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# Bacteria in decomposing wood and their interactions with wood-decay fungi

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**Running head**: Bacteria in wood

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**Single-sentence summary:**

Bacteria and fungi both live in wood, but not much is known about how they interact - this article reviews what is already known about them and what is yet to be found out.

## Abstract

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

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

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

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

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

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

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

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

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

It has been suggested (Greaves 1971; Frey-Klett *et al.* 2011) that bacterial activity in the earliest stages of decay renders the wood more accessible to fungi. Whilst bacteria may detoxify certain compounds inhibitory to fungi, notably in treated wood (Greaves 1971; Clausen 1996), experiments using fresh, sterile wood show that fungi are competent wood decayers in the absence of bacterial conditioning (*e.g.* Hiscox *et al.* 2010). Similarly, although it is sometimes suggested that bacteria are the earliest colonists of dead wood, there is little evidence on whether or not that is the case (van der Wal *et al.* 2007). Given that wood decay fungal propagules are latently present in functional wood (Parfitt *et al.* 2010), for this to hold true bacteria would likewise have to be latently present, and/or colonise wood very rapidly once conditions were favourable.

## 3. Bacterial colonisation of wood

The provenance of saproxylic bacteria communities and their means of colonisation are largely unknown. Bacteria have limited motility and are unable to cross air voids, meaning that colonisation is likely to be slow without some means of carriage into the wood. Essentially, bacteria in a woody resource have four possible points of origin: the soil; the air; the wood itself; and fungi or other organisms colonising the wood. The relative importance of these sources is likely to vary under different conditions; the bacterial community in attached dead branches is probably very different from that in wood on the forest floor.

### *3.1. Edaphic and atmospheric sources of bacteria*

Soil represents a rich source of potential colonists for wood in ground contact, and the underlying assumption of many studies is that it is the main point of origin for bacteria in wood (*e.g.* van der Wal *et al.*, 2007; Folman *et al.*, 2008; Hervé *et al.*, 2014). These show that a subset of soil bacteria are competent to colonise wood, but do not indicate to what extent this occurs under natural conditions. Underlying soil type was a good predictor of bacterial assemblage in experimental wood blocks (Sun *et al.* 2014), which suggests either an edaphic origin of saproxylic bacteria, or an indirect influence of soil: for example, via an altered fungal community.

Movement via airborne spores and other propagules is a major means of bacterial dispersal, which has led to the widespread view that all bacteria are, or have the potential to be, ubiquitous: a view that has since been challenged (Green and Bohannan 2006). Nevertheless, the air could represent another means for bacteria to arrive at decomposing wood. In woodlands, rainfall creates bio-aerosols of bacteria and fungal spores, presumably contributing to their dispersal (Huffman *et al.* 2013). An experiment on bacteria in woodland pools indicated that the community composition was not dispersal limited, suggesting that airborne dispersal is effective at least across local scales (Bell 2010).

### *3.2 Bacterial endophytes*

As mentioned above, wood decay fungi exist in living trees as latent propagules which spread as mycelia when the branch or trunk is no longer functional in water conduction (Parfitt *et al.* 2010). Scanning electron microscopy indicates that at least some, if not all, living trees also host abundant bacterial endophytes in their wood (Jeremic *et al.* 2004). However, there is very little literature on bacterial endophytes in wood, and the identity of these endophytes is as yet uncertain.

### *3.3. Bacteria co-colonisation with other organisms*

*Burkholderia terrae* BS001 has been shown to migrate across soil with wood-decay fungi, including the aggregated mycelial cords of *Phanerochaete velutina* (Nazir *et al.* 2014). This demonstrates active bacterial movement, as the apical growth of fungal hyphae rules out the possibility of passive carriage. *P. velutina* had a lower bacterial 'carrying capacity' than several other fungi, suggesting that cords may be less conducive to migration than are fine hyphae. The presence of *B. terrae* BS001 can also facilitate the movement of other bacteria which would otherwise not be competent to migrate along hyphae (Warmink *et al.* 2011). Independent hyphal migration has also been observed for several other members of the Burkholderiales and some strains of *Dyella japonica* (Warmink and van Elsas 2009; Nazir *et al.* 2012). It has been suggested that other fungus-associated bacteria such as *Collimonas* may share this migratory ability (Leveau *et al.* 2010). Such behaviour raises the possibility that when fungi colonise a resource, they bring a suite of bacterial travelling companions. In this manner saproxylic bacteria could use foraging fungal mycelium as a conduit to new resources.

Other saproxylic organisms, particularly invertebrates, may transfer bacteria from one woody resource to another. Bark beetles can carry bacteria phoretically (Mercado *et al.* 2014), and introduce them into trees during the construction of galleries. This has also been suggested as a source of nitrogen-fixing bacteria in wood (Griffiths *et al.* 1993).

## 4. Wetwood

The presence of bacteria in living trees is most obvious in bacterial wetwood. Wetwood, also known as wet-heartwood or watermark, is a condition where the heartwood of a living tree becomes saturated and discoloured. This change may be accompanied by blocked vessels, gas build up, and the presence of a fetid liquid. The term refers to a suite of phenomena, probably with multiple causal agents but broadly similar manifestations, making it hard to disentangle the exact role bacteria play. Whilst in some tree species (*e.g. Salix sachalinensis*) wetwood is a serious disease, spreading to sapwood and ultimately killing the tree (Sakamoto and Sano 2000), in others it seems to be an almost-ubiquitous part of maturation (*e.g. Ulmus americana*) (Murdoch and Campana 1983). Wetwood is often attributed to bacterial activity, but there is no clear evidence whether this is true for this latter form, where there are no apparent ill-effects to the tree: it could equally be caused by physical processes, and bacteria secondarily colonise and modify the habitat.

Wetwood is frequently associated with the presence of anaerobic, methanogenic, pectinolytic prokaryotes, which could account for many of the observed symptoms (Schink *et al.* 1981a). Although wetwood can form around the site of fungal infections, within the wetwood itself fungi are likely to be excluded by low $O_2$ concentrations, large amounts of organic acids and inhibitory metabolites (Worrall and Parmeter 1983). If fungi are indeed absent from wetwood, it represents an almost unique wood habitat in this respect.

## 5. Bacterial metabolism in wood

### *5.1 Bacterial nitrogen fixation in wood*

It has long been recognised that dead wood plays host to nitrogen-fixing (diazotrophic) bacteria, which provide an independent source of nitrogen to the system (Cornaby and Waide 1973; Sharp and Millbank 1973). Many studies have focussed on coniferous forests of the Pacific Northwest, and used acetylene reduction as a measure of nitrogenase activity (reviewed by Son, 2001). Interpretation and comparison of these results requires caution, as the exact methodology used varies; acetylene reduction has been criticised for its sensitivity to experimental parameters (Giller 1987), although the effects may not be as serious as suggested (Son 2001). Additionally, the conversion factor used to calculate N-fixation from acetylene reduction is not consistent (Son 2001; Brunner and Kimmins 2003), and there is evidence to suggest that the true conversion rate may vary between sample types (Hicks *et al.*, 2003b). In light of these difficulties, acetylene reduction should perhaps be regarded as semi-quantitative, suitable for comparison within but not between studies.

The picture that emerges of N-fixation in dead wood suggests a highly dynamic process, influenced by many factors (Hicks *et al.*, 2003a). Wood water content is consistently positively correlated with N-fixation (Larsen *et al.*, 1978; Jurgensen *et al.*, 1984; Brunner & Kimmins, 2003; Hicks *et al.*, 2003a), possibly because it creates better microhabitats for the anaerobic/microaerophilic diazotrophs (Spano *et al.*, 1982; Hicks *et al.*, 2003a). The optimum temperature for fixation is 30°C (Hicks *et al.,* 2003a), which may explain higher N-fixing activity in summer than in winter (Jurgensen *et al.* 1984; Sollins *et al.* 1987). The requirements for high temperature and high moisture suggest an interplay of factors that determine seasonal fixation patterns (Hicks *et al.*, 2003a). The effect of tree species on fixation is unclear, with some authors reporting significant differences between species (Jurgensen *et al.* 1989; Griffiths *et al.* 1993; Hoppe *et al.* 2014), and others reporting none (Sollins *et al.*, 1987; Hicks *et al.*, 2003b). Nitrogen fixation in forest ecosystems is likely to be limited by the availability of molybdenum, which is necessary for nitrogenase synthesis, and possibly also by other micronutrients (Silvester, 1989).

Nitrogen fixation increases as decay proceeds (Larsen *et al.* 1978; Spano *et al.* 1982; Jurgensen *et al.* 1984), although two studies found that fixation peaked before dropping off in the most advanced stage of decay, perhaps because the latter studies included more decayed wood than the former (Hicks *et al.* 2003b). An experiment using a finer resolution time-series over 6 years revealed considerable variation within the overall increase in N fixation (Griffiths *et al.,* 1993). In the very early stages of decay, N fixation will be limited by the rate at which diazotrophs can colonise the resource. If nitrogen-fixing bacteria rely on carbon from fungal activity (see section 6.1), they may also experience a growth lag whilst fungi colonise and start to decompose the wood. Diazotrophic activity is higher in sapwood than in heartwood, and higher again in bark; low fixation has been recorded in heartwood, possibly because it is the fraction most refractory to decomposition (Griffiths *et al.*, 1993; Brunner & Kimmins, 2003; Hicks *et al.*, 2003b) and often contains inhibitory extractives.

The identity of the saproxylic diazotrophs is underexplored, but *Clostridium* and *Klebsiella* have been cultured (Spano *et al.* 1982). A survey of *nifH* nitrogenase genes in decaying wood indicated Rhizobiales was the predominant identifiable order, with Rhodocyclales, Pseudomonadales, Rhodospirillales, Sphingomonadales and Burkholderiales also present; however, most of the saproxylic *nifH* variants could not be matched to known bacteria (Hoppe *et al.* 2014). These bacterial orders have also been identified previously in 16S rRNA gene surveys of decaying wood (Folman *et al.* 2008; Valášková *et al.* 2009).

### *5.2 Bacterial wood decomposition*

Bacteria are well known to be capable of cellulose decomposition, although their contribution to overall wood decay is restricted by small size and limited movement (Greaves 1971; Clausen 1996) unlike fungi with mycelial growth (de Boer *et al.* 2005). Various bacteria from woodland soil possess enzymes involved in the breakdown of cellulose/ cellulose products, including members of the Acidobacteria, a common phylum in dead wood (Lladó *et al.* 2016; Table 1). Some cellulolytic bacteria apparently use new, uncharacterised means of metabolising cellulose without expressing the usual enzymes (López-Mondéjar *et al.* 2016). Certain bacteria in wood break down pectin (Schink *et al.* 1981b; Clausen 1996), although in some cases this may be a strategy to access cellulose (Lynd *et al.* 2002). Evidence has emerged of bacteria with lignin-decomposing abilities, albeit to a lesser extent than fungi (Bugg *et al.* 2011; Brown and Chang 2014). An Actinobacterium, *Amycolatopsis* sp. 75iv2, can use lignin as a sole carbon source (Brown and Chang 2014). Previously-unknown ligninolytic bacterial enzyme systems have been found, unlike those deployed by fungi, and environmental metagenomics may reveal more (Brown and Chang 2014). Lignin-model compounds are frequently used to screen for activity, and whilst they may not be fully representative, there is also evidence of bacterial depolymerisation of natural lignin (Salvachúa *et al.* 2015).

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

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

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

Bacterial wood decomposition is often described based on the physical patterns produced in the wood ultrastructure, and is can be grouped into four main types (reviewed Greaves, 1971; Clausen, 1996; Kim & Singh, 2000). These categories are based on the morphology of wood substratum following decay, rather than the taxonomic affiliations of the bacteria involved. ‘Tunnelling’ bacteria decay a convoluted path inside the cell walls (Kim and Singh 2000), which they may enter via pit chambers (Greaves 1969). They can act on all components of the cell wall and may be able to degrade/modify lignin, at least to some extent (Kim and Singh 2000). ‘Erosion’ bacteria create depressions in the wall from inside the lumen, which follow the path of cellulose microfibrils; they rarely affect the middle lamella, and probably lack the ability to degrade lignin (Greaves 1969; Kim and Singh 2000). ‘Pitting’ bacteria produce small, shallow indentations (Greaves 1971); the term is somewhat confusing, given that bacteria often are associated with pits connecting cells. ‘Cavitation’ bacteria cause diamond-shaped cavities inside cell walls, possibly involving the production of diffusible enzymes (Kim and Singh 2000). Bacteria are often associated with pits between wood cells, and decomposition activity in these areas can greatly increase the permeability of the wood (Greaves 1969).

## 6. Bacterial-fungal community interactions

### *6.1 Community competition and co-operation*

It is not difficult to envisage why fungal-bacterial co-existence in wood could lead to conflict. Both may compete for the same substrates; bacteria may remove the products of fungal extracellular enzyme decomposition (effectively microbial kleptoparasitism); and either group may regard the other as a food resource. Certainly wood-decay fungi have an arsenal of competitive strategies capable of deployment (Boddy 2000). Bacteria described as closely associated with *Phanerochaete chrysosporium* were all proficient at utilising lignin breakdown products *in vitro*, supporting the idea that they gained nutrition from fungal activity (Seigle-Murandi *et al.* 1996).

Studies show that the introduction of *Hypholoma fasciculare* can alter the abundance and community composition of bacteria within wood (Folman *et al.*, 2008; de Boer *et al.*, 2010), and rapid pH change has been suggested as a possible mechanism (de Boer *et al.* 2010). Whether this is representative of a natural situation is uncertain: bacteria were very abundant in more decayed *H. fasciculare*-colonised wood in a field scenario, which could be due to bacterial recovery over time, or the proliferation of fungus-tolerant bacteria (Valášková *et al.* 2009). Inoculation with *Phlebiopsis gigantea* lowered bacterial species richness in stumps after 12 months, although the effect had disappeared by 6 years post-inoculation (Sun *et al.* 2013). It is possible that direct fungal-bacterial competition occurs in wood similar to that observed in soil, where experimental inhibition of bacteria results in accelerated fungal growth indicative of competitive release (Rousk *et al.*, 2008, 2010). Intriguingly, the competitive outcome appears to depend on the ambient pH: fungi prevail at low pH, bacteria at higher pH (Rousk *et al.* 2010). This is salient in light of the major pH modification – typically lowering – that fungi effect in wood (de Boer *et al.* 2010).

There is much *in vitro* evidence for antagonism amongst saproxylic micro-organisms, both of fungi against bacteria (Janes *et al.* 2006; Popova *et al.* 2009) and of bacteria against fungi (Murray and Woodward 2003; de Boer *et al.* 2007; Caldeira *et al.* 2008; Boaisha 2012). This is not necessarily evidence for antagonism *in situ*, given that the effect can depend on the culture medium (Murray and Woodward 2003; Boaisha 2012). In addition, soil bacteria with little or no anti-fungal activity on their own (*Pedobacter* sp. and *Pseudomonas* sp.) can show considerable fungal inhibition when in combination, either as a collaborative effort or a by-product of antagonism towards each other (de Boer *et al.* 2007).

Conclusive evidence of fungal-bacterial mutualism requires demonstration of a benefit to both partners. Although this has been demonstrated in a variety of habitats (Frey-Klett *et al.* 2011), there is a shortage of clear examples in terrestrial deadwood. There are reports of basidiomycetes gaining more biomass and decaying wood faster in the presence of yeasts and nitrogen-fixing bacteria than in their absence (Blanchette and Shaw 1978). Moreover, SEM revealed close physical association between the mycelial fungi, yeasts and bacteria (Blanchette and Shaw 1978). *H. fasciculare* and *Resinicium bicolor* decompose wood significantly faster in the presence of bacteria than alone; *Heterobasidion annosum* displayed the same effect only if bacteria were added after the fungus had become established (Murray and Woodward 2003). However, in other instances, bacteria had no effect on *H. fasciculare* decomposition (Weißhaupt *et al.* 2013). Such variability could be influenced by bacterial community composition, fungal intra-specific variation, or environmental conditions. Increased decomposition may be attributable to bacterial nutrient provision (*e.g.* vitamin production (Ghignone *et al.* 2012) or N-fixation), or up-regulation of fungal enzymes due to the removal of breakdown products (Murray and Woodward 2003; de Boer *et al.* 2005). Whilst the latter scenario would represent facilitation in ecosystem process terms, the benefit to the fungus is questionable, depending on whether faster decomposition translates to increased fungal growth, or simply to decreased efficiency due to bacterial consumption of breakdown products. This could be particularly disadvantageous to fungi with an ecological strategy that involves slowly decomposing wood over a long period, as with some xylariacious ascomycetes (Boddy *et al.* 1989).

There are examples of bacterial-fungal interactions that benefit at least one party with (currently) no evidence of harm to the other, suggesting at least a commensal association. There is also *in vitro* evidence for growth enhancement, which should be regarded with the same caveats as *in vitro* antagonism. For example, a bacterium of the *Burkholderia cepacia* complex, isolated from a *Pleurotus ostreatus* fruit body, showed increased growth in the presence of *P. ostreatus* mycelium (Yara *et al.* 2006). A *Curtobacterium* sp. from dead wood promoted growth of *Stereum* sp. – although it was the only one out of 24 culturable strains to do so (Kamei *et al.* 2012). Notably, *Streptomyces* from woodland soil showed negative or neutral influences on mycorrhizal and pathogenic fungi, yet all consistently and markedly promoted growth of the white-rot fungus *Phanerochaete chrysosporium*; it may be salient that none of these *Streptomyces* strains showed ligninolytic activity themselves (Bontemps *et al.* 2013).

The fungal-migratory bacterium *Burkholderia terrae* BS001 (see section 3.3) has been demonstrated to protect a (non-wood decay) fungus from inhibition by the fungicide cycloheximide or metabolites from the antagonistic bacterium *Pseudomonas fluorescens* strain CHA0 (Nazir *et al.* 2014). This raises the possibility of a true mutualism, whereby *B. terrae* BS001 gains access to resources and in return affords protection to its fungal host. The level of protection depended on the identity of the fungus, and, for reasons unknown, the timing of bacterial arrival (Nazir *et al.* 2014).

### *6.2 Bacterial endosymbiosis and intimate hyphal associations*

Bacteria co-exist endosymbiotically with arbuscular mycorrhizal fungi (Bonfante and Anca 2009), but such an association has yet to be conclusively demonstrated for wood-decay fungi. It is likely that bacteria do occur within the hyphae of wood decay fungi, as they have also been found inside ectomycorrhizal hyphae (Bertaux *et al.* 2005), plant-pathogenic fungi (Partida-Martinez and Hertweck 2005), a range of endophytic fungi (Hoffman and Arnold 2010) and the soil saprotroph *Mortierella elongata* (Sato *et al.* 2010). Intrahyphal existence does not necessarily indicate true endosymbiosis, which implies active interaction between living cells (Lumini *et al.* 2006). Rather, bacteria may enter compromised or senescent hyphae opportunistically and without vertical transmission (de Boer *et al.* 2005; Lumini *et al.* 2006). This distinction is not always made clearly in the literature on fungal 'endosymbionts', nor is it always recognised that bacteria may associate intimately but extracellularly with mycelium.

Where true endosymbiosis does occur, the extent of its implications are illustrated by the well-characterised association between the plant-pathogenic fungus *Rhizopus microsporus* and its bacterial endosymbiont, *Burkholderia rhizoxinica* (Partida-Martinez and Hertweck 2005). The toxin rhizoxin forms a key part of *R. microsporus* pathogenicity, yet is synthesised not by the fungus but by the bacteria within it. Vertical transmission of the bacteria is guaranteed, as *R. microsporus* has lost the ability to sporulate in the absence of *B. rhizoxinica* (Partida-Martinez *et al.*, 2007). *B. rhizoxinica* is also highly competent to colonise *R. microsporus* hyphae from the outside by localised chitinase activity that does not cause fungal lysis (Moebius *et al.* 2014). Colonisation relies on both the type II and type III secretion systems (Lackner *et al.* 2011; Moebius *et al.* 2014). Pertinently, these secretion systems have been implicated in other fungal-bacterial interactions, such as mycorrhiza formation, described as the 'helper bacteria effect' (Cusano *et al.* 2011); mycophagy (Mela *et al.* 2012); co-migration (Warmink & van Elsas, 2009; Nazir *et al.*, 2012, 2013, 2014); and an undefined bacteria-fungus association (Warmink and van Elsas 2008).

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

### *6.3 Mycophagy and predation*

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

An increasing body of evidence suggests that glycerol is a favoured carbon source for many mycophagous bacteria, although so far none of this evidence is derived directly from wood. In liquid culture *Burkholderia terrae* strain BS001 stimulates glycerol release by the fungus *Lyophyllum* sp. strain Karsten, and the glycerol is apparently consumed by the bacteria (Nazir *et al.* 2013). Whilst this ability has not yet been tested in an ecologically realistic situation, the *B. terrae* BS001 genome encodes glycerol transporters that are unique among the *Burkholderia*, possibly linked to its fungus-associated lifestyle (Haq *et al.* 2014). *B. rhizoxinica*, the *Rhizopus* endosymbiont, likewise possesses genes involved in glycerol metabolism and can utilise glycerol as a carbon source (Partida-Martinez *et al.*, 2007; Lackner *et al.*, 2011). The mycophagous bacterium *Collimonas fungivorans* is also capable of metabolising glycerol (de Boer *et al.* 2004). In several *Burkholderia* species glycerol induces production of antibiotics, including the antifungal pyrrolnitrin (Depoorter *et al.,* 2016).

True mycophagy can be difficult to demonstrate, as many bacteria may feed saprotrophically on dead hyphae or passively on fungal exudates, whilst others may lyse hyphae for reasons other than nutrition (Leveau and Preston 2008). Evidence for mycophagy in wood is limited. A strain of *Streptomyces violaceusniger* isolated from bark inhibited fungi by endochitinase production, but only after being 'conditioned' by exposure to chitin (a major component of fungal cell walls) (Shekhar *et al.* 2006). This is an example of probable mycophagy where it has not yet been demonstrated that the bacteria fulfil the criterion of using fungal material for growth. Bacteria closely related to the mycophagous *Collimonas fungivorans* have been found on mycelial cords of the white-rot fungus *R. bicolor* (Folman *et al.* 2008). *Collimonas* is able to use fungal hyphae as a sole carbon source, and apparently uses the fungal exudate oxalic acid as a signal molecule to locate hyphae (Rudnick *et al.* 2015). The abundance of *Collimonas* cells has been observed to be higher in forest soils than in either grassland or ex-arable soils (Höppener-Ogawa *et al.* 2007). The further observation that collimonads can alter the fungal community composition in soil microcosms indicates their potentially far-reaching importance (Höppener-Ogawa *et al.* 2009).

Conversely, there is also evidence for wood decay fungi feeding on bacteria; for example, the physical disappearance of bacteria cells following fungal inoculation, and/or the appearance of bacterial nutrients in mycelium under starvation conditions (Folman *et al.* 2008; Weißhaupt *et al.* 2011). Wood decay fungi have been observed to lyse bacterial colonies in culture, including consumption of the bacteria that were decomposing dead nematodes (Tsuneda and Thorn, 1994). Again, such associations could be mutualistic if the fungus, despite consuming some bacterial cells, provided bacteria with nutrition and/or habitat. This seems to be the case for the soil saprotroph *Morchella crassipes*, which showed reciprocal carbon exchange with *Pseudomonas putida* but lysed some of the bacteria to feed the nutrient-intensive process of sclerotia formation: a situation that has been described, controversially, as fungal farming of bacteria (Pion *et al.* 2013).

## 7. Conclusions and future perspectives

Despite the many gaps in our knowledge of wood-dwelling bacteria, a picture emerges of a diverse and dynamic community, intimately linked to their physical habitat and the fungi they share it with. The complexity of these potential interactions, and the challenges associated with wood as a study system, mean that gaining a clear understanding of this environment will require the assembly of many 'jigsaw pieces' of information. The ultimate goal in researching fungal-bacterial interactions in wood is a functional understanding of how fungi and prokaryotes interact, in terms of outcome for each partner, mechanisms of interaction, and effects on the process of wood breakdown. Before such questions can be addressed, it is first necessary to ascertain which organisms are present and their activities within the deadwood environment. Outstanding questions include:

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

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

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

The overwhelming conclusion regarding the current state of knowledge is that, despite the work already done on saproxylic bacteria and their interactions with fungi, we have still barely scratched the surface. Results can be disparate or even contradictory depending on the environmental conditions, identity of the organisms involved, or methods employed, frustrating the chance of drawing together a robust theoretical framework. With so much ground still to cover, the microbiota of dead wood remains a lively and under-explored area of ecological research, but one that is likely to be highly rewarding and will be furthered by deployment of modern genomic and post-genomic approaches.

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

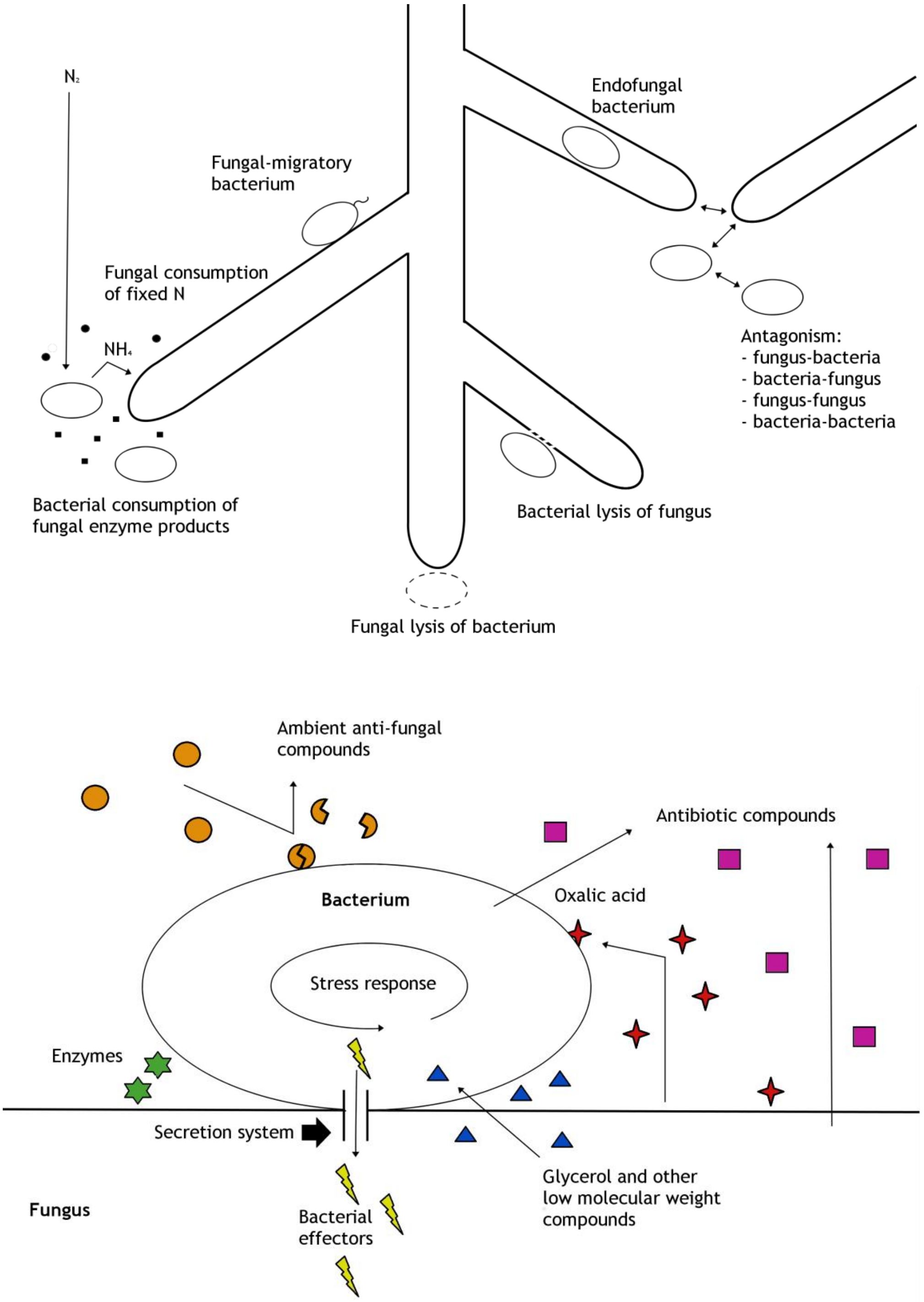

**Table 1** Marker gene-based studies of bacteria in wood, and the major phyla reported in each.

| **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 |

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