed using R packages: phyloseq (McMurdie and Holmes, 2013), ape  
303 (Paradis et al., 2004) and vegan (Oksanen et al., 2007). The data were visualized  
304 using ggplot 2 (Wickham, 2009). The distribution of the bacterial communities  
305 according to their compartments was analyzed by performing multidimensional  
306 scaling (MDS) ordination using Bray Curtis matrix distance. Permutational  
307 multivariate analysis of variance (PERMANOVA) based on Bray-Curtis distances with  
308 999 permutations were performed on the OTU table using the vegan package and  
309 the Adonis command. Analysis of variance and subsequent post-hoc Tukey's tests to  
310 compare taxonomic and functional differences between the compartments were also  
311 performed. Relative abundance values at all taxonomic levels (phylum, class, order,  
312 family, genus, OTU) were compared for taxa or OTUs presenting a minimum of 100  
313 sequences. These values were transformed using the arcsine square root to achieve  
314 a normal distribution. When required, a p adjustment was done using the false-  
315 Discovery-Procedure (FDR) of Benjamini and Hochberg (1995) to determine the p-  
316 value threshold. Correlations between the major wood phyla and wood chemistry  
317 was assessed using Pearson correlations ( $P < 0.05$ ).

318

### 319 **3. Results**

#### 320 **3.1. Chemical analyses and wood decay stage assessment**

321 The chemical analyses were done after nine months of incubation on the BS and  
322 wood samples (SW and HW sawdust) (Table SI-1A, B). The analysis of the pH  
323 revealed that BS harbored similar pH to the SW. The C/N ratio was 10 to 26 times  
324 lower in BS than in the wood compartments (Tab. SI-1A, B). The comparison of the  
325 wood compartments revealed that SW was characterized by significantly higher pH  
326 (pH-SW=4.63 vs pH-HW=3.75), higher amounts of N, P and Mg (ANOVA,  $P<0.05$ )  
327 and lower (approximately two times) C/N ratio (ANOVA,  $P<0.05$ ). The content of  
328 cellulose and lignin (expressed on percentage relative to the global composition)  
329 appeared similar in HW and SW (ANOVA,  $P>0.05$ ) (Tab. SI-1A).

330 After nine months of *in situ* decay, the visual inspection showed a strong fungal  
331 colonization of the SW compartment, while the HW compartment appeared poorly  
332 colonized (Fig. 1A, B). Significant variations of the wood density were observed  
333 based on the pilodyn measurements. Indeed, the penetration increased from T0  
334 (25.8%  $\pm$  7.4 depth penetration in SW vs 16.4%  $\pm$  3.2 in HW) to T9 months (55.8%  $\pm$   
335 12.6 in SW vs 30.4%  $\pm$  4.5 in HW). A significantly higher decay stage was measured  
336 for SW from T3 to T9 months compared to HW (t-Test;  $P<0.05$ ) (Fig. 1C). According  
337 to these data, the decay stage of the oak discs after nine months of decay was  
338 considered as a middle stage.

#### 339 **3.2. OTUs distribution and diversity indices in soil and decaying wood**

340 From the 213,672 16SrRNA sequences retained after bioinformatics treatments  
341 (Tab. SI-2), a total of 2,901 OTUs were generated. Comparison between the  
342 compartments revealed that the BS harbors significantly more OTUs (1,141  $\pm$  189)

343 than the wood compartments (SW:  $548 \pm 127$  and HW:  $351 \pm 123$ ) (ANOVA,  
344  $P < 0.05$ ). No difference was observed between the SW and HW (ANOVA,  $P > 0.05$ )  
345 (Tab. SI-3). Among the total number of OTUs obtained, 9.8% were shared between  
346 the three compartments, while some were compartment specific (BS: 50.2%; SW:  
347 14.3% and HW: 7.7% OTUs). Furthermore, 7.4% of the OTUs were shared between  
348 the two wood compartments and ca 5% of the OTUs were shared between BS and  
349 the wood compartments (Fig. SI-2). The observed (i.e., number of OTU observed)  
350 and the estimated richness (i.e., chao1, ACE) as well as the alpha-diversity (i.e.,  
351 Shannon, Inv. Simpson) were significantly higher in BS compared to the wood  
352 compartments (ANOVA,  $P < 0.05$ ). These indices tended to be higher in the SW than  
353 in the HW, but only significantly for the Shannon values (Tab. SI-3). Most of the  
354 OTUs obtained were assigned at the phylum (99.8%-23 different phyla) to family  
355 (88.1%) level, but only 66.4% of these sequences were assigned at the genus level.  
356 A global description of the taxonomic affiliation of these OTUs is presented in the  
357 table SI-4.

### 358 **3.3. Structure and taxonomic composition of the bacterial communities in** 359 **soil and decaying wood.**

360 The properties of the different compartments considered (BS, SW and HW)  
361 strongly determined the bacterial community structure (i.e., BS vs HW vs SW;  
362  $F=10.6$ ;  $R^2=0.70$ ;  $P=0.01$ ; PERMANOVA and Fig. 2A). Noticeably, the bacterial  
363 community structure significantly differed between SW and HW (i.e.,  $F=3.17$ ;  $R^2=$   
364  $0.35$ ;  $P=0.03$ ; PERMANOVA). A MDS analysis done on the OTUs confirmed this  
365 compartment effect (Fig. 2A, B). A detailed analysis of the taxonomic composition  
366 revealed that the wood-inhabiting bacterial community was significantly dominated by  
367 the *Proteobacteria* (59 to 73.4% of the 16S rRNA sequences in SW and HW,



368 respectively) compared to the BS compartment (ANOVA,  $P < 0.05$ ) (Fig. 2A, Tab. SI-  
369 5). *Alphaproteobacteria* was the most abundant class in wood with 28.8% in SW and  
370 41.7% in HW. A similar trend was observed for *Betaproteobacteria* (wood > BS;  
371 ANOVA,  $P < 0.05$ ). Notably, our analyses revealed that the SW was significantly  
372 enriched in *Bacteroidetes* (ANOVA,  $P < 0.05$ ). In contrast, the BS bacterial community  
373 was mainly represented by *Acidobacteria*, followed by *Proteobacteria* (with  
374 *Alphaproteobacteria* being the most represented class) and *Verrucomicrobia*  
375 (12.8%). The relative abundance of *Acidobacteria* and *Verrucomicrobia* was  
376 significantly higher in BS than in HW and SW (ANOVA,  $P < 0.05$ ). Noticeably, the  
377 relative abundance of the *Bacteroidetes* (mainly present in SW) was positively  
378 correlated with wood pH ( $P = 0.01$ ) and P concentration ( $P = 0.02$ ), while it was  
379 negatively correlated with the C/N ratio ( $P = 0.02$ ). At the opposite, the relative  
380 abundance of the *Alphaproteobacteria* (dominant in HW) was positively correlated  
381 with the C/N ratio ( $P = 0.001$ ) but negatively correlated with P concentrations ( $P = 0.02$ )  
382 and the total amount of N ( $P = 0.02$ ) (Fig. SI-3).

383 Differences, at the OTUs level, between wood compartments and between wood  
384 and BS were also observed. Dominant and specific OTUs of each compartment are  
385 presented in Fig. 3, Table SI-6 and 7.

### 386 **3.4. Taxonomic affiliation of the bacterial isolates and relationship with** 387 **their ecological origin**

388 The quantification of the culturable bacteria by dilution-plating revealed that BS  
389 harbored significantly higher densities ( $9.03 \pm 0.14 \text{ Log}_{10} \text{ CFU g}^{-1}$  of soil) than the  
390 SW ( $8.09 \pm 0.08$ ; ANOVA,  $P < 0.05$ ), but was not different from HW ( $8.80 \pm 0.30$ ;  
391 ANOVA,  $P > 0.05$ ). HW harbored slightly higher densities than the SW (ANOVA,  
392  $P < 0.05$ ).

393 The taxonomic affiliation of the bacterial isolates revealed that most of them were  
394 affiliated to *Proteobacteria* (77.7%), *Actinobacteria* (15.5%), *Acidobacteria* (4.5%)  
395 and *Bacteroidetes* (2.3%). The relative distribution of the 30 genera detected is  
396 presented in Figure 4 and Table SI-8. Notably, ca. 10% of the bacterial isolates had  
397 less than 97% similarity with the 16S rRNA gene sequence of a known culturable  
398 bacterial strain deposited in NCBI. Most of these particular isolates came from SW.

399 When the taxonomic affiliation of the bacterial isolates was linked to their  
400 ecological niche (BS vs HW vs SW), our analysis showed that isolates belonging to  
401 the *Beta*- and *Gammaproteobacteria* were common to the three compartments (Fig.  
402 4; Tab. SI-8). However, we noticed that the SW compartment was significantly  
403 enriched in *Gammaproteobacteria* isolates (SW, 57.8% of the isolates; BS, 24.4%  
404 and HW, 17.8%;  $\text{Chi}^2$ ,  $P < 0.002$ ), whereas, the BS compartment was significantly  
405 enriched in *Betaproteobacteria* isolates (BS, 49.6% of the isolates; SW, 30.6% and  
406 HW, 19.8%;  $\text{Chi}^2$ ,  $P < 0.003$ ). Interestingly, all the isolates belonging to *Acidobacteria*  
407 and *Bacteroidetes* (*Alphaproteobacteria*) were exclusively found in wood (SW and/or  
408 HW). Several genera were strongly represented and only detected in wood. This is  
409 the case of *Sphingomonas*, *Mucilaginibacter* and *Luteibacter* in SW, and *Acidocella*,  
410 *Acidipila* and *Novosphingobium* in HW. At the opposite, *Actinobacteria* strains  
411 assigned to *Streptomyces*, *Kitasatospora*, *Arthrobacter* and *Streptacidiphilus* were  
412 dominant in BS. Strains assigned to the *Paraburkholderia* and *Dyella* genus were  
413 recovered in the three compartments considered, with *Paraburkholderia* representing  
414 34.7% of the total of the isolates.

### 415 **3.5. Metabolic and functional potentials of the culturable bacterial** 416 **communities**

417 The metabolic bioassays based on Biolog plates revealed that the BS bacterial  
418 isolates harbored significantly higher metabolic potentials than those of the decaying-  
419 wood compartments (BS>SW=HW) (ANOVA;  $P<0.05$ ) (Fig. SI-4). Such difference  
420 was observed at both substrate and substrate category levels (Fig. SI-4 and Fig 5).  
421 Comparisons between wood bacterial isolates showed that HW isolates had a higher  
422 metabolic potentials for substrates belonging to (i) the carboxylic acid group (ca. 2  
423 fold higher than SW for quinic acid, sebacic acid, succinamic acid, D-glucosaminic  
424 acid, glycyl-L-glutamic acid) (ii) the amino acid group (ca. 2 fold higher than SW for  
425 L-histidine); (iii) the miscellaneous group (15 fold higher than SW for thymidine); and  
426 finally, (iv) the amine-amide group (aminoethanol, 3 fold higher). Beta-methyl-  
427 D-glucoside was almost the unique substrate more effectively metabolized by the SW  
428 bacterial isolates (4 fold higher than HW isolates) (Fig. 5).

429 Among the functional bioassays performed, the cellulose decomposition assay  
430 gave the highest number of positive bacterial isolates. Indeed, ca. 12.6% of the  
431 bacterial isolates tested appeared capable of degrading cellulose (Fig. SI-5).  
432 Significantly more bacteria isolated from HW (ca. 28.6%) were capable of degrading  
433 cellulose, than those coming from SW (13.4%) and BS (10.3%) ( $\chi^2$ ;  $P<0.0001$ ). In  
434 term of effectiveness, the BS and HW bacterial isolates tended to be more effective  
435 at degrading cellulose (halo diameters:  $0.21\text{cm}\pm 0.04$  for BS isolates and  
436  $0.20\text{cm}\pm 0.02$  for HW isolates) than the SW isolates ( $0.12\text{cm}\pm 0.02$ ; ANOVA  
437  $P=0.07$ ). In the wood compartments, the ability to degrade cellulose was mainly  
438 encountered for bacterial isolates assigned to the genus *Mucilaginibacter* (relative  
439 efficacy to degrade cellulose (halo diameter):  $0.81\text{cm}\pm 0.51$ ), *Novosphingobium*  
440 ( $0.50\text{cm}\pm 0.51$ ) and *Acidisoma* ( $0.48\text{cm}\pm 0.41$ ). Noticeably, 71.4% of the  
441 *Mucilaginibacter* isolates were capable of hydrolyzing cellulose, while only 54.5% for

442 *Acidisoma* and 38.5% for *Novosphingobium* (Fig. 6). In contrast, in the BS  
443 compartment this function was mainly encountered for bacterial isolates assigned to  
444 the genus *Kitasatospora* ( $2.05\text{cm}\pm 1.61$ ) and *Streptomyces* ( $0.73\text{cm}\pm 1.21$ ) (Fig. 6).

445 Concerning the ability to degrade chitin, only 8.5% of the bacterial isolates were  
446 capable of degrading this compound and were mainly isolated from BS (45.5% of the  
447 isolates capable to hydrolyze chitin were originated from BS) (Fig. SI-5). The BS and  
448 HW bacterial isolates tended to be more effective at degrading chitin. The bacterial  
449 genera presenting the highest potential were assigned to the genus *Collimonas* (halo  
450 diameter:  $1.83\text{cm}\pm 0.49$ ; only recovered from the BS compartment), *Luteibacter*  
451 ( $0.91\pm 0.74$ ; only recovered from the HW compartment) and *Streptomyces*  
452 ( $0.62\text{cm}\pm 0.72$ ; only recovered from the BS compartment). While all of the *Collimonas*  
453 isolates were effective at hydrolyzing chitin, only 57.1% and 25% of the *Luteibacter*  
454 and *Streptomyces* isolates did it (Fig. 6).

455 For the other functions related to organic matter decomposition, the ability to  
456 degrade xylan was only detected for seven bacterial isolates (2.8% of the bacterial  
457 collection) (Fig. 6) and none of the bacterial isolates was capable of degrading lignin.  
458 Concerning the ability to mobilize iron, ca. 24% of the bacterial isolates tested were  
459 positives (Fig. SI-5). Significantly higher proportions were observed in BS (72.9% of  
460 the isolates mobilizing iron) than in HW (18.6%) or SW (8.5%) ( $\text{Chi}^2$ ;  $P < 0.001$ ). The  
461 bacterial genera presenting the highest potentials to mobilize iron were assigned to  
462 the genus *Pseudomonas* (relative efficacy to mobilize iron (halo diameter):  
463  $2.08\text{cm}\pm 0.98$ ) and in a lesser extend to *Collimonas* isolates ( $1.48\text{cm}\pm 0.69$ ) with  
464 respectively 85.7% and 100% of the isolates of these genera being effective for iron  
465 mobilization. The two other genera: *Paraburkholderia* ( $0.7\text{cm}\pm 0.93$ ) and

466 *Streptomyces* (0.2cm±0.49) were significantly less efficient than *Pseudomonas*  
467 isolates (ANOVA,  $P<0.05$ ) (Fig.6).

### 468 **3.6. Comparison of the Sanger and Illumina 16S RNA gene sequences**

469 This analysis highlighted that all the 308 bacterial isolates of our collection had a  
470 minimum of 97% of sequence homology with at least one OTU of the Illumina  
471 sequence dataset. When the highest homology was considered, this analysis  
472 revealed that the bacterial isolates corresponded to 65 OTUs identified in the Illumina  
473 dataset. These OTUs represented 55.4% of the total number of Illumina 16S rRNA  
474 sequences. Interestingly, the most represented bacterial isolates in our collection (at  
475 the genus level) were also among the dominant in the OTUs (Rank in the collection /  
476 rank in the OTU table; *Paraburkholderia* (1/1), *Acidisoma* (2/5), *Dyella* (3/12),  
477 *Acidocella* (6/4) and more distantly *Pseudomonas* (7/114) (data not shown).

478

## 479 **4. Discussion**

480 To date, a few studies have investigated the taxonomic diversity and especially  
481 the metabolic and functional role of decaying wood-inhabiting bacteria (Murray and  
482 Woodward 2007; Folman et al., 2008; Hervé et al., 2016). However, this aspect is of  
483 importance to better understand the decaying wood process and how bacteria  
484 contribute to the recycling of the nutritive elements accumulated in the tree biomass.  
485 Therefore, to investigate the structure and the potential role of the bacterial  
486 communities, we used oak discs installed on the floor of an oak forest site, mimicking  
487 partially in this way the conditions when trees fall on the soil and initiate a  
488 decomposition process. A combination of culture-dependent and -independent  
489 approaches combined with chemical analyses were used and comparisons with bulk  
490 soil bacteria was made to highlight the soil-decaying wood continuum.

#### 491 **4.1. Edaphic origin of decaying oak wood-inhabiting bacteria.**

492 How decaying-wood is colonized by microorganisms is rather difficult to  
493 determine. Indeed, part of the microorganisms found in decaying wood may originate  
494 from the tree tissues (i.e., endophytes), atmospheric deposits or from the surrounding  
495 BS (Baldrian et al., 2016). Previous analyses provided clear evidences that whether  
496 or not an initial microbiota exists inside trees during their life, its biomass and  
497 diversity changed importantly and quickly between the early and later stages of wood  
498 decay (Tláškal et al., 2017). The Venn diagram analyses done in our study suggest  
499 that a part of the wood-inhabiting bacterial communities is also part of the BS  
500 bacterial communities (10.6%). The other OTUs only detected in wood may  
501 represent minor groups occurring in BS but not detected by sequencing or may be  
502 coming from other locations (i.e., atmosphere, endophytes of the oak discs, woody  
503 debris fallen on the oak discs). Interestingly, Probst et al. (2018) also observed that a  
504 significant part of the OTUs identified in decaying wood were shared with the  
505 surrounding BS, supporting an edaphic origin of wood-inhabiting bacteria. The  
506 detailed analysis of the culture-dependent approach also indicated that several  
507 bacterial genera were detected in both the BS and wood compartments including  
508 bacterial isolates assigned to *Paraburkholderia*, *Dyella* and *Pseudomonas*. These  
509 bacterial genera have frequently been described in forest soils, underlying their ability  
510 to adapt to various habitats such as decaying wood (Folman et al., 2008; Lladó et al.,  
511 2016; Nicolitch et al., 2017). Supporting this hypothesis, Hervé et al. (2014)  
512 demonstrated that *Burkholderia* and *Dyella* strains isolated from BS and inoculated in  
513 wood sawdust microcosms became dominant after a succession of enrichment  
514 steps. Those results support the idea that the BS in forest ecosystem represents a  
515 seedbank with a high diversity of functional groups capable of decomposing wood.

516 This is not surprising as the topsoil is continuously in contact with litter and wood  
517 debris. Consequently, the abundance of wood-inhabiting bacteria depends on the  
518 turnover of tree biomass (wood, litter) with bacteria returning to the surrounding soil  
519 at the end of the wood decomposition process (Probst et al., 2018).

#### 520 **4.2. Metabolic and functional potential of decaying oak wood-inhabiting** 521 **bacterial communities differ from soil bacterial communities.**

522 Decaying oak wood-inhabiting bacterial communities may directly or indirectly  
523 contribute to wood decomposition. In this study, we tested the hypothesis of a direct  
524 role of bacteria in wood decay. To do it, a functional screening targeting the main  
525 wood constituents (cellulose, xylan and lignin) was performed. We also focused on  
526 chitin because it is an essential constituent of fungal cell walls, and the  
527 decomposition of the wood is characterized also by a dynamic succession of different  
528 fungi during the whole process (Rajala et al., 2015), generating an important fungal  
529 necromass potentially recyclable by bacteria. Mobilization of iron was also studied  
530 because iron plays a role in the oxidative process of wood decomposition (Goodell et  
531 al., 1997). Our results showed that cellulose degrading bacteria were more frequent  
532 in wood, especially in HW, than in the surrounding BS. However, BS was  
533 characterized by bacterial isolates presenting a higher effectiveness at degrading  
534 cellulose, xylan or chitin or at mobilizing iron than HW and SW. These results differ  
535 from those obtained by Hervé et al. (2016) where there was no functional difference  
536 observed between bacteria isolated from BS and those adapted to beech decaying  
537 wood. However, our experiment was done *in situ* and over a longer period, which  
538 may explain the differences we observed. Looking at the taxonomic identity of the  
539 strains, we found that representatives of the *Kitasatospora* genus, isolated from BS,  
540 were among the most effective at degrading cellulose. Nonetheless, representatives

541 of the *Mucilaginibacter*, *Acidisoma* and *Novosphingobium* genera, all isolated from  
542 wood (SW and HW), were also effective. Except for *Novosphingobium*, these genera  
543 were previously identified as important cellulose decomposers (Štursová et al., 2012;  
544 Lladó et al., 2016; Lopez-Mondejar et al., 2016; Nicolitch et al., 2016). The absence  
545 of bacterial strains able to degrade lignin confirms the results obtained by Murray and  
546 Woodward (2007) and Hervé et al. (2016). Those results suggest that the ligninolytic  
547 activity is probably mainly attributable to fungi (Janusz et al. 2017). However,  
548 bacterial strains affiliated to the *Actinomycetes*, *Alphaproteobacteria* and  
549 *Gammaproteobacteria* have been reported for their ability to break down lignin (Bugg  
550 et al., 2011; Janusz et al., 2017). The metabolic assays (strain by strain) revealed  
551 significant differences between wood and soil bacteria. The decaying wood-inhabiting  
552 bacteria presented weaker metabolic potentials than the BS isolates, but a conserved  
553 ability to consume cellulose and hemicellulose derived monomers such as alpha D-  
554 glucose, D-mannitol, D-mannose, L-arabinose, N-acetyl-D-glucosamine, L-fucose, D-  
555 sorbitol, D-fructose, L-rhamnose and beta-methyl-D-glucoside. The preferential use  
556 of these wood monomers by bacterial strains isolated from a wood environment was  
557 also demonstrated by Hervé et al. (2016). Another hypothesis explaining the  
558 enrichment of particular bacteria in decaying wood is their indirect role in the wood  
559 decay process (Clausen 1996; Johnston et al., 2016). Indeed, it was proposed that  
560 wood-colonizing fungi enrich their habitat with bacteria capable of fixing nitrogen or  
561 providing them other services, promoting fungal development and wood  
562 decomposition. Although this hypothesis was not tested in our study, the chitin  
563 degradation assay performed evidenced that some HW bacteria may have a  
564 negative impact on fungi. Altogether, the results obtained through the functional  
565 bioassays suggest that the decaying wood-inhabiting bacteria are adapted to their



566 environment where substrates are less diverse than in the soil and potentially more  
567 recalcitrant to decomposition.

#### 568 **4.3 Wood properties determine the structure of the bacterial communities.**

569 Intrinsic (chemistry) and extrinsic (environment) factors may impact the  
570 structuration of the wood-inhabiting bacterial communities. Indeed, wood is not a  
571 homogenous material and it is established that HW and SW present different  
572 properties. In our study, the chemical analyses done on the SW and HW  
573 compartments clearly evidenced that SW presented significantly higher content of N,  
574 P and Mg, and lower C/N ratio than HW. In addition, HW presented a significantly  
575 more acidic pH than SW. Interestingly, C/N ratio and pH have been proposed as  
576 driving factors governing the composition of the wood-inhabiting bacterial  
577 communities (Folman et al., 2008; Hoppe et al., 2015). In this sense, the correlation  
578 analyses done between the Illumina 16S rRNA sequences and the wood properties  
579 revealed that the C/N ratio was negatively correlated with the abundance of  
580 *Bacteroidetes*, while it was positively correlated with *Alphaproteobacteria*. In addition,  
581 pH appeared positively correlated with the abundance of *Bacteroidetes*. These two  
582 wood chemical properties were also reported as main drivers of bacterial community  
583 structures in decaying wood (Tláškal et al., 2017; Moll et al., 2018). The HW  
584 compartment is also known to present a higher phenolic extractives content (up to  
585 10%), which may have an antimicrobial activity (Kebbi-Benkeder et al., 2015;  
586 Miranda et al., 2017; Moll et al., 2018). The colonization of the wood compartments  
587 by other types of decaying wood organisms (i.e., fungi and insects; Kubartova' et al.,  
588 2012; Ulyshen, 2016; Leonhardt et al., 2019) may also be important extrinsic factors  
589 driving the structure of the bacterial communities. Indeed, fungi and insects are  
590 known to be important wood decomposers (Baldrian et al., 2016; Ulyshen, 2016) and

591 their interactions with bacteria are known to have an important ecosystemic role  
592 (Folman et al., 2008; Frey-Klett et al., 2011; Hervé et al., 2016; Johnston et al.,  
593 2016). Notably, bacterial–fungal consortia were shown to degrade wood more  
594 effectively than fungi alone (Murray and Woodward, 2003; Hervé et al., 2016). In our  
595 study, a visual inspection of the oak discs at the end of the incubation revealed an  
596 important fungal development (i.e., hyphae) on the surface of SW, which was not  
597 visible on HW. Consequently, it is strongly plausible that differences between HW  
598 and SW in term of bacterial community structure are also due to the presence of  
599 fungal communities. Whether the density of culturable bacteria was similar between  
600 the HW and BS, the taxonomic structuration differed. Although it was only significant  
601 for the richness index (i.e., Shannon), all the richness and diversity indices tended to  
602 have lower values for the HW compartment compared to SW. A similar trend was  
603 obtained by Moll et al. (2018) considering 30 tree species, including oak tree. In our  
604 study, the decrease of diversity and richness corresponded to higher abundances of  
605 *Alpha-* and *Betaproteobacteria* and lower abundance of *Bacteroidetes* in HW than in  
606 SW. These results differ from the study of Moll et al. (2018) where the major  
607 difference between HW and SW of decaying oak wood relied on an increase of  
608 *Acidobacteria* in SW. The culturable approach also permitted to differentiate the SW  
609 and HW compartments. Notably, several genera were only isolated in HW (i.e.,  
610 *Acidocella*) or only in SW (i.e., *Mucilaginibacter*, *Luteibacter*, *Yersinia*), evidencing a  
611 relatively good overlap between the conclusions obtained by the culture-dependent  
612 and -independent approaches. Together, these results highlight that specific bacterial  
613 communities are enriched in each wood compartment, potentially according to the  
614 wood properties as well as due to the interactions with the fungal communities  
615 occurring in decaying wood.

616

## 617 **5. Conclusion**

618 Our study highlighted that at middle stage of decay, a significant part of the  
619 microbiota enriched in wood is shared with the surrounding bulk soil (BS), evidencing  
620 that decaying wood is not disconnected from its environment. At the same time, ca  
621 30% of the OTUs identified in our study were only detected in the wood  
622 compartments, highlighting the enrichment of particular taxa and/or functional group  
623 in decaying wood. Interestingly, the bacterial communities occurring in the SW and  
624 HW compartments significantly differed in their taxonomic and functional  
625 compositions. The distribution of some taxa appeared significantly correlated to the  
626 wood chemical properties. This differentiation clearly evidences that the colonization  
627 of decaying wood by bacteria is based on a deterministic process linking extrinsic  
628 and intrinsic factors. Although the bacteria isolated from the wood compartments  
629 presented lower metabolic potentials compared to those of the surrounding BS, they  
630 were characterized by a conserved ability to decompose cellulose/hemicellulose and  
631 derivatives. In contrast, no bacteria capable of decomposing lignin was identified.  
632 Those results suggest that wood-inhabiting bacteria are capable of degrading the  
633 easily degradable part of wood, but that the decomposition of the recalcitrant part  
634 (i.e., lignin) is probably done by fungi (van der Wal et al., 2015; Hoppe et al., 2015;  
635 Leonhardt et al., 2019; Tláskal et al., 2017). Another hypothesis may be that these  
636 bacterial communities indirectly contribute to wood decay through the promotion of  
637 fungal growth, highlighting a possible functional complementation between bacteria  
638 and fungi (Frey-Klett et al., 2011). Future work will need to focus i) on the functional  
639 structuration of the bacterial communities at different stage of decay, ii) on bacterial-  
640 fungal interactions and/or co-occurrence studies during wood decay and iii) on the

641 indirect role of bacteria such as nitrogen fixation during wood decay. Combination of  
642 field experiments and controlled microcosms will be necessary to better understand  
643 the role of microbial interactions and community successions in wood decay.

644

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651

## 652 **7. Author contributions**

653 S.M., S.U. and M.B., conceived and designed the study; S.M., C.B., P.R. and  
654 C.L., performed field and laboratory work; S.M. and S.U., analyzed the data and  
655 wrote the paper. All authors reviewed and edited the manuscript.

656

657 **Figure Legends:**

658

659 **Figure 1. Evidence of the oak decay stage.** **A.** Picture of a freshly cut oak disc at  
660 the beginning of the experiment (T0 month). **B.** Picture of an oak disc after 9 months  
661 of *in situ* decay (T9 months) showing the side in direct contact with the soil. **C.** Mean  
662 percentage of the penetration depth for the sapwood (SW) and heartwood (HW)  
663 compartments assessed using a Pilodyn penetrometer at T0 ( $n=3$ ) and T3, T6 and  
664 T9 months ( $n=20$  per time point). Significant differences between the two wood  
665 compartments for each time point are presented using a star ( $P<0.05$  according to a  
666 t-test).

667

668 **Figure 2. A. Relative abundance of the Illumina Miseq 16S rRNA sequences**  
669 **from bacterial taxonomic phyla for each compartment (oak decaying sapwood**  
670 **(SW) and heartwood (HW) and bulk soil (BS)).** The ten most abundant phyla were  
671 considered. **B. Multidimensional scaling (MDS) ordination using Bray Curtis**  
672 **matrix distance representing the distribution of the bacterial communities**  
673 **according to each compartment (oak decaying sapwood (SW) and heartwood**  
674 **(HW) and bulk soil (BS)).**

675

676 **Figure 3. Heatmap showing the relative abundance of the 10 most important**  
677 **OTUs per compartment (oak decaying sapwood (SW) or heartwood (HW) or**  
678 **bulk soil (BS)) expressed in percentage.**

679

680 **Figure 4. Neighbor-joining tree showing the phylogenetic relationships of**  
681 **bacterial isolates depending on their compartment (oak decaying sapwood**  
682 **(SW) and heartwood (HW), Bulk soil (BS)), based on PCR sequencing of a**  
683 **portion of the 16S rRNA gene.** A bootstrap analysis was performed with 1000  
684 repetitions. Due to the relatively large number of sequences analysed, we have  
685 chosen one representative sequence in each genus for the tree representation. Pie  
686 chart on the right side of each genus represents the distribution of bacterial isolates  
687 assigned to this genus according to their origin (blue: BS, red: SW, green: HW).  
688 Number accessions for reference strains from NCBI are indicated in brackets.

689

690 **Figure 5. Heatmap analysis of the metabolic potentials obtained using Biolog**  
691 **GN2 microplates.** A total of 304 bacterial isolates was tested. Detailed analysis of  
692 the substrates for which metabolic potentials varied significantly among the bacterial  
693 isolates depending on the compartment (oak decaying sapwood (SW) and heartwood  
694 (HW) and bulk soil (BS)) was done. Statistics on the right of the heatmap were  
695 obtained according to a one-way ANOVA followed by a Tukey test (NS: not  
696 significant,  $*P<0.05$ ,  $**P<0.01$ ,  $***P<0.001$ ).

697

698 **Figure 6. Relationship between functional efficacy and taxonomic affiliation.**  
699 For each bacterial genus represented by a minimum of three effective isolates, their

700 relative efficacy to hydrolyze cellulose, xylan and chitin and to mobilize iron is  
701 presented, whatever their compartment (oak decaying sapwood (SW) and heartwood  
702 (HW) and bulk soil (BS)) of the bacterial isolates. Lowercase letters (a, b, c, d or e)  
703 indicate significant differences between genus efficacies. Statistics were obtained  
704 according to a one-way ANOVA followed by a Tukey test ( $P < 0.05$ ). The number of  
705 isolates tested per genus as well as their repartition according to their compartment  
706 (BS, SW or HW) is presented under the bars. The error bars indicate the standard  
707 error.

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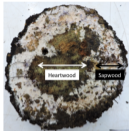
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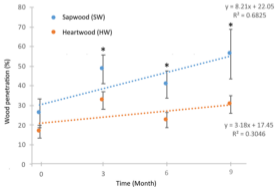
A.



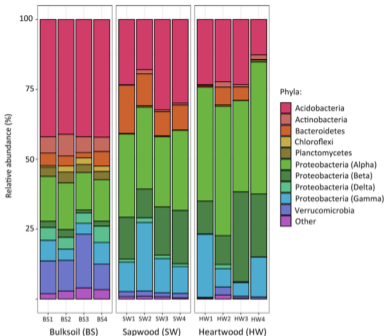
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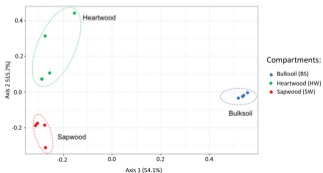
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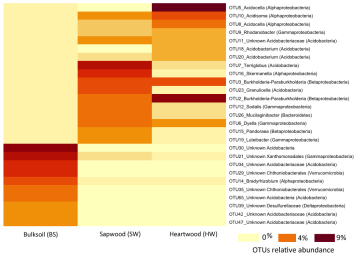


A. Composition within bacteria (top 10 phyla)



B. MDS+BC







Bacteroidetes  
Actinobacteria  
Acidobacteria  
Alphaproteobacteria  
Gammaproteobacteria  
Betaproteobacteria

