Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Chemical Biology

Lignocellulose deconstruction in the biosphere

Yannick J Bomble[1], Chien-Yuan Lin[1], Antonella Amore[1], Hui Wei[1], Evert K Holwerda[2], Peter N Ciesielski[1], Bryon S Donohoe[1], Stephen R Decker[1], Lee R Lynd[2] and Michael E Himmel[1]

Microorganisms have evolved different and yet complementary mechanisms to degrade biomass in the biosphere. The chemical biology of lignocellulose deconstruction is a complex and intricate process that appears to vary in response to specific ecosystems. These microorganisms rely on simple to complex arrangements of glycoside hydrolases to conduct most of these polysaccharide depolymerization reactions and also, as discovered more recently, oxidative mechanisms via lytic polysaccharide monooxygenases or non-enzymatic Fenton reactions which are used to enhance deconstruction. It is now clear that these deconstruction mechanisms are often more efficient in the presence of the microorganisms. In general, a major fraction of the total plant biomass deconstruction in the biosphere results from the action of various microorganisms, primarily aerobic bacteria and fungi, as well as a variety of anaerobic bacteria. Beyond carbon recycling, specialized microorganisms interact with plants to manage nitrogen in the biosphere. Understanding the interplay between these organisms within or across ecosystems is crucial to further our grasp of chemical recycling in the biosphere and also enables optimization of the burgeoning plant-based bioeconomy.

Addresses
[1] Biosciences Center, National Renewable Energy Laboratory, Golden, CO, USA
[2] Thayer School of Engineering, Dartmouth College, Hanover, NH, USA

Corresponding author: Bomble, Yannick J (yannick.bomble@nrel.gov)

Current Opinion in Chemical Biology 2017, **41**:61–70

This review comes from a themed issue on **Energy**

Edited by **Matthew W. Kanan**

For a complete overview see the Issue and the Editorial

Available online 2nd November 2017

http://dx.doi.org/10.1016/j.cbpa.2017.10.013

Introduction

Photosynthesis and the resulting plant biomass is the only significant source of organic compounds in the terrestrial biosphere. The primary product of photosynthesis, cellulosic biomass, has evolved to be recalcitrant to deconstruction by microorganisms and their enzymes. This recalcitrance is due to natural barriers in plant mesostructure (bark, rind, and vascular networks); as well as the composition, structure, and chemical linkages in the plant cell wall. Cellulose crystallinity can be itself a barrier to enzymatic deconstruction, but the complexity and heterogeneity of the xylan matrix covering microfibrils further restricts enzyme accessibly and requires a large suite of xylan degrading enzymes [1,2]. Finally, lignification of the plant cell wall that provides rigidity to the plant is an impediment to efficient deconstruction by further reducing accessibly.

To overcome this natural recalcitrance, fungi and bacteria have developed a diverse set of enzymes and strategies suited for the ecosystem in which they occur. These strategies are primarily based on the use of glycoside hydrolases (GHs) (more than 140 GH families to date) [2]. Additionally, some fungi and bacteria can deploy oxidative processes that assist GHs in the deconstruction of biomass [3]. These enzymes are efficient enough for the microorganisms to grow on biomass as their sole carbon source, but have rather low turnover rates compared to other enzymes. Additionally, they are often more efficient in the presence of the microbe that produces them [4].

Biomass degrading microbes also rely on inter-microbial synergy to thrive in their natural environment where these interactions depend on the composition of microbial communities and the specific environmental conditions encountered. Moreover, these interactions can be crucial to the survival of these microorganisms and represent a vast resource of knowledge that can help us understand the chemical biology of carbon/nitrogen recycling and biomass deconstruction in the biosphere.

Plant cell wall structure

Plant biomass is composed of several energy-rich biopolymers that are arranged into a hierarchical structure to form the fiber reinforced matrix of plant cell walls. This material, termed lignocellulose, displays impressive structural complexity and robust functionality. During the lifetime of the plant, specialized cells in plant stems provide physical support and also form the conduits through which water and nutrients are transported. The mature cell walls in these supportive and conductive tissues typically comprise three ultrastructural domains: the middle lamella, the primary wall, and the secondary wall. The middle lamella of vascular cells

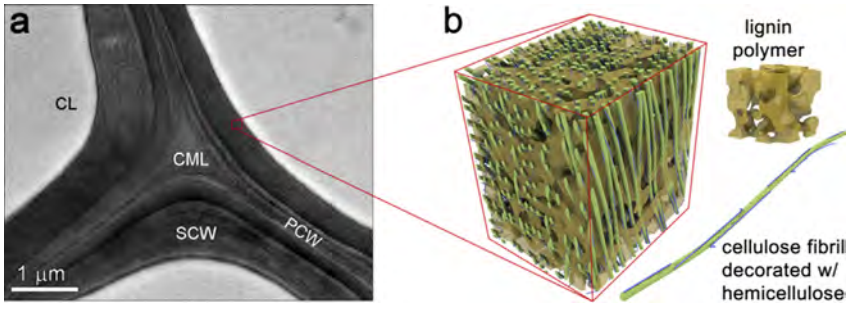
is heavily lignified and serves to adhere neighboring cells. The primary wall is the first layer of the wall to be synthesized during plant growth and consists of several layers of differently oriented cellulose microfibrils [1]. The secondary wall is synthesized after the primary wall is completed and provides substantial mechanical reinforcement to the vascular tissue. The secondary wall is distinct from the primary wall in that it is synthesized by the individual cell that it encapsulates whereas synthesis of the primary wall is achieved jointly by both cells that boarder the wall. By the time the secondary cell wall has been produced and the cell wall has lignified it is difficult to delineate the primary cell wall from the middle lamella. The term compound middle lamella (CML) is used to refer to these two layers collectively (Figure 1a).

Although the precise architectural details of lignocellulose nanostructure vary among plant species and tissues and remain an active area of research, some general agreement exists and informs future studies of efficient plant deconstruction. Aggregates of cellulose chains form strong and highly ordered bundles of cellulose micro-fibrils and macrofibrils [5], which serve as the rigid scaffolding structure and are deposited in discrete layers or lamella in the cell wall. These cellulose fibrils are decorated and interconnected with hemicellulose, which is a structurally diverse, branched polymer composed of various sugars including xylose, arabinose and mannose (Figure 1b). In the case of cells that produce a secondary cell wall, lignin, an amorphous polymer of different phenylpropanoid units, fills much of the remaining void volume of the cell wall [6]. Lignin provides additional mechanical strength to the composite and increases the hydrophobicity of the walls to aid in transport of water. In addition, lignin serves as a defense mechanism to prevent deconstruction by the hydrolytic enzymes secreted by pathogens. In most land plants, most of the cellulose is found in such lignified secondary cell walls, which poses a considerable challenge to biochemical deconstruction.

Hydrolytic and oxidative mechanisms of enzymatic cell wall deconstruction

In Nature, bacteria and fungi commonly deconstruct biomass by producing and secreting a combination of synergistically acting enzymes [7••]. The most abundant enzymes in these mixtures are hydrolytic glycoside hydrolases (GHs) and carbohydrate esterases. Other less abundant enzymes include polysaccharide lyases, 'auxiliary activity' enzymes (AA) [2], and cellodextrin phosphorylases. In the system used to classify carbohydrate active enzymes based on sequence and structure (CAZy), the GHs are represented by more than 140 different families [2]. Based on their mechanism and role in lignocellulose deconstruction there are three main classes of GHs, exoglucanases, endoglucanases, and cellobiases. Exoglucanases are processive enzymes and can cleave a cellulose polymer from either the reducing or non-reducing end of the polysaccharide chain. Endoglucanases typically hydrolyze cellulose chains nonprocessively anywhere along the polysaccharide chain. However in some cases endoglucanases can be processive exhibiting high cellulolytic activity [8–10]. Cellobiases primarily hydrolyze the cellobiose dimer into glucose monomers. These GHs cleave glycosidic bonds using one of two different types of catalytic mechanisms: Firstly, inverting, (i.e. inversion of anomeric configuration), wherein the catalytic acid and base residues generally achieve hydrolysis in a one-step mechanism [11,12]; or secondly, retaining, (i.e. retaining of anomeric configuration), wherein there is a general acid/base residue and a potential nucleophile used to conduct a Koshland type hydrolysis mechanism [13]. In this two-step mechanism, the first step is glycosylation (formation of a glycosyl enzyme intermediate) and the second step is deglycosylation (the glycosyl enzyme is hydrolyzed by water). The diversity of these GHs represents a vast arsenal of specific activities for the efficient deconstruction of biomass in the biosphere. However, microorganisms have also evolved ways to increase substrate specificity and enzyme kinetics by physically linking polysaccharidases in close proximity, increasing efficiency.

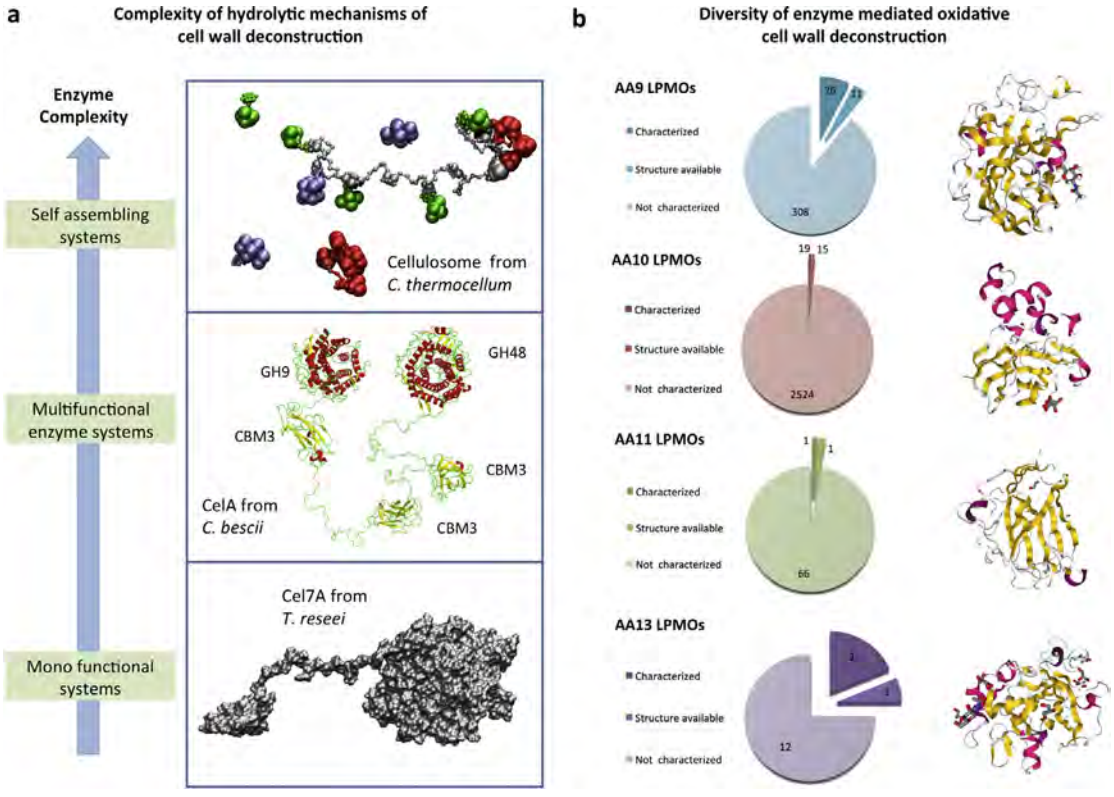
Figure 1



(**a**) Transmission electron micrograph of cell walls from vascular tissue in maize. CL, cell lumen; CML, compound middle lamella; SCW, secondary cell wall; PCW, primary cell wall. (**b**) Depiction of the structure of the lignocellulose composite in secondary cell walls.

In addition to producing different mixtures of enzymes with a range of activities it is interesting to hypothesize that microbes have tailored strategies to the biomass available and the chemical environment in their ecosystems by evolving different enzyme architectures. These enzyme architecture strategies that are found in Nature rely on the following general schemes of increasing complexity for biomass deconstruction: mono-functional enzymes, multi-functional enzymes, and highly aggregated (cellulosomal) enzyme systems (Figure 2a). The most common strategy used by lignocellulose degrading fungi and bacteria involves mono-functional enzymes, wherein GHs with a single catalytic domain are combined with carbohydrate binding modules (CBMs) [7••,14]. CBMs are non-catalytic, functionally independent domains, able to target a specific polysaccharide [15,16]. CBMs have been shown to help substrate recognition and increase catalytic activity on insoluble substrates at low solids loadings. This strategy relies entirely on synergism between CAZymes as first reported by Reese *et al.* [17]. It is the strategy most commonly used in commercial enzyme preparations and the major component, Cel7A (cellobiohydrolase/exoglucanase) from *Trichoderma reesei*, is one of the most studied cellulases to date. A strategy described thus far in thermophilic bacteria also takes advantage of synergism within biomass degrading enzymes that include more than one catalytic domain within a single gene product, often with complementary activities. A notable example of a multi-functional cellulase is CelA from *Caldicellulosiruptor bescii* that couples an exoglucanase (GH48) with a processive endoglucanase (GH9-CBM3) and two additional CBMs to efficiently degrade biomass [9••,18]. This combination can be quite potent as CelA is to date the most active cellulose-solubilizing enzyme reported. Finally, in contrast to the mono-functional or multi-functional cellulase systems, some cellulolytic anaerobic bacteria and fungi utilize CAZymes that self-assemble onto a protein scaffold attached to the cell surface, forming a superstructure called the cellulosome. Cellulosomes take advantage of a proximity effect by coupling enzymes with complementary functionalities. This paradigm was first identified in *Clostridium thermocellum*, which is still to date the most promising and studied cellulosomal system reported

Figure 2



(a) Examples of the complexity of the three main hydrolytic mechanisms used in the biosphere for cell wall deconstruction: Cel7A from *T. reesei* is the most studied cellulase representing the free enzyme/mono functional system, CelA from *C. bescii* is a prime example of an efficient multifunctional cellulase, highly active on crystalline cellulose, and the cellulosome from *C. thermocellum* was the first scaffolded CAZyme system to be studied and shown to be highly active on biomass. **(b)** Examples of the diversity of oxidative enzyme families so far reported in the biosphere: AA9 LPMO from *Neurospora crassa* (4EIR), AA10 LPMO from *Thermofibida fusca* (4GBO), AA11 LPMO from *Aspergillus oryzae* (4MAH), and AA13 LPMO from *Aspergillus oryzae* (4OPB).

[19,20,21,22•]. *C. thermocellum* is one of the best plant cell wall biomass degraders in the biosphere. Moreover, its cell-free cellulosomes are equally potent, inspiring several important studies of synthetic cellulosomes aiming to emulate the efficacy of the native system [22•,23].

Despite the diversity of families and the range of architectures, all of the enzymes discussed above share the common feature of employing a hydrolytic mechanism. In addition to hydrolytic deconstruction, aerobically favored oxidative reactions were shown by Eriksson *et al.* [3] to be involved in cellulose deconstruction in *Sporotrichum pulverulentum*. More recent studies have continued to reveal the role of oxidative processes in lignocellulose deconstruction; a departure from the classic paradigm of endocellulase, exocellulase, and cellobiase mediated cellulose hydrolysis. Lytic polysaccharide monooxygenases (LPMOs) are the primary class of enzymes responsible for oxidative biomass deconstruction and have been the most studied oxidative route over the past decade. Organisms such as brown rot fungi however, also rely on non-enzymatic Fenton chemistry-based pathways [24,25].

The LPMOs are metalloenzymes capable of directly attacking cellulose, chitin, starch, and hemicellulose through oxidative reactions, utilizing a mechanism involving a divalent metal ion (usually copper), molecular oxygen, and an electron donor [26•,27,28]. LPMOs have been re-classified from GH61 and CBM33 into four main families of auxiliary activity (AA) enzymes [2]. The AA9 and AA10 families comprise LPMOs mainly from fungi and bacteria, respectively [29], with AA9s oxidizing various carbon positions (C1, C4, or C6) of the glucose ring structure in the presence of reducing equivalents, and AA10s acting on both crystalline chitin and cellulose to produce aldonic acids. AA11 proteins cleave chitin chains with oxidation of C1 [29], whereas AA13 LPMOs are able to oxidatively cleave starch [27] (Figure 2b).

Several important aspects of LMPO activity, specificity, and binding have only recently been studied. Jung *et al.* [30] examined the properties of an LPMO from *Gloeophyllum trabeum* (*Gt*), demonstrating that the addition of *Gt*GH61 increases conversion of pretreated oak and kenaf by 12% and 11%, respectively, due to synergy with *G. trabeum* family 10 xylanase (Xyl10G) and family 5 cellulase (Cel5B). *Gt*LPMO9A-2 from the same fungus was recently shown to be active on cellulose and carboxymethyl cellulose and was able to fragment xyloglucan anywhere along the β-glucan backbone, regardless of substitutions [31]. Recently, Bennati-Granier *et al.* [32] reported insights into the mechanism of action of AA9 LPMOs found in the exoproteome of *Podospora anserine (Pa)*. Among the seven LPMOs considered in this study, those harboring a CBM1 domain were able to release higher amounts of oxidized oligosaccharides. The *Pa*LPMO9A and *Pa*LPMO9H were shown to fragment oligosaccharides at both C1 and C4, whereas *Pa*LPMO9E acted only at C1. Of industrial importance, when LPMOs from *Thermoascus aurantiacus* were added to a commercial cellulase formulation, a 60% increase in cellulose solubilization was observed in the presence of oxygen and a reductant [33•]. Similarly, several AA10 LPMOs from *Streptomyces coelicolor* (*Sc*) were produced and characterized by Forsberg *et al.* [34], showing that LPMOs complement the cellulolytic machinery of this microbe during biomass deconstruction by deploying both C1 (*Sc*LPMO10C) and C1/C4 (*Sc*LPMO10B) oxidative activities.

It should be noted that the complete role and mode of action of LPMOs *in vivo* are not yet clear.

For example, LPMOs are thought to perform cellulose fragmentation at the cellulose microfibril surface, relying on GHs to conduct the majority of cell wall polysaccharide depolymerization, which may not explain the ~10–15% boost in cellulose solubilization reported for LPMO addition.

Microbially mediated deconstruction of plant cell walls

In the biosphere, the action of biomass deconstruction enzymes on plant cell walls is closely associated with the production and presentation of such enzymes by microorganisms. Understanding microbially mediated plant cell wall deconstruction requires consideration of a number of fundamental factors in addition to those associated with the action of the enzymes. Lignocellulose solubilization by anaerobic microorganisms has received intensive study in recent years and is addressed here, focusing on work with *C. thermocellum*.

The kinetics of microbially mediated cellulose deconstruction in batch culture tends to be dominated by biocatalyst concentration during the initial stages, and by substrate concentration during the later stages. As a result, the specific growth rate of cellulolytic microbes goes through a maximum and varies continuously during batch culture [35]. Based on cell-free enzymatic hydrolysis studies, it was long thought that the universal mechanism of plant cell wall solubilization relied primarily on the action of cellobiose-producing cellobiohydrolases. However, studies with live cultures of *C. thermocellum* provided strong evidence that cellodextrins are assimilated (an average degree of polymerization of 4), with approximately three quarters of β-D-glucosidic bonds cleaved intracellularly via a phosphorolytic rather than hydrolytic mechanism. As a result of phosphorolytic cleavage combined with an inverse relationship between cellodextrin chain length and the energy required for substrate uptake, *C. thermocellum* realizes cellulose-specific bioenergic benefits greater than the bioenergetic cost of cellulase synthesis [36]. Bioenergetic understanding of

cellulolytic anaerobes continues to progress, with recent indications that glycolysis may be more reversible than in other microbes [37] and that the ATP conserved per glucose moiety metabolized may be ≥4 [38••].

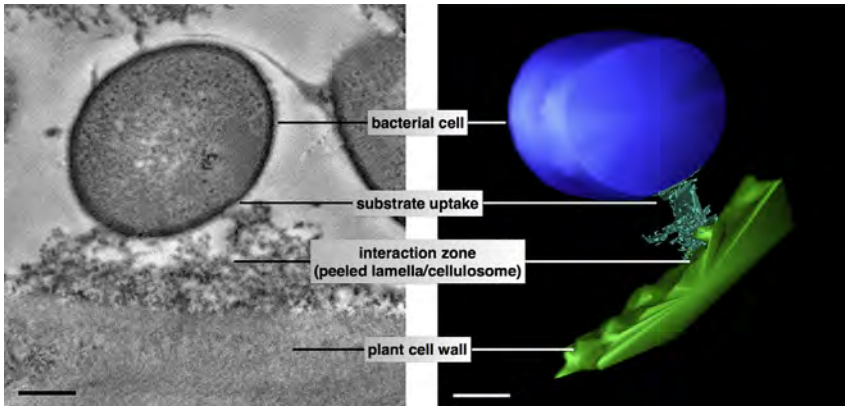
The presence of metabolically active cells appears to enhance the effectiveness of plant cell wall solubilizing enzymes by several-fold [4]. Although the mechanism of such enzyme-microbe synergy is not well-understood, it likely involves the distinctive local chemical environment in which microbially presented biomass deconstruction enzyme systems function. Cellulolytic anaerobic microbes typically express some cellulase enzymes on the cell surface and form a near-continuous monolayer on plant biomass particles [39,40]. As a result, cellulase enzyme systems act in the space between the closely adjacent surfaces of the substrate and the cell, and have evolved in response to opportunities and constraints in this distinctive milieu. The properties of water are known to be radically different at surfaces in general [41], and the surfaces of lignocellulose in particular [42]. The same is no doubt true of the cell surfaces of biomass degrading microbes, which have been subject to evolutionary pressure to maximize the effectiveness of plant cell wall deconstruction and capture of these products (Figure 3).

Recent comparative studies indicate substantial differences in the extent of plant cell wall solubilization mediated by various microorganisms in pure culture. Paye [43•] found that total fractional carbohydrate solubilization of mid-season switchgrass under controlled conditions varied from 0.24 for *C. bescii* to 0.66 for *C. thermocellum*, with intermediate values obtained for *C. cellulolyticum*, *Clostridium clariflavum*, a commercial fungal cellulase preparation (Ctec2 and Htec2), and an enrichment obtained from horse manure compost. Lynd *et al.* [38••] found total carbohydrate solubilization by *C. thermocellum* cultures to be substantially higher than a commercial cellulase preparation over a broad range of reaction conditions and feedstocks, and to be about threefold higher for the most recalcitrant feedstocks.

Progressing to yet a higher level of aggregation and complexity, anaerobic plant cell wall solubilization by mixed microbial enrichments or 'microbiomes' have received considerable recent study. Considerable progress has been made at identifying the composition and structure of undefined microbial communities in environments, such as the rumen [44], termite guts [45], and biogas digesters [46]. Interspecies synergy in deconstructing plant cell walls has been inferred based on genes and transcripts for complementary enzymatic activities [45,47]. However, pure cultures were found to exhibit comparable extents of carbohydrate solubilization to mixed enrichments by Paye *et al.* [43•] and Reed *et al.* [48].

Cellulolytic microorganisms could in principle be used for industrial processing, and have received considerable attention in the context of one-step consolidated bioprocessing (CBP) without added enzymes. Exposure of plant cell walls to some combination of heat and chemicals prior to biologically mediated solubilization, thermochemical pretreatment, has generally thought to be necessary in order to make plant cell walls accessible to cellulase enzymes. However, total fractional carbohydrate solubilization of 0.88, comparable to that obtained using thermochemical pretreatment, has recently been reported using *C. thermocellum* in the presence of continuous ball milling [49•]. Industrial processing of cellulosic feedstocks by cellulolytic microbes with milling during fermentation, although still nascent and speculative, has potential to offer transformative cost reductions

Figure 3



Bacterial mediated deconstruction by *Clostridium thermocellum.* Cellulolytic bacteria express CAZymes directly near the cell wall surface. This confinement likely enhances deconstruction and product uptake. **(a)** Tomographic slice from a 3D transmission electron tomogram showing a cross section of a bacterial cell attached to and actively deconstructing a plant cell wall surface. **(b)** Tomographic surface rendering of similar bacterial cell (blue) near a plant cell wall surface (green) shows evidence for peeling of cell wall lamella. Scale bars = 200 nm.

compared to technology based on the thermochemical pretreatment and added enzymes [49•]. As pointed out by Weimer *et al.* [50], 'the cow employs a similar strategy of alternating biological and physical attack.'

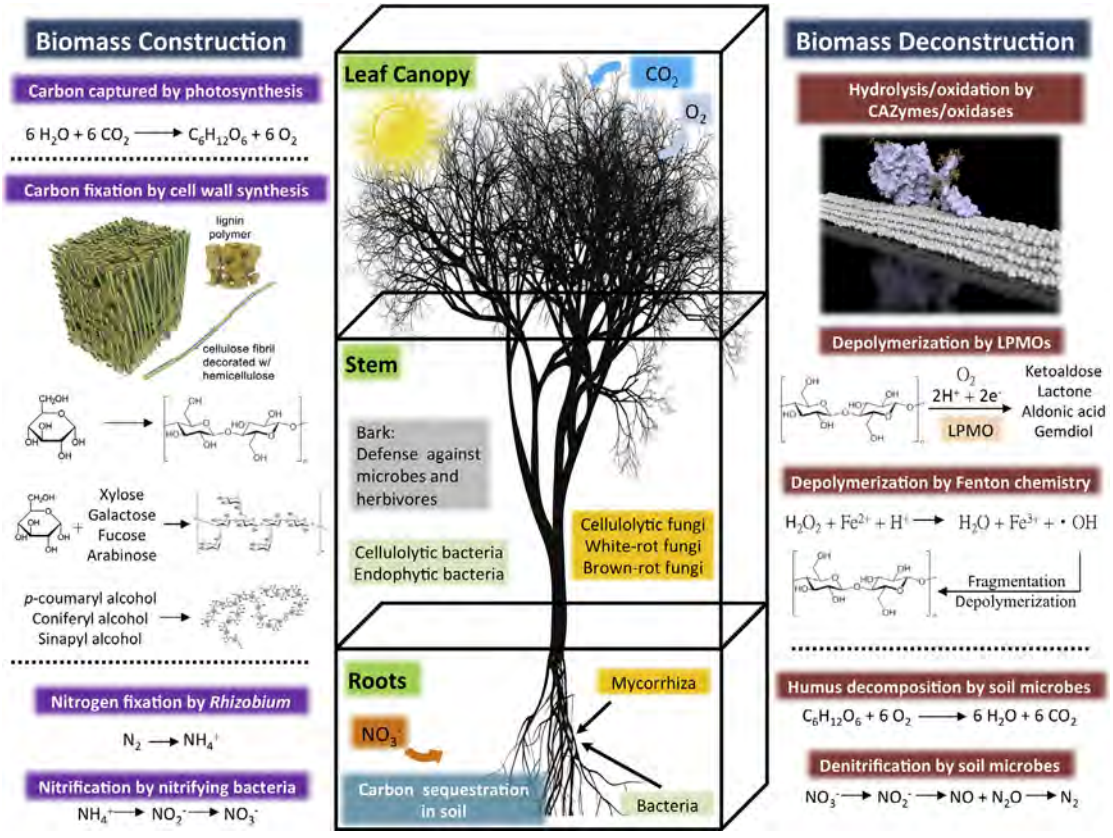
To be used for industrial processes, naturally occurring cellulolytic microbes need to be modified so that they produce desired products at high yields and titers and exhibit robustness under industrial conditions. The status of consolidated bioprocessing using thermophilic bacteria has recently been reviewed [38••,51].

Lignocellulose deconstruction at the level of the biosphere

Maintaining equilibrium in ecosystems through organic carbon and nitrogen recycling relies on the deconstruction by natural microbial communities and sometimes mutualistic symbiotic/saprotrophic microorganisms [52,53••,54,55] (Figure 4). Here we mainly focus on the microbial deconstruction on lignocellulose materials.

Cellulolytic Bacteria: In terrestrial ecosystems, soil is the principal carbon reservoir because of the accumulation of humus, wood/leaf-litter and natural composts. Although soil is generally considered the major habitat of cellulolytic bacteria in terrestrial ecosystems [56•], many aerobic and anaerobic bacteria can also be found in aquatic ecosystems [57]. Cellulolytic bacteria are commonly identified in several Phyla, such as Actinobacteria, Bacteroides, Fibrobacteres, Firmicutes and Proteobacteria [58,59•]. As mentioned above, one of the most promising anaerobic bacteria for realizing cost effective biofuel production is *C. thermocellum*, due to its high biomass degrading efficiency and the ethanol titers reported in optimized strains [51]. Several proteomic and transcriptomic analyses reported that *C. thermocellum* relies heavily on families GH5, GH8, GH9, GH11 and GH48 for biomass deconstruction [21]. These GH families seem to be preferred by many thermophilic bacteria [2]. Recent meta-transcriptomic studies of the rumen of dairy cattle revealed the relative abundance of Bacteroides, Fibrobacteres and Firmicutes species producing enzymes from

Figure 4



Carbon/nitrogen cycle and deconstruction at the level of the biosphere. In the biosphere, the recycling of carbon and nitrogen relies on biomass construction and deconstruction. Biomass construction is mainly driven by photosynthesis of the plant for cell wall. Soil microbes provide nitrate (NO_3^-) to plants by nitrogen fixation and facilitate the nutrients uptake for plant biomass construction. In contrast, the deconstruction of the biomass largely depends on the microbes. Cellulolytic microbes evolved enzymatic and chemical routes to break down the recalcitrance of plant cell wall and gain energy for their survivals. In soil, denitrifying bacteria recycle the nitrogen back to the atmosphere.

families important for the depolymerization of cellulose (GH5, GH9, GH48 and GH74) and hemicellulose (GH10, GH11, and GH43) and the high levels of GH94 (cellobiose phosphorylase) suggested a putative role of phosphorylation during the oligosaccharides deconstruction [60••]. Going beyond polysaccharides, bacteria with the ability to degrade lignin have been investigated for biomass deconstruction and high-value chemical production [61]. Additionally, recently engineered bacterial candidates for simultaneous lignin depolymerization and product generation are now considered as promising routes for lignin consolidated bioprocessing ('L'CBP) [62].

Cellulolytic fungi: Most fungi in terrestrial ecosystems are Ascomycota and Basidiomycota, and many of them possess the ability to degrade cellulose aerobically [63]. Although the majority of fungi are in terrestrial ecosystems, several aquatic fungi, mostly Chytridiomycota and Ascomycota, have been identified in both marine and fresh water [64]. Similarly to bacteria, fungi are identified as symbionts (parasitism/mutualism/commensalism) or saprotrophs [65] in both aerobic and anaerobic environments [66]. A distinct feature of anaerobic fungi is the presence of hydrogenosome, which couple the metabolism of glucose to cellular energy production without the need for oxygen [67]. In the terrestrial biosphere, *Aspergillus*, *Chaetomium*, *Fusarium*, *Penicillium* and *Trichoderma* have been reported as the main fungal species responsible for cellulosic biomass deconstruction [7••,58]. Among the cellulolytic fungi, white-rot and brown-rot fungi (basidiomycete) are considered the most prevalent and efficient decomposers of lignocellulosic materials [68]. For carbon recycling, white-rot fungi accounts for 90% and brown rot fungi constitutes about 7% of all wood rotting basidiomycete fungi in Nature [68]. White-rot fungi (e.g. *Phanerochaete chrysosporium*) cause progressive erosion of the wood polymers by degrading cellulose, hemicellulose and lignin under 'simultaneous' or 'selective' patterns and typically colonize hardwoods; brown-rot fungi (e.g. *Oligoporus* (*Postia*) *placenta*) cause the selective removal of cellulose and hemicellulose along with slight modification of lignin and are prevalent on softwoods [7••,24]. The biochemical mechanism of wood decay is fundamentally different between white-rot and brown-rot fungi. Besides endo-glucanases and exo-glucanases and cellobiases for cellulose deconstruction, white-rot fungi simultaneously produce oxidases (e.g. lignin peroxidase, manganese peroxidase, versatile peroxidase, laccase) for delignification of the biomass [69]. In contrast, brown-rot fungi deploy non-enzymatic methods to open up the amorphous regions of the cellulose microfibrils and use endoglucanases and hemicellulases to breakdown the overall holocellulose component [70]. Non-enzymatic deconstruction results in the depolymerization/fragmentation of the wood polysaccharides and is presumably done by hydroxyl free radicals, generated from the proposed extracellular Fenton chemistry ($H_2O_2 + Fe^{2+} + H^+ \rightarrow H_2O + Fe^{3+} + \bullet OH$) [25,71]. Furthermore, recent research combining genomic data and biochemical methods has demonstrated that the availability of extracellular electron donors is essential to fuel the fungal oxidative attack on polysaccharides [72••].

Bacterial–fungal interaction and lignocellulose–microbes interaction: Bacterial–fungal interaction (BFI) creates an important consortium with unique chemistries and metabolic associations (planktonic, mixed biofilm and intrahyphal colonization), which may promote the opportunistic strategy of cellulolytic bacteria [73]. These interactions allow and facilitate the antibiosis, signal molecule exchanges, and metabolite conversions between the two species. A consortia of bacteria with the white-rot fungus (*Phanerochaete chrysosporium*) showed proficient utilization of lignin breakdown products under the symbiotic lifestyle [74]. In the rumen, anaerobic fungi belonging to *Neocallimastigaceae* are often identified along with bacterial communities [60••,75] and the percentage of fungi to total microbial mass was shown to be 18–67% across various pasture and forest soils [76]. The BFI is a factor that could shape the performance of microbial communities and the concerted actions of their cellulolytic enzymes could further promote efficiency toward biomass deconstruction [56•,77••].

Additionally, synergism between bacterial and fungal cellulases has been demonstrated, which may indicates their interactions in natural processes [78]. Also, bacterial–fungal consortia can perform novel catabolic reactions for the deconstruction of high-molecular-weight polycyclic aromatic hydrocarbons [79]. The type 3 secretion systems of Gram-negative bacteria (*Burkholderia rhizoxinica*) was shown to facilitate the intrahyphal survival of bacteria itself and the sporulation of the fungal host (*Rhizopus microspores*) [80]. Similarly, BFI and the formation of fungal-bacterial biofilm (FBB) have both been shown to have better growth and colonization abilities than the monoculture of fungi or bacteria [81,82]. Although FBB does not necessarily increase sugar yields compared to fungal monoculture [83], the presence of bacteria can significantly promote the wood decomposition ability of certain fungi (*Hypholoma fasciculare* and *Resinicium bicolor*) [84].

Concluding remarks

We are beginning to understand that chemical recycling in the biosphere involves all of Earth's ecosystems. For millennia, mankind has observed the terrestrial biosphere primarily from the perspective of its emergent properties – those that can be somewhat understood. Now that descriptive science has set the stage, new trends toward multi-scale science will enable new levels of understanding. It is apparent that plants and microorganisms have co-evolved in a way that ensures efficient

recycling of not only carbon, but also other nutrients and micronutrients. This system is robust – as terrestrial ecosystems recover from historical trauma, such as glacial epochs, climate change, and the ever-changing array of foraging animals.

To enable deeper understanding of the fate of carbon in the biosphere, we must be able to observe and describe all (or most) of the chemical reactions, either biotic or abiotic, that impact the synthesis and deconstruction of plant biomass. Microbiota excels at using enzymes and carefully mediated chemistry to depolymerize plant cell wall polymers. Terrestrial plants excel at capturing carbon via photosynthesis and storing it in the complex polymers of cell walls. Most plants defend against microbes, using a many-length scale system of protective tissues (rind and bark), toxic secretions, thickened cell walls, and an array of recalcitrant polymers. Microbes have responded to these defenses by producing or adapting numerous enzymatic solutions – and are often aided in this process by collaborations with insects (termites) and animals (ruminants) providing mechanical disruption.

Once this natural complexity is sufficiently understood, it may become possible to better harness chemical recycling in the biosphere to enhance farming practices for food, production and conversion of energy crops, and ecosystem stability.

Acknowledgments

This work was supported by the BioEnergy Science Center (BESC). BESC is a U.S. Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the U.S. DOE Office of Science. C-Y.L, H.W, P.N.C, were supported as part of the Center for Direct Catalytic Conversion of Biomass to Biofuels (C3Bio), an Energy Frontier Research Center funded by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, Award Number DE-SC0000997.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Cosgrove DJ: **Growth of the plant cell wall**. *Nat Rev Mol Cell Biol* 2005, **6**:850-861.

2. Lombard V, Golaconda Ramulu H, Drula E, Coutinho PM, Henrissat B: **The carbohydrate-active enzymes database (CAZy) in 2013**. *Nucleic Acids Res* 2014, **42**:D490-D495.

3. Eriksson K-E, Pettersson B, Westermark U: **Oxidation: an important enzyme reaction in fungal degradation of cellulose**. *FEBS Lett* 1974, **49**:282-285.

4. Lu Y, Zhang Y-HP, Lynd LR: **Enzyme–microbe synergy during cellulose hydrolysis by *Clostridium thermocellum***. *Proc Natl Acad Sci U S A* 2006, **103**:16165-16169.

5. Li S, Bashline L, Zheng Y, Xin X, Huang S, Kong Z *et al.*: **Cellulose synthase complexes act in a concerted fashion to synthesize highly aggregated cellulose in secondary cell walls of plants**. *Proc Natl Acad Sci U S A* 2016. 201613273.

6. Vanholme R, Demedts B, Morreel K, Ralph J, Boerjan W: **Lignin biosynthesis and structure**. *Plant Physiol* 2010, **153**:895-905.

7. •• Payne CM, Knott BC, Mayes HB, Hansson H, Himmel ME, Sandgren M *et al.*: **Fungal cellulases**. *Chem Rev* 2015, **115**:1308-1448.
Comprehensive and detailed review of structure/function in fungal cellulases.

8. Gilad R, Rabinovich L, Yaron S, Bayer EA, Lamed R, Gilbert HJ *et al.*: **Cell, a noncellulosomal family 9 enzyme from *Clostridium thermocellum*, is a processive endoglucanase that degrades crystalline cellulose**. *J Bacteriol* 2003, **185**:391-398.

9. •• Brunecky R, Alahuhta M, Xu Q, Donohoe BS, Crowley MF, Kataeva IA *et al.*: **Revealing nature's cellulase diversity: the digestion mechanism of *Caldicellulosiruptor bescii* CelA**. *Science* 2013, **342**:1513-1516.
Results highlight the discovery of a new paradigm for cellulose deconstruction by a multifunctional cellulase with GH9 and GH48 catalytic domains.

10. Sakon J, Irwin D, Wilson DB, Karplus PA: **Structure and mechanism of endo/exocellulase E4 from *Thermomonospora fusca***. *Nat Struct Mol Biol* 1997, **4**:810-818.

11. McCarter JD, Withers SG: **Mechanisms of enzymatic glycoside hydrolysis**. *Curr Opin Struct Biol* 1994, **4**:885-892.

12. Tiels P, Baranova E, Piens K, De Visscher C, Pynaert G, Nerinckx W *et al.*: **A bacterial glycosidase enables mannose-6-phosphate modification and improved cellular uptake of yeast-produced recombinant human lysosomal enzymes**. *Nat Biotechnol* 2012, **30**:1225-1231.

13. McIntosh LP, Hand G, Johnson PE, Joshi MD, Korner M, Plesniak LA *et al.*: **The pKa of the general acid/base carboxyl group of a glycosidase cycles during catalysis: a 13C-NMR study of bacillus circulans xylanase**. *Biochemistry* 1996, **35**:9958-9966.

14. Wilson DB: **Microbial diversity of cellulose hydrolysis**. *Curr Opin Microbiol* 2011, **14**:259-263.

15. Boraston AB, Bolam DN, Gilbert HJ, Davies GJ: **Carbohydrate-binding modules: fine-tuning polysaccharide recognition**. *Biochem J* 2004, **382**:769-781.

16. Guillen D, Sanchez S, Rodriguez-Sanoja R: **Carbohydrate-binding domains: multiplicity of biological roles**. *Appl Microbiol Biotechnol* 2010, **85**:1241-1249.

17. Reese ET, Siu RG, Levinson HS: **The biological degradation of soluble cellulose derivatives and its relationship to the mechanism of cellulose hydrolysis**. *J Bacteriol* 1950, **59**:485-497.

18. Zverlov V, Mahr S, Riedel K, Bronnenmeier K: **Properties and gene structure of a bifunctional cellulolytic enzyme (CelA) from the extreme thermophile '*Anaerocellum thermophilum*' with separate glycosyl hydrolase family 9 and 48 catalytic domains**. *Microbiology* 1998, **144**:457-465.

19. Bayer EA, Belaich JP, Shoham Y, Lamed R: **The cellulosomes: multienzyme machines for degradation of plant cell wall polysaccharides**. *Annu Rev Microbiol* 2004, **58**:521-554.

20. Bayer EA, Shimon LJ, Shoham Y, Lamed R: **Cellulosomes-structure and ultrastructure**. *J Struct Biol* 1998, **124**:221-234.

21. Xu Q, Resch MG, Podkaminer K, Yang S, Baker JO, Donohoe BS *et al.*: **Dramatic performance of *Clostridium thermocellum* explained by its wide range of cellulase modalities**. *Sci Adv* 2016, **2**:e1501254.

22. • Artzi L, Bayer EA, Morais S: **Cellulosomes: bacterial nanomachines for dismantling plant polysaccharides**. *Nat Rev Microbiol* 2017, **15**:83-95.
Comprehensive review of the concept of the cellulosome and synthetic cellulosome applications.

23. Morais S, Stern J, Kahn A, Galanopoulou AP, Yoav S, Shamshoum M *et al.*: **Enhancement of cellulosome-mediated deconstruction of cellulose by improving enzyme thermostability**. *Biotechnol Biofuels* 2016, **9**:164.

24. Arantes V, Goodell B: **Current understanding of brown-rot fungal biodegradation mechanisms: a review**. *Deterioration and*

Protection of Sustainable Biomaterials. American Chemical Society; 2014:3-21.

25. Arantes V, Jellison J, Goodell B: **Peculiarities of brown-rot fungi and biochemical Fenton reaction with regard to their potential as a model for bioprocessing biomass**. *Appl Microbiol Biotechnol* 2012, **94**:323-338.

26. • Frandsen KE, Simmons TJ, Dupree P, Poulsen JC, Hemsworth GR, Ciano L *et al.*: **The molecular basis of polysaccharide cleavage by lytic polysaccharide monooxygenases**. *Nat Chem Biol* 2016, **12**:298-303.
Authors have reported the crystal structure of an LPMO–oligosaccharide complex, paving the way forward for the understanding of molecular basis of polysaccharide cleavage by LPMOs.

27. Lo Leggio L, Simmons TJ, Poulsen JC, Frandsen KE, Hemsworth GR, Stringer MA *et al.*: **Structure and boosting activity of a starch-degrading lytic polysaccharide monooxygenase**. *Nat Commun* 2015, **6**:5961.

28. Morgenstern I, Powlowski J, Tsang A: **Fungal cellulose degradation by oxidative enzymes: from dysfunctional GH61 family to powerful lytic polysaccharide monooxygenase family**. *Brief Funct Genom* 2014, **13**:471-481.

29. Hemsworth GR, Henrissat B, Davies GJ, Walton PH: **Discovery and characterization of a new family of lytic polysaccharide monooxygenases**. *Nat Chem Biol* 2014, **10**:122-126.

30. Jung S, Song Y, Kim HM, Bae HJ: **Enhanced lignocellulosic biomass hydrolysis by oxidative lytic polysaccharide monooxygenases (LPMOs) GH61 from *Gloeophyllum trabeum***. *Enzyme Microb Technol* 2015, **77**:38-45.

31. Kojima Y, Varnai A, Ishida T, Sunagawa N, Petrovic DM, Igarashi K *et al.*: **A lytic polysaccharide monooxygenase with broad xyloglucan specificity from the brown-rot fungus *Gloeophyllum trabeum* and its action on cellulose-xyloglucan complexes**. *Appl Environ Microbiol* 2016, **82**:6557-6572.

32. Bennati-Granier C, Garajova S, Champion C, Grisel S, Haon M, Zhou S *et al.*: **Substrate specificity and regioselectivity of fungal AA9 lytic polysaccharide monooxygenases secreted by *Podospora anserina***. *Biotechnol Biofuels* 2015, **8**:90.

33. • Muller G, Varnai A, Johansen KS, Eijsink VG, Horn SJ: **Harnessing the potential of LPMO-containing cellulase cocktails poses new demands on processing conditions**. *Biotechnol Biofuels* 2015, **8**:187.
Results highlight the importance of LPMOs in enzyme cocktails and the need to rethink bioprocess procedures based on the key determinants of LPMOs activity, that is, electron donors and molecular oxygen.

34. Forsberg Z, Mackenzie AK, Sorlie M, Rohr AK, Helland R, Arvai AS *et al.*: **Structural and functional characterization of a conserved pair of bacterial cellulose-oxidizing lytic polysaccharide monooxygenases**. *Proc Natl Acad Sci U S A* 2014, **111**:8446-8451.

35. Holwerda Evert K, Ellis Lucas D, Lynd Lee R: **Development and evaluation of methods to infer biosynthesis and substrate consumption in cultures of cellulolytic microorganisms**. *Biotechnol Bioeng* 2013, **110**.

36. Zhang Y-HP, Lynd LR: **Cellulose utilization by *Clostridium thermocellum*: bioenergetics and hydrolysis product assimilation**. *Proc Natl Acad Sci U S A* 2005, **102**:7321-7325.

37. Rabinowitz JD, Aristilde L, Amador-Noguez D: *Metabolomics of Clostridial Biofuel Production. No. SC0006839_FTR*. 2015.

38. •• Lynd LR, Guss AM, Himmel ME, Beri D, Herring C, Holwerda EK *et al.*: **Advances in consolidated bioprocessing using *Clostridium thermocellum* and *Thermoanaerobacter saccharolyticum***. *Industrial Biotechnology: Microorganisms*. Wiley-VCH Verlag & Co. K; 2016:365-394.
Includes consideration of bioenergetics, an update of organism development for CBP a detailed comparison of the biomass deconstruction capability of *C. thermocellum* and commercial fungal cellulase.

39. Dumitrache A, Wolfaardt G, Allen G, Liss SN, Lynd LR: **Form and function of *Clostridium thermocellum* biofilms**. *Appl Environ Microbiol* 2013, **79**:231-239.

40. Wang Z-W, Lee S-H, Elkins JG, Morrell-Falvey JL: **Spatial and temporal dynamics of cellulose degradation and biofilm formation by *Caldicellulosiruptor obsidiansis* and *Clostridium thermocellum***. *AMB Express* 2011, **1**:30.

41. Pollock GH, Pollock GH: *The Fourth Phase of Water: Beyond Solid, Liquid, and Vapor*. Seattle: Ebner and Sons; 2013.

42. Matthews JF, Skopec CE, Mason PE, Zuccato P, Torget RW, Sugiyama J *et al.*: **Computer simulation studies of microcrystalline cellulose Iβ**. *Carbohydr Res* 2006, **341**:138-152.

43. • Paye JMD, Guseva A, Hammer SK, Gjersing E, Davis MF, Davison BH *et al.*: **Biological lignocellulose solubilization: comparative evaluation of biocatalysts and enhancement via cotreatment**. *Biotechnol Biofuels* 2016, **9**:8.
A controlled comparison of the biomass deconstruction effectiveness among various biocatalysts finds substantial differences.

44. Chaucheyras-Durand F, Ossa F: **REVIEW: the rumen microbiome: composition, abundance, diversity, and new investigative tools**. *Prof Anim Sci* 2014, **30**:1-12.

45. Brune A: **Symbiotic digestion of lignocellulose in termite guts**. *Nat Rev Micro* 2014, **12**:168-180.

46. Ortseifen V, Stolze Y, Maus I, Sczyrba A, Bremges A, Albaum SP *et al.*: **An integrated metagenome and -proteome analysis of the microbial community residing in a biogas production plant**. *J Biotechnol* 2016, **231**:268-279.

47. Zhou Y, Pope PB, Li S, Wen B, Tan F, Cheng S *et al.*: **Omics-based interpretation of synergism in a soil-derived cellulose-degrading microbial community**. *Sci Rep* 2014, **4**:5288.

48. Reed PT, Izquierdo JA, Lynd LR: **Cellulose fermentation by *Clostridium thermocellum* and a mixed consortium in an automated repetitive batch reactor**. *Bioresour Technol* 2014:155.

49. • Balch ML, Holwerda EK, Davis MF, Sykes RW, Happs RM, Kumar R *et al.*: **Lignocellulose fermentation and residual solids characterization for senescent switchgrass fermentation by *Clostridium thermocellum* in the presence and absence of continuous in situ ball-milling**. *Energy Environ Sci* 2017.
High solubilization yields are reported when milling during fermentation, cotreatment, is used in lieu of thermochemical pretreatment.

50. Weimer PJ, Russell JB, Muck RE: **Lessons from the cow: what the ruminant animal can teach us about consolidated bioprocessing of cellulosic biomass**. *Bioresour Technol* 2009, **100**:5323-5331.

51. Olson DG, Sparling R, Lynd LR: **Ethanol production by engineered thermophiles**. *Curr Opin Biotechnol* 2015, **33**:130-141.

52. Fabian J, Zlatanovic S, Mutz M, Premke K: **Fungal-bacterial dynamics and their contribution to terrigenous carbon turnover in relation to organic matter quality**. *ISME J* 2017, **11**:415-425.

53. •• García-Palacios P, McKie BG, Handa IT, Frainer A, Hättenschwiler S: **The importance of litter traits and decomposers for litter decomposition: a comparison of aquatic and terrestrial ecosystems within and across biomes**. *Funct Ecol* 2016, **30**:819-829.
The authors re-analyzed the published data from aquatic and terrestrial ecosystems to investigate the major drivers for decomposition between these two systems.

54. Wu S, Ren Y, Peng C, Hao Y, Xiong F, Wang G *et al.*: **Metatranscriptomic discovery of plant biomass-degrading capacity from grass carp intestinal microbiomes**. *FEMS Microbiol Ecol* 2015, **91**:fiv107.

55. Jetten MSM: **The microbial nitrogen cycle**. *Environ Microbiol* 2008, **10**:2903-2909.

56. • Wei H, Tucker MP, Baker JO, Harris M, Luo Y, Xu Q *et al.*: **Tracking dynamics of plant biomass composting by changes in substrate structure, microbial community, and enzyme activity**. *Biotechnol Biofuels* 2012, **5**:20.
The first time-course correlation study reveals the dynamic composting of yellow poplar biomass.

57. Amore A, Pepe O, Ventorino V, Aliberti A, Faraco V: **Cellulolytic *Bacillus* strains from natural habitats-a review**. *Chim Oggi-Chem Today* 2013, **3**:49-52.

58. Gupta P, Samant K, Sahu A: **Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential**. *Int J Microbiol* 2012, **2012**:5.

59. • Koeck DE, Pechtl A, Zverlov VV, Schwarz WH: **Genomics of cellulolytic bacteria**. *Curr Opin Biotechnol* 2014, **29**:171-183.
This review compiles a complete list of genome-sequenced and annotated cellulolytic bacteria in the CAZy-database.

60. •• Comtet-Marre S, Parisot N, Lepercq P, Chaucheyras-Durand F, Mosoni P, Peyretaillade E *et al.*: **Metatranscriptomics reveals the active bacterial and eukaryotic fibrolytic communities in the rumen of dairy cow fed a mixed diet**. *Front Microbiol* 2017, **8**:67.
This study conduct a metatranscriptomic analysis to reveal the relative contribution of the microbiota members and enzymes in the rumen of dairy cow. Their results show the important role of fibrolytic bacteria and support a substantial contribution of fungi and ciliate protozoa to polysaccharide degradation.

61. Brown ME, Chang MCY: **Exploring bacterial lignin degradation**. *Curr Opin Chem Biol* 2014, **19**:1-7.

62. Salvachua D, Karp EM, Nimlos CT, Vardon DR, Beckham GT: **Towards lignin consolidated bioprocessing: simultaneous lignin depolymerization and product generation by bacteria**. *Green Chem* 2015, **17**:4951-4967.

63. Boer Wd, Folman LB, Summerbell RC, Boddy L: **Living in a fungal world: impact of fungi on soil bacterial niche development**. *FEMS Microbiol Rev* 2005, **29**:795-811.

64. Bärlocher F, Boddy L: **Aquatic fungal ecology – how does it differ from terrestrial?** *Fungal Ecol* 2016, **19**:5-13.

65. Dighton J: *Fungi in Ecosystem Processes*. CRC Press; 2016.

66. Griffith GW, Baker S, Fliegerova K, Liggenstoffer A, van der Giezen M, Voigt K *et al.*: **Anaerobic fungi: *Neocallimastigomycota***. *IMA Fungus: Glob Mycol J* 2010, **1**:181-185.

67. Gruninger RJ, Puniya AK, Callaghan TM, Edwards JE, Youssef N, Dagar SS *et al.*: **Anaerobic fungi (phylum *Neocallimastigomycota*): advances in understanding their taxonomy, life cycle, ecology, role and biotechnological potential**. *FEMS Microbiol Ecol* 2014, **90**:1-17.

68. Singh T, Singh AP: **White and brown rot fungi as decomposers of lignocellulosic materials and their role in waste and pollution control**. In *Fungal Applications in Sustainable Environmental Biotechnology.* Edited by Purchase D. Cham: Springer International Publishing; 2016:233-247.

69. Daniel G: **Fungal degradation of wood cell walls**. In *Secondary Xylem Biology: Origins, Functions, and Applications.* Edited by Yoon S, Kim RF, Singh AP. Elsevier Inc.; 2015:131-167.

70. Mester T, Varela E, Tien M: **Wood degradation by brown-rot and white-rot fungi**. In *Genetics and Biotechnology.* Edited by Kück U. Berlin, Heidelberg: Springer Berlin Heidelberg; 2004:355-368.

71. Eastwood DC, Floudas D, Binder M, Majcherczyk A, Schneider P, Aerts A *et al.*: **The plant cell wall – decomposing machinery underlies the functional diversity of forest fungi**. *Science* 2011, **333**:762-765.

72. •• Kracher D, Scheiblbrandner S, Felice AKG, Breslmayr E, Preims M, Ludwicka K *et al.*: **Extracellular electron transfer systems fuel cellulose oxidative degradation**. *Science* 2016.
This study demonstrates the availability of extracellular electron sources for fungal oxidative degradation of cellulose.

73. Frey-Klett P, Burlinson P, Deveau A, Barret M, Tarkka M, Sarniguet A: **Bacterial–fungal interactions: hyphens between agricultural, clinical, environmental, and food microbiologists**. *Microbiol Mol Biol Rev* 2011, **75**:583-609.

74. Seigle-Murandi F, Guiraud P, Croize J, Falsen E, Eriksson KL: **Bacteria are omnipresent on Phanerochaete chrysosporium burdsall**. *Appl Environ Microbiol* 1996, **62**:2477-2481.

75. Dai X, Tian Y, Li J, Su X, Wang X, Zhao S *et al.*: **Metatranscriptomic analyses of plant cell wall polysaccharide degradation by microorganisms in the cow rumen**. *Appl Environ Microbiol* 2015, **81**:1375-1386.

76. Wei H, Xu Q, Taylor LE II, Baker JO, Tucker MP, Ding SY: **Natural paradigms of plant cell wall degradation**. *Curr Opin Biotechnol* 2009, **20**:330-338.

77. •• Hoppe B, Kahl T, Karasch P, Wubet T, Bauhus J, Buscot F *et al.*: **Network analysis reveals ecological links between N-fixing bacteria and wood-decaying fungi**. *PLoS ONE* 2014, **9**:e88141.
The authors assessed the diversity of nifH (dinitrogenase reductase) genes in tree species *Fagus sylvatica* and *Picea abies* from different managed forest plots. The co-occurrence patterns analysis revealed at least commensal interactions among fungi and N-fixing bacteria in the dead wood.

78. Baker JO, Adney WS, Thomas SR, Nieves RA, Chou Y-C, Vinzant TB *et al.*: **Synergism between purified bacterial and fungal cellulases**. *Enzymatic Degradation of Insoluble Carbohydrates*. American Chemical Society; 1996:113-141.

79. Boonchan S, Britz ML, Stanley GA: **Degradation and mineralization of high-molecular-weight polycyclic aromatic hydrocarbons by defined fungal-bacterial cocultures**. *Appl Environ Microbiol* 2000, **66**:1007-1019.

80. Lackner G, Moebius N, Hertweck C: **Endofungal bacterium controls its host by an hrp type III secretion system**. *ISME J* 2011, **5**:252-261.

81. Seneviratne G, Zavahir JS, Bandara WMMS, Weerasekara MLMAW: **Fungal-bacterial biofilms: their development for novel biotechnological applications**. *World J Microbiol Biotechnol* 2007, **24**:739.

82. Nazir R, Tazetdinova DI, van Elsas JD: ***Burkholderia terrae* BS001 migrates proficiently with diverse fungal hosts through soil and provides protection from antifungal agents**. *Front Microbiol* 2014, **5**:598.

83. Gunathilake K, Ratnayake R, Kulasooriya S, Karunaratne D: **Evaluation of cellulose degrading efficiency of some fungi and bacteria and their biofilms**. *J Natl Sci Found Sri Lanka* 2013, **41**:155-163.

84. Murray AC, Woodward S: **In vitro interactions between bacteria isolated from Sitka spruce stumps and *Heterobasidion annosum***. *Forest Pathol* 2003, **33**:53-67.