Ecological Entomology

Ecological Entomology (2015), **40** (Suppl. 1), 97–112 DOI: 10.1111/een.12176

INSECTS AND ECOSYSTEM SERVICES SPECIAL ISSUE

Insect-mediated nitrogen dynamics in decomposing wood

MICHAEL D. ULYSHEN USDA Forest Service, Athens, Georgia, U.S.A.

Abstract. 1. Wood decomposition is characterised by complex and poorly understood nitrogen (N) dynamics with unclear implications for forest nutrient cycling and productivity. Wood-dwelling microbes have developed unique strategies for coping with the N limitations imposed by their substrate, including the translocation of N into wood by cord-forming fungi and the fixation of atmospheric nitrogen (N_2) by bacteria and Archaea.

2. By accelerating the release of nutrients immobilised in fungal tissues and promoting N_2 fixation by free-living and endosymbiotic prokaryotes, saproxylic insects have the potential to influence N dynamics in forests.

3. Prokaryotes capable of fixing N_2 appear to be commonplace among wood-feeding insects, with published records from three orders (Blattodea, Coleoptera and Hymenoptera), 13 families, 33 genera and at least 60 species. These organisms appear to play a significant role in the N economies of their hosts and represent a widespread solution to surviving on a diet of wood.

4. While agricultural research has demonstrated the role that termites and other insects can play in enhancing crop yields, the importance of saproxylic insects to forest productivity remains unexplored.

Key words. Arthropods, diazotroph, ecosystem services, Isoptera, mineralisation, saproxylic, symbiosis.

Introduction

Nitrogen (N) is the limiting nutrient in many systems (Vitousek & Howarth, 1991; LeBauer & Treseder, 2008) and this is especially true for dead wood where concentrations typically range from just 0.03% to 0.1% by dry weight (Käärik, 1974). Woody debris nevertheless supports a large biomass of living organisms with tissues comparatively rich in N. The N concentrations of wood-dwelling fungal and insect tissues, for example, are in the ranges 1.5–7% and 8–13%, respectively (Cowling & Merrill, 1966; Higashi *et al.*, 1992). Although fungi and insects possess mechanisms for conserving N as well (Brune & Ohkuma, 2011), many researchers have suspected that the abundance of such organisms in decomposing wood and the constant export of N in fungal spores, dispersing insects, and so on, can only be possible if external sources of N are utilised (Cowling & Merrill, 1966; Higashi *et al.*, 1992). Early support for this idea came from efforts to calculate the amount of wood required to support the N-rich tissues of particular insect and fungal species. For example, Baker (1969) reported that *Anobium punctatum* (De Geer) developing in dry wood acquired 2.5 times the amount of N provided by the wood itself. Similarly, Merrill and Cowling (1966) estimated that 40.3 kg (0.07 m^3) of wood would be required to supply enough N to produce the amount of spores released by a single fruiting body of *Fomes fomentarius* (L. ex Fr.) Kickx over a 20-day period. More recently, Thompson (2013) estimated that the volume of wood consumed by larvae of the wood-feeding sawfly *Sirex noctilio* F. provides no more than 10% of the total N required by these insects. Higashi *et al.* (1992) suggested that termites face a ‘carbon-nitrogen balance problem’ and must either add N to inputs or selectively eliminate C (e.g. via CO_2, CH_4 or C-rich waste products). Two major biotic inputs of N to decomposing wood are now recognised. The first involves the translocation of N from other sources by cord-forming fungi (Boddy & Watkinson, 1995; Boddy, 1999), thus resulting in the redistribution and concentration of N within the existing N pool. The second involves N_2 fixation [the reduction of atmospheric nitrogen (N_2) to ammonia (NH_3)] by prokaryotes, thus increasing the total amount of available N in the system.

Correspondence: Michael D. Ulyshen, USDA Forest Service, Southern Research Station, Athens, Georgia 30602, U.S.A. E-mail: mulyshen@fs.fed.us

Woody debris supports an extremely diverse assemblage of invertebrates, with an estimated 20–30% of all forest insect species being ‘saproxylic’, or dependent on dying or dead wood (Stokland *et al.*, 2012). Major functional groups include phloem and wood feeders, fungus feeders, detritus feeders and predators, all of which have the potential to affect decomposition (Ulyshen, 2015) as well as nutrient cycling (Fig. 1). Termites are among the most abundant and best-studied wood-feeding insects in many parts of the world and exert an especially strong influence on ecological processes. Members of the more basal families (non-termitids or ‘lower-termites’) are predominantly wood-feeders, whereas the termitids (‘higher termites’) exhibit more diverse feeding habits (e.g. grass, soil, cultivated fungi), including many species that never feed on wood (Donovan *et al.*, 2001). One important distinction between these two groups is that the guts of non-termitids (as in wood-feeding *Cryptocercus* cockroaches) contain symbiotic protists that play an important role in lignocellulose digestion and other functions (Brune & Ohkuma, 2011; Brune, 2014). The degree of N limitation faced by wood-feeding termites varies widely depending on the feeding habits of the particular groups. Subterranean termites (Rhinotermitidae) feeding in wood on the forest floor benefit from the high N contents of fungal tissues (Rouland-Lefèvre, 2000), for example, whereas dry-wood termites (Kalotermitidae) feeding above ground on dead limbs face more severe limitations. Regardless of feeding habit, termites tightly conserve nutrients within their colonies, owing to their habits of proctodeal trophallaxis and cannibalism (Wood, 1976; Lee, 1983). Nitrogen immobilised in termites or other wood-dwelling insects can enter higher trophic levels, be returned locally upon the death of an individual or be dispersed widely as subsequent generations leave in search of new resources. Insects may also indirectly affect the N economy of woody debris through interactions with fungi or other organisms. By disrupting the mycelial networks of fungi, for example, insects may act to release nutrients immobilised in microbial tissues, thereby accelerating the transition of wood from being a sink to a source of soil nutrients. Insects also have the potential to promote the fixation of atmospheric N through interactions with free-living and endosymbiotic prokaryotes, thus resulting in an input of N to the system.

Even at the coarsest levels of understanding, much remains unclear about N dynamics in decomposing wood. Many studies have shown increases in nutrient concentrations (especially N and phosphorus) as wood decomposes, for example, but it remains uncertain to what extent such patterns reflect inputs of nutrients as opposed to losses of carbon through respiration (Krankina *et al.*, 1999; Laiho & Prescott, 2004). Many abiotic and biotic factors influence the N content of decomposing wood at any given time (Fig. 1), but few efforts have been made to measure their relative impacts (Yamada *et al.*, 2006). While the role of invertebrates in nutrient cycling within organic matter has received a great deal of interest, much of this work has focused on their activities in soil (Lavelle *et al.*, 1997) and leaf litter (Seastedt, 1984). Less attention has been paid to the effects of these organisms in decomposing wood despite great interest in the diversity and conservation of this fauna (Stokland *et al.*, 2012). In the current article, I review how these organisms may indirectly affect N dynamics through interactions with fungi, bacteria and other microbes. I explore their role in accelerating the release of nutrients from decomposing wood, how they may promote N_2 fixation by both free-living and symbiotic prokaryotes and end with a discussion on the implications for forest productivity. Because termites have been the most intensively studied insects associated with dead wood, these organisms necessarily dominate many sections of this review.

Accelerating nutrient release

Woody debris is widely believed to act as a nutrient sink during the early stages of decomposition as nutrients are imported from the soil and immobilised within fungal tissues (Swift *et al.*, 1979; Boddy & Watkinson, 1995), only becoming a source years or decades later (Palviainen *et al.*, 2010; Garrett *et al.*, 2012). Boddy and Watkinson (1995) suggested that insects play a critical role in the release of these nutrients by grazing on or otherwise damaging fungal mycelia. Several researchers have reported an association between insect activity and the release of nutrients from wood. In England, for instance, Swift (1977) found the concentrations of N and other nutrients to be lower in branch wood that had been invaded by arthropods than in uninvaded branches [see Swift and Boddy (1984) for a graphical depiction of these results]. It was suggested that wood comminution and bark fragmentation caused by these organisms might have increased nutrient loss through leaching. Similarly, decomposing logs in Malaysia were shown to have higher C : N ratios when termites were present than when they were excluded, suggesting that termites accelerate the release of N (Takamura & Kirton, 1999). Also in Malaysia, Takamura (2001) found the N content of wood to increase over time when termites were excluded but to decrease when termites were present. This pattern was not seen in a species of wood less preferred by termites, however. In the boreal forests of Canada, Cobb *et al.* (2010) sampled mineral soil beneath logs in which varying numbers of *Monochamus scutellatus scutellatus* (Say) had been artificially inserted. The researchers found a positive and significant relationship between larval abundance and total soil N, presumably due to inputs of frass. They also estimated that nearly half the forest floor in post-fire forests would be likely to receive direct inputs of N in the form of *Monochamus* frass.

By contrast, some studies do not support the idea that insects accelerate the release of nutrients from decomposing wood. In north-western North America, for example, Edmonds and Eglitis (1989) found little difference in the N concentrations of logs from which insects had or had not been excluded for 10 years. Because multiple imports and exports determine the N content of decomposing wood, differences in N concentrations over time or between treatments are difficult to interpret. For example, insects have the potential to simultaneously accelerate the release of N immobilised in fungal tissues and input significant amounts of N by promoting the activities of N_2-fixing prokaryotes (see the following section). Interpreting the net effect of insects on N concentrations will therefore require more detailed knowledge about the influence of these organisms on the various pathways of N gain and loss in decomposing wood.

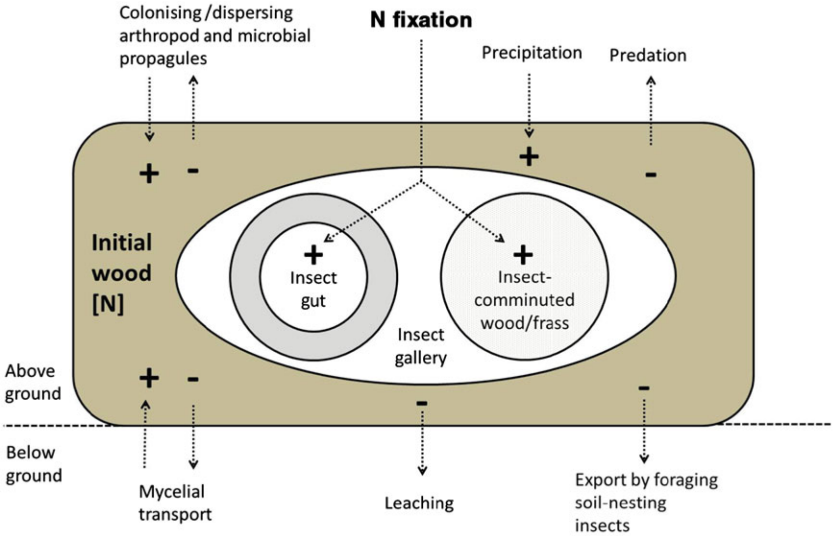

Fig. 1. The nitrogen balance of decomposing wood (shaded rectangle) is determined by various inputs and outputs (relative weights unknown; but see Yamada *et al.*, 2006), including N_2 fixation by prokaryotes. Insects promote N_2 fixation by providing conditions conducive to prokaryote activity in their guts as well as in comminuted wood.

Promoting N fixation

Until recently, research on biological N_2 fixation has focused largely on free-living prokaryotes or those living in association with plants. Nitrogen-fixing endosymbionts appear to be common among invertebrates feeding on organic matter as well, however, and these organisms may contribute significantly to the processing of C and N within ecosystems (Nardi *et al.*, 2002). While much remains unknown about biological N_2 fixation, three major advances have been made in our ability to study this process over the past 50 years. First, the discovery that C_2H_2 (i.e. acetylene) is reduced to C_2H_4 (ethylene), in a reaction analogous to the reduction of N_2 to NH_3, led to the development of the acetylene reduction assay for testing for nitrogenase activity in biological samples (Hardy *et al.*, 1968). Many studies have since used this method to indirectly demonstrate that N_2-fixing bacteria are common in decomposing organic matter, including dead wood. The second major advance involves screening for the *nifH* gene which encodes a major component of nitrogenase (Zehr *et al.*, 2003). This has provided considerable insight into the diversity of organisms potentially involved in N_2 fixation. Finally, ^{15}N isotope analysis provides a means for directly confirming N_2-fixing activity by an organism or associated species (Robinson, 2001). One approach involves the ^{15}N tracer technique whereby candidate organisms are incubated under elevated concentrations of $^{15}N_2$ and then tested for the incorporation of ^{15}N into their tissues. A variant method considers the natural abundance of ^{15}N in different N pools. Because the nitrogenase reaction results in little isotope fractionation, the ^{15}N concentration of organisms heavily dependent on N_2 for their N needs should more closely match that of the atmosphere. In other words, the difference in ^{15}N between the tissues of such organisms and the atmosphere ($\delta^{15}N$) should be closer to zero than for organisms less dependent on N_2 fixation (Tayasu *et al.*, 1997).

A large but scattered body of literature suggests that wood-feeding insects may play an especially important role in promoting N_2 fixation in forested ecosystems. Much of this literature focuses on symbioses between insects and N_2-fixing prokaryotes where gut microbes benefit from the stable environmental conditions and supply of substrates provided by their hosts (Nardi *et al.*, 2002). Although less attention has been paid to free-living N_2-fixing prokaryotes in dead wood, the activities of these organisms are also thought to be promoted by saproxylic insects. Interactions between free-living and endosymbiotic N_2-fixing prokaryotes and wood-feeding insects are reviewed separately in the following sections.

Free-living prokaryotes

A large proportion of free-living prokaryotes in wood appear capable of N_2 fixation. Of 130 bacteria strains isolated from white fir decay by Aho *et al.* (1974), for example, over half were found capable of fixing atmospheric N_2. Although the importance of wood to N_2-fixing bacteria relative to other substrates is not fully understood, the highest N_2 fixation rates are generally expected where N is most limiting (Reed *et al.*, 2011). Because the N content of decomposing wood is generally lower than other major pools of organic matter (e.g. leaf litter), woody debris may potentially represent a hotspot of N_2 fixation. Indeed, inputs of N into decomposing wood by free-living N_2-fixing bacteria can be substantial (Sollins *et al.*, 1987; Griffiths *et al.*, 1993; Brunner & Kimmins, 2003; Chen & Hicks, 2003). In north-western North America, for instance, Sollins *et al.* (1987) estimated that asymbiotic bacteria in fallen logs fixed about 1 kg N ha^{-1} $year^{-1}$ compared with inputs from precipitation and dry deposition of 2–3 kg N ha^{-1} $year^{-1}$. Estimates from north-western Canadian forests were similar, ranging from 1 to 2.1 kg N ha^{-1} $year^{-1}$ (Brunner & Kimmins, 2003).

These rates were shown to vary greatly between and within logs (e.g. rates higher in sapwood than in heartwood in the early stages of decay) and to increase as decomposition proceeds. Many free-living prokaryotes, including those capable of N_2 fixation, are likely to benefit from the actions of wood-feeding insects. These organisms are thought to be favoured by the production of particulate matter, such as the comminuted wood produced by wood-feeding insects. Ausmus (1977) compared N_2-fixation rates among various stages of wood decomposition and found the highest rates in the 'channelisation' phase, during which termites, ants, passalid beetles and other insects were actively tunnelling through wood. Nitrogen fixation appeared to be highest in the frass-filled tunnels of these insects, suggesting that frass may be a preferred substrate for N_2-fixing bacteria. Subsequent laboratory studies provided some support for this idea, showing that C : N ratios decrease substantially when termites are present (Kitchell *et al.*, 1979). While gut-dwelling endosymbionts have been the focus of most research on insect-mediated N_2 fixation (see the following section), the contributions of free-living prokaryotes should not be overlooked. Indeed, in some cases, free-living species have been shown to contribute more to the process than endosymbionts. For example, Hewitt *et al.* (1987) attributed most of the N_2 fixation occurring within intact colonies of the grass-feeding termite *Hodotermes mossambicus* (Hagen) to free-living bacteria inhabiting nest materials, whereas the insects themselves exhibited low rates of acetylene reduction. Frass appeared to be an especially important substrate to N_2-fixing bacteria in that study, with acetylene reduction rates rising over the first several days before dropping sharply after around 10 days. Past studies on wood-feeding insects have sometimes overlooked the potential contributions of free-living N_2-fixing prokaryotes. For example, Prestwich and Bentley (1981) compared rates of acetylene reduction measured from intact nests of *Nasutitermes corniger* (Motschulsky) with that of individuals removed from the nest. Individuals separated from the nest exhibited *c.* 10-fold lower rates of acetylene reduction. While the authors attributed this reduction to the effects of disturbing the termites, the possible role of free-living bacteria associated with the nest material, including frass, was not considered.

Gut-dwelling microbes

Although the use of atmospheric N was suspected by some earlier researchers (Cleveland, 1925; Peklo & Satava, 1949; Baker, 1969), the acetylene reduction assay permitted the first definitive demonstrations of N_2 fixation by wood-feeding insects (Benemann, 1973; Breznak *et al.*, 1973). It was clear that N_2 fixation occurred primarily within the guts of these organisms [although the process may also occur in the salivary glands of some wood-feeding insect species (Gomathi *et al.*, 2005; Thompson, 2013)]. For example, Breznak *et al.* (1973, 1974) showed that guts removed from termites or wood-feeding cockroaches were still able to reduce acetylene, whereas the degutted bodies were not. The same studies also showed that acetylene reduction could be abolished by feeding the insects antibiotics, suggesting that bacteria were the organisms responsible. Nitrogen fixation appears to be widespread among phloem- and wood-feeding insects, with evidence from at least 60 species of termites, beetles and a woodwasp (Table 1). By contrast, Breznak *et al.* (1973) subjected a wide range of non-wood-feeding insect taxa to the acetylene reduction assay and all tested negative, indicating that N_2 fixation is not ubiquitous among insects. Because N_2 fixation is an energy-demanding process, requiring 20–30 molecules of ATP for each molecule of N_2 fixed (Breznak, 2000), it is expected to occur most commonly where other sources of N are not available. This has been largely borne out by research on termites showing that acetylene reduction rates increase as the N content of the food decreases (Breznak *et al.*, 1973). Accordingly, some of the highest N_2-fixation rates have been reported from wood-feeding termites, whereas lower rates are seen from those feeding on other, more N-rich substrates. Many soil-feeding and fungus-farming termite species, for example, appear to be incapable of fixing N_2 or do so at very low rates (Rohrmann & Rossman, 1980; Prestwich & Bentley, 1982; Tayasu *et al.*, 1997, 1998; Ohkuma *et al.*, 1999; Yamada *et al.*, 2006). Similarly, Prestwich *et al.* (1980) reported higher rates of acetylene reduction from wood-feeding termites compared with litter-feeders in Costa Rica. Nitrogen-fixing gut microbes may also explain why certain wood-feeding beetle species produce frass with elevated N concentrations relative to the food consumed (Jönsson *et al.*, 2004; Micó *et al.*, 2011). Although N_2 fixation appears to be more common among wood-feeding insects, it should be noted that some species appear to lack the ability. For example, Breznak (1975) reported that the beetles *Lyctus carbonarius* (=*planicollis*) Waltl and *Xyletinus peltatus* (Harris) tested negative for acetylene reduction. Nardi *et al.* (2002) summarised some early research by Becker (published in German), which suggested that wood-boring beetles with gut symbionts develop independently of the N content of wood, whereas those without symbionts [e.g. the cerambycid *Hylotrupes bajulus* (L.)] develop at rates proportional to the wood N content.

In addition to the interspecific differences reviewed here, considerable intraspecific variation in N_2-fixation rates has also been reported. Waller *et al.* (1989) showed that acetylene reduction rates can vary considerably (up to 19-fold) among colonies of *Coptotermes formosanus* Shiraki in Louisiana, U.S.A. Similarly, Pandey *et al.* (1992) found considerable differences in acetylene reduction rates among colonies of *Reticulitermes* in Virginia, as did Prestwich and Bentley (1981) for *N. corniger* in Costa Rica and Yamada *et al.* (2006) for *Globitermes suphureus* (Haviland) in Thailand. Significant differences in acetylene reduction rates have been reported between different habitats as well, possibly resulting from differences in resource quality. In Thailand, Yamada *et al.* (2006) reported higher rates of acetylene reduction from dry deciduous forests than from dry evergreen forests and attributed this to higher C : N ratios in the deciduous forests. Similarly, Sylvester-Bradley *et al.* (1978) found acetylene reduction rates to be higher from termites in a Brazilian pasture than in forests where higher-quality food may have been available. Even within a single termite colony, the N_2-fixation rate varies in space and time and is influenced, in part, by caste and developmental stage. Regarding temporal variability, Prestwich and Bentley (1981) suggested that N_2-fixation rates by soldier termites may vary seasonally, being highest when resources are directed at the production of

Table 1. Wood-feeding insect taxa (whether obligatory or facultative, including fungus-farming termites) thought to possess gut microbes capable of N_2 fixation. Termite classification follows Krishna *et al.* (2013).

Order		Positive acetylene reduction assay				
Family	Species	Living insects	Cultured bacteria from gut	*nifH* gene present	Isotope analysis (^{15}N)	Comment(s)
Blattodea						
Archotermopsidae	*Hodotermopsis sjostedti* Holmgren	Workers (Ohkuma *et al.*, 1999)	–	(Ohkuma *et al.*, 1999)	–	–
	Zootermopsis angusticollis (Hagen)	Unspecified stage (Benemann, 1973)	*Treponema* sp. (Lilburn *et al.*, 2001)	(Lilburn *et al.*, 2001)	–	–
	Zootermopsis nevadensis (Hagen)	–	–	(Yamada *et al.*, 2007)	–	–
	Zootermopsis sp.	Workers and reproductive nymphs (Breznak *et al.*, 1973)	–	–	–	–
Cryptocercidae	*Cryptocercus punctulatus* Scudder	(Breznak *et al.*, 1974)	*Desulfovibrio* sp. (Kuhnigk *et al.*, 1996)	(Yamada *et al.*, 2007)	–	–
Kalotermitidae	*Cryptotermes brevis* (Walker)	Unspecified stage (Benemann, 1973); reproductive nymphs (Breznak *et al.*, 1973)	–	–	–	–
	Cryptotermes domesticus (Haviland)	Workers (Ohkuma *et al.*, 1999)	–	(Ohkuma *et al.*, 1999)	–	–
	Cryptotermes longicollis Banks	Workers (Desai & Brune, 2012)	–	(Desai & Brune, 2012)	–	–
	Cryptotermes primus (Hill)	–	–	–	–	*Enterobacter* from gut grew on an N-free medium (Eutick *et al.*, 1978)
	Glyptotermes fuscus Oshima	Workers (Ohkuma *et al.*, 1999)	–	(Ohkuma *et al.*, 1999)	–	–
	Incisitermes marginipennis (Latreille)	Workers (Desai & Brune, 2012)	–	(Desai & Brune, 2012)	–	–
	Incisitermes minor (Hagen)	Workers, soldiers and reproductives (Benemann, 1973)	–	–	–	–
	Kalotermes flavicollis (Fabricius)	Workers (Desai & Brune, 2012)	–	(Desai & Brune, 2012)	–	–
	Neotermes castaneus (Burmeister)	Workers (Golichenkov *et al.*, 2006); workers (Desai & Brune, 2012)	–	(Desai & Brune, 2012)	–	–

Table 1. continued

Order		Positive acetylene reduction assay				
Family	Species	Living insects	Cultured bacteria from gut	*nifH* gene present	Isotope analysis (^{15}N)	Comment(s)
	Neotermes koshunensis (Shiraki)	Workers (Ohkuma *et al.*, 1999)	–	(Noda *et al.*, 1999; Ohkuma *et al.*, 1999)	Natural abundance method (Tayasu *et al.*, 1994)	–
Mastotermitidae	*Mastotermes darwiniensis* Froggatt	Workers (French *et al.*, 1976)	*Citrobacter freundii* (French *et al.*, 1976); *Desulfovibrio* sp. (Kuhnigk *et al.*, 1996)	(Yamada *et al.*, 2007)	–	*Enterobacter* from gut grew on an N-free medium (Eutick *et al.*, 1978)
Rhinotermitidae	*Coptotermes acinaciformis* (Froggatt)	–	–	–	–	*Enterobacter* from gut grew on an N-free medium (Eutick *et al.*, 1978)
	Coptotermes formosanus Shiraki	Workers and soldiers (Breznak *et al.*, 1973); workers (Waller *et al.*, 1989); workers (Ohkuma *et al.*, 1999)	*Enterobacter agglomerans* (Potrikus & Breznak, 1977)	(Ohkuma *et al.*, 1999; Hongoh *et al.*, 2008)	–	Bacteroidales endosymiont of a gut protist expresses *nifH* gene (Hongoh *et al.*, 2008)
	Coptotermes gestroi (Wasmann)	Workers (Yamada *et al.*, 2006)	–	(Yamada *et al.*, 2007)	–	–
	Coptotermes lacteus (Froggatt)	Workers (French *et al.*, 1976); workers (Lovelock *et al.*, 1985)	*Citrobacter freundii* (French *et al.*, 1976)	–	–	*Enterobacter* from gut grew on an N-free medium (Eutick *et al.*, 1978)
	Heterotermes ferox (Froggatt)	–	–	–	–	*Enterobacter* from gut grew on an N-free medium (Eutick *et al.*, 1978)
	Heterotermes indicola (Wasmann)	–	*Desulfovibrio termitidis* (Kuhnigk *et al.*, 1996)	–	–	–
	Heterotermes sp.	Workers (Sylvester-Bradley *et al.*, 1978)	–	–	–	–
	Reticulitermes chinensis Snyder	–	–	(Du *et al.*, 2012)	–	–
	Reticulitermes flavipes (Kollar)	Workers and soldiers (Breznak *et al.*, 1973); workers (Ayayee *et al.*, 2014)	*Desulfovibrio desulfuricans* (Kuhnigk *et al.*, 1996)	–	Natural abundance method (Meuti *et al.*, 2010)	–

Table 1. continued

Order		Positive acetylene reduction assay				
Family	Species	Living insects	Cultured bacteria from gut	*nifH* gene present	Isotope analysis (^{15}N)	Comment(s)
	Reticulitermes lucifugus (Rossi)	(Vecherskii *et al.*, 2008)	–	–	–	–
	Reticulitermes speratus (Kolbe)	Workers (Ohkuma *et al.*, 1999)	–	(Ohkuma *et al.*, 1996, 1999; Kudo *et al.*, 1998)	Tracer technique (Fujita & Abe, 2006)	–
	Reticulitermes tibialis Banks	Unspecified stage (Schaefer & Whitford, 1979)	–	–	–	–
	Reticulitermes spp.	Workers (Pandey *et al.*, 1992); workers (Curtis & Waller, 1995); workers (Curtis & Waller, 1996); workers (Curtis & Waller, 1997); workers, soldiers, presoldiers, larvae and alates (Curtis & Waller, 1998); workers (Waller & Curtis, 2003)	–	–	–	Combinations of *R. flavipes* and *R. virginicus* were potentially used in these studies
	Schedorhinotermes intermedius (Brauer)	–	–	–	–	*Enterobacter* from gut grew on an N-free medium (Eutick *et al.*, 1978)
	Schedorhinotermes medioobscurus (Holmgren)	–	–	(Yamada *et al.*, 2007)	–	No acetylene reduction detected (Yamada *et al.*, 2006)
Stolotermitidae	*Stolotermes ruficeps* Brauer	–	–	(Reid & Lloyd-Jones, 2009)	–	–
Termitidae	*Amitermes wheeleri* (Desneux)	Unspecified stage (Schaefer & Whitford, 1979)	–	–	–	–
	Amitermes spp.	Workers and soldiers (Sylvester-Bradley *et al.*, 1978)	–	–	–	–
	Armitermes spp.	Workers and soldiers (Sylvester-Bradley *et al.*, 1978)	–	–	–	–
	Constrictotermes sp.	Workers (Sylvester-Bradley *et al.*, 1978)	–	–	–	–
	Cornitermes spp.	Workers and soldiers Sylvester-Bradley *et al.*, 1978)	–	–	–	–

Table 1. continued

Order		Positive acetylene reduction assay				
Family	Species	Living insects	Cultured bacteria from gut	*nifH* gene present	Isotope analysis (^{15}N)	Comment(s)
	Globitermes sulphureus (Haviland)	Workers (Yamada *et al.*, 2006)	–	(Yamada *et al.*, 2007)	–	–
	Gnathamitermes tubiformans (Buckley)	(Schaefer & Whitford, 1981)	–	–	–	–
	Microcerotermes crassus Snyder	Workers (Yamada *et al.*, 2006)	–	(Yamada *et al.*, 2007)	–	–
	Microcerotermes parvus (Haviland)	–	–	–	Natural abundance method (Tayasu *et al.*, 1997)	–
	Microcerotermes sp.	Workers (Yamada *et al.*, 2006)	–	(Yamada *et al.*, 2007)	–	–
	Nasutitermes corniger (Motschulsky)	Intact colonies (Prestwich & Bentley, 1981); mixed workers and soldiers (Bentley, 1984)	–	–	Tracer technique (Bentley, 1984)	–
	Nasutitermes dimorphus Ahmad	Workers (Yamada *et al.*, 2006)	–	(Yamada *et al.*, 2007)	–	–
	Nasutitermes ephratae (Holmgren)	Workers and soldiers (Prestwich *et al.*, 1980)	–	–	–	Prestwich and Bentley (1981) state that this species was probably misidentified, being *N. corniger* instead. They also claim, based on preliminary data, that *N. ephratae* is capable of fixing N_2 at comparable rates
	Nasutitermes exitiosus (Hill)	Workers (French *et al.*, 1976); workers (Lovelock *et al.*, 1985)	–	–	–	Acetylene reduction detected when workers fed filter paper but not wood (French *et al.*, 1976)
	Nasutitermes graveolus (Hill)	–	–	–	–	*Enterobacter* from gut grew on an N-free medium (Eutick *et al.*, 1978)
	Nasutitermes macrocephalus (Silvestri)	Mixed workers and soldiers (Sylvester-Bradley *et al.*, 1983)	–	–	–	No acetylene reduction following termite decapitation

Table 1. continued

Order		Positive acetylene reduction assay				
Family	Species	Living insects	Cultured bacteria from gut	*nifH* gene present	Isotope analysis (^{15}N)	Comment(s)
	Nasutitermes matangensis (Haviland)	–	–	(Yamada *et al.*, 2007)	–	–
	Nasutitermes takasagoensis (Nawa) (termitidae)	Workers (Ohkuma *et al.*, 1999)	–	(Ohkuma *et al.*, 1999)	–	Low rates of acetylene reduction relative to non-termitids tested in the same study
	Nasutitermes walkeri (Hill)	Workers (Lovelock *et al.*, 1985)	–	–	Tracer technique (Chappell & Slaytor, 1986)	–
	Nasutitermes spp.	Workers and soldiers (Sylvester-Bradley *et al.*, 1978)	–	(Warnecke *et al.*, 2007)	–	–
	Neocapritermes sp.	Workers (Sylvester-Bradley *et al.*, 1978)	–	–	–	–
	Odontotermes formosanus (Shiraki)	–	–	(Ohkuma *et al.*, 1999)	–	Acetylene reduction not detected
	Odontotermes sp.	Workers (Yamada *et al.*, 2006)	–	–	–	Very low rates of acetylene reduction
	Termes sp.	Workers (Sylvester-Bradley *et al.*, 1978)	–	–	–	–
Coleoptera						
Cerambycidae	*Anoplophora glabripennis* (Motschulsky)	Larvae (Ayayee *et al.*, 2014)	–	(Ayayee *et al.*, 2014)	Tracer technique (Ayayee *et al.*, 2014)	–
	Prionoplus reticularis White	–	Gut homogenate (Reid *et al.*, 2011)	–	–	–
Curculionidae	*Dendroctonus frontalis* Zimmerman	–	(Bridges, 1981)	–	–	–
	Dendroctonus rhizophagus Thomas & Bright	–	*Raoultella terrigena* (Morales-Jiménez *et al.*, 2013)	(Morales-Jiménez *et al.*, 2013)	–	–
	Dendroctonus terebrans (Olivier)	–	*Enterobacter agglomerans* and *Enterobacter* spp. (Bridges, 1981)	–	–	Acetylene reduction not detected for living larvae or adults
	Dendroctonus valens LeConte	Larvae and adults (Morales-Jiménez *et al.*, 2009)	*Raoultella terrigena* (Morales-Jiménez *et al.*, 2013)	(Morales-Jiménez *et al.*, 2013)	–	–
	Ips avulsus (Eichhoff)	–	(Bridges, 1981)	–	–	–

Table 1. continued

Order		Positive acetylene reduction assay				
Family	Species	Living insects	Cultured bacteria from gut	*nifH* gene present	Isotope analysis (^{15}N)	Comment(s)
Lucanidae	*Dorcus rectus* (Motschulsky)	Larvae (Kuranouchi *et al.*, 2006)	–	–	–	–
Passalidae	*Odontotaenius disjunctus* (Illiger)	–	–	(Ceja-Navarro *et al.*, 2014)	Tracer technique (Ceja-Navarro *et al.*, 2014)	N_2 fixation attributed to various bacteria
Scarabaeidae	*Cetonia aurata* L.	Larvae or adults (Citernesi *et al.*, 1977)	–	–	–	–
Hymenoptera						
Siricidae	*Sirex noctilio* F.	–	–	–	Tracer technique and natural abundance method (Thompson, 2013)	–

reproductive individuals. In North America, Curtis and Waller (1998) reported higher acetylene reduction rates by *Reticulitermes* spp. in autumn and spring than in winter and summer. In Thailand, Yamada *et al.* (2006) detected higher rates of acetylene reduction during the early rainy season than during the late rainy season or dry season. Considerable variability has been reported over shorter time frames as well. For example, Sylvester-Bradley *et al.* (1983) found termites from a single nest to exhibit high rates of acetylene reduction one week but none a week later. The opposite response was observed in other nests, with a week of no detectable acetylene reduction being followed by a week of high activity. Sylvester-Bradley *et al.* (1983) reported that different sections taken from a single *Nasutitermes* sp. nest exhibited dissimilar rates of acetylene reduction. It is not clear how termite abundance may have differed among these sections, however, and no explanation for these results was provided.

Many researchers have reported dissimilar rates of acetylene reduction among termite castes, but no consistent pattern has emerged. Whereas studies involving non-termitids (kalotermitids and rhinotermitids) suggest workers play a more important role in N_2 fixation than soldiers or other castes (Benemann, 1973; Breznak *et al.*, 1973; Curtis & Waller, 1998), studies involving *Nasutitermes* have shown higher rates from soldiers than workers (Prestwich *et al.*, 1980; Sylvester-Bradley *et al.*, 1983). As suggested by Prestwich *et al.* (1980), the higher relative N_2-fixation rates reported from *Nasutitermes* soldiers may relate to the fact that soldiers are more common in nasutitermine colonies than in other termite subfamilies. Less nutritionally dependent soldiers may allow for higher soldier:worker ratios. Prestwich *et al.* (1980) reported higher acetylene reduction rates from *Nasutitermes* workers within the nest compared with foraging workers, possibly reflecting differences in food quality. Yamada *et al.* (2006) associated higher rates of acetylene reduction with major workers of *Microcerotermes crassus* Snyder than with minor workers in Thailand. Benemann (1973) suggested that larval age may influence rates of N_2 fixation in termites. Support for this came from Breznak (1975), who reported a 317-fold greater rate of acetylene reduction by young larvae of the termite *C. formosanus* than by older larvae used in a previous experiment. Breznak (1975) suggested that N_2-fixation rates may be higher in young, growing larvae due to higher N demands and this may also be the case for other wood-feeding insects. Consistent with this view, nearly half the bark beetle (*Ips avulsus* Eichhoff) larvae tested by Bridges (1981) exhibited acetylene reduction activity, whereas none of the adults was found to possess this ability. However, elevated rates of acetylene reduction in younger developmental stages have not been shown in all studies on wood-feeding insects (Rohrmann & Rossman, 1980; Curtis & Waller, 1998). Among fully grown *C. formosanus* workers, Waller *et al.* (1989) reported a positive relationship between biomass and the acetylene reduction rate, indicating that larger individuals fix more N_2 than smaller individuals. Another important source of variability in the acetylene reduction rate is the amount of time elapsed between sample collection and the measurement. Numerous researchers have shown a rapid decline in acetylene reduction by termites when held under laboratory conditions (Breznak *et al.*, 1973; Prestwich *et al.*, 1980; Sylvester-Bradley *et al.*, 1983; Lovelock *et al.*, 1985; Pandey *et al.*, 1992; Curtis & Waller, 1995), thus complicating the interpretation of results from the acetylene reduction assay. Such declines are thought to represent a response of termites to disturbance. Curtis and Waller (1995) showed that *Reticulitermes* can recover or even exceed (depending on when the termites were initially collected) initial rates of acetylene reduction after several months of laboratory confinement.

A number of early efforts attempted to identify the organisms responsible for N_2 fixation in the guts of wood-feeding insects by culturing them on N-free media and testing for acetylene reduction (French *et al.*, 1976; Potrikus & Breznak, 1977; Eutick *et al.*, 1978; Bridges, 1981; Kuhnigk *et al.*, 1996). Because only a small proportion of gut microbes can be cultured, however, this approach provides limited information. Recent years have therefore seen a switch to non-culture techniques. Most notably, surveys of *nifH* diversity in termite guts suggest that a wide array of prokaryotes are involved, including both bacteria (e.g. Spirochaetes, Clostridia, Proteobacteria, Bacteroidales, etc.) and Archaea (Ohkuma *et al.*, 1996, 1999; Noda *et al.*, 1999; Lilburn *et al.*, 2001; Hongoh *et al.*, 2008; Du *et al.*, 2012). While much of this diversity remains unknown (Ohkuma *et al.*, 1996), it appears that microbial communities in closely related termite species are similar (Ohkuma *et al.*, 1999; Yamada *et al.*, 2007; Reid & Lloyd-Jones, 2009), consistent with the view that termite gut microbes have co-evolved with their hosts (Hongoh *et al.*, 2005). The obligate nature of some relationships between N_2-fixing prokaryotes and their hosts is demonstrated particularly well by the principal N_2-fixing bacteria associated with *C. formosanus*, a species of Bacteroidales which is an endosymbiont of an endosymbiotic protist found within the gut of this insect (Hongoh *et al.*, 2008). The protists found within the guts of certain dry-wood termites (Kalotermitidae) also have Bacteroidales symbionts and these organisms are thought to play an especially important role in the N metabolism of these insects (Desai & Brune, 2012). Such findings suggest that the species composition of the protist community may largely determine which species of N_2-fixing microbes predominate in the guts of lower termites (Desai & Brune, 2012). Although less well-studied, the guts of wood-feeding beetles also contain a diversity of *nifH* genes. A detailed study by Ceja-Navarro *et al.* (2014) detected a variety of *nifH* sequences from the different gut regions of the passalid *Odontotaenius disjunctus* (Illiger), for example, with sequences related to a species of *Paludibacter* (Bacteroidetes) dominating. While possessing a relatively low diversity of gut microbes, phloem-feeding bark beetles of the genus *Dendroctonus* appear to support populations of at least one *nifH*-encoding (and acetylene-reducing) bacterium, *Raoultella terrigena* (Morales-Jiménez *et al.*, 2009, 2012, 2013). Intriguingly, Ayayee *et al.* (2014) detected *nifH* genes and transcripts in the surface-sterilised eggs of the cerambycid *Anoplophora glabripennis* (Motschulsky) and raised the possibility that N_2-fixing bacteria are vertically transmitted in this species. It should be mentioned that the presence of *nifH*, by itself, does not confirm N_2-fixing capabilities. For example, (Ohkuma *et al.*, 1999) showed that most *nifH* clones from the guts of termitid species belonged to the 'pseudo nif group', which is believed to function in some process other than N_2 fixation. The acetylene reduction assay showed lower rates of activity in these species than in the non-termitid species tested. Moreover, even when a particular *nifH* sequence encodes a functional nitrogenase, expression of the gene cannot be assumed. For example, only a few of the many *nifH* sequences detected by Noda *et al.* (1999) from *Neotermes koshunensis* Shiraki gut microbes were preferentially transcribed. By amplifying cDNA from mRNA by reverse transcription PCR, the researchers showed that the level of expression of the *anf* genes (i.e. a group of *nifH* genes) largely determines the level of N_2-fixation activity within *N. koshunensis*.

Many researchers have employed the ^{15}N isotope tracer technique or the natural ^{15}N abundance method to demonstrate that fixed N_2 gets incorporated into the tissues of wood-feeding insects (Table 1), thus confirming that N_2-fixing endosymbionts provide relief to their hosts from the high C : N ratios encountered in wood (Nardi *et al.*, 2002). Bentley (1984) was the first to do this, showing that N_2 fixed by gut symbionts of the termite *N. corniger* is assimilated and incorporated into the tissues of the host. Newly fixed N_2 was also shown to be transferred between workers and soldiers by trophallaxis. Tayasu *et al.* (1994) estimated that at least 30%, and probably more than 50%, of the N in tissues of the dry-wood termite *N. koshunensis* is derived from the atmosphere. More recently, Thompson (2013) estimated that the wood-feeding sawfly *S. noctilio* F. derives >90% of its larval N budget from N_2 fixation. The N_2-fixation rate for termites is sometimes expressed in terms of the N doubling time, i.e. the amount of time required for a termite colony to double its N content. This figure varies widely among termite species, ranging from just a few years to several centuries (Breznak, 2000). Nitrogen fixation represents a major source of N for many wood-feeding termites, especially when the contributions of free-living species are included. For example, Prestwich and Bentley (1981) estimated the N doubling time for *N. corniger* colonies (including the nest material) to be 207–618 days, meaning the entire nest population could be replaced around one to two times per year.

Implications for productivity

While many researchers have speculated about a connection between the actions of saproxylic insects and forest productivity (Adamson, 1943; Breznak *et al.*, 1973; Sylvester-Bradley *et al.*, 1978; Reid & Lloyd-Jones, 2009), no experimental efforts, to my knowledge, have been made to formally test this question. As reviewed here, however, termites and other wood-feeding insects appear to hasten the release of N immobilised in fungal tissues and promote N_2 fixation. Because N is the most limiting nutrient in many forests, both processes have the potential to influence productivity. Although research in forests is lacking, a considerable body of evidence indicates that termites promote agricultural productivity in areas with low soil fertility (Jouquet *et al.*, 2011). Indeed, farmers in Africa and South America have long recognised the benefits of these insects to crop production and sometimes incorporate them into their farming practices. Depending on the crop, type of soil and species of termite, this can include planting crops on or near termite mounds (or in circular fields surrounding the mounds), spreading soil from mounds across fields to increase fertility, and placing food resources (e.g. manure, woody debris, etc.) to encourage termite activity (Nyamapfene, 1986; Logan, 1992; Batalha *et al.*, 1995; Wood, 1996; Black & Okawol, 1997; Brouwer & Bouma, 1997; Holt & Lepage, 2000). In Australian wheat fields, Evans *et al.* (2011) reported a 70% reduction in mineral N concentrations in soil from which termites and ants had been excluded compared with control plots. Regarding the benefits of wood-feeding termites, Burnett (1948) described a cultural practice in Africa where degraded fields were intentionally covered in woody branches to provide food for termites. He noted that badly eroded land quickly became fertile after this treatment, but he was unsure whether the effect was due to the release of nutrients or to improved soil aeration brought about by the tunnelling activities of termites. Although beyond the scope of the current review, the role of termites in improving soil aeration, drainage and turnover represents a second major mechanism by which wood-feeding insects may act to boost forest productivity (Adamson, 1943; Holt & Lepage, 2000). Evans *et al.* (2011) concluded that a combination of increased N availability and improved soil aeration explained the benefits of termites and ants to crop yield in Australia.

Several studies have attempted to quantify the amount of N inputed to forest ecosystems by termites and their endosymbionts. Pandey *et al.* (1992) estimated that *Reticulitermes* colonies in the south-eastern United States fix $0.13–0.45\,\mathrm{kg\,N_2\,ha^{-1}\,year^{-1}}$. Similarly, Yamada *et al.* (2006) estimated the amount of N_2 fixed by termites in dry deciduous forests and dry evergreen forests in Thailand to be 0.21 and $0.28\,\mathrm{kg\,ha^{-1}\,year^{-1}}$, respectively, comprising 7–22% of the total N_2 fixation occurring in plant debris on the forest floor. Reid and Lloyd-Jones (2009) estimated that within a range of $1–100$ termite colonies $\mathrm{ha^{-1}}$, N_2-fixing microbes found in *Stolotermes ruficeps* Brauer guts may contribute between 0.7 and 110 Mg of N to New Zealand forests. Curtis and Waller (1998) estimated that the gut symbionts of *Reticulitermes* fix several grams of N per log per year in the south-eastern United States and suggested that it may be most ecologically relevant to view termite-infested logs as N ‘hotspots’, rather than thinking of inputs in terms of per unit area. The authors also suggest that the uneven input of N by termites may contribute to the nutrient patchiness of forest soils. This may be especially true for mound-building termites that nest in the same location for long periods of time. Salick *et al.* (1983) provided an excellent demonstration of this in Venezuela, where termite nests represent rich nutrient patches compared with the highly weathered soil along the Rio Negro. While termites limited the establishment of plants on inhabited nests, seedlings quickly established on the nests once they were abandoned, suggesting that there may be localised effects of termites on forest productivity. Similarly, termites attacking the cores of living trees may result in localised inputs of nutrients to the soil below, with the potential to stimulate tree growth (Janzen, 1976; Apolinário & Martius, 2004). This possibility remains largely untested, although Werner and Prior (2007) reported a negative correlation between the relative size of termite-created tree hollows and tree growth in northern Australia. In Canada, Cobb *et al.* (2010) showed that the larvae of large cerambycid beetles (*Monochamus s. scutellatus*) increase the total amount of N in the soil beneath infested trees in post-fire boreal forests by expelling frass. In a laboratory study, the same researchers showed that additions of *Monochamus* frass reduced the germination and growth of several plant species that commonly colonise post-fire forests in their study area. These findings may reflect altered N dynamics in frass-augmented soils.

Although termites and other wood-feeding insects act to concentrate N in dead wood or in their mounds, the element is more widely disseminated by dispersing individuals or those consumed by and incorporated into higher trophic levels. The annual production of winged reproductive individuals (i.e. alates) represents a major loss of N and other resources for many termite species. Wood and Sands (1978) summarised estimates of alate production by different species of termites. In some cases, over a quarter of all individuals leave as alates and these individuals are typically heavier and have a higher caloric value than other castes. A wide range of insect (especially ants) and vertebrate predators consume termites (Wood & Sands, 1978) and these organisms probably play an especially important role in releasing nutrients immobilised in termite tissues. In a desert system, Schaefer and Whitford (1981) found predation to represent the major release of nutrients from termites to the soil, amounting to approximately $0.4\,\mathrm{kg\,ha^{-1}\,year^{-1}}$. Lee (1983) estimated that N returned to the ecosystem through predation on termites generally ranges from <1 to $25\,\mathrm{kg\,ha^{-1}\,year^{-1}}$, but can possibly exceed $100\,\mathrm{kg\,ha^{-1}\,year^{-1}}$. Tayasu *et al.* (1994) speculated about the importance of termites in the flow of N from the atmosphere to higher trophic levels. The effects of wood-feeding insects on N cycling thus appear to have two components. The first involves the concentration of N in wood and the localised release of nutrients immobilised in fungal tissues, and the second involves the diffuse release of N over wide areas by dispersing individuals and higher trophic levels.

Conclusions

Wood represents an abundant resource for organisms possessing the enzymes necessary to break down cellulose and lignin. The activities of these organisms are restricted by the N-limiting nature of wood, however, with important implications for decomposition rates and nutrient cycling. The translocation and fixation of N by cord-forming fungi and prokaryotes, respectively, represent key mechanisms by which these organisms supplement their needs with external sources of N. These processes create opportunities for other organisms as well; for example, it is clear from the 40 years of research reviewed herein that wood-feeding insects commonly live in symbiosis with N_2-fixing prokaryotes. The possibilities that saproxylic insects accelerate nutrient cycling, result in increased rates of N_2 fixation and promote forest productivity remain speculative in the absence of experimental evidence. Research in these areas is needed to more fully understand the ecological roles of these organisms and to properly recognise the ecosystem services they provide.

Acknowledgements

I am grateful to two anonymous reviewers and the associate editor for comments that greatly improved an earlier version of the manuscript.

References

Adamson, A.M. (1943) Termites and the fertility of soils. *Tropical Agriculture*, **20**, 107–112.

Aho, P., Seidler, R.J., Evans, H.J. & Raju, P.N. (1974) Distribution, enumeration, and identification of nitrogen-fixing bacteria associated with decay in living white fir trees. *Phytopathology*, **64**, 1413–1420.

Apolinário, F.E. & Martius, C. (2004) Ecological role of termites (Insecta, Isoptera) in tree trunks in central Amazonian rain forests. *Forest Ecology and Management*, **194**, 23–28.

Ausmus, B.S. (1977) Regulation of wood decomposition rates by arthropod and annelid populations. *Ecological Bulletins (Stockholm)*, **25**, 180–192.

Ayayee, P., Rosa, C., Ferry, J.G., Felton, G., Saunders, M. & Hoover, K. (2014) Gut microbes contribute to nitrogen provisioning in a wood-feeding cerambycid. *Environmental Entomology*, **43**, 903–912.

Baker, J.M. (1969) Digestion of wood by *Anobium punctatum* DeGeer and comparison with some other wood boring beetles. *Proceedings of the Royal Entomological Society of London C*, **33**, 31.

Batalha, L.S., Da Silva Filho, D.F. & Martius, C. (1995) Using termite nests as a source of organic matter in agrosilvicultural production systems in Amazonia. *Scientia Agricola*, **52**, 318–325.

Benemann, J.R. (1973) Nitrogen fixation in termites. *Science*, **181**, 164–165.

Bentley, B.L. (1984) Nitrogen fixation in termites: fate of newly fixed nitrogen. *Journal of Insect Physiology*, **30**, 653–655.

Black, H.I.J. & Okawol, M.J.N. (1997) Agricultural intensification, soil biodiversity and agroecosystem function in the tropics: the role of termites. *Applied Soil Ecology*, **6**, 37–53.

Boddy, L. (1999) Saprotrophic cord-forming fungi: meeting the challenge of heterogeneous environments. *Mycologia*, **91**, 13–32.

Boddy, L. & Watkinson, S.C. (1995) Wood decomposition, higher fungi, and their role in nutrient redistribution. *Canadian Journal of Botany*, **73**, S1377–S1383.

Breznak, J.A. (1975) Symbiotic relationships between termites and their intestinal microbiota. *Symposia of the Society for Experimental Biology*, **29**, 559–580.

Breznak, J.A. (2000) Ecology of prokaryotic microbes in the guts of wood- and litter-feeding termites. *Termites: Evolution, Sociality, Symbioses, Ecology* (ed. by T. Abe, D. Bignell and M. Higashi), pp. 209–231. Kluwer Academic Publishers, Dordrecht, Netherlands.

Breznak, J.A., Brill, W.J., Mertins, J.W. & Coppel, H.C. (1973) Nitrogen fixation in termites. *Nature*, **244**, 577–580.

Breznak, J.A., Mertins, J.W. & Coppel, H.C. (1974) Nitrogen fixation and methane production in wood-eating cockroach, *Cryptocercus punctulatus* Scudder (Orthoptera: Blattidae). *University of Wisconsin Forestry Research Notes*, **184**, 1–2.

Bridges, J.R. (1981) Nitrogen-fixing bacteria associated with bark beetles. *Microbial Ecology*, **7**, 131–137.

Brouwer, J. & Bouma, J. (1997) *Soil and Crop Growth Variability in the Sahel: Highlights of Research (1990–1995) at ICRISAT Sahelian Center*. Information Bulletin no. 49. International Crops Research Instituted for the Semi-Arid Tropics, Andhra Pradesh, India.

Brune, A. (2014) Symbiotic digestion of lignocellulose in termite guts. *Nature Reviews Microbiology*, **12**, 168–180.

Brune, A. & Ohkuma, M. (2011) Role of the termite gut microbiota in symbiotic digestion. *Biology of Termites: A Modern Synthesis* (ed. by D. E. Bignell, Y. Roisin and N. Lo), pp. 439–475. Springer, New York, New York.

Brunner, A. & Kimmins, J.P. (2003) Nitrogen fixation in coarse woody debris of *Thuja plicata* and *Tsuga heterophylla* forests on northern Vancouver island. *Canadian Journal of Forest Research*, **33**, 1670–1682.

Burnett, J.R. (1948) Crop production. *Agriculture in the Sudan* (ed. by J. D. Tothill), pp. 275–301. Oxford University Press, London, U.K.

Ceja-Navarro, J.A., Nguyen, N.H., Karaoz, U., Gross, S.R., Herman, D.J., Andersen, G.L. *et al.* (2014) Compartmentalized microbial composition, oxygen gradients and nitrogen fixation in the gut of *Odontotaenius disjunctus*. *ISME Journal*, **8**, 6–18.

Chappell, D.J. & Slaytor, M. (1986) Nitrogen fixation in the higher termite, *Nasutitermes walkeri*. *The Eighth Australian Nitrogen Fixation Conference* (ed. by W. Wallace and S. E. Smith), pp. 119–120. The Australian Institute of Agricultural Science, University of Adelaide, Adelaide, Australia.

Chen, H. & Hicks, W. (2003) High asymbiotic N_2 fixation rates in woody roots after six years of decomposition: controls and implications. *Basic and Applied Ecology*, **4**, 479–486.

Citernesi, U., Neglia, R., Seritti, A., Lepidi, A.A., Filippi, C., Bagnoli, G. *et al.* (1977) Nitrogen fixation in the gastro-enteric cavity of soil animals. *Soil Biology and Biochemistry*, **9**, 71–72.

Cleveland, L.R. (1925) The ability of termites to live perhaps indefinitely on a diet of pure cellulose. *Biological Bulletin*, **48**, 289–293.

Cobb, T.P., Hannam, K.D., Kishchuk, B.E., Langor, D.W., Quideau, S.A. & Spence, J.R. (2010) Wood-feeding beetles and soil nutrient cycling in burned forests: implications of post-fire salvage logging. *Agricultural and Forest Entomology*, **12**, 9–18.

Cowling, E.B. & Merrill, W. (1966) Nitrogen in wood and its role in wood deterioration. *Canadian Journal of Botany*, **44**, 1539–1554.

Curtis, A.D. & Waller, D.A. (1995) Changes in nitrogen fixation rates in termites (Isoptera: Rhinotermitidae) maintained in the laboratory. *Annals of the Entomological Society of America*, **88**, 764–767.

Curtis, A.D. & Waller, D.A. (1996) The effects of decreased pO_2 and increased pCO_2 on nitrogen fixation rates in termites (Isoptera: Rhinotermitidae). *Journal of Insect Physiology*, **42**, 867–872.

Curtis, A.D. & Waller, D.A. (1997) Variation in rates of nitrogen fixation in termites: response to dietary nitrogen in the field and laboratory. *Physiological Entomology*, **22**, 303–309.

Curtis, A.D. & Waller, D.A. (1998) Seasonal patterns of nitrogen fixation in termites. *Functional Ecology*, **12**, 803–807.

Desai, M.S. & Brune, A. (2012) Bacteroidales ectosymbionts of gut flagellates shape the nitrogen-fixing community in dry-wood termites. *ISME Journal*, **6**, 1302–1313.

Donovan, S.E., Eggleton, P. & Bignell, D.E. (2001) Gut content analysis and a new feeding group classification of termites. *Ecological Entomology*, **26**, 356–366.

Du, X., Li, X., Wang, Y., Peng, J., Hong, H. & Yang, H. (2012) Phylogenetic diversity of nitrogen fixation genes in the intestinal tract of *Reticulitermes chinensis* Snyder. *Current Microbiology*, **65**, 547–551.

Edmonds, R.L. & Eglitis, A. (1989) The role of the Douglas-fir beetle and wood borers in the decomposition of and nutrient release from Douglas-fir logs. *Canadian Journal of Forest Research*, **19**, 853–859.

Eutick, M.L., O'Brien, R.W. & Slaytor, M. (1978) Bacteria from the gut of Australian termites. *Applied and Environmental Microbiology*, **35**, 823–828.

Evans, T.A., Dawes, T.Z., Ward, P.R. & Lo, N. (2011) Ants and termites increase crop yield in a dry climate. *Nature Communications*, **2**, 262.

French, J.R.J., Turner, G.L. & Bradbury, J.F. (1976) Nitrogen fixation by bacteria from the hindgut of termites. *Journal of General Microbiology*, **95**, 202–206.

Fujita, A. & Abe, T. (2006) Atmospheric nitrogen assimilation by a wood-feeding termite, *Reticulitermes speratus* (Isoptera: Rhinotermitidae). *Sociobiology*, **47**, 175–188.

Garrett, L.G., Kimberley, M.O., Oliver, G.R., Pearce, S.H. & Beets, P.N. (2012) Decomposition of coarse woody roots and branches in managed *Pinus radiata* plantations in New Zealand – A time series approach. *Forest Ecology and Management*, **269**, 116–123.

Golichenkov, M.V., Kostina, N.V., Ul'yanova, T.A., Kuznetsova, T.A. & Umarov, M.M. (2006) Diazotrophs in the digestive tract of termite *Neotermes castaneus*. *Biology Bulletin*, **33**, 508–512.

Gomathi, V., Ramalakshmi, A. & Ramasamy, K. (2005) Isolation of nitrogen fixing bacteria from fungus termites. *Entomological Research*, **35**, 75–78.

Griffiths, R.P., Harmon, M.E., Caldwell, B.A. & Carpenter, S.E. (1993) Acetylene reduction in conifer logs during early stages of decomposition. *Plant and Soil*, **148**, 53–61.

Hardy, R.W.F., Holsten, R.D., Jackson, E.K. & Burns, R.C. (1968) The acetylene-ethylene assay for N_2 fixation: laboratory and field evaluation. *Plant Physiology*, **43**, 1185–1207.

Hewitt, P.H., Van der Westhuizen, M.C., Van der Linde, T.C. & Adam, R.A. (1987) Acetylene reduction by the harvester termite *Hodotermes mossambicus* (Hagen). *Journal of the Entomological Society of Southern Africa*, **50**, 513–520.

Higashi, M., Abe, T. & Burns, T.P. (1992) Carbon-nitrogen balance and termite ecology. *Proceedings of the Royal Society B*, **249**, 303–308.

Holt, J.A. & Lepage, M. (2000) Termites and soil properties. *Termites: Evolution, Sociality, Symbioses, Ecology* (ed. by T. Abe, D. Bignell and M. Higashi), pp. 389–407. Kluwer Academic Publishers, Dordrecht, Netherlands.

Hongoh, Y., Deevong, P., Inoue, T., Moriya, S., Trakulnaleamsai, S., Ohkuma, M. *et al.* (2005) Intra- and interspecific comparisons of bacterial diversity and community structure support coevolution of gut microbiota and termite host. *Applied and Environmental Microbiology*, **71**, 6590–6599.

Hongoh, Y., Sharma, V.K., Prakash, T., Noda, S., Toh, H., Taylor, T.D. *et al.* (2008) Genome of an endosymbiont coupling N_2 fixation to cellulolysis within protist cells in termite gut. *Science*, **322**, 1108–1109.

Janzen, D.H. (1976) Why tropical trees have rotten cores. *Biotropica*, **8**, 110.

Jönsson, N., Méndez, M. & Ranius, T. (2004) Nutrient richness of wood mould in tree hollows with the scarabaeid beetle *Osmoderma eremita*. *Animal Biodiversity and Conservation*, **27**, 79–82.

Jouquet, P., Traoré, S., Choosai, C., Hartmann, C. & Bignell, D.E. (2011) Influence of termites on ecosystem functioning. Ecosystem services provided by termites. *European Journal of Soil Biology*, **47**, 215–222.

Käärik, A.A. (1974) Decomposition of wood. *Biology of Plant Litter Decomposition*, Vol. **1** (ed. by C. H. Dickinson and G. J. F. Pugh), pp. 129–174. Academic Press, London, U.K.

Kitchell, J.F., O'Neill, R.V., Webb, D., Gallepp, G.W., Bartell, S.M., Koonce, J.F. *et al.* (1979) Consumer regulation of nutrient cycling. *Bioscience*, **29**, 28–34.

Krankina, O.N., Harmon, M.E. & Griazkin, A.V. (1999) Nutrient stores and dynamics of woody detritus in a boreal forest: modeling potential implications at the stand level. *Canadian Journal of Forest Research*, **29**, 20–32.

Krishna, K., Grimaldi, D., Krishna, V. & Engel, M.S. (2013) Treatise on the Isoptera of the world, volume 1: introduction. *Bulletin of the American Museum of Natural History*, **377**, 1–200.

Kudo, T., Ohkuma, M., Moriya, S., Noda, S. & Ohtoko, K. (1998) Molecular phylogenetic identification of the intestinal anaerobic microbial community in the hindgut of the termite, *Reticulitermes speratus*, without cultivation. *Extremophiles*, **2**, 155–161.

Kuhnigk, T., Branke, J., Krekeler, D., Cypionka, H. & König, H. (1996) A feasible role of sulfate-reducing bacteria in the termite gut. *Systematic and Applied Microbiology*, **19**, 139–149.

Kuranouchi, T., Nakamura, T., Shimamura, S., Kojima, H., Goka, K., Okabe, K. *et al.* (2006) Nitrogen fixation in the stag beetle, *Dorcus* (*Macrodorcus*) *rectus* (Motschulsky) (Col., Lucanidae). *Journal of Applied Entomology*, **130**, 471–472.

Laiho, R. & Prescott, C.E. (2004) Decay and nutrient dynamics of coarse woody debris in northern coniferous forests: a synthesis. *Canadian Journal of Forest Research*, **34**, 763–777.

Lavelle, P., Bignell, D., Lepage, M., Wolters, V., Roger, P., Ineson, P. *et al.* (1997) Soil function in a changing world: the role of invertebrate ecosystem engineers. *European Journal of Soil Biology*, **33**, 159–193.

LeBauer, D.S. & Treseder, K.K. (2008) Nitrogen limitation of net primary producivity in terrestrial ecosystems is globally distributed. *Ecology*, **89**, 371–379.

Lee, K.E. (1983) The influence of earthworms and termites on soil nitrogen cycling. *New Trends in Soil Biology* (ed. by P. Lebrun, H. M. André, A. de Medts, C. Grégoire-Wibo and G. Wauthy), pp. 35–48. Imprimeur Dieu-Brichart, Ottignies-Louvain-la-Neuve, Belgium.

Lilburn, T.G., Kim, K.S., Ostrom, N.E., Byzek, K.R., Leadbetter, J.R. & Breznak, J.A. (2001) Nitrogen fixation by symbiotic and free-living Spirochetes. *Science*, **292**, 2495–2498.

Logan, J.W.M. (1992) Termites (Isoptera): a pest or resource for small farmers in Africa? *Tropical Science*, **32**, 71–79.

Lovelock, M., O'Brien, R.W. & Slaytor, M. (1985) Effect of laboratory containment on the nitrogen metabolism of termites. *Insect Biochemistry*, **15**, 503–509.

Merrill, W. & Cowling, E.B. (1966) Role nitrogen in of wood deterioration: amount and distribution of nitrogen in fungi. *Phytopathology*, **56**, 1083–1090.

Meuti, M.E., Jones, S.C. & Curtis, P.S. (2010) ^{15}N discrimination and the sensitivity of nitrogen fixation to changes in dietary nitrogen in *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). *Environmental Entomology*, **39**, 1810–1815.

Micó, E., Juárez, M., Sánchez, A. & Galante, E. (2011) Action of the saproxylic scarab larva *Cetonia aurataeformis* (Coleoptera: Scarabaeoidea: Cetoniidae) on woody substrates. *Journal of Natural History*, **45**, 2527–2542.

Morales-Jiménez, J., Zúñiga, G., Villa-Tanaca, L. & Hernández-Rodríguez, C. (2009) Bacterial community and nitrogen fixation in the red turpentine beetle, *Dendroctonus valens* LeConte (Coleoptera: Curculionidae: Scolytinae). *Microbial Ecology*, **58**, 879–891.

Morales-Jiménez, J., Zúñiga, G., Ramírez-Saad, H.C. & Hernández-Rodríguez, C. (2012) Gut-associated bacteria throughout the life cycle of the bark beetle *Dendroctonus rhizophagus* Thomas and Bright (Curculionidae: Scolytinae) and their cellulolytic activities. *Microbial Ecology*, **64**, 268–278.

Morales-Jiménez, J., León, V.-P.d., García-Domínguez, A., Martínez-Romero, E., Zúñiga, G. & Hernández-Rodríguez, C. (2013) Nitrogen-fixing and uricolytic bacteria associated with the gut of *Dendroctonus rhizophagus* and *Dendroctonus valens* (Curculionidae: Scolytinae). *Microbial Ecology*, **66**, 200–210.

Nardi, J.B., Mackie, R.I. & Dawson, J.O. (2002) Could microbial symbionts of arthropod guts contribute significantly to nitrogen fixation in terrestrial ecosystems? *Journal of Insect Physiology*, **48**, 751–763.

Noda, S., Ohkuma, M., Usami, R., Horikoshi, K. & Kudo, T. (1999) Culture-independent characterisation of a gene responsible for nitrogen fixation in the symbiotic microbial community in the gut of the termite *Neotermes koshunensis*. *Applied and Environmental Microbiology*, **65**, 4935–4942.

Nyamapfene, K.W. (1986) The use of termite mounds in Zimbabwe peasant agriculture. *Tropical Agriculture*, **63**, 191–192.

Ohkuma, M., Noda, S., Usami, R., Horikoshi, K. & Kudo, T. (1996) Diversity of nitrogen fixation genes in the symbiotic intestinal microflora of the termite *Reticulitermes speratus*. *Applied and Environmental Microbiology*, **62**, 2747–2752.

Ohkuma, M., Noda, S. & Kudo, T. (1999) Phylogenetic diversity of nitrogen fixation genes in the symbiotic microbial community in the gut of diverse termites. *Applied and Environmental Microbiology*, **65**, 4926–4934.

Palviainen, M., Finér, L., Laiho, R., Shorohova, E., Kapitsa, E. & Vanha-Majamaa, I. (2010) Carbon and nitrogen release from decomposing Scots pine, Norway spruce and silver birch stumps. *Forest Ecology and Management*, **259**, 390–398.

Pandey, S., Waller, D.A. & Gordon, A.S. (1992) Variation in acetylene-reduction (Nitrogen fixation) rates in *Reticulitermes* spp. (Isoptera; Rhinotermitidae). *Virginia Journal of Science*, **43**, 333–338.

Peklo, J. & Satava, J. (1949) Fixation of free nitrogen by bark beetles. *Nature*, **163**, 336–337.

Potrikus, C.J. & Breznak, J.A. (1977) Nitrogen-fixing *Enterobacter agglomerans* isolated from guts of wood-eating termites. *Applied and Environmental Microbiology*, **33**, 392–399.

Prestwich, G.D. & Bentley, B.L. (1981) Nitrogen fixation by intact colonies of the termite *Nasutitermes corniger*. *Oecologia*, **49**, 249–251.

Prestwich, G.D. & Bentley, B.L. (1982) Ethylene production by the fungus comb of macrotermitines (Isoptera, Termitidae): a caveat for the use of the acetylene reduction assay for nitrogenase activity. *Sociobiology*, **7**, 145–152.

Prestwich, G.D., Bentley, B.L. & Carpenter, E.J. (1980) Nitrogen sources for neotropical nasute termites: fixation and selective foraging. *Oecologia*, **46**, 397–401.

Reed, S.C., Cleveland, C.C. & Townsend, A.R. (2011) Functional ecology of free-living nitrogen fixation: a contemporary perspective. *Annual Review of Ecology and Systematics*, **42**, 489–512.

Reid, N.M. & Lloyd-Jones, G. (2009) Symbiotic nitrogen fixation in the New Zealand dampwood termite (*Stolotermes ruficeps*). *New Zealand Journal of Ecology*, **33**, 90–95.

Reid, N.M., Addison, S.L., Macdonald, L.J. & Lloyd-Jones, G. (2011) Biodiversity of active and inactive bacteria in the gut flora of wood-feeding Huhu beetle larvae (*Prionoplus reticularis*). *Applied and Environmental Microbiology*, **77**, 7000–7006.

Robinson, D. (2001) δ15N as an integrator of the nitrogen cycle. *Trends in Ecology & Evolution*, **16**, 153–162.

Rohrmann, G.F. & Rossman, A.Y. (1980) Nutrient strategies of *Macrotermes ukuzii* (Isoptera: Termitidae). *Pedobiologia*, **20**, 61–73.

Rouland-Lefèvre, C. (2000) Symbiosis with fungi. *Termites: Evolution, Sociality, Symbioses, Ecology* (ed. by T. Abe, D. Bignell and M. Higashi), pp. 289–306. Kluwer Academic Publishers, Dordrecht, Netherlands.

Salick, J., Herrera, R. & Jordan, C.F. (1983) Termitaria: nutrient patchiness in nutrient-deficient rain forests. *Biotropica*, **15**, 1–7.

Schaefer, D.A. & Whitford, W.G. (1979) Nitrogen fixation in desert termites. *Bulletin of the Ecological Society of America*, **60**, 128.

Schaefer, D.A. & Whitford, W.G. (1981) Nutrient cycling by the subterranean termite *Gnathamitermes tubiformans* in a Chihuahuan desert ecosystem. *Oecologia*, **48**, 277–283.

Seastedt, T.R. (1984) The role of microarthropods in decomposition and mineralization processes. *Annual Review of Entomology*, **29**, 25–46.

Sollins, P., Cline, S.P., Verhoeven, T., Sachs, D. & Spycher, G. (1987) Patterns of log decay in old-growth Douglas-fir forests. *Canadian Journal of Forest Research*, **17**, 1585–1595.

Stokland, J.N., Siitonen, J. & Jonsson, B.G. (2012) *Biodiversity in Dead Wood*. Cambridge University Press, Cambridge, U.K.

Swift, M.J. (1977) The roles of fungi and animals in the immobilisation and release of nutrient elements from decomposing branch-wood. *Ecological Bulletins*, **25**, 193–202.

Swift, M.J. & Boddy, L. (1984) Animal-microbial interactions in wood decomposition. *Invertebrate-microbial Interactions* (ed. by J. M. Anderson, A. D. M. Rayner and D. W. H. Walton), pp. 89–131. Cambridge University Press, Cambridge, U.K.

Swift, M.J., Heal, O.W. & Anderson, J.M. (1979) *Decomposition in Terrestrial Ecosystems*. University of California Press, Berkeley, California.

Sylvester-Bradley, R., Bandeira, A.G. & de Oliveira, L.A. (1978) Fixaçao de nitrogênio (reduçao de acetileno) em cupins (Insecta: Isoptera) da Amazonica central. *Acta Amazonica*, **8**, 621–627.

Sylvester-Bradley, R., de Oliveira, L.A. & Bandeira, A.G. (1983) Nitrogen fixation in *Nasutitermes* in central Amazonia. *Social Insects in the Tropics* (ed. by P. Jaisson), Vol. 2, pp. 235–244. Universite Paris-Nord, Paris, France.

Takamura, K. (2001) Effects of termite exclusion on decay of heavy and light hardwood in a tropical rain forest of peninsular Malaysia. *Journal of Tropical Ecology*, **17**, 541–548.

Takamura, K. & Kirton, L.G. (1999) Effects of termite exclusion on decay of a high-density wood in tropical rain forests of peninsular Malaysia. *Pedobiologia*, **43**, 289–296.

Tayasu, I., Sugimoto, A., Wada, E. & Abe, T. (1994) Xylophagous termites depending on atmospheric nitrogen. *Naturwissenschaften*, **81**, 229–231.

Tayasu, I., Abe, T., Eggleton, P. & Bignell, D.E. (1997) Nitrogen and carbon isotope ratios in termites: an indicator of trophic habit along the gradient from wood-feeding to soil-feeding. *Ecological Entomology*, **22**, 343–351.

Tayasu, I., Inoue, T., Miller, L.R., Sugimoto, A., Takeichi, S. & Abe, T. (1998) Confirmation of soil-feeding termites (Isoptera; Termitidae; Termitinae) in Australia using stable isotope ratios. *Functional Ecology*, **12**, 536–542.

Thompson, B.M. (2013) *Community ecology and* Sirex noctilio: *interactions with microbial symbionts and native insects*. PhD dissertation, University of Maryland, College Park, Maryland.

Ulyshen, M.D. (2015) Wood decomposition as influenced by invertebrates. *Biological Reviews*. DOI: 10.1111/brv.12158.

Vecherskii, M.V., Kostina, N.V., Gorlenko, M.V., Dobrovol'skaya, T.G. & Umarov, M.M. (2008) Specific features of nitrogen fixation in the termite *Reticulitermes lucifugus*. *Biology Bulletin*, **35**, 446–451.

Vitousek, P.M. & Howarth, R.W. (1991) Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry*, **13**, 87–115.

Waller, D.A. & Curtis, A.D. (2003) Effects of sugar-treated foods on preference and nitrogen fixation in *Reticulitermes flavipes* (Kollar) and *Reticulitermes virginicus* (Banks) (Isoptera: Rhinotermitidae). *Annals of the Entomological Society of America*, **96**, 81–85.

Waller, D.A., Breitenbeck, G.A. & La Fage, J.P. (1989) Variation in acetylene reduction by *Coptotermes formosanus* (Isoptera: Rhinotermitidae) related to colony source and termite size. *Sociobiology*, **16**, 191–196.

Warnecke, F., Luginbühl, P., Ivanova, N., Ghassemian, M., Richardson, T.H., Stege, J.T. *et al.* (2007) Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. *Nature*, **450**, 560–565.

Werner, P.A. & Prior, L.D. (2007) Tree-piping termites and growth and survival of host trees in savanna woodland of north Australia. *Journal of Tropical Ecology*, **23**, 611–622.

Wood, T.G. (1976) The role of termites (Isoptera) in decomposition processes. *The Role of Terrestrial and Aquatic Organisms in Decomposition Processes* (ed. by J. M. Anderson and A. Macfadyen), pp. 145–168. Blackwell Scientific, Oxford, U.K.

Wood, T.G. (1996) The agricultural importance of termites in the tropics. *Agricultural Zoology Reviews*, **7**, 117–155.

Wood, T.G. & Sands, W.A. (1978) The role of termites in ecosystems. *Production Ecology of Ants and Termites* (ed. by M. V. Brian), pp. 245–292. Cambridge University Press, Cambridge, U.K.

Yamada, A., Inoue, T., Wiwatwitaya, D., Ohkuma, M., Kudo, T. & Sugimoto, A. (2006) Nitrogen fixation by termites in tropical forests, Thailand. *Ecosystems*, **9**, 75–83.

Yamada, A., Inoue, T., Noda, S., Hongoh, Y. & Ohkuma, M. (2007) Evolutionary trend of phylogenetic diversity of nitrogen fixation genes in the gut community of wood-feeding termites. *Molecular Ecology*, **16**, 3768–3777.

Zehr, J.P., Jenkins, B.D., Short, S.M. & Steward, G.F. (2003) Nitrogenase gene diversity and microbial community structure: a cross-system comparison. *Environmental Microbiology*, **5**, 539–554.

Accepted 21 November 2014