

MINIREVIEW

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

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One 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.

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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 dead wood 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 minireview 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.

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

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. dead wood-inhabiting) organisms (Stokland, Siitonen and Jonsson 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, Yang and Tu 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 en-

vironment. 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 surrounds and interacts with a fungus effectively constitutes its microbiome, and as such, they must be considered together. The aim of this minireview 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.

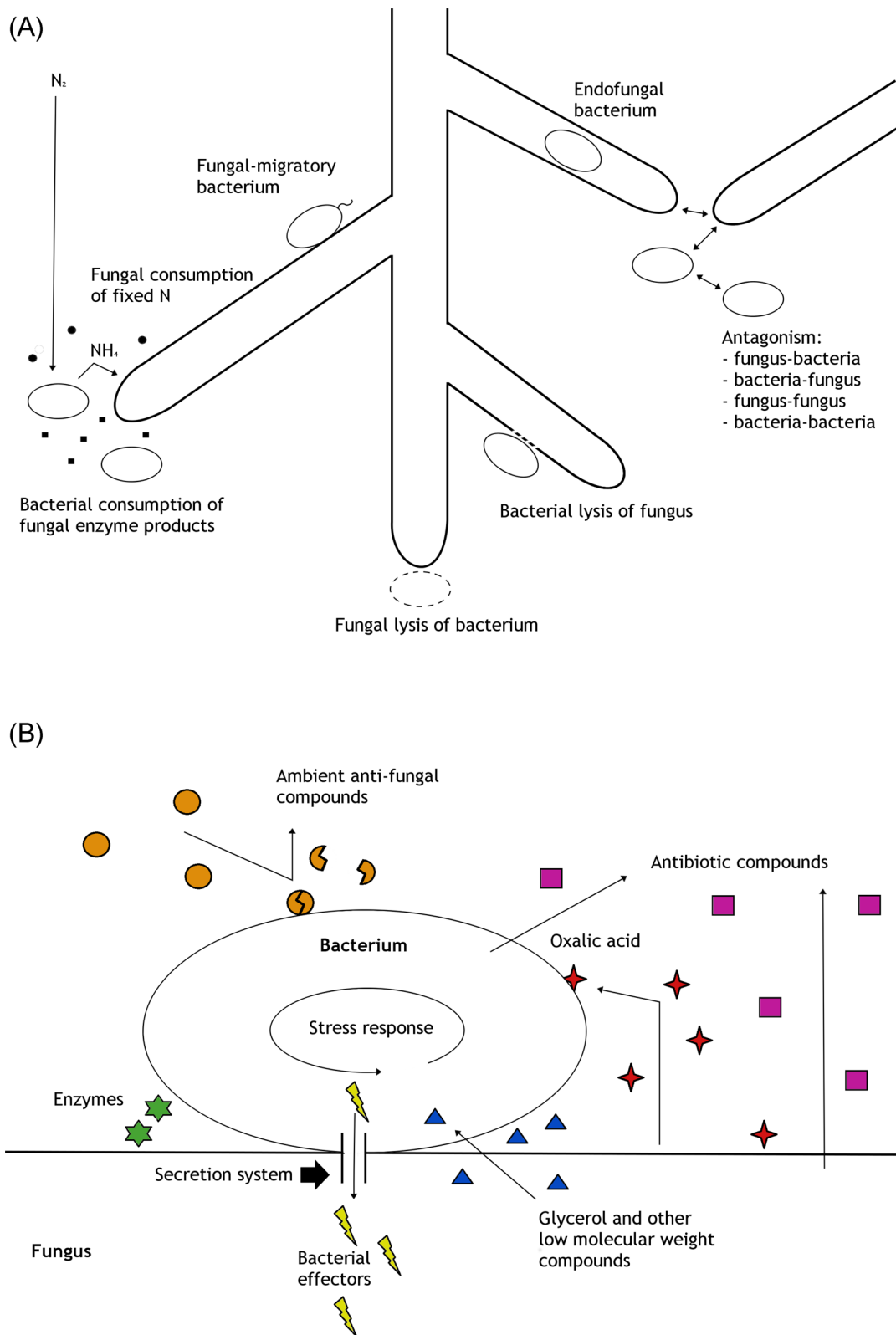


Figure 1. Potential fungus–bacteria interactions occurring in wood; not all have so far been observed. The interactions may be obligate (e.g. some endosymbioses) or facultative (e.g. predation). In many cases it is not clear whether a given interaction is beneficial to one, both or neither of the partners. 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 preying on nematodes, etc. that had in turn preyed 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)	<i>Fagus sylvatica</i>	7–10 months' colonisation	Lab	16S rRNA	DGGE and sequencing	Betaproteobacteria; Gammaproteobacteria; Acidobacteria; Bacilli
Hervé et al. (2014)	<i>Fagus sylvatica</i>	Sawdust, 3–5 months' colonisation	Lab	16S rRNA	NGS amplicons	Alpha-, beta- and gamma-proteobacteria
Hoppe et al. (2014)	<i>Fagus sylvatica</i> , <i>Picea abies</i>	Not reported	Temperate woodland (Germany)	<i>nifH</i>	NGS amplicons	Alphaproteobacteria
Hoppe et al. (2015)	<i>Fagus sylvatica</i> , <i>Picea abies</i>	Kahl et al. (2012) decay class 1–4 (3–27 years)	Temperate woodland (Germany)	16S rRNA	NGS amplicons	Alphaproteobacteria; Actinobacteria; Acidobacteria
Kielak et al. (2016b)	<i>Pinus sylvestris</i>	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)	<i>Picea abies</i>	Range from 'recently dead' to 'almost decomposed' (Mäkinen et al. 2006)	Boreal forest (Finland)	16S rRNA	NGS amplicons	Alphaproteobacteria; Acidobacteria
Sun et al. (2014)	<i>Picea abies</i>	2–4 months' colonisation	Boreal forest (Finland)	16S rRNA	NGS amplicons	Proteobacteria; Bacteroidetes; Acidobacteria; Actinobacteria
Valášková et al. (2009)	<i>Betula sp.</i> , <i>Fagus sylvatica</i> , <i>Quercus robur</i> , <i>Pinus sylvestris</i>	Reported only as 'high'	Temperate woodland (The Netherlands)	16S rRNA	Clone library	Alpha-, beta- and gamma-proteobacteria; Acidobacteria; Firmicutes
Zhang, Yang and Tu (2008)	<i>Keteleeria evelyniana</i>	Not reported	Not reported; possibly sub-tropical?	16S rRNA	Clone library	Alpha-, beta-, gamma- and delta-proteobacteria; Actinobacteria; Acidobacteria

DIVERSITY OF BACTERIAL COMMUNITIES IN WOOD

Information on bacterial communities in decomposing wood is surprisingly scarce, 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, Bardsley and Gibbon 1987). In contrast, whilst there are studies that have looked at the culturable fraction of saproxylic bacteria (e.g. Murray and Woodward 2007; van der Wal et al. 2007), a large and variable proportion is 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, Tazetdinova and van Elsas 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 open 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 intrasite heterogeneity (Sun et al. 2014). The bacterial community varies depending 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, Yang and Tu 2008); nonetheless, bacteria may be more abundant in sapwood (Jeremic, Cooper and Srinivasan 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 (Rinta-Kanto et al. 2016; Kielak et al. 2016b). 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

fungus 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, Šnajdr and Baldrian 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, van Veen and de Boer 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. 2015; Valášková et al. 2009; Leveau, Uroz and de Boer 2010; Sato et al. 2010; Hervé et al. 2014; Prewitt et al. 2014; Sun et al. 2014), a widespread and versatile family of bacteria that 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, specialised associations between fungi and the genus *Burkholderia* involving collabora-

tive 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, Tazetdinova and van Elsas 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 *Burkholderia 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.

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.

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 bioaerosols of bacterial 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).

Bacterial endophytes

As mentioned above, wood-decay fungi exist in living trees as latent propagules that 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, Cooper and Srinivasan 2004). However, there is very little literature on bacterial endophytes in wood, and the identity of these endophytes is as yet uncertain.

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, Tazetdinova and van Elsas 2014). This demonstrates active bacterial movement, as the apical growth of fungal hyphae rules out the possibility of passive carriage. *Phanerochaete 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, Uroz and de Boer 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).

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, Ward and Zeikus 1981b). Although wetwood can form around the site of fungal infections, within the wetwood itself fungi are likely to be excluded by low O₂ 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.

BACTERIAL METABOLISM IN WOOD

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 require 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, Harmon and Myrold 2003). 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 from N-fixation in dead wood suggests a highly dynamic process, influenced by many factors (Hicks, Harmon and Griffiths 2003). Wood water content is consistently positively correlated with N-fixation (Larsen, Jurgensen and Harvey 1978; Jurgensen et al. 1984; Brunner and Kimmins 2003; Hicks, Harmon and Griffiths 2003), possibly because it creates better microhabitats for the anaerobic/microaerophilic diazotrophs (Spano et al. 1982; Hicks, Harmon and Griffiths 2003). The optimum temperature for fixation is 30°C (Hicks, Harmon and Griffiths 2003), 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, Harmon and Griffiths 2003). 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, Harmon and Myrold 2003). 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, Jurgensen and Harvey 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, Harmon and Myrold 2003). 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 'Community competition and co-operation'), 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 and Kimmins 2003; Hicks, Harmon and Myrold 2003) 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 that 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).

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, Ward and Zeikus 1981a; 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 (Björödal 2012).

Bacterial wood decomposition is often described based on the physical patterns produced in the wood ultrastructure, and can be grouped into four main types (reviewed Greaves 1971; Clausen 1996; Kim and 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).

BACTERIAL-FUNGAL COMMUNITY INTERACTIONS

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; Rousk, Brookes and Bååth 2010). Intriguingly, the competitive outcome appears to depend on the ambient pH: fungi prevail at low pH, bacteria at higher pH (Rousk, Brookes and Bååth 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 microorganisms, both of fungi against bacteria (Janes, Umek and Kreft 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 antifungal 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 dead wood. 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). *Hypholoma 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 intraspecific 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 upregulation 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 xylariaceous ascomycetes (Boddy, Owens and Chapela 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 *B. terrae* BS001 (see section ‘Bacteria co-colonisation with other organisms’) 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, Tazetdinova and van Elsas 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, Tazetdinova and van Elsas 2014).

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, *B. 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. 2007b). *Burkholderia 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, Moebius and Hertweck 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 and van Elsas 2009; Nazir et al. 2012, 2013; Nazir, Tazetdinova and

van Elsas 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 10 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, *B. sordidicola* was isolated from two strains of *Phanerochaete sordida*, and bacteria-free fungal cultures could not be established (Lim et al. 2003).

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, *B. 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). *Burkholderia rhizoxinica*, the *Rhizopus* endosymbiont, likewise possesses genes involved in glycerol metabolism and can utilise glycerol as a carbon source (Partida-Martinez et al. 2007a; 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 *C. 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, van Veen and de Boer 2015). The abundance of *Collimonas* cells has been observed to be higher in for-

est 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, Pritzkow and Noll 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).

CONCLUSIONS AND FUTURE PERSPECTIVES

Despite 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, means 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 dead wood environment. Outstanding questions include:

- (i) What is the origin of bacteria in wood; how and over what timescale does colonisation occur?
- (ii) What are the major biotic and abiotic determinants of bacterial communities, and by what mechanisms do these operate?
- (iii) Are interactions with fungi predominantly beneficial or antagonistic? Does one partner consistently benefit at the expense of the other?
- (iv) 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 outcompete 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 underexplored 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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