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1 **Bacteria in decomposing wood and their interactions with wood-decay fungi**

2

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10

11 **Running head**: Bacteria in wood

12

13 **Keywords:** bacteria; fungi; wood; decomposition; community ecology; wetwood

14

15 **Single-sentence summary:**

16 Bacteria and fungi both live in wood, but not much is known about how they interact - this article

17 reviews what is already known about them and what is yet to be found out.

18 **Abstract**

19 The fungal community within dead wood has received considerable study, but far less
20 attention has been paid to bacteria in the same habitat. Bacteria have long been known to inhabit
21 decomposing wood, but much remains underexplored about their identity and ecology. Bacteria
22 within the deadwood environment must interact with wood decay fungi, but again, very little is
23 known about the form this takes; there are indications of both antagonistic and beneficial
24 interactions within this fungal microbiome. Fungi are hypothesised to play an important role in
25 shaping bacterial communities in wood, and conversely, bacteria may affect wood-decay fungi in a
26 variety of ways. This mini-review considers what is currently known about bacteria in wood and their
27 interactions with fungi, and proposes possible associations based on examples from other habitats.
28 It aims to identify key knowledge gaps and pressing questions for future research.

29

30 *141 words*

1. Introduction

Globally, fallen wood stores more than 73 billion tonnes of carbon (Pan *et al.* 2011) and provides habitat for a wide range of saproxylic (*i.e.* deadwood-inhabiting) organisms (Stokland *et al.* 2012). Understanding the rate, mechanisms and control of wood decomposition is of major ecological and economic importance, and the key to doing so lies in understanding the microbial communities that effect and regulate decomposition. Fungi are the dominant agents of wood decomposition, but it has long been known that bacteria also inhabit dead wood (Greaves, 1971). There are indications of great bacterial diversity within wood (Zhang *et al.* 2008; Větrovský *et al.* 2011; Sun *et al.* 2014; Hoppe *et al.,* 2015), but bacteria are very poorly understood compared with fungi in the same environment. Wherever bacteria and fungi co-occur they must interact with and influence each other (Fig.1), yet although wood-decay fungi are well-known for being highly competitive (Boddy 2000) relatively little attention has been paid to the fungus-bacteria relationship (de Boer *et al.* 2005). Fungal-bacterial interactions have already been studied in other contexts for their importance in medicine, agriculture, and food and drink (Frey-Klett *et al.* 2011), but have been explored far less with respect to decomposition. The suite of bacteria that surround and interact with a fungus effectively constitute its microbiome, and as such, they must be considered together. The aim of this mini-review is to synthesise the current state of knowledge about bacteria in wood and how they interact with wood decay fungi, so as to identify key areas for future exploration.

2. Diversity of bacterial communities in wood

Information on bacterial communities in decomposing wood is surprisingly scare, given how well saproxylic fungal communities have been studied. This disparity is doubtless partially due to the greater propensity of fungi to enter culture – there is a long history of successful isolation of fungal decay communities from wood (*e.g.* Boddy *et al.* 1987). In contrast, whilst there are studies that have looked at the culturable fraction of saproxylic bacteria (*e.g.* Murray & Woodward 2007; van der Wal *et al.* 2007), a large and variable proportion are unculturable (Folman *et al.* 2008). Culture-based studies can thus, at best, only indicate part of the bacterial community. Because the culturable *proportion* of total bacteria varies, plate counts can never be used for quantitative comparison in this context. For example, a microcosm experiment recorded that in the absence of wood-decay fungi, 61% of bacteria colonising wood blocks could be cultured; when a white-rot fungus was introduced, the culturable proportion dropped to 1% (Folman *et al.* 2008). Unfortunately, the limitations of culture-based surveys mean that much older literature in this field is of restricted usefulness. Whilst culture-based approaches can no longer be used for whole-community characterisation, they remain highly useful for exploring specific relationships (*e.g.* Nazir *et al.*,

65 2014). Culture-based studies have also succeeded in isolating new genera from dead wood, including
66 members of the difficult-to-culture phylum Acidobacteria (*e.g.* Folman *et al.*, 2008). The accessibility
67 and high throughput of next-generation DNA sequencing and associated metagenomics opens the
68 door to more comprehensive study of saproxylic bacterial communities. This review will therefore
69 pay special attention to studies that have used molecular methods to assess bacterial diversity in
70 wood (Table 1), drawing on culture-based studies as well where applicable.

71 Bacterial diversity is far lower in wood than in soil (Hervé *et al.* 2014; Sun *et al.* 2014), and is
72 highly influenced by the underlying soil type; nonetheless, there is a high level of intra-site
73 heterogeneity (Sun *et al.* 2014). The bacterial community varies dependent on the wood's state of
74 decay, with bacterial richness increasing as the wood decomposes (Hoppe *et al.* 2015). Heartwood
75 and sapwood contain markedly different bacterial communities, but communities in heartwood are
76 apparently more diverse (Zhang *et al.* 2008); nonetheless, bacteria may be more abundant in
77 sapwood (Jeremic *et al.* 2004). There are indications that bacterial communities differ between tree
78 species (Folman *et al.* 2008; Hoppe *et al.* 2014, 2015; Prewitt *et al.* 2014). The water content, pH,
79 and C:N ratio of the wood affect the bacterial community, as does the forest management regime
80 (Hoppe *et al.* 2015). Bacterial abundance and richness is highest at advanced stages of wood decay,
81 but does not show a clear pattern for phylum-level community composition (Kielak *et al.* 2016b;
82 Rinta-Kanto *et al.* 2016). These studies offer a tantalising insight into saproxylic bacterial
83 communities, but the field is still young and the conclusions are tentative.

84 There are parallels between the microbial decomposer communities in wood and leaf litter.
85 In leaf litter, bacterial and fungal communities show linked dynamics and both are also influenced by
86 the same abiotic drivers of C:N ratio, nutrient availability, water content and pH (Purahong *et al.*,
87 2016). In another study on deciduous leaf litter, fungi showed less dependence than bacteria on
88 environmental variables, such as water availability and ambient temperature (Liu *et al*. 2016).
89 However, it is very difficult to extrapolate results from one habitat or taxonomic group to another.
90 For example, dominant tree identity is a major fungal community driver in both soil and litter, but
91 less important for bacteria in litter and unimportant for bacteria in soil (Urbanová *et al.* 2015).

92 In functional terms, Greaves (1971) classified saproxylic bacteria into four groups based on
93 their role in decomposition: bacteria that make wood more water-permeable without affecting its
94 structural integrity; bacteria with (albeit limited) decomposition ability; bacteria that stimulate
95 fungal decomposition; and bacteria that inhibit fungal decomposition. These classes maintain their
96 relevance today, but present a challenge: how best to relate broad-scale, whole sample taxonomic
97 information from sequencing to fine-scale functional abilities. Even the bacteria amenable to culture
98 can show vastly different properties depending on medium, *etc*. (Murray and Woodward 2003).

99 Truly making sense of the bacterial communities in wood will depend upon linking their identity and
100 function, even for species that can be cultivated.

101 Any consideration of bacteria in wood should take into account the presence and identity of
102 wood decay fungi, as current evidence strongly indicates that they greatly influence the bacterial
103 community (Folman *et al.* 2008; Sun *et al.* 2013; Hoppe *et al.* 2014). In the soil environment, areas
104 under close fungal influence have distinctive bacterial communities (Warmink and van Elsas 2008).
105 In *Picea abies* logs, fungal diversity correlated negatively with bacterial abundance, and there are
106 indications that certain bacterial taxa co-occur preferentially with particular fungi (Rinta-Kanto *et al.*
107 2016). Discerning ecologically realistic patterns is challenging, due to the huge number of potentially
108 significant variables; for example, the effects of fungal inoculation on bacteria alter over time (Sun *et*
109 *al.* 2013). It is also virtually impossible to establish fungus-free bacterial controls when inoculating
110 wood from soil (de Boer *et al.* 2010).

111 Fungi profoundly influence the wood physical environment by lowering the pH, excreting
112 metabolites such as oxalic acid and translocating nitrogen and phosphorus into the resource
113 (Watkinson *et al.* 2006; de Boer *et al.* 2010; Rudnick *et al.* 2015). It should be noted that whilst
114 translocation increases the bulk N and P content of the wood, they are contained within hyphae and
115 only available to bacteria that can access the hyphal contents. Experimental evidence shows that
116 dead mycelium also provides a rich and largely labile nutrient source which supports a distinct
117 bacterial community (Brabcová *et al.* 2016). However, in the environment fungi recycle cytoplasm
118 from senescent hyphae to other parts of the mycelium, so not all of these nutrients are available to
119 other decomposers (Watkinson *et al.* 2006).

120 Wood-decay fungi have repeatedly been associated with Burkholderiaceae (Seigle-Murandi
121 *et al.* 1996; Lim *et al.* 2003; Yara *et al.* 2006; Folman *et al.* 2008; Valášková *et al.* 2009; Leveau *et al.*
122 2010; Sato *et al.* 2010; Hervé *et al.* 2014; Prewitt *et al.* 2014; Sun *et al.* 2014). A widespread and
123 versatile family of bacteria, it crops up alongside fungi with remarkable regularity, and not only in
124 wood (de Boer *et al.* 2005; Frey-Klett *et al.* 2011). Moreover, there are indications of close,
125 specialized associations between fungi and the genus *Burkholderia* involving collaborative
126 pathogenicity (Partida-Martinez and Hertweck 2005); intimate mycelial associations (Lim *et al.*
127 2003); endosymbiosis (Sato *et al.* 2010); co-migration and detoxification of antimicrobials (Nazir *et*
128 *al.* 2014); and successional persistence (Hervé *et al.* 2014). An analysis of global soil microbiota
129 found significant co-occurrence between *Burkholderia* and fungi (Stopnisek *et al.* 2015). The same
130 study also analysed the proteome of *B. glathei* when grown alone or with fungi, and found that
131 when fungi were present the bacteria expressed fewer proteins associated with starvation, but

132 upregulated its stress response, suggesting that the bacteria gained nutrients from the fungus but
133 experienced antibiosis and/or unfavourable chemical conditions (Stopnisek *et al.* 2015).
134 It has been suggested (Greaves 1971; Frey-Klett *et al.* 2011) that bacterial activity in the
135 earliest stages of decay renders the wood more accessible to fungi. Whilst bacteria may detoxify
136 certain compounds inhibitory to fungi, notably in treated wood (Greaves 1971; Clausen 1996),
137 experiments using fresh, sterile wood show that fungi are competent wood decayers in the absence
138 of bacterial conditioning (*e.g.* Hiscox *et al.* 2010). Similarly, although it is sometimes suggested that
139 bacteria are the earliest colonists of dead wood, there is little evidence on whether or not that is the
140 case (van der Wal *et al.* 2007). Given that wood decay fungal propagules are latently present in
141 functional wood (Parfitt *et al.* 2010), for this to hold true bacteria would likewise have to be latently
142 present, and/or colonise wood very rapidly once conditions were favourable.
143
144 **3. Bacterial colonisation of wood**
145 The provenance of saproxylic bacteria communities and their means of colonisation are
146 largely unknown. Bacteria have limited motility and are unable to cross air voids, meaning that
147 colonisation is likely to be slow without some means of carriage into the wood. Essentially, bacteria
148 in a woody resource have four possible points of origin: the soil; the air; the wood itself; and fungi or
149 other organisms colonising the wood. The relative importance of these sources is likely to vary under
150 different conditions; the bacterial community in attached dead branches is probably very different
151 from that in wood on the forest floor.
152 *3.1. Edaphic and atmospheric sources of bacteria*
153 Soil represents a rich source of potential colonists for wood in ground contact, and the
154 underlying assumption of many studies is that it is the main point of origin for bacteria in wood (*e.g.*
155 van der Wal *et al.*, 2007; Folman *et al.*, 2008; Hervé *et al.*, 2014). These show that a subset of soil
156 bacteria are competent to colonise wood, but do not indicate to what extent this occurs under
157 natural conditions. Underlying soil type was a good predictor of bacterial assemblage in
158 experimental wood blocks (Sun *et al.* 2014), which suggests either an edaphic origin of saproxylic
159 bacteria, or an indirect influence of soil: for example, via an altered fungal community.
160 Movement via airborne spores and other propagules is a major means of bacterial dispersal,
161 which has led to the widespread view that all bacteria are, or have the potential to be, ubiquitous: a
162 view that has since been challenged (Green and Bohannan 2006). Nevertheless, the air could
163 represent another means for bacteria to arrive at decomposing wood. In woodlands, rainfall creates
164 bio-aerosols of bacteria and fungal spores, presumably contributing to their dispersal (Huffman *et al.*
165 2013). An experiment on bacteria in woodland pools indicated that the community composition was

166 not dispersal limited, suggesting that airborne dispersal is effective at least across local scales (Bell
167 2010).
168 *3.2 Bacterial endophytes*
169 As mentioned above, wood decay fungi exist in living trees as latent propagules which
170 spread as mycelia when the branch or trunk is no longer functional in water conduction (Parfitt *et al.*
171 2010). Scanning electron microscopy indicates that at least some, if not all, living trees also host
172 abundant bacterial endophytes in their wood (Jeremic *et al.* 2004). However, there is very little
173 literature on bacterial endophytes in wood, and the identity of these endophytes is as yet uncertain.
174 *3.3. Bacteria co-colonisation with other organisms*
175 *Burkholderia terrae* BS001 has been shown to migrate across soil with wood-decay fungi,
176 including the aggregated mycelial cords of *Phanerochaete velutina* (Nazir *et al.* 2014). This
177 demonstrates active bacterial movement, as the apical growth of fungal hyphae rules out the
178 possibility of passive carriage. *P. velutina* had a lower bacterial 'carrying capacity' than several other
179 fungi, suggesting that cords may be less conducive to migration than are fine hyphae. The presence
180 of *B. terrae* BS001 can also facilitate the movement of other bacteria which would otherwise not be
181 competent to migrate along hyphae (Warmink *et al.* 2011). Independent hyphal migration has also
182 been observed for several other members of the Burkholderiales and some strains of *Dyella japonica*
183 (Warmink and van Elsas 2009; Nazir *et al.* 2012). It has been suggested that other fungus-associated
184 bacteria such as *Collimonas* may share this migratory ability (Leveau *et al.* 2010). Such behaviour
185 raises the possibility that when fungi colonise a resource, they bring a suite of bacterial travelling
186 companions. In this manner saproxylic bacteria could use foraging fungal mycelium as a conduit to
187 new resources.
188 Other saproxylic organisms, particularly invertebrates, may transfer bacteria from one
189 woody resource to another. Bark beetles can carry bacteria phoretically (Mercado *et al.* 2014), and
190 introduce them into trees during the construction of galleries. This has also been suggested as a
191 source of nitrogen-fixing bacteria in wood (Griffiths *et al.* 1993).
192
193 **4. Wetwood**
194 The presence of bacteria in living trees is most obvious in bacterial wetwood. Wetwood, also
195 known as wet-heartwood or watermark, is a condition where the heartwood of a living tree
196 becomes saturated and discoloured. This change may be accompanied by blocked vessels, gas build
197 up, and the presence of a fetid liquid. The term refers to a suite of phenomena, probably with
198 multiple causal agents but broadly similar manifestations, making it hard to disentangle the exact
199 role bacteria play. Whilst in some tree species (*e.g. Salix sachalinensis*) wetwood is a serious disease,

200 spreading to sapwood and ultimately killing the tree (Sakamoto and Sano 2000), in others it seems
201 to be an almost-ubiquitous part of maturation (*e.g. Ulmus americana*) (Murdoch and Campana
202 1983). Wetwood is often attributed to bacterial activity, but there is no clear evidence whether this
203 is true for this latter form, where there are no apparent ill-effects to the tree: it could equally be
204 caused by physical processes, and bacteria secondarily colonise and modify the habitat.
205 Wetwood is frequently associated with the presence of anaerobic, methanogenic,
206 pectinolytic prokaryotes, which could account for many of the observed symptoms (Schink *et al.*
207 1981a). Although wetwood can form around the site of fungal infections, within the wetwood itself
208 fungi are likely to be excluded by low O_2 concentrations, large amounts of organic acids and
209 inhibitory metabolites (Worrall and Parmeter 1983). If fungi are indeed absent from wetwood, it
210 represents an almost unique wood habitat in this respect.
211
212 **5. Bacterial metabolism in wood**
213 *5.1 Bacterial nitrogen fixation in wood*
214 It has long been recognised that dead wood plays host to nitrogen-fixing (diazotrophic)
215 bacteria, which provide an independent source of nitrogen to the system (Cornaby and Waide 1973;
216 Sharp and Millbank 1973). Many studies have focussed on coniferous forests of the Pacific
217 Northwest, and used acetylene reduction as a measure of nitrogenase activity (reviewed by Son,
218 2001). Interpretation and comparison of these results requires caution, as the exact methodology
219 used varies; acetylene reduction has been criticised for its sensitivity to experimental parameters
220 (Giller 1987), although the effects may not be as serious as suggested (Son 2001). Additionally, the
221 conversion factor used to calculate N-fixation from acetylene reduction is not consistent (Son 2001;
222 Brunner and Kimmins 2003), and there is evidence to suggest that the true conversion rate may vary
223 between sample types (Hicks *et al.*, 2003b). In light of these difficulties, acetylene reduction should
224 perhaps be regarded as semi-quantitative, suitable for comparison within but not between studies.
225 The picture that emerges of N-fixation in dead wood suggests a highly dynamic process,
226 influenced by many factors (Hicks *et al.*, 2003a). Wood water content is consistently positively
227 correlated with N-fixation (Larsen *et al.*, 1978; Jurgensen *et al.*, 1984; Brunner & Kimmins, 2003;
228 Hicks *et al.*, 2003a), possibly because it creates better microhabitats for the
229 anaerobic/microaerophilic diazotrophs (Spano *et al.*, 1982; Hicks *et al.*, 2003a). The optimum
230 temperature for fixation is 30°C (Hicks *et al.,* 2003a), which may explain higher N-fixing activity in
231 summer than in winter (Jurgensen *et al.* 1984; Sollins *et al.* 1987). The requirements for high
232 temperature and high moisture suggest an interplay of factors that determine seasonal fixation
233 patterns (Hicks *et al.*, 2003a). The effect of tree species on fixation is unclear, with some authors

234 reporting significant differences between species (Jurgensen *et al.* 1989; Griffiths *et al.* 1993; Hoppe
235 *et al.* 2014), and others reporting none (Sollins *et al.*, 1987; Hicks *et al.*, 2003b). Nitrogen fixation in
236 forest ecosystems is likely to be limited by the availability of molybdenum, which is necessary for
237 nitrogenase synthesis, and possibly also by other micronutrients (Silvester, 1989).
238 Nitrogen fixation increases as decay proceeds (Larsen *et al.* 1978; Spano *et al.* 1982;
239 Jurgensen *et al.* 1984), although two studies found that fixation peaked before dropping off in the
240 most advanced stage of decay, perhaps because the latter studies included more decayed wood
241 than the former (Hicks *et al.* 2003b). An experiment using a finer resolution time-series over 6 years
242 revealed considerable variation within the overall increase in N fixation (Griffiths *et al.,* 1993). In the
243 very early stages of decay, N fixation will be limited by the rate at which diazotrophs can colonise the
244 resource. If nitrogen-fixing bacteria rely on carbon from fungal activity (see section 6.1), they may
245 also experience a growth lag whilst fungi colonise and start to decompose the wood. Diazotrophic
246 activity is higher in sapwood than in heartwood, and higher again in bark; low fixation has been
247 recorded in heartwood, possibly because it is the fraction most refractory to decomposition
248 (Griffiths *et al.*, 1993; Brunner & Kimmins, 2003; Hicks *et al.*, 2003b) and often contains inhibitory
249 extractives.
250 The identity of the saproxylic diazotrophs is underexplored, but *Clostridium* and *Klebsiella*
251 have been cultured (Spano *et al.* 1982). A survey of *nifH* nitrogenase genes in decaying wood
252 indicated Rhizobiales was the predominant identifiable order, with Rhodocyclales,
253 Pseudomonadales, Rhodospirillales, Sphingomonadales and Burkholderiales also present; however,
254 most of the saproxylic *nifH* variants could not be matched to known bacteria (Hoppe *et al.* 2014).
255 These bacterial orders have also been identified previously in 16S rRNA gene surveys of decaying
256 wood (Folman *et al.* 2008; Valášková *et al.* 2009).
257 *5.2 Bacterial wood decomposition*
258 Bacteria are well known to be capable of cellulose decomposition, although their
259 contribution to overall wood decay is restricted by small size and limited movement (Greaves 1971;
260 Clausen 1996) unlike fungi with mycelial growth (de Boer *et al.* 2005). Various bacteria from
261 woodland soil possess enzymes involved in the breakdown of cellulose/ cellulose products, including
262 members of the Acidobacteria, a common phylum in dead wood (Lladó *et al.* 2016; Table 1). Some
263 cellulolytic bacteria apparently use new, uncharacterised means of metabolising cellulose without
264 expressing the usual enzymes (López-Mondéjar *et al.* 2016). Certain bacteria in wood break down
265 pectin (Schink *et al.* 1981b; Clausen 1996), although in some cases this may be a strategy to access
266 cellulose (Lynd *et al.* 2002). Evidence has emerged of bacteria with lignin-decomposing abilities,
267 albeit to a lesser extent than fungi (Bugg *et al.* 2011; Brown and Chang 2014). An Actinobacterium,

268 *Amycolatopsis* sp. 75iv2, can use lignin as a sole carbon source (Brown and Chang 2014). Previously-
269 unknown ligninolytic bacterial enzyme systems have been found, unlike those deployed by fungi,
270 and environmental metagenomics may reveal more (Brown and Chang 2014). Lignin-model
271 compounds are frequently used to screen for activity, and whilst they may not be fully
272 representative, there is also evidence of bacterial depolymerisation of natural lignin (Salvachúa *et al.*
273 2015).

274 Many bacteria are thought to favour easily accessible, low molecular weight compounds
275 present during early decay, or released by fungal activity (de Boer and van der Wal 2008). Under
276 such a scenario, it would be expected that bacteria would be most numerous at the start of decay,
277 and would be displaced by fungi as the latter become established and the most labile components
278 are used up (Clausen 1996). Conversely, the absolute number of bacteria may be maintained or even
279 increase, but shift towards bacteria adapted to fungal co-existence, living on the products of fungal
280 decomposition. Fungi may affect bacterial decomposition in other ways, too: for example, *in vitro* a
281 forest soil bacterium, *Clostridium phytofermentans*, lyses fungal hyphae to increase its own cellulose
282 decomposition, presumably due to acquisition of fungal nutrients (Tolonen *et al.* 2015).

283 Again, work on forest soils and leaf litter can offer clues as to the roles of fungi and bacteria
284 in complex polymer decomposition. Acidobacteria from a forest soil showed a range of enzymatic
285 abilities, including the capacity to break down chitin (a fungal cell wall component) and cellobiose (a
286 cellulose breakdown product) (Lladó *et al*. 2016). Importantly, the dominant taxa in terms of DNA
287 abundance do not necessarily match the most active taxa based on RNA transcripts (Žifčáková *et al*.
288 2016). Fungal and bacterial biomass in soil does not vary greatly between seasons, but their patterns
289 of transcription activity do show strong seasonal effects (Žifčáková *et al*. 2016).There is evidence for
290 some degree of functional redundancy in litter-decomposing communities (Purahong *et al.* 2014).

291 Mixed communities of bacteria show greater decomposition ability in wood than individual
292 species (Schmidt and Liese 1994), which implies that bacterial contributions to wood decomposition
293 may have been underestimated. Nonetheless, total bacterial decomposition is likely to remain
294 negligible compared to fungi, due to the latter's size and superior access to material: factors which
295 would also allow fungi to decompose wood at a faster rate. One situation in which bacteria do play a
296 major role in wood decomposition is in wet/waterlogged wood such as cooling towers and
297 archaeological structures; the low oxygen concentrations under these conditions are inhibitory to
298 most fungi, leaving bacteria as major agents of decomposition (Kim and Singh 2000). A fluid-filled
299 environment is also far more conducive to bacterial movement (facilitating colonisation) than a dry
300 material. Bacterial wood decomposition is usually slow and incomplete, and thus wooden artefacts
301 can be preserved for centuries under these conditions (Björdal 2012).

302 Bacterial wood decomposition is often described based on the physical patterns produced in
303 the wood ultrastructure, and is can be grouped into four main types (reviewed Greaves, 1971;
304 Clausen, 1996; Kim & Singh, 2000). These categories are based on the morphology of wood
305 substratum following decay, rather than the taxonomic affiliations of the bacteria involved.
306 'Tunnelling' bacteria decay a convoluted path inside the cell walls (Kim and Singh 2000), which they
307 may enter via pit chambers (Greaves 1969). They can act on all components of the cell wall and may
308 be able to degrade/modify lignin, at least to some extent (Kim and Singh 2000). 'Erosion' bacteria
309 create depressions in the wall from inside the lumen, which follow the path of cellulose microfibrils;
310 they rarely affect the middle lamella, and probably lack the ability to degrade lignin (Greaves 1969;
311 Kim and Singh 2000). 'Pitting' bacteria produce small, shallow indentations (Greaves 1971); the term
312 is somewhat confusing, given that bacteria often are associated with pits connecting cells.
313 'Cavitation' bacteria cause diamond-shaped cavities inside cell walls, possibly involving the
314 production of diffusible enzymes (Kim and Singh 2000). Bacteria are often associated with pits
315 between wood cells, and decomposition activity in these areas can greatly increase the permeability
316 of the wood (Greaves 1969).
317
318 **6. Bacterial-fungal community interactions**
319 *6.1 Community competition and co-operation*
320 It is not difficult to envisage why fungal-bacterial co-existence in wood could lead to conflict.
321 Both may compete for the same substrates; bacteria may remove the products of fungal
322 extracellular enzyme decomposition (effectively microbial kleptoparasitism); and either group may
323 regard the other as a food resource. Certainly wood-decay fungi have an arsenal of competitive
324 strategies capable of deployment (Boddy 2000). Bacteria described as closely associated with
325 *Phanerochaete chrysosporium* were all proficient at utilising lignin breakdown products *in vitro*,
326 supporting the idea that they gained nutrition from fungal activity (Seigle-Murandi *et al.* 1996).
327 Studies show that the introduction of *Hypholoma fasciculare* can alter the abundance and
328 community composition of bacteria within wood (Folman *et al.*, 2008; de Boer *et al.*, 2010), and
329 rapid pH change has been suggested as a possible mechanism (de Boer *et al.* 2010). Whether this is
330 representative of a natural situation is uncertain: bacteria were very abundant in more decayed *H.*
331 *fasciculare*-colonised wood in a field scenario, which could be due to bacterial recovery over time, or
332 the proliferation of fungus-tolerant bacteria (Valášková *et al.* 2009). Inoculation with *Phlebiopsis*
333 *gigantea* lowered bacterial species richness in stumps after 12 months, although the effect had
334 disappeared by 6 years post-inoculation (Sun *et al.* 2013). It is possible that direct fungal-bacterial
335 competition occurs in wood similar to that observed in soil, where experimental inhibition of

336 bacteria results in accelerated fungal growth indicative of competitive release (Rousk *et al.*, 2008,
337 2010). Intriguingly, the competitive outcome appears to depend on the ambient pH: fungi prevail at
338 low pH, bacteria at higher pH (Rousk *et al.* 2010). This is salient in light of the major pH modification
339 – typically lowering – that fungi effect in wood (de Boer *et al.* 2010).
340 There is much *in vitro* evidence for antagonism amongst saproxylic micro-organisms, both of
341 fungi against bacteria (Janes *et al.* 2006; Popova *et al.* 2009) and of bacteria against fungi (Murray
342 and Woodward 2003; de Boer *et al.* 2007; Caldeira *et al.* 2008; Boaisha 2012). This is not necessarily
343 evidence for antagonism *in situ*, given that the effect can depend on the culture medium (Murray
344 and Woodward 2003; Boaisha 2012). In addition, soil bacteria with little or no anti-fungal activity on
345 their own (*Pedobacter* sp. and *Pseudomonas* sp.) can show considerable fungal inhibition when in
346 combination, either as a collaborative effort or a by-product of antagonism towards each other (de
347 Boer *et al.* 2007).
348
349 Conclusive evidence of fungal-bacterial mutualism requires demonstration of a benefit to
350 both partners. Although this has been demonstrated in a variety of habitats (Frey-Klett *et al.* 2011),
351 there is a shortage of clear examples in terrestrial deadwood. There are reports of basidiomycetes
352 gaining more biomass and decaying wood faster in the presence of yeasts and nitrogen-fixing
353 bacteria than in their absence (Blanchette and Shaw 1978). Moreover, SEM revealed close physical
354 association between the mycelial fungi, yeasts and bacteria (Blanchette and Shaw 1978). *H.*
355 *fasciculare* and *Resinicium bicolor* decompose wood significantly faster in the presence of bacteria
356 than alone; *Heterobasidion annosum* displayed the same effect only if bacteria were added after the
357 fungus had become established (Murray and Woodward 2003). However, in other instances,
358 bacteria had no effect on *H. fasciculare* decomposition (Weißhaupt *et al.* 2013). Such variability
359 could be influenced by bacterial community composition, fungal intra-specific variation, or
360 environmental conditions. Increased decomposition may be attributable to bacterial nutrient
361 provision (*e.g.* vitamin production (Ghignone *et al.* 2012) or N-fixation), or up-regulation of fungal
362 enzymes due to the removal of breakdown products (Murray and Woodward 2003; de Boer *et al.*
363 2005). Whilst the latter scenario would represent facilitation in ecosystem process terms, the benefit
364 to the fungus is questionable, depending on whether faster decomposition translates to increased
365 fungal growth, or simply to decreased efficiency due to bacterial consumption of breakdown
366 products. This could be particularly disadvantageous to fungi with an ecological strategy that
367 involves slowly decomposing wood over a long period, as with some xylariacious ascomycetes
368 (Boddy *et al.* 1989).

369 There are examples of bacterial-fungal interactions that benefit at least one party with
370 (currently) no evidence of harm to the other, suggesting at least a commensal association. There is
371 also *in vitro* evidence for growth enhancement, which should be regarded with the same caveats as
372 *in vitro* antagonism. For example, a bacterium of the *Burkholderia cepacia* complex, isolated from a
373 *Pleurotus ostreatus* fruit body, showed increased growth in the presence of *P. ostreatus* mycelium
374 (Yara *et al.* 2006). A *Curtobacterium* sp. from dead wood promoted growth of *Stereum* sp. –
375 although it was the only one out of 24 culturable strains to do so (Kamei *et al.* 2012). Notably,
376 *Streptomyces* from woodland soil showed negative or neutral influences on mycorrhizal and
377 pathogenic fungi, yet all consistently and markedly promoted growth of the white-rot fungus
378 *Phanerochaete chrysosporium*; it may be salient that none of these *Streptomyces* strains showed
379 ligninolytic activity themselves (Bontemps *et al.* 2013).
380 The fungal-migratory bacterium *Burkholderia terrae* BS001 (see section 3.3) has been
381 demonstrated to protect a (non-wood decay) fungus from inhibition by the fungicide cycloheximide
382 or metabolites from the antagonistic bacterium *Pseudomonas fluorescens* strain CHA0 (Nazir *et al.*
383 2014). This raises the possibility of a true mutualism, whereby *B. terrae* BS001 gains access to
384 resources and in return affords protection to its fungal host. The level of protection depended on the
385 identity of the fungus, and, for reasons unknown, the timing of bacterial arrival (Nazir *et al.* 2014).
386 *6.2 Bacterial endosymbiosis and intimate hyphal associations*
387 Bacteria co-exist endosymbiotically with arbuscular mycorrhizal fungi (Bonfante and Anca
388 2009), but such an association has yet to be conclusively demonstrated for wood-decay fungi. It is
389 likely that bacteria do occur within the hyphae of wood decay fungi, as they have also been found
390 inside ectomycorrhizal hyphae (Bertaux *et al.* 2005), plant-pathogenic fungi (Partida-Martinez and
391 Hertweck 2005), a range of endophytic fungi (Hoffman and Arnold 2010) and the soil saprotroph
392 *Mortierella elongata* (Sato *et al.* 2010). Intrahyphal existence does not necessarily indicate true
393 endosymbiosis, which implies active interaction between living cells (Lumini *et al.* 2006). Rather,
394 bacteria may enter compromised or senescent hyphae opportunistically and without vertical
395 transmission (de Boer *et al.* 2005; Lumini *et al.* 2006). This distinction is not always made clearly in
396 the literature on fungal 'endosymbionts', nor is it always recognised that bacteria may associate
397 intimately but extracellularly with mycelium.
398 Where true endosymbiosis does occur, the extent of its implications are illustrated by the
399 well-characterised association between the plant-pathogenic fungus *Rhizopus microsporus* and its
400 bacterial endosymbiont, *Burkholderia rhizoxinica* (Partida-Martinez and Hertweck 2005). The toxin
401 rhizoxin forms a key part of *R. microsporus* pathogenicity, yet is synthesised not by the fungus but by
402 the bacteria within it. Vertical transmission of the bacteria is guaranteed, as *R. microsporus* has lost

403 the ability to sporulate in the absence of *B. rhizoxinica* (Partida-Martinez *et al.*, 2007). *B. rhizoxinica*
404 is also highly competent to colonise *R. microsporus* hyphae from the outside by localised chitinase
405 activity that does not cause fungal lysis (Moebius *et al.* 2014). Colonisation relies on both the type II
406 and type III secretion systems (Lackner *et al.* 2011; Moebius *et al.* 2014). Pertinently, these secretion
407 systems have been implicated in other fungal-bacterial interactions, such as mycorrhiza formation,
408 described as the 'helper bacteria effect' (Cusano *et al.* 2011); mycophagy (Mela *et al.* 2012); co-
409 migration (Warmink & van Elsas, 2009; Nazir *et al.*, 2012, 2013, 2014); and an undefined bacteria-
410 fungus association (Warmink and van Elsas 2008).

411 Aside from true endosymbiosis, there is evidence that wood decay fungi form intimate
412 mycelial associations with bacteria (Seigle-Murandi *et al.* 1996; Lim *et al.* 2003; Yara *et al.* 2006). For
413 example, bacteria have been observed to co-exist with ten strains of *Phanerochaete chrysosporium*
414 (Seigle-Murandi *et al.* 1996), although Janse *et al.*, (1997) failed to isolate bacteria from five strains
415 of the same fungus, including one described by Seigle-Murandi *et al.* (1996). Thirty-two other wood-
416 rot fungi tested negative, but where bacteria were present on *P. chrysosporium*, pure cultures of the
417 fungus could not be established even from conidiospores, suggesting that bacteria may be within the
418 hyphae and vertically transmitted (Seigle-Murandi *et al.* 1996). Similarly, *Burkholderia sordidicola*
419 was isolated from two strains of *P. sordida*, and bacteria-free fungal cultures could not be
420 established (Lim *et al.* 2003).

421 *6.3 Mycophagy and predation*

422 From mycelial associations, it is a small step to bacterial mycophagy (fungus-eating): the
423 active utilisation of living fungal matter for bacterial growth (Leveau and Preston 2008). Given that
424 bacteria are smaller than fungi, and do not kill the entire host organism, bacterial mycophagy is
425 more analogous to parasitism than predation. There is also potential for mutualistic mycophagy,
426 where bacteria 'pay their way' by provision of specific nutrients or degrading toxins (Leveau and
427 Preston 2008). Endosymbiosis can be regarded as a specialised form of mycophagy (Leveau and
428 Preston 2008).

429 An increasing body of evidence suggests that glycerol is a favoured carbon source for many
430 mycophagous bacteria, although so far none of this evidence is derived directly from wood. In liquid
431 culture *Burkholderia terrae* strain BS001 stimulates glycerol release by the fungus *Lyophyllum* sp.
432 strain Karsten, and the glycerol is apparently consumed by the bacteria (Nazir *et al.* 2013). Whilst
433 this ability has not yet been tested in an ecologically realistic situation, the *B. terrae* BS001 genome
434 encodes glycerol transporters that are unique among the *Burkholderia*, possibly linked to its fungus-
435 associated lifestyle (Haq *et al.* 2014). *B. rhizoxinica*, the *Rhizopus* endosymbiont, likewise possesses
436 genes involved in glycerol metabolism and can utilise glycerol as a carbon source (Partida-Martinez

437 *et al.*, 2007; Lackner *et al.*, 2011). The mycophagous bacterium *Collimonas fungivorans* is also
438 capable of metabolising glycerol (de Boer *et al.* 2004). In several *Burkholderia* species glycerol
439 induces production of antibiotics, including the antifungal pyrrolnitrin (Depoorter *et al.,* 2016).
440 True mycophagy can be difficult to demonstrate, as many bacteria may feed saprotrophically
441 on dead hyphae or passively on fungal exudates, whilst others may lyse hyphae for reasons other
442 than nutrition (Leveau and Preston 2008). Evidence for mycophagy in wood is limited. A strain of
443 *Streptomyces violaceusniger* isolated from bark inhibited fungi by endochitinase production, but only
444 after being 'conditioned' by exposure to chitin (a major component of fungal cell walls) (Shekhar *et*
445 *al.* 2006). This is an example of probable mycophagy where it has not yet been demonstrated that
446 the bacteria fulfil the criterion of using fungal material for growth. Bacteria closely related to the
447 mycophagous *Collimonas fungivorans* have been found on mycelial cords of the white-rot fungus *R.*
448 *bicolor* (Folman *et al.* 2008). *Collimonas* is able to use fungal hyphae as a sole carbon source, and
449 apparently uses the fungal exudate oxalic acid as a signal molecule to locate hyphae (Rudnick *et al.*
450 2015). The abundance of *Collimonas* cells has been observed to be higher in forest soils than in
451 either grassland or ex-arable soils (Höppener-Ogawa *et al.* 2007). The further observation that
452 collimonads can alter the fungal community composition in soil microcosms indicates their
453 potentially far-reaching importance (Höppener-Ogawa *et al.* 2009).
454 Conversely, there is also evidence for wood decay fungi feeding on bacteria; for example,
455 the physical disappearance of bacteria cells following fungal inoculation, and/or the appearance of
456 bacterial nutrients in mycelium under starvation conditions (Folman *et al.* 2008; Weißhaupt *et al.*
457 2011). Wood decay fungi have been observed to lyse bacterial colonies in culture, including
458 consumption of the bacteria that were decomposing dead nematodes (Tsuneda and Thorn, 1994).
459 Again, such associations could be mutualistic if the fungus, despite consuming some bacterial cells,
460 provided bacteria with nutrition and/or habitat. This seems to be the case for the soil saprotroph
461 *Morchella crassipes*, which showed reciprocal carbon exchange with *Pseudomonas putida* but lysed
462 some of the bacteria to feed the nutrient-intensive process of sclerotia formation: a situation that
463 has been described, controversially, as fungal farming of bacteria (Pion *et al.* 2013).
464
465 **7. Conclusions and future perspectives**
466 Despite the many gaps in our knowledge of wood-dwelling bacteria, a picture emerges of a
467 diverse and dynamic community, intimately linked to their physical habitat and the fungi they share
468 it with. The complexity of these potential interactions, and the challenges associated with wood as a
469 study system, mean that gaining a clear understanding of this environment will require the assembly
470 of many 'jigsaw pieces' of information. The ultimate goal in researching fungal-bacterial interactions

471 in wood is a functional understanding of how fungi and prokaryotes interact, in terms of outcome
472 for each partner, mechanisms of interaction, and effects on the process of wood breakdown. Before
473 such questions can be addressed, it is first necessary to ascertain which organisms are present and
474 their activities within the deadwood environment. Outstanding questions include:

475 - What is the origin of bacteria in wood; how and over what time-scale does colonisation occur?
476 - What are the major biotic and abiotic determinants of bacterial communities, and by what
477 mechanisms do these operate?
478 - Are interactions with fungi predominantly beneficial or antagonistic? Does one partner
479 consistently benefit at the expense of the other?
480 - How, and to what extent, do bacteria influence ecosystem-level flows of carbon and nitrogen in
481 the context of dead wood?

482

483 One of the major features that emerges with regard to fungal-prokaryote interactions is just
484 how hard it can be, in any given case, to distinguish the exact identity of the association. If fungal
485 growth increases in the presence of a bacterium (or *vice versa*), is it mutualism, commensalism or
486 parasitism? Do we truly see mycophagy rather than saprotrophy, endosymbiosis rather than
487 opportunism? When fungi alter the bacterial community, are they selecting for their specific
488 symbionts or simply unable to out-compete the remaining bacteria – or a mixture of the two? Such
489 associations can be deceptively hard to disentangle.

490 Happily, a suite of methods is coming of age that will hopefully assist in answering such
491 questions. Metagenomics gives a snapshot not just of the taxonomic identities of the community,
492 but also of their genomic potential (although it still has limited ability to marry the two).
493 Metatranscriptomics and metaproteomics offer insight into which of these potential abilities are
494 realised in a particular situation. Metabolomics explores the complete metabolic signature of a
495 microbial community under given conditions. At the same time, new culture methods offer hope for
496 isolating key community players, allowing physiological characterisation and manipulative
497 experiments (Ling *et al.,* 2015; Oberhardt *et al.,* 2015; Kielak *et al.* 2016a). Each of these techniques
498 comes with associated limitations, pitfalls and benefits, and it will require judicious use of these
499 approaches, combined with appropriate statistical and mathematical methods, to pick apart fungal-
500 prokaryote associations.

501 The overwhelming conclusion regarding the current state of knowledge is that, despite the
502 work already done on saproxylic bacteria and their interactions with fungi, we have still barely
503 scratched the surface. Results can be disparate or even contradictory depending on the
504 environmental conditions, identity of the organisms involved, or methods employed, frustrating the

505 chance of drawing together a robust theoretical framework. With so much ground still to cover, the
506 microbiota of dead wood remains a lively and under-explored area of ecological research, but one
507 that is likely to be highly rewarding and will be furthered by deployment of modern genomic and
508 post-genomic approaches.

509

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513

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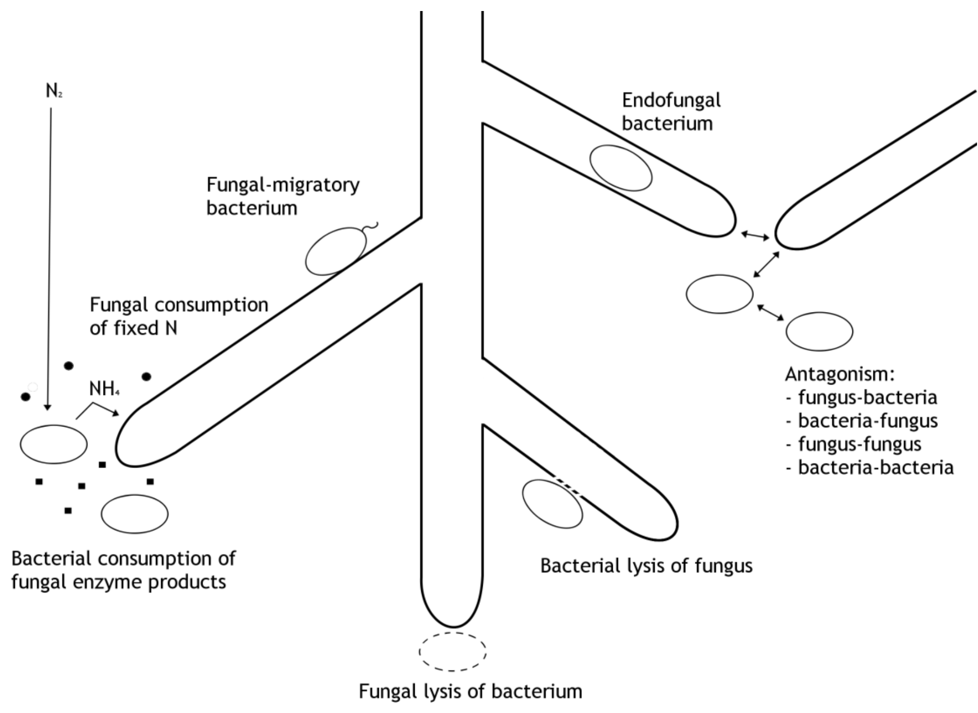
517

518 **Fig. 1** Potential fungus-bacteria interactions occurring in wood; not all have so far been observed.
519 The interactions may be obligate (*e.g.* some endosymbioses) or facultative (*e.g.* predation). In many
520 cases it is not clear whether a given interaction is beneficial to one, both or neither of the partners.
521 The outcomes indicated may occur via more than one route: for example, fungi could receive fixed N
522 by mutualism with diazotrophs, by lysing the bacteria, or by predating on nematodes *etc*. that had in
523 turn predated on bacteria. A) Whole-organism level interactions. B) Molecular mechanisms of
524 interaction; see main text for details of each.



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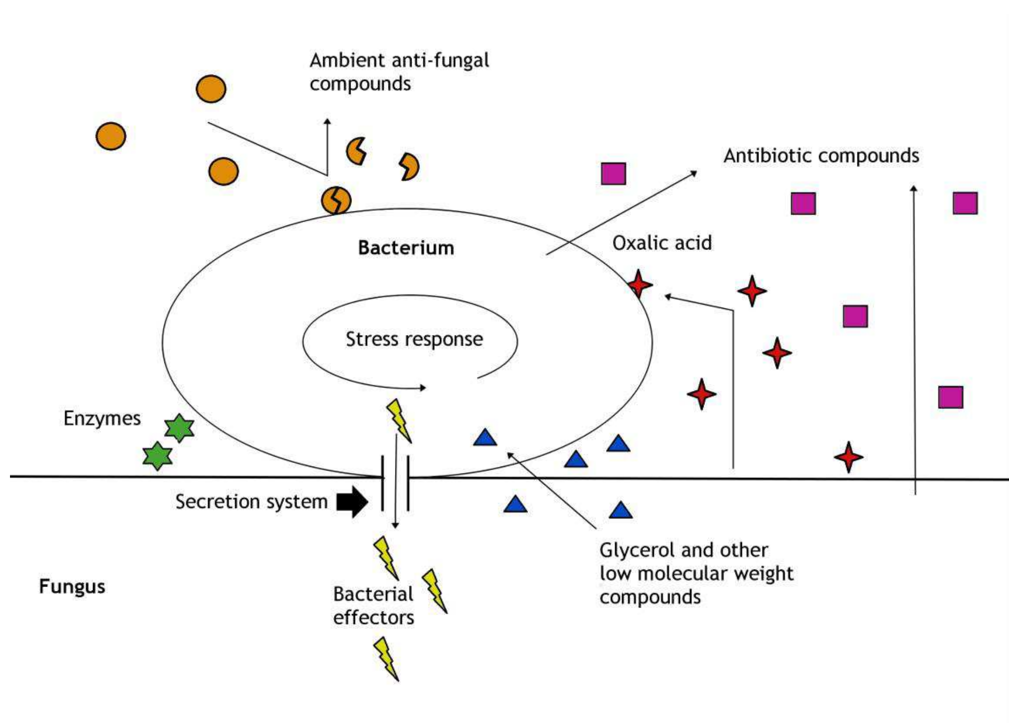

526

527 **Table 1** Marker gene-based studies of bacteria in wood, and the major phyla reported in each.

Study	**Wood species**	**State of decay**	**Location**	**Marker gene**	**Method**	**Major bacterial phyla**
Folman *et al.*, 2008	*Fagus sylvatica*	7-10 months' colonisation	Lab	16S rRNA	DGGE and sequencing	Betaproteobacteria; Gammaproteobacteria; Acidobacteria; Bacilli
Hervé *et al.*, 2014	*Fagus sylvatica*	Sawdust, 3-5 months' colonisation	Lab	16S rRNA	NGS amplicons	Alpha-, beta- and gamma-proteobacteria
Hoppe *et al.*, 2014	*Fagus sylvatica, Picea abies*	Not reported	Temperate woodland (Germany)	*nifH*	NGS amplicons	Alphaproteobacteria
Hoppe *et al.*, 2015	*Fagus sylvatica, Picea abies*	Kahl (2012) decay class 1-4 (3-27 years)	Temperate woodland (Germany)	16S rRNA	NGS amplicons	Alphaproteobacteria; Actinobacteria; Acidobacteria
Kielak *et al.*, 2016	*Pinus sylvestris*	Classified as early, middle or late decay based on density	Temperate woodland (The Netherlands)	16S rRNA	NGS amplicons	Alphaproteobacteria; Acidobacteria
Rinta-Kanto *et al.*, 2016	*Picea abies*	Range from '"recently dead" to "almost decomposed" (Mäkinen *et al.*, 2006)	Boreal forest (Finland)	16S rRNA	NGS amplicons	Alphaproteobacteria; Acidobacteria

Sun *et al.*, 2014	*Picea abies*	2-4 months' colonisation	Boreal forest (Finland)	16S rRNA	NGS amplicons	Proteobacteria; Bacteroidetes; Acidobacteria; Actinobacteria
Valásková *et al.*, 2009	*Betula sp., Fagus sylvatica, Quercus robur, Pinus sylvestris*	Reported only as "high"	Temperate woodland (The Netherlands)	16S rRNA	Clone library	Alpha-, beta- and gamma-proteobacteria; Acidobacteria; Firmicutes
Zhang *et al.*, 2008	*Keteleeria evelyniana*	Not reported	Not reported; sub-tropical?	16S rRNA	Clone library	Alpha-, beta-, gamma- and delta-proteobacteria; Actinobacteria; Acidobacteria

528

529

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