Symbiotic digestion of lignocellulose in termite guts

Andreas Brune

Abstract | Their ability to degrade lignocellulose gives termites an important place in the carbon cycle. This ability relies on their partnership with a diverse community of bacterial, archaeal and eukaryotic gut symbionts, which break down the plant fibre and ferment the products to acetate and variable amounts of methane, with hydrogen as a central intermediate. In addition, termites rely on the biosynthetic capacities of their gut microbiota as a nutritional resource. The mineralization of humus components in the guts of soil-feeding species also contributes to nitrogen cycling in tropical soils. Lastly, the high efficiency of their minute intestinal bioreactors makes termites promising models for the industrial conversion of lignocellulose into microbial products and the production of biofuels.

Department of Biogeochemistry, Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch-Strasse 10, 35043 Marburg, Germany.
e-mail: brune@mpi-marburg.mpg.de
doi:10.1038/nrmicro3182
Published online
3 February 2014

Termites are found on all continents, except Antarctica, but are most abundant in the tropics and subtropics. They are small insects, but the combination of their sociality with their ability to efficiently digest lignocellulose led to a tremendous evolutionary success. Today, termites contribute up to 95% of the insect biomass in tropical soils, and in the African savannah, their biomass densities can surpass that of grazing mammals[1,2]. Almost 3,000 termite species have been described but, contrary to common belief, relatively few pose a danger to wooden structures. Nevertheless, their economic impact in the United States alone amounts to billions of US$ per year[3], and termite damage in tropical agriculture is considerable[4]. However, in general, termite activity increases soil fertility and crop yield[5,6] and makes important contributions to ecosystem processes, particularly in arid climates[7]; these are beneficial effects that are difficult to express on a monetary basis.

All termites feed on lignocellulose, which is the principal cell wall component of woody plants, and it is consumed either in the form of sound wood or in different stages of decomposition[2,8]. The intimate complex of cellulose, hemicelluloses and lignin is highly recalcitrant to enzymatic attack, and rapid mineralization of lignocellulose by termites contrasts with its slow and often incomplete breakdown in soil. With the removal of 74–99% of the cellulose and 65–87% of the hemicellulose components, the digestion of wood by termites is far more efficient than that of the less lignified forage grasses by ruminants[1,9].

In the nineteenth century, the American naturalist Joseph Leidy suspected that the ability of termites to thrive on a diet of wood was related to the conspicuous presence of 'parasites' in their hindgut paunch and concluded that 'the infestation … is so habitual and constant that it appears to be their normal condition' (REF. 10). We now know that the 'parasites' are in fact mutualistic symbionts that make essential contributions to the digestive process and that comprise representatives from all three domains of life[11]. Whereas bacteria and archaea are present in all termites, cellulolytic flagellates occur exclusively in the evolutionarily basal lineages, which are referred to as 'lower termites' (BOX 1).

Even 130 years after Leidy's observations, the subject has not lost its fascination. In this Review, I mostly cover the work of the past decade, which has greatly illuminated the role of the termite gut microbiota in symbiotic digestion. After outlining the different digestive strategies of the major termite lineages, I explain how termites efficiently break down lignocellulose by combining their own mechanical and enzymatic contributions with the catalytic capacities of their respective microbial partners. Focusing on the prokaryotic symbionts, I describe the diversity of microorganisms in the hindgut bioreactor and the functions of the major microbial populations, which not only contribute to the hydrolysis and subsequent fermentation of plant fibre but which also compensate for the severe nutritional deficits of the lignocellulosic diet.

Digestive strategies

Whereas the foregut and midgut of termites are relatively small, the hindgut is always enlarged, forming a paunch that houses the bulk of the symbionts (FIG. 1). However,

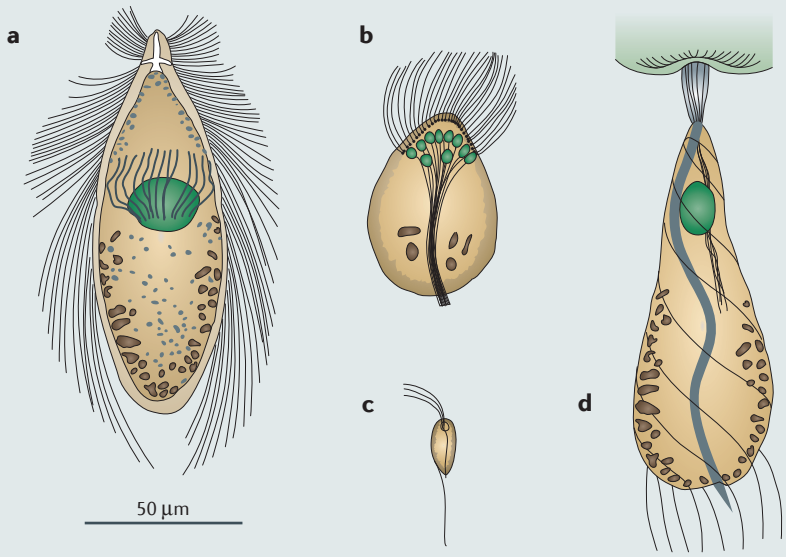
Box 1 | **Termite gut flagellates**

All lower termites harbour flagellate protists that fill up the bulk of the hindgut paunch. Their origin is obscure, but it is generally assumed that a common ancestor of termites and their sister group (which are cockroaches of the family Cryptocercidae) acquired an ancestral set of these protists, which then evolved together with their termite host. Despite occasional losses of flagellates and their horizontal transfer among members of different termite families, the flagellate assemblages are shaped by co-speciation and are characteristic for each termite species[12,123,134].

The flagellate assemblages can be simple (three species in *Coptotermes formosanus*) or quite complex (19 species in *Hodotermopsis japonica*), and each species seems to have a specific role in digestion. The large (see the figure, part **a**) and medium-sized (part **b**) flagellates are cellulolytic and xylanolytic, whereas many smaller species (part **c**) do not ingest wood but probably take up soluble substrates from the hindgut fluid[123,135]. It is assumed that cellulases from glycoside hydrolase family 7 (GHF7) were present in flagellates before they became associated with termites, whereas enzymes from other GHFs may have been acquired by lateral gene transfer from other termite gut microbiota, either at ancestral (for example, GHF5) or more recent stages (for example, GHF10 or GHF11) of the symbiosis[136].

Termite gut flagellates belong to two eukaryotic phyla — the Parabasalia and the Preaxostyla (order Oxymonadida)[18]. Parabasalid flagellates (parts **a**–**c**) comprise several lineages that convergently evolved to have huge cells with multiple flagella[134]. They are often gigantic in size and highly mobile in the viscous gut environment, which prevents washout and helps them to compete for wood particles that are phagocytized as they pass through the enteric valve. The presence of hydrogenosomes suggests that hydrogen is a typical fermentation product of all parabasalid species. Oxymonadid flagellates lack hydrogenosomes. They are either highly motile or attached to the inside of the hindgut wall[17,123] (part **d**). Many of the larger species contain wood particles, but their cellulolytic or hemicellulolytic capacities remain to be studied.

The illustrations show parabasalid flagellates of the genera *Trichonympha* (part **a**), *Calonympha* (part **b**) and *Tricercomitus* (part **c**), and an oxymonadid flagellate of the genus *Pyrsonympha* (part **d**).



Hydrogenosomes
The hydrogen-producing organelles of many anaerobic protists; they share a common origin with mitochondria but only generate ATP by substrate-level phosphorylation.

the major termite lineages differ substantially in the nature of their diet, their primary cellulolytic partners and the microbial communities that colonize the different compartments of their digestive tracts.

Lower termites — a tripartite symbiosis. The hallmark of termite evolution was the acquisition of cellulolytic gut flagellates during the Late Jurassic period (~150 million years ago), which gave a presumably omnivorous, ancestral cockroach the capacity to digest wood[12]. The seminal work of Cleveland in the early 1920s[1] showed the obligate nature of this symbiosis. If termites are 'cured' of these anaerobic protists either by starvation or by treatment with hyperbaric oxygen (which are procedures that render the entire hindgut paunch oxic)[13], they continue to feed on wood but die of starvation within a few weeks. However, they remain viable if they are switched to a starch diet or re-inoculated with gut flagellates during contact with untreated nestmates. More than two decades later, Hungate's equally inspiring experiments clarified that the flagellates are responsible not only for the hydrolysis of cellulose but also for the generation of the bulk of the fermentation products that are eventually resorbed by the host[1].

The most characteristic members of the bacterial microbiota are spirochaetes (phylum Spirochaetes) (FIG. 2), owing to their high abundance and eye-catching morphology and motility. They seem to be almost completely absent from omnivorous cockroaches but form by far the largest group of the microbiota — in both abundance and species richness — in the hindgut of most wood-feeding termites, where they can make up as much as one-half of all prokaryotes[14]. Although associations with flagellates are not uncommon, most spirochaetes are free in the hindgut fluid. Their high mobility in viscous media might enable them to maintain a favourable position in this dynamic environment, and the high surface-to-volume ratio of their cells may function to overcome the diffusion limitation of their metabolic rates, contributing to their success in this habitat[14].

Many of the smaller bacteria and archaea are associated with the hindgut cuticle or colonize filamentous microorganisms that are themselves attached to the hindgut wall[15–17]. However, the most prominent habitats for bacteria and archaea in lower termites are the cytoplasm and external surface of the flagellates[18–20]. The endosymbionts of the larger flagellates often make up a substantial fraction of the bacterial community in the hindgut, as illustrated by a large proportion of Elusimicrobia (specifically, *Candidatus* Endomicrobium)[21–23] in *Reticulitermes* spp. and the clear dominance of Bacteroidetes (specifically, *Candidatus* Azobacteroides)[24,25] over Spirochaetes in *Coptotermes* spp. (FIG. 2).

Dietary diversification in higher termites. Sometime in the Eocene period (~60 million years ago), the complete loss of flagellates in all higher termites meant that new strategies for cellulose digestion were required[12]. The ensuing dietary diversification was an enormous success, both from an evolutionary and an ecological perspective. Today, higher termites represent >80% of all termite species and comprise a variety of different feeding guilds. Whereas the evolutionary lower termites are generally restricted to wood, higher termites (family Termitidae) have greatly enlarged the scope of their diet, which includes dry grass or other plant litter and herbivore dung or organic matter in advanced stages of humification[2,8]. Substantial changes in the composition of the gut microbiota suggest that it gained new functions in the digestive process (FIG. 2).

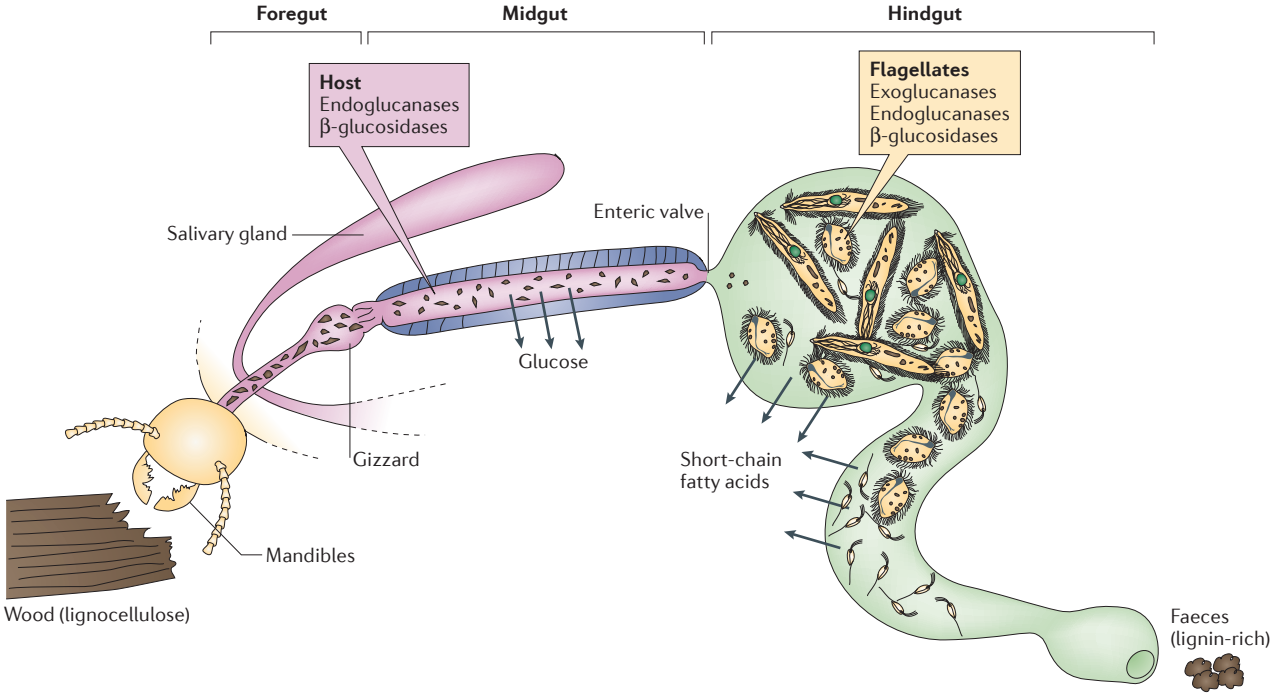


Figure 1 | **The dual-cellulolytic systems of termites.** Lignocellulose digestion in termites involves activities of both the host and its gut microbiota; it is best investigated in lower termites[44,139]. In the foregut, the wood particles that are produced by the mandibles are mixed with enzymes of the salivary glands and further comminuted by the muscular gizzard. Any glucose that is released in the midgut is resorbed via the epithelium, whereas the partially digested wood particles pass through the enteric valve into the voluminous hindgut paunch. They are immediately phagocytized by cellulolytic flagellates, which hydrolyse the remaining polysaccharides using powerful cellulases and hemicellulases that are secreted into their digestive vacuoles. The microbial fermentation products (which are mainly short-chain fatty acids) are resorbed by the host, and the lignin-rich residues are voided as faeces. In higher termites, hindgut bacteria apparently took over the role of the flagellates in cellulose degradation.

The earliest innovation was symbiosis with a basidiomycete fungus, *Termitomyces* spp., which is cultivated on lignocellulosic biomass inside the nest[26]. Such fungus gardens are exclusively found in members of the subfamily Macrotermitinae, which is a relatively small (in terms of species diversity) but highly abundant group. The foraging workers collect plant litter that is only incompletely digested but that is mixed with fungal spores during a first gut passage. The lignocellulose-rich faeces are deposited onto the fungus gardens, which are groomed by their nestmates. The gardens provide their keepers with both fungal biomass and preprocessed plant fibre, which is completely digested during a second gut passage. The specific roles of termite, fungus and intestinal microbiota in the digestive process are not clear. The bacterial communities in *Macrotermes* spp. and *Odontotermes* spp. are dominated by the Bacteroidetes and Firmicutes and thereby deviate substantially from the communities of wood-feeding higher termites[27,28] (FIG. 2), which probably results from their preprocessed and fungus-rich diet[29]. Analysis of the microbial processes in the gut of fungus-cultivating termites is complicated by large differences in the composition of ingested material not only between termite species but also among the worker castes[26,30], which might affect the density and community structure of the bacterial microbiota[27,31].

In all other subfamilies of higher termites, symbiotic digestion is independent of fungal symbionts. In this case, dietary diversification was accompanied by extensive anatomical modifications[8]. Whereas Macrotermitinae still have the simple gut structure of lower termites, which only have a single paunch, the more derived lineages show further elongation and compartmentation of the hindgut and an increased alkalinity in its anterior compartments (FIG. 3). In representatives of both Nasutitermitinae and Termitinae that have cellulose-rich diets, the hindgut is abundantly colonized by spirochaetes and members of the Fibrobacteres and the related candidate phylum TG3 (REFS 32–34). However, in the dung-feeding and humus-feeding lineages of Termitinae, which have adopted a more nitrogen-rich diet, their abundance decreases in favour of firmicutes[35–37], which suggests that both host phylogeny and diet can be important determinants of bacterial community structure in termite guts.

At least three subfamilies of higher termites have evolved to be true soil feeders that thrive exclusively on the humic substances of mineral soil[8]. It had long been assumed that they either hydrolyse residual polysaccharides or degrade polyphenolic components of soil organic matter. However, in feeding trials using radiolabelled model compounds, *Cubitermes* spp. did not

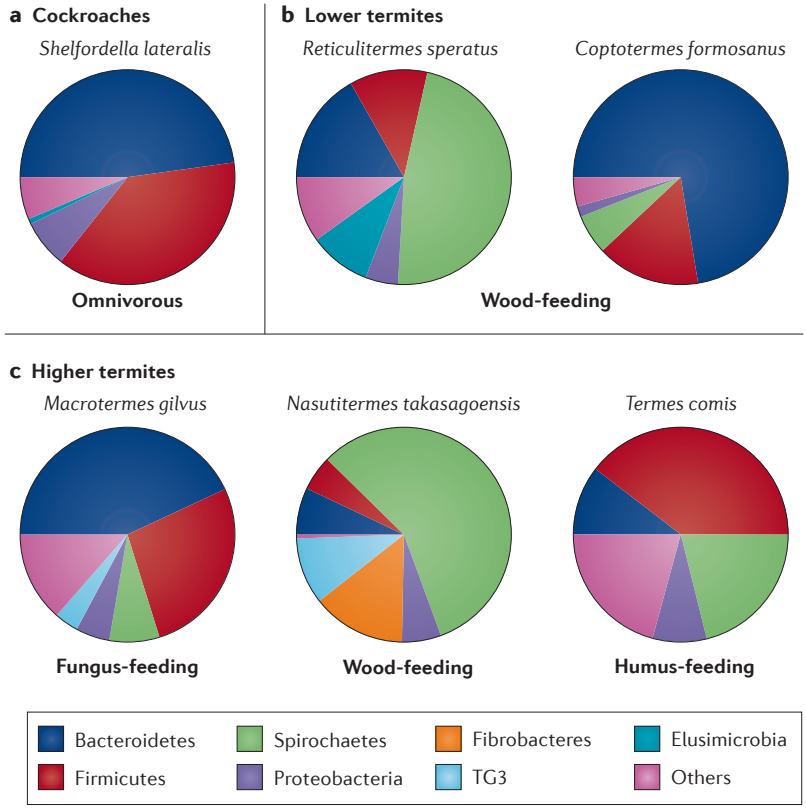


Figure 2 | **Diversity of the bacterial microbiota in termite guts.** Phylum-level classification of bacterial 16S rRNA genes in the hindgut of selected termites from different feeding groups and a closely related omnivorous cockroach (*Shelfordella lateralis*[140], *Reticulitermes speratus*[21], *Coptotermes formosanus*[141], *Macrotermes gilvus*[27], *Nasutitermes takasagoensis*[33], and *Termes comis*[36]). The communities are very diverse and comprise hundreds of different phylotypes, which comprise many lineages of mostly uncultivated bacteria that exclusively occur in this habitat[18]. Recent deep-sequencing efforts indicate that the diversity is even higher than initially thought[37,73]. Spirochaetes (phylum Spirochaetes) are absent in cockroaches (part **a**) but are a characteristic element of the termite gut microbiota in most wood-feeding termites. A notable exception is *C. formosanus*, in which the bacterial community is dominated by Bacteroidetes that colonize the cytoplasm of its largest gut flagellate[24]. Although the bacterial gut microbiota of lower termite species (part **b**) is shaped by the specific symbionts of their gut flagellates, the differences among higher termites (part **c**) may reflect functional adaptations of the community to different diets. The bacterial community in the gut of fungus-feeding termites is most similar to that of omnivorous cockroaches, whereas wood-feeding species harbour large proportions of Fibrobacteres and members of the TG3 phylum, and humus-feeding species are characterized by an abundance of Firmicutes.

Endoglucanases
Cellulases that randomly hydrolyse β-1,4-glycosidic bonds within the amorphous regions of cellulose. This generates additional chain ends, which increases the activity of exoglucanases in a synergistic manner.

β-glucosidases
Enzymes that hydrolyse cellobiose and the oligomeric degradation products of cellulose (such as cellotriose and cellotetraose).

mineralize polyphenols but efficiently digested peptidic or other nitrogenous residues of humic substances (such as chitin and peptidoglycan)[38,39], which are derived from microbial biomass but which are generally protected from degradation by covalent linkage to polyphenols and an intimate association with clay minerals[40,41]. The ability to mobilize such recalcitrant humus constituents is accompanied by an even more pronounced elongation and extreme alkalization (to >pH 12) of the anterior hindgut[42].

Lignocellulose degradation

The glycosidic bonds of the cellulose microfibrils are only accessible to cellulases from the chain ends or in the less-ordered, amorphous regions. A network of hemicelluloses that connects the microfibrils hinders access of the enzymes to the crystalline core. The recalcitrance of plant fibres to degradation is further increased by lignification, in which the interfibrillar space is filled with a non-hydrolysable phenolic resin that is formed by free-radical polymerization of phenylpropanoid precursors[9,43]. As a consequence, lignocellulose digestion requires not only efficient cellulases and a wide range of other glycoside hydrolases that break down the associated cell wall components but also a mechanism that overcomes the barrier that is imposed by the lignin matrix. In termites, this is accomplished by a dual system that combines activities of both the host and its intestinal symbionts (FIG. 1).

Host activities in foregut and midgut. Leidy recognized the termite as "a powerful mill that reduces the ligneous food to a pulpy condition, adapted to the more delicate constitution of its occupants" (REF. 10). Comminution of the ingested wood to small fragments (of 10–20 μm in diameter) by the mandibles and the gizzard is a prerequisite for phagocytosis by the gut flagellates, and the enlarged surface area increases the efficiency of enzymatic digestion[9,44]. The hydrolysis of cellulose is initiated by endoglucanases that are secreted by the salivary glands (in lower termites) or the midgut epithelium (in higher termites)[45,46]. The high enzyme concentrations in the midgut enable the rapid breakdown of the amorphous regions of the cellulose fibres that are exposed by the grinding process, and the synergistic action of β-glucosidases prevents product inhibition by cleaving the resulting oligosaccharides to glucose[9,44]. It is now firmly established that endoglucanases of glycoside hydrolase family (GHF) 9 were present in insects long before termites developed their wood-feeding habit[47], which puts an end to the long-lasting dogma that cellulase activities in animals are always contributed by microbial symbionts.

Symbiont activities in the hindgut. Hungate observed in the 1940s that the digestion of sawdust by foregut and midgut extracts of lower termites (such as *Zootermopsis* spp.) was incomplete, and only the cellulose component was partially degraded. However, hindgut extracts also attacked the hemicelluloses and completely hydrolysed even crystalline cellulose[1]. This is accomplished by the dense assemblage of flagellates that are housed in the hindgut, which have diverse sets of powerful glycoside hydrolases in their digestive vacuoles. The flagellates of lower termites and *Cryptocercus punctulatus* have been shown to produce not only cellobiohydrolases (which are exoglucanases), endoglucanases and β-glucosidases from various GHFs but also numerous other glycoside hydrolases that are required for hemicellulose digestion (such as xylanases, arabinosidases, mannosidases and arabinofuranosidases). Although representative enzymes have been purified and their genes have been heterologously expressed, most of these enzymes have been identified by metatranscriptome analysis of the hindgut contents[11,20,44].

The bacterial microbiota of lower termites seems to have no major role in fibre digestion. This is plausible as all wood particles that enter the hindgut are immediately

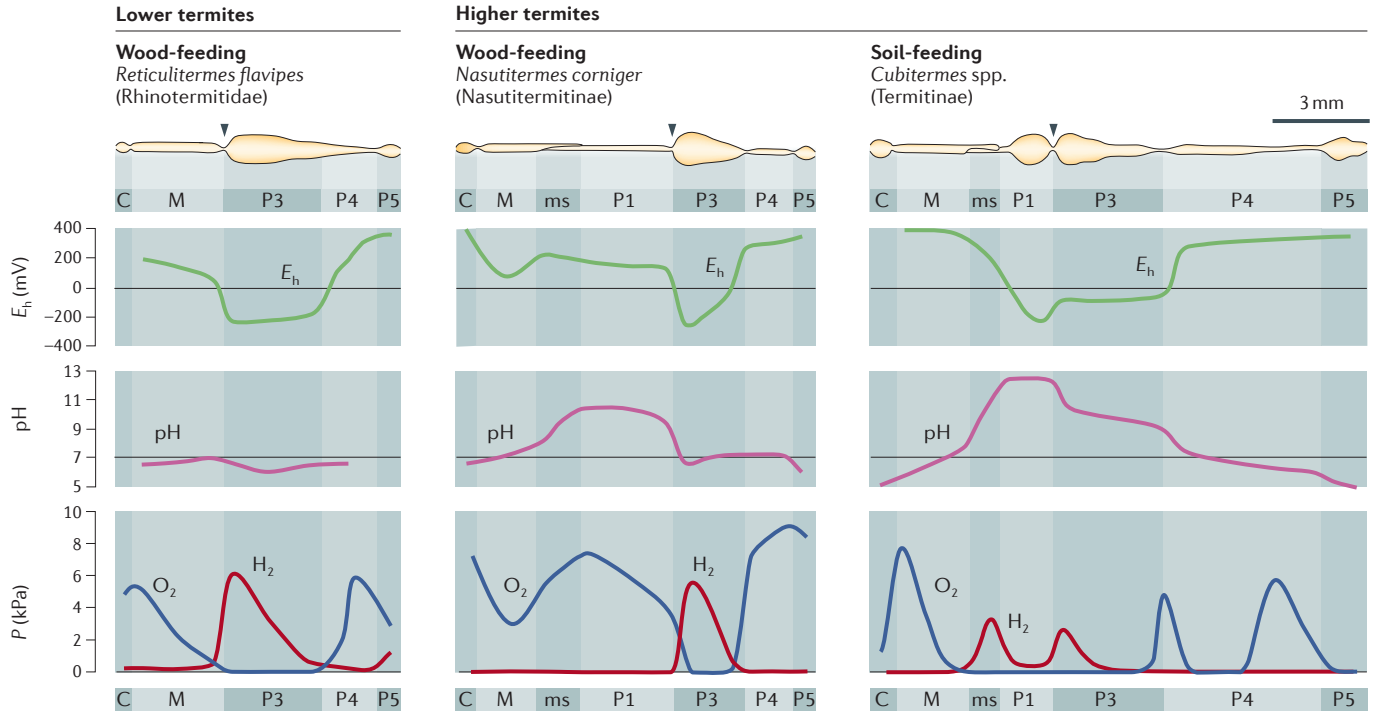


Figure 3 | **Increasing gut compartmentation in higher termites.** The guts of lower termites are relatively short and resemble those of cockroaches and fungus-cultivating higher termites (such as Macrotermitinae) in both morphology[8] and physicochemical conditions[65,140]. In other higher termites, the hindgut is longer and more compartmentalized. The pH sharply increases in the mixed segment (which is an anatomical innovation of these lineages)[8]. Alkalinity in the anterior hindgut of soil feeders matches the highest values that have been reported for biological systems[42]. The dilated compartments are always anoxic and accumulate hydrogen (with exceptions[72,120]). Oxygen and hydrogen partial pressures (*P*), intestinal pH and apparent redox potential (E_h) were measured using microsensors along the central axis of agarose-embedded guts in *Reticulitermes flavipes*[13], *Nasutitermes corniger*[73] and *Cubitermes* spp.[42,63,75]. The arrowhead indicates the position of the enteric valve (P2). C, crop; M, midgut; ms, mixed segment; P1–P5, proctodeal segments.

Glycoside hydrolase family (GHF). A family of glycosidases or related enzymes. There are more than 130 different GHFs, and many of them comprise enzymes that are involved in the digestion of plant fibre (for example, cellulases, hemicellulases, pectinases and carbohydrate esterases).

Cellobiohydrolases
Cellulases that act unidirectionally from the ends of the cellulose chain (and thus are exoglucanases), yielding cellobiose as a product; they are more active than endoglucanases against crystalline cellulose.

Cellulosomes
Extracellular multi-enzyme complexes of anaerobic cellulolytic bacteria that are composed of cellulases, other glycoside hydrolases and carbohydrate-binding modules, which are held together and adhere to the cell surface via scaffold proteins that have cohesin and dockerin domains.

sequestered into the food vacuoles of the flagellates. Although free wood particles are abundant in the flagellate-free hindgut of wood-feeding higher termites, older work reported only low cellulase activities in the hindgut fluid. This apparent contradiction was resolved by more recent work on several *Nasutitermes* spp., which discovered substantial cellulase activities targeting crystalline cellulose in the particulate fraction of the hindgut content[48]; these activities remained undetected when only the clarified supernatant was tested.

The presence of cellulolytic bacteria in the hindgut paunch of *Nasutitermes* spp. was substantiated by metagenomic analyses of the paunch contents, which identified many genes encoding glycoside hydrolases that are relevant to the degradation of plant fibre[34,37]. Many putative cellulases, xylanases and other glycoside hydrolases were tentatively assigned to Fibrobacteres, which, although also present in other termite lineages, are most abundant in wood-feeding higher termites[32,33].

A major role of Fibrobacteres in cellulose degradation in the hindgut of *Nasutitermes* spp. is supported by the identification of numerous homologues of *Fibrobacter succinogenes* genes in the metagenomes, encoding proteins putatively involved in binding to cellulose[34,37]. *F. succinogenes* is one of the most important bacteria in the rumen, but lacks both soluble cellulases and the scaffoldin proteins and dockerin domains typical of clostridial cellulosomes. This is consistent with the absence of the corresponding genes in the metagenomes and the relatively low recovery of endoglucanases in the metaproteomic analyses of the hindgut fluid of *Nasutitermes* spp.[34,49].

The situation differs fundamentally in the dung-feeding *Amitermes wheeleri*, in which the gut microbiota contains few Fibrobacteres and is dominated by Clostridiales[37]. Metagenomic analysis of the hindgut contents identified cohesin and dockerin genes that were most similar to components of clostridial cellulosomes[37]. The near total absence of such genes in the metagenomes of *Nasutitermes* spp. hindguts highlights the fundamental differences between these termites with respect to their cellulolytic partners. In the fungus-cultivating *Odontotermes yunnanensis*, in which the gut microbiota is dominated by Bacteroidetes, cellulases and hemicellulases seem to be strongly underrepresented. Instead, metagenomic analysis showed that there was an abundance of debranching and oligosaccharide-degrading enzymes and an overrepresentation of genes with functions involved in the digestion of fungal cell walls and the metabolism of monoaromatic compounds[29], which corroborates the important role of the fungal partner in the preprocessing of lignocellulose in the fungus garden[50].

Advantage of a dual-cellulolytic system. The relative contributions of the two consecutive systems to cellulose digestion vary between termite species[44]. An abundance of wood particles in the hindgut indicates that fibre digestion by host enzymes is always far from complete. This is most probably due to the absence of cellobiohydrolases and hemicellulolytic activities and the short residence time of the digesta (<30 min) in the tubular midgut. The complete breakdown of the wood particles by the more potent cellulose-digesting and hemicellulose-digesting enzymes of the gut flagellates (or bacterial symbionts in higher termites) is facilitated by the increased residence time of the digesta in the voluminous hindgut, which is further extended by the segregation of wood particles into the digestive vacuoles of the gut flagellates[1] (and, presumably, by sorting processes at the enteric valve in higher termites[8]).

The maintenance of this dual strategy over a long evolutionary time indicates that both systems fulfil essential functions. It has been suggested that a resorption of glucose in the midgut is necessitated by the inability of termites to use the products of their hindgut microbiota (mainly acetate) for gluconeogenesis[51]. Conversely, the inevitable energy loss that is caused by the microbial fermentations might be more than balanced by the nutritional value of their microbial biomass (see below).

Overcoming the lignin barrier. Whereas Macrotermitinae benefit from the lignin-degrading capacity of their fungal partner, all other termites must overcome the lignin barrier during gut passage. The possibility of discovering new principles of lignin degradation in termite guts has stimulated research for several decades[1]; however, the subject remains controversial, not least because of many methodological pitfalls. Older reports claiming that there is a substantial decrease in lignin content between ingested wood and faeces must be regarded with caution[1]. Also, more recent studies found that there were only small differences in the mass of lignin or its spectroscopic properties[52–55], and the lack of convincing evidence for a substantial degradation of core lignin during gut passage is consistent with the fact that lignin is the major constituent of the faeces of wood-feeding termites.

However, the question of whether lignin is efficiently degraded does not touch the heart of the matter. Any structural modifications that improve the accessibility of polysaccharides to glycoside hydrolases will increase the efficiency of digestion. Several studies have reported modifications to both aromatic ring substituents and side chains of lignin during gut passage in lower[53–56] and higher termites[57], whereas the linkages between the aromatic units were found to be conserved and the original aromatic properties retained[53,55,58]. It is possible that such modifications are caused by endogenous enzymes, such as the phenol-oxidizing laccases that are secreted by the salivary glands of *Reticulitermes flavipes*[59,60]. Carboxyl esterases that are expressed in the midgut might increase the digestibility of plant fibre by cleaving the linkages that 'pointweld' the lignin matrix to the hemicellulose chains[61].

Furthermore, non-enzymatic processes might help to unlock the crosslinks between the fibre fraction and lignin. Lignocellulose degradation by brown-rot fungi involves Fenton reactions that chemically oxidize lignin and depolymerize cellulose — a strategy so powerful that the fungus requires neither exoglucanases nor endoglucanases that have cellulose-binding domains[62]. In termites, such an iron-mediated production of hydroxyl radicals would be feasible in the slightly acidic foregut, which contains substantial concentrations of reactive (that is, ferrous) iron[63–65]. By contrast, the strongly increased pH in the anterior hindgut of many higher termites should promote autoxidative processes, which have been shown to cleave lignin–carbohydrate complexes during alkaline pulping and might also be responsible for modifications of humic acids in the extremely alkaline gut compartments of soil feeders[63,66].

Finally, it cannot be ignored that mechanical grinding by the mandibles and the gizzard increases the digestibility of lignocellulose[53]. Ball-milling of wood to a fine powder cleaves aryl ether linkages, particularly the common β-*O*-4 structures, and liberates phenolic hydroxyl groups[67,68], which leads to a substantial decrease in the molecular weight of lignin and facilitates enzymatic digestion[69].

The hindgut microbial bioreactor

The introduction of microsensors in termite gut research fundamentally changed the concept of the hindgut bioreactor[70]. Termite hindguts are not simple anoxic fermenters that are analogous to the mammalian rumen but are structured microenvironments that fundamentally differ in the prevailing physicochemical conditions and the microbial processes that they accommodate (FIG. 4).

Fermentative processes. The microbiota converts wood fibres to short-chain fatty acids, which accumulate in the hindgut fluid and are eventually resorbed by the host. In the lower termites, the bulk of the fermentation products is attributed to the flagellates. The few termite gut flagellates that are studied in axenic culture convert cellulose to acetate, H_2 and CO_2 (REFS 11,14). It is not known whether the same products are formed by all parabasalid species (particularly those that ferment the pentose units of hemicelluloses), and the metabolism of the oxymonadid flagellates is entirely unclear.

The flagellates are probably also responsible for the production of lactate, which accounts for 10% of the respiratory electron flow[71,72]. It does not accumulate but is rapidly converted to acetate by bacteria that are located in the gut periphery in an oxygen-dependent process. Furthermore, formate is formed in the hindgut of many termite species. Depending on the termite species, it either accumulates, is oxidized to CO_2 or is reduced to acetate, presumably by homoacetogenic bacteria[71,72]. Little is known about the fermentation processes in the flagellate-free higher termites. The products that are detected in their gut fluid are essentially the same as in lower termites[31,71,73], and all species that have been investigated emit at least some hydrogen[74,75].

Fenton reactions
Iron-mediated reactions in which hydroxyl radicals are formed ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO\cdot + HO^-$). These non-selectively oxidize many organic compounds.

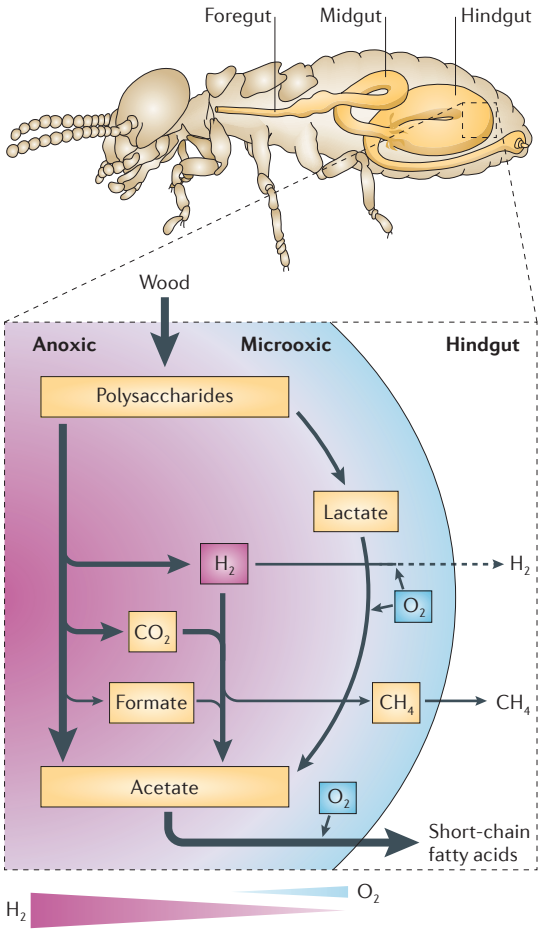


Figure 4 | **Major microbial processes in the hindgut of lower termites.** The fermentation of wood polysaccharides by the gut flagellates yields acetate and other short-chain fatty acids, which are resorbed by the host. Hydrogen is an important intermediate that drives the reduction of CO_2, which yields additional acetate (via homoacetogenesis) and some methane[14]. Although H_2 may strongly accumulate at the gut centre, most of it is consumed before it can escape from the gut[13,72]. The high surface-to-volume ratio of the microlitre-sized hindgut compartment causes an enormous influx of oxygen across the gut wall. Its efficient removal by the gut microbiota within fractions of a millimetre results in steep gradients in the hindgut periphery[11,70]. Oxygen is consumed by both microaerobic and anaerobic bacteria and methanogenic archaea that use acetate, lactate or hydrogen as the electron donor[71,72,100,104].

Hydrogen is a key metabolite and strongly accumulates in the hindgut paunches of most lower termites[13,72] and of all higher termites[65,73,75] that have been investigated so far. Hydrogen turnover in lower termites is up to threefold higher than in the rumen (per volume unit)[72], but its production and consumption by the gut microbiota are typically closely coupled. The hydrogen emission rates of termites rarely exceed those of methane production[74]. A notable exception is *Coptotermes formosanus*, in which a substantial fraction of the hydrogen that is formed in the primary fermentations (0.75 H_2 per glucose unit) was found to escape from the gut[76].

Reductive acetogenesis. Reductive acetogenesis from H_2 and CO_2 is the major hydrogen sink in wood-feeding termites[1,14]. *In situ* rates in lower termites account for about 25% of the respiratory electron flow[71,72]. There is substantial evidence that the process is catalysed by spirochaetes. Termite gut spirochaetes are represented by two immensely diverse but monophyletic clades in the genus *Treponema*[77,78]. *Treponema primitia*, which was the first spirochaete to be isolated from termite guts, was also the first homoacetogenic member of the phylum to be identified[79–81]. Formyltetrahydrofolate synthase (FTHFS) and other marker genes for the Wood–Ljungdahl pathway of *T. primitia* cluster with many homologues obtained from the guts of both lower termites[82–85] and higher termites[34,86,87]. In addition, homologues of [FeFe]-hydrogenases, which are widely distributed in both lower and higher termites, have been assigned to spirochaetes[34,88,89].

Spirochaetes are mostly absent in omnivorous cockroaches, in which firmicutes seem to catalyse reductive acetogenesis[90], and might have lost their importance in reductive acetogenesis in the humivorous lineages of higher termites[86,87]. Termite gut spirochaetes probably also have a role in primary fermentations. Non-homoacetogenic *Treponema* spp. that were isolated from lower termites have the capacity to use cellobiose[80,91]. In the hindgut metagenomes of *Nasutitermes* spp. and *Amitermes* spp., an abundance of glycoside hydrolases that putatively target oligosaccharides has been assigned to spirochaetes[34,37], which indicates that they have an important role in processing the oligomeric products of fibre digestion.

Methanogenesis. Although the density of bacteria in termite guts typically exceeds that of archaea by almost two orders of magnitude, most termites emit substantial amounts of methane[14,92]. All methanogens that have been isolated from termite guts belong to the genus *Methanobrevibacter* (order Methanobacteriales)[15,93]. Lower termites seem to be almost exclusively colonized by members of this genus, which are either attached to the gut wall or associated with flagellates[18,19]. The methanogenic communities in higher termites are much more diverse and include hitherto uncultured members of the orders Methanomicrobiales, Methanosarcinales and the recently discovered Methanoplasmatales[18,94,95]. The increased diversity may be related to the availability of additional methanogenic substrates. Although all *Methanobrevibacter* species that have been isolated from termites have a hydrogenotrophic metabolism and grow only poorly (if at all) on formate, methanogenesis in *Cubitermes* spp. is strongly stimulated by formate[75], and enrichment cultures of Methanoplasmatales obligately require methanol[95]. Aceticlastic methanogens seem to be absent from the termite gut[14,92].

Although methanogens typically out-compete homoacetogens for hydrogen for thermodynamic reasons, reductive acetogenesis predominates over methanogenesis in most wood-feeding termites. The explanation for this phenomenon seems to lie in the spatial organization of the responsible populations[14,92]. Although the

homoacetogens (that is, highly motile spirochaetes) are able to colonize the hydrogen-rich lumen, the methanogens are typically attached to the hindgut wall, which places them downstream in the hydrogen gradient and precludes direct competition with the homoacetogens. It is not clear why methanogens replace homoacetogens as the major hydrogen sink in most fungus-cultivating and humivorous termites. Although methanogenesis is also strongly limited by hydrogen in these species, a cross-epithelial transfer of hydrogen and other methanogenic substrates from the anterior to the posterior compartments[75] may favour populations that are located in the periphery of the gut.

The discovery of methanogenesis in termites by Breznak in 1974 has triggered numerous studies concerning its implications for the global greenhouse budget[14,92]. Although the counter-gradients of methane and oxygen in the periphery of the hindgut provide seemingly ideal conditions for aerobic methane oxidation, there is no evidence for the presence of methanotrophic bacteria or their activities[96]. However, except for mounds that have a well-developed ventilation system[97], methane oxidation in the nest material or the surrounding soil may strongly mitigate the production of methane by its inhabitants[74]. Current estimates attribute <3% of the global methane source strength to termites, and large uncertainties arise from unaccounted differences in the emission rates among species, their regional biomass and a lack of scaling factors to correct for reoxidation within the mounds[92].

The oxygen status of termite guts. Owing to the high surface-to-volume-ratio of the microlitre-sized hindgut compartments, the influx of oxygen across the gut wall is enormous[70]. Efficient consumption of oxygen by the gut microbiota gives rise to steep oxygen gradients in the periphery of the paunch, which maintains anoxia of the luminal contents and poises the redox potential at values low enough to enable methanogenesis and reductive acetogenesis[13]. The microbial communities that colonize the hindgut wall of *Reticulitermes* spp. differ from those in the lumen[23,98]. Microaerophiles, such as *Stenoxybacter acetivorans*[99,100] and *Diplosphaera colotermitum*[101], are autochthonous members of the termite gut microbiota. They possess high-affinity cbb_3-type terminal oxidases as specific adaptations to hypoxic conditions and can contribute up to 5% of the acetate oxidation rate of intact guts[100]. Furthermore, higher termites harbour bacterial lineages that have a clear preference for this habitat[73].

Anaerobic, fermenting bacteria that have been isolated from termite guts reduce oxygen at astonishingly high rates, which shifts their metabolism towards increased acetate production[102] — a phenomenon that is also relevant *in situ*[71]. Even notoriously oxygen-sensitive methanogens and homoacetogens are capable of hydrogen-dependent oxygen reduction[15,103]. The ability of *Methanobrevibacter* species to remain metabolically active as long as the oxygen flux does not exceed their capacity for oxygen removal[104] might be the metabolic basis for their survival in the hindgut periphery[92]. The flagellates, which are attached to the hindgut wall of many termite species, might also substantially contribute to oxygen reduction[100].

Lastly, the termite itself might also facilitate the colonization of the hindgut periphery by a generally oxygen-sensitive microbial community. Whereas many other insects practice a regular, discontinuous gas exchange, *Zootermopsis nevadensis* reportedly maintains a continuous hypoxia in its tracheal system by partially opening or closing its spiracles in response to environmental changes[105] — a behaviour that might function to prevent strong temporal fluctuations of oxygen partial pressure that would damage the more oxygen-sensitive among the symbionts in the microbial biofilm attached to the hindgut wall.

Microbial processes in humivorous species. The strong mineralization of nitrogen in the gut of soil-feeding termites indicates that a large proportion of their substrates is derived from peptides and other nitrogenous humus components. It has been estimated that nitrogen-rich humus constituents substantially contribute (20–40%) to the dietary carbon that is oxidized by *Cubitermes* spp.[106], which is consistent with the unusual nitrogen isotope ratios of soil-feeding termites[107], the depletion of peptides in soil organic matter during gut transit[52] and the high ammonia concentrations in the posterior hindgut (which are up to 130 mM)[108].

Decreasing fibre and increasing nitrogen contents during the humification of lignocellulose are consistent with an underrepresentation of functions in cellulose digestion and nitrogen fixation in the hindgut metagenome of a dung-feeding *Amitermes* sp. (relative to the wood-feeding *N. corniger*)[37]. Information on humivorous termites is limited, but both *Termes* spp. (which are wood–soil interface feeders) and *Cubitermes* spp. (which are true soil feeders) harbour bacterial communities that fundamentally differ from those of other termites; they lack many lineages that are typical of wood-feeding higher termites and contain only a few spirochaetes[35,36]. The highly alkaline anterior gut regions are mostly colonized by firmicutes, including several lineages of Clostridiales that also occur in the alkaline gut compartment of wood feeders[36,73], which indicates that both the physicochemical conditions and the availability of substrates affect microbial community structure in termite guts.

Nitrate that is ingested by soil-feeding termites is microbially reduced to both N_2 and ammonia[106]. The reoxidation of ammonia in the posterior hindgut of *Cubitermes* spp. might stimulate nitrogen cycling, which would explain the substantial N_2O emissions of soil-feeding termites[109]. The hindgut of *Cubitermes* spp. is densely colonized by several deep-branching lineages of Planctomycetes[110], and the archaeal community comprises uncultured crenarchaeotes[111]; however, their involvement in the oxidation of ammonia has not been studied. Nevertheless, mounds of soil-feeding termites have been identified as hot spots of N_2O emission in the African savannah[112], which highlights the fact that methanogenesis might not be the only microbial process in termite guts that substantially contributes to greenhouse-gas production.

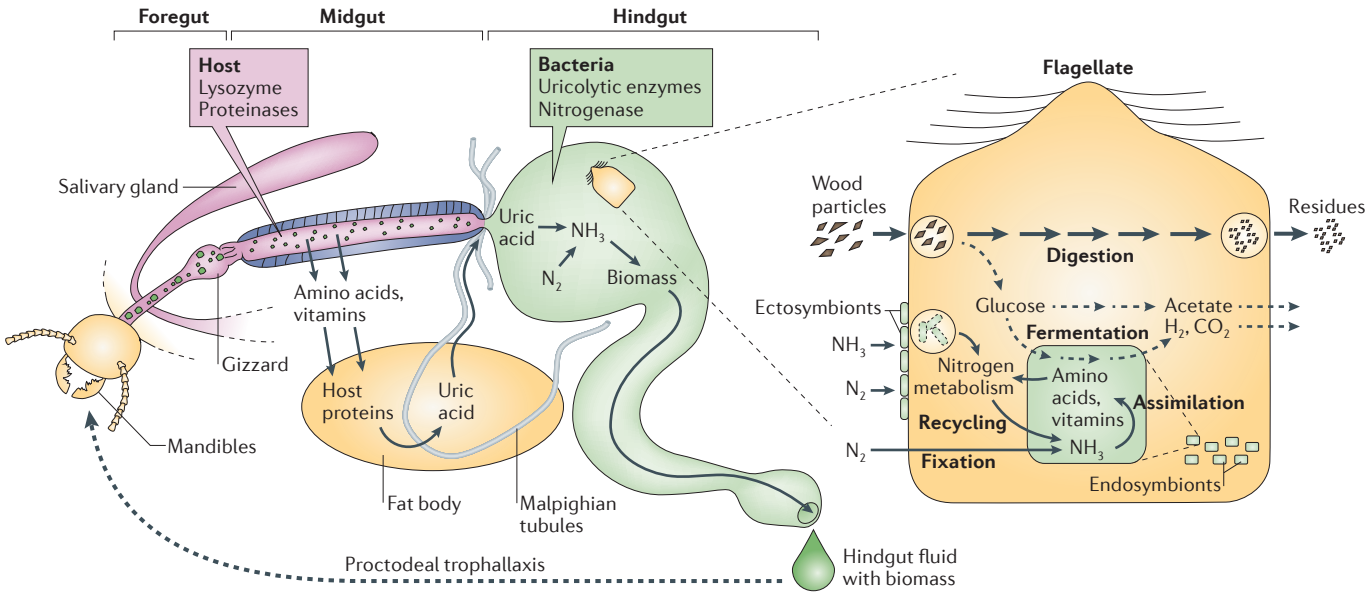


Figure 5 | **Nitrogen cycling in lower termites.** The low nitrogen content and poor nutritional value of their wood diet forced termites to develop an efficient system for conserving and upgrading dietary nitrogen. Whereas the lignin-rich residues of wood digestion are voided as faeces, the nutrient-rich microbial biomass that is produced in the hindgut is passed on to nestmates via proctodeal trophallaxis[14]. It is digested by salivary enzymes in the foregut and the midgut[113] and amino acids and vitamins are resorbed by the host. The major waste product of nitrogen metabolism is uric acid[14]. It is formed in the fat body and secreted into the hindgut via the Malpighian tubules, where it is rapidly mineralized by the gut microbiota. The assimilation of ammonia into new microbial biomass completes the nitrogen cycle. Dinitrogen fixation by hindgut bacteria introduces additional nitrogen into the system[14]. The bacterial symbionts of the flagellates seem to have important roles in nitrogen fixation, the assimilation of ammonia and the synthesis of amino acids and vitamins[18,20] — activities that benefit the host cell either directly (for endosymbionts) or after phagocytosis (for ectosymbionts).

The nutritional role of the gut microbiota

The termite gut microbiota not only makes essential contributions to the digestion of plant fibre but also has important roles in nutrition. Lignocellulose is notoriously low in nitrogen and contains only negligible amounts of amino acids and vitamins. Gut bacteria efficiently recycle the nitrogenous waste products of the termite, assimilate ammonia into nutritionally valuable microbial biomass and amend the nitrogen budget by dinitrogen fixation[11,14,20].

Recycling and upgrading of nitrogen. The major waste product of nitrogen metabolism in most terrestrial insects is uric acid. It is secreted into the hindgut via the Malpighian tubules and typically voided with the faeces. However, in termites, uricolytic hindgut bacteria convert uric acid nitrogen to ammonia, which is subsequently assimilated into microbial cells[11,14]. Since the enteric valve prevents the reflux of hindgut contents to the midgut, termites practice proctodeal trophallaxis to access the nutritionally valuable microbial biomass, which is digested by the enzymes that are produced by the salivary glands and midgut[113]. The resorption of amino acids and vitamins by the midgut epithelium completes the nitrogen cycle (FIG. 5).

The establishment of proctodeal trophallaxis in the common ancestor of termites and wood-feeding cockroaches (family Cryptocercidae) is considered to have been a prerequisite for the evolution of symbiotic digestion, as it ensures that freshly molted individuals are consistently colonized with the same set of symbionts[12]. However, proctodeal trophallaxis also has an important role in nutrition. This is highlighted by the fate of *Blattabacterium cuenoti*, which is a fat-body endosymbiont that is present in (and co-evolves with) all cockroach lineages[12]. It is essential for the development of cockroaches as it presumably recycles urea (but not uric acid), assimilates ammonia and provides essential amino acids to its host[114]. However, *B. cuenoti* experienced a progressive gene loss in its biosynthetic pathways in *Cryptocercus punctulatus* and its sister group, which is the most primitive termite *Mastotermes darwiniensis*[115,116], and has entirely disappeared in all other termites. Its functions were apparently no longer required after proctodeal trophallaxis was established, which led to relaxed selection and progressive genome erosion[116].

Nitrogen fixation. The fixation of atmospheric N_2 in termite guts was independently discovered by Benemann and Breznak in 1973 (REFS 11, 14). Its importance differs among termite lineages, but species that feed on intact wood can acquire 30–60% of their nitrogen via this pathway[14,107]. Numerous strains of nitrogen-fixing bacteria have been isolated from termite guts, but the most important diazotrophs among the gut microbiota remain uncultivated. The diversity of *nifH* genes in termite guts indicates that the capacity for nitrogen fixation is present among Spirochaetes, Clostridia, Bacteroidetes

Proctodeal trophallaxis
A social behaviour of termites, which solicit and imbibe droplets of hindgut fluid from nestmates.

nifH genes
Genes that encode the catalytic subunit of nitrogenase reductase; they are commonly used as a molecular marker for studying the diversity and community structure of nitrogen-fixing bacteria (also known as diazotrophs).

Box 2 | **Termites and biofuels**

Although the industrial fermentation of sugar-rich and starch-rich crops to ethanol is well established, the production of so-called second-generation biofuels from agricultural wastes is still inefficient[43]. A better understanding of lignocellulose digestion by termites may help to overcome challenges in the conversion of lignified plant cell walls into soluble sugars.

Models for technical processes

The strategies that termites use for the breakdown of lignified plant cell walls resemble technical processes much more closely than those found in other environments. Mandibles and gizzards are powerful mechanical mills, the midgut is an enzymatic reaction chamber with a permeation filter (the peritrophic membrane) for product recovery and the hindgut paunch is an anaerobic digester that converts polymers to microbial products. The consecutive gut compartments of higher termites form sequential reactors that use the same alkaline pretreatment of lignocellulose as the paper industry[9,137].

However, other properties of the digestive system are more difficult to mimic. In particular, the minute size of the hindgut bioreactor cannot be scaled up without loss of its intrinsic properties[70]. It creates a delicate balance between the influx and removal of oxygen, which enables oxidative processes and anaerobic fermentations to occur in close juxtaposition. Interactions between the gut lumen, periphery and epithelium do not require radial mixing; diffusion alone suffices as a means of metabolite transport. Understanding the basis for the suppression of methanogenesis in the wood-feeding species may hold the key to increasing the yields of hydrogen or other valuable products in technical fermentations of plant biomass.

Sources of novel enzymes

Although termites probably cannot be directly used in the processing of agricultural wastes, they are a promising reservoir of microbial symbionts and enzymes that have biotechnological potential. Most research has been done on the endogenous endoglucanases of termites. They have been heterologously expressed, and their thermostability and catalytic properties have been improved by genetic engineering[9,44]. Transgenic enzymes with proper glycosylation and catalytic activities that are superior to those of endoglucanases from bacteria or fungi have been produced in eukaryotic expression systems. In addition, some cellulases from gut flagellates have been expressed in different hosts[44]; however, they may require codon optimization to avoid premature polyadenylation[138]. Except for a few xylanases, enzymes of bacterial origin have only been poorly investigated.

and possibly Fibrobacteres[25,34,117,118], but only a subset of these homologues seems to be expressed[119,120]. Inferences regarding nitrogenase activity from transcriptional profiles have to be made with particular caution as nitrogenase activity in bacteria and archaea is subject to complex regulation also at the post-translational level[121]. As nitrogenase activity in most diazotrophs is switched off in the presence of a readily utilizable nitrogen source, it is also not clear whether the active populations in termite guts are adapted to higher ammonia concentrations (which are in the millimolar range, even in wood-feeding termites)[96] or colonize nitrogen-poor microhabitats.

In several lower termites, the bulk of the nitrogen-fixing activity has been ascribed to the bacterial symbionts of their gut flagellates. The most important diazotroph in the gut of *Coptotermes formosanus* (and the first member of the Bacteroidetes to be shown to possess *nif* genes) is *Candidatus* Azobacteroides pseudotrichonymphae[25], which is an abundant endosymbiont of *Pseudotrichonympha* species[122]. Ectosymbiotic Bacteroidetes that are associated with other gut flagellates even possess a second paralogue of *nifH*, which is preferentially expressed over the conventional variant[120]. It was discovered in the dry-wood termite *Neotermes koshunensis* and is part of an operon that encodes an alternative nitrogenase lacking molybdenum and vanadium cofactors[119]. However, the evolutionary origin of the diverse nitrogenase genes in termite gut symbionts remains unclear[118,120].

Nutrition drives co-speciation of flagellates and their symbionts. There is increasing evidence that the frequent associations of gut flagellates with bacterial symbionts have a nutritional basis[20] (FIG. 5). Although little is known about their physiology, the phagocytosis of bacterial cells (or the need for heat-killed bacteria in axenic cultures)[14,123] suggests that the nutritional requirements of termite gut flagellates are quite complex. Despite a considerable reduction in genome size, the endosymbionts *Candidatus* Endomicrobium trichonymphae[124] and *Candidatus* Azobacteroides pseudotrichonymphae[25] conspicuously retained the capacity to synthesize most amino acids and various cofactors. The provision of such essential nutrients to the host cell would explain the general specificity of such symbioses[22,24,125,126] and the strict co-speciation of the partners[122,127,128].

The individual lineages of flagellate symbionts are typically embedded in larger clusters of putatively free-living relatives, which suggests that the symbionts were recruited from the gut microbiota long after the flagellates had established their symbiosis with termites[125,129]. Flagellates of the genus *Trichonympha*, which were probably already present in the common ancestor of termites and *Cryptocercus* spp.[130,131], were independently colonized at least twice by endosymbionts that subsequently co-evolved with their flagellate host[126,127].

It is also possible that the proximity of bacteria and archaea in the biofilms of the gut wall or their attachment to larger, filamentous prokaryotes[15,16] facilitates the cross-feeding of nutrients among prokaryotic cells, but such interactions are more difficult to map. Cryptic interdependencies among keystone species might also explain why disturbance of the gut microbiota by antibiotic treatment can have long-term consequences for the fitness of a termite colony[132].

Conclusions

Joseph Leidy correctly assumed that the microorganisms in the hindgut of termites are not parasites but contribute to the well-being of their host[10]. It is now clear that they are not only an essential component of the dual-cellulolytic system but also have an important role in nutrition. Although fundamental changes in the digestive strategies of termites seem to have caused major shifts in the gut microbiota, the evolutionary patterns in microbial community structure and the underlying ecological drivers are just emerging. The nitrogen transformation processes in the guts of humivorous species are an entirely novel aspect of the digestive symbioses in termite guts. Although their nature is still in the dark, they deserve attention because of their potential impact on nitrogen metabolism in tropical soils.

The mechanisms underlying the efficient digestion of lignocellulose and humus, which are highly relevant to applied research, remain poorly understood. Their unique gut conditions and an abundance of digestive enzymes

provided by both the symbionts and the host make termites a 'treasure trove' for biotechnological applications, particularly the industrial conversion of lignified plant fibres to biofuels and other valuable chemicals (BOX 2).

Recent progress in our understanding of symbiotic digestion was driven by the introduction of high-throughput sequencing techniques. They provide sufficient resolution and sampling depth to illuminate the distribution patterns of microbial lineages across the wide range of host species and their highly different microhabitats, enabling the distinction between phylogenetic and environmental drivers of community structure. Large-scale metagenomic and metatranscriptomic studies will facilitate the identification of key functions in the intestinal processes of different feeding guilds, and single-cell sequencing approaches will help to pinpoint the information to individual taxa. Nevertheless, the relevance of such findings should be corroborated by further investigation of the key activities of the microbiota *in vivo*.

Another important goal on the agenda of termite gut microbiologists should be the isolation of key members of the gut microbiota. Many of the microbial lineages that are unique to the gut microbiota of termites do not have any cultured representatives. Despite progress in metagenomics, it remains impossible to reliably predict the functional and catalytic characteristics of many putative gene products on the basis of sequence annotations, let alone those with functions that are still unknown even in the most intensively studied model organisms. Any representative of the gut microbiota that is brought into pure culture will be an invaluable asset for ecophysiological studies, enabling us to characterize phenotypic properties that are not evident from its genome but that help to explain important functions in the gut microecosystem. The successful enrichment and isolation of novel gut microorganisms by selective substrates[95,100] or unconventional cultivation strategies[79,133] highlights the potential of efforts based on a rational design.

1. Breznak, J. A. & Brune, A. Role of microorganisms in the digestion of lignocellulose by termites. *Annu. Rev. Entomol.* **39**, 453–487 (1994).
2. Bignell, D. E. & Eggleton, P. in *Termites: Evolution, Sociality, Symbioses, Ecology* (eds Abe, T., Bignell, D. E., Higashi, M.) 363–387 (Kluwer Academic Publishers, 2000).
3. Su, N.-Y. & Scheffrahn, R. H. A review of subterranean termite control practices and prospects for integrated pest management programmes. *Integ. Pest. Manag. Rev.* **3**, 1–13 (1998).
4. Rouland-Lefèvre, C. in *Biology of Termites: A Modern Synthesis* (eds Bignell, D. E., Roisin, Y. & Lo, N.) 499–517 (Springer, 2011).
5. Wood, T. G. The agricultural importance of termites in the tropics. *Agr. Zool. Rev.* **7**, 117–155 (1996).
6. Evans, T. A., Dawes, T. Z., Ward, P. R. & Lo, N. Ants and termites increase crop yield in a dry climate. *Nature Commun.* **2**, 262 (2011).
7. Jouquet, P., Traoré, S., Choosai, C., Hartmann, C. & Bignell, D. Influence of termites on ecosystem functioning. Ecosystem services provided by termites. *Eur. J. Soil Biol.* **47**, 215–222 (2011).
8. Eggleton, P. in *Biology of Termites: A Modern Synthesis* (eds Bignell, D. E., Roisin, Y. & Lo, N.) 1–26 (Springer, 2011).
9. Watanabe, H. & Tokuda, G. Cellulolytic systems in insects. *Annu. Rev. Entomol.* **55**, 609–632 (2010).
This review provides an excellent overview of the endogenous cellulolytic system of termites and was authored by the two scientists who contributed most essentially to its discovery.
10. Leidy, J. The parasites of the termites. *J. Acad. Nat. Sci. (Phila.)* **8**, 425–447 (1881).
11. Brune, A. & Ohkuma, M. in *Biology of Termites: A Modern Synthesis* (eds Bignell, D. E., Roisin, Y. & Lo, N.) 439–475 (Springer, 2011).
12. Lo, N. & Eggleton, P. in *Biology of Termites: A Modern Synthesis* (eds Bignell, D. E., Roisin, Y. & Lo, N.) 27–50 (Springer, 2011).
13. Ebert, A. & Brune, A. Hydrogen concentration profiles at the oxic–anoxic interface: a microsensor study of the hindgut of the wood-feeding lower termite *Reticulitermes flavipes* (Kollar). *Appl. Environ. Microbiol.* **63**, 4039–4046 (1997).
14. Breznak, J. A. in *Termites: Evolution, Sociality, Symbiosis, Ecology* (eds Abe, T., Bignell, D. E. & Higashi, M.) 209–231 (Kluwer Academic Publishers, 2000).
This chapter is the last comprehensive review written by the long-time leader in the field of termite gut microbiology and provides important insights into the nitrogen economy of the gut, with many links to the older literature.
15. Leadbetter, J. R. & Breznak, J. A. Physiological ecology of *Methanobrevibacter cuticularis* sp. nov. and *Methanobrevibacter curvatus* sp. nov., isolated from the hindgut of the termite *Reticulitermes flavipes*. *Appl. Environ. Microbiol.* **62**, 3620–3631 (1996).
16. Thompson, C. L., Vier, R., Mikaelyan, A., Wienemann, T. & Brune, A. '*Candidatus* Arthromitus' revised: segmented filamentous bacteria in arthropod guts are members of *Lachnospiraceae*. *Environ. Microbiol.* **14**, 1454–1465 (2012).
17. Tamschick, S. & Radek, R. Colonization of termite hindgut walls by oxymonad flagellates and prokaryotes in *Incisitermes tabogae, I. marginipennis* and *Reticulitermes flavipes*. *Eur. J. Protistol.* **49**, 1–14 (2013).
18. Ohkuma, M. & Brune, A. in *Biology of Termites: A Modern Synthesis* (eds Bignell, D. E, Roisin, Y. & Lo, N.) 413–438 (Springer, 2011).
19. Hongoh, Y. & Ohkuma, M. in *(Endo)symbiotic Methanogenic Archaea* (ed. Hackstein, J.H.P) 55–80 (Springer, 2010).
20. Hongoh, Y. Toward the functional analysis of uncultivable, symbiotic microorganisms in the termite gut. *Cell. Mol. Life Sci.* **68**, 1311–1325 (2011).
This paper is an important review by one of the experts in the field, with more details on the recent advances in lignocellulose digestion and nitrogen metabolism by termite gut symbionts.
21. Hongoh, Y., Ohkuma, M. & Kudo, T. Molecular analysis of bacterial microbiota in the gut of the termite *Reticulitermes speratus* (Isoptera; Rhinotermitidae). *FEMS Microbiol. Ecol.* **44**, 231–242 (2003).
22. Stingl, U. Radek, R., Yang, H. & Brune, A. '*Endomicrobia*': cytoplasmic symbionts of termite gut protozoa form a separate phylum of prokaryotes. *Appl. Environ. Microbiol.* **71**, 1473–1479 (2005).
23. Yang, H., Schmitt-Wagner, D., Stingl, U. & Brune, A. Niche heterogeneity determines bacterial community structure in the termite gut (*Reticulitermes santonensis*). *Environ. Microbiol.* **7**, 916–932 (2005).
24. Noda, S. *et al.* Endosymbiotic *Bacteroidales* bacteria of the flagellated protist *Pseudotrichonympha grassii* in the gut of the termite *Coptotermes formosanus*. *Appl. Environ. Microbiol.* **71**, 8811–8817 (2005).
25. Hongoh, Y. *et al.* Genome of an endosymbiont coupling N_2 fixation to cellulolysis within protist cells in termite gut. *Science* **322**, 1108–1109 (2008).
This paper reports the second genome of a flagellate symbiont, documenting a crucial role in nitrogen fixation and the capacity for ammonia assimilation and amino acid upgrading.
26. Nobre, T., Rouland-Lefèvre, C. & Aanen, D. K. in *Biology of Termites: A Modern Synthesis* (eds Bignell D. E., Roisin, Y. & Lo, N.) 193–210 (Springer, 2011).
27. Hongoh, Y. *et al.* Intracolony variation of bacterial gut microbiota among castes and ages in the fungus-growing termite *Macrotermes gilvus*. *Mol. Ecol.* **15**, 505–516 (2006).
28. Shinzato, N., Muramatsu, M., Matsui, T. & Watanabe, Y. Phylogenetic analysis of the gut bacterial microflora of the fungus-growing termite *Odontotermes formosanus*. *Biosci. Biotechnol. Biochem.* **71**, 906–915 (2007).
29. Liu, N. *et al.* Metagenomic insights into metabolic capacities of the gut microbiota in a fungus-cultivating termite (*Odontotermes yunnanensis*). *PLoS ONE* **8**, e69184 (2013).
30. Hyodo, F. *et al.* Differential role of symbiotic fungi in lignin degradation and food provision for fungus-growing termites (Macrotermitinae: Isoptera). *Funct. Ecol.* **17**, 186–193 (2003).
31. Anklin-Mühlemann, R., Bignell, D. E., Veivers, P. C., Leuthold, R. H. & Slaytor, M. Morphological, microbiological and biochemical studies of the gut flora in the fungus-growing termite *Macrotermes subhyalinus*. *J. Insect Physiol.* **41**, 929–940 (1995).
32. Hongoh, Y. *et al.* Intra- and interspecific comparisons of bacterial diversity and community structure support coevolution of gut microbiota and termite host. *Appl. Environ. Microbiol.* **71**, 6590–6599 (2005).
33. Hongoh, Y. *et al.* Phylogenetic diversity, localization and cell morphologies of the candidate phylum TG3 and a subphylum in the phylum Fibrobacteres, recently found bacterial groups dominant in termite guts. *Appl. Environ. Microbiol.* **72**, 6780–6788 (2006).
34. Warnecke, F. *et al.* Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. *Nature* **450**, 560–565 (2007).
This paper reports the first metagenome of a termite gut microbiota, providing evidence for a major role of Fibrobacteres in cellulose degradation in the hindgut of higher termites and a 'treasure trove' for other important gene functions.
35. Schmitt-Wagner, D., Friedrich, M. W., Wagner, B. & Brune, A. Phylogenetic diversity, abundance, and axial distribution of bacteria in the intestinal tract of two soil-feeding termites (*Cubitermes* spp.). *Appl. Environ. Microbiol.* **69**, 6007–6017 (2003).
36. Thongaram, T. *et al.* Comparison of bacterial communities in the alkaline gut segment among various species of higher termites. *Extremophiles* **9**, 229–238 (2005).
37. He, S. *et al.* Comparative metagenomic and metatranscriptomic analysis of hindgut paunch microbiota in wood- and dung-feeding higher termites. *PLoS ONE* **8**, e61126 (2013).
38. Ji, R., Kappler, A. & Brune, A. Transformation and mineralization of synthetic ^{14}C-labeled humic model compounds by soil-feeding termites. *Soil Biol. Biochem.* **32**, 1281–1291 (2000).
39. Ji, R. & Brune, A. Transformation and mineralization of ^{14}C-labeled cellulose, peptidoglycan, and protein by the soil-feeding termite *Cubitermes orthognathus*. *Biol. Fertil. Soils* **33**, 166–174 (2001).
40. Vairavamurthy, A. & Wang, S. Organic nitrogen in geomacromolecules: insights on speciation and transformation with K-edge XANES spectroscopy. *Environ. Sci. Technol.* **36**, 3050–3056 (2002).
41. Miltner, A., Bombach, P., Schmidt-Brücken, B. & Kästner, M. SOM genesis: microbial biomass as a significant source. *Biogeochemistry* **111**, 41–55 (2012).

42. Brune, A. & Kühl, M. pH profiles of the extremely alkaline hindguts of soil-feeding termites (Isoptera: Termitidae) determined with microelectrodes. *J. Insect Physiol.* **42**, 1121–1127 (1996).
43. Himmel, M. E. *et al.* Biomass recalcitrance: engineering plants and enzymes for biofuels production. *Science* **315**, 804–807 (2007).
44. Ni, J. & Tokuda, G. Lignocellulose-degrading enzymes from termites and their symbiotic microbiota. *Biotechnol. Adv.* **31**, 838–850 (2013).
This paper is the most detailed current review of the literature on the dual cellulolytic system of termites and includes a critical review of the numerous studies of lignin modification during gut passage.
45. Watanabe, H., Noda, H., Tokuda, G. & Lo, N. A cellulase gene of termite origin. *Nature* **394**, 330–331 (1998).
46. Tokuda, G. *et al.* Major alteration of the expression site of endogenous cellulases in members of an apical termite lineage. *Mol. Ecol.* **13**, 3219–3228 (2004).
47. Lo, N., Watanabe, H. & Tokuda, G. in *Biology of Termites: A Modern Synthesis* (eds Bignell, D. E., Roisin, Y. & Lo, N.) 51–67 (Springer, 2011).
48. Tokuda, G. & Watanabe, H. Hidden cellulases in termites: revision of an old hypothesis. *Biol. Lett.* **3**, 336–339 (2007).
49. Burnum, K. E. *et al.* Proteome insights into the symbiotic relationship between a captive colony of *Nasutitermes corniger* and its hindgut microbiome. *ISME J.* **5**, 161–164 (2011).
50. Johjima, T., Taprab, Y., Noparatnaraporn, N., Kudo, T. & Ohkuma, M. Large-scale identification of transcripts expressed in a symbiotic fungus (*Termitomyces*) during plant biomass degradation. *Appl. Microbiol. Biotechnol.* **73**, 195–203 (2006).
51. Slaytor, M. Cellulose digestion in termites and cockroaches: what role do symbionts play? *Comp. Biochem. Physiol.* **103**, 775–784 (1992).
52. Griffiths, B. S., Bracewell, J. M., Robertson, G. W. & Bignell, D. E. Pyrolysis–mass spectrometry confirms enrichment of lignin in the faeces of a wood-feeding termite, *Zootermopsis nevadensis* and depletion of peptides in a soil-feeder, *Cubitermes ugandensis*. *Soil Biol. Biochem.* **57**, 957–959 (2012).
53. Hyodo, F., Azuma, J. & Abe, T. Estimation of effect of passage through the gut of a lower termite, *Coptotermes formosanus* Shiraki, on lignin by solid-state CP MAS C-13 NMR. *Holzforschung* **53**, 244–246 (1999).
54. Katsumata, K. S., Jin, Z., Hori, K. & Iiyama, K. Structural changes in lignin of tropical woods during digestion by termite, *Cryptotermes brevis*. *J. Wood Sci.* **53**, 419–426 (2007).
55. Li, H., Lu, J. & Mo, J. Physicochemical lignocellulose modification by the Formosan subterranean termite *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) and its potential uses in the production of biofuels. *BioResources* **7**, 675–685 (2012).
56. Geib, S. M. *et al.* Lignin degradation in wood-feeding insects. *Proc. Natl Acad. Sci. USA* **105**, 12932–12937 (2008).
57. Hopkins, D. W. *et al.* Application of ^{13}C NMR to investigate the transformations and biodegradation of organic materials by wood- and soil-feeding termites, and a coprophagous litter-dwelling dipteran larva. *Biodegradation* **9**, 423–431 (1998).
58. Ke, J., Laskar, D. D., Singh, D. & Chen, S. *In situ* lignocellulosic unlocking mechanism for carbohydrate hydrolysis in termites: crucial lignin modification. *Biotechnol. Biofuels* **4**, 17 (2011).
59. Tartar, A. *et al.* Parallel metatranscriptome analyses of host and symbiont gene expression in the gut of the termite *Reticulitermes flavipes*. *Biotechnol. Biofuels* **2**, 25 (2009).
60. Coy, M. R. *et al.* Phenol-oxidizing laccases from the termite gut. *Insect Biochem. Molec. Biol.* **40**, 723–732 (2010).
61. Wheeler, M. M., Tarver, M. R., Coy, M. R. & Scharf, M. E. Characterization of four esterase genes and esterase activity from the gut of the termite *Reticulitermes flavipes*. *Arch. Insect Biochem. Physiol.* **73**, 30–48 (2010).
62. Martinez, D. *et al.* Genome, transcriptome, and secretome analysis of wood decay fungus *Postia placenta* supports unique mechanisms of lignocellulose conversion. *Proc. Natl Acad. Sci. USA* **106**, 1954–1959 (2009).
63. Kappler, A. & Brune, A. Dynamics of redox potential and changes in redox state of iron and humic acids during gut passage in soil-feeding termites (*Cubitermes* spp.). *Soil Biol. Biochem.* **34**, 221–227 (2002).
64. Vu, A. T., Ngyen, N. C. & Leadbetter, J. R. Iron reduction in the metal-rich guts of wood-feeding termites. *Geobiology* **2**, 239–247 (2004).
65. Li, H. *et al.* Physiochemical conditions and metal ion profiles in the gut of the fungus-growing termite *Odontotermes formosanus*. *J. Insect Physiol.* **58**, 1368–1375 (2012).
66. Kappler, A. & Brune, A. Influence of gut alkalinity and oxygen status on mobilization and size-class distribution of humic acids in the hindgut of soil-feeding termites. *Appl. Soil Ecol.* **13**, 219–229 (1999).
67. Chang, H.-M., Cowling, E. B., Brown, W., Adler, E. & Miksche, G. E. Comparative studies on cellulolytic enzyme lignin and milled wood lignin of sweetgum and spruce. *Holzforschung* **29**, 153–159 (1975).
68. Hu, Z., Yeh, T.-F., Chang, H.-M., Matsumoto, Y. & Kadla, J. F. Elucidation of the structure of cellulolytic enzyme lignin. *Holzforschung* **60**, 389–397 (2006).
69. Matsumura, Y., Sudo, K. & Shimizu, K. Enzymatic hydrolysis of woods. II. Effect of grinding and alkali treatment on hydrolysis of woods by *Trichoderma viride* cellulase. *Mokuzai Gakkaishi* **23**, 562–570 (1977).
70. Brune, A. Termite guts: the world's smallest bioreactors. *Trends Biotechnol.* **16**, 16–21 (1998).
71. Tholen, A. & Brune, A. Impact of oxygen on metabolic fluxes and *in situ* rates of reductive acetogenesis in the hindgut of the wood-feeding termite *Reticulitermes flavipes*. *Environ. Microbiol.* **2**, 436–449 (2000).
72. Pester, M. & Brune, A. Hydrogen is the central free intermediate during lignocellulose degradation by termite gut symbionts. *ISME J.* **1**, 551–565 (2007).
This paper reports the *in situ* assessment of hydrogen turnover and other metabolic fluxes in the hindgut of several lower termites, combining microsensor and isotope dilution techniques.
73. Köhler, T., Dietrich, C., Scheffrahn, R. H. & Brune, A. High-resolution analysis of gut environment and bacterial microbiota reveals functional compartmentation of the gut in wood-feeding higher termites (*Nasutitermes* spp.). *Appl. Environ. Microbiol.* **78**, 4691–4701 (2012).
This is the first study that combines microsensor techniques with high-throughput sequencing and is the first evidence for hydrogen accumulation in the gut of wood-feeding higher termites.
74. Sugimoto, A. *et al.* Methane and hydrogen production in a termite–symbiont system. *Ecol. Res.* **13**, 241–257 (1998).
75. Schmitt-Wagner, D. & Brune, A. Hydrogen profiles and localization of methanogenic activities in the highly compartmentalized hindgut of soil-feeding higher termites (*Cubitermes* spp.). *Appl. Environ. Microbiol.* **65**, 4490–4496 (1999).
76. Inoue, J.-I., Saita, K., Kudo, T., Ui, S. & Ohkuma, M. Hydrogen production by termite gut protists: characterization of iron hydrogenases of parabasalian symbionts of the termite *Coptotermes formosanus*. *Eukaryot. Cell* **6**, 1925–1932 (2007).
77. Lilburn, T. G., Schmidt, T. M. & Breznak, J. A. Phylogenetic diversity of termite gut spirochaetes. *Environ. Microbiol.* **1**, 331–345 (1999).
78. Iida, T., Ohkuma, M., Ohtoko, K. & Kudo, T. Symbiotic spirochetes in the termite hindgut: phylogenetic identification of ectosymbiotic spirochetes of oxymonad protists. *FEMS Microbiol. Ecol.* **34**, 17–26 (2000).
79. Leadbetter, J. R., Schmidt, T. M., Graber, J. R. & Breznak, J. A. Acetogenesis from H_2 plus CO_2 by spirochetes from termite guts. *Science* **283**, 686–689 (1999).
This study reports the isolation of termite gut spirochaetes in pure culture and the basis for their identification as the major hydrogen sink in wood-feeding termites; it was a milestone in termite gut research.
80. Graber, J. R., Leadbetter, J. R. & Breznak, J. A. Description of *Treponema azotonutricium* sp. nov. and *Treponema primitia* sp. nov., the first spirochetes isolated from termite guts. *Appl. Environ. Microbiol.* **70**, 1315–1320 (2004).
81. Graber, J. R. & Breznak, J. A. Physiology and nutrition of *Treponema primitia*, an H_2/CO_2-acetogenic spirochete from termite hindguts. *Appl. Environ. Microbiol.* **70**, 1307–1314 (2004).
82. Salmassi, T. M. & Leadbetter, J. R. Molecular aspects of CO_2-reductive acetogenesis in cultivated spirochetes and the gut community of the termite *Zootermopsis angusticollis*. *Microbiology* **149**, 2529–2537 (2003).
83. Pester, M. & Brune, A. Expression profiles of *fhs* (FTHFS) genes support the hypothesis that spirochaetes dominate reductive acetogenesis in the hindgut of lower termites. *Environ. Microbiol.* **8**, 1261–1270 (2006).
84. Matson, E. G., Gora, K. G. & Leadbetter, J. R. Anaerobic carbon monoxide dehydrogenase diversity in the homoacetogenic hindgut microbial communities of lower termites and the wood roach. *PLoS ONE* **6**, e19316 (2011).
85. Zhang, X., Matson, E. G. & Leadbetter, J. R. Genes for selenium dependent and independent formate dehydrogenase in the gut microbial communities of three lower, wood-feeding termites and a wood-feeding roach. *Environ. Microbiol.* **13**, 307–323 (2011).
86. Ottesen, E. A. & Leadbetter, J. R. Formyltetrahydrofolate synthetase gene diversity in the guts of higher termites with different diets and lifestyles. *Appl. Environ. Microbiol.* **77**, 3461–3467 (2011).
87. Zhang, X. & Leadbetter, J. R. Evidence for cascades of perturbation and adaptation in the metabolic genes of higher termite gut symbionts. *mBio* **3**, e00223-12 (2012).
88. Ballor, N. R. & Leadbetter, J. R. Patterns of [FeFe] hydrogenase diversity in the gut communities of lignocellulose-feeding higher termites. *Appl. Environ. Microbiol.* **78**, 5368–5374 (2012).
89. Ballor, N. R., Paulsen, I. & Leadbetter, J. R. Genomic analysis reveals multiple [FeFe] hydrogenases and hydrogen sensors encoded by treponemes from the H_2-rich termite gut. *Microb. Ecol.* **63**, 282–294 (2012).
90. Ottesen, E. A. & Leadbetter, J. R. Diversity of formyltetrahydrofolate synthetases in the guts of the wood-feeding cockroach *Cryptocercus punctulatus* and the omnivorous cockroach *Periplaneta americana*. *Appl. Environ. Microbiol.* **76**, 4909–4913 (2010).
91. Dröge, S., Rachel, R., Radek, R. & König, H. *Treponema isoptericolens* sp. nov., a novel spirochaete from the hindgut of the termite *Incisitermes tabogae*. *Int. J. Syst. Evol. Microbiol.* **58**, 1079–1083 (2008).
92. Brune, A. in *Handbook of Hydrocarbon and Lipid Microbiology* (ed. Timmis, K. N.) 707–728 (Springer, 2010).
93. Deevong, P. *et al.* Isolation and detection of methanogens from the gut of higher termites. *Microb. Environ.* **19**, 221–226 (2004).
94. Brune, A. in *(Endo)symbiotic Methanogenic Archaea* (ed. Hackstein, J.H.P) 81–100 (Springer, 2011).
95. Paul, K., Nonoh, J. O., Mikulski, L. & Brune, A. 'Methanoplasmatales': *Thermoplasmatales*-related archaea in termite guts and other environments are the seventh order of methanogens. *Appl. Environ. Microbiol.* **78**, 8245–8253 (2012).
96. Pester, M., Tholen, A., Friedrich, M. W. & Brune, A. Methane oxidation in termite hindguts: absence of evidence and evidence of absence. *Appl. Environ. Microbiol.* **73**, 2024–2028 (2007).
97. Darlington, J. P. E. C., Zimmerman, P. R., Greenberg, J., Westberg, C. & Bakwin, P. Production of metabolic gases by nests of the termite *Macrotermes jeanneli* in Kenya. *J. Trop. Ecol.* **13**, 491–510 (1997).
98. Nakajima, H., Hongoh, Y., Usamib, R., Kudo, T. & Ohkuma, M. Spatial distribution of bacterial phylotypes in the gut of the termite *Reticulitermes speratus* and the bacterial community colonizing the gut epithelium. *FEMS Microbiol. Ecol.* **54**, 247–255 (2005).
99. Wertz, J. T. & Breznak, J. A. *Stenoxybacter acetivorans* gen. nov., sp. nov., an acetate-oxidizing obligate microaerophile among diverse O_2-consuming bacteria from termite guts. *Appl. Environ. Microbiol.* **73**, 6819–6828 (2007).
100. Wertz, J. T. & Breznak, J. A. Physiological ecology of *Stenoxybacter acetivorans*, an obligate microaerophile in termite guts. *Appl. Environ. Microbiol.* **73**, 6829–6841 (2007).
101. Wertz, J. T., Kim, E., Breznak, J. A., Schmidt, T. M. & Rodrigues, J. L. M. Genomic and physiological characterization of the *Verrucomicrobia* isolate *Diplosphaera colotermitum* gen. nov., sp. nov. reveals microaerophily and nitrogen fixation genes. *Appl. Environ. Microbiol.* **78**, 1544–1555 (2012).
102. Boga, H. I., Ji, R., Ludwig, W. & Brune, A. *Sporotalea propionica* gen. nov. sp. nov., a hydrogen-oxidizing, oxygen-reducing, propionigenic firmicute from the intestinal tract of a soil-feeding termite. *Arch. Microbiol.* **187**, 15–27 (2007).
103. Boga, H. I. & Brune, A. Hydrogen-dependent oxygen reduction by homoacetogenic bacteria isolated from termite guts. *Appl. Environ. Microbiol.* **69**, 779–786 (2003).

104. Tholen, A., Pester, M. & Brune, A. Simultaneous methanogenesis and oxygen reduction by *Methanobrevibacter cuticularis* at low oxygen fluxes. *FEMS Microbiol. Ecol.* **62**, 303–312 (2007).
105. Lighton, J. R. B. & Ottesen, E. A. To DGC or not to DGC: oxygen guarding in the termite *Zootermopsis nevadensis* (Isoptera: Termopsidae). *J. Exp. Biol.* **208**, 4671–4678 (2005).
106. Ngugi, D. K., Ji, R. & Brune, A. Nitrogen mineralization, denitrification, and nitrate ammonification by soil-feeding termites — a ^{15}N-based approach. *Biogeochemistry* **103**, 355–369 (2011).
107. Tayasu, I., Abe, T., Eggleton, P. & Bignell, D. E. Nitrogen and carbon isotope ratios in termites: an indicator of trophic habit along the gradient from wood-feeding to soil-feeding. *Ecol. Entomol.* **22**, 343–351 (1997).
108. Ji, R. & Brune, A. Nitrogen mineralization, ammonia accumulation, and emission of gaseous NH_3 by soil-feeding termites. *Biogeochemistry* **78**, 267–283 (2006).
109. Ngugi, D. K. & Brune, A. Nitrate reduction, nitrous oxide formation, and anaerobic ammonia oxidation to nitrite in the gut of soil-feeding termites (*Cubitermes* and *Ophiotermes* spp.). *Environ. Microbiol.* **14**, 860–871 (2012).
This paper analyses nitrogen metabolism in soil-feeding termites using isotope tracers and provides evidence for the production of N_2O and an unusual ammonia-oxidizing activity in the posterior hindgut.
110. Köhler, T., Stingl, U., Meuser, K. & Brune, A. Novel lineages of *Planctomycetes* densely colonize the alkaline gut of soil-feeding termites (*Cubitermes* spp.). *Environ. Microbiol.* **10**, 1260–1270 (2008).
111. Friedrich, M. W., Schmitt-Wagner, D., Lueders, T. & Brune, A. Axial differences in community structure of *Crenarchaeota* and *Euryarchaeota* in the highly compartmentalized gut of the soil-feeding termite *Cubitermes orthognathus*. *Appl. Environ. Microbiol.* **67**, 4880–4890 (2001).
112. Brümmer, C., Papen, H., Wassmann, R. & Brüggemann, N. Termite mounds as hot spots of nitrous oxide emissions in South-Sudanian savanna of Burkina Faso (West Africa). *Geophys. Res. Lett.* **36**, L09814 (2009).
113. Fujita, A. Lysozymes in insects: what role do they play in nitrogen metabolism? *Physiol. Entomol.* **299**, 305–310 (2004).
114. Sabree, Z. L., Kambhampati, S. & Moran, N. A. Nitrogen recycling and nutritional provisioning by *Blattabacterium*, the cockroach endosymbiont. *Proc. Natl Acad. Sci. USA* **106**, 19521–19526 (2009).
115. Neef, A. *et al.* Genome economization in the endosymbiont of the wood roach *Cryptocercus punctulatus* due to drastic loss of amino acid synthesis capabilities. *Genome Biol. Evol.* **3**, 1437–1448 (2011).
116. Sabree, Z. L. *et al.* Genome shrinkage and loss of nutrient-providing potential in the obligate symbiont of the primitive termite *Mastotermes darwiniensis*. *Appl. Environ. Microbiol.* **78**, 204–210 (2012).
117. Lilburn, T. G. *et al.* Nitrogen fixation by symbiotic and free-living spirochetes. *Science* **292**, 2495–2498 (2001).
118. Yamada, A., Inoue, T., Noda, Y., Hongoh, H. & Ohkuma, M. Evolutionary trend of phylogenetic diversity of nitrogen fixation genes in the gut community of wood-feeding termites. *Mol. Ecol.* **16**, 3768–3777 (2007).
119. Noda, S., Ohkuma, M., Usami, R., Horikoshi, K. & Kudo, T. Culture-independent characterization of a gene responsible for nitrogen fixation in the symbiotic microbial community in the gut of the termite *Neotermes koshunensis*. *Appl. Environ. Microbiol.* **65**, 4935–4942 (1999).
120. Desai, M. S. & Brune, A. *Bacteroidales* ectosymbionts of gut flagellates shape the nitrogen-fixing community in dry-wood termites. *ISME J.* **6**, 1302–1313 (2012).
121. Leigh, J. A. & Dodsworth, J. A. Nitrogen regulation in bacteria and archaea. *Annu. Rev. Microbiol.* **61**, 349–377 (2007).
122. Noda, S. *et al.* Cospeciation in the triplex symbiosis of termite gut protists (*Pseudotrichonympha* spp.), their hosts, and their bacterial endosymbionts. *Mol. Ecol.* **16**, 1257–1266 (2007).
This paper is an elegant case study of co-evolution between the partners of a tripartite symbiosis involving a lineage of termites, their major gut flagellate and its intracellular symbiont.
123. Inoue, T., Kitade, O., Yoshimura, T. & Yamaoka, I. in *Termites: Evolution, Sociality, Symbiosis, Ecology* (eds Abe, T., Bignell, D. E. & Higashi, M.) 275–288 (Kluwer Academic Publishers, 2000).
124. Hongoh, Y. *et al.* Complete genome of the uncultured Termite Group 1 bacteria in a single host protist cell. *Proc. Natl Acad. Sci. USA* **105**, 5555–5560 (2008).
This paper reports the first genome analysis of a flagellate endosymbiont that maintained its biosynthetic pathways for vitamins and amino acids despite considerable genome erosion.
125. Noda, S., Hongoh, Y., Sato, T. & Ohkuma, M. Complex coevolutionary history of symbiotic Bacteroidales bacteria of various protists in the gut of termites. *BMC Evol. Biol.* **9**, 158 (2009).
126. Strassert, J. F. H. *et al.* '*Candidatus* Ancillula trichonymphae', a novel lineage of endosymbiotic *Actinobacteria* in termite gut flagellates of the genus *Trichonympha*. *Environ. Microbiol.* **14**, 3259–3270 (2012).
127. Ikeda-Ohtsubo, W. & Brune, A. Cospeciation of termite gut flagellates and their bacterial endosymbionts: *Trichonympha* species and '*Candidatus* Endomicrobium trichonymphae'. *Mol. Ecol.* **18**, 332–342 (2009).
128. Desai, M. S. *et al.* Strict cospeciation of devescovinid flagellates and *Bacteroidales* ectosymbionts in the gut of dry-wood termites (*Kalotermitidae*). *Environ. Microbiol.* **12**, 2120–2132 (2010).
129. Ikeda-Ohtsubo, W., Faivre, N. & Brune, A. Putatively free-living '*Endomicrobia*' — ancestors of the intracellular symbionts of termite gut flagellates? *Environ. Microbiol.* **2**, 554–559 (2010).
130. Ohkuma, M., Noda, S., Hongoh, Y., Nalepa, C. A. & Inoue, T. Inheritance and diversification of symbiotic trichonymphid flagellates from a common ancestor of termites and the cockroach *Cryptocercus*. *Proc. R. Soc. B* **276**, 239–245 (2009).
131. Carpenter, K. J., Chow, L. & Keeling, P. J. Morphology, phylogeny, and diversity of Trichonympha (Parabasalia: Hypermastigida) of the wood-feeding cockroach *Cryptocercus punctulatus*. *J. Eukaryot. Microbiol.* **56**, 305–313 (2009).
132. Rosengaus, R. B., Zecher, C. N., Schultheis, K. F., Brucker, R. M. & Bordenstein, S. R. Disruption of termite gut microbiota and its prolonged consequences for fitness. *Appl. Environ. Microbiol.* **77**, 4303–4312 (2011).
133. Geissinger, O., Herlemann, D. P. R., Mörschel, E., Maier, U. G. & Brune, A. The ultramicrobacterium "*Elusimicrobium minutum*" gen. nov., sp. nov., the first cultivated representative of the Termite Group 1 phylum. *Appl. Environ. Microbiol.* **75**, 2831–2840 (2009).
134. Noda, S., Hongoh, Y., Sato, T. & Ohkuma, M. Molecular phylogeny and evolution of Parabasalia with improved taxon sampling and new protein markers of actin and elongation factor-1α. *PLoS ONE* **7**, e29938 (2012).
135. Brugerolle, G. & Radek, R. in *Intestinal Microorganisms of Termites and Other Invertebrates* (eds König, H. & Varma, A.) 243–269 (Springer, 2006).
136. Todaka, N. *et al.* Phylogenetic analysis of cellulolytic enzyme genes from representative lineages of termites and a related cockroach. *PLoS ONE* **5**, e8636 (2010).
137. Bayané, A. & Guiot, S. R. Animal digestive strategies versus anaerobic digestion bioprocesses for biogas production from lignocellulosic biomass. *Rev. Environ. Sci. Biotechnol.* **10**, 43–62 (2011).
138. Sasaguri, S. *et al.* Codon optimization prevents premature polyadenylation of heterologously-expressed cellulases from termite-gut symbionts in *Aspergillus oryzae*. *J. Gen. Appl. Microbiol.* **54**, 343–351 (2008).
139. Nakashima, K., Watanabe, H., Saitoh, H., Tokuda, G. & Azuma, J.-I. Dual cellulose-digesting system of the wood-feeding termite, *Coptotermes formosanus* Shiraki. *Insect Biochem. Molec. Biol.* **32**, 777–784 (2002).
140. Schauer, C., Thompson, C. L. & Brune, A. The bacterial community in the gut of the cockroach *Shelfordella lateralis* reflects the close evolutionary relatedness of cockroaches and termites. *Appl. Environ. Microbiol.* **78**, 2758–2767 (2012).
141. Shinzato, N., Muramatsu, M., Matsui, T. & Watanabe, Y. Molecular phylogenetic diversity of the bacterial community in the gut of the termite *Coptotermes formosanus*. *Biosci. Biotechnol. Biochem.* **69**, 1145–1155 (2005).

Acknowledgements
The current concept of symbiotic digestion in termite guts is based on countless contributions from many colleagues. The author tried his best to identify the mentors of important advances but regrets that space restrictions did not always permit references to the original work.